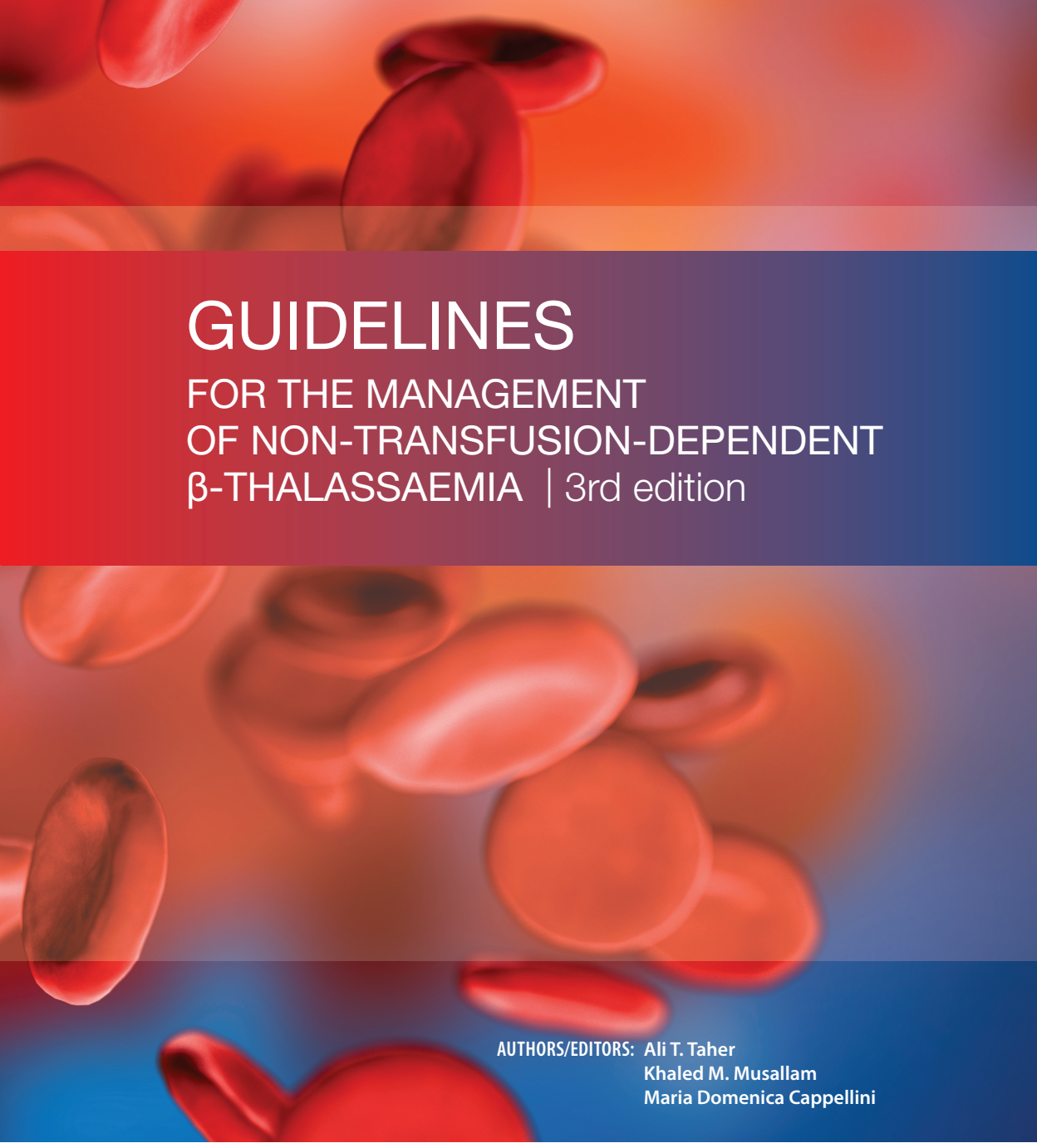

GUIDELINES

FOR THE MANAGEMENT OF NON-TRANSFUSION-DEPENDENT β-THALASSAEMIA | 3rd edition

AUTHORS/EDITORS: Ali T. Taher
Khaled M. Musallam
Maria Domenica Cappellini

THALASSAEMIA INTERNATIONAL FEDERATION

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“Cure sometimes, treat often, comfort always.”

Hippocrates (460-357 B.C.)

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CONTENTS

FOREWORD

It has long been recognised that thalassaemia has a spectrum of clinical severity, in fact with such differences that allow for a separate description of symptoms and outcomes. The most severe forms of β-thalassaemia are those that require regular, lifelong blood transfusion for survival – what is referred to as the transfusion-dependent β-thalassaemia (TDT). Over many years, our attention has been indeed focussed on this form, on account of its severity and development of morbidities leading to early premature death if left unaddressed or sub-optimally managed.

Hence all efforts have been directed to improve the management of these patients both as far as the medical treatment is concerned but also concerning service development for social and other care and psychosocial support. The recognition that the so called chronic 'milder' forms, those with which one can survive without frequent blood transfusions referred to in current years as non-transfusion dependent β-thalassaemia (NTDT) forms were in the past regarded by parents, patients, and doctors as a 'blessing'. Experience and studies however through the years, have in fact shown that they are not as benign as once thought and that over time they can develop serious to life-threatening complications which may even be different in frequency, severity and/or organ involvement from those experienced by TDT patients, making them almost a distinct clinical syndrome.

It is this syndrome that has been studied and described comprehensively in this book by three primary contributors and key medical advisors and friends of the Thalassaemia International Federation (TIF), Professor **Ali Taher** of the American University of Beirut in Lebanon, Professor **Khaled Musallam** of Burjeel Medical City in Abu Dhabi, the United Arab Emirates, and Professor **Maria Domenica Cappellini** of the University of Milan in Italy. The Editors have stellar international contributions in the improvements achieved through the years in effectively managing haemoglobin disorders, especially NTDT forms. Their dedicated work has indeed brought light and hope to patients across the world for better health, future and quality of life.

And indeed my very first duty as the President of the Board of Directors of TIF, is to express the immense gratitude of the Federation and the patients' associations that it represents globally in over 60 countries across the six regions of the world, to these academics who have been in the forefront of the struggle to control these lifelong congenital haemoglobin disorders over so many years with hard work and undivided commitment towards the patients and their families across the world. Their contribution to TIF's educational efforts and activities goes beyond that and is directed to the huge impact this work has had on the education and disease specific knowledge of our health professionals as well in every part of the world.

This new edition of the NTDT guidelines is an invaluable contribution to the efforts focused by scientists to upgrade patient care of the individuals with NTDT that was for years underestimated in importance and clinical significance. For many decades NTDT was considered by health care professionals of less concern

with regards to the early and accurate diagnosis and the implementation of regular and expert monitoring and management that could prevent the development of a heterogeneous range of morbidities related to poor health and quality of life.

In this effort, the Editors of this book have drawn not only on their vast experience and studies but also on a detailed and long review of published literature. This is a collective, comprehensive, highly committed work, for which indeed countless hours of very hard work have been devoted.

The TIF Board of Directors and the whole global thalassaemia community wish to express their gratitude and sincere respect to the Editors and are certain that through these guidelines patients across the world will benefit from better management and care and will lift up their hopes for better quality of life – an important component for which TIF is fighting for.

One can safely sate that TIF's Guidelines for the management of TDT and later NTDT, a major deliverable of its educational programme published since 1999 (updated, upgraded regularly, translated in many languages and distributed worldwide as a free of charge service) is one that has a truly measurable, direct impact on the knowledge of healthcare professionals. This is undoubtedly a service closely and significantly related to the provision of better care of our patients. Indeed, patient outcomes reflected through TIF's work have seen dramatic improvements albeit considerable work is still needed in many countries mainly of the developing world.

Ending, on behalf of the Board of Directors of the TIF I wish to express my gratitude to every member of TIF's Medical Advisory Board, to every single scientist, clinician, researcher at the country and international level who contributed and continues to contribute to TIF's educational programmes.

Last but not least, I invite every physician, healthcare professional caring for patients with thalassaemia to take advantage of what the Editors suggest or recommend in this book and join TIF's efforts in distributing it as widely as possible amongst the national relevant competent authorities and healthcare professionals' community across the world.

Panos Englezos
President,
Thalassaemia International Federation

Dr Androulla Eleftheriou
Executive Director,
Thalassaemia International Federation

[**Knowledge** is our **strength.**]

1 INTRODUCTION

The thalassaemias are inherited haemoglobin disorders characterized by defective synthesis of the α-globin (α-thalassaemia) or β-globin (β-thalassaemia) chains of adult haemoglobin A. A variety of β-thalassaemia phenotypes can result from heterozygous, compound heterozygous, or homozygous inheritance of β-globin gene mutations, or their co-inheritance with structural haemoglobin variants such as haemoglobin E or other secondary genetic modifiers [1-5]. Transfusion-dependence has recently become an essential factor in classifying the various phenotypes of β-thalassaemia. Patients requiring lifelong regular transfusion therapy for survival are considered as having transfusion-dependent β-thalassaemia (TDT), such as patients with β-thalassaemia major or sever haemoglobin E/β-thalassaemia. On the other hand, non-transfusion-dependent β-thalassaemia (NTDT) – the focus of these guidelines – is the term used **for patients who do not require lifelong regular transfusions for survival, such as patients with β-thalassaemia intermedia or mild-moderate haemoglobin E/β-thalassaemia;** although they may require occasional or even frequent transfusions in certain clinical settings and for defined periods of time **(Figure 1-1)** [4, 6].

There are several aspects to note for the NTDT/TDT classification. It mainly relates to clinically significant forms of β-thalassaemia, so phenotypes such as β-thalassaemia silent carrier or trait/minor are not considered among NTDT in the medical literature. The classification has mainly been adopted to move away from using the terms β-thalassaemia major and intermedia which may give a false impression of disease severity. It also allowed standardization of research and clinical management based on transfusion-requirement, a key driver in pathophysiology.

It should be stressed, however, that transfusion receipt is not always a measure of underlying disease severity but can also be attributed to access to blood products or reflect a physician or patient choice, with varying practices worldwide especially in patients with moderate phenotypes. In recent clinical trials, the use of the NTDT/TDT classification has commonly been associated with the patient's transfusion profile in the past six months (e.g., <6 red blood cell units denoting NTDT). This may be practical when taking immediate management decisions especially as relates to anaemia and iron overload. **However, the natural course of a patient's disease course should always be taken into consideration since many patients with NTDT go on to become TDT following permanent morbidity development while also some patients with TDT can become NTDT following interventions that decrease transfusion requirement [7].** Disease severity and prognostic scoring systems have been recently developed for β-thalassaemia but further validation and data from real-world feasibility may be needed before wide utilization [8, 9].

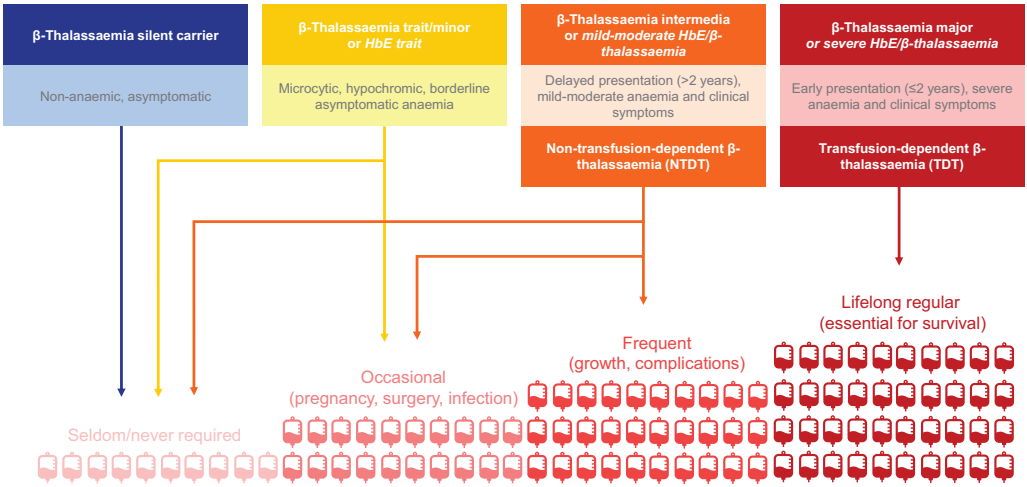

Figure 1-1. *Transfusion requirement in various β-thalassaemia phenotypes [4, 6]. Hb, haemoglobin.*

EPIDEMIOLOGY OF NTDT

Inherited haemoglobin disorders primarily exist in the low- or middle-income countries of the tropical belt stretching from sub-Saharan Africa, through the Mediterranean region and the Middle East, to South and Southeast Asia [10]. This is primarily attributed to the high frequency of consanguineous marriages in these regions as well as a conferred resistance of carriers to severe forms of malaria in regions where the infection had been or is still prevalent [10, 11]. Improvements in public health standards in these regions have also helped improve survival of affected patients. Moreover, continued migration has greatly expanded the reach of these diseases into large, multi-ethnic cities in Europe and North America [10, 12].

Data on the global epidemiology of the thalassaemias in general, and NTDT in specific, are scarce [12, 13]. Around 68,000 children are born with various thalassaemia syndromes each year [10, 14, 15]. β-thalassaemia is highly prevalent, with 80 to 90 million people reported to be carriers across the world (1.5% of the global population). Approximately half of these carriers originate from South East Asia [16]. Some 23,000 children are born with TDT each year, while a smaller ill-defined number have β-thalassaemia intermedia [1, 10, 15]. Irrespectively, β-thalassaemia intermedia has a varying prevalence in every population with a high frequency of β-thalassaemia carriers and is particularly common in parts of the Eastern Mediterranean and Africa where mild β-thalassaemia alleles predominate [1].

The highest prevalence of the structural variant haemoglobin E is observed throughout India, Bangladesh, Thailand, Laos, and Cambodia where carrier frequencies may reach as high as 80% [17, 18]; as well as through regions of China, Malaysia, Indonesia and Sri Lanka [10, 19]. Haemoglobin E/β-thalassaemia currently affects around 1,000,000 people worldwide [20]. In North America, it has become the most common form of β-thalassaemia identified in many new-born screening programs [21]. In California, 1 in 4 Cambodian births and 1 in 9 Thai/Laotian births are haemoglobin E carriers [22]. Globally, more than 19,000 children are born each year with haemoglobin E/β-thalassaemia, with half of them having a severe transfusion-dependent form (which represents around 30%-50% of all severe forms of β-thalassaemia [18]) while the remaining half fall into the category of NTDT (mild-moderate haemoglobin E/β-thalassaemia) [10, 15].

GENOTYPE-PHENOTYPE ASSOCIATIONS IN NTDT

Distinction of the various phenotypes of thalassaemia is mostly based on clinical parameters, although a genotype-phenotype association is established in both α- and β- thalassaemia syndromes [23].

β-Thalassaemia intermedia

In patients with β-thalassaemia intermedia, the primary modifier of phenotype is the broad diversity of mutations that usually affect the β-globin gene in the homozygous or compound heterozygous state (>300 disease-causing mutations) [2, 4, 24-26]. These range from mild promoter mutations that cause a slight reduction in β-globin chain production (mild β+) to those leading to more significant deficiency (β+) to the many different mutations that result in complete absence of β-globin chain synthesis (β0). Deletions of the β-globin gene are uncommon. Primary mutation severity has been linked to clinical outcomes in β-thalassaemia [27, 28]. The resulting free α-globin chains are unstable and generate cytotoxic reactive oxidant species and cellular precipitates that impair the maturation and viability of red-cell precursors, resulting in ineffective erythropoiesis and premature haemolysis of circulating red cells [29].

The diversity of mutations and the consequent variable degree of α/β-globin chain imbalance and ineffective erythropoiesis are the main determinants for milder anaemia in β-thalassaemia intermedia than β-thalassaemia major. Secondary modifiers are those that are involved directly in modifying the degree of α/β-globin chain imbalance including coinheritance of different molecular forms of α-thalassaemia [30], changes in ubiquitin-proteasome system or expression of α-haemoglobin stabilizing protein [29, 31-34], and effective synthesis of γ-chains and foetal haemoglobin in adult life.

Several genes have been uncovered which could modify γ-chain production and ameliorate phenotype, some that are encoded in the β-globin gene cluster (δβ0-thalassaemia or point mutations at A-γ or G-γ promoters), others that are on different chromosomes (BCL11A, KLF1, HBS1L-MYB) [26]. BCL11A has gained most attention as a key regulator of the foetal-to-adult haemoglobin switch and foetal haemoglobin silencing [35-38] Genetic variation in the expression of BCL11A and persistence of foetal haemoglobin production was shown to reduce clinical severity in β-thalassaemia [39, 40]. β-thalassaemia intermedia may

also result from the increased production of α-globin chains by a triplicated or quadruplicated α-genotype associated with β-heterozygosity [41-46]. Less commonly, only a single β-globin locus is affected, the other being completely normal, so in these instances, β-thalassaemia intermedia is dominantly inherited [47, 48]. Tertiary modifiers include polymorphisms that are not related to globin chain production but may have an ameliorating effect on specific complications of the disease like iron absorption, bilirubin metabolism, bone metabolism, cardiovascular disease, and susceptibility to infection [49, 50]. **Table 1-1** illustrates common genotypes leading to a β-thalassaemia intermedia phenotype [6].

***Table 1-1**. Genotype-phenotype associations in β-thalassaemia. Reproduced with permission from [6].*

Phenotype	Genotype	Clinical severity
Silent carrier	• silent β/β	• Asymptomatic • No haematological abnormalities
Trait/minor	• β^0/β, β^+/β, or mild β^+/β	• Borderline asymptomatic anaemia • Microcytosis and hypochromia
Intermedia	• β^0/mild β^+, β^+/mild β^+, or mild β^+/mild β^+ • β^0/silent β, β^+/silent β, mild β^+/silent β, or silent β/silent β • β^0/β^0, β^+/β^+, or β^0/β^+ **and** deletion or non-deletion α-thalassaemia • β^0/β^0, β^+/β^+, or β^0/β^+ **and** increased capacity for γ-chain synthesis • Deletion forms of δβ-thalassaemia and HPFH • β^0/β or β^+/β **and** $\alpha\alpha\alpha$ or $\alpha\alpha\alpha\alpha$ duplications • Dominant β-thalassaemia (inclusion body)	• Late presentation • Mild-moderate anaemia • Transfusion-independent • Clinical severity is variable and ranges between minor to major
Major	• β^0/β^0, β^+/β^+, or β^0/β^+	• Early presentation • Severe anaemia • Transfusion-dependent

HPFH, hereditary persistence of foetal haemoglobin.

Haemoglobin E/β-thalassaemia

Haemoglobin E is caused by a G-to-A substitution in codon number 26 of the β-globin gene, which produces a structurally abnormal haemoglobin and an abnormally spliced non-functional mRNA. Haemoglobin E is synthesized at a reduced rate and behaves like a mild β+-thalassaemia. Patients with haemoglobin E/β-thalassaemia co-inherit a β-thalassaemia allele from one parent, and the structural variant, haemoglobin E, from the other [30, 51]. Haemoglobin E/β-thalassaemia is further classified into severe (haemoglobin level as low as 4-5 g/dl, transfusion-dependent, clinical symptoms similar to β-thalassaemia major), moderate (haemoglobin levels between 6 and 7 g/dl, transfusion-independent, clinical symptoms similar to β-

thalassaemia intermedia), and mild (haemoglobin levels between 9 and 12 g/dl, transfusion-independent, usually do not develop clinically significant problems) clinical forms; with the latter two falling into the category of NTDT [52]. A disease scoring system that helps classify patients into mild, moderate, and severe has been proposed (**Table 1-2**) [53].

Table 1-2. *Mahidol score for haemoglobin E/β-thalassaemia severity classification. Reproduced with permission from [53].*

Criteria	Value	Score	Value	Score	Value	Score
Steady-state haemoglobin, g/dL	>7	0	6-7	1	<6	2
Age of onset, years	>10	0	2-10	0.5	<2	1
Age at 1st blood transfusion, years	>10	0	4-10	1	<4	2
Requirement for transfusion	None/rare	0	Occasionally	1	Regularly	2
Size of spleen, cm	<4	0	4-10	1	>10	2
Growth retardation	-	0	+/-	0.5	+, s/p	1

For each criterion, a score is given depending on the value. The total sum of all scores is then interpreted as follows: mild haemoglobin E/β-thalassaemia (severity score <4); moderate haemoglobin E/β-thalassaemia (severity score 4-7); and severe haemoglobin E/β-thalassaemia (severity score >7).

Similar to patients with β-thalassaemia intermedia, modifiers of disease severity in haemoglobin E/β-thalassaemia include the type of β-thalassaemia mutation, co-inheritance of α-thalassaemia and determinants that increase foetal haemoglobin production *(BCL11A and HBS1L-MYB),* as well as tertiary modifiers of complications like the inherited variability in the function of the gene for UDP-glucuronosyltransferase-1 underlying the more severe chronic hyperbilirubinemia and an increased occurrence of gallstones observed in some patients [19, 20, 30, 54-60]. It should be noted that patients with haemoglobin E/β-thalassaemia also show different phenotypic severity at particular stages of development. Advancing age has an independent and direct effect on the background level of erythropoietin production in response to anaemia [61-63]. A notable environmental factor influencing phenotype in patients with haemoglobin E/β-thalassaemia is infection with malaria, particularly *Plasmodium vivax* [64].

SCREENING AND LABORATORY DIAGNOSIS OF NTDT

The approach to screening for NTDT is dependent on the frequency of the specific mutations in the region, the available resources, cultural and religious issues, and the age of the targeted population. **Public awareness and education, public surveillance and population screening, extended family screening of first-born child, premarital screening and genetic counselling, prenatal diagnosis, and family planning are among the strategies commonly applied in screening programs.** It should be part of a generalized program to educate and screen the at-risk population for thalassaemia disorders and improve the quality of life and management of affected patients [10]. Although migration is occurring towards the richer, more developed countries, screening and prevention strategies need implementation in the countries

of origin. In areas with a high incidence of thalassaemia, universal screening of neonates is recommended for both α- and β-thalassaemia disorders [65, 66]. Advances in capillary electrophoresis and molecular testing improve the specificity and availability of diagnosing individuals outside the neonatal period [65, 66]. While the cost of specific testing has decreased, it is not yet universally applicable. Advances in algorithms utilizing red cell indices, haemoglobin, and reticulocyte count have a high sensitivity and specificity for both α- and β-thalassaemia mutations [67]. These discriminating formulas have excellent diagnostic efficacy and are very economical. The best initial screening approach is to combine finding from history and physical examination with analysis of red blood cells ((low mean corpuscular volume, low mean corpuscular haemoglobin level, and normal red-cell distribution width), followed by haemoglobin electrophoresis or high-performance liquid chromatography to confirm the diagnosis, and DNA analysis to confirm the genotype. The level of the minor haemoglobin component, haemoglobin A2, is nearly always elevated in β-thalassaemia carriers [13].

It should be noted that many patients co-inherit α- and β-thalassaemia mutations. The detection of an α-thalassaemia mutation therefore does not exclude the concomitant β-thalassaemia mutation. These observations are important for clinical prognosis as well as genetic counselling. Haemoglobin E testing with electrophoresis might be difficult since it migrates with many other β-globin variants. It is better separated on isoelectric focusing and high-pressure liquid chromatography. The optimal diagnosis of haemoglobin E disorders is thus DNA-based [68]. Algorithms to guide the clinical suspicion at primary care (**Figure 1-2**) and laboratory diagnosis of NTDT (**Figure 1-3**) have been recently published [69].

