



Hemoglobin

Reference range

Men	140–180 g/L
Women	120–160 g/L

Hemoglobin forms in the normal adult:

	Amount	Structure
Hb A ₁	96–98%	α2β2
Hb A ₂	1.5–3.2%	α2δ2
Hb F	0.5–0.8%	α2γ2

Color of the hemoglobin

Oxyhemoglobin absorbs light in the range 650–750 nm markedly less strongly than desoxyhemoglobin. This is why oxygen-rich arterial blood is bright red and venous blood dark red.

Hemoglobin determination with hematology machines

The hemoglobin concentration can be measured using a photometer thanks to its color.

To do so, one must first release the hemoglobin from the erythrocytes by completely lysing them.

Important: If the lysis solution in the hematology machine is empty or no longer good, the solution to be measured will be turbid due to the insufficiently lysed erythrocytes, leading to falsely high Hb readings.

The dissolved hemoglobin is stabilized in the lysis solution by further additives, such that it can no longer change color. Formerly potassium cyanide was used. Nowadays cyanide-free lysis reagents such as SLS or Biolyse are used for environmental protection reasons.

Introduction

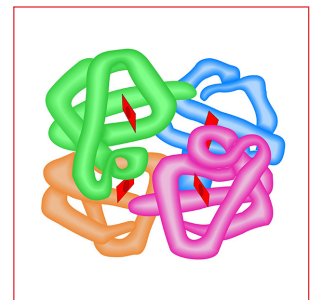
Hemoglobinopathies are disorders of hemoglobins formation. They are amongst the commonest single gene inherited disorders worldwide. They are especially widespread in the Mediterranean regions, the Near East, parts of Asia and West Africa. However, migration means that the hemoglobinopathies are also becoming increasingly important today in our latitudes. In addition, there are variants, e.g. disturbances of the O₂ transport function, which occur in all ethnicities. Diagnostic work-up generally occurs in microcytic anemias without iron deficiency, chronic hemolytic anemias, occlusive vascular crises of uncertain etiology, known familial hemoglobinopathy or in couples trying for children where one partner has a known hemoglobinopathy.

There is a distinction between quantitative disturbances with reduced formation of normal globin chains (thalassemias) and qualitative disturbances in which structurally abnormal hemoglobins is formed (e.g. HbS in sickle cell disease or HbC). Mixed forms are also common. Our inter-laboratory test preparation 2015-03 H3A derives from a 48-year-old female patient with compound heterozygosity for HbS/HbC (HbSC).

Normal hemoglobins

Hemoglobin is the protein which permits the erythrocytes to transport oxygen from the lungs to the tissues. There are 640 million hemoglobin molecules in a normal erythrocyte, which gives blood its red color.

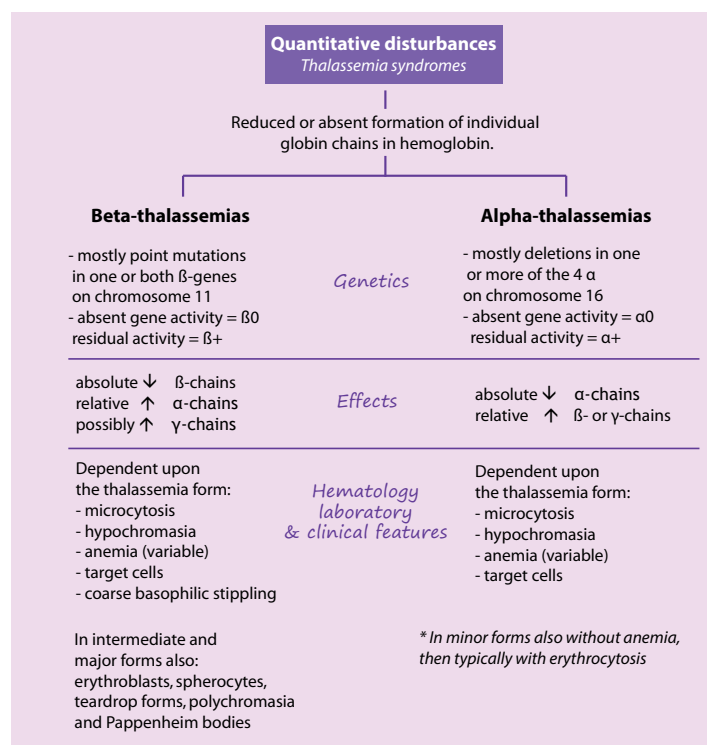
Hemoglobin is made from four protein chains, which each contain a heme group. This heme group is a disc-shaped organic molecule, which contains an iron ion (Fe²⁺) in its center. On one side, the iron is bound to the protein, on the other side it can bind to oxygen.



Humans produce four different types of protein subunit, which are identified by Greek letters (α,β,γ,δ). In normal adults, hemoglobin consists mainly of Hb A, a combination of two alpha- and two beta-chains.

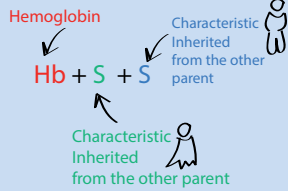
Hemoglobin which does not contain any oxygen is referred to as deoxyhemoglobin. As soon as it finds a molecule of oxygen, it undergoes a structural transformation, which allows the other three sites to bind oxygen more readily. The hemoglobin is then referred to as oxyhemoglobin.

Thalassemia syndromes





Hemoglobin anomalies Inheritance and notation



Normal	HbA _{1A2}
Heterozygosis	HbAS
Homozygosis	HbSS
Compound Heterozygosis	HbSC (z.B.)

Heterozygosis

The defect is inherited from one parent.

Homozygosis

Both parents pass on the same defect in the same gene.

Compound heterozygosis

Both parents pass different defects, which affect the same genetic locus

Diagnosing the hemoglobinopathies

- complete hematogram (specifically: Ec, Hb, Ec indices, RDW)
- Differential blood picture, Ec morphology
- Hemoglobin chromatography
- possibly DNA analysis

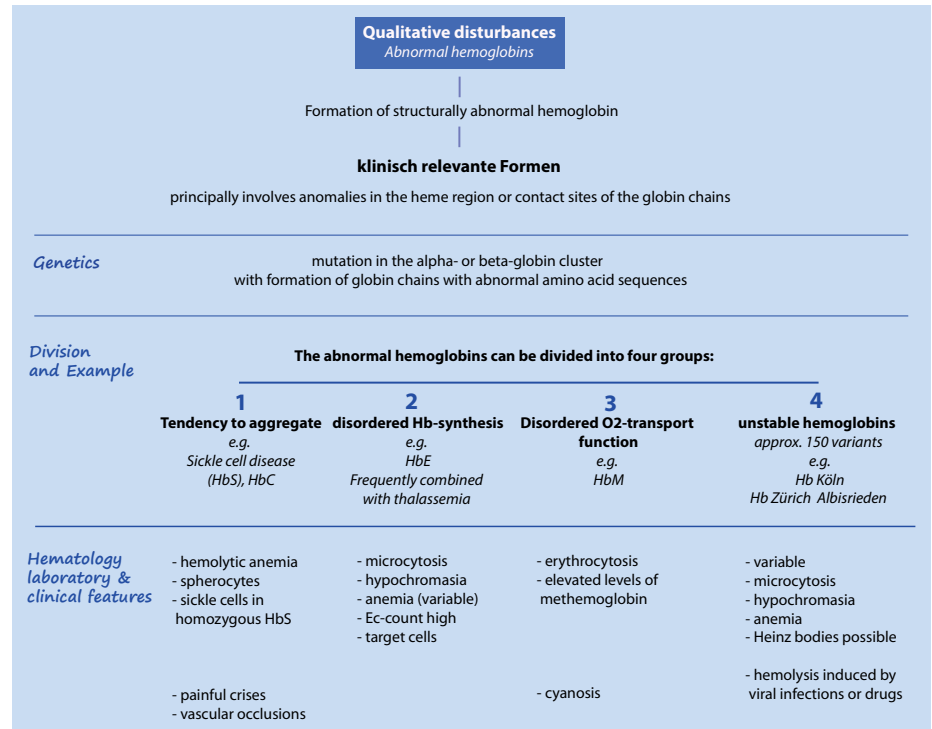
The hematogram values in iron-deficiency anemia and hemoglobinopathies can be very similar. The microscopic blood picture differentiation on the basis of erythrocyte morphology often gives valuable clues to the probable cause in this setting and so permits targeted prescription of additional laboratory diagnostic tests.

About
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To date, approaching 500 abnormal hemoglobins are recognized. Many of them cause no clinically detectable illness. The clinically relevant forms can be divided into 4 different groups (see table above). The forms in groups 3 and 4 can already cause severe illnesses in heterozygotes and homozygous carriers may, depending upon the abnormal hemoglobin, be incompatible with life. The commonest abnormal hemoglobins are HbS, HbC and HbE.

Hemoglobin SC disease

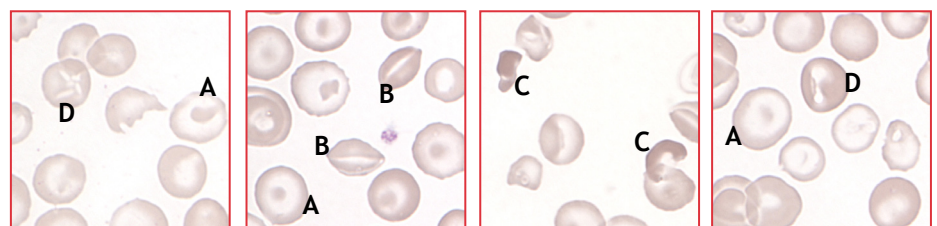
The patients have a combination of two abnormal hemoglobin chains. One half of the beta-chains contains the sickle cell mutation, the other half the hemoglobin-C mutation.

In the homozygous sickle cell disease (Hb SS), the 6th amino acid in the beta chain of the hemoglobin is valine instead of glutamic acid. This tiny change results in the hemoglobin molecules sticking to each other in the deoxy form to make polymers.

In the homozygous hemoglobin-C disease (Hb CC) the 6th amino acid of the beta chain of the hemoglobin is lysine instead of glutamic acid. The patients mostly have mild microcytosis and anemia, and with increasing age also mild splenomegaly, but they are often asymptomatic.

The blood picture shows neither the typical sickle cells nor HbC crystals as are seen in HbC disease. Morphological features in the erythrocytes arise, however, as a result of an altered erythrocyte surface area/volume ratio. Thus, one frequently sees target cells, bizarre folded erythrocyte forms and elongated stomatocytes, which resemble a folded pitta bread optically.

SC-poikilocytes can also occur, and are irregularly formed, darkly pigmented (hemoglobin conglomerates) and contain crystalline material (paler inclusions). Occasionally HbS-induced boat-shaped forms occur.



Pictures of MQ 2015-3 H3A

A: Target cells, B: elongated stomatocytes (pitta bread forms), C: SC-poikilocyte, D: bizarre folded Ec