GUIDELINES
FOR THE MANAGEMENT OF NON TRANSFUSION DEPENDENT THALASSAEMIA (NTDT) 2ND EDITION

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CHAPTER 01
INTRODUCTION

Inherited haemoglobin disorders can be divided into two main groups. The first group includes the α- and β-thalassaemias, which result from the defective synthesis of the α- or β-globin chains of adult haemoglobin A. The second group includes structural haemoglobin variants like haemoglobin S, C, and E. A variety of thalassaemia phenotypes can result from the simultaneous inheritance of two different thalassaemia mutations from each parent or the co-inheritance of thalassaemia together with structural haemoglobin variants [1, 2].

Transfusion-dependence is one essential factor in distinguishing the various thalassaemia phenotypes and their severity. For example, the diagnosis of ß-thalassaemia major entails life-long regular transfusion requirement for survival, and the term transfusion-dependent thalassaemia (TDT) is conventionally used to describe such forms [3]. Non-transfusion-dependent thalassaemias (NTDT) is a term used to label patients who do not require such lifelong regular transfusions for survival, although they may require occasional or even frequent transfusions in certain clinical settings and for defined periods of time (Figure 1-1) [4]. It should be noted, however, that TDT and NTDT are fluid categories, based on clinical parameters, variations and advances in the clinical management, as well as other modifiers of disease, which may shift a patient from one group to another during their lives. Thus, the TDT and NTDT designations should primarily represent patients' 'current' clinical status and entail the understanding that these two designations are interchangeable.

NTDT encompasses three clinically distinct forms: ß-thalassaemia intermedia, haemoglobin E/ß-thalassaemia (mild and moderate forms), and α-thalassaemia intermedia (haemoglobin H disease) [5]. Although patients with haemoglobin S/ß-thalassaemia and haemoglobin C/ß-thalassaemia may have transfusion requirements similar to those of NTDT patients, these forms have other specific characteristics and management peculiarities [6, 7], and will not be covered in these guidelines.

Figure 1-1:
Transfusion requirement in various thalassaemia forms. Reproduced with permission from reference [4].
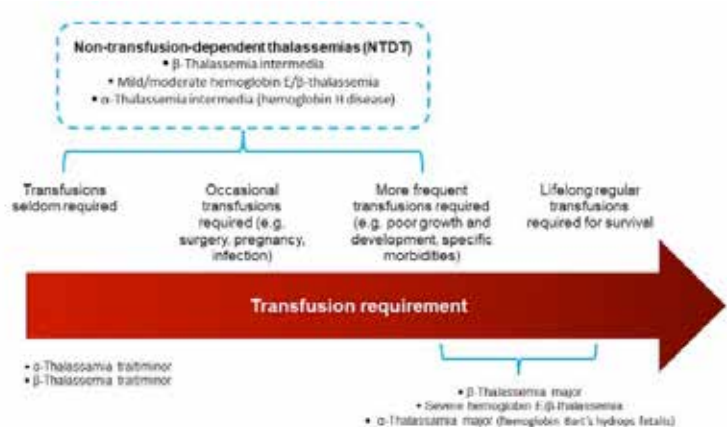
EPIDEMIOLOGY OF NTDT

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 [8]. 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 has been or is still prevalent [8, 9]. Improvements in public health standards in these regions have helped to improve survival of affected patients. However, continued migration has greatly expanded the reach of these diseases into large, multi-ethnic cities in Europe and North America [8].

Data on the global epidemiology of the thalassaemias, especially NTDT, are scarce [5]. Approximately 68,000 children are born with various thalassaemia syndromes each year [8, 10, 11]. ß-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 [12]. Some 23,000 children are born with transfusion-dependent ß-thalassaemia major each year, while a smaller ill-defined number have the NTDT form ß-thalassaemia intermedia [1, 8, 11]. Irrespectively, this form has a relatively low and varying prevalence in every population with a high frequency of ß-thalassaemia carriers and is particularly common in parts of the Eastern Mediterranean and Africa where mild ß-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% [13, 14]; as well as through regions of China, Malaysia, Indonesia and Sri Lanka [8, 15]. Haemoglobin E/ß-thalassaemia currently affects approximately 1,000,000 people worldwide [16]. In North America, it has become the most common form of ß-thalassaemia identified in many newborn screening programmes [16]. In California, 1 in 4 Cambodian births and 1 in 9 Thai/Laotian births are haemoglobin E carriers [18]. Globally, more than 19,000 children are born each year with haemoglobin E/ß-thalassaemia, with half of them having a severe transfusion-dependent form (which represents approximately 30%-50% of all severe forms of ß-thalassaemia [14]) while the remaining half fall into the category of NTDT (mild and moderate forms) [8, 11].

α-Thalassaemia is the most common inherited disorder of haemoglobin, with approximately 5% of the world's population being carriers and an approximate of 1,000,000 patients affected with the various α-thalassaemia syndromes worldwide [19, 20]. It occurs at a particularly high frequency in populations from sub-Saharan Africa through the Mediterranean region and Middle East, to the Indian sub-continent and East and Southeast Asia [1]. Recent global population

movements have also led to increasing incidences in other areas of the world, such as North Europe and North America, previously relatively unaffected by this condition [21]. Of 530,000 newborns screened for haemoglobinopathies between January 1998 and June 2006 in California; sickle cell disease was the most frequent (1 in 6600 births) followed by α-thalassaemia in 11.1 per 100,000 infants screened or roughly 1 in 9000 births [17, 22]. α-Thalassaemia syndromes are also increasingly being diagnosed outside of California, but since most states do not screen for these disorders, the diagnosis is not made until clinical complications occur. Globally, more than 5,000 children are born each year with severe forms of α-thalassaemia, α-thalassaemia major or haemoglobin Bart's Hydrops Foetalis but only few children survive facing a lifelong requirement for transfusions. The annual number of births for the NTDT form of α-thalassaemia, α-thalassaemia intermedia or haemoglobin H disease, is approximately 10,000 [8, 11]. However, these numbers might even be underestimated since there are limited genetic epidemiology studies of α-thalassaemia syndromes in several parts of the world in the Far East, in particular.

PHENOTYPE DISTINCTION AND GENOTYPE-PHENOTYPE ASSOCIATIONS IN NTDT

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

β-THALASSAEMIAA INTERMEDIA

In patients with β-thalassaemia intermedia, the primary modifier of phenotype is the broad diversity of mutations that affect the β-globin gene in the homozygous or compound heterozygous state (>200 disease-causing mutations, updated list available http://globin.cse.psu.edu) [24]. These range from mild promoter mutations that cause a slight reduction in β-globin chain production to the many different mutations that result in the β°-thalassaemias; that is, a complete absence of β-globin chain synthesis. Deletions of the β-globin gene are uncommon. 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 and phenotype 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 [25], increased expression of α-haemoglobin stabilizing protein [26, 27], and effective synthesis of γ-chains 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) [24]. Tertiary modifiers include polymorphisms that are not related to globin

chain production but may have an ameliorating effect on specific complications of the disease such as iron absorption, bilirubin metabolism, bone metabolism, cardiovascular disease, and susceptibility to infection [28, 29]. ß-thalassaemia intermedia may also result from the increased production of α-globin chains by a triplicated or quadruplicated α-genotype associated with ß-heterozygosity [30-35]. Less commonly, only a single ß-globin locus is affected, the other being completely normal, so in these instances, ß-thalassaemia intermedia is dominantly inherited [36, 37]. Table 1-1 illustrates common genotypes leading to a ß-thalassaemia intermedia phenotype [4].

Table 1-1: Genotype-phenotype associations in ß-thalassemia. Reproduced with permission from reference [4].

Phenotype	Genotype	Clinical severity
Silent carrier	• silent ß/ß	• Asymptomatic • No hematological abnormalities
Trait/minor	• ß⁰/ß, ß⁺/ß, or mild ß⁺/ß	• Borderline asymptomatic anemia • Microcytosis and hypochromia
Intermedia	• ß⁰/mild ß⁺, ß⁺/mild ß⁺, or mild ß⁺/mild ß⁺ • ß⁰/silent ß, ß⁺/silent ß, mild ß⁺/silent ß, or silent ß/silent ß • ß⁰/ß⁰, ß⁺/ ß⁺, or ß⁰/ ß+ and deletion or nondeletion α-thalassemia • ß⁰/ß⁰, ß⁺/ ß⁺, or ß⁰/ ß⁺ and increased capacity for γ-chain synthesis • Deletion forms of δß-thalassemia and HPFH • ß⁰/ß or ß⁺/ß and ααα or αααα duplications • Dominant ß-thalassemia (inclusion body)	• Late presentation • Mild-moderate anemia • Transfusion-independent • Clinical severity is variable and ranges between minor to major
Major	• ß⁰/ß⁰, ß⁺/ ß⁺, or ß⁰/ ß⁺	• Early presentation • Severe anemia • Transfusion-dependent

HPFH, hereditary persistence of fetal hemoglobin.

HAEMOGLOBIN E/ß-THALASSAEMIA

Haemoglobin E is caused by a G-to-A substitution in codon number 26 of the ß-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 ß+-thalassaemia. Patients with haemoglobin E/ß-thalassaemia co-inherit a ß-thalassaemia allele from one parent, and the structural variant, haemoglobin E, from the other [25, 38]. Haemoglobin E/ß-thalassaemia is further classified into severe (haemoglobin level as low as 4-5 g/dl,

transfusion-dependent, clinical symptoms similar to ß-thalassaemia major), moderate (hae-moglobin levels between 6 and 7 g/dl, transfusion-independent, clinical symptoms similar to ß-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 [39]. A disease scoring system that helps classify patients into mild, moderate, and severe has been proposed (Table 1-2) [40].

Table 1-2: Mahidol score for haemoglobin E/ß-thalassaemia severity classification. Repro-duced with permission from reference [40]

Criteria	Value	Score	Value	Score	Value	Score
Steady-state hemoglobin, 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/ß-thalassaemia (severity score <4); moderate haemoglobin E/ß-thalassaemia (severity score 4-7); and severe haemoglobin E/ß-thalassaemia (severity score >7).

Similar to patients with ß-thalassaemia intermedia, modifiers of disease severity in hae-moglobin E/ß-thalassaemia include the type of ß-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 [15, 16, 25, 41-46]. It should be noted that patients with haemoglobin E/ß-thalassaemia also show different phenotypic severity at particular stages of development. Advancing age has an inde-pendent and direct effect on the background level of erythropoietin production in response to anaemia [47-49]. A notable environmental factor influencing phenotype in patients with hae-moglobin E/ß-thalassaemia is infection with malaria, particularly Plasmodium vivax [50].

α-THALASSAEMIA INTERMEDIA (HAEMOGLOBIN H DISEASE)
Unlike ß-thalassaemia, deficient synthesis of α-globin chains in α-thalassaemia is typically caused by deletions within the α-globin gene cluster on chromosome 16. Approximately 128 dif-ferent molecular defects are known to cause α-thalassaemia [21, 51]. There are many different sized deletions of the α-globin genes. Southeast Asian deletion (-SEA) is the most common and involves both α-genes, but not embryonic globin genes. Larger deletions such as (-THAI) affect embryonic genes and may be more severe [52, 53]. The different phenotypes in α-thalassaemia

are primarily attributed to whether one (α+-thalassaemia) or both (α0-thalassaemia) α-globin genes are deleted in each of the two loci (Table 1-3) [4].

Table 1-3: Genotype-phenotype associations in α-thalassaemia. Modified with permission from reference [4].

Phenotype	Genotype	Clinical severity
Silent carrier	-α/αα	• Asymptomatic • No hematological abnormalities
Trait/minor	-α/-α --/αα	• Borderline asymptomatic anemia • Microcytosis and hypochromia
Deletional hemoglobin H disease	--/-α	• Mild-moderate anemia • Transfusion-independent • Clinical severity is variable and ranges between minor to major
Non-deletional hemoglobin H disease	--/α^{T}α	• Moderate-Severe anemia • May require occasional or frequent transfusions
Major (hemoglobin Bart's hydrops fetalis)	--/--	• Most develop hydrops fetalis syndrome and die in utero during pregnancy, or shortly after birth • Survivors are transfusion-dependent

Haemoglobin Bart's hydrops foetalis (α-thalassaemia major) is caused by deletion of all four α-globin genes, and is a condition that is primarily incompatible with life. Deletion of three α-globin genes results in haemoglobin H disease (α-thalassaemia intermedia) [21, 51]. In addition to deletional forms, there are at least 70 forms of non-deletional haemoglobin H disease (α-thalassaemia intermedia), which are typically associated with a more severe phenotype (Table 1-4) [54] and may require occasional or frequent transfusions. The most commonly described non-deletional haemoglobin H disease forms are haemoglobin H Constant Spring and also including haemoglobin H Paksé, Quong Sze, and Suan Dok [20, 25, 55-57]. These deletional and non-deletional haemoglobin H diseases (α-thalassaemia intermedia) are the NTDT forms covered in these guidelines. It should be noted that the most severe forms of non-deletional haemoglobin H disease may become completely transfusion-dependent (haemoglobin H hydrops) in which case these should be managed as with β-thalassaemia major patients. These rare forms usually result from interaction of α0-thalassaemia and rare non-deletional mutations such as αCd 59Gly-Asp, αΔCd 30, αCd 66 Leu-Pro, αCd 35Ser-Pro, and haemoglobin H Pak Num Po. Studies on the role of modifiers of disease severity in α-thalassaemia are limited. Genetic modification may occur with co-inheritance of mutations in β-globin genes resulting in β-thalassaemia, also referred to as haemoglobin H/β-thalassaemia trait [20].

In non-deletional haemoglobin H disease there may be a role for the α-haemoglobin stabilizing protein in ameliorating disease severity although this warrants further study [25].

Table 1-4: Clinical and haematologic manifestation of deletional and non-deletional forms of haemoglobin H. Reproduced with permission from reference [54].

Clinical manifestation	Deletional (Hemoglobin H disease)	Non-deletional (Hemoglobin H Constant Spring)
Hemoglobin, g/dl	8.5 (range 6.9–10.7)	7.2 (range 3.8–8.7)
Mean corpuscular volume, fl	54.0 (range 46.0–76.0)	65.2 (range 48.7–80.7)
Mean corpuscular hemoglobin, pg	16.6 (range 14.3–24.7)	18.6 (range 14.8–24.8)
Anemia	Microcytic	Normocytic
Reticulocytosis	+	++
Hypochromia	++	+
Age at first transfusion, years	11 ± 5.5	1.5 ± 2.1
History of blood transfusion, %	3-29	24-80
Splenomegaly	+	+++
Gallstones	+	++
Growth retardation	Rare	Common
Decreased bone density	Rare	Common

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 [8]. 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 [22, 58]. Advances in capillary electrophoresis and molecular testing improve the specificity and availability of diagnosing individuals outside the neonatal period [22, 58]. 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 [59].

These discriminating formulas have excellent diagnostic efficacy and are very economical.

The best initial screening approach is to combine an analysis of red blood cells (mainly to detect reduction in the size of red cells and haemoglobin content) together with a measurement of the level of the minor haemoglobin component, haemoglobin A2, which is nearly always elevated in β-thalassaemia carriers [5]. However, the existence of two types of α-thalassaemia raises screening challenges. α0-Thalassaemia in which both α-genes are deleted is associated with typical thalassaemic red blood cell changes, while α+-thalassaemia in which only one of these genes is deleted exhibits minimal red blood cell changes. Thus, the only absolutely secure approach to screening for α-thalassaemia may be by DNA analysis [60]. The easiest time to diagnose haemoglobin H disease is at birth. Haemoglobin Bart's is commonly seen on haemoglobin electrophoresis, but rapidly disappears after birth [22]. Bart's haemoglobin level of 25% indicates haemoglobin H disease. Bart's haemoglobin level at birth of 1-4% and 4-10% indicate α+- and α0-thalassaemia carriers, respectively. Definitive diagnosis requires molecular genetic testing [53, 61]. 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. The structural variants (haemoglobin E, S, and C) are easily identified by various forms of haemoglobin analysis [5]. 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 [62]. Algorithms to guide the clinical suspicion (Figure 1-2) and laboratory diagnosis of NTDT (Figure 1-3) have been recently published [63].

Table 1-2:
Clinical suspicion
of NTDT at primary
care. Reproduced
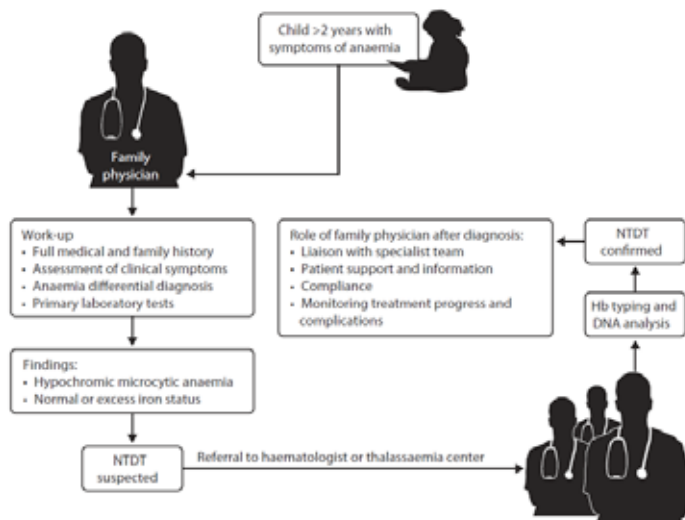with permission from
reference [63].

