Compendium of Hemoglobinopathies

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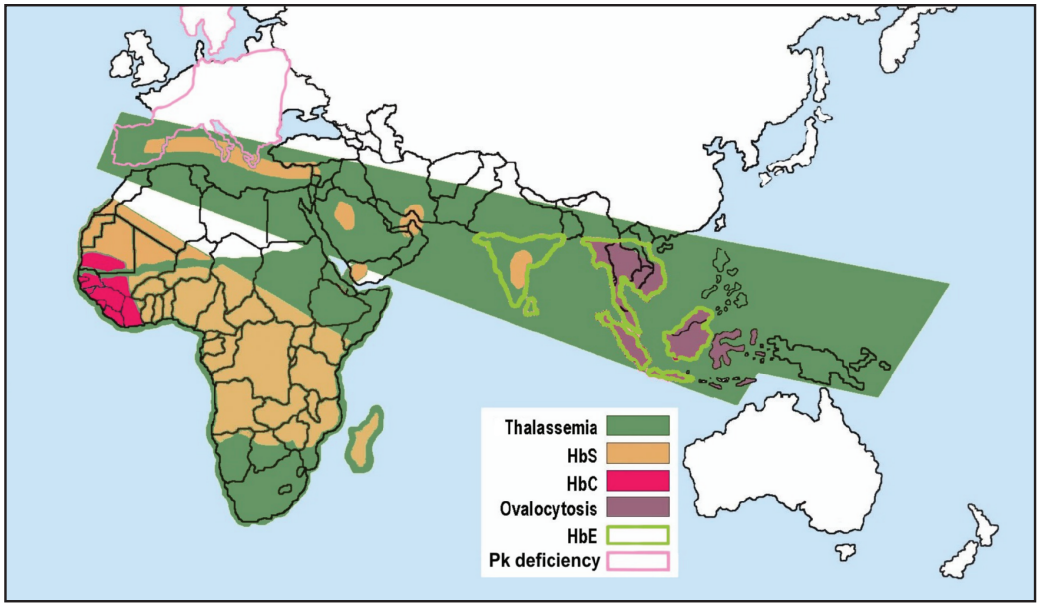
Introduction and basics

With approximately 7% of the worldwide population being carriers, hemoglobinopathies are the most common monogenic diseases and one of the world's major health problems. They were originally found mainly in the Mediterranean area and large parts of Asia and Africa (Fig. 1). International migration has spread them from those areas all over the world. In many parts of Europe today, hemoglobin defects are classified as endemic diseases (Tab. 1).

Germany is one of the countries in which this group of diseases has a rapidly growing importance. There are no epidemiological studies on their frequency. The following statements can be made regarding gene carriers: prevalence among the approximately 9 million immigrants from countries with a high risk of hemoglobinopathies as a whole is 5%, giving a figure of 450 000 affected. To date the number of patients diagnosed with severe hemoglobinopathies is higher than 6,000 (author's own findings). See Fig. 2 for the most important countries of origin in the last 3 decades.

The prevalence of hemoglobinopathies among the total population living in Germany today is approximately 1%. It is very low among the ethnic German population and is estimated to be about 0.01%. Two development processes have taken place in German medicine in recent years which are increasingly redefining the significance of hemoglobinopathies in the country. On one hand, immigrants from regions with a high incidence of hemoglobinopathy have immensely increased medical care requirements and on the other hand, the focus of medical care has expanded considerably from pediatrics to adult medicine. As a result of this, internists, laboratory physicians, human geneticists and gynecologists in addition to pediatricians are now dealing intensively with the matter. Its significance in the local spectrum of diseases is also gaining importance because an increasing number of patients are affected by severe illnesses, impending disability, and high inheritance risks. The care of these patients imposes important requirements. This includes qualified diagnostic including the assessment of results, understanding of the clinico-hematological manifestations and genetic bases as well as adequate knowledge of therapeutical guidelines.

Fig. 1: Original main affected areas of hemoglobinopathies



Significance of hemoglobinopathies in global medicine

• Most widespread genetic diseases among the world population; ·• 6 - 7% genetic carriers; almost half of them thalassemias and the other half - Hb abnormalities; • annual birthrate of children with severe Hb deficiencies is approximately 400,000; • with increasing prosperity this is expected to double in 10 years.

Significance of hemoglobinopathies in German medicine

• Special field with increasing importance
- 15 million inhabitants of Germany have a migration background
- of which 9 million come from high risk countries of Hb defects ⟶ the number of genetic carriers is estimated to be 450,000; • number of severely ill patients is > 6000

Fig. 2: Countries of origin and frequency distribution of patients with hemoglobinopathies examined in Ulm hemoglobin laboratory

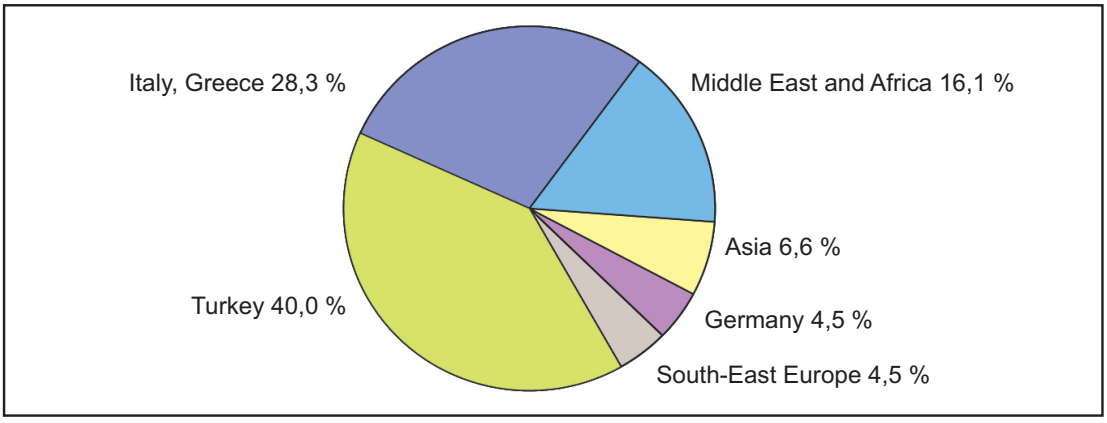


Table 1: Prevalence of hemoglobinopathy carriers in the world population with indication of the arithmetical frequency in Germany

Region	Genetic carriers
Africa	5 - 30 %
Arabic countries	5 - 40 % regional 60 %
Central Asia and India South-East Asia	10 - 20 % 5 - 40 % regional 70 %
USA and Central America	5 - 20 %
Italy Greece Turkey	7 - 9 % 6 - 7 % 7 - 10 %
Germany, Great Britain, Spain, France, Netherlands, Belgium, Scandinavia	Total population up to 1 % Immigrants 5 %
Balkan countries Russia Trans-Caucasus countries	2 - 5 % Seldom up to 5 %

Particularities of German medicine

What is remarkable and typical for immigration countries such as Germany is the great variety of hemoglobin defects and hematological symptoms, in accordance with the variegated ethnic composition of the population. For the most important hemoglobinopathy forms, refer to Tab. 2. The manifestations vary from slight hypochromia and microcytosis with or without anemia up to complex severe disease patterns with diverse complications.

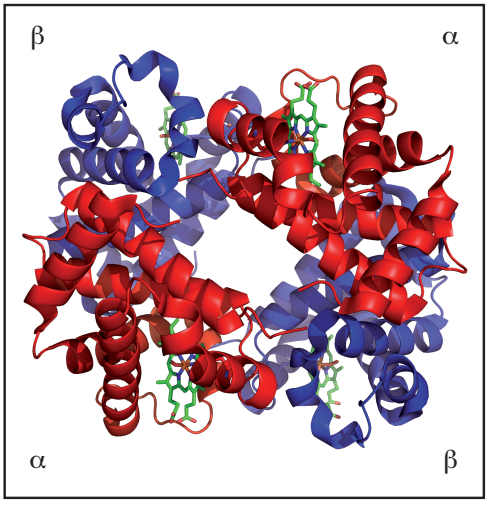
Table 2: The major hemoglobinopathies in Germany in order of frequency

Persons with a migration background	
1. β-thalassemia syndromes	65,8 %
2. Hemoglobin S	21,2 %
3. α-thalassemia syndromes	7,8 %
4. HbE	2,1 %
5. HbC	1,8 %
6. Unstable Hb-abnormalities	0,2 %
Reference: 35 831 laboratory diagnoses = 100 %	

The normal human hemoglobin

The human red blood pigment consists of a tetrameric globin complex (Fig. 3) and four Heme groups which are joined together via chemical and physical bonds in a way that the stability of the molecular structure and the oxygen transport function are guaranteed in an optimum manner. Each globin molecule contains four polypeptide chains of which two are identical (i.e. 2 α and 2 β chains). The specificity of the chains results from their different sequence and number of amino acids.

Fig. 3: Model of the hemoglobin molecular structure



A characteristic feature of hemoglobin is its composition from different components: the major component HbA, the minor component HbA_2 and small quantities of fetal Hb = HbF. The embryonic hemoglobins Hb Gower 1, Gower 2 and Hb Portland are no longer present after birth (Tab. 3).

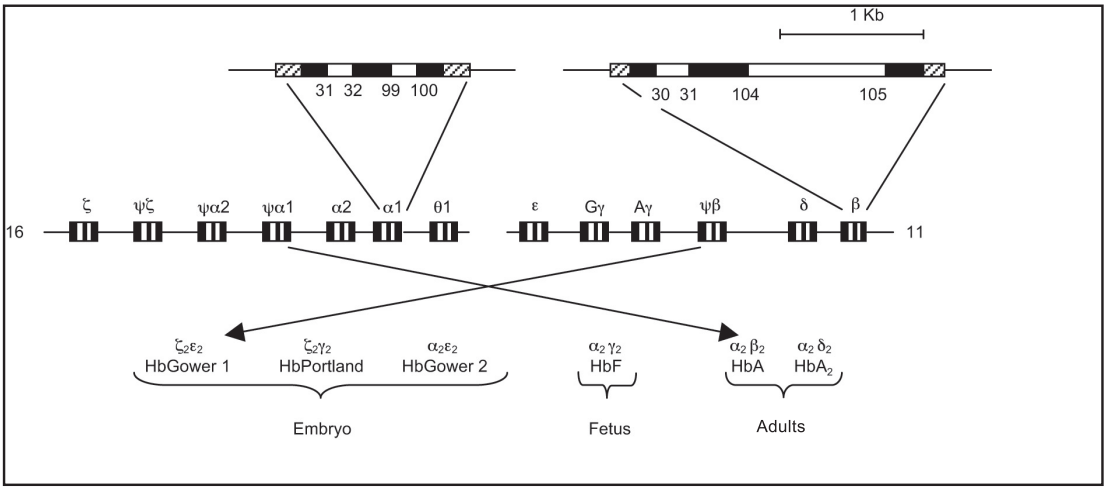
Table 3: Normal human hemoglobins with their chain formula and quantitative distribution

Hb-type	Chain formula	Occurence	Percentage
HbA	$\alpha_2\beta_2$	Children > 1/2 years Adults	97,0 %
HbA_2	$\alpha_2\delta_2$	Children > 1 year Adults	2,5 - 3,0 %
HbF	$\alpha_2\gamma_2$	Fetal/neonatal period Children < 1 year	Children: age-related, Adults: up to 0,5 %
Embryonic hemoglobins		**Normally no longer detectable after birth**	
HbGower 1	$\zeta_2\,\varepsilon_2$		
HbGower 2	$\alpha_2\,\varepsilon_2$		
HbPortland 1	$\zeta_2\,\gamma_2$		

Molecular genetics

The genetic information controlling the α-globin-complex synthesis is encoded on the short arm of chromosome 16, whereas the β-globin locus is located on the short arm of chromosome 11. The regulation of hemoglobin biosynthesis consists of a series of steps which can be interrupted or altered in many different ways due to mutations resulting in quantitative and qualitative changes in hemoglobin.

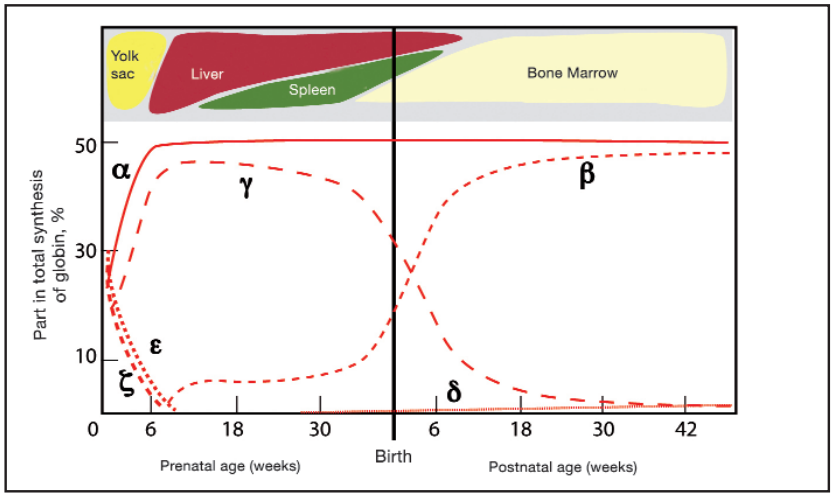
Fig. 4: Topography of the α and β globin structural genes on chromosomes 16 and 11 and the hemoglobins controlled by the respective genes



Ontogenetic development

During embryonic development, fetal and adult hemoglobins are successively superseded. The production of HbA and HbA_2 begins in significant quantities a few weeks before birth and it gradually increases postpartum as part of the hemoglobin switching so that in the first half year of life a complete change from HbF to HbA takes place (Fig. 5).This explains an important insight into the understanding of hemoglobinopathies: diseases caused by mutations in the β-globin gene develop no earlier than the 4th-6th month of life. On the other hand, the α-Hb abnormalities and α thalassemias appear right after birth or even before.

Fig. 5: Ontogenetic development of globin synthesis



Basic forms of hemoglobinopathies

The collective term hemoglobinopathy summarizes all genetically caused hemoglobin diseases (Tab. 4). They are sub-divided into two main groups:

1. Thalassemia syndromes, and
2. Hemoglobin structural variants = abnormal hemoglobins.

Common causes are mutations in the α– or β globin genes or other genomic regions which influence the synthesis of hemoglobin. If the genetic defects cause a disorder of Hb synthesis, thalassemia arises. In this case the structure of hemoglobin is (in most cases) normal. If they give rise to changes in the Hb-structure, abnormal hemoglobins are formed. There are numerous mixed combinations between the individual groups; e.g. β^0/ β^+-thalassemias, HbS- β thalassemias, HbSC disease or HbE- α thalassemias.

Table 4: Overview of genetic defects of hemoglobin

1. Thalassemia syndromes
• α–, β–, γ– and δ– thalassemias
• Thalassemic hemoglobin variants
2. Hemoglobin structure abnormalities
• Abnormalities of the α–, β–, γ– and δ–globin chains e.g. HbS, HbC, HbE
3. Combination and interaction forms
e.g. HbS-β thalassemias HbSC-disease HbE -α thalassemias

Thalassemia syndromes

Basic information

The term thalassemia syndromes refers to a clinically and genetically heterogeneous group of diseases with autosomal recessive inheritance in most cases, whose common feature is an altered synthesis ratio between α and β globin chains. This results in a deficit of one globin chain type and excess of the other, which characterizes the hematological disease pattern depending on the extent of the imbalance and the genetic abnormality.

Corresponding to the different globin chains, there are four basic types of thalassemias: α-, β-, γ- and δ thalassemia. These can be further classified in groups where synthesis defects are partial (e.g. β+-thalassemia) or complete (e.g. β°-thalassemia). On the other hand, all possible combinations of the thalassemias among themselves as well as with abnormal structural variants in the form of "interacting thalassemias" and non-interacting thalassemias" are present depending on frequencies within different ethnic groups. This gives rise to the clinically and genetically large and heterogeneous group of diseases of thalassemia syndromes, whose most important basic types and sub-types are listed in the following table (Tab. 5).

In particular α- and β thalassemias have clinical significance because the affected α- or β globin chains are the main components of hemoglobin HbA (α2β2). The rare γ and δ thalassemias do not cause any disease by themselves although they can be clinically relevant in the combination forms such as δβ or γδβ thalassemia.

Heterozygous thalassemia carriers are not completely healthy, but they require individual clarification as they generally present symptoms like iron-refractory or hypochromic microcytic anemia. Major homozygous forms are associated with severe hypochromic hemolytic anemias and complex diseases.

Table 5: Basic forms and sub-types of thalassemia syndromes

1 α thalassemia syndromes
- α^{o}-thalassemias
- α^{+}-thalassemias
  - Deletion forms $(-\alpha)$
  - Non-deletion forms (α^{T})

2 β thalassemia syndromes
- β^{o}-thalassemias
- β^{+}-thalassemias
  - Asymptomatic β^{++} thalassemias
- δβ-thalassemias
- Hb-Lepore-syndromes

4 Thalassemic hemoglobin variants *

- Hereditary HbF persistence
- δ thalassemias *
- γ thalassemias *

3 Combination and interaction forms

* Refer to specialized literature

Abnormal hemoglobins

Basic information

The term abnormal hemoglobins cover a large group of hemoglobin structural variants. A common designation in clinical medicine is 'Hb abnormalities'. The mode of inheritance is mostly autosomal recessive.

- Clinically significant abnormal hemoglobins are characterized by an amino acid change in the α- or β globin chain
- Molecular causes include point mutations and very rarely deletions/insertions of the α- or β globin genes
- The description is given with capital letters: HbS, HbC, HbE or according to the location of the first description: HbKöln, HbLeiden

Abnormal hemoglobins cause a disease or hematological changes only if the underlying molecular defect is accompanied by a defect in the function, solubility, stability or a reduced synthesis of the hemoglobin molecule. This explains why only a small amount of the considerable number of structural variants are medically relevant. The most common and clinically significant are the hemoglobins S, C and E.

There is also a variety of rare variants associated with clinically relevant and sometimes serious syndromes. Overviews of the Hb abnormalities among ethnic German and immigrant population found in the Hemoglobin Laboratory at Ulm University are specified in 2 tables in the Appendix of this compendium. This reference is especially important for the laboratory medicine as it gives information about the frequency of rare hemoglobin variants that can be expected in Germany.

The pathogenic abnormal hemoglobins can be sub-divided into several, clearly definable groups according to pathophysiological criteria (Tab. 6).

Table 6: Classification of the most important pathological hemoglobin variants

- **Variants with aggregation tendency and subsequent vascular occlusion and hemolysis: e.g. HbS, HbC**
- **Variants with the phenotype of a thalassemia: e.g. HbE, HbLepore**
- **Unstable hemoglobins with hemolysis: e.g. HbKöln, HbLeiden**
- **Variants with impaired oxygen transport function = familial erythrocytosis: e.g. HbOhio**
- **Variants with pathological methemoglobin = familial cyanosis: e.g. HbM Iwate**

Laboratory diagnosis

Indications for hemoglobin analysis

General aspects

Laboratory medicine makes an important and usually decisive contribution to the diagnosis of hemoglobin diseases. Tab.7 contains a list of indications for hemoglobin analysis. Some practical information is given below and the relevant hemoglobin tests are indicated for each case. Molecular genetic analysis (DNA analysis) is used if required for some given indications, see Tab. 8.

