

# A Short Guide for the Management of Transfusion Dependent Thalassaemia (TDT)



## **Prepared by:**

Farmakis, D.  
Angastiniotis, M.  
Eleftheriou, A.

Based on Cappellini, MD., Cohen, A., Porter, J., Taher, A., Viprakasit, V. (2014). eds., Guidelines for the Management of Transfusion Dependent Thalassaemia. 3<sup>rd</sup> edition. Thalassaemia International Federation: TIF Publication No. 20.

## **Reviewer:**

Cappellini, MD



**THALASSAEMIA  
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\* This publication is part of TIF's educational programme, aiming to ensure that physicians and other health professionals have all the necessary knowledge to best treat their patients.

## FOREWORD

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The Thalassaemia International Federation (TIF) has for many years produced guidelines for the clinical management of Transfusion Dependent Thalassaemia (TDT) with the clear aim to present treating physicians with a guide for their daily practice in the management of TDT, based on current scientific evidence, and assist them in making clinical decisions that will be most beneficial for the patient. The first edition of these guidelines was published in the year 2000.

Guidelines for thalassaemia management give emphasis to standards concerning basic treatment, which involve blood transfusion and iron chelation, on account of the fact that good clinical practices in these areas of thalassaemia management have led to the increased patient survival in the last few decades. However, as patients grow in age, systemic complications affecting vital organs, including heart, liver and endocrines, may become more common and should be prevented and appropriately treated at the very onset. With the aim to always achieve the best possible outcomes for all patients, guidelines also include instructions on diagnostic and monitoring procedures on the whole spectrum of complications given that in chronic diseases such as thalassaemia, good clinical practice includes the prevention of complications, and the need for different specialists to collaborate within a multidisciplinary team.

Maintaining good standards for clinical practice is not always possible in under-resourced countries and so national standards need to be developed to reflect the specifics of the environment they will be implemented in. However, a universal standard is also needed to form the basis of practitioners' and health services' decisions. TIF is acutely aware of the fact that standards of care vary across the world and that many, if not the

majority of the global patient population does not benefit from optimum care. By providing a short pocket guide describing the best possible practices in the treatment and management of TDT, TIF seeks to support physicians worldwide in making good clinical decisions that can benefit individual patients and enable them to advocate for more and better resources for thalassaemia care at the level of national health authorities. This effort is also supported in TIF's educational programme, aiming to ensure that physicians and other healthcare professionals have all the necessary knowledge to support their patients.

The guidelines published herein is the joint effort of two teams of experts, who respectively (1) pulled together, carefully assessed the latest scientific evidence on TDT treatment and management, currently available in international literature, and developed recommendations on the topic; (2) reviewed and finalised the guidelines, ensuring the validity and quality of the content.

The third edition of the original full-length guidelines was published in 2014 in the form of a long textbook and include a full review of the evidence supporting each recommendation, which physicians can consult in order to know all the facts underlying their clinical decisions. As an alternative to the long version, difficult to use on a daily basis, the present volume is meant to serve as an easy-to-carry pocket reference for physicians in their daily consultations. Physicians reading this book should keep in mind that a shortened version of guidelines, such as the present one, can only summarize and, therefore, may not be exhaustive of all clinical circumstances.



It is up to each individual physician to use his/her best judgement while interpreting the guidelines for each patient, based however, on the principles included herein. Physicians are encouraged to revisit the long version of the guidelines for additional facts and a thorough review of the evidence supporting each recommendation included in this manuscript. TIF hopes that this manuscript will be of useful and practical support for physicians around the world during the daily clinical management of TDT, and will have a global impact on the improvement of practices within treatment and management of TDT in the long-run.

On behalf of the Board of Directors,

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## **\*\* Guide for Interpreting Summary Recommendations**

The Latin characters I, II, III indicate the Class of Recommendation of the reviewed literature.

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

The letters A, B, C indicate the level of evidence available in the reviewed literature, i.e., the strength of the results described in the guideline.

- A: Data is derived from multiple well-designed randomized controlled trials or meta-analysis with a number of participants of sufficient statistical power.
- B: Data is derived from at least one large well-designed clinical trial with or without randomization, from cohort or case-control analytic studies, or well-designed meta-analysis.

## Chapter 1 GENETIC BASIS, PATHOPHYSIOLOGY AND DIAGNOSIS

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  $\alpha$ -,  $\beta$ -,  $\gamma$ -,  $\delta$ -,  $\delta\beta$ -, or  $\epsilon\gamma\delta\beta$ -thalassaemias.

The phenotypic classification of thalassaemia syndromes based on clinical severity and transfusion requirements is outlined in Figure 1 below:

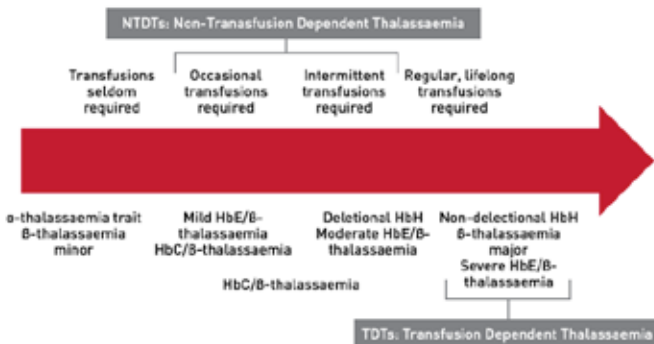


Figure 1: Types and transfusion requirements in Transfusion-Dependent and Non-Transfusion-Dependent Thalassaemia

### The genetic basis of $\beta$ -thalassaemia

The basic defect in  $\beta$ -thalassaemia is a reduced or absent production of  $\beta$ -globin chains with relative excess of  $\alpha$ -chains. The end result is the extensive premature destruction of red cell precursors in the bone marrow, which is referred to as “ineffective erythropoiesis” and is the hallmark of  $\beta$ -thalassaemia, as well as to peripheral haemolysis, hence ultimately leading to anaemia, typically presented with

hypochromic microcytosis. The first response to ineffective erythropoiesis and anaemia is an increased production of erythropoietin, causing a marked erythroid hyperplasia, which may, in turn, produce skeletal deformities, osteoporosis, and occasionally extramedullary masses, and contribute to splenomegaly. Further consequences include growth retardation, increased iron absorption and cardiac dilatation and failure, which is still the main cause of death.

### Pathophysiology

The pathophysiology of the disorder is summarized in Figure 2:

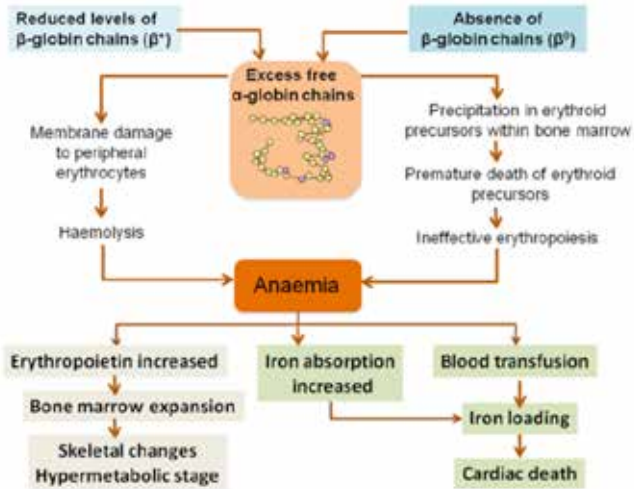


Figure 2: Pathophysiology of  $\beta$ -thalassaemia

The clinical presentation of  $\beta$ -thalassaemia major usually occurs between 6 and 24 months of age with severe microcytic anaemia, mild jaundice, and hepatosplenomegaly.  $\beta$ -thalassaemia intermedia should be suspected in individuals who, at a later age, present similar but milder clinical findings.

## Diagnosis

The diagnostic algorithm for individuals with hypochromic microcytosis is summarized in Figure 3, while the diagnostic criteria for thalassaemia and other haemoglobinopathies (or Hb Disorders) are outlined in Figure 3.

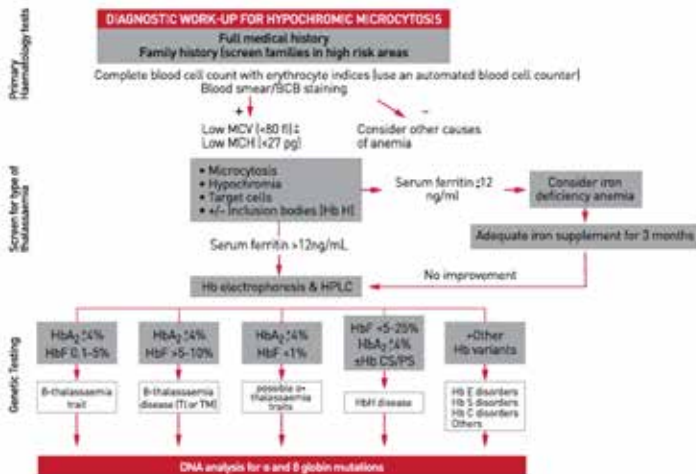


Figure 3: Diagnostic algorithm for hypochromic microcytosis

## Diagnostic measures for thalassaemia and other haemoglobin variants

		$\beta$ -TM	$\beta$ -TI	HBE/ $\beta$ -Thal	HbH	
Hb levels		<5 g/dL	~7-10 g/dL	Mild Moderately Severe Severe	9-12 g/dL 6-7 g/dL 4-5 g/dL	2.6-13.3 g/dL
BLOOD SMEAR	Low Hb production	Red cell hypochromia microcytosis, Target cells				
	Haemolysis	Irregularly crenated RBC, increased reticulocytes (5-10%)				
	Ineffective erythropoiesis	Nucleated RBC, Basophilic 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 ↓	HbF 10-50% (up to 100%) HbA2>4%	HbE [40-60%] HbF [60-60%] ±Hb A (with $\beta$ -thal) HbA2 ↓	Variable HbH [0.8-40%] HbA2 ↓ + the presence of $\alpha$ -variants i.e. Hb CS, Hb PS etc.		
DNA analysis	<ul style="list-style-type: none"> <li>Common known mutations of both <math>\beta^D</math> and <math>\beta^E</math>-thal mutations in population specific set can be done by PCR based methods</li> <li>For rare or unusual mutations, a direct sequencing or array analysis required</li> <li>Other analysis for <math>\beta</math>-TI included <math>\alpha</math> and <math>\beta</math>-globin rearrangements, Xmn I polymorphism and other QTLs for <math>\gamma</math>-globin expression</li> </ul>				Gap-PCR developed for 7 common $\alpha$ -thal deletions and RDB for non-deletional mutations For unknown mutations, Southern blotting or MLPA analysis and sequencing required	

*Figure 4: Summary of diagnostic measures for thalassaemia and other haemoglobin variants*

For a more detailed, laboratory diagnostic workout, please refer to: Cappellini, MD., Cohen, A., Porter, J., Taher, A., Viprakasit, V. (2014). eds., Guidelines for the Management of Transfusion Dependent Thalassaemia. 3<sup>rd</sup> ed. Thalassaemia International Federation: TIF Publication No. 20. Available online at <http://thalassaemia.org.cy/publications/tif-publications/>.

### **Pillars of Clinical Management of $\beta$ -thalassaemia:**

1. Blood Transfusion
2. Iron Chelation
3. Multidisciplinary Care - mainly but not limited to:
  - a. Heart
  - b. Liver
  - c. Endocrine
  - d. Infection

### **Goals of Blood Transfusion Therapy**

The major goals of blood transfusion therapy are the following:

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

### **Quality and Adequacy of Blood**

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

### **Whom to Transfuse**

The decision to start blood transfusion therapy is based on the following:

- Confirmed diagnosis of thalassaemia.

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

### **Recommended Blood Product**

Patients with thalassaemia major (TM) should receive leukoreduced packed red blood cells with a minimum haemoglobin content of 40g.

Reduction to  $1 \times 10^6$  or less leucocytes per unit is taken as the critical threshold for eliminating adverse reactions attributed to contaminating white cells.

Pre-storage filtration of whole blood is the preferred method for leukoreduction.

### **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.
- *Cryopreserved (frozen) red cells* are used to maintain a supply of rare donor units for patients who have unusual red cell antibodies or patients who are missing common red cell antigens.
- *Red cells obtained by donor apheresis* (collection of two units of red cells from the same donor for transfusion of the same patient) may decrease the risk of transmission of



infections, as well as the risk of developing alloimmunization and other transfusion-related complications, but creates significant logistical and organizational problems.

- **Neocyte transfusions** (using only the younger fraction of red cells) may reduce transfusion requirements but increase the cost and the risk of infections and alloimmunization.

### Storage of Donor Red Cell Units

The anticoagulant preservative solutions used in blood collection have been developed to prevent coagulation and to permit the storage of red cells without losing 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). The maximum duration of storage, as noted on each unit varies according to the type of preparation, as shown in Table 1 below:

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

Table 1: Maximum duration of storage of CPD, CP2D, CPDA-1, CPD, CP2D, CP2D or CPDA-1 with AS-1, AS-3, AS-5

### Compatibility Testing

The development of one or more specific red cell antibodies (alloimmunization) is an important complication of chronic transfusion therapy.

