DIAGNOSIS, TREATMENT AND SURVEILLANCE OF DIAMOND BLACKFAN ANEMIA (DBA) SYNDROME: INTERNATIONAL CONSENSUS STATEMENT

Marcin W. Wlodarski, MD, PhD[1,2*#], Prof Adrianna Vlachos, MD[3,4,5*], Jason E. Farrar, MD[6*], Prof Lydie M. Da Costa, MD, PhD[7,8,9], Prof Antonis Kattamis, MD[10], Irma Dianzani, MD, PhD[11], Cristina Belendez, MD[12, 13,14,15], Prof Sule Unal, MD[16], Prof Hannah Tamary, MD[17,18], Ramune Pasauliene, MD[19], Prof Dagmar Pospisilova, MD, PhD[20], Josu de la Fuente, PhD, FRCP [21,22], Deena Iskander, MD, PhD, FRCPath[21,22], Prof Lawrence Wolfe, MD[3,5], Prof Johnson M. Liu, MD[23], Prof Akiko Shimamura, MD, PhD[24], Katarzyna Albrecht, MD, PhD[25], Birgitte Lausen, MD, PhD[26], Anne Grete Bechensteen, MD, PhD[27], Ulf Tedgard, MD, PhD[28], Alexander Puzik, MD[2], Paola Quarello, MD[29], Prof Ugo Ramenghi, MD[29], Marije Bartels, MD, PhD[30], Heinz Hengartner, MD[31], Prof Roula A. Farah, MD[32], Mahasen Al Saleh, MD[33], Prof Amir Ali Hamidieh, MD[34], Wan Yang, MD[35], Etsuro Ito, MD, PhD[36], Prof Hoon Kook, MD, PhD[37], Galina Ovsyannikova, MD, PhD[38], Prof Leo Kager, MD, PhD[39], Prof Pierre-Emmanuel Gleizes, PhD[40], Prof Jean-Hugues Dalle, MD, PhD[41], Brigitte Strahm, MD[2], Prof Charlotte M. Niemeyer, MD[2,42], Prof Jeffrey M. Lipton, MD, PhD[3,4,5*], Thierry M. Leblanc, MD[41*].

On behalf of the international DBA syndrome guideline panel (additional participants: appendix 1).

*Equally contributing authors

Author affiliations:

1. Department of Hematology, St. Jude Children's Research Hospital, Memphis, TN, USA.
2. Division of Pediatric Hematology and Oncology, Department of Pediatrics and Adolescent Medicine, Medical Center, Faculty of Medicine, University of Freiburg, Freiburg, Germany.
3. Cohen Children's Medical Center, Hematology/Oncology and Cellular Therapy, New Hyde Park, New York, USA.
4. Feinstein Institutes for Medical Research, Manhasset, New York, USA.
5. Zucker School of Medicine at Hofstra/Northwell, Hempstead, New York, USA.
6. Arkansas Children's Research Institute and Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA.
7. Hôpital R. DEBRE, Groupe Hospitalier Universitaire, Assistance Publique-Hôpitaux de Paris Nord, Université de Paris Cité, F-75019, Paris, France
8. HEMATIM, EA4666, UPJV, F-80000, Amiens, France
9. Laboratoire d'excellence des globules rouges - GR-Ex, France.
10. First Department of Pediatrics, National and Kapodistrian University of Athens, Greece.
11. Department of Health Sciences, Università del Piemonte Orientale, Novara, Italy.
12. Pediatric Hematology and Oncology Department, Hospital Universitario Gregorio Marañón, Madrid, Spain.
13. Facultad de Medicina, Universidad Complutense de Madrid, Spain.
14. Instituto Investigación Sanitaria Gregorio Marañón, Madrid, Spain.
15. Instituto Nacional de Investigación Biomédica en Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain.
16. Hacettepe University, Department of Pediatric Hematology and Research Center for Fanconi Anemia and Other Inherited Bone Marrow Failure Syndromes, Ankara, Turkey
17. The Rina Zaizov Hematology-Oncology Division, Schneider Children's Medical Center of Israel.
18. Felsenstain Medical Research Center, Sackler School of Medicine, Tel-Aviv University, Israel.
19. Vilnius University Hospital Santaros Klinikos, Lithuania.
20. Department of Pediatrics, Faculty Hospital of Palacky University, Czech Republic.
21. Department of Paediatrics, St. Mary's Hospital, Imperial College Healthcare NHS Trust, London, UK.
22. Department of Immunology & Inflammation, Imperial College London, London, UK.
23. Division of Hematology/Oncology, Icahn School of Medicine at Mount Sinai, The Tisch Cancer Institute, New York, NY, USA
24. Dana Farber/Boston Children's Cancer and Blood Disorders Center, Harvard Medical School, Boston, USA.
25. Department of Oncology, Paediatric Haematology, Clinical Transplantology and Paediatrics, Medical University of Warsaw, Warsaw, Poland.
26. Department of Pediatrics and Adolescent Medicine, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark.
27. Department of Pediatric Hematology and Oncology, Oslo University Hospital, Oslo, Norway.
28. Department of Pediatric Hematology and Oncology, Skåne University Hospital, Lund, Sweden.
29. Department of Pediatric and Public Health Sciences, University of Turin, Turin, Italy.
30. Pediatric Hematology Department, University Medical Center Utrecht, Utrecht, The Netherlands.
31. Pediatric Hospital of Eastern Switzerland St. Gallen, Switzerland.
32. Department of Pediatrics, LAU Medical Center, Rizk Hospital- Beirut, Lebanon.
33. King Faisal Hospital and Research Center Riyadh, Saudi Arabia.
34. Pediatric Cell and Gene Therapy Research Center, Gene, Cell & Tissue Research Institute, Tehran University of Medical Sciences, Iran.
35. State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology and Blood Diseases Hospital, Chinese Academy of Medical Sciences, Peking Union Medical College, China.
36. Department of Pediatrics, Hirosaki University Graduate School of Medicine, Japan
37. Chonnam National University Hwasun Hospital, Gwangju, South Korea.
38. Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russia.
39. St. Anna Children's Hospital, Department of Pediatrics, Medical University Vienna; Children's Cancer Research Institute, Vienna, Austria.
40. MCD, Centre de Biologie Intégrative, Université de Toulouse, CNRS, UT3, 31000 Toulouse, France.
41. Pediatric Immunology and Hematology Department, and CRMR aplasies médullaires. Robert Debré Hospital, Groupe Hospitalier Universitaire, Assistance Publique-Hôpitaux de Paris Nord, Université de Paris Cité, Paris, France
42. German Cancer Consortium (DKTK), Freiburg, Germany. German Cancer Research Center (DKFZ), Heidelberg, Germany.

Corresponding author:
Marcin W. Wlodarski, MD, PhD
Department of Hematology, St. Jude Children's Research Hospital, Memphis, TN, USA
Tel: +1901-907-1947, Marcin.wlodarski@stjude.org

Running title: **2024 DBA Syndrome International Consensus**

Key words: Diamond Blackfan anemia, DBA, ribosomopathy, clinical guidelines, consensus
Word count: 5365

ABSTRACT

Diamond Blackfan anemia (DBA), first described over 80 years ago, is a congenital disorder of erythropoiesis with a predilection for birth defects and cancer. Despite scientific advances, this chronic, debilitating, life-limiting disorder continues to exact a substantial physical, psychological, and financial toll on patients and their families.

The highly complex medical needs of affected patients require specialized expertise and multidisciplinary care. However, gaps remain in effectively bridging scientific discoveries to clinical practice and disseminating the latest knowledge and best practices to providers. Following the publication of the first international consensus in 2008, advances in our understanding of the genetics, natural history and clinical management of DBA have strongly supported the need for new consensus recommendations. In 2014 in Freiburg, Germany, a panel of 53 experts including clinicians, diagnosticians, and researchers from 27 countries convened. With support from patient advocates, the panel met repeatedly over subsequent years, engaging in ongoing discussions. This led to the development of these new 2024 consensus recommendations, replacing the previous guidelines. To account for the diverse phenotypes including presentation without anemia, the panel agreed to adopt the nomenclature "DBA syndrome". We propose new simplified diagnostic criteria, describe the genetics of DBA syndrome and its phenocopies, and introduce major changes in therapeutic standards, including lowering the prednisone maintenance dose to maximum 0.3mg/kg/day, raising the pre-transfusion hemoglobin to 9-10g/dL independent of age, recommending early aggressive chelation, broadening indications for hematopoietic stem cell transplantation, and recommending systematic clinical surveillance including early colorectal cancer screening. In summary, the current practice guidelines standardize the diagnostics, treatment, and long-term surveillance of patients with DBA syndrome of all ages worldwide.

Funding

DBA Foundation USA, DBA UK Charity, Fritz-Thyssen Stiftung and Eva Uth Foundation, Germany (support for in-person meetings), R01HL079571 to JML/AV and R01HL150194 to JEF.

MAIN

1. RATIONALE

Diamond Blackfan anemia (DBA) is a rare, clinically and genetically heterogeneous inherited bone marrow failure syndrome (IBMFS).[1,2] Because of its rarity, advances in clinical care must extend to settings where the disorder is only infrequently encountered. In 2008 our first clinical consensus was developed to review the criteria for diagnosis and evaluate the available treatment options.[3] Advances in clinically relevant research (including discovery of new DBA-associated genes, new advances in iron burden assessment, and new knowledge on the epidemiology of cancers) and disparities in management and surveillance across centers strongly supported the need for a new set of recommendations. The present guidelines were developed to standardize the diagnostic process and management and to improve long-term outcomes for patients with DBA worldwide. They are not an absolute standard applicable to all clinical scenarios and resources. However, resource limitations should not reduce efforts to deliver the best possible care.

2. METHODS

An international panel of 53 representatives from 27 countries, recognized as key opinion leaders in DBA diagnosis and management, was appointed by the leaders of the European DBA (EuroDBA) consortium and the DBA Registry of North America (DBAR), see authors and extended authors (**appendix 1**). The objective was to revise and replace the previous 2008 guidelines (**appendix 2**). Panel composition, search strategy and evidence level grading are outlined in **appendix 3.** As no level A or B evidence exists for the DBA, the panel relied on level C evidence, including published work, unpublished updates from registries[4-17] and participant expertise/experience. We used a modified Delphi technique involving iterative voting on key topics identified from expert judgments and available data until >85% consensus was reached.[18] Items lacking consensus were left with multiple options, as detailed. An outline of the contents and tables is shown in **Figure 1.**

3. DEFINITION OF THE SYNDROME

DBA has historically been defined as a macrocytic anemia with reticulocytopenia and a paucity of bone marrow (BM) erythroid precursors, presenting at less than one year of age.[19] However, we acknowledge that i) variable phenotypes exist within and among DBA genotypes, ii) some individuals with DBA-associated gene mutations paradoxically lack anemia, and iii) the diagnosis may occur in adulthood.[20-22] Therefore, we adopt the term "Diamond Blackfan anemia (DBA) syndrome", encompassing classic DBA, with an incidence of approximately 5-10 cases per million live births and an equal gender ratio, and a broader range of phenotypes. Most patients present with a reticulocytopenic (hyporegenerative/hypoplastic) anemia +/- additional findings. However, a variety of phenotypes may be encountered with/without anemia or other cytopenias, B-cell lymphopenia, congenital anomalies, or cancer. For example, congenital heart disease can manifest in ribosomal protein (RP) gene mutation carriers without anemia.[21] We restrict the genetic nosology to genes encoding either small (RPS) or large (RPL) subunit-associated RP or their chaperones resulting in RP haploinsufficiency (ribosomopathy), the erythroid transcription factor gene *GATA1*[23] and gain-of-function mutations in *TP53*.[24,25] Phenocopies with different pathophysiology are mentioned but remain distinct from DBA syndrome.[24,26-29]

4. CLINICAL PRESENTATION

The hematological presentation of DBA syndrome was initially termed congenital hypoplastic anemia, a description preferred by Dr. Diamond.[19] Classically, macrocytic anemia and reticulocytopenia present initially, with 90% of patients becoming symptomatic in the first year of life (median age 3 months).[30] However, anemia can manifest in utero as hydrops fetalis[31,32] or, as late as the sixth decade of life, where DBA syndrome could be misdiagnosed as acquired pure red cell aplasia (PRCA) or myelodysplastic syndrome (MDS).[30] There is a shift towards an older age at diagnosis due to an improved index of suspicion in adults. In unpublished UK DBA syndrome analylsis, only 77% (112/146) of patients presented with anemia in infancy. Other hematopoietic abnormalities at diagnosis including thrombocytosis (only in infants), thrombocytopenia or neutropenia are generally clinically insignificant. A study evaluating 38 children, found that 21 had thrombocytosis, while 12 had thrombocytopenia, with 3 also exhibiting leukopenia.[33] Patients with *RPL35A* genotype often present with severe neutropenia and immunodeficiency.[34] Impaired cellular and humoral immunity is observed, with up to 55% of patients showing quantitative deficits in serum immunoglobulins and/or circulating T, natural killer and B lymphocytes, arising independently of steroid treatment or RP genotype.[35] Patients with *GATA1* mutation may present with thrombocytopenia and neutropenia,[23] with some exhibiting dyserythropoiesis and dysmegakaryopoiesis, representing a distinct disease subset.[36] Hemolysis, hepatomegaly or splenomegaly are not characteristic. In patients with anemia, diagnostic BM evaluation shows absent or diminished erythroid activity with left shifted erythroid precursors, rarely progressing beyond the pronormoblast stage, with normal erythroid morphology. Dyserythropoietic features (outside of *GATA1*), ring sideroblasts or vacuoles are not observed, and other lineages lack morphologic abnormalities at presentation. Although initially normocellular, the marrow often becomes increasingly hypocellular with age, as demonstrated in steroid-refractory patients, of whom 75% developed moderate to severe BM hypoplasia over time, with 43% and 29% of those patients developing neutropenia and thrombocytopenia, respectively.[37] Our unpublished experience confirms the findings of hypocellular BM in the majority of DBA syndrome patients over time. However, severe hypocellularity and pancytopenia as seen in severe aplastic anemia is not a feature of DBA syndrome. Increased hematologic toxicity (e.g., delayed count recovery) has been reported in patients receiving radio/chemotherapy.[38]

5. ESTABLISHING THE DIAGNOSIS

Diagnosis involves assessing patient history, clinical evaluation, laboratory testing of peripheral blood and BM, and genetic analysis. To capture variable presentations, we agreed on streamlined diagnostic criteria requiring only one of the following: 1) Pathogenic/likely pathogenic mutation in a DBA syndrome–associated gene, or 2) hematologic features consistent with DBA syndrome, after exclusion of other known differential diagnoses (**Table 1**). The presence of a pathogenic or likely pathogenic variant according to ACMG/AMP classification[39] is sufficient for diagnosis and allows for inclusion of patients with DBA syndrome who are currently asymptomatic. Establishing a diagnosis based on genetics alone has limitations as current gene panels may lack newly discovered genes and some genetic variants are difficult to interpret. Additionally, 20-30% of cases have an unknown genetic origin. Therefore, DBA syndrome can also be diagnosed by the presence of characteristic hematologic features in peripheral blood and BM after ruling out other differential diagnoses (**Table 1**). Other common findings such as elevated erythrocyte adenosine deaminase (eADA) activity[40] and rRNA processing

defect[41,42] are not required for diagnosis. Recommended diagnostic tests agreed on by our panel are shown in **Table 2**. In addition to essential tests, further initial evaluations are recommended in all patients to fully characterize the clinical phenotype and guide management. While a diagnosis can be made based on hematologic features alone after excluding other conditions, genetic testing should be promptly performed. Patients diagnosed via genetics alone (e.g., infants with typical manifestations, those with a positive family history and segregation of the mutation) should undergo confirmatory BM evaluation, though this is typically deferred in infants until prior to starting steroid treatment. DBA syndrome should also be considered in those with family history, macrocytosis, DBA syndrome-associated congenital anomalies and cancers, or excessive chemotherapy-associated hematopoietic toxicity, regardless of anemia.

