Beta Thalassemia Major: Overview of Molecular Etiology, Pathophysiology, Current and Novel Therapeutic Approaches

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Review Article

ABSTRACT

Major beta thalassemia is a severe form of thalassemia caused by the alteration of two beta globin genes resulting in a defective synthesis of hemoglobin.
It is characterized by chronic severe anemia, ineffective erythropoiesis (IE) and iron overload. However although the thransfusion and chelation asssociated constitute the basis of the traitement curently recommended, they do not allow always to control the iron overload induced by pathology and repeated transfusions.

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Hematopoietic stem cell transplantation (HSCT) has proven to be a definitive treatment for beta thalassemia. However, this procedure is confronted to immunological complications and the small nomber of histocompatible donors. In the face of these therapeutic blocks, much research has been undertaken in recent years leading to the development of a number of promising therapeutic strategies in order to reduce the constraints linked to current chronic treatments, and to move towards an access to healing for all patients. Among other three approaches are envisaged and are in the experimental phase: Gene therapy to restore globin chain imbalance, Improve ineffective erythropoiesis and Improve iron dysregulation. In this article we give a view on the pathophysiology, clinical manifestations, genetic origin of beta-thalassaemia major. The second part presents the therapeutic arsenal currently used, and its limits leading to therapeutic impasse. The last part explores the scientific tracks that present a real therapeutic potential in β-Thalassemia.

1. INTRODUCTION

Beta thalassemia major is an autosomal recessive disorders resulting from reduced or absent production of β-globin chains of haemoglobin [1]. It is characterized by ineffective erythropoiesis, chronic haemolytic anaemia and subsequent clinical complications. Beta-thalassemia major or COOLAY anemia was first discovered in the Mediterranean Basin and is highly prevalent in countries also affected by malaria, but marriage and human migration have expanded their area of presence around the world [2]. It has been estimated that about 1.5% of the global population (80 to 90 million people) are carriers of betathalassemia, with the average worldwide incidence of the number of new cases per year is 1 in 100,000 people [3]. In Morocco, it is estimated that nearly 3,000 new beta thalassemia (homozygous) are born each year, which presupposes a health problem [4]. Patients with beta-thalassemia major require regular transfusions of red blood cells to survive [5]. However, repeated transfusions cause iron overload, with life-threatening complications, such as endocrine dysfunction, cardiomyopathy, liver disease and, ultimately, premature death. In the absence of transfusion, patients with beta thalassemia major die within the first five years of life, and even with transfusions, only 50-65% of patients live beyond the age of 35 years in high-income countries [6]. The advances in haematopoietic stem cell transplantation (HSCT) techniques have provided a potentially curative option for some patients. It is unfortunately not a success or can be envisaged for all patients and can be fatal in the case of incompatible donors [7]. Faced with this finding of therapeutic deadlock, numerous researches have been undertaken in recent years in order to improve patient care, to reduce the constraints linked to current chronic treatments which impose high costs on health systems, and to tend towards access to healing for all patients with β-Thalassemia. This article covers three parts: The first provide an overview of the pathophysioloy, genetic origin and clinical manifestation of beta-thalassemia major. The second parthighlight the therapeutic arsenal currently used and its limits leading to the therapeutic deadlock. Finally, the last part explores the scientific tracks which present a real therapeutic potential in β-Thalassemia; between improvements in current tools and real advances leading to the elimination of the pathological gene, governed by the limits of science and ethics.

2. PATHOPYSIOLOGY

Haemoglobin is a tetramer, made up of 4 identical protomers 2 to 2. Each protomer is composed of a globin (alpha or beta chain of globular glycoproteins) and heme which carrying an iron atom [8].

Haemoglobin synthesis is controlled by two multigene clusters, on chromosome 16 (is composed of 3 homologous genes, the zeta (ζ) gene, alpha 1 (HBA1) and alpha 2 genes (HBA2)) and on chromosome 11 containing five functional genes, (ε (*HBE*), Gγ (*HBG2*), Aγ (*HBG1*), δ (*HBD*), and β (*HBB*)), which are arranged along the chromosome in the order of their developmental expression to produce different Hb tetramers sequentially at each stage of embryonic and fetal development : Embryonic (Hb Gower-1 ($\zeta_2\varepsilon_2$), Hb Gower-2 ($\alpha_2\varepsilon_2$) and Hb Portland ($\zeta_2\beta_2$)), fetal ($\alpha_2\gamma_2$) and adult (HbA, $\alpha_2\beta_2$ and HbA$_2$, $\alpha_2\delta_2$) [9] (Fig. 1).

Upstream of the entire β globin complex is the locus control region (LCR), which consists of five DNase hypersensitive (HS) sites (designated HS 1–5). The LCR plays a critical role in β globin gene expression by maintaining an open chromatin state and acting as a powerful enhancer of globin gene transcription; in its absence, the level of β globin gene expression is low. Four of the sites (HS 1–4) are erythroid-specific, encompassing binding sequences for erythroid-restricted transcription factors (GATA-1 and NF-E2), while HS5 is ubiquitous [10].

The physiological situation is characterized by a balanced production of theα and the non-α globin chains that ensures a reciprocal pairing into the normal tetramers. In bêta-thalassemia, this equilibrium is perturbed by the defective production of bêta globin chains resulting from mutations in the beta-globin gene leading to accumulation of α globin chains and causing anemia and an increase in HbF and HbA2 as there are decreased beta chains for HbA formation [8].

