

GUIDELINES FOR THE MANAGEMENT OF TRANSFUSION DEPENDENT THALASSAEMIA (TDT)

3RD EDITION

Editors

Cappellini MD

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Porter J

Taher A

Viprakasit V



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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)

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FOREWORD

It is now established that the hereditary haemoglobin disorders, previously lethal from childhood, can now be treated as chronic conditions, in which the patient may aspire to a long and productive life. This requires optimum lifelong care, which in turn requires physician experience and patient cooperation. Both depend on adherence to internationally accepted and evidence based guidelines. It is an ethical requirement to provide accurate and unbiased information and the quality of information is an essential element in offering good quality patient care.

Thalassaemia International Federation (TIF) through a panel of experts has published guidelines both for transfusion dependent thalassaemia as well as non-transfusion dependent thalassaemia. This is one of the main objectives of the creation of the Thalassaemia International Federation, which was established in 1987 with the objective to promote care which will result in improved survival and a good quality of life. Through these guidelines quality, evidence based information is offered for the clinicians to help them in their effort to provide the best possible care, in a chronic disease that becomes more complex as the patient grows in years.

Well treated thalassaemia will lead the patient beyond childhood, to an age where there is multiple organ involvement. The consequences, mainly of iron overload, cannot be totally prevented even by present day iron chelation treatment. This means that health professionals at several levels and different specialties need to be expert in all aspects of patient care. For this reason these guidelines target health professional of various disciplines.

Patients and families must deal with a difficult and lifelong treatment schedule, disability, emotional and other psychological reactions which require adaptation and coping, complex as well as difficult medication regimens (such as the daily subcutaneous infusions of chelating agents in Thalassaemia major), difficult life style and social adjustments and the need for specialized multidisciplinary care. The patients must therefore have the confidence and skills to manage their condition under the appropriate guidance and contribute to treatment decisions. Health professionals, in their turn, need to keep up with the various advances and require continuing education.

Thalassaemia care in many countries has achieved survival of Thalassaemia patients well into adult life mainly by adopting good blood transfusion and chelation practices but also by adopting follow up protocols which aim to detect early and prevent if possible complications to vital organs. Optimum treatment will also improve quality of life which in addition to avoiding complications can be achieved by making psychosocial support a priority in management.

The development of nuclei of expert medical specialists allows sharing of knowledge through networking at the national and international level. The establishment of expert centres for these anaemias which in most countries are considered rare, has been shown to improve survival and such centres can become reference centres for professionals to refer cases for periodic monitoring and trouble shooting. This concept of sharing expertise is particularly important where the haemoglobin disorders have a low prevalence. The guidelines therefore have the dual purpose of increasing skills and knowledge, but also to improve communication between centres and professionals by emphasising the need for multi-disciplinary care.

Another motive for the publication of the guidelines is to encourage research activity. A guideline is of value as the quality of the scientific evidence behind it is scrutinized by experts and accepted. New advances, improved approaches, are steps for achieving better survival and will hopefully lead, step by step, to a final cure. The doctor working outside an academic unit will be asked to contribute to this process by taking part with patients in clinical trials. He needs to be aware where science is taking patient care and cannot continue to be a passive bystander. TIF and the patients it serves are acutely aware of this and expect physicians to be ready to adopt new approaches where these are assessed, by scientific evidence as being effective and safe.

Research also increases the number of stakeholders that the thalassaemia patient requires to support aspirations for a better life. Apart from the clinical team, with its interdisciplinary nature, basic science, pharmaceutical industries, international organizations, non-governmental organizations, sociological and legal approaches become more active in influencing decisions. The adult now patient has to contribute along with his/her physician to a suitable treatment plan. Patient information is now a part of doctors duties and the patient's rights.

For these reasons the new guideline takes a holistic approach to patient care, not neglecting lifestyle and the issue of psychosocial support.

TIF, as a patient centered organization which has been striving for decades to support effective prevention and optimum patient care, wishes to thank and is greatly indebted to each and everyone of the dedicated scientists and medical specialists who have offered their time and labour freely to this vast and challenging work of updating the existing Guidelines. Their knowledge and scientific judgment are now disseminated across the world and TIF will exert every possible effort to ensure that health authorities in all countries and regions of the world adopt and apply these recommendations even in times of economic austerity, for the benefit of citizens who, despite their inherited position, are willing and able to return the investment through their own labour and positive contribution to society if kept healthy and with an acceptable quality of life.

Today we can safely state that thalassaemia is considered to be both effectively prevented and appropriately treated and no Government or national health authority at the national or international level has the right to deprive the basic human right of everyone for health.

On behalf of the Board of Directors

Panos Englezos

President

Dr Androulla Eleftheriou

Executive Director

Thalassaemia International Federation

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). 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 the 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. These changes have challenged health 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 require 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 counseling programs established to educate populations about the risks of having affected children; 2. population screening or screening in prenatal clinics where if a women is carrier the partner is screened and if positive, following counseling they are offered a prenatal diagnosis and termination of affected fetuses. Prenatal diagnosis programs well established in the Mediterranean region resulting in a major reduction in newborns with severe forms of thalassaemias are now available in several other countries such as China, India, Iran, Lebanon, Pakistan, Singapore, Thailand and several other countries are establishing similar programs. Whatever are 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:

- Standards and guidelines for laboratory services
- National guidelines for the management of thalassaemia
- Epidemiological information and surveillance
- Establishment of an educational programme for health 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 harmonize and optimize the quality of treatment as much as possible. The need for management guidelines for Transfusion Dependent Thalassaemias (TDT) is clear. Throughout the past four years, six major TDT management guidelines became 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 light of a 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.

This updated third 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
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1

GENETIC BASIS, PATHOPHYSIOLOGY AND DIAGNOSIS

Authors Vip Viprakasit and Raffaella Origa
 Reviewer Suthat Fucharoen

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 α -globin cluster, comprising the ζ - and α -globin chains, and the β -globin cluster, comprising the globin chains ϵ , γ , β and δ . 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. "Foetal" haemoglobin (HbF), which constitutes the predominant oxygen carrier during pregnancy and is a $\alpha 2\gamma 2$ molecule;
3. Adult" haemoglobin (HbA $\alpha 2\beta 2$), which replaces HbF shortly after birth; and
4. A minor adult component, HbA₂ ($\alpha 2\delta 2$).

The process of different haemoglobin species being produced and stop at certain period 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 HbA, 2-3% of HbA₂ and traces of HbF.

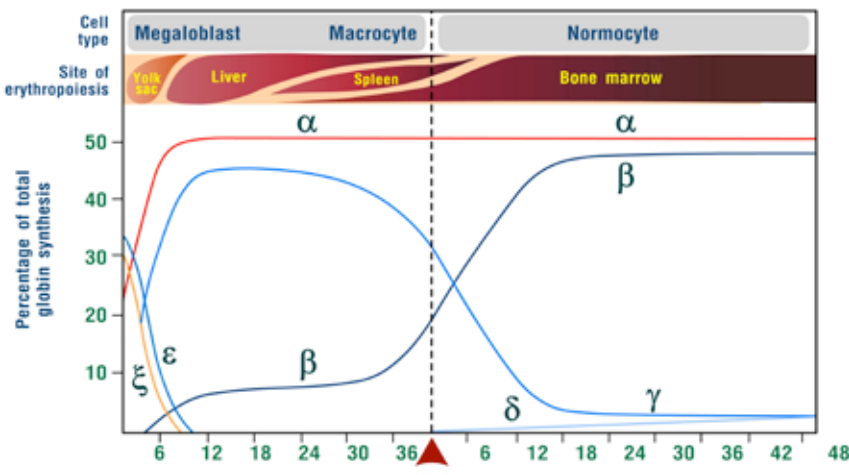


Figure 1. Globin synthesis at various stages of embryonic, foetal and adult erythroid development.

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 α gene cluster) and 11 (the β 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 the erythroblasts in the bone marrow. Globin chains must have the correct structure and be trimmed in such a way that the number of α -chains precisely matches that of the β -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: Definitions And Worldwide Distribution

The term "**thalassaemia**" refers to a group of blood diseases characterised by decreased or absent synthesis of normal globin chains. According to the chain whose synthesis is impaired, the thalassaemias are called α -, β -, γ -, δ -, $\delta\beta$ -, 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 HbE and Hb Lepore). From a clinical point of view, the most relevant types are α - and β -thalassaemias, resulting from the decrease of one of the two types of polypeptide chains (α or β) that form the normal adult human haemoglobin molecule (HbA, $\alpha_2\beta_2$).

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 β thalassaemia is reported in 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 where thalassaemia was previously absent. As for α -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, Gulf region, the Middle East, Greece, Italy, and Northern Europe. In Southeast Asia the frequency of is so high to cause a major public health problem because of the elevated number of patients with severe HbH disease and of fetuses with HbBart's hydrops foetalis.

As autosomal recessive condition, heterozygotes of either α - or β -thalassaemia are usually asymptomatic and require no treatment. Homozygotes and compound heterozygotes of thalassaemia alleles result in thalassaemia syndromes or diseases. In addition, interactions of thalassaemia and corresponding haemoglobinopathies e.g. HbE, Hb C or Hb S with β -thalassaemia or Hb Constant Spring (Hb CS) with α -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**.

Spectrum of Thalassaemia Syndromes

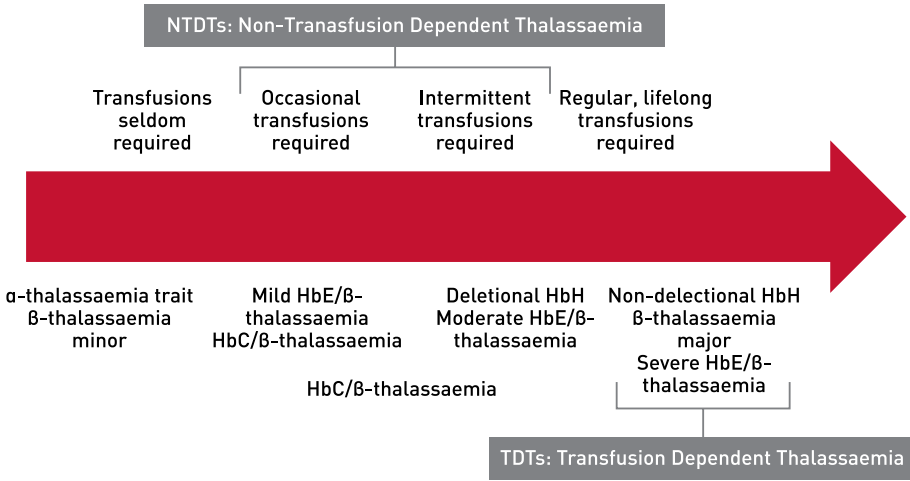


Figure 2. Phenotypic classification of thalassaemia syndromes based on clinical severity and transfusion requirement.

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 β -thalassaemia major, severe HbE/ β -thalassaemia, transfusion dependent HbH disease or HbH hydrops and surviving HbBart's hydrops. This TDT group is the main focus of this present clinical practice guideline (CPG). The groups of NTDT patients include β -thalassaemia intermedia, HbE/ β -thalassaemia, and HbH disease. The CPG for this category of patients has been separately prepared and published recently by TIF (2013).

β -Thalassaemia

Phenotypic heterogeneity

β -thalassaemia includes three main forms: Thalassaemia Major variably referred to as "Cooley's Anaemia" and "Mediterranean Anaemia", Thalassaemia Intermedia and Thalassaemia Minor also called " β -thalassaemia carrier", " β -thalassaemia trait" or "heterozygous β -thalassaemia". Apart from the rare dominant forms, subjects with β -thalassaemia major are homozygotes or compound heterozygotes for β^0 or β^+ genes, subjects with thalassaemia intermedia are mostly homozygotes or compound heterozygotes and subjects with thalassaemia minor are mostly heterozygotes.

Pathophysiology

The basic defect in β -thalassaemia is a reduced or absent production of β -globin chains with relative excess of α -chains. The direct consequences are a net decrease of the haemoglobin production and an imbalance of the globin chain synthesis. The former is more evident in carriers, leading to a reduction of mean cell haemoglobin and mean cell volume, and has a minor clinical significance. The latter has dramatic effects on the red cell precursors, ultimately resulting in their extensive premature destruction in the bone marrow and in the extramedullary sites. This process is referred to as "**ineffective erythropoiesis**" and is the hallmark of β -thalassaemia. Peripheral haemolysis contributing to anaemia is less

prominent in thalassaemia major than in thalassaemia intermedia, and occurs when insoluble α -globin chains induce membrane damage to the peripheral erythrocytes. The first response to ineffective erythropoiesis and anaemia is an increased production of erythropoietin, causing a marked erythroid hyperplasia, which, in turn, may produce skeletal deformities, osteoporosis, and occasionally extramedullary masses, and contributes to splenomegaly. Untreated or undertreated thalassaemia major patients 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. Ineffective erythropoiesis is also associated with increased iron absorption, which occurs mainly from increased intestinal absorption of iron caused by deficiency of hepcidin, a 25-amino acid peptide produced by hepatocytes that plays a central role in the regulation of iron homeostasis. The pathophysiology of β -thalassaemia is summarised in **Figure 3**.

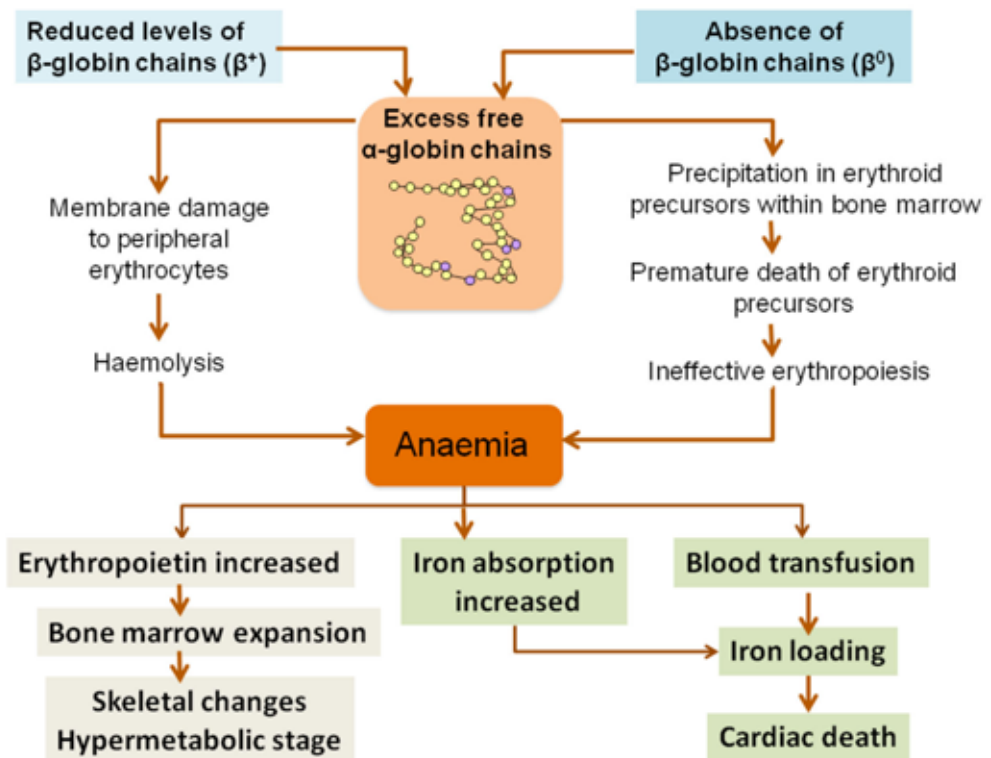


Figure 3. Effects of excess production of free α -globin chains in β -thalassaemia.

The degree of globin chain imbalance is determined by the nature of the mutation of the β -gene. β^0 refers to the complete absence of production of β -globin on the affected allele. β^+ refers to alleles with some residual production of β -globin (around 10%). In β^{++} the reduction in β -globin production is very mild. More than 200 β -thalassaemia mutations have been reported to date.

Table 1 summarises the common types of β -thalassaemia mutations according to ethnic distribution and severity. A comprehensive list of β mutations can be found on the internet at <http://globin.cse.psu.edu/globin/html/huisman>.

Table 1. Common types of β -thalassaemia, their severity and ethnic distribution

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

HGVS, Human Genome Variation Society.

Clinical diagnosis

Since the activity of the normal β gene on the allelic chromosome makes enough stable globin, under normal circumstances, β -thalassaemia trait has no important clinical effects.

Clinical presentation of β -thalassaemia major usually occurs between 6 and 24 months 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 hypermetabolic state or inter-current infection, and progressive enlargement of the abdomen caused by spleen and liver enlargement may occur. In some resource-limited settings, 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 facie (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.

β -thalassaemia intermedia should be suspected in individuals who present at a later age with similar but milder clinical findings. At the severe end of the clinical spectrum, patients present between the ages of 2 and 6 years and although they are capable of surviving without regular blood transfusion, growth and development are retarded. 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 pathologic fractures of long bones and formation of erythropoietic masses that primarily affect the spleen, liver, lymph nodes, chest and spine. Enlargement of the spleen is also a consequence of its major role in clearing damaged red cells from the bloodstream. Leg ulcers are frequent. While in β -thalassaemia major haemosiderosis is secondary to the chronic transfusions, individuals with β -thalassaemia intermedia are also at risk of iron overload secondary to increased intestinal iron absorption.

Haematologic diagnosis

Heterozygous carriers of β -thalassaemia, usually display a low mean cellular haemoglobin (MCH), low mean cell volume (MCV), and an increased level of HbA₂, which may be associated with low normal or slightly subnormal haemoglobin levels. Peripheral blood smear shows less severe erythrocyte morphologic changes than affected individuals and erythroblasts are normally not seen. β -thalassaemia major is characterised by reduced haemoglobin level (<7 g/dl), MCV >50 and <70 fl and MCH >12 and <20 pg. Thalassaemia intermedia is characterised by Hb level between 7 and 10 g/dl, MCV between 50 and 80 fl and MCH between 16 and 24 pg. Affected individuals show microcytosis, hypochromia, anisocytosis, poikilocytosis (spiculated tear-drop and elongated cells), target cells and erythroblasts. The number of erythroblasts (nucleated red blood cell) is related to the degree of anaemia and is markedly increased after splenectomy. In general, these abnormal red blood cell morphology and features share among different types of thalassaemia syndromes even interactions with haemoglobin variants such as HbE/ β -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.

In β^0 thalassaemia homozygotes, HbA is absent and HbF constitutes the 92-95% of the total Hb. In β^+ thalassaemia homozygotes and β^+/β^0 genetic compounds HbA levels are between 10 and 30% and HbF between 70-90%. HbA₂ is variable in β thalassaemia homozygotes and it is enhanced in β thalassaemia minor. HbF can readily be detected by acid elution test (F-cell staining) and alkali denaturation.

Molecular analysis

Commonly occurring mutations of the β globin gene are detected by 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. If targeted mutation analysis fails to detect the mutation, β globin gene sequence analysis can be used to detect mutations in the β globin gene.

Correlation genotype-phenotype

The extent of globin chain imbalance is the main determinant of clinical severity in β -thalassaemia. Therefore, the presence of factors able to reduce the globin chain imbalance results in a milder form of thalassaemia. One of the most common and consistent mechanisms is homozygosity or compound heterozygosity for two β^+ -thalassaemia mild and 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 α -thalassaemia or of genetic determinants that increase gamma-chain production. Deletion and non-deletion HPFH mutations, associated with a high HbF level in carriers, when in genetic compounds with severe β -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 β 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 HbF levels in healthy adults and are associated with the mild thalassaemia intermedia phenotype and with a delayed need of transfusions in patients with homozygous β zero thalassaemia. Furthermore, Bcl11A seems to be involved in the regulation of the haemoglobin switching process.

In some instances, heterozygous β -thalassaemia may lead to the thalassaemia intermedia phenotype instead of the asymptomatic carrier state. Most of these patients have excess functional α globin genes (α gene triplication or quadruplication) which increases the imbalance in the ratio of α /non- α globin chain synthesis. Moreover, rare mutations that result in the synthesis of extremely unstable β 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 the recent years. The most studied is the presence of (TA)₇ polymorphism in the promoter region of the uridine diphosphate-glucuronosyltransferase gene, which in the homozygous state is associated with the Gilbert syndrome, and was associated with the development of cholelithiasis in thalassaemia major and intermedia. Other candidate genes are the apolipoprotein E ϵ 4 allele, which seems to be a genetic risk factor for left ventricular failure in homozygous β -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

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

HbE is characterised by the substitution of lysine for glutamic acid at position 26 of the β -globin chain. The mutation G→A at codon 26 of the β -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 (HbE); HbE constitutes of 25-30% of total haemoglobin in HbE carrier, instead of expected 50%. In other words, the codon 26 G→A mutation results both in a qualitative and quantitative β -globin gene defect.

HbE is the most common abnormal haemoglobin in South East Asia, with a carrier frequency of up to 50% in some regions. It is also prevalent in parts of the Indian subcontinent, including India, Pakistan, Bangladesh and Sri Lanka. Heterozygotes for HbE are clinically normal and manifest only minimal changes in red blood cell indices, with a presence HbE on haemoglobin analyses. HbE can be easily detected using a special dye: dichlorophenolindophenol (DCIP). Homozygotes for HbE are clinically silent and may be only mildly anaemic. The peripheral blood smear examination shows microcytosis with 20-80% of target red cells, while Hb electrophoresis shows 85-95% of HbE and 5-10% of HbF. Few individuals with homozygous HbE with up to 20% of HbF have been identified.

Genetic compounds for HbE and β -thalassaemia, which are also common in South East Asia, have clinical manifestations variable in severity—from thalassaemia intermedia to severe transfusion-dependent thalassaemia major. These can be classified into three categories:

- 1) Mild HbE/ β -thalassaemia: It is observed in about 15% of all cases in Southeast Asia. This group of patients maintains Hb levels between 9 and 12 g/dl and usually does not develop clinically significant problems in early age. However some patients can suffer from growth failure, iron overload and other complications similar to those of NTDT patients.
- 2) Moderately severe HbE/ β -thalassaemia: The majority of HbE/ β -thalassaemia cases fall into this category. The Hb levels remain at 6-7 g/dl and the clinical symptoms are similar to β -thalassaemia intermedia or NTDT. Transfusions are not required unless infections precipitate further anaemia. Iron overload may occur.
- 3) Severe HbE/ β -thalassaemia: The Hb level can be as low as 4-5 g/dl. Patients in this group manifest symptoms similar to β -thalassaemia major and are treated as thalassaemia major or TDT patients.

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

Hb Lepore is another structural β variant resulting from a fusion of the δ and β globin genes. The homozygous state of Hb Lepore or Hb Lepore and β -thalassaemia can result in moderate to severe transfusion-dependent β -thalassaemia syndromes.

Haemoglobin S (HbS) disorders are the most common haemoglobin variant in the world, results from a substitution of valine for glutamic acid at position 6 of the β -globin chain. The interaction of β -thalassaemia with HbS results in a syndrome that most closely resembles the sickling disorders. This syndrome typically does not require transfusions and is not associated with iron overload. The management of sickle thalassaemia should follow the existing NIH guidelines for management of sickle cell disorders.

(See <http://www.nhlbi.nih.gov/health/prof/blood/sickle/sick-mt.htm> for more information.)

α -Thalassaemia

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

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

α -Thalassaemia trait: Subjects with two residual functional α -genes either by deletions that remove two linked α -globin genes from the same chromosome or α^0 - (--/aa) or combination of deletional α^+ -thalassaemia (-a/-a), have mild hypochromic and microcytosis. Their MCV and MCH are usually lower than 80 fl and 27 pg, respectively.

Less commonly, mutations caused by single or few nucleotide deletions or alterations known as non-deletional α -thalassaemia (α Ta/ or α T/) have been identified in several populations from Mediterranean countries to Southeast Asia and China. Some non-deletional mutations also generate abnormal haemoglobin specie such as Hb Constant Spring (Hb CS) or Hb Paksé (Hb PS). These α -globin variants causes by mutations at the termination codon of the α -globin gene resulting in an extended α -globin chain with 31 extra amino acid residues and ineffectively synthesised. Heterozygotes for these non-deletional α -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: Deletions or abnormalities of three globin genes such as deletional type (--/-a) or non-deletional type (--/ α^+ Ta or --/ α T) result in HbH disease, 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 HbH disease are

usually more severe than patients with deletional HbH disease. For example, co-inheritance of Hb Constant Spring and the deletion of two α -genes results in a severe form of HbH disease in which up to 20% of these patients require frequent blood transfusion and splenectomy. Most patients with HbH disease can be managed as recommended in the NTDT's guideline.

Rarely, few HbH patients with specific non-deletional mutations i.e. Hb Pak Num Po ($\alpha\alpha^{\text{PNP}}$), Hb Quong Sze ($\alpha^{\text{QZ}}\alpha$), Hb Adana ($\alpha^{\text{CD59}}\alpha$) have a severe phenotypes mimic those of α -thalassaemia major; early onset of anaemia (at birth or within 6 month of age), marked anaemia (Hb <5 g/dL), huge hepatosplenomegaly and failure to thrive. This condition is known as **transfusion dependent HbH disease or HbH hydrops** since some patients even developed severe anaemia and hydropic changes in utero.

HbBart's hydrops foetalis, the most severe clinical manifestation of α -thalassaemia, is generally associated with the absence of all four α -globin genes, severe foetal anaemia and death in utero. In addition, several maternal complications including preeclampsia, ante partum haemorrhage, dystocia etc. are common in pregnant women with Hb Bart's hydrops. Absence of α -globin genes in "cis" position in the same chromosome (α^0 -thalassaemia, --/) is common in South East Asia and the Far East, while it is rare in the Mediterranean area and very rare in Africa. This different distribution explains why HbBart's hydrops foetalis syndrome and HbH disease are common in South East Asian countries and China, rare in Mediterranean populations and almost absent in the African population. The complete loss of α -globin production from foetal stage results in a tetramer formation of unpaired α -globin chains (α_4) and presents as HbBart's by haemoglobin analyses. With advanced foetal medicine including intrauterine transfusion, several affected foetus with HbBart's hydrops have survived their intrauterine ordeals. The number of these surviving HbBart's hydrops is increasingly recognised. However these individuals would become transfusion dependent and require life-long blood transfusion. Therefore patients with HbBart's or HbH hydrops or transfusion dependent HbH disease must be treated as TDT patients and follow the recommendations of this present guideline.

Pathophysiology

Reduction of α -globin synthesis results in a decrease production of HbA ($\alpha_2\beta_2$) and reduced haemoglobinisation. In addition, excess unpaired β -globin chains can form tetramers (β_4) that do not physically stable and precipitate to the red cell membrane surface causing oxidative damage and shortening of red cell survival. The formation of β -globin tetramers can be detected by haemoglobin analysis and identified as HbH. The presence of HbH increases during acute febrile illness due to increase body temperature. In non-deletional α -thalassaemia in particular mutations that generate β -globin variants such as Hb CS, this variant can directly precipitate at the membrane surface and generate reactive oxygen species even at the steady state. Therefore, patients with non-deletional HbH disease are usually more severe than those with deletional HbH.

Hematologic diagnosis

Similar to β -thalassaemia syndromes, patients with HbH disease have hypochromic microcytic anaemia with a baseline of haemoglobin of 4-13 g/dl. Increased polychromasia and reticulocytosis are observed and can further be augmented during acute infectious episode or haemolytic crisis. Nucleated and basophilic stippling positive red blood cells are commonly present in a more severe phenotypes such as HbBart's hydrops and severe non-deletional HbH disease. Detection of HbH as HbH inclusion body in peripheral blood smear using a 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 HbH (β_4) and Bart's (β_4) is characteristic of α -thalassaemia syndromes. The levels of HbH measured can be varied from < 1% to up to 40% (normally range 10-15%) due to sensitivity of tests, laboratory expertise, type of instruments and the quality of blood samples. HbH might not be readily identified through some platforms of liquid chromatography; a manual identification using the presence of haemoglobin specie at a specific retention time (RT) is required. Due to a lack of available α -globin chains, HbA₂ ($\alpha_2\delta_2$) is reduced. In patients with non-deletional HbH disease especially HbH/Hb CS, Hb CS variant can be detected with a very low amount (1-4%).

Molecular diagnosis

Similar to β -thalassaemia, common α -thalassaemia mutations are readily detected using PCR based technology. As the majority of α -thalassaemia caused by gene deletions, a multiplex set of "GAP-PCR" to amplify the breakpoint fragments are widely used. The most commonly used methods for known non-deletional mutations are reverse dot blot analysis, primer specific amplification or PCR following by enzymatic digestion. The complete both α^1 and α^2 -globin gene sequencings are required in cases that the common PCR panels fail to identify mutation. For rare or unknown deletions, a set of multiplex ligation-dependent probe amplification (MLPA) can be used to map the α -globin gene cluster. This technique has completely replaced the conventional chromosome mapping using Southern blot analysis due to its high reliability, sensitivity without the need of radioactive detection.

Since almost all thalassaemia conditions as aforementioned presented with hypochromic microcytic anemia, therefore diagnosis of thalassaemia should be considered in allthose who have such abnormal red blood cell features. However, it is important to exclude the possible cause of iron deficiency anemia that remains common in several part of the world. Summary of diagnostic measures for patients with hypochromic and microcytosis and further with diagnostic features of common thalassaemia syndromes are shown in **Figures 4, 5, 6.**

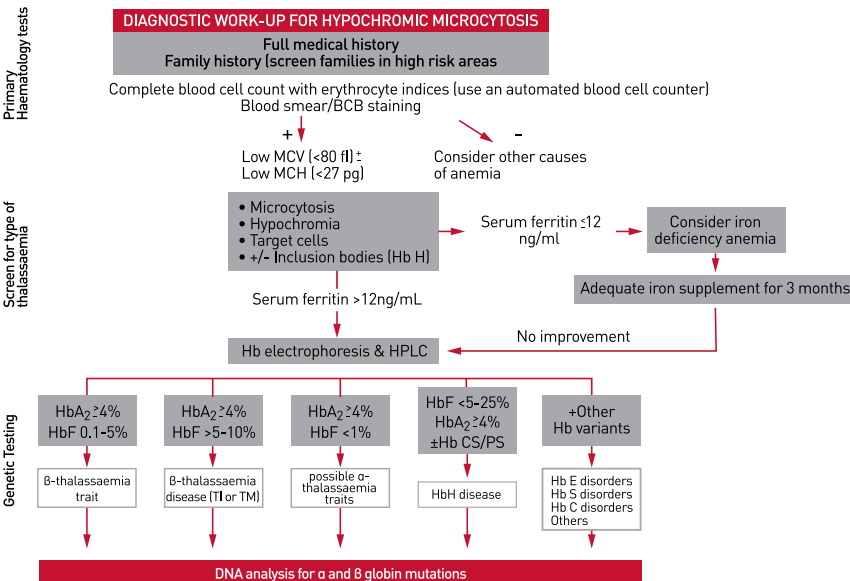


Figure 4. Diagnostic algorithm for individuals with hypochromic microcytosis.

		β -TM	β -TI	HbE/ β -Thal	HbH	
Hb levels		<5 g/dL	~7-10 g/dL	Mild	9-12 g/dL	2.6-13.3 g/dL
				Moderately Severe	6-7 g/dL	
				Severe	4-5 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 stippling				
	Specific features	+Numerous F-cells/acid elusion	+F- cells/acid elusion	+ DCIP staining (Hb E) + F-cells/acid elusion	HbH inclusion bodies	
Hemoglobin study	HbF up to 100% HbA2 \uparrow	HbF 10-50% (up to 100%) HbA2>4%	HbE (40-60%) HbF (60-40%) \pm Hb A (with β^+ -thal) HbA2 \uparrow	Variable HbH (0.8-40%) HbA2 \downarrow + the presence of α -variants i.e. Hb CS, Hb PS etc.		
DNA analysis	<ul style="list-style-type: none"> Common known mutations of both β^0 and β^+ -thal mutations in population specific set can be done by PCR based methods For rare or unusual mutations, a direct sequencing or array analysis required Other analysis for β-TI included α and β-globin rearrangements, Xmn I polymorphism and other QTLs for γ-globin expression 			Gap- PCR developed for 7 common α -thal deletions and RDB for non- deletional mutations For unknown mutations, Southern blotting or MLPA analysis and sequencing required		

Figure 5. Summary of diagnostic measures for thalassaemia and haemoglobinopathies.

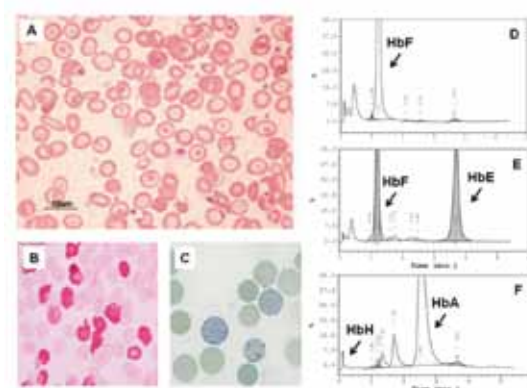


Figure 6. Haematological features and haemoglobin profiles in common thalassaemia syndromes. (A) Peripheral blood smear from a patient with thalassaemia syndrome showing marked hypochromic microcytosis with anisopoikilocytosis, target cells and polychromasia. (B) F-cell staining test positive in β -thalassaemia. (C) Positive HbH inclusion body in α -thalassaemia. Liquid chromatography showed haemoglobin profiles in β -thalassaemia major (D), HbE/ β thalassaemia (E) and HbH disease (F).

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2

BLOOD TRANSFUSION

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Reviewer John Porter

This chapter will address five of the most common questions related to the transfusion therapy of patients with thalassaemia major:

- Whom to transfuse and when to initiate transfusion therapy?
- How blood is processed and chosen for effective and safe transfusion therapy?
- What is the optimal haemoglobin level for effective transfusion?
- How do transfusion requirements affect the success of iron chelation therapy?
- What are the most common or most serious transfusion-related reactions?
- What are the correct methods of administration and monitoring during transfusion episodes?

Goals of Blood Transfusion Therapy

Appropriate goals of transfusion therapy and optimal safety of transfused blood are the key concepts in the protocol for routine administration of red blood cells to patients with thalassaemia. The major goals are:

- Use of donor erythrocytes with an optimal recovery and half-life in the recipient.
- Achievement of appropriate haemoglobin level.
- Avoidance of adverse reactions, including transmission of infectious agents.

Quality and Adequacy of Blood

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

Adherence to the directives from the European Union (EU), World Health Organisation (WHO), American Association of Blood Banks (AABB) and other or other international groups, with additional consideration of national needs, resources and prevalence of infectious agents, should safeguard the quality of blood transfusion services. 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, 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 <http://www.edqm.eu/en/blood-transfusion-mission-65.html> while additional WHO guidelines and American Standards are available at www.who.int/bloodsafety/gcbs/structure/en/ and <http://www.aabb.org/content>, respectively.

Whom to Transfuse

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

- Confirmed diagnosis of thalassaemia.
- Laboratory criteria:
 - Haemoglobin level (Hb) <7 g/dl on 2 occasions, > 2 weeks apart (excluding all other contributory causes such as infections) **OR**
- Clinical criteria irrespective of haemoglobin level:
 - Haemoglobin > 7 g/dl with any of the following:
 - > Facial changes
 - > Poor growth
 - > Fractures
 - > Clinically significant extramedullary haematopoiesis

Recommended Blood Product

Patients with thalassaemia major should receive leucoreduced packed red blood cells with a minimum haemoglobin content of 40g.

Reduction to 1×10^6 or less leucocytes per unit is considered the critical threshold for eliminating adverse reactions attributed to contaminating white cells (**Table 1**) (Klein 2007).

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 leucoreduction include:

- Pre-storage filtration of whole blood is the preferred method for leucoreduction. 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 leucoreduced 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 which 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 usually does not result in adequate leucocyte reduction and should not be used as a substitute for leucoreduction. 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 such as glycerol, and then stored at -60°C to -80°C . 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 resuspended 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 many centres they are kept for 10 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 alloimmunisation 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 organisational 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 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.

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.

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.

Taking all of these issues into consideration and especially in view of the fact that in thalassaemia major decreased recovery and a shortened red cell half-life may increase transfusion requirements and as a consequence the rate of transfusional iron loading, the current practice is to use red cells stored in additive solutions for less than two weeks. In patients with cardiac disease and in small children, particular attention should be paid to the increased volume resulting from additive solutions. In general, for all patients, the lower haematocrit of red cell units containing newer additive solutions should be taken into consideration when calculating the annual rate of transfusional iron loading (see below).

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

Compatibility Testing

Development of one or more specific red cell antibodies (alloimmunisation) is an important complication of chronic transfusion therapy (Thompson 2011, Singer 2000, Spanos 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 and to eliminate donors with the corresponding antigens. 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 C, c, D, E, e, and Kell, (though preferably a full red cell phenotype/genotype panel) in order to help identify and characterise antibodies in case of later immunization.
- If the patient is already transfused, antigen typing can be performed using molecular rather than serologic testing.
- All patients with thalassaemia should be transfused with ABO and Rh(C, c, D, E, e) and Kell compatible blood in order to avoid alloimmunisation against these antigens.

Some centres use even more extended antigen matching, including full Rh matching, or focus on specific antigens that are more likely to cause alloimmunization in a particular population (Cheng 2012).

Most blood banks currently perform a screen for new antibodies and a full crossmatch before each transfusion. In some centres, blood banks use a newer approach in which the initial approach is an antibody screen only. This alternative approach to formal crossmatch, often referred to as an electronic crossmatch, is only appropriate in blood banks that adhere to strict regulations regarding computer systems, sample labelling and other critical issues. (Milkins 2013). Using either approach, new antibodies must be identified so that blood lacking the corresponding antigen(s) can be used if needed. Not all antibodies are clinically significant and may not be able to destroy apparently incompatible red cells at body temperature. Therefore blood does not need to be antibody negative if the antibody is not reactive at certain temperatures and the blood is crossmatch compatible e.g. Anti N or if they are non significant e.g., CL1 related antibodies.

A complete and detailed record of antigen typing, 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. The primary concern related to the time between the antibody screen and transfusion is the appearance of new and therefore undetected antibodies during this interval.

Transfusion Programmes

The recommended treatment for thalassaemia major involves lifelong regular blood transfusions, usually administered every two to five weeks, to maintain the pre-transfusion haemoglobin level above 9-10.5 g/dl. This transfusion regimen promotes normal growth, allows normal physical activities, adequately suppresses bone marrow activity in most patients, and minimises transfusional iron accumulation (Cazzola 1997, Cazzola 1995). A higher target pre-transfusion haemoglobin level of 11-12 g/dl 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 take into account 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 1997, Cazzola 1995). The optimal regime 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 regimes achieve the same outcomes in such patients, the same approach as for other patients is currently recommended.

The decision to initiate lifelong transfusion therapy should be based on a definitive diagnosis of thalassaemia. This diagnosis should take into account the molecular defect, the severity of anaemia on repeated measurements, the level of ineffective erythropoiesis, and clinical criteria such as failure to thrive or bone changes. The initiation of regular transfusion therapy for severe thalassaemia genotypes usually occurs in the first two years of life. 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. The risk of alloimmunisation appears to be greater in patients who begin transfusion therapy after the first few years of life (Spanos 1990, Michail-Merianou 1987, see **Table 3**). Presence of alloantibodies and autoantibodies (see below) may severely compromise transfusion therapy in patients with thalassaemia intermedia, for example, who receive their first transfusions in adolescence or later.

Table 3. Age and alloimmunisation in thalassaemia.

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

Recommendations regarding the volume of transfused red cells are complicated by the use of different anticoagulant-preservatives and additive solutions. For CPD-A units with a haematocrit of approximately 75%, the volume per transfusion is usually 10-15 ml/kg. Units with additive solutions usually have lower haematocrits in the range of 60-70%, and consequently larger volumes are needed to administer the same red cell mass (see **Table 4**). For most patients, it is usually easier to avoid these differences in red cell concentration by ordering a certain number of units (e.g., one or two) rather than a particular volume of blood. 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 calculation is generally used (Davies Transfusion 2007):

$(\text{Desired} - \text{actual Hb}) \times \text{weight} \times 3 / \text{haematocrit of transfused unit} = \text{ml to be transfused}$

Most transfusions of 2 or 3 donor units are administered over 3-4 hours. However, an ongoing study in two London thalassaemia centres suggests that in very carefully selected patients free of cardiac disease and not receiving large volumes, transfusions can be administered at the rate of one unit per hour. Patients with cardiac failure or very low initial haemoglobin levels should always receive smaller amounts of red cells and/or have slower rates of infusion.

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

		HAEMATOCRIT OF DONOR RED CELLS			
		50%	60%	75%	80%
Target increase in haemoglobin level	2 g/dl	12 ml/kg	10 ml/kg	8 ml/kg	7.5 ml/kg
	3 g/dl	18 ml/kg	15 ml/kg	12 ml/kg	11.2 ml/kg
	4 g/dl	24 ml/kg	20 ml/kg	16 ml/kg	15 ml/kg

As an example, to raise haemoglobin level by 4 g/dl in a patient weighing 40 kg and receiving AS1 blood with a haematocrit of 60% would require 800 ml. This calculation assumes a blood volume of 70 ml/kg body weight.

The post-transfusion haemoglobin should not be greater than 14-15 g/dl as higher post transfusion haemoglobin values risk hyperviscosity and stroke. Post transfusion haemoglobin should be measured occasionally to assess the rate of fall in the haemoglobin level between transfusions. This decline may be helpful in evaluating the effects of changes in the transfusion regimen, the degree of hypersplenism, or unexplained changes in response to transfusion.

Thus the currently accepted mean target is 12 g/dl with a post-transfusion haemoglobin of 14-15 g/dl and a pre-transfusion haemoglobin of 9.0-10.5 g/dl. 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 minimise transfusional iron accumulation in most patients (Cazzola 1997).

Although erythrocytapheresis, or automated red cell exchange, has been shown to reduce net blood requirements and thus the rate of transfusional iron loading (Friedman 2003, Berdoukas 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 –transmitted infections and development of alloimmunisation. In addition there are financial constraints with such a procedure and logistic issues surrounding the need for suitable venous access.

A careful record of transfused blood should be maintained for each patient, including 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 is 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 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 and **Table 5** shows the relationship between the annual transfusion requirements and the daily rate of iron loading at two common haematocrits for donor blood. The rate of transfusional iron loading may be very important in choosing the appropriate dose of an iron chelator. For example, the recommended dose of the chelator deferasirox is based in part on the daily or annual rate of transfusional iron loading.

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

Annual blood requirement: 13 transfusions x 600 ml/40 kg = 195 ml/kg
 Annual pure red cell requirement: 195 ml/kg/yr x 60% (average haematocrit) = 117 ml/kg/yr

Annual transfusional iron loading:
 117 ml/kg/yr of pure red cells x 1.08 mg iron per ml pure red cells = 126 mg iron
 Daily transfusional iron loading: 126 mg iron/yr/365 days = 0.34 mg/kg

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

Table 5. Relationship between annual blood requirements and rate of daily iron loading.

ANNUAL BLOOD REQUIREMENT (HAEMATOCRIT 60%)	ANNUAL BLOOD REQUIREMENT (HAEMATOCRIT 75%)	VOLUME OF PURE RBCS/KG (HAEMATOCRIT 100%)	DAILY IRON LOADING
100-150 ml/kg	80-120 ml/kg	60-90 ml/kg	0.18-0.27 mg/kg
150-200 ml/kg	120-160 ml/kg	90-120 ml/kg	0.27-0.36 mg/kg
200-250 ml/kg	160-200 ml/kg	120-150 ml/kg	0.36-0.44 mg/kg
250-300 ml/kg	200-240 ml/kg	150-180 ml/kg	0.44-0.53 mg/kg

Knowing the annual transfusion requirements is also valuable in identifying changes that may constitute important evidence of hypersplenism or accelerated destruction of donor red cells.

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 1981). More recently, work has shown that 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 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 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).

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. Moreover, the decision to proceed to splenectomy must take into consideration the individual patient's ability to control iron stores with chelation therapy at a given level of transfusional iron loading. 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 the rate of iron-loading.

Adverse Reactions

Blood transfusion exposes the patient to a variety of risks and adverse events (**see Table 6**). Thus, it is vital to continue to improve blood safety and to find ways of reducing transfusion requirements and the number of donor exposures.

Nonhaemolytic febrile transfusion reactions were common in past decades, but have been dramatically reduced by leucoreduction, especially pre-storage leucoreduction, which sharply reduces cytokine accumulation and leucocyte alloimmunisation. In the absence of effective leucoreduction, 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. Occasional mild allergic reactions often can be prevented by the use of 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 characterised by the abrupt onset of fever, chills, lower back pain, a sense of impending death, dyspnea, 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 full crossmatching of donor units 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. 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. Some evidence also suggests that new alloantibodies develop more frequently after splenectomy (Thompson 2011). The use of extended antigen matched donor blood is effective in reducing the rate of alloimmunization.