6. GENETICS (gene table in Table 3)

Currently, published data indicate 70-80% of DBA syndrome patients have an identifiable genetic defect. The panel defined two genetic categories of DBA syndrome: 1) **Ribosomopathy**: mutations in genes directly involved in ribosome biogenesis, including 11 *RPS* and 13 *RPL* genes, and the RP chaperones *TSR2* (*RPS26* chaperone) and *HEATR3* (nuclear import of *RPL5*); and 2) **Other**: *GATA1* and *TP53* with gain-of-function mutations (unlike the loss-of-function mutations found in Li-Fraumeni syndrome) because these diseases can manifest with hyporegenerative anemia and have altered pathways directly connected to DBA-ribosomopathy. Additionally, 7 RP candidate genes require functional validation. We excluded erythropoietin (*EPO*) mutation[26] and ADA2 deficiency as distinct phenocopies with different mechanisms and associated clinical features from DBA syndrome.[43,44] The majority of DBA syndrome-associated genes are autosomal dominant, with 8 affecting 50-65% of cases (*RPS19* [~25%], *RPL5* [7-10%], *RPL11* [~5%], *RPS26* [3-6%], *RPS10* [3-6%], *RPS24* (2-4%), *RPL35A* [2-4%], and *RPS17* [1-3%]). X-linked inheritance is seen with *GATA1* and *TSR2* and autosomal recessive inheritance with *HEATR3*. DBA syndrome is de novo or sporadic in two-thirds and familial in one-third of cases, with variable expressivity and penetrance within families.[4,20,45,46]

Most genetic alterations affect RP genes and are frequently due to single nucleotide variants at the start codon, nonsense, indels or splice-site mutations inducing nonsense-mediated transcript decay, or genomic deletions (individual exons, whole gene, or multigenic deletions). Missense coding changes are less frequently implicated and appear enriched in small subunit RP genes.[17] While nonsense mutations in RP genes almost always result in DBA,[47] missense variants can be challenging to interpret and may require additional validation (e.g., rRNA processing studies or animal models).[48]

The panel recommends genetic analysis for every patient with suspected DBA syndrome. Due to variable penetrance, family studies should also be pursued after identifying a pathogenic variant in the proband. Testing laboratories offer either stepwise or all-in-one testing to identify mutations, exon/gene deletions, or contiguous gene microdeletions using gene panel, array, whole exome or whole genome sequencing.[49,50] Genetic testing can lead to a revised diagnosis distinct from DBA syndrome (e.g.. ADA2 deficiency, or Shwachman-Diamond syndrome), thus some panelists prefer simultaneous testing for DBA syndrome genes, phenocopies and other inherited IBMFS genes. Variant interpretation should follow ACMG/AMP guidelines.[39] The ClinGen consortium establishes disease-specific variant specification rules; similarly standardized, DBA syndrome-specific rules are needed to enable accurate and uniform variant interpretation.

7. CONGENITAL ABNORMALITIES

More than half of DBA syndrome patients have a congenital abnormality, including craniofacial dysmorphisms (cleft lip and/or palate and others), cardiac, radial ray (thumb), urogenital, and eye malformations **(Table 4)**.[4,45,51-53] More than one anomaly occurs in ~25% of patients.[7] Careful physical examination and imaging are vital, as subtle anomalies may not be apparent on initial evaluation, leading to delayed detection and management of consequential defects. Clinical expressivity varies within kindreds. Patients with large genomic deletions often have complex malformations and developmental delay.[34]

Most congenital anomalies are genotype-nonspecific with only few established associations, including abnormal thumbs in *RPL5*/*RPL11*, cleft lip and/or palate in *RPL5*/*RPL11*/*RPS26* genotypes.[17,54,55] More than one anomaly more frequently occurs with *RPL5/RPL11* mutations compared to *RPS19*.[55] Congenital anomalies overall are more frequent with *RPL* than *RPS* genotypes.[17,47] Short stature is common, but confounded by steroid use, chronic anemia, and iron overload.[56] Severe colitis recently emerged as a new association in DBA syndrome.[57-60] Many issues are disease-intrinsic, but some may be therapy-related (**Table 5**).

8. DIFFERENTIAL DIAGNOSES

Differential diagnoses include acquired or genetic conditions resulting in isolated erythroid failure (**Table 1**). Steroid treatment should be avoided in the absence of a DBA syndrome diagnosis. Transient erythroblastopenia of childhood (TEC), although rare,[61] remains the leading differential diagnosis. Viral infections may inhibit erythropoiesis, with parvovirus B19 (B19V), due to its tropism for the erythroid CFU-E progenitor, specifically causing suppression of erythropoiesis (for details see **appendix 4**).[62] Most diagnostic tests can wait until after a life-saving red blood cell (RBC) transfusion except measuring eADA activity (done pre-transfusion).[63] The mechanism underlying elevated eADA activity in DBA syndrome remains unclear. However, eADA testing helps differentiate DBA syndrome from other anemias/IBMFS, with 84% sensitivity and 95% specificity.[64] Substantially elevated eADA strongly suggests DBA syndrome, but normal eADA does not rule it out, particularly in patients with *GATA1* mutations[65]. If genetics is inconclusive, other hereditary conditions mimicking DBA syndrome should be excluded (**Table 1**).[28,66] ADA2 deficiency is a common phenocopy (accounting for 6% of genetically undiagnosed patients in the German DBA registry).[29] An important differential in adults is acquired *RPS14*-haploinsufficient 5q deletion associated with PRCA/macrocytosis.[67]

9. THERAPY RECOMMENDATIONS

The primary goal is to ensure an acceptable hemoglobin (Hb) level, generally defined as ≥9-10g/dL (≥90-100g/L), and to enable adequate growth and development in children and good quality of life in adults. However, the goal Hb must be individualized to ensure asymptomatic status. Advances are being made in the development of new therapies. However, as of 2024, the 3 therapeutic options remain: RBC transfusions with iron chelation, corticosteroid therapy, and HSCT. Supplementation with folate, or other vitamins or trace elements is not indicated. Birth defects must be repaired to the extent possible.

Red blood cell transfusions

Our panel unanimously agreed that restrictive transfusion strategies (with a "transfusion trigger" at Hb 6-7/dL) as applied in other fields **are harmful to patients with DBA syndrome.** Many patients will require life-long RBC transfusions to maintain Hb levels sufficient for normal growth, development, and quality of life. We agreed that the therapeutic nadir (pre-transfusion) Hb should be maintained at **≥9-10/dL** life-long (**Table 6**). This usually requires 10-15 ml/kg packed RBC in children or 2-3 RBC units in adults, every ~3 weeks, although some patients need higher volumes (~20ml/kg) or "catch-up" transfusions. A higher Hb nadir may be needed for good quality of life with regard to school, work, social life, and exercise tolerance. Every transfused patient should be vaccinated against hepatitis B and regularly monitored for hepatitis B, C, and HIV. RBC antigen typing by serologic/molecular methods and RBC leukoreduction should be performed per local standards. Although alloimmunization is not common in DBA syndrome, some panelists prefer repeating extended RBC antibody screening before each transfusion, with crossmatching as needed. Patients developing atypically high transfusion needs should undergo testing to rule out increased RBC destruction or blood loss.

Oral steroids

Corticosteroids (steroids) have been successfully used in treating DBA syndrome for >70 years. The standard are equally potent oral prednisone or prednisolone.[68] The panel agreed on the following indications, timing, and therapeutic considerations (**Table 7**: **1) Starting criteria:** Steroids can be initiated in any transfusion-dependent patient, after reaching the age of 1 year, and optimally after administration of the first live vaccines (MMRV) and a diagnostic BM evaluation. **2) Starting dose:** Initial prednisone dose is 2mg/kg/day (maximum 80mg per day) in children or 80mg/day in adults, given once daily in the morning or divided into two equal doses. The panel strongly opposed the use of higher doses. There was no consensus on when to initiate prednisone in relation to the last transfusion: starting concurrently (e.g., one day after), or delaying ~10-14 days post transfusion are both acceptable approaches. Extending the initial dose beyond 4 weeks is strongly discouraged due to lack of added benefit and harmful side effects. **3) Response assessment and tapering:** To assess for initial response, reticulocytes and Hb are measured 10-14 days after starting steroids. If a significant reticulocytosis (≥50-100x10^9/L) is observed and Hb is stable/increasing, tapering should start within 2-4 weeks, decreasing by 0.5mg/kg/day every ~2 weeks until reaching 0.5mg/kg/day. Thereafter tapering should proceed over months to find the lowest effective maintenance dose. The panel agreed the maximum long-term maintenance dose should not exceed 0.3mg/kg/day or 0.6mg/kg on alternate days. Based on experience and published evidence from other conditions, alternate-day dosing seems equally effective to daily dosing[69] A more restrictive threshold for maximum maintenance dose of 0.2mg/kg/day was suggested by some panelists, without consensus. Experience with adult patients is limited, but the panel agreed the maintenance dose in adults should not exceed 10-15mg/day.[68] There was no consensus on the optimal approach for further tapering to a minimally effective dose sufficient for Hb ≥9g/dL. Some providers prefer "active weaning" with gradual dose reduction to the minimally effective level, with Hb monitoring. Others do "passive weaning", allowing the patient to outgrow the dose while closely monitoring linear growth and side effects. Importantly, many patients require minuscule doses (e.g., 0.05mg/kg/day) for continued response. If the response is lost during weaning, the dose should be immediately increased to the previous level at which the Hb was ≥9g/dL. Failure to do so may result in a total loss of response, requiring the process be restarted at 2mg/kg/day. If the Hb cannot be sustained at ≥9g/dL with ≤0.3mg/kg/day, steroids should be discontinued. If a patient does not respond to 2mg/kg/day prednisone within 4 weeks, the

drug must be discontinued promptly (there was no consensus whether to recommend gradual tapering versus abrupt stopping, so providers should adhere to local standard). Endocrinologist should be involved for monitoring of adrenal insufficiency during tapering and long-term maintenance therapy. **4) Response definitions:** Initial prednisone responsiveness (**Table 7** is achieved in ~60-80% of cases.[7,11,45] Over time patients may lose responsiveness or prednisone must be discontinued due to unacceptable side effects. Across age groups, ~30-40% of patients remain on steroids, while maintaining a durable response[45,70]. A steroid non-responsive patient is defined as a patient who cannot reach Hb ≥9g/dL after 4 weeks of 2mg/kg/day prednisone, or requires ≥0.3mg/kg/day to maintain a Hb ≥9g/dL. **5) Steroid holiday**: Some patients may benefit from a temporary pause in steroid administration for 1-3 years during or before puberty to optimize growth. The panel agreed based on their clinical experience that the majority of patients regain steroid responsiveness after a "steroid holiday", although definitive evidence is lacking. **6) Subsequent steroid trials**: In non-responders, a second trial (>1-2 years later) is a reasonable option, especially before a planned HSCT. Toxicity monitoring and supportive care recommendations are outlined in **Table 7**

Treatment-independence (formerly "remission").

Approximately 20% of patients previously treated with steroids or transfusions may become "treatment-independent", able to discontinue all therapy for anemia. For instance, analysis of 222 treated DBA syndrome patients in the French registry showed a 21% rate of treatment independence, with 30% for initially steroid responsive and 5% steroid non-responsive patients with approximately 70% achieving this during the first decade of life.[4] While treatment-independence often persists long-term[71], anemia can return and emerging registry data suggests that cancer risk persists. Thus, the term "clinical remission" should be avoided. All patients require life-long surveillance as neither steroid response nor genetics predict treatment-independence.

Chelation therapy

Iron overload in DBA syndrome

Transfusion-associated iron overload and cancer are the leading causes of death in non-transplanted patients.[72] Increased iron absorption is not expected; RBC transfusions are the main source of iron. Without adequate chelation, even modest transfusion volumes lead to significant iron accumulation, and organ dysfunction, especially in the liver, heart, and endocrine glands. Iron overload develops early during chronic transfusions[73] and the non-transferrin bound iron (NTBI), present when transferrin saturation exceeds 60–70%, plays a key role in tissue iron deposition. NTBI levels are increased in transfused patients with DBA syndrome compared to thalassemia or sickle cell disease,[74] likely because iron is not utilized in RBC production. Consequently, patients with DBA syndrome develop iron overload more rapidly compared to patients with thalassemia and frequently develop cardiac complications.[75] Early pancreatic iron loading is also more prominent in DBA syndrome.[76]

Evaluation of iron overload

Serum ferritin is not a reliable indicator of iron overload in DBA syndrome. Besides low accuracy it may be affected by factors such as inflammation.[77] High ferritin together with elevated transferrin saturation can confirm initial iron overload before starting chelators (**Table 8**. Liver iron concentration (LIC), measured by MRI using T2* or R2 (FerriScan) methods accurately reflects total body iron burden[78] and should be strongly

advocated as the standard for chelation guidance. The target LIC for transfusion-dependent patients with DBA syndrome is as close as possible to normal, i.e., lower than 3mg Fe/g dry weight.[79] Cardiac iron measurement using MRI T2* has been shown to be highly sensitive and reproducible.[80] In healthy volunteers, a mean T2* value >40 ms has been considered normal.[81] Cardiac T2* <20msec is associated with decreased cardiac function and a worse survival, while T2* values <10msec show clear association with an imminent risk of heart failure.[82] Emerging data supports that T2* >35msec indicates no relevant cardiac iron load and reflects little risk for heart dysfunction.[83,84] Thus, the panel recommended that the cardiac T2* should remain as close to normal as possible, with an acceptable range of 20-35 msec. Many of the panelists argued to aim for a stricter T2* >25msec. Hemosiderosis of the pancreas and the pituitary gland is predictive of the development of endocrinopathies. However, pancreatic, and pituitary iron loading is difficult to measure due to the size, shape, and location of these organs.

The panel recommends initiating MRI-based liver and cardiac iron assessment as early as feasible, by age 5 years at the latest, and repeating yearly (or more often if needed) in all chronically transfused patients. While MRI can be performed without sedation starting ~5 years old, earlier evaluation under procedural sedation should be considered to allow for early detection and monitoring of iron burden in young transfusion-dependent children.