More than 200 β-thalassaemia mutations have been described on the β gene cluster affecting any of the stages from transcription to RNA processing to translation of β-globin mRNA. The large majority of mutations are simple nucleotide substitutions or deletions or insertions of oligonucleotides leading to frame shift. Rarely the β-thalassemias are the result of gross gene deletions [11].

The mutations are categorized into β0, β+ and β++ thalassemia alleles according to the degree of reduction of the β-globin chain output. Mutations that completely abolish the production of β-globin are known as β0 - thalassemia (severe) or beta thalassemia major, whilst mutations which result in milder reduction in β-globin are known as β+ - thalassemia. A group of mutations which are labelled as β++-thalassemia results in only slight effects on the β-globin chain synthesis [12].
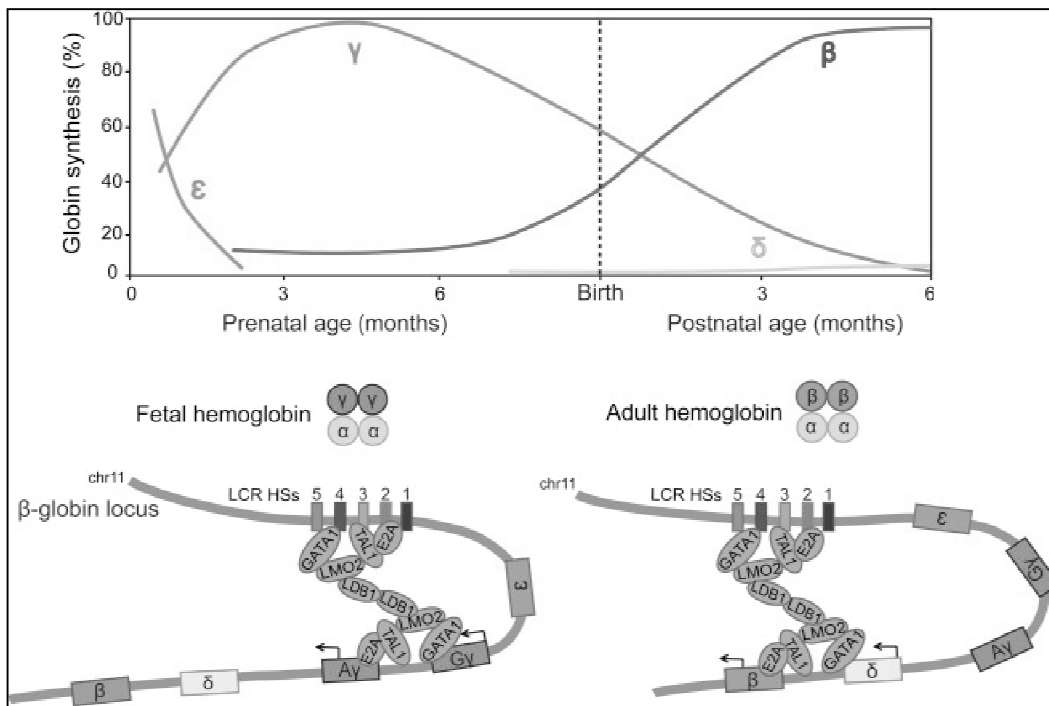

Fig. 1. The human β-like globin genes and their patterns of expression during development: the human β-like globin gene locus contains five β-like globin genes. The ε-globin is expressed during the embryonic stage and replaced by γ-globin during fetal life. Around time of birth, a γ-to-β-globin switch occurs and the β-globin is predominantly expressed in the adult life. The adult δ-globin gene is poorly expressed. A pentameric complex mediates long-range interactions between the LCR and γ- and β-globin promoters in fetal and adult erythroblasts, respectively
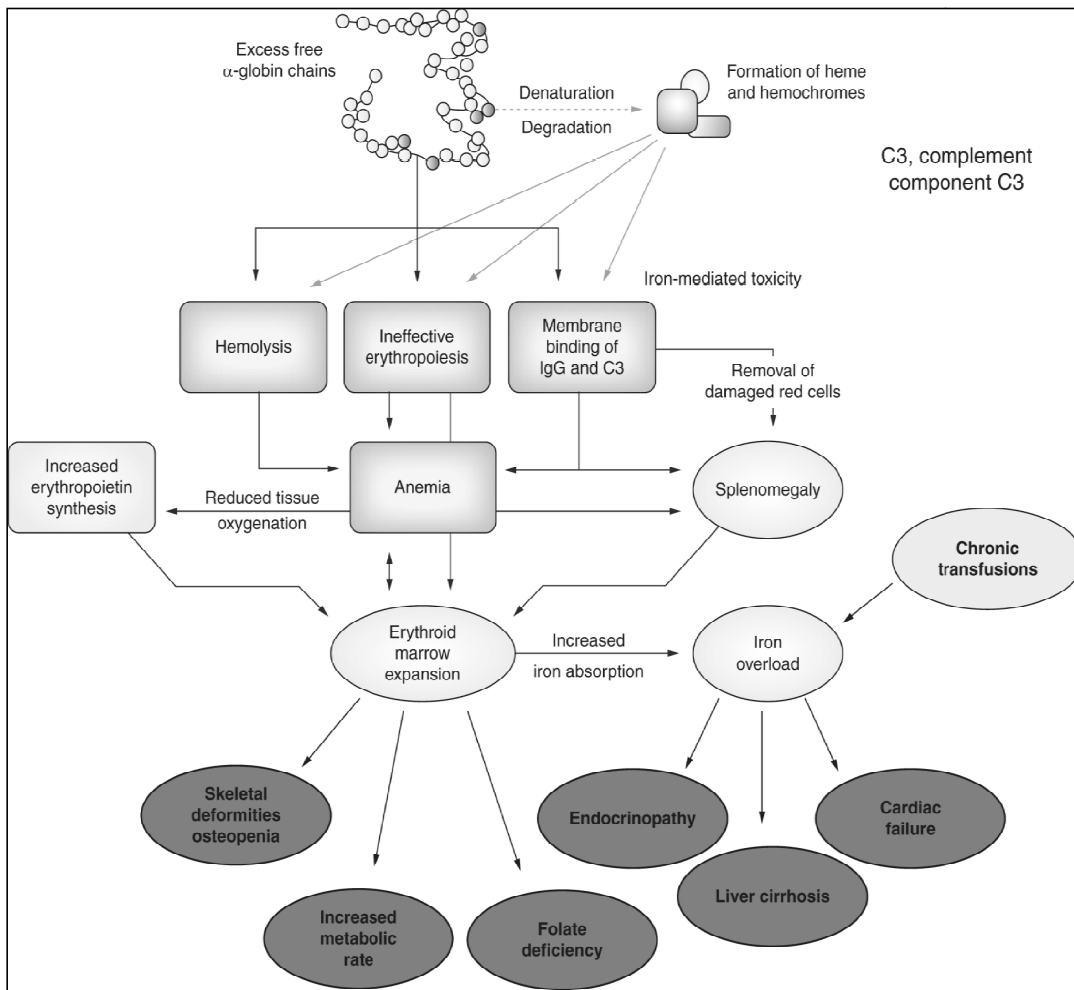
Fig. 2. Pathophysiology of b-thalassemia