It is recommended that:

- Before embarking on transfusion therapy, patients should have an 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 immunization later on.

- 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 alloimmunization against these antigens.

### **Transfusion Programmes**

The recommended treatment for TDT involves lifelong blood transfusions on a regular basis, usually administered every two to five weeks to maintain a ***pre-transfusion haemoglobin level of 9-10.5 g/dl*** and achieve a ***post-transfusion haemoglobin of 14-15 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.

A higher target pre-transfusion haemoglobin level of 11-12 g/dl may be appropriate for patients with heart disease, significant extramedullary haematopoiesis or patients who do not achieve adequate suppression of bone marrow over-activity at a lower haemoglobin level.

The use of different anticoagulant-preservatives and additive solutions complicates recommendations regarding the ***volume of transfused red cells*** (Table 2). 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. ***For younger children***, the following calculation is generally used:

**(Desired - actual Hb) x weight x 3/haematocrit of transfused unit = ml to be transfused**

Most transfusions of 2 or 3 donor units are administered over 3-4 hours.

The guidelines for calculating how much blood to transfuse is summarized in Table 2 below:

		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

Table 2: Calculation of the amount of blood to transfuse

The relationship between annual blood requirements and rate of daily iron loading is depicted in Table 3 below:

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

Table 3: The relationship between annual blood requirements and iron loading

## Transfusion and the Spleen

Transfusion requirements in non-splenectomized patients are generally higher than in splenectomised patients. Specific thresholds of annual transfusion requirements that would lead to consideration of splenectomy as a valid treating option are difficult to establish, but in cases where annual transfusion requirements rise above 200 ml/kg/year of pure red cells, splenectomy may be considered as one out of several strategies to reduce the rate of iron-loading.

## Adverse Reactions

Blood transfusion exposes the patient to a variety of risks and adverse effects, which are listed in the following:

- ***Non haemolytic febrile transfusion reactions*** were common in past decades, but have dramatically decreased due to leukoreduction.
- ***Allergic reactions*** are usually due to plasma proteins and range from mild to severe. Occasional mild allergic reactions can be prevented with the use of antihistamines or corticosteroids before transfusion. Recurrent allergic reactions can be 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 after a transfusion has been initiated and are characterised by the abrupt onset of fever, chills, lower back pain, a sense of impending death, dyspnoea, haemoglobinuria and shock, arising from errors in patient identification or blood typing and compatibility testing. 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 may require additional measures such as the use of 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.

- **Alloimmunization** occurs in 10-20% of patients with thalassaemia and is more common in children who begin transfusion therapy after 1-3 years of age than in those who begin transfusion therapy earlier. The use of extended antigen matched donor blood is effective in reducing the rate of alloimmunization.
- **Delayed transfusion reactions** usually occur within 5-14 days after transfusion and are characterised by unexpected levels of anaemia, as well as malaise and jaundice and are usually caused by an alloantibody not detectable at the time of transfusion or by 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 units.
- **Autoimmune haemolytic anaemia** is a serious complication of transfusion therapy that usually but not always occurs in patients with alloantibodies and more frequently in patients who begin transfusion therapy later in life. Steroids, immunosuppressive drugs and intravenous immunoglobulin and rituximab are used for the clinical management of autoimmune haemolytic anaemia.
- **Transfusion-related acute lung injury (TRALI)** is a potentially severe complication caused by specific anti-neutrophil or anti-HLA antibodies that activate patient's neutrophils or due to non-antibody related accumulation of pro-inflammatory mediators during storage of donor red cells. It is characterised by dyspnoea, tachycardia, fever and hypotension during or within six hours of transfusion, hypoxaemia and chest radiograph with bilateral infiltrates typical of pulmonary oedema. Management includes oxygen, 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 and is often fatal. It occurs within 1-4 weeks of transfusion and is characterized by fever, rash, liver dysfunction, diarrhoea and pancytopenia due to bone marrow failure. To reduce the risk, donated blood from a family member should be avoided or, if used, should always be irradiated before transfusion.
- **Transfusion-associated circulatory overload** may occur in the presence of cardiac dysfunction, or when the rate of transfusion is inappropriately fast. Signs and symptoms include dyspnoea and tachycardia, and the chest radiograph shows the typical 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 on Infections).

The broad categorisation of immune-mediated transfusion reactions and reported frequencies are presented in Table 4 below:

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		

*Table 4: Categorisation of immune-mediated transfusion reactions and reported frequencies*

## Summary recommendations 1

- 1) Confirm the diagnosis of thalassaemia and run appropriate clinical and laboratory tests for transfusion (IIA).
- 2) Use careful donor selection and screening, favouring voluntary, regular, non-remunerated blood donors (IIA).
- 3) Before the first transfusion, perform extended red cell antigen typing of patients, at least for C, E, and Kell (IIA).
- 4) On each transfusion, give ABO, Rh(D) compatible blood. Matching for C, E and Kell antigen is highly recommended (IIA).
- 5) 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).
- 6) Use leukoreduced 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).
- 7) Use washed red cells for patients who have severe allergic reactions (IIA).
- 8) 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).
- 9) Transfuse every 2-5 weeks, maintaining pre-transfusion haemoglobin levels above 9-10.5 g/dl or higher (11-12 g/dl) for patients with cardiac complications (IA).
- 10) Keep a record of red cell antibodies, transfusion reactions and annual transfusion requirements for each patient (IIA).
- 11) Keep the post-transfusion haemoglobin level below 14-15 g/dl (IIA).

### Pathophysiology of iron overload

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.

Table 5 below shows the rates of iron loading in the absence of iron chelation and in relation to the patient's weight.

Patient's weight	20kg	35kg	50kg	65kg
Pure red cells Vol. ml/year	2000-4000	3500-7000	5000-10000	6500-13000
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

*Table 5: Iron loading rates in the absence of chelation*

### Initiation of Iron Chelation treatment

- Iron chelation therapy usually starts once all of the following criteria have been met:
  - 10 - 12 transfusions or
  - Serum ferritin >1000 µg/l on two consecutive readings, the first taken two weeks before the second
  - Age > 2 years old
- For children transfused from a very young age, consideration might be given to starting earlier than this, if the serum transferrin saturation exceeds 90%, and/or when 1000 g of pure red cells have been transfused.



- According to licensing in Europe, children < 6 years old should initially be offered sub-cutaneous DFO infusions (while US labelling permits DFX from 2years of age). If there is failure of adherence or if the patient is intolerant to DFO, then DFX should be started as soon as possible to prevent worsening iron loading. The patient must be monitored very closely particularly in the first weeks after starting treatment.

## Requirements

### *Monitoring of transfusion iron overload*

Monitoring is essential for effective and safe iron chelation, tailored to individuals' specific needs.

- The age of starting regular transfusions and iron chelation therapy should be documented for each patient.
- An annual record of blood usage (ml/kg pure red cells) and daily iron loading (mg/kg/day) should be maintained for each patient.
- Serum ferritin (SF) - measured at least every 3 months (1-3 months). Target value is currently between 500-1000  $\mu\text{g/L}$ .

Measuring the trends in SF over a period of at least 3 months provides a more reliable indicator for adjusting therapy rather than the use of single values. When ferritin is  $>4000\mu\text{g/L}$ , it is much harder to see a trend in ferritin.

It is recognized that SF may not reflect total body iron levels or organ specific levels in some patients. SF needs to be interpreted together with LIC and myocardial iron since it fluctuates in response to inflammation, abnormal liver functions, and metabolic deficiencies. The ferritin trend is as valuable as a single ferritin reading. A falling ferritin is usually due to falling levels of total bod iron. By contrast, a persistently high ferritin or increasing ferritin levels could be due to inflammation or liver disease and sequential measurement of LIC will help avoid over or under treatment.

- Liver iron concentration (LIC) - (As measured by MRI based methods).

Liver iron concentration (LIC) should be assessed using an externally validated and standardised MRI technique. R2 (Ferriscan®) is preferable to R2\* because the methodology is more robustly standardised and has been licensed for use in routine clinical practice. MRI LIC methods should not be used interchangeably. In particular, sequential MRI LIC estimations in an individual patient should be done with the same methodology.

LIC of 3-7 mg/g dw is an acceptable therapeutic goal in TM patients. It is recommended that levels are kept towards the lower part of this range.

The frequency of LIC assessment should be guided by LIC and rate of change in LIC.

- Stable levels in the range 3-7 mg/g dw: Every one or two years
- Levels >7 mg/g dw: yearly
- Levels falling rapidly or <3 mg/g dw: 6 -12 monthly

LIVER IRON CONCENTRATION THRESHOLDS IN TRANSFUSIONAL IRON OVERLOAD	
LIC RANGE	CLINICAL RELEVANCE
0-17-18 mg Fe/g dw	Normal range in the healthy population
3.2-7.0 mg Fe/g dw	Suggested optimal range of LIC for chelation therapy in transfusional iron loading
7.0-15.0 mg Fe/g dw	Increased risk of complications
>15.0 mg Fe/g dw	Greatly increased risk of cardiac disease and early death in patients with transfusional iron overload

Table 6: Liver Iron Concentration Thresholds in Transfusional Iron Overload

- Myocardial iron (As measured by MRI based methods with a specific software T2\*).

Myocardial iron should be assessed by T2\* Cardiac MRI, using an externally validated protocol and software which

should undergo at least annual external calibration and other measurements to ensure validation of the measurement process.

*A grading scheme for assessing myocardial iron and guiding changes in chelation therapy.*

Risk (If untreated)	T2*
No cardiac iron, low risk of heart failure (HF)	≥ 20 milliseconds
Mild to moderate cardiac iron, low risk of HF	10 - 19 milliseconds
High cardiac iron, moderate risk of HF	6 - 9 milliseconds
High cardiac iron, high risk of HF	< 6 milliseconds

*Table 7: Standards for the Clinical Care of Children and Adults with Thalassaemia in the UK (3rd Edition, 2016)*

- The frequency of cardiac MRI scan should be guided by myocardial iron level for example:
  - Stable T2\* > 20 milliseconds: two yearly
  - T2\* 10-20 milliseconds: yearly
  - T2\* < 10 milliseconds: 6 monthly
  - It is particularly important to measure left ventricular function when cardiac iron is high (e.g. T2\* <10ms) as this is associated with a high risk of deteriorating function which requires urgent intensification of chelation (see below).
- LIC and myocardial iron should be monitored regularly in patients from age 9 or younger if they are able to tolerate MRI scanning without sedation.
- Other organ function and iron-mediated damage (tested as per recommendations in Appendix I): diabetes, hypothyroidism, hypoparathyroidism and hypogonadotropic hypogonadism.

## **Treatment of Iron Overload**

The aims of iron chelation therapy include:

### 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) in order to minimize the advent of iron-medicated complications.

### 2) Rescue therapy:

Once iron has accumulated as a result of frequent blood transfusions, more iron must be removed. Removal of storage iron is slow and inefficient because only a small proportion of body iron is available for chelation at any given point in time. Once iron has been 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, as shown by a falling Left Ventricular (LV) ejection fraction or if there is a clear downward trend in LV function, urgent action is required that usually involves intensifying the treatment within a hospital setting using continuous intravenous desferrioxamine, which may be combined with deferiprone.

### 4) Dose and regime adjustment of therapy:

Correct dosing and frequency of administration are critical to treatment success and require adjustment to changing circumstances. These can be identified through careful monitoring of iron overload and its distribution. Without monitoring the trends of iron load (liver iron and ferritin) and iron distribution (heart iron and function), patients are at risk of suffering from either a) under-chelation with increased iron toxicity; or b) over-chelation and increased chelator toxicity. The dosing and regime must be adjusted periodically to accommodate these factors.

### 5) Adherence to therapy:

Failure of chelation is most frequently due to infrequency of

dosing and so good adherence is essential. Furthermore, while intermittent high dose chelation can induce negative iron balance in some patients, it does not provide continuous protection from labile iron and also risks increased toxicity from the iron chelator. Poor adherence can result from practical issues such as difficulty with DFO infusions, intolerance of a particular chelator, or often from psychological / psychosocial or life-style issues. A key role that the treating centre needs to assume is the monitoring and encouragement of the patient's adherence, alongside support from their family and the patients. However, encouraging the patients to take control or 'self-manage' their conditions is often a useful approach of long-term benefit.

#### 6) Use of combination therapies

Combination therapies are not specifically licensed but have been widely used when iron overload or its consequences, such as cardiac iron, cannot be well controlled with single agent therapies. In principle, chelators can be combined either at the same time or following one another (sequentially) and there are theoretical advantages to each. In practice, the regime that is given often involves a conversation with the patient about the chelators they are best able to take regularly and how often. Various combinations of DFP and DFO are those where most experience has been accumulated. A typical regime would involve DFP at standard doses with subcutaneous DFO, as many nights a week as the patient is willing or able to self-administer the DFO.

- Other combinations have recently been evaluated in prospective trials, such as DFO with DFX (Aydinok *et al.*, 2015)<sup>1</sup> or DFP with DFX (Elalfy, M. S. *et al.*, 2015)<sup>2</sup>. No new toxicity issues were identified in these studies and these combinations may have value in removing iron in selected circumstances. These are discussed in the next pages.