Treatment of iron overload

The goal of iron chelation therapy is to eliminate enough iron to reduce the harmful effects of excess iron from transfusions, control NTBI and achieve neutral or negative total body iron balance. Three drugs are available (**Table 8**. **Deferoxamine/Desferrioxamine (DFO)** is a parenteral chelator with a half-life of 20 minutes, requiring prolonged (usually subcutaneous) administration. Growth retardation and bone changes occur in young children with thalassemia receiving higher DFO doses. Although such data do not exist for DBA syndrome, the panel agreed that DFO should be given at a lower dose (≤30mg/kg) in patients <3 years old. Ototoxicity (sensineuronal hearing loss, tinnitus) is a significant side effect of DFO that is likely related to individual susceptibility as there are no reliable predictive variables.[85] Despite good compliance some patients still develop cardiac iron loading. Intensive chelation with 24 hours per day intravenous DFO can reduce cardiac iron, often in conjunction with another chelator. **Deferasirox (DFX)** is an oral chelator with a half-life of 8-16 hours allowing once-daily dosing. DFX has shown linear dose-dependent effects on iron excretion, accomplished primarily through the biliary tract. The efficacy of DFX in achieving a significant reduction of iron load was demonstrated across a wide range of patients with transfusion-dependent anemias.[86] The most common adverse events are dose-dependent, transient and of mild to moderate severity, including gastrointestinal symptoms (nausea, vomiting, ulcers, diarrhea), transient skin rash, increase in creatinine and transaminases and ototoxicity. Severe toxicity has been reported, i.e., gastrointestinal bleeding, nephrotoxicity including reversible Fanconi syndrome,[87] and hepatic failure.[88] **Deferiprone (DFP)** is an oral chelator with a half-life of 2-3 hours showing high efficacy in removing excess cardiac iron. Common but less severe side effects include gastrointestinal symptoms, arthralgia, zinc deficiency and fluctuating transaminase levels.[89] Agranulocytosis is the most serious adverse event, occurring in 1% of patients with hemoglobinopathies, and ~10% in DBA syndrome.[90] A recent analysis from France confirmed that 10% (3/23) of patients with DBA syndrome treated with DFP develop non-fatal agranulocytosis which resolved after DFP discontinuation.[91] The

panel agreed that DFP as primary chelation is indicated as first-line therapy in DBA syndrome for severe cardiac iron overload or cardiac failure[92] or third-line therapy with persistent cardiac iron loading or iron loading with failure/toxicity of other chelators (**Table 8**. Patient education, monitoring of neutrophil counts, and emergency plan for agranulocytosis are necessary.

Practical considerations

Published data on chelation in DBA syndrome are very limited. The panel agreed that early initiation of chelation is essential for long-term success. Generally, chelation is initiated after ~10 transfusions or evidence of iron load (ferritin >500ng/ml, transferrin saturation >60%, or elevated LIC), which in children typically coincides with the first failed steroid trial. Reduced chelator dose should be given when initiating therapy in children under labeled age limits (3 years for DFO and 2 years for DFX), as routinely practiced across expert centers. In patients <3 years old, DFO dose should not exceed 30mg/kg/day. In older children and adults, 50-60mg/kg/day of DFO can effectively control iron overload. Importantly, chelation therapy in transfusion-dependent patients with DBA syndrome must be given continuously. The panel highlighted that a combination of 2 chelators is a standard approach often required in DBA syndrome. Examples are: i) DFO 5 days per week at night (~12 hours) and DFX for 2 remaining days, or ii) more intensified regime with DFO 7 days per week at night and DFX during the day. Panel members unanimously emphasized that chronically transfused patients with DBA syndrome require early, aggressive chelation and iron overload monitoring by MRI liver/heart as the gold standard. Chelators have higher toxicity when body iron is low. Thus, doses should be lowered promptly to prevent overchelation toxicity. Patients with high tissue iron but low ferritin pose a challenge, requiring cautious low-dose chelation and close toxicity monitoring. There is also risk of overchelation toxicity if the decrease in ferritin occurs too rapidly. As iron overload often persists in patients becoming steroid-responsive, therapy-independent, or post-HSCT, ongoing monitoring and chelation or phlebotomy (only post-HSCT) may be indicated.

Hematopoietic stem cell transplantation

HSCT represents the only option for hematopoietic cure, preventing long-term side effects of steroids and transfusion/chelation in DBA syndrome. In our previous consensus,[3] HSCT from HLA-matched sibling donors (MSD) was considered for transfusion-dependent patients, while unrelated donor (UD) HSCT was reserved only for severe multi-lineage cytopenia and/or progression to MDS or acute myeloid leukemia (AML) due to previously poor overall survival (OS) after UD HSCT.[7] However, recent data show significantly improved OS of 85% for HLA-matched UD (MUD) transplants since 2000.[70,93,94] Similarly improved alternative donor results come from China,[95] Austria[96] and the Netherlands.[13] A large analysis from Germany and France found comparable 5-year OS for MSD (91%) or MUD (92%) transplants, with a similar graft-versus-host-disease (GVHD)-free OS at 89% or 83%, respectively.[94] Given these improvements, the panel recommends HSCT from MSD or 10/10 HLA matched UD for transfusion-dependent children (**Table 9**.

Considerations prior to HSCT

Iron overload is one of the major risk factors for an unfavourable HSCT outcome thus chelation should be optimized prior to HSCT. Although there are no data clearly indicating that high iron load (measured by LIC) portends poorer survival after HSCT in DBA syndrome, the panel agreed that LIC values should be optimally

lowered before HSCT to be as close as possible to the 3mg Fe/g threshold and not exceed 7mg/g. These assumptions rely on expert opinion due to the absence of controlled studies. Evaluation of liver fibrosis/cirrhosis might be indicated in patients with very high LIC. Infertility remains a relevant late effect following HSCT thus families should be counselled accordingly. In post-pubertal patients, cryopreservation of sperm or stimulated oocytes/ovarian tissue is routinely offered; in contrast cryopreservation of gonadal tissue from prepubertal patients remains experimental.

Age at HSCT

Since studies have shown that the OS in patients transplanted before the age of 10 years is superior to that seen in older patients,[70,93,94] HSCT should be performed preferentially below the age of 10 years, since in this patient group the morbidity from therapy-related complications is lower (potential adverse factors: iron overload, liver/kidney damage from chelation, infections from underlying immunodeficiency). For patients ≥10 years, HSCT may be considered for transfusion dependence after individual evaluation (i.e., short transfusion history, limited iron overload, no organ dysfunction). Adults should generally not undergo HSCT only for transfusion dependence, however, exceptions may be considered after discussing the patient's specific situation, including iron overload, health status, and donor options.

Indications and type of donor

MSD or MUD donors with a 10/10 HLA match can be considered in transfusion-dependent patients <10 years, unable to maintain adequate Hb on tolerable steroid dose. For clinically significant pancytopenia, immunodeficiency with frequent severe infections, alloimmunization, severe toxicity or intolerance to chelators, or MDS/AML, HSCT from the most suitable donor should be considered. In patients who respond to low dose steroids without relevant toxicity, HSCT should generally not be considered because the risks may outweigh the benefits. However, each patient should be assessed and counseled individually. Sibling donors, even if clinically asymptomatic, require testing for the presence of the genetic mutation identified in the patient.

Preparative regimen

Important considerations for the type of conditioning regimen are the acute toxicity and late effects, including infertility and a possibly increased malignancy risk. Regimens utilizing total body irradiation should be avoided. We recommend myeloablative conditioning with treosulfan or busulfan in combination with fludarabine.[97] Addition of thiotepa might be considered based upon the thalassemia experience.[98] Reduced intensity regimens should be currently limited to clinical trials pending more data to establish sufficient evidence for safety and efficacy.

Stem cell source and GVHD prophylaxis

Acute and chronic GVHD significantly contribute to HSCT toxicity. Since DBA syndrome patients do not benefit from GVHD effect, BM remains the first choice for MSD or MUD transplants. Results following MSD cord blood HSCT are excellent and a good choice for all indications. Unmanipulated peripheral blood stem cells should be avoided. GVHD prophylaxis in MUD and MSD settings includes serotherapy with a calcineurin inhibitor and methotrexate or mycophenolate.

Considerations following HSCT

DBA syndrome-specific cancer risk may be increased following HSCT.[72] Therefore, in addition to standard post-transplant follow-up, physicians must closely monitor for subsequent cancers. Recommendations from the European Blood and Marrow Transplantation group provide excellent guidance.[99]

Other treatments for anemia

The amino acid L-leucine has been shown to induce erythroid response and linear growth in some transfusion-dependent patients with DBA syndrome.[100] While not defined as standard of care, a few panelists use L-leucine routinely. Studies with L-leucine for steroid-responsive DBA syndrome are underway. Other drugs like sotatercept, immunosuppressive (rituximab or cyclosporine), and hematopoietic growth factors like erythropoietin have proven ineffective and are not recommended for anemia outside of clinical trials.[3]

10. CANCER RISK AND SURVEILLANCE

There is a significantly increased cancer risk in patients with DBA syndrome.[101,102] Shimamura and Alter[30] described 15 cases of MDS/AML and 19 solid tumors among 970 cases of DBA syndrome. Of the 19 solid tumors, 6 were osteogenic sarcoma. In 2012 the DBAR reported a quantitative assessment of cancer risk, firmly establishing DBA syndrome as a cancer predisposition syndrome.[103] The update from 2018 identified an overall fivefold increase of observed-to-expected ratio (O/E) for all cancers, with colon cancer and osteogenic sarcoma as the most prevalent with O/E ratios of 45 and 42, respectively.[72] The risk of AML (O/E 29) and MDS (O/E 350) was also increased. Recent analysis of 62 patients identified 3 MDS cases (at 25-29 years) and single cases of colorectal cancer, breast cancer, lymphoma, and multiple myeloma (ages 28-70 years).[14] Another registry reported one case of MDS (age 4 years) and single cases of gastric cancer, thyroid cancer, lymphoma, and osteosarcoma (11-48 years); there were also 2 cases of osteosarcoma and a cardiac Purkinje cell tumor post-HSCT (ages 19, 18 and 9 years).[16] As improvements in management allow patients to live longer, the number of malignancies in the DBAR cohort continuously increases, allowing better risk characterization.[104] The short post-HSCT cancer latency in some cases suggests that respective tumors are DBA syndrome-related, rather than-therapy related. Various malignancies (breast, testicular and skin cancers, i.e., melanoma/squamous cell carcinoma, Wilms tumor) indicate general susceptibility.[14,16,72,103] There is no known genotype-cancer association, except for *GATA1*-mutations associated with MDS with monosomy 7.[16,103]

Our panel reached consensus to recommend colorectal cancer screening in DBA syndrome starting at age 20 years (with follow-up every 5 years if initially normal), supported by increased prevalence of early onset colorectal cancer in DBA syndrome.[105] For patients with abnormal findings, national guidelines for follow-up intervals are recommended.[106] There was no consensus post-HSCT colonoscopy timing, but starting before age 20 years is reasonable. Data are still limited specifically post-HSCT and prospective studies are warranted. Hematologic surveillance includes complete blood counts (CBC) every 3-4 months, with significant change triggering a BM examination for MDS/AML. BM examination should be considered in any patient before transition to adult care to assess for baseline changes. Broad screening like whole-body MRI is currently not

recommended. Imaging should be readily performed for any bone/joint pain or injury given osteosarcoma risk. Specific cancer screening like mammography should follow national preventive standards.

11. LONG-TERM MANAGEMENT AND SURVEILLANCE

Recommendations are discussed in detail in **appendix 5**.

Major pediatric care goals include optimizing growth and development while monitoring for hormone issues, steroid toxicity, iron overload, and considering HSCT after initial steroid failure. Key adult care priorities are transition from pediatric care, adjusting anemia therapy, and monitoring for various significant malignant and non-malignant complications that contribute to excess mortality. Other priorities include genetic counseling for family planning, high-risk pregnancy management, colon cancer screening and comprehensive supportive care. DBA patient organizations (**appendix 6**) are key resources for health literacy.

Lastly, DBA syndrome may be diagnosed in adults, particularly in family members. Rather than "silent carrier", a term used in the past, individuals with no symptoms should be classified as **DBA syndrome currently without phenotype** since presentations including cancers may arise later in life.

12. CONCLUSIONS

In the 16 years since our first consensus, major advances have emerged in understanding DBA syndrome pathology, genetics, and treatment. These updated international recommendations synthesize extensive accumulated knowledge to further improve care of children and adults with DBA syndrome.

ACKNOWLEDGMENTS

We thank patient group representatives, physicians, and scientists facilitating the discussion (appendix 6).

DECLARATION OF INTERESTS

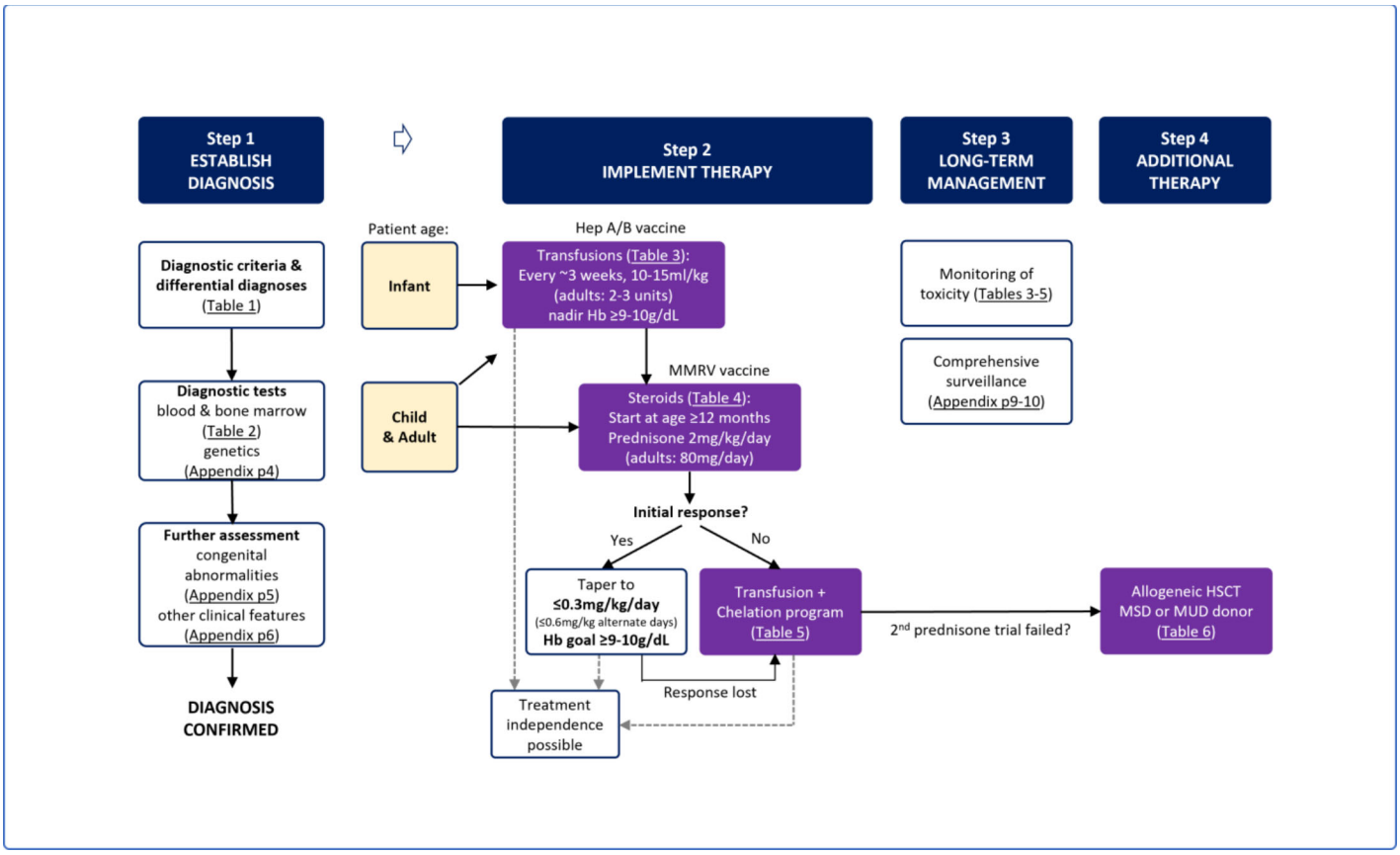
Antonis Kattamis (Chiesi: Honoraria, advisory board; Novartis: Honoraria, advisory board, research grant), Jeffrey M Lipton (Chiesi: Honoraria, advisory board), Leo Kager (advisory board Agios, Amgen, Bayer, Novartis).

