



THALASSAEMIA  
INTERNATIONAL  
FEDERATION

**4TH EDITION** (Version 2.0)

# **2021 GUIDELINES**

## FOR THE MANAGEMENT OF TRANSFUSION DEPENDENT THALASSAEMIA (TDT)

PUBLISHERS THALASSAEMIA INTERNATIONAL FEDERATION

### **EDITORS:**

Cappellini, M.D.

Farmakis, D.

Porter, J.

Taher, A.

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***“Cure sometimes, treat often,  
comfort always”***

**Hippocrates (460-357 B.C.)**

***“The good physician  
treats the disease;  
the great physician treats the  
patient who has  
the disease.”***

**Sir William Osler (1849-1919)**

TIF's Board of Directors dedicates this edition to Sir David John Weatherall and Prof. George Stamatoyannopoulos.

**SPECIAL CONTRIBUTION**

Bain Barbara, Imperial College London (language editing)

Eleftheriou Androulla, Thalassaemia International Federation (co-ordination)

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## FOREWORD

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Dear doctors,

On behalf of the Board of Directors of the Thalassaemia International Federation (TIF), it is my great privilege to write the foreword to the 4th edition of the Guidelines for the Management of Transfusion-Dependent Thalassaemia (TDT).

One can today, and this has been so for some time now, safely state that haemoglobin disorders especially thalassaemia – characterised in the very early years and perhaps until the late 1970s as a fatal childhood disease – can be effectively prevented and appropriately managed.

Published data on increased survival, reduced annual number of deaths and morbidity rates coupled with improved quality of life and social integration demonstrated beyond any doubt that the fight to address and effectively control this disorder has nearly been won. Particularly if one includes in the equation the dramatic progress achieved in the last few years in the development and authorisation of two long awaited innovative therapies and in strengthening research of many other drugs today in the pipeline at different stages of development.

Sadly however, such progress has occurred only in countries, mainly with high disease prevalence, where national health and social care systems are providing universal coverage to their populations including importantly patients with chronic diseases, and where robust medical/public health, haematology and paediatric infrastructures existed to support the development of disease-specific services.

Unfortunately however, this picture is seen only in a few countries mainly with developed economies and the naked truth is that of these huge medical and scientific advances happening through the years, only a very small portion of the global patient population benefits and overall this is estimated by TIF to be well less than 20%.

It is evident that countries, mainly with high disease prevalence, need to be further empowered to recognise the value of strengthening disease-specific policies of prevention and health/social care of patients with these disorders. The World Health Organization (WHO) through its disease-specific resolutions – but also a number of other relevant resolutions and programmes, including Blood Safety, Patient Safety, Congenital Anomalies and Birth Defects, Human Genomics in Global Health, Access to medicines and health products just to name a few – can provide considerable support to national efforts. In addition, the work of other regional health fora and initiatives can contribute to national actions; And in this context the European Union's (EU) work on Rare Diseases since the 1990s, is indeed exemplary and many related recommendations as well as directives and regulations give food for thought and provide a basis for others beyond the EU on which to build. As countries become more and more engaged in promoting the UN 2030 SDGs (Sustainable Development Goals), it is hoped that progress in this field, albeit to a different extent across countries and regions of the world, will be achieved.

TIF remains committed to continuing to support, with its work, the national patients'/parents' organisations, the health care professionals' community and the competent authorities. The preparation, publication, translation and free distribution across countries of Guidelines, an important deliverable of its educational programme, has been widely recognised as a significant contribution to the care of patients with these disorders.

Their adoption by competent health authorities and/or medical bodies as published, or as content on which to build national Guidelines, has been a most valuable tool in the education and/or training of treating physicians across the world, and is one of TIF's activities considered to have the highest measurable impact on the health and lives of patients with thalassaemia across the globe.

National Thalassaemia patients/parents associations, members of TIF, across the world have contributed significantly to the work of TIF and have made a true and impressive difference with their voluntary work towards national improvements, complementing and on occasions undertaking Governments' commitments. To this end therefore, TIF poses a humble but clear request to Governments all over the world for their highly needed support.

The engagement and contribution of patient advocates in national, regional and international efforts in promoting progress for the control and care of thalassaemia are indeed invaluable elements. TIF has been fighting since its establishment in 1986 to bring to the forefront the value of the meaningful involvement of patients at the decision-making level. This updated 4th edition of the Guidelines for the Management of Transfusion-Dependent Thalassaemia proudly incorporates an additional chapter dedicated to the value of patient engagement. This chapter aims to cover comprehensively this topic, underscoring the progress that has been achieved through the years in 'making' patients "expert" advocates of their needs as well as the many and multiple benefits of their meaningful involvement in decision making at national, regional and international level.

The huge economic, social and health challenges that have arisen as a result of the recent COVID-19 pandemic, have undoubtedly added to the already difficult and challenging conditions of many countries across the world with regard to the care of these disorders. The significant contradiction here is that parallel to the pandemic tragedy, research and science presented to the global thalassaemia community, patients and health care professionals alike, an invaluable and long awaited gift: the completion of clinical trials and the authorisation by the FDA (Food and Drug Administration) and the EMA (European Medicines Agency) in 2019 and 2020 of two innovative products for the care and cure of thalassaemia. It is thus more than ever essential for every involved stakeholder in the care of patients, Governments, health care professionals and very importantly patients and parents themselves through their associations, to become more actively and productively engaged in the strengthening of those tools, policies and collaborations that would ensure the access of patients to such medical advancements. At the same time and very importantly, all of us jointly should safeguard carefully the existing prevention and disease-specific policies, medical and public health infrastructures including transfusion services, against any regression, threat or risk, in the name of the pandemic, that would jeopardise the quality and/or standard of care and services a country has achieved for these patients through the years.

TIF remains committed to continuing and strengthening its support to the patients it represents through their national associations and its collaboration with decision-making bodies, health care professionals, industry and other relevant stakeholders at national and international level.

My very special and humble request on behalf of the Board of Directors of the Thalassaemia International Federation, through this foreword to every treating professional studying these Guidelines, is to make every effort with his/her national competent authority to support the development and/or strengthening of those policies and programmes that would allow the adoption and implementation at national level of

these valuable and evidenced-based recommendations made by international medical/scientific experts.

In this context, allow me on behalf of the Board, to express my deepest and most sincere gratitude to each one of the key authors, the reviewers/editors and to every contributing author of this book, without the work of whom, these Guidelines as well as any other publication or educational activity of TIF would not have been possible to deliver.

**Finally, taking this opportunity, the Board of Directors of TIF, wishes to dedicate this 4th edition of the Guidelines to those two medical specialists and researchers who are no longer with us and whom we lost in recent years but who, with their work and devotion, have made history and set milestones in this field – to Sir David John Weatherall and to Professor George Stamatoyannopoulos.**

On behalf of the Board of Directors

Panos Englezos  
President





## THE NEED FOR GUIDELINES AND THEIR IMPLEMENTATION

The inherited haemoglobin disorders are the commonest diseases attributable to single defective genes. They fall into two main groups: the structural haemoglobin variants, including sickle cell disease (SCD) and the thalassaemias, which are caused by defective globin production. Carrier numbers of >270 million and more than three hundred thousand children born each year with one of the thalassaemia syndromes or one of the structural haemoglobin variants have been estimated (WHO 1989, 1994, 2008). The extremely high frequency of the haemoglobin disorders compared with other monogenic diseases reflects natural selection mediated by the relative resistance of carriers against *P. falciparum* malaria. Other factors that may be involved include the widespread practice of consanguineous marriage, increased maternal age in the poorer countries, and gene drift and founder effects. For these reasons the thalassaemias are most frequent in Southeastern and Southern Asia, in the Middle East, in Mediterranean countries and in North and Central Africa. However, as the result of mass migrations of populations from high prevalence areas, thalassaemias are now encountered in most countries.

Such countries include the USA, Canada, Australia, South America, the United Kingdom and France, where migration occurred up to a century ago and where large ethnic minority groups are now entering their fourth and even fifth generation.

More recent migration movements from highly endemic countries have been to Northern and Western Europe, where the prevalence of haemoglobin disorders in the indigenous population was very low, including Germany, Belgium, the Netherlands and, more recently, Scandinavia (Kattamis A, Forni GL, Aydinok Y, Viprakasit V. Changing Patterns in the Epidemiology of beta-Thalassemia. *Eur J Haematol* 2020). These changes have challenged health care professionals and policy-makers throughout the region in providing equitable access to quality services for the prevention and treatment of haemoglobin disorders. The epidemiological data available mainly in endemic countries underestimate the future health burden resulting from inherited haemoglobin disorders; effectively addressing the control of these disorders in these countries requires considerable work, financial backing and certainly political commitment. The main difficulty is that the populations of these countries are not homogeneous, as was the case in the Mediterranean countries where the earliest control programmes were successfully established. Programmes to reduce the number of seriously affected individuals follow two approaches: 1. population screening and counselling programmes established to educate populations about the risks of having affected children and, 2. population screening or screening in prenatal clinics where if a woman is a carrier the partner is screened and if positive, following counselling they are offered a prenatal diagnosis and termination of affected fetuses. Prenatal diagnosis programmes well established in the Mediterranean region resulted in a major reduction in newborns with severe forms of thalassaemia. Such programmes are now available in other countries including China, India, Iran, Lebanon, Pakistan, Singapore and Thailand and several other countries are establishing similar programmes. Whatever the results of the screening programmes, they require a proper education of the population about the nature of inherited haemoglobin disorders. This education requires input from many sectors of society, including the media, public health workers, local volunteer societies and the medical community (Weatherall DJ: Disease control priority in developing countries).

Beside prevention, a main objective is to offer to subjects affected by haemoglobin disorders the most efficacious treatment. Studies evaluating thalassaemia major cohorts in both developed and developing countries continue to show a progressive improvement in life expectancy. For this reason there is an urgent need to bridge a wide gap until every

patient in every part of the world has equal access to quality medical care. An essential means of doing so is through global collaboration on haemoglobin disorders, enabling all countries to benefit from each other's experience. Health authorities need to recognise haemoglobin disorders as a significant threat to public health – one that deserves the development and implementation of national policies for treatment and prevention.

**The instruments required to support such policies include:**

- Standardised guidelines for laboratory services
- National guidelines for the management of thalassaemia
- Epidemiological information and surveillance
- Establishment of an educational programme for health care professionals, patients, parents and the community

The full costs of treating patients with inherited disorders of haemoglobin is extremely variable among countries depending on different health care systems, varying methods of obtaining blood, different practices in screening for blood pathogens and different costs of drugs and equipment. It is evident that all countries would benefit from the sharing of experience and expertise in order to harmonise and optimise the quality of treatment as much as possible. The need for management guidelines for transfusion-dependent thalassaemias (TDT) is clear. During the past four years, six major TDT management guidelines have become available for use by thalassaemia care givers (TIF, US, Canadian, UK, Italian and Australian Guidelines). A comparison among those guidelines has been recently published (Musallam KM et al. *Acta Haematologica* 2013). In the light of swiftly evolving evidence, the need for revisiting and updating TDT management recommendations remains crucial. More importantly, ensuring access to such guidelines and careful application and implementation should only help arriving at early diagnosis of morbidity to allow prompt and effective management. It would also allow early prediction of risk and would enable preventive measures to be set in place saving unnecessary health care costs. Moreover in view of novel therapies that are under development or already approved such as gene therapy and molecules targeting ineffective erythropoiesis (Luspatercept) it is mandatory to guarantee to thalassaemia patients all over the world an optimal standard of care in order to face in good clinical status the future scenario of thalassaemia cure.

*This updated fourth edition of the TIF guidelines will offer valuable information to all allied healthcare professionals involved in the treatment of patients with TDT. It includes updated information on new approaches for more effective, safe and less laborious treatment, and an overview of the progress achieved to-date towards a total cure using methods such as gene therapy and stem cell transplantation.*

**Maria Domenica Cappellini**

*Professor of Internal Medicine University of Milan*

## CLASS OF RECOMMENDATION

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# DESIGNATION USED TO INDICATE WHETHER A THERAPY IS RECOMMENDED OR NOT AND THE CERTAINTY SURROUNDING THAT RECOMMENDATION

### Class of Recommendation

- I:** There is evidence and/or general agreement that a given treatment or procedure is beneficial, useful, effective
- II:** There is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of the given treatment or procedure
- IIa:** The weight of evidence is in favour of usefulness/efficacy and therefore should be considered
- IIb:** The usefulness/efficacy is less well established by evidence/opinion and therefore may be considered
- III:** There is evidence or general agreement that the given treatment or procedure is not useful/effective, and in some cases may be harmful

### Level of Evidence

- A:** Data derived from multiple randomized clinical trials or meta-analyses.
- B:** Data derived from a single randomized clinical trial or large non-randomized studies.
- C:** Consensus of opinion of the experts and/or small studies, retrospective studies, registries.



Author: Raffaella Origa | Reviewer: John Porter

## CHAPTER 1

### Genetic basis, pathophysiology and diagnosis of thalassaemias

#### Haemoglobin Types

Oxygen is transported from the lungs to the tissues by a highly specialised protein molecule, haemoglobin, which is located in the red cells of the blood. Each red blood cell contains approximately 300 million molecules of this protein, totalling about 30 picograms in weight per cell. Each molecule of haemoglobin is formed by two pairs of identical sub-units, the globin chains. These chains are named with letters of the Greek alphabet and belong to two groups: the  $\alpha$  globin cluster, comprising the  $\zeta$  and  $\alpha$  globin chains, and the  $\beta$  globin cluster, comprising the globin chains  $\epsilon$ ,  $\gamma$ ,  $\beta$  and  $\delta$ . The globin chains appear sequentially during ontogeny and, after pairing, form the following four major types of haemoglobin:

1. 'Embryonic' haemoglobins, which are detectable from the 3rd to the 10th week of gestation and represent  $\zeta 2\epsilon 2$  (Hb Gower 1),  $\alpha 2\epsilon 2$  (Hb Gower 2),  $\zeta 2\gamma 2$  (Hb Portland 1) and  $\zeta 2\beta 2$  tetramers (Hb Portland 2);
2. 'Fetal' haemoglobin (Hb F), which constitutes the predominant oxygen carrier during pregnancy and is a  $\alpha 2\gamma 2$  molecule;
3. 'Adult' haemoglobin (Hb A  $\alpha 2\beta 2$ ), which replaces Hb F shortly after birth; and
4. A minor adult component, Hb A2 ( $\alpha 2\delta 2$ ).

The process of different haemoglobin species being produced and stopping production at certain periods of human development is known as 'haemoglobin switching' as shown in Figure 1. Under normal conditions, the red cells of the adult human contain approximately 97-98% of Hb A, 2-3% of Hb A2 and traces of Hb F.

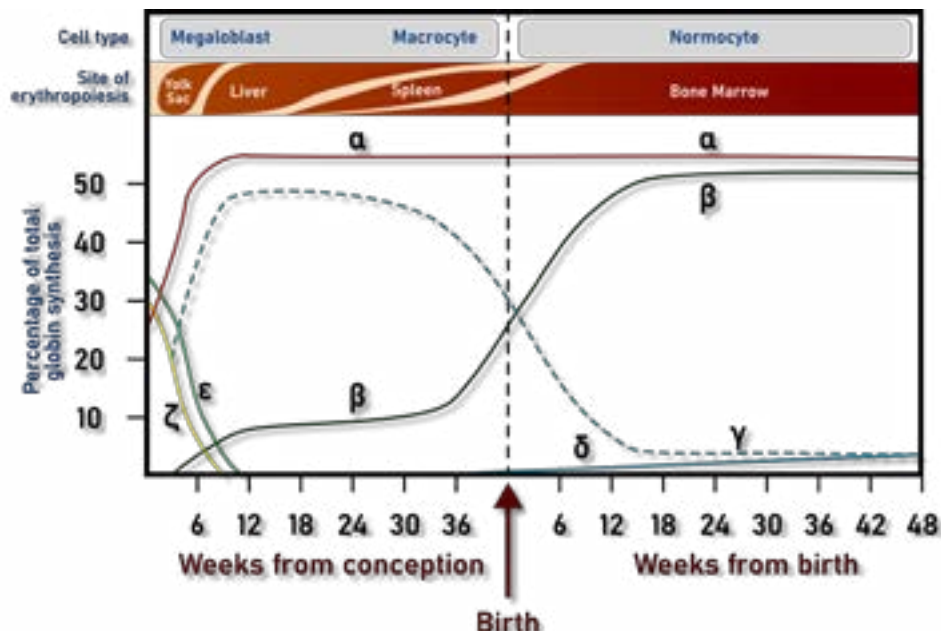


Figure 1. Globin synthesis at various stages of embryonic, fetal and adult erythroid

## Globin genes and globin synthesis

The globin chains have an extremely precise structure, ensuring their prompt loading with oxygen in the lung alveoli and the controlled gradual delivery of the gas into the tissues. The precise structure of the globin chains is coded by genes contained in the DNA of chromosomes 16 (the  $\alpha$  gene cluster) and 11 (the  $\beta$  gene cluster). Flanking the structural genes, i.e. in front (on the 5' side of the DNA sequence, 'upstream') and following them (on the 3' side of the DNA sequence, 'downstream'), lie several nucleotide sequences which have a 'regulatory' role, i.e. they determine which gene is to be turned on and which is turned off, as well as how efficient its expression will be. In adult life, most of the globin synthesis occurs in erythroblasts in the bone marrow. Globin chains must have the correct structure and be trimmed in such a way that the number of  $\alpha$  chains precisely matches that of the  $\beta$  chains. When the above conditions are not met, the result is a complete or partial defect in one or both 'allelic' globin genes.

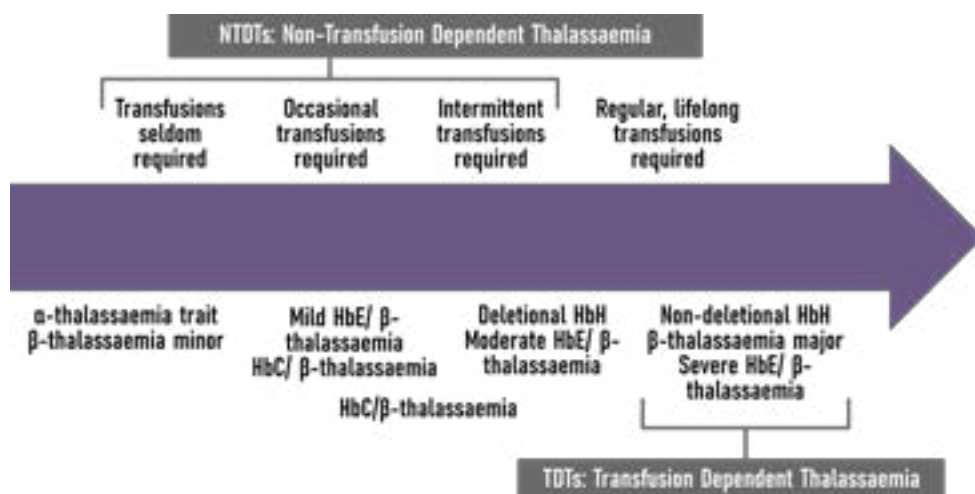
## The thalassaemias

The term '**thalassaemia**' refers to a group of blood diseases characterised by **decreased or absent synthesis of one or more of the normal globin chains**. According to the chain whose synthesis is impaired, the thalassaemias are called  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$ ,  $\delta\beta$  or  $\epsilon\gamma\delta\beta$  thalassaemias. Most thalassaemias are inherited as recessive traits. These primary quantitative defects are no longer rigidly differentiated by the structural variants produced at reduced rate (such as Hb E and Hb Lepore). From a clinical point of view, the most relevant types are  $\alpha$  and  $\beta$  thalassaemias, resulting from the decrease of one of the two types of polypeptide chains ( $\alpha$  or  $\beta$ ) that form the normal adult human haemoglobin molecule (Hb A,  $\alpha_2\beta_2$ ).

The present book mainly addresses the latter group of thalassaemias, which constitute a major problem in the countries around the Mediterranean Sea, the Middle East and Trans-Caucasus, India and the Far East. The highest carrier frequency of  $\beta$  thalassaemia is reported in the Maldives (18%), Cyprus (14%), Sardinia (10.3%) and Southeast Asia (3-5%). The high gene frequency in these regions is most likely related to the selective pressure from *Plasmodium falciparum* malaria. However, population migration and intermarriage between different ethnic groups has introduced thalassaemia in almost every country of the world, including Northern Europe, North and South America, the Caribbean and Australia. As for  $\alpha$  thalassaemia, it is commonly encountered in Southeast Asia and China with up to 40% of the regional population being carriers, and less commonly in India, the Gulf region, the Middle East, Greece, Italy and Northern Europe. In Southeast Asia the frequency is so high as to cause a major public health problem because of the elevated number of patients with severe Hb H disease and of fetuses with Hb Bart's hydrops fetalis.

As autosomal recessive conditions, heterozygotes of either  $\alpha$  or  $\beta$  thalassaemia are usually asymptomatic and require no treatment. Homozygotes and compound heterozygotes for thalassaemia alleles result in thalassaemia syndromes or diseases. In addition, interactions of thalassaemia and corresponding haemoglobinopathies e.g. Hb E, Hb C or Hb S with  $\beta$  thalassaemia or Hb Constant Spring (Hb CS) with  $\alpha$  thalassaemia also give rise to various thalassaemia syndromes. Currently, based on their clinical severity and transfusion requirement, these thalassaemia syndromes can be classified phenotypically into two main groups; 1. Transfusion-Dependent

Thalassaemias (TDTs) and 2. Non-Transfusion-Dependent Thalassaemias (NTDTs) as shown in Figure 2.



**Figure 2.** Phenotypic classification of thalassaemia syndromes based on clinical severity and transfusion requirement.

The TDTs require regular blood transfusion to survive and without adequate transfusion support, they would suffer several complications and a short life span. This category includes patients with  $\beta$  thalassaemia major, severe Hb E/ $\beta$  thalassaemia, transfusion -dependent Hb H disease or Hb H hydrops fetalis and surviving Hb Bart's hydrops fetalis. This TDT group is the main focus of this present clinical practice guideline (CPG). The groups of NTDT patients include  $\beta$  thalassaemia intermedia, Hb E/ $\beta$  thalassaemia and Hb H disease. The CPG for this category of patients has been separately prepared and published recently by TIF (2013 & 2017).

## **$\beta$ thalassaemia**

$\beta$  thalassaemia includes three main forms: thalassaemia major variably referred to as 'Cooley's anaemia' and 'Mediterranean anaemia', thalassaemia intermedia and thalassaemia minor also called ' $\beta$  thalassaemia carrier', ' $\beta$  thalassaemia trait' or 'heterozygous  $\beta$  thalassaemia'. Apart from the rare dominant forms, subjects with  $\beta$  thalassaemia major are homozygotes or compound heterozygotes for  $\beta^0$  or  $\beta^+$  genes, subjects with thalassaemia intermedia are mostly homozygotes or compound heterozygotes and subjects with thalassaemia minor are mostly heterozygotes.

## **Pathophysiology**

The basic defect in  $\beta$  thalassaemia is a reduced or absent production of  $\beta$  globin chains with relative excess of  $\alpha$  chains which accumulate and precipitate in the erythroid precursors forming inclusion bodies that, bound to the membrane skeleton, cause oxidative membrane damage and extensive premature destruction by apoptosis of the red blood cell precursors in the bone marrow (ineffective erythropoiesis). Haemolysis plays a secondary role and is less prominent in thalassaemia major than in thalassaemia intermedia. The first response to ineffective erythropoiesis and anaemia is an increased production of erythropoietin which, in turn, may produce hyper-

plasia of erythroid marrow in medullary and extramedullary sites with characteristic deformities of the skull and face, cortical thinning and pathological fractures of long bones, extramedullary erythropoietic tissue masses and splenomegaly. The lipid membrane composition of abnormal red blood cells may result in thrombotic complications, especially in splenectomised patients. Moreover, untreated or undertreated thalassaemia major patients may have retarded growth as a result of anaemia and the excessive metabolic burden imposed by erythroid expansion. Anaemia may produce cardiac enlargement and sometimes severe cardiac failure. In non-transfused patients, erythropoiesis, anaemia, and hypoxia downregulate production of hepcidin, a 25-amino acid peptide produced by hepatocytes that plays a central role in the regulation of iron homeostasis, with increased intestinal iron absorption and resulting iron overload. The pathophysiology of  $\beta$  thalassaemia is summarised in Figure 3.

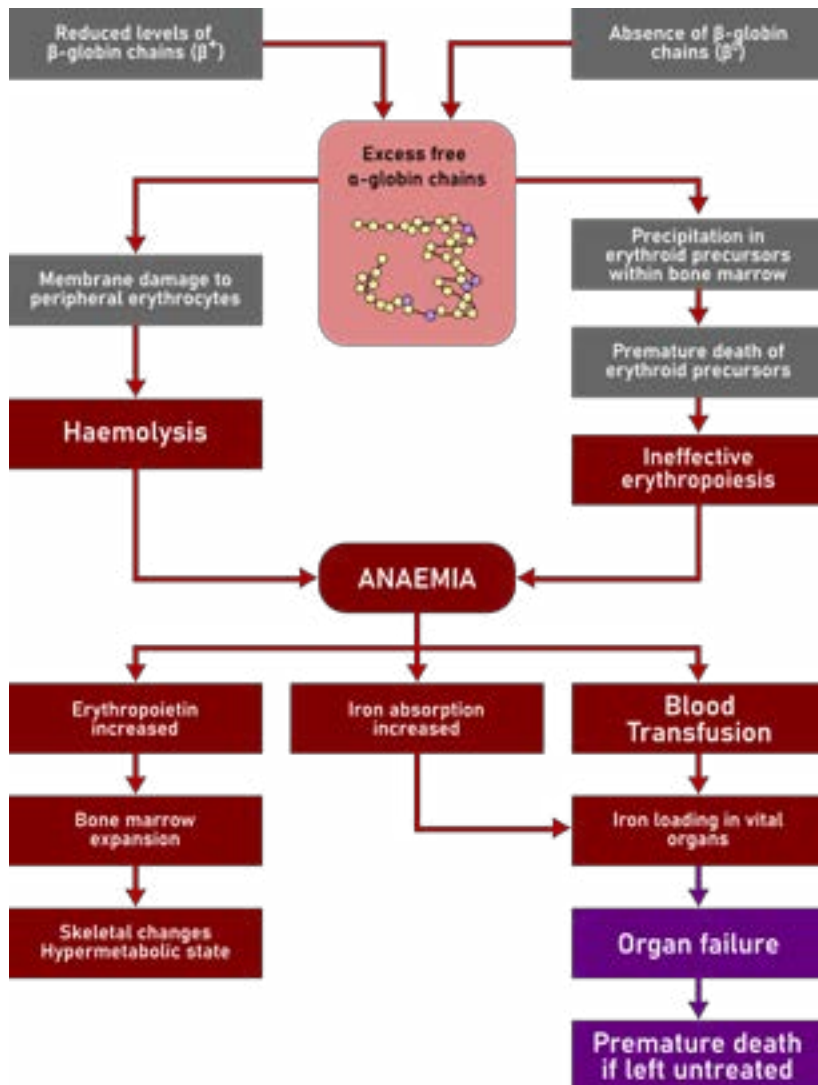


Figure 3. Effects of excess production of free  $\alpha$  globin chains in  $\beta$  thalassaemia.



The degree of globin chain imbalance is determined by the nature of the mutation of the  $\beta$ -gene.  $\beta^0$  refers to the complete absence of production of  $\beta$  globin directed by the affected allele.  $\beta^{++}$  refers to alleles with some residual production of  $\beta$  globin (often around 10%). In  $\beta^+$  the reduction in  $\beta$  globin production is very mild. Almost 300 beta thalassaemia alleles have now been characterised. A complete, current list is available at the Globin Gene Server (<http://globin.cse.psu.edu/>). The largest majority are missense, nonsense or frameshift variants. Rarely, the  $\beta$  thalassaemias are the result of gross gene deletion. Despite marked molecular heterogeneity, the prevalent molecular defects are limited in each at-risk population, in which 4 to 10 variants usually account for most of HBB disease-causing alleles.

Table 1 summarises the most common types of  $\beta$  thalassaemia mutations according to their ethnic distribution and severity.

**Table 1.** Common types of  $\beta$  thalassaemia, their severity and ethnic distribution

Population	B-gene Mutation	HGVS nomenclature	Severity
Indian	-619del	NG_000007.3:g.71609_72227del619	$\beta^0$
Mediterranean	-101	HBB:c.-151C>T	$\beta^{++}$
Black	-88	HBB:c.-138C>T	$\beta^{++}$
Mediterranean, African	-87	HBB:c.-137C>T HBB:c.-137C>G HBB:c.-137C>A	$\beta^{++}$
Japanese	-31	HBB:c.-81A>G	$\beta^{++}$
African	-29	HBB:c.-79A>G	$\beta^{++}$
Southeast Asian	-28	HBB:c.-78A>G	$\beta^{++}$
Black	-26	HBB:c.-76A>C	$\beta^{++}$
Mediterranean	codon 5	HBB:c.17_18delCT	$\beta^0$
Mediterranean, African-American	codon 6	HBB:c.20delA	$\beta^0$
Southeast Asian	Codon 17	HBB:c.52A>T	$\beta^0$
Mediterranean, Asian, Indian	IVS1-nt1	HBB:c.92+1G>A	$\beta^0$
Mediterranean, Asian, Indian	IVS1-nt5	HBB:c.92+5G>A	$\beta^0$
Mediterranean	IVS1-nt6	HBB:c.92+6T>C	$\beta^{+/+}$
Mediterranean	IVS1-nt110	HBB:c.93-21G>A	$\beta^+$
Southeast Asian	HbE	HBB:c.79G>T	$\beta^{++}$
Mediterranean	HbKnossos	HBB:c.82G>T	$\beta^{++}$
Mediterranean	codon 39	HBB:c.118C>T	$\beta^0$
Southeast Asian	codon 41/42	HBB:c.126_129delCTTT	$\beta^0$
Chinese	IVS2-nt654	HBB:c.316-197C>T	$\beta^+$
Mediterranean	IVS2-nt745	HBB:c.316-106C>G	$\beta^+$
African-American	AATAAA to AACAAA	HBB:c.*+110T>C	$\beta^{++}$
Mediterranean	AATAAA to AACGAA	HBB:c.*+111A>G	$\beta^{++}$

HGVS, Human Genome Variation Society.

## Clinical diagnosis

Carriers of thalassaemia are usually clinically asymptomatic but sometimes have mild anaemia. Clinical presentation of  $\beta$  thalassaemia major usually occurs between 6 and 24 months of age with severe microcytic anaemia, mild jaundice and hepatosplenomegaly. Affected infants fail to thrive and become progressively pale. Feeding problems, irritability, recurrent bouts of fever due to a hypermetabolic state or intercurrent infection, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur. In developed countries, if prenatal diagnosis has not been performed, the diagnosis of  $\beta$  thalassaemia major is established at this stage and a regular transfusion program is initiated. The classic clinical picture of  $\beta$  thalassaemia major is currently seen only in some developing countries in which the resources for performing long-term transfusion programmes are not available. The clinical picture in patients who are untreated or poorly transfused, is characterised by growth retardation, pallor, jaundice, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary haematopoiesis, and skeletal changes resulting from expansion of the bone marrow. Skeletal changes include deformities in the long bones of the legs and typical craniofacial changes: thalassaemic facies (bossing of the skull, prominent malar eminence, depression of the bridge of the nose and hypertrophy of the maxillae, which tends to expose the upper teeth). If a chronic transfusion regimen is not started, patients with thalassaemia major usually die within the first few years of life from high-output heart failure.

Individuals with thalassaemia intermedia present later than those with thalassaemia major, have milder anaemia and, by definition, do not require or only occasionally require transfusions. At the severe end of the clinical spectrum, patients are brought to medical attention between the ages of 2 and 6 years with retarded growth and development. At the other end of the spectrum are patients who are completely asymptomatic until adult life with only mild anaemia. Hypertrophy of erythroid marrow with the possibility of extramedullary haematopoiesis (EMH) is common. Its consequences are characteristic deformities of the bone and face, osteoporosis with pathological fractures of long bones, formation of erythropoietic masses that primarily affect the spleen, liver, lymph nodes, chest and spine and a tendency to thrombotic complications. Enlargement of the spleen is also a consequence of its major role in clearing damaged red cells from the bloodstream. Leg ulcers are frequent. Cardiac involvement in thalassaemia intermedia is mainly characterised by a high-output state and pulmonary hypertension, with systolic left ventricle function usually preserved. Myocardial siderosis is rare. Without appropriate treatment, the incidence of the comorbidities increases with advancing age. Iron overload in non-transfused patients mainly occurs because of increased intestinal iron absorption due to greatly expanded but ineffective erythropoiesis. Although the rate of iron loading is slower in thalassaemia intermedia than in thalassaemia major, patients with thalassaemia intermedia can eventually develop complications similar to those of patients with thalassaemia major, including hepatic, endocrine and cardiac dysfunction.

## Haematological diagnosis

Heterozygous carriers of  $\beta$  thalassaemia, usually display a low mean cell haemoglobin (MCH), low mean cell volume (MCV) and an increased level of Hb A<sub>2</sub>, which may be associated with low normal or slightly subnormal haemoglobin levels. A peripheral blood film shows less severe erythrocyte morphological changes than affected individuals and erythroblasts are normally not seen.  $\beta$  thalassaemia major is characterised by reduced haemoglobin level ( $<70$  g/l), MCV  $>50$  fl and  $<70$  fl and MCH  $>12$

and <20 pg. Thalassaemia intermedia is characterised by Hb level between 70 and 100 g/l, MCV between 50 and 80 fl and MCH between 16 and 24 pg. The peripheral blood film in affected individuals demonstrates red blood cell morphological changes with microcytosis, hypochromia, anisocytosis, poikilocytosis and nucleated red blood cells (erythroblasts, NRBC). The number of erythroblasts is related to the degree of anaemia and is markedly increased after splenectomy. In general, these abnormal red blood cell morphological features are shared among different types of thalassaemia syndromes including interactions with haemoglobin variants such as Hb E/ $\beta$  thalassaemia (see below).

## Qualitative and quantitative haemoglobin analysis

Cellulose acetate electrophoresis or capillary electrophoresis (CE) and DE-52 microchromatography or high pressure liquid chromatography (HPLC) identify the amount and type of haemoglobin present. The Hb pattern varies by  $\beta$  thalassaemia type. In  $\beta^0$  thalassaemia homozygotes, Hb A is absent and Hb F constitutes 92-95% of the total Hb. In  $\beta^+$  thalassaemia homozygotes and  $\beta^+/\beta^0$  compound heterozygotes Hb A levels are between 10 and 30% according to the variable degree of reduction of  $\beta$  globin chain synthesis and Hb F is 70-90%. Hb A2 is variable in  $\beta$  thalassaemia homozygotes and it is enhanced in  $\beta$  thalassaemia minor. Hb F can readily be detected by an acid elution test (F-cell staining) and alkali denaturation.

## Molecular analysis

Since the prevalent pathogenic variants are limited in each at-risk population, targeted analysis for pathogenic variants based on ancestry may be considered first. Commonly occurring mutations of the  $\beta$  globin gene are detected by polymerase chain reaction (PCR)-based procedures. The most commonly used methods are reverse dot blot analysis or 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.  $\beta$  globin gene sequence analysis may be considered first if the affected individual is not of an ancestry at high risk or if targeted analysis reveals only one or no pathogenic variant. If the results are inconclusive, gene-targeted deletion/duplication analysis can follow.

## Correlation genotype-phenotype

The extent of globin chain imbalance is the main determinant of clinical severity in  $\beta$  thalassaemia. Therefore, any factor capable of reducing this imbalance results in a lesser degree of  $\alpha$  globin chain precipitation and may ameliorate the clinical picture. One of the most common and consistent mechanisms is homozygosity or compound heterozygosity for two  $\beta^+$  thalassaemia mild or silent mutations. Examples of these alleles are the silent -101 C T and the mild IVS-1-6 T C mutation in the Mediterranean population, the -28 A G in Southeast Asian population and the -29 A G in Africans.

Other factors able to ameliorate the phenotype are the coinheritance of  $\alpha$  thalassaemia or of genetic determinants that increase gamma chain production. Deletional and non-deletional hereditary persistence of fetal haemoglobin (HPFH) mutations, associated with a high Hb F level in carriers, when in genetic compounds with severe  $\beta$  thalassaemia alleles, result in mild thalassaemia intermedia. A mild phenotype may also be determined by co-inheritance of genetic determinants associated with gamma chain production, mapping outside the  $\beta$  globin cluster. Recently, several studies using genome-wide association studies (G-WAS) have identified two quantitative trait loci (BCL11A on chromosome 2p16 and HBS1L-MYB intergenic region on chromosome

6q23) that account for 20%-30% of the common variation in Hb F levels in healthy adults and that are associated with the mild thalassaemia intermedia phenotype and with a delayed need for transfusion in patients with homozygous  $\beta^0$  thalassaemia. Furthermore, BCL11A seems to be involved in the regulation of the haemoglobin switching process.

In some instances, heterozygous  $\beta$  thalassaemia can lead to the thalassaemia intermedia phenotype instead of the asymptomatic carrier state. Most of these patients have excess functional  $\alpha$  globin genes ( $\alpha$  gene triplication or quadruplication) which increases the imbalance in the ratio of  $\alpha$ /non- $\alpha$  globin chain synthesis. In addition, rare mutations that result in the synthesis of extremely unstable  $\beta$  globin variants which precipitate in erythroid precursors causing ineffective erythropoiesis may be associated with thalassaemia intermedia in the heterozygotes (dominant thalassaemia).

Several secondary genetic modifiers able to modify the clinical expression of the thalassaemia syndrome have been identified in recent years. The most studied is the presence of (TA)<sub>7</sub> polymorphism in the promoter region of the uridine diphosphate-glucuronosyltransferase gene, which in the homozygous state is associated with Gilbert syndrome, and is associated with the development of cholelithiasis in thalassaemia major and intermedia. Other candidate genes are the apolipoprotein E  $\epsilon$ 4 allele, which seems to be a genetic risk factor for left ventricular failure in homozygous  $\beta$  thalassaemia. Less defined modifying factors are genes coding for HFE-associated hereditary haemochromatosis and genes involved in bone metabolism.

## **$\beta$ structural haemoglobin variants relevant to thalassaemia management**

### **1. Haemoglobin E disorder is the most common structural variant with thalassaemic properties.**

**Hb E** is characterised by the substitution of lysine for glutamic acid at position 26 of the  $\beta$  globin chain. The mutation G-A at codon 26 of the  $\beta$  globin genes not only produces the amino acid substitution but also activates a cryptic splice site at codon 24-25, leading to an alternative splicing pathway. The overall result is the production of reduced amounts of the variant haemoglobin (Hb E); Hb E constitutes 25-30% of total haemoglobin in Hb E carriers, instead of the expected 50%. In other words, the codon 26 G-A mutation results both in a qualitative and a quantitative  $\beta$  globin gene defect.

Hb E is the most common abnormal haemoglobin in Southeast Asia, with a carrier frequency of up to 50% in some regions. It is also prevalent in parts of the Indian sub-continent, including India, Pakistan, Bangladesh and Sri Lanka. Heterozygotes for Hb E are clinically normal and manifest only minimal changes in red blood cell indices, with the presence of Hb E on haemoglobin analyses. Hb E can be easily detected using a special dye: dichlorophenolindophenol (DCIP). Homozygotes for Hb E are clinically silent and may be only mildly anaemic. The peripheral blood film shows microcytosis with 20-80% of target cells, while Hb electrophoresis or HPLC shows 85-95% Hb E and 5-10% Hb F. However, a few individuals with homozygous Hb E with up to 20% of Hb F have been identified. Genetic compounds for Hb E and  $\beta$  thalassaemia, which are also common in Southeast Asia, have clinical manifestations that are variable in severity – from thalassaemia intermedia to severe transfusion-dependent thalassaemia major. These can be classified into three categories:

- **Mild Hb E/ $\beta$  thalassaemia:** this is observed in about 15% of all cases in Southeast

Asia. This group of patients maintain Hb levels between 90 and 120 g/l and usually do not develop clinically significant problems at an early age. However some patients suffer from growth failure, iron overload and other complications similar to those of NTDT patients.

- **Moderately severe Hb E/ $\beta$  thalassaemia:** the majority of Hb E/ $\beta$  thalassaemia cases fall into this category. The Hb levels remain at 60-70g/l and the clinical symptoms are similar to  $\beta$  thalassaemia intermedia or NTDT. Transfusions are not required unless an infection precipitates further anaemia. Iron overload may occur.

- **Severe Hb E/ $\beta$  thalassaemia:** The Hb level can be as low as 40-50 g/l. Patients in this group manifest symptoms similar to those of  $\beta$  thalassaemia major and are treated as thalassaemia major or TDT patients.

The reasons for this variability have only partially been defined including type of  $\beta$  thalassaemia mutation ( $\beta^+$  or  $\beta^0$  thalassaemia), coinheritance of  $\alpha$  thalassaemia and an innate propensity to produce post natal  $\gamma$  globin expression, and subjects with seemingly identical genotypes may have clinical manifestations very different in severity.

**2. Hb Lepore** is another structural  $\beta$  variant resulting from a fusion of the  $\delta$  and  $\beta$  globin genes. The homozygous state of Hb Lepore or Hb Lepore coinherited with  $\beta$  thalassaemia can result in moderate to severe transfusion-dependent  $\beta$  thalassaemia syndromes.

**3. Haemoglobin S (Hb S) is the most common haemoglobin variant in the world,** resulting from a substitution of valine for glutamic acid at position 6 of the  $\beta$  globin chain. The interaction of  $\beta$  thalassaemia with Hb S results in a syndrome that closely resembles other sickling disorders. The management of sickle/ $\beta$  thalassaemia should follow the existing National Institutes of Health (NIH) guidelines for management of sickle cell disorders (See <http://www.nhlbi.nih.gov/health/prof/blood/sickle/sick-mt.htm> for more information.)

## **$\alpha$ thalassaemia**

$\alpha$ -thalassaemias are inherited disorders characterised by reduced or suppressed production of  $\alpha$  globin chains. The human  $\alpha$  globin genes are duplicated and located at the telomeric end of the short arm of chromosome 16.  $\alpha$  thalassaemia is caused most commonly by deletions of large DNA fragments that involve one or both  $\alpha$  globin genes.

**Silent carrier state:** the presence of a single  $\alpha$  globin gene deletion or deletional  $\alpha^+$  thalassaemia results in the silent carrier state. Heterozygotes for one missing  $\alpha$  globin gene are not anaemic and have normal red blood cell indices. Two major types of this deletional  $\alpha^+$  thalassaemia, 3.7 and 4.2 kb deletions, are wide spread throughout the globe and they have been identified even in the population of the Pacific.

**$\alpha$  thalassaemia trait:** subjects with two residual functional  $\alpha$  genes either by deletions that remove two linked  $\alpha$  globin genes from the same chromosome or  $\alpha^0$  ( $-/-\alpha\alpha$ ) or combination of deletional  $\alpha^+$  thalassaemia ( $-\alpha/-\alpha$ ), have mild hypochromia and microcytosis. Their MCV and MCH are usually lower than 80 fl and 27 pg, respectively. Less commonly, mutations caused by single or a few nucleotide deletions or alterations known as non-deletional  $\alpha$  thalassaemia ( $\alpha\text{T}\alpha/$  or  $\alpha\alpha\text{T}/$ ) have been

identified in several populations from Mediterranean countries to Southeast Asia and China. Haemoglobin Constant Spring (Hb CS) and Hb Paksé, two abnormal Hbs characterised by elongated alpha globin chains resulting from mutations of the termination codon in the alpha2 globin gene, are the most prevalent non-deletional alpha thalassaemias in Southeast Asia. Heterozygotes for non-deletional  $\alpha$  thalassaemia have borderline MCV and MCH therefore they might not be detected in most of the programmes for thalassaemia prevention and control that use red blood cell indices as a screening tool.

**Hb H disease:** When there are deletions or non-deletional abnormalities of three globin genes, the affected individual would have only one functional gene and this hereditary disorder is known as HbH disease. It is usually characterised by a moderate haemolytic anaemia, splenomegaly and acute haemolytic crisis in response to oxidant drugs and infections. In general, patients with non-deletional Hb H disease have more severe disease than patients with deletional Hb H disease. For example, co-inheritance of Hb Constant Spring and the deletion of two  $\alpha$  genes results in a severe form of Hb H disease in which up to 20% of patients require frequent blood transfusion and splenectomy. Most patients with Hb H disease can be managed as recommended in the NTDT guideline.

Rarely, Hb H disease patients with certain non-deletional mutations, specifically Hb Pak Num Po ( $\alpha\alpha$ PNP), Hb Quong Sze ( $\alpha$ QZ $\alpha$ ) or Hb Adana ( $\alpha$ CD59 $\alpha$ ) have a severe phenotype that mimics that of  $\alpha$  thalassaemia major: early onset of anaemia (at birth or within 6 month of birth), marked anaemia (Hb <50 g/l), huge hepatosplenomegaly and failure to thrive. This condition is known as transfusion-dependent Hb H disease or Hb H hydrops fetalis since some patients even develop severe anaemia and hydropic changes in utero.

**Hb Bart's hydrops fetalis**, the most severe clinical manifestation of  $\alpha$  thalassaemia, is generally associated with the absence of all four  $\alpha$  globin genes, severe fetal anaemia and death in utero. In addition, several maternal complications including preeclampsia, ante partum haemorrhage and dystocia are common in pregnant women with Hb Bart's hydrops fetalis. Absence of  $\alpha$  globin genes in 'cis' position in the same chromosome ( $\alpha^0$  thalassaemia, – –/) is common in Southeast Asia and the Far East, while it is rare in the Mediterranean area and very rare in Africa. This different distribution explains why the Hb Bart's hydrops fetalis syndrome and Hb H disease are common in Southeast Asian countries and China, rare in Mediterranean populations and almost absent in the African population. The complete loss of  $\alpha$  globin production from the fetal stage results in formation of a tetramer of unpaired  $\alpha$  globin chains ( $\alpha_4$ ) that is designated Hb Bart's. With advanced fetal medicine including intrauterine transfusion, several affected fetuses with Hb Bart's hydrops have survived their intrauterine ordeals. The number of these surviving Hb Bart's hydrops is increasing as the practice of IVT increases. However, these individuals will remain transfusion dependent and require life-long blood transfusion. Therefore patients with Hb Bart's or Hb H hydrops or transfusion-dependent Hb H disease must be treated as TDT patients and follow the recommendations of these present guidelines.

## Pathophysiology

Reduction of  $\alpha$  globin synthesis results in decreased production of Hb A ( $\alpha_2\beta_2$ ) and reduced haemoglobin synthesis. In addition, excess unpaired  $\beta$  globin chains can form tetramers ( $\beta_4$ ) that are not physically stable and precipitate, attaching to the red cell surface membrane and causing oxidative damage, thus shortening

of red cell survival. The formation of  $\beta$  globin tetramers (Hb H) can be detected by haemoglobin analysis. The presence of Hb H increases during acute febrile illness due to increase body temperature. In non-deletional  $\alpha$  thalassaemia and in particular in the case of mutations that generate  $\alpha$  globin variants such as Hb CS, Hb H can directly precipitate at the membrane surface and generate reactive oxygen species even in the steady state. Therefore, patients with non-deletional Hb H disease are usually have more severe disease than those with deletional Hb H disease. Thalassaemia intermedia and Hb H disease may have similar degrees of anaemia, but haemolysis rather than ineffective erythropoiesis is the primary mechanism in Hb H disease. Indeed, iron loading is much more common in thalassaemia intermedia than in Hb H disease.

### Haematological diagnosis

Similarly to  $\beta$  thalassaemia syndromes, patients with Hb H disease have a hypochromic microcytic anaemia with a baseline of haemoglobin of 40-130 g/l. Increased polychromasia and reticulocytosis are observed and can be further augmented during acute infectious episode or haemolytic crises. Nucleated red blood cells and basophilic stippling are commonly present in more severe phenotypes such as Hb Bart's hydrops and severe non-deletional Hb H disease. Detection of Hb H as Hb H inclusion bodies in a peripheral blood film using supravital staining (brilliant cresyl blue) is the hallmark of this condition.

### Qualitative and quantitative haemoglobin analysis

Identification of fast moving haemoglobin species by electrophoresis representing Hb H ( $\beta_4$ ) and Bart's ( $\gamma_4$ ) is characteristic of  $\alpha$  thalassaemia syndromes. The levels of Hb H measured can vary from < 1% up to 40% (usual range 10-15%) due to sensitivity of tests, laboratory expertise, type of instruments and the quality of blood samples. Hb H might not be readily identified through some platforms of liquid chromatography; a manual identification using the presence of haemoglobin species at a specific retention time (RT) is required. Due to a lack of available  $\alpha$  globin chains, Hb A2 ( $\alpha_2\delta_2$ ) is reduced. In patients with non-deletional Hb H disease especially Hb H/Hb CS, Hb CS variant can be detected at a very low level (1-4%).

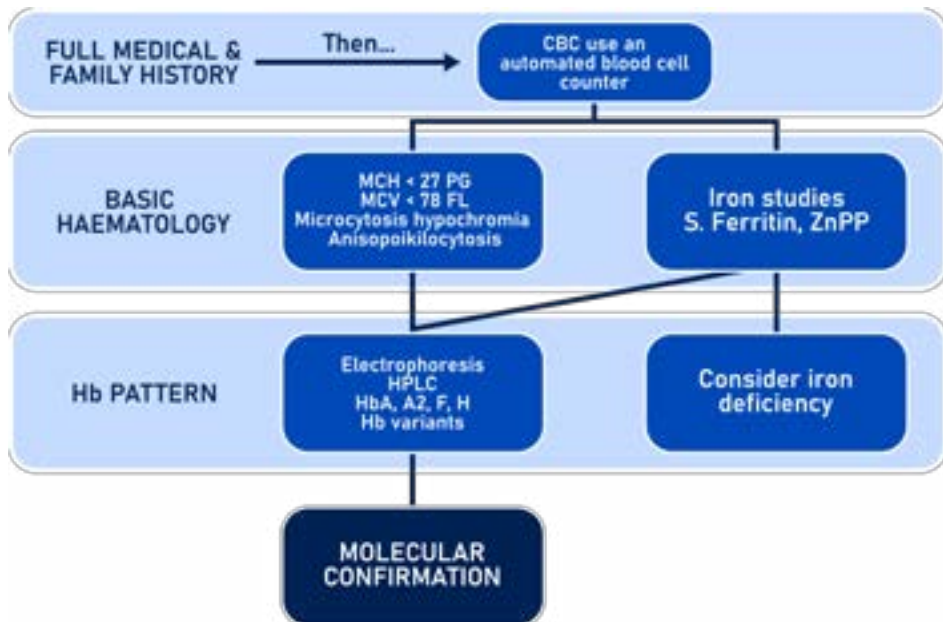
### Molecular diagnosis

Molecular testing approaches can include targeted deletion analysis for common deletions, sequence analysis, and deletion analysis of the  $\alpha 1$  and  $\alpha 2$  globin genes and the HS -40 regulatory region (LCRA). Targeted deletion analysis for specific deletions within the  $\alpha$  globin gene cluster can be performed first. The method commonly used to target a deletion is GAP-PCR. The most commonly used methods for known non-deletional mutations are reverse dot blot analysis, primer-specific amplification or PCR following by enzymatic digestion. Sequence analysis of the  $\alpha 1$  and  $\alpha 2$  globin genes can be performed next if a common deletion is not identified. Typically, exon or whole-gene deletions/duplications are not detected. Deletion analysis of  $\alpha 1$  and  $\alpha 2$  globin genes and the HS -40 regulatory region located 40 kb upstream from the  $\alpha$  globin cluster can be performed next to detect uncommon deletions associated with  $\alpha$  thalassaemia if pathogenic variants have not been identified by targeted deletion testing or sequence analysis. Methods that may be used to detect rare or unknown deletions include: Southern blotting (now fallen into abeyance), quantitative PCR, long-range PCR and, above all, multiplex ligation-dependent probe amplification (MLPA). The same methods may be used to detect duplications of the  $\alpha$  cluster. Further testing for genes associated with genetic disorders similar to  $\alpha$  thalassaemia, such as ATRX and HBB



may also be considered if clinically indicated.

Since almost all thalassaemic conditions as aforementioned, present with hypochromic microcytic anaemia, a diagnosis of thalassaemia should be considered in all those who have such abnormal red blood cell features. However, it is important to exclude iron deficiency anaemia as a possible cause since it remains common in several parts of the world. A summary of diagnostic measures for patients with **hypochromia and microcytosis** and further with diagnostic features of common thalassaemia syndromes are shown in Figures 4, 5 and 6.

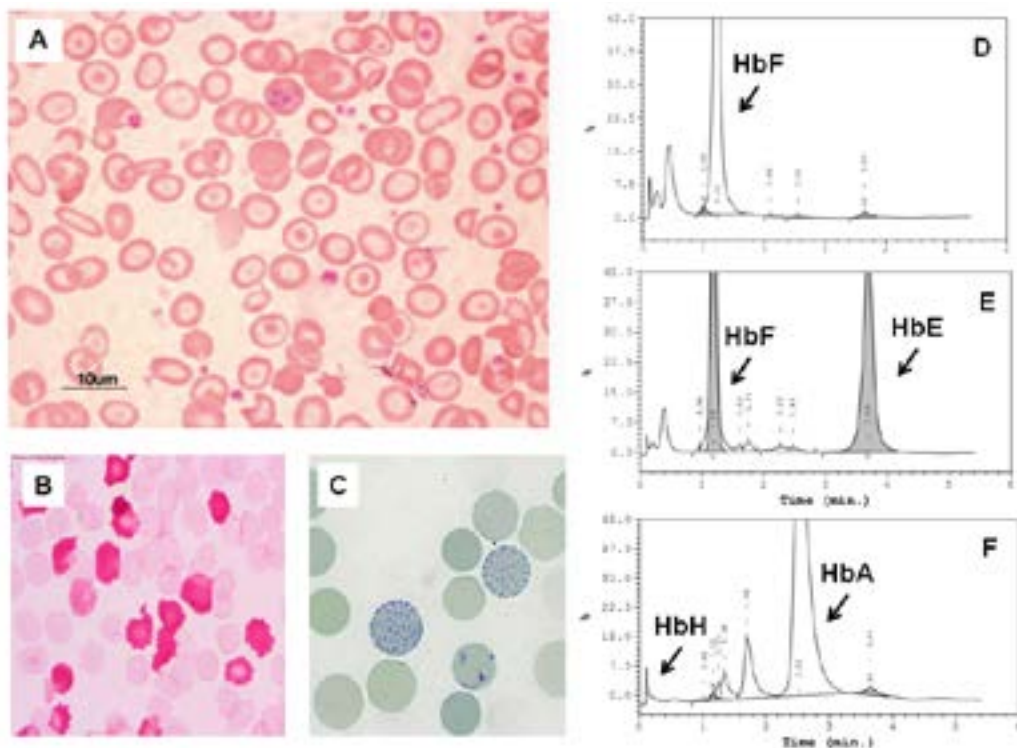


**Figure 4.** Flow chart for the diagnosis of haemoglobin disorders; steps in carrier screening and disease diagnosis. (MCH, mean cell haemoglobin; MCV, mean cell volume; ZnPP, zinc protoporphyrin).



		$\beta$ -TM	$\beta$ -TI	HbE/ $\beta$ -Thal	HbH
Hb levels		< 5 g/dL	- 7 g/dL	Mild 9 - 12 g/dL Moderately Severe 6 - 7 g/dL Severe 4 - 5 g/dL	2.6 - 13.3 g/dL
BLOOD SMEAR	Low Hb Production	Red cell hypochromia microcytosis, target cells			
	Haemolysis	Irregularly crenated RBC, increased reticulocytes [5 - 10%]			
	Ineffective erythropoiesis	Nucleated RBC, Basophilic strippling			
	Special Features	+ Numerous F-cells/ Acid elution	+ F-cells/ Acid elution	+ DCIP staining [HbE] + F-cells/ Acid Elution	HbH inclusion bodies
Haemoglobin study		HbF up to 100% HbA2 $\Delta$	HbF 10 - 50% [up to 100%] HbA2 $\Delta$ 4%	HbE [40 - 60%] HbF [60 - 40%] + HbA [with $\beta$ -thal] HbA2 $\Delta$	Variants HbH [0.8 - 40%] HbA2 $\Delta$ the presence of $\alpha$ -variants e.g. Hb CS, Hb PS etc.
DNA analysis		<ul style="list-style-type: none"><li>Commonly known mutations of both <math>\beta^0</math> and <math>\beta</math>-thal mutations in population specific set can be done by PCR based methods.</li><li>For rare or unusual mutations, direct sequencing or array analysis is required.</li><li>Other analysis for <math>\beta</math>-TI included <math>\alpha</math>- and <math>\beta</math>-globin rearrangements, Xmn I polymorphism and other QTLs for <math>\gamma</math>-globin expression.</li></ul>			Gap-PCR developed for 7 common $\alpha$ -thal deletions and ROB for non-deletional mutations. For unknown mutations, MLPA analysis and sequencing required.

Figure 5. Summary of diagnostic measures for thalassaemia and haemoglobinopathies. MLPA, multiplex ligation-dependent probe amplification; QTL, quantitative locus; TI, thalassaemia intermedia; TM, thalassaemia major



**Figure 6.** Haematological features and haemoglobin profiles in common thalassaemia syndromes. (A) Peripheral blood film from a patient with a thalassaemia syndrome showing marked hypochromic microcytosis with anisopoikilocytosis, target cells and polychromasia. (B) Positive F-cell staining test in  $\beta$  thalassaemia. (C) Positive Hb H inclusion test in  $\alpha$  thalassaemia. Liquid chromatography showed haemoglobin profiles in  $\beta$  thalassaemia major (D), Hb E/ $\beta$  thalassaemia (E) and Hb H disease (F).

## Summary

The term 'thalassaemia' refers to a group of autosomal recessive blood diseases characterised by decreased or absent synthesis of normal globin chains. From a clinical point of view, the most relevant types are  $\alpha$  and  $\beta$  thalassaemias, resulting from decreased synthesis of one of the two types of polypeptide chains ( $\alpha$  or  $\beta$ ) that form the normal adult human haemoglobin.

The basic defect in  $\beta$  thalassaemia is a reduced or absent production of  $\beta$  globin chains with a relative excess of  $\alpha$  chains.  $\alpha$  globin chains accumulate and precipitate in the erythroid precursors forming inclusion bodies that cause oxidative membrane damage and ineffective erythropoiesis.

Reduction of  $\alpha$  globin synthesis results in a decreased production of Hb A ( $\alpha_2\beta_2$ ) and reduced haemoglobinisation. In addition, excess unpaired  $\beta$  globin chains can form tetramers that are not physically stable and precipitate and attach to the red cell surface membrane causing oxidative damage and shortening of red cell survival. Individuals with  $\beta$  thalassaemia major have severe anaemia and hepatosplenomegaly; they usually come to medical attention within the first two years of life. Without treatment, affected children have severe failure to thrive and shortened life expectancy. Individuals with  $\beta$  thalassaemia intermedia present later and have milder anaemia that only rarely requires transfusion. Hb H disease (deletions or abnormalities of three  $\alpha$  globin genes) is usually characterised by a moderate haemolytic anaemia, splenomegaly and acute haemolytic crisis. Hb Bart's hydrops fetalis, the most severe clinical manifestation of  $\alpha$  thalassaemia, is generally associated with the absence of all four  $\alpha$  globin genes, severe fetal anaemia and death in utero.

The diagnosis of thalassaemias relies on measuring red blood cell indices and haemoglobin analysis and assessing the clinical severity of anaemia. Molecular genetic testing is available in clinical laboratories and may be useful for predicting the clinical phenotype in some cases as well as enabling presymptomatic diagnosis of at-risk family members and prenatal diagnosis.

## Recommendations

- Diagnosis of thalassaemia should be considered in all those who have hypochromic microcytic anaemia (grade A)
- In the diagnostic work-up for hypochromic microcytosis, iron deficiency anaemia should be always be excluded (grade A)
- Molecular analysis is not required to confirm the diagnosis of a  $\beta$  carrier, but it is necessary to confirm the  $\alpha$  thalassaemia carrier status (grade A)
- An  $\alpha$  gene triplication or quadruplication should be taken into consideration in heterozygous  $\beta$  thalassaemia subjects with a thalassaemia intermedia phenotype (grade A)
- The haematological parameters including red cell indices and morphology, followed by separation and measurement of Hb fractions are the basis for the identification of  $\beta$  thalassaemia carriers (grade A)
- Detection of Hb H as Hb H inclusion body in a peripheral blood film using supravital staining (brilliant cresyl blue) is the hallmark of this condition (grade A)
- Since the prevalent pathogenic variants of the  $\beta$  globin gene are limited in each at-risk population, a PCR method designed to detect the common specific mutation simultaneously should be used initially (grade B)
- $\beta$  globin gene sequence analysis may be considered first if the affected individual is not of an ancestry at high risk or if targeted analysis reveals only one or no pathogenic variant (grade B)
- $\alpha$  thalassaemias are mainly due to deletions of different length and they can be detected preferentially by reverse dot blot and Gap-PCR (grade B)
- Methods that may be used to detect rare or unknown deletions include: Southern blotting (now fallen into abeyance), quantitative PCR, long-range PCR and, above all, MLPA (grade B)

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## CHAPTER 2

### Blood transfusion

This chapter will address the important considerations related to transfusion therapy in patients with transfusion dependent thalassaemia:

- Aims of blood transfusion
- Haemovigilance
- Blood Donation
- Blood component specification
- Compatibility testing and alloimmunisation
- Criteria for initiating transfusion therapy
- Transfusion thresholds and frequency
- Volume to be transfused
- Transfusion and the spleen
- Adverse reactions

#### Aims of blood transfusion

The aim of blood transfusion in thalassaemia is to deliver a safe and effective transfusion regimen whilst minimising the burden of transfusion therapy on everyday life.

An effective transfusion regimen will result in:

- Good growth and development
- Good energy levels
- **Sufficient suppression of intra and extramedullary haematopoiesis**

A safe transfusion regimen will

- Use a product that is collected, tested, selected, issued and administered adherent to established quality and safety regulations and guidance
- Be administered by staff trained in blood transfusion
- Involve informed patient consent
- Be performed in a system with a good haemovigilance structure

#### Haemovigilance

**“Haemovigilance is the set of surveillance procedures covering the entire blood transfusion chain, from the donation and processing of blood and its components, through to their provision and transfusion to patients, and including their follow-up”.**

It includes the monitoring, reporting, investigation and analysis of adverse events related to the donation, processing and transfusion of blood, and taking action to prevent their occurrence or recurrence. The reporting systems play a fundamental role in enhancing patient safety by learning from failures and then putting in place system changes to prevent them in future.

The haemovigilance system should involve all relevant stakeholders and should be coordinated between the blood transfusion service, hospital clinical staff and transfusion laboratories, hospital transfusion committees, the national regulatory agency and national health authorities.

The resulting modifications to transfusion policies, standards and guidelines, as well as improvements to processes in blood services and transfusion practices in hospitals, lead to improved patient safety” (WHO, 2021b).

Good haemovigilance is key to the delivery of safe and effective transfusion in any setting and must be in place in the delivery of blood transfusion to those with thalassaemia.

## Blood donation

To safeguard the health of patients with thalassaemia, **blood should be obtained from carefully selected voluntary, non-remunerated donors** and should be collected, processed, stored and distributed, by dedicated blood transfusion centres with established quality assurance systems in place.

Adherence to the directives from the European Union (EU), World Health Organisation (WHO), American Association of Blood Banks (AABB) or other international groups, with additional consideration of national needs, resources and prevalence of infectious agents, should safeguard the quality of blood transfusion services particularly to prevent transfusion transmitted infections (TTI). Clearly patients who have repeated donor exposure are at greater risk of such infection. Blood donation practices, donor selection (e.g., through questionnaires) and specific product screening for hepatitis B, hepatitis C, HIV, syphilis and, in some countries, other infectious diseases such as HTLV I/ II, malaria, toxoplasma, Hepatitis A, Hepatitis E, West Nile virus and Chagas disease constitute some of the most important strategies that contribute to the safety and adequacy of blood. For more information on EU directives visit <https://www.edqm.eu/en/blood-transfusion-mission.html> while additional WHO guidelines and American Standards are available at <https://www.who.int/bloodsafety/gcbs/structure/en/> and <https://www.aabb.org/standards-accreditation/standards>.

## Blood component specification

### *Leucodepletion*

Reduction to  $1 \times 10^6$ /l or less leucocytes per unit is considered the critical threshold for eliminating adverse reactions attributed to contaminating white cells (Table 1) (Klein, Spahn & Carson, 2007) and in countries where variant Creutzfeld Jakob Disease was prevalent (e.g. the United Kingdom), it is universally used for all cellular products to decrease the risk of transmission **through blood products**.

**Table 1.** Adverse effects of leucocytes in blood products.

REACTION	CAUSATIVE AGENT
Febrile non-haemolytic transfusion	HLA-antibodies in patients, cytokine reactions produced by donor leucocytes
HLA- alloimmunisation of recipients	HLA- alloimmunisation of recipients HLA
Transfusion-transmitted infections	Cell-associated infectious agents such as cytomegalovirus



### **Methods for leucodepletion include:**

- Pre-storage filtration of whole blood is the preferred method for leuco-reduction. This method of leucocyte removal offers high efficiency filtration and provides consistently low residual leucocytes in the processed red cells and high red cell recovery. Packed red cells are obtained by centrifugation of the leucodepleted whole blood.
- Pre-transfusion, laboratory filtration refers to the filtration at the blood bank laboratory of packed red cells, prepared from donor whole blood.
- Bedside filtration refers to the packed red cell unit that is filtered at the bedside at the time of transfusion. This method may not allow optimal quality control because the techniques used for bedside filtration may be highly variable.

### **Blood Products for Special Patient Populations**

**Washed red cells** may be beneficial for patients with thalassaemia who have repeated severe allergic transfusion reactions or for patients with immunoglobulin A (IgA) deficiency, in which the recipient's pre-formed antibody to IgA may result in an anaphylactic reaction. Washing of the donor product removes plasma proteins that constitute the target of antibodies in the recipient. Washing may be accomplished using manual or automated techniques. Washed red cells that are not suspended in storage solution must be transfused within 24 hours, and this shorter shelf-life creates the possibility of wastage if patients are not available for transfusion at the time the blood is prepared. Suspension in SAGM after washing allows for shelf life as long as 14 days if a closed circuit is used.

Washing alone usually does not result in adequate leucocyte reduction and should not be used as a substitute for leuco-reduction. Instead, washing should be used in conjunction with filtration. In addition, washing of red cell units removes some erythrocytes from the transfusion product, and it is therefore valuable to monitor post-transfusion haemoglobin levels to ensure attainment of the targeted haemoglobin level.

**Cryopreserved (frozen) red cells** is the component derived from whole blood in which red cells are frozen, preferably within 7 days of collection and using a cryopreservant, and can be stored at  $-60^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  in an electrical freezer, when a high-glycerol method is used or alternatively at  $-140^{\circ}\text{C}$  to  $-150^{\circ}\text{C}$  if stored in vapour phase liquid nitrogen, when a low-glycerol method is used.

This product is used to maintain a supply of rare donor units for patients who have unusual red cell antibodies or who are missing common red cell antigens. Their shelf life of 1-7 days depends on whether they were washed in an open or closed system and whether they were re-suspended in SAGM. The shorter shelf life again creates the possibility of wastage. Approximately 20% of the donor cells are lost in the washing after the freezing process. There is no good evidence about how long these can be stored though - in NHS Blood and Transplant they are now kept for 30 years.

**Red cells obtained by donor apheresis** refers to the collection of two units of red cells from the same donor for transfusion of one patient. The reduction of donor exposures may decrease the risk of transmission of infections and developing alloantibodies and other transfusion-related complications. This approach creates significant logistical problems as the donors need higher haematocrits, can

attend less regularly for donation and the collections are performed using more invasive apheresis techniques. In addition, the collection of two separate bags may create an organizational challenge in ensuring that both units go to the same donor.

**Neocyte transfusions** may modestly reduce blood requirements by using only the younger fraction of red cells from the donor units (Spanos et al., 1996). However, patients are exposed to a higher number of donors, with a consequent increase in cost, risk of transmission of infections, and risk of developing alloantibodies.

Additional selection or processing of products may be necessary in certain clinical situations e.g. CMV negative products for pregnant women, irradiation if issues with T-cell function e.g. Hodgkin Lymphoma.

### ***Storage of Donor Red Cell Units***

The anticoagulant preservative solutions used in blood collection (Table 2) have been developed to prevent coagulation and to permit storage of red cells without loss of metabolic integrity. All of these solutions contain sodium citrate, citric acid and glucose, and some of them also contain adenine, guanosine and phosphate (e.g., CPD-A). As shown in Table 2, the introduction of additives such as AS-1, AS-3 and AS-5 permits storage of red cells for up to 42 days.

**Table 2.** Storage time for anticoagulant- preservative solutions with and without additive solution

Solution type	Shelf-Life (days)
CPD	21
CP2D	21
CPDA-1	35
CPD, CP2D or CPDA-1 with AS-1 (Adsol), AS-3 (Nutricell), AS-5	35-42

The maximum duration of storage, as noted on each unit varies with the type of preparation. However, all of the storage solutions should achieve a mean 24-hour post-transfusion survival of no less than 75% of the transfused red cells. The actual half-life of donor red cells after transfusion is not routinely tested for different additives and for different lengths of storage.

The haemoglobin oxygen release function which is extremely important in thalassaemia major is impaired during normal storage due to progressive loss of 2, 3-biphosphoglycerate (2, 3-BPG, previously known as 2, 3- diphosphoglycerate, DPG). However, the rapid repletion of 2,3-BPG after transfusion generally compensates for the loss of function during storage.

In TDT, decreased recovery and a shortened red cell half-life may increase transfusion requirements and as a consequence the rate of transfusional iron overload; **the current practice is to use red cells stored in additive solutions for less than two weeks where this is available and does not lead to an unacceptable delay in transfusion.**

## Compatibility Testing

Development of one or more specific red cell antibodies (alloimmunisation) is an important complication of chronic transfusion therapy (Thompson et al., 2011; Singer et al., 2000; Spanos et al., 1990). However, the prevalence of alloantibodies varies widely among centers and may be related to the homogeneity of the population, strategies for antigen matching and other factors. It is important to monitor patients carefully for the development of new antibodies. Anti-E, anti-C and anti-Kell alloantibodies are the most common. However, 5-10% of patients develop alloantibodies against other erythrocyte antigens or develop warm or cold antibodies of unidentified specificity.

It is recommended that:

- **Before embarking on transfusion therapy, patients should have extended red cell antigen typing that includes at least A, B, O, C, c, D, E, e, and Kell, (though preferably a full red cell phenotype/genotype panel)**
- **If the patient is already transfused, antigen typing can be performed using molecular rather than serological testing.**
- **All patients with thalassaemia should be transfused with ABO and Rh (C, c, D, E, e) and Kell compatible blood to avoid alloimmunisation against these antigens.**
- **There should be a valid group and antibody screen available prior to transfusion being administered**

It may be appropriate to extend antigen matching in line with specific local population requirements (Cheng, Lee & Lin, 2012).

Most blood banks currently perform a screen for new antibodies and an IAT (indirect antiglobulin test) crossmatch before each transfusion. Where patients are not pregnant and have no history of alloimmunisation, a newer approach in which the initial approach known as an electronic issue may be used. Here, in eligible patients with a negative antibody screen, blood is issued without an IAT crossmatch being performed. This is only appropriate in blood banks that adhere to strict regulations regarding computer systems, sample labelling and other critical issues (Milkins et al., 2013). Using either approach, new clinically significant antibodies must be identified so that blood lacking the corresponding antigen(s) is selected.

**A complete and detailed record of antigen typing, current and historical red cell antibodies and transfusion reactions should be maintained for each patient, and should be readily available if the patient is transfused at a different centre. Transfusion of blood from first-degree relatives should be avoided because of the risk of developing antibodies that might adversely affect the outcome of a later stem cell transplant and the risks of transfusion associated graft versus host disease.**

The length of time between the sample acquisition and antibody screen and the transfusion of blood for regularly transfused patients is usually **72 hours but may be as long as one week in centres** with full Rh and Kell antigen matching in patients who are regularly transfused (Trompeter et al., 2015). In patients who are irregularly transfused or start transfusion later in life (Table 3) the risk of allomunisation may be greater (Spanos et al., 1990; Michail-Merianou et al., 1987, see Table 3), so that the interval between sample for antibody screen and transfusion should preferably not be longer than 72h.

**Table 3.** Age and alloimmunisation in thalassaemia.

AGE AT FIRST TRANSFUSION (YRS)	ALLOIMMUNISATION RATE (%)	REFERENCE
<1	7.7	(Michail-Merianou et al., 1987)
<1	27.9	
<3	20.9	(Spanos et al., 1990)
<3	47.5	

### Criteria for initiating transfusion therapy

For deciding whom to transfuse, the following should be included in the investigations:

- **Confirmed diagnosis of thalassaemia.**
- **Laboratory criteria:**
  - Haemoglobin level (Hb) <70 g/l on 2 occasions, > 2 weeks apart (excluding all other contributory causes such as infections) AND/OR
- **Clinical criteria irrespective of haemoglobin level:**
  - > Significant symptoms of anaemia
  - > Poor growth / failure to thrive
  - > Complications from excessive intramedullary haematopoiesis such as pathological fractures and facial changes
  - > Clinically significant extramedullary haematopoiesis

The decision to initiate a long-term transfusion regimen should be based on a definitive diagnosis of transfusion dependent thalassaemia. This diagnosis should consider the molecular defect, the severity of anaemia on repeated measurements, the level of ineffective erythropoiesis, and clinical criteria such as failure to thrive or significant symptoms or bone changes. It must be established that the severity of anaemia is not transient to issues such as an infection, in which case a one-off transfusion may be sufficient.

The initiation of regular transfusion therapy for severe thalassaemia genotypes usually occurs in the first two years of life and is in this setting usually due to severe anaemia or significant anaemia with accompanying symptoms such as not being able to feed or failure to thrive. Some patients with milder forms of thalassaemia who only need sporadic transfusions in the first two decades of life may later need regular transfusions because of a falling haemoglobin level or the development of serious complications.

### Transfusion thresholds and frequency

The recommended treatment for transfusion dependent thalassaemia is lifelong regular blood transfusions, usually administered every two to four weeks, to maintain the **pre-transfusion haemoglobin level 95-105 g/l**. This transfusion regimen promotes normal growth, allows normal physical activities, adequately suppresses bone marrow activity in most patients, and minimizes transfusion-

al iron accumulation (Cazzola et al., 1997, 1995). **A higher target pre-transfusion haemoglobin level of 110-120 g/l** may be appropriate for patients with heart disease, clinically significant extramedullary haematopoiesis or other medical conditions, and for those patients who do not achieve adequate suppression of bone marrow activity at the lower haemoglobin level. Sometimes back pain occurs prior to blood transfusion and may also respond to a higher pre-transfusion haemoglobin level. Although shorter intervals between transfusions may reduce overall blood requirements, the choice of interval must consider other factors such as the patient's school or work schedule and other lifestyle issues.

The schedule outlined above has been shown to minimize iron loading while suppressing bone marrow expansion in Italian patients with thalassaemia major (Cazzola et al., 1997, 1995). The optimal regimen with other transfusion dependent phenotypes such as E-Beta thalassaemia has not been formally studied and may not be the same, as there is some evidence that lower haemoglobin values may be tolerated in patients with E-Beta thalassaemia. However, in the absence of prospective data to show that low transfusion regimens achieve the same outcomes in such patients, the same approach as for other patients is currently recommended.

### Volume to be transfused

It is difficult to make clear recommendations regarding the volume of blood to be infused as the number of red cells per unit and the haematocrit will differ depending on local policies. These relate to the acceptable haematocrit of the donors, the volume collected at donation, whether it is whole or packed red cells and the type of anticoagulant used (see Table 4). To limit donor exposure, a certain number of units (e.g. one or two) rather than a particular volume of blood is ordered. Younger children may require a fraction of a unit to avoid under- or over- transfusion. For such children or for others who may need a specific volume, the following calculations is generally be used:

**(i) (Desired – actual Hb (g/l)) x weight (kg) x 0.3 = ml to be transfused assuming the haematocrit of the unit is 0.58 (Davies et al., 2007)**

The right regimen is one in which the haematological targets are met and that achieve the clinical aims of the transfusion regimen.

**Table 4.** Guidelines for choosing how much blood to transfuse.

		HAEMATOCRIT OF DONOR RED CELLS			
Target increase in haemoglobin level		50%	60%	75%	80%
	20 g/l	12 ml/kg	10 ml/kg	8 ml/kg	7.5 ml/kg
	30 g/l	18 ml/kg	15 ml/kg	12 ml/kg	11.2 ml/kg
	40 g/l	24 ml/kg	20 ml/kg	16 ml/kg	15 ml/kg

As an example, using the above Guidelines (Table 4), in order to raise haemoglobin level by 4g/l in a patient weighing 40 kg and receiving blood with a haematocrit of 60%, 800 ml would be required. This calculation assumes a blood volume of 70 ml/kg body weight.

Post transfusion haemoglobin can be measured when evaluating the effects of changes in the transfusion regimen, the degree of hypersplenism, or unexplained changes in response to transfusion.

**To achieve pre-transfusion haemoglobin of 95-105 g/l it is often usual to aim for a post transfusion haemoglobin of 130-150g/l. This overall approach to transfusion has been shown to promote normal growth, to allow normal physical activities, to adequately suppress bone marrow activity and to minimize transfusional iron accumulation in most patients (Cazzola et al., 1997).**

Although erythrocytapheresis, or automated red cell exchange, has been shown to reduce net red cells infused, and thus the rate of transfusional iron loading (Friedman et al., 2003; Berdoukas, Kwan & Sansotta, 1986), its use may be limited due to a two- to three-fold increase in donor blood utilization and donor exposure resulting in increased costs, and increased risk of transmission. Thalassaemia settings audits have not shown to-date the benefit seen in sickle cell disease. Lastly, there are financial constraints with such a procedure and logistic issues surrounding the need for suitable venous access.

## Rate of transfusion

The rate of transfusion has not been subjected to a prospective study and again will depend on the component issued. Leucodepletion decreases systemic reactions, packed cells are smaller volume. The British Society of Haematology Guidelines state that for adults, a unit of blood (here, packed red cells of a mean volume of 260ml) can be **infused over 90 minutes, however, the clinical state of the patient needs to be ascertained to see whether this is suitable. However, an ongoing study in two London thalassaemia centres suggests that in very carefully selected adults >45kg, free of cardiac disease and receiving up to 3 units of mean volume of 260ml can be administered at the rate of one unit per hour. Particular caution should be taken with smaller patients, particularly children, patients with cardiac failure or very low initial haemoglobin levels.**

There are international guidelines regarding the keeping of transfusion records (e.g. EU directives). Historically for patients with thalassaemia additional records would be kept. These included the volume or weight of the administered units, the haematocrit of the units or the average haematocrit of units with similar anticoagulant-preservative solutions, and the patient's weight. With this information, it was possible to calculate the annual blood requirements as volume of transfused blood or pure red cells (haematocrit 100%) per kg of body weight. The latter (pure red cells per kg of body weight) when multiplied by 1.08, the estimated amount of iron per ml of RBC (see Chapter 3: Iron Overload and Chapter 4: Iron Chelation), yields an approximate value for the amount of transfusional iron that the patient receives per kilogram of body weight in a year. Figure 1 shows a detailed example of how the daily rate of iron loading (mg/kg/day) is calculated. The rate of transfusional iron loading may be very important in choosing the appropriate dose of an iron chelator among other indicators of iron overload. For example, the recommended dose of the chelator deferasirox is based in part on the daily or annual rate of transfusional iron loading. Nowadays, this level of calculation is not often done, although may be useful in situations where there has been a change in blood requirement, development of hypersplenism or where access to accurate MRI measurements of iron loading is poor.