Table 1-3: Laboratory work-up to facilitate diagnosis of 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 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/βthalassaemia. These diagnoses can be confirmed using appropriate globin genotyping. Reproduced with permission from reference [63].
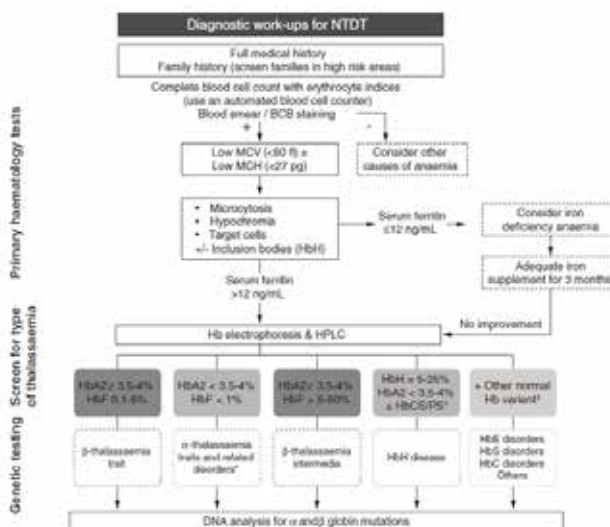
SCOPE OF THESE GUIDELINES

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 combating 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, the editors provide, through consensus, management recommendations using their clinical expertise in treating NTDT patients. Lastly, we would like to note that a new disease scoring system that can guide management decisions in NTDT has been developed, although validation is still awaited before wide recommendation as part of these guidelines [64]. 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 [65].

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CHAPTER 02
BLOOD TRANSFUSION

ILLUSTRATIVE CASE

A four-year old girl undergoes routine medical checkup by her local physician. The parents had noted that the child is lethargic and that she reaches her development milestones slightly late. She is short for her age, although not quite below the 5th percentile line of the growth chart. Clinical examination shows mild scleral icterus, tachycardia with regular rhythm, II/VI flow murmur over the left sternal border, and hepatosplenomegaly. Laboratory test results were as follows: serum ferritin level 327 ng/ml, iron 155 µg/dl, total haemoglobin level 7.1 g/dl, Chem-10 within normal limits, and total bilirubin level 5.2 mg/dl. Using a supravital staining by brilliant cresyl blue, numerous golf ball-like red blood cell inclusions (haemoglobin H inclusion body) were detected. This finding was further confirmed by haemoglobin electrophoresis that showed 15% haemoglobin H (ß4) and haemoglobin Bart's (γ4) with haemoglobin A as the majority of total haemoglobin. In addition, a slow moving haemoglobin species was detected with a rather small amount (~2%) suggesting the presence of haemoglobin H Constant Spring or haemoglobin H Pakse'. The diagnosis was subsequently confirmed using DNA testing which showed the patient is compound heterozygote for SEA type α-thalassaemia deletion and a non-deletional α-thalassaemia mutation due to a nucleotide substitution at the termination codon; haemoglobin H Constant Spring (--^{SEA}/α^{CS}α). The haematologist recommends blood transfusions to overcome growth retardation.

CONTEXT AND EVIDENCE

BENEFITS OF TRANSFUSION THERAPY IN NTDT
In 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 methemoglobin and hemichromes with eventual separation of haem from globin. The free iron released from haem disintegration in thalassaemia 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) or peripheral circulation (haemolysis) [1-5]. Ineffective erythropoiesis and peripheral haemolysis in NTDT patients lead to a multitude of subsequent patho-physiologies and clinical complications throughout the course of the disease (Figure 2-1) [6-8], which will be featured in later chapters. Without appropriate treatment, the incidence of these morbidities increases with advancing age [9, 10]. Recent work has identified and independent and direct link between anaemia and morbidities in NTDT, with a change of 1 g/dl of haemoglobin being significantly associated with morbidity risk. Patients living with total haemoglobin values <7 g/dl appeared to definitely develop

morbidity across the course of their disease while those with values >10 g/dl were commonly protected from developing any severe morbidity [11].

Figure 2-1: Pathophysiologic mechanisms and clinical complications in NTDT. Reproduced with permission from reference [6].
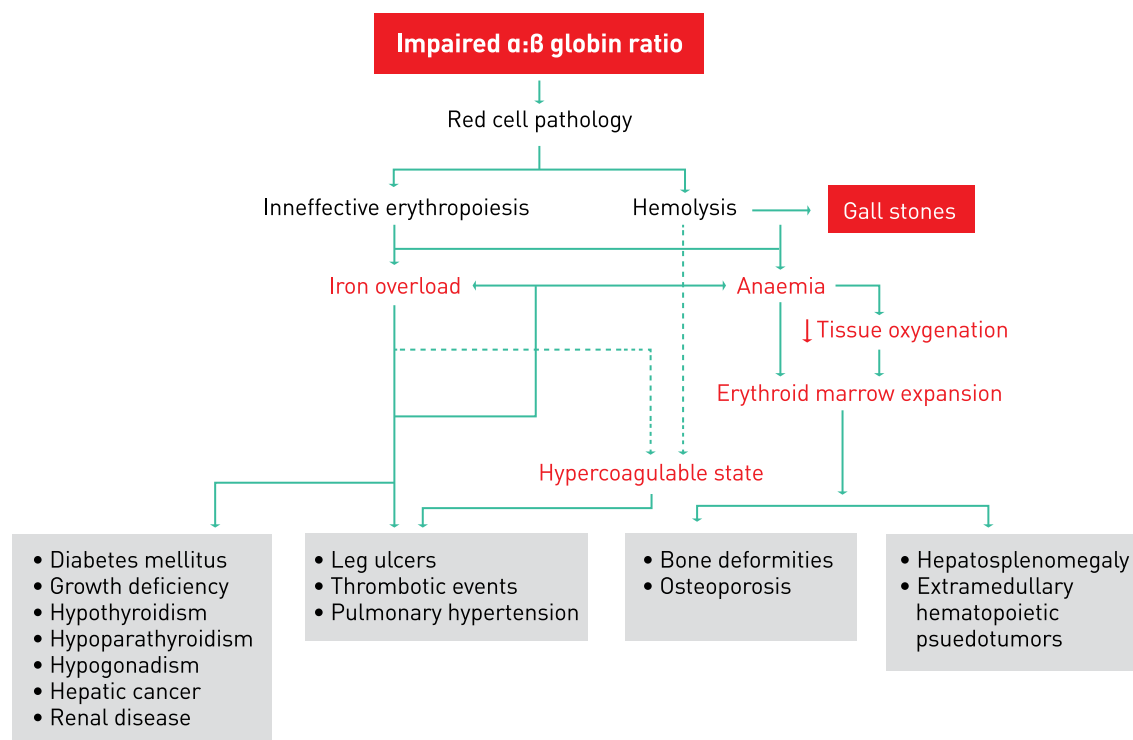
Transfusion therapy is effective in supplying normal erythrocytes and suppressing ineffective erythropoiesis [12, 13]. Upon transfusion therapy, erythroid activity decreases to 1-2 times normal levels with pre-transfusion haemoglobin values between 10 and 11 g/dl, 1-4 times normal levels with values between 9 and 10 g/dl, and 2-6 times normal levels for values between 8.6 and 9 g/dl [14]. Thus, despite transfusion-independence in patients with NTDT, transfusion therapy can theoretically ameliorate many of the pathophysiologic mechanisms and morbidities emanating from anaemia, haemolysis, and ineffective erythropoiesis; and it is logical to consider blood transfusions for the prevention or management of complications in this group

of patients. 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 treatment modality, especially transfusion therapy, too hastily. Many patients with NTDT, who may not need regular transfusion, embark on a life of unnecessary treatment of this kind, particularly if they present with an unusually low haemoglobin level during a period of inter-current infection. Even if a few transfusions have been administered in the acute situation, immediate commitment to a transfusion programme is not recommended and haemoglobin level should not be a sole determinant of transfusion need. In fact, some children with NTDT, specifically with haemoglobin E/ß-thalassaemia, have a remarkable facility to adaptation for low haemoglobin levels [15, 16]. Instead, the patient's well-being, particularly with respect to activity, growth, development, and the early appearance of skeletal changes are the factors to be taken into consideration [17, 18]. A beneficial role of transfusion therapy on growth parameters in NTDT patients has been objectively identified (Vip Viprakasit, Unpublished Data). It should also be noted that in patients with haemoglobin H disease, transfusion requirement is often encountered in non-deletional forms (e.g. haemoglobin H Constant Spring), while deletional form often grow relatively well without the need for transfusion therapy [19].

Although clinical trials evaluating the role of transfusion therapy in NTDT patients are lacking, observational studies also suggest a role for blood transfusions in the prevention and management of the following clinical morbidities frequently encountered in adult NTDT patients: leg ulcers, thrombotic events, pulmonary hypertension, silent brain infarcts, and extramedullary hematopoietic pseudotumours [17, 20-25]. These observations may also partly explain the higher prevalence of these morbidities in certain NTDT forms compared to regularly trans-fused ß-thalassaemia major patients [6, 26, 27].

Rather than enforcing the lifelong regular transfusion regimens implemented in ß-thalassemia major patients; blood transfusion, if initiated in patients with NTDT will require closer monitoring and should be individually tailored to meet patient's needs. Although some patients may require frequent transfusions, these are usually temporary and can be tailored or withdrawn when the desired outcomes are achieved. Patients having sustained frequent transfusions for extended periods of time should be managed as per the guidelines for transfusion-dependent ß-thalassaemia major patients [28].

ADVERSE OUTCOMES ASSOCIATED WITH TRANSFUSION THERAPY

The concern with transfusion therapy in NTDT patients is the risk of iron overload (see Chapter 5) and alloimmunization. The risk of alloimmunization is highest in minimally transfused and newly transfused patients, in splenectomized patients, and during pregnancy (see Chapter 10). The risk of alloimunization is 1 to 1.6% after transfusion of one blood unit [29-31]. The risk of alloimmunization can be minimized by extended genotype and antibody screening, and the use

of fully-phenotyped matched blood should be given. Concomitant administration of steroids for 3 to 5 days as prophylaxis against alloimmunization has been reported but the efficacy of this intervention is controversial [30].

Novel therapies that can ameliorate ineffective erythropoiesis and improve haemoglobin in NTDT

Gene therapy is based on the idea that if there is a defect in production of ß-globin in ß-thalassaemia, exogenous production of ß-like globins could correct the disorder. The hematopoietic system is convenient for such approaches, since hematopoietic stem cells from an individual with ß-thalassaemia can be isolated and transduced with viruses to introduce exogenous genetic material, such as ß-like globin transgenes that can allow for such exogenous gene expression. Following successful application in few thalassaemia cases [32-34], clinical trials have been initiated (NCT01745120, NCT02151526, NCT02140554) but mostly in transfusion-dependent patients. Several years are expected before efforts in the area of gene therapy will widely benefit patients, and this may be largely restricted to resource-rich countries and transfusion-dependent patients. Another limitation of such approaches for correction in ß-thalassemia is that it is challenging to fully compensate for the significant deficit of ß-globin expression using exogenous genetic material introduced through a viral vector. Improved viral vectors that have enhanced globin gene expression and other technological develops are showing promise to improve upon such approaches [35].

In the past few years, a group of ligand-traps were developed that have been shown to potently stimulate erythropoiesis and improve ineffective erythropoiesis in ß-thalassaemia [33]. These ligand-traps, which have been named Sotatercept and Luspatercept, bind TGF-ß superfamily members including growth differentiation factor 11 (GDF11) and promote late-stage erythropoiesis [36, 37]. In an ongoing phase II open-label ascending dose study of Luspatercept administered subcutaneously every three weeks, adult patients with NTDT (ß-thalassaemia intermedia or haemoglobin E/ß-thalassemia) showed improvement in haemoglobin level associated with improved patient reported outcomes as well as improvement in liver iron concentration and amelioration of some morbidities like leg ulcers. No serious adverse events were noted (NCT01749540); these data are paving the road to further clinical development.

PRACTICAL RECOMMENDATIONS

- Haemoglobin level should not be an indicator for initiation of transfusion therapy, except in patients with considerably severe anemia (haemoglobin level <5 g/dl)

- Occasional blood transfusions should be considered in NTDT patients within any setting with anticipated acute stress, haemoglobin drop, or blood loss; such as:

> Pregnancy
> Surgery
>Infections

- More frequent transfusions should be considered in the following settings, with reassessment for tapering or withdrawal when a sustained clinical benefit is achieved:
  > Declining hemoglobin level in parallel with profound enlargement of the spleen (at a rate exceeding 3 cm/year in periods of maximal growth and development)
  > Growth failure (height is more indicative of growth pattern than weight)
  > Poor performance at school
  > Diminished exercise tolerance
  > Failure of secondary sexual development in parallel with bone age.
  > Signs of bony changes
  > Frequent haemolytic crisis (haemoglobin H disease)
  > Poor quality of life

- Transfusions may be considered for the primary prevention (in high-risk populations), management, or secondary prevention of the following complications:
  > Thrombotic or cerebrovascular disease (see Chapter 6)
  > Pulmonary hypertension with or without secondary heart failure (see Chapter 7)
  > Extramedullary haematopoietic pseudotumours (see Chapter 11).
  > Leg ulcers (see Chapter 12)

  When transfusion therapy is considered, careful attention to the potential risks of iron overload should be made (see Chapter 5)

- The risk of alloimmunization should be considered in the following subgroups of patients:
  > Pregnant women (see Chapter 10)
  > Splenectomized patients
  > Never or previously minimally transfused patients

- Blood processing and administration characteristics should be similar to those applied in transfusion-dependent β-thalassaemia major, generally:
  > Blood storage for <2 weeks, conditioning to achieve mean 24-hour post-transfusion RBC survival ≥75%
  > Leucoreduced packed red blood cells (≤1 x 10^6 leucocytes/unit) with hemoglobin content ≥40 g (pre-storage filtration preferred)
  > ABO and Rh(D) matched blood
  > Rh (C, c, E, e) and Kell matching highly recommended
  > Appropriate infections and viral vaccinations and screening of donor and recipient

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CHAPTER 03
SPLENECTOMY

ILLUSTRATIVE CASE

A patient was diagnosed with ß-thalassaemia intermedia at the age of four years, after presenting to his paediatrician with mild jaundice and anaemia (haemoglobin level: 8.0 g/dl). On molecular analysis, he was found to be heterozygous for the ß+ mutation IVSI-110 G>A along with a full duplication of the α-globin locus (α-genotype: αα, αα/αα, αα). At the age of ten years, his physician recommended splenectomy in view of a total haemoglobin level drop to 7.0 g/dl and a finding on physical examination of asymptomatic moderate splenomegaly (largest dimension 18 cm) which was confirmed by ultrasonography. The complete blood count at this time revealed a platelet count of 400 x10⁹/l and a nucleated red blood cell count of 150 x106/l. He thus underwent a laparoscopic procedure and his spleen was removed, along with the gall bladder which had evidence of gallstones. His medical course was later uneventful and he maintained a total haemoglobin level between 7.5-8.0 g/dl. At the age of 17 years, he presented again to his physician with pain and swelling of the left arm, and a diagnosis of thrombophlebitis was confirmed with ultrasonography. At this time, his complete blood count revealed a platelet count of 900 x10⁹/l and his nucleated red blood cell count was 400 x106/l. The work up did not reveal any conventional risk factors for venous thrombosis.

CONTEXT AND EVIDENCE

ADVERSE OUTCOMES FOLLOWING SPLENECTOMY IN NTDT

Splenectomy is associated with a variety of adverse outcomes in patients with thalassemia, especially in NTDT patients for whom this intervention is a common practice aiming to increase the total haemoglobin level by 1-2 g/dl and to avoid blood transfusions [1-6].

