

Part IV

NON-TRANSFUSION DEPENDENT THALASSEMIA (NTDT)

Principles

- To diagnose NTDT at early stages of life and perform all necessary investigations.
- To be able to screen for complications and prevent them before development.
- To be able to treat complications including iron overload in patients with NTDT.
- To recognize patients with NTDT that needs blood transfusion or other disease specific therapies as Hydroxyurea.

Recommendation

A. Diagnosis

- NTDT should be suspected in patients with a chronic microcytic anemia, often in the range of 70-90 g/L, presenting with symptoms secondary to chronic hemolysis and/or ineffective erythropoiesis. Such patients include beta-thalassemia intermedia, Hemoglobin H disease and Hemoglobin E/Beta-thalassemia and rare forms of unstable hemoglobins.
- Laboratory diagnostic approach should include:
 - Red cell and hemolytic markers.
 - Hemoglobinopathy investigations (HPLC, electrophoresis).
- Confirmatory DNA analysis of alpha and or beta globin genes as appropriate for the suspected diagnosis.
 - Consider further DNA analysis in cases where alpha tri/or quadriplection is suspected to cause NTDT in the presence of a simple beta-thalassemia trait.

B. Complications

Extramedullary hematopoiesis

- Screening:
 - Clinical examination for organomegaly and bony deformities (ex. frontal bossing, zygomatic bone prominence, depression base of nose, shortening of long bones) at each clinic visit.
 - Screen for neurologic symptoms or back pain at regular clinic visits.
- Prevention:
 - There is insufficient evidence to recommend specific primary prevention strategies but treatment with hydroxyurea and red cell transfusions may be of benefit in select populations.
- Treatment:
 - Consider red blood cell transfusions, hydroxyurea, radiation therapy, and surgical decompression depending on severity.

Hypercoagulable state and thromboembolic events

- Screening:
 - Have a high index of suspicion for thromboembolic events.
- Prevention and Treatment:
 - Acute VTE management is generally in accordance with standard TE guidelines.
 - Further evidence is required to adequately provide recommendations regarding prophylactic anticoagulation or anti-platelet therapy as well as long-term management of patients with thrombotic or cerebrovascular disease.
 - Consider low-dose aspirin in splenectomized NTDT patients with platelets $> 500 \times 10^9/L$.

Pulmonary Hypertension

- Screening:
 - Annual routine echographic assessment of TRV.
 - In asymptomatic patients with a TRV $>3.2\text{m/s}$ or $>2.5\text{ m/s}$ if they are symptomatic, confirmation by right heart catheterization.
 - If pulmonary hypertension is found, rule out other causes of pulmonary hypertension, particularly thromboembolic causes.
- Treatment:
 - Further studies are required for providing adequate evidence for management of pulmonary hypertension in the context of NTDT but referral to a cardiologist/pulmonary hypertension specialist is recommended.
 - Consider hydroxyurea and/or transfusions. Selection of therapeutic strategy may depend upon the severity of the pulmonary hypertension, as well as physician and patient preference.

Leg ulcers

- Screening:
 - Inspection of the skin (particularly of the lower limbs) during each clinic visit.
- Prevention:
 - There is insufficient evidence to recommend specific primary prevention strategies.
 - Consider leg elevation and /or sleeping with the end of the bed elevated.
- Treatment:
 - Referral to dermatology or plastic surgery is suggested.
 - Further studies are required for adequate evidence in specific management of leg ulcers but treatment considerations include 1) local management with leg elevation, occlusive dressings, topical antibiotics, sodium nitrite cream, skin grafting, 2) systemic treatments such as red blood cell transfusions, hydroxyurea, arginine butyrate.

Iron overload

- Screening:
 - Initiate iron burden screening by imaging (ex. MRI T2*) by 10 years of age. One can consider delaying this to 15 years of age in patients with deletional HbH disease as they accumulate iron more slowly.
 - NTDT patients requiring frequent blood transfusions over a prolonged period of time should be screened according to TDT guidelines.
- Treatment:
 - Consider starting chelation treatment in NTDT patients ≥ 10 years of age with an LIC ≥ 5 mg Fe/g dry weight. Start at half the usual starting dose in TDT (DT deferasirox 10mg/kg/day.)
 - Adjust dosing according to LIC and ferritin after 6 months.
 - Ongoing monitoring by imaging yearly and serum ferritin every 3 months.
 - Suggested parameters for discontinuation of therapy: LIC < 3 mg Fe/g dry weight or (serum ferritin < 300 μ g/L if LIC not available).

Low bone density and other bone diseases

- Follow recommendations outlined for osteoporosis in TDT
- Screening:
 - Monitor for changes in facial bone structure and head circumference.
 - Assess bone mineral density of the spine, hips, radius, and ulna by dual-energy X-ray absorptiometry (DXA) starting at age 10 years.
 - Screen for bone pain, particularly back pain and neurologic symptoms. If present, imaging is necessary to look for fractures.
 - Hormone and nutritional profile screening.
- Prevention:
 - Vitamin D and Calcium.
- Treatment:
 - If osteopenia or osteoporosis is present, referral to an endocrinologist for management (ex bisphosphonates).

Endocrinopathies

- Screening:
 - In children, growth parameter monitoring with particular attention to growth velocity, especially in young children and adolescents where it is expected to be at its highest.
 - In children ≥ 10 years of age, yearly Tanner staging. Puberty delay defined by lack of breast bud development in girls ≥ 13 years or no testicular enlargement in boys ≥ 14 years. If present, consider: gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone/estradiol, pelvic ultrasound, zinc level, thyroid-stimulating hormone (TSH) and T4.
 - Annual screening in patients ≥ 10 years: free T4, thyroid-stimulating hormone, calcium, phosphate, vitamin D, and fasting blood glucose.