Delayed transfusion reactions usually occur 5-14 days after transfusion and are characterised 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 2003). 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 immunoglobulin are used for the clinical management of this complication, although they may give little benefit. Some patients have also been treated with rituximab, but the effectiveness of its use in this situation is still not well defined. Autoimmune haemolytic anaemia occurs more frequently in patients who begin transfusion therapy later in life (Rebulla, 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 proinflammatory mediators during storage of donor red cells (Vlaar 2013, Swanson 2006). This complication is characterised 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-induced graft versus host disease (TI-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 TI-GVHD may also occur in immunocompetent recipients of red cells from a haploidentical donor such as a family member. TI-GVHD usually occurs within 1-4 weeks of transfusion and is characterised by fever, rash, liver dysfunction, diarrhoea and pancytopenia due to bone marrow failure. To reduce the risk of TI-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 may occur in the presence of recognised or unrecognised 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.

Transmission of infectious agents including viruses, bacteria and parasites, are a major risk in blood transfusion (see **Chapter 7: Infections**). 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, highly virulent influenza strains and prions may constitute serious threats,;
- Absence of widely accepted or routinely used tests for bacterial, viral and other pathogens (e.g., *Yersinia enterocolitica*, hepatitis A, toxoplasmosis, malaria and babesiosis).

While pathogen inactivation systems for red cell products are under development (Solheim 2008, Pelletier 2006), these are not yet available in routine practice. 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 6. Broad categorisation of immune-mediated transfusion- reactions and reported frequencies.

ACUTE	FREQUENCY	DELAYED	FREQUENCY
Haemolytic (intravascular)	1/25,000	Alloimmune	1/100
Anaphylactic	1/50,000	Haemolytic (extravascular)	1/2,500
Febrile non-haemolytic	1/100	Graft vs Host Disease	Rare
Allergic (Urticarial)	1/100		
TRALI	1/10,000		

Summary and Recommendations

- Confirm the diagnosis of thalassaemia and appropriate clinical and laboratory for transfusion (IIA).
- 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 C, E, and Kell (IIA).
- At each transfusion, give ABO, Rh(D) compatible blood. Matching for C, E and Kell antigen is highly recommended (IIA).
- Before each transfusion, perform a full cross-match and screen for new antibodies, or in centres that meet regulatory requirements, perform an electronic cross-match (IA).
- Use leucoreduced 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 two weeks of collection. (IA)
- Transfuse every 2-5 weeks, maintaining pre-transfusion haemoglobin above 9-10.5 g/dl or higher levels (11-12 g/dl) 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 14-15 g/dl (IIA).

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3

IRON OVERLOAD AND CHELATION

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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 which 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 a lifetime, as even short periods of interruption to treatment can have damaging effects. While the convenience and tolerability of individual chelators is important in achieving this goal, other factors such as psychological wellbeing, family and institutional support also impact on adherence and outcomes.

In this chapter we first describe the effects of iron overload and the tools for monitoring excess iron. We then cover the general goals of chelation therapy, and the mechanisms by which chelators work. Recommendations for the dosing of three licensed chelators are then described, based on evidence on their efficacy. The potential toxicities of each chelation regime and how to minimise their risks are given in **Appendix 1**. Finally, guidelines for monitoring chelation therapy so as to minimize the risks of toxicity from iron chelation are discussed.

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 per mg/mL of blood can therefore be estimated from $1.16 \times$ the haematocrit of the transfused blood product. In cases where organizational systems or other difficulties prevent such estimations to be 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 (**Chapter 2**), 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 cells vol. 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 gastro-intestinal 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 9 g/dl have been shown to prevent such expansion (Cazzola 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 III and iron II – 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 their capacity to bind iron is exceeded both within cells and in the plasma compartment. The resulting ‘free iron’, either within cells or within plasma, damages many tissues in the body or is fatal unless treated by iron chelation therapy. Free iron also increases the risk of infections (**Chapter 7**) and neoplasia. A summary of the mechanisms for toxic effects of iron overload is shown in **Figure 1**.

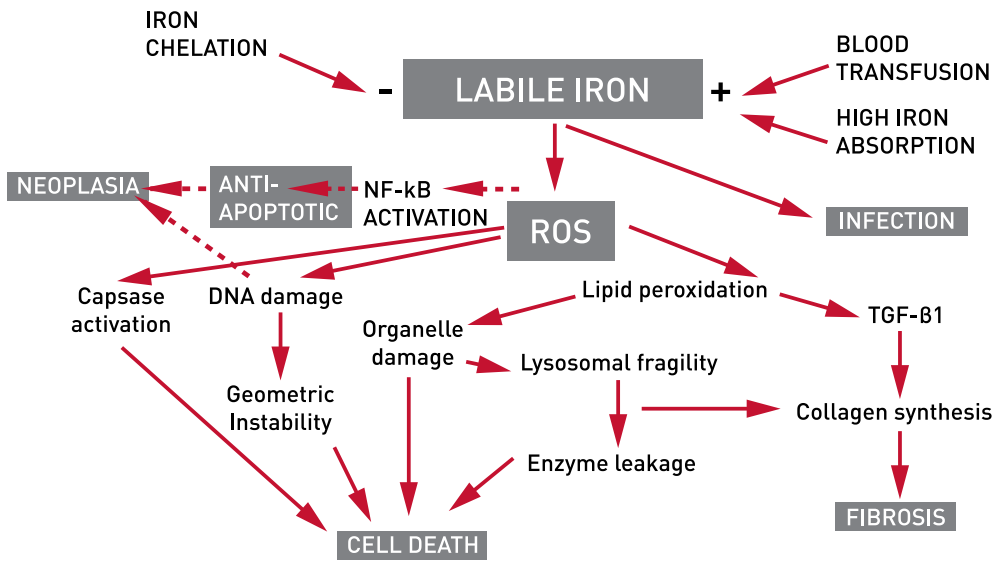


Figure 1. Pathological mechanisms and consequences of iron overload. In iron overload resulting from repeated blood transfusions or long-term increased iron absorption, iron that is not bound to naturally occurring molecules such as transferrin, or ferritin or to therapeutic iron chelators, generates a variety of reactive oxygen species (ROS), most notably hydroxyl radicals. This occurs in cells where labile plasma iron is taken up and accumulated as storage iron (ferritin and haemosiderin). ROS generate lipid peroxidation, 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).

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 other such myocardial muscle, endocrine tissue and hepatocytes take up NTBI rapidly. This iron is then stored as ferritin or haemosiderin which are visible by MRI. The myocardial iron overload induces 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, hypothyroidism and hypoparathyroidism are also seen. Liver disease with fibrosis and eventually cirrhosis and hepatocellular carcinoma, particularly if concomitant chronic hepatitis is present, are also serious complications (see **Chapter 5**).

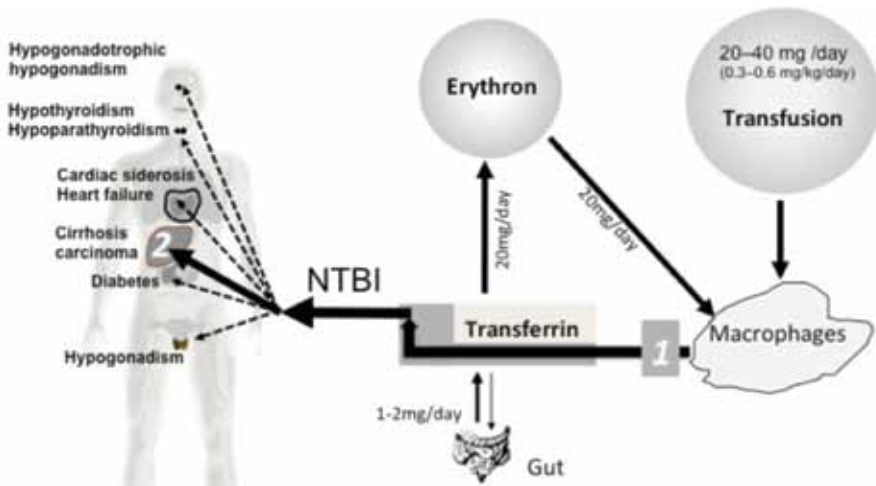


Figure 2. The main routes of iron turnover and uptake are shown by solid black arrows on the right panel: 20 mg of iron is delivered daily to the erythron in health. This increases several fold in untransfused thalassaemias but can be inhibited by hypertransfusion. NTBI is generated when transferrin (which is about 30% saturated in healthy adults) becomes saturated. Transferrin saturation occurs either following iron overload of the macrophage system, but also as a result of decreased clearance of transferrin iron in hyper-transfused patients. The organs in which NTBI are taken up and retained as storage are shown on the left, with >80% cleared by hepatocytes. Despite variable and low 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 TM; many studies have shown an association between the control of serum ferritin and prognosis [Borgna-Pignatti 2004, Davis 2004, Gabutti 1996, Olivieri 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 $\mu\text{g/L}$ (with Deferoxamine, or DFO) over a period of a decade or more [Olivieri 1994]. Observations with larger patient numbers show that maintenance of an even lower serum ferritin of 1,000 $\mu\text{g/L}$ may be associated with additional clinical advantages [Borgna-Pignatti 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 plasma ferritin (Brittenham 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 plasma 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 2009), and body iron can fall considerably from a high starting point (e.g. LIC >30 mg/g dry wt) 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 2004, Worwood 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 2010) and by duration of chelation therapy (Fischer 2003).

Table 2. Use of SF 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 levels
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

Liver iron concentration (LIC) measurement

Uses for liver iron concentration monitoring

- **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 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 1997) or liver function abnormalities (Jensen 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 2003, Buja 1971). However, the relationship between LIC and extrahepatic iron is complicated by chelation therapy as iron tends to be accumulate 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 2008, Anderson 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 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 µg/L), the relationship to LIC is not linear and patients may show 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 1997). Inadequate sample size (4 mg dry wt or about a 2.5 cm core length) or uneven distribution of iron, particularly in the presence of cirrhosis (Villeneuve 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 standardization is not trivial and differences between laboratories, for example in wet

to dry weight ratios, can mean that results from different labs may not be equivalent.

- SQUID

Magnetic biosusceptometry (SQUID) (superconducting quantum interference device) determines the paramagnetism of the liver which is proportional to LIC (Brittenham 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 the same, 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 1994). However, this is not 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 (is the same as $1/T1$) and the R2 rate (is the same as $1/T2$). A variation of this principle are Gradient Echo techniques, achieved by applying a strong graded magnetic field to the radio-frequency (rf) pulse that is used for spin echo. This relies on multiple echoes over a shorter acquisition time period than spin echo techniques. The shorter acquisition 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. 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 2008, Storey 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. Specialized 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 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 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 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 analyzed). 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 heart 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 artifact
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

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 breathold (Kirk 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 2001). More recently the relationship between biochemically measured myocardial iron concentration

and myocardial T2* has been shown using post mortem myocardial material (Carpenter, 2011). Here, mean myocardial iron causing severe heart failure in 10 patients at post mortem was 5.98 mg/g dry weight (ranging from 3.2 to 9.5 mg/g); levels that in the liver would not be regarded as harmful to the liver. The relationship of myocardial iron concentration (MIC) to T2* is: $MIC \text{ (mg/g dry wt)} = 45 * (T2^* \text{ ms})^{-1.22}$ (Kirk 2009b). 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 heart failure in the next 12 months (Kirk 2009b). 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 year period while taking deferasirox (DFX) and desferrioxamin (DFO) combination therapy (Ayidinok 2014).

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. The value of T2* monitoring is supported by a recent report in a cohort of TM patients monitored for 10 years using T2*, in which iron mediated cardiomyopathy was no longer the leading cause of death, and the proportion of patients with T2* <20 ms fell from 60% to 1% over the decade (Thomas 2010). Alternative factors such as improved chelation options may also have contributed to these improvements in outcome. T2* monitoring has now been established and validated internationally (Kirk 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 the method adopted in a given centre undertakes measurements to independently validate and calibrate measurements, otherwise inappropriate assessment of heart failure prognosis may result. **Table 4** summarizes advantages and disadvantages of using T2* MRI for monitoring cardiac iron overload.

Heart function

Sequential monitoring of LVEF has been shown to identify patients at high risk of developing clinical heart failure (Davis 2004, Davis 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 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 hypogonadotropic hypogonadism (growth and sexual development and biochemical markers of HH), diabetes mellitus (yearly OGTT), hypothyroidism and hypoparathyroidism. There has been recent interest in using MRI as a way of identifying the risks of 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 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 in the pituitary and pancreas (Noetzli 2009, Au 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 desferioxamine (about half of total iron excreted in urine) (Pippard 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 2012a, Mourad 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 unbound 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 HPLC (Singh 1990), or by colorimetric analysis (Gosriwatana 1999) or indirectly by exploiting the impact of labile iron species to oxidised fluochrome, such as in the labile plasma iron (LPI) assay (Zanninelli 2009, Cabantchik 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 2009). Whilst some loose associations of NTBI (Piga 2009) or LPI (Wood 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 1996) after an iron chelator has been cleared (Zanninelli 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 2011) adding to the complexity of interpreting levels (Hod 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 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.

Treatment of Iron Overload

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 accumulated, more iron must be removed than accumulates as a result of blood transfusion. Removal of storage iron is slow and inefficient, because only a small proportion of body iron is available for chelation at any given time. Once iron has deposited in some tissues, damage is often irreversible. Prevention is therefore preferable to rescue therapy. 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. These can be identified by careful monitoring of iron and its distribution. Without monitoring of trends in iron load (liver iron and ferritin) and iron distribution (heart iron and function) patients are at risk of either a) underchelation with increased iron toxicity; or b) overchelation 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 effectively. This requires good adherence to the chelation regime. Intermittent high dose chelation can induce negative iron balance but does not provide continuous protection from labile iron and risks increased toxicity from the iron chelator. Poor adherence can result from practical issues such as difficulty with DFO infusions, intolerance of a particular chelator, or from psychological / psychosocial issues. A key role of the treating centre is the monitoring and encouragement of adherence to chelation, alongside support from their family. However, encouraging a patient to take control or 'self-manage' is often a useful approach of long-term benefit.

Sources of chelatable iron

Only a very small fraction of body iron is available for iron chelation at any moment of time. This is because iron chelators interact with low molecular weight 'labile' iron pools better than with iron stored as ferritin or haemosiderin. Labile iron is constantly being generated, so that the efficiency of chelation is better when a chelator is available at all times (chelator present 24 hours a day). 24h chelation also has the potential to remove toxic labile iron pools within cells continuously, which is particularly important in reversing heart failure. Chelatable iron is derived from two major sources: iron derived from the breakdown of red cells in macrophages (about 20 mg/day in healthy adults), and iron derived from the catabolism of stored ferritin iron within cells. Most of the storage iron in the body is in hepatocytes, and the ferritin in these cells is turned over less frequently (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 extent to which this chelated iron is eliminated in faeces or urine varies with each chelator. With DFO about half is excreted in urine and half in faeces, whilst with DFX excretion is mainly through the urine and DFP through faeces. Urinary excretion of iron chelated by DFO is derived mainly from macrophage catabolism of red cells, whereas urine iron chelated by DFP is derived from macrophage and hepatocyte pools. Small quantities of storage iron are also deposited in the endocrine system and in the heart. Because these are not designed as cells for iron storage and release, unlike hepatocytes, storage iron is turned over in the lysosome compartment less frequently and a lower proportion of cellular iron is available for chelation at any moment. Thus it generally takes longer to remove iron from these tissues than from the liver.

Chemical and pharmacological properties of licensed chelators

Three iron chelators are currently licensed for clinical use and their iron binding properties, routes of absorption, elimination and metabolism differ. These are summarized in **Table 5**. Of note, the majority of information presented refers to prototype formulations of the chelators.

Chemistry: The number of chelator molecules required to bind iron differs with each of

these chelators. DFO binds iron in a 1:1 ratio, which results in a very stable iron chelate complex but also a large molecule that cannot be absorbed from the gut. DFX binds iron in a 2:1 chelator to iron ratio, and is small enough for oral absorption. DFP is smaller still and requires 3 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 chelate-iron complexes are shown in **Table 5**. Iron free DFO is eliminated rapidly in urine and faeces (short $T_{1/2}$) if it does not bind iron, but the elimination of iron complexes are slower. Iron free DFP has a short plasma half-life, requiring it to be given 3 times a day. It is rapidly metabolized at its iron binding site in hepatocytes. DFX has a longer plasma half-life, typically requiring only once daily dosing and providing 24 clearance of labile plasma iron. Plasma drug levels differ between the chelators. DFO levels rarely exceed 10 μM when given as an infusion at night, and negligible levels of iron-free chelator are present during the day. DFP levels fluctuate with peaks exceeding 100 μM at approximately 2h after ingestion but with negligible levels at night, if the 3 doses are given during the day (Aydinok 2012a, Limenta 2011). DFX and its iron complex are eliminated in faeces (**Table 5**) (Nisbet-Brown 2003), and about 10% of plasma DFX is bound to iron (Galanello 2003). Metabolism is mainly by glucuronidation to iron binding metabolites, with less than 10% of metabolism being oxidative, by cytochrome p450 (Waldmeier 2010).

Table 5. Chemical and pharmacological properties of licensed chelators.

COMPOUND	Desferrioxamine (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Molecular weight (daltons)	560	373	139
Log 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	Oral, 3 times daily
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 2005b)	80 (Galanello 2003)	90-450 (Kontoghiorghes 1990)
Concentration of iron complex	Complex remains similar (about 7 μM) with ascending doses but the iron-free drug and metabolites increase (Porter 2005b)	Complex accounts for about 10% of plasma drug in steady (Waldmeier 2010)	Complex correlates with urine iron excretion and predicts response to therapy (Aydinok 2005)

COMPOUND	DEFERRIOXAMINE (DFO)	Deferasirox (DFX)	Deferiprone (DFP)
Min. plasma level (μM) 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 2005b, Porter 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 2010)	Glucuronide formed in liver does not bind iron (Kontoghiorghe 1990)
Recommended dose mg/kg/d	30-60 5-7 x/week	20-40 once daily	75-100 in 3 divided doses
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

In general, as with any therapy, the potential benefits of chelation therapy must be balanced against occasional unwanted adverse effects which 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. Unfortunately the combination of chelation therapies is not specifically licensed, so there is no prescribing information provided by licensing authorities in this respect. However, the clinical and research experience with combination therapies will be described as are used in many treatment centres when monotherapy is inadequate. Appendix 1 summarises the particular prescribing information from licensing authorities which act as a guide for prescribing individual monotherapies.

Desferrioxamine monotherapy (Desferal® or deferoxamine; DFO)

DFO is licensed for the treatment of chronic iron 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 clinically. A large body of literature has since emerged on the changing complications and improved survival, predominantly from retrospective cohort analysis. As no treatment alternatives were available at the time of its introduction, the benefits of its long term use are clearer than for newer chelators, where patients have often received more than one chelation treatment over a lifetime. The main disadvantages of the treatment are that it is costly and it must be administered parenterally which is uncomfortable and time consuming. Also because of its short half live it typically only chelates iron during the time infused, therefore leaving 12 or more hours with no active chelator with standard regimens. The increased toxicity of DFO at low levels of body iron (see **Appendix 2**) means that guidelines for its use have been conservative, generally recommending that therapy not be started until SF levels reach 1000 µg/L, and with care to avoid 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 2500µg/L (Olivieri 1994) with even better outcomes at levels <1000µg/L (Borgna-Pignatti 2004). Four decades of clinical experience clearly show that ferritin can be controlled with DFO monotherapy at 40-50 mg/kg administered as an 8-10-h infusion at least 5 times a week. In children however, mean daily doses should not exceed 40 mg/g because of the effects on growth and skeletal development. Guidelines about the dosing required to control iron overload were based on retrospective data until recently. A randomised study in 290 TM patients identified the doses required to stabilize or decrease SF, with a mean daily dose of 42 mg/kg resulting in a small decrease in serum ferritin of 364µg/L at one year, whereas 51 mg/kg resulted in an average decrease of approximately 1,000 µg/L (Cappellini 2006). Further analysis shows that response is also linked to the transfusion rate and that larger doses are required in patients requiring higher transfusions (see below) (Cohen 2008). Thus the effectiveness of DFO at controlling SF is related to dose, frequency and duration of exposure and transfusion rate.

Effects on liver iron

Administered at least 5 times a week in sufficient doses, DFO is effective in controlling liver iron and hence total body iron stores (Brittenham 1993). In a prospective randomized study (Cappellini 2006), a mean dose of 37 mg/kg stabilised LIC for patients with baseline LIC values of between 3 and 7 mg/g dry wt. For patients with LIC values between 7 and 14 mg/g dry wt, a mean dose of 42 mg/kg resulted in a small decrease of 1.9 mg/kg dry wt over a 1 year interval. 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 (giving a mean daily dose of $50 \times 5/7 = 36$ mg/kg) is recommended if a significant decrease in optimal LIC levels is required (see above). It should be emphasised that these are average changes and that the dose required may increase or decrease depending on transfusion requirements (Cohen 2008).

Effects on heart function

Subcutaneous therapy has long been known to prevent (Wolfe 1985) or improve asymptomatic cardiac disease in thalassaemia major (Aldouri 1990, Freeman 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 2004, Brittenham 1994). Symptomatic heart disease can be reversed by high dose intravenous treatment (Davis 2000, Cohen 1989, Marcus 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 2004, Davis 2000). Continuous intravenous doses of 50-60 mg/kg/day typically normalise LVEF in a period of three months (Anderson 2004), significantly before liver or heart iron stores have been normalised. However, if advanced heart failure has developed before treatment is intensified, the chances of successful rescue are reduced. Early intervention for decreased LV function is therefore recommended. Once heart function has improved, sustained compliance is critical to improve outcomes, especially while myocardial iron remains increased (Davis 2004).

Effects on heart iron (mT2*)

Myocardial iron can improve with either subcutaneous or intravenous therapy provided treatment is given in adequate doses and frequency. Improvement in mild to moderate cardiac T2*, even at low intermittent doses (5 days a week) has been confirmed by prospective randomised studies (Pennell 2014, Pennell 2006b, Tanner 2006). For patients with established mild to moderate myocardial iron, a simple increase in dose or frequency of use may be sufficient to improve the mT2*. For example at relatively low doses of 35 mg/kg, an average improvement in T2* of 1.8 ms over one year has been shown (Pennell 2006b). At a slightly higher dose of 40-50 mg/kg five days a week, patients showed an improvement of 3 ms over one year (Porter 2005a). When mT2* is < 10 ms, as with other iron chelators, it will take several years of sustained and compliant therapy to normalise myocardial iron (Porter 2002). For T2* values <10 ms, a simple 5 day a week s.c. DFO at standard doses is unlikely to be sufficient, and treatment intensification is indicated. This could involve higher dose continuous DFO or more likely switching to another chelation regime in the absence of heart failure (see below).

Effects on long term outcome

DFO has been in clinical use since the 1970s and widely used as subcutaneous infusions since about 1980. The most powerful evidence for the effectiveness of DFO and indeed for chelation as a treatment modality is the improving survival and decreased morbidity in patients treated with DFO since this time (**Table 6**). This benefit is clearly shown in successive cohorts born since this time. Only patients born after 1980 will have started treatment at an early age, and age of starting treatment is a key factor in outcome (Borgna-Pignatti 2004, Brittenham 1993). Regular subcutaneous therapy started before the age of 10 years reduces co-morbidities such as the incidence of hypogonadism (Bronspiegel-Weintrob 1990), as well as other endocrine disturbances, including diabetes mellitus (Borgna-Pignatti 2004, Olivieri 1994, Brittenham 1993). Adherence to therapy has been the main limiting factor to successful outcomes; failure to take treatment at least 5 times a week at adequate doses and subsequent failure to control serum ferritin in the long term leads to increased mortality (Gabutti 1996). Up until 2000, 50% of UK patients still died by age 35 years (Modell 2000), reflecting difficulties with DFO use and other issues such as the variable support patients on chelation therapy received in centres where only small numbers of patients attended. It is important to recognize that toxicity from iron overload is a long-term phenomenon, so the entire chelation history of an individual is important for outcomes, rather than simply the treatment a patient is taking when an event happens.

Table 6. Decreasing complications in cohorts of Italian patients born after DFO became available. Reproduced with permission from (Borgna-Pignatti 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)		

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 and frequency to obtain iron balance**

The standard aim is to balance iron input from transfusions with iron excretion through urine plus faeces. The recommended method is slow subcutaneous infusion over 8-12 hours of a 10% DFO solution, using an infusion pump a minimum of 5 days per week. In countries where pre-filled balloon infusers are available, this has been found to ease the convenience of adhering with DFO chelation. In general, average doses should not exceed 40 mg/kg until growth has ceased. The standard dose is 20-40 mg/kg for children, and up to 50-60 mg/kg for adults, as an 8-12-hour subcutaneous infusion for a minimum of 5-6 nights per week. To achieve negative iron balance in patients with average transfusion requirements, a dose of 50 mg/kg/day at least 5 days a week is required. It is important that patients with high degrees of iron loading, or those at increased risk of cardiac complications receive adequate doses, advice about compliance or consideration of alternative chelator regimens.

- **Use with vitamin C**

Vitamin C increases iron excretion by increasing the availability of chelatable 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 supplements, taken at the time of the DFO infusion so that liberated iron is rapidly chelated. Where a patient has just started on DFO and it has been decided to administer vitamin C, the vitamin supplement should not be given until after several weeks of treatment.

- **Dose adjustment to avoid DFO toxicity**

At low ferritin levels, the dose of DFO 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 $\mu\text{g/L}$) to keep this < 0.025 (Porter, 1989): Although a tool in protecting the patient from excess chelator, this index is not a substitute for careful clinical monitoring. Liver iron concentration has recently been advocated as a more reliable alternative to serum ferritin at low levels of body iron loading (see below).

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 7** 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 35-50 mg/kg, while >50 mg/kg are required to achieve negative iron balance.

Table 7. % of responders (% in negative iron balance) by dose and transfusion rate. Adapted from (Cohen 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 daily 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 cardiac iron of 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 ($T2^*$ <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 9 and 12**), intensification of therapy may be

helpful to minimize the levels of iron overload. The optimal regime has not been studied systematically 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 (NTBI), which returns to pre-treatment levels within minutes of stopping a continuous intravenous infusion (Porter 1996). The route of administration is not critical, provided that as close to 24-hour exposure to chelation as possible is achieved. Intensification of treatment through continuous, 24-hour intravenous administration of DFO via an implanted intravenous delivery system (e.g. Port-a-cath) (Davis 2000), or subcutaneously (Davis 2004) has been shown to normalise heart function, reverse heart failure, improve myocardial T2* (Porter 2013b, Anderson 2004) and lead to long-term survival, provided treatment is maintained. Some studies have included cases where for operational reasons, intensification was undertaken without continuous infusion. Continuous infusion is usually given through an indwelling line for long-term management. 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 mls of saline to avoid damage to the veins where the drug is infused. A dose of at least 50 mg/kg/day and not exceeding 60 mg/kg/day is recommended as a 24-hour infusion (Davis 2004, Davis 2000). Higher doses have been used by some clinicians, however, DFO is not licensed at these 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 2004). As ferritin falls, the dose but preferably not the duration of treatment can be reduced - in line with the therapeutic index (see above).

The question of whether to add DFP to intensified DFO needs to be considered. This is partly because DFP at high doses (90-100 mg/kg) was found to increase the T2* more than conventional s.c. DFO 5 days a week (Pennell 2006b) and because combined DFP + DFO has also been found to improve T2* more rapidly than conventional doses of DFO (Tanner 2007). However, these patients have baseline LVEF in the normal range and were not in heart failure but showed greater LVEF increases with the DFP containing regimes. Furthermore, these studies compared conventional intermittent non-intensified DFO with the DFP containing regimes; such low DFO doses should not be recommended for patients in heart failure. The only randomised study to examine the effect of additional DFP to intensified DFO found no difference between the two study arms with or without DFP, either with respect to LVEF or to improvements in T2* (Porter 2013b). Nevertheless, this study also showed no major extra toxicities in the study arm containing DFP, so the addition of DFP to intensified DFO would seem a reasonable course of action for patients in heart failure, provided that patient can tolerate oral administration of DFP.

Deferiprone monotherapy (Ferriprox®, Kelfer®, GPO-L-ONE®; DFP)

Deferiprone (DFP) is an orally absorbed bidentate iron chelator that began clinical trials in the UK in the 1980s. DFP was licensed in several countries from the 1990s and more recently in the US (October 2011) (Traynor 2011) for the treatment of iron overload in TM patients. The indication for treatment differs slightly in different countries (see below):

Effects on ferritin

Randomised trials comparing the effects of DFP on serum ferritin at baseline and at follow-up have been reported from the 1990s (Pennell 2006b, Ha 2006, Gomber 2004, Maggio 2002, Olivieri 1997). Pooled analysis shows a statistically significant decrease in serum ferritin at six months in favour of DFO, with no difference between the two drugs at 12 months (Pennell 2006b, Gomber 2004). There are numerous non-randomised cohort studies demonstrating a lowering of serum ferritin at doses of 75 mg/kg/day administered in three doses. The effect on SF at this dose appears greater at higher baseline ferritin values. In these studies significant decreases in serum ferritin are seen in patients with baseline values above 2,500 µg/L (Viprakasit 2013, Olivieri 1995, Al-Refaie 1992, Agarwal 1992) but not with values below 2,500 µg/L (Cohen 2000, Hoffbrand 1998, Olivieri 1995). In a recent study from Thailand, only 45% of paediatric thalassaemia patients (age > 2 years) had significant reduction of serum ferritin after 1 year at doses of over 79 mg/kg/day (Viprakasit 2013). In this study, baseline SF was the major factor that predicted clinical efficacy; patients with baseline SF > 3,500 µg/L had the most significant fall of SF at 1 year. The FDA licensing agreement in 2011 concluded that "data from a total of 236 patients were analyzed, of the 224 patients with thalassaemia who received DFP monotherapy and were eligible for serum ferritin analysis...the endpoint of at least a 20% reduction in serum ferritin was met in 50% (of 236 subjects), with a 95% confidence interval of 43% to 57%".

Effects on liver iron

Change in LIC from baseline after various periods of treatment with DFP has been compared with DFO in randomised studies (El-Beshlawy 2008, Ha 2006, Pennell 2006a, Maggio 2002, Olivieri 1998) and also with combination of DFP plus DFO (Aydinok 2007). One study showed initial LIC decreases at 1 year but increases in LIC at 33 months of 5 mg/g dry wt with DFP (n=18) and 1 mg/g dry wt with DFO (n=18) (Olivieri 1998). In another study an average decrease in LIC at 30 months was reported with both DFP (n=21) and DFO (n=15) (Maggio 2002). A greater 1 year decrease in LIC with DFO than DFP monotherapy was reported in several studies (Aydinok 2007, Pennell 2006a). A decrease of 0.93 mg/g dry wt with DFP (n=27) and 1.54 mg/g dry wt with DFO (n=30) (Pennell 2006) was observed at 1 year. Another study reported initial decreases in LIC at six months with both DFP and DFO, but LIC had increased by the end of the trial (Ha 2006), consistent with earlier observations (Olivieri 1998). In a randomised 1 year comparison of DFP with DFP + DFO, there was no decrease in LIC with DFP monotherapy but a decrease with combination therapy or in the DFO comparison group (Aydinok 2007). In a non-randomised prospective study using DFP, LIC increased with DFP by 28% at two years and by 68% at three years (Fisher 2003). In a recent study of paediatric patients, decrease in LIC was seen in those patients who showed a clinical response by reduction of serum ferritin and in those with a higher baseline LIC (Viprakasit 2013). In observational studies where only single biopsies were performed after several years of DFP treatment, LIC was found to be above 15 mg/g dry wt in variable proportions of patients, ranging between 11% (Del Vecchio 2002), 18% (Tondury 1998) and 58% (Hoffbrand 1998). Overall negative iron balance (decrease in LIC) with standard transfusion rates using DFP monotherapy is achieved in only about 1/3 of patients receiving 75 mg/kg (Fischer 1998).

Effects on myocardial iron

The effect of DFP monotherapy on myocardial iron has been reported in randomised studies. One compared high dose DFP (92 mg/kg/day) with s.c. DFO 5-7 days a week in patients with mild to moderate myocardial iron (mT2* 8-20 ms). The actual dose prescribed for DFO was 43 mg/kg for 5.7 days/week (or a mean daily dose of 35 mg/kg/day). The increase in mT2*

from 13 ms to 16.5 ms in the DFP group was greater than that seen in the DFO group, where an increase from 13.3 to 14.4 ms at 1 year was observed (Pennell 2006b). In another 1 year randomised study of DFP and DFO, no change in heart iron estimated by MRI (signal intensity ratio) was reported for either drug. Lower doses of DFP (75 mg/kg/day) were used in this study (Maggio 2002). In a retrospective study, higher mT2* values were seen using a multislice technique and with higher global systolic ventricular function, in patients with DFP monotherapy (n=42) than those with DFO (n=89) or DFX (n=24) monotherapies, although the mean values were in the normal range in each monotherapy category (Pepe 2011).

Effects on heart function

The effects of DFP on heart function have been documented in patients with normal baseline function (Pennell 2006b, Maggio 2002) but not in patients with baseline LVEF below the normal reference range. In a one-year randomised study of patients with normal LVEF, DFP given at high doses (92 mg/kg) increased LVEF (Pennell 2006b). In another 1 year randomised study, no difference in LVEF or other measures of LV function were seen with either DFP at 75 mg/kg/day or DFO (Maggio 2002). In a three year retrospective reanalysis of patients in the one year prospective study, follow up data showed that DFP monotherapy was associated with significant increase in LVEF in patients with LVEF in the normal range at baseline (Maggio 2012). Another retrospective study of 168 patients with thalassaemia major and baseline mean LVEF within the normal range were followed for at least 5 years while receiving monotherapy with DFO or DFP. LVEF increased in both groups but was higher in the DFP group at 3 years. However the subgroup of patients with LVEF <55% at baseline was greater in the DFO than in the DFP group (Filosa 2013).

Compliance with DFP

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 2013). As with other oral chelators, two important points should be taken into consideration: (i) compliance with any treatment 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.

Evidence of long term benefits of DFP monotherapy

Several retrospective studies have reported a survival advantage of DFP either alone (Borgna-Pignatti 2006) or with DFO (Telfer 2006) (see below), compared with DFO alone. For example, in a retrospective cohort analysis of patients treated with DFP or DFO, no deaths were reported (n=157) in the DFP arm (n=157), in contrast to 10 in DFO-treated patients (Borgna-Pignatti 2006). Other retrospective or observational studies have drawn inferences about potential advantages of DFP over DFO based on surrogate markers for survival, such as SF, myocardial T2* or LVEF (though not liver iron) (Filosa 2013, Maggio 2012, Pepe 2011). However, two systematic analyses have not found clear evidence of survival advantages of any particular chelator regime (Fisher 2013b, Maggio 2011). The Cochrane systematic review concluded: "earlier trials measuring the cardiac iron load indirectly by measurement of the magnetic resonance imaging T2* signal had suggested DFP may reduce cardiac iron more quickly than DFO. However, meta-analysis of two trials showed a significantly lower left ventricular ejection fraction (at baseline) in patients who received DFO alone compared with those who received combination therapy using DFO with DFP" (Fisher 2013b). Another

systematic study concluded “There is no evidence from randomised clinical trials of different chelators or regimes to suggest that any has a greater reduction of clinically significant end organ damage, although in two trials, combination therapy with DFP and DFO showed a greater improvement in left ventricular ejection fraction than DFO used alone” (see below). Thus while retrospective analyses encourage the view of a survival advantage with DFP monotherapy compared with DFO, this has not been confirmed by in systematic meta-analysis.

Unwanted effects with DFP

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

Recommended treatment regimens with DFP

According to the FDA, Ferriprox® “is indicated for the treatment of patients with transfusional iron overload due to thalassaemia syndromes when current chelation therapy is inadequate” (FDA 2011). FDA approval is ‘based on a reduction in serum ferritin levels’. The European licensing Agency (EMA) states ‘Ferriprox is indicated for the treatment of iron overload in patients with thalassaemia major when DFO therapy is contraindicated or inadequate’. In Thailand and many Asian countries, DFP was registered for similar indications and is licensed for use from the age of 6.

Standard dosing and frequency

The daily 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. The drug’s labeling 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

Adjustments may be made on the basis of the patient’s response but should never exceed 33 mg three times daily. Doses of 100 mg/kg/day have been given in at least one prospective study (Pennell, 2006), with no increase in reported side-effects. The relation of dose to iron balance or serum ferritin has not been reported in a single study. High dose monotherapy with DFP has not yet been prospectively evaluated for safety and effectiveness for patients with abnormal heart function, and thus combination therapy with DFP and DFO (see below) or intensive therapy with DFO as a 24-hour infusion should be recommended for this group of patients.

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 pediatric patients, age range 3-19 years (Viprakasit 2013), as well as a similar study involving 100 children of 1-10 years old who received the liquid formulation of DFP found no specific tolerability issues that have not been previously reported in adults.

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 (Exjade®, Asunra®; DFX)

Deferasirox (DFX) was developed as a once-daily oral monotherapy for the treatment of transfusional iron overload. The drug has been 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 Appendix 1).

Chemistry and Pharmacology

Deferasirox is an orally absorbed tridentate iron chelator, with two molecules binding each iron atom. The chemical properties and pharmacology are summarized in Table 5. The tablet is dispersed (not dissolved) in water or apple juice using a non-metallic stirrer and consumed as a drink once daily, preferably before a meal. The drug is rapidly absorbed, reaching peak concentrations of 80µM at 20 mg/kg and the long half-life of this iron-free drug allows trough concentrations of about 20 µM, providing 24hr protection from plasma labile iron (Nisbet-Brown 2003, Galanello 2003), with about 90% in the free drug form and 10% as iron complexes (Waldmeier 2010). The lipid solubility allows entry into cells, including cardiomyocytes. The majority of the drug is excreted in faeces, and metabolism is mainly to an acyl-glucuronide that retains its ability to bind iron (Waldmeier 2010). Metabolic iron balance studies show iron to be excreted almost entirely in the faeces, with less than 0.1% of the drug eliminated in urine (Nisbet-Brown 2003). The main pathway of DFX metabolism is via glucuronidation to the acyl glucuronide and the 2-O-glucuronide metabolites. Oxidative metabolism by cytochrome 450 enzymes is minor (10% of the dose) (Waldmeier 2010). The efficiency of chelation is 28% over a wide range of doses and levels of iron loading.

Evidence of effectiveness of DFX

Dose effect on serum ferritin

A dose-dependent effect on serum ferritin has been observed in several studies (Porter 2008, Cappellini 2006, Piga 2006). A prospective randomised study comparing the effects of DFX in 296 thalassaemia major patients with DFO in 290 patients, found that 20 mg/kg daily stabilized serum ferritin close to 2,000µg/L and at 30 mg/kg, serum ferritin was reduced with an average fall of 1,249 µg/L over one year (Cappellini 2006). Longer-term analysis of ferritin trends show that the proportion of patients with ferritin values <1,000 µg/L and less than 2,500 µg/L is decreasing progressively with time. At 4-5 years follow up in 371 patients, median SF had fallen to < 1500 µg/L (Cappellini 2011) and the increase in mean dose from an initial value of < 20 mg/kg to 25 mg/kg was associated with a significant fall in serum ferritin. Overall, 73% of patients attained serum ferritin levels ≤2500 µg/L and 41% of patients achieved serum ferritin levels of ≤1000 µg/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 large scale studies involving 1,744 transfusion-dependent anaemias, including 1,115 with TM (Cappellini 2010). The initial dose of deferasirox 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 adjustment 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. In a recent substudy, the largest SF decrease of

-1,496 µg/L/year was noted in patients with the highest baseline SF values (baseline median SF 6,230 µg/L) (Porter 2013a). These patients were treated with DFX at high dosage (35-40 mg/kg/day), which are therefore doses now recommended for heavily iron overloaded patients.

Dose effect on liver iron and iron balance

Metabolic balance studies showed that excretion averaged 0.13, 0.34 and 0.56 mg/kg/d at DFX doses of 10, 20 and 40 mg/kg/d respectively, predicting equilibrium or negative iron balance at daily doses of 20 mg and above (Nisbet-Brown 2003). In a longer term randomised prospective study in 586 thalassaemia patients aged 2 to 53 years (with half of patients <16 years old), iron balance with DFX (n=290) assessed by serial LIC determination was achieved at 20 mg/kg/day, with mean LIC remaining stable over one year (Cappellini 2006). Negative iron balance was achieved at 30 mg/kg/day, with a mean LIC decrease of 8.9 mg/g dry wt (equivalent to a decrease in body iron of 94 mg/kg body weight) over one year. These are average trends and a closer analysis shows that the blood transfusion rate influences the response to treatment (Cohen 2008) (Table 8). This shows that negative iron balance over 1 year (response rate) is increased as doses increase, and that the response rate is less at high transfusion rates, who therefore required higher doses.

Table 8. % of responders (% in negative iron balance) by dose and transfusion rate. Adapted from (Cohen 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
10	29	14	0
20	76	55	47
30	96	83	82

At 4-5 years of follow up, the percentage of patients with LIC values <7 mg/g dry wt by biopsy increased from 22% at baseline to 44% (Cappellini 2011). A more moderate reduction in LIC occurred in children under six years old, despite the administration of an average dose of 21.9 mg/kg in this subgroup. However, these patients had the highest mean transfusional iron intake. In a recent liver MRI analysis of 374 patients enrolled on the EPIC study (Porter 2013a), response to DFX was analyzed according to baseline levels of iron overload. In patients with a high baseline LIC of 27.5 mg/g dry wt, LIC decreased by 6.9 mg/g dry wt at one year at doses of 25-35 mg/kg/day. In patients with LIC of 32 mg/g dry wt the decrease was 7.3 mg/g dry wt and 35-40 mg/kg/day, respectively. Thus, provided adequate doses are given, there is a good response to DFX across the full range of baseline LIC values (Porter 2013a).

Iron balance and safety in children

DFX was the first chelator to be formally assessed in children as young as 2 years old. Approximately 50% of patients in 5 clinical studies that included 703 patients were children

aged <16 years. The drug appears to be tolerated in children as well as in adults. Importantly, no adverse effects on growth or skeletal development were observed at a dose of 10 or 20 mg/kg /day (Piga 1988). In another observational study of chelation-naïve transfusion-dependent children (aged < 5 years) with SF > 1000 µg/L at baseline, DFX or DFO was prescribed to maintain serum ferritin levels between 500 and 1000 µg/L. With a median follow up of 2.3 years for DFX (n = 71) and 2.8 years for DFO (n = 40), DFX was shown to be well tolerated and at least as effective as DFO in maintaining safe serum ferritin levels and normal growth progression (Aydinok 2012b).

Effect on myocardial T2*

Improvement in mT2* was first reported in a retrospective analysis of effects on myocardial T2* after 1 and 2 years (Porter 2010, Porter 2005a). Prospective data demonstrated the efficacy of DFX in improving myocardial T2* over a range of mT2* from 5-20 ms, with 41% having severe myocardial iron loading <10 ms at baseline (Pennell 2010). In a prospective trial, 114 patients with high mean baseline LIC (mean 28 mg/g dry wt) were treated with DFX for up to 3 years (Pennell 2012), receiving mean actual doses of 33, 35, and 34 mg/kg/day during the 1st, 2nd and 3rd years, respectively. Higher mean doses of 37 mg/kg per day were received by patients with baseline T2* between 5 and <10 ms, compared with those between 10 and 20 ms (32 mg/kg per day). Of the 114 patients initially enrolled, 101 continued into the 2nd year, 86 completed two years of treatment and 71 entered into a third year. There was year by year significant improvements in mT2*; from 12.0 ms at baseline to 17.1 ms at 3 yrs, corresponding to a decrease in cardiac iron concentration (from 2.43 mg/g dry wt at baseline, to 1.80 mg/g dry wt. After three years, 68 % of patients with baseline T2* between 10 and <20 ms benefited from normalization of T2*, and 50% of patients with baseline T2*>5 to <10 ms at baseline improved to 10 to <20 ms. There was no significant variation in left ventricular ejection fraction over the three years and no deaths occurred. Tolerability was similar to other DFX studies in TM over the doses up to 40 mg/kg/day.