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<sup>1</sup> Aydinok *et al.* (2015). Effects of deferasirox-deferoxamine on myocardial and liver iron in patients with severe transfusional iron overload. *Blood*, 125(25), pp. 3868-77.

<sup>2</sup> Elalfy, M. S. *et al.* (2015). Efficacy and safety of a novel combination of two oral chelators deferasirox/deferiprone over deferoxamine/deferiprone in severely iron overloaded young beta thalassaemia major patients. *European Journal of Haematology*, 95, pp. 411-20.

The licensed indications for commercially available iron chelators are summarized in Table 8 below:

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, 60 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	- Pregnancy (but has been used in 3rd trimester) - Hypersensitivity	- Pregnancy - History of neutropenia or condition with underlying risk of cytopenia - Hypersensitivity including Henoch Schönlein purpura: urticaria and periorbital oedema with skin rash	- Pregnancy - Hypersensitivity - Estimated creatinine clearance <60 ml/min - Hepatic impairment or renal failure

Table 8: Licensed indications for commercially available iron chelators

- DFX is indicated for children with NTDT > 10 yrs (not 6 yrs).
- For DFX, a film coated tablet is now available in several countries.
- For DFX, a mild to moderate skin rash is not an absolute contraindication for patients especially patients of Asian origins. This should be noted regarding DFX's hypersensitivity.

A liquid preparation of deferiprone is now available in the USA.

The main precautions during the use of licensed chelators are reviewed in Table 9 below:

CATEGORY	DFO [DESFERIOXAMINE]	DFP [DEFERIPRONE]	DFX [DEFERASIROX]
Precautions	<ul style="list-style-type: none"> <li>- Monitor ferritin: if it falls to &lt;1000 µg/L, reduce dose (so mean daily dose/ferritin remains &lt;0.025)</li> <li>- Monitor audiometry regularly, particularly as ferritin falls</li> <li>- Monitor eyes regularly including electroretinography if on high doses</li> <li>- Fever suggestive of septicemia with organisms that used ferrioxamine (yersinia, klebsiella)</li> <li>- Renal failure or diminishing renal function with other comorbidities</li> </ul>	<ul style="list-style-type: none"> <li>- Measure neutrophil count (ANC) before starting and monitor ANC weekly</li> <li>- For neutropenia: ANC &lt; 1.5 x 10<sup>9</sup>/L] interrupt treatment</li> <li>- For agranulocytosis [ANC &lt; 0.5 x 10<sup>9</sup>/L], consider hospitalization</li> <li>- Advise patients to report immediately symptoms of infection. Interrupt if fever develops</li> <li>- Monitor for symptoms of arthropathy</li> <li>- Monitor liver function regularly</li> <li>- No guidance on dose adjustment at low ferritin</li> </ul>	<ul style="list-style-type: none"> <li>- Monitor creatinine trends for 1st 4 weeks after starting or after dose escalation then monthly</li> <li>- If rapid fall in serum ferritin to &lt;1000 µg/L- dose reduce. If ferritin 500 µg/L consider very low doses<sup>a</sup></li> <li>- Proteinuria may occur: occasionally with renal tubular acidosis. Monitor urine protein regularly</li> <li>- 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]</li> <li>- Hypersensitivity reactions</li> <li>- Monitor liver function regularly</li> </ul>

Table 9: Main precautions during the use of licensed chelators

## Monitoring of chelation therapy

	DFO	DFP	DFX
Neutrophil count	Not required	Weekly during therapy	Not required
Creatinine	Not required	Not required	Twice before start, then weekly during first month after initiation and change of dose. Thereafter monthly
ALT	Monthly	Monthly	Twice before start, then 2 weekly for first month after initiation of therapy. Thereafter monthly
Urinalysis	Not required	Not required	Twice before start, then weekly during first month after initiation and change of dose. Thereafter monthly
Pure tone audiometry	Annual	6-12 monthly for combination DFO and DFP, not if used as single agent	Annual
Ophthalmology	Annual	6-12 monthly for combination DFO and DFP, not if used as single agent	Annual

Table 10: Current recommendations for toxicity monitoring, from the respective SPCs

Taken from "Standards for the Clinical Care of Children and Adults with Thalassaemia in the UK", 3<sup>rd</sup> Edition, 2016.



The main drug interactions concerning the licensed chelators are reviewed in Table 11:

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

Table 11: Main drug interactions concerning the licensed chelators

For best results, the chelation regime should be formally reviewed with the patient every 3 months, and decisions regarding dose adjustment or agents should take into account the following:

- Problems with adherence, as reported by the patient.
- Clinical evidence of adverse effects.
- Biochemical and haematological evidence of toxicity, although results of monitoring tests should be checked more often in case of substantial derangements.
- Trend in Serum Ferritin (SF).
- The annual monitoring tests for tissue iron (Liver Iron Concentration - LIC) and cardiac iron concentration using MRI).

- Past history of iron-related tissue damage, including liver, endocrine, and heart disease.
- For children and adolescents, additional monitoring of height centile, height velocity, and symptoms such as joint pains, stiffness or swelling.

Some guideline for suitable dose, frequency or regimen change for different levels of SF and varying levels of iron in the myocardium and liver are detailed below. It is important to understand that the **direction of change** in ferritin and liver iron levels, Cardiac T2\* is a very important tool for deciding what treatment to recommend.

1. **Adjustments to chelation with acceptable iron stores (SF consistently 500 - 1500 µg/l, LIC 3 - 7 mg/g dw, myocardial T2\* > 20 ms).** There is no indication to modify the chelation regime. A switch to DFX can be considered if adherence to DFO infusions is difficult.
2. **Adjustments to chelation with Increasing or high total body iron stores and no cardiac iron (or high absolute levels high > 1500 µg/l, LIC > 7 mg/g dw, myocardial T2\* > 20 ms).**

The aim of chelation therapy in this group is to reverse a rising trend in body iron overload (conformed by increasing LIC) and bring SF and LIC down to acceptable levels. Options would be as follows:

Patient on DFO:

- Optimise adherence.
- Optimise DFO dosage and infusion regime by:
  - Increasing the frequency of infusions to 6 or 7 times per week.
  - Increasing the duration of infusion to at least 12 hours.
  - Increasing DFO dose to 50 mg/kg per infusion, if an adult.
  - Consider switching to either DFX or to DFP/DFO combination.
- Patient on DFP or combined DFP/DFO:
- Optimise adherence.

- Increase DFP dosage up to 100 mg/kg/day and/or increase frequency and dosage of DFO.
- Consider switching to DFX.

#### Patient on DFX:

- It may take several years for SF and LIC to reach target levels in patients with severe body iron burden (ferritin >4000µg/L) (as with other chelation regimes) so the direction of change is very important. If there is a trend to decreasing SF and LIC, the current regime can be continued. The trend may not be clear when the SF is >4000µg/L and regular liver iron monitoring is recommended (*Porter et al, 2017*)<sup>3</sup>. Higher doses of DFX above 30 mg/kg/day should be considered if increasing trend of LIC and/or ferritin. Dose can be increased every 3 months within the licensed range (20-40mg/kg/day) in increments of 5 mg/kg.
  - If the patient is unable to take DFX regularly for reasons of tolerability (e.g., high serum creatinine) or adherence, consider switching to either DFO or DFO/DFP combination. In exceptional circumstances where other options are not possible, DFX/DFO combination can also be considered (*Aydinok et al, 2015*)<sup>4</sup>. The latter has been shown to produce a rapid decrease in liver iron stores.
- 3. Adjustments to chelation regime with moderately increased cardiac iron and satisfactory body iron stores (SF consistently 500 - 1500 µg/l, LIC < 7 mg/g dw, myocardial iron 10 - 20 ms).**
- If the cardiac T2\* is improving and the ferritin is also improving, the current regime can often be continued.

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<sup>3</sup> Porter et al, (2017). Limitations of serum ferritin to predict liver iron concentration responses to deferasirox therapy in patients with transfusion-dependent thalassaemia. *European Journal of Haematology*, 98(3), pp.280-288.

<sup>4</sup> Aydinok et al, (2015). Effects of deferasirox-deferoxamine on myocardial and liver iron in patients with severe transfusional iron overload. *Blood* 125, pp. 3868-77.

- If the cardiac T2\* is worsening, carefully examine patient's adherence. If the patient is unable to tolerate DFX or the patient is already on maximum doses, consider switching to DFP 75 - 100 mg/kg/day, seven days per week with escalated dose.

**4. Adjustments to chelation regime with moderately increased myocardial and high body iron stores (SF consistently > 1500 µg/l, and/or LIC > 7 mg/g dw, myocardial iron, Cardiac T2\* 10 - 20 ms)**

Considerations are similar to 3 above, except at higher levels of body iron, DFO-containing regimes are better tolerated.

Options to be considered will depend on the direction of the T2\* change (also ferritin and LIC).

- Escalating DFX at maximal dosage (35-40 mg/kg/day)
- Daily combination of DFO 40 - 50mg/kg, (initially at least 4-5 infusions per week) plus DFP 75-100mg/kg/day, seven days per week. The dose of DFP should be determined by the cardiac T2\* value.
- Intensive DFO chelation at 50-60mg/kg, 6-7 days per week with optimal adherence. Continuous subcutaneous (SC) or continuous IV DFO infusion through an indwelling venous device would also be options.

**5. Adjustments to chelation regime with severe and worsening myocardial iron loading (Myocardial T2\* < 10 ms), normal left ventricular function.**

- If iron stores are low (SF < 1000 µg/l, LIC < 7 mg/g dw), DFP monotherapy has usually been considered as first line therapy. The dose should be escalated to 100 mg/kg/day.
- If iron stores are high (SF > 1000 µg/l, LIC > 7 mg/g dw), DFO/DFP combination DFP monotherapy. DFP

can be given at 85-100 mg/kg/day and combined with DFO infusions (40 – 60mg/kg/day), either continuous IV, continuous SC, or intermittent SC infusions over 12 hours 5 – 7 times per week, with careful monitoring for adverse effects, depending on iron levels.

Under these circumstances, progressive improvements in mT2\* and iron load have also been demonstrated in prospective studies with DFX monotherapy at daily high doses (30-40mg/kg) where mT2\* is >6ms (Pennell, D. J., 2012)<sup>5</sup>.

Combinations of DFX with DFO has recently been reported to be effective and well tolerated in patients with low T2\* and high levels of body iron (Aydinok, 2015)<sup>6</sup>.

#### **6. Adjustments to chelation regime with severe myocardial iron loading (Myocardial T2\* < 10 ms), and low LVEF.**

- DFO at a dose of 50-60 mg/kg should be started immediately via a peripheral line and given as a continuous 24 hour IV infusion. A long-term intravenous line should be inserted to facilitate long-term therapy. Simultaneous addition of DFP (75-100mg/kg/day) should also be considered (*Porter et al, 2013*)<sup>7</sup>.

#### **7. Adjustments to chelation regime with falling iron levels**

- With DFO, chelator toxicity is more likely when body iron stores are either low (SF persistently < 1000 µg/dl and/or DFO mg/kg to serum ferritin ratio > 0.025, or when LIC is < 3 mg/g dw) or when SF is decreasing rapidly. This link is less clear with DFP and DFX but precautions should nevertheless be taken, particularly in SF < 500 µg/L).

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<sup>5</sup> Pennell, D. J., (2012). Deferasirox for up to 3 years leads to continued improvement of myocardial T2\* in patients with beta-thalassaemia major. *Haematologica*, 97, pp. 842-8.

<sup>6</sup> Aydinok, (2015) Effects of deferasirox-deferoxamine on myocardial and liver iron in patients with severe transfusional iron overload, *Blood*, 125, pp. 3868-77

<sup>7</sup> Porter, J. et al., (2013). Treatment of heart failure in adults with thalassaemia major: response in patients randomised to deferoxamine with or without deferiprone. *Journal of cardiovascular magnetic resonance*, pp. 15-38.

- When used alone, ferritin trends particularly at high levels > 4000µg/L can be misleading, and LIC monitoring is advised with rapidly falling ferritin trends to avoid over-chelation.
- Dose reduction is generally preferable to dose interruption (e.g. DFO 10 - 20mg/kg per infusion 5 per week; or DFX 5 - 10mg/kg/day, or DFP 50 - 75 mg/kg/day). While DFX licensing recommends interruption when SF is <500µg/dl, this may not be the best strategy in transfused patients as they will continue to load iron and Non-Transferrin-Bound Serum Iron (NTBI) will continue to be present.
- The chelator dose should be reduced if there is a rapid SF decline (of > 500 µg/dl over a three month period at absolute levels <2000 µg/L), and SF is persistently < 1000 µg/dl, or when LIC is <3 mg/g dw, and cardiac T2\* is > 20ms.
- With DFO monotherapy, it is the dose per infusion that should be reduced rather than the infusion frequency. With combination therapies containing DFO, the infusion frequency can be reduced.
- Conversion from DFO to oral chelation should be considered if SF is consistently in the range 500 -750 µg/l, or if LIC is < 3 mg/g dw.
- Special Monitoring at low iron loads:
  - when on DFO, patients should be closely monitored for audiometric toxicity. Retinal toxicity is rare, unless continuous infusion is given or doses are not reduced.
  - when on DFX,-, trends in serum creatinine and urine protein/creatinine ratio and ALT should be carefully followed.
  - when on DFP, increased monitoring of White Blood Count (WBC) and Alanine Aminotransferase (ALT) is advisable although formal studies are lacking.

