

Hematology

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Hematology

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Sickle cell anaemia

General

Sickle cells are much less flexible than normal erythrocytes. They therefore have difficulty in passing through capillary vessels, the diameter of which is often less than half the diameter of a red blood cell. Janet Watson noted that symptoms appeared in infants only after concentrations of fetal haemoglobin (Hb F) had fallen, establishing the notion of the beneficial effect of Hb F on disease manifestations.

Sickle cell anaemia, haemoglobin

Haemoglobin structure

Each molecule of haemoglobin consists of a tetramer consisting of 2 pairs of polypeptide chains to which a total of 4 haem groups (one haem group per globin chain) is linked. One molecule of haemoglobin therefore contains 4 proteins. Hb A contains 2 globin chains of one type (alpha) and 2 globin chains of another type (beta). Depending on which 4 chains are present in the haemoglobin tetramer, the molecule is given its name.

There are many haemoglobin variants:

Normal

Hb A: $\alpha_2\beta_2$

Hb A2: $\alpha_2\delta_2$

Hb F: $\alpha_2\gamma_2$

Pathological

Hb S: $\alpha_2\beta_2^S$

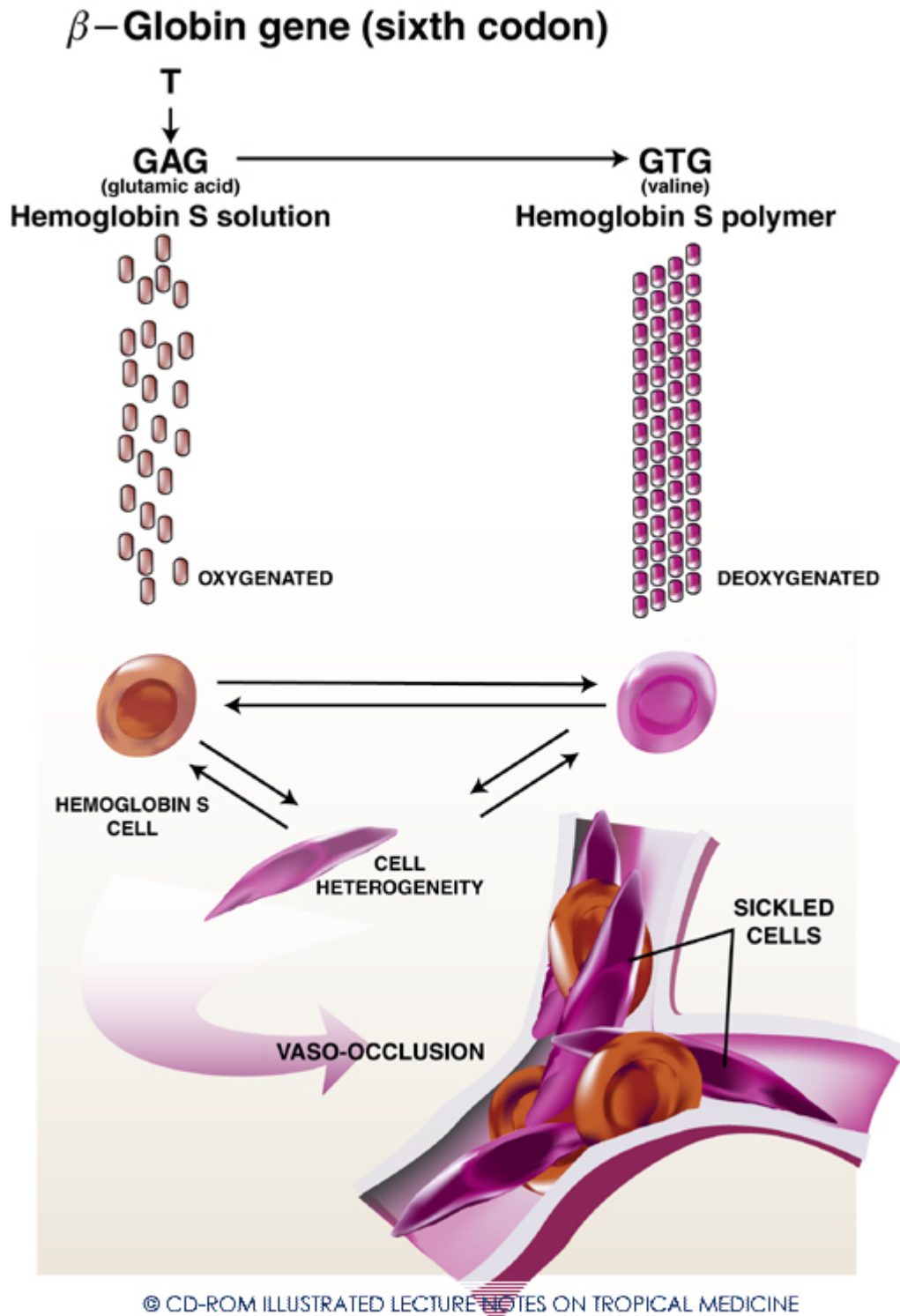
Hb C: $\alpha_2\beta_2^C$

Hb E: $\alpha_2 \beta_2^E$

Hb H: β_4 (see alpha-thalassemia)