AUTHOR CONTRIBUTIONS

MWW and TML: initial conceptualization and funding acquisition. MWW, AV, JEF, JML, and TML: identification of key opinion leaders, data curation and validation, formal analysis, development of tables and manuscript writing, access and verification of the underlying data reported. All authors: literature search, participation in selection of discussion items and iterative voting, manuscript writing, review, editing, and approval of the final manuscript.

FIGURE LEGEND

Figure 1. Approach to diagnosis, therapy, and long-term management of patients with DBA syndrome. Abbreviations: Hep A/B, hepatitis A and B; MMRV, mumps, measles, rubella, varicella; Hb, hemoglobin; HSCT, hematopoietic stem cell transplantation; MSD, matched sibling donor; MUD, matched unrelated donor.



REFERENCES

1. Diamond L, Blackfan K. Hypoplastic anemia. *Am J Dis Child* 1938; **56**: 464-7.
2. Josephs HW. Anaemia of Infancy and Early Childhood. *Medicine* 1936; **15**(3): 307-451.
3. Vlachos A, Ball S, Dahl N, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol* 2008; **142**(6): 859-76.
4. Willig TN, Niemeyer CM, Leblanc T, et al. Identification of new prognosis factors from the clinical and epidemiologic analysis of a registry of 229 Diamond-Blackfan anemia patients. DBA group of Societe d'Hematologie et d'Immunologie Pediatrique (SHIP), Gesellshaft fur Padiatrische Onkologie und Hamatologie (GPOH), and the European Society for Pediatric Hematology and Immunology (ESPHI). *Pediatr Res* 1999; **46**(5): 553-61.
5. Orfali KA, Ohene-Abuakwa Y, Ball SE. Diamond Blackfan anaemia in the UK: clinical and genetic heterogeneity. *Br J Haematol* 2004; **125**(2): 243-52.
6. Chen S, Warszawski J, Bader-Meunier B, et al. Diamond-blackfan anemia and growth status: the French registry. *J Pediatr* 2005; **147**(5): 669-73.
7. Lipton JM, Atsidaftos E, Zyskind I, Vlachos A. Improving clinical care and elucidating the pathophysiology of Diamond Blackfan anemia: an update from the Diamond Blackfan Anemia Registry. *Pediatr Blood Cancer* 2006; **46**(5): 558-64.
8. Tamary H, Nishri D, Yacobovich J, et al. Frequency and natural history of inherited bone marrow failure syndromes: the Israeli Inherited Bone Marrow Failure Registry. *Haematologica* 2010; **95**(8): 1300-7.
9. Kim SK, Ahn HS, Back HJ, et al. Clinical and hematologic manifestations in patients with Diamond Blackfan anemia in Korea. *Korean J Hematol* 2012; **47**(2): 131-5.
10. Delaporta P, Sofocleous C, Stiakaki E, et al. Clinical phenotype and genetic analysis of RPS19, RPL5, and RPL11 genes in Greek patients with Diamond Blackfan Anemia. *Pediatr Blood Cancer* 2014; **61**(12): 2249-55.
11. Smetanina NS, Mersiyanova IV, Kurnikova MA, et al. Clinical and genomic heterogeneity of Diamond Blackfan anemia in the Russian Federation. *Pediatr Blood Cancer* 2015; **62**(9): 1597-600.
12. Wan Y, Chen X, An W, et al. Clinical features, mutations and treatment of 104 patients of Diamond-Blackfan anemia in China: a single-center retrospective study. *Int J Hematol* 2016; **104**(4): 430-9.
13. van Dooijeweert B, van Ommen CH, Smiers FJ, et al. Pediatric Diamond-Blackfan anemia in the Netherlands: An overview of clinical characteristics and underlying molecular defects. *Eur J Haematol* 2018; **100**(2): 163-70.
14. Volejnikova J, Vojta P, Urbankova H, et al. Czech and Slovak Diamond-Blackfan Anemia (DBA) Registry update: Clinical data and novel causative genetic lesions. *Blood Cells Mol Dis* 2020; **81**: 102380.
15. Vogel N, Schmugge M, Renella R, Waespe N, Hengartner H, Swiss Paediatric Oncology Group Hematology Working G. The landscape of pediatric Diamond-Blackfan anemia in Switzerland: genotype and phenotype characteristics. *Eur J Pediatr* 2021; **180**(12): 3581-5.
16. Quarello P, Garelli E, Carando A, et al. A 20-year long term experience of the Italian Diamond-Blackfan Anaemia Registry: RPS and RPL genes, different faces of the same disease? *Br J Haematol* 2020; **190**(1): 93-104.
17. Iskander D, Wang G, Heuston EF, et al. Single-cell profiling of human bone marrow progenitors reveals mechanisms of failing erythropoiesis in Diamond-Blackfan anemia. *Sci Transl Med* 2021; **13**(610): eabf0113.
18. Jackson DJ, Butler C, Chaudhuri R, et al. Recommendations following a modified UK-Delphi consensus study on best practice for referral and management of severe asthma. *BMJ Open Respir Res* 2021; **8**(1).
19. Diamond LK, Wang WC, Alter BP. Congenital hypoplastic anemia. *Adv Pediatr* 1976; **22**: 349-78.
20. Carlston CM, Afify ZA, Palumbos JC, et al. Variable expressivity and incomplete penetrance in a large family with non-classical Diamond-Blackfan anemia associated with ribosomal protein L11 splicing variant. *Am J Med Genet A* 2017; **173**(10): 2622-7.
21. Vlachos A, Osorio DS, Atsidaftos E, et al. Increased Prevalence of Congenital Heart Disease in Children With Diamond Blackfan Anemia Suggests Unrecognized Diamond Blackfan Anemia as a Cause of Congenital Heart Disease in the General Population: A Report of the Diamond Blackfan Anemia Registry. *Circ Genom Precis Med* 2018; **11**(5): e002044.
22. Simkins A, Bannon SA, Khoury JD, et al. Diamond-Blackfan Anemia Predisposing to Myelodysplastic Syndrome in Early Adulthood. *JCO Precis Oncol* 2017; **1**(1): 1-5.

23. Sankaran VG, Ghazvinian R, Do R, et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. *J Clin Invest* 2012; **122**(7): 2439-43.
24. Toki T, Yoshida K, Wang R, et al. De Novo Mutations Activating Germline TP53 in an Inherited Bone-Marrow-Failure Syndrome. *Am J Hum Genet* 2018; **103**(3): 440-7.
25. Fedorova D, Ovsyannikova G, Kurnikova M, et al. De novo TP53 germline activating mutations in two patients with the phenotype mimicking Diamond-Blackfan anemia. *Pediatr Blood Cancer* 2022; **69**(4): e29558.
26. Kim AR, Ulirsch JC, Wilmes S, et al. Functional Selectivity in Cytokine Signaling Revealed Through a Pathogenic EPO Mutation. *Cell* 2017; **168**(6): 1053-64 e15.
27. Hashem H, Egler R, Dalal J. Refractory Pure Red Cell Aplasia Manifesting as Deficiency of Adenosine Deaminase 2. *J Pediatr Hematol Oncol* 2017; **39**(5): e293-e6.
28. Gagne KE, Ghazvinian R, Yuan D, et al. Pearson marrow pancreas syndrome in patients suspected to have Diamond-Blackfan anemia. *Blood* 2014; **124**(3): 437-40.
29. Szvetnik EA, Klemann C, Hainmann I, et al. Diamond-Blackfan Anemia Phenotype Caused By Deficiency of Adenosine Deaminase 2. *Blood* 2017; **130**(Suppl 1): 874-.
30. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. *Blood Rev* 2010; **24**(3): 101-22.
31. Da Costa L, Chanoz-Poulard G, Simansour M, et al. First de novo mutation in RPS19 gene as the cause of hydrops fetalis in Diamond-Blackfan anemia. *Am J Hematol* 2013; **88**(2): 160.
32. Wlodarski MW, Da Costa L, O'Donohue MF, et al. Recurring mutations in RPL15 are linked to hydrops fetalis and treatment independence in Diamond-Blackfan anemia. *Haematologica* 2018; **103**(6): 949-58.
33. Buchanan GR, Alter BP, Holtkamp CA, Walsh EG. Platelet number and function in Diamond-Blackfan anemia. *Pediatrics* 1981; **68**(2): 238-41.
34. Gianferante MD, Wlodarski MW, Atsidaftos E, et al. Genotype-phenotype association and variant characterization in Diamond-Blackfan anemia caused by pathogenic variants in RPL35A. *Haematologica* 2021; **106**(5): 1303-10.
35. Iskander D, Roberts I, Rees C, et al. Impaired cellular and humoral immunity is a feature of Diamond-Blackfan anaemia; experience of 107 unselected cases in the United Kingdom. *Br J Haematol* 2019; **186**(2): 321-6.
36. van Dooijeweert B, Kia SK, Dahl N, et al. GATA-1 Defects in Diamond-Blackfan Anemia: Phenotypic Characterization Points to a Specific Subset of Disease. *Genes (Basel)* 2022; **13**(3).
37. Giri N, Kang E, Tisdale JF, et al. Clinical and laboratory evidence for a trilineage haematopoietic defect in patients with refractory Diamond-Blackfan anaemia. *Br J Haematol* 2000; **108**(1): 167-75.
38. Kimura K, Shimazu K, Toki T, et al. Outcome of colorectal cancer in Diamond-Blackfan syndrome with a ribosomal protein S19 mutation. *Clin J Gastroenterol* 2020; **13**(6): 1173-7.
39. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; **17**(5): 405-24.
40. Willig TN, Perignon JL, Gustavsson P, et al. High adenosine deaminase level among healthy probands of Diamond Blackfan anemia (DBA) cosegregates with the DBA gene region on chromosome 19q13. The DBA Working Group of Societe d'Immunologie Pediatrique (SHIP). *Blood* 1998; **92**(11): 4422-7.
41. Quarello P, Garelli E, Carando A, et al. Ribosomal RNA analysis in the diagnosis of Diamond-Blackfan Anaemia. *Br J Haematol* 2016; **172**(5): 782-5.
42. Farrar JE, Quarello P, Fisher R, et al. Exploiting pre-rRNA processing in Diamond Blackfan anemia gene discovery and diagnosis. *Am J Hematol* 2014; **89**(10): 985-91.
43. Ben-Ami T, Revel-Vilk S, Brooks R, et al. Extending the Clinical Phenotype of Adenosine Deaminase 2 Deficiency. *J Pediatr* 2016; **177**: 316-20.
44. Claassen D, Boals M, Bowling KM, et al. Complexities of genetic diagnosis illustrated by an atypical case of congenital hypoplastic anemia. *Cold Spring Harb Mol Case Stud* 2018; **4**(6).
45. Vlachos A, Klein GW, Lipton JM. The Diamond Blackfan Anemia Registry: tool for investigating the epidemiology and biology of Diamond-Blackfan anemia. *J Pediatr Hematol Oncol* 2001; **23**(6): 377-82.
46. Moetter J, Kartal M, Meerpohl J, et al. Analysis of ribosomal protein genes associated with Diamond Blackfan anemia (DBA) in German DBA patients and their relatives. *ASH Annual Meeting Abstracts* 2011; **118**(21): 729.