## Iron chelation in special circumstances

### Pregnancy

- Planning pregnancies should be preceded by a period of intensive chelation to reduce SF, LIC and myocardial iron to optimal levels before attempting to become pregnant.
- In patients with a previous or current history of increased cardiac iron, monitoring of LV function and cardiac MRI is advisable during pregnancy.
- All chelator drugs should be discontinued as soon as the pregnancy is diagnosed.

When the risk of cardiac complications is judged to be high, DFO has been given in the final trimester (*Singer and Vichinsky 1999*<sup>8</sup>; *Howard, Tuck, Eissa, Porter, 2012*)<sup>9</sup>. Some centres recommend continuous IV DFO, 20 mg/kg/24 hours during labour to reduce the possible risk of dysrhythmia or cardiac failure during and after childbirth. There are insufficient human data in humans to recommend the use of DFP or DFX at any stage during pregnancy. However there have been several case reports of successful pregnancies without evidence of foetal toxicity with DFX during the early stages of pregnancy.

- Women of childbearing potential must be advised to avoid pregnancy while taking DFP, take contraceptive measures and immediately stop taking deferiprone, if they become pregnant or plan to become pregnant.
- A common practical approach for planned or assisted pregnancy is to stop oral chelators three months before likely conception, continuing with desferrioxamine until the time of ovulation.

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<sup>8</sup> Singer, S.T. and Vichinsky, E.P. (1999). Deferoxamine treatment during pregnancy: is it harmful? *American Journal of Hematology* 60, pp. 24-6.

<sup>9</sup> Howard J., Tuck S., Eissa A., Porter J. (2012). Haemoglobinopathies in pregnancy. In: H., Cohen and P., O'Brien, eds., *Disorders of Thrombosis and Hemostasis in pregnancy*. Springer - Verlag: London pp. 197 - 217.

## Renal impairment

- DFO should be used with caution in patients with renal impairment because the kidneys are one of the routes for excretion of DFO. At normal doses of DFO given intermittently, renal toxicity is unlikely but with continuous DFO regimes, increments in serum creatinine >25% have been reported in up to 60% of patients, independently of heart failure (El-Sharkawi, Davis, and Porter, 2008)<sup>10</sup>.
- DFO can be given to patients on dialysis as the DFO is eliminated in the liver as well as in the kidneys. In dialysed patients, the iron complexes formed in plasma can only be eliminated by dialysis so DFO should be ideally given on the days before dialysis three times a week.
- There are no data available on the use of DFP in patients with renal impairment. DFP is not reported to be nephrotoxic but should be used with caution in patients with renal failure because free drug, DFP metabolites and DFP iron complexes are excreted predominantly via the kidneys (Ferriprox™ SPC).
- An increase in serum creatinine greater than 30% is seen in about one third of patients and can usually be managed by dose reductions. DFX is potentially nephrotoxic and is contraindicated in patients with estimated creatinine clearance < 60 ml/min. Renal tubular acidosis has been occasionally identified in patients. All patients should have serum creatinine and urine protein/creatinine ratio monitored on each visit. Urine protein/creatinine ratio can oscillate so that regular monitoring is necessary to identify a trend and dose reduce accordingly.
- For patients with end stage renal failure on renal replacement therapy, it is reasonable to use DFX at low therapeutic doses (20 - 25 mg/kg), and also for patients on haemodialysis as both the free drug and its complex are eliminated by the liver.

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<sup>10</sup> El-Sharkawi, D, Davis, B. and Porter, J. (2008). The Effects of 24 Hour Continuous Deferoxamine Intensification on Serum Creatinine. Blood (ASH Annual Meeting Abstracts) 112 (1), p. 1851.



## Summary recommendations 2

- 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 (B)
- (3) Serum ferritin is an approximate marker of LIC, but real trends in body iron may be missed, particularly at high levels of iron overload (B)
- 4) Chelation therapy is an effective treatment modality in improving survival, decreasing the risk of heart failure and decreasing morbidities from transfusional iron overload (A).
- 5) 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 based on 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 that the centre performing the measurement uses a validated method, which has been independently calibrated (B).
- 13) Chelation can rapidly reverse iron mediated heart dysfunction (within weeks) by rapid chelation of labile iron, if 24h chelation cover is achieved (A).
- 14) Chelation therapy removes myocardial storage iron slowly compared with liver iron (A)

Heart disease constitute a major determinant of prognosis in patients with thalassaemia. Cardiovascular complications of thalassaemia can be considered in relation to iron overload (Figure 5). Prior to effective chelating and monitoring care, iron-related heart complications of thalassaemia were the leading cause of death in the western countries but also across the world. With appropriate chelation programmes, mainly, and although heart disease is no longer the case in the western world, it is still a problem elsewhere outside the western developed countries and remain one of the leading causes of morbidity across the world.

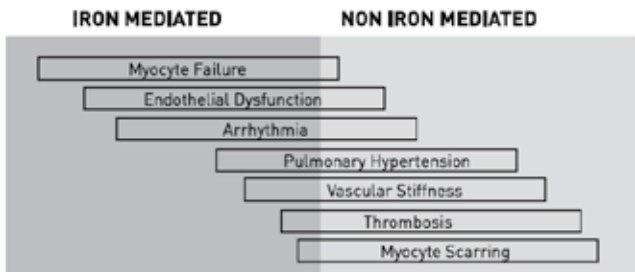


Figure 5: Cardiovascular complications of thalassaemia in relation to iron overload

### Cardiac dysfunction

#### Pathophysiology

##### Iron overload

- Cardiac iron accumulation is the single greatest risk factor for cardiac dysfunction in TDT. Cardiac iron loading occurs when the heart is exposed to high circulating Non-Transferrin-Bound Iron (NTBI) species for long periods of time. Once labile iron levels rise in the myocyte, they produce oxidative damage to cardiomyocytes, triggering cardiac dysfunction, arrhythmias and if not reversed, myocardial fibrosis.

- 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 goal, since, once symptomatic heart failure occurs there is a high immediate risk of death.

### *Other causes*

- In addition to iron, deficiencies in nutrients such as carnitine, thiamine, vitamin D and selenium as well as endocrine disorders such as hypothyroidism, hypoparathyroidism and hypogonadism can also contribute to cardiac dysfunction. Acute myocarditis can precipitate severe heart failure, arrhythmias and heart block, although this complication is not commonly seen in many countries.

### **Clinical Manifestations**

- Patients with considerable cardiac iron overload may remain free of symptoms for long. Symptoms are generally related to the degree of ventricular impairment, and may include left heart failure features (dyspnoea on exertion, orthopnoea, pulmonary rales) and/or right heart failure features (neck vein distension, hepatomegaly, peripheral oedema). The development of heart failure implies generally advanced disease with poor prognosis.

### **Assessment**

- The regular assessment of cardiac status helps physicians to recognize the early stages of heart disease and allows prompt intervention.
- Basic assessment includes a thorough medical history, physical examination, 12-lead electrocardiogram and a detailed echocardiogram. Cardiac magnetic resonance imaging (CMR), used to quantitatively estimate cardiac iron overload ( $T2^*$ ), is an invaluable tool in the estimation of risk for heart complications and in guiding chelation therapy. Additional tests in individual patients may include Holter or 24-hour ECG or functional assessment by exercise tests.

## Management

### *Prevention strategy*

- Prevention strategy to diminish the risk of heart complications involves blood transfusion to maintain a pre-transfusion haemoglobin of at least 10 g/dl and regular chelation therapy to maintain a CMR T2\* >20 ms.

### *Patients with mild cardiac dysfunction*

- A mild impairment of ventricular function merits aggressive escalation of iron chelation therapy, even if patients are completely asymptomatic. Combined therapy with deferiprone 75-100 mg/kg and deferoxamine 40-50 mg/kg/day is the best option.
- 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.

### *Patients hospitalized for heart failure*

- The presence of symptomatic heart failure should trigger hospital admission, ideally to a tertiary hospital with experience in managing thalassaemia patients or at least communication between treating physician and cardiac consultants with experience in thalassaemia.
- Patients should be given continuous deferoxamine therapy at 50 mg/kg/day as long as they have 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.
- In-hospital assessment should include bedside echocardiography to assess cardiac function and possible pericardial effusion or pulmonary hypertension, cardiac enzymes for possible myocarditis and D-dimers for possible pulmonary embolism in patients with right heart symptoms. Thyroid and parathyroid dysfunction should be identified and corrected if present and blood glucose should be controlled, as necessary, by insulin infusion.
- 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.

- Pressor medications should be used cautiously as they worsen iron-mediated oxidative stress and because thalassaemia patients typically operate with lower diastolic and mean blood pressures. Thus, blood pressure support should not be guided by clinical measures of renal and cerebral perfusion.
- Arrhythmias can be difficult to control. Amiodarone therapy is the drug of choice in the acute setting.
- Cardiac CMR 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.

#### *Heart failure-specific therapy*

- 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, including angiotensin converting enzyme inhibitors (ACE inhibitors), beta-blockers and aldosterone antagonists, gradually titrated to the maximum tolerated according to heart failure guidelines.
- Implantable cardio-defibrillators are generally discouraged because life-threatening arrhythmias are reversible with aggressive iron chelation therapy. External defibrillator vests can serve as useful bridge.
- 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.

## Arrhythmias

The context within which the arrhythmia occurs generally determines the clinical response and the risk to the patient.

- Arrhythmias usually present as palpitations, but may sometimes be asymptomatic.
- Arrhythmias are life-threatening in the presence of heart failure, but can also be ominous harbingers of pending cardiac decompensation in patients with normal cardiac function, but high iron overload.
- In older patients, even without any evidence of current iron overload, there is a high incidence of Atrial Fibrillation (up to 40% of those over 40 years), which carried a high risk of stroke in this group of individuals who may carry increased thrombotic tendencies due to the main disease.
- Sudden death is rare in the modern era, but historical data suggests an association with increased QT dispersion, consistent with Torsades de Pointes as a possible mechanism.
- 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; now it is rare, but may occasionally occur in severe iron load.

## Management

- **Any arrhythmia associated with haemodynamic compromise, syncope or pre-syncope must be considered a medical emergency, until fully characterized.**
- Management of iron overload is a main therapeutic goal.
- The symptomatic treatment of the documented arrhythmias is a secondary strategy.
- For most supraventricular arrhythmias, reassurance of the patient is generally appropriate.
- Many arrhythmias reverse over time and thus antiarrhythmic therapy can often be short term (less than one year).
- Ventricular couplets and non-sustained ventricular tachycardia are highly specific for iron overload cardiomyopathy and require attention to address high myocardial iron load via intensified chelation.

- Amiodarone is the drug of choice in the acute setting. Long term amiodarone therapy increases the risk of hypothyroidism because of pre-existing iron toxicity to the thyroid gland.
- Beta-blockers are generally well tolerated, if titrated slowly, and can be useful in controlling ectopic rhythms.
- Catheter based interventions for intra-atrial reentrant and ventricular tachycardia should be avoided as thalassaemia patients usually lack a true anatomic substrate.
- For the primary or secondary prevention of potentially life-threatening ventricular arrhythmias, conventional criteria for device therapy do not apply as arrhythmias are often reversible.
- 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 a safety net in these situations.

### **Atrial Fibrillation**

- Atrial Fibrillation (AF) occurring in an acute context, often precipitating heart failure, may be treated with immediate cardioversion by synchronized DC shock, if its duration is <48hr and the patient is fully anti-coagulated or 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, 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 patients with permanent or persistent AF radiofrequency isolation of the pulmonary veins may be considered.
- Anti-coagulation should be undertaken in all patients with significant episodes of AF.

## Heart block and conduction disturbances

- Heart block generally, but not always, responds to adequate chelation, but response may be slow and thus patients may require a pacemaker.
- It is essential that MRI conditional pacemakers and leads be used.

## Pulmonary hypertension

Pulmonary hypertension is primarily encountered in thalassaemia intermedia.

### Pathophysiology

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 (Figure 6).

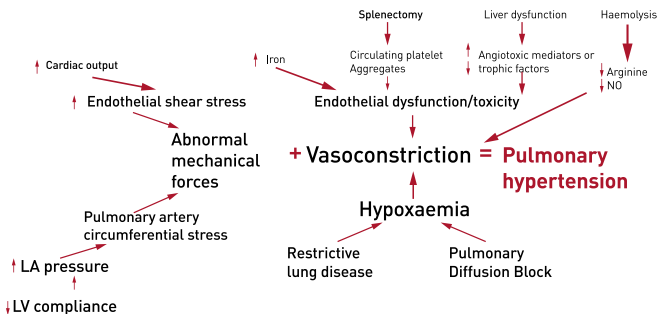


Figure 6: The pathophysiology of pulmonary hypertension

### Assessment

- 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.
- 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 haematological status.
- Brain natriuretic peptide and six-minute walk tests are useful for trending response to therapy.
- Overnight pulse oximetry is indicated to screen for nocturnal desaturation in all patients. However symptoms of obstructive sleep apnoea should provide a formal sleep evaluation.

