Literature Review

Therapeutic Trends and Perspectives in Diamond Blackfan Anemia

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Abbreviations:
DBA – Diamond Blackfan anemia, **DBAR** – Diamond Blackfan Anemia Registry of North America, **HbF** – fetal hemoglobin, **eADA** – erythrocyte adenosine deaminase, **CHH-AD** – cartilage hair hypoplasia - anauxetic dysplasia, **TCS** – Tracher Collins syndrome, **PRCA** – pure red cell aplasia, **RPS** – ribosomal protein small subunit, **RPL** – ribosomal protein large subunit, **AML** – acute myeloid leukemia, **MDS** – myelodysplastic syndrome, **IBMF** – inherited bone marrow failure, **HSCT** – hematopoietic stem cell transplantation, **RBC** – red blood cells, **GCs** – glucocorticoids, **GCR** – glucocorticoid receptor, **DFX** – Deferasirox, **DFO** – Deferoxamine, **DFP** – Deferiprone, BFU-E – burst forming unit-erythroid, **CFU-E** – colony forming unit-erythroid, **cGvHD** – chronic grafts versus host disease, **aGvHD** – acute grafts versus host disease, **HLA** – human leukocyte antigen, **AIEOP** – Italian Association of Paediatric Haematology and Oncology Registry, LVs – lentiviral vectors, **EFS** – elongation factor 1α short, **ZFNs** – Zinc finger nucleases, **TALENs** – transcription activator-like effector nucleases, **CRISPR/Cas9** – clustered regularly interspaced short palindromic repeats-associated Cas9, **mTOR** – mammalian target of rapamycin, **SMER** – small molecule enhancers of rapamycin.

Abstract

Blackfan Diamond anemia is a rare clinical entity caused by mutations in genes encoding ribosomal proteins.

Erythrogenesis defects, somatic malformations, and increased risk of developing hematological malignancies or solid tumors place the affected pediatric population in the focus of specialists.

While classical approaches such as blood transfusions and stem cell transplantation aim to relieve anemia and correct bone marrow dysfunction, therapeutic strategies based on *L-Leucine*, *Sotatercept*, *Trifluoperazine*, *SMER28*, and *Eltrombopag* seek to improve the production of erythroid precursors.

Even if in its nascent stages, therapeutic directions focused on processing genetic material aim to remedy abnormal gene expression.

This literature review aims to highlight treatments used to improve blood-forming function and therapeutic prospects that could increase the life expectancy of patients with Diamond Blackfan anemia.

Introduction

Blackfan Diamond Anemia (DBA) is an inherited bone marrow failure syndrome characterized by deficient production of erythroid precursors in association with craniofacial, upper limb, genitourinary, and cardiac malformations.

According to studies conducted by Diamond Blackfan Anemia Registry of North America (DBAR), half of patients are diagnosed within the first 3 months of life, while 90% of cases are identified by the age of one year.[1]

DBA usually manifests as a macrocytic and a regenerative anemia.

Even if other cell lines are normal, some patients may present with neutropenia, thrombocytopenia, or thrombocytosis.

Patients often have increased fetal hemoglobin (HbF) and increased erythrocyte adenosine deaminase activity (eADA).[2]

The clinical picture of DBA includes pallor, growth disorders[3], and multiple congenital malformations such as microcephaly, micrognathia, microtia, epicanthus, palatoschisis, short neck, absence of kidney, horseshoe kidney, hypospadias, coarctation of the aorta, tetralogy of Fallot.

Some patients may also see absent, hypoplastic, bifid, or triphalangeal thumb and flat thenar eminence.[1]

Differential diagnosis may include a multitude of syndromes that sum up a range of organic or limb malformations such as Fanconi Anemia, Shwachman-Diamond syndrome, Pearson syndrome, dyskeratosis congenita[1], cartilage-hair hypoplasia-anauxetic dysplasia (CHH-AD) and Treacher Collins syndrome (TCS).[4]

DBA is included in the category of rare diseases, being estimated between 1 and 4 cases /500,000 live births in a year, similarly affecting both sexes and different racial groups.[5]

According to studies, DBA is a form of congenital pure red cell aplasia (PRCA)[6], resulting from monoallelic mutations in ribosomal genes: RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPL5, RPL11, RPL26, RPL35A.