In β -thalassaemia major the excess unpaired and insoluble α globin chains precipitate at the red cell precursors membrane and Free iron catalyses the formation of reactive oxygen species leading to oxidative cell damage and premature cell death by apoptosis. This happens within the erythropoietic tissue and so results in ineffective erythropoiesis (IE) and haemolysis of mature red cells [13].

The severe anemia increases production of erythropoietin leading to bone marrow expansion and results in characteristic deformities of the skull and face, pathological fractures of long bones, and may lead to the formation of extramedullary erythropoietic tissue. Substances released from degenerating red cells increase iron absorption, which contributes to iron overload (Fig. 2).

3. DIAGNOSIS

3.1 Clinical Diagnosis

The clinical diagnosis is most often between 6 and 24 months with severe microcytic anemia associated with, irritability, poor weight gain, abdominal distension and hepatosplenomegaly.

3.2 Haematologic and Biochimic Diagnosis

The typical findings of RBC indices show reduced haemoglobin (30-70 g/l), haematocrit, red blood cell count, mean corpuscular volume (MCV) (50-60 fL), mean Corpuscular Haemoglobin (MCH) (12e18 pg), raised biliribune and LDH. The iron deficiency (iron content) and the iron stores represented by ferritinemia are greatly increased [14]. The blood film will show

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microcytosis, hypochromia, anisopoikilocytosis, basophilic stippling, pappenheimer bodies, target cells and nucleated red blood cells (NRBCs).

3.3 Haemoglobin Analysis

Diagnosis of β-thalassaemia is confirmed by qualitative and quantitative Hb analysis, either by high-performance liquid chromatography or capillary electrophoresis. In β0/β0 homozygote thalassemia, HB A is almoste nule, HbF representes the majority fraction with 95–98% and HbA2 representes (2–5%) of the total hemoglobin. In β 0 /β+ - or β+ /β+ -thalassemia, HbA is 10–30%, HbF is 70–90% and HbA2 is 2–5%15 of the total hemoglobin.

3.4 Differencial Diagnocis

The major differential diagnosis in hypochromic microcytic anemia is either iron deficiency anemia or a haemoglobinopathy. Iron deficiency is easily ruled out with reference range iron studies (serum iron, total iron binding capacity, percent transferrin saturation). Haemoglobinopathies typically require haemoglobin electrophoresis or HPLC for diagnosis:

   - The Iron deficiency, sideroblastic anemia, or inflammatory anemia is therefore differentiated from β-Thalassemias by their normal hemoglobin balance.
   - It can also be confused with other nearby haemoglobinopathies, such as sickle cell disease or α-Thalassemia. Moreover, when these other haemoglobinopathies are associated with β-Thalassemia, the quantitative imbalance of the β and α chains can be compensated. Subsequently the clinical and biological picture presented is no longer suggestive of a β-Thalassemia, complicating the diagnosis.
   - Hyperthyroidism, some type of anemia (megaloblastic and dyserythropoietic congenital) and some treatments (tritherapies) can notably evoke a β-Thalassemia on electrophoresis causing the false positives results.

3.5 Molecular Genetic Analysis

The prevalence of a limited number of mutations in each population has greatly facilitated molecular genetic testing. Commonly occurring mutations of HBB gene are detected by polymerase chain reaction–based procedures [15]. The most commonly used methods are reverse dot blot analysis and primer-specific amplification, with a set of probes or primers complementary to the most common mutations in the population from which the affected individual originated. If targeted mutation analysis fails to detect the mutation, HBB sequence analysis can be used to detect mutations in HBB gene [16].