Patient weight: 40 kg  
 Transfusion amount and schedule: 600 ml every 4 weeks  
 Average haematocrit of transfused red cells: 60%

Annual blood requirement:  $13 \text{ transfusions} \times 600 \text{ ml}/40 \text{ kg} = 195 \text{ ml/kg}$   
 Annual pure red cell requirement:  $195 \text{ ml/kg/yr} \times 60\%$   
 (average haematocrit) =  $117 \text{ ml/kg/yr}$

Annual transfusional iron loading:  
 $117 \text{ ml/kg/yr of pure red cells} \times 1.08 \text{ mg iron per ml pure red cells} = 126 \text{ mg iron}$   
 Daily transfusional iron loading:  $126 \text{ mg iron/yr}/365 \text{ days} = 0.34 \text{ mg/kg}$

Figure 1. Calculation of annual blood requirements and transfusional iron loading.

## Transfusion and the spleen

The transfusion requirements in unsplenectomised patients are generally higher than splenectomised patients. In a study of thalassaemia major patients who required more than 250 ml of packed red cells/kg/yr, splenectomy decreased the annual iron loading by an average of 39% (Graziano et al., 1981).

Average transfusion requirements are about 30% higher in unsplenectomised (0.43 mg/kg/day) than splenectomised thalassaemia major patients (0.33 mg/kg/day) (Cohen, Glimm & Porter, 2008). With modern chelation regimes, this is seldom a justification for splenectomy unless blood transfusion rates increase into unmanageable ranges, in the context of an enlarging spleen. Hypertransfusion decreases the rate of splenic enlargement (O'Brien, Pearson & Spencer, 1972) and the introduction of a hypertransfusion regimen may diminish the extent to which the spleen contributes to an increased blood transfusion requirement (Modell, 1977) thus preventing the need for a splenectomy.

Specific thresholds of annual transfusion requirements that would lead to consideration of splenectomy are difficult to establish because earlier studies did not specify the haematocrit levels of the transfused blood and because the potential reduction in transfusional iron loading after splenectomy must be weighed against the long-term consequences of asplenia including sepsis, thrombosis and pulmonary hypertension. With access and tolerance to good chelation regimens, splenectomy is not often needed for iron control. Nevertheless, **as the annual transfusion requirements rise above 200 ml/kg/year of pure red cells, splenectomy may be considered** as one of several strategies to reduce transfusion requirements.

## Adverse Reactions

Blood transfusion exposes the patient to a variety of risks and adverse events including immune (see Table 5) and non-immune related. Thus, it is vital to continue to improve blood safety and to find ways of reducing transfusion requirements and the

number of donor exposures. Equally **adverse events reporting should be embedded within the haemovigilance framework**. The Serious Hazards of Transfusion (SHOT) yearly reports are an excellent resource for those interested in adverse events, and are frequently accompanied by a chapter on haemoglobinopathies (SHOT, 2021).

**Non-haemolytic febrile transfusion reactions** were common in past decades, but have been dramatically reduced by leuco-reduction, especially pre-storage leuco-reduction, which sharply reduces cytokine accumulation and leucocyte alloimmunization. In the absence of effective leuco-reduction, patients experiencing such reactions should be given antipyretics before their transfusions. Since fever may accompany a haemolytic transfusion reaction or the administration of a unit with bacterial contamination, these other causes should always be considered in a patient who develops fever during administration of red cells.

**Allergic reactions** are usually due to plasma proteins and range from mild to severe. Milder reactions include urticaria, itching and flushing, and they are generally mediated by IgE. More severe reactions, such as stridor, bronchospasm, hypotension or other symptoms of anaphylaxis may occur, especially in patients with IgA deficiency and anti-IgA antibodies. Allergic reactions have been reported in patients who receive units of blood from donors who have been exposed to something that the patient is allergic to e.g. a donor eating strawberries donating blood to someone who is allergic to strawberries.

Occasional mild allergic reactions often can be prevented using antihistamines or corticosteroids before transfusion. Recurrent allergic reactions can be markedly reduced by washing the red cells to remove the plasma. Patients with IgA deficiency and severe allergic reactions may require blood from IgA -deficient donors.

**Acute haemolytic reactions** begin within minutes or sometimes hours of initiating a transfusion and are characterized by the abrupt onset of fever, chills, lower back pain, a sense of impending death, dyspnoea, haemoglobinuria and shock. These unusual reactions most commonly arise from errors in patient identification or blood typing and compatibility testing. The risk of receiving the wrong blood is greater for a patient with thalassaemia who travels to another centre or is admitted to a hospital not familiar with his/her case and medical history. Haemolytic reactions in these patients can still be avoided by (1) the use of optimal methods for identifying the patients and labeling of the sample when blood is obtained for crossmatch, (2) proper linkage of the sample to the donor unit in the blood bank, (3) adherence to standard protocols for screening for antibodies and carrying out the necessary compatibility protocols and, (4) use of multiple patient identifiers before transfusing the blood. In many transfusion units, two staff members check the identification of the unit and the recipient prior to beginning the transfusion although this has been replaced in resource rich countries by electronic forms of patient identification. If signs and symptoms suggest an acute haemolytic reaction, the transfusion should be stopped immediately and intravenous fluids should be administered to maintain intravascular volume. Diuretics may help to preserve renal function. Disseminated intravascular coagulation (DIC) may require additional measures such as heparin. The identification of the patient and the donor unit should be re-checked. The blood bank should also be alerted to the possibility of an undetected alloantibody.

**Alloimmunisation**, as described above, is a common complication of transfusion therapy, occurring in as many as 10-20% of patients with thalassaemia. Alloimmunisation is more common in children who begin transfusion therapy



after 1-3 years of age than in those who begin transfusion therapy earlier. This may reflect the fact that such people are often transfused in an emergency (therefore often not at the hospital where they are known to have thalassaemia and therefore inadequately matched) or when immune activated i.e. when they are unwell. Some evidence also suggests that new alloantibodies develop more frequently after splenectomy (Thompson et al., 2011). The use of extended antigen matched donor blood is effective in reducing the rate of alloimmunisation.

**Delayed transfusion reactions** usually occur 5-14 days after transfusion and are characterized by unexpected levels of anaemia, as well as malaise and jaundice. These reactions may be due to an alloantibody that was not detectable at the time of transfusion or to the development of a new antibody. A sample should be sent to the blood bank to investigate the presence of a new antibody and to repeat cross-matching of the last administered unit(s).

**Autoimmune haemolytic anaemia** is a very serious complication of transfusion therapy that usually but not always occurs in patients with alloantibodies (Ameen et al., 2003) although may be unrelated to transfusion. Even red cells from seemingly compatible units (i.e., those units that do not contain the antigen to which there is a known alloantibody) may demonstrate markedly shortened survival, and the haemoglobin concentration may fall well below the usual pre-transfusion level because of destruction of both the donor's and the recipient's red cells. The serologic evaluation by the blood bank usually shows an antibody that reacts with a wide range of test cells and fails to show specificity for a particular antigen. Steroids, immunosuppressive drugs and intravenous immunoglobulins are used for the clinical management of this complication (British Society for Haematology, 2016; Hill et al., 2017; Jäger et al., 2020).

Autoimmune haemolytic anaemia occurs more frequently in patients who begin transfusion therapy later in life (Rebulla & Modell, 1991), and this complication should be carefully considered before instituting transfusion therapy for teenagers and adults with thalassaemia intermedia.

**Transfusion-related acute lung injury (TRALI)** is a potentially severe complication that is usually caused by specific anti-neutrophil or anti-HLA antibodies that activate the patient's neutrophils, but may also be due to non-antibody related accumulation of pro-inflammatory mediators during storage of donor red cells (Vlaar & Juffermans, 2013; Swanson et al., 2006). This complication is characterized by dyspnoea, tachycardia, fever and hypotension during or within six hours of transfusion. Hypoxemia is present and the chest radiograph shows bilateral infiltrates typical of pulmonary oedema although there is no reason to suspect volume overload. Management includes oxygen, administration of steroids and diuretics, and, when needed, assisted ventilation.

**Transfusion-associated graft versus host disease (TA-GVHD)** is caused by viable lymphocytes in donor red cell units. It is a rare but often fatal complication of transfusion. Immunosuppressed patients are at particular risk, but TA-GVHD may also occur in immunocompetent recipients of red cells from a haploidentical donor such as a family member. TA-GVHD usually occurs within 1-4 weeks of transfusion and is characterized by fever, rash, liver dysfunction, diarrhoea and pancytopenia due to bone marrow failure. To reduce the risk of TA-GVHD, donated blood from a family member should be avoided or if used should always be irradiated before transfusion. Leucodepletion alone is inadequate for the prevention of this complication.

**Transfusion-associated circulatory overload (TACO)** may occur in the presence of recognized or unrecognized cardiac dysfunction, or when the rate of transfusion is inappropriately fast. Signs and symptoms include dyspnoea and tachycardia, and the chest radiograph shows the classic findings of pulmonary oedema. Treatment focuses on volume reduction and cardiac support, as required.

**Transfusion transmitted infections (TTI)** including viruses, bacteria and parasites, are a major risk in blood transfusion (see Chapter 9: Infections). Blood traceability rules are important and enshrined in law in several countries so that “look back” can occur when blood is contaminated (ref EU directive). Even in countries where residual risk of transmission through blood transfusion of clinically significant pathogens (HIV, HBV, HCV and syphilis) has been reduced to minimal levels, problems continue to exist or emerge because:

- Laboratory tests may fail to identify viruses during the window period or because of imperfect sensitivity.
- The clinical significance of newly identified infectious agents is not always completely clarified and donors are not screened for these agents.
- Newly emerging infectious agents such as coronaviruses, hepatitis E, highly virulent influenza strains and prions may constitute serious threats.
- There is currently no evidence that SARS-COV-2 is transmitted by blood transfusions, however, donor deferral due to recent illness or the logistics of donation during a pandemic has affected blood stocks in many countries.
- Absence of widely accepted or routinely used tests for bacterial, viral and other pathogens (e.g., *Yersinia enterocolitica*, hepatitis A, toxoplasmosis, malaria and babesiosis).

Although the standard of care to prevent TTI is through donor specific questionnaire and sample screening, there is growing interest in the use of pathogen inactivation/reduction technologies. These have enjoyed greater development in platelet and plasma products and there are ongoing studies in the use of such technologies for red cell products (please see Chapter 9: Infectious disease).

In many regions of the developing world in which thalassaemia is most common, continued transmission of hepatitis B, hepatitis C and HIV underscores the importance of promoting the quality of national blood transfusion services, including voluntary blood donations, careful donor selection and donor blood screening, and the consistent use of immunizations such as hepatitis B vaccine.

**Table 5.** Broad categorization of immune-mediated transfusion reactions and reported frequencies (Spanos et al., 1990).

ACUTE	FREQUENCY	DELAYED	FREQUENCY
HTR	1/25,000	Alloimmune	1/100
Anaphylactic	1/50,000	NHFTR	1/2,500
Febrile non-haemolytic	1/100	TAGVHD	Rare
Allergic (Urticarial)	1/100		
TRALI	1/10,000		

## Summary and Recommendations

- Confirm the diagnosis of thalassaemia and appropriate clinical and laboratory assessment for transfusion (IIA).
- Blood transfusion requires informed consent.
- Haemovigilance and adverse events reporting are key to the safety of blood transfusion.
- Use careful donor selection and screening, favoring voluntary, regular, non-remunerated blood donors (IIA).
- Before first transfusion, perform extended red cell antigen typing of patients at least for D, C,c, E,e and Kell (IIA) and if available a full red cell pheno/genotype.
- At each transfusion, give ABO, Rh(D) compatible blood. Choosing units compatible units for ABO, C,c, E,e and Kell antigens is highly recommended (IIA).
- Before each transfusion, perform a screen for new antibodies and an IAT cross-match, or in centres that meet regulatory requirements, perform an electronic cross-match where allowed (IA).
- Use leucodepleted packed red cells. Pre-storage filtration is strongly recommended, but blood bank pre-transfusion filtration is acceptable. Bedside filtration is only acceptable if there is no capacity for pre-storage filtration or blood bank pre-transfusion filtration (IA).
- Use washed red cells for patients who have severe allergic reactions (IIA).
- Transfuse red cells stored in CPD-A within one week of collection and red cells stored in additive solutions within less than two weeks of collection where available (IA).
- Transfuse every 2-4 weeks, maintaining pre-transfusion haemoglobin above 95-105 g/l or up to 110-120 g/l for patients with cardiac complications (IA).
- Keep a record of red cell antibodies, transfusion reactions and annual transfusion requirements for each patient (IIA).
- Keep the post-transfusion haemoglobin below 130-150 g/l (IIA).

**European Blood Directive 2002/98/EC, Commission Directive 2004/33/EC, Commission Directive 2005/61/EC, Commission Directive 2005/62/EC, 2009/135/EC, 2011/38/EU, 2014/110/EU, 2016/1214**

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## CHAPTER 3

### Iron overload: Pathophysiology, diagnosis and monitoring

Iron overload occurs when iron intake is increased over a sustained period of time, either as a result of red blood cell transfusions or increased absorption of iron through the gastrointestinal (GI) tract. Both of these occur in thalassaemias, with blood transfusion therapy being the major cause of iron overload in thalassaemia major and increased GI absorption being more important in non-transfusion dependent thalassaemia (NTDT). When thalassaemia major patients receive regular blood transfusion, iron overload is inevitable because the human body lacks a mechanism to excrete excess iron. Iron accumulation is toxic to many tissues, causing heart failure, cirrhosis, liver cancer, growth retardation and multiple endocrine abnormalities.

Chelation therapy aims to balance the rate of iron accumulation from blood transfusion by increasing iron excretion in urine and /or faeces with chelators. If chelation has been delayed or has been inadequate, it will be necessary to excrete iron at a rate that exceeds this. Because iron is also required for essential physiological purposes, a key challenge of chelation therapy is to balance the benefits of chelation therapy with the unwanted effects of excessive chelation. Careful dose adjustment is necessary to avoid excess chelation as iron levels fall. The second major challenge in chelation therapy is to achieve regular adherence to treatment regimens throughout life, as even short periods of treatment interruption can have damaging effects. While the convenience and tolerability of individual chelators is important in achieving this goal, other factors such as psychological well-being and family and institutional support also impact on adherence and outcomes.

### The Rate of Iron Loading

#### *Blood transfusion*

Gaining the most accurate information on the rate of iron loading from transfusion therapy is important in assisting selection of the best chelation therapy for each patient. **A unit processed from 420 ml of donor blood contains approximately 200 mg of iron, or 0.47 mg/ ml of whole donor blood.** For red cell preparations with variable haematocrits, **the iron in mg/ml of blood can therefore be estimated from 1.16 x the haematocrit** of the transfused blood product. In cases where organisational systems or other difficulties prevent such estimations from being calculated, a rough approximation can be made based on the assumption that 200 mg of iron is contained in each donor unit. Irrespective of whether the blood used is packed, semi-packed or diluted in additive solution, if the whole unit is given, this will approximate to 200 mg of iron intake. According to the recommended transfusion scheme for thalassaemia major, the equivalent of 100–200 ml of pure red blood cell (RBC) per kg body weight per year are transfused. This is equivalent to 116–232 mg of iron/ kg body weight/year, or 0.32–0.64 mg/kg/day. Regular blood transfusion therapy therefore increases iron stores to many times the norm unless chelation treatment is provided. If chelation therapy is not given, Table 1 shows how iron will accumulate in the body each year, or each day.

Table 1. Iron loading rates in the absence of chelation.

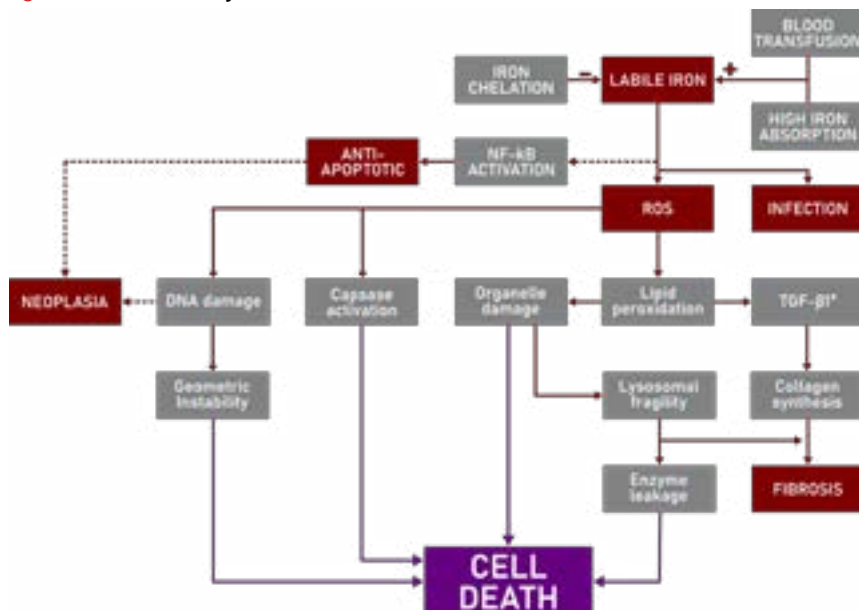
PATIENTS WEIGHT	20 kg	35 kg	50 kg	65 kg
Pure red cell volume ml/year	2,000-4,000	3,500-7,000	5,000-10,000	6,500-13,000
Yearly iron loading (g)	2.3-4.6	4.1-8.2	5.8-11.6	7.5-15.1
Daily Iron loading (mg)	6.3-12.6	11.2-22.5	15.9-31.8	20.5-41.4

**Increased gastrointestinal absorption of iron**

In transfusion-dependent thalassaemia (TDT), the contribution of iron absorbed from the diet is small compared with blood transfusion. Normal intestinal iron absorption is about 1-2 mg/day. In patients with thalassaemia who do not receive any transfusion, iron absorption increases several-fold. It has been estimated that iron absorption exceeds iron loss when expansion of red cell precursors in the bone marrow exceeds five times that of healthy individuals. **Transfusion regimens aimed at keeping the pre-transfusion haemoglobin above 90 g/l (95-105 g/l) have been shown to prevent such expansion (Cazzola et al., 1997). In individuals who are poorly transfused, absorption rises to 3-5 mg/day or more, representing an additional 1-2 g of iron loading per year.**

**Toxicity from Iron Overload****Mechanisms of iron toxicity**

Iron is highly reactive and easily alternates between two states – iron<sup>3+</sup> and iron<sup>2+</sup> – in a process which results in the gain and loss of electrons, and the generation of harmful free radicals (atoms or molecules with unpaired electrons). These can damage lipid membranes, organelles and DNA, causing cell death and the generation of fibrosis. In health, iron is ‘kept safe’ by binding to molecules such as transferrin, but in iron overload the capacity to bind iron is exceeded both within cells and in the plasma compartment. The resulting ‘free iron’, either within cells or in plasma, damages many tissues in the body or is fatal unless treated by iron chelation therapy. Free iron also increases the risk of infections and neoplasia.

**Figure 1.** A summary of the mechanisms for toxic effects of iron overload

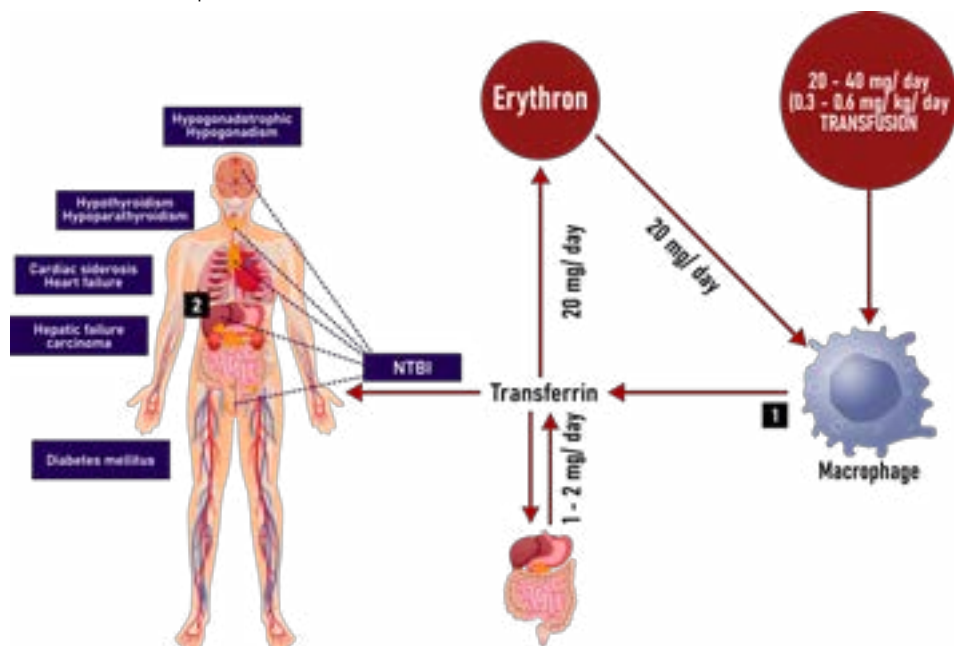
*Pathological mechanisms and consequences of iron overload. In iron overload resulting from repeated blood transfusions or long-term increased iron absorption, iron that is not bound to naturally occurring molecules such as transferrin, or ferritin or to therapeutic iron chelators, generates a variety of reactive oxygen species (ROS), most notably hydroxyl radicals. This occurs in cells where labile plasma iron is taken up and accumulates as storage iron (ferritin and haemosiderin). ROS generate lipid peroxidation and organelle and DNA damage and dysregulate mechanisms involved in apoptotic cell death, increasing the risk of neoplasia such as hepatoma. Labile iron is also more available to microorganisms that iron bound to transferrin or ferritin, thereby increasing the risk of infection.*

*Reproduced with permission from (Porter 2014). [ROS, Reactive Oxygen Species. \*TGF-β1, transforming growth factor β1].*

### **Distribution and consequences of transfusional iron overload**

In the absence of iron overload, uptake of iron into cells is controlled by the interaction of transferrin with its receptors – mainly on red cell precursors, hepatocytes and dividing cells. In iron overload, transferrin becomes saturated and iron species that are not bound to transferrin are present in plasma (plasma non-transferrin bound iron, or NTBI). The distribution of NTBI uptake is fundamentally different from transferrin uptake, and is thought to involve calcium channels. Organ damage in transfusional iron overload reflects the pattern of tissue iron uptake from NTBI. Some tissue are spared from iron loading through this mechanism (such as skeletal muscle), while others such myocardial muscle, endocrine tissue and hepatocytes take up NTBI rapidly. This iron is then stored as ferritin or haemosiderin which are visible by magnetic resonance imaging (MRI). The myocardial iron overload can induce heart failure from cardiomyopathy in patients without chelation in as early as the second decade of life. Iron overload also causes

pituitary damage, leading to hypogonadism, growth retardation and delayed puberty. Endocrine complications, namely diabetes mellitus, hypothyroidism and hypoparathyroidism are also seen. Liver disease with fibrosis and eventually cirrhosis and hepatocellular carcinoma, particularly if concomitant chronic viral hepatitis is present, are also serious complications.



**Figure 2.** The main routes of iron turnover and uptake are shown by solid black arrows on the right panel: 20 mg of iron is delivered daily to the erythron in health. This increases several fold in untransfused thalassaemias but can be inhibited by hypertransfusion. Non-transferrin bound iron (NTBI) is generated when transferrin (which is about 30% saturated in healthy adults) becomes saturated. Transferrin saturation occurs following iron overload of the macrophage system, but also as a result of decreased clearance of transferrin iron in hypertransfused patients. The organs in which NTBI is taken up and retained as storage iron are shown on the left, with >80% cleared by hepatocytes. Despite variable and lower quantities of iron taken into other tissues (represented by broken lines), serious and often irreversible iron-mediated damage may occur. Iron excretion by chelation therapy acts mainly at sites (1) the interception of iron released from macrophages after red cell catabolism, and (2) iron released by the catabolism of ferritin within hepatocytes.

## Monitoring of Iron Overload

Monitoring is essential in establishing effective iron chelation regimes, tailored to individuals' specific needs. However, some general principles of monitoring iron overload apply to all.

### Serum ferritin

#### Why measure serum ferritin?

Serum ferritin (SF) generally correlates with body iron stores, and is relatively easy and inexpensive to determine repeatedly. Serum ferritin is most useful in

identifying trends. A decreasing trend in SF is good evidence of decreasing body iron burden but absence of a decreasing trend does not exclude a decreasing iron burden. However, an increasing SF trend implies an increasing iron burden but may also be due to inflammation or tissue damage, so clinical judgment must be used to interpret these trends.

Long term control of SF is also a useful guide to the risk of complications from iron overload in thalassaemia major (TM); many studies have shown an association between the control of serum ferritin and prognosis (Borgna-Pignatti et al., 2004; Davis et al., 2004; Gabutti & Piga, 1996; Olivieri et al., 1994). Studies have identified a significantly lower risk of cardiac disease and death in at least two-thirds of cases where serum ferritin levels have been maintained below 2,500 µg/l (with deferoxamine – desferrioxamine, DFO) over a period of a decade or more (Olivieri et al., 1994). Observations with larger patient numbers show that maintenance of an even lower serum ferritin of 1,000 µg/l may be associated with additional clinical advantages (Borgna-Pignatti et al., 2004) (see Table 2).

### ***What are the limitations of serum ferritin measurements?***

Most SF assays were developed mainly for detecting iron deficiency, and the linear range of the assay at high SF values needs to be known. SF must be performed in a laboratory that has established how to dilute samples with high values, to give readings within the linear range of the assay. SF measures do not always predict body iron or trends in body iron accurately. In TM, variation in body iron stores accounts for only 57% of the variability in serum ferritin (Brittenham et al., 1993). This variability is in part because inflammation increases serum ferritin, and partly because the distribution of liver iron between macrophages (Kupffer cells) and hepatocytes in the liver has a major impact on serum ferritin. **A sudden increase in serum ferritin should prompt a search for hepatitis, other infections, or inflammatory conditions. A lack of fall in SF with chelation does not therefore necessarily prove that the patient is a 'non responder' to the chelation regime.**

As outlined above, this can be because inflammation may have falsely raised SF, or because the relationship between body iron and SF is not always linear, particularly in the context of inflammation or tissue damage (Adamkiewicz et al., 2009), and body iron can fall considerably from a high starting point (e.g. liver iron concentration >30 mg/g dry weight) before a change in ferritin is clear. Below 3000 µg/l SF values are influenced mainly by iron stores in the macrophage system, whereas above 3000 µg/l they are determined increasingly by ferritin leakage from hepatocytes (Davis et al., 2004; Worwood et al., 1980). Day-to-day variations are particularly marked at these levels. The relationship between serum ferritin and body iron stores may also vary depending on the chelator used (Ang et al., 2010) and by duration of chelation therapy (Fischer et al., 2003).

**Table 2.** Use of serum ferritin for monitoring chelation treatment.

ADVANTAGES	DISADVANTAGES
Easy to assess repeatedly	Indirect estimate of iron burden
Inexpensive	Increased by inflammation
Trend identification possible with repeat samples	Cannot determine iron balance directly
Long term control linked to outcome	Non-linear response to iron load at high
Useful for dose adjustment as iron levels fall	Absence of decrease doesn't exclude response
	Relationship to iron load varies with chelator
	Relationship to LIC differs in different diseases

*LIC, liver iron concentration.*

### **Liver iron concentration (LIC) measurement**

#### **Why monitoring liver iron concentration?**

- *To identify whether body iron is adequately controlled.*

Adequate control of LIC is linked to the risk of hepatic damage as well as the risk of extrahepatic damage. **Normal LIC values are up to 1.8 mg/g dry weight (wt), with levels of up to 7 mg/g dry wt seen in some non-thalassaemic populations without apparent adverse effects. Sustained high LIC (above 15-20 mg/g dry wt) have been linked to worsening prognosis, liver fibrosis progression (Angelucci et al., 1997) or liver function abnormalities (Jensen et al., 2003).** In the absence of prior iron chelation therapy, the risk of myocardial iron loading increases with the number of blood units transfused and hence with iron overload (Jensen et al., 2003; Buja & Roberts, 1971). However, the relationship between LIC and extra-hepatic iron is complicated by chelation therapy as iron tends to be accumulated initially in the liver and later in the heart but also is removed more rapidly from the liver than the heart by chelation therapy (Noetzli et al., 2008; Anderson et al., 2004). Thus, in patients receiving chelation therapy, whilst high LIC increases the risk of cardiac iron overload, the measurement of LIC will not predict myocardial iron and hence cardiac risk reliably, and myocardial iron may be found in some patients despite currently well controlled LIC.

- *To determine iron balance: is body iron increasing or decreasing on current therapy?*

LIC is the most reliable indicator of body iron load, which can be derived from the following formula: Total body iron stores in mg iron /kg body wt = 10.6 x the LIC (in mg/g dry wt) (Angelucci et al., 2000). Sequential measurement of LIC is the best way to determine whether body iron is increasing or decreasing with time (iron balance). While serum ferritin is simple, relatively inexpensive and can be repeated frequently, LIC determination should be considered for those patients whose serum ferritin levels deviate from expected trends (i.e. those with suspected co-existing hepatitis, or patients on chelation regimens with variable or uncertain responses), as

this may reduce the risk of giving either inadequate or excessive doses of chelation therapy. Since the relationship of SF to iron overload and iron balance has not yet been established, assessment of LIC may be particularly useful when new chelating regimes are being used. At high levels of SF ( $>4000 \mu\text{g/l}$ ), the relationship to LIC is not linear and patients may show a fall in LIC (negative iron balance) without a clear trend in SF in the first 6-12 months. When a patient fails to show a fall in SF over several months the change in LIC can identify whether the current regime is adequate or need to be modified (increased frequency or adherence, increased dose or change in regime).

## **Methods for measuring LIC**

### **- Biopsy**

Measurement of LIC was initially done by chemical determination on a liver biopsy sample (fresh, fixed or from dewaxing of paraffin-embedded material) (see Table 3). Biopsy is an invasive procedure, but in experienced hands has a low complication rate (Angelucci et al., 1997). Inadequate sample size (less than 4 mg dry wt or less than about a 2.5 cm core length) or uneven distribution of iron, particularly in the presence of cirrhosis (Villeneuve et al., 1996), may give misleading results however. Biopsy also allows the evaluation of liver histology which cannot yet be reliably estimated by non-invasive means. Laboratory standardisation is not trivial and differences between laboratories, for example in wet to dry weight ratios, can mean that results from different laboratories may not be equivalent.

### **- SQUID**

Magnetic biosusceptometry (SQUID) (superconducting quantum interference device) determines the paramagnetism of the liver which is proportional to LIC (Brittenham et al., 1993). Current methodology requires liquid helium which is very expensive. Furthermore, the SQUID apparatus needs to be in an environment away from paramagnetic forces (e.g. lifts, cars) which is often impractical. For these reasons, the current generation of SQUID devices are unlikely to be used outside a small number of well-resourced centres. Surprisingly not all SQUID devices have been calibrated in the same way, so comparison of results from different centres must be interpreted with caution unless the relevant machines have been cross-validated.

### **- MRI**

MRI techniques are now becoming the most widely used methods for LIC determination. The first techniques compared the signal in the liver or heart with that of skeletal muscle, which does not accumulate iron (Jensen et al., 1994). However, this is not in widespread use today and has been superseded by better methods. The principle shared by all MRI techniques currently used is that when a radio-frequency (rf) magnetic field pulse is applied to the tissue (e.g. liver or myocardium), protons take up energy, altering their spin orientation and they later relax returning to their original state. With spin echo, after the pulse the nuclei take time to relax in the 'relaxation time'; T1 in the longitudinal plane, and T2 in the transverse plane. Values may also be expressed as relaxation rates, the R1 rate (the same as  $1/T1$ ) and the R2 rate (the same as  $1/T2$ ). A variation of this principle are Gradient Echo techniques, achieved by applying a strong graded magnetic field to the rf pulse that is used for spin echo. This relies on multiple echoes over a shorter acquisition time than spin echo techniques. The shorter acquisition time may improve sensitivity and can be measured as  $T2^*$  (in ms), where  $1/T2^* = 1/T2 + 1/T2'$ , and T2 is the tissue relaxation time and T2' is the magnetic inhomogeneity of the tissue. An important point is that tissue iron concentration is not linearly related to  $T2^*$  or the T2, but is linearly related to  $1/T2^*$  or  $1/T2$  ( $R2^*$  or R2). Both gradient and/or spin echo techniques have been used in clinical practice.  $T2^*$  (or  $R2^*$ ) can be achieved with a



single breath hold, while T2 or R2 take a little longer to acquire data. Manufacturers of suitable MRI scanners are: Siemens (Erlangen, Germany); GE Healthcare (Milwaukee, WI, USA); Philips Healthcare (Amsterdam Netherlands). The strength of the magnetic field applied by these scanners is measured in Tesla (T) units. Most imaging is done on 1.5T machines but 3T machines give a better signal to noise ratio. However, 3T machines have greater susceptibility to artefacts, and the maximum detectable iron level is also halved (which is too low for many patients) (Wood & Ghugre, 2008; Storey et al., 2007). At present only 1.5T machines are widely used with reliable precision and accuracy based on standardised validation procedures. Liver packages (including standard sequences and analysis of the data) are included in the software provided with these MRI machines. Specialised LIC analysis software can also be bought separately.

**A note of caution** is that the different MRI techniques may not be equivalent – at least in the manner they are currently calibrated and practiced. The first widely used technique was the T2\* technique (Anderson et al., 2001), where liver biopsy was used to calibrate the method. Although this demonstrated the principle of T2\* to measure liver iron, unfortunately due to factors such as long echo times (TE 2.2-20.1 ms), and multi breath-hold acquisition, the calibration differs from later techniques, and can underestimate LIC by two-fold. Therefore, studies using this calibration may underestimate LIC (Garbowski et al., 2009). The R2 based FerriScan® technique appears to have acceptable linearity and reproducibility up to LIC values of about 30 mg/g dry wt (St Pierre et al., 2005), with an average sensitivity of >85% and specificity of >92% up to an LIC of 15 mg/g dry wt, and has been registered in the EU and US. For calibration of FerriScan®, the MRI machine must use a Phantom supplied by the company, while the data acquired is sent via internet for analysis by dedicated FerriScan® software (payment per scan analysed). A particular advantage of this technique is that it can be applied with little training, at any centre with a reasonably up-to-date MRI machine (see Table 3).

**Table 3.** Rationale, advantages and disadvantages of (LIC) determination by (MRI) and biopsy.

ADVANTAGES	DISADVANTAGES
Gives the most reliable estimate of body iron	Expensive (either by biopsy or MRI)
Allows calculation of iron balance (LIC change)	Cannot be repeated as frequently as SF (cost with MRI or inconvenience with biopsy)
Long term LIC control – linked to prognosis	LIC unreliable as predictor of cardiac iron in chelated patients
LIC not affected by inflammation (unlike SF)	Biopsy risks complications (low in expert centre)
Biopsy shows degree of liver damage	Biopsy method affected by sampling artefact
MRI non-invasive with good patient acceptance	MRI method is not universally available
MRI method can readily be set up and standardised across different centres	MRI method requires external validation
	MRI determination unreliable above LIC of 30 mg/g dry wt

*LIC, liver iron concentration; MRI, magnetic resonance imaging; SF, serum ferritin.*



## Myocardial iron estimation: T2\* and other tools

The physical principles of iron measurement for the heart by MRI are the same as for the liver (see above), with the additional challenge of measuring a moving object – the myocardium. The T2\* (or R2\*) techniques have the advantage over T2 or R2 in that they require shorter acquisition times and can be achieved with a single breathhold (Kirk et al., 2010). The utility of myocardial T2\* (mT2\*) MRI was originally identified on the basis of shortened T2\* values <20 ms in patients with decreased left ventricular ejection fraction (LVEF) (Anderson et al., 2001). More recently the relationship between biochemically measured myocardial iron concentration and myocardial T2\* has been shown using post-mortem myocardial material (Carpenter et al., 2011). Here, mean myocardial iron causing severe heart failure in 10 patients at post-mortem was 5.98 mg/g dry wt (ranging from 3.2 to 9.5 mg/g); levels that in the liver would not be regarded as harmful. The relationship of myocardial iron concentration (MIC) to T2\* is: MIC (mg/g dry wt) =  $45 * (T2^* \text{ ms}) - 1.22$  (Kirk et al., 2009). This relationship is non-linear so small changes in mT2\* at values <10 ms may indicate relatively large changes in MIC. **The risk of developing heart failure increases with T2\* values <10 ms, which are associated with a 160 fold increased risk of heart failure in the next 12 months (Kirk et al., 2009).** This risk further increases progressively with T2\* values <10 ms, so that the proportion of patients developing heart failure in the next 12 months at T2\* of 8-10 ms, 6-8 ms and <6 ms was 18%, 31%, and 52% respectively. These risks were derived from patients whose chelation therapy and adherence was not reported, so this risk may be less in patients taking regular chelation. For example, in a recent prospective study in patients with severe myocardial iron loading (T2\* values <10 ms), no patients developed heart failure over a 2 years period while taking deferasirox (DFX) and deferoxamine (DFO) combination therapy (Aydinok et al., 2013).

In centres where the T2\* method has been validated, the T2\* value may have predictive value in identifying patients at high risk of developing deterioration in LVEF, thus allowing targeted intensification of treatment before heart failure develops. Prompt identification of patients at risk by MRI, timely therapeutic intervention and improved chelation options have contributed to decreasing number of cardiac deaths (Thomas et al., 2010; Voskaridou et al., 2019). T2\* monitoring has now been established and validated internationally (Kirk et al., 2010), and is now recommended as part of yearly monitoring of multi-transfused patients at risk of developing myocardial iron loading. However, **it is very important that a given centre undertakes procedures to independently validate and calibrate measurements of the method adopted, otherwise inappropriate assessment of heart failure prognosis may result.** Table 4 summarizes advantages and disadvantages of using T2\* MRI for monitoring cardiac iron overload.

## Cardiac function

Sequential monitoring of LVEF has been shown to identify patients at high risk of developing clinical heart failure (Davis et al., 2004; Davis, O'Sullivan & Porter, 2001). When LVEF fell below reference values, there was a 35-fold increased risk of clinical heart failure and death, with a median interval to progression of 3.5 years, allowing time for intensification of chelation therapy. This approach required a reproducible method for determination of LVEF (such as MUGA (Multigated Acquisition) scan or MRI), while echocardiography was generally too operator-dependent for this purpose. Furthermore, there is a clear need to identify high risk patients before there is a decline in LVEF. Myocardial T2\* by MRI can achieve this and has additional predictive value (see above). However, as only a subset of patients with T2\* values between 10 and 20 ms, or even with T2\* less than 10 ms have abnormal heart function, sequential measurement of LVEF can identify the subset of patients who have developed

decompensation of LV function and are therefore at exceptionally high risk and require very intensive chelation therapy (see below).

**Table 4.** MRI T2\* method to assess myocardial iron.

ADVANTAGES	DISADVANTAGES
Rapidly assessed iron content in myocardial septum	Indirect non-linear relationship with myocardial iron
Reproducible method	Requires a validated centre with dedicated methods
Linked to heart iron (reciprocal relationship)	Technically demanding
Potential to measure heart function at same visit	Methodology requires standardisation worldwide
Potential to measure LIC at same visit	Does not predict liver body iron overload
Linked to LVEF at time of measurement	Requires continuous quality assurance such as regular phantom scanning
Linked to risk of heart failure in next year	

### ***Monitoring of other organ function and iron-mediated damage***

The monitoring of organ function as a marker of damage from iron overload is discussed more fully in other chapters. In general, by the time diabetes, hypothyroidism, hypoparathyroidism or hypogonadotropic hypogonadism (HH) have been identified, irreversible damage has set in and the focus then becomes replacing hormones. These are late effects and the primary aim of chelation therapy is to prevent such damage. Iron overloaded patients should be monitored for evidence of HH (growth and sexual development and biochemical markers of HH), diabetes mellitus (yearly oral glucose tolerance test (OGTT)), hypothyroidism and hypoparathyroidism. There has been recent interest in using MRI as a way of identifying iron-mediated damage to the endocrine system. Early work in this area showed good correlation between MRI findings (loss of pituitary volume) and biochemical markers of pituitary damage (Chatterjee et al., 1998). With improved MRI imaging, other endocrine organs have also been evaluated (Wood, 2007). It is of interest, that there is generally a close correlation between iron deposition in the heart and deposition in endocrine tissues, such as those of the pituitary and pancreas (Noetzli et al., 2009; Au et al., 2008). This supports the notion of shared uptake mechanisms for NTBI in heart and endocrine systems and supports clinical observations of shared risks in cardiac and endocrine systems once iron begins to escape from the liver.

### ***24h Urinary iron estimation***

Measurement of the urinary iron excretion has been used in assessing the effect on iron excretion by deferoxamine (about half of total iron excreted in urine) (Pippard,

Callender & Finch, 1982) or deferiprone (over 80% of iron excreted in urine), but is not useful in patients treated with deferasirox, as nearly all the iron is excreted in faeces. Urine iron has also been used to compare effects of combination and monotherapy regimes containing deferiprone (DFP) (Aydinok et al., 2012; Mourad et al., 2003). The inherent variability in daily iron excretion necessitates repeated determinations and this is not widely used in routine monitoring.

### ***Plasma non-transferrin bound iron and labile plasma iron***

As plasma iron that is not bound to transferrin (NTBI), is considered to be the main route through which iron is distributed to liver and extrahepatic targets of iron-overloaded thalassaemia major patients, levels of NTBI might be expected to correlate with the risk of damage to these tissues. Assays may estimate NTBI directly using a chelation capture method followed by high performance liquid chromatography (HPLC) (Singh, Hider & Porter, 1990) or by colorimetric analysis (Gosriwatana et al., 1999) or indirectly by exploiting the impact of labile iron species to oxidised fluoro-chrome, such as in the labile plasma iron (LPI) assay (Zanninelli, Breuer & Cabantchik, 2009; Cabantchik et al., 2005). A potential advantage of the LPI assay is that it is better suited to measurements when iron chelators are present in the plasma (Zanninelli, Breuer & Cabantchik, 2009). Whilst some loose associations of NTBI (Piga et al., 2009) or LPI (Wood et al., 2011) with some markers of cardiac iron or response to chelation have been found by some investigators, thus far measurements have not been sufficiently strongly predictive of cardiac risk to be recommended for routine clinical practice. This is partly because NTBI and LPI are highly labile, rapidly returning or even rebounding (Porter et al., 1996) after an iron chelator has been cleared (Zanninelli, Breuer & Cabantchik, 2009). Although NTBI correlates loosely with iron overload, it is affected by other factors such as ineffective erythropoiesis, the phase of transfusion cycle, and the rate of blood transfusion (Porter et al., 2011) adding to the complexity of interpreting levels (Hod et al., 2010). It is also not clear which methods identify the iron species that are most strongly linked to myocardial iron uptake. Therefore, although the measurement of NTBI (or LPI) has proved to be a useful tool for evaluating how chelators interact with plasma iron pools, its value as a guide to routine treatment or prognosis has yet to be clearly demonstrated.

## Summary and Grade of Evidence

- Uncontrolled transfusional iron overload increases the risks of heart failure, endocrine damage, liver cirrhosis and hepatocellular carcinoma (B).
- Liver iron concentration can be used to calculate total body iron, and serum ferritin is an approximate marker of LIC (B).

## Key Recommendations

Monitoring is essential in establishing effective iron chelation regimes, tailored to individuals' specific needs. However, some general principles of monitoring iron overload may apply to all including:

- Keeping a pre-transfusion haemoglobin above 90g/l (95-105 g/l) is recommended to prevent expansion of red cell precursors and reduce iron absorption from the gut.
- Serum ferritin should be aimed to be:
- Regularly monitored (please refer to the General Timetable for Clinical and Laboratory Investigation). Serum ferritin measurement is most useful in identifying trends.
- Maintained at levels of no greater than 1000µg/l (a level that may be associated with additional clinical advantages).
- For Liver Iron Concentration (LIC) measurement/assessment:
- Normal LIC values are up to 1.8 mg/g dry weight (wt), with levels of up to 7 mg/g dry wt seen in some non-thalassaemic populations without apparent adverse effects. Sustained high LIC (above 15-20 mg/g dry wt) have been linked to worsening prognosis, liver fibrosis progression (Angelucci et al., 1997) or liver function abnormalities (Jensen et al., 2003).
- Although measurement of LIC was initially done by chemical determination on a liver biopsy sample, MRI techniques are now becoming the most widely used methods for LIC determination. Different MRI techniques may not be equivalent. The first widely used tool was the T2\* method which due to factors such as long echo times and multi breath-hold acquisition, the calibration differs from later techniques and can underestimate LIC by two-fold.
- The R2 based MRI technique FerriScan® approved by EMA and FDA appears to have acceptable linearity and reproducibility up to LIC values of about 30mg/g dry wt with an average sensitivity of >85% and specificity of >92% up to a LIC of 15mg/g dry wt.

FerriSmart trained by FerriScan® uses artificial intelligence to perform automated analysis and provide instantaneous results by delivering a report back to the clinician and patient in real-time and it has a 7 to 10 minutes scan time. It is approved by the FDA in the USA and has a CE Mark in Europe covering 27 countries.

- For myocardial iron estimation
- The T2\* (or R2\*) techniques are commonly used as they require shorter acquisition times and can be achieved with a single breathhold. These measurements have indirect non-linear relationship with myocardial iron. The risk of developing heart failure increases with T2\* values of <10ms, which are associated with 160 - fold increased risk of heart failure in the next 12 months.

All MRI based measurements should require standardisation and continuous quality assurance such as regular phantom scanning. Generally such measurements should be performed in a validated centre with dedicated methods

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## CHAPTER 4

### Iron chelation

#### Aims of iron chelation therapy

##### 1. Prevention therapy:

The primary goal of chelation therapy is to maintain safe levels of body iron at all times, by balancing iron intake from blood transfusion with iron excretion by chelation (iron balance).

##### 2. Rescue therapy:

Once iron overload has occurred, the rate of iron excretion with chelation must exceed that accumulated from transfusion. Removal of storage iron is slow and inefficient, because only a small proportion of body iron is available for chelation at any moment. Once iron has been deposited in some tissues, damage is often irreversible. Prevention is therefore preferable to rescue. Chelation therapy should therefore be initiated before toxic levels of iron have accumulated.

##### 3. Emergency therapy:

If heart failure develops, urgent action is required, which usually requires changing and/or intensifying the treatment.

##### 4. Dose adjustment of therapy:

Dosing and treatment regimens require adjustment to changing circumstances. Articles that refer only to whether a patient is 'on' or 'off' a particular chelation regime miss the critical importance of tailoring dosing to the current needs of the patient. These can be identified by careful monitoring of body iron and its distribution. Monitoring is important to avoid: a) under-chelation with increased iron toxicity; or b) over-chelation and increased chelator toxicity. The dosing and regime must be adjusted periodically to take these factors into account.

##### 5. Adherence to therapy:

Chelation must be taken regularly for it to work. This requires good adherence. Intermittent high dose chelation can induce negative iron balance but does not provide continuous protection from labile iron and also risks increased toxicity from the iron chelator. Poor adherence can result from practical issues such as difficulty with deferoxamine (DFO) infusions, intolerance of a particular chelator, psychological/ psychosocial issues or limited accessibility. A key role of the treating centre is the monitoring and encouragement of adherence to chelation, alongside support from their family.

#### Sources of chelatable (chelateable) iron

Only a small fraction of body iron is available for iron chelation at any moment. This is because iron chelators interact with low molecular weight 'labile' iron pools more rapidly than with iron stored as ferritin or haemosiderin. **Labile iron is constantly being generated, so that the efficiency of chelation and protective effects are better when a chelator is also continuously available (chelator present 24 hours a day).** Chelatable iron is derived from two major sources: from the breakdown of red cells in macrophages (about 20 mg/day in healthy adults) and from the catabolism of stored ferritin within cells. In iron overload, most of the storage iron in the body is in hepatocytes, and the ferritin in these cells is turned over less frequently than in the

absence of iron overload (every few days). Iron chelated within the liver is excreted through the biliary system, or circulates back into plasma and is excreted in the urine. The proportion of excreted iron in urine and faeces varies with each chelator. Although other cells, such as cardiomyocytes, contain about one tenth of the storage iron concentration of hepatocytes, iron from these cells is cleared more slowly by chelators.

## Chemical and pharmacological properties of licensed chelators

**Iron binding:** Three iron chelators are currently licensed for clinical use. Their iron binding properties, routes of absorption, elimination and metabolism differ (summarized in Table 1).

**Chemistry:** DFO binds iron in a 1:1 ratio, which results in a very stable chelator-iron complex but is a large molecule that cannot be absorbed from the gut. Deferasirox (DFX) binds iron in a 2:1 chelator to iron ratio, and is small enough for oral absorption. Deferiprone (DFP) is even smaller than DFX and is suitable for oral absorption, but requires three molecules to bind iron, resulting in a less stable iron complex and a lower efficiency of iron binding at low chelator concentrations (low pM).

**Pharmacology:** The patterns of elimination of the chelator-iron complexes are shown in Table 1 and discussed for each relevant chelator.

**Table 1.** Chemical and pharmacological properties of licensed chelators.

COMPOUND	Deferoxamine (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Molecular weight (Daltons)	560	373	139
Long iron binding affinity(pM)	26.6	22.5	19.9
Delivery	s.c.or i.v. 8-12 hours 5 days/week	Oral, once daily	(i) Oral, three times daily (EMA/FDA approved) (ii) Oral Twice-A-Day (TAD) Formulation Ferriprox® (FDA approved)
Half-life of iron-free drug	20-30 minutes	12-16 hours	3-4 hours
Lipid solubility	Low	High	Intermediate
Route of iron excretion	Urinary and faecal	Faecal	Urinary
Max plasma levels (µM) of iron-free drug	7-10 (Porter et al., 2005a)	80 (Galanello et al., 2003)	90-450 (Kotoghiorghes et al., 1990)

COMPOUND	Deferoxamine (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Concentration of iron complex	Complex remains similar (about 7 $\mu\text{M}$ ) with ascending doses but the iron-free drug and metabolites increase (Porter et al., 2005a)	Complex accounts for about 10% of plasma drug in steady state (Waldmeier et al., 2010)	Complex correlates with urine iron excretion and predicts response to therapy (Aydinok et al., 2005)
Minimum plasma level ( $\mu\text{M}$ ) with daily dosing	0	20	0
Elimination of iron complex	Urine + faeces Iron complex removed more slowly than free drug	Faeces	Urine
Metabolism	Intrahepatic to metabolite B which binds iron (Porter et al., 2005a, 1998)	>90% eliminated in faeces, 60% unmetabolised. Metabolism mainly in liver to glucuronides. Oxidative metabolism by cytochrome 450 accounts for <10%. Most metabolites bind iron (Waldmeier et al., 2010)	Glucuronide formed in liver does not bind iron (Kontoghiorghe et al., 1990)
Recommended dose mg/kg/d	30-60 5-7 times/week	20-40 g once daily of dispersible tablet or 14-28 of film-coated tablet	(i) 75-100 in three divided doses (ii) 75-99 Twice-A-Day (TAD) Formulation Feriprox® (FDA approved only)
Chelation efficiency (% of drug excreted) iron	13	27	7
Main adverse effects (For more see details Appendix 2)	Ocular, auditory, bone growth retardation local reactions, allergy	Gastrointestinal, increased creatinine, increased hepatic enzymes	Gastrointestinal, arthralgia, agranulocytosis/neutropenia

## Practical prescribing of individual chelators

The potential benefits of chelation therapy must be balanced against occasional unwanted adverse effects that are generally more likely when doses are high relative to the level of iron overload. These typically take time to develop, so that careful monitoring should reduce these risks. **Appendix 1 summarises the particular prescribing information from licensing authorities that act as a guide for prescribing individual monotherapies.**

### Deferoxamine monotherapy (DFO)

DFO is licensed for the treatment of chronic transfusional iron overload worldwide for affected patients above the age of 2 years, reflecting its long-standing clinical use. There are some small differences in age of treatment commencement and maximum doses recommended in different countries.

#### Evidence of beneficial effects

DFO was the first chelator introduced in the late 1960s to early 1970s and it reduced the complications of iron overload and, when taken sufficiently regularly and at sufficient doses, improved survival progressively. Only patients born after 1980 will have started treatment at an early age, a key factor affecting survival (Borgna-Pignatti et al., 2004; Brittenham et al., 1993) as well as co-morbidities such as hypogonadism (Bronsiegel-Weintrob et al., 1990) and other endocrine disturbances, including diabetes mellitus (Borgna-Pignatti et al., 2004; Olivieri et al., 1994; Brittenham et al., 1993). However, treatment is costly and inconvenient, requiring subcutaneous or intravenous infusion over at least 8 hours a day at least 5 days a week in regularly transfused patients. Adherence to therapy has been the main limiting factor to successful outcomes (Gabutti & Piga, 1996). Over-chelation can also be a problem, particularly in children or when doses are not modified for age or level of iron overload (see Appendix 2). Because of such concerns, guidelines have been conservative, generally recommending that therapy not be started until serum ferritin (SF) levels reach 1000 µg/l, and with downward adjustment of dosing over chelation below this SF value.

#### Effects on serum ferritin

Long-term control of SF has been linked to protection from heart disease and to improved survival if levels are consistently less than 2500 µg/l (Olivieri et al., 1994) with even better outcomes at levels <1000 µg/l (Borgna-Pignatti et al., 2004). Control of SF is dependent on dose, frequency of use and transfusional iron loading rate (Cohen, Glimm & Porter, 2008). For example a mean daily dose of 42 mg/kg resulted in a small mean decrease in SF over one year, whereas 51 mg/kg resulted in a decrease of approximately 1,000 µg/l (Cappellini et al., 2006).

#### Effects on liver iron

In a prospective randomised study, a mean dose of 37 mg/kg stabilised liver iron concentration (LIC) for patients with baseline LIC values of between 3 and 7 mg/g dry weight (wt) (Cappellini et al., 2006). In patients with LIC values >14 mg/g dry wt, a mean dose of 51 mg/kg resulted in LIC decreases of an average of 6.4 mg/g dry wt. Thus, a dose of 50 mg/kg at least 5 days a week is recommended, if a significant decrease to optimal LIC levels is required (see above). It should be emphasised that these are average changes and that the dose required varies with the transfusional

iron rate (Cohen, Glimm & Porter, 2008).

### Effects on heart function

Subcutaneous DFO has long been known to prevent (Wolfe et al., 1985) or improve asymptomatic cardiac disease in thalassaemia major (Aldouri et al., 1990; Freeman et al., 1983). After the introduction of DFO, the incidence of iron-induced heart disease in different cohorts of patients fell progressively – with a key factor being the age of starting treatment (Borgna-Pignatti et al., 2004; Brittenham et al., 1994). Symptomatic heart disease can be reversed by high dose intravenous treatment (Davis & Porter, 2000; Cohen, Mizanin & Schwartz, 1989; Marcus et al., 1984). The same results can be obtained with excellent long-term prognosis with lower doses (50-60 mg/kg/day – see below), and consequently less drug toxicity using continuous dosing (Davis et al., 2004; Davis & Porter, 2000). Continuous intravenous (IV) doses of 50-60 mg/kg/day can normalise left ventricular ejection fraction (LVEF) over three months (Anderson et al., 2004), significantly earlier than normalisation of liver or cardiac iron stores. However, if advanced heart failure has developed before treatment is intensified, the chances of successful rescue are reduced. Early intervention for decreased left ventricular (LV) function is therefore recommended. Once heart function has improved, sustained compliance is critical to improve outcomes, especially while myocardial iron remains increased (Davis et al., 2004).

### Effects on cardiac iron (mT2\*)

Myocardial iron estimated by T2\* (mT2\*) can improve with either subcutaneous or intravenous DFO, provided that treatment is given at adequate doses, frequency and duration. Improvement in mild to moderate cardiac iron, even at low intermittent doses (5 days a week) has been confirmed (Pennell et al., 2014, 2006; Pennell, 2006).

**Table 2.** Decreasing complications in cohorts of Italian patients born after DFO became available. Reproduced with permission from (Borgna-Pignatti et al., 2004).

	BIRTH 1970-74*	BIRTH 1980-84†
Death at 20 years	5%	1%
Hypogonadism	64.5%	14.3%
Diabetes	15.5%	0.8%
Hypothyroidism	17.7%	4.9%

**\*IM DFO introduced in 1975**

**†SC DFO introduced in 1980**

**In 1995, 121 patients switched to DFP (censored at the time)**

**DFO, deferoxamine; DFP, deferiprone; IM, intramuscular; SC, subcutaneous.**

## Recommended treatment regimens for DFO monotherapy

### Standard therapy

#### - When to start DFO therapy?

Provided that treatment is 1) begun within 2-3 years of beginning transfusion therapy, (2) administered regularly (at least 5 times a week) and 3) administered in adequate doses, DFO has a well-established impact on survival and on cardiac and other complications of iron overload described above. In thalassaemia major, this should start before transfusions have deposited enough iron to cause tissue damage. This

has not been formally determined, but **current practice is to start after the first 10-20 transfusions, or when the ferritin level rises above 1,000 µg/l**. If chelation therapy begins before 3 years of age, particularly careful monitoring of growth and bone development is advised, along with reduced dosage.

#### - **Standard dose regimen**

The recommended method is slow subcutaneous infusion of a 10% DFO solution over 8-12 hours, a minimum of 5 days per week. In countries where pre-filled balloon infusors are available, this has been found to ease the convenience of adhering to DFO chelation. In general, **average daily doses should not exceed 40 mg/kg until skeletal growth has been completed: 20-40 mg/kg for children and up to 50-60 mg/kg for adults, as an 8-12-hour subcutaneous infusion**. To achieve negative iron balance in patients with average transfusion rates, a dose of 50 mg/kg/day at least 5 days a week is required. It is important that patients with high degree of iron loading and those at increased risk of cardiac complications receive adequate doses, are advised about compliance or are considered for alternative chelator regimens.

#### - **Use with vitamin C**

Vitamin C increases iron excretion by increasing the availability of chelateable iron, but, if given in excessive doses, may increase the toxicity of iron. **It is recommended not to give more than 2-3 mg/kg/day as a supplement, taken at the time of the DFO infusion**, so that the liberated iron is rapidly chelated. Should a patient have been started on DFO, the vitamin C supplement should not be given until after several weeks of treatment.

#### - **Dose adjustment to avoid DFO toxicity**

At low serum ferritin levels (<100 µg/l), the DFO dose needs to be reduced and DFO-related toxicities monitored particularly carefully (see below). **Dose reductions can be guided using the therapeutic index (= mean daily dose (mg/kg)/SF µg/l) to keep this < 0.025** (Porter et al., 1989): This index is not a substitute for careful clinical monitoring. Liver iron concentration may be a more reliable alternative to serum ferritin in monitoring response.

### **Rescue therapy**

#### - **Rescue to achieve negative iron balance**

If iron has already accumulated to harmful levels (see monitoring), negative iron balance is necessary. Dose adjustment is critical to the success of chelation therapy; increased frequency, duration and dose when rescue therapy is required, and decreased dosing when body iron is well controlled. Table 3 shows how the dose can be adjusted to achieve negative iron balance, depending on the transfusion rate. **At transfusion rates > 0.5 mg/kg/day only about half of patients will be in negative iron balance at doses of 35-50 mg/kg/day, while >50 mg/kg/day are required to achieve negative iron balance.**

**Table 3.** % of responders (% in negative iron balance) by dose and transfusion rate. Adapted from (Cohen, Glimm & Porter, 2008).

Dose (mg/kg)	Low transfusion rate <0.3 mg/kg/day	Intermediate transfusion rate 0.3-0.5 mg/kg/day	High transfusion rate >0.5 mg/kg/day
35 - <50	76	75	52
≥ 50	100	86	89

**- Rescue to remove cardiac iron**

For patients with mild to moderate myocardial iron ( $T2^*$  10-20 ms), increasing the mean dose to 50-60 mg/kg/day may be sufficient to improve the  $T2^*$  provided that adherence to therapy can be achieved. For patients with higher cardiac iron ( $T2^*$  6-10 ms), other chelation regimes have been shown to be effective, such as combination of DFP with DFO or DFX monotherapy (see below). For severe cases of cardiac iron overload ( $T2^*$  <6 ms), other regimes need to be considered (see below). For patients with abnormal LVEF, emergency therapy is recommended.

**- Intensive therapy for other reasons**

Prior to pregnancy or bone marrow transplantation, when avoidance of high levels of iron overload is desirable (see Chapters 13 and 16), intensification of therapy may be helpful to minimise the degree of iron overload. The optimal regime has not been systematically studied, but may include dose adjustment, as described above, with attention to adherence through goal setting.

**Emergency therapy**

In high-risk cases with decreased LVEF, continuous infusion is potentially more beneficial than periodic infusions because it reduces the exposure to toxic free iron (non-transferrin-bound iron – NTBI) (Porter et al., 1996). Intensification of treatment through continuous, 24-hour intravenous administration of DFO via an implanted intravenous delivery system (e.g. Port-a-cath) (Davis & Porter, 2000), or subcutaneously (Davis et al., 2004) has been shown to normalise heart function, reverse heart failure, improve myocardial  $T2^*$  (Porter et al., 2013b; Anderson et al., 2004) and lead to long-term survival, provided treatment is maintained. For emergency management before a central line can be inserted, DFO can be given through a peripheral vein, provided it is diluted in at least 100 ml of saline to avoid damage to the veins where the drug is infused. **At least 50 mg/kg/day (not exceeding 60 mg/kg/day) is recommended as a 24-hour infusion** (Davis et al., 2004; Davis & Porter, 2000). Higher-doses have been used by some clinicians but DFO is not licensed at such doses and the risk of retinopathy increases. Addition of vitamin C is recommended only when acute heart dysfunction has settled, which usually occurs by three months of continuous treatment (Anderson et al., 2004). Addition of DFP to DFO has been advocated by the American Heart Association consensus statement (Pennell et al., 2013). This recommendation was based mainly on a randomised study in patients without heart failure whose LVEF improved more on the combination arm (Tanner et al., 2007). Although another randomised study (DFO monotherapy vs same regimen with added DFP) in patients with more severe decrements in LVEF showed no difference in rate of improvement in  $T2^*$  or LVEF (Porter et al., 2013b), as there were no additional side effects in the combination arm, the addition of DFP would appear a reasonable approach in these patients.

**Deferiprone monotherapy (DFP)**

DFP is an orally absorbed iron chelator that began clinical trials in the UK in the 1980s. DFP was licensed in several countries and the European Union from the 1990s and in the US in October 2011 (Traynor, 2011). The accepted indications for treatment differ slightly in different countries (Musallam et al., 2013) (see below).

**Effects on serum ferritin**

DFP, when used as monotherapy, will maintain SF in transfused patients at desirable levels in about one third of patients. The effect on SF is generally greater when starting SF levels are high (>2500 µg/l). For example, at 75 mg/kg/day (in

three divided doses), the decreasing effect on SF was greater in patients with high baseline ferritin values > 2,500 µg/l (Viprakasit et al., 2013; Olivieri et al., 1995; al-Refaie et al., 1992; Agarwal et al., 1992) but there was no decrease, when baseline values were < 2,500 µg/l (Cohen et al., 2000; Hoffbrand et al., 1998; Olivieri et al., 1995). In paediatric Thai patients (age > 2 years), about 45% had significant reduction of serum ferritin after 1 year at mean doses of over 79 mg/kg/day (Viprakasit et al., 2013). The FDA licensing agreement in 2011 concluded that with DFP monotherapy a minimum 20% reduction in SF was met in only 50% of patients, (CI 43% - 57%). Compared with DFO at various doses pooled analyses (Pennell et al., 2006; Ha et al., 2006; Gomber, Saxena & Madan, 2004; Maggio et al., 2002; Olivieri & Brittenham, 1997) suggested no difference between the two drugs at 12 months. A more recent single study (Maggio et al. 2020) also found no difference. It is important to understand that the relative responses of LIC and ferritin to different chelators will depend to some extent on prior chelation therapy and baseline values of individual patients.

### ***Effects on liver iron***

Change in LIC with DFP has been compared with DFO in various studies (El-Beshlawy et al., 2008; Ha et al., 2006; Pennell, 2006; Maggio et al., 2002; Olivieri et al., 1998). After long-term monotherapy, variable levels of high residual LIC values (>15 mg/g dry wt) have been found (11% (Del Vecchio et al., 2002), 18% (Töndury et al., 1998) and 58% (Hoffbrand et al., 1998). Overall negative iron balance (decrease in LIC) at standard transfusion rates is achieved in only about a third of patients receiving 75 mg/kg (Fischer et al., 1998).

### ***Effects on myocardial iron***

In a randomised study, higher dose DFP (92 mg/kg/day) was compared with DFO at 43 mg/kg 5-7 days/week (mean daily dose 35 mg/kg/day). The increase in mT2\* was greater than that seen in the DFO group although relatively low doses of DFO were used as the comparator (Pennell et al., 2006).

### ***Effects on heart function***

In one study the LVEF in patients with normal baseline function in patients treated with DFP monotherapy showed a better improvement compared to patients treated with DFO (Pennell et al., 2006). This was confirmed in another trial (Maggio et al. 2002) and results in a 3-year study with administration of DFP demonstrated significant enhancement of LVEF over time (Maggio et al., 2012). Retrospective studies suggest that DFP monotherapy offers superior cardiac protection compared to DFO (Borgna-Pignatti et al., 2006).

### ***Compliance with DFP***

This varies between studies: one study comparing compliance with DFP and DFO found rates of 95% and 72% respectively (Olivieri, 1990), while another reported rates of 94% and 93%, respectively (Pennell 2006b). The similar rate of compliance with DFP has been observed in other populations (Viprakasit et al., 2013). As with other oral chelators, two key points should be taken into consideration: (i) compliance which tends to be higher in the context of clinical studies than in routine use, and (ii) although compliance with oral treatment is expected to be better, the importance of constant supervision and patient support as provided when administering DFO, should not be overlooked.

### ***Long-term benefits of DFP monotherapy***

Retrospective studies have reported a survival advantage of DFP either alone (Borgna-Pignatti et al., 2006) or with DFO (Telfer et al., 2006) (see below), over DFO



alone. Other retrospective or observational studies used surrogate markers, such as SF, myocardial T2\* or LVEF (though not liver iron) (Filosa et al., 2013; Maggio et al., 2012; Pepe et al., 2011). However, two systematic analyses have not found clear evidence of survival advantages of any particular chelator regime (Fisher et al., 2013; Maggio et al., 2011).

### ***Unwanted effects with DFP***

The unwanted effects of DFP and their monitoring and management are described in Appendix 2.

### ***Recommended treatment regimens with DFP***

#### ***Standard dosing and frequency***

**The dose of DFP that has been evaluated most thoroughly is 75 mg/kg/day, given in three doses.** In the EU, the drug is licensed for doses up to 100 mg/kg/day but formal safety studies of this dose are limited. The standard dose of 75 mg/kg/day administered in three separate doses is therefore recommended as a starting dose. The drug's labelling includes charts stating how many tablets and half tablets to use per dose for patient weights ranging from 20 to 90 kg. Each 500 mg tablet is scored to facilitate tablet splitting. An oral solution is also available for paediatric use.

#### ***Dose escalation with DFP***

While the relationship of dose to iron balance or serum ferritin trends has not been reported within a single study, the presumption is that higher doses will increase iron excretion and response rate. Doses of 100 mg/kg/day have been given in at least one prospective study, with no increase in side-effects reported (Pennell, 2006) and the drug is licensed up to this dose. Patients without iron overload may be more likely to develop agranulocytosis.

#### ***Age of commencement***

There is less experience on the safety and efficacy of DFP in children under 6 years of age than in adults. A recent open label prospective study examined efficacy and tolerability in 73 paediatric patients, age range 3-19 years (Viprakasit et al., 2013), and a similar study involving 100 children 1-10 years old who received the liquid formulation of DFP found no specific tolerability issues that have not been previously reported in adults. A randomised trial between DFP and DFX in paediatric patients aged 0-18 years showed similar efficacy and tolerability between the two regimens, but with higher study discontinuation rate in the DFP arm. This study along with a retrospective study on the use of DFP in the same population confirms that DFP can be a treatment option in children (Botzenhardt et al., 2018; Maggio et al., 2020).

#### ***Use of vitamin C***

The effect of vitamin C on iron excretion with DFP is not clear and is thus not recommended.

### ***Safety monitoring, precautions and interactions***

These are summarised in Appendix 1 and described in Appendix 2.

## **Deferasirox (DFX)**

DFX was developed as a once-daily oral monotherapy for the treatment of iron overload. The drug is licensed as first-line monotherapy for thalassaemia major in over 100 countries worldwide, although the earliest age at which deferasirox qualifies as first-line treatment differs somewhat between the FDA and the EMEA (see also Ap-

pendix 1) (Musallam et al., 2013).

### **Chemistry and Pharmacology**

Deferasirox is an orally absorbed iron chelator binding iron in a 3:1 ratio: the chemical properties and pharmacology are summarised in Table 1. The original formulation, a tablet dispersible in water (DT) has been replaced by a film-coated tablet (FCT) formulation that is better absorbed, and preferred by patients (Taher et al., 2017, 2018). Due to enhanced absorption, doses of the FCT formulation need to be decreased to  $0.7 \times$  of those previously recommended with DT. In this respect, reporting dosing in studies published before 2018 need to be converted to the equivalent dosing of film-coated tablet.

### **Evidence of effectiveness of DFX**

#### ***Dose effect on serum ferritin***

A dose-dependent effect on serum ferritin has been observed in several studies (Porter et al., 2008; Cappellini et al., 2006; Piga et al., 2006). A randomised study found that 20 mg/kg of the original formulation daily stabilised serum ferritin close to 2,000  $\mu\text{g/l}$  and at 30 mg/kg, serum ferritin was reduced with an average fall of 1,249  $\mu\text{g/l}$  over one year (Cappellini et al., 2006). Longer-term analysis show that the proportion of patients with ferritin values  $<1,000 \mu\text{g/l}$  and less than 2,500  $\mu\text{g/l}$  is decreasing progressively with time. At 4-5 years follow up median SF fell to  $<1500 \mu\text{g/l}$  (Cappellini et al., 2011). Overall, 73% of patients attained serum ferritin levels  $\leq 2500 \mu\text{g/l}$  and 41% of patients achieved serum ferritin levels of  $\leq 1000 \mu\text{g/l}$ , compared with 64% and 12% at baseline respectively. A large-scale prospective study (EPIC) has examined the interaction between dose and SF response in over 1000 patients with thalassaemia major (TM) (Cappellini et al., 2010). The initial dose of deferasirox (original formulation) was 20 mg/kg/day for patients receiving 2-4 packed red blood cell units/month, and 10 or 30 mg/kg/day for patients receiving less or more frequent transfusions, respectively. Dose adjustments were made on the basis of ferritin trends at 3 monthly intervals. A significant though modest overall fall in ferritin was seen at 1 year. The largest SF decrease of 1,496  $\mu\text{g/l/year}$  was noted in patients with the highest baseline SF values (baseline median SF 6,230  $\mu\text{g/l}$ ) (Porter et al., 2013a) but who were treated at high dosages (35-40 mg/kg/day). Therefore, these doses are now recommended for heavily iron overloaded patients. For the new formulation (FCT or Jadenu®) equivalent doses are  $0.7 \times$  these doses.

#### ***Dose effect on iron balance***

The descriptions of dose effects below refer to the original dispersible tablet formulation unless otherwise stated. On approximate dose conversion to the new formulation dose can be obtained by a correction factor of 0.7 (i.e. a lowering of the target dose when the new formulation is given). Detailed short-term studies examining the effect of dose on iron balance are available (Nisbet-Brown et al., 2003). In a longer-term large randomised study, iron balance was determined by changes in LIC over 1 year (Cappellini et al., 2006). Negative iron balance was achieved at 30 mg/kg/day, with a mean LIC decrease of 8.9 mg/g dry wt. These are average trends and a closer analysis shows that the blood transfusion rate influences the response to treatment (Cohen, Glimm & Porter, 2008) (Table 4).

**Table 4.** Percentage of responders (% in negative iron balance) by dose and transfusion rate. Adapted from (Cohen, Glimm & Porter, 2008).

\* The equivalent dose of the new formulation (non dispersible film coated tablet) is put in brackets for ease of reference.

Dose (mg/kg)	Low transfusion rate <0.3 mg/kg/day	Intermediate transfusion rate 0.3-0.5 mg/kg/day	High transfusion rate >0.5 mg/kg/day
10 (7*)	29	14	0
20 (14*)	76	55	47
30 (21*)	96	83	82

### **Effect on myocardial T2\*(mT2\*)**

Improvement in mT2\* was first reported in a retrospective analysis of effects on mT2\* after 1 and 2 years (Porter & Shah, 2010; Porter et al., 2005b). Prospective data demonstrated the efficacy of DFX in improving mT2\*, over 1 year, with a baseline mT2\* range of 5-20 ms (Pennell et al., 2010), while, a steady, significant mT2\*improvement, from mean of 12.0 ms at baseline to 17.1 ms, was observed after 3 years of treatment, despite a high mean baseline LIC (mean 28 mg/g dry wt) (Pennell et al., 2012). In this latter study, 68% of patients with baseline mT2\* from 10 ms to <20 ms normalized mT2\*, and 50% of patients with baseline mT2\*>5 to <10 ms at baseline improved to 10 to <20 ms, while there was no significant variation in left ventricular ejection fraction and no deaths. A randomised 1 year comparison of DFO (50-60 mg/kg/day subcutaneously for 5-7 days/week) with DFX (target dose 40 mg/kg/day) for patients with mT2\* of 6-20 ms (Pennell et al., 2014) and high mean baseline LIC (mean 29.8 mg/g dry wt) found improvements in mT2\* with both drug regimens, establishing non-inferiority of DFX vs. DFO for cardiac iron removal. Taken together, these studies show that DFX is an effective treatment for patients with increased heart iron with mT2\* >5-20 ms. As with other chelation regimes, high levels of baseline heart iron (mT2\* <10 ms) will typically take several years to clear, but the risk of developing heart failure during this time appears very low provided treatment is monitored.

### **Effects on heart function and long term survival**

In the above studies, even though mT2\* values at baseline were as low as mT2\* of 5-6 ms and the proportion of patients with mT2\* <10 ms was significant (17.2-33%), LVEF remained stable, and there were neither deaths nor episodes of symptomatic heart failure observed. Only one case of atrial fibrillation and one case of cardiomyopathy were reported. According to risk analysis of heart failure in TM from other cohorts, the risk of developing cardiac failure was expected to be substantial, with a relative risk 160 fold higher for patients with mT2\* <10 ms (Kirk et al., 2009). The stability of LVEF and the absence of heart failure in this high risk group of patients suggests that DFX renders effective prophylaxis for heart failure, even in patients with mT2\* values of 5-10 ms. This may be related to the 24 hour 'protection time' against labile iron that results from the long plasma half-life of DFX (Daar et al., 2009). Other smaller studies show similar stability over 5 years (Vlachaki et al., 2015). Recent reports of a 12 year 'real world' follow up study also show no cases of heart failure on treatment but for the first time also show progressive improvement in LVEF, even in patients with decreased LVEF at baseline (Casale et al., 2019). However, as DFX has not been evaluated in

formal trials for patients with symptomatic heart failure or LVEF <56%, at this time other chelation options are recommended for such patients.

### ***Convenience and impact on quality of life***

Convenience and quality of life, as with other oral chelation regimes, are expected to impact on adherence and hence survival. This is likely to have a greater impact outside formal clinical studies, where adherence is generally better. In the large scale EPIC study, patients reported improved quality of life (estimated by SF36 scores) and greater adherence to chelation therapy compared with baseline before starting DFX (Porter et al., 2012). Recent studies with the new formulation show further improvements in adherence and patient reported acceptability (Taher et al., 2017, 2018).

### ***Recommended standard dosing***

The original dispersible tablet formulation was taken orally as a suspension in water once daily. Due to increased absorption of the new formulation (FCT or Jadenu®) this should be taken at 0.7 x the dose shown to be effective and safe with the original dispersible tablet. Thus, **the starting dose for FCT is 14 mg/kg/day for thalassaemia major patients who have received 10-20 transfusion episodes**. Optimal maintenance doses will depend on the transfusional iron-loading rate (see table). For patients with high rates of transfusional iron loading (>21 ml/kg/month of packed red blood cells) a higher dose of 21 mg/kg day will be necessary. **For patients with already severe iron overload, doses of 21-28 mg/kg /day may be required**. Due to the rapid fall in serum ferritin that can occur with improved adherence using the FCT, careful monitoring of ferritin trends and serum creatinine, with downward adjustment of dose when ferritin values fall below 1000 µg/l is advisable (Cheesman et al., 2018). The FCT is recommended to be swallowed whole before food or with a light meal.

### ***Rescue therapy for patients with mild to moderate myocardial iron (mT2\* 5-20 ms)***

On the basis of prospective studies these patients can be successfully treated with DFX, resulting in preservation and stabilisation of LV function. Doses of up to 40 mg/kg have been used and are advisable in patients with very high levels of liver iron or serum ferritin.

### ***Rescue therapy for patients with severe myocardial iron (mT2\* < 6 ms)***

Prospective clinical trials with DFX monotherapy have been confined to patients with mT2\* values ≥6 ms. For patients with mT2\* <6 ms, other alternative chelation regimes are recommended.

### ***Emergency therapy for patients with reduced LVEF or symptomatic heart failure***

DFX has not been formally evaluated in prospective trials for such patients and is therefore not recommended.

### ***Other indications and contraindications***

**DFX is contraindicated in patients with renal failure or significant renal dysfunction (see below). Caution is recommended for patients with advanced liver disease and hepatic decompensation.**

### ***Age of commencement***

The labelling for age of commencement differs in countries which follow US licensing from those that adhere to EU licensing (Appendix 1). However, based on prospectively randomised studies in children as young as two years of age, some general recommendations can be made. A fall in LIC has been seen across all age groups analysed,

with no age-related adverse effects. In particular, no adverse effects on growth, sexual development or bones have been observed (Aydinok et al., 2012; Piga et al., 2006). On the basis of present knowledge, the criteria for starting treatment (ferritin level, age, number of transfusions) are similar to those of DFO. However, a target of 500-1000 µg/l appears to be achievable with DFX without additional toxicity issues, provided that doses are adjusted downwards as SF values fall towards 500 µg/l. The safety and efficacy of the FCT in children from birth to 23 months of age have not yet been established but trials are ongoing.

### ***Unwanted effects with DFX***

Unwanted effects of DFX and their monitoring and management are described in Appendix 2.

## **Combination therapies**

### ***Concept and pharmacology of combination therapies***

The term 'combination therapy' has been used to cover a variety of approaches to improve outcomes if monotherapy proves inadequate. In principle, two chelators can be given at the same time (simultaneously) or one after the other (sequentially). True combination, where two chelators are present in the blood or tissues at the same time, has been used relatively rarely compared with sequential regimes. If the drugs are given simultaneously, they may interact in a process that involves the 'shuttling' of iron, which may lead to additional chelation of iron from cells or plasma NTBI (Evans et al., 2010) and so improved efficiency of iron chelation in plasma (Lal et al., 2013). Simultaneous exposure to two chelators may also result in synergistic removal of cellular iron, which was demonstrated in cell culture with combinations of all three chelators (Vlachodimitropoulou, Maciej & Porter, 2013). The most commonly used regimes have tended to give DFP daily at standard divided doses, combined with varying frequency and dosing of DFO. More recently, combinations of DFX with DFO or DFX with DFP have been evaluated.