- Treatment:
 - Patients with diagnosed endocrinopathies should be managed by an endocrinologist and managed according to recommendations for TDT with regards to chelation therapy and hormone supplementation.
 - If presence of growth failure, consider initiating chronic transfusion therapy with weaning and or discontinuation as appropriate once catch up growth has been achieved.

Infections, hemolytic and aplastic crises

- Manage asplenic patients as per asplenia guidelines.
- During periods of infection, monitor closely for acute drops in hemoglobin that could indicate a hemolytic or aplastic crisis.
 - Hemolytic crisis: maintain Hb > 70-90 g/L, hydrate adequately and monitor renal function and electrolytes closely. Consider looking for bacterial (or parasitic) causes as appropriate.
 - Aplastic crisis: send parvovirus IgM to diagnose. Maintain Hb > 70 g/L.

Cholelithiasis

- Screening:
 - Screen yearly by ultrasound for the presence of gallstones.
- Treatment:
 - Symptomatic cholelithiasis should be managed with a cholecystectomy.

Liver disease

- Screening:
 - Liver enzymes/function tests should be monitored every 6 months in NTDT patients ≥10 years.
 - Consider measuring liver fibrosis by transient elastography in patients with an LIC ≥ 5mg Fe/g dry weight or serum ferritin ≥800ng/ml.
 - Albumin, INR and PTT monitoring in patients with cirrhosis or ≥ 40 years old.
- Prevention:
 - Adequate iron chelation therapy as described in iron overload section.
 - Immunizations for Hepatitis A and B prior to starting blood transfusion therapy as well as regular monitoring of Hepatitis B titers.
 - Annual serologic monitoring for Hepatitis B and C in transfused patients. Positive results should be confirmed by polymerase chain reaction and managed by sub-specialists as per institutional guidelines.
- Treatment:
 - Patients with evidence of hepatic disease should be referred to a hepatologist for further management.

NTDT-directed therapies

- All patients should receive folic acid supplementation.
- Red blood cell transfusions
 - Occasional transfusions should be considered in periods of (anticipated) acute stress or hemoglobin drop
 - Pregnancy
 - Surgery
 - Infections
 - Chronic transfusions: The goal of chronic transfusion is to improve anemia and decrease effects from ineffective erythropoiesis. Benefits must be balanced with long term complications including iron overload and red cell alloimmunisation. Patients should be considered for chronic blood transfusion if they develop:
 - Growth failure
 - Poor school performance / developmental concerns
 - Delayed puberty
 - Bone deformities
 - Patients with symptomatic anemia and secondary decreased quality of life / poor exercise tolerance
 - Rapid splenic enlargement (> 3cm/year) accompanied by drop in hemoglobin, particularly during periods of rapid growth
 - Primary prevention in high-risk patients as well as management and or secondary prevention of NTDT complications:
 - Pulmonary hypertension
 - Chronic leg ulceration
 - Thrombotic or cerebrovascular disease
 - Extramedullary hematopoietic pseudotumors
 - If chronic transfusions are initiated, monitor for red cell alloimmunization and manage the expected iron overload.
- Hydroxyurea
 - While there are no randomized clinical trials to provide strong evidence for use of hydroxyurea, observational studies have suggested some benefit in certain β NTDT subgroups and for management of NTDT complications (pulmonary hypertension, leg ulcers, extramedullary hematopoietic pseudotumors.)
 - Starting dose 10mg/kg/day, escalating to a maximum of 20mg/kg/day with concomitant folic acid supplementation.
 - Assess for response by 6 month and discontinue if no clinical benefit. In patients having responded, continue to monitor for sustained response.
 - Monitor for adverse effects: CBC+diff, renal function, liver enzymes.
 - Contraindicated in pregnant women as well as patients with hepatic or renal failure.
- Splenectomy
 - Routine splenectomies should not be performed but rather reserved for specific clinical situations such as:
 - Worsening anemia with poor growth/development and chronic transfusions or iron chelation therapy are not possible.
 - Hypersplenism with worsening cytopenias resulting in clinical problems, ex bleeding or infection.
 - Massive splenomegaly with risk of splenic rupture significant left upper quadrant pain or early satiety.

- o Immunize pre-splenectomy and manage post-splenectomy as per asplenia guidelines.
- o There is a lack of evidence to provide a recommendation on post-splenectomy thromboprophylaxis. Based on expert opinion, consider low-dose aspirin in splenectomized NTDT patients with platelets $> 500 \times 10^9/L$.

Background

The term Non-Transfusion Dependent Thalassemia (NTDT) refers to a thalassemic spectrum of disease that is milder than transfusion-dependent thalassemia but more severe than trait. By definition, these patients do not require transfusions to sustain life; however, they may require transfusional support in certain clinical settings such as poor growth, excessive extramedullary hematopoiesis, and periods of acute stress or profound anemia (hemolytic crises, aplastic crises, pregnancy, infection, surgery). The baseline hemoglobin often lies between 70-90 g/dL.¹

By referring to NTDT, we primarily include forms of:

- Beta Thalassemia Intermedia, where there is an excess of α -globin chains in relation to available β -globin chains
- Hemoglobin E/Beta thalassemia
- Hemoglobin H disease, where there is an excess of β -globin chains in relation to available α -globin chains²
- Rare forms of unstable hemoglobin.

The degree of severity of NTDTs is quite variable and is largely related to the underlying molecular defects, the presence of additional polymorphisms, and the resultant degree of chain imbalance³. Therefore, the specific genotype may provide some information; however, the diagnosis of NTDT is a clinical one and the genotype may not necessarily correlate with the phenotype. Despite its genotypic variability, all manifestations of NTDT are primarily due to ineffective erythropoiesis, hemolytic anemia, and iron overload.