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 6) [7-9]. These abnormalities become more prominent following splenectomy considering the beneficial role of the spleen in scavenging these pro-coagulant platelets and red blood cells; and thus putting this subgroup of patients at a higher risk of thrombotic and vascular events [10, 11]. For instance, about 80% of damaged red blood cells are removed extravascularly by macrophages present mainly in the spleen [12]. Moreover, it has been proposed that removal of the spleen in ß-thalassemia patients with an absolute excess of α-chains, such as the case described herein, is associated with a more notable persistence of damaged erythroblasts and erythrocytes in the blood stream compared to patients with defective ß-chain production and a relative excess of α-chains [13]. Clinically, observational studies, especially in patients with ß-thalassaemia 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 [2, 10, 11, 14-28]. Splenectomised β-thalassaemia intermedia patients who experience thrombotic events are characterized by high nucleated red blood cell counts (≥300 x106/l) and platelet counts (≥500 x 10^9/l), are more likely to have a history of pulmonary hypertension, and to have never received any transfusions [29]. The median time to thrombosis following splenectomy is around eight years [29]. 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 [30, 31], and the observation that splenectomised patients have a higher rate of iron-related organ morbidity than their non-splenectomised peers [14].

Splenectomy places NTDT patients of all ages at risk of morbidity and mortality due to infection [1]. These infections could have an overwhelming, fatal course such as with meningitis and sepsis [32]. In older studies, the risk of post-splenectomy sepsis in thalassaemia patients was increased more than 30-fold in comparison with the normal population [33]. Modern preventative measures, however, have reduced this risk but the overall impact of these measures is unclear. The pathogens most commonly associated with post-splenectomy sepsis are encapsulated organisms [34], 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 [35]. Characteristics of overwhelming post-splenectomy 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 [32]. Early intervention on the basis of clinical suspicion, even in the absence of many of the above findings, is critical. The risk of overwhelming post- splenectomy 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 [36]. 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 [37]. 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 [38]. Antibiotic prophylaxis with oral penicillin or other antibiotics reduces the risk of post-splenectomy 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 post-splenectomy sepsis. The risk of death from febrile illnesses remains and rapid evaluation of a febrile episode is critical [39].

SPLENECTOMY TECHNIQUE

Laparoscopic splenectomy has become the gold standard for the removal of the spleen in benign haematological conditions including the thalassaemias. 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 [40-43]. 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 [42, 44-46] and even superiority [43, 47-50] of laparoscopic over open splenectomy even in patients with massive or supra-massive 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 [51]. 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 [43, 52-55]. In some centres, partial splenectomy has been used to preserve some immune function while reducing the degree of hyper-splenism [56, 57]. The long-term success of this approach is still under evaluation [58]. 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 [59]. This approach has not gained wide acceptance and may be complicated by fever, pain, and a subsequent need for splenectomy.

GALLSTONES IN NTDT

Gallstones are more common in NTDT than in transfusion-dependent thalassemia patients due to increase haemolysis [60]. 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 [1, 3, 6, 61-67]. 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 [68].

NOVEL APPROACHES TO COMBAT SPLENOMEGALY
JAK2 inhibition, an established therapy in patients with myeloproliferative disorders, was shown to ameliorate ineffective erythropoiesis and decrease spleen size in thalassaemia mice [69, 70]. This has motivated ongoing clinical trials in transfusion-dependent patients, which showed considerable reduction in spleen volume (NCT02049450). Data in NTDT patients are awaited.

PRACTICAL RECOMMENDATIONS

• Splenectomy should generally be avoided in NTDT patients younger than 5 years

• Splenectomy should be reserved for cases of:
  > Worsening anaemia leading to poor growth and development
  - When transfusion therapy is not possible or iron chelation therapy is unavailable
  > Hypersplenism
  - Leading to worsening anaemia, leucopenia, or thrombocytopenia and causing clinical problems such as recurrent bacterial infections or bleeding
  > Splenomegaly
  - Accompanied by symptoms such as left upper quadrant pain or early satiety
  - Massive splenomegaly (largest dimension >20 cm) with concern about possible splenic rupture

• Post-splenectomy sepsis remains a risk in all splenectomized thalassaemia patients. Therefore, febrile splenectomized patients should undergo rapid evaluation and treatment

• Whenever splenectomy is indicated, patients should receive the following vaccines:
  > 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
  > 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
> 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
> Influenza vaccine, annually

- Splenectomized patients should receive prophylactic antibiotic therapy for at least two years following splenectomy

   > 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
   > Oral penicillin, 125 mg twice daily for children under two years, and 250 mg twice daily for children two years and over is recommended
   > Alternative antibiotics for patients unable to take penicillin include amoxicillin, trimethoprim-sulfomethoxazole, and erythromycin
   > 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

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

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

- For recommendations on thromboprophylaxis relevant to splenectomized patients see Chapter 6

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CHAPTER 04
FOETAL HAEMOGLOBIN INDUCTION

ILLUSTRATIVE CASE

A 12-year-old female was diagnosed at the age of two years to have ß-thalassaemia intermedia (homozygous for the δß fusion haemoglobin variant, haemoglobin Lepore). Monthly red cell transfusions were commenced at the age of six years to manage growth and developmental retardation and splenectomy was performed at age eight years to reduce red cell requirements. Transfusions were discontinued after three years because of red cell alloimmunization, after which the total haemoglobin level varied between 5 and 7 g/dl. The anaemia led to poor exercise tolerance, growth failure, delayed sexual maturation and extensive bone marrow hyperplasia, resulting in progressive skeletal deformities. the patient was started on hydroxyurea 10 mg/kg/day, increased over six months to 15 mg/kg/day. The patient showed an increase of total hemoglobin level of 2.5 g/dl at the maximum used dose. Improvement in anaemia was associated with considerable improvement in growth parameters and functional status.

CONTEXT AND EVIDENCE

Increased production of the foetal ß-like globin molecule, γ-globin, can bind excess α-chains to produce foetal haemoglobin, leading thus to improvements in α/ß-globin chain imbalance and more effective erythropoiesis [1]. This partly explains the more favourable phenotype in some patients with ß-thalassaemia intermedia and haemoglobin E/ß-thalassaemia compared with transfusion-dependent ß-thalassaemia major [1]. Data from recent observational studies confirm that increased foetal haemoglobin production improves the clinical course in a number of patients with NTDT [2-8]. Thus, the use of foetal haemoglobin inducers in patients with NTDT has been trialled in several studies throughout the past couple of decades [1].

Hydroxyurea (or hydroxycarbamide) is the foetal haemoglobin inducer for which the most data in NTDT have been generated. Hydroxyurea is a cytotoxic, anti-metabolic, and antineoplastic agent that was identified as a potent foetal haemoglobin inducer [9], 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 [10]. 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 [11-14]. Hydroxyurea therapy exerts a 2 to 9-fold increase in γ-mRNA expression in ß-thalassaemia patients [15-19] leading to improvement in the α/non-α chain imbalance and more effective erythropoiesis [20]. There is good correlation between in vitro γ-mRNA fold

increase and in vivo foetal haemoglobin fold increase [18, 21]; 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-thalassaemia patients receiving hydroxyurea [22, 23]. 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 [22].

In splenectomised patients with NTDT, there is also evidence that hydroxyurea diminishes phosphatidylserine externalization on the red cell [24]. Whether this is attributed to foetal haemoglobin induction and an associated decrease in α-globin aggregates remains to be elucidated [24]. 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 [25]. 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-thalassaemia patients treated with hydroxyurea, several studies evaluated the efficacy and safety of the drug in NTDT patients [1]. However, data mostly comes from single-arm trials or retrospective cohort studies; 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% [1]. An association between the degree of foetal haemoglobin level increase and improved haematological outcomes was noted in some studies[26, 27], 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 [1]. 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 [1]; which is comparable to findings in patients with sickle cell disease [14, 28]. 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% [1]. Such increases may be essential since a difference between a severe and mild haemoglobin E/β-thalassaemia patient, for example, is only 1-2 g/dl [29]. Improvement in anaemia was usually associated with better exercise tolerance, appetite, and sense of well-being [1].

Hydroxyurea therapy was also found to decrease the frequency of certain morbidities in patients with NTDT. A beneficial role in patients with pulmonary hypertension was suggested,

especially upon combination with the antioxidant L-carnitine [30-32]. Hydroxyurea therapy was also associated with improvements in leg ulcers [33] and extramedullary haematopoietic pseudo-tumours [34], in smaller studies. These findings are further confirmed through a recent cross-sectional study of 584 ß-thalassaemia intermedia patients from the Middle East and Italy, where hydroxyurea therapy was associated with reduced adjusted odds of extramedullary haematopoietic pseudo-tumours (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) [35]. 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 [1]. These doses remain lower than those used in patients with sickle cell disease, which are often in excess of 20 mg/kg/day [14, 28]. 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 [15].

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 [1]. 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 [1]. However, some studies noted a decline in haematological response beyond 12 months [21, 36]. 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 hematopoietic stem cells to effectively give rise to erythroid lineage cells [21].

Alongside dose and duration of therapy, several other factors were assessed for their association with haematological response in patients with NTDT [1]. Findings regarding the role of age and foetal haemoglobin level at the start of treatment are conflicting. Moreover, although some studies found certain ß-globin genotypes to be predictors of a favourable response, others failed to establish such an association. Similar discrepancies are noted for ß-globin haplotypes. Patients with Lepore or δß-thalassaemia genotypes usually showed a better response. Co-inheritance of α-thalassaemia 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 Gγ) was a strong predictor of favourable responses, although the case was different in some studies especially those including patients with haemoglobin E/ß-thalassaemia. The rs766432

polymorphism at intron 2 of the BCL11A gene also correlates strongly with response to hydroxyurea therapy [1].

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 [1]. The rate of myelotoxicity ranged between 2% and 30% while some studies did not report any haematological toxicities [1]. Myelotoxicity was usually dose-dependent, especially when doses >20 mg/kg/day were used, and could be reversed upon dose reduction [1]. The bone marrow of NTDT patients may be more sensitive to myelosuppression by hydroxyurea than occurs in other disorders, possibly due to medullary inflammation [1]. There is only one report of leukemic transformation in a ß-thalassaemia intermedia patient following three years of hydroxyurea therapy at 19 mg/kg/day [37]. The rate of gastrointestinal adverse events ranged between 1% and 30% [1]. 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 [1]. 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 [1]. Interestingly, two patients got pregnant while on hydroxyurea and delivered normally without any congenital malformations [1]. Nonetheless, evidence from patients with sickle cell disease points out that hydroxyurea therapy can transiently decrease sperm numbers and viability [38].

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.' [39]

Other foetal haemoglobin inducers have also been evaluated in few small studies in NTDT patients [1]. A pilot study on five patients with ß-thalassaemia 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 [40]. 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 [41-45]. The orally bio available butyrate derivative 2,2-dimethylbutyrate sodium salt (HQK-1001) was recently 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 [46]. 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 [47]. 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 [48, 49].

NOVEL APPROACHES FOR ALTERING FOETAL HAEMOGLOBIN EXPRESSION IN NTDT
Recent approaches have been developed to directly correct genetic mutations in the endogenous DNA of the cell or to disrupt specific DNA sequences in the genome. This approach is known as genome editing and has been facilitated through the identification of a number of enzymes, including CRISPR/Cas9, which can introduce DNA breaks in specific regions of the genome [50, 51]. While it is challenging to envision correcting every one of the hundreds to thousands of mutations causing ß-thalassaemia, disruption of factors that silence the γ-globin genes, such as BCL11A, may allow more immediate treatment through foetal haemoglobin induction with current genome editing approaches [51-53]. Clinical trial data are awaited.

PRACTICAL RECOMMENDATIONS

• There are no randomized clinical trials to recommend an evidence-based use of hydroxyurea in NTDT patients. However, based on data from a large body of observational cohort studies and small clinical trials, the agent may be considered in the following groups of NTDT patients:
  > ß-Thalassaemia intermedia homozygous for the XmnI polymorphism
  > Patients with Lepore or δß-thalassaemia
  > Patients for which a transfusion course is required but are alloimmunized
  > Patients with the following clinical morbidities
  - Pulmonary hypertension (see Chapter 7)
  - Extramedullary haematopoietic pseudotumours (see Chapter 11)
  - Leg ulcers (see Chapter 12)

• Hydroxyurea 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

• Response should be evaluated after 3 and 6 months of therapy and should be defined as a total hemoglobin level increase of >1 g/dl at 6 months. The drug should be discontinued in patients not showing response. Patients showing response should be re-evaluated at 12, 18, and 24 months to ensure maintenance of response

- Other response parameters that could be evaluated as indicated:
  - > Improvement in growth measures
  - > Improvement in functional status and exercise tolerance
  - > Improvement in quality of life
  - > Improvement in clinical morbidities (pulmonary hypertension, extramedullary hematopoietic pseudotumors, leg ulcers)

- The following safety measures should be evaluated and treatment discontinued or tailored accordingly. These include:
  - > Complete blood counts, every two weeks for the first three months then monthly
  - > Hepatic and renal function studies, every two weeks for the first three months then monthly
  - > History and physical examination evaluating for gastrointestinal, neurologic, or dermatologic side-effects, monthly
  - > Gonadal function follow-up
  - > Hydroxyurea should not be used in pregnant women or patients with hepatic or renal failure

- The use of other fetal hemoglobin inducers or erythropoietic stimulants should be restricted to clinical trials and research study settings, until more data become available

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CHAPTER 05
IRON OVERLOAD AND CHELATION THERAPY

ILLUSTRATIVE CASE

A 21-year-old man of Middle Eastern origin was diagnosed with ß-thalassaemia intermedia at 3 years of age, after presenting with pallor, mild anaemia (total hemoglobin level 10.5 g/dl), and splenomegaly. He was transfused once at 7 years of age prior to splenectomy. He had migrated to Italy at the age of 18 to complete his studies. He presented to an outpatient clinic in his neighbourhood for a regular check-up. His total hemoglobin level was 9.8 g/dl. Among the laboratory work-up, his serum ferritin level appeared to be 870 ng/ml. His primary care physician advised him to refer to a nearby thalassaemia centre to do further testing for iron overload. Although initially hesitant in view of his serum ferritin level being <1000 ng/ml and his almost-negative transfusion history, he eventually presented to a specialized thalassemia care centre and had further assessment of iron overload status through T2* magnetic resonance imaging of the liver and heart. His liver iron concentration was 5.8 mg Fe/g dry weight (normal <1.8 mg Fe/g dry weight) and his cardiac T2* was 36.5 ms (normal <20 ms). The patient was started on subcutaneous deferoxamine therapy. After 6 months, the patient was re-evaluated using liver T2* magnetic resonance imaging and his liver iron concentration remained at 6 mg Fe/g dry weight. He admitted he was skipping most of his doses because the idea of carrying a pump around at college created major discomfort. Patient was started on deferasirox 10 mg/kg/day. His liver iron concentration after 12 months from starting deferasirox dropped from 6 to 4.2 mg Fe/g dry weight. His serum ferritin level was 480 ng/ml. Patient did not experience any adverse events.

CONTEXT AND EVIDENCE

MECHANISM AND CONSEQUENCES OF IRON OVERLOAD IN NTDT

In NTDT, ineffective erythropoiesis leads to inappropriately low hepcidin levels and increased intestinal iron absorption [1, 2]. Earlier studies, proposed several regulators for hepcidin production including twisted gastrulation factor-1 [3], hypoxia inducible transcription factors [4], transmembrane protease serine-6 [5, 6], and arguably growth differentiation factor-15 [7-10]. More recently, an erythroid factor, erythroferrone, was established as the key regulator of hepcidin synthesis and iron homeostasis in ß-thalassemia mice [11, 12]. Clinical studies also illustrated correlation between erythron expansion biomarkers (soluble transferrin receptor, growth differentiation factor-15, nucleated red blood cells) and iron overload biomarkers [13]. A correlation between the severity of anaemia and iron overload has also been illustrated in NTDT patients [14]. 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 [15]. This in turn leads to depletion of macrophage iron, relatively lower levels of serum ferritin (than what would be seen in transfusion-dependent ß-thalassemia intermedia patients), and preferential portal and hepatocyte iron loading (increased liver iron concentration) [16], with subsequent release into the circulation of free iron species that can cause target-organ damage [17]. Aside from this primary source, NTDT can eventually accumulate a minor fraction of this iron overload from occasional or more frequent transfusions [13] which may be indicated as illustrated in Chapter 2. The mechanism of iron overload in NTDT patients is illustrated in Figure 5-1 [18].