3.6 Genetic Counseling and Prenatal Diagnosis

The Prevention programs have reduced the birth prevalence of thalassemia in some countries and possibly saved resources for patient care. Such programs require planning and investment in order to: Include public awareness, screening to identify carriers, genetic counseling aiming to assist couples in making informed choices, and finally making available solutions such as prenatal diagnosis. Prenatal diagnosis for higher risk pregnancies is possible through the analysis of DNA extracted from fetal cells obtained by amniocentesis, usually performed approximately 15 to 18 weeks of gestation. The analysis of fetal cells in maternal blood and the analysis of fetal DNA in plasma maternal can also be realized [17].

4. CLINICAL MANIFESTATION

Individuals with TM are usually homozygous or compound heterozygous for one of the 200 β - thalassaemia mutations which are characterized by severe anemia (range, 1-7 g/dL of Hb), hemolysis, and massive *ineffective erythropoiesis* (IE) [18].

Untreated or poorly transfused patients show growth retardation, Tired, essoufflement, Pallor, Irritability, Facial bone deformation, Mucocutaneous jaundice, slowdown in growth, black discoloration of urine ,protruding abdomen, leg ulcers, formation of masses due to extramedullary hematopoiesis, and skeletal changes including deformities in the long bones of the legs [3]. In conseaquence they will die in the 1st or 2nd decade of life. The typical clinical picture of beta-thalassemia with significant splenomegaly and bone deformities is no longer observed today because of early management with regular transfusions. The clinical diagnosis is most often between 6 and 24 months in front of severe anemia. However, In regularly transfused patients (to maintaining a minimum hemoglobin (Hb) concentration of 9.0 to 10.5 g/dl), growth and development tend to be normal

until age 10 or 11 years when the complications related to iron overload will manifest, depending on their compliance with chelation therapy, including growth retardation and failure or delay of sexual maturation. Later-onset iron overload complications include dilated myocardiopathy, arrhythmias, liver fibrosis and cirrhosis, diabetes mellitus, and insufficiency of the parathyroid, thyroid, pituitary, and, less commonly, adrenal glands [19]. Other complications are related to *ineffective erythropoiesis* (IE) and chronic haemolysis increasing the red blood cells prothrombotic potential that can cause hypercoagulability. Vascular manifestations such as venous thrombosis and pulmonary hypertension are now well recognized in patients with beta-thalassaemia, especially those who have undergone splenectomy [20].

5. GENETIC MODIFIERS

The β-thalassemia is caused by more than 200 point mutations and, rarely, by deletions. However, genotypic variability at known loci is often insufficient to explain the phenotypic variability between individuals with the same genotype [21]. This interpatient clinical variability in the β-thalassemia syndromes has swayed researchers toward identifying genetic modifiers of severity for these disorders. Such genetic modifiers could potentially lead to the development of more specific and effective therapies [22]. According to Sonja Pavlovi and al [23] the Genetic modifiers exert their potential on three levels: Primary modifiers usually refer to a type of alterations affecting β-globin gene. Location of the mutations within different gene regions determines the phenotypic severity, therefore the point mutations affecting the β-globin expression belong to three different categories: mutations leading to defective β-globin gene transcription (promoter and 5' UTR mutations); mutations affecting mRNA processing (splice-junction and consensus sequence mutations, polyadenylation and other 3' UTR mutations); and mutations resulting in abnormal mRNA translation (nonsense, frameshift, and initiation codon mutations). Mutations affecting transcription usually result in a mild deficit of β-globin production that reflects the relatively mild phenotype of these β+ -thalassemias. The example of transcription affecting mutation is the C>T mutation at position -101 to the β-globin gene which appears to cause an extremely mild deficit of β-globin, such that it is asymptomatic in heterozygotes who have normal HbA2 (α2δ2) levels [6]. A wide

variety of mutations interfere with processing of the primary mRNA transcript. Those that affect the invariant dinucleotide GT or AG sequences at exon-intron splice junctions prevent normal splicing altogether, causing β0 thalassemia. Mutations affecting β-globin mRNA processing are located within 5'- and 3'-splice junction (donor and acceptor site), as well as within splice junctions' consensus sequences. Mutations altering the donor splice as IVS2-2-T and acceptor splice site as IVS2- 850 G>T lead to deficiency of functional mRNA production resulting in complete absence of β-globin polypeptide chains and, hence, to β0 - thalassemia. On the other hand, mutations affecting consensus sequences surrounding splice-junction, decrease the efficiency of the normal splicing to varying degrees, hence producing β-thalassemia phenotype that ranges from mild to severe. Mutations disrupting the mRNA translation ether in initiation or elongation phase, result in β0-thalassemia phenotype. Most of these defects result from the introduction of premature termination codons (PTCs) due to frameshift or nonsense mutations and nearly all terminate within first and second exon [24]. Other mutations of RNA translation involve the initiation (ATG) codon. Nine of these have been described; of these, all are single base substitutions apart from one mutation of 45 bp insertion and they result in β0 thalassemia [12].