1. Diseases

Beta Thalassemia Intermedia

Beta thalassemia intermedia describes a spectrum of disease in which there is an increase in the ratio of alpha globin to beta globin. Diverse genetic modifiers contribute to this ratio of alpha/beta globin resulting in the phenotypic variability of beta thalassemia intermedia. These genetic modifiers include :

- The degree of deficit in β -globin chain production. There are greater than 200 β -globin gene mutations described ranging in decreased to absent β -globin production. These may be present in the homozygous or compound heterozygous state. Larger deletions may affect other globin genes (ex. hereditary persistence of fetal hemoglobin (HPFH), $\delta\beta$ thalassemia, Hb Lepore.) There also exist rare dominant β -globin mutations. In addition, β -globin synthesis can be modulated by various transcription factors (ex. TFIIH, GATA-1).^{3,4}
- The presence of α gene deletions or duplications results in variations in the α - to β -globin chain imbalance (deletions improve the ratio of α - to β -globin whereas triplications and quadruplications worsen it.)
- Polymorphisms and mutations in genes modulating γ -globin production (ex. Xmn1-HBG2, HBS1L-MYB, and BCL11a) can alter the capacity for continued γ chain synthesis after birth.³⁻⁵ Increased γ -globin chains reduces the imbalance of α to β -like globins.
- Excess α -globin chains, whether through decreased γ/β -globin and/or increased α -globin production, precipitate as hemichromes forming inclusion bodies in early erythroid precursors as they are unable to form stable tetramers.⁶ These inclusions damage the membranes of red blood cells and their organelles as well as trigger the formation of reactive oxygen species, which further damage the red blood cells.^{7,8} This process of red blood cell injury leads to the intramedullary destruction of erythroid precursors and results in the ineffective erythropoiesis that is a hallmark of beta thalassemias. In comparison to transfusion dependent thalassemia (TDT) where there is little to no β globin, the residual β globin production as well as the variable γ -globin synthesis in NTDT moderate the consequences of the excess α -globin, which leads to the less severe phenotype of NTDT.

Hemoglobin E/ beta thalassemia

Hemoglobin E /beta thalassemia is present in high frequency in South East Asia and Bangladesh, and accounts for approximately half of all severe beta thalassemia patients globally.^{9,10} It is caused by compound heterozygosity for beta thalassemia and the hemoglobin variant HbE. HbE is the result of a single point mutation substituting G to A in codon 26 of the β -globin gene. This point mutation creates in an alternate mRNA splicing site, decreasing β -globin production. In addition, the resultant beta-globin binds more weakly to α -globin leading to instability in high oxidative states.¹¹ This thalassemia subgroup's disease severity can range from entirely transfusion independent to transfusion dependent and, like thalassemia intermedia, multiple other genetic factors will affect a patient's phenotype.

Hemoglobin H disease

Alpha thalassemia caused by deletion and or mutation of alpha globin genes (on chromosome 16p13.3) is the most common genetic disorder affecting up to 5% of the world's population.¹² When three of the four alpha globin genes are inactivated, unpaired beta globin chains form beta tetramers (hemoglobin H) in red cells, ultimately resulting in a chronic hemolytic anemia. This form of alpha thalassemia was first described in 1955 and is called Hemoglobin H disease (HbH disease).¹³ This condition is most prevalent in Southern/Western China, Indochina, and South-East Asia; however, its prevalence has increased worldwide due to population migration. It is estimated that at least 10,000 babies are born every year with this condition.¹⁰ A review of a Canadian cohort of HbH disease patients in Ontario showed that the majority of patients were of Chinese, Filipino, Laotian, and Vietnamese descent, with a minority of Mediterranean, Middle Eastern or East Indian descent.¹⁴

While HbH disease is generally thought to be one of the milder forms of NTDT, clinical severity can be heterogeneous but can largely be divided into the less severe "deletional" and more severe "non-deletional" forms.¹⁵ The type and location of the alpha globin molecular defect(s) play into the disease severity modifying the α - to β -globin ratio and α -globin stability.¹⁶

- Deletional mutations: Deletional mutations are more frequent than non-deletional. They can occur in cis resulting in α^0 or no production of α -globin from one chromosome (ex. --SEA, --FIL, --THAI, --MED). Deletions can also affect a single α -globin gene on a chromosome leading to α^+ or reduced alpha globin production (ex. $-\alpha^{3.7}$, $-\alpha^{4.2}$.)
- Non-deletional mutations: There are >70 forms of non-deletional mutations (ex. Constant Spring (CS), Quong Sze, Pakse, and polyA.) The majority of these mutations involve the $\alpha 2$ gene which is located closest to the α -globin regulatory region and has the stronger gene expression resulting in 2-3:1 gene product production over $\alpha 1$.¹⁶ Hence, an $\alpha 2$ mutation will have more significant impact on α -globin production.
- In the deletional type, the HbH amount is variable but is usually less than 30%. HbH is unstable and is oxidized to form intracellular inclusion bodies in older red blood cells causing oxidative damage, membrane dysfunction, and shortened red cell survival with premature destruction in the spleen resulting in moderate to severe hemolysis. Hemoglobin H has high oxygen affinity, lacks heme-heme interaction and has poor oxygen delivery capacity. Hence, patients are functionally more anemic than their hemoglobin may indicate.^{17,18} In non-deletional types, the situation is made worse by the instability of the mRNA transcribed, which results in the production of hyper-unstable globin variants. They are unable to assemble in stable tetramers and thus rapidly degraded, further aggravating the globin chain imbalance resulting in increased hemolysis.¹⁸ The oxidative damage and membrane dysfunction results in rigid overhydrated cells with a shortened red cell survival. The loss of phospholipid asymmetry with phosphatidylserine exposure increases the risk of thrombosis.¹⁹