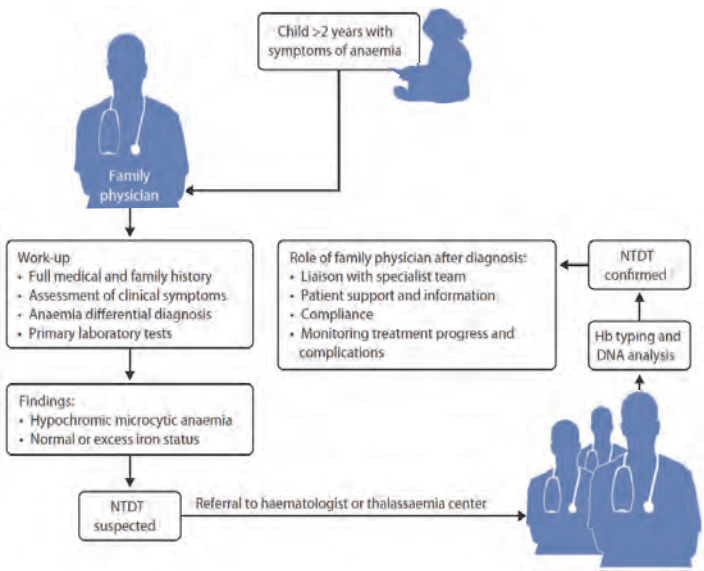

***Figure 1-2**. Clinical suspicion of non-transfusion-dependent β-thalassaemia (NTDT) at primary care. Reproduced with permission from [69]. Hb, haemoglobin.*

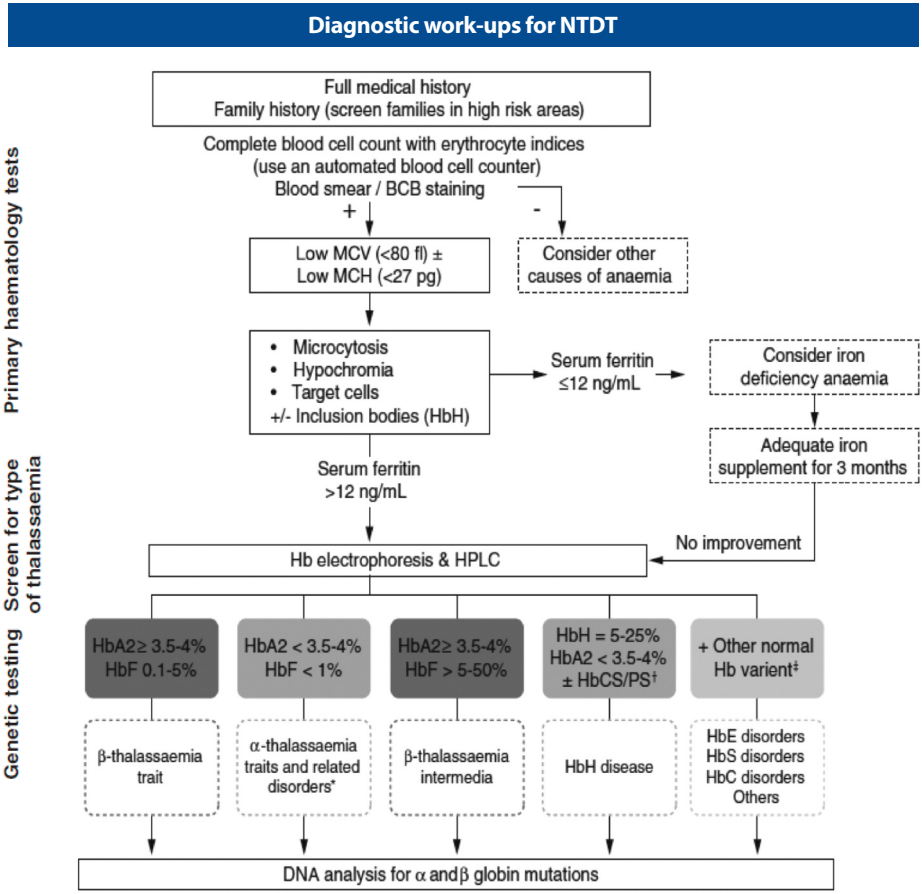

Figure 1-3. *Laboratory work-up to facilitate diagnosis of non-transfusion-dependent β-thalassaemia (NTDT). *α-thalassaemia traits and related disorders include α0 and α+-thalassaemia by deletions and non-deletional α-thalassaemia mutations. †There are two main types of HbH disease: 1) deletional HbH due to deletions (- -/-α) and; 2) non-deletional HbH disease caused by α0-thalassaemia and non-deletional mutation (--/αTα). ‡The common disorders associated with Hb structural variants include homozygous HbE, HbE/β-thalassaemia and HbE with other variants such as HbE/HbS or HbE/HbC or HbE/HbD, HbS (sickle), HbS/β-thalassaemia, homozygous HbC and HbC/β-thalassaemia. These diagnoses can be confirmed using appropriate globin genotyping. Reproduced with permission from [69]. Hb, haemoglobin; HbF, foetal haemoglobin; HbCS, haemoglobin Constant Spring; HbPS, haemoglobin Pakse; BCB, brilliant cresyl blue; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; HPLC, high-performance liquid chromatography.*

SURVIVAL IN NTDT

In a recent large global study of 2033 NTDT patients (mostly from Italy), the crude mortality rate was 5.6% over a median follow-up time of 33.9 years (median age at death was 46.3 years). Cumulative survival estimates by age 18, 50, 65, 75, and 85 years were 99.4%, 93.4%, 81.8%, 66.2%, and 25.4%, respectively – lower in comparison to the normal Italian population. **Cardiovascular disease**, mostly non-iron related, was the leading cause of early death (36.3%, at a median age of 34.2 years), while **hepatic disease** was the leading cause of death in older patients (20.4%, at a median age of 55.4 years) [70]. A recent study of patients with haemoglobin E/β-thalassaemia from Sri Lanka observed shortened survival compared with that reported in high-resource countries for β-thalassaemia major and intermedia not involving an allele for haemoglobin E, with severe anaemia and iron overload being the most notable risk factors [71].

SCOPE OF THESE GUIDELINES

In these updated guidelines, we continue to feature the most prominent pathophysiologic mechanisms and clinical morbidities commonly encountered in NTDT patients and provide practical recommendations for managing these morbidities. Our recommendations stem from the most recent evidence delivered through published observational studies or clinical trials. In areas where evidence is unavailable or insufficient, we provide management recommendations using our clinical expertise in treating NTDT patients. Moreover, with improved survival, NTDT patients are expected to experience common diseases of the older population such as malignancies and cardiovascular disease; thus work up for such disorders should follow conventional guidelines in non-thalassaemic patients [72-75]. Finally, in most instances, treatment of NTDT needs to be individualized and patient expectations need to be managed considering the false premise that NTDT represents a mild and clinically 'innocent' form of thalassaemia.

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2 INEFFECTIVE ERYTHROPIESIS AND ANAEMIA

In non-transfusion-dependent β-thalassemia (NTDT), erythropoiesis is ineffective due to the imbalance in the production of α- and β-globin chains. Unstable globin chain tetramers precipitate and undergo oxidation into methaemoglobin and haemichromes with eventual separation of haeme from globin. The free iron released from haeme disintegration in thalassemia erythroid cells eventually catalyses the formation of reactive oxygen species, which lead to oxidation of membrane proteins, structural membrane defects, and exposure of red-cell senescence antigens like phosphatidylserine causing premature cell death within the bone marrow (ineffective erythropoiesis) and early haemolysis for those released into the circulation [1-8].

Ineffective erythropoiesis in NTDT patients leads to a multitude of subsequent pathophysiology including chronic anaemia, primary iron overload (see Chapter 3), and hypercoagulability (see Chapter 4) (Figure 2-1) [6, 9-12]. Without appropriate treatment, the magnitude of pathophysiology and incidence of clinical morbidities increase with advancing age, especially as patient enter into adulthood [13-16].

Ineffective erythropoiesis and medullary expansion can directly lead to skeletal deformity and osteoporosis as well as extramedullary haematopoiesis [6, 9]; while chronic anaemia has been linked to morbidities affecting almost every organ system in children and adults [17-19]. In untreated patients with NTDT, a recent cross-sectional analysis identified a haemoglobin level of >10 g/dL as the best predictor for absence of morbidity (liver disease, extramedullary haematopoiesis, endocrine and bone disease, leg ulcers, thrombosis, pulmonary hypertension) [20]. In a more recent retrospective cohort study following untreated NTDT patients for 10 years, a haemoglobin level <10 g/dL was associated with a significantly worse (~4-fold) morbidity-free survival than ≥10 g/dL [20]. Overall survival was also significantly worse (~8-fold) in patients with a haemoglobin level ≤10 g/dL than those with >10 g/dL in a separate large global cohort [21]. Low haemoglobin levels have recently been recognized as a significant predictor for mortality in the Thalassemia International Prognostic Scoring System (TIPSS) [22]. Low haemoglobin levels have also been linked to cerebrovascular disease in NTDT patients [23]. Increases by 1 g/dL of haemoglobin were found to significantly ameliorate the risk of morbidity [24]. **These findings reflect the impact of chronic anaemia and hypoxia on tissue health in various organs. Moreover, anaemia is a direct marker of ineffective erythropoiesis and all its associated sequelae (primary iron overload and hypercoagulability) [25, 26].** For instance, a correlation between haemoglobin level and serum ferritin values has been observed in both cross-sectional and longitudinal studies [27, 28]. In addition to increased risks of long-term morbidity and mortality, chronic anaemia can also impact patients' well-being in the short-term with symptoms of fatigue and decreased exercise tolerance, leading to poor quality of life and mental health [12, 29-32].

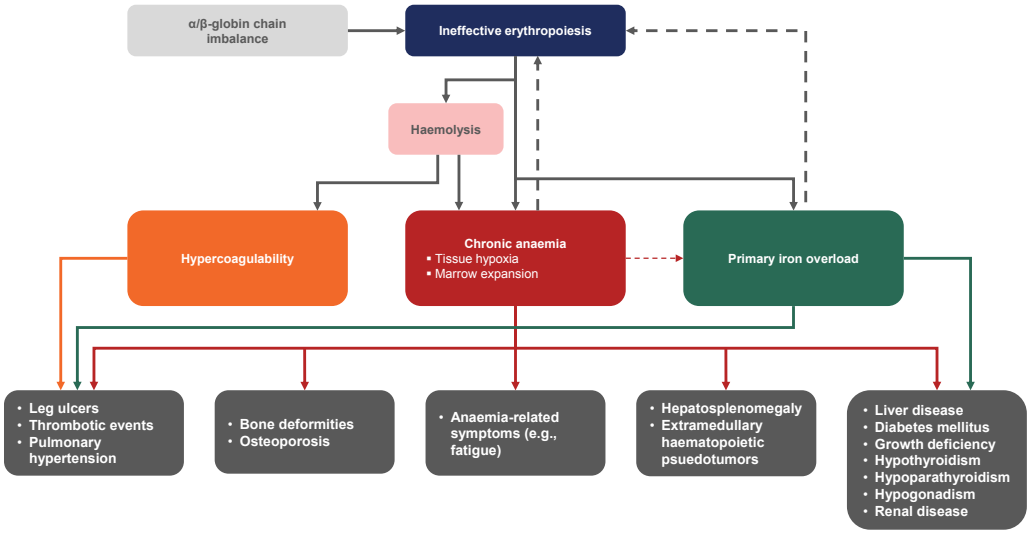

***Figure 2-1**. Ineffective erythropoiesis and anaemia at the core of pathophysiology and clinical complications in non-transfusion-dependent β-thalassemia (NTDT) [6, 9, 12].*

MANAGEMENT OF INEFFECTIVE ERYTHROPIESIS AND ANAEMIA

General principles

Despite transfusion-independence being an inherent characteristic of NTDT, it is now evident that associated anaemia needs to be regularly monitored with management indicated in various acute and chronic clinical settings. Irrespectively, management of chronic anaemia in patients with NTDT needs to be individualized with careful assessment of benefits and risks of intervention. As mentioned earlier, it is now established that patients with a haemoglobin level ≤10 g/dL are at highest risk of morbidity and mortality from chronic anaemia and underlying ineffective erythropoiesis, and thus may be most ideal candidates for long-term intervention to raise haemoglobin level and prevent future serious and often irreversible clinical complications [12]. Patients with any degree of anaemia combined with ineffective erythropoiesis/anaemia-related symptoms or complications may also be considered for short/limited-term intervention to raise haemoglobin levels and alleviate their symptoms, especially in instances where evidence of benefit is established.

In newly diagnosed children with NTDT, it is very essential to assess the patient carefully over the first few months after the diagnosis is established and not to embark on any long-term treatment modality, especially transfusion therapy, too hastily. Many patients with NTDT embark on a life of unnecessary treatment, particularly if they present with an unusually low haemoglobin level during a period of intercurrent infection.

Even if treatment has been administered in the acute clinical setting, immediate commitment to long-term intervention is not recommended. Instead, the patient's steady-state haemoglobin level and well-being, particularly with respect to activity, growth, development, and the early appearance of skeletal changes are the factors to be taken into consideration [33, 34].

Lastly, it should be stressed that management of anaemia in NTDT guided by improvement in haemoglobin level also reflects control of the underlying ineffective erythropoiesis and prevention of associated pathogenesis such as iron overload and hypercoagulability, which should ultimately have an impact on overall morbidity, mortality, and quality of life [12]. Patients who already have established iron overload and hypercoagulability should also be managed as described in **Chapter 3** and **Chapter 4**, respectively.

Transfusion therapy

Transfusions remain the ideal intervention in acute clinical settings that require immediate improvement of haemoglobin level or sustaining it in view of anticipated blood loss or physiologic changes, such as during acute infection, pregnancy, and surgery [6, 9].

Transfusion therapy is effective in supplying normal erythrocytes and suppressing ineffective erythropoiesis [35, 36]. Upon transfusion therapy, erythroid activity decreases to 1-2 times normal levels with pretransfusion haemoglobin values between 10 and 11 g/dL, 1-4 times normal levels with values between 9 and 10 g/dL, and 2-6 times normal levels for values between 8.6 and 9 g/dL [37]. There is a direct correlation between linear growth and development and the magnitude of transfusion therapy NTDT patients receive during childhood [38]. Although clinical trials evaluating the role of transfusion therapy in NTDT patients are lacking, cross-sectional studies have consistently showed that patients who were receiving regular transfusions had lower rates of leg ulcers, thrombotic events, pulmonary hypertension, silent brain infarcts, and extramedullary haematopoietic pseudotumours [18, 33, 39-43]. A recent analysis from Italy showed that NTDT patients who started transfusion therapy in adulthood had a slowed down progression of disease [44]. Another recent survival analysis of a global cohort of 2,033 patients with NTDT identified a subset of 254 patients (12.5%) who were eventually placed on regular transfusion programs, starting at a median age of 10 years. The remaining 1,779 patients received only sporadic or no transfusions at all. Survival was significantly worse in non-regularly transfused patients compared to regularly transfused patients for all-cause and cardiovascular disease-related mortality [38]. Such cardioprotective effects of regular transfusions in NTDT has also been reported from Oman [45].

Rather than enforcing a long-term transfusion regimen, regular blood transfusion, if initiated in patients with NTDT, should ideally be for a short/limited-term and can be tailored or withdrawn when the desired outcomes are achieved. Patients having long-term regular transfusions should be managed as per the guidelines for transfusion-dependent β-thalassemia (TDT) patients. **The concern with long-term regular transfusion therapy in NTDT patients is the risk of secondary iron overload and end-organ failure [46]. Transition to transfusion-dependence may also have an impact on patient's mental well-being and quality of**

life, as it may implicate disease progression and require complete lifestyle modification. The magnitude of transfusion load is directly linked to healthcare resource utilization as well as clinical and economic burden [47-49]. Patients with NTDT who begin transfusions as adults are also at very high risk for developing red cell alloimmunization and serious haemolytic transfusion reactions [50, 51]. The risk of alloimmunization is highest in splenectomised patients and during pregnancy [52-55].

Splenectomy

Splenectomy was previously a common practice in patients with NTDT to increase the haemoglobin level by 1-2 g/dL and avoid blood transfusions [9, 33, 34, 56, 57]. However, recent evidence detailed herein indicates multiple serious adverse events associated with splenectomy, which explains the decline in its use over the years in most Western countries, especially for patients who can be placed on regular transfusion therapy [58].

Peculiar abnormalities of platelets and red blood cells are believed to be the key factors causing a hypercoagulable state in patients with NTDT (see **Chapter 4**) [26, 59-61]. These abnormalities become more prominent following splenectomy considering the beneficial role of the spleen in scavenging these procoagulant platelets and red blood cells and thus putting this subgroup of patients at a higher risk of thrombotic and vascular events [62, 63].

For instance, about 80% of damaged red blood cells are removed extravascularly by macrophages present mainly in the spleen [64]. Moreover, it has been proposed that removal of the spleen in β-thalassemia patients with an absolute excess of α-chains is associated with a more notable persistence of damaged erythroblasts and erythrocytes in the blood stream compared to patients with defective β-chain production and a relative excess of α-chains [65]. Clinically, observational studies, especially in patients with β-thalassemia intermedia, confirm that splenectomised NTDT patients have a higher risk of venous thromboembolism (~5-fold), pulmonary hypertension (~4-fold), leg ulcers (~4-fold), and silent cerebral infarction than non-splenectomised patients [18, 39, 40, 43, 62, 63, 66-77]. Splenectomised β-thalassemia intermedia patients who experience thrombotic events are characterized by high nucleated red blood cell counts (≥300 x106/L) and platelet counts (≥500 x 109/L), are more likely to have a history of pulmonary hypertension, and to have never received any transfusions [78]. The median time to thrombosis following splenectomy is around eight years [78]. This delay indicates that thrombosis in splenectomised NTDT patients is not necessarily an acute complication, but a manifestation of a chronic underlying process, further emphasizing the need for long-term treatment modalities for prevention.

It has also been suggested that the spleen may be a reservoir of excess iron and may have a possible scavenging effect on iron free fractions including non-transferrin-bound iron, which may explain the higher serum level of this toxic iron species in splenectomised NTDT patients [79, 80], and the observation that splenectomised patients have a higher rate of iron-related organ morbidity than their non-splenectomised peers [18].

Splenectomy places NTDT patients of all ages at risk of morbidity and mortality due to infection [34, 81]. These infections could have an overwhelming, fatal course such as with meningitis and sepsis [82]. In older studies, the risk of postsplenectomy sepsis in thalassemia patients was increased more than 30-fold in comparison with the normal population [83]. Modern preventative measures, however, have reduced this risk but the overall impact of these measures is unclear. The pathogens most commonly associated with postsplenectomy sepsis are encapsulated organisms [84], particularly Streptococcus pneumoniae, Haemophilus influenza, and Neisseria meningitides. Infections with gram negative, rod-shaped bacteria, notably Escherichia coli, Klebsiella and Pseudomonas aeroginosa occur with increased frequency in asplenic patients and are often associated with high mortality. Protozoan infections due to Babesia have been implicated in a fulminant haemolytic febrile state in splenectomised patients. Malaria is also reportedly more severe in asplenic people and carries an increased risk of death [85]. **Characteristics of overwhelming postsplenectomy sepsis include the sudden onset of fever, chills, vomiting, and headache. The illness rapidly progresses to hypotensive shock and is commonly accompanied by disseminated intravascular coagulation. The mortality rate is approximately 50%, despite intensive supportive measures, and is highest amongst children [82].** Early intervention on the basis of clinical suspicion, even in the absence of many of the above findings, is critical. The risk of overwhelming postsplenectomy infection varies with age (risk is very high in children under two years of age), time since splenectomy (the greatest risk appears to be from one to four years after surgery), and immune status of patient. Vaccination against Streptococcus pneumoniae is a critical step in preventing overwhelming infection after splenectomy [86]. The protection rate with the 23-valent polysaccharide vaccine is 70-85%. The Haemophilus influenzae and meningococcal polysaccharide vaccines are also essential in the splenectomised patient [87]. Vaccination against the influenza virus helps prevent this febrile illness that might otherwise require intensive evaluation and management of a febrile episode in the splenectomised host [88].

Antibiotic prophylaxis with oral penicillin or other antibiotics reduces the risk of postsplenectomy sepsis. The optimal duration of antibiotic prophylaxis is still controversial with some clinicians continuously treating all splenectomised patients with prophylactic antibiotics, irrespective of age, while others treat patients whose spleens are removed after the age of five years only for the first two years. Irrespectively, antibiotic prophylaxis does not entirely prevent postsplenectomy sepsis. The risk of death from febrile illnesses remains and rapid evaluation of a febrile episode is critical [89].

When it comes to technique in indicated cases, such as in cases of hypersplenism or symptomatic splenomegaly, laparoscopic splenectomy has become the gold standard for the removal of the spleen. Patients who undergo laparoscopic splenectomy generally have lower rates of intraoperative blood loss, postoperative morbidity and mortality, a shorter length of hospital stay, as well as a more favourable body image and cosmesis than patients who undergo open splenectomy [90-93]. Nonetheless, doubts have been

raised regarding the suitability of laparoscopic splenectomy for patients with splenomegaly because of limited exposure and complex vascular control that could potentially lead to an increased risk of intraoperative bleeding and transfusion use. Recent studies, however, continue to confirm the comparativeness [92, 94-96] and even superiority [93, 97-100] of laparoscopic over open splenectomy even in patients with massive or supramassive spleens. There have also been concerns that laparoscopic splenectomy might increase the risk of developing splenic or portal vein thrombosis, which is relevant to patients with NTDT, as it reduces the blood flow in the portal system due to the pneumoperitoneum [101]. However, laparoscopic splenectomy is also associated with less postoperative modifications of coagulation parameters than open splenectomy. In fact, several studies confirm that the rate of postoperative venous thrombosis, including splenic or portal, remain similar in laparoscopic compared with open splenectomy, both in patients who received and those who do not receive anticoagulation [93, 102-105]. In some centres, partial splenectomy has been used to preserve some immune function while reducing the degree of hypersplenism [106, 107]. The long-term success of this approach is still under evaluation [108]. The likelihood of splenic re-growth and the volume of splenic tissue required to preserve immune function remain unknown. Reduction of splenic tissue by embolization is an alternative to complete or partial splenectomy [109]. This approach has not gained wide acceptance and may be complicated by fever, pain, and a subsequent need for splenectomy.

Removal of the gallbladder during splenectomy is a common practice, especially if stones are considered symptomatic. This is particularly important as cholecystitis can have serious consequences in the splenectomised patient [110]. Gallstones are more common in NTDT than in TDT patients due to increased haemolysis [111]. Unrelated genetic factors such as inherited variability in the function of the gene for UDP-glucuronosyltransferase-1 have also been reported to increase gallstone formation in patients with NTDT [9, 34, 56, 112-118].

Foetal haemoglobin induction

Increased production of the foetal β-like globin molecule, γ-globin, can bind excess α-chains to produce foetal haemoglobin, leading to improvements in α/β-globin chain imbalance, ineffective erythropoiesis, and associated anaemia [119]. Several genes are involved in modifying the γ-chain response, some that are encoded in the β-globin gene cluster, others that are on different chromosomes. Genome-wide association studies examining common variation in foetal haemoglobin levels identified the multi-zinc finger containing transcriptional regulator, BCL11A, as a key regulator of the foetal-to-adult haemoglobin switch and foetal haemoglobin silencing [120-123]. Data from several observational studies confirm that persistent production of foetal haemoglobin improves the clinical course in patients with NTDT [124-130]. Thus, the use of foetal haemoglobin inducers in patients with NTDT has been evaluated in several studies throughout the past couple of decades, with mixed experiences [119, 131].

Hydroxyurea (or hydroxycarbamide) is the foetal haemoglobin inducer for which most data in NTDT have been generated [132, 133]. Hydroxyurea is cytotoxic, antimetabolic, and antineoplastic agent that was identified as a potent foetal haemoglobin inducer [134], and became one of the key therapeutic agents for the management of patients with sickle cell disease. The exact mechanisms by which hydroxyurea induces foetal haemoglobin production are not fully understood.

A cytotoxic effect resulting in stress erythropoiesis with increased foetal haemoglobin levels occurring as a result is most commonly proposed [135]. More complex effects involving the production of nitric oxide and the soluble guanylyl cyclase and cyclic guanosine monophosphate-dependent protein kinase pathway gene have been proposed as being responsible for this activity [136-139]. Hydroxyurea therapy exerts a 2 to 9-fold increase in γ-mRNA expression in β-thalassemia patients [140-144]; leading to improvement in the α/non-α chain imbalance and more effective erythropoiesis [145]. There is good correlation between in vitro γ-mRNA fold increase and in vivo foetal haemoglobin fold increase [143, 146]; however, increases in foetal haemoglobin level did not always correlate with increases in total haemoglobin level in clinical studies. This may be best explained by findings from earlier studies showing increases in the α/β but not the α/γ biosynthetic ratio in β-thalassemia patients receiving hydroxyurea [147, 148]. Thus, in addition to its known effects in stimulating γ-globin production during stress erythropoiesis, hydroxyurea may have a more general role in augmenting globin synthesis, including β-globin in some NTDT patients who maintain the capacity to express normal β-globin chains [147].

In splenectomised patients with NTDT, there is also evidence that hydroxyurea diminishes phosphatidylserine externalization on the red cell [149]. Whether this is attributed to foetal haemoglobin induction and an associated decrease in α-globin aggregates remains to be elucidated [149]. Irrespectively, phosphatidylserine membrane exposure is not only associated with reduced red cell survival but also increased thrombin generation leading to hypercoagulability and subsequent morbidity in patients with NTDT [62]. Thus, hydroxyurea therapy theoretically has the potential of ameliorating the hypercoagulable state and subsequent vascular disease in patients with NTDT.

After early case reports documented haematological improvements in β-thalassemia patients treated with hydroxyurea, several studies evaluated the efficacy and safety of the drug in NTDT patients [119]. However, data mostly come from single-arm trials or retrospective cohort studies, and randomized clinical trials are lacking. Reported elevations in foetal haemoglobin level from baseline showed substantial variability, ranging between 1% and 90%, and averaging at 20% [119]. An association between the degree of foetal haemoglobin level increase and improved haematological outcomes was noted in some studies [150, 151], while others failed to document such an association, further supporting the idea that the effects of hydroxyurea in NTDT patients could extend beyond foetal haemoglobin induction [119]. In studies including NTDT patients, the primary haematological outcome was improvement in total haemoglobin level. Mean increases within studies ranged approximately between 0.5 and 2.5 g/dL with an average of around 1.5 g/L [119]; which is comparable to findings in patients with sickle cell disease [139, 152]. However, a high variance

is noted in total haemoglobin response in most studies, indicating that although some patients achieve considerable elevations, others have minimal or no change. The proportion of patients having total haemoglobin increases of >1.0 g/dL ranged between 40% and 70% [119]. Such increases may be essential since for example a difference between a severe and mild haemoglobin E/β-thalassemia patient is only 1-2 g/dL [153]. Improvement in anaemia was usually associated with better exercise tolerance, appetite, and sense of well-being [119].

Hydroxyurea therapy was also found to decrease the frequency of certain morbidities in patients with NTDT [133]. A beneficial role in patients with pulmonary hypertension was suggested, especially upon combination with the antioxidant L-carnitine [154-156]. Hydroxyurea therapy was also associated with improvements in leg ulcers [157] and extramedullary haematopoietic pseudotumours [42] in smaller studies. These findings are further confirmed through a large cross-sectional study of 584 β-thalassemia intermedia patients from the Middle East and Italy, where hydroxyurea therapy was associated with reduced adjusted odds of extramedullary haematopoietic pseudotumours (0.52, 95% CI: 0.30-0.91), pulmonary hypertension (0.42, 95% CI: 0.20-0.90), leg ulcers (0.10, 95% CI: 0.02-0.43), hypothyroidism (0.05, 95% CI: 0.01-0.45), and osteoporosis (0.02, 95% CI: 0.01-0.09) [18]. These effects were independent of total haemoglobin level or transfusion status, which further suggests that the benefit from hydroxyurea could extend beyond foetal haemoglobin induction and subsequent improvement of anaemia.

Responses in NTDT patients were observed at hydroxyurea doses ranging between 10 and 20 mg/kg/day, with most investigators opting to use a fixed low dose (10 mg/kg/day) while others escalated the dose according to toxicity (maximal tolerated dose) up to a maximum of 20 mg/kg/day [119]. These doses remain lower than those used in patients with sickle cell disease, which are often in excess of 20 mg/kg/day [139, 152]. Whether dose increments above 20 mg/kg/day could lead to more favourable responses warrants further study; however, one recent report suggests that a dose increase to 30 mg/kg/day in a small group of non-responsive patients did not provide any additional benefit [140].