### *Management*

- Treatment for pulmonary hypertension in thalassaemia is multifaceted and depends upon its severity and aetiology.
- Chronic anticoagulation is the treatment of choice in thromboembolic disease.
- Early pulmonary hypertension in thalassaemia major patients often responds to shortening transfusion intervals.
- Hydroxyurea use has been used with benefit in non-transfused thalassaemia syndromes and is effective in some patient populations (Banan, 2013)<sup>11</sup>.
- In refractory patients, PDE inhibitors or endothelin antagonists may be considered.
- Continuous positive airway pressure should be used in the presence of obstructive sleep apnoea.

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<sup>11</sup> Banan, M. (2013). Hydroxyurea treatment in beta-thalassaemia patients: to respond or not to respond? *Annals of Hematology* 92, pp. 289-99.

## **Peripheral vascular disease**

### *Pathophysiology*

- Many factors in thalassaemia accelerate the vascular ageing 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.
- An acquired pseudoxanthoma elasticum-like disorder, a degenerative process of elastin fibres of unknown mechanism more commonly observed in patients who are inadequately transfused or poorly chelated, may also contribute to vascular disease.

### *Assessment and management*

- No consensus exists for routine screening of systemic vascular disease.
- Prevention of vascular disease in thalassaemia primarily consists of properly controlling transfusion therapy and iron chelation.

### Summary recommendations 3

- 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 cannot 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).

## Chapter 5 LIVER DISEASE

In thalassaemia patients, the liver is a major target for several adverse factors leading to cellular injury, with major consequences starting from fibrosis and leading to cirrhosis. Increasingly, there is progression to hepatocellular carcinoma (HCC).

The causative factors leading to hepatic injury in thalassaemia - whether transfusion dependent or not - are:

1. Iron overload
2. Viral infections – mainly hepatitis B and C viruses
3. Biliary obstruction and cholecystitis especially in those patients rarely or not transfused at all
4. Portal vein thrombosis and portal hypertension
5. Medications
6. Non-alcoholic fatty liver disease

Table 12: Factors leading to liver injury in thalassaemia

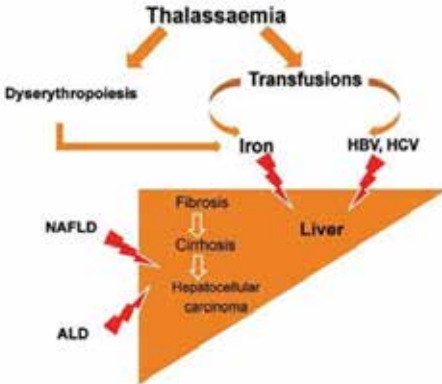


Figure 7: Causative factors leading to hepatic injury in thalassaemia (TDT and NTDT)

## **Causative factors**

Iron overload in the liver is the major causative factor of liver disease in thalassaemia. Hepatitis C and B viruses (HCV and HBV respectively) remain a concern even though preventive measures have reduced the new cases of infection. Other possible hepatotoxic co-factors should be kept in mind. Chronic liver disease may lead to cirrhosis and HCC.

## **Iron overload**

Iron overload is the result of regular transfusions as described in Chapter 3. Iron saturation of plasma transferrin, normally 45%, rapidly increases often reaching 100%. This leads to the appearance of non- transferrin bound iron (NTBI), which affects the parenchymal cell of liver, heart and pancreas. Dyserythropoiesis, a characteristic of thalassaemia, also reduces the production of hepcidin, leading to an increased absorption of iron from the gut, contributing to the iron overload.

As the storage proteins become saturated, damage to hepatocytes occurs, leading to their replacement by fibrous tissue and loss of liver function, evidenced by a rise in serum transaminase levels, when hepatic iron concentration reaches  $300\mu\text{mol/g}$ .

Monitoring and diagnosing hepatic iron overload is, therefore, of vital importance if the damage to the liver is to be controlled. The recommended tests are:

- Clinical approaches, such as observing skin pigmentation and iron overload in other organs, mainly the heart.
- Serum ferritin measurements. Increase in levels may be affected by causes other than iron overload, such as inflammation (check reactive protein levels), and hepatic cytolysis from other causes (e.g. dysmetabolic syndrome). The trend of rising or falling ferritin levels is a strong indicator, however of the iron load over time and the effectiveness of iron chelation.

- Plasma transferrin saturation provides information on the degree of biologically available iron. When over 75%, it is an indicator of the presence of NTBI.
- Magnetic resonance imaging (MRI) is the major non-invasive method for quantifying liver iron.
- Liver biopsy permits quantitation of iron, and allows histological examination. It is invasive, however and combining MRI and serum ferritin is the preferred strategy.
- Calculating the amount of transfused iron is precise but a retrospective method.

### **Diagnosis of hepatic disease**

- Clinical approaches: hepatomegaly (size and consistency), signs of hepatocellular failure, signs of portal hypertension (these are unusual since iron overload does not produce significant liver dysfunction).
- Biochemical markers:
  - (1) Increase in serum transaminase to less than x2-3 the upper limit of normal (in the absence of raised prothrombin time, alkaline phosphatase,  $\gamma$ Gt and conjugated bilirubin).
  - (2) Hyaluronic acid levels (if cirrhosis is suspected).
  - (3) Alpha-foetoprotein: every 6 months if cirrhosis is diagnosed or if HCC is suspected
- Imaging:
  - (1) Ultrasound for liver morphology and homogeneity. Also looking for signs of portal hypertension. This is recommended to be performed annually in thalassaemia patients, and every 6 months if cirrhosis is present.
  - (2) Transient elastography: evaluates the degree of liver fibrosis.
  - (3) Liver biopsy: to quantify fibrosis and to identify other lesions such as fatty deposits and inflammatory lesions.

## **Chronic Hepatitis C in thalassaemia**

In the past, up to 85% of regularly transfused patients were infected with HCV. Where specific prevention measures have been established, the incidence has been considerably reduced. In some developing countries, HCV screening of blood donors has not been established, at least not all blood banks have conformed to the requirement, and so the risk for patients is still high. In conjunction with iron overload, Chronic Hepatitis C (CHC) is an important contributor to liver disease, making the availability of effective treatment essential.

### *Diagnosis of Chronic Hepatitis C in thalassaemia*

- a) Suspected if transfusions were started before 1991.
- b) Serum transaminases raised for more than 6 months.
- c) Anti-HCV antibodies and HCV-RNA positivity.
- d) Firm hepatomegaly associated with signs of liver dysfunction. Cirrhosis is often already established.
- e) Biological data:
  - i) prothrombin time, leukopenia and thrombopenia (indicating portal hypertension through hypersplenism)
  - ii) aspartate transaminase/platelet ratio (APRI)
  - iii) Fibrosis<sup>4</sup> index (based on platelets, aspartate transaminase, alanine transaminase and ag
  - iv) Fibrotest
- f) Imaging:
  - i) Ultrasound
  - ii) Transient elastography
  - iii) Liver biopsy - using histological scores (Metavir score, fibrosis score, Ishak's score)

## **Treatment of Chronic Hepatitis C in thalassaemia**

The referral of the patient to a hepatologist early on in the detection of chronic liver disease is essential. Collaboration with the multidisciplinary throughout the management process of this complication may determine the final outcome.

To decide on the treatment, it is essential to know:

- 1) the HCV genotype and the presence of the IL28B polymorphism (If Peg-Inf and ribavirin are to be used in the treatment).
- 2) the HCV activity (increased serum transaminases) and the viral load, measured by a real-time PCR-based assay with a lower limit of detection of  $\leq 15$  IU/ml. This measurement will be used to monitor levels during treatment and assess the effectiveness of treatment.
- 3) the patients with significant fibrosis (Metavir score F2, 3 or F4) or cirrhosis

The treatment choices cannot be made by the thalassaemia unit alone and should be made by hepatologist familiar with the latest EASLE recommendations.

### **Chronic Hepatitis B in thalassaemia**

Vaccinations and blood donor screening have considerably reduced the incidence of HBV infections.

Chronic HBV carriers can be active or inactive, and co-infection with HCV and HDV (Hepatitis D virus) are possible. Chronic hepatitis from this virus (HBV) may also lead to cirrhosis or HCC.

#### **Diagnosis of active HBV:**

- 1) The presence of circulating HBV DNA
- 2) Non- invasive markers of fibrosis
- 3) Transient elastography
- 4) High levels of serum transaminases when the disease is highly active
- 5) Histological inflammation

#### **Treatment of HBV:**

As in the case of CHC, referral and collaboration with a liver specialist is imperative. Oral nucleoside and nucleotide analogues are effective drugs.



## Summary recommendations 4

Hepatic iron excess should be evaluated using a non-invasive strategy, combining serum ferritin values with MRI imaging. Liver biopsy is often not necessary.

Reversal of hepatic iron excess is a key objective not only to protect the liver but also the rest of the body.

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, based on serum markers which are predictive of fibrosis, as well as on transient elastography of the liver. Biopsy is not usually needed.

Serum transaminase and HBV DNA levels are the main means of differentiating inactive from active HBV.

Oral nucleoside and nucleoside analogs are well tolerated and effective drugs for HBV-chronic hepatitis, although AgHBs seroconversion remains a rare event.

## Chapter 6 THE SPLEEN

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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 it. Current strict transfusion regimen and chelation has considerably reduced the incidence of splenomegaly and iron overload in TDT patients.

The indications for splenectomy in TDT patients are summarized in Table 13 below:

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

*Table 13: Indications for splenectomy in TDT patients*

There are currently 4 approaches to splenectomy: open and laparoscopic total splenectomy, partial splenectomy and reduction of splenic tissue by embolization. Total splenectomy is the most commonly used method and the laparoscopic approach seems to be the most favourable one.

Splenectomy leads to thrombocytosis, while major adverse events include sepsis, thrombophilia, pulmonary hypertension and iron overload. To prevent splenectomy-related complications, thromboprophylaxis, chemoprophylaxis and immune-prophylaxis are recommended.

### Thromboprophylaxis

Post-operative thrombocytosis is common, with platelet counts often reaching 1,000,000-2,000,000/mm<sup>3</sup>. All guidelines recommend thromboprophylaxis perioperatively in patients with thrombocytosis:

- low-dose aspirin (80 mg/kg/d) for patients with high platelet counts.
- anticoagulation for patients with a history of previous thrombosis or other risk factors.

### Chemoprophylaxis and immune-prophylaxis

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.

The recommended chemoprophylaxis for splenectomised patients is summarized in Figure 8 below:

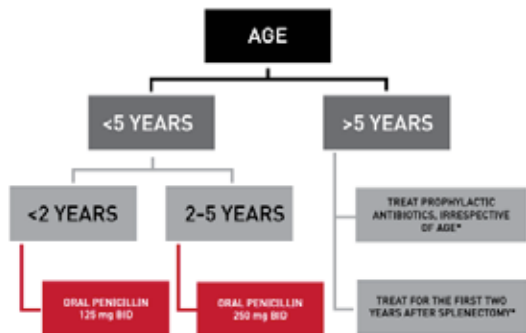


Figure 8: Recommended chemoprophylaxis for splenectomised patients

*The recommended immune-prophylaxis for splenectomised patients is summarized in Table 14 below:*

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"> <li>• Rate of protection is 70-85%</li> <li>• The immune response is poor in children less than two years of age</li> </ul>
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"> <li>• To prevent this febrile illness that might otherwise require intensive evaluation and management of a febrile episode in the splenectomised host</li> </ul>
<p>*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.</p>		

*Table 14: Recommended immunoprophylaxis for splenectomised patients*

## Summary recommendations 5

- 1) At present, we do not recommend splenectomy as a standard procedure in thalassaemic individuals (C). There is a 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 of iron overload with chelation therapy, hypersplenism and symptomatic splenomegaly (C).
- 2) When performing the splenectomy, the laparoscopic seems to be the most favourable approach (B).
- 3) The most frequent pathogens that cause infections in splenectomised patients are *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis*, immunoprophylaxis is therefore recommended against these agents 2 weeks prior to the operation and 3-5 years post op. Additionally, an annual influenza vaccine is encouraged.
- 4) Chemoprophylaxis with oral penicillin depends on the age of the individual and the treating physician's opinion (C).
- 5) 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 increased risk of many disease-related morbidities and should be monitored more closely.

Infections have been always a leading cause of morbidity and death in thalassaemia. The aetiology of infections is summarized in Table 15 below:

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

Table 15: Aetiology of infections in thalassaemia

### Prevention and management

#### *Transfusion transmitted infections (TTIs)*

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 include:

- The deferral of high risk prospective donors.
- Donor recruitment through voluntary non-remunerated blood donor.
- Routine testing of donor blood for HBV, HCV, HIV and syphilis by advanced generation technology safeguarding specificity and sensitivity of detection in very early stages of donor infection.

- HBV and HAV Vaccination (including booster HBV dose in individuals with decreasing anti-HBV titer for all patients with thalassaemia).
- Annual testing for TTIs markers including HBV, HCV and HIV.