### ***Combined DFO and DFP***

A variety of regimens involving combinations of DFP and DFO have been used, either in the context of a formal trial or on an ad hoc basis, usually when monotherapy with DFO or DFP has failed to control iron overload or its effects. If compliance with DFO is poor, the use of DFP can improve overall exposure to chelation. Alternatively, if control of ferritin or LIC is inadequate with DFP monotherapy, DFO can be added to increase iron excretion.

### ***Evidence of efficacy of combined regimes***

#### ***- Effects of combined use on serum ferritin***

A randomised study (Tanner et al., 2007), showed that serum ferritin (SF) was decreased more with combined treatment (DFO five days a week plus DFP seven days a week) than with standard DFO monotherapy (40 mg/kg five times a week). However, in another randomised study (Aydinok et al., 2007), SF decreased more with combination therapy than DFP monotherapy but similarly to DFO monotherapy 5 nights a week. Alternate therapy with 5 days of DFP and 2 days of DFO/week showed comparable efficacy to DFO monotherapy at 5-7 days/week (Galanello et al., 2006). On the other hand, a 5-year randomized clinical trial also showed a greater SF reduction with sequential DFP (75 mg/kg/day-4 days/week)-DFO (50mg/kg/day-3days/week) compared with DFP alone, but with no clear survival trend benefit

(Maggio et al., 2009). While much of the variability between trials may relate to variable compliance in the DFO monotherapy group, taken together, these studies show that serum ferritin can be controlled with a relatively low frequency of DFO given twice a week when combined with DFP at standard doses (75 mg/kg/day), while sequential therapy may benefit some patients.

#### **- Effects of combined use on liver iron**

There is less evidence examining effects on LIC and results are variable but overall support combination therapy being more effective than DFP monotherapy. DFO monotherapy or combination therapy DFP + DFO (two times weekly) appear more effective than DFP monotherapy at controlling LIC (Aydinok et al., 2007). Another randomised study showed that combination was more effective than DFO monotherapy (Tanner et al., 2007). Similarly, in patients with heart failure who received DFO with or without DFP, there was decrease in LIC and ferritin in the combination arm but not with DFO monotherapy (Porter et al., 2013b).

#### **- Effects on heart function and mT2\***

In a randomised study LVEF increased within the normal range by approximately 2.5% in a combination arm vs. 0.5% in the DFO monotherapy arm (Tanner et al., 2007). For high-risk patients with decreased LVEF or with symptomatic heart disease, a randomised study showed equivalent improvement in LVEF and mT2\* in patients receiving either DFO intensification or DFO intensification plus DFP (Porter et al., 2013b). Observational studies have also reported improvements in heart function within the normal range using combined treatment over 12-57 months (Origa et al., 2005) or 3 years (Kattamis et al., 2006). If the imperative is to have improvement as rapidly as possible (for example in preparation for or prior to bone marrow transplant), then high dose DFP monotherapy (>90 mg/kg) or regular doses of DFP in combination with standard DFO (5 days a week) should be used, as combination of DFP and DFO may result in more rapid improvement in cardiac iron load and LVEF increases.

#### **- Long term effects on survival**

Some retrospective analyses have reported a benefit in survival compared with the previously prescribed DFO alone (Telfer, 2009; Telfer et al., 2006). In these studies, 75 of 344 patients discontinued chronic combination therapy because of agranulocytosis (5%), recurrent neutropenia (2.9%), gastrointestinal disturbances (5.6%), arthralgia (1.6%), allergic reactions (0.7%), weight gain (0.7%), increased liver enzymes (0.3%), non-adherence to DFO (3.3%), pregnancy (2.6%) and other reasons (2%). The achievement of very low serum ferritin values using a 'flexible' approach to combination therapy has been also reported without any adverse effects (Farmaki et al., 2010). However, controlled trials and meta-analysis demonstrating improvement in disease-related symptoms, organ function, or increased survival are lacking (Maggio et al., 2011).

#### **- Safety of combined DFO + DFP treatment**

Formal safety data on combined treatment are limited. The side effects described above are largely consistent with the known effects of the individual chelating agents, with the possible exception of cerebellar syndrome in a single case. Tolerability of simultaneous combinations may differ from sequential use, but this has not been formally studied.

#### **- Conclusions and possible treatment regimens**

Combinations of DFP and DFO are useful, especially when various monotherapy regimes have not been adhered to and/or have failed to control either liver iron or

cardiac iron. If a patient is not doing well with DFP monotherapy, combined treatment offers an additional option to improve iron balance. For patients not doing well with DFO monotherapy for reasons of compliance, and where dose intensification has failed, combined treatment has been used as a way of decreasing the frequency at which DFO is needed to maintain SF and iron balance. For patients with very high levels of cardiac iron (8 <ms) and/or cardiac dysfunction without frank heart failure, 24-hour treatment with DFO and daily therapy with DFP should be strongly considered.

### ***Combined DFX and DFO***

This combination has been evaluated in prospective studies and shown rapid removal of liver iron as well as improvement in cardiac iron (Lal et al., 2013; Aydinok et al., 2013). In a prospective study of 60 patients with severe liver and heart iron overload (cardiac T2\* 5-10 ms) treated with DFX 20-40 mg/kg/d 7 days per week plus DFO 40 mg/kg/d 5 days per week, a significant reduction in ferritin and LIC, of 44% and 52%, respectively, and an increase in cardiac T2\* of 33% were achieved (Aydinok et al., 2014). Importantly, improvement in mT2\* were greater in patients with baseline LIC <30 than those with LIC >30 mg/g dry wt. LVEF remained stable during the study. Tolerability was consistent with that seen with monotherapy regimes.

### ***Combined DFX and DFP***

This combination appears particularly effective at synergistically mobilising cardiomyocytic iron in cell culture (Vlachodimitropoulou Koumoutsea, Garbowski & Porter, 2015). Clinical case reports suggested that this can be an effective approach (Voskaridou, Christoulas & Terpos, 2011). In 16 patients studied over 2 years serum ferritin, LIC and mT2\* improved without additional adverse effects (Farmaki et al., 2010) with reversal of cardiac dysfunction in 2/4 patients. In a larger 1 year randomised trial (Elalfy et al., 2015) of 96 paediatric patients, two combination regimes were compared: DFP 75 mg/kg in two divided doses was given in both regimes and combined with either DFX 20 mg/kg once daily, or with overnight DFO at 40 mg/kg. SF, LIC and mT2\* improved significantly in both groups with greater improvement in mT2\* and in quality of life in the oral combination group with no serious adverse events. In a smaller study, which did not recruit sufficient patients for clear conclusions, common adverse events reported included arthralgia and gastrointestinal symptoms, while no episodes of neutropenia/agranulocytosis occurred (Hammond et al., 2019). This combination may be valuable in selected patients where other approaches have failed.

## **Which chelation regime, when and how much?**

### ***Standard therapy for obtaining iron balance***

The licensing of individual chelators, specified in the country where the treatment is prescribed should act as an initial guide on when to start the therapy and at what dose (see Appendix 1). Standard first line doses have been discussed above, and depend in part on the rate of transfusional iron loading. Starting chelation before overload has built up, or irreversible damage has occurred, is critical to success. With DFO, chelation was often withheld until the SF had reached 1000 µg/l because of fears toxicity would have on growth, ears and eyes at low levels of body iron. If a patient is failing on first line therapy, dose adjustment and attention to adherence (practical as well as psychological support) are the next steps. If this fails, then regime adjustment can be considered, depending on the circumstances – some of which described below.



### ***Iron load too high or increasing – rescue therapy to achieve negative iron balance***

If body iron load builds up because of a delays in starting chelation therapy, inadequate dosing, and poor adherence or because of poor response to an individual monotherapy, rescue therapy is required by one or more of the following:

1. ***Increasing the daily dose of chelation (see a, b, c)***
2. ***Increasing the frequency of the chelator (improving adherence (see d) or increasing prescription advice)***
3. ***Switching chelator regimen***
4. ***Rotating (see or combining chelators) (see e, f)***
  - a. DFO monotherapy is effective at producing negative iron balance if it is given in sufficient doses and sufficient frequency, but adherence is often a problem.
  - b. Dose escalation of DFX is effective at producing negative iron balance (see Table 3). Doses >35 mg/kg and up to 40 mg/kg (up to 28 mg/kg of new formulation – FCT) are effective in patients with high LIF or SF.
  - c. DFP monotherapy is likely to achieve iron balance at 75 mg/kg in only about one third of patients, with average transfusion rates. It may be increased up to 100 mg/kg with close monitoring. DFO is often added.
  - d. If adherence is the major reason for a regime not working, every effort should be made by the health team to support the patient and their family in achieving better adherence.
  - e. Rotation of individual monotherapies (sequential chelation) can be helpful in managing individual patients, often for reasons of adherence as much as for specific complications.
  - f. True ‘combination therapy’ (where two chelators are combined with some degree of overlap pharmacologically) is widely practiced, although not specifically licensed. This can be useful when monotherapy is inadequate, either to control iron balance or to control iron distribution, particularly in the heart.

### ***Mild increase in cardiac iron (mT2\* 10-20 ms) - rescue therapy to remove cardiac iron***

DFO, DFP and DFX monotherapy can all be effective at decreasing cardiac iron, but need to be given without interruption for optimal effects and at adequate doses. The immediate risk of heart failure is low, provided that the patient remains on chelation therapy without interruption (Kirk et al., 2009). Regular daily monotherapy at optimal doses (often an increase from current dose or frequency) will usually improve heart iron but can take several years of consistent therapy to normalise. Monotherapy with DFX is usually effective at improving mT2\* across a full range on LIC concentrations (Pennell et al., 2014, 2012). If the imperative is to do this as rapidly as possible (for example in preparation for pregnancy or prior to bone marrow transplant), then high dose DFP monotherapy (>90 mg/kg) or regular doses of DFP in combination with standard DFO 5 days a week should be used, rather than DFO alone when given subcutaneously 5 days a week. DFX has not been compared directly with DFP either alone or in combination with DFO. If there is no trend of improvement in mT2\* with DFO, DFP or DFX monotherapy, then combined DFP and DFO should be considered.

### ***Cardiac T2\* < 10 ms – rescue therapy for cardiac iron overload***

The risk of developing heart failure increases with lower cardiac T2\*, especially when mT2\* values drop below 10 ms (i.e. higher cardiac iron). However, if continuous chelation therapy is given, heart failure may be prevented even before the mT2\* is



corrected. This has been shown for continuous 24h DFO, with high dose DFX in a population where mT2\* was 6-10 ms, and in patients treated with different combination regimes (DFO+DFP, DFO+DFX). Patients with mT2\*  $\leq$  6 ms, are a very high-risk group for developing heart failure. This group has not been evaluated extensively with interventional studies (except people with heart failure). There is some experience of treating these patients with DFO + DFP, but randomised trials did not include patients with mT2\* values  $<8$  ms (Tanner et al., 2007). In the absence of formal comparisons with other regimes, the combination of DFO (given as often and as continuously as possible) with DFP at standard doses is recommended. DFX monotherapy at doses  $>30$  mg/kg/day ( $>21$ mg/kg of FCT formulation) has also been shown to be effective for patients with T2\*  $>5$  ms and normal heart function. If patients also have high levels of body iron (high LIC) as well as heart iron, it is important that the regime also reduces total body iron.

### ***Patients with heart failure - reverse heart failure***

If chelation therapy is taken regularly, clinical heart failure is now rare. Reversal of heart failure requires continuous DFO therapy and can occur within a few weeks of starting treatment. This will not succeed in all cases, but if started early in the development of heart failure, is usually effective. The addition of DFP in these circumstances may be beneficial, although a small randomised comparison did not show a difference with or without DFP (Porter et al., 2013b). Once reversal of heart failure has been demonstrated both clinically and with myocardial MRI or echocardiography, continuation of the same therapy is recommended until the cardiac T2\* improves to mT2\*  $>8$  ms, which may take many years, depending on the starting mT2\*. The key to success is the timely introduction of intensification and the maintenance of intensive treatment after the heart failure has been corrected.

### ***Downward adjustment of chelator dose if body iron falls rapidly or reaches low levels***

An increasingly common challenge for patients who respond well to a chelation regime is that the clinician does not recognize this and/or does not adjust the dose downward soon enough to prevent toxicity from over-chelation. This is more likely in centres without long term or regular experience in monitoring and prescribing chelation. Regular monitoring for SF trends (1-3 times monthly) and for the known toxicities of each chelator are minimum requirements. The general principle of downward dose adjustment with rapidly falling body iron loads is clear, but the specifics as regards how much and when are less so. In general, the risk of over chelation with DFO increases when the SF is low relative to the dose. This has not been analysed systematically with other chelation regimes. With DFX, low levels of SF, even below 500  $\mu\text{g/l}$ , can be safely achieved, even in patients not receiving transfusion, provided that the doses of DFX are low (5-10 mg/kg of DT formulation) (Taher et al., 2013). Cases of toxicity from over-chelation have been observed even at SF  $>500$   $\mu\text{g/l}$ , or if the rate of decrease is rapid. DFX dose adjustment should be made at the first sign of increasing serum creatinine values. With DFP it is not clear whether to or how to adjust dosing at low levels of SF or with rapid decrements in SF.

## Summary, Recommendations and Grade of Evidence

1. Chelation therapy is an effective treatment modality in improving survival, decreasing the risk of heart failure and decreasing morbidities from transfusion-induced iron overload (A).
2. Chelation therapy at the correct doses and frequency can balance iron excretion with iron accumulation from transfusion (A).
3. Absolute change in total body iron in response to chelation can be calculated from change in LIC (B).
4. Direction of change in body iron in response to transfusion and chelation can usually but not always be estimated from the trend in serum ferritin (B).
5. Prevention of iron accumulation using chelation therapy is preferable to rescue treatment because iron-mediated damage is often irreversible, and removal of storage iron by chelation is slow - particularly after it has escaped the liver (B).
6. Response to chelation is dependent on the dose applied and the duration of exposure (A).
7. Response to chelation is affected by the rate of blood transfusion (B).
8. Cardiac iron accumulates later than liver iron, and is rare before the age of 8 years, affecting a subset of patients (B).
9. Chelation of storage iron from the liver tends to be faster than from myocardium (B).
10. Cardiac storage iron concentration is directly related to the risk of heart failure, which can be reliably estimated by MRI (e.g. cardiac T2\*), provided the centre performing the measurement uses a validated method that has been independently calibrated (B).
11. Chelation can reverse iron-mediated cardiac dysfunction rapidly (within weeks) by rapid chelation of labile iron, if 24 h chelation cover is achieved (A).
12. Chelation therapy removes myocardial storage iron slowly (months or years) (A).
13. Over-chelation increases side effects from chelation therapy, and doses should therefore be decreased as serum ferritin or liver iron levels fall (demonstrated most clearly with DFO) (B).
14. The optimal chelation regime must be tailored for the individual and will vary with their current clinical situation.
15. Chelation therapy will not be effective if it is not taken regularly – a key aspect of chelation management is to work with patients to optimise adherence (B).

## Appendix 1

CATEGORY	DFO (DEFEROXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
Children age 2-6 years	First line for TM	<p>Under European licensing, there are limited data available on the use of deferiprone in children under six years.</p> <p>Under USA licensing, Ferriprox® (deferiprone) Oral Solution is an iron chelator indicated for the treatment of transfusional iron overload in adult and paediatric patients 3 years of age and older with thalassaemia syndromes, sickle cell disease and other anemias.</p>	<p>First line in USA. Under European Licensing (EMA) the drug is approved for:</p> <ul style="list-style-type: none"> <li>• children aged 2 to 5 years with beta thalassaemia major who receive frequent blood transfusions, when deferoxamine cannot be used or is inadequate and for</li> <li>• patients from 2 years of age with beta thalassaemia major who receive infrequent blood transfusions, when deferoxamine cannot be used or is inadequate;</li> </ul>
Children age >6 years and adults	First line for TM	<p>Approved if other chelator or DFO are not tolerated or inadequate (European Medicines Agency (EMA)).</p> <p>Under USA licensing, Ferriprox® Tablets are an iron chelator indicated for the treatment of transfusional iron overload in adult and paediatric patients 8 years of age and older with thalassaemia syndromes, sickle cell disease and other anemias.</p>	<p>First line in USA (FDA). Under European Licensing (EMA) the drug is used as first line for:</p> <ul style="list-style-type: none"> <li>• patients from 6 years of age who have beta thalassaemia major and who receive frequent blood transfusions; and for</li> <li>• patients from 10 years of age with non-transfusion-dependent thalassaemia syndromes, when deferoxamine cannot be used or is inadequate</li> </ul>

CATEGORY	DFO (DEFEROXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
Route	s.c./i.m. or i.v injection	Oral, tablet or liquid	Oral, dispersed tablet
Dosage and frequency	20-60 mg/kg 5-7 x /week, 50 mg/kg Children's dose up to 40 mg/kg	(i) 75 -100 mg/kg/day in 3 divided doses daily (ii) 75-99 mg/kg/dg Oral Twice-A-Day (TAD) Formulation Ferriprox® (FDA approved )	14-28 mg/kg/day once daily for film coated tablet. Lower doses in NTDT.
Contra-indications	- Pregnancy (but has been used in 3rd trimester)  - Hypersensitivity	- Pregnancy  - History of neutropenia or condition with underlying risk of cytopenia  - Hypersensitivity including Henoch Schönlein purpura: urticaria and periorbital oedema with skin rash	- Pregnancy  - Hypersensitivity  - Estimated creatinine clearance <60 ml/min  - Hepatic impairment or renal failure
Precautions	- Monitor ferritin; if it falls to <1000 µg/l , reduce dose (so mean daily dose/ferritin remains <0.025)  - Monitor audiometry regularly, particularly as ferritin falls  - Monitor eyes regularly including electroretinography if on high doses	- Measure neutrophil count (ANC) before starting and monitor ANC weekly  Under European licensing, the patient's absolute neutrophil count (ANC) should be monitored every week during the first year of therapy. For patients whose Ferriprox® has not been interrupted during the first year of therapy due to any decrease in the neutrophil count, the frequency of ANC monitoring may be	- Monitor creatinine trends for 1st 4 weeks after starting or after dose escalation, then monthly  - If rapid fall in serum ferritin to <1000 µg/l, dose reduce. If ferritin 500 µg/l consider very low doses.  - Proteinuria may occur, occasionally with renal tubular acidosis.  - Monitor urine protein regularly

## Appendix 1

CATEGORY	DFO (DEFEROXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
	<ul style="list-style-type: none"> <li>- Fever suggestive of septicaemia with organisms that used ferrioxamine (Yersinia, Klebsiella)</li> <li>- Renal failure or diminishing renal function with other comorbidities</li> </ul>	<p>extended to the patient's blood transfusion interval (every 2-4 weeks) after one year of deferiprone therapy</p> <p>In the USA licensing, the below is mentioned: Due to the risk of agranulocytosis, monitor ANC before and during Ferriprox® therapy. Test ANC prior to start of Ferriprox® therapy and monitor on the following schedule during treatment:</p> <ul style="list-style-type: none"> <li>•First six months of therapy: Monitor ANC weekly;</li> <li>•Next six months of therapy: Monitor ANC once every two weeks;</li> <li>•After one year of therapy: Monitor ANC every two to four weeks (or at the patient's blood transfusion interval in patients)</li> </ul> <ul style="list-style-type: none"> <li>- For neutropenia : <math>ANC &lt; 1.5 \times 10^9 / l</math> interrupt treatment</li> <li>- For agranulocytosis (<math>ANC &lt; 0.5 \times 10^9 / l</math>), consider hospitalisation</li> <li>- Advise patients to report im-</li> </ul>	<ul style="list-style-type: none"> <li>- Prescribing to the elderly; non-fatal gastrointestinal bleeding, ulceration, and irritation may occur; caution with drugs of known ulcerogenic or haemorrhagic potential, (e.g. NSAIDs, corticosteroids, oral bisphosphonates, and anticoagulants)</li> <li>- Hypersensitivity reactions</li> <li>- Monitor liver function regularly</li> </ul>

CATEGORY	DFO (DEFEROXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
		<ul style="list-style-type: none"> <li>- For agranulocytosis (ANC &lt; 0.5 x 10<sup>9</sup>/l), consider hospitalisation</li> <li>- Advise patients to report immediately symptoms of infection; interrupt if fever develops</li> <li>- Monitor for symptoms of arthropathy</li> <li>- Monitor liver function regularly</li> <li>- No guidance on dose adjustment at low ferritin</li> </ul>	
Potential drug interactions	<ul style="list-style-type: none"> <li>- Co-administration with prochlorperazine: may lead to temporary impairment of consciousness</li> <li>- Gallium-67: Imaging results may be distorted by rapid urinary excretion of deferoxamine-bound gallium-67. Discontinuation 48 hours prior to scintigraphy is advisable</li> </ul>	<ul style="list-style-type: none"> <li>- Theoretical interactions with UGT1A6 inhibitors (e.g. diclofenac, probenecid or silymarin (milk thistle))</li> <li>- Avoid concomitant use with drugs associated with neutropenia</li> <li>- Gallium-67 as with DFO</li> <li>- Oral preparations containing polyvalent cations (e.g., aluminium containing antacids, and zinc) allow at least a 4-hour interval</li> </ul>	<ul style="list-style-type: none"> <li>- Theoretical interactions with drugs metabolized by CYP3A4 e.g. midazolam</li> <li>- Theoretical interactions with drugs metabolized by CYP1A2: e.g. theophylline</li> <li>- Gallium-67 as with DFO</li> <li>- Oral preparations containing polyvalent cations as with DFP</li> </ul>

## Appendix 2

### ***Unwanted Effects of Iron Chelators; Monitoring and Management***

Unwanted effects of chelation therapy are generally more likely at high chelator doses and at low levels of iron overload, and possibly in association with high rates of reduction in body iron. There is more information about the relationship of these variables with DFO than with DFX, and little information is available about the effect of DFP dosing on unwanted effects. Although the licensing of each chelator includes some recommendations about how to monitor for unwanted effects, in this appendix we have included the recommendations in the context of overall management of TM patients.

### **Unwanted effects with DFO and their management**

#### ***General tolerability and frequency of adverse effects***

The unwanted effects of DFO are seen mainly when doses are given that are too high in relation to the level of iron overload, and typically take weeks or months to develop (over-chelation). Some effects are largely independent of the dose given, however. Limited data on the frequency of adverse effects at currently recommended doses are available,

#### ***Unwanted effects related to excessive chelation***

- ***Hearing problems***

High frequency sensorineural loss, tinnitus and deafness may occur when DFO is given in high doses, particularly to young children whose iron burden is low (Olivieri et al., 1986), and when the therapeutic index is exceeded ( $>0.025$ ) (Porter et al., 1989). Annual monitoring is particularly important in patients where SF values fall rapidly, or are  $<1000 \mu\text{g/l}$ , and in patients where the therapeutic index has been exceeded.

- ***Effects on the eye***

Visual disturbances are rare if dosage guidelines are not exceeded, and may include retinal effects and cataracts. Symptoms may include night-blindness, impaired colour vision, impaired visual fields and reduced visual acuity. Severe cases may show signs of retinitis pigmentosa on fundoscopy, whereas milder cases are only demonstrable with electroretinography. The main risk factor appears to be high dosing (Olivieri et al., 1986) but complications are also more likely in patients who have diabetes (Arden 1984) and in those receiving concomitant phenothiazine treatment (Blake et al., 1985). Treatment with DFO should be temporarily suspended in patients who develop complications.

- ***Growth retardation***

Growth retardation may occur if DFO is administered at too high a dose. Another risk factor is a young age of starting treatment ( $<3$  years) (De Virgiliis et al., 1988; Piga et al., 1988). Growth velocity resumes rapidly when the dose is reduced to  $<40 \text{ mg/kg}$  day, while it does not respond to hormonal treatment. It is therefore recommended that doses do not exceed  $40 \text{ mg/kg}$  until skeletal growth has been completed. Regular monitoring of growth is essential in all children (see Chapter 8 for details on Endocrine complications).

- ***Skeletal changes***

Skeletal changes are more common in cases of excessive dosage of DFO, (Gabutti & Piga, 1996; Olivieri et al., 1992; De Virgiliis et al., 1988). Rickets-like bony lesions and genu valgum may be seen in association with metaphyseal changes, particularly in

the vertebrae, giving a disproportionately short trunk. Radiographic features include vertebral demineralisation and flatness of vertebral bodies. Patients should be regularly observed for such changes, as they are irreversible. Careful monitoring of growth charts for important toxic effects of DFO should be considered in the differential diagnosis if this falls away from previous growth curves.

- **Rare complications**

Renal impairment may occur at high doses and renal monitoring is therefore recommended. Interstitial pneumonitis has been reported at very high doses of 10 mg/ kg/h or more. Neurological complications have also been described; in patients without iron overload, DFO has induced reversible coma when used with a phenothiazine derivative (Blake et al., 1985). Hypotension can also occur with rapid intravenous injection, and may occur during flushing of a line containing DFO, which should therefore be avoided.

- **Use in pregnancy**

The risk of teratogenicity after clinical DFO usage in pregnancy is unclear (Singer & Vichinsky, 1999). If no chelation is given in pregnancy, particularly in those with high iron levels pre-pregnancy, myocardial iron can accumulate during pregnancy with worsening heart function (Perniola et al., 2000) or even fatal heart failure (Tuck et al., 1998; Tsironi, Karagiorga & Aessopos, 2010). DFO has therefore been used in some higher risk pregnancies, particularly in the final trimester (Tsironi et al., 2005; Singer & Vichinsky, 1999; Bajoria & Chatterjee, 2009).

### **Unwanted effects not related to excessive chelation**

- **Local skin reactions**

Reactions such as itching, erythema, induration and mild to moderate discomfort are common and may be due to inadequate dilution of DFO. Ulceration at the site of a recent infusion results from an intradermal infusion of DFO and should be addressed by deeper placement of the needle in subsequent infusions. The % solution infused should not exceed 10% or the risk of skin reactions increases.

- **Infection with *Yersinia enterocolitica***

This is an important risk associated with DFO treatment (described in detail in Chapter 9). Such infections may be difficult to diagnose. However, where there is reasonable clinical suspicion of infection by *Yersinia enterocolitica*, treatment with DFO should be temporarily discontinued.

- **Severe hypersensitivity**

This is a rare event and can be treated by careful desensitisation, carried out under close medical supervision (Bousquet et al., 1983; Miller et al., 1981). Desensitisation is usually successful but may need to be repeated. If unsuccessful, an alternative chelator, such as DFP or DFX may be considered (see below).

### **Unwanted effects with DFP and their management**

- **General tolerability and frequency of adverse effects**

Adverse reactions based on pooled data collected from 642 patients showed nausea (13%), vomiting, abdominal pain (10%), elevations in alanine aminotransferase (8%), arthralgia (10%) and neutropenia (7%). Other unwanted effects >1% were back pain (2%), arthropathy (1%), agranulocytosis (1.7%), change in appetite (5%), diarrhoea (3%), dyspepsia (2%) and headache (3%) (FDA, 2011).



- ***Relationship to dose and levels of iron overload***

Most studies where tolerability has been reported have used 75 mg/kg in three divided doses. The drug is licensed up to 100 mg/kg/day but insufficient numbers have been reported to know whether the incidence of the most serious complication, namely agranulocytosis, is increased at these higher doses.

- ***Neutropenia, agranulocytosis and thrombocytopenia***

Agranulocytosis (absolute neutrophil count or ANC  $<0.5 \times 10^9/l$ ) is a serious and potentially fatal adverse event. This may be preceded by neutropenia and is reported in approximately 1.7% of patients. **Each patient's absolute neutrophil count should be measured before starting DFP therapy and every 1-2 weeks during treatment.** Recent addition from EMA Summary of Product Characteristics (SmPC) suggest that it may be reasonable patients whose Ferriprox® has not been interrupted during the first year of therapy due to any decrease in the neutrophil count, the frequency of ANC monitoring may be extended to the patient's blood transfusion interval (every 2-4 weeks) after one year of DFP therapy (See Annex 1, Precautions<sup>1</sup>). DFP therapy should be interrupted and the patient's neutrophil count closely monitored if an infection develops. It is not clear whether this effect is dose related. If severe neutropenia or agranulocytosis develops, the drug should be stopped and not reintroduced, and the use of granulocyte colony-stimulating factor (G-CSF) should be considered in the case of agranulocytosis; off-label use of the drug should be avoided. Agranulocytosis and neutropenia have also been reported in patients taking combinations of DFP and DFO.

- ***Effects on liver***

In a summary of available clinical studies, the FDA noted that 7.5% of 642 subjects developed increased ALT values. 0.62% of subjects discontinued the drug due to increased serum alanine transaminase (ALT) levels, and 0.16% due to an increase in both ALT and aspartate transaminase (AST) (FDA, licensing information 2011). In a recent retrospective study, mean ALT increases 6 fold above baseline were reported in chronically treated patients on DFP but not with DFX (Olivieri NF, et al. 2019). Fluctuation of liver enzymes more than twice the upper limit of normal should prompt investigation of the cause and consideration of interrupting DFP therapy.

- ***Arthropathy***

The frequency of arthropathy varies greatly between studies, from as low as 4.5% at one year (Cohen et al., 2000) to 15% after four years (Cohen et al., 2003) in a predominantly European patient group, and as high as 33-40% in studies of patients from India (Sharma et al., 2013; Choudhry et al., 2004; Agarwal et al., 1992). Symptoms range from mild non-progressive arthropathy, typically in the knees and controllable with non-steroidal anti-inflammatory drugs to (more rarely) severe erosive arthropathy that may progress even after treatment is stopped.

- ***Neurological effects***

DFP penetrates the blood brain barrier but neurological complications are very rare in thalassaemia treatment and have been typically associated with unintentional overdosing ( $>230$  mg/kg/day).

<sup>1</sup> FDA has also made certain suggestions with regards for the frequency of monitoring neutrophil count (see Appendix 1)

- ***Effects on ears and eyes***

One study reported continued audiometric deterioration after switching from DFO to DFP (Chiodo et al., 1997). A more recent study found hearing impairment and audiometric abnormalities in 56% of children with TM receiving DFP or DFO, with no difference between the two chelation groups, with the main shared risk factor being a low ferritin (Chao et al., 2013). It may therefore be advisable to monitor audiometric function in patients on regimes containing DFP as well as DFO. There have been isolated reports of loss of vision (central scotoma). A regular eye examination including retinal evaluation at least once yearly is therefore advisable.

- ***Other effects***

Zinc deficiency has also been observed in some patients, especially those with diabetes (al-Refaie et al., 1994). Zinc deficiency is difficult to assess from plasma samples, which need to be taken during fasting, in the absence of chelator in the blood. Some clinicians routinely add zinc supplementation with DFP monotherapy or combination therapy (not given at the same time as the DFP) (Porter et al., 2013b).

- ***Pregnancy***

DFP is teratogenic in animals and must never be given to patients attempting to conceive. Until more is known, potentially fertile sexually active women and men taking DFP must use contraception. DFP should not be used in pregnant women.

## **Unwanted effects with DFX and their management**

- ***General tolerability and frequency of adverse effects***

DFX has been given in the context of prospective trials to over 5900 patients with over 5 years of follow up in some prospective studies, so the relationship between DFX and unwanted effects are relatively well documented and defined.

- ***Relationship to dose and iron overload***

Although drug labelling suggests interrupting dosing when the serum ferritin reaches 500 µg/l, this leads to a 'stop-start' approach, which risks rebounding of NTBI and labile iron pools. Many clinicians therefore operate a dose reduction policy; giving very low doses (3.5-7 mg/kg) to those patients who continue to be transfused. Some effects are notable by their absence, such as effects on growth, bone and joints.

- ***Gastrointestinal effects***

Gastrointestinal events are relatively frequent with DFX therapy but are typically mild to moderate and include diarrhoea, abdominal pain, nausea and vomiting, occurring in approximately 15-26% of patients (Vichinsky et al., 2010). These symptoms rarely require dose adjustment or discontinuation, and decrease year on year over 5 years of follow up (Cappellini et al., 2011). Special attention should be given to patients taking concomitant medications that can increase the possibility of gastric ulceration. The film-coated tablet can be given with food and this seems to improve gastrointestinal (GI) disturbances.

- ***Skin rashes***

Skin rashes occurred in 7-11% of patients, and were typically pruritic, maculopapular and generalised, but occasionally confined to palms and soles of the feet. Rash typically develops within two weeks of starting treatment. A minority of patients require permanent discontinuation of therapy, and mild rashes often resolve without dose modification, and became very rare after year 1 of treatment (Cappellini

et al., 2011). For moderate to severe rashes, treatment should be stopped and later restarted at a very low dose (<5 mg/kg), slowly increasing to therapeutic doses.

#### • **Renal effects**

- An increase in serum creatinine  $\geq 30\%$  on at least two consecutive readings was observed in 38% of patients receiving DFX, most frequently at doses of 20 mg/kg and 30 mg/kg of DT (Cappellini et al., 2006). These increases were sometimes transient and generally within the normal ranges, never exceeding two times the upper limit of normal (ULN), and were more frequent in the population of patients having the most dramatic decrease in LIC and serum ferritin. At 5 years of follow up, no evidence of progressive renal dysfunction had been reported where the above doses and modifications were used (Cappellini et al., 2011). Other causes of increasing creatinine should also be considered in patients on DFX therapy, such as renal stones or concomitant use of non-steroidal anti-inflammatory agents (NSAIDs). If a patient becomes acutely unwell for another reason, such as septicæmic shock or severe acute vaso-occlusive complications in sickle cell disease (SCD), it is probably wise to interrupt chelation therapy until the general condition stabilises.
- Proteinuria may be present in about a quarter of thalassaemia major patients, irrespective of the underlying chelation therapy, with average values about three times that of healthy controls (Economou et al., 2010). Elevation of urine calcium and cystatin C are also seen in patients on DFX or DFP and DFO, whereas elevation of  $\beta 2$  microglobulin was seen in patients on DFX only (Economou et al., 2010). It is recommended that urine is monitored regularly for protein, and this can be conveniently performed at the time of visits for cross-matching blood. Although proteinuria can fluctuate considerably, if there is a clear upward trend in the protein/creatinine ratio above 1 mg/g, interruption or dose reduction should be considered. Current drug labelling recommends monthly urine testing for protein, which is helpful in establishing trends in proteinuria, as isolated estimates can be misleading. Case reports of renal tubular acidosis (Fanconi syndrome) with electrolyte imbalance, and metabolic acidosis due to tubular dysfunction have been rarely reported in adults and children taking DFX (Rheault et al., 2011; Grangé et al., 2010). All cases recovered following withdrawal of DFX.

#### • **Hepatic effects**

Generally liver enzymes improve in line with falling LIC. However, increases in liver transaminases are occasionally seen: In the EPIC study 0.6% of 1115 TM patients showed an increase of AST  $>10\times$  the upper limit of normal (Cappellini et al., 2010). Checking ALT approximately monthly is recommended. Abnormal liver function tests are more frequent in children receiving DFX, and in such instances chelation should be stopped and ALT levels carefully monitored to ensure they return to normal. Reintroducing DFX using a slow escalation schedule has been reported in some cases; improvements in the liver pathology of 219 patients with  $\beta$  thalassaemia treated with DFX for at least 3 years has been reported in a prospective trial (Deugnier et al., 2011).

#### • **Eyes and ears**

These are very rare and their significance is uncertain: current labelling recommends yearly auditory and eye assessments (EMA, 2013). Early lens opacity was reported in the DFX core registration trials, but the incidence (0.3%) did not significantly differ from the control group of DFO-treated patients (Cappellini et al., 2006). The electroretinographic effects previously seen with DFO have not been described; possible audiometric effects were identified in early studies but this has not been reported systematically.

- ***Pregnancy and DFX***

DFX has been shown to have teratogenic effects in animal studies and its use is not recommended in pregnancy.

- ***Post-marketing experience***

A number of additional adverse reactions have been reported with post marketing experience (see highlights of prescribing information in section 6.2 of FDA, 2011). Because these are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or to establish a causal relationship to drug exposure.

## **Appendix 3**

### ***Practical Issues with DFO Infusions***

#### **Practical issues with subcutaneous infusion**

Because regular use of DFO is critical to a good outcome, every effort should be made with each individual to help him or her to find the most convenient way to infuse the drug.

- ***Strength of infusion***

The manufacturers of DFO recommend that each 500 mg vial of the drug is dissolved in at least 5 ml of water, giving a 10% solution. Concentrations in excess of this may increase the risk of local reactions at the site of infusion.

- ***Site of infusion***

Care must be taken to avoid inserting needles near important vessels, nerves or organs. The abdomen is generally the best place. However, because of local reactions such as erythema, swelling and induration, it is often necessary to 'rotate' the sites used for injection (see Figure 1). Some patients find that the skin over the deltoid or the lateral aspect of the thigh provides useful additional, alternative sites. The best needle to use will depend on the individual. Many patients are happy with butterfly needles of 25 gauge or smaller, which are inserted at an angle of about 45 degrees to the skin surface. The needle tip should move freely when the needle is waggled. Other patients prefer needles that are inserted vertically through the skin and are fixed with an adhesive tape attached to the needle. Patient preference is highly variable and clinicians should explore the best type of needle for each patient, to help maximise compliance.

- ***Types of infuser***

There are many types of infuser now available. Newer devices, including balloon pumps, are smaller, lighter, and quieter than their predecessors. For patients who find dissolving, mixing and drawing up DFO a problem, pre-filled syringes or balloons may be useful. Some pumps are designed to monitor compliance.

- ***Local reactions***

Persistent local reactions may be reduced by varying injection sites, lowering the strength of infusion, or in severe cases, by adding 5-10 mg of hydrocortisone to the infusion mixture. Application of topical low potency corticosteroid cream after injection can reduce local reactions.

### **Practical details for intravenous infusions**

10% solutions of DFO given to peripheral veins will damage and sclerose the vein. If infused (as an emergency) into a peripheral vein, the solution must be diluted – for example in 200-500 ml of saline.

- Management of in-dwelling intravenous lines

Infection and thrombosis of catheters may occur. Careful aseptic procedures must be followed in order to prevent possible infection by *Staphylococcus epidermidis* and *aureus*, which when established are difficult to eradicate, and often removal of the infusion system becomes necessary. The risk of thrombosis and infection is likely to be greater in centres that do not have regular experience in the use of long-term in-dwelling lines (Piga et al., 2006). Use of prophylactic anticoagulation is advised, as line-thrombosis is relatively common in thalassaemia major (Davis & Porter, 2000). As development of a thrombus can occur at the tip of the catheter, it is advisable, if possible, to avoid placing the tip in the right atrium.

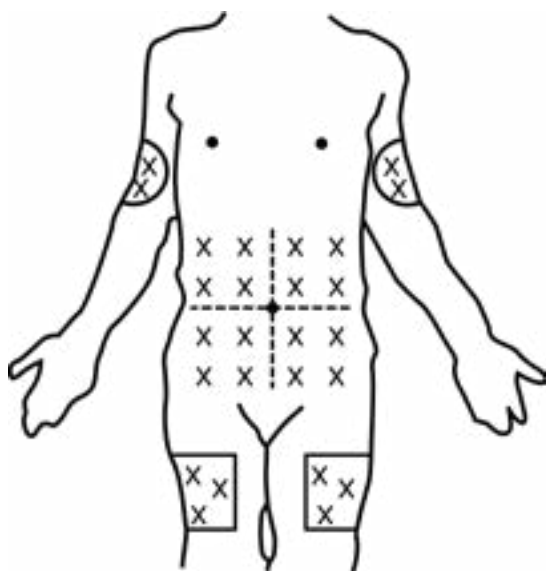
- Intravenous DFO with blood transfusion

This has been used as a supplement to conventional therapy (e.g. 1 g over 4 hours piggy- backed into the infusion line), but its contribution to iron balance is very limited and not recommended as a standard procedure. Special attention must be given to avoiding accidental boluses due to DFO collecting in the dead space of the infusion line.

- Use of DFO by subcutaneous bolus

If an infusion pump is not available or if 10-hour infusions are not tolerated, bolus subcutaneous treatment may be considered if the patient is not at high risk of heart disease (Yarali et al., 2006). However, this technique may be impractical in the clinic – particularly in paediatric patients, due to the painful nature of bolus infusions.

**Figure 1.** Rotation of infusion sites.



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## CHAPTER 5

### Cardiovascular disease

#### Introduction

Cardiovascular (CV) complications represent the leading cause of mortality in patients with thalassaemia, including thalassaemia major (TM) and thalassaemia intermedia (TI) (Zurlo et al., 1989; Borgna-Pignatti et al., 1998, 2004). In contemporary cohort studies, however, CV mortality has significantly declined, reflecting the overall mortality reduction observed in the same cohorts (Voskaridou et al., 2012). This improvement has resulted from the effective implementation of modern diagnostic and therapeutic modalities. Among other advances, magnetic resonance imaging (MRI)-guided chelation therapy with the use of the T2\* technique has been estimated to account for 71% reduction of mortality due to iron overload and 62% reduction of all-cause mortality since 2000 (Modell et al., 2008). This progress is not however the case in thalassaemia populations with limited access to modern therapy and therefore the global burden of CV disease in thalassaemia remains high, affecting 42% of patients according to a recent meta-analysis (Koochi, Kazemi & Miri-Moghaddam, 2019).

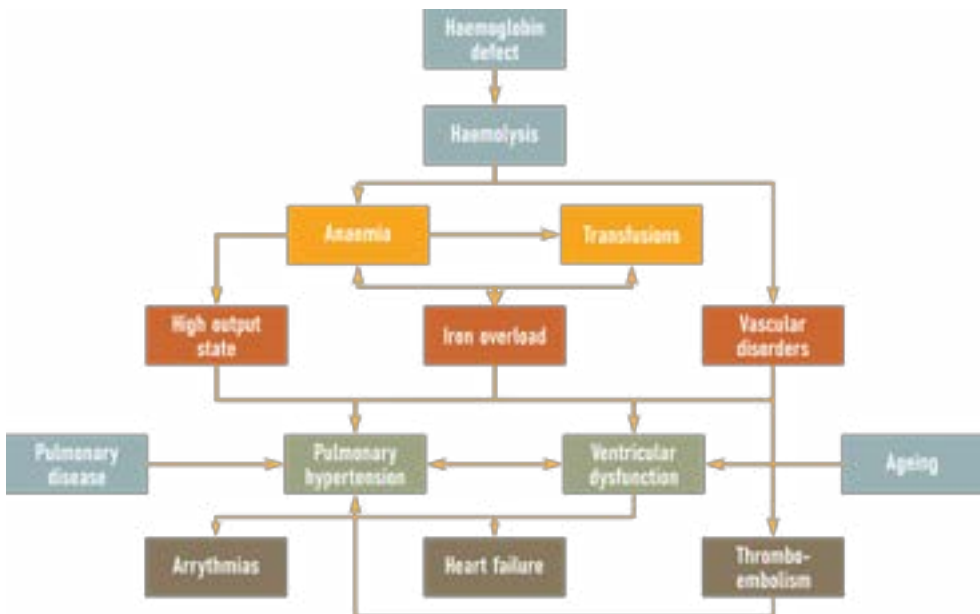
The present chapter provides an overview of CV complications that may arise in thalassaemia, focusing on the particularities of CV disease in these patients that need to be taken into consideration during their CV assessment and treatment.

#### Pathophysiology

The pathophysiology of CV disease in thalassaemia has been reviewed in detail elsewhere (Figure 1) (Farmakis et al., 2017). Importantly, the pathophysiology is primarily determined by two main factors, the severity of the haematological defect, which is dictated by the genetic background, and the applied therapy, including blood transfusions and iron chelation, which is determined by physicians' therapeutic choices as well as patients' access to and compliance with the prescribed regimens.

A third factor with growing impact on the pathophysiology of CV disease in thalassaemia is ageing (Farmakis et al., 2020). Ageing of thalassaemia patients, a notable accomplishment of modern therapy, is expected to modify the clinical spectrum of the disease by increasing the occurrence of age-related conditions. This may be particularly true for CV disease that is largely age-dependent.

**Figure 1.** Pathophysiology of cardiovascular disease in thalassaemia (modified from Farmakis et al, Eur J Heart Fail 2017;19: 479-489).



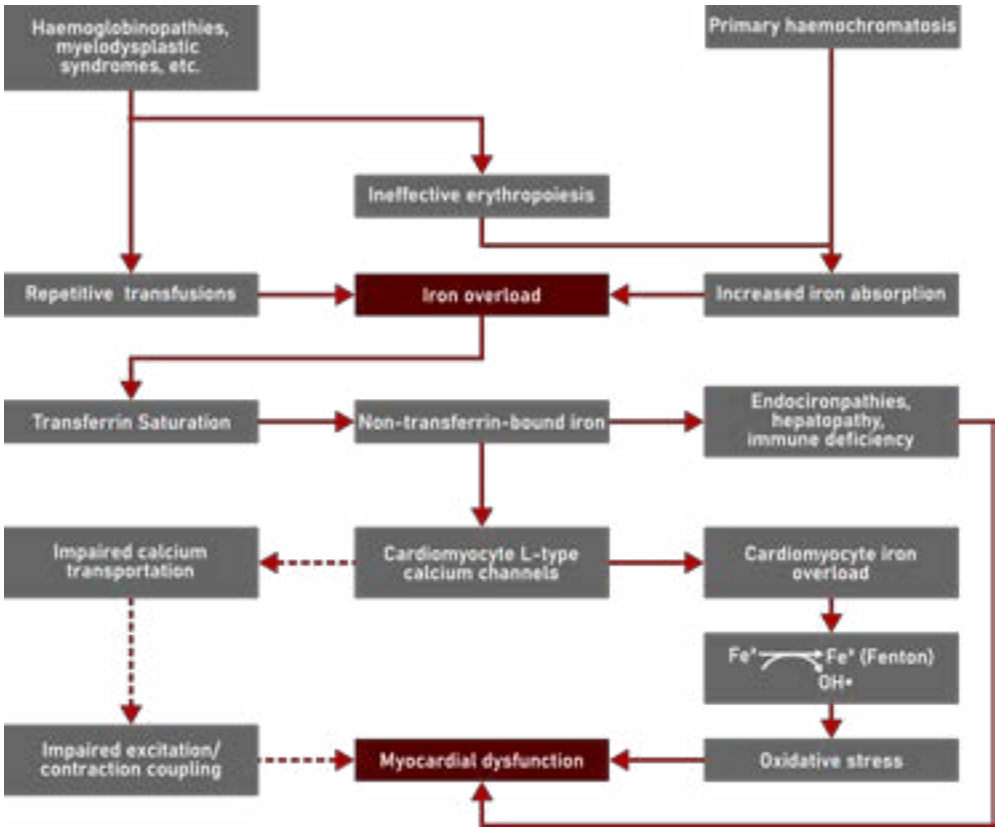
## Forms and phenotypes

A wide range of CV abnormalities are seen in patients with thalassaemia. The spectrum of cardiac disease includes left and/or right ventricular dysfunction, with or without heart failure, pulmonary hypertension, tachyarrhythmias such as atrial fibrillation, bradyarrhythmias such as atrioventricular block, valvular disease, pericarditis and myocarditis (Farmakis et al., 2017). Further CV disorders include, thromboembolic events, resulting from either venous or arterial thrombosis (Taher et al., 2006), cerebrovascular disease, manifested as either ischaemic or haemorrhagic stroke (Aessopos et al., 1997), and vascular abnormalities, including endothelial dysfunction and increased arterial stiffness (Cheung, Chan & Ha, 2002).

### *Iron overload cardiomyopathy*

In patients on regular blood transfusions who are not adequately chelated because of the lack of compliance with, or access to, iron chelation regimens, iron overload cardiomyopathy constitutes the main form of heart disease (Kremastinos & Farmakis, 2011; Kremastinos et al., 2010; Aessopos et al., 2004). The pathophysiology of iron overload cardiomyopathy has been reviewed elsewhere (Figure 2) (Kremastinos & Farmakis, 2011). This form of cardiomyopathy may be manifested as two different phenotypes, either as hypokinetic cardiomyopathy with reduced left ventricular (LV) contractility, with or without LV dilatation, leading to heart failure with reduced LV ejection fraction (HFrEF), or as restrictive cardiomyopathy with severely impaired LV diastolic function and preserved contractility, leading to heart failure with preserved LV ejection fraction (HFpEF). Many patients with severe iron loading persist with normal or near normal systolic LV function, sometimes for prolonged periods, but are at risk

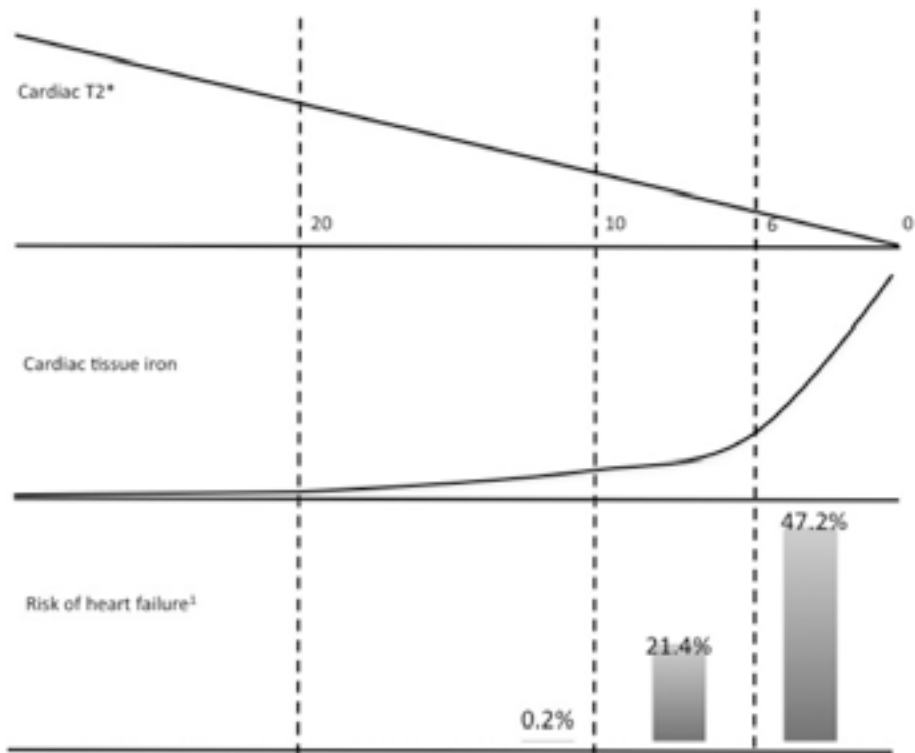
of acute decompensation, which may be rapid and often precipitated by intercurrent illness. Reduction in systolic function in the presence of significant myocardial iron overload ( $T2^* < 20\text{ms}$ ), from the often supra-normal ejection fraction (EF) associated with thalassaemia (EF  $> 60\%$ ), have a potentially dire prognosis.



**Figure 2.** Pathophysiology of iron overload cardiomyopathy (from Kremastinos et al., Circulation 2011;124: 2253-2263).

Rhythm disorders, including conduction abnormalities and ventricular arrhythmias have also been associated with severe myocardial iron overload. The risk of LV ejection fraction decline, heart failure and arrhythmias increases with the severity of cardiac iron overload, as evaluated by MRI  $T2^*$  (Figure 3) (Kremastinos & Farmakis, 2011; Kirk et al., 2009). Although the prevalence of cardiac abnormalities is very low in well-treated patient populations (Aessopos et al., 2004), the global prevalence of cardiac iron overload remains high, affecting 25% of TM globally (Koochi, Kazemi & Miri-Moghaddam, 2019). Importantly, iron overload cardiomyopathy may be reversed by intensive iron chelation therapy (Tanner et al., 2007, 2008).

**Figure 3.** Schematic representation of the relationship among cardiac iron overload, as estimated by magnetic resonance imaging T2\*, left ventricular ejection fraction and the risk of heart failure in patients with thalassaemia (from Kremastinos et al., *Circulation* 2011;124:2253-2263).



### **High-output failure**

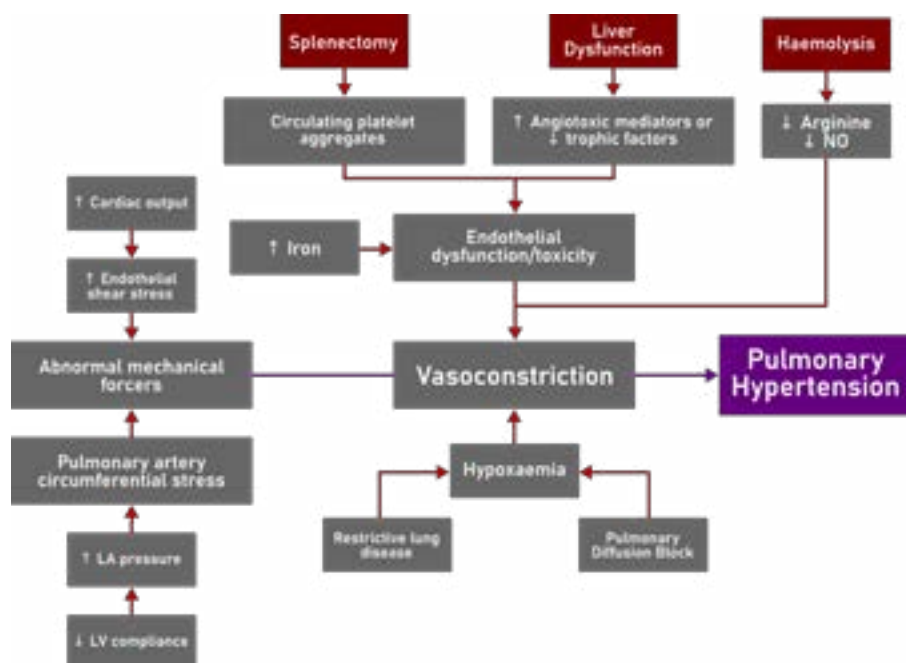
In the absence of regular transfusions able to maintain an adequate pre-transfusional haemoglobin concentration, chronic anaemia leads to a compensatory increase in cardiac output (Aessopos, Kati & Farmakis, 2007). High-output failure was actually the single leading cause of death in thalassaemia patients before the era of regular transfusion therapy (Engle, Erlandson & Smith, 1964). In addition, high-output state also contributes to heart disease in contemporary thalassaemia populations, including TI or sub-optimally transfused TM patients, to an extent that is directly related to the severity of residual chronic anaemia (Aessopos et al., 2004, 2005).

### **Pulmonary hypertension**

In non-regularly treated patients with TI, age-related pulmonary hypertension, leading to HFpEF, has been reported as the main form of heart disease, accounting for up to 60% of these patients in previous cohorts (Aessopos et al., 2005, 2001; Farmakis & Aessopos, 2011). In contrast, the prevalence of pulmonary hypertension in contemporary regularly treated thalassaemia populations is considerably lower approximating 2% (Derchi et al., 2014). The pathophysiology of pulmonary hypertension is multifac-

torial and summarised in Figure 4. In non-transfused TI patients, particularly those with previous splenectomy, a high occurrence of **thromboembolic complications** is also observed, including deep vein thrombosis, pulmonary embolism, stroke, portal vein thrombosis and others (Taher et al., 2006; Cappellini et al., 2000). The prevalence of thromboembolic disease is reported to be considerably lower in regularly treated TM patients (5%) compared to TI patients with a history of splenectomy (29%) (Cappellini et al., 2000).

**Figure 4.** Pathophysiology of pulmonary hypertension in thalassaemia. LA, left atrium.



### Other forms of cardiovascular disease

A high prevalence of **pericarditis** of nearly 50% has been reported in a historical cohort of young, poorly treated thalassaemia patients, before the era of any regular treatment (Engle, Erlandson & Smith, 1964). The reported prevalence has been considerably lower in more recent cohorts, including 5% in well-treated TM patients (Aessopos et al., 2004), and 8% in TI patients not receiving transfusion therapy (Aessopos et al., 2001).

A 4% prevalence of clinically suspected **myocarditis** has been reported by a large study in thalassaemia, with half of these cases having histologically confirmed myocarditis according to Dallas criteria (Kremastinos et al., 1995). Myocarditis was associated with acute or chronic heart failure and arrhythmias in these patients.

**Valvular heart disease** concerns an increasing prevalence of mainly mild to moderate disorders including mitral valve prolapse, mitral and aortic valve regurgitation and some sporadic cases of severe aortic stenosis (Aessopos et al., 2004, 2005, 2001; Farmakis et al., 2006). These lesions have been partly related to cardiac remodelling in the context of a high output state and, interestingly, to a coexisting disorder of elastic

tissue resembling hereditary pseudoxanthoma elasticum (PXE) (Aessopos, Farmakis & Loukopoulos, 2002). This disorder is primarily seen in middle-aged or elderly patients, usually with non-regularly treated TI, and is followed by cutaneous, ocular, vascular and valvular lesions.

**Cerebrovascular disease** in the form of either ischaemic or haemorrhagic stroke has been reported in patients with thalassaemia (Aessopos et al., 1997). Ischaemic strokes have been associated with underlying atrial fibrillation in patients with TM, while haemorrhagic strokes with the aforementioned PXE-like elastic tissue disorder in patients with TI, with PXE-related vascular lesions comprising calcification and increased risk of rupture and bleeding (Aessopos, Farmakis & Loukopoulos, 2002).

As previously stated, ageing in combination with coexisting CV risk factors such as diabetes mellitus, which may arise by suboptimal iron chelation, and smoking, may modify the clinical spectrum of the disease with the increasing occurrence of **age-related CV complications**, including atrial fibrillation, diastolic LV dysfunction, aortic valve stenosis, and perhaps vascular diseases such as systemic hypertension and coronary and cerebrovascular disease that have hitherto been uncommon in thalassaemia (Farmakis et al., 2020). In addition, already known disease-related complications such as pulmonary hypertension, endothelial dysfunction, increased vascular stiffness and PXE-like lesions also increase with age (Aessopos et al., 2001; Aessopos, Farmakis & Loukopoulos, 2002; Aessopos et al., 2007a).

## Assessment and monitoring

### *When to refer?*

**The burden and prognostic impact of CV disease have imposed the regular CV assessment of thalassaemia patients as an indispensable part of the multidisciplinary monitoring programs.** Regular CV monitoring should be performed on an annual basis in all thalassaemia patients, regardless of the presence of history or symptoms of CV disease. In the presence of CV disease, the frequency and content of CV assessment should be tailored to meet each patient's needs and shorter intervals (e.g., 3 or 6 monthly) may be applied according to severity.

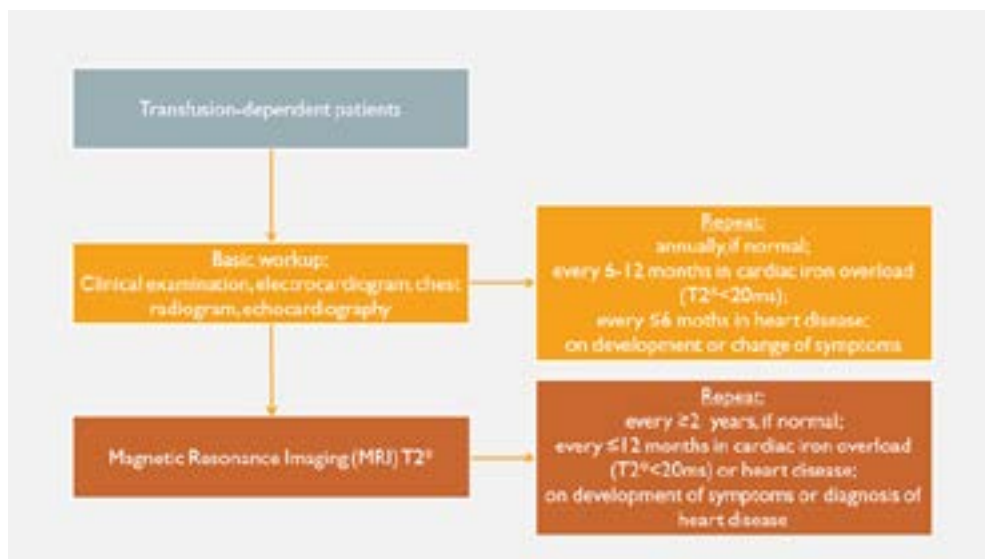
In addition, the development of new symptoms potentially suggestive of CV disease, such as dyspnoea, chest discomfort, frequent palpitations, syncope or fainting, lower limb oedema, fatigue or exercise intolerance should prompt immediate referral for CV evaluation. Importantly, symptoms such as fatigue, exercise intolerance or palpitations may also result from the anaemia caused by the main disease, while hepatic congestion due to right heart failure may be confused with disease-related hepatomegaly or other causes of abdominal discomfort. In these cases, the diagnosis of CV disease may be overlooked or delayed. Given the particularities of CV disease in thalassaemia, CV assessment and monitoring should ideally be performed either in a specialised cardiac clinic for haemoglobinopathies or in close consultation with such a clinic or a cardiologist with experience in thalassaemia heart disease.

### *How to assess?*

The regular annual basic CV assessment consists of (Figure 5):

- history taking;
- physical examination;
- resting electrocardiogram (ECG);
- transthoracic echocardiography (TTE).

**Figure 5.** Basic algorithm for the cardiac evaluation of patients with thalassaemia (modified from Farmakis et al, Eur J Heart Fail 2017;19:479-489). DFO, deferoxamine; DFP, deferiprone; HfrEF, heart failure with reduced left ventricular ejection fraction; MRA, mineralocorticoid receptor inhibitor; RAASi, renin-angiotensin-aldosterone system inhibitor.



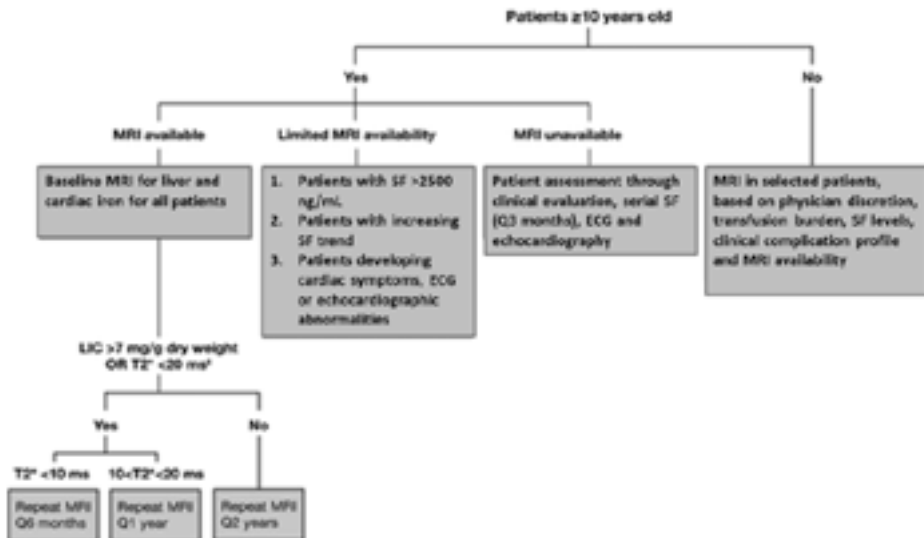
A typical TTE examination should assess and report cardiac cavity dimensions, LV wall thickness, LV systolic and diastolic function indices, right ventricular systolic function indices, tricuspid regurgitant flow velocity (TRV) to screen for pulmonary arterial hypertension, cardiac valve morphology and function and presence of pericardial fluid or other abnormalities such as shunts or intracavity masses. It should be stressed that increased Doppler velocities may reflect a high-output state due to chronic anaemia and not true heart disease. It is increasingly recognised that newer modalities such as strain measurements may be better at detecting subtle changes in function, predating changes in EF and thus give warning that increased chelation is advised.

Evaluation of cardiac iron content with cardiac MRI T2\* should be performed simultaneously with hepatic MRI T2\* in time intervals determined by the degree of iron load of the patient and the local protocols. Typically, the first MRI T2\* scan is performed 7–10 years after the initiation of blood transfusions and repeated every 2 years thereafter. Although the cardiovascular magnetic resonance (CMR) T2\* has developed into the 'gold standard' metric for iron assessment there is increasing realisation that newer CMR sequences and parameters may provide advantages in the future by drastically reducing scan times and cost and so increasing availability (Abdel-Gadir et al., 2016).

It should be stressed that, serum ferritin concentration, although widely available and used as a predictor of total iron load, correlates quite poorly with cardiac iron content and should not be used as a surrogate in this regard (Aessopos, Kati & Farmakis, 2007). In the absence of access to MRI T2\*, worsening of LV diastolic or systolic function indices by serial echocardiography, may serve as red flags for possible cardiac iron overload (Aessopos et al., 2007b; Maggio et al., 2013). It should be kept in mind, however, that cardiac dysfunction generally lags behind cardiac iron deposition by

several years, while cardiac iron clearance is also a very slow process requiring years to complete (Carpenter et al., 2011; Anderson et al., 2004). A proposed algorithm to guide MRI T2\* use according to availability is depicted in Figure 6 (Viprakasit et al., 2018).

**Figure 6.** A proposed algorithm to guide magnetic resonance imaging (MRI) T2\* use according to local availability (LIC, liver iron concentration; MRI, magnetic resonance imaging; SF, serum ferritin; modified from Viprakasit et al., Am J Hematol 2018;93: E135-E137)



Additional CV investigations may be needed in the presence of known CV abnormalities, symptoms suggestive of CV disease or abnormal findings during basic CV assessment. Such investigations may include (but are not limited to):

- ambulatory ECG monitoring for the evaluation of frequent palpitations or known arrhythmias or to assess the arrhythmogenic risk of patients with systolic LV dysfunction or heart failure;
- cardiac biomarkers, including cardiac troponins (e.g., in suspected myocarditis) or natriuretic peptides (e.g., for the evaluation of patients with known or suspected heart failure);
- cardiac magnetic resonance imaging for the more accurate assessment of cardiac cavities, systolic LV function and myocardial tissue characterization;
- exercise testing such as exercise ECG or ergospirometry for the assessment of functional capacity or arrhythmias;
- right cardiac catheterization for the evaluation of pulmonary artery pressure in patients with elevated TRV (e.g., >3 m/s, with optimal transfusion therapy and a pre-transfusional haemoglobin level close to 100 g/l) ;
- lung function tests, high-resolution chest computed tomography (CT), CT pulmonary angiography or lung scanning along with careful LV ventricular function evaluation are required for the comprehensive diagnostic assessment of confirmed pulmonary hypertension.



Assessment should always take into consideration the parameters of the main disease, such as blood transfusion and iron chelation programme, pre-transfusional haemoglobin level and serum ferritin concentration, as well as parameters related to other systems such as liver or endocrine disease. In addition, CV assessment should always be performed in close collaboration and communication with the thalassaemia physician who oversees the whole patient's monitoring and treatment.

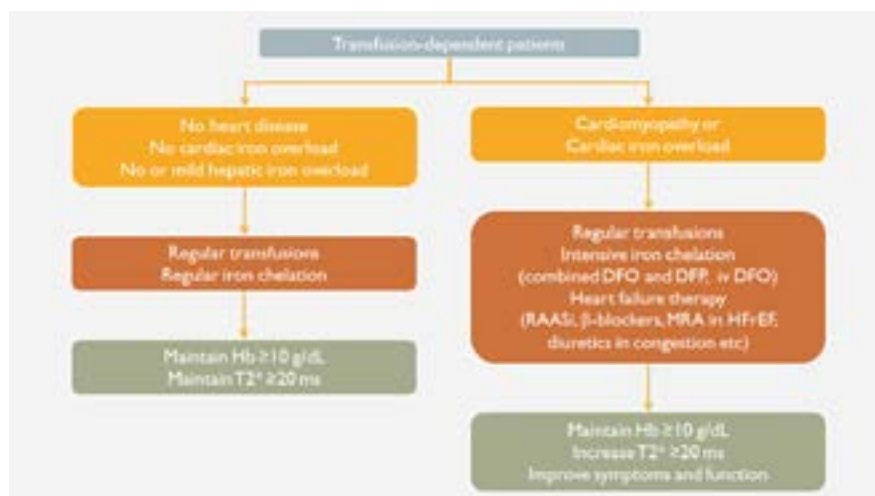
## Prevention and treatment

Prevention and treatment of CV disease in thalassaemia consists of two pillars, disease-specific therapy and cardioactive therapy.

### Disease specific therapy

The management of CV abnormalities in thalassaemia patients should take under consideration the pathophysiology and characteristics of the main underlying disease. As a result, disease-specific therapy, including regular blood transfusions aiming at a pre-transfusional haemoglobin level of 100 g/l and iron chelation regimens aiming at a cardiac T2\* value greater than 20 ms, hold the key role for the prevention and management of CV disease (Figure 7). Patients with high levels of serum ferritin or hepatic iron overload with or without cardiac iron overload should be treated with combined chelation therapy (e.g., deferoxamine and deferiprone), (Porter et al., 2013) while those with acute or advanced iron overload-induced heart failure may require continuous intravenous infusion of deferoxamine. (Tanner et al., 2008). It should further be stressed that cardiac dysfunction generally lags cardiac iron deposition by several years, while cardiac iron clearance is also a very slow process requiring several years to complete (Carpenter et al., 2011; Anderson et al., 2004). In the presence of significant cardiac iron overload (T2\* <20 ms), patients are at risk of rapid deterioration, even in the presence of normal or near normal systolic LV function, while a drop in LV EF may often carry a dire prognosis. As a result, chelation regimes must be adjusted to ensure a rapid fall in cardiac iron content (Pennell et al., 2013).

**Figure 7.** A basic algorithm for the management of thalassaemia patients on regular blood transfusions (DFO: deferoxamine; DFP: deferiprone; DFX: deferasirox; Hb: haemoglobin concentration; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin II receptor blockers; AFib: atrial fibrillation; modified from Farmakis et al, Eur J Heart Fail 2017;19:479-489).



## Cardioactive therapy

### CV Prevention

Undertaking a healthy lifestyle, in terms of diet, regular exercise, body weight control and smoking abstinence, in accordance with general guidelines on CV prevention is crucial for the prevention of CV disease in combination with proper disease-specific therapy. In addition, the management of CV risk factors and of complications arising from other systems or organs such as diabetes, thyroid disease, or liver disease, is also of key importance. CV prevention is becoming even more important today in view of the increasing risk of age-related complications in ageing thalassaemia patients.

### CV treatment

Diagnosis of a specific form of CV disease will prompt the initiation of the recommended therapeutic modalities, in accordance with the corresponding guidelines published by cardiology societies or associations such as the European Society of Cardiology (ESC) or the American Heart Association (AHA); the description of the management of each form of CV disease falls outside the scope of this guideline. The CV management of patients should take under consideration the following tips:

- CV disease should primarily prompt optimisation of disease-specific therapy, including blood transfusions and iron chelation as well as investigation and treatment of comorbid conditions such as endocrine or metabolic disease.
- Cardiac dysfunction and heart failure due to iron overload may be reversed with intensified iron chelation therapy and this possibility should be considered in decision making regarding more permanent CV interventions such as implantable cardioverter defibrillator (ICD) implantation (using MRI conditional devices), catheter ablation of arrhythmias, permanent ventricular assist device implantation or cardiac transplantation.
- A pacemaker may be needed for the management of atrioventricular block, often related to iron overload; in this case leads should be MRI conditional to allow periodic evaluation of iron overload by the T2\* technique.
- Thalassaemia patients generally have low blood pressure levels, and therefore use of blood pressure-lowering medications such as renin-angiotensin-aldosterone system inhibitors should be cautious. In addition, the use of vasopressors and other blood support therapies in patients with hypotensive heart failure should rather be targeted to renal perfusion and other surrogates instead of blood pressure values.
- Thalassaemia patients often have restrictive cardiac physiology, usually due to iron overload, and increased vascular stiffness, that may render them sensitive to hypovolaemia during diuresis.
- Anticoagulation in patients with atrial fibrillation may be challenging; non-regularly treated patients with previous splenectomy carry an increased risk of thromboembolism, while patients with pseudoxanthoma elasticum-like lesions may carry an increased risk of bleeding (Aessopos, Farmakis & Loukopoulos, 2002).
- The use of general risk prediction scores such as the CHA2DS2-VASc may be inappropriate for thalassaemia patients, as it may underestimate their potential risk.

## Summary and Recommendations

CV disease has been the single leading cause of mortality in thalassaemia, but is now significantly declining in patient populations with access to modern multi-disciplinary care and monitoring. The pathophysiology and phenotypes of CV disease depend on the interaction between the main disease and the applied therapy. The CV spectrum consists of a wide range of CV disorders. Among them, iron overload cardiomyopathy has long been the main form of heart disease in thalassaemia patients treated with transfusions but is now being effectively prevented and managed with MRI-guided contemporary chelation therapy. Regular CV assessment should be part of a multi-disciplinary monitoring programme and should ideally be performed by or in consultation with clinics or physicians with experience in CV disease in haemoglobinopathies and in close collaboration with the attending thalassaemia physician. The prevention and treatment of CV disease, besides cardioactive therapies and interventions, relies crucially on the optimisation of disease-specific therapy and the successful management of comorbid conditions. A lifestyle that promotes CV health is an important part of CV prevention, while the particularities of CV disease in thalassaemia should be taken under consideration in the management of patients. Most importantly, cardiac dysfunction and heart failure may be reversible by timely therapy.

## Recommendations and grade of evidence

1. Regular CV assessment should be part of a patient's multidisciplinary monitoring programme (C).
2. CV assessment and management should ideally be performed by or in consultation with clinics or physicians with experience in CV disease in haemoglobinopathies and in close collaboration with the attending thalassaemia physician (C).
3. MRI T2\* guided chelation therapy represents the best available approach to prevent cardiac dysfunction related to iron overload (B).
4. In places lacking CMR T2\* assessments, worsening of LV function in serial echocardiograms in transfusion-dependent patients may be used as a red flag for iron toxicity and should prompt aggressive and sustained escalation of chelation therapy (B).
5. Echocardiographic screening for pulmonary hypertension should be part of annual CV assessment. Patients having a TRV greater than 3 m/s should undergo cardiac catheterisation to confirm the diagnosis of pulmonary hypertension if a proximate cause cannot be identified and corrected (B).
6. Combined therapy with deferoxamine and deferiprone represents the best available intensive chelation for thalassaemia major patients with cardiac iron overload, with or without overt cardiac dysfunction or heart failure (B).
7. Diagnosis of CV disease should prompt optimisation of disease-specific therapy in addition to cardioactive treatment (C).
8. Screening and treatment of endocrine and metabolic comorbidities is crucial for the prevention and management of CV disease (C).
9. Management of cardioactive therapies must account for a patient's unique physiology compared with the general population (C).
10. Cardiac abnormalities including ventricular dysfunction, heart failure and arrhythmias, are often reversible following intensification of disease-specific therapy, albeit after several weeks or months (C).
11. Lifestyle choices that promote CV health (absence of smoking, physical exercise, weight control, healthy diet) should be vigorously promoted in thalassaemia patients (C).

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## CHAPTER 6

### Liver disease

#### Introduction

**Iron overload and viral hepatitis are the two main causes of liver disease in patients with thalassaemia leading to chronic inflammation, fibrosis and ultimately cirrhosis.**

The approach to liver disease includes clinical parameters, biochemical/serological/molecular tests and imaging techniques in order to identify the aetiological factor(s) and assess the severity of the injury.

**Clinical examination** may reveal signs of systemic iron excess such as skin pigmentation and hepatomegaly while in later stages stigmata of chronic liver disease (i.e. palmar erythema, spider naevi), ascites and encephalopathy may follow.

**Laboratory tests** may show moderate elevations (2-3 times higher than the upper limit of normal) of the aminotransferases, aspartate transaminase (AST) and alanine transaminase (ALT) and a mild increase of alkaline phosphatase and gamma glutamyl transferase (γGT). In the presence of severe hepatic impairment, prolonged prothrombin time, low albumin and high serum bilirubin are found.

With regard to **imaging techniques**, ultrasonography (US) remains the gold standard to evaluate hepatic morphology and to recognise signs of fibrosis and cirrhosis. Portal hypertension can be also assessed by duplex ultrasonography or spectral Doppler imaging and colour Doppler imaging (Procopet & Berzigotti, 2017). Hepatic transient elastography (TE), a pulse-echo ultrasound technique, has replaced biopsy as a non-invasive diagnostic method for staging liver fibrosis. Liver stiffness, measured by TE, has also been shown to correlate with stages of fibrosis in patients with thalassaemia (Ferraioli et al., 2016; Paparo et al., 2013; Di Marco et al., 2010a), although specific cut-off values for the stage of fibrosis need to be defined. Moreover, it is believed that iron overload may affect the accuracy of TE in assessing liver fibrosis (Fraquelli et al., 2010). As compared to liver biopsy which is the gold standard examination for the evaluation of liver fibrosis but consists an invasive technique with potential complications, TE is an easy-to-perform diagnostic tool with moderate to high accuracy, especially in thalassaemic patients with chronic HCV infection (Poustchi et al., 2013).

#### A) Hepatic Iron Overload

##### 1. Pathophysiology

Hepatocytes are the main site of iron storage and also the principal site of synthesis of hepcidin, a hormone responsible for the regulation of iron transport to the extracellular space through activation of the cellular iron exporter, ferroportin. In cases of thalassaemia, the excess iron due to frequent transfusions initially accumulates in macrophages and later in hepatocytes, while hypoxia caused by dyserythropoiesis intensifies erythropoietin production leading to further suppression of hepcidin production and increased transferrin bound iron (TBI) and non-transferrin bound iron (NTBI) plasma levels. (Chaston et al., 2011).

Excessive levels of iron in the blood stream exceed the capacity of plasma transferrin to bind iron and lead to the appearance of NTBI in the plasma which



consists a highly toxic iron species. The liver rapidly clears NTBI from the serum through divalent metal transporter 1 (DMT1) and incorporates it into ferritin. However, once the protective storage efficiency of ferritin is exceeded, unbound iron accumulates in the hepatocytes and leads to severe oxidative stress and overproduction of toxic reactive oxygen species (ROS), which cause lipid peroxidation and protein damage (Figure 1). The subsequent hepatic inflammation and necrosis lead to fibrosis and cirrhosis (Sikorska, Bernat & Wroblewska, 2016). Development of fibrosis in the absence of inflammation has also been found to be associated with iron accumulation in the liver and relates to direct activation of stellate cells (Philippe, Ruddell & Ramm, 2007).

## 2. Iron overload monitoring

Magnetic resonance imaging (MRI) R2 or R2\* using liver iron content (LIC) (mg of iron/g dry weight) is considered the method of choice for iron load monitoring in thalassaemic patients. A yearly LIC assessment should be performed in all patients in order to monitor chelation therapy effectiveness (Brittenham, 2011). LIC values greater than 7mg Fe/g dry weight and 15mg/g dry weight are associated with moderate and severe iron overload respectively (Allali et al., 2017) while LIC>16mg/g dry weight is associated with an increased risk of hepatic fibrosis. MRI iron assessment of the liver is also mandatory as a pre- and post-transplant evaluation in cases of haematopoietic stem cell transplantation (HSCT) (Mavrogeni et al., 2018).

Serum ferritin (SF) is the most widely used marker of total body iron load. SF values higher than 2000 ng/ml are associated with liver iron overload (Krittayaphong et al., 2018). However, SF measurement has low specificity and should be interpreted with caution since it can be elevated also in the case of concomitant inflammation, malignancy or oxidative stress (Chirico et al., 2015; Puliyl et al., 2014).