47. Ulirsch JC, Verboon JM, Kazerounian S, et al. The Genetic Landscape of Diamond-Blackfan Anemia. *Am J Hum Genet* 2018; **103**(6): 930-47.
48. Aspesi A, Monteleone V, Betti M, et al. Lymphoblastoid cell lines from Diamond Blackfan anaemia patients exhibit a full ribosomal stress phenotype that is rescued by gene therapy. *Sci Rep* 2017; **7**(1): 12010.
49. Clarke SR, Vlachos A, Lichtenberg J, et al. Whole Genome Sequencing of Diamond Blackfan Anemia Syndrome Patients Detects Mutations That Alter mRNA Splicing. *Blood* 2021; **138**(Supplement 1): 863-.
50. Da Costa L, O'Donohue MF, van Dooijeweert B, et al. Molecular approaches to diagnose Diamond-Blackfan anemia: The EuroDBA experience. *Eur J Med Genet* 2018; **61**(11): 664-73.
51. Pospisilova D, Cmejlova J, Ludikova B, et al. The Czech National Diamond-Blackfan Anemia Registry: clinical data and ribosomal protein mutations update. *Blood Cells Mol Dis* 2012; **48**(4): 209-18.
52. Campagnoli MF, Garelli E, Quarello P, et al. Molecular basis of Diamond-Blackfan anemia: new findings from the Italian registry and a review of the literature. *Haematologica* 2004; **89**(4): 480-9.
53. Pali M, Moetter J, Meerpohl J, et al. Identification of Novel Mutations In Ribosomal Genes In Patients with Diamond Blackfan Anemia (DBA) In Germany and Genotype-Phenotype Correlation Analysis. *ASH Annual Meeting Abstracts* 2010; **116**(21): 2244.
54. Doherty L, Sheen MR, Vlachos A, et al. Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. *Am J Hum Genet* 2010; **86**(2): 222-8.
55. Gazda HT, Sheen MR, Vlachos A, et al. Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. *Am J Hum Genet* 2008; **83**(6): 769-80.
56. Wan Y, Gong X, Cheng S, et al. Short Stature in Patients with Diamond-Blackfan Anemia: A Cross-Sectional Study. *J Pediatr* 2022; **240**: 177-85.
57. Bhar S, Zhou F, Reineke LC, et al. Expansion of germline RPS20 mutation phenotype to include Diamond-Blackfan anemia. *Hum Mutat* 2020; **41**(11): 1918-30.
58. Lezzerini M, Penzo M, O'Donohue MF, et al. Ribosomal protein gene RPL9 variants can differentially impair ribosome function and cellular metabolism. *Nucleic Acids Res* 2020; **48**(2): 770-87.
59. O'Donohue MF, Da Costa LM, Lezzerini M, et al. HEATR3 variants impair nuclear import of uL18 (RPL5) and drive Diamond-Blackfan anemia. *Blood* 2022.
60. Nissen KB, Masmas TN, Nielsen RG, Christiansen M, Wlodarski M, Hasle H. Congenital pure red cell anemia and idiopathic very early onset of severe colitis cured by allogeneic hematopoetic stem cell transplantation. *Pediatr Blood Cancer* 2023; **70**(11): e30525.
61. van den Akker M, Dror Y, Odame I. Transient erythroblastopenia of childhood is an underdiagnosed and self-limiting disease. *Acta Paediatr* 2014; **103**(7): e288-94.
62. Young NS, Brown KE. Parvovirus B19. *N Engl J Med* 2004; **350**(6): 586-97.
63. Glader BE, Backer K, Diamond LK. Elevated erythrocyte adenosine deaminase activity in congenital hypoplastic anemia. *N Engl J Med* 1983; **309**(24): 1486-90.
64. Fargo JH, Kratz CP, Giri N, et al. Erythrocyte adenosine deaminase: diagnostic value for Diamond-Blackfan anaemia. *Br J Haematol* 2013; **160**(4): 547-54.
65. Narla A, Davis NL, Lavasseur C, Wong C, Glader B. Erythrocyte adenosine deaminase levels are elevated in Diamond Blackfan anemia but not in the 5q- syndrome. *Am J Hematol* 2016; **91**(12): E501-E2.
66. Bluteau O, Sebert M, Leblanc T, et al. A landscape of germ line mutations in a cohort of inherited bone marrow failure patients. *Blood* 2018; **131**(7): 717-32.
67. Vlachos A, Farrar JE, Atsidaftos E, et al. Diminutive somatic deletions in the 5q region lead to a phenotype atypical of classical 5q- syndrome. *Blood* 2013; **122**(14): 2487-90.
68. Liu D, Ahmet A, Ward L, et al. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. *Allergy Asthma Clin Immunol* 2013; **9**(1): 30.
69. Chaia-Semerena GM, Vargas-Camano ME, Alonso-Bello CD, et al. The Effects of Alternate-Day Corticosteroids in Autoimmune Disease Patients. *Autoimmune Dis* 2020; **2020**: 8719284.
70. Vlachos A, Muir E. How I treat Diamond-Blackfan anemia. *Blood* 2010; **116**(19): 3715-23.
71. Vlachos A, Blanc L, Lipton JM. Diamond Blackfan anemia: a model for the translational approach to understanding human disease. *Expert Rev Hematol* 2014; **7**(3): 359-72.
72. Vlachos A, Rosenberg PS, Atsidaftos E, et al. Increased risk of colon cancer and osteogenic sarcoma in Diamond-Blackfan anemia. *Blood* 2018; **132**(20): 2205-8.
73. Berdoukas V, Nord A, Carson S, et al. Tissue iron evaluation in chronically transfused children shows significant levels of iron loading at a very young age. *Am J Hematol* 2013; **88**(11): E283-5.

74. Porter JB, Walter PB, Neumayr LD, et al. Mechanisms of plasma non-transferrin bound iron generation: insights from comparing transfused diamond blackfan anaemia with sickle cell and thalassaemia patients. *Br J Haematol* 2014; **167**(5): 692-6.
75. Roggero S, Quarello P, Vinciguerra T, Longo F, Piga A, Ramenghi U. Severe iron overload in Blackfan-Diamond anemia: a case-control study. *Am J Hematol* 2009; **84**(11): 729-32.
76. Pfeifer CD, Schoennagel BP, Grosse R, et al. Pancreatic iron and fat assessment by MRI-R2* in patients with iron overload diseases. *J Magn Reson Imaging* 2015; **42**(1): 196-203.
77. Puliyel M, Sposto R, Berdoukas VA, et al. Ferritin trends do not predict changes in total body iron in patients with transfusional iron overload. *Am J Hematol* 2014; **89**(4): 391-4.
78. Wood JC, Enriquez C, Ghugre N, et al. MRI R2 and R2* mapping accurately estimates hepatic iron concentration in transfusion-dependent thalassemia and sickle cell disease patients. *Blood* 2005; **106**(4): 1460-5.
79. Coates TD. Iron overload in transfusion-dependent patients. *Hematology Am Soc Hematol Educ Program* 2019; **2019**(1): 337-44.
80. Carpenter JP, He T, Kirk P, et al. On T2* magnetic resonance and cardiac iron. *Circulation* 2011; **123**(14): 1519-28.
81. Lota AS, Gatehouse PD, Mohiaddin RH. T2 mapping and T2* imaging in heart failure. *Heart Fail Rev* 2017; **22**(4): 431-40.
82. Patton N, Brown G, Leung M, et al. Observational study of iron overload as assessed by magnetic resonance imaging in an adult population of transfusion-dependent patients with beta thalassaemia: significant association between low cardiac T2* < 10 ms and cardiac events. *Intern Med J* 2010; **40**(6): 419-26.
83. Kamperidis V, Vlachou M, Pappa Z, et al. Prediction of long-term survival in patients with transfusion-dependent hemoglobinopathies: Insights from cardiac imaging and ferritin. *Hellenic J Cardiol* 2021; **62**(6): 429-38.
84. Bonios MJ, Fountas E, Delaporta P, et al. Left ventricular deformation mechanics over time in patients with thalassemia major with and without iron overload. *BMC Cardiovasc Disord* 2021; **21**(1): 81.
85. Styles LA, Vichinsky EP. Ototoxicity in hemoglobinopathy patients chelated with desferrioxamine. *J Pediatr Hematol Oncol* 1996; **18**(1): 42-5.
86. Cappellini MD, El-Beshlawy A, Kattamis A, et al. Efficacy and Safety of Deferasirox (Exjade®) in Patients with Transfusion-Dependent Anemias: 1-Year Results from the Large, Prospective, Multicenter EPIC Study
Blood 2008; **112 Suppl**(3875a).
87. Dee CM, Cheuk DK, Ha SY, Chiang AK, Chan GC. Incidence of deferasirox-associated renal tubular dysfunction in children and young adults with beta-thalassaemia. *Br J Haematol* 2014; **167**(3): 434-6.
88. Menaker N, Halligan K, Shur N, et al. Acute Liver Failure During Deferasirox Chelation: A Toxicity Worth Considering. *J Pediatr Hematol Oncol* 2017; **39**(3): 217-22.
89. Cohen AR, Galanello R, Piga A, Dipalma A, Vullo C, Tricta F. Safety profile of the oral iron chelator deferiprone: a multicentre study. *Br J Haematol* 2000; **108**(2): 305-12.
90. Tricta F, Uetrecht J, Galanello R, et al. Deferiprone-induced agranulocytosis: 20 years of clinical observations. *Am J Hematol* 2016; **91**(10): 1026-31.
91. Lecornec N, Castex MP, Reguerre Y, et al. Agranulocytosis in patients with Diamond-Blackfan anemia treated with deferiprone for post-transfusion iron overload: a retrospective study of the French DBA cohort. *Br J Haematol* 2022; **In Press**.
92. Porter JB, Wood J, Olivieri N, et al. Treatment of heart failure in adults with thalassemia major: response in patients randomised to deferoxamine with or without deferiprone. *J Cardiovasc Magn Reson* 2013; **15**: 38.
93. Fagioli F, Quarello P, Zecca M, et al. Haematopoietic stem cell transplantation for Diamond Blackfan anaemia: a report from the Italian Association of Paediatric Haematology and Oncology Registry. *Br J Haematol* 2014; **165**(5): 673-81.
94. Strahm B, Loewecke F, Niemeyer CM, et al. Favorable outcomes of hematopoietic stem cell transplantation in children and adolescents with Diamond-Blackfan anemia. *Blood Adv* 2020; **4**(8): 1760-9.
95. Li Q, Luo C, Luo C, et al. Disease-specific hematopoietic stem cell transplantation in children with inherited bone marrow failure syndromes. *Ann Hematol* 2017; **96**(8): 1389-97.

96. Crazzolara R, Kropshofer G, Haas OA, Matthes-Martin S, Kager L. Reduced-intensity conditioning and stem cell transplantation in infants with Diamond Blackfan anemia. *Haematologica* 2017; **102**(3): e73-e5.
97. Peffault de Latour R, Peters C, Gibson B, et al. Recommendations on hematopoietic stem cell transplantation for inherited bone marrow failure syndromes. *Bone Marrow Transplant* 2015; **50**(9): 1168-72.
98. Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. *Haematologica* 2014; **99**(5): 811-20.
99. Miano M, Eikema DJ, de la Fuente J, et al. Stem Cell Transplantation for Diamond-Blackfan Anemia. A Retrospective Study on Behalf of the Severe Aplastic Anemia Working Party of the European Blood and Marrow Transplantation Group (EBMT). *Transplant Cell Ther* 2021; **27**(3): 274 e1- e5.
100. Vlachos A, Atsidaftos E, Lababidi ML, et al. L-leucine improves anemia and growth in patients with transfusion-dependent Diamond-Blackfan anemia: Results from a multicenter pilot phase I/II study from the Diamond-Blackfan Anemia Registry. *Pediatr Blood Cancer* 2020; **67**(12): e28748.
101. Wasser JS, Yolken R, Miller DR, Diamond L. Congenital hypoplastic anemia (Diamond-Blackfan syndrome) terminating in acute myelogenous leukemia. *Blood* 1978; **51**(5): 991-5.
102. Lipton JM, Federman N, Khabbaze Y, et al. Osteogenic sarcoma associated with Diamond-Blackfan anemia: a report from the Diamond-Blackfan Anemia Registry. *J Pediatr Hematol Oncol* 2001; **23**(1): 39-44.
103. Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. *Blood* 2012; **119**(16): 3815-9.
104. Lipton JM, Molmenti CLS, Desai P, Lipton A, Ellis SR, Vlachos A. Early Onset Colorectal Cancer: An Emerging Cancer Risk in Patients with Diamond Blackfan Anemia. *Genes (Basel)* 2021; **13**(1).
105. Lipton JM, Molmenti CLS, Hussain M, et al. Colorectal cancer screening and surveillance strategy for patients with Diamond Blackfan anemia: Preliminary recommendations from the Diamond Blackfan Anemia Registry. *Pediatr Blood Cancer* 2021; **68**(8): e28984.
106. NCCN colorectal cancer screening practice guidelines. National Comprehensive Cancer Network. *Oncology (Williston Park)* 1999; **13**(5A): 152-79.

Table 1. Diagnostic criteria and differential diagnoses of DBA syndrome

DIAGNOSTIC CRITERIA		
• **Pathogenic or likely pathogenic mutation in a DBA syndrome gene (Table 3),** **OR** • **Hematologic features consistent with DBA:** macrocytic anemia[1] with reticulocytopenia and BM erythroblastopenia; absence of dysplasia, dyserythropoiesis[2], and sideroblasts AND: exclusion of known differential diagnoses (below)		
TYPICAL FINDINGS (NOT MANDATORY FOR DIAGNOSIS) [3]		
• Age at onset less than 1 year • Elevated eADA activity (prior first transfusion; in non-transfused patients and/or parents) • Elevated HbF (reliably assessed in patients > 6 months of age) • Positive family history or unexplained history of anemia during infancy or childhood • Congenital abnormalities **(Table 4)** • Abnormal rRNA processing in patient cells [4]		
DIFFERENTIAL DIAGNOSES		
ACQUIRED	Transient erythroblastopenia of childhood	• Onset usually > 1 year of age • Normal MCV, eADA, HbF • Negative family history and no congenital abnormalities • Transient course: erythroid recovery in days to weeks
	Viruses: Specific to red cell lineage (Parvovirus B19) Non-specific (HIV, CMV, EBV and others)	• Positive PCR and/or serology • Normal eADA and HbF • Normal MCV (except Parvovirus B19) • Negative family history and no congenital abnormalities • Concomitant immune deficiency or chronic hemolysis
	Myelodysplastic syndrome [5], specifically with 5q deletion (acquired RPS14 haploinsufficiency)	• Typical BM findings (morphology, histology, karyotype, FISH, MDS-related somatic mutations) • Normal eADA
	Drugs Autoimmunity (SLE, acquired PRCA) Lymphoproliferative diseases Malignancy: CLL[5], LGL[5], acute leukemias and some solid tumors	• Typical BM and immunologic findings • Normal eADA • No congenital abnormalities • Features of malignancy
	Thymoma with concomitant PRCA[5]	• Typical imaging (chest x-ray, CT or MRI) • No congenital abnormalities • Mostly in adults, unlikely in children
GENETIC	IBMFS (specifically FA, SDS, DC)[6] Pearson syndrome Congenital sideroblastic anemia Congenital dyserythropoietic anemia	• Classical clinical presentation, laboratory findings • BM morphology consistent with respective condition • MCV and HbF can be elevated, eADA normal • Syndrome-specific diagnostic findings and genetics
	ADA2 deficiency	• Onset at any age, vasculopathy often absent • Low B cells and hypogammaglobulinemia • Normal eADA and HbF; MCV can be high • Low ADA2 enzyme activity and *ADA2* mutations • Typically, no congenital abnormalities
	Erythropoietin dysfunction	• Homozygous *EPO* R150Q mutation

[1] Additional cytopenia can be encountered (neutropenia more often than thrombocytopenia), transient thrombocytosis in infants.
[2] Except for cases with *GATA1* mutations.
[3] Highly suggestive of DBA syndrome; however not specific enough to make the diagnosis.
[4] Research test in specialized labs only; useful in cases with ambiguous or uninformative genetics.
[5] Typically presenting in adults.
[6] These IBMFS typically demonstrate multi-lineage cytopenia and often present with other disease-specific abnormalities affecting multiple organ systems. Such distinguishing features can help differentiate these conditions from DBA syndrome, which initially characteristically manifests with isolated erythroid hypoplasia.

Abbreviations: BM; bone marrow; eADA, erythrocyte adenosine deaminase; HbF, fetal hemoglobin; SLE, systemic lupus erythematosus; PRCA, pure red cell aplasia; CLL, chronic lymphocytic leukemia; LGL, large granular lymphocytic leukemia; CT, computer tomography; MRI, magnetic resonance imaging; IBMFS, inherited bone marrow failure syndromes; FA, Fanconi anemia; SDS, Shwachman Diamond syndrome; DC, dyskeratosis congenita.

