Endo-Thal
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Diagnosis and Current Challenges in the Management of Hemoglobinopathies: The Equality Plus Project (3rd Part)

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Abstract

Hemoglobinopathies, including severe β-thalassemia and sickle cell disease (SCD), represent the most common monogenic disorders worldwide. With migration they are becoming more common worldwide, making their management and care an increasing concern for health care systems. All hemoglobinopathies result from a genetic mutation in one or more genes that affect hemoglobin synthesis. Regardless of the mutation encountered, hematologists divide hemoglobinopathies into two categories: structural defects (qualitative) and thalassemias (quantitative). Definitive diagnosis of hemoglobinopathies requires a comprehensive workup from complete blood count, hemoglobin analysis, and molecular studies to identify mutations of globin genes. Thalassaemias and SCD are complex lifelong haematological disorders which are complicated over time with multi-organ involvement. Multidisciplinary management is recommended by international guidelines. Herein, the authors report the third part of Equality Plus project on the current diagnostic tehniques and conventional therapies for the two main hemoglobinopathies, β-thalassemias and SCD, and present very briefly the emerging therapies to cure the diseases.

Key words: Thalassemias, sickle cell disease, diagnosis, treatment, Equality Plus project.

Introduction

Approximately 300,000 children are born each year with some form of inherited hemoglobin disorder. All hemoglobinopathies result from a genetic mutation in one or more genes that affect hemoglobin synthesis. Regardless of the mutation encountered, hematologists divide hemoglobinopathies into two categories: structural defects (qualitative) and thalassemias (quantitative). Sickle cell disease (SCD) is a wide-spread inherited hemolytic anemia that is due to a point mutation leading to a valine/glutamic acid substitution in the beta-globin chain, causing a spectrum of clinical manifestations in addition to hemolysis and anemia. Acute painful crisis is a common sequela that can cause significant morbidity and negatively impact the patient's quality of life. The basic defect in the thalassaemia syndromes is reduced globin chain synthesis with the resultant red cells having inadequate haemoglobin content. The reduction in the amount of hemoglobin synthesized produces an anemia and stimulates the production of other hemoglobins not affected by the mutation in an attempt to compensate for the anemia. Herein, the authors report the third part of Equality Plus project on the current diagnostic tehniques and conventional therapies for the two main hemoglobinopathies, β-thalassemias and SCD, and present very briefly the emerging therapies to cure the diseases.

Diagnosis of Hemoglobinopathies

The hemoglobin molecule is composed of 4 globin chains linked to 4 porphyrin rings. In adult life, there are 95% HbA1, 3.5% HbA2 and 1.5% HbF. Hb A1 is composed of 2 α and 2 β chains (α2β2). Hb A2 is composed of 2 α chains and 2 δ chains (α2δ2). Hb F or Fetal hemoglobin is composed of 2 α chains and 2 γ chains (α2γ2). There are 3 embryonic hemoglobins seen in the early embryonic period (Hb Portland I (ζ2 γ2), Hb Gower I (ζ2 ε2), Hb Gower II (α 2 ε2).

The structure of all globin genes is similar, being composed of a promoter region, 3 exons with 2 intervening introns. The α globin gene complex is located on chromosome 16, whereas the β globin gene complex is located on chromosome 11. The α globin gene complex consists of 2 functional α globin genes (α 1 and α 2) as well as the embryonic zeta chain and several pseudo genes. The beta globin gene complex consists of 2 γ chains, the δ globin gene, the β globin gene, the embryonic epsilon gene and 1 pseudo gene (1, 2).
Hemoglobinopathies are genetic hemoglobin disorders caused by mutations and/or deletions in the α-globulin or β-globulin genes. These are divided into 2 main categories: thalassemias and

Table 1. *Classification of the hemoglobinopathies.*

Type	Group	Clinic	Genetic structure
Thalassemia	α-thalassemias	Heterozygous α^+ thalassemia	- α/αα
		Heterozygous αο thalassemia	-α/-α or - -/αα
		Thalassemia Intermedia Hb H diseases	- - /-α
		Homozygous α thalassemia (Hb Bart's hydrops fetalis	- - / - -
	β-thalassemias	Heterozygous β -thalassemia	β^{++}/β, β^{+}/β, β^{0}/β
		Thalassemia Intermedia Mild homozygous or compound heterozygous β -thalassemia	β^{++}/β^{++}, β^{+}/β^{++}, β^{++}/β^{0}, β^{0}/β^{0} + α^{0} or α+ or + β modifier genes
		Homozygous β -thalassemia	β^{+}/β^{+}, β^{+}/β^{0}, β^{0}/β^{0}
	Gamma	Heterozygous/ Homozygous	Gγ, Aγ
	Delta	Heterozygous/ Homozygous	δ^{0} or δ^{+}
	Delta beta:	Hb Lepore	$\delta\beta^{0}$ or $\delta\beta^{+}$
Structural variants	Sickle cell syndromes	Heterozygous Sickle cell Sickle cell disease Sickle cell /Beta Sickle cell / Hb C	S/A S/S S/ β S/C
	Variants with multiple effects	Heterozygous HbE Homozygous Hb E	E / A E / E
	Unstable variants	Hemolytic anemia Normal	Hb Brockton Hb Khartoum
	High affinity variants	Erythrocytosis	Hb Kempsey
	Low affinity variants	Cyanosis	Hb Kansas
	Globin chain elongation variants	Microcytosis	Hb Constant Spring

structural hemoglobin variants (3). The hemoglobinopathies classified according to the genotype are shown in Table 1.

Thalassemias

The synthesis defects of hemoglobin chains cause thalassemias, classified as α, β, γ or δ thalassemia depending on globin or globins synthesised at a reduced rate. Thalassemia genotype consists of point mutations in beta thalassemia, and deletions in alpha thalassemia. As a result of these mutations, sufficient amount of HbA1 cannot be formed in individuals.

α-thalassemias: α-thalassemias are caused by an α-globin chain synthesis defect. At the molecular level, they result from partial (α^{+}) or total (α^{0}) deletions, or more rarely mutations, of one or more of the four α-globin genes (αα/αα).

Clinically, there are three types of α thalassemias:
1. α-thalassemia minor (heterozygous α0-thalassemia,--/αα, or homozygous α+-thalassemia, (-α/-α) with mild anemia, hypochromia, microcytosis.
2. HbH disease (compound heterozygous α+/α0-thalassemia with three inactive α-genes, --/-α),moderate hypochromic hemolytic anemia with splenomegaly.
3. Hb Bart's hydrops fetalis (homozygous α0-thalassemia) with very serious hemolytic anemia already present in utero and marked by a lack of any α-globin chain synthesis (--/--), with hydrops and ascites. This is fatal if not treated during intrauterin period (4, 5).

β-thalassemias: β-thalassemia syndromes are the result of less (β^{++}) insufficient (β^{+}), absent (β^{0}) or dominant production depend on mutation types of β-globin chains.

There are also three types of β-thalassemias:
1. ***Thalassemia minor*** (heterozygous β-thalassemia): The clinical picture of thalassemia minor varies according to the type of mutation as silent, normal or dominant, from the normal clinical picture to the need for transfusion.
2. ***Thalassemia intermedia*** (mild homozygous or mixed heterozygous β-thalassemia): The clinical picture of thalassemia intermedia shows a very different clinical picture according to the type of mutation, association with alpha thalassemia and the current state of beta gene modifiers. Moderate severity and with a varying need for transfusions; typical complications are skeletal deformities and tumorous masses as a result of massive hyperplastic erythropoiesis.
3. ***Thalassemia major*** (severe homozygous or mixed heterozygous β-thalassemia) with long-term, transfusion-dependent anemia untreated children die before the age of 10. Optimally treated patients have a projected life span of 50 to 60 years (6).