Most studies of hydroxyurea in NTDT patients evaluated outcomes after 6, 12, or 24 months of therapy, although results from longer follow-up were also reported [119]. Response to hydroxyurea therapy was commonly noted in the first 3 to 6 months, with further improvements noted up to 12 months of therapy, and sustained responses observed over long-term follow up [119]. However, some studies noted a decline in haematological response beyond 12 months [146, 158]. As a result of these observations, it has been theorized that long-term treatment with hydroxyurea may result in impairment in the ability of certain haematopoietic stem cells to give rise effectively to erythroid lineage cells [146].

Alongside dose and duration of therapy, several other factors were assessed for their association with haematological response in patients with NTDT [119]. Findings regarding the roles of age and foetal haemoglobin level at the start of treatment are conflicting. Moreover, although some studies found certain

β-globin genotypes to be predictors of a favourable response, others failed to establish such an association. Similar discrepancies are noted for β-globin haplotypes. Patients with Lepore or δβ-thalassemia genotypes usually showed a better response. Co-inheritance of α-thalassemia was described as a predictor of good response in some studies but found to have no effect in others. Homozygosity for the XmnI polymorphism (–158 C→T $^{G}\gamma$) was a strong predictor of favourable responses, although the case was different in some studies especially those including patients with haemoglobin E/β-thalassemia. The rs766432 polymorphism at intron 2 of the BCL11A gene also correlates strongly with response to hydroxyurea therapy [119].

Hydroxyurea therapy was generally well tolerated at the doses used in NTDT studies, with some studies reporting no adverse events at all even with long-term therapy, as recently reviewed [119, 159]. The rate of myelotoxicity ranged between 2% and 30% while some studies did not report any haematological toxicities [119]. Myelotoxicity was usually dose-dependent, especially when doses >20 mg/kg/day were used, and could be reversed upon dose reduction [119]. The bone marrow of NTDT patients may be more sensitive to myelosuppression by hydroxyurea than occurs in other disorders, possibly due to medullary inflammation [119]. There are some reports of leukemic transformation in a β-thalassemia intermedia patient following three years of hydroxyurea therapy at 19 mg/kg/day [160]. The rate of gastrointestinal adverse events ranged between 1% and 30% [119]. Some studies also reported dermatological (hyperpigmentation, alopecia, maculopapular rash, or facial erythema) and neurological (headache or dizziness) adverse events on long-term therapy, although others did not observe such symptoms or attributed them to other disease-related risk factors [119]. No renal or hepatic side effects were reported with hydroxyurea therapy. Although some reports suggested that hydroxyurea may adversely affect gonadal function, others failed to document such an association even on long-term therapy [119]. Interestingly, two patients got pregnant while on hydroxyurea and delivered normally without any congenital malformations [119]. Nonetheless, evidence from patients with sickle cell disease points that hydroxyurea therapy can transiently decrease sperm numbers and viability [161].

In a recent Cochrane review on the use of hydroxyurea in NTDT, the authors concluded that 'there is no evidence from randomized controlled trials to show whether hydroxyurea has any effect compared with controls on the need for blood transfusion.' However, 'administration of 10 mg/kg/day compared to 20 mg/kg/day of hydroxyurea resulted in higher haemoglobin levels and seems safer with fewer adverse effects. Large well-designed randomized controlled trials with sufficient duration of follow up are recommended.' [162]

Other foetal haemoglobin inducers have also been evaluated in few small studies in NTDT patients [119]. A pilot study on five patients with β-thalassemia intermedia showed that **the subcutaneous demethylating agent decitabine** given at 0.2 mg/kg two times per week for 12 weeks increased total haemoglobin level by an average of 1 g/dL. Favourable changes in red blood cell indices were also noted and the drug was generally well-tolerated [163]. Similar effects were also seen in haemoglobin E/β-thalassaemia [164]. Favourable responses to **short-chain fatty acid (butyrate derivatives)** inducers of foetal haemoglobin in

small studies involving NTDT patients have also been documented, although effects were less notable on long-term therapy [165-169]. The orally bio available butyrate derivative 2,2-dimethylbutyrate sodium salt (HQK-1001) was also evaluated in a phase 2 study of 10 adults with NTDT. Foetal haemoglobin increased in all subjects, with peak increase occurring after a mean of 14 weeks of therapy and total haemoglobin increased in 7 subjects, with a mean increase of 4.7 g/L [170]. The use of recombinant human erythropoietin or the newer erythropoietic stimulating agent darbepoetin alfa in patients with NTDT is associated with increases in total haemoglobin level [171]. When such agents were combined with foetal haemoglobin inducers in NTDT patients, an additive effect on total haemoglobin augmentation was noted, although mostly at high doses [172, 173]. Thalidomide has also been associated with haematologic responses in patients with NTDT in observational studies and small trials from India and China, often in combination with hydroxyurea [131, 174, 175]. None of these agents currently have active/announced clinical development programs in NTDT.

More recently, **tovinontrine (IMR-687;** a potent, specific, and highly selective small molecule inhibitor of phosphodiesterase 9 which mediates cellular signalling pathways by degrading cyclic guanosine monophosphate to its inactive or monophosphate form) was shown to increase intracellular cGMP levels and stimulates the production of foetal haemoglobin as mostly evident in pre-clinical models of sickle cell disease [176]. A phase 2, randomized, double-blind, placebo-controlled study (FORTE) was initiated to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of IMR-687 administered once daily for 36 weeks in 120 adult subjects with NTDT or TDT (NCT04411082) [131]. The primary objective was to assess the safety and tolerability of IMR-687 while secondary objectives in NTDT patients included increase in total and foetal haemoglobin in the absence of a transfusions. Interim results showed no improvement in most disease-related markers and the program was discontinued through a company announcement in April 2022 [177].

Benserazide, a peripheral decarboxylase inhibitor used in patients with Parkinson's disease, was recently shown to activate the γ-globin gene transcription in a high throughput screen, and subsequent studies confirmed foetal haemoglobin induction in erythroid progenitors from haemoglobinopathy patients, transgenic mice, and anaemic baboons [178-181]. A phase 1bA sequential, open-label, dose-ranging study (BENeFiTS) is currently evaluating the safety, pharmacokinetics, and preliminary activity (total and foetal haemoglobin level) of benserazide in 36 adult patients with NTDT and a baseline haemoglobin of 6-10 g/dL (NCT04432623) [131].

Genome editing and insertion techniques aimed at expression of adult or foetal haemoglobin are currently only being developed in patients with TDT [131].

Novel agents targeting ineffective erythropoiesis and anaemia

Luspatercept: Luspatercept (ACE-536) is a recombinant fusion protein comprising a modified extracellular domain of the human activin receptor type IIB fused to the Fc domain of human IgG1. The domains bind to select transforming growth factor (TGF) β superfamily ligands, block SMAD2/3 signalling, and enhance

erythroid maturation [182, 183]. The murine analog of luspatercept, RAP-536, was shown to enhance erythroid maturation by restoring nuclear levels of the transcription factor GATA-1 in erythroid precursors [184]. In β-thalassemia mouse models, treatment with RAP-536 reduced α-globin chain aggregation and haemolysis, while increasing erythrocyte life span and improving iron overload [183]. Additionally, RAP-536 increased red-blood cell parameters, as well reduced comorbidities associated with β-thalassemia, such as decreased bone mineral density and splenomegaly [182]. A multicentre, open-label, dose-ranging phase 2 study of luspatercept in adults with β-thalassemia (NCT01749540, with 5-year extension NCT02268409) confirmed its safety and effectiveness in reducing transfusion requirement in TDT and improving haemoglobin level in NTDT [185, 186].

BEYOND (NCT03342404) was a phase 2, double-blind, randomized (2:1), placebo-controlled, multicentre study evaluating the efficacy and safety of luspatercept in 145 adult patients with NTDT and a haemoglobin level ≤10 g/dL [187]. Luspatercept was started at 1.0 mg/kg with titration up to 1.25 mg/kg, or reduction in **the event of toxicity or excessive haemoglobin concentration increase. The trial met its primary endpoint with 74 (77.1%) of patients in the luspatercept arm vs 0 placebo patients achieving a mean haemoglobin increase of ≥1.0 g/dL from baseline over a continuous 12-week interval during weeks 13-24 in the absence of transfusions (52.1% of patients on luspatercept actually had ≥1.5 g/dL increase) [187].** The key secondary endpoint was a change in a patient-reported outcome measure of tiredness/weakness specifically developed and validated for patients with NTDT (NTDT-PRO T/W) [188, 189]. Improvement in NTDT-PRO T/W favoured luspatercept and correlated with improvement in haemoglobin level [187]. The proportion of patients with serious adverse events was lower in the luspatercept group than in the placebo group (11 [12%] vs 12 [25%]). **Treatment-emergent adverse events most commonly reported with luspatercept were bone pain (35 [37%]), headache (29 [30%]), and arthralgia (28 [29%]). No thromboembolic events or deaths were reported during the study [187].** The application for regulatory approval in the USA (Food and Drug Administration) for the treatment of anaemia in NTDT was withdrawn in June 2022 for lack of agreement on benefit/risk [190].

However, luspatercept received European Commission approval in March 2023 as a treatment for adult patients with anaemia associated with NTDT [191]. Luspatercept is already approved in the USA and Europe for the treatment of anaemia in TDT based on data from the BELIEVE trial showing significant reduction in transfusion burden [192].

Mitapivat: Mitapivat (AG-348) is a first-in-class oral, small-molecule, allosteric activator of the red blood cell-specific form of pyruvate kinase (PK) which has shown efficacy and safety and received approval in the USA and Europe for the treatment of anaemia in adult patients with PK deficiency [193]. In thalassemia mouse models, it reduced markers of ineffective erythropoiesis and improved anaemia, red blood cell survival, and indices of iron overload [194]. Data have recently become available from an open-label, multicentre, phase 2 study of mitapivat in 20 adults with non-transfusion-dependent thalassemia (median age 44 years, 50% identifying as Asian; 15 with β-thalassemia and 5 with α-thalassemia) and a haemoglobin level ≤10 g/dL

evaluating safety and efficacy in achieving a haemoglobin increase by ≥1.0 g/dL. Sixteen (80%) patients had a response (5/5 in α-thalassemia and 11/15 in β-thalassemia). Favourable changes in markers of erythropoiesis and haemolysis were also noted. The most common treatment-emergent adverse events were initial insomnia (50%), dizziness (30%), and headache (25%) [195]. Mitapivat (100 mg orally, twice daily) is currently being evaluated in two phase 3 trials in non-transfusion-dependent and transfusion-dependent thalassemia [196]. ENERGIZE (NCT04770753) is a phase 3, double-blind, randomized, placebo-controlled, multicentre, phase 3 trial conducted in adult patients with non-transfusion-dependent thalassemia (α- and β-thalassemia) which plans to enrol 171 patients over 24 weeks with an open-label extension for 5 years.

The primary endpoint is haemoglobin response defined as a ≥1.0 g/dL increase in average haemoglobin concentration from week 12 through week 24 compared with baseline. Changes in Functional Assessment of Chronic Illness Therapy (FACIT) - Fatigue Subscale will also be assessed [197].

Other agents: Several other agents have also been evaluated for improvement of ineffective erythropoiesis and anaemia in NTDT. The oral agent **bitopertin (RO-4917838)**, a potent and selective glycine transporter 1 (GLYT1) inhibitor, resulted in reduced anaemia and haemolysis, enhanced in vivo survival of erythrocytes, and diminished ineffective erythropoiesis in β-thalassemia mice models [198]. However, a proof-of-mechanism phase 2 study of bitopertin in adults with NTDT (NCT03271541) was prematurely terminated due to lack of benefit in preliminary efficacy analysis (mean total haemoglobin reduction) [199].

Agents targeting the hepcidin or ferroportin pathways have also been evaluated for improvement in anaemia in NTDT considering the bidirectional relationship between iron dysregulation and ineffective erythropoiesis [25].

Anti-sense oligonucleotides (ASO) and small interfering RNA (siRNA) targeting downregulation of transmembrane serine protease 6 (TMPRSS6) have been effectively used to stimulate hepcidin expression, reduce iron burden, and improve ineffective erythropoiesis and red blood cell survival in mouse models of NTDT, with synergistic effects noted with the use of iron chelation therapy [200-209].

Sapablursen (IONIS-TMPRSS6-LRx) is a generation 2+ ligand-conjugated ASO targeting TMPRSS6. It is delivered as a subcutaneous drug (given every 4 weeks). It is being evaluated in a randomized, open-label, phase 2 trial (NCT04059406) in 36 adults with NTDT and baseline haemoglobin ≤10 g/dL for its effect on increasing haemoglobin level by ≥1.0 g/dL and decreasing liver iron concentration [131]. No data are currently publicly available. **SLN124** is a GalNAc conjugated double-stranded fully modified siRNA targetting TMPRSS6 messenger RNA. It is delivered as a subcutaneous drug and is being evaluated in a phase 1, randomized, single-blind, placebo-controlled, single-ascending and multiple-dose study (GEMINI II, NCT04718844) in 112 adults with non-transfusion-dependent thalassemia (α- and β-thalassemia) and myelodysplastic syndromes to investigate safety and measure effects on hepcidin, iron parameters, and

haemoglobin level [131]. No data are currently publicly available. **Vamifeport (VIT-2763)** is a small molecular weight oral ferroportin inhibitor which competes with hepcidin for binding to ferroportin, displaces hepcidin bound to recombinant ferroportin, and reducing cellular iron efflux. In NTDT mouse models, it restricted iron availability, ameliorated anaemia, and reversed the dysregulated iron homeostasis [210, 211]. In a phase 1 study in 72 healthy adult volunteers, vamifeport administered at single oral doses up to 240 mg or multiple oral doses up to 120 mg twice daily was well tolerated compared with placebo [212]. VITHAL (NCT04364269) is a randomized, double-blind, placebo-controlled, phase 2a trial evaluating the safety and preliminary efficacy of vamifeport in improving haemoglobin and iron indices in 36 adult NTDT patients with a baseline haemoglobin of ≤11 g/dL. Recent data from 25 patients (vamifeport once daily n=9, twice daily n=12, placebo n=4) treated over 12 weeks indicated a favourable safety and tolerability profile and showed promising target engagement and pharmacodynamic effects on serum iron and transferrin saturation but no clinically significant effects on haemoglobin level [213]. Continued development plans of all three agents in NTDT are currently unclear.

Data on combinations of several agents targeting ineffective erythropoiesis may be needed to understand the ideal approach to fully control or reverse the underlying disease process in NTDT [202, 214].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. Curative therapies such as bone marrow transplantation and gene manipulation techniques have not been evaluated in patients with NTDT.
2. Unless contraindicated, blood transfusions can be considered to manage anticipated haemoglobin drop in acute clinical settings such as during acute infection, pregnancy, blood loss, or surgery.
3. Patients with NTDT should be considered for short/limited- or long-term intervention targeting ineffective erythropoiesis and anaemia according to the patient profiles summarized in **Table 2-1.**
4. Luspatercept should be used according to the local product prescribing information for indication, dosing, response, and adverse event monitoring and management.
5. When transfusion therapy is considered:
    a. Monitoring and management of iron overload (see **Chapter 3**)
    b. The risk of alloimmunization should be considered, especially in the following subgroups of patients: pregnant women, splenectomised patients, never or previously minimally transfused patients
    c. Blood processing and administration characteristics should be similar to those applied in TDT, generally:
        i. Blood storage for <2 weeks, conditioning to achieve mean 24-hour post-transfusion red blood cell survival ≥75%
        ii. Leucoreduced packed red blood cells (≤1 x 10^6 leucocytes/unit) with haemoglobin content ≥40 g (pre-storage filtration preferred)

iii. ABO and Rh(D) matched blood
iv. Rh (C, c, E, e) and Kell matching highly recommended
v. Appropriate infections and viral vaccinations and screening of donor and recipient

6. When hydroxyurea is considered:
   a. It should be used at a starting dose of 10 mg/kg/day with dose escalation by 3-5 mg/kg/day every 8 weeks to the maximal tolerated dose, but not exceeding 20 mg/kg/day. Concomitant folic acid supplementation is recommended
   b. The following safety measures should be evaluated and treatment discontinued or tailored accordingly. These include
      i. Complete blood counts, every two weeks for the first three months then monthly
      ii. Hepatic and renal function studies, every two weeks for the first three months then monthly
      iii. History and physical examination evaluating for gastrointestinal, neurologic, or dermatologic side-effects, monthly
      iv. Gonadal function follow-up
      v. Hydroxyurea should not be used in pregnant women or patients with hepatic or renal failure
7. The spleen size should be examined in clinical visits and splenectomy should generally be avoided in NTDT patients younger than 5 years of age, and otherwise reserved for cases of:
   a. When other interventions to manage anaemia are contraindicated
   b. Hypersplenism leading to worsening anaemia, leukopenia, or thrombocytopenia and causing clinical problems such as recurrent bacterial infections or bleeding
   c. Splenomegaly accompanied by symptoms such as left upper quadrant pain or early satiety
   d. Massive splenomegaly (largest dimension >20 cm) with concern about possible splenic rupture
8. When splenectomy is considered:
   a. Post-splenectomy sepsis remains a risk in all splenectomised thalassemia patients. Therefore, febrile splenectomised patients should undergo rapid evaluation and treatment
   b. Splenectomised patients should receive the following vaccines
      i. Pneumococcal 23-valent polysaccharide vaccine
         - It can be given subcutaneously or intramuscularly two weeks prior to splenectomy and then three to five years later
         - Children vaccinated under the age of two should be re-vaccinated at age two
         - Patients who underwent splenectomy without being given the vaccine may still benefit from vaccination postsplenectomy
      ii. Haemophilus influenzae vaccine
         - If not administered as part of routine childhood immunizations, it should be given to patients before they undergo splenectomy
         - Patients who underwent splenectomy without being given the vaccine may still benefit from vaccination postsplenectomy

iii. Meningococcal polysaccharide vaccine
   - It should be given to patients before they undergo splenectomy
   - Patients who underwent splenectomy without being given the vaccine may still benefit from vaccination postsplenectomy

iv. Influenza vaccine, annually

v. Covid-19 vaccine as per local standards

c. Splenectomised patients should receive prophylactic antibiotic therapy for at least two years following splenectomy:

   i. Longer durations may be applied at the discretion of the treating physician, especially in very young children which should be covered until older than five years of age

   ii. Oral penicillin, 125 mg twice daily for children under two years, and 250 mg twice daily for children two years and over is recommended

   iii. Alternative antibiotics for patients unable to take penicillin include amoxicillin, trimethoprim-sulfamethoxazole, and erythromycin

   iv. The importance of compliance with prophylactic antibiotic therapy should be stressed repeatedly to patients and parents while explaining that it does not entirely prevent postsplenectomy sepsis and immediate presentation in cases of febrile illness is essential

d. The gall bladder should be inspected and removed during splenectomy if there is evidence of gallstones. A liver biopsy may also be considered at the time of splenectomy

e. Laparoscopic splenectomy is preferred to the open procedure unless otherwise indicated by the responsible surgeon

f. For recommendations on thromboprophylaxis relevant to splenectomised patients see **Chapter 4.**

***Table 2-1**. Considerations for management of ineffective erythropoiesis and anaemia in non-transfusion-dependent β-thalassaemia (NTDT).*

Haemoglobin level	Ineffective erythropoiesis/ anaemia-related symptoms or morbidities	Ineffective erythropoiesis/ anaemia-related intervention considerations and treatment objectives*
<10 g/dL	No	Long-term intervention to raise haemoglobin level by ≥1 g/dL and prevent symptoms or morbidities
	Yes	• Short/limited-term intervention to reverse/alleviate symptoms or morbidities per physician's judgement, and • Long-term intervention to raise haemoglobin level by ≥1 g/dL and prevent progression or recurrence of symptoms or morbidities
≥10 g/dL	No	None
	Yes	• Short/limited-term intervention to reverse/alleviate symptoms or morbidities per physician's judgement, and • Long-term intervention to prevent progression or recurrence of symptoms or morbidities per physician's judgement
Guide		
Ineffective erythropoiesis/anaemia-related interventions		• Luspatercept (in patients ≥18 years) • Blood transfusion (careful consideration of secondary iron overload [especially in patients with iron-related morbidity such as hepatic and endocrine disease] with long-term intervention and risk of alloimmunization) • Hydroxyurea (in patients with *XmnI* polymorphism or Lepore or δβ-thalassemia, careful consideration of adverse events and loss of response with long-term intervention) • Clinical trials
Response		• Achievement of treatment objective to be evaluated at 3-6 monthly intervals or per physician's judgement
Ineffective erythropoiesis/ anaemia-related symptoms or morbidities		• Fatigue, tiredness, weakness, shortness of breath, poor exercise tolerance, poor development, poor performance at work/school, diminished quality of life, diminished mental well-being • Growth failure (height is more indicative of growth pattern than weight), failure of secondary sexual development in parallel with bone age, thrombotic disease, cerebrovascular disease, pulmonary hypertension with or without secondary heart failure, extramedullary haematopoietic pseudotumours, hepatosplenomegaly, leg ulcers, osteoporosis or skeletal changes, endocrine disease, liver disease, renal disease

**In conjunction with other management and prevention strategies indicated for specific symptoms or morbidities.*

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3 IRON OVERLOAD

In non-transfusion-dependent β-thalassaemia (NTDT), ineffective erythropoiesis and hypoxia lead to inappropriately low hepcidin levels, increased intestinal iron absorption, and primary iron overload [1-3]. Erythroferrone, a hormone secreted by erythroblasts as a consequence of EPOR/JAK2/STAT5 pathway activation, has been identified as the main erythroid regulator of this process [4, 5]. Clinical studies also illustrated correlation between erythron expansion biomarkers (soluble transferrin receptor and nucleated red blood cells) and iron overload biomarkers in NTDT [6]. Another independent factor involved in hypoxia-induced hepcidin suppression is platelet-derived growth factor-BB (PDGF-BB) [7, 8]. Growth differentiation factor 15 (GDF15) and twisted-gastrulation 1 (TWSG1) have also been proposed as potential erythroid regulators of hepcidin, but their roles were debated [9-13]. A correlation between the severity of chronic anaemia and primary iron overload has also been illustrated in NTDT patients in cross-sectional and longitudinal studies [14, 15]. Regardless of the signalling mechanism, the end result is suppression of hepcidin levels, increased intestinal iron absorption, and increased release of recycled iron from the reticuloendothelial system [16]. This in turn leads to depletion of macrophage iron and preferential portal and hepatocyte iron loading (increased liver iron concentration) with relative low levels of serum ferritin [17], and subsequent

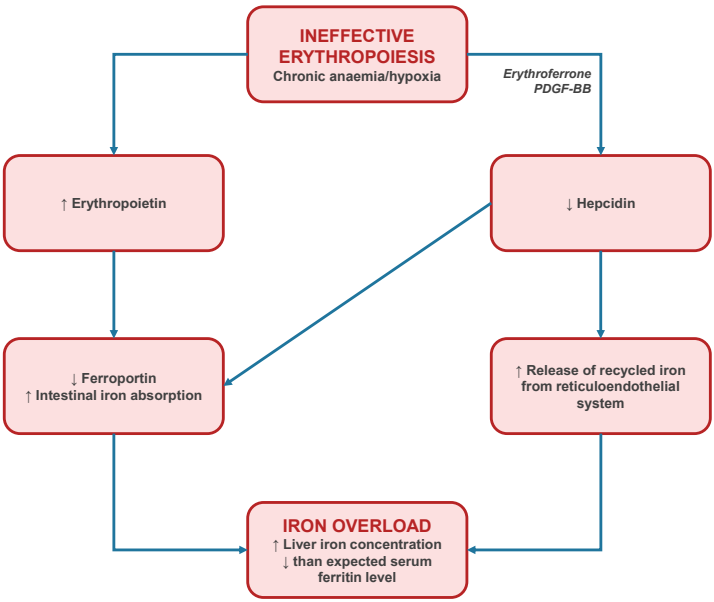


***Figure 3-1**. Mechanism of primary iron overload in non-transfusion-dependent β-thalassaemia (NTDT) [19, 20]. PDGF-BB, platelet-derived growth factor-BB.*

release into the circulation of free iron species that can cause target-organ damage [18]. Occasional transfusions can also lead to added iron burden, while in patients who end up on long-term regular transfusion programs will mainly suffer from secondary iron overload similar to patients with transfusion-dependent β-thalassaemia (TDT) [19]. The mechanism of iron overload in NTDT patients is illustrated in **Figure 3-1** [19, 20].

The accumulation of iron from intestinal absorption in NTDT patients is slower than that observed in transfusional siderosis and may reach 3-4 mg/day or as much as 1,000 mg/year [21]. A mean annual increase in liver iron concentration of 0.38 ± 0.49 mg Fe/g dry weight was observed in clinical trials including NTDT patients receiving placebo [22]. Nonetheless, iron overload in NTDT patients is a cumulative process as evident from longitudinal studies [23] and cross-sectional studies documenting positive correlations between iron overload indices and older age [18, 24-26]. Thus, a considerable proportion of NTDT patients eventually accumulate iron to liver iron concentration thresholds of clinical significance [17, 22, 27-29], and can start experiencing iron-related morbidity beyond 10 years of age [21, 24].

Although cardiac siderosis is a major cause of morbidity and mortality and a key factor in management decisions in patients with TDT, it does not seem to be a major concern in NTDT patients, even those with considerably elevated total body iron [30-33]. However, an association between iron loading evident form longitudinal elevations in serum ferritin level and worsening of hepatic function and fibrosis has been confirmed [34-36]. Reports documenting the occurrence of hepatocellular carcinoma in viral hepatitis negative patients with NTDT and iron overload also continue to emerge [37-44].

Liver disease remains one of the leading causes of death in this patient population [45]. Moreover, in a study of 168 non-chelated patients with NTDT, higher liver iron concentration values on magnetic resonance imaging were associated with a significantly increased risk of developing thrombosis, pulmonary hypertension, hypothyroidism, hypogonadism, and osteoporosis [28]. Liver iron concentration levels ≥5 mg Fe/g dry weight were associated with a considerable risk of morbidity and mortality in both patients with β-thalassaemia intermedia and haemoglobin E/β-thalassaemia [46, 47].