In a 1 year randomised prospective study (CORDELIA) 197 patients with T2* of 6-20 ms and no signs of cardiac dysfunction were randomised to DFX (target dose 40 mg/kg/day) or subcutaneous DFO treatment (50-60 mg/kg/day for 5-7 days/week) (Pennell 2014). Baseline LIC was high in both DFX (mean 29.8 mg/g dry wt) and DFO treated patients (30.3 mg/g dry wt), with 73% of patients having baseline LIC >15 mg/g dry wt. The geometric mean (Gmean) myocardial T2* improved with DFX from 11.2 ms at baseline to 12.6 ms at 1 year (Gmeans ratio 1.12) and with DFO (11.6 ms to 12.3 ms, Gmeans ratio 1.07). This study established non-inferiority of DFX vs. DFO for cardiac iron removal in this patient population. LVEF remained stable in both arms and the frequency of drug-related adverse events was comparable between DFX (35.4%) and DFO (30.8%). Taken together, these studies show that DFX is an effective treatment for patients with increased heart iron with mT2* >5-20 ms. It also demonstrates response in patients with high levels of baseline mT2* (5-10 ms), as well as those with high levels of baseline LIC or SF. As with other chelation regimes, high levels of baseline heart iron (<10 ms) will typically take several years to clear, but the risk of developing heart failure during this time appears very low (see below), provided treatment is monitored.

Effects on heart function

In the above studies, even though mT2* values at baseline were as low as 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 $T2^* < 10$ ms (Kirk 2009a). The stability of LVEF and the absence of heart failure in this otherwise high risk group of patients suggests that DFX renders effective prophylaxis for heart failure, even in patients with $T2^*$ values of 5-10 ms. This may be related to the 24hour 'protection time' against labile iron that results from the long plasma half-life of DFX (Daar 2009). Deferasirox has not been evaluated in formal trials for patients with symptomatic heart failure or LVEF $< 56\%$, therefore at this time other chelation options are recommended for such patients.

Evidence of long term survival benefits of DFX

More than 5,900 patients have been enrolled in prospective trials but these, with some exceptions, have typically been designed for short term evaluation. Up to 5 years of follow up have now been reported in one prospective clinical trial from the initial registration studies, which provides useful information about risk and benefit with this treatment (Cappellini 2011). Other prospective data of patients with myocardial $T2^*$ of 5-20 ms and high levels of liver iron but without complications, provides further insight into comorbidities and mortality in high-risk subjects. The stability of left ventricular function, lack of progression to heart failure and absence of any deaths are notable features of the prospective 3-year EPIC and 1-2 year CORDELIA cardiac studies, despite including patients at high risk of cardiac decompensation, with $mT2^*$ levels as low as 5 ms (Pennell 2012) or 6 ms (Pennell 2014).

Convenience and impact on quality of life

Convenience and quality of life on DFX, 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 than in routine clinical use. Studies comparing satisfaction and convenience of DFX with DFO in thalassaemia major show a significant and sustained preference for DFX (Cappellini 2007). In a randomised comparison, total withdrawals in DFX-treated patients was 6% at one year, compared with 4% with DFO (Cappellini 2006). This compares with a dropout rate of 15% at one year with DFP, although these are not matched populations (Cohen 2000). 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 2012).

Recommended treatment regimens with DFX

Recommended standard dosing

Deferasirox is taken orally as a suspension in water once daily, and preferably before a meal. A starting dose of 20 mg/kg is recommended for thalassaemia major patients who have received 10-20 transfusion episodes and currently receive standard transfusion at rates of 0.3-0.5 mg of iron/kg/day. In those patients in whom there is a higher rate of iron intake from transfusion (>0.5 mg/kg/day), or in patients with pre-existing high levels of iron loading where a decrease in iron loading is clinically desirable, 30 mg/kg/day is recommended. For patients with a low rate of iron loading (<0.3 mg/kg/day), a dose of 10-15 mg/kg may be sufficient to control iron loading.

Age of commencement

The labeling for age of commencement differs in countries that follow US licensing from those that adhere to EU licensing (**Appendix 1**). However, based on prospectively randomised

studies of DFX in children as young as two years of age, some 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 (Piga 2006). Deferasirox also appears to be palatable to children at this young age. 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.

Rescue therapy to achieve negative iron balance

When body iron has accumulated to high levels (see monitoring), negative iron balance is required. The proportion of patients in negative iron balance at a given dose is partially dependent on the rate of iron loading (see above). Doses of up to 40 mg/kg/day are recommended for patients with LIC or SF values and are now licensed at this dose (Porter 2013a). Dividing the dose as a twice daily dose has been used in some patients who fail to achieve negative iron balance, despite these higher doses (Pongtanakul 2013). Some patients have taken DFX after rather than before food, with apparently improved efficacy. This is consistent with the known effects of food on GI absorption (Galanello 2008).

Rescue therapy for patients with mild to moderate myocardial iron (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 (< 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.

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 at the same time, has been used relatively rarely

compared with sequential regimes. Some investigators have used the term 'alternating therapy' to describe the use of two drugs administered on alternate days, reserving the term 'sequential therapy' for when DFO is given at night and DFP during the day. In practice, regimes may involve both a component of 'sequential' and 'alternating' therapy such as when DFO is given three times a week (alternate nights) and DFP every day. Most commonly used regimes have tended to give DFP daily at standard doses, combined with varying frequency and dosing of DFO. More recently, combinations of DFX with DFO, or DFX with DFP have been evaluated. In these instances, both drugs may be present in plasma or intracellularly for at least part of the time owing to the half-life of DFX and its extended time in the plasma - for up to 24hr.

The pharmacology and mechanisms of action in combining chelators is dependent on whether the drugs are present in cells or plasma at the same time. By giving DFO at night and DFP by day, 24-hrs of exposure to iron chelation can be achieved (similar exposure to that achieved with 24-hrs desferrioxamine infusion, or once daily deferasirox). This has the theoretical advantage of 24hr protection from labile (redox active) iron (Cabantchik 2005). If the drugs are given at the same time (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 2010) and so improved efficiency of iron chelation. On the other hand, there is also the possibility of chelation from metalloenzymes, leading to increased drug-related toxicity, but this has not been an issue clinically. The use of DFX, which is present in plasma 24hrs/day, together with DFO by intermittent infusion provides 24hr chelation, with decreases in LPI and NTBI (Lal 2013). Simultaneous exposure to two chelators may also result in synergistic removal of cellular iron. This has been demonstrated in cell culture with combinations of all three chelators (Vlachodimitropoulou 2013).

Combined DFO and DFP

Combinations of these chelators has been studied more extensively than other chelator combinations so far. 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. These have been detailed elsewhere (Porter and Hershko 2012). Here some of the key studies providing useful evidence are described.

Evidence of efficacy of combined regimes

- Effects of sequential use on serum ferritin

One study (Mourad 2003) found the decrease in SF achieved using five days of DFO monotherapy (n=11) to be similar to that achieved with two nights of DFO plus seven days of DFP at 75 mg/kg (n=14). Another randomised study involving 30 patients and three different treatments (Gomber 2004) found that the decrease in SF was greatest with five nights of DFO, albeit not significantly different from that achieved with a combined treatment of DFO two nights a week, plus DFP seven days a week. A further randomised study involving 60 patients receiving 'alternating' therapy (Galanello 2006) found no difference in SF in patients randomised to combined treatment (two days DFO at 33 mg/kg + seven days DFP at 75 mg/kg), or to DFO five nights a week at 33 mg/kg. In another randomised study from Turkey (Aydinok 2007), SF decreased more with combination therapy than DFP monotherapy but similarly to DFO monotherapy. In a randomized study of 65 patients (Tanner 2007), serum ferritin was decreased more by

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). A 5-year follow up randomized clinical trial (Maggio 2009) also showed a greater SF reduction with sequential DFP (75 mg/kg/day-4 days/week)-DFO (50 mg/kg/day-3days/week) compared with DFP alone on reducing serum ferritin, with comparable adverse effects and cost. However, survival trend of both groups was not significantly different. 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).

- **Effects of sequential use on liver iron**

When an alternating DFP + DFO regime was compared to DFO monotherapy, a baseline LIC of <7 mg/g dry wt was maintained on average in both arms of the study (Galanello 2006). A different prospective randomised study found LIC reduction in either DFO monotherapy or DFP + DFO (2 times weekly) combination therapy, but not with DFP monotherapy at 75 mg/kg/day (Aydinok 2007). In another study comparing LIC changes using DFP + DFO or DFO monotherapy (5 times a week), a greater improvement in liver T2* (as a surrogate measure of LIC) was seen with combination therapy than with DFO alone (Tanner 2007).

In another randomised study 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 monotherapy (Porter 2013b). These studies did not give definitive results with respect to the comparative LIC effects of combination therapy relative to other therapies, although they do support combination therapy being more effective than DFP monotherapy. The relative efficacy of combination compared with DFO monotherapy most likely depends on the dosing and frequency of DFO used in the different regimes.

- **Effects on heart function and T2***

In a randomised controlled study of 65 patients without heart failure but with moderate heart iron loading (T2* 8-20 ms) and LVEF in the normal reference range, changes in myocardial T2* with combined DFP at 75 mg/kg seven days a week plus DFO five days a week were compared with patients on standard DFO treatment five times a week (Tanner 2007). The study showed that LVEF increased within the normal range by approximately 2.5% in the combination arm and 0.5% in the DFO monotherapy arm. Myocardial T2* improvements were seen with both treatments, but were greater in the combination arm. For high risk patients with decreased LVEF or with symptomatic heart disease, a prospective randomised study showed significant improvement in LVEF and T2* in patients receiving either DFO intensification or DFO intensification plus DFP (Porter 2013b). No statistical difference between the two study arms was found with respect to these variables, although larger study numbers would be required to show a 5% difference in LVEF response. Observational studies have also reported changes in heart function with combined treatment. In 79 patients with SF>3000 µg/L treated with a variable DFO regimen plus DFP at 75 mg/kg seven days a week for 12 to 57 months, there was an increase in LVEF by echocardiography (Origa 2005). In an observational study of 42 patients with sequential use of treatment over three to four years (DFP 75 mg/kg/day plus DFO two to six days a week), the LV shortening fraction improved (Kattamis 2006). In another observational multicentre study, changes in mT2* and LVEF were examined in patients who were receiving combined DFO + DFP (N=51), DFP (N=39) or DFO (N=74) monotherapy (Pepe 2013). The proportion of patients that maintained a

normal heart T2* value was comparable between DFP and DFO when compared with both monotherapy groups. Increase in mT2* was greater in patients on DFO + DFP, or DFP than with DFO monotherapy, but did not differ between DFO + DFP and DFP, with combination therapy not showing an additional effect on heart function over DFP alone. Monotherapy with DFX is usually effective at improving T2* across a full range of LIC levels (Pennell 2012). The effects of DFX on T2* has not been compared directly with DFP either alone, or in combination with DFO. If the imperative is to have improvement 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, as combination of DFP and DFO may result in more rapid improvement in cardiac iron load and increases in LVEF, which has not been observed with other chelation regimes.

- **Long term effects on survival**

Several retrospective analyses have reported a benefit in survival compared with DFO alone. In one such analysis, of 544 patients with β -thalassemia from Cyprus treated between 1980 and 2004, 304 switched to combination chelation therapy from 1999 as part of their regular treatment. The authors reported a worsening survival in Cyprus up until 2000, followed by an improved subsequent survival which they attributed to switching to combination therapy (Telfer 2009, Telfer 2006). 75 patients came off 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%). Some authors have achieved very low serum ferritin values using a 'flexible' approach to combination therapy without any reported side effects, although the reasons for giving combination therapy were not clear (Farmaki 2010). Controlled trials demonstrating improvement in disease-related symptoms, organ function, or increased survival are, however, lacking. Meta-analysis reviewing multiple trials found no statistically significant variations in heart T2* signal during associated or sequential versus mono-therapy treatment ($p=0.46$ and $p=0.14$, respectively) (Maggio 2011). This analysis did find "improved ejection fraction during combination associated or sequential [combination] versus monotherapy treatment ($p=0.01$ and $p=0.00001$, respectively)". However "these findings do not support any specific chelation treatment. The literature shows risks of bias, and additional larger and longer trials are needed" (Maggio 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 cerebella 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 these two drugs are useful, especially when various monotherapy regimes have failed to control either liver iron or cardiac iron. This is most commonly a consequence of pharmacological differences with DFP, or for reasons of poor adherence to DFO. In general, 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 heart iron 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

Experience with this combination is relatively limited compared with the above regimes. Two prospective studies have evaluated this combination. In the first, 22 patients were studied over 12 months of DFX at 20-30 mg/kg daily plus DFO at 35-50 mg/kg on 3-7 days/week. Median LIC was shown to decrease by 31% and median ferritin by 24%. All 6 subjects with elevated myocardial iron showed improvements in MRI T2*. Both NTBI and LPI fell significantly. Tolerability was consistent with that seen previously with individual treatments (Lal 2013). A larger prospective study has examined 60 patients with severe liver and heart iron overload (cardiac T2* 5-10 ms) given DFX 20-40 mg/kg/d 7 days per week, plus DFO 40 mg/kg/d 5 days per wk for ≥ 8 hrs/d (Aydinok 2013). Results up to 2 years show a reduction in SF of 44% and 52% in LIC, and an increase in cardiac T2* of 33% (Aydinok 2014). Improvement in mT2* were greater in patients with baseline LIC <30 than those >30 mg/g dry wt. LVEF remained stable during the study. Tolerability was consistent with that seen with monotherapy regimes.

Combined DFX and DFP

Experience with the combination of these two drugs is currently even more limited. Single case reports suggest that this is an effective regime (Voskaridou 2011). One study reported combined use in 16 patients for a period of up to 2 years with decrease of total body iron load as estimated by serum ferritin, LIC and MRI T2* indices (Farmaki 2010). The incidence of adverse events was minor compared to the associated toxicity of monotherapy of each drug. No new onset of iron overload-related complications was demonstrated, with reversal of cardiac dysfunction in 2/4 patients and significant increase in mean LVEF. More recently, preliminary results of a larger randomised trial have been presented comparing this combination with DFP monotherapy (Elalfy 2013). In 96 patients in Egypt, two combination regimes were compared over 1 year: 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 (the frequency of DFO is not stated in the abstract). SF, LIC, and mT2* improved significantly in both groups and no serious adverse events were reported during the study in either treatment group. The authors reported improvement in quality of life in a greater number of patients in the arm containing DFX, than those treated with DFO. These findings are encouraging but further studies are needed to clarify the tolerability of this approach, and to determine how this might be used most effectively and safely. The optimal relative doses and frequency of each drug also need to be determined, which may vary depending on the degree of cardiac or overall iron overload.

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 in detail 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 $\mu\text{g/L}$ because of fears toxicity would have on growth, ears and eyes

at low levels of body iron. It may be that with new chelator regimes that chelation can be started earlier, however, information about this is limited at present. In practice, the exact timing of starting chelation is currently constrained to some extent by the licensing of the compound by regulatory authorities, which differs somewhat between countries. 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 is increasing – rescue therapy to achieve negative iron balance

If body iron load builds up because of a delayed 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:

- (a) Increasing the dose of chelation a, b, c
- (b) Increasing frequency of the chelator (improving adherence d, or increasing prescription advice)
- (c) Switching chelator regimen
- (d) Rotating e or combining f chelators
 - 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 7). Doses >35 mg/kg and up to 40 mg/kg 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. DFO is often added to this.
 - 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 has been shown to be useful in individual patients when monotherapy is inadequate, either to control iron balance or to control iron distribution, particularly in the heart.

Mild increase in cardiac iron (T2* 10-20 ms) - rescue therapy to remove heart iron

DFO, DFP and DFX monotherapy are all effective at decreasing heart 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 2009b). Regular daily monotherapy at optimal doses (often an increase from current dose or frequency) will usually improve heart iron but normalisation of heart iron can take several years of consistent therapy and sequential monitoring of T2* is required. Monotherapy with DFX is usually effective and recent work shows that DFX is effective at improving T2* across a full range on LIC concentrations (Pennell 2014, Pennell 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 s.c. 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 T2* with DFO, DFP or DFX monotherapy, then combined DFP and DFO should be considered.

Cardiac T2* < 10 ms - rescue therapy for heart iron

The risk of developing heart failure increases with lower cardiac T2*, especially when values drop below 10 ms (i.e. higher heart iron). However, if continuous chelation therapy is given, heart failure may be prevented even before the T2* is corrected. This has been shown for continuous 24h DFO, with high dose DFX in a population where T2* was 6-10 ms, and in patients treated with different combination regimes (DFO+DFP, DFO+DFX). Patients with T2* ≤ 6 ms, are a very high risk group for developing heart failure. This group has not been evaluated extensively with interventional studies (expect people with heart failure). There is some experience of treating these patients with DFO + DFP, but randomised trials did not include patients with T2* values < 8 ms (Tanner 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 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 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 2013b). Once reversal of heart failure has been demonstrated clinically and with myocardial MRI or echocardiography, continuation of the same therapy is recommended until the cardiac T2* improves to T2* above 8 ms, depending on the starting T2* this may take as long as a year. 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 problem 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 iron 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 clear with some of the newer chelator regimes, and these are discussed in the respective sections on individual chelators. 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 can be achieved even in patients not receiving transfusion, provided the doses are low (5-10 mg/kg), as SF values fall below 500µg/L (Taher 2013). Cases of toxicity from over chelation have been observed when SF are higher than this, if the rate of decrease is rapid. DFX dose adjustment consideration should be made at the first sign of increasing serum creatinine values (as a late event associated with falling SF or LIV 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 and Recommendations

- 1) Uncontrolled transfusional iron overload increases the risks of heart failure, endocrine damage, liver cirrhosis and hepatocellular carcinoma **(B)**.
- 2) Liver iron concentration can be used to calculate total body iron, and serum ferritin is an approximate marker of LIC **(B)**.
- 3) Chelation therapy is an effective treatment modality in improving survival, decreasing the risk of heart failure and decreasing morbidities from transfusional iron overload **(A)**.
- 4) Chelation therapy at the correct doses and frequency can balance iron excretion with iron accumulation from transfusion **(A)**.
- 5) Absolute change in total body iron in response to chelation can be calculated from change in LIC **(B)**.
- 6) 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)**.
- 7) 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)**.
- 8) Response to chelation is dependent on the dose applied and the duration of exposure **(A)**.
- 9) Response to chelation is affected by the rate of blood transfusion **(B)**.
- 10) Heart iron accumulates later than liver iron, and is rare before the age of 8 years; affecting a subset of patients **(B)**.
- 11) Chelation of storage iron from the liver tends to be faster than from myocardium **(B)**.
- 12) Heart 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)**.
- 13) Chelation can reverse iron mediated heart dysfunction rapidly (weeks) by rapid chelation of labile iron, if 24h chelation cover is achieved **(A)**.
- 14) Chelation therapy removes myocardial storage iron slowly (months or years) **(A)**.
- 15) 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)**.
- 16) The optimal chelation regime must be tailored for the individual and will vary with their current clinical situation.
- 17) Chelation therapy will not be effective if it is not taken regularly – a key aspect of chelation management is to work with patients to optimize adherence **(B)**.

Appendix 1

Table A1. Licensed indications, and precautions for chelation in thalassaemia.

CATEGORY	DFO (DESFERRIOXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
Children age 2-6	First line for TM	Insufficient information for licensing	First line in USA Second line when DFO contra-indicated or inadequate in Europe
Children age > 6 and adults	First line TM	If other chelation (FDA 2011) or DFO not tolerated or ineffective	First line TM First line NTDT
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 in EU Children's dose up to 40 mg/kg	75 -100 mg/kg/day in 3 divided doses daily	20-40 mg/kg/day once daily. Lower doses in NTDT
Contra-indications	<ul style="list-style-type: none"> - Pregnancy (but has been used in 3rd trimester) - Hypersensitivity 	<ul style="list-style-type: none"> - Pregnancy - History of neutropenia or condition with underlying risk of cytopenia - Hypersensitivity including Henoch Schönlein purpura: urticaria and periorbital oedema with skin rash 	<ul style="list-style-type: none"> - Pregnancy - Hypersensitivity - Estimated creatinine clearance <60 ml/min - Hepatic impairment or renal failure

CATEGORY	DFO (DESFERRIOXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
Precautions	<ul style="list-style-type: none"> - Monitor ferritin: if it falls to $<1000 \mu\text{g/L}$, 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 - Fever suggestive of septicemia with organisms that used ferrioxamine (yersinia, klebsiella) - Renal failure or diminishing renal function with other comorbidities 	<ul style="list-style-type: none"> - Measure neutrophil count (ANC) before starting and monitor ANC weekly - For neutropenia : $\text{ANC} < 1.5 \times 10^9/\text{L}$) interrupt treatment - For agranulocytosis ($\text{ANC} < 0.5 \times 10^9/\text{L}$), consider hospitalization - Advise patients to report immediately symptoms of infection: Interrupt if fever develops - Monitor for symptoms of arthropathy - Monitor liver function regularly - No guidance on dose adjustment at low ferritin 	<ul style="list-style-type: none"> - Monitor creatinine trends for 1st 4 weeks after starting or after dose escalation then monthly - If rapid fall in serum ferritin to $<1000 \mu\text{g/L}$- dose reduce. If ferritin $500 \mu\text{g/L}$ consider very low doses[^] - Proteinuria may occur: occasionally with renal tubular acidosis. Monitor urine protein regularly - Prescribing to the elderly: non-fatal gastrointestinal bleeding, ulceration, and irritation may occur: caution with drugs of known ulcerogenic or hemorrhagic potential, (e.g. NSAIDs, corticosteroids, oral bisphosphonates, and anticoagulants) - Hypersensitivity reactions - Monitor liver function regularly

CATEGORY	DFO (DEFERRIOXAMINE)	DFP (DEFERIPRONE)	DFX (DEFERASIROX)
Potential drug interactions	<ul style="list-style-type: none"> - Co-administration with prochlorperazine: may lead to temporary impairment of consciousness. - Gallium-67: Imaging results may be distorted by rapid urinary excretion of Desferal bound gallium-67. Discontinuation 48 hours prior to scintigraphy advisable 	<ul style="list-style-type: none"> - Theoretical interactions with UGT1A6 inhibitors (e.g. diclofenac, probenecid, or silymarin (milk thistle)) - Avoid concomitant use with drugs associated with neutropenia - Gallium-67 as with DFO - Oral preparations containing polyvalent cations (e.g., aluminum containing antacids, and zinc) allow at least a 4-hour interval 	<ul style="list-style-type: none"> - Theoretical interactions with drugs metabolized by CYP3A4 e.g. midazolam - Theoretical interactions with drugs metabolized by CYP1A2: e.g. Theophylline - Gallium-67 as with DFO - Oral preparations containing polyvalent cations as with DFP

^ Drug labelling recommends stopping when ferritin 500 µg/L but this risks rebound labile iron and see-saw pattern of iron overload. Consider gradual dose reduction as ferritin falls < 1000µg/L.

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 this appendix we have placed these in the context of overall management of TM patients.

Evidence for the relative frequency of adverse events in randomised studies have been aggregated from 18 trials by systematic review (Fisher 2013a, Fisher 2013b). This concluded "Adverse events are increased in patients treated with DFP compared with DFO and in patients treated with combined DFP and DFO compared with DFO alone. People treated with all chelators must be kept under close medical supervision and treatment with DFP or DFX requires regular monitoring of neutrophil counts or renal function".

Unwanted effects with desferrioxamine

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, as most data were accumulated in the 1970s and 1980s, when optimal dosing was not fully understood. In a 1 year randomised clinical trial comparing DFO with DFX, abnormalities of hearing were reported as adverse events irrespective of drug relationship in 2.4% on DFO. Cataracts or lenticular opacities were reported as adverse events irrespective of drug relationship in 1.7% on DFO. A similar percentage of patients receiving DFX and DFO experienced cardiac adverse events (DFX 5.1%, DFO 6.9%).

Unwanted effects related to excessive chelation

- **Hearing problems**

High frequency sensory neural loss, tinnitus and deafness may occur when DFO is given in high doses, particularly to young children whose iron burden is low (Olivieri 1986), and when the therapeutic index is exceeded (>0.025) (Porter 1989). Minor sensory neural deficit has been reversible in some cases, but significant hearing loss is usually permanent. Tinnitus may also occur. It is therefore advisable to monitor audiometry yearly, bearing in mind that audiometric changes due to excessive DFO are usually symmetrical; asymmetry suggests other pathology. Annual monitoring is particularly important in patients where SF values fall rapidly, or are $<1000 \mu\text{g/L}$, or 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. Retinal effects were first noted when very high doses ($>100 \text{ mg/kg/day}$) were given (Davies 1983). 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. Examination may also include scotoma and optic neuritis. The main risk factor appears to be high dosing (Olivieri 1986) but complications are also more likely in patients who have diabetes (Arden 1984), or those receiving concomitant phenothiazine treatment (Blake 1985). Treatment with DFO should be temporarily suspended in patients who develop complications, to be reintroduced at lower doses once investigations indicate resolution of the problem. Formal monitoring with electroretinography is recommended in patients on continuous DFO infusions, or in patients where high doses have been given relative to the iron load.

- **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 ($<3 \text{ yrs}$) (De Virgillis 1988, Piga 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 mg/kg until growth has ceased. 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, where patients have a low level of iron loading (Gabutti 1996, Olivieri 1992, De Virgillis 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 demineralization 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 have 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 1985). Hypotension can also occur with rapid intravenous injection, as may occur during flushing of a line containing DFO, which should therefore be avoided.

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 increase.

- **Infection with *Yersinia enterocolitica***

This is an important risk associated with DFO treatment (described in detail in **Chapter 7**). Such infections may be difficult to diagnose. However, where there is reasonable clinical suspicion of infection of *Yersinia enterocolitica*, treatment with DFO should be temporarily discontinued. Infection should be considered in any patient with a febrile illness, especially when associated with abdominal pain, diarrhoea or joint pains, and should be treated as a medical emergency. DFO can usually be reintroduced once symptoms have subsided and a full course of antibiotics completed. Other infections such as *Klebsiella* may also be exacerbated by continued treatment with DFO. It is therefore recommended to cease administration of DFO in anyone with an unexplained fever, until the cause has been identified and effective antibiotic treatment begun. The decision as to when to recommence treatment with DFO requires clinical judgment and a careful balancing of the potential risks and benefits. For example, a patient with high cardiac iron or poor heart function may be at high risk if DFO is withheld during a septic episode, outweighing the risks of infection once antibiotics have been commenced.

- **Severe hypersensitivity**

This is a rare event and can be treated by careful desensitization, carried out under close medical supervision (Bosquet 1983, Miller 1981). Desensitization 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**

In clinical trials, the rates of adverse reactions based on pooled data collected from 642 patients who participated in single arm or active-controlled clinical studies are available. The most common were chromaturia (red in colour due to urine in iron), 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%), diarrhea (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 3 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 or indeed decreased at lower doses. Because there are no formal studies examining dose in relation to unwanted effects in DFP, it is not clear which of the effects described below are due to over-chelation and which are independent of this mechanism. These are therefore described together. As a result of the various unwanted effects, 20-30% of patients are unable to sustain long-term treatment with DFP (Hoffbrand 1998).

- **Neutropenia, agranulocytosis and thrombocytopenia**

The labeling for DFP includes a boxed warning stating that the drug can cause agranulocytosis (absolute neutrophil count or ANC <500/mm³), which can lead to serious infections and death as a consequence of infection. This agranulocytosis may be preceded by neutropenia. The frequency reported incidence is approximately 1.7% of patients. Each patient's absolute neutrophil count should be measured before starting DFP therapy and weekly during treatment. The labeling states that DFP therapy should be interrupted and the patient's neutrophil count closely monitored if an infection develops. The mechanism for DFP's adverse effects on leukocytes is not known according to the labeling and is unpredictable in humans. Dose related effects on bone marrow hypoplasia and neutropenia has been seen in animal studies. Reported timing of onset of agranulocytosis is variable, from a few months to nine years. The condition may occur with thrombocytopenia, but isolated thrombocytopenia has also been occasionally reported, particularly in patients of Asian origin with probable hypersplenism.

In a prospective trial where weekly neutrophil counts were undertaken and where DFP was discontinued when ANC was <1,500/mm³, agranulocytosis developed in 0.2/100 patient years and milder forms of neutropenia (ANC 500-1500/mm³) occurred in about 2.8/100 patient years (Cohen 2003, Cohen 2000). Neutropenia is more common in patients with intact spleens and commonly occurs in the first year of therapy. Recently, a continuation of DFP in patients with mild neutropenia (ANC 1000-1500/mm³) has been advocated by some investigators, with daily blood counts until resolution (El-Beshlawy 2013). However, it can be difficult in clinical practice to distinguish whether a low neutrophil count is part of a pattern of underlying neutropenia, or the first sign of agranulocytosis developing, which can be fatal. In 46 cases of agranulocytosis reported in Europe there were nine related deaths (Swedish Orphan 2006). Five cases were in patients who had been prescribed the drug for an unspecified 'off label' indications,

and several were not receiving weekly blood count monitoring. Swedish Orphan subsequently issued the following recommendations on the use of DFP: "ANC should be monitored every week or more frequently if there are signs of infection; concomitant treatment that could affect the white cell count should be avoided; if severe neutropenia or agranulocytosis develop, the drug should be stopped and not reintroduced, and the use of GM-CSF should be considered in the case of agranulocytosis; off-label use of the drug should be avoided". Agranulocytosis and neutropenia has also been reported in patients taking combinations of DFP and DFO.

- **Gastrointestinal symptoms**

Nausea, vomiting, gastric irritation and change in appetite (loss or gain) occurs in 3-24% of patients (Ceci 2002, Cohen 2000). A proportion of patients have to stop treatment for these reasons, which varies between studies. A liquid preparation is available which may be more palatable in children 1-10 years of age (EIAly 2010).

- **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 ALT levels, and 0.16% due to an increase in both ALT and AST (FDA, licensing information 2011). The frequency varied considerably between studies; about a quarter of patients show ALT fluctuations of twice the normal upper limit (Cohen 2000). By contrast, one prospective randomised study reported no significant end-of-study changes in liver enzymes with DFP or DFO (Pennell 2006b). An observational report of fibrosis after treatment of three or more years (Olivieri 1998) has not been supported by other reports (Wanless 2002, Hoffbrand, Tondury 1998). A relevant prospective randomised study investigating the progression to fibrosis using DFP for one year showed no difference as compared with DFO over the same period, and no difference in baseline and end-of-treatment liver function tests (Maggio 2002). A reduction of increased liver transaminase was observed in patients who were responding to DFP monotherapy, accompanied with a decreased LIC (Viprakasit 2013). Fluctuation of liver enzymes more than twice the upper limit of normal should prompt investigation of the cause and consideration for interrupting DFP therapy.

- **Arthropathy**

The frequency of arthropathy varies greatly between studies, from as low as 4.5% at one year (Cohen 2000) to 15% after four years (Cohen 2003) in a predominantly European patient group, and as high as 33-40% in studies of patients from India (Sharma 2013, Choudhry 2004, Agarwal 1992). It is not yet clear whether these differences reflect environmental or genetic differences, or differences in iron overload between populations at the start of treatment. 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. Cases involving other joints, such as wrists, ankles and elbows and avascular necrosis of the hips have also been described. Radiologic evaluations revealed bony dysplasia, deformation and impaired growth of ulnar epiphyses in 13 out of 40 thalassaemia children who have received DFP for an average of 84 months (range 12-128 months) (Sharma 2013). It is recommended that treatment should be stopped where joint symptoms continue despite a reduction in DFP dose and are not controlled by non-steroidal anti-inflammatory drugs.

- **Neurological effects**

DFP penetrates the blood brain barrier and has been evaluated for the treatment of conditions associated with brain iron deposition such as Friedreich's ataxia (Tsou 2009). Neurological complications are very rare in thalassaemia treatment and have been typically associated with unintentional overdosing (>230 mg/kg/day). Reports of rare neurological effects have included stroke, cognitive effects, nystagmus, walking disorders, ataxia, dystonia and impaired psychomotor skills. These effects appear to improve on cessation of treatment. Cerebellar syndrome has been described with combination therapy.

- **Effects on ears and eyes**

One study reported continued audiometric deterioration after switching from DFO to DFP (Chiodo 1997). A 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 low ferritin (Chao 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 1994). Zinc deficiency is difficult to measure in plasma samples, and needs to be taken during fasting, in the absence of chelator in the blood. Zinc deficiency has been linked to toxicity of DFP in animal studies and were abrogated by zinc supplementation (Maclean 2001). Some clinicians routinely add zinc supplementation with DFP monotherapy or combination therapy is given (not given at the same time as the DFP) (Porter 2013b).

- **Frequency of adverse events compared with DFO**

Adverse effects have been reported in four randomised studies comparing DFP with DFO. One trial has reported data that allows comparison of the probability of an adverse event with DFP and DFO (Maggio 2002), establishing a statistically significant two-fold difference between DFP (34%) and DFO (15%), but no difference between temporary or permanent treatment withdrawal.

- **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.

- **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 (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.

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. The frequency of adverse effects appears somewhat higher at doses of 25 to < 35 mg/kg/day (n = 136; 39.4%) than at 15 to < 25 mg/kg/day (n = 118; 31.1%) or < 15 mg/kg/day (n = 101; 29.4%).

- **Relationship to dose and iron overload**

In general it is helpful to divide these effects into those which result from excess dosing with respect to the degree of iron overload and those which do not. Gastrointestinal, skin and renal effects can all be affected by dosing, although the exact relationship to body iron load has not been determined. It has however been shown in NTDT that with suitably low dosing (not exceeding 5-10 mg/kg) and low levels of ferritin or LIC, that low ferritin and LIC levels can be achieved without renal or other toxicities. Although a therapeutic index (as with DFO) has not been calculated, the general principle of reducing the dose, either as serum ferritin falls rapidly, or drops below 1000 µg/L for the first time would be adhered to. 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 (5 mg-10 mg/kg) to those patients who continue to be transfused. Some effects are notable by their absence, such as effects on growth, bone and arthropathy. The relationship of unwanted effects to iron load (LIC) has also been reported in 373 patients (Porter 2013a). Here, drug-related gastrointestinal AEs - mostly mild to moderate - were more frequently reported in patients with baseline LIC <7 versus those ≥7 mg/g dry wt, and were not confounded by diagnosis, dosing, ethnicity or a history of hepatitis B or C. Reported serum creatinine increases did not increase in low versus high-iron cohort patients. The smaller ESCALATOR trial however, found no clear trends in the type or frequency of drug-related AEs between the LIC <7 and ≥7 mg/g dry wt cohorts (Taher 2009). Tolerability of DFX tends to improve with long-term treatment (Cappellini 2011, Cappellini 2010). A practical guidance paper has been published which includes a summary of DFX-associated adverse events and a set of proposed management strategies (Vichinsky 2008).

- **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 2010). These symptoms rarely require dose adjustment or discontinuation, and decrease year on year over 5 years of follow up (Cappellini 2011). In the EPIC study, these symptoms were more common in patients with low baseline LIC values, as were abnormal LFTs (Porter 2013a). It is unclear to what extent the lactose component of the DFX formulation affects gastrointestinal tolerability in lactose-intolerant patients but this requires clarification, as lactose intolerance is common - particularly in South-East Asia. The role of co-administration of acidophilus or lactobacillus probiotic yoghurt to aid lactose has not been systematically studied. Hematemesis and melena due to gastric and/or duodenal ulceration has been reported in patients taking DFX (Yadav 2013, Bauters 2010). These patients should be investigated and managed appropriately, including *Helicobacter pylori* eradication therapy if

required. Special attention should be taken in patients taking concomitant medications that can increase the possibility of gastric ulceration. Although the manufacturer does not recommend taking DFX with food or dividing the doses, some clinical experience has found that more flexible dosing schedules may be more suitable in patients who experience gastrointestinal disturbances. While the drug should still be taken at the same time each day, administration of DFX in the evening with or after food can potentially improve gastrointestinal tolerability and compliance. For example, a recent study found that palatability and gastrointestinal tolerability of DFX was improved when patients were allowed to take treatment with a soft food at breakfast, or with a beverage of choice (Goldberg 2013). Another option is to divide the DFX dose, which can help to reduce gastrointestinal events without adversely affecting iron excretion (Otto-Duessel 2007).

- **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. Skin rash is more common in Asian population (up to 18%), often mild in severity and rarely developing into severe drug-hypersensitivity (Viprakasit 2011). 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 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. Severe skin rash associated with angioedema is rare, and unlike the more common rash seen with DFX, may not respond to interruption and reintroduction at a lower dose. Here, DFX therapy may need to be halted completely as the angioedema may be evidence of an immune sensitisation response.

- **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 (Cappellini 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. In a randomized study, dose reduction of 33-50% was planned if at least two consecutive increases in serum creatinine were $>33\%$ above baseline. As the creatinine spontaneously normalised in a number of cases, dose reductions were instituted in only 13%. In about 25% of those cases, the creatinine then returned to baseline, while in the rest it remained stable or fluctuated between baseline and the maximum increase observed prior to dose reduction. At 5 years of follow up, no evidence of progressive renal dysfunction had been reported where the above doses and modifications were used (Cappellini 2011). Other causes of increasing creatinine should also be considered in patients on DFX therapy, such as renal stones or concomitant use of NSAIDs. If a patient becomes acutely unwell for another reason, such as septicemic shock or severe acute vaso-occlusive complications in 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 2010). Elevation of urine calcium and cystatin C are

also seen in patients on DFX or DFP and DFO, whereas elevation of B2microglobulin was seen in patients on DFX only (Economou 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 ration 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 2011, Grange 2010). All cases recovered following withdrawal of DFX and appropriate electrolyte supplementation. Symptoms of renal tubular acidosis can be non-specific but may include polyuria, polydipsia and dehydration. Investigations may show proteinuria, hypokalemia, hypophosphatemia, hyperchloremic metabolic acidosis with excessive loss of substances in the urine (e.g. amino acids, glucose, phosphate and bicarbonate). Some patients, especially children, have intercurrent infections associated with Fanconi syndrome. Renal impairment due to DFX may also develop as part of a generalized delayed hypersensitivity reaction. It is recommended that kidney and proximal tubular function be periodically monitored in patients receiving DFX throughout their course of therapy.

- **Hepatic effects**

Generally speaking 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 >10x the upper limit of normal (Cappellini 2010). These changes are commonly observed within 1 month of initiating DFX therapy, although this may occur later, particularly at high doses in patients with low iron burdens. Careful baseline assessment of alanine transaminase levels (ALT) should therefore be performed before starting treatment, and monitored every two weeks for the first month. Thereafter, 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 such cases, where the patient is started on DFX 125 mg, with the dose increased every 2–3 weeks provided the ALT levels remain stable with weekly monitoring. 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 2011). By the end of the study, stability of Ishak fibrosis staging scores (change of -1, 0, or +1) or improvements (change of ≤ -2) were observed in 82.6% of patients. DFX treatment for 3 or more years reversed or stabilized liver fibrosis in 83% of patients with iron-overloaded beta-thalassaemia.

- **Effects on hematology**

Although hematological adverse effects such as thrombocytopenia and agranulocytosis have been added to the product information since DFX was approved, only a few cases have been published as case reports thus far. In two patients, thrombocytopenia due to DFX developed as part of a generalized delayed hypersensitivity reaction, in combination with a rash, fever, eosinophilia, hepatic and renal impairment (Wei 2011, Gutiérrez Macías 2010).

- **Arthropathy and growth failure**

No cases of arthropathy or growth failure have been associated with DFX administration. Comparing 296 patients who received DFX in a one-year prospective randomised study with 290 patients receiving DFO, deafness, neurosensory deafness or hypoacusis were reported as adverse events in eight patients on DFX, and seven on DFO.

- **Eyes and ears**

These are very rare and their significance is uncertain, however, current labelling recommends yearly auditory and eye assessments (Novartis 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 2006). The electroretinographic effects previously seen with DFO have not been described, and the frequency at which electroretinography assessment is indicated has not been formally assessed. Possible audiometric effects were identified in early studies but this has not been reported systematically. One investigator has reported lens opacities in 3 out of 12 patients (Bloomfield 1978), which would approximate to 80-times the incidence observed in the large-scale trials, the reason for which is presently unclear (Ford 2008).

- **Pregnancy and DFX**

DFX has been shown to have teratogenic effects in animal studies. However, there has been a report of a healthy male baby delivered to a 38-year-old mother with thalassemia major who has unintentionally conceived during DFX therapy (Anastasi 2011). However, at present it is recommended that thalassaemia patients who plan to conceive should avoid the use of iron chelation for at least 3 months before.

- **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 3**). 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 (see **Figure 4**). Patient preference is highly variable and clinicians should explore the best type of needle for each patient, to help maximize compliance.

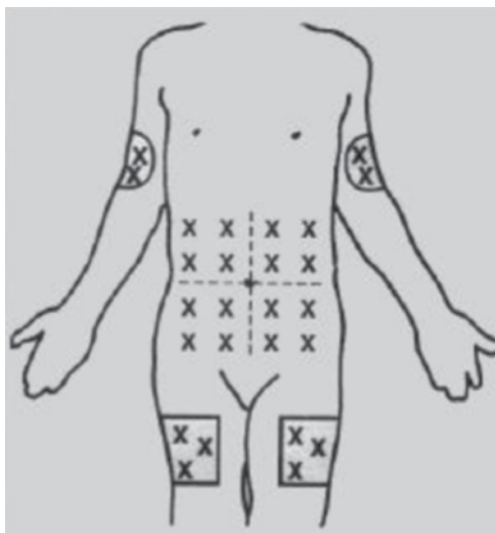


Figure 3. Rotation of infusion sites.

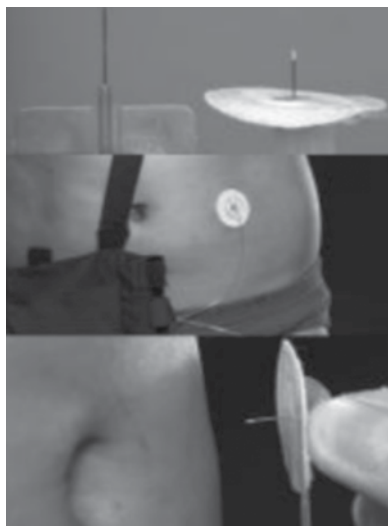


Figure 4. Insertion of needles for desferrioxamine infusions.

- **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 mls 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 2006). Use of prophylactic anticoagulation is advised, as line-thrombosis is relatively common in thalassaemia major (Davis 2000). As development of thrombosis 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. 1g 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. Co-administration of DFO and blood can lead to errors in interpreting side effects, such as acute fever, rashes, anaphylaxis and hypotension during blood transfusion. DFO should never be added directly into the blood unit.

- **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. A randomised study has shown that serum ferritin and liver iron can be controlled equally effectively by giving an equivalent total dose (45 mg/kg x 5 per week) either as two subcutaneous 'boluses' or as a nightly 10-hour subcutaneous infusion (Yarali 2006). However, this technique may be impractical in the clinic – particularly in paediatric patients, due to the painful nature of bolus infusions.

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CARDIAC COMPLICATIONS IN THALASSAEMIA MAJOR

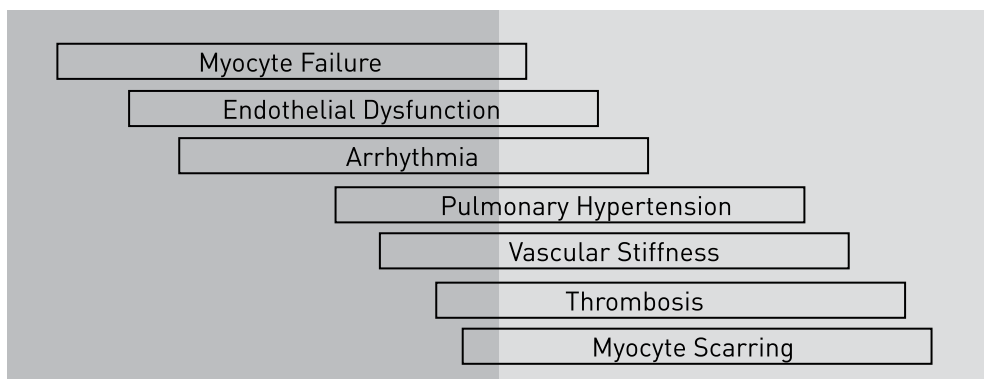
Authors Malcolm Walker and John Wood
Reviewer Ali Taher

The quality and duration of life of transfusion-dependent patients with thalassaemia has been transformed over the last decade (Borgna-Pignatti 2010, Modell 2008, Telfer 2006). It should now be expected that with well organized care a patient with thalassaemia will live a good quality life into middle age and beyond, including the possibility of raising a family of their own. Although historically the major complication affecting the heart was heart failure due to accumulation of iron within heart muscle cells (myocytes), with increased survival other manifestations of thalassaemia have become apparent. Thus the cardiovascular complications of thalassaemia can be considered in two major clinical categories:

1. **Iron overload complications**
 - a. Reversible myocyte failure.
 - b. Arrhythmia, including heart block.
 - c. Arterial changes - loss of vascular compliance.
2. **Non-iron overload complications**
 - a. Pulmonary hypertension.
 - b. Arrhythmia – particularly Atrial Fibrillation (AF) later in life.
 - c. Thrombotic stroke, linked to AF.
 - d. Cardiac function changes due to restriction / diastolic dysfunction / fibrosis.
 - e. Arterial changes - loss of vascular compliance.

