*InTheName Of God*

# Management of Hemoglobin H

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# Alpha thalassemia

α-thalassaemias are inherited disorders characterised by reduced or suppressed production of α globin chains.

The human α globin genes are duplicated and located at the telomeric end of the short arm of chromosome 16.

α thalassaemia is caused most commonly by deletions of large DNA fragments that involve one or both α globin genes.

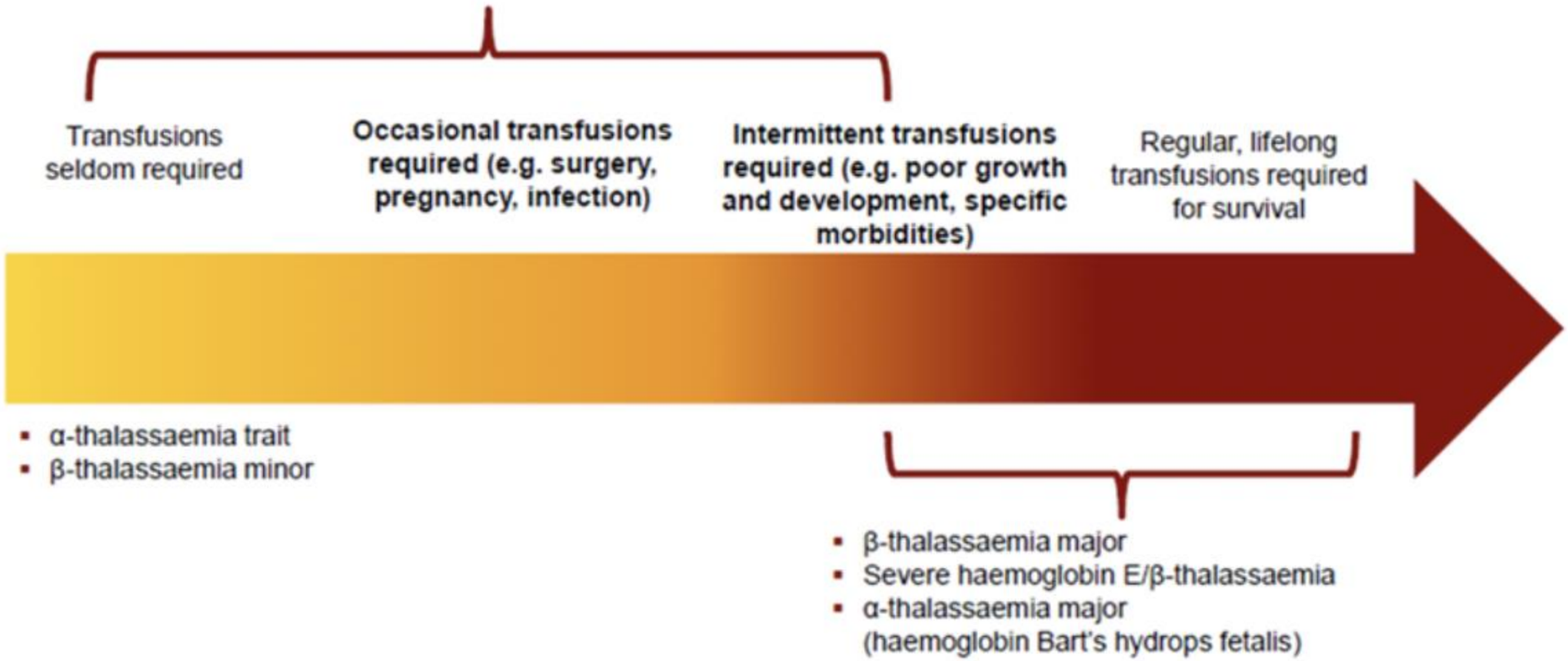
Silent carrier state:

- The presence of a single α globin gene deletion or deletional α+ thalassaemia results in the silent carrier state.
- Heterozygotes for one missing α globin gene are not anaemic and have normal red blood cell indices.

- Two major types of this deletional α+ thalassaemia, **3.7 and 4.2 kb** deletions, are wide spread throughout the globe and they have been identified even in the population in the Pacific.