A more recent longitudinal follow-up over a 10-year period confirmed these findings, and **a serum ferritin level of ≥800 ng/mL was the threshold after which all patients became at risk of developing morbidity**, while patients with values ≤300 ng/ml did not develop any morbidity [23]. An independent global database also identified a serum ferritin level of >800 ng/mL as an independent risk factor for death in NTDT [48]. An association between iron overload and renal glomerular and tubular dysfunction as evident from proteinuria has also been reported in NTDT patients [49, 50], with some patients observed to progress to end-stage renal disease [51]. Several studies have also documented a high prevalence of silent brain infarction, large cerebral vessel disease, and decreased neuronal function primarily in NTDT patients; and such findings were more prevalent in patients with iron overload [52-54].

An association between iron overload and bone marrow stress with the development of haematologic malignancies has also been suggested but not confirmed [55, 56]. Collectively, these observations clearly demonstrate that iron overload in NTDT patients should be promptly diagnosed and managed to prevent the occurrence of serious clinical morbidities.

ASSESSMENT OF IRON OVERLOAD IN NTDT

The same modalities available for the assessment of iron overload in transfusion-dependent β-thalassaemia major patients have been used in NTDT studies. Assessment of liver iron concentration remains the gold standard for quantification of total body iron [57]. Liver iron concentration in NTDT patients has been measured directly by needle biopsy [17]; however, several risks are associated with the procedure. The most common adverse event with liver biopsy is pain at the needle site. More serious complications can include haemorrhage or sepsis, although these are rare [58]. Moreover, liver iron accumulation has been shown to be uneven in cirrhotic patients, resulting in a risk of sampling error [59-61]. Furthermore, different tissue processing methods can produce variable liver iron concentration measurements [62]. Studies evaluating liver iron concentration in NTDT patients using magnetic resonance imaging are numerous [21]. Magnetic resonance imaging using either hepatic R2 or T2* pulse sequences are reliable, internationally reproducible, and non-invasive methods for assessing liver iron concentration, and have been validated against liver biopsy [63-71].

The upper limit to reliably estimate liver iron concentration by magnetic resonance imaging is approximately 30–40 mg Fe/g dry weight, depending on the scanner specifications [64]. Of note, the T2* technique was originally developed to estimate myocardial iron but the first description of the method also demonstrated a clear relationship between liver T2* and liver iron concentration measured by biopsy [72]. However, it was later evident that the original T2* method underestimated liver iron concentration by a factor of about two-fold, and a new calibration showing acceptable linearity and reproducibility over a liver iron concentration range up to 30 mg Fe/g dry weight was demonstrated [73]. Devices that estimate the magnetic susceptibility can also be used to quantify liver iron concentration non-invasively. The superconducting quantum imaging device and the magnetic iron detector are such devices [29]. However, their use is usually limited by availability. In addition, superconducting quantum imaging device is not particularly accurate for measurements of liver iron concentration ranging between 3 and 10 mg Fe/g dry weight. Newer devices, such as the room-temperature magnetic iron detector offer promise for low-cost, non-invasive quantification of liver iron concentration in the future. Cardiac siderosis is measured using T2* magnetic resonance imaging, and the technique is now validated as a true measure of cardiac iron, correlating with chemical measurement on post-mortem cardiac biopsies [74]. However, as previously mentioned, current evidence suggests that patients with NTDT are less likely to show iron deposits in the heart at any liver iron concentration value [30-33].

In resource-poor countries, serum ferritin measurement may be the only method available for the assessment of iron overload. Observational studies continue to confirm a positive correlation between serum ferritin level and liver iron concentration in NTDT patients [22, 25, 29, 75]; however, the ratio of serum ferritin to liver iron concentration is lower relative to patients with TDT [17, 25, 29, 31, 76]. Thus, spot measurements of serum ferritin level may underestimate iron overload and delay therapy in patients with NTDT if they are to be interpreted like with TDT patients [20, 21] (for e.g., the projected liver iron concentration from a serum ferritin level of 1000 ng/mL would be approximately 9 compared to 15 in a TDT vs. NTDT, respectively [25]). Data on the practical use of other iron overload indices, such as transferrin saturation or non-transferrin-bound iron in NTDT patients are still limited [21].

MANAGEMENT OF IRON OVERLOAD IN NTDT

Phlebotomy is not an option in NTDT considering that the disease is already complicated with anaemia. Some simple measures may be of benefit, like tea consumption which decreases iron absorption and has antioxidant properties [77-79]. Curcumin supplementation has also been linked to decreased iron burden and oxidative stress in NTDT [80, 81].

However, iron chelation therapy is an inevitable option in iron overloaded patients with NTDT. As iron overload has been an 'overlook' condition in NTDT in the past, only few, mostly small, studies determined the efficacy and safety of iron chelation therapy in NTDT patients, with larger clinical trials only recently becoming available [82, 83]. An overview of previous studies investigating iron chelation therapy in patients with NTDT is shown in **Table 3-1** [22, 79, 84-94].

Subcutaneous deferoxamine therapy was the first iron chelator to be studied in NTDT patients since 1980s [93, 94]. Both studies, although with a very small number (total of 14) of patients, demonstrated that deferoxamine could generate significant urinary iron excretion in the majority of enrolled patients (mainly β-thalassaemia intermedia). However due to a limitation of iron overload evaluation, in particular tissue iron monitoring, and a short study duration, an appropriate chelation regimen with optimal dosage, duration, and administration interval to achieve clinical efficacy of deferoxamine on iron overload in NTDT remained unclear. Moreover, due to the cumbersome subcutaneous administration of deferoxamine together with pain and inconvenience, it might be difficult for NTDT patients in whom iron overload has been treated as a 'silent' but 'morbid' condition to accept and comply for such chelation regimen. Indeed, recent data from studies of deferasirox in patients with NTDT [86, 87], noted that recruited patients have not been successfully treated previously with deferoxamine due to poor compliance (sporadic use). **Thus, there could be a challenge for deferoxamine therapy to be evaluated in clinical trials and data to be translated into real clinical practice for patients with NTDT, although real-world evidence studies indicate effectiveness in decreasing liver iron concentration [95]. Therefore, the use of oral iron chelators for iron overload management in NTDT patients seemed to be logically advantageous to deferoxamine and considered more preferable for adoption in the clinics.**

Previous investigational studies have shown reduction in serum ferritin level in NTDT (mainly haemoglobin E/β-thalassaemia) patients using the oral iron chelator deferiprone (total 39 patients) [89, 90]. However, only nine patients were demonstrated to reduce liver iron concentration by direct liver biopsy [90]. Interestingly, removal of iron haemichrome from erythrocyte cell membranes and an increase in erythropoietin production was noted in some patients. These might be associated with prolongation of red blood cell survival and improvement of ineffective erythropoiesis, respectively, resulting in reduction of any transfusion requirement in some patients during the course of the study [90]. One splenectomised Thai haemoglobin E/β-thalassaemia patient died during the study period from inter-current infection [90]. Another four patients with β-thalassaemia intermedia have been previously treated with deferiprone and showed reduction in iron overload indices [91, 92]. More recently, a randomized, open-label trial compared 47 thalassaemia intermedia (phenotypes not listed) patients receiving deferiprone (75 mg/kg/day) to 41 patients receiving deferoxamine (50 mg/kg/day over 5 days – deferasirox was not approved at that time). All patients had to have baseline serum ferritin 800-3000 ng/mL and be ≥13 years old. The study showed comparable reduction of serum ferritin over 5 years; although it should be noted that patients had a considerable transfusion history and chelator doses used are more commonly used for TDT patients. The major adverse events observed included gastrointestinal symptoms and joint pain or arthralgia. Neutropenia and agranulocytosis were also detected [88].

Promising results with the oral iron chelator deferasirox in small studies recruiting NTDT patients (β-thalassaemia intermedia) were observed [86, 87]. Subsequently, data from the largest and first randomized clinical trial of iron chelation therapy in 166 patients with non-transfusion-dependent thalassaemia (β and α) became available (THALASSA) [22]. The trial showed that deferasirox dispersible tablets (DT) therapy results in significant reduction of liver iron concentration compared with placebo following 12 months of therapy in patients ≥10 years of age and a baseline liver iron concentration ≥5 mg Fe/g dry weight. Liver iron concentration decreased by a mean of 2.33 ± 0.70 and 4.18 ± 0.69 mg Fe/g dry weight in patients receiving starting doses of 5 mg/kg/day and 10 mg/kg/day, respectively. Doses were doubled at 24 weeks for patients with liver iron concentration >7 mg Fe/g dry weight and <15% reduction from baseline; and were suspended when liver iron concentration was <3 mg Fe/g dry weight at any visit. The frequency of adverse events in patients receiving deferasirox DT was similar to placebo. The most common drug-related adverse events were nausea (6.6%), rash (4.8%), and diarrhoea (3.6%) [22]. The analyses also showed that greater reductions in liver iron concentration were achieved in patients who were dose-escalated at 6 months from deferasirox DT 10 mg/kg/day starting dose to 20 mg/kg/day. The deferasirox DT safety profile remains consistent as patients approach the chelation interruption target of liver iron concentration 3 mg Fe/g dry weight [96].

Table 3-1. *Key studies evaluating iron chelation therapy in non-transfusion-dependent β-thalassaemia (NTDT) patients.*

Study	Disease type	Description				Results
		Drug investigated	N (age, years)	Design	Study objectives	
Deferasirox						
Taher 2016, Lai 2022 [84, 99] (THETIS)	NTDT (including α-thal)	Deferasirox DT (starting dose 10 mg/kg/day) with escalation at 4 weeks (max 20 mg/kg/day) or 24 weeks (max 30 mg/kg/day)	134 (≥10)	Prospective, single-arm, open-label trial	Efficacy (measured by change in LIC and SF) and safety of deferasirox over 52 weeks in iron overloaded patients with NTDT (Core study) with five year extension (ongoing)	Significant† decrease in both LIC and SF after 12 months, maintained up to 5 years
Taher 2012, 2013 [22, 85] (THALASSA)	NTDT (including α-thal)	Deferasirox DT (starting dose 5 or 10 mg/kg/day) with escalation at 24 weeks (max 20 mg/kg/day)	166 (≥10)	Prospective, randomized, double-blind, placebo-controlled trial	Efficacy (measured by change in LIC and SF) and safety of deferasirox over 52 weeks in iron overloaded patients with NTDT (Core study) with one year extension	Significant† decrease in both LIC and SF after 12 months, compared to placebo, with continued improvement over 24 months
Ladis 2010 [86]	β-TI	Deferasirox DT (starting dose 10 or 20 mg/kg/day)	11 (25–40)	Prospective, single-arm, open-label trial	Efficacy (measured by changes in hepatic and cardiac iron, and SF) and safety of deferasirox to 24 months	Significant† decrease from baseline in LIC and SF after 12 and 24 months
Voskaridou 2010 [87]	β-TI	Deferasirox DT (starting dose 10 or 20 mg/kg/day)	11 (28–53)	Prospective, single-arm, open-label trial	Efficacy and safety of deferasirox in sporadically transfused, iron overloaded patients with β-TI over 12 months	Significant† improvement in both liver T2* and mean SF after 12 months
Deferiprone						
Calvaruso G 2015 [88]	TI	Deferiprone (75 mg/kg/day) vs deferoxamine (50 mg/kg/day for 5 days)	47 deferiprone 41 deferoxamine (≥13 years)	Prospective, randomized, open-label trial	Efficacy and safety over 5 years	Comparable decrease in serum ferritin in both deferiprone and deferoxamine

Study	Disease type	Description				Results
		Drug investigated	N (age, years)	Design	Study objectives	
Akrawinthawong 2011 [89]	Hb E/β-thal	Deferiprone (starting dose 50 mg/kg/day)	30 (18–50)	Prospective, single-arm, open-label trial	Efficacy of deferiprone in reducing possibility of cardiac complications over 1 year in Hb E/β-thal patients receiving intermittent transfusions	Significant† decrease in mean pulmonary arterial pressure and pulmonary vascular resistance and significant† decrease in SF after 1 year
Pootrakul 2003 [90]	Hb E/β-thal or β-TI	Deferiprone (starting dose 25 or 50 mg/kg/day)	9 (20–48)	Prospective, single-arm, open-label trial	Efficacy and toxicity of deferiprone over 17–86 weeks	Significant† decreases in SF, LIC, red cell membrane iron and NTBI; reduced transfusion requirements in four patients
Rombos 2000 [91]	β-TI	Deferiprone (75 mg/kg/day)	3 (>18)	Prospective, single-arm, open-label trial	Efficacy (change in SF and urinary iron excretion) and safety of deferiprone over 2 years	Decline in SF in all patients within 6 months and was maintained over 24 months; arthropathy and agranulocytosis were not observed
Olivieri 1992 [92]	β-TI	Deferiprone (75 mg/kg/day)	1 (29)	Case study	Change in iron status of a 29-year-old man with deferiprone treatment over 9 months	Decrease in SF from 2174 ng/ml to 251 ng/ml after 6 months; Decrease in LIC from 14.6 mg Fe/g dw to 1.9 mg Fe/g dw after 9 months
Deferoxamine						
Pippard 1988 [93]	β-TI	Deferoxamine (150 mg/kg over 24 hours)	4 (18–27)	Prospective, placebo-controlled crossover trial	Effect of deferoxamine on iron balance in β-TI patients with positive iron balance	All patients achieved negative iron balance after 6 days of deferoxamine treatment
Cossu P 1981 [94]	β-TI	Deferoxamine (3 days each of 20, 40, 60, 80 and 100 mg/kg/day)	10 (1.2–17.3)	Prospective, single-arm open-label trial	Urinary iron excretion over 24 hours and change in SF over 6 months	Significant† increases in urinary iron excretion; non-significant decreases in SF

†p≤0.05; Hb, haemoglobin; SF, serum ferritin; LIC, liver iron concentration; dw, dry weight; thal, thalassaemia; β-TI, β-thalassaemia intermedia; DT, dispersible tablet.

Sub-analyses from THALASSA showed that reduction in liver iron concentration with deferasirox DT 5 and 10 mg/kg/day starting dose groups is consistent irrespective of baseline liver iron concentration, serum ferritin, age, gender, race, splenectomy status, and underlying phenotype [97]. An extension of the core study showed that deferasirox progressively decreases iron overload over 2 years with a safety profile consistent with that in the core study. Of 166 patients enrolled, 64 (38.6 %) and 24 (14.5 %) patients achieved liver iron concentration <5 and <3 mg Fe/g dry weight by the end of the study, respectively [85]. Considering that liver iron concentration measurement may not always be available, the study also established serum ferritin thresholds that best predict liver iron concentration values used to initiate or suspend therapy (initiation: serum ferritin of ≥800 ng/mL corresponds to a liver iron concentration of ≥5 mg Fe/g dry weight and suspension: serum ferritin of ≤300 ng/mL corresponds to a liver iron concentration of ≤3 mg Fe/g dry weight) [98]. More recently, THETIS, an open-label, single-arm, multicentre, phase 4 study, added to the evidence from THALASSA by investigating earlier deferasirox DT dose escalation by baseline liver iron concentration (week 4: escalation according to baseline liver iron concentration; week 24: adjustment according to liver iron concentration response, maximum dose 30 mg/kg/day). Liver iron concentration decreased significantly from 15.1 at baseline to 8.5 mg Fe/g dry weight at week 52 ($p<0.0001$). Most common drug-related adverse events were gastrointestinal: abdominal discomfort, diarrhoea and nausea (n=6 each). These data support earlier escalation with higher deferasirox DT doses in iron-overloaded patients [84]. The five-year data from the same study were recently published and confirmed continued iron burden benefit [99].

On January 23rd 2013, deferasirox received the US Food and Drug Administration (FDA) approval as first-line and only approved therapy for the management of iron overload in NTDT patients 10 years and older. Deferasirox had also received the European Medicines Agency (EMA) approval on November 16th 2012 for the treatment of chronic iron overload requiring chelation therapy when deferoxamine therapy is contraindicated or inadequate in patients aged 10 years and older with NTDT syndromes. It should be noted, however, that deferasirox remains the only iron chelator 'specifically' approved for NTDT patients. The deferoxamine label in most European countries allows for its use in NTDT patients, although this was not based on any specific clinical studies from NTDT patient populations.

It should also be noted that recently, a new deferasirox formulation was developed. The deferasirox film-coated tablet (FCT) formulation received FDA approval in 2015 and EMA approval in 2016. Deferasirox FCT provides some flexibility with regard to preparation and administration, as it does not require any preparation or mixing, and can be taken with a light meal. The tablet can also be crushed and sprinkled on soft food. In addition, deferasirox FCT does not contain any lactose or sodium lauryl sulfate, which may be associated with gastro-intestinal disturbances. Because of higher bioavailability, the deferasirox FCT dose is set to be 30% lower than with dispersible tablets (7, 14, 21 and 28 mg/kg/day for FCT correspond to 10, 20, 30 and 40 mg/kg/day for DT). The monitoring schedule for patients on deferasirox FCT is the same as that in patients taking deferasirox dispersible tablets. In a randomized phase 2 trial, the deferasirox FCT

showed greater adherence and satisfaction, better palatability and fewer concerns with than dispersible tablets as reported by TDT patients [100].

Thus, safe and effective chelation therapy is now available for NTDT patients ≥10 years, which is the age at which iron-related morbidity starts to be of concern [24]; as well as for patients with a liver iron concentration ≥5 mg Fe/g dry weight or serum ferritin ≥800 ng/mL, which again represent thresholds after which the risk of serious iron-related morbidity and mortality is increased [23, 28, 48]. The latter remains essential since data on the ideal serum ferritin of liver iron concentration thresholds for initiation and tailoring of chelation therapy in NTDT cannot be extrapolated from TDT patients (e.g., the serum ferritin of 1000 and 2500 ng/mL or the liver iron concentration of 7 and 15 mg Fe/g dry weight), as these were established using cardiac endpoints irrelevant in NTDT patients and also considering the aforementioned discrepancy in serum ferritin interpretation for the two patient groups [20].

Chelation target is to achieve a liver iron concentration of 3 mg Fe/g dry weight or serum ferritin of 300 ng/mL. In instances where patients present with a serum ferritin level of 300-800 ng/mL and liver iron concentration cannot be determined, the concern is that around 50% of those patients may still show a liver iron concentration of ≥5 mg Fe/g dry weight [98]. In such instances, a probability curve to determine the likelihood of having such a liver iron concentration in patients with a serum ferritin of 300-800 ng/mL has been proposed, although the decision might likely remain individualized based on observation of other laboratory or clinical markers that indicate a state of iron overload [83, 101-103].

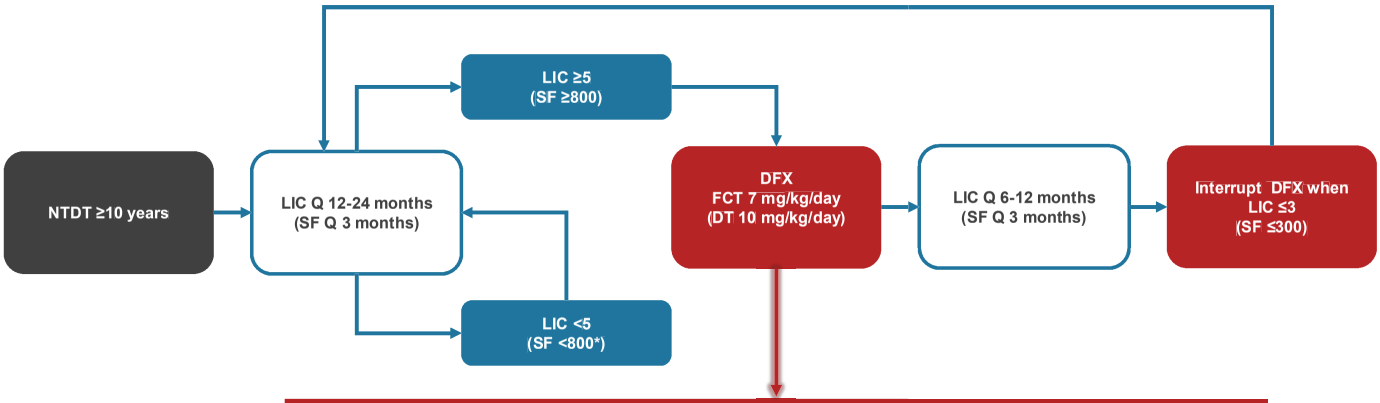
A beneficial effect of iron chelation therapy in reducing clinical morbidity risk in patients with NTDT is also suggested by observational studies [34, 104]. Survival data from a large cohort (n = 2033) of patients with NTDT also confirmed that patients who received iron chelation therapy were less likely to die from liver disease [45].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. All patients with NTDT ≥10 years of age should be frequently assessed for iron overload status.
2. Assessment of iron overload status in NTDT patients should be done through liver iron concentration or serum ferritin measurement:
    a. Magnetic resonance evaluation is recommended. Other non-invasive liver iron concentration measurement techniques may be used when magnetic resonance imaging is unavailable
    b. Serum ferritin levels should be considered the primary index of iron overload status when liver iron concentration measurement is unavailable
    c. Assessment of serum ferritin level should be done at baseline and every 3 months
    d. Assessment of liver iron concentration should be done at baseline and every 12-24 months in patients who are not receiving iron chelation therapy (levels lower than those indicated for chelation, or chelation interrupted after achieving treatment goal) and every 6-12 months (or earlier as needed for iron chelator dose modification) in patients receiving iron chelation therapy
    e. The use of other conventional or experimental iron studies as well as clinical indicators of the severity of ineffective erythropoiesis may be used to support interpretation of serum ferritin level when it is the only index of iron overload status
    f. The use of cardiac T2* magnetic resonance imaging in NTDT patients cannot be widely recommended. It may be considered in older patients with severe iron overload or as clinically indicated
3. Iron chelation therapy with deferasirox should be initiated in NTDT patients ≥10 years of age if any of the below is evident:
    a. Liver iron concentration ≥5 mg Fe/g dry weight
    b. Serum ferritin level ≥800 ng/mL
    c. Serum ferritin level >300 to <800 ng/mL (liver iron concentration measurement is not possible) and other clinical or laboratory measures indicative of iron overload
4. Deferasirox therapy should be administered as follows:
    a. Starting dose: FCT 7 mg/kg/day (DT 10 mg/kg/day)
    b. Dose escalation after 1 month as follows
        i. Baseline liver iron concentration ≥5 to ≤7 mg Fe/g dry weight or serum ferritin ≥800 to ≤1500 ng/mL: no escalation
        ii. Baseline liver iron concentration >7 to ≤15 mg Fe/g dry weight or serum ferritin >1500 to ≤3000 ng/mL: escalate to FCT 11.5 mg/kg/day (DT 15 mg/kg/day)
        iii. Baseline liver iron concentration >15 mg Fe/g dry weight or serum ferritin >3000 ng/mL: escalate to FCT 14 mg/kg/day (DT 20 mg/kg/day)

c. Further dose escalation after 6 months as follows
    i. 6 months liver iron concentration ≥3 to ≤7 mg Fe/g dry weight or serum ferritin ≥300 to ≤1500 ng/mL: same dose (maximum FCT 7 mg/kg/day [DT 10 mg/kg/day])
    ii. 6 months liver iron concentration >7 to ≤15 mg Fe/g dry weight or serum ferritin >1500 to ≤3000 ng/mL: increase by FCT 3.5 mg/kg/day (DT 5 mg/kg/day) (maximum FCT 14 mg/kg/day [DT 20 mg/kg/day])
    iii. 6 months liver iron concentration >15 mg Fe/g dry weight or serum ferritin >3000 ng/mL: increase dose by FCT 3.5-7 mg/kg/day (DT 5-10 mg/kg/day) (maximum FCT 21 mg/kg/day [DT 30 mg/kg/day]). Deferasirox is not currently approved at doses higher than FCT 14 mg/kg/day (DT 20 mg/kg/day) in patients with NTDT; the recommendation is based on clinical expert opinion guided by data from the THETIS trial [84]
d. Deferasirox therapy should be discontinued when patients reach a liver iron concentration value of 3 mg Fe/g dry weight or serum ferritin level 300 ng/mL and patients should continue to be monitored for iron overload as indicated earlier
e. Safety monitoring and management should follow local product prescribing information
f. Compliance should be closely monitored

5. **Figure 3-2** illustrates an iron overload assessment and management algorithm for NTDT patients in accordance with the above recommendations.
6. The use of other iron chelators cannot be recommended until larger, randomized studies evaluating effects on liver iron concentration are available.
7. Tea consumption should be encouraged in NTDT patients, as it may have some benefit in decreasing iron absorption from the gut.
8. Patients with NTDT who require long-term regular blood transfusions should be managed for secondary iron overload similar as per guidelines for patients with TDT.



DFX dose escalation after 1 month			
Baseline LIC	≥5 to ≤7	>7 to ≤15	>15
Baseline SF	≥800 to ≤1500	>1500 to ≤3000	>3000
Dose modification	None	Escalate to FCT 11.5 or DT 15 mg/kg/day	Escalate to FCT 14 or DT 20 mg/kg/day
DFX dose escalation after 6 months			
6 months LIC	≥3 to ≤7	>7 to ≤15	>15
6 months SF	≥300 to ≤1500	>1500 to ≤3000	>3000
Dose modification	Same dose (max FCT 7 or DT 10 mg/kg/day)	Escalate by FCT 3.5 or DT 5 mg/kg/day (max FCT 14 or DT 20 mg/kg/day)	Escalate by FCT 3.5-7 or DR 5-10 mg/kg/day (max FCT 21.5 or DT 30 mg/kg/day)†

***Figure 3-2**. Iron overload assessment and management algorithm for patients with non-transfusion-dependent β-thalassaemia (NTDT). Q, every; SF, serum ferritin level in ng/mL; LIC, liver iron concentration in mg Fe/g dry weight; DFX, deferasirox; FCT, film-coated tablets; DT, dispersible tables. *If serum ferritin level >300 to <800 ng/mL and liver iron concentration measurement is not possible, initiate chelation if other clinical or laboratory measures are indicative of iron overload. †Deferasirox is not currently approved at doses higher than FCT 14 mg/kg/day (DT 20 mg/kg/day) in patients with NTDT; the recommendation is based on clinical expert opinion guided by data from the THETIS trial [84].*

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4 HYPERCOAGULABLITY AND THROMBOTIC DISEASE

A hypercoagulable state has been identified in patients with non-transfusion-dependent β-thalassaemia (NTDT), which can be present since childhood [1-4]. It has been primarily attributed to ineffective erythropoiesis and chronic haemolytic anaemia leading to pathological red blood cells, although several other factors are believed to be involved ultimately leading to clinical thrombosis (**Figure 4-1**) [5-11]. The severity of anaemia is linked to increased overall morbidity risk including thrombotic disease [12-14].