Structural hemoglobin variants

This group of hemoglobin disorders is caused by structural defects resulting from an altered amino acid sequence in the globin chains. Today, about 1830 variant was determined in HbVar: A Database of Human Hemoglobin Variants and Thalassemias (http://globin.bx.psu.edu/hbvar). The distribution of 1830 variants according to genes is as follows; 362 in α 1 gene, 456 in α 2 gene, 940 in β gene,138 in δ gene, 63 in γ gene 76 in G γ gene.
These variants alter hemoglobin structure and biochemical properties with physiological effects ranging from insignificant to severe. Studies of these mutations in patients and in the laboratory have produced a wealth of information for hemoglobin biochemistry and hematology practice.
Clinicians must distinguish between clinically harmless Hb abnormalities and those that cause illness. Some variants with a tendency to aggregate and with sickle cell formation, e.g. the sickle syndromes. Some variants with abnormal hemoglobin synthesis like thalassemia ,e.g. HbE. Some variants with a tendency to precipitate and with hemolysis (unstable hemoglobins, e.g. Hb Brockton) Some variants have abnormal oxygen transportation and congenital polycythemia, (Hb Kempsey) or with congenital cyanosis (Hb Kansas) (7, 8) (Table 1).
Clinical indications for laboratory testing to investigate potential Hb variants are listed below.
- Routine newborn testing for common hemoglobinopathies (i.e., HbS, HbC, thalassemias)
- Cyanosis with adequate arterial oxygenation and no apparent cardiopulmonary disease
- Erythrocytosis with normal or elevated erythropoietin levels
- Unexplained hemolytic anemia
- Unexplained thalassemia phenotype
- Family history consistent with an Hb variant (9)

Laboratory Methods in Diagnosis of Hemoglobinopathies

A. Conventional methods

1. ***Baseline hematological tests***: Complete blood count (CBC), blood smear and reticulocyte counts perform for RBC parameters suc as Red Blood Count (RBC), Hemoglobin(Hb), Hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW).
Other diagnostic tests are iron status parameters (iron, transferrin, ferritin) and hemolysis parameters (bilirubin, LDH, haptoglobin) for differeantial diagnosis.

2. ***Electrophoretic methods***: Different hemoglobin electrophoresis tests used to detect and quantify normal hemoglobin fractions and abnormal hemoglobins for many years (**Figure 1**).

a. Cellulose acetate electrophoresis: This also called alkaline electrophoresis, as it is performed at a pH of 8.6;
b. Citrate Agar Electrophoresis: Also called acid electrophoresis, as it is performed at a pH of 6.2. This method is very useful for the confirmation of Hb S, Hb C, and Hb E;
c. Isoelectric Focusing: This electrophoretic method utilizes carrier ampholytes, small proteins which are able to carry both current and pH;
d. Capillary electrophoresis:This is a relatively new method that has been gaining in popularity over recent years. This method are used in many centers today.

3. ***Chromatographic methods***: As the chromatographic method, high performance liquid chromatography (HPLC) is the most commonly used method today. HPLC instruments have become widely available that are compact, user-friendly, and dedicated to the detection of hemoglobins and their variants (**Figure 2**).

4. ***Chemical methods***: There are some classical chemical methods for separating Hb variants.

a. Solubility test use in order to distinguish HbS from other variants with the same electrophoretic migration characteristics.
b. Classic alkali denaturation performs for the quantitative determination of HbF .
c. Isopropanol test: Special denaturation tests use for the simple detection of unstable hemoglobins included heat denaturation at 69.5°C.

5. ***Cytological methods***: Some of the cellular tests are still practically used in centers where other methods are not available.

a. Acid elution method use the demonstration of HbF cells on the carriers.
b. Intra-erythrocytic inclusion bodies (Heinz bodies) is detected by the incubation of 0.1 mL of blood with brilliant cresyl blue solution followed for some unstabil variants.
c. Sickle-cell testing: By squeezing the fingertip, the oxygen flow is reduced, then a drop of blood taken from the fingertip is examined under a microscope and sickle cells are seen.

6. ***Functional methods***: The function of normal and pathological hemoglobins are tested as follows:

a. Hb-O2 binding curve: This test, performed on whole red blood cells or hemolysate, indicates the percent (%) oxygenated Hb at a given O2 partial pressure. Hemoglobin variants with an abnormally high O2 affinity or reduce O2 affinity is determined by this method.
b. Visible wavelength spectroscopy: Hemoglobin variants with amino acid substitutions in the heme pocket affect visible light absorbance. For example, M-type Hbs Show characteristic spectra that can distinguish them from methemoglobinemia caused by an enzyme deficiency in the metHb reductase system (7, 9).
c. Spectral analysis method: spectral detection of variants of sickle cell disease and β-thalassemia trait is an innovative technique, which when made accurate and reliable could be an effective alternative method (10).

7. ***Mass spectrometry method***: Mass spectrometry (MS) systems have been present in clinical laboratories for at least 35-40 years but only more recently have been applied to the analysis of protein.MS has the ability to provide very precise identification of even rare variants (11).

Multiple biophysical, biochemical, and genetic assays are available to provide phenotypic or genotypic evidence of pathology. Today, Hb analysis may be carried out by either automatic high-performance liquid chromatography (HPLC) or capillary zone electrophoresis (CE)

Figure 1. *Different electrophoresis methods.*

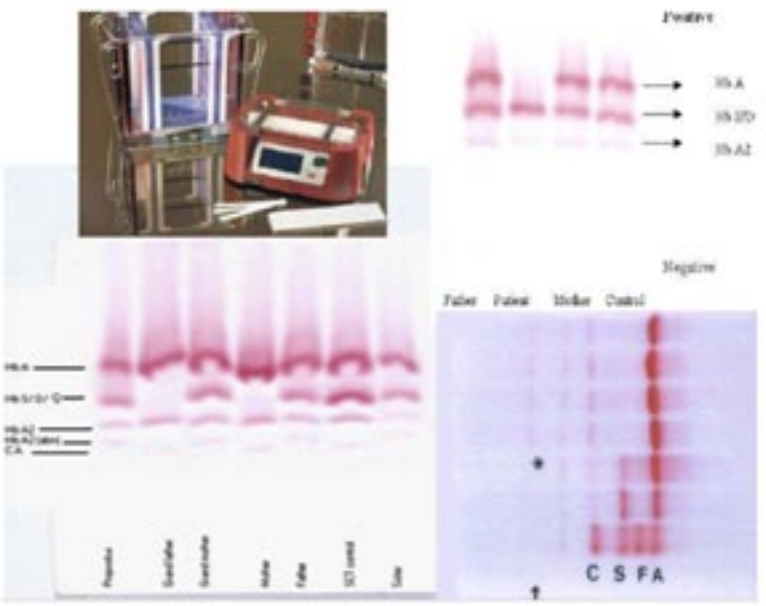
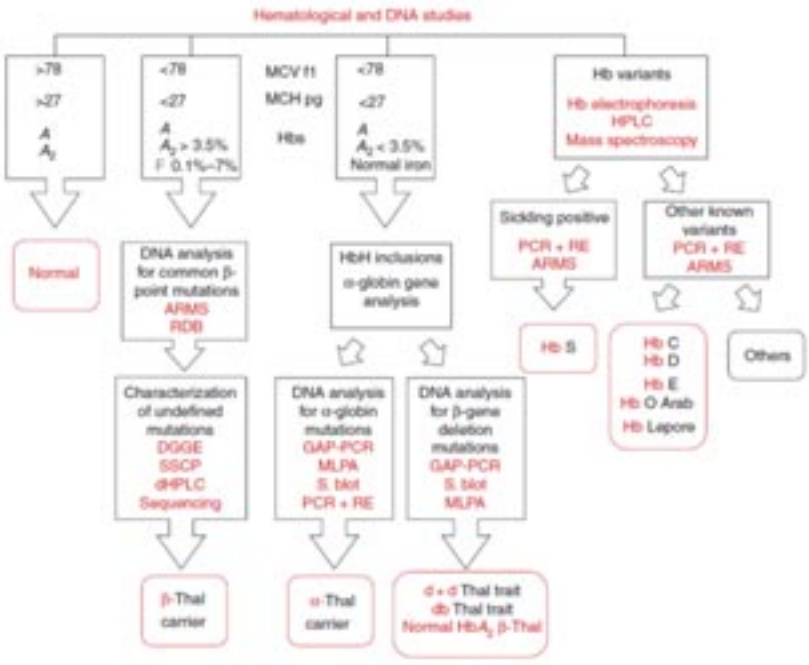
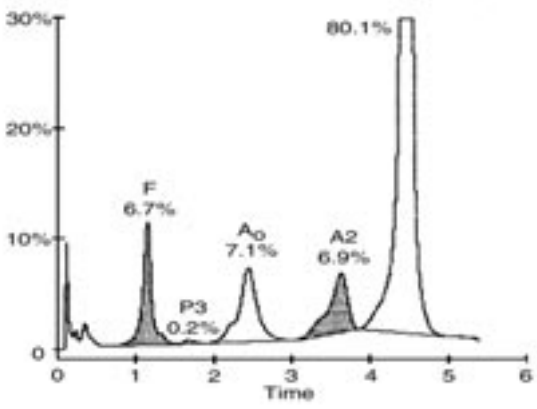