## Non-transfusion-dependent thalassaemias (NTDT)

- β-thalassaemia intermedia
- Mild/moderate HbE/β-thalassaemia
- α-thalassaemia intermedia (HbH disease)




HbE, haemoglobin E; HbH, haemoglobin H.

Musallam KM, et al. Haematologica. 2013;98:833-44.

# α thalassaemia trait:

- Subjects with two residual functional α genes either by deletions that remove two linked α globin genes from the same chromosome or α0 (– –/αα) or combination of deletional α+ thalassaemia (–α/–α), have mild hypochromia and microcytosis.
- Their **MCV and MCH are usually lower than 80 fl and 27 pg,** respectively.
- Less commonly, mutations caused by single or a few nucleotide deletions or alterations known as non-deletional α thalassaemia (αTα/ or ααT/) have been identified in several populations from Mediterranean countries to Southeast Asia and China.
- Haemoglobin Constant Spring (Hb CS) and Hb Paksé, two abnormal Hbs characterised by elongated alpha globin chains resulting from mutations of the termination codon in the alpha2 globin gene, **are the most prevalent non- deletional alpha thalassaemias in Southeast Asia.**

- For example, co-inheritance of Hb Constant Spring and the deletion of two α genes results in a severe form of Hb H disease in which up to 20% of patients require frequent blood transfusion and splenectomy.
- Most patients with Hb H disease can be managed as recommended in the NTDT guideline.

Heterozygotes for non-deletional α thalassaemia have borderline MCV and MCH therefore they might not be detected in most of the programmes for thalassaemia prevention and control that use red blood cell indices as a screening tool.

## Haematological diagnosis

- When there are deletions or non-deletional abnormalities of three globin genes , the affected individual would have only **one functional gene** and this hereditary disorder is known as HbH disease.
- It is usually characterised by a **moderate haemolytic anaemia, splenomegaly and acute haemolytic crisis in response to oxidant drugs and infections.**
- In general, patients with **non-deletional Hb H disease** have more severe disease than patients with deletional Hb H disease.

# Qualitative and quantitative haemoglobin analysis

- Identification of fast moving haemoglobin species by electrophoresis representing Hb H (β4) and Bart's (γ4) is characteristic of α thalassaemia syndromes.
- The levels of Hb H measured can vary f**rom < 1% up to 40% (usual range 10-15%) due to sensitivity of test**s, laboratory expertise, type of instruments and the quality of blood samples.
- Hb H might not be readily identified through some platforms of liquid chromatography; a manual identification using the presence of haemoglobin species at a specific retention time (RT) is required.

- Due to a lack of available α globin chains, Hb A2 (α2δ2) is reduced. In patients with non-deletional Hb H disease especially Hb H/Hb CS, Hb CS variant can be detected at a very low level (1-4%).

- Molecular testing approaches can include targeted deletion analysis for common deletions, sequence analysis, and deletion analysis of the α1 and α2 globin genes and the HS -40 regulatory region (LCRA).

| | | ß-TM | ß-TI | HßE/ß-Thal | | HbH |
|---|---|---|---|---|---|---|
| Hb levels | | <5g/dL | ~7- | Mild | 9-12 | 2.6-13.3 g/dL |
| | | | | Moderately Severe | 6-7 g/dL | |
| | | | | Severe | 4-5 g/ | |
| BLOODSMEAR | Low Hb Production | Red cell hypochromia microcytosis, Target cells | | | | |
| | Haemolysis | Irregularly crenated RBC, increased reticulocytes [5- | | | | |
| | Ineffective erythropoiesis | Nucleated RBC, Basophilic stippling | | | | |
| | Special Features | +Numerous F-cells/acid elusion | +F-cells/acid elusion | + DCIP staining[HbE] | | HbH inclusion |
| Hemoglobin study | | HbF up to 100% HbA2↑ | HbF 10-50% [up to 100%] HbA2 >4% | HbE [40-60%] HbF (60-40%) ± HbA [with ß⁺-thal] HbA2 ↑ | | Variable HbH (0,8-40%) HbA2↓ ⁺ the presence of a-varaints i.e. Hb CS, Hb PS etc. |
| DNA analysis | | • Common known mulations of both ß⁰ and ß⁺- thal mulations in population specific set can be done by PCR based methods.<br>• For rare or unusual mulations, a direct sequencing or array analysis required<br>• Other analysis for ß-TI included α and ß- globin rearrangements, Xmn l polymorphism and other | | | | Gap-PCR developed for 7 common α- thal delections and RDB for non-deltional mulations For inknown mulations, Southern blotting or MLPA analysis and sequencing required |

