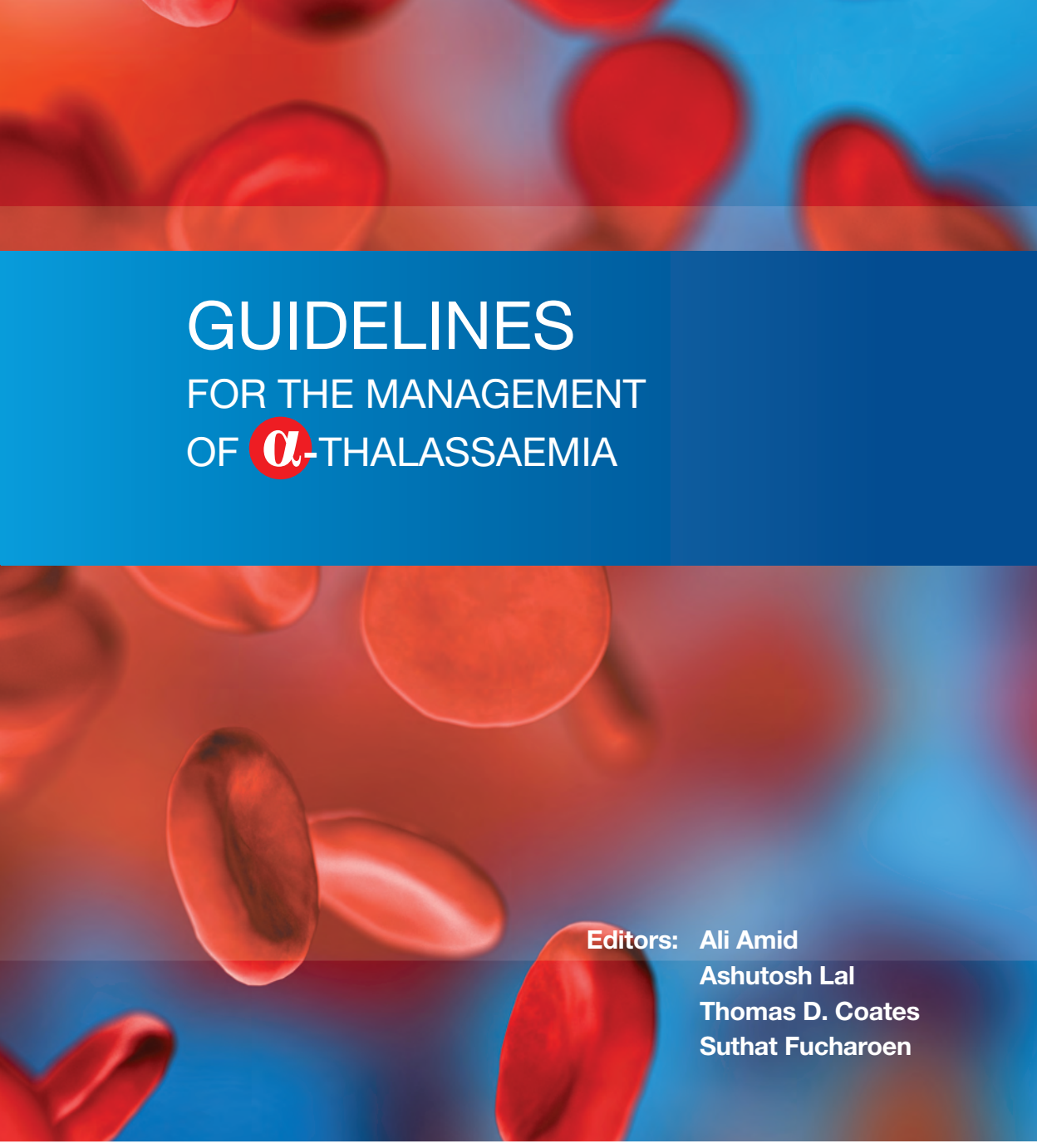

GUIDELINES
FOR THE MANAGEMENT OF α-THALASSAEMIA

Editors: Ali Amid
Ashutosh Lal
Thomas D. Coates
Suthat Fucharoen

THALASSAEMIA INTERNATIONAL FEDERATION

PUBLISHERS: THALASSAEMIA INTERNATIONAL FEDERATION

“Cure sometimes, treat often, comfort always.”

Hippocrates (460-357 B.C.)

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CONTENTS

FOREWORD

It is our great honour and privilege to introduce the **first-ever** *Guidelines for the Management of α-thalassaemia,* a comprehensive and indispensable resource for healthcare professionals, researchers, and anyone involved or interested in the care of individuals with α-thalassaemia.

α-Thalassaemia is a complex genetic and hereditary haemoglobinopathy characterized by a wide range of clinical presentations and as such poses significant challenges in its timely and accurate diagnosis, treatment, and patient management. Quite often, individuals with α-thalassaemia, based on their early years' milder clinical manifestations and absence or very confined awareness regarding their haemoglobin disorder, remain undiagnosed and very randomly monitored, if at all, and reach out to clinical centres and haematologists at rather advanced stages of medical complications.

This is an invaluable contribution to the efforts of healthcare professionals to upgrade patient care of individuals with the so-called Non-Transfusion Dependent α-Thalassaemia. Despite the huge advances in the molecular and genetic understanding of this syndrome leading to improvements in early diagnosis, the value of systematic follow-up and prompt management of developed complications has been severely underestimated for decades to-date. In the absence of registries, particularly those that include the incidence of α-thalassaemia and its contribution to the national and international disease burden, quality of life and social integration of patients is still largely unknown.

These Guidelines therefore come at a turning-point in the history of thalassaemia, where there is improved understanding and more accumulated data, and thus they represent a critical step forward in the better management of this disorder focusing on both preventing and addressing complications which contribute significantly to the quality of life and social integration of individuals with this disorder.

It is estimated that 5% of the worlds' population is a carrier of a defective α-thalassaemia gene,[1] and approximately **1 million patients are affected by the various α-thalassaemia syndromes globally,** albeit in the absence of registries and methodologically sound epidemiological studies these numbers are considered to be gross underestimations. Although found in varying rates across the entire thalassaemia belt, α-thalassaemia is predominantly found in populations of South-East Asian origin, where α-thalassaemia (HbH disease) is more clinically severe in comparison to other countries. Furthermore, due to population movements, an increasing number of carriers and patients with α-thalassaemia are now born in countries of Northern Europe and America. Indeed, the few newborns that survive birth with the severe form of α-thalassaemia major (also known as haemoglobin Bart's Hydrops Foetalis) require lifelong blood transfusions. Moreover, those born with non-transfusion dependent (NTD) forms of α-thalassaemia (known as α-thalassaemia intermedia or HbH Disease) demonstrate a considerable variability of clinical severity as does their requirement for blood transfusions – noting of course that the more severe forms of HbH Disease may eventually deem the patient entirely transfusion-dependent. [2]

These Guidelines have been meticulously developed by a diverse panel of medical experts in the field, bringing together their collective knowledge and experience to produce an invaluable educational resource that provides evidence-based recommendations for the diagnosis, treatment, and ongoing care of individuals affected by α-thalassaemia.

Offering a comprehensive overview of α-thalassaemia, covering its genetic basis, epidemiology, and clinical manifestations, these Guidelines provide a thorough understanding of the condition's complexities, aiding healthcare professionals in accurate diagnosis and risk assessment. Moreover, the Guidelines delve into the intricacies of clinical management and supportive care, further highlighting the significance of a multidisciplinary care team and the importance of patient education. In the rapidly evolving field of medical science, these guidelines also touch upon emerging and novel therapies that are on the horizon for improving patient care.

This publication represents a milestone in TIF's quest to improve the lives of individuals affected by all thalassaemia syndromes.

On behalf of the TIF Board of Directors, I extend our heartfelt gratitude to all those who have contributed to the development of these Guidelines, commending their dedication to advancing the understanding and management of α-thalassaemia, a largely "neglected" haemoglobinopathy and whose work will undoubtedly make a lasting impact on the lives of patients and their families.

It is our hope that these Guidelines serve as a valuable resource for healthcare professionals, researchers, and policymakers worldwide, fostering excellence in the care and management of α-thalassaemia. Together, we can work towards a future where individuals with α-thalassaemia, particularly those in low- and middle-income countries, can enjoy a longer and improved quality of life.

On behalf of the Thalassaemia International Federation

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[1]- Piel, FB, Weatherall, DJ. The α-Thalassaemias. N Engl J Med 2014;371:1908-16. DOI: 10.1056/NEJMra1404415

[2]- Taher A et al. Guidelines for the management of non-transfusion dependent thalassaemia. 2nd Edition TIF publication 22 2017

1 EPIDEMIOLOGY, PATHOPHYSIOLOGY AND DIAGNOSIS OF α-THALASSAEMIA

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Introduction

Haemoglobin (Hb) is a vital respiratory carrier protein that facilitates the transport of oxygen from the lungs to the body's tissues and the return of carbon dioxide from the tissues to the lungs. Each haemoglobin molecule is structured in a tetrameric form, composed of two α-like and two β-like globin chains. Each globin chain contains a heme group with a ferrous iron atom, which binds with oxygen [1].

The synthesis of globin proteins is controlled by the α-gene cluster on chromosome 16 and the β-gene cluster on chromosome 11 (see **Figure 1**). The genes within these two clusters are coordinately expressed to ensure an equal amount of α-like and β-like globins are produced. The various globin genes are situated on α- and β-gene loci in the order they are developmentally expressed, leading to the production of different haemoglobins during the stages of human development. In adults, haemoglobin primarily consists of adult haemoglobin (HbA, $\alpha_2\beta_2$), with a smaller proportion of foetal haemoglobin (HbF, $\alpha_2\gamma_2$), and a minor component, HbA_2 ($\alpha_2\delta_2$). The expression of globin genes is regulated by enhancer elements located in the upstream regions of the α-gene and β-gene clusters, known as HS-40 and LCR, respectively.

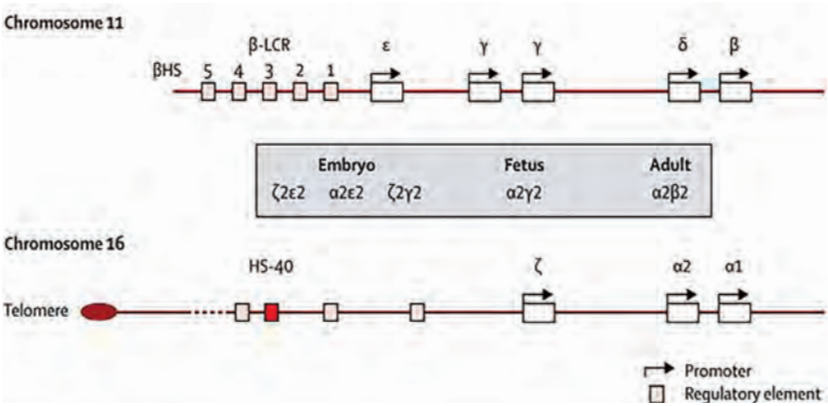
Epidemiology and global burden of α-thalassaemias

α-Thalassaemia is characterized by a quantitative reduction or absence of α-globin chain production, or rarely, by the production of abnormal (variant) α-globin chains. α-thalassaemia is one of the most common inherited blood disorders, with approximately 5% of the world's population being carriers, and around 1,000,000 patients affected by various forms of α-thalassaemia syndromes worldwide [2-4]. The α-thalassaemia is most prevalent in Southeast Asia, Southern China, the Middle East, the Mediterranean Region, and Africa **(Figure 3)**. In certain areas, the carrier frequency can be as high as 80-90% of the population [1]. α-thalassaemia carrier state offers varying degrees of resistance to severe malaria and is believed to have evolved as a genetic adaptation to protect against this parasitic disease [5]. Thalassaemias, including α-thalassaemia, have spread globally due to population migrations, making them a significant global health concern. In regions where the carrier state is common, two clinically significant diseases can arise: HbH disease and Hb Bart's hydrops foetalis.

Genetic basis of α-thalassaemias

The α-globin gene cluster lies in a 135-155 kb GC-rich, Alu repeat dense and gene-dense genomic DNA region approximately 150 kb from the telomere of chromosome 16 (16p13.3). It contains three functional globin genes, i.e. the embryonic ζ-gene (*HBZ*) and duplicated foetal/adult α-genes (*HBA1* and *HBA2*), three pseudogenes, i.e. the pseudo ζ (*HBZps*), the pseudo α1 (*HBA1ps*), and pseudo α2 (*HBM*) and the θ (*HBQ*)-gene of unknown function (see **Figure 1**). Mutations in the *HBA2* gene are associated with more prominent effect on α-globin production than mutations in the *HBA1* gene [6].

Figure 1. *Schematic representation of the chromosomal location of the α- and β-globin clusters on 16p and 11p respectively.*

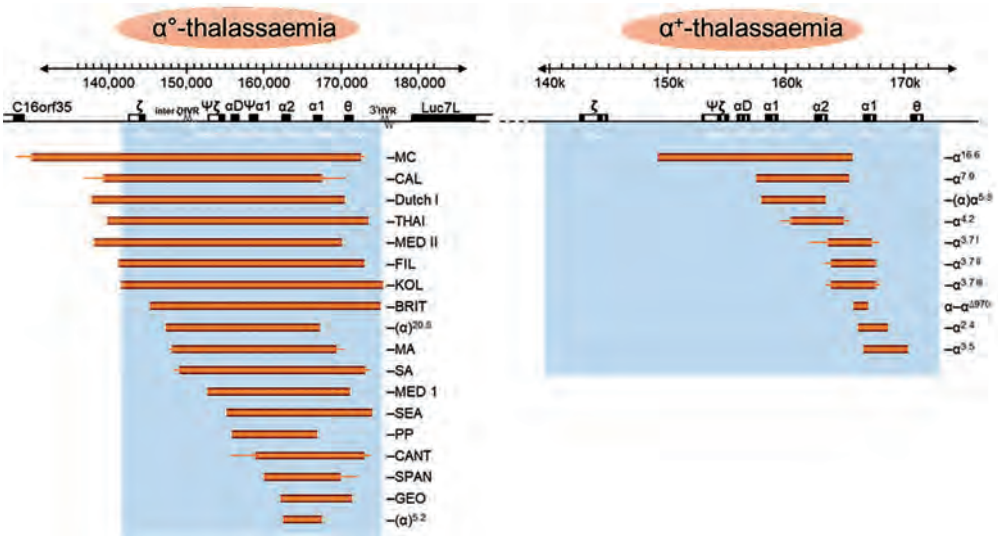


As previously mentioned, normal haemoglobin consists of two α-globin chains and two β-globin chains. In normal individuals, there are four functional α-globin genes, with two inherited from each parent (αα/αα), and two β-globin genes, with one inherited from each parent (β/β).

α-Thalassaemia primarily arises from large fragment deletions (known as copy number variations or CNV) or point mutations (referred to as single nucleotide variations or SNV) in the regions that encode the α-globin chains. These genetic changes lead to a varying degree of reduced or absent production of α-globin chains. Deletions that result in the loss of duplicated α-genes lead to the absence of α-globin production from that chromosome, and they are called α^0-thalassaemia deletions (--), and those that result in the loss of single a gene and decreased production of α-globin from that chromosome are called α^+-thalassaemia deletions (-α). Less frequently, α-thalassaemia can occur due to point mutations in either *HBA1* or *HBA2,* resulting in the production of abnormal or unstable variant α-globin chains ($\alpha^T\alpha$ or $\alpha\alpha^T$).

The spectrum of α-thalassaemia mutations has been extensively documented over the past few decades, with more than 370 mutations currently catalogued in the public IthaGenes database [7]. Among these mutations, over 130 are deletions, while more than 220 are non-deletional mutations, with the remaining mutations falling into other categories. Detailed information about these variations is regularly recorded and updated on the IthaNet portal (http://www.ithanet.eu). **Figure 2** provides a summary of the most common α^+-thalassaemia and α^0-thalassaemia deletions.

Figure 2. *Molecular basis of α-thalassaemia. The most common α-thalassaemia deletions are shown in orange bars indicating the length and the annotation of the deletion and subdivided into α^{+}- and α^{0}- thalassaemia deletions (adapted from Harteveld, CL and Higgs DR, Orphanet Journal of Rare Diseases, 2010 [1], with permission).*

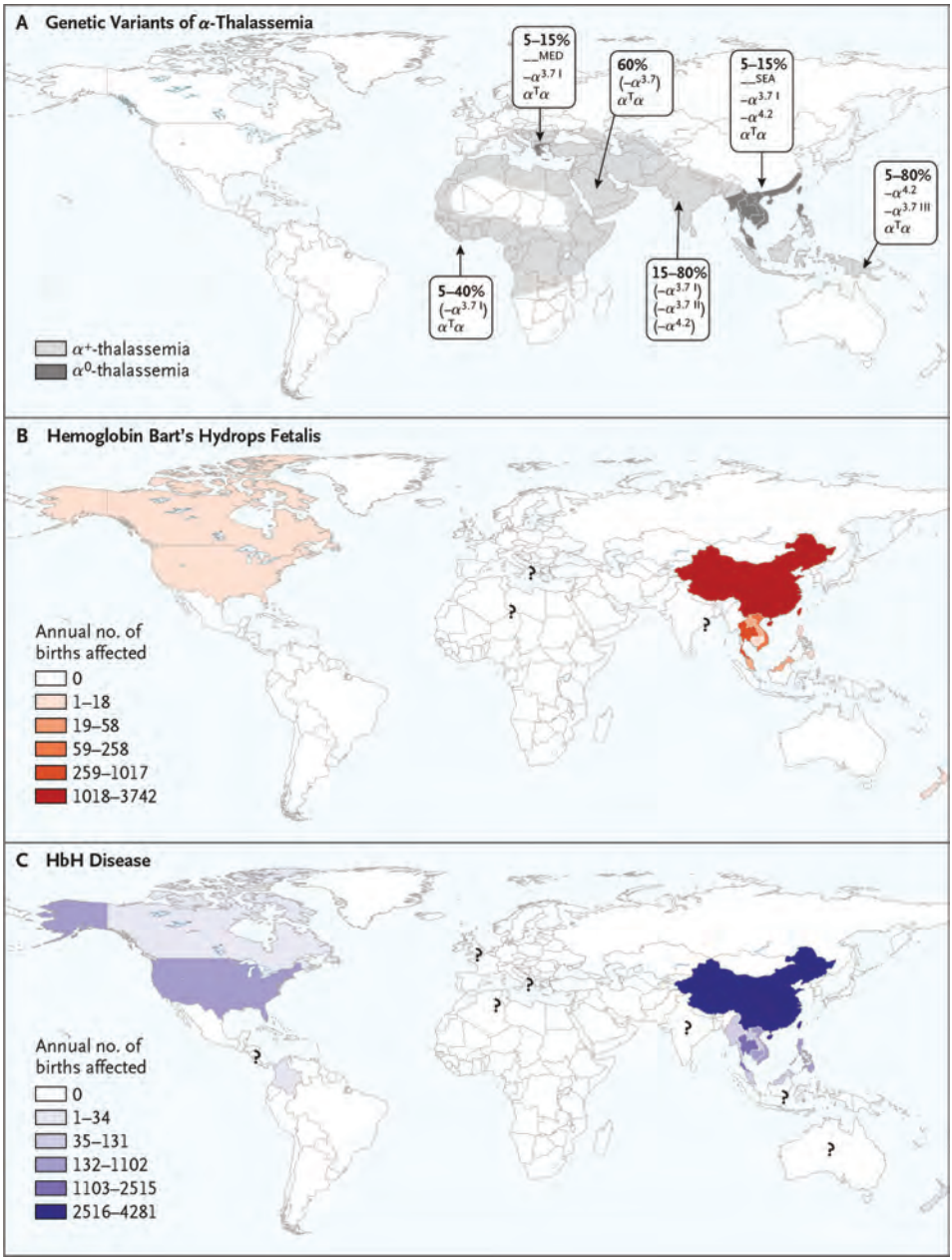


The most prevalent deletional α^{+}-thalassaemias result from unequal homologous recombination of duplicated α-genes during meiosis, leading to the $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions. Conversely, α-gene triplications (ααα anti 3.7 and ααα anti 4.2) represent the complementary events [1, 8]. Among these, $-\alpha^{3.7}$ is the most common α^{+}-thalassaemia deletion and its compound heterozygosity with α^{0}-thalassaemia deletions is the most common genotype observed in HbH disease.

Larger deletions that eliminate both duplicated α-genes in *cis* constitute α^{0}-thalassaemias and occur less frequently but may be prevalent in specific populations. If the deletion leaves the *HBZ* gene intact (e.g. --SEA deletion), homozygotes develop Hb Bart's hydrops foetalis syndrome, characterized by development of non-immune hydrops in the second or third trimester (as discussed below). However, when *HBZ* is also deleted (e.g. --FIL deletion), homozygotes are unlikely to survive even in the earliest developmental stages. The most common α^{0}-deletional type encountered is the --SEA deletion, which is prevalent in Southeast Asia, while the --MED deletion is the most common α^{0}-thalassaemia mutation in the Middle East and Mediterranean region.

Point mutations, small deletions, or insertions that affect sequences controlling gene expression fall under the category of non-deletional α^{+}-thalassaemias ($\alpha^{T}\alpha$ or $\alpha\alpha^{T}$) and are less common than their deletional counterparts. However, some of these non-deletion types can lead to a more pronounced reduction in α-globin synthesis compared to single α-gene deletions. These mutations have been documented to affect gene expression, mRNA splicing, and globin chain stability. Important examples of these mutations are Hb Constant Spring and the IVS I-1 (-5 bp) (HBA2:c.95+2_95+6delTG) [7].

Figure 3. *Map showing the worldwide geophraphic distribution of a-thalassaemia carriers, HbH disease and haemoglobin Bart's hydrops foetalis (reproduced with permission from Piel FB and Weatherall DJ. N Engl J Med. 2014 [13]).*



Classification of α-thalassaemia

α-Thalassaemia is highly heterogeneous at both clinical and molecular levels. The clinical course of α-thalassaemia is generally correlated with the number of affected α-globin genes. There are four primary clinical types of α-thalassaemia syndromes (see **Figure 4**). :

1. Silent carrier: This type is defined as heterozygous α^+-thalassaemia (-α/αα) resulting from the deletion or dysfunction of one of the four normal α-globin genes. Individuals with this condition are generally healthy and exhibit a normal hematological profile.
2. α-Thalassaemia trait: This includes two subtypes:

   i) Heterozygous α^0-thalassaemia (--/αα), resulting from the deletion of two α-genes in cis.
   ii) Homozygous α^+-thalassaemia (-α/-α), resulting from deletion of two α-genes in trans.
   Individuals with α-thalassaemia trait are healthy and asymptomatic, although they may experience mild anaemia or microcytosis. Identification of individuals with α^0 trait (--/αα genotype) is a critical step in a screening and prevention programme as inheritance of these deletions is necessary for more severe forms of α-thalassaemia disease.
3. HbH disease: This category encompasses compound heterozygous α^0 and α^+-thalassaemia mutations (--/-α or --/α^Tα). Additionally, individuals homozygous for non-deletional α^+-thalassaemia mutations (α^Tα/α^Tα) can also be classified within this group. The severity of haemolytic anaemia and ineffective erythropoiesis varies depending on the specific mutations involved.
4. Hb Bart's hydrops foetalis: This type is defined by homozygous α^0-thalassaemia (--/--) with the complete absence of functioning α-genes.

Haemoglobin H disease

Haemoglobin H (HbH) disease is a clinical condition that arises when only one residual functioning α-globin gene is present, resulting in genotypes of (--/-α) or (--/α^Tα). Consequently, there is a relative excess of β-globin chains, which combine to form β_4 tetramers known as HbH. These HbH molecules typically constitute 3-30% of the total haemoglobin in patients with HbH disease.

The phenotypic severity of HbH disease does not merely correlate with the degree of α-chain deficiency. HbH has very high oxygen affinity, making it unable to efficiently deliver oxygen to the body's tissues. Additionally, HbH is relatively unstable, which leads to its precipitation within red blood cells. These precipitated cells are then prematurely destroyed in the spleen, resulting in moderate extravascular hemolysis and anaemia. When compared to β-thalassaemias, the underlying pathophysiology of most forms of HbH disease primarily involves peripheral hemolysis, with a lesser degree of ineffective erythropoiesis.

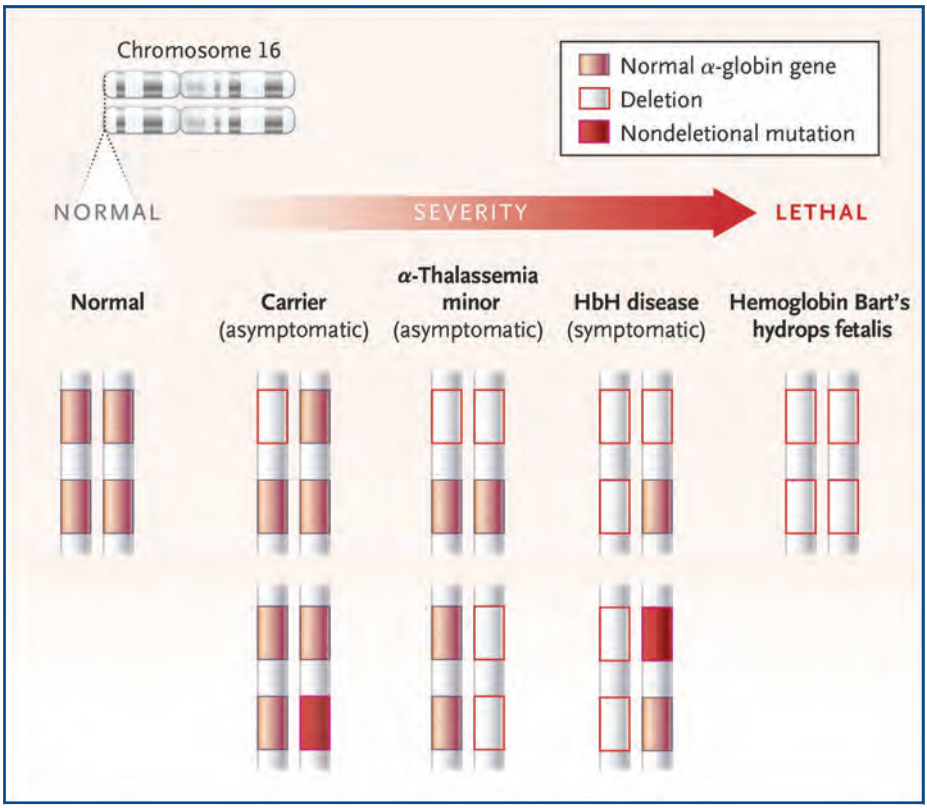
HbH disease exhibits significant variability in clinical and hematological severity. Typically, patients with deletional HbH disease, resulting from deletional mutations, maintain good health and often do not require treatment. However, in rare instances, red blood cell transfusions may become necessary if there is a sudden drop in haemoglobin levels, especially during pregnancy or due to acute haemolytic or aplastic events triggered primarily by viral infections.

Conversely, patients with non-deletional HbH disease experience a more severe clinical phenotype. This severity arises from the production of abnormal or unstable α-globin variants, which, when combined with the presence of unstable HbH due to globin chain imbalance, contributes to additional red cell pathobiology. This combined abnormality can lead to increased peripheral hemolysis and ineffective erythropoiesis, resulting in a more pronounced clinical phenotype.

Clinically, a wide variety of phenotypes is observed as a result of homozygosity for non-deletional α^+-thalassaemia mutations. For instance, homozygous poly(A) mutations are a common cause of HbH disease, particularly among populations in the Middle East and Central Asia. In contrast, homozygosity for Hb Constant Spring or Hb Koya Dora mutations tends to result in milder thalassaemia syndromes.

Rarely, the combination of specific non-deletional α^+-thalassaemia mutations and α^0-thalassaemia deletions can give rise to an exceptionally severe phenotype, akin to that of β-thalassaemia major. In its most severe forms, this condition can lead to hydrops foetalis (HbH hydrops foetalis).

Figure 4. *Classification of α-thalassaemia defects (reproduced with permission from Piel FB and Weatherall DJ. N Engl J Med. 2014 [13])*



Haemoglobin Bart's hydrops foetalis syndrome

Hb Bart's hydrops foetalis syndrome represents the most severe form of α-thalassaemia and is characterized by the absence of all four α-globin genes (--/--), as depicted in **Figure 4**. In the absence of α-globin production, γ-globin chains combine to form Hb Bart's (γ_4) during the foetal period, which switches to HbH (β_4) after birth, both of with are non-functional haemoglobins [1]. Foetuses with Hb Bart's hydrops foetalis (homozygous α°-thalassaemia) typically exhibit 80–90% Hb Bart's [9].

These affected foetuses experience hypoxia, heart failure, and hydrops foetalis, often succumbing in utero during the second or third trimester of gestation, or they may pass away within hours after birth. This condition is notably the most common cause of hydrops in Southeast Asia. In recent decades, due to population migrations, there has been an increase in the prevalence of this syndrome in other parts of the world [10]. With improvement of antenatal and prenatal care and availability of intrauterine transfusion, an increasing number of long-term survivors with this condition are being reported.

Hb Bart's hydrops foetalis syndrome is inherited in a recessive manner, requiring the inheritance of two α^0-thalassaemia alleles. Given the severity of the syndrome to both the fetus and the mother, prenatal diagnosis is strongly recommended for carrier couples of α^0-thalassaemia.

Unusual forms of α-thalassaemia

ATR-16 is a rare genetic condition that arises from large chromosomal abnormalities at the telomere end of chromosome 16, which encompasses the α-globin genes. Affected individuals demonstrate an unusual association of α-thalassaemia, cognitive impairment and dysmorphic features [11].

ATR-X syndrome is another rare genetic condition that is associated with a distinct and recognizable dysmorphic appearance in boys that is also associated with α-thalassaemia with severe mental cognitive impairment. It is inherited due to deletions or mutations of *ATRX* gene located on chromosome X (X-linked). *ATRX* plays an important role in the incorporation of the histone variant H3.3 into telomere and pericentromeric DNA. When the gene is mutated, among many other effects, this leads to down-regulation of expression of the α-globin genes on the telomere end of chromosome 16 [11].

α-Thalassaemia myelodysplastic syndrome (ATMDS) is an acquired form of α-thalassaemia that results from somatic mutations in ATRX gene or very rarely from acquired loss of telomere end of chromosome 16 during myelodysplastic syndrome or haematological malignancies [11].

Laboratory and genetic diagnosis of α-thalassaemias

Diagnosis of α-thalassaemias requires a combination of laboratory tests, including the measurement of red blood cell indices using automatic haematology analyzers, haemoglobin analysis, and quantification of HbA_2 and HbF. Two widely adopted automatic methods for this purpose are High-Performance Liquid Chromatography (HPLC) and Capillary Zone Electrophoresis (CE). These systems provide both qualitative and quantitative analyses of haemoglobin components with high precision and reproducibility, enabling both prenatal and postnatal thalassaemia diagnoses within minutes.

Haematology and haemoglobin analysis

Initial laboratory testing to identify α-thalassaemia carriers involves a complete blood count (CBC), with determination of mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). Carriers with genotypes -α/-α and --/αα typically exhibit reduced MCV and MCH values. In contrast, -α/αα carriers may have normal red cell indices or only slightly reduced MCV and MCH levels.

Patients with HbH disease display significant variations in haemoglobin, MCV, and MCH values, which can vary among individuals (see **Table 1**).

Table 1. *Clinical and haematologic manifestation of deletional and non-deletional forms of haemoglobin H (reproduced with permission from Guidelines for the Management of Non Transfusion Dependent Thalassaemia (NTDT), 2nd edition)*

Clinical manifestation	Deletional HbH disease	Non-deletional HbH disease
Haemoglobin (g/L)	85 (range 69 - 107)	72 (range 38 – 87)
Mean corpuscular volume (MCV) (fl)	54.0 (range 46.0 – 76.0)	65.2 (range 48.7 – 80.7)
Mean corpuscular haemoglobin (MCH) (pg)	16.6 (range 14.3 – 24.7)	18.6 (range 14.8 – 24.8)
Reticulocytosis	+	++

Automatic haemoglobin analyzers

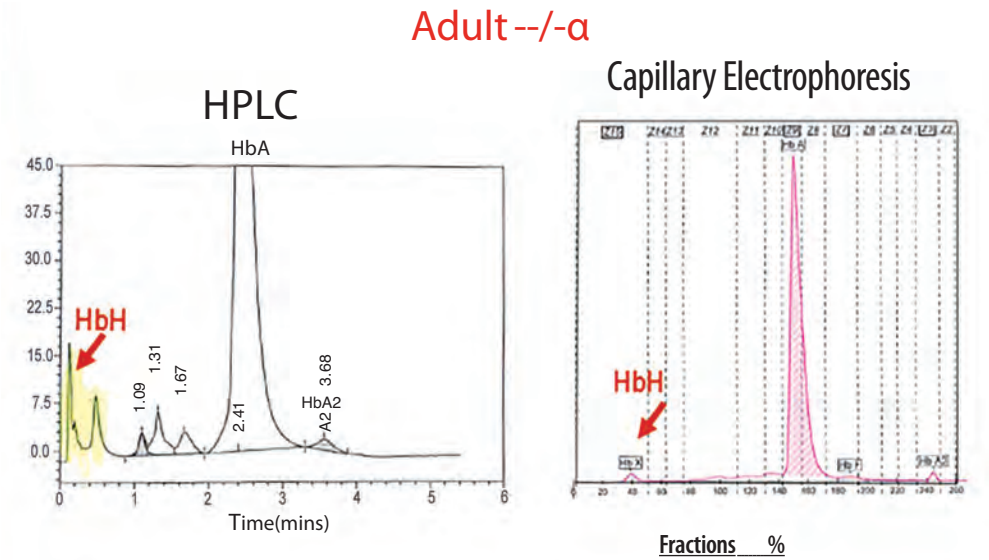
High-performance liquid chromatography: The HPLC system is cation exchange and use two dual piston pumps to set gradient sodium phosphate buffers of increasing ionic strength to pass through a column spherical cation exchange resin during a 6.5 minutes. Hemolysate samples are determined by spectrophotometer that read double wavelengths at 415 and 690 nm. The resulting chromatograms are separated in retention time (RT).

Capillary electrophoresis: The CE system is based on capillary electrophoresis in free solution from cathode to anode. Haemoglobin components are separated in silica capillaries by their electroosmotic flow and at a high voltage (9,800 V) in electrophoretic mobility in an alkaline buffer. The photometry at an absorbance wavelength 415 nm is used to directly detect Hb fractions.

Automatic haemoglobin analyzers are unable to reliably identify individuals who are heterozygous carriers for α-thalassaemia deletions, although they can identify great majority of β-haemoglobinopathies or carriers for some non-deletional α^+-thalassaemia mutations.

Patients with HbH disease have reduced (<2%) HbA_2, but the characteristic finding is the presence of variable amounts (up to 30%) of HbH. HbH is easily detected as an early-eluting peak in HPLC (**Figure 5**). In neonates, those with HbH disease can be detected through haemoglobin electrophoresis due to elevated levels (approximately 25% or greater) of Hb Bart's, making this feature useful for neonatal screening.

Figure 5. *High-performance liquid chromatography (HPLC) and capillary haemoglobin electrophoresis patterns of an adult with HbH (adapted from Harteveld, CL and Higgs DR, Orphanet Journal of Rare Diseases, 2010 [1], with permission)*



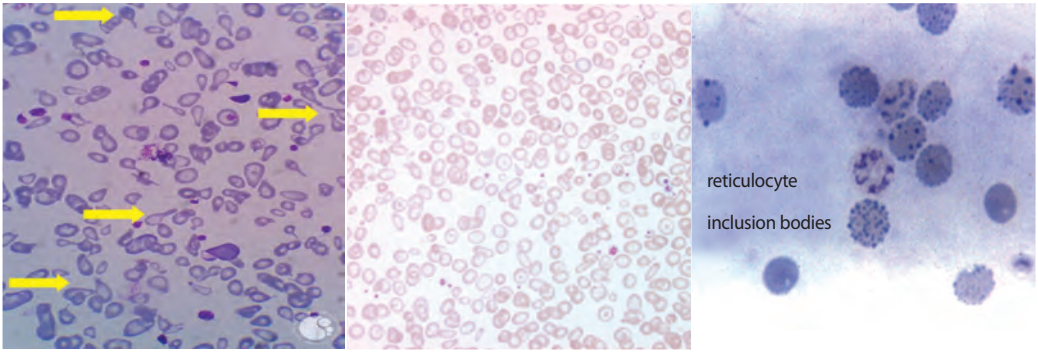
Peak Name	Calibrated Area	Area %	Retention Time (min)	Peak Area
F	1.5	---	1.09	22685
P2	---	4.0	1.31	64164
P3	---	2.5	1.67	140698
Ao	---	90.4	2.41	1459868
A2	1.7*	---	3.56	26662

Red blood cell morphology

Peripheral blood smears of patients with HbH disease typically exhibit variable degrees of microcytic hypochromic anaemia, a distinctive feature of this condition. **Figure 6** illustrates several haematological anomalies commonly observed in individuals with HbH disease, including severe hypochromia, anisocytosis (variation in cell size), poikilocytosis (abnormal cell shapes), microcytosis (small cell size), fragmentation, anisochromia (variation in cell colour), polychromasia (presence of polychromatic cells), target cells, and teardrop cells.

Red cell inclusion bodies (precipitated β_4 tetramers or H bodies) can often be detected under a microscope in a significant portion of red blood cells. This detection is facilitated by using supravital staining dyes such as methylene blue or brilliant cresyl blue. These inclusion bodies are a characteristic feature of HbH disease and play a key role in its diagnosis.

Figure 6. *Red blood morphology in patients with HbH disease (Panel A and B). Red cell inclusion body is induced after the incubation of red cell with brilliant cresyl blue (Panel C).*



Molecular analysis

Molecular genetic testing plays a pivotal role in definitively diagnosing α-thalassaemia and is a critical component in identifying couples at risk of having severe forms of the condition. Over the past decades, molecular genetic analysis has evolved significantly, enabling precise diagnoses of α-thalassaemia and accurate determination of the genetic defects responsible for these disorders.

Since these genetic tests can often be costly, it is crucial to accurately characterize hematologic features, such as haemoglobin levels, MCV, and MCH to guide the selection of samples for genetic analysis. While low MCV and MCH are characteristic of thalassemic red blood cells, these indices alone cannot distinguish between thalassaemia trait and iron deficiency and the hematological parameters in these two conditions may closely resemble each other, leading to confusion between α-thalassaemia trait and iron-deficiency anaemia. In such cases, assessing iron status (e.g., serum iron, transferrin saturation, or red blood cell zinc protoporphyrin levels) can be helpful in making an accurate diagnosis although it may not be sufficient in those where the two conditions co-exist.

Hence, it is crucial and strongly advised to assess haematological parameters and, in certain cases, family history when determining the most appropriate molecular analysis. This approach is vital to avoid misinterpretation of results [12]. Occasionally, the clinical manifestations of α-thalassaemia are influenced by the specific type of variant present in an individual and its location within the gene. For instance, in α-thalassaemia, non-deletional variants of α-globin genes tend to be associated with more severe clinical phenotypes when compared to large deletions. Therefore, it is essential to strategically select the appropriate molecular analysis based on the type of variant linked to the clinical phenotype, and sometimes, this choice may also depend on specific population characteristics and knowledge of common regional mutations.

Molecular testing for α-thalassaemia typically entails a battery of methods aimed at detecting and characterizing both known and unknown mutations, with the majority of these methods relying on polymerase chain reaction (PCR). Amplifying the α-globin gene cluster poses particular challenges due to the sequence homology shared among genes within this cluster, such as *HBA1* and *HBA2*. Therefore, careful attention must be given to assay design to ensure specific amplification and differentiation of these homologous genes.

These testing strategies can be categorized into two groups based on the type of variants being analyzed. The first group involves copy number variations (CNVs), encompassing large deletions and duplications, while the second group focuses on single nucleotide variations (SNVs), which include non-deletional variants.

The gap-PCR (gap polymerase chain reaction) method serves as a simple, rapid and inexpensive technique for identifying common deletions within specific populations. This method employs primers designed to flank known deletion breakpoints, allowing for the targeted detection of deletions. Common single α-globin gene deletions include --MED, $-\alpha^{20.5}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{THAI}$, $--^{SEA}$, $--^{FIL}$, as well as the triplication, anti-$\alpha^{3.7}$ (ααα).

In cases where gap-PCR analysis yields a negative result for individuals showing haematological indications that suggest the presence of a deletional variant, further investigation is warranted using multiplex ligation-dependent probe amplification (MLPA). MLPA is another technique employed to characterize deletions in thalassaemia. This method relies on the ligation of multiple probe-pairs hybridized across the entire locus of interest, facilitating the quantification of gene copy numbers. MLPA represents a valuable alternative or supplementary method to gap-PCR, particularly when examining both known and unknown deletions and duplications underlying α-thalassaemia.

For the detection of single nucleotide variants (SNVs), **Sanger sequencing is the most practical method**, enabling comprehensive detection of all variants without prior knowledge of family history. However, sequencing α-globin genes presents a unique challenge due to the near-complete homology shared by the two α-globin genes (*HBA1* and *HBA2*). Therefore, specialized design and optimization of PCR conditions are necessary for accurate sequencing. In instances where the specific carrier mutation within a family is known, direct mutation detection methods, such as restriction enzyme digestion PCR (RED-PCR), designed for that particular variation, may be employed.

Nevertheless, this approach can be time-consuming, labour-intensive, and it sometimes carries the risk of not detecting certain variants. The landscape of human globin gene mutation detection methods has significantly evolved with the **advent of next generation sequencing (NGS) platforms.** NGS has revolutionized genetic diagnosis by enabling rapid, highly multiplexed, and high-throughput detection of genetic variants. To simplify traditional strategies, some molecular testing laboratories have introduced targeted NGS testing for the genetic analysis of thalassaemias. Commercial targeted NGS kits now allow for the comprehensive analysis of the entire spectrum of thalassaemias, detecting variations, SNVs, indels, and copy number variations (CNVs) in *HBA1*, *HBA2*, and *HBB* using a single one-tube NGS assay. This fast, straightforward, and robust NGS workflow replaces complex multi-step protocols and eliminates the need for maintaining multiple thalassaemia assays in your laboratory.

Summary and recommendations

- α-Thalassaemia is one of the most commonly inherited blood conditions. Initially prevalent in areas where malaria was most common, α-thalassaemia is now considered a global health concern due to population migration.
- Clinically significant forms of α-thalassaemia, HbH disease and haemoglobin Bart's hydrops foetalis result from compound heterozygosity for α^0-thalassaemia deletions with other mutations or deletions of α-globin genes. Hence, identification of carriers of α^0-thalassaemia deletions is pivotal for population control and prenatal diagnosis.
- HbH disease is the most common clinically significant form of α-thalassaemia, which is most prevalent in Southeast Asia, South China and also in some areas in the Middle East or Mediterranean region.
- HbH disease has a considerable heterogeneity, both genetically and clinically. Individuals with deletional forms of HbH disease have generally a benign course but those who harbour a non-deletional mutation have a higher rate of transfusion requirement and experience more frequent thalassaemia-related complications. As a result, identification of the underlying genetic abnormality has clinical implications.
- Accurate diagnosis of α-thalassaemia syndromes requires application of a range of diagnostic techniques, including complete blood count (CBC) with reticulocyte count, haemolytic panel, peripheral blood smears, automatic haemoglobin analyzers, and different modalities of molecular analysis.
- Knowledge of the patient's clinical phenotype and the prevalence of specific mutations in the region is essential to strategically select the appropriate molecular analysis.

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2 CLINICAL PRESENTATION AND MANAGEMENT OF DELETIONAL HAEMOGLOBIN H DISEASE

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Introduction

The clinical expression of haemoglobin H (HbH) disease extends from mild asymptomatic anaemia to severe anaemia with transfusion-dependence and hydrops foetalis [1, 2]. Since phenotype is largely determined by underlying genetic changes, it is important to specify the subtype of HbH disease with the terms deletional (deletion of three α-globin genes) and non-deletional (two or three affected α-globin genes, of which one or more have point mutations)[3]. Individuals with deletional HbH disease usually have mild asymptomatic anaemia that remains unsuspected in many cases until an incidental laboratory finding of anaemia prompts diagnostic workup.

In this chapter, we review clinical presentation and management of deletional HbH disease while the more severe form of HbH disease (non-deletional HbH disease) will be discussed separately in the next chapter (Please see Chapter 3: Clinical presentation and management of non-deletional haemoglobin H disease).

Genetic basis of deletional HbH disease

The most frequent genotype in deletional HbH disease results from various combinations of α^+-thalassaemia deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, other) with α^0-thalassaemia deletions ($--^{SEA}$, $--^{FIL}$, $--^{THAI}$, $--^{MED}$, other). Regardless of the underlying mutations, patients with deletional HbH disease have a relatively homogenous clinical phenotype. Co-inheritance of the β-thalassaemia trait or particularly the haemoglobin E (HbE) trait is common in regions where both conditions have high prevalence [4,5], but there is no significant impact on disease severity. Similarly, due to their evolutionary contribution to protection against severe malaria disease, HbH disease is common in areas where glucose-6-phosphate dehydrogenase (G6PD) deficiency was prevalent and their coinheritance is also common. In deletional HbH disease, relative deficiency of α-globin chains lead to formation of tetramers of β-globin (β_4, HbH) leading to ineffective erythropoiesis and chronic haemolysis, with exacerbation of anaemia during periods of infections, fever, surgical procedures, or pregnancy.