IRON MEDIATED

NON IRON MEDIATED



An important consensus document on cardiac management in thalassaemia was recently published (Pennell 2013). Previously published consensus documents (Cogliandro 2008) and review articles (Walker 2012, Wood 2005) may also serve as valuable references.

Key commentary: Iron-related heart complications of thalassaemia were once the leading cause of death and remain one of the leading causes of morbidity.

Cardiac Dysfunction

Pathophysiology

Cardiac iron accumulation is the single greatest risk factor for cardiac dysfunction in thalassaemia. Cardiac iron loading occurs when the heart is exposed to high circulating non-transferrin bound iron species for long periods of time. The exact transport mechanisms remain controversial, although animal studies suggest a role for L and T-type calcium channels. The duration of chelator exposure appears to be an important determinant of cardiac iron accumulation, independent of total body iron balance. As a result chelation strategies that deliver high drug doses, sporadically, should be avoided, even if this strategy can successfully control liver iron and serum ferritin.

Once inside the heart, labile iron is quickly bound to ferritin and degraded to hemosiderin. This buffering mechanism is vital to survival and creates a clinically-silent condition where cardiac iron stores are increased but toxic labile iron species are not present (Anderson 2001). MRI assessment of cardiac T2* can identify and quantitate cardiac iron stores (Carpenter 2011), allowing modification of iron chelation before cardiac symptoms develop.

Eventually, iron buffering mechanisms in the heart fail. The greater the stored iron, the higher the probability this will occur (Kirk 2009). Once labile iron levels rise in the myocyte, they produce oxidative damage to membranes, iron transporters, and DNA, triggering cardiac dysfunction, arrhythmias and if not reversed, eventual fibrosis. Dysregulation of calcium homeostasis, particularly the ryanodine channel, is believed to play an important role in iron cardiomyopathy.

From the clinical point of view, the key feature of iron overload complications, even when severe, is that with intensive chelation therapy they may be reversible. However, prevention of excessive iron overload remains the primary responsibility of the clinicians in charge of thalassaemia patients, since, once symptomatic heart failure occurs there is a high immediate risk of death.

Despite undoubted improvements in care, cardiovascular disorders remain crucially important and their early recognition mandates intensified chelation therapy, with specific cardiac interventions and medication taking second place in priority. Prevention of early life iron load will also impact on at least some of the more troublesome “non-iron overload” complications in later life, such as atrial fibrillation (AF).

Although iron is the most important cause of cardiac dysfunction, deficiencies in carnitine, thiamine, vitamin D, and selenium can worsen cardiac function; these nutrients are commonly deficient in thalassaemia (Claster 2009, Wood 2008). Hypothyroidism, hypoparathyroidism (DeSanctis 2008), and hypogonadism can also exacerbate cardiac dysfunction. Acute myocarditis can precipitate severe heart failure, arrhythmias and heart block (Kremastinos 1995), although this complication is not commonly seen in many countries.

Iron-negative cardiac dysfunction is also encountered in older thalassaemia patients from the Mediterranean region. Patchy delayed hyperenhancement, consistent with fibrosis, has also been described in the same study population (Pepe 2009), raising the possibility that longstanding hepatitis C infection may produce smoldering myocarditis and myocardial dysfunction (Matsumori 2006).

Key commentary: Even after significant toxic effects on heart muscle have prevailed, aggressive iron chelation can restore myocardial function to normality.

Clinical manifestations: symptoms and signs

Patients with considerable iron overload of the heart may remain free of symptoms. Once myocardial dysfunction develops, symptoms are related to the degree of ventricular impairment. Subtle early signs may be confused with the effects of the underlying condition. For example, breathlessness during exercise may be attributed to anaemia. In more advanced stages of heart failure, clinical presentations are equivalent to those seen with any severe heart muscle disease and may include dyspnoea, peripheral oedema, hepatic congestion and severe exercise limitation.

Clinical presentation of heart failure is variable. Classic left heart failure features, including rales, or crackles, dyspnea on exertion, and orthopnea are a late finding. Right heart failure symptoms, including neck vein distension, hepatomegally, and peripheral edema, often are the first clinical signs (Pennell 2013). Rapid decompensation, with predominant right heart failure features, may mimic an acute abdomen, with tender hepatomegally mistaken for cholangitis or biliary obstruction. The development of the signs of classical heart failure implies advanced disease with a poor prognosis, until the acute situation is resolved, by intensive chelation. It must be emphasised that the patient may require support of the failing circulation for a period of several weeks in order to achieve a recovery.

Key commentary: An important distinguishing feature of heart failure due to iron overload is the capacity of heart function to make a complete recovery with appropriate chelation therapy - a fact that may not be widely appreciated by physicians and cardiologists unaccustomed to dealing with patients with thalassaemia.

Symptoms of palpitations are common in patients with thalassaemia, and are a frequent cause for anxiety - both for patients and their physicians. In brief, the prognostic implications of arrhythmia are related to the degree of myocardial iron-overload and any associated myocardial dysfunction. Thus in the case of a non-iron overloaded patient, the development of an arrhythmia such as atrial fibrillation (AF) deserves simple investigation and possible pharmacological treatment including anti-coagulation, but does not necessarily imply an adverse outcome. The same arrhythmia in a heavily iron overloaded heart, particularly if cardiac dysfunction is present, may be the harbinger of severe decompensation and requires immediate response and probable hospitalisation. Chest pain is uncommon in thalassaemia, but may accompany intercurrent illnesses including pericarditis or myocarditis. The frequency of these complications appears to differ between countries.

Key commentary: Management of the patient with palpitations depends on the clinical situation taken as a whole, including iron loading status and cardiac function.

Clinical examination

A thorough medical history and physical examination are required for a basic cardiological assessment, which should also include: 12-lead electrocardiogram and a detailed echocardiogram, undertaken according to published guidelines. Cardiac magnetic resonance imaging (CMR), used to quantitatively estimate cardiac iron overload (T2*), has become an invaluable tool in the estimation of clinical risk for the development of heart complications in thalassaemia. Additional tests may also be valuable for the detailed assessment of individual clinical problems, such as the investigation of cardiac arrhythmia

(Holter or 24-hour ECG) or functional assessment by exercise tests.

Key commentary: The regular assessment of cardiac status helps physicians to recognise the early stages of heart disease and allows prompt intervention.

Cardiovascular investigations

- **Electrocardiogram – the ECG or EKG**

- The electrocardiogram is frequently abnormal, but changes are typically non-specific. These changes commonly include depolarisation changes in the T-waves and ST segments of the anterior chest leads, the T wave axis and QT interval (Detterich 2012), and sometimes a preponderance of right ventricular voltages. Occasionally P-waves are also affected, suggesting bi-atrial enlargement. First degree heart block and conduction disturbance in the forms of bundle branch block may be seen but higher degrees of conduction disturbance are rare. Given that ECG changes are nonspecific and reversibility with iron chelation has not been established, it is important to begin regular monitoring in childhood to detect new onset ECG changes.

When new ECG abnormalities appear during follow-up, further investigation is required in order to detect the cause. This is particularly the case for changes suggesting an increase in right heart forces. These may reflect the development of pulmonary hypertension, which is a common complication in thalassaemia intermedia, but less common in thalassaemia major, where it is often iatrogenic in nature due to embolisation from non-anti-coagulated implanted catheters and lines (PICC and Port-a-Cath devices).

- **Ambulatory monitoring of ECG**

- The standard method for detecting and investigating cardiac arrhythmia is via Holter ECG recording for 24 or more hours. There are now many types of recorders suited to the detection of intermittent cardiac arrhythmia. However, the yield in asymptomatic, well chelated patients is fairly low.

- **Exercise ECG**

- Exercise testing, by treadmill or cycle ergometer, may be of value in identifying patients at risk for cardiac arrhythmias or for assessing functional capacity. Adequacy of treatment of cardiac disease can also be gauged by exercise test performance. An exercise test with gas-exchange evaluation allows verification of: $\dot{V}O_2$ peak (maximal O_2 utilisation at the peak of the stress) and $\dot{V}O_2$ AT (anaerobic threshold), which are parameters closely related to the functional status and prognosis of patients with left-ventricular dysfunction.

- **Echocardiography**

- Echocardiography is widely available, relatively inexpensive and easy to perform. A large number of parameters can be obtained from the cardiac ultrasound investigation but even the simplest measurements of chamber size can provide immediate and valuable data on cardiac status and clinical progress, as long as they are obtained by a skilled practitioner following a standardised protocol. A minimum data set should include:

1. **Dimensions**

- a. LV in diastole & systole.

- b. Atrial dimensions & areas.
 - c. Pulmonary artery and Aortic root.
 - d. Ventricular thickness.
 - e. LV and RV dimensions/ volumes.
2. Function
 - a. LV EF by standardized methods that should include: Teicholz and Simpson's methods.
 - b. Diastolic function.
 - i. Mitral Doppler.
 - ii. Tissue Doppler annular velocities.
 - iii. Pulmonary vein Doppler profiles.
 3. Doppler flow assessments
 - a. Tricuspid regurgitant jet velocity (TR)Vmax).
 - b. Pulmonary artery flows, acceleration/ diastolic jet velocity
 4. Morphology
 - a. Structure and function of valves.
 - b. Exclusion of thrombus in right atrium in patients with implanted lines.
 - c. Chamber morphology.
 - d. Presence of shunts or foramen ovale.

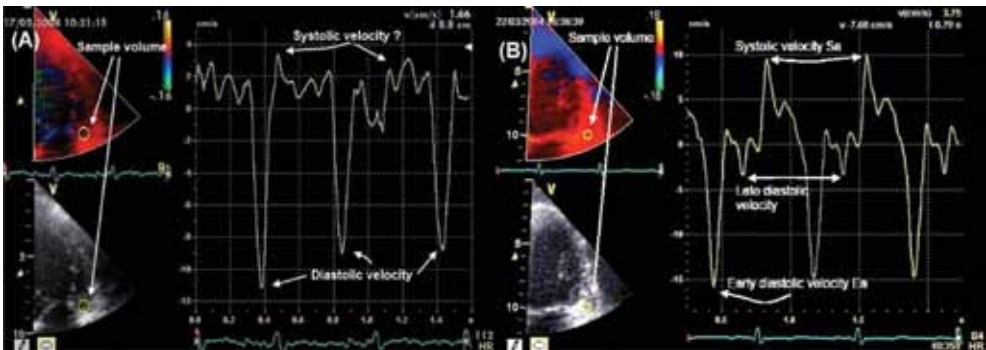


Figure 1. Examples of echocardiography in thalassaemia patients

This is not an exhaustive list, but includes most of the parameters, which characterize cardiac function in thalassaemic patients. If collected longitudinally subtle changes in parameters may become evident, highlighting the need to pursue more vigorous investigation with CMR imaging. A recent publication has illustrated the value of simple echocardiographic follow-up in patients with thalassaemia (Maggio 2013). Iron cardiomyopathy presents first with increased end-systolic volumes and borderline ejection fractions; progression to dilated cardiomyopathy is a late and ominous finding. A combination of conventional and tissue Doppler should be used to evaluate diastolic function. Isolated diastolic dysfunction can occur but is relatively rare.

A simple database for each patient can easily be developed for each patient to aid longitudinal follow up. Newer echo methods may also increase the sensitivity of the echo in detecting pre-clinical disease (Vogel 2003).

Key commentary: The important point is that each centre should develop a specified protocol, for their patients that can be used for long term surveillance and early appreciation of change.

Examination by echocardiography of the ventricular response to exercise may also be useful, highlighting individuals with sub-clinical disease in whom the ejection fraction fails to rise, or even falls, in response to exertion or simulated exercise using intravenous (i.v.) dobutamine

- **Cardiac Magnetic Resonance Imaging (cMR or MRI)**

- For more than a decade it has been possible to measure tissue iron load using non-invasive magnetic resonance imaging (MRI), (Anderson 2001). The cardiac T2* parameter has been validated as an accurate reflection of cardiac iron content and its usefulness in clinical management cannot be over-emphasized (Modell 2008, Wood 2009). It is now a matter of basic clinical standards that cardiac T2* should be undertaken in every transfused thalassaemia patient from as early an age as practicable, 10 years in most centres, but as early as 7 years in some cases, if there exists a suspicion of a high iron burden.

Key commentary: The value of the T2* parameter is that it identifies those individuals at risk of developing cardiac complications, before they become evident by changes in function detected by simpler non-invasive methods, such as echocardiography.

Monitoring the effectiveness of chelation in individual patients has proven to be critical in benefiting patient motivation in adhering to demanding treatment programmes and thus to outcomes. Cardiac MRI represents the gold standard for monitoring patients with thalassaemia major, not only because it allows estimation of cardiac iron burden (Carpenter 2011, Kirk 2009), but because it provides consistency in detecting preclinical changes in ejection fraction. Studies are recommended at 24, 12, and 6 month intervals for low, standard, and high risk patients. As a result of chronic anaemia, norms for cardiac volumes and ejection fraction are different for thalassaemia patients and must be taken into account when evaluating results (Westwood 2007).

Management of cardiovascular complications

The therapeutic strategy to diminish the risk of heart complications in patients with thalassaemia involves a number of general measures including the maintenance of a pre-transfusion Hb of at least 10 g/dl, along with particular cardiovascular interventions. The primary emphasis must be to encourage regular chelation therapy and maintenance of a CMR T2* > 20 ms. Monitoring cardiac function can be a useful guide to a patient's overall prospects. Impaired myocardial function may require specific cardiac treatment, but it also calls attention to the immediate need for much stricter adherence to chelation protocol or the initiation of a more intensive chelation programme, in order to prevent an inexorable progression to severe cardiac dysfunction.

Cardiac dysfunction generally lags cardiac iron deposition by several years (Carpenter 2011). Unfortunately, cardiac iron clearance is an extremely slow process, often requiring 3 or more years to clear severe cardiac iron deposition (Anderson 2004). Therefore, prevention of cardiac iron accumulation and early recognition of preclinical disease (through MRI) have a much greater likelihood of success than waiting for echocardiographic or clinical evidence of heart dysfunction or clinical symptoms to appear (Chouliaras 2010, Modell 2008).

Key commentary: Mild decreases in ventricular function, merit aggressive escalation of iron chelation therapy, even if patients are completely asymptomatic (Davis 2004).

Combined therapy with deferiprone 75-100 mg/kg and deferoxamine 40-50 mg/kg/day represent the best option to clear cardiac iron and stabilize ventricular function (Porter 2013). Deferoxamine should be given continuously, either subcutaneously or through a percutaneous intravenous catheter, until the ventricular function normalizes (Anderson, 2004, Davis 2000, Tanner 2008). An important practical point is that intra-venous lines pose a considerable risk of thrombosis and iatrogenic pulmonary hypertension, through chronic pulmonary thromboembolism and should mandate formal anticoagulation, particularly in chronically implanted lines.

Patients with cardiac T2* values below 6 ms are at high risk for symptomatic heart failure (Kirk 2009) and should be treated with intensive chelation, even if cardiac function remains normal. The presence of symptomatic heart failure should trigger admission to a tertiary hospital with experience in managing thalassaemia patients. If this is not possible, then communication between the treating physician and cardiac consultants with experience in thalassaemia major is strongly advised because of key differences between iron cardiomyopathy and other forms of cardiomyopathy. A summary of recommendations is as follows (Pennell 2013):

- Patients should be given continuous deferoxamine therapy at 50 mg/kg/day as long as the patient has adequate urine output. Deferiprone at 75 mg/kg/d, divided TID, should be added as soon as the patient is capable of tolerating oral medications.
- Pressor medications should be used cautiously because they worsen iron-mediated oxidative stress. Thalassaemia patients typically operate with lower diastolic and mean blood pressures than other patients. Hence blood pressure support should not be targeted to any given level, but to clinical measures of renal and cerebral perfusion.
- Cardiac enzymes should be sent to screen for possible myocarditis. D-dimers should be sent for detection of possible pulmonary embolism in patients with right heart symptoms. Bedside echocardiography should also be performed to look for pericardial effusion, and pulmonary hypertension.
- Gentle diuresis will alleviate congestive symptoms but over-diuresis can precipitate acute renal failure. Thalassaemia patients in heart failure often have restrictive physiology and stiff vasculature, making them sensitive to hypovolemia. In the acute setting, furosemide drips can be easier to titrate than bolus diuretics.
- Patients with liver damage from iron overload, hepatitis C, and passive congestion may have impaired synthetic function and low oncotic pressures. Albumen replacement is helpful.
- Arrhythmia's can be difficult to control. Amiodarone therapy is the drug of choice in the acute setting because of its broad spectrum of action and relatively modest impact on ventricular function.
- Patients should be assumed to have adrenal insufficiency until proven otherwise, with stress dose steroids initiated empirically after cortisol levels have been drawn.
- Thyroid and parathyroid dysfunction should be identified and corrected if present.
- Many patients with iron cardiomyopathy have type II diabetes. Glucose should be controlled, as necessary, by insulin infusion.
- Cardiac T2* should be performed as soon as practical. Cardiac dysfunction in the absence of a T2* < 20 ms should prompt alternative diagnoses. Contrast-enhanced cardiac MRI can also be used to screen for myocarditis.

- Maintenance of urinary output is imperative, since both deferoxamine and deferiprone are eliminated primarily by the kidney. Dialysis should be promptly initiated if kidney function fails despite optimal medical management.
- Placement of implantable cardio-defibrillators is discouraged because life-threatening arrhythmias are reversible with aggressive iron chelation therapy. External defibrillator vests can serve as useful bridge.
- It can be useful to use biochemical markers of heart failure (BNP or pro-N-terminal BNP). Values are high in decompensated heart failure and fall in response to treatment. Data support delaying hospital discharge in decompensated heart failure until BNP levels have reverted to normal.
- Heart transplant remains a treatment of last resort. Iron cardiomyopathy is often completely reversible if organ function can be supported long enough for iron chelation therapy to work.

Clinical stabilization can occur as soon as 2 weeks but can also take months. Clinical improvement precedes cardiac iron clearance. Removal of longstanding cardiac iron is quite slow, with a half-life of fourteen months (Anderson 2004). The iron chelator, deferasirox, has not been evaluated in heart failure patients and may be ill-advised in patients with marginal renal perfusion. Its use later in the convalescent phase is reasonable (Pennell 2010, Wood 2010).

Over recent years there has been a trend towards treating patients with thalassaemia exhibiting mild ventricular dysfunction with agents known to improve myocardial function in other forms of cardiomyopathy (see published guidelines: (McMurray 2012, Yancy 2013)). All these agents have a tendency to lower blood pressure, making their use in thalassaemia difficult and usually limited by the development of hypotension.

Key commentary: Adjunctive treatment of thalassaemia patients at risk of developing or having ventricular dysfunction with medications known to improve survival in other forms of ventricular dysfunction should be strongly considered.

Treatment of myocardial dysfunction is best undertaken using a group of drugs (see **Table 1**), including angiotensin converting enzyme inhibitors (ACE inhibitors). In controlled trials, these agents as well as beta-blockers and aldosterone antagonists, have been shown to reduce mortality in patients with cardiomyopathy and to reduce the rate of appearance of heart failure in those with asymptomatic left-ventricular dysfunction.

These results are very promising, and while their extension to heart failure in thalassaemia remains conjectural, it is widely applied in clinical practice. The usual precautions for initiating treatment in patients who are well hydrated and starting at low doses are recommended. The dose should be increased to the maximum tolerated, limited by hypotension in patients with thalassaemia. Certain patients are unable to tolerate ACE inhibitors due to the development of chronic cough. These individuals should be treated with angiotensin II receptor antagonists (ARB), such as losartan.

Table 1. Common drugs and dosing regimens used in the treatment myocardial dysfunction, including heart failure in thalassaemia.

CLASS	AGENT	DOSING
ACE inhibitor	Ramipril	1.25 - 10 mg once daily
	Captopril	6.25 - 50 mg three times daily
	Enalapril	2.5 - 20 mg twice daily
	Lisinopril	2.5 - 40 mg once daily
	Perindopril	2 - 16 mg once daily
ARB	Losartan	25 - 150 mg once daily
	Valsartan	20 - 160 mg twice daily
	Candesartan	4 - 32 mg once daily
Beta-adrenoceptor blockade	Bisoprolol	1.25 - 10 mg once daily
	Carvidelol	3.125 - 50 mg twice daily
Aldosterone antagonist	Spironolactone	12.5 - 50 mg twice daily
	Eplerenone	25 - 50 mg once daily

Arrhythmias

These usually present as palpitations, but sometimes may be asymptomatic. The context within which the arrhythmia occurs generally determines the clinical response and the risk to the patient. Arrhythmias are life-threatening in the presence of heart failure (Mancuso 2009), but can also be ominous harbingers of pending cardiac decompensation in patients with normal cardiac function, but high iron overload. Palpitations must therefore be investigated and treated in the context of the patient as a whole. Ectopic activity, usually supra-ventricular but occasionally ventricular, can produce symptoms requiring prophylactic drug treatment (often with beta-blockers), especially as these transient events can trigger more sustained arrhythmias, particularly AF. Arrhythmias that produce symptoms of haemodynamic compromise (dizziness, syncope or pre-syncope) pose a significant clinical risk and are almost always associated with significant myocardial iron-overload. In the absence of a CMR iron load measurement, clinicians should assume significant arrhythmias are due to iron overload and respond by intensifying chelation therapy, as a matter of urgency if the symptoms include syncope or pre-syncope. Treatment is directed towards the relief of iron overload, with a secondary strategy of symptomatic treatment of the documented arrhythmia. Arrhythmias in thalassaemia major can often be reduced or eliminated by aggressive iron chelation (Anderson 2004). In many instances, the use of drugs to treat relatively benign but symptomatic arrhythmias may produce greater problems than the symptoms deserve. The decision to treat arrhythmias in patients with thalassaemia must therefore be carefully considered, bearing in mind that iron toxicity is the primary cause of this complication. For most supraventricular arrhythmias, reassurance of the

patient is generally appropriate; frequent premature ventricular contractions, by themselves, are not suggestive of iron toxicity but couplets and non-sustained ventricular tachycardia are highly specific for iron cardiomyopathy and require urgent attention to address associated high myocardial iron load, via intensified chelation.

In older patients with thalassaemia, even without any evidence of current iron overload, there appears to be a high and increasing incidence of atrial fibrillation (up to 40% of those over 40 years in one large clinic experience). This may pose a future management problem and risk of stroke in this group of individuals who may carry increased thrombotic tendencies (Walker 2013). Sudden death is relatively rare in thalassaemia major, in the modern era, but historical data suggests an association with increased QT dispersion (Russo 2011), consistent with Torsades de Pointes as a possible mechanism.

Key commentary: Any arrhythmia associated with cerebral symptoms or collapse must be considered a medical emergency, until fully characterized.

Management of arrhythmia

Since many arrhythmias reverse over time, antiarrhythmic therapy can often be relatively short term (less than one year). Amiodarone is the drug of choice in the acute setting because of its broad spectrum of action and modest compromise of cardiac function (Pennell 2013). Long term amiodarone therapy is associated with an increased risk of hypothyroidism because of pre-existing iron toxicity to the thyroid gland (Mariotti 1999), however therapy can often be terminated after 6-12 months. Beta-blockers are also generally well tolerated, if titrated slowly, and can be useful in controlling ectopic rhythms.

Atrial fibrillation may occur in an acute context, particularly in situations of heavy iron load, where it may precipitate heart failure. Immediate cardioversion by synchronized DC shock should be considered, if the duration of the episode is known to be <48hr, if the patient is already fully anti-coagulated or if a simultaneous trans-oesophageal echocardiogram confirms the absence of atrial clot. Less acute presentations can be conventionally managed with anti-coagulation and introduction of parenteral amiodarone (via a central vein), simultaneously with intensive chelation. Cardioversion should be considered in patients who fail to revert to sinus rhythm with iron chelation therapy and pharmacological intervention. In view of the likelihood of an associated pro-thrombotic tendency, anti-coagulation should be undertaken in all patients with significant episodes of AF.

Thalassaemia patients with permanent or persistent AF may respond to radiofrequency isolation of the pulmonary veins but catheter based interventions for intra-atrial reentrant and ventricular tachycardia should be avoided. In thalassaemia, these procedures have a low success rate because the rhythms lack a true anatomic substrate (there is no scar, only functional conduction impairment). Treatment of potentially life threatening ventricular arrhythmias, such as Torsades De Pointes, may pose management problems in thalassaemia because they are often reversible and conventional criteria for device therapy do not apply to this unusual group of patients with a "toxic cardiomyopathy". Implantable cardio-defibrillators could provide vital rescue shocks while the heart is being de-ironed but their placement permanently precludes future MRI interrogation. External defibrillation vest can provide an important alternative safety net in these situations.

Heart block and conduction disturbance

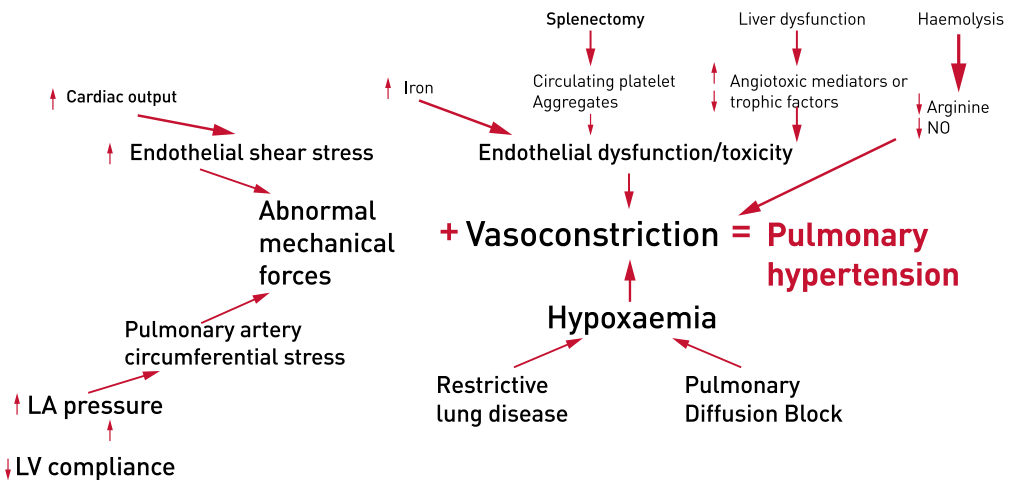
Historically, before the availability of chelation therapy, complete heart block was relatively common in thalassaemia patients, occurring in up to 40% of those aged over 15 years. It is now rare in most communities, but may occasionally be encountered in the context of severe iron load. The heart block generally, but not always, responds to adequate chelation, but the speed of this response may be slow. Thus these patients may require a pacemaker. It is essential that MRI conditional pacemakers and leads be used. Placing the pacemaker on the right may also be advantageous in allowing better unrestricted imaging of the ventricular walls and septum, to allow for continued monitoring of myocardial iron content (T2*).

Pulmonary Hypertension

Pulmonary hypertension is quite common in thalassaemia intermedia syndromes but reports on the prevalence in thalassaemia major vary (Vlahos 2012, Morris 2010,). Local differences in chelation and transfusion practices as well as the use of splenectomy undoubtedly impact reported prevalence rates. Splenectomy, transfusion intensity (frequency and pre-transfusion haemoglobin), and severity of iron overload appear to be the strongest predictors of pulmonary hypertension (Vlahos 2012, Morris 2011, Musallam 2011).

Mechanisms

Pulmonary hypertension represents the interaction of multiple mechanical and biochemical interactions to produce impaired endothelial function, smooth muscle proliferation, and eventual vascular obliteration in the pulmonary vasculature (Morris 2008). Mechanical forces include increase vascular shear stress from high cardiac output as well as increased vascular distending pressures resulting from left ventricular diastolic dysfunction. Biochemical stressors include circulating free haemoglobin, non-transferrin bound iron, vasoactive membrane fractions (Singer 2006) and erythropoietic stress hormones. Increased arginase activity and low nitric oxide bioavailability has been implicated, demonstrating some overlap with the pulmonary hypertension found in sickle cell disease (Morris 2013, Hagar 2006), but multiple pathways are likely operational. Lung disease and hypoxia likely contribute to pulmonary hypertension in thalassaemia as well, similar to the general population. Chronic pulmonary embolic disease must be considered in all patients, particularly those with implanted central venous lines.



Diagnosis

Echocardiographic screening for pulmonary hypertension should be performed annually or biannually. Tricuspid regurgitation (TR) and pulmonary insufficiency jets provide estimates of pulmonary artery systolic and diastolic pressure, respectively. TR velocity below 2.5 m/s represents a negative screening test, 2.5 – 3.0 m/s a borderline finding and TR velocity > 3 m/s a positive finding. Borderline and abnormal TR velocities should prompt a review of transfusion practices to determine whether ineffective erythropoiesis is adequately suppressed. Left ventricular systolic and diastolic function should be carefully evaluated to screen for possible mechanisms of post-capillary pulmonary hypertension. Overnight pulse oximetry is indicated to screen for nocturnal desaturation in all patients. However symptoms of obstructive sleep apnea should provide a formal sleep evaluation. Complete pulmonary function testing, including diffusing capacity, should be obtained to exclude restrictive lung disease. High resolution CT and CT angiogram to exclude pulmonary fibrosis and thromboembolic disease is warranted. Cardiac catheterization is indicated in patients with persistent elevated TR velocity greater than 3 m/s despite optimization of haematologic status. Brain natriuretic peptide and six-minute walk tests are useful for trending response to therapy.

Management

Treatment for pulmonary hypertension in thalassaemia is multifaceted and depends upon its severity and etiology. Continuous positive airway pressure should be used in the presence of obstructive sleep apnea. Nasal cannula may be sufficient in the presence of nocturnal desaturation without airway obstruction. Chronic anticoagulation is the treatment of choice in thromboembolic disease and should be considered as prophylaxis against thrombosis in-situ in patients with severe pulmonary hypertension. Early pulmonary hypertension in thalassaemia major patients often responds to shortening transfusion intervals, by suppressing proinflammatory cytokines such as PLGF. Hydroxyurea use in thalassaemia major has never been systematically studied, but has been used with benefit in non-transfused thalassaemia syndromes and is effective in some patient populations (Banan 2013). In patients refractory to more conservative measures, sildenafil has been effective in small series and is generally well tolerated (Morris 2013, Derchi 2005). Successful use of the endothelin 1 blocker, bosentan, has been described in a single thalassaemia intermedia patient but careful consideration must be given to hepatic function in patients with hepatitis C or hepatic iron overload.

Peripheral Vascular Disease

Mechanisms

Progressive vascular disease is part of normal aging. Many factors contribute, but abnormal free radical signaling is central to the decreased endothelial reactivity, intimal proliferation, increased cellular adhesion, and vascular inflammation observed in senescent vessels. However, many factors in thalassaemia accelerate this process, including iron overload, circulating microparticles, circulating haemoglobin, chronic anaemia, oxidized lipoproteins, and inflammatory cytokines. Insulin resistance and diabetes mellitus also increase vascular oxidative stress. The underlying systemic vessel pathophysiology is similar to that observed in pulmonary hypertension. Thalassaemia patients are also at risk for acquired pseudoxanthoma elasticum (Aessopos 2002), a degenerative process of elastin fibers of unknown mechanism more common in patients who are inadequately transfused or poorly chelated.

Diagnosis

No consensus exists for routine screening of systemic vascular disease. Flow mediated dilatation (FMD), while a sensitive marker of endothelial health, is not well suited to clinical

practice. Carotid intimal thickness can be performed routinely but norms are laboratory and patient population specific. Furthermore, no clear risk thresholds or identified interventions have been characterized. Oxidized low density lipoprotein correlates with vascular stiffness in thalassaemia (Stakos 2009), but it is not widely available.

Routine surveillance of ascorbate sufficiency is recommended because ascorbate deficiency causes impaired collagen formation in elastic arteries. However, ascorbate replacement in iron overload syndromes must be performed in conjunction with iron chelation therapy to prevent increases in labile iron. Pseudoxanthoma elasticum typically presents with specific cutaneous manifestations. Valvular and pericardial manifestations of this condition can be identified during routine echocardiographic screening. However, computed tomography angiography is advisable to evaluate vascular calcification and possible aneurysm formation in patients with cutaneous lesions.

Management

Prevention of vascular disease in thalassaemia primarily consists of properly controlling transfusion therapy and iron chelation. Splenectomy may be a factor, because of the spleen's essential role in removing prematurely senescent red blood cells and vasoactive membrane fragments (Morris 2011, Singer 2006). Iron chelation therapy should be aimed at controlling non-transfusion bound iron, as well as lowering mitochondrial oxidative stress. Different chelators have different relative strengths in these regards, however both deferasirox and deferiprone improve endothelial function over time (Cheung 2008, Tanner 2007).

Summary and Recommendations

The prospects for patients with thalassaemia have improved with a greater understanding of the disease and with better individualised regimes of management. Close co-operation between the medical disciplines is called for. At the same time, the fundamental treatment aim remains to provide regular, effective iron chelation, in forms that encourage patients to comply with treatment - which must be allied to more precise definition of tissue-specific iron loads, so that patient and physician alike have a better idea of individualised risk. Similar to vascular disease in non thalassaemic patients, lifestyle choices can have a major impact. Obesity is less common in thalassaemia patients than the general population, but no less toxic to the vasculature. Regular exercise improves vascular health by restoring endothelial reactivity and lowering vascular inflammation. While there have been no controlled studies of diet and exercise in the thalassaemia population, there is sufficient shared pathophysiology to extrapolate results from the general population.

Below is a summary of the key recommendations discussed in this chapter. The level of evidence associated with each respective point is included:

- 1) Thalassaemia major patients with heart failure should be managed at (or in close consultation with) a tertiary center experienced in thalassaemia **(C)**.
- 2) Management of diuretics, pressors, and antiarrhythmic therapies in thalassaemia patients with heart failure must account for their unique physiology compared with the general population **(C)**.
- 3) Screen and treat endocrine and metabolic co-morbidities in thalassaemia major patients with ventricular dysfunction **(C)**.

- 4) Futility of supportive care should not be prematurely determined in thalassaemia patients because ventricular arrhythmias and heart failure are often reversible following intensive chelation, albeit after weeks or months of therapy **(C)**.
- 5) Any arrhythmia associated with cerebral symptoms must be considered a medical emergency until fully characterized **(C)**.
- 6) Combined therapy with deferoxamine and deferiprone represent the best available intensive chelation for thalassaemia major patients with severe cardiac iron deposition, with or without over heart failure **(B)**.
- 7) Routine cardiac T2* assessment represents the best available tool to prevent cardiac dysfunction **(B)**.
- 8) In places lacking cardiac T2* assessments, preclinical reductions in cardiac systolic function can also be used to detect cardiac iron toxicity prior to cardiac failure if standardized protocols are used and data are tracked meticulously over time **(B)**.
- 9) Even mild decreases in ventricular function warrant aggressive and sustained escalation of therapy **(B)**.
- 10) Echocardiographic screening for pulmonary hypertension should be performed annually. Patients having a TR velocity greater than 3 m/s should undergo cardiac catheterization if proximate cause can not be identified and corrected **(B)**.
- 11) Lifestyle choices that promote vascular health (absence of smoking, regular physical activity, weight control, vegetable and nitrate rich diet) should be vigorously promoted in thalassaemia patients **(C)**.

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5

LIVER DISEASE

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Reviewer Maria Domenica Cappellini

Among the different organs susceptible to damage in thalassaemia patients, the liver represents a major target. Iron overload is the main causative factor (Voskaridou 2012, Lobo 2011, Porter 2009). Hepatitis viruses, especially hepatitis C virus (HCV) and hepatitis B virus (HBV), remain an important concern (Lai 2013, Triantos 2013, Di Marco 2010, Ragab 2010), although recently preventive measures have significantly reduced new cases of infection. The potentially aggravating role of hepatotoxic co-factors, such as dysmetabolism and alcohol, should also be kept in mind. The main risk of chronic liver disease is the development of cirrhosis (Li 2002) with its risk of hepatocellular carcinoma (HCC) (Maakaron 2013, Mancuso 2010), complications which are becoming more frequent due to overall improvements in thalassaemia outcomes. The diagnosis of both type and severity of hepatic disease in thalassaemia has benefited from the availability of non-invasive techniques. The prognosis of liver disease in thalassaemia should continue to improve thanks to increasingly effective therapeutic modalities for treating both iron overload and virus-related chronic hepatitis.

Hepatic Iron Overload in Thalassaemia

Repeated transfusions represent the major cause of iron overload in thalassaemia major. Each unit of blood represents 200-250 mg of iron. Considering that total body iron stores are approximately 4 g, and that normal daily iron losses are of the order of 1-2 mg (with a very limited capacity for the body to regulate these losses), one can understand that, when a given individual needs for instance one unit of blood every 2 weeks, body iron overload develops rapidly. Since red blood cells are degraded in the reticulo-endothelial system (macrophages, essentially within the spleen), iron overload will primarily affect the spleen and, to a lesser degree, hepatic macrophages (called Kupffer cells) which are much less numerous than the parenchymal cells (hepatocytes) within the liver. Thereafter, this intra-macrophagic iron will be released progressively into the blood stream, reaching the bone marrow and leading to the production of new red blood cells. During this release process the iron saturation of plasma transferrin, normally less than 45%, increases rapidly, often reaching 100%. This leads to the appearance of plasma non-transferrin bound iron (Brissot 2012), an iron species which is rapidly taken up by parenchymal cells of the liver, heart and pancreas, therefore contributing subsequently to overload these organs. This is especially true for the liver which is, for circulating iron, both the first line target and the main storage organ.

Dyserythropoiesis is another important mechanism accounting for iron excess. It has been shown to result from the decreased production of the iron regulatory hormone hepcidin by the liver. Hepcidin deficiency, through activation of the cellular iron exporter ferroportin (Ganz and Nemeth 2012), leads to an increase in entry of iron into the plasma at two major sites: on one hand, the duodenum corresponding to an increased intestinal absorption of iron and on the other, and quantitatively 10 to 20 times more important, at the splenic level. These two simultaneous processes lead to increased transferrin saturation. The intimate mechanism whereby dyserythropoiesis favours iron excess through hepcidin deficiency has recently seen a major breakthrough with the discovery of the hormone erythroferrone, which very likely represents

the long-sought “erythropoietic factor” (Kautz 2013, Biolron, London). In thalassaemia major the role of dyserythropoiesis as a cause for iron excess can be considered relatively accessory as compared to that resulting from blood transfusions, though it may explain why these patients can develop significant iron overload even before any transfusions. In contrast, dyserythropoiesis is the primary iron overloading factor in non-transfusion dependent thalassaemia (NTDT) (Taher 2013b), where iron pathophysiology is in fact very close to that seen in hepcidin deficient hereditary haemochromatosis (Brissot 2011) (such as type 1, 2, 3 or 4B haemochromatosis).

Anaemia and hypoxia also contribute to iron overload by decreasing the impact of erythropoietin on hepcidin synthesis.

These mechanisms are summarised in **Figure 1**.

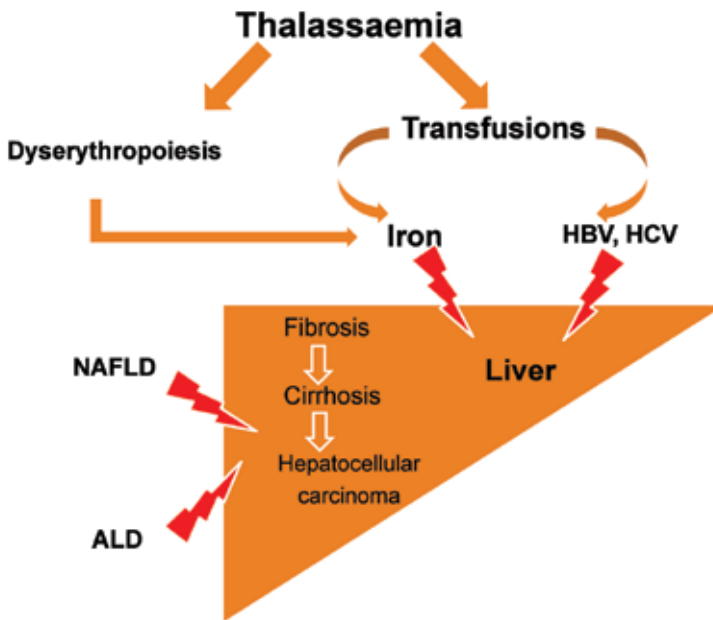


Figure 1. Main causes of hepatic iron damage in thalassaemia. NAFLD, non-alcoholic fatty liver disease; ALD, alcoholic liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus.

As far as macrophagic iron excess is concerned, hepatic damage seems relatively limited because iron is less toxic when deposited within the reticuloendothelial cells. Hepatocytic iron overload, in contrast, is highly damaging. As soon as the protective effect of the iron storage protein ferritin is exceeded, hepatocyte damage occurs leading to cellular necrosis (biologically expressed by increase serum transaminase activities: alanine aminotransferase and aspartate aminotransferase) followed by the progressive development of scarring (called fibrosis), the ultimate stage of which is cirrhosis. It has been reported that increased serum transaminase levels are observed with hepatocyte iron concentrations (HIC) $>300 \mu\text{mol/g}$ (Jensen et al. 2003) and hepatic fibrosis with HIC $>400 \mu\text{mol/g}$ (Angelucci 2002). The way iron toxicity develops is largely dependent upon the level of plasma non-transferrin bound iron (NTBI). Indeed, part of this iron species is in the form of labile plasma iron (Esposito 2003, Hershko 2010) which has a high propensity to produce reactive oxygen species. These are known to damage membrane lipids, affecting not only hepatocyte plasma membranes but also the membranes of intracellular organelles, including cell nuclei.

Diagnosis of hepatic iron overload

Diagnosis rests upon several, more or less, combined approaches. Acquisition of clinical data remains an essential first step of the diagnostic process. This includes signs of systemic iron excess such as skin pigmentation and associated iron-related organ damage, especially at the cardiac and endocrine levels.

Assessment of biochemical parameters represents the second step. The most informative test is the level of serum ferritin (with normal being <300 ng/ml in men and <200 ng/ml in women), provided the result is correctly interpreted. Increased serum ferritin must be rigorously interpreted for two reasons. Firstly, increased serum ferritin can be seen in several situations unrelated to iron excess in thalassaemia. Among these, the inflammatory syndrome (hence the importance of checking serum C-reactive protein levels), hepatic cytolysis (thus importance of checking serum transaminases) and in combination with co-factors, especially the dysmetabolic (or polymetabolic) syndrome, are key. Once elevated, serum ferritin levels are related to the degree of iron excess and especially with HIC. However, it is important to keep in mind that this correlation depends on the cellular localisation of stored iron. Thus, the absolute increase in serum ferritin will be relatively more important when iron deposits are located in the reticuloendothelial system rather than in parenchymal cells. This means, for instance, that a level of 1000 ng/ml has a different meaning depending on whether iron is deposited in the parenchymal cells (in this case, it is a critical threshold in terms of toxicity) or in the macrophagic system (where this value may correspond to a much less deleterious situation).

Plasma transferrin saturation is another important parameter. It provides information on the degree of biologically available iron, i.e. iron which will be delivered to the cells. Moreover, when over 75% it usually means the presence of NTBI (Pootrakul 2004). Plasma NTBI, and especially LPI, are not yet routinely assessed but represent promising iron parameters which correspond to the potentially toxic form of circulating iron. Normalizing NTBI/LPI is an important goal of treatment (Zanninelli 2009). Whether serum hepcidin determination may assist clinical monitoring warrants further studies.

Magnetic resonance imaging (MRI, **Figure 2**) is today the major non-invasive method used to confirm and quantify organ iron excess (Wood 2011). It allows the determination of HIC, which is globally related to total body iron stores, even where there is extra-hepatic iron deposition (Wood 2011), notably in the spleen. Ultrasound examination and computed tomography have no practical value for estimating hepatic iron load, the former technique being unable to detect iron and the latter lacking sensitivity (Wood 2011)

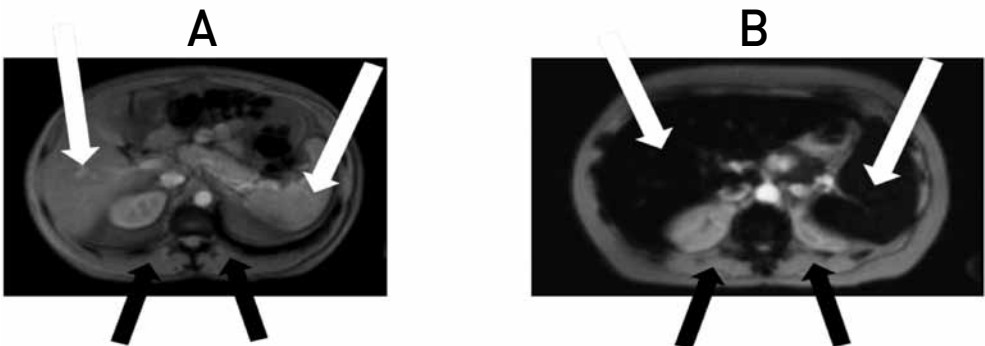


Figure 2. Illustration of the diagnostic value of magnetic resonance imaging in hepatic and splenic iron overload. (A): Normal hepatic and splenic iron concentrations (white arrows): the signal is identical to that from the spinal muscles (black arrows). (B): Massive hepatic and splenic iron overload (white arrows): the signal is very low as compared to that from the spinal muscles (black arrows).