Transient elastography has been used for the assessment of liver fibrosis in thalassaemic patients with initially promising results. Its value as a means of estimation of iron overload has been examined in recent studies. TE values were found to be strongly correlated to MRI T2\* results (Pipaliya et al., 2017). LIC values, SF levels and TE stiffness measurements in patients with sickle cell anaemia were also correlated and found to improve after successful chelation therapy (Delicou et al., 2018), although this correlation was not confirmed by other studies (Ou et al., 2017). Further studies focusing on the effectiveness of TE in assessing liver iron overload need to be conducted in order to draw robust conclusions.

## 3. Treatment

Three chelators are currently available for the treatment of iron overload in thalassaemic patients: deferoxamine (DFO), deferiprone (DFP) and deferasirox (DFX). They are all proven to effectively reduce LIC (Maggio et al., 2011, 2002; Taher et al., 2009), while specifically DFX may have a positive effect on liver fibrosis as well (Deugnier et al., 2011; Maira et al., 2017; Sousos et al., 2018).

## B) Viral Hepatitis

### Hepatitis C virus infection

Chronic infection with hepatitis C virus (HCV) in combination with iron load hepatotoxicity remains a major risk factor for acceleration of liver fibrosis in thalassaemic patients. Antiviral treatment used to be quite challenging, mainly due to the haemolytic effect of ribavirin and interferon (IFN)-induced cytopenias (Kowdley, 2005). Recent advances in HCV therapies have improved treatment success rates and minimised



adverse events significantly.

## 1. Hepatitis C virus epidemiology

The prevalence of HCV infection among thalassaemic patients shows great variation in published data (Di Marco et al., 2010b), ranging between 19 and 75% and depending on the region where it was studied (Behzadifar, Gorji & Bragazzi, 2018; Jang et al., 2017; Kountouras et al., 2013; Triantos et al., 2013). The most frequent HCV genotype (GT) in thalassaemic patients is GT 1b, reflecting the distribution of genotypes in the general population per geographical area (Di Marco et al., 2010b; Dodd, Notari & Stramer, 2002). More recent epidemiological studies in specific eastern Mediterranean countries describe the increase of GT3 prevalence (Ahmadi-Ghezeldasht et al., 2018) as well. The implementation of systematic blood donor screening with molecular methods in the last two decades has dramatically decreased HCV transmission through transfusions in Western countries (Dodd et al. 2002), although such methods may not yet be extensively applied in developing countries.

## 2. Hepatitis C virus infection, diagnosis and treatment

All thalassaemic patients who were transfused before 1991 should be screened for HCV infection. HCV diagnosis requires two steps: a) blood testing for anti-HCV antibodies, b) HCV-RNA by polymerase chain reaction (PCR) testing in the case of positive anti-HCV result (Figure 2). The diagnosis of chronic HCV infection must be followed by fibrosis stage evaluation with TE, hepatic function assessment and genotype determination when a pangenotypic regimen is not available in order to select the best treatment option (EASL HCV Guidelines, 2020).

The HCV treatment landscape has changed dramatically in recent years. Direct acting anti-viral agents (DAAs) have emerged (Table 1), yielding > 95% rates of sustained virological response (SVR) which is the equivalent of complete cure. DAAs have replaced interferon in the treatment of HCV infection and have combined efficacy with a very safe profile. Treatment duration varies between 8 and 12 weeks, with ribavirin co-administration not being needed in many cases. Pangenotypic regimens that provide high SVR rates across genotypes are also available and have further simplified therapy (Table 1). As a result, the indication for HCV treatment in the thalassaemic population is similar to that of the general population (EASL HCV Guidelines, 2020). Specific phase 3 (Hézode et al., 2017), randomised (Mangia et al., 2017) and real-life studies (Sinakos et al., 2017) have confirmed DAAs effectiveness in thalassaemic patients, showing SVR rates comparable to those in the general population and more significantly without any additional safety concerns. It should be noted that before treatment initiation, drug-drug interactions between concomitant medications and DAAs should be checked. Liverpool University site for Hepatitis Drug interactions (<https://hep-druginteractions.org/query>) is a very useful tool for everyday clinical practice. In general, co-administration of anti-viral agents with chelation treatment is not contra-indicated, while specific caution should be taken with certain anti-arrhythmic drugs that share common metabolic paths with DAAs with the consequence that co-administration may affect drug levels (EASL HCV Guidelines, 2020).

## Hepatitis B virus infection

Chronic hepatitis B virus (HBV) infection was reported to be present in 5% of thalassaemic patients based on an Italian registry (Borgna-Pignatti et al., 2014). This rate could be higher in countries where blood donors are not adequately assessed. Diagnosis is made by the presence of detectable HBV surface antigen

(HBsAg) in serum for more than 6 months. HBV DNA levels in serum detected by PCR, presence of HBV e antigen (HBeAg), fibrosis stage and transaminase levels guide the decision for treatment. Currently approved drugs for treatment of chronic HBV infection include pegylated interferons and oral nucleoside/nucleotide analogues (EASL HBV Guidelines, 2017), but interferons should be avoided due to their myelosuppressive effect (Kowdley, 2005). Prevention is mandatory and HBV vaccination is strongly recommended. Immunisation of thalassaemic children for HBV has been proven to be effective without any tolerance issues (Sharifi, Milani & Shooshtari, 2010).

## **Hepatitis E virus (HEV) infection**

Hepatitis E virus (HEV) infection rates have been increasing lately, even in high-income countries (Capai, Charrel & Falchi, 2018). Despite the fact that the predominant route of HEV transmission is consumption of contaminated food, it is estimated that 1/5000 blood donations could be contaminated (Capai, Charrel & Falchi, 2018). Since acute HEV infection may have severe complications in patients with pre-existing liver disease, testing for IgM anti-HEV antibodies and HEV viral load assessment should be performed in cases of acute hepatitis or unexplained flares of chronic liver disease (EASL HEV Guidelines, 2018) in thalassaemic patients. A case of acute hepatitis E infection in a thalassaemic patient has been already reported (Politis et al., 2018). In severe cases, treatment guidelines include ribavirin and IFN, although further investigation is needed (EASL HEV Guidelines, 2018). Universal screening of blood donors is not a worldwide strategy due to cost and differences in the frequency of immunoglobulin (Ig) G anti-HEV antibodies in the general population.

## **C) Hepatocellular Carcinoma**

### **1. Incidence and Pathophysiology**

The incidence of hepatocellular carcinoma (HCC) in thalassaemic patients has increased lately due to the prolonged survival achieved by effective iron chelation therapies and decrease of heart-associated complications (Borgna-Pignatti et al., 2014). Co-existence of specific risk factors such as iron overload and HCV or HBV infection contribute to the development of HCC and provide an explanation for the reported younger age of HCC diagnosis (Mancuso et al., 2006) in this population. Furthermore, there are reported cases of HCC in non-cirrhotic thalassaemic patients (Deugnier & Turlin, 2001; Papadopoulos et al., 2020). HCV infection and iron overload are reported to work in a synergistic way towards HCC development. More specifically, it has been described that HCV induces reduction of serum hepcidin (Girelli et al., 2009) promoting further accumulation of total iron, while unbound iron has been connected to carcinogenesis through the production of ROS. Moreover, frequent blood transfusions cause continuous antigenic stimulation which could potentially have an immunomodulatory effect (Refaai & Blumberg, 2013).

### **2. Screening for HCC**

Screening patients with thalassaemia for HCC has a pivotal role in their clinical management. Abdominal US should be performed every 6 months in cirrhotic patients (EASL HCC Guidelines, 2018), while alpha fetoprotein (AFP) is not considered a reliable marker in general and especially in thalassaemic patients (Fragatou, Tsourveloudis & Manesis, 2010). The early onset of HCC and the observation of HCC cases even in non-cirrhotic patients indicate that biannual screening should be extended not

only to cirrhotic patients, but also to every patient with high risk factors such as HCV and/or HBV infection, non-transfusion-dependent thalassaemia (NTDT) with LIC $\geq$  5 mg Fe/g dry weight, transfusion-dependent thalassaemia (TDT) with LIC $\geq$  7 mg Fe/g dry weight or serum ferritin  $\geq$ 1000 ng/ml (Moukhadder et al., 2017). Annual MRI R2 or R2\* to evaluate LIC also contributes on early HCC prevention through strict iron overload monitoring and treatment (Taher et al., 2008).

### 3. Treatment

Few data exist on efficacy of HCC treatments in patients with thalassaemia. Treatment strategies according to stage of HCC and severity of liver disease used for the treatment of HCC in the general population (i.e. surgical resection, transarterial chemoembolisation (TACE), percutaneous radiofrequency ablation (RFA) and ethanol injection) have been used with success (Mancuso, 2010; Mancuso et al., 2006, 2005). However, the administration of sorafenib, a multi-kinase inhibitor indicated for advanced HCC did not yield adequate response in three thalassaemic patients with HCC in an Italian study (Restivo Pantalone et al., 2010). Further multi-centre prospective studies need to be conducted in order to clarify the effectiveness of the new immunomodulatory anti-cancer drugs in thalassaemic patients. Liver transplantation for the treatment of HCC in thalassaemic patients remains controversial. Two studies by Manusco et al. and Restivo Pantalone et al. have shown promising results, but larger studies are needed to clarify the role of liver transplantation in these patients (Mancuso, 2010; Restivo Pantalone et al., 2010).

Figure 1.

#### Causes of liver disease in thalassaemia.

[DMT1, divalent metal transporter 1; FPN, ferroportin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; ROS, reactive oxygen species, NTBI, non-transferrin bound iron; TBI, transferrin bound iron; TFR1 transferrin receptor 1].

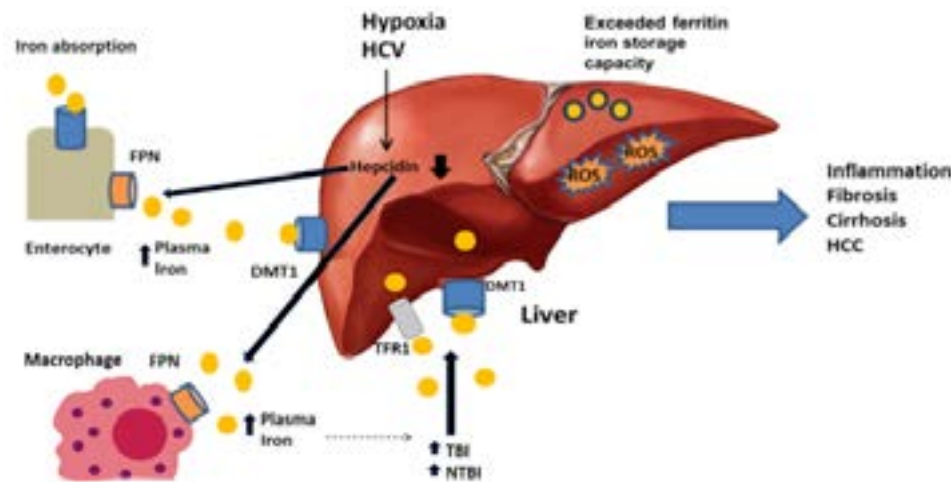


Figure 2. Hepatitis C virus (HCV) diagnosis algorithm



**Table 1.** Summary of approved Direct Anti-viral Agents(DAAs) per Hepatitis C Virus Genotype (EASL 2020)

DAA Regimen	Approved Genotypes
Grazoprevir/elbasvir	1b
Sofosbuvir/velpatasvir	1, 2, 3, 4, 5, 6
Glecaprevir/pibrentasvir	1, 2, 3, 4, 5, 6
Sofosbuvir/velpatasvir/voxilaprevir	1, 2, 3, 4, 5, 6 (2nd line)

## Summary

Iron overload and viral hepatitis are the two main causes of liver disease in patients with thalassaemia leading to chronic inflammation, fibrosis and ultimately to cirrhosis. In iron overload states, unbound iron accumulates in the hepatocytes and leads to severe oxidative stress with overproduction of toxic ROS which lead to severe hepatic inflammation and fibrosis (Sikorska, Bernat & Wroblewska, 2016). LIC estimated by MRI R2 or R2\* is currently the method of choice to monitor iron overload specifically in this organ (Brittenham, 2011). Current iron chelators reduce significantly LIC and slow down liver fibrosis (Borgna-Pignatti et al., 2004; Cappellini et al., 2006; Maggio et al., 2011).

Hepatitis C virus (HCV) or hepatitis B virus (HBV) may further accelerate liver fibrosis in thalassaemic patients. Adequate blood donor checking, widespread vaccination for HBV and access to anti-viral treatments are mandatory to minimise this risk (Borgna-Pignatti et al., 2014; Di Marco et al., 2010b). More specifically, a new generation of highly effective and safe DAAs have currently replaced interferon-based regimens in the HCV treatment landscape and have simplified treatment approaches for thalassaemic patients with chronic HCV infection (EASL HCV Guidelines, 2020).

The achievement of prolonged survival of thalassaemic patients in recent years is associated with effective iron overload treatments and lower risk of heart disease. However, there are several reports of a recent increase in HCC incidence (Borgna-Pignatti et al., 2014). Based on this observation, biannual screening for HCC with ultrasound for all thalassaemic patients is strongly recommended.

## Recommendations and grade of evidence

1. MRI R2 or R2\* is the method of choice to assess liver iron concentration (LIC) and monitor chelation therapy effectiveness (A).
2. Deferoxamine, deferiprone and deferasirox are effective in decreasing total body iron burden as well as LIC (A).
3. Screening for HCV and HBV chronic infection is recommended in thalassaemic patients (A).
4. Vaccination against hepatitis B is recommended in all patients with thalassaemia who are seronegative for HBV markers (A).
5. If anti-HCV antibodies are detected, the presence of HCV-RNA in serum or plasma should be determined to identify patients with chronic infection (A).
6. The new IFN-free, ribavirin-free direct acting anti-viral drugs for hepatitis C are effective and safe in patients with thalassaemia (B).
7. Serum transaminases, HBV DNA levels and liver fibrosis assessment by transient elastography are the main tools to guide treatment decision in HBV-chronic hepatitis (A).
8. Oral nucleoside and nucleotide analogues are well tolerated and effective drugs for HBV-chronic hepatitis, though loss of HBsAg remains a rare event (A).
9. Biannual ultrasound screening for hepatocellular carcinoma should be performed in all thalassaemic patients (B).

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# CHAPTER 7

## Growth abnormalities

### Introduction

Growth failure in  $\beta$  thalassaemia major (TM) has been recognised for many years, and has persisted despite major therapeutic advances (De Sanctis et al., 2013; Tumba et al., 2007).

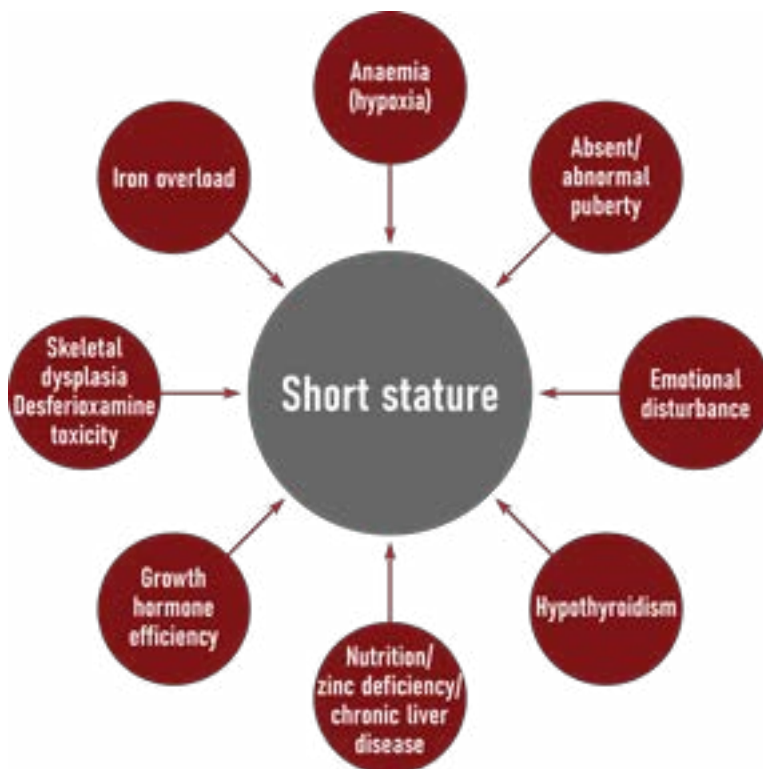
In the past, prevalence of growth failure and short stature in children with thalassaemia varied from 30 to 60% in most studies (Table 1). In the current era, the adherence to modern transfusion and iron chelation protocols and avoidance of iron chelator overdosage has clearly reduced the risk of short stature and may have potentially enhanced endocrine development in children with TM.

Country survey	Growth Delay
Cyprus	35%
Greece	32%
Italy	---
Egypt	62%
Qatar	42%
Romania	54%
N. America	----
TIF	30.8%

**Table 1.** Prevalence of short stature (< 3° percentile) in thalassaemia

The short stature encountered in thalassaemia is often disproportionate with a low upper segment to lower segment ratio. The exact reason is not clear and an interplay of multiple factors is responsible, factors such as iron overload (impaired cartilage growth), early use of desferrioxamine (DFO) for chelation and delayed puberty/hypogonadism (De Sanctis et al., 2013).

The child with TM has a particular growth pattern, which is relatively normal until age 9-10 years; after this age a slowing down of growth velocity and reduced or absent pubertal growth spurt are observed. The pathogenesis of growth failure is multifactorial. The fundamental problem is the free iron and haemosiderosis-induced damage of the endocrine glands. Additional factors may contribute to the aetiology of growth delay including chronic anaemia and hypoxia, chronic liver disease, zinc, folic acid and other nutritional deficiencies, intensive use of chelating agents, emotional factors, endocrinopathies (hypogonadism, delayed puberty, hypothyroidism, disturbed calcium homeostasis and bone disease) and dysregulation of the growth hormone – insulin-like growth factor 1 axis (GH-IGF-1) (Figure 1) (Skordis & Kyriakou, 2011).



Three phases of growth disturbances according to age of presentation are well recognised, and have different aetiologies: in the first phase growth disturbance is mainly due to hypoxia, anaemia, ineffective erythropoiesis and nutritional factors. During late childhood (second phase), growth retardation is mainly due to iron overload affecting the GH-IGF-1 axis and other potential endocrine complications (Table 2). After the age of 10-11 years (third phase), delayed or arrested puberty is an important contributing factor to growth failure in adolescent thalassaemics, who do not exhibit a normal growth spurt (Figure 2).

Country	Prevalence %
Qatar – Doha (n = 81)	45%
Egypt – Alexandria (n = 120)	40%
Egypt – Cairo (n = 30)	33%
Greece – Athens	38%
Turkey – Izmir	40%
Iran – Shiraz (n = 138)	(53.5%) boys, (46.5%) girls
Italy – Ferrara (adults)	8% males, 9% females
Italy – Rome (adults)	32%
Italy – Milan (adults)	22% (severe) 19% (partial)

**Table 2.** Prevalence of growth hormone deficiency in children, adolescents and young adults with thalassaemia

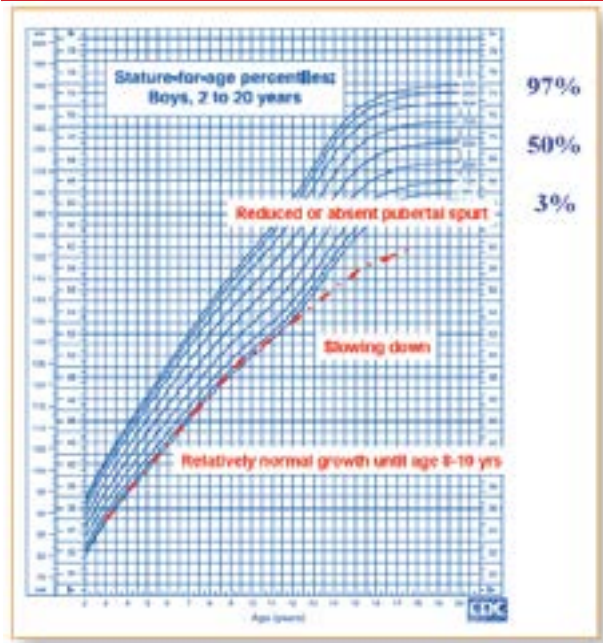


Figure 2. Typical growth curve in a boy with thalassaemia major

### Assessment of personal history in a thalassaemic child with short stature or poor growth velocity/year

The following points should be taken into consideration:

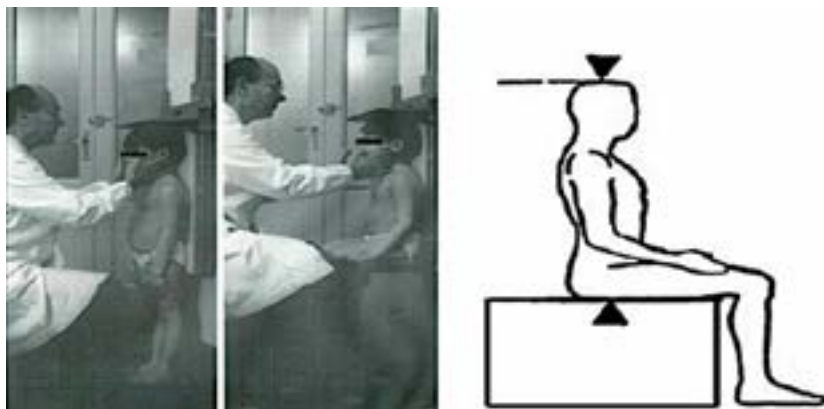
- Onset of disease and the need for blood transfusions. Patients with the  $\beta^0/\beta^0$  genotype have a significantly higher prevalence of growth retardation compared to those with the  $\beta^0/\beta^+$  and  $\beta^+/\beta^+$  genotypes.
- Pre-transfusion haemoglobin level
- Annual packed red blood cells requirement
- Chelation therapy (type, dose, compliance)
- Serum ferritin levels
- Associated comorbidities (endocrine complications, chronic liver disease, chronic cardiac failure, human immunodeficiency virus (HIV) infection).

### Diagnosis and investigations

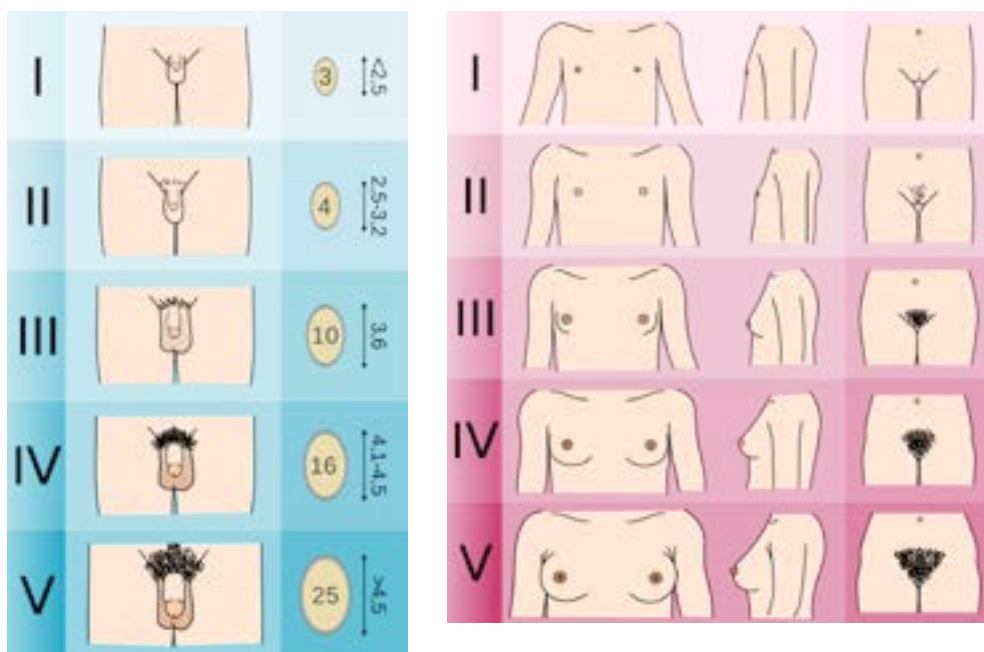
Diagnosis requires careful clinical evaluation to establish:

- Short stature – height below the 3rd centile for sex and age (based on national growth charts), and/or
- Slow growth rates – growth velocity expressed in cm/year, below the 10th centile for age and sex (based on growth velocity charts), and /or
- Signs of other pituitary hormone deficiencies (e.g. gonadotrophins, growth hormone, central hypothyroidism)
- Signs of other possible causes of retarded growth (nutritional deficiencies, chronic hepatic disease, chronic heart failure).

The first step in the management of short stature or retarded growth is the regular (six-month intervals) and accurate measurement of standing and sitting height (Figure 3) and pubertal staging (Figure 4). **Growth data are plotted on ethnically adjusted charts or internationally (World Health Organization) adjusted charts.** Interpretation of absolute height must consider the height of the parents.

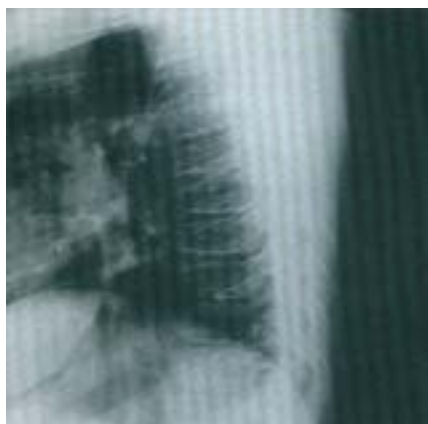


**Figure 3.** Measurements of standing height and sitting height with Harpenden stadiometer in absence of sitting height table.



**Figure 4.** Pubertal assessment in males and females according to Tanner. For the male figure, the (average) size of the testis [cm] and the capacity in mL is indicated. (Source: Dr Michał Komorniczak (Poland)).

In subjects with disproportionate short stature, radiographs of tibia and spine to exclude the presence of platyspondylosis or metaphyseal cartilaginous dysplasia changes (Figure 5).



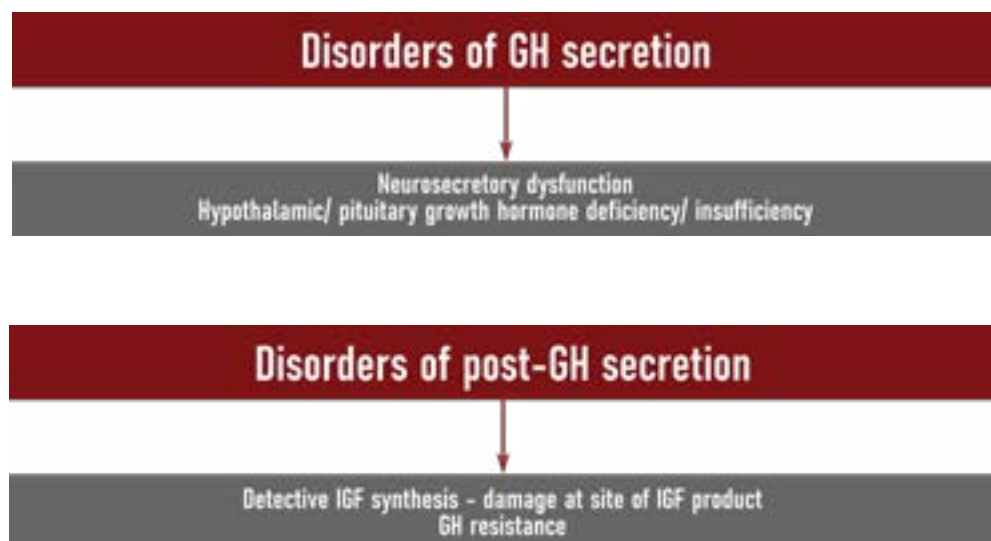
**Figure 5.** Platyspondylosis of the vertebral bodies in a thalassaemia major patient

### **Annual growth screening**

This should be started from the age of 9 years, or earlier if clinically indicated. The following tests and assessments are recommended:

1. Serum thyroid stimulating hormone (TSH) and free thyroxine (FT4).
2. Serum calcium, ionised calcium, inorganic phosphate, magnesium and alkaline phosphatase.
3. Serum IGF-1 and insulin-like growth factor-binding protein 3 (IGF BP-3) in growth screening are useful indicators of growth hormone secretion and nutrition, bearing in mind that chronic liver diseases and malnutrition may interfere with their secretion.
4. Serum zinc (in selected cases).
5. Screening for coeliac disease.
6. X- ray of wrist and hand, tibia and spine should be evaluated in patients with TM who have body disproportion to exclude the presence of platyspondylosis or metaphyseal cartilaginous dysplasia changes.
7. Assessment of growth hormone (GH) secretion: significant GH insufficiency may be diagnosed by a reduced response of GH to two provocative tests (GH peak <10 ng/ml) in children and adolescents and 3 ng/ml in adults). A list of the classification of the GH-IGF disorders is given in Figure 7.
8. Magnetic resonance imaging (MRI) of the hypothalamic–pituitary region is useful to evaluate pituitary iron overload as well as the size of pituitary gland (atrophy).
9. Luteinising hormone (LH), follicle-stimulating hormone (FSH) and sex steroids, starting from the pubertal age.





**Figure 6.** Level of defect in the GH-IGF 1 axis in thalassaemia. GH, growth hormone; IGF, insulin-like growth factor.

At present, there are no guidelines in the literature for the assessment of GH in adult patients with TM. The American Association of Clinical Endocrinologists (AACE) advises that one GH stimulation test is sufficient if clinical suspicion is high, such as in patients with at least one other pituitary hormone deficiency and low or low-normal IGF 1 level. However, in patients with TM and chronic liver disease, IGF 1 levels are low and may have a low diagnostic sensitivity for growth hormone deficiency (GHD) (Soliman et al., 1999).

GHD in adults is a clinical syndrome associated with lack of positive well-being, depressed mood, feelings of social isolation, decreased energy, alterations in body composition with reduced bone and muscle mass, diminished exercise performance and cardiac capacity and altered lipid metabolism with increase in adiposity (Soliman et al., 2013).

In patients with chronic diseases, the clinical evaluation of GHD is difficult because signs and symptoms may be subtle and nonspecific, and universal provocative testing in all patients is difficult because the approach is cumbersome and expensive. In addition, TM patients with GHD may have deficiencies of other pituitary hormones, further complicating the clinical picture. This contrasts with childhood-onset GHD where growth failure acts as a useful biological marker of GHD.

### **Criteria for the assessment of GH secretion in adult patients with thalassaemia**

The following recommendations for GH testing in adults with TM have been reported by Soliman et al (Soliman et al., 2014, 2013):

- Short stature (height  $\geq 2.5$  SD below the mean),
- Severe and/or prolonged iron overload,
- Dilated cardiomyopathy,



- Very low IGF 1 levels, especially in those patients with childhood onset GHD, in the presence of pituitary iron deposition and/or atrophy,
  - Severe osteoporosis and/or serum IGF 1 level ( $\geq 2$  SD below the mean),
- Furthermore, in adult TM patients with normal liver function and IGF-1 level < 50th centile, for the standards available in adult TM patients, a GH assessment should be also considered (De Sanctis et al., 2014).

## Treatment

Prevention and treatment of growth abnormalities in patients with TM should include (Figure 7):

- Adequate blood transfusion to maintain pretransfusion haemoglobin level >90 g/l.
- Adequate chelation to attain serum ferritin < 1,000 ng/ml.
- Use of new iron-chelators with lower toxicity on the skeleton and with better patient compliance.
- Correction of nutritional deficiencies (protein-calorie, folate, vitamin D, vitamin A, zinc, carnitine) when suspected.
- Oral zinc sulphate supplementation should be given to patients with proven deficiency.
- Correction of hypersplenism.
- Appropriate and timely management of pubertal delay in boys and girls with TM and appropriate induction of puberty to attain normal pubertal growth spurt and normal bone accretion.
- Accurate diagnosis and early management of hypothyroidism and abnormal glucose homeostasis (impaired glucose tolerance and diabetes mellitus).
- The management of GHD has been a matter of debate. The linear growth velocity attained after exogenous GH administration in children with thalassaemia is reported to be lower than that seen in children with primary GH deficiency, possibly due to GH insensitivity (De Sanctis & Urso, 1999; Soliman, Khalafallah & Ashour, 2009). Therefore, large well-designed randomised controlled trials over a longer period with sufficient duration of follow up are needed (Ngim et al., 2017).
- At present, there are no guidelines in the literature for the use of recombinant human GH in adult patients with TM and GHD. However, due to the possible positive effects of GH on the heart, it could be speculated that GH treatment may be useful in some patients with cardiac failure (Erfurth et al., 2004; Smacchia et al., 2012).
- During GH treatment, patients should be monitored at 3-4 monthly intervals with a clinical assessment and an evaluation for parameters of GH response and adverse effects (De Sanctis & Urso, 1999).

## How to prevent growth retardation in thalassaemia major

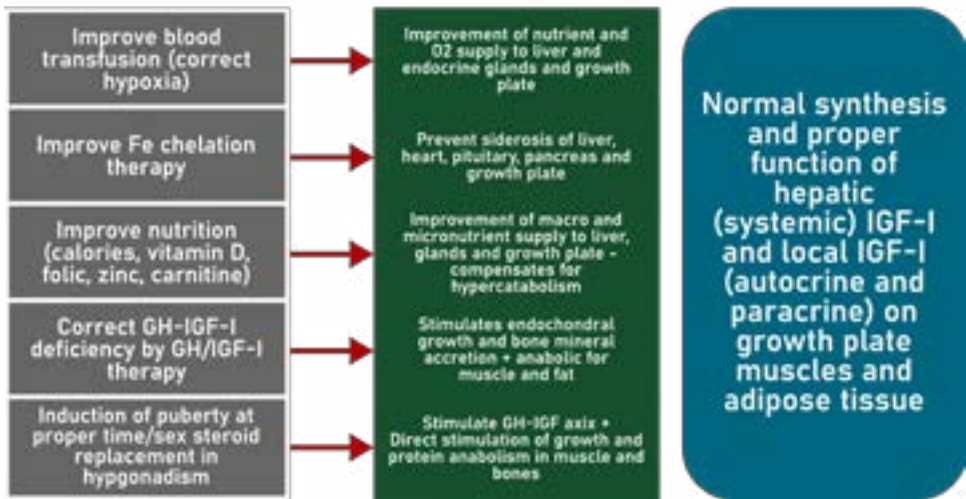


Figure 7. Practical approach to the treatment of growth retardation in thalassaemia. Reproduced with permission from Soliman et al. (2013).

## Summary

The pathogenesis of growth failure is multifactorial. Key contributing factors to stunted growth in patients with TM include chronic anaemia, transfusion related iron overload and chelation toxicity.

Other contributing factors include hypothyroidism, hypogonadism, GH deficiency/insufficiency, zinc deficiency, chronic liver disease, under-nutrition and psychosocial stress. Patients' growth should be assessed every 6 months by accurate measurement of standing and sitting height and weight. Management consists of optimising blood transfusion; improving nutrition with high caloric balanced diet. Optimising iron chelation can be achieved by using the new oral chelators and/or intensive and combined chelation therapy in patients with severe iron overload. Also, an early diagnosis and treatment of associated endocrinopathies is important. Recombinant GH (rhGH) treatment is not always as effective as expected in non-thalassaemic children with GHD.

## Short statement and grade of evidence

- The adherence to modern transfusion and iron chelation protocols and avoidance of iron chelator overdosage has clearly reduced the risk of short stature and may have potentially enhanced endocrine development in children with TM (Evidence B).
- Although the cause of short stature in children with TM is not well understood, it is believed to be multifactorial (Evidence A).
- The hormonal cause of growth retardation in TM patients is complex. Besides hypothyroidism and hypogonadism it has become apparent that growth hormone (GH) deficiency also plays a role in their subnormal growth (Evidence A).
- Excessive iron deposition in the pituitary and liver appear to be the major aetiology for GH and/or IGF-I deficiency (Evidence A).
- The efficacy of rhGH treatment in the management of children with TM with growth failure secondary to GH deficiency has been a matter of debate (Evidence A).
- The linear growth velocity attained after exogenous GH administration in children with thalassaemia is reported to be lower than that seen in children with primary GH deficiency, possibly due to GH insensitivity (Evidence B).
- Essentially there is no pathognomonic clinical feature to lead to suspicion of GH deficiency in adults (Evidence B).
- GH treatment may be useful in some patients with cardiac failure (Evidence C).

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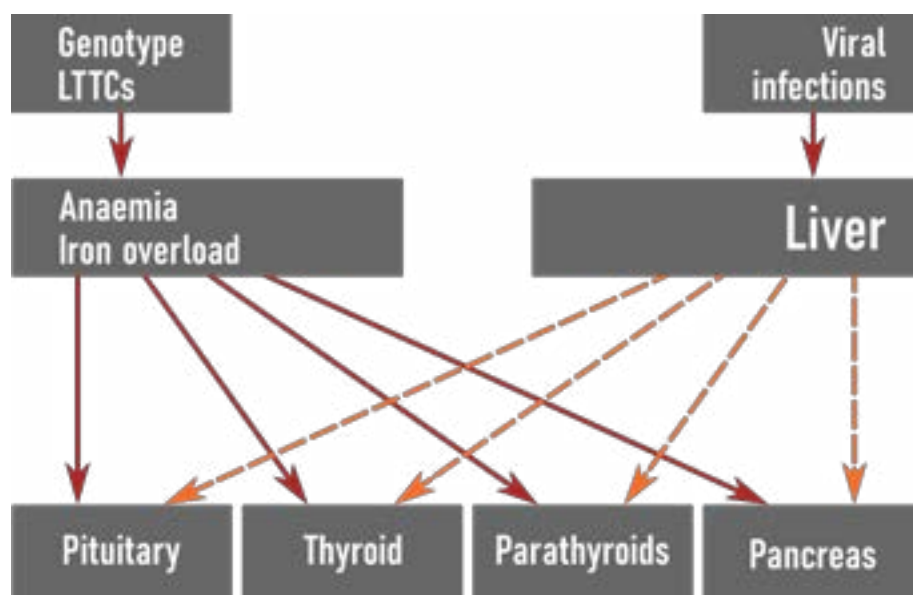
Authors: Vincenzo De Sanctis, Ashraf T. Soliman and Nikos Skordis | Reviewers: Ali Taher, John Porter

## CHAPTER 8

### Endocrine Disease

#### Introduction

Endocrine abnormalities are the most common complications of transfusion-dependent  $\beta$  thalassaemia (TM). Prevalence varies because of the different levels of treatment followed by centres across the world, particularly the severity of the defective genetic background, the haemoglobin concentration and the degree of iron load in various patient groups (Figure 1). Another contributing factor is that of the increased survival to adulthood (De Sanctis et al., 2016a).



**Figure 1.** Aetiopathogenesis of endocrine complications in thalassaemia. [LTTCs, L-type  $\text{Ca}^{2+}$  channels]

#### Aims of blood transfusion

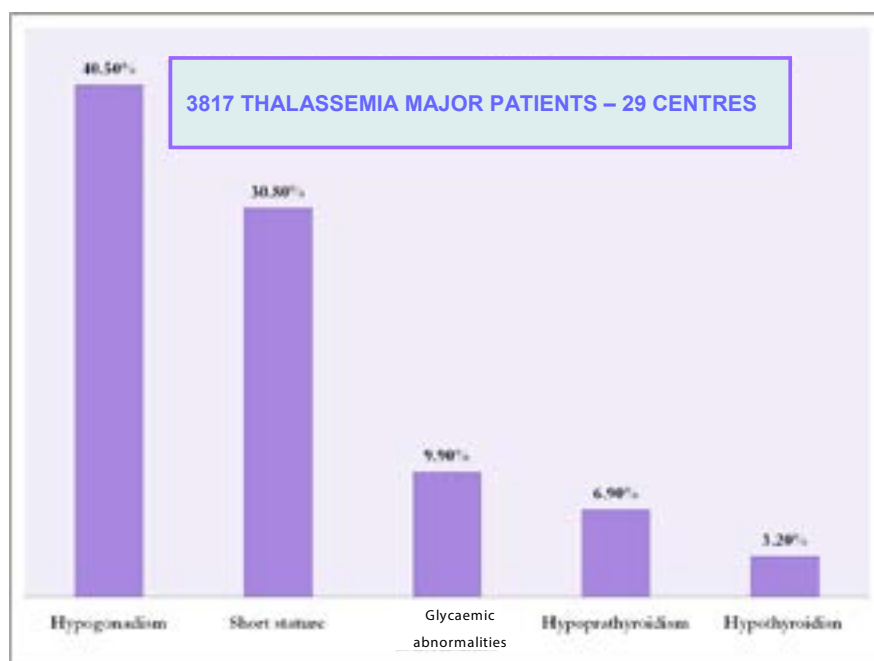
#### The prevalence of different endocrine-related complications:

1. Delayed puberty and hypogonadism: the prevalence of hypogonadotropic hypogonadism in both sexes varies considerably between countries and between different centres. It ranges from 50% to 100 %. The reported prevalence of adult-onset hypogonadism (AOH) in TM patients ranges between 8.3% and 12%.
2. Hypothyroidism: the prevalence varies from 6 to 35% in different studies.
3. Impaired glucose tolerance and diabetes mellitus: the prevalence increases with age and varies from 10 to 24% in different studies.
4. Hypoparathyroidism: the prevalence varies from 1% to 19%.
5. Adrenal insufficiency: prevalence of 'biochemical adrenal insufficiency' varies up to 45%, but clinical adrenal insufficiency is rare (De Sanctis et al., 2016b).

Genetic factors influence the susceptibility to hypogonadism in patients with thalassaemia, possibly because of differences in transfusional iron input and/or the vulnerability to free radical damage. Patients with the  $\beta^0/\beta^0$  genotype have a significantly higher prevalence of growth retardation, hypogonadism, hypothyroidism and hypoparathyroidism compared to those with the  $\beta^0/\beta^+$  and  $\beta^+/\beta^+$  genotypes.

## Delayed Puberty and Hypogonadism

Delayed puberty and hypogonadism are the most obvious clinical consequences of iron overload. Delayed puberty is defined as the complete lack of pubertal development in girls by the age of 13, and in boys by the age of 14. Hypogonadism is defined in boys as the absence of testicular enlargement (less than 4 ml) and in girls as the absence of breast development by the age of 16 (De Sanctis et al., 2013). Arrested puberty is a relatively common complication in moderately or grossly iron overloaded patients with TM and is characterised by a lack of pubertal progression over a year or more. In such cases, the testicular size remains 6-8 ml, and breast size at B3. In such cases annual growth velocity is either markedly reduced or completely absent (De Sanctis et al., 2013). Hypogonadism in adolescents and adults with TM has a prevalence of 38% in females and 43% in males (Figure 2).



**Figure 2.** The prevalence of endocrine- related complications in thalassaemia major. [Short stature (< 3rd centile) ] (De et al. Sanctis 2004).

- Routine investigations include biochemical analysis, thyroid function tests (thyroid stimulation hormone (TSH) and free thyroxine (FT4), bone age (X-ray of wrist and hand) and bone mineral density (BMD).
- Testing the hypothalamic-pituitary gonadal axis (hypogonadotropic hypogonadism) – patients with TM and delayed puberty/hypogonadism have:

- Lower basal follicle stimulating hormone (FSH) and luteinising hormone (LH) secretion.
  - Low LH/FSH response to gonadotropin releasing hormone (GnRH) and variable disturbance of the spontaneous pulsatile pattern of LH and FSH secretion.
  - Low basal sex steroid levels (estradiol and testosterone).
  - In some cases, low testosterone secretion in response to human chorionic gonadotropin (HCG).
- Pelvic ultrasound to assess ovarian and uterine size in females.

## Treatment

The treatment of delayed or arrested puberty and of hypogonadotropic hypogonadism depends on factors such as age, severity of iron overload, damage to the hypothalamic-pituitary-gonadal axis, chronic liver disease and the presence of psychological problems resulting from hypogonadism. Collaboration between endocrinologists and other doctors is critical.

For girls, therapy may begin with the oral administration of ethinyl estradiol (2.5-5 µg daily) for six months, followed by hormonal reassessment. If spontaneous puberty does not occur within six months from the end of treatment, oral oestrogen is re-introduced in gradually increasing dosages (ethinyl estradiol from 5-10 µg daily) for another 12 months. If breakthrough uterine bleeding does not occur, low oestrogen-progesterone hormone replacement is the recommended treatment (De Sanctis et al., 2013).

For delayed puberty in males, low dosages of intramuscular depot-testosterone esters (30-50 mg) are given monthly for six months, followed by hormonal re-assessment. In patients with hypogonadotropic hypogonadism, treatment at a dose of 50 mg per month can be continued until growth rates wane. The fully virilising dose is 75-100 mg of depot-testosterone esters every 10 days, administered intramuscularly after growth is almost completed and afterwards. The same effects can be achieved with topical testosterone gel (De Sanctis et al., 2013).

For pubertal arrest, the treatment consists of testosterone esters or topical testosterone gel, administered as for the treatment of delayed puberty and hypogonadotropic hypogonadism.

It is important that the treatment of pubertal disorders is considered on a patient-by-patient basis, taking account of the complexity of the issues involved and the many associated complications (De Sanctis et al., 2013).

## Hypothyroidism

This complication is mainly attributed to iron overload and is uncommon in optimally treated patients. Central hypothyroidism is uncommon (Soliman et al., 2013a). The frequency of hypothyroidism in TM patients ranges from 6 to 30%. A lower prevalence is found among patients with evidence of lower iron load as measured by ferritin levels. The wide variations in different reports can be attributed to differences in patient genotypes, differences in patients' ages, ethnic variations and different treatment protocols, including differing transfusion rates and chelation therapies (De Sanctis et al., 2013).

## Laboratory tests

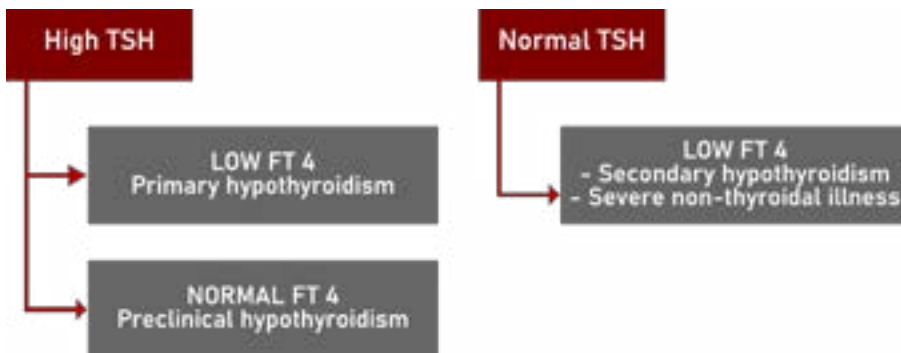
Investigation of thyroid function should be performed annually, beginning at the age of nine years (unless symptomatic hypothyroidism is observed earlier) (Rindang et al., 2011). Free T4 and TSH are the key investigations. Additional tests may include the following:

- Thyroid autoantibodies: anti-thyroid peroxidase and antithyroglobulin autoantibodies. Thyroid antibodies to exclude autoimmunity are usually negative and are performed in selected cases. Ultrasonography, which may show different echo patterns, to evaluate structural thyroid abnormalities.
- Bone age, in selected cases.
- Biochemistry including lipid profile.
- Serum ferritin.
- Electrocardiogram (ECG) and echocardiogram (especially in severe cases).
- Hypothalamic-pituitary magnetic resonance imaging (MRI), in patients with central hypothyroidism.

## Assessment of thyroid function

The following grades of hypothyroidism have been identified (De Sanctis et al., 2012):

- Sub-clinical hypothyroidism is a combination of high TSH with normal FT4 levels.
- Two types of sub-clinical hypothyroidism have been reported:
  - Type A (normal FT4, TSH 5-10  $\mu\text{U/ml}$ )
  - Type B (normal FT4, TSH > 10  $\mu\text{U/ml}$ )
- Overt hypothyroidism is a combination of high TSH with low FT4.
- Diagnosis of central hypothyroidism is usually made on a biochemical basis showing low circulating concentrations of thyroid hormone associated with inappropriately low TSH levels (Figure 3).



**Figure 3.** Flow chart for the assessment of thyroid function. [TSH, thyroid stimulating hormone; FT4, free thyroxine].



## Clinical examination

The classical clinical signs of hypothyroidism in TM patients are not easy to identify because most of the symptoms, especially in mild cases, are nonspecific and are frequently attributed to anaemia or associated diseases (Sabato et al., 1983). Thalassaemic patients with overt hypothyroidism have been reported to exhibit stunted growth, delayed puberty, cardiac failure, and pericardial effusion (De Sanctis et al., 2012). They are shorter with more delayed bone age than euthyroid TM patients.

## Treatment

- Overt and central hypothyroidisms are treated with levothyroxine (L-thyroxine).
- If subclinical hypothyroidism is detected, chelation should be intensified and the patient carefully monitored (De Sanctis et al., 2012).
- Subclinical hypothyroidism is treated with L-thyroxine when TSH is equal to or above 8 mUI/ml (Figure 4).

Treatment with amiodarone may result in the rapid progression from subclinical hypothyroidism to severe hypothyroidism, which in turn causes deterioration of cardiac function (Alexandrides et al., 2000).

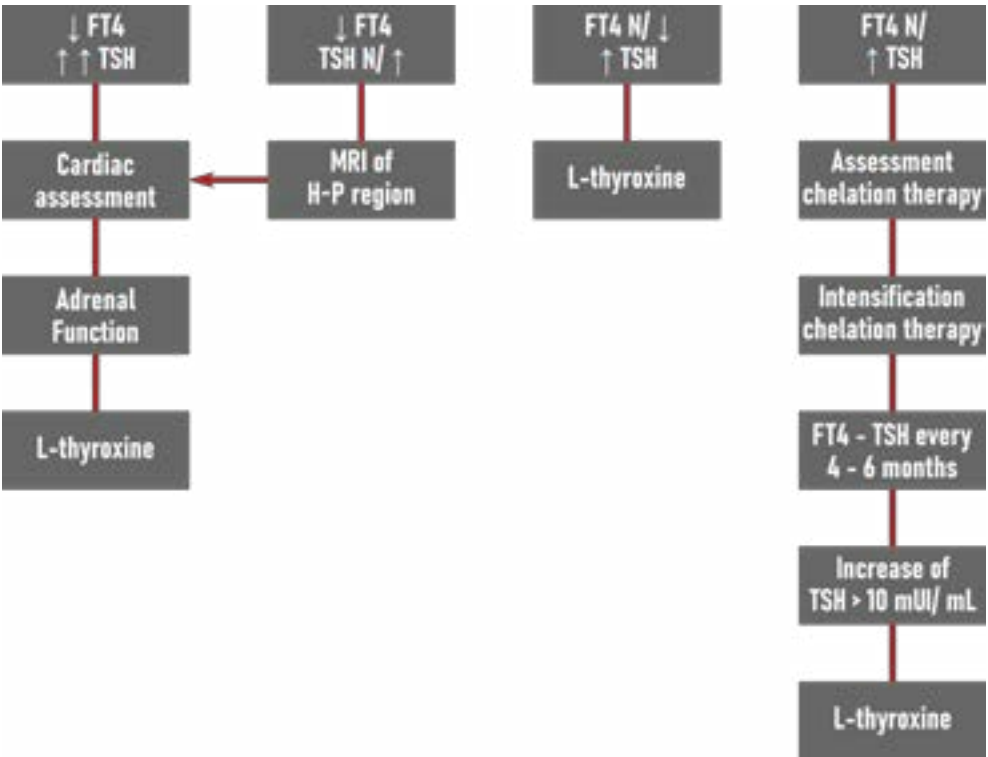


Figure 4. Flow chart for the treatment of thyroid dysfunction in thalassaemia. [FT4, free thyroxine; RM of hypothalamic-pituitary region; TSH, thyroid stimulating hormone]

## Impaired Glucose Tolerance (IGT) and Insulin-dependent Diabetes Mellitus (IDDM)

IGT and IDDM are relatively common complications in patients who have been inadequately iron chelated, although these abnormalities have also been observed in well transfused and regularly chelated TM patients, suggesting that the development of diabetes might be caused by other factors such as: individual sensitivity to iron damage, chronic anaemia, zinc deficiency and increased collagen deposition secondary to increased activity of the iron-dependent procollagen proline hydroxylase enzyme, with subsequent disturbed microcirculation in the pancreas (De Sanctis et al., 2004; Iancu, Ward & Peters, 1990).

The prevalence of IGT and IDDM in adolescents and young adults with TM treated mainly with deferoxamine (desferrioxamine) varies in different series from 0 to 17% (Skordis, 2012). IDDM is uncommon during the first years of life and rates progressively increase with age. Impaired glucose tolerance may start early in the second decade of life in parallel with puberty. The combined adverse effects of puberty and thalassaemia-associated risk factors on insulin action may partly explain the increase of insulin resistance in adolescent thalassaemics (Skordis, 2012).

### Pathogenesis of IDDM in $\beta$ thalassaemia patients

The initial abnormality appears to be iron-mediated insulin resistance rather than defective insulin production, but pancreatic  $\beta$ -cell damage and insulin deficiency subsequently develop as a result of direct toxic damage from iron deposition (Skordis, 2012).

Pancreatic  $\beta$ -cell function in thalassaemia is characterised by the following sequence (Figure 5):

- Insulin - resistance with hyperinsulinaemia and normal glucose tolerance.
- Insulin-resistance with IGT and progressive impairment of  $\beta$ -cell function with reduction of insulin secretion, and
- Insulin dependent diabetes mellitus.

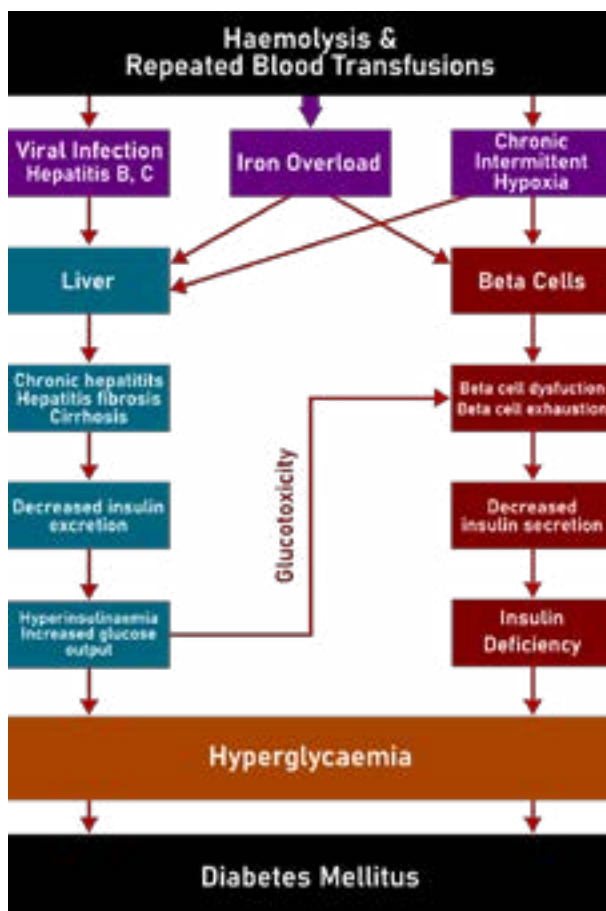


Figure 5.

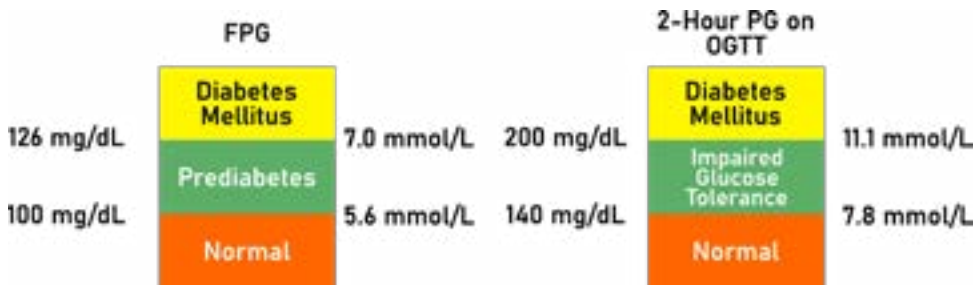
Pathogenesis of abnormal glucose homeostasis in thalassaemia (Reproduced with permission from Soliman AT).

Both liver and pancreatic  $\beta$ -cell siderosis and glucose toxicity may impair glucose tolerance. The interplay between liver siderosis and hepatitis C facilitates and accelerates the progression to IDDM, at least in adulthood (De Sanctis et al., 2016c). Early recognition of glucose abnormalities is essential. The oral glucose tolerance test (OGTT) should be done in every patient with thalassaemia after the age of ten or earlier if needed (Skordis, 2012).

## Diagnosis

The diagnostic criteria for glucose intolerance (Figure 6) are as follows:

- Fasting glucose  $>126$  mg/dl is diagnostic of diabetes mellitus.
- OGTT serum glucose at 2 hours  $>200$  mg/dl is diagnostic of diabetes mellitus.
- OGTT serum glucose at 2 hours  $>140$   $<200$  mg/dl indicates glucose intolerance (Figure 6).



**Figure 6.** The diagnostic criteria for the glucose tolerance. FPG: fasting plasma glucose; OGTT, oral glucose tolerance test; PG, plasma glucose.

Pancreatic iron is the strongest predictor of  $\beta$ -cell toxicity and can be evaluated by MRI of the pancreas (Noetzli et al., 2009), although this technique is yet to be standardised for use in routine clinical practice. MRI and fasting glucose/insulin are complementary screening tools and if proven valid, they may identify high-risk patients before irreversible pancreatic damage occurs. Nevertheless, oral glucose tolerance testing remains the gold standard test for assessing glucose homeostasis. Screening for viral hepatitis and regular chelation therapy are important measures in preventing the development of diabetes.

## Management

Management of impaired glucose tolerance and diabetes (De Sanctis et al., 2016c; Skordis, 2012) is based on:

- Intensive iron-chelation therapy and prevention and treatment of chronic hepatitis C infection are now the most important issues in managing impairment of glucose homeostasis in patients with transfusion-dependent  $\beta$ -thalassaemia. Intensive chelation therapy is effective to normalise  $\beta$ -cell function and may improve insulin secretion and glucose tolerance and reduce liver iron deposition (Berdoukas et al., 2012).
- Management of IDDM should be individualised
- Healthy diet suitable for IDDM (as assessed and advised by expert dietician)
- Regular physical activity

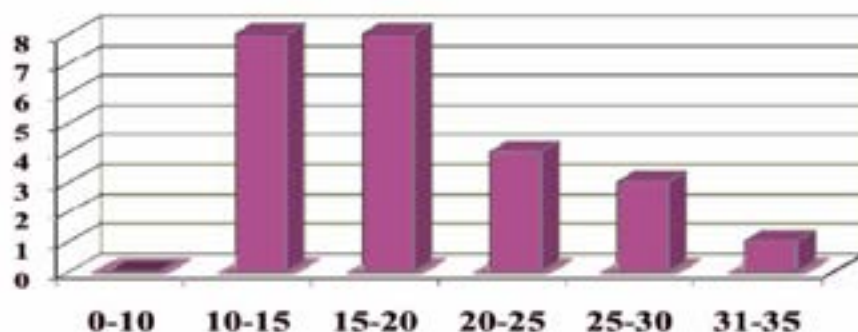
- There is very limited published data on the efficacy and safety of oral antidiabetic agents in patients with TM. The only drugs used in small studies in this context with good effect were metformin, glibenclamide, sitagliptin and acarbose.
- When overt IDDM develops, patients require daily subcutaneous injections of insulin to normalise blood sugar levels.
- Diabetic patients with TM should be seen regularly by a specialised multidisciplinary team with expertise in both diabetes and TM. There should be ongoing diabetes self-management education. The team should include an endocrinologist and dietician with experience in TM.
- TM women with pre-existing diabetes should have pre-pregnancy counselling and planning to aim for optimal glycaemic control before and throughout pregnancy to minimise adverse pregnancy outcomes.

Monitoring glycaemic control in thalassaemia patients is the same as with non-thalassaemic patients with IDDM (De Sanctis et al., 2016c):

- Self-glucose monitoring (SGM) at home using glucometers.
- Urine ketones if blood sugar >250 mg/dl.
- Fructosamine determination is useful for monitoring diabetes in these patients (De Sanctis et al., 2016c).
- Periodic assessment of renal function.
- A microalbumin test is used to detect early signs of kidney damage in people who are at risk of developing kidney disease (once a year). If albumin in the urine (micro-albuminuria) is detected, it should be confirmed by retesting twice within a 3-6 month period.
- Evaluation of retinopathy.

## Hypoparathyroidism (hypoPT)

HypoPT has been considered as a typical complication of the second decade of life in transfusion-dependent patients with thalassaemia major (Figure 7). The incidence of hypoPT varies from centre to centre (from 1.2% to 19%) and hypoPT seems to affect men more frequently (male/female ratio = 1.35) (De Sanctis et al., 2018; Sleem, Al-Zakwani & Almuslahi, 2007; Vogiatzi et al., 2009). Recently, abnormal cerebral computed tomography findings have been reported in a high percentage of patients with thalassaemia and hypoPT (Karimi et al., 2009; Soliman et al., 2008). An ECG can detect an abnormality in the electrical activity of the heart.



**Figure 7.** Age at presentation of hypoparathyroidism and number of thalassaemia major patients (De Sanctis V, personal experience).

## Signs and symptoms

Most patients show a mild form of the disease accompanied by paraesthesia and prolonged QTC interval (Figure 8). More severe cases may demonstrate tetany, seizures or cardiac failure (Skordis, 2012).



**Figure 8.** Prolonged QTC interval in a male thalassaemic patient with hypoparathyroidism.

## Investigations

Investigations should begin from the age of 16 years and should include serum calcium, serum phosphate and phosphate balance. In cases with low serum calcium and high phosphate levels, parathyroid hormone should also be measured (Skordis, 2012).

## Management

Treatment of hypoPT aims to prevent acute and chronic complications of hypocalcaemia. The primary goals of management include: control of symptoms, maintaining serum calcium in the low to normal range, maintaining serum phosphorus within normal limits, maintaining 24-hour urine calcium under 7.5 mmol/day (300 mg/day) and maintaining a calcium-phosphate product under 55 mg/dl (4.4 mmol/l) to guard against the development of nephrolithiasis, nephrocalcinosis and soft-tissue calcification (Skordis, 2012).

### Treatment includes:

- Oral administration of vitamin D or one of its analogues. Some patients require high doses of vitamin D to normalise their serum calcium levels. This should be carefully monitored, as hypercalcaemia is a common complication of this treatment (De Sanctis et al., 2018, 2013).
- Calcitriol, 0.25-1.0 µg, twice daily, is usually sufficient to normalise plasma calcium and phosphate levels. At the start of the treatment, weekly blood tests are required. These are followed by quarterly plasma and 24-hour urinary calcium and phosphate measurements.
- In patients with persistently high serum phosphate levels, a phosphate binder (except aluminium) may be considered.
- Tetany and cardiac failure due to severe hypocalcaemia require intravenous administration of calcium, under careful cardiac monitoring, followed by oral vitamin D.
- In some studies, synthetic human parathormone 1-34 (PTH), once or twice daily, has been shown to effectively treat children with hypoPT. However, this therapy is not yet approved for the treatment of hypoPT and no data are available in the literature in subjects with thalassaemia.
- In some patients with hypoPT treated with calcium and vitamin D, the development of hypercalciuria is a potential unwanted effect, due to the anticalciuric effect of PTH. In these cases, restriction of sodium intake, use of thiazide diuretics or reduction in the doses of calcium or 1 alpha-hydroxylated vitamin D may be required. Such measures may also be employed at the beginning of treatment to prevent hypercalciuria (De Sanctis et al., 2018, 2013).

### Dietary steps

No special diet is required, but some doctors recommend consulting a dietician, who is likely to advise a diet that is:

- Rich in calcium. This includes dairy products, green leafy vegetables, broccoli, kale, fortified orange juice and breakfast cereals.
- Low in phosphorus-rich items. This means avoiding carbonated soft drinks, which contain phosphorus in the form of phosphoric acid. Eggs and meats also tend to be high in phosphorus.

### Adrenal Insufficiency

Several studies reported a significant prevalence of 'biochemical' adrenal insufficiency in patients with thalassaemia ranging from 0 to 45%. 'Clinical' adrenal insufficiency, i.e. adrenal crisis, on the other hand, is extremely rare (El Kholi, 2012; Soliman et al., 2013b).

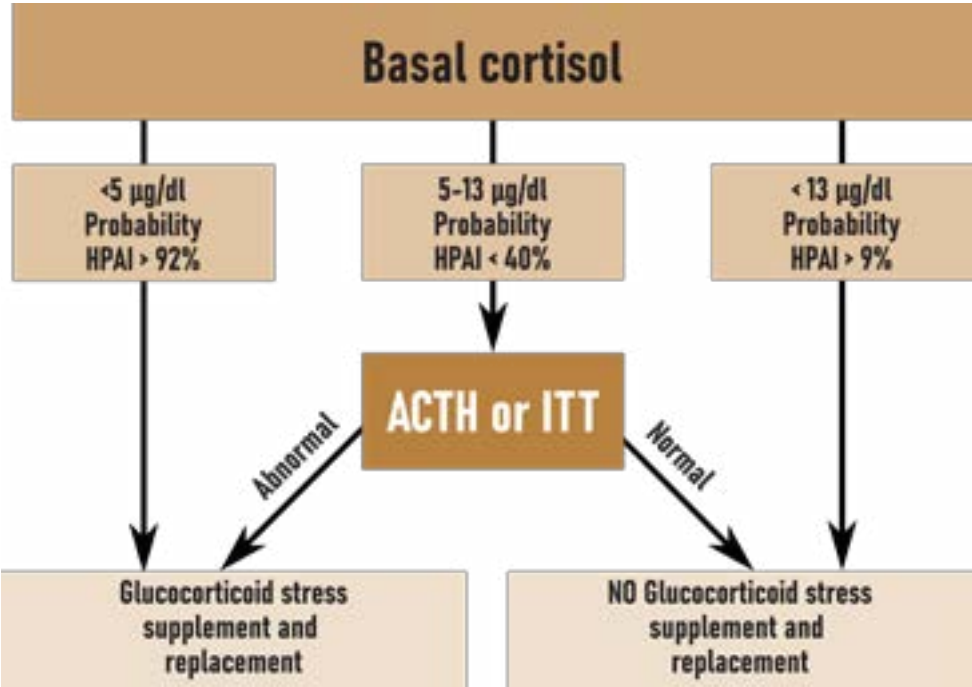
### Diagnosis

Manifestations of mild adrenal hypofunction might be masked by symptoms that are commonly complained of by thalassaemic patients, such as asthenia, muscle weakness, arthralgias and weight loss.

### Laboratory tests

Cortisol levels both basal and 30-60 minutes after adrenocorticotrophic hormone (ACTH) or insulin stimulation, are used for the assessment of adrenal function (Figure 9).

It is advised that adrenal function be tested every 1–2 years, especially in growth hormone (GH) deficient patients during recombinant human growth hormone (rhGH) therapy (El Kholy, 2012; Soliman et al., 2013b), because patients with GH deficiency may have additional anterior pituitary hormone deficits and are at risk of developing complete or partial corticotropin (ACTH) deficiency.



**Figure 9.** Optimal testing strategy for evaluating patients with potential hypothalamic-pituitary –adrenal insufficiency (HPAI). [ACTH, adrenocorticotrophic hormone; ITT, insulin tolerance test].

### Treatment

Subclinical impairment of adrenocortical function in patients with TM is not uncommon; however, it has little or no clinical impact under basal conditions although it may have a potential relevance during stressful events. Accordingly, glucocorticoid treatment coverage might be advised only for stressful conditions (El Kholy, 2012; Soliman et al., 2013b). Clinical adrenal insufficiency and adrenal crisis are rare.

## Summary

Endocrine complications, growth and pubertal delay are common manifestations of iron overloading in TM and carry significant morbidity. As such, patients with TM need regular monitoring for signs and symptoms of endocrine complications. Prevention remains the priority, and there are limited data to support a role for chelation therapy in this. Once endocrine complications have developed, management should focus on halting the progression of such complications and treating associated symptoms.

### Short statements and level of evidence

- Endocrine complications are very common in multi-transfused TM patients (Evidence A).
- Periodic evaluation of these problems should be carried out in TM patients with iron overload, particularly after the age of 11 years (Evidence B).
- Delayed puberty and hypogonadism are the most obvious clinical consequences of iron overload (Evidence A).
- The aetiology of IDDM is multifactorial (genetic factors, insulin deficiency, insulin resistance and liver dysfunction secondary to viral hepatitis) (Evidence A).
- Sub-clinical hypothyroidism – basal TSH 5 to 8 mUI/ml – requires regular follow-up and optimising chelation therapy (Evidence B).
- Subclinical impairment of adrenocortical function in patients with TM is not uncommon, but clinical adrenal insufficiency and adrenal crisis are very rare (Evidence A).
- Most patients with hypoparathyroidism show a mild form of the disease (Evidence B).
- Normalisation of total body iron load with very intensive combined chelation (deferrioxamine plus deferiprone) reverses cardiac and endocrine complications of TM (Evidence B).
- Monitoring of growth, pubertal development, reproductive ability and endocrine functions in general are essential to achieve a good quality of life in TM (Evidence B).



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# CHAPTER 9

## Infectious disease

### Introduction

Infections and their complications were previously the second commonest cause of death in transfusion-dependent thalassaemia (TDT), prior to the new millennium (Borgna-Pignatti et al., 2004). Infections are becoming the leading cause of death in western countries due, in part, to a significant reduction in the number of deaths from iron-induced cardiac disease (Modell et al., 2008). Infections have already been reported as the primary cause of mortality among E-beta thalassaemia patients in Thailand years ago (Wanachiwanawin, 2000).

The variability in the epidemiology of infections, differences in socio-economic level, preventative strategies and accessibility to healthcare in each country should have an impact on variability in rates of infection-related morbidity and mortality in TDT throughout the world. In TDT, allogeneic packed red cell transfusions (pRBC) carry significant burden, including direct exposure to risk of transfusion-transmitted infections (Vamvakas & Blajchman, 2009), indirect risks of transfusion-related immunomodulation (TRIM) (Blajchman, 2005) and iron overload (IOL) (Marx, 2002). Underlying pathophysiological mechanisms of disease such as ineffective erythropoiesis (IE), haemolysis and anaemia may also have deleterious effects on the immune system and contribute to susceptibility to infections (Wanachiwanawin et al., 1993). Furthermore, some other therapeutic interventions such as iron chelation therapy, splenectomy, central venous catheters, and stem cell transplantation may contribute to infectious complications with resultant to morbidity and mortality (Figure 1).

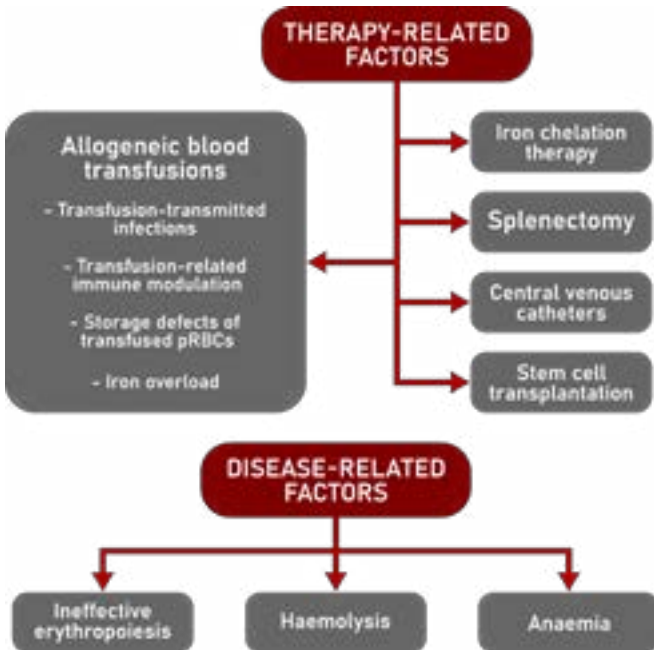


Figure 1. Factors contributing to infection risk in TDT.

## Therapy-Related Risks of Infections in TDT and Preventive Measures

Allogeneic blood transfusion-related risks of infections

### A. Transfusion-transmitted infections (TTIs)

The risk of transfusion-transmitted infections (TTI) in patients with transfusion-dependent thalassaemia does not differ from other multi-transfused patients. Hepatitis C virus (HCV), hepatitis B virus (HBV), human immunodeficiency virus (HIV) and syphilis are the most common infectious agents that may be transmitted via pRBC transfusions. There has also been a steadily increasing number of reported cases of transfusion-transmitted hepatitis E virus in blood donation recipients (Domanović et al., 2017). Transfusion transmitted malaria remains one of the most common TTI on a global scale (Kitchen & Chiodin, 2006). West-Nile virus (WNV) is also recognized as a transfusion-transmitted virus requiring preventive blood safety measures (Domanovic et al., 2019). Despite improved donor testing, long-term transfusion support has a substantial cumulative life-time residual risk of TTI (Kleinman & Stassinopoulos, 2015), due to low burden pathogens undetected by testing (e.g., Plasmodia, Babesia), emerging pathogens for which tests are not available (e.g., dengue, chikungunya), and unrecognised bacterial contamination (e.g., *Yersinia enterocolitica*) (Stramer & Dodd, 2013; Damgaard et al., 2015). Further, the blood safety chain (donor selection, TTI testing, and haemovigilance, including post-transfusion surveillance) is suboptimal in low-income countries (Shyamala, 2014).

### Fundamental principles for providing safe blood:

- The deferral of high risk prospective donors is the first level of defence against TTIs.
- Strategies regarding donor recruitment through voluntary non-remunerated blood donation (VNRBD) should be implemented because such donors have been found to have lower risk for TTIs.
- The routine testing of donor blood for HBV, HCV, HIV and syphilis by validated technology should be implemented in Blood Banks (Bloch, 2012).

Testing of donations for infectious agents is a key factor in ensuring that the risk of disease transmission is minimized and that blood components are suitable for their intended purpose. Current tests are based on the detection of relevant antigens and/or antibody and gene sequences.

The minimum mandatory serological blood donor screening tests are:

- antibody to HIV-1 and HIV-2 including outlying types e.g., HIV-1 type O,
- antibody to hepatitis C virus,
- hepatitis B surface antigen (HBsAg) assay,
- *Treponema pallidum* haemagglutination assay (TPHA),
- Elisa for syphilis.

**Additional Serological Screening Tests.** These may be required by the national authorities for specific components or in particular epidemiological conditions, e.g.

- Anti-HTLV-I and HTLV-II,
- Antibody to hepatitis B core antigen (anti-HBc),
- Testing for CMV antibodies for the transfusion of highly susceptible patients and in cases of candidates for HSCT,
- Chagas testing for *Trypanosoma cruzi* antibodies is employed in endemic areas and elsewhere for travellers returning from an endemic area.

### **Molecular testing**

Nucleic Acid Screening or Nucleic Acid Amplification Techniques (NAT) for HCV-RNA, HIV-RNA and HBV-DNA in mini pools or single donations represents the state of the art in many countries. It was developed in order to remedy limitations in serological testing, e.g., the window period - the time lapse from the appearance of the virus in blood until the detectability of a given marker (antibody, antigen or nucleic acid) (Candotti & Allain, 2013). This method is technologically demanding and costly. Unfortunately, it has not yet gained worldwide application even where most needed.

### **Silent /occult infections**

Apart from seronegative donors during the infectious window period, the greatest threat to the safety of the blood supply using serological markers is posed by the occult period of HBV. This period is characterised by very low viral load and undetectable HBsAg at the tail end of chronic carriage or the occurrence of escape mutants interfering with HBsAg synthesis and the interference of other viruses in HBV replication (Paraskevis et al., 2013).

All patients with TDT should be protected by vaccination against HBV. Since the protection offered by vaccination is not absolute, patients should be tested annually for HBV markers as well as the other TTIs such as HCV and HIV. Booster dose of HBV vaccine is considered if anti-HBs titre decreases (Singh 2003) (A).

- The diversity of blood-borne infectious agents transmitted through transfusion of infected blood donated by apparently healthy and asymptomatic blood donors also includes human T-cell lymphotropic viruses (HTLV-1/2), cytomegalovirus (CMV), parvovirus B19, West Nile Virus (WNV), dengue virus, *Babesia* spp., *Plasmodium* spp., *Trypanosoma cruzi* and the prions that cause variant Creutzfeldt-Jakob disease (CJD) (Allain et al., 2009). The national authorities may require additional Serological Screening Tests for specific components or in particular epidemiological conditions, e.g. CMV antibodies for the transfusion of highly susceptible patients and in cases of candidates for HSCT or Chagas testing for *Trypanosoma cruzi* antibodies is employed in endemic areas and elsewhere for travelers returning from an endemic area.

- Haemovigilance is another crucial pillar for safeguarding blood safety through a system of epidemiological surveillance of adverse reactions and adverse events in donors and in recipients (European Commission Directive 2005/61/EC). Its ultimate goal is to prevent the recurrence of adverse events and reactions. To accomplish this task, haemovigilance must be a shared responsibility of the professionals in the field and the Competent Authorities for blood safety (Politis et al., 2016).

### **Preventative measures include:**

- Prestorage leucodepletion of pRBC units reduces the transmission of CMV, and may also be effective in reducing the risk of a number of additional transfusion-transmitted infections, including infections due to herpes viruses (e.g., Epstein-Barr virus [EBV] and human herpesvirus-8 [HHV-8]), retroviruses (e.g., HTLV-1 and HIV), bacteria (e.g., *Yersinia enterocolitica*), protozoa (e.g., *Leishmania* species and *Trypanosoma cruzi*) and infectious prions.

- It should be noted that leucodepletion does not provide 100% risk prevention from these infections, but it may provide an additional and justified measure of caution (Cervia, Wenz & Ortolano, 2007) (C).

Bacterial sepsis associated with transfusion of contaminated pRBC units is associated with high fever, rigors and hypotension, beginning during or shortly after the transfusion. The pRBC unit is presumably contaminated by transient donor bacteraemia due to a recent infection. Causative bacteria are most often Gram-negative bacilli – mainly *Yersinia enterocolitica* and *Serratia marcescens* (Lindholm, Annen & Ramsey, 2011).

### **The potential safeguard to mitigate the risk of TTI:**

Pathogen reduction treatment (PRT) of pRBC, which is referred to the inactivation of all viruses, bacteria, parasites and any replicating structures, could be a method to achieve the goal of almost absolute blood safety, albeit there are some limitations of these technologies. Some pathogens (Parvovirus B19, HAV, and HEV) show partially intrinsic resistance to the inactivation process constituting a limitation to the use of the PI-technology (Kleinman & Stassinopoulos, 2015). Two methods are currently under development for supplying pathogen reduced pRBC: Photochemical inactivation of whole blood using Riboflavin (vitamin B2) and ultraviolet light energy (@Mirasol Pathogen Reduction System) has been investigated in a phase-III clinical trial for whole blood use (Allain, 2016) but not yet been evaluated for RBC use. Chemical inactivation of RBC using Amustaline-glutathione (@Intercept Blood System) has been evaluated in Phase-III clinical trials for supporting chronic transfusion program in patients with TDT (Aydinok, 2019). In the latter study, PRT of pRBC appeared to be well-tolerated and logistically feasible for chronic transfusion therapy without significant increase in pRBC utilization in TDT patients.

The universal utilization of an approved PRT pRBC could allow donor testing to be significantly revised and may ultimately be less complex and less expensive than continued assay development. However, the cost will be regarded as an important factor for implementing PRT technology, particularly in low-income countries.