Clinical presentations and complications of deletional HbH disease

Newborn infants diagnosed at birth through a screening programme have asymptomatic perinatal period and infancy. Microcytic, hypochromic anaemia is present at birth with haemoglobin generally at 110-130 g/L. On haemoglobin analysis using high performance liquid chromatography, haemoglobin Bart's fraction of 25-35% of total haemoglobin can be identified [6, 7]. Haemoglobin follows the expected

trend with nadir around 3 months of age (usually staying >75 g/L), following which it slowly increases to reach 85 - 95 g/L by 4 years. After a child reaches puberty, haemoglobin generally is at 100-120 g/L in men and 90 -105 g/L in women [1-3, 6, 8-11].

In those who are identified later in life, laboratory tests also show anaemia, microcytosis, hypochromia, anisocytosis with increase in red cell width (RDW) and target cells while nucleated red blood cells are few or absent on a peripheral blood smear. Such patients may be misdiagnosed with iron deficiency anaemia and prescribed supplemental iron, which can increase the risk of iron overload. However, there is evidence of mild haemolysis with slight increase in bilirubin and reticulocyte count. Haemoglobin electrophoresis demonstrates presence of fast-moving HbH 5-15% of the total haemoglobin. The peaks of HbH and Hb Bart's are also identified by automatic high-performance liquid chromatography (HPLC) and capillary electrophoresis system, however, the percentage may not be accurately quantitated. HbH inclusion bodies can be visualized with a supravital stain such as methyl violet or brilliant cresyl blue. Genetic testing is essential in every patient to exclude presence of non-deletional HbH disease. Gastrointestinal iron absorption is increased, which leads to a mild iron overload in the 3rd decade or later manifesting as elevation in serum ferritin, but with normal transferrin saturation [3].

Patients with HbH disease can present with episodic exacerbation of haemolysis or transient aplastic events during infections. While haemolytic (or aplastic) episodes can occur in individuals with deletional HbH disease, various publications from different regions have uniformly recognized the milder natural history of deletional HbH disease compared with non-deletional HbH disease. However, heterogeneity in clinical manifestations of deletional HbH disease is observed due to unexplained reasons. When compared with non-deletional HbH disease, in the absence of other disease modifiers, clinically severe acute haemolytic episodes requiring transfusions are not characteristic of deletional HbH disease and blood transfusion is seldom necessary [2,3]. Even during significant infections, the haemoglobin level usually stays over 70 g/L and is unlikely to drop below 60 g/L. It is noteworthy that the risk of requiring blood transfusion in deletional HbH disease varies in different regions. Published case series report that 29% of patients in Thailand, 14% in Italy, and 3% in California were transfused on one or more occasions [2,3,6]. It is possible that environmental variables (recurrent infections, malaria, or nutrition) modify the phenotypic expression of HbH disease [3], and they increase the probability of needing blood transfusion [2,3,9,12].

Children with deletional HbH disease demonstrate normal growth and development based on their constitutional trajectory, and their physical activity is generally indistinguishable from peers [3]. Skeletal changes that are commonly seen in β-thalassaemia syndromes are not observed in deletional HbH disease. Splenomegaly is either absent or mild [2, 3].

In keeping with the benign disease course, cholelithiasis is observed in 15-20% of adults, though a significant increase in thrombotic and vascular complications is not reported [6]. Women can go through pregnancy without an increase in obstetrical complications or the need for regular transfusion

support [6, 13]. An increase in adverse perinatal outcome and foetal growth restriction is reported by some groups [13], though this was not observed in a different series [6]. The risk of haemoglobin Bart's hydrops foetalis must be considered if the partner has α^0 thalassaemia trait. Some adults may develop fatigue, although quality of life has not been formally evaluated in HbH disease [6].

Management of deletional HbH disease

The aim is to provide comprehensive haematology care and follow up to patients with deletional HbH disease at all stages of life, while recognizing that the initial diagnosis may be made in different settings and at any age.

Routine care

The initial clinic visit is used to review complete blood count, electrophoresis, and genetic tests to ensure that results are consistent with deletional HbH disease. DNA testing is strongly recommended in all cases to exclude non-deletional mutants that make the disease course more severe. While routine testing for co-existing G6PD deficiency is not recommended, this should be considered when a patient experiences haemolytic crisis. The patient and family are counselled about the expected mild clinical course. Subsequent visits are conducted every three months for the first year and then every 12 months. A complete blood count with reticulocyte count is obtained at each visit, while bilirubin and ferritin are checked once a year. All childhood vaccinations are given according to the normal schedule and patients should receive the seasonal influenza vaccine. At each visit, height and weight percentiles are checked to document normal growth that is expected in deletional HbH. Folic acid 0.4 to 1 mg per day is recommended to all patients starting around 6 months. Vitamin D status is checked to maintain sufficiency, using supplements if needed. No dietary modifications are needed and there are no specific medications or foods to avoid. In particular, there is no evidence that oxidant drugs should be avoided in the absence of known G6PD deficiency. Adhering to a reduced-iron diet is not necessary, however, supplements containing iron are discouraged unless laboratory tests show presence of iron deficiency. Starting at 10 years of age, the child's bone mineral density is evaluated by dual-energy X-ray absorptiometry (DXA) every 2–3 years, or more frequently if needed. Maintenance of physical activity and participation in sports is encouraged.

Children with deletional HbH have similar incidences of common paediatric febrile illnesses compared with their peers. Patients with fever can be seen in the clinic on the next day unless an urgent visit is warranted by the reported symptoms. Checking haemoglobin level urgently is not necessary as a sudden drop in haemoglobin level is not expected. However, if there is concern based on severity or duration of illness or the development of pallor with or without jaundice, a complete blood count, reticulocyte count, and haemolytic tests should be obtained. While aplastic crisis from parvovirus infection is possible, it has been rarely reported to cause severe anaemia in deletional HbH disease.

As severe anaemia is rare even during febrile illness, the need for transfusion should be assessed based on the clinical status of the patient. In otherwise uncomplicated situations, it is appropriate to observe the patient and transfuse if the haemoglobin drops <60 g/L in younger children, or <65 g/L in adults. Avoidance of transfusions is a desirable goal in the management of deletional HbH disease in view of the risk of alloimmunization and other complications. Chronic transfusion therapy or splenectomy is not recommended for patients with typical clinical course of deletional HbH disease (Please see Chapter 9: Blood transfusion and Chapter 10: Splenomegaly and splenectomy).

Iron overload

Iron overload due to increased absorption of gastrointestinal iron develops with age in adults and can become significant (liver iron concentration >3 mg/d) in the fourth or fifth decade. Serum ferritin gradually increases, earlier in men than women, although transferrin saturation stays in the normal range. Iron overload is monitored by checking serum ferritin annually. In patients with ferritin >300 ng/mL, we recommend an MRI for measuring liver iron concentration (LIC). There are no reports of iron-induced cardiac or endocrine complications in patients with deletional HbH disease, however, the hepatic iron overload could be associated with fibrosis and liver injury in older adults [14,15]. In individuals with an unusual degree of iron overload, co-existing hereditary haemochromatosis or excessive alcohol intake should be considered. Options for chelation therapy should be discussed if LIC >5 mg/g dry weight. Lower level of iron overload may be treated in some cases based on comorbidities that affect liver health. Treatment is continued until ferritin < 300 ng/mL and LIC <3 mg/g dry weight and then stopped with regular monitoring of iron overload.

Other complications

The risk of developing gallstones is less than observed in non-deletional HbH disease. Cholecystectomy is recommended in patients with recurrent symptoms attributable to gallstones. The role of echocardiograms to monitor pulmonary artery systolic pressure is not certain as the risk of pulmonary hypertension is very low or none.

Pregnant women with deletional HbH disease should be followed by maternal-foetal medicine due to a higher incidence of adverse foetal outcomes in some regions and the risk of Bart's hydrops foetalis. They generally do not require additional haematology care during pregnancy. A decline in haemoglobin is anticipated, and an occasional transfusion for worsening of anaemia may be needed, but regular transfusion support is not recommended.

Older patients should be monitored for deterioration in quality of life from fatigue or difficulty in coping at work or the impact of age-related comorbidities.

Genetic counselling

Reproductive counselling is very important and partner testing is routinely advised to identify the risk for transmission of a significant haemoglobin disorder. If the partner is carrier of the α^0-thalassaemia trait, then there is a 25% risk of pregnancy with Hb Bart's hydrops foetalis (α-thalassaemia major).

Summary and recommendations

Domain	Management
Frequency of clinic visits	Every 3 months for first 2 years, then annually for life.
Laboratory testing	Complete blood count, reticulocyte count, liver function: Every 6 months for first 2 years, then annually. Ferritin, transferrin saturation (TSAT): Annually starting at 1 year. TSAT is not needed after 3 years, but should be checked if ferritin >200 ng/mL.
Febrile illness	Evaluation by paediatrician as for children without HbH disease. Check CBC if severe symptoms or development of pallor or jaundice.
Transfusions	Regular transfusions are not required. Episodic transfusions are not needed during most febrile illnesses, unless the haemoglobin level drop below 60 g/L in young children or 65 g/L in adolescents and adults. Transfusion may be needed for surgery or other specific indications.
Red cell phenotyping	Not needed unless there is a need for recurrent transfusions
Iron overload assessment	Mild to moderate iron overload is observed in the 4th -5th decades of life, earlier in males than females. Check ferritin annually, check liver MRI for liver iron concentration (LIC) if ferritin >200 ng/mL.
Iron chelation	Start if LIC >5 mg/g dry weight or ferritin >500 ng/mL. Treat until LIC <3 mg/g dry weight and ferritin <300 ng/mL, then stop. Treat at lower level of LIC in presence for specific indications.
Splenectomy	Not indicated.
Endocrinology evaluation	If onset of puberty is delayed >2 years or if concern for slow growth. Obtain family history and consider x-ray for bone age.
DXA scan	Every 3 years starting at 12 years.
ECHO	Check at 10-12 years to assess for pulmonary artery pressure. If normal, repeat every 3-5 years.
Pregnancy and genetic counselling	Anticipate mild decline in haemoglobin, but routine transfusions are not needed. Partner testing to evaluate foetal risk for Bart's hydrops foetalis.

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3 CLINICAL PRESENTATION AND MANAGEMENT OF NON- DELETIONAL HBH DISEASE

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Introduction

Clinical phenotypes are diverse among affected individuals with non-deletional haemoglobin H (HbH) disease (--/$\alpha^T\alpha$). This mainly depends on the type of mutation in α-globin genes, whether the mutation is in the *HBA1* or the *HBA2* gene, as well as co-inheritance of β-thalassaemia. Nevertheless, patients with non-deletional HbH disease with identical genotypes can exhibit substantially different clinical severity. Homozygosity for non-deletional α-globin mutations and compound heterozygosity of two different non-deletional mutations ($\alpha^T\alpha/\alpha^T\alpha$) or non-deletional mutation and deletional α^+-thalassaemia (-α/$\alpha^T\alpha$) can also result in HbH disease.

In the past, HbH disease was generally thought to be a mild form of thalassaemia. A number of patients with more severe phenotypes, especially those with non-deletional HbH disease, were left undertreated. In this chapter, clinical presentations of common and less common non-deletional HbH disease, as well as special forms of non-deletional α-thalassaemia will be described. In addition, management of affected individuals with non-deletional HbH disease and monitoring of the disease-associated complications from early childhood to adulthood will be covered.

General clinical presentation of non-deletional HbH disease

A variable extent of anaemia, ranging from mild asymptomatic anaemia to the most severe foetal anaemia (hydrops foetalis), can be observed in individuals with non-deletional HbH disease. This is unlike patients with deletional HbH disease, in which the degree of anaemia is generally mild and uniform. Haemoglobin Constant Spring (CS) appears to be the most prevalent form of non-deletional α-globin variant worldwide [1, 2]. This results in HbH-CS (--/$\alpha^{CS}\alpha$) being the most common form of non-deletional HbH disease, of which clinical phenotypes were extensively described. Clinical symptoms of non-deletional HbH are generally more severe than those of deletional forms. This is because some of the common non-deletional mutations affect expression of the HBA2 gene which produces the majority of the α-globin chains [2, 3]. Some mutations lead to production of highly unstable α-globin chains that precipitate in red blood cell precursors, leading to more pronounced ineffective erythropoiesis and haemolysis [4, 5]. Comparison of clinical and laboratory characteristics between individuals with deletional and non-deletional HbH disease is shown in **Table 1**. The data were derived from multiple key publications on paediatric [2, 6, 7] and adult patients with HbH disease [8, 9] and from a recent study of 246 patients with HbH disease from Thailand [10]. Neonates with non-deletional HbH disease can develop haemolytic jaundice and anaemia. In rare cases, hydrops foetalis is observed.

Coinheritance of HbE with HbH-CS or HbH Pakse (HbH-PS) are often identified in Southeast Asia. Coinheritance of HbH with heterozygous HbE has been referred to as AEBart's disease, whereas coinheritance of HbH with homozygous HbE has been called EFBart's disease. Clinical presentation of individuals with non-deletional AEBart's and EFBart's disease is comparable to non-deletional HbH without coinherited E trait or EE [11, 12]. However, patients with non-deletional AEBart's and EFBart's diseases have lower mean corpuscular volume as compared to their non-deletional HbH counterparts (MCV of ~50-60 fL vs. 65-75 fL), although the Hb levels are comparable [11, 12]. Acute haemolytic episodes following infection or inflammation were reported to be less frequently observed in patients with non-deletional HbH disease who coinherited HbE [13]. Unlike coinheritance of E trait or EE, coinheritance of non-deletional HbH disease with HbE/β-thalassaemia results in severe anaemia [13]. The severity of anaemia in patients with non-deletional HbH disease who are also carriers for β-thalassaemia mutations is generally less than those with non-deletional HbH disease alone [9].

Table 1. *Clinical and laboratory characteristics of individuals with deletional and non-deletional HbH*

Characteristics	Deletional HbH	Non-deletional HbH (mainly HbH-CS)
Symptomatic patients (%)	20-40	40-60
Age at diagnosis (years)	4-22	2.5-10
History of blood transfusion by age of 20 years (%)	3-29	24-80
Age at first transfusion (years)	5.5-12	1.5-3.5
Transfusion dependent (%)	Rare	28-33
Facial bone changes (%)	2-3	20-30
Growth retardation (%)	Rare	~15%
Spleen size below costal margin (cm)	0-1.5	3-4.5
Liver size below costal margin (cm)	0-1	1-2
Gallstones	+	++
Acute haemolytic episode following infection/inflammation	Rare	Common
Baseline Hb level (g/L)	85-100	72-96
Reticulocyte count (%)	2.5-4	5.5-6.5
Aspartate aminotransferase (U/L)	23-25	37-38
Lactate dehydrogenase (U/L)	175-440	284-900
Ferritin	Increases after 18 years of age	Increases from birth to the age of >30 years

Average data from multiple key studies as described in the text

Uncommon non-deletional HbH diseases

To date, up to 400 non-deletional α-globin mutations have been reported in the database of Hb variants (https://globin.bx.psu.edu/hbvar/menu.html). The vast majority of these mutations are extremely rare and were reported as family-specific mutations [2]. Only a few mutations are recurrent or prevalent in limited population groups. These latter α-globin mutations usually affect mRNA transcription, splicing or protein translation by creating novel truncated or elongated polypeptide, sometimes leading to highly unstable variants [14]. Examples of regional common non-deletional mutations are poly A mutations found in Greece, Türkiye and the Middle East [15] and Hb Adana frequently found in Malaysia, Indonesia, Saudi Arabia and China [16]. In Southeast Asia, besides HbCS and HbPS, other non-deletional mutations, such as Hb Quang Sze (HbQS) and Hb Pak Num Po (HbPNP) are also seen but their prevalence is much lower.

All of these non-deletional mutations can result in various forms of α-thalassaemia, depending on whether they interact with deletional or other non-deletional α-globin mutations in *trans* or whether they are inherited in homozygous form. Non-deletional HbH (--/$\alpha^{T}\alpha$) occurs in the regions where these non-deletional variants and centres-thalassaemia alleles (--/αα) are both prevalent. **Table 2** summarizes clinical phenotypes of selected uncommon non-deletional HbH. Non-deletional mutations leading to other forms of α-thalassaemia are described at the end of this chapter.

Individuals with certain uncommon genotypes of non-deletional HbH present with very severe clinical course that includes life-long transfusion dependency and hydrops foetalis (HbH hydrops foetalis syndrome). Foetuses affected with HbH hydrops foetalis syndrome almost always harbour α^{0}-thalassaemia on one allele and a non-deletional mutation involving the HBA2 gene on the other allele producing an extremely unstable Hb variant in utero [17]. These foetuses suffer from severe intrauterine anaemia and hypoxia, which result in various degrees of oedema, ascites, pleural and pericardial effusions, as well as foetal growth restriction. Additionally, associated congenital anomalies, such as hypospadias, ambiguous genitalia and undescended testes, were observed in HbH hydrops foetalis syndrome [17] similar to that found in Hb Bart's hydrops foetalis syndrome (BHFS), which is caused by deletion of all four α-globin genes (18). The affected foetuses are typically born premature and die shortly after birth, unless given prenatal and postnatal transfusions. Most survivors with HbH hydrops foetalis syndrome become transfusion-dependent from early infancy [17]. Rare non-deletional HbH genotypes reported to cause hydrops foetalis include $--^{Tot}/\alpha^{CD30(delGAG)}\alpha$, $--^{SEA}/\alpha^{CD66(CTG->CCG)}\alpha$, $--^{FIL}/\alpha^{CD35(TCC->CCC)}\alpha$, $--^{MED}/\alpha^{TSaudi}\alpha$ and $--^{SEA}/\alpha^{Zurich-Albisrieden}\alpha$ [17, 19-22]. More frequently observed genotypes causing such hydrops include $--^{SEA}/\alpha^{Adana}\alpha$ [17], $--^{FIL}/\alpha^{Adana}\alpha$ [23] and $--^{SEA}/\alpha^{QS}\alpha$ [24, 25]. Affected foetuses with HbH-CS and HbH-PS were previously thought to not develop severe hydrops foetalis, despite having severe anaemia in utero [17, 26]. However, very rare cases of HbH hydrops foetalis associated with compound heterozygosity of SEA deletion and HbCS [27] or HbPS [28] have recently been described.

Table 2. *Summary of selected non-deletional HbH (--/$\alpha^T\alpha$) phenotypes*

Mutations Affected gene HbH syndrome (--^{DEL}/$\alpha^T\alpha$)	Regional prevalence	Phenotype of --^{DEL}/$\alpha^T\alpha$
Hb Constant Spring (CS) *HBA2* HbH-CS	SEA, China, Mediterranean	Mild to moderate anaemia in most cases, ~30% have more severe phenotypes requiring regular transfusion, rare report of hydrops foetalis
Hb Pakse (PS) *HBA2* HbH-PS	Laos, Thailand	Clinical and haematologic features similar to HbH-CS, except higher proportion of HbH, rare report of hydrops foetalis
Hb Quong Sze (QS) *HBA2* HbH-QS	SEA, China	Range from mild anaemia to hydrops foetalis
Polyadenylation site mutation (Poly A1 or A2) *HBA2* HbH-Poly A	Greece, Cyprus, Iran, Saudi Arabia, Türkiye	Range from mild to moderate anaemia requiring occasional transfusion, transfusion-dependent from early infancy in limited cases
Hb Adana *HBA2* or *HBA1* HbH-Adana	Indonesia, Malaysia, China	Hydrops foetalis or transfusion-dependent from early infancy in most cases
IVS I (-5 nt) *HBA2* --/$\alpha^{-5nt}\alpha$ or --/$\alpha^{Hph}\alpha$	Mediterranean, Iran, Türkiye	Range from mild to moderate anaemia requiring occasional transfusion to severe anaemia requiring frequent transfusion in limited cases
Codon 19 (-G) *HBA2* --/$\alpha^{CD19(-G)}\alpha$	Iran	~40% have moderate to severe anaemia requiring occasional or regular transfusion
Hb Pak Num Po (PNP) *HBA1* HbH-PNP	Thailand	Severe anaemia requiring regular transfusion
Hb Q-Thailand *HBA1* (linked with $-\alpha^{4.2}$) HbQ-H disease	Thailand, China	Mild to moderate anaemia, usually transfusion -independent
Hb Suan Dok (SD) *HBA2* HbH-SD	Thailand	Severe anaemia requiring regular transfusion, possibility of hydrops foetalis

Management of non-deletional HbH disease during childhood

General management and follow-up monitoring

Supplementation with folic acid ranging from 1 to 5 mg per day is generally recommended for all patients with non-deletional HbH disease, as it is required for increased erythropoietic activity [6]. Non-iron-containing multivitamin is suggested [6, 7] especially in paediatric patients who may not attain proper dietary intakes. Iron accumulation occurs from early life in non-deletional HbH disease [7], mainly from increased gastrointestinal iron absorption, regardless of whether they have received blood transfusions. Iron supplements should therefore be avoided. Although specific dietary restrictions are not deemed necessary, it is recommended to exercise moderation when consuming iron-rich foods and excessive intake is not advisable.

As compared to patients with deletional HbH disease, those with non-deletional forms are more likely to experience acute worsening of anaemia during infection/inflammation, leading to an urgent need of blood transfusion [7]. This more commonly occurs during childhood; therefore, care should be provided to avoid such events. These preventive measures include prompt treatment of acute illness and alertness to symptoms of severe anaemia. Furthermore, it is advisable to follow scheduled immunizations in accordance with the national guidelines for each individual (see Chapter 10).

As most patients with non-deletional HbH disease are non-transfusion dependent (NTD), the follow-up interval for the affected paediatric subgroup may be considered according to baseline Hb levels. Ideally, those with Hb>80 g/L should be seen in clinic every six months while patients with Hb≤80 g/L should be followed more frequently. Assessment of growth, as well as monitoring of facial bone changes and spleen size are crucial during childhood and should be performed at every clinic visit. Monitoring of pubertal development is essential for affected adolescent patients. For those patients with more severe phenotypes, initiation of regular blood transfusion should be considered. Serum ferritin levels should be assessed every six months, although the need for iron chelation therapy is relatively rare within the first two decades of life unless the patient relies on regular transfusions. For pediatric patients with non-deletional HbH disease who are transfusion-dependent, clinical and laboratory monitoring should align with the protocols typically used for patients with β-thalassaemia major (β-TM).

Transfusion management

Patients should receive blood transfusions in cases of acute exacerbation of anaemia, typically occurring after episodes of acute illness. This intervention is recommended when the haemoglobin level drops below 70 g/L or when there is accompanying symptoms of anaemia, with an aim to restore Hb to 80-90 g/L [2].

The decision to initiate regular transfusions in non-deletional HbH disease should be approached differently in children and adults. Failing to provide adequate transfusion support for children with more severe anaemia or ineffective erythropoiesis can lead to undesirable outcomes, including stunted growth and changes in facial bone structure. In cases of massive splenomegaly, hypersplenism may eventually develop, necessitating splenectomy. Unlike some of paediatric patients with NTD β-thalassaemia/HbE who can remarkably adapt to low Hb levels [29, 30], a similar level of haemoglobin may not be adequate to facilitate optimal growth, pubertal development and daily activities for those with non-deletional HbH. This is because total measured haemoglobin in patients with non-deletional HbH disease is not all functional as the total haemoglobin consists of a variable proportion of Hb Bart's (γ4) and HbH (β4), which are unable to deliver oxygen to tissues, and patients with higher proportion of these non-functional Hb are likely to encounter more severe clinical symptoms [10].

Recent observational study shows the beneficial role of regular transfusion therapy on the growth parameter of severely affected paediatric patients with non-deletional HbH disease [10]. Nevertheless, careful assessment of individual patients before initiation of regular blood transfusion is essential to avoid overtreatment. In general, a regular transfusion regimen should be considered in patients with the following condition: declining Hb level in parallel with progressive enlargement of spleen, failure of growth or secondary sexual development, poor performance at school, decreased exercise tolerance, presence of bone changes, frequent haemolytic crisis, or poor quality of life [31]. Since paediatric patients with non-deletional HbH disease might be cared for by general paediatricians or less-experienced haematologists in many countries, a simple objective score to aid the decision to initiate regular transfusions is shown in **Table 3**. A transfusion regimen is recommended for those patients with a persistent score of ≥4 (severe phenotype) over a period of 3 to 6 months [10].

Table 3. *Score for paediatrics non-deletional HbH severity classification (10).*

Criteria	Value	Score	Value	Score	Value	Score
Age at diagnosis (y)	<2	1	≥2	0		
Spleen size (cm below costal margin)	≥3	1	<3	0		
Hb at steady-state (g/L)	<70	4	70-80	3	>80	0

For each criterion, a score is given according to the value. Total sum of all scores is interpreted as follows: severity score <4, non-severe disease; severity score ≥4, severe disease likely requiring regular blood transfusion for better outcomes.

Once initiated, blood transfusions should be scheduled, usually every 3 to 6 weeks, with pre-transfusion haemoglobin aimed at a slightly lower level (80-90 g/L) in comparison to that aimed for β-TM [32, 33]. This is because there is generally less degree of ineffective erythropoiesis to be suppressed in non-deletional HbH. It is important to periodically re-assess these patients for tapering off or withdrawing blood transfusions when a sustained clinical benefit is achieved. This is to minimize unnecessary transfusional iron overload. Most often, discontinuation of blood transfusions is considered when secondary sexual characteristics are fully developed or maximum adult height is reached, unless the patients have other indications for continuing regular transfusions into adulthood.

It is important to keep in mind that transfusion requirements of individual patients can be dynamic. Children and adolescents who do not require transfusions initially may later become transfusion dependent. Therefore, regular assessment of clinical symptoms and haemoglobin (Hb) levels at each visit (every 3–6 months, as discussed earlier) is imperative during childhood and early adulthood. By contrast, affected patients who are born with hydrops foetalis or require frequent transfusions during the first 6 months of life usually remain transfusion-dependent, unless cured through haematopoietic stem cell transplantation.

In certain transfusion-dependent patients with rare genotypes of non-deletional HbH, the proportion of non-functional Hb Bart's and HbH can remain high (>20%), even when following a conventional transfusion regimen [34, 35]. Similar to that identified in survivors of BHFS [36], these patients exhibited subtle improvements in growth and the persistence of massive hepatosplenomegaly despite standard transfusion treatments. Such cases may necessitate a more aggressive transfusion regimen, akin to that employed in BHFS survivors, which aims to achieve a pre-transfusion functional Hb level of 90-100 g/L [37]. Functional Hb is calculated as total Hb x (1- [HbH % + Hb Bart's %] / 100). In BHFS survivors, maintaining this level of pre-transfusion functional Hb was found to reduce haemolysis and enhance tissue oxygenation, resulting in improved clinical symptoms [36, 37]. However, it is of utmost importance to carefully evaluate whether the benefits of this aggressive transfusion regimen outweigh the increased risk of iron overload for individual patients with severe non-deletional HbH disease.

Splenectomy

Splenectomy should generally be avoid in patients younger than 5 years [31].

Management of adult patients with non-deletional HbH disease

General management and follow-up monitoring

Folic acid supplementation and non-iron-containing vitamins are also recommended for all adult patients, and avoidance of iron supplementation should be emphasized unless iron deficiency is confirmed. Regular follow-up visits remain vital for adult patients, and their frequency should be determined based on the patient's condition, severity, and treatment plan. For individuals with non-deletional HbH and mild anaemia (Hb > 80 g/L), follow-up visits every 6–12 months are appropriate.

For those with moderate anaemia (Hb ≤ 80 g/L), visits every 3–6 months are recommended. A complete blood count with reticulocyte count, haemolytic profile, and serum ferritin should be monitored with every visit and a quantitative iron assessment of liver iron concentration (LIC) should be obtained with MRI if ferritin is elevated. Infections often trigger acute haemolysis, so patients are advised to take precautions. Routine vaccinations against common infections, such as influenza, pneumococcus, and SARS-CoV-2 are strongly recommended to prevent infections.

Transfusion management

The majority of adult patients with non-deletional HbH disease have mild to moderate anaemia and do not require blood transfusions. However, episodic (on-demand) transfusions may be necessary in cases of haemolytic crisis to achieve a target Hb level of 80-90 g/L. Leucocyte-depleted red blood cells should be administered at a volume of 10-15 ml/kg (1–2 units for adults) one or more times based on the severity of anaemia [2, 6]. To manage haemolysis effectively, it is crucial to identify and address the underlying causes of inflammation and infection, such as pregnancy, oxidative stress, hypersplenism, and pyrexia. Frequent transfusions may be considered in more severely affected adult patients for prevention of the disease-related complications, such as significant bone deformities and, in rare instances, extramedullary haematopoiesis (EMH), and for improvement of their quality of life. Regular blood transfusions should be considered for secondary prevention or treatment of thromboembolic diseases, pulmonary hypertension, and EMH pseudotumours [31].

Splenectomy

Splenectomy is a traditional treatment option for patients with β-thalassaemia, but its use has declined due to the risk of postoperative complications, such as hypercoagulability, thrombotic events, and infections [38, 39]. However, in patients with non-deletional HbH disease, splenomegaly with hypersplenism or severe anaemia, splenectomy is commonly performed [7, 40]. Studies have shown that splenectomy can increase haemoglobin levels by 10-30 g/L and decrease or eliminate the need for blood transfusions in HbH-CS [7, 9, 41]. Despite the potential benefits, several complications can arise after splenectomy, including venous thromboembolism, pulmonary hypertension, and sepsis [42-45]. Therefore, splenectomy is recommended in selected patients with non-deletional HbH disease. These indications include severe anaemia that impedes growth and development in the patients with limited access or poor adherence to regular blood transfusions and/or iron chelation, hypersplenism with anaemia, leukopenia, or thrombocytopenia that results in infections or bleeding, or massive splenomegaly with left upper quadrant pain that increases the risk of splenic rupture. Low-dose aspirin is routinely given to patients who have undergone splenectomy to prevent post-splenectomy thromboembolic events. Benefit of splenectomy in patients with other uncommon genotypes of non-deletional HbH remains unclear and mostly based on a limited number of case reports.

Complications in non-deletional HbH diseases

Gallstones

The incidence of gallstones is high among individuals with HbH disease, which is possibly caused by the unstable haemoglobin precipitation in erythrocytes leading to chronic haemolysis. Patients with non-deletional HbH disease carry a greater risk of gallstone formation than those with deletional HbH disease [2]. Moreover, more than two-thirds of patients with thalassaemia have asymptomatic gallstones [46]. Prior studies have found that aging and splenectomy are significant factors associated with gallstone formation in adult patients with thalassaemia [44, 47]. While most thalassaemia-related complications increase with age and are typically observed during the second and third decades of life [48], gallstones present a unique complication that may arise earlier due to ongoing haemolysis. Therefore, patients with abdominal symptoms compatible with gallstones should have a detailed evaluation that includes abdominal ultrasonography. Although the incidence of gallstones is high among individuals with thalassaemia, a significant proportion of affected patients remain asymptomatic. Thus, cholecystectomy is typically indicated only in the presence of recurrent symptoms, biliary obstruction or cholecystitis.

Extramedullary haematopoiesis (EMH)

Reports of EMH in patients with α-thalassaemia are limited, with most cases reported in patients with β-thalassaemia. EMH is a compensatory process that occurs secondary to ineffective erythropoiesis in patients with thalassaemia. It is a time-dependent complication, and aging is a significant risk factor for developing EMH in patients with thalassaemia [47]. Typically, EMH is found in the second and third decades of life, except for those patients with severe anaemia who may experience it earlier. Additionally, the degree of ineffective erythropoiesis is also a significant risk factor for EMH. This is consistent with studies on ineffective erythropoiesis biomarkers that have found that non-deletional HbH disease leads to more severe ineffective erythropoiesis than deletional HbH disease [4, 49]. EMH is commonly found in the spleen, liver, and lymph nodes, but it can also occur in other locations, including the pleural space, sinonasal tract, paravertebral, and mesentery. Several treatment modalities are available for EMH, including hypertransfusion, radiation therapy, surgery, and hydroxyurea [50-52]. Hypertransfusion is the preferred treatment for EMH, and it may also help prevent this complication. Radiotherapy has also been shown to be effective in treating EMH because the EMH mass is radiosensitive. In cases where other treatments are ineffective, surgery may be considered. It is important to note that surgery carries the risk of significant bleeding, which must be carefully evaluated before deciding to proceed with the procedure.

Iron overload

Iron overload is a frequently encountered complication among older patients with non-deletional HbH, even in the absence of regular blood transfusions, due to an increase in intestinal iron absorption. Although serum ferritin represents a convenient tool for assessing iron burden in thalassaemia patients, studies have shown that it may underestimate the extent of iron overload in those with non-deletional HbH disease

[53, 54]. Thus, quantitative evaluation of LIC should be conducted if serum ferritin is elevated in order to indicate the necessity for iron chelation therapy (see Chapter 11: Iron overload in α-thalassaemia).

Endocrine complications

Most studies on endocrine complications have been conducted in patients with transfusion-dependent β-thalassaemia, while data on patients with α-thalassaemia are limited. Unlike other complications associated with thalassaemia, endocrine issues can manifest early in young thalassaemia patients [55], but their prevalence appears to increase with age [44, 47]. These endocrine complications, which include hypogonadism, hypothyroidism, growth retardation, and diabetes mellitus, are more frequently reported in patients with transfusion-dependent thalassaemia compared to those with non-transfusion-dependent forms [47]. Iron overload resulting from chronic red blood cell transfusions is believed to be the primary risk factor for developing these complications, although several other factors contribute, such as chronic anaemia, nutritional deficiencies, and chronic liver disease [56, 57].

Hypogonadism is a common endocrine complication in patients with transfusion dependent thalassaemia, affecting both males and females at rates between 40% and 80% [58, 59]. Hypogonadotropic hypogonadism, resulting from iron deposition in the pituitary gland, is more common than excess iron deposition in the ovaries or testes. Therefore, hormone replacement therapy can alleviate symptoms and prevent long-term complications of hormone deficiency [60].

Impaired glucose tolerance (IGT) and diabetes mellitus (DM) are more common in individuals with transfusion-dependent thalassaemia. The primary pathogenesis of glucose abnormalities is pancreatic iron overload, which leads to insulin deficiency and increased insulin resistance [61]. The prevalence of IGT and DM ranges from 7% to 14%, and this prevalence tends to increase with age [47, 62].

Growth failure is also common in transfusion-dependent thalassaemia. In young patients with α-thalassaemia, the prevalence of growth retardation varies from 8% to 68.5% [55, 63]. Insulin-like growth factor-1 (IGF-1) concentrations were significantly lower in individuals with β-thalassaemia major compared to healthy children and can be used as a primary screening test [64]. Contributing factors to the reduced synthesis of IGF-1 in patients with thalassaemia include chronic anaemia, iron overload, and nutritional deficiencies [65].

Hypothyroidism is another complication that can be detected in 4% to 14% of patients with α-thalassaemia [55, 63], but its rate is considerably higher in transfusion dependent patients [66]. Variations in prevalence could arise from differences in genotype and treatment protocols.

A case-control study conducted in young patients with α-thalassaemia revealed a significant increase in the prevalence of hypogonadism, growth retardation, and hypoparathyroidism compared to the control group. Interestingly, it was observed that non-deletion α-thalassaemia (HbH-CS) had notably higher rates of growth retardation and hypogonadism compared to deletion α-thalassaemia [63].

At present, there are no established evidence-based guidelines for managing these endocrinopathies in individuals with thalassaemia. An appropriate approach to addressing endocrine complications in thalassaemia patients involves early detection through a multidisciplinary team, which includes endocrinologists and nutritionists. Until further data becomes available, we recommend surveillance testing and monitoring, similar to those employed for β-thalassaemia.

Pregnancy complications

Patients with non-deletional HbH disease are at increased risk of maternal and foetal complications during pregnancy. This will be reviewed in Chapter 6: Fertility and pregnancy in α-thalassaemia.

Thromboembolic events

Thalassaemia is associated with a state of hypercoagulability, which can increase the risk of thromboembolic events. There are several factors that contribute to this predisposition, including chronic haemolysis, platelet activation, endothelial damage, decreased natural anticoagulants, and splenectomy [67]. In non-deletional HbH disease, the accumulation of excess β-globin chains in erythroid cells can result in ineffective erythropoiesis and abnormal red cell membrane, leading to the exposure of phosphatidylserine (PS) on the outer surface of red blood cells. This can initiate blood coagulation and thrombin generation, triggering platelet activation and increasing β-thromboglobulin (β-TG) levels in the blood circulation [68-72]. Moreover, the accumulation of excess iron in thalassaemia can generate reactive oxygen species, leading to endothelial damage and the expression of intercellular adhesion molecules (ICAMs). This can promote adhesion and transmigration of white blood cells from blood vessels to extravascular tissue [73]. All of these factors can contribute to thrombotic complications in thalassaemia. Previous research has shown that thromboembolic events are more frequent in thalassaemia intermedia (TI) than in thalassaemia major (TM), with more venous thrombosis in TI and more arterial thrombosis in TM. Thrombosis is more common among TI patients who underwent splenectomy and have anaemia (Hb < 90 g/L) [74]. Although research on thrombotic events in patients with α-thalassaemia is limited, previous studies suggest an association between thalassaemia and thrombotic events, particularly in elderly patients who have undergone splenectomy and received inadequate blood transfusions [44]. A small-scale study revealed an elevation of ICAM-1, tumor necrosis factor α (TNFα), β-TG, and PS levels in patients with HbH disease [75]. Given that splenectomy is a well-established risk factor for thrombosis in patients with thalassaemia, prophylactic use of low-dose aspirin is recommended for patients with non-deletional HbH following splenectomy.

Special forms of non-deletional α-thalassaemia

Homozygous HbCS

In certain regions with a high prevalence of HbCS, such as Southeast Asia, homozygous HbCS ($\alpha^{CS}\alpha/\alpha^{CS}\alpha$) is often encountered. Adults affected by this condition typically exhibit mild HbH phenotypes, which may include normocytic or mild microcytic haemolytic anaemia, jaundice, and splenomegaly [76, 77].

Acute episodes of increased haemolysis following infection/inflammation can occur during childhood [78]. While some patients may be diagnosed incidentally in their adulthood, some present in utero with hydrops foetalis [76, 79-81]. Premature birth and associated congenital anomalies, including urogenital and mild cardiac defects similar to those identified in BHFS and HbH hydrops foetalis, can be observed. Neonatal jaundice is common and often requires treatment. Some infants may experience severe haemolytic anaemia necessitating blood transfusions in the first few months of life [80]. After the stormy pre- and post-natal periods, the infants spontaneously recover, usually after the age of 3–4 months, and by the time the patients reach 1–2 years of age, they often have haemoglobin levels exceeding 90 g/L, and no longer require blood transfusions [80]. This is unlike the survivors of BHFS and other HbH hydrops foetalis who almost always remain transfusion-dependent. The mechanisms underlying such clinical courses of affected infants with homozygous HbCS remain unclear. Compound heterozygosity for HbCS and HbPS has also been reported to exhibit a clinical course similar to that observed in individuals with homozygous HbCS [81].

Therefore, homozygous HbCS should be one of the differential diagnoses in foetuses and neonates presenting non-immune hydrops foetalis or haemolytic jaundice/anaemia requiring treatments, especially in the areas where the variant is prevalent. Once diagnosed in utero, prompt intrauterine transfusions to maintain the affected foetus as close to term as possible is recommended. Monitoring of bilirubin and prompt phototherapy should be provided to prevent kernicterus. Blood transfusions should be administered as frequently as needed during the first few months of life. Once the clinical symptoms of the patient improve, general supportive care similar to that provided to patients with deletional HbH is recommended.

Less common non-deletional variants in homozygosity or compound heterozygosity with α^+-thalassaemia

Polyadenylation site (poly A) mutations in the HBA2 gene affect the polyadenylation signal site and impede the efficiency of globin protein synthesis. Poly A site mutations also interfere with the expression of the HBA1 gene in *cis*, contributing to the severity of the phenotype observed [82, 83]. There are two common poly A site mutations, namely α-Poly A1 (AATAAA > AATAAG) and α-Poly A2 (AATAAA > AATGAA) [82, 84]. Clinical disease is observed in individuals who are homozygous for poly A mutation ($\alpha^T\alpha/\alpha^T\alpha$) or compound heterozygous with α^0 deletion ($--/\alpha^T\alpha$).

The α-^{5nt} mutation is a pentanucleotide deletion in the intron 1 region of the HBA2 gene. This mutation has a significant impact on mRNA processing, and affected patients have a phenotype similar to α^+-thalassaemia. Since poly A and α-^{5nt} mutations are regional common variants, their homozygosity and compound heterozygosity with other α^+-thalassaemia are often identified in the areas. **Table 4** summarizes clinical phenotypes of such combinations of selected non-deletional α-globin mutations.

Table 4. *Summary of clinical phenotypes of selected less common non-deletional variants in homozygosity ($\alpha^T\alpha/\alpha^T\alpha$) or compound heterozygosity with α^+-thalassaemia ($-\alpha^+/\alpha^T\alpha$)*

α-Genotype Affected gene	Specific regions found	Clinical phenotypes
$\alpha^{polyA1}\alpha/\ \alpha^{polyA1}\alpha\alpha2$	Greece, Iran, Saudi Arabia, Türkiye	Mild to moderate anaemia, Hb H in infancy, requiring occasional transfusion
$\alpha^{polyA2}\alpha/\ \alpha^{polyA2}\alpha\alpha2$	Greece, Iran, Saudi Arabia, Türkiye	Mild to moderate anaemia , Hb H in infancy, requiring occasional transfusion
$-\alpha^{-3.7}/\ \alpha^{polyA1}\alpha\alpha2$	Iran	Mild to moderate anaemia , requiring occasional transfusion
$-\alpha^{3.7}/\alpha^{CD19(-G)}\alpha\alpha2$	Iran	Mild to moderate anaemia , requiring occasional transfusion
$\alpha^{CD19(-G)}\alpha/\alpha^{CD19(-G)}\alpha\alpha2$	Iran	Mild anaemia, rarely requiring blood transfusion
$\alpha^{-5nt}\alpha\ /\ \alpha^{-5nt}\alpha\alpha2$	Mediterranean, Iran	Mild anaemia, rarely requiring blood transfusion

Summary and recommendations

- Clinical presentations of patients with non-deletional HbH disease are highly variable and are generally more severe than those of deletional HbH (see Table 1). Majority of patients need occasional transfusions, and some become transfusion-dependent.
- HbH hydrops foetalis and transfusion-dependency from early infancy are observed in certain non-deletional HbH genotypes, including HbH-QS, HbH-PolyA, HbH-Adana, HbH-PNP and HbH-SD (see **Table 2**).
- Supplementation of folic acid 1-5 mg/day and a non-iron-containing multivitamin are recommended in affected patients of all age groups.
- Prompt treatment of acute illness and alertness to symptoms of severe anaemia and haemolysis are essential for the prevention and management of acute haemolytic crisis. Patients with fever should be evaluated by a physician.
- All immunization according to the national programmes and regular vaccinations against common infections, such as influenza, pneumococcus, SARS , and hepatitis B, are strongly recommended to prevent infections.
- Recommended follow-up intervals for NTD patients:
  - Baseline Hb>80 g/L: every 6 months
  - Baseline Hb≤80 g/L: every 3 to 6 months
- Assessment of growth, bone changes, spleen size and pubertal development are essential at every clinic visit during childhood and adolescence.
- Complete blood count with reticulocyte count, haemolytic markers, serum ferritin, transferrin saturation, and liver enzymes should be checked with every visit.
- Red blood cell transfusion at a volume of 10-15 ml/kg should be considered one or more times to manage acute haemolytic episodes in patients of all ages when Hb <70 g/L with an aim to restore Hb to 80-90 g/L.
  - It is recommended that "effective" haemoglobin be measured at steady state. Effective - - haemoglobin can be calculated as total Hb x (1- [HbH % + Hb Bart's %] / 100).
- Regular blood transfusions are considered for prevention of significant growth failure, facial bone changes, failure of secondary sexual development, and massive splenomegaly in paediatric patients. They should also be considered for patients with following:
  - Hb at steady-state <70 g/L
  - Hb at steady-state 70-80 g/L with the presentation of symptoms before 2 years of age and/or spleen size ≥3 cm below costal margin
- A pre-transfusion haemoglobin target of 80-90 g/L is acceptable in most patients. However, those with high proportion of circulating HbH and those with ineffective erythropoiesis may require higher pre-transfusion haemoglobin targets.
- Periodic re-assessment of TD paediatric and young adult patients is critical for tapering off or withdrawing blood transfusion when a sustained clinical benefit is achieved.