Liver biopsy (**Figure 3**) has, for a long time, been considered as the “gold standard” for hepatic iron load evaluation. It permits a quantitative approach through the determination of HIC (either biochemically, or today by atomic absorption spectrophotometry). Normal HIC is $<40 \mu\text{mol/g}$ (dry liver weight). HICs of $40\text{--}120 \mu\text{mol/g}$, between 120 and $240 \mu\text{mol/g}$, and $>240 \mu\text{mol/g}$ may be considered mild, moderate and heavy, respectively [Berdoukas 2012]. Moreover, it allows the semi-quantitative histological differentiation of the cell types affected by iron excess (Deugnier 2011). MRI comparison of hepatic versus splenic iron load is a new way to evaluate the respective involvement of the parenchymal and macrophagic systems (dominant splenic iron meaning dominant macrophagic deposition). Given that liver biopsy is invasive, one can understand why today it is less and less performed for hepatic iron load evaluation: combining serum ferritin and MRI data has become the preferred strategy.

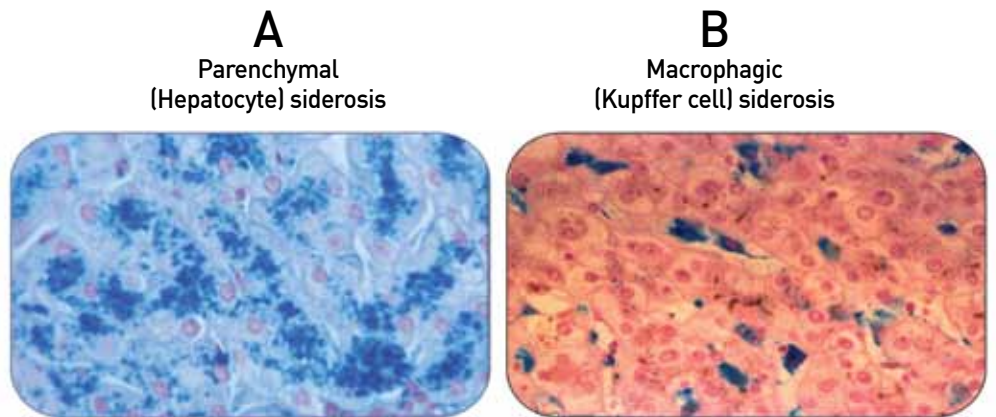


Figure 3. Histological liver iron overload (shown by blue intra-cellular deposits using Perl's staining). (A): Parenchymal (hepatocyte iron excess) as seen in dyserythropoiesis. (B): Macrophagic (Kupffer cell) iron deposits as seen in transfusional iron overload.

Calculating the amount of transfused iron is obviously a precise method to evaluate body iron stores, and therefore, to predict hepatic iron excess. However, it is a retrospective approach which loses much of its value once an effective iron-depleting treatment has been started.

Diagnosis of hepatic disease in general

Diagnosis of hepatic disease is also based upon both clinical and related parameters. The clinical approach must always be the first step, searching essentially for hepatomegaly (size and consistency). It should be remembered that hepatic iron overload by itself, even when massive and longstanding, does not produce significant liver dysfunction so signs of hepatocellular failure or portal hypertension are not usually present (even in case of true iron-related cirrhosis).

Biochemical parameters have value in two main areas. Firstly, to provide a functional evaluation of the liver. In case of substantial hepatic iron excess, a moderate increase in serum

transaminase activities (to less than 2-3 times the upper limits of normal) can be observed in the absence of hepatocellular failure (normal prothrombin time) or cholestasis (normal serum alkaline phosphatase, gammaglutamyltransferase and conjugated bilirubin levels). Secondly, to assess morphology. When cirrhosis is suspected, biochemical markers such as hyaluronic acid levels can be assayed (El-Shabrawi 2012), although their diagnostic value remains uncertain. Whenever HCC is suspected (this is so when cirrhosis is present, especially if general health is altered and cholestatic enzymes are elevated), alpha-foetoprotein (AFP) should be checked, knowing however that normal levels do not exclude the diagnosis.

There is clear value in imaging. Ultrasound examination is a key method for determining liver morphology and homogeneity (diffuse heterogeneity due to cirrhosis, focal lesion possibly related to HCC) and confirming the absence of signs of portal hypertension. Hepatic transient elastography is more and more commonly performed to evaluate, in a non-invasive way, the degree of hepatic fibrosis. It is a measure of hepatic stiffness, based on a mechanical wave generated by vibration. There are two main limitations regarding its interpretation. On one hand it is mostly interesting to differentiate between the extreme situations of cirrhosis versus absence of (or no significant) fibrosis: it is much more difficult to appreciate the intermediate fibrosis stages. On the other hand, this technique has essentially only been validated in chronic HCV. This said, it may be a reliable tool in both transfusion dependent and non-transfusion dependent thalassaemias (NTDT) (Musallam 2012).

Liver biopsy remains the key method to establish and quantify fibrosis, with the limitations of invasiveness and the possibility that a sample will not be representative (which may make it challenging to diagnose cirrhosis, particularly of the macronodular type). Liver biopsy also permits assessment for possible associated lesions (especially fat deposition and inflammatory lesions).

The main concern, when evaluating hepatic complications of iron overload, is to know whether there is or not cirrhosis. Indeed, the presence of cirrhosis carries a sufficient risk of HCC to justify checking the hepatic ultrasound appearance and the serum AFP level every 6 months. Even though this complication remains rare in thalassaemia, it may become more frequent due to the lengthening of patient lifetimes.

Treatment of hepatic iron overload

The liver being the main iron storage organ, it is essential to remove hepatic iron excess as early as possible in order both to limit the risk of liver damage (especially cirrhosis) and to protect the other organs, notably the heart, endocrine organs and bone (Musallam 2011). The level of hepatic iron overload indicating when treatment should be initiated remains unclear. Classically, a serum ferritin threshold of 1000 ng/ml was used but, considering the above-mentioned data, serum ferritin concentration should be interpreted depending on the type of cellular iron excess. It is likely that, when parenchymal iron overload related to dyserythropoiesis is the main cellular feature, this threshold is far too high. HIC is the most reliable parameter: it is likely that a HIC >40 $\mu\text{mol/g}$ dry weight should be considered as already reflecting significant tissue iron excess.

The therapeutic modalities are based on chelation (Hoffbrand 2012). This topic is covered in detail in **Chapters 3**, here we focus on data around the impact of chelation on the liver. In thalassaemia major chelation is usually started between 2 and 4 years of age, after 20-25 transfusions. Desferrioxamine, administered by prolonged subcutaneous infusion at a dosage of 30-40 mg/kg 5 days a week, has been shown to be effective. The major problem with desferrioxamine therapy is constraints in terms of quality of life: these substantially impact compliance, especially in

young patients. Deferiprone, given orally three times a day at an average dosage of 75-100 mg/kg body weight, has been shown to effectively decrease HIC when given either with desferrioxamine (Berdoukas 2012) or as monotherapy (Viprakasit 2013). Its main limitation is an exceptional but unpredictable risk of agranulocytosis, leading to a requirement to systematically check the white cell count on a weekly basis. Deferasirox has been the most widely studied oral chelator (Deugnier 2011). Given in 219 thalassaemia patients for 3 years or more, deferasirox reversed or stabilized liver fibrosis in 83% of patients. Unexpectedly, the positive effect on fibrosis was not strictly correlated with the degree of iron depletion, raising the issue of a specific anti-fibrogenic effect of this compound, independent of its iron chelating properties. Moreover, this therapeutic effect was independent of previous exposure to HCV. In NTDT patients, deferasirox (5 to 20 mg/kg/day) also resulted in dose-dependent reductions in liver iron concentration: efficacy was consistent across patient subgroups (Taher 2013a). Combining deferasirox and desferrioxamine could also be an interesting option (Lal 2013). It should be mentioned that a meta-analysis of 1520 patients with thalassaemia major included in randomized trials suggested that HIC was lower with combined as compared to single-drug therapy. However, only 7% of the trials were free of bias, and the data did not support any specific chelation treatment (Maggio 2011).

As regards stem cell transplantation (Elborai 2012) and iron overload in thalassaemia, two points are important. Firstly, decreasing iron overload before the procedure is likely important (Khalil 2012). Secondly, after successful transplantation venesection therapy becomes an effective and well-tolerated option for reducing iron excess (Angelucci 2000). For the future, therapies targeting Tmprss6 (Guo 2013, Schmidt 2013), a hepatic protein playing a key role in maintaining iron balance, represent potential innovative approaches.

Chronic Hepatitis C in Thalassaemia

Epidemiological aspects

HCV infection, essentially if of transfusional origin, remains a major concern in the field of thalassaemia. 4 – 85% of thalassaemia patients are positive for anti-HCV antibodies (Di Marco 2010). This concern is mostly related to the large number of infected patients in a past period where specific preventive measures in the transfusion process were not yet applied (essentially before 1991, the date of the HCV discovery) (Azarkeivan 2012). However, in a number of developing countries, the transfusion system has not yet integrated the key requirement of HCV screening of blood donors. The concerns in thalassaemia patients are also related to the potential prognostic severity of chronic HCV infection for two main reasons. Firstly, the association with the damaging effect of hepatic iron overload (Angelucci 2000) and secondly limitations around the use of anti-viral treatments in this population due to their side effects. Moreover, improvement in life expectancy for thalassaemia patients will make iron-related hepatic complications, and the development of severe hepatic lesions, including cirrhosis and HCC, more likely.

Diagnosis of chronic hepatitis C in thalassaemia

The overall diagnostic procedure is not different from that used in non-thalassaemia patients. HCV infection should be suspected when transfusions began before 1991 and serum transaminase activities are higher than those expected when considering the sole role of excess body iron. Diagnosis rests upon a combination of clinical, biological, imaging and biopsy data.

Key clinical data include an increase in transaminase for more than 6 months. While this usually defines chronic infection, this chronological argument may not be necessary when clinical examination indicates obvious signs of a chronic hepatic damaging process. Indeed,

cirrhosis is often already established when the following signs are observed: firm hepatomegaly (associated with signs of liver dysfunction), hepatocellular failure (bruising, palmar erythema, spider naevi and finger clubbing), portal hypertension (abdomen collateral venous circulation and splenomegaly, although the latter sign must be of course interpreted with caution given the haematological context), decompensated cirrhosis (ascites, encephalopathy, chronic jaundice unrelated to haemolysis and gastrointestinal haemorrhage due to oesophageal varices). Other, non-liver related signs of HCC may also be the mode by which the diagnosis is revealed.

Biological data are also important. From a functional standpoint, prothrombin time may be increased and combined leucopenia and thrombopenia may reflect portal hypertension (through hypersplenism). From a morphological viewpoint, blood markers of hepatic fibrosis have are important in predicting the severity of fibrosis. In thalassaemia, the aspartate transaminase-to-platelet ratio index (APRI) and Fibrosis 4 test (based on platelets, aspartate transaminase, alanine transaminase and age) have shown some value, although splenectomy may interact with the platelet parameter (Poustchi 2013). The fibrotest appears promising (Elalfy 2013).

Imaging and biopsy then represent important next steps. Ultrasound examination is a routine procedure to search for direct (hepatic) and indirect (portal hypertension features) features of cirrhosis. Transient elastography, as discussed above, is of interest in thalassaemia patients (Poustchi 2013) and should become a routine investigation for evaluating the presence and severity of hepatic fibrosis. Though much less often performed nowadays due to the availability of non-invasive techniques, liver biopsy remains justified when other investigations either do not provide convergent results or are not feasible. The main histological scores permitting to define hepatic status are the Metavir score, which evaluates both activity (0-3) and fibrosis (0-4, grade 4 corresponding to cirrhosis) and the Ishak's score.

Treatment of chronic hepatitis C in thalassaemia

The presence of significant HCV activity, as expressed by biological cytolysis (increased serum transaminase activities) or histological inflammation, together with mild or severe liver fibrosis, represent the usual basic criteria for proposing antiviral therapy. Two other important criteria for deciding the type of therapy offered are the HCV genotype and presence of the IL28B polymorphism.

The current standard of care mainly depends on HCV genotype. In case of genotype 1 (the most common), triple therapy with ribavirin, pegylated interferon and a protease inhibitor (boceprevir or telaprevir) is recommended. The mean overall period of treatment is one year (the decision to continue after 3 months of therapy depends on efficacy to that point). Tolerance of this treatment may be problematic, especially in terms of asthenia, depression, skin problems, leucopenia, thrombopenia and dysthyroidism. The specific issue when considering thalassaemia patients is the haematological side-effects of ribavirin, which include acquired haemolytic anaemia, a most undesirable effect in a disease which is a chronic haemolytic anaemia of genetic origin. In practice, however, a number of studies indicate that the global tolerance of ribavirin therapy is acceptable in thalassaemia patients provided careful follow-up is undertaken and that the requirement for increased transfusion frequency is satisfied (Di Marco 2010): erythropoietin therapy is not advised. As to interferon-related leucopenia, severe neutropenia (<500/mm³) should prompt the administration of granulocyte colony-stimulating factor. Concomitant treatment with deferiprone is discouraged due to an increased risk of leucopenia and agranulocytosis (Ricchi 2010). A sustained complete response (defined by the disappearance of HCV RNA and normalization of transaminases persisting more than 6 months after the end of therapy) with dual therapy (ribavirin and pegylated inteferon) is seen in between 40% and 60%

of patients (Di Marco 2010). It is likely that triple therapy will, as in non-thalassaemia patients, significantly increase cure rates. The presence of the IL28B polymorphism appears to affect response rates in non-thalassaemia patients, though its importance in thalassaemia patients has yet to be specifically studied (Di Marco 2012).

In case of genotype 2 or 3 disease, the standard protocol remains dual therapy with ribavirin and pegylated interferon. In non-thalassaemia patients expected response rates exceed 90% after only 6 months of treatment: no large studies for these genotypes are yet available in thalassaemia.

Therapeutic approaches to chronic HCV hepatitis are rapidly evolving. Novel active oral compounds will be available in the near future and are highly likely to transform therapeutic strategies. In practice, this perspective is so real that, in the absence of pronounced fibrosis (stage 0, 1 or 2 of the Metavir score), it is often suggested that one should wait for the arrival of the new drugs. During this waiting period hepatic status should be followed carefully on an annual basis, with special attention to transient elastography data.

Chronic Hepatitis B in Thalassaemia

Epidemiological aspects

HBV infection affects 0.3% - 5.7% of thalassaemia patients worldwide (Di Marco 2010). Where HBV screening of blood donors and vaccination against HBV have been implemented, new cases of HBV infection have considerably decreased. Chronic HBV-carriers can be either inactive or active carriers, the latter situation corresponding to chronic hepatitis. In case of chronic hepatitis, the evolution of the disease may lead to cirrhosis and HCC. The possibility of co-infection with HCV must always be remembered (Tyson 2013).

Diagnostic aspects

In the inactive HBV carrier state there are no clinical symptoms and no increases in serum transaminases. Virologically, patients typically demonstrate the presence of serum HBsAg, the absence of HBeAg, the presence of anti-HBe antibody, and either the absence or the low abundance of circulating HBV DNA. Follow-up is based on yearly evaluation of transaminases and HBV DNA levels. Differentiating the inactive carrier state from chronic infection can be difficult. One must look carefully for underlying cirrhosis, especially given that clinical, biological, ultrasound, and even elastographic data may be unreliable. When in doubt, a liver biopsy should be performed.

A diagnosis of the active HBV carrier state is reached in the same way as for HCV chronic hepatitis. Differences include that: i) viral replication is expressed by the presence of circulating HBV DNA; ii) non-invasive serum markers of fibrosis, such as the fibrotest have not been validated and iii) transient elastography can underestimate the degree of fibrosis present. Moreover, when the disease is highly active (high levels of serum transaminases or the presence of histological inflammation) but HBV replication levels are low, one must not forget to look for co-existing hepatitis delta virus (HDV) infection.

Therapeutic aspects

Anti-HBV drugs include interferon (mainly pegylated interferon), nucleoside analogs (lamivudine and entecavir) and nucleotide analogs (adefovir and tenofovir). Nucleoside and nucleotide analogs are administered by the oral route. Interferon therapy is usually confined to patients with high serum transaminase activity and low HBV replication. The key therapeutic target is negativity for

HBV DNA, HBeAg and HBsAg by 48 weeks of treatment. Nucleoside and nucleotide analogs are either used as second line treatments (after interferon failure) or, given their favourable safety profile and antiviral efficacy, more and more frequently as a first line treatment. Due to the resistance profile of lamivudine and adefovir, the best options today are entecavir or tenofovir. These drugs are expensive and may not be available in all settings, however. Importantly, they are prescribed for long-term treatment because, if HBV DNA suppression is readily obtained, negativity for HBeAg and HBsAg are rarely achieved. As yet, data in the setting of thalassaemia are not available.

Non-Alcoholic Fatty Liver Disease In Thalassaemia

Non-Alcoholic Fatty Liver Disease (NAFLD) is an increasing problem in children. It has been reported that, in the United States, 17% of children are overweight and 3% have NAFLD. Knowing that this situation can lead both to some degree of iron excess (corresponding to so-called dysmetabolic iron overload) and to hepatic damage (through the development of non-alcoholic steatohepatitis), special attention should be paid to the presence of polymetabolic features in young thalassaemia patients. In the frame of this nutritional domain, excessive alcohol consumption must be avoided in order to eliminate one further important co-factor for hepatotoxicity.

Summary and Recommendations

- Hepatic iron excess should be evaluated using a non-invasive strategy based on combined serum ferritin values and MRI data. Liver biopsy is often not necessary.
- Serum ferritin interpretation should be rigorous, excluding causes of false positive results such as inflammation, cytotoxicity, dysmetabolism and alcoholism.
- Serum ferritin levels indicating the need for iron depletive treatment depend on both the degree of tissue iron excess and cellular iron distribution, as documented non-invasively by MRI.
- Reversal of hepatic iron excess is a key objective not only to protect the liver but also the rest of the body.
- Deferasirox is effective in producing a negative iron balance and decreasing hepatic damage.
- Diagnosis and treatment of HCV and/or HBV chronic hepatitis remain important.
- Non-invasive strategies should be used to evaluate hepatic status in HCV hepatitis. These are based on serum markers which are predictive of fibrosis, as well as on transient elastography of the liver. Liver biopsy is not usually needed.
- When treating HCV patients special attention should be paid to the side effects of drugs, especially ribavirin, given the risk of provoking (acquired) haemolytic anaemia.
- Provided there is no significant hepatic fibrosis, treatment of HCV hepatitis can often be delayed until new oral highly effective compounds become available: these are expected in the very near future.
- Serum transaminase and HBV DNA levels are the main means of differentiating inactive from active HBV
- Oral nucleoside and nucleotide analogs are well tolerated and effective drugs for HBV-chronic hepatitis, though AgHBs seroconversion remains a rare event.

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6

THE SPLEEN

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Thalassaemia represents a heterogeneous group of inherited diseases characterised by the lack or reduced production of haemoglobin β -chains. The common pathophysiology bedrock is an increased destruction of red blood cells by reticuloendothelial system, in particular by the spleen, resulting in its enlargement (splenomegaly). Many patients with thalassaemia require splenectomy. The main therapeutic rationale for splenectomy in transfusion-dependent patients with β -thalassaemia major (TM) is to decrease blood consumption and transfusion requirement with the ultimate goal of reducing iron overload (Rachmilewitz 2011, Cohen 2008). However, current transfusion regimens are setting more adequate pretransfusional haemoglobin levels and more correct intervals between transfusions, has considerably reduced the incidence of splenomegaly and splenectomy in TM patients. The probability to undergo surgery within the first 10 years of life was 57, 22, 6, and 7%, respectively, for thalassaemic patients born in the 1960s, 1970s, 1980s, and 1990s (Piga 2011).

Throughout the care of the patient with thalassaemia, the size of the spleen should be carefully monitored on physical examination and, as needed, by ultrasonography.

Indications for Splenectomy

All 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 (Taher 2010).

Splenomegaly due to periods of under-transfusion with blood of inappropriately low haemoglobin may be reversible. Before considering splenectomy in this situation, the patient should be placed on an adequate transfusion program for several months and then re-evaluated.

Splenectomy should be avoided in children <5 years of age because of a considerably greater risk of fulminant postsplenectomy sepsis. The main indications for splenectomy are highlighted in **Table 1**.

Table 1. Indications for splenectomy in thalassaemia major.

INDICATION	COMMENT
Increased blood requirement that prevents adequate control with iron chelation therapy	<ul style="list-style-type: none"> • Annual transfusion volume (75% haematocrit) used to flag an increased blood requirement (200–220 ml/kg/ year) • Alloimmunization, concurrent infections, suboptimal transfusion therapy should be ruled out
Hypersplenesim	Cytopenias
Symptomatic splenomegaly	<ul style="list-style-type: none"> • Accompanied by symptoms such as left upper quadrant pain or early satiety • Massive splenomegaly causes concern about possible splenic rupture

Splenectomy and Peri-operative Complications

There are currently 4 approaches to splenectomy; open and laparoscopic total splenectomy, partial splenectomy and reduction of splenic tissue by embolization. Splenectomy is the recommended intervention to reduce excessive blood consumption and consequent severe iron overload. The two surgical techniques most commonly employed for total splenectomy are the 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 2013). Doubts have been raised regarding the suitability of LS for patients with splenomegaly however recent studies confirm the superiority of LS to OS in patients with massive and even supramassive spleens (Koshenov 2012).

Partial splenectomy is used to preserve some of the immune function of the spleen while reducing the degree of hypersplenism (De Montalembert 1990). 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 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. Any surgery on the spleen should include a careful search for accessory spleens.

Reduction of splenic tissue by embolization is a less invasive approach to hypersplenism than complete or partial surgical splenectomy (Pringle 1982). However, this approach has not gained wide acceptance and may be complicated by fever, significant pain and the possible need for a subsequent total splenectomy. Embolization does not permit a search for accessory spleens.

Concomitant Cholecystectomy

An evaluation for gallstones should be performed prior to surgery, 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.

Splenectomy Adverse Events

Peri-operative complications include bleeding, atelectasis and subphrenic abscess. Postoperative thrombocytosis is common, with platelet counts often reaching 1,000,000 -2,000,000/mm³, all guidelines recommend thromboprophylaxis perioperatively in patients with thrombocytosis.

Special consideration should be given to the use of low-dose aspirin (80 mg/kg/d) for patients with high platelet counts, or the use of anticoagulation for patients with a history of previous thrombosis or other risk factors.

Major adverse effect of splenectomy are Sepsis, Thrombophilia, Pulmonary hypertension and Iron overload.

Sepsis

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

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*, and *Klebsiella pneumonia* (Koren 1984). The introduction of routine anti-pneumococcal vaccination and prophylactic antibiotics can prevent severe pneumococcal infections in the first 2-4 critical post-splenectomy years.

Protozoan infections due to *Babesia* have been implicated in a fulminant haemolytic febrile state in splenectomised patients. Malaria is reportedly more severe in asplenic people and carries an increased risk of death (Boone 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. Postsplenectomy 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.

The risk of overwhelming postsplenectomy infection varies with:

- Age, risk is very high in children <2 years of age. However, fulminant bacteraemia has been reported in adults as much as 25-40 years after splenectomy.
- Time since splenectomy, the greatest risk appears to be in the period 1-4 years after surgery

- Immune status of patient

Immune prophylaxis in splenectomised patients is summarised in **Table 2**.

Table 2. Immune prophylaxis in splenectomised patients.

VACCINE	SCHEDULE	COMMENT
Streptococcus pneumoniae	At least 2 weeks in advance of a splenectomy and then in 3-5 years	<ul style="list-style-type: none"> • Rate of protection is 70-85% • The immune response is poor in children less than two years of age
Haemophilus influenzae type B	At least 2 weeks in advance of a splenectomy and then in 3-5 years	-
Neisseria meningitides	At least 2 weeks in advance of a splenectomy and then in 3-5 years	-
Influenza virus vaccination	Annual	<ul style="list-style-type: none"> • To prevent this febrile illness that might otherwise require intensive evaluation and management of a febrile episode in the splenectomised host

*Children vaccinated under the age of two should be re-vaccinated at age two.
 *Patients who underwent splenectomy without being given pneumococcal vaccine may still benefit from vaccination post- splenectomy.
 *These vaccines can be given at the same time in different syringes at different sites.

Chemoprophylaxis in splenectomised patients is summarised in **Figure 1**.

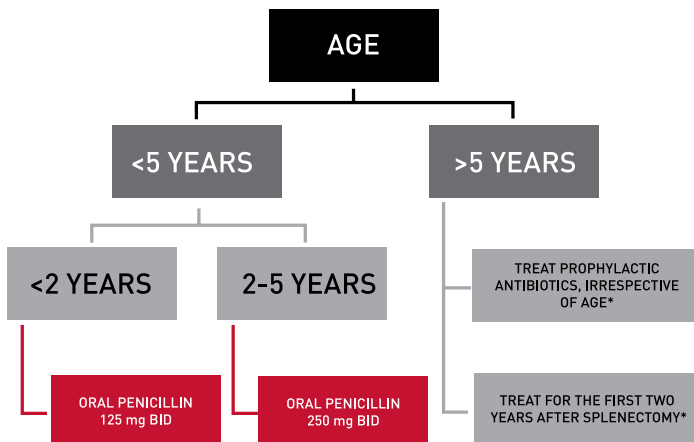


Figure 1. Chemoprophylaxis for splenectomised patients. *Depends on opinion of treating physician.

Alternative antibiotics for patients unable to take penicillin include amoxicillin, trimethoprim-sulfomethoxazole and erythromycin. The use of prophylactic antibiotics will need to be regularly re-evaluated as improved vaccines 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 recognize 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 recognizing 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 an antimicrobial regimen effective against *Streptococcus pneumoniae* and *Neisseria meningitidis*.
- If bacteraemia is suspected, the patient should be treated with parenteral antibiotics and observed in a medical facility until the cultures are evaluated.
- 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.

Hypercoagulability

Thromboembolic complications are frequent in thalassaemia, and even more frequent in splenectomised patients. 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 triggered by the absence of the spleen [Cappellini 2005, Borgna-Pignatti 1998].

Once they persist in the circulation they trigger mechanisms of Thrombin generation. In postsplenectomy patients markers of thrombin generation such as thrombin AT III (TAT) complexes, prothrombin fragments [F1,2] fibrinopeptide A (FPA) and D-dimer should be assessed annually, and anti-coagulant prophylaxis prescribed where indicated.

Pulmonary hypertension

This complication is more frequent in thalassaemia intermedia, but is also increasingly identified in thalassaemia major.

Advancing age and a history of splenectomy are major risk factors in this population [Morris 2010]. For more information on this complication please refer to Chapter 4 on cardiovascular disease.

Iron overload

In many patients the size and 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 ferric kinetic profile of iron overload and toxicity in β -TM patients. Following splenectomy the total body iron storage capacity is reduced. 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 2011, Aessopos 2005, Fiorelli 1990)

In a separate study splenectomised patients had a higher incidence of myocardial iron load (48%) and higher myocardial iron by comparison to non-splenectomised patients (28%) (Aydinok 2011).

Summary and Recommendations

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 strict transfusion regimen and chelation has considerably reduced the incidence of splenomegaly and iron overload in transfusion-dependent thalassaemia patients.

- At the current time we do not recommend splenectomy as a standard procedure in thalassaemic individuals (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. We have come to consider splenectomy in thalassaemic patients in three clinical scenarios. Increased blood requirement that prevents adequate control with iron chelation therapy, hypersplenism and symptomatic splenomegaly (C).
- When performing the splenectomy, laparoscopic approach seems to be the most favorable (B).
- The most frequent pathogens that cause infections in splenectomised patients are *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis*, therefore immunoprophylaxis is recommended against these agents 2 weeks prior to the operation and 3-5 years post op. Additionally, an annual influenza vaccine is encouraged.
- Chemoprophylaxis with oral penicillin depends on the age of the individual and the treating physician's opinion (C).
- In current practice, due to the strict transfusion and chelation protocols, the disease is very well controlled and we are seeing less splenectomies than before. Nevertheless a large bulk of the thalassaemic population is already splenectomised. These patients are at an increased risk of many disease related morbidities and should be monitored more closely.

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INFECTIONS

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Infections and their complications were previously the second commonest cause of death in transfusion-dependent thalassaemia (TDT), prior to the new millennium (Borgna-Pignatti 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 B, 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 health care 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 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 anemia may also have deleterious effects on the immune system and contribute to susceptibility to infections (Wanachiwanawin 1993). Further, 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 (**Table 1**).

Table 1. Etiology of risks of infections in transfusion dependent thalassaemia.

THERAPY RELATED FACTORS

Allogeneic blood transfusions

- Transfusion transmitted infections
- Transfusion related immune modulation
- Iron overload

Splenectomy

Iron chelation therapy

Central venous catheters

Stem cell transplantation

DISEASE RELATED FACTORS

Ineffective erythropoiesis

Haemolysis

Anemia

Therapy Related Risks of Infections in TDT and Preventive Measures

Allogeneic blood transfusions 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.

Fundamental principles for providing safe blood:

- The deferral of high risk prospective donors is the first level of defense against TTIs.
- Strategies regarding donor recruitment through voluntary non-remunerated blood donor (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 a valid technology should be implemented in Blood Banks (Bloch 2012).
- 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 titer 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-I/II), Cytomegalovirus (CMV), Parvovirus B19, West Nile Virus (WNV), Dengue virus, Babesia spp., Plasmodium spp., trypanosoma cruzi, and the prions that cause variant CJD (Allain 2009).

Preventative measures include:

- Prestorage leucodepletion of pRBC units reduces the transmission of cytomegalovirus (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., human T cell lymphotropic virus type 1 [HTLV-1] and HIV), bacteria (e.g., Yersinia enterocolitica), protozoa (e.g., Leishmania species and Trypanosoma cruzi), and infectious prion.
- 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 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 bacteremia due to a recent infection. Causative bacteria are most often a Gram -negative bacilli - mainly Yersinia enterocolitica and Serratia marcescans. (Lindholm 2011).

Suspicion and approach to transfusion related bacterial sepsis:

- If bacterial contamination is suspected, the transfusion should be halted immediately.
- Intravenous infusion of third generation cephalosporin (cefotaxime 2 g every 8 h 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's 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 septicemia. 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 1992) (C).

B. Transfusion related immune modulation (TRIM)

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 1986).

C. Storage defects of transfused pRBCs

It is suggested that free haeme 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 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 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 less than 14 days may provide benefit to avoid deleterious effects of storage defects (C).

Table 2. Pathogens isolated from infections in thalassaemia patients:

Pathogen	Number of cases (%)
Staphylococcus aureus	9 (15%)
Staphylococcus pneumonia	8 (13%)
Escherichia coli	7 (11%)
Klebsiella pneumonia	6 (10%)
Salmonella sp.	4 (7%)
Pseudomonas aeruginosa	3 (5%)
Brucella sp.	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 G et al. 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). 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 (Pietrangelo 2003). Further, iron overload is associated with faster HIV-1 disease progression and poor outcome in TDT patients (Gordeuk 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 unfavorable outcomes in many types of infection (Nairz 2010).

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

Splenectomy

Splenectomy has 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 2011).

Overwhelming post-splenectomy infection (OPSI), is defined as fulminating sepsis, meningitides or pneumonia triggered mainly by *S. pneumoniae* followed by *H. influenzae* type B and *N. meningitides*. The risk of OPSI is more than 50 times higher than in the general population and is a permanent life-long condition (Hansen 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, hypoglycemia, and, commonly, disseminated intravascular coagulation and massive adrenal gland hemorrhage (Waterhouse-Friderichsen syndrome), progressing to multiorgan failure and death (Brigden 1999). The mortality rate is around 50 to 70% and most death occurs within the first 24 hours; only prompt diagnosis and immediate treatment can reduce mortality (Holdsworth 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 for 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 1999).
- While waiting results of blood culture, bacteria can be visualised in gram staining.
- A RT-PCR test for simultaneous identification of 3 main encapsulated bacteria (*S. pneumoniae*, *H. influenzae* type B and *N. meningitides*) is available (Di Sabatino 2011) **(A)**.

The preventive strategy based on penicillin prophylaxis and vaccination is extremely important and has been discussed in **Chapter 6** (The Spleen).

Iron chelation therapy

The control of systemic iron and withholding iron from invading microbes are important strategies of host defense. As a siderophore, some benefits of DFO has been demonstrated in particular infections; for example, DFO was able to promote recovery from coma in children with cerebral malaria (Gordeuk 1992) and experimental studies indicate beneficial effects of DFO in infections with *H.Capsulatum* & *T.cruzi* (Arantes 2011). This is partly attributable to the immuno-modulatory 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 ROS generation (Collins 2002). Further, some pathogens, including *Y. enterocolitica* or *V. vulnificus* and *Mucorales*, can utilise DFO as 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 siderophore or taken under control is strongly advised **(B)**.

Nonsiderophoric iron chelators such as DFX and DFP 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, 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. 2007; Paradkar 2008). However, the latter could not be supported by a subsequent double blinded, 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 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 phagocytise all 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 1996) and consequently overwhelms pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) (Ozinsky 2000). In the clinical setting, severe anemia, itself, has also been observed as a risk factor for bacterial infections in thalassaemia (Wanachiwanawin 2000).

- Deleterious effects of anemia, IE and Haemolysis on the host defense mechanisms may be taken under control by maintenance of pretransfusional haemoglobin levels between 9.0-9.5 g/dl that corrects anemia while suppressing erythroid marrow **(C)**.

Infectious Agents in Thalassaemia - Diagnosis and Treatment

Bacterial infections

Yersinia enterocolitica

Y. enterocolitica are 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* septicemia has been reported as a common infectious risk in DFO-treated thalassaemic patients from western countries (Adamkiewicz 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 polyarthritis are also other clinical manifestations of infection.

The mortality can reach to 50% in septicemia with complications including hepatic and splenic abscesses, osteomyelitis, intussusception, nephritis, meningitides 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 for enabling correct culture conditions. Serological tests may display cross-reactivity. However, four fold rises in IgG titers in samples obtained 15 days apart may be suggestive of recent infection.

Treatment: The basic and most important point is that a patient with thalassemia 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 septicemia) 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). Ciproflaxacin (400 mg every 12 h) is also an active antibiotic **(A)**.

Klebsiella spp.

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

Clinical manifestations: Infection is presented with sinusitis, intracranial infections, septicemia 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 gram 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 meningitides, pneumonia and sepsis caused by encapsulated bacteria (*S. pneumoniae*, *Hemophilus influenzae* type B, *Neisseria meningitidis*). Other pathogens responsible for post-splenectomy infections include; *E.coli*, *P. aeruginosa*, group B streptococci, *Enterococcus* spp., *V. vulnificus* [Cullingford 1991].

Treatment:

- Thalassaemic patients with fever and/or other signs of bacterial infection, particularly whom underwent 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 2002). DFP and DFX chelation may not be interrupted in the suspicion of *Y. enterocolitica* infection **(C)**.

Viral infections

Human Parvovirus B19 (HPV B19)

Clinical manifestations: HPV B19 typically causes erythema, infections or fifth disease in children with the clinical course of a flu-like syndrome. HPV B19 DNA presents 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 could not be present in immunocompromised subjects that lead to persistence of viral DNA.

HPV B19 particularly infects erythroid progenitors complicated by a transient red cell aplasia. Because of high erythroid turnover, patients with thalassemia may develop, severe anemia with low reticulocyte counts during the course of HPV B19 infection (Ricerca 2009). The patients require intensification of transfusion regimen during acute infection. HPV B19 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 by pRBC collected from persistently infected blood donors play a secondary role (Lefrère 2005).

Human immunodeficiency virus (HIV)

HIV virus leads to CD4+ lymphocytes depletion that renders the individual at risk for many types of opportunistic infections. Due to continuous implementation and improvement of more sensitive serologic methods and nucleic acid amplification test (NAT), the residual risk of viral transmission decreased in less than 1:1.3 million for HIV in the European Union and US (Velati 2008, Allain 2002). 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 Africa foundation 2008).

In a large multicenter study composed of 79 HIV positive thalassemia patients from different countries, the progression to overt 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 1992]. However, a significant inverse relationship between disease progression and the dose of DFO administration was reported; the rate of progression decreases as the mean daily DFO dose increases [Costagliola 1994].

A large spectrum of therapeutic options are currently available in HIV infected patients that have also been used in patients with TDT. Since iron overload can have an adverse effects on HIV-1 disease progression such as faster progression of HIV in patients with low doses of DFO and high serum ferritin [Gordeuk 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 a caution in an HIV-1 positive patient.

Cytomegalovirus infection (CMV)

Cytomegalovirus (CMV) 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 stem cell transplantation in thalassemia.

- The use of blood products from CMV sero-negative donors has been shown effective in preventing transmission. However, it does not completely eliminate the risk of transmission. Moreover, as CMV sero-prevalence reaches 50 to 100% in different geographical regions and the availability of CMV sero-negative 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 1995] **(A)**.

Fungal infections

Mucor species

Mucormycosis or Zygomycoses are opportunistic infections and 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 the DFO itself may act as a siderophore for the fungi. Observations that DFX chelation may be a useful adjunct to antifungal treatment [Ibrahim, 2007], led to a trial of DFX combined with liposomal amphotericin B (AmBisomew) 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 insidiosus

Pythiosis is a very rare human infection caused by *Phytiosum insidiosus*, 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 2006]. Pythiosis has been reported in Thailand, Australia, Haiti, India, New Zealand and US. The systemic form was common in patients with thalassemia and associated with a high morbidity and mortality (most patients die within 6 months) [Prasertwitayakij 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 2004).

Malaria

There is evidence that carriers of haemoglobinopathy are associated with 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 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 2005).

Summary and Recommendations

There is a lack of properly controlled studies evaluating infections in thalassaemia. The knowledge about 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.

Key recommendations include:

- Infection-related mortality used to be the second leading cause of death and has gradually become the leading cause of death in thalassaemia in the modern era.
- Physicians must be aware of the potential life threatening infections in thalassaemia and patients should be educated to seek early care when fever develops.
- Control of iron homeostasis may have therapeutic benefit against infections.
- Temporary discontinuation of DFO and prompt initiation of antibiotics are strongly advised; whereas, iron loaded patients can continue to use synthetic oral iron chelators such as deferiprone (DFP) and deferasirox (DFX) during febrile episodes.
- Transfusion of pre-storage leucodepleted RBCs that have been stored <14 days may have therapeutic benefit against infections.
- Quality assurance guidelines and strict regulatory standards should be established for enhancing transfusion safety.
- Splenectomy indications and preventive measures for post-splenectomy risk of sepsis should be revisited.

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8

ENDOCRINE DISEASE

Authors Vincenzo De Sanctis, Nikos Skordis and Ashraf. T. Soliman
Reviewer Alan Cohen

Endocrine abnormalities are among the most common complications of β -thalassaemia major (TM). Despite early establishment of appropriate chelation therapy, problems such as delayed growth and sexual maturation and impaired fertility may persist. Determining the prevalence of endocrine complications is difficult because of the considerable differences in the age of first exposure to chelation therapy, the degree and type of chelation, the haemoglobin level attained before blood transfusion, and the continuing improvement in survival in well-chelated patients. The growth rates and endocrine complications of a sample of 3,817 TM patients in 29 countries are reported in **Figure 1** [De Sanctis 2004].

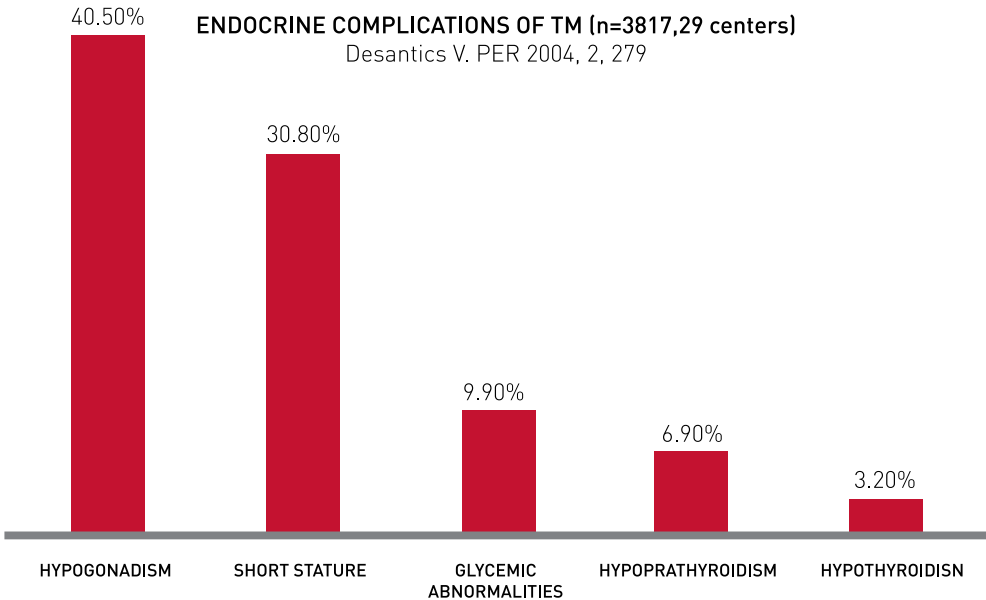


Figure 1. Growth and endocrine complications in thalassaemia. Reproduced from Thalassaemia International Federation Study Group on Growth and Endocrine Complications in Thalassaemia (De Sanctis 2004).

Short Stature and Retarded Growth

Growth retardation occurs almost invariably in TM subjects. Significant size retardation is observed in stature, sitting height, weight, and biacromial (shoulder) and bicristal (iliac crest) breadths. After the age of 4 years, the longitudinal growth patterns display rates of growth consistently behind those of normal controls. The bone age is frequently delayed after the age of 6–7 years. Growth retardation becomes severe with the failure of the pubertal growth spurt. Key contributing factors to stunted growth in patients with thalassaemia include chronic

anaemia, transfusional iron overload and chelation toxicity (De Sanctis 2013a). Other important contributing factors include nutritional deficiencies (protein-calorie malnutrition, vitamin D and A, zinc and carnitine deficiencies), growth hormone deficiency (GHD)/insufficiency (GHI), insulin-like growth factor-I (IGF-I) deficiency, chronic liver disease, hypogonadism, hypothyroidism and psychosocial stress.

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) (see **Appendix**), and/or
- Slow growth rates- growth velocity expressed in cm/year, below 1SD for age and sex (based on growth velocity charts), and /or
- Signs of other pituitary hormone deficiencies (e.g., gonadotrophins, GHD, TSH deficiency).
- 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-monthly intervals) and accurate measurement of standing and sitting height, pubertal staging (**Table 1**) and bone age, including examination of metaphyses. Interpretation of absolute height must take into account the height of the parents.

Table 1. Pubertal assessment according to Tanner.

PENILE DEVELOPMENT	BREAST DEVELOPMENT	GROWTH OF PUBIC HAIR
P1: Prepubertal	B1: Prepubertal	PH1: Prepubertal
P2: Early puberty (enlargement of scrotum and testes, 4-5 ml, little or no enlargement of penis)	B2: Early puberty (breast bud stage)	PH2: Early puberty (sparse growth)
P3: Mid-puberty (enlargement of penis and further growth of testes, 8-12 ml, and scrotum)	B3: Mid-puberty (breast and areolar enlargement)	PH3: Mid-puberty (hair extends over the pubic junction)
P4: Advanced puberty (enlargement of penis in length and breadth. Increased pigmentation of scrotal skin and enlargement of testicles, (14-25 ml)	B4: Advanced puberty (areola and nipple project separately from the contour of the breast)	PH4: Advanced puberty (hair corresponds to adult growth but less extensive)
P5: Adult	B5: Adult (Fully developed breast, the areola no longer projects separately from the breast contour)	PH5: Adult

Initial endocrine studies that may be helpful include: thyroid function tests (FT4, TSH), assessment of the pituitary gonadal axis (testosterone, estradiol, LH, FSH) and pituitary growth axis (IGF-I) , Insulin Growth Factor Binding Protein-3 (IGFBP-3) and Growth Hormone (GH) stimulation test when needed. Additional studies include: calcium homeostasis (calcium, phosphate, alkaline phosphatase, parathormone and 25-OH vitamin D levels), glucose tolerance tests and IgA transglutaminase antibodies to exclude coeliac disease. The secretion of GH is normal in the majority of patients with thalassaemia. Testing the hypothalamic-pituitary growth axis can be accomplished by directly measuring IGF-I level and performing a GH stimulation test (using clonidine, glucagon, or GHRH).