Preventive measures include:

- Pre-storage leucodepletion of pRBC units reduces the transmission of cytomegalovirus (CMV), and may also reduce the risk of other transfusion-transmitted infections, including herpes viruses (e.g., Epstein Barr virus), retroviruses (e.g., human T cell lymphotropic virus type1 [HTLV-1] and HIV), bacteria (e.g., *Yersinia enterocolitica*), protozoa (e.g., *Leishmania* species and *Trypanosoma cruzi*), and infectious prion.
- Leucodepletion does not provide 100% risk prevention from these infections, but it may provide an additional and justified measure of caution (C).

**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 2g every 8h or ceftriaxone 2g every 12h) or carbapenem (meropenem or imipenem 2g every 8h) combined with vancomycin (1-1.5g every 12h).
- Gram's stain and blood culture are obtained from both the blood bag and the recipient (A).

Preventive measures for bacterial sepsis:

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

### *Transfusion-related immune modulation (TRIM)*

TRIM may contribute to all immunological alterations observed in TDT patients. Pre-storage leucodepletion of pRBC units has no protective effect on immune alterations observed in patients with thalassaemia. Storage of pRBC for more than 14 days has been 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) that include a decrease in antioxidant capacity.

### *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.

- 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 creating susceptibility to infections in thalassaemia, since the spleen has a crucial function in the body's immune defence as a phagocytic filter for blood borne microorganisms, and the production of antibodies. Overwhelming Post-Splenectomy Infection (OPSI) is a medical emergency, defined as fulminating sepsis, meningitidis or pneumonia, triggered mainly by *S. pneumonia*, and followed by *H. influenza* type B and *N. meningitidis*. The risk of OPSI is more than 50 times higher than in the general population and is a permanent life-long condition.

Suspicion and approach to OPSI:

- Physicians must be aware of the potential life threatening infections in TDT patients who underwent splenectomy and patients should be educated to seek early care when fever develops.
- In patients at risk and with indicative symptoms, prompt initiation of empirical antibiotics is essential. Intravenous



infusion of third generation cephalosporin (cefotaxime 2 g every 8 h or ceftriaxone 2 g every 12 h), combined with gentamicin (5-7 mg/kg every 24 h) or ciprofloxacin (400 mg every 12 h) or vancomycin (1-1.5 g every 12 h) (Brigden 1999).

- While waiting results of blood culture, bacteria can be visualised in gram staining.
- An RT-PCR test for simultaneous identification of 3 main encapsulated bacteria: *S.pneumonia*, *H. influenza* type B and *N. meningitides*, is available (Di Sabatino 2011) (A).

### *Iron chelation therapy*

The control of systemic iron and withholding iron from invading microbes are important strategies of host defence.

Pathogens, including *Y. enterocolitica* or *V. vulnificus* and *Mucorales*, can utilise DFO as siderophore for increasing their pathogenicity.

- 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 can be continued during febrile episodes(C).

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

Ineffective Erythropoiesis (IE) and haemolysis result in hyperplasia of monocyte/macrophages, which phagocytise all defective erythroid precursors and erythrocytes; this increased phagocytic activity may reduce the capacity of the phagocytic system to defend against microorganisms. Severe anaemia, on the other hand, is a risk factor for bacterial infections in thalassaemia.

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

## **Specific infectious agents in thalassaemia**

### *Yersinia enterocolitica*

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

Treatment:

- Stop DFO chelation.
- Obtain suitable laboratory samples.
- Commence effective antibiotic treatment immediately. Intravenous trimethoprim-sulfamethoxazole (400 mg sulfamethoxazole every 12 h) for 7 days (14 days in the case of septicaemia) plus gentamicin (5-7 mg/kg every 24 h) should be used for the treatment. Intramuscular ceftriaxone (2 g every 12 h) is an alternative in focal infections (e.g., enteritis, pharyngitis, tonsillitis). 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 thalassaemia from the Far East (Wanachiwanawin 2000).

Clinical manifestations include sinusitis, intracranial infections, septicaemia and pyogenic abscesses in the liver, lung, kidney and parathyroid gland, which are associated with high rates of morbidity and mortality.

### *Other bacterial infections*

Thalassaemia 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. pneumonia*, *Haemophilus 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 those who underwent splenectomy should be considered as emergency to treat.
- Stop DFO chelation.
- Obtain suitable laboratory samples.
- Commence effective antibiotic treatment immediately (A).

## Summary recommendations 6

- 1) Infection-related mortality used to be the second leading cause of death globally and has gradually become, albeit only in the Western world countries, the leading cause of death in thalassaemia in the modern era.
- 2) Physicians must be aware of the potential life threatening infections in thalassaemia and patients should be educated to seek early care when fever develops.
- 3) Control of iron homeostasis may have therapeutic benefit against infections.
- 4) 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.
- 5) Transfusion of pre-storage leukodepleted RBCs that have been stored for <14 days may have therapeutic benefit against infections.
- 6) Quality assurance guidelines and strict regulatory standards should be established for enhancing transfusion safety.
- 7) Splenectomy indications and preventive measures for post-splenectomy risk of sepsis should be revisited.

Endocrine abnormalities are the most common complications of TDT. Prevalence varies because of the different levels of treatment offered by centres across the world, the severity of the genetic background, the levels of haemoglobin and the level of iron load in various patient groups, as well as the degree and type of chelation therapy. Another contributing factor is that of increased survival to adulthood.

**The incidences of different endocrine related complications are:**

1. Growth retardation, commonly reported in children and adolescents with TDT. Significant retardation is observed in stature, sitting height, and biacromial (shoulder) and bicristal (iliac crest) breadths. By the age of 4 years, height is significantly below normal controls and bone age is delayed after the age of 6-7 years. There is failure or attenuation of their pubertal growth spurt. Short stature is reported in 30.8% in a multicentre study conducted by TIF in 2004.
2. Delayed puberty and hypogonadism: the incidence rate of hypogonadotropic hypogonadism in both sexes varies considerably between countries and among special centres. It ranges between 50% and 100 % (40.5% in the TIF study). The reported prevalence of adult-onset hypogonadism (AOH) in TM patients ranges between 8.3% and 12%.
3. Hypothyroidism: the prevalence varies from 6 to 35% across studies, although in the TIF study (2004), it was as low as 3.2%.
4. Impaired glucose tolerance and diabetes mellitus: the prevalence increases with age and varies from 10 to 24% in different studies.
5. Hypoparathyroidism: the prevalence varies between 1% and 19%.
6. Adrenal insufficiency: "biochemical adrenal insufficiency" varies to up to 45%, but clinical adrenal insufficiency is extremely rare.

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

### **Short stature and retarded growth**

Clinically identified as:

- Shortness in stature, height below the 3rd centile, also standing and particularly sitting height, shoulder and iliac crest breadths (during childhood and adolescence). Growth velocity in cms/year is below 1SD for age and sex (based on growth velocity charts).
- Bone age is delayed after the age of 6-7 years.
- Failure or attenuated pubertal growth spurt.

Contributing factors to growth retardation include:

- a) Chronic anaemia
- b) Iron overload damaging endocrine glands (especially growth hormone insufficiency, insulin-like growth factor deficiency, hypothyroidism and hypogonadism)
- c) Chelation toxicity (desferrioxamine)
- d) Nutritional deficiencies (malnutrition, vitamin D, zinc and carnitine deficiencies)
- e) Chronic liver disease
- f) Psychosocial factors

Based on these factors, growth retardation can be prevented if a good management protocol is followed from early life.

## HOW TO PREVENT GROWTH RETARDATION IN THALASSAEMIA MAJOR

Improve blood transfusion [correct hypoxia]	→	Improvement of nutrient and O <sub>2</sub> supply to liver and endocrine glands and growth plate
Improve Fe chelation therapy	→	Prevent siderosis of liver, heart, pituitary, pancreas and growth plate
Improve nutrition [calories, vit. D, folic, zinc, carnitine]	→	Improvement of macro and micronutrient supply of liver, glands and growth plate: compensates for hypercatabolism
Correct GH-IGF-1 deficiency by GH/IGF-1 therapy	→	Stimulates endochondral growth and bone mineral accretion + anabolic for muscle and fat
Induction of puberty at proper time/sex steroid replacement in hypogonadism	→	Stimulate GH-IGH axis + Direct stimulation of growth and protein anabolism in muscle and bones

**Normal synthesis and proper function of hepatic (systemic) IGF-1 and local IGF-1 (autocrine and paracrine) on growth plate muscles and adipose tissue**

Table 16: Practical approach to treatment of growth retardation in thalassaemia (Soliman 2013)

### Initial endocrine studies

Endocrine screening should start at the age of 9 years or earlier, if clinically indicated (e.g., short stature or decreased growth velocity (GV) per year).

The following tests and assessments are recommended annually:

- a) Maintain a growth chart for each patient (based on national data if available):
  - Height below the 3<sup>rd</sup> centile is an indicator of poor growth
  - Slowing of growth velocity, expressed as cm/year, below 1SD for age (using growth velocity chart based on national data).

- Crossing centiles over a 1 year period.
  - Standing and sitting heights are measured every 6 months.
  - Consider the height of both parents (Mid-parental height)
- b) Examine bone age radiologically in subjects with short stature, and bone mineral density during late childhood. Skeletal survey is useful to assess skeletal abnormalities (in selected cases).
- c) Assess pubertal stage (see Tanner's stages in Table 17 below)
- d) Study the following endocrine tests:
- i. Thyroid function tests (FT4, TSH)
  - ii. Pituitary gonadal axis: Measure LH, FSH, testosterone (males) and oestradiol (females), in the pubertal age group.
  - iii. Pituitary growth axis: measure IGF-1, Insulin growth factor binding protein -3, and perform growth hormone stimulation test (if needed).
- e) Assess calcium homeostasis (measure serum calcium, phosphate, alkaline phosphatase, parathormone, and 25-OH vitamin D levels).
- f) Measure IgA transglutaminase antibodies to exclude coeliac disease in patients with short stature or reduced GV/year.
- g) Assess glucose tolerance: fasting glucose every three months, glucose tolerance test annually.

The goals of prevention and treatment of growth retardation are met by the following practical steps:

- Maintaining pre-transfusion Hb above 9g/dl.
- Proper iron chelation to keep serum ferritin below 1000ng/ml.
- Correction of nutritional deficiencies. Oral zinc supplements to be given if there is proven deficiency.
- Correction of hypersplenism.



- Timely diagnosis of growth delay and pubertal delay as well as other hormone deficiencies.

### **Delayed puberty and hypogonadism**

- Delayed puberty is defined as the complete lack of pubertal development by the age of 13 in girls and 14 in boys.
- Hypogonadism is defined as the absence of testicular enlargement (less than 4ml) in boys, and the absence of breast development in girls by the age of 16.
- Arrested puberty is defined as the lack of pubertal progression over a year or more. In boys, there is usually an arrest of testicular size at 6-8ml, and in girls arrest in breast size at B3.
- Secondary hypogonadism appears later in life and is manifested in men by a decline in sexual drive, decrease in spontaneous erections, reduced shaving, infertility, low bone mineral density, reduced physical energy and a worsened sense of wellbeing leading to a degradation of the patient's quality of life. Other manifestations may include loss of muscle mass and strength, increased body fat, gynaecomastia, fatigue, depression and diminished physical and work performance.
- Secondary amenorrhoea commonly develops over time, and is particularly likely to occur in cases where female patients are poorly compliant with iron chelation therapy or start iron chelation late in life.

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

*Table 17: Pubertal assessment according to Tanner*

Investigations for diagnosing hypogonadism:

- a) Biochemistry and routine examinations (as for growth retardation above).
- b) Testing of hypothalamic-pituitary -gonadal axis. Findings include:
  - i. Low basal FSH and LH secretion
  - ii. Low LH/FSH response to gonadotropin releasing hormone (GnRH)
  - iii. Variable disturbance of the pulsatile pattern of the LH and FSH secretion
  - iv. Low basal sex steroid levels (oestradiol and testosterone)
  - v. Low testosterone in response to human chorionic gonadotrophin (hCG), in some cases
  - vi. Human menopausal gonadotrophin (hMG) test has been done in tertiary care centres to assess the gonadal function in females

- c) Pelvic ultrasound to assess ovarian and uterine size in females.
- d) Bone mineral density (in both sexes).

Treatment of hypogonadism in TM:

Treatment depends on age, severity of iron overload, the coexistence of chronic liver disease, psychological factors as well as the degree of damage to the hypothalamo-pituitary-gonadal axis.

*For girls:*

- Ethinyloestradiol (2.5-5 $\mu$ g daily) for 6 months and then hormonal assessment.
- If puberty does not occur, gradually increase dose to 5-10 $\mu$ g daily for 12 months.
- If breakthrough uterine bleeding does not occur, then low oestrogen-progesterone hormone replacement is recommended.

*For boys:*

- Low dose IM depot-testosterone esters (25-50mg) monthly, for 6 months followed by hormonal assessment.
- If no response, 50mg per month is continued until growth rate wanes.
- The full virilising dose of depot testosterone is 75-100mg every 10 days, after growth is completed (the same effect is can be achieved with topical gel).
- If there is pubertal arrest, treatment is carried out with the use of testosterone esters or gel.

Treatment for these pubertal disorders is considered for each patient individually because of the complexity of the contributing factors.