- Adult patients with non-deletional HbH typically do not require regular blood transfusion due to mild to moderate anaemia.
- Frequent transfusions may be considered in more severely affected adult patients for primary prevention of disease-related complications and for improvement of their quality of life.
- Regular blood transfusion should be considered for managing complications such as thrombotic diseases, cerebrovascular complications, and pulmonary hypertension.
- Monitoring and management of transfusion-dependent (TD) patients should be performed similar to patients with transfusion-dependent β-thalassaemia.
- Splenectomy can increase Hb levels and decrease or eliminate the need for transfusions in patients with HbH-CS. Splenectomy should be avoided in patients younger than 5 years. The procedure should be reserved for patients with severe anaemia and limited access to blood transfusions, hypersplenism with anaemia, leukopenia or thrombocytopenia resulting in infections or bleeding, or massive splenomegaly with left upper quadrant pain that increases the risk of splenic rupture.
- Prophylactic use of low-dose aspirin is recommended for all patients who have undergone splenectomy to prevent post-splenectomy thromboembolic events.
- Patients with non-deletional HbH disease are at a higher risk of developing gallstones due to chronic haemolysis. Cholecystectomy is typically indicated only in the presence of symptoms or cholecystitis.
- Iron overload is a common complication in non-deletional HbH disease due to increased gastrointestinal absorption.
- For non-transfusion dependent patients, monitor serum ferritin levels with every clinical visit and measure LIC with MRI if ferritin is >300 ng/mL. Iron chelation should be started if LIC >5 mg/g dry weight or ferritin >500 ng/mL.
- Patients with α-thalassaemia experience a higher prevalence of endocrine complications compared to the general population. Iron overload is a primary risk factor for the development of endocrine complications in thalassaemia. Patients with non-deletional HbH disease should be monitored similar to those with β-thalassaemia.
- If onset of puberty is delayed >2 years or if there is concern for slow growth, obtain family history and consider taking an x-ray for bone age. DXA scan should be done every 3 years (or more frequently if indicated) starting at 12 years.
- ECHO should be offered to patients starting at 10-12 years to assess for pulmonary artery pressure. If normal, repeat every 3-5 years. More frequent assessments may be needed at older ages.
- Splenectomy, aging, and inadequate blood transfusion are well-established risk factors for thrombosis in patients with thalassaemia.
- Exacerabtion of anaemia requiring transfusion is common during pregnancy and risk of preterm birth, foetal growth restriction and low birthweight are increased in pregnant mothers with non-deletional HbH disease. Partners should be screened for α-thalassaemia to evaluate foetal risk for haemoglobin Bart's hydrops foetalis.
- Homozygous HbCS may present with hydrops foetalis and/or neonatal haemolytic jaundice/anaemia requiring treatments during the first few months of life. These symptoms spontaneously recover to become a mild HbH-like phenotypes with Hb > 90 g/L.
- Other regional common non-deletional variants in homozygote forms or in compound heterozygotes with α^{+}-thalassaemia most often result in mild to moderate HbH-like phenotypes.

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4 PRENATAL MANAGEMENT OF HAEMOGLOBIN BART'S HYDROPS FOETALIS

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Introduction

Haemoglobin Bart's hydrops foetalis is the most severe form of α-thalassaemia. It results from homozygosity for deletion of both α-globin genes (--/--) on chromosome 16.

Following the completion of primitive erythropoiesis in the first 8–10 weeks of embryonal life, embryonic ζ-genes (HBZ) are silenced and α-globin replaces the ζ-globin for the production of foetal haemoglobin (HbF, α2γ2). With the deletion of all four α-globin genes in a foetus with haemoglobin Bart's hydrops foetalis, α-globin chains are not being produced. In the absences of α-globins, HbF is not formed and, instead, the excess γ-globins form a tetramer haemoglobin (γ4), referred to as haemoglobin (Hb) Bart's. The Hb Bart's has extremely high oxygen affinity, impeding the release of oxygen to the developing foetal tissues and is essentially non-functional. The profound anaemia and tissue hypoxia leads to development of foetal hydrops in the second or third trimester, as well as other complications like neurodevelopmental compromise and congenital birth defects. **Consequently, most pregnancies that are not treated with foetal transfusions are nonviable and will result in foetal demise or neonatal death shortly after delivery [1–4]. Rare survivors who have not received intrauterine transfusion have been reported after a medically unstable neonatal period. It is thought that continued low-level expression of embryonic ζ-gene and persistence of Hb Portland (ζ2γ2) throughout pregnancy may provide some degree of tissue oxygenation in these patients. As such, homozygosity for deletions that also encompass HBZ (e.g. --FIL or --THAI deletions) are incompatible with life, and affected mothers may present with recurrent early pregnancy loss [5].**

In addition to severe clinical consequences of haemoglobin Bart's hydrops foetalis on the foetus, this condition also affects mothers. Maternal mirror syndrome is a known complication in pregnancies affected with hydrops foetalis, regardless of etiology. This complication is characterized by the development of maternal oedema, proteinuria, and hypertension [6]. Dystocia and postpartum hemorrhage due to placental enlargement are among other maternal complications. In addition, the significance of the psychological burden of pregnancies affected by this condition cannot be understated.

Diagnosis

Affected pregnancies that have not been diagnosed through screening process (see Chapter 14: Prevention and control of alpha-thalassaemia diseases) may present with pregnancy loss, or features of hydrops in the second or third trimester. The diagnosis of hydrops can be reflected by ultrasound findings through increased cardiothoracic ratio (≥0.5), enlarged placenta (>18mm before 15 weeks and >30mm after 18 weeks), other signs of hydrops (fluid collection in any one compartment including pericardial effusion, pleural effusion, ascites, or skin edema), elevated middle cerebral artery peak systolic velocity (MCA-PSV), amniotic fluid abnormalities (oligohydramnios and polyhydramnios), fetal growth restriction and echogenic bowel. MCA-PSV >1.5 multiples of the median (MoM) for gestational age is suggestive of moderate to severe anaemia. Of note, MCA-PSV is less reliable for assessing anaemia in haemoglobin Bart's hydrops foetalis compared with other anaemias, especially in early stages of hydrops [7-9].

Other ultrasound observations that may be identified even before development of hydrops include urogenital abnormalities in males. In addition, digital and limb anomalies may be seen, and a subset of patients are found to have atrial septal defect. Other anomalies have less frequently been reported and are not clearly associated with haemoglobin Bart's hydrops foetalis [10].

A diagnosis of haemoglobin Bart's hydrops foetalis is confirmed by molecular testing by chorionic villus sampling, amniocentesis or foetal blood demonstrating the deletion of all four α-globin genes or presence of >80% of Hb Bart's, < 20% Hb Portland, and complete absence of HbF on haemoglobin electrophoresis performed on foetal cord blood obtained from the percutaneous umbilical blood sampling (PUBS) [11, 12]. In the absence of prenatal diagnosis, observation of hydrops with or without elevated MCA-PSV on foetal ultrasound during the second or third trimester and microcytosis or hypochromia in both parents are highly suggestive of this diagnosis. Haemoglobin Bart's hydrops foetalis is the most common cause of foetal hydrops in Southeast Asia, accounting for 60% to 90% of the cases [2].

Decision making process

Once an affected pregnancy has been identified, detailed and non-directive counselling should be provided and all management options should be discussed. Possible options include foetal therapy with intrauterine transfusions, continuation of pregnancy without intrauterine transfusion, or termination of affected pregnancy [13].

Active management of affected pregnancies with early prenatal diagnosis and intrauterine transfusions (IUT) can produce viable offspring. Outcomes in the foetus and neonate correlate with the adequacy of prenatal transfusion management. Active management with IUT may resolve the complication of foetal hydrops, improve the delivery outcomes, and should in turn reduce the overall risk for maternal complications due to foetal hydrops [3, 5, 14–18]. Intrauterine transfusions have not been demonstrated to affect the incidence of structural findings, which suggests that hypoxia during organogenesis may

be a factor. Furthermore, concerns regarding neurodevelopmental compromise due to sustained in utero hypoxia persist [5]. There is an emerging body of evidence that supports the impact of IUT to preserve neurodevelopment [17]. A review of outcomes following IUT in 14 pregnancies with hydrops foetalis demonstrated generally favourable outcomes, with mild delays in four (29%) and normal neurodevelopmental assessment in 10 (71%) [15].

For those patients that elect to proceed with foetal transfusions, the expectations regarding outcomes including the delivery of a child with lifelong chronic transfusion needs should be discussed. Furthermore, the possible effect of structural findings or neurodevelopmental compromise on patient's function and quality-of-life should be reviewed. Counselling should be done in the context of available resources in the community. Referral to paediatric haematology is strongly recommended for detailed counselling regarding long-term prognosis and management [13].

Given risks for severe maternal complications in the setting of untreated hydrops foetalis, expectant management should be discouraged, however for patients electing for expectant management, maternal risks due to mirror syndrome should be emphasized. Close surveillance to mitigate these risks are essential [13].

Regardless of the decision, extensive psychosocial support should be offered. Education about options for future pregnancy should also be provided, including the use of in vitro fertilization (IVF) with preimplantation genetic testing (PGT-M).

Prenatal management

If the family elects to pursue IUTs, they should be referred to a centre with expertise in this technique if it is not available locally [13].

Data from case series in which pregnancies with haemoglobin Bart's hydrops foetalis were actively managed provide support for early initiation of optimally provided IUT to improve outcomes. In a 2021 report of outcomes in a cohort of pregnancies with haemoglobin Bart's hydrops foetalis diagnosed prenatally, the majority of cases in which IUT was used prior to 28 weeks resulted in survival with full resolution of hydrops at delivery. Of the foetuses not treated with IUT, none survived. Four fetuses were treated with IUT starting after 28 weeks; two survived and two died due to complications of hydrops at delivery [15].

If patients elect to proceed with foetal therapy, IUT should be initiated as soon as technically possible (18 weeks at most foetal treatment centers) to minimize the long-term impacts of foetal hypoxia [13]. The aim of IUT regimen should be to prevent or reverse foetal hydrops, allow for normal growth and development of the foetus, and eliminate maternal complications that are associated with hydrops. While the protocol for IUTs is similar to standard protocols used for haemolytic disease of the foetus and newborn (HDFN) [19], there is one important difference: in haemoglobin Bart's hydrops foetalis, the foetal haemoglobin concentration does not represent the amount of functional haemoglobin in foetal

red blood cells. This is because the predominant haemoglobin (Hb Bart's), which accounts for > 80% of haemoglobin in the faetus, is functionally useless in oxygen delivery due to its extremely high oxygen affinity [2, 5]. Thus, in addition to the need to correct anaemia, one should also consider the amount of non-functional Hb Bart's and, ideally, this should be kept at <20% of total haemoglobin. Nevertheless, the optimal protocol establishing the transfusion volume or their frequency for the treatment of foetuses affected with haemoglobin Bart's hydrops foetalis remains an area of continued research. It is likely that more frequent transfusions are needed shortly after the diagnosis of hydrops while less frequent transfusions may be needed with next transfusions. We do not administer phenobarbital, as is done by some experts for foetuses with HDFN because severe hyperbilirubinemia does not occur in those who have received sufficient transfusions.

Intrauterine haploidentical haematopoietic stem cell transplant using maternal bone marrow enriched for CD34+ cells is being studied in a phase I clinical trial. Maternal stem cells are administered as a one-time infusion immediately before the RBC transfusion through the umbilical vein at the time of IUT (see Chapter 13: Novel and emerging therapies for alpha-thalassaemia).

Delivery considerations

Delivery should occur in a facility that can provide high-level critical care. Typically, this involves a tertiary care centre with specialized perinatology, paediatric haematology, and/or neonatology teams that can provide emergency management if needed. Some neonates may need aggressive resuscitation and mechanical ventilation at birth, although this can generally be precluded by an adequate IUT programme. Most of these neonatal complications (except congenital anomalies) can be prevented with adequate IUT starting early during gestation [13].

Cord blood haemoglobin and Hb Bart's are tested at birth. Hb Bart's can be measured by electrophoresis, high-performance liquid chromatography (HPLC), or capillary electrophoresis [20]. This may be helpful in appreciating the impact of foetal transfusion to reduce haemoglobin Bart's and calculate the functional haemoglobin. The cord blood haemoglobin value will vary based upon the interval from the last IUT. The proportion of Hb Bart's at birth depends upon adequacy of IUT and the duration between last transfusion and birth.

Delayed cord clamping should not be performed due to ineffective haemoglobin in the foetal blood.

Postnatal care

The neonatal course and outcome in the setting of haemoglobin Bart's hydrops foetalis is greatly affected by the timing and effect of the IUT approach [15, 17]. Early delivery may be indicated when the foetus remains hydropic, with limited or no foetal transfusions accomplished. Alternatively, it may occur at or near-term gestation following resolution of hydrops foetalis.

Overall, the neonatal course is frequently complicated by varying degrees of respiratory distress, pulmonary hypertension, and hyperbilirubinemia. Newborns are also often small for gestational age due to early foetal hypoxia, and they may have associated birth defects, as noted above. The primary approach to treatment of the newborn with haemoglobin Bart's hydrops foetalis is serial red cell transfusions, but the specific transfusion approach will be highly dependent on the timing of delivery in relationship to the last IUT and the degree of illness. Acute cardiopulmonary illness is often responsive to increasing oxygen delivery through decreasing ineffective Hb Bart's fraction and increasing effective haemoglobin with transfused red blood cells, which may require exchange transfusion in some cases. **For these reasons, planned delivery at a centre with expertise in neonatal resuscitation and paediatric haematology, and with a blood bank and clinical laboratories that can support urgent neonatal transfusions and the indicated laboratory evaluations is important.**

Neonatal resuscitation and early management

Infants born with hydrops are likely to also be affected by prematurity, with critical illness due to cardiopulmonary failure. If urgent delivery is indicated at this point in pregnancy, every effort should be made for delivery to occur in an experienced neonatal centre with ready access to blood products appropriate for neonatal transfusion as emergent transfusion may be required. If time allows, emergency packed (high haematocrit) red blood cells should be prepared and available prior to delivery, to allow for urgent administration during neonatal resuscitation if needed.

Initial management of the newborn with hydrops includes early/immediate intubation, evacuation of pleural effusions, if present, and surfactant administration, given the effect of hydrops on delayed lung maturation. If ascites has been documented and the abdomen is taut or thought to be impeding diaphragmatic excursion, paracentesis can be performed. Pulmonary hypertension may complicate respiratory disease. Rapid placement of umbilical arterial and venous lines facilitates resuscitation and provides access for transfusion. Initial transfusion consists of packed red blood cells ~10 mL/kg (using estimated dry weight, and potentially divided into 5 mL/kg aliquots) after sampling of blood to document baseline haemoglobin and haematocrit, complete blood count (CBC) with manual differential for assessment of nucleated red blood cell (NRBC) count and haemoglobin electrophoresis for haemoglobin Bart's fraction. Blood sample can be sent directly from cord blood at the time of delivery.

Importantly, baseline haemoglobin alone does not determine the need for initial transfusion, and additional transfusion or possible exchange transfusion may be required as total haemoglobin includes a fraction of haemoglobin Bart's, which is ineffective for oxygen transfer. "Effective" haemoglobin is the target for red cell transfusions and its calculation requires the quantification of Hb Bart's and HbH fraction [21]. Effective haemoglobin is calculated as follows:

Effective haemoglobin (g/L) = (1 - % Hb Bart's + % HbH) x total haemoglobin (g/L)

As noted, the approach to transfusion should be based on the history and timing of in utero red cell transfusions, while also considering the level of acute illness. By increasing oxygen carrying capacity, red cell transfusion often results in decreased severity of cardiopulmonary illness in newborns with haemoglobin Bart's hydrops foetalis, with improved markers of oxygenation and decreased metabolic acidosis, if present. Those newborns with only a single in utero transfusion and more severe critical illness should be considered for urgent transfusion (as above), and a more aggressive postnatal transfusion approach utilizing direct or exchange transfusion, even if Hb Bart's fraction result is not yet available. Those newborns with two or more in utero transfusions may be able to be managed more judiciously, even if hydrops has not fully resolved. Additional transfusions may await laboratory results with respect to Hb Bart's fraction. Alternatively, given that haemoglobin electrophoresis is not necessarily available every day from the clinical laboratory, even at quaternary paediatric centres, the kinetics of response to in utero transfusions (i.e., haemoglobin Bart's fraction from baseline PUBS results and its change with subsequent transfusions) can be used to inform additional empiric transfusions by estimating the baseline haemoglobin Bart's fraction until laboratory results are available.

For term and near-term infants born without hydrops foetalis or following its resolution, a neonatal team should be prepared to provide resuscitation if needed. Placement of umbilical lines is useful for initial haematological management and/or in case of acute cardiopulmonary illness. Respiratory support should be provided as needed with consideration of surfactant administration based on risk factors for lung immaturity. Baseline haematological labs should be sent from cord blood at the time of delivery (preferably), or soon after from the newborn if cord blood is not obtained. Early red cell transfusion should be anticipated (see section on Transfusion, below), even in the case of a newborn without acute illness.

As noted, newborns with haemoglobin Bart's hydrops foetalis are at risk for pulmonary hypertension. Newborns with respiratory distress or cyanosis should have pre- and post-ductal oxygen saturations monitored to assess for physiology consistent with persistent pulmonary hypertension of the newborn (PPHN). A pre-ductal (usually right upper extremity) oxygen saturation > 5% higher than a post-ductal (lower extremity) saturation raises concern for PPHN, which can be further investigated by echocardiography. Increase in effective haemoglobin via direct or exchange transfusion improves severity of pulmonary hypertension. Although extracorporeal life support (ECLS) is not necessarily contraindicated in term and near-term newborns, transfusion may result in clinical improvement to a level that averts the need for ECLS and should be strongly considered prior to ECLS cannulation unless the clinical course is complicated by other factors that exacerbate the level of cardiopulmonary illness (e.g., sepsis, meconium aspiration syndrome). Lung hypoplasia has been reported, which could preclude recovery despite ECLS support.

Transfusion

Serial red blood cell transfusions are the foundation of treatment for haemoglobin Bart's hydrops foetalis. Goal effective haemoglobin nadir (prior to transfusion) is 90-100 g/L and chronic haemoglobin Bart's fraction < 20%. This minimal effective haemoglobin goal results in sufficient oxygen carrying capacity to support normal infant growth and development and it suppresses endogenous erythropoiesis, thereby limiting dysfunctional haemoglobin Bart's production. As a result, the post-transfusion target of effective haemoglobin is 110-120 g/L. In the neonatal period, this is typically accomplished with early (in the first several hours of life) and subsequent packed red blood cell transfusions of 5-10 mL/kg in an infant without critical illness, or following resolution of critical illness. Serial CBC with manual differential to quantify NRBC should be collected to measure total haemoglobin and follow the trend of NRBC counts, which should fall with increasing suppression of erythropoiesis, due to the effective haemoglobin from transfused RBC. Haemoglobin electrophoresis should be obtained to aid clinicians in the decision for red blood cell transfusion and to monitor for chronic response to transfusion therapy. Consultation with Paediatric Haematology will inform the timing of transfusions.

Exchange transfusion may be considered for treatment of critical illness, or if direct transfusion to meet the goal of 90-100 g/L effective haemoglobin results in a total haemoglobin > 220 g/L or symptoms of polycythemia. Continued monitoring of CBC with NRBC counts and haemoglobin electrophoresis should follow exchange transfusion.

Hyperbilirubinemia

Hyperbilirubinemia requiring phototherapy is common due to haemolysis of haemoglobin Barts-containing endogenous RBC and repeated red cell transfusions. Elevated risk for kernicterus may also be present due to prematurity, low birth weight and acute illness, prompting use of "high-risk" thresholds for initiation of phototherapy from published nomograms. Serum bilirubin levels should be followed closely.

Echocardiography

Echocardiography should be performed during the neonatal hospitalization. It can support the diagnosis of PPHN or identify less severe pulmonary hypertension, and it can be used to monitor resolution of high cardiac output and increased chamber size with increasing effective haemoglobin. Regardless of level of illness, echocardiography is important for identifying and monitoring of any structural heart disease (particularly patent ductus arteriosus or secundum atrial septal defect, which cannot be diagnosed by foetal echocardiogram).

Neurologic evaluation and brain imaging

Brain injury due to foetal hypoxia, perturbed circulation with hydrops foetalis and postnatal illness, as well as other central nervous system findings, have been described. A neurological evaluation and brain MRI should be considered prior to hospital discharge to provide additional information for the family and to direct early developmental intervention, as infants and children with haemoglobin Bart's hydrops foetalis are at risk for developmental delay.

Associated birth defects

For the most part, the anomalies associated with haemoglobin Bart's hydrops foetalis do not require intervention during the neonatal hospitalization. While the exact underlying pathophysiology has not been fully elucidated, many of these structural defects are thought to be due to profound foetal hypoxia starting from the early foetal period. Limb anomalies, with nail hypoplasia, digital hypoplasia, or aplasia and distal limb amputation have been described in multiple reports, with more severe limb amputation described in cases of foetal demise. Genitourinary anomalies include hypospadias and cryptorchidism, but more severe forms including severe chordae and micropenis have been observed [10, 15]. These genitourinary anomalies are commonly described in male newborns with haemoglobin Bart's hydrops foetalis. Jejunal atresia, atrial septal defect, and neuronal migration abnormalities have also been described.

Ongoing care and post-discharge follow-up

Once the newborn has stabilized from a cardiopulmonary and hematological standpoint, enteral feeds can be initiated and advanced consistent with gestational age and centreapproaches. Newborns are ready for discharge to home when they meet standard criteria and have established an appropriate growth trajectory.

In addition to a primary paediatrician, follow up should include referral to an experienced thalassaemia centre as infants undergo serial red cell transfusions with consideration for bone marrow transplant as definitive cure. Paediatric subspecialty follow-up for any anomalies should be arranged (e.g., urology, orthopedics). As infants and children with haemoglobin Bart's hydrops foetalis (α-thalassaemia major) are at risk for developmental delay, close surveillance of the neurodevelopmental trajectory should occur by the child's paediatrician, with consideration of referral to paediatric neurology, a high-risk infant follow-up clinic or other developmental screening centre, and physical and occupational therapy.

Hydrops foetalis due to rare forms of non-deletional haemoglobin H disease or homozygosity for mutational α^{+}-thalasssaemia

While individuals with deletional haemoglobin H (HbH) disease and most patients with non-deletional forms of HbH disease do not require intensive foetal management, rarely, compound heterozygosity for a^{0}-thalassaemia deletion and rare α^{+}-thalassaemia mutations result in a very unstable haemoglobin. This can result in severe ineffective erythropoiesis and anaemia starting from the foetal period, leading to hydrops foetalis (HbH hydrops foetalis). Similarly, homozygosity for some non-deletional α^{+}-thalassaemia mutations (e.g., Hb Constant Spring) can present with hydrops, although the majority of these patients will become non-transfusion dependent later in life [22, 23]. Prenatal screening and diagnosis of these patients are discussed in Chapter 14. Management of hydrops in these patients is similar to those with haemoglobin Bart's hydrops foetalis, as discussed above.

Summary and recommendations

- Hydrops foetalis in the setting of a pregnancy in parents who are known carriers for α-thalassaemia is suggestive of foetal haemoglobin Bart's hydrops foetalis in most cases. In this situation, the diagnosis should be confirmed as quickly as possible so that parents can be counselled in a timely manner.
- If the family wishes to pursue intervention, an initial IUT can be performed while awaiting the diagnosis.
- In a pregnancy with a confirmed diagnosis of haemoglobin Bart's hydrops foetalis, nondirective counselling should include the option of expectant management, pregnancy termination, or foetal therapy with IUTs. Parents should be offered the opportunity to consult with a paediatric haematologist to understand the long-term outcomes, prognosis, and the requirements of postnatal and childhood management.
- If the family elects to pursue IUTs, they should be referred to a centre with expertise in this technique if it is not available locally.
- For patients electing to proceed with foetal therapy, IUTs should begin as soon as technically possible.
- Delivery should be planned at a tertiary care centre with availability of perinatology, paediatric haematology, and neonatology teams.
- While general principle of long-term management of patients with haemoglobin Bart's hydrops foetalis is similar to those for transfusion dependent β-thalassaemia, the transfusion requirement for patients with haemoglobin Bart's hydrops foetalis is different given the presence of non-functional Hb Bart's. **Table 1** summarizes the steps in management of newborn with diagnosis of haemoglobin Bart's hydrops foetalis.

Table 1. *Steps in management of newborn with diagnosis of haemoglobin Bart's hydrops foetalis (α-thalassaemia major)*

Delivery at high-level paediatric centre Expertise in neonatal resuscitation Access to emergency blood products Ideally, clinical laboratories to support ongoing hematologic evaluation and therapy. If not available at delivery centre, transfer to appropriate centershould be arranged following initial resuscitation.
Stabiliazation and management of acute illness
Serial transfusion/exchange transfusion and monitoring haematology labs in consultation with paediatric haematologist
Monitoring and treatment of hyperbilirubinemia
Evaluation of associated anomalies and brain injury with paediatric subspecialty consultation if indicated
Referral to thalassaemia centre for follow up care after hospital discharge

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5 LONG-TERM MANAGEMENT OF α-THALASSAEMIA MAJOR (HEMOGLOBIN BART'S HYDROPS FOETALIS)

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In this chapter, we will review the specifics of long-term management for patients with α-thalassaemia major (haemoglobin Bart's hydrops foetalis). We advocate for using the term "α-thalassaemia major" for long-term survivors, as these patients no longer exhibit the features of hydrops or produce Hb Bart's. Perinatal management of patients with haemoglobin Bart's hydrops foetalis is discussed in Chapter 4, while curative therapy for patients with α-thalassaemia major is addressed in Chapter 12.

Introduction

Once an infant with α-thalassaemia major (haemoglobin Bart's hydrops foetalis) survives the neonatal period, they will become lifelong transfusion-dependent, unless they are cured by haematopoietic stem cell transplantation. Generally, the management of patients with α-thalassaemia major follows the same principles as those for patients with transfusion-dependent β-thalassaemia, involving regular blood transfusions and effective iron chelation. However, there are important differences in the pathophysiology of α-thalassaemia major and β-thalassaemia major, necessitating a specific approach.

1. Due to the physiologic switching of γ-to-β globin synthesis within the first few months of life, tetramers of β-globin (β_4, HbH) gradually replace the Hb Bart's (γ4), which was the predominant haemoglobin in a foetus with haemoglobin Bart's hydrops foetalis. Consequently, endogenous erythrocytes of a patient with α-thalassaemia major are almost completely composed of HbH [1]. These HbH-containing erythrocytes are susceptible to early haemolysis leading to intramedullary erythroid cell death (ineffective erythropoiesis), but more prominently, haemolysis in peripheral circulation [2, 3]. As a result, patients with transfusion-dependent α-thalassaemia major exhibit significant reticulocytosis in contrast to patients with β-thalassaemia major [3].

2. Similar to Hb Bart's, HbH has an extremely high oxygen affinity and does not participate in transportation of oxygen to the tissues [4]. Thus, in patients with transfusion-dependent α-thalassaemia major, the total haemoglobin concentration does not accurately represent tissue oxygenation. When regularly transfused according to protocol that are developed for β-thalassaemia major, patients with α-thalassaemia major continue to have a high proportion of circulating non-functional HbH and display features of hypoxia, haemolysis, and ineffective erythropoiesis [3]. Ultimately, the resulting tissue hypoxia and increased haemolysis lead to

chronic organ dysfunction and clinical complications. As the result, patients with α-thalassaemia major require a more aggressive transfusion regimen. This "hypertransfusion" approach aims to reduce the production of endogenous HbH-containing erythrocytes and improve "functional" haemoglobin (non-HbH haemoglobin). This approach has been shown to be associated with reduced haemolysis and improved tissue oxygenation. However, such a regimen results in a higher degree of iron load and its associated costs and complications [3].

3. All infants with α-thalassaemia major are transfusion-dependent from birth, and most likely they have already received transfusions in the prenatal period. This is in contrast to the majority of patients with β-thalassaemia major who do not require transfusion until at least six to nine months of age. The early initiation of blood transfusions, coupled with the need for more aggressive transfusion, results in early iron overload and inevitably the need for early iron chelation [5, 6]. Both early iron overload and early initiation of iron chelation at a young age have been shown to be associated with long-term complications [7-9]. In a small cohort of adolescents with α-thalassaemia major, a high rate of endocrinopathies and growth failure has been observed [5].

4. Patient with α-thalassaemia major exhibit an increased rate of congenital birth defects and almost all males have genitourinary abnormalities with a varying degree of severity. In addition, a higher rate of neurodevelopmental compromise has been observed in patients with α-thalassaemia major [10].

Transfusion for patients with α-thalassaemia major

Due to preserved reticulocytosis and high proportion of circulating non-functional HbH, patients with α-thalassaemia major require a more aggressive transfusion strategy as compared to patients with β-thalassaemia major. However, the best transfusion targets for these patients are not well defined. One case-series has suggested that maintaining pre-transfusion functional haemoglobin (non-HbH haemoglobin) > 105 g/L and HbH proportion < 15% are required to suppress ineffective erythropoiesis. These targets however were associated with significant increase in the required transfusion volume and consequently transfusional iron load, thus its haematological benefit may be offset by complications of iron overload [11]. **It is likely that pre-transfusion functional haemoglobin of 90-100 g/L and HbH at < 15% are sufficient to offer long-term clinical benefit without excessive iron load. Nevertheless, it is important to calculate the non-functional haemoglobin periodically (ideally with every transfusion) and adjust transfusions as required. Functional haemoglobin can be calculated as follows:**

Functional Hb = Total Hb x (1- [HbH % + Hb Bart's %] / 100)

We recommend HbH (and HbH Bart's) be measured using capillary zone electrophoresis, ideally on a fresh sample. HPLC can also be used; however, it is less accurate as bilirubin may interfere with the measurement of HbH or Hb Bart's on HPLC [12]. If an automated haemoglobin analyzer is not available, peripheral blood smear using a supravital stain can be used to calculate the proportion of endogenous HbH-containing cells vs. HbA-containing transfused red blood cells. This method is not completely

accurate as HbH-containing red blood cells have lower mean corpuscular haemoglobin (MCH) as compared to transfused red cells; nevertheless, this can be helpful.

If HbH cannot be reduced to < 15% with simple transfusions, sessions of exchange transfusion can be considered. Alternatively, some centres have used regular exchange transfusions to reduce the burden of iron overload [13]. Such an approach is associated with higher volume of blood consumption and may not be feasible in most settings. Centres lacking access to precise HbH measurements and, as a result, are unable to calculate functional haemoglobin may consider maintaining pre-transfusion total haemoglobin at 105-110 g/L and reticulocyte count < 500 x 10e9/L.

Some centres have advocated for a more aggressive transfusion protocol for the first few months of life, in order to effectively supress erythropoiesis and reduce splenomegaly with the intention to eventually decreased the volume of transfusions in the long-term [14]. In this approach, total pretransfusion haemoglobin is maintained at >120 g/L (non-functional haemoglobin at 15–20% and the functional >100 g/L) for the first few months of life. In this approach, the transfusion interval is initially two weeks and gradually increases to three weeks. After the first six months, infants will be transitioned to the chronic transfusion protocol, as reviewed above. The long-term benefit of this approach needs to be further studied as such an aggressive transfusions in infants may be associated with expedited iron overload.

Finally, RBC antigens should be determined by DNA testing so that antigen-matched blood can be provided to reduce the risk of alloimmunization. For detailed review of principles of transfusions, please refer to the *Thalassaemia International Federation Guidelines for the Management of Transfusion Dependent Thalassaemia* [15].

Iron overload and chelation

As reviewed previously, in patients with α-thalassaemia major, transfusional iron overload occurs early, within a few months of birth. The management of iron overload in α-thalassaemia major, especially in younger infants and children is not well-defined. Until further data becomes available, it is advisable to postpone chelation until at least 12 months of age given concerns over inflammation of the liver and immaturity of the kidneys and the lack of experience with iron chelators during the first year of life. Iron chelating agents are not approved for use in children under 2 years, but delaying chelation in α-thalassaemia major until 2 years of age could be harmful.

The goal of chelation is to maintain liver iron concentration to **< 7 mg/g of dry weight, and ideally between 2-5 m/g dry weight,** measured through magnetic resonance imaging (MRI). When available, we recommend measuring LIC from first year of life and on an annual basis and assessing cardiac iron through cardiac T2*MRI if LIC is > 7 mg/g dry weight, or starting at 10 years of life and, again, annually. Note that there are no data on the iron overload in patients with α-thalassaemia major.

Serum ferritin is not a reliable marker for the degree of iron overload, especially in the first few months of life [14]. Furthermore, ferritin can underestimate the degree of iron overload in transfused patients who continue to have ineffective erythropoiesis [16]. Its trend is however a useful tool for evaluating the effectiveness of iron chelation. In the absence of access to non-invasive imaging modalities for assessment of LIC, serum ferritin should be in the range of 1000 to 2000 ng/mL in the second year of life, and 800-1500 ng/mL thereafter. An attempt to achieve lower ferritin levels in the second year of life may increase the risk of toxicity from chelation. Transaminitis is the limiting toxicity of deferasirox in infants, and it can be difficult to distinguish from other causes of transaminase elevation such as iron overload or acute viral infection.

We recommend starting iron chelation with deferasirox at low dose (Jadenu, 3 to 5 mg/kg, usually 45 mg, crushed tablet, or equivalent dosing for Exjade) and increase the dose by 45 mg every two months as long as transaminases are stable. The dose is increased up to a maximum of 14 mg/kg until the child is 2 years old, following which the general guidelines for deferasirox are followed. Please refer to Chapter 11 (Iron Overload in α-thalassaemia) and the *Thalassaemia International Federation Guidelines for the Management of Transfusion Dependent Thalassaemia* for more detailed review [15]. We do not recommend aggressive iron chelation before 2 years of life.

Deferiprone should be reserved for where deferasirox is not available or accessible, or when deferasirox is not tolerated at therapeutic doses. Dosing of deferiprone in young children follows the same approach as for deferasirox, as reviewed above. Deferiprone can be added as a second chelator at a dose of 50 to 75 mg/kg. Safety and efficacy of deferiprone in children <2 years with β-thalassaemia major, sickle cell disease, and other anaemias have been reported [17, 18] but no data are available for patients with α-thalassaemia major.

Deferoxamine can be used in combination with one of the other chelators in children with severe hepatic iron overload however, it is not the preferable chelation in infants and toddlers given potential long-term side effects. The dose of deferoxamine is limited to 20 to 30 mg/kg as a subcutaneous infusion on three to five days per week to reduce the risk of toxicity affecting bones and growth [8, 9, 19].

Once on iron chelation, patients should be monitored regularly for changes associated with iron overload or chelation. Liver enzymes and biochemical markers of kidney function should be checked with every interaction. Urinalysis for measurement of urine protein should be done every three months. Hearing test and retinal examinations should be performed annually.

Other issues

Growth

High rates of growth delay and growth failure has been reported in patients with α-thalassaemia major [5]. Close monitoring of growth, regular surveillance with biochemical tests, routine assessment of bone age (X-ray of wrist and hand), and early referral to specialists are recommended. Consideration should be given to optimizing transfusions and iron chelation if these have not been achieved yet due to limitations in resources or suboptimal patient adherence.

Endocrinopathies and bone health

A study conducted on a small cohort of adolescents with α-thalassaemia major has reported a high rate of endocrine abnormalities and decreased bone mineralization in these patients [5, 10]. While the exact cause of this observation is not clear, several factors such as chronic hypoxia, iron overload, early initiation of iron chelation, or periods of foetal hypoxia could have contributed to these outcomes.

Patients with α-thalassaemia major should be strongly encouraged to ensure they have a sufficient daily intake of vitamin D and calcium.

Routine investigations should include semi-annual measurements of extended electrolytes, including serum calcium and phosphate. Additionally, thyroid function tests (thyroid-stimulating hormone and free thyroxine), assessment of the hypothalamic-pituitary-gonadal axis, glucose levels and HbA_{1c}, measurements of bone age (X-rays of the wrist and hand), and bone mineral density (BMD) should be conducted at least annually, starting at around 10 years of age. These measures will help in early detection and management of potential endocrine and bone health issues in patients with α-thalassaemia major.

Fertility

There is no report of fertility in adults with α-thalassaemia major. Chronic anaemia and iron overload are known risk factors for reduced fertility. Genitourinary anomalies in males may also contribute to reduced fertility. Referral to endocrinologist for assessment of puberty and fertility is recommended.

Management of congenital abnormalities

Children diagnosed with α-thalassaemia major should undergo comprehensive evaluations for genitourinary, bone, and cardiac anomalies. We recommend that all children with this condition undergo ultrasonography of the genitourinary system, a bone survey, and echocardiography.

Early consultation with appropriate surgical teams, such as plastic surgery or urology, should be arranged to plan for the correction of congenital anomalies.

The most common surgical interventions for urogenital abnormalities include urethroplasty and orchidopexy. Depending on the severity of urogenital defects, staged procedures may be necessary. Transfusion should be offered a few days prior to surgery to ensure adequate oxygenation.

Management of neurodevelopmental compromise

Emerging evidence suggests that children treated with intrauterine transfusions (IUT) generally exhibit improved long-term neurodevelopmental outcomes compared to those who did not receive IUT [5, 20]. However, it's important to note that even children treated with IUT may have experienced a period of severe anaemia early in gestation. This early anaemia could potentially have an adverse impact on neurocognitive development [10]. Additionally, suboptimal post-natal transfusions can lead to a state of chronic hypoxia and haemolysis, which may affect neurodevelopment. In fact, a high rate of ischemic white matter changes has been observed in children and adolescents with α-thalassaemia major [5].

All infants with α-thalassaemia major should be closely monitored by developmental specialists and undergo neurologic assessments. Neurodevelopmental assessments should be repeated during school-age or if there are concerns regarding academic achievements.

If a child with α-thalassaemia major is diagnosed with neurodevelopmental compromise, they should be promptly referred for specialized intervention and appropriate support. The management plan will depend on the extent of intellectual disability and whether any visual, hearing, or motor deficits are present.

Haematopoietic stem cell transplant

As discussed earlier, patients with α-thalassaemia major necessitate more intensive transfusion and iron chelation regimens and experience higher rate of thalassaemia or treatment-related complications compared to individuals with transfusion-dependent β-thalassaemia. Consequently, the option of haematopoietic stem cell transplant should be considered early as a potential curative option for patients with α-thalassaemia major (please refer to Chapter 12: Curative therapies for α-thalassaemia for further details).

Summary and recommendations

- The management of patients with α-thalassaemia major follows the same principles as those for patients with transfusion-dependent β-thalassaemia, involving regular blood transfusions and effective iron chelation. However, patients with α-thalassaemia major require more aggressive transfusion strategies due to preserved reticulocytosis and high proportion of circulating non-functional HbH.
- The goal of a transfusion regimen is to maintain functional haemoglobin (HbA) >90 g/L. Functional haemoglobin should be calculated ideally prior to each transfusion by testing haemoglobin fractions. Centres lacking access to precise HbH measurements and, as a result, are unable to calculate functional haemoglobin may consider maintaining pre-transfusion total haemoglobin at 105-110 g/L and reticulocyte count < 500 x 10e9/L.
- Iron chelation is started at approximately one year of age, with careful monitoring for toxicities. Initiation of iron chelation before 1 year of age is not recommended. Aggressive iron chelation prior to 2 year of age is not recommended.
- Patients with α-thalassaemia major have increased rate of congenital anomalies and neurocognitive compromise. Similarly, a higher rate of growth delay, reduced bone density and endocrinopathies have been reported. Appropriate diagnostic and surveillance testing, and referral to surgical or medical specialist are prudent to improve the outcome of patients with α-thalassaemia major.
- Given the high rate of complications, the option of curative therapy with stem cell transplant should be reviewed early.

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6 FERTILITY AND PREGNANCY IN α-THALASSAEMIA

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Introduction

Although patients with haemoglobin H (HbH) disease are mostly asymptomatic, during periods of stress, such as pregnancy, anaemia may be aggravated and patients may require transfusion [1, 2]. Therefore, regular prenatal care is recommended to improve pregnancy outcomes in women with HbH disease. Furthermore, patients with more severe forms of HbH disease (non-deletional HbH disease) have generally a more pronounced anaemia, require frequent, or rarely regular, transfusions. Chronic anaemia and iron overload may have adverse effect on endocrine function and, consequently, on an individual's fertility. Finally, genetic counselling may be necessary in either pre-conception and prenatal periods for couples at risk of having an offspring with severe forms of α-thalassaemia and some couples seek preimplantation genetic diagnosis for selecting an unaffected offspring.

HbH disease and fertility

Studies on the fertility outcomes of patients with α-thalassaemia are scarce. Individuals with HbH disease have a variable degree of anaemia and ineffective haematopoiesis, which can cause bone changes, and secondary iron overload [3–6]. In general, individuals with the most common form of HbH disease, the deletional form, have a mild degree of anaemia and ineffective erythropoiesis and rarely require red blood cell transfusions in the first decades of life. As a result, iron overload does not develop until adulthood, with little effect on fertility. Patients with more severe forms of HbH disease (non-deletional HbH disease), however, may require frequent or regular transfusions leading to more expedited iron overload. In addition, these patients may have a higher degree of ineffective erythropoiesis, leading to increased gastrointestinal absorption of iron and earlier iron overload. In such patients, endocrine complications can develop, which include growth failure, hypogonadotropic hypogonadism, hypothyroidism, and poor glycemic control or diabetes mellitus. [7–11].

Female fertility

Studies of other conditions that are associated with iron overload have demonstrated that women with severe iron overload are at increased risk of infertility due to the effect of iron deposition in various organs involved in reproduction. Iron overload leads to a disruption at the hypothalamic-pituitary level and limits the pituitary sensitivity to GnRH. Iron free radicals may act as reactive oxygen species causing chronic oxidative stress on the hypothalamic–pituitary axis [12–14]. Apart from endocrine dysfunction

affecting the hypothalamic-pituitary-gonadal axis, oocyte quality and anatomical abnormalities of the genital tract have an effect on female fertility. The anterior pituitary gland is stimulated by gonadotrophin-releasing hormone (GnRH) secreted by the hypothalamus. This creates a pulsatile release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which in turn stimulates the ovary to promote follicular genesis and ovulation, respectively. Several studies showed lower anti-mullerian hormone (AMH) in the transfusion-dependent β-thalassaemia patient, resulting from iron overload on the follicular pool and leading to impaired ovarian function [12, 15]. While there is a lack of data on the fertility outcomes of women with HbH disease, extrapolating from data on other iron loading conditions, it is possible that iron overload also has a negative effect on fertility in HbH disease.

Male fertility

The hypothalamic-pituitary-gonadal axis also has an effect on male fertility. Spermatogenesis is a process that is hormonally driven by FSH and LH. Iron plays an important role in spermatogenesis, including the synthesis of DNA and germ cell growth. Same as many cells, spermatozoa are sensitive to oxidative stress [16, 17]. One study showed that oligospermia or azoospermia in men with transfusion-dependent thalassaemia (TDT) was directly correlated with seminal plasma iron levels [16]. Furthermore, high seminal plasma iron levels were found to be associated with a 10% reduction in sperm motility. Another study reported that a rate of sperm DNA damage was higher in men with TDT than in a control group ($p<0.01$) [18]. This result was similar in men who had delayed chelation therapy. Adequate transfusion have protective effects on male fertility, including improved spermatogenesis, increased sperm counts, improved morphology, and progressive motility. The mechanism is not clearly indicated, but improving erythropoiesis by blood transfusion and oxygenation can be related to this observation.

As reviewed, infertility with iron-overload is mostly reported in patients with β-thalassaemia. In contrast, data on infertility in HbH disease are scarce. However, it is possible that anaemia, ineffective erythropoiesis, and iron overload, as well as other factors, affect the success of pregnancy. Therefore, managing HbH disease (especially those with non-deletional forms or those who require frequent or regular transfusions) and its potential impact on fertility and pregnancy requires a multidisciplinary approach that includes haematologists, fertility specialists, obstetricians, and mental health professionals. Adult patients with HbH disease who wish to start a family should have an early evaluation, especially when there is a prior history of iron overload. If a female with HbH disease with normal menstruation or a male with HbH has not been able to conceive within six months, they should be evaluated by a fertility specialist.

Typically, a fertility specialist will thoroughly assess all possible factors contributing to infertility, regardless of whether they are connected to thalassaemia. This evaluation usually involves an examination of both partners, including deficiency of hormone levels, ovulation process or spermatogenesis, irregularity of sperm movement, or genital tract abnormalities.

Pregnancy outcomes and transfusion during pregnancy in HbH disease

Pregnancy with HbH diseases is often associated with an increased severity of anaemia and adverse maternal and foetal outcomes. Some patients with HbH disease may require blood transfusion during pregnancy [1, 19]. The physiologic changes in pregnancy leading to an expansion of blood volume can aggravate the severity of anaemia. Maternal anaemia is associated with adverse pregnancy and neonatal outcomes, such as intrauterine hypoxia leading to fetal growth restriction, low birth weight, and preterm birth [1, 2, 20, 21]. Noticeably, non-deletional HbH disease has worse pregnancy outcomes than deletional HbH [22, 23].

Transfusion during pregnancy

Individuals with HbH disease who get pregnant should be monitored for exacerbation of anaemia during pregnancy. Supplementation with folic acid is provided prior to and during pregnancy. Iron supplement does not have any benefit in improving maternal anaemia, and can worsen the iron overload in individuals with HbH disease.

It is recommended that maternal haemoglobin be maintained above 80 g/L. When required, transfusion of red blood cells should be performed every 3 to 4 weeks with the aim of maintaining pre- and post-transfusion haemoglobin levels at 90 and 120 g/L, respectively. Few studies have explored the use of recombinant human erythropoietin (rhEPO) if blood transfusions are not safe [1, 19, 24, 25].