Figure 3. *Conventional and molecular methods in screening of hemoglobinpathies (From Cao A and Kan YW, modified).*



system. These two systems give both qualitative and quantitative analysis of Hb components and help to do thalassemia prenatal and postnatal diagnoses within a short period.

Figure 2. *HPLC system show HbF, HbA1, HbA2 and Abnormal Hb.*



Algorithms in screening program

Comprehensive prevention programs involve public education, screening of carrier, molecular diagnostics, genetic counseling, and preconception diagnosis, preimplanttion genetic diagnosis and prenatal diagnosis (12).

Screening may be preoperative, neonatal, antenatal, preconceptual, premarriage or targeted at specific groups perceived to be at risk. Screening in the setting of haemoglobinopathies may be directed at optimising management of a disorder by early diagnosis, permitting informed reproductive choice or preventing a serious disorder by offering termination of pregnancy (13).

Screening programs consist of conventional and molecular methods step by step (**Figure 3**).

B. Molecular methods

Various molecular techniques have been used for point mutation detection in β-thalassemia and large-deletion detection in α-thalassemia. All of these techniques have some advantages and disadvantages. Recently, screening for both α- and β-thalassemia genes by next-generation sequencing (NGS) has been introduced. This technique gives an accurate diagnosis of thalassemia that may be misdiagnosed by other conventional techniques. The major limitation for using NGS in the screening of thalassemia is its cost which is still expensive (14).

Amplification refractory mutation system (ARMS): This technique employs two primers identical in sequence except for the 30-terminus base, one of which is complementary to the wild-

type and the other for the mutant base; a common primer for the opposite strand must of course be used as well. With a normal individual, PCR product will be seen only in the reaction employing the wild-type primer set. A heterozygote will generate a band using both wild-type and mutant primer set, and an individual with homozygous mutation will be negative with the normal and positive with the mutant primer set (14) (Figure 4).

Reverse Dot Blot Analysis: The suspected mutation can be identified by hybridization of an allele-specific oligomer (ASO) DNA probe with detection methods quite a routine procedure. For each mutation, two hybridization reactions need to be conducted, one with the probe for the mutant sequence (14) (Figure 5).

Gap-polymerase chain reaction (gap-PCR): The real-time PCR or quantitative PCR (qPCR) is widely used to detect, characterize, and quantify nucleic acids. Currently, the application of real-time PCR with melting curve analysis for thalassemia diagnosis is based on two general approaches, intercalating dye assays and probe- based assays, obtaining a fluorescent signal from the synthesis of product in PCR. In first, the multiplex GAP-PCR with melting curve analysis are developed for large deletion mutations in a and b-thalassemia genotyping. In second, probe-based assays are now widely used for detection of point mutations. This technique can apply for beta-thalassemia diagnosis, the multiplex probe-based fluorescence melting curve analysis (FMCA) which is a powerful tool for point mutations detection (14).

Multiple ligation-dependent probe amplification (MLPA): is a multiplex PCR method that allows the detection of any deletions or duplications in the screened regions. This technique has been proven to find known and unknown deletions in unsolved cases after performing conventional techniques (14).

DNA sequencing analysis methods: Unknown point mutations can be identified by sequencing the PCR product, usually employing the Sanger's dideoxy termination method. This requires the production of a single DNA strand as a template (14) (Figure 6).

Next Generation Sequencing (NGS): NGS technologies have gained the capacity to sequence entire human genome in an ultra-high throughput, scalability, and speed manner at a level that is not possible using Sanger sequencing technology. Most NGS platforms have three general steps: first library preparation using random fragmentation of DNA followed by ligation with custom linkers. Second, library amplification using clonal amplification methods and PCR. Third, sequencing using incorporation of fluorescent-labelled nucleotides by DNA polymerases or ligation processes.
A comprehensive NGS-based test that offers advantages over the traditional screening/ molecular testing methods. To our knowledge, this is among the first large-scale population study to systematically evaluate the application of an NGS technique in carrier screening and molecular diagnosis of hemoglo-

Figure 4. *Allel spesific PCR.*

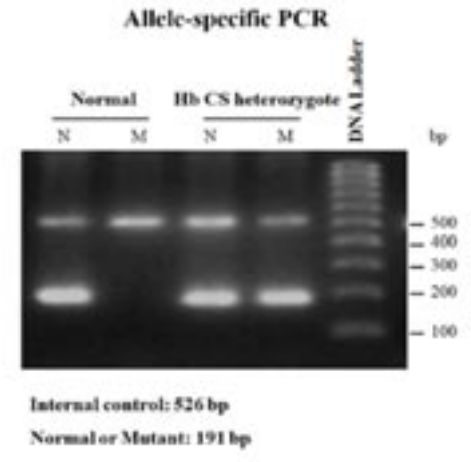


Figure 5. *Reverse Dot Blot Analysis.*

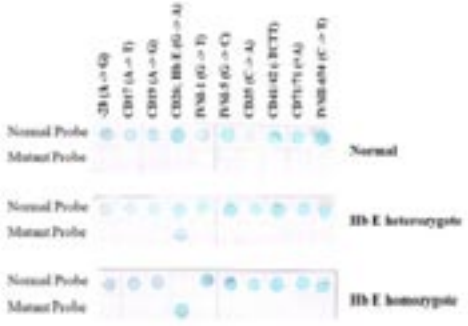
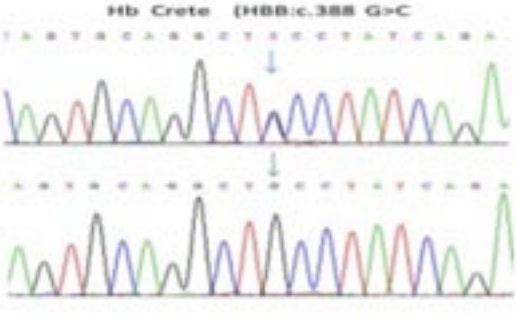


Figure 6. *Hb Crete was detected by DNA Sequencing Analysis in AGTC Genetic Center.*



binopathies. NGS has enabled researchers to diagnose and understand complex diseases through whole-genome sequencing, exome sequencing, or targeted gene panels.
Target NGS approach was designed to cover entire globin genes coding regions, their key regulatory regions, and modifier genes such as KLF1, BCL11A, HBS1L, and MYB (14, 15).
Whole-exome sequencing and whole-genome sequencing is useful for the identification of non-coding causative mutations, which might account for the disruption of transcriptional factor occupancy sites and cis-regulatory elements (16).
In recent years, with the introduction of NGS technology, clinic exom, whole exome and whole genome analysis, the success of prenatal and postnatal diagnosis of hemoglobinopathies has increased.