Figure 5-1: Mechanism of iron overload in NTDT. TWGF-1, twisted gastrulation factor-1; HIFs, hypoxia inducible transcription factors; TMPRSS6, transmembrane protease, serine 6; GDF-15, growth differentiation factor-15. Modified with permission from reference [18].
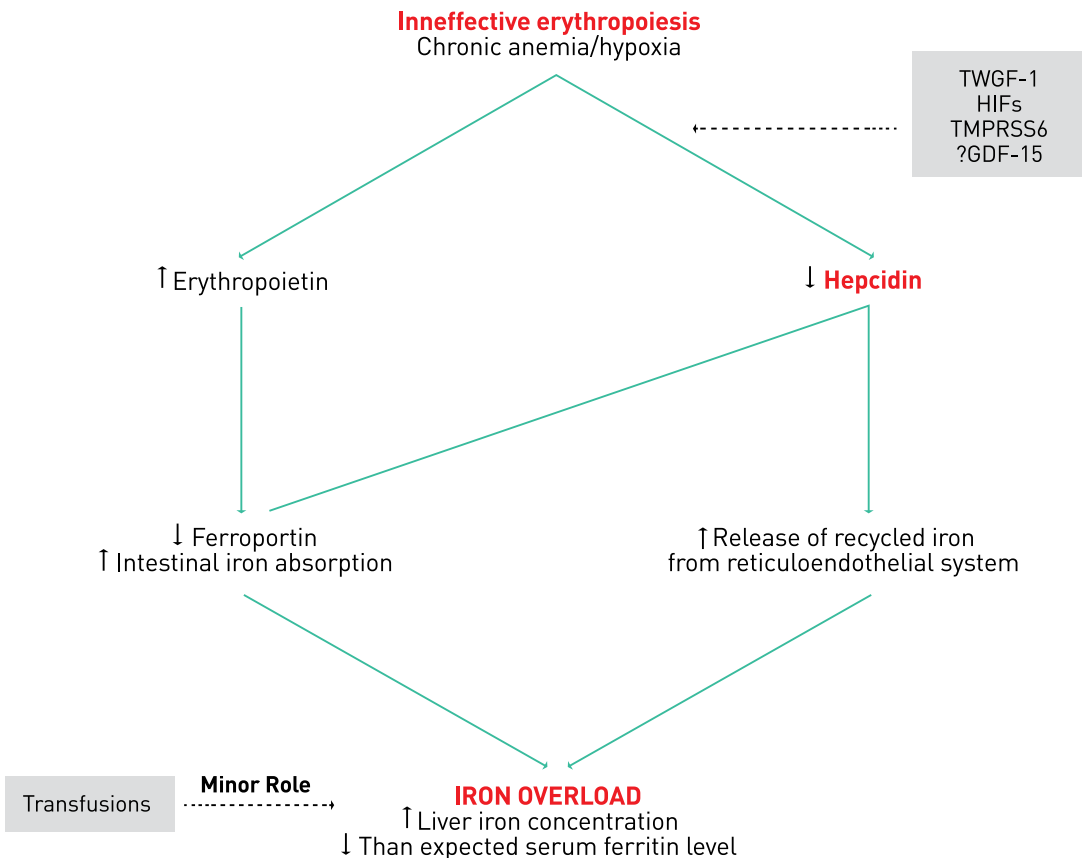
The accumulation of iron from intestinal absorption in NTDT patients is slower than that observed in transfusional siderosis and may reach 3-4 mg/day or as much as 1,000 mg/year [19]. A mean annual increase in liver iron concentration of 0.38 ± 0.49 mg Fe/g dry weight was observed in a recent trial including NTDT patients [20]. Nonetheless, iron overload in NTDT patients is a cumulative process as evident from studies documenting positive correlations between iron overload indices and advancing age [17, 21-24]. Thus, a considerable proportion of NTDT patients eventually accumulate iron to liver iron concentration thresholds of clinical significance [16, 20, 23, 25-27], and can start experiencing iron-related morbidity beyond 10 years of age [19, 21]. Of note, evidence suggests that patients with deletional haemoglobin H disease accumulate iron much more slowly than other NTDT patients and considerable iron overload or related morbidity may only start beyond 15 years of age [23, 28, 29]. Although cardiac siderosis is a major cause of morbidity and mortality and a key factor in management decisions in patients with transfusion-dependent ß-thalassaemia major, 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 fibrosis in non-chelated patients with NTDT has been recently confirmed [34]. Reports documenting the occurrence of hepatocellular carcinoma in hepatitis-negative patients with NTDT and iron overload also continue to emerge [35-40]. Moreover, in a recent 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 [26]. Liver iron concentration levels ≥5 mg Fe/g dry weight were associated with a considerable morbidity risk increase [41]. 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 [42]. An association between iron overload and renal tubular dysfunction as evident from proteinuria has also been recently reported in NTDT patients [43], with some patients observed to progress to end-stage renal disease [44]. Recent 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 [45-47]. 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 ß-thalassaemia major patients have been used in NTDT studies. Assessment of liver iron concentration remains the gold standard for quantification of total body iron [48]. Liver iron concentration in NTDT patients has been measured directly by needle biopsy [16]; 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 [49]. Moreover, liver iron accumulation has been shown to be uneven in cirrhotic patients, resulting in a risk of sampling error [50-52]. Furthermore, different tissue processing methods can produce variable liver iron concentration measurements [53]. Studies evaluating liver iron concentration in NTDT patients using magnetic resonance imaging are numerous [19]. Magnetic resonance imaging using either R2 (1/T2) or R2* (1/T2*) pulse sequences are reliable, internationally reproducible, and non-invasive methods for assessing liver iron concentration, and have been validated against liver biopsy [54-61]. 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 [55]. 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 [62]. 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 [63]. 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 [27]. 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 [64]. However, as previously mentioned current evidence suggests that patients with NTDT are less likely to show iron deposits in the heart along 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 [20, 22, 23, 27]; however, the ratio of serum ferritin to liver iron concentration is lower relative to patients with transfusion-dependent β-thalassaemia major [16, 22, 27, 31]. Thus, spot measurements of serum ferritin level may underestimate iron overload, and delay therapy in patients with NTDT, if they are to be interpreted as in the case of transfusion-dependent β-thalassaemia major patients [19, 65] (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 transfusion-dependent β-thalassemia major vs. a transfusion-independent β-thalassaemia intermedia patient, respectively [22]). Data on the use of other iron overload indices, such as transferrin saturation or non-transferrin-bound iron in NTDT patients are still limited [19].

TREATMENT 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 [66, 67]. 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 [68]. An overview of previous studies investigating iron chelation therapy in patients with NTDT is shown in Table 5-1 [69].

Table 5-1: Studies evaluating iron chelation therapy in NTDT patients. Updated with permission from reference [69].

Study	Disease type	Description				Results
		Drug investigated	N (patient ages, years)	Type of study	Study objectives	
Deferasirox						
Taher 2016 [70] (THETIS)	NTDT	Deferasirox (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,
Taher 2012, 2013 [20, 71] (THALASSA)	NTDT	Deferasirox (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 [72]	β-TI	Deferasirox (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 [73]	β-TI	Deferasirox (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 [74]	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 (patient ages, years)	Type of study	Study objectives	
Akrawinthawong 2011 [75]	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
Chan 2006 [76]	Hb H disease	Deferiprone (starting dose 50 mg/kg/day)	17 (29–76)	Prospective, control-matched, open-label trial	Efficacy and toxicity of deferiprone in Hb H patients with gross iron overload over 18 months, compared with age- and Hb H genotype-matched controls without iron overload	Significant[†] reduction in SF after 6 and 18 months
Pootrakul 2003 [77]	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 [78]	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 [79]	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 [80]	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 a 6 days of deferoxamine treatment
Cossu P 1981 [81]	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

†$p \leq 0.05$; Hb, haemoglobin; SF, serum ferritin; LIC, liver iron concentration; dw, dry weight; β-thal, β-thalassaemia; β-TI, β-thalassaemia intermedia.

Subcutaneous deferoxamine therapy was the first iron chelator to be studied in NTDT patients since 1980s [80, 81]. 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 [72, 73], 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. 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 transfusion-independent haemoglobin E/ß-thalassaemia patients using the oral iron chelator deferiprone (total 39 patients) [75, 77]. However, only nine patients were demonstrated to reduce liver iron concentration by direct liver biopsy [77]. Interestingly, removal of iron hemichrome 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 transfusion requirement in some patients during the course of the study [77]. One splenectomised Thai haemoglobin E/ß-thalassaemia patient died during the study period from inter-current infection [77]. Another four patients with ß-thalassaemia intermedia have been previously treated with deferiprone and showed reduction in iron overload indices [78, 79]. There has been only one study of iron chelation using deferiprone in 17 patients with haemoglobin H disease [76]. A significant reduction of serum ferritin was observed from 6 months of deferiprone therapy (starting with 50 and increased up to 75 mg/kg/day in the majority of patients) and throughout the study period (up to 24 months). However, interpretation of the efficacy of deferiprone on liver iron removal from this study remains difficult considering the nature of the assessment technique (magnetic resonance T1 and T2-signal intensity ratio) and the control group (age and genotype-matched patients). Most 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 transfusion-dependent patients. The major adverse events observed included gastrointestinal symptoms and joint pain or arthralgia. Neutropenia and agranulocytosis were also detected [74].

Promising results with the oral iron chelator deferasirox in small studies recruiting NTDT patients (ß-thalassaemia intermedia) were observed [72, 73]. Subsequently, data from the largest and first randomized clinical trial of iron chelation therapy in 166 patients with NTDT became available (THALASSA) [20]. The trial showed that deferasirox 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 was similar to placebo. The most common drug-related adverse events were nausea (6.6%), rash (4.8%), and diarrhoea (3.6%) [20]. The analyses also showed that greater reductions in liver iron concentration were achieved in patients dose-escalated at 6 months from deferasirox 10 mg/kg/day starting dose to 20 mg/kg/day. The deferasirox safety profile remains consistent as patients approach the chelation interruption target of liver iron concentration 3 mg Fe/g dry weight [82]. Sub-analyses from the study showed that reduction in liver iron concentration with deferasirox 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 NTDT form [83]. An extension of the core study showed that deferasirox progressively decreases iron overload over 2 years in NTDT patients with a safety profile consistent with that in the core study. Of 166 patients enrolled, 64 (38.6 %) and 24 (14.5 %) patients achieved LIC <5 and <3 mg Fe/g dry weight by the end of the study, respectively [71]. 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) [84]. More recently, THETIS, an open-label, single-arm, multicentre, phase IV study, added to the evidence from THALASSA by investigating earlier deferasirox 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, diarrhea and nausea (n=6 each). These data support earlier escalation with higher deferasirox doses in iron-overloaded NTDT patients [70].

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 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 parallel 10, 20, 30 and 40 mg/kg/day for dispersible tablets). 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 transfusion-dependent thalassaemia patients [85].

Thus, safe and effective chelation therapy is now available for NTDT patients who (a) are ≥10 years, which is the age at which iron-related morbidity starts to be of concern [21]; (b) have a liver iron concentration ≥5 mg Fe/g dry weight or (c) have serum ferritin ≥800 ng/ml, which represent thresholds after which, the risk of serious iron-related morbidity is increased [26, 42].

The latter threshold for serum ferritin remains essential, since data on the ideal serum ferritin in relation to liver iron concentration as guides for the initiation and tailoring of chelation therapy in NTDT, cannot be extrapolated from transfusion-dependent ß-thalassaemia major patients (such as 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 [65].

The target of chelation therapy 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 [84]. 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 [69, 86-88].

A beneficial effect of iron chelation therapy in reducing clinical morbidity risk in patients with NTDT is also suggested by observational studies and further long-term studies in this direction are awaited [34, 89].

Novel therapies targeting iron dysregulation in NTDT

Part of the challenge with NTDT, is that none of the iron chelators targets the naturally existing mechanisms for iron handling in the body. Recent developments suggest alternative avenues by which, iron overload can be more effectively dealt with, especially in NTDT patients with primary iron overload. As noted earlier, hepcidin plays a key role in limiting iron absorption and utilization [90]. In β-thalassaemia intermedia mouse models, moderate transgenic hepcidin expression decreased iron loading in the liver, and resulted in prolonged red cell life span, increased haemoglobin levels and amelioration of splenomegaly suggesting a bidirectional relationship between ineffective erythropoiesis and iron overload [91]. Recent pre-clinical studies have suggested that long-acting hepcidin analogues (minihepcidins) may be beneficial to restrict iron absorption and utilization in the setting of iron overload, with beneficial effects on ineffective erythropoiesis [92-94]. An alternative approach is to stimulate endogenous hepcidin production. One effective way to accomplish this is through the downregulation of a metalloprotease, TMPRSS6, which plays a key role in hepcidin expression from the liver, and whose inactivation leads to increased hepcidin levels, ameliorated iron overload, and improved ineffective erythropoiesis [5, 93, 95]. Anti-sense oligonucleotides and siRNAs targeting TMPRSS6 have been effectively used to stimulate hepcidin, reduce iron burden, and improve ineffective erythropoiesis and red cell survival using this approach in pre-clinical iron overload models [95-97]. This may be an alternative approach for further restricting iron burden. Clinical trial data from all such novel therapies are awaited.

PRACTICAL RECOMMENDATIONS

- All patients with NTDT ≥10 years of age should be frequently assessed for iron overload status (an age threshold of ≥15 years may be used in patients with deletional haemoglobin H disease)

- Assessment of iron overload status in NTDT patients should be done through liver iron concentration measurement:
  > Magnetic resonance evaluation is recommended. Other liver iron concentration measurement techniques may be used when magnetic resonance imaging is unavailable
  > Serum ferritin levels should be considered the primary index of iron overload status when liver iron concentration measurement is unavailable
  > Assessment of serum ferritin level should be done at baseline and every 3 months
  > 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
  > The use of other conventional or experimental iron studies as well as clinical indicators of the
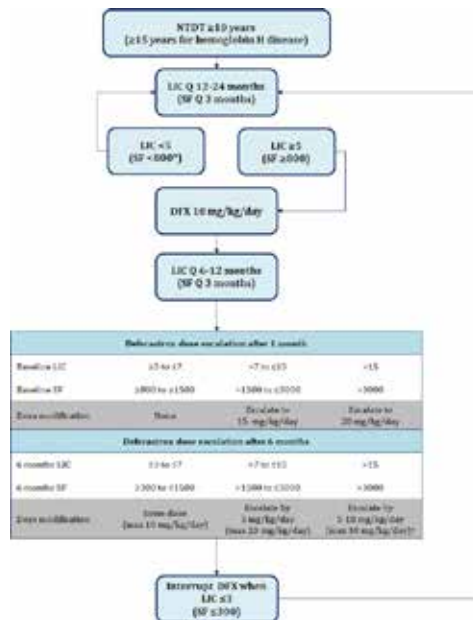
severity of ineffective erythropoiesis may be used to support interpretation of serum ferritin level when it is the only index of iron overload status
> The use of cardiac T2* magnetic resonance imaging in NTDT patients cannot be widely recommended. It may be considered in elderly patients with severe iron overload or as clinically indicated

- Iron chelation therapy with deferasirox should be initiated in NTDT patients ≥10 years of age if:
  > Liver iron concentration ≥5 mg Fe/g dry weight
  > Serum ferritin level ≥800 ng/ml
  > Serum ferritin level >300 to <800 ng/ml (if liver iron concentration measurement is not possible) and other clinical or laboratory measures indicative of iron overload

- Deferasirox therapy should be administered as follows:
  > Starting dose: 10 mg/kg/day
  > Dose escalation after 1 month as follows:
      - Baseline liver iron concentration ≥5 to ≤7 mg Fe/g dry weight or serum ferritin ≥800 to ≤1500 ng/ml: no escalation
      - Baseline liver iron concentration >7 to ≤15 mg Fe/g dry weight or serum ferritin >1500 to ≤3000 ng/ml: escalate to 15 mg/kg/day
      - Baseline liver iron concentration >15 mg Fe/g dry weight or serum ferritin >300 ng/ml: escalate to 20 mg/kg/day
  > Further dose escalation after 6 months as follows:
      - 6 months liver iron concentration ≥3 to ≤7 mg Fe/g dry weight or serum ferritin ≥300 to ≤1500 ng/ml: same dose (maximum 10 mg/kg/day)
      - 6 months liver iron concentration >7 to ≤15 mg Fe/g dry weight or serum ferritin >1500 to ≤3000 ng/ml: increase by 5 mg/kg/day (maximum 20 mg/kg/day)
      - 6 months liver iron concentration >15 mg Fe/g dry weight or serum ferritin >300 ng/ml: increase dose by 5-10 mg/kg/day (maximum 30 mg/kg/day). Deferasirox is not currently approved at doses higher than 20 mg/kg/day in patients with NTDT; the recommendation is based on clinical expert opinion guided by data from the THETIS trial [70]
  > 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
  > For deferasirox film-coated tablets, appropriate doses should be used: 3.5, 7, 11.5, 14, 21 mg/kg/day for film-coated tablets correspond to 5, 10, 15, 20, 30 mg/kg/day for dispersible tablet
  > Safety monitoring should follow standard guidelines used in patients with transfusion-dependent ß-thalassaemia major
  > Compliance should be closely monitored

- Figure 5-2 illustrates an iron overload assessment and management algorithm for NTDT patients in accordance with the above recommendations

- The use of other iron chelators cannot be recommended until larger, randomized studies are available

- Tea consumption should be encouraged in NTDT patients, as it may have some benefit in decreasing iron absorption from the gut

- Patients with NTDT who require frequent blood transfusions for sustained durations of time should be managed for iron overload similar to patients with transfusion-dependent ß-thalassaemia major

Figure 5-2: Iron overload assessment and management algorithm for patients with NTDT. SF, serum ferritin level in ng/ml; LIC, liver iron concentration in mg Fe/g dry weight; DFX, deferasirox. For Deferasirox film-coated tablets doses of 3.5, 7, 11.5, 14, 21 mg/kg/day replace 5, 10, 15, 20, 30 mg/kg/day of dispersible tablet. *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 20 mg/kg/day in patients with NTDT; the recommendation is based on clinical expert opinion guided by data from the THETIS trial [70].