Splenectomized patients should be provided prophylactic antibiotics and infection precautions, while low-dose aspirin or other anticoagulants may be used for thrombosis prevention [26].

Iron chelation during pregnancy

Currently, three iron chelators are available for the treatment of iron overload: deferoxamine (DFO), deferiprone (DFP) and deferasirox (DFX), alone or in combination [27–29]. Indications and management of chelation during pregnancy are similar to those for transfusion-dependent β-thalassaemia. Iron chelators are usually not recommended during pregnancy due to the lack of data about foetal development. Deferoxamine is assigned to pregnancy category C (there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks) as delayed ossification and skeletal anomalies were observed in pre-clinical studies in pregnant mice and rabbits. Some studies reported that using DFO did not lead to adverse effects on the foetus or cause major pregnancy complications, though it should be avoided in the first trimester if possible. Deferiprone (pregnancy category D) and deferasirox (pregnancy category C) are not recommended to use during pregnancy due to limited data [28].

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) involves a process to determine if an embryo is affected with the disease in question prior to implantation. It has the benefit that the woman can start her pregnancy knowing that the foetus would unlikely be affected [30, 31]. Currently, PGD or prenatal genetic testing

(PGT) is of three types: (1) PGT-A for aneuploidies; (2) PGT-M for monogenic disorders (including thalassaemia); and (3) PGT-SR for structural chromosomal rearrangements [32]. The main steps of PGT include in vitro fertilization to produce embryos to be tested, sampling of the embryonic cells for the test (embryo biopsy), and laboratory tests. Finally, the embryos with an unaffected result are candidates to be transferred into the uterine cavity (which has been appropriately prepared) for implantation [33–35]. These steps are shortly discussed as follows:

1. ***In vitro fertilization***. This needs an ovarian stimulation protocol to bring about adequate growth of several ova. These ova are then picked up. For the PGT purpose, each ovum is fertilized by only one sperm with the process of intracytoplasmic sperm injection (ICSI) using micromanipulator. The resulting embryos are cultured in special environment.

2. ***Embryo biopsy***. Originally, embryo biopsy was performed at day 3 after fertilization, yielding 1–2 cells for analysis. These cells are blastomeres. More recently, with the improvement of culture technique, embryos can survive longer. At present, the majority of embryo biopsies are performed on day 4 or 5 at blastocyst stage, when embryos have developed further and have formed trophectoderm, providing 5–10 cells for the tests. This is called trophectoderm biopsy.

3. ***Laboratory testing.*** The obtained embryonic cells are subjected to genetic testing according to clinical risk. At its infancy, PGT was mainly used to screen or diagnose major chromosome aneuploidies using fluorescence in situ hybridization (FISH). Later on, with advances in molecular technologies, it became possible to analyse a greater number of chromosomes and single gene disorders.

4. ***Embryo transfer.*** Embryos with an unaffected result are candidates for transfer that can be performed in the same cycle or can be frozen for transferring later. Usually, the transfer cycle is planned and the uterine cavity, including endometrium, needs to be prepared for a suitable milieu for implantation.

In some institutes, polar body biopsy is used as another available technique for PGDT. However, this gives information of maternal derived genetic material only [36, 37]. It might be considered in cases where maternal alleles are the important factor for the disease to develop. The interpretation is that the ovum would contain the allele that is not found in the polar body. A drawback of this procedure is that it is somewhat complicated as polar bodies are tiny. In addition, if only the first polar body is analysed, meiotic recombination may invalidate the PGT diagnosis if it takes place at the gene of interest and it is not known which allele is with the ovum/zygote and which allele is with the second polar body after fertilization [37, 38].

That said, some obstacles still exist with PGT. With only a small number of cells (even with biopsy at the blastocyst stage), errors could easily arise. Molecular techniques that need amplification of genomic materials are vulnerable to failures, as the initial amount, or template, of DNA is small, especially if the

biopsy is performed at the cleavage stage. The obtained cells may be lost during the transfer from one container to another. The cells may not be healthy and the nuclei may not be amplified properly. As a result, no diagnosis may not be obtained from the test [34, 35, 39].

Another problem with PGT is allele dropout. This occurs when one of the alleles at the locus of interest area fails to amplify. Consequently, the allele is not identified leading to a wrong result or diagnosis. Moreover, with the sensitivity of polymerase chain reaction (PCR) amplification, a small amount of DNA contamination can be amplified and can lead to misdiagnosis. Additional amplification of polymorphic markers that are linked to the genes of interest has been suggested to alleviate these problems [40, 41].

Preimplantation genetic diagnosis for HbH disease

Severe forms of thalassaemia include β-thalassaemia major, β-thalassaemia/haemoglobin E disease and Hb Bart's hydrops foetalis. The option of termination of pregnancy may be offered to couples who have a foetus affected by one of these conditions. Before conceiving, PGT is also an option for these couples in order to avoid this dilemma.

Genetic counselling is important, and a complete thalassaemia screening is necessary in both partners to identify the risk of having a baby with HbH disease or haemoglobin Bart's hydrops foetalis (see Chapter 14).

The majority of individuals with HbH disease (i.e., deletional HbH disease) have a mild course, thus termination of pregnancy is usually not indicated in affected cases. As such, prenatal diagnosis programmes are not generally offered to identify pregnancies at risk for deletional forms of HbH disease, especially considering the risk and benefit of the procedure. Furthermore, while PGT is not harmful to pregnancy, it is costly. Various possible complications can still occur; such as ovarian hyperstimulation, and complications associated with ovum pick up [42, 43]. Not many cases of PGT for deletional HbH have been reported. However, non-deletional HbH disease is typically more severe and can cause moderate to severe anaemia with more frequent need for blood transfusions. PGT may be an option for couples at risk of having a child with non-deletional HbH disease.

Once the genotype of couples or the affected pregnancy is identified, counselling of the clinical course and the risk and benefit of prenatal diagnosis, or PGT, should be discussed. Some couples might opt to go ahead with the procedure to select an unaffected embryo or choose to end the pregnancy.

Laboratory techniques for PGT for α-thalassaemia

Laboratory techniques for PGT for α-thalassaemia are complex. Usually, gap-PCR with primers designed for parental mutations is used. With a small amount of samples, the multiplex PCR is preferable with the trade off being the chances of amplifying failures especially in mutations with a large deletion [44–46].

Chen et al. in Singapore described multiplex PCR of intra-deletion markers together with flanking microsatellite markers, an added amplicon to 9 closely linked microsatellite markers. Their strategy was able to identify $-\alpha^{3.7}$, $-\alpha^{4.2}$ and α^{0} deletions. Unfortunately, of the 6 embryos that were diagnosed to be unaffected and were transferred, only 1 continued to a successful pregnancy resulting in a healthy baby [47].

In another group, Chen et al. in China used multiple displacement amplification for whole genome amplification of the biopsied trophectoderm from blastocysts [48]. Gap-PCR was able to diagnose one blastocyst as $-\alpha^{3.7}/\alpha\alpha$ genotype. The remaining embryos either had allele drop out or amplification failure with gap-PCR. The group also used NGS-based SNP haplotyping with the informative SNPs derived from the family to determine the genotype of the blastocysts by linkage analysis.

Pregnancy and fertility in α-thalassaemia major

The data on pregnancy and fertility patients on α-thalassaemia major are limited, but the management of transfusion-dependent β-thalassaemia can be applied. If individuals with α-thalassaemia major are concerned about their fertility or are planning to have children, it is recommended to consult with specialists in thalassaemia and reproductive medicine.

Pregnancy in individuals with α-thalassaemia trait

Alpha thalassaemia trait is not associated with significant fertility issues or reproductive problems [21, 49, 50]. However, it is essential to identify both partners carrying α-thalassaemia trait, as there is a risk of having a baby with haemoglobin Bart's hydrops foetalis.

Summary and recommendations

- Some individuals with HbH disease require regular blood transfusions leading to iron overload, which can diminish their fertility. These patients will benefit from a multidisciplinary approach that includes haematologists, fertility specialists, obstetricians, and mental health professionals. An early fertility evaluation, especially in those with more severe anaemia or a prior history of iron overload may be warranted.
- Individuals with HbH disease may occasionally require blood transfusion during pregnancy. Transfusions are conducted every 3–4 weeks with the goal to maintain the haemoglobin level at 90 g/L pre-transfusion and at 120 g/L post-transfusion.
- In splenectomized patients, thrombosis prevention with low-dose aspirin (or other anticoagulants that are safe during pregnancy) is recommended.
- While chelation should be generally avoided during pregnancy, deferoxamine (pregnancy category C) can be used for severe iron overload.
- Pre-conceptional and prenatal testing should be recommended to couples at risk of having an offspring with haemoglobin Bart's hydrops foetalis or non-deletional forms of HbH disease after assessment of both partners and explanation of risks and benefits.

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7 INFECTIONS AND HAEMOGLOBIN H DISEASE

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Introduction

Infection remains one of the leading causes of morbidity and mortality in patients with thalassaemia, although its incidence has decreased over decades, owing to early recognition, availability of effective treatments, and preventive measures. The incidence and causative organisms of infections also vary depending on the socioeconomic status and epidemiology of individual regions. The pathogenesis of thalassaemia itself, iron overload, blood transfusion, and splenectomy contribute to immunological abnormalities and susceptibility to infection in these patients [1]. Such immunological abnormalities include impaired chemotaxis and phagocytic activities of macrophages and neutrophils [2, 3], alteration in T-lymphocyte subsets [4, 5] decreased numbers and activity of natural killer cells [6], increased numbers and activity of B-lymphocytes [5, 7], impaired immunoglobulin secretion, as well as impaired function of the complement system [8]. Recently, red blood cells (RBCs) were also discovered to play a vital role in the immune system by expressing toll-like receptor 9 (TLR9), which binds pathogens' cell-free mitochondrial DNA. This subsequently triggers innate immune response and the production of cytokines [9]. Further studies are required to demonstrate whether such immune response is impaired in thalassaemic RBCs or the RBC-mediated immune process actually aggravates anaemia in thalassaemia patients with ongoing infection.

While there are numerous reports describing infections in β-thalassaemia major, the study of infections observed specifically in α-thalassaemia, for which the majority are non-transfusion-dependent thalassaemia (NTDT), is very limited. A recent study in Thailand reported a moderate prevalence (~5%) of serious bacterial infections among NTDT, of which 40% were those with haemoglobin (Hb) H diseases [10]. The most commonly isolated organism was *Klebsiella* species, which was similar to that reported in other studies of Asian transfusion-dependent-thalassaemia (TDT) patients [11, 12], but different from that observed in studies performed in Western countries [13–15], where *Yersinia* species is the most common causative organism. To date, there is no solid evidence supporting whether patients with α-thalassaemia are more or less susceptible to infections compared to those with β-thalassaemia. However, among patients with α-thalassaemia, those affected with non-deletional forms may be more susceptible to infections than those with deletional forms. This is because the former group of patients experience a more severe degree of ineffective erythropoiesis, haemolysis, and anaemia compared to the latter group [16, 17]. This underlying pathophysiology mainly contributes to an impaired phagocytic system, which in turn impairs the ability to defend against pathogenic microorganisms [1]. Affected patients with non-

deletional α-thalassaemia are also more likely to require occasional or frequent blood transfusions, as well as splenectomy, which can further lead to additional immunological abnormalities, as mentioned above.

In this chapter, the practical management of disease-related and therapy-related infections will be described. General and specific preventive measures will also be covered.

Disease-related infectious issues

Acute haemolytic crisis

A haemolytic crisis is considered a dominant clinical feature of α-thalassaemia, and it is characterized by acute worsening of anaemia, frequently in combination with jaundice and/or dark-coloured urine. Infections and resulting pyrexia are one of the most common precipitating factors of this condition [18–20], and clinical symptoms of acute haemolysis may occur during or after the febrile episodes [20]. Acute haemolytic crisis is more commonly observed in non-deletional HbH disease, although it can also occur in the deletional form [19, 20]. The Hb levels may drop to as low as 30-40 g/L overnight since the red cells with precipitated HbH are rapidly destroyed [21, 22]. Additionally, further generation of HbH inclusion bodies by high temperatures can induce abundant oxidative damage to the red cells and extramedullary haemolysis. The acute haemolytic crisis may be as brisk as that found in glucose-6-phosphate deficiency (G6PD) [20], and the episode can be even worse in HbH patients who coinherit G6PD deficiency mutations. These latter patients are at very high risk of progressing to shock and acute renal failure owing to massive haemoglobinuria. An acute haemolytic crisis is a major complication in HbH disease that requires emergency management. This includes early recognition of the episode, prompt blood transfusion, treatment for infections, and prevention of acute renal failure. The latter can be achieved by adequate intravenous hydration with urine alkalinization in the presence of haemoglobinuria [20]. **Table 1** summarizes the guidelines for the management of HbH disease in haemolytic crisis.

The common causative organisms leading to an acute haemolytic crisis are gram-negative bacteria, including *Salmonella, Shigella*, and *Klebsiella* species, as well as unspecified viruses [21, 23]. Other gram-positive organisms, such as *Streptococcus* species, could be prevalent in some regions [21]. In tropical areas, diagnosis and management of patients with HbH disease with dengue infection are challenging. These patients encounter acute haemolysis during the febrile stage, leading to anaemia (Hb lower than baseline) rather than haemoconcentration typically found [24, 25]. Moreover, thalassaemia patients tend to have more chance of developing severe dengue haemorrhagic fever grade 3 and 4 and severe liver involvement compared to non-thalassaemic individuals [24]. Almost all patients exhibit haemoglobinuria and required blood transfusion, regardless of bleeding episodes. Thus, in endemic regions, dengue infection should be suspected in affected patients with HbH disease who present with fever and symptoms of viral syndromes in the presence of progressive anaemia, leucopenia, and thrombocytopenia, with or without haemoglobinuria. Diagnosis during the early febrile stage can be made using the rapid dengue nonstructural protein 1 antigen (NS1 Ag) test, and all patients with HbH disease diagnosed with dengue infection should be hospitalized for close monitoring [25]. Failure to provide diagnosis and prompt treatments to these patients may lead to fatal outcomes.

Table 1. *Guidelines for the management of HbH disease in acute haemolytic crisis (reproduced with permission from Haematology by ASH. Education Program 2009, Fucharoen S and Viprakasit V).*

1. Restore patient's Hb to 80-90 g/L by red cell transfusion
   - Provide filtered red blood cells or leucocyte-depleted blood at a volume of around 5-15 ml/kg (1-2 units in adults) depending on the patient's clinical severity and anaemia levels.
   - A close monitoring on total body fluid and cardiovascular status is highly recommended.
   - Serial Hb and Hct evaluation should be done at least daily since haemolysis could be continued if the cause has not been removed or properly treated.
2. Give adequate hydration
   - Intravenous fluid therapy should be provided to maintain circulation but withheld during transfusion support.
   - The amount and rate should be carefully calculated to avoid possible heart failure from volume overload.
3. Check blood electrolytes and provide appropriate correction
   - Metabolic acidosis is usually observed but mostly resolved by transfusion support and fluid therapy. Only rare cases require alkali therapy.
4. Try to control body temperature by various means
   - Frequent tepid sponge.
   - Paracetamol 10-12 mg/kg every 4-6 hrs.
   - The usage of NSAIDs in haemolytic crisis of HbH has limited data.
5. Identify the cause of infection/inflammation and provide appropriate treatment
   - Blood and urine cultures should be done.
   - Empirical antibiotics 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.

Hb, haemoglobin; Hct, haematocrit; NSAIDs, nonsteroidal anti-inflammatory drugs.

An aplastic crisis caused by parvovirus B19 infection can occur in affected patients with HbH disease, although there have only been a few reports [26, 27]. Since the viruses selectively target erythroid precursors and induce apoptosis [28], infected patients exhibit reticulocytopenia instead of reticulocytosis in the presence of ongoing acute haemolysis [20]. The white blood cell and platelet count may also decrease [29]. These clinical features can differentiate parvovirus B19-induced aplastic crisis from the typical acute haemolytic crisis in patients with HbH disease. The diagnosis can be made by viral serology testing or demonstration of the viral DNA [29]. The main route of transmission of parvovirus B19 is respiratory. However, there is evidence that it can be transmitted through blood transfusions [30, 31]. Infection with parvovirus B19 is usually self-limited, while supportive care, including prompt blood transfusions, should be given as needed. In cases of persistent infection and prolonged haematopoietic suppression, specific therapy with intravenous immunoglobulin should be considered [29].

Malaria

Strong micro-epidemiological evidence generated over decades supports that α-thalassaemia has arisen as an evolutionary mechanism of human RBCs to protect against the ravages of falciparum malaria [32, 33]. The mechanisms underlying this protection have been extensively studied but remain unclear. Nevertheless, evidence demonstrates that individuals affected with α^+-thalassaemia homozygotes and heterozygotes are protected against only severe forms of *Plasmodium* (P.) *falciparum* but not against uncomplicated infections [33, 34]. Paradoxically, younger children affected with α^+-thalassaemia were reported to have increased susceptibility to uncomplicated *P. vivax* compared to normal children in some populations [35]. There is no evidence demonstrating whether patients affected with HbH disease and other forms of non-deletional α-thalassaemia are less susceptible to malarial infections. Given acute malaria infection leads to high-grade fever and the pre-existing tendency of acute haemolysis, it is strongly recommended that affected patients with HbH disease take a malaria chemoprophylaxis regimen, as normal persons do, when travelling to endemic areas. Prompt management of acute haemolytic crisis and extreme caution regarding renal damage caused by massive haemoglobinuria, as well as prompt administration of specific anti-malarial drugs, is required for those with acute infection.

COVID-19

As of April 2023, the latest evidence from Italy shows that the prevalence of SARS-CoV-2 infection among thalassaemia patients is 5.5%, which is similar to, or lower than that of the general population [36]. However, thalassaemia patients face a 5-fold greater risk of having age-standardized mortality. Older age and comorbidities, such as chronic lung, heart, or liver diseases, are risk factors for developing severe COVID-19 disease. Nevertheless, most thalassaemia patients experience a self-limited clinical course without the requirement of specific antiviral therapy [37]. Affected patients with HbH disease should be monitored for any acute haemolytic crisis during the illness. In patients with haemodynamic instability, adrenal insufficiency should be investigated. Low-dose corticosteroids can be prescribed if necessary while keeping in mind that the treatment prolongs clearance of the viral DNA from the respiratory tract and may increase the complication rates [38]. There is limited data on whether splenectomy increases the risk of severe SARS-CoV-2 infection. Splenectomized thalassaemia patients with COVID-19 and who have fever should be investigated for possible bacterial co-infection, and initial empirical antibiotics should be administered if necessary. To date, there is no evidence showing transmission of SARS-CoV-2 viruses through blood products.

Therapy-related infectious issues

Infections in splenectomized patients

The spleen serves as a critical organ of the reticuloendothelial system with its ability to trigger innate and adaptive immune responses to pathogens, including encapsulated bacteria [39]. Splenectomized patients encounter more than a 50-fold greater risk of having an overwhelming post-splenectomy infection (OPSI) compared to the general population [40]. OPSI is characterized by fulminant sepsis,

meningitis, or pneumonia caused mainly by *Streptococcus pneumoniae (S. pneumoniae), Haemophilus influenzae* type B (Hib), and *Neisseria meningitidis (N. meningitidis)*. Severe infections caused by gram-negative bacteria, such as *Escherichia coli* (E. coli) and *Pseudomonas* species, are also commonly observed [41]. Affected patients with OPSI may initially present a mild viral-like illness, which rapidly progresses to fulminant and fatal sepsis within 24–48 hours. Therefore, alertness to symptoms of infections, early antibiotic treatments, as well as the implementation of associated preventive measures, are extremely necessary for splenectomized patients.

The risk of severe bacterial infections is greatest in the first 2–3 years following splenectomy, although it remains high throughout life [42–44]. Splenectomized thalassaemia patients should carry medical-alert cards or wear medical-alert bracelets. The patients and their family members should be educated regarding prompt recognition of infections and the need for immediate treatment. The patients should have emergency oral antibiotic supplies on hand to be taken as an initial treatment before arriving at an accident and emergency department (**Table 2**). An empirical intravenous antibiotic with a third-generation cephalosporin (ceftriaxone) should be administered as soon as the patient arrives at the hospital [39]. Further evaluation and diagnostic investigations for the source of infections should be performed in parallel.

Table 2. *Antibiotic prophylaxis for patients with splenectomy*

Intervention	Age at splenectomy	
	<5 years	**≥5 years**
Routine prophylaxis	-Age <2 years: oral penicillin 125 mg BID -Age 2-5 years: oral penicillin 250 mg BID	-Oral penicillin 250 mg BID for at least 2-3 years following splenectomy -Lifelong oral penicillin 250 mg BID in high-risk patients
Emergency supply	Amoxycillin or amoxycillin-clavulanate upon initial symptoms of febrile illness	Amoxycillin or amoxycillin-clavulanate given upon initial symptoms of febrile illness

Daily oral antibiotic prophylaxis is recommended in all splenectomized patients, as studies have shown a significant reduction in *S. pneumoniae* infections with its use [45, 46]. However, there is little consensus on the duration of use, and recommendations vary among individual countries. In general, patients undergoing splenectomy at age <5 years should receive antibiotic prophylaxis at least until the age of 5. Those who undergo splenectomy at age ≥5 years should receive the oral prophylactic regimen at least in the first 2–3 years following the procedure (**Table 1**) [39, 41]. High-risk patients, such as those who have already experienced an episode of severe infection, those with diabetes mellitus, or those receiving immunosuppressive agents, should receive lifelong prophylaxis. Vaccination against *S. pneumoniae, N. meningitidis,* and Hib can help prevent OPSI and it is recommended in splenectomized patients. In general, initial vaccinations should be completed at least two weeks prior to the planned

splenectomy. In cases of urgent splenectomy, the vaccinations given within two weeks after splenectomy remain beneficial [41]. Annual immunization against influenza is also recommended in all splenectomized patients to prevent the risk of subsequent bacterial co-infection, particularly by *S. pneumoniae* [39]. The vaccine recommendations vary among different countries, and this probably depends on epidemiological data of individual causative organisms and the availability of the vaccines. **Table 3** provides a general recommendation for immune prophylaxis in splenectomized patients [39, 41]. Antibiotic prophylaxis with amoxicillin should be prescribed when a splenectomized patient undergoes oral procedure, sinus surgery, or airway procedure [39].

Table 3. *Immune prophylaxis for patients with splenectomy*

Vaccine	Schedule	Comment
Pneumococcal	-Complete vaccination preferably 4-6 weeks pre-splenectomy, or at least 2 weeks in advance	-13-valent conjugated pneumococcal vaccine followed by 23-valent polysaccharide pneumococcal vaccine should be used for initial vaccination
	-Repeat 5 yearly thereafter	-23-valent polysaccharide pneumococcal vaccine should be used for the booster dose
Meningococcal	-Complete vaccination preferably 4-6 weeks pre-splenectomy, or at least 2 weeks in advance	-Quadrivalent conjugated meningococcal vaccine (MenACWY) is recommended for initial and booster doses in all age groups
	-Repeat 3-5 yearly thereafter if risk remains	-Recombinant meningococcal B vaccine (MenBV) is recommended for initial and booster doses in patients aged ≥10 years
Haemophilus influenzae type B	-Complete vaccination preferably 4-6 weeks pre-splenectomy, or at least 2 weeks in advance	Currently, there is no recommendation for the booster dose
Influenza	Annually	This should also be recommended to non-splenectomized thalassaemia patients

It is essential to keep in mind that these preventive measures will reduce but not abolish the risk of severe infection. Therefore, awareness of the need for early treatment of any febrile illness remains the utmost important task for the patients and their families. Currently, there is no separate recommendation for patients undergoing partial splenectomy given these patients may ultimately require the entire spleen removed. Thus, partial splenectomized patients should follow the guidelines for total splenectomized individuals.

Surveillance of patients for transfusion-transmitted infections (TTIs)

The pathogens involved in TTIs of packed red cell products are mainly viruses, including Human Immunodeficiency Virus (HIV) and Hepatitis B and C viruses (HBV and HCV). Although the nucleic acid test has been introduced to improve limitations of serological testing to detect these viruses in the serologically silent window period [47], this method has not yet been applied in many low-income countries owing to its cost and technologically demanding characteristic. Blood donor selection and post-transfusion surveillance also remain sub-optimal in some regions. Routine surveillance of TTIs is strongly recommended for α-thalassaemia patients who require either occasional or regular blood transfusions to early detect seroconversion. The surveillance should be performed annually in transfusion-dependent patients and annually in the years in which the occasionally transfused patients are exposed to blood products. This should initially include antibody testing of HIV and HCV, and all markers of HBV. All α-thalassaemia patients requiring occasional or regular transfusions should be vaccinated against HBV, although the protection offered by the vaccine is not absolute [48]. Booster dose of the HBV vaccine is considered if anti-HBs titer decreases [48].

Other common regional or less common TTIs include, but are not limited to, malaria, West Nile virus, *Trypanosoma cruzi,* parvovirus B19, and cytomegalovirus infections. Preventive measures for these TTIs mostly involve careful donor selection and haemovigilance in affected areas. Routine post-transfusion surveillance of such TTIs is not generally recommended.

HIV

A recent 30-year (1988–2015) multicentre study of 3,000 TDT in Greece demonstrated that 45 patients tested positive for HIV infections [49]. Of these, 42 were infected by the end of 1987 and only an additional three patients were seroconverted by 2005. Among 32 deceased patients, progression to acquired immunodeficiency syndrome (AIDS) occurred at a mean age of 16.5±9.2 years. The remaining survivors were 43±7.1 years old by the time of the study. HBV and HCV were higher in HIV seropositive than in seronegative. Iron overload, as demonstrated by serum ferritin, was associated with the duration of survival after HIV diagnosis in these patients. This supported a finding of a direct relationship between HIV disease progression and ferritin values in previous studies in the 1990s [50, 51]. As such, an optimal iron chelation regimen for adequate control of body iron burden in thalassaemia patients with HIV infection is recommended.

To date, there is a wide range of antiretroviral (ARV) therapy regimens and other therapeutic options [52], which aim to improve the survival and quality of life of HIV-infected patients. Similar treatments can also be used in thalassaemia patients. There is no evidence that splenectomy results in adverse outcomes of HIV infection, however, one should be highly cautious in deciding to perform splenectomy in HIV-infected α-thalassaemia patients.

HBV

Affected patients with HBV infection may present as acute hepatitis, of which the episode can range from asymptomatic to severe and fulminant hepatic necrosis, resulting in death [53]. Following the acute phase of infection, ~2–10% of patients evolve to chronic liver disease, and subsequently, end-stage liver disease, cirrhosis, and hepatocellular carcinoma [54]. Treatment of acute viral hepatitis B includes general management, such as bed rest and avoiding alcohol. Treatment of chronic hepatitis B involves antiviral medications, such as entecavir, tenofovir, lamivudine, adefovir, and telbivudine, and/or injection of interferon α-2b to reduce viral replication and its damage to the liver [55]. Although the virus cannot be completely cleared, the treatment is considered effective when liver fibrosis does not progress to cirrhosis [56].

HCV

In the acute phase of HCV infection, the symptoms of viral illness and hepatitis can be mild, and in a high percentage of cases, the episode goes unnoticed. However, HCV infection is fearsome, as up to 80% of acute cases subsequently develop chronic disease of HCV hepatitis [57]. Many studies consistently demonstrate a negative correlation between iron overload and the outcome of HCV infection [58–61]. A study of thalassaemia patients who survived haematopoietic stem cell transplantation also showed that either HCV or iron overload are independent risk factors for the progression of liver fibrosis and they have an additive effect [62].

Affected patients with initial HCV infection do not always require treatment, as the immune response in some people will clear the infection. However, when the HCV infection becomes chronic, treatment is necessary with the goal of curing the disease. The currently recommended therapy for children ≥3 years old, adolescents and adults with chronic hepatitis C is pan-genotypic direct-acting antivirals (DDAs), which offer a cure in most patients with a short treatment duration (~12 to 24 weeks) [63, 64]. The most widely used and low-cost DDA regimen is sofosbuvir and daclatasvir. Nevertheless, access to HCV treatment remains limited in some countries. Although there is currently no effective vaccine against HCV, immunization against HBV and HAV is recommended in affected patients with α-thalassaemia who require occasional or regular blood transfusions,to prevent the additional risk of liver diseases.

Iron overload-related risks of infection

Not only transfusion-dependent but also non-transfusion-dependent α-thalassaemia patients encounter iron excess when they age, regardless of whether they have received blood transfusions. Numerous pathogens such as *Yersinia enterocolitica, Klebsiella species, E. coli, S. pneumoniae, Pseudomonas aeruginosa, Listeria monocytogenes*, and *Legionella pneumophilia* increase their virulence and pathogenicity in the presence of iron overload [65]. Additionally, many studies have demonstrated that immunological function is largely and negatively influenced by iron excess [66]. This immunological dysfunction mainly involves impaired phagocytosis and neutrophil function [67, 68]. Similar to the related observation in

transfusion-dependent β-thalassaemia, iron overload (serum ferritin >1,000 ng/mL) was also identified as a potential predictive factor of severe bacterial infection in affected patients with NTDT, including α-thalassaemia [10]. Furthermore, iron overload is associated with faster progression from HIV infection to AIDS in TDT patients [50, 51, 69], aggravates evolution to liver fibrosis in affected patients with chronic HCV infection [62], and impairs clinical response to HCV antiviral therapy [70].

Therefore, optimal control of iron overload in all α-thalassaemia patients for clinical benefits against severe infections is recommended.

Risks of infections related to iron chelators

Deferoxamine (DFO) therapy has been identified as a clinical factor for a severe bacterial infection in TDT, especially due to *Yersinia enterocolitica* in Western countries [14, 15, 71, 72], possibly because the pathogens can utilize DFO as a siderophore for enhancing their pathogenicity. In vitro studies also demonstrated that DFO therapy enhanced the growth of *Klebsiella* [13], the major causative bacterial organism in Asia [11, 12, 73]. Similar to related studies in transfusion-dependent β-thalassaemia, DFO was shown as one of the clinical factors potentially associated with severe bacterial infection in NTDT patients, including those with α-thalassaemia [10]. Association of deferiprone (DFP) and deferasirox (DFX) with the risks of severe infection caused by *Yersinia enterocolitica* and other pathogens has been studied. However, there is no evidence supporting adverse infectious outcomes in thalassaemia patients using these two iron chelators [13, 71].

Therefore, discontinuation of DFO during a febrile illness until the likely causative pathogen is identified or the infection is under control is recommended. Discontinuation of DFP or DFX is not necessary during a febrile episode unless there is a presence of other chelator-associated adverse effects, such as agranulocytosis or neutropenia in the patients using DFP.

Summary and recommendations

- Patients with both transfusion-dependent and non-transfusion-dependent α-thalassaemia are susceptible to various kinds of infection, because of the disease pathophysiology itself and related treatments.
- Understanding the specific characteristics of the patients (requirement for blood transfusion, splenectomy, type of chelator used) and regional epidemiology of causative pathogens will lead to appropriate preventive measures, early recognition of infection, and prompt treatments.
- A haemolytic crisis is the dominant clinical feature of α-thalassaemia, which may occur during or after a febrile episode.
- A haemolytic crisis in patients with HbH who also have G6PD deficiency can be critical and result in massive haemoglobinuria and acute renal failure.
- Patients with HbH should be educated to promptly take antipyretic medication to decrease their body temperature, recognize signs and symptoms of acute haemolysis early, and seek medical help in the event of their occurrence.
- During an acute haemolytic episode, blood transfusion should be provided to restore Hb to 80-90 g/L, along with adequate intravenous hydration. If haemoglobinuria is present, urine alkalinization should also be administered (see **Table 1**).
- HbH patients with dengue infection experience haemolytic anaemia, rather than haemoconcentration, during the febrile stage, which can pose a problem in diagnosis.
- In endemic regions, dengue infection should be suspected in HbH patients who present with symptoms of viral illness and progressive anaemia, leucopenia, and thrombocytopenia. All HbH patients with dengue infection should be hospitalized for close monitoring.
- An aplastic crisis caused by parvovirus B19 infection can be differentiated from the typical acute haemolytic crisis by the presence of reticulocytopenia, instead of reticulocytosis.
- Individuals affected with α^{+}-thalassaemia homozygotes and heterozygotes are protected against severe forms of *P. falciparum*. However, this is not the case for other forms of α-thalassaemia diseases.
- Affected patients with HbH disease are strongly advised to take a malaria chemoprophylaxis regimen when travelling to endemic areas.
- The latest evidence suggested the prevalence of SARS-CoV-2 infection among thalassaemia patients was similar or might be lower than that of the general population. However, thalassaemia patients encountered a 5-fold greater risk of having age-standardized lethality.
- HbH patients should be monitored for haemolytic crisis during the clinical course of COVID-19 and adrenal insufficiency should be investigated in the patients with haemodynamic instability. Possible bacterial co-infections must be kept in mind among splenectomized patients and initial empirical antibiotics administration is recommended.
- Splenectomized patients encounter >50-fold greater risk of having OPSI, caused mainly by S. pneumoniae, Hib, and N. meningitidis.
- In the presence of any febrile illnesses, splenectomized patients should take emergency oral antibiotic supplies they have on hand (amoxicillin or amoxicillin-clavulanate, see **Table 2**) and rapidly proceed to an accident and emergency department.

- Empirical intravenous antibiotic with a third-generation cephalosporin (ceftriaxone) should be administered as soon as the patient arrives at the hospital.
- Daily oral antibiotic prophylaxis with penicillin is strongly recommended in all splenectomized patients and this should be continued until the patients reach the age of 5 or at least in the first 2-3 years following splenectomy in older children and adult patients. (see **Table 2**)
- High-risk patients should continue daily oral antibiotic prophylaxis for lifelong.
- Initial vaccination against *S. pneumoniae, N. meningitidis*, and Hib should be completed at least 2 weeks in advance of the planned splenectomy. (see **Table 3**)
- In case of urgent splenectomy, administration of these vaccines within 2 weeks following the procedure remains beneficial.
- Annual influenza vaccine is recommended for both splenectomized and non-splenectomized patients with α-thalassaemia.
- The main pathogens involved in TTIs are HIV, HBV, and HCV.
- Antibody testing of HIV and HCV, and testing of all markers of HBV are recommended annually in transfusion-dependent α-thalassaemia and once within the year in which the occasionally transfused patients are exposed to blood products.
- A protective level of immunity against HBV should be ensured in all patients with α-thalassaemia, who require occasional or regular transfusions. A booster dose of the HBV vaccine is recommended if anti-HBs titer decreases.
- Transfusion-associated HIV infection has decreased dramatically in the past two decades.
- A wide range of ARV regimens offer improved survival and quality of life for HIV-infected individuals and this therapy can be used in thalassaemia patients.
- Acute viral hepatitis B infection can be managed with only supportive care. Treatment of chronic hepatitis B involves antiviral medications and/or injection of interferon α-2b to reduce viral replication and its damage to the liver.
- Up to 80% of acute HCV infections evolve into chronic hepatitis C, for which DDAs are currently the treatment of choice with the goal to cure the disease.
- There is no effective vaccine against HCV, however, immunization against HBV and HAV is strongly recommended in both regularly and occasionally transfused α-thalassaemia patients, to prevent additional risk of liver diseases.
- Iron overload is a risk factor for severe bacterial infection in patients with α-thalassaemia and is associated with faster progression of HIV disease, evolution to liver fibrosis in affected patients with chronic HCV infection, and impaired clinical response to HCV antiviral therapy.
- Optimal control of iron overload in all α-thalassaemia patients for clinical benefits against severe infections is advised.
- DFO, but not DFP or DFX therapy, is associated with severe bacterial infection, especially due to Yersinia enterocolitica and Klebsiella species.
- Discontinuation of DFO during a febrile illness is strongly advised.
- DFP or DFX can be continued during a febrile episode, unless there is a presence of other chelator-associated adverse effects, such as agranulocytosis or neutropenia in the patients using DFP.

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8 LIVER DISEASE IN α-THALASSAEMIA

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Introduction

The liver plays an important role in iron homeostasis and is the major storage site of excess iron in the body. Similar to β-thalassaemia, excessive liver iron deposition secondary to ineffective erythropoiesis in haemoglobin H (HbH) disease could lead to chronic inflammation of hepatocytes, fibrosis, and ultimately cirrhosis. Furthermore, some patients with more severe forms of non-deletional HbH disease may require frequent on-demand transfusions or even become transfusion-dependent. This will eventually lead to transfusional iron overload. Concomitant liver diseases especially viral hepatitis would further aggravate liver damage. Cirrhosis is an important risk factor for hepatocellular carcinoma (HCC), which is a sinister complication with high mortality risk. HCC has been reported in patients with β-thalassaemia even in the absence of cirrhosis.

Prevalence and burden of liver disease in HbH disease

HbH disease was once considered a relatively benign disorder with only few patients who were thought to develop clinically significant iron overload. However, in the early 1990s, Hsu *et al.* reported that iron overload occurred in 74% of a series of Chinese patients with HbH disease who did not require regular blood transfusions. Hepatic iron deposition was found in all seven patients who underwent liver biopsy and three of them had moderate to severe liver fibrosis, suggesting that liver disease in HbH disease has been under-recognized [1]. In recent studies, liver iron overload was detected by MRI T2* in 48% to 85% of Chinese patients with HbH disease, and 27% to 56% of patients had moderate to severe liver iron overload (>7mg/g Fe dry weight [DW]) [2–4]. In one study, 9% of patients were found to have cirrhosis in a cohort of adults with non-transfusion dependent HbH disease who had a low prevalence of hepatitis B (HBV) and HCV infection [3].

Transfusion-transmitted viral infections are major complications in patients with thalassaemia, especially those on chronic transfusions. The prevalence of viral infections in patients with β-thalassaemia has been reported to range from 0.3–5.7% for HBV and up to 85% for HCV in different populations. The seroprevalence of HBV surface antigen and anti-HCV in patients with HbH disease has not been reported in detail, likely due to the fact that only a small portion of patients with HbH disease require frequent or regular transfusions. The risk of acquiring HBV and HCV from transfusion of blood products has been largely mitigated nowadays by regular donor screening and nucleic acid testing.

Similarly, there is a paucity of data in regards to the prevalence of HCC in patients with HbH disease. Macuso *et al.* identified two cases of HCC out of 105 evaluated patients with β-thalassaemia in a study performed in 2003 [5]. Since then, a significant increase in the incidence of HCC in adults with β-thalassaemia has been reported in both transfusion-dependent and non-transfusion dependent patients. However, data on the risk of HCC in patients with HbH disease are quite scarce [6–8]. It is important to note that HCC occurs more frequently in non-transfusion dependent β-thalassaemia (NTDT) than in transfusion-dependent β-thalassaemia (TDT) [6, 9].

Despite improvements in the screening of blood products for viral pathogens, liver disease has emerged as a significant cause of morbidity and mortality in thalassaemia, mostly due to HCC followed by cirrhosis [10]. This is possibly related to the improved survival of patients so that they live long enough to develop complications that usually occur later in life [6].

Pathophysiology of liver disease in HbH disease

Liver iron overload in HbH disease

Iron overload is the most important cause of liver damage in HbH disease. Unlike in TDT where excess iron is mostly contributed by blood transfusions, the major mechanism of iron accumulation in NTDT, including HbH disease, is increased intestinal iron absorption resulted from hepcidin suppression by ineffective erythropoiesis [11]. More importantly, absorbed iron directly deposits in hepatocytes, whereas iron from transfused blood would first saturate the reticuloendothelial system [6, 10]. Though both conditions are categorized as NTDT, ineffective erythropoiesis is less marked in HbH disease than in β-thalassaemia intermedia while chronic haemolysis plays a more significant role in the pathogenesis of the former [12]. A recent study proposed that this phenomenon makes iron loading less pronounced in HbH disease, as evidenced by a higher hepcidin and lower serum ferritin level than that in β-thalassaemia intermedia. However, further study is required to compare the burden of liver disease in these two distinct types of NTDT [13]. HbH disease with non-deletional types of mutation (e.g., HbH Constant Spring or HbH Quong Sze) usually has a more severe phenotype including a lower baseline haemoglobin, higher transfusion requirement, and more prominent hepatosplenomegaly [14]. A higher liver iron concentration (LIC) was also observed in non-deletional than in deletional forms of HbH disease [3, 15]. Patients with more severe disease phenotype including those with lower baseline haemoglobin level, higher transfusion requirement, and history of splenectomy are at higher risk of developing liver iron overload [3, 4, 15]. The spleen may act as a reservoir of excess iron, accounting for the higher LIC in splenectomised patients [16].

Some studies showed that advancing age had a direct correlation with serum ferritin level and LIC in NTDT including HbH disease, suggesting that patients could develop clinically significant liver iron overload over time [4, 16, 17]. Tantiworawit *et al.* demonstrated that age >20 years was a significant risk factor of liver iron overload in NTDT (OR 30.2, 95 % CI 4.5-203). Huang *et al.* reported similar findings in a more recent study. The odd ratio of liver iron overload in patients >30 years of age was 77.8 (95%

CI: 8.8–690.5). The proportions of patients with HbH disease were 40.7% and 55.6% respectively in these two studies [4, 15]. On the other hand, some groups did not demonstrate a correlation between age and LIC in NTDT [3, 18]. This could be partly attributed to the prior use of iron chelation therapy in some patients. Further investigations are warranted to study whether age affects LIC in HbH disease.

Hepatic fibrosis and cirrhosis in HbH disease

Iron toxicity to the liver and other organs is largely dependent on non-transferrin bound iron (NTBI) especially labile plasma iron, which appears when the iron binding capacity of transferrin is exceeded [19]. NTBI is efficiently taken up by the liver and mainly targets hepatocytes. It exerts toxicity by its ability to catalyse reactions that generate free hydroxyl radicals responsible for lipid peroxidation of cellular organelles [20, 21]. Clearance of debris from iron-laden hepatocytes induces inflammatory and profibrogenic signals, resulting in fibrinogenesis and ultimately cirrhosis [10, 19, 22–24]. As previously discussed, iron overload in NTDT is mostly related to hepcidin suppression secondary to ineffective erythropoiesis. This mechanism results in saturation of transferrin and emergence of NTBI earlier in the course of disease than TDT, in which NTBI only appears in the presence of severe iron overload after multiple blood transfusions, exceeding the protective effect of storage protein ferritin [16, 25, 26]. Prolonged duration of hepatic iron exposure significantly increases the risk of advanced liver fibrosis and cirrhosis [27, 28]. A study in Hong Kong showed that age ≥65 years (OR 5.0, 95% CI 1.5–17.5) and moderate-to-severe liver iron overload (OR 3.5, 95% CI 1.01–12.1) were independently associated with advanced liver fibrosis in HbH disease [3].

Concomitant HCV infection has a synergistic role in liver injury and fibrosis progression in thalassaemia patients [29]. One of the possibilities is the chronic HCV infection further suppressed hepcidin level and exacerbated hepatic iron overload [29, 30]. Alcoholism and non-alcoholic fatty liver disease also increased the risk of liver fibrosis in patients with NTDT [24, 31-33].

Hepatocellular carcinoma

Most HCC in NTDT patients occurred after 45 years of age [10]. Liver cirrhosis is the strongest risk factor for HCC irrespective of its etiology. Thus when one reaches the cirrhotic stage, HCC surveillance should be considered [34, 35]. Hepatic iron overload promotes hepatocarcinogenesis via several mechanisms. First, excess iron triggers the generation of reaction oxygen species, which disrupt DNA and impair protein synthesis, leading to inactivation of tumour suppressor genes (e.g. TP53) or their products. As previously mentioned, NTBI would accelerate fibrinogenesis and indirectly increase the risk of HCC. Finally, immune dysregulation in iron overloaded liver would attenuate anti-cancer immune surveillance [35–37]. **Importantly, the occurrence of hepatocellular carcinoma in the absence of liver cirrhosis in thalassaemia patients has been recurrently reported. A common feature in these patients is the considerably high liver iron burden [5, 9, 38, 39]. This suggests the necessity of extending HCC surveillance to NTDT patients who have not yet reached cirrhotic stage.**

Concomitant chronic HCV infection could induce necroinflammation and significantly increase the risk of HCC [5, 39]. Although chronic HBV infection is implicated in around half of HCC cases worldwide, its

effect on HCC development in NTDT patients is not well characterized [35]. Alcohol and non-alcoholic liver disease are cofactors that possibly worsen liver damage and promote the development of HCC in iron loading disorders including thalassaemias [33].

Biliary tract disease

Hyperbilirubinaemia is a common feature found in more than half of the patients with HbH disease owing to chronic haemolysis [40]. Consequently, like other thalassaemia syndromes, cholelithiasis is also common in HbH disease. The reported prevalence of gallstones in HbH disease was 28–42%, which was 8-fold higher than the background population though lower than patients having β-thalassaemia [40, 41]. This risk increased with concomitant Gilbert's genotype. The possible complications of gallstones include cholangitis and cholecystitis [14, 25, 40].