### **Hypothyroidism**

This is mainly due to iron overload and is uncommon in optimally chelated patients. Patients are routinely monitored

from the age of 9. Symptoms and signs in thalassaemia are non-specific and usually overlap with those caused by anaemia. However, hypothyroidism in TM may contribute to stunted growth, delayed puberty, heart failure and pericardial effusion.

Investigations for hypothyroidism:

It is recommended that annual investigation of thyroid function begins at the age of 9 unless symptomatic hypothyroidism is observed. The diagnosis is easily made by measuring serum T4 and TSH levels.

- a) Subclinical hypothyroidism is diagnosed when FT4 is normal and TSH is between 5-10  $\mu\text{U/ml}$ .
- b) Overt hypothyroidism is when TSH is high  $>10 \mu\text{U/ml}$  and FT4 is low ( $<10 \mu\text{U/ml}$ ).
- c) Diagnosis of central hypothyroidism is usually made on a biochemical basis showing low circulating concentrations of thyroid hormone associated with inappropriately low TSH levels.
- d) Thyroid antibodies (anti-thyroid peroxidase, anti-thyroglobulin auto-antibodies).
- e) Ultrasonography to evaluate structural thyroid gland abnormalities.
- f) ECG and echocardiography in severe cases of iron overload.
- g) MRI to view the hypothalamic- pituitary region in selected cases of central hypothyroidism.

Physical examination findings include stunted growth, delayed puberty, cardiac failure and pericardial effusion. The classical signs of hypothyroidism are not usually found.

Treatment for hypothyroidism:

- If subclinical hypothyroidism is detected, chelation should be intensified and the patient should be carefully monitored. Treatment is required if TSH is equal or above  $10\text{mU/ml}$ .

- If there is cardiomyopathy, treatment with amiodarone may result in rapid progression to severe hypothyroidism and further affect heart function.
- Overt and central hypothyroidism are treated with L-thyroxin.
- Subclinical hypothyroidism-basal TSH from 5 to 7  $\mu\text{UI/ml}$ -requires regular follow-up and optimizing chelation therapy.

### **Impaired glucose tolerance (IGT) and diabetes mellitus (DM)**

Although inadequate iron chelation is a prominent cause in the development of DM, other factors, such as zinc deficiency and liver disease are known to contribute.

Impaired glucose metabolism may occur early on in the second decade of life and its incidence may progressively increase with age.

### **Pathogenesis of abnormal glucose homeostasis in thalassaemia**

The development of DM in thalassaemia is attributed to impaired insulin excretory function, secondary to chronic iron overload in the pancreas, selective immune system activation against pancreatic  $\beta$ -cells leading to cell damage, and/or pancreatic cell death due to fat transformation.

Pancreatic  $\beta$ -cell function in thalassaemia is characterised by the following sequence (Figure 9):

- Insulin resistance with hyper-insulinaemia and normal glucose tolerance.
- Insulin resistance with IGT and progressive impairment of  $\beta$ -cell function.
- Insulin dependent DM.

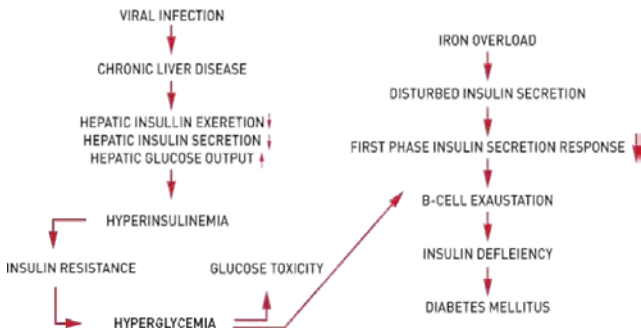


Figure 9. Pathogenesis of abnormal glucose homeostasis in thalassaemia (De Sanctis, Dubai 2006)

#### Diagnosis:

- Fasting blood glucose >126mg/dl.
- Oral Glucose Tolerance Test (OGTT) serum glucose at 2 hours >200mg/dl is diagnostic of diabetes mellitus.
- OGTT serum glucose at 2 hours >140 <200mg/dl indicates glucose intolerance.
- Pancreatic iron is the strongest predictor of  $\beta$ -cell toxicity, which can be evaluated by MRI - this can identify high risk patients before irreversible pancreatic damage occurs.

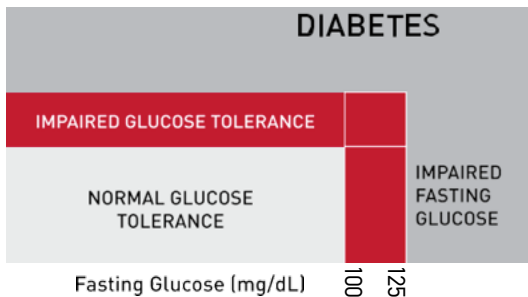


Figure 10: The diagnostic criteria for the glucose tolerance

### Management:

- Intensive iron-chelation therapy (see chapter 3) can normalise  $\beta$ -cell function and may improve insulin secretion, and reduce liver iron deposition.
- Healthy diet, suitable for DM should be adopted (advised by an expert dietician following assessment).
- Regular physical activity.
- Oral antidiabetic agents: oral hypoglycaemic drugs in the early stages of DM, may be beneficial. Limited data on their effect have been reported.
- When overt DM develops, with persistently elevated blood glucose, patients require daily subcutaneous injections of insulin to normalise blood sugar levels.
- Diabetic patients with TM should regularly see a specialised multidisciplinary team with expertise in both diabetes and TM, and engage in ongoing diabetes self-management education. The team should include an endocrinologist and dietician with experience in TM.
- TM women with pre-existing diabetes should have pre-pregnancy counselling and planning to aim for optimal glycaemic control before and throughout pregnancy in order to minimize adverse pregnancy outcomes.

**Monitoring glycaemic control in thalassaemia patients follows the same procedures as in non-thalassaemic patients with DM.**

Diabetic patients with TM should regularly see a specialised multidisciplinary team with expertise in both diabetes and TM, and engage in ongoing diabetes self-management and continuous education. The team should include an endocrinologist and dietician with experience in TM.

### Indicated tests and procedures:

- Self-glucose monitoring (SGM) at home using glucometers (daily monitoring).
- Monitoring of urine ketones if blood sugar is  $>250\text{mg/dl}$
- Fructosamine determination every month is useful for monitoring diabetes in these patients.

- Periodic assessment of renal function.
- A microalbumin test is used to detect early signs of kidney damage. If albumin in the urine (micro-albuminuria) is detected, it should be confirmed by retesting twice within a 3-6 month period of time.
- Evaluation of retinopathy.

## **Hypoparathyroidism**

This uncommon complication presents equally in both sexes after the age of 16. The majority of the patients present with mild hypocalcaemia and, rarely, present with tetany and cardiac failure.

### Signs and symptoms

The majority of patients have mild disease with paraesthesia as the only symptom. However, severe cases may present with severe symptoms such as tetany, seizures, or cardiac failure.

### Investigations:

- a) The diagnosis is based on low serum calcium, high phosphate and low PTH levels, in respect to serum calcium level.
- b) ECG to detect any abnormality in the electrical activity of the heart

### Management:

Aims to control symptoms and prevent acute and chronic complications of hypocalcaemia and maintain normal levels of calcium and phosphate, and to keep a calcium x phosphate solubility product under 55mg/dl (4.4mmol/l). Also 24 hour urine calcium should be under 7.5mmol/day (300mg/day).

- Oral administration of Vitamin D (monitoring serum calcium levels to avoid hypercalcaemia).
- Administration of Calcitriol 0.25-1.0 µg, twice daily (monitor plasma calcium and phosphate and 24-hour urinary calcium and phosphate levels).
- IV calcium if tetany or heart failure (with careful cardiac monitoring).



- Dietary recommendations (foods rich in calcium and low in phosphorus).
- If hypercalciuria develops in patients treated with calcium and vitamin D, then restriction of sodium intake and the use of thiazide diuretics and/or the reduction of the dose of calcium and vitamin D may be required.

### **Adrenal insufficiency**

Secondary haemosiderosis due to excess iron has the potential to disrupt adrenal function by affecting the hypothalamic-pituitary-adrenal axis at the hypothalamic or pituitary and/or adrenal level.

#### Diagnosis

Clinical manifestations of mild adrenal hypofunction might be masked by symptoms commonly reported by TDT patients, such as asthenia, muscle weakness, arthralgia and weight loss. In the subclinical deficiency, there is no clinical impact under basal conditions but there may be a potential relevance in stressful events (such as acute cardiac decompensation, stress, or sepsis).

#### Investigations:

Cortisol levels (basal and after ACTH or insulin stimulation) measured every 1-2 years, especially in patients with iron overload and/or growth hormone deficiency (which may indicate additional anterior pituitary deficits).

#### Management

Subclinical impairment of adrenocortical function in patients with thalassaemia is of little or no clinical impact under basal conditions and may have a potential relevance during stressful events. Accordingly, glucocorticoid treatment coverage might be advised only for stressful conditions.

## Summary recommendations 7

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 the role of iron chelation in this.

Once endocrine complications have developed, management should focus on halting the progression of these complications and treating associated symptoms.

Expecting to have a family is a key aspect of quality of life and an important aspiration for many patients with thalassaemia. Although spontaneous fertility can occur in well-transfused and well-chelated patients with spontaneous puberty and normal menstrual function, the majority of patients are sub-fertile mainly due to hypogonadotrophic hypogonadism (HH) as a consequence of transfusional haemosiderosis. 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 thalassaemia are high risk for both the mother and the baby. However, these risks can be minimized through pre-pregnancy counselling involving the various members of the multidisciplinary team: the haematologist, the reproductive medicine specialist, the cardiologist and the obstetrician, in conjunction with the specialist nurse.

### **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 hypothalamic- pituitary- gonadal (HPG) axis.

Careful assessment, not only of endocrine function, but also of general and cardiac health, aiming for pre-pregnancy counselling, should precede any fertility treatment. The partner should also be evaluated. The carrier status of the partners and whether they are both homozygotes must be included in the assessment so that their options are presented in the counselling session.

- Induction of ovulation with pulsatile GnRH infusion is only possible at the early stage of HPG damage, when gonadotrophins (FSH, LH) are pulsatile.
- Most patients with HH are pulsatile but with functional gonads, and are therefore likely to benefit from gonadotrophin- therapy (80% success rate).
- Patients with endometrial or Fallopian tube damage respond better to IVF programmes. Induction of ovulation may be indicated in women:
  - a. with primary and secondary amenorrhoea or
  - b. those with normal menstrual function who fail to conceive.
  - c. in planned pregnancy when both partners are thalassaemic.

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.

- Ovulation induction protocols use standard medications, including FSH, SH and clomiphene citrate (to stimulate the development of follicles), and HCG and LH to trigger ovulation.
- About 1-2% of women undergoing induction of ovulation develop severe hyperstimulation syndrome causing abdominal pain, dyspnoea, vomiting and rapid weight gain. Induction of ovulation should therefore only be undertaken by a specialist reproductive team, according to Human Fertilization and Embryology Authority (HFEA) guidelines.

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.

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 a sample.
- If azoospermia persists, STOP treatment.

Collaboration between endocrinologists and other members of the multidisciplinary team is essential. Treatment is demanding and may take up to 2 years.

- Initial regimen with 1-2000 IU of hCG administered IM, twice weekly. For those patients with onset of hypogonadism before the completion of pubertal development, human menopausal gonadotrophin or FSH are added. The clinical response is monitored by testosterone levels every 2-3 months. Dosage adjustments of hCG may be needed.
- If this treatment does not result in adequate sperm production after 2 years then there is no indication to continue.
- Sperm banking even in subjects with reduced sperm count, is recommended.

## **Pre-pregnancy counselling**

Before embarking on fertility treatment, it is important that patients and their partners attend pre-pregnancy counselling to evaluate eligibility and feasibility and discuss potential risks associated with induced fertility and pregnancy.

Eligibility evaluation includes the following elements:

- Cardiac function: ECG, Echocardiogram (the cardiac load is increased during pregnancy by 25-30% at least, because of increased heart rate and stroke volume). If left ventricular dysfunction is demonstrated or significant arrhythmias have occurred, then women should be strongly advised against pregnancy
- Liver function tests, MRI and Ultrasound of the liver to evaluate liver disease. If HCV RNA positive, then anti-viral treatment should be given before pregnancy
- Bone health should be assessed by radiography and DEXA of spine and hip
- 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

The main points regarding the potential risks associated with pregnancy include:

- Pregnancy does not alter the natural history of the disease.
- Requirement for intense/vigilant monitoring.
- Risk of cardiac complications: the most important issue
- Risk of pregnancy-specific complications same as background population
- Risk of miscarriage is the same as background population
- No increased risk of foetal malformation
- A two-fold increase in the risk of foetal growth restriction
- Preterm labour risk: two-fold increase
- Risk of Hepatitis B/C and HIV transmission to the foetus/ baby.
- Risk of iso-immunisation
- Increased risk of pre-maturity and growth restriction (in multiple births).