Prenatal and preimplantation genetic diagnosis in hemoglobinopathies

Biological tests analyzed involve adult/newborn subjects, whereas genetic analyses involve adult thalassemia patients, newborns, embryos/fetuses (including non-invasive prenatal diagnosis), preimplantation embryos, and pre-fertilization oocytes (17).
Prenatal diagnosis of hemoglobinopathies enables couples at risk to have a healthy child. Currently used fetal sampling procedures are invasive with some risk of miscarriage. A non-invasive approach to obtain fetal deoxyribonucleic acid (DNA) for diagnosis would eliminate this risk. A non-invasive prenatal diagnostic approach for hemoglobinopathies using cell-free fetal DNA circulating in the maternal plasma was developed in some centers.
An accepted and widely adopted approach to reduce the number of new cases involves carrier-screening programs, with the option of prenatal diagnosis (PND) or preimplantation diagnosis (preimplantation genetic testing for monogenic disease, PGT-M) for carrier couples.
The aim of PND is to provide an accurate result as early in pregnancy as possible, which necessitates prior identification of the parental disease-causing mutations, as well as safe and timely biopsy of fetal material. PGT-M aims to characterize the genetic status of in vitro fertilized embryos during assisted reproductive technology (ART), in a few cells biopsied from oocytes/zygotes or embryos, in order to initiate an unaffected pregnancy. Another application of PGT-M is preimplantation genetic diagnosis for human leukocyte antigen (PGD-HLA), which, in addition to identifying unaffected embryos, also characterizes the embryos that are HLA compatible with an existing affected child requiring a hemopoietic stem cell transplantation (HSCT) (18, 19).
As conclusion, There are three options for PND and PGD. In the first case, if couples have β^0/β^0; β^0/β^+; β^+/β^+; β^0/S; β^+/S; --/$\alpha\alpha$; α-α/-α; S/S and E/E, it should definitely be recommended, in second case, if couples have β^{++}/β^+; β^{++}/β^0; β0/E; β^+/E; β^0/C; β^0/C, plus -$\alpha/\alpha\alpha$ and beta modifier genes it should be discussed with family for decision of PND and PGD, in third case, if couples have β^{++}/β^{++}; $\alpha\alpha/\beta^0$, $\alpha\alpha/\beta^+$, and plus beta modifier genes, PND and PGD tests are not required.

Management of β-Thalassemias

Thalassemia is due to decreased production of at least one globin polypeptide chain (beta, alpha, gamma, delta) which results in unbalanced hemoglobin synthesis. Inheritance of thalassemia is autosomal. β-thalassemia arises from mutations in human globin genes encoding for α- and β-globin polypeptide chains of haemoglobin. Two α- and 2 β-globin chains, each conjugated with an iron containing haem moiety, form adult haemoglobin (HbA). Molecular defects of thalassaemia lead to either reduced or absent production of β-globin chains resulting in β-thalassemia or defective synthesis of α-globin leading to α-thalassemia (**Figure 7**).
More than 200 different β-globin gene mutations have been characterized. Most of the β-thalassemia mutations are caused by point mutations, small deletions or insertions within the coding regions and the exon-intron junctions. The types of the mutation are typically ethnic specific (20-22).
Based on the extent of β -chain imbalance, anemia severity, and clinical presentation, β-thalassemia lie on a spectrum of severity with different clinical phenotypes, complications, and strategies for treatment. Coinheritance of α- and γ-mutations as well as coinheritance of other

hemoglobinopathies (eg, HbE, Hb Lepore, Constant Spring, sickle cell hemoglobin, or HbS) may modify the clinical manifestations.
Clinically, β-thalassemias can be classified as transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT) according to the severity of the phenotype, which is caused by a wide spectrum of mutations in a homozygous or compound heterozygous state (Table 2) (23, 24).
Current treatment of TDT consists of regular transfusions that lead to iron overload, requiring iron chelation to prevent iron-related organ toxicity. NTDT patients do not require transfusions or only occasionally require them; however, they develop iron overload as well because of increased intestinal iron absorption caused by chronic anemia (23, 24).

1. Blood transfusion

The purpose of transfusion is twofold: to improve the anemia and to suppress the ineffective erythropoiesis. Blood transfusions compensate for chronic anemia, prevent bone deformities, facilitate normal growth and activity levels, and allow patients to have a good quality of life (QoL).
The decision to start transfusion in patients with a confirmed diagnosis of thalassaemia should be based on the presence of severe anaemia (Hb < 7 g/dL on 2 occasions, more than two weeks apart, excluding all other contributory causes such as infections). However, even in patients with haemoglobin > 7 g/dL, other factors should be considered, including age at presentation of first symptoms, facial changes, poor growth, evidence of bony expansion and increasing splenomegaly untreated (24, 25).
Patients with NTDT may need only sporadic blood transfusions, although their transfusion requirement may increase later in life (24, 25). The decision to start regular transfusions in these patients depends on clinical and laboratory assessment: worsening anemia, inability to tolerate anemia, massive splenomegaly, worsening bone disease, increasing nucleated red blood cells and dropping hemoglobin. Skeletal malformation can be severe in NTDT patients and should be considered in the decision to start transfusion. Patients starting transfusions at adult age are at very high risk for developing red cell alloimmunization and serious hemolytic transfusion reactions.

Figure 7. *Globin chain synthesis after conception.*

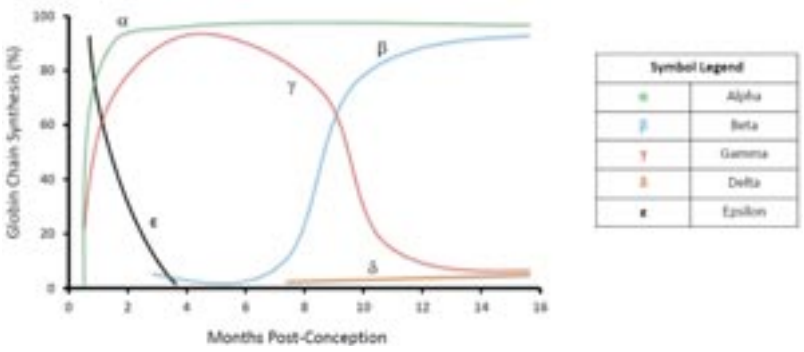
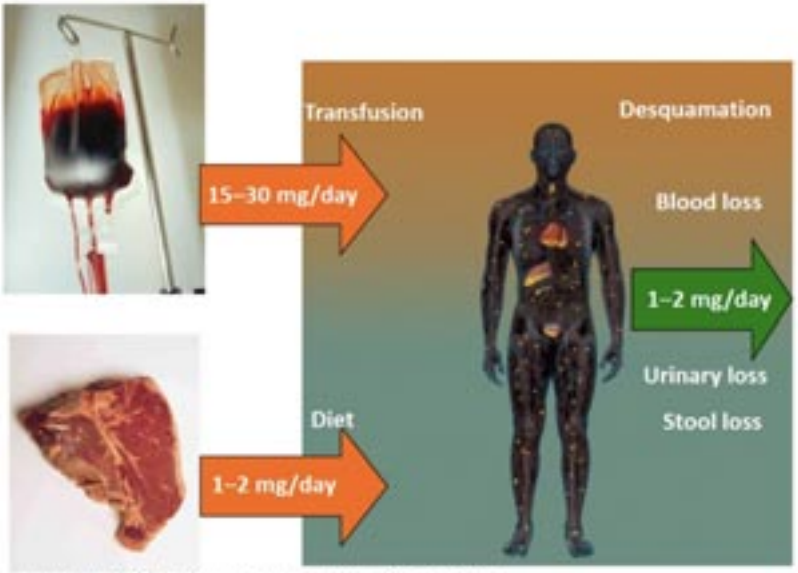


Table 2. *Thalassemias : Quantitative defects of globin chain synthesis.*

Thalassemia	Thalassemia Trait	Thalassemia Disease
Inherited anemia Inability to synthesize normal amounts of globin proteins	Heterozygote Mild anemia that has insignificant effect on health	Heterozygote, Homozygote or Compound Heterozygote Moderate or severe anemia
α thal: Decrease or absence of α-globin chains	α thal trait	May not require transfusion: Thalassemia intermedia NTDT
β thal: Decrease or absence of β-globin chains	β thal trait	Requires regular transfusions: Thalassemia major TDT

Figure 8. *Iron balance in TDT patients not treated with iron chelating agents.*



1 mL of pure RBCs contains approximately 1.08 mg of iron;
1 unit of packed RBCs contains approximately 200–220 mg of iron.