Clinically, HbH disease manifests as microcytic anemia present from birth. Most individuals with deletional HbH disease remain relatively asymptomatic, and hence may not be diagnosed until later on in life. Some may be misdiagnosed and treated inappropriately for iron deficiency anemia. Their hemoglobin level after infancy is approximately 95g/L, MCH 16pg.¹⁵ There may be mild pallor with no or minimal jaundice and usually mild splenomegaly from childhood. This condition is 1) most often compatible with a normal unrestricted physical life 2) rarely requires red blood cell transfusions 3) may result in the development of gallstones in adulthood 4) results in a slower rate of iron accumulation than other NTDT patients.^{15,20}

In comparison, individuals with non-deletional HbH disease have a more severe hemolytic anemia with average hemoglobin

around 72g/L, MCH 18.6pg, and a higher reticulocyte count.¹⁵ Other findings include mild to moderate pallor with jaundice as well as early onset splenomegaly, which can progress quite significantly later on in life. Growth delay, intermittent transfusion requirements particularly around infections, and iron overload in the first decade of life are additional fairly frequent features.¹⁵ Rarely and in its most severe form, hydrops fetalis can result.

In summary, the heterogeneous clinical features of HbH disease, like NTD beta thalassemias, underscore the importance of early and accurate diagnosis with proper management.

2. Complications

The imbalance in the α and β chains in this spectrum of disease results in a number of complications, primarily as a result of ineffective erythropoiesis, hemolytic anemia, and iron overload. Various studies have suggested risk factors for morbidity to include increased age, lower hemoglobin, and prior splenectomy.²¹⁻²⁶ Transfusion support with adequate chelation and hydroxyurea use appears to reduce the frequency of NTDT-associated complications in patients who are on the more severe end of the spectrum.^{21,26}

Extramedullary hematopoiesis

As a result of chronic hemolytic anemia and ineffective erythropoiesis, there is a physiologic response with erythroid hyperplasia leading to expansion of hematopoietic tissue. The expansion of hematopoietic tissue results in osteoporosis, bone deformities, and extramedullary hematopoiesis, which can manifest as hepatosplenomegaly and or hematopoietic pseudotumors.²⁷ Extramedullary hematopoiesis can arise in any tissue normally contributing to hematopoiesis in the developing fetus. While hematopoiesis normally ceases in these sites at birth, the vascular connective tissue retains the ability to be activated to produce red cells under conditions of chronic ineffective erythropoiesis.²⁸ Age, more severe ineffective erythropoiesis and anemia, as well as lower HbF levels are thought to increase the risk for developing this complication.^{22,25,29} It is hypothesized that the chronic red cell therapy in TDT patients shuts down the erythropoietic drive, reflecting some of the differences in morbidity observed between NTDT and TDT patients.^{1,26} Indeed hematopoietic pseudotumors arise in 20% of NTDT patients as opposed to <1% of adequately transfused TDT patients.^{1,26}

One of the most concerning regions for hematopoietic pseudotumors is the paraspinal region as serious neurologic sequelae can result from spinal compression.²⁸ These account for 11-15% of extramedullary hematopoietic pseudotumors.^{30,31} Management strategies for spinal pseudotumors as well as other areas of extramedullary hematopoiesis include red blood cell transfusions, hydroxyurea, radiation therapy, and surgical decompression depending on severity.^{28,30-33}

Hypercoagulable state and thromboembolic events

Venous and arterial thromboembolic events are fairly common in patients with thalassemia.^{23-25,34,35} The pathogenesis of hypercoagulability in thalassemia remains to be fully elucidated. However, contributing factors are thought to include oxidative stress, red cell membrane abnormalities, endothelial injury and dysfunction, as well as platelet activation and thrombin generation.³⁶⁻⁴⁴

The prevalence of thromboembolic events has been reported to range from 4 to 14 % in NTD thalassemia, with an approximately 2 to 4 fold increased risk as compared to TD thalassemia patients.^{23,26,34,35} Identified risk factors include splenectomy, age > 35 years, serum ferritin > 1000 ng/ml, Hb level of < 90g/L.²³⁻²⁶ In fact, in one study over a 10 year period, 29% of splenectomized NTD patients had developed a venous thrombotic event.²³

In addition, while strokes are less common in NTDT patients as compared to TD patients³⁴, there is an increased risk for silent cerebral infarcts in NTDT patients. White matter lesions in otherwise asymptomatic individuals with NTDT have been reported in 16-60%, with a higher risk of these in older, transfusion naïve, and splenectomized patients.⁴⁵⁻⁴⁹ Interestingly, the presences of arterial stenosis, particularly of the internal carotid⁴⁹, as well as increased velocities by transcranial Doppler (as compared to normal controls but below the conditional threshold defined in sickle cell disease)⁵⁰⁻⁵¹ have been

identified, although the significance of these findings is unclear.

Further evidence is required to adequately provide recommendations regarding prophylactic anticoagulation or anti-platelet therapy as well as long-term management of patients with thrombotic or cerebrovascular disease. Acute management is generally in accordance with standard VTE guidelines. Expert opinion suggests considering low-dose aspirin in splenectomized NTDT patients with platelets $> 500 \times 10^9/L$.⁵²

Pulmonary hypertension

Even though NTDT patients are not generally affected by severe cardiac hemosiderosis, multiple cardiac anomalies have been described in NTDT patients. These include congestive heart failure, pericarditis, pericardial changes, valvular defects, and most importantly for this group of patients, pulmonary hypertension.⁵³