Radiological evaluation of the skeleton including assessment of bone maturation (bone age) and measuring bone mineral density during late childhood and early adolescence is recommended because of high prevalence of skeletal abnormalities and osteoporosis in TM patents. It is important to bear in mind that although the use of desferrioxamine has declined, it remains a cause of delayed growth (see **Chapter 3** on Iron Overload and Chelation) as well as skeletal abnormalities.

Treatment

Prevention and treatment of growth abnormalities in patients with TM should include (see **Figure 2**):

- Proper blood transfusion to maintain pretransfusion haemoglobin level > 9 g/dl.
- Proper chelation to attain serum ferritin < 1000 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 zinc deficiency.
- Correction of hypersplenism.
- Proper diagnosis and management of growth delay and GH treatment in patients with GHD is beneficial in most of the cases.
- Proper 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.
- Proper diagnosis and early management of hypothyroidism and abnormal glucose homeostasis (impaired glucose tolerance and diabetes mellitus).

HOW TO PREVENT GROWTH RETARDATION IN THALASSAEMIA MAJOR

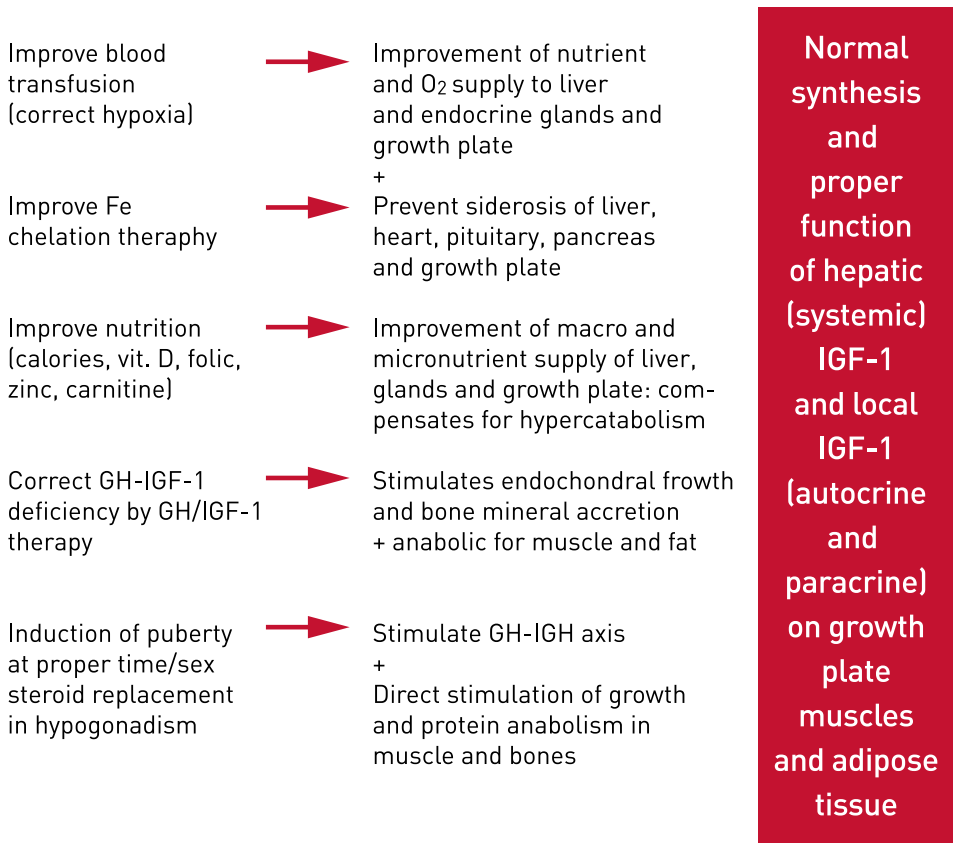


Figure 2. Practical approach to the treatment of growth retardation in thalassaemia. Reproduced with permission from [Soliman 2013].

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 2013a]. 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 2013a]. Hypogonadism in adolescents and adults with TM has prevalence of 38% in females and 43% in males (**Figure 1**).

- Routine investigations include: biochemical analysis, thyroid function (TSH and 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 FSH and LH secretion.
- Low LH/FSH response to GnRH (gonadotropin releasing hormone) 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 hypogonadotrophic hypogonadism depends on factors such as age, severity of iron overload, damage to the hypothalamo-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 after 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.

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 hypogonadotrophic 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.

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

Hypothyroidism

This complication is mainly attributed to iron overload and is uncommon in optimally treated patients. Central hypothyroidism is uncommon (De Sanctis 2012a). 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 2012a).

Laboratory tests

Investigation of thyroid function should be performed annually, beginning at the age of 9 years (unless symptomatic hypothyroidism is observed) (Rindang 2011). Free T4 and TSH are the key investigations. Additional tests may include the following:

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

Grades of thyroid dysfunction

The following grades of hypothyroidism have been identified (De Sanctis 2012a):

- 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 microU/ml)
 - Type B (normal FT4, TSH > 10 microU/ml)
- Overt hypothyroidism is a combination of high TSH with low FT4.

Clinical examination

The classical clinical signs of hypothyroidism in TM patients are not easy because most of the symptoms, especially in mild cases, are nonspecific and are frequently attributed to anaemia or associated diseases (Sabato 1983). Thalassaemic patients with overt hypothyroidism have been reported to exhibit stunted growth, delayed puberty, cardiac failure and pericardial effusion (De Sanctis 2013a). They are shorter with more delayed bone age than euthyroid TM patients.

Treatment

Treatment depends upon the severity of organ failure. Good compliance with chelation therapy may prevent or improve hypothyroidism (sub-clinical hypothyroidism - basal TSH 5 to 10 mUI/ml).

Subclinical hypothyroidism requires regular medical follow-up and intensive iron chelation therapy. Patients with overt hypothyroidism should be given L-thyroxine (De Sanctis 2013a). A notable caution in thalassaemics with subclinical hypothyroidism and cardiomyopathy: treatment with amiodarone may result in the rapid progression to severe hypothyroidism, which in turn causes deterioration of cardiac function (Alexandrides 2000).

Impaired Glucose Tolerance (IGT) and Diabetes Mellitus (DM)

IGT and DM are relatively common complications in patients who have been inadequately iron chelated, although these abnormalities have been also 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 protocollagen proline hydroxylase enzyme, with subsequent disturbed microcirculation in the pancreas (De Sanctis 2013b, De Sanctis 2004, Iancu 1990).

The prevalence of IGT and insulin-dependent diabetes mellitus (IDDM) in adolescents and young adults with TM treated mainly with desferrioxamine varies in different series from 0 to 17 % (Skordis 2013). DM 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 both puberty and thalassaemia associated risk factors on insulin action may partly explain the increase of insulin resistance in adolescent thalassaemics (Skordis, 2013).

Pathogenesis of DM in β -thalassaemia patients

The initial insult appears to be due to iron-mediated insulin resistance rather than defective insulin production, but pancreatic β -cell damage and insulin deficiency subsequently develop as a result of direct toxic damage from iron deposition (Skordis 2013).

Pancreatic β -cell function in thalassaemia is characterised by the following sequence (Figure 3):

- Insulin-resistance with hyperinsulinemia and normal glucose tolerance.
- Insulin-resistance with IGT and progressive impairment of β -cell function with reduction of insulin secretion, and
- Insulin dependent DM.

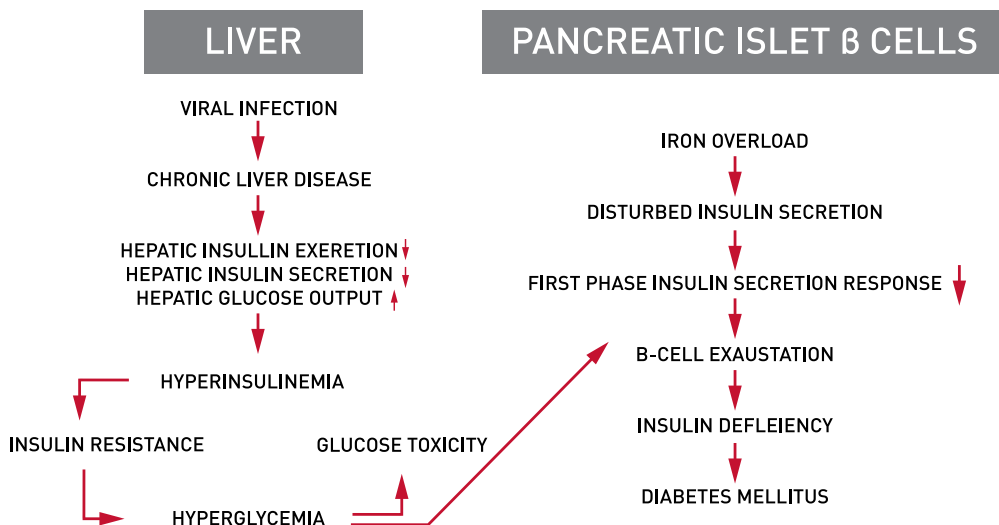


Figure 3. Pathogenesis of abnormal glucose homeostasis in thalassaemia. Reproduced with permission from (De Sanctis V. TIF Congress, Dubai – 2006).

Both liver and pancreatic β -cell siderosis and glucose toxicity may impair glucose tolerance. The interplay between liver siderosis and hepatitis C facilitates and accelerates the progression to DM, at least in adulthood (De Sanctis 2013a). 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 2013).

Diagnosis

The diagnostic criteria for glucose tolerance (Figure 4) 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.

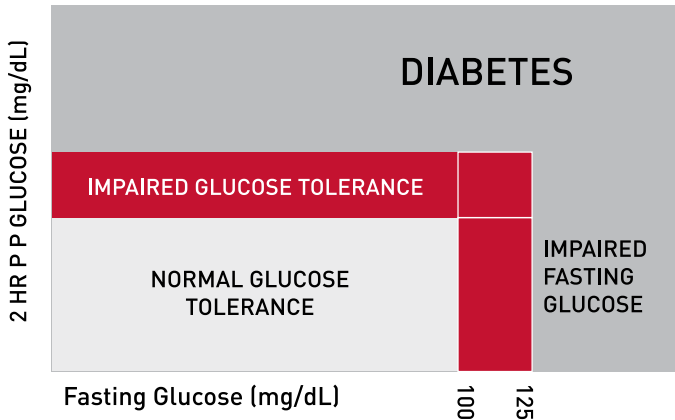


Figure 4. The diagnostic criteria for the glucose tolerance.

Pancreatic iron is the strongest predictor of β cell toxicity, which can be evaluated by the MRI of the pancreas (Noetzli 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, they may identify high-risk patients before irreversible pancreatic damage occurs. Nevertheless oral glucose tolerance testing still remains the gold standard test for glucose homeostasis. Screening for hepatitis infections and use of regular chelation therapy are important measures in preventing the development of diabetes.

Management

Management of impaired glucose tolerance and diabetes (De Sanctis 2013a, De Sanctis 2013b, Skordis 2013) is based on:

- Strict diabetic diet.
- Regular physical activity.
- Intensive chelation therapy: enhanced iron chelation therapy with desferrioxamine and deferiprone is effective to normalise β -cell function and may improve insulin secretion and glucose tolerance and reduce liver iron deposition (Berdoukas 2012).
- Oral hypoglycemic drugs: introducing oral hypoglycemic drugs in the early stage of DM before dependence on insulin may be beneficial, although limited data on the effect of oral antidiabetic drugs are reported (**Figure 5**).
- Insulin- symptomatic patients or patients with persistently elevated blood glucose despite other measures will need more definitive treatment with insulin therapy (**Figure 5**).

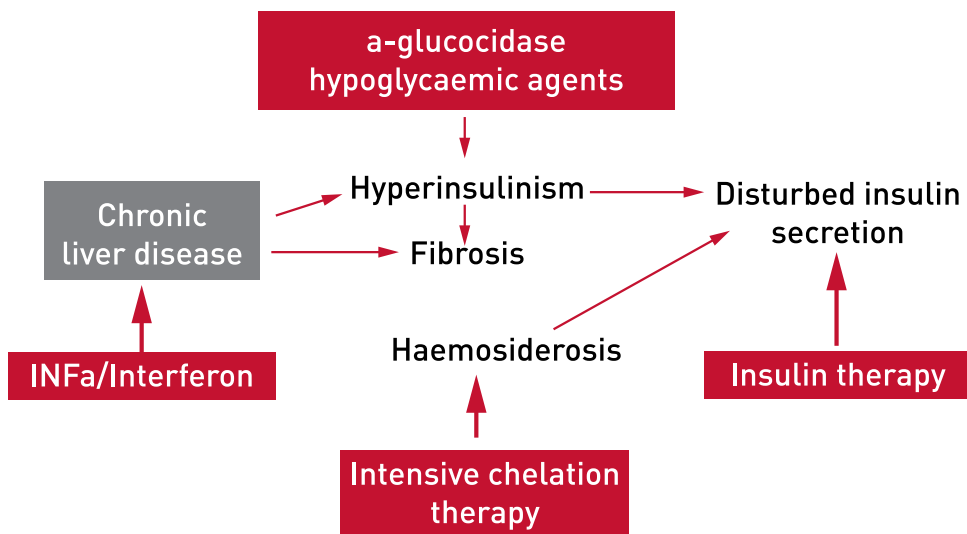


Figure 5. Therapeutic approach to abnormal glucose tolerance and diabetes in thalassaemia
 Reproduced with permission from [De Sanctis V. TIF Congress, Dubai – 2006].

Monitoring glycaemic control in thalassaemic patients with DM is not different from that in the general diabetic population [De Sanctis 2013a, De Sanctis 2013b]:

- Daily home capillary glucose monitoring.
- Urine ketones if blood sugar is above 250 mg/dl.
- Fructosamine estimation every month. HbA1c is not a reliable indicator of glycaemic control because of reduced red cell lifespan, ineffective haemopoiesis and frequent blood transfusions, all of which may potentially affect the validity of the HbA1c result [De Sanctis 2013a].
- Assessment of renal function.
- Urinary microalbumin and protein.
- Evaluation of retinopathy.

Hypoparathyroidism (HPT)

HPT has been considered as a typical complication of the second decade of life in transfusion dependent patients with thalassaemia major. The incidence of HPT varies from centre to centre (from 1.2% to 19 %) and HPT seems to affect men more frequently (male/female ratio = 1.35) [Vogiatzi 2009, Sleem 2007, De Sanctis 2004]. Recently, abnormal cerebral computed tomography findings have been reported in a high percentage of patients with thalassaemia and HPT [Karimi 2009, Soliman 2008]. An electrocardiogram (ECG) can detect an abnormality in the electrical activity of the heart.

Signs and symptoms

The majority of patients show a mild form of the disease accompanied by paraesthesia. More severe cases may demonstrate tetany, seizures or cardiac failure [Skordis 2013].

Investigations

Investigations should begin from the age of 16 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 2013).

Management

Treatment of HPT 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 calcifications (De Sanctis 2013a). 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 2012b).
- Calcitriol, 0.25-1.0 µg, twice daily, is usually sufficient to normalize 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 PTH 1-34, both once and twice daily, has been shown to effectively treat children with hypoparathyroidism. However, this therapy is not yet approved for the treatment of hypoparathyroidism and no data are available in literature in subjects with thalassaemia (De Sanctis 2012b).
- In some patients with HPT 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 2012b).

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, and 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 thalassemia ranging from 0-45%. “Clinical” adrenal insufficiency, i.e. adrenal crisis, on the other hand, is extremely rare (El Kholy 2013).

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 ACTH or insulin stimulation, are used for assessment of adrenal function. It is advised to test adrenal function every 1-2 years, especially in GHD patients during rhGH therapy (El Kholy, 2013), because patients with GH deficiency may have additional anterior pituitary hormone deficits, and are at risk of developing complete or partial corticotropin (ACTH) deficiency.

Treatment

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

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9

FERTILITY AND PREGNANCY

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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 of them. 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 hypogonadotrophic hypogonadism (HH) as a consequence of transfusional haemosiderosis (Skordis 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 counseling involving the various members of the multidisciplinary team: the haematologist, the reproductive medicine specialist, the cardiologist and the obstetrician, in conjunction with 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 intact hypothalamic- pituitary- gonadal (HPG) axis (Chatterjee 2000). Furthermore their management during pregnancy is different in that TI patients have an increased thrombotic risk and may need transfusion during pregnancy to decrease this risk (Nassar 2006). In addition to complications specific to iron overload, TM patients also face the risk of thromboembolism: this is particularly after splenectomy and in those with autoimmune antibodies.

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 gonadotrophin therapy, 'bypassing' the HPG axis (De Sanctis 1988a, De Sanctis 1988b). However, other endocrine disorders, namely diabetes and hypothyroidism, may also influence the outcome of fertility treatment and need to be corrected by standard care. Successful spontaneous pregnancies, as well those resulting from the induction of gametogenesis, have been documented in TM females and males (Aessopos 1999, Skordis 2004).

Management of subfertility requires careful planning and preparation (a thorough work-up), including pre-pregnancy counseling of the couple (see below). Fertility assessment of patients with thalassaemia should also include evaluation of the partner according to standard criteria (see <http://www.rcog.org.uk>). 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 GnRH infusion is only possible at the early stage of HPG damage, when gonadotrophins (FSH, LH) are pulsatile. But most of patients with HH are apulsatile but with functional gonads, and are therefore likely to benefit from gonadotrophin- therapy (80% success rate) (Skordis 2004). Patients with endometrial or Fallopian tube damage respond better to IVF programmes. The drugs used however are powerful, and can often induce growth of two or more follicles, with 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 severe 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 counseled 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 an informed consent.

Carefully documented notes should be kept throughout. Induction of ovulation may be indicated in women with primary amenorrhoea, secondary amenorrhoea, or those with normal menstrual function who fail to conceive and 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 hCG and LH to trigger ovulation at the end of follicular development. Adjuvant medications, such as GnRH analogues (agonists and antagonists) 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 estradiol. hCG is administered when at least two follicles reach 17 mm size and 36 hours after the administration of hCG egg collection is performed.

Key points in induction of ovulation include:

- Careful monitoring of the cycle by serial vaginal ultrasound scans is needed.
- Therapy should be continued until hCG is injected/biochemical pregnancy is confirmed.
- Luteal support with progesterone may be required.
- After a maximum of six cycles, the physician should reassess and refer for in vitro fertilization (IVF).

Male Fertility and Induction of Spermatogenesis

The induction of spermatogenesis in male patients with thalassaemia is more difficult than the induction of ovulation in their female counterparts, with a success rate of only 10-15% in moderate to severely iron loaded patients (Skordis et al 2004). The induction process must be undertaken according to HFEA guidelines, with an emphasis on consent and counseling [Deech, 1998]. An established protocol for induction of spermatogenesis is described below:

- 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 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 human menopausal gonadotropin (or FSH) to induce spermatogenesis.

The treatment process is demanding and may take up to 2 years. The initial regimen of hCG is usually 1,000 to 2,000 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 [De Sanctis 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 cryopreserved should be considered in all subjects with a stated wish to have children in future unless already azoospermic, to better preserved fertility and so the chance of conception. However, recent literature on sperm DNA damage in males with thalassaemia (De Sanctis 2008, Perera 2002) raises anxiety about mutagenic risks in these individuals, especially after ICSI, where natural protective barrier against gamete selection during fertilization is lost.

Pre-Pregnancy Counseling

Before embarking on fertility treatment, it is important that patients and their partners attend pre-pregnancy counseling, 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 should have cardiac assessment by echocardiography (left ventricular ejection fraction >65%; fractional shortening >30%), by electrocardiogram (both at rest and with exercise) and by 24 hour tape recording to check for rhythm disorders. 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 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 l 2001). If the facility exists, cardiac MRI should be performed with the aim of identifying a T2* of less than 20 ms.
2. Liver function should be evaluated by biochemical tests, with the possibility of iron overload status being assessed by liver biopsy and MRI. Liver biopsy can also provide information on fibrosis and cirrhosis (RCOG Clinical Green Top Guidelines 2004). With respect to Hepatitis C positive cases, these women should be given a course of antiviral agents to attain Hepatitis C RNA negative status.
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**, Osteoporosis).

4. All patients should be screened for the human immunodeficiency virus (HIV), Hepatitis B, Hepatitis C 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. Patients should also be screened for diabetes, thyroid function and acquired red cell antibodies. Both partners should be screened for haemoglobinopathies (Galanello 2010).

Eligibility evaluation includes the following elements:

- Cardiac function: ECG, Echocardiogram.
- Liver function tests, Ultrasound of the liver.
- Vessels: Clotting factors, Doppler.
- Endocrine: Thyroid function, Calcium homeostasis, Vitamin D levels.
- Pancreas: Glucose Tolerance test.
- Viral infections: HBV, HCV, HIV.
- Iron status.

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 (deferasirox or deferoxamine) should be advised to switch to desferrioxamine prior to induction of ovulation/spermatogenesis (Singer 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 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, ribovar and hydroxyurea. Hypothyroid patients receiving thyroid replacement therapy should receive increased doses 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.

Medication review for pregnancy focus points:

- Emphasize folic acid supplementation.
- Stop DFX and Vitamin C.
- Stop ACE inhibitors.
- Can safely continue Metformin, but may need to change oral hypoglycemic drugs to Insulin.
- Stop biphosphonates 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 foetal outcome is usually improved with a slight reduction in incidence of growth restriction (Aessopos 1999, Tuck 2005, Ansari 2006). It has been shown that the risks of pregnancy-specific complications such as ante-partum haemorrhage and pre-eclampsia in thalassaemia are similar to that in the background population. It has also been shown that deferoxamine is not required during pregnancy in patients that are not iron overloaded and have adequate cardiac function prior to pregnancy. Serum ferritin is likely to alter by 10%, despite increases in frequency of blood transfusion (Daskalakis 1998, Aessopos 1999, Butwick 2005, Tuck 2005). The aim during pregnancy is to maintain pre-transfusion haemoglobin concentrations above 10 g/dl. Once pregnancy is confirmed, the patient should be managed in a multidisciplinary setting with a team consisting of an obstetrician, midwife, physician, haematologist and anaesthetist. The patient should be made aware that although pregnancy is high risk, the outcome is usually favourable.

Identified 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 foetal malformation: no increase.
- Risk of foetal 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 pre-maturity and growth restriction is increased in multiple births.

It is important to note that the main risk to the mother is cardiac complications, which can be minimised by ensuring optimal cardiac function and good control of iron overload before initiation of pregnancy.

Management of Pregnancy

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 showed by the deterioration of mitral valve 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* and before initiation of pregnancy in women with TM provided they have started early on proper treatment and have a normal resting cardiac performance (Kypris 2011).

All patients should be screened for gestational diabetes at 16 weeks and, if normal, screening should be repeated at 28 weeks. Serial ultrasound scans from 24-26 weeks onwards must be undertaken to monitor foetal growth. In selected cases, and particularly in those with TI, thromboprophylaxis with low molecular weight heparin is required from mid-trimester (Nassar 2006, Eldor 2002). Serial ultrasound scans from 24-26 weeks onwards must be undertaken to monitor foetal growth. Although there is a predisposition to venous thrombosis in post-splenectomy patients, no reports of thrombotic episodes have been noted in the literature (Daskalakis 1998, Tuck 1998). Folate demand in pregnancy is normally increased: this may be relevant in patients with thalassaemia due to bone 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 β -thalassaemia minor (carriers) (Leung 1989).

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 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 2010, Tuck 1998). Deferoxamine has therefore been used in some higher risk pregnancies, particularly in the final trimester (Bajoria 2009, Tsironi 2005, Singer 1999). With respect to the newer oral chelating agents, data on foetotoxicity 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 foetal 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 labor with acidosis may increase the risk of cardiac decompensation.

With respect to the management of labour, if pregnancy is non-complicated one can await the spontaneous onset of labor. Similarly though to the reported data the authors' experience suggests that 80% of women with thalassaemia will require Caesarean section because of higher frequency of cephalopelvic disproportion, largely due to short stature and skeletal deformity combined with normal foetal growth. It is desirable to use epidural anaesthesia wherever feasible, to avoid the risk of difficult intubation and trauma associated with general anesthesia because of severe maxillo-facial deformity in some TM patients. If the mother has pre-pregnancy morbidities such as diabetes or cardiac disease then the prolonged pregnancy should be avoided. Low dose deferoxamine may be used during prolonged labour in patients with cardiac disease.

Although most skeletal deformities are largely preventable by regular transfusion, spinal

abnormalities associated with TM are related to regional blockade. 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 (Borgna-Pignatti 2006a, Borgna-Pignatti 2006b). It is therefore important to correct osteoporosis prenatally by hormone replacement and with bisphosphonates, where 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. After delivery, in principle deferoxamine can be recommenced because concentrations are very low in breast milk and because it is not absorbed from by the oral route (Howard 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 HBsAg positive because of the risk of vertical transmission via breast milk.

All patients should be offered counseling 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 most cases, the progesterone-only pill or barrier methods are usually appropriate. Male patients with HH are not fertile spontaneously and therefore contraception is not required. Calcium and vitamin D supplements should be continued during breastfeeding, however bisphosphonate therapy for osteoporosis should only be resumed after cessation of breastfeeding (Howard et al 2012).

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 10 g/dl.
- 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 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.

Women with TM appear to have premature ovarian aging 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 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 for 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 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.

Assessment of women with thalassaemia seeking pregnancy should include:

- Assessment of cardiac function with electrocardiogram and echocardiogram.
- Liver function test and ultrasound.
- Status of viral infections (HCV, HBV, HIV).
- Vessels: clotting factors, Doppler.
- Oral glucose tolerance test - optimize diabetic control.
- Iron status - optimize chelation.
- Thyroid function
- Virology – Rubella – Toxoplasmosis
- Review medication
- Screen for acquired red cell antibodies (risk of haemolytic disease)
- Check male for haemoglobinopathy
- Arrange genetic counseling if necessary

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 gonadotrophin 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.

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Cooley and Lee described the first series of splenomegaly in non-transfused children with anaemia and peculiar bone changes with mongoloid appearance caused by the enlargement of the cranial and facial bones (Cooley 1925). Other bone abnormalities have also been described in patients with thalassaemia, such as spinal deformities, scoliosis, nerve compression, spontaneous fractures, osteopenia and osteoporosis.

In osteoporosis the bone mineral density (BMD) is reduced, bone microarchitecture is disrupted and the amount and variety of non-collagenous proteins in bone is altered (**Figure 1**). 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 consequential increase in fracture risk. Osteoporosis is a prominent cause of morbidity in patients with thalassaemia major (TM).

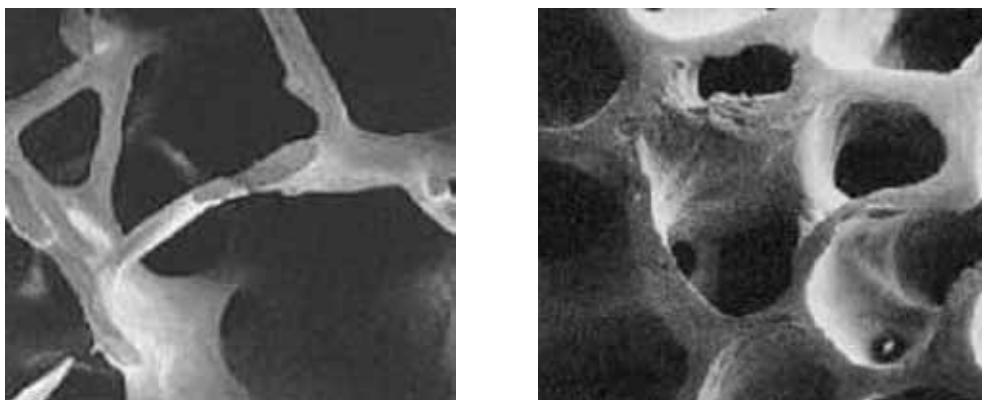


Figure 1. Osteoporotic (A) and Normal (B) bone.

The cut-off of 2.5 standard deviations below the normal mean in bone mineral density (BMD) for the respective age is used for the definition of osteoporosis, whereas the decrease of BMD between 1.5 and 2.5 standard deviations below the normal mean for the respective age is defined as osteopenia.

Treatment with transfusion programmes and chelation therapy have significantly prolonged survival in thalassaemia patients. Thus, osteopenia and osteoporosis represent prominent causes of morbidity in young adults of both genders with TM or thalassaemia intermedia and the incidence of osteopenia or osteoporosis in well-treated TM patients has been found to be approximately 40–50% (Voskaridou 2004). Bone mineral density (BMD) is a widely used and well-established measure of skeletal health. DXA is the gold standard for the measurement of BMD. It is a non-invasive technique and can be performed at the hip, lumbar spine, and distal radius (**Figure 2**).



Figure 2. Typical DXA equipment.

Definitions

- Osteoporosis: is defined as BMD T-score < -2.5 leading to higher risk of fracture.
- Osteopenia: is defined as BMD T-score between -1 and -2.5 .
- Normal BMD: T-score > -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.

Biology of Normal Bone Metabolism

It is a common misconception that bones are static in nature and hardly change once an individual becomes an adult. On the contrary, bones are continuously undergoing a dynamic process of resorption and deposition known as bone metabolism (**Figure 3**).

The cells responsible for bone metabolism are known as osteoblasts, which secrete new bone, and osteoclasts, which break bone down. The structure of bones, as well as adequate supply of calcium, requires close cooperation between these two types of cells. It relies on complex signaling pathways to achieve proper rates of growth and differentiation. These signaling pathways include the action of several hormones, including parathyroid hormone (PTH), vitamin D, growth hormone, Wnt signaling, steroids, and calcitonin, as well as several cytokines. It is in this way that the body is able to maintain proper levels of calcium required for physiological processes.

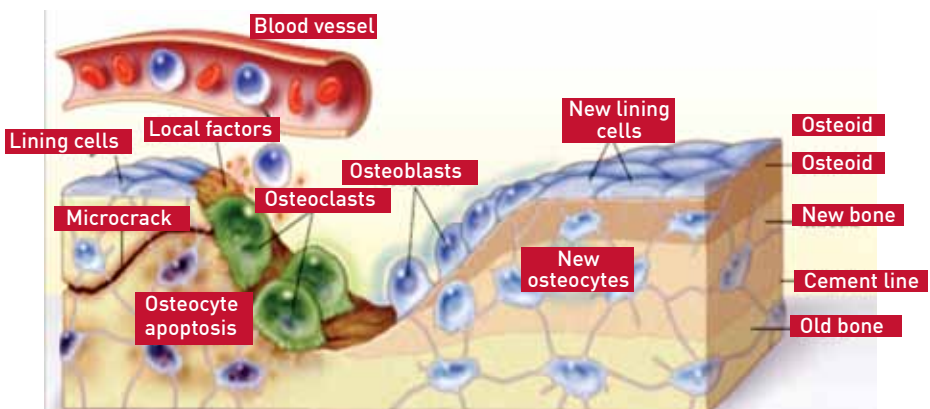


Figure 3. Several microcracks are developed every minute in our bones. The damaged bone is resorbed by the osteoclasts, and rebuilt by osteoblasts, both of which communicate through cytokine signaling. Osteocytes are old osteoblasts that occupy the lacunar space and are surrounded by the bone matrix; osteocytes are considered the key cell for bone remodeling.

Pathogenesis

In thalassaemia, marrow expansion causes mechanical interruption of bone formation, leading to cortical thinning, and is considered to-date as a main reason of distortion and fragility of the bones in thalassaemia patients. However, the pathogenesis of osteoporosis in TM is very complex. In addition to the development of bone distortion due to ineffective haemopoiesis and progressive marrow expansion, there are several genetic and acquired factors are implicated in bone destruction in TM (Wonke 1998).

Genetic factors

The polymorphism at the Sp1 site of the collagen type Ia1 (COLIA 1) gene (collagen type I is the major bone matrix protein) has been associated with reduced BMD in postmenopausal osteoporosis, and predisposes women to osteoporotic fractures. In a study by Wonke et al, approximately 30% of the TM patients were heterozygotes (Ss) and 4% were homozygotes (SS) for the Sp1 polymorphism. The authors concluded that male patients with TM carrying the Sp1 mutation may develop severe osteoporosis of the spine and the hip more frequently than patients who do not carry this mutation. Other genetic factors that have been reported to correlate with low BMD in adult patients with TM include the vitamin D receptor (VDR) BsmI BB polymorphism, the loss of-function mutations in the gene of the vitamin D receptor, the sequence variation of 713-8delC of transforming growth factor- β 1, the presence of restriction fragment length polymorphisms for the calcitonin receptor gene, estrogen receptor and interleukin-6 gene loci.

Acquired factors

Endocrine complications: Hypothyroidism, hypoparathyroidism, diabetes mellitus, and mainly hypogonadism (as delayed puberty and/or secondary hypogonadism) are considered as major causes of osteopenia/osteoporosis in TM. Hemosiderosis of the pituitary gonadotrophic cells and iron deposition in the testes and ovaries are involved in the pathogenesis of hypogonadism in TM.

Iron overload and desferrioxamine: Iron deposition in the bone impairs osteoid maturation and inhibits mineralisation locally, resulting in focal osteomalacia. The mechanism by which iron overload interferes in osteoid maturation and mineralisation includes the incorporation of iron into crystals of calcium hydroxyapatite, which consequently affects the growth of hydroxyapatite crystals and reduces the bone metabolism unit tensile strength. Desferrioxamine inhibits DNA synthesis, osteoblast, and fibroblast proliferation, osteoblast precursors differentiation, and collagen formation, although enhances osteoblast apoptosis, especially in patients who receive inappropriately high doses of desferrioxamine.

Vitamin deficiencies: Vitamin C deficiency in iron overload patients with low levels of serum ascorbic acid induces the risk of osteoporotic fractures. Vitamin D deficiency is also implicated in the pathogenesis of osteoporosis in TM patients due to the regulatory effect of vitamin D in both osteoclasts and osteoblasts.

Physical activity: Patients with TM have reduced physical activity due to the complications of the disease and occasionally overprotection by their parents who do not encourage muscle activity. This reduced physical activity predisposes to bone loss and subsequent osteoporosis.

All the above factors can lead to bone loss by increasing the osteoclast function and/or reducing the osteoblast activity.

Increased osteoclast function in thalassaemic patients with osteoporosis

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 type 5b (TRACP-5b) that correlate with BMD of the lumbar spine in these patients (Voskaridou, 2001) (**Table 1**). 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 2004), which is of great importance for the activation and proliferation of osteoclast precursors, and the overproduction of cytokines that are involved in the osteoclast differentiation and function. The ratio of sRANKL/OPG in the serum as well as IL-1 α , TNF- α , IL-6 and TGF- β are increased in thalassaemia patients with osteopenia/osteoporosis, suggesting their involvement in the pathogenesis of bone loss in TM. Activin-A, a member of the TGF- β superfamily that promotes osteoclastic activity in vitro is also elevated in the serum of patients with TM and correlates with low BMD.

Reduced osteoblast function in thalassaemic patients with osteoporosis

There is evidence of reduced osteoblast function in TM mainly due to iron poisoning in osteoblasts and/or the result of reduced function of growth hormone (GH) and insulin-like growth factor-1 (IGF-1) axis. However, novel molecules seem to be implicated in osteoblast dysfunction in TM. 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 2012, Voskaridou 2009). Wnt inhibition seems to be a major pathway that implicated in bone loss in TM. Furthermore, activin-A, which is elevated in the serum of TM patients, does not only activate osteoclast function but also inhibits osteoblast activity.

Table 1. Markers of biochemical of bone metabolism that can be used in patients with thalassaemia.

MARKERS OF BONE RESORPTION	MARKERS OF BONE FORMATION
NTX*	bALP*
CTX*	OC*
ICTP*	PINP*
RANKL	
Activin-A	
Dickkopf-1	
Sclerostin	

*These markers can easily be performed in biochemical laboratories routinely.

NTX, N-terminal cross-linking telopeptide of collagen type-I; CTX, C-terminal cross-linking telopeptide of collagen type-I; ICTP, carboxyterminal cross-linking telopeptide of collagen type-I; RANKL, receptor-activator of nuclear factor-kappa B ligand; bALP, bone-specific alkaline phosphatase; OC, osteocalcin; PINP, Procollagen I Intact N-terminal.

Management of Thalassaemia-Associated Osteoporosis

Prevention and general principles

Prevention and treatment of early bone loss make the best policy:

- Annual checking of BMD starting in adolescence is considered indispensable. BMD is a widely used and well-established measure of skeletal health and it is evaluated with DXA scans. DXA is the gold standard for measurement of BMD. It is a non-invasive technique and can be performed at the hip, lumbar spine, and distal radius.
- Physical activity must always be encouraged.
- Smoking should be discouraged.
- Adequate calcium intake during skeleton 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.
- Early diagnosis and treatment of diabetes mellitus
- Adequate iron chelation may prevent iron toxicity in the bone and sufficient blood transfusions may inhibit uncontrolled bone marrow expansion.

Hormonal replacement

Prevention of hypogonadism seems to be a very effective way for preventing osteoporosis and other bone deformities in thalassaemia patients. Continuous hormonal replacement therapy with transdermal estrogen for females or human chorionic gonadotrophin for males improves bone density parameters.

Calcitonin

Parenteral and intranasal instillations of calcitonin, a potent inhibitor of osteoclasts, are available. Calcitonin relieves bone pain, improves radiological findings of osteoporosis, decreases the number of fractures with no important side effects. However, very limited clinical data on TM have been reported in the literature

Bisphosphonates

Bisphosphonates are potent inhibitors of osteoclastic bone resorption and remain one of the cornerstones for the management of TM-associated osteoporosis (**Table 2**). Bisphosphonates inhibit osteoclastic recruitment and maturation, prevent the development of monocyte precursors into osteoclasts, induce osteoclast apoptosis and interrupt their attachment to the bone. Almost all generations of bisphosphonates have been used in an attempt to increase the BMD in thalassaemia induced osteoporosis.

Oral administration of alendronate but not intramuscular clodronate normalises the rate of bone turnover and results in a rise in BMD of the spine and the hip. Pamidronate, a second-generation aminobisphosphonate, has been given intravenously in patients with TM and osteoporosis with a significant improvement in BMD in most patients, a clear decrease of markers of bone resorption (NTX and TRACP-5b), and significant reduction of pain (Voskaridou 2003).

Neridronate is a third generation bisphosphonate which was given intravenously in TM patients with osteoporosis. Neridronate was associated with reduction of bone resorption, increase of BMD, reduction of back pain and improved quality of life (Forni 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 2006). Zoledronic acid continues to act at least 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. However, more trials must be conducted to clarify the exact role of each bisphosphonate, their exact dosage, their long-term benefit and safety as well as the effects of the combination of bisphosphonates with other effective agents in TM-induced osteoporosis. There is no experience, based on clinical studies, for the effects of bisphosphonates following fractures or hip surgery.

Table 2. The bisphosphonates used in patients with thalassaemia and osteoporosis.

BISPHOSPHONATES*	ROUTE OF ADMINISTRATION	DOSE & DURATION
Alendronate	Per os	10 mg / day**
Pamidronate	IV	30 mg / month**
Zoledronate	IV	4 mg / 3 months**
Neridronate	IV	100 mg / 6 months**

*All patients should take 500-1000 mg/d of elemental calcium and 400 IU cholecalciferol. IV, intravenous.

**The use of bisphosphonates should not exceed 12 months administration. The patient should then be monitored with DXA each year and depending on the findings, the therapy is repeated. There is no experience greater than 2-3 years of treatment.

Other novel agents

Teriparatide, a recombinant peptide fragment of parathyroid hormone, and strontium ranelate, a second anabolic agent, that seem to prevent osteoporotic fractures in postmenopausal women, are being studied but their effects in TM-induced osteoporosis remains to be proven. Antibodies against RANKL, such as denosumab, which has just been licensed by the US Food and Drugs Administration for the treatment of postmenopausal osteoporosis, and antibodies against Dkk-1 or against sclerostin may be future agents for the effective management of this difficult complication of thalassaemia. Sotatercept, a chimeric protein containing the extracellular domain of the activin receptor 2A (ActRIIA) inhibits activin-A, and it increased bone mineral density as well as haemoglobin levels in TM animal models. The phase II study in TM and thalassaemia intermedia patients has just been started.

Summary and Recommendations

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 the 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-A 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.
- Biochemical markers of bone metabolism that can be done every year: NTX, CTX, bALP.
- Physical activity must always be encouraged.
- Smoking should be discouraged.
- Adequate calcium intake during skeleton 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.
- Early diagnosis and treatment of diabetes mellitus.
- Adequate iron chelation may prevent iron toxicity in the bone and sufficient blood transfusions may inhibit uncontrolled bone marrow expansion.
- Hormonal replacement where it is needed.
- Bisphosphonates should be given concomitantly with calcium and vitamin D and not for longer than two years.

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DENTAL CARE

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Reviewer John Porter

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 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 β -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 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 protrusive premaxilla.

The dental arch parameter characteristics of patients with β -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 2011, Al-Wahadni 2005, Hattab 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 2011, Amini 2007). These changes may have implications for orthodontic treatment. Delayed dental development with associated physical growth retardation has also been noted in patients with β -thalassaemia major (Hattab 2013b).

The caries prevalence has been found to be significantly higher in thalassaemic patients than in healthy controls (Hattab 2001, Siamopoulou-Mavridou 1992). The higher dental caries experience in β -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 β -thalassaemia

major patients (Hattab 2001), a lower concentration of salivary immunoglobulin A (Siamopoulou-Mavridou 1992) and higher levels of salivary *Streptococcus mutans* (Luglie 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 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 2006). Iron deposition in the parotid glands can result in painful facial swelling but is rare (Hattab 2012, Goldfarb 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 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 inpatient 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 2003). Multiple immune abnormalities (Vento 2006), defective neutrophils, macrophage chemotaxis (Skoutelis 1984) and increased oral *Candida albicans* colonization (Van Dis 1984) 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 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 (Caliskan 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 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 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 β -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 2011). 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 2009).

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12

HAEMOPOIETIC STEM CELL TRANSPLANTATION

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Reviewer Maria Domenica Cappellini

Thirty years have passed since the first haemopoietic stem cell transplants (HSCT) in thalassaemia and this procedure now stands today as a widely applied treatment for the definitive cure of thalassaemia major, with more than 3000 HSCTs performed worldwide (Angelucci 2010). Even at a time when we are finally entering the long awaited gene therapy era, to this day HSCT remains the only available curative option for thalassaemia major. This chapter shall provide an overview of the evidence, current recommendations, practical applications and health considerations of HSCT in thalassaemia major.

Overview of Evidence

HLA identical sibling transplant

In the final 20 years of the last millennium more than 1000 thalassaemia patients underwent HLA identical sibling HSCT in the transplant center of Pesaro. In a series of 900 consecutive patient transplants, Pesaro's Center reported a 20 years probability of thalassaemia-free survival at 73% (Angelucci 2008). This provides the best evidence for the potential of HSCT as a curative therapy for thalassaemia major.

In recent years a number of factors – including better prophylaxis against graft versus host disease, more effective treatment of cytomegalovirus infection, improved aseptic techniques, better HLA typing and the evolution of systemic antibiotic therapy, have led to a remarkable improvement in outcomes for bone marrow transplantation procedures (Angelucci 2010), with cure of thalassaemia achieved in between 80% to 90% of subjects.

Categories of risk

In the 80s during the “deferoxamine only” era, the Pesaro group developed a prognostic scheme to predict transplant outcomes in patients younger than 17 years of age (Lucarelli 1993, Lucarelli 1990). This prognostic scheme included three variables all related to iron burden:

1. Lifetime quality of chelation received prior to transplantation (regular versus non-regular).
2. Hepatomegaly (defined as more than 2 centimeters below the costal margin).
3. Presence of liver fibrosis pre-transplant, as determined by hepatic biopsy examination.

These variables stratified patients into three groups: Class I patients in whom there were none of the adverse risk factors, Class II in which one or two adverse risk factors were present, and Class III who exhibited all three. Outcomes were found to be remarkably different between these three groups. The classification was developed at a time when deferoxamine was the only available chelation therapy, and “irregular

chelation” represented a failure of conventional therapy. Nevertheless, the important and still applicable concept stemming from the Pesaro classification is that constant life-long control of iron overload, with prevention of iron-related tissue damage, is crucial for successful transplantation. In the last decade, almost all transplant centers have followed this simple classification for predicting the risks and benefits of HSCT in thalassaemia and performed HSCT in the first years of life before iron-related complications have developed [Baronciani 2011].