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CHAPTER 06
THROMBOTIC DISEASE

ILLUSTRATIVE CASE

A 30-year-old never transfused woman known to have ß-thalassaemia intermedia (diagnosed at 5 years, IVSI-6/CD39) presented to the thalassaemia clinic with severe thigh pain of a few days duration. The pain was located in her mid-right thigh and radiated to her knee. The pain started two days prior to presentation and intensified gradually. It increased with walking and was relieved by lying down. The pain was unresponsive to paracetamol. She denied any trauma to the area and any fever or chills. She also reported a hot sensation in her right lower limb. She denies any dyspnoea, cough, or chest pain. Her past medical and surgical history were positive for splenectomy at 8 years of age and a postpartum left ileo-femoral deep vein thrombosis 5 years prior to current presentation which was treated by low-molecular weight heparin. She had a negative genetic thrombophilia profile and a negative family history of thrombosis. She also reported having two previous spontaneous early second trimester abortions. She was only receiving folic acid supplementation at the time of presentation. On physical examination, she had diffuse erythema in her right thigh, which was hot and tender to palpation. She had a negative Homan's sign, normal capillary refill time, and no oedema was noted. Her laboratory work-up revealed a total haemoglobin level of 7.6 g/dl and a platelet count of 1,071 x 109/l. Duplex ultrasonography revealed partial recanalization of the right common and superficial femoral veins with thrombosis of branches of long saphenous veins bilaterally. The patient was started on a treatment dose of low-molecular weight heparin, and was advised to start a blood transfusion course every 3 months as well as baby aspirin.

CONTEXT AND EVIDENCE

HYPERCOAGULABILITY IN NTDT
A hypercoagulable state has been identified in NTDT patients, especially those with ß-thalassaemia syndromes, which can be present since childhood [1-3]. The hypercoagulable state in patients with NTDT has been primarily attributed to abnormalities in platelets and pathological red blood cells, although several factors are believed to be involved, ultimately leading to clinical thrombosis (Figure 6-1) [4-9].

Figure 6-1: Factors contributing to a hypercoagulable state and subsequent thrombotic events in NTDT. RBC, red blood cells. Reproduced with permission from reference [8].
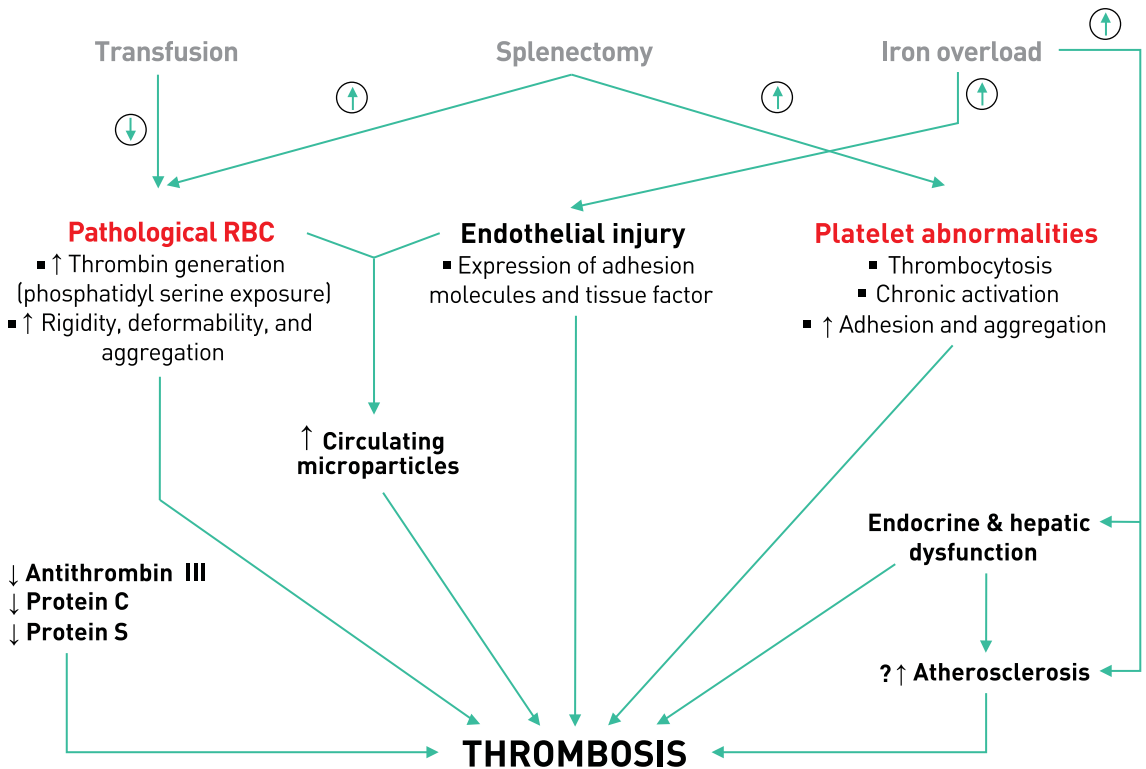
Patients with NTDT have chronically activated platelets, and enhanced platelet aggregation [10], as confirmed by the increased expression of CD62P (P-selectin) and CD63, markers of in vivo platelet activation [11, 12]. It has been demonstrated that NTDT patients have 4 to 10 times higher metabolites of prostacyclin (PG I2) and thromboxane A2, both markers of haemostatic activity, than healthy individuals [13]. Splenectomised NTDT patients also have high platelet counts [14, 15], but with a shorter life-span due to enhanced consumption [16]. A recent study demonstrated that increased platelet adhesion is a common finding in splenectomised ß-thalassaemia patients, which is induced by mechanisms involving both platelets and red blood cells, and is a strong contributor to occlusive thrombus formation in the carotid arteries of thalassaemic mice [17, 18].

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 hemichromes [19], which precipitate, instigating haem disintegration and the eventual release of toxic iron species [20]. 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 [21], which cause the thalassaemic red blood cell to become rigid, deformed, and to aggregate, resulting in premature cell removal [22]. Thalassaemic red blood cells with such negatively charged phospholipids increase thrombin generation [23, 24], as evidenced by studies using annexin V, a protein with high affinity and specificity for anionic phospholipids [24]. Splenectomised patients have a substantially higher number of these negatively charged pathological red blood cells and in turn show higher thrombin generation [25, 26]. NTDT patients were also found to have higher levels of procoagulant microparticles of red blood cell, leukocytic, and endothelial origins compared to controls [27]; the contribution of these fragments to thrombotic events in NTDT is under investigation.

The presence of other peripheral blood elements in thalassaemics 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 [28, 29]. In fact, studies have demonstrated that red blood cells from NTDT patients show increased adhesion to cultured endothelial cells [30]. Inherited thrombophilia does not have a role in the hypercoagulable state of NTDT [31, 32], but low levels of antithrombin III, proteins C, and protein S have been documented [33]. The presence of hepatic or endocrine dysfunction in older patients with severe iron overload may also contribute to hypercoagulability [33].

It has been demonstrated that the presence of non-transferrin-bound iron in iron overload states can cause oxidative vessel injury [34]. 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 [35]. In fact, recent studies support the idea that NTDT patients do exhibit a proatherogenic biochemical phenotype [36, 37].

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 ß-thalassaemia major and 9.6% of 52 patients with ß-thalassaemia intermedia had experienced a thrombotic event [38]. In a cohort study including 83 splenectomised patients with ß-thalassaemia intermedia followed for over 10 years, 29% of patients experienced a venous thrombotic event [26]. Con-

ventional risk factors (described in the non-thalassaemic population) for venous thrombosis were usually absent in such patients [39], further highlighting the unique pathophysiology of hypercoagulability in NTDT. Thrombotic events were also documented in case series of pregnant women with β-thalassaemia intermedia (see Chapter 10) [40]. 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 regularly-transfused β-thalassaemia major [41]. It was 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 β-thalassaemia intermedia patients [41].

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 β-thalassaemia intermedia at six comprehensive care centres (Lebanon, Italy, Iran, Egypt, United Arab Emirates, and Oman) 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 year, iron overload (serum ferritin level ≥1000 ng/ml), and a haemoglobin level <9 g/dl [42]. A sub-study of the OPTIMAL CARE determined that splenectomised β-thalassaemia intermedia patients who experience thrombosis are characterized by high nucleated red blood cell (≥300 x 106/l) and platelet counts (≥500 x 109/l) [43], 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 [43]. 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 severe ineffective erythropoiesis were also observed in separate studies evaluating β-thalassemia intermedia patients [44-48].

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% [41, 43, 49]. Few case reports also describe a frequent occurrence of overt strokes in β-thalassaemia intermedia patients with moyamoya syndrome [50-53]. However, a higher prevalence of silent strokes has been consistently documented in this group of patients [54]. 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-thalassemia intermedia (mean age 29 years) who were neurologically intact and had no conventional stroke-related risk factors (e.g., diabetes, smoking, hypertension, cardiac thrombi) [55]. 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) [56]. It was noted that increasing age and having never received any transfusions were both independently associated with a higher occurrence and multiplicity of lesions [56]. Around the same time, another cross-sectional study was conducted in Iran on 30 randomly selected ß-thalassaemia intermedia adults (mean age 24 years) who were splenectomised, had a haemoglobin level >7 g/dl, and a platelet count ≥500 x 109/l. The authors noted eight patients (26.7%) with silent ischemic lesions [57]. The harmful roles of splenectomy and thrombocytosis in this setting were confirmed in a more recent study [58]. 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 [56]. Of note, similar observations were not noted in children with ß-thalassaemia intermedia [59]. Only one study evaluated the prevalence of silent stroke in patients with haemoglobin E/ß-thalassemia (mean age 31 years), and the rate was also high (24%) [60].

Three independent studies evaluated intracranial blood flow velocity in neurologically asymptomatic patients (evaluated by a neurologist) with ß-thalassaemia intermedia using transcranial Doppler Ultrasonography. Both studies revealed that mean flow velocities in the intracranial circulation of patients with ß-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) [59, 61, 62]. Brain magnetic resonance angiography (MRA) and positron emission tomography-computed tomography (PET-CT) studies have also been recently conducted in ß-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) [63]. The risk of abnormality on MRA increased with declining haemoglobin level and increased non-transferrin-bound iron [63]. 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 [64]. The risk of abnormality on PET-CT increased with higher

liver iron concentration values [64].

There are currently no data to determine whether the observed silent brain abnormalities in ß-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 [54].

THE ROLE OF INTERVENTION
This 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 [43].

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 [65]. Transfusion therapy may in fact explain the lower rate of thrombotic events in regularly-transfused ß-thalassaemia major patients than NTDT [8, 41, 66]. In observational studies from patients with ß-thalassaemia intermedia, transfusion therapy has been associated with lower rates of thromboembolic events and silent strokes [42, 56]. Successful use of transfusion therapy for the prevention of silent strokes in subgroups of patients with sickle cell disease has been established [67].

Although an independent association between iron overload and thrombotic disease in patients with NTDT is suggested by observational studies [42, 44, 45, 68], 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 this setting has not been evaluated.

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 [69]. Hydroxyurea may also decrease haemostatic activation by its effect in decreasing the white blood cell count and particularly monocytes that express tissue factor [6]. 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 ß-thalassaemia intermedia patients [58]. Similar evaluations in patients with sickle cell disease were not encouraging [70].

There are no available results from clinical trials on anticoagulant or antiplatelet therapy for the prevention of thrombotic or cerebrovascular disease in thalassaemia patients. However, an association between high platelet counts and thrombosis as well as a lower recurrence rate of thrombotic events in splenectomised ß-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 [41, 43]. Moreover, the high prevalence of silent strokes in splenectomised patients with elevated platelet counts also suggests a potential role for aspirin therapy [57, 58]. Even in patients with normal platelet counts, observations have suggested that aspirin therapy could delay occlusive thrombus formation in carotid arteries of thalassaemic mice [17, 18].
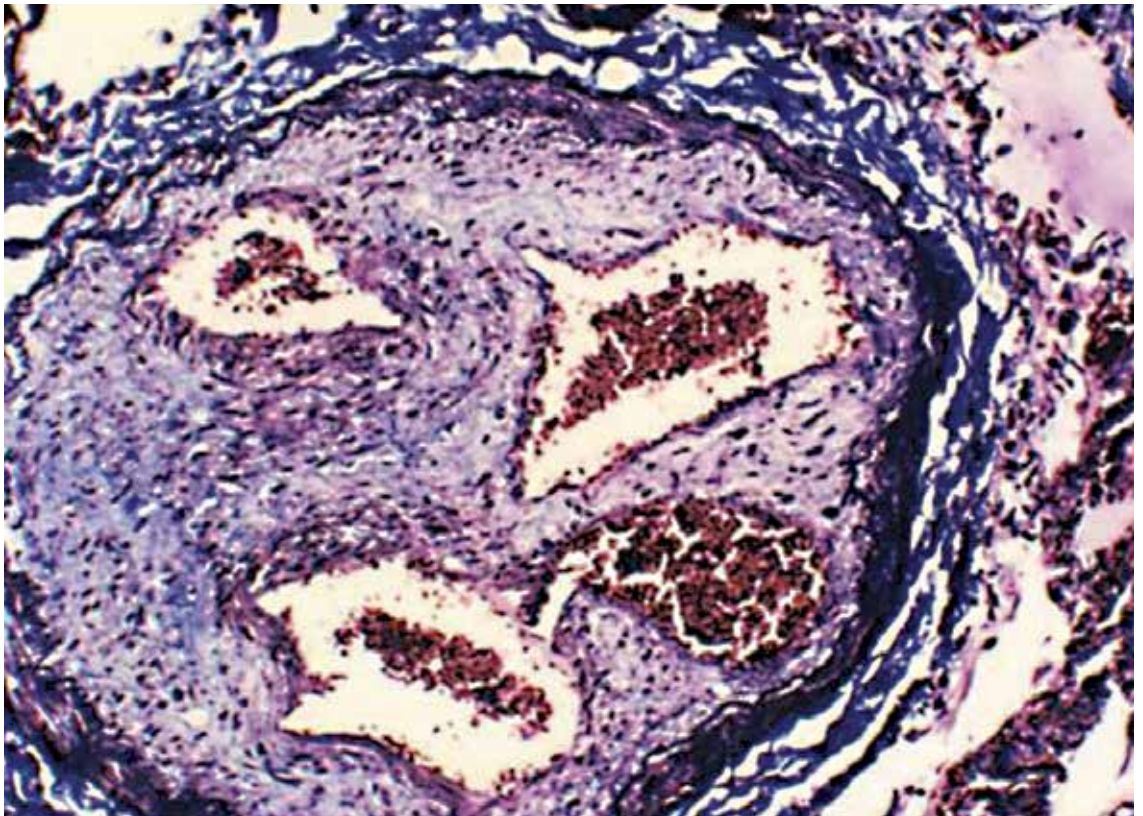
PRACTICAL RECOMMENDATIONS

- 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:
  > Patients with a diagnosis of ß-thalassaemia intermedia
  > Adult patients
  > Splenectomized patients
  > Never or previously minimally transfused patients
  > Patients with elevated platelet counts ($\geq$500 x 10^9/l)
  > Patients with elevated nucleated red blood cell counts ($\geq$300 x 10^6/l)
  > Patients with a haemoglobin level <9 g/dl
  > Patients with a history of pulmonary hypertension (see Chapter 7)
  > Patients with iron overload (liver iron concentration $\geq$5 mg Fe/g dry weight or serum ferritin level $\geq$800 ng/ml)
  > Pregnant patients (see Chapter 10)
  > Patients with a personal or family history of thrombosis
  > Patients with other conventional risk factors for thrombosis or cerebrovascular disease

- Assessment with brain or cerebrovascular imaging for high-risk patients may be considered, although routine assessment or management of positive findings in asymptomatic patients cannot yet be recommended unless deemed necessary by the treating physician

- Patients who develop thrombotic or cerebrovascular disease should be treated as per standard local or international guidelines

- Prophylactic intervention with anticoagulants or antiaggregants in high-risk patients should follow standard local or international guidelines

- Aspirin therapy should be considered in splenectomized NTDT patients with elevated platelet counts ($\geq$500 x 10^9/l)

- The use of transfusion therapy for the primary or secondary prevention of thrombotic or cerebrovascular disease in high-risk patients should be considered

- There is no sufficient evidence to recommend iron chelation or hydroxyurea therapy for the primary or secondary prevention of thrombotic or cerebrovascular disease, although when used for different indications a beneficial effect may be observed

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CHAPTER 07
PULMONARY HYPERTENSION

ILLUSTRATIVE CASE

A 36-year old Thai woman known to have haemoglobin E/ß-thalassaemia (moderate severity) presented with progressive dyspnoea of a 3-week duration. She was diagnosed with haemoglobin E/ß-thalassaemia (IVSI-5 ß-mutation and homozygous for the XmnI polymorphism) at 6 years of age after presenting with pallor and a total haemoglobin level of 5.9 g/dl. She tolerated her anaemia in childhood and never required transfusions for growth or development. She had marked symptomatic splenomegaly (>10 cm) and underwent splenectomy at 9 years of age. She had no prior history of cardiac disease. Her laboratory studies revealed a total haemoglobin level of 6.8 g/dl, a platelet count of 918 x 10^9/l, and a serum ferritin level of 1453 ng/ml. On continuous-wave Doppler transthoracic echocardiography, she had a peak tricuspid-valve regurgitant jet velocity of 3.3 m/s. She was referred to an interventional cardiologist and underwent a right heart cardiac catheterization, which revealed a mean pulmonary arterial pressure of 46 mm Hg. She also underwent a ventilation/perfusion lung scan, which showed irregular distribution of radioactivity and a triangular defect in the posterior segment of the left lower lobe. She was started on anticoagulant therapy and referred back to her primary physician for consideration of blood transfusion, iron chelation, and antiplatelet therapy.