To reduce the risk of allo-immunisation, before commencing the first blood transfusion, it is essential to perform extended phenotyping of red blood cell (RBC) antigens (minor blood groups) to minimise future problems. Knowing the red cell antigen profile of the patient is very useful when antibody screens are positive and multiple auto- or alloantibodies are identified. Where multiple alloantibodies are found and the chances of finding a compatible unit from routine blood stocks are low, a panel of regular blood donors, negative for the antigens is instead identified for continued transfusion support of the patient.

Patients with TDT require regular transfusions usually 2-5 weekly, to maintain a target pre-transfusion Hb level of 9-9.5 g/dL. The currently accepted protocol is to achieve a post-transfusion Hb level of 13-14 g/dL by transfusing washed leuco-depleted (reduced to <1 x 106 leucocytes per unit) packed red blood cells (PRBCs). All patients with thalassaemia should receive fresh blood which is less than 2 weeks old. The volume of blood to be transfused is calculated using the following formula: volume (ml) of blood to be transfused = (14g/dl –pre-transfusion Hb) x weight (kg) x 3 / hematocrit of transfused blood (26, 27). For patients maintaining a pretransfusion Hb of 9.5 g/dL, the increase in transfusion requirement is represented by a consumption of more than 200 mL of RBC/kg/year (assuming that the Hct of the unit of red cells is 75%).

Increased transfusion volumes and frequencies are signs of poor red cell survival which may be due to hypersplenism, red cell antibodies or poor red cell quality and appropriate interventions need to be taken.

2. Iron overload

The predominant mechanisms driving the process of iron loading include increased iron burden secondary to transfusion therapy in TDT and enhanced intestinal absorption secondary to ineffective erythropoiesis and hepcidin suppression in NTDT. Different organs are affected differently by iron overload in TDT and NTDT owing to the underlying iron loading mechanism and rate of iron accumulation (20, 25-27).

Each unit of transfused packed red blood cells contains 200 to 250 mg elemental iron. In TDT, transfusional iron usually amounts to 0.3 to 0.6 mg/kg per day with an assumed monthly transfusion rate of 2 to 4 U packed red blood cells (**Figure 8**). Excess accumulation of iron in organ (hemosiderosis) leads to oxidative damage as a result of generation of reactive oxygen species (ROS). Oxidative damage by reactive oxygen species (generated by free globin chains and labile plasma iron) is believed to be one of the main contributors to cell injury, tissue damage, and hypercoagulability in patients with thalassemias (28).

In TDT patients, iron accumulation in organ tissues starts early and leads to organ toxicity and dysfunction. Excess iron can lead to cardiomyopathy, and untreated patients may die from heart failure before 20 years of age. In NTDT patients, iron overload is cumulative with advancing age, and starts usually beyond the age of 10 years (28, 29).

3. Monitoring of iron overload

Serum ferritin (SF) is the most widely used marker to assess iron overload and is measured 3-monthly in thalassaemia patients. It is generally a reliable indicator of total body iron stores, provided that iron burdens are low as in early transfused, non-chelated patients, but can be falsely elevated with concomitant inflammatory conditions and liver disease.

Use of magnetic resonance imaging (MRI) to noninvasively assess liver and myocardial iron concentration is an important advance in thalassaemia care. Liver iron concentration (LIC) can be measured both by T2* and R2 (also known as FerriScan®) techniques whereas, cardiac iron is assessed by T2* MRI (**Table 3**). While SF is a convenient measure of iron status; its trend is unable to predict changes in LIC in individual patients. Therefore, SF trends need to be interpreted with caution and confirmed by direct measurement of LIC. Cardiac T2* values < 20 ms are indicative of declining left ventricular function whilst values below 10 ms are associated with heart failure and very high mortality (20, 24, 30).

4. Iron chelation therapy (ICT)

Iron chelation therapy (ICT) is the standard method of choice in thalassemia management, decreasing morbidity and mortality in this patient population. Chelation should be considered after one to two years of transfusion therapy, when the SF is greater than 1,000 ng/mL, or when the hepatic iron is approximately 7 mg/g dry weight, or after received of 10-20 times RBCs transfusions. A SF level above 2,500 ng/mL is associated with increased risk of cardiac and

endocrine disease. In patients with NTDT, ICT is indicated when SF rises above 800 ng/mL (31). However, despite increased knowledge, there are still uncertainties about the level of body iron at which iron chelation therapy should be started and about the appropriate degree of iron stores' depletion.
Three iron chelators are currently available for the treatment of iron overload: deferoxamine (DFO) in subcutaneous or intravenous injection; oral deferiprone (DFP) in tablet or solution form; and oral deferasirox (DFX), in dispersible tablet (DT) and - more recently - film-coated tablet (FCT) forms (Table 4) in monotherapy or in combination with subcutaneous DFO associate to oral DFP, mainly used for patients in whom chelation with DFO is inadequate or intolerable. Recently there is a tendency for oral monotherapy with DFX, as it is equally effective to DFO and DFP.
Adherence to therapy is crucial for good results. Timely initiation, close monitoring and continuous adjustment are the cornerstones of optimal chelation therapy in children, who have a higher transfusional requirements compared to adults in order to reach haemoglobin levels adequate for normal growth and development (32, 33). The timing, choice of single or combination, and optimal dosing regimen for iron chelation is still debatable.

5. Splenectomy

Current recommendations for splenectomy are limited to patients with hypersplenism and clinically symptomatic splenomegaly. Hypersplenism is associated with leukopenia, thrombocytopenia, and increased requirement for transfusions. Splenectomy should be considered on an individual basis in patients with very high transfusion requirements, particularly when the annual transfusion requirement rises above 200-250 ml/kg/year, to reduce the need for red cell transfusion (Table 5) (34).

6. Antioxidants and vitamin supplements

Oxidative stress may be ameliorated by endogenous and exogenous antioxidants. Their effects include scavenging and inactivating ROS and correcting their damage to cellular components. Many antioxidants are supplied by nutrition. Antioxidants can also be taken as food additives, either as pure compounds, such as vitamins C and E and Q10, or as crude extracts, such as the fermented papaya preparation and curcumin (35).

Table 3. *Hb Crete was detected by DNA Sequencing Analysis in AGTC Genetic Center.*

LIC threshold (1) (mg Fe/g dry weight)	Clinical relevance (1)	Sensitivity (2)	Specificity (2)
1.8	Upper 95% of normal	94% (86–97)	100% (88–100)
3.2	Suggested lower limit of optimal range for LICs for chelation therapy in transfusional iron overload	94% (85–98)	100% (91–100)
7.0	Suggested upper limit of optimal range for LICs for transfusional iron overload and threshold for increased risk of iron-induced complications	89% (79–95)	96% (86–99)
15.0	Threshold for greatly increased risk for cardiac disease and early death in patients with transfusional iron overload	85% (70–94)	92% (83–96)

From: 1. Olivieri NF, Brittenham GM. Blood. 1997;89:739-761. 2. St Pierre TG, et al. Blood. 2005;105:855-661.