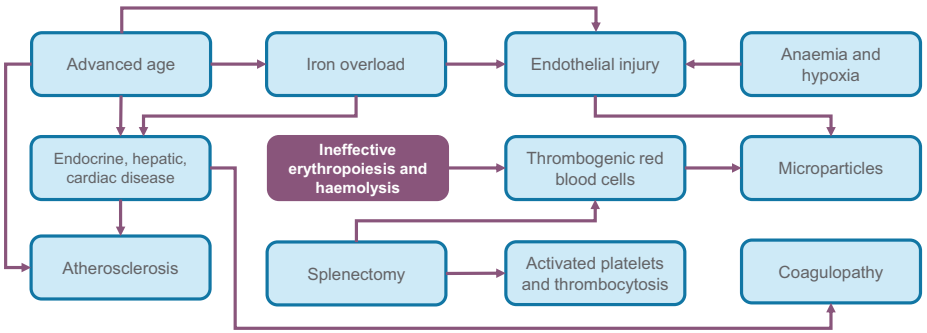

Figure 4-1. Factors contributing to a hypercoagulable state and subsequent thrombotic events in non-transfusion-dependent β-thalassaemia (NTDT) [9, 11]. RBC, red blood cells.

Patients with NTDT have chronically activated platelets, and enhanced platelet aggregation [15], as confirmed by the increased expression of CD62P (P-selectin) and CD63, markers of in vivo platelet activation [16, 17]. It has been demonstrated that NTDT patients have 4 to 10 times higher metabolites of prostacyclin (PG I2) and thromboxane A2, both markers of haemostatic activity, than healthy individuals [18]. Splenectomised NTDT patients also have high platelet counts [19, 20], but with a shorter life-span due to enhanced consumption [21]. Increased platelet adhesion is a common finding in splenectomised β-thalassaemia patients that is induced by mechanisms involving both platelets and red blood cells, and is a strong contributor to occlusive thrombus formation [22-24].

The role of red blood cells in the hypercoagulability of NTDT has received great attention. The oxidation of globin subunits in thalassaemia erythroid cells leads to the formation of haemichromes [25], which precipitate, instigating haeme disintegration and the eventual release of toxic iron species [26]. The free iron in turn catalyses the formation of reactive oxygen species, leading to oxidation of membrane proteins and formation of red-cell senescence antigens like phosphatidylserine [27], which cause the thalassaemic red blood cell to become rigid, deformed, and to aggregate, resulting in premature cell removal [28]. Thalassaemic red blood cells with such negatively charged phospholipids increase thrombin generation [29, 30], as evidenced by studies using annexin V, a protein with high affinity and specificity for anionic

phospholipids [30]. Splenectomised patients have a substantially higher number of these negatively charged pathological red blood cells and in turn show higher thrombin generation [31, 32]. NTDT patients were also found to have higher levels of procoagulant microparticles of red blood cell, leukocytic, and endothelial origins compared to controls [33]; the contribution of these fragments to thrombotic events in NTDT was illustrated [34].

The presence of other peripheral blood elements in NTDT such as E-selectin, intercellular adhesion molecule-1, von Willebrand factor, and vascular cell adhesion molecule-1 indicates that endothelial injury or activation may be an aspect of the disease, aiding in the recruitment of white and red blood cells, and promoting thrombosis [35, 36]. In fact, studies have demonstrated that red blood cells from NTDT patients show increased adhesion to cultured endothelial cells [37]. Inherited thrombophilia does not have a role in the hypercoagulable state of NTDT [38, 39], but low levels of antithrombin III, proteins C, and protein S have been documented [40]. The presence of hepatic or endocrine dysfunction in older patients with severe iron overload may also contribute to hypercoagulability [40]. Heparanase levels are also elevated in thalassaemia, which may contribute to thrombotic phenomena in these patients [41].

It has been demonstrated that the presence of non-transferrin-bound iron in iron overload states can cause oxidative vessel injury [42]. Free radicals act directly on the endothelial cells and have a close interaction with lipid peroxidation, causing a modification of low-density lipoprotein and facilitating its deposition, with the consequent formation of atherosclerotic plaques [43]. Few studies also support the idea that NTDT patients do exhibit a proatherogenic biochemical phenotype [44-47].

CLINICAL THROMBOSIS IN NTDT

Data on the incidence of thrombotic events in NTDT patients are limited. In one study including nine Italian paediatric thalassaemia centres, 4% of 683 patients with β-thalassaemia major and 9.6% of 52 patients with β-thalassaemia intermedia had experienced a thrombotic event [48]. In a cohort study including 83 splenectomised patients with β-thalassaemia intermedia followed for over 10 years, 29% of patients experienced a venous thrombotic event [32]. Conventional risk factors (described in the non-thalassaemic population) for venous thrombosis were usually absent in such patients [49], further highlighting the unique pathophysiology of hypercoagulability in NTDT. Thrombotic events were also documented in case series of pregnant women with β-thalassaemia intermedia [50]. The largest study to date, examined data from 8,860 thalassaemia patients in the Mediterranean area and Iran, and observed that thrombotic events, mostly venous, occurred 4.38 times more frequently in NTDT patients (β-thalassaemia intermedia) than transfusion-dependent β-thalassaemia (TDT, β-thalassaemia major) [51]. It was also found that 14% of mortalities in the whole group were attributed to thrombotic events. Age above 20 years, splenectomy, personal or family history of thrombotic events were identified as the main risk factors for thrombosis in NTDT patients [51].

The OPTIMAL CARE (Overview on Practices in Thalassaemia Intermedia Management Aiming for Lowering Complication rates Across a Region of Endemicity) study evaluated 584 patients with NTDT (β-thalassaemia intermedia) at six comprehensive care centres (Lebanon, Italy, Iran, Egypt, United Arab Emirates, and Oman) and established that thrombotic disease, mostly venous, was the 5th most common complication, affecting 14% of the patient population. The main independent risk factors for thrombotic events were splenectomy, age >35 years, iron overload (serum ferritin level ≥1000 ng/mL), and a haemoglobin level <9 g/dL [52]. A sub-study of the OPTIMAL CARE determined that splenectomised patients who experience thrombosis are characterized by high nucleated red blood cell (≥300 x 10^6/L) and platelet counts (≥500 x 10^9/L) [53], further confirming the dual role of platelets and red blood cells in this setting. The study further examined how long it took for a thrombosis to develop following splenectomy and found the median time to thrombosis to be eight years [53]. Higher rates of thrombosis with advancing age, iron overload (liver iron concentration ≥5 mg Fe/g dry weight or serum ferritin level ≥800 ng/mL), and anaemia (≤10 g/dL) were also observed in several separate studies evaluating β-thalassaemia intermedia patients [12-14, 54-59].

In a recent large, global cohort of 2033 patients with NTDT, thrombotic disease was among the most common causes of death in this patient population [60].

There is currently no sufficient evidence to indicate an increased risk of thrombotic events in β-thalassaemia patients during Covid-19 infection [61, 62].

CEREBROVASCULAR DISEASE IN NTDT

The prevalence of overt strokes in NTDT patients (β-thalassaemia intermedia) with a history of thrombosis ranges between 5% to 9% [51, 53, 63]. Few case reports also describe a frequent occurrence of overt strokes in β-thalassaemia intermedia patients with moyamoya syndrome [64-67]. However, a higher prevalence of silent strokes has been consistently documented in this group of patients [68]. The earliest study was conducted in 1999 and showed a 37.5% rate of ischemic lesions on brain magnetic resonance imaging in 16 patients with β-thalassaemia intermedia (mean age 29 years) who were neurologically intact and had no conventional stroke-related risk factors (e.g., diabetes, smoking, hypertension, cardiac thrombi) [69]. More recently, a cross-sectional brain magnetic resonance imaging study was conducted in Lebanon on 30 splenectomised adults with β-thalassaemia intermedia (mean age 32 years) that were selected from a larger cohort of patients based on absence of neurological or gross cognitive signs or symptoms and any stroke-related risk factors. None of the patients were receiving antiplatelet or anticoagulant therapy. Eighteen patients (60%) had evidence of one or more ischemic lesions all involving the subcortical white matter. Most patients had evidence of multiple lesions. The frontal subcortical white matter was nearly always involved followed by the parietal and occipital subcortical white matter. The vast majority of patients (94%) had evidence of small to medium (<1.5 cm) lesions with only one patient showing evidence of a large lesion (>1.5 cm) [70]. It was noted that increasing age and having never received any transfusions were both independently associated with a higher occurrence and multiplicity of lesions [70].

Around the same time, another cross-sectional study was conducted in Iran on 30 randomly selected β-thalassaemia intermedia adults (mean age 24 years) who were splenectomised, had a haemoglobin level >7 g/dl, and a platelet count ≥500 x 10^9/L The authors noted eight patients (26.7%) with silent ischemic lesions [71]. The harmful roles of splenectomy and thrombocytosis in this setting were confirmed in a more recent study [72]. The variability in the observed frequency and multiplicity of silent stroke in available studies could be primarily attributed to the strength of the magnetic field used (Tesla units). Although none of these studies included a control group, the incidence of silent strokes discovered incidentally on brain scans of healthy individuals of a similar age group (<50 years) ranges from zero to a maximum of 11%, suggesting that the described changes are pathological rather than normal variations [70]. Of note, similar observations were not noted in children with β-thalassaemia intermedia [73]. Only one study evaluated the prevalence of silent stroke in patients with haemoglobin E/β-thalassaemia (mean age 31 years), and the rate was also high (24%) [74].

Three independent studies evaluated intracranial blood flow velocity in neurologically asymptomatic patients (evaluated by a neurologist) with β-thalassaemia intermedia using transcranial Doppler ultrasonography. They revealed that mean flow velocities in the intracranial circulation of patients with β-thalassaemia intermedia are higher than healthy controls, but were lower than those associated with ischemic stroke risk in patients with sickle cell disease (>2 m/s) [73, 75, 76]. Brain magnetic resonance angiography (MRA) and positron emission tomography-computed tomography (PET-CT) studies have also been recently conducted in β-thalassaemia intermedia. In one study including 29 asymptomatic, splenectomised adults, 27.6% had evidence of arterial stenosis on MRA. Two patients had more than one artery involved and the internal carotid artery was the most commonly involved artery. Among the twelve identified stenotic lesions, two were severe (>75% stenosis), one was moderate (51-75% stenosis), and the remaining nine were mild (≤50% stenosis) [77]. The risk of abnormality on MRA increased with declining haemoglobin level and increased non-transferrin-bound iron [77]. The same findings of increased cerebrovascular involvement were not replicated in other studies [78]. PET-CT scanning revealed that decreased neuronal function is also a common finding (63.3%) in this patient population; that is primarily left sided, multiple, and most commonly in the temporal and parietal lobes [79]. The risk of abnormality on PET-CT increased with higher liver iron concentration values [79]. A recent study, however, did not find evidence of increase brain iron deposition on MRI, although such technique is not widely used or validated [80].

There are currently no data to determine whether the observed silent brain abnormalities in β-thalassaemia intermedia are truly 'silent'. In the general population and in patients with sickle cell disease, silent strokes, arterial stenosis on MRA, and decreased neuronal function on PET-CT have all been associated with subsequent risk of overt stroke and neurocognitive decline [68]. That said, brain functional impairment in NTDT patients has been reported [81].

PREVENTIVE STRATEGIES

A thalassaemia-related thrombosis risk scoring system **(TRT-RSS)** has been recently proposed to identify patients' thrombosis risk profile based on age, serum ferritin level, haemoglobin level, transfusion requirement, and splenectomy status [82]. Irrespectively, patients with NTDT, especially splenectomised adults, should always be considered at high risk of thrombotic events during medical and surgical settings and during pregnancy.

The delay in thrombotic events in splenectomised patients with NTDT further highlighted that such manifestation is a result of a chronic underlying process, and emphasized the need for long-term preventive strategies [53].

Prevention strategies should primarily aim at ameliorating ineffective erythropoiesis and the chronic haemolytic anaemia. The role of blood transfusion in the primary or secondary prevention of thrombotic events in NTDT patients has not been evaluated in clinical trials. However, blood transfusions may control the hypercoagulability in NTDT patients by improving ineffective erythropoiesis and decreasing the levels of pathological red blood cells with thrombogenic potential (wash out) [83]. Transfusion therapy may in fact explain the lower rate of thrombotic events in β-thalassaemia major compared to intermedia patients in most studies [9, 51, 84]. Patients with β-thalassaemia intermedia who were placed on transfusion therapy were also less likely to have thromboembolic events and silent strokes in observational studies [52, 70]. NTDT patients who eventually became regularly transfused were also less likely to die from cardiovascular disease including thrombosis than those who did not [60].

The foetal haemoglobin inducer hydroxyurea was shown to decrease plasma markers of thrombin generation and coagulation activation in NTDT patients by reducing phospholipid expression on the surface of red blood cells [85]. Hydroxyurea may also decrease haemostatic activation by its effect in decreasing the white blood cell count and particularly monocytes that express tissue factor [7]. The role of hydroxyurea in the prevention of thrombotic disease in NTDT patients has not been evaluated. One study, however, suggested an association between hydroxyurea use and lower rates of silent strokes in β-thalassaemia intermedia patients [72].

Although an independent association between iron overload and development of thrombotic disease in patients with NTDT is suggested by observational studies [52, 54, 55], further studies are needed to confirm that such observation is not confounded by the role of ineffective erythropoiesis. The role of iron chelation therapy in prevention of thrombotic disease in NTDT has not been evaluated.

There are no data from clinical trials on anticoagulant or antiplatelet therapy for the primary or secondary prevention of thrombotic or cerebrovascular disease in NTDT patients in particular. However, an association between high platelet counts and thrombosis as well as a lower recurrence rate of thrombotic events in splenectomised β-thalassaemia intermedia patients who took aspirin after their first event, when compared

to those who did not, could suggest a potential role for aspirin in the prevention of thrombotic disease [51, 53, 58]. Moreover, the high prevalence of silent strokes in splenectomised patients with elevated platelet counts also suggests a potential role for aspirin therapy [71, 72]. Even in patients with normal platelet counts, a role for aspirin therapy is suggested by observations that it could delay occlusive thrombus formation in carotid arteries of thalassaemic mice [22, 23].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. Within medical or surgical risk-assessment settings, NTDT patients should be considered at higher risk of thrombosis or cerebrovascular disease than normal individuals, especially the following patient subgroups:
   a. Adult patients
   b. Splenectomised patients
   c. Minimally- or never-transfused patients
   d. Patients with a haemoglobin level ≤10 g/dL
   e. Patients with elevated platelet counts (≥500 x 10^9/L)
   f. Patients with elevated nucleated red blood cell counts (≥300 x 10^6/L)
   g. Patients with iron overload (liver iron concentration ≥5 mg Fe/g dry weight or serum ferritin level ≥800 ng/mL)
   h. Patients with a history of pulmonary hypertension
   i. Pregnant patients
   j. Patients with limited mobility
   k. Patients with a history of malignancy
   l. Patients with a personal or family history of thrombosis
   m. Patients with other conventional risk factors for thrombosis or cerebrovascular disease
2. Patients who present with unprovoked, spontaneous thrombosis at unusual sites should also be worked up for thrombophilia especially in regions with high prevalence of common mutations.
3. High-risk patients should be closely monitored and managed for ineffective erythropoiesis/anaemia (see **Chapter 2**) and iron overload (see **Chapter 3**).
4. Prophylactic intervention with anticoagulant or antiaggregant therapy in high-risk patients should follow local standards or international guidelines:

a. Enoxaparin or newer oral anticoagulants may be considered, while acknowledging lack of data in thalassaemia, especially if long-term prophylaxis is needed

5. Aspirin therapy should be considered in splenectomised NTDT patients with elevated platelet counts (≥500 x 10^9/L).
6. Patients who develop thrombotic or cerebrovascular disease should be treated as per local standards or international guidelines in non-thalassaemic patients.

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5 PULMONARY HYPERTENSION

Among the sequelae associated with a diagnosis of non-transfusion-dependent β-thalassaemia (NTDT), pulmonary hypertension has received great attention in recent years [1-3]. Studies relying primarily on echocardiographic parameters, reported prevalence rates ranging between 10% and 78.8% (averaging at ~30%), with higher prevalence generally noted in NTDT (β-thalassaemia intermedia and haemoglobin E/β-thalassaemia) than transfusion-dependent β-thalassaemia (TDT, β-thalassaemia major) patients [4-21]. The diagnosis was usually established based on a tricuspid-valve regurgitant jet velocity (TRV) exceeding 2.5-2.8 m/s corresponding to a pulmonary arterial systolic pressure exceeding 30-35 mm Hg, with some studies including symptomatology within the definition [4, 6, 8, 10, 12, 14-17, 19-21].

The main concern with such high prevalence rates is that most available studies established the diagnosis of pulmonary hypertension solely based on echocardiographic criteria, without systematic confirmation on right heart catheterization, a procedure that is recommended in international guidelines as the standard of care [22-24].

Evidence from patients with sickle cell disease echoed earlier studies in other conditions which established that the use of echocardiography alone results in a considerable number of false positive diagnoses that are not confirmed on right heart catheterization [25]. Thus, a proportion of identified patients may not have a confirmed clinical diagnosis of pulmonary hypertension, although they may be at high-risk of developing such morbidity. In a large study (1309 β-thalassaemia patients) from Italy, the prevalence of pulmonary hypertension was considerably lower when more strict echocardiographic criteria (which took into consideration the chronic anaemic state of patients) and confirmatory right heart catheterization were used (5.7% for a TRV >3.0 m/s, 3.6% for a TRV >3.2 m/s, and 2.1% on right heart catheterization [pre-capillary pulmonary arterial **hypertension]). Patients with β-thalassaemia intermedia had a 5-fold increased prevalence of pulmonary hypertension on right heart catheterization than patients with β-thalassaemia major (4.8% vs. 1.1%).** When a threshold TRV of 3.2 m/s was used to define pulmonary hypertension, the positive predictive value of echocardiography was as high as 93.9% [26]. The same threshold was also associated with an increased risk of mortality [27]. In a follow up of 24 patients from this cohort with confirmed pulmonary hypertension on right heart catheterization, the median survival time was 9 years with rates of mortality being 54.2% and 41.7% for overall and pulmonary hypertension-related death, respectively [28]. In fact, survival analysis of a large cohort of patients with NTDT (n = 2033) identified non-iron mediated cardiovascular disease, including pulmonary hypertension, as the leading cause of death in this patient population [29].

ETIOLOGY AND RISK FACTORS FOR PULMONARY HYPERTENSION IN NTDT

Although the exact mechanisms implicated in the pathogenesis of pulmonary hypertension in NTDT remain unclear, its association with several risk factors has been illustrated [1, 30]. Pulmonary hypertension in NTDT patients is pulmonary arterial hypertension which is characterized by the presence of pre-capillary pulmonary hypertension in the absence of left-sided heart disease, lung disease, or chronic thromboembolism [22, 23, 31]. In newer classification, it would belong to Group 5 pulmonary hypertension associated with chronic haemolytic anaemia with unclear/multifactorial mechanism [32]. Irrespectively, the possibility of pulmonary hypertension occurring secondary to chronic thromboembolic disease cannot be fully excluded in the NTDT patient with a hypercoagulable state; and this has been previously reported [33, 34]. Hypercoagulability can also still play a major role in the aetiology of pulmonary arterial hypertension; where thrombi may be present in both the small distal pulmonary arteries and the proximal elastic pulmonary arteries [22]. The association of advancing age, splenectomy, history of thrombosis, thrombocytosis (platelet count ≥500 x 10^6/L), increased platelet activation, high nucleated red blood cell counts (≥300 x 106/L), and other markers of hypercoagulability with increased pulmonary hypertension risk further supports the role of a hypercoagulable state, even in patients with no evidence or history of pulmonary embolic disease, in the aetiology of pulmonary hypertension in NTDT and could explain its higher prevalence compared with TDT patients [6, 7, 10, 12, 13, 20, 21, 26, 34-40].

Other factors are also implicated in the aetiology of pulmonary hypertension in NTDT. Associations between chronic anaemia/hypoxia (especially a haemoglobin level ≤10 g/dL) and increased markers of haemolysis with this morbidity have been reported [10, 13, 37, 38, 41-43]. The process of haemolysis disables the arginine-nitric oxide pathway through the simultaneous release of erythrocyte arginase and cell-free haemoglobin. Both nitric oxide and its obligate substrate arginine are rapidly consumed. The biological consequences of haemolysis on nitric oxide bioavailability ultimately translate into pulmonary vasoconstriction and the clinical manifestations of pulmonary hypertension [30, 44]. Pulmonary hypertension is also associated with increased expression of endothelin receptors in pulmonary microvascular endothelial cells and monocytes, due to intrinsic high levels of placenta growth factor [45]. Decreased arginine bioavailability and nitric oxide depletion secondary to haemolysis have been recently directly associated with pulmonary hypertension in patients with NTDT [44, 46, 47]. Similar to thrombotic disease, an association between iron overload (liver iron concentration ≥5 mg Fe/g dry weight or serum ferritin level ≥800 ng/mL) and pulmonary hypertension has also been observed in patients with β-thalassaemia intermedia [19, 40, 48, 49]. The aetiology of pulmonary hypertension in NTDT is thus most likely multifactorial, involving a complex interaction of platelets, the coagulation system, erythrocytes, and endothelial cells along with inflammatory and vascular mediators [30, 50].

PREVENTION AND MANAGEMENT

NTDT patients with pulmonary hypertension show functional limitation and considerable decrease in the 6-minute walk distance [26]. Several studies confirm that pulmonary hypertension in NTDT is a serious morbidity associated with subsequent right heart failure [16, 18, 33, 51-54] and death [28, 29]. Thus, preventive strategies and regular monitoring for prompt detection and management are warranted.

Prevention strategies should primarily aim at ameliorating ineffective erythropoiesis, chronic haemolytic anaemia, hypercoagulability, and iron overload; especially considering the multifactorial aetiology of pulmonary hypertension in NTDT. NTDT patients have higher pulmonary hypertension rates than TDT patients, and among patients with NTDT, the administration of transfusion therapy is associated with lower pulmonary hypertension rates [20, 34, 55]. Patients with NTDT who are eventually transitioned to regular transfusion programs were also less likely to die from cardiovascular disease including pulmonary hypertension [29]. Transfusions not only improve anaemia and haemolysis but may also have a role in ameliorating the hypercoagulable state in NTDT through washout of pathogenic red blood cells. Similar protective effects were also noted with the use of hydroxyurea [11, 20, 34, 56-58] and iron chelation therapy in observational studies [20, 34]. A role for such therapies in ameliorating pulmonary hypertension in patients with established disease has not been formally evaluated, but it is common practice for patients to receive transfusion therapy with the intention of improve TRV.

No drugs have been specifically approved for the management of pulmonary hypertension in thalassaemia [59]. Sildenafil citrate, a potent inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase-5 and a selective smooth muscle relaxant, showed promising results for the management of pulmonary hypertension in small studies in β-thalassaemia patients [60-62]. A multicentre trial including patients with β-thalassaemia major and intermedia showed that sildenafil therapy may improve cardiopulmonary haemodynamics in patients with a TRV >2.5 m/s [47]. Bosentan (endothelin receptor antagonist) and epoprostenol (prostacyclin) were also reported to be effective in some patients [63-65]. In practice, several pharmacologic agents are being used based on data from other conditions, including bosentan, ambrisentan, sildenafil, tadalafil, macitentan, riociguat, and other angiotensin-converting enzyme inhibitors, calcium channel and b-blockers, and anticoagulants. Overall, patients with >25% reduction in systolic artery pressure with pharmacologic therapy were at lowest risk of mortality, although dual therapy may be needed to achieve treatment targets [27, 28].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. Patients with NTDT should undergo routine echocardiographic assessment (annually) for the assessment of TRV, especially the following patient subgroups:
   a. Adult patients
   b. Splenectomised patients
   c. Minimally- or never-transfused patients
   d. Patients with a haemoglobin level ≤10 g/dL or elevated markers of haemolysis
   e. Patients with elevated platelet counts (≥500 x 10^9/L)
   f. Patients with elevated nucleated red blood cell counts (≥300 x 10^6/L)
   g. Patients with iron overload (liver iron concentration ≥5 mg Fe/g dry weight or serum ferritin level ≥800 ng/mL)
   h. Patients with a history of thrombosis
   i. Patients with other conventional risk factors for pulmonary hypertension
2. Echocardiographic TRV values should be interpreted as follows:
   a. TRV >2.5 m/s and asymptomatic: 'possible' to have pulmonary hypertension
   b. TRV >2.5 m/s and symptomatic or with other echocardiographic criteria suggestive of pulmonary hypertension: 'likely' to have pulmonary hypertension
   c. TRV >3.2 m/s: 'likely' to have pulmonary hypertension
3. Patients 'likely' to have pulmonary hypertension on echocardiography should undergo right heart catheterization to confirm the diagnosis. Ventilation/perfusion lung scan testing is also recommended to rule out pulmonary thromboembolic disease.
4. Patients with confirmed pulmonary hypertension should be referred to a cardiologist and managed as per local standards or international guidelines for the treatment of pulmonary hypertension.
5. Patients with possible, likely, or confirmed pulmonary hypertension should be closely monitored and managed for ineffective erythropoiesis/anaemia (see **Chapter 2**), iron overload (see Chapter 3), and hypercoagulability (see **Chapter 4**).
6. The algorithm in **Figure 5-1** summarizes the approach to pulmonary hypertension in patients with NTDT.