The precise pathophysiology of pulmonary hypertension in NTDT remains unclear and is likely multifactorial. Hemolysis with resulting endothelial dysfunction is likely a dominant contributing factor to pulmonary hypertension.^{54,55} Additionally, hypercoagulability and thromboembolic disease may play a role in causing secondary pulmonary hypertension; however, further research is needed to clarify their role in NTDT patients.⁵⁶ The prevalence of pulmonary hypertension in beta NTDT has varied widely across studies, ranging between 11-59%. However, these findings are based largely on echocardiogram findings: a tricuspid-valve regurgitant jet velocity (TRV) > 2.5 - 2.8 m/s corresponding to a pulmonary arterial systolic pressure > 30 - 35 mm Hg, without confirmation by cardiac catheterization.^{6, 57-59} In one study, the prevalence of pulmonary hypertension by cardiac catheterization in beta NTDT patients was 4.8%, up to 5 times more frequent than in patients with TDT.⁶⁰ Risk factors include age, prior splenectomy, hydroxyurea naïve, iron chelation naïve, transfusion naïve, prior TE event, increased WBC, higher number of transfusions, higher nRBCs, increased non transferrin bound iron (NTBI), and hypoxia.^{56,59-62} While fewer studies have been performed in alpha thalassemia patients with regard to pulmonary hypertension, the prevalence of pulmonary hypertension is reported to be 4.7 to 7% by similar echographic criteria.^{63,64}

Patients with NTDT should be screened annually for pulmonary hypertension by echocardiogram with measurement of the TRV. A TRV threshold of > 3.2 m/s has positive predictive value of 93.3%.⁵⁹ Therefore, asymptomatic patients with a TVR > 3.2 m/s or symptomatic patients with a TVR > 2.5 m/s, should undergo right heart catheterization for confirmation of pulmonary hypertension. Patients with confirmed pulmonary hypertension should be referred to a cardiologist and treated according to pulmonary hypertension guidelines. For management of pulmonary hypertension in the context of NTDT, further studies are required to study the response to various therapies including hydroxyurea⁶⁵⁻⁶⁷ and chronic transfusions.⁶⁸

Leg ulcers

Leg ulcers are a more common and problematic issue in patients with NTDT than in those with TDT.^{1,25,26} Factors thought to contribute to their development include friable tissues from chronic tissue hypoxia as well as hypercoagulability, abnormal red cell rheology, increased venous pressure, and perhaps non-transferrin bound iron injury.^{16,23,26,69-71}

Managing ulcers is generally difficult and inspection for these at each clinic visit is recommended. Based on expert opinion, referral to dermatology or plastic surgery is suggested and treatment approaches can include leg elevation, topical antibiotics and occlusive dressings, sodium nitrite cream, chronic blood transfusions, hydroxyurea, skin grafting, arginine butyrate, platelet-derived wound healing factors.^{16,52}

Iron overload

Iron overload is seen across the spectrum of NTDT subgroups⁷²; however, it occurs more slowly in patients with deletional HbH disease.¹⁵ Iron overload in NTDT is a consequence of ineffective erythropoiesis and anemia leading to inappropriately decreased hepcidin levels. Decreased hepcidin results in increased intestinal absorption of iron via ferroportin.⁷³⁻⁷⁵ As a consequence, in these patients, the rate of iron absorption is approximately 2-4 times faster as compared to the normal population.^{75,76} The distribution of iron deposition is different from TD thalassemia with preferential loading of iron in hepatocytes and slower loading in cardiac myocytes and endocrine cells in NTDT patients^{75,77-80}. This may perhaps be

related to the higher episodic dose of iron during transfusions in TDT as opposed to slow but increased iron absorption from the gut in NTDT.

Iron overload in NTDT predisposes patients to liver complications such as liver fibrosis, cirrhosis, and hepatocellular carcinoma^{75,81-83} as well as endocrinopathies.^{25,70} Other complications linked with increased NTBI include thrombotic disease, pulmonary hypertension, cerebrovascular disease such as silent cerebral infarcts, as well as osteoporosis.^{25,70,74}

In addition, unlike TDT, serum ferritin levels appear to correlate more poorly with iron burden as measured by liver biopsy liver iron content (LIC) or MRI assessment.⁸¹ In NTDT, ferritin underestimates LIC. Moreover, ferritin was not effective in predicting the development of iron-related morbidities.⁷⁰ Therefore, iron overload assessment by imaging modalities is imperative to more accurately evaluate an NTDT patient's iron burden.

Treatment for iron overload in NTDT patients consists of iron chelation therapy with a few studies demonstrating improvement in the LIC and or serum ferritin.⁸⁴⁻⁹⁴ Based on the period of onset of iron-related morbidities, monitoring and, when needed, treatment of iron overload should begin at the age of 10 years in NTDT patients.²⁵ However, there remain important gaps in existing literature, specifically with regards to the long-term safety and efficacy as well as the threshold at which to begin iron chelation therapy in these patients.