One focus of this guide is the information about laboratory parameters which are decisive for the respective diagnoses, and their value for the assessment of hemoglobin defects.

The type and extent of the laboratory diagnosis is determined depending on the question arising from the case history (congenital disorder/disease, family, ethnic descent), and also from clinical and hematological data. If the values of all hematological parameters are located within the reference range, a clinically significant hemoglobinopathy is excluded. On the other hand, if the hemoglobin analysis indicates values within the expected range of particular hemoglobinopathy, an extended and more specific laboratory diagnosis program must be considered.

Table 7: Indications for hemoglobin analysis

These arise mainly from the following findings:
• microcytic-hypochromic anemias after exclusion of iron deficiency,
• chronic hemolytic anemia,
• vaso-occlusive crises of unclear etiology in patients from regions where HbS and/or HbC are widespread,
• drug-induced anemia,
• erythrocytosis and/or cyanosis caused by hematological factors,
• hydrops fetalis syndrome of unclear etiology,
• prevention: testing of family members, diagnosis of partners for genetic counseling
• prenatal diagnosis

Carrying out hemoglobin electrophoresis indiscriminately for all cases of anemia is not reasonable and economically unjustifiable, especially in people without migration background.

Indications for DNA analysis

DNA analysis should only be performed in cases that cannot be clarified by conventional Hb analysis (see also page 17).The regulations of the Genetic Diagnostics Law must also be respected.

Table 8: Indications for DNA analysis

Thalassemia syndromes • genetic typing of β thalassemia major, • molecular diagnosis of β thalassemia intermedia, • combination forms of different hemoglobinopathies, • suspicion of genetic carriers, • diagnostic of α-thalassemias, • with respect to genetic questions: family, partner, prenatal diagnostics
Abnormal hemoglobins • identification of rare abnormalities, • for clarification in case of lack of electrophoretic or chromatographic separation, • within the scope of genetic questions (families, partner, prenatal diagnostics), • combination forms of different hemoglobinopathies

Methods of determination

The laboratory tests for hemoglobinopathies consist of basic hematological diagnostics, clinical-chemical tests, hemoglobin analyses and, when indicated, molecular genetic tests. The standard program is described in table 9. A complete flowchart for the laboratory diagnostics of the most important hemoglobin diseases can be found at the end of the chapter “Laboratory diagnosis” (see Fig. 12).

Table 9: **Standard program for the laboratory diagnostics of hemoglobinopathies**

Hematology	Red blood cell count or complete blood count with Ery-Indices (MCV, MCH) and reticulocytes
Clinical chemistry	Iron status: ferritin, transferrin saturation Hemolysis parameters: Bilirubin, Haptoglobin, LDH
Hemoglobin analysis	Capillary electrophoresis, agarose gel electrophoresis, HPLC. If necessary, HbF cell stain, solubility test in case of HbS diagnostics.
Molecular genetics	MLPA DNA sequencing

Basic hematological diagnostics

If a hemoglobinopathy is suspected, the determination of the complete red blood cell count (Tab. 10) with reticulocytes count, Hb value, number of erythrocytes and the erythrocyte indices MCH, MCV and MCHC is mandatory. The RDW value (erythrocyte distribution width) is also a good indication, which is mostly increased in cases of iron deficiency as a measure for anisocytosis, while normal RDW values are seen in cases of minor thalassemias (Tab. 24).

Table 10: Parameters of the red blood cell count

Hemoglobin	g/dl
Erythrocytes	$X10^{12}$/L
Hematocrit	Vol %
MCV	fl
MCH	pg
MCHC	g/dl
Reticulocytes	‰

Clinical chemistry

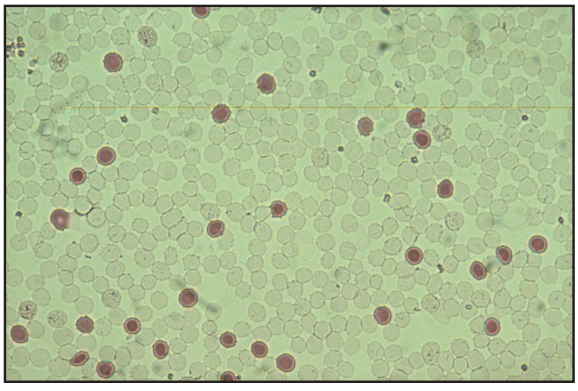
See table 9; iron status tests, ferritin value and examination of hemolysis parameters (bilirubin, haptoglobin, LDH and Coombs' test) are conducted using the usual methods.

Hemoglobin analysis

HbF determination and HbF cell detection

The classic method for exact quantification of the HbF percentage is alkali denaturation. HbF can also be determined with great accuracy by means of capillary electrophoresis and HPLC; here the measured values are usually higher than those obtained with alkali denaturation. The acid elution method (Kleihauer test) helps to prove the presence of HbF cells in blood smear preparation in situations like fetomaternal transfusion and unclear increase of HbF (Fig. 6).

Fig. 6: HbF cell staining for cytological determination



Hb electrophoresis

The basic diagnostics of the hemoglobinopathies is based on the separation and quantification of normal hemoglobin fractions and/or abnormal hemoglobins. Among different methods established for quantitative as well as qualitative analyses, the fully automatic capillary electrophoresis (CE) has proved to be the optimum technique, among the preferred methods in modern laboratory, (Fig. 7 and 8). Capillary electrophoresis is equal in its efficiency with respect to sensitivity and specificity of the HPLC. The advantages of CE are the efficient

automation, and revealing the classic thalassemia syndromes as well as the most important pathological Hb variants, in addition to supporting the interpretation of the findings.

The diagnostic examples indicated in this compendium were mainly created using the capillary electrophoresis system Capillarys by Sebia, whereas the agarose gel electrophoresis were carried out using the automatic Hydrasys system by Sebia using Hydragel Hemoglobin gels.

The increasingly widespread method of Hydragel Hb electrophoresis allows the separation of normal and abnormal hemoglobins into alkaline (pH 8.5), see Fig. 9, and acidic (pH 6.0) buffer systems. Another frequently used Hb electrophoresis is the microzone electrophoresis on cellulose acetate layers; a separation system for normal and abnormal Hb fractions is shown in Fig. 10.

Fig. 7: CE-separation and quantification of the normal hemoglobins HbA and HbA_2 in a normal adult

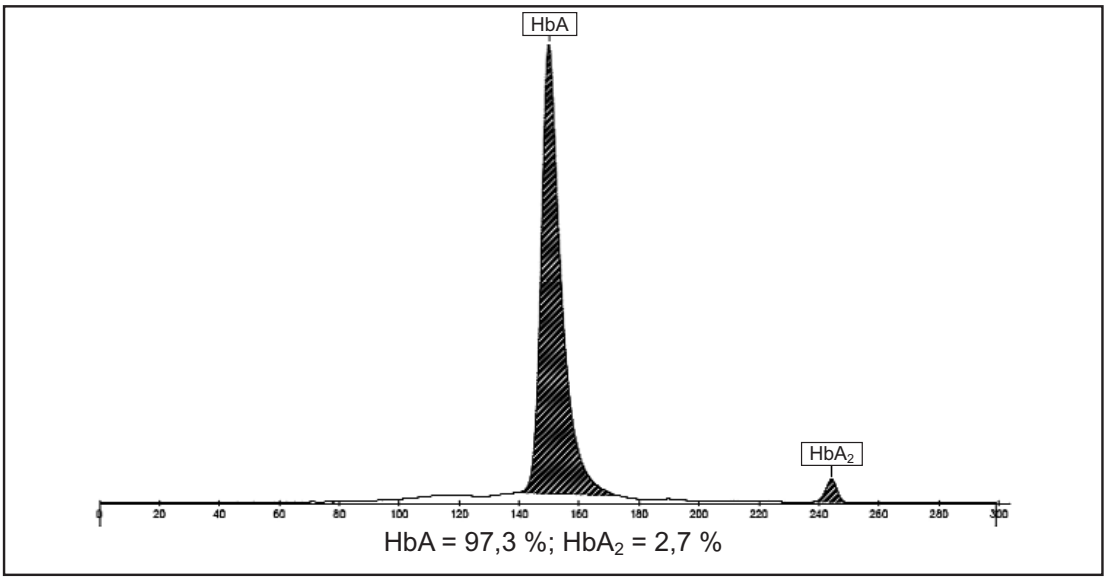


Fig. 8: CE-separation and quantification of the hemoglobins HbA, HbF and HbA_2 in case of a three-week old infant

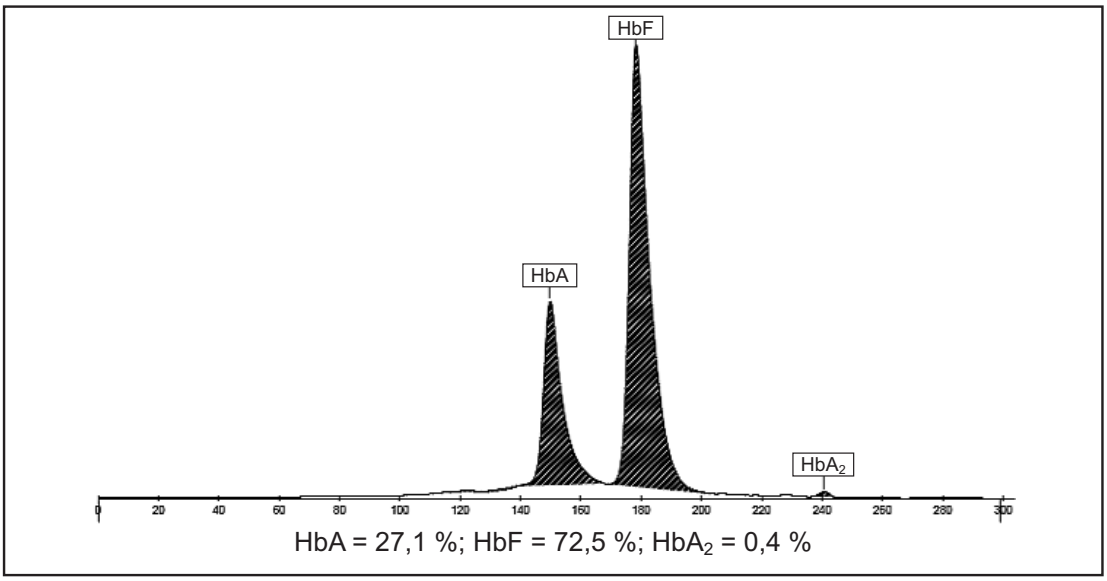


Fig. 9: **Alkaline electrophoresis on Hydragel Hb agarose gel. 1 and 4 = normal adults, 2 and 3 = children aged 2 months**

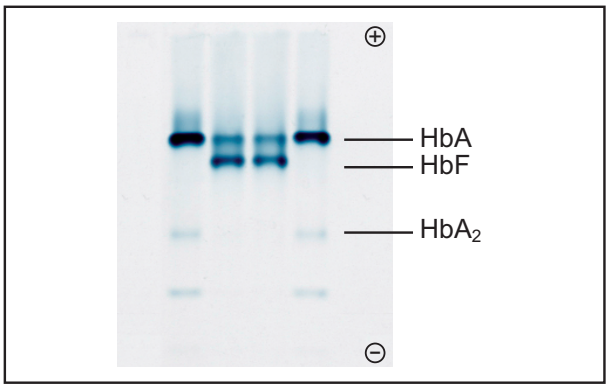
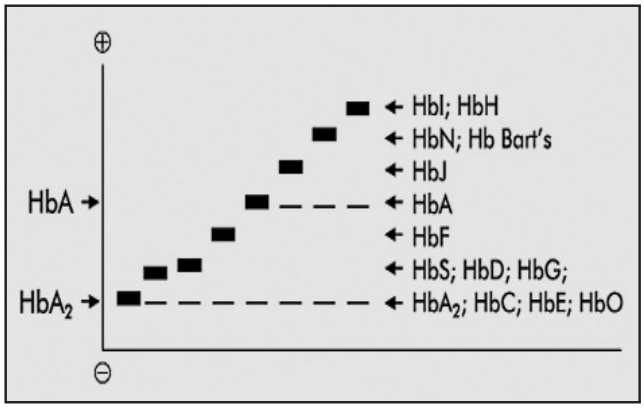


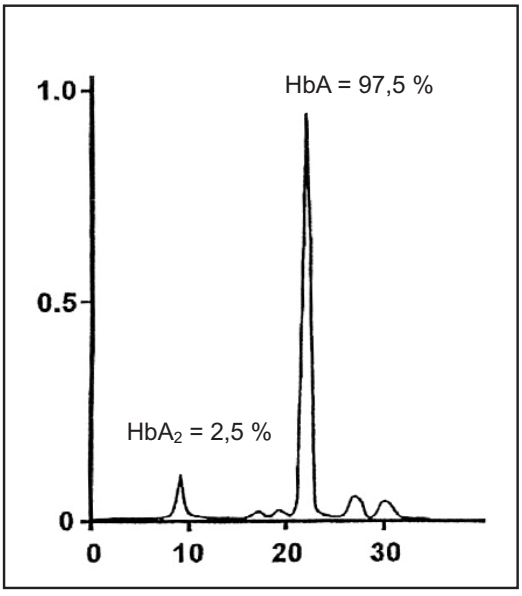
Fig. 10: **Separation scheme for normal and abnormal hemoglobins in different electrophoresis systems at pH 8.6**

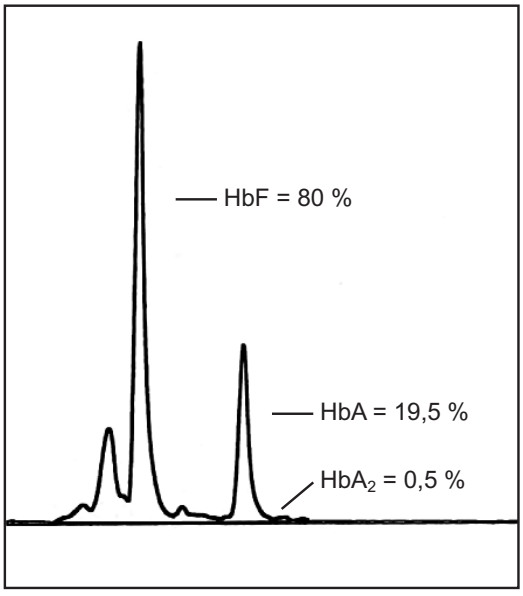


HPLC

In many laboratories, hemoglobin testing is performed on HPLC, which can be used to do qualitative as well as quantitative determinations. Examples for the separation of normal hemoglobin fractions are shown in Fig. 11.

Fig. 11: **Examples for HPLC separations for quantitative determination of HbA_2, HbA and HbF. Left panel: normal Hb fractions of an adult (anion exchanger). Right panel: Hb fractions of a new-born (cation exchanger). The measurement units are valid for both diagrams.**





Diagnostics of abnormal hemoglobin variants

The frequent variants HbS, HbC and HbE represent more than 90% of all structural hemoglobin abnormalities identified in daily laboratory practice. Generally, these can be diagnosed directly by evaluating the electrophoretic separation pattern, especially such as the one obtained by capillary electrophoresis, and if necessary by including chemical test procedures, e.g. the solubility test. The geographical or ethnic origin of the patient, the hematological data and the clinical picture provide essential information.

DNA analysis is used to identify rare pathological hemoglobin variants. Molecular genetic testing should however be restricted to those Hb abnormalities which cannot be clarified by conventional methods of Hb analysis.

HbS solubility test

The solubility test is necessary to differentiate HbS from abnormal hemoglobins with identical migration on electrophoresis, such as HbD or HbG.

Test material and reference ranges

Anticoagulated erythrocytes are required. Optimally EDTA blood (in coated sample tubes) should be used, which is also suitable for testing the blood count including erythrocyte morphology and for erythrocyte enzyme determination. EDTA blood can be stored for a few days without any significant loss in quality. For routine tests, 5 ml blood is sufficient most of the times; a greater volume is required in case of severe anemia. The reference ranges for the normal hemoglobin components = the values for HbA, HbA_2 and HbF in children and adults can be seen in Tab. 11 and 12.

Table 11: Reference ranges* for HbA and HbA_2 in infants and adults. Method: Capillary electrophoresis or HPLC

	New-borns	Children < 1 year	Children 1-2 years of age	Children > 2 years of age and adults
HbA	17,7 % (13,1 - 22,3)	Age-dependent	96,0 % (95,0 - 98,2)	97,5 % (97 - 98,5)
HbA_2	0,19 - 0,60 %	1,6 - 2,4 %	2,5 - 3,0 %	2,5 - 3,0 %

*Taken from the Book Lothar Thomas: Labor und Diagnose, TH_BOOKS, 2012

Table 12: Reference values for HbF at different ages. Method: Capillary electrophoresis or HPLC

Age (months)	HbF (%)
Birth	70 - 90
1	50 - 75
2	25 - 60
3	10 - 35
4	5 - 20
6	< 8
9	< 5
12	< 2
Adults	not detectable (< 0,4)

Molecular genetic diagnostics of hemoglobinopathies

DNA analysis plays an increasingly important role in the identification or diagnosis of hemoglobinopathies. An important area of application is the prenatal diagnostics of thalassemias or clinically severe hemoglobinopathies, e.g. the sickle cell disease. In addition, a DNA analysis is always advisable when specific findings in the clinico-hematological disease pattern give rise to a strong suspicion of abnormal hemoglobin even if all methods of protein analysis yield negative results.

In case of prenatal diagnostics, the sought mutation is often known by examination of affected siblings and their parents, so that a targeted analysis can be conducted in such cases. The molecular genetic examination is conducted using a fetal DNA sample usually taken in the 10th to 12th week of pregnancy by chorionic villus sampling.

See Tab. 13 for the molecular genetic methods and their principles.

Table 13: List of the most important molecular genetic methods

DNA preparation	Basic material
Polymerase chain amplification	Technique for quick amplification (multiplication) of specific DNA sequences of varied size
DNA sequencing	Analysis of the nucleotide sequence of DNA molecules or fragments by means of enzymatic or chemical methods. It is done using automatic sequencing machines
MLPA Deletion diagnostics	Multiplex Ligation-dependent Probe Amplification, molecular genetic method used to search for large genomic deletions or duplications individual locus regions or entire genes.

Evaluation of the laboratory findings

Generally, significant criteria include the age of the patient, the ethnic origin, the family history and the clinico-hematological findings.

The evaluation of hemoglobin analysis consists of:

• the assessment of quantitative changes in the normal hemoglobin components, like the typical increase of HbA_2 and/or HbF in thalassemias.

• the exclusion or confirmation of an abnormal variant, as well as its identification and quantification in a positive case. In any case, it must be clarified if a detected abnormal hemoglobin is responsible for the disease symptoms or represents an incidental finding without pathogenic significance.