Although the most common mutation detected appears to be in the RPS19 gene, there are families whose mutant genes also include mutations in RPL3, RPL7, RPL9, RPL14, RPL18, RPL19, RPL23A, RPL26, RPL35, RPL36, RPS8, RPS15, RPS27A.[7]

Beyond ribosomal gene defects, in some cases, the disease can be caused by mutations in GATA1, also called erythroid transcription factor.

This transcription factor is required for normal hematopoiesis.[5,8]

Although DBA is inherited in an autosomal dominant manner in 40-50% of cases, recent studies do not rule out the possibility that the disease occurs independently of family history.[5]

Cases of DBA require increased attention from clinicians because this syndrome is associated with a predisposition to developing malignancies such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia, Hodgkin's and non-Hodgkin's lymphoma, breast cancer and hepatocellular carcinoma.[9]

As opposed to other inherited bone marrow failure (IBMF), DBA predisposes to the development of solid tumors such as osteosarcoma, colon cancer, and lung cancer.[10]

Materials and methods

Reviews, clinical cases, and studies relevant to the topic from specialized sources (PubMed, Wiley Online Library, MPDI, Elsevier) were used for this article.

The information presented was selected from documents published between 2016-2022, except for three publications published in 2008, 2010 and 2014.

The keywords used for the search were "Diamond Blackfan anemia", "therapy" and "treatment".

For the description of therapeutic strategies have been included papers exposing the benefits of therapy, adverse reactions, risks, and their complications.

Papers with unavailable full text and those dealing with outdated therapies or without notable benefits were excluded.

Current therapeutic methods

First-line therapy includes blood transfusions, corticotherapy, and hematopoietic stem cell transplantation (HSCT).

Given the deficient production of erythroid precursors, blood transfusions are an effective choice for correcting anemia.

Generally, clinicians aim to maintain hemoglobin levels above 80g/l by administering 10-15 ml/kg red blood cells (RBC) every 3-5 weeks.

To promote growth and development, it is especially important to keep hemoglobin levels over 90 g/l in newborns and young children.
Beyond trying to treat anemia, transfusion therapy is closely related to corticosteroid therapy.

Transfusions are the best method to treat patients under one year of age who do not respond to glucocorticoid therapy or who need higher doses of the drug, while also avoiding the side effects of glucocorticoids.[3]

The establishment of chronic transfusion therapy requires prophylaxis of iron overload.

In this regard, oral or parenteral administration of chelators such as *deferasirox* (DFX) or *deferoxamine*

(DFO) is of benefit to patients with repeated transfusions.

Unlike other congenital anemias, patients with DBA develop secondary hemochromatosis more rapidly, often associated with cardiac complications through the deposition of large amounts of iron in the heart muscle, which predisposes them to early heart failure.[11]

Even if *deferiprone* (DFP) is an effective chelator in removing excess cardiac localized iron, its induced agranulocytosis makes its use in DBA difficult.[11,12]

According to DBAR studies, an increased percentage of patients respond to *prednisone* or *prednisolone* therapy.

While complete blood count and reticulocyte count are monitored weekly, maintaining hemoglobin values above 9 g/dl in the absence of transfusions proves the effectiveness of treatment.[1]

Administration of glucocorticoids (GCs) activates glucocorticoid receptors (GCRs) and causes transcription of *Myb*, *Kit*, and *Lmo2* factors, involved in the proliferation of burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E).[13]

It is important to note that during treatment, patients are prone to infections with opportunistic bacteria, such as *Pneumocystis jirovecii*, which requires prophylactic administration of *Sulfamethoxazole-trimethoprim.*[1]

During long-term cortisone therapy, side effects such as demineralization and bone fractures, osteoporosis, hypertension, diabetes mellitus, glaucoma, cataracts, vascular necrosis, and growth deficiency are highly possible, probable, and of increased intensity.[1,14]

Nowadays, hematopoietic stem cell transplantation (HSCT) is a therapeutic method that offers a chance to cure patients with DBA.

At the same time, patients who develop myelodysplastic syndromes (MDS) secondary to DBA, resistant to steroids, and dependent on transfusions can obtain improvement in the quality of life after HSCT.[15]

Recent studies show that stem cell transplantation should be performed before the patient is 10 years old because post-transfusional iron overload is associated with the development of chronic graft-versus-host disease (cGvHD).[16]

Even if the cells can be taken from a donor unrelated to the patient, an HLA-matched sibling without genetic mutations or specific DBA manifestations is the best option.

If the donor is not related to the patient, the transplant can be carried out provided the marrow is a perfect match.