Table 4. *Iron chelators currently available and their properties for the treatment of iron overload.*

Property	Deferoxamine	Deferiprone	Deferasirox	
Usual Dose For TDT	25 – 50 mg/kg/day	75 – 99 mg/kg/day	20 – 40 mg/kg/day	14 – 28 mg/kg/day
Route	SC or IV	Oral tablet or oral solution	Dispersible tablet	Film-coated tablet or sprinkles
Dosing frequency	Over 8 to 24 hours	3 times daily	Once daily	
Adverse Effects	Local reactions Audiologic Ophthalmologic Bone abnormalities Pulmonary disease	Gastrointestinal Neutropenia Agranulocytosis Arthralgia Hepatic	Gastrointestinal Hepatic Renal Rash	
Availability	Licensed worldwide 1st line agent	Licensed in Europe, U.S. as 2nd line	Licensed in Europe- 6y+ (2 - 5y as 2nd line) U.S.- 2y+	
Challenges	Adherence with parenteral	Weekly blood count monitoring	GI side effects may limit optimal dosing	

Adapted from Kwiatkowski, Hematology, 2011

7. Hematopoietic stem cell transplantation

The only potential cure for β -thalassaemia is haematopoietic stem cell transplantation (HSC). The major source of HSC for transplant is still derived from HLA-identical sibling bone marrow. In this procedure, autologous hematopoiesis is eradicated through chemotherapy. The major source of haematopoietic stem cells (HSC) for transplant is still derived from HLA-identical sibling bone marrow. The success of stem cell transplantation depends on the amount of erythrocyte transfusions received and the severity of

Table 5. *Indications for splenectomy in TD and NTDT patients.*

Worsening anemia leading to poor growth and development	When transfusion therapy is not possible or iron chelation therapy is unavailable
Hypersplenism	Leukopenia or thrombocytopenia causing clinical problems such as recurrent bacterial infection or bleeding
Splenomegaly	Accompanied by symptoms such as left-upper-quadrant pain or early satiety Massive splenomegaly (largest dimension >20 cm) with concern about possible splenic rupture

1. Taher A et al. Guidelines for the management of NTDT. 2013 TIF Publication No. 19.
2. Taher AT et al. Br J Haematol 2011;152:512–523.

iron overload. The recent developments of new chemotherapeutic conditioning regimens and improved supportive care have decreased the rate of fatal complications considerably (36). HLA-identical sibling, cord blood transplants, when available has shown encouraging results comparable to bone marrow-derived HSCT with less graft-versus-host disease (GVHD) occurrence. Families, who do not have HLA-identical siblings available for HSCT, may be able to resort to unrelated cord blood transplantation although studies are still limited (37).
Alternatively, HSC obtained from adult matched unrelated donors may be option and has shown considerable success especially with improvements in high resolution molecular HLA typing and stringent selection of best-matched donors. Overall survival and thalassemia-free survival of 79% and 66%, respectively, have been reported among a group of group of paediatric and adult patients transplanted with a matched unrelated donor (38).

8. Emerging therapies

Several new therapies, both pharmacological and gene-based are currently being investigated for thalassaemia. Most of these aim to restore globin chain imbalance in thalassaemia RBCs. Induction of γ-globin with an aim to increase fetal haemoglobin production using pharmacological agents has been tried for several years (39). Despite numerous drugs having reached clinical trials, none has been efficacious enough to be recommended for routine clinical use.

New therapies like erythroid maturation agents [e.g. first in class sotatercept (40) and luspatercept (41)], and gene therapy (42) are emerging, while others are in investigation [e.g. genome editing (43)].

Management of Sickle cell disease (SCD)

Background

SCD is a systemic condition, with complications that affect almost all organs of the human body and clinical manifestations depend on many factors and not only from the genetic condition. Genotype-phenotype association studies have identified the existence of genetic modifiers that can modulate complications. One example is fetal hemoglobin (HbF), in which several known alleles prevent the physiologic switch from fetal to adult hemoglobin that ordinarily occurs shortly after birth (44, 45). The potential life span of SCD has markedly improved as a result of the implementation of several prophylactic measures like newborn screening, immunizations, the improved detection and treatment of infections, and the use of disease-modifying drugs like hydroxyurea (46).
In Europe, the mortality rates of SCD during early childhood have decreased up to 95% due to the implementation of newborn screening, improvements in vaccination, especially with the pneumococcal vaccine, and the use of prophylactic and therapeutic antibiotics (47). Although increased survival to adulthood is certainly progress, people with SCD die at a much younger age than race-matched peers and the overall median survival is 58 years. Adolescents and young adults in the second and third decades of life suffer significant morbidity with higher rates of SCD-related complications and higher health care costs (48).
This is in part due to observations showing that these young adult patients receive fewer transfusions and are less likely to be on hydroxyurea and/or chelation therapy when eligible for such treatments as they transition from pediatric to adult care (49). The most common cause of death is cardiac, respiratory, renal, infectious, neurologic, gastrointestinal, and hepatobiliary disease in descending order, and leukocytosis remains a key predictor of poor outcome along with renal insufficiency recurrent

episodes of acute chest syndrome, low Hb F concentration, severe anemia higher rates of hemolysis, and dactylitis before 1 year of age (50).
The first symptoms of SCD may be expected a few months after birth when HbS level rises. While in less severe sickle cell disorders, clinical problems may develop later in life, SCD is a chronic disease characterized by anemia and multiorgan damage, but punctuated by acute painful episodes. These random crises are of variable severity and triggered by different factors such as cold weather, infection, or dehydration. Chronic organ damages, as well as acute, random painful crises, can be life-threatening. They also can have a profound effect on all aspects of life; as a consequence, psychological and social problems are very common in these patients and their families. Genetic counseling and psychosocial support are pivotal at all stages of development and into adulthood. As mentioned before, SCD is a chronic disease, characterized by chronic hemolytic anemia associated with painful vaso-occlusive crises, progressive organic injures due to vascular disease, infections, and severe complications affecting the chest, spleen, and kidney. The most important SCD clinical manifestations are the following (50):

1. ***Hemolysis***: The existence of hemolysis in SCD has been documented by both indirect and direct methods. The existence of bone-marrow erythroid hyperplasia, reticulocytosis, indirect hyperbilirubinemia, and elevations of plasma hemoglobin and serum lactic acid dehydrogenase (LDH) values show hemolytic disease.

2. ***Vase-occlusive crisis***: These are highly painful crises and the main characteristic of the disease. They can appear in any location, and their frequency and intensity are variable: 1/3 of the patients do not suffer pain crises while 1% present more than 6 episodes per year. Pain crises represent 50% to 60% of consultations and 60% to 80% of hospitalizations. Infants are protected from these crises during the first months of life due to the high Hb F levels. The first episode of pain is usually dactylitis in the small bones of the hands and feet, and about 50% of the children present this manifestation at the age of 2-years-old.

3. ***Infections***: These are the most common cause of child mortality because they are at high risk for encapsulated germ infections: pneumococcus, Haemophilus, and meningococcus.
This elevated risk of infections is the consequence of functional asplenia (partial loss of splenic function) and the presence of plasma complement (C) and/or opsonization disorders. 80% of patients with HbSS and HbSβ^0 have functional asplenia before 1 year of life, and a complete loss of spleen function (autosplenectomy) at 5 years. The risk of fatal invasive pneumococcal disease is very high during the first 5 years of life as well as the increased risk of *staph aureus* infections, *viridian's streptococcus*, *E. Coli*, and *Salmonella*.

4. ***Acute thoracic syndrome***: Acute thoracic syndrome (ATS) is a frequent, and sometimes fatal, SCD complication, characterized by fever, respiratory distress, pain, hypoxemia, and pulmonary infiltrates, easily identified on chest X-ray. The most frequent causes are infections by atypical bacteria (mycoplasma and chlamydia), viruses and pulmonary fat embolism coming from long bones infarction. The highest incidence occurs in the first decade, between 1 and 7 years. Moreover, more than 30% of patients suffer at least 1 episode, and it the second important cause of children's death.

5. ***Cerebral vascular accidents (CVAs)***: CVAs in SCD, are the main cause of morbidity, and leads to ischemic and hemorrhagic attacks. It is 300 times more frequent than in the normal population, and the peaks of maximum incidence are between 2 and 8 years and over 50 years. 10% of children between 2 and 10-years-old have clinical infarcts and 17% have silent infarcts associated with occlusion of the internal carotid and middle cerebral arteries.
Alterations in the blood flow of the internal carotid and middle brain arteries can be detected by transcranial eco Doppler (TCED) and the risk of stroke is between 0.5% and 1%. However, if the blood speed in the middle cerebral artery is higher than 200 cm/sec, the risk increases to 10 to 13%. Cerebral infarction can be prevented with periodic transfusions every 3-4 weeks to maintain HbS <30%. Once a patient has presented a heart attack the risk of recurrence is 50%.