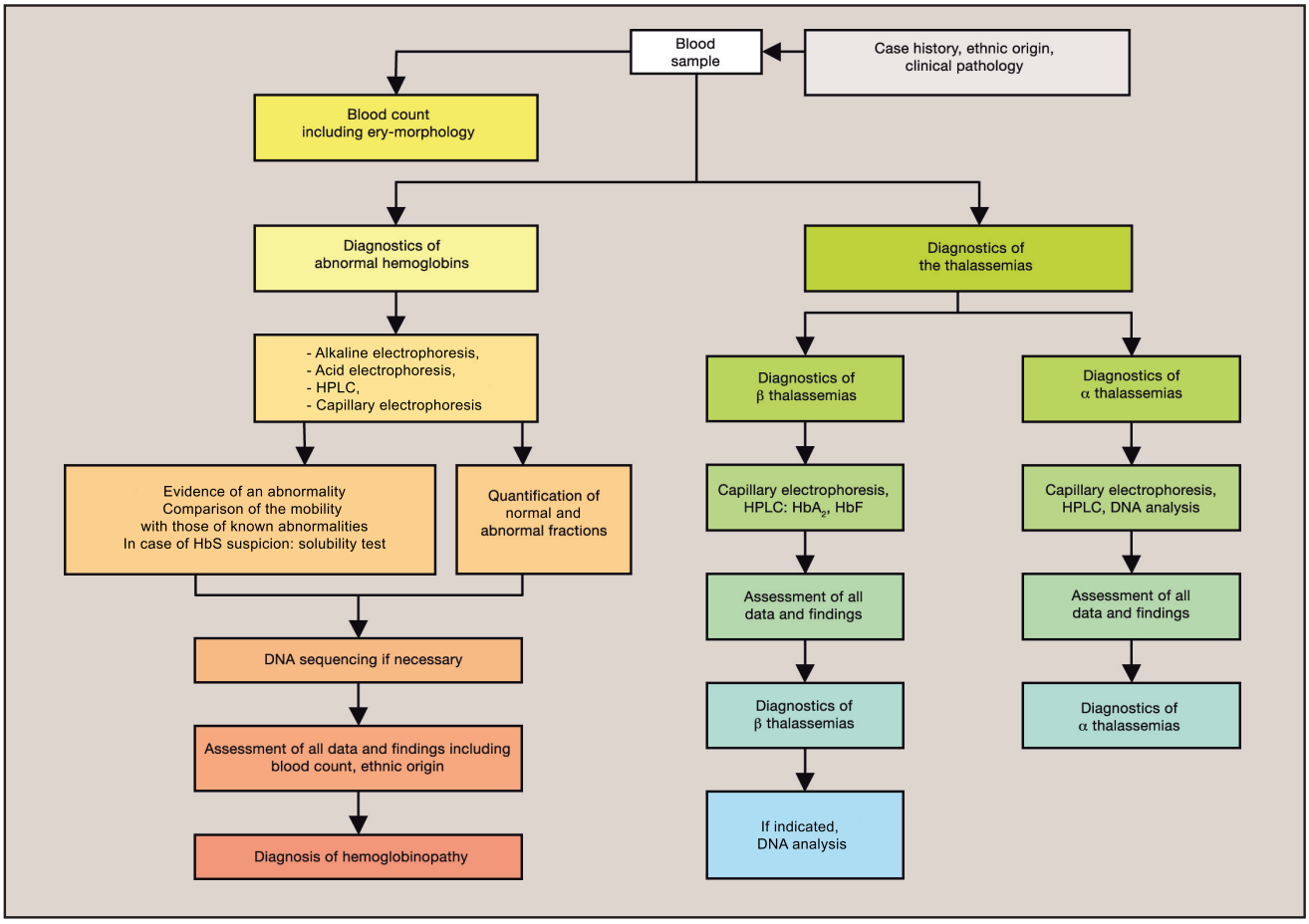
Quality assurance, error prevention

- The guidelines for quality control must be strictly respected (round robin tests, accreditation, certification)
- Hemoglobinopathies are genetic diseases with significant consequences for the patient
  - the diagnosis must be conclusive, indicating details of the clinical manifestation
  - accurate written reporting and archiving of data is essential
- In case of doubt a checkup must be made, if necessary with the help of a specialized laboratory.

Gene Diagnostics Law

- The regulations of the Gene Diagnostics Law must be respected.

Fig. 12: Flowchart for the laboratory diagnostics of hemoglobinopathies



Pathophysiology. Clinical presentations. Laboratory findings. Treatment

Thalassemia syndromes

α-thalassemias

Basic information and pathophysiology:

α thalassemias are diseases caused by an insufficience or lack of synthesis of the α chains of normal hemoglobin. At the molecular level, they result from partial (α^+) or total (α°) deletions (Fig. 13) or rare mutations of one or more of the four α globin genes ($\alpha\alpha/\alpha\alpha$). They originally occurred mainly in Africa, Arab nations, and more frequently in South-East Asia, but have now spread all over the world.

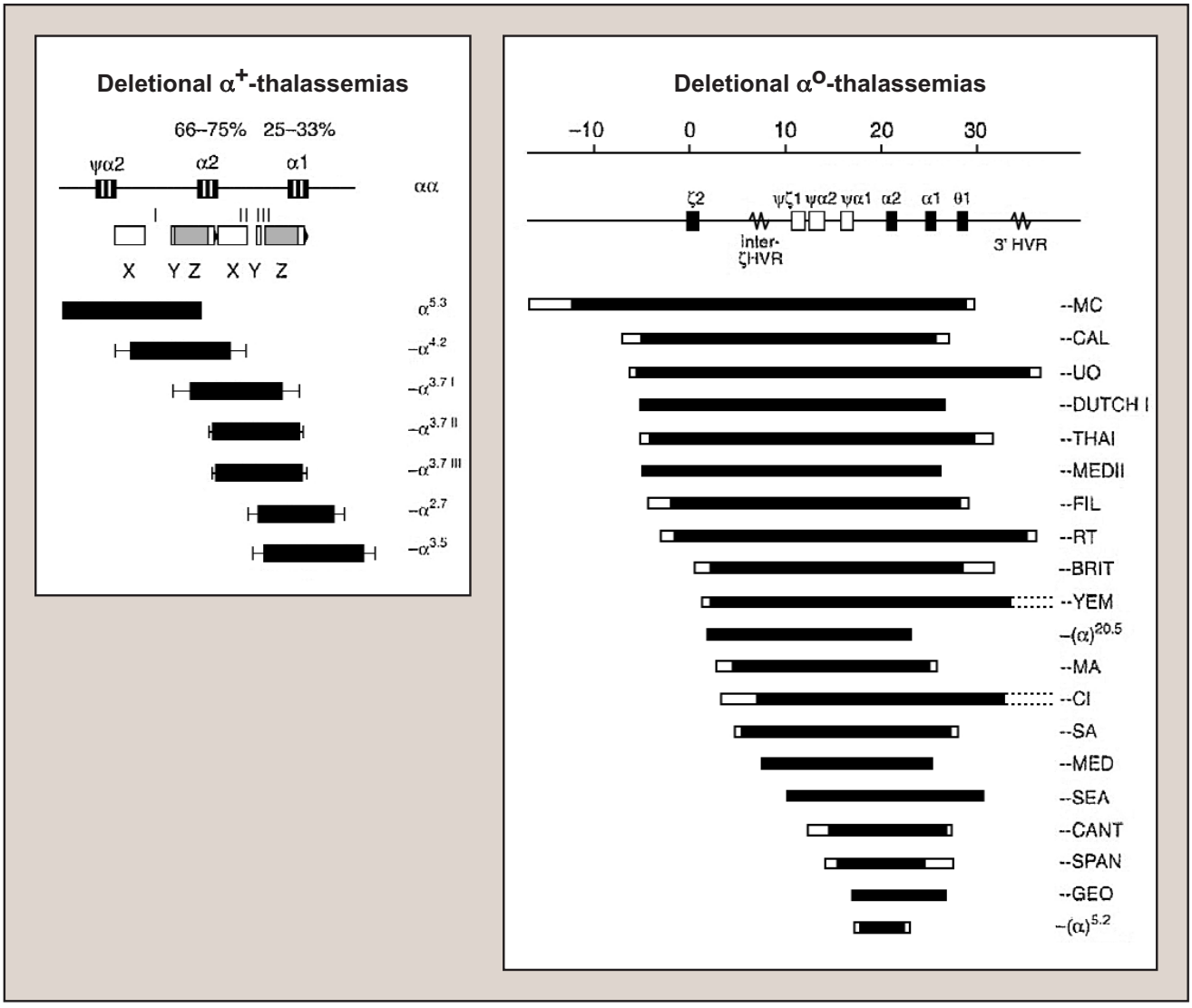
Consequences of the pathophysiology:

• Adverse effects on hemoglobin production	Anemia, microcytosis, hypochromias
• α : β chain imbalance	Production of surplus hemoglobins HbH and HbBart's Hemolytic anemia

Molecular pathology

About 95% of the molecular defects of α–thalassemias that are known so far are caused by deletions. These can include sections of varying size of the α-globin gene cluster (Fig. 13). For a detailed characterization, the size of the deletion is specified in kilobases, e.g. $-\alpha^{3.7}/\alpha\alpha$ corresponds to a 3.7 kb α thalassemia deletion. Depending on the effect of the deletion, α^{+} or α^{o} thalassemias develop. In the case of some frequently occurring α^{o}-thalassemias, the geographical region is also specified, e.g. $--^{SEA}$ = South-East Asia- α-thalassemia.
About 5% of the α-thalassemias are non-deletional forms which are caused by point mutations in the α-globin gene cluster. For these forms, the notation T is used (formula = $\alpha^{T}\alpha/\alpha\alpha$).

Fig. 13: Different α-thalassemia deletions



Clinic and cardinal symptoms

Depending on the number of genes affected by the loss of function, there are four α-thalassemic disease patterns; all of them manifest perinatally (see Tab. 14-18):

- **The clinically inapparent α thalassemia minima** = heterozygous a^+-thalassemia = (-α/αα) identifiable on the basis of mild hypochromia revealed in a blood count with a barely measurable reduction in Hb values.
- **α-thalassemia minor** = heterozygous a^o-thalassemia (--/αα) or homozygous a^+-thalassemia (-α/-α) with mild anemia, hypochromia and microcytosis.
- **HbH disease** = compound heterozygous α^+/α^o-thalassemia with three inactive α-genes (--/-α), moderately severe hypochromic hemolytic anemia with splenomegaly. Anemic crises are caused by viral infections and oxidants (drugs). Complications include cardiac problems, gallstones, lower leg ulcers and folic acid deficiency.
- **Hb Bart's hydrops fetalis syndrome** = homozygous α^o-thalassemia is a very serious hemolytic anemia already present in utero and marked by a lack of any α globin chain synthesis (--/--) with hydrops and ascites. This is fatal if not treated.

An overview including the genotype-phenotype correlation can be seen in table 14.

Table 14: Diagnosis, genotypes, hematological data and cardinal symptoms of α-thalassemias

Genetic status/diagnosis	Structure of the α-globin genes	Red blood cell count	Qualitative hemoglobin pattern	Cardinal symptoms
Normal finding	■■ / ■■ αα / αα	Hb normal MCH normal	normal	no symptoms
Heterozygous α^+-thalassemia = α-thalassemia minima	■■ / ■■ –α / αα	Hb normal MCH < 27 pg	normal	no symptoms, minor changes in the blood count
Homozygous α^+-thalassemia = α-thalassemia minor	■■ / ■■ –α / –α	Hb low MCH < 26 pg	normal	mild anemia, noticeable changes in the blood count
Heterozygous α^o-thalassemia = α-thalassemia minor	■■ / ■■ – – / α α	Hb low MCH < 24 pg	normal	mild anemia, noticeable changes in the blood count
Compound heterozygosity α^+/α^o-thalassemia = HbH disease	■■ / ■■ – – / –α	Hb 8-10 g/dl MCH < 22 pg	HbH ≈ 10 - 20 %	variable, chronic hemolytic anemia
Homozygous α^o-thalassemia = HbBart's Hydrops fetalis syndrome	■■ / ■■ – – / – –	Hb < 6 g/dl MCH < 20 pg	HbBart's 80 - 90 % HbPortland ≈ 10 - 20 % HbH < 1 %	life-threatening fetal anemia, generalized hydrops

■ active α-genes ■ deleted α-genes

Laboratory findings in α-thalassemias

The specific laboratory tests comprise the hematological and clinico-chemical basic diagnostics, special hemoglobin analyses and molecular genetic tests.
The respective diagnostic criteria of the different α-thalassemia types are compiled in tables 15 to 18.

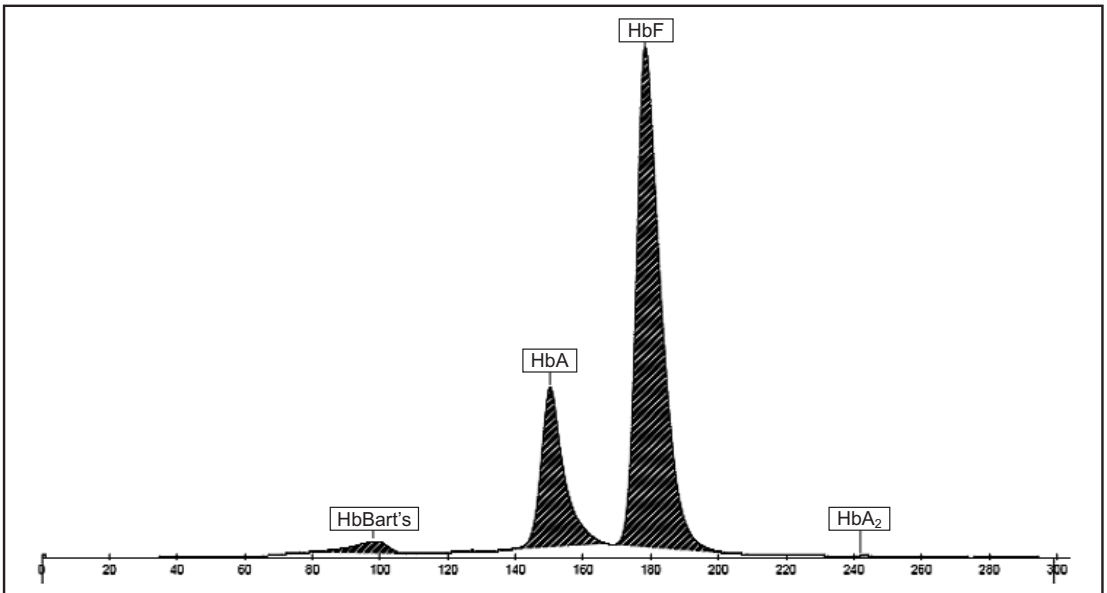
Table 15: Laboratory findings in the heterozygous α^+-thalassemia -α/αα (α-thalassemia minima)

Hematology	Hb	normal
	MCV	≈ 76 - 77 fl
	MCH	≈ 25 - 27 pg
Clinical chemistry	All values within the normal range	
Hemoglobin analysis		
Normal hemoglobin pattern:		
HbA_2	≈ 2,5 %	
HbA	≈ 97,5 %	
Molecular genetics	Presence of a α^+-deletion e.g $-\alpha^{3.7}/\alpha\alpha$ or $-\alpha^{5.2}/\alpha\alpha$	

Table 16: Laboratory findings in homozygous α^+-thalassemia or heterozygous α^o-thalassemia (α-thalassemia minor)

Hematology	Hb	slightly decreased
	MCV	< 74 - 76 fl
	MCH	< 24 - 26 pg
Clinical chemistry	Normal findings	
Hemoglobin analysis		
Normal hemoglobin pattern:		
HbA_2	≈ 2,5 %	
HbA	≈ 97,5 %	
Molecular genetics	Presence of a small double α-deletion e.g $-\alpha^{3.7}/-\alpha^{3.7}$ or a bigger α-deletion e.g $--^{SEA}/\alpha\alpha$	

Fig. 14: **Hemoglobin pattern (CE) of a newborn with homozygous α^+-thalassemia**



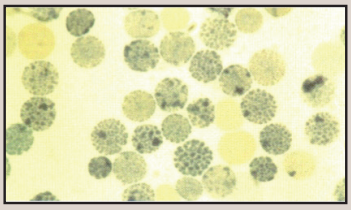
HbBart's = 2,3 %; HbA = 22,0 %; HbF = 75,6 %; HbA_2 = 0,1 %

Table 17: **Laboratory findings in HbH disease = compound heterozygous α^+/α^o-Thalassemia**

Hematology

Hb	8 – 9 g/dl
MCV	< 72 fl
MCH	< 22 pg
HbH- cells	abundant
Extreme Poikilocytosis	
Many target cells	
Many reticulocytes	

Typical HbH cells in case of HbH disease



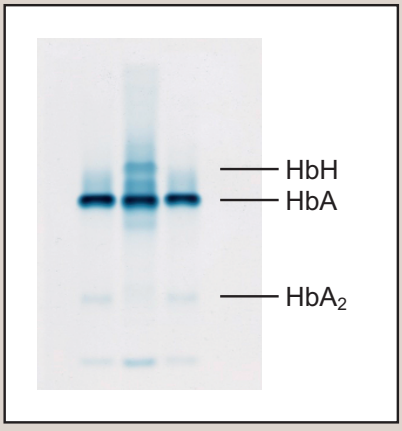
Clinical chemistry

Hemolysis parameters	positive

Hemoglobin analysis

HbH	10 – 20 %
HbBart's	negative or variable
HbA_2	decreased

Separation of HbH in Hydragel Hb electrophoresis



Molecular genetics

Presence of a compound α^o/α^+-thalassemia
e.g $--^{SEA}/-\alpha^{3.7}$

Table 18: Laboratory findings in case of new-borns with HbBart's Hydrops fetalis syndrome = homozygous α^o-thalassemia

Hematology

Hb	< 6 g/dl
MCV	< 65 fl
MCH	< 20 pg
Extreme poikilocytosis	
Extreme erythroblastosis	

Clinical chemistry

Pathological functional tests in terms of multi-organ failure

Hemolysis parameters	positive

Hemoglobin analysis

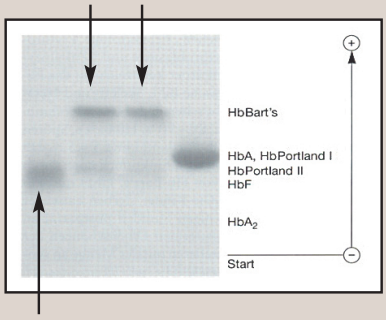
HbBart's	80 - 90 %
HbPortland	≈ 10 - 20 %
HbH	≈ 1 %

Molecular genetics

Proof of the complete deletion of all α-genes

e.g. $--SEA/--SEA$

Hemoglobin-electrophoresis of two prematures infants with HbBart's syndrome



normal premature infant 28th week of pregnancy

α-thalassemia and mental retardation

The heterogeneous group of the combination of mental retardation and α-thalassemia includes, among others, the ATRX syndrome, which is particularly noticeable due to a symptomatology with mental retardation and striking facial dysmorphism in addition to other changes. For details, refer to the specialized literature.