About the source of stem cells, studies show that stem cell collection from bone marrow has a lower risk of cGvHD compared to cells from peripheral blood.[3]

Cord blood is also a source of stem cells associated with satisfactory results in related donors.[15]

According to studies conducted by DBAR and the Italian Association of Paediatric Haematology and Oncology Registry (AIEOP), total body irradiation used in unrelated donor HSCT and *busulfan*-based conditioning therapy used in related donor HSCT caused osteosarcoma.

In this regard, *fludarabine* and *treosulfan* therapy, an agent with lower hematological toxicity, has been tried for a lower intensity of the preparative regimen.[17]

In opposition to the excellent results of HSCT are the risk of acute and chronic graft versus host disease, infertility, and death caused by multiple viral infections.[15]

Alternative therapies

According to a multicenter study by A. Vlachos, supplementation of *L-Leucine* increased reticulocyte counts and improved growth deficits in children with DBA.[18]

While a phase II study expresses the efficacy of *Sotatercept (ACE-011)* in correcting chemotherapy-induced anemia, another trial conducted by A. Vlachos supports the safety of its administration in DBA patients.[19,20]

According to a recent study, administration of *Eltrombopag* to patients with DBA resulted in improved red blood cell production due to chelating properties and the ability to correct disorders of heme and globin synthesis.[21]
In addition, recent papers mention the possibility of preventing pancytopenia in elderly patients treated with *Eltrombopag.*[22]

Furthermore, DBA patients treated with *trifluoperazine*, a calmodulin inhibitor, experienced better erythroid differentiation and decreased p53 protein activity.[23]

Designed as a mechanistic target of rapamycin (mTOR), *SMER28* is an autophagy-inducing

molecule used to ameliorate neurodegenerative processes.

In DBA patients, the introduction of *SMER28* into the therapeutic plan resulted in the multiplication of erythrocytes, due to its action on immature erythroid precursors and erythroblasts.

Last but not least, *SMER28* was able to correct deficient globin and heme expression caused by the RPS19 gene defect.[24]

Strategies based on genetic material processing

Gene therapy

Because 25% of patients have the RPS19 gene mutation, gene therapy is an attractive treatment option.[25]

This approach aims to proliferate erythroid colonies by transferring cDNA into CD34+ cells using lentiviral (LVs) or oncoretroviral vectors.[26]

Although bone marrow failure could be treated with lentiviral vectors, therapy does not exclude the risk of insertional mutagenesis.

In a study conducted on a RPS19-deficient mouse model, the use of the EFS-RPS19 vector caused increased expression of mRNA RPS-19 and contributed to the differentiation of erythroid precursors, without generating hematological abnormalities and increased the risk of insertion mutagenesis.[25,27]

Genome editing

By correcting aberrant genetic sequences, editing the human genome offers the chance to treat congenital or acquired disorders and allows the management of hematological diseases.[28]

Among ZFNs, TALENs, and CRISPR/Cas9, the most commonly used genome editing technologies, CRISPR/Cas9 is the most recent and promising method.

Since the DNA repair system in DBA is unaffected, CRISPR/Cas9 technology should be more effective than in diseases associated with DNA repair deficiency.[26]

Although the risk of oncogenesis is lower than with lentiviral vector gene transfer, off-target nuclease activity can disrupt the response of genes involved in tumor suppression.[28]

Discussions

Although DBA is listed as a rare disease, further research into early diagnosis and curative treatment without major side effects is extremely important.

Even though the life expectancy of these children has increased significantly, it is very important to have a well-established program of screening for potential oncological abnormalities at risk of developing as well as adverse reactions due to chronic medication.

Also, within the family, members should be advised to consult a geneticist for genetic advice.

Because the incidence of occurrence of the disease is extremely low, the diagnosis of DBA is often missed when the clinician is faced with an anemic child with various associated congenital malformations, mainly because other differential diagnoses are more likely and more common.

Conclusions

Research findings on DBA highlight that the management of these patients remains a challenge for clinicians.

Beyond the favorable prognosis of the disease, complications from conventional therapies can alter the quality of life, while the risk of neoplasia contributes to reduced life expectancy.

Although alternative therapies and strategies based on the processing of genetic material seem to have a promising future, further research is needed into their feasibility as a curative treatment for DBA.

Author contributions

The author contributed to the reviewing of the previous literature on the subject, the collection of data from specialized sources and the drafting of the article.

Conflict of interest

The author declares that there are no conflicts of interest concerning this article.

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