Diagnosis and treatment of liver disease in HbH disease

Evaluation and monitoring of liver iron overload

Monitoring of liver iron burden and early detection of complications are the cornerstones of managing liver disease in HbH disease. Serum ferritin is an easily available test, which has reasonable correlation to LIC in thalassaemia. However, it is very important that serum ferritin level is significantly lower in NTDT than in TDT despite similar LIC [42]. The lower serum ferritin to LIC ratio than TDT is also observed in HbH disease [43, 44]. This is likely the effect of preferential iron absorption by the liver in NTDT (please see Chapter 11). Another pitfall of serum ferritin is the lack of specificity as it also increases in response to inflammation [45]. MRI T2* has replaced liver biopsy as the gold standard of LIC quantification in thalassaemia because its non-invasive, rapid, accurate, and reproducible nature [46, 47]. It may however be less available in resource-limited settings. In non-transfusion dependent patients with HbH disease, LIC monitoring could be started at around 10 years of age [48] or when the ferritin exceeds >300 ng/dl. Some patients especially those with deletional type HbH disease have milder disease phenotype and slow kinetics in iron accumulation. In these patients, assessment of LIC could be commenced at 15 years of age, or at the start of adulthood [49, 50]. LIC should be measured after 8–10 transfusions for those who are on a regular transfusion programme. The frequency of liver iron quantification by MRI T2* should be individualized according to the baseline value and serum ferritin level (please see Chapter 11) [10, 51, 52].

Detection of hepatic complications in HbH disease

Since cirrhosis is a significant risk factor of HCC development, early detection of liver fibrosis would be useful to identify high risk patients warranting close monitoring and early intervention. Transient elastography, which measures the velocity of an elastic shear wave propagating through the liver parenchyma, is an extensively validated method to detect liver fibrosis and cirrhosis [53]. Liver stiffness measurement by transient elastography correlates with different stages of liver fibrosis in various liver diseases including thalassaemia [28, 54]. Being non-invasive, highly reliable and reproducible, and more easily available nowadays, the test should be considered especially when risk factors of liver fibrosis are present [55].

USG is able to detect early HCC amenable to curative treatment [56, 57]. Regular USG screening is therefore recommended every 6 months in patients with risk factors, including patients with concomitant viral HBV and/or HCV infection, established liver cirrhosis irrespective of the aetiology and moderate to severe liver iron overload [35, 56]. Alpha-fetoprotein is not sensitive enough to be used alone to screen for or diagnose HCC [6]. **Table 1** summarizes the monitoring strategy of liver disease in HbH disease.

Table 1. *Strategy of liver disease monitoring in HbH disease*

	Baseline	Frequency
Liver iron overload		
Serum ferritin	TDT: at the start of transfusions NTDT: 10 years of age	TDT: at each transfusion NTDT: every 6-12 months. More frequent if on iron chelation
Liver MRI T2*	TDT: after 8-10 transfusions NTDT: 10 years of age to adulthood, or when ferritin > 300 ng/dl	TDT: Every 1 year (every 6 months if LIC ≥15mg/g DW) NTDT: every 1-2 years. Every 1 year if ferritin > 300 ng/dl or LIC > 5 mg/g DW
Detection of concomitant liver disease		
HbsAg	When starting regular transfusion therapy	Every 1 year
Anti-HCV antibody	When starting regular transfusion therapy	Every 1 year. Positive result to be confirmed with HCV RNA
Liver complications		
Liver enzymes	10 years of age	TDT: Every 3 months NTDT: Every 6-12 months. More frequent if on iron chelation
Transient elastography	LIC >5mg/g dry weight	Every 1-2 years
Liver ultrasonography	Risk factors of HCC present: • HBV infection • HCV infection • Cirrhosis • LIC ≥5mg/g dry weight • SF ≥800ng/mL	Every 6 months

LIC, liver iron concentration. SF, serum ferritin. TDT, transfusion-dependent thalassaemia. NTDT, non-transfusion dependent thalassaemia. DW, dry weight.

Iron chelation therapy for hepatic iron overload

Evidence suggests that all the three available iron chelating agents, namely deferoxamine, deferiprone and deferasirox were able to improve hepatic iron overload in thalassaemia [10, 58, 59]. Reduction in body iron burden by chelation therapy was associated with improvement in liver enzymes, liver stiffness measurement, and stage of liver fibrosis in β-thalassaemia intermedia [60, 61]. Patients with β-thalassaemia intermedia treated with deferasirox for at least 3 three years had stabilization or improvement in fibrosis stage regardless of the change in LIC [62]. The use of chelation therapy could also potentially prevent carcinogenesis induced by iron toxicity but clinical data is required to confirm the hypothesis [63]. Although we are lacking in direct evidence showing the beneficial effects of iron chelators on liver outcomes in HbH disease, treatment of liver iron overload is still recommended based on the favourable findings in studies including other NTDT patients.

Management of concomitant liver diseases and cirrhosis

Consultation with a hepatologist is recommended in the management of viral hepatitis in patients with thalassaemia. The availability of direct-acting antiviral (DAA) drugs (e.g., combination therapy with sofosbuvir and velpatasvir, or ledipasvir and sofosbuvir) has remarkably improved the outcomes of thalassaemia patients with HCV infection. Studies confirmed the excellent tolerability of DAAs in patients with thalassaemia and the rate of achieving sustained virological response was 90–100% [64, 65]. HCV treatment should be sought to prevent the development of complications including HCC [66]. Management of chronic HBV infection in patients with HbH disease should follow the standard of care for the general population. HBV vaccination is recommended in patients who are seronegative for HBV and who are planned for transfusion therapy. When liver cirrhosis is diagnosed, evaluation by a hepatologist is recommended. Screening of variceal disease should be considered [67].

Management of hepatocellular carcinoma

Early diagnosis remains crucial to decrease mortality resulting from HCC. Once diagnosed, patients should be managed according to the severity of liver disease and the stage of HCC. Patients should be referred to appropriate oncology, surgery, or radiation oncology specialists, but the haematology team should be involved in optimizing the care of patients. Treatment modalities including surgical resection and thermoablation in early stage disease should follow the same indications applied to the general population with HCC [56]. Patients with thalassaemia without significant comorbidities should not be precluded from liver transplantation if indicated [35]. The use of chemoembolization in intermediate stage and systemic treatments in advanced stage HCC should also follow the same indications in non-thalassaemia patients [56].

Summary

Liver disease is one of the leading causes of morbidity and mortality in thalassaemia syndromes. Iron overload due to ineffective erythropoiesis, which promotes fibrinogenesis and carcinogenesis, is the major mechanism of disease manifestation. Iron often accumulates slowly in HbH disease and thus liver complications often occur in adulthood or even at advanced age. Concomitant liver disease, especially chronic HCV infection, further increases the risk of cirrhosis and hepatocellular carcinoma. Liver iron burden should be regularly monitored by serum ferritin and MRI T2*. Iron chelation is effective to remove excess hepatic iron and to reduce the risks of developing complications. Viral hepatitis should be managed according to the respective guidelines applied to the general population. USG liver in patients with risk factors enable early detection of HCC to decrease mortality.

Recommendations

- Regular monitoring of liver iron burden by serum ferritin and MRI T2* should commence at 10 years of age or older for patients with milder disease phenotype.
- Iron chelation therapy is recommended in patients with LIC ≥5mg/g dry weight or serum ferritin ≥500 ng/mL in non-transfusion dependent patients with HbH disease.
- Vaccination against the hepatitis B virus in seronegative patients prior to blood transfusion therapy is recommended.
- Annual screening of HBV and HCV infection is recommended in patients receiving blood transfusions, and positive anti-HCV results should be followed by HCV-RNA to identify chronic infection.
- Consultation with a hepatologist is recommended for management of concomitant HBV and/ or HCV infection, or when liver cirrhosis is established.
- Evaluation for liver fibrosis and cirrhosis with transient elastography is recommended in patients with liver iron concentration ≥5mg/g dry weight or serum ferritin ≥500 ng/mL.
- Biannual ultrasonography of liver for HCC surveillance is recommended in patients with HbH disease when risk factors (liver iron overload, cirrhosis, HBV and/ or HCV infection) are present.

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9 BLOOD TRANSFUSION

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Introduction

In contrast to people with β-thalassaemia syndromes, individuals with α-thalassaemia are largely non-transfusion dependent. This is, in part, due to the intrauterine demise of patients with the most severe form of α-thalassaemia, haemoglobin Bart's hydrops foetalis (or α-thalassaemia major), while other less severe forms of α-thalassaemia (e.g., most patients with haemoglobin H disease) are generally non-transfusion dependent. Nevertheless, episodic (on-demand) blood transfusion remains an important component of management for most patients with HbH disease. In some patients with more severe forms of HbH disease and all surviving patients with haemoglobin Bart's hydrops foetalis, regular blood transfusion is the mainstay of treatment [1–4]. Furthermore, intrauterine blood transfusion can be the lifesaving intervention for foetuses with haemoglobin H hydrops foetalis or haemoglobin Bart's hydrops foetalis syndromes [5, 6].

Overall, the general principles of transfusion in α-thalassaemia are based on those of β-thalassaemia. In this chapter, we do not intend to duplicate these recommendations, and readers and clinicians are encouraged to refer to *Thalassaemia International Federation Guidelines for the Management of Transfusion Dependent Thalassaemia* [7] for a more in-depth review and recommendations. Here, however, we provide an overview of these recommendations, but more specifically, we highlight some aspects of transfusion that are specific for α-thalassaemia.

Aims of blood transfusion in HbH disease and haemoglobin Bart's hydrops foetalis syndrome (α-thalassaemia major)

In individuals with α-thalassaemia, blood transfusion can be required either on-demand or occasionally as part of a regular transfusion programme.

On-demand transfusions

Despite having moderate anaemia, most patients with HbH disease are asymptomatic and do not require transfusions. However, during periods of physiologic stress or illness, anaemia can become severe enough to necessitate on-demand transfusion [1–4]. These events can occur following an infection, febrile episode, an oxidative challenge, surgical procedure, or during pregnancy. Acute haemolytic episodes are more common in individuals with non-deletional forms of HbH disease, but they can also occur in those with deletional HbH disease, especially in younger children [4, 8]. Haemolysis can be brisk

leading to acute haemodynamic compromise requiring urgent intervention [1]. Similarly, patients may experience an acute aplastic event following viral infections. Patients who require frequent on-demand transfusions may be considered for a disease-modifying approach (e.g., regular transfusion, splenectomy, or other novel treatments).

The decision to offer on-demand transfusion is based on a patient's clinical status rather than a haemoglobin number. The underlying clinical indication, expected course of acute illness, and preference of patient or their caregivers plays a role. Most patients with an acute drop of haemoglobin to less than 60-70 g/L will benefit from correction of acute anaemia. On-demand transfusion can be considered during pregnancy to ensure normal development of the foetus and mother's health [9, 10]. On-demand transfusion can also be offered to patients with more severe anaemia prior to surgeries as per discretion of the surgeon or anaesthesiologist.

If blood transfusion is required during an acute haemolytic event (or acute aplastic event), the haemoglobin should be increased to baseline, or slightly higher if it is expected that the haemolytic process is ongoing. In general, clinicians are advised not to expose patients to extra units of blood if not needed, but the patients' clinical status should allow for transfusion of the whole unit so valuable donated blood is not discarded if the volume of transfusion does not exceed 20 ml/kg.

Patients with an acute event should be monitored closely to ensure indications for further transfusion have resolved. Supportive therapies should include adequate hydration to preserve renal function, but excessive hydration should be avoided to prevent volume overload. Addressing the underlying cause of an acute event is paramount.

Recommendations

- Identify the underlying cause of an acute event (e.g., infection/inflammation/aplasia) and provide appropriate treatment. Empiric antibiotics with the coverage against encapsulated bacteria should be initiated in splenectomized patients with fever.
- The decision to offer on-demand transfusion should be based on patient's clinical status, underlying clinical indication of expected course of acute illness, and preference of patient or their caregivers.
- The aim of blood transfusion is to restore patient's haemoglobin to baseline or slightly higher.
- Patients experiencing an acute event should be monitored closely to ensure indications for further transfusion have resolved.
- Serial assessment of haemoglobin concentration, haemolytic markers, electrolytes, and renal function should be done, since continuing hemolysis could lead to worsening anaemia, electrolyte and acid-base disturbances, and renal damage.
- Adequate hydration should be provided to maintain circulation and renal perfusion, but excessive hydration should be avoided to prevent volume overload.
- Other supportive measures should be offered as needed (e.g. antipyretics).
- If clinically indicated, on-demand transfusion can be offered prior to surgeries, or during pregnancy.

Regular transfusion programme

Regular blood transfusion is less commonly utilized in individuals with HbH disease. In general, individuals with deletional forms of HbH disease will rarely require starting regular transfusion unless there are associated disease modifiers or other aetiologies exacerbating their anaemia. In patients with non-deletional forms of HbH disease, regular blood transfusions are intended to improve the haemoglobin level in symptomatic patients, prevent or reduce the long-term sequelae of chronic haemolytic anaemia, suppress ineffective erythropoiesis, allow normal growth and development in children and adolescents, and improve an individual's quality of life [1-4].

Severe anaemia can be observed from early childhood in patients with certain forms of non-deletional HbH disease (e.g., compound heterozygosity for α^{0}- thalassaemia deletion and haemoglobin Pak Num Po [11], or haemoglobin Suan Dok $\alpha^{109leu\rightarrow arg}\alpha$ mutations). In the most severe forms of HbH disease, profound anaemia starts in utero leading to hydrops foetalis (e.g., due to interaction of α^{0}-thalassaemia deletions and rare non-deletional mutations such as haemoglobin $\alpha^{Cd\ 59Gly\text{-}Asp}\alpha$ (Adana), $\alpha^{\Delta Cd\ 30}\alpha$, $\alpha^{Cd\ 66\ Leu\text{-}Pro}\alpha$ (Dartmouth), $\alpha^{Cd\ 35Ser\text{-}Pro}\alpha$ (Evora). Patients with this form of α-thalassaemia (HbH hydrops foetalis) require intrauterine and then regular lifelong transfusions, although, rarely, some patients may no longer require regular transfusions as they age [9]. Interestingly, individuals who are homozygote for $\alpha^{142\ STOP\rightarrow Gln;TAA\rightarrow CAA}\alpha$ (Hb Constant Spring), $\alpha^{PA1(AATAAG)}\alpha$ (Poly A), or Hb Taybe can present with hydrops during pregnancy and severe anaemia requiring transfusion during first few months of life, which will resolve by age 1 [3, 9]. These patients will remain non-transfusion dependent thereafter.

Regular transfusion is the cornerstone of treatment in long-term survivors of haemoglobin Bart's hydrops foetalis (α-thalassaemia major). Intrauterine transfusion is associated with improved prenatal outcomes in patients with haemoglobin Bart's hydrops foetalis, although their effect on the long-term outcomes is not entirely clear [5].

It is important to identify any other modifiable cause of anaemia (e.g., folate deficiency, vitamin B12 deficiency, or G6PD enzyme deficiency) prior to embarking on a chronic transfusion programme.

When to start regular transfusions?

In individuals with HbH disease, deciding to start regular transfusions and defining transfusion targets is complex and requires experience and clinical judgment. This complexity arises from several factors. First, there is little data available to systematically study and compare the long-term outcomes of regular transfusions versus other treatment approaches (e.g., continuing on-demand transfusions or splenectomy), and recommendations in this setting are based on expert opinions. Second, there is considerable heterogeneity in clinical presentation and underlying disease process in HbH disease, especially in those with non-deletional types. Patients can have predominantly a haemolytic disease (majority of patients), while some genotypes can lead to a significant apoptosis of early erythroid cells leading to ineffective erythropoiesis similar to β-thalassaemia [1]. Third, in patients with HbH disease,

the need for regular transfusions is a dynamic process, and occasionally, patients who require transfusions may no longer be transfusion-dependent and *vice versa*. Fourth, there is a heterogeneity of the proportion of circulating HbH in different subtypes of HbH disease [4, 9]. As HbH is a non-functional haemoglobin due to its extremely high oxygen affinity, the total haemoglobin may not be a true representation of tissue oxygen delivery in those patients who have a high level of circulating HbH (> 20%) [12]. Finally, a regular transfusion programme should be decided per individual case as this requires significant resources and is associated with considerable burden on both patients and health care systems.

In general, if a disease modifying therapy is required, regular transfusion is the intervention of choice for young children, and early splenectomy is not recommended. This is because of the higher risk of overwhelming sepsis in young children. Furthermore, some of these children may no longer require transfusion later in life even without splenectomy.

For patients with genotypes that are associated with severe anaemia (effective Hb < 70 g/L) and considerable ineffective erythropoiesis, a transfusion approach similar to those with β-thalassaemia is desired [1, 13] and splenectomy is not generally recommended (please see Chapter 10 Splenomegaly and Splenectomy). In contrast, patients with a predominantly haemolytic disease, those with effective Hb >70 g/L, or those with symptomatic splenomegaly or hypersplenism may be considered for splenectomy if the resources are scarce or if a regular transfusion programme is associated with significant burden on patients' quality of life. In this setting, long-term complications of splenectomy (e.g., risk of thrombotic events, pulmonary hypertension) should be carefully reviewed [14, 15].

In patients who are being considered for transfusion, lower pre-transfusion haemoglobin targets as compared to β-thalassaemia may be acceptable (80-90 g/L) as generally there is less degree of ineffective erythropoiesis in HbH disease in comparison to β-thalassaemia [16, 17]. However, those with a high proportion of circulating HbH or those with genotypes associated with significant ineffective erythropoiesis may require higher pre-transfusion haemoglobin targets to adjust for the non-functional HbH [18, 19] or to suppress ineffective erythropoiesis. The decision to start regular transfusion should be made through a patient-centred approach. A severity scoring system to predict the need for regular transfusions in children has been recently developed and validated [13].

Recommendations

- The criteria to start regular transfusion in individuals with HbH disease are not well defined and are generally based on expert opinion.
- It is recommended that "effective" haemoglobin be measured at a steady state. Effective haemoglobin can be calculated as total Hb x (1- [HbH % + Hb Bart's %] / 100). Please note that the current technology of Hb analysis by automatic high-performance liquid chromatography (HPLC) such as Variant (Bio Rad) could not accurately quantitate the percentage of HbH and Hb

Bart's. The percentage of HbH and Hb Bart's can be more accurately measured by capillary electrophoresis system; however, caution should be exercised that this is not done during an acute haemolytic event.

- Initiations of regular transfusion should be individualized and be offered through a patient-centred approach.
- Regular transfusions should be considered in the following settings:
  - Patients with Hb Bart's hydrops foetalis (α-thalassaemia major).
  - Patients with baseline "effective" haemoglobin < 70 g/L (or < 80 g/L if effective haemoglobin cannot be calculated).
  - Declining haemoglobin concentration or development of symptomatic anaemia in those who have not been on regular transfusions.
  - To prevent or reduce the long-term sequelae of chronic haemolytic anaemia or to suppress ineffective erythropoiesis.
  - Frequent acute haemolytic events requiring on-demand transfusions
  - Children and adolescents with growth failure, poor academic performance, diminished exercise tolerance, or delayed puberty.
  - Poor quality of life due to anaemia.
- When regular transfusion is being considered, alternative therapeutic interventions, such as splenectomy, should also be reviewed.
- Careful monitoring and treatment of iron overload is paramount, although, as compared to β-thalassaemia, ferritin may underestimate the degree of tissue iron due to the difference in the pathophysiology of α- vs. β-thalassaemia.
- In low-resource settings, or if a regular transfusion programme and iron chelation are challenging or not feasible, splenectomy can be considered in those who are older than 5 years of age with a predominantly haemolytic HbH disease. Pre-splenectomy vaccination for encapsulated organisms is highly recommended. Patients with severe anaemia requiring transfusion from early childhood or those with significant ineffective erythropoiesis should be treated similar to β-thalassaemia and in general, splenectomy is not recommended.
- A pre-transfusion haemoglobin target of 80-90 g/L is acceptable in most patients. However, those with high proportion of circulating HbH and those with ineffective erythropoiesis may require higher pre-transfusion haemoglobin targets.
- Patients with Hb Bart's hydrops foetalis (α-thalassaemia major) require an aggressive transfusion approach to suppress significant erythropoiesis and improve oxygenation through increasing functional (non-HbH) haemoglobin. In these patients, "functional" haemoglobin should be calculated for a pre-transfusion functional Hb target of 90-100 g/L.
- The need for ongoing regular transfusion should be regularly evaluated, as some patients may become transfusion independent.

Haemovigilance and blood conservation

According to the World Health Organization, "haemovigilance is the set of surveillance procedures covering the entire blood transfusion chain, from the donation and processing of blood and its components, through to their provision and transfusion to patients, and including their follow-up. It includes the monitoring, reporting, investigation and analysis of adverse events related to the donation, processing and transfusion of blood, and taking action to prevent their occurrence or recurrence" [20].

In general, principals of haemovigilance for blood transfusion in α-thalassaemia is similar to β-thalassaemia and readers are referred to *Thalassaemia International Federation Guidelines for the Management of Transfusion Dependent Thalassaemia* for further review [7].

One of the important aspects of transfusion in thalassaemia is the risk of alloimmunization, which is the development of specific red cell antibodies following frequent exposure to blood products [21]. The development of antibodies to red cell antigens is a significant event and has major implications for the transfusion therapy [22]. Here, we would like to highlight that the data related to prevalence of alloimmunization and its risk factors in α-thalassaemia are quite scarce, and limited studies have included transfusion dependent thalassaemia patients as a single group [21–24]. In the absence of further specific data, it is reasonable to follow similar recommendations for β-thalassaemia. Risk of alloimmunization is likely higher during pregnancy, in splenectomized patients, and in those who have had minimal transfusion exposure [7]. Patients with α-thalassaemia should have extended red cell antigen typing that includes at least A, B, O, C, c, D, E, e, and Kell antigens and preferably a full red cell phenotype/genotype panel, and all patients should be transfused using A, B, O, C, c, D, E, e, and Kell compatible blood [7].

Recommendations

- Use a product that is collected, tested, selected, issued, and administered in adherence to established quality and safety regulations and guidance.
- Blood transfusion should be administered by staff trained in blood transfusion.
- Involve informed patient consent.
- The transfusion should be performed in a system with a good haemovigilance structure.
- If possible, do not waste valuable donated blood.
- Blood storage for <2 weeks, conditioning to achieve mean 24-hour post-transfusion RBC survival ≥75%.
- Leucoreduced packed red blood cells (≤1 x 106 leucocytes/unit) with haemoglobin content ≥40 g (pre-storage filtration preferred).
- Patients with α-thalassaemia should have extended red cell antigen typing that include at least A, B, O, C, c, D, E, e, and Kell antigens and preferably a full red cell phenotype/genotype penal should be done prior to first transfusion.
- All patients should be transfused with A, B, O, C, c, D, E, e, and Kell compatible blood.
- Patients are advised to have appropriate vaccinations against transfusion-transmitted infections.

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10 SPLENOMEGALY AND SPLENECTOMY

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Introduction

The spleen is a unique lymphoid organ that plays important roles in innate and adaptive immunity and clearance of senescent, or abnormal, blood cells. It is also a part of the reticuloendothelial system and contributes to the recycling of iron. The spleen also hosts haematopoietic cells and participates in erythropoiesis when the capacity of bone marrow to meet erythroid demand is exhausted [1–3].

In haematological disorders, pathophysiology of splenomegaly is diverse, and it is mainly driven by the underlying disease process. For example, in hereditary spherocytosis, splenomegaly represents chronic congestion of splenic cords by rigid spherocytes and splenic hyperplasia [4]. In β-thalassaemia, in addition to the increased splenic hyperplasia due to extravascular haemolysis, ineffective erythropoiesis and elevated erythropoietin lead to extramedullary haematopoiesis in the spleen, further contributing to enlargement of the spleen. Ineffective erythropoiesis, in turn, further increases the metabolic demand, exacerbates the plasma volume expansion, and imposes greater load on the already-burdened myocardium [5]. Ineffective erythropoiesis also contributes to defective iron metabolism [6].

Splenomegaly is a common complication in patients with HbH disease (mainly the non-deletional forms) and the spleen is quite enlarged in patients with haemoglobin Bart's hydrops foetalis (α-thalassaemia major) [7]. In HbH disease, most of erythrocyte destructions occur in the spleen, while the spleen also contributes to ineffective erythropoiesis, although to a lesser degree as compared to β-thalasssaemia [8-9].

Clinically, splenomegaly may be associated with a variety of complications, like sense of heaviness and discomfort, abdominal pain, early satiety, and features associated with hypersplenism, leading to worsening of anaemia, thrombocytopenia and leukopenia. A very large spleen poses the risk of acute rupture with abdominal trauma.

Splenectomy for HbH disease

Splenectomy is a treatment option for many benign haematological conditions, including immune thrombocytopenia (ITP) and heredity or acquired haemolytic anaemias like hereditary spherocytosis [10]. While improvements in surgical techniques have resulted in lower perioperative complications and mortality, there is increasing evidence of long-term complications due to the loss of spleen function.

Immune dysfunction and increased susceptibility to infection, particularly with encapsulated bacteria, are well-described complications of splenectomy. The risk of infection is highest within the first 2–3 years following splenectomy. Young children and older adults [11] and those with other underlying medical conditions (e.g., diabetes mellitus) may be at higher risk of infection. Effective vaccination against encapsulated bacteria prior to splenectomy is protective. Please refer to Chapter 7: Infections and haemoglobin H disease for further recommendations.

Other long-term complications of splenectomy are observed in a variety of haematological conditions. The rate of these complications vary according to the underlying condition. For example, splenectomized patients with β-thalassaemia are at particularly higher risk of venous thromboembolism or pulmonary hypertension [10]. While these complications have been observed in splenectomized patients with HbH disease [12–15], this risk is likely lower in patients with HbH disease as compared to splenectomized patients with β-thalassaemia. Furthermore, the risk of alloimmunization is likely higher in splenectomized patients [16].

In HbH disease, splenectomy is effective in increasing the haemoglobin levels, often by 20-30 g/L [17–19]. The actual clinical benefit of this increase in haemoglobin levels for patients with milder forms of HbH disease may be negligible; thus, splenectomy is not generally recommended for these patients [17]. Similarly, while in patients with very severe forms of HbH disease, who require early initiation of transfusion, or those with severe anaemia (functional Hb < 70 g/L), splenectomy may raise the haemoglobin level, it may not eliminate the need for transfusions and may be associated with a higher degree of complications. Thus, splenectomy is not ideal in this group of patients. For patients with an intermediate degree of anaemia (e.g., non-deletional HbH disease with moderate–severe anaemia and episodes of acute hemolysis requiring frequent transfusions), or those who have significant and symptomatic hepatosplenomegaly, splenectomy might be considered [17, 20]. Even in these patients, the decision to proceed with splenectomy (an irreversible procedure with long-term complications) is complicated. In addition to the severity of symptoms due to α-thalassaemia, existing risk factors that can increase or exacerbate complications (e.g., underlying risk of thrombosis, history of prior infection, presence of risk factors for pulmonary hypertension) and the availability or convenience of other therapeutic options (e.g., chronic transfusion programme and iron chelation) are important to be considered. Most importantly, a patient's values and preference should be valued. Splenectomy is not recommended in very young children because of the higher risk of overwhelming sepsis in young children.

In patients with HbH disease, the effect of splenectomy on iron balance and distribution is not well-studied [21]. Thus, until further data becomes available, splenectomy is not recommended for iron management in patients who do not require regular or frequent transfusions and do not have other indications for splenectomy.

Splenectomy for haemoglobin Bart's hydrops foetalis (α-thalassaemia major)

Patients with haemoglobin Bart's hydrops foetalis (α-thalassaemia major) have significant splenomegaly, even when they are on an aggressive transfusion protocol. This is likely due to extravascular haemolysis and significantly increased erythropoiesis that cannot be fully abrogated by chronic transfusions in this severe haemolytic condition. In patients with haemoglobin Bart's hydrops foetalis (α-thalassaemia major), removing the spleen would likely prolong the lifespan of endogenous cells that are exclusively made of non-functional haemoglobin H (see Chapter 5: Long-term management of α-thalassaemia major (haemoglobin Bart's hydrops foetalis)). As such, although the total haemoglobin concentration may increase, this would not lead to any physiological improvement due to the higher proportion of nonfunctional haemoglobin H, and instead would make effective transfusion even more challenging [22]. In contrast, splenectomy may be associated with the development of a life-threatening thromboembolic complication [23]. Thus, currently, splenectomy is not recommended in patients with haemoglobin Bart's hydrops foetalis [7].

Methods of splenectomy

Splenectomy can be performed via a laparotomy or a laparoscopic approach. Although there are no extensive data on the comparison of these two approaches in patients with HbH disease, in other conditions (including β-thalassaemias), laparoscopic splenectomy has been shown to be associated with a lower bleeding rate, earlier hospital discharge, less post-operative pain, and lower risk of infections. It is, however, associated with the possibility of conversion to open splenectomy [24, 25]. The choice of splenectomy approach should be individualized for the patient and the expertise of the surgical team.

Another approach to splenectomy is the complete removal of spleen tissue (total splenectomy), or partial removal of the spleen tissue (partial splenectomy) with the intent to preserve some of its desired function, mainly the immune function. While partial splenectomy may be considered in some patients with HbH disease, especially those who may be at higher risk of infection or other complication of asplenia (e.g., high risk of thrombosis), overall, the safety and effectiveness of partial splenectomy compared to total splenectomy in patients with HbH disease has not been studied.

Reduction of splenic tissue by embolization is a less invasive approach that has been used for other indications. One drawback is the risk of post-embolization syndrome (fever, nausea, significant pain) and the possible need for a subsequent total splenectomy. More significant complications include abscess formation, pleural effusion, portal vein thrombosis, and liver failure, especially with the larger areas of embolization [26, 27]. The effectiveness of splenic embolization has not been studied in α-thalassaemia.

Gallstones, including intrahepatic bile duct stones, are detected in over 30% of asymptomatic patients with HbH disease, which can cause abdominal discomfort and hepatobiliary infection [28]. It is thus reasonable to consider cholecystectomy, if splenectomy is being considered in patients with HbH disease.

Summary of recommendations

- Splenectomy may be considered in patients with HbH disease who have moderately severe anaemia, those with long-term complications of chronic haemolytic anaemia, or patients who experience frequent acute haemolytic events requiring on-demand transfusions, or those with symptomatic splenomegaly.
- Splenectomy is not recommended for: children younger than 5, those with severe anaemia (functional Hb < 70 g/L) or those requiring transfusion from early childhood, and those with significant ineffective erythropoiesis with a β-thalassaemia-like phenotype. In these patients, chronic transfusion is the more appropriate disease modifying approach.
- Splenectomy is not recommended for management of patients with haemoglobin Bart's hydrops foetalis (α-thalassaemia major).
- The risks and benefits of splenectomy vs. alternative therapeutic interventions (e.g., chronic transfusions with iron chelation or novel emerging disease modifying therapies) should be carefully reviewed.
- Patient preference should be valued and available resources should be considered.
- Patient should receive vaccination against encapsulated bacteria as per local vaccination guidelines for patients with asplenia.
- While there is little consensus on the recommended duration of use, daily oral antibiotic prophylaxis with activity against encapsulated bacteria is recommended in all splenectomized patients. In general, patients undergoing splenectomy at age <5 years should receive antibiotic prophylaxis at least until the age of 5, and all patients should receive prophylactic regimen at least in the first 2–3 years following the procedure. High-risk patients should receive lifelong prophylaxis.
- In splenectomized patients who present with fever or signs or symptoms of infection, appropriate parenteral antibiotics against encapsulated bacteria should be started while awaiting culture results.
- Low-dose aspirin should be considered in splenectomized patients as long as there are no contraindications. This is particularly important for patients with a history of previous thrombosis or other risk factors.
- Clinicians should offer preventative measures, surveillance tests or appropriate prevention and treatments for post-splenectomy complications.
- There is limited data to suggest the effectiveness and safety of partial splenectomy or splenic embolization in patients with HbH disease. Thus, they are not recommended except in a research setting or as part of structured observational cohort.
- The choice of laparoscopy vs. laparotomy procedure is based upon the patient's preference and the surgical team's expertise.

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11 IRON OVERLOAD AND IRON CHELATION IN α-THALASSAEMIA

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Introduction

Patients with thalassaemia develop iron overload due to increased iron absorption secondary to ineffective erythropoiesis and repeated red cell transfusions. Regardless of the source, excess iron can lead to significant organ damage. The goals of treatment of iron overload with chelation therapy in particular are (1) to prevent or reverse organ damage due to iron toxicity and (2) to remove excess iron. These concepts apply to all diseases at risk of iron overload.

Total body iron content is accurately reflected by measurement of the liver iron concentration (LIC), which is expressed in mg per gram dry weight of liver (mg/g DW)[1]. and is usually measured by magnetic resonance imaging (MRI) [2, 3]. The iron detected by MRI is non-reactive Fe^{3+} [2]. Toxic, reactive $Fe2^{+}$ is not seen by MRI but can be inferred from transferrin saturation > 60% [4, 5] and by presence of iron in the pancreas, pituitary, or heart [6].

Thalassaemia and ineffective erythropoiesis

Thalassaemia is characterized by abnormal globin gene expression resulting in total absence or quantitative reduction of globin chain synthesis [7,8]. Globin chain imbalance causes apoptosis of erythroid precursors, leading to both medullary and intravascular haemolysis [9]. Thalassaemia creates a state of ineffective erythropoiesis (IE), which refers to the inability to produce an adequate number of mature red blood cells (RBC) in the presence of increased immature erythroid precursors driven by anaemia [10]. Intramedullary apoptosis of erythroid precursors results in an erythroid maturation arrest and perturbation of normal iron regulatory mechanisms, leading to increased iron absorption and the release of iron from storage pools that is out of proportion to the degree of iron loading [10].

Normally, the iron regulatory peptide hepcidin increases in the presence of iron overload, blocking export of absorbed iron from enterocytes and stored iron from macrophages to reduce plasma iron [6]. Erythroferrone produced by the increased RBC progenitors seen in thalassaemia lowers hepcidin, increasing circulating iron [11]. As the result, hepcidin levels are lower than expected for the degree of iron loading. The erythroferrone-mediated suppression of hepcidin, coupled with the inability to use the iron to make mature RBC because of IE, results in iron overload [9, 10]. In transfusion-dependent thalassaemia (TDT), the iron input is much higher, and severe hepatic and, more importantly, severe endocrine and cardiac iron loading occurs because the binding capacity of transferrin is exceeded and labile plasma iron (LPI) goes up, leading to unregulated iron loading.

Iron toxicity and role of Fe^{2+}

Iron toxicity is related to exposure to reactive ferrous iron (Fe^{2+}), also referred to as labile plasma/cellular iron (LPI/LCI). Importantly, pathologic loading of iron into extra-hepatic sites (endocrine organs and heart) only occurs when LPI is elevated. In the absence of transfusion, the amount of iron loading is determined by the degree of IE. With transfusion, hepatic and reticuloendothelial iron loading is much greater and linearly related to the number of RBC transfused [1]. Extra-hepatic distribution of iron occurs because the iron binding capacity of transferrin is exceeded, the marrow cannot use iron to make RBC, and LPI rises. Elevated levels of LPI are pathologic and are seen in conditions with IE, such as thalassaemia [12], in contrast to conditions with effective erythropoiesis like sickle cell disease or hereditary spherocytosis where LPI is low [13, 14].

IE is an important feature of non-transfusion-dependent β-thalassaemia (NTDT-β) [15, 16] and haemoglobin H (HbH) disease (NTDT-α) [10,16], and it also occurs in TDT when the marrow is not well suppressed.

Iron toxicity and subsequent organ dysfunction is related to the amount of exposure to the reactive form of iron (Fe^{2+}), the duration of exposure, and the protective effects of antioxidant mechanisms [6]. Ferrous iron (Fe^{2+}) reacts with hydrogen peroxide to form hydroxyl radical and singlet oxygen (Fenton reaction), which dramatically amplifies oxidant damage secondary to inflammatory processes [17]. The duration or "area under the curve" exposure to these oxidant species is the most important cause of iron-related organ damage (reviewed elsewhere [18]).

As there are many different α- and β-thalassaemia gene mutations and combinations, there are subsequently different degrees of IE and associated exposure to LPI.

The biology of iron transport explains the relation of extra-hepatic iron loading to LPI exposure. The major source of iron in humans is recycling of senescent endogenous or transfused red cells by macrophages in the reticuloendothelial system, where internalized heme is degraded by heme oxygenase and Fe^{2+} is produced [15]. Intracellular Fe^{2+}, also called labile cellular iron (LCI), is either stored in ferritin, which converts Fe^{2+} to non-reactive ferric iron (Fe^{3+}), or is released into the plasma through the iron export protein, ferroportin [6]. Ferrous iron is then converted to non-toxic Fe^{3+} by plasma ceruloplasmin upon exiting the cell through ferroportin, where it binds to the transport protein transferrin. Ferritin production is driven by high levels of Fe^{2+} in the cytoplasm (LCI), as well as the inflammatory cytokine IL-6 [19].

Transferrin binds two molecules of Fe^{3+} and transferrin-bound iron (TBI) enters the cell through receptor-mediated endocytosis after binding to transferrin receptor-1 (TfR1) [6,10], which is expressed on the surface of nearly every cell type. Normally, in the presence of excess LCI, TfR1 is downregulated, preventing intracellular entry of TBI and protecting cells from iron overload [20]. Liver is one of the few cell types that also possess transferrin receptor-2 (TfR2), which is not downregulated by high LCI, allowing the liver to load via transferrin even when intracellular Fe^{2+} is high and additionally allowing TfR2 to serve as the iron sensor. Since humans have no way to excrete excess iron, and there is substantial iron in transfused RBC

(0.8 mg/mL), iron levels achieved by recurrent transfusion are significantly higher than those achieved by the increased iron absorption due to IE alone. Under normal circumstances, this elegant system keeps iron absorption and losses in balance.

Normally, about 20% to 30% of transferrin is bound to Fe^{3+}. Non-transferrin-bound iron (NTBI) can be detected in plasma as soon as transferrin saturation reaches 35%, rising significantly when transferrin saturation exceeds 70% to 80% [4, 5], making transferrin saturation a reasonable clinical surrogate for NTBI/LPI. NTBI is mainly ferric-citrate and is in equilibrium with the loosely bound and highly reactive Fe^{2+} subspecies of NTBI, LPI [4]. The heart and endocrine organs are protected from import of transferrin-bound iron through downregulation of TfR1. However, if LPI (Fe^{2+}) is present, it can enter these organs non-physiologically through divalent ion transporters for Ca^{2+} and Zn^{2+} that are not regulated by iron. Importantly, pathologic extra-hepatic iron deposition (pancreas, pituitary, heart) only develops when NTBI/LPI is present, making iron loading of extra-hepatic sites an indicator of exposure to toxic LPI [6, 21]. When iron loading occurs due to iron absorption only, as in NTDT, it takes decades for enough iron to load so that transferrin saturation is exceeded and NTBI/LPI appears, and the heart and endocrine organs load. In TDT, this can happen within a much shorter time such that extra-hepatic loading may be seen by a year or two of life with severe IE or no erythropoietic activity [22, 23].

This physiology is very important clinically, because iron chelators immediately drop circulating NTBI (Fe^{3+}) near zero [24], and therefore drop reactive LPI, preventing loading through divalent ion transporters and markedly reducing tissue exposure to toxic iron. Thus, for effective protection against iron toxicity, the chelator needs to circulate all the time. This also means that chelators can protect from Fe^{2+} toxicity even if the tissue iron measured by MRI (which is non-reactive Fe^{3+}) is still high.

α-Thalassaemia and iron overload

The α-thalassaemia syndromes differ from β-thalassaemias in that excess α-homotetramers in β-thalassaemia are more unstable than excess β-homotetramers in α-thalassaemia, causing earlier damage to red cell precursors in the marrow and extramedullary haematopoiesis in β-thalassaemia [25]. Marrow expansion and extramedullary haematopoiesis seems to be less in α-thalassaemia, but IE is still a problem. Reduction of α-globin leads to extracellular precipitates of γ-homotetramers (γ4, Hg Bart's) in the foetus and newborn, while β-homotetramers (β4, HbH) affect older children and adults [26]. Both Hb Bart's and HbH have increased oxygen affinity, leading to poor oxygen delivery as well as premature destruction of mature RBC and maturing erythroblasts.

With the exception of haemoglobin Bart's hydrops foetalis (α-thalassaemia major) and some severe forms of HbH disease, most α-thalassaemia syndromes do not require chronic transfusion. The iron physiology in NTDT-α seems to be similar to that of NTDT-β. However, the degree of IE appears to be less in α-thalassaemia than in β-thalassaemia, as evidenced by lower levels of iron loading and less bone deformity in the absence of transfusion [27, 28]. The iron physiology of TDT-α appears to be the same as that of TDT-β.

Because of anaemia and poor oxygen carrying ability of the haemoglobin that is made, infants with four α-gene deletion (haemoglobin Bart's hydrops foetalis or α-thalassaemia major) require in utero transfusions to survive until delivery [29], providing an in-utero source of iron overload. There was presence of pituitary and pancreatic iron loading by 1.5 years of age as well as increased LIC in a small series of children with α-thalassaemia major but there was no evidence of organ dysfunction in this group [23]. They had moderate to severe iron overload that responded well to chelation, but the more concerning finding was that nearly all patients had pancreatic and pituitary iron deposition prior to four years of age suggesting exposure to toxic $Fe2^{+}$. However, this is not significantly different than other chronically transfused young children [22]. Ferritin levels were quite a bit higher at 12 months of age in α-thalassaemia major infants than in those with β-thalassaemia major, likely due to the aggressive transfusions required to suppress fast migrating haemoglobin. However, overall, the ferritins were lower that TDT-β after time in this group [30]. Interestingly, while the relation between ferritin and LIC was linear in both groups, the slopes of the relations were very different with ferritin in TDT-α (α-thalassaemia major) being significantly lower than in TDT-β at a given LIC [30]. Very interestingly, when marrow suppression was increased by increasing transfusion, the ferritin relative to LIC increased [30, 31], consistent with the increase in hepcidin relative to iron loading predicted by the biology described above [10, 11]. Patients with TDT-α seem to have intellectual, growth, and brain MRI differences at older ages [32], but these are not likely related to iron and there is no evidence that supports or refutes possible importance of in utero iron exposure and subsequent toxicity. The role of α-globin gene expression in small arterioles, its interaction with oxidants and in regulation of blood flow [33–36] and of high oxygen binding of HbH and Hb Bart's [37] make untangling causes of organ damage in α-thalassaemia syndromes very difficult.

The development of iron overload in transfusion-dependent and non-transfusion dependent patients with α-thalassaemia syndromes appears to parallel that in transfusion-dependent and non-transfusion dependent patients with β-thalassaemia; however, there are not enough comparative data. In a series of 27 β-thalassaemia intermedia and 10 haemoglobin H disease (HbH) individuals, LIC and the relation between LIC and ferritin were the same by age 50 years in NTDT-β, although LIC was slightly lower in HbH [38]. About 46% of NTDT HbH over 18 years old had a ferritin over 800 ng/mL, and 64% had LIC greater than 5 mg/g DW compared to 56% and 72% for NTDT-β and 78% and 95% for haemoglobin E-β-thalassaemia [39]. Patients with HbH Constant Spring seem to be particularly affected, with 37% having a ferritin between 800 and 2500 ng/mL and 7% with ferritin greater than 2500, and 60% being TDT [40]. A longitudinal study of 86 patients with HbH disease demonstrated that ferritin is fairly stable throughout childhood and adolescence, then increases with age after 18 years old [41]. Patients with non-deletional HbH disease, on the other hand, often have elevated ferritin at a young age that continues to increase over time due to their transfusion dependence. Similar to what has been shown in NTDT-β and consistent with the iron biology we have described, patients with deletional HbH disease demonstrate preferential iron storage in the liver, sparing the heart [38]. **The α-thalassaemia syndromes in general are NTDT, but some non-deletional α-thalassaemia and all α-thalassaemia major patients are transfusion-dependent. The rate and pattern of iron accumulation in NTDT-β is much slower from**

that of TDT-β [42], and the same seems to be true of NTDT-α and TDT-α. The complications of thalassaemia differ between TDT and NTDT, likely because transfusion suppresses IE, and thus IE-related complications like osteoporosis, bony deformity, and pulmonary hypertension are less observed. At least in NTDT-β, serum ferritin over 800 ng/mL is a strong predictor of decreased morbidity-free survival [43]. It is likely that ferritin is a reflection of severity of IE and does not imply that iron overload is causing the morbidity. The iron loading, of course, is much greater in TDT and results in more cardiac iron loading and endocrine failure. Ferritin is generally lower in NTDT than in TDT at the same LIC [44].

Clinical measurement and management of iron overload

Liver iron concentration (LIC) is the best measure of total body iron loading, as it is linearly related to total body iron content ($r=0.98$, $p < 0.01$) [1]. MRI measurement of LIC only detects aggregated ferritin-Fe^{3+} (haemosiderin) present in tissue; thus, while MRI-LIC is an excellent measure of total body iron, it represents the non-reactive Fe^{3+}. Pancreatic and cardiac iron is an indicator of reactive Fe^{2+} exposure as extra-hepatic organs only load pathologically when there is circulating Fe^{2+}. Of note, the pancreas loads before the heart and pituitary gland [21]. Additionally, the spleen in thalassaemia can be 30% of the volume of the liver and can contribute significantly to total body iron. There is some evidence that in the presence of treatments that modulate IE and differentially affect splenic iron, the LIC may not reflect total iron accurately [45]. Clinically relevant iron and ferritin levels are presented in **Table 1**.