### **Suspicion and approach to transfusion-related bacterial sepsis:**

- If bacterial contamination is suspected, the transfusion should be halted immediately.
- Intravenous infusion of a third generation cephalosporin (cefotaxime 2 g every 8h or ceftriaxone 2 g every 12 h) or carbapenem (meropenem or imipenem 2 g every 8 h) combined with vancomycin (1–1.5 g every 12 h).
- Gram stain and blood culture are obtained from both the blood bag and the recipient (A).

### **Preventative measures for bacterial sepsis:**

- Transfusion of pRBC units stored less than 2 weeks reduces the risk of transfusion-associated *Yersinia septicaemia*. It has been demonstrated that *Yersinia* grows in the contaminated RBC unit after a lag time of 2 weeks (C).
- Leucodepletion is able to eliminate or markedly reduce the growth of the bacterium in processed blood. However, it is not capable of providing 100% protection from the risk of these infections. It may provide an additional and justified measure of caution (Kim et al., 1992) (C).

### **B. Transfusion-related immune modulation**

TRIM may contribute to all immunological alterations observed in TDT patients and it is assumed that either allogeneic mononuclear cells in the pRBC unit, or the soluble substances that are released during storage, play a central role in pathogenesis of TRIM. Pre-storage leucodepletion of pRBC units has no protective effect on immune alterations observed in patients with thalassaemia (Sirchia et al., 1986).

### **C. Storage defects of transfused pRBCs**

It is suggested that free haem compounds released from the lysis of transfused red cells can readily provide iron for bacteria and promote infection (Griffiths, 1999). This hypothesis could be augmented by evidence suggesting that low molecular mass iron complexes occur in pRBC units stored for more than 10 days (Marwah et al., 2002). In fact, marked increases in non-transferrin bound iron and a decrease in antioxidant capacity have been observed in pRBCs stored for more than 14 days (Ozment & Turi, 2009). A large comparative study is required to reveal whether prolonged storage of pRBC is associated with an increased risk of nosocomial infection.

- Transfusions with pRBC units that have been stored for less than 14 days may provide benefit to avoid deleterious effects of storage defects (C).

**Table 1.** Pathogens isolated from thalassaemic patients with infection:

Pathogen	Number of cases (%)
Staphylococcus aureus	9(15%)
Staphylococcus pneumoniae	8(13%)
Escherichia coli	7(11%)
Klebsiella pneumoniae	6(10%)
Salmonella spp.	4(7%)
Pseudomonas aeruginosa	3(5%)
Brucella spp.	3(5%)
Giardia lamblia	3(5%)
Haemophilus influenzae	3(5%)
Group A streptococcus	3(5%)
Campylobacter jejuni	2(3%)
Gram-negative bacteria	8(13%)
Others	2(3%)

From: Rahav Getal. Br J Haematol. 2006;133(6)

### **D. Transfusional iron overload**

Iron overload is suggested to be a risk factor predisposing to infections, since all groups of protozoa, fungi, Gram-positive and negative bacteria require iron for survival and replication, with the only exception being pathogenic *Borrelia burgdorferi* which use manganese in place of iron. Some pathogens such as *Yersinia enterocolitica*, *Klebsiella* species, *Escherichia coli*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Legionella pneumophila* increase their virulence and pathogenicity in the presence of excess iron (Weinberg, 2000). A

gram-negative bacterium, *V. vulnificus* can be transmitted by ingestion of uncooked warm seawater fish, crustaceans, and mollusks and can cause a lethal infection in 20-50% of iron-overloaded patients (Kuo et al., 2009). Although viruses do not require iron, studies have reported that iron increases the risk of viral infections (Weinberg, 2009) and impairs the clinical response to antiviral therapy in HCV infection (Pietrangelo, 2003). Further, iron overload is associated with faster HIV-1 disease progression and poor outcome in TDT patients (Gordeuk et al., 2001). Iron availability is linked to pathogenicity of *Candida albicans* and *Aspergillum fumigates*. Iron has subtle effects on cell-mediated immune effector pathways and systemic iron overload is associated with unfavourable outcomes in many types of infection (Nairz et al., 2010).

- Despite the lack of properly controlled studies, control of iron overload may have therapeutic benefit against infections (C).

### **Splenectomy**

Splenectomy plays a significant role in susceptibility to infections in thalassaemia, since the spleen has a crucial function in immune defence as a phagocytic filter for blood-borne microorganisms, and also produces antibodies (Di Sabatino, Carsetti & Corazza, 2011).

Overwhelming post-splenectomy infection (OPSI) is defined as fulminating sepsis, meningitis or pneumonia triggered mainly by *S. pneumoniae* followed by *H. influenzae* type B and *N. meningitidis*. The risk of OPSI is more than 50 times higher than in the general population and is a permanent life-long condition (Hansen & Singer, 2001).

OPSI is a medical emergency. Following brief prodromal symptoms such as fever, shivering, myalgia, vomiting, diarrhoea and headache, septic shock develops in just a few hours, with anuria, hypotension, hypoglycaemia and, commonly, disseminated intravascular coagulation and massive adrenal gland haemorrhage (Waterhouse–Friderichsen syndrome), progressing to multiorgan failure and death (Brigden & Pattullo, 1999). The mortality rate is around 50 to 70% and most deaths occur within the first 24 hours; only prompt diagnosis and immediate treatment can reduce mortality (Holdsworth, Irving & Cuschieri, 1991).

### **Suspicion and approach to OPSI:**

- Physicians must be aware of the potential life-threatening infections in TDT patients who underwent splenectomy and patients should be educated to seek early care when fever develops.
- In patients at risk and with indicative symptoms, prompt initiation of empirical antibiotics is essential. Intravenous infusion of third generation cephalosporin (cefotaxime 2 g every 8 h or ceftriaxone 2 g every 12 h), combined with gentamicin (5–7 mg/kg every 24 h) or ciprofloxacin (400 mg every 12 h) or vancomycin (1–1.5 g every 12 h) (Brigden & Pattullo, 1999).
- While awaiting results of blood culture, bacteria can be visualised by Gram staining.
- A reverse transcriptase-polymerase chain reaction (RT-PCR) test for simultaneous identification of three main encapsulated bacteria (*S. pneumoniae*, *H. influenzae* type B and *N. meningitidis*) is available (Di Sabatino, Carsetti & Corazza, 2011) (A).



The preventive strategy based on penicillin prophylaxis and vaccination is extremely important and has been discussed in Chapter 12 (Splenomegaly and Splenectomy).

### Iron chelation therapy

The control of systemic iron and withholding iron from invading microbes are important strategies of host defence. As a siderophore, some benefits of deferoxamine (DFO) have been demonstrated in particular infections; for example, DFO was able to promote recovery from coma in children with cerebral malaria (Gordeuk et al., 1992) and experimental studies indicate beneficial effects of DFO in infections with *H. capsulatum* and *T. cruzi* (Arantes et al., 2011). This is partly attributable to the immunomodulatory role of iron chelation via increased nitric oxide (NO) and decreased interleukin-4 (IL-4) production in DFO-treated patients. However, a certain amount of iron is important for the formation of oxygen radicals by the Fenton reaction and via the catalytic action of phagocyte oxidase (phox) while iron overload has immune-debilitating effects. In fact, treatment of *Salmonella*-infected mice with DFO impairs pathogen clearance due to reduced reactive oxygen species (ROS) generation (Collins, Kaufmann & Schaible, 2002). Furthermore, certain pathogens, including *Y. enterocolitica*, *V. vulnificus* and *Mucorales*, can utilise DFO as a siderophore for increasing their pathogenicity.

- As a measure, temporary discontinuation of DFO during a febrile illness until establishing whether the episode is caused by a pathogen that can use DFO as a siderophore or taken under control is strongly advised (B).

Nonsiderophoric iron chelators such as deferasirox are being studied for possible anti-infective properties. In an in vitro study, it has been observed that *V. vulnificus* was stimulated by DFO, whereas orally bioavailable iron chelators such as deferasirox (DFX) and deferiprone (DFP) had an inhibitory effect on the growth of *V. vulnificus* (Neupane & Kim, 2009). Further, DFX and DFP limit the growth of *Chlamydia psittaci*, *C. trachomatis* and *L. pneumophila* and may be suitable as add-on therapies in mucormycosis (Ibrahim et al., 2007; Paradkar et al., 2008). However, the latter could not be supported by a subsequent double-blind, placebo-controlled Phase II trial that aimed to define the safety and efficacy of short-term therapy with DFX for patients with acute mucormycosis (Spellberg et al., 2012).

- DFX or DFP can be continued during febrile episodes (C).

### Disease-Related Risks of Infections in TDT and Preventive Measures

Ineffective erythropoiesis and haemolysis result in hyperplasia of monocyte/macrophages, which phagocytose defective erythroid precursors and erythrocytes. The increased phagocytic activity resulting from clearance of defective erythrocytes may reduce the capacity of the phagocytic system to defend against microorganisms (Wiener et al., 1996) and consequently overwhelms pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) (Ozinsky et al., 2000). In the clinical setting, severe anaemia, itself, has also been observed as a risk factor for bacterial infections in thalassaemia (Wanachiwanawin, 2000).

- Deleterious effects of anaemia, IE and haemolysis on the host defence mechanisms may be brought under control by the maintenance of pretransfusional haemoglobin levels between 90 and 95 g/l. This corrects anaemia while suppressing erythroid marrow (C).

## Infectious Agents in Thalassaemia – Diagnosis and Treatment

### Bacterial infections

#### *Yersinia enterocolitica*

*Y. enterocolitica* is of low pathogenicity and restricted to the gastrointestinal tract in an immune competent host. The availability of large amounts of iron in those with iron overload or undergoing DFO chelation increases the virulence of *Y. enterocolitica*. Fulminant *Y. enterocolitica* septicaemia has been reported as a common infectious risk in DFO-treated thalassaemic patients from western countries (Adamkiewicz et al., 1998), rather than eastern countries.

Clinical manifestations: Fever is the most common presenting feature, often associated with abdominal pain and enterocolitis. Pharyngitis-tonsillitis, acute respiratory distress syndrome and polyarthritides are other clinical manifestations of infection.

The mortality can reach 50% in septicaemia with complications including hepatic and splenic abscesses, osteomyelitis, intussusception, nephritis, meningitis and endocarditis.

Laboratory diagnosis: specific culture conditions (at 22°C for 48 hours) for blood and stool samples are necessary. The microbiology laboratory should be informed to enable correct culture conditions. Serological tests may display cross-reactivity. However, fourfold rises in IgG titres in samples obtained 15 days apart may be suggestive of recent infection.

#### Treatment:

The basic and most important point is that a patient with thalassaemia manifesting the above symptomatology should be managed as follows:

- Stop DFO chelation\*.
- Obtain suitable laboratory samples.
- Commence effective antibiotic treatment immediately.

Intravenous trimethoprim-sulfamethoxazole (400 mg sulfamethoxazole every 12 h) for 7 days (14 days in the case of septicaemia) plus gentamicin (5–7 mg/kg every 24 h) should be used for the treatment. Intramuscular ceftriaxone (2 g every 12 h) is an alternative in focal infections (e.g., enteritis, pharyngitis, tonsillitis). Ciprofloxacin (400 mg every 12 h) is also an effective antibiotic (A).

#### *Klebsiella* spp.

*Klebsiella* spp. has been reported as the major cause of severe bacterial infections in patients with thalassaemia from the Far East (Wanachiwanawin, 2000).

Clinical manifestations: infection presents with sinusitis, intracranial infections, septicaemia and pyogenic abscesses in liver, lung and kidney and parathyroid gland that are associated with high rates of morbidity and mortality.

#### Treatment:

- Stop DFO chelation\*.
- Obtain suitable laboratory samples.
- Commence effective antibiotic treatment immediately.
- Ceftazidime (2 g every 8 h) plus gentamicin (5–7 mg/kg every 24 h) should be used for treatment. Meropenem, imipenem and fluoroquinolones are alternative antibiotics for resistant species.

- Early surgical intervention should be considered (A).

### **Other bacterial infections**

Thalassaemic patients appear to be at high risk of severe bacterial infections, particularly after splenectomy. The most common OPSIs are meningitis, pneumonia and sepsis caused by encapsulated bacteria (*S. pneumonia*, *Haemophilus influenzae* type B, *Neisseria meningitidis*). Other pathogens responsible for post-splenectomy infections include: *E. coli*, *P. aeruginosa*, group B streptococci, *Enterococcus* spp. and *V. vulnificus* (Cullingford et al., 1991).

### **Treatment:**

- Thalassaemic patients with fever and/or other signs of bacterial infection, particularly those who have undergone splenectomy, should be considered as having an emergency medical condition.
- Stop DFO chelation\*.
- Obtain suitable laboratory samples.
- Commence effective antibiotic treatment immediately (A).

*\* Deferiprone does not have the virulence-enhancing effect observed with deferoxamine during experimental Y. enterocolitica infection in mice (Lesic, Foulon & Carniel, 2002). DFP and DFX chelation need not be interrupted on the suspicion of Y. enterocolitica infection (C).*

### **Viral infections**

#### **Human parvovirus B19 (HPVB19)**

Clinical manifestations: HPVB19 typically causes erythema infectiosum or fifth disease in children with the clinical course of a flu-like syndrome. HPVB19 DNA is present in the circulation for almost one week and disappears during the production of neutralizing antibodies (IgM for 6-8 weeks and IgG afterwards). This protective mechanism would not be present in immunocompromised subjects, leading to persistence of viral DNA.

HPVB19 particularly infects erythroid progenitors complicated by a transient red cell aplasia. Because of high erythroid turnover, patients with thalassaemia may develop severe anaemia with low reticulocyte counts during the course of HPV B19 infection (Ricerca, Di Girolamo & Rund, 2009). The patients require intensification of the transfusion regimen during acute infection. HPVB19 infection should be suspected in patients with increased blood consumption once other responsible factors (e.g. allo-immunization or hypersplenism) are excluded.

Although the main route of transmission is respiratory, transfusions of pRBC collected from persistently infected blood donors play a secondary role (Lefrère et al., 2005).

#### **Human immunodeficiency virus (HIV)**

HIV virus leads to CD4+ lymphocyte depletion that renders the individual at risk for many types of opportunistic infections. Due to continuous implementation and improvement of more sensitive serological methods and a nucleic acid amplification test (NAT), the residual risk of viral transmission decreased to less than 1:1.3 million for HIV in the European Union and the US (Velati et al., 2008; Allain, Thomas & Saulea, 2002). No case has been recorded since implementation of NAT screening (August 2008-2015). However, in Africa, higher prevalence and less comprehensive testing still results in an estimated 10% to 15% of cases of HIV linked to unsafe blood transfusion (Safe Blood for Africa Foundation, 2008).

In a large multicentre study comprising 79 HIV-positive thalassaemia patients from different countries, the progression to overt acquired immune deficiency syndrome (AIDS) after seroconversion was 1.4% after 3 years and 9% after five years. There was no statistically significant relationship between disease progression and age, sex, acute infection or splenectomy (Costagliola et al., 1992). However, a significant inverse relationship between disease progression and the dose of DFO administered was reported; the rate of progression decreases as the mean daily DFO dose increases (Costagliola et al., 1994).

A 30-year multicentre study of about 3000 TDT patients (3-65 years old, 45% splenectomised) examined data on the survival of HIV infected patients along with national epidemiological data on donor blood. In 1982-2015, 43 patients tested positive for HIV infection (Politis et al., 2017). Forty-two who had received 819,000 units of RBCs (1:19,000) were infected by the end of 1987. TT-HIV infection risk was reduced significantly by 2005 with seroprevalence in only three patients transfused with seronegative blood donated from three different donors during the serologically silent window period. Therefore, the residual risk of TT-HIV in these patients in 1988-2015 was estimated at 1:290,666 serologically tested blood units. The corresponding figure in the blood donor population was 1:1,833,333 units. Of the 32 patients (74%) who died up to 2015, 26 (81%) progressed to AIDS at a mean age of  $16.5 \pm 9.2$  years. One deceased patient was co-infected with HBV and HCV. Hepatitis B and C were higher in HIV seropositive patients than in seronegative. Of the 13 survivors (mean age  $43 \pm 7.1$  years), two have a history of splenectomy and two are anti-HCV positive with PCR HCV RNA negative. Most patients have negative viral load and are free of HIV symptoms. A multivariate analysis demonstrates that serum ferritin levels are statistically associated with the duration of survival after diagnosis of HIV infection in this group. This 30-year study findings confirm Costagliola et al.'s earlier findings (1992,1994) about the inverse relationship between the rate of disease progression and iron chelation therapy.

A large spectrum of therapeutic options is currently available for HIV-infected patients, which have also been used in patients with TDT. Since iron overload can have an adverse effect on HIV-1 disease progression such as faster progression of HIV in patients with low doses of DFO and high serum ferritin (Gordeuk et al., 2001), optimal control of body iron burden with iron-chelation regimens is recommended in HIV-1-positive TDT patients. Although there is no evidence that splenectomy facilitates the progression of HIV infection, a splenectomy treatment strategy should be decided with caution in an HIV-1 positive patient.

### ***Cytomegalovirus infection***

Cytomegalovirus can be transmitted by fresh blood components containing leukocytes. It is estimated that approximately 2-12% of CMV-positive healthy donors can transmit the virus by blood donation to the recipients. Consequences of CMV infection are serious in immunocompromised patients such as thalassaemia patients who have had stem cell transplantation.

- The use of blood products from CMV-seronegative donors has been shown to be effective in preventing transmission. However, it does not completely eliminate the risk of transmission. Moreover, CMV seroprevalence reaches 50 to 100% in various geographical regions and the availability of CMV-seronegative products is limited.
- Pre-deposit leukodepletion of cellular blood products achieving a residual leukocyte count  $<5 \times 10^6$  per unit allows the reduction of CMV transmission to a level at least

equivalent to the transfusion of sero-negative blood components for those patients at major risk of severe CMV transfusion-associated disease (Bowden et al., 1995) (A).

### **West Nile virus**

West Nile virus is a mosquito-borne flavivirus that primarily causes an asymptomatic or mild disease. However, in <1% it causes neurological disease, such as encephalitis, meningitis or - more rarely - acute flaccid paralysis. Elderly and immunocompromised persons are at higher risk of developing severe disease and having a fatal outcome. The risk of transmission through blood transfusion (TT-WNV) has been recognized and preventive blood safety measures have been implemented in the affected areas. The paucity of reported TT-WNV infections and the blood screening results in the USA and in Europe suggest that blood safety interventions are effective (Domanovic et al., 2019).

Investigation of WNV infection in 369 TDT patients in affected areas of a European country showed that 1.9% of the patients were positive for antibodies against the virus with mild clinical course. Transmission of TT-WNV before the implementation of blood screening with NAT-RNA was confirmed in two thalassaemia patients (Politis et al., 2011).

### **Hepatitis E**

Hepatitis E is caused by infection with a non-enveloped, single-stranded RNA virus (HEV). Although underdiagnosed worldwide, it is responsible for 20 million infections yearly. Four major genotypes infect humans. Genotypes 1 and 2, endemic in many developing countries, are responsible for water-borne epidemics. Genotypes 3 and 4 are associated with zoonotic HEV infections causing sporadic infections in industrialised countries; they are transmitted to humans through consumption of uncooked infectious pork and game products, or by contact with infected animals. Transmissions through transfusion (TT-HEV) and transplantation have also been reported (Perez-Garcia et al., 2015; Schlosser et al., 2012).

HEV mainly causes acute self-limiting infections, but chronic infections may occur in immunocompromised patients and can lead to fulminant hepatitis and death. Locally-acquired HEV cases have been observed across Europe where genotype 3 infections have raised questions for Public Health and blood safety. HEV genotype 3 infection is commonly asymptomatic or mild and self-limiting without chronic sequelae. As acute phase viraemia persists for 6-8 weeks and because most cases are asymptomatic, it is possible for infected blood donors to donate blood while viraemic. A multicentre study in blood donors and thalassaemic patients in a European country, found an overall prevalence of 2.9% for HEV IgG antibodies (significantly greater in males and older donors, as well as in one particular city) and prevalence of 3.6% in thalassaemic patients (Zervou et al., 2015). The increasing incidence of TT-HEV has led several European countries to include blood screening in preventive strategies for this infection. The risk of TT-HEV in groups such as thalassaemia patients with heavy exposure to donor RBCs has raised new issues in their clinical management.

Studies in donor blood in several European countries using NAT testing have shown a high frequency of viraemic donations of up to 1:726 (Hogema, 2016). However, the number of TT-HEV has until now been very low, probably due to under-reporting and under-recognition mainly because of asymptomatic infections in transfused patients.

## Fungal infections

### ***Mucor species***

Mucormycosis or zygomycoses are opportunistic infections that may affect thalassaemics who have undergone stem cell transplantation. Iron is a key nutrient for fungi as well as bacteria. The notion that iron chelation may serve as an effective antifungal modality was proposed more than 30 years ago. However, administration of DFO resulted in exacerbation of mucormycosis. This was attributed to the fact that the DFO itself may act as a siderophore for the fungi. Observations that DFX chelation may be a useful adjunct to antifungal treatment (Ibrahim et al., 2007) led to a trial of DFX combined with liposomal amphotericin B (AmBisome) as short-term therapy for mucormycosis. The results were disappointing as patients treated with DFX had a higher mortality rate at 90 days, leading the authors to conclude that the data did not support a role for initial, adjunctive DFX therapy for mucormycosis.

### ***Phytiosum insidiosum***

Pythiosis is a very rare human infection caused by *Phytiosum insidiosum*, a fungus-like organism. Three forms of human pythiosis are recognised: 1) cutaneous form affecting the periorbital area, face and limbs as a granulomatous, ulcerating abscess-like cellulitis; 2) ophthalmic pythiosis affecting the eyes as corneal ulcers and keratitis; 3) systemic pythiosis affecting vascular tissue and resulting in arterial occlusions leading to gangrene and amputation (Vento, Cainelli & Cesario, 2006). Pythiosis has been reported in Thailand, Australia, Haiti, India, New Zealand and the US. The systemic form was common in patients with thalassaemia and associated with a high morbidity and mortality (most patients die within 6 months) (Prasertwitayakij et al., 2003).

Serological tests and PCR methods are being developed for diagnosis. Antifungal drugs are ineffective for providing disease control. Medical treatment alone is insufficient to salvage patients with systemic infections.

Two vaccines for pythiosis have been prepared. One vaccine has been prepared that has been prepared from soluble concentrated *P. insidiosum* antigen and is administered intradermally in the first, and subcutaneously in the following three injections and at 2 weekly intervals in patients with life threatening systemic infections. The vaccine was curative in a substantial number of cases (Wanachiwanawin et al., 2004).

## Parasitic infections

### ***Malaria***

With reference to transfusion-transmitted diseases, parasitic infections play a very important role. Malaria is believed to be the most important parasitic disease currently facing humans (Angachaisuksiri et al., 2014, World Health Organization. Fact sheet: 2016). Malaria is a life-threatening disease caused by parasites that are transmitted to humans through the bites of infected female *Anopheles* mosquitoes (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale*). The WHO estimates that 3.2 billion people live in areas at risk of malaria transmission in 106 countries and territories. In 2015, out of 91 countries and areas with ongoing malaria transmission, Africa was home to 90% of malaria cases and 92% of malaria deaths (mostly among children) (WHO, 2017). Transfusion transmitted malaria (TTM) is also reported sporadically in non-endemic areas, where the risk of transmission arises from travellers to endemic areas and permanent residents with origin in endemic areas. However, autochthonous transmis-

sions are possible due to the presence of competent vectors.

An outbreak of locally acquired *Plasmodium vivax* malaria in Greece in 2009 which peaked in 2011 raised the question of how to define spatial boundaries of an affected area and when to trigger specific blood safety measures targeted to affected areas with ongoing local transmission. In this context, the ECDC advises the following expert opinion that could help the EU national blood safety authorities in developing a preventive strategy during malaria outbreaks (Domanovic et al., 2016).

- a) Suspension of blood sessions in the affected areas and in surrounding locations up to a radius of 6 km. The distance criterion is based in the dispersal range of the mosquito vector.
- b) Temporary deferral for 6 months from blood donation of asymptomatic persons residing or working in the above areas and visitors from the beginning of an outbreak up to 4 months after the end of the season of mosquito activity.
- c) Blood screening for malarial antibodies or for malarial DNA might be considered instead of temporary deferral that might jeopardize the blood supply. Pathogen reduction could be considered if a suitable methodology is available (Henschler et al., 2011, El Chaar et al., 2013).

There is evidence that carriers of certain haemoglobinopathies have a reduced risk of severe and fatal falciparum malaria. However, the same is not true for the homozygous state including thalassaemia major and intermedia (Vento, Cainelli & Cesario, 2006). The evolving patterns of drug resistance in malaria parasites and changes in recommendations for malaria prevention should be taken into account by physicians who advise chemoprophylaxis to patients before and during periods of travel into endemic areas (Chen & Keystone, 2005).

## Summary and Recommendations

There is a lack of properly controlled studies evaluating infections in thalassaemia. The knowledge of infections depends more on anecdotal reports and experimental studies. The mechanisms of susceptibility to infections in thalassaemia have yet to be clarified completely.

Better understanding of underlying mechanisms and their impact on evolving infections, regional and community-based differences in infectious risks and preventative measures may contribute to a reduction in infection-related mortality in thalassaemia.

Infection-related mortality has become the leading cause of death in thalassaemia in the modern era.

Key recommendations include:

- Physicians must be aware of the potential life threatening infections in thalassaemia and react promptly to investigate and treat I
- Parents and patients should be educated to seek early care when fever develops. I
- Control of iron homeostasis may have therapeutic benefit against infections.
- Prompt initiation of antibiotics and temporary discontinuation of DFO are strongly advised unless the patient has a severe cardiac risk or acute heart failure; whereas, iron-loaded patients can continue to use synthetic oral iron chelators such as deferiprone (DFP) and deferasirox (DFX) during febrile episodes. I
- Transfusion of pre-storage leucodepleted red cells that have been stored <14 days may have therapeutic benefit against infections. IIA
- Quality assurance guidelines, strict regulatory standards and vigilance procedures should be established for safeguarding the quality of donor blood and enhancing the safety of the transfusion process I
- Splenectomy indications and preventive measures for post-splenectomy risk of sepsis should be revisited.



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## CHAPTER 10

### Bone disease

#### Introduction

Bone abnormalities are often seen in patients with thalassaemia major (TM) and thalassaemia intermedia (TI) and have become a major cause of skeletal complications. These unusual skeletal changes include decreased bone mineral density (BMD), spontaneous fractures and spinal deformities with compression of the vertebrae and nerves often causing severe pain and discomfort (Kyriakou et al., 2008; Toumba & Skordis, 2010; Tzoulis et al., 2014; Voskaridou & Terpos, 2004). Children and adolescents with TM have lower BMD than the healthy population and suboptimal peak bone mass and fracture rates are increased and rise with age (Vogiatzi et al., 2009). TM patients have lower trabecular volumetric BMD, cortical area, cortical thickness, and periosteal circumference compared to controls, and these deficits remain unchanged after adjustment for gender, lean body mass and growth deficits (Fung et al., 2011). It has been suggested that males with TM are more frequently and severely affected from bone disease in contrast to the well-known predominance of females among patients with osteoporosis in the general population (Kyriakou et al., 2008; Wong et al., 2014). It is not clear if there are gender differences in the prevalence and severity of bone disease in TM and the exact mechanisms that may underlie a possible gender variation are not known.

According to the World Health Organization, osteoporosis is characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk. In osteoporosis, BMD is reduced, and bone microarchitecture is disrupted. BMD is the most commonly used and well-established measure of bone health. Dual Energy X-ray Absorptiometry (DXA), which is a non-invasive technique and can be performed on the hip, lumbar spine, and distal radius, is still the gold standard for the measurement of BMD. Based on DXA findings, osteoporosis is defined as a BMD T-score  $\leq -2.5$  leading to higher risk of fracture and osteopenia as a BMD T-score between  $-1$  and  $-2.5$ , with normal values of T-score being  $\geq -1.0$ . T-score is defined as the number of standard deviations (SD) that a patient's bone mass is above or below the mean peak bone mass for a 30-year-old healthy woman.

As differences in growth and pubertal timing introduce great variability in skeletal mineral mass and density, interpretation of DXA-derived results of BMD in TM patients is challenging. DXA results are strongly influenced by the child's body size and skeletal maturation; therefore, BMD values must be adjusted for height, bone age, the Tanner stage and bone area. All DXA-derived parameters must be compared with reference data specific to the patient's age, sex, ethnicity and the measuring equipment. The diagnosis of osteoporosis in children should not be made on the basis of densitometric criteria alone. The presence of at least one vertebral compression fracture is indicative of osteoporosis. In the absence of vertebral compression fractures, the diagnosis of osteoporosis is indicated by the presence of both a clinically significant fracture history and a BMD Z-score  $< -2.0$  (adjusted for age and sex as appropriate) (Bishop et al., 2014).

Short stature is common in adults with TM. Children with TM are shorter than their healthy peers and often have delayed pubertal maturation. Both factors contribute to

low BMD values in DXA measurements, and unless properly adjusted for, they lead to over diagnosis of osteoporosis. An additional contributing factor that interferes with BMD readings by DXA in TM is the presence of spinal degenerative skeletal changes, which can be detected only by magnetic resonance imaging (MRI) and most likely interfere with BMD values given by DXA, resulting in false diagnosis of bone disease. Moreover, in high concentrations, iron is radiographically dense; thus, excessive hepatic and cardiac iron stores may lead to potential errors in accurate BMD assessment (Drakonaki et al., 2007).

Peripheral quantitative computed tomography (pQCT) is an alternative bone densitometry technique that is able to assess volumetric BMD at peripheral sites and estimate bone geometry. pQCT has the additional advantage that peripheral sites are assessed, where iron is not accumulated. The radiation dosage to patients is slightly lower than DXA, making it appropriate for use in paediatric populations (Fung et al., 2011).

## Pathogenesis

The pathogenesis of bone disease in TM is multifactorial, complicated, and still unclear. Patients with TM display an unbalanced bone turnover with an increased resorption phase and decreased formation phase, resulting in severe bone loss (Voskaridou & Terpos, 2008). Several contributing factors seem to be involved, acting independently or in concert. The primary disease causes bone marrow expansion due to ineffective erythropoiesis, leading to mechanical interruption of bone histology, cortical thinning, increased distortion and, finally, bone fragility (De Sanctis et al., 1998). Direct iron toxicity on bone impairs osteoid maturation and inhibits mineralization. Iron-chelating agents inhibit osteoblast proliferation and collagen formation and promote osteoblast apoptosis (Skordis et al., 2008). Iron chelation with Desferoxamine may induce dysplastic bone changes in the long bones that are associated with short stature and characteristic x-ray appearances. Deferasirox, at therapeutic doses, has been implicated in the increased incidence of hypercalcuria, nephrolithiasis and bone loss since it has been introduced with seemingly dose-dependent effects on hypercalcuria (Wong P, 2016).

In addition, genetic factors have been shown to be involved in the pathogenesis (Wonke et al., 1998). One of the most important candidate genes for predisposition to osteoporosis is the collagen type Ia1 gene (COL1A1), which encodes type I collagen, the major protein of bone. The polymorphism at the Sp1 site of COL1A 1 has been associated with severe osteoporosis and pathological fractures of the spine and hip in TM patients in addition to the effects of the vitamin D receptor gene (VDR) polymorphisms (Voskaridou & Terpos, 2008).

Failure to progress normally through puberty is associated with failure of adequate bone mineralization and achievement of peak bone mass (Bielinski et al., 2003). In TM patients, BMD, which is already low in childhood, decreases further during and after puberty, especially in patients with absent or delayed puberty. Association between hypogonadotropic hypogonadism and osteoporosis in adult patients with TM is clearly documented (Skordis et al., 2006; Tzoulis et al., 2014). The contribution of other than sex steroid hormones on the acquisition and maintenance of bone mass is well known. BMD at the lumbar spine is lower in adult patients with growth hormone/insulin-like growth factor 1 (GH/IGF-I) deficiency, suggesting that GH/IGF-I deficiency plays an important role in the multifactorial aetiology of bone disease (Soliman et



al., 2014). Additional aetiological factors include parathyroid gland dysfunction, impaired calcium homeostasis, nutritional deficiencies, hypothyroidism, diabetes mellitus, chronic hepatitis and liver disease and the lack of physical activity (Skordis et al., 2008; Voskaridou & Terpos, 2004).

Vitamin D deficiency or insufficiency may start early in TM patients, and its prevalence increases after the first decade and remains, despite vitamin D supplementation (Tzoulis et al., 2014), contributing to bone disease. Potential explanations of the high prevalence of low vitamin D are impaired 25-hydroxylation of vitamin D due to liver haemosiderosis, intestinal malabsorption of vitamin D, lack of sun exposure and impaired synthesis of vitamin D by the skin because of jaundice. However, the prevalence of vitamin D deficiency in patients with TM and the extent of its contribution to bone disease are still under debate. Severe vitamin D deficiency, defined by a vitamin D level less than 25nmol/L with accompanying elevated parathyroid hormone (PTH) and alkaline phosphatase, is rare but can cause osteomalacia (adults) and rickets (children) which in turn may increase the risk of fracture. Optimising vitamin D and calcium through diet +/- supplements is recommended, as a modifiable risk factor, in both osteoporosis and before treatment with bisphosphonate.

## Bone Markers

Patients with TM and osteoporosis have elevated biochemical markers of bone resorption, such as the N-terminal or C-terminal cross-linking telopeptide of collagen type-I (NTX or CTX, respectively) and tartrate-resistant acid phosphatase type5b (TRACP-5b) that correlate with BMD of the lumbar spine in these patients (Voskaridou, 2001). This increased osteoclast activity seems to be at least partially due to an imbalance in the receptor-activator of nuclear factor-kappa B ligand (RANKL)/osteoprotegerin (OPG) system (Morabito et al., 2004), which is of great importance for the activation and proliferation of osteoclast precursors. The ratio of RANKL/OPG is increased in patients with TM and osteopenia/osteoporosis, suggesting their involvement in the pathogenesis of bone loss.

There is also evidence of reduced osteoblast function in TM mainly due to iron poisoning in osteoblasts and/or the result of reduced function of the GH/IGF-1axis. Wnt signaling inhibitors dickkopf-1 (Dkk-1) and sclerostin, which block osteoblast differentiation and function, are increased in the serum of TM patients with osteoporosis and inversely correlate with BMD (Voskaridou et al., 2012, 2009). Wnt inhibition seems to be a major pathway implicated in bone loss in TM.

Biochemical markers of bone metabolism that can be used in patients with TM include:

### Bone formation markers

1. bALP, bone-specific alkaline phosphatase
2. OC, osteocalcin
3. PINP (Procollagen I Intact N-terminal)

### Bone resorption markers

1. NTX, N-terminal cross-linking telopeptide of collagen type-I
2. CTX, C-terminal cross-linking telopeptide of collagen type-I
3. RANKL, receptor-activator of nuclear factor kappa B ligand
4. Dickkopf – 1

## 5. Sclerostin

Assessment of bone health in TM patients should begin at the age of 10 years, with annual assessment of serum calcium, phosphate, alkaline phosphatase, vitamin D, PTH and urinary calcium and phosphate excretion. In clinical practice, biomarkers of bone turnover should include one marker of bone formation, commonly bone-specific alkaline phosphatase and one marker of bone resorption, dependant on local availability of assay, to provide information on bone homeostasis. Assessment of BMD by DXA should be performed every 2 years after the age of 10 years, accompanied by vertebral fracture assessment. Lateral spinal radiographs for vertebral morphometry should be performed at longer intervals, unless there are clinical indications. MRI of the spine may be considered to exclude degenerative spinal skeletal changes.

**Recommendations for the prevention and treatment of early bone loss** include the following:

- Annual checking of BMD starting in adolescence
- Encourage physical activity
- Smoking should be discouraged
- Adequate calcium and vitamin D intake
- Early diagnosis and treatment of diabetes mellitus
- Adequate iron chelation to prevent iron toxicity in the bone
- Sufficient blood transfusions to inhibit bone marrow expansion.
- Hormonal replacement of gonadal failure: Prevention of hypogonadism seems to be a very effective way for preventing osteoporosis and other bone deformities in TM patients. Continuous hormonal replacement therapy with transdermal oestrogen for females or human chorionic gonadotrophin formula improves bone density parameters.

## Medical treatment

**Bisphosphonates (BPs)** are the medications that have been used for the treatment of osteoporosis in thalassaemia in an attempt to increase BMD and alleviate symptomatology. Almost all generations of bisphosphonates have been used in various studies. Bisphosphonates inhibit osteoclastic bone resorption, by inhibiting osteoclastic recruitment and maturation, therefore preventing further bone destruction. Most of the studies did not include any control group; therefore, the results should be interpreted cautiously. The main outcomes of the studies were increase in BMD, normalization of bone markers, improved quality of life, and a decrease in the incidence of fractures. The safety of the medication was also taken into consideration. Current evidence supports the use of BPs in patients with TM-associated osteoporosis to prevent bone loss and/or to improve BMD in those with established osteoporosis.

The BPs that have been used in randomized controlled studies (Dede & Callan, 2018; Giusti, 2014; Bhardwaj et al., 2016) are:

1. Oral alendronate 10 mg/day
2. IV zoledronic acid 4 mg every 3 months
3. IV neridronate 100 mg every 6 months
4. IV pamidronate 30 mg every month

Oral administration of alendronate normalizes the rate of bone turnover and results in a rise in BMD of the spine and the hip. Pamidronate results in a significant improvement in BMD in most patients, a clear decrease of markers of bone resorption (NTX and TRAP-5b) and significant reduction of pain (Voskaridou et al., 2003). Neridronate was also associated with reduction of bone resorption, increase of BMD, reduction of back pain and improved quality of life (Forni et al., 2012). Zoledronic acid is the most potent third generation bisphosphonate to-date and has been found to be extremely efficacious in increasing BMD in TM patients (Voskaridou et al., 2006). Zoledronic acid continues to act for one more year after its discontinuation. All bisphosphonates have to be given in higher doses in TM patients with osteoporosis than the dose used in post-menopausal osteoporosis in order to produce similar effects, due to the complex aetiology of TM-associated osteoporosis.

Although there is no consensus it is recommended that the use of bisphosphonates should not exceed a period of 24 to 36 months. Patients on BPs should receive in addition to vitamin D, elemental calcium supplementation at a dose 200–1000 mg daily and they need periodical measurement of urine calcium excretion to avoid hypercalciuria that may predispose to nephrolithiasis. Patients who receive BPs are monitored for improvement of their symptoms and increase in the BMD findings based on DXA scans. A suggested treatment schedule would include Zoledronate (0.05mg/kg 6 monthly) whilst there is remaining growth potential, and the BMD remains outwith what is normal for population (lumbar spine or total body BMD <-2.0 SDS).

Further research is warranted however to establish the efficacy of medications on fracture prevention, the safety and efficacy of long-term therapy, the drug free periods and the optimal duration of treatment. In addition, more trials must be conducted to clarify the exact role of each bisphosphonate, their exact dosage and their long-term benefit as well as the effects of the combination of bisphosphonates with other effective agents in TM-induced osteoporosis. Hypercalcuria is common in TM, reported up to 50%, thought to be a consequence of iron chelation, and is part of recommended annual monitoring. Bisphosphonates, in all children, have been shown to decrease 24hr urinary excretion of calcium through reduction in bone resorption and therefore may reduce the incidence of hypercalcuria in TM. To avoid problems of interpretation of urinary calcium relative to the timing of bisphosphonate dose we would recommend performing measurements immediately pre-dose.

Other novel agents that could be effective in TM associated bone disease include: teriparatide, a recombinant peptide fragment of parathyroid hormone; strontium ranelate, a second anabolic agent that seems to prevent osteoporotic fractures in postmenopausal women; and Denosunab, which is an antibody against RANKL. In a randomized, placebo-controlled, double-blind study Denosunab increased lumbar spine and wrist bone mineral density and reduced pain and bone remodeling markers, hence being another valuable option for the management of osteoporosis in TM (Voskaridou et al., 2018).

Prevention should undoubtedly be the first step in the management of bone disease in TM. Induction of puberty at a proper age and management of hypogonadism are very important steps. However, there is conflicting evidence for the clinical effectiveness of hormone replacement therapy (HRT) in maximizing bone mass in TM patients (Voskaridou et al., 2001). Effective iron chelation, improvement of haemoglobin levels, calcium and vitamin D supplementation, physical activity and smoking cessation are the main preventative measures. During the last two decades, new evidence has suggested that the reduced osteoblastic activity, which is believed to be the basic

mechanism of bone loss in TM, is accompanied by a comparable increase in bone resorption. The presence of unbalanced bone turnover with an increased resorption phase has justified the use of bisphosphonates, aiming in preventing further loss of BMD. Combined therapy with bisphosphonate and HRT has also been used with encouraging results (Terpos & Voskaridou, 2010).

Osteonecrosis of the jaw (ONJ) and atypical fractures are rare, no documented case of ONJ in childhood, and are primarily related to the duration of bisphosphonate treatment. There is no evidence of any one bisphosphonate that is more likely to be implicated in either aetiology. Additional risk factors for ONJ in adults include age, the use of concomitant medication that affect bone turnover, underlying medical conditions and history of recent dental procedures.

## Summary and Recommendations

I-CET (International Network on Growth Disorders and Endocrine Complications in Thalassaemia) (De Sanctis et al., 2013)

Osteoporosis is a prominent cause of morbidity in patients with thalassaemia major; it is present in approximately 40–50% of patients. The pathogenesis includes genetic factors as well as endocrine complications (mainly hypogonadism), iron overload, bone marrow expansion, vitamin deficiencies and lack of physical activity. These factors can lead to bone destruction through the increase of osteoclast function and/or the reduction of osteoblast activity. Management of thalassaemia-associated osteoporosis consists of adequate calcium and vitamin D intake, sufficient iron chelation, hormone replacement and inhibition of the osteoclast function mainly by bisphosphonates. Intravenous administration of pamidronate or zoledronic acid seems to be more efficient than oral bisphosphonates. Other novel agents such as the novel osteoclast inhibitor, denosumab, teriparatide and the activin antagonist, sotatercept, are under investigation but their effects in TM-induced osteoporosis remains to be proven.

Key recommendations include:

- Annual checking of BMD starting in adolescence is considered indispensable (IC)
- Biochemical markers of bone metabolism that can be done every year: NTX, CTX, Balp (IlbC)
- Physical activity must always be encouraged (IC)
- Smoking should be discouraged (IC)
- Adequate calcium intake during skeletal development can increase bone mass in adult life and in combination with administration of low doses of vitamin D may prevent bone loss and fractures (IC)
- Early diagnosis and treatment of diabetes mellitus (IC)
- Adequate iron chelation may prevent iron toxicity in the bone and sufficient blood transfusions may inhibit uncontrolled bone marrow expansion (IA)
- Hormonal replacement where it is needed (IA)
- Bisphosphonates should be given concomitantly with calcium and vitamin (IA)

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Figure 1. Normal and osteoporotic bone morphology

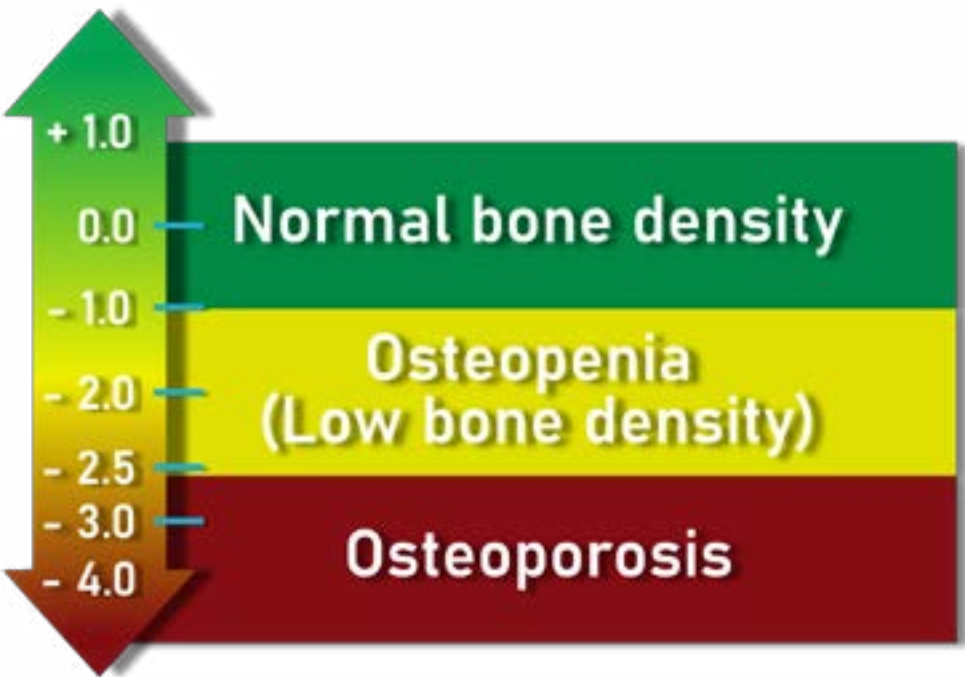


Figure 2. Classification of bone disease based on BMD measurements

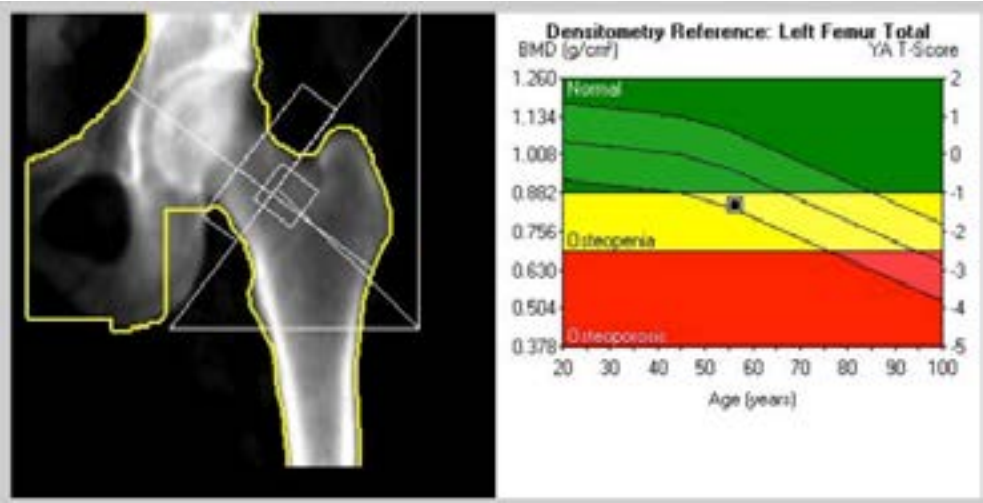


Figure 3. Interpretation of DXA findings

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## CHAPTER 11

### Dental Care

Thalassaemia is one of the most common genetic disorders worldwide and presents significant public health and social challenges in areas where incidence is high. The manifestations of the condition are modulated by several genetic, racial, and environmental factors. Thalassaemia almost exclusively affects people of particular ethnic origins and is characteristic in its distribution. As a result, there are geographical variations in dental awareness of the oro-facial manifestations of thalassaemia and many dentists may lack experience in treating patients with this condition (Hattab, 2012; Duggal et al., 1996). As a consequence patients may experience difficulties in accessing appropriate dental care. When dental treatment is provided, the dentist may not be fully aware of the implications of thalassaemia on dental management, and may not liaise with haematology colleagues when appropriate. Conversely, fear of the unknown may be associated with a reluctance to provide anything beyond basic dental care. Indeed, many general dentists may prefer to refer these patients to either specialist dental services, or to hospital-based specialised dental units, especially when dental extractions are required. This chapter shall review key considerations in the dental care of patients with thalassaemia, and provide guidance on best management and also provision of optimal care in respect to healthcare systems, organisation and referral pathways.

### Oro-facial Features

Many oro-facial features have been described in thalassaemia, and these are summarised in Table 1. It is important that both patients and dentists are aware that if these features are present, they may be associated with the underlying thalassaemia disease process, so that appropriate management can be initiated. It is known that thalassaemia may result in changes in the bones; the extent of which depends on the severity of the anaemia, the patient's age, duration of the clinical symptoms, and the timing of both therapeutic blood transfusion and splenectomy. When bone changes are present, the main oral change that has been reported in the literature is malformation of the facial bones due to bone marrow hyperplasia caused by rapid red cell turnover, particularly in  $\beta$ -thalassaemia major. The hyperplasia of bone marrow in the maxilla (upper jaw) exceeds that of the mandible, and results in a characteristic appearance known as 'chipmunk face' (Abu Alhaija, Hattab & al-Omari, 2002), as illustrated in Figure 1. This may be associated with spacing of the upper teeth, forward drift of the maxillary incisors and increased overjet (see Figure 2). Where there is misalignment of teeth due to maxillary expansion, orthodontic treatment or cosmetic dentistry may be required to correct alignment.

**Table 1.** Summary of the main oro-facial features described in thalassaemia.

### **ORO-FACIAL MANIFESTATIONS OF THALASSAEMIA**

- Enlargement of the upper jaw (chipmunk face)
- Migration and spacing of upper anterior teeth
- Increase in dental decay
- Delayed dental development
- Change in dental morphology
- Alveolar bone may have a 'chickenwire-like' radiological appearance
- Delayed pneumatization of maxillary sinuses
- Painful swelling of parotids and xerostomia (due to iron deposits)
- Mucosal Pallor and dental discolouration
- Sore or burning tongue due to folate deficiency
- Oral ulceration (very rare)
- Necrotizing gingivostomatitis (very rare)



**Figure 1.** Profile view of a 13-year-old boy with thalassaemia major showing typical facial features of thalassaemia major; characterized by frontal bossing, bulging cheekbone, saddle nose, and pro-trusive premaxilla.

The dental arch parameter characteristics of patients with  $\beta$ -thalassaemia major may include a narrower maxilla, a shorter maxilla and mandible, reduced ramus length and width, and smaller tooth crown size (Hattab, 2013a; Hattab & Yassin, 2011; Al-Wahadni, Qudeimat & Al-Omari, 2005; Hattab, Abu-Alhaija & Yassin, 2000).

The reduced tooth size may render the dentoalveolar bone housing the teeth to be more deficient. An increased incidence of mild Class II skeletal pattern and prominent vertical growth direction of the mandible has also been noted (Toman et al., 2011; Amini et al., 2007). These changes may have implications for orthodontic treatment. Delayed dental development with associated physical growth retardation has also been noted in patients with  $\beta$ -thalassaemia major (Hattab, 2013b). The caries prevalence has been found to be significantly higher in thalassaemic patients than in healthy controls (Hattab et al., 2001; Siamopoulou-Mavridou et al., 1992). The higher dental caries experience in  $\beta$ -thalassaemia major patients may be attributed to poor oral hygiene, improper dietary habits, lack of dental awareness, reduced salivary flow rate, and neglected dental care. In addition to the reduced salivary flow rate in  $\beta$ -thalassaemia major patients (Hattab et al., 2001), a lower concentration of salivary immunoglobulin A (Siamopoulou-Mavridou et al., 1992) and higher levels of salivary *Streptococcus mutans* (Lugliè et al., 2002) have been described, compared with controls. Thalassaemic patients show a tendency for higher plaque rates, gingivitis and periodontitis scores than control subjects (Hattab, 2012; Mehdizadeh, Mehdizadeh & Zamani, 2008).

Changes in dental morphology have been consistently noted and include short roots, taurodonts and attenuated lamina dura. Radiographic changes (shown in Figure 2) include thickened frontal bone, thinned cortex of the mandible, small maxillary sinuses, faint inferior dental canal and enlarged marrow spaces (Hattab, 2012; Hazza'a & Al-Jamal, 2006). Iron deposition in the parotid glands can result in painful facial swelling but is rare (Hattab, 2012; Goldfarb, Nitzan & Marmary, 1983). Dental and jaw pain, pallor oral mucosa, oral ulceration and burning tongue may also be present, secondary to chronic anaemia. Necrotising stomatitis, possibly linked to agranulocytosis due to deferiprone has also been described in thalassaemia (Tewari et al., 2009).



**Figure 2.** Cephalometric radiograph of a 15-year-old boy with thalassaemia major disclosing prominent premaxilla, thickened frontal bone, thinned inferior border of the mandible and partially obliterated maxillary sinus.

## Risk Assessment for Delivery of Dental Care

Due to the great clinical variability in systemic signs and symptoms with which patients with thalassaemia present, the most important aspect of dental care is the need to deliver it through a coordinated team approach, ensuring close liaison with the haematologist, and where appropriate the paediatrician. In order to undertake a complete risk assessment, information from the haematology team on the patient's clinical status and recent blood test results should be accessed to ensure risk is minimised when planning dental care. The appropriate setting for provision of care should be determined, namely whether in the setting of primary or secondary (hospital-based) care.

### Type of anaesthesia

Most people with thalassaemia can receive routine dental treatment in the primary care setting, using local anaesthesia without problems. There is a theoretical risk associated with giving local anaesthetic containing adrenaline, as it may lead to impairment of local circulation in patients with thalassaemia. In view of this, consideration may be given to using a local anaesthetic without a vasoconstrictor for short dental procedures, with 2% Lidocaine and 1/100,000 epinephrine used for longer procedures requiring more profound anaesthesia.

Sedation should be used with caution in patients with thalassaemia due to the presence of chronic, potentially severe anaemia and the risk of respiratory depression. For this reason, inhalation sedation is preferable to intravenous sedation. The use of general anaesthesia is best avoided due to the risks associated with underlying anaemia. When general anaesthesia is absolutely necessary, it should be carried out as an in-patient procedure, with the patient admitted under the care of the haematology team.

### Co-morbidities that may impact on dental care

Individuals with thalassaemia often experience multiple secondary effects from their disorder. These can impact on the delivery of dental care in a number of ways, as summarised below:

- **Chronic anaemia**

- In addition to the oro-facial manifestations associated with chronic anaemia, patients may appear to be fatigued, lethargic and poorly motivated. Dental care should be adapted according to their tolerance of the planned procedure on the day of treatment.

- **Infections**

- Infections are major complications and represent one of the main causes of morbidity in patients with thalassaemia. Patients who have undergone a splenectomy are at higher risk of significant infection following a bacteremia (Wang et al., 2003). Multiple immune abnormalities (Vento 2006), defective neutrophils, macrophage chemotaxis (Bassaris et al., 1984) and increased oral *Candida albicans* colonization have been noted in patients with thalassaemia. The increased infection risk should be taken into account when providing dental care.

- **Depression**

- Lifelong adherence to a complicated medical regimen can potentially impact on the emotional functioning of patients with thalassaemia. This can further impact patient motivation and willingness to accept dental interventions (Mednick et al., 2010).

- **Transfusion-transmitted infections**

- Prior to screening of blood products, people with thalassaemia were at increased risk of carriage of Hepatitis B, C, G viruses and HIV. Appropriate cross-infection protocols should be in place and precautions taken when providing care. In the case of associated hepatic disease / liver cirrhosis, caution must be used when prescribing medication. For all patients with thalassaemia receiving regular exchange transfusion, invasive dental care should be delivered in the week following a planned exchange, as the patient's blood counts will be optimal. Invasive dental procedures should be avoided on the same day as the exchange, as the patient is often fatigued following transfusion.

- **Iron overload and tissue deposition**

- Iron accumulation in hepatic, cardiac and endocrine tissues is well documented for patients with thalassaemia major. Dentists need to take additional precautions to compensate for potential complications such as impaired liver function and diabetes. Iron deposits have also been found in the gingivae (Calışkan et al., 2011). Incorporation of blood pigment bilirubin; a product of haemoglobin breakdown, has been described in the dentinal tubules resulting in yellow discoloration of teeth (Hattab, Qudeimat & al-Rimawi, 1999). Although the impact of iron deposits on periodontal health is unknown, further studies investigating the use of gingival biopsies for diagnosis of iron overload are needed.

- **Cardiomyopathy**

- Chronic anaemia can result in cardiomyopathy and is further exacerbated by cardiac iron overload. Although patients may be asymptomatic with their cardiac dysfunction, when anxious and / or undergoing a stressful dental procedure, they may precipitate their cardiac symptoms. Dentists need to be aware of the degree of cardiac involvement and implement precautions as appropriate.

- **Bisphosphonate-related complications**

- Bisphosphonates are commonly used in thalassaemia patients to stabilise bone remodelling. However, in recent years there have been an increasing number of cases of bisphosphonate (BP)-related osteonecrosis of the jaw (BRONJ). This is characterised by trans-mucosal exposure of necrotic bone, often triggered by surgical trauma such as dental extractions (see Figure 3). There is currently no clear evidence for the efficacy of any intervention to manage BRONJ (Fedele et al., 2009). In view of this, dental extractions are avoided where at all possible.



**Figure 3.** Area of BRONJ: Exposed bone 3 months after dental extraction of a lower left molar.



## Practical Management

Patients with  $\beta$ -thalassaemia major are at increased risk of developing dental caries and periodontal disease. Furthermore, there may be increased risk when delivering invasive dental treatment due to the multiple potential co-morbidities that are associated with thalassaemia. In view of this, patients should be maintained closely on a preventive programme with regular follow-up. Oral hygiene instructions, dietary advice and preventive measures including prophylaxis, fluoride application, and fissure sealants should be implemented to minimize the need for invasive dental procedures. Dentists also need to be aware of the oro-facial manifestations of thalassaemia so that they can be identified early and appropriately managed. Close liaison with the haematology team is required to determine the potential complications when delivering invasive dental treatment and measures put in place to reduce risk. The severity of the thalassaemia, the degree of anaemia - as determined by recent blood test results, and the extent of multi-system involvement / co-morbidities should be established so that risk can be reduced and care provided in the appropriate setting.

### Dental infections and abscesses

Predisposing factors for infections in thalassaemic patients include severe anaemia, iron overload, splenectomy, and a range of immune abnormalities. As a result, these patients are at potential risk of infection following any dental procedures associated with bacteraemia (most notably dental extractions or scaling). Guidelines regarding antibiotic prophylaxis vary from country to country with some recommending prophylaxis similar to that used for the prevention of bacterial endocarditis. Patients presenting with acute dental infections / abscesses should receive urgent dental care and antimicrobial therapy as required.

### Maxillofacial deformity

Patients with thalassaemia may have bone marrow expansion leading to malformations of the facial bones. This is more common for those individuals who are under-transfused or begin transfusion at a later stage. Correction of drifted maxillary anterior teeth and increased overjet should be undertaken to improve aesthetics, reduce susceptibility to trauma, avoid gingival inflammation, and improve functional ability. It is recommended that orthodontic treatment be initiated as early as possible, concentrating on preventive and interceptive approaches.

### Management of patients on bisphosphonates

All patients should ideally have a comprehensive dental assessment with their local dentist prior to the commencement of bisphosphonate therapy, to ensure that they are as dentally fit as feasible. Emphasis is on reduction of mucosal trauma and avoidance of subsequent dental extractions. Preventive dental advice should be given, emphasizing the importance of reporting any symptoms such as loose teeth, pain, or swelling, as soon as possible (SDCEP Guidance, 2017). If a patient has spontaneous or chronic bone exposure, referral to an oral surgery/oral and maxillofacial surgery specialist should be considered. When a patient is already on bisphosphonates and a dental extraction is unavoidable, straightforward extractions can be undertaken in primary care, although a second opinion can be sought when necessary. Surgical extractions should be undertaken by a specialist in oral surgery / maxillofacial surgeon. All patients should be advised of the risk pre-operatively and closely monitored post-operatively. There is no evidence supporting the discontinuation of bisphosphonates temporarily, as the drugs persist in the skeletal tissues for years. There is also no conclusive evidence supporting the use of antibiotics or topical antiseptic prophylaxis in reducing the risk of BRONJ (Fedele et al., 2009).



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## CHAPTER 12

### Splenomegaly and splenectomy

#### Introduction

Thalassaemia represents a heterogeneous group of inherited diseases characterised by the lack, or reduced production, of haemoglobin  $\beta$  chains. One feature of the common pathophysiological bedrock is an increased destruction of red blood cells by the reticuloendothelial system, in particular the spleen and this, together with extramedullary haemopoiesis, results in splenic enlargement (splenomegaly) (Cappellini et al., 2018). Many patients with thalassaemia require splenectomy, although it is more commonly needed in non-transfusion dependent thalassaemia (NTDT). The main therapeutic rationale for splenectomy in transfusion-dependent patients with  $\beta$  thalassaemia major (TM) has been to decrease blood consumption and transfusion requirements with the ultimate goal of reducing iron overload (Rachmilewitz & Giardina, 2011). **However, current transfusion guidelines setting more adequate pretransfusional haemoglobin levels (90-105 g/l), usually achieved by more frequent transfusions, have considerably reduced the incidence of splenomegaly and the need for splenectomy in TM patients.** The probability of undergoing surgery within the first 10 years of life is 57, 22, 6 and 7%, for patients with thalassaemia born in the 1960s, 1970s, 1980s and 1990s respectively (Piga et al., 2011).

Throughout the care of the patient with thalassaemia, the size of the spleen should be carefully monitored and recorded on physical examination and, as needed, by ultrasonography. Splenomegaly due to periods of under-transfusion may be reversible. Before considering splenectomy in this situation, the patient should be placed on an adequate transfusion programme for several months and then re-evaluated. The spleen may increase in size during pregnancy and these patients require careful follow up.

#### Steps that can be taken to reduce or delay the onset of splenomegaly include

- Adequate transfusion, maintaining pre-transfusion haemoglobin concentration (Hb) 90 g/l as a minimum. Splenomegaly may be reversible by increasing transfusion, albeit at the cost of increased iron load.
- Transfusion of red cells with a minimum haemoglobin content of 40 g.
- Use of the freshest possible red cells but, at the least, those that have been stored for less than 2 weeks.
- In certain cases whereby the pre-transfusion Hb is kept at adequate levels and yet the spleen size is increasing, a short period of a few months of hypertransfusion with the aim of increasing the trough Hb and reducing further the extramedullary erythropoiesis might be needed.

#### Indications for Splenectomy

**All current guidelines agree that physicians should adopt a guarded approach and restrict splenectomy to certain indications in view of the observation of an increased risk of venous thrombosis and pulmonary hypertension, alongside overwhelming infections after splenectomy** (Vichinsky et al., 2012; Sayani et al., 2009; Taher, Weatherall & Cappellini, 2018).

**Splenectomy should be avoided in children less than 5 years of age because of a considerably greater risk of fulminant post-splenectomy sepsis.**

The main indications for splenectomy are highlighted in Table 1.

**Table 1.** Indications for Splenectomy in Thalassaemia Major.

INDICATION	COMMENT
Increased blood requirements that prevent adequate control with iron chelation therapy, having made sure that the increased requirements are not due to allo/auto-antibodies or blood loss.	Annual transfusion volume used to flag an increased blood requirement (200-275 ml/kg/yr red cells)*. However, if effective chelation therapy continues to be maintained despite increased transfusion, splenectomy may not be necessary.
Hypersplenism	Cytopenias causing clinical problems
Symptomatic splenomegaly	<ul style="list-style-type: none"> <li>Accompanied by symptoms such as left upper quadrant pain or early satiety.</li> <li>Massive splenomegaly causing concern about possible splenic rupture.</li> </ul>

\* Guidelines vary slightly as to what constitutes increased requirements: (US: 225-250 ml/kg/yr, Vichinsky et al., 2012, Canada: 250 -275 ml/kg/yr, Sayani et al., 2009, UK: 200-220 ml/kg/yr, Yardumian et al., 2016). Physicians should make a decision based on all clinical parameters.

## Splenectomy and Peri-operative Complications

Any surgery on the spleen should include a careful search for accessory spleens. There are currently four approaches to splenectomy; open and laparoscopic total splenectomy, partial splenectomy and reduction of splenic tissue by embolization. The two surgical techniques most commonly employed for total splenectomy are open splenectomy (OS) and laparoscopic splenectomy (LS) approaches. LS is associated with a significant reduction in 30-day postoperative mortality, shorter hospital stay, and significantly fewer pulmonary, wound and infectious complications (Musallam et al., 2013). Although there have been doubts raised regarding the suitability of LS for patients with splenomegaly, recent studies have demonstrated the superiority of LS to OS in patients with massive and even supramassive spleens (Koshenkov, Németh & Carter, 2012; Tsamalaidze et al., 2017), although the hand-assisted approach may be necessary (Kercher et al., 2002; Habermalz et al., 2008).

Partial splenectomy is used to preserve some of the immune function of the spleen while reducing the degree of hypersplenism. Because of a lack of randomised trials, no conclusive findings can be drawn about the comparative effectiveness of partial splenectomy compared with total splenectomy (Rice et al., 2012). The long-term success of this approach is still undergoing evaluation. In particular, the likelihood of splenic re-growth and the volume of splenic tissue required to preserve immune function are two questions outstanding.

Reduction of splenic tissue by embolization is a less invasive approach to hypersplenism than complete or partial surgical splenectomy. The exact splenic volume necessary is still undefined but optimal results can be obtained by 50%-70% reduction (Ahuja, Farsad & Chadha, 2015). Common complications are fever, nausea, significant pain and the possible need for a subsequent total splenectomy. Antibiotics should be given before and after the procedure. More significant complications reported include abscess formation, pleural effusion, portal vein thrombosis and liver failure. It should be noted that most of the serious complications occur when the volume embolised is 70% or greater (Koconis, Singh & Soares, 2007). Splenic embolisation has not gained wide acceptance but is certainly worth exploring as an option at centres where there is expertise in this procedure. Embolization does not permit a search for accessory spleens.

## Concomitant Cholecystectomy

An evaluation for gallstones should be performed prior to splenectomy, especially if the patient has experienced symptoms suggestive of biliary tract disease. In some cases, positive findings will lead to cholecystectomy at the same time as splenectomy. Removal of the appendix at the time of splenectomy may prevent later problems in distinguishing infection with *Yersinia enterocolitica* from appendicitis. Splenectomy also provides a good opportunity for a liver biopsy to assess the liver histology and iron concentration.

## Adverse Events of Splenectomy

Peri-operative complications include bleeding, atelectasis and subphrenic abscess. Major immediate and long term adverse effects of splenectomy are sepsis, thrombosis and pulmonary hypertension.

### A. Sepsis:

The major long-term risk after splenectomy is overwhelming sepsis. The spleen provides important host defence functions by removing circulating antigens and synthesising opsonising antibodies, tuftsin, and immunoglobulins, principally immunoglobulin M (IgM) (Darzi 2015). Removal of the spleen is associated with an increased predisposition to severe infections and mortality.

The risk of overwhelming post-splenectomy infection varies with:

Age: the risk is greatest in children up to the age of 16 years and in adults over 50 years (reviewed by William & Corazza, 2007).

Time since splenectomy: although the risk of sepsis is life-long, the greatest risk appears to be in the period 1-4 years after surgery. However, fulminant bacteraemia has been reported in adults as much as 25-40 years after splenectomy.

## Immune status of patient

The most frequent pathogens that cause infections in splenectomised patients are *Streptococcus pneumoniae*, *Haemophilus influenzae* type B, and *Neisseria meningitidis*, all of which are associated with a high mortality rate. Other organisms associated with systemic infection in splenectomised patients are *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp, and *Klebsiella pneumoniae* (Castagnola & Fioredda, 2003; Yapp et al., 2009). The introduction of routine anti-pneumococcal immunisation and prophylactic antibiotics can prevent severe pneumococcal infec-

tions in the first 2-4 critical post-splenectomy years. Immune prophylaxis in splenectomised patients is summarised in Table 2. Protozoan infections due to *Babesia* have been implicated in a fulminant haemolytic febrile state in splenectomised patients. Malaria is more severe in asplenic people and carries an increased risk of death (Boone & Watters, 1995).

Characteristics of overwhelming post-splenectomy sepsis include the sudden onset of fever and chills, vomiting and headache. The illness rapidly progresses to hypotensive shock, and is commonly accompanied by disseminated intravascular coagulation. Post-splenectomy sepsis has many of the features of adrenal haemorrhage (Waterhouse–Friederichsen syndrome). The mortality rate for such infections is approximately 50%, despite intensive supportive measures. Therefore, early intervention on the basis of clinical suspicion, even in the absence of many of the above findings, is critical.

**Table 2.** Immune prophylaxis in splenectomised patients (modified from Davies, 2011)

VACCINE*	SCHEDULE	COMMENT
Streptococcus Pneumoniae Pneumococcal polysaccharide (23 valent) vaccine [Pneumovax 11] 1 dose 0.5ml IM <sup>1</sup>	4-6 weeks pre-splenectomy or at least 2 weeks in advance <sup>2</sup> . Repeat 5 yearly thereafter.	Rate of protection is 70-85% • The immune response is poor in children less than two years of age. In this age group PCV7 may be used
Haemophilus influenzae type B	4-6 weeks pre-splenectomy or at least 2 weeks in advance <sup>2</sup> .	There are currently no recommendations for repeat vaccinations
Meningococcal Group C conjugate vaccine	4-6 weeks pre-splenectomy or at least 2 weeks in advance <sup>2</sup> . MenACWY 2 months later Repeat 5 yearly thereafter.	
Influenza virus vaccination	Yearly	This should also be offered to non-splenectomised patients

\* Children vaccinated under the age of two should be re-vaccinated at age two.

<sup>1</sup> CDC (USA) guidelines additionally advise that a second pneumococcal vaccine, PCV13, should also be given 2 months prior to the 23 valent vaccine.

<sup>2</sup> Patients undergoing emergency splenectomy can still benefit from vaccination TWO WEEKS post splenectomy.

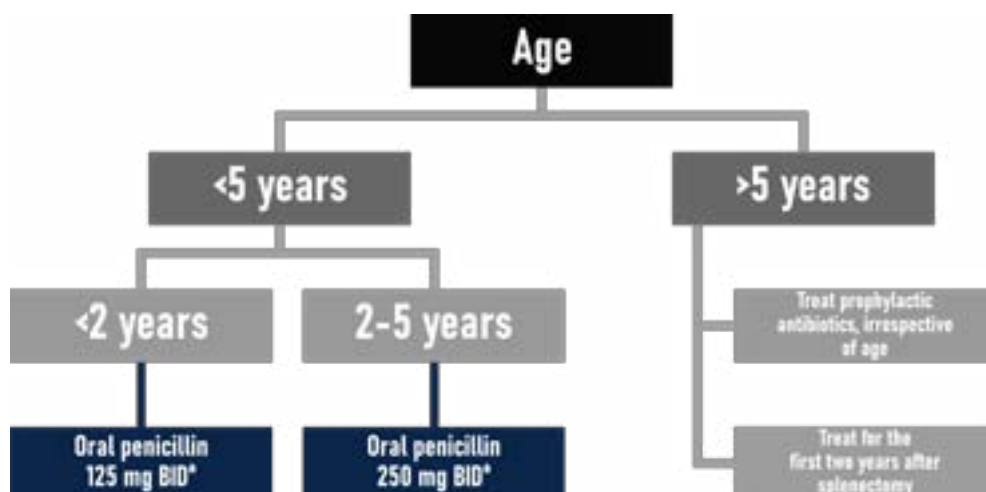
## Chemoprophylaxis in splenectomised patients (summarized in Figure 1)

The increased risk of infection after splenectomy remains throughout life, but is highest in the first few years post-operatively.

A broad spectrum antibiotic should be given pre- and continued post-operatively. There are currently no RCTs regarding the length of time post-splenectomy penicillin should be continued. The use of life-long prophylactic penicillin against pneumococcal disease has been BCSH policy for more than 20 years (BCSH, 1996). Penicillin prophylaxis is highly effective in children with sickle cell disease and this experience provides the main evidence for continuing prophylaxis in other at risk groups (Riddington & Owusu-Ofori, 2002; Cummins, Heuschkel & Davies, 1991). British Society of Haematology guidelines 2011 recommend that life-long prophylactic antibiotics should be offered to patients considered at continued high risk of pneumococcal infection using oral penicillins or macrolides. This advice should be regularly reviewed in the light of local pneumococcal resistance patterns. The 2011 BCSH guidelines recommend **penicillin prophylaxis post-splenectomy up to the age of 16 years** and over the age of 50 years. **In addition, high-risk patients such as diabetics, should have life-long prophylaxis. Other authors recommend prophylaxis in children for 5 years and adults 2 years post-splenectomy** (Habermalz et al., 2008).

Antibiotic prophylaxis should be prescribed when a splenectomised patient undergoes invasive dental procedures and vigilance should be applied for antimicrobial cover pre- or post- any other invasive procedure.

Figure 1. Chemoprophylaxis in splenectomised patients



*\*Depends on opinion of treating physician and patient characteristics/comorbidities*

Alternative antibiotics for patients unable to take penicillin include amoxicillin, trimethoprim-sulfamethoxazole and erythromycin. The use of prophylactic antibiotics will need to be regularly re-evaluated as improved immunisations become available and as new data regarding antibiotic-resistant bacteria are developed.

**The importance of compliance with prophylactic antibiotics should be stressed repeatedly to patients and parents.** However, the limitations of antibiotic prophylaxis must also be emphasised. Patients and parents should recognise that chemoprophylaxis does not prevent all cases of post-splenectomy sepsis: the risk of death from febrile illnesses remains, and rapid evaluation of febrile episode is essential. Patient and parent education can be highly effective in preventing overwhelming post-splenectomy infection.

**Physicians should emphasise to the patient and parents the importance of recognising and reporting febrile illnesses and seeking immediate medical attention.**

For all febrile episodes, the physician should strongly consider:

- Evaluating the patient, including a complete physical examination, obtaining blood and other cultures as indicated.
- Beginning treatment with antimicrobials effective against *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Klebsiella pneumoniae* while waiting for cultures to be evaluated.
- Liver abscess should always be screened for, particularly in diabetic patients.
- Patients also need to be made aware of the potential for travel-related infections such as babesiosis and malaria, as well as the risk inherent in travel to an area where medical care is not readily accessible. In the latter case, an appropriate antibiotic should be made available for the patient to carry with him/her.
- Patients should be reminded always to alert consulting physicians about their splenectomised status. Patients should be given a card with their splenectomy status and contact number of their primary treatment centre/team.

### **B. Hypercoagulability**

Thromboembolism is more common both in the peri-operative period and post-splenectomy (Kristinsson et al., 2014; Lee et al., 2015). In addition, patients with thalassaemia, particularly those who have been splenectomised, have an increased tendency for both venous and arterial embolism (Taher et al., 2006). One of the main factors is the procoagulant effect of anionic phospholipids on the surface of altered red blood cells and erythroblasts, as the number of these circulating cells is dramatically increased by the absence of the spleen (Taher, Weatherall & Cappellini, 2018). Once they persist in the circulation they trigger mechanisms of thrombin generation. In post-splenectomy patients, markers of thrombin generation such as thrombin antithrombin (TAT) complexes, prothrombin fragments (F1,2) fibrinopeptide A (FPA) and D-dimer should be assessed annually, while decisions about anticoagulation should be discussed on a case by case basis. Portal vein thrombosis post-splenectomy can also occur despite prophylactic anticoagulation and physicians should have a high index of suspicion (Pietrabissa et al., 2004; Winslow et al., 2002).

Following splenectomy, postoperative thrombocytosis is common, with platelet counts often reaching  $1,000\text{--}2,000 \times 10^9/\text{l}$ . The UK thalassaemia guidelines state that peri-operative thromboprophylaxis should be routinely given and continued until the patient is fully mobile (Yardumian et al., 2016). We would recommend that all post-splenectomy patients receive low dose aspirin as long as there are no contraindications (Taher et al., 2018). This is particularly important for patients with a history of previous thrombosis or other risk factors.



**C. Pulmonary hypertension**

This complication is more frequent in non-transfusion dependent thalassaemia, but is also increasingly identified in thalassaemia major. Advancing age and a history of splenectomy are major risk factors in this population (Morris & Vichinsky, 2010). Chronic thromboembolic pulmonary hypertension (CTEPH) is a consequence of delayed resolution of pulmonary artery thromboembolism; a recent meta-analysis has shown that CTEPH may be associated with splenectomy (Zhang et al., 2021). For more information on this complication, please refer to Chapter 5: Cardiovascular disease.

**D. Iron overload**

In many patients, the iron load of the spleen approaches that of the liver, highlighting the importance of the spleen as a major organ of iron storage in TM. Splenectomy causes major changes in the ferrokinetic profile of iron overload and toxicity in TM patients. Following splenectomy, the total body iron storage capacity is reduced (Ca-sale et al., 2013). Iron will be redirected and accumulated in the liver, heart and other organs and, unless effective chelation protocols are introduced, the iron concentration in these organs will increase (Aydinok et al., 2011; Kolnagou et al., 2013). In addition, splenectomised patients had a higher incidence of myocardial iron load (48%) and higher myocardial iron compared to non- splenectomised patients (28%) (Aydinok et al., 2011).

## Summary and Recommendations with Grade of Evidence

Splenectomy is the recommended intervention to reduce excessive blood consumption and consequent severe iron overload. However, physicians should keep a guarded approach towards splenectomy because of the high disease burden associated with splenectomy. Current optimal transfusion regimens and iron chelation have considerably reduced the incidence of splenomegaly and iron overload in transfusion-dependent thalassaemia patients.

1. At the current time, we do not recommend splenectomy as a standard procedure in patients with thalassaemia(C). There is large amount of evidence that links splenectomy to a variety of complications such as pulmonary hypertension, silent brain infarcts, venous thrombosis and sepsis to name a few. Splenectomy should be considered in patients with thalassaemia in three clinical scenarios: increased blood requirement that prevents adequate control with iron chelation therapy, hypersplenism and symptomatic splenomegaly (C).

2. When performing splenectomy, the laparoscopic approach seems to be the most favourable (B).

3. The most frequent pathogens that cause infections in splenectomised patients are *Streptococcus pneumoniae*, *Haemophilus influenzae* type B, and *Neisseria meningitidis*; therefore immune prophylaxis is recommended against these agents at least 2 weeks prior to the operation and repeated post-operatively as recommended. Additionally, an annual influenza vaccination is encouraged.

4. Chemoprophylaxis with oral penicillin depends on the age of the individual and the treating physician's opinion (C). Life-long prophylactic antibiotics should be offered to patients considered at continued high risk of pneumococcal infection using oral penicillins or macrolides

5. In current practice, due to strict transfusion and chelation protocols, the disease is very well controlled and we are seeing fewer splenectomies than before. Nevertheless, a large percentage of the thalassaemia population (particularly in the older age group), is already splenectomised. These patients are at an increased risk of many disease-related morbidities and should be monitored closely.

- ADHERE to current transfusion guidelines to prevent or delay splenomegaly.
- If splenectomy cannot be avoided, make sure IMMUNISATION protocols are followed.
- Be aware of the dangers of THROMBOSIS in the peri-and post-operative period.
- Discuss post-splenectomy CHEMOPROPHYLAXIS with patient/family and make sure they are aware of the dangers of post-splenectomy sepsis.
- START appropriate parenteral antibiotics in case of suspected sepsis while awaiting culture results.

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## CHAPTER 13

### Fertility and Pregnancy

#### Introduction

Advances in the primary care of thalassaemia major (TM) including optimal blood transfusion and chelation therapy have improved patient survival into adulthood. At the same time, patients' quality of life has also significantly increased and the expectation of having a family – a key aspect of quality of life – is consequently an important aspiration for many. Although spontaneous fertility can occur in well-transfused and well-chelated patients with spontaneous puberty and normal menstrual function, the majority are subfertile mainly due to hypogonadotropic hypogonadism (HH) as a consequence of transfusional haemosiderosis (Skordis et al., 1998). Those who fail to achieve pregnancy spontaneously require assisted reproductive techniques (ART).