CONTEXT AND EVIDENCE

PREVALENCE AND DIAGNOSTIC CHALLENGES

Among the sequelae associated with a diagnosis of NTDT, pulmonary hypertension has received great attention in recent years, especially in patients with ß-thalassaemia syndromes [1]. 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 (ß-thalassaemia intermedia and haemoglobin E/ ß-thalassaemia) than ß-thalassaemia major patients [2-19]. 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 [2, 4, 6, 8, 10, 12-15, 17-19]. 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 [20-22]. More importantly, recent 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 [23]. 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 more recent large study on 1309 ß-thalassaemia patients from Italy, (which took into consideration the chronic anaemic state of patients), the prevalence of pulmonary hypertension was considerably lower when more strict echocardiographic criteria 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). Patients with ß-thalassaemia intermedia had a 5-fold increased prevalence of pulmonary hypertension on right heart catheterization than patients with ß-thalassaemia major (4.8% vs. 1.1%). When a threshold TRV of 3.2 m/s is used to define pulmonary hypertension, the positive predictive value of echocardiography was as high as 93.9% [24].

AETIOLOGY 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, 25]. Similar to patients with sickle cell disease and other chronic haemolytic anaemias, pulmonary hypertension in NTDT patients is commonly classified as pulmonary arterial hypertension (Class 1) in international guidelines; which is characterized by the presence of pre-capillary pulmonary hypertension in the absence of left-sided heart disease, lung disease, or chronic thromboembolism [20, 21, 26]. However, the possibility of pulmonary hypertension occurring secondary to chronic thromboembolic disease cannot be fully excluded in the NTDT patient with a hyper-coagulable state (see Chapter 6); and this has been previously reported [27, 28]. Moreover, hypercoagulability can still play a major role in the aetiology of Class I pulmonary arterial hypertension; where thrombi may be present in both the small distal pulmonary arteries and the proximal elastic pulmonary arteries [20]. The association of advancing age, splenectomy, history of thrombosis, thrombocytosis (platelet count $\geqslant$500 x 106/l), increased platelet activation, high nucleated red blood cell counts ($\geqslant$300 x 106/l), and other markers of hypercoagulability with increased pulmonary hypertension risk further supports the role of hyper-coagulable 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 ß-thalassaemia major patients [4, 5, 8, 10, 11, 18, 19, 24, 28-34].

Other factors are also implicated in the aetiology of pulmonary hypertension in NTDT. Associations between lower haemoglobin levels and increased markers of haemolysis with this morbidity have been reported [8, 11, 31, 32]. 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 [25, 35]. PH is also associated with increased expression of endothelin receptors in pulmonary micro-

vascular endothelial cells and monocytes, due to intrinsic high levels of placenta growth factor in patients with SCD and ß-thalassaemia [36]. Decreased arginine bioavailability and nitric oxide depletion secondary to haemolysis have been recently directly associated with pulmonary hypertension in patients with ß-thalassaemia including NTDT [35, 37, 38]. Similar to thrombotic disease (see Chapter 6), 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 ß-thalassaemia intermedia [17, 34, 39, 40]. 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 [25, 41].

THE ROLE OF INTERVENTION

NTDT patients with pulmonary hypertension show functional limitation and considerable decrease in the 6-minute walk distance [24]. Studies confirm that pulmonary hypertension in ß-thalassaemia patients including NTDT is a serious morbidity associated with subsequent right ventricular dysfunction [14, 16, 27, 42-45], warranting prompt intervention.

Despite the absence of dedicated clinical trials, the aforementioned higher prevalence of pulmonary hypertension in NTDT compared with regularly-transfused ß-thalassaemia major patients, suggests a role for transfusion therapy in this setting. Even among patients with NTDT, the administration of transfusion therapy is associated with lower pulmonary hypertension rates [18, 28, 46]. Transfusions not only improve anaemia and haemolysis, but may also have a role in ameliorating the hypercoagulable state in NTDT (see Chapter 6). Similar protective effects were noted with hydroxyurea [9, 18, 28, 47-49] and iron chelation therapy in observational studies [18, 28]. 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 ß-thalassaemia patients [50-52]. More recently, a multicentre trial including patients with ß-thalassaemia major and intermedia showed that sildenafil therapy may improve cardiopulmonary haemodynamics in patients with a TRV >2.5 m/s. Bosentan (endothelin receptor antagonist) and epoprostenol (prostacyclin) were also reported to be effective in some patients [53-55].

PRACTICAL RECOMMENDATIONS

- Patients with NTDT should undergo routine echocardiographic assessment (annually) for the assessment of TRV, especially the following patient subgroups:
  > Patients with ß-thalassemia intermedia and hemoglobin E/ß-thalassemia
  > Adult patients
  > Splenectomized patients
  > Never or previously minimally transfused patients
  > Patients with elevated platelet counts (≥500 x 10^9/l)
  > Patients with elevated nucleated red blood cell counts (≥300 x 10^6/l)
  > Patients with a hemoglobin level <9 g/dl or elevated markers of haemolysis
  > Patients with iron overload (liver iron concentration ≥5 mg Fe/g dry weight or serum ferritin level ≥800 ng/ml)
  > Patients with a history of thrombosis
  > Patients with other conventional risk factors for pulmonary hypertension

- Echocardiographic TRV values should be interpreted as follows:
  > TRV >2.5 m/s, asymptomatic: 'possible' to have pulmonary hypertension
  > TRV >2.5 m/s, symptomatic or with other echocardiographic criteria suggestive of pulmonary hypertension: 'likely' to have pulmonary hypertension
  > TRV >3.2 m/s: 'likely' to have pulmonary hypertension

- 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

- Patients with confirmed pulmonary hypertension should be referred to a cardiologist and managed as per standard local or international guidelines for the treatment of pulmonary hypertension

- Patients with 'possible', 'likely', or confirmed pulmonary hypertension may benefit from the following interventions
  > Blood transfusion
  > hydroxyurea
  > Sildeanfil citrate
  > Adequate control of iron overload status
  > Anticoagulant therapy

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CHAPTER 08
LIVER DISEASE

ILLUSTRATIVE CASE

A 54-year-old man was diagnosed with ß-thalassaemia intermedia at the age of 5 years. He had been transfusion-independent for most of his life, and only received sporadic transfusions during a surgery and an episode of infection. He underwent splenectomy for splenomegaly in childhood and had been maintained on hydroxyurea therapy with a stable haemoglobin level between 7 and 8 g/dl. The patient presented to his physician with right upper quadrant pain that has been persistent for two months. Initial imaging with ultrasonography and computed tomography showed foci in his liver, which were initially interpreted by the radiologist as extramedullary hematopoietic pseudotumours. Liver function tests were within normal range. His ferritin level was 1100 ng/ml and his liver iron concentration was also measured by R2 magnetic resonance imaging and was 14.3 mg Fe/g dry weight. A computed tomography-guided biopsy was recommended and showed the lesions to be compatible with multifocal hepatocellular carcinoma. Hepatitis B and C testing by polymerase chain reaction were negative. The patient received palliative treatment. He developed hepatorenal syndrome and rapidly succumbed to his illness.

CONTEXT AND EVIDENCE

Although viral hepatitis is mainly a concern in the subgroup of NTDT patients who eventually require blood transfusion therapy (see Chapter 2), primary iron overload in patients with NTDT leads to preferential portal and hepatocyte iron loading and considerably elevated liver iron concentration (see Chapter 5). Data from patients with hereditary hemochromatosis and other acquired liver disease states continue to confirm the role of chronic hepatocellular iron deposition in promoting liver fibrogenesis and cirrhosis [1]. A longer duration of hepatic iron exposure is associated with a higher risk of significant fibrosis [2], while liver cirrhosis can develop within a decade in severely iron overloaded patients [3]. 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 [4, 5]. A recent longitudinal study of a cohort of 42 never-transfused ß-thalassemia intermedia adults followed for four years (median age 38 years) evaluated the association between longitudinal changes in serum ferritin levels and transient elastography values [6]. 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

ß-thalassemia, and has a good inter-observer and intra-observer reproducibility [7]. 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 [6]. Another recent 10-year follow-up study confirmed an association between higher serum ferritin levels (≥800 ng/ml) and liver disease in non-chelated ß-thalassaemia intermedia patients [8].

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 [1, 9-12]. 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 [13, 14]. Several cases of hepatocellular carcinoma in patients with NTDT (primarily ß-thalassemia intermedia) have been described with malignancy sometimes occurring in the absence of viral hepatitis or cirrhosis while having considerably high iron overload indices [12, 15-19].

Liver disease is responsible for around 10% of causes of death in NTDT patients, further confirming the essential role of monitoring and intervention [20].
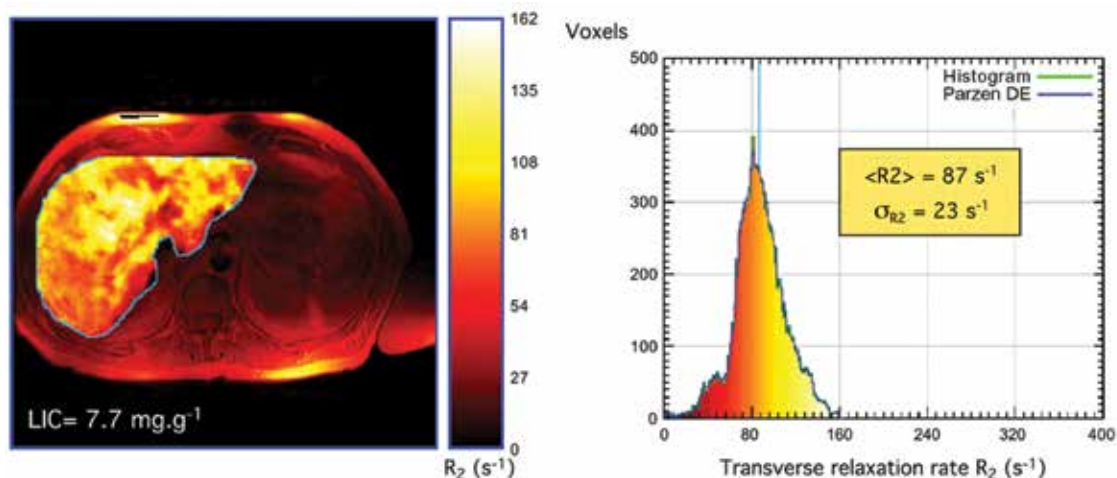
PRACTICAL RECOMMENDATIONS

- Patients with NTDT ≥10 years should be screened for hepatic function and disease as follows:
  - > Liver function tests: every 3 months, all patients
  - > Liver ultrasound: annually in patients with liver iron concentration ≥5 mg Fe/ g dry weight or serum ferritin level ≥800 ng/ml
  - > Alpha-feto protein: annually in cirrhotic patients or patients >40 years
  - > Transient elastography monitoring may be administered when available, annually in patients with liver iron concentration ≥5 mg Fe/ g dry weight or serum ferritin level ≥800 ng/ml

- NTDT patients with evidence of hepatic disease should be referred to a hepatologist for further work-up and management

- NTDT patients should be closely monitored and adequately managed for iron overload (see Chapter 5)

- Vaccination against hepatitis B prior to the initiation of any planned blood transfusion therapy, with regular monitoring of antibody titers, is recommended

- Vaccination against hepatitis A virus is recommended

- Patients receiving blood transfusions should undergo annual serologic monitoring for hepatitis B 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

- In patients with confirmed hepatitis B and C infection on polymerase chain reaction, management should follow guidelines from transfusion-dependent β-thalassemia major patients. A hepatologist should be consulted to decide on the indications for treatment, drug choices, dosing, and safety monitoring

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CHAPTER 09
ENDOCRINE AND BONE DISEASE

ILLUSTRATIVE CASE

A 35-year-old woman with haemoglobin E/ß-thalassaemia presents to a primary physician with symptoms of fatigue, depression, and mild weight gain. The patient's medical history included splenectomy at the age of 6 years. Physical examination demonstrated a heart rate of 58 beats per minute, coarse dry skin, and bi-lateral eyelid oedema. Her laboratory work-up revealed: total haemoglobin level 7.4 g/dl, serum ferritin level 1300 g/dl, serum free thyroxine (FT4) 6.44 pmol/l (normal range: 9.00-23.12 pmol/l), and thyroid-stimulating hormone 40 mIU/l (normal range: 0.35-6.20 mIU/l). She was started on levothyroxine 100 mg daily and the patient's symptoms improved. Repeat testing six weeks later revealed a normal thyroid-stimulating hormone (5 mIU/l). The patient was maintained on this dose and repeat thyroid-stimulating hormone testing is planned yearly or if symptoms recur. She was also referred back to her thalassaemia specialist for the management or iron overload.

CONTEXT AND EVIDENCE

Growth retardation and skeletal deformity attributed to ineffective erythropoiesis, medullary expansion, and anaemia may be encountered in children with NTDT. However, these are less commonly encountered compared to patients with more severe forms of thalassaemia (ß-thalassaemia major and severe forms of haemoglobin E/ß-thalassaemia) [1-4]; and can usually be managed with blood transfusion therapy during childhood (see Chapter 2). Nonetheless, NTDT patients remain at risk of morbidity from endocrine gland and bone pathology.

An association between iron overload and target-endocrine gland toxicity has been established from biological, clinical, and radiological studies in ß-thalassaemia major patients [5-7]. Although the prevalence of endocrinopathy in NTDT patients is relatively lower than regularly-transfused patients with ß-thalassaemia major, reported prevalence rates of diabetes mellitus, hypothyroidism, hypoparathyroidism, hypogonadism, and adrenal insufficiency remain considerably high, especially as patients advance in age (Vip Viprakasit, Unpublished Data) [1, 8, 9]. In patients with ß-thalassaemia intermedia, associations between elevated liver iron concentration or serum ferritin level and the risk of diabetes mellitus, hypothyroidism, hypoparathyroidism, and hypogonadism have been reported [8, 10-13]. Lower rates of such morbidities with iron chelation therapy use have also been suggested by observational studies [8]. The possibility that other pathophysiologic mechanisms may be implicated in the development of endocrinopathies in NTDT patients cannot be dismissed, especially that splenectomy, severe ineffective erythropoiesis, and low foetal haemoglobin levels have been associated with endocrine disease in NTDT patients [8, 14, 15]. Lower rates of endocrinopathies have also been

noted in patients receiving hydroxyurea therapy compared with those who do not [8].

Similar to patients with ß-thalassaemia major [16], 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 [14, 17]. Moreover, nutritional imbalances or hormonal alterations due to other endocrinopathies, as described above, can aid in the development of osteopenia and osteoporosis in NTDT [16]. 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 [16, 18-22]. Clinically, osteoporosis is a common finding in ß-thalassaemia intermedia [8] and haemoglobin E/ß-thalassaemia (Vip Viprakasit, Unpublished Data), especially as they advance in age [8, 9]. Female gender, iron overload, splenectomy, and low foetal haemoglobin levels have been associated with increased rates of osteoporosis in ß-thalassaemia intermedia patients [8, 10, 11, 13, 15]. 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 ß-thalassaemia major patients [16, 23, 24]. 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 [25, 26]. Although the efficacy and safety of bisphosphonates has been proven in patients with ß-thalassaemia major, data on patients with NTDT are limited [27]. Lower rates of osteoporosis were noted in ß-thalassaemia intermedia patients receiving iron chelation and hydroxyurea therapy than those who do not [8].