Magnetic resonance imaging (MRI) can assess LIC with greater accuracy and safety than liver biopsy, which has fallen out of favour due to sampling error and bleeding risk [2]. An estimate of total body iron by MRI assessment of LIC is the main parameter used to initiate and modify iron chelation therapy. Presence of cardiac iron or pancreatic iron means there have been prolonged periods when chelation has not been present or adequate to lower NTBI/LPI. We mention pancreatic and pituitary iron measurement here because they are helpful, even if non-essential, for management. Though the measurement does not require special equipment, the techniques are not established in more than a few centres worldwide.

Ferritin levels are routinely available, correlate with total iron in populations of patients and can be used to infer total iron and change in iron. However, they can be misleading in individuals because of significant measurement-to-measurement variability and dramatic effects of inflammation on ferritin levels [46, 47]. Ferritin trends can be useful for therapeutic decision-making but with particular caution at near-normal total iron levels (e.g., LIC 3-5 mg/g DW) [18, 46]. Ferritin cut-off levels have been published for using ferritin alone to estimate LIC if MRI methods are not available [48–50]. In general, for TDT, ferritin greater than 800 ng/mL is highly predictive of LIC greater than 5 mg/g, whereas ferritin between 300 and 500 ng/mL is highly predictive of LIC greater than 5 mg/g DW for NTDT [39,49–51].

LIC measurement by MRI is the gold standard for assessing iron overload and regulating chelator dosing. Monitoring of cardiac iron by MRI is critical in all TDT patients with thalassaemia or other disorders associated with IE [2, 3].

Iron chelators are effective in reducing iron [52, 53], and they improve clinical outcome in thalassaemia [54]. Humans have no iron export mechanism, and iron overload cannot resolve in the absence of chelation therapy or chronic blood loss. Phlebotomy is not an appropriate approach in thalassaemia patients. Iron toxicity is known to cause cardiac failure, arrythmia, abnormal cardiac beat-to-beat variability, autonomic dysfunction, endocrine failure, elevated liver enzymes, and bone marrow suppression. These complications can be reversed with chelation and removal of NTBI/LPI, even when significant organ loading by Fe^{3+} measured by MRI persists. NTBI/LPI levels drop to near zero immediately upon starting chelation and return immediately when chelators stop [24]. NTBI/LPI are not seen by MRI but can be inferred by elevation of transferrin saturation and presence of iron in extra-hepatic sites (heart, pancreas). This biology informs the clinical approach to protection from iron toxicity, namely, chelator should be in circulation all the time to keep NTBI low. Removal of stored iron takes longer. Chelation takes 4 to 6 months to decrease the LIC by 50% and up to 14 months to reduce cardiac iron by 50% [55]. The presence of circulating chelator prevents entry of Fe^{2+} into extra-hepatic sites and protects from Fe^{2+}-mediated oxidant damage [18 ,56–58].

The protective effect of chelators in the presence of high tissue Fe^{3+} is a clinically important concept and is clearly demonstrated in a study of thalassaemia patients with severe cardiac iron loading (T2* < 5.6 ms) and low ejection fraction at baseline, who were treated with continuous infusion of deferoxamine [55]. The ejection fraction normalized by 3 months after initiation of deferoxamine, despite persistent severe cardiac iron (T2* 8.1 ms) after one year. Chelation therefore protects organ function and prevents Fe^{2+} damage and loading even in the face of existing severe Fe^{3+} deposition. Clinically this means that organ dysfunction is a significant driver of the urgency to correct iron overload.

Differential iron unloading is very important clinically because it means total iron reflected by LIC can be normalized while there is still significant cardiac iron. Increasing chelator dose in this circumstance can lead to chelator toxicity, especially with deferasirox or deferoxamine where toxicity is greater at low total iron. The solution is to ensure there is circulating chelator all the time but at a dose that maintains the LIC in a low-safe range (2–3 mg/g DW). The extra-hepatic sites should clear with time. If they do not, it is likely that a significant number of days of chelation per month are being missed.

Chelation is rarely started prior to 2 years of age due to concerns about effects of chelation on cognitive development in infants. With a poor blood-brain barrier in pre-terms and newborns, chelator in the brain could cause severe local iron deficiency, which is known to have profound effects on neurocognitive development [59]. While there is no clear data on this point with respect to chelation, iron overload at 2 years old is usually easily controlled within a year or two with no known negative effects.

There are many guidelines and suggestions in the literature for administration of iron chelators. We do not think there is one prescriptive answer and prefer a common-sense approach based on the principles discussed here. The most important point is to arrive at an effective dose and combination of one or more chelators that the patient will take, and to monitor the results of treatment and toxicity. The primary goal of chelation is to protect from iron toxicity, which means exposure to reactive Fe^{2+}. The amount of toxicity depends on the amount of toxic iron in tissue, the duration of exposure, and effectiveness of antioxidant mechanisms. Since the survival of all thalassaemia syndromes now is long, approaching that of the normal population, patients with even low levels of iron can develop complications after decades

of exposure to low amounts of iron. While heart failure was the major focus of chelation in thalassaemia, the focus is now shifted to endocrine failure and increased propensity for malignant transformation based on β-thalassaemia [56]. There is very little data on this for the α-thalassaemia syndromes.

It seems prudent to keep the LIC between 2.5 and 3.5 mg/g DW at all times with no pancreatic or cardiac iron. You cannot achieve this without MRI monitoring and by carefully monitoring for chelator toxicity. Toxicity is particularly an issue at low LIC with deferasirox, and less so with deferiprone. If all you have is ferritin with no access to MRI iron measures, try to keep it between 300 and 800 for NTDT, and between 800 and 1500 for TDT.

Treatment guidelines for monitoring and treating iron overload in NTDT are available [51]. Basically, chelation should be started when the LIC is greater than 5 mg/g DW or the serum ferritin is greater than 500 ng/mL with a goal of keeping the LIC between 2 to 5 mg/g DW or the ferritin between 300 and 800 ng/mL [49]. Deferasirox doses in the range of 10 to 15 mg/kg/day have been suggested for NTDT [60]. We would not suggest trying to target low levels of iron if MRI measurement of LIC is not available.

It is certainly true that patients with higher transfusion burden need more chelator, and experienced thalassaemia experts have recommended modulating the dose of chelator based on amount of transfusion. The major variable in chelation efficacy is adherence to treatment, so in practice, we do not recommend these schemata even if theoretically pleasing. We would reduce chelator doses 50% in NTDT patients and then adjust based on LIC. As long as the LIC is over 5 to 7 mg/g DW, we maximize the chelator dosage based on tolerance and chelator toxicity and monitor the response closely. We would reduce the chelator when the LIC decreases below 5 and certainly below 3 mg/g DW, and titrate the doses every 3 to 6 months to maintain LIC around 3 mg/g DW. The organ toxicity from iron takes many months and even years to develop and there is protection from circulating chelator so there is time to make these adjustments. Certainly, we would like to achieve this goal as quickly as is safe.

The exception to this is severe cardiac iron loading (T2* less than 8 to 10 ms) and presence of heart failure or serous arrythmia. This is primarily a risk with TDT patients, and it is a medical emergency. Cardiomyopathy due to iron is almost always reversible if properly and aggressively treated. Importantly, there are very few cardiologists who are aware that this cardiomyopathy is reversible, or who are aware of the treatment, so immediately contacting a cardiologist familiar with iron cardiomyopathy is critical. The treatment is outlined in "Cardiovascular function and treatment in beta-thalassaemia major: a consensus statement from the American Heart Association" [61]. Start continuous (24/7) infusion of deferoxamine 50 mg/kg/24 hours and find a thalassaemia cardiologist. Delay in starting this chelation can be life-threatening [61].

A key issue with chelation is to work with the patient to come up with a regimen that they can tolerate and will take. We suggest combinations of medications to mitigate toxicity of the drugs. The main cause of chelation failure is adherence to prescribed medication.

Organ damage from iron should be routinely monitored. There are no clear issues that differentiate α-thal NTDT/TDT from β-thalassaemia with respect to iron. We suggest monitoring growth velocity in children as a measure of pituitary function and iron loading; annual TSH, T4, fasting blood sugar and insulin to determine insulin resistance; and, testosterone, LH, FSH post puberty. Bone density by DEXA scan can be obtained in late adolescence and then every few years for NTDT. Note that "osteopenia" is likely from marrow expansion and means the haemoglobin needs to be increased and IE suppressed. We do recommend anti-Mullerian hormone measurement in late adolescent females to assess egg reserve with referral to reproductive specialist. It is not clear the early loss of fertility is due to iron or thalassaemia itself [62, 63]. We also recommend a two-week ECG recording after age 40, as the risk of intermittent atrial fibrillation is increased in thalassaemia.

Table 1 provides key points regarding iron overload monitoring and chelation based on our experience.

Three licensed iron chelators are available in the United States and Europe, with key differences in route of administration, half-life, and toxicities, which are summarized in **Table 2**. All three chelators are very effective at controlling iron individually or in combination [18]. Deferiprone is most effective at protecting and restoring cardiac function [64]. Severe renal complications with deferasirox are rare, but can be fatal and they are preventable by monitoring. Of course, adherence to therapy is more important than the chelator's mechanism of action in removing iron deposition and protecting from iron toxicity [65], so the primary goal is to apply an effective plan that the patient can closely follow.

Summary and recommendations

- The primary goal of treatment of iron overload is to protect against organ toxicity from exposure to reactive ferrous (Fe^{2+}) iron.
- Organ toxicity is related to the magnitude and duration of ferrous iron exposure so the goal of treatment is to keep ferrous iron levels at zero all the time.
- Transferrin saturation > 50% and certainly > 70% on multiple measures is a reasonable surrogate for circulating NTBI/LPI.
- MRI only measures non-reactive Fe^{3+}, though high levels are somewhat related to toxicity. Presence of high MRI detected iron in pancreas, pituitary or heart indicates prolonged exposure to high levels of NTBI/LPI.
- Circulating chelators lower NTBI/LPI to zero immediately, prevent/reduce toxicity, and block entry into endocrine and cardiac tissue as long as they are circulating. Chelators can preserve organ function even in the presence of very high tissue iron levels by reducing NTBI/LPI.
- Increased iron absorption due to ineffective erythropoiesis as seen in NTDT-α or β takes decades before extra-hepatic organs load.
- Iron loading in TDT happens rapidly and linearly with the number of transfusions. Loading of endocrine organs and the heart can occur within a few years, but organ dysfunction takes longer.
- The main reason for failure of iron chelation treatment is poor adherence to prescribed medications.

Table 1. *Monitoring iron overload and chelation*

	[2] Ferritin ng/mL)	[1] LIC (mg/g DW)	[1,4] Pancreas R2* (sec[1])	[1] Cardiac T2* (ms)
Key iron values Normal Low Moderate High Very high	 25-300 300-800 800-1700 1700-2500 >2500	 0.8-1.5 1.5-5.0 5.0-10.0 10-20 >20	<27 27-40 40-100 100-300 >300	>30 > 20 10-20 8-10 <8
First evaluation	TDT: At start of transfusions NTDT: 10 years of age	TDT: after 8 to 10 transfusions NTDT: Ferritin >300 ng/mL	[4] With each LIC	TDT: Starting at age 10 years. NTDT: Ferritin >1000 ng/mL, or LIC >10 mg/g DW, or [4] Pancreas R2* >100
Start chelation	TDT: >800 NTDT: >500	TDT: >3.5 NTDT: >5.0		
Monitoring	TDT: at each transfusion NTDT: every 6-12 months. More frequent if on iron chelation	TDT: Yearly NTDT: Every 1-2 years. Every 1 year if ferritin > 500 ng/mL or LIC > 5 mg/g DW	[4] With each LIC	TDT: Annual NTDT: Periodically, or annual if ferritin > 2000 ng/mL
Consider combination chelation	No apparent response after 6-12 months of therapy	LIC ≥10 mg/g DW with apparent inadequate response		Add deferiprone if <10 ms
Optimal target	[3] TDT: 800-1500 NTDT: 300-800	2-5	< 27	>30 ms

1. Measured by MRI
2. Ferritin cutoff differ for TDT and NTDT. Conservative values listed for simplicity (see 39).
3. LIC by MRI is the primary value for chelator dose decisions. Use MRI LIC for dosing regardless of ferritin level unless MRI not available. Ferritin levels listed reduce risk of over chelating someone if the implied LIC may be less than 3-5 mg/g DW.
4. Pancreatic iron can be helpful but not required for management. Pancreas R2* > 100 sec-1 is pathologic.
TDT, transfusion dependent. NTDT, non-transfusion dependent. LIC, liver iron concentration. DW, dry weight.

Table 2. *Currently available iron chelators (adapted from Coates & Wood, 2017 [57])*

	[1] [2] [3] Deferoxamine (DFO)	[1] [2] [3] [4] **Deferiprone (DFP)**	[1] [2] [3] **Deferasirox (DFX)**
Route	Continuous IV or SQ	PO (or IV)	PO
Half life	30 min	3 hrs	8-16 hrs
Usual dose	40-50 mg/kg/24 hrs	TDT: 75-99 mg/kg/day. Start at 50 mg but increase to 99 mg/kg/day. Lower dose not effective for TDT. *NTDT:* dosing not established but 25 to 50 mg/kg would be reasonable.	20-40 mg/kg/day (Exjade) 14-28 mg/kg/day (Jadenu)
Administration	Continuous over 10-24 hrs (TDT)	[5] Oral every 8-12 hrs	[6] Daily or BID
Toxicity	Allergy, vision loss, hearing loss, osteoporosis	Agranulocytosis (1.5%) is idiosyncratic, not dose related and not predicted by CBC. More common in first 6 months of use. Transient ↑ AST and ALT common (7.7%) [8]	[7] Renal toxicity, allergy, GI bleed Renal Fanconi's syndrome can be fatal. ↑ AST and ALT (5%) [8] ↑ toxicity with LIC<5 mg/g DW.
Monitoring	Audiogram, bone density	Fever → stop drug, present to ED for fever/neutropenia protocol. CBC/diff weekly for 6 months. Then every 2 weeks for 6 months, then with transfusion only. (package insert recommendation)	Serum creatinine, electrolytes, urine protein/creatinine ratio monthly. Severe abdominal pain → stop drug, call MD

1. Adjust for continuous chelator exposure, adherence, no toxicity
2. Assess efficacy every 6 to 12 months: no iron related organ toxicity, corrected iron parameters. It may take 6 to 12 months to see clear effect of dose change.
3. All three chelators have been used off-label in combination.
4. Deferiprone is contraindicated in pregnancy or while trying to become pregnant.
5. Deferiprone has two dosage forms, one for every 8 hour administration and one for every 12 hours.
6. Some patients rapidly metabolize deferasirox so once daily dosing results in very low chelator levels in the second half of the day [66, 67]. Dividing the dose every 12 can improve response [68].
7. Renal monitoring is critical for DFX especially at LIC < 3-5 mg/g DW. Reduce or hold if > 30% increase in creatinine over baseline even if normal, or > 2.5 X ULN urine protein/creatinine on more than one measure. Monitor electrolytes.
8. LIC > 3 to 5 mg/g DW can cause chemical hepatitis and can cause transaminases in the 50 to 300 IU/L range but does not cause high direct bilirubin. High transaminases in this range are not a contraindication to chelators. They will come down as the iron comes down.

IV, intravenous. SQ, subcutaneous. PO, per oral. TDT, transfusion dependent thalassaemia. NTDT, non-transfusion dependent thalassaemia. Min, minutes. Hrs, hours. BID, twice daily. GI, gastrointestinal. LIC, liver iron concentration. ULN, upper limit of normal.

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12 CURATIVE THERAPIES FOR α-THALASSAEMIA

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Introduction

α-Thalassaemia is one of the most common monogenic disorders, where an estimated 5% of the global population carry an α-thalassaemia variant [1, 2]. In Southeast Asia, the prevalence of α-thalassaemia is 23% [3], reaching up to 50% in countries such as United Arab Emirates, Oman, and Saudi Arabia [4]. Historically, haemoglobin Bart's hydrops foetalis (homozygous α^0-thalassaemia) was considered a universally fatal condition due to the intrauterine demise of affected foetuses.

In contrast, most patients with haemoglobin H (HbH) disease, do not experience significant symptoms. However, some patients with non-deletional mutations may experience a more severe clinical course and become transfusion-dependent, making them candidates for curative therapy [5].

In haemoglobin Bart's hydrops foetalis, the absence of α-globin chain production leads to the development of erythroid cells that primarily consist of Hb Bart's (γ_4) during foetal life, or HbH (β_4) in long-term survivors. Both HbH and Hb Bart's have an exceptionally high affinity for oxygen, rendering them ineffective oxygen transporters [6]. Consequently, circulating HbH cells are considered non-functional. Unlike the ineffective erythropoiesis seen in β-thalassaemia patients, the predominant mechanism of anaemia in α-thalassaemia is extravascular haemolysis of erythroid cells within the splenic microvasculature [7, 8]. Therefore, even with regular transfusion regimens, patients with haemoglobin Bart's hydrops foetalis (α-thalassaemia major) continue to display features of hypoxia and severe haemolysis.

Despite the advances in pre-natal management of foetuses with haemoglobin Bart's hydrops foetalis, surviving infants continue to face major and unique challenges after birth. Even with intrauterine transfusions, chronic transfusions are still required shortly after birth, in contrast to infants with α-thalassaemia, in which transfusions usually start a few months later. As the diagnosis of haemoglobin Bart's hydrops foetalis incurs a more intensive transfusion regimen, patients inevitably carry a higher rate and earlier onset of iron overload and its complications, as compared to α-thalassasemia patients. Furthermore, chelation therapy is typically commenced earlier resulting in greater long-term toxicity [9, 10]. All these challenges make curative therapy a more appealing and desirable strategy for these children.

In the past, parents who received a prenatal diagnosis of haemoglobin Bart's hydrops foetalis typically elected to terminate their pregnancy due to the severity of the disease and the limited universal availability of therapeutic options [11]. Over the past three decades, however, improvements in early prenatal

diagnoses, such as the advent of in-utero transfusions (IUT), and advances in perinatal intensive care have enabled the survival of a growing number of children with this condition. This has led to an increase in the demand for more conclusive therapy.

In contrast to α-thalassaemia, there is tremendous experience garnered with transplantation for β-thalassaemia over the past few decades. Despite the differences in the underlying pathophysiology, the current approach for transplant in α-thalassaemia is extrapolated from protocols developed for β-thalassaemia. In this chapter, we first discuss specific aspects of haematopoietic stem cell transplant in β-thalassaemia as a framework for transplantation in α-thalassaemia. We will then review the transplant experience gained in α-thalassaemia.

Allogeneic haematopoietic stem cell transplantation

Allogeneic haematopoietic stem cell transplantation (HSCT) is currently the only available curative option for patients with transfusion dependent α-thalassaemia. The principle of HSCT in thalassaemia is to substitute the ineffective or abnormal erythropoiesis with donor derived red blood cells that will produce adequate functional haemoglobin. The European Society for Blood and Marrow Transplant (EBMT) outlines transfusion dependency as a currently accepted indication for transplant in thalassaemia [12, 13], particularly when matched sibling donors are available. HSCT provides a potential cure for patients with thalassaemia and is a more cost-effective treatment than lifelong blood transfusion and chelation therapy [14].

Risk allocation

The largest reported study of patients with β-thalassaemia who had HSCT occurred in Pesaro, Italy. In 1990, Guido Lucarelli and the Pesaro group developed a pre-transplantation risk assessment scoring system to predict outcomes post-HSCT in thalassaemia, a hallmark for diseases with iron overload [15–17]. This system stratified patients into three risk classes based on the presence of hepatomegaly by physical examination, biopsy proven liver fibrosis, and adherence to regular iron chelation [18, 19] (**Table 1**). Several early reports showed outcomes after transplantation were significantly affected by the pre-transplantation risk status, where patients with Pesaro class III had the lowest overall survival and thalassaemia-free survival rates.

Pesaro scoring system

The Pesaro system has several drawbacks when assessing patients prior to transplant, specifically: (1) it lacked quantitative direct indicators of iron overload such as serum ferritin, liver iron concentration, and myocardial iron loading; (2) it relied on the antiquated practice of using liver biopsies to evaluate the presence of liver fibrosis; and (3) there was a potential for intraobserver and interobserver variations regarding liver size and the effectiveness of iron chelation. Nonetheless, for the past three decades, this scoring system continues to significantly impact transplantation strategies [20, 21].

Table 1. *Pesaro risk classification for predicting outcome of haematopoietic stem cell transplantation for thalassaemia major patients.*

	Hepatomegaly[a]	Liver fibrosis[b]	Chelation history[c]	Overall survival/Thalassaemia-free survival (%)*
Class I	No	No	Regular	94/87
Class II	No/Yes	No/Yes	Regular/Irregular	84/81
Class III	Yes	Yes	Irregular	50/47

a Defined as >2 cm below the costal margin

b Fibrosis at any degree proven by biopsy

c Adequate iron chelation was defined as chelation with deferoxamine starting within 18 months of the first transfusion and administered regularly as an 8-hour continuous infusion for at least 5 days/week

** Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassaemia. N Engl J Med. 1990;322: 417–421.*

** Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in thalassaemia. Hematol Oncol Clin North Am. 1991;5: 549–556.*

Over the past 20 years, we have gained a better understanding of iron homeostasis, primarily as a result of treating patients with haemoglobinopathies, whose main morbidity is caused by toxicity from iron loading. Of the most significant advancement in clinical practice has been the development of MRI techniques to non-invasively and objectively evaluate iron levels in the liver and other organs such as the heart, pancreas, and pituitary [22, 23, 24]. The capacity to employ MRI to provide liver iron quantification as well as non-invasive serologic indicators for fibrosis prediction (serum ferritin, platelet count, transaminases, etc.) has impacted the utility of liver biopsies in iron metabolism disorders [25].

Outcomes of allogeneic HSCT in transfusion-dependent β-thalassaemia

The results of allogeneic HSCT for β-thalassaemia major have been thoroughly examined in large studies. Despite the differences in pathophysiologies between α- and β-thalassaemia, the valuable transplant experiences gained from β-thalassaemia, provides us with a framework when designing the transplant approach for patients with α-thalassaemia. The 2-year overall (OS) and event-free survival (EFS) in almost 1500 β-thalassaemia patients undergoing HSCT between 2000 and 2010 were 88 and 81%, respectively (Baronciani 2016). A more recent study demonstrated that OS and EFS were statistically higher in patients transplanted at ≤6 years vs ≥ 16 years (90% vs 63%, 86% vs 63%, respectively) [26].

Many institutions involved in thalassaemia transplantation have adopted transplants at an earlier age since initiating the transplant process before end-organ damage develops has been proven to improve outcomes. Considering thalassaemia is a progressive disease characterized by ongoing tissue and organ damage, the impact of age correlates with declining clinical status in keeping with the Pesaro risk classification. These findings are consistent with earlier studies that have highlighted the impact of age on outcomes and survival [27, 28].

Alternative donors and conditioning regimens

Although an HLA-matched sibling is the preferred donor, about 70% of patients who need allogeneic HSCT do not have a matched sibling and must rely on alternative donors [29]. Racial disparities influencing donor availability have been described where the probability of finding a match within the US registry is estimated to be 0.93 for Whites, 0.82 for Hispanics, 0.77 for Asian Americans and 0.58 for Blacks [30, 31]. More recently, as ethnic diversity continues to increase, there has been an increase in transplants utilizing unrelated and haploidentical donors [32].

Recent advances in the haploidentical transplant (haplo-HSCT) platforms, particularly the development of *in vivo* and *ex vivo* T cell depletion, and the application of post-transplantation cyclophosphamide (PTCy) have drastically improved outcomes [33–35]. Such strategies have resulted in equivalent if not superior outcomes, with OS and EFS values up to 94% of those using matched related and unrelated donors [36, 37].

Traditionally, the standard conditioning regimen implemented was a myeloablative dose of busulfan (Bu) and cyclophosphamide (Cy) which resulted in sustained donor myeloid engraftment. This however was associated with infertility as well as potential significant organ toxicity as well as a long-term burden on HSCT survivors. Reduced toxicity conditioning (RTC) regimens have since been trialled using the addition of fludarabine (Flu) and/or thiotepa (TT) to Bu and Cy or treosulfan with good results [38–40]. Pharmacokinetic model-based dosing of conditioning agents has also been used to reduce cumulative drug exposure and lower toxicity [41].

Sequential immunoablative pre-transplant regimen of fludarabine and dexamethasone, in conjunction with hyper-transfusion and chelation, is one of the novel approaches developed by Anurathapan *et al.* to establish stable graft function while lowering toxicity [39]. This regimen has demonstrated outstanding results in class III patients who typically had higher transplant related mortality (40%) and rejection rates (~16%). These results were similarly seen when using both matched unrelated and haploidentical donors [39, 42]. Using the risk features identified in previous studies has allowed tailoring donor choice and conditioning regimens in high-risk recipients. The results of transplantation have consequently grown more comparable across risk categories [43].

GVHD prophylaxis

Improvements in the prevention and management of graft-versus-host disease (GVHD) and induction of graft tolerance have encouraged the use of alternative donors as well. Newer targeted agents, such as co-stimulation blockade, are being studied to prevent GVHD especially in non-malignant transplants with promising outcomes [44, 45].

Table 2. *Summary of patients who received allogeneic haematopoietic stem cell transplantation for alpha-thalassaemia.*

Patient no.	Age at HSCT/Sex	Mutation	IUT (Y/N)	Donor Source, Graft	Conditioning regimen	GVHD prophylaxis	Complications	Outcome	Authors
1	21 months/F	NA	N	MSD, BM	Bu,Cy, horse ATG	MTX, CSA	None	Neutrophil engraftment at day +17. Stable mixed chimerism (75%-90%). Transfusion independent.	Chik et al 1998 [46]
2	21 months/F	Homozygous SEA deletion	N	MSD, 8/8 BM	Bu/Cy	CSA, MTX, horse ATG	None	Neutrophil engraftment at day +17. Donor chimerism 99%. Transfusion independent.	Chan et al (2021) [58]
3	20 months/F	Homozygous SEA deletion	N	MSD, 5/6 CB	Bu/Cy	CSA, MTX, horse ATG	Grade 2 skin aGVHD resolved with systemic steroid, HHV–6 viremia resolved with foscarnet	Neutrophil engraftment at day +26. Donor chimerism 100%. Transfusion independent.	Chan et al (2021) [58]
4	22 months/F	Homozygous SEA deletion	Y	MUD, 12/12 PBSC	Bu/Cy	CSA, MTX, horse ATG	Grade 2 aGVHD of skin and grade 3 aGVHD of gut resolved with systemic steroid, HHV–7 viremia spontaneously resolved	Neutrophil engraftment at day +15. Donor chimerism 100%. Transfusion independent.	Chan et al (2021) [58]

Patient no.	Age at HSCT/Sex	Mutation	IUT (Y/N)	Donor Source, Graft	Conditioning regimen	GVHD prophylaxis	Complications	Outcome	Authors
5	28 months/M	Homozygous SEA deletion	Y	MUD, 12/12 PBSC	HU/AZA/Cy/Bu/TT/Flu	CSA, MTX, MMF, rabbit ATG	Grade 2 skin aGVHD resolved with systemic steroid, EBV and HHV–6 viremia resolved spontaneously.Line sepsis.	Neutrophil engraftment at day +11. Donor chimerism 98%. Transfusion independent.	Chan et al (2021) [58]
6	60 months/M	Homozygous SEA deletion	Y	Haplo, PBSC (TCRαβ/CD45RA depleted)	HU/AZA/Cy/TT/Flu/Treo	Rabbit ATG	Klebsiella bacteremia cleared with antibiotics. Grade 2 skin aGVHD resolved by topical steroid, grade 2 gut aGVHD resolved with steroid and ruxolitinib	Neutrophil engraftment at day +13. Donor chimerism 99%. Transfusion independent.	Chan et al (2021) [58]
7	12 months/NA	NA	Y	MSD/NA	NA	NA	NA	Transfusion independent by 18 months	Kreger et al 2016 [2]
8	24 months/NA	NA	N	NA	NA	NA	NA	Transfusion independent by 3 years	Kreger et al 2016 [2]
9	20 months/F	Homozygous SEA deletion	N	MSD/CB	Bu/Cy/ATG	MTX,CSA	HHV-6 viremia, grade 2 skin aGVHD resolved with steroids, ITP resolved with IVIG.	Neutrophil engraftment at Day +26. Donor chimerism 100%. Transfusion independent.	Zhou et al 2001[59]
10	23 months/M	Homozygous SEA deletion, Hb E trait	Y	MSD/BM	Cy/ 14Gy TBI	MTX, CSA	Mild VOD	Neutrophil engraftment at Day +27. Stable mixed donor chimerism 66%. Transfusion independent.	Thornley et al, 2003 [60]

Patient no.	Age at HSCT/Sex	Mutation	IUT (Y/N)	Donor Source, Graft	Conditioning regimen	GVHD prophylaxis	Complications	Outcome	Authors
11	5 months/M	Homozygous deletion	Y	MUD, 10/10 BM	Bu/Cy/rabbit ATG	MTX, CSA	Moderate VOD treated with Antithrombin, Grade 4 mucositis, intubated x 18 days.	Neutrophil at Day +25. Stable mixed donor chimerism (78-95%). Transfusion independent.	ElSaid et al, 2016 [61]
12	13 years/M	Homozygous SEA deletion	N	MMUD 9/10	RIC (details NA)	NA	Invasive fungal infection, grade 4 skin and gut GVHD	Died 4 months post transplant from transplant related morbidity.	Pecker et al, 2016 [62]
13	10 years/M	Homozygous SEA deletion	Y	MUD 9/10	NA	NA	None	Donor 100% chimerism. Transfusion independent.	Pecker et al, 2016 [62]
14	8 months/M 2nd transplant at 18 months	Homozygous SEA deletion	Y	#1 MUD, 4/6 CB #2 MUD, 6/6 CD34 selected PBSC	#1 Bu/Flu/ATG, TLI 750 cGy #2 Bu/Flu/ATG/TBI 200cGy	#1 CSA, MMF #2 CSA, MMF	#1 Graft failure #2 EBV associated PTLD treated with ganciclovir, Rituximab and reduced IS. No GVHD.	#1 Graft failure #2 Neutrophil engraftment at Day +18. Stable mixed donor chimerism 97%. Transfusion independent.	Yi et al, 2009 [63]
15	2 years/M	Homozygous SEA deletion	Y	MSD	Bu/Flu/ATG/TLI	NA	NA	Secondary graft failure at 7 months. Transfusion dependent.	Joshi et al, 2004 [64]

Patient no.	Age at HSCT/Sex	Mutation	IUT (Y/N)	Donor Source, Graft	Conditioning regimen	GVHD prophylaxis	Complications	Outcome	Authors
16	44 months/F	Homozygous SEA deletion	N	MUD, 5/6 CB	Bu/Cy/horse ATG	Tacrolimus, MMF	Candida krusei sepsis, treated with liposomal amphotericin, grade 2 acute skin GVHD resolved with steroids.	Neutrophil engraftment at Day +12. Donor chimerism 100%. Transfusion independent.	Gumuscu et al, 2013 [65]
17	19 months/F	Homozygous SEA deletion	N	MSD, BM	Bu/Cy	CSA, MTX	Febrile neutropenia, viral pneumonia, mucositis.	Neutrophil engraftment at Day +17. Mixed donor chimerism 59%. DLI x 5 then full donor chimerism achieved. Transfusion independent.	Pongtanakul et al, 2013 [66]

NA: not available, HSCT: haematopoietic stem cell transplantation, IUT: intrauterine transfusions, GVHD: graft-versus-host-disease, F: female, M: male, SEA: Southeast Asian, MSD: matched sibling donor, MUD: matched unrelated donor, MMUD: mismatched unrelated donor, Haplo: haploidentical donor, BM: bone marrow, CB: cord blood, PBSC: peripheral blood stem cells, Bu: busulfan, Cy: cyclophosphamide, Hu: hydroxyurea, Aza: azathioprine, TT: thiotepa, Flu: fludarabine, Treo: treosulfan, CSA: cyclosporine, MTX: methotrexate, ATG: anti-thymocyte globulin, MMF: mycophenolate mofetil, aGVHD: acute graft-versus-host-disease, HHV-6: human herpes virus-6, EBV: Epstein-Barr virus, ITP: immune thrombocytopenia, IVIG: intravenous immunoglobulin, TBI: total body irradiation, VOD: veno-occlusive disease, Hb: haemoglobin, RIC: reduced intensity conditioning, TLI: total lymphoid irradiation, PTLD: post-transplant lymphoproliferative disease, ID: immunosuppression, DLI: donor lymphocyte infusion.

Transplant in α-thalassaemia

In 1998, the first transplant in severe α-thalassaemia was conducted in a 21-month-old girl from a matched sibling donor after conditioning with busulfan (Bu) and cyclophosphamide (Cy). Transfusion independence was achieved post-transplant despite residual recipient haematopoietic stem cells (HSCs) [46]. Since then, just a few case reports describing successful transplant in α-thalassaemia have been published, in contrast to thousands of transplants conducted for β-thalassaemia. This observation highlights the significance and particular challenges we are encountering as we work to create a curative therapy option for patients with α-thalassaemia.

Summary of all previous transplants in α-thalassaemia

After the first case published in 1998, 16 additional children have undergone HSCT for α-thalassaemia (**Table 2**). Due to inadequate prenatal care or parental refusal to test, 6 of the 16 patients (38%) did not receive an antenatal diagnosis of thalassaemia. All these patients were born premature, had evidence of hydrops, and required intensive neonatal support. The remaining 10 patients, who had been antenatally diagnosed with Hb Bart's, got intrauterine transfusions (IUTs); the earliest recorded start time was 24 weeks gestation. Five of these patients were delivered at term. Among the 10 patients, 6 were delivered vaginally, 2 were via C-sections, and the other 2 had no known method of delivery. In comparison to the patients who had not had IUTs, these patients had less hydrops, needed fewer interventions after delivery, and had higher median birth weights (2100g).

Ten patients (63%) of the 16 recipients of a HSCT were younger than 24 months, with the youngest recipient being 5 months old (range, 5 months–13 years). Nine patients (53%) received a myeloablative, busulfan-based conditioning regimen with different agents including cyclophosphamide alone (5 patients), fludarabine/cyclophosphamide/thiotepa (1 patient), and fludarabine alone (1 patient). The remaining patients were divided into the following groups: 3 received total body or lymphoid irradiation-based conditioning, 1 received a regimen based on treosulfan, 2 were reported as receiving reduced intensity conditioning, but details were not available, and 3 were not reported. Regarding the stem cell source, 5 patients had a matched sibling donor (3 bone marrow grafts, 2 unknown), 5 had matched unrelated donors (2 peripheral blood stem cells, 1 bone marrow, 2 unknown), 2 received mismatched related cord blood cells, 2 received mismatched unrelated cord blood cells, 1 received a haploidentical TCRαβ/CD45 RA depleted maternal graft and 1 patient had an unknown donor and graft source. Most of the patients (62%) were reported to have received serotherapy with anti-thymocyte globulin (ATG). Seven patients were given cyclosporine and methotrexate based GVHD prophylaxis regimen, 1 received tacrolimus and mycophenolate mofetil (MMF), 1 received cyclosporine and MMF, 1 was only given ATG, and 6 were not reported. The eldest patient in this cohort, a 13-year-old, was the only reported death from transplant-related complications.

The median time to neutrophil engraftment was 19 days (range, 11–27 days). One patient (patient 14), who experienced primary graft failure, engrafted using CD34-selected peripheral blood stem cells on day 18 following the second transplant. Time to engraftment was not reported in 6 patients. Six patients achieved full donor chimerism, while 6 patients had stable mixed chimerism, and 4 did not have their chimerism data reported. One patient (patient 17) had declining chimerism down to 59% by day 112 but responded to 5 sessions of donor lymphocyte infusions ($1x10^7$ cell/kg). One patient (patient 15) experienced secondary graft failure 7 months post-transplant and continued chronic transfusion and chelation. Otherwise, all the patients that engrafted, even those with mixed chimerism (lowest reported was 66.5%), attained transfusion independence. Of note, only 1 patient (patient 14) required 2 transplants: the first with matched unrelated donor cord blood and the second with CD34 selected peripheral blood stem cells. Corticosteroids were effective in treating the grade III-IV GVHD in 2 patients and grades 1-2 GVHD in 6 patients. Only 1 patient was reported to have developed severe and one patient to have developed mild veno-occlusive disease of the liver from those patients with known iron overload prior to transplant.

Long-term outcomes, post-transplant, were only reported in a subset of patients. Four of 14 (29%) patients that underwent successful engraftment and survived had short stature, and 4 of 14 (29%) had normal growth. Four patients showed mild developmental delays (intellectual disability and speech/language delay), and 3 patients had gross motor delays, 1 of which was stated to have improved by the age of 4. Six patients, of whom 5 had received previous IUTs, were able to reach normal developmental milestones. Five patients needed treatment for iron overload after transplant, the longest of which lasted for 4 years.

A minority of patients with other alpha thalassaemia mutations, such as HbH disease, may similarly benefit from curative therapies [5]. Most patients with HbH disease live normal lives. A small subset however may require regular transfusions for survival. There is a scarcity of data on such patients. Surapolchai *et al.* reported a case of an 8-year-old boy with transfusion-dependent non-deletional severe HbH disease (--SEA and α2 polyA deletions) who received conventional myeloablative conditioning regimen for HLA matched related HSCT, with a favorable outcome [47].

Due to the variable phenotype of HbH, some people may develop transfusion dependence later in life despite a benign history in childhood. Additional manifestations including iron overload, osteopenia, splenomegaly, and biliary disease may still occur among non-transfusion dependent patients and in those with deletional disease [48]. This raises the question of whether these patients should pursue transplant in childhood in pursuit for a better prognosis.

It is noteworthy that the data presented above is made available from published case reports, which are inherently subject to publication bias. We recognize that there is a subset of patients with inferior transplant outcomes that may not have been captured in the literature.

Mixed chimera

The level of donor chimerism needed to achieve transfusion independence in patients with thalassaemia post-transplant is not defined. Mixed chimerism (MC) is characterized by the simultaneous presence of donor derived cells and residual host cells (RHCs) which has been frequently observed at an incidence of 32% to 46% after HSCT for β-thalassaemia [49]. It is unclear why in some patients MC can be transient before evolving towards complete chimerism or graft rejection, while, in others, it eventually become persistent.

The persistence of RHCs at >25% in the early post-transplant period, within 2 months, has been linked with a 40% increased risk of thalassaemia recurrence [50, 51]. Nevertheless, patients with persistent mixed chimerism (PMC), which is defined as a stable mixed chimerism for more than two years, were able to become transfusion independent even with >25% RHCs, without the risk of graft failure. PMCs are observed in about 10%–15% of thalassaemic transplanted patients, and even with as little as 20–30% donor-derived nucleated cells, these patients are clinically cured [52].

In a study on split chimerism, Andreani et al. found that although the proportion of donor derived cells was equally distributed in the different cell lineages in both the peripheral blood and bone marrow, that was not the case for the erythrocyte compartment. Despite the presence of few donor-engrafted nucleated cells, the erythrocytes were almost completely of donor origin. This enrichment of donor RBC in the blood was not observed in erythroid precursors from the marrow, suggesting that the ineffective erythropoiesis that is presumably responsible for this phenomenon works at a later stage of erythroid development. Long-term observation demonstrated that even in the presence of very low percentage donor engrafted nucleated cells (<20%), patients may achieve a functioning graft status.

Extrapolating from the β-thalassaemia data, complete chimerism is likely not required for long term engraftment or attaining transfusion independence post-transplant in α-thalassaemia [53]. However, a distinctive feature in α-thalassaemia is the presence of high degree of erythropoiesis and futility of endogenous HbH cells in the setting of mixed chimerism. While theoretically mixed chimera would entail a lower level of "functional" haemoglobin than the value reported, there is still no existing data to recommend an acceptable chimerism threshold in the clinical setting in the absence of transfusion dependence.

HSCT is the only curative option to date for α-thalassaemia survivors. Patients with α-thalassaemia may require earlier intervention than those with β-thalassaemia as adverse effects of α-thalassaemia starts as early as in utero. As findings from studies conducted on patients with β-thalassaemia demonstrated that it is unnecessary to reach full donor chimerism to achieve transfusion independence, reduced toxicity conditioning can be utilized to minimize transplant-related morbidity and mortality. In order to reduce the likelihood of graft failure and enable the adoption of less toxic preparative regimens, the concept of pre-transplant immunosuppression can be similarly implemented in patients with α-thalassaemia. Recent advances and comparable outcomes seen when using human leukocyte antigen

(HLA) matched and mismatched related and unrelated donor sources have also expanded the donor pool. The advent of newer targeted GVHD preventive therapy, which may be added to the conventional regimens, will be particularly useful for patient populations of diverse ethnic backgrounds. All these aspects should be taken into consideration while designing and performing transplants tailored for patients with α-thalassaemia.

Long-term follow-up after HSCT

Haematopoietic stem cell transplantation, as opposed to supportive blood transfusions, gives patients with thalassaemia the possibility of definitive cure. Caocci *et al.* reported that the 30-year survival of thalassaemia patients after HSCT was similar to that expected in thalassaemia patients treated with blood transfusions and iron chelation (85.3% vs 82.6%) [54]. Most patients surviving HSCT were cured from thalassaemia (94.2%) [54]. A systematic review by Zhai *et al.*, found that the quality of life (QOL) of patients with β-thalassaemia after HSCT from a sibling donor is higher than that of patients that received blood infusion and iron-chelating therapy [55]. HSCT survivors were found to have a QOL as good as that of a healthy population and the ability to return to normal life. These studies however did not include a large sample size and were limited in terms of donor type and source [55].

The survival of patients who have received treatment has significantly improved because of improvements in transplantation platforms and supportive care practices. However, HSCT survivors remain at risk for developing long-term complications, such as effects on growth, fertility and endocrinopathies among others. The risk of these complications is influenced by pre-HSCT health status and therapeutic exposures, transplantation-related conditioning, and post-transplantation management of GVHD [56].

Systematic monitoring and follow-up are essential for managing potential long-term consequences of HSCT and the residual symptoms of pre-HSCT disease. Late complications vary with age and disease status at HSCT and with transplant variables such as preparative regimen, donor source, human leukocyte antigen (HLA) compatibility, and immune reconstitution. Patients may still require iron reduction therapy post-HSCT with either regular phlebotomy or iron chelators to prevent complications related to iron overload. An international guideline, published in July 2018 and titled "Late Effects Screening Guidelines after Haematopoietic Cell Transplantation (HCT) for Haemoglobinopathy: Consensus Statement from the Second Pediatric Blood and Marrow Transplant Consortium International Conference on Late Effects after Pediatric HCT", summarizes the consensus on long-term follow-up guidelines after HCT for haemoglobinopathy [57].

Conclusion

Haemoglobin Bart's hydrops foetalis (α-thalassaemia major) was once considered to be fatal. The recent advancement of transplant-care has transformed the landscape for these patients, with transfusion independence and provision of an improved quality of life. With allogeneic HSCT currently being the only potential cure, it is crucial to continue to develop strategies to improve transplant-related outcomes. Meanwhile, transplant of children with haemoglobin Bart's hydrops foetalis (α-thalassaemia major) should be performed early to reduce the risk of transplant-related morbidity and other complications. The clinical expertise in transplant for α-thalassaemia remains derived from β-thalassaemia protocols despite a clear distinction in disease pathobiology. A different approach for α-thalassaemia may perhaps be warranted and may prove to be even more successful. Until further data is available, however, it is advised that the transplant approach for α-thalassaemia should be based on what has been established for β-thalassaemia.

Summary and recommendations

- Antenatal counselling of parents involving obstetricians, neonatologists, hematologists, and stem cell transplant physicians is imperative.
- Surviving patients with haemoglobin Bart's hydrops foetalis require lifelong transfusion therapy and iron chelation, and as compared to their β-thalassaemia counterparts, they are at higher risk of disease or treatment-related complications.
- Allogeneic haematopoietic stem cell transplantation is the only curative option currently available for long term survivors of haemoglobin Bart's hydrops foetalis.
- Transplant for thalassaemia should be offered as early as possible as outcomes are superior prior to the onset of organ dysfunction secondary to iron overload.
- Several case reports have been published to date describing the success of transplant in patients with α-thalassaemia. All patients who have engrafted and survived were able to achieve transfusion independence.
- Similar to patients with complete chimerism, those with persistent mixed chimerism were still able to be clinically cured. However, the acceptable threshold for residual host cells post-transplant is yet to be defined.
- Improvements in transplantation platforms such as *ex vivo* or *in vivo* graft manipulation and graft versus host disease prophylaxis have expanded the use of alternative donors (haploidentical, unrelated) for patients with thalassaemia with comparable outcomes to matched sibling donors.
- Outcomes of transplant have improved significantly over the last decade including those of high-risk patients.
- HSCT survivors will require lifelong monitoring and follow-ups for long-term consequences of HSCT and the residual symptoms of pre-HSCT disease.

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13 NOVEL AND EMERGING THERAPIES FOR α-THALASSAEMIA

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Disease modifying therapies

Different pharmacological agents that may improve anaemia in α-thalassaemia by acting in different stages of the thalassaemia pathogenesis are being evaluated. These agents are not expected to have any benefit in patients with haemoglobin Bart's hydrops foetalis who do not have the ability to produce functional haemoglobin. The patients that will benefit the most from these agents are patients with moderate to severe forms of haemoglobin H (HbH) disease, who require red blood cell transfusions and/or exhibit thalassaemia-related morbidities. These patients exhibit a high degree of haemolysis and/or ineffective erythropoiesis, and they usually carry non-deletional α-thalassaemia mutations.