6. ***Splenic sequestration crisis***: They start during the 2nd or 3rd month of life and exhibit high mortality. Their recurrence is estimated at 50% after the first episode and can occur in 30% of children before 6 years.
They create a life emergency episode when there is a sudden increase of the spleen (splenomegaly) associated with a hypovolemic shock. For this reason, it is of vital importance to educate the patient's family to aid in the prevention of these episodes. As mentioned before, 80% of patients with HbSS and HbSβ^0 have functional asplenia before 1 year of age, and complete autosplenectomy at 5 years, but the patients with HbSC and HbS β^+ are at risk of splenic sequestration throughout their life.

7. ***Aplastic crisis (AC)***: AC is due to the infection by parvovirus B-19 that blocks the erythropoiesis and therefore the production of RBCs in the bone marrow. This leads to severe non-regenerative anemia with very low values of hemoglobin concentration and reticulocytes.
All these mentioned disorders are complex, and their prevalence is highly variable. For this reason, it is unlikely that all the services necessary for the best diagnosis, follow-up, and treatment of SCD patients can be offered by only one health care provider (HCP), and a multi-disciplinary team of health and social services with local centers networking together with are the most convenient to offer a full range of services, including specialist access and supervision when required.
In reality, however, such healthcare organization HCP is rarely available, even in developed countries, and in the majority of care has to be delivered close to the patient's home by a local team or clinicians, with expertise in SCD and available for an in-person consultation or by telephone/internet communication. Regardless, it is of utmost importance that patients are educated on infection prevention, pain management, and early detection of complications starting with general measures that are beneficial to maintain health and avoid acute disease events. These measures include avoiding overexertion, excessive temperatures, hypoxia, and maintain an adequate water intake. It is always convenient to prevent megaloblastic erythropoiesis with the folic acid intake (50, 51).

Clinical management of SCD

Management of SCD patients must include prevention programs, curative, symptomatic and psychosocial interventions, from childhood to adult life and, can be summarised in 7 key issues (51):

- Education, information, and advice regarding sickle cell disease (given to health workers, parents, and/or patients);
- Prevention of infections, i.e. extended vaccinations, penicillin prophylaxis and pneumococcal vaccination;
- Follow-up with patients to identify those at risk for certain adverse outcomes such as a) For stroke by monitoring them by transcranial Doppler scanning and b) For a severe disease by monitoring the number of painful events per year;
- Prevention of acute events related to a surgical procedure or pregnancy.
  a) Treatment of acute events, i.e. blood transfusion for acute stroke or acute chest syndrome, antibiotics for the infection, tailored analgesia for a painful crisis, and others;
- Prevention of acute or chronic events, i.e. treatment by chronic blood transfusion or hydroxycarbamide (Hydrea):
- Treatment of some chronic complications, by chronic blood transfusion (not for chronic anemia) or Hydrea;
- Monitoring and treatment of iron overload;
- If applicable due to severe disease, curative therapy by hematopoietic stem cells transplantation (HSCT).

Genetic counseling and psychosocial support are pivotal at all stages of development and into adulthood transition. At the same time, routine clinical visits allow for the acquisition of baseline laboratories that can help differentiate crisis events. This can be achieved with the following key points for the management of patients with SCD (51, 52):

- Universal neonatal screening.
- Early prophylaxis with penicillin
- Vaccination
- Hydroxyurea administration in both children and adolescents
- Blood transfusions
- Iron Chelation
- Hematopoietic Stem Cell Transplantation (HSCT)
- Treatments in development

1. Universal Neonatal Screening

To avoid acute complications and delay organ damage, it is essentially an early diagnosis, through neonatal screening programs that aim to be able to initiate preventive measures and care for the baby as early as possible. In this sense, it is essential, from as early as the first month of life, to initiate health education to the family, anti-infective vaccination programs, and prophylaxis with penicillin, to avoid the most serious complications of childhood such as invasive pneumococcal disease, which is the first cause of death in infancy and splenic sequestration. The early start of these measures is only possible through early neonatal diagnosis programs.

The European Commission's action on Rare Diseases by co-financing the European Network for Rare and Congenital Anemias (ENERCA) has implemented neonatal screening programs in Europe that are financed by the local or national public health authorities. For this, a survey has been undertaken by ENERCA in 2013 to know the situation of hemoglobinopathies in Europe and has been published as an EC Health and Migration policy report (53). As a first conclusion, it has been demonstrated that in the EU, at least five Countries have implemented a universal newborn screening program for SCD (Table 6).

Table 6. *Results of neonatal/newborn screening for sickle cell disease (SCD) within the European Union (EU).*

Implementation	Year	SCD prevalence
England (UK) Universal	1985	1:2000
France Targeted	440,465	1:700
Belgium (Brussels) Universal	416,531	1:1600
The Netherlands Universal	290,681	1:4200
Madrid (Spain) Universal	237,047	1:6250
Catalonia (Spain) Universal	167,859	1:3909 (Ref. 54, 55)

2. Early prophylaxis with penicillin

The main goal of universal neonatal screening is to initiate prophylaxis with penicillin before the child's 2 months of life. The recommended doses of penicillin are Children <3 years: 125 mg / 12h; Children between 3 and 5 years: 250 mg / 12h and Children> 5 years: do not require routine penicillin.

3. Vaccination

Moreover, it is advisable to start the vaccination program early with vaccines against encapsulated germs:

- Pneumococcus: 13-valent conjugate vaccine: at 2, 4, 6, and 12 months - 23-valent unconjugated vaccine at 2 years and 5 years. Revaccinate every 5 years;
- Haemophilus b: at 2, 4, and 6 months
- Meningococcus: - Quadrivalent conjugate vaccine (A, C, Y, W135) between 12-24 months.- Meningococcal B vaccine: at 2,4, 6, and between 12-23 months
- Hepatitis B vaccine
- Seasonal Influenza vaccine

4. Hydroxyurea administration

Hydroxyurea (HU) is a ribonucleotide synthetase inhibitor that promotes the production of Hb F. A high concentration of Fetal Hb prevents the formation of Hb S polymers reducing the severity of clinical manifestations of sickle cell disease. Hydroxyurea in children aged 9 to 18 months has been shown to decrease pain crisis, dactylitis, acute chest syndrome, and the requirements transfusion. It is also indicated in all children over 9 months of age. In adults, it is indicated in patients with 3 pain attacks every 12 months, when pain interferes with daily activity when there is a history of the acute thoracic syndrome (ATS), severe chronic anemia that interferes with daily life, or chronic kidney disease. The recommended doses of HU are:

- In children: 20 mg/kg/day. It can be increased by 5 mg/kg/day every 8 weeks until clinical improvement is achieved or toxicity occurs
- In adults: 10 - 15 mg / kg / day. In the event of hematological toxicity, the treatment has to be interrupted until it disappears. Later treatment can be continued at a dose 5 mg/kg/day, a little lower than the initial dose.