**Planned pregnancy is essential both in spontaneous and ART conceptions, since pregnancies in patients with TM are high risk for both the mother and the baby.** However, these risks can be minimized through **pre-pregnancy counselling** involving the various members of the **multidisciplinary team**: the haematologist, the reproductive medicine specialist, the cardiologist and the obstetrician, in conjunction with the endocrinologist and the specialist nurse.

The management of patients with thalassaemia intermedia (TI) is similar to that of TM, with minor adjustments. Older patients with TM usually have HH and are unlikely to conceive spontaneously, whereas patients with TI are potentially fertile with an intact hypothalamic-pituitary-gonadal (HPG) axis (Chatterjee & Katz, 2000). Furthermore their management during pregnancy is different in that TI patients have an increased thrombotic risk, compared to regularly transfused patients and may need transfusion during pregnancy to decrease this risk (Nassar et al., 2006). In addition to complications specific to iron overload, TM patients also face the risk of thromboembolism; this is particularly so after splenectomy and in those with auto-antibodies.

Women with TM appear to have premature ovarian ageing compared with non-thalassaemic women, raising a concern around the maximum age at which ovarian reserve is sufficient for hormonal stimulation to be successful. Ovarian reserve reflects the capacity of the ovary to provide eggs that are capable of fertilization resulting in a healthy and successful pregnancy. It also determines the risk of miscarriage (Singer et al., 2011). In ovarian reserve testing, ultrasound techniques are used to indirectly measure of the size of the residual ovarian follicle pool. Reproductive aging is directly related to the decline in the number of antral follicles. Low gonadotropin secretion in women with TM results in reduced ovarian antral follicle count and ovarian volume, though levels of anti-Müllerian hormone (AMH), a sensitive marker for ovarian reserve independent of gonadotropin effect, are mostly normal. AMH, which prevents recruitment of non-dominant follicles and reduces the responsiveness of ovarian follicles to FSH during cyclic recruitment, is produced by the pre-antral and early antral follicles. Low ovarian reserve is considered predictive of low chances of spontaneous pregnancy and for poor ovarian response to hormonal stimulation. AMH is the earliest marker of change with age and has very little intercycle and intracycle variability and therefore emerges as an important biomarker for assessment of reproductive capacity in TM, demonstrating that fertility is preserved in the majority of those younger than 30 to 35 years. AMH can be useful in future studies aiming at improved chelation for fertility

preservation (Leung & Lao, 2012).

Spontaneous pregnancies in women with a preserved hypothalamic-pituitary-gonadal axis, who have normal menstrual cycles, are common. On the other hand women with primary or secondary amenorrhea are able to conceive following ovulation induction therapy. However, most of the other potential complications in TM must be seriously considered before and during pregnancy.

## Management of Subfertility in Females

Although 80-90% of patients have HH, gonadal function is intact in the majority of patients, indicating that fertility is usually salvageable, i.e. ovulation in females and spermatogenesis in males can be induced by exogenous gonadotropin therapy, 'bypassing' the HPG axis (De Sanctis et al., 1988a, 1988b). However, other endocrine disorders, namely diabetes mellitus and hypothyroidism, may also influence the outcome of fertility treatment and need to be corrected by standard care. Successful spontaneous pregnancies, as well as those resulting from the induction of gametogenesis, have been documented in TM females and males (Aessopos et al., 1999; Skordis et al., 2004).

Management of subfertility requires careful planning and preparation (a thorough-work-up), including pre-pregnancy counselling of the couple (see below). Fertility assessment of patients with thalassaemia should also include evaluation of the partner according to standard criteria (see <https://www.rcog.org.uk/>, 2021). The fertility options are dependent on two factors: (a) her partner's carrier status and (b) the site of damage to the HPG axis. If both partners are homozygous for thalassaemia the use of donor gametes, preferably donor sperm, is the ideal option as sperm can be more easily available from sperm banks, whereas the use of donor eggs is technically more complicated with an unpredictable success rate (Deech, 1998). If the partner is heterozygous, then pre-implantation genetic diagnosis (PGD) is another option, where diagnosis can be made prior to conception. This method may be more acceptable to certain communities with religious beliefs against termination of affected pregnancies. Lastly, in patients with severe organ damage or where both partners have TM, an alternative option may be adoption. When considering adoption, the family environment and competencies need to be taken into consideration.

### **Methods for induction of ovulation**

Induction of ovulation with pulsatile gonadotropin-releasing hormone (GnRH) infusion is only possible at the early stage of HPG damage, when gonadotropins, follicle stimulating hormone (FSH) and luteinizing hormone (LH) are pulsatile. But most of patients with HH are a pulsatile but with functional gonads, and are therefore likely to benefit from gonadotropin therapy (80% success rate) (Skordis et al., 2004). The drugs used, however, are powerful and can often induce growth of two or more follicles, with the risk of twin or triplet pregnancy and often result in ovarian hyperstimulation syndrome. In this condition the ovarian blood vessels become more permeable and leak fluid into the abdomen causing ascites and dehydration. About 1-2% of women undergoing induction of ovulation develop severe hyperstimulation syndrome causing abdominal pain, dyspnoea, vomiting and rapid weight gain. Severe cases are admitted to hospital to manage serious complications such as electrolyte imbalance, hypovolaemic shock, renal and respiratory insufficiencies and arterial thromboembolism, which can be life threatening. Induction of ovulation should therefore only be undertaken by a specialist reproductive team, according to Human Fertilization and Embryology Authority



(HFEA) guidelines (Deech, 1998). Patients should be counselled regarding the risk of hyperstimulation syndrome, multiple pregnancy, ectopic pregnancy and miscarriage. The risk of hyperstimulation and multiple births can be minimized by vigilant monitoring of the induced cycle by endovaginal ultrasound scans. For such procedures, it is important to obtain informed consent.

Carefully documented notes should be kept throughout. Induction of ovulation may be indicated in women with primary amenorrhea, secondary amenorrhea, or those with normal menstrual function who fail to conceive, and also in planned pregnancy where both partners are thalassaemics. Stimulation of follicular development to retrieve mature oocytes is essential in these cases, because of the greater chance of pregnancy occurring following the transfer of more than one embryo. The induction of the growth of follicles necessitates the administration of the ovulation induction drugs and different induction protocols. The regime to be followed will be dependent on the team's local protocol (see Figure 1 for an established protocol).

Most ovulation induction protocols for thalassaemia patients use standard medications. Agents include gonadotropins (FSH and LH) and clomiphene citrate, which are used to stimulate development of follicles, and human chorionic gonadotropin (HCG), that mimics LH to trigger ovulation at the end of follicular development. Adjuvant medications, such as GnRH analogues for ovarian suppression are not used in thalassaemia as the hypothalamic–pituitary axis is not intact. The dose and frequency of gonadotropin injections depend on the woman's response, which is evaluated by the number and size of the growing follicles and levels of oestradiol. hCG is administered when at least two follicles reach 17 mm in size and 36 hours after the administration of hCG egg collection is performed.

It should be remembered to counsel the patient for egg collection and storage if haemopoietic transplant or gene therapy are contemplated. This of course, usually is done at an earlier age, in adolescent patients.

**Table 1. Key points in induction of ovulation include:**

- Careful monitoring of the cycle by serial vaginal ultrasound scans and oestradiol measurement is needed.
- Therapy should be continued until hCG is given to induce ovulation.
- Pregnancy is confirmed by testing.
- Luteal support with progesterone may be required.
- Refer for in vitro fertilization (IVF) if required.

***Male Fertility and Induction of Spermatogenesis***

The induction of spermatogenesis in male patients with thalassaemia is more challenging than the induction of ovulation in their female counterparts, with a success rate of only 10-15% in moderate to severely iron loaded patients and advanced aged patients (Skordis et al., 2004). The induction process must be undertaken according to HFEA guidelines, with an emphasis on consent and counselling (Deech, 1998). An established protocol for induction of spermatogenesis is described below:

**Table 2: Induction of spermatogenesis**

- Baseline testosterone and semen analysis.
- hCG 2000 units twice-weekly for 6 months.
- Monitor testosterone level.
- Repeat semen analysis – no sperm.
- Continue hCG with combined HMG (Human Menopausal Gonadotrophin) 75 units or recombinant FSH three times weekly for additional 6 months.
- If semen analysis is satisfactory SAVE.
- If azoospermia persists, STOP treatment.

Hormonal treatment of pubertal disorders in thalassaemia is a complex issue due to the many associated complications. Therefore, each patient has to be assessed individually. Collaboration between endocrinologists and other doctors is crucial. Male patients with onset of HH before completion of pubertal development generally have testes smaller than 5 ml in volume and usually require therapy with both hCG and HMG or recombinant FSH to induce spermatogenesis.

The treatment process is demanding and may take up to 2 years. The initial regimen of hCG is usually 2,000 – 3,000 IU administered intramuscularly twice a week. The clinical response is monitored, and testosterone levels are measured every 2 to 3 months. Dosage adjustments of hCG may be needed to determine an optimal response. If the patient is fully virilised and 8-12 months of HCG therapy has not resulted in the production of sperm, then FSH therapy should be initiated. Sperm banking procedures, even in subjects with reduced sperm count and motility, are recommended. Once pregnancy has occurred FSH therapy can be stopped and spermatogenesis can be maintained with HCG alone (Sanctis, Soliman & Yassin, 2012). If this treatment regimen does not result in adequate sperm production after a maximum of 2 years, there is no indication to continue.

The recent advent of micromanipulation techniques such as intra-cytoplasmic sperm injection (ICSI) has improved conception rates, even in oligo-asthenospermic patients. Therefore, sperm cryopreservation should be considered in all subjects with a stated wish to have children in future unless already azoospermic, to better preserve fertility and so the chance of conception. However, recent literature on sperm DNA damage in males with thalassaemia (De Sanctis et al., 2008) raises anxiety about mutagenic risks in these individuals, especially after ICSI, where natural protective barrier against gamete selection during fertilization is lost however no birth defects were reported in children of thalassaemics fathers.

## **Pre-Pregnancy Counselling**

Before embarking on fertility treatment, it is important that patients and their partners attend pre-pregnancy counselling, which has a three-fold purpose: (a) evaluation of eligibility, (b) an opportunity for physicians to review the medications involved and (c) time for a discussion between physician/s, patient and partner regarding the risks associated with induced fertility and pregnancy.

### **Evaluation of eligibility**

Each patient should be assessed regarding suitability to embark on pregnancy with optimum outcome both for the mother and the fetus. There are at least three important factors that must be cautiously taken into consideration before encouraging

women with TM to embark on pregnancy: degree of cardiac impairment, liver dysfunction and the risk of vertical transmission of viruses.

1. The most important issue is that of **cardiac function** because cardiac complications remain the leading cause of death in transfused patients. The cardiac load is increased during pregnancy by at least 25-30% due to increased heart rate and stroke volume. This, along with iron load, has a real potential for premature death from cardiac failure. Therefore it is prudent that all patients with TM have cardiac assessment by echocardiography (left ventricular ejection fraction >65%; fractional shortening >30%), by electrocardiogram (ECG), both at rest and with exercise, and by 24 hour tape recording to check for rhythm disorders (Aessopos et al., 1999). If left ventricular dysfunction can be demonstrated in patients under stressful conditions or if significant arrhythmias have occurred, then women should be strongly advised against planning pregnancy (Hui et al., 2002). Most of the non-invasive cardiac investigations are relatively insensitive for detecting early cardiac iron loading. Modified magnetic resonance imaging (MRI) using gradient T2\* measurements, can quantify iron levels, and can accurately relate these to left ventricular dimensions assessed using the same technique (Anderson et al., 2001). If the facility exists, cardiac MRI should be performed with the aim of identifying a T2\* of less than 20 ms. If cardiac iron load is detected and especially if complications are detected, it is strongly advised to intensify iron chelation therapy before embarking on a pregnancy so that the cardiac T2\* > 20 ms wherever possible. There is evidence that cardiac function can be restored to a great extent (refer to Chapter 3) by aggressive chelation, which however, may require several months and up to 2 years.

2. **Liver function** should be evaluated by biochemical tests, with the possibility of iron overload status being assessed by MRI. With respect to hepatitis C-positive cases, these women should be given a course of antiviral agents to attain hepatitis C RNA-negative status. Iron overload in the liver should be treated before pregnancy and optimisation of the iron burden is advisable as much as possible in the pre-conception period for liver iron of < 7 mg/g (dry weight) (dw). Liver and gall bladder (and spleen if present) ultrasound should be used to detect the presence of gallstones.

3. Before embarking on pregnancy, it is also important to establish **bone health** by plain radiography of the spine and dual-energy X-ray absorptiometry scanning of the hip and spine (bone mineral density scoring) and correction of osteoporosis/osteopenia by institution of appropriate therapy (see Chapter 10, Bone Disease). In addition all women should have vitamin D levels optimised before pregnancy and thereafter maintained in the normal range.

4. All patients should be screened for the **human immunodeficiency virus (HIV), hepatitis B (HBV), hepatitis C (HCV) and rubella**. The opportunity should not be missed to ensure rubella immunity prior to pregnancy. If the patient is HIV positive and wishes to have a family, she should be advised of the usual recommendations for care which include appropriate antiviral agents, delivery by Caesarean section and the avoidance of breast feeding to reduce the risk of vertical transmission.

5. Patients should also be screened for **diabetes, thyroid hypofunction and acquired red cell antibodies**.

6. **Both partners should be screened for haemoglobinopathies** (Galanello & Origa, 2010).

**Table 3. Eligibility evaluation includes the following examinations:**

- Cardiac function: ECG, echocardiogram.
- Liver function tests, ultrasound of the liver.
- Vessels : clotting factors ( Prothrombin time, INR, Protein C , Protein S, Homocysteine, Thrombophilia panel) and doppler.
- Endocrine: thyroid function, calcium homeostasis, vitamin D levels.
- Pancreas: glucose tolerance test and optimisation of diabetic control.
- Status of viral infections: HBV, HCV, HIV, Rubella. Test for other possible infections including toxoplasmosis.
- Iron status assessment and optimisation of iron chelation therapy.
- Review medications.
- Screen for acquired red cell antibodies (risk of haemolytic disease of the fetus and newborn).
- Check partner for haemoglobinopathy.
- Arrange genetic counselling if necessary.

**Table 4. Feasibility evaluation includes the following elements:**

- Hypothalamic-pituitary-gonadal axis.
- Assessment of ovulation.
- Ultrasound of the uterus and ovaries.
- Post-coital test.
- Hysterosalpingography.
- Complete endocrine assessment.

## Review of medications

This is a good opportunity to review medications and to advise patients about their dietary habits, smoking and alcohol, and to commence supplements of folic acid, calcium and vitamin D. Patients on oral chelators (DFX or DFP) should be **advised to switch to DFO**, prior to induction of ovulation/spermatogenesis (Singer & Vichinsky, 1999). Hormone replacement therapy should also be terminated at least 4-6 weeks prior to induction of gametogenesis. **Bisphosphonates are contraindicated** during pregnancy and breast-feeding is not advised because of the considerable negative calcium balance associated with these states. Given the long biological half-life of bisphosphonates, ideally they should be stopped at least 6 months prior to conception, although there are no consensus guidelines. It is of paramount importance to ensure adequate calcium and vitamin D intake before and throughout pregnancy. **Other medications that should be discontinued for at least six months prior to fertility treatment include interferon, ribavirin and hydroxycarbamide (hydroxyurea).**

Hypothyroid patients receiving thyroid replacement therapy should receive increased doses, as needed to ensure they are euthyroid. Hyperthyroidism is rare in patients with thalassaemia. However, **if a patient is receiving anti-thyroid medication such as carbimazole, they should be switched to propyl thiouracil.**

**Table 5. Medication review for pregnancy focus points:**

- Emphasize folic acid supplementation even before conception.
- Oral iron chelating agents (DFP, DFX) should be discontinued 3 months before conception.
- Stop angiotensin-converting enzyme (ACE) inhibitors.
- Can safely continue metformin, but may need to change oral hypoglycaemic drugs to insulin.
- Stop bisphosphonates at least 6 months prior to planned pregnancy.
- Give calcium and vitamin D supplementation.

**Risks Associated with Pregnancy**

All patients should be made aware that pregnancy per se does not alter the natural history of thalassaemia. If pregnancy is managed in a multidisciplinary setting, the fetal outcome is usually improved with a slight reduction in incidence of growth restriction (Aessopos et al., 1999; Ansari et al., 2006; Tuck, 2005). It has been shown that the risks of pregnancy-specific complications such as antepartum haemorrhage and pre-eclampsia in thalassaemia are similar to that in the background population. It has also been shown that DFO is not required during pregnancy in patients who are not iron overloaded and have adequate cardiac function prior to pregnancy. Serum ferritin is likely to increase by 10%, despite increases in frequency of blood transfusion (Aessopos et al., 1999; Butwick, Findley & Wonke, 2005; Daskalakis et al., 1998; Tuck, 2005).

**The aim during pregnancy is to maintain pre-transfusion haemoglobin concentration above 100 g/l.** Once pregnancy is confirmed, the patient should be managed in a multidisciplinary setting with a team consisting of an obstetrician, midwife, physician, haematologist, cardiologist and endocrinologist. The patient should be made aware that although pregnancy is high risk, the outcome is usually favourable.

The risks of bone deformities may affect pregnancy and labour management, especially if cephalo-pelvic disproportion complicates delivery. Although most skeletal deformities are largely preventable by regular transfusion, spinal abnormalities associated with TM are related to regional blockade (Petrakos, 2016). Osteoporosis and scoliosis are common in TM, despite transfusion therapy. Patients with osteoporosis usually have vertebral bodies with reduced height and the segmental position of the conus may be lower than predicted. It is therefore important to correct osteoporosis prenatally by hormone replacement and with bisphosphonates, when required, to increase bone density so that spinal anaesthesia becomes feasible. Bisphosphonates however have to be stopped at least 6 months prior to pregnancy due to their long biological half-life.

**Table 6. Potential risks associated with pregnancy include:**

- Pregnancy does not alter the natural history of the disease.
- Requires intense/vigilant monitoring.
- Cardiac complications.
- Risk of pregnancy-specific complications same as background population.
- Risk of miscarriage same as background population.
- Risk of fetal malformation: no increase.
- Risk of fetal growth restriction: two-fold increase.
- Preterm labour risk: two-fold increase.
- Risk of transmission to the fetus/baby of hepatitis B/C, HIV.
- Risk of iso-immunisation.
- Risk of prematurity and growth restriction is increased in multiple births.
- Thrombotic risk may be increased.

It is important to note that the main risk to the mother are possible cardiac complications, which can be minimised by ensuring optimal cardiac function and good control of iron overload before the initiation of pregnancy.

## Management of Pregnancy

### **Monitoring the heart:**

The key points include evaluation of cardiac function by echocardiography, and of liver and thyroid function, in each trimester. Echocardiographic follow up in pregnant women with TM in our centre has revealed that there is often mild diastolic dysfunction during the third trimester, as shown by the deterioration of mitral valve early filling/late filling (E/A) ratio, which corresponds to the elevated filling pressures due to the increased vascular volume in pregnancy. No significant cardiac complications were encountered provided they started pregnancy with optimal iron load. Hence clinicians should ensure good control of iron overload with optimal cardiac function and myocardial T2\* before initiation of pregnancy in women with TM provided they have started early on proper treatment and have a normal resting cardiac performance (Kypris, Simamonian & Efsthathiou, 2011).

### **Diabetes:**

All patients should be screened for gestational diabetes at 16 weeks and, if normal, screening should be repeated at 24-28 weeks. Women with thalassaemia in whom diabetes is detected, should have monthly assessment of serum fructosamine concentrations and review in the specialist diabetic pregnancy clinic.

### **Fetal growth:**

Serial ultrasound scans from 24-26 weeks onwards must be undertaken to monitor fetal growth. Chronic anaemia will affect fetal growth so maintaining a pre-transfusion Hb of at least 100g/L is necessary. This is particularly true in NTDT (Nassar et al., 2008; Luewan, Srisupundit & Tongsong, 2009), but has also been reported in TDT (Bajoria & Chatterjee, 2009).

### **Thromboprophylaxis:**

Pregnancy increases the risk of thrombosis from three-fold to four-fold. Thalassaemia, is a hypercoagulable state with an enhanced risk of thromboembolic complications especially in splenectomised patients. Although there is a predisposition to venous

thrombosis no reports of thrombotic episodes have been noted in women receiving low molecular weight heparin (Origa et al., 2010; Tuck et al., 1998). For this reason in patients who have undergone splenectomy, and particularly in those with TI, thromboprophylaxis with low molecular weight heparin is required from mid-trimester (Eldor & Rachmilewitz, 2002; Nassar et al., 2006). Many splenectomised women may already be using low dose aspirin (75mg) if the platelet count is  $>600000/\text{cmm}$ ; these patients should also be given low molecular weight heparin in addition. It is added that a regular transfusion regimen aiming to reduce endogenous erythropoiesis and so reducing the circulation of abnormal red cell fragments, especially in splenectomised patients, is also a measure to avoid thrombotic phenomena since these fragments predispose to the formation of blood clots.

### ***Folic acid supplementation:***

Folate demand in pregnancy is normally increased: this may be relevant in patients with thalassaemia due to bone marrow overactivity. Regular folic acid supplementation is recommended in mothers with TM to prevent superimposed megaloblastic anaemia, although this has only been demonstrated in individuals with  $\beta$  thalassaemia minor (carriers) (Leung, Lao & Chang, 1989). In addition, as in other pregnancies, folic acid should start before conception to reduce the incidence of spina bifida.

### ***Iron chelation during pregnancy:***

**If cardiac function deteriorates during pregnancy, deferoxamine may be used with caution after the first trimester.** This is because the literature supporting teratogenicity with this agent is equivocal (Singer & Vichinsky, 1999). However myocardial iron can accumulate during pregnancy and cases of worsening heart function (Perniola et al., 2000) and fatal heart failure have been described (Tsironi, Karagiorga & Aessopos, 2010; Tuck et al., 1998). Deferoxamine has therefore been used in some higher risk pregnancies, particularly in the final trimester (Bajoria & Chatterjee, 2009; Singer & Vichinsky, 1999; Tsironi et al., 2005). With respect to the newer oral chelating agents, data on fetotoxicity are insufficient. However, the manufacturer's product information for deferoxamine includes risk of skeletal anomalies in animal pregnancies. Although there are currently no reports regarding human fetal anomalies from this drug, patients should be informed about this possible risk prior to its administration during pregnancy. Therefore, in patients with a history of previous myocardial iron deposition or borderline myocardial cardiac function, deferoxamine may be considered in the final trimester or in the peri-delivery period, as a prolonged labour with acidosis may increase the risk of cardiac decompensation. It is emphasised again that these management decisions are taken in common with the haematologist and the cardiologist.

### ***Thyroid function:***

Thyroid function should be determined periodically throughout pregnancy and if hypothyroid the dose of thyroxine should be adjusted.

### ***Managing delivery:***

With respect to the management of labour, if the pregnancy is non-complicated, one can wait for the spontaneous onset of labour. There is no current consensus on the mode and timing of delivery in this population. Individual assessment should be made by the multidisciplinary team and according to the woman's wishes. Similarly to the reported data, the author's experience suggests that 80% of women with thalassaemia will require Caesarean section because of a higher frequency of cephalopelvic disproportion, largely due to short stature and skeletal deformity

combined with normal fetal growth. It is desirable to use epidural anaesthesia wherever feasible, to avoid the risk of difficult intubation and trauma associated with general anaesthesia because of severe maxillofacial deformity in some TM patients.

If the mother has pre-pregnancy morbidities such as diabetes or cardiac disease then prolonged pregnancy should be avoided. Low dose deferoxamine may be used during prolonged labour in patients with cardiac disease.

If the haemoglobin is less than 100 g/l, cross-match 2 units on admission to the labour ward.

### **Postpartum care:**

After delivery, in principle deferoxamine can be recommenced because concentrations are very low in breast milk and because it is not absorbed by the oral route (Howard et al., 2012). Experience with breastfeeding in patients receiving deferoxamine is scant, however, and has not been examined in formal clinical trials. Breastfeeding should be encouraged in all cases except in those who are HIV and/or hepatitis C RNA-positive and/or HBV surface antigen (HbsAg) positive because of the risk of vertical transmission via breast milk.

***Women with thalassaemia should be considered at high risk for venous thromboembolism and should receive low-molecular-weight heparin prophylaxis while in hospital. In addition this treatment should be administered for 7 days post discharge following vaginal delivery or for 6 weeks following caesarean section.***

In case of miscarriage or termination of pregnancy the danger of thromboembolism is still present and low-molecular-weight heparin prophylaxis must be provided during and following the loss for at least 7 days.

All patients should be offered counselling regarding contraception. Intrauterine devices should be avoided because of the risk of infection. Taking oestrogen-containing birth control pills is also not advisable because of the risk of thromboembolism, in the author's opinion. In most cases, the progesterone-only pill or barrier methods are appropriate. Male patients with HH are not spontaneously fertile and therefore contraception is not required.

Calcium and vitamin D supplements should be continued during breastfeeding, but bisphosphonate therapy for osteoporosis should only be resumed after cessation of breastfeeding (Howard et al., 2012).



**Table 7. Key points for pregnancy care include:**

- Check cardiac, liver and thyroid function once each trimester.
- Screen for gestational diabetes.
- Increase frequency of blood transfusion to maintain pre-transfusion haemoglobin above 100 g/l.
- Serial ultrasound scans to monitor fetal growth.
- Higher incidence of caesarean section.
- Encourage breastfeeding unless HIV positive and/or HCV RNA and/or HBsAg positive.
- Resume DFO after delivery.
- Discuss contraception, where appropriate with either the progesterone-only pill (POP) or barrier method.
- Avoid intrauterine devices and oestrogen-containing preparations.
- Implement a multidisciplinary approach with all specialists involved in the medical care of thalassaemic women.

## Summary and Recommendations

- Iron overload in the pituitary is the main cause of infertility in females.
- Successful pregnancy can be achieved in thalassaemia major though ovulation induction because ovarian function is usually preserved.
- Ovulation in females and spermatogenesis in males can be induced by exogenous gonadotropin therapy.
- Management of infertility requires careful planning and preparation.
- Induction of ovulation should only be undertaken by a specialist reproductive team.
- Several factors must be taken into consideration before encouraging women with thalassaemia major to embark on pregnancy. These include the degree of pre-existing cardiac impairment and of liver dysfunction, as well as the possibility of vertical transmission of viruses.
- Pregnancy per se does not alter the natural history of thalassaemia – it is safe, provided they have started early on proper treatment and have normal resting cardiac function. If cardiac function deteriorates during pregnancy, deferoxamine may be used cautiously after the first trimester.
- Pre-transfusion haemoglobin should be kept above 100 g/l.
- Fetal growth must be carefully monitored since anaemia may result in fetal growth retardation.
- Thrombosis is a major concern in thalassaemic pregnancies and so thromboprophylaxis with low-molecular-weight heparin is recommended as the main treatment from mid-trimester in all cases. Aspirin is provided mainly when there is a high platelet count.
- Multidisciplinary management is required before and during pregnancy and delivery, monitoring and managing organ function, particularly the heart.

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## CHAPTER 14

### Lifestyle and Quality of Life

#### Introduction

**Patients with optimally treated thalassaemia can now enjoy a near-normal life and lifestyle, and experience full physical and emotional development from childhood to adulthood.**

According to the WHO definition (set out in the constitution, WHO, 2021), health is a state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity. Healthcare professionals should, beyond following clinical protocols, have the clear aim of reducing as far as possible the degree to which the disease interferes with the patients' personal and social lives. This is achieved by recognising the limitations that the disease imposes but also the effect that the treatment regimens have on the patients' lifestyles, and the time that these treatments steal from normal living. Recognition of these challenges comes with knowledge of all aspects of the disease, with experience, by learning from patients, and through providing a holistic approach to patients. Beyond managing the physical condition, healthcare staff should be willing to listen to any queries that the patients may bring up and be able to advise on all lifestyle issues.

Leading a 'normal' life is an often-expressed priority for patients. This includes social integration (Politis et al., 1990), connecting and interacting with people and contributing to society, despite counter forces that the disease and its treatment bring, which can lead to isolation and, in some societies, stigmatisation. Marginalisation will lead to depression and possibly increase health risks. These issues, and the broader concept of quality of life, become more prominent as longer survival and minimisation of complications are achieved through modern treatment. The concepts of quality of life, social integration, living and experiencing life beyond health preservation, are interwoven. Psychosocial support as described in Chapter 15 is a necessary component of management, as is high quality and well organised holistic care. The healthcare team should have these concepts in mind and be able to guide patients on a variety of lifestyle matters. Below are some of the issues that patients may seek answers to. Although the thalassaemia healthcare team is responsible for providing the answers to such matters, all too often no answers or misleading answers are provided. This chapter aims to provide a foundation from which healthcare professionals can respond with confident and informed guidance to their patients.

- Activities – sports – how far can I go?
- Social life – dancing, smoking, and alcohol – what is allowed?
- Education – can treatment and clinic visits interfere with the education programme? Can clinic times be adjusted to my needs?
- Employment – what jobs can I accept? Is there employer prejudice? Will I have absences?
- Can I get married? Can I have children?
- Can I support a family? For how long?
- Can I have life insurance? Health insurance?
- Will I be able to get a mortgage? Can I borrow?

## Exercise and Participation in Sports

A frequent enquiry from patients concerns their ability to work and participate in sports. In general, physical and recreational activities should be encouraged, as this is an important aspect of healthy and normal living, as well as a means towards social integration. Limitations in chronic disease must however be recognised. Physical capacity can be influenced primarily by the degree of anaemia, and cardio-circulatory and pulmonary function, which are important to the oxygenation of tissues. Additionally, in a chronic condition such as thalassaemia, other co-morbidities may be present, such as cardiac, liver and endocrine dysfunction. For the most part, clinical evaluation focuses on the contribution of respiratory and cardiovascular function to exercise tolerance. Ergometry is used in the majority of studies (mainly cycle ergometry or treadmill), with respiratory function tests to measure aerobic capacity (e.g. VO<sub>2</sub>max) and cardiovascular investigations using echocardiography and cardiac magnetic resonance to examine cardiac function through assessment of maximum heart rate responses, stroke volume reserve, and the effects of iron overload (Marinov et al., 2008; Nanas et al., 2009; Vasileiadis et al., 2009). Collectively, all existing studies have concluded that there is exercise limitation in transfusion-dependent thalassaemia patients. Factors contributing to this limitation include the degree of anaemia, iron overload affecting heart function – especially through vascular inflammation (Sohn et al., 2013) – and even restrictive lung dysfunction (Piatti et al., 2006).

The global thalassaemia population is not homogeneous, with thousands of patients surviving and functioning with low haemoglobin levels and poor adherence to chelation therapy. The prevalence of complications therefore varies, depending largely on the quality of treatment. A universal and all-encompassing guideline on how much exercise can be taken cannot therefore be formulated, and an individualised approach based on a comprehensive clinical assessment (which may include ergometry) is advisable. In general, pre-adolescent children are allowed to exercise without restrictions, if treated according to accepted standards. If maintained at low haemoglobin levels, careful cardiorespiratory assessment is necessary. From early adolescence, iron accumulation and tissue damage may be evident in the heart and endocrine glands if chelation has been sub-optimal. For this reason, even though routine daily activity is unrestricted, exercise tolerance should be assessed at regular intervals. We have seen from previous chapters that annual cardiological assessment is already recommended (see Chapter 5) for all thalassaemia patients from an early age since iron accumulation may occur early in their lives (Berdoukas et al., 2013). Careful evaluation is recommended if athletic activity is contemplated.

In addition to the above, consideration must be given to all co-morbidities, including bone disease, which is common at all ages in thalassaemia patients. In addition to pain, which may limit mobility, the propensity to fractures must also be considered whenever giving advice concerning exercise and sport. In a well-publicised case, a patient with thalassaemia major ran the London marathon on two occasions. This is an inspiration for all patients across the world, and proof that modern treatment can lead to a normal quality of life. However, in the spectrum of thalassaemia care that exists across the world, this example is sadly relevant to only a minority of optimally treated patients.



## Education

In a recent survey involving thalassaemia and sickle cell patients from across Europe, 21% of patients over the age of 18 years had completed university education (Corrons et al., 2014). In a more recent review of 230 patients from Cyprus 53.5% had completed tertiary education (Sitarou, unpublished data), which compares well with the general population of Cyprus in which 39.4% are university graduates. This should dispel any doubts concerning the ability of patients to achieve a full education, despite reports of a cognitive impairment in patients (Tartaglione et al., 2019). Factors associated with chronic illness, rather than the disease per se, could play a potential role in the development of cognitive dysfunction (Economou et al., 2006). In the USA, 82% percent of school age children were at expected grade level (Pakbaz et al., 2010). The main limiting factor expressed by patients is the need to interrupt educational sessions in order to meet clinic and transfusion appointments, which are in most clinical services, during daytime working hours. Adjusting day transfusion services to include evening and weekend sessions, to suit patients in full-time education or employment, will greatly assist their achievement of social integration and their contribution to society (see Chapter 15) and this should be a priority for healthcare professionals and managers offering the service. In addition, medical teams should be ready to liaise with educational services and especially schoolteachers, to provide information and education concerning thalassaemia, and the ability of patients to perform in school, recognising that concerns and sometimes even prejudice from teachers may adversely affect student performance. Prejudice is also a feature in the playground, where bullying and negative behaviour can make the young thalassaemia patient feel different and isolated, which can have a lasting effect on their self-image. Feeling different can also have personal consequences in countries where thalassaemia is viewed as an 'immigrant disease', potentially leading to racial and ethnic issues (Dyson et al., 2010). These issues require educational intervention for and by teachers.

## Employment

Many adult thalassaemia patients are employed without difficulty. However, problems do still exist due to several factors, which originate in part from patients but also from employers and the social environment more generally. Many patients who have not benefitted from adequate psychosocial support still have low self-esteem and feel that 'poor health' does not allow them to work. Even in Europe where overall services are regarded as achieving a high level, these problems remain prevalent. In the survey referred to above (Enerca 2014), of more than 300 patients over 20 years of age, half were fully employed, while 19% were working part time and 31% were unemployed. These figures far exceed national unemployment statistics, although it should be noted that 14% of those unemployed were so through their own choice. This indicates that a significant number of patients are having difficulties and need support from their healthcare team as well as social services. Prejudice from employers is however still an issue in many parts of the world (Wahab et al., 2011). The reasons given include repeated absences, again reinforcing the need for out-of-hours clinic and transfusion sessions, but also a fear that 'something may happen' as a result of the disease, and the belief that such an employee may not be able to do the job.

The United Nations Convention on the Rights of Persons with Disabilities (United Nations, 2006) clearly states in Article 27 that parties must recognise the right of persons with disabilities to work on an equal basis with others, prohibiting discrimination on the basis of disability, assuring equal remuneration for work of equal value as well as safe and healthy working conditions. In view of this, the thalassaemia care team have a duty for advocacy on behalf of their patients, educating the public in general but also individual potential employers. The team should also instil a positive attitude

in their patients concerning their ability to work. For manual tasks it may be necessary to assess the individual's ability according to the same protocol used in assessment for exercise and sports, exercising caution if heart disease or osteoporosis is present.

## Marriage and Reproductive Life

Getting married and having a family is widely accepted as a key goal in one's life, and thalassaemia patients have a good record in forming relationships. In an updated report from Cyprus, (2020 unpublished data) of 318 patients over the age of 18 years, only 35% had not married. In the same group of patients, 64% had children. These figures cannot be achieved where the majority of patients are children, and where stigmatisation limits patient opportunities to form stable relationships. Several studies have reported that as many as 51% to 80% of transfusion dependent thalassaemia (TDT) patients may have pubertal failure, sexual dysfunction and/or infertility, due to hypogonadism (De Sanctis et al., 2018). From an early age, patients should be seen by an endocrinologist to avoid hypogonadism as detailed in Chapters 8 and 13. The thalassaemia team should also provide general support and encouragement to the patients and has a duty to coordinate the multidisciplinary team which oversees both the preconception optimisation of both men and women and cares for the woman through her pregnancy and delivery.

## Nutrition

Questions relating to nutrition are often posed by patients and their parents, since daily needs may influence the patient's health. Indeed, it has been suggested that the growth failure observed in children may be partially related to undernutrition (Skordis & Kyriakou, 2011). To test this hypothesis, the effect of a high calorie diet on partial or complete correction of impaired growth in thalassaemia major children who were unaffected by endocrinopathy or cardiomyopathy has been studied (Soliman et al., 2004). The results showed that increasing caloric intake significantly increased insulin growth factor 1 (IGF-1), skin fold thickness, mid-arm circumference and body mass index (BMI), thus at least partially improving growth. This observation is consistent with other studies (Fuchs et al., 1997). It therefore seems logical to recommend a high caloric intake during growth, especially as the difference between intake and expenditure is greater in young children (Fung et al., 2012). In addition, there are many reports of vitamin and micronutrient deficiencies in thalassaemia, which may also affect growth (Goldberg et al., 2018; Ozturk, Genc & Gumuslu, 2017). It is important therefore to look at some of the important vitamin and minerals in more detail.

## Zinc

Zinc is an essential element which in thalassaemia can be reduced either because it is removed by iron chelating drugs (Erdoğan et al., 2013) or because of inadequate dietary intake, poor absorption (Fung et al., 2012) or increased urinary loss with prolonged use of thiazide diuretics that are sometimes used in patients with cardiac failure. Zinc deficiency has been demonstrated in thalassaemia and has been shown to affect growth, bone health, sexual maturation, and glucose homeostasis (Fung et al., 2015). Zinc deficiency may also cause hair loss, diarrhoea, skin disorders and loss of appetite. In addition, it is also essential for the immune system – particularly for lymphocyte function (Tienboon, 2003). There have been few studies examining the effects of supplementation in thalassaemia major. Some show improvement in both bone health and linear growth in the short term and glucose homeostasis (Matter et al., 2020). However, the long-term safety and acceptability of continued supplementation need to be evaluated (Fung, 2016).

Monitoring zinc levels every 6 months, especially in patients on regular chelation is recommended and, if indicated, supplements should be prescribed (the normal range in blood is 70-120 µg/dl). However, plasma zinc is a sensitive measurement when there is a major deficiency but if the deficiency is moderate this may not be registered and so a normal result does not exclude a deficiency. The levels in plasma may also be influenced by the level of plasma proteins, liver disease, infections, pregnancy and also the time of sampling in relation to meals (Sandström, 2001); above all the level will be influenced by the iron chelating agents which have been shown to increase excretion (Erdoğan et al., 2013) and by the taking zinc supplements. For these reasons interpretation of results should take these factors into consideration and supplements should be avoided 24 hours before the test; the same may be argued concerning the iron chelating agents even though there is no study to support this at present. Leukocyte zinc may be another approach to estimate deficiency but this has not been properly investigated in thalassaemia patients.

Zinc supplements are available in various formulations, with different contents of elemental zinc. It is recommended that whichever formulation is chosen, 15 mg up to a maximum of 40 mg/day of elemental zinc is prescribed, according to individual patient's needs. Caution is needed for high doses, as toxicity can occur – including gastrointestinal irritation – as well as interactions with other minerals and medication.

## Iron

In regularly transfused thalassaemia major patients the contribution of dietary intake of iron is not significant when compared with transfusional iron intake (see Chapter 3). However, intestinal absorption becomes more significant at low pre-transfusion levels. Globally, many transfusion-dependent patients do not receive blood transfusions before haemoglobin levels fall to 60 or 70 g/l, and iron absorption may rise up to 5 mg/day compared to 1-2 mg and so contribute to iron loading. In this group of patients on low blood transfusion in particular, dietary restriction of iron is important. Taking black tea with meals may reduce iron absorption, while foods rich in vitamin C will increase absorption. Needless to say that iron supplements have no place in the thalassaemia syndromes, TDT or NTDT; this includes pregnancy during which routine supplements are often given which include iron along with folic acid.

## Calcium and Vitamin D

Calcium and vitamin D are the most commonly prescribed supplements for thalassaemia patients. Calcium homeostasis is intimately related to vitamin D, and deficiency of this vitamin in thalassaemia ranges in different reports from Thailand from 50.8% (Dejkharnon et al., 2018) to 90% (Nakavachara & Viprakasit, 2013). Vitamin D deficiency is defined as D25 <20 ng/ml (50 nmol/l) and insufficiency D25 = 20–30 ng/ml (50-75 nmol/l) (WMA, Statement on Vitamin D Insufficiency, 2015; Wood et al., 2008). Vitamin D deficiency is also associated with poor bone mineralisation, contributing to thalassaemic bone disease and muscle weakness and, more importantly, can affect the heart muscle, causing left ventricular dysfunction associated with cardiac iron uptake (Wood et al., 2008). Low consumption of calcium (Fung et al., 2012), and decreased intestinal calcium transport (Lertsuwan et al., 2018) found in thalassaemia patients, contribute to the disturbance in calcium homeostasis, particularly if hypoparathyroidism is present (see Chapter 8).

Hypercalciuria has been demonstrated in thalassaemia related to renal tubular dysfunction (Quinn et al., 2011) and possibly to the use of deferasirox (Wong et al., 2016) with cases of nephrocalcinosis reported. Hypercalciuria with nephrocalcinosis was also previously described in desferrioxamine treated patients, although a mechanis-

tic link has not been established. Hypercalciuria does not seem to be related to dietary calcium intake (Quinn et al., 2011).

Vitamin D supplementation is recommended for all patients at a dose of at least 2000 iu/day (Fung et al., 2012). It is also recommended that vitamin levels are monitored every 6 months in thalassaemia patients (Nakavachara & Viprakasit, 2013). A diet high in calcium, including milk, cheese, and oily fish is also recommended. However, calcium at around 1 g /day is also recommended along with vitamin D in selected cases.

### **Folic acid**

Folic acid is an essential element for the synthesis of nucleoproteins and since these are lost in the cell destruction of ineffective erythropoiesis, a deficiency of this vitamin is expected. Patients on high transfusion regimens, which suppress endogenous erythropoiesis in thalassaemia major, rarely develop folate deficiency, in contrast to those on low transfusion regimens. In view of the fact that many patients with thalassaemia major are transfused at low haemoglobin levels (and their folate status is unknown), as well as possible benefits from folic acid supplementation in reducing risks of thrombosis related to homocysteine levels and atherosclerosis (Baghersalimi et al., 2018; Qin et al., 2012), the possibility of providing folic acid supplements at 1-5 mg/day especially to patients on low transfusions, should be considered. This is especially so since we now have an ageing thalassaemia population more prone to thrombotic complications and atherosclerosis (Farmakis et al., 2020). The chances of toxic effects are low.

### **Vitamin E**

Vitamin E, also known as  $\alpha$ -tocopherol, is a fat-soluble vitamin, which is often depleted in thalassaemia patients. The main reason is that iron load in the liver, and associated liver damage, results in a reduction of serum lipids (Livrea et al., 1996), although reduced dietary intake has also been demonstrated (Fung et al., 2012). Supplements of vitamin E have been shown to reduce oxidative stress in thalassaemia (Pfeifer et al., 2008) and to reduce lipid peroxidation of red cell membranes (Sutipornpalangkul et al., 2012). However, these trials, using 400 iu/day, were for relatively short durations of treatment and with small patient numbers. Prolonged use, especially at high doses, requires more extensive trials in thalassaemia. However, a diet rich in foods that contain vitamin E can be recommended, with intake of foods including eggs, vegetable oils (e.g. olive oil, corn oil, safflower and sunflower oil), nuts and cereals.

### **Vitamin C**

Vitamin C has antioxidant properties and can also be depleted in conditions in which there are increased free iron radicals causing oxidative damage. However, caution in recommending supplementation has been expressed because:

- Vitamin C is known to promote the absorption of dietary iron, and even regularly transfused patients should control their intake of iron.
- Vitamin C increases labile iron and therefore contributes to iron toxicity. However, the increased availability of chelatable iron allows deferoxamine (desferrioxamine) to excrete more iron. In order to avoid toxicity, the vitamin is given at the time of deferoxamine infusion at a dose not exceeding 2-3 mg/kg. This benefit is not seen with the other oral chelating agents and so its use with these agents is not recommended.

## Anti-oxidant therapy

Iron overload and especially non-transferrin bound iron results in the production of a variety of reactive oxygen species (ROS), which lead to cell and tissue damage. Levels of antioxidant compounds such as vitamin A, carotenoids and vitamin E are decreased in  $\beta$  thalassaemia major patients. In view of the antioxidant properties of vitamins and other mostly plant-derived substances, there have been several investigations of the possible benefits and especially of the effects on free iron and liver function (Elalfy et al., 2013; Livrea et al., 1996). Most studies, using combinations of substances, are on small numbers of patients and short duration of treatment, with one exception where the duration was 1 year. In transfusion-dependent patients, some benefit is demonstrated but there is also possible long-term toxicity, especially where vitamin C is concerned. For this reason there is no agreed guideline on their use in the everyday life of patients. However, encouraging the use of natural antioxidants such as curcuminoids, quercetin and green tea may be of some help, even though larger and longer duration studies are required to assess benefits and long term effects.

## Supportive Treatments

Various substances, often derived from herbal sources, have been proposed to enhance treatment in thalassaemia. These often attract the attention of patients, and professionals should therefore be able to respond to any questions and be aware of the potential benefits, limitations or even dangers of these substances. Some of these are supported by clinical trials and should be considered in more detail.

### **L-Carnitine**

Carnitine is a butyrate derivative – beta-hydroxy-gamma-trimethylaminobutyric acid – with potential benefits in thalassaemia, since it is believed to have anti-oxidant and cardioprotective properties. It is known to be essential for the metabolism of long-chain fatty acids and it is present in high energy demanding tissues such as skeletal muscle, cardiac muscle and the liver. In clinical trials, L-carnitine at a dose of 50 mg/kg/day given to small groups of patients for 3-6 months, the following benefits have been reported:

- Improved heart function and improvements in exercise performance.
- Significant improvement in pulmonary artery systolic pressure in patients with pulmonary hypertension (El-Beshlawy et al., 2008).
- An increase in transfusion intervals (El-Beshlawy et al., 2007).
- Reduced bone pain and muscle weakness (Tabei et al., 2013).

However, caution is needed in patients with seizures and those with hypothyroidism, since L-carnitine inhibits triiodothyronine (T3) and thyroxine (T4) entry into the cell nuclei (Benvenga et al., 2004).

### **Wheat grass**

This is a popular health food prepared as a juice from the leaf buds of the wheat grass plant. It contains chlorophyll, vitamins, minerals and several enzymes. Wheat grass is believed to increase the production of red cells and increase the interval between transfusions, which has been demonstrated in a small number of patients and confirmed more recently (Singh et al., 2010). There do not appear to be side effects, but long-term benefit is uncertain.

### **Silymarin**

A derivative of milk thistle (*Silybum marianum*), silymarin, is a flavonolignan complex which has antioxidant properties and has been investigated extensively as a hepatoprotective agent. In recent publications, this role of silymarin has been confirmed and it has additionally been found to inhibit hepatitis C virus entry into hepatocytes (Blaising et al., 2013; Polyak, Ferenci & Pawlotsky, 2013). These benefits may be of use in thalassaemia patients who have liver damage from iron overload, and/or hepatitis B (HBV) or C (HCV) infection. There are also reports that it can potentiate the effect of iron chelating agents (Darvishi-Khezri et al., 2018). It is available in capsular form and usually dosed at 140 mg three times a day (420 mg) with no recorded side effects.

### **Alcohol**

Patients with thalassaemia should be discouraged from consuming alcohol. Alcohol can potentiate the oxidative damage of iron and aggravates the effect of the hepatitis viruses on liver tissue. If the liver is iron loaded and/or infected by HCV or HBV, alcohol consumption may further promote progression to cirrhosis and hepatocellular carcinoma. Excessive alcohol consumption may also affect bone formation and is a risk factor for osteoporosis. In addition, alcohol may have unexpected interactions with medications.

### **Smoking**

Tobacco must also be avoided since it may directly affect bone remodelling, which is associated with osteoporosis. In view also of the doubts concerning cardiorespiratory fitness for exercise (see the discussion above), it can be assumed that smoking will make matters worse and, of course, it carries all the adverse effects described in the general population.

### **Drug abuse**

Substance abuse is common in most societies and a special danger among adolescents and young people. Thalassaemia patients attempting to 'fit in' and be accepted into peer groups are potentially vulnerable to experimentation with these drugs. There are no published studies on the prevalence of drug abuse in this cohort, but many clinicians have encountered isolated cases. Treating staff should be able to recognise patients who have a problem and be ready for transparent and frank discussions around these issues. Substance abuse can have serious consequences in thalassaemia patients with tissue damage affecting many vital organs.

## **Quality of Life**

All the issues discussed in this chapter are addressed by a service which offers patient-centred and holistic care, alongside accepted clinical standards of care. The aim is to achieve autonomy in life, and to allow patients to satisfy their personal ambitions. In considering whether a healthcare team has been successful in its efforts, quality of life should be a major outcome measure. In an editorial, the Communication Committee of the European Hematology Association notes: *"Quality of Life will, very soon, become completely integrated into patient care....In times when some haematological diseases are turning from acute, life threatening diseases into lifelong chronic conditions, assessing and maintaining Quality of Life becomes even more important for patients"* (Chomienne et al., 2013).

**How then is quality of life assessed?** The concept of quality of life involves each patient's perception of his or her own life and wellbeing, and since wellbeing includes psychological and social functions, which in turn are influenced by the physical state

of health, any assessment must include all these dimensions. Several measures have been developed to evaluate quality of life, which explore domains such as physical state, emotional state and social circumstances. These domains are incorporated in questionnaires – of which several have been tested, validated and used in thalassaemia. The following are examples of the main instruments used:

- The WHOQoL questionnaire (Telfer et al., 2005).
- The PedsQoL Generic Core Scales (Clarke et al., 2010).
- The Dartmouth Primary Care Cooperative Information Chart System (Pakbaz et al., 2005).
- The Short Form Health Survey- SF36 (Musallam et al., 2011; Sobota et al., 2011).
- The Specific Thalassaemia Quality of Life Instruments (STQOLI), (Lyraeos et al., 2012).
- TranQoL, is also a thalassaemia-specific instrument (Klaassen et al., 2013).

It is not the aim of this chapter to recommend any one instrument in particular, but to strongly urge thalassaemia clinics to adopt and use an instrument of their choice and apply it over time to their patients. Clinics should follow changes in their patients' own evaluations and views, as each patient's situation in each domain changes with alterations in treatment, or the appearance of complications (Gollo et al., 2013). These instruments can be used to monitor and evaluate individuals, as well as groups of patients, thus allowing them to evaluate service quality and clinic performance, and identify any weaknesses that need to be addressed.

Health related quality of life as estimated by these various tools cannot be used to make comparisons between care in different geographical regions. Variables include the disease severity of patient groups, past management of patients, the onset of complications, whether on oral versus parenteral chelation, the age of patients, and whether parents or children are responding (Gollo et al., 2013). Monitoring patient groups over time using the same instrument in the same clinical setting can, however, provide invaluable information on how patients are faring with the care they are receiving.



## Summary and Recommendations

- A holistic approach to patient care includes recognition of the need for social integration and a 'normal' life. [I]
- The treating physician should be able to provide advice on lifestyle issues. [I]
- Physical activity should be encouraged but the condition of each individual patient should be recognised and co-morbidities identified. Cardiovascular assessment and ergometry if available should be undertaken before recommending exercise and activity. [I]
- Clinic and transfusion times should be flexible and take into consideration the patient's commitments, such as their education and work. Availability of some evening and weekend clinic and transfusion sessions is highly recommended. [IIb]
- Liaison with teachers and employers to provide understanding of the condition and its management may be necessary. [IIb]
- Routine monitoring of growth is necessary, and nutritional factors such as caloric intake and micronutrient deficiencies should be considered in instances of poor growth. The services of a dietician are helpful in this respect. [I]
- Zinc supplements may be given in cases of deficiency, poor growth and reduced bone mass, but are not recommended as routine for all patients. Zinc levels in plasma are measured every 6 months. [IIa]
- Dietary iron restriction is recommended in patients on low transfusion regimens with low pre-transfusion haemoglobins. [IIa]
- Calcium and vitamin D supplements are recommended for all patients at a dose of 2000 iu per day, along with measurements of vitamin D levels every 6 months. A diet high in calcium is also recommended (through milk, fish, cheese etc.). [IIb]
- Folic acid supplements of up to 1 mg/day are needed for all patients with low haemoglobin levels [I]. Supplementation for all patients may be considered, since the risk of thrombosis may be reduced and toxicity is low. [IIb]
- A diet rich in foods with high vitamin E content, such as eggs and vegetable oils is recommended. Prolonged use of supplements requires further research. [IIb]
- Vitamin C supplements are recommended, in conjunction with deferoxamine infusions at a dose of 2-3 mg/kg/day, or if deficiency is proven. [IIa]
- L-carnitine may be beneficial at a dose of 50 mg/kg/day, although caution should be exercised in patients with thyroid dysfunction. [IIb]
- There is insufficient evidence on any long term benefits from wheat grass.
- Silymarin at a dose of 140 mg three times daily is recommended if liver involvement is detected, and on consultation with a hepatologist. [IIa]
- Consumption of alcohol and tobacco and substance abuse should be avoided.
- A quality of life assessment should form part of the regular evaluation of each patient's progress, and may usefully also highlight issues in service delivery. [IIa]



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## CHAPTER 15

### Psychological support

The need for continuity of care and psychological support for chronic disease is widely accepted (Falvo, 2014; Lubkin & Larsen, 2013), as is the negative impact of psychological issues on chelation adherence (Beratis, 1989; Evangelaki, Mughal & Porter, 2010; Panitz, 1999; Porter, Evangelaki & El-Beshlawy, 2011) and the quality of life (Rikos et al., 2020) in thalassaemia major patients. This chapter will (1) provide a comprehensive review of the published social and behavioural problems in thalassaemia, with a specific focus on any suggested interventions, and (2) articulate the social and psychological support interventions that have been successfully used for similar problems in other diseases.

Unfortunately, there is a surprising lack of published evidence for psychological support interventions in thalassaemia. A 2001 Cochrane Review of psychological therapies for thalassaemia (Anie & Massaglia, 2001), assessed as “up-to-date” in 2014, concludes that “no randomised controlled trials employing psychological therapies ... were identified” and “no trials, where quasi-randomization methods such as alteration are used, were found.” This is particularly concerning since a standard observation in many clinical reviews of thalassaemia over the past 25 years is that patient behaviour, primarily with adherence to iron chelation therapy (ICT), is a significant variable in long-term outcome and increased financial burden due to management of long term health complications (Borgna-Pignatti et al., 2004; Efthimiadis et al., 2006; Modell, Khan & Darlison, 2000; Olivieri et al., 1994; Porter & Davis, 2002; Vekeman et al., 2016). Non-adherence is episodic in many patients but over time probably just as damaging and further research is needed to see how this influences interventions (Vosper et al., 2018).

### The Challenge of Psychological Support: What Does the Literature Tell Us?

The challenge of ‘psychological support’ in thalassaemia is not a simple construct. Psychological support encompasses a complex set of defined responses to a diverse set of problems that have become apparent in thalassaemia over the past 30 years. This is illustrated by a simple PubMed Title/Abstract search for thalassaemia/thalassaemia and only “psychological support”. The first of eleven reports (including the Cochrane review) appears in 1985 identified the need for psychological support in a child care centre in Italy (Colombino & Bonzano, 1985), but it took over a decade before a second report described how psychosocial problems impacted chelation adherence, despite an expansion of clinical support services (Politis, 1998). This was restated in 2003 with a characterisation of adult patients (Galanello, 2003) and a cross sectional patient survey (Vardaki, Philalithis & Vlachonikolis, 2004). A small cluster of subsequent articles looked at “psychological burdens” in different patient groups including children and caregivers (Aydinok et al., 2005; Prasomsuk et al., 2007), adolescents (Roy & Chatterjee, 2007), and adults (Gharaibeh, Amarneh & Zamzam, 2009; Mednick et al., 2010). A single, non-randomised interventional study in 2009 used cognitive behavioural family therapy to try and alter adherence to chelation therapy (Mazzone et al., 2009). These results suggest a wide diversity in the application of psychological support in the clinical effort to manage the patient developmental pathway and their long-term survival associated with ICT adherence.

This finding suggests that “psychological support” is an undefined response to a clinical need that requires specification. In order to develop a more complete understanding of the component elements of psychological support in thalassaemia, we conducted a comprehensive review of the 371 articles identified by a broad search of the “behavioural and social science research” (BSSR) literature (Figure 1). A full-text review determined that 9% (35) of the articles were either specific to BSSR or personal narratives. Another 11% (39) focused on clinical problems that happened to include a BSSR component (e.g. pregnancy in adult patients requires additional support services), and did not further an understanding of psychological support. The remaining articles are organised around the following clinical domains:

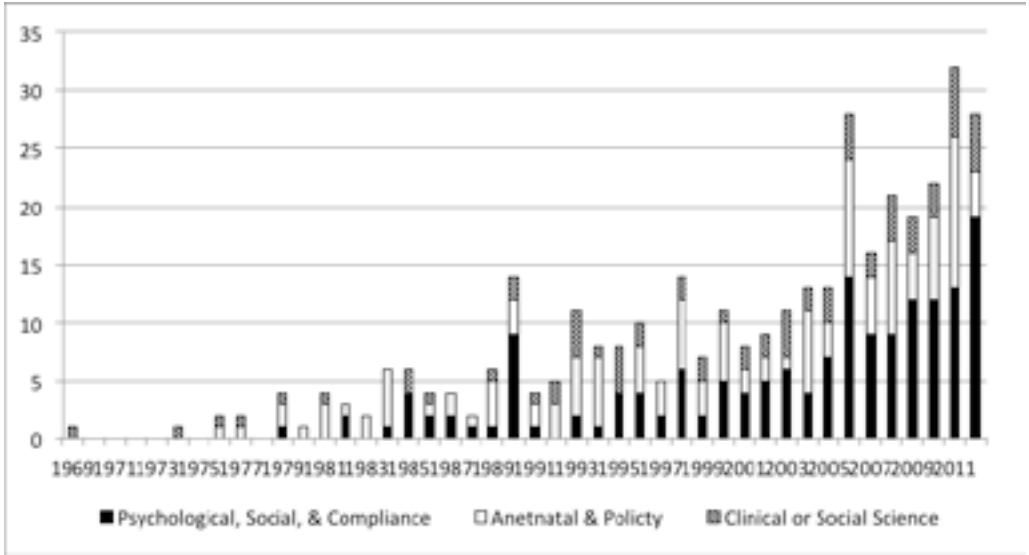
- **Antenatal screening** (30% of articles): these articles show a well-organised response to the problem of introducing antenatal screening in an at-risk population. They illustrate the complexity of creating a comprehensive solution that includes governmental support, legislation, community education, and face-to-face interaction. These reports tend to be post hoc celebrations of an arduous ad hoc process (TIF grade: D). The efforts to replicate this success have yielded some articles that identify specific complications associated with community demographic diversity in migrant populations. These articles identify the challenges this presents for implementing interventional strategies (TIF grade: C). Experience from antenatal screening that led to successful implementation were in relatively small and homogenous environments. However, the challenges when implementing clinical intervention in complex heterogeneous populations have not been fully considered. A few articles have addressed elements of this complex environment (Vichinsky et al., 2005) by looking at the economics of ICT (Payne et al., 2007; Riewpaiboon et al., 2010), clinical outreach to the communities of affected patients (Choy et al., 2000) and addressing the needs of culturally different patients (Banerjee et al., 2011) (TIF grade: C).

- **Iron chelation therapy** (10% of articles): most of these investigations either measure adherence (Matsui et al., 1994) or assess patient experience with treatment (Payne et al., 2007; Porter et al., 2012; Taher et al., 2010) (TIF grade: B). Over half of these articles appeared in the past 10 years with the introduction of new oral chelators and lay a scientific foundation for assessing the patient-reported health outcome as one step in understanding the patient’s ICT practices (Evangeli, Mughal & Porter, 2010; Mednick et al., 2010; Porter et al., 2012; Porter, Evangeli & El-Beshlawy, 2011; Sobota et al., 2011). These reports tend to have a very good scientific basis (TIF grade: A), because they are associated with other kinds of clinical investigations. They do not attempt to solve observed behavioural or social problems.

- **Psychological problems** (14% of articles): there appears to be a wide-ranging cross-national recognition that patients with thalassaemia are vulnerable to psychiatric problems (Aydinok et al., 2005; Cakaloz et al., 2009; Pradhan et al., 2003; Sadowski et al., 2002; Saini et al., 2007; Shaligram, Girimaji & Chaturvedi, 2007b, 2007a). These articles look at the psychological problems within the context of patient adherence to therapy, with the implied connection that failure to adhere reflects a patient’s psychological or cognitive makeup. The early reports tended to be at the level of clinical descriptive studies (TIF grade: C). More recent studies have shifted to identifying the neuropsychological investigation of cognitive deficits (Armstrong, 2005; Duman et al., 2011; Monastero et al., 2000; Zafeiriou, Economou & Athanasiou-Metaxa, 2006) (TIF grade: B). Angastiniotis points out that the problem of observed psychological problems in thalassaemia could actually be a function of the level and nature of support services that are available to patients (Angastiniotis, 2002) and not simply a problem of patient’s psychological makeup.



• Social support (20% of articles): these studies address the range of needs of families and patients. The effort to scientifically specify these needs began with Ratip's work to develop disease-specific standardised assessments of these domains (Canatan et al., 2003; Ratip et al., 1995; Ratip & Modell, 1996) and has continued with other studies (Tsiantis et al., 1996; Zani, Di Palma & Vullo, 1995). This domain appears to have the most interventional studies that include targeting changes in institutional organisation practices (Marovic & Snyders, 2008), patient group sessions (Marovic & Snyders, 2008; Yamashita, Foote & Weissman, 1998), family therapy (Mazzone et al., 2009) and patient chelation camps (Treadwell & Weissman, 2001). While these reports suggest some success, they all lack a robust analytical assessment (TIF grade: C).



**Figure 1.** 1979-2012: BSSR articles on psychological aspects of thalassaemia by type. A comprehensive database of the available literature was constructed from title & abstract searches of thalassaemia (thalassaemia) in a number of bibliographic databases: PubMed, biological abstracts, psycINFO, CINHAL, sociological abstracts, social services, and JSTOR. This collection was then searched using a variety of truncated terms (e.g. psych\*, soc\*, quality of life), and relevant problems (e.g. counsel\*, compl\*, adher\*, econ\*, etc.). An abstract review for relevance was conducted since many clinical articles invoke BSSR terminology as a conclusion (e.g. the outcome improves patient quality of life) and do not substantively use it in the study.

As a whole this literature suggests that patients with thalassaemia and their caregivers are faced with many distinct psychological and social challenges which have an impact on emotional functioning and may result in increased vulnerability to symptoms of psychiatric illnesses, such as depression and anxiety (Angastiniotis, 2002; Aydinok et al., 2005; Duman et al., 2011; Galanello, 2003; Gharaibeh, Amarneh & Zamzam, 2009; Marovic & Snyders, 2008; Politis, 1998; Prasomsuk et al., 2007; Ratip et al., 1995; Ratip & Modell, 1996; Roy & Chatterjee, 2007; Vardaki, Philalithis & Vlachonikolis, 2004; Zafeiriou, Economou & Athanasiou-Metaxa, 2006). Psychological support appears to be a loose reference to a broad mix of organisational responses to clinical needs, and not a coherent interventional strategy. Thus, there are no well-developed interventional trials aimed at providing psychological support to improve overall well-being of patients and their families (TIF grade: F). The few, small interven-

tional studies are descriptive reports of clinic-level responses (TIF grade: C). They lack analytical rigor because standardised behavioural and social science research instruments were not used. Recent reports show an effort to develop the needed rigorous, scientific understanding of patient-reported outcomes within on-going studies of iron chelation therapy (Evangelaki, Mughal & Porter, 2010; Haines et al., 2013; Porter et al., 2012; Porter, Evangelaki & El-Beshlawy, 2011; Sobota et al., 2011; Trachtenberg et al., 2011, 2012b, 2012a). Most are designed to inform a clinical response to underlying clinical problems. These efforts should establish the analytical foundation for future interventional studies in psychological support.

In the meantime, we can only offer recommendations for psychological support based on existing best practices and research done with other disease populations.

## Practical Considerations

Recommendations for standards of care for psychological support require a practical organisational model. As the specific challenges associated with being a patient with thalassaemia differ throughout development, a clinical pathway model that starts with the functional landmarks that define the patient and family experience is helpful (diagnosis-treatment). There are two modifiers to the clinical experience. Firstly, because thalassaemia is a chronic disease presenting shortly after birth, the natural growth from infant to adult will shape how patients learn to live with their disease. In the early stages, patients are dependent on their family caregivers, and as they develop, the patient must learn to successfully manage their own care. The second is the institutional organisation of clinical medicine. Paediatrics typically works with the patient and their family and adult medicine works with the individual patient. This situation complicates any psychological support recommendations. At each of the landmarks along the pathway (e.g. point of diagnosis, start of transfusion, initiation of chelation, transition into more self-care in adolescence, and transition to adult care), patients and families may be more vulnerable to experiencing psychological sequelae associated with the disease management and developmental challenges commonly experienced during that period of time. Our model of the 'clinical pathway of thalassaemia' is illustrated in Figure 2.

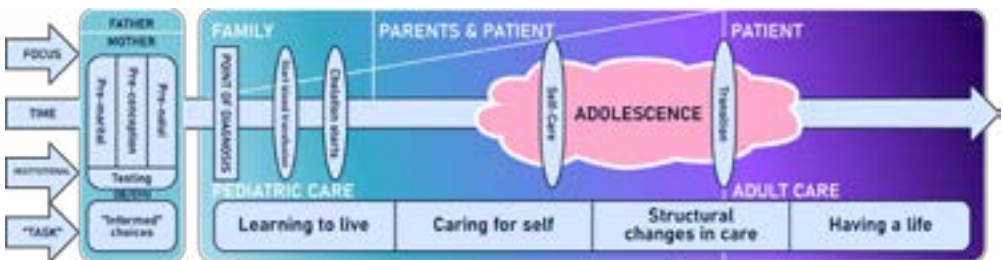


Figure 2. Clinical pathway diagram.

Systematic studies to examine different intervention modalities that may help patients and families effectively cope with the particular challenges inherent at each time point are needed. These should address how early 'upstream' familial experiences impact 'downstream' patient adherence adaptations and long term survival. As most of the existing literature consists of descriptive reports and cross-sectional studies, the fol-

lowing practical recommendations are largely based on what we know from our clinical work and/or research with other chronic illnesses.

### ***Point of diagnosis***

Parents will undergo a series of changes after their child is diagnosed with thalassaemia (shock, denial, sadness/anger, adaptation, reorganisation) (Drotar et al., 1975). One of their most important immediate concerns is getting reliable information (Starke, 2002). Learning the additional tasks associated with caring for a child with thalassaemia can be overwhelming to the parent and lead to psychological distress (Galanello, 2003; Politis, 1998; Yamashita, Foote & Weissman, 1998). Importantly, if parents feel overwhelmed with caring for their child, effective management of the illness may become compromised (Otsuki et al., 2010). To minimise these feelings, effective psychological support of parents around the time of diagnosis should include:

- Providing necessary information about thalassaemia. This may need to be repeated several times for full comprehension.
- Opportunities to ask questions and share concerns.
- Occasions to meet parents of older children diagnosed with thalassaemia, as this can help increase social support and confidence, while decreasing feelings of helplessness and hopelessness.
- Access to psychosocial clinicians who can help them explore and manage their feelings of loss in a constructive manner.

It is especially important to help parents accept and learn to effectively cope with their child's chronic medical condition at this early stage. This is because parental behaviours and attitudes throughout development will lay the groundwork for how children will cope with their condition. Parents who demonstrate healthy coping and understand that a well-managed patient who adheres to his/her therapy can live a successful life (Pakbaz et al., 2010) will help their children to learn to make thalassaemia a piece of who they are, rather than what defines them. Introducing the family to an appropriately experienced family with a child who has thalassaemia can be a helpful learning experience for parents of young children.

### ***Start of blood transfusion***

The best ways to provide psychological support aimed at helping children effectively cope with invasive medical procedures has been widely studied (Brown, 1999; Brown, Daly & Rickel, 2007; Edwards & Titman, 2011; Hayman, Mahon & PhD, 2002; Hymovich & Hagopian, 1992; Thompson, 2009). It is essential to help parents and children engage in effective coping strategies as soon as developmentally appropriate, as the experience of distress during a medical procedure has been found to be predictive of distress during future procedures (Frank et al., 1995).

Starting at a very young age, children often look to their parents for signals on how they should react in anxiety-provoking, novel situations. In one study, parent behaviour during an invasive medical procedure accounted for 53% of the variance in child distress behaviour (Frank et al., 1995). Providing information about the procedure prior to the actual procedure and giving the parent a job to do (e.g., distract the child), is likely to reduce parental anxiety, with positive indirect benefits for their children. However, if parents are not able to remain calm in front of their children during procedures such as blood transfusion, it is helpful for clinicians to give parents' permission' to leave the room and instead consider including the presence of another supportive adult.

Specific coping strategies aimed directly at the child have been particularly useful in helping children cope effectively with invasive medical procedures. In a meta-analysis of psychological interventions for needle-related procedural distress in children and adolescents, distraction was found to be one of the most efficacious coping techniques (Uman et al., 2008). In fact, a recent study conducted with patients with thalassaemia found that bubble blowing during an injection helped reduce anxiety (Bagherain et al., 2012). Importantly, distraction techniques should be adapted to the child's interest and age/developmental level. It is particularly useful to encourage parents who engage in excessive reassurance to instead focus on distracting their child, as reassurance often amplifies fear and distress (Manimala, Blount & Cohen, 2000), likely due to refocusing the child's attention on the fearful and painful aspects of the situation.

As children get older, they may ask for more information about transfusions or other invasive medical procedures (e.g., magnetic resonance imaging). Fostering trust, reducing uncertainty, correcting misconceptions, enhancing the belief in their ability to cope with a procedure, and minimising distress are some of the potential benefits of providing advance information about a procedure to a child (Jaaniste, Hayes & Baeyer, 2007; Jipson & Melamed, 2007). Effective pre-procedural information should include:

- A developmentally appropriate verbal explanation of what the child will see, hear, feel and smell during, before and after the procedure.
- Minimally threatening, but accurate information, as children who are given information that turns out not to be true (e.g., "you will not feel a thing" when in fact the child is liable to experience some pain), are more likely to develop a distrustful relationship with their parents and/or the medical team, which may negatively affect future interactions.
- Use of visual aids (e.g., books, pictures, models, videos).
- Where possible, medical play can help young children understand their therapeutic regimen (Bandstra et al., 2008; Bolig, Yolton & Nissen, 1991; Burns-Nader, 2011; McCue, 1988).
- Time for the child to ask questions.

In ideal clinical settings, a child-life specialist should be included on the treatment team. Child life specialists are health care professionals who have the specific training to understand the impact transfusion-dependent thalassaemia (TDT) has on children and can help them and their families navigate the complex processes needed to not only understand their treatment, but provide them with the necessary life-long skills not just to manage their clinical needs but to live with their disease.

### ***Initiation of chelation***

Parents need to be provided with support and guidance about choosing which type of chelation is best for their child. For example, although oral chelators are associated with less distress and better quality of life in older patients, due to specific developmental characteristics of very young children (e.g., transient food preference, oppositional behaviour, unpredictability), this may not be true for some children in this age group (Fiese, Wamboldt & Anbar, 2005). Parents of very young children need to be encouraged to carefully consider their chelation options, and determine which option best fits with their own capacities and their child's personality characteristics. When starting chelation therapy, parents should be encouraged to develop consistent routines around medication taking. Developing predictable routines around a child's medical regimen makes these tasks part of the typical daily schedule, thereby fos-

tering good adherence by minimising several of the problems often associated with adherence difficulties (e.g., forgetting, conflicts about when to take the medication) (Fiese, Wamboldt & Anbar, 2005; Rand, 2005).

Behavioural interventions which include increased monitoring and incentives for meeting goals have been shown to be successful at improving adherence in patients with thalassaemia (Koch et al., 1993). The use of incentives may be particularly useful for paediatric patients who don't yet understand the intrinsic value of adhering to an undesirable medical regimen. These may include verbal praise, stickers or small toys or other incentives earned either immediately or over time, for cooperating with daily chelation. By pairing a positive outcome (e.g., sticker, toy) with an aversive stimulus (chelation) the child develops a positive association with the aversive event, increasing the likelihood that the child will perform the behaviours again in the future.

At various times along the clinical pathway, patients may struggle with chelation adherence (Evangelii, Mughal & Porter, 2010). When this occurs, it is essential to identify why the patient is having difficulty following the prescribed plan. Interventions that do not consider the specific barrier to adherence will have limited success (see Table 1 for common barriers and suggested interventions). In general, effective interventions aimed at improving adherence usually:

- Incorporate behavioural or multiple strategies;
- Include patients (and parents) in the development;
- Start from where the patient is at, gradually increasing goals, while working towards the ideal;
- Revise over time.

**Table 1. Common barriers to adherence and suggested interventions.**

Barrier	Intervention
Lack of understanding concerning regimen implementation or importance	Provide age-appropriate education by other more junior physicians according to the number of patients
Forgetfulness	Set alarms; use visual reminders
Inconvenience	Work with the medical team to change the regimen to fit better with the patient's lifestyle
Inconsistent schedule of medication	Implement a reminder system (e.g., alarms); use a self-monitoring chart to document completion of tasks
Side effects of treatment	Find ways to help minimise or cope with the side effects
Length of treatment	Help the patient find activities to do during the treatment
Complicated regimen	Simplify regimen (with medical team); create a self-monitoring chart to document completion of each task

Barrier	Intervention
Social Stigma	Engage the patient in treatment aimed at improving self-esteem; encourage the patient to meet other individuals with similar medical conditions
Poor supervision	Increase adult involvement and monitoring
Cultural or religious beliefs	Work with family to understand their beliefs and when possible adapt treatments to fit within their values
Psychiatric illness	Treat underlying psychiatric illness
Family psychopathology	Work with caretakers to create an environment that is conducive to encouraging adherence (e.g., decreased conflict, increased communication)
Poor social support	Help the patient/family find resources within their community; encourage the patient to meet other individuals with similar medical conditions

Older patients often make remarks such as “I am tired of doing this,” “I don’t want to,” or “I need a break.” Many often take the break. This ‘voluntary non-adherence’ is difficult to assess and many treating physicians do not often attempt to study this in their patients. It is important to continually monitor every patient’s adherence to ICT.

### ***Additional opportunities for psychological support during childhood***

As children with thalassaemia frequently miss school for medical appointments and transfusions (Gharaibeh, Amarneh & Zamzam, 2009), which can negatively impact school functioning (Thavorncharoensap et al., 2010), parents should be encouraged to educate the school about their child’s condition and to set-up plans which support the child when he/she needs to miss school. Further, patients with thalassaemia may be vulnerable to experiencing cognitive deficits (Armstrong, 2005; Duman et al., 2011; Economou et al., 2006; Lücke, Pfister & Dürken, 2005; Monastero et al., 2000; Nevruz et al., 2007; Zafeiriou, Economou & Athanasiou-Metaxa, 2006; Zafeiriou et al., 2004). If there are concerns from parents or the school, it may be valuable for patients to participate in neuropsychological testing to assess for any concerns and provide recommendations that could help support the patients learning potential.

### ***Importance of social support throughout development***

As social support has been found to play an important role in the psychological functioning of children and their families (Lewandowski & Drotar, 2007), starting from an early age, patients and their families would benefit from:

- Deciding how to present information about the patient’s medical condition to friends and family;
- Learning about the harmful effects (e.g., feelings of shame) of keeping thalassaemia a secret;
- Relying on existing friend, family, religious and community supports;
- Meeting other patients and families with chronic medical conditions through attending camps, events sponsored by specific illness foundations, or one-to-one meeting facilitated by a clinician.

### ***Adolescence and transition to increased self-care***

Adolescence is a time when adherence to daily medical regimens often declines (Trachtenberg et al., 2011). Frequently the transition of responsibility from the parent to the adolescent occurs before the patient is emotionally ready, resulting in poor adherence. Because adolescents are vulnerable to having their decision making driven by their desire to be independent and to fit-in with peers, parents need to continue to play an active role in monitoring adolescents self-care. Shared responsibility between the patient and caregiver has been found to be associated with better adherence (Evangeli, Mughal & Porter, 2010; Treadwell & Weissman, 2001).

Also, to avoid the negative consequences of abrupt shifts in responsibility, the transition of responsibility needs to:

- Occur gradually over time, starting when children are young (e.g., help gathering supplies) and increasing their involvement as they mature;
- Teach older patients how to take over responsibility for often-overlooked tasks, such as ordering supplies and making medical appointments.

### ***Transition to adult care***

One reason why adherence may be lowest in young adults (Trachtenberg et al., 2011) is because of insufficient psychosocial support as patients transition from paediatric to adult medical providers. Often the transition to adult care providers happens in an abrupt manner, leaving the patient unprepared for the shift to adult medicine (Bryant & Walsh, 2009). Discussions about transitions should occur well in advance of the actual transfer in care and should include an exploration of the patients concerns and how they will prepare for and manage the changes inherent in moving from a paediatric to an adult medicine clinic. This plan can result in fewer cases being 'lost to follow-up' (Allemang et al., 2019). A well-coordinated transition plan includes:

- Long-term plan that starts with orienting pre-adolescent patients for the change;
- Annual assessments of a patient's preparation for transition;
- Multiple opportunities to orient the patient to an adult clinic, adult care practices, and the adult care system;
- Multiple overlapping visits with paediatric and adult haematologists.

### ***Pain in thalassaemia patients***

Pain is a concern for patients with thalassaemia. However, reports of pain in thalassaemia are relatively recent. Pain appears to increase in intensity and frequency with age (Haines et al., 2013; Trachtenberg et al., 2010). Because pain appears more frequently in older transfusion-dependent patients, pain in thalassaemia presents a new TDT problem.

The presence of pain usually indicates an underlying organic source, but in thalassaemia there is a lack of understanding of pain symptoms and its organic cause. The presence of pain in the non-thalassaemia adults is associated with decreased social function and increased depression (Avlund, Rantanen & Schroll, 2007; Burckhardt, 1985; Dunn & Croft, 2004; Garber et al., 2010; Koenig, 1997; Ozminkowski et al., 2012). For most patients who report the presence of pain, their response and choice of clinical intervention solutions is predicated on how they understand the source of the pain. If chronic pain symptoms are not modulated, patients are at risk of choosing non-clinically managed pharmacological solutions.



Because there is a lack of understanding of the relationship between thalassaemia pain symptoms and its organic cause, effective pain management strategies do not exist. If thalassaemia presents with pain symptoms, an effective treatment plan requires conducting a careful clinical evaluation to identify the underlying cause. Depending on the site of pain, there should be a consideration of clinical studies such as: bone density assessment, MRI/ X-ray evaluation to assess musculoskeletal deformities or recent injuries, and an evaluation of average haemoglobin levels to elucidate the sources of pain (Lal, 2016; Piga, 2017). Based on these findings, a pain management strategy can be developed. Pain management plans can include pharmacological and nonpharmacological interventions; however, because most thalassaemia pain appears to be chronic, strong consideration should be given to nonpharmacological interventions such as:

- Physical Therapy
- Acupuncture/acupressure
- Massage
- Deep breathing
- Guided imagery
- Progressive muscle relaxation
- Hypnosis
- Biofeedback
- Mindfulness training

Until the underlying organic studies of thalassaemia pain are conducted, clinicians need to routinely ask if the TDT patient has the presence of pain. If they report on having the presence of pain, then patient pain symptoms management strategies need to be routinely assessed. Clinicians should encourage patients with pain to engage in a variety of empirically validated (Eccleston et al., 2009; Palermo et al., 2010; Shega et al., 2012) cognitive and behavioural coping strategies which have been shown to successfully help patients manage their pain and distress through learning how to regulate their emotional and physical responses to pain.