Non-deletional forms of α-thalassemia

In addition to the deletions, there are so-called non-deletional forms in which the globin genes are structurally intact but functionally inactive. Causes for the changed expression are mostly single base substitutions or frameshifts caused by insertions or deletions of nucleotide bases. The symbol T is used for the mutations not based on a deletion. The notation is $\alpha^T\alpha/$ or $\alpha\alpha^T$.

The α-thalassemias due to structural defects also include, in the broadest sense, the α-thalassemic structural variants, which phenotypically create the pattern of an α-thalassemia. This group includes the Hb-Constant Spring syndromes for instance. For details, refer to the specialized literature.

Treatment for α-thalassemias

The minima and minor forms of α-thalassemias do not require treatment. Iron supplements (except in cases of iron deficiency) are contraindicated.

Treatment for the HbH disease depends on the severity of the disease pattern which can vary widely. Transfusions are rarely indicated. Anemia requires a regular substitution with folic acid (e.g. 5 mg/week). Iron supplements (unless there is simultaneous iron deficiency) are contraindicated.

For HbBart's syndrome transfusions are required in utero and continuously after birth. Where possible, stem-cell transplantation is performed.

β-thalassemias

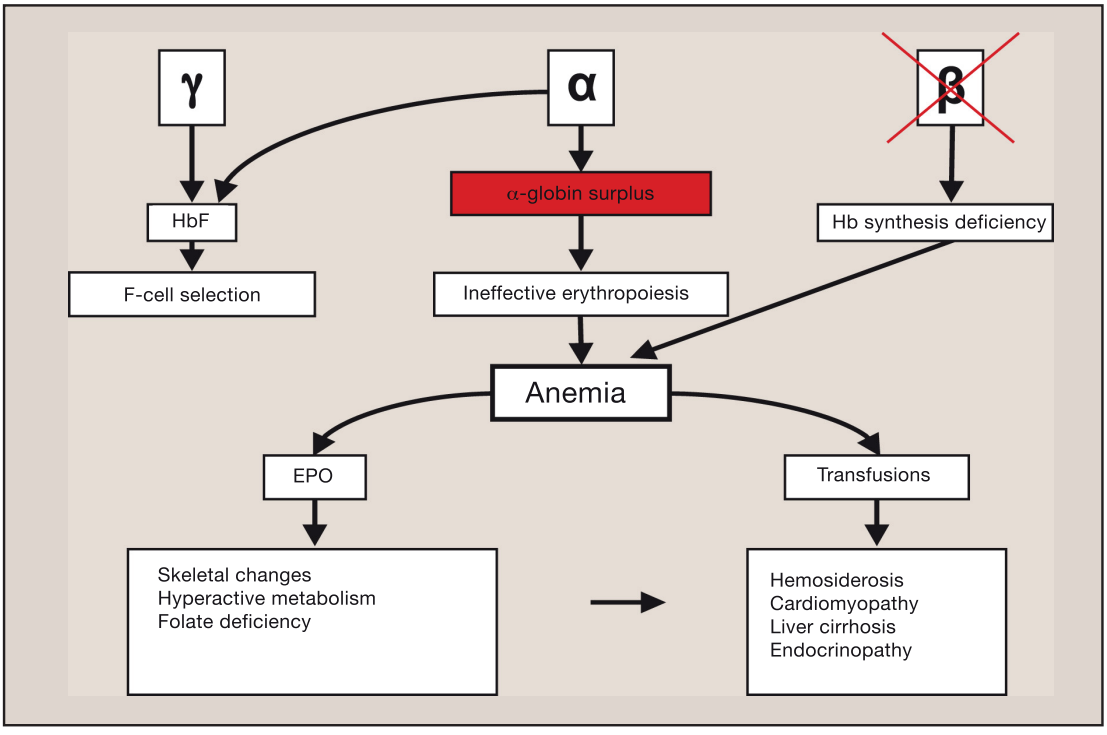
Basic information and pathophysiology:

β-thalassemias are the result of insufficient (β^+) or absent (β^o) synthesis of β–chains of the normal hemoglobin. Molecular cause is β-globin gene mutations. Most patients come from Mediterranean countries, South-East Europe, Arab nations and Asia. The hematological changes become manifest not earlier than the age of three to six months.

Consequences of pathophysiology (Fig. 15):

- α : β-chain imbalance → hemolytic anemia
  → ineffective erythropoiesis
- Interference in the hemoglobin production → hypochromic microcytic anemia

Fig. 15: Pathophysiological characteristics of β-thalassemia major



Molecular pathology

- Most of the β-thalassemias are caused by point mutations in the β-globin gene
- Deletions are rare
- The gene expression can be disturbed at all levels from the gene to the β-chain (Tab. 19)
  - different mutations
  - extremely variable symptoms

β^{++} = mild anemias
β^{+} = slight anemias
β^{o} = severe anemia

Table 19: Examples of different β-thalassemia mutations*

Level of gene expression	Example of mutation	Phenotype	Geographic Region
Transcription			
Promoter mutations	-28 A → C	β^{++}	Turkey, Africa
Capping	ACA → CCA	β^{+}	Asia
Polyadenylation	AATAAA → AACAAA	β^{+}	African American
Splicing			
Mutation of normal	IVS1 Pos. 1 G → A	β^{o}	Mediterranean
splicing signals	IVS1 Pos. 6 T → C	β^{++}	Mediterranean
Activation of hidden splicing signals	IVS1 Pos. 110 G → A	β^{+}	Mediterranean
Translation			
Nonsense Mutations	Codon 39 CAG → TAG	β^{o}	Mediterranean
DNA deletions	619 bp	β^{o}	India

* For more details, refer to Hentze, Kulozik, Bartram:
Introduction to Medical Molecular Biology, Springer publishing house 1990.

Clinic and cardinal symptoms

- **β-thalassemia minor** = heterozygous β-thalassemia is the clinically mildest, often asymptomatic or slightly symptomatic form. The hematological changes are identified by a hypochromic-microcytic mild to moderate anemia.
- **β-thalassemia major** = homozygous or compound heterozygous β-thalassemia is a serious, long-term, transfusion-dependent anemia with hemolysis, microcytosis and hypochromia, hyperferremia and a complex clinical manifestation.
- **β-thalassemia intermedia** = mild homozygous or compound heterozygous β-thalassemia is a moderately severe, extremely variable hypochromic-microcytic, hemolytic anemia; initially without need of transfusion (but over the course transfusion is often necessary), hyperferremia and complex clinical manifestation.

An overview including the genotype-phenotype correlation can be found in table 20.

Table 20: Diagnosis, genotypes, hematological findings and cardinal symptoms of β–thalassemias

Genetic status/diagnosis		Red blood cell count	Hemoglobin pattern	Cardinal symptoms
Heterozygous β-thalassemia	β^{++}	Hb ♂ 9 - 15 g/dl	HbA_2 > 3,2 %	mild anemia
= β-thalassemia minor	β^{+}	Hb ♀ 9 - 13 g/dl	HbF 0,5 - 6 %	without any disease
	β^{o}	MCV 55 - 75 fl MCH 19 - 25 pg		
Homozygous β–thalassemia	β^{+}/β^{+}	Hb < 7 g/dl	HbA_2 variable	severe disease
= β-thalassemia major	β^{o}/β^{o}	MCV 50 - 60 fl	HbF 70 - 90 %	with long-term
Compound heterozygous β-thalassemia	β^{+}/β^{o}	MCH 14 - 20 pg		transfusion-dependent anemia
Mild homozygous	β^{+}/β^{+}	Hb 6-10 g/dl	HbA_2 variable	moderately severe
or	β^{+}/β^{++}	MCV 55 - 70 fl	HbF up to 100 %	disease, variable
compound heterozygote β-Thal.	β^{+}/β^{o}	MCH 15 - 23 pg		transfusion requirement
= β-thalassemia intermedia	β^{o}/β^{o} (In combination with additional factors)			

Laboratory findings in β-thalassemias

The specific laboratory tests consist of hematological and clinico-chemical basic diagnostics, hemoglobin analysis and, depending on the problem, molecular genetic tests as well.

The respective diagnostic parameters are described in tables 21 to 30.

β-thalassemia minor

The red blood cell count with the erythrocyte indices MCV and MCH forms the starting point for the assessment. The significant laboratory parameters of β-thalassemia minor from the 4th-5th month of life include increased HbA_2 and/or increased HbF values (refer to Fig. 16 for an example). If the MCH value is < 27 pg and the HbA_2 value is above 3.5%, the diagnosis of heterozygous β-thalassemia = thalassemia minor is made. The majority of the β-thalassemia genetic carriers have MCH reductions to 19 - 23 pg and HbA_2 values of 4.0 - 6.0%, increased HbA_2 of 6.5 - 8.0% can be seen. In about 30% of patients, an increased HbF of 1 - 3% and rarely of > 3.0 - 15% is found at the same time. The age-dependent higher HbF values in case of young children with β-thalassemia minor must be considered. The iron status (ferritin, iron saturation) is generally normal. Exceptions, i.e. an iron deficiency in case of β-thalassemia minor, can occur in children and during pregnancy. A simultaneous iron deficiency can temporarily make the HbA_2 value appear erroneously too low. Thus, in cases of doubt it is necessary to check iron deficiency compensation requirements.

Table 21: Laboratory findings in β-thalassemia minor

Clinical symptoms	no relevant disease symptoms
Hematology	
Hb concentration	9 - 15 g/dl for men 9 - 13 g/dl for women
Erythrocyte count	significantly increased in relation to the Hb value
MCH	19 - 25 pg
MCV	55 - 75 fl
MCHC	Normal to slightly reduced
Erythrocyte morphology	Microcytosis, anisocytosis, hypochromia, target cells
Clinical chemistry	
Ferritin	normal to increased
Iron saturation	increased
Hemolysis parameters	mostly normal
Hemoglobin analysis	
HbA_2	3,5 - 7,5 (- 10) %
HbF	in case of > 50 % of the patients, increased to 2 - 5 % (or higher)
Molecular genetics	
not applicable	
Attention: No reliable diagnostic features before the 4th-5th month of life	

Fig. 16: **Case study for β-thalassemia minor**

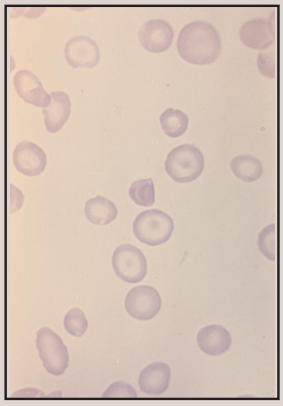
Patient: **GO. 5 yo**
Origin: **Turkish**
Case history: **“very pale”**

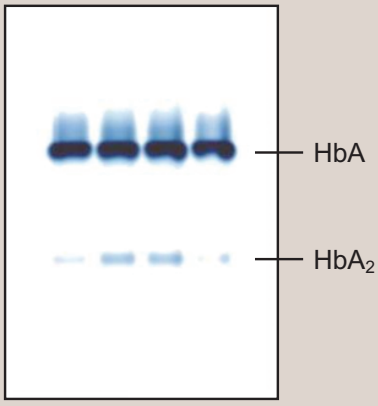
Red blood cell count:

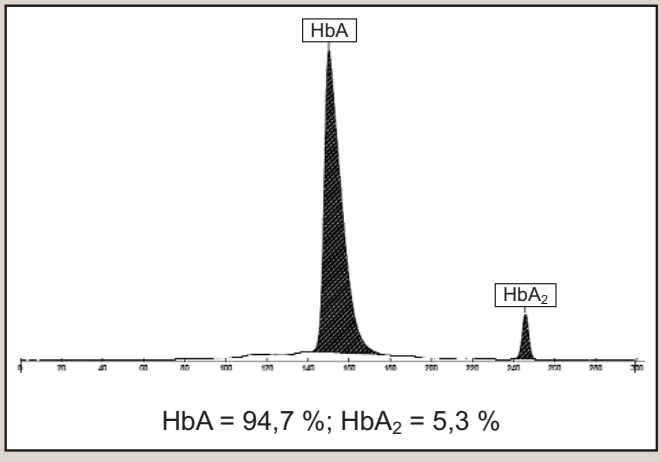
Hb	10.5 g/dl	[11.1 - 14.3]
Ery	5 x 10^{12}/L	[3.9 - 4.6]
MCV	56 fl	[77 - 89]
MCH	21 pg	[27 - 31]
Target cells		

Hb analysis:

Ferritin	59 µg/L	[up to 20]
HbA_2	= 5,3 %	







β-thalassemia minor with normal HbA_2 (asymptomatic genetic carriers)

This type of case concerns a relatively rare special form of heterozygous β-thalassemia with minimal β synthesis disorder (β^{++}-thalassemia), in which the hematological findings are characterized by minimal changes in the blood count (MCV, MCH). The HbA_2 and HbF values are within the normal range. The differential diagnosis includes the heterozygous α-thalassemias, but also iron deficiency and eventually sideroachrestic anemias.

Table 22: Parameters of the asymptomatic β-thalassemia minor with normal HbA_2 = "silent carrier"

Genetics	Heterozygous β^{++}-thalassemia
Hematology	Slight reduction in MCH and MCV Clearly distinguishable from the normal blood count
Hb analysis	HbA_2 normal, HbF negative
DNA analysis	Proof of a β^{++}-mutation

Special notes regarding the α and β minor thalassemias

With a percentage of more than 70%, minor thalassemias are by far the largest hemoglobinopathy (or thalassemia group) in Central Europe. They are the most common differential diagnosis in case of hypochromic microcytic anemia among immigrants. Minor thalassemias carry the risk of serious illness in the offsprings of two affected partners.

The physician has the important task to detect the disease and provide genetic counseling, and carrying out prenatal diagnosis if necessary.

The differential diagnostic criteria of the entire group of mild microcytic hypochromic anemias are summarized in a few tabular overviews below.

Table 23: Differential diagnostics of mild microcytic hypochromic anemia

	Heterozygous α-thalassemias	**Heterozygous β-thalassemias**	**Iron deficiency anemias**
Blood count			
Hb g/dl	12,0 - 13,7	10,1 - 14,0	8,6 - 11,8
Ery-count $x10^{12}$/L	5,1 - 5,9	4,4 - 6,4	4,24 - 5,10
MCV fl	63,2 - 75,5	55,2 - 66,2	60,4 - 72,6
MCH pg	20,2 - 26,5	18,2 - 23,1	18,9 - 24,7
Hb-analysis			
HbA_2 %	normal or reduced	> 3,2 %	slight decrease but within the normal range
HbF %	negative	0,2 - 5 (- 8) %	< 1 %
HbBart's	often positive	negative	negative
DNA analysis	α-gene deletion, rarely mutation	not applicable	not applicable

The microcytic anemias in case of chronic inflammatory diseases are not considered.

Table 24: Classification of microcytic hypochromic anemias on the basis of MCV and RDW

Microcytic isocytic		Microcytic anisocytic		Normocytic isocytic	
MCV	RDW	MCV	RDW	MCV	RDW
Reduced	Normal	Reduced	High	Normal	Normal
β-thalassaemia minor		**Iron deficiency anemia**		**Anemia in chronic diseases**	

Table 25: Iron metabolism serum parameters of hypochromic microcytic anemias

	Serum iron	Serum ferritin	Storage iron
Iron deficiency	reduced	reduced	reduced
Secondary Anemia in case of tumor or inflammation	reduced	normal to high	high
Thalassemias	normal to high	high	high
Sideroblastic (sideroachrestic) anemias	normal to high	high	high
Lead poisoning	normal	normal	normal

Table 26: HbA_2 level in case of iron deficiency

- in rare cases of β–thalassemia with simultaneous iron deficiency, the HbA_2 level can be temporarily normal
- mostly in small children and during pregnancy
- in such a case: a rather strong anemia with high erythrocytes level
- recommendation: Checking the HbA_2 level after therapeutic compensation of the iron deficiency

Table 27: Differential diagnosis of increased HbA_2 values

Genetically caused hemoglobin defects
- Heterozygous β-thalassemia
- HbSS-β^o-thalassemia
- Unstable hemoglobins

Hereditary diseases
- Hereditary spherocytosis

Acquired diseases
- Hyperthyroidism
- Megaloblastic anemias
- Wilson's disease

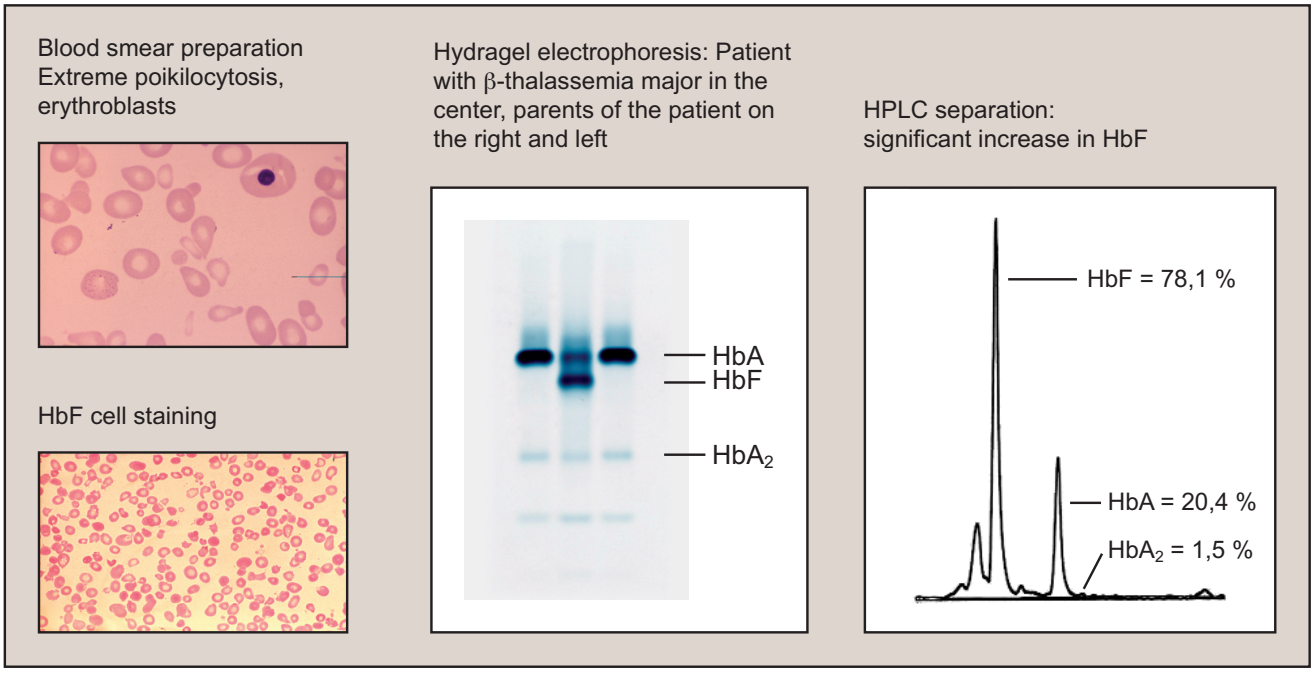
β-thalassemia major

This disease manifests itself at the age of about three to five months. The anemia is variable during diagnosis; the Hb value is mostly less than 8 g/dl. The anemia is always hypochromic with a MCH value < 22 pg. The MCV lies between 50 and 60 fl. Thalassemic erythrocyte morphology can be seen in the blood smear with a significant poikilocytosis. The hemoglobin analysis shows variable proportions of HbA, HbF and HbA_2 (for more details, see Fig. 17 and 18). It can generally be assumed that in case of an increase of HbF between 20 - 98% along with the typical clinico-hematological pathology, a thalassemia major (or thalassemia intermedia) is present. The transfusion status of the patient must be included in the assessment. Generally, a DNA analysis is conducted to confirm the β-thalassemia mutations. A family checkup (see Tab. 28) and genetic counseling are part of the diagnostic program. The life expectancy of well treated patients can be 50-60 years.