PRACTICAL RECOMMENDATIONS

- Patients with NTDT who are ≥10 years, should undergo the following routine testing:
  > Growth retardation
    - Standing and sitting height: every 6 months
    - Bone age
    - 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
  > Hypogonadism
    - Tanner staging: 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
    - Adults: routine assessment for infertility, secondary hypogonadism, impotence
  > Hypothyroidism:
    - Free thyroxine (FT4) and thyroid-stimulating hormone: annually
  > Hypoparathyroidism
    - Calcium, phosphate, vitamin D: annually
    - Parathyroid hormone: if indicated
    - Diabetes mellitus
    - Fasting blood sugar: annually
    - Oral glucose tolerance test: if indicated
  > Adrenal insufficiency
    - Adrenocorticotropic hormone stimulation test: annually
  > Osteoporosis
    - Bone Mineral Density spine, hips, radius, ulna (dual- energy X-ray absorptiometry): annually
    - Other hormonal and nutritional imbalances
    - Spine imaging: for back pain or neurological findings

- Standards for prevention of osteoporosis (behavioral, hormonal, vitamins and supplements) in patients with NTDT should follow guidelines and recommendations in transfusion-dependent ß-thalassaemia major patients

- Careful consideration of transfusion (see Chapter 2) and iron chelation (see Chapter 5) requirements should be maintained in NTDT patients.

- Patients with established endocrine disease or osteoporosis should be referred to a paediatric or adult endocrinologist for management according to standard local or international guidelines or as per recommendations in transfusion-dependent ß-thalassaemia major patients.

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CHAPTER 10
PREGNANCY

ILLUSTRATIVE CASE

A 29 year-old woman at 24 weeks of gestation presented for a regular check-up visit to her obstetrician. She was diagnosed with ß-thalassaemia intermedia at 5 years of age (IVSI-6/IVSI-6) when she had presented with anaemia (total haemoglobin level 9.5 g/dl) and splenomegaly. She had an uncomplicated disease course since diagnosis and receives no treatment. On routine laboratory studies, she showed a total haemoglobin level of 8 g/dl. The patient was thus started on blood transfusions to elevate the total haemoglobin level to >10 g/dl. She developed alloantibodies and worsening anaemia. She underwent Caesarean section at 31 weeks of gestation for intrauterine growth restriction and non-reassuring foetal heart monitoring and had splenectomy postpartum.

CONTEXT AND EVIDENCE

Although delayed puberty may be common in NTDT patients, fertility is usually preserved. Few case series reported pregnancy outcomes in women with NTDT (ß-thalassaemia intermedia) [1-4]. All reported pregnancies were spontaneous [1-4]. Abortion, pre-term delivery, intrauterine growth restriction (IUGR), Caesarean section delivery, thromboembolic events, and splenectomy were generally common in such women [1-4].

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 [2]. 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 [5]. The first reported large case series of pregnant ß-thalassaemia intermedia (83 pregnancies in 44 women) reported that 22% of patients still had IUGR despite abiding by this standard [1]. A recent 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 [4]. Another recent ß-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 [3]. Transfusions were not only administered based on total haemoglobin level but also general and cardiac maternal status and foetal growth [3]. The main concern with administering blood transfusion during pregnancy especially to previously never- or minimally-transfused patients is alloimmunization. Such patients commonly had al-

loimmunization in available studies and usually had adverse outcomes (abortion, IUGR, cardiac failure, Caesarian section delivery) [1-3]. Thrombotic disease was also a common occurrence, especially in women with additional prothrombotic risk factors [1, 4, 6]. In one series were all splenectomised patients received aspirin and all patients were given low-molecular-weight heparin the peripartum period, no thrombotic events occurred in any patient [3]. 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 [4]. Splenomegaly can interfere with the enlargement of the uterus and can be complicated by hypersplenism necessitating splenectomy during gestation or after delivery [1, 2].

PRACTICAL RECOMMENDATIONS

- NTDT patients planning to get pregnant need comprehensive counseling regarding the risk of having an affected child and prenatal diagnosis

- Pregnancy in NTDT patients should be considered a high-risk one, and care should be achieved through close collaboration between the hematologist, obstetrician, cardiologist, and other concerned specialists

- Introduction of blood transfusions for pregnant patients with NTDT should rely on
  > Total hemoglobin level
  > Maternal general and cardiac status
  > Fetal growth status

- 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 (see Chapter 2)

- Splenectomy should be considered for patients complicated with hypersplenism or splenomegaly before conception or postpartum

- 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 6) may be considered for anticoagulant therapy (low-molecular-weight heparin) all throughout pregnancy. Splenectomized patients should also be considered for aspirin therapy

- The following general monitoring and management standards should also be considered:
  > Pre-pregnancy
    - Assess iron overload status and ensure adequate management of iron overload (see Chapter 5)
    - Assess cardiac status and manage accordingly (echocardiogram, , cardiac stress test, electrocardiogram, Holter monitoring)
    - Assess endocrine (see Chapter 9), bone (see Chapter 9), and liver function (see Chapter 8) and manage accordingly
    - Assess viral status (hepatitis B and C, HIV, rubella) and ensure appropriate vaccinations (hepatitis B, Pneumovax, seasonal influenza)

  - Screen for red blood cell antibodies
  - Initiate folic acid
> During pregnancy
  - Cardiac, hepatic, and thyroid monitoring
  - Serial ultra sound to monitor growth restriction
  - Gestational diabetes monitoring (16 and 28 weeks)
  - Discontinue: iron chelators (deferoxamine may be used if needed in second and third trimesters, no other chelators are so far suggested), hormone replacement therapy, hydroxyurea, bisphosphonates (6 months prior), interferon/ribavirin, warfarin (switch to heparin), oral hypoglycemic (switch to insulin)
  - May be resumed: calcium, vitamin D, penicillin in splenectomized patients
> During delivery
  - Vaginal vs. Caesarian section: assessment depending on pelvic and cardiac status
  - Epidural anesthesia in case of Caesarian section
> Post-delivery
  - Restart iron chelation (post breast feeding, deferoxamine can be resumed immediately)
  - Resume calcium and vitamin D
  - Restart bisphosphonates (post breast feeding)
  - Restart hormone replacement therapy (post breast feeding)
  - Avoid breast feeding if positive for HIV, hepatitis B or C
  - Discuss contraception

• In NTDT patients with hypogonadism, ovulation or spermatogenesis may need to be induced and should be done by an experienced fertility centre.

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CHAPTER 11
EXTRAMEDULLARY HAEMATOPOIESIS

ILLUSTRATIVE CASE

A 25-year-old never-transfused man known to have haemoglobin E/ß-thalassaemia (moderate phenotype) presented to the emergency room with inability to walk for 3 days. His symptoms started 3 weeks prior to presentation with mild weakness that has been increasing progressively. He has become bedridden for the previous two days. He concomitantly felt a lower thoracic burning pain and breathing discomfort associated with the start of the weakness. He also reported pain (4/10), non-radiating, constant over time, and not relieved by paracetamol. His lower limbs sensation has also been affected, with a worsening tingling sensation. He also complained of difficulty with urination (not able to initiate a flow) in the past 3 days. He had 3 episodes of stool incontinence in the past 2 days, and denied any fever, chills, nausea, or vomiting. He also denied any history of discopathy, trauma to the back, or any previous episodes of back pain or prior limitation of movement. He had a negative family history of neurologic disease. His neurologic exam was consistent with spastic paraplegia. His muscle power was 3/5 in right leg and 2/5 in left leg. He also showed decreased sensation below the T10 level and deep tendon reflexes were increased. The patient was given dexamethasone for spinal cord syndrome and an emergency magnetic resonance imaging of the spine was done, which showed a posterior extradural mass compressing the spinal cord from levels T4 to T8. The patient was managed with a combination of radiotherapy and blood transfusion, and follow-up magnetic resonance imaging after 30 days showed resolution of the mass with no residual weakness.

CONTEXT AND EVIDENCE

PATHOPHYSIOLOGY AND CLINICAL ASPECTS

Expansion of the erythron in the bone marrow in NTDT 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, 2].

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 termed extramedullary haematopoietic pseudotumours. The prevalence of extramedullary haematopoietic pseudotumours is considerably higher in NTDT (~20%) than regularly-transfused ß-thalassaemia major patients (<1%) [3-5], and is mainly reported in

ß-thalassaemia intermedia and haemoglobin E/ ß-thalassaemia patients with few cases reported in haemoglobin H disease [6]. The prevalence is higher in older patients [7], as well as those with more severe ineffective erythropoiesis [8], and low foetal haemoglobin levels [9]. 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 [10-15]. 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 [10].

Among the various body regions reported, paraspinal involvement received special attention due to the debilitating clinical consequences secondary to neural element compression [10]. 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 [16, 17]. 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 [18], while others still attribute the masses to embolic phenomena [19, 20]. 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 [16]. 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 [21, 22], 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 [23].

A paraspinal location for the haematopoietic tissue occurs in 11 to 15% of cases with extramedullary haematopoietic pseudotumours [24, 25], and a large number of cases have been reported in the literature as reviewed recently [10]. 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 [22, 26, 27]. 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 [28], although few reports

described presentation as early as the first decade of life [19, 29, 30]. The male to female ratio reaches 5:1 [28]. Various clinical presentations have been reported including: back pain, lower extremity pain, parasthesia, 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 [10, 31].

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 [10, 31]. 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 [10, 31]. 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 [32-34] 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 [35, 36]. Bony destruction or pathological fractures are usually absent (Figure 11-1-A) [10]. In the early 1980s, several reports demonstrated that computed tomography was a more preferred diagnostic imaging method (Figure 11-1-B) [10]. 99 mTc bone scan has also been used to diagnose paraspinal extramedullary haematopoietic pseudotumours [37] but the diagnosis within the epidural space may be difficult due to the proximity to bone marrow [38]. Myelography is declining in popularity due to its invasiveness, the need for cisternal puncture in cases of complete block preventing passage of radiographic contrast [37, 39] and reports of neurological deterioration following the procedure [20, 40].

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 [10]. 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 [16, 38, 41]. If the patient is treated with blood transfusions, the lesion may decrease in size and appear on magnetic resonance imaging with massive iron deposition [41]. Fatty degeneration is most probably related to oxidative stress leading to lipid peroxydation of cell membranes and production of oxygen free radicals. This is probably the reason why foci with fatty content are ob-

served 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 [10]. 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 [41]. Active lesions show intermediate signal intensity in both T1- and T2- weighted magnetic resonance images (Figure 11-1-C). Gadolinium enhancement is minimal or absent differentiating it from other epidural lesions such as abscesses or metastases [38, 42]. 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 [43, 44]. 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 [41]. 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 [10].

Figure 11-1: 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 reference [10].
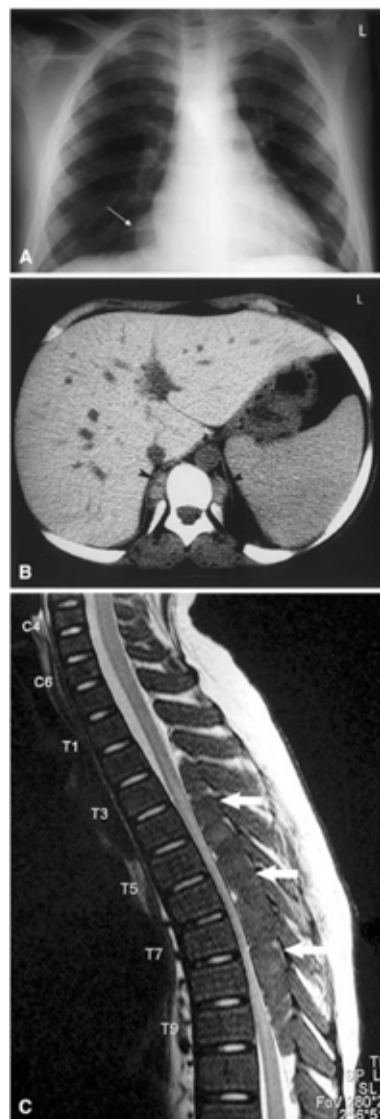
A recent report on 57 NTDT patients has also shed light on additional imaging findings related to spinal involvement of extramedullary haematopoiesis. Twenty-seven patients (47.4%) were found to have cortical bone invasion alongside extramedullary haematopoiesis with the most common location being the 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%) [45].

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 [3, 10-12]. A beneficial role of Janus Kinase 2 (JAK2) inhibitors on extramedullary haematopoiesis in the spleen is suggested by animal studies, and further clinical evaluation is underway [1, 46-49].

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 [10]. 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 [10]. 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 [22, 50]. 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 [22, 50-55]. The target haemoglobin level was usually >10 g/dl [10]. 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 [22]. However, several reports also showed that improvement may be slow, insufficient and only temporary [19, 29, 35, 42, 56]. 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 [22, 55]. 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 [28, 38, 40, 51].

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 [23, 35, 39, 57, 58]. Haematopoietic tissue is extremely radiosensitive and undergoes shrinkage after radiotherapy [59]. Dosages reported in the literature range from 900 to 3500 cGy [23, 28, 32, 39, 52]. A high risk of recurrence up to 19-37%, is the main drawback of radiotherapy [32, 39]. These recurrences, however, are often amenable to further doses. The risks of radiotoxicity on an already compressed and injured spinal cord remain a concern [35]. Tissue edema associated with radiation can sometimes result in neurological deterioration during the initial phase of treatment which is minimized by concomitant high-dose steroid therapy [40]. The immunosuppressive effect of radiotherapy is usually monitored with frequent peripheral blood counts as the resultant pancytopenia may further aggravate the condition [24, 32, 54]. 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 [23, 25, 28, 38].

NTDT 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 [60, 61].
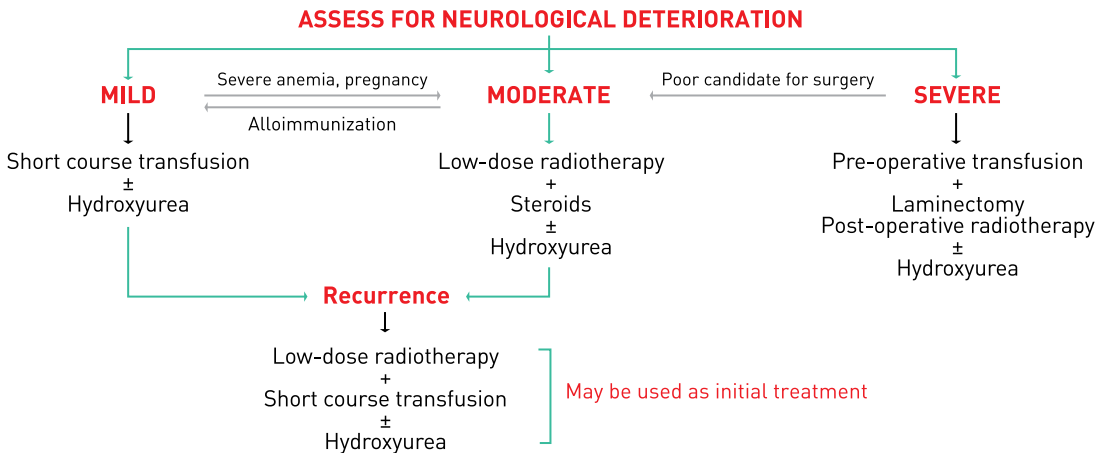
Successful combination therapy of any two of the three modalities (low-dose radiation, blood transfusion, and hydroxyurea) has been reported as a therapeutic option, either for cases of recurrence after using a single treatment method alone or as an initial treatment regimen [22, 30, 32, 35, 39, 52, 60, 62-65].

Laminectomy is usually reserved for cases of acute presentation which do not respond to adequate transfusion or radiotherapy [24, 38, 39]. Surgery confers the benefits of immediate relief of cord compression and histological diagnosis [38, 66, 67]. 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 [20, 28, 34, 40, 42]. 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 [39].