Figure 5. Summary of diagnostic measures for thalassaemia and haemoglobinopathies. MLPA, multiplex ligation-dependent probe amplification; QTL, quantitative locus; TI, thalassaemia intermedia; TM, thalassaemia major

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ORIGINAL ARTICLE

# Heterogeneity of Hemoglobin H Disease in Childhood

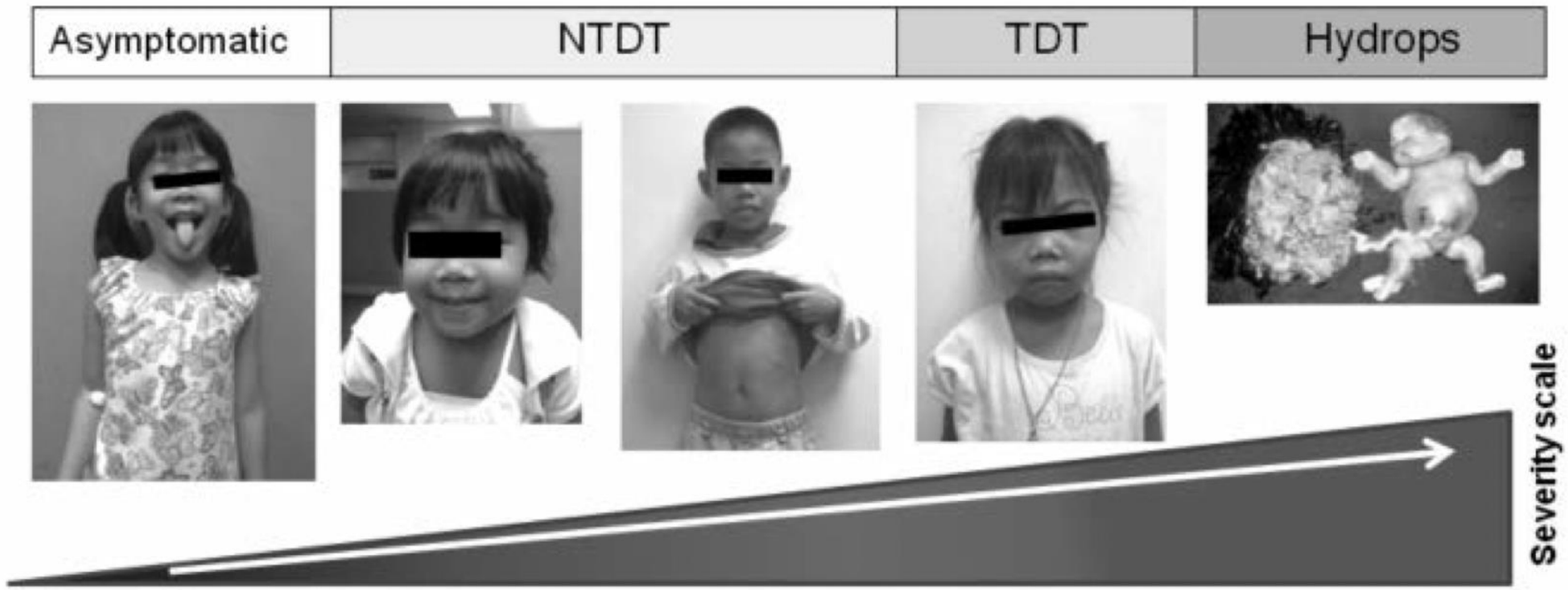
Ashutosh Lal, M.D., Michael L. Goldrich, B.A., Drucilla A. Haines, P.N.P., Mahin Azimi, C.L.S., Sylvia T. Singer, M.D., and Elliott P. Vichinsky, M.D.

We analyzed longitudinal clinical data for patients with hemoglobin H disease arising from the deletion of three of four α-globin genes (HbH) and from hemoglobin H Constant Spring (HCS), caused by the deletion of two α-globin genes and the Con- stant Spring mutation.