Table 28: Clinical symptoms and laboratory parameters in case of β-thalassemia major

Clinical symptoms	• Start from the 2nd half year of life; frequent infections • Severe disease, pallor, jaundice • Enlarged liver and spleen • Impaired growth, skeletal changes
Hematology	Hb < 7 g/dl MCH < 20 (< 15) pg, MCV 50 - 60 fl Reti 30 - 40 ‰ Normoblasts; extreme poikilocytosis
Clinical chemistry	Ferritin ↑ Hemolysis parameters positive
Hemoglobin analysis	HbA_2 normal to increased HbF 20 - > 80 %
Molecular genetics	Presence of β-thalassemic mutations Homozygous β^+ or β^o or compound heterozygous
Parents:	Presence of the thalassemia carrier status: - mild hypochromic anemia - HbA_2 > 3.5%, HbF variable - if necessary, proof of the thalassemic mutations - genetic consultation

Fig. 17: Description of the most important laboratory findings in case of β-thalassemia major



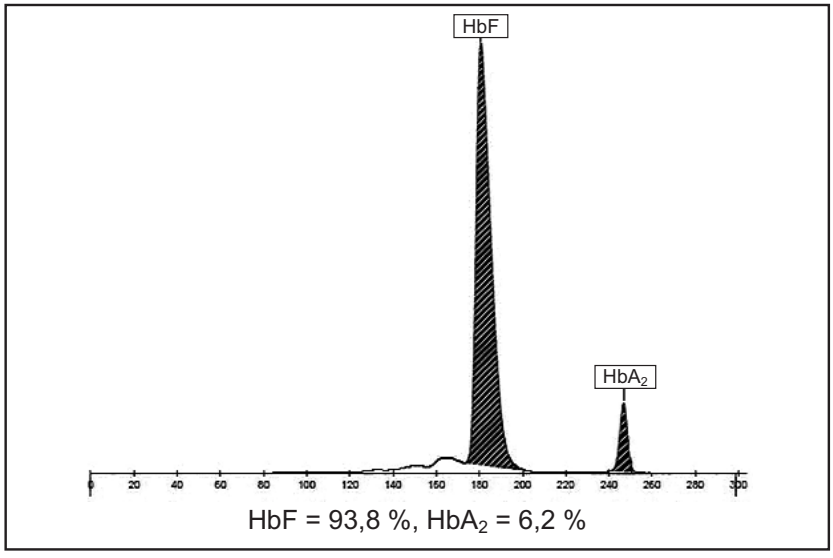
Notes regarding the diagnostic problems in cases of patients with major thalassemia under long-term transfusion therapy: The question mainly relates to young patients and young adults, who come to Germany as a part of family reunification.

- The patients are to a great extent clinically inconspicuous
- The blood count cannot be used diagnostically
- The hemoglobin analysis results in almost normal values

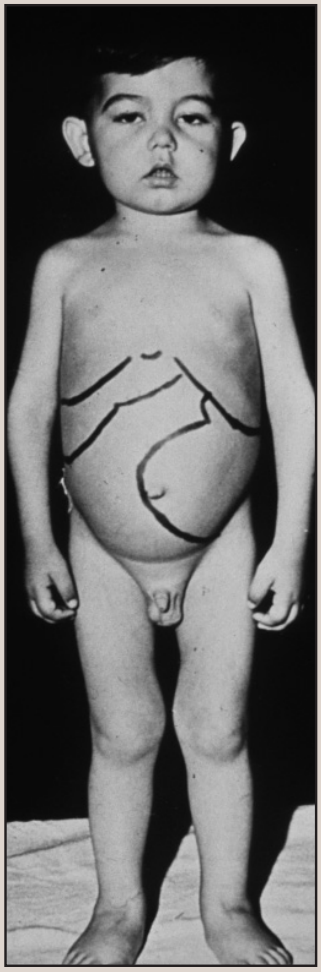
Result

The diagnosis is based on the case history and can be traced only with molecular genetics.

Fig. 18: Hemoglobin pattern in case of β-thalassemia major (capillary electrophoresis)



Laboratory diagnostics and findings in a Turkish patient with β-thalassemia major



Clinical symptoms

- Start from the 2nd half year of life: serious disease, pallor, jaundice, enlarged liver and spleen, growth impairment, skeletal changes

Hematology

Hb	6 g/dl
MCH	20 pg
MCV	50 fl
Reti	40 ‰

Normoblasts; Poikilocytosis

Clinical chemistry

Increased ferritin
Hemolysis parameters positive

Hemoglobin analysis

HbF	= 78,1 %
HbA_2	= 1,5 %

Molecular genetics

Presence of the mutation
IVS-I-1 G → A

= β^o/β^o-thalassemia homozygous

β-thalassemia intermedia:

The term basically refers to a clinical diagnosis in patients with a hemoglobin pattern as seen in thalassemia major, who stand out due to a reduced or even no need for transfusion. The diagnostic differentiation from thalassemia major takes some time due to regular clinico-hematological controls. DNA analysis is conducted if necessary. In the process, either a high residual activity of the β-globin genes is proven or a classic genetic thalassemia major is found, in other cases, a genetic thalassemia minor but with additional influencing factors. In homozygous β-thalassemia, this is mainly a hereditary HbF persistence or an α-thalassemia, and in the heterozygous β-thalassemia, triplicated α-genes (see Table 30).

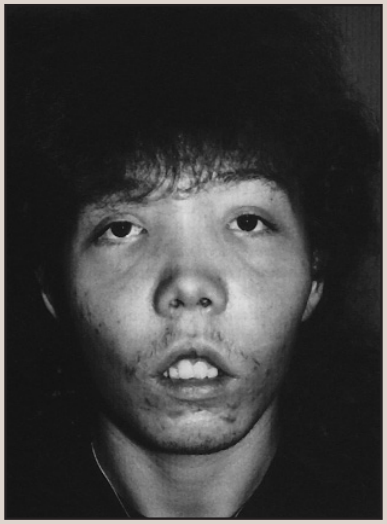
Table 29: Clinical symptoms and laboratory parameters in case of β-thalassemia intermedia

Clinical symptoms	• Moderately severe thalassemic disease • There is often a subsequent deterioration in the course of the disease • Initially and for a long time no transfusion requirement in case of Hb ≈ 8 g/dl • Subsequently, a pattern similar to major thalassemia
Hematology	Hb 6 - 10 g/dl MCH reduced MCV reduced
Clinical chemistry	Ferritin normal to high Iron saturation high Hemolysis parameter slightly positive
Hemoglobin analysis	HbA_2 normal to increased HbF 10 - 80 %
Molecular genetics	Proof of the following abnormalities: Homozygous β^+/β^+-thalassemia or compound heterozygous β^+/β^+-thalassemia or β^o/β^o-thal. or β^+/β^o-thal., often with additional influencing factors
Parents:	Proof of the thalassemia carrier status
Complications:	Skeletal deformations. Huge spleen; hypersplenism. Tumorous masses ⟶ Compression syndromes. Cardiac problems.

Table 30: Overview of the most important etiologies of thalassemia intermedia

- Homozygous β-thalassemia with high residual activity of one or both of the β-globin genes
- Homozygous β-thalassemia with high HbF production (HPFH mutations)
- Homozygous β-thalassemia with coexisting α-thalassemia
- Combination of heterozygous β-thalassemia with specific β-globin variants (e.g. HbE, Hb Knossos, unstable variants)
- Combination of heterozygous β-thalassemia with triplicated α-globin genes
- Thalassemic hemoglobinopathies (dominant β-thalassemias)
- Combination of heterozygous β-thalassemia with hereditary enzyme or membrane disorders
- α-thalassemia: HbH disease

Findings in a case of β-thalassemia intermedia



Patient:	**A.CH. 22 yo**
Origin:	**Greek**
Case history:	**similar to thal. major without need for transfusion**
Problems:	**tumor masses due to extramedullary hematopoiesis, organopathies**

Clinical symptoms	• moderately severe disease similar to β-thal. major, transfusion not necessarily needed
Hematology	Hb 8,5 g/dl MCV 61,5 fl MCH 21,5 pg Target cells, erythroblasts
Clinical chemistry	Ferritin ↑ LDH-↑ Bili ↑
Hemoglobin analysis	HbA_2 = 4,4 % HbF = 59,9 % HbA = 35,7 %
Molecular genetics	Proof of mutation -87 (C⟶G) = β^+/β^+-thalassemia homozygous = thalassemia intermedia

Peculiarity:

The indication for transfusion therapy arises from clinical conditions and not on the basis of laboratory data.

Treatment for β-thalassemias

Treatment for β-thalassemia major

After diagnosis, patients should be referred to a hematology center for consulting and decision on treatment and, if necessary, regular diagnostic monitoring.

The current international standard treatment is based on the results of studies conducted at major centers in England and the USA and is available in the child and adolescent medicine guidelines (AWMF/II/025-017.htm).

Curative treatment

Hematopoietic stem-cell transplantation is the first-line of treatment if a donor can be found.

Symptomatic treatment

Symptomatic treatment for thalassemia major includes lifelong regular transfusions combined with effective iron removal. Hemosiderosis-related organ damage requires specific treatment.

- **Transfusion therapy**

  Repeated hemoglobin concentration levels of less than 8 g/dL are an indication for beginning transfusion therapy. The target baseline hemoglobin level is 9 to 10.5 g/dL. The recommended frequency of transfusion is usually once every three weeks. Transfusion amount is usually 12 to 14 ml/kg body weight of an RBC concentrate with 60% of hematocrit. Target Hb levels are 13 to 13.5 g/dL.

- **Drug treatment to remove iron (chelation therapy)**

  Iron removal is indicated when the serum ferritin concentration repeatedly exceeds 1000 ng/mL.

  - **Iron removal using deferoxamine**

  Standard deferoxamine treatment is a daily subcutaneous infusion (over several hours) with a dose of (20) - 40 – (50) mg/kg BW, 5 to 7 days per week. Dose adjustment is based on monthly tests of serum ferritin concentration. Patients must be closely monitored for potential side effects of deferoxamine (reduced growth, bone damage, high frequency hearing loss, retinal damage).

  - **Iron removal with deferasirox**

  Deferasirox is a well-tolerated iron chelator in tablet form and has taken on a central role in iron removal therapy. However, this is a relatively new drug of which no long-term studies have been conducted.

  A standard dose of 20 mg/ kg BW/day is recommended for patients with β-thalassemia major who are receiving long-term transfusion therapy. This dose must be adjusted on the basis of monthly measurement of ferritin levels. The main side effects (close monitoring required!) are kidney failure, agranulocytosis, and liver failure.

- **Splenectomy**

  Splenectomy is indicated for tumorous enlargement of the spleen with increased need for transfusions and hypersplenism.

β-thalassemia intermedia treatment

Transfusion therapy is indicated for patients with complications as a result of strongly increased erythropoiesis, and patients with anemia and an inability to maintain stable hemoglobin levels of more than 8 g/dL. In such cases, lifelong continuous transfusion therapy should be considered, rather than transfusions at intervals, combined with appropriate chelation therapy.

β-thalassemia minor treatment

In case of an increased anemia, the supplement of folic acid (0.5 mg/day orally) may be considered. Iron supplements are contraindicated unless there is a simultaneous iron deficiency.

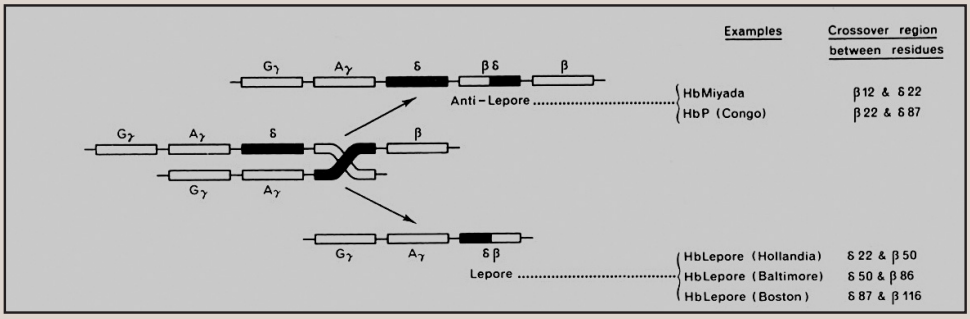
Rare forms of thalassemia

HbLepore syndromes

HbLepore variants are the product of a δβ gene fusion. For more details, see Tab. 31.

Table 31: Characteristic features of HbLepore syndromes

- Hb Lepore variants are so-called fusion hemoglobins, in which a δβ globin chain or rarely a βδ chain (= Hb anti-Lepore) instead of the β chain is present.
- The molecular defect consists in βδ fusion or hybrid genes.



- The phenotypical manifestations correspond to thalassemia minor in case of heterozygosity and thalassemia major in case of homozygosity (see Tab. 32 and 33).

Table 32: Clinical symptoms and laboratory parameters in cases of HbLepore heterozygosity

Manifestation	same as in β-thalassemia minor	
Laboratory diagnostics and findings		
Hematology	Hb	10 - 14 g/dl
	MCV	< 85 fl
	MCH	< 26 pg
Clinical Chemistry	Iron status/Ferritin	normal
	Hemolysis parameters	positive
Hemoglobin analysis	HbA_2	≈ 2 %
	HbLepore	= 5 - 15 %
	HbF	= 1 - 5 %
Molecular genetics In addition to the conventional hemoglobin analysis, a molecular genetic test can be conducted to prove the δβ gene fusion	Presence of HbLepore band in electrophoresis	

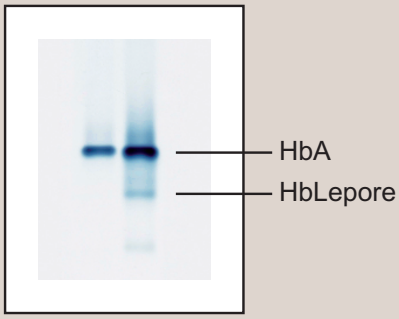


Table 33: Clinical symptoms and laboratory parameters in case of HbLepore homozygosity

Manifestation	- Variable, severe to moderately severe disease - Symptoms like those in case of thalassemia major or intermedia	
Laboratory diagnostics and findings		
Hematology	Hb MCV MCH	4 - 8 g/dl < 75 fl < 22 pg
Clinical chemistry	Hemolysis parameter positive	
Hemoglobin analysis	HbA HbA_2 HbLepore HbF	= 0 = 0 = 10 - 30 % = 70 - 90 %
Molecular genetics	In addition to the conventional hemoglobin analysis, a molecular genetic test can be conducted to prove the δβ gene fusion	

HbLepore and β- and/or δβ-thalassemia

The combinations of HbLepore with β^+ and β^o thalassemia generally give rise to the clinical and hematological pattern of a transfusion-dependent thalassemia major. Depending on the type of β-thalassemia, the HbF values fluctuate between 40 and 90%; the HbLepore percentage is 10, the HbA_2 more or less nomal and the remainder is HbA. There are just very few well-documented patients with double heterozygosity of HbLepore and δβ-thalassemia. The clinical-hematological disease pattern corresponds to thalassemia intermedia. The hemoglobin pattern consists of HbF (> 90%) and HbLepore (< 10%) only.

δβ-thalassemias

A relatively rare form of thalassemia, δβ-thalassemia (or F-thalassemia) is characterized by a lack of synthesis of δ and β-globin chains and a persistence of synthesis of γ-globin chains right up to adulthood. In the heterozygous status, it corresponds to a heterozygous β-thalassemia with normal HbA_2, but increased HbF values (5-20%) from the clinico-hematological point of view. Homozygosity is accompanied by one of the disease patterns comparable to thalassemia intermedia. In this case, the hemoglobin consists only of HbF.

Hereditary HbF persistence and diagnostic significance of an increase in HbF

Hereditary HbF persistence is a clinically harmless increase in HbF. The specific diagnostic significance of increased HbF values in case of β-thalassemias was already pointed. δβ-thalassemias are also characterized by high HbF values. In case of sickle cell disease a high HbF value has a positive prognostic significance. Irrespective of the hemoglobinopathies, an increase in HbF can occur as a secondary phenomenon in numerous hematological diseases (Tab. 34). In many cases, HbF increase is an integral part of another diagnosis and do not need to be examined further by DNA analysis.

Table 34: Criteria for hereditary HbF persistence (HPFH)

These are synthesis defaults in the non-α-globin chains based on the different molecular defects in the γ–δ–β-gene cluster

Symptom

Persistent synthesis of γ-globin chains = HbF

Manifestation

Generally, the HbF persistence is not accompanied by clinical or hematological changes.

Diagnostic significance

- Distinction of HbF increase values in case of HPFH from HbF increase in case of β-thalassemias or other hematological diseases
- Identifying the impact of high HbF percentage in relation to abnormal hemoglobins and/or thalassemia intermedia

Table 35: Differential diagnosis of increased HbF values

Defective hemoglobin synthesis	**HbF values**
β and δβ-thalassemias and severity of the thalassemia	Variable depending on the type
Hereditary persistence of HbF (HPFH) of HPFH	Variable depending on the type
Hemoglobin abnormalities influences	Variable, depending on genetic
Different acquired and hereditary diseases	**HbF values 2 - 85%**
• Iron deficiency anemias	
• Hemolytic anemias	
• Nephrogenic anemias	
• Aplastic anemias	
• Acute myeloid leukemias	
• Chronic myeloid leukemias	
• Erythroleukemias	
• Myelodysplasias	
• Vitamin B12 and/or folic acid deficiencies	
• Other diseases	

Abnormal hemoglobins

HbS and sickle cell disease

Basic information

The term sickle cell disease includes the entire spectrum of the hemoglobin diseases caused by pathological HbS with an HbS percentage of >50 %. The main distribution areas are Africa, India, Arabia, the South Mediterranean countries and Turkey.

The following diseases and syndromes caused in relation to the sickle cell hemoglobin are summarized under the term sickle cell disease:

- The homozygous HbS hemoglobinopathy = HbSS.
- HbS-β thalassemias = HbS-β^+ thalassemia or HbS-β^o thalassemia.
- The sickle cell HbC disease = HbSC disease.