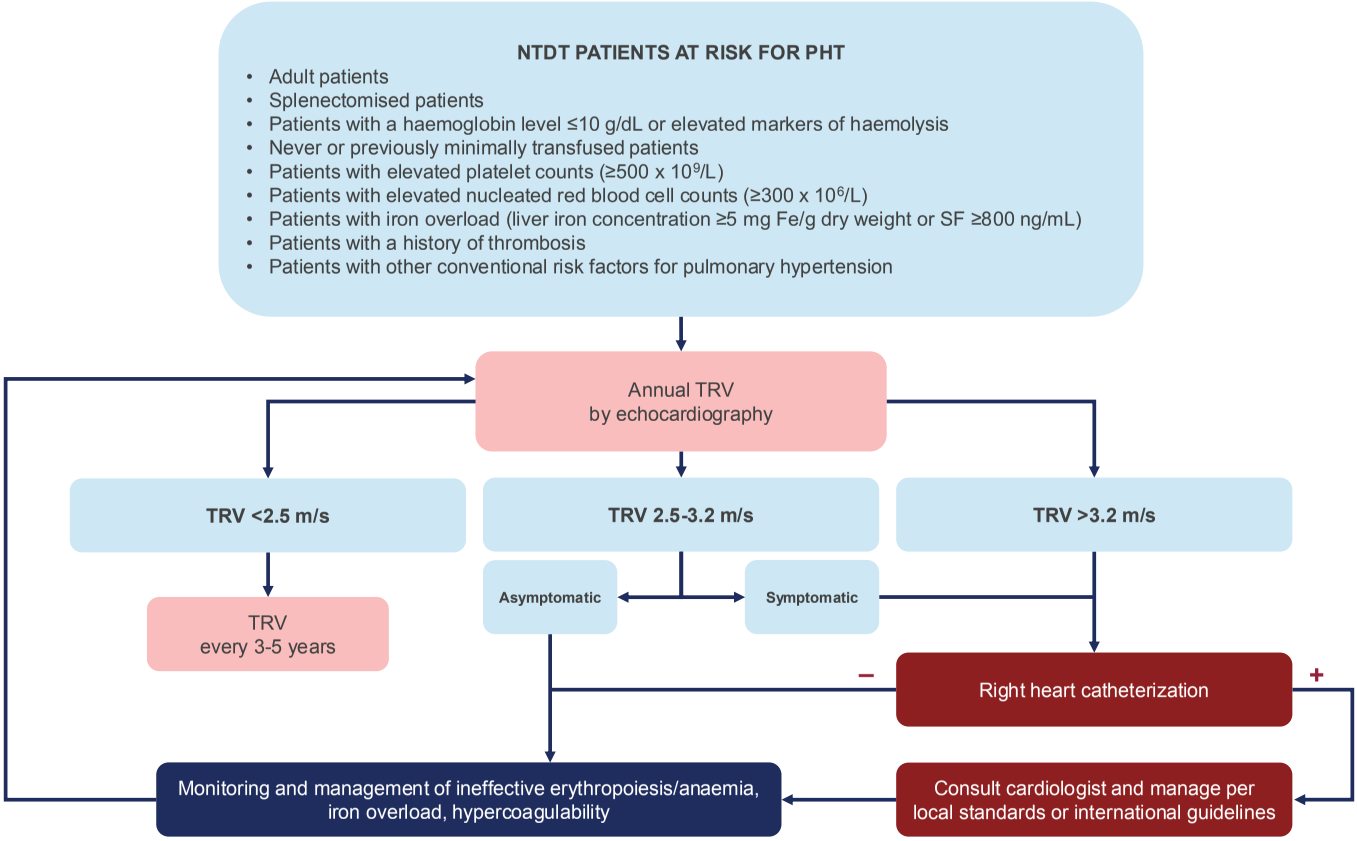

Figure 5-1. *Algorithm for the prevention and management of pulmonary hypertension (PHT) in non-transfusion-dependent β-thalassaemia (NTDT). TRV, tricuspid-valve regurgitant jet velocity; SF, serum ferritin.*

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5 LIVER DISEASE

Primary iron overload in patients with non-transfusion-dependent β-thalassaemia (NTDT) leads to preferential portal and hepatocyte iron loading and considerably elevated liver iron concentration (see **Chapter 3**) [1-4]. Data from patients with hereditary haemochromatosis and other acquired liver disease states continue to confirm the role of chronic hepatocellular iron deposition in promoting liver fibrogenesis and cirrhosis [5]. A longer duration of hepatic iron exposure is associated with a higher risk of significant fibrosis [6], while liver cirrhosis can develop within a decade in severely iron overloaded patients [7]. In the subset of patients who end up receiving frequent transfusions, hepatic disease may also be caused or exacerbated by hepatitis C (and less commonly B) virus infection. Hepatitis C virus infection and iron overload are independent risk factors for hepatic fibrosis and cirrhosis, although their co-existence is synergistic in injury [8].

Studies on hepatic outcomes in patients with NTDT remain scarce. Abnormal liver function tests (alanine transaminase >50 IU/L) are noted in iron overloaded and older patients with NTDT, especially those with a liver iron concentration ≥5 mg Fe/g dry weight [9-12]. A longitudinal cohort study of 42 never-transfused β-thalassaemia intermedia adults followed for four years (median age 38 years) evaluated the association between longitudinal changes in serum ferritin levels and transient elastography values [13]. Transient elastography is a recently developed, rapid, non-invasive technique designed to predict hepatic fibrosis, based on a mechanical wave generated by vibration. The measurement of the speed of propagation of the wave across the hepatic parenchyma provides an estimate of the liver elasticity, which is a surrogate marker of liver fibrosis. When compared with liver biopsy, transient elastography shows a satisfactory sensitivity and specificity for identifying fibrosis in patients with chronic liver disease including β-thalassaemia and has a good inter-observer and intra-observer reproducibility [14]. A significant increase was observed in both serum ferritin levels (+81.2 [ng/mL]/year) and transient elastography values in non-chelated patients (n=28) (+0.3 kPa/year), with two patients worsening their fibrosis stage. Chelated patients (n=14) had a significant decrease in both measures (-42.0 [ng/mL]/year and -0.9 kPa/year, respectively), with two patients improving their fibrosis stage. There was a strong correlation between the rate of change in serum ferritin level and the rate of change in transient elastography value (R2: 0.836, $p<0.001$); noted in both non-chelated and chelated patients [13]. Another 10-year follow-up study confirmed an association between higher serum ferritin levels (≥800 ng/mL) and liver disease in non-chelated β-thalassaemia intermedia patients [2]; while chronic anaemia and hypoxia have also been implicated in chronic liver damage [15, 16].

The proliferative and mutagenic effects of excess iron are established, which converge in determining an increased susceptibility to hepatocellular carcinoma, even in the absence of pre-existing liver cirrhosis [5, 17-20]. Iron overload produces oxygen-free radicals. Oxidative damage can in turn give rise to neoplastic

clones through genetic or epigenetic alterations. Iron overload can also suppress tumoricidal action of macrophages and alteration of cytokine activities. Iron chelation therapy, however, can exert protective effects on cell cycle control molecules and on nuclear factor-κB and has a protective effect against the oxygen radicals and proto-oncogene expression [21, 22]. Several cases of hepatocellular carcinoma in patients with NTDT have been described with malignancy sometimes occurring in the absence of viral hepatitis or cirrhosis while having considerably high iron overload indices [20, 23-31]. Additional factors such as obesity or alcohol consumption may also increase steatosis and oxidative stress, which accelerate liver iron uptake and increase risk of liver fibrosis, cirrhosis, and cancer in NTDT patients [32, 33].

In a small series from Lebanon, liver disease including hepatocellular carcinoma was responsible for around 10% of causes of death in NTDT patients [34]. In a more recent global cohort of 2033 NTDT patients, liver disease including hepatocellular was the second-leading cause of death in this patient population, with a lower risk noted in patients receiving iron chelation therapy [35].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. Patients with NTDT ≥10 years should be screened for hepatic function and disease as follows:
   a. Liver function tests: every 3 months, all patients
   b. Liver ultrasound: annually in patients with liver iron concentration ≥5 mg Fe/ g dry weight or serum ferritin level ≥800 ng/mL, or every six months in case of abnormal findings
   c. Alpha-feto protein: annually in cirrhotic patients or patients >40 years
   d. Transient elastography monitoring when available, every 12-24 months, in patients with liver iron concentration ≥5 mg Fe/ g dry weight or serum ferritin level ≥800 ng/mL
2. NTDT patients with evidence of hepatic disease should be referred to a hepatologist for further work-up and management.
3. NTDT patients should be closely monitored and adequately managed for ineffective erythropoiesis/anaemia (see **Chapter 2**) and iron overload (see **Chapter 3**).
4. Vaccination against hepatitis B prior to the initiation of any planned blood transfusion therapy, with regular monitoring of antibody titers, is recommended.
5. Vaccination against hepatitis A virus is recommended.
6. Patients receiving blood transfusions should undergo annual serologic monitoring for hepatitisB and C infections. In patients with evidence of hepatitis B and C infection upon serologic testing, confirmatory tests with a polymerase chain reaction should be done.
7. In patients with confirmed hepatitis B and C infection on polymerase chain reaction, management should follow guidelines for transfusion-dependent β-thalassaemia (TDT). A hepatologist should be consulted to decide on the indications for treatment, drug choices, dosing, and safety monitoring.

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7 ENDOCRINE AND BONE DISEASE

Growth retardation and skeletal deformity attributed to ineffective erythropoiesis, medullary expansion, and chronic anaemia may be encountered in children with non-transfusion-dependent β-thalassaemia (NTDT), although to a lesser extent than in sub-optimally treated patients transfusion-dependent β-thalassaemia (TDT) [1-3].

An association between secondary iron overload and endocrine gland toxicity has been established from biological, clinical, and radiological studies in TDT patients [4-6]. Although the prevalence of endocrinopathy in NTDT patients is relatively lower than TDT, reported prevalence rates of diabetes mellitus, hypothyroidism, hypoparathyroidism, hypogonadism, and adrenal insufficiency remain considerably high, especially as patients advance in age [1, 7-11]. In patients with β-thalassaemia intermedia, associations between elevated liver iron concentration (≥5 mg Fe/g dry weight) or serum ferritin level (≥800 ng/mL) and the risk of diabetes mellitus, hypothyroidism, hypoparathyroidism, and hypogonadism have been reported [7, 12-15]. Lower rates of such morbidities with iron chelation therapy use have also been suggested by observational studies [7]. Ineffective erythropoiesis and anaemia (especially haemoglobin level ≤10 g/dL) have also been associated with endocrine disease in NTDT patients, indicating a potential role for chronic tissue hypoxia [7, 16-19]. Lower rates of endocrinopathies have been noted in patients receiving hydroxyurea therapy compared with those who do not while patients receiving frequent transfusions showed higher prevalence rates in observational studies [7, 20].

Similar to patients with β-thalassaemia major [21], the pathophysiology of low bone mineral density (osteopenia and osteoporosis) in patients with NTDT is probably multifactorial. Ineffective erythropoiesis and expansion of the erythron in the bone marrow is directly implicated in the pathophysiology of osteoporosis [16, 22]. Moreover, nutritional imbalances or hormonal alterations due to other endocrinopathies, as described above, can aid in the development of osteopenia and osteoporosis in NTDT [21]. There is also biological evidence that iron can lead to alterations in bone metabolism. There is evidence of reduced bone formation through direct iron toxicity on osteoblasts. Iron deposition in bone impairs osteoid maturation and inhibits mineralization locally, resulting in focal osteomalacia. Iron deposits appear along mineralization fronts and osteoid surfaces, whereas focal thickened osteoid seams are found together with focal iron deposits. Incorporation of iron into crystals of calcium hydroxyapatite also affects the growth of hydroxyapatite crystals and reduces bone metabolism unit tensile strength [21, 23-27].

Clinically, osteoporosis is a common finding in β-thalassaemia intermedia [7] and haemoglobin E/β-thalassaemia, more so in adulthood than during childhood [7, 8, 28]. Advanced age, female gender, anaemia, iron overload, splenectomy, and low foetal haemoglobin levels have been associated with increased rates

of osteoporosis in β-thalassaemia intermedia patients [7, 12, 13, 15, 17-20, 29, 30]. Although only few studies evaluated consequences of osteoporosis in NTDT patients, it is commonly associated with bone pain, skeletal and spinal deformities, and fractures as in β-thalassaemia major patients [21, 31, 32]. There are also no studies on the role of prevention or management of osteoporosis in NTDT patients. Different regimens of vitamin D and calcium are frequently prescribed to patients with NTDT, but with careful monitoring of renal function [33, 34]. Although the efficacy and safety of bisphosphonates has been proven in patients with β-thalassaemia major, data on patients with NTDT are limited [35]. Lower rates of osteoporosis were noted in β-thalassaemia intermedia patients receiving iron chelation and hydroxyurea therapy than those who do not [7].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. Patients with NTDT, should undergo the following routine testing:
   a. Growth retardation (<18 years)
      i. Standing and sitting height: every 6 months
      ii. Bone age: every 12 months if delayed puberty or growth
      iii. In patients who fall-off the growth curve (5%), have decreased height velocity, or delayed bone age: growth hormone stimulation, insulin-like growth factor (IGF)-1 level, IGF-BP3 level, deferoxamine toxicity, other hormonal and nutritional imbalances
   b. Hypogonadism
      i. Tanner staging (10-17 years): annually
         - Delayed puberty: girls 13 years, boys 14 years
         - Hypogonadism: absence of testicular development in boys and breast development in girls by 16 years
         - Evidence of pubertal delay: gonadotropin-releasing hormone, luteinizing hormone, follicle-stimulating hormone, testosterone, oestradiol, pelvic ultrasound, zinc deficiency, growth retardation, hypothyroidism
      ii. Adults: routine assessment for infertility, secondary hypogonadism, impotence
   c. Hypothyroidism (≥10 years)
      i. Free thyroxine (FT4) and thyroid-stimulating hormone: annually
   d. Hypoparathyroidism (≥10 years)
      i. Calcium, phosphate, vitamin D: annually
      ii. Parathyroid hormone: if indicated
   e. Diabetes mellitus (≥10 years)
      i. Fasting blood sugar: annually
      ii. Oral glucose tolerance test: if indicated`

f. Adrenal insufficiency (≥10 years)
    i. Adrenocorticotropic hormone stimulation test: annually
g. Osteoporosis (≥10 years)
    i. Bone Mineral Density spine, hips, radius, ulna (dual- energy X-ray absorptiometry): every 24 months or 12 months with abnormality
    ii. Other hormonal and nutritional imbalances
    iii. Spine imaging: for back pain or neurological findings

2. Standards for prevention of osteoporosis (behavioural, hormonal, vitamins and supplements) in patients with NTDT should follow guidelines and recommendations in TDT patients.
3. NTDT patients should be closely monitored and managed for ineffective erythropoiesis/ anaemia (see **Chapter 2**) and iron overload (see **Chapter 3**).
4. Patients with established endocrine disease or osteoporosis should be referred to a paediatric or adult endocrinologist for management according to local standards or international guidelines or as per recommendations in TDT patients.

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8 PREGNANCY

Although delayed puberty may be common in non-transfusion-dependent β-thalassaemia (NTDT) patients, fertility is usually preserved, and many patients aim for pregnancy [1]. Few case series reported pregnancy outcomes in women with NTDT (β-thalassaemia intermedia) [2-5]. All reported pregnancies were spontaneous [2-5]. Abortion, pre-term delivery, intrauterine growth restriction (IUGR), Caesarean section delivery, thromboembolic events, and splenectomy were generally common in such women [2-5].

Blood transfusion therapy is a common consideration in pregnant NTDT women due to intensification of anaemia during gestation. The physiologic anaemia of pregnancy becomes exaggerated in NTDT patients, and the increased oxygen demand by the foetus makes blood transfusion a tempting option [3]. Data from non-thalassaemic cohorts suggest that keeping haemoglobin level above 10 g/dL is optimal for the development of the foetus and to avoid intrauterine growth IUGR, intrauterine foetal demise (IUFD), or preterm delivery [6]. The first reported large case series of pregnant β-thalassaemia intermedia (83 pregnancies in 44 women) observed that 22% of patients still had IUGR despite abiding by this standard [2].

Keeping haemoglobin level above 10 g/dL is optimal for the development of the foetus and to avoid intrauterine growth IUGR, intrauterine foetal demise (IUFD), or preterm delivary.

An update of pregnancy outcomes on 85 pregnancies in 48 women reported much lower rates of IUGR (1.3%), no IUFD, and 2.5% preterm delivery noting that 56% of women received transfusion during pregnancy [5]. Another β-thalassaemia intermedia case series from Italy with trials of random transfusion regimens (1 to 1 per week, mean total haemoglobin level from 7.6 to 9.3 g/dl) in 11 out of 17 pregnancies showed that most babies (except in two cases) were appropriate for gestational age [4]. Transfusions were not only administered based on total haemoglobin level but also general and cardiac maternal status and foetal growth [4].

The main concern with administering blood transfusion during pregnancy especially to previously never- or minimally-transfused patients is alloimmunization. Such patients commonly had alloimmunization in available studies and usually had adverse outcomes (abortion, IUGR, cardiac failure, Caesarean section delivery) [2-4]. Thrombotic disease was also a common occurrence, especially in women with additional prothrombotic risk factors [2, 5, 7]. In one series where all splenectomised patients received aspirin and all patients were given low-molecular-weight heparin in the peripartum period, no thrombotic events occurred in any patient [4]. However, in another recent series where all patients received aspirin therapy and those with a history of recurrent miscarriage or thrombosis received heparin, thrombotic events (placental thrombosis and deep vein thrombosis) were still noted in up to 6% of patients [5]. Splenomegaly can interfere with the enlargement of the uterus and can be complicated by hypersplenism necessitating splenectomy during gestation or after delivery [2, 3].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. NTDT patients planning to get pregnant need comprehensive counselling regarding the risk of having an affected child and prenatal diagnosis.
2. **Pregnancy in NTDT patients should be considered a high-risk one, and care should be achieved through close collaboration between the haematologist, obstetrician, cardiologist, and other relevant specialists.**
3. Introduction of blood transfusions for pregnant patients with NTDT should rely on:
   a. Total haemoglobin level (maintain >10 g/dL)
   b. Maternal general and cardiac status
   c. Foetal growth status
4. Pregnant women with NTDT who were previously never- or minimally-transfused, should be considered at high risk of alloimmunization if blood transfusions are to be administered during pregnancy. If blood transfusion is deemed necessary, extended genotype and antibody screening should be performed before giving any transfusion and fully-phenotyped matched blood should be given.
5. Splenectomy should be considered for patients complicated with hypersplenism or splenomegaly before conception or postpartum.
6. Pregnant women with NTDT should receive prophylactic dose anticoagulant therapy (low-molecular-weight heparin) in the peripartum. Patients with a history of recurrent abortions or who are at increased risk of thromboembolic events (see **Chapter 4**) may be considered for anticoagulant therapy (low-molecular-weight heparin) all throughout pregnancy. Splenectomised patients should also be considered for aspirin therapy.
7. The following general monitoring and management standards should also be considered:
   a. Pre-pregnancy
      i. Assess iron overload status and ensure adequate management of iron overload (see **Chapter 3**)
      ii. Assess cardiac status (echocardiogram, cardiac stress test, electrocardiogram, Holter monitoring) and manage accordingly
      iii. Assess liver function (see **Chapter 6**) and manage accordingly
      iv. Assess for gall bladder with ultrasound and manage accordingly
      v. Assess viral status (hepatitis B and C, HIV, rubella) and ensure appropriate vaccinations (hepatitis B, Pneumovax, seasonal influenza)
      vi. Assess endocrine function, calcium, vitamin D, and bone health (see **Chapter 7**) and manage accordingly

vii. Screen for red blood cell antibodies
viii. Initiate folic acid

b. During pregnancy
   i. Serial serum ferritin (monthly)
   ii. Cardiac, hepatic, and thyroid monitoring (every trimester)
   iii. Serial ultrasound to monitor growth restriction (monthly)
   iv. Gestational diabetes monitoring (16 and 28 weeks)
   v. Prenatal diagnosis
   vi. Discontinue: iron chelators (deferoxamine may be used if needed in second and third trimesters, no other chelators are so far suggested), hormone replacement therapy, ACE inhibitors, hydroxyurea, bisphosphonates (6 months prior), interferon/ribavirin, warfarin (switch to heparin), oral hypoglycaemic (switch to insulin), luspatercept
   vii. May be resumed: calcium, vitamin D, penicillin in splenectomised patients

c. During delivery
   i. Vaginal vs. Caesarean section: assessment depending on pelvic and cardiac status
   ii. Epidural anaesthesia in case of Caesarean section

d. Post-delivery
   i. Restart iron chelation (post breast feeding, deferoxamine can be resumed immediately)
   ii. Resume calcium and vitamin D
   iii. Restart bisphosphonates (post breast feeding)
   iv. Restart hormone replacement therapy (post breast feeding)
   v. Avoid breast feeding if positive for HIV, hepatitis B or C
   vi. Discuss contraception

8. Fertility assessment can be done through menstrual history, antral follicle count, and antimullerian hormone measurement.
9. In patients with hypogonadism, ovulation or spermatogenesis may need to be induced and should be done by an experienced fertility centre.

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9 EXTRAMEDULLARY HAEMATOPOIESIS

Expansion of the erythron in the bone marrow of non-transfusion-dependent β-thalassaemia (NTDT) patients during ineffective erythropoiesis is not only associated with osteoporosis and bone deformities but is also associated with homing and proliferation of erythroid precursors in the spleen and liver as a physiological compensatory phenomenon termed extramedullary haematopoiesis, which leads to hepatosplenomegaly [1-3].

Ineffective erythropoiesis in NTDT patients also forces expansion of the haematopoietic tissue (extramedullary haematopoiesis) in areas other than the liver and spleen, mostly in the form of masses commonly termed extramedullary haematopoietic pseudotumours [3]. The prevalence of extramedullary haematopoietic pseudotumours is considerably higher in NTDT (>10%) than transfusion-dependent β-thalassaemia (TDT) patients (<5%) [4-7]. The prevalence is higher in older patients [8], as well as those with more severe ineffective erythropoiesis [9], haemoglobin levels ≤10 g/dL [10, 11], and low foetal haemoglobin levels [12]. The level of soluble transferrin receptor has also been proposed to be a predictor of extramedullary haematopoiesis development in NTDT patients [13].

Almost all body sites may be involved including the lymph nodes, thymus, heart, breasts, prostate, broad ligaments, kidneys, adrenal glands, pleura, retroperitoneal tissue, skin, peripheral and cranial nerves, brain, and the spinal canal [14-19]. These sites are believed to normally engage in active haematopoiesis in the foetus during gestation. This pathway normally stops at birth, but the extramedullary haematopoietic vascular connective tissues retain the ability to produce red cells under conditions of longstanding ineffective erythropoiesis [14].

Among the various body regions reported, paraspinal involvement received special attention due to the debilitating clinical consequences secondary to neural element compression [14]. The origin of the spinal epidural haematopoietic tissue is still controversial. It has been hypothesized that this tissue could be extruded through the trabecular bone of the vertebral body with a circumferential involvement of the vertebra, or it may have extended through the thinned trabeculae at the proximal rib ends [20, 21]. Others have proposed some embryological haematopoietic cell remnants within the epidural space, which would be stimulated along the course of chronic anaemia. Development of haematopoietic tissue from branches of the intercostal veins has also been suggested [22], while others still attribute the masses to embolic phenomena [23, 24]. Early in its evolution, the paraspinal extramedullary site of haematopoiesis reveals immature and mature cells mainly of the erythroid and myeloid series and dilated sinusoids containing precursors of red cells. The lesions eventually become inactive and reveal some fatty tissue and fibrosis or massive iron deposits [20]. There is some predilection for the site of spinal cord involvement by the

haematopoietic tissue. The thoracic region and to a lesser extent the lumbar region are the most commonly involved sites. The reason for this predilection is uncertain, but because the subarachnoid space and the spinal canal are narrow in the thoracic region, which also has limited mobility [25, 26], small intraspinal haematopoietic tissue may cause compression of the spine at this level. This is in contrast with other regions of the cord in which such tissues must reach larger sizes to exert enough pressure on the spinal cord and cause symptoms [27].

A paraspinal location for the haematopoietic tissue occurs in 11 to 15% of cases with extramedullary haematopoietic pseudotumours [28, 29], and a large number of cases have been reported in the literature as reviewed recently [14]. Paraspinal extramedullary haematopoietic pseudotumours may cause a variety of neurological symptoms due to spinal compression. However, it is believed that more than 80% of cases may remain asymptomatic and the lesions are usually discovered incidentally by radiologic techniques [26, 30, 31]. The development of neurologic symptoms depends on the chronicity of the disease with neurologic symptoms most frequently being reported during the third and fourth decades of life [32], although few reports described presentation as early as the first decade of life [23, 33, 34]. The male to female ratio reaches 5:1 [32]. Various clinical presentations have been reported including: back pain, lower extremity pain, paraesthesia, abnormal proprioception, exaggerated or brisk deep tendon reflexes, Babinski response, Lasegue sign, paraparesis, paraplegia, ankle clonus, spastic gate, urgency of urination, and bowl incontinence. The size and location of lesions and the extent of spinal cord involvement determine the severity, acuteness and multiplicity of signs and symptoms [14, 35].

DIAGNOSIS OF PARASPINAL INVOLVEMENT

Early diagnosis of paraspinal extramedullary haematopoietic pseudotumours will affect the course of management and may reduce the incidence of irreversible neurologic damage that would otherwise occur with prolonged undiagnosed cord compression [14, 35]. The medical history remains important to rule out other entities in the differential diagnosis of epidural masses including metastatic malignant disease, lymphoma, multiple myeloma, vascular anomalies, or an epidural abscess [14, 35]. In the past, the diagnosis of paraspinal extramedullary haematopoietic pseudotumours in patients with NTDT was suspected from the typical osseous abnormalities found on chest radiographs [36-38] or was confirmed after surgical removal of the mass [63]. Plain radiographs often reveal well demarcated paraspinal masses and bony changes associated with chronic anaemia such as trabeculation, widened ribs, or thickened calvaria [39, 40]. Bony destruction or pathological fractures are usually absent (**Figure 9-1-A**) [14]. In the early 1980s, several reports demonstrated that computed tomography was a more preferred diagnostic imaging method (**Figure 9-1-B**) [14]. 99 mTc bone scan has also been used to diagnose paraspinal extramedullary haematopoietic pseudotumours [41] but the diagnosis within the epidural space may be difficult due to the proximity to bone marrow [42]. Myelography is declining in popularity due to its invasiveness, the need for cisternal

puncture in cases of complete block preventing passage of radiographic contrast [41, 43] and reports of neurological deterioration following the procedure [24, 44].