HCS should be recognized as a distinct thalassemia syndrome with a high risk of life-threatening anemia during febrile illnesses.

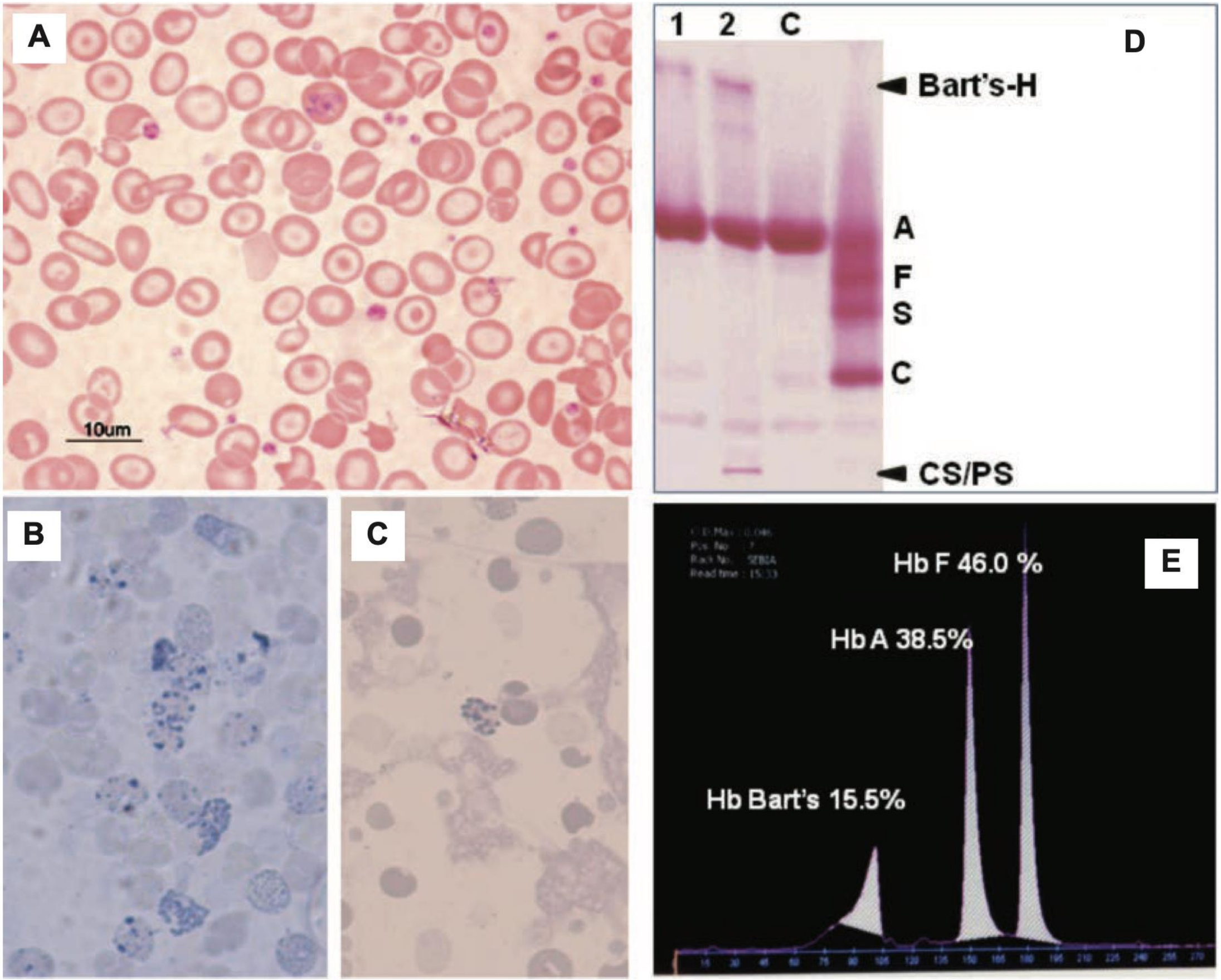
HbH was not associated with an increased rate of severe anemia with infections and was managed without blood transfusions.