Table 2. Recommended diagnostic tests in patients with suspected DBA syndrome

Essential diagnostic tests	• CBC (including differential, red cell indices and reticulocyte count) • eADA and HbF [1] • BM morphology/cellularity at initial manifestation or prior to starting steroids • Parvovirus B19 PCR and/or serology in BM or blood • DBA genetic testing (**appendix p4**) • Evaluation for congenital abnormalities: physical examination, echocardiography, abdominal ultrasound; additional imaging as indicated[2]
Additional baseline evaluations (all patients)	• Laboratory parameters: ferritin, LDH, bilirubin, transaminases, creatinine, vitamin B12/MMA, folate • DAT (direct Coombs test), blood group antigens, RBC antibodies to guide transfusion management • Immunoglobulin levels (>6 months of age) and lymphocyte immunophenotyping • HLA typing of patient and family members[3]
Further tests in selected patients	• BM cytogenetics and BM biopsy[4] • Suspicion of IBMFS: chromosome breakage (Fanconi anemia), telomere length (dyskeratosis congenita), fecal elastase (Shwachman Diamond syndrome), mitochondrial DNA genetics (Pearson syndrome), ADA2 genetics or enzyme activity (ADA2 deficiency), genetics for other IBMFS[5] • Erythropoietin (EPO) level[6]

[1] Prior to first transfusion or ≥6 weeks (or as far away as possible) after last transfusion, HbF reliably assessed in patients >6 months of age.
[2] Neuro-imaging, hand x-ray and other imaging studies as clinically indicated.
[3] Not required for diagnosis, but essential for long-term therapeutic planning.
[4] In patients with suspicion of MDS or leukemia.
[5] In patients with clinical suspicion of respective syndromes.
[6] In suspected renal dysfunction; note, EPO levels are elevated in patients with DBA syndrome.

Abbreviations: CBC, complete blood counts; ADA, erythrocyte adenosine deaminase; HbF, fetal hemoglobin; BM; bone marrow; MMA, methylmalonic acid; DAT (direct antiglobulin test); IBMFS, inherited BM failure syndromes.

Table 3. Genes associated with DBA syndrome and genetic phenocopies

Gene symbol	Inheritance	Chromosome location	New protein symbol	Approximate frequency	References (see manuscript)
DBA SYNDROME: RIBOSOMOPATHY[1]					
Small ribosomal subunit (11 genes)					
RPS7	AD	2p	eS7	< 1%	107
RPS10	AD	6p	eS10	3%	54
RPS15A	AD	16p	uS8	<1%	108
RPS17	AD	15q	eS17	1%	109
RPS19	AD	19q	eS19	25%	110
RPS20	AD	8q	uS10	< 1%	57,111
RPS24	AD	10q	eS24	2.4%	112
RPS26	AD	12q	eS26	6.6%	54
RPS27	AD	1q	eS27	< 1%	113
RPS28	AD	19p	eS28	< 1%	114
RPS29	AD	14q	uS14	< 1%	115
Large ribosomal subunit (13 genes)					
RPL4	AD	15q	uL4	< 1%	116
RPL5	AD	1p	uL18	7%	55
RPL8	AD	8q	uL2	< 1%	117
RPL9	AD	4p	uL6	< 1%	13,54
RPL11	AD	1p	uL5	5%	55
RPL15	AD	3p	eL15	< 1%	32,118
RPL17	AD	18q	uL22	< 1%	15
RPL18	AD	19q	eL18	< 1%	119
RPL26	AD	17P	uL24	< 1%	120
RPL27	AD	17q	eL27	< 1%	113
RPL31	AD	12q	el31	< 1%	42
RPL35	AD	3q	uL29	< 1%	119
RPL35A	AD	9q	eL33	3%	121
Ribosomal protein chaperones (2 genes)					
TSR2	X	X		< 1%	114
HEATR3	AR	16q		< 1%	59
DBA SYNDROME OTHER[2]					
GATA1	X	X		< 1%	23,122-124
TP53 (GOF)	AD	AD		< 1%	24,25
CANDIDATE GENES[3]					
RPS11	AD	19q	uS17	< 1%	47
RPL3	AD	22q	uL3	< 1%	
RPL10	AD	X	uL16	< 1%	
RPL10A	AD	6p	uL11	< 1 %	
RPL19	AD	17q	eL19	< 1%	
RPL34	AD	4q	eL34	< 1%	
RPL0	AD	12q	uL10	< 1%	
GENETIC PHENOCOPIES[4]					
ADA2	AR	22q11.1			27,29,43
EPO	AR	7q22.1			26

[1] Bona fide ribosomopathy genes validated functionally (ribosomal biogenesis defect or presence of somatic genetic rescue).

[2] Genes affecting pathways implicated in DBA syndrome and associated with hyporegenerative anemia.

[3] Considered putative due to lack of studies demonstrating impaired ribosomal biogenesis.

[4] Diseases with different pathomechanisms that can manifest with pure red cell aplasia.

Abbreviations: AD, autosomal dominant; X, X-linked recessive; AR, autosomal recessive; GOF, gain-of-function

Table 4. Common congenital abnormalities associated with DBA syndrome

ORGAN SYSTEM	Frequency, median (range)	FINDINGS
Any type	54.4% (40.6-71.8)	(Including short stature, small for gestational age/intrauterine growth retardation)
Craniofacial and neck	21.6% (14.5-25) *Cleft palate: 4.26% (3.5-5.8)*	Hypertelorism, microcephaly, micrognathia (Pierre-Robin), microtia, broad flat nasal bridge, epicanthus, cleft lip, cleft palate, shorted/webbed neck, Sprengel deformity, Klippel-Feil deformity, low set ears, prominent ears, low hair line, ptosis, mandibulofacial dysostosis (Treacher-Collins syndrome phenocopy)
Cardiac	11.6% (6.9-15)	Ventricular septal defect, atrial septal defect, coarctation of the aorta, tetralogy of Fallot, bicuspid aortic valve, pulmonary stenosis, anomalous venous return, other complex cardiac defects
Thumb and skeletal	18.5% (17.9-19) *Thumbs: 7.6% (6-9.2)*	Thumb (absent, atypical, duplex, bifid, triphalangeal), flat thenar eminence, polydactyly, syndactyly, absence of radial artery, acetabular dysplasia, pectus excavatum
Urogenital	10.7% (6.3-19.5)	Absent or horseshoe kidney, duplicated collecting systems, hypospadias, inguinal hernias
Ophthalmological	Rare	Congenital glaucoma or cataracts, strabismus
Skin	Rare	Café au lait spots, congenital nevi, hemangioma, dermatofibroma
Neurodevelopmental	3% (1.3-4.6)	Learning difficulties, mild to severe developmental delay

Data on frequency are from DBA syndrome registry papers cited in the manuscript.

Table 5. Summary of clinical features in patients with DBA syndrome

	INTRINSIC (DISEASE RELATED)	THERAPY RELATED
Hematologic	Macrocytic anemia with reticulocytopenia Leukopenia, neutropenia Thrombocytopenia Thrombocytosis (infants) Hypocellular marrow (mostly in adults, sometimes in children)	Alloimmunization with subsequent increased transfusion requirement Agranulocytosis (DFP)
Immunologic	Lymphocytopenia Decreased B-cell numbers Hypogammaglobulinemia Recurrent infections	Port catheter infections Transfusion-related pathogens Viral infections (steroids) Lymphocytopenia (steroids) Infections: campylobacter and other bacteria (associated with iron overload), yersiniosis, mucor mycosis (exacerbated by DFX)
Endocrinologic	Intrauterine growth restriction Failure to thrive Short stature Congenital abnormalities	>50% patients experience endocrine problems related to steroids and iron overload: • Adrenal insufficiency • Sex hormone insufficiency • Growth hormone dysfunction • Hypogonadism • Thyroid and parathyroid problems • Pancreatic insufficiency, diabetes mellitus
Orthopedic	Short stature Congenital abnormalities Osteosarcoma (osteogenic sarcoma)	Osteopenia and bone fractures (steroids)
Hepatic, Urogenital, Cardiac	Congenital abnormalities	Liver toxicity (DFX) Liver cirrhosis (iron) Cardiomyopathy with arrhythmias (iron) Nephrotoxicity, phosphate loss (DFX) Urinary stones (DFX, DFO)
Gastrointestinal	Colitis	Diarrhea, esophagitis, nausea (DFX)
Otolaryngologic, ophthalmologic	Congenital abnormalities	Cataract (steroids, DFX) Ototoxicity (DFO)
Neurologic and psychosocial	Developmental delay	High psychosocial burden from chronic illness
Oncologic	Cancer risk: MDS, osteosarcoma, colorectal cancer, and other cancers	Increased risk after HSCT
Obstetric	High risk pregnancies	Infertility (iron overload, HSCT)

Abbreviations: DFO, deferoxamine; DFP, deferiprone; DFX, deferasirox; HSCT, hematopoietic stem cell transplantation.

Table 6. Recommendations for transfusion support

GENERAL PRINCIPLES	
Indications and timing	**Therapeutic considerations**
• Any patient with severe anemia • Patient within 12 months of life • Patient not responding to steroids or experiencing significant side effects • Patient responding to steroids and experiencing acute Hb drop (e.g., due to viral illness) • Patient on steroid holiday (to improve growth during adolescence) • Pregnant patient with anemia	General: • Hepatitis B vaccination • RBC antigen typing and repeat RBC antibody screening Hb goal prior transfusion (nadir Hb): • **≥9-10g/dL** or a higher level at which the patient is asymptomatic, independent of age Transfusion process: • [1]Volume: 10-15ml/kg (children), ~2-3 RBC units (adults) • [2]Interval: every 3 (2-4) weeks
ADVERSE EFFECTS AND CLINICAL PROBLEMS	
Iron overload	Start early chelation (**Table 5**)
Clinically significant anemia, especially days before transfusion	Increase transfusion volume or decrease transfusion interval, "catch-up" transfusion
Blood-transmitted pathogens	Hepatitis B vaccine Virus testing (HIV, Hepatitis B and C) at least yearly
Higher transfusion requirements	Rule out alloimmunization and hypersplenism (rare in DBA syndrome), hemorrhage

[1] Higher transfusion volumes occasionally required across all ages (i.e., ~20ml/kg).
[2] Interval may be longer in patients with some erythropoiesis who maintain Hb $\geq$9-10g/dL for longer period of time.

Table 7. Recommendations for steroid treatment

GENERAL PRINCIPLES	
Indications and timing	**Therapeutic considerations**
First trial: • Patient with chronic transfusions: Start ≥12 months old. Possible start at 15-18 months in children with failure to thrive. Earlier start (~9 months) if unable to provide safe venous access or safe transfusions Second trial: • In previous non-responders (1-2 years after first unsuccessful trial), recommended before planned HSCT Additional trials: Not recommended	**Before:** • Live viral vaccines (1st dose MMRV) given optimally ≥ 3 weeks before first steroid trial **Dosing:** • Drug: Oral prednisone or prednisolone (equal potency) • Start dose: 2mg/kg per day in children (max 80mg); 80mg per day in adults • When to start: one day or ~10-14 days after last transfusion • Initial response assessment: reticulocytes and Hb at day 10-14 **Tapering principles and stopping rule:** • Initial response: start taper after 2 weeks but not later than 4 weeks: reduce by 0.5mg/kg every ~2 weeks. • From 0.5mg/kg slow taper to arrive at maximum maintenance dose (0.3mg/kg per day or 0.6mg/kg alternate days) • Further passive/active taper to reach minimally effective dose • Non-response after 4 weeks: stop initial dose without unnecessarily extending therapy **Definitions of steroid response:** • Initial response: significant reticulocytosis (≥50-100x10^9/L) and stable/increasing Hb (expected within 2-4 weeks). • Long-term response: maximum maintenance dose resulting in Hb ≥9g/dl without transfusions
CLINICAL SCENARIOS AND MANAGEMENT	
Loss of efficacy	• Acute Hb drop (e.g., viral illness): single RBC transfusion • Persistent Hb drop: consider increasing dose; if dose too high, declare non-response and switch to RBC transfusions
Estrogen-containing oral contraception	• May limit steroid response
Pregnancy, systemic disease (including cancer)	• Discontinue steroids and switch to RBC transfusions
Preadolescence/adolescence	• Consider steroid holiday (1-3 years) to improve growth
Immunosuppression, lymphopenia with risk of opportunistic infections	• Taper/discontinue steroids if clinically relevant infection • Avoid live vaccines during initial high dose steroids
Classic side effects: hypertension, diabetes, adrenal insufficiency, and others	• Monitoring toxicity with endocrinologist • Annual eye exam (cataracts?) • Annual bone densitometry scan (osteopenia?)
SUPPORTIVE CARE	
Vitamin D and calcium supplementation	• All patients on long-term steroids
Proton pump inhibitors or H2 antagonists	• During initial high dose of steroids or if symptomatic
Pneumocystis jirovecii pneumonia prophylaxis	• No consensus reached on antibiotic prophylaxis during initial high dose steroids (2mg/kg). Adapt to local standard

Abbreviations: HSCT, hematopoietic stem cell transplantation; Hb, hemoglobin; MMRV, mumps, measles, rubella, varicella; RBC, red blood cells

Table 8. Recommendations for chelation therapy

	DEFEROXAMINE, DESFERRIOXAMINE (DFO)	DEFERASIROX (DFX)	DEFERIPRONE (DFP)
Indications	First line: DFO or DFX (off label in children <2 years old[1]) Second line: switch between or combine both • Start after 10 transfusions or evidence of iron load (transferrin saturation >60%, serial ferritin >500ng/ml) • Infant with DBA: wait until after first failed steroid trial, then start with low dose and close monitoring		Third line in patients with cardiac iron overload or failure /intolerance to other chelators First line in patients with severe cardiac iron overload or cardiac failure (in combination with DFO)
Formulation	Subcutaneous (SQ) or intravenous (IV): 500mg/vial or 2 g/vial	a) film-coated oral tablet or granules (90, 180, 360mg); b) dispersible oral tablet (125, 250, 500mg)	Oral tablet: 500mg, 1g Oral syrup: 100mg/ml
Dose	30-60mg/kg/day (max 30mg/kg/day in children <3 years), as (10-)12h SQ infusion 5-7 days/week or 24h continuous IV infusion	a) 14-28 mg/kg/day b) 20-40 mg/kg/day once daily	75mg/kg/d, 3 times daily Combination with DFO is standard, with DFX possible
Benefits	Longest experience, liver>heart iron removal	Most effective in liver iron removal	Most effective in heart iron removal
Relevant side effects	Ototoxic, skeletal abnormalities	Renal, hepatic, and gastrointestinal toxicity	Agranulocytosis[2], zinc deficiency, arthralgia
Monitoring of iron overload	• **Diagnostic gold standard: MRI for liver and cardiac iron assessment** o Start by age 5 years at the latest; earlier if possible (especially when evidence of high iron load and when planning HSCT) o Follow up: annual MRI liver iron (more or less often according to iron status). Annual MRI heart iron (more frequently if cardiac iron load present) • Serial ferritin levels and transferrin saturation[3]		
Goals and adjustment plan	• Adjust therapy frequently, based on efficacy/toxicity (typically every 3-6 months) • Optimal target values for iron overload[4]: o MRI liver iron content <3mg/g[5] dry weight; MRI heart T2* >20-35 msec[6] o Serial ferritin: <500ng/ml • Reduction/stopping rules based on ferritin if MRI not available (not standard)[3] o Ferritin 500-1000ng/ml: consider dose reduction o Ferritin 300-500ng/ml: dose reduction or temporary pause required o Ferritin <300ng/ml: temporary pause required • Patients with low ferritin (<500ng/ml), but high liver iron by MRI (>5mg/g dry weight): consider chelation at lower dose and with intensified monitoring for toxicity		
Toxicity monitoring	• DFO and DFX: Annual audiometry (sensineuronal hearing loss?) • DFX: Annual eye exam (cataracts?) • DFX: Monitor for renal (creatinine increase in serum, Fanconi syndrome: phosphate loss, protein in urine), and hepatic injury (transaminitis), gastrointestinal symptoms • Pancreatic iron overload: regularly assess endocrine pancreatic function by fasting glucose, oral glucose tolerance test, fructosamine (instead Hba1c) • Consider hemochromatosis gene testing in patients with rapid/severe iron overload		

[1] Approval status in most countries: DFO first line >3 years old, DFX in 2-6 years old when DFO cannot be used.
[2] DFP prescription should come from an experienced provider. Patient/primary care team must receive emergency protocol for agranulocytosis and fever (immediate drug cessation, antibiotics, G-CSF if needed).
[3] Ferritin and transferrin saturation have limited value in chelation monitoring. Ferritin often inaccurately reflects true iron burden in DBA syndrome (elevated levels may be observed despite low iron burden, while some patients with severe iron overload by MRI can have deceptively low ferritin). Given potential discordance with true tissue iron, these biomarkers alone are inferior to MRI for quantifying actual iron. MRI should be strongly advocated as the standard method for optimal chelation management.
[4] There is a risk of chelator toxicity if treatment is continued too aggressively when MRI liver iron content is <3mg/g, or when serial ferritin is below 500ng/ml (in case MRI measurement is not available).
[5] Unit conversion: mg/g x 18 = µmol/g
[6] Some panel members suggest a more restrictive threshold of >25 msec.