Luspatercept, a recombinant fusion protein consisting of a modified form of the extracellular domain of the activin receptor type IIB (ActRIIB) linked to the human immunoglobin G1 Fc domain, is the first-in-class erythroid maturation agent that has been approved for the treatment of anaemia in adult patients with either β-transfusion dependent thalassaemia (TDT) (US Food and Drug Administration-FDA and European Medicines Agency-EMA) or with β-non-transfusion dependent thalassaemia (NTDT) (EMA) [1]. Luspatercept seems to restore normal erythropoietic differentiation and improve anaemia by binding some transforming growth factor-β (TGF-β) ActRIIB ligands. Results from the studies in β-thalassaemia have shown a decrease in transfusion burden in patients with TDT and an increase in haemoglobin levels in patients with NTDT. Of note, the improvement of the haematological status in NTDT did not correlate with significant changes in all the patient-reported outcome measures used in the trial, which partially reflects the lack of sensitive tools for measuring respective mild changes. The drug was well tolerated, though an increase in the incidence of thromboembolic events and in increase in extramedullary haemopoietic sites, mainly in patients with TDT, were observed. Long-term data on the use of luspatercept have verified persistent efficacy with manageable toxicity profile. As the pathogenetic mechanisms differ between β- and α-thalassaemia, with haemolysis playing a more significant role than ineffective erythropoiesis in the majority of patients with HbH disease, the efficacy of the treatment with luspatercept in this condition may be more variable and dependent to underlying genotypes. A clinical trial on both α-TDT and α-NTDT (NCT05664737) is currently ongoing.

Thalassaemic red blood cells have substantially increased metabolic oxidative stress, leading to increased haemolysis and early cell death. Increasing the erythrocytic ATP levels will counterbalance the augmented intracellular energy demands and decrease the vulnerability of the thalassaemic erythrocytes. Allosteric

activators of pyruvate kinase have been shown to increase ATP concentrations and ameliorate haemolysis in different chronic haemolytic anaemias. The first-in-class of these medications, mitapivat, has been shown to increase haemoglobin levels in a few patients with haemoglobin H disease in a phase 2 study [2]. Mitapivat is currently studied in phase 3 clinical trials for either α-TDT (NCT04770779) or α-NTDT (NCT04770753). A phase 2 clinical trial with the use a second pyruvate kinase activator, Etavopivat, is also currently ongoing (NCT04987489). Based on their mode of action, which targets mainly to the increased haemolysis, the allosteric activators of pyruvate kinase may be shown to have a more robust and homogeneous efficacy in individuals with α-thalassaemia compared to luspatercept.

Iron chelation medications

Iron homeostasis is deranged in thalassaemia both secondary to red blood cell transfusions and to increased gastrointestinal iron absorption. Agents that increase hepcidin levels or block ferroportin may improve erythropoiesis and anaemia and enhance the efficacy of iron chelation. Different agents are being studied in this setting. So far, despite initial encouraging preclinical observations, results have not been positive.

Gene therapy

The use of autologous gene therapy using lentiviral vectors carrying β-globin gene has been successfully validated for β-thalassaemia treatment [3]. Similarly, *ex-vivo* lentiviral gene therapy to introduce α-globin gene is a potential strategy to cure α-thalassaemia. However, no gene therapy clinical trial is currently available for patients with α-thalassaemia.

Clinical trials evaluating gene editing approaches for the treatment of β-thalassaemia are currently ongoing. The most advanced studies have been using CRISPR/Cas9 methods to reactivate γ-globin expression [4]. These strategies are based in altering the evolutionary process of haemoglobin switching and are not applicable for the α-gene complex and α-thalassaemia. Gene editing for correction of specific point mutations mainly by base editing are currently being evaluated for haemoglobinopathies (sickle cell disease, β-thalassaemia) and may be applicable for specific non-deletional types of α-thalassaemia. The specificity of this gene editing method to a particular mutation affecting a small proportion of the respective patients' population is a significant limiting factor for its development and its widespread applicability.

In-utero transplantation

In-utero haematopoietic stem cell transplantation (IUHSCT) allows introduction of donor non-HLA-matched haematopoietic stem cells (HSCs) without conditioning by taking advantage of the naïve foetal immune system. Barriers to engraftment include a competitive disadvantage of donor cells compared to endogenous foetal HSCs for available haematopoietic niches and an immune barrier between the foeto-maternal immune systems.

IUHSCT has been proposed for the treatment of haemoglobin Bart's hydrops foetalis, as it can be detected early in foetal life and poses detrimental effects in the absence of intrauterine intervention with blood transfusions. In an ongoing phase 1 clinical trial (NCT02986698), maternal bone marrow derived CD34+ cells are in-utero transplanted via the umbilical vein, between 18 and 26 weeks of gestation [5]. Interim data demonstrated a sustained maternal cells tolerance albeit minute levels of maternal chimerism. These patients continued to rely on regular transfusions both pre- and postnatal. The results showed that IUHSCT can be safely administered and can achieve prenatal tolerance induction, while low levels of engraftment confirmed the need for a post-natal "boost" with possibly minimal conditioning to establish definitive cure.

Brief summary and recommendations

- Different agents are studied in α-thalassaemia, with the most promising being luspatercept and mitapivat. Luspatercept is an erythroid maturation agent with good efficacy in decreasing transfusion dependency in β-TDT and improving anaemia in β-NTDT. Mitapivat is an activator of pyruvate kinase which increases haemoglobin levels in patients with HbH disease.
- Results of the studies on the use of these novel agents in α-thalassaemia are expected within the next few years. Should they be positive, they may offer an alternative to transfusions for improving anaemia in patients with α-thalassaemia. Before the completion of the studies, the use of these agents outside the setting of clinical trials is not recommended.
- Novel curative approaches like gene addition of α-globin gene and in-utero haematopoietic stem cell transplantation are currently in early phases of development.

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14 PREVENTION AND CONTROL OF α-THALASSAEMIA DISEASES

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Introduction

α-Thalassaemias result from deletions or mutations involving α-globin genes (HBA1 and HBA2) on chromosome 16, causing either the absence or reduced production of α-globin chains [1]. Additionally, non-deletional mutations of the α-globin genes can lead to the production of α-globin variants, which may have further adverse effects on erythroid differentiation and cell metabolism. Clinically significant α-thalassaemias can be broadly categorized into two main conditions: HbH disease and haemoglobin Bart's hydrops foetalis.

The prevention and control of α-thalassaemias aims to identify couples at risk of conceiving a foetus with the most severe form of α-thalassaemia, Hb Bart's hydrops foetalis, or other clinically severe forms of HbH disease. Identifying these at-risk couples serves multiple purposes, including informed family planning decisions and offering couples with affected pregnancies a range of management options, such as the possibility of terminating the pregnancy or foetal therapy. It also facilitates the monitoring of maternal complications.

This chapter will cover the prenatal screening for a couple at risk of having a foetus with Hb Bart's hydrops foetalis and foetal diagnostic methods. Additionally, screening process for severe forms of HbH disease (HbH hydrops foetalis) and homozygous non-deletional forms of α-thalassaemia (e.g., homozygous Hb Constant Spring (CS) will be reviewed.

Haemoglobin Bart's hydrops foetalis

Haemoglobin Bart's hydrops foetalis or α-thalassaemia major, results from homozygosity or compound heterozygosity of *in-cis* deletions of duplicated α-globin genes (α^0-thalassaemia). The deletion of all four α-globin genes (--/--) results in an absent α-globin production. Fetuses with Hb Bart's hydrops foetalis from a^0-thalassaemia deletions that spare the ζ-globin gene (*HBZ*), such as the Southeast Asian a^0-thalassaemia deletion (--SEA), typically presents with features of hydrops in the second or third trimester. Their haemoglobin mainly consist of Hb Bart's (γ_4) and Hb Portland I ($\zeta_2\gamma_2$) [2-8]. Other haemoglobins that may be present in small amount include Hb Portland II ($\zeta_2\beta_2$), Hb epsilon4 (e_4), Hb Gower I and HbH (β_4) [2, 6, 7]. Hb Bart's has an extremely high oxygen affinity and is non-functional for delivering oxygen to foetal tissue. Hb Portland I is a functional Hb that delivers oxygen in the earlier gestation to the foetuses. Foetuses with Hb Bart's hydrops foetalis present with severe anaemia, enlarged liver and spleen, developmental abnormalities, and heart failure. If left untreated, hydrops foetalis will

ensue and will ultimately leads to foetal demise in utero, stillbirth, or early neonatal death. It also affects the mother, potentially causing preeclampsia or eclampsia (mirror syndrome), and increasing maternal mortality [9]. Foetuses who are homozygous for larger α^0-thalassaemia deletions involving the ζ-globin gene (*HBZ*), such as the --FIL or --THAI α^0-thalassaemia deletions are unable to produce any functional haemoglobin and result in early pregnancy loss [9, 10]. Additionally, foetuses who are compound heterozygous for --THAI and --SEA α^0-thalassaemia deletions have been reported with earlier onset of hydrops foetalis and few long-term survivors [11].

While it is rare, some affected pregnancies may result in childbirth and long-term survival without any foetal therapy. However, with the availability of intensive perinatal care and intrauterine transfusion, there has been increasing number of surviving patients with Hb Bart's hydrops foetalis [12, 13]. An international registry reported outcomes of 69 patients with Hb Bart's hydrops foetalis who survived into childhood and adulthood [12]. All patients require lifelong red cell transfusion and iron chelation. About two-thirds of the patients have congenital abnormalities, most frequently urogenital or limb abnormalities [12]. The congenital abnormalities are compatible with a vascular disruption defect caused by vascular occlusion or hypoxia associated with Hb Bart's hydrops foetalis [14]. Intrauterine transfusion contributes to better outcome during the perinatal period. However, growth retardation and neurodevelopmental delay remain the major problems in 40–50% and 20% of the patients, respectively [12]. Haematopoietic stem cell transplantation resulted in transfusion independency in 14 out of 18 patients who underwent the treatment but did not improve the growth retardation or neurodevelopmental delay in some patients. Despite intrauterine transfusion, there are associated maternal complications, such as amniotic fluid abnormalities, preeclampsia, and preterm birth [12]. A report of foetuses with Hb Bart's hydrops foetalis from Canada showed that all 12 Foetuses who did not receive intrauterine transfusion died within the first week of life [13]. Nine out of 13 foetuses who received intrauterine transfusion survived, were transfusion-dependent and experienced earlier iron overload, and had more frequent endocrinopathies and short stature. Neurocognitive outcome was not significantly affected in 5 patients who were assessed. MRI of the brain showed silent ischemic infarcts in 3 patients [13]. Given this information, clinical characteristics, intrauterine management, long-term complications, especially growth retardation, neurodevelopmental delay, and congenital abnormalities, should be discussed in detail with parents during counselling for management [12, 13].

Due to the lethal nature of Hb Bart's hydrops foetalis and its adverse effects on foetal and maternal health, genetic counselling and prenatal screening for couples at risk of having a foetus with Hb Bart's hydrops foetalis should be offered to all couples. Detailed and non-directive counselling should be provided and management options should be discussed. In cases where parents wish to continue with the pregnancy, management should be provided at centres with experience in caring for foetuses with Hb Bart's hydrops foetalis. Early ultrasounds to detect hydrops and associated congenital abnormalities should be performed. Early intrauterine transfusion and close follow-up on foetal and maternal status should be offered. The care team should be coordinated between obstetrics and neonatology for postnatal intensive care and haematology for long-term management.

Prenatal screening for carriers of α^0-thalassaemia

Both individuals in a couple at risk of foetal Hb Bart's hydrops foetalis must carry α^0-thalassaemia allele. They are either α^0-thalassaemia carriers or individuals with HbH disease. Carriers of α^0-thalassaemia have microcytic and hypochromic red blood cells. The commonly used cut-off mean corpuscular volume (MCV) and mean corpuscular Hb (MCH) for screening of α^0-thalassaemia is typically <78-80 fL and <27 pg, respectively [15-17]. HbH disease can be detected by hypochromic, microcytic anaemia in blood count and presence of HbH and/or Hb Bart's during Hb analysis. Haemoglobin analysis can also reveal co-inheritance of β-thalassaemias or other Hb variants but it is not able to identify α^0-thalassaemia or some unstable globin variants.

When both individuals in a couple meet the MCV or MCH criteria, they undergo DNA-based testing for α^0-thalassaemia. The test for α^0-thalassaemia is required regardless of β-thalassaemia status or iron deficiency as coinheritance of α-thalassaemia with β-thalassaemia, other β-haemoglobinopathies, and iron deficiency is common [18]. The exception is in the African population where α^+-thalassaemia is common but α^0-thalassaemia is rare [16, 19-21]. Genetic testing for α^0-thalassaemia should be performed when both partners have low MCV or MCH and β-thalassaemia carrier state has been ruled out through Hb analysis [16].

DNA-based method for identification of α-thalassaemia

Approximately 5% of the world's population carries α-thalassaemia mutations [22]. Common α^0-thalassaemias and their prevalent regions are summarized in **Table 1**. Ethnicity information is crucial for selecting the types of deletions to be tested.

Table 1. *Common α^0-thalassaemias and the prevalent regions*

Type of a^0-thalassaemias	Prevalent regions
Thai ($--^{THAI}$)*	Southeast Asia, China
Filipino ($--^{FIL}$)*	Philippines
Mediterranean II ($--^{MED\ II}$)*	Mediterranean
Southeast Asian ($--^{SEA}$)	Southeast Asia, China
Mediterranean I ($--^{MED\ I}$)	Mediterranean
-20.5 kb deletion ($-(a)^{-20.5}$)	Mediterranean

*Deletions involved the *HBZ* gene

Gap-polymerase chain reaction (gap-PCR) technique is employed for detection of common types of α^0-thalassaemia. The technique is particularly useful for identifying large gene deletions. PCR primers are designed to flank known deletion breakpoints that are specific for each deletion. In cases of α^0-thalassaemia deletions with unknown breakpoints, multiplex ligation-dependent probe amplification (MLPA) of α-globin gene cluster is utilized to determine the region of deletion.

Couples at risk of foetal Hb Bart's hydrops foetalis

Pregnant women are strongly encouraged to seek early antenatal care. During the initial antenatal care visit, couples should receive counselling regarding the risk of foetal thalassaemia diseases, and prenatal testing to identify those at risk should be conducted at this stage [23]. For pregnant women at risk of foetal Hb Bart's hydrops foetalis, the first ultrasound examination should be scheduled for 12-13 weeks of gestation. Couples facing this risk should receive counselling on the clinical presentation of foetuses with Hb Bart's hydrops foetalis, foetal diagnostic methods, and management options.

There are two foetal diagnostic approaches available:

1. The conventional approach: This method involves obstetric procedures to obtain foetal DNA or blood for molecular and/or Hb analysis. It is invasive and carries a slightly higher risk of procedure-related foetal loss or complications, although the rates of complications are generally low. The primary advantage of this approach is that it provides a definitive diagnosis early in pregnancy, allowing for earlier management and reducing parental anxiety.
2. The non-invasive approach or ultrasound algorithm: The second approach involves a serial ultrasound algorithm. Its main advantage is the reduced need for invasive procedures. However, this method requires continuous follow-up of the pregnant woman, which may result in increased parental anxiety. Ultrasound algorithm can avoid invasive diagnostic procedures in approximately 70% of cases and can detect nearly all affected cases during the pre-hydropic phase [24].

Conventional foetal diagnosis of Hb Bart's hydrops foetalis: analysis of foetal DNA or blood

The conventional foetal diagnostic approach entails obstetric procedures to obtain foetal DNA or blood for molecular and/or Hb analysis. the details of each obstetric procedure are as shown in **Table 2.**

DNA-based diagnosis of the foetus is performed using gap-PCR to detect the α^0-thalassaemia deletions that may have been inherited from the parents. In cases where the specifics of α^0-thalassaemia deletion is unknown, the foetal cord blood should be utilized for Hb analysis. In foetuses with Hb Bart's hydrops foetalis, the predominant haemoglobins are Hb Bart's (75-90%) and Hb Portland (10-15%) [2-8]. The possibility of maternal DNA or blood contamination should be assessed using standard methods, as described elsewhere [27].

Table 2. *Obstetric procedures for prenatal diagnosis*

	Chorionic villi sampling (CVS)	Amniocentesis	Cordocentesis
Gestational age (wks)	10-13	16-18	18-22
Size/volume	2 mm	10 mL	0.5-1 mL
Samples	Chorionic villi	Amniotic fluid	Fetal blood
Diagnostic technique	DNA analysis	DNA analysis	Hb analysisDNA analysis
Limitation	Maternal tissue (decidua) contamination	Lower DNA yield than CVS sample	Relatively delayed diagnosis
		Maternal cell contamination	
Advantage	Early diagnosis	Simple procedure	Rapid result
Miscarriage risk	weighted procedure-related risk 0.20%	weighted procedure-related risk 0.30%	
	(95% CI, −0.13 to 0.52%)[25]	(95% CI, 0.11–0.49%)[25]	1.6% (vs 1.0% in controls)[26]

Noninvasive foetal diagnosis of Hb Bart's hydrops foetalis: ultrasound

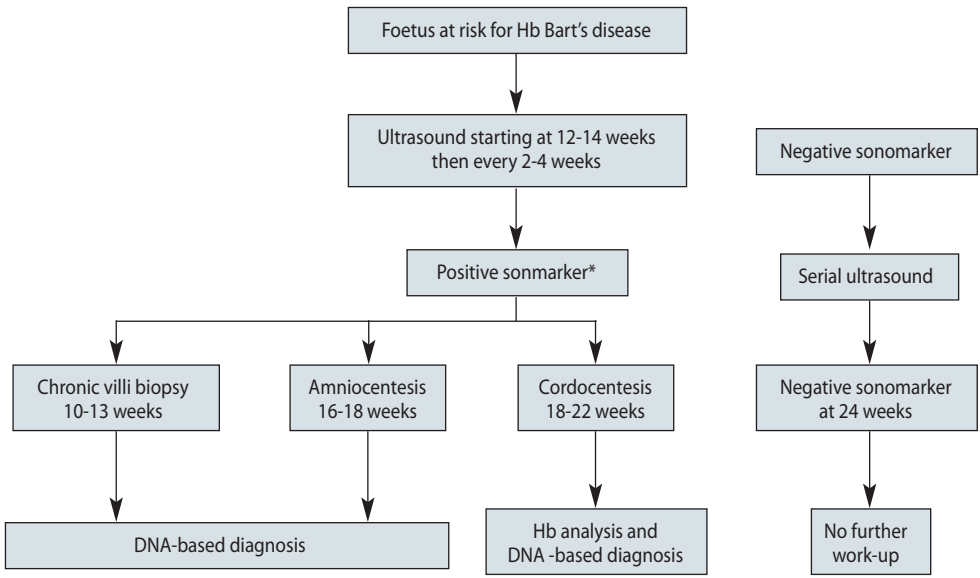
Ultrasound can be used to evaluate signs of foetal anaemia and hydrops in an affected foetus in cases that the couple opts against invasive prenatal diagnosis, or when the risk identification is incomplete, such as a single mother who is an a^0-thalassaemia carrier. If signs of foetal anaemia or hydrops are present, DNA-based foetal diagnosis needs to be done to confirm the final diagnosis.

The ultrasound approach for the known couple at risk of having a foetus with Hb Bart's hydrops foetalis is as follows [24, 28]:

1. The mother should undergo serial foetal ultrasound scans starting from 12–14 weeks of pregnancy. The ultrasound is aimed to identify the pre-hydropic signs in the foetus: (a) an increase in foetal cardiac size, represented by cardio-thoracic diameter ratio: CTR or cardiac circumference; and (b) middle cerebral artery-peak systolic velocity (MCA-PSV). It is also used to identify hydropic signs such as ascites, pleural effusion, or pericardial effusion. It is important to note that MCA-PSV alone may underestimate the degree of tissue hypoxia and fail to identify hydrops early in pregnancy.
2. Serial ultrasound examination is to be scheduled every 2-4 weeks if there are no pre-hydropic signs.
3. In cases of absent pre-hydropic signs, serial ultrasound scans can be discontinued after 24 weeks of gestational age. An additional scan before labour may be performed.
4. If any pre-hydropic or hydropic signs is identified on serial ultrasound examinations, the invasive diagnosis is offered to obtain a definite diagnosis.

The ultrasound algorithm for prenatal diagnosis of Hb Bart's hydrops foetalis is shown in **Figure 1**. The ultrasound algorithm is based on the evidence that [24, 29]: (1) Ultrasound is highly effective in early detection of Hb Bart hydrops foetalis in pre-hydropic phase. (2) All of the affected foetuses can be detected before 24 weeks of gestation. (3) The most sensitive parameters in predicting foetal anaemia in Hb Bart's hydrops foetalis appear to be the cardiac diameter to thoracic diameter ratio (CTR), followed by middle cerebral artery peak systolic velocity (MCA-PSV), and placental thickness, respectively. (4) On serial ultrasound examination, either increased CTR or increased MCA-PSV are highly suggestive of hydrops and confirmation of diagnosis through obtaining foetal blood is strongly recommended. (5) Several other ultrasound markers are helpful in increasing specificity, such as hepatosplenomegaly.

Figure 1. *Ultrasound algorithm for prenatal diagnosis of Hb Bart's hydrops foetalis among at-risk pregnancies*



The ultrasound algorithm is adapted from reference [24]. (with permission)

*Positive sonomarker [28]:

- Abnormally increased cardiac diameter-to-thoracic diameter ratio (CTR) defined as the CTR at 12-14, 15-17, 18-20, 21-22 and 23-25 weeks of gestation that were greater than 0.48, 0.49, 0.50, 0.51 and 0.52, respectively
- Abnormally increased middle cerebral artery peak systolic velocity (MCA-PSV) defined as any values greater than 1.5 MoM
- Increased placental thickness, ascites, pleural effusion, pericardial effusion, or hepatosplenomegaly

Other consideration: noninvasive prenatal testing of Hb Bart's hydrops foetalis by analysis of cell-free foetal DNA

Noninvasive prenatal testing (NIPT), which involves the analysis of cell-free foetal DNA (cff-DNA), has found widespread use in prenatal diagnosis of foetal aneuploidies and autosomal dominant diseases [30]. NIPT using cff-DNA has also been employed for prenatal diagnosis of Hb Bart's hydrops foetalis [31–38]. Various highly sensitive molecular detection techniques have been researched for this purpose, including real-time quantitative PCR, digital PCR, next-generation sequencing, and mass spectrometry methods. The sensitivity and specificity of the tests are approaching 100% with the advanced techniques. With the advancement of these techniques, the sensitivity and specificity of the tests are approaching nearly 100%. As these methods continue to improve, NIPT for Hb Bart's hydrops foetalis using cff-DNA should be considered as a screening test and used in conjunction with other established methods for foetal diagnosis.

HbH hydrops foetalis

HbH disease arises from mutations of three out of four α-globin genes, typically involving a compound heterozygosity of α^0-thalassaemia on one chromosome and either a deletion or a point mutation of one α-globin gene on the other. Deletional HbH disease (genotype --/-α) is generally associated with mild and non-transfusion-dependent haemolytic anaemia. In contrast, non-deletional HbH disease (genotype --/α^Tα or --/αα^T) result in a more severe phenotype [39, 40]. While the majority of these patients remain non-transfusion dependent, on demand transfusion is common and some will require regular transfusions.

HbH hydrops foetalis represents a subtype of HbH disease characterized by severe foetal haemolytic anaemia, which can lead to hydrops foetalis [41–43]. HbH hydrops foetalis typically results from a compound heterozygosity involving α^0-thalassaemia and a hyper-unstable α-globin variant. Foetuses with HbH hydrops foetalis suffer from severe haemolytic anaemia and may exhibit congenital abnormalities, similar to those observed in Hb Bart's hydrops foetalis. Their Hb analysis reveals Hb Bart's levels of 30–60%, alongside the presence of HbF and HbA [41, 43]. Intrauterine transfusion can help improve perinatal outcomes. Patients with HbH hydrops foetalis who survive the perinatal period typically remain transfusion-dependent in the long term.

Given the rarity of HbH hydrops foetalis and the limited available data, the monitoring for foetal anaemia in pregnancies with HbH hydrops foetalis is based on guidelines established for Hb Bart's hydrops foetalis. It is crucial to obtain a definitive molecular diagnosis and identify potential couples at risk for appropriate management.

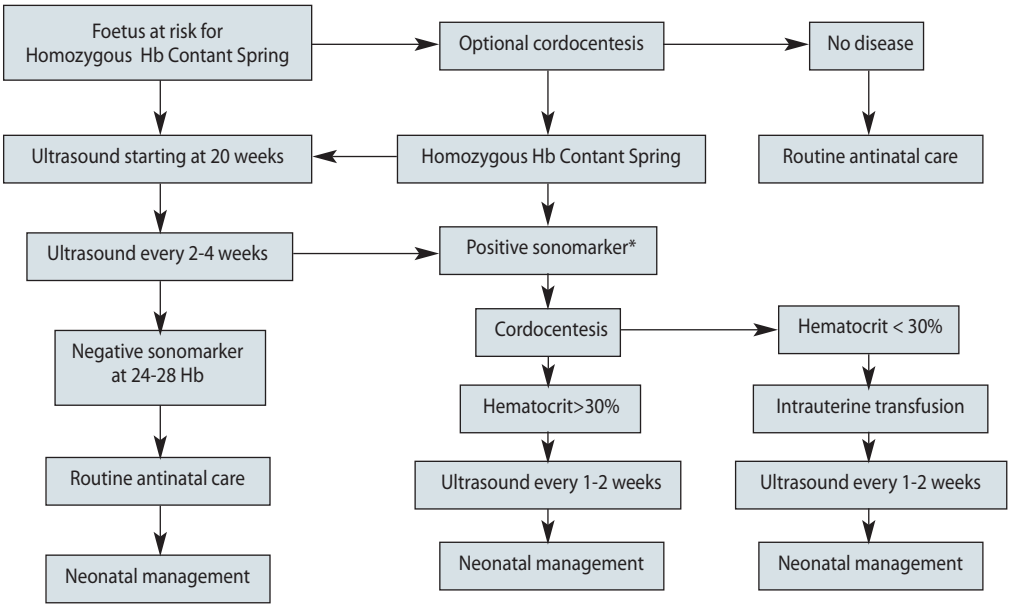
Hydrops foetalis due to homozygous Hb Constant Spring mutation or other rare non-deletional α-thalassaemia mutations

Homozygous HbCS ($\alpha^{CS}\alpha/\alpha^{CS}\alpha$) is associated with mild anaemia in older children and adults, although the condition is an emerging cause of severe foetal anaemia and hydrops [44-48]. Foetuses with hydrops foetalis associated with homozygous HbCS and other rare non-deletional α^{+}-thalassaemia mutations (e.g. Hb Taybi and Hb Pakse) present from mid-gestation with varying degree of anaemia and/or hydropic signs. Haemoglobin analysis in foetuses with homozygous HbCS shows presence of HbF, HbA, Hb Bart's and HbCS (Hb F/A/Bart's/CS pattern) with Hb Bart's of 7–17%, lower than those seen in HbH hydrops foetalis [8, 44, 45]. Intrauterine transfusion helps to reverse the anaemia and hydropic changes. After intrauterine transfusion, patients with homozygous HbCS usually have mild anaemia at birth and are non-transfusion dependent thereafter. The finding of anaemia in foetal period that improves after birth suggests an adverse interaction between the CS variant and the γ-globin, although this has not been documented. Compound heterozygosity of HbCS and Hb Pakse can result in foetal anaemia with comparable findings as homozygous HbCS and should be managed similarly [8].

As intrauterine transfusion can improve the perinatal outcome in foetuses with hydrops foetalis associated with homozygous HbCS, the ultrasound monitoring for foetal anaemia will be beneficial. Based on limited evidence, the ultrasound algorithm for prenatal diagnosis of Hb Bart's hydrops foetalis is as shown in **Figure 2**.

Severe foetal anaemia or hydrops associated with HbH/CS disease has also been reported [49, 50]. The findings emphasize the variable clinical severity of HbH/CS disease. A small case series of 4 foetuses with HbH/CS disease shows that the patients present with signs of anaemia detected by ultrasound in late second trimester. Intrauterine transfusion is beneficial, and anaemia may improve without treatment toward late gestation [49]. The study suggests that a foetus with HbH/CS disease be monitored for signs of anaemia in the second half of pregnancy [49]. Further studies are needed to illustrate the incidence and factors leading to severe foetal anaemia in patients with HbH/CS disease.

Figure 2. *Ultrasound algorithm for foetuses at risk of homozygous Hb Constant Spring*



The ultrasound algorithm is adapted from reference [48]. (with permission)

*Positive sonomarker [28]:

- Abnormally increased cardiac diameter-to-thoracic diameter ratio (CTR) defined as the CTR at 12-14, 15-17, 18-20, 21-22 and 23-25 weeks of gestation that were greater than 0.48, 0.49, 0.50, 0.51 and 0.52, respectively
- Abnormally increased middle cerebral artery peak systolic velocity (MCA-PSV) defined as any values greater than 1.5 MoM
- Increased placental thickness, ascites, pleural effusion, pericardial effusion, or hepatosplenomegaly

Summary and recommendations

- Genetic counselling and prenatal screening for couples at risk of foetal Hb Bart's hydrops foetalis should be offered to all couples.
- Pregnant women are encouraged to attend early antenatal care. During the first visit of antenatal care, the couple should be counselled regarding the risk of foetal thalassaemia diseases. Prenatal testing to identify couple at risk is performed in the first visit.
- For prenatal screening of thalassaemia carriers, when both individuals in a couple meet the MCV or MCH criteria, they need to be tested for α^0-thalassaemia by DNA-based methods, along with Hb analysis, to detect coinheritance of β-thalassaemia carrier and other Hb variants. The test for α^0-thalassaemia is required regardless of β-thalassaemia status as coinheritance of α-thalassaemia and β-thalassaemia is common, except for the African population where α^+-thalassaemia is common but α^0-thalassaemia is rare. In the African population α^0-thalassaemia is to be tested for when MCV or MCH is low and β-thalassaemia carrier state has been excluded by Hb analysis.
- Prenatal diagnosis should be offered to all couples at risk of having a foetus with Hb Bart's hydrops foetalis.
- The pregnant woman at risk of Hb Bart's hydrops foetalis should undergo the first ultrasound examination at 12-13 weeks of gestation. The couple at risk should be counselled about the clinical presentation of Hb Bart's hydrops foetalis, foetal diagnostic methods, and options for management.
- The options of foetal diagnosis are: 1) the conventional approach, which involves an obstetric procedure to obtain foetal DNA or blood for molecular and/or Hb analysis and 2) the non-invasive approach or ultrasound algorithm.
- Ultrasound can be used to evaluate signs of foetal anaemia and hydrops in an at-risk foetus in cases that the couple opts against invasive prenatal diagnosis, or when the risk identification is incomplete such as a single mother who is an α^0-thalassaemia carrier. If signs of foetal anaemia or hydrops are present, DNA-based foetal diagnosis needs to be done to confirm the final diagnosis.
- In pregnancies affected by Hb Bart's hydrops foetalis, detailed and non-directive counselling and discussion should be given and the option to terminate the pregnancy should be discussed.
- In cases in which parents wish to continue with the affected pregnancy, the management should be given in centers with expertise in caring for Hb Bart's hydrops foetalis. Early ultrasound to detect associated congenital abnormalities should be performed and results should be discussed with the parents. Early intrauterine transfusion and close follow-up on foetal and maternal status should be offered. The care team should be coordinated between obstetrics and neonatology for postnatal intensive care and haematology for long-term management.
- Noninvasive prenatal testing for Hb Bart's hydrops foetalis using cff-DNA should be considered as a screening test and should be used in conjunction with other established methods for foetal diagnosis.
- For HbH hydrops foetalis, the monitoring for foetal anaemia is based on the guideline for Hb Bart's hydrops foetalis. The decision of intrauterine transfusion should be discussed with the family and is based on the molecular diagnosis and the long-term prognosis.
- For foetal homozygous Hb CS, the ultrasound monitoring for foetal anaemia is recommended. Intrauterine transfusion is beneficial.

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15 GENETIC COUNSELLING FOR FAMILIES AT RISK FOR α-THALASSAEMIA

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Genetic counsellors operate as a part of the multi-disciplinary team in the care of patients in reproductive and family planning [1–3]. They serve as a critical resource for the identification and education of individuals and their families that are at risk for α-thalassaemia. Genetic counsellors assist families identified to be at risk for thalassaemia to clarify their family specific priorities for reproductive planning by detailing the current understanding of the diagnosis including prognosis, management, and recurrence risks.

The genetic counsellor will facilitate genetic evaluation for confirmation of carrier status, and/or diagnostic testing in a pregnancy for identification of affected foetuses. Genetic counsellors may also serve as a resource to identify regional experts including haematologists to enrich family understanding regarding the diagnosis of α-thalassaemia including the long-term outcomes and how the needs of the diagnosis would impact the family based on their regional resources.

The care of patients with clinically severe forms of α-thalassaemia (haemoglobin Bart's hydrops foetalis, HbH hydrops foetalis, and some severe forms of HbH disease) is complex, depending on both Obstetric/Maternal Fetal Medicine expertise for the prenatal management, as well as haematology, and access to safe blood for chronic transfusion needs. The resources to support this care vary globally, and consultation with local providers is essential for families to understand the implications of the diagnosis and long-term care based on their locality. In addition, given the development of newer therapies in the setting of thalassaemia, the genetic counsellor may also identify and make patients aware of opportunities to participate in clinical trials.

In the event of a pregnancy diagnosis, supporting the family through understanding their immediate management options and pregnancy decision-making is essential [4]. This involves both the logistical care coordination as well as the psychosocial aspects in adjusting to a diagnosis in their foetus. This support will include the acute needs to manage the family's feelings of potential grief and loss of the anticipated healthy or unaffected child, as well as the long-term needs of navigating the future, which could include identifying a path forward to mitigate those risks in a future pregnancy. Identification of resources, including individual therapy and regional support groups related to pregnancy loss, pregnancy termination, and/or families and individuals with clinically significant forms of α-thalassaemia, may be essential to offer these families for on-going needs.

A genetic consultation will include a review of known family history by obtaining a 3-generation pedigree (family tree). This information is valuable to understand family history that may be related to thalassaemia in the family, including a history of recurrent miscarriage, in utero foetal demise, stillbirth, and hydrops foetalis. Often patients will report a personal history of anaemia. Clarification regarding the impact of iron supplementation on their anaemia may be a clue to identifying a carrier as iron supplementation will not correct the anaemia seen in an α-thalassaemia carrier. Confirmation of carrier status through molecular testing is essential for these patients. The family history may also indicate needs for additional carrier screening or evaluations as the possibility of multiple risks exist for a couple and it is important to recognize and clarify all risks in the reproductive planning for families.

Ideally, screening for thalassaemia occurs during the preconception period, although most couples begin a pregnancy unaware of their risk. Identification of potential carriers can be ascertained through the complete blood count (CBC) assessment. This is often recognized with the initial pregnancy evaluation, though evidence of a decreased mean corpuscle volume (MCV) or mean corpuscle haemoglobin (MCH) can be misdiagnosed as iron deficiency anaemia. While screening for iron deficiency or iron deficiency anaemia is a routine component of prenatal care, specific evaluation for genetic etiologies remains essential in patients at increased risk due to geographic ancestry. Importantly, iron deficiency and α-thalassaemia are not mutually exclusive and may co-occur in a patient. **Geographic ancestry of the patient is key, and screening through molecular testing of the α-globin locus should be considered in an individual with haematologic values suggestive of microcytic, hypochromic anaemia and reporting origins in Southeast Asia, China, South Asia, the Pacific Islands, the Middle East, Mediterranean, Latin America or Africa. Evaluation of α-thalassaemia status should include complete blood count and peripheral blood smear as well as haemoglobin electrophoresis or high-performance liquid chromatography (HPLC) to rule out other haemoglobinopathies. Simultaneous α-globin gene testing to detect common α-thalassaemia deletions and point mutations is critical. This is because normal haemoglobin electrophoresis or HPLC results do not exclude most common forms of α-thalassaemias, and it is important to distinguish between α^{+}-thalassaemia trait from α^{0}-thalassaemia trait, which determines the risk of clinically severe disease (e.g., haemoglobin Bart's hydrops foetalis) in the foetus. When a patient is identified to be a carrier for α-thalassaemia, emphasizing the implications for other family members is also important and a discussion including identifying those that could be at risk and support for educating these relatives should be a part of the genetic counselling purview (see Chapter 1 and Chapter 14).**

Depending on the pregnant person and the partner's carrier status there is a spectrum of clinically meaningful outcomes for a pregnancy and child. Haemoglobin Bart's hydrops foetalis is the result when both parents have α^{0}-thalassaemia trait, which deletes both α-globin genes on the same chromosome (--/αα), the affected foetus inherits the deleted chromosomes from each parent (--/--) [5]. In pregnancies at risk for haemoglobin Bart's hydrops foetalis, due to the known eventual development of anaemia and hydrops, early confirmation of a diagnosis is critical for optimal management in those patients that elect to pursue foetal therapy. Foetal hydrops due to other forms of α-thalassaemia include non-deletion or

unstable mutations in the α-gene even in the presence of one or two normal α-globin genes (--/$\alpha^T\alpha$ or -α^T/-α^T). This condition, known as haemoglobin H hydrops foetalis, is more severe in the intrauterine period and foetal rescue by active in utero management may also be considered [6-8]. The postnatal course is highly variable depending upon the type of α-globin gene mutation, ranging from a non-transfusion dependent moderate anaemia to transfusion dependency. Therefore, knowledge of the parental genotypes is critical for counseling regarding prenatal risks, management options and prognosis.

Partner testing is critical in the determination of reproductive risks. Couples should be made aware of the spectrum of outcomes with this diagnosis. Haematology management after birth may be essential with varying transfusion needs. A less-severe form of thalassaemia remains possible depending on the parental genotypes.

If the father does not have α^0-thalassaemia trait and paternity is assured, then the foetus will not have haemoglobin Bart's hydrops foetalis, but may still be at risk for severe prenatal presentation with anaemia and hydrops or an attenuated form where presentation may occur in childhood or later in life.

All reproductive planning options should be reviewed with couples at risk for α-thalassaemia disease spectrum including the risks, benefits, and limitations of in vitro fertilization (IVF) with preimplantation genetic testing (PGT-M) to mitigate risks of an affected pregnancy. Families should also be provided an overview of options in an affected pregnancy, including termination of pregnancy, active foetal management with in-utero transfusions, and expectant management. It is appropriate to refer these couples to meet with an obstetric/maternal foetal medicine (MFM) specialist for preconception consultation to discuss these options in detail and review the patient's medical history for a specific risks/benefit discussion of these options should a pregnancy occur [4].

Prenatal diagnosis should be offered to a patient whose foetus is at risk for haemoglobin Bart's hydrops foetalis. For patients electing to proceed with active management, given the relatively low risk of invasive testing, the benefits of early prenatal diagnosis to allow for early introduction of foetal therapy should be emphasized.

Procedures for foetal testing include chorionic villus sampling (CVS), amniocentesis, and percutaneous umbilical cord blood sampling (PUBS; cordocentesis) [9–11]. The CVS procedure is performed between 10 and 13 weeks gestation by the sampling of placental tissue. This procedure is performed by ultrasound guidance either transcervically or transabdominally depending on placental positioning. The amniocentesis procedure removes a sample of the amniotic fluid surrounding the foetus and is performed ideally between 15 to 20 weeks gestation. This procedure will be performed under ultrasound guidance using a needle into the amniotic cavity.

After either the CVS or amniocentesis procedure the cells will require culturing which can take two weeks or more depending on the sample. Setting clear patient expectations is critical in the decisions regarding choosing the prenatal diagnosis procedure, this includes discussion regarding turn-around time for results in the context of gestational age. These details will be regionally specific but turn-around time for foetal diagnostic results will often exceed four weeks.

For a pregnancy demonstrating evidence of foetal anaemia, where initiation of foetal therapy sooner would benefit the foetal course, foetal blood sampling should be considered. This procedure, referred to as either PUBS (percutaneous umbilical blood sampling) or cordocentesis is performed under direct ultrasound guidance using a needle in the umbilical vein to obtain foetal blood. This procedure may be used to simultaneously confirm foetal anaemia and initiate treatment by infusion of red blood cells. The earliest this is usually performed is the 18th week of gestation. Molecular testing can be performed on any of the above specimens, including foetal DNA extracted from the foetal cord blood sample. Evaluation of Hb Bart's (>80%) from the cordocentesis sample may also be used in the diagnosis of an affected foetus (Please see Chapter 14).

Finally, for those patients at risk for having a foetus with haemoglobin Bart's hydrops foetalis who do not proceed with prenatal diagnosis, ultrasound may be used as a tool to detect features consistent with a diagnosis of haemoglobin Bart's hydrops foetalis [12]. These findings include developments of foetal effusions consistent with hydrops spectrum, increased cardio-thoracic diameter ratio, and elevated middle cerebral artery (MCA) peak systolic velocity on Doppler (see Chapter 14).

Options in the setting of a foetal diagnosis include continuation with foetal therapy, pregnancy surveillance alone or pregnancy termination. For those patients that choose to continue in pregnancy, counselling by a MFM provider is necessary to ensure clear understanding of the implications of initiating foetal transfusions versus electing to carrying a foetus with haemoglobin Bart's hydrops foetalis without foetal therapy. Both maternal and foetal risks should be weighed and a defined treatment and/or surveillance plan should be determined [4].

For those patients that elect to proceed with foetal transfusions the expectations regarding outcomes including the delivery of a child with chronic transfusion needs should be discussed. Ultimately, the option for a curative treatment by bone marrow transplantation could be considered with multiple variables impacting the likelihood of success including identification of a donor. Referral to paediatric haematology for extensive counselling regarding long-term prognosis and management is crucial to set the appropriate expectations and understanding for these families [4].

Summary and recommendations

- Comprehensive family history is essential to understand what, if any, reproductive risks may be present and should be addressed. This may include a family or personal history of anaemia, recurrent pregnancy loss or hydrops foetalis.
- Preconception/Early in pregnancy: clarification of carrier status in parents is essential.

  Couples at risk for Hb Bart's Hydrops foetalis should be referred to paediatric haematology for comprehensive education regarding long term prognosis and management options.
- Couples at risk for a pregnancy affected by Hb Bart's Hydrops foetalis or nondeletional Hb H should be educated regarding all pregnancy options including pregnancy termination, in utero transfusions or expectant management to make informed reproductive decisions.
- Prenatal diagnosis (CVS or amniocentesis) is critical in identifying affected pregnancies prior to presentation of hydrops.
- Patients carrying a pregnancy diagnosed with hydrops foetalis and/or elevated MCA Doppler concerning for foetal anaemia may consider PUBS to confirm anaemia and the option to initiate in utero transfusions. Hb Bart's evaluation of foetal blood sample may be used to confirm or exclude the diagnosis.

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16 PATIENT CENTRED MULTIDISCIPLINARY CARE IN A-THALASSAEMIA

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HbH disease

It is clear from the preceding chapters that the clinically significant forms of α-thalassaemia present a wide spectrum of severity. Nevertheless, in all cases age brings with it morbidities that cannot be explained by iron overload alone since hypoxemia and haemolysis may play an equal if not greater role. Even though these complications may be less common in HbH disease compared to β-thalassaemia [see **Table 1**], they are still to be expected. Even more frequent or severe complications are to be expected in non-deletional forms. In one study from Thailand, comparing the deletional and non-deletional HbH, patients with the non-deletional type of HbH disease had more symptoms at a younger age, more severe haemolytic anaemia, more growth deficiency, more dysmorphic facial features, larger spleens, larger livers, and higher serum ferritin levels, and they required more transfusions than patients with deletional HbH disease [1]. Similar observations were described in Middle Eastern populations [2]. The clinical severity is highly variable even among patients with an identical genotype in HbH disease [3].

For all these reasons HbH disease requires careful follow up from childhood, irrespective of the initial clinical picture and irrespective of the genotype; only the frequency of clinic visits may vary, especially in childhood, between those with mild anaemia and those with more severe anaemia. Patients who are in a steady state have haemoglobin levels around 90 to 100 g/L, and they may be seen once a year. Patients with non-deletional HbH disease may be more anaemic with significant splenomegaly, and they may require regular blood transfusions, thus requiring more frequent visits [4]. As patients grow, however, the need for vigilance concerning iron overload and other complications will necessitate more frequent monitoring and a more strict protocol of investigations.