5. Blood Transfusions

Transfusions play a very important role in the treatment of some events acute symptoms of sickle cell disease and in situations where they can lead to a risk to the patient. The main indications are:

- Acute chest syndrome
- Acute stroke
- Liver sequestration crisis
- Splenic sequestration crisis
- Aplastic crisis (parvovirus B19 infection)
- Pre-surgical intervention, to achieve Hb 10 mg / dL
- In symptomatic anemia

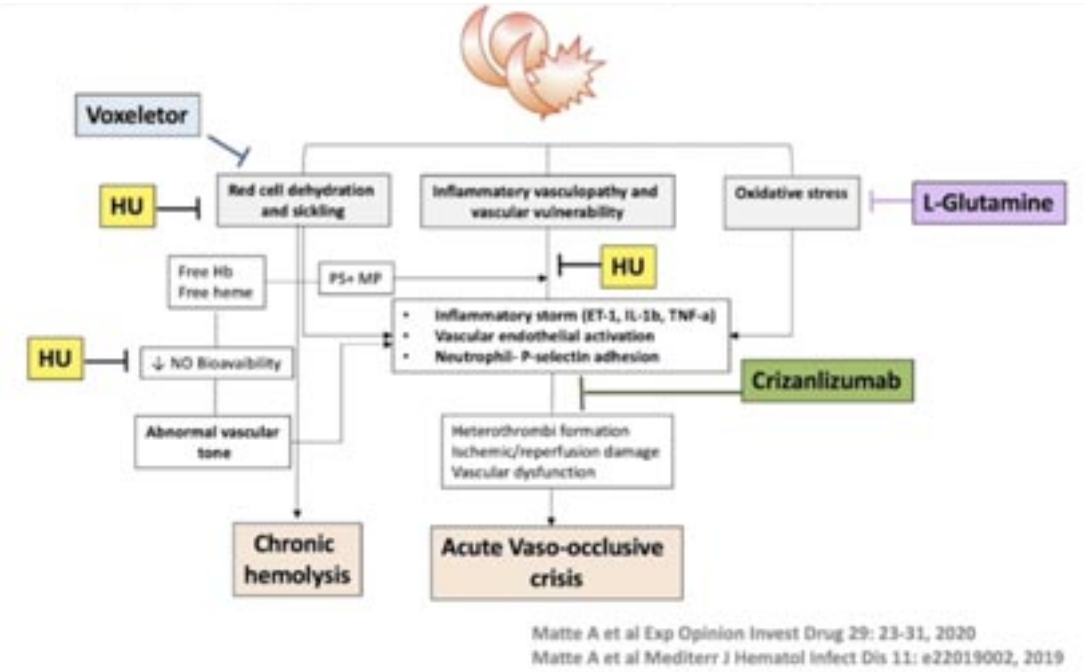
Transfusions are also indicated prophylactically in the prevention of primary and recurrent cerebral infarction in children with blood velocity in the middle cerebral artery higher than 200 cm/sec measured by transcranial Echo-Dopler. There is not yet enough evidence whether prophylactic transfusions could prevent symptomatic cerebral infarction in patients with silent infarcts. In the case of administration of transfusions, these should be given periodically at intervals between 3 and 4 weeks, to maintain an HbS concentration <30% that is associated with a lower risk of ischemic events.
Transfusion prophylaxis can be administered through simple transfusions, however, when the patient's starting Hb is ≥10 g/dL, the risk of Increased blood viscosity with transfusion may increase the HbS polymerization. In these cases, it is advisable to perform erythrocytopheresis that consists of doing a blood exchange through an apheresis procedure to achieve an HbS of <30% without increasing blood viscosity risk.

6. Iron chelation
Each unit of RBCs used for blood transfusion introduces between 200 to 250 mg of elemental iron into the body, and the risk of iron overload is high when the patient has to be regularly transfused. Iron overload can give rise to significant morbidity through the development of hepatic, cardiac, or endocrine impairment. For this reason, iron chelation therapy is an adjunct to any transfusion-based treatment regimen. Serum ferritin is a rough estimation of body iron stores and is typically obtained quarterly. Accuracy of ferritin in estimating iron stores is jeopardized by chronic inflammation, relatively frequent in patients with SCD. For this reason, liver biopsy is the gold-standard method to evaluate the body iron burden, but it is invasive and currently replaced by non-invasive techniques such as magnetic resonance R2* or T2* MRI, or liver susceptometry with a SQUID magnetometer (BSL) also known as ferrometer (56, 57). Unfortunately, these procedures have limited availability due to their centralization in highly specialized centers but here, serum ferritin can be used to monitor the effectiveness of chelation. Three iron chelators are currently commercially available: deferoxamine, deferiprone, and deferasirox and local experience, availability and cost of each chelator, age of the patient, drug side effects, patient comorbidities, and preference largely inform the decision of what agent to initiate. Although it is typically well-tolerated, deferoxamine requires a daily subcutaneous infusion that can be difficult for patients to manage long-term treatments and for this, deferiprone and deferasirox are often preferred by patients.

7. Hematopoietic stem cell transplantation
Although RBC transfusions and hydroxyurea play an important role in the treatment of SCD, their primary impact is a reduction in morbidity and mortality. Currently, the only cure for SCD is Hematopoietic Stem Cell Transplantation (HSCT) or Bone Marrow Transplantation (BMT). In this procedure, a sick patient is transplanted with bone marrow from healthy, genetically compatible sibling donors. However only about 18 percent of children with sickle cell disease have a healthy, matched sibling donor, and the risk of graft-versus-host disease and failure to engraft is relatively high and must be weighed against the risk of non-transplanted SCD (58).

8. Treatments in development
8.1 New approaches for medical treatament of SCD: Although hydroxyurea is the cornerstone of treatment for SCD and remains the only FDA-approved medication for its management, drugs aiming to target several other key elements of the complex pathophysiology of the disease are actively being investigated in humans today (59). Very recently there are new approaches for drug medication of SCD that alone or in combination with HU are the subject of clinical trials in both USA and Europe. Up to now, three main new drugs are under clinical trials for the treatment of SCD: Voxeletor,Crizanlizumab and L-Glutamine Voxeletor blocks intermolecular contacts to prevent HbS fiber generation. It ameliorates invitro RBC deformability and viscosity and improves mouse Sickle RBCs survival with reduction in reticulocyte count. In phase III randomized double-bind placebo controled

Table 7. *Pathophysiology Based New Therapeutic Options for SCD.*



multicentric study it has been shown that the primary endpoint is an increase of hemoglobin and the secondary endpoint is a reduction of VOC and hemolysis. Crianzlizumab is well tolerated and induces a neutrophil p-selectin block, reducing the pain crisis and increasing the time between pain crisis. In Europe it has been approved by the European Medicines Agency under the name of Adakveo.

L-Glutanine is involved in GSH metabolism since it prevents NADPH levels required for GSH recicling. Multicenter randomize placebo-controled double-blind phase III clical trial with L-Glutamine (0.3 g/kg twice a day) involving 230 SS/S-b0thal patients. Supplementation with L-Glutamine decreases the number and lenght of hospitalisation and increases the mean time to the first crisis. However since no information is still available on long-term use of L-glutamine, Sicle-cell scientific community should use cautin in prescribing this drug supplementation in both children and adults wit SCD.

All these new therapeutic approaches involve pathophysiology-based targets and are directed to modify natural history of the disease such as the VOC and related organ complications Accordingly a new field is open for combinatioral therapy for SCD that will require an holistic approach considerring the improvement of patient's quality of live (QoL) as an important outcome in designing new clinical studies oriented to a pesonalised medicine.

8.2 Genetic therapy: The most promising new generation treatment for SCD is genetic therapy. This offers a promise of a total cure of the disease and researchers are experimenting with attempts to correcting the defective gene and inserting it into the bone marrow of those with sickle cell to stimulate the production of normal hemoglobin. Recent several experiments have used bioengineering to create mice with a human gene that produces the defective hemoglobin causing sickle cell disease. Bone marrow containing the defective hemoglobin gene was removed from the mice and genetically "corrected" by the addition of the anti-sickling human beta-hemoglobin gene.

The corrected marrow was then transplanted into other mice with sickle cell disease. The genetically corrected mice began producing high levels of normal red blood cells and showed a dramatic reduction in sickled cells. Scientists are hopeful that the techniques can be applied to human gene transplantation using autologous transplantation, in which some of the patient's bone marrow cells would be removed and genetically corrected (60).

Conclusions

Approximately 80% of the annual births of babies with hemoglobinopathies occur in low-or middle-income countries, many of which have extremely limited facilities for their control and management. With migration they are becoming more common worldwide, making their management and care an increasing concern for health care systems. Thalassaemias and SCD are complex lifelong hematological disorders that are complicated over time with multi-organ involvement. The advances in prevention and early intervention have improved their survival and quality of life. The first step is the screening and identification of asymptomatic carriers, so that appropriate genetic counseling and preventive measures can be undertaken, while the second is directed to afflicted symptomatic individuals who require medical attention to prevent complications associated with both the condition and its treatment. Moreover, hemoglobinopathy registries are essential, providing necessary resources, for raising awareness of the diseases among health authorities.

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