Table 9. Re o endations for allogeneic hematopoietic stem cell transplantation

General	• Assessment of iron overload (MRI liver/heart) before planning HSCT • Iron overload: chelation prior HSCT, consider phlebotomies post HSCT
Age	• In general, before the age of 10 years in chronically transfused patients • If possible, preferably at the pre-school age (~2–5 years) to minimize risk of toxicities • In individual patients, HSCT for transfusion dependence can be considered after the age of 10 years (low transfusion burden, optimal iron balance, adequate organ function) • In adults, HSCT is generally not advised solely for the avoidance of transfusion dependence[1]
Indications	**Listed in order of increasing urgency and clinical necessity:** • Chronic transfusions in patients not responding to steroids • Chronic transfusions in patient with non-manageable iron overload (significant toxicity or chelator failure) • Chronic transfusions in patient with alloimmunization to RBC • Severe immunodeficiency and/or multilineage cytopenia • MDS/AML
Donor choice	**Donors listed with most optimal first:** • MSD: after exclusion of DBA syndrome in potential donor (genetic testing, CBC, eADA) • MUD: 10/10 HLA match based on molecular testing • MMUD and MMFD[2]: only in the absence of alternative therapies (patients with MDS/AML) or in context of clinical trials
Conditioning regimen	• Myeloablative (busulfan or treosulfan) regimen combined with fludarabine • Consider addition of thiotepa • Avoid irradiation
Stem cell source	• Bone marrow (any donor) • Cord blood (healthy sibling donor) • Avoid unmanipulated mobilized peripheral blood stem cells
GVHD prophylaxis	• Standard GVHD prophylaxis i.e., calcineurin inhibitor plus MTX or MMF and serotherapy (also for MSD)

[1] to be considered on a case-by-case basis for transfusion-dependent young adults in good health, after weighing the risks and benefits.
[2] includes haplo-donors.

Abbreviations: HSCT, hematopoietic stem cell transplantation; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; MSD, HLA-matched sibling donor; MUD, HLA-matched unrelated donor; MMUD, HLA-mismatched unrelated donor; MMFD, HLA-mismatched family donor; CBC, complete blood counts; eADA, erythrocyte adenosine deaminase; MTX, methotrexate; MMF, mycophenolate mofetil.

Table 10. Comprehensive long-term surveillance in children and adults with DBA syndrome

Clinical scenario	Surveillance recommendations
HEMATOLOGY	
Any patient (including therapy-independent) Monitor changes in blood counts (Hb, other blood lineages): viral infection, drug induced, MDS/AML	• CBC and reticulocyte count at regular intervals (once yearly if therapy-independent) • Bone marrow aspirate if: more severe anemia without explanation, unexpected reticulocytosis, worsening of neutropenia or thrombocytopenia, abnormal cells

Patient receiving steroids
 	
Monitor efficacy (Hb and reticulocyte count) and treatment toxicity (see also **Table 7**)
 	
Involve endocrinology
	• CBC every ~3-4 months in stable patients
	• LFTs, creatinine, vitamin D levels regularly
	• Vitamin D and calcium supplementation as needed
	• Proton pump inhibitors (or H2 antagonists) during initial high dose prednisone therapy, or when symptomatic
	• Disclose that estrogen-containing oral contraceptives might weaken steroid effect
	• Steroid toxicity requires repeat endocrine evaluation
	  ○ At least yearly testing for diabetes/ metabolic syndrome, bone health (densitometry scan), eye exam (cataract exclusion)
	  ○ Bisphosphonates as therapy option in patients with significant osteoporosis
	  ○ Joint/bone pain must be investigated being mindful of steroid-induced avascular necrosis and risk of osteogenic sarcoma (MRI may be warranted)

Patients on transfusions and chelation
 	
Monitor efficacy (nadir Hb before transfusion) and toxicity (see also **Table 6 and 8**)
 	
Patients with poor iron balance
	• Before every transfusion: CBC with reticulocyte counts, RBC antibodies (if possible)
	• Ferritin and transferrin saturation trend (i.e., every 1-3 months before transfusion)
	• Routine transaminases, creatinine, electrolytes (phosphate if on DFX) virus serology (hepatitis B/C)
	• Annual MRI evaluation or more often according to iron status:
	  ○ Liver iron content (LIC) by T2* or R2
	  ○ Heart iron by T2*
	• Echocardiography, ECG evaluation every 1 to 3 years according to iron status. Consider Holter monitor for patients with cardiac iron overload. Intensify chelation; consider DFP
	• Pancreas and pituary glands: specific endocrine tests: fructosamine (instead of HbA1c in transfused patients), TSH, PTH
	• Growth hormone replacement when indicated
	• Consultation for medically assisted reproduction
	• Dose adjustments and combination of two chelators are frequently required, emphasize importance of medication adherence, facilitate networking with patient groups

Patients on DFO Monitor for toxicity: hearing loss, osteopenia, renal lithiasis	• Bone densitometry (every 1 to 3 years) • Regular audiogram and eye exam (at least annually or more often with dose changes) • Regular renal ultrasound surveillance

	• Higher risk of toxicity is present in patients with low ferritin
Patients on DFX: monitor for toxicity: renal (glomerular or tubular damage including Fanconi syndrome), hepatic toxicity, transaminitis, gastrointestinal issues	• Frequent evaluation of liver and kidney parameters. Patients with toxicity: decrease dose • Regular renal ultrasound surveillance • Audiogram and eye exam (yearly) • Higher risk of toxicity is possibly present in patients with low ferritin
Patients on DFP: screening for neutropenia/agranulocytosis	• Weekly CBC at treatment initiation and during any fever episode, monitor counts often and discontinue DFP for any sign of unusual or progressive neutropenia • Patient information & education (drug passport for emergencies with established plan)
For transplanted patients	• Standard surveillance recommendations. • Higher cancer risk in DBA syndrome patients must be taken into account
IMMUNOLOGY / INFECTIONS	
Hypogammaglobulinemia, Lymphopenia, recurrent infections	• Ig G, A, M levels and lymphocyte subsets (regularly if indicated) • Antibody responses, discuss immunizations and immunoglobulin treatment • For severe T-cell lymphopenia: consider pneumocystis jirovecii pneumonia prophylaxis • Additional prophylaxis and diagnostics according to local standard
Transfusion-related pathogens	• Virus testing at least once yearly (hepatitis B/C, HIV)
Vaccinations	• No restrictions on vaccines: Hepatitis B vaccine especially in patients receiving transfusions; live vaccines: first dose ideally before start prednisone, following doses after steroid reduction. • Patient with significant hypogammaglobulinemia: measure specific vaccine antibody titers
ONCOLOGY	
Solid tumors, MDS/AML	• Patient education, healthy lifestyle (avoid smoking, alcohol, toxins, unprotected sun exposure) • HPV vaccination • Patient adherence to screening procedures as in the general population • Colonoscopy beginning age 20 years, every 5 years or more often if clinically indicated • Bone marrow analysis: consider as baseline in adolescents/ young adults before transitioning to adult care, otherwise in any patient with significant unexplained cytopenia or rise in reticulocytes • Unexplained joint/bone pain: risk of osteogenic sarcoma (low threshold for x-ray / imaging)
FAMILY PLANNING, PREGNANCY	
Genetic risk (transmission)	• Patient education and genetic counselling • Discuss medically assisted reproduction for individuals asking for prenatal or pre-implantation diagnostics (according to national legal regulations)
Pregnancies in DBA syndrome: high risk obstetric care required	• Intensification of chelation prior planned pregnancy to optimize iron balance • Blood support frequently needed to maintain Hb >10.0-10.5 g/dL during pregnancy • Screening for fetal anemia • Detailed recommendations reviewed elsewhere (reference 136)

appendix 1. EXTENDED AUTHOR LIST

additional members of the international DBA syndrome guideline panel (alphabetical order):

Name	Affiliation
Alter, Blanche P.	Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA.
Atsidaftos, Eva	Feinstein Institutes for Medical Research, Manhasset, New York, USA.
Darrigo Junior, Luiz Guilherme	Division of Pediatric Hematology and Oncology, Department of Pediatrics. Ribeirão Preto School of Medicine, Univ. of São Paulo. Ribeirão Preto, Brazil.
Flygare, Johan	Division of Molecular Medicine & Gene Therapy, Department of Laboratory Medicine, Lund Stem Cell Center, BMC A12, Lund University, Sweden.
Giri, Neelam	Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland
Glader, Bertil E.	Stanford University Departments of Pediatrics and Pathology, Stanford Medicine Children's Health, USA.
Grönroos, Marika H.	Department of Pediatric Hematology and Oncology, Turku University Hospital, Turku, Finland.
Hainmann, Ina	Department of Pediatric Hematology and Oncology, University Hospital Bonn, Bonn, Germany.
Hutnik, Lukasz	Medical University of Warsaw, Warsaw, Poland.
Karlsson, Stefan	Molecular Medicine and Gene Therapy, Lund University, Lund, Sweden.
McInnes, Alyson W.	Byondis, B.V., Nijmegen, The Netherlands.
Shi, Lihong	State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China.
Sieff, Colin A.	Division of Hematology/Oncology, Boston Children's Hospital and Dana Farber Cancer Institute Boston, Massachusetts, USA.
Smetanina, Nataliya S.	Dmitry Rogachev National Research Medical Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russian Federation.

Appendix 2. SYNOPSIS OF MAJOR CHANGES

(compared to 2008 consensus - Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference, PMID 18671700):

1. Nomenclature
    a. Previously classical and non-classical DBA / and clinical remission
    b. Now DBA syndrome (to account for individuals without anemia, or atypical presentations). Use of 'treatment-independence" rather than remission in patients with count normalization.
2. Diagnostic criteria
    a. Previously diagnostic and supporting (major and minor) criteria
    b. Now simplified: 1 of 2 diagnostic criteria based on genetics and phenotype
3. Major role of genetics
    a. Previously 6 genes (*RPS17, RPS19, RPS24, RPL5, RPL11, RPL35A*), in ~50% patients
    a. Now 26 genes in ~80% patients and new genetic classification established
2. Hemoglobin prior transfusion
    a. Previously 8g/dL
    b. Now ≥9-10g/dL or a higher level at which the patient is asymptomatic, independent of age across life span
3. Steroid treatment – starting rules
    a. Previously when Hb 9-10g/dL (typically 1-2 weeks after last transfusion)
    b. Now timing independent of last transfusion
4. Steroid treatment – definition of non-response
    a. Previously when Hb <8g/dL
    b. Now when Hb <9g/dL
5. Steroid treatment - maximum maintenance dose
    a. Previously prednisone 0.5mg/kg per day or 1.0mg/kg alternate days
    b. Now prednisone 0.3mg/kg per day or 0.6mg/kg alternate days
6. Chelation therapy – starting rules
    a. Previously when liver iron content 6-7mg/g or ferritin 1000-1500µg/l
    b. Now after 10 transfusions or evidence of iron load (infants: wait until after first failed steroid trial)
7. Chelation therapy – drugs
    a. Previously deferoxamine and deferasirox
    b. Now deferoxamine and deferasirox (first line or second line as combination) and deferiprone (third line)
8. Hematopoietic stem cell transplantation – donor choice
    a. Previously only HLA-matched related (family) donors
    b. Now addition of HLA-matched unrelated donors as comparable donor choice
9. Toxicity monitoring and surveillance
    a. Previously general statements
    b. Now with specific recommendations according to age and therapy status
10. Cancer risk
    a. Previously rudimentary knowledge not allowing to make recommendations
    b. Now specific recommendations based on evidence from registries (including new recommendation on colorectal cancer screening starting age 20 years old)

Appendix 3. METHODS

Panel composition

An international task force of content experts consisting of 53 representatives from 27 countries who are recognized key opinion leaders in clinical management and diagnosis of DBA and ribosome research was appointed by the leaders of the European DBA (EuroDBA) Consortium and the DBA Registry of North America (DBAR) and met for the first time in person in 2014 in Freiburg, Germany. The panel, consisted of clinical providers collectively caring for >2500 children, adolescents, and adults with DBA syndrome, including pediatric hematologists and oncologists, pathologists, endocrinologists, geneticists, and experts in transfusion medicine, hematopoietic stem cell transplantation (HSCT), iron management, adult medicine, in addition to researchers in ribosome biology and patient group representatives. At the first meeting in 2014, clinically relevant discussion items were selected (see below). After 2 additional meetings in Europe 2015 (Vienna, Austria) and 2017 (Freiburg, Germany), the task force met for a 4th time in the Atlanta USA in 2018 to agree on the final items and develop a final structure of the consensus manuscript. The manuscript was refined through continued discussion at 4 additional virtual meetings between 2019 and 2022 resulting in this international guideline document.

Search strategy and consensus methodology

We sourced all publications on PubMed database (https://pubmed.ncbi.nlm.nih.gov) through 30th June 2022, with the relevant search terms (including but not limited to: Diamond Blackfan anemia, congenital hypoplastic anemia, congenital anemia, pure red cell aplasia, bone marrow failure, congenital abnormalities, hematopoietic stem cell transplantation, transfusion, steroids, prednisone, chelators, deferoxamine, deferasirox, deferiprone, iron overload, liver iron content, heart iron, MRI, cancer risk, colorectal cancer, osteosarcoma, MDS, AML, cancer screening, toxicity, long-term management, surveillance) and made use of unpublished observations and updates from participating experts, particularly those from national registries. We took into account the expertise and experience of the participants in addition to published evidence.