PRACTICAL RECOMMENDATIONS

- There is no sufficient evidence to recommend blood transfusion or hydroxyurea therapy for the prevention of extramedullary hematopoietic pseudotumors in NTDT patients, although when used for different indications a beneficial effect may be observed, especially in the following subgroups of patients:
  > Patients with β-thalassemia intermedia or hemoglobin E/β-thalassemia
  > Adult patients
  > Patients with severe anaemia
  > Minimally- or never-transfused patients
  > Patients with low foetal haemoglobin levels

- 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

- NTDT patients with evidence of paraspinal extramedullary hematopoietic pseudotumors should be promptly managed and followed by a dedicated team including a neurologist, a neurosurgeon, and a radiation specialist

- Figure 11-2 presents a proposed algorithm for the management of paraspinal extramedullary haematopoietic pseudotumours

Figure 11-2: Algorithm for the management of paraspinal extramedullary hematopoietic pseudotumors in patients with NTDT. Reproduced with permission from reference [10]

ASSESS FOR NEUROLOGICAL DETERIORATION

MILD	MODERATE	SEVERE
Severe anemia, pregnancy	Poor candidate for surgery	
Alloimmunization		
Short course transfusion ± Hydroxyurea	Low-dose radiotherapy + Steroids ± Hydroxyurea	Pre-operative transfusion + Laminectomy Post-operative radiotherapy ± Hydroxyurea

Recurrence

Low-dose radiotherapy
+
Short course transfusion
±
Hydroxyurea

May be used as initial treatment

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CHAPTER 12
LEG ULCERS

ILLUSTRATIVE CASE

A 40-year-old man with ß-thalassaemia intermedia presented to his physician complaining of a painful lateral right ankle lesion. The lesion appeared a month ago and started progressively expanding and becoming more painful. He went to a primary care physician who prescribed topical antibiotics and advised continuous protection of lesion with a gauze, but the lesion did not improve. His past medical history included splenectomy at the age of 9 years and a deep vein thrombosis in his right arm at the age of 29 years. He was not taking any medications. On physical examination he had a 1 x 4 cm lesion on the lateral area of the right ankle, which was painful to palpation. The lesion did not look infected but the surrounding skin had a black hue. His laboratory work-up revealed a total haemoglobin level of 8.1 g/dl and a platelet count of 988 x 10⁹/l. The patient was started on a once per month transfusion regimen. His leg ulcer progressively improved, and complete resolution was achieved by one year. His blood transfusions were later tapered and he was advised to start aspirin.

CONTEXT AND EVIDENCE

Leg ulcers are more common in NTDT compared with regularly-transfused ß-thalassaemia major patients [1-5]. The risk of leg ulcers in NTDT patients increases with advancing age [5-7]. 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 levels have been described as risk factors for the development of leg ulcers [3, 8-10]. The hypercoagulable state and deformability of red blood cells in NTDT patients (see Chapter 6) has been incriminated in leg ulcer formation since this might cause ischemia to the skin and consequently friability and ulceration [11, 12]. High venous pressure as a consequence, in the subgroup of patients with right-heart failure and venous insufficiency, may also be exacerbating factors [12, 13]. 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 than low foetal haemoglobin levels [14]. Higher rates of leg ulcers have also been reported in NTDT patients with iron overload [7, 10, 15, 16]. Local iron overload is also thought to be a perpetuating factor causing chronicity of lesions especially when the haem from the degraded red blood cells accumulates locally and gives a dark hue [17].

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 [3, 10, 13, 18]. The

beneficial effects of hydroxyurea on leg ulcers in NTDT patients are not limited to foetal haemo-globin induction and improvement of anaemia but also include improvement of red blood cell pathology, deformability, and hypercoagulability [19]. Pentoxifylline, which alters the rheologi-cal properties of the red blood cell, was also shown to accelerate the healing of leg ulcers [20]. The use of an oxygen chamber was also shown to provide moderate relief where tissue hypoxia may be an underlying cause of the ulceration [11]. The vasodilator dialzep (adenosine reuptake inhibitor) was shown to have some benefit in a trial of eight patients with haemoglobin E/ß-thalassaemia and chronic leg ulcers (three patients had total healing and four had improve-ment) [21]. Skin grafts have been tried by some plastic surgeons [11]. Both platelet derived wound healing factors and granulocyte macrophage colony-stimulating factor have been suc-cessfully used in some patients [22]. There is limited evidence on the benefit of anticoagulation for the management of leg ulcers in NTDT patients [12]. A recent trial has established benefit of sodium nitrite cream in patients with sickle cell disease and refractory leg ulcers [23].

Thalassemia patient with leg ulcer

PRACTICAL RECOMMENDATIONS

• There is no sufficient evidence to recommend blood transfusion, iron chelation or hydroxyurea therapy for the prevention of leg ulcers in NTDT patients, although when used for different indications a beneficial effect may be observed

• The skin of NTDT patients should always be inspected on routine physical examination

• Patient with evidence of leg ulcers should be treated in close collaboration with a dermatologist and a plastic surgeon

• 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.

• Topical antibiotics and occlusive dressing should be applied

• Topical sodium nitrite cream may be considered

• Blood transfusion should be considered as the first treatment option

• The following treatment measures may also be considered in patients who have persistent leg ulcers, although no clinical trials to supporting their use exist:
  > Hydroxyurea
  > Dialzep (vasodilators)
  > Oxygen chamber
  > Skin grafts
  > Platelet derived wound healing factors and granulocyte macrophage
  > Anticoagulation

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CHAPTER 13
HAEMOLYTIC CRISIS

ILLUSTRATIVE CASE

A 12-year-old Thai girl presented to the emergency department with fever and acute abdominal pain. The patient was diagnosed with haemoglobin H disease (with 20% haemoglobin H) at 9 years of age after presenting with jaundice and microcytic anaemia (total haemoglobin level 9.2 g/dl). Molecular diagnosis revealed that she was compound heterozygote of SEA type and 3.7 kb deletions. She had normal growth and development in early childhood and was transfusion-independent. Laboratory work-up at the emergency department revealed severe hypochromic anaemia (total haemoglobin level 4.5 g/dl) and the peripheral blood smear showed extreme anisocytosis and poikilocytosis as well as teardrops, target cells, and marked reticulocytosis. Haemoglobinuria was detected, together with increased indirect bilirubin, lactase dehydrogenase and aspartate transaminase suggesting acute haemolytic crisis in the patient. Her oxygen saturation was lower than 90% at room air using a pulse oximeter. White blood cell counts showed marked leucocytosis and predominant neutrophils with numerous toxic granulation and vacuolization. The patient was started on blood transfusions (10-12 ml/kg/dose), intravenous hydration, and alkalinisation of the urine. Antipyretics and empiric antibiotics were commenced pending bacterial culture results. Her clinical status and total haemoglobin level improved and she was admitted to the hospital for monitoring.

CONTEXT AND EVIDENCE

Haemolytic crisis can occur in both deletional and non-deletional forms of haemoglobin H disease [1-2]. During haemolytic crisis, the haemoglobin level in patients with haemoglobin H disease may drop significantly. Occurrence is more commonly noted in non-deletional than deletional forms, although they are observed in both conditions. There are several factors that can contribute to haemolytic crisis including infection and pyrexia (during or after), oxidative challenge, hypersplenism, or pregnancy [2-4]. Jaundice is common during haemolytic crisis [3]. With haemolytic crisis the total haemoglobin level may drop down to 3 g/dl overnight because the red cells with precipitated haemoglobin H are rapidly destroyed [5]. In addition, it has been previously shown that increased body temperature can further induce the generation of haemoglobin H inclusion bodies that can induce oxidative damage to the red blood cells and cause further extramedullary haemolysis. However, the acute haemolytic crisis may be as brisk as that found in Glucose-6-phosphate dehydrogenase deficiency, with evidence of haemoglobinuria and haemoglobinuria (intravascular haemolysis), which results in renal damage and renal insufficiency. Patients may ultimately go into shock with acute renal failure. This is probably the most serious complication in haemoglobin H disease that requires immediate intervention [3].

The majority of causative pathogens leading to haemolytic crisis are gram-negative bacteria including Salmonella, Shigella, and Klebsiella species. However, other gram-positive organisms such as Streptococcus species could also be prevalent [5]. Therefore, empirical antibiotics are usually commenced at first instant until the culture results have verified the causative pathogens. In the tropics, dengue haemorrhagic fever is probably one of the most lethal inter-current infections that cause haemolytic crisis in patients with haemoglobin H disease [6]. Contrary to clinical dengue haemorrhagic fever or dengue shock syndrome in normal children, haemoglobin H patients have no evidence of haemoconcentration. Instead, they develop haemolytic crisis with decreased haemoglobin levels and in most instances this is misdiagnosed as having gram-negative septicaemia [6]. Moreover, fragmented red blood cell vesicles from haemolysis can cause a factitious count of the platelets if an automated cell counter is used resulting in a delay of detecting thrombocytopenia [6]. Therefore, correct identification of causative organisms in haemoglobin H patients with haemolytic crisis is considered the most important challenge and usually requires careful clinical evaluation. More importantly, patients show evidence of poor tissue oxygenation or hypoxia and thus require immediate intervention [3]. Adequate intravenous hydration with urine alkalinisation often becomes necessary to prevent possible kidney damage from the precipitation of haemoglobin passing through the renal glomeruli and tubules [3].

Aplastic crisis due to infection of parvovirus B19 may also occur in patients with haemoglobin H disease and these are different from haemolytic crisis [7-8]. This virus targets active hematopoietic cells, particularly erythroblasts, and results in selective disruption of erythropoiesis and decrease in red blood cell count while in haemolytic crises, red blood cell count is usually increased [9-10]. The white blood cells and platelet counts may also be reduced. Parvovirus B19 infection is self-limited and usually resolves spontaneously, although some may require blood transfusion support or intravenous immunoglobulin therapy [3] .

PRACTICAL RECOMMENDATIONS

- Patients with haemoglobin H disease should be monitored closely for severe anemia during acute infections and pregnancy

- Patients showing acute drops in total haemoglobin level and increased markers of hemolysis should be promptly treated

- The distinction between haemolytic and parvovirus B19-induced aplastic crisis should be made by observing reticulocyte counts (reticulocytopenia favours Parvovirus B19-induced aplastic crisis). The diagnosis of parvoviral infection requires viral serology testing or demonstration of the virus by DNA technology

- Patients with evidence of haemolytic crisis should be managed as follows:
  > Restoring total haemoglobin level to 8-9 g/dl by red cell transfusion
    - Filtered red blood cells or leucocyte-depleted blood 5-12 ml/kg/dose should be given depending on patient's clinical severity and levels of anemia
    - Monitoring tissue oxygenation using pulse oxymetry and peripheral blood gas is recommended
    - Close monitoring of total body fluid and cardiovascular status is highly recommended
    - Serial total haemoglobin and haematocrit evaluation should be done at least daily as haemolysis may persist if the cause has not been removed or properly treated
  > Adequate hydrations should be given
    - Intravenous fluid support (at least a maintenance rate with 5-10% volume deficit) should be provided to maintain circulation and withheld during transfusion support
    - The amount and rate should be carefully calculated to avoid possible heart failure from volume overload
  > Blood electrolytes should be checked and correction of abnormalities made
    - Metabolic acidosis is usually observed but mostly resolved by transfusion support and fluid therapy; only rare cases with evidence of hemoglobinurea may require alkali therapy
  > Body temperature should be controlled
    - Frequent tepid sponge
    - Paracetamol 10-12 mg/kg every 4-6 hours
    - There are no data to support the use of Non-steroidal anti-inflammatory drugs in this setting
  > The cause of infection/inflammation should be identified and treatment
    - Blood and urine culture should be done
    - Empirical antibiotic with the coverage of gram-negative bacteria and/or encapsulated bacteria (depending on splenic condition) such as Streptococcus and Salmonella species, as

well as meningococci, should be promptly provided

- Parvovirus B19 infection is self-limited and usually resolves spontaneously, although some patients may require blood transfusion support. In cases with chronic infection and prolonged hematopoietic suppression, specific treatment with intravenous immunoglobulin should be considered

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CHAPTER 14
QUALITY OF LIFE

ILLUSTRATIVE CASE

A 32-year-old ß-thalassaemia intermedia patient presented to his physician for a routine follow-up. When the doctor asked the patient how he is doing, he answered the following: "I haven't been sleeping well lately. I have constant worry about what's coming next. I feel I have been betrayed, but I don't know how or by whom. I remember when I was a child my mother used to always tell me that I should be thankful that I was not diagnosed with ß-thalassaemia major and that I do not have to spend most of my time at the hospital taking transfusions. But that was not the case. For the past 10 years, I have been in and out of the hospital so many times that I lost count. I suffered serious complications that almost got me killed [referring to pulmonary embolism] and had a major surgery that almost got me paralyzed [referring to laminectomy for paraspinal extramedullary pseudotumours]! And worse, now I can no longer play any sports from this ulcer on my foot that refuses to go away. My friends are pushing me to get married, but how can I take care of a family if I'm spending most of my time taking care of myself?"

CONTEXT AND EVIDENCE

Global public health efforts alongside advances in medical management surely translated into prolonged survival and lower morbidity in patients with ß-thalassaemia major [1-3]. Despite these favourable effects, the burden of regular therapy poses a negative impact on patients' health-related quality of life (HR-QoL). HR-QoL in patients with ß-thalassaemia major remains lower than that reported for normal individuals and these effects become more relevant as patients transition from childhood to adulthood [4, 5]. In patients with NTDT, the situation is also far from ideal. It is now apparent that the diagnosis of NTDT carries higher morbidity than previously recognized especially as patients advance in age [6]. Moreover, NTDT patients may suffer from chronic pain, which appears multifactorial and also increases with age [7]. Hence, both the NTDT patient and the caring physician may be faced with 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 ß-thalassaemia intermedia (non-transfused, non-chelated) and 48 ß-thalassaemia major patients [8]. Patients with ß-thalassaemia intermedia and major were comparable with age, gender, and socioeconomic parameters; but patients with ß-thalassaemia major had a significantly longer median duration with a known thalassaemia diagnosis while patients with ß-thalassaemia intermedia had a higher prevalence of multiple complications. The mean Total, Physical Health, and Mental Health Scores were significantly lower in patients with

ß-thalassaemia intermedia compared with ß-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 [8]. Data from another study including paediatric patients also confirmed a higher proportion of ß-thalassaemia intermedia children having impaired HR-QoL than ß-thalassaemia major [9]. In another study, it was also apparent that a considerable proportion of adult patients with both ß-thalassaemia major and intermedia show evidence of depression (Beck Depression Inventory) and anxiety (State-Trait Anxiety Inventory). Patients with ß-thalassaemia intermedia, however, seemed more liable to state anxiety (feeling 'right now, at this moment') than ß-thalassaemia major patients of a similar age, which was attributed to a shorter duration of living with a known thalassaemia diagnosis (poorer adaptation to disease) [10]. 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 [11]. Similar data from patients with haemoglobin E/ß-thalassaemia and haemoglobin H disease are limited. However, in a recent study, patients with deletional forms of haemoglobin H who were evaluated for HR-QoL measures, did not suffer chronic fatigue, limitation of physical activity, or evidence of learning problems. Patients with non-deletional forms (haemoglobin H Constant Spring), however, did experience some limitations and had an increased number of annual clinic visits an increased number of annual hospital than deletional haemoglobin H patients [12]. Data from studies on haemoglobin E/ß-thalassaemia illustrate the beneficial effects of appropriate treatment on HR-QoL [13].

PRACTICAL RECOMMENDATIONS

- Patients with NTDT should be closely followed and appropriately managed as per the guidelines enclosed herein

- Frequent assessment of patients HR-QoL and mental health status is recommended, preferably by standardized instruments

- The following interventions should be considered
  > Patient level
    - Working on self-image conception
    - Helping the patient to understand illness and accept it
    - Involving the patient in his treatment to become responsible and autonomous
    - Psycho-social intervention in patients with poor HR-QoL or mental health problems
  > Family level
    - Helping the family to accept the situation and live with the diseased child
    - Arranging for meetings with other parents
    - Genetic counseling for better family planning
    - Psycho-social counseling to support the adolescent crisis of their children
  > Community level
    - Integration of patients into their society
    - Awareness campaigns about the disease
    - Highlighting patients' normal intellectual capacities

- NTDT patients should receive appropriate nutritional, vitamin, and supplements support as per guidelines from transfusion-dependent ß-thalassaemia major patients

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ABOUT TIF

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

TIF works in official relations with the World Health Organisation (WHO) since 1996 and with a number of other official health bodies and patient oriented organizations.
(www.thalassaemia.org.cy)

MISSION: The development of National Control Programmes, including both components of prevention and management and the promotion of their establishment across 'affected' countries.

VISION: Establishment of equal access to quality health care for every patient with thalassaemia wherever he or she may live.

OBJECTIVES: The objectives of the Federation in addressing effectively the needs of the world thalassaemia family have since its establishment remained the same and include:
• The establishment of new and promotion of existing National Thalassaemia Patient/Parents Associations
• Encouraging, motivating and supporting studies and research for further improving prevention strategies, clinical care and for achieving the long-awaited final cure and
• Extending the knowledge and experiences gained from countries with successful control programmes to those in need.

TODATE: TIF has developed into an umbrella federation with 102 member associations, from 60 countries of the world, safeguarding the rights of patients for quality health care.

Its educational programme, focused on the needs of patients/parents, medical health professionals and the community at large, has been, and still is, amongst its strongest tools towards achieving its objectives.

TIF since 1990 has organised 60 national/local, 6 regional workshops and 14 international conferences and has prepared, published, translated and distributed more than 15 books todate in more than 50 countries worldwide.

OTHER TIF PUBLICATIONS

"Please note that some of the publications are translated to number of languages.
Please visit TIF website at http://thalassaemia.org.cy/ or TIF mobile app (TIF Digital Library)".

"KNOWLEDGE IS OUR POWER"
"UNITY IS OUR STRENGTH"