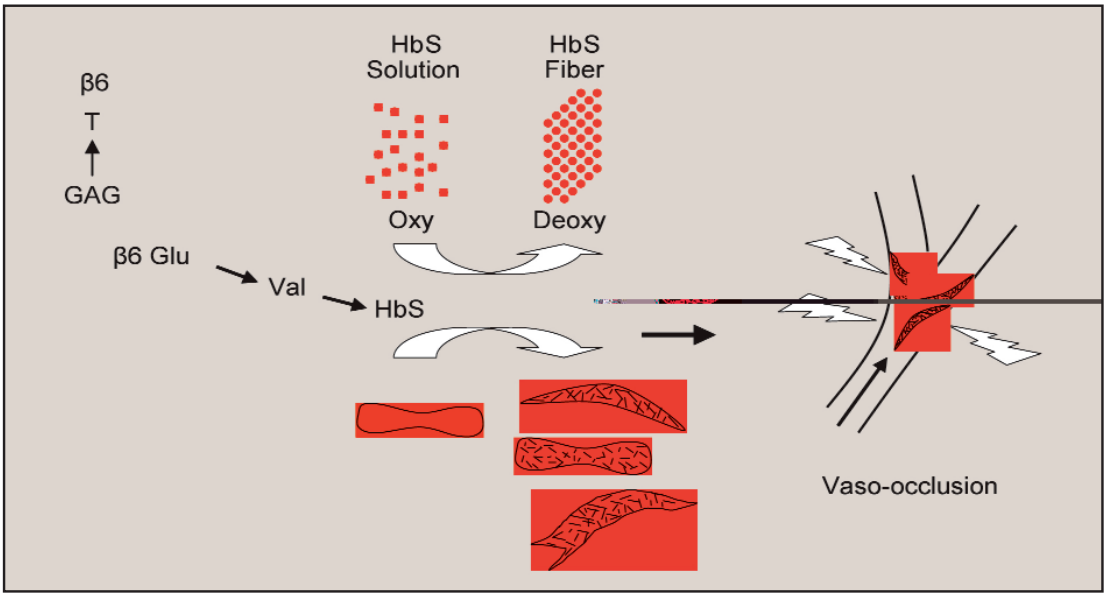
In addition, the rare combination forms HbSD, HbSO-Arab, HbS-Lepore and HbSE disease are part of the sickle cell syndrome group.

In accordance with the international nomenclature the formerly usual name sickle cell anemia should no longer be used because the vascular occlussion and the resulting organ damage dominate the disease, not the anemia.

Pathophysiology

Among all hemoglobinopathies, HbS is the most threatening variant; the affected patients are exposed to a very stressful suffering. Induced by deoxygenation, the sickle cells cause vascular occlussions (Fig. 19) whereby it results in infarctions with tissue destruction in almost all organs (skin, liver, spleen, bones, kidneys, retina, CNS). The chronic hemolytic anemia is usually well tolerated. Aplastic crises with severe anemia should be checked for virus infections.

Fig. 19: Pathophysiology of the sickle cell disease



Clinic and cardinal symptoms

The pathology begins in the first year of life with chronic hemolytic anemia and developmental disorders (for initial diagnostics, see Table 37). The main problem is pain crises (sickle cell crises), which mainly affect the back, limbs, chest, abdomen and CNS. In addition, there is a dangerous susceptibility to infection, mainly due to *Pneumococcus*, *Haemophilus*, *Salmonella,* and also *Klebsiella* and *Mycoplasma*. Sepsis, osteomyelitis and meningitis, also with cardiac involvement, are often causes of death. Spleen crises are often fatal; acute thorax syndromes and strokes are also life endangering. This subsequently results in serious organ damage.

The life expectancy can be 50 - 60 years if all the treatment options are utilized.

Laboratory findings

HbS heterozygosity

Heterozygous HbS carriers are not clinically affected and have a normal blood count. The diagnosis is based on the presence of HbS fraction in a typical position on capillary electrophoresis and agarose gel electrophoresis (see Tab. 36), whose quantitative percentage is less than that of HbA and constitutes about 35 - 40% of the total hemoglobin on average. HbS values below 30% lead to a suspicion: a) of a simultaneously existing iron deficiency or b) of a coexisting α-thalassemia. In both the cases, the MCH value is reduced.

Table 36: Laboratory diagnostics and findings in case of HbS heterozygosity

Hematology

Normal blood count
- no anemia, MCV and MCH normal
- no signs of hemolysis

Clinical chemistry

No pathological laboratory values

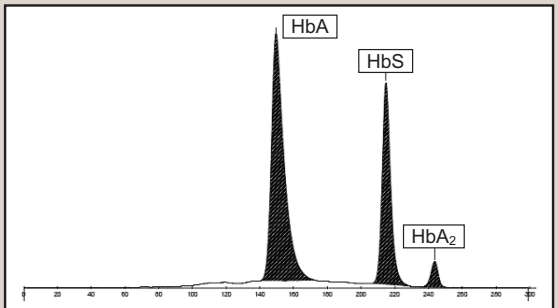
Hemoglobin analysis

HbS ≈ 35 - 40 %
HbA_2 often > 3,2 %
Solubility test: positive

Molecular genetics

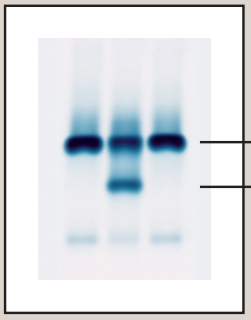
omitted

Capillary electrophoretic separation of the Hb fractions in case of HbS heterozygosity



HbA = 62,4 %; HbS = 34,0 %; HbA_2 = 3,6 %

Hydragel Electrophoresis



Special note for HbS heterozygosity:

Heterozygous HbS trait carriers are not affected clinically and hematologically.

HbS homozygosity

The hemoglobin concentration in the blood count is mostly 6 - 9 g/dl. Sickle cells and target cells are found in the blood smear. Normal HbA is not found on hemoglobin electrophoresis in case of HbSS (homozygous form). The percentage of HbF varies and usually lies between 5 - 15%. Higher HbF percentages can often be seen. HbF values of above 15% have a prognostically favorable significance (see Tab. 37).

Table 37: Laboratory diagnostics and findings in case of sickle cell diseases

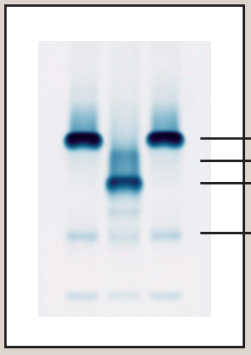
Hematology
- Hemolytic anemia; Hb 6 - 9 g/dl
- Ery-indices:
  - normochromic or hypochromic
  - normocytic or microcytic
- Morphology:
  Sickle cells, poikilocytosis

Clinical chemistry
- Hemolysis parameter positive
- Functional tests (liver, kidney, etc) positive

Hemoglobin analysis

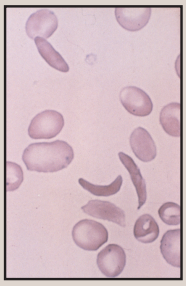
Electrophoresis and/or HPLC:
HbS > 50 % (75 - 95 %)
HbF 3 - 10 %
HbA_2 often increased
Positive solubility test

Molecular genetics
- Necessary only in case of special indications



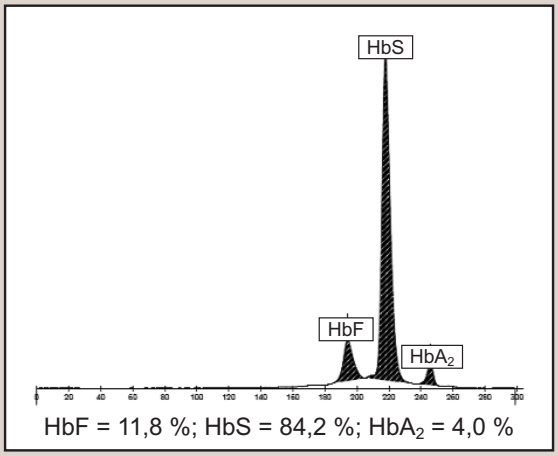
Hydragel Electrophoresis

Sickle cells in the blood smear



Positive solubility test (sample on the right)

Capillary electrophoretic separation of the Hb fractions in case of HbS homozygosity



HbF = 11,8 %; HbS = 84,2 %; HbA_2 = 4,0 %

Table 38: Indication for the molecular genetic diagnostics in case of sickle cell diseases

HbS is primarily diagnosed with the conventional Hb analysis

Special indications for the DNA test are:

1. Combination forms of HbS
   - with other abnormalities
   - with β-thalassemias: e.g. HbS-β^o-thalassemia or HbS-β^+- thalassemia
2. For prenatal diagnostics

HbS-β-thalassemias

These hemoglobinopathies belonging to the group of sickle cell diseases are not a rarity in the immigrated population. For the laboratory diagnostic parameters, see Tab. 39. The hematological manifestation is similar to that of the sickle cell disease. Microcytosis and hypochromia are the discriminative features from HbS homozygosity. The HbS-β^o forms are easily differentiated from the HbS-β^+ forms by the presence of HbA fraction in the HbS-β^+ combination, while HbA is not present in the HbS-β^o form. The diagnosis can be achived using Hb analysis or a molecular genetic analysis (also see Tab. 39 as well as Fig. 20 and 21).

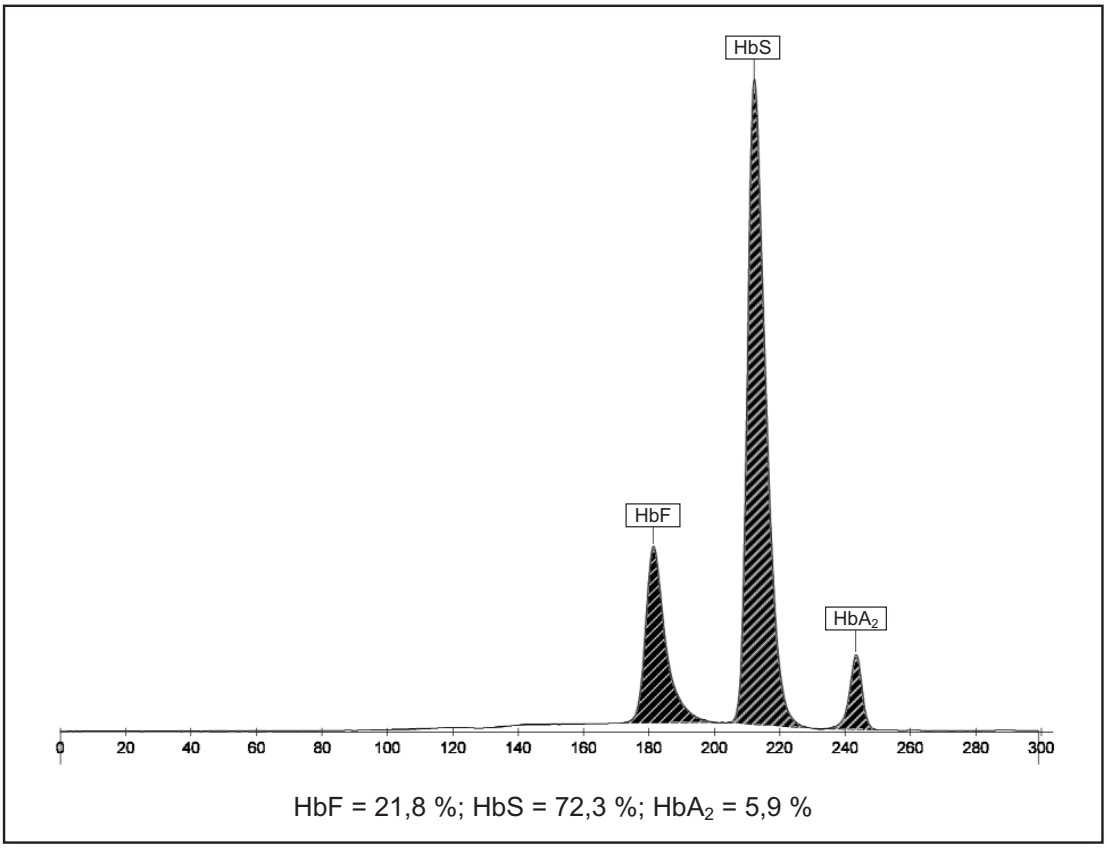
Table 39: Diagnostic features of sickle cell β-thalassemias

HbS-β^o-thalassemia	**HbS-β^+-thalassemia**
Manifestation:	Manifestation:
same as a severe sickle cell disease	depending on the β-globin gene residual activity = mild to severe sickle cell disease
Laboratory diagnostics and findings:	Laboratory diagnostics and findings:
Hematology: Hb 6 - 10 g/dl MCH < 22 pg	Hematology: Hb 9 - 12 g/dl MCH < 24 pg
Clinical chemistry:	Clinical chemistry:
Hemolysis parameter positive	Hemolysis parameter positive
Hemoglobin analysis:	Hemoglobin analysis:
HbA_2 > 3,5 % HbS > 80 % HbF 1 - 15 % HbA 0 %	HbA_2 > 3,5 % HbS 55 - 75 % HbF 1 - 20 % HbA 3 - 30 %
Molecular genetics:	Molecular genetics:
Presence of the following mutations:	Presence of the following mutations:
1. HbS = β codon 6 GAG ⟶ GTG heterozygous and 2. β^o-thalassemic mutation heterozygous = compound heterozygosity	1. HbS = β codon 6 GAG ⟶ GTG heterozygous and 2. β^+-thalassemic mutation heterozygous = compound heterozygosity

HbS-α-thalassemias

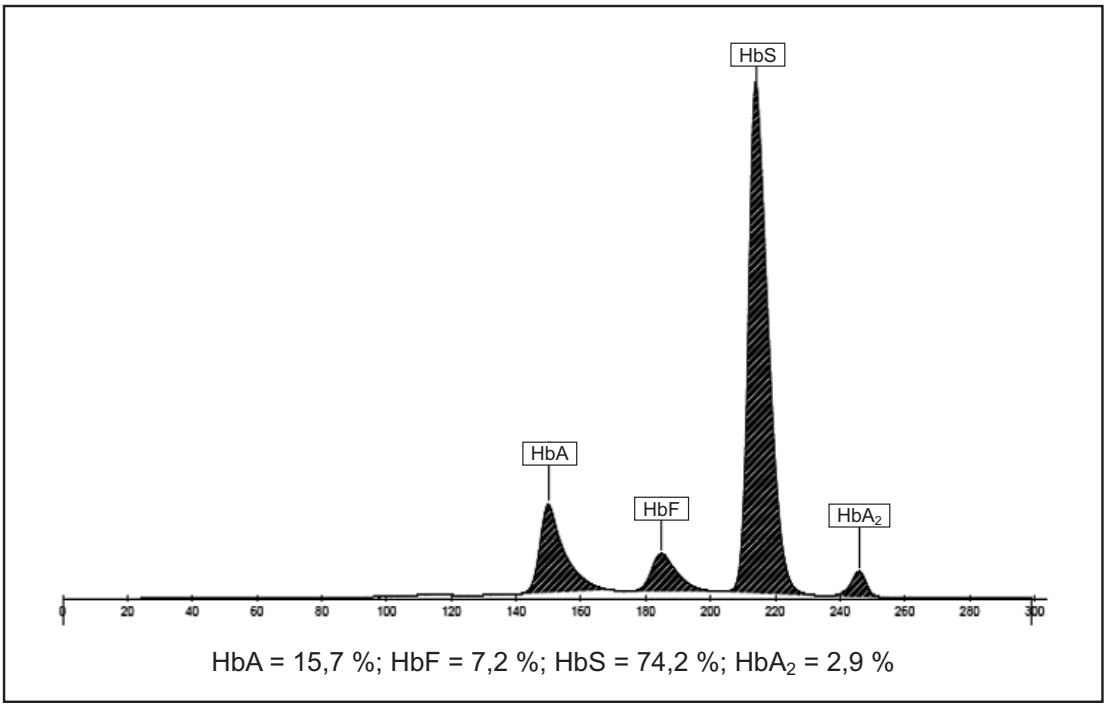
The combination of a HbS-homozygosity with α-thalassemia minor forms occurs in up to 30% of the HbS patients. Positive as well as negative influences appear in this case. One comes across a less severe anemia and fewer CNS-related incidents. The incidence of pain crises is significantly higher.
In case of heterozygous HbS carriers with microcytic hypochromic erythrocyte indices, less HbS is present depending on the number of inactive α genes.

Fig. 20: Hemoglobin pattern in case of HbS-β^0-thalassemia (capillary electrophoresis)



HbF = 21,8 %; HbS = 72,3 %; HbA_2 = 5,9 %

Fig. 21: Hemoglobin pattern in case of HbS-β^+-thalassemia (capillary electrophoresis)



HbA = 15,7 %; HbF = 7,2 %; HbS = 74,2 %; HbA_2 = 2,9 %

Sickle cell hemoglobin D-(HbSD-) disease

The clinical pathology of the HbSD disease is variable, but less pronounced than in case of homozygous sickle cell disease (HbSS). In the foreground stands a chronic hemolytic anemia with hemoglobin concentrations of about 9 g/dl. There can be crises. Different HbD variants in double heterozygosity with HbS result in disease patterns of various severity. For instance, the combination of HbS with HbD Ibadan is practically asymptomatic. In comparison to that, the compound heterozygosity of HbS and HbD Los Angeles (HbD Punjab) has a severe course. The frequency of the HbSD disease depends on the geographic occurrence of both the abnormalities. The most frequent is the combination HbD Los Angeles (HbD Punjab) in the population of Pakistan and North-West India; on the other hand it is rare in the African population.

Treatment for sickle cell disease

Following diagnosis, patients should be referred to a hematology center for counseling and a decision on treatment, and, if necessary, also for regular diagnosis appraisal. The current standard treatment (also see Tab. 40) is based on the results of studies conducted at major centers in England and the USA and is stated in the available guidelines (AWMF/II/025-016.htm). The life expectancy is given as 50-60 years.

Table 40: Important contents of the treatment concept for sickle cell disease

1. General measures and long-term monitoring within the scope of the routine check-up:
2. Treatment of the sickle cell crises (pain crises)
3. Treatment of infections; pyretotherapy
4. Treatment with hydroxyurea (Litalir®)
5. Transfusion therapy; individual transfusions; exchange transfusions; long-term transfusion program
6. Treatment for specific complications: Splenic sequestration; priapism; cerebral infarctions; acute thorax syndrome
7. Surgical treatment measures: Splenectomy; cholecystectomy; osteotomy

Curative treatment

In children < 16 years, allogenic stem cell transplantation is considered. Indications include a cerebral infarction, especially serious and frequent pain crises and frequent acute thorax syndromes. Transplantation in case of older patients in conflictive due to the lack of donors and high risks.

Symptomatic treatment

- **Analgesic:** first-line treatment for pain crises consists of sufficient administration of fluids and analgesics appropriate to the level of pain (paracetamol, metamizol, possibly also codeine or tramal, or even morphine).
- **ACE inhibitors** can inhibit progression of glomerulonephritis or glomerulosclerosis with proteinuria above 0.5 g/24 hours.
- **Antibiotics:** are also administered, particularly for pneumococcal infection with suspected sepsis and for *Salmonella* infection with suspected osteomyelitis.
- **Hydroxyurea** is the only substance to date that can reduce the number and severity of pain crises (in 70% to 75% of patients) and decrease the number of episodes of acute thoracic syndrome and mortality.