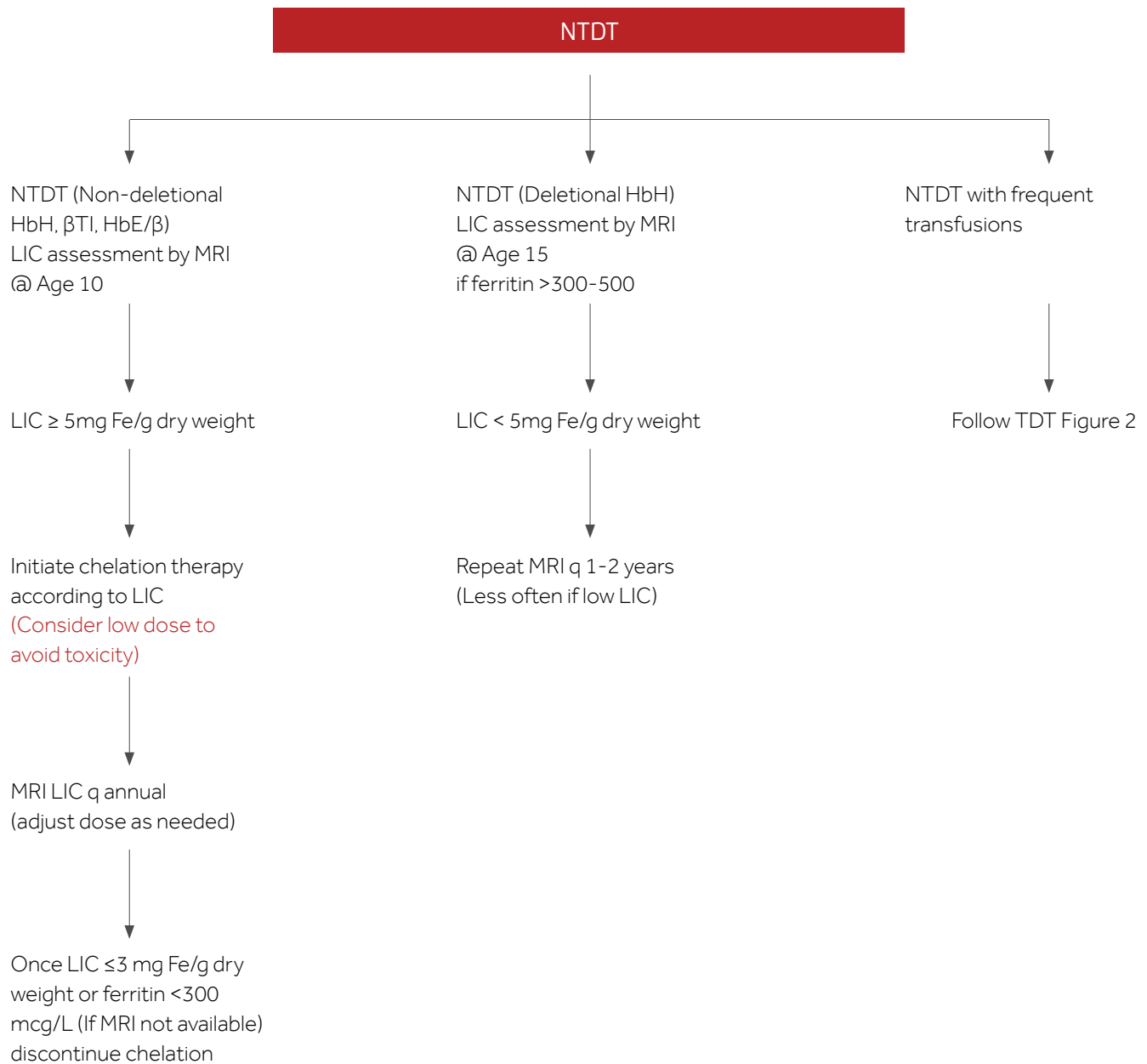


Figure 3: Iron Overload Assessment and Management in NTDT patients

Low bone density and other bone abnormalities

Bone abnormalities are a frequent complication in NTDT. Bony changes can include frontal bossing, prominence of the zygomatic bones, depression of the base of the nose, shortening of long bones, excessive thinning of cortices and dilatation of the medullary cavities.^{16,95} These changes are a consequence of ineffective erythropoiesis and resulting bone marrow hyperplasia, which can expand up to 30 times the normal volume.⁹⁶

This process also contributes in part to the development of osteopenia. Other contributing factors to bone disease in NTDT can include iron toxicity directly impacting bone metabolism and/or causing endocrinopathies, vitamin D deficiency, and chelation therapy resulting in micronutrient deficiencies.⁹⁷⁻⁹⁹ In fact, osteopenia and/or osteoporosis are nearly universal and often present by age 10 in patients.^{100,101} Pain and fractures are also common.¹⁰²

Therefore, bone mineral density screening should be initiated by age 10. There are few studies on the prevention and management of bone disease in NTDT. The OPTIMAL care study found that osteoporosis was less frequent in patients receiving hydroxyurea and chelation therapy.²⁶ Vitamin D and calcium are frequently prescribed^{15,101} although the efficacy of this supplementation has not been well studied. Therefore, supplementation should be based on general practice guidelines for vitamin D and calcium replacement. Bisphosphonates have been shown, in a small number of studies, to improve thalassemia patients' (NTDT and TDT) back pain symptoms, to increase their bone mineral density, and to be well-tolerated.^{103,104}

Endocrinopathies

Endocrine complications, while less prevalent in NTDT than TDT patients, are still a frequent complication in NTDT patients.^{1,15,97} While this difference in prevalence has not been fully studied, the differential rate, degree, and location of iron loading between the two are thought to play a role.⁹⁸ Indeed, endocrinopathies in NTDT, like TDT, have been mainly attributed to iron overload.^{26,70} Other contributing factors include splenectomy, severity of ineffective erythropoiesis, and lower HbF.^{26,29,105}

The endocrinopathies most frequently reported in NTDT patients are growth retardation, delayed puberty, hypogonadism, diabetes mellitus, dyslipidemia as well as thyroid, parathyroid, and adrenal dysfunction.^{26,101,106} Short stature is reported in 7-46% of NTDT patients^{97,107,108}, although final height difference from mid-parental height was -0.72 ± 1.23 standard deviations (SD) in one study.⁹⁷ Growth failure is likely multifactorial in etiology. Chronic anemia/hypoxia, increased metabolism, nutritional deficiencies, and iron overload resulting in growth hormone - insulin like growth factor axis dysregulation as well as hypogonadism are some of the potential factors impacting growth.¹⁰¹

Screening for endocrine disorders should include monitoring of growth parameters well as annual Tanner staging in children. Other suggested annual testing for NTDT patients ≥ 10 years includes: free T4, thyroid-stimulating hormone, calcium, phosphate, vitamin D, and fasting blood glucose.⁵²