Table 1. *Reproduced from Lee YC et al 2022: Differences between HbH and β-thalassaemia intermedia [5].*

Complications %	HbH, n = 72	β-TI, n = 80	<18 Years (n = 104)	>18 Years (n = 48)
Short stature	22.2	42.5		
Growth retardation	34.7	50		
Osteoporosis	18	30	24	25
Extramedullary hematopoiesis	5.6	17.5	12.5	10.4
Heart failure	0	13.8	6.7	8.3
Pulmonary hypertension	1.4	5	2	3.3
Gallstones	1.4	2.5	0	6.3
Cholecystectomy	0	7.5	1	10.4
Deep vein thrombosis	0	2.5	0	4.1
Leg ulcers	1.4	3.8	2	4.1
Iron overload	11.1	45	30.8	25

Like β-thalassaemia, HbH disease becomes, over time, a multi-organ pathology with considerable variation in severity and frequency of complications. An individualized programme of clinical monitoring and management by various specialists is therefore inevitable. It is noted that although most clinical studies describing the clinical features are based on small numbers of patients and are retrospective, a pattern of clinical evolution emerges:

A history of blood transfusion is described in more than double the patients with non-deletional types of HbH disease compared to deletional. Iron overload, as reflected by serum ferritin measurements, increased with age in a study from Hong Kong [6], and there was increased liver iron content, indicated by MRI measurements, with a danger of fibrotic changes. In three out of 25 patients from this study, heart failure developed as a result of iron overload, unrelated to transfusion history; diastolic function was found abnormal by echocardiography in these patients. In conclusion, the authors propose that patients with HbH disease should be closely monitored for tissue damage due to iron overload and chelation therapy should be initiated once such damage is detected. Confirming these conclusions, a study from Thailand indicated that patients with non-deletional HbH disease had higher liver iron concentration (LIC) compared to patients with deletional HbH disease [7]. The serum ferritin levels are relatively low and at the same levels of LIC in patients with non-deletional HbH disease compared to deletional HbH disease. Regular screening for liver complications should be standard in the management of HbH disease [8].

Blood transfusion is usually a response to haemolytic episodes which are triggered usually by infections. If, however, there is declining baseline Hb, rapid enlargement of the spleen, failure of growth or secondary sexual development, signs of bone changes, poor quality of life, and frequent haemolytic crises, then regular blood transfusions should be considered [9]. During haemolytic episodes, red cell transfusion, at a volume of 5 to 10 ml/kg (or 1–2 units in adults) depending on levels of anaemia, should be provided to restore Hb to 80 to 90 g/L.

Role of nutrition: Patients with HbH disease may have excessive iron absorption triggered by ineffective erythropoiesis and haemolysis. In general, all patients with HbH should receive folic acid supplementation at a dose of 1–5 mg/day, as this is required to increase erythropoietic activity. Biannual monitoring of vitamin D levels and supplementation of vitamin D and calcium as needed is recommended, even though data on vitamin deficiency or bone disease are limited.

The care of patients with HbH disease should also include complete immunization of vaccine-preventable diseases, prompt treatment of fever and infections, and alertness to acute anaemic symptoms. Psychosocial support may be required as in all chronic conditions, and physicians and nurses should be alert to such needs. At the same time more studies on the quality of life of these patients are needed, especially in cases where there is a more severe clinical burden.

Conclusion

From all published clinical observations, there is concern of underdiagnosed end-organ damage. The management of HbH disease, according to these observations, should be based on inter-professional teams that consist of primary care providers, haematologists, geneticists, nurses, pharmacists, dietitians, and specialists since the condition may affect multiple organs. Close monitoring is required, which must be increased in frequency in non-deletional HbH disease and as the deletional forms increase in age. Patients must also be monitored carefully for complications of chronic transfusion and given iron chelation support as needed, especially during the second and third decades of life [10]. TIF's recommendation is for a coordinated multidisciplinary service for these patients, provided in specialized thalassaemia centres by experienced staff. This holds true for α-thalassaemia as much as for β-thalassaemia.

Hb Bart's hydrops foetalis

Management of this condition is inter-professional from the moment a pregnancy is recognized as being a possible case of Hb Bart's hydrops foetalis. The parents require careful counselling on the genetic aspects, the possible clinical course for both the mother and baby, and the postnatal course and needs of the affected parents. The first dilemma that parents have to face is whether to terminate the pregnancy because of concerns about foetal prognosis, including survival and the risk of obstetric complications for the mother. Indeed maternal complications such as preeclampsia may be life threatening. This is a process that demands the attention of competent genetic counsellors to ensure informed choice in the options offered, an experienced obstetric team, including an ultrasonographer, and an expert haematologist. This can be a traumatic period for the parents and psychosocial support should not be neglected [11].

The pregnancy will be complicated by polyhydramnios, preeclampsia, abruptio placenta, and possible intrauterine infections. Such a high-risk pregnancy should be cared for in a competent centre that is able to perform prenatal diagnoses and has the expertise to perform intrauterine transfusions if the parents choose to have this treatment for their unborn child. Intrauterine transfusions have been shown to reverse hydrops foetalis by improving anaemia and oxygenation, enabling the pregnancy to be carried

to term or near term and avoiding the consequences of the condition to both the foetus and the mother [12]. Nevertheless, the outcomes of the surviving newborn are not always as hoped for.

Experience of surviving neonates is increasing and an international registry has been created to collect this experience [13]. Prematurity is prevalent in around 70% of pregnancies and perinatal respiratory depression are frequent, and as a consequence resuscitation, intubation, and mechanical ventilation are needed [14]. This means that along with expertise from the obstetric service, expert neonatal paediatric services and intensive care will also be required to ensure the best possible outcomes. During the neonatal period several interventions may be needed to correct anaemia (blood transfusions), jaundice (exchange transfusions), and respiratory distress syndrome and pulmonary hypertension. Added to this are several congenital abnormalities such as urogenital abnormalities in males (hypospadias and undescended testes), skeletal malformations, congenital heart defects (mainly atrial septal defect), jejunal atresia, and neonatal hepatitis. All these require specialist interventions from paediatric surgery and heart surgery.

Cerebral palsy in the form of spastic diplegia has been described in the post-neonatal period, along with developmental delay. All patients will require regular blood transfusions [15]. As a consequence, all the multi-organ complications arising from iron overload as experienced in β-thalassaemia are to be expected while iron overload is manifest earlier in life as their transfusions had started in utero [16].

Conclusion

Overall management of Hb Bart's hydrops foetalis is a complex process which starts from the moment prospective at-risk parents select to initiate or continue a pregnancy which will lead to the production of foetal anaemia and heart failure due to homozygosity for α^0–thalassaemia deletions. Expert counselling, specialized obstetric care, experienced neonatal intensive care services, paediatric surgery, paediatric neurodevelopmental services, paediatric and, later, adult haematology services, and the multi-disciplinary team that is experienced in thalassaemia care are all part of a collaborative team that, according to individual needs, will be called upon to serve the clinical and rehabilitation needs of the survivors of this syndrome, which in the past was almost universally lethal. Holistic care demands that psychosocial professional support is part of the whole process from even before a pregnancy ensues and continues throughout the patient's life.

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ABOUT THE THALASSAEMIA INTERNATIONAL FEDERATION (TIF)

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

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

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

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

Our Work:

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

Our Partners:

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

Our Motto: Unity & Knowledge constitute our Strength!

A COMPREHENSIVE EDUCATIONAL PROGRAMME

EDUCATIONAL EVENTS

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

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

RENZO GALANELLO FELLOWSHIP PROGRAMME

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

e-ACADEMY

- **eThalED Course for Medical Specialists**

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

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

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

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

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

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

EDUCATIONAL RESOURCES

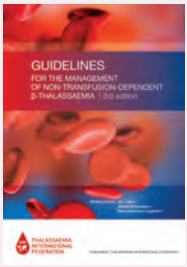
▶ TIF's LIbrary eXtended (TIFLIX)

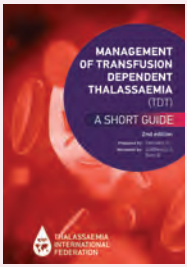
TIF's Library has been extended to provide its users with premium, on-demand educational video content on a variety of topics relevant to thalassaemia and other haemoglobin disorders. TIFLIX contains an extensive library of case studies and lectures addressed to healthcare professionals with an interest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

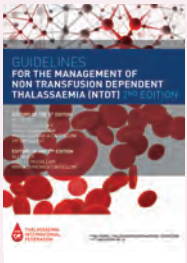
▶ Publications

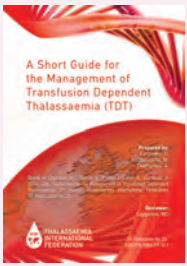
Since 1996, TIF has issued a vast number of diverse publications on thalassaemia and sickle cell disease, many of which have been and are still used as reference texts for academics, healthcare professionals, patient-support organisations and individual patients. New editions are regularly produced to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

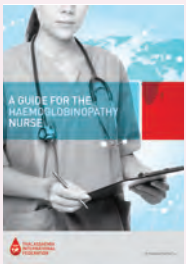
TIF PUBLICATIONS | HEALTH CARE PROFESSIONALS

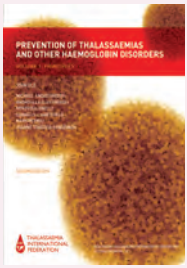


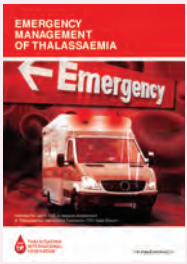






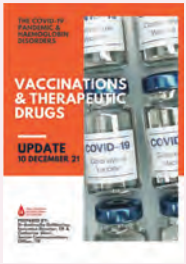


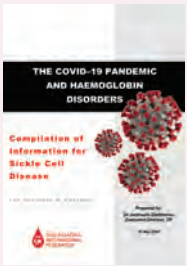


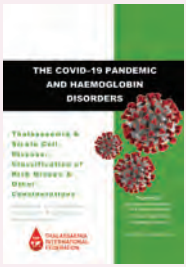


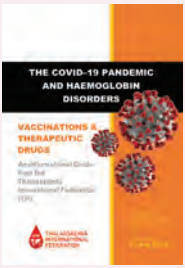
Please note that some of our publications are translated to a number of languages.

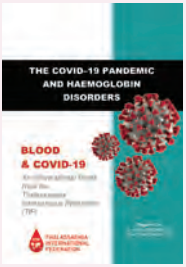
TIF PUBLICATIONS | **PATIENTS**

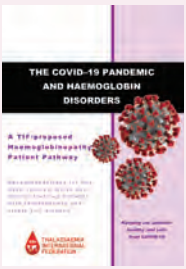



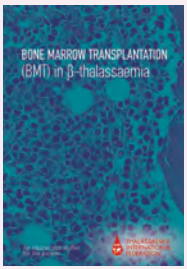



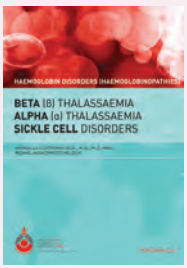


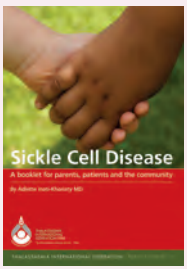


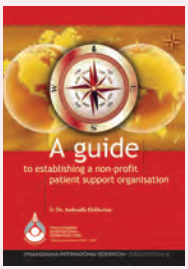


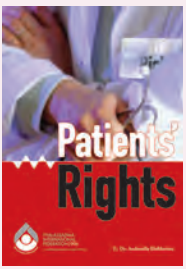










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GUIDELINES
FOR THE MANAGEMENT OF α-THALASSAEMIA

Summary and recommendations

1 EPIDEMIOLOGY, PATHOPHYSIOLOGY AND DIAGNOSIS OF α-THALASSAEMIA

Summary and recommendations

- α-Thalassaemia is one of the most commonly inherited blood conditions. Initially prevalent in areas where malaria was most common, α-thalassaemia is now considered a global health concern due to population migration.
- Clinically significant forms of α-thalassaemia, HbH disease and haemoglobin Bart's hydrops foetalis result from compound heterozygosity for α^0-thalassaemia deletions with other mutations or deletions of α-globin genes. Hence, identification of carriers of α^0-thalassaemia deletions is pivotal for population control and prenatal diagnosis.
- HbH disease is the most common clinically significant form of α-thalassaemia, which is most prevalent in Southeast Asia, South China and also in some areas in the Middle East or Mediterranean region.
- HbH disease has a considerable heterogeneity, both genetically and clinically. Individuals with deletional forms of HbH disease have generally a benign course but those who harbour a non-deletional mutation have a higher rate of transfusion requirement and experience more frequent thalassaemia-related complications. As a result, identification of the underlying genetic abnormality has clinical implications.
- Accurate diagnosis of α-thalassaemia syndromes requires application of a range of diagnostic techniques, including complete blood count (CBC) with reticulocyte count, haemolytic panel, peripheral blood smears, automatic haemoglobin analyzers, and different modalities of molecular analysis.
- Knowledge of the patient's clinical phenotype and the prevalence of specific mutations in the region is essential to strategically select the appropriate molecular analysis.

2 CLINICAL PRESENTATION AND MANAGEMENT OF DELETIONAL HAEMOGLOBIN H DISEASE

Summary and recommendations

Domain	Management
Frequency of clinic visits	Every 3 months for first 2 years, then annually for life.
Laboratory testing	Complete blood count, reticulocyte count, liver function: Every 6 months for first 2 years, then annually. Ferritin, transferrin saturation (TSAT): Annually starting at 1 year. TSAT is not needed after 3 years, but should be checked if ferritin >200 ng/mL.
Febrile illness	Evaluation by paediatrician as for children without HbH disease. Check CBC if severe symptoms or development of pallor or jaundice.
Transfusions	Regular transfusions are not required. Episodic transfusions are not needed during most febrile illnesses, unless the haemoglobin level drop below 60 g/L in young children or 65 g/L in adolescents and adults. Transfusion may be needed for surgery or other specific indications.
Red cell phenotyping	Not needed unless there is a need for recurrent transfusions
Iron overload assessment	Mild to moderate iron overload is observed in the 4th -5th decades of life, earlier in males than females. Check ferritin annually, check liver MRI for liver iron concentration (LIC) if ferritin >200 ng/mL.
Iron chelation	Start if LIC >5 mg/g dry weight or ferritin >500 ng/mL. Treat until LIC <3 mg/g dry weight and ferritin <300 ng/mL, then stop. Treat at lower level of LIC in presence for specific indications.
Splenectomy	Not indicated.
Endocrinology evaluation	If onset of puberty is delayed >2 years or if concern for slow growth. Obtain family history and consider x-ray for bone age.
DXA scan	Every 3 years starting at 12 years.
ECHO	Check at 10-12 years to assess for pulmonary artery pressure. If normal, repeat every 3-5 years.
Pregnancy and genetic counselling	Anticipate mild decline in haemoglobin, but routine transfusions are not needed. Partner testing to evaluate foetal risk for Bart's hydrops foetalis.

3 CLINICAL PRESENTATION AND MANAGEMENT OF NON-DELETIONAL HBH DISEASE

Summary and recommendations

- Clinical presentations of patients with non-deletional HbH disease are highly variable and are generally more severe than those of deletional HbH (see **Table 1** p. 30). Majority of patients need occasional transfusions, and some become transfusion-dependent.
- HbH hydrops foetalis and transfusion-dependency from early infancy are observed in certain non-deletional HbH genotypes, including HbH-QS, HbH-PolyA, HbH-Adana, HbH-PNP and HbH-SD (see **Table 2** p. 32).
- Supplementation of folic acid 1-5 mg/day and a non-iron-containing multivitamin are recommended in affected patients of all age groups.
- Prompt treatment of acute illness and alertness to symptoms of severe anaemia and haemolysis are essential for the prevention and management of acute haemolytic crisis. Patients with fever should be evaluated by a physician.
- All immunization according to the national programmes and regular vaccinations against common infections, such as influenza, pneumococcus, SARS , and hepatitis B, are strongly recommended to prevent infections.
- Recommended follow-up intervals for NTD patients:
  - Baseline Hb>80 g/L: every 6 months
  - Baseline Hb≤80 g/L: every 3 to 6 months
- Assessment of growth, bone changes, spleen size and pubertal development are essential at every clinic visit during childhood and adolescence.
- Complete blood count with reticulocyte count, haemolytic markers, serum ferritin, transferrin saturation, and liver enzymes should be checked with every visit.
- Red blood cell transfusion at a volume of 10-15 ml/kg should be considered one or more times to manage acute haemolytic episodes in patients of all ages when Hb <70 g/L with an aim to restore Hb to 80-90 g/L.
  - It is recommended that "effective" haemoglobin be measured at steady state. Effective - - haemoglobin can be calculated as total Hb x (1- [HbH % + Hb Bart's %] / 100).
- Regular blood transfusions are considered for prevention of significant growth failure, facial bone changes, failure of secondary sexual development, and massive splenomegaly in paediatric patients. They should also be considered for patients with following:
  - Hb at steady-state <70 g/L
  - Hb at steady-state 70-80 g/L with the presentation of symptoms before 2 years of age and/or spleen size ≥3 cm below costal margin
- A pre-transfusion haemoglobin target of 80-90 g/L is acceptable in most patients. However, those with high proportion of circulating HbH and those with ineffective erythropoiesis may require higher pre-transfusion haemoglobin targets.
- Periodic re-assessment of TD paediatric and young adult patients is critical for tapering off or withdrawing blood transfusion when a sustained clinical benefit is achieved. ▶▶▶

- Adult patients with non-deletional HbH typically do not require regular blood transfusion due to mild to moderate anaemia.
- Frequent transfusions may be considered in more severely affected adult patients for primary prevention of disease-related complications and for improvement of their quality of life.
- Regular blood transfusion should be considered for managing complications such as thrombotic diseases, cerebrovascular complications, and pulmonary hypertension.
- Monitoring and management of transfusion-dependent (TD) patients should be performed similar to patients with transfusion-dependent β-thalassaemia.
- Splenectomy can increase Hb levels and decrease or eliminate the need for transfusions in patients with HbH-CS. Splenectomy should be avoided in patients younger than 5 years. The procedure should be reserved for patients with severe anaemia and limited access to blood transfusions, hypersplenism with anaemia, leukopenia or thrombocytopenia resulting in infections or bleeding, or massive splenomegaly with left upper quadrant pain that increases the risk of splenic rupture.
- Prophylactic use of low-dose aspirin is recommended for all patients who have undergone splenectomy to prevent post-splenectomy thromboembolic events.
- Patients with non-deletional HbH disease are at a higher risk of developing gallstones due to chronic haemolysis. Cholecystectomy is typically indicated only in the presence of symptoms or cholecystitis.
- Iron overload is a common complication in non-deletional HbH disease due to increased gastrointestinal absorption.
- For non-transfusion dependent patients, monitor serum ferritin levels with every clinical visit and measure LIC with MRI if ferritin is >300 ng/mL. Iron chelation should be started if LIC >5 mg/g dry weight or ferritin >500 ng/mL.
- Patients with α-thalassaemia experience a higher prevalence of endocrine complications compared to the general population. Iron overload is a primary risk factor for the development of endocrine complications in thalassaemia. Patients with non-deletional HbH disease should be monitored similar to those with β-thalassaemia.
- If onset of puberty is delayed >2 years or if there is concern for slow growth, obtain family history and consider taking an x-ray for bone age. DXA scan should be done every 3 years (or more frequently if indicated) starting at 12 years.
- ECHO should be offered to patients starting at 10-12 years to assess for pulmonary artery pressure. If normal, repeat every 3-5 years. More frequent assessments may be needed at older ages.
- Splenectomy, aging, and inadequate blood transfusion are well-established risk factors for thrombosis in patients with thalassaemia.
- Exacerabtion of anaemia requiring transfusion is common during pregnancy and risk of preterm birth, foetal growth restriction and low birthweight are increased in pregnant mothers with non-deletional HbH disease. Partners should be screened for α-thalassaemia to evaluate foetal risk for haemoglobin Bart's hydrops foetalis.
- Homozygous HbCS may present with hydrops foetalis and/or neonatal haemolytic jaundice/anaemia requiring treatments during the first few months of life. These symptoms spontaneously recover to become a mild HbH-like phenotypes with Hb > 90 g/L.
- Other regional common non-deletional variants in homozygote forms or in compound heterozygotes with α^{+}-thalassaemia most often result in mild to moderate HbH-like phenotypes.

4 PRENATAL MANAGEMENT OF HAEMOGLOBIN BART'S HYDROPS FOETALIS

Summary and recommendations

- Hydrops foetalis in the setting of a pregnancy in parents who are known carriers for α-thalassaemia is suggestive of foetal haemoglobin Bart's hydrops foetalis in most cases. In this situation, the diagnosis should be confirmed as quickly as possible so that parents can be counselled in a timely manner.
- If the family wishes to pursue intervention, an initial IUT can be performed while awaiting the diagnosis.
- In a pregnancy with a confirmed diagnosis of haemoglobin Bart's hydrops foetalis, nondirective counselling should include the option of expectant management, pregnancy termination, or foetal therapy with IUTs. Parents should be offered the opportunity to consult with a paediatric haematologist to understand the long-term outcomes, prognosis, and the requirements of postnatal and childhood management.
- If the family elects to pursue IUTs, they should be referred to a centre with expertise in this technique if it is not available locally.
- For patients electing to proceed with foetal therapy, IUTs should begin as soon as technically possible.
- Delivery should be planned at a tertiary care centre with availability of perinatology, paediatric haematology, and neonatology teams.
- While general principle of long-term management of patients with haemoglobin Bart's hydrops foetalis is similar to those for transfusion dependent β-thalassaemia, the transfusion requirement for patients with haemoglobin Bart's hydrops foetalis is different given the presence of non-functional Hb Bart's. **Table 1** (p. 57) summarizes the steps in management of newborn with diagnosis of haemoglobin Bart's hydrops foetalis.

5 LONG-TERM MANAGEMENT OF α-THALASSAEMIA MAJOR (HEMOGLOBIN BART'S HYDROPS FOETALIS)

Summary and recommendations

- The management of patients with α-thalassaemia major follows the same principles as those for patients with transfusion-dependent β-thalassaemia, involving regular blood transfusions and effective iron chelation. However, patients with α-thalassaemia major require more aggressive transfusion strategies due to preserved reticulocytosis and high proportion of circulating non-functional HbH.
- The goal of a transfusion regimen is to maintain functional haemoglobin (HbA) >90 g/L. Functional haemoglobin should be calculated ideally prior to each transfusion by testing haemoglobin fractions. Centres lacking access to precise HbH measurements and, as a result, are unable to calculate functional haemoglobin may consider maintaining pre-transfusion total haemoglobin at 105-110 g/L and reticulocyte count < 500 x 10e9/L.
- Iron chelation is started at approximately one year of age, with careful monitoring for toxicities. Initiation of iron chelation before 1 year of age is not recommended. Aggressive iron chelation prior to 2 year of age is not recommended.
- Patients with α-thalassaemia major have increased rate of congenital anomalies and neurocognitive compromise. Similarly, a higher rate of growth delay, reduced bone density and endocrinopathies have been reported. Appropriate diagnostic and surveillance testing, and referral to surgical or medical specialist are prudent to improve the outcome of patients with α-thalassaemia major.
- Given the high rate of complications, the option of curative therapy with stem cell transplant should be reviewed early.

6 FERTILITY AND PREGNANCY IN α-THALASSAEMIA

Summary and recommendations

- Some individuals with HbH disease require regular blood transfusions leading to iron overload, which can diminish their fertility. These patients will benefit from a multidisciplinary approach that includes haematologists, fertility specialists, obstetricians, and mental health professionals. An early fertility evaluation, especially in those with more severe anaemia or a prior history of iron overload may be warranted.
- Individuals with HbH disease may occasionally require blood transfusion during pregnancy. Transfusions are conducted every 3–4 weeks with the goal to maintain the haemoglobin level at 90 g/L pre-transfusion and at 120 g/L post-transfusion.
- In splenectomized patients, thrombosis prevention with low-dose aspirin (or other anticoagulants that are safe during pregnancy) is recommended.
- While chelation should be generally avoided during pregnancy, deferoxamine (pregnancy category C) can be used for severe iron overload.
- Pre-conceptional and prenatal testing should be recommended to couples at risk of having an offspring with haemoglobin Bart's hydrops foetalis or non-deletional forms of HbH disease after assessment of both partners and explanation of risks and benefits.

7 INFECTIONS AND HAEMOGLOBIN H DISEASE

Summary and recommendations

- Patients with both transfusion-dependent and non-transfusion-dependent α-thalassaemia are susceptible to various kinds of infection, because of the disease pathophysiology itself and related treatments.
- Understanding the specific characteristics of the patients (requirement for blood transfusion, splenectomy, type of chelator used) and regional epidemiology of causative pathogens will lead to appropriate preventive measures, early recognition of infection, and prompt treatments.
- A haemolytic crisis is the dominant clinical feature of α-thalassaemia, which may occur during or after a febrile episode.
- A haemolytic crisis in patients with HbH who also have G6PD deficiency can be critical and result in massive haemoglobinuria and acute renal failure.
- Patients with HbH should be educated to promptly take antipyretic medication to decrease their body temperature, recognize signs and symptoms of acute haemolysis early, and seek medical help in the event of their occurrence.
- During an acute haemolytic episode, blood transfusion should be provided to restore Hb to 80-90 g/L, along with adequate intravenous hydration. If haemoglobinuria is present, urine alkalinization should also be administered (see **Table 1** p. 77).
- HbH patients with dengue infection experience haemolytic anaemia, rather than haemoconcentration, during the febrile stage, which can pose a problem in diagnosis.
- In endemic regions, dengue infection should be suspected in HbH patients who present with symptoms of viral illness and progressive anaemia, leucopenia, and thrombocytopenia. All HbH patients with dengue infection should be hospitalized for close monitoring.
- An aplastic crisis caused by parvovirus B19 infection can be differentiated from the typical acute haemolytic crisis by the presence of reticulocytopenia, instead of reticulocytosis.
- Individuals affected with α^+-thalassaemia homozygotes and heterozygotes are protected against severe forms of *P. falciparum*. However, this is not the case for other forms of α-thalassaemia diseases.
- Affected patients with HbH disease are strongly advised to take a malaria chemoprophylaxis regimen when travelling to endemic areas.
- The latest evidence suggested the prevalence of SARS-CoV-2 infection among thalassaemia patients was similar or might be lower than that of the general population. However, thalassaemia patients encountered a 5-fold greater risk of having age-standardized lethality.
- HbH patients should be monitored for haemolytic crisis during the clinical course of COVID-19 and adrenal insufficiency should be investigated in the patients with haemodynamic instability. Possible bacterial co-infections must be kept in mind among splenectomized patients and initial empirical antibiotics administration is recommended.
- Splenectomized patients encounter >50-fold greater risk of having OPSI, caused mainly by S. pneumoniae, Hib, and N. meningitidis.

- In the presence of any febrile illnesses, splenectomized patients should take emergency oral antibiotic supplies they have on hand (amoxicillin or amoxicillin-clavulanate, (see **Table 2** p. 79) and rapidly proceed to an accident and emergency department.
- Empirical intravenous antibiotic with a third-generation cephalosporin (ceftriaxone) should be administered as soon as the patient arrives at the hospital.
- Daily oral antibiotic prophylaxis with penicillin is strongly recommended in all splenectomized patients and this should be continued until the patients reach the age of 5 or at least in the first 2-3 years following splenectomy in older children and adult patients. (see **Table 2** p. 79).
- High-risk patients should continue daily oral antibiotic prophylaxis for lifelong.
- Initial vaccination against *S. pneumoniae, N. meningitidis*, and Hib should be completed at least 2 weeks in advance of the planned splenectomy. (see **Table 3** p.80).
- In case of urgent splenectomy, administration of these vaccines within 2 weeks following the procedure remains beneficial.
- Annual influenza vaccine is recommended for both splenectomized and non-splenectomized patients with α-thalassaemia.
- The main pathogens involved in TTIs are HIV, HBV, and HCV.
- Antibody testing of HIV and HCV, and testing of all markers of HBV are recommended annually in transfusion-dependent α-thalassaemia and once within the year in which the occasionally transfused patients are exposed to blood products.
- A protective level of immunity against HBV should be ensured in all patients with α-thalassaemia, who require occasional or regular transfusions. A booster dose of the HBV vaccine is recommended if anti-HBs titer decreases.
- Transfusion-associated HIV infection has decreased dramatically in the past two decades.
- A wide range of ARV regimens offer improved survival and quality of life for HIV-infected individuals and this therapy can be used in thalassaemia patients.
- Acute viral hepatitis B infection can be managed with only supportive care. Treatment of chronic hepatitis B involves antiviral medications and/or injection of interferon α-2b to reduce viral replication and its damage to the liver.
- Up to 80% of acute HCV infections evolve into chronic hepatitis C, for which DDAs are currently the treatment of choice with the goal to cure the disease.
- There is no effective vaccine against HCV, however, immunization against HBV and HAV is strongly recommended in both regularly and occasionally transfused α-thalassaemia patients, to prevent additional risk of liver diseases.
- Iron overload is a risk factor for severe bacterial infection in patients with α-thalassaemia and is associated with faster progression of HIV disease, evolution to liver fibrosis in affected patients with chronic HCV infection, and impaired clinical response to HCV antiviral therapy.
- Optimal control of iron overload in all α-thalassaemia patients for clinical benefits against severe infections is advised.
- DFO, but not DFP or DFX therapy, is associated with severe bacterial infection, especially due to Yersinia enterocolitica and Klebsiella species.
- Discontinuation of DFO during a febrile illness is strongly advised.
- DFP or DFX can be continued during a febrile episode, unless there is a presence of other chelator-associated adverse effects, such as agranulocytosis or neutropenia in the patients using DFP.

8 LIVER DISEASE IN α-THALASSAEMIA

Summary

Liver disease is one of the leading causes of morbidity and mortality in thalassaemia syndromes. Iron overload due to ineffective erythropoiesis, which promotes fibrinogenesis and carcinogenesis, is the major mechanism of disease manifestation. Iron often accumulates slowly in HbH disease and thus liver complications often occur in adulthood or even at advanced age. Concomitant liver disease, especially chronic HCV infection, further increases the risk of cirrhosis and hepatocellular carcinoma. Liver iron burden should be regularly monitored by serum ferritin and MRI T2*. Iron chelation is effective to remove excess hepatic iron and to reduce the risks of developing complications. Viral hepatitis should be managed according to the respective guidelines applied to the general population. USG liver in patients with risk factors enable early detection of HCC to decrease mortality.

Recommendations

- Regular monitoring of liver iron burden by serum ferritin and MRI T2* should commence at 10 years of age or older for patients with milder disease phenotype.
- Iron chelation therapy is recommended in patients with LIC ≥5mg/g dry weight or serum ferritin ≥500 ng/mL in non-transfusion dependent patients with HbH disease.
- Vaccination against the hepatitis B virus in seronegative patients prior to blood transfusion therapy is recommended.
- Annual screening of HBV and HCV infection is recommended in patients receiving blood transfusions, and positive anti-HCV results should be followed by HCV-RNA to identify chronic infection.
- Consultation with a hepatologist is recommended for management of concomitant HBV and/ or HCV infection, or when liver cirrhosis is established.
- Evaluation for liver fibrosis and cirrhosis with transient elastography is recommended in patients with liver iron concentration ≥5mg/g dry weight or serum ferritin ≥500 ng/mL.
- Biannual ultrasonography of liver for HCC surveillance is recommended in patients with HbH disease when risk factors (liver iron overload, cirrhosis, HBV and/ or HCV infection) are present.

9 BLOOD TRANSFUSION

Recommendations

- Identify the underlying cause of an acute event (e.g., infection/inflammation/aplasia) and provide appropriate treatment. Empiric antibiotics with the coverage against encapsulated bacteria should be initiated in splenectomized patients with fever.
- The decision to offer on-demand transfusion should be based on patient's clinical status, underlying clinical indication of expected course of acute illness, and preference of patient or their caregivers.
- The aim of blood transfusion is to restore patient's haemoglobin to baseline or slightly higher.
- Patients experiencing an acute event should be monitored closely to ensure indications for further transfusion have resolved.
- Serial assessment of haemoglobin concentration, haemolytic markers, electrolytes, and renal function should be done, since continuing hemolysis could lead to worsening anaemia, electrolyte and acid-base disturbances, and renal damage.
- Adequate hydration should be provided to maintain circulation and renal perfusion, but excessive hydration should be avoided to prevent volume overload.
- Other supportive measures should be offered as needed (e.g. antipyretics).
- If clinically indicated, on-demand transfusion can be offered prior to surgeries, or during pregnancy.
- The criteria to start regular transfusion in individuals with HbH disease are not well defined and are generally based on expert opinion.
- It is recommended that "effective" haemoglobin be measured at a steady state. Effective haemoglobin can be calculated as total Hb x (1- [HbH % + Hb Bart's %] / 100). Please note that the current technology of Hb analysis by automatic high-performance liquid chromatography (HPLC) such as Variant (Bio Rad) could not accurately quantitate the percentage of HbH and Hb Bart's. The percentage of HbH and Hb Bart's can be more accurately measured by capillary electrophoresis system; however, caution should be exercised that this is not done during an acute haemolytic event.
- Initiations of regular transfusion should be individualized and be offered through a patient-centred approach.
- Regular transfusions should be considered in the following settings:
  - Patients with Hb Bart's hydrops foetalis (α-thalassaemia major).
  - Patients with baseline "effective" haemoglobin < 70 g/L (or < 80 g/L if effective haemoglobin cannot be calculated).
  - Declining haemoglobin concentration or development of symptomatic anaemia in those who have not been on regular transfusions.

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- To prevent or reduce the long-term sequelae of chronic haemolytic anaemia or to suppress ineffective erythropoiesis.
- Frequent acute haemolytic events requiring on-demand transfusions
- Children and adolescents with growth failure, poor academic performance, diminished exercise tolerance, or delayed puberty.
- Poor quality of life due to anaemia.

- When regular transfusion is being considered, alternative therapeutic interventions, such as splenectomy, should also be reviewed.
- Careful monitoring and treatment of iron overload is paramount, although, as compared to β-thalassaemia, ferritin may underestimate the degree of tissue iron due to the difference in the pathophysiology of α- vs. β-thalassaemia.
- In low-resource settings, or if a regular transfusion programme and iron chelation are challenging or not feasible, splenectomy can be considered in those who are older than 5 years of age with a predominantly haemolytic HbH disease. Pre-splenectomy vaccination for encapsulated organisms is highly recommended. Patients with severe anaemia requiring transfusion from early childhood or those with significant ineffective erythropoiesis should be treated similar to β-thalassaemia and in general, splenectomy is not recommended.
- A pre-transfusion haemoglobin target of 80-90 g/L is acceptable in most patients. However, those with high proportion of circulating HbH and those with ineffective erythropoiesis may require higher pre-transfusion haemoglobin targets.
- Patients with Hb Bart's hydrops foetalis (α-thalassaemia major) require an aggressive transfusion approach to suppress significant erythropoiesis and improve oxygenation through increasing functional (non-HbH) haemoglobin. In these patients, "functional" haemoglobin should be calculated for a pre-transfusion functional Hb target of 90-100 g/L.
- The need for ongoing regular transfusion should be regularly evaluated, as some patients may become transfusion independent.
- Use a product that is collected, tested, selected, issued, and administered in adherence to established quality and safety regulations and guidance.
- Blood transfusion should be administered by staff trained in blood transfusion.
- Involve informed patient consent.
- The transfusion should be performed in a system with a good haemovigilance structure.
- If possible, do not waste valuable donated blood.
- Blood storage for <2 weeks, conditioning to achieve mean 24-hour post-transfusion RBC survival ≥75%.
- Leucoreduced packed red blood cells (≤1 x 106 leucocytes/unit) with haemoglobin content ≥40 g (pre-storage filtration preferred).
- Patients with α-thalassaemia should have extended red cell antigen typing that include at least A, B, O, C, c, D, E, e, and Kell antigens and preferably a full red cell phenotype/genotype penal should be done prior to first transfusion.
- All patients should be transfused with A, B, O, C, c, D, E, e, and Kell compatible blood.
- Patients are advised to have appropriate vaccinations against transfusion-transmitted infections.

10 SPLENOMEGALY AND SPLENECTOMY

Summary of recommendations

- Splenectomy may be considered in patients with HbH disease who have moderately severe anaemia, those with long-term complications of chronic haemolytic anaemia, or patients who experience frequent acute haemolytic events requiring on-demand transfusions, or those with symptomatic splenomegaly.
- Splenectomy is not recommended for: children younger than 5, those with severe anaemia (functional Hb < 70 g/L) or those requiring transfusion from early childhood, and those with significant ineffective erythropoiesis with a β-thalassaemia-like phenotype. In these patients, chronic transfusion is the more appropriate disease modifying approach.
- Splenectomy is not recommended for management of patients with haemoglobin Bart's hydrops foetalis (α-thalassaemia major).
- The risks and benefits of splenectomy vs. alternative therapeutic interventions (e.g., chronic transfusions with iron chelation or novel emerging disease modifying therapies) should be carefully reviewed.
- Patient preference should be valued and available resources should be considered.
- Patient should receive vaccination against encapsulated bacteria as per local vaccination guidelines for patients with asplenia.
- While there is little consensus on the recommended duration of use, daily oral antibiotic prophylaxis with activity against encapsulated bacteria is recommended in all splenectomized patients. In general, patients undergoing splenectomy at age <5 years should receive antibiotic prophylaxis at least until the age of 5, and all patients should receive prophylactic regimen at least in the first 2–3 years following the procedure. High-risk patients should receive lifelong prophylaxis.
- In splenectomized patients who present with fever or signs or symptoms of infection, appropriate parenteral antibiotics against encapsulated bacteria should be started while awaiting culture results.
- Low-dose aspirin should be considered in splenectomized patients as long as there are no contraindications. This is particularly important for patients with a history of previous thrombosis or other risk factors.
- Clinicians should offer preventative measures, surveillance tests or appropriate prevention and treatments for post-splenectomy complications.
- There is limited data to suggest the effectiveness and safety of partial splenectomy or splenic embolization in patients with HbH disease. Thus, they are not recommended except in a research setting or as part of structured observational cohort.
- The choice of laparoscopy vs. laparotomy procedure is based upon the patient's preference and the surgical team's expertise.

11 IRON OVERLOAD AND IRON CHELATION IN α-THALASSAEMIA

Summary and recommendations

- The primary goal of treatment of iron overload is to protect against organ toxicity from exposure to reactive ferrous (Fe^{2+}) iron.
- Organ toxicity is related to the magnitude and duration of ferrous iron exposure so the goal of treatment is to keep ferrous iron levels at zero all the time.
- Transferrin saturation > 50% and certainly > 70% on multiple measures is a reasonable surrogate for circulating NTBI/LPI.
- MRI only measures non-reactive Fe^{3+}, though high levels are somewhat related to toxicity. Presence of high MRI detected iron in pancreas, pituitary or heart indicates prolonged exposure to high levels of NTBI/LPI.
- Circulating chelators lower NTBI/LPI to zero immediately, prevent/reduce toxicity, and block entry into endocrine and cardiac tissue as long as they are circulating. Chelators can preserve organ function even in the presence of very high tissue iron levels by reducing NTBI/LPI.
- Increased iron absorption due to ineffective erythropoiesis as seen in NTDT-α or β takes decades before extra-hepatic organs load.
- Iron loading in TDT happens rapidly and linearly with the number of transfusions. Loading of endocrine organs and the heart can occur within a few years, but organ dysfunction takes longer.
- The main reason for failure of iron chelation treatment is poor adherence to prescribed medications.

12 CURATIVE THERAPIES FOR α-THALASSAEMIA

Summary and recommendations

- Antenatal counselling of parents involving obstetricians, neonatologists, hematologists, and stem cell transplant physicians is imperative.
- Surviving patients with haemoglobin Bart's hydrops foetalis require lifelong transfusion therapy and iron chelation, and as compared to their β-thalassaemia counterparts, they are at higher risk of disease or treatment-related complications.
- Allogeneic haematopoietic stem cell transplantation is the only curative option currently available for long term survivors of haemoglobin Bart's hydrops foetalis.
- Transplant for thalassaemia should be offered as early as possible as outcomes are superior prior to the onset of organ dysfunction secondary to iron overload.
- Several case reports have been published to date describing the success of transplant in patients with α-thalassaemia. All patients who have engrafted and survived were able to achieve transfusion independence.
- Similar to patients with complete chimerism, those with persistent mixed chimerism were still able to be clinically cured. However, the acceptable threshold for residual host cells post-transplant is yet to be defined.
- Improvements in transplantation platforms such as *ex vivo* or *in vivo* graft manipulation and graft versus host disease prophylaxis have expanded the use of alternative donors (haploidentical, unrelated) for patients with thalassaemia with comparable outcomes to matched sibling donors.
- Outcomes of transplant have improved significantly over the last decade including those of high-risk patients.
- HSCT survivors will require lifelong monitoring and follow-ups for long-term consequences of HSCT and the residual symptoms of pre-HSCT disease.

13 NOVEL AND EMERGING THERAPIES FOR A-THALASSAEMIA

Brief summary and recommendations

- Different agents are studied in α-thalassaemia, with the most promising being luspatercept and mitapivat. Luspatercept is an erythroid maturation agent with good efficacy in decreasing transfusion dependency in β-TDT and improving anaemia in β-NTDT. Mitapivat is an activator of pyruvate kinase which increases haemoglobin levels in patients with HbH disease.
- Results of the studies on the use of these novel agents in α-thalassaemia are expected within the next few years. Should they be positive, they may offer an alternative to transfusions for improving anaemia in patients with α-thalassaemia. Before the completion of the studies, the use of these agents outside the setting of clinical trials is not recommended.
- Novel curative approaches like gene addition of α-globin gene and in-utero haematopoietic stem cell transplantation are currently in early phases of development.

14 PREVENTION AND CONTROL OF ALPHA-THALASSAEMIA DISEASES

Summary and recommendations

- Genetic counselling and prenatal screening for couples at risk of foetal Hb Bart's hydrops foetalis should be offered to all couples.
- Pregnant women are encouraged to attend early antenatal care. During the first visit of antenatal care, the couple should be counselled regarding the risk of foetal thalassaemia diseases. Prenatal testing to identify couple at risk is performed in the first visit.
- For prenatal screening of thalassaemia carriers, when both individuals in a couple meet the MCV or MCH criteria, they need to be tested for α^0-thalassaemia by DNA-based methods, along with Hb analysis, to detect coinheritance of β-thalassaemia carrier and other Hb variants. The test for α^0-thalassaemia is required regardless of β-thalassaemia status as coinheritance of α-thalassaemia and β-thalassaemia is common, except for the African population where α^+-thalassaemia is common but α^0-thalassaemia is rare. In the African population α^0-thalassaemia is to be tested for when MCV or MCH is low and β-thalassaemia carrier state has been excluded by Hb analysis.
- Prenatal diagnosis should be offered to all couples at risk of having a foetus with Hb Bart's hydrops foetalis.
- The pregnant woman at risk of Hb Bart's hydrops foetalis should undergo the first ultrasound examination at 12-13 weeks of gestation. The couple at risk should be counselled about the clinical presentation of Hb Bart's hydrops foetalis, foetal diagnostic methods, and options for management.
- The options of foetal diagnosis are: 1) the conventional approach, which involves an obstetric procedure to obtain foetal DNA or blood for molecular and/or Hb analysis and 2) the non-invasive approach or ultrasound algorithm.
- Ultrasound can be used to evaluate signs of foetal anaemia and hydrops in an at-risk foetus in cases that the couple opts against invasive prenatal diagnosis, or when the risk identification is incomplete such as a single mother who is an α^0-thalassaemia carrier. If signs of foetal anaemia or hydrops are present, DNA-based foetal diagnosis needs to be done to confirm the final diagnosis.
- In pregnancies affected by Hb Bart's hydrops foetalis, detailed and non-directive counselling and discussion should be given and the option to terminate the pregnancy should be discussed.
- In cases in which parents wish to continue with the affected pregnancy, the management should be given in centers with expertise in caring for Hb Bart's hydrops foetalis. Early ultrasound to detect associated congenital abnormalities should be performed and results should be discussed with the parents. Early intrauterine transfusion and close follow-up on foetal and maternal status should be offered. The care team should be coordinated between obstetrics and neonatology for postnatal intensive care and haematology for long-term management. ▶▶▶

- Noninvasive prenatal testing for Hb Bart's hydrops foetalis using cff-DNA should be considered as a screening test and should be used in conjunction with other established methods for foetal diagnosis.
- For HbH hydrops foetalis, the monitoring for foetal anaemia is based on the guideline for Hb Bart's hydrops foetalis. The decision of intrauterine transfusion should be discussed with the family and is based on the molecular diagnosis and the long-term prognosis.
- For foetal homozygous Hb CS, the ultrasound monitoring for foetal anaemia is recommended. Intrauterine transfusion is beneficial.

15 GENETIC COUNSELING FOR FAMILIES AT RISK FOR α-THALASSAEMIA

Summary and recommendations

- Comprehensive family history is essential to understand what, if any, reproductive risks may be present and should be addressed. This may include a family or personal history of anaemia, recurrent pregnancy loss or hydrops foetalis.
- Preconception/Early in pregnancy: clarification of carrier status in parents is essential.
  Couples at risk for Hb Bart's Hydrops foetalis should be referred to paediatric haematology for comprehensive education regarding long term prognosis and management options.
- Couples at risk for a pregnancy affected by Hb Bart's Hydrops foetalis or nondeletional Hb H should be educated regarding all pregnancy options including pregnancy termination, in utero transfusions or expectant management to make informed reproductive decisions.
- Prenatal diagnosis (CVS or amniocentesis) is critical in identifying affected pregnancies prior to presentation of hydrops.
- Patients carrying a pregnancy diagnosed with hydrops foetalis and/or elevated MCA Doppler concerning for foetal anaemia may consider PUBS to confirm anaemia and the option to initiate in utero transfusions. Hb Bart's evaluation of foetal blood sample may be used to confirm or exclude the diagnosis.

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

Sir William Osler (1849-1919)

THALIA

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