### ***Non-Adherence to Chelation Therapy***

Patient adherence to their chelation therapy is the single-most important action that improves long-term outcome in TDT. Advances in chelation therapy have changed the expectation of patient long-term survival, and published studies suggest adherence rates as high as 90% (Pinto et al., 2018), 63% (Vekeman et al., 2016) and as low as 42% (Rofail et al., 2009). A recent Cochrane review of "Interventions for improving adherence to iron chelation therapy in people with sickle cell disease or thalassaemia" (Fortin et al., 2018) suggests that the higher adherence reports with ICT could be an artefact of trial participation. Critically, the "quality of evidence" for adherence interventions was "low to very low across all" studies in the review, and only 3 trials "measured quality of life with validated instruments" and they "reported no difference in QoL" but "provided no analysable data."

Regardless of any flaws in the published data, the introduction of new interventions has changed the life-expectancy for TDT patients. For patients who need life-saving chronic blood transfusions, it is incumbent on practitioners to be alert to changes in the patient's ICT adherence practices. Change needs to be evaluated at several levels.



**First**, evaluation needs to start with measurable organic changes.

- Evaluating trends in iron loading: such as serial increases in ferritin levels in conjunction with increasing indications of organ involvement (using MRI reports).
- Other clinical tests that can be a useful indicator of iron burden: for example, measuring labile plasma iron (LPI), even though this is largely a research tool, can indicate iron burden (Steinberg-Shemer et al., 2018).
- Bioassays of chelator drug levels can provide an 'objective' measure of actual chelator use, but the tests are expensive for routine application

**Second**, patient reported adherence needs to be evaluated. Self-reports of adherence are inherently unreliable since patient subjective responses are highly variable and dependent on the patient-provider relationship. Most reported assessment methods are described in research studies but can be adjusted for clinical use:

- Basic standardised questionnaires have been devised to assess patient medication adherence. The most well-known is probably the Morisky Medication Adherence Scale (MMAS) (Moon et al., 2017).
- Pill counts or empty vial counts (in the case of deferoxamine) have been practiced with variable results. A refinement on this approach is to use the medication possession ratio (MPR), which is the proportion of days of medication supply a given drug in a particular time period, divided by the number of days in the time period (Sperber, Samarasinghe & Lomax, 2017).
- Medication Monitoring Devices have also been introduced. These include 'digital pill boxes' that have a 'smart' vial cap that electronically records the date and time of bottle opening. This is an expensive tool and studies have failed to show superiority to self-reporting questionnaires (Badawy et al., 2019; Monnette et al., 2018; Shi et al., 2010).
- Text messaging and mobile phone app interventions are another approach but there is a need to establish the long-term benefit.

**Third**, and most important, the patient needs to be engaged in developing solutions for their practices. A paternalistic (top down) approach, where the physician tells a patient that they have to do their therapy, will not work. As with parenting, where every parent knows that telling an adolescent or young adult what they have to do rarely yields the appropriate long-term action. With TDT, clinicians need to recognise that patients live their thalassaemia every day, many (especially older patients) know more about the disease burden than clinical practitioners. The lived experience frames their understanding and ultimately their actions. Finding effective long-term solutions requires building the patient/provider relationship. The relationship is built around trust and respect, allows for creative solutions aimed at helping a patient develop a coherent response to a long-term problem.

- Taking time to listen to concerns and provide support is the most essential action supporting patient adherence (Zolnieriek & Dimatteo, 2009).
- Increasing patient knowledge and understanding of their condition results in patient empowerment which can encourage adherence to prescribed treatment (Náfrádi, Nakamoto & Schulz, 2017). As patients age, discussing laboratory results such as serum ferritin or MRI results, can help engage patients. These discussions not only enable patients to understand why the tests are needed, but they also inform

them about their overall health trajectory.

- Utilising and trusting the input of the clinical team (other physicians, nurses and allied healthcare workers). The team gets to know the patient in different ways. Their insight into the social factors that shape adherence practice (illustrated, in part, in Table 1) provide a more comprehensive understanding of the patient's circumstance.

Whatever other method is used to engage patient adherence, it is important to remember that thalassaemia is no longer a 'disease of childhood', it is a life long chronic disease. Patient adherence requires constant, periodic assessment. This lifelong relationship has negative side effects on the provider and the provider team. The amount of time required to build and sustain the relationship, can result in staff fatigue, burn-out and information overload. Often, the time commitment to build and sustain the patient-provider relationship runs against the artificial limits imposed by the healthcare reimbursement system. Developing a workable solution requires a non-combative and collaborative 'monitoring' and 'quality control' approach by healthcare administrators (Wagner et al., 2001).

### ***Psychosocial support throughout the lifespan as part of standard care***

As social and emotional concerns can occur anywhere along the lifespan and such concerns can have an impact on the patient's quality of life and physical health, opportunities for regular psychological support should be part of the treatment plan of all patients with thalassaemia. This is best accomplished through a comprehensive team approach used by thalassaemia centres of excellence found in high-income countries. The teams include multidisciplinary allied health specialists including skilled nursing staff, psychologists, social workers and other specialists such as family or child-life specialists. These health providers are better equipped to assess any social, emotional or cognitive concerns and intervene with additional support when necessary. If the team members regularly meet with patients and their families, it allows multiple communication pathways to be built. An effective team that is centred on improving patient response to therapy can take advantage of the multi-dimensional understanding of a patient, to create interventions that work for the patient. This is especially important when the team tries to build a novel adherence strategy for a patient. The different information pathways are also important for monitoring commonly seen anxiety and depression symptoms, and determining when those symptoms become psychiatric disorders that need early psychotherapy in order to prevent long-term health consequences. Importantly, the inclusion of psychological support as part of the standard care, some of the stigmatization associated with seeing a therapist may be removed.

Because TDT has become a chronic condition that begins at birth, there is a need for a comprehensive care framework that offers multidisciplinary care that spans both paediatrics and adult care. Because costs are a challenge, there are generally distinct choices of where national investments are made. Paediatric multidisciplinary teams are common in the US model of healthcare delivery for TDT. The US paediatric centres exert a significant level of energy to provide young patients with the tools they need to survive into adulthood. As young adults, they are then transitioned to systems where comprehensive care is not available where patients become 'lost to follow up'. In the UK, healthcare resources are aimed at supporting the workforce. Multidisciplinary Teams are accessible for adults, but non-existent in paediatrics. The UK centres have to commit a high level of psychological resources aimed at providing the TDT patient with the necessary tools that allow them to learn to become a functional (working) adult. For many, learning the skills are often challenging, because they were not intro-

duced to them at an earlier age. Efforts to bridge the UK and US models can be found, but finite resources usually require rationing access to multi-disciplinary centres of excellence, or by forcing a limit on the services provided by these 'centres'. This situation is clearly suboptimal because thalassaemia is no longer a disease of childhood, but a clearly described life-long chronic condition that begins at birth. We need a new model for healthcare resource allocation and delivery to address these types of health problems.

## Summary, Recommendations and Grade of Evidence

Overall, despite a general lack of large scale, randomised, controlled trial evidence conducted with patients with thalassaemia, there are innumerable cohorts of case-controlled analytical studies to suggest that psychological well-being has an impact on adherence to treatment for chronic disease in general (B). In thalassaemia, the published reports to demonstrate this linkage are mainly descriptive studies (C). A meta-analysis would suggest that more recent efforts are more towards B grade investigations (usually ancillary studies attached to robust controlled trials in other clinical areas). However, the lack of uniform instruments and standardised measurements weakens this assessment. The findings to date suggest that:

- Psychological well-being impacts on adherence to chelation treatment in thalassaemia major and hence on survival (C).
- Patients with thalassaemia are vulnerable to experiencing psychological challenges (C).
- Patient-reported health outcomes show that oral chelation therapy has a beneficial impact, relative to parenteral chelation (B).
- Neuropsychological investigation of cognitive deficits shows that there are clear intellectual and psychopathological problems in a very limited number of thalassaemia patients (B).
- Benefits of psychological support have been suggested using a variety of approaches (C) which include:
  - targeting changes in institutional organisation practices
  - patient group sessions
  - family therapy
  - patient chelation camps
- In all chronic illness, continuity of comprehensive care across the lifespan is essential for long-term, beneficial health outcome (A). Institutional organisational support for multidisciplinary teams is essential (A). There is a growing body of evidence that highlight the problems associated with transition from paediatric care to adult internal medicine in inherited chronic disease (B). Rare and neglected diseases complicate resource allocation models and lead to notable health disparities (A). In thalassaemia, these problems are known and reports from expert committees recommend addressing them, but there are no formal studies of the problems, much less any standardised evidence (F).

While A and B grade evidence for psychological support in thalassaemia is scarce, experience in several large thalassaemia centres strongly suggests that psychological well-being is key to adherence and to outcome.

- Expert psychological support has to be available at all centres specialising in thalassaemia care (C).
- Additional psychosocial support provided by trained specialists (e.g. social workers or family or child health specialists) should be tailored to the patient's age
  - Children (in general, A, thalassaemia C)
  - Adolescents – transition (in general, B, thalassaemia C)
  - Older adults – pain issues (in general, A, thalassaemia C)

Funding for clinical psychological support services could be more widely achieved if well-designed, multi-centre, interventional studies using common standardised instruments were undertaken to evaluate the benefit of psychological and psychosocial support to treatment adherence. The use of established behavioural and social science approaches in such studies need to identify the active components of 'psychological support' that are most applicable to patients with thalassaemia.

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# CHAPTER 16

## Haematopoietic Stem Cell Transplantation for Thalassaemia

### Introduction

To date, allogeneic haematopoietic stem cell transplantation (HSCT) has been the only curative treatment option for thalassaemia major, with more than 3000 HSCTs having been performed worldwide (Angelucci, 2010). It may be possible in the future to transplant autologous CD34+ stem cells transduced ex vivo with a vector harbouring a normal globin gene (= gene therapy), but this cost-intensive approach will not be used in routine clinical practice within the next few years. In recent years, a number of factors including improved conditioning regimens, improved prevention of graft-versus-host disease (GvHD) and more effective antibacterial, antiviral and antifungal treatment have resulted in a significant improvement in outcomes for HSCT with cure of thalassaemia achieved in 80% to 90% of patients today. This chapter will provide an overview of the current state of the art of HSCT in  $\beta$  thalassaemia major.

### Risk class approach for patient and protocol selection

In the 1980s the influence of pre-transplant characteristics on the outcome of HSCT was analysed in 161 patients less than 17 years of age (Storb et al., 1977; Lucarelli et al., 1990). Multivariate analysis showed that (i) hepatomegaly of more than 2 cm below the costal margin, (ii) portal fibrosis and (iii) irregular chelation history were associated with a significantly reduced probability of survival.

On the basis of these risk factors, a prognostic scheme was developed by the Pesaro group: patients were categorised into three risk classes influencing the probability of survival.

**Table 1.** Expected 5 y probability of overall survival (OS) and thalassaemia-free survival (TFS) after HSCT in  $\beta$  thalassaemia major

Stem cell source	Patient Age	Pesaro Class	OS	TFS	Reference
MSD	<17 y	Class 1 + 2	97%	≥89%	Isgrò et al., 2010 Sabloff et al., 2011
		Class 3	92%	92%	Gaziev et al., 2016
	Adult	Class 3	65%	65%	Lucarelli et al., 1992, 1999; Lucarelli & Gaziev, 2008 Gaziev et al., 2005
MUD	<17 y	Class 1 + 2	97%	80%	La Nasa et al., 2005a
		Class 3	66%	55%	La Nasa et al., 2005a
	Adult	Class 3	70%	70%	La Nasa et al., 2005a
Haploidentical (MMFD) TCR $\alpha$ /B+/- CD19+ depleted graft	<17 y	Class 1,2,3	84%	69%	Gaziev et al., 2018
	Median age 12 y	Class 1,2,3	96%	96%	Anurathapan et al., 2020

MMFD, miss matched family donor; MSD, matched sibling donor; MUD, matched unrelated donor; y, years.

### *Pre-transplant evaluation*

Particular attention has to be paid to an appropriate pre-transplant work-up. In addition to classical pre-HSCT evaluations, this should include accurate iron studies based on magnetic resonance imaging (MRI) analysis to evaluate liver iron load and cardiac MRI to evaluate cardiac iron load and function. The key to reducing graft rejection is to reduce erythroid expansion prior to HSCT.

### ***HSCT from HLA-matched sibling donors (MSD)***

In the past 30 years more than 2,000 thalassaemia patients have undergone HLA-matched related donor HSCT, predominantly in the transplant centres of Pesaro and Rome (Lucarelli & Gaziev, 2008; Isgrò et al., 2010).

In a large EBMT survey of 1061 cases of MSD HSCT performed between 2000 and 2010 in 132 centres in 28 countries with a median patient age of 7 years, long-term overall survival (OS) and thalassaemia-free survival (TFS) were 91% and 83%, respectively (Baronciani et al., 2016).

### *Conditioning regimen for class 1 and class 2 patients*

Preparatory regimens for HSCT must achieve two objectives: elimination of the disordered marrow and establishment of a tolerant environment that will permit the transplanted marrow to survive and thrive. There is considerable evidence and decades of experience for the use of busulfan (BU) and its derivatives for ablating marrow in patients undergoing HSCT for the treatment of non-malignant conditions (Parkman et al., 1978; Kapoor et al., 1981; Hobbs et al., 1986). Cyclophosphamide (CY) is a well-established agent for providing adequate immunosuppression for allogeneic engraftment (Thomas et al., 1972; Storb et al., 1991). The combination of 14–16 mg/kg BU and 200 mg/kg CY can eradicate thalassaemia and facilitate sustained allogeneic engraftment. Treosulfan-based conditioning has been introduced as a well-tolerated alternative (Mathews et al., 2013) and should be analysed in comparison to BU-based conditioning in a future randomised trial. When the allogeneic graft starts to proliferate in the recipient, an immunological reaction against the recipient (GvHD) may occur. Therefore, the prophylactic administration of ciclosporin (an immunosuppressive drug) is an important part of the pre-transplant and post-transplant treatment. Three doses of methotrexate (MTX) may also be given in addition to ciclosporin during the first 15 days after transplant.

### *Conditioning regimen for class 3 patients*

To further decrease graft rejection in class 3 patients, the initial conditioning regimen was modified by the Pesaro group with the addition of thiotepea (TT) – a drug with potent myelosuppressive and immunosuppressive potential, but mild extra-haematological toxicity. This improvement in the treatment protocol resulted in a 5-year OS and TFS probability of 92% (95% CI, 77–97%) compared to 85% OS (95% CI, 64–94%) and 73% TFS (95% CI, 51–86%) achieved with the previous protocol (Gaziev et al., 2016).

### ***HSCT from HLA-matched unrelated donors***

A major obstacle to successful transplantation is the limited number of HLA-matched related donors within families. Approximately 60% of patients lack a suitable sibling

donor. Some of these patients could benefit from HSCT from a matched unrelated donor (MUD). A number of studies with a few hundred patients transplanted worldwide have shown that MUD can cure a large proportion of patients with thalassaemia, provided that the donor is selected using high-resolution molecular typing for both HLA class I and II molecules. The risk of rejection can be reduced by selecting unrelated donors who do not have non-permissive mismatches at the HLA-DPB1 locus in the host-versus-graft direction (Fleischhauer et al., 2006). For example, a study of MUD HSCT in 68 patients with thalassaemia major who received BU/CY or BU/FLU (fludarabine) and/or thiotepea (TT) as a conditioning regimen reported 96.7% OS and 80% TFS for class 1 and 2 patients, whereas class 3 patients only had a OS of 65.5% and a TFS of 54.5% (La Nasa et al., 2005a). Outcome is affected by the risk classes; so that in the group of 30 thalassaemic patients in risk classes 1 and 2, the probability of OS and DFS were 96.7% (CI 90-100%) and 80.0% (CI 65-94%), respectively, whereas in the 38 patients in class 3 OS was 65.2% (CI 49-80%) and DFS was 54.5% (CI 38-70%). In another study that included 21 children who received MUD transplants: the 2-year TFS was 71% compared with 82% for patients who received MSD transplants (Hongeng et al., 2007).

***Thus when donor selection is based on stringent compatibility criteria and appropriate selection of class risk category in the recipient, the results of unrelated transplantation in thalassaemia patients are comparable to those obtained when the donor is a compatible sibling.***

### ***HSCT from phenotypically matched related donors***

In 2005, the Mediterranean Institute of Hematology (IME), Rome, adopted a new transplant approach for related donor HSCT in thalassaemia. This study was a prospective, single-centre investigation of the safety and efficacy of a novel preparative regimen for HSCT in patients with thalassaemia from related donors, who were not HLA-matched siblings.

Between 2005 and 2012, 16 patients with thalassaemia received their first HSCT from related donors who were phenotypically matched or 1-antigen-mismatched. The treatment protocol was based on BU/TT/CY/antithymocyte globulin (ATG) conditioning. Rejection incidence was 0% with a TFS probability of 94% and a transplant-related mortality of 6% (Gaziev et al., 2013).

Together, these data strongly suggest that improvements in donor selection and transplantation preparation improve the safety of unrelated and related donor HSCT for thalassaemia treatment (Gaziev et al., 2013).

### ***Haploidentical HSCT***

Haploidentical HSCT in principle may have the potential to extend the use of this treatment option to the 50%–60% of patients who lack a matched sibling donor or an HLA-identical unrelated donor. Initially high levels of graft failure of around 27% were seen (Sodani et al., 2010). A novel graft manipulation method that removes  $\alpha\beta^+$  T lymphocytes while retaining  $\gamma\delta^+$  T lymphocytes, natural killer (NK) cells, and other accessory cells was proposed (Chaleff et al., 2007) to reduce the graft failure rate and improve outcomes. Recent advances in graft engineering with effective ex vivo T-cell depletion through positive selection of CD34<sup>+</sup> cells, depletion of CD3<sup>+</sup>/CD19<sup>+</sup> cells or T-cell receptor  $\alpha\beta^+$  (TCR $\alpha\beta^+$ )/CD19<sup>+</sup> depletion significantly improved the outcome (Aversa



et al., 1998; Federmann et al., 2012; Diaz et al., 2016). Since June 2012, we have been using this new graft manipulation technique with selective depletion of TCRαβ+ and CD19+ lymphocytes. The use of TCRαβ+/CD19+-depleted grafts have been associated with significantly reduced graft failure (Gaziev et al., 2018). The haploidentical HSCT protocol was based on a BU/TT/CY/ATG conditioning regimen as previously published (Sodani et al. 2010). Patients received GvHD prophylaxis with cyclosporin and methylprednisolone or mycophenolate mofetil. The 5-year probabilities of OS and TFS were 84% and 69%, respectively (Gaziev et al. 2018). The incidence of graft failure was 14%. The incidence of grade 2 to 4 acute GvHD was 28% and 21% for extensive chronic GvHD.

While extensive T-cell depletion can significantly reduce the incidence of GvHD, it has been associated with delayed immune recovery and an increased risk of graft rejection, especially in non-malignant diseases (Willasch et al., 2006). Delayed immune reconstitution and associated morbidity and mortality remain significant problems in this setting. Novel pharmacologic and cell therapy approaches to enhance immune reconstitution and improve outcome after T-cell-depleted haplo-HCT are warranted.

A recently developing alternative platform for haploidentical HSCT uses T-cell-replete grafts and post-transplant cyclophosphamide (PT-Cy). Bolanos-Meade et al. reported a high graft failure rate (46%) among 14 sickle cell disease (SCD) patients who underwent haplo-identical HSCT following nonmyeloablative conditioning regimen and PT-Cy (Luznik et al., 2008). An intensive preconditioning immunoablation followed by a myeloablative conditioning regimen and PT-Cy in the haploidentical setting for thalassaemia showed promising results with an OS and disease-free survival (DFS) of 95% and 94%, respectively (Anurathapan et al., 2016). In the most recent report (Anurathapan et al., 2020) of 83 consecutive transfusion-dependent patients with thalassaemia (median age, 12 years; range, 1 to 28 years) with a minimum follow-up of 6 months (median, 15 months; range, 7 to 53 months) the 3-year projected overall and event-free survival is over 96%, and there have been no secondary graft failures.

### ***Mixed chimaerism following HSCT for thalassaemia***

Mixed haematopoietic chimaerism (MC) is an interesting phenomenon that sometimes occurs after HSCT for thalassaemia. The incidence of MC in a study of 335 patients who received MSD HSCT for thalassaemia was 32.2% 2 months after transplantation (Lucarelli, Andreani & Angelucci, 2002). Of the 227 patients with complete donor chimaerism, none rejected their grafts, whereas graft loss occurred in 35 of 108 patients (32.4%) with MC, indicating that MC is a significant risk factor for graft rejection in thalassaemia patients. The percentage of residual host haematopoietic cells (RHCs) determined 2 months after transplant was predictive for graft rejection, with nearly all patients experiencing graft rejection when RHCs exceeded 25%. The risk of graft rejection was only 13% in patients with <10% RHCs and was 41% in patients with 10%–25% RHCs (Andreani et al., 1996, 2000). Of patients receiving HSCT for thalassaemia following myeloablative conditioning, 10% became persistent mixed chimaeras and became transfusion independent, suggesting that once donor-host tolerance is established, a limited number of engrafted donor cells might be sufficient to provide significant improvement of disease phenotype in patients with thalassaemia major.



## **Adult thalassaemia patients**

Adult thalassaemia patients generally have more advanced disease, with both disease- and treatment-related organ complications that are mainly due to prolonged exposure to iron overload consequently, worse outcomes with transplantation have been seen in adults. From 1988 to 1996, 107 patients more than 16 years of age received transplants from matched donors at the Pesaro Hospital. The median age for this population was 20 years, with a range of 17 to 35 years. The probabilities of overall survival, thalassaemia-free survival, rejection mortality and non-rejection mortality for this entire group were 66%, 62%, 4% and 37%, respectively (Lucarelli et al., 1992, 1999).

From April 1997, 15 high-risk adult patients were prepared for transplantation with a reduced total dose of 90 mg/kg cyclophosphamide. The probabilities of OS, TFS, rejection mortality and non-rejection mortality were 65%, 65%, 7% and 28%, respectively (Gaziev et al., 2005).

Encouraging results have been reported in adult thalassaemia patients who received bone marrow from matched unrelated donors, with rates of OS, TFS, transplant-related mortality (TRM) and rejection of 70%, 70%, 30% and 4%, respectively (La Nasa et al., 2005b).

## **Follow up after HSCT**

Adequate post-transplant clinical follow-up is of particular importance. Within the first year, careful monitoring of haematological and engraftment parameters, infectious complications and GvHD is essential. Appropriate immunisation is necessary in the second year, if there is no GvHD. Long-term follow-up is of particular interest with respect to monitoring the evolution of multi-system problems (iron overload, pubertal development, growth and endocrine deficiencies) related to thalassaemia. A number of reports indicate that iron overload, chronic hepatitis, cardiac function and endocrine deficiencies can be managed more easily after transplantation, sometimes permitting the healing of severely damaged organs (Muretto, Angelucci & Lucarelli, 2002). It is also important to remove excess iron after transplantation by phlebotomy (venesection) or chelation therapy. All iron removal treatments should be started only once the graft is stabilised, and the patient free from any immunosuppressive treatment or prophylaxis, and in the absence of chronic GvHD. Endocrine dysfunction and infertility require specific expertise and follow up after HSCT.

## **Cost and cost effectiveness**

Thalassaemia medical care is a complex, multidisciplinary and expensive process requiring dedicated and experienced units. An Italian study based on cost/benefit estimations from a societal perspective quantified tariffs, expenses and net earnings in 2006 for thalassaemia patients. The mean costs were €1242/patient/month, of which 55.5% was attributed to iron chelation therapy and 33.2% to transfusions (Scalone et al., 2008). These data compare to total overall median costs of HSCT from MSD of approximately 150,000 US \$, which would translate to 1,900 US \$ per expected life year after HSCT (Matthes-Martin et al., 2012). However, the cost of transplantation can vary significantly around the world. When considering the very significant combined costs of life-long blood transfusions, chelation and the management of complications for optimal thalassaemia care (which clearly exceed the healthcare resources available in most non-industrialised countries), transplantation is certainly a cost-effective option, if adequate expertise exists, even in developing countries.

## Summary and Recommendations

- HSCT should be offered to thalassaemia patients and their parents at an early age, before complications due to iron overload have developed, if an HLA identical sibling is available.
- Either bone marrow or cord blood from an HLA-identical sibling can be used.
- A matched unrelated donor can be used, provided that high compatibility criteria for both HLA class I and II loci are met.
- Haploidentical HSCT in thalassaemia can be considered in experienced HSCT centres in the context of well-designed clinical trials.
- Myeloablative conditioning regimens should always be used for standard transplantation
- Post-transplant care should include all transplant and thalassaemia-related complications.
- In thalassaemia patients, HSCT is cost-effective when compared to life-long supportive therapy.

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## CHAPTER 17

### Gene therapy for thalassaemia

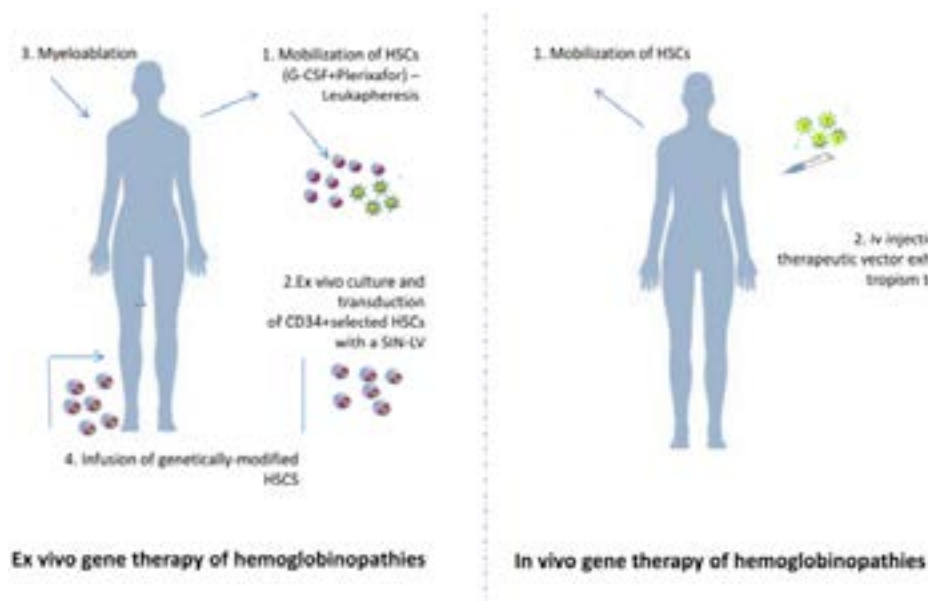
#### Introduction

Despite major advances in the treatment of thalassaemia, resulting in prolongation of the life expectancy of affected patients, a definitive cure available to all patients remained elusive until very recently. For years, the only curative treatment has been allogeneic bone marrow transplantation, limited however by its availability only to young patients with a well-matched donor (Lucarelli, Andreani and Angelucci, 2002) and the requirement for long-term immunosuppression to prevent or treat transplant-related immunological complications such as graft-vs-host-disease (GvHD) and rejection, carrying a non-negligible risk of mortality (Galanello and Origa, 2010).

Given the widespread elucidation of the genetic basis of haemoglobinopathies (Stamatoyannopoulos et al., 2001; Weatherall and Clegg, 2001), gene therapy approaches have long been envisioned by pioneers such as late Dr George Stamatoyannopoulos (Higgs, Engel and Stamatoyannopoulos, 2012). Gene therapy aspires to provide a cure for thalassaemia through the manipulation of the genome of haematopoietic stem cells, thus compensating for the inadequate or faulty function of mutated genes. This can be achieved either by gene addition via a semi-random insertion of a healthy copy of the therapeutic gene into the cells using viral vectors (Dong and Rivella, 2017) or gene editing via a precisely directed mutation that repairs the gene in situ or induces a disease-modifying effect (i.e. reactivation of Hb F synthesis) using site-specific nucleases (Porteus, 2017; Antoniani et al., 2018; Boulad et al., 2018).

**Ex vivo gene therapy for thalassaemia, either by gene addition or gene editing, is a haematopoietic stem cell (HSC) transplantation-based procedure.** The autologous (patient-derived) HSCs are mobilised with granulocyte colony-stimulating factor (G-CSF) plus plerixafor, harvested by cytopheresis, CD34+cell-enriched by immunomagnetic separation and genetically modified ex vivo (Yannaki et al., 2012; Psatha et al., 2014; Karponi et al., 2015). The patient undergoes myeloablative conditioning followed by infusion of the gene-modified cells and remains hospitalised until the haematopoietic recovery (Finotti et al., 2015; Malik, 2016; Mansilla-Soto et al., 2016; Ferrari, Cavazzana and Mavilio, 2017; Biffi, 2018; Cavazzana and Mavilio, 2018) (Figure 1, left panel).

Recently, in vivo approaches for gene therapy of thalassaemia, either by gene addition or gene editing, have also been preclinically pursued, aiming to overcome the current limitations of ex vivo gene therapy including the need for myeloablative conditioning, the need for transplantation expertise and high costs (Li et al., 2018; Wang et al., 2019) (Figure 1, right panel).



**Figure 1.** Ex vivo and in vivo gene therapy for haemoglobinopathies: the concept. G-CSF, granulocyte colony-stimulating factor; HSCs, haematopoietic stem cells; iv, intravenous; SIN-LV, self-inactivating lentiviral vectors.

## Gene therapy by gene addition

Gene therapy by gene addition, in other words, the transfer of a healthy copy of the  $\beta$  (or  $\gamma$ ) globin gene along with its important regulatory genomic elements into target-cells, using modified viral constructs as delivery vehicles (Higgs, Engel and Stamatoyannopoulos, 2012; Mansilla-Soto et al., 2016; Cavazzana, Antoniani and Miccio, 2017), has been the most developed gene therapy approach up-to-now. After many years of preclinical evaluation, self-inactivating lentiviral vectors (SIN-LVs) have proven to be the safest and most effective means of delivery (Zufferey et al., 1997; Naldini, 2011; Naldini, Trono and Verma, 2016). In contrast to  $\gamma$ -retroviral vectors favouring integration in transcription start sites (TSS) and having been associated with insertional mutagenesis in gene therapy trials of X-linked severe combined immunodeficiency (SCID) (Hacein-Bey-Abina et al., 2010; Zhang, Thrasher and Gaspar, 2013) and Wiskott–Aldrich syndrome (Boztug et al., 2010; Braun et al., 2014), SIN-LVs, which preferentially integrate into actively transcribed genes rather than TSS, have not been associated with genotoxicity so far (Montini et al., 2006, 2009; Cesana et al., 2014). Extensive integration site analysis from preclinical and clinical studies involving SIN-LVs and in various disease settings, has demonstrated polyclonal haematopoietic reconstitution patterns (Aiuti et al., 2013; Biffi et al., 2013; Thompson et al., 2018; Marktel et al., 2019). Short genomic elements termed chromatin insulators have been used (Chung, Whiteley and Felsenfeld, 1993; Cavazzana-Calvo et al., 2010) or are under evaluation (Emery et al., 2000; Liu et al., 2015), concerning their ability to act as additional safety features, shielding the transgene from the influence of its surrounding genomic environment (Rivella et al., 2000).

## Gene therapy by Gene editing

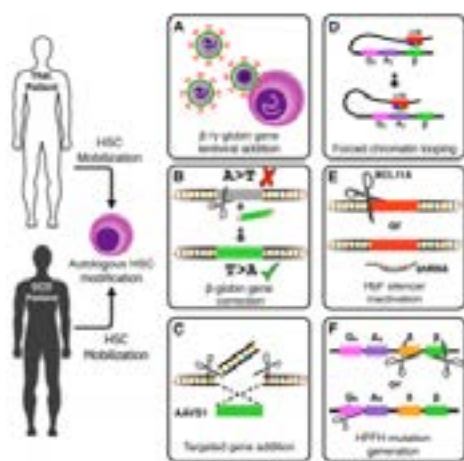
In gene editing approaches, site-specific nucleases – (zinc-finger nucleases (ZFN) (Carroll, 2011), transcription activator-like effector nucleases (TALEN) (Ma et al., 2013)



and clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) complex (Dever et al., 2016) are engineered to precisely identify specific genomic sequences where they create a double-strand break (DSB), which can subsequently be repaired by endogenous cellular repair mechanisms: i) in non-homologous end joining (NHEJ), a direct ligation of the two ends takes place, in an error-prone process that causes insertions or deletions (indels) leading to frameshift mutations and rendering the locus non-functional (i.e. gene knockout); ii) in homology directed repair (HDR), a donor DNA template is used and high fidelity, albeit substantially less efficient, repair of the DSB takes place, due to the resistance of haematopoietic stem cells to HDR; iii) another approach is the targeted integration of a therapeutic gene in a predetermined genomic 'safe harbour' that supports long-term expression without interfering with the transcriptional activity of endogenous loci (Osborn et al., 2016) (Figure 2).

Genome editing eliminates the need for semi-randomly integrating viral vectors and, reasonably, the risk of insertional mutagenesis; however, the main safety concern with this approach is the unintended 'off target' mutagenesis that could generate additional mutations in undesired genomic loci with potential unexpected consequences.

Gene editing strategies for  $\beta$  thalassaemia have primarily focused on inducing reactivation of the  $\gamma$  globin gene (Wienert et al., 2018), aiming to correct the imbalance of the  $\alpha$ -like/ $\beta$ -like globin chain ratio, the main pathophysiological cause of the disease. This can be achieved by inhibiting factors that repress the expression of the  $\gamma$  globin gene (i.e. BCL11A), thus mimicking hereditary persistence of fetal haemoglobin (HPFH) in which high levels of haemoglobin (Hb) F are maintained throughout adult life and compensate for the low levels of  $\beta$  globin expression in patients carrying thalassaemic mutations. Disrupting the binding site or the enhancer of BCL11 causes upregulation of HbF to therapeutic levels (Psatha et al., 2018). Both CRISPR/Cas9 and ZFNs are being used in ongoing gene editing clinical trials for  $\beta$  thalassaemia and sickle cell disease (SCD). Several other interventions at the molecular level that may provide a therapeutic result (e.g. RNA interference and forced chromatin looping) (Yannaki, Emery and Stamatoyannopoulos, 2010) are also being currently tested (Figure 2).



**Figure 2.**

Therapeutic approaches involving genetic modification of HSCs in patients with haemoglobinopathies. a) LV addition of a therapeutic  $\beta$ - or  $\gamma$ -globin gene into HSCs b) Mutation correction by a nuclease-template genome editing method, c) Targeted integration of the therapeutic gene into a 'safe harbour locus' (AAVS1), by a site-specific nuclease and a DNA template, d) Forced chromatin looping reactivating the endogenous HbF through Ldb1-specific binding on the  $\gamma$ -globin locus, e) BCL11A enhancer inactivation increasing HbF by either nuclease- or shRNA-mediated knockdown, f) Introduction of deletional or non-deletional HPFH mutations by genome editing. Adapted from (Psatha, Papayanni and Yannaki, 2018), HPFH, hereditary persistence of fetal haemoglobin; HSCs, haematopoietic stem cells; LV, lentivirus

## Gene therapy clinical trials

### A. Gene addition

#### **Bluebird bio trials**

The first clinical trial phase I/II was conducted in 2007, in Paris, using myeloablative conditioning (busulfan 14 mg/kg) and a SIN-LV (HPV569, developed by Ph. Leboulch) flanked with the cHS4 insulator and encoding a  $\beta$  globin gene with antisickling properties (T87Q). One treated patient with compound  $\beta^E/\beta^0$  thalassaemia became transfusion independent 12 months later, after a dominant clone bearing an integration site near the HMGA2 gene emerged, contributing to a third of the total haemoglobin with the rest consisting of one third of endogenous HbE and one third of HbF (Cavazzana-Calvo et al., 2010). Fortunately, this clone subsided over time without causing oncogenesis (Cavazzana, Antoniani and Miccio, 2017). Subsequently, Bluebird bio modified the vector by removing the cHS4 insulator and replacing the 5'LTR with a cytomegalovirus (CMV) promoter (vector BB305). Further clinical phase I/II studies were initiated including adults and adolescents with  $\beta$  thalassaemia (Northstar HGB-204), SCD (Northstar HGB-206, still ongoing) or either (Northstar HGB-205). In HGB-204 and HGB-205 trials (Mansilla-Soto et al., 2016), 15 of 22 patients with  $\beta$  thalassaemia became transfusion independent whereas seven had a median 73% reduction (range 19-100%) in transfusion requirements. The majority of patients (6/7) in whom best response was reduction in the number or/and the volume of transfusions belonged to the  $\beta^0$  genotype. In contrast, transfusion independence was achieved predominantly in patients having a non- $\beta^0/\beta^0$  genotype (12/13) over those carrying  $\beta^0/\beta^0$  / IVSI-110 or 2 copies of IVSI-110 mutations (3/9). Lower vector copy number in the drug product was associated with lower therapeutic benefit in  $\beta^0$  patients, suggesting that improved transductions can help in achieving transfusion idendency in these patients. (Thompson et al., 2018). A subsequent transduction refinement was introduced in the manufacturing process of the phase III Northstar-2 HGB-207 (non- $\beta^0/\beta^0$  genotypes) and HGB-212 ( $\beta^0/\beta^0$  genotypes or double IVSI-110 mutations) clinical trials, in which enrolment of 23 and 15 patients, respectively, was anticipated in USA, France, Germany, Italy, Thailand, UK and Greece.

Recent data on HGB212 and HGB207, as well as data from LTF-303, the long-term follow up study of patients having completed 2 years follow up in the parental studies, have been published recently. A total of 41 patients across all age groups (kids, adolescents and adults) and spanning a broad spectrum of TDT severity genotypes ( $\beta^+/\beta^+$ ,  $\beta^+/\beta^0$ ,  $\beta^0/\beta^0$ ,  $\beta^0$ /IVSI-110 or 2 copies of IVSI-110 mutations) have been treated with beti-cel in the two Phase III clinical studies (Locatelli et al., 2021). Transfusion independence (TI) (defined as weighted average Hb  $\geq$  9 g/dl without any pRBC transfusions for  $\geq$  12 months) was achieved in 89% (32/36) of evaluable patients. All patients who achieved TI maintained TI with median duration of ongoing TI 25 months (min-max: 12.5 – 38.5 months). Median weighted average Hb during TI was 11.6 (min-max: 9.3 – 13.7) g/dl, mainly driven by HbAT87Q (the globin vector-derived Hb). Biomarkers of ineffective erythropoiesis trended towards normal over time in patients who achieved TI. The patients who complete Phase I/II and Phase III studies are invited to enroll in a 13-year long term follow up study, LTF-303 (Yannaki et al., 2021a). As of March 2021, 51 patients have been enrolled in the long-term follow up study, LTF-303 with a median follow-up of 44.2 months (min – max: 22.9 – 86.5 months) after beti-cel infusion. All TI patients maintained TI in LTF-303 with up to 7 years of follow-up. Among all patients treated with beti-cel, the safety profile was consistent with the known effects of mobilization with G-CSF and plerixafor and myeloablation with single-agent busulfan. No graft failure, GVHD, or deaths occurred. No vector-mediated replication-competent

lentivirus, insertional oncogenesis or malignancy were detected (Locatelli et al., 2021; Yannaki et al., 2021a).

### ***San Raffaele-TIGET***

This phase I/II clinical trial was conducted in Milan and recruited 9 patients, stratified into three age groups (three adults, three adolescents, three young children). Interestingly, a myeloablative, but reduced-toxicity conditioning (treosulfan–thiotepa) was administered and the  $\beta$  globin SIN-LV (GLOBE)-transduced cells were infused intra-osseously. Four of the total six paediatric patients became transfusion independent whereas all three adults demonstrated significant reductions (33-80%) in – but not independence from – transfusions. Integration site analysis revealed highly polyclonal haematopoietic reconstitution without emergence of clonal expansion (Marktel et al., 2019).

### ***Other gene addition trials for thalassaemia***

A phase I/II clinical trial was conducted in MSKCC, New York, in 2012 with the TNS9.3.55  $\beta$  globin vector. The use of partial myeloablation (8 mg/kg busulfan) in three of four enrolled patients, provided stable engraftment but low in vivo VCN, thus resulting, at best, in reduction in transfusion requirements in one patient (Boulad et al., 2014, 2018). Those data highlighted the importance of the depth of myeloablation to obtain clinically meaningful outcomes in thalassaemia gene therapy with the currently existing globin vectors.

## ***B. Gene editing trials***

**CRISPR Therapeutics** uses the CRISPR/Cas9 technology for HbF reactivation in two phase I/II clinical trials for  $\beta$  thalassaemia (CTX001-111) and SCD (CTX001-121), recruiting 45 patients each, aged 18-35 years. A putative  $\gamma$ - $\delta$  intergenic HbF silencer, binding site of BCL11A, is targeted and disrupted by CRISPR/Cas9 (Antoniani et al., 2018). One  $\beta$  thalassaemia patient has been treated and reported transfusion-free for over 4 months post-treatment.

**Sangamo** in another clinical trial (Thales), exploits ZFNs to disrupt the BCL11A erythroid enhancer. The study anticipates the enrolment of six patients with  $\beta$  thalassaemia (Holmes et al., 2018), one of whom has been reported transfusion-free for 5 weeks post-treatment with a significant increase in HbF (31%) and stable total haemoglobin levels (~90 g/l).

### ***The long-term safety of gene therapy***

Patients with haemoglobinopathies undergoing gene therapy by gene addition or gene editing need to be prospectively evaluated for at least 15 years, so as both the sustainability of clinical benefit and the long-term safety be addressed. Such patients maybe at an increased risk of developing malignancies for several reasons; first, host factors generated by the disease background or/and treatment, as in SCD, the chronic hypoxia and inflammation, clonal hematopoiesis of indeterminate potential (CHIP)-related mutations (Ghannam et al., 2020) and chronic exposure to hydroxyurea or as in thalassaemia solid organ hemosiderosis, may create a permissive environment for the development of malignancies. Second, the transplant-procedure itself, either the conditioning regimen (myeloablative or non-myeloablative) or/and a low engraftment of gene-corrected HSCs allowing for clonal proliferation of the endogenous cells, constitute additional risk factors for oncogenesis. Finally, gene therapy by gene addition is associated with a low, but existing risk of insertional mutagenesis while gene therapy by gene editing has the potential to cause double strand breaks at other than the de-

sired locations (“off-target” effects).

Up to date, in the Bluebird Bio trials with the longest follow up, there have been 3 SCD patients who have developed myeloid malignancies following gene therapy by gene addition. The malignancies developed in the HGB-206 trial, at 3 and 5.5 years (MDS progressing to AML and AML, respectively) or 6 months (MDS) post gene therapy. In the first case of MDS that subsequently progressed to acute leukemia, the investigation demonstrated that the event was not gene therapy-related due to absence of vector integration in the CD34+ blast cells (Hsieh et al., 2020). In the recent case of AML, investigation has demonstrated that the vector integrated within the vesicular-associated membrane protein 4 (VAMP4) gene which has no known role in the development of malignancies whereas the patient presented several well-known genetic mutations and chromosomal abnormalities commonly observed in AML. In the early onset post-gene therapy MDS event, the diagnosis was based on trisomy 8, an MDS-related cytogenetic abnormality, but no blast cells were seen in the bone marrow and the patient is followed for the true impact of this abnormality.

Data so far suggest that in SCD patients who developed hematologic malignancies, it is unlikely the integration site of BB305 lentiviral vector to have played a role and more likely malignancies have been triggered by host factors and been exacerbated by the busulfan conditioning probably in combination with the chronic use of Hydroxyurea. Such risks may not be directly relevant to thalassaemia patients; indeed there is accumulating evidence that SCD patients may be more prone to the development of myeloid malignancies (Brunson et al., 2017).

Gene editing clinical studies are still in their beginning and longer follow-up in this setting also, will address the safety and the sustainability of the clinical effect.

### ***Regulatory approval and marketing authorisation***

Based on the successful clinical data in Bluebird bio's clinical trials, the European Commission granted conditional approval, in June 2019, for the Bluebird bio gene therapy product (autologous CD34+ cells encoding the  $\beta$  A-T87Q-globin gene) under the commercial name Zynteglo®, to treat transfusion-dependent  $\beta^+$  thalassaemia in patients 12 years and older, who are eligible for stem cell transplantation but do not have a matched related donor.

The recent marketing authorization of Zynteglo® and also other gene therapy products in different disease settings, with burdensome price tags has economically challenged the health system payers – even in higher income countries – who are tasked to pay a large price once, instead of paying smaller amounts repeatedly for a life-time. To encourage patient access to such highly expensive genetic treatments, new models of payment have been proposed by the stakeholders. Bluebird bio, on an outcome-based model of payment, suggested that the total cost of Zynteglo® is only realized if the patient achieves transfusion independence and maintains it through 5 years. After the first payment, subsequent payments (five annual payments in total) are made only if the patient remains transfusion independent.

Nevertheless, it remains to be shown in the long run and on the basis of the percentage of patients becoming transfusion independent, developing a thalassaemia intermedia phenotype or failing to respond, whether indeed, such an one-time expense will be cost-effective overall, compared to the cumulative costs of life-long conventional treatment of the disease and its complications.

***The current challenge of applying gene therapy***

After almost 4 decades of strenuous research on haemoglobinopathies, the long-awaited gene therapy is entering the realm of clinical practice. With one gene therapy product already officially approved for  $\beta$  thalassaemia in Europe and more under development with promising and encouraging results in clinical trials so far, the future looks brighter than ever (Yannaki, et al., 2021b). However, new challenges emerge for the thalassaemia community globally, including the costs of application of these expensive therapies on a massive scale, selection of patients clinically capable of undergoing such procedures, global access to the treatment and need for transplantation expertise to administer these sophisticated cellular treatments.

Moreover, the striking imbalance between developed and developing countries, both in terms of patient demographics (i.e. large populations of young patients in low-income countries and of adult, aged patients in high-income countries) and financial potential of health systems, makes the situation an even more complicated conundrum. **To enable global patient access to such transformative therapies, there is an urgent need for drastic changes in market, access policies for gene therapy products, further supported by the recent decision of Bluebird Bio to wind down their operations in Europe and focus on the US market due to failure of finding a financial platform for Zynteglo<sup>®</sup>, acceptable to both payers and the company. Close collaboration among all stakeholders involved (healthcare professionals, patients, the pharmaceutical industry, policy makers and healthcare providers) in making such scientific advances accessible and available to the patients, is urgent and mandatory.**

## Summary

The new era in the treatment of haemoglobin disorders provides patients with more than one therapeutic option (Psatha et al., 2018). Among the novel gene therapies, lentiviral vector gene therapy is the most mature intervention, shown to provide clinical efficacy and safety (Locatelli et al., 2021; Yannaki et al., 2021a) as an one-off, life-changing treatment. Nevertheless, the long-term safety and sustainability of the response needs also to be demonstrated; thus treated patients are followed in follow up trials lasting overall 15 years. Precision medicine with genome editing tools has enabled overcoming some of the obstacles associated with gene addition gene therapy; however, clinical experience with gene editing is currently limited and more clinical data and large scale trials are needed to demonstrate that gene editing is a potential safe and curative treatment for haemoglobinopathies.

While waiting for the long-term clinical data on gene therapy for  $\beta$  thalassaemia, currently, and on the basis of existing indications, patients with  $\beta$  thalassaemia major have potentially the following options for treatment

- i) allogeneic HSC transplantation: young patients ( $\leq 17$  years old) with a  $\beta^+$  or  $\beta^0$  genotype having an HLA-compatible sibling or a 10/10 matched volunteer donor
- ii) gene therapy with Zynteglo<sup>®</sup>: young patients ( $>12 <17$  years old) with a  $\beta^+$  genotype who do not have an HLA-compatible sibling donor
- iii) gene therapy with Zynteglo<sup>®</sup>: patients  $>17 \leq 55$  years old with a  $\beta^+$  genotype who do not have severe comorbidities and are at risk or ineligible to undergo an allo-HSC transplant but can otherwise undergo an autologous gene therapy procedure with an acceptable risk.

The recent successful clinical data of patients with  $\beta^0$  genotypes created optimism for the approval of Zynteglo<sup>®</sup> for  $\beta^0$  patients also. Unfortunately, the current situation with Bluebird Bio leaving Europe, places the thalassaemia community in uncertainty and suspense as regards a widely applicable thalassaemia gene therapy. Until things change, only allogeneic HSC transplantation remains an indicated and available treatment.

**Table 1.** Gene therapy and gene editing clinical trials for  $\beta$ -thalassaemia. TDT: transfusion dependent thalassaemia; PK pharmacokinetics; hHSPCs: Human Hematopoietic Stem and Progenitor Cells; ZFN: zinc finger nuclease <sup>§</sup>as of March 3, 2021 from ClinicalTrials.gov

Trial identifier	Phase/ Status	Enrolled Patients	Conditioning	Drug product	Sponsor/ Center
NCT01639690	Phase 1 Active, not recruiting <sup>§</sup>	TDT ≥18 years of age Estimated enrollment: 10 participants	Busulfan	TNS9.3.55: Lentiviral Vector Encoding the Normal Human $\beta$ -Globin Gene	Memorial Sloan Kettering Cancer Center New York, New York, United States
NCT02453477	Phase 1/2 Active, not recruiting <sup>§</sup>	TDT 10 (3 adults, 7 children)	Treosulfan and Thiotepa	Autolo- gous HSCs genetically modified with GLOBE len- tiviral vector encoding for the human $\beta$ -globin gene	IRCCS San Raffaele Fondazione Telethon Institute for Gene Therapy (SR-TIGET), Milan, Italy
NCT01745120 (HGB-204)	Phase 1/2 Completed <sup>§</sup>	TDT 18 (between 12 and 35 years of age)	Busulfan	Autologous HSCs trans- duced with LentiGlobin BB305 lenti- viral vector encoding the human $\beta$ A- T87Q-globin gene	Bluebird bio
NCT02151526 (HGB-205)	Phase 1/2 Completed <sup>§</sup>	TDT 4 (between 5 and 35 years of age)	Busulfan (adjusted based on daily PK monitoring)	Autologous HSCs trans- duced with LentiGlobin BB305 lenti- viral vector encoding the human $\beta$ A- T87Q-globin gene	Bluebird bio

Trial identifier	Phase/ Status	Enrolled Patients	Conditioning	Drug product	Sponsor/ Center
NCT02906202 (HGB 207)	Phase 3 Active, not recruiting <sup>§</sup>	TDT non $\beta^0/\beta^0$ , $\leq 50$ years of age Estimated enrollment: 23 participants	Busulfan	Autologous HSCs trans- duced with LentiGlobin BB305 lenti- viral vector encoding the human $\beta$ A- T87Q-globin gene	Bluebird bio
NCT03207009 (HGB 212)	Phase 3 Active, not recruiting <sup>§</sup>	TDT $\beta^0/\beta^0$ , $\beta^0$ /IVS-I-110, or IVS-I-110/IVS- I-110 $\leq 50$ years of age Estimated enrollment: 18 participants	Busulfan	Autologous HSCs trans- duced with LentiGlobin BB305 lentiviral vector encoding the human $\beta$ A- T87Q-globin gene	Bluebird bio
NCT03432364	Phase 1/2 Active, not recruiting <sup>§</sup>	TDT $\geq 18$ and $\leq 40$ years of age Estimated enrollment:	Busulfan	ST-400: autologous CD34+ HSPCs genetically modified with ZFN technology at the erythroid- specific enhancer of the BCL11A gene	Sangamo Therapeutics
NCT03655678	Phase 1/2 Recruiting <sup>§</sup>	TDT 12-35 years, eli- gible for HSCT but no matched sibling donor Estimated enrollment: 45 participants	Busulfan	CTX001: autologous CD34+ hHSPCs modified with CRISPR-Cas9 at the erythroid lineage- specific enhancer of the BCL11A gene	Vertex Phar- maceuticals Incorporated



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## CHAPTER 18

### Novel and Emerging Therapies – Pharmaceutical approaches

An improved understanding of the pathophysiology of  $\beta$  thalassaemia has helped optimise disease management, and has paved the way for the development of novel therapies (Bou-Fakhredin et al., 2020; Motta et al., 2020). These can be classified into three major categories based on their efforts to address different aspects of the underlying pathophysiology of  $\beta$  thalassaemia: (i) correction of the  $\alpha/\beta$  globin chain imbalance (already covered in the dedicated 'Gene Therapy' chapter), (ii) targeting ineffective erythropoiesis, and (iii) iron dysregulation. In this chapter focus will be given on (ii) and (iii).

#### Targeting Ineffective Erythropoiesis and Iron Dysregulation

Many preclinical studies have provided evidence on the role of **ruxolitinib** (JAK1/JAK2 inhibitor) as a potential target to improve ineffective erythropoiesis. The inhibition of JAK2 in TDT and non-transfusion-dependent thalassaemia (NTDT) mouse models was shown to not only improve ineffective erythropoiesis but also to decrease splenomegaly (Casu et al., 2018). A phase 2a study assessed the efficacy and safety of ruxolitinib in TDT patients with spleen enlargement. Ruxolitinib was overall well tolerated in this study population, and the safety profile was consistent with the previous reports. However, because the major purpose of reducing spleen size in patients with TDT is to improve pre-transfusion haemoglobin and related reduction in transfusion needs where ruxolitinib had shown a limited effect, no further studies were conducted.

**Sotatercept** or ACE-011 has also been shown to correct ineffective erythropoiesis by acting as a ligand trap to inhibit negative regulators of late-stage erythropoiesis in the transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily. A phase 2 study conducted on 16 TDT patients showed that the majority of TDT patients (66%) treated with higher doses of sotatercept (0.75-1.0 mg/kg) achieved reductions of  $\geq 33\%$  in red cell transfusion requirements. The increase in haemoglobin and reduction in red cell transfusion correlated with increased serum exposure to sotatercept (Cappellini et al., 2019). Sotatercept exhibited an overall good safety profile and was tolerated by most patients. Treatment discontinuation due to adverse events was rare, and the incidence of grade 3-4 adverse events was low. A decision, however, was made not to advance trials of sotatercept in  $\beta$  thalassaemia due to binding of sotatercept to activin A.

Preliminary data with sotatercept led to the initiation of similar trials in TDT patients using luspatercept. **Luspatercept** or ACE-536 is a recombinant fusion protein that binds to specific ligands of the TGF- $\beta$  superfamily and enhances erythroid maturation. It is the most recently approved therapy (FDA and EMA) for the management of TDT. Pre-clinical data on murine models showed that treatment with RAP-536 reduced  $\alpha$  globin chain aggregation and haemolysis, while increasing erythrocyte life span and improving iron overload (Suragani et al., 2014b). Additionally, RAP-536 increased haemoglobin concentration and red cell count (RBC), and reduced comorbidities associated with  $\beta$  thalassaemia, such as decreased bone mineral density and splenomegaly (Suragani et al., 2014a). In the phase 1 study, 32 healthy volunteers were randomized 3:1 to receive 2 doses of luspatercept (0.0625–0.25 mg/kg) or placebo subcutaneously every 2 weeks (ClinicalTrials.gov number NCT01432717). Luspatercept was well-tolerated and dose-dependent and increases in haemoglobin concentration and RBC were observed after the first dose (Attie et al., 2014). A phase 2, open-label, non-

randomised, uncontrolled, dose-finding study was then conducted to evaluate the effects of luspatercept in  $\beta$  thalassaemia patients. The study enrolled 33 NTDT patients and 31 TDT patients (ClinicalTrials.gov number NCT01749540) (Piga et al., 2019). Luspatercept was administered subcutaneously every 21 days (0.2–1.25 mg/kg) in dose escalation and expansion cohorts. The primary endpoint of mean increase in haemoglobin concentration from baseline of  $\geq 15$  g/l for  $\geq 2$  weeks (in the absence of red cell transfusions) was achieved by 58% (95% confidence interval [CI] 39.1 to 75.5) of NTDT patients receiving the higher dose range of luspatercept (0.6–1.25 mg/kg). In TDT patients, the primary endpoint of a transfusion-burden reduction of  $\geq 20\%$  over any 12 weeks vs baseline was achieved by 81% (95% CI 63.6, 92.8) of patients receiving the higher dose range of luspatercept. These findings, including the achievement of secondary endpoints, prompted a randomised Phase 3 clinical trial (BELIEVE Trial) to assess efficacy and safety. It is the most recently approved therapy [Food and Drug Administration (FDA) and European Medicines Agency (EMA)] for the management of TDT.

**The approval of luspatercept was based on the results the BELIEVE trial**, a phase 3, randomised, double-blind, placebo-controlled trial which showed that subcutaneous administration of luspatercept ( $n=224$ ) at doses of 1–1.25 mg/kg led to a reduction in the transfusion burden of at least 33% from baseline during weeks 13 through 24. Moreover, a reduction of at least 2 red-cell units over this 12-week interval was significantly greater in the luspatercept group than in the placebo group (21.4% vs. 4.5%) (Cappellini et al., 2020b). During any 12-week interval, the percentage of patients who had a reduction in transfusion burden of at least 33% was greater in the luspatercept group than in the placebo group (70.5% vs. 29.5%), as was the percentage of those who had a reduction of at least 50% (40.2% vs. 6.3%). Parallel reductions in serum ferritin levels were also observed. Adverse events more commonly seen in the luspatercept group compared to placebo included bone pain, arthralgia, dizziness, hypertension and hyperuricaemia. Data on the long-term use of luspatercept, its real-life application and its use in the paediatric population are awaited.

The effects of luspatercept on iron loading over time and the impact of baseline iron parameters on response to luspatercept was also evaluated and showed that luspatercept treatment resulted in clinically meaningful and maintained reductions in serum ferritin levels (Porter et al., 2019). Baseline iron overload did not seem to affect response rates with luspatercept. Treatment with luspatercept resulted in clinically meaningful reductions in red cell transfusion burden regardless of baseline serum ferritin level. There was a trend for decrease in liver iron concentration (LIC) with longer follow up at 96 weeks; among responders, the decrease was more pronounced compared with non-responders (Porter et al., 2019). In a sub-analysis on long-term efficacy and safety of luspatercept, it was shown that luspatercept treatment was associated with prolonged periods of clinically meaningful reductions in transfusion burden, including in patients who crossed over from the placebo arm (Taher et al., 2020a). The safety profile of crossover patients was consistent with that reported in the luspatercept arm. Another sub-analysis study explored the association between  $\beta$  globin genotype and response to luspatercept in adult patients with  $\beta$  thalassaemia in the BELIEVE trial (Cappellini et al., 2020a). It was found that although response rates were lower in patients with the most severe disease ( $\beta^0/\beta^0$ ), clinically meaningful reductions in transfusion burden were observed across all genotypes also (Cappellini et al., 2020a). Other agents targeting ineffective erythropoiesis and iron dysregulation also exist.

These include VIT-2763 (an oral ferroportin inhibitor), TMPRSS6-LRx (anti-sense oligonucleotides downregulating TMPRSS6), and Mitapivat (AG-348) (an oral, small molecule, allosteric activator of the red cell-specific form of pyruvate kinase). Ongoing clinical trials on the use of these agents are currently underway in NTDT patients only.

## Summary and Recommendations

Luspatercept, now known as Reblozyl®, after official authorisation by EMA (European Medicines Agency) and FDA (Food and Drug Administration), seems to be quite promising, with significant improvement in haemoglobin levels and reduction in transfusion requirements. Moreover, this reduction in transfusion burden decreases ongoing iron intake and, thus, the iron-chelation therapy requirements. The reduction in the serum ferritin level observed with luspatercept also suggests favourable early effects on iron balance. This could be due to improved iron utilisation (by reducing ineffective erythropoiesis and promoting red cell production), reduced transfusional iron intake augmenting the efficiency of iron-chelation therapy, or both (Suragani et al., 2014b, 2014a). Patients with TDT living in resource poor areas with limited access to regular and safe blood transfusions as well as those patients who were previously transfusion independent and are currently under transfusion are likely to be the ones to benefit considerably from this drug.

In conclusion, several novel therapeutic approaches are currently under development for TDT patients, with the aim of improving outcomes in thalassaemia care and cure and overall quality of life. Once the efficacy and safety of all these novel therapies are established, long-term, head-to-head, and comparison trials are necessary to determine the optimal management of TDT patients, which is becoming more and more personalised. Moreover, the optimal use of these novel therapies on their own or in combination with other conventional therapeutic modalities also warrants future clinical studies. Healthcare systems all over the world, with the support of treating physicians, health academia, health economists, industry, other relevant stakeholders and importantly the patients, will need to work very closely to identify those tools and solutions that will make these novel therapies accessible and available, as far as possible, to patients.

### Basic recommendations regarding the use of Reblozyl®

- Reblozyl® can be considered for:
  - Patients who require regular red blood cell transfusions
  - ≥18 years of age
- The recommended starting dose is 1 mg/kg once every 3 weeks by subcutaneous injection
- If the pre-dose haemoglobin level is  $\geq 115$  g/l and is not influenced by recent transfusion, consider delaying dosing of Reblozyl® until the level is  $\leq 110$  g/l
- Before administration of Reblozyl® haemoglobin level, and liver function tests including alanine transferase and aspartate transferase levels should be monitored to ensure proper dosing and metabolism of the medication.
- Blood pressure must be monitored prior to each administration of luspatercept.

- If a TDT patient does not achieve a reduction in red cell transfusion burden after at least 2 consecutive doses (6 weeks) at the 1 mg/kg starting dose, increase the Reblozyl® dose to 1.25 mg.
- If a patient experienced a response followed by a lack of or lost response to Reblozyl®, consider initiating a search for causative factors
- Reblozyl® should be discontinued if a patient does not experience a decrease in transfusion burden after 9 weeks of treatment (administration of 3 doses) at the maximum dose level or if unacceptable toxicity occurs at anytime. However, recent data by Piga et.al., has showed that these patients who did not respond to Reblozyl® treatment received clinical benefit from continuing therapy through week 48. Initial data from the 5-year open label extension phase of the BELIEVE trial showed that luspatercept was associated with sustained reductions in RBC transfusion units and visits in responders and non-responders during the first 48 weeks vs placebo. Moreover, patients on luspatercept continued to experience sustained reductions in transfusion burden and events over 2 years of therapy (Taher et al, 2020b). Further data from the long-term study is awaited, however.
- As thromboembolic events were reported in 8/223 (3.6%) of Reblozyl® -treated patients, it is important to monitor any TDT patient receiving this drug for signs and symptoms of thromboembolic events and initiate treatment accordingly.
- Hypertension was reported in 10.7% (61/571) of Reblozyl® -treated patients. It is therefore recommended that blood pressure be monitored prior to each administration.
- Reblozyl® may cause foetal harm. While no data are currently available on its use in pregnant women, all pregnant women should be advised of the potential risk to a foetus.
- Safety and efficacy of Reblozyl® in paediatric patients has not yet been established. Its use in paediatric patients is therefore not currently recommended.

For more details regarding Reblozyl®, the treating doctor can refer to the EMA (European Medicines Agency - <https://www.ema.europa.eu/en>) and/or the FDA (Food and Drug Administration - <https://www.fda.gov>) summaries of product characteristics. For facilitation purposes, some extracts selected from these summaries, can be found in ANNEX I.



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ANNEX I

Product characteristics of Reblozyl®. Extracts from EMA (European Medicines Agency) and the FDA (Food and Drug Administration) summaries.

NAME OF THE MEDICINAL PRODUCT

Reblozyl 25 mg powder for solution for injection Reblozyl 75 mg powder for solution for injection (EMA)

Table 5: Reconstitution Volumes

Vial Size	Amount of Sterile Water for Injection, USP required for reconstitution	Final Concentration	Deliverable Volume
25 mg vial	0.68 mL	25 mg/0.5 mL	0.5 mL
75 mg vial	1.6 mL	75 mg/1.5 mL (50 mg/mL)	1.5 mL

(FDA)

- This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions (EMA)

POSODOLOGY

Prior to each Reblozyl administration, the haemoglobin (Hb) level of patients should be assessed. In case of a red blood cell (RBC) transfusion occurring prior to dosing, the pre-transfusion Hb level must be considered for dosing purposes. (EMA)

**Table 1: Beta Thalassemia - REBLOZYL Dose Titration for Response**

	<b>REBLOZYL Dosing Recommendation*</b>
<b>Starting Dose</b>	<ul style="list-style-type: none"> <li>1 mg/kg every 3 weeks</li> </ul>
<b>Dose Increases for Insufficient Response at Initiation of Treatment</b>	
No reduction in RBC transfusion burden after at least 2 consecutive doses (6 weeks) at the 1 mg/kg starting dose	<ul style="list-style-type: none"> <li>Increase the dose to 1.25 mg/kg every 3 weeks</li> </ul>
No reduction in RBC transfusion burden after 3 consecutive doses (9 weeks) at 1.25 mg/kg	<ul style="list-style-type: none"> <li>Discontinue treatment</li> </ul>
<b>Dose Modifications for Predose Hemoglobin Levels or Rapid Hemoglobin Rise</b>	
Predose hemoglobin is greater than or equal to 11.5 g/dL in the absence of transfusions	<ul style="list-style-type: none"> <li>Interrupt treatment</li> <li>Restart when the hemoglobin is no more than 11 g/dL</li> </ul>
Increase in hemoglobin greater than 2 g/dL within 3 weeks in the absence of transfusions and <ul style="list-style-type: none"> <li>current dose is 1.25 mg/kg</li> <li>current dose is 1 mg/kg</li> <li>current dose is 0.8 mg/kg</li> <li>current dose is 0.6 mg/kg</li> </ul>	<ul style="list-style-type: none"> <li>Reduce dose to 1 mg/kg</li> <li>Reduce dose to 0.8 mg/kg</li> <li>Reduce dose to 0.6 mg/kg</li> <li>Discontinue treatment</li> </ul>

\* Do not increase the dose if the patient is experiencing an adverse reaction as described in Table 2.

(FDA)

**Table 2: Beta Thalassemia - REBLOZYL Dosing Modifications for Adverse Reactions**

	<b>REBLOZYL Dosing Recommendation*</b>
Grade 3 or 4 hypersensitivity reactions	<ul style="list-style-type: none"> <li>Discontinue treatment</li> </ul>

Other Grade 3 or 4 adverse reactions

- Interrupt treatment
- Restart when the adverse reaction resolves to no more than Grade 1

\*Grade 1 is mild, Grade 2 is moderate, Grade 3 is severe, and Grade 4 is life-threatening.

(FDA)

**Missed doses**

In case of a missed or delayed scheduled treatment administration, the patient should be administered Reblozyl as soon as possible and dosing continued as prescribed with at least 3 weeks between doses. (EMA)

**Patients experiencing a loss of response**

If patients experience a loss of response to Reblozyl, causative factors (e.g. a bleeding event) should be assessed. If typical causes for a loss of haematological response are excluded, dose increase should be considered as described above for the respective indication being treated (EMA)

**Discontinuation**

Reblozyl should be discontinued if patients do not experience a reduction in transfusion burden after 9 weeks of treatment (3 doses) at the maximum dose level if no alternative explanations for response failure are found (e.g. bleeding, surgery, other concomitant illnesses) or if unacceptable toxicity occurs at any time. (EMA)

**WARNINGS AND PRECAUTIONS (FDA)****5.1 Thrombosis/Thromboembolism**

In adult patients with beta thalassemia, thromboembolic events (TEE) were reported in 8/223

(3.6%) REBLOZYL-treated patients. Reported TEEs included deep vein thromboses, pulmonary embolus, portal vein thrombosis, and ischemic strokes. Patients with known risk factors for

Reference ID: 4586176 8 thromboembolism, e.g. splenectomy or concomitant use of hormone replacement therapy, may be at further increased risk of thromboembolic conditions. Consider thromboprophylaxis in patients with beta thalassemia at increased risk of TEE. Monitor patients receiving REBLOZYL for signs and symptoms of thromboembolic events and institute treatment promptly.

**5.2 Hypertension**

Hypertension was reported in 10.7% (61/571) of REBLOZYL-treated patients. Across clinical studies, the incidence of grade 3-4 hypertension ranged from 1.8% to 8.6%. In adult patients with beta thalassemia with normal baseline blood pressure, 13 (6.2%) patients developed systolic blood pressure (SBP)  $\geq 130$  mm Hg and 33 (16.6%) patients developed diastolic blood pressure (DBP)  $\geq 80$  mm Hg. In adult patients with MDS with normal baseline blood pressure, 26 (29.9%) patients developed SBP  $\geq 130$  mm Hg and 23 (16.4%) patients developed DBP  $\geq 80$  mm Hg.

Monitor blood pressure prior to each administration. Manage new-onset hypertension or exacerbations of preexisting hypertension using anti-hypertensive agents.

**5.3 Embryo-Fetal Toxicity**

Based on findings from animal reproductive studies, Reblozyl® may cause fetal harm when administered to a pregnant woman. In animal reproduction studies, administration of luspatercept-aamt to pregnant rats and rabbits during organogenesis resulted in adverse developmental outcomes including increased embryo-fetal mortality, alterations to growth, and structural abnormalities at exposures (based on area under the curve [AUC]) above those occurring at the maximum recommended human dose (MRHD) of 1.75 mg/kg. Advise pregnant women of the potential risk to a fetus. Advise

females of reproductive potential to use an effective method of contraception during treatment with REBLOZYL and for at least 3 months after the final dose [see Use in Specific Populations (8.1, 8.3)].

### Tabulated list of adverse reactions

The highest frequency for each adverse reaction that was observed and reported in the two pivotal studies in MDS and  $\beta$ -thalassaemia is shown in Table 3 below. The adverse reactions are listed below by body system organ class and preferred term. Frequencies are defined as: very common ( $\geq 1/10$ ), common ( $\geq 1/100$  to  $< 1/10$ ), uncommon ( $\geq 1/1,000$  to  $< 1/100$ ), rare ( $\geq 1/10,000$  to  $< 1/1,000$ ) and very rare ( $< 1/10,000$ ). (EMA)

**Table 3. Adverse drug reactions (ADRs) in patients treated with Reblozyl for MDS and  $\beta$ -thalassaemia**

System organ class	Preferred term	Frequency (all grades) for MDS	Frequency (all grades) for $\beta$ -thalassaemia
Infections and infestations	bronchitis	Very common	Common
	urinary tract infection	Very common	Common
	upper respiratory tract infection	Common	Very common
	influenza	Common	Common
Immune system disorders	hypersensitivity*	Common	Common
Metabolism and nutrition disorders	hyperuricaemia	Common	Common
Nervous system disorders	dizziness	Very common	Very common
	headache	Very common	Very common
	syncope/pre-syncope	Common	Common
Ear and labyrinth disorders	vertigo/vertigo positional	Common	Common
Vascular disorders	hypertension <sup>†</sup>	Common	Common
	thromboembolic events <sup>‡</sup>	Common	Common
Respiratory, thoracic and mediastinal disorders	dyspnoea	Very common	Common
Gastrointestinal disorders	diarrhoea	Very common	Very common
	nausea	Very common	Common
Musculoskeletal and connective tissue disorders	back pain	Very common	Very common
	arthralgia	Common	Very common
	bone pain	Common	Very common
General disorders and administration site conditions	fatigue	Very common	Very common
	asthenia	Very common	Common
	injection site reactions <sup>§</sup>	Common	Common

\* Hypersensitivity includes eyelid oedema, drug hypersensitivity, swelling face, periorbital oedema, face oedema, angioedema, lip swelling, drug eruption.

<sup>†</sup> Hypertension reaction includes essential hypertension, hypertension and hypertensive crisis.

<sup>‡</sup> Injection site reactions include injection site erythema, injection site pruritus, injection site swelling and injection site rash.

<sup>§</sup> Thromboembolic events include deep vein thrombosis, portal vein thrombosis, ischaemic stroke and pulmonary embolism.

## CLINICAL TRIALS EXPERIENCE (FDA)

The median age of patients who received REBLOZYL was 30 years (range: 18, 66); 59% female; 54% White and 36% Asian.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The data in the WARNINGS AND PRECAUTIONS reflect exposure to REBLOZYL as a single agent administered across a range of doses (0.125 mg/kg to 1.75 mg/kg) in 571 patients in 4 trials.

Serious adverse reactions occurred in 3.6% of patients on REBLOZYL. Serious adverse reactions reported in 1% of patients were cerebrovascular accident and deep vein thrombosis. A fatal adverse reaction occurred in one patient treated with REBLOZYL who died due to an unconfirmed case of AML (M6).

Permanent discontinuation due to an adverse reaction (Grades 1-4) occurred in 5.4% of patients who received REBLOZYL. Most frequent adverse reactions requiring permanent discontinuation in patients who received REBLOZYL included arthralgia (1%), back pain (1%), bone pain (<1%), and headache (<1%).

Dosage reductions due to an adverse reaction occurred in 2.7% of patients who received REBLOZYL. Most frequent adverse reactions requiring dosage reduction in >0.5% of patients who received REBLOZYL included hypertension and headache.

Dosage interruptions due to an adverse reaction occurred in 15.2% of patients who received REBLOZYL. Most frequent adverse reactions requiring dosage interruption in >1% of patients who received REBLOZYL included upper respiratory tract infection, ALT increase, and cough.

The most common adverse reactions (at least 10% for REBLOZYL and 1% more than placebo) were headache (26%), bone pain (20%), arthralgia (19%), fatigue (14%), cough (14%), abdominal pain (14%), diarrhea (12%), and dizziness (11%).

## DESCRIPTION OF SELECTED ADVERSE REACTIONS (EMA)

**Bone pain.** Bone pain was reported in 19.7% of  $\beta$ -thalassaemia patients treated with luspatercept (placebo 8.3%). In  $\beta$ -thalassaemia patients treated with luspatercept, bone pain was most common in the first 3 months (16.6%) compared to months 4-6 (3.7%). Most events (41/44 events) were Grade 1-2, with 3 events Grade 3. One of the 44 events was serious, and 1 event led to treatment discontinuation.

**Arthralgia.** Arthralgia was reported in 19.3% of  $\beta$ -thalassaemia patients treated with luspatercept (placebo 11.9%). In the  $\beta$ -thalassaemia patients treated with luspatercept, arthralgia led to treatment discontinuation in 2 patients (0.9%).

**Hypertension.** Patients treated with luspatercept had an average increase in systolic and diastolic blood pressure of 5 mmHg from baseline not observed in patients receiving placebo. Hypertension was reported in 8.1% of  $\beta$ -thalassaemia patients treated with luspatercept (placebo 2.8%).

In  $\beta$ -thalassaemia patients, Grade 3 events were reported in 4 patients (1.8%) treated with luspatercept (0.0% placebo). No patient discontinued due to hypertension.

**Hypersensitivity.** Hypersensitivity-type reactions (including eyelid oedema, drug hypersensitivity, swelling face, periorbital oedema, face oedema, angioedema, lip swelling, drug eruption) were reported in 4.5% of  $\beta$ -thalassaemia patients treated with luspatercept (1.8% placebo). In clinical studies, all events were Grade 1/2. In  $\beta$ -thalassaemia patients treated with luspatercept, hypersensitivity led to treatment discontinuation in 1 patient (0.4%).

**Injection site reactions.** Injection site reactions (including injection site erythema, injection site pruritus, injection site swelling and injection site rash) were reported in 2.2% of  $\beta$ -thalassaemia patients receiving luspatercept (placebo 1.8%). In clinical studies, all events were Grade 1 and none led to discontinuation.

**Thromboembolic events.** Thromboembolic events (including deep vein thrombosis, portal vein thrombosis, ischaemic stroke and pulmonary embolism) occurred in 3.6% of  $\beta$ -thalassaemia patients receiving luspatercept (placebo 0.9%). All events were reported in patients who had undergone splenectomy and had at least one other risk factor.

Reporting of suspected adverse reactions. Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via the national reporting system.

## OVERDOSE (EMA)

Overdose with luspatercept may cause an increase of Hb values above the desired level. In the event of an overdose, treatment with luspatercept should be delayed until Hb is  $\leq 11$  g/dL.

## CONDITIONS OR RESTRICTIONS WITH REGARD TO THE SAFE AND EFFECTIVE USE OF THE MEDICINAL PRODUCT (EMA)

### • Risk management plan (RMP)

The marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

An updated RMP shall be submitted by CHMP agreed deadline.



### • Additional risk minimisation measures

Prior to launch of Reblozyl in each Member State the Marketing Authorisation Holder (MAH) must agree about the content and format of the educational programme, including communication media, distribution modalities, and any other aspects of the programme, with the National Competent Authority.

The MAH shall ensure that in each member state where Reblozyl is marketed, all HCPs who intend to prescribe Reblozyl are provided with an HCP Information Pack, containing the following:

1. Information on where to find latest SmPC;
2. HCP Checklist;

### HEALTHCARE PROFESSIONAL CHECKLIST (EMA)

The HCP Checklist is to be used before initiating treatment, at each administration, and then at regular intervals when performing follow-up. The HCP Checklist shall contain the following key messages:

- Information on studies in animals showing luspatercept reproductive and embryo-foetal toxicity and is therefore contraindicated during pregnancy.
- Reminder that luspatercept is contraindicated during pregnancy and in WCBP not using effective contraception.
- Need to provide counselling before treatment initiation and regularly there after regarding the potential teratogenic risk of luspatercept and required actions to minimise this risk.
- A pregnancy test must be carried out and negative results verified by the prescriber before starting treatment. It must be repeated at suitable intervals.
- Patients must use highly effective contraception during the treatment with luspatercept.
- While on treatment, women must not become pregnant. If a woman becomes pregnant or wants to become pregnant, luspatercept should be discontinued. Women of childbearing potential must use highly effective contraception during treatment with luspatercept and for at least 3 months following discontinuation of treatment with luspatercept.
- Need to provide counselling in the event of pregnancy and evaluation of the outcome of any pregnancy.
- Should a pregnancy occur during treatment or within 3 months following discontinuation of treatment with luspatercept, remind the patient that it should be reported to the HCP, NCA, and/or to Celgene by contacting the local e-mail address or visiting the URL provided in the material, irrespective of adverse outcomes observed.

### USE IN SPECIFIC POPULATIONS (FDA)

#### ***Pregnancy Risk Summary***

Based on findings in animal reproduction studies, REBLOZYL may cause fetal harm when administered to a pregnant woman. There are no available data on REBLOZYL use in pregnant women to inform a drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes. In animal reproduction studies, administration of luspatercept-aamt to pregnant rats and rabbits during the period of organogenesis resulted in adverse developmental outcomes including embryo-fetal mortality, alterations to growth, and structural abnormalities at exposures (based on

area under the curve [AUC]) above those occurring at the maximum recommended human dose (MRHD) (see Data). Advise pregnant women of the potential risk to a fetus.

The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

### **Lactation**

#### **Risk Summary**

Luspatercept-aamt was detected in milk of lactating rats. When a drug is present in animal milk, it is likely that the drug will be present in human milk. There are no data on the presence of REBLOZYL in human milk, the effects on the breastfed child, or the effects on milk production. Because of the potential for serious adverse reactions in the breastfed child, advise patients that breastfeeding is not recommended during treatment with REBLOZYL, and for 3 months after the last dose.

### **Females and Males of Reproductive Potential**

#### **Pregnancy Testing**

Pregnancy testing is recommended for females of reproductive potential before starting REBLOZYL treatment.