### **Pre-pregnancy assessment and management of pregnancy**

Assessment of women with thalassaemia seeking pregnancy should include:

- Assessment of cardiac function with electrocardiogram and echocardiogram.
- Liver function test and ultrasound.
- Checking the 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 of medication
- Screen for acquired red cell antibodies (risk of haemolytic disease)
- Checking male for haemoglobinopathy
- Arranging genetic counselling, if necessary

Key points for pregnancy care include:

- Checking cardiac, liver and thyroid function once each trimester.
- Screening for gestational diabetes.
- Increasing the frequency of blood transfusion to maintain pre-transfusion haemoglobin above 10 g/dl.
- Serial ultrasound scans to monitor foetal growth.
- Higher incidence of caesarean section.
- Encouraging breastfeeding unless the mother is HIV positive and/or HCV RNA and/or HBsAg positive.
- Resuming DFO after delivery.
- Discussing contraception, where appropriate with either the POP or barrier method.
- Avoiding intrauterine devices and oestrogen-containing preparations.
- Implementing a multidisciplinary approach with all specialists involved in the medical care of thalassaemic women.

Medication review for pregnancy - focus points:

- Emphasize folic acid supplementation.
- Stop DFX and Vitamin C.
- Stop ACE inhibitors.
- Metformin may be continued, but oral hypoglycaemic drugs may need to be changed to Insulin.
- Stop bisphosphonates at least 6 months prior to planned pregnancy.
- Give Calcium and Vitamin D supplementation.



## Summary and recommendations 8

- 1) Iron overload in the pituitary is the main cause of infertility in females.
- 2) Successful pregnancy can be achieved in thalassaemia major through ovulation induction because ovarian function is usually preserved.
- 3) Ovulation in females and spermatogenesis in males can be induced by exogenous gonadotrophin therapy.
- 4) Management of infertility requires careful planning and preparation.
- 5) Induction of ovulation should only be undertaken by a specialist reproductive team.
- 6) 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.
- 7) 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.

## Chapter 10 OSTEOPOROSIS

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Osteoporosis is a prominent cause of morbidity in patients with thalassaemia major, being present in 40-50% of patients.

### 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) by which a patient's bone mass is above or below the mean peak bone mass for a 30-year-old healthy woman.

### Pathogenesis

The pathogenesis includes genetic factors, 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.

### Diagnosis and monitoring

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.

Biochemical markers of bone metabolism that can be used in thalassaemia are summarized in Table 18 below:

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

\*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.

*Table 18: Markers of Bone Resorption and Bone Formation*

## Prevention and management

Prevention and treatment of early bone loss is the best policy and should include:

- 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.

**Hormone replacement therapy** for the prevention of hypogonadism is very effective in preventing osteoporosis and consists of transdermal oestrogen for females or human chorionic gonadotrophin for males.

**Bisphosphonates** are potent inhibitors of osteoclast function. Intravenous administration of pamidronate or zoledronic acid seems to be more efficient than oral bisphosphonates.

The bisphosphonates used in patients with TDT and osteoporosis are summarized in Table 19 below:

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.

*Table 19: Bisphosphonates used in patients with TDT and osteoporosis*

**Calcitonin** in parenteral and intranasal instillations is another potent inhibitor of osteoclasts, but data in thalassaemia is very limited.

**Other novel agents** such as the novel osteoclast inhibitor denosumab, teriparatide and the activin-A antagonist sotatercept are under investigation but their effects in thalassaemia-induced osteoporosis remains to be proven.

## Summary recommendations 9

- 1) Annual checking of BMD starting in adolescence is indispensable.
- 2) Biochemical markers of bone metabolism that can be done every year include NTX, CTX and bALP.
- 3) Physical activity must always be encouraged.
- 4) Smoking should be discouraged.
- 5) 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.
- 6) Early diagnosis and treatment of diabetes mellitus.
- 7) Adequate iron chelation may prevent iron toxicity in the bone and sufficient blood transfusions may inhibit uncontrolled bone marrow expansion.
- 8) Hormonal replacement where it is needed.
- 9) Bisphosphonates should be given concomitantly with calcium and vitamin D and not for longer than two years.

## Chapter 11 HAEMOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

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Thirty years since the first haemopoietic stem cell transplants (HSCT) in thalassaemia, this procedure stands today as a widely applied treatment for the definitive cure of thalassaemia major, with more than 3000 HSCTs performed worldwide.

When considering the very significant combined costs of life-long blood transfusions, chelation and the management of complications for optimal thalassaemia care, HSCT is certainly a cost-effective option if adequate expertise exists.

The currently accepted and experimental transplantation approaches for TDT are summarized in Table 20 below:

APPROACH	STATUS
HLA-identical sibling HSCT	Accepted
HLA-well matched, unrelated donor HSCT	Accepted
HLA-matched unrelated Cord Blood HSCT	*Experimental
HLA-mismatched related donor transplant	*Experimental

*Table 20: Currently accepted and experimental transplantation approaches*

\* Experimental in this context means that the procedure described must be conducted in well-specialised transplantation centres, within the context of well-designed, controlled clinical trials

### Outcomes

A prognostic scheme developed in the 80s included three risk factors related to iron burden:

- Lifetime quality of chelation therapy received prior to transplantation (regular versus non- regular).

- Hepatomegaly (defined as more than 2 centimetres below the costal margin).
- Presence of liver fibrosis pre-transplant, as determined by hepatic biopsy examination.

These variables stratified patients into three groups:

- Class I in the absence of the above factors
- Class II in the presence of one or two factors
- Class III in the presence of all three factors

The expected probability of overall survival and thalassaemia free survival after HSCT in TDT, according to the above classification is summarized in Table 21 below:

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

*Table 21: Expected probability of overall survival and thalassaemia free survival after HSCT in TDT*

### **Pre-transplant evaluation**

Pre-transplant evaluation should include (in addition to classical pre-HSCT evaluations):

- accurate iron studies, including cardiac iron / function, and liver iron / function.
- assessment of the degree of fibrosis by liver biopsy (preferred over liver elastography).

The ideal situation would be one of constant and regular lifelong chelation therapy achieving a negative iron balance.

Endocrine function should also be studied to allow accurate post-transplant follow up.

## **Follow up**

Post-transplant follow-up should include:

- Careful monitoring of haematological and engraftment parameters, infectious complications and graft versus host disease (during the first year)
- Appropriate immunization, if there is no graft versus host disease (during the second year)
- Monitoring of multi-system problems related to primary disease e.g., iron overload, pubertal development, growth and endocrine deficiencies (in the long term).

Removal of excess iron by repeated venesections (6 ml/kg blood withdrawn at 14-day intervals) or oral chelation with standard dosing schedule is essential and should be started only after graft stabilization and discontinuation of any immunosuppressive treatment or prophylaxis and in the absence of chronic graft versus host disease. Agranulocytosis risk following deferiprone warrants caution.

Endocrine dysfunction and infertility in post bone marrow transplantation require specific expertise and follow up.



## Summary recommendations 10

If an HLA identical sibling is available, 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 (B).

Either bone marrow or Cord Blood (CB) 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 Umbilical Cord Blood Transplantation (UCBT) in thalassaemia should only be considered in the case of 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).

With increasing survival, gained by today's availability of optimal management, healthcare services should aim to reduce as far as possible the degree to which the disease and its treatment interferes with the patients' personal and social life. The aim is to achieve the patients' social integration, as included in the concept of wellbeing.

### **Exercise and participation in sports**

Physical and recreational activities should be encouraged in thalassaemia but the limitations posed by this chronic disease should also be recognised. Physical capacity is influenced by the degree of anaemia, as well as the cardio-circulatory and pulmonary function and the presence of other co-morbidities such as endocrine and hepatic complications.

These factors are identified through the following tests and procedures:

- general monitoring of patients (see monitoring protocol)
- ergometry to assess respiratory and cardiac function, using a bicycle or treadmill with respiratory function tests and aerobic capacity (e.g.  $\text{VO}_2\text{max}$ )
- echocardiography
- cardiac magnetic resonance
- cardiac function through the assessment of maximum heart rate responses and stroke volume reserve.

This type of assessment is essential if athletic activity is contemplated, but not for routine daily activity.

The presence and degree of bone disease with a predilection to fractures, as well as the limitations imposed by pain must be taken into account in any advice given to thalassaemia patients concerning physical activity.

## **Education and employment**

Many patients who have benefitted from good clinical care have now progressed to tertiary education and are involved in productive employment. Factors which can facilitate good achievements in the field of education, apart from good clinical management, include:

- Family support.
- Psychosocial support.
- Clinic and transfusion appointments which reduce visits during school and working hours (afternoon, evening or weekends), avoiding repeated absences.
- Collaboration of the clinic with teachers and employers when necessary.
- Raising community awareness in order to avoid prejudice, bullying and social isolation of the patients.
- The recognition of the patients' rights to work (UN Convention on the rights of persons with disabilities to work, article 27, 2007).

## **Marriage and reproduction**

Well treated patients have a good record in the field of developing stable relationships although stigmatisation is still an issue in many situations.

To achieve this important goal in life, the following aspects should be kept in mind:

- Good psychosocial support
- Good clinical care, which includes endocrinological monitoring from adolescence, and hormone replacement therapy where necessary. This aims to promote pubertal maturation as well as reproductive competency. Management of subfertility in both sexes is discussed chapter 9.
- Pre-pregnancy counselling to the potential mothers, which includes:
  - i. a cardiological assessment, since the cardiac load is increased during pregnancy

- ii. advice as to the use of any drugs, including iron chelating agents during pregnancy.
- iii. liver function evaluation by biochemical tests and MRI
- iv. screening for HIV, HCV, HBV and rubella
- v. if HCV positive, the potential mother should be treated
- vi. establishing bone health by DEXA and treat, if necessary

Assessment and management for reproduction should involve the entire multidisciplinary team.

## **Nutrition**

- During growth, a high calorie diet is recommended.
- Zinc - deficiency may affect growth and sexual maturation, as well as cause hair loss, diarrhoea, skin rashes and loss of appetite. Zinc is essential for the health of the immune system and bones. Zinc levels are recommended to be monitored every 6 months, especially in patients taking deferiprone. Supplements in the form of Zn-sulphate 220mg three times daily are recommended for haemoglobin disorders.
- Calcium and Vitamin D: deficiency is associated with poor bone mineralisation, muscle weakness and left ventricular dysfunction. Vitamin D levels are measured every 6 months. Supplements are recommended for all thalassaemia patients, along with a diet high in calcium (i.e., milk, cheese and oily fish). Vitamin D dose is at 2000 IU daily.
- Folic acid deficiency develops in patients on low transfusion regimens. In this sub-group of patients, 1mg/day is recommended.
- Vitamin E is often depleted in thalassaemia patients due to reduced dietary intake and liver disease. A diet rich in Vitamin E is recommended (i.e., eggs, vegetable oils, nuts and cereals). Some studies suggest that supplementation of 400 IU/day will reduce oxidative stress.
- Vitamin C increases labile iron, and despite its anti-oxidant properties, it is potentially toxic. It is given only with desferrioxamine infusions at a dose not exceeding 2-3mg/kg, aiming to enhance iron excretion.

- L-carnitine has potential benefits in thalassaemia as an anti-oxidant and a cardioprotective agent. A dose of 50mg/kg is recommended with caution in patients with hypothyroidism and patients with seizures.

**Alcohol** (which can aggravate liver disease), smoking (which can affect bone remodelling and cardiorespiratory fitness) and drug abuse, are all out of bounds for thalassaemia patients.

### **Health Related Quality of life**

The concept of quality of life involves each patient's perception of their own life and wellbeing. Several measures have been developed to evaluate this, exploring domains which include physical health, emotional state and social circumstances. Several measurements (questionnaires) have been developed and used by researchers to examine the responses of thalassaemia patients. Most are general, also used in other medical conditions (e.g. The World Health Organisation Quality of Life - WHOQoL, the Short Form Health Survey – SF36). Disease specific instruments for TDT patients have also been developed (e.g., the Specific Thalassaemia Quality of Life Instrument - STQOLI and the Transfusion Dependent Thalassaemia Quality of Life - TranQol).

It is strongly recommended that patient reported outcomes, as discerned through such questionnaires are periodically used in all thalassaemia treating centres to assess the success of each clinic's treatment efforts and to monitor changes brought about by new developments in management.

### **Patient adherence to prescribed treatment**

Poor patient adherence remains a major obstacle in the care of all chronically ill patients and thalassaemia patients are no exception. Monitoring adherence is not a simple task, but clinicians are urged to periodically assess the level of patients' adherence, using recognised tools such as the validated Morisky questionnaire, an electronic method or an outcome related measure.

## General Timetable for Clinical and Laboratory Evaluation

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

every X months			As clinically indicated	Initially	Comments
6	12	24			
				X	
				X	
				X	
X	X				
			X		
	X				
X					After the age of 10 years
X					
	X				
					Every 1-2 weeks for those taking Deferiprone
			X		
					From the age of 8 years. If there is evidence of hepatitis, histology is necessary.
	X		X		
	X		X		
	X				
			X		
			X		
					Monthly if on deferasirox
	X				
	X				
	X		X		If serology if positive. When hepatitis is active, do this test as per international protocol.
	X				
	X		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		X		From age 8 years. If there is evidence of hepatitis, histology is necessary.
	X				
		X	X		
			X		
					Monthly if on Deferasirox
X					
X					
	X				
	X				Start at 5 years.
	X				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		
	X		X		
	X		X		
	X		X		
	X		X		From age 8 years







## NOTES

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