The initial dose of 15 mg/kg/day can be increased to 35 mg/kg/day. Due to severe side effects, sickle cell patients may only be treated with hydroxyurea when it is strictly indicated, following patient education, if birth control is used (for women of reproductive age), with regular blood counts (initially every two weeks and then monthly), and with careful documentation of side effects. Side effects include cytopenia revealed in a blood count, hyperpigmentation, weight gain, opportunistic infections, azoospermia in approximately 80% of men (even years after the end of treatment), and marked hypomagnesemia. It is also believed that there is a teratogenic effect.

- **Transfusion therapy**

  Transfusion therapy is subject to strict indications. Unfortunately, these rules are often broken. Single transfusions are indicated for major splenic sequestration, aplastic crises, and acute thoracic syndrome, as well as before major surgery (Hb must be increased to 10 g/dL!). Partial exchange transfusions to reduce the proportion of HbS are indicated for acute organ failure or vascular occlusions, but rarely for refractory pain crises.

  The main indication for long-term transfusion programs (to maintain low proportions of HbS in the blood long-term) is a cerebral infarction. Sickle cell patients receiving frequent transfusions must receive chelation therapy.

- **Splenectomy**

  Homozygous sickle cell diseases lead to spleen sclerosis and functional asplenia even in childhood. Patients with HbS β-thalassemia undergo splenectomy following splenic sequestrations or in the event of hypersplenism.

Prevention

- Affected children must receive all recommended vaccinations and 7-valent pneumococcal vaccinations from the age of two months onwards (also see guidelines AWMF/ll25-016.htm).
- Prophylactic penicillin must also be administered from the age of three months onwards, for at least five years.

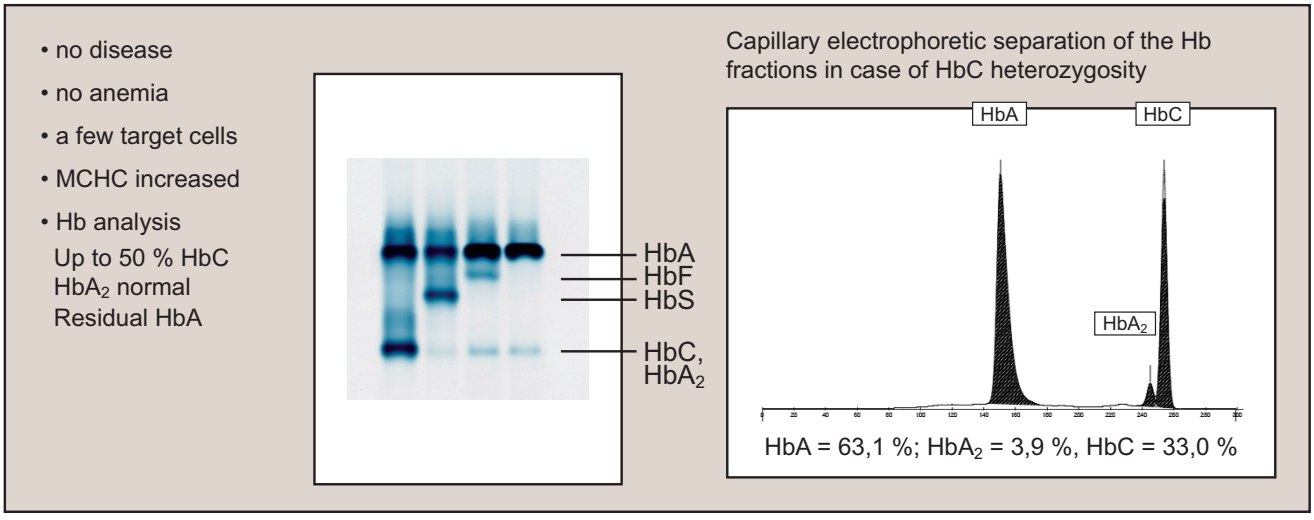
HbC abnormality and HbC syndromes

As in the case of HbS, the pathophysiology of HbC is also based on a disordered intracellular solubility, which in the case of HbC is accompanied by intraerythrocytic crystallization and an increased tendency towards cell aggregation. HbC can be seen mainly in Africa.

HbC heterozygosity

HbC genetic carriers are not ill; there are no hematological changes (Fig. 22).

Fig. 22: Symptoms and findings (agarose gel and capillary electrophoresis) in case of HbC heterozygosity



HbC-homozygosity = HbC disease

The HbC-homozygosity is a mild to moderately severe hemolytic anemia. Target cells can be predominantly seen in the blood count. The Hb value is about 10 - 12 g/dl. The MCHC value is above 35 g/dl. Almost 100% HbC is found in hemoglobin electrophoresis (Tab. 41).

Table 41: Laboratory diagnostics and findings in case of HbC homozygosity

Manifestation

Mild to moderately severe pathology just like in the case of sickle cell disease.
Normal hemolytic anemia

Laboratory diagnostics and findings:

Hematology

Hb 10 - 12 g/dl
Target cells, microcytes
Crystal cells
MCV decrease
MCHC increase

Clinical chemistry

Hemolysis parameters positive

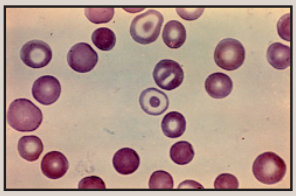
Hemoglobin analysis

HbC > 97 %
HbA_2 2,5 %
HbF slightly increased

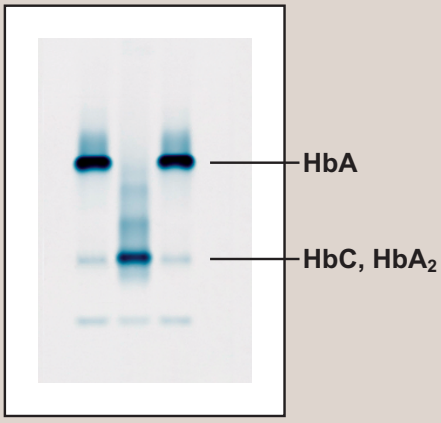
Molecular genetics

not applicable

Erythrocyte morphology with target cells and rod cells



Electrophoretic separation of HbC on Hydragel electrophoresis



HbC-β-thalassemias

In case of combination of HbC with β-thalassemia, the severity of the disease depends on whether there is a β^o- or β^+-thalassemia. The corresponding hematological changes are similar to those seen in HbC disease. The mild to serious hemolytic anemia is accompanied by a hypochromia (Tab. 42).

The HbSC disease

is similar (with respect to the treatment as well) to a mild sickle cell disease with a clearly milder expression of the acute symptoms. The following laboratory findings can be seen in this case: moderate anemia, a few target cells, increased MCHC. Hb analysis: about 45% of HbS and HbC (Fig. 23 and 24). HbF can sometimes be increased. HbA_2 is normal.

Fig. 23: Electrophoretic separation (Hydragel electrophoresis) in case of HbSC disease

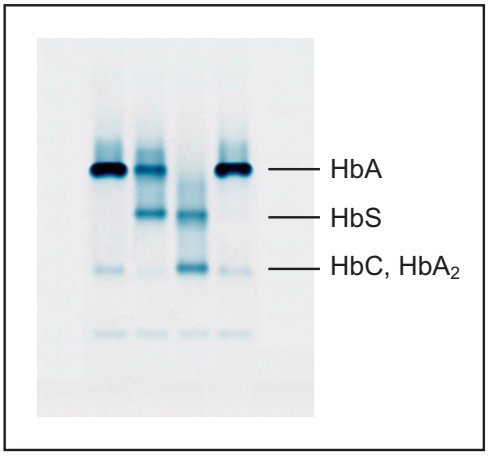
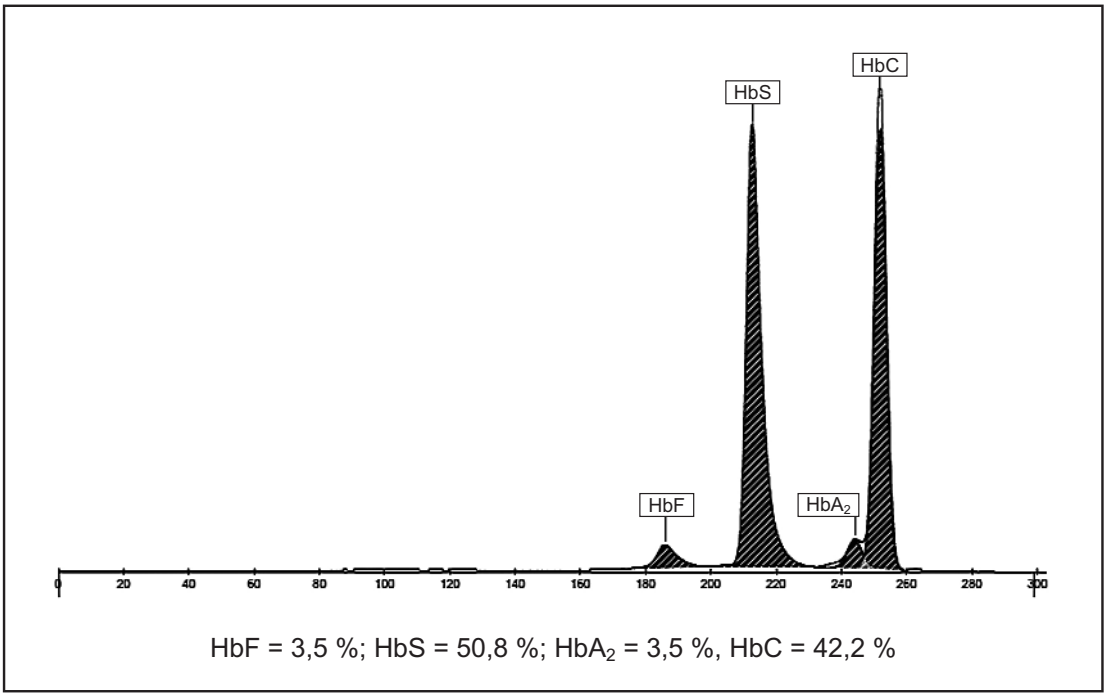


Fig 24: **Hemoglobin pattern in capillary electrophoresis in case of the HbSC disease**



HbF = 3,5 %; HbS = 50,8 %; HbA_2 = 3,5 %, HbC = 42,2 %

Table 42: **Differential diagnosis of the homozygosity for HbC and HbC-β-thalassemias**

	HbC-Homozygosity	HbC-β^+-Thal.	HbC-β^o-Thal.
Anemia	mild to moderate	mild	moderate to high
Blood smear	Target, rod, crystal cells	Microcytic, hypochromic, target cells	Anisocytosis, hypochromic, microcytic target cells
Reticulocytes	increased	mildly increased	increased
MCV fl	≈ 70 - 72	60 - 70	55 - 70
HbA %	0	20-30	0
HbC (+ HbA_2) %	> 97	65 - 80	90 - 95
Splenomegaly	yes	no	yes

Treatment for the HbC syndromes

The treatment for the HbC disease is restricted to symptomatic measures if necessary. The treatment of complications in the HbSC disease (e. g. retinopathies, renal symptoms) depends on the seriousness of the symptoms and follows the principles for treating sickle cell disease. Pregnancies must be monitored very carefully. HbC-β-thalassemias are also treated with symptomatic measures if necessary.

HbE abnormality and HbE syndromes

HbE is most common in people of South-East Asia with more than 50 million abnormality carriers.

Basic information

- In respect to frequency, HbE tops the world list among all Hb variants
- Endemic in Asian countries
- Strong increase in Germany due to migrants from Asia

Clinical significance

- HbE heterozygosity clinically corresponds to a β-thalassemia minor
- HbE-homozygosity and HbE-β^+-thalassemia are mild to intermediate hemoglobinopathies
- HbE-β^o thalassemia clinically corresponds to a thalassemia major.
- The combination forms of HbE and α-thalassemias are also very common

Pathophysiology

A characteristic feature is quantitatively reduced hemoglobin production and instability of HbE such that hemolysis is triggered under the influence of oxidative substances.
Heterozygous genetic carriers are not affected.

Laboratory diagnostic criteria and cardinal symptoms of the HbE-hemoglobinopathies

HbE-heterozygosity

Hematologically, there is a mild, variable microcytic hypochromic anemia (Tab. 43).

HbE-homozygosity = HbE disease

- Hypochromia with microcytosis is typical (see Tab. 44), the number of reticulocytes is normal.
- Unlike heterozygosity, several target cells combined with mild to normal anemia can be seen in HbE homozygosity. The quantity of HbE is approximately 95%; the remaining is HbF and HbA_2.

In HbE heterozygosity and homozygosity and combination forms, there is a risk of an acute Heinz body anemia in case of medication with oxidizing substances (see Tab. 48).

Table 43: HbE heterozygosity

Manifestation

- Mild hypochromic anemia
- Like β-thalassemia minor

Laboratory diagnostics and findings:

Hematology

Hb	10 - 14 g/dl
MCH	20 - 25 pg
MCV	55 - 70 fl
individual target cells	

Clinical chemistry

Iron status	normal
Hemolysis parameters	negative

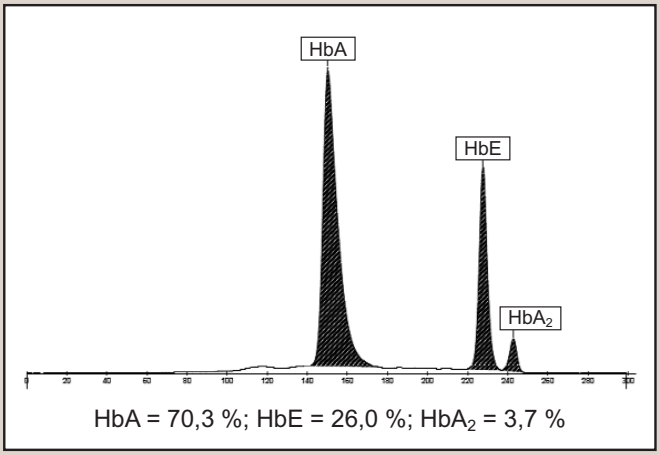
Hemoglobin analysis

HbE	25 - 35 %
HbA	60 - 75 %
HbA_2	≈ 3,4 %
HbF	not increased

Molecular genetics

not applicable

Capillary electrophoretic separation of the Hb fractions in case of HbE heterozygosity



HbA = 70,3 %; HbE = 26,0 %; HbA_2 = 3,7 %

Table 44: HbE homozygosity = HbE disease

Manifestation

- Variable hypochromic anemia
- Hemolysis due to oxidative influences
  = virus infections, drugs, environmental pollutants

Laboratory diagnostics and findings:

Hematology

Hb	9 - 14 g/dl
MCV	60 fl
MCH	20 pg
Morphology: target cells, hypochromia, microcytosis	

Clinical chemistry

Iron status	normal
Hemolysis parameters	mostly positive

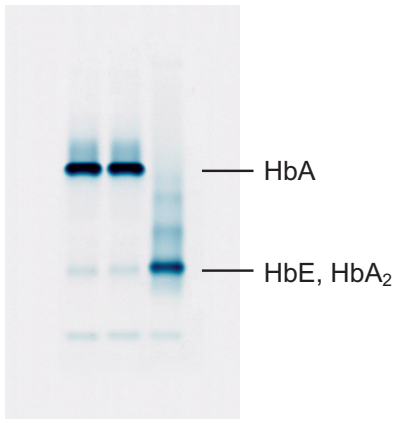
Hemoglobin analysis

HbE	> 95 %
HbF	< 3 %
HbA_2	≈ 4,4 %

Molecular genetics

not applicable, except in case of compound heterozygosities

Electrophoretic migration of HbE (Hydragel Hb electrophoresis) in case of HbE homozygosity



HbE-β-thalassemias and HbE-α-thalassemias

The combination of HbE with β-thalassemia forms, frequent in the Asian and Arabic population, results in a moderately serious to serious hypochromic and dyserythropoietic anemia. Especially influenced by the instability of HbE and depending on the effect of the β-thalassemia mutation, the clinical picture corresponds to the thalassemia intermedia or thalassemia major.

The combination of HbE with α-thalassemia forms is found frequently in South-East Asia. As expected, the HbE percentage is significantly reduced. For example, in the combination of α^o-thalassemias and HbE heterozygosity it is 15-22% HbE as compared to 25-30% HbE in simple heterozygosity for HbE. Reduced values for the abnormal HbE are also associated with an iron deficiency. It has to be observed, however, that the abnormal HbE synthesis is primarily reduced, causing a hypochromic anemia in the heterozygous status, which aggravates in combination with α-thalassemia. **The combination of HbH disease with HbE heterozygosity**, which clinically corresponds to the described pattern of a HbH disease, is often found in South-East Asia. (For details, see Tab. 45 and Tab. 46).

Table 45: Differential diagnosis of the homozygosity for HbE and HbE-β-thalassemias

	HbE-homozygosity	HbE-β^+-Thal.	HbE-β^o-Thal.
Anemia	mild	extremely variable	corresponds to thal. major
Blood smear	target cells, microcytic	target cells, microcytic	anisocytosis, microcytic
Reticulocytes	normal	high	high
MCV fl	62 - 67	55 - 62	52 - 58
HbA %	0	5-60	0
HbF %	< 3	6-50	15 - 25
HbE (+ HbA_2) %	> 95	25 - 80	75 - 85
Splenomegaly	no	yes	yes
In need of transfusion	no	no	always

Table 46: Hemoglobin patterns and manifestations of HbE abnormalities and HbE α-thalassemias

Number of active α-globin genes	Heterozygosity ($\beta^A\beta^E$) Hb-pattern %	Phenotype	HbE Homozygosity (β^E/β^E) Hb pattern %	Phenotype
αα/αα (normal)	HbE: 25 - 35 HbA: 75 - 65	hypochromia	HbE: Remainder: > 97 HbA_2, HbF	mild hypochromic anemia
–α/αα	HbE: 22 - 30 HbA: 78 - 68	hypochromia	HbE: Remainder: > 95 HbA_2, HbF	mild hypochromic anemia
–α/–α or – –/αα	HbE: 15 - 25 HbA: 85 - 75	hypochromic anemia splenomegaly	HbBart's traces	hypochromic anemia
– –/–α (α^o/α^+-Thal)	HbE: 10 - 17 HbBart's: 5 - 10 HbH: traces HbA: = 80	similar to severe HbH disease	HbE: 85 HbF: 13 HbBart's: 2	similar to severe HbH disease

Therapy for different HbE syndromes

Treatment of HbE homozygosity is not required in most cases; it is restricted to supportive measures if necessary. Depending on the severity, the combination of HbE with β-thalassemia can be similar to a thalassemia major or thalassemia intermedia with the corresponding need for treatment. Under the influence of oxidative damages or agents (e.g. drugs (Tab. 48), infections), there is a risk of acute hemolytical crises (also in case of HbE heterozygosity) right up to serious Heinz body anemias. According treatment measures are necessary in this case.

Unstable abnormal hemoglobins

This group of extremely rare abnormal hemoglobins with autosomal dominant modes of inheritance is accompanied by the clinical and hematological disease pattern of the so-called congenital hemolytical Heinz body anemia. The most common and frequent variant of this type is HbKöln, also called **HbKöln disease** which occurs throughout the world.

The clinical manifestation forms of unstable hemoglobins are extremely variable and range from chronic hemolytic anemia with acute phases to long-term diseases with transfusion requirement to mild anemias. The unstable hemoglobin precipitates in the erythrocytes either spontaneously or under oxidative stress to form inclusion bodies (Heinz bodies), with subsequent spleen sequestration and hemolysis. Jaundice of varying intensity and an increasing splenomegaly as well as discharge of dark urine in phases (urobilinuria-stercobilinuria) occurs in almost all patients. The number of reticulocytes varies significantly (40 – 250 ‰). The erythrocyte morphology shows anisocytosis and macrocytosis, with spherocytes (occasionally) and basophil punctured erythrocytes (very frequently). After a splenectomy, clear inclusion bodies are visible in erythrocytes treated with brilliant cresyl blue. Many of the unstable Hb variants cannot be separated.

In case of infections, high fever and after taking certain drugs (Tab. 48), the hemolysis can increase significantly.

Table 47: Clinical symptoms and laboratory findings of unstable hemoglobins

Name of the disease:

= Congenital hemolytic Heinz body anemia

Prototype: HbKöln disease

- variable hemolytic anemia
- jaundice, splenomegaly, dark urine
- crises due to oxidative damage

Laboratory diagnostics and findings:

Hematology

Hb 9 - 13 g/dl
MCH reduced
MCV increased
Spherocytes, basophilic stippling
Reticulocytes 40 - 250 ‰
Heinz inclusion bodies, especially after splenectomy

Clinical chemistry

Hemolysis parameters positive

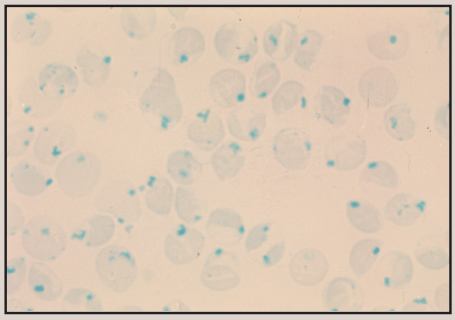
Hemoglobin analysis

Unclearly separated Hb fraction approx.10%

Molecular genetics

Presence of the mutation in β Codon 98 GTG → ATG

Heinz body preparation (brilliant cresyl blue staining) in case of HbKöln disease



Electrophoretic separation of HbKöln with detection of the unclear bands in case of HbKöln (Hydragel Hb electrophoresis)

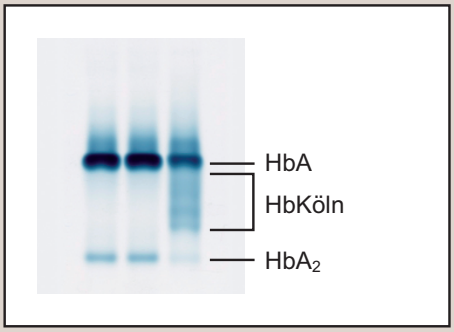
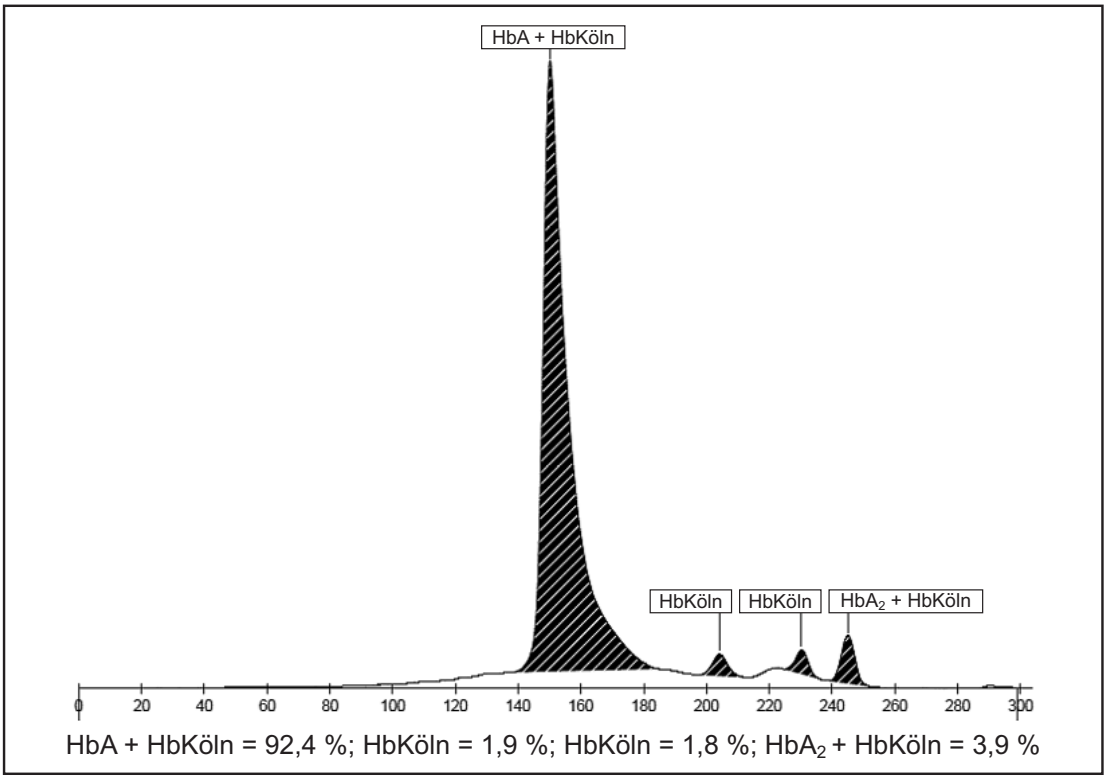


Fig. 25: Capillary electrophoresis hemoglobin pattern of HbKöln disease



HbA + HbKöln = 92,4 %; HbKöln = 1,9 %; HbKöln = 1,8 %; HbA_2 + HbKöln = 3,9 %

Treatment for severe hemoglobinopathies in case of unstable hemoglobins

The therapeutic options are limited. Stronger anemias require an erythrocyte substitution especially in case of a critical drop in the hemoglobin concentration. Extremely serious forms can be improved by splenectomy, but cannot be cured. Splenectomy is also indicated in case of hypersplenism due to a large spleen tumor. Possible post-splenectomy risks like sepsis, thromboembolisms and pulmonary embolisms must be considered. Stem cell transplantation must be considered in case of patients with a long-term transfusion requirement.

The prophylactic measures include a dose of folic acid (1 mg/day) and above all the prohibition of exposure to oxidative substances and taking drugs with oxidizing properties. The iron metabolism must be monitored with respect to the development of a hemosiderosis, in order to start chelation therapy at the right time.

Table 48: Selection of drugs and chemicals which can cause hemolytic anemia in case of unstable hemoglobins

• Acetanilide	• Phenylhydrazine
• Methylene blue	• Primaquine
• Nalidixic acid	• Sulfacetamide
• Naphthalene	• Sulfamethoxazole
• Niridazole	• Sulfanilamide
• Nitrofurantoin	• Sulfapyridine
• Pamaquine	• Thiazosulfone
• Pentaquine	• Toluidine blue
• Phenazone	• Trinitrotoluene

Abnormal hemoglobins with impaired oxygen transport function

Variants with reduced O_2-affinity

Characteristic of this extremely rare group of hemoglobinopathies is cyanosis and in some cases altered oxygen saturation, sometimes with mild anemia. Some of these Hb variants are accompanied by methemoglobinemia. Treatment is not required.

Variants with increased O_2-affinity

As a result of the increased oxygen affinity of hemoglobin, the delivery of oxygen to the tissue is hampered resulting in tissue hypoxia, which in turn causes the release of erythropoietin leading finally to **erythrocytosis**. Common examples of this extremely rare but important for the differential diagnosis hemoglobinopathy group are Hb York, Hb Johnstown or Hb Vila Real, among others.

Classification of primary and secondary erythrocytosis

Erythrocytosis (older term - polycythemia) is characterized by increased hemoglobin values and/or hematocrit values. An overview of the differential diagnosis of the different erythrocytosis forms is given in table 49.

Table 49: Differential diagnosis of erythrocytosis

Primary erythrocytosis, low plasma EPO	
Congenital forms:	EPO-receptor mutations
Acquired forms:	Polycythemia vera
Secondary erythrocytosis, normal plasma EPO	
Acquired forms	- Cardiac and pulmonary diseases - Sleep apnea; massive adiposities - Altitude adaptation
Congenital forms:	- Hemoglobinopathies with high O_2-affinity - 2,3-diphosphoglycerate mutase deficiency - Chronic CO-poisoning
Secondary erythrocytosis, increased plasma EPO	
	- EPO-producing tumors - Renal anemia with EPO ↑ - Von Hippel-Lindau gene defects (= Chuvash polycythemia) - Rare defects

HbM abnormalities

This is also a very rare special group of Hb abnormalities whose specific amino acid substitution changes the hem-iron globin bond so that the iron is permanently present in trivalent form (methemoglobin). The structural and functional pathological methemoglobins can be differentiated from normal methemoglobin by their characteristic properties. The inheritance is autosomal dominant.

The only symptom is the permanent cyanosis without affecting the physical ability.

The abnormalities of the α-globin chain are manifested from birth itself, since fetal ($\alpha 2\gamma 2$) as well as adult hemoglobin ($\alpha 2\beta 2$) have α-globin chains.

The abnormalities of the β-globin chain become symptomatic only postnatally, parallel with the disappearance of HbF. The HbM cyanosis does not disappear after administration of redox dyes.

The activity of cytochrome-b5-reductase is normal.

The diagnosis is made using specific hemoglobin analyses or molecular genetic analyses.

The treatment of the HbM abnormalities is not possible and is unnecessary. The psychological burden of the patients due to cyanosis is normally low.

Annex: Toxic and enzymopenic methemoglobinemias

Methemoglobinemia is an increase in the methemoglobin level above the standard value of 1% of total hemoglobin. A cyanosis is visible after about 10% of MetHb.

In addition to the HbM abnormalities, there are two causes for a methemoglobinemia:

- An increased oxidation rate of the normal hemoglobin through oxidizing substances = toxic methemoglobinemia.
- Defects in the enzymatic reduction systems = enzymopenic methemoglobinemias

Toxic methemoglobinemia

This acquired methemoglobinemia is either acute or subacute under the effect of oxidizing substances, mainly

- Nitrate and/or nitrite
- Aniline dyes
- Local anesthetics
- Drugs (see product information if necessary)

Cyanosis develops at different speeds. A poor general condition with dyspnea and tachycardia is typical. MetHb values of 70-80% are fatal.

A redox dye treatment is carried out, e.g. 1% methylene blue solution i.v.: Dose - 1-2 mg/kg body weight. Cyanosis disappears after 30 - 60 min.; the injection can be repeated. Further treatment with 0.5 - 1 g/day ascorbic acid (peroral) can be useful. In case of serious toxic forms, transfusions and exchange transfusions are necessary, especially in the case of new-borns.

Enzymopenic methemoglobinemia

This is a heterogeneous group of diseases with autosomal recessive inheritance. It is caused by a deficiency of NADH-cytochrome-b5-reductase. There are different structural variants of the enzyme based on the different genetic defects, which lead to two different types of enzymopenic methemoglobinemias:

- **Type I**: The defect is restricted to the erythrocytes. Homozygous or compound heterozygous patients are affected by methemoglobinemia; heterozygotes are clinically inapparent.
- **Type II** is the generalized and fatal form. In addition to the hereditary methemoglobinemia, there are progressive neurological symptoms with the risk of most severe psychomotor disorder and death in early childhood.

Treatment

The methemoglobin can be reduced using ascorbic acid (0.5 g daily, new-borns 0.2 g daily) or using vitamin C-rich food.

The neurological problems in case of type II cannot be treated curatively.

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Appendix: Rare anormal hemoglobins

eTABLE 1

Rare anomalous hemoglobins: native German population

Designation of Hb anomaly	Manifestations	Number of patients
Hb Cologne	chronic hemolytic anemia	103
HbE	mild hypochromic anemia; hemolysis induced by medications, viral infections, oxidative damage	84
HbD Ibadan, Iran, Los Angeles, Neath	normal	50
HbM Milwaukee, Iwate, Saskatoon, Boston	cyanosis, methemoglobinemia, erythrocytosis	45
Hb Okayama	Falsely elevated HbA_{1C}, hemat. and clinically healthy	39
HbJ Meerut, Iran, Chicago, Camagüey, Baltimore, Auckland, Paris, Cambridge, Amiens, Wenchang Wuming, Bangkok	normal	22
Hb Hammersmith	severe hemolytic anemia	1
HbA_2 anomalies	normal	17
Hb Andrew Minneapolis	erythrocytosis	17
Hb York	erythrocytosis	10
Hb Cheverly	chronic hemolytic anemia	9
Hb Camperdown	normal	8
Hb Little Rock	erythrocytosis	7
Hb Presbyterian	normal	7
Hb Vanderbilt	severe erythrocytosis	6
Hb Johnstown	mild erythrocytosis	6
HbI Interlaken, Philadelphia	normal	5
Hb Freiburg	cyanosis, hemolytic anemia	5
Hb Ohio	erythrocytosis	5
HbN Baltimore, Seattle, Timone	normal	4
Hb Nottingham	severe hemolytic anemia	3
Hb Saint Mandé	cyanosis	3
Hb Shepherds Bush	hemolytic anemia	3
Hb Zurich	Episodic drug-induced hemolytic anemia	3
Hb San Diego	erythrocytosis	3
Hb Hasharon	hemolytic anemia on exposure to toxic substances	2
Hb Malmö	erythrocytosis	2
Hb Tübingen	episodic hemolytic anemia, cyanosis, methemoglobinemia	2
Hb Athens GA	normal to mild erythrocytosis	4
Hb Nagoya	chronic hemolytic anemia	2
Hb Palmerstone North	erythrocytosis	1
Hb Savannah	hemolytic anemia	1
Hb Pyrgos	mild erythrocytosis	2
Hb Olympia	erythrocytosis	1
Hb Saitama	hemolytic anemia	1
Hb Limassol	normal	1
Hb Shaare Zedek	normal	1
Hb Yokohama	severe hemolytic anemia	1
Hb Syracuse	erythrocytosis	1
Hb Strasbourg	normal	1
Hb Atlanta	mild hemolytic anemia	1
Hb Utrecht	mild hypochromic hemolytic anemia	1
Hb Osu Christiansborg	normal	1
Hb Seattle	chronic hemolytic anemia	1
Hb Buenos Aires	hemolytic anemia	1
HbE Saskatoon	normal	1
HbF Catalonia	incidental finding	1
HbK Woolwich	hematologically normal	1
Hb Agenogi	mild anemia	1
Hb Alesa	hemolytic anemia	1
Hb Camden	normal	1
Hb Lufkin	normal	1
Hb Mainz	severe hemolytic anemia	1
Hb Radcliffe	erythrocytosis	1
Hb Regina	mild erythrocytosis	1
Hb Riverdale Bronx	hemolytic anemia	1
Hb Titusville	normal	1
Hb Rothschild	mild anemia	1
Hb Mozhaisk	hemolytic anemia	2
Hb Higaschitochigi	cyanosis, methemoglobinemia	1
Hb Beth Israel	cyanosis, hematologically normal	1
Hb Vila Real	mild erythrocytosis	6
Hb Hanamaki	mild erythrocytosis	1
Hb Leiden	hemolytic anemia, episodes induced by drugs or viral infection; see HbE, above	2
Hb South Florida	normal	1
Hb Villaverde	severe erythrocytosis	1
Hb Cagliari	hemolytic hypochromic anemia	1
Hb Louisville	mild hemolytic anemia	1
Hb Mito	mild erythrocytosis	1
Hb Hoshida	normal	1

Hb, hemoglobin; HbF, fetal hemoglobin

eTABLE 2

Rare anomalous hemoglobins: immigrant population

Designation of Hb anomaly	Manifestations	Number of patients
HbD Punjab, Iran, Ibadan, Ouled Rabah, Los Angeles, Camperdown, Neath	normal	183
HbA_2 anomalies	normal	67
HbO Arab, Padova, Indonesia	mild anemia	69
HbG Copenhagen, Coushatta, Ferrara, Honolulu, San José, Szuhu, Taipei, Philadelphia/ HbC, Philadelphia, Accra	normal	40
Hb Cologne	hemolytic anemia	25
HbJ Auckland, Baltimore, Calabria, Camagüey, Havana, Iran, Paris, Sardinia	normal	18
HbI Interlaken, Philadelphia	normal	11
HbM Iwate, Milwaukee, Saskatoon	congenital methemoglobinemia	6
Hb Setif	mild hemolysis	7
HbQ Iran	normal	4
Hb Hasharon	hemolytic anemia induced by drugs, oxidative damage	3
Hb Moabit	hemolytic anemia	3
Hb Quin Hai	normal	3
HbN Baltimore	normal	3
Hb Sallanches	unknown	3
Hb Freiburg	hemolysis, cyanosis	2
Hb Stanleyville II	falsely elevated HbA_{1C}	3
Hb Hamadan	normal	2
HbK Woolwich	normal	2
HbP Nilotic	normal	1
Hb Alberta	erythrocytosis	1
Hb Agenogi	anemia	1
Hb Bethesda	erythrocytosis	1
Hb Hofu	mild anemia	1
Hb Corfu	normal	1
Hb Ohio	erythrocytosis	1
Hb Okayama	falsely elevated HbA_{1C}	3
Hb Providence	erythrocytosis	1
Hb Pyrgos	erythrocytosis	1
Hb St. Louis	hemolysis, cyanosis	1
HbO Padova	normal	1
Hb Andrew Minneapolis	erythrocytosis	2
Hb Mizushi	normal	1
Hb Hope	normal to mild anemia	1
Hb Stanleyville I	normal	1
Hb Osu Christiansborg	normal	1
Hb Beckman	chronic hemolytic anemia microcytosis	1
Hb La Lamentin	normal	1
Hb Westmead	mild anemia	1
Hb Bunbury	erythrocytosis	1
Hb Fontainebleau	normal	1
Hb Bicêtre	hemolytic anemia	1
Hb Camden	normal	1
Hb Chesapeake	erythrocytosis	2
Hb Ernz	normal	2
Hb Handsworth	normal	1
Hb Paksé	mild anemia	1
Hb Agrinio	mild anemia, microcytosis	1
Hb Saint Etienne	hemolytic anemia	1
Hb Siriaj	normal	1
Hb Evanstone	mild anemia, microcytosis	1
Hb Pitié Salpêtrière	erythrocytosis	1
Hb Bronovo	mild microcytic anemia	1
Hb West One	normal	1
Hb Khartoum	normal	1
Hb Knossos	mild anemia, microcytosis	1
Hb Buenos Aires	normal	1
Hb Waimonolo	normal	1

Hb, hemoglobin

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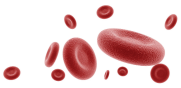

Memo

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