#### **Contraception**

##### **Females**

REBLOZYL may cause embryo-fetal harm when administered to pregnant women [see Use in Specific Populations (8.1)]. Advise female patients of reproductive potential to use effective contraception during treatment with REBLOZYL and for at least 3 months after the last dose.

#### **Infertility**

##### **Females**

Based on findings in animals, REBLOZYL may impair female fertility [see Nonclinical Toxicology (13.1)]. Adverse effects on fertility in female rats were reversible after a 14-week recovery period.

#### **Pediatric Use**

Safety and effectiveness in pediatric patients have not been established. Based on findings in juvenile animals, REBLOZYL is not recommended for use in pediatric patients [see Non-Clinical Toxicology (13.1)].

#### **Geriatric Use**

Clinical studies of REBLOZYL in beta thalassemia did not include sufficient numbers of patients age 65 years and older to determine whether they respond differently from younger patients.

Clinical studies of REBLOZYL for treatment of anemia in MDS-RS and MDS/MPN-RS-T. included 206 (79%) patients  $\geq$  65 years of age and 93 (36%) patients  $\geq$  75 years of age. No differences in safety or effectiveness were observed between older ( $\geq$  65 years) and younger patients.

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## CHAPTER 19

### The value of patients' engagement

#### Introduction

Over the last 10 years, health care systems as well as academic, research and industry stakeholders have been increasing their efforts to integrate the patient voice into their work and decisions being taken.

The provision of a patient centred element in a health care system requires the development of an environment that will truly foster engagement between patients and the health care team.

**Transforming health care** in the 21st Century is a difficult and an extremely challenging task due to the many intricate layers that form part of and influence the system. Political, economic and cultural factors are often constrained by value conflicts and resistance to change. In 2013, an international patient movement referred to as 'Patient Revolution' was established to enlist patients who live and experience the health-care system on an everyday basis to help in designing care services that was better suited to their collective needs. Such initiatives mirror actions launched in other fields that rely on citizen science methods. The collective intelligence of large groups of people has been known to help address complex problems more effectively. Engaging patients with chronic conditions such as those living with haemoglobin disorders, will contribute significantly to the identification of those components including quality standards and protocols of care that require improvement and aid in the development of new initiatives that may positively impact patient care and improve their overall quality of life.

The valuable contribution of patients has been demonstrated through a number of published studies but also unpublished information including that compiled by patient oriented organisations such as Thalassaemia International Federation (TIF) through their work with patients. TIF for example, has worked since 1986 with patients in different parts of the world, in countries with different economies, different health and social care systems, cultures, religions and social beliefs and has evidenced the invaluable contribution of the patients' active involvement in achieving significant improvements in care policies.

Patients often have great insight into many aspects within the health care provision and how services directly and indirectly can substantially affect the care they receive. They are essential key players in assessing service needs and are instrumental in finding ways in which these can be improved. It is necessary thus to discover and leverage the huge untapped resource of patients' knowledge and experience to better understand and recognize those components of their care that are less than obvious to medical specialists. One of the most important drivers for change is to promote and implement a sort of 'cultural shift' on the part of medical specialists, clinicians and scientists, in order to eventually forget the old figure of the patient under the paradigm of the paternalistic medicine and to accept what has been clearly demonstrated nowadays by many qualified 'expert patients' that their involvement constitutes an added value to healthcare system improvements. In the field of haemoglobin disorders many paediatricians and haematologists across the world from the old school are

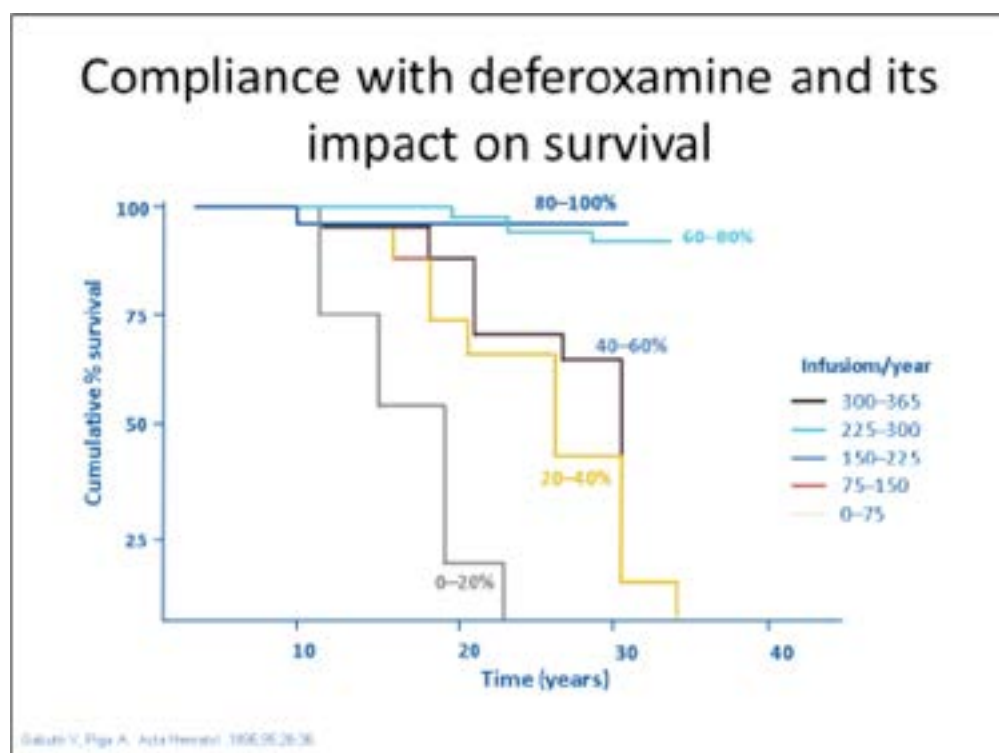
still involved and lead patients' organisations, thus this change is difficult to achieve. Specialists in many fields of the healthcare system, in regulatory and decision-making institutions, have reported how important can be the full involvement of patients for implementing inspired and creative outcomes with a mutual benefit. It has been shown in almost every hospital, clinic or centre that has been successful in reforming and improving the care services in particular of its chronic patients including those living with haemoglobin disorders, that top-down strategies related to restructuring of the care services are not the sole proponents to improve the quality of care. The creation of a truly patient-centred care system is particularly favourable to the chronic, 'frequent' visitors of the services such as the multiply transfused patients with haemoglobin disorders.

An obvious outcome of their 'satisfaction' with health services provided to them for example is **their better concordance to the often difficult, on many occasions painful, lifelong protocols of treatment they are receiving**. Concordance to treatment which is related to better survival and quality of life (Truglio-Londrigan et al., 2012; Náfrádi, Nakamoto & Schulz, 2017) has indeed been for many decades, and still is, amongst patients with transfusion-dependent thalassaemia (TDT), across all ages, sexes, of different educational level, ethnicities and cultures across all regions of the world, a huge concern and a documented cause of negative clinical outcome (Gabutti & Piga, 1996; Vekeman et al., 2016). Better quality interaction between treating physicians and patients is significant in improving concordance (Zolnieriek & Dimatteo, 2009), albeit the competent health authorities should be interested and willing to facilitate such practices.

**Quality interaction between patient and parents** particularly on complex and challenging issues including innovative and 'difficult' therapies such as Haematopoietic Stem Cell Transplantation (HSCT), gene based curative ones (and not confined to) constitutes a key pillar in the patients' better understanding of the benefit and the risks involved. This kind of interaction makes patients involved in shared decision, feeling a kind of ownership and responsibility of their choice. Treatments that can potentially affect their future, e.g. fertility or timely recognising the risk of an infection are only very few examples of the goals of patients in establishing with their health care professionals close and 'open' interactions.

For most high income countries in particular where advanced care is available and accessible to almost all patients, non-adherence to treatment protocols particularly iron chelation therapy constitutes an important remaining challenge experienced by treating physicians.

**Figure 1.** The relation between adherence and concordance and outcomes demonstrated (Gabutti V, Piga A 1996)



Good concordance with lifelong treatment protocols can only happen if and when the patient is truly pleased with all aspects of the care he/she is receiving and when she/he is involved in the solution-finding processes. “Treatment is for living and not living to be treated” is a phrase often used by one of TIF’s lead patient advocates. Health care professionals should thus not wait for their institution or hospital or clinics to act in engaging patients in discussions, as they can identify together, in a timely manner, key areas of their care that they can improve without massive administrative intervention.

### How engaging patients can be of benefit

Engaging patients in identifying gaps and weaknesses in the care that is provided to them even when this is in accordance with disease specific guidelines, has proved a very strong tool for the Thalassaemia International Federation (TIF). This is particularly seen in, but not confined to, countries of the developing economies, where the absence or very limited existence of national registries, reference centres and published information could not facilitate TIF’s understanding of the challenges patients with haemoglobin disorders are facing in their individual countries. Obtaining a reliable picture of the situation of a country or a region or even part of a county with regards to the quality of care provided to chronic patients, can be extremely challenging without the active and meaningful involvement and participation of patient organisations themselves. Such information is crucial for TIF in order to support its work and to better tailor its activities, and projects at the national, regional and international level based on the needs and concerns as expressed by the patients themselves.

Needless to underscore the fact that the contribution and collaboration of treating physicians and generally the health care professionals' community in identifying gaps and in supporting the promotion of measures and policies for improvement through well-structured and documented recommendations to policy makers, remains invaluable.

The different perspective between patients and physicians was very well described in an informal survey circulated among European Blood Network (EuroBloodNet) members, in the very early stage of the start of the project (in 2016), in which it was clearly shown that in the context of seeking their opinion on the structure of the network, what was mostly relevant for patients was less important for clinicians and vice versa (unpublished data communicated by a patient representative in the Eurobloodnet project). This is to confirm that indeed every contribution is important for policy-makers.

### ***Engaging patients in the work of decision makers at national and international level***

Patient supported proposals for improvements may complement those of the national competent authorities and health care professionals and can and have been documented (Hertel et al., 2019; Bombard et al., 2018) to be of truly great value. In addition to national health authorities, patient involvement can be of benefit to regional or international health related bodies including the World Health Organization (WHO). Patients' views and perspective can greatly facilitate the better understanding of the hugely heterogeneous unmet needs of patients with different chronic conditions, in a country, across countries, across different Regions of the world. WHO and other official health related bodies are in need of reliable, real world data to tailor more effectively their work and actions including revisiting general health or disease specific resolutions and recommendations with the support and interaction of their member states. Data on policy related outcomes cannot be obtained in the absence of patients' involvement and without truly 'capturing' the patients' perspective and position.

### ***The example of the European Union (EU)***

For many years now, in Europe a very strong effort has been undertaken to support Rare Diseases through a number of European Union (EU) official Recommendations, Regulations, Directives, and Decisions. All of these include as a prerequisite for their preparation and later implementation at national level, the full and meaningful engagement of the patients and the clear expression of their position. In fact, the empowerment and recognition, by the European Community (EC), of the added value involved in addressing the huge unmet needs of Rare Diseases (RDs) (Aymé, Kole & Groft, 2008) came initially in the 1990s from the active, persistent and very well-structured engagement of the RD patients themselves through their European umbrella association, the European Organisation for Rare Diseases (EURORDIS) (De Santis et al., 2019). The methodology used by the EU in interacting and collaborating with the patient, and the weight given to the patient's position could indeed constitute a fine example of how official stakeholders can truly involve the patient's perspective beyond Europe and across the world (TNS Qual+, 2012).

<sup>1</sup> EuroBloodNet results from a joint effort of the European Hematology Association (EHA), the European Network on Rare and Congenital Anaemias (ENERCA), the European haematology patient organisations represented in both the EURORDIS European Patient Advocacy Groups (ePAGS) and the EHA Patient Organisations Workgroup and it encompasses oncological and non-oncological rare haematological diseases including rare anaemias. Its main goal is to improve the healthcare and overall quality of life of patients with a Rare Haematological Disease.

## ***Where and how patients can be engaged - examples***

In another field, engaging the patient's perspective in developing new or in reforming existing official legislations and policies relevant mainly but not confined to health, social care and education, has proved, in every case and in every country, that has 'allowed' or encouraged such practices, extremely beneficial. The involvement of patients within the health care provision has been in place for quite a long time in Cyprus, with the Pancyprrian Thalassaemia Association in the lead since the 1960s. Patient involvement (PI) however, was made mandatory in Cyprus in 2016 through a new law [46(I)/2016]) that officially recognised patients as a valuable and equal stakeholder to the Government. This has allowed their full and active engagement in almost every step of the very extensive health care reforms the Cyprus Government undertook to implement in recent years. The contribution of the patient's engagement has been extremely valuable and greatly appreciated by Government and all other official stakeholders involved in this reform acknowledging the very fact that Cyprus was committed to establishing a new truly patient centred health care system.

Patients in many countries today are involved at different levels in national health committees that may be disease specific and/or public health/ health care system related.

The involvement of patients living with haemoglobin disorders in some countries across the world has resulted in the inclusion of their condition in a number of important disease specific social, and where patients felt essential, disability oriented policies/legislations including (and not confined to) early retirement, travel remuneration, quotas for university admission and employment and many others, different in each country.

The engagement of patients in many, if not all, decision making committees of the EC/ EU is mandatory and has thus contributed significantly to having truly patient centred decisions, recommendations, directives and regulations. The participation of patients is clearly described in most of these and an illustrative example is the European Directive (transposed into the national legislation of every EU member country) 2011/24/EU (and its two Addenda 2014/286/EU and 2014/287/EU) for safeguarding the rights of patients with RDs in obtaining cross-border healthcare. In this, the establishment of the European Reference Networks (ERNs) for RDs (including rare anaemias and haemoglobin disorders) constitutes a key recommendation and the full patient's involvement is a prerequisite.

One must not ignore that further to the patient's wellbeing and social integration, which constitute the two major goals for an effective health and social care system in a country, practices which involve the patient's perspective contribute greatly to the system's sustainability through for example their participation in Health Technology Assessment (HTA) bodies (Single et al., 2019) and which in many instances, has been pivotal for price negotiations of many available expensive drugs.

**A 2019 Deloitte Report** on patient access to innovative medicines in Europe (Deloitte Centre for Health Solutions, 2019), points out that **early dialogue and partnership with regulatory and important stakeholders including patients can provide a number of benefits to all and can aid pharma product pricing.** Similarly, outside Europe, pharma organisations, more and more in current years, are engaging with payers and patients earlier, in much more constructive, collaborative and valuable ways. **Such engagement helps pharma develop products that meet the priorities of the health care system and the unmet needs of patients and importantly enter into pricing ne-**



**gotiation to model** and discuss a variety of potential contracting solutions to achieve market access. **New innovative drug products and therapies need to be made available and to reach the patients in a timely way particularly those with RDs.**

European based pharmaceutical industry has attempted in 2015 a qualitative interview study to identify the value and challenges of Patient Involvement (PI). In the conclusions of this work, many were uncertain about when, how and which patients to involve (Parsons et al., 2016).

Patients and the public's lack of knowledge and interest in medicines R&D, and the pharmaceutical industry's lack of knowledge, interest and receptivity to PI were believed to be key challenges to increasing PI. Interviewees also believed that relationships between the pharmaceutical industry, patient organisations, patients and the public needed to change to facilitate PI in medicines R&D. Existing pharmaceutical industry codes of practice and negative media reporting of the pharmaceutical industry were also seen as negative influences on these relationships.

On the same argument, the authors of a 2018 publication (Levitan et al., 2018) argue that risk-adjusted financial models can actually assess the impact of patient engagement particularly in the context of clinical trials. A combination of empirical data and subjective parameter estimates shows that engagement activities with the potential to avoid protocol amendments and/or improve enrolment, adherence and retention may add considerable financial value.

**In the context of the new Regulation (EU) No. 536/2014 for clinical trials of medicinal products for human, there is (in addition to many other benefits) a significant increase in transparency on clinical trial data and data generated with a greater involvement of the public and patients with the mandatory introduction of a patient into the testing teams and the publication of a final report in language (Tenti et al., 2018) dedicated to the public and not the workforce.**

In more recent years, patients' involvement along the whole chain of Research and Drug Development (R&D) has been greatly strengthened and placed on a more professional basis by the two major drug regulatory authorities including the European Medicines Agency (EMA), National Institute for Health and Care Excellence (NICE) and the Food and Drug Administration (FDA).

There is unequivocal evidence collected by EMA (EMA, Stakeholders & Communication Division, 2014) and FDA on the valued contribution of patients' views and involvement in medicines R&D. Already EMA, has taken significant steps in this direction through a number of its committees, in which patients participate including the Committee for Orphan Medicinal Products (COMP), Paediatric Committee (PDCO), the Committee for Advanced Therapies (CAT) and Pharmacovigilance Risk Assessment Committee (PRAC). In these bodies, patients' representatives may assume the status of full members including having the right to vote for the drugs' assessment and approval processes. Patients involved however, should be free from any conflict of interest and no association at any level with the pharma industries so as to ensure the unbiased nature and full transparency of the approval process. In 2018, EMA reported that one in five scientific advice procedures involved patients and the scientific committee members (SAWAP) considered that in almost every case, patients provided an added value to the scientific advice and in about one in four cases, the scientific advice recommended that the development plan be modified to reflect patient advice (EMA, 2019).



EMA is now proposing the fostering of earlier contact with patient/consumer organisations. It proposes to reach out to patients at the start of the evaluation of new Marketing Authorisation Application (MAAs) so that patients can share their experience and concerns about their condition(s) and key aspects that are important for them in order for this to be taken into account in a timely manner during the assessment process. Aspects that are of particular to patients/ carers such as quality of life, standard treatments on how acceptable they are, how these interfere with their lives, therapeutic unmet needs, what their expectations are with regards to the benefits they hope to have, the level of side effects they would consider acceptable. It will greatly facilitate the better understanding of whether there are large differences between groups of patients/carers of the same disease area or concerns are similar across the condition and certainly to note anything else that patients/carers feel is important for EMA to know. (EMA/372554/2014-29/11/2020).

Similarly, and along the same lines, earlier in 2012, as part of the reauthorization of the Prescription Drug Use Fee Act (PDUFA V), the US FDA (Perfetto et al., 2015), established a programme to help ensure patients' experiences, perspectives, needs and priorities are captured and meaningfully incorporated into the development and review process. This was formalized as Patient-Focused Drug Development (PFDD). A number of activities and meetings are involved in this novel approach so as to gather input from patients who are willing to share their personal experiences of living with a disease or condition.

Patients and patients' organisations should be meaningfully engaged at all stages from defining research priorities to trial design, review of proposals, trial implementation and participation. And indeed, this has become, especially in the last decade, a routine and in most cases a mandatory practice both by the academic research community and the industry.

In this context, published literature even provides description of a possible road map of patient involvement (PI) across the whole spectrum of R&D life cycle (Figure 2) and key areas and opportunities for PI within early stage are identified (Geissler et al., 2017). In the course of such involvement, both the patients and research scientists gain a number of different for each party benefits, some of which are mentioned below including for patients (A) and research (B):

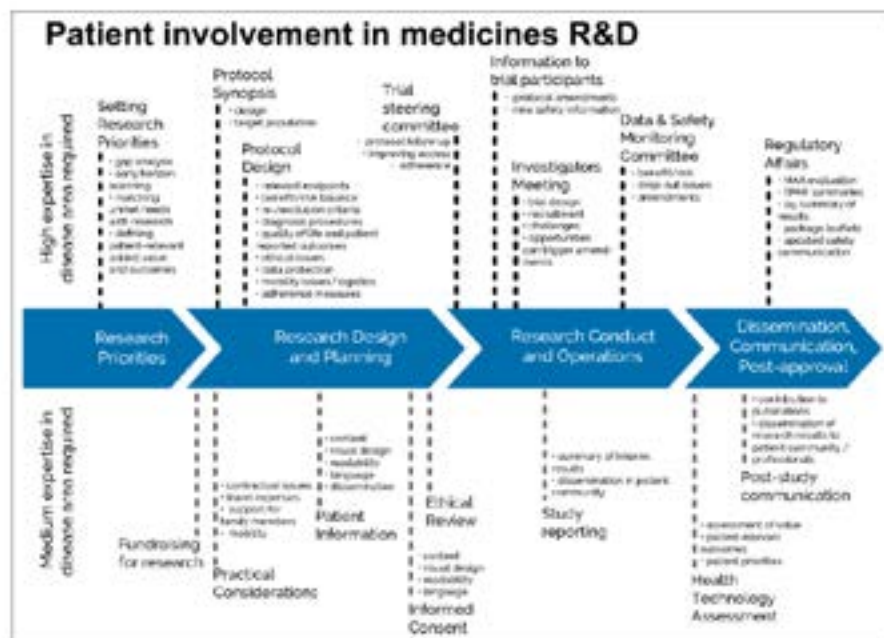
#### **A. Patients**

- Gaining knowledge and research skills;
- Increased understanding of the nature and purpose of a clinical trial;
- Greater self-esteem and confidence of patient representation involved in the process;
- Acceptance of patients as equal partners;
- Utilizing patient experience and knowledge on their condition, leading to development of health care and therapies that are more representative of patients' needs;
- Data and information exchange between users and industry during post marketing period and in the context of pharmacovigilance commitments.

#### **B. Research**

- Ensuring research and research outcomes address patients' real unmet need;
- Increased response and participation rates;
- Productive changes in study design, wider dissemination of results.

Figure 2



*Practical roadmap for patient involvement in medicines R&D. DIA Therapeutic Innovation & Regulatory Science 2018. Vol 52(2) 220-229. (With permission)*

It is true that many on the development side often fail to take into account real-world challenges when developing a clinical trial. They are more interested in determining the efficacy and safety of a drug rather than taking into consideration quality of life issues. The contribution of the patient's perspective is in more recent years not only recognized but very importantly sought after and considered essential. The collection of real patient data, for example is a prerequisite in the context of certain types of authorization licenses granted by EMA and FDA and mainly regarding innovative drugs/therapies, which may be granted accelerated procedures or authorization under exceptional circumstances or conditional marketing authorization.

These types of licenses are granted to address unmet medical needs of patients. Either for patients with unmet needs to facilitate their accelerated access to a new medicine or because the drugs cannot be approved under a standard authorization as comprehensive data cannot be obtained either due to the rarity of the disease, or because there are gaps in the scientific knowledge. These drugs are subject to specific post-authorisation obligations and monitoring and they are authorized on the basis of less comprehensive data than normally required to address unmet medical needs. Although the applicant needs to present data that indicate that the medicine's benefit outweighs its risk, the applicant should be in a position to provide the comprehensive clinical data in future.

For such data to be collected, the continued, well-structured and coordinated patient engagement is absolutely essential. PI has steadily gained increased recognition, not only by the EU and the WHO which had since the early 1990s official collaboration with patient oriented Non-Government Organizations (NGOs), but also

by an increasing number of national health authorities across all regions of the world. An expert patient advocate Jan Geissler, leading the EUPATI (European Patients' Academy on Therapeutic Innovation), an excellent educational initiative originally launched by the Innovative Medicines Initiative (IMI) and hosted by the European Patients' Forum (EPF), believes that "involving patients while designing trials and developing drugs will help so that non-scientific factors that are still crucial to evaluating a drugs' efficacy can be taken into consideration" (Chakradhar, 2015).

### ***Engaging patients in educational programmes***

Significant progress has also been made by medical bodies with respect to the recognition of the value of engaging patients in their programmes, mainly educational ones. The European Hematology Association (EHA) is a fine example in the case of haematological diseases, including haemoglobin disorders. Since 1992 EHA has promoted, in a very structured way, initially with scepticism and reservations to a certain level, a patients' advocacy group (in which TIF participates), the work of which through the years has proved to be an added value to the work of EHA. In the 2018 the 'EHA Research Roadmap on Haemoglobinopathies and Thalassaemia: An update', includes "the development of Patient Reported Outcomes (PRO) tools to support the work and collaboration with patient organisations" amongst its key recommendations (Iolascon et al., 2019). Such collaborations have developed through the years or are in development with other medical orientated groups including the International Society of Blood Transfusion (ISBT), the European Blood Alliance (EBA), the European Association for the Study of the Liver (EASL) just to mention a few relevant to the work of TIF.

### ***Acknowledging the value of patient outcome in healthcare delivery***

Patient reported outcomes is a major and invaluable tool developed to 'capture' in a more structured way patients' information and views that can be appropriately analysed and assessed in order to better understand their needs and expectations for the health, social and other specialised care they are receiving (Lavalley et al., 2016).

Collecting patient experiences and expectations in routine care is crucial in developing services that focus on patient centred care. Often, changes with regards to service provisions are made by those who have the best intention but no true or life experience of the condition. As a result, their perceived goals as to what their patients want may differ to that of those which live with the condition. Having pragmatic insights into patients' experiences of symptoms, quality of life, values, preferences and goals in life are essential in providing any health care service that is effective for a medical condition.

Previously embraced in the research realm, patient-reported outcomes have started to play a role in successful shared decision making, which can enhance the safe and effective delivery of health care. Present and future challenges need to be analyzed and examined so as to provide the opportunity to health care systems to maximize the use of patient-reported outcomes in the clinic/hospital.

Reported outcomes as a tool, therefore, can be both disease specific or general health care oriented one. It can play a role in shared decision making which can in turn enhance safe and effective delivery of healthcare. Emerging practices consequent to patient reported outcomes have provided value to both patients and clinicians and have improved care services albeit this tool is not to date extensively applied.

### ***The key...***

**However, the key to meaningful and productive patient involvement lies largely on the very good knowledge and often relevant experience that the patient has in the particular area he/she is assigned to interact.** In this context, European umbrella organisations such as the European Patients' Forum (EPF), the European Organisation for Rare Diseases (EURORDIS) and a number of disease specific organisations at the European level have been very actively involved in developing very comprehensive educational programmes for patients with different diseases on a variety of health, drug and other research related topics in collaboration with experts and other stakeholders including the industry. These programmes aim at building a competent patient community that is knowledgeable enough to interact productively and advocate effectively for the rights in quality and safe care at all levels and importantly at decision-making level at country and European levels.

TIF, the International Alliance of Patients' Organizations (IAPO) and other international disease specific organisations on the other hand have been struggling for decades now in the international arena and have been, and still are, actively involved in safeguarding patient safety, drug and blood safety and equity of all to quality health and social care. TIF, particularly since its establishment in 1986, with the development and continual updating and upgrading of its educational programmes aims to strengthen the knowledge of patients across the world and 'transform' them to valuable and equal partners at the decision making level.

TIF in this context has developed since 1989, an educational programme which is constantly strengthened and is based on three pillars:

1. Preparation, publication, translation (into many languages) and distribution of educational and informational material: books/factsheets etc.;
2. The organization of events: workshops, seminars, conferences, symposia, meetings, courses, fellowships;
3. The development of electronic educational platforms for patients and health care professionals and more currently the organisation of virtual educational events including webinars.

In addition, in more recent years, TIF established in 2019, the TIF Patient Advocacy Group (T-PAG) comprised of 198-member patient advocates from 62 countries. Many of these have developed adequate competency to actively advocate and interact productively at different levels of decision making at national, regional or international level and still many others are under training and working mostly at national level. The vision of TIF being to 'create' a large group of competent patient advocates across countries and regions of the world to make the voice and position of the patients with haemoglobin disorders when involved, strong and effective.

Below Figure 2 and Tables 1 & 2 describe TIF's Patient Advocacy Groups (TIF PAGs) project, its structure and membership (Figure 2), the eligibility criteria for the inclusion of patients in the PAGs (Table 1) and briefly their role as TIF PAG members (Table 2) and TIF's responsibilities in supporting their educational work and meaningful engagement at all levels (Table 2).

Figure 3

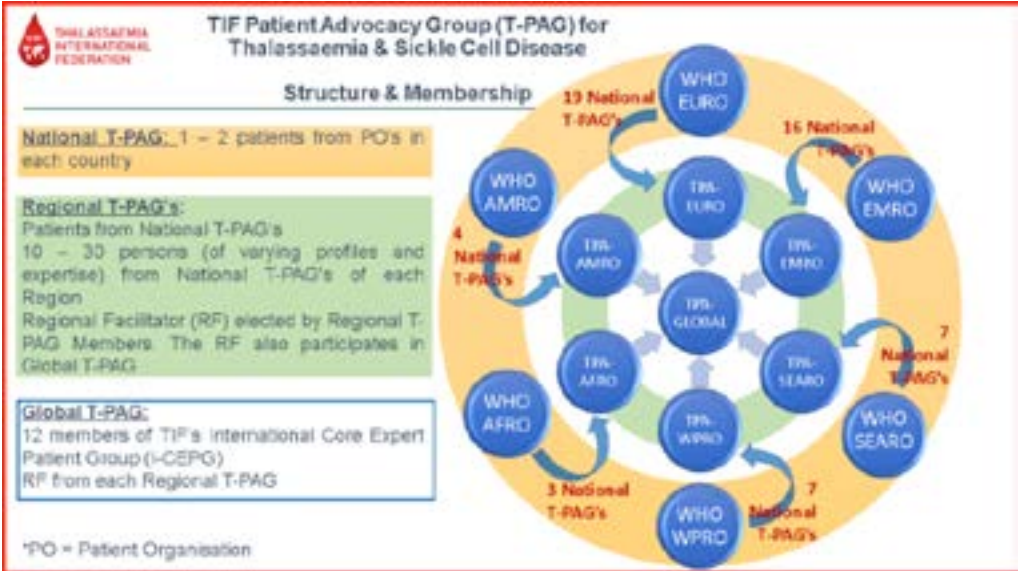


TABLE 1

**Thalassaemia Patient Advocates (TPA) Programme: Eligibility Criteria**

- ✓ Be patients with thalassaemia or sickle cell disease;
- ✓ Are over 18 years of age;
- ✓ Belong to a National Thalassaemia Association, preferably;
- ✓ Understand the needs of patients both in the country, but also across the region, and globally (if he/she belongs to TPA –Global);
- ✓ Understand TIF's mission, vision, strategic objectives, plan of activities, policies and positions;
- ✓ **Successfully completed the Thal e-course** (scoring >80%);
- ✓ Must acquire knowledge on drug development, clinical trials, drug regulatory affairs and research in the field through webinars, educational meetings and courses offered by TIF and others;
- ✓ Continue to enrich and consolidate the knowledge obtained through the Thal e-course through active involvement in webinars, personal communications and interviews initiated by TIF;
- ✓ Have knowledge of the health system public/private of the country they represent;
- ✓ Have knowledge of the strengths and weaknesses of the clinical/social system of the country they represent.



TABLE 2

Thalassaemia Patient Advocates (TPA) Programme: Responsibilities & Benefits of Participations	
The Role of TPA Members:	TIF's Responsibilities Towards TPA Members
<ul style="list-style-type: none"><li>i. To advocate for TIF's mission and vision in accordance to TIF's strategic objectives, through the materialisation of specific activities, in alignment with TIF policies and positions on a wide range of issues;</li><li>ii. Maintain an up-to-date understanding of the needs of patients both in their home country, but also across the region, and globally (if he/she belongs to TPA -Global);</li><li>iii. May represent TIF in preassigned missions;</li><li>iv. Provide their opinion / perspective as patients, when this is requested, and participate in consultations on issues concerning haemoglobinopathies;</li><li>v. Be in close communication with the TIF Office, using official channels (i.e. email, fax or post);</li><li>vi. Must discuss and receive the consent of TIF prior to undertaking any mission in the context of their role as TPA Members.</li></ul>	<ul style="list-style-type: none"><li>i. Responses to communication received from TPA Members will not exceed 2 working days;</li><li>ii. Sustain interactions with the TIF International Scientific Advisory Committee, seeking input and scientific knowledge in order to fulfil responsibilities below;</li><li>iii. Ensure that the content of the Thal e-course is up-to-date;</li><li>iv. Develop targeted and specific preparatory and / or training materials as required for TPA Members prior to each mission (event or visit) and consultation;</li><li>v. Organise and coordinate teleconferences with TPA Members to further explain the preparatory materials, ensure understanding and confirm alignment with TIF's position on the matters to be discussed;</li><li>vi. Cover costs of any travelling or participation in meetings and events organised in the context of TPA.</li></ul>
* TPA Members are required to adhere to the Terms of Reference Agreement for CONFIDENTIALITY and COORDINATION	

**Who are really patient advocates?**

Patient advocates can be defined as patients who have invested the time and energy needed to acquire a high level of disease specific knowledge i.e. on the condition they represent, the research activity around it and the authorized for their condition of therapies/drugs. These are patients themselves who know and truly represent, the views of the patients they represent, have deep knowledge of the unmet needs as expressed by the patients they represent in their country. They certainly need to be well versed with the functioning and services offered by the health care system in their countries, as well as what is happening in countries of their region but also globally. To achieve this they need to be aware of the work of official health related bodies in the region and globally including importantly the WHO, network with others in their country, region and internationally while keeping close and productive relations with the relevant health care professionals' communities at all levels. The support of TIF to its members in the context of education and provision of reliable and updated information is immense and the collaboration of every National Thalassaemia Association with TIF should be safeguarded and highly embraced.

**TIF and its work on PI**

TIF, since its establishment in 1986, has strongly advocated for patient engagement and respect to the patient's perspective and position and has involved patients in every aspect of its work and activity at all levels. TIF was amongst the first, if not the first patient organization, that was organizing annually, since 1989, patient associations meetings, what we today describe as as 'training programmes' for improving patients' knowledge, skills and competencies for advocacy.

The national Thalassaemia Associations in UK, Italy, Greece, Cyprus and USA, founding members of TIF, are fine examples in exercising very active PI since the very early years of their establishment in the 1960s/1970s and it is indeed the successful outcome of such involvement that gave TIF the strength and empowerment to extent this work across the world. Today many patients through the 137 National Thalassaemia

Associations in 59 countries members of TIF are actively involved at national and some at regional level and the outcomes of such involvement as reported to and evidenced by TIF, are very encouraging and in many cases truly impressive.

Engagement of patients has been happening for many years now in the field of haemoglobin disorders, albeit in a less structured way and with greater scepticism and slower pace in many countries outside Europe and other Western countries and the PI and its achievements in some of the developing countries are indeed quite remarkable. Patients must however continue to act through the strong, united voice of their national associations which bear the responsibility to build up educational programmes for strengthening the knowledge and advocacy skills of their patients, some of whom will reach that advocacy competency that would allow their interaction at national or international decision making level.

TIF as a patients umbrella association, through its work has achieved the establishment of very important collaborations with valuable stakeholders whose support is substantial for achieving its mission, including:

- the World Health Organisation (WHO) with which has been in official relations since 1996,
- the United Nations Economic and Social Council (ECOSOC) with which it has had an active consultative status since 2017,
- the European Commission as a strategic partner since 2018
- the INGOs Conference of the Council of Europe as a member since 2019.

In addition, throughout the years TIF has achieved to establish valuable collaborations and to network productively with over 200 medical/scientific national and international experts and with almost every relevant medical/scientific association or body. Importantly, TIF has gained the respect of competent national health authorities in more than 60 of its member countries around the world, with many of which, it works in the context of special collaborations and/or official agreements.

## Conclusions

In conclusion, the national health/social and every other competent authority in a country, needs to 'invest' in developing official, well-structured channels of PI at all levels of decision making if the aim is to honour the many and important relevant resolutions/declarations signed by all members of the WHO and in achieving the UN2030 SDG. These include but are not confined to: **patient rights, universal health coverage, quality health care, respect for patients and human rights for equal access to quality health and other care** and last but not least patient centred health care systems across the globe.

Finally, as this chapter is a new addition to one of the key TIF's publications – the Guidelines for the Management of Transfusion Dependent Thalassaemia - where recommendations by medical experts are incorporated, below are briefly listed some important disease specific areas of possible patient engagement at national level.

Table 3

**Patient engagement in the:**

1. Design of national disease specific registries and/or patient health records;
2. Design of educational or informational material that is prepared by health professionals and is focused on patient care, new drugs, research etc;
3. Planning of the transfusion services and the related whole chain of the transfusion process. TD patient spend considerable amount of their time throughout their lives in hospitals/centres/wards/clinics for their one or two or more monthly transfusions. The timings of consultation and transfusion therapy are expected by patients to be less burdensome and more patient friendly allowing the least interruption in their lives and profession; In the context of research and clinical trials at all stages of the process – prior, during and post;
4. Context or research and clinical trials at all stages of the process – prior, during and post;
5. Post authorisation process of drugs facilitating the collection of real patients' data on the value, effectiveness and safety of a drug;
6. Negotiations and discussions for pricing of and access to drugs and therapies;
7. Plans of actions prepared by national competent authorities for addressing an epidemic or pandemic crisis (e.g. COVID-19 pandemic);
8. Revisiting, revising or developing new recommendations and/or legislations that are related to their care and quality of lives;
9. Identifying gaps and weaknesses in the care provided for their condition in their particular hospital/ward/clinic/centre and in the solutions proposed;
10. Interactions concerning the designing of national studies on the cost effectiveness versus added value of a new drug/therapy;
11. Preparation of protocols and/or Guidelines the aim being to integrate their views and experience and to bring forward in any equation the element of quality of life, which is often 'forgotten' or underestimated in importance, by medical/scientific specialists and competent health/social authorities;
12. Revisiting process or development of new social care policies;
13. National health committees for raising awareness on the disease and blood donation campaigns.



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## CHAPTER 20

### Multidisciplinary Care and Reference Centres in addressing haemoglobin disorders

#### Introduction

The lifelong and multi-organ nature of transfusion-dependent thalassaemia is well reflected in the very contents of this book. As patients advance in years, the basic needs in blood transfusion and iron chelation, even if and when provided appropriately and in accordance to international standards, gradually become inadequate to sustain life, maintain wellbeing, and achieve social integration. For this reason, specialists in several medical disciplines including but not confined to heart, liver and endocrine, are called upon to contribute by monitoring and offering proactive management of iron toxicity and organ dysfunction in their field of expertise (Angastiniotis & Eleftheriou, 2009). These considerations and needs lead to significant and multiple challenges in the organisation of integrated services so that the best possible conditions for patient care are achieved.

Historically, and based on TIF's experience through its work globally, the need for establishing day transfusion centres for patients with transfusion-dependent thalassaemia (and other haemoglobin disorders), separate from the main haematology or paediatric wards, where they were 'naturally' admitted initially, arose first in areas and places where patients with these disorders were treated in sufficient numbers. This prompted policy-makers and healthcare professionals to recognise and respect that the needs of these patients are quite different from those of patients with malignant haematological conditions. So through the years, different arrangements (as seen in Table 1 below) were promoted with regards to providing more targeted, specialised services to patients with haemoglobin disorders.

Table 1

1. Within haematology departments: Development of RBC (non-malignant) clinics separate from malignant haematology services
2. Dedicated space within paediatric units where patients remained well into their adolescence and in many cases into their adulthood
3. Dedicated space within haematology units (amongst malignant haematology/ oncology patients) or transfusion centres of hospitals.
4. Development of "independent" thalassaemia units/centres based on outpatient principle attached to/associated with tertiary level hospitals
5. Within general paediatric or paediatric/oncology or general haematology wards/ clinics without any arrangement for a dedicated space or development of specialised services.
6. Development of centres run by patient NGOs in collaboration with pharmacists/Red Cross/ health care professionals

Provision of specialised care extended beyond the basic care of transfusion and iron chelation began to be recognised as an absolute necessity in those centres where:

- i. There was high disease(s) prevalence amongst the population whether in the indigenous (Cyprus, Greece, Italy) or in the well-established and integrated migrant population (e.g. UK, France);
- ii. The scientific community and the national health competent authorities recognised the important disorders' related medical, public health, social and economic repercussions, in the absence of any effective national policies for their prevention and appropriate management.

As a result, these disorders were prioritised on the country's national health agenda and disease-specific policies and programmes under national coordination and support were developed, allowing patients to grow, age and have a good quality of life. The integration of specialised multidisciplinary care services into the management of patients with these disorders brought about the need for specialised, disease-specific training/education of the different medical specialties, and many and multiple organisational challenges to ensure appropriate coordination. Where such steps were taken and where these led to successful, meaningful integration of services, including active research activity, Reference or Expert Centres began to spring up.

Such centres first developed in the 1980s in Mediterranean countries including Cyprus, Greece and Italy, and subsequently in the UK and France in the latter more in the context of Rare Disease (RD) national strategies; however in all, at least initially, without any structured specific criteria. Both the knowledge and experience of what 'optimal' care was for these disorders as well as the research activity around the better understanding of their pathophysiology and medical needs were in those early days quite limited. The development of Reference/Expert Centres was guided almost solely by the needs of the growing, ageing patients; the services, patient care pathways and quality standards that these centres developed through the years became the core and solid basis for setting the criteria in later years for defining the role a Reference/Expert Centre for Haemoglobin Disorders should be fulfilling. Gradually these first centres were officially as well and not only by reputation, assigned by the country's national competent authorities as Reference or Expert Centres undergoing regular and professional reviewing of their quality standards. Patients with haemoglobin disorders within and gradually from outside these countries began to be referred to these centres by their treating physicians for consultation, second opinion or for receiving specialised services that did not exist in their own area, region or country.

As national competent authorities and healthcare specialists across the world began to acknowledge the value of effectively addressing these disorders and to gather knowledge and experience at national level, clinics/centres of variable level of expertise, offering a different range of services of variable quality standards began to spring up, particularly in the 1990s, in various countries across the world. In most of these however, mainly basic care was provided including blood transfusion and iron chelation, while Multidisciplinary Care (MDC), which is an essential element of the care of growing patients and a major component of a Reference/Expert centre, was and still is, largely lacking.

In most low and middle income countries, where more than 80% of the patients with these disorders live, the poor/weak medical and public health infrastructures (including haematology and transfusion services), the lack of universal health and social care

coverage that is related mainly to their weak economies, and very importantly their focus on other health priorities, including communicable and common non-communicable diseases (NCDs) have not allowed such advances to take place. In a few of these countries for example, even Non-Government Organisations (NGOs), including the Red Cross and patient/parent oriented ones, were encouraged by competent national authorities to contribute to the management of haemoglobin disorders. These have developed through the years in collaboration with medical and other healthcare professionals' important services for patients, confined however only to the provision of transfusion services and iron chelation therapies. Certainly in such settings the provision of any extended specialised treatment is not possible. Therefore, patients need to be referred to hospital settings; in many instances in an uncoordinated manner and to medical specialists who (in the greatest majority) do not have specialised knowledge of the treatment of haemoglobin disorders.

The work of TIF for more than 35 years in over 60 countries across all regions of the world has exposed the naked truth: the development and integration of MDC services into the management of patients with these disorders and the promotion and establishment of Reference/Expert Centres are to-date components of care that are far from being adopted or implemented. Such advances can only happen if and when the basic, essential medical care in a centre or across a country has reached those quality levels that allows patients to grow satisfactorily, achieve social integration and have a reasonably acceptable, decent quality of life. This prerequisite can only be accomplished when the medical and public health infrastructures and quality standards are adequately strengthened in the context, as previously mentioned, of a healthcare system based on universal coverage. To-date such improvements are still to happen in most of the countries, and particularly in the developing ones where the majority of patients with these disorders live. The young age of the patients in these countries, who in their greatest majority do not reach ages beyond twenty or thirty years, confirms the fact that they are still receiving suboptimal basic care.

Bringing fragmented services together in an organised, collaborative manner and adopting international guidelines and standards of care is indeed very challenging, but it has been demonstrated beyond any doubt that such an effort maximises the patients' benefits and facilitates their very cumbersome treatment pathways with timely interventions. Such an approach is reflected in the improvement of outcomes (Allen, Gillen & Rixson, 2009), encompassing both clinical and social outcomes that have already happened in some countries, while at the same time, and very importantly, leads to the creation of cost-effective services which are of benefit both to the healthcare system and public health (Rocks et al., 2020).

Imperative to the success of multidisciplinary or interdisciplinary, integrated management of thalassaemia patients is the quality of the relationship between the haematology (or paediatric) team and the various supporting physicians across medical disciplines. The latter ones must be well organised, well-coordinated, interested in becoming involved, in acquiring experience in these conditions and very importantly they need to be supported and facilitated by the healthcare system of the country. Random consultations do not contribute to efficiency and effectiveness because accumulation of sufficient experience and knowledge requires a dedicated group of medical experts in the MDC, who will indeed be willing to receive education and better understanding of the management of haemoglobin disorders for those aspects that fall within their medical specialty.

**In many centres the multidisciplinary approach to thalassaemia is even misunderstood as having a specialist to refer to once a complication has arisen.** The whole concept of MDC and of a Reference Centre lies on the availability of **proactive, quality and specialised interventions** and in this context different medical specialists should be involved well before the appearance of complications i.e. regular involvement early in the patients' life. In a multi-organ disease like thalassaemia there is no doubt as to the necessity and benefits of this approach and TIF has focused considerable attention on promoting this concept and on creating those tools and advisory groups that could support it.

Furthermore, it is more functional if all members of the MDC belong/have medical attachments to the same hospital. This is because meetings of the members of the MDC group must occur regularly, and also when an emergency decision for the health/condition of a patient needs to be taken. It is understood however that this may not be possible, but technology through teleconferences and telemedicine tools have given us valuable solutions for such challenges.

### ***The role of the co-ordinator/leadership***

In order to achieve effective functioning of the interdisciplinary team that could be reflected in the desired outcomes of early detection and reversal or minimisation of organ damage, the principles of teamwork and good communication must include first of all a competent team coordinator who is an expert physician in the clinical management of haemoglobin disorders. This should best be the lead physician (haematologist, paediatrician etc.) who is responsible for the routine, everyday care and who sees each patient most often. Mature leadership will ensure that scheduled visits to each specialist are implemented and that in turn different specialists communicate timely and comprehensively their results to allow the treating specialist to reach a decision on any treatment adjustments.

As the issue of communication is central to the functioning of the MDC team (Eleftheriou, 2017) ensuring its effectiveness is important and this can be achieved in a number of ways and through for example:

- Joint clinics
- Regular team meetings
- Case conferences
- Sharing of results, along with interpretation and discussion for joint decisions
- Using electronic disease-specific medical records with full access to all members of the team

**• Involving patients in the discussions and decisions which concern their lives is critical and every effort should be made by all to establish a well-structured and regular programme of active and meaningful patients' engagement.**

Some of the key barriers therefore that may prevent the achievement of effectiveness of the work of the MDC include (i) lack of appropriate and structured coordination and efficient communication, (ii) lack or poor involvement of dedicated physicians across medical disciplines, (iii) inadequate implementation of common shared decisions, (iv) lack of sufficient time given by the involved physicians to interact productively and discuss comprehensively the cases and (v) inadequate access to, or lack of existence of, well-informed patient records.

An example of the structure of interdisciplinary team for the care of haemoglobin disorders as extracted from some well-established European Reference Centres with successful patient outcomes is presented in the Table below (Table 2) (European Commission, 2016):

**Table 2**

### **Interdisciplinary team for the care of haemoglobin disorders**

Specialty	Description
Haematologist/ paediatrician/ internist	Usually the physician in charge of the routine care, including monitoring of iron load. Usually coordinates the multidisciplinary team. Is supported by other more junior physicians according to the number of patients
Specialised nurses	Specially trained and experienced haemoglobinopathy nurses who, apart from routine duties like supervising blood transfusions and triage of patients, because of their closer contact with patients, have a significant role in counselling and psychosocial support
Cardiologist	A special interest and experience in the cardiac complications of thalassaemia. Monitors all patients from childhood, collaborates with the lead physician on any management modifications and takes charge when complications like arrhythmias arise
Endocrinologist	Monitors all patients usually from early adolescence for these very common complications of thalassaemia. Apart from liaison with the multidisciplinary team, there is collaboration with gynaecologists in the case of infertility and pregnancy. Also part of the team managing bone disease
Hepatologist	Liver disease is increasing in the aging thalassaemia population. Liver function is monitored from an early age by the clinic team but persisting disturbance of liver function, iron overload and viral hepatitis require a consultation with a liver specialist
Psychologist/ social worker	These are essential supportive services and should be part of team at all times. Many issues may not be recognised by other physicians or nurses and periodic visits of the psychologist could bring matters to the surface for all patients. They are not just for referral when acute emotional problems arise. Especially where universal health coverage is not available, financial hardship and social isolation can contribute to negative patient outcomes. Support for the healthcare team may also be necessary
Obstetricians	A member of the collaborative team in intended and ongoing pregnancies. Pre-pregnancy counselling along with mainly the haematologist, endocrinologist and cardiologist is essential but teamwork during gestation is also necessary for good outcomes
Dental care	All patients should be routinely monitored for dental and maxillary complications at least yearly

Many other important health care specialists are needed particularly when the centres are treating sickle cell disease (SCD) patients as well, which is the usual case in most centres across countries. Some of the key specialist services to which treating physicians and patients should have timely and well-co-ordinated access in this case are included in Table 3 below (which is by no means exhaustive):

**Table 3**

- Erythrocytapheresis
- Pulmonary hypertension team
- Fertility, contraception and sexual health services
- Consultant neurologist
- Consultant ophthalmologist
- Consultant nephrologist
- Consultant urologist with expertise in managing priapism and erectile dysfunction
- Orthopaedic service
- Specialist imaging including:
  - MRI tissue iron quantification of the heart and liver with regularly standardise software to ensure accuracy and reliability of iron measurement
  - Trans-cranial Doppler ultrasonography (children)
- Polysomnography and ENT surgery
- Bone marrow transplantation services

## **The place and role of the patient in the MDC of his/her condition**

In the multidisciplinary team, one essential member, constituting the central component, is most frequently forgotten or his or her importance is under-recognised. This is the patient (or parent in the case of children). The patient's involvement is important for the carer/patient relationship and in particular with regards to the need to support his/her self-management and for the continued patient concordance to prescribed treatment. The patient however, requires education, continued provision of reliable and up-to-date information along with strong encouragement and empowerment to take charge of his/her life under specialist guidance. Treatment planning should take into consideration, as far as possible, patient preferences, choices and lifestyle so that every effort to reach concordance is made. The active and meaningful engagement of patients is substantial in the better understanding of the patients' needs, and thus in the better planning and more appropriate reforming of related policies. The aim for every government and treating physician is certainly to achieve good health and quality of life of their patients associated with a high level of social integration; and indeed this can only happen if and when the patient remains at the centre of decisions.

## **Reference or Experts' Centres for Haemoglobin Disorders**

Considerable work on this topic has been conducted mainly by the European Commission in the context of its work on promoting quality services for RDs across the EU. The many and complex challenges faced by patients/families and treating physicians in the early and accurate diagnosis, and management and monitoring of RDs are similar to those that characterise haemoglobin disorders, which in many countries are RDs. However, contrary to the many thousands of



other RDs there is (and has existed for some time now) ample and reliable knowledge as well as experience for haemoglobin disorders with regards to early and accurate diagnosis, specialised monitoring, appropriate management and effective prevention.

The European Commission recognised RDs as a priority action area since the mid-1990s and since then the different EU initiatives addressing RDs have predominantly focused on bringing together scattered resources and expertise across Member States. This is an effort that is certainly needed in the case of haemoglobin disorders as well – both across Europe, and more importantly across countries with developing economies where the majority of patients with these disorders live. In addition, EU initiatives and policies aimed to strengthen and empower research activities in order to provide more innovative drugs and therapies for RDs, as well as the development of national plans in every EU Member State to more effectively addressing the needs of RDs. Within this work, the European Commission established a special committee of experts, the EUCERD (European Union Committee of Experts on Rare Diseases), which focused on developing quality criteria for centres of expertise for RDs in Member States (2011) and recommendations on establishing RD European Networks (ERNs) (2013) (EUCERD, 2011). In this context, the idea of ERNs was integrated into an EU Directive (2011/24/EU) which is related to the application of EU patients' rights in cross-border healthcare acknowledging that this is a major step towards more effectively promoting the sharing of knowledge/expertise and best practices and the creation of clearer structures and networks in the area of RDs by bringing together highly specialised providers across the EU (European Parliament, 2011).

Within the 24 ERNs that were established to cover 24 different RDs or families of RDs, aiming to share best practices for their care and cure, the ERN on haematological diseases (EuroBloodNet) is the one focused on rare blood disorders including haemoglobin disorders. Considerable work is being undertaken by this network to pool together knowledge and expertise from across the EU on these disorders (EuroBloodNet, 2021).

In the context of the above work of the EU, a number of relevant projects were launched including the European Network for Rare and Congenital Anaemias (ENERCA) which was relevant to haemoglobin disorders and other rare anaemias, and in which TIF was a major partner. Through this activity, TIF contributed along with other European and International medical and scientific experts in rare anaemias to the completion, amongst other important deliverables, of a book titled: "The Recommendations for Centres of Expertise in Rare Anemias: A White Book" (Corrons et al., 2014). This document reflects the extensive work that the EU has undertaken for over two decades for the benefit of rare anaemias including haemoglobin disorders, and indeed represents a major contribution towards the creation of a much needed European infrastructure of expertise around rare anaemias. The authors of this Chapter of the 4th edition of the Guidelines for the Management of Transfusion Dependent Thalassaemia, have felt that the above description was essential to be included, as the experience gained through the years by EU Member States in this field can be extended and constitute a sound basis for the countries outside and well beyond Europe to build upon.

Some of the benefits outlined below in Table 4 and in the context of the EU directive (2011/24/EU) (mentioned above) on facilitating and safeguarding the rights of patients with RDs, including rare anaemias and haemoglobin disorders, for obtaining cross-border healthcare, underscore the importance of developing and pooling together specialised knowledge and experience as well as of networking between centres of expertise. From these ideas and policies, if and when tailored to the

<sup>†</sup> <https://eurobloodnet.eu/>

needs and prevailing situation across any country or region of the world, patients with haemoglobin disorders, the healthcare specialists and the healthcare systems at large could benefit significantly, as has been the case with the RDs across the EU.

**Table 4**

- Providing patients and healthcare professionals access to experts and expertise throughout all European member states, regardless of the country of origin or practice, thereby reducing inequalities and maximising the cost-effective use of resources;
- Implementing epidemiological surveillance throughout the EU that gathers comparable data on patients affected by RAs and launching preventive programmes for tackling RAs;
- Fostering best practices for prevention, diagnosis and clinical management;
- Promoting the dissemination of knowledge, the sharing of expertise, supporting research, and increasing awareness of RAs;
- Facilitating the transposition of the Directive 2011/24/EU of 9 March 2011 on the application of patients' rights in cross-border healthcare. The European Reference Networks (ERN) between healthcare providers and Centres of Expertise is a main point of interest of the directive, especially for rare diseases. The networks will be a tool to improve the access to diagnosis and the provision of high-quality healthcare to all patients who have conditions requiring a particular concentration of resources or expertise, and could also be focal points for medical training and research, information dissemination and evaluation, especially for rare diseases.

TIF, as a patient-driven umbrella organisation, has a constitutional mandate to continually identify ways and tools to promote the quality of care provided to patients with haemoglobin disorders (Angastiniotis & Eleftheriou, 2014). It has thus focused particular attention and considerable work on its educational programme since its establishment in 1986 and in this context TIF has initiated in 2017, a new project titled "TIF's Certification Programme" focused on: the empowerment of national competent authorities, healthcare professionals and patient communities to dedicate work on promoting the MDC component and the establishment of Reference/Expert centres into their management strategy (Soteriades et al., 2017).

TIF's vision through this project is to first identify and develop an extended list of centres/clinics within a country that treat patients with haemoglobin disorders, followed by an effort supported by TIF's International Expert Advisors to 'classify' them based on set criteria (see below) including the range and quality standards of the services provided by each of them (TIF, 2017). The aim of TIF is to (i) identify those centres already qualified to perform the role of a Reference Centre today, (ii) to provide through its scientific advisors support to those treating centres that have the potential to upgrade their services, (iii) to support other treating centres to reach at least acceptable levels of quality basic care for their patients and (iv) very importantly to support the networking between them at national level and of the Reference/Expert Centres at regional and international level.

The programme focuses on the application of specific quality standards for reference centres involved in the provision of care for patients with thalassaemia and other haemoglobin disorders. The TIF Quality Standards are based on the general principles already developed by the relevant organisations as outlined in Table 5 below:

Table 5

1. The Joint Commission International (JCI): "Survey process Guide for Ambulatory Care (3rd Edition, 2015) European Union Committee of Experts on Rare Diseases (EUCERD): Quality Criteria for Centres of Expertise for Rare Diseases in Member States (2011) and EUCERD recommendations on Rare Disease European Networks (2013)
2. Guidelines for Good Clinical Practice
3. US Institute of Medicine: Quality Improvement
4. US Department of Health and Human Services, Health Resources and Service Administration: Quality Improvement
5. UK NHS, Peer review of health Services for People with Haemoglobin Disorders: (2015 Review)
6. TIF "Guidelines for the management of transfusion dependent thalassaemia" 3rd edition 2014
7. TIF "Guidelines for the management of non-transfusion dependent thalassaemia" 2013
8. Specific standards, such as the "International Collaboration for Transfusion Medicine (ICTMG): "Red blood cell specifications for patients with haemoglobinopathies: a systematic review and guideline" 2017
9. ENERCA White Book
10. European Guidelines for the certification of Haemophilia Centres EUHANET 2013.
11. Current literature reviews

The criterion for recognizing any centre as a reference/ expert centre is certainly the quality of services and its patient-centred care, and not just availability of various technical components necessary for thalassaemia (and other haemoglobin disorders) care. It includes following national or international evidence-based guidelines, which allow for good patient outcomes.

A Reference / Expert Centres must, for example:

1. Have the capacity to provide expert diagnosis of the disease as well as its long term complications.
2. Have the capacity to provide expert case management, based on best practice guidelines including a multidisciplinary approach and psychosocial support. These requirements imply experienced healthcare personnel in adequate numbers to ensure continuity of care.

3. Ensure that health care professionals work in a structured environment with clearly defined roles and hierarchy.
4. Maintain a patient registry with the ability to report patient outcomes and other epidemiological information. Electronic information systems must be regarded as essential tools for the provision of quality services.
5. Have regular auditing of clinical and laboratory guidelines.
6. Serve a sufficient number of patients (at least 50 transfusion-dependent-thalassaemia patients) to maintain staff experience. What is a sufficient number of patients is not clear but a consensus should be reached.
7. Provide patients with sufficient knowledge and information to promote partnership models and self-management support.
8. Have a significant contribution to research as evidenced by peer reviewed publications.
9. Establish networking with
  - i. Secondary treatment centres to provide education and share knowledge and expertise as well as expert opinion on challenging cases and
  - ii. Other centres of expertise nationally.
10. Establish networks/collaborations with other Reference Centres outside the country – regional and international – to share best practices.
11. Maintain close links with patient organisations and other community resources at national, regional and international level.
12. Make a major contribution to educational activities.
13. Provide evidence of the improvement of patients' survival, clinical outcomes and quality of life.

In addition, (i) there must be evidence of government and more specifically health system support, (ii) free access of patients to treatment modalities, (iii) the centres' administrative structure, working hours and clinical space availability must also be taken into consideration, with the patient experience in mind, (iv) deficiencies and gaps must be promptly identified and corrected, (v) regularly assess the experience and knowledge of professional staff and (vi) the patient perceptions of the quality of the services and the relationship with the staff should be monitored regularly through professional tools and taken into account in quality assessment.

TIF Standards as described in TIF's project for assessing the quality of the services provided to patients with haemoglobin disorders in the different domains comprising a Reference Centre are outlined in Tables 6 – 12 below:

Table 6

**1. Governance**

- The existence of a hierarchical structure, ordained by law and policy. This should include a chief executive/ managing director and a professional team which is co-ordinated and includes multidisciplinary services, recognising the complex pathology of haemoglobin disorders.
- A clear definition of the centre's mission and the existence of policies and programmes to fulfil the mission.
- Ensuring staff qualifications, experience and continual education
- Monitoring and evaluating the functions of the centre by the management, including staff performance and patient safety.
- The existence of plans for quality improvement and advocacy to health authorities
- Connection with patient support associations, with patient representation on advisory bodies. Taking into account all stakeholders views regarding matters of priority and focus in any quality improvement activity.
- All decisions are based on data, obtained through patient records and outcomes, as well as any new developments that have been noted through publications and trials.
- A culture promoting ethical practices in all aspects of administration and clinical care. Considering internationally accepted patients' rights.

Table 7

**2. Safety Concerns**

- Staff education on safety is programmed.
- Patient identification is clear in individual records (electronic or paper based), of blood transfusions and laboratory results.
- There is effective patient communication and explanation of all interventions.
- Haemovigilance and pharmacovigilance are practised, including drug safety alerts.
- There are evidence-based hand hygiene guidelines.
- There are measures to reduce accidents, such as falls in the centre. A secure environment is planned and regularly inspected. Hazardous material handling and disposal (such as needles), is part of the centre's daily procedures.
- There are treatment rooms, and resuscitation equipment.
- Fire safety and certification by the country's fire services is available. This includes regular testing of any devices required for fire control.
- Cigarette and other smoking is forbidden on the premises.
- Emergency procedures are in place in the event of power and water cuts or contamination. Monitoring water quality is performed regularly.

Table 8

**3. Access to care**

- The centre clearly serves benign haematology patients and does include malignancies as they constitute a dangerous and vulnerable cohabitor.
- Patient flow: there must be adequate numbers of patients of each diagnostic group: at least 50 thalassaemia patients and/or 50 SCD patients for the centre to be regarded as experienced.
- Continuity of care is safeguarded by low staff turnover and the presence of experienced and qualified caregivers.
- Clinical records with lifetime data are kept.
- Multidisciplinary care is provided with a referral system where necessary, and collaboration with in-patient services.
- Networking with secondary centres as well as with other centres of excellence, nationally or internationally is an added value. A twinning programme with an academic centre is also an additional advantage.
- Any existing electronic health record must fulfil all the requirements of patient safety, including patient consent, confidentiality and anonymisation in data storage and sharing of data for research.
- Barriers to patient access, including distance, language, cultural or religious barriers are considered and dealt with.
- Respect for patient rights and time is a must in all cases.
- Informed consent for all procedures is obtained.

Table 9

**4. Partnership model**

- Adequate information to patients/families about the disease and any treatment decisions, including possible side effects, is always provided.
- Patients are given choices about their treatment.
- Self management is encouraged.
- Special attention to patient adherence is given and the patients supported appropriately.
- Workshops for patients/families are held regularly, at least once a year.

Table 10

**5. Guidelines and standards for clinical care**

- Evidence based national guidelines, put together by experts in the field or international guideline (e.g. TIF's) are used in the centre and adhered to.
- Pain screening is performed and a pain management system is in place.
- Assessing the quality of laboratory and other technologies used to monitor patients is the responsibility of the clinical team which must alert the providers of any divergent or inaccurate results.
- Infection control procedures are part of the clinical standards of the centre.
- Availability of food during day care is necessary and the quality and nutritional value must be monitored.
- Blood transfusion procedures and standards according to international directives are kept.
- Any medical treatment, such as IV fluids and exchange transfusions, are provided according to standards that ensure patient safety.
- Continual medical and other professional education are part of the centres long term programme.
- Staff qualifications, skills, knowledge and experience are defined and described along with the job description of each.
- Staff/patient ratio is defined approximately as 1 doctor per 100 patients and 1 nurse per 50 patients.

Table 11

**6. Quality improvement**

- Having surveyed all aspects of the service, and noted all strengths and weaknesses, the survey team will present a report and also make suggestions for quality improvement where necessary.
- Quality improvement is a systematic approach to changes aiming to upgrade services and correct any deficiencies in the governance, structure and functions of the service. "Quality improvement includes better patient experience and outcomes, by changing provider behaviour" (Dr John Ovretveit: "Does improving quality save money?").
- The way in which change is introduced and implemented is a matter of concern and may require expert advice. In this process the following are considered:
- External influences, such as governmental policies or interest, budgetary support, professional requirements.
- Understanding the issues involved at all levels, including why a problem exists.
- Setting goals and monitoring progress.
- Choosing the tools to bring about change. These could be skills development, computerisation, updating guidelines etc.
- Full staff engagement is necessary. There often needs to be a multidisciplinary approach to change making.
- The patient's voice must be involved in all stages of quality improvement. Patient/families can also effectively monitor the effects and benefits of change since they experience the whole 'patient pathway'.
- Studying other centres experience in change making: have the changes been successful elsewhere?

Table 12

**7. Information Management**

- Patient records (paper or electronic) are kept with due consideration to confidentiality, security and accuracy of data.
- The retention time of records in a haemoglobinopathy setting is lifelong, since the current clinical condition may be influenced by past events and disease control (such as iron levels).
- Standard diagnosis codes are kept (e.g. ICD10).
- E-health systems are assessed and tested prior to implementation, for quality and patient safety.
- Protection against loss, unauthorised access or use is ensured.
- Policies and procedures concerning record keeping are clearly directed to all the staff, through documents and training.
- The patient should be clearly identified on each record.
- Those authorised to have access to clinical records are clearly defined.

Through this Certification Programme, TIF may provide:

- On-site audit of the centre's performance by external reviewers with regards to the quality of the processes, outcomes and structures involved in the care it provides to haemoglobinopathy patients.
- Technical support and recommendations for improvement to reach desired outcomes.
- Networking opportunities with other regional and international centres for the exchange of knowledge and expertise.

Successful centres are granted the Certificate of TIF Collaborating Reference Centre for Haemoglobinopathies, and provided with needs-based technical support and personalised recommendations for continuous improvement in order to reach the desired outcomes.

The Certificate is valid for a period of 2 years before the evaluation team is called back to the centre to ensure that quality of care is maintained.

The key goals of this programme are to:

- Provide authoritative scientific opinions and advice on key topics in clinical management including accurate diagnostic techniques, blood safety, correct iron monitoring and dealing with complications through a multidisciplinary approach in order to achieve continuous improvement within the healthcare delivery system for haemoglobinopathies worldwide;
- Provide a mechanism for internal and external peer evaluation towards excellence and ensure each centre's accountability for the service they provide to thalassaemia patients;
- Establish an international network of reference centres for the delivery of quality healthcare services for haemoglobinopathies worldwide;
- Improve patient and programme safety in all activities and initiatives;
- Facilitate all patients' access to expert management and contribute to the reduction of inequalities in the care that patients receive;
- Provide educational and training outlets for the centre's staff for stimulating the organization's quality improvement efforts. Secondary centres will have the opportunity to send staff for training and continuing medical education to the certified centres;



- Provide networking opportunities with other regional and international treating centres and benefit from staff training and tele-consultation to enable stakeholders to promote quality services from central level (government) and;
- Enhance community confidence in thalassaemia care in all affected countries.

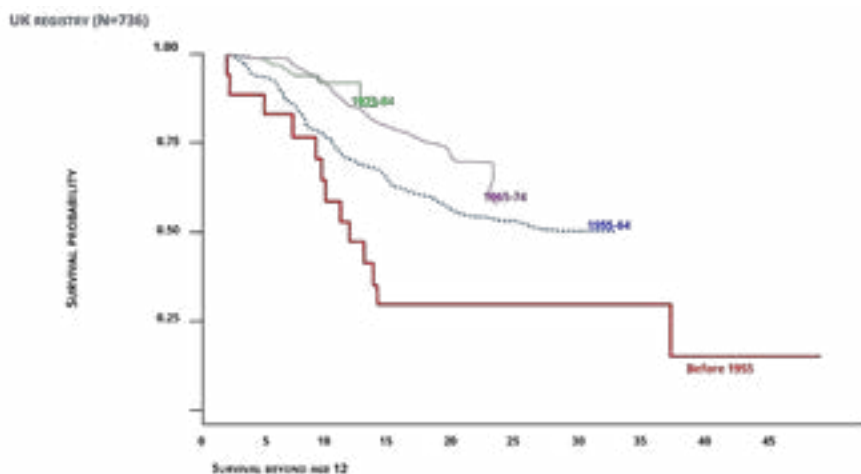
Certainly, such upgrading of a centre's services and quality standards requires considerable national collaboration, support and funding and this can only be achieved if and when its value to the patients, the healthcare professionals and the healthcare system itself are well recognised by the competent authorities; it must be emphasised that TIF's close and official collaboration with the national authorities is a prerequisite for any activity related to this project.

This initial work of TIF cannot and is neither meant to replace the value of quality assessment tools, including simple but valuable ones, such as audit and peer review, already practiced in many countries mainly of the Western world. Moreover, it is certainly not meant to replace those dedicated accrediting organisations which offer their work at a cost to assess and establish quality standards in the services provided by health institutions – hospitals, clinics or centres, public or private.

Indeed, TIF strongly encourages competent authorities to adopt such a methodology where and when possible.

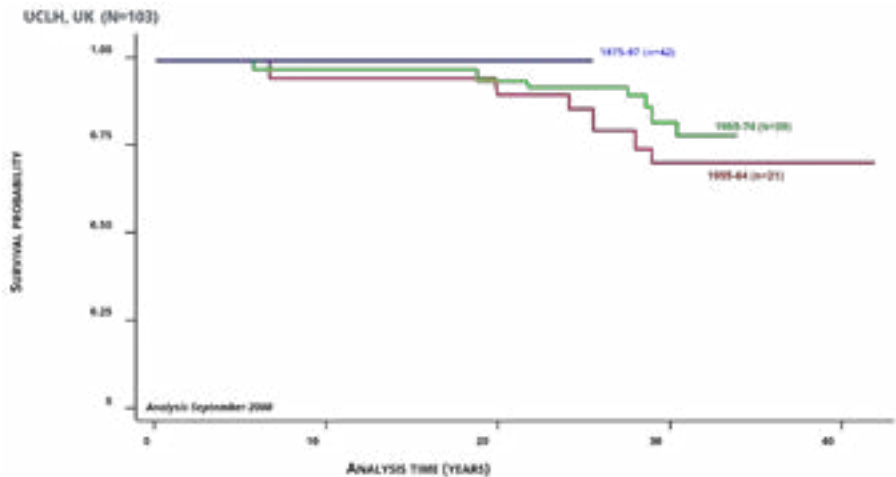
TIF through its programme outlined above, mainly aims to initiate an effort towards raising awareness on the value of MDC and Reference Centres in improving survival and quality of life of patients with these disorders, as has already been documented in a few countries (Figures 1-3). It aims to offer a simpler methodology as a first step to support the upgrading of services provided by treating centres particularly, but not only, of the developing economies by introducing the practice of MDC and by better acknowledging the value of the idea of pooling knowledge and experience and sharing best practices through the existence of Reference/Expert Centres.

Figure 1



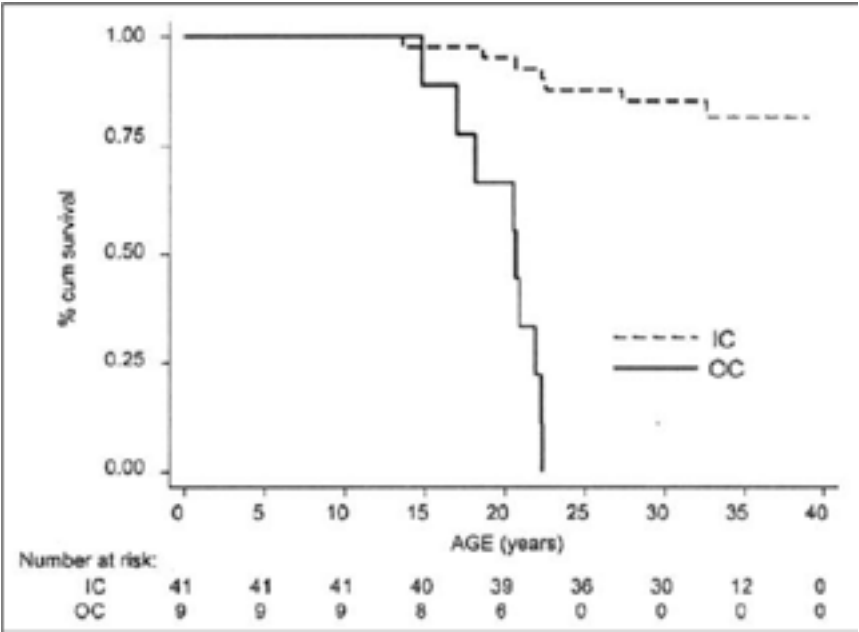
Modell et al. Lancet. 2000;355:2051.

Figure 2



Porter & Davis *Best Pract Res Clin Haematol*, 15, 328-68 2002

Figure 3



Kaplan-Meier overall survival curves of patients referred to specialised centres (IC) versus patients referred to non-specialised centres (OC). Log-rank  $P$ -value  $<0.0001$ ; hazard ratio of OC versus IC adjusted for sex (Cox model): 18.1, 95% confidence interval = 4.7-69.0;  $P<0.001$ . IC, specialised centre; OC, non-specialised centre.

Reproduced with permission from (Forni 2009).

## Discussion and Conclusions

Patients globally are indeed faced with huge unmet needs both in basic medical care and to an even larger extent with regards to accessing MDC and expert review of their clinical status in Reference Centres with accumulated expertise. Both of these latter elements are unfortunately largely missing and according to TIF's records, accumulated through its 35 years' work at country level in over 60 countries across the world, such components are provided to less than 2% of the patients globally, constituting a severe violation of their rights both as humans and as patients.

It is hoped that the work of every country around the world towards promoting the UN Sustainable Development Goals 2030 (United Nations, 2021) and the work of the WHO on disease-specific but also many other relevant resolutions [WHO EB118.R1 on Thalassaemia & other Hemoglobinopathies (WHO, 2006b) and Resolution WHA59.20 on sickle cell anaemia (WHO, 2006a)], recommendations and programmes including blood and patient safety, will contribute towards achieving significant progress in the prevention and management of these disorders and will 'allow' them to further improve and introduce more specialised care, as described in this Chapter.

Healthcare services are becoming increasingly strained and healthcare authorities worldwide need to invest in integrated care particularly in the case of chronic, complex diseases such as the haemoglobin disorders, to first and above all deliver higher quality services for the patients while at the same time containing costs. Unfortunately, existing evidence of the cost-effectiveness of integrated care is limited particularly with regards to haemoglobin disorders. Future economic evaluation should target methodological issues to aid policy decisions with more robust evidence based on reliable, nationwide data (Aguilar Martinez et al., 2014).

It is also hoped that the contribution of this updated 4th edition of TIF's Guidelines for the Management of Transfusion Dependent Thalassaemia, and the work of TIF at large, greatly supported by the WHO, the UN, the United Nations Economic and Social Council (ECOSOC), the EU, a large group of medical and scientific bodies and experts including the authors of this book, and very importantly by patients and families themselves, at national and international level, will contribute to the efforts of every country in providing a better future and ensuring more equity for patients with thalassaemia and other haemoglobin disorders.

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GENERAL TIMETABLE FOR CLINICAL AND LABORATORY INVESTIGATION			
INITIAL ASSESSMENT / DIAGNOSIS			ROUTINE TESTS
<b>Laboratory Tests</b> <b>Recommended before 1st Blood Transfusion</b>			
CBC Hb; Indices; Nucleated Red Cells; Platelets	Hb Fractionation	Quantification HbA; HbA2; HbF	
		Quantification Hb Variants	Monthly
	Molecular Mapping	α-Globin Gene Cluster	Transfusion Reactions
		β-Globin Gene Cluster	Weight
	Blood Group Antigen Typing	ABO System	Blood Pressure
		Rh System and Subgroups	Complete Blood Count Nucleated Red Cell Count
	HLA Type		New Red Cell Antibodies
	G6PD Quantification		Temperature
Clinical Tests (Repeat at each visit and/or as indicated)			Pulse
<div> <div>Clinical History</div> <div>Physical examination</div> <div>Symptoms</div> <div>Height; Weight; BMI</div> <div>BP; HR; SpO2</div> <div>Vaccinations</div> <div>Size - Liver Spleen</div> </div>			O2 Saturation

GENERAL TIMETABLE FOR CLINICAL AND LABORATORY INVESTIGATION			
ROUTINE TESTS	Clinical Testing	Laboratory Testing	Imaging Testing
Children and Adolescents			
3-Monthly	6-Monthly	12-Monthly	As Clinically Indicated
Height	Growth Velocity	Bone Deformity	
Weight	Tanner Stage (Pubertal Development; from 10 years)	Bone Age (for the first 10 years of life)	
Adults			
3-Monthly	6-Monthly	12-Monthly	As Clinically Indicated
Serum Ferritin	Zinc Levels	Audiology	Holter ECG
Transferrin Saturation	Magnesium Levels	Ophthalmology	Right Cardiac Catheterisation (if Elevated TRV)
Transaminases	Vitamin D Levels	Dental	Liver Biopsy (Optional)
γ-GT, ALP, LDH	Cholesterol / Triglycerides	ECG	Duplex Abdominal Ultrasonography
Bilirubin (t,d)	Urinalysis	Ergospirometry (if clinically indicated)	MRI of Hypothalamic - Pituitary Region
Total Protein		Echocardiography	MRI Pancreas
Albumin		MRI Cardiac T2*	Thyroid Ultrasound
Blood Urea		MRI Liver Iron (LIC)	PQCT
Creatinin		Abdominal Ultrasound (every 6 ms if liver disease)	Lateral Spinal X-Ray for Spinal Fractures
Uric Acid		DEXA Scan	Direct Coomb's Test
Electrolytes		Fibroscan (6ms if liver disease)	C-Reactive Protein
Calcium Ionised		HAV Serology (unless vaccinated)	Serum Folate
Phosphate		HBV Serology/PCR (unless vaccinated)	Creatinine Clearance
Fasting Sugar		HCV Serology/PCR	PT, PTT, Fibrinogen (if Active Disease)
Creatine Kinase		HIV Serology	Alpha Fetoprotein
		OGTT	Troponins
		Thyroid Function (>9yrs)	Nitrouretic Peptides
		Parathormone (>16yrs)	Fructosamine (if Diabetes)
		LH-ICMA (>12-14yrs)	Fasting Plasma Insulin
		FSH (12-14yrs)	GH-Secretion
		Estradiol/Testosterone (12-14yrs)	Osteocalcin
		IGF-1, IGF BP-3	Test for Bone Formation e.g. Bone Specific ALP
		Cortisol + after ACTH Stimulation	Test for Bone Resorption e.g. RANKL

## ABOUT THE THALASSAEMIA INTERNATIONAL FEDERATION (TIF):

Thalassaemia International Federation, a non-governmental, patient driven umbrella organisation, established in 1986, supports today, the rights of patients for access to quality health, social and other care through its work with over 200 national thalassaemia associations in 62 countries across the world. It was founded by a small group of doctors and patients/parents who represented National Patient Associations, mainly from Cyprus, Greece, Italy, UK and USA i.e. Countries where thalassaemia had been recognized early as a genetic, hereditary disorder with huge medical, public health, social and economic repercussions if left unaddressed in terms of both effective prevention and management. Thus, these were the countries where strong research activity was initiated and the first control programmes were implemented in the early 1980s, with measurable success. The rationale of these founding members lay on the establishment of an international umbrella organisation to build on the accumulated experience and the knowledge gained, aiming to support the efforts of other countries since by the mid-1980s the worldwide prevalence of the disease had been well verified.

**Our Mission:** The prioritisation of thalassaemia on national health agendas and the development and implementation of effective disease – specific control (prevention and clinical management) programmes within national healthcare systems based on universal coverage

**Our Vision:** To support the provision of equal access of every patient with thalassaemia to high quality health, social and other care in a truly patient-centred healthcare setting

**Our Values:** Transparency, reliability, ethos, accountability, independence and patient-centredness

### **Our Work:**

- Education • Advocacy • Collaborations/ Networking
- Research • Raising Awareness

### **Our Partners:**

- **World Health Organisation:** in official relations since 1996
- **United Nations:** in special consultative status with the United Nations Economic and Social Council (ECOSOC) since 2017
- **Council of Europe:** participatory status in the Conference of International NGOs since 2019
- **European Union:** official partners of the European Commission in the field of Health since 2018

**Our Motto:** Unity & Knowledge constitute our Strength!

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