Patients with diagnosed endocrinopathies should be managed by an endocrinologist and managed according to TDT recommendations for chelation therapy and hormone supplementation. The presence of growth failure would be an indication to initiate chronic/frequent transfusion therapy with weaning and/or discontinuation as appropriate once catch up growth has been achieved.⁵²

Infections, Hemolytic and Aplastic Crises

Infection is a significant cause of morbidity and mortality in TDT patients. Patients with NTDT are also at increased risk of infection and secondary complications from these infections. Predisposing factors include anemia, iron overload, splenectomy, and immune abnormalities due to nitric oxide depletion, presence of plasma iron, as well as macrophage dysfunction due to iron.¹⁰⁹ In addition, many patients have undergone a splenectomy, increasing their risk for infection by encapsulated organisms. Infections in asplenic patients can be reduced through the use of immunizations to *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*, prophylactic antibiotics, as well as urgent fever management with intravenous antibiotics.

Patients with NTDT are also at risk for hemolytic and aplastic crises. This is particularly true for patients with HbH disease and is generally worse for patients with non-deletional HbH due to the more rapid rate of hemolysis. Triggers for hemolytic crises include infection, pyrexia, oxidative challenge, hypersplenism, and pregnancy. The hemolytic crises can result in hemolysis as brisk as one might expect with glucose-6-phosphate dehydrogenase deficiency with risk for severe anemia as well as renal dysfunction.

Aplastic crises are a result of exposure to parvovirus B19. The virus targets red cells including erythroblasts, resulting in a halt in erythropoiesis. Most recover spontaneously but some patients require red blood transfusion(s) and/or immunoglobulin therapy¹².

Cholelithiasis

Similarly to other chronic hemolytic conditions, patients with NTDT are predisposed to forming cholelithiasis due to increased production and excretion of bilirubin. Studies report a prevalence of 15.7 to 63%¹¹⁰⁻¹¹⁴ NTDT is occasionally co-inherited with Gilbert's syndrome, which further increases the risk for development of cholelithiasis.¹¹⁴

Gallstones can be screened for by abdominal ultrasound. Symptomatic gallstones should be managed with a cholecystectomy.

Liver disease

Liver complications such as liver fibrosis, cirrhosis, and hepatocellular carcinoma have been described in patients with NTDT.⁸¹⁻⁸³ While viral hepatitis may contribute to liver disease in a subgroup of patients with NTDT who require transfusions^{82,83}, the majority of patients are at risk for liver disease due to chronic hepatocellular iron deposition. Based on extrapolations from other liver diseases such as hereditary hemochromatosis, iron is likely key in the development of liver fibrosis and cirrhosis and possibly carcinogenesis in NTDT patients.¹¹⁵⁻¹¹⁸ Iron-related liver damage is supported by the correlation seen between higher serum ferritin levels in NTDT patients and higher transient elastography values measuring liver fibrosis as well as increased transaminases.^{25,26,119} In addition, hepatocellular carcinoma has been described in some NTDT patients without viral hepatitis.^{120, 121}

Based on expert opinion, liver function tests should be monitored every 3 months in NTDT patients ≥ 10 years as well as liver ultrasounds yearly in patients with an LIC ≥ 5 mg Fe/g dry weight or serum ferritin ≥ 800 ng/ml.⁵² For measuring liver fibrosis and cirrhosis, transient elastography has been demonstrated to be comparable to a liver biopsy with the advantage of being non-invasive.¹²² Patients with evidence of hepatic disease should be referred to a hepatologist for further management.

3. Treatment

Management of NTDT is complex and must take into consideration the screening and prevention of complications, as well as the treatment of established complications, as described in previous sections. The decision to initiate treatment in NTDT patients is often a difficult one to make given the wide array of clinical presentations and should be patient-specific.^{1,2,6,10,11,14,15,19-21, 123-129.}

NTDT treatment modalities include:

- Observation
- Blood transfusion
- Fetal Hemoglobin induction
- Splenectomy
- Erythropoietin (EPO) stimulating agents

Observation

Due to the wide clinical variability of NTDTs, a number of patients are simply mildly anemic, asymptomatic or minimally symptomatic, and may be brought to clinical attention only later in adult life.^{29,105} It is imperative to screen regularly for potential complications and treat them accordingly. All patients should receive folic acid supplementation.

Blood transfusions

By definition, patients with NTDT do not need chronic red cell transfusions to sustain life, but periodic transfusions may be required in specific situations, such as surgery, pregnancy and aggravated anemia (acute hemolysis, viral-induced pure red cell aplasia, sepsis, etc.) In addition, chronic transfusions may be necessary in certain circumstances after weighing the risks and benefits. These can include but are not limited to growth failure, developmental concerns, extramedullary hematopoiesis (excessive splenomegaly, facial changes, paraspinal pseudotumors.), pulmonary hypertension, and poor quality of life. Hemoglobin level should not be the sole determining factor for implementing chronic transfusions.

More specifically, in the pediatric setting, patients with growth failure, as evidenced by inadequate growth velocity, should be started on chronic transfusion therapy⁵². Such patients are often identified early, sometimes requiring chronic transfusions as early as 2 years of age, and are closer on the spectrum of disease to TDT. They may also present with complications such as delayed puberty, developmental concerns, bone deformities, symptomatic anemia with decreased quality of life, and worsening splenomegaly.