Although this classification works well, there are limitations when applied to patients who have been poorly chelated. For such high-risk patients, an approach based on age (above or below 7 years) and size of the liver (whether or not 5 centimeters below the costal margin) seems to discriminate risk categories well and correlates with outcome [Mathews 2007]. This approach has also been validated in an analysis by the CIBMTR [Sabloff 2011]. In addition to this, transplantation techniques have improved and transplantation-related mortality (TRM) has fallen to 5%, or even lower in young low-risk children transplanted from an HLA-matched sibling [Angelucci 2010]. In a large EBMT survey of 1061 cases of matched-sibling donor transplantations performed in the last decade (consisting of 132 centers in 28 countries, with median patient age 7 years), long-term overall survival and thalassaemia-free survival were 91 ± 0.01 and 83 ± 0.01 , respectively [Baronciani 2011]. Treosulfan based conditioning has helped improve overall and thalassaemia-free survival even in high risk patients to 88% and 77%, respectively [Mathews 2013]. **Table 1** reports HSCT predictable outcomes in the present day. It is worth noting that no significant differences have been registered in the published reports between transplants performed in industrialized countries and those performed in other countries, where today more than 30% of procedures are regularly performed [Baronciani 2011]. A detailed analysis of the recent worldwide-published results have been reported elsewhere [Angelucci 2014].

Table 1. Expected probability of overall survival and thalassaemia free survival after HSCT in thalassaemia major.

CLASS	OVERALL SURVIVAL	THALASSAEMIA-FREE SURVIVAL
Class 1	95%	90%
Class 2	85%	80%
Class 3	75-80%	65-70%
Adult	70-75%	75%

Similar results have not been achieved in adult patients, where overall survival and thalassaemia-free survival peaks at 66% to 62% respectively, with transplant mortality rates of 35% and very limited risk of disease recurrence. We currently lack any large experience data in adults from the last decade, with only a single study reporting a 25% transplant-related mortality. As regard haemopoietic stem cell source, a large retrospective study has recently demonstrated that HLA identical sibling bone marrow, or cord blood derived haemopoietic stem cells can be used with similar probability of success, provided that an adequate number of nucleated cells are harvested and infused [Locatelli 2013].

Alternative donors

As most patients with thalassaemia lack a compatible sibling donor, there is interest in using alternative donors (see **Table 2** for approved and experimental HSCT indications in thalassaemia major). In this context three approaches have been reported:

1. Matched unrelated donors

- A number of studies have shown that unrelated-donor (UD)-HSCT can cure a large proportion of patients with thalassaemia, provided that the UD is selected using high-resolution molecular typing for both HLA class I and II molecules, and according to stringent criteria of compatibility with the recipient (i.e. identity or single allelic disparity for the loci for HLA-A, B, C, DRB1, and DQB1 loci). Using this approach, a suitable donor can be found in approximately one third of Caucasian patients with thalassaemia major. Additionally, 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 2006). However, much needs to be learnt with regard to suitable donor selection for MUD transplants in different risk categories. The major limitation to this approach is the limited experience, with only a few hundred patients transplanted globally. The distribution of donor registries is also limited mostly to industrialized countries and Caucasian donors, as the costs of such transplants as well as establishing new donor registries are significant.

2. Matched unrelated cord blood

- At present only two reports have examined this approach, and with contrasting results. Jaing et al reported results of UCBT in 35 thalassaemia patients, where overall survival was found to be 88%, with a thalassaemia free survival of 74%. The cumulative incidence of TRM was 11% (Jaing 2012). Combining data from 3 different registries, Ruggeri found the outcome of unrelated cord blood transplantation in thalassaemia to be much less favourable. In 35 thalassaemia patients, an overall survival of 62% was reported; with a thalassaemia free survival of only 21% (Ruggeri 2011).

3. Mismatched related donors

- The experience of HSCT from HLA-disparate relatives is thus far limited, and the results are inferior to those obtained with an HLA-identical sibling donor. In a consecutive series of 29 patients, the probability of overall survival and disease-free survival was 65% and 21% respectively, with a median follow-up of 7 years (Gaziev 2000). Better results have been reported in a limited series (n = 22) of heterogeneous thalassaemia patients using a haploidentical related donor and a "megadose" of positively-selected CD34+ cells (Sodani 2010).

Table 2. Accepted and experimental transplantation approaches for thalassaemia major.

APPROACH	STATUS
HLA identical sibling HSCT	Accepted
HLA well match unrelated donor HSCT	Accepted
HLA matched unrelated cord blood HSCT	Experimental
HLA mismatch related donor transplant	Experimental

Pre transplant evaluation

In children and adults particular attention should be reserved to an appropriate pre-transplant work-up. This should include (in addition to classical pre-HSCT evaluations) accurate iron studies, including cardiac iron / function, and liver iron / function, as well as histology - with particular attention to the degree of fibrosis (for this purpose liver biopsy remains the preferred tool, over liver elastography). No specific chelation regimen is recommended before HSCT, beyond the general aim that iron should be removed as much as possible. The intensive intravenous deferoxamine pre transplant regimen applied by the Pesaro group for high-risk patients was developed in association with a hyper-transfusional regimen to decrease erythroid expansion. It was part of the conditioning regimen and not intended for intensive chelation therapy to decrease previous existing iron load. However, it is important to note that the ideal situation would be one of constant and regular lifelong chelation therapy achieving a negative iron balance, rather than an intensive pre transplant regimen. Endocrine dysfunction has no relevance on HSCT outcomes, but should nonetheless be studied to allow accurate post transplant follow up.

Follow up

Post-transplant clinical follow-up of BMT is of particular importance. Within the first year, careful monitoring of hematological and engraftment parameters, infectious complications and graft versus host disease is essential. Appropriate immunization is necessary in the second year, if there is no graft versus host disease. 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 the primary disease. A number of reports indicate that iron overload, chronic hepatitis, cardiac function and endocrine deficiencies can be managed more easily after transplant, sometimes permitting the healing of severely damaged organs (Muretto 2002). It is also particularly important to remove excess iron after transplant. This can usually be achieved by repeated venesections (6 ml/kg blood withdrawn at 14-day intervals) (Angelucci 1997), or by chelation therapy. If venesections are not feasible, oral chelation can be proposed with standard dosing schedule. The reported agranulocytosis events with Defेरiprone warrant caution when using this drug. All iron removal treatments should be started only once the graft is stabilized, 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, although several spontaneous and successful conceptions have been registered after HSCT in paternal and maternal treated subjects.

Cost and cost effectiveness

Thalassaemia medical care is a complex, multidisciplined and expensive process which requires dedicated and experienced units. From a global health perspective, thalassaemia represents an enormous burden of care in certain regions. 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 2008). These data compare to total overall median costs of HSCT from sibling donors for non-malignant diseases, of 112,000 – 150,000 USD, which would translate to 1,900 USD per expected life year in cases of HSCT

performed in infancy (Matthes-Martin 2012). However, the cost of transplantation can vary significantly around the world, and is about USD 20,000 in India, for example (Chandy 2008).

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-industrialized countries), transplantation is certainly a cost-effective option if adequate expertise exist, even in developing countries.

Summary and Recommendations

- Haemopoietic stem cell transplantation should be offered to thalassaemia patients (and families) at an early age, or before complications due to iron overload have developed if an HLA identical sibling is available **(B)**.
- Either bone marrow or cord blood from an HLA identical sibling can be used **(B)**.
- A matched unrelated donor can be selected as a haemopoietic stem cell transplant donor for thalassaemia, provided that high compatibility criteria for both HLA class I and II loci are present **(B)**.
- Unrelated UCBT in thalassaemia should only be considered in low risk patients and if the CB unit is HLA compatible and contains an appropriate cell number, in the context of well-designed experimental clinical trials **(C)**.
- HSCT from an HLA-mismatched family member in thalassaemia should still be considered an experimental approach and should be conducted only in the context of well-designed clinical trials **(C)**.
- Myeloablative conditioning regimens (without irradiation) should always be used for standard transplantation **(B)**. Reduced-toxicity regimens are under investigation and may be used in the context of experimental clinical trials **(C)**.
- Post transplant care should include all thalassaemia related complications present from the moment of transplantation. After HSCT, iron overload can be completely removed by sequential phlebotomies **(B)**.
- In thalassaemia patients, HSCT is cost-effective when compared to life-long supportive therapy **(B)**.

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13

ALTERNATE AND NOVEL APPROACHES

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Reviewer Ali Taher

While there is currently no definitive treatment for the major haemoglobin disorders, with the exception of bone marrow transplantation, the potential of correcting the globin chain imbalance in β -thalassaemia by reactivation of the foetal haemoglobin genes is an approach that holds tremendous promise and could lead to widespread therapeutic options for patients.

The human β -globin locus on chromosome 11 is developmentally regulated (Sankaran 2010). In the first few weeks of gestation, there is predominant expression of an embryonic β -like globin chain, ϵ -globin. This is then replaced throughout much of gestation by the foetal haemoglobin or γ -globin genes that form the predominant β -like globin chain until after the time of birth (Sankaran 2010). Around that period of time, a developmental switch takes place where the foetal γ -globin genes are silenced and the adult β -globin chain is activated. In individuals without anemia, this switch is nearly complete by 1 year of age. This process has been studied at the molecular level and some regulators of this process have been defined (Sankaran 2011).

Increased production of γ -globin chains past the period of infancy can compensate for the defective production of β -globin that characterises β -thalassaemia (Weatherall 2001). As a result, globin chain imbalance is reduced and the clinical symptoms of this disease are ameliorated. Early clinical observations demonstrating the ameliorating effect of increased foetal haemoglobin (HbF) production in rare patients with β -thalassaemia who are clinically asymptomatic (such as those with Hereditary Persistence of Foetal Haemoglobin) have now been substantiated by larger epidemiological studies showing the quantitative ameliorating effect of increased γ -globin production leading to more HbF (Musallam 2012, Weatherall 2000).

Overview of Evidence

We have recently comprehensively reviewed the clinical literature of trials involving HbF inducers in β -thalassaemia patients (Musallam 2013). Overall, while there has been success in a limited number of trials with the use of HbF inducers, there has been no single agent that has demonstrated universal success. Moreover, the studies of various HbF inducers are confounded by the heterogeneity of study end points and populations of patients with β -thalassaemia (**Table 1**) (Musallam 2013). Here we summarise these findings briefly and make further recommendations for the use of these agents in clinical settings.

DNA methylation inhibitors (5-azacytidine and decitabine)

The earliest clinical studies of HbF inducers were done with 5-azacytidine (Ley 1982). In short-term studies, there was a robust HbF induction observed in patients with β -thalassaemia. Further studies have been limited because of concerns over the long-term adverse effects of using this drug. Decitabine (5-aza-2'-deoxycytidine) is a

5-azacytidine analog with a safer side effect profile. A single pilot study has shown that subcutaneous decitabine given at 0.2 mg/kg two times per week for 12 weeks increased total haemoglobin from 78.8 to 90.4 g/l (two patients had elevations ≥ 15 g/l) and absolute foetal haemoglobin from 36.4 to 42.9 g/l in five patients with β -thalassaemia intermedia (Olivieri 2011). Further studies are needed to examine whether this agent will be effective and have minimal toxicity with long-term use.

Hydroxyurea

The largest body of literature on HbF inducer use in β -thalassaemia stems from the use of hydroxyurea, a cytotoxic agent that inhibits ribonucleotide reductase and therefore slows progression through the cell cycle (Musallam 2013). The exact mechanism by which hydroxyurea increases HbF is not clear, but this is thought to be attributable to its action upon the differentiation of erythroid cells in the bone marrow (Platt 2008). No randomised studies have been performed with this agent, but larger cohort and case-control studies of this agent have been done in various β -thalassaemia patient populations (Musallam 2013). Moreover, hydroxyurea has been extensively used in sickle cell disease patients, where it has shown clinical effectiveness in both short and long-term follow-up studies (Ware 2010, Platt 2008). Studies of this agent have been performed in both transfusion-dependent and independent populations with variable forms of β -thalassaemia. We have recently reviewed all studies using this agent that have been performed to date (Musallam 2013). While the end-points studied and populations examined have been heterogeneous and the results have been variable, a number of studies have shown a clear benefit from the use of this agent in some patients. This includes a decreased need for transfusion, an increase in haemoglobin levels, decreased markers of ineffective erythropoiesis, and decreased morbidities (Musallam 2013). While the studies of this agent have been heterogeneous, the largest body of evidence stems from the use of hydroxyurea and therefore this has the best evidence grade of all HbF inducers tested in β -thalassaemia patient populations (**Table 1**).

Short-chain fatty acids

The observation that infants of diabetic mothers had a delayed switch from foetal-to-adult haemoglobin led to the hypothesis that short-chain fatty acids, such as butyrate could act as inducers of HbF. This led to an initial trial involving a 2-3 week infusion of arginine butyrate (at a dose of 500 mg/kg/day) in three β -thalassaemia patients (two transfusion-dependent) that showed promise with a decrease in globin chain imbalance observed (Perrine 1993). However, a follow up trial extending this therapy to 9-13 weeks with doses of arginine butyrate escalating from 500 to 2000 mg/kg/day for six days per week failed to achieve a primary haematological outcome of an increase in haemoglobin by 20 g/l among five β -thalassaemia patients (Sher 1995). A separate cohort study of oral sodium phenylbutyrate therapy at a dose of 20 g/day over the course of 41 to 460 days showed that four of 11 β -thalassaemia patients had increased levels of total haemoglobin of greater than 10 g/l (mean increase 21 g/l), along with an increased production of HbF (Collins 1995). Two cohort studies have examined the efficacy of the oral butyrate derivative isobutyramide to induce HbF and have shown variable responses in β -thalassaemia patients (Cappellini 2000, Reich 2000). Very recently, the oral short-chain fatty acid, 2,2-dimethylbutyrate, has been studied in patients with β -thalassaemia intermedia and the initial results show some promise (Fucharoen 2013), although further trials of this agent are needed. Further studies of these short-chain fatty acids are warranted, but currently the evidence of clinical effectiveness is rather limited for these agents.

Erythropoietin Stimulating and Other Agents

The use of recombinant human erythropoietin or the newer erythropoietic stimulating agent darbepoetin alfa in patients with β -thalassaemia is associated with increases in total haemoglobin level (Singer 2011). It has been shown that the combination of hydroxyurea and erythropoietin (50,000 U three times a week) is associated with higher increments in total haemoglobin level than hydroxyurea alone (17 versus 2 g/l after 6 months of therapy) in patients with β -thalassaemia intermedia (Loukopoulos). Limited further studies of such combination therapies have been performed. Newer erythropoietic stimulating agents that act independently of erythropoietin are also showing promise in pre-clinical studies and clinical trials of such agents are currently being planned or are underway.

Thalidomide, a drug known for its immunomodulatory and anti-angiogenic properties, has recently been suggested to induce γ -globin gene expression and to increase the proliferation of erythroid cells using in vitro culture models (Aerbajinai 2007). Two case reports reported that thalidomide therapy at 75-100 mg/kg/day caused a progressive and rapid increase in total haemoglobin and HbF levels in β -thalassaemia major patients (Maserà 2010, Aguillar-Lopez 2008). Whether this agent will have efficacy in larger studies remains to be seen.

In addition, recent pre-clinical studies have suggested that agents that block the activity of certain TGF- β family cytokines, particularly sotatercept (ACE-011), as well as JAK2 kinase inhibitors may be useful agents in the treatment of patients with β -thalassaemia. At the time that this chapter was written, there are no peer-reviewed publications reporting the use of these agents in clinical trials, although promising early reports from the clinical trial testing sotatercept (Clinical trial registration NCT01571635) suggest that such publications will be forthcoming in the near future. At the current time, it is too early to determine whether or not these agents will show efficacy in clinical trials.

Table 1. Summary of HbF inducer studies in patients with β -thalassaemia. Modified with permission from [Musallam 2013].

AGENT	MAIN POSITIVE FINDINGS	LIMITATIONS	EVIDENCE BASED QUALITY GRADE
DNA methylation inhibitors			
5-azacytidine	<ul style="list-style-type: none"> • Marked haematological responses achieved 	<ul style="list-style-type: none"> • Few studies • Small sample sizes • Safety concerns 	C
Decitabine	<ul style="list-style-type: none"> • Haematological responses achieved • Favorable effects on red cell indices • Well-tolerated 	<ul style="list-style-type: none"> • Few studies • Small sample sizes 	C
Hydroxyurea	<ul style="list-style-type: none"> • Haematological responses achieved • Favorable effects on red cell, haemolysis, and hypercoagulability indices • Favorable effects on clinical morbidities • Well-tolerated 	<ul style="list-style-type: none"> • Heterogonous phenotypes studied together • Heterogeneous study endpoints evaluated together • Ideal dose and duration of therapy still controversial • Lack of efficacy on long-term therapy • Data on predictors of response remain inconsistent 	B
Short-chain fatty acids	<ul style="list-style-type: none"> • Haematological responses achieved • Favorable effects on red cell and haemolysis indices • Well-tolerated 	<ul style="list-style-type: none"> • Small sample sizes • Lack of efficacy on long-term therapy 	C

AGENT	MAIN POSITIVE FINDINGS	LIMITATIONS	EVIDENCE BASED QUALITY GRADE
Erythropoietic stimulating agents	<ul style="list-style-type: none"> • Haematological responses achieved • Favorable effects on combination with hydroxyurea • Well-tolerated 	<ul style="list-style-type: none"> • Few studies • Small sample sizes • High doses required • No additive effects with short-chain fatty acids 	C

Summary and Recommendations

At the current time, we would not recommend the use of HbF inducing agents or other erythropoiesis stimulating agents outside the context of clinical trials. However, we would note that there is a large body of literature suggesting that hydroxyurea can be effective and help certain patients with β -thalassaemia, including those who are regularly transfused and those who only require intermittent transfusions or who are transfusion-independent (C). As such, if a patient is not showing appropriate responses to other therapies and they have a desire to try another therapeutic agent, a trial of hydroxyurea may be useful. It is important to monitor for signs of hydroxyurea toxicity, in particular by monitoring for leukopenia. In addition, gastrointestinal discomfort and hyperpigmentation or other skin changes can be associated with the use of hydroxyurea and should be monitored closely. We recommend that hydroxyurea be initiated at a dose of 15 mg/kg/day orally with blood count monitoring every 4 weeks. We recommend dose escalation by 2.5 – 5 mg/kg/day every 8 weeks with close monitoring performed at 4 week intervals. We generally aim to determine a maximum tolerated dose by ensuring that the absolute neutrophil count remains $> 2.0 \times 10^9 / L$. Clinical effectiveness can be assessed by measuring transfusion frequency in patients on regular transfusions or by monitoring total haemoglobin levels in those who are intermittently or never transfused.

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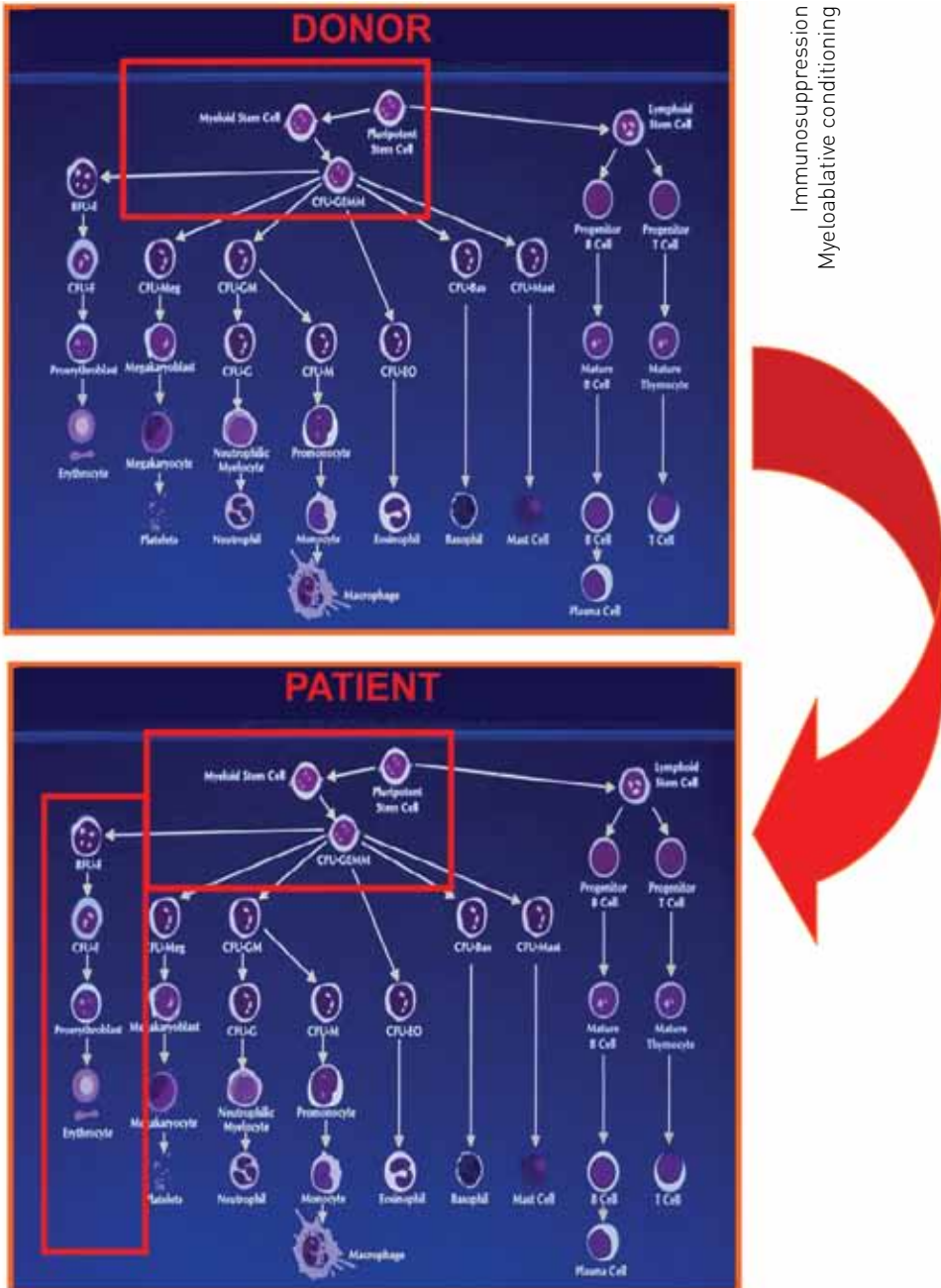
Rationale for Globin Gene Transfer and Stem Cell Engineering

Beta-thalassaemia major is treated with life-long transfusions of donor-derived normal red blood cells (RBCs), which the thalassaemic bone marrow is unable to produce. Iron chelation is required to curb the iron overload that inexorably builds up in chronically transfused patients. The only means to cure rather than treat severe beta-thalassaemias is to provide the patient with healthy hematopoietic stem cells (HSCs), which are cells that reside in the bone marrow and give rise to all blood cell types, including 20 billion RBCs per day in adults. The HSCs have to be harvested from a donor with wild-type beta-globin genes to yield long-lived RBCs with a normal content in haemoglobin. The successful transplantation of donor HSCs in thalassaemic patients can indeed be curative, but this option is not available to the vast majority of thalassaemic subjects, for whom a suitably matched related donor cannot be found (Sadelain 2007). Given the greater risks associated with matched-unrelated or mismatched transplants, most thalassaemia patients have to settle for life-long transfusion therapy, which does not correct ineffective erythropoiesis and exacerbates systemic iron accumulation. Moreover, despite the considerable improvement in life expectancy in the last decades (Borgna-Pignatti 2004, Telfer 2009, Ladis 2011), the risk of some serious complications arising over the long term from viral infections, iron toxicity and liver cirrhosis, remain (Mancuso 2006). These medical risks, together with the socio-economic cost of chronic beta-thalassaemia, warrant the pursuit of curative therapies.

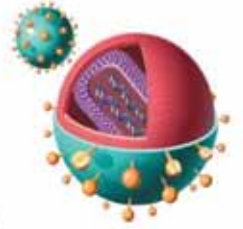
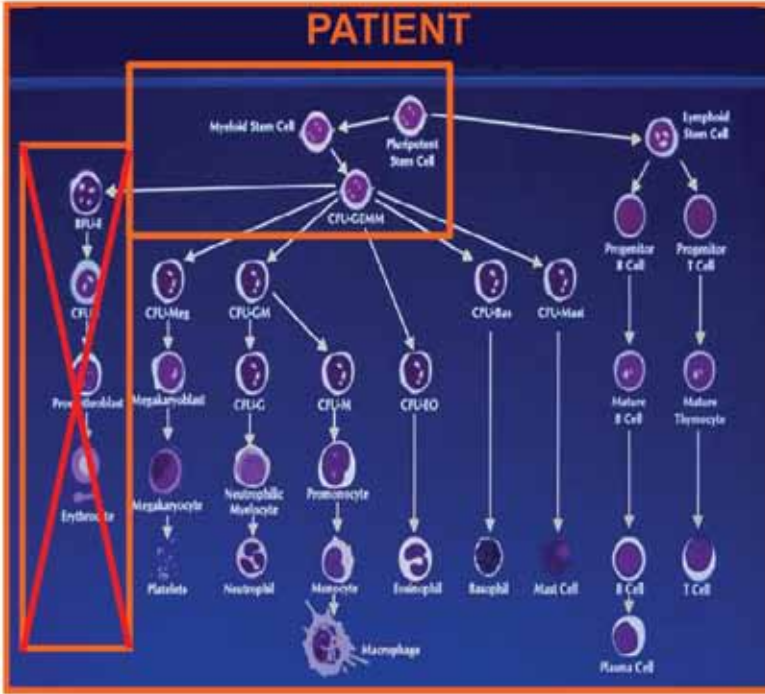
The goal of globin gene transfer is to restore the capacity of the thalassaemic subject's own blood-forming stem cells to generate RBCs with a normal haemoglobin content (Persons 2004, Sadelain 2006, Sadelain 2006). The patient's own HSCs are the cells in which the vector carrying the globin gene must be transferred in order to achieve long-term benefits because these cells can persist for years and are the only cells capable of continuously producing all haematopoietic lineages including the erythroid lineage and RBCs (**Figure 1**). The goal of this therapy is thus to achieve transfusion-independence without incurring the risks of bone marrow transplantation from a sub-optimally matched donor. For patients who lack an HLA-matched donor and thus have a higher risk of mortality following allogeneic HSC transplantation, globin gene transfer in autologous stem cells offers the prospect of a curative stem cell-based therapy.

Figure 1. Allogeneic hematopoietic stem cell transplantation versus genetic engineering of autologous hematopoietic stem cells. In allogeneic transplantation (A), the donor HSCs harbor healthy globin genes, but successful engraftment requires immunosuppression to prevent or treat graft rejection and graft-versus-host disease. In autologous HSC engineering (B), a vector is required to introduce healthy genes into the patient's own hematopoietic stem cells. Engraftment does not require immunosuppression because the cells are the patient's own; a myeloablative conditioning regimen is nonetheless needed to facilitate their engraftment.

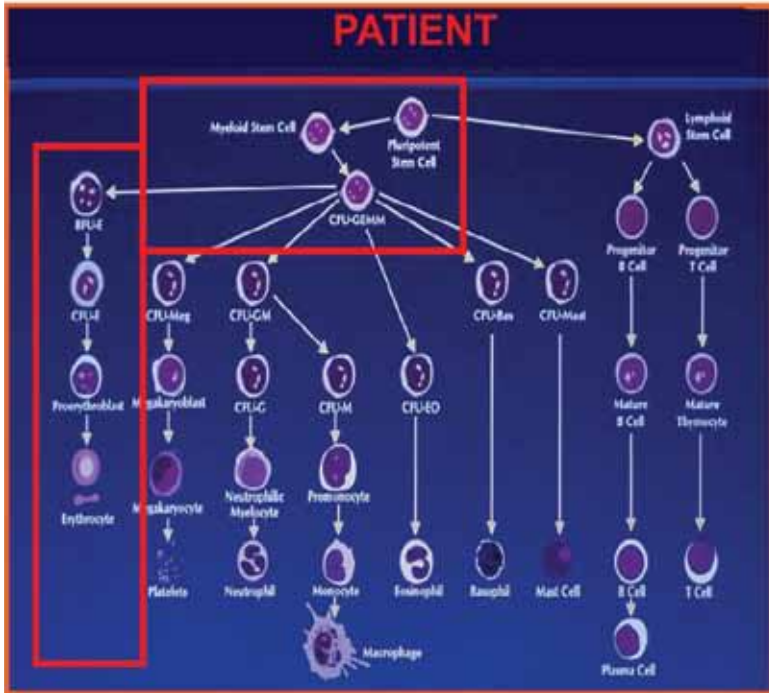
A. Allogeneic Stem Cell Transplantation



B. Autologous Globin Gene Transfer



HIV virus



James A. Perkins 2001

Preclinical Proof-of-principle and Safety Studies

The implementation of globin gene transfer for the treatment of severe beta-thalassaemia requires the efficient introduction of a regulated human β - or β -like globin gene in HSCs. The beta-globin gene must be expressed in an erythroid-specific fashion and at a high level, especially for the treatment of transfusion-dependent beta-zero thalassaemias. Following very extensive and systematic testing of different vector designs, we identified in the late 1990's several combinations of genomic sequences that could be stably transferred in murine HSCs and express the human beta-globin gene at therapeutic levels (May 2000). This study opened up the field, which for over a decade had failed to achieve this goal despite best efforts by many international groups. The lentiviral vector termed TNS9, which encodes a particularly potent combination of promoter, intron, enhancer and locus control region elements (Figure 2), effectively corrected the thalassaemia syndrome in beta-thalassaemic mice (May 2000). In this and subsequent studies (Rivella 2003, May 2002, May 2000), we demonstrated that mice harboring the TNS9 vector in their blood and bone marrow cells exhibited correction of anaemia, regression of extra-medullary haematopoiesis and absence of iron accumulation in peripheral tissues and organs (May 2002). In a lethal model of beta-zero thalassaemia major we established, in which mice succumb within 60 days of birth to severe anaemia, massive splenomegaly, extra-medullary hematopoiesis and hepatic iron overload, we showed rescue and long-term survival following TNS9 transfer in fetal liver HSCs (Rivella 2003). In large cohorts of mice, we did not observe evidence of vector silencing over time, neither in primary, secondary nor even tertiary chimeras (unpublished observations), indicating that the TNS9 vector could function continuously for over 2 years. Several groups subsequently generated variants of the TNS9 vector and also reported curative responses in murine models of beta-thalassaemia or sickle/beta-thalassaemia (reviewed in Persons 2004, Sadelain 2008, Sadelain 2007, Sadelain 2006). In view of the high performance of the TNS9 vector and the vast amount of data collected over several years with this vector, including extensive safety data summarised below, we selected the TNS9 transcription unit for clinical investigation. Philippe Leboulch's group in Paris evaluated a variant vector of TNS9 called β 87 (Bank 2005).

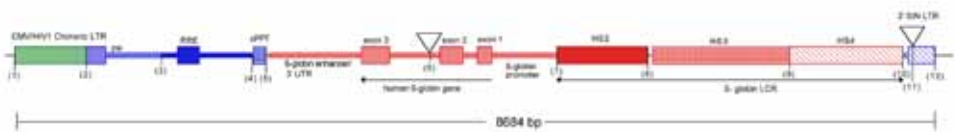


Figure 2. Schema of the integrated TNS9.3.55 globin vector. The vector encodes the human β -globin gene, including its promoter (p) and distal enhancer (e) and three fragments of the human locus control region HS2, HS3 and HS4. The inverted triangles represent deletions in the second intron of the human β -globin gene and in the enhancer/promoter region of the long terminal repeats. The β -globin gene is oriented in anti-sense relative to vector transcription.

Another critical aspect of designing and selecting a vector for therapeutic application is its safety profile. The major concern in this regard is the potential for “insertional oncogenesis”, which in its extreme form may lead to leukemia. Leukemia formation is caused by a combination of events involving the action of the vector on endogenous

oncogenes and the accumulation of additional mutations in the same clone. This complication has been observed in patients with severe combined immunodeficiency who were treated with HSCs modified with retroviral vectors comprising “long terminal repeats”, which are genetic elements including a promoter and enhancer that are active in all cell types. Globin vectors, in contrast, are erythroid-specific, i.e. they are not transcribed in HSCs themselves or in all hematopoietic lineages other than the erythroid lineage (Chang 2007). Furthermore, globin vectors strongly activate in late stage erythroid cells shortly prior to enucleation, a naturally occurring process in maturing erythroid cells that precludes oncogenic transformation and clonal expansion. These advantageous features of globin vectors notwithstanding, we conducted, as part of our Investigational New Drug (IND) application to the United States (US) Food and Drug Administration (FDA), extensive safety studies evaluating a series of TNS9-like vectors and control vectors in large cohorts of thalassaemic mice (unpublished observations). In nearly 300 recipient mice, including primary and secondary recipient animals, followed for an average 12 months and 20 months, respectively, we did not observe a single case of leukaemia. While such results cannot guarantee the safety of these vectors, the safety data are far superior to those obtained with vectors encoding long terminal repeats and as encouraging as can possibly be. Based on the above efficacy and safety data, in 2012 the FDA granted us the first approval in the US for a clinical trial investigating globin gene transfer in thalassaemia patients. This critical approval paved the way for other groups to obtain FDA approval for globin gene transfer studies for the treatment of beta-thalassaemia and sickle cell disease.

First Clinical Steps

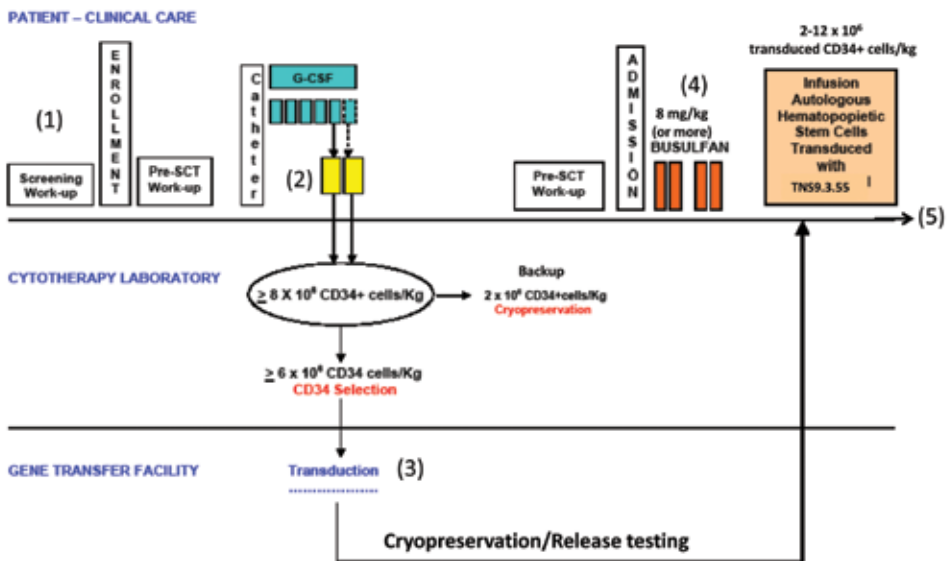
Multiple studies in several animal models have established that correction of anaemia and secondary organ damage due to iron accumulation is feasible using lentiviral vectors encoding a regulated human beta- or beta-like globin gene. These robust results strongly support the merit of transferring a human globin gene in autologous HSCs as a rational alternative to high-risk non matched-related donor transplantation in patients with severe beta-thalassaemia. Before proceeding to clinical studies, we decided to first assess the safety and feasibility of harvesting HSCs from thalassaemia patients and further utilise these thalassaemia patient CD34+ cells to optimise globin gene transfer under “current Good Manufacturing Practice” (cGMP) conditions. Performing gene transfer to patient HSCs following rigorous, FDA-approved conditions, within a purpose-built facility (which we have constructed and validated at MSKCC), is essential to properly implement this therapy.

We conducted a pilot trial to investigate the safety and effectiveness of mobilising CD34+ HSCs in adults with beta-thalassaemia major. A secondary objective of this clinical study was to assess whether these CD34+ HSCs could be transduced under cGMP conditions at levels sufficient for proceeding to a therapeutic clinical trial, utilising the TNS9.3.55 lentiviral vector. All five patients enrolled tolerated G-CSF well with minimal side effects, confirming prior mobilisation studies in paediatric (Li et al. 1999) and adult (Yannaki et al. 2012) thalassaemic subjects. All CD34+ cell collections achieved the minimum targeted dose of 8×10^6 CD34+ cells/kg following leukaphereses on days 5 and 6. Using clinical grade TNS9.3.55 vector stock, we demonstrated gene transfer in the range of 0.2 to 1.5 vector copies per cell in patient CD34+ cells in our optimisation studies, averaging 0.55 in three validations performed under cGMP conditions-corresponding to an excellent range for transduction efficiency with a high

expressing vector such as TNS9.3.55. The transduced CD34+ cells maintained their potential to engraft NOD/scid- γ cnnull mice, maintaining a stable vector copy number 6 months post-transplant (Boulad 2014). This validated procedure for stem cell collection and globin gene transduction was approved as part of our IND application and is now implemented in the first US trial to evaluate globin gene transfer in patients with severe inherited globin disorders (NCT01639690 at clinicaltrials.gov). Our risk/benefit analysis was supported by the Recombinant DNA Advisory Committee, which reviewed in detail our trial proposal and unanimously voted in favour (RAC meeting minutes - Biomedical Technology Assessment at the NIH 2006)

This clinical trial, which opened in late 2012, is enrolling adult subjects with transfusion-dependent beta-thalassaemia major who lack an HLA-matched donor. The treatment is based on the administration of autologous CD34+ hematopoietic cells transduced with the TNS9.3.55 vector, a lentiviral vector encoding the normal human β -globin gene. The cells are transduced ex vivo, frozen down, tested for transduction efficiency, sterility and other release criteria, and returned to the donor after reduced intensity conditioning (Figure 3). The ultimate goal is to restore the subject's ability to produce RBCs with an increased content of normal adult haemoglobin and achieve transfusion-independence. Short of this, a marked decrease in transfusion requirements would still represent a significant progress.

Figure 3. Schematic of the New York clinical trial. The trial proceeds in the following five key steps: (1) eligibility review and informed consent; (2) CD34+ cell mobilisation and collection; (3) globin gene transfer and biosafety assessment; (4) conditioning and engineered CD34+ cell infusion; (5) post-infusion monitoring.



The essential safety end-points of this and other comparable studies is to assess the safety and tolerability of autologous CD34+ cells that have been transduced with TNS9.3.55 (Table 1). This is achieved by carefully measuring the level of engrafted HSCs, which is reflected in the vector copy number in blood cells, and the multiplicity

of HSCs that contribute to blood cell formation at different time points, which is achieved by high throughput analysis of vector integration sites. The latter provides important information on the possible emergence of clonal expansion, which reflects massive expansion of a single cell and may be a prelude to leukemic transformation. Such an occurrence has been observed in one thalassaemia patient treated with the globin vector $\beta 87$ at the Necker Hospital in Paris, France. In this case, a single clonal progenitor cell bearing the $\beta 87$ vector the integrated into an endogenous gene termed HMGA2, provided the largest fraction of genetically modified myeloid and erythroid cells [Cavazzana-Calvo 2010]. These expanded cells contributed to about a third of total haemoglobin one year into the therapy, with another third coming from haemoglobin F induced after transplantation and the last third corresponding to the patient's own haemoglobin E. In aggregate, these three haemoglobin sources added up to 9-10 g/dl and rendered this patient trans-fusion-independent – a positive outcome that, in view of the low level of gene transfer, would not have occurred without the unexpected emergence of a single transduced clone. Fortunately, this clonal expansion has not progressed to leukaemic transformation and the patient remains healthy 5 years into his therapy. The use of this vector has now been discontinued.

Table 1. Key end-points in globin gene transfer and other engineered stem cell-based clinical trials.

EFFICACY EXPECTATIONS	Peripheral blood gene marking >10% (myeloid cells)
	Polyclonal haematopoietic reconstitution
	Transfusion-independence
	Long-term benefit, ideally life-long
SAFETY CONCERNS	Conditioning toxicity (mucositis, neutropenia, bleeding, amenorrhea)
	Immune response to vector components
	Recombination of a replication-competent lentivirus
	Clonal expansion, which may precede leukemic transformation

Beyond the intrinsic efficacy and safety attributes of the vector itself, the therapeutic responses is largely determined by three key parameters: the CD34+ cell dose, the level of globin gene transfer in the infused CD34+ cells, and the conditioning regimen administered to the patient prior to CD34+ cell infusion. The latter is a short course of cytotoxic chemotherapy that is necessary to promote the engraftment of the infused CD34+ cells. A key question is whether a maximum dose, known as myeloablation, is required, or whether a reduced intensity conditioning, associated with lesser toxicity and more rapid hematopoietic recovery, is sufficient. The Paris study utilised the former; the TNS9.3.55 study in New York is evaluating the latter. There is broad consensus that a non-myeloablative conditioning regimen would have many advantages, including decreased toxicity, rapid hematopoietic recovery and shortened hospitalisation, but it may not be sufficient to ensure optimal engraftment. Thoughtful studies will be needed to determine if a more “gentle” conditioning enables a therapeutic engraftment in thalassaemia patients.

Summary and recommendations

Globin gene transfer in autologous CD34+ cells is beginning to be evaluated in thalassaemia patients. The $\beta 87$ study opened first in 2008 but, due to low efficiency gene transfer, vector genomic instability and the occurrence of a concerning clonal expansion, the clinical evaluation of this vector has been discontinued. After completing additional in-depth work to optimise CD34+ cell collection, improve vector production and increase gene transfer efficiency in thalassaemia patient CD34+ cells, we submitted the first protocol to the US FDA, which granted us approval to evaluate the TNS9.3.55 vector in 2012 (**Figure 4**). As per FDA recommendations, the current study is restricted to adults; paediatric patients will be included at a later date after reviewing safety and efficacy data obtained in adults. The MSKCC trial functions as an international consortium, including (but not limited to) collaborating centers in Italy, Greece, Lebanon and Thailand (Sadelain 2010). Other studies based on globin gene transfer are expected to start treating patients in 2014 or later in the US and France (Genetix Pharmaceuticals, now Blue Bird Bio, with a vector replacing $\beta 87$), in Milano, Italy (MolMed), Cincinnati, USA (Children's Hospital), Memphis, USA (Saint Jude Hospital) and Los Angeles (UCLA). The next 5 years are poised to determine the safety, efficacy and ultimate potential of this therapy. It is encouraging that simpler vectors, which do not afford regulated or high level transgene expression, have shown promising clinical results in some immunodeficiency syndromes (Boztug 2010, Aiuti 2009, Hacein-Bey-Abina 2002, Cavazzana-Calvo 2000) and metabolic disorders (Biffi 2013, Cartier 2009). However, the challenge is greater in thalassaemia, as in sickle cell disease, where complex vectors with lower titers are evaluated in adult patients rather than the paediatric patients treated for these other disorders.

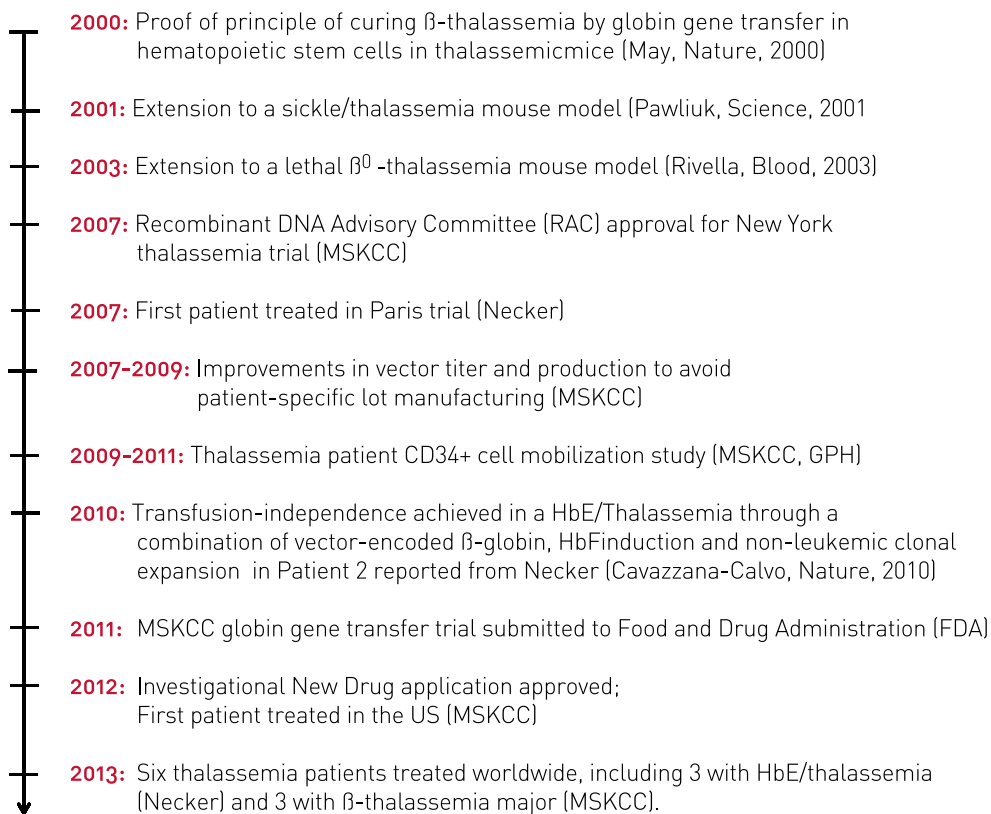


Figure 4. Milestones in the evolution of Globin Gene Therapy. The field opened up following the report by May et al. in 2000, which demonstrated the feasibility and efficacy of globin gene transfer in a mouse model of beta-thalassaemia. Six patients were treated by 2013, three in Paris, France, at Hôpital Necker, and three in New York, USA, at Memorial Sloan-Kettering Cancer Center.