Currently, magnetic resonance imaging has eventually replaced all these methods and is considered the method of choice for the diagnosis and follow-up evaluation of spinal cord compression cases resulting from paraspinal extramedullary haematopoietic pseudotumours [14]. Magnetic resonance imaging can clearly show anatomical details with high quality including both site and extent of the masses within the spinal canal, while producing soft tissue delineation with high sensitivity. Active recent haematopoietic extramedullary lesions have rich vasculature while inactive older lesions have more fatty tissue and iron deposits [20, 42, 45]. If the patient is treated with blood transfusions, the lesion may decrease in size and appear on magnetic resonance imaging with massive iron deposition [45]. Fatty degeneration is most probably related to oxidative stress leading to lipid peroxidation of cell membranes and production of oxygen free radicals. This is probably the reason why foci with fatty content are observed in non-transfused, non-chelated NTDT patients in whom conditions of oxidative stress occur more often than in transfused and iron-chelated β-thalassaemia major patients [14]. Although iron deposition and fatty replacement of the foci are inactivity procedures, they seem to never coexist, probably because of the different oxidative stress conditions [45]. Active lesions show intermediate signal intensity in both T1- and T2- weighted magnetic resonance images (**Figure 9-1-C**). Gadolinium enhancement is minimal or absent differentiating it from other epidural lesions such as abscesses or metastases [42, 46]. Older inactive lesions show high signal intensity in both T1 and T2 weighted magnetic resonance images due to fatty infiltration or low signal intensity in both T1 and T2 weighted magnetic resonance images due to iron deposition [47, 48]. Differential diagnosis is often very easy, when the lesion is multifocal (paravertebral and epidural) or bilateral, due to characteristic iron deposition or fatty replacement and the characteristic topography. The only diagnostic problem exists with the solitary, unilateral active lesion. Mesenchymal tissue tumours or tumours from neural tissue elements are in the differential diagnosis but the clinical history of congenital haemolytic anaemia usually helps correct diagnosis [45]. Although biopsy remains the gold standard for establishing a tissue diagnosis, it is an invasive procedure that carries the risk of catastrophic haemorrhage and is therefore not usually advocated. It may be of value reserved for older patients with a high probability of malignant disease and for cases in which the clinical and radiological picture is equivocal [14].

A recent report on 57 NTDT patients has also shed light on additional imaging findings related to spinal involvement of extramedullary haematopoiesis. Twenty-seven patients (47.4%) were found to have cortical bone invasion alongside extramedullary haematopoiesis with the most common location being the thoracic spine. Splenectomy and lower haemoglobin level were found to be independent risk factors for its development. Most lesions were homogenous (70%), had predominant red marrow signal (67%), and well-defined margins (89%) [49].

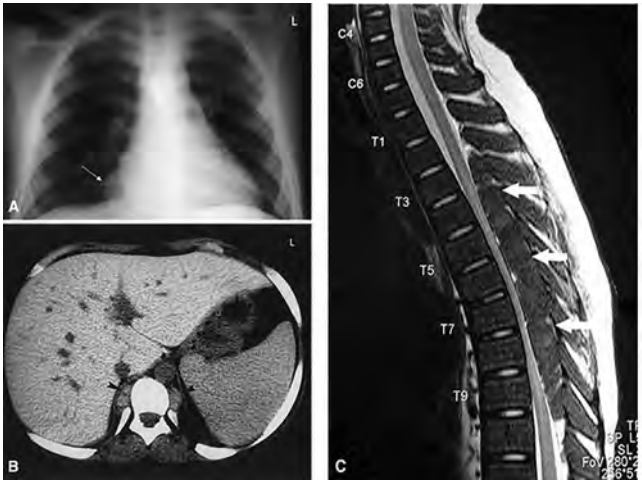

***Figure 9-1**. Representative images of paraspinal extramedullary haematopoietic pseudotumours. **(A)** Chest X-ray demonstrating expanded anterior rib ends consistent with medullary hyperplasia. A paraspinal mass is seen in the right lower zone (white arrow). **(B)** Computed tomography scan showing inactive paraspinal extramedullary haematopoietic lesion with increased density compared to soft tissue due to iron deposition (black arrowheads). **(C)** Magnetic resonance image of cervical and thoracic spine. T2-weighted sagittal image showing thoracic cord compression by extramedullary intraspinal epidural haematopoietic mass from T2 to T10 (white arrows). Reproduced with permission from [14].*

THE ROLE OF INTERVENTION

Observational studies, case series, and case reports confirm that both transfusion and hydroxyurea therapy may have a role in the prevention and management of extramedullary haematopoietic pseudotumours [4, 14-16]. A beneficial role of Janus Kinase 2 (JAK2) inhibitors on extramedullary haematopoiesis in the spleen leading to splenomegaly is suggested by animal studies and small clinical trials [1, 50-55].

Aside from blood transfusions and hydroxyurea, management options of paraspinal extramedullary haematopoietic pseudotumours may also include radiotherapy or surgical decompression, or any combination of these modalities [14]. Therapy usually depends on the severity of symptoms, size of the mass, patient's clinical condition, and previous treatment. Because the extramedullary haematopoiesis in NTDT patients is only a compensatory mechanism for ineffective erythropoiesis and chronic anaemia, initiation of blood transfusions can decrease the need for extramedullary haematopoiesis; thus resulting in relative inactivity of these tissues, and leading to the shrinkage of the mass size, decompression of the spinal cord, and neurologic improvement [14]. The initial response results primarily from a decrease in blood flow to these tissues even before reduction in the size of the mass can be detected [26, 56].

Thus blood transfusion (commonly hypertransfusion) is commonly used as the principal treatment modality. Some authors have reported cases treated exclusively by this modality as a first choice or in cases where surgical decompression or radiotherapy were contraindicated e.g., pregnancy or severe anaemia [26, 56-61]. The target haemoglobin level was usually >10 g/dL [14].

Blood transfusion was even considered of diagnostic value since only cases of cord compression secondary to extramedullary haematopoiesis, and not other entities on the differential diagnosis, could respond to transfusion therapy [26]. However, several reports also showed that improvement may be slow, insufficient, and only temporary [23, 33, 39, 46, 62]. Moreover, while blood transfusion may prevent further progression of the mass, it may be unable to reverse pre-existing cord compression. Its role in the management of patients with symptoms of acute onset may therefore be limited [26, 61]. Thus, many advocate using blood transfusion only as an adjunct to surgery (preparation and/or postoperative course) as correction of the haemoglobin level can be helpful in the immediate pre-operative period in order to insure an optimal oxygenation of the spinal cord during surgery [32, 42, 44, 57].

Data on the use of other modalities that target ineffective erythropoiesis and anaemia for the management of extramedullary haematopoietic pseudotumours in NTDT patients is limited. Patients with paraspinal extramedullary haematopoietic pseudotumours have been successfully treated with hydroxyurea alone especially in thalassaemic patients who are unable to receive blood transfusions due to alloimmunization [63, 64].

Low-dose radiation as a monotherapy has been reported to yield excellent results in up to 50% of patients with neurological improvement observed as soon as 3 to 7 days after initiation of treatment [27, 39, 43, 65, 66]. Haematopoietic tissue is extremely radiosensitive and undergoes shrinkage after radiotherapy [67]. Dosages reported in the literature range from 900 to 3500 cGy [27, 32, 36, 43, 58]. A high risk of recurrence up to 19-37%, is the main drawback of radiotherapy [36, 43]. These recurrences, however, are often amenable to further doses. The risks of radiotoxicity on an already compressed and injured spinal cord remain a concern [39]. Tissue oedema associated with radiation can sometimes result in neurological deterioration during the initial phase of treatment which is minimized by concomitant high-dose steroid therapy [44]. The immunosuppressive effect of radiotherapy is usually monitored with frequent peripheral blood counts as the resultant pancytopenia may further aggravate the condition [28, 36, 60]. In patients who need rapid therapeutic response due to severe neurological symptoms, radiotherapy is usually considered the primary treatment. In addition to primary treatment, radiotherapy is commonly employed as a post-operative adjunct following laminectomy to reduce the likelihood of recurrence [27, 29, 32, 42].

Successful combination therapy of any two of the three modalities (low-dose radiation, blood transfusion, and hydroxyurea) has been reported as a therapeutic option, either for cases of recurrence after using a single treatment method alone or as an initial treatment regimen [26, 34, 36, 39, 43, 58, 63, 68-71].

Laminectomy is usually reserved for cases of acute presentation which do not respond to adequate transfusion or radiotherapy [28, 42, 43]. Surgery confers the benefits of immediate relief of cord compression and histological diagnosis [42, 72, 73]. Disadvantages include risk of bleeding associated with the high vascularity of the mass in question and the risks of operating on anaemic individuals who are predisposed to shock, incomplete excision in cases of diffuse involvement, instability and kyphosis associated with multilevel laminectomy [24, 32, 38, 44, 46]. Another drawback is that the procedure is not always possible or desirable due to diffuse nature of the mass and the possibility of recurrence. Moreover, immediate total resection of extramedullary haematopoietic pseudotumours can lead to clinical decompensation and deterioration because these masses play a crucial role in maintaining an adequate haemoglobin level [43].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. NTDT patients at highest risk of developing extramedullary haematopoietic pseudotumours include:
   a. Adult patients
   b. Minimally- or never-transfused patients
   c. Patients with a haemoglobin level ≤10 g/dL
   d. Patients with low foetal haemoglobin levels
2. High-risk patients should be closely monitored and managed for ineffective erythropoiesis/ anaemia (see **Chapter 2**).
3. Patients with NTDT presenting with symptoms and signs of spinal cord compression, should be promptly evaluated for paraspinal extramedullary haematopoietic pseudotumours, preferably with magnetic resonance imaging of the spine, unless other diagnoses are suspected.
4. NTDT patients with evidence of paraspinal extramedullary haematopoietic pseudotumours should be promptly managed and followed by a dedicated team including a neurologist, a neurosurgeon, and a radiation specialist.
5. **Figure 9-2** illustrates a proposed algorithm for the management of paraspinal extramedullary haematopoietic pseudotumours.

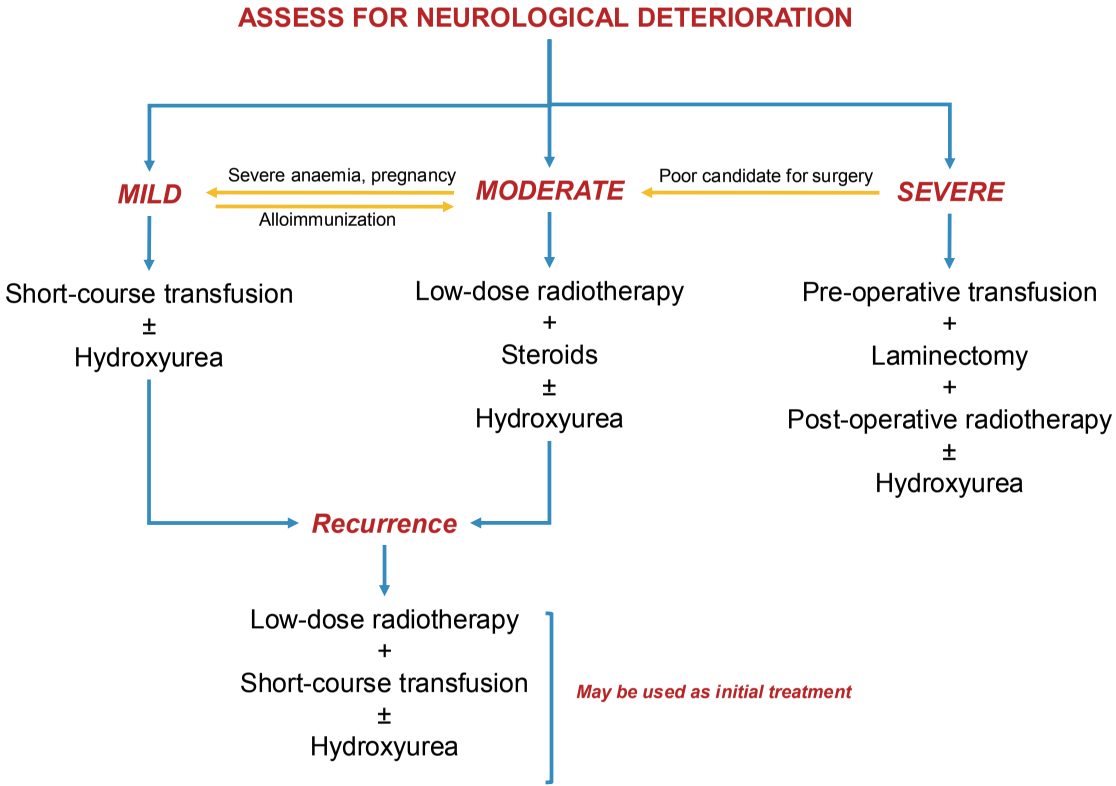

***Figure 9-2**. Algorithm for the management of paraspinal extramedullary haematopoietic pseudotumours in patients with non-transfusion-dependent β-thalassaemia (NTDT). Reproduced with permission from [14].*

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10 LEG ULCERS

Leg ulcers are more common in non-transfusion-dependent β-thalassaemia (NTDT) compared with transfusion-dependent β-thalassaemia (TDT) patients [1-4]. The risk of leg ulcers in NTDT patients increases with advancing age [4-6]. The skin at the extremities of elderly patients can be thin due to reduced tissue oxygenation making the subcutaneous tissue fragile and increasing the risk of ulceration after minimal trauma. **Severe anaemia and ineffective erythropoiesis, as well as splenectomy and hypercoagulability have all been described as risk factors for the development of leg ulcers** [3, 7-11]. The hypercoagulable state and deformability of red blood cells in NTDT patients has been incriminated in leg ulcer formation since this might cause ischemia to the skin and consequently friability and ulceration [12, 13]. High venous pressure in the subgroup of patients with right-heart failure and venous insufficiency may also be exacerbating factors [13, 14]. Data on the role of foetal haemoglobin levels are conflicting. Although some propose that high foetal haemoglobin levels, by virtue of its oxygen retaining capacity, increase the risk of ulcers, other studies showed lower rates of leg ulcers in patients with high vs low foetal haemoglobin levels [15]. Higher rates of leg ulcers have also been reported in NTDT patients with iron overload [6, 9, 16, 17]. Local iron overload is also thought to be a perpetuating factor causing chronicity of lesions especially when the haeme from the degraded red blood cells accumulates locally and gives a dark hue [18].

Leg ulcers are often very painful and indolent. Observational studies indicate that blood transfusion or hydroxyurea therapy with or without erythropoietin may have a role in prevention and management [3, 9, 14, 19]. The beneficial effects of hydroxyurea on leg ulcers in NTDT patients are not limited to foetal haemoglobin induction and improvement of anaemia but also include improvement of red blood cell pathology, deformability, and hypercoagulability [20]. Pentoxifylline, which alters the rheological properties of the red blood cell, was also shown to accelerate the healing of leg ulcers [21]. The use of an oxygen chamber was also shown to provide moderate relief where tissue hypoxia may be an underlying cause of the ulceration [12]. The vasodilator dialzep (adenosine reuptake inhibitor) was shown to have some benefit in a trial of eight patients with haemoglobin E/β-thalassaemia and chronic leg ulcers (three patients had total healing and four had improvement) [22]. Skin grafts have been tried by some plastic surgeons [12]. Both platelet derived wound healing factors and granulocyte macrophage colony-stimulating factor have been successfully used in some patients [23]. There is limited evidence on the benefit of anticoagulation for the management of leg ulcers in NTDT patients [13]. One trial has established benefit of sodium nitrite cream in patients with sickle cell disease and refractory leg ulcers [24]. Observations of healing leg ulcers have also been reported in clinical trials evaluating luspatercept for the treatment of anaemia in NTDT [25].

PRACTICAL RECOMMENDATIONS AND EXPERT OPINION

1. NTDT patients at highest risk of developing leg ulcers include:
   a. Adult patients
   b. Splenectomised patients
   c. Minimally- or never-transfused patients
   d. Patients with a haemoglobin level ≤10 g/dL
   e. Patients with iron overload (liver iron concentration ≥5 mg Fe/g dry weight or serum ferritin level ≥800 ng/mL)
   f. Patients with low foetal haemoglobin levels
2. High-risk patients should be closely monitored and managed for ineffective erythropoiesis/anaemia (see **Chapter 2**), iron overload (see **Chapter 3**), and hypercoagulability (see **Chapter 4**).
3. The skin of NTDT patients should always be inspected on routine physical examination.
4. Patient with evidence of leg ulcers should be treated in close collaboration with a dermatologist and a plastic surgeon:
   a. Simple measures may be beneficial, such as keeping the patient's legs and feet raised above the level of the heart for 1-2 hours during the day or sleeping with the end of the bed raised.
   b. Topical antibiotics and occlusive dressing should be applied
   c. Topical sodium nitrite cream may be considered
5. The following treatment measures may be considered in patients who have persistent leg ulcers, although no clinical trials supporting their use in this setting exist:
   a. Blood transfusion
   b. Hydroxyurea
   c. Luspatercept
   d. Dialzep (vasodilators)
   e. Oxygen chamber
   f. Skin grafts
   g. Platelet derived wound healing factors and granulocyte macrophage
   h. Anticoagulation

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11 QUALITY OF LIFE

It is now apparent that the diagnosis of non-transfusion-dependent β-thalassaemia (NTDT) carries higher morbidity than previously recognized especially as patients advance in age [1-3]. Chronic anaemia can also impact patients' mental and physical well-being with symptoms of fatigue, tiredness, weakness, and shortness of breath leading to poor health-related quality of life (HR-QoL) [4-10]. Moreover, NTDT patients may suffer from chronic pain, which appears multifactorial and also increases with age [11]. Hence, both the NTDT patient and the caring physician may be faced with multiple challenges towards understanding the true burden of the disease and its optimal management.

A recent cross-sectional study compared HR-QoL using the RAND short form (SF)-36 survey in 32 adult β-thalassaemia intermedia (never-transfused, non-chelated) and 48 β-thalassaemia major patients [12]. Patients with β-thalassaemia intermedia and major were comparable with age, gender, and socioeconomic parameters; but patients with β-thalassaemia major had a significantly longer median duration with a known thalassaemia diagnosis while patients with β-thalassaemia intermedia had a higher prevalence of multiple complications. The mean Total, Physical Health, and Mental Health Scores were significantly lower in patients with β-thalassaemia intermedia compared with β-thalassaemia major indicating poorer HR-QoL. A longer duration with a known thalassaemia diagnosis was the only independent variable correlating with higher (better) Mental Heath Scores; while multiplicity of clinical complications was the only independent variable correlating with lower (poorer) Physical Heath Scores. The study clearly indicated that multiplicity of complications in the non-transfused patient is a risk factor for compromised HR-QoL. Moreover, the shorter duration with a known diagnosis is also a risk factor (NTDT patients are usually diagnosed at an older age). This could be attributed to the diagnosis being made in adolescent years (period with high emotional stress), lower chance to adapt to the disease psychosocially, lower chance to understand disease, or fewer interactions with comprehensive care centres and staff [12].

Data from more recent studies including paediatric and adult patients also confirmed a high proportion of β-thalassaemia intermedia patients exhibiting lower than expected HR-QoL (similar or lower than β-thalassaemia major) [5, 13-15]. It was also apparent that a considerable proportion of adult patients with both β-thalassaemia major and intermedia show evidence of depression (Beck Depression Inventory) and anxiety (State-Trait Anxiety Inventory). Patients with β-thalassaemia intermedia, however, seemed more liable to state anxiety (feeling 'right now, at this moment') than β-thalassaemia major patients of a similar age, that was attributed to a shorter duration of living with a known thalassaemia diagnosis (poorer adaptation to disease) [16]. However, an organic cause of mental health could not be fully excluded, especially in light of the high prevalence of silent cerebral infarcts in these patients [17].

Data from patients with haemoglobin E/β-thalassaemia illustrate the beneficial effects of appropriate treatment on HR-QoL [18]. Improvements in haemoglobin levels have also been linked to improvements in patient reported outcomes in recent novel therapy trials [19]; and similar endpoints continue to be evaluated in NTDT studies [20].

PRACTICAL RECOMMENDATIONS AND EXPERT INSIGHTS

1. Patients with NTDT should be closely followed and appropriately managed as per the guidelines enclosed herein.
2. Frequent assessment of patients' HR-QoL and mental health status is recommended, preferably by standardized instruments and specialized staff.
3. The following interventions should be considered:
    a. Patient level
        i. Working on self-image conception
        ii. Helping the patient to understand illness and accept it
        iii. Involving the patient in his treatment to become responsible and autonomous
        iv. Psycho-social intervention in patients with poor HR-QoL or mental health problems
    b. Family level
        i. Helping the family to accept the situation and live with the diseased child
        ii. Arranging for meetings with other parents
        iii. Genetic counselling for better family planning
        iv. Psycho-social counselling to support the adolescent crisis of their children
    c. Community level
        i. Integration of patients into their society
        ii. Awareness campaigns about the disease
        iii. Highlighting patients' normal intellectual capacities
4. NTDT patients should receive appropriate nutritional, vitamin, and supplements support as per guidelines for transfusion-dependent β-thalassaemia.

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12 THE VALUE OF PATIENTS' ENGAGEMENT

Over the last 10 years, healthcare systems as well as academic, research and industry stakeholders have been increasing their efforts to integrate the patient voice into their work and decisions being taken. The provision of a patient centred element in a health care system requires the development of an environment that will truly foster engagement between patients and the healthcare team.

Transforming healthcare in the 21st century is a difficult and an extremely challenging task due to the many intricate layers that form part of and influence the system. Political, economic, and cultural factors are often constrained by value conflicts and resistance to change. In 2013, an international patient movement referred to as 'Patient Revolution' was established to enlist patients who live and experience the healthcare system on an everyday basis to help in designing care services that were better suited to their collective needs. Such initiatives mirror actions launched in other fields that rely on citizen science methods. The collective intelligence of large groups of people has been known to help address complex problems more effectively. Engaging patients with chronic conditions, such as those living with haemoglobin disorders, will contribute significantly to the identification of those components including quality standards and protocols of care that require improvement and aid in the development of new initiatives that may positively impact patient care and improve their overall quality of life.

The valuable contribution of patients has been demonstrated through a number of published studies but also unpublished information including that compiled by patient-oriented organisations such as the Thalassaemia International Federation (TIF) through their work with patients. TIF for example, has worked since 1986 with patients in different parts of the world, in countries with different economies, different health and social care systems, cultures, religions and social beliefs, and has evidenced the invaluable contribution of the patients' active involvement in achieving significant improvements in care policies.

Patients often have great insight into many aspects within the healthcare provision and how services directly and indirectly can substantially affect the care they receive. They are essential key players in assessing service needs and are instrumental in finding ways in which these can be improved. It is necessary thus to discover and leverage the huge untapped resource of patients' knowledge and experience to better understand and recognize those components of their care that are less than obvious to medical specialists. One of the most important drivers for change is to promote and implement a sort of 'cultural shift' on the part of medical specialists, clinicians, and scientists, in order to eventually forget the old figure of the patient under the paradigm of the paternalistic medicine and to accept what has been clearly demonstrated nowadays by many qualified 'expert patients' that their involvement constitutes an added value to healthcare system

improvements. In the field of haemoglobin disorders, many paediatricians and haematologists across the world from the old school are still involved and lead patients' organisations, thus this change is difficult to achieve. Specialists in many fields of the healthcare system, in regulatory and decision-making institutions, have reported how important can be the full involvement of patients for implementing inspired and creative outcomes with a mutual benefit. It has been shown in almost every hospital, clinic or centre that has been successful in reforming and improving the care services, in particular of its chronic patients including those living with haemoglobin disorders, that top-down strategies related to restructuring of the care services are not the sole proponents to improve the quality of care. The creation of a truly patient-centred care system is particularly favourable to the chronic, 'frequent' visitors of the services such as the multiply transfused patients with haemoglobin disorders.

HOW ENGAGING PATIENTS CAN BE OF BENEFIT

Engaging patients in identifying gaps and weaknesses in the care that is provided to them even when this is in accordance with disease specific guidelines, has proved a very strong tool for TIF. This is particularly seen in, but not confined to, countries of the developing economies, where the absence or very limited existence of national registries, reference centres and published information could not facilitate TIF's understanding of the challenges patients with haemoglobin disorders are facing in their individual countries. Obtaining a reliable picture of the situation of a country or a region or even part of a county with regards to the quality of care provided to chronic patients, can be extremely challenging without the active and meaningful involvement and participation of patient organisations themselves. Such information is crucial for TIF in order to support its work and to better tailor its activities, and projects at the national, regional and international level based on the needs and concerns as expressed by the patients themselves.

Needless to underscore the fact that the contribution and collaboration of treating physicians and generally the health care professionals' community in identifying gaps and in supporting the promotion of measures and policies for improvement through well-structured and documented recommendations to policy makers, remains invaluable.

THE KEY…

The key to meaningful and productive patient involvement lies largely on the very good knowledge and often relevant experience that the patient has in the particular area he/she is assigned to interact. In this context, European umbrella organisations such as the European Patients' Forum (EPF), the European Organisation for Rare Diseases (EURORDIS), and a number of disease specific organisations at the European level have been very actively involved in developing very comprehensive educational programmes for patients with different diseases on a variety of health, drug, and other research related topics in collaboration with experts and other stakeholders including the industry. These programmes aim at building a competent patient community that is knowledgeable enough to interact productively and advocate effectively for the

rights in quality and safe care at all levels and importantly at decision-making level at country and European levels.

TIF, the International Alliance of Patients' Organizations (IAPO), and other international disease specific organisations on the other hand have been struggling for decades now in the international arena and have been, and still are, actively involved in safeguarding patient safety, drug and blood safety and equity of all to quality health and social care. TIF, particularly since its establishment in1986, with the development and continual updating and upgrading of its educational programmes aims to strengthen the knowledge of patients across the world and 'transform them to valuable and equal partners at the decision making level'.