Secondary modifiers include variations in genes affecting α/β globin chain equilibrium such as α- and γ-globin genes, as well as genes involved in the γ-globin gene expression (HBS1- MYB, BCL11A (B-cell CLL/lymphoma 11A), KLF1 (Krüppel-like factor), C1orf77) and genes affecting the amount and stability of α-globin chains (AHSP: αHb-stabilizing protein) [23,25]. These genetic modifiers could be located within (α- and γ-globin genes) or outside globin gene cluster. In the recent years, there has been significant advancement in the fields of secondary genetic modifiers ameliorating the clinical phenotype of β-thalassemia syndromes. Specifically, production of fetal hemoglobin (HbF) trough adulthood could ameliorate the severity of β-thalassemia phenotype since γ-globin polypeptide chains compensate for the lack of the functional β-globin polypeptide chains However Other mutations increasing HbF production are those associated with deletional and non-deletional HPFH linked to the beta globin gene cluster [3]. Recently, the genome-wide association approach, particularly studying quantitative trait loci (QTL) which cause elevated

HbF, have revealed genetic elements (i.e. polymorphism in BCL11A gene and in the HBS1LCMYB intergenic region) unlinked to beta globin gene cluster, able to modify the severity of the homozygous beta zero thalassemia [26]. This is why β-globin genes, along with other secondary modifiers, represent the most common targets for modern therapeutic.

Tertiary modifiers are gene variations affecting the phenotype with regard to some of the complications caused by beta-thalassemia syndromes such as hyperbilirubinemia, propensity to gallstone formation, bone diseases, thrombophilia and cardiopathies [25].

Hyperbilirubinemia and gallstone formation occurs with variable incidence in homozygous beta-thalassemia with the reported variation being partly related to the age of the patients and to its clinical severity, as it is more common in thalassemia intermedia than thalassemia major. Studies have shown that the levels of bilirubin and the predisposition to gallstones in beta-thalassemia is related to a polymorphic variant in the promoter of the uridine diphosphate glucuronyl transferase A1 (*UGT1A1*) gene, also referred to as Gilbert's syndrome. Individuals who are homozygous for the this (TA)7 variant instead of the usual (TA)6, tend to have higher levels of bilirubin and increased predisposition to gallstone [27]. Progressive osteoporosis and osteopenia is another increasingly common complication observed in young adults with beta-thalassemia and it is determined by a combination of genetic and environmental factors. Anemia and bone marrow expansion which are prevalent in beta- thalassemia are major contributors in inadequately treated patients. Bone mass, the main indicator of the osteoporosis and osteopenia, is another quantitative trait known to be under strong genetic control involving multiple loci including estrogen receptor gene, vitamin D receptor (*VDR*), collagen type a1 and type a2 genes (*COL1A1*, *COL1A2*), and transforming growth factor (*TGFβ1*) [28]. Also genetic risk factors for thrombosis, such as mutations in the gene for Factor II and Factor V, as well as variations in the *MTHFR* gene, could significantly influence the phenotype of the *β*-thalassemia syndromes [19]. Cardiac diseases are the main cause of death in *β*-thalassemia patients and are attributed to iron overload. One of the studies depicted apolipoprotein E4 and it decreased antioxidant activity, as a risk factor for left ventricular failure (LVF) in thalassemia patients.

However, the presence of this E4 allele does not guarantee. Recently, a polymorphism in glutathione-Stransferase M1 gene has been associated with an increased risk of heart iron overload in thalassemia major.

6. CURRENT MANAGEMENT OF BETA-THALASSAEMIA

Patients with severe beta-thalassaemia require lifelong therapy to prevent and manage the clinical consequences of disease [29]. Current management strategies for Transfusion dependant thalassemia TDT comprise blood transfusion, iron chelation, splenectomy (less common than in the past) and for a subset of patients and hematopoietique stem cell transplant (HSCT).

6.1 Transfusion Therapy

The objectives of transfusion therapy is to correct anemia, suppress erythropoiesis and inhibit gastrointestinal iron absorption that occurs in untransfused patients as a result of increased, albeit ineffective, erythropoiesis. The decision to start transfusion therapy in patients with confirmed diagnosis of beta-thalassemia should be based on the presence of severe anemia with hemoglobin (Hb) levels < 7 g/dL and any following clinical criteria: Facial changes, poor growth, spontaneous fractures and clinically significant extramedullary hematopoiesis [16]. The recommended treatment for beta-thalassemia major involves regular red blood cell transfusions throughout life, usually administered every two to five weeks depending on the transfusion needs of each individual in order to maintain the pre-transfusion level of hemoglobin between 9 and 10.5 g/dL [3] or higher (11–12 g/dL) for patients with cardiac complications and keep post-transfusion hemoglobin levels below 15 g/dL. The need for transfusions can start as early as six months old [30], to avoid the risk of developing red blood cell alloantibody and subsequent difficulty in finding compatible units for transfusion [30-32]. Patients starting regular transfusion regimens should be vaccinated against hepatitis A and B (depending on the age) [30] and their cytomegalovirus status should be assessed. The development of one or more specific anti-red blood cell antibodies (alloimmunization) is an important complication of regular transfusion therapy [16,32]. Before starting transfusion therapy, the patient's blood type must be determined as well as red blood cell phenotyping for at least the C, c, D, E, e and

Kell antigens in order to help identify and characterize antibodies in the case of subsequent immunization. Preferably, extended phenotyping should be made including antigens of other blood systems such as Duffy and Kidd and genotyping [16] as well as The use of extended antigen-matched donor blood reduces alloimmunization rates [30]. The risk of transfusion-transmitted infections in thalassemia patients has been greatly reduced since screening for human immunodeficiency virus infections began in 1985 and for hepatitis C in 1991 [33]. However, new agents, such as West Nile Virus and babesiosis, which are not screened for, may contaminate the blood supply from asymptomatic donors [34]. The use of packed washed leukocyte depleted/filtered red blood cells is recommended for all patients to reduce allergic reactions and febrile non-hemolytic transfusion reactions, as well as cytomegalovirus infection [35]. The washing of packed red blood cells is in fact indicated for patients with repeated allergic reactions and IgA-deficient patients. The amount of red blood cells to be transfused depends on several factors, such as patient weight, target Hb level and hematocrit of the blood bag. In clinically stable patients, approximately 8–15 mL/kg body weight of red blood cells can be infused over a period of 1–2 h [16].