At the same time, some new technologies are emerging, which will build on and extend the globin gene transfer experience. Retroviral-mediated gene transfer in bone marrow, mobilised peripheral blood, or umbilical cord blood HSCs is efficient but subject to the vagaries of semi-random vector integration. The inclusion of genetic elements with enhancer-blocking and chromatin barrier activity may enhance the performance and safety of current vectors (Emery 2011). An alternative to globin gene transfer is to depress the gamma-globin gene by targeting the BCL11A transcription factor (Uda 2008). This approach would not require high-level transgene expression, but its efficacy, alone, is unproven. The inability to propagate primary HSCs available in limited numbers precludes the screening of vector insertion sites prior to therapeutic cell infusion. Two alternative approaches are emerging to circumvent this limitation (Riviere 2012). One is to target globin gene delivery, using a targeted nuclease to increase the efficiency of gene delivery (Li 2013) – which, however, remains sub-clinical to date. This approach may be used for gene repair or for gene addition to a predetermined site, ideally a validated genomic safe harbor (Sadelain 2012). The other is to capitalise on

the proliferative potential of pluripotent stem cells, from which HSCs may be derived in principle – although this goal too remains elusive at the present. As uncertain as these new approaches may be in the present, it is heartwarming that further options are being developed for the cure of beta-thalassaemia, should lentiviral-mediated globin gene transfer not fulfill its enormous potential.

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15

PSYCHOLOGICAL SUPPORT

Authors Robert C. Yamashita, L Lauren Mednick and Dru Haines
Reviewer John Porter

The need for continuity of care and psychological support for chronic disease is widely accepted (Falvo 2014, Lubkin 2014), as is the negative impact of psychological issues on chelation adherence in thalassaemia major (Porter 2011, Evangelini 2010, Panitz 1999, Beratis 1989). This chapter will (1) provide a comprehensive review of the published social and behavioral 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.

However, there is a surprising lack of published evidence for psychological support interventions in thalassaemia. A 2001 Cochrane Review of psychological therapies for thalassaemia (Anie 2001), assessed as “up-to-date” in 2011, 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 behavior, primarily with adherence to iron chelation therapy (ICT), is a significant variable in long-term outcome (Efthimiadis 2006, Borgna-Pignatti, 2004, Porter 2002, Modell 2000, Olivieri, 1994).

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 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 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 characterization of adult patients (Galanello 2003) and a cross sectional patient survey (Vardaki 2004). A small cluster of subsequent articles looked at “psychological burdens” in different patient groups including children and caregivers (Prasosmuk 2007, Aydinok, 2005), adolescents (Roy 2007), and adults (Mednick, 2010, Gharaibeh 2009). A single, non-randomised interventional study in 2009 used cognitive behavioral family therapy to try and alter adherence to chelation therapy (Mazzone 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 “behavioral 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. The challenges when implementing clinical intervention within complex heterogeneous populations have not been fully considered however. A few articles have addressed elements of this complex environment (Vichinsky 2005) by looking at the economics of ICT (Payne, 2007; Riewpaiboon 2010), clinical outreach to the communities of affected patients (Choy 2000), and addressing the needs of culturally different patients (Banerjee 2011) (**TIF grade: C**).
- Iron Chelation Therapy (10% of articles): most of these investigations either measure adherence (Matsui 1994), or assess patient experience with treatment (Porter 2012, Taher 2010, Payne 2007) (**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 to assess the patient reported health outcome as one step in understanding the patient’s ICT practices (Porter 2012, Porter 2011, Sobota 2011, Evangelii 2010, Mednick 2010). 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 behavioral 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 experiencing psychiatric problems (Cakaloz 2009, Saini 2007, Shaligram 2007a, Shaligram 2007b, Aydinok 2005, Pradhan 2003, Sadowski 2002). 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 (Duman 2011, Zafeiriou, 2006, Armstrong 2005, Monastero 2000) (**TIF grade: B**). Angastiniotis points out that the problem of observed psychological problems in thalassaemia could actually be a function of the levels and kinds 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 (Canastan, 2003, Ratip 1996, Ratip 1995) and has continued with other studies (Tsiantis 1996, Zani 1995). This domain appears to have the most interventional studies that include targeting changes in institutional organization practices (Marovic 2008), patient group sessions (Marovic 2008, Yamashita 1998), family therapy (Mazzone, 2009), and patient chelation camps (Treadwell 2001). While these reports suggest some success, they all lack a robust analytic assessment (TIF grade: C).

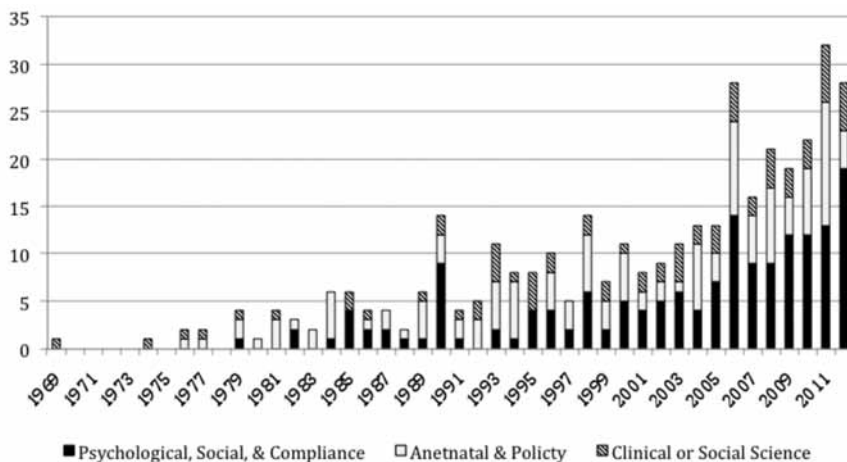


Figure 1. 1979-2012: BSSR articles on psychological aspects of thalassaemia by type. A comprehensive database of the available literature was constructed from title & abstract searches of thalassaemia (thalassaemia) in a number of bibliographic databases: PubMed, biological abstracts, pscyINFO, CINHALL, 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 impact emotional functioning and may result in increased vulnerability for experiencing symptoms of psychiatric illnesses, such as depression and anxiety (Duman 2011, Gharaibeh 2009, Marovic 2008, Prasomsuk 2007, Roy 2007, Zafeiriou 2006, Aydinok 2005, Vardaki 2004, Galanello 2003, Angastiniotis 2002, Politis 1998, Ratip 1996, Ratip 1995). Psychological support appears to be loose reference to a broad mix of organizational 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 bold: F). The few, small interventional studies are descriptive reports of clinic-level responses (TIF bold: C). They lack analytic rigor because standardised behavioral and social science research instruments were not used. Recent reports show an effort to develop the needed rigorous, scientific understanding of patient reported outcome within on-going studies of iron chelation therapy (Haines 2013, Porter 2012, Trachtenberg 2012a, Trachtenberg 2012b, Porter 2011, Sobota, 2011, Trachtenberg 2011, Evangelii 2010). Most are designed to inform a clinical response to underlying clinical problems. These efforts should establish the analytic 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 organizational model. As the specific challenges associated with being a patient with thalassaemia differs 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 organization of clinical medicine. Pediatrics 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**.

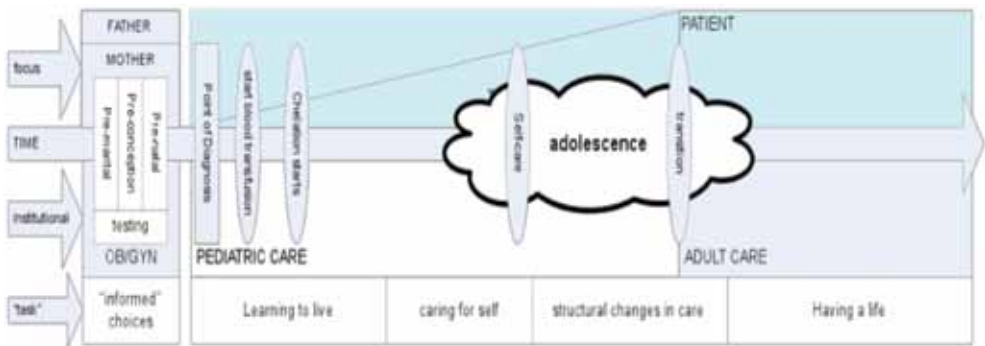


Figure 2. Clinical pathway diagram.

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 following 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, reorganization) (Drotar, 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 (Politis, 1998; Galanello, 2003; Yamashita, 1998). Importantly, if parents feel overwhelmed with caring for their child, effective management of the illness may become compromised (Otsuki, 2010). To minimize 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 behaviors 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 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 (Edwards 2010, Thompson 2009, Brown 2007, Hayman 2002, Brown, 1999, Hymovich, 1992). 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 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 behavior during an invasive medical procedure accounted for 53% of the variance in child distress behavior (Frank 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 2008). In fact, a recent study conducted with patients with thalassaemia found that bubble blowing during an injection helped reduce anxiety (Bagherain 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 2000), likely due to refocusing the child's attention onto 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., MRI). Fostering trust, reducing uncertainty, correcting misconceptions, enhancing the belief in their ability to cope with a procedure, and minimizing distress are some of the potential benefits in providing advance information about a procedure to a child (Jaaniste 2007; Jipson 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 (Burns-Nader 2012, Bandstra 2008, Bolig 1991, McCue 1988).
- Time for the child to ask questions.

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 behavior, unpredictability), this may not be true for some children in this age group (Fiese 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 fostering good adherence by minimizing several of the problems often associated with adherence difficulties (e.g., forgetting, conflicts about when to take the medication) (Fiese 2005, Rand 2005).

Behavioral interventions which include increased monitoring and incentives for meeting goals have been shown to be successful at improving adherence in patients with thalassaemia (Koch 1993). The use of incentives may be particularly useful for pediatric 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 behaviors again in the future.

At various times along the clinical pathway, patients may struggle with chelation adherence (Evangelini 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 behavioral 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.
- Need revision 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
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 minimize 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
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

BARRIER	INTERVENTION
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

Additional opportunities for psychological support during childhood

As children with thalassaemia frequently miss school for medical appointments and transfusions (Gharaibeh 2009), which can negatively impact school functioning (Thavorncharoensap 2010), parents should be encouraged to educate the school about their child's conditions 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 (Duman 2011, Nevruz 2007, Economou 2006, Zafeiriou 2006, Armstrong 2005, Lucke 2005, Zafeiriou 2004, Monastero 2000). 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.

Adolescence and transition to increased self-care

Adolescence is a time when adherence to daily medical regimens often declines (Trachtenberg 2011). Frequently the transition of responsibility from the parent to 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 2010, Treadwell 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 2011) is because of insufficient psychosocial support as patients transition from pediatric 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 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 pediatric to adult medicine clinic. Further, a well-coordinated transitional plan should be developed, which includes:

- Opportunities to orient the patient to an adult clinic and the adult care system.
- Overlapping visits with pediatric and adult hematologists.

An emerging concern that is common in adult patients with thalassaemia is the experience of pain (Haines 2013, Trachtenberg 2010). The presence of pain in the non-thalassaemia adults is associated with decreased social function and increased depression (Ozminkowski 2012, Garber 2010, Avlund 2007, Dunn 2004, Koenig, 1997, Burckhardt 1985). Clinicians should encourage patients with pain to engage in a variety of empirically validated (Shega 2012, Palermo 2010, Eccleston 2009) cognitive and behavioral 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. Effective pain management includes a combination of pharmacologic and non pharmacologic approaches including and not limited to:

- Deep breathing
- Guided imagery
- Progressive muscle relaxation
- Hypnosis
- Biofeedback

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 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.

Psychosocial support throughout the lifespan as part of standard care

As social and emotional concerns can occur anywhere along the clinical pathway and such concerns can impact the patient's quality of life, as well as 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 multidisciplinary team approach, which include nurses, social workers and psychologists who meet with the patient and families on a regular basis as part of their standard care. These clinicians are best suited to assess for any social, emotional, or cognitive concerns and intervene with additional support when necessary. This could be especially useful for getting patients who experience significant symptoms of psychiatric disorders such as anxiety and depression, engaged in psychotherapy early on in an effort to prevent long-

term health consequences. Importantly, by including psychological support as part of standard care, some of the stigmatization associated with seeing a therapist may be removed.

Summary and Recommendations

Overall, despite a general lack of large scale, randomised, controlled trial evidence conducted with patients with thalassaemia, there are innumerable cohorts of case-controlled analytic studies to suggest that psychological well-being impacts 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 outcome shows that oral chelation therapy has a beneficial impact, relative to parenteral chelation **(B)**.
- Neuropsychological investigation of cognitive deficits show 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 organization 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 organizational support for multidisciplinary teams is essential **(A)**. There is a growing body of evidence that highlight the problems associated with transition from pediatric 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 specializing in thalassaemia care **(C)**.
- Psychological support should be tailored to the patients 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 support to treatment adherence. The use of established behavioral 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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16

LIFESTYLE AND QUALITY OF LIFE

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Patients with optimally treated thalassaemia can now enjoy a near-normal life and lifestyle, and experience regular physical and emotional development from childhood to adulthood. According to the WHO definition, 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 life. This is achieved by recognising the limitations that the disease imposes but also the effect that the treatment regimens have on the patients' lifestyle, and the time that these treatments steal from normal living. Recognition of these needs comes with knowledge of all aspects of the disease, with experience, 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, 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 quality and 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 will aim to provide a foundation from which healthcare professionals can provide 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 program?
- 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 assumed to be influenced primarily by the degree of anaemia, and cardio-circulatory and pulmonary function, which are key to oxygenation of tissues. Additionally, in a chronic condition such as thalassaemia, other co-morbidities may be present, such as cardiac and endocrine dysfunction, as well as chronic hepatitis. These factors have been investigated in several studies, which have in the most part focused on the contribution of respiratory and cardiovascular function on exercise tolerance. Ergometry was used in the majority of studies (mainly cycle ergometry or treadmill), with respiratory function tests to measure aerobic capacity (e.g. VO₂max) 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, amongst others (Nanas 2009, Vasileiadis 2009, Marinov 2008). 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 2013), and even restrictive lung dysfunction (Piatti 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 quality of treatment. A universal and all-encompassing guideline is therefore not useful, and an approach of individualised tests and advice according to comprehensive clinical assessment (which should include ergometry) is more practical and more widely applicable. 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 are evident in the heart and endocrine glands. For this reason, even though routine daily activity is unrestricted, exercise tolerance should be assessed – preferably at regular intervals. We have seen from previous chapters that annual cardiological assessment is already recommended (see **Chapter 7**) for all thalassaemia patients after the age of 8-10 years. If athletic activity is contemplated, then ergometric tests must be included.

In addition to the above, consideration must be given for all co-morbidities, including bone disease which is common at all ages in thalassaemia patients. In addition to pain, this may limit mobility, and 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 privileged and 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 (Enerca in-print). This should dispel any doubts concerning the ability of patients to achieve an education. The main limiting factor expressed by patients is the need to interrupt educational sessions in order to meet clinic and transfusion appointments, which are most often during working hours. This feedback should be taken seriously when planning services (see **Chapter 17**). 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 2010). These issues require educational intervention for teachers, and may require the involvement and assistance of the thalassaemia centre. Ultimately, thalassaemia patients must be treated in the same manner as any other students, and should never be regarded as 'weak' members of the class, or society.

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 in print), 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 decision. 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. In a TIF survey (unpublished 2009) involving patients from the Middle East, 80% (of 96 patients) stated that they had difficulty getting a job mostly because of reluctance, or in some cases even blatant refusal by employers. The reasons given include repeated absences, again reinforcing the point that clinic visits during working hours may play a role, but also 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 2007) clearly states in Article 27 that state parties 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 individuals ability according to the same protocol used in assessment for exercise and sports but with special cautions if heart disease or osteoporosis are present.

Marriage and Reproductive Life

Getting married is widely accepted as a key goal in ones life, and thalassaemia patients have a good record in forming relationships. In Cyprus, from a presentation of the Nicosia Thalassaemia Centre [Christou 2012], of 284 patients over the age of 18 years, 52% were married, whilst 4% were divorced. In the same group of patients, 78% had children, while 194 children had been born to thalassaemic mothers. These figures may be in contrast to thalassaemia populations where the majority of patients are children, and where stigmatisation limits patient opportunities to form stable relationships. The role of the treatment team in this respect is to ensure that from an early age that patients are seen by an endocrinologist to avoid hypogonadism as much as possible and to initiate treatment early, so that any delay or absence in sexual development is avoided, as detailed in Chapter 8 and **Chapter 9**. The team should also provide general support and encouragement, and the thalassaemia service has a duty to coordinate the multidisciplinary team which oversees the thalassaemic mother 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. 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 2004]. The results showed that increasing caloric intake significantly increased insulin growth factor I (IGF-I), skin fold thickness, mid arm circumference and BMI, thus at least partially improving growth. This observation is consistent with other studies [Fuchs 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 2012]. In addition, there are many reports of vitamin and micronutrient deficiencies in thalassaemia, which may also affect growth [Fung 2010, Claster 2009]. It is important therefore to look at look at some of the important vitamin and minerals in more detail.

Zinc

Zinc is an essential element which in thalassaemia can be either removed by iron chelating drugs [Erdogan 2013] as well as from inadequate dietary intake, poor absorption [Fung 2012], or increased urinary loss with prolonged use of thiazide diuretics. Zinc deficiency has been shown to affect growth and sexual maturation, and 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 a recent Cochrane Review on zinc supplementation in thalassaemia and sickle cell disease (Swe 2013) it was concluded that despite some effect of supplementation on height velocity, there is no evidence from randomised controlled trials to indicate benefit with regards to serum zinc level in thalassaemia patients. The authors did recommend further trials examining this question, and a subsequent randomised, double-blind and placebo controlled trial (Fung 2013) of young patients (10-30 years) with low bone mass, zinc supplementation did result in significantly greater increases in bone mineral content and areal bone mineral density. Monitoring zinc levels, especially in patients on regular chelation with Deferiprone, is recommended and if indicated supplements should be prescribed. Zinc supplementation is usually delivered in the form of zinc sulphate, although other formulations are also available. The usual dose is 125mg 1-3 times daily, although doses of 220mg 3 times daily have been quoted for haemoglobin disorders. Caution is however needed for high doses, as toxicity can occur - including gastrointestinal irritation, as well as interactions with other minerals and drugs.

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, in patients who are transfused, oral intake becomes more significant at low pre-transfusion levels. Globally, many transfusion dependent patients do not receive blood transfusions before haemoglobin levels fall to 6 or 7 g/dl, and iron absorption may rise up to 5 g/dl/day. In this group of patients 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.

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 from 85% (Mirhosseini 2013) to 100% (Soliman 2008). Vitamin D deficiency is even found in sunny environments, where the majority of patients with thalassaemia live (Nakavachara 2013). Low consumption of calcium (Fung 2012) and hypercalciuria (Quinn 2011) that are found in thalassaemia patients contribute to the disturbance in calcium homeostasis, particularly if hypoparathyroidism is present. The effects of vitamin D deficiency are well known. Increased calcium and phosphorus absorption in the gut, and regulation of parathyroid hormone levels can help increase levels (Wacker 2013). Deficiency results in poor bone mineralisation, which contributes to thalassaemic bone disease. Deficiency is also associated with muscle weakness, and more importantly can affect the heart muscle, causing left ventricular dysfunction associated with cardiac iron uptake (Wood 2008).

Vitamin D and Calcium supplementation is recommended for all patients at a dose of 2000IU/day (Fung EB 2011). It is also suggested that vitamin levels are monitored every 6 months in thalassaemia patients (Nakavachara 2013, Fung 2011). A diet high in calcium, including milk, cheese, and oily fish is also recommended.

Folic acid

Patients on high transfusion regimes 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 homocystein levels and atherosclerosis (Qin 2012), the possibility of providing folic acid supplements at up to 1mg/day to all patients may be considered. The chances of toxic effects are low.

Vitamin E

Vitamin E is a fat-soluble vitamin which is often depleted in thalassaemia patients. The main reason is that iron load in the liver, with the associated liver damage, results in a reduction of serum lipids (Livrea 1996), although reduced dietary intake has also been demonstrated (Fung 2012). Supplements of vitamin E have been shown to reduce oxidative stress in thalassaemia (Pfeifer 2008) and to reduce lipid peroxidation of red cell membranes (Sutipornpalangkul 2012). However these trials, using 400 IU/day, were for relatively short durations of treatment and with small patients numbers. Prolonged use, especially at high doses, has potential dangers and more extensive trials are therefore needed 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, cornoil, 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 due to the following:

- 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. The increased availability of chelatable iron allows desferrioxamine to excrete more iron. In order to avoid toxicity, the vitamin is given at the time of desferrioxamine infusion at a dose not exceeding 2-3mg/kg. This benefit is not seen with the other chelating agents.

Supportive Treatments

Various substances, often derived from herbal sources, have been proposed to enhance treatment in thalassaemia. These often draw 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 50mg/kg/day resulted in the following benefits:

- Improved diastolic function and improvements in exercise performance.
- Significant improvement in pulmonary artery systolic pressure in patients with

- pulmonary hypertension (El-Beshlawy 2008)
- An increase in transfusion intervals (El-Beshlawy 2007)

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 (Benvenega 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 2010).

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 2013, Caciapuoti 2013, Polyak 2013). These benefits may be of use in thalassaemia patients who have liver damage from iron overload, and many are infected by hepatitis C. It is available in capsular form and usually dosed at 140mg three times a day.

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 as well as being 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 bring 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 discussions around these issues. Substance abuse will 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 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 Haematology Association mentions the following: "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 2012).

How then is quality of life assessed? The concept of quality of life involves each patient's perception of their own life and wellbeing, and since wellbeing includes psychological and social functions, which in turn are influenced 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 2005).
- The PedsQoL Generic Core Scales (Clarke 2010).
- The Dartmouth Primary Care Cooperative Information Chart System (Pakbaz 2005).
- The Short Form Health Survey- SF36 (Musallam 2011, Sobota 2011).

More recently, the Specific Thalassaemia Quality of Life Instruments (STQOLI) have been developed and validated. The STQOLI, has been published in Greece to take into account experiences such as the dependency on regular iron chelation by various regimes (Lyrakos 2012). TranQol has also been developed in Canada (Klaassen 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 2013). These instruments can be used to monitor and evaluate individuals, as well as groups of patients, thus allowing them to evaluate clinic performance, and identifying 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 the state of care between different geographical regions. Variables include the disease severity of patient groups (Musallam 2011), past management of patients, the onset of complications, whether on oral versus parenteral chelation (Porter 2012), the age of patients, and whether parents or children are responding (Coacci 2012). Monitoring patient groups over time using the same instrument can, however, provide invaluable data on measures of outcome and clinic performance.

Summary and Recommendations

- A holistic approach to patient care includes recognition of the need for social integration and a 'normal' life.
- The treating physician should be able to provide advice on lifestyle issues.
- Physical activity should be encouraged but the condition of each individual patient should be recognised and co-morbidities identified. Ergometry and cardiovascular assessment may be necessary according to the activity proposed.
- Clinic and transfusion times should be flexible and take into consideration the patient's commitments, such as their education and work.
- Liaison with teachers and employers to provide understanding of the condition and its management may be necessary.
- 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 may be helpful in this respect.
- Zinc supplements may be given in cases of deficiency, poor growth and reduced bone mass, but are not recommended as routine. The usual dose is 125mg of zinc sulphate 1-3 times daily.
- Dietary iron restriction is recommended in patients on low transfusion regimens with low pre-transfusion haemoglobins.
- Calcium and Vitamin D supplements are recommended for all patients at a dose of 2000 IU, along with measurements of vitamin D levels every 6 months. A diet high in calcium is also recommended (through milk, fish, cheese etc.).
- Folic acid supplements of up to 1mg/day are needed for all patients with low haemoglobin levels. Supplementation for all patients may be considered, since the risk of thrombosis may be reduced and toxicity low.
- 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.
- Vitamin C supplements are recommended, in conjunction with Desferrioxamine infusions at a dose of 2-3mg/kg/day, or if deficiency is proven.
- L-carnitine may be beneficial at a dose of 50mg/kg/day, although caution should be exercised in patients with thyroid dysfunction.
- There is insufficient evidence on any long term benefits from wheat grass.
- Silymarin at a dose of 140mg three times daily is recommended if liver involvement is detected, and on consultation with a hepatologist.
- Routine dental care is a must for all thalassaemia patients. Adequate blood transfusions from an early age will prevent maxillary deformities and reduce the need for orthodontic interventions.
- Consumption of alcohol, tobacco and substance abuse should be avoided.

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17

ORGANISATION AND PROGRAMMING OF THALASSAEMIA CARE

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In many hospitals patients with thalassaemia are managed in general haematology, paediatric and oncology inpatient departments and day care units, alongside a heterogeneous groups of patients with a variety of unrelated disorders. It is not uncommon to have adult patients being transfused alongside children in many centres. This may be justified when patient numbers are small, but in areas of high prevalence, separate units were created many years ago in recognition of the need for patient privacy and safety, and to facilitate multidisciplinary care (Angastiniotis 1988). An ideal thalassaemia centre may share space and services with other red cell disorders such as sickle cell disease and the more rare congenital and chronic anaemias, since they share common complications and needs. The key is that these units be dedicated but not isolated. This chapter shall examine how healthcare systems can be best organised to deliver optimal care to patients with thalassaemia.

The Multidisciplinary Team

The multi-organ involvement seen in thalassaemia and other transfusion dependent anaemias has been made clear in these guidelines, and to a great degree it is these complications that dictate the composition of the multidisciplinary team. It is expected that a haematologist, or an experienced paediatrician or internist will supervise the provision of basic care to these patients (see **Table 1**), including the monitoring of iron overload and assessment of organ damage that inevitably result. The support team should include the following:

- **Specialised nurses**
 - The important and wide-ranging responsibilities and competences of haemoglobinopathy nurses include the supervision of blood transfusions, practical aspects of iron chelation therapy, patient support and communication, provision of information, encouragement of self management, and symptom control, amongst others (Anionwo 2012, Aimiwu 2012, Tangayi 2011). To develop the kind of expertise required there is need for continuity of care and not the frequent rotation of staff that is often witnessed in hospital services. The specialist nurse is an asset to the haemoglobinopathy service, representing the closest contact to the patient, and usually acting as liaison between the patient and medical team.
- **Cardiologist**
 - In view of the importance of cardiologic monitoring and timely management of heart complications (see **Chapter 4**), the cardiologist forms a central figure in the team. In many centres, the patient is often referred to a cardiologist only once symptoms manifest. It is strongly recommended that a cardiologist with specialist knowledge of thalassaemia care becomes a regular member of the team. Heart complications are closely linked to issues of control in haemoglobin levels, iron overload, iron chelation, endocrinopathies, nutritional factors and

other issues in this multi-organ condition. It is therefore important that cardiology colleagues involved in the care understand the broader issues of concern, and are able to discuss these not only with colleagues on the same team but also with patients. For these reasons, the cardiologist should be kept well informed on issues such as patient compliance and psychosocial states, to permit them to contribute to the complete care of the patient. Cardiologists with special interest in thalassaemia should therefore be identified and invited to supervise monitoring and treatment of patients in close collaboration with the team.

- **Hepatologist**

- Although heart complications remain the primary cause of death, improved control of cardiac complications with early intervention has resulted in an older thalassaemia population and increasing development of liver complications, which are responsible for an increasing proportion of morbidity and mortality. Management of liver disease is also complicated by the presence of iron overload, with or without the contribution of chronic viral hepatitis (Di Marco 2010). Matters such as the role of intensifying iron chelation, controlling haemoglobin levels when anti-viral agents are used, and dealing with the complications of anti-viral treatments make it imperative that the team work in close collaboration with the hepatologist.

- **Endocrinologist**

- Endocrine complications are almost universal in thalassaemia patients. They affect quality of life as well as having serious consequences to physical wellbeing (see **Chapter 8**). It is therefore important from an early age that all transfusion dependent patients be reviewed by an endocrinologist to supervise all treatment that may be necessary. An international group of experts in the endocrinological aspects of thalassaemia has been set up in recent years, which encourages and trains endocrinologists in thalassaemia care (De Sanctis 2013). The importance of the endocrinologist in the multidisciplinary team is wide-reaching, as illustrated by the psychological impact of endocrine disorders such as delayed puberty and the need for frequent liaison of the team.

- **Diabetes specialist**

- The prevalence of diabetes is high in thalassaemia and rising as patients grow older, reaching rates as high as 20% (see **Chapter 8**). Although this is usually dealt with by the team's endocrinologist, the benefits of a dedicated diabetic clinic which has its own multidisciplinary team may be advantageous. Recently in the UK, a joint diabetes/thalassaemia clinic has been functioning with success and may be recommended as being of added benefit to thalassaemia patients (Tzoulis 2013).

- **Psychologist**

- The complexities of psychosocial support are explained in **Chapter 15**. The need for presence of a psychologist on the team should not require further emphasis. The role of the psychologist is also to support and advise the care team, including the patients' families. All relevant staff need training in dealing with chronic diseases, especially as they are frequently asked for advice well in advance of being seen by a professional psychologist. In addition, the feeling of helplessness in managing an incurable illness may lead to emotional exhaustion, and so discussion with the care team should be part of the psychologist's role in the centre. Psychiatric interventions are not frequently needed but teams should be alert to this possibility and make prompt referrals when necessary.

- **Social worker**
 - The role of a social worker frequently overlaps with that of a psychologist. There are however specific problems that arise in the family, financial and social settings which fall clearly in the realm of the social worker, depending on the role of the social and welfare system in each country. It is the role of the care team to decide whether there is a need for input from social workers according to the individual circumstances of the case, and to ensure their presence when appropriate.
- **Obstetricians**
 - In cases of pregnancy, obstetric specialists should be involved and collaborate closely with the thalassaemia team from the stage of pre-pregnancy counselling right through to labour and post-partum management (see **Chapter 9**).

Table 1. Summary of roles, desired characteristics and responsibilities of members making up the thalassaemia care team.

SPECIALTY	DESCRIPTION
Haematologist/Paediatrician/ Internist	Usually the physician in charge of routine care who also coordinates the whole team
Specialised nurses (haemoglobinopathy nurses)	Supervision of transfusions, patient education, support, and symptom control. Ensures continuity of care
Cardiologist	Preferably with special interest in haemoglobin disorders. Monitors all patients from childhood and takes charge of treatment when complication arise. Liaison with other team members on iron chelation needs
Endocrinologist	Ideally with a special interest in haemoglobin disorders. Monitors all patients from early adolescence through to adulthood. Suggests individual treatment of complications and acts as liaison with the whole team, as well as with gynaecologist in case of infertility or pregnancy
Liver specialist (hepatologist)	The liver specialist is called in when the need arises, often when hepatic viral infections require treatment
Obstetricians	Liaise with the haematology team mainly during pregnancy, which requires multidisciplinary care
Psychologist and social worker	Essential supportive services for patients and families. Provide education and support to staff in providing holistic care
Dentist	Routinely monitor for dental and maxillary complications and provide early intervention when needed
Pharmacists	Knowledge of the haemoglobinopathies will allow informed pharmacists to be advisors to the team on drug therapy and to ensure the ready supply of essential drugs

SPECIALTY	DESCRIPTION
Dietician	Represent infrequent members of the team, as the primary members are able to deal with dietary needs. Professional dietetic input may help in answering queries and giving advice when complications relating to diabetes and liver disease necessitate specialist advice

It is crucial that teams are well coordinated, and this is the role of the primary haematologist or other physicians in charge of basic therapy and care. For the team to fulfil its role there should be frequent meetings and shared decision making, with each specialty contributing its expert view on the clinical and psychosocial issues raised by individual cases, but also concerning the group of patients under their care. According to EH Wagner (Wagner 2000), the team's responsibility begins with a protocol or guideline that defines the components of high quality care. Other functions of the team include:

- Treatment planning. This should take into consideration patient preferences. Concordance between the team and the patient may improve patient adherence (Haynes 2002).
- Evidence based management and the availability of decision support tools so that all staff follow consistent protocols.
- Self-management support. This requires information, guidance and encouragement to the patient by various specialties.
- Offering joint interviews with patients when a new complication arises or a change of treatment is needed. For example, when a heart complication is detected, a common interview with the haematologist and cardiologist involved will be informative and also reassure the patient that there is continuity of care and experts have discussed their management and come to agreements on decisions regarding their care.
- Assurance to the patient that teamwork is not theoretical, but that follow up by one specialty results in shared information by all groups and disciplines involved, so that decisions take into account all relevant organ systems and consequences to the patient.

Programming of Treatment

The general organisation of a thalassaemia unit is illustrated in **Figure 1**. Visits to treatment centres are frequent and related to preparing and conducting blood transfusions, reviews by doctors - including various specialist consultations, conduction of specialised tests, as well as visiting other specialist units such as magnetic resonance imaging centres. Complying with the appropriate follow up is therefore time consuming for patients, and can interfere with other important activities - such as attending school and work. This is compounded by the fact clinic hours often conflict with working hours; a point which has been highlighted by patients at international meetings. In a survey conducted by TIF and published in the Enerca White Book (in print), of 415 patients from across Europe almost 20% stated that they have to wait over 2 hours for transfusion to be prepared, and 62% transfused in the mornings - inevitably leading to their missing

school or work. These considerations should be taken into account in a patient centred service aiming towards greater patient integration. To achieve a better service, the thalassaemia centre needs to closely collaborate with hospital administration in order to arrange afternoon, evening or weekend transfusions and consultations. The centre should also build a close cooperation with the blood bank and other laboratories to provide out of hours services, to better cater for the needs of thalassaemia patients. The ideal day care centre should be designed to allow for privacy - for adult patients in particular.

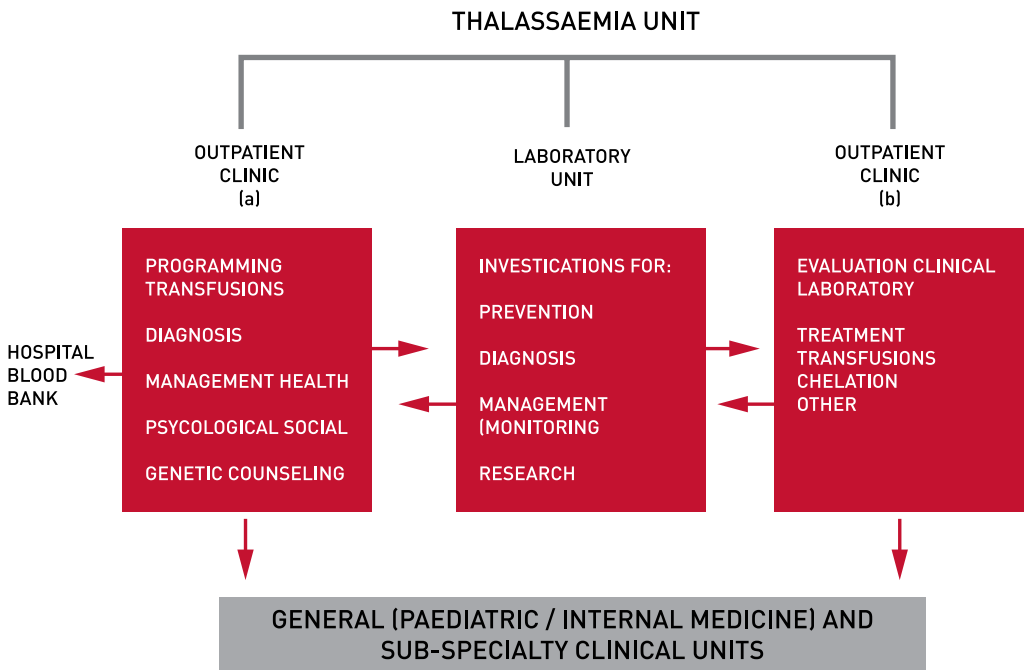


Figure 1. An example of organisational interaction of the thalassaemia unit with other hospital facilities. Reproduced with permission from (Kattamis 1989).

Reference Centres

According to the number of patients that they serve, thalassaemia centres may be divided into reference or secondary/peripheral centres. A reference centre should achieve internationally recognised standards for expert centres. Such standards include:

- The quality criteria for centres of expertise for rare diseases, recommended by the European Union Committee of Experts on Rare Diseases (EUCERD). Among the 16 main criteria that EUCERD proposes (EUCERD Recommendations on Quality Criteria for Centres of Expertise for Rare Diseases in Member States 2011), it is stated that a centre of expertise should bring together, or coordinate within the specialised healthcare sector, multidisciplinary competences/skills including paramedical skills and social services. They should collaborate with patient organisations to bring in the patients' perspective. They should contribute to elaboration of good practice guidelines, contribute to research and provide

education and training to healthcare professionals.

- The Chronic Care Model (CCM) developed in the United States (Epping-Jordan 2004). This model includes the promotion of self-management and changes in the organisation of care delivery - one example could be the timing of clinic visits, as suggested above. The use of evidence based practice guidelines must be integrated into a decision support system, which can assist the staff in following the various protocols. In addition, clinical information systems to organise data and to develop patient registries are strongly recommended.
- Accreditation of healthcare centres by an internationally recognised body, such as the ISO Technical Committee, the ISQua and the Joint Commission International (JCI), would also be an added asset.

Networking of Centres

An additional requirement is that reference centres should network with secondary or peripheral centres and offer clinical support. This is also a strong recommendation of the EUCERD group (EUCERD Recommendations to the European Commission and the Member States on European Reference Networks for Rare Diseases 2013). It is not possible to maintain all the expertise and technology required for thalassaemia care, as described in this book, in all centres within a country, especially if small numbers of patients are involved. Networking, which includes sharing of patient information, providing monitoring technology and advising on clinical decisions, is an important service of the reference centre. These aims can be achieved through periodic visits of patients to the reference centre. **Figure 2** provides evidence that patients managed at or in close collaboration with a reference centre (through networking) have improved survival (Forni 2009).

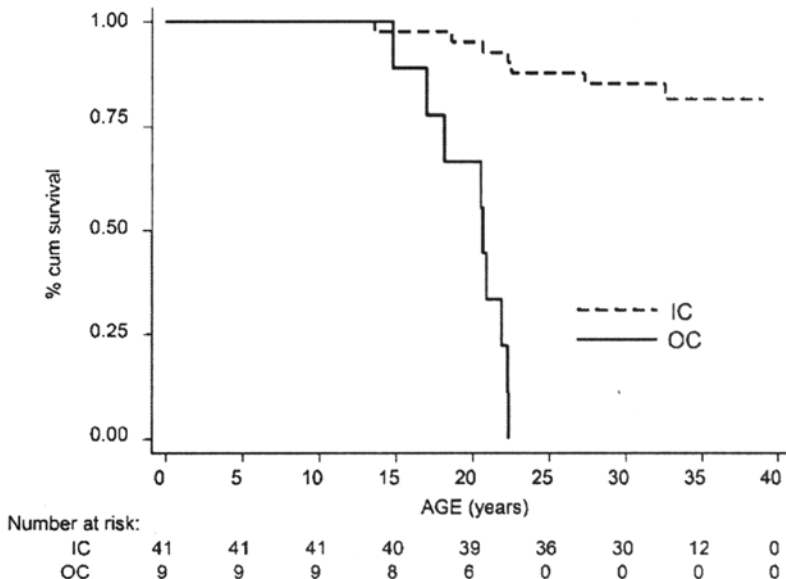


Figure 2. Kaplan-Meier overall survival curves of patients referred to specialised centres (IC) versus patients referred to nonspecialised centres (OC). Log-rank P-value <0.0001; hazard ratio of OC versus IC adjusted for sex (Cox model): 18.1, 95% confidence interval = 4.7-69.0; P<0.001. Reproduced with permission from (Forni 2009). IC, specialised centre; OC, non-specialised centre.

Summary and Recommendations

A thalassaemia centre should provide the following:

- Day care with access to inpatient facilities if needed.
- Facilitates equal access to quality care for every thalassaemia patient. This may be achieved through networking of centres.
- Close collaboration with necessary services, such as the blood bank and other laboratories.
- Follows evidence based guidelines / standards, providing comprehensive and holistic care.
- Actively involved in research.
- Close collaboration with patient support groups.
- Provides advocacy to health authorities for service development and patients rights.

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GENERAL TIMETABLE FOR CLINICAL AND LABORATORY EVALUATION

Category	Measurement	Check every (months)				
		1	3	6	12	24
Baseline information	Haemoglobin fraction					
	DNA mapping (alpha and beta)					
	Red blood cell phenotype (at baseline)					
Blood Transfusion	Volume of packed red blood cells transfused			X	X	
	Coombs (direct)					
Growth and Development in children	Height		X			
	Sitting height		X			
	Weight		X			
	Bone age				X	
	Growth velocity			X		
	Tanner Stage			X		
Body measurements in adults	Height				X	
	Weight		X			
Haematology	CBC	X				
	Coombs (direct)					
Iron	Ferritin		X			
	Liver iron				X	
	Audiology evaluation				X	
	Ophthalmology evaluation				X	
	Iron, TIBC					
	Transferrin saturation					
Liver function and disease	AST, ALT, γ GT		X			
	Bilirubin (total)		X			
	Bilirubin (direct)		X			
	Hepatitis A serology				X	
	Hepatitis B serology				X	
	Hepatitis B PCR				X	
	Hepatitis C serology				X	
	Hepatitis C PCR				X	
	PT, PTT					
	Albumin		X			
	Liver MRI				X	
	Ultrasound				X	
	Fibroscan					X
	Liver biopsy					
Periodic	Chemistry panel urea, creatinine		X			
	Urinalysis			X		
	Zn levels			X		
	Dental				X	
Endocrine	T3, free T4, TSH				X	
	PTH				X	
	Calcium, ionized calcium		X			
	Fasting glucose		X			
	Glucose tolerance test				X	
	IGF-1, IGF BP-3					
	LH-ICMA					
	FSH					
	Estradiol					
Osteoporosis	DEXA					X
	Vitamin D level			X		
Cardiology	ECG				X	
	ECG Holter					
	Exercise ECG (Ergometry)				X	
	Echocardiography				X	
	CMRI T2*				X	

As clinically indicated	Initially	Comments
	X	
	X	
	X	
X		
		After the age of 10 years
		Every 1-2 weeks for those taking Deferiprone
X		
X		From the age of 8 years. If there is evidence of hepatitis, histology is necessary.
X		
X		
X		
		Monthly if on deferasirox
X		If serology if positive. When hepatitis is active, do this test as per international protocol.
X		If serology if positive. When hepatitis is active, do this test as per international protocol.
X		Do this before a liver biopsy. Patients with active hepatitis should have the test more frequently.
X		From age 8 years. If there is evidence of hepatitis, histology is necessary.
X		
X		
		Monthly if on Deferasirox
		Start at 5 years.
		Start at 5 years.
		Start at 5 years.
		Start at 5 years.
X		Perform Annually after the age of 10 years
X		Perform at noted growth delay.
X		Perform at noted delay in puberty:12 years for girls and 14 years for boys
X		Perform at noted delay in puberty:12 years for girls and 14 years for boys
X		Perform at noted delay in puberty:12 years for girls and 14 years for boys
X		
X		
X		
X		From age 8 years

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ABOUT THALASSAEMIA INTERNATIONAL FEDERATION

The Thalassaemia International Federation (TIF) is a non-profit, non-governmental organization, founded in 1987 by a small group of patients and parents representing mainly National Thalassaemia Associations in Cyprus, Greece, UK, USA and Italy - countries where Thalassaemia was first recognized as an important public health issue and where the first programmes for its control, including prevention and clinical management had started to be promoted and implemented.

MISSION

Promotion of control programmes and access to quality treatment for every patient with Thalassaemia where ever he or she may live”.

OBJECTIVES

The objectives of the Federation in addressing effectively the needs of the world Thalassaemia family have since its establishment remained the same and include:
The creation of new and the promotion of existing National Thalassaemia Patients / Parents Associations; encouraging, motivating and supporting studies and research for further improving prevention strategies, clinical care and for achieving the long-awaited final cure;



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