Milder forms of NTDT can present with distinct challenges for which starting chronic transfusion should be considered: symptomatic extramedullary hematopoiesis, symptomatic anemia with decreased quality of life, pulmonary hypertension, bone deformities, and chronic leg ulceration.^{68,130-133} The decision to start chronic transfusion therapy needs to be balanced with the risks of 1) chronic iron overload and need for iron chelation therapy 2) red cell alloimmunization. In spite of these risks, the prevention of NTDT complications linked to chronic anemia and hemolysis is desirable, and chronic transfusion therapy may be considered in certain patients even before these occur.¹³⁰

Fetal Hemoglobin Induction

Hydroxyurea, known to induce hemoglobin F, is a common medication used in sickle cell disease as well as in certain myeloproliferative diseases. Its use has been reported in patients with beta thalassemia intermedia as well as Hemoglobin E/beta thalassemia. It is theorized that, by increasing γ globin production, there is a decrease in the α/β globin imbalance resulting in decreased ineffective erythropoiesis.¹³⁴ While there have not been any large randomized controlled trials comparing hydroxyurea use versus placebo or standard of care, hydroxyurea therapy has been associated with decreased blood transfusion requirements, total and fetal hemoglobin rise, decreased splenomegaly, decreased ferritin, as well improvement in some NTDT complications such as pulmonary hypertension and extramedullary hematopoietic pseudotumors.^{26,32,65-67,114,134-153} There appears to be an association between hematological response to hydroxyurea and certain genotypes and polymorphisms (MAP3K5, KLF10)¹³⁸⁻¹⁴⁰; however, there is however not enough evidence thus far to screen patients for certain polymorphisms prior to hydroxyurea use.

Hemoglobin F induction with hydroxyurea may be an attractive option when chronic transfusions are not desired and treatment for NTDT is warranted. In all trials, hematologic responses were seen at dosages from 8 to 20mg/kg/day.¹³⁴⁻¹⁵³ Overall, hydroxyurea is well tolerated, particularly at the lower dose of 10mg/kg/day. In comparison, there was a higher risk of developing neutropenia in patients receiving 20mg/kg/day.¹³⁷ No additional side effects to hydroxyurea have been described in this particular population. Standard hydroxyurea monitoring and follow up is recommended in any patient receiving this medication.

Given the correlation between disease severity and HbF level as well as response to hydroxyurea for some patients, other HbF inducers are being examined and developed. These include butyrate derivatives, decitabine, as well as genetic therapeutic blockade with knockdown of γ globin gene repressors.^{5,154-156} At this time, there are insufficient data to recommend the use of other hemoglobin F inducers in the NTDT population.

Splenectomy

Due to chronic hemolysis and ineffective erythropoiesis resulting in extramedullary hematopoiesis and chronic splenic congestion, patients with NTDT generally have splenomegaly, which at times may be massive. Historically, splenectomies were routinely performed in NTDT patients to increase their hemoglobin level and decrease symptoms attributed to chronic severe anemia; however, it should no longer be routinely performed, as it is associated with a number of potential adverse outcomes.⁵²

In all patients, there is an increased risk for sepsis and thrombosis post splenectomy.¹⁵⁷⁻¹⁵⁹ The risk of sepsis can be in part mitigated by pre-splenectomy immunization to *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* as well as by following asplenia guidelines with regard to antibiotic prophylaxis and fever management. However, with regards to thrombosis risk, patients with NTDT are already hypercoagulable and have evidence of endothelial dysfunction. Not surprisingly, patients with NTDT who have undergone a splenectomy are at further increased risk for thrombosis, as compared to the general population and even other NTDT patients.^{23,24,26,59,64} Careful prophylaxis must be employed in the peri-operative setting. It is unknown whether prolonged prophylaxis, and if so which therapeutic agent reverses those risks. Therefore, further evidence is required to adequately provide recommendations regarding prophylactic anticoagulation or anti-platelet therapy. Expert opinion suggests considering low-dose aspirin in splenectomized NTDT patients with platelets $> 500 \times 10^9/L$.⁵²

In addition, an increase in other NTDT complications have been reported post-splenectomy, including pulmonary hypertension as well as iron overload, ulcers, nephrolithiasis, and some endocrinopathies.^{26,72,159-161}

Therefore, splenectomies should be reserved for patients with significant complications such as 1) worsening anemia with poor growth/development and chronic transfusions or iron chelation therapy are not possible/available 2) hypersplenism with worsening cytopenias resulting in clinical problems: bleeding, infection, severe anemia with lack of post-transfusion hemoglobin increment 3) massive splenomegaly with risk of splenic rupture, significant left upper quadrant pain, or early satiety.^{52,162} Thus the decision to perform a splenectomy in NTDT patients must be carefully balanced between indications such as these and the potential risks described above.

Erythropoietin Stimulating Agents

The level of endogenous erythropoietin (EPO), has been reported to be low relative to the degree of anemia and hemolysis in NTDT.¹⁶³ In addition, EPO levels also tend to decrease with age.¹⁶⁴ Based on this premise, erythropoietic stimulating agents (ESAs) have been assessed in few studies. While some improvement in hemoglobin level was observed, there was concern for potentially worsening marrow expansion and which could result in symptomatic extramedullary disease.¹⁶⁵ Hence, hydroxyurea in combination with ESA may be a desirable option; however, such regimens cannot be routinely recommended at this time due to lack of published data confirming long-term benefit.¹⁶⁶

Novel interventions

As the pathophysiology of NTDT is better understood, possible targets for interventions are being examined. These novel therapies aim to improve various aspects of NTDT including anemia, ineffective erythropoiesis, red cell deformities, HbF levels, and iron loading. Examples of these include activin receptor ligand traps,^{167,168} HbF inducers,¹⁵⁴⁻¹⁵⁶ altered iron metabolism with decreased TMPRSS6,^{169,170} hepcidin,¹⁷¹ transferrin,¹⁷² as well as gene editing.^{5,173} These interventions are at various stages of development ranging from animal to clinical trials and demonstrate the exciting advances in the field; however, at this time, there are insufficient data to recommend these therapies outside of clinical trials.

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