TIF in this context has developed since 1989, an educational programme which is constantly strengthened and is based on three pillars:

1. Preparation, publication, translation (into many languages), and distribution of educational and informational material such as books and factsheets.
2. The organization of events including workshops, seminars, conferences, symposia, meetings, courses, and fellowships.
3. The development of electronic educational platforms for patients and health care professionals and more currently the organisation of virtual educational events including webinars.

In addition, in more recent years, TIF established in 2017 the TIF Patient Advocacy Group (T-PAG) comprised of 198-member patient advocates from 62 countries. Many of these have developed adequate competency to actively advocate and interact productively at different levels of decision making at national, regional, or international levels and still many others are under training and working mostly at the national level. The vision of TIF being to 'create' a large group of competent patient advocates across countries and regions of the world to make the voice and position of the patients with haemoglobin disorders when involved, strong and effective.

THE PATH FORWARD

The national health/social and every other competent authority in a country need to 'invest' in developing official, well-structured channels of patient involvement at all levels of decision making if the aim is to honour the many and important relevant resolutions and declarations signed by all members of the WHO and in achieving the UN2030SGDI. These include but are not confined to patient rights, universal health coverage, quality healthcare, respect for patients and human rights for equal access to quality health and other care, and last but not least patient centred healthcare systems across the globe. These are all goals which cannot be effectively achieved without active involvement and official engagement of the patients (and their families) themselves. **Panel 12-1** summarizes possible patients' contribution in activities and policies relevant to their disease and its effective care, taking the example of TIF's work.

***Panel 12-1**. Patient engagement is probably advisable and invaluable in the following areas.*

1. Design of national disease specific registries and/or patient health records.
2. Design of educational or informational material that is prepared by health professionals and is focused on patient care, new drugs, research, and other.
3. Planning of the transfusion services and the related whole chain of the transfusion process. Transfused patients spend considerable amount of their time throughout their lives in hospitals/centres/wards/clinics for their one or two or more monthly transfusions. The timings of consultation and transfusion therapy are expected by patients to be less burdensome and more patient friendly allowing the least interruption in their lives and profession.
4. In the context of research and clinical trials at all stages of the process prior, during and post
5. Post drug authorisation period in facilitating the collection of real patients' data on the value, effectiveness, and safety of a drug.
6. Interactions concerning the design of national studies on the cost effectiveness versus added value of a new drug/therapy.
7. Negotiations and discussions for pricing of and access to drugs and therapies.
8. Plans of actions prepared by national competent authorities for addressing an epidemic or pandemic crisis (e.g., COVID-19 pandemic).
9. Revisiting, revising, or developing new recommendations and/or legislations that are related to their care and quality of lives.
10. Identifying gaps and weaknesses in the care provided for their condition in their particular hospital/ward/clinic/centre and in the solutions proposed.
11. Preparation of protocols and/or guidelines, the aim being to integrate their views and experience and to bring forward in any equation the element of quality of life, which is often 'forgotten' or underestimated in importance, by medical/scientific specialists and competent health/social authorities.
12. Revisiting process or development of new social care policies.
13. National health committees for raising awareness on the disease and blood donation campaigns.

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13 MULTIDISCIPLINARY CARE & REFERENCE CENTRES IN ADDRESSING HAEMOGLOBIN DISORDERS

The lifelong and multi-organ nature of non-transfusion dependent β-thalassaemia (NTDT), similar to transfusion-dependent β-thalassaemia (TDT), is well reflected in the very contents of this book. The early and accurate diagnosis of individuals with NTDT is of substantial significance, allowing close and well-structured monitoring of their progress aiming to prevent, as far as possible, the development of medical complications which may vary from mild to severe levels and which may eventually lead to transfusion dependence and poor quality of life. As patients advance in age, the basic needs in monitoring and medical attention even if and when provided appropriately, timely, and in accordance with international standards, gradually become inadequate to sustain life, maintain wellbeing, or achieve social integration. For this reason, specialists in several medical disciplines including but not confined to cardiovascular, liver, and endocrine disease are called upon to contribute by monitoring and offering proactive management of organ dysfunction in their field of expertise. These considerations and needs lead to significant and multiple challenges in the organization of integrated services so that the best possible conditions for patient care are achieved.

Historically, and based on the Thalassaemia International Federation (TIF)'s experience through its work globally, the need for establishing day transfusion centres for patients with TDT and NTDT (and other haemoglobin disorders such as sickle cell disease), separate from the main haematology or paediatric wards, where they were 'naturally' admitted initially, arose first in areas and places where patients with these disorders were treated in sufficient numbers. This prompted policy makers and healthcare professionals to recognise and respect that the needs of these patients are quite different from those of patients with malignant haematological conditions. So, through the years, different arrangements and development of specialized services (**Panel 13-1**) were promoted with regards to providing more targeted, specialised services to patients with haemoglobin disorders.

***Panel 13-1**. Arrangement options for specialised services for haemoglobin disorders.*

1. Within haematology departments: development of red blood cell (non-malignant) clinics separate from malignant haematology services.
2. Dedicated space within paediatric units where patients remain well into their adolescence and in many cases into their adulthood.
3. Dedicated space within haematology units (amongst malignant haematology/oncology patients) or transfusion centres of hospitals.
4. Development of "independent" thalassaemia units/centres based on outpatient principle attached to/associated with tertiary level hospitals.
5. Within general paediatric or paediatric/oncology or general haematology wards/clinics without any arrangement for a dedicated space or development of specialized services.
6. Development of centres run by patient non-government organizations in collaboration with pharmacists/red cross or crescent/healthcare professionals.

The need for the development and integration of specialised multidisciplinary care services into the management protocols to address more effectively haemoglobin disorders brought about the idea and vision for specialised, disease-specific training/education of healthcare professionals across different medical specialties. At the same time, however, it brought many and multiple organisational challenges to the healthcare system in order to ensure and achieve appropriate and effective coordination. Where such steps were taken and where these led to successful and meaningful integration of services, including active research activity, Reference or Expert Centres began to spring up.

Such centres first developed in the 1980s in Mediterranean countries including Cyprus, Greece, and Italy, and subsequently in the UK and France - in the latter more in the context of rare disease national strategies; although, at least initially, without any structured specific criteria. Both the knowledge and experience of what 'optimal' care was for these disorders as well as the research activity around the better understanding of their pathophysiology and medical needs were in those early days quite limited. The development of Reference/Expert Centres was guided almost solely by the needs of the growing, ageing patients with both TDT and NTDT; the services, patient care pathways and quality standards that these centres developed through the years became the core and solid basis for setting the criteria in later years for defining the role a Reference/Expert Centre for haemoglobin disorders should be fulfilling. Gradually these first centres, which in the meantime became deeply engaged in research, clinical trials, and educational programmes were officially as well and not only by reputation, assigned by the country's national competent authorities as Reference or Expert Centres undergoing regular and professional review of their quality standards. Patients with haemoglobin disorders within and gradually from outside these countries began to be referred to these centres by their treating physicians for consultation, second opinion, or for receiving specialised services that did not exist in their own area, region, or country.

As national competent authorities and healthcare specialists across the world began to acknowledge the value of effectively addressing these disorders and to gather knowledge and experience at the national level, clinics/centres of variable level of expertise, offering a different range of services of variable quality standards started emerging, particularly in the 1990s, in various countries across the world. In most of these, however, basic care was primarily provided in the form of blood transfusion and iron chelation for TDT patients. Care for NTDT patients was often less structured and more random. Multidisciplinary care, an essential component of care for hereditary, chronic, complex diseases such as haemoglobin disorders and a major component of a Reference/Expert Centre was, and still is, largely lacking in the majority of countries worldwide.

In most low- and middle-income countries, where more than 80% of the patients with these disorders live, the suboptimal medical and public health infrastructures (including haematology and transfusion services), the lack of universal health and social care coverage due to struggling economies and focus on other health priorities including communicable and common non-communicable diseases have not allowed such advances to take place. Particularly in the case of NTDT, for many decades to date, very little attention was given to its inclusion in the national strategies or health programmes for addressing haemoglobin disorders in almost all countries worldwide despite the recognition in more recent years of the high morbidity related to NTDT that needs to be addressed effectively to allow improvements of the health and quality of life and social integration.

The work of TIF for more than 35 years in over 60 countries across all regions of the world has exposed the naked truth: the development and integration of multidisciplinary care services into the management of patients with these disorders and the promotion and establishment of Reference/Expert Centres are to-date components of care that are far from being adopted or implemented. Such advances can only happen if and when the basic, essential medical care in a centre or across a country has reached those quality levels that allow patients to grow satisfactorily, achieve social integration, and have a reasonably acceptable, decent quality of life. This prerequisite can only be accomplished when the medical and public health infrastructures and quality standards are adequately strengthened in the context, as previously mentioned, of a healthcare system based on universal coverage. To-date such improvements are still to happen in most of the countries, and particularly in the developing ones where the majority of patients with these disorders live. The young age of the patients with TDT and the high rates of morbidity in those with NTDT in these countries, confirm the fact that they are still receiving suboptimal basic care and are devoid of well-coordinated monitoring and multidisciplinary care.

An example of the structure of interdisciplinary team for the care of haemoglobin disorders as extracted from some well-established European Reference Centres with successful patient outcomes initially and for many years for TDT but in more current years for NTDT patients includes (but is not confined to) the following medical health disciplines:

- Haematologist / paediatrician / internist
- Specialised nurses
- Cardiologist
- Endocrinologist
- Hepatologist
- Psychologist / social worker
- Obstetrician
- Dental care

Many other important heath care specialists are needed particularly when the centres are also treating sickle cell disease patients, which is the usual case in most centres across countries. Some of the key specialist services to which treating physicians and patients should have timely and well-co-ordinated access in this case are summarized in **Panel 13-2.**

Panel 13-2. *Additional specialist services for the management of haemoglobin disorders.*

1. Erythrocytapheresis.
2. Pulmonary hypertension team.
3. Fertility, contraception and sexual health services.
4. Consultant neurologist.
5. Consultant ophthalmologist.
6. Consultant nephrologist.
7. Consultant urologist with expertise in managing priapism and erectile dysfunction.
8. Orthopaedic service.
9. Specialist imaging including: a) MRI tissue iron quantification of the heart and liver with regularly standardised software to ensure accuracy and reliability of iron measurement, b) trans-cranial Doppler ultrasonography (children).
10. Polysomnography and head and neck surgery.
11. Bone marrow transplantation services.

REFERENCE/EXPERT CENTRES FOR HAEMOGLOBIN DISORDERS

Considerable work to-date on this topic has been conducted mainly by the European Commission in the context of its work on promoting quality services for rare diseases across the European Union. The many and complex challenges faced by patients, families, and treating physicians in the early and accurate diagnosis, management, and monitoring of rare diseases are similar to those that characterise haemoglobin disorders, which in many countries are rare diseases. However, contrary to the many thousands of other rare disease there is (and has existed for some time now) ample and reliable knowledge as well as experience

for haemoglobin disorders with regards to early and accurate diagnosis, specialised monitoring, appropriate management, and effective prevention.

The European Commission recognised rare diseases as a priority action area since the mid-1990s and since then the different European Union initiatives addressing rare diseases have predominantly focused on bringing together scattered resources and expertise across member states. This is an effort that is certainly needed in the case of haemoglobin disorders as well – both across Europe, and more importantly across countries with developing economies where the majority of patients with these disorders live. Through this work, the idea and vision of developing Reference/Expert Centres and networks arose by defining quality criteria and standards.

Within the 24 European Reference Networks (ERNs) that were established to cover different rare diseases or families of rare diseases and share best practices for their care and cure, the ERN on haematological diseases (EuroBloodNet) is the one focused on rare blood disorders including haemoglobin disorders. Considerable work is being undertaken by this network to pool together knowledge and expertise from across the European Union on these disorders. Some of the benefits of such actions are outlined in **Panel 13-3**.

Panel 13-3. *Benefits of the European Reference Networks initiative.*

1. Providing patients and healthcare professionals access to experts and expertise throughout all European member states, regardless of the country of origin or practice, thereby reducing inequalities and maximising the cost-effective use of resources.
2. Implementing epidemiological surveillance throughout the European Union that gathers comparable data on patients affected by rare diseases and launching preventive programmes for tackling rare diseases.
3. Fostering best practices for prevention, diagnosis, and clinical management.
4. Promoting the dissemination of knowledge, the sharing of expertise, supporting research, and increasing awareness of rare diseases.
5. Facilitating the transposition of the Directive 2011/24/EU of 9 March 2011 on the application of patients' rights in cross-border healthcare. The European Reference Networks between healthcare providers and Centres of Expertise is a main point of interest of the directive, especially for rare diseases. The networks will be a tool to improve the access to diagnosis and the provision of high-quality healthcare to all patients who have conditions requiring a particular concentration of resources or expertise and could also be focal points for medical training and research, information dissemination and evaluation, especially for rare diseases.

TIF, as a patient-driven umbrella organisation, has a constitutional mandate to continually identify ways and tools to promote the quality of care provided to patients with haemoglobin disorders. It has thus focused particular attention and considerable work on its educational programme since its establishment in 1986 and in this context TIF has initiated in 2017, a new project titled "TIF's Certification Programme of Reference Centres" focused on: the empowerment of national competent authorities, healthcare professionals. and patient communities to dedicate work on promoting the multidisciplinary care component and the establishment of Reference/Expert Centres into their management strategy.

The programme focuses on the application of specific quality standards for reference centres involved in the provision of care for patients with thalassaemia and other haemoglobin disorders. The TIF Quality Standards are based on the general principles already developed by the relevant organisations (**Panel 13-4**).

Panel 13-4. *TIF Quality Standards sources.*

1. The Joint Commission International (JCI): "Survey process Guide for Ambulatory Care (3rd Edition, 2015) European Union Committee of Experts on Rare Diseases (EUCERD): Quality Criteria for Centres of Expertise for Rare Diseases in Member States (2011) and EUCERD recommendations on Rare Disease European Networks (2013).
2. Guidelines for Good Clinical Practice.
3. US Institute of Medicine: Quality Improvement.
4. US Department of Health and Human Services, Health Resources and Service Administration: Quality Improvement.
5. UK National Health Services (NHS): Peer Review of Health Services for People with Haemoglobin Disorders (2015)
6. TIF "Guidelines for the management of transfusion dependent thalassaemia".
7. TIF "Guidelines for the management of non-transfusion dependent thalassaemia".
8. Specific standards, such as the "International Collaboration for Transfusion Medicine (ICTMG): Red blood cell specifications for patients with haemoglobinopathies: a systematic review and guideline" (2017).
9. European Network for Rare Congenital Anaemias (ENERCA) white book.
10. European Guidelines for the Certification of Haemophilia Centres (EUHANET 2013).
11. Current literature reviews.

The criterion for recognizing any centre as a Reference/Expert Centre is certainly the quality of services and its patient-centred care, and not just availability of various technical components necessary for thalassaemia (and other haemoglobin disorders) care. It includes following national or international evidence-based guidelines, which allow for good patient outcomes (**Panel 13-5**)

Panel 13-5. *Components of a Reference/Expert Centre.*

1. Have the capacity to provide expert diagnosis of the disease as well as its long-term complications.
2. Have the capacity to provide expert case management, based on best practice guidelines including a multidisciplinary approach and psychosocial support. These requirements imply experienced healthcare personnel in adequate numbers to ensure continuity of care.
3. Ensure that health care professionals work in a structured environment with clearly defined roles and hierarchy.
4. Maintain a patient registry with the ability to report patient outcomes and other epidemiological information. Electronic information systems must be regarded as essential tools for the provision of quality services.
5. Have regular auditing of clinical and laboratory guidelines.
6. Serve a sufficient number of patients (for example at least 50 transfusion-dependent thalassaemia patients) to maintain staff experience. Noting that what is a sufficient number of patients is not clear, but a consensus should be reached.
7. Provide patients with sufficient knowledge and information to promote partnership models and self-management support.
8. Have a significant contribution to research as evidenced by peer-reviewed publications.
9. Establish networking with a) Secondary treatment centres to provide education and share knowledge and expertise as well as expert opinion on challenging cases, and b) Other centres of expertise nationally.
10. Establish networks/collaborations with other Reference/Expert Centres outside the country – regional and international – to share best practices.
11. Maintain close links with patient organisations and other community resources at national, regional, and international levels.
12. Make a major contribution to educational activities.
13. Provide evidence of the improvement of patients' survival, clinical outcomes, and quality of life.

In addition, (i) there must be evidence of government and more specifically health system support, (ii) free access of patients to treatment modalities must be ensured, (iii) the centres' administrative structure, working hours and clinical space availability must also be taken into consideration, with the patient experience in mind, (iv) deficiencies and gaps must be promptly identified and corrected, (v) experience and knowledge of professional staff should be regularly assesses, and (vi) the patient perceptions of the quality of the services and the relationship with the staff should be monitored regularly through professional tools and taken into account in quality assessment.

FINAL THOUGHTS

Patients both with TDT and NTDT globally face huge unmet needs both in close and appropriate monitoring as well as basic medical care. Access to multidisciplinary care and expert review of their clinical status in Reference/Expert Centres is even more problematic. Both of these latter elements are unfortunately largely missing and according to TIF's records, accumulated through its 35 years' work at country level in over 60 countries across the world, such components are provided to less than 2% of the patients globally, constituting a severe violation of their rights as humans and as patients.

Healthcare services are becoming increasingly strained and healthcare authorities worldwide need to invest in integrated care particularly in the case of chronic, complex diseases such as the haemoglobin disorders, to first and above all deliver higher quality services for the patients while at the same time containing costs, safeguarding their sustainability and resilience to withstand continuity of quality care of chronic diseases such as thalassaemia and to addressing health crisis such as the recent COVID-19 pandemic. Unfortunately, existing evidence of the cost effectiveness of integrated care is limited particularly with regards to haemoglobin disorders where reliable data through well-designed and kept national registries encompassing the component of patient health records are largely absent. Future economic evaluation should target methodological issues to aid policy decisions with more robust evidence based on reliable, nationwide data.

It is also hoped that the contribution of this edition of TIF's Guidelines for the Management of Non-Transfusion Dependent β-Thalassaemia and the work of TIF at large, greatly supported by the World Health Organisation, the United Nations, the European Union, and many other regulatory and scientific/medical/professional bodies and individuals (at national, regional, and international levels) and importantly the patients and their families themselves where these may live, will contribute to the efforts of every country and TIF in providing a better future and ensuring more equity for patients with these complex, highly demanding disorders. Existence of inequalities and unmet needs constitutes unacceptable violation of our patients' rights.

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ABOUT THE THALASSAEMIA INTERNATIONAL FEDERATION (TIF)

The Thalassaemia International Federation, a non-governmental, patient-driven umbrella organisation, established in 1986, supports to-date, the rights of patients for access to quality health, social and other care through its work with over 200 national thalassaemia associations in 64 countries across the world. It was founded by a small group of doctors and patients/parents who represented National Patient Associations, mainly from Cyprus, Greece, Italy, UK and USA, i.e. countries where thalassaemia had been recognized early as a genetic, hereditary disorder with huge medical, public health, social and economic repercussions if left unaddressed in terms of both effective prevention and management. Thus, these were the countries where strong research activity was initiated and the first control programmes were implemented in the early 1980s, with measurable success. The rationale of these founding members lay on the establishment of an international umbrella organisation to build on the accumulated experience and the knowledge gained, aiming to support the efforts of other countries since by the mid-1980s the worldwide prevalence of the diseases had been well verified.

Our Mission: The prioritisation of thalassaemia on national health agendas and the development programmes within national healthcare systems based on universal coverage

Our Vision: To support the provision of equal access of every patient with thalassaemia to high quality health, social and other care in a truly patient-centred healthcare setting

Our Values: Transparency, reliability, ethos, accountability, independence and patient-centredness

Our Work:

- Education
- Advocacy
- Collaborations / Networking
- Research
- Raising Awareness

Our Partners:

- World Health Organisation:
- United Nations: in special consultative status with the United Nations Economic and Social Council (ECOSOC) since 2017
- Council of Europe: participatory status in the Conference of International NGOs since 2019
- European Union: official partners of the European Commission in the field of Health since 2018

Our Motto: Unity & Knowledge constitute our Strength!

A COMPREHENSIVE EDUCATIONAL PROGRAMME

EDUCATIONAL EVENTS

TIF organises physical and virtual educational events (conferences, seminars and workshops) held at local, national, regional and international levels.

- TIF's International Conferences on Thalassaemia and Other Haemoglobinopathies constitute the biggest educational events in the field of haemoglobin disorders, attracting over 2,000 participants from over 60 countries around the world.
- TIF's Pan-European, Pan-Asian and Pan-Middle East Regional Conferences are held in the years between the international conferences to shed light onto regional challenges and actively engage local actors in disease-specific education.

RENZO GALANELLO FELLOWSHIP PROGRAMME

Offered each year through the Joint Red Cell Unit, Haematology Department of the University College London NHS Foundation Trust in London, UK, under the leadership of Dr Perla Eleftheriou, Consultant Haematologist, the Renzo Galanello Fellowship programme covers all aspects of the clinical management of haemoglobinopathies and is addressed to physicians, specialists in the field of haematology, paediatrics or internal medicine.

e-ACADEMY

- **eThalED Course for Medical Specialists**

  The eThalED course offers specialised knowledge on the prevention and clinical management of thalassaemia to medical specialists who have an interest and/or are involved in these areas. Based on the "Guidelines for the Management of Transfusion-Dependent Thalassaemia (4th edition, 2021)", the course offers valuable insights on a number of topics, incl. genetic counselling, patient adherence and the changing doctor-patient relationship.

- **SCD e-Course for Healthcare Professionals**

  The Sickle Cell Disease Course is an online educational course for healthcare professionals around the world. This course covers all aspects of SCD clinical management, with content developed by eminent international medical experts, with extensive experience in treating patients with SCD. The course has been reviewed and endorsed by the European Hematology Association (EHA).

- **HPLC Screening in the Service of Prevention and Diagnosis**

  The course, HPLC Screening in the Service of Prevention and Diagnosis, offers specialised knowledge on interpreting HPLC chromatograms to identify haemoglobinopathy cases. This course is comprised of a series of educational videos covering in-depth key issues related to understanding and analysing HPLC chromatograms.

All courses are offered free-of-charge, attested by a certificate issued by TIF, and can be accessed through a simple registration on TIF's e-Academy.

EDUCATIONAL RESOURCES

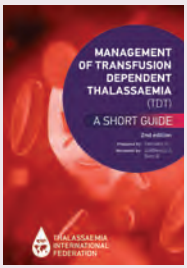
▶ TIF's LIbrary eXtended (TIFLIX)

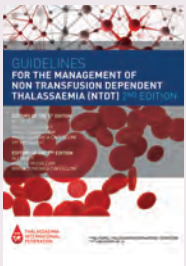
TIF's Library has been extended to provide its users with premium, on-demand educational video content on a variety of topics relevant to thalassaemia and other haemoglobin disorders. TIFLIX contains an extensive library of case studies and lectures addressed to healthcare professionals with an interest in the clinical management of haemoglobin disorders.

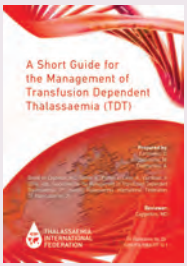
▶ Publications

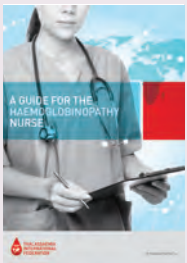
Since 1996, TIF has issued a vast number of diverse publications on thalassaemia and sickle cell disease, many of which have been and are still used as reference texts for academics, healthcare professionals, patient-support organisations and individual patients. New editions are regularly produced to keep up with scientific progress and novel concepts.

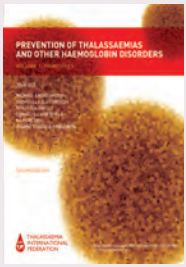
TIF PUBLICATIONS | HEALTH CARE PROFESSIONALS

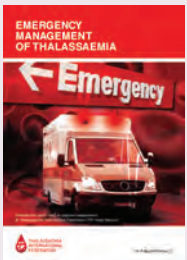












Please note that some of our publications are translated to a number of languages.

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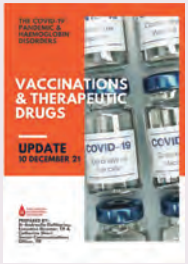

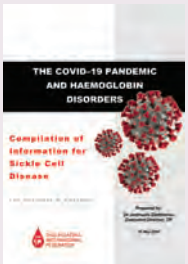

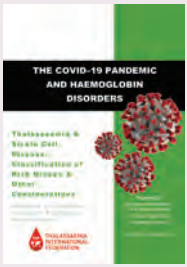

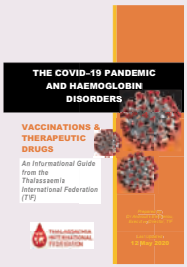

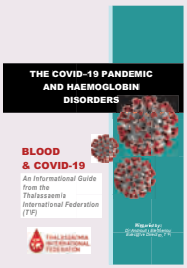

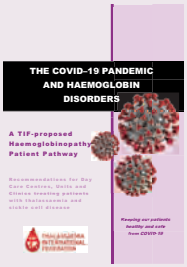

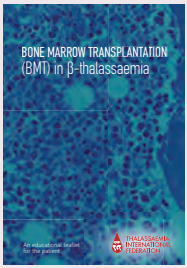

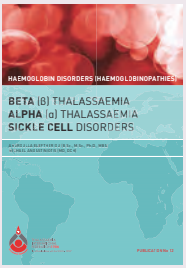

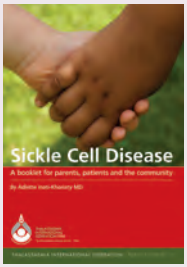

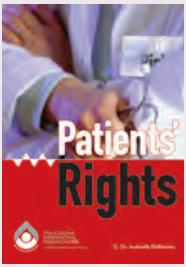

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“The good physician treats the disease; the great physician treats the patient who has the disease.”

Sir William Osler (1849-1919)

Notes

Notes

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