If Hb levels are < 5 g/dL and/or in the presence of heart failure, smaller aliquots of RBCs (5 mL/kg) should be administered to prevent volume overload until the Hb level is gradually increased to 9 g/dL [5]. A clinical record of all transfusion events should be monitored annually to identify hypersplenism. A record of weight, the amount of blood transfused at each visit, and the pretransfusion Hb level is needed to calculate the annual transfusion requirement [5].

6.2 Iron Overload and Chelation

Blood transfusions are responsible for iron accumulation, as iron cannot be excreted physiologically. Iron accumulation is already evident in children with (Transfusion dependent Thalassemia) TDT from 2 years of age. Adults with TDT receive an average of 0.3–0.6 mg of iron per kg per day and, without effective chelation, will accumulate approximately 6–12 g of iron each year [36]. Excess iron is toxic to many tissues, including the liver, endocrine organs and heart, leading to a series of complications which cause morbidity and mortality in these patients [37]. Although iron

chelation therapy is available, death due to iron overload remains an issue. Serum ferritin is the most widely used marker to assess iron overload and is measured 3-monthly in thalassaemia patients. In patients with TDT, serum ferritin above 1000 µg/l is an indication to start iron chelation and serum ferritin above 2500 µg/l is associated with increased risk of cardiac and endocrine disease. In patients (Non transfusion dependent thalassemia) NTDT, iron chelation is indicated when serum ferritin rises above 800 µg/l [38]. However use of magnetic resonance imaging (MRI) to noninvasively assess liver and myocardial iron concentration is an important advance in thalassaemia care but its availability varies by country [5].

To date, there are 3 major classes of iron chelators: Hexadentate (deferoxamine [DFO], Desferal), in which 1 atom of iron is bound to 1 DFO molecule; bidentate (deferiprone, [DFP]), in which 1 atom of iron is bound to 3 DFP molecules; and tridentate (deferasirox [DFX], Exjade), in which 1 atom of iron is bound to 2 DFX molecules [39]. Deferoxamine (DFO), the first commercially available iron chelator is , a naturally occurring sideraphore derived from *Streptomyces pilosus* with a high molecular weight of 657 and a very short half-life of 8-10 minutes, and is requires intravenous or subcutaneous parenteral administration for 8–12 hours, 5–7 nights per week . DFO enters hepatic parenchymal cells, chelates iron and appears in the serum and bile as the iron chelator feroxamine. It also chelates iron released after catabolism of senescent RBCs and is excreted in the urine. The proportions and the long-term patient survival of DFO-chelated iron vary from patient to patient and are related to the degree of iron loading, chelator dose, frequency or duration and IE activity [40]. The initial recommended dose is 30-40 mg/kg per day for daily use 5-7 days each week in regularly transfused thalassemia patients. Chelation generally begins between 2 and 4 years of age, after 20-25 RBC units are transfused, with a serum ferritin level > 1000 µg/*dL* and an liver iron concentration (LIC) > 3 mg Fe/g dry weight as measured by liver biopsy or by noninvasive hepatic T2*MRI. The efficiency of chelation can be relatively low during the first few years and may warrant gradual escalation of the daily DFO dose to 50 mg/kg and subsequently to 60 mg/kg in adolescents and adults. DFO was the firstly developed and most efficacious iron chelator for many years. However, unavailability of an oral dosage form and the need for subcutaneous

infusion for long hours markedly limited its use. Deferiprone DFP is a synthetic compound originally identified in the 1980 years in London. It is absorbed by the gastrointestinal (GI) tract and has a plasma half-life of 1.5-4 hours. The recommended daily dose is 75 mg/kg per day, which can be increased to 100 mg/kg per day, given orally in 3 divided doses [5]. DFP has a higher chelating ability of myocardial iron compared to other drugs but is not recommended as monotherapy. It found to be 70 -100% as effective as desferrioxamine and leads to effective reduction in both serum ferritin and tissue iron overload. Deferasirox (DFX), an oral chelator with a similar efficacy to DFO, has been the first line iron chelator for iron overload in thalassaemia patients in many countries worldwide. It is absorbed in the GI tract and because of its dose-dependent half-life of 12-18 hours, it can be taken once a day (20 à30 mg/kg/j). It is found to be nearly five times as effective as subcutaneous desferrioxamine and ten times more potent than deferiprone in animal studies [41]. The Combination of deferasirox and desferrioxamine is used when a single chelator is unable to control iron overload and combination of desferrioxamine and deferiprone is used especially when cardiac iron chelation becomes a priority. However, the combination of deferasirox and deferiprone was be proved to be effective in decrease both cardiac and hepatic siderosis without noticeable side effect in (Thalassemia Major) TM patients [42].

6.3 Splenectomy

Splenectomy has been performed conventionally as a supplement or alternative to transfusion therapy. It is rarely indicated in the TM and It is nonetheless proposed in case of hypersplenism (thrombocytopenia, neutropenia, splenomegaly) or to lower blood transfusion requirements when these exceed 200 mL / kg / yr (calculated volume) for globular concentrates at 75% hematocrit).She has for a long time been frequently proposed in independent transfusion (IT), to reduce the degree of anemia and thus limit or stop occasional transfusions. Nevertheless, the infectious risks and associated thrombo embolisms tend to limit the resort to splenectomy in recent years.