The modified Delphi technique employed in this study involved a systematic, multi-round process designed to achieve consensus among an expert panel.[18] At an initial face-to-face meeting, we formed separate working groups focused on diagnosis, therapies, and surveillance in children and adults. In the first Delphi round, we conducted an extensive literature review and synthesis of existing data on the topic, which informed the development of a list containing key discussion items and claims for each working group. Each member of the expert panel independently provided judgments and critiques on each item. In subsequent iterative rounds, we had additional meetings and rediscussed the list items, with the goal of obtaining consensus, defined as >85% agreement on each item. This process continued until consensus was reached on most items. However, there were a few items where agreement remained below the 85% threshold; these areas of non-consensus are transparently acknowledged and discussed in the manuscript. Overall, the modified Delphi technique enabled systematic convergence of expert opinions on this complex topic through structured, iterative data collection and discussion within each focused working group.

Evidence level grading

Both levels of evidence A and B (data from randomized trials, meta-analyses, or large non-randomized studies) are lacking in DBA syndrome. Because of that and due to the rarity of disease, level of evidence C (expert consensus statement, retrospective analyses, and registry data) can be used for guideline development on DBA syndrome. The task force sourced published work (level of evidence C) and made use of expertise and clinical experience of the participants and unpublished observations, in particular those from national registries (level of evidence C).

Appendix 4. PARVOVIRUS B19 TESTING

In individuals with normal red blood cell turnover, short interruption of erythropoiesis by Parvovirus B19 infection does not lead to anemia, however in infants and patients with chronic hemolysis or immunodeficiency state, severe anemia can develop. Modern diagnostics of B19 infection usually include the combination of serology (B19 IgG and IgM antibodies in blood) and PCR for B19 DNA in blood or BM. Morphologically, BM aspirates show no mature erythroid precursors and with characteristic giant pronormoblasts at time of acute infection. In infected individuals, the positive rates of parvovirus B19 genome were shown to be significantly higher in the bone marrow (22%) vs. peripheral blood cells (0.8%).[125] B19V infection is usually self-limited, resolving in days to weeks. However, B19 DNA can be found by PCR in 2% of healthy individuals in the BM (but not in blood) despite seroconversion after previous infection. Thus, persistency of B19 DNA may represent both infectious virus and residual DNA from remote infection.[126]

Appendix 5. LONG-TERM MANAGEMENT AND SURVEILLANCE

Children

Major management goals are optimizing physical and cognitive development. Birth defects require by appropriate follow-up. Routine growth monitoring is essential, with an endocrinologist guiding hormone testing and steroid toxicity management (**Table 7**). To improve growth, steroids can be stopped before/during puberty during which transfusions are given. Growth hormone (GH) therapy can effectively treat DBA syndrome-associated growth deficiency[127] There are no data demonstrating increased cancer risk from GH in DBA syndrome and results from childhood cancer survivors studies are reassuring.[128,129] The panel agreed that GH is reasonable if needed - either during pubertal growth (i.e., steroid holiday) or earlier. As steroids may impair efficacy of GH, optimal GH use would be with transfusions, although combining with steroids is not contraindicated. Early and aggressive chelation with toxicity monitoring (**Table 8**) and MRI-based evaluation of iron burden are critical. HSCT has excellent outcomes in DBA syndrome and should be discussed after initial steroid failure (**Table 9**).

Adults

Adult patient care should involve adjusting anemia-directed therapy, monitoring therapy toxicities and comorbidities including cancer predisposition, addressing family planning and pregnancy monitoring, and planning careful transition from pediatric care. Adults with DBA syndrome are at risk for both non-malignant and malignant complications, resulting in a cumulative incidence of death of 23% by age 45 years, as noted in the DBAR; mainly of HSCT complications, iron overload, infections, and leukemia/solid tumors.[130] Treatment-related organ dysfunction is common (**Table 5, Table 10)** Adrenal insufficiency and hypogonadism require specific testing. Psychosocial support and comprehensive services enable optimal possible care. Many adults require higher Hb for a healthy daily life and work. Frequent chelator adjustments are needed with long-term chelator toxicity. CBC monitoring to detect early MDS/AML signs is recommended,[131] as is baseline BM assessment before transition to adult care. Colonoscopy is recommended from 20 years old. Associated immunodeficiency (decreased B/NK lymphocyte counts and immunoglobulins)[132,133] warrants immune parameter monitoring. Increased autoimmunity has not been reported. Regular immunizations are warranted. Genetic counseling and family planning are of concern to adolescents and adults. DBA syndrome has mainly autosomal dominant inheritance, but genetic penetrance and expressivity vary significantly, and no predictions can be made about disease severity in the next offspring. Decreased fertility has not been reported in males with DBA syndrome. In female patients, pregnancy complications occur, requiring expert obstetrical care (for detailed guidelines see [134]). Iron chelation should be optimized in transfusion-dependent females prior to conception since chelating drugs are contraindicated during pregnancy (although data from pregnant women with thalassemia suggest that chelation with DFO could be considered in severely iron-overloaded women during the last trimester[135]). In a study evaluating 64 pregnancies, vascular-placental complications were noted in 66% of pregnant women with DBA syndrome.[136] The panel agreed that the nadir Hb should be maintained at $\geq$10.0-10.5g/dL as recommended for pregnancy in thalassemia[137]. Thus, many patients with DBA syndrome, including those responsive to steroids, will require transfusions during pregnancy. Due to placental vasculopathy, prophylaxis with acetylsalicylic acid may be considered.[134,136]

Appendix 6. ACKNOWLEDGMENTS

Clinicians and scientists facilitating the discussion:
Amina Szvetnik, Regine Grosse, and Doris Steinemann (Germany), Polyxeni Delaporta (Greece), Vijay Sankaran and Steven Ellis (USA), Elsbeth Payne (UK), Susana Navarro and Juan Bueren (Spain), Marieke Von Lindern and Marc Bierings (The Netherlands), Isabelle Marie and Marie-Françoise O'Donohue (France), Denis LJ Lafontaine (Belgium), Jan Valka (Czech Republic), Maryam Behfar (Iran), Nurten Akarsu (Turkey), Anupama Narla (USA), Alan Warren (UK), Anna Aspesi (Italy), Anna Beatriz Willemes Batalha and Simone de Castro Resende Franco (Brazil), Lukasz Dembinski (Poland), Amee George and Ross Hannan (Australia).

Patient group representatives:

Dawn M. Baumgardner and Kathleen Kilcoyne Vroman Marie Arturi	USA	DBA Foundation: dbafoundation.org Daniella Maria Arturi Foundation: https://www.facebook.com/daniellafoundation/
Leisa Batkin, Martin Winter and Peter Redmond	UK	DBA UK Charity: diamondblackfan.org.uk
Wilma Rzany, Rosemarie Behling and Beate Buchholz	Germany	Diamond-Blackfan-Anämie-Selbsthilfegruppe: diamond-blackfan.de
Marcel Hibert	France	Association Francophone de la Maladie de Blackfan-Diamond: afmbd.org
Maria Elisabetta Villa	Italy	DBA Italia ODV www.fondazionedba.org
Carlos Carrión Martín and Susana Pont Quesada	Spain	Asociación Afectados Blackfan Diamond España: anemiablackfandiamond.org.es
Sofia Thoren, Patrick and Camilla Fors	Sweden	Swedish DBA patient group: www.facebook.com/groups/472777966082065
Boris Marte and Marianne Marte-Hofbauer	Austria	Austrian support group: https://dbaexperiment.org
Tingting Deng and Xiaomei Jia	China	Organization for Rare Disorders and Ai You Foundation
Ivana Kyselkova and Jiri Heczko	Czech Republic	
Jukka von Hertzen	Finland	
Hans Bruun Dabelsteen and Heidi Mia Kirkebye	Denmark	
Janet Pereira and Carol Manfini	Canada	DBA Canada: www.dbacanada.com
Jessica Bond	Australia	Maddie's Vision: www.mrv.org.au

APPENDIX REFERENCES

107. Gerrard G, Valganon M, Foong HE, et al. Target enrichment and high-throughput sequencing of 80 ribosomal protein genes to identify mutations associated with Diamond-Blackfan anaemia. *Br J Haematol* 2013; **162**(4): 530-6.

108. Ikeda F, Yoshida K, Toki T, et al. Exome sequencing identified RPS15A as a novel causative gene for Diamond-Blackfan anemia. *Haematologica* 2017; **102**(3): e93-e6.

109. Cmejla R, Cmejlova J, Handrkova H, Petrak J, Pospisilova D. Ribosomal protein S17 gene (RPS17) is mutated in Diamond-Blackfan anemia. *Hum Mutat* 2007; **28**(12): 1178-82.

110. Draptchinskaia N, Gustavsson P, Andersson B, et al. The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. *Nat Genet* 1999; **21**(2): 169-75.

111. Nieminen TT, O'Donohue MF, Wu Y, et al. Germline mutation of RPS20, encoding a ribosomal protein, causes predisposition to hereditary nonpolyposis colorectal carcinoma without DNA mismatch repair deficiency. *Gastroenterology* 2014; **147**(3): 595-8 e5.

112. Gazda HT, Grabowska A, Merida-Long LB, et al. Ribosomal protein S24 gene is mutated in Diamond-Blackfan anemia. *Am J Hum Genet* 2006; **79**(6): 1110-8.

113. Wang R, Yoshida K, Toki T, et al. Loss of function mutations in RPL27 and RPS27 identified by whole-exome sequencing in Diamond-Blackfan anaemia. *Br J Haematol* 2015; **168**(6): 854-64.

114. Gripp KW, Curry C, Olney AH, et al. Diamond-Blackfan anemia with mandibulofacial dystostosis is heterogeneous, including the novel DBA genes TSR2 and RPS28. *Am J Med Genet A* 2014; **164A**(9): 2240-9.

115. Mirabello L, Macari ER, Jessop L, et al. Whole-exome sequencing and functional studies identify RPS29 as a novel gene mutated in multicase Diamond-Blackfan anemia families. *Blood* 2014; **124**(1): 24-32.

116. Jongmans MCJ, Diets IJ, Quarello P, Garelli E, Kuiper RP, Pfundt R. Somatic reversion events point towards RPL4 as a novel disease gene in a condition resembling Diamond-Blackfan anemia. *Haematologica* 2018; **103**(12): e607-e9.

117. Lebaron S, O'Donohue MF, Smith SC, et al. Functionally impaired RPL8 variants associated with Diamond-Blackfan anemia and a Diamond-Blackfan anemia-like phenotype. *Hum Mutat* 2022; **43**(3): 389-402.

118. Landowski M, O'Donohue MF, Buros C, et al. Novel deletion of RPL15 identified by array-comparative genomic hybridization in Diamond-Blackfan anemia. *Hum Genet* 2013; **132**(11): 1265-74.

119. Mirabello L, Khincha PP, Ellis SR, et al. Novel and known ribosomal causes of Diamond-Blackfan anaemia identified through comprehensive genomic characterisation. *J Med Genet* 2017; **54**(6): 417-25.

120. Gazda HT, Preti M, Sheen MR, et al. Frameshift mutation in p53 regulator RPL26 is associated with multiple physical abnormalities and a specific pre-ribosomal RNA processing defect in diamond-blackfan anemia. *Hum Mutat* 2012; **33**(7): 1037-44.

121. Farrar JE, Nater M, Caywood E, et al. Abnormalities of the large ribosomal subunit protein, Rpl35a, in Diamond-Blackfan anemia. *Blood* 2008; **112**(5): 1582-92.

122. Klar J, Khalfallah A, Arzoo PS, Gazda HT, Dahl N. Recurrent GATA1 mutations in Diamond-Blackfan anaemia. *Br J Haematol* 2014; **166**(6): 949-51.

123. Ludwig LS, Gazda HT, Eng JC, et al. Altered translation of GATA1 in Diamond-Blackfan anemia. *Nat Med* 2014; **20**(7): 748-53.

124. Parrella S, Aspesi A, Quarello P, et al. Loss of GATA-1 full length as a cause of Diamond-Blackfan anemia phenotype. *Pediatr Blood Cancer* 2014; **61**(7): 1319-21.

125. Watanabe K, Otabe K, Shimizu N, et al. High-sensitivity virus and mycoplasma screening test reveals high prevalence of parvovirus B19 infection in human synovial tissues and bone marrow. *Stem Cell Res Ther* 2018; **9**(1): 80.

126. Heegaard ED, Petersen BL, Heilmann CJ, Hornsleth A. Prevalence of parvovirus B19 and parvovirus V9 DNA and antibodies in paired bone marrow and serum samples from healthy individuals. *J Clin Microbiol* 2002; **40**(3): 933-6.

127. Howell JC, Joshi SA, Hornung L, Khoury J, Harris RE, Rose SR. Growth hormone improves short stature in children with Diamond-Blackfan anemia. *Pediatr Blood Cancer* 2015; **62**(3): 402-8.

128. Boguszewski MCS, Cardoso-Demartini AA, Boguszewski CL, et al. Safety of growth hormone (GH) treatment in GH deficient children and adults treated for cancer and non-malignant intracranial tumors-a review of research and clinical practice. *Pituitary* 2021; **24**(5): 810-27.
129. Pollock NI, Cohen LE. Growth Hormone Deficiency and Treatment in Childhood Cancer Survivors. *Front Endocrinol (Lausanne)* 2021; **12**: 745932.
130. Vlachos A, Rosenberg PS, Atsidaftos E, et al. Increased risk of colon cancer and osteogenic sarcoma in Diamond-Blackfan anemia. *Blood* 2018; **132**(20): 2205-8.
131. Simkins A, Bannon SA, Khoury JD, et al. Diamond-Blackfan Anemia Predisposing to Myelodysplastic Syndrome in Early Adulthood. *JCO Precis Oncol* 2017; **1**(1): 1-5.
132. Khan S, Pereira J, Darbyshire PJ, et al. Do ribosomopathies explain some cases of common variable immunodeficiency? *Clin Exp Immunol* 2011; **163**(1): 96-103.
133. Iskander D, Roberts I, Rees C, et al. Impaired cellular and humoral immunity is a feature of Diamond-Blackfan anaemia; experience of 107 unselected cases in the United Kingdom. *Br J Haematol* 2019; **186**(2): 321-6.
134. Taher AT, Iolascon A, Matar CF, et al. Recommendations for Pregnancy in Rare Inherited Anemias. *Hemasphere* 2020; **4**(4): e446.
135. Origa R, Comitini F. Pregnancy in Thalassemia. *Mediterr J Hematol Infect Dis* 2019; **11**(1): e2019019.
136. Faivre L, Meerpohl J, Da Costa L, et al. High-risk pregnancies in Diamond-Blackfan anemia: a survey of 64 pregnancies from the French and German registries. *Haematologica* 2006; **91**(4): 530-3.
137. Vlachos A, Ball S, Dahl N, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. *Br J Haematol* 2008; **142**(6): 859-76.