Many patients with these disorders had mixed ethnic backgrounds, which highlights the need for **extended newborn screening in populations that are traditionally considered to be at low risk for hemoglobin H disease.**




| Genotypes | Silent HbH | Deletional HbH | Non-deletional HbH | Transfusion – dependent HbH | | HbH hydrops |
|---|---|---|---|---|---|---|
| 1⁰ | --/-α<br>--/α$^{T}$α | --/-α | --/α$^{T}$α<br>--/αα$^{T}$ | --/-α | --/α$^{T}$α<br>--/αα$^{T}$<br>α$^{T}$α/α$^{T}$α | --/α$^{T}$α<br>α$^{T}$α/α$^{T}$α |
| 2⁰ | β thal | +β globin variants such as Hb E, Hb C, Hb S, homozygous Hb E, β thal/Hb E etc. | | - | | - |
| 3⁰ | - | | | KLF-1 | | - |

**Figure 2. Heterogeneous clinical presentation and severity of patients with α-thalassemia syndromes from Hb H hydrops to silent Hb H disease. The table shows three levels of partially known genetic basis underlying the clinical heterogeneity of Hb H disease. Primary defects are based on the types of α-globin mutations (deletional and non-deletional α-thalassemias) and their interaction. The secondary level of genetic control is the co-inheritance of β-thalassemia[62-64] or β-hemoglobinopathy such as Hb E.[65,66] The presence of β-thalassemia generally causes more balanced globin synthesis resulting in a milder phenotype with possible absence of Hb H (silent Hb H), while inheritance of unstable β-globin variants in particular Hb E or homozygous Hb E causing AE Bart's and EF Bart's disease results in a more severe phenotype than simple deletional Hb H disease.[67] The tertiary level involves other genetic modifiers outside the globin gene clusters. At present, only KLF-1 was found to deteriorate the clinical course of patients with deletional and non-deletional Hb H disease.[48] Other genetic modifiers that might affect other complications such as bone disease (vitamin D receptor gene), iron overload (Hfe and others), jaundice and gall stone formation (UGT1A1 and others) are not shown and were reviewed previously.[18] NTDT: non-transfusion dependent thalassemia; TDT: transfusion-dependent thalassemia.**


A
10um
1 2 C
D
Bart's-H
A
F
S
C
CS/PS
B
C
Hb F 46.0 %
E
Hb A 38.5%
Hb Bart's 15.5%

- Reduction of α globin synthesis results in decreased production of Hb A (α2β2) and reduced haemoglobin synthesis.
- In addition, excess unpaired β globin chains can form tetramers (β4) that are not physically stable and precipitate, attaching to the red cell surface membrane and causing oxidative damage, thus shortening of red cell survival.
- The formation of β globin tetramers (Hb H) can be detected by haemoglobin analysis.

- Similarly to β thalassaemia syndromes, patients with Hb H disease have a hypochromic microcytic anaemia with a baseline of haemoglobin of 40-130 g/l.
- Increased **polychromasia and reticulocytosis** are observed and can be further augmented during acute infectious episode or haemolytic crises.
- **Nucleated red blood cells and basophilic stippling** are commonly present in more severe phenotypes such as **Hb Bart's hydrops and severe non- deletional** Hb H disease.
- Detection of Hb H as Hb H inclusion bodies in a peripheral blood film using **supravital staining (brilliant cresyl blue)** is the **hallmark** of this condition

- The presence of Hb H increases during acute febrile illness due to increase body temperature.

In non-deletional α thalassaemia and in particular in the case of mutations that generate α globin variants such as Hb CS, Hb H can directly precipitate at the membrane surface and generate reactive oxygen species even in the steady state.

- Therefore, patients with non-deletional Hb H disease are usually have more severe disease than those with deletional Hb H disease.
- Thalassaemia intermedia and Hb H disease may have similar degrees of anaemia, but haemolysis rather than ineffective erythropoiesis is the primary mechanism in Hb H disease. Indeed, iron loading is much more common inthalassaemia intermedia than in Hb H disease.

Data on growth among patients with HbH during the first decade of life were reassuring, since there was no significant deviation in mean weight or height from the normal population.

In contrast, growth deficits in patients with HCS were identified early and were persistent

The genotypic data are similar to those in earlier studies from Asia,showing that the Southeast Asian double-gene deletion with the 3.7-kb deletion was the most frequent genotype for HbH and that there is an almost exclusive

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# Thank You