6.4 Haematopoietic Stem Cell Transplant-ation

Despite the remarkable improvements in medical therapy for TM, [36]. The allogeneic hematopoietic stem cell transplantation (HSCT) still remains the only available curative approach [43]. The first HSCT for thalassaemia was performed in 1982 and over 10 000 HSCT have been performed for thalassaemia up to now. However it is still limited in developing countries because of the lack of equipment and the very expensive cost. According to the First Meeting of African Blood and Marrow Transplantation Group held in Casablanca in 2018, of the 71,000 transplants performed in 2013 worldwide, only 3% were performed in Africa and the Middle East [44]. Donor selection is of great importance because transplantations may fail or be lethal resulting from immunologic complications. The best results have been obtained with HLA-matched siblings. The preparatory regimen includes administration of busulfan fludarabine (14 mg/kg) and cyclophosphamide ((120 à 200 mg/kg), which in combination can eradicate the thalassemia clone, enhance immunosuppression and facilitate sustained allogeneic engraftment. Several factors have been shown to affect patient outcome: Severity criteria before transplantation (hepatomegaly, portal fibrosis and irregular chelation history), age at transplantation, stem cell source (peripheral blood, bone marrow, cord blood), histocompatibility (related matched,unrelated matched, mismatched, haploidentical), preparative conditioning regimen and pretransplant eradication of marrow hyperplasia [45]. This has been formalized by the classification Lucarelli who, depending on the presence or not of the 3 Risk factors that are hepatomegaly, portal fibrosis and insufficient chelation, classifies patients into three risk classes for transplant success [46] ((class 1= no risk factor, class 2 =one or two risk factors and class 3= three risk factors). Children without any of these risk factors have survival and disease-free survival rates of greater than 90% 3 years after transplantation. On the other hand, for patients with all 3 risk factors, and in most adults, the rates are about 60%. The best clinical outcomes of HSCT among patients with thalassaemia are reported in those aged under 14 years at transplantation [47]; this is likely to be because older patients have existing morbidity related to iron overload and other complications Young patients with TDT who have a human leukocyte antigen (HLA)-matched sibling donor should be offered HSCT at an early age [48] and have survival and disease-free survival rates of greater than 90% 3 years after transplantation. In a large European

Blood and Marrow Transplantation (EBMT) survey of 1061 cases of HLA-matched sibling donor (MSD) transplantation performed in the last decade, overall survival (OS) and disease-free survival (DFS) were 91±0.01 and 83±0.01 months, respectively [7]. Adults with a matched sibling donor may be offered HSCT within a clinical trial context [48]. Other techniques are required for the 70–75% of patients worldwide who do not have an existing suitably HLA-matched related donor [7]. Outcomes using matched unrelated donor HSCT are improving and may be attempted providing that the donor is selected using high-resolution molecular typing for both HLA class I and II loci and according to stringent compatibility criteria [7].

Donor cells can also be obtained from cord blood (CB) samples or pripheral blood. however it 's avoid to use HSC obtained from peripheral blood due to increased risk of chronic graft versus the host [49]. The transplantation of CB cells originating from HLA-identical siblings is a viable alternative to the use of adult samples in class I/II patients. Moreover, these cells have the theoretical advantages of tolerating a higher degree of HLA incompatibility than adult cells and causing a lower incidence of acute and chronic Graft versus host disease (GVHD) [50].

7. NOVEL STRATEGIES FOR TREATMENT OF BETA-THALASSAEMIA

Despite immense achievement in the treatment of *β*-thalassemia, including transfusion and drug therapy, until recently, a definitive cure for these disorders could only be achieved by bone marrow transplantation (BMT) from related or unrelated donors. However, BMT is available for only a small fraction of beta-thalassemia patients and is characterized by relatively high mortality and morbidity, especially in the case of unrelated donors [27]. In fact new therapies, both pharmacological and gene-based are now in advanced stages of clinical trials and offer the promise of a cure for a much larger proportion of patients with thalassemia. Most of these aim to restore globin chain imbalance, Improve ineffective erythropoiesis or Improve iron dysregulation.

7.1 Improving Globin Chain Imbalance

Gene therapy technology has potential to correct the underlying alpha/beta-globin chain imbalance in beta-thalassaemia.

7.1.1 Gene therapy

Gene therapy using autologous stem cells could offer an alternative curative approach to HSCT, which is limited to patients with an appropriately matched donor. Most commonly, gene therapy is accomplished by first harvesting a patient's HSPCs (progenitors cells) from either bone marrow, peripheral blood, or umbilical cord blood. Traditional gene therapies currently in clinical trials consist of inserting an additional globin gene *via* a lentiviral vector that integrates into the host cell's genome. After full or partial myeloablation, the genetically modified autologous HSPCs are returned to the patient where the modified cells repopulate the haematopoietic compartment [51]. While engraftment of only a small population of corrected HSPCs can result in amelioration of a hemoglobinopathy, highly efficient gene transfer must occur in order to modify a sufficient number of cells able to achieve long-term engraftment. Current gene therapies in clinical trials achieve this by using a lentiviral vector to insert an additional globin gene [52]. Even with the ability of current vectors to improve the hemoglobin synthesis in patients affected by hemoglobinopathies, additional efforts are now focusing on improving the ability of these vectors to express curative hemoglobin levels with a reduced number of gene integrations per cell. Reducing integrations minimizes the chance of oncogenic random integration and limits the level of myeloablation required for these patients to receive the corrected HSCs. In order to improve this approach, additional strategies are being explored [53].

7.1.2. Gene editing

Recently, genome editing, a new form of gene therapy, has shown promise in pre-clinical studies. Contrary to traditional gene therapy, genome editing can be used to geneticaly modify the endogenous DNA of haematopoietic stem cells using programmable nucleases to create an edit in a pre-determined target site in the human genome. Genome editing has been used to upregulate *β*-globin by mutating the *β*-globin suppressor BCL11A gene or to downregulate *β*-globin by mutating its enhancers [54].

7.1.3 Induction of fetal Hb synthesis

Induction of fetal Hb synthesis can reduce the severity of *β*-thalassemia by improving the imbalance between *β*-globin and non *β*-globin

chains. Several pharmacologic compounds including 5-azacytidine, decytabine, and butyrate derivatives have had encouraging results in clinical trials. These agents induce HbF by different mechanisms that are not yet well defined. Their potential in the management of beta-thalassemia syndromes is under investigation [55].

7.2 Improving Ineffective Erythropoiesis

A number of agents that target ineffective erythropoiesis are currently under investigation for the treatment of anaemia due to beta-thalassaemia. Of these, the Janus kinase 2 (JAK2) inhibitor, ruxolitinib, and the activin receptor-II ligand traps, sotatercept (ACE-011) and luspatercept (ACE-536), are at the most advanced stages of development. Recent work has elucidated the roles of JAK2 and the transforming growth factor (TGF)-beta superfamily in the control of erythropoiesis [56]. Binding of erythropoietin to its cell membrane receptor activates the cytoplasmic JAK2, which in turn activates multiple signal transduction pathways to increase proliferation, differentiation, and survival of erythroid progenitors. JAK2 is the only intracellular signal transductor of erythropoietin and is, therefore, a potential target to treat conditions caused by disordered and ineffective erythropoiesis [56]. JAK2 inhibitors have been shown to improve ineffective erythropoiesis and reduce splenomegaly in a mouse model of NTDT [57]. Recent data from mouse models of NTDT and TDT also support the ability of JAK2 inhibitors to reduce splenomegaly [57]. However, this positive effect on spleen size was associated with suppression of endogenous erythropoiesis that was not improved by blood transfusion. Activins, members of TGF-β family signaling, are key regulators of human hematopoiesis and modulate various cellular responses such as proliferation, differentiation, migration and apoptosis. A modified activin type IIB receptor inhibiting signaling induced by some members of the TGF-β superfamily promotes maturation of terminally differentiating erythroblasts. In thalassemic mice, it ameliorates hematologic parameters as well as comorbidities that develop as a consequence of the erythroid hyperplasia [58]. Sotatercept (ACE-011) acts as a ligand trap to inhibit negative regulators of late-stage erythropoiesis in the transforming growth factor β superfamily, correcting ineffective erythropoiesis [57]. Sotatercept is a ligand trap that inhibits transforming growth factor beta (TGF-β)

superfamily members including growth differentiation factor 11 (GDF-11) and activin B. GDF-11 is overexpressed in immature erythroblasts in β-thalassemia. Aberrant GDF-11 production may induce expansion of erythroid progenitors and increase oxidative stress, leading to maturation arrest of late erythroid precursors and ineffective erythropoiesis. Preclinical work has shown that administration of an activin receptor IIA (ActRIIA) ligand trap decreases GDF11 concentration, reduces reactive oxidative stress levels, and promotes terminal maturation in immature erythroblasts [59].

7.3 Improving Iron Dysregulation

Recent studies have shown that interrupting the vicious cycle between ineffective erythropoiesis and iron overload may be of therapeutic benefit in thalassemias. Body iron balance is controlled by the 25–amino acid peptide hormone hepcidin (HAMP), which is produced by the liver in response to plasma and intracellular iron levels. In normal erythropoiesis, hepatocytes respond to elevated iron levels by increasing hepcidin production. Hepcidin is then released into the circulation and subsequently binds to its target ferroportin (FPN-1), inducing its internalization and degradation within lysosomes. Several studies have demonstrated that hepcidin is chronically suppressed in thalassemia [60]. It may, therefore, be possible to prevent primary iron overload in beta-thalassaemia, and perhaps reduce the existing iron burden, by manipulating circulating levels of hepcidin. In fact, it has been already demonstrated that $th3 +$ thalassemic mice moderately over expressing hepcidin show reduced iron levels in the serum, liver, spleen, and kidney and improvement of IE, RBC survival, and morphology [61]. Motivating preclinical data on mini-hepcidins and transmembrane protein serine 6 (TMPRSS6) inhibitors have also been reported recently. TMPRSS6 is a transmembrane serine protease that reduces production of hepcidin [53]. Thus, endogenous hepcidin production can be stimulated by reducing expression of TMPRSS6 [62]. It has been shown that administration of these molecules in a thalassemic mouse model results in reduced iron absorption and increased iron retention in splenic macrophages [61]. Induction of iron restriction by means of transferrin infusions, prevents iron overload, redistributes iron from parenchymal cells to macrophage stores, and partially controls anemia in β-thalassemic mice [63].

8. CONCLUSION

In conclusion, despite the progress made in the management of thalassemia, future research must focus on the discover a less expensive therapeutic strategy, which would be accessible to all patients and would lead to definitive cure.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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