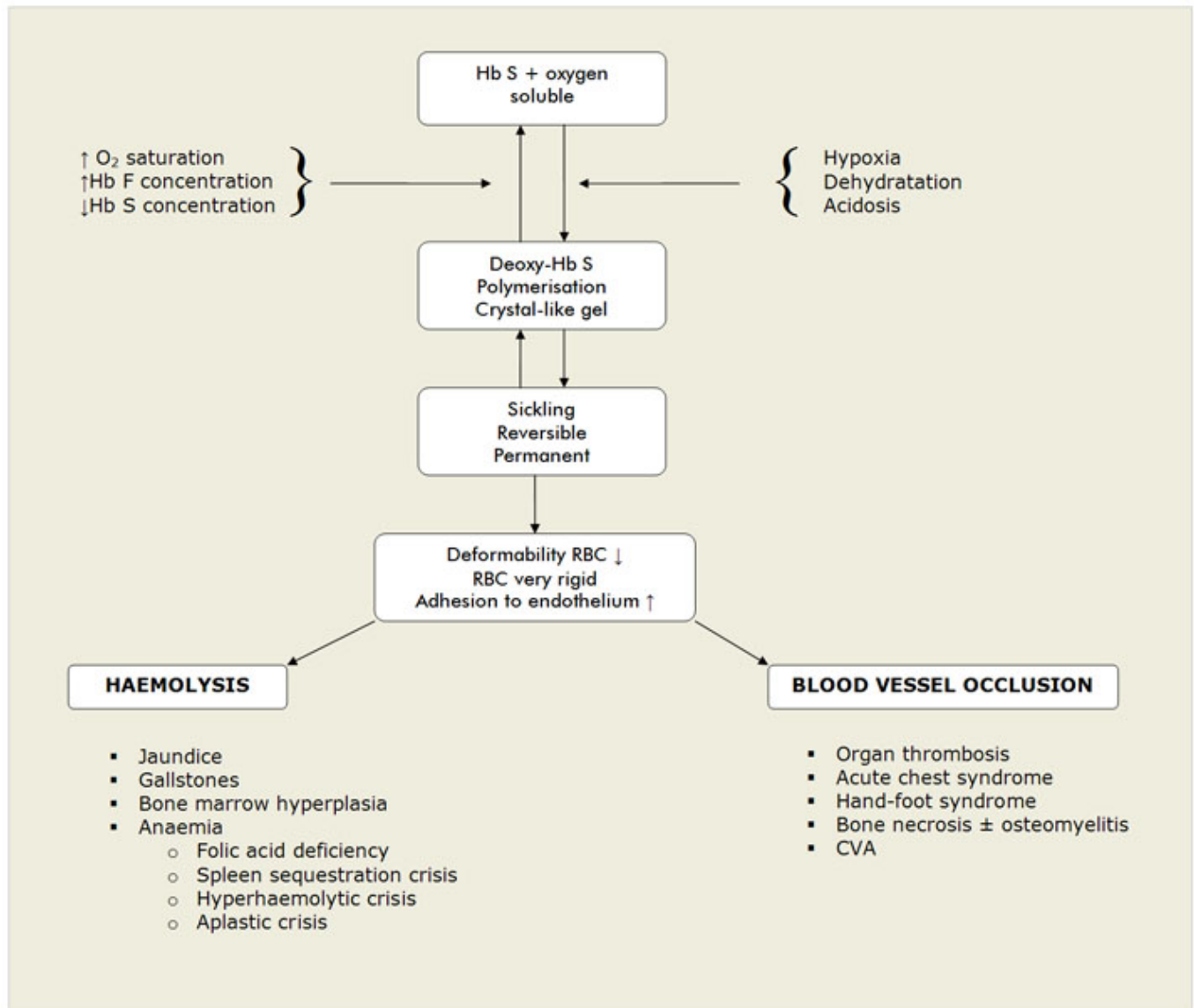
Hb Barts: γ_4

Physiopathology



Pathophysiology of sickle cell anaemia. Deoxy-haemoglobin S undergoes time-dependent polymerisation. This changes the shape of the red blood cells, which can block the microcirculation.

Adapted from a drawing in New England Journal of Medicine, 2002.



The normal haemoglobin of a child or an adult are Hb A (97%), Hb A₂ (2%) and Hb F (1%). Hb A contains 2 alpha-chains and 2 beta-chains. If by mutation, the 6th amino acid of the β-chain (glutamic acid, negatively charged) is replaced by a different amino acid (valine, hydrophobic), Hb S is formed. As a result a hydrophobic site is formed on the outside of the folded mutated beta chain. With normal arterial oxygen tension there is no problem and the molecule transports the oxygen. In the capillary bed in the tissues the oxygen is released and deoxyhaemoglobin S is formed. This latter substance has several different properties. In deoxy-Hb S there is a second hydrophobic site on the surface. This

site is concealed in oxy-Hb S. The site is complementary to the first. These two hydrophobic regions adhere to each other, resulting in a kind of polymerization of the deoxyhaemoglobin S molecules. The hydrophobic valine on the surface makes the haemoglobin molecule somewhat less water-soluble if the molecule is not bound to oxygen. The concentration of haemoglobin in the erythrocyte (32-34 g %) does however require a very water-soluble molecule. The deoxyhaemoglobin S molecules start to come out of solution (precipitate). At low oxygen concentrations the deoxyhaemoglobin S molecules adhere to each other, forming long, rigid strands and thus deform the red blood cells making them more rigid. The molecules stick to each other in a definite pattern (like a crystal). This polymerization reaction is relatively slow, giving a “delay time” or T_d . The slower the circulation and therefore the longer the time before reoxygenation in the lungs, the more sickling occurs. Usually the transit time of a red blood cell in the microcirculation is less than T_d and a major catastrophe is avoided.

The main variables that affect sickling are the intracellular haemoglobin concentration, pH, the level of oxygenation and the percentage of Hb F. Sickling is accelerated by lack of oxygen, slow blood circulation, acidification and dehydration (a situation which is common with infections). The formation of rigid Hb SS strands is counteracted by Hb F (efficiency of polymerization is reduced). People with high concentrations of Hb F have far fewer symptoms than patients with low Hb F concentrations.

The sickling process also causes damage to various membrane proteins of the erythrocyte, thus promoting adhesion to the vascular endothelium. This makes circulation even more difficult. The degree of adherence is closely correlated to the severity of the disease. If there is inflammation, this “stickiness” can increase even more.

Additional factors play a part in the pathophysiology of sickle cell disease: endothelial cells can be “activated” i.e. they can be induced to express all kinds of molecules on their membrane, after exposure to various inflammatory substances (cytokines, prostacyclin’s, etc). Such cells become “sticky” and promote local haemostasis and possibly thrombosis. There is also an increase in the number of adhesion molecules on the red blood cells. The local production of Nitric oxide (NO) by the damaged endothelium falls.

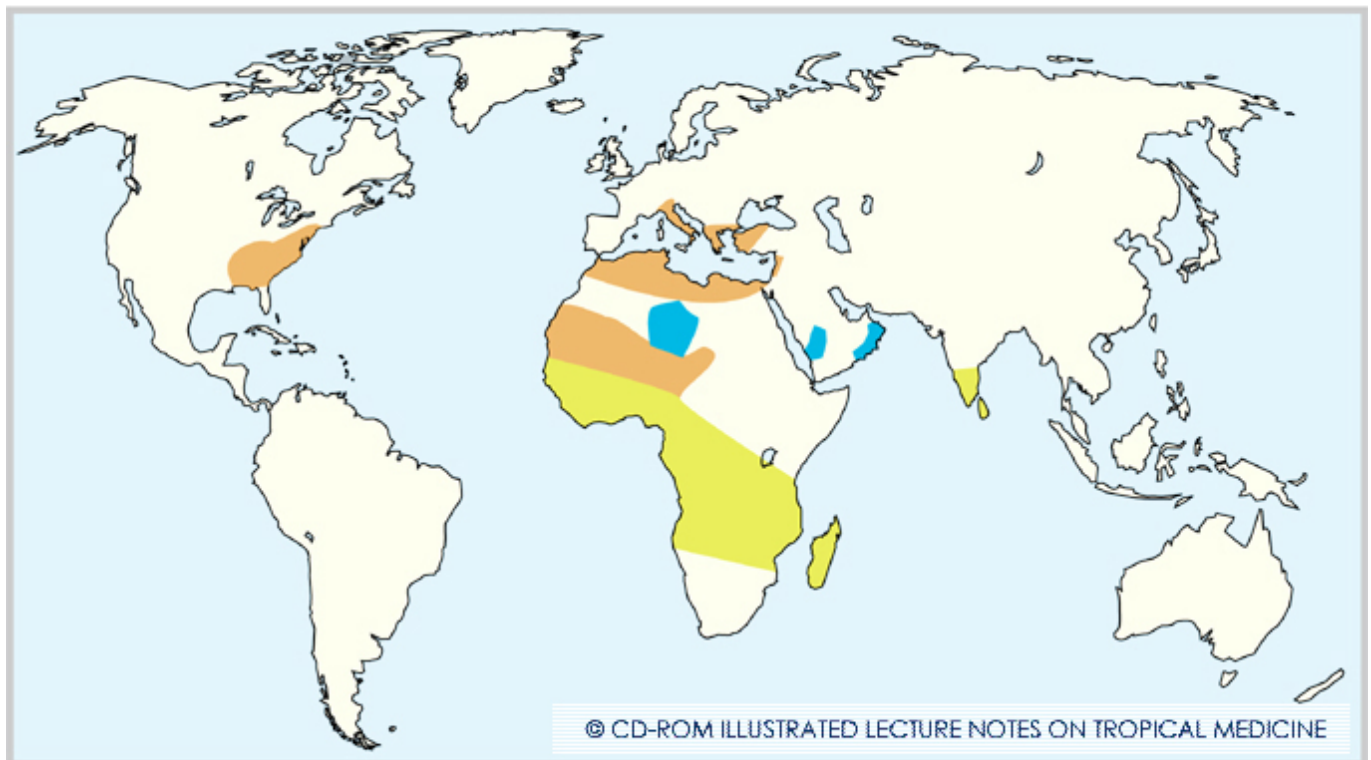
Nitric oxide function

Nitric oxide (NO) produced by endothelial cells causes vasodilatation (effect is concentration dependent). Free haemoglobin in plasma will capture NO, thereby diverting nitric oxide from its homeostatic vascular function.

What are the clinical consequences of sickling?

- Sick cells rapidly haemolyse. As a result, anaemia occurs: sickle cell anaemia.
- Sick cells are rigid and obstruct the microcirculation. As a result, small or large infarctions can occur.
- Tissues with poor blood circulation can be infected more easily.
- Due to splenic atrophy, resistance to certain pathogens is reduced.

Geographical distribution



**Geographic
distribution of Hb S (1961)**



Map sickle cell disease (drepanocytosis). Due to the slave trade, the disease also exists in North America, but is especially common in areas where *Plasmodium falciparum* is frequent. Copyright ITM

The sickle cell gene occurs in large parts of Africa and to a somewhat lesser extent in the Middle East (Saudi Arabia) and India. In West Africa, 5 to 25% of the population are carriers of the gene. In Central and East Africa, heterozygotes occur with a frequency of from 20 to 40%. If 20% of the population are carriers of the gene, it follows that 1% of newborn children will be homozygous. Through the slave trade the sickle cell gene also found its way to North and South America.

Heterozygous carriers are relatively protected against fatal *P. falciparum* malaria. They are infected just as often, but are less likely to die from the infection. If the malaria parasite is present within the erythrocyte, the red blood cell acidifies slightly. This is enough to promote sickling. Because of the damage to the membrane; potassium flows out of the red blood cell, which is damaging to the parasite and the erythrocyte. The red blood cell is rapidly destroyed, for example in the spleen (heterozygotes have a normal spleen). Since heterozygotes in an endemic malaria area have a longer life expectancy than people with normal haemoglobin, it is thought that this has promoted the occurrence of sickle cell haemoglobin in Africa over the course of evolution. On the other hand, homozygous Hb S people have a very low life expectancy. There will therefore be a genetic equilibrium.

Sickle cell anaemia, genetics and heredity

Sickle cell anaemia is a genetically determined disease. A distinction is made between three main groups: homozygotes, heterozygotes and double heterozygotes.

Hb SS disease: classic sickle cell anaemia

Hb AS: sickle cell trait, heterozygote

Hb S/Beta⁰-thalassemia; Hb SC: severe double heterozygote; phenotypical similar as Hb SS

Heterozygosity (“sickle cell trait”)

If someone has both a normal gene (from one parent) and a mutated gene (from the other parent), they produce both the normal haemoglobin (Hb A) and also the sickle cell haemoglobin (Hb S). One would expect a heterozygote Hb AS to have about 50% haemoglobin A and about 50% haemoglobin S, but for a variety of reasons, the average patient has about 2/3 Hb A and 1/3 Hb S. The person is an asymptomatic carrier and each red blood cell contains both Hb A and Hb S. Such erythrocytes are functionally normal and have the advantage that they provide relative protection against fatal *Plasmodium falciparum* infection. Heterozygotes lead a normal life. But they may well pass the gene

on to their children

Probability per child of having the different haemoglobins:

Parent Hb AA x Parent Hb AS → 50% probability of Hb AA and 50% probability of Hb AS

Parent Hb AS x Parent Hb AS → 25% probability of Hb AA, 25% probability of Hb SS and 50% probability of Hb AS

Homozygosity

If a patient has two identical mutated genes (homozygote) they cannot produce Hb A. After birth, the Hb F concentration falls and after 3 to 6 months, the red blood cells contain mainly haemoglobin Hb S. This will lead to sickle cell disease.

Double heterozygotes

Certain double heterozygotes can display a sickle cell phenotype.

1. haemoglobin SC
2. haemoglobin SD
3. haemoglobin SO Arab
4. haemoglobin S beta thalassemia
5. haemoglobin S with haemoglobin New York

Sometimes a child has both a sickle cell gene and also a gene for haemoglobin C. It then has both haemoglobin S and haemoglobin C (Hb SC). Doubly heterozygous people suffer a less serious course of the disease than homozygous sickle anaemia patients. They have a clearly increased risk of eye damage (retinitis proliferans), avascular necrosis of the head of the femur, haematuria and complications during pregnancy (pulmonary infarction and risk of fat embolism after bone marrow infarction).

A little caveat: patient who are homozygous for Hb SS can have Hb A in their blood after a blood transfusion. Don't be misled by this.

Clinical aspects

It is possible to distinguish two clinical phenotypes of sickle cell disease. The first is dominated by haemolysis and is characterized by severe haemolytic anaemia, leg ulcers (especially lower legs and around ankles) and pulmonary hypertension. The second is dominated by vaso-occlusion incidents, with episodic painful crises, acute chest syndrome, splenic infarction leading to functional asplenia, stroke and avascular necrosis of joints (hip, humerus) predominate.

Vaso-occlusive complications

Pain episodes	In more than 70% of patients.
CVA	In 10% of children; "silent" lesions with cognitive damage in 50-90%.
Acute chest syndrome	In 40% of patients, more often in children.
Priapism	In 10-40% of men. Severe cases lead to permanent dysfunction.
Liver disease	In <2%. Multiple causes: hep B, C, iron overload.
Spleen sequestration	In children < 6 years of age. Often preceded by infection.
Spontaneous abortion	In 6% of pregnant women.
Skin ulcers (leg)	In 20% of adults
Osteonecrosis	In 10-50% of adults (often femur, humerus).
Proliferative retinopathy	Rare in sickle cell anaemia; in 50% with Hb SC.
Renal insufficiency	In 5-20% of adults, often with severe anaemia.

Complications of haemolysis

Anaemia	Haematocrit often 15-30%.
Gallstones	In the majority of adults, usually asymptomatic.
Red bone marrow	Expansion leads to weakened cortical bone.

Infectious complications

Streptococcus pneumoniae	Sepsis in 10% of children < 5 years.
Osteomyelitis	Often by Salmonella or Staphylococcus aureus.
Escherichia coli sepsis	In adults often originating from infection of the urinary tract.
Acute aplastic crisis	Due to parvovirus B19. Sudden severe anaemia.

Individuals with sickle cell trait are generally asymptomatic and have no abnormal physical findings. Their laboratory evaluation often shows microcytosis but is otherwise normal with no anaemia, no evidence of haemolysis and no laboratory abnormalities other than haemoglobin AS on haemoglobin electrophoresis. Complications such as splenic infarction, pain episodes and sudden death may be induced by severe hypoxia, severe dehydration, and exertion at the limits of human endurance, e.g. at high altitudes.

Homozygous (Hb SS) children with little Hb F have the clearest symptoms. The symptoms result from haemolysis, thromboses, infections and acute haematological crises. In rural Africa only a few survive beyond puberty. In the first few months after birth the baby is virtually normal (the Hb F concentration is still high). The first problems start at about 3 to 6 months.

Haemolysis



Suana Ferdinand. Radiographie du
(23-avril 1952)
Crâne en grosse. (profil)

FIGURE -3.

"Hair-on-ends" image caused by bone marrow expansion in the diploic space in chronic haemolytic diseases, such as sickle cell anaemia or more commonly in beta thalassemia major. The major trabecular spicules in the diploë are aligned perpendicular to the inner table in an effort to support the soft outer table. Photo ITM



Fish vertebrae in sickle cell anaemia. Marrow expansion makes the vertebrae more susceptible to compression, leading to this diabolo-shape. Copyright ITM

Chronic haemolysis manifests itself as pallor, mild jaundice, dark urine and retarded growth. There is hypertrophy of the bone marrow, which can often be seen in the cranium and the maxillae. But the expansion of the bone marrow is less pronounced than in homozygous β -thalassemia, possibly because less erythropoietin is produced than expected due to repeated kidney damage. Due to the constant haemolysis and the production of bilirubin; bilirubin gallstones are produced at a very young age (such stones are often not radio-opaque). There is splenomegaly up to about 5 years, afterwards there is atrophy because of the repeated infarctions of the spleen. The expansion of the bone marrow can usually be seen clearly by frontal “bossing”, a pronounced curving of the forehead and by widely spaced teeth in the jaws. On an X-ray of the cranium, small canals in the diploë of the vault of the cranium and are known as a “hair-on-end” appearance (see Fig).

Acute haematological crisis

Haematological crises sometimes occur. In children aged from 6 months to 3 years the spleen can sometimes swell acutely (sequestration of blood in the spleen), with sudden anaemia, hypovolemia and shock as a result. Many do not survive this. Due to infections, such as malaria for example, hyperhaemolysis can occur. After certain viral infections (for example parvovirus B19) a period may follow during which the bone marrow does not form any new red blood cells (aplastic crisis). Bone marrow arrest can also occur if there is a marked folic acid deficiency.

Thrombosis

Thrombosis is manifested most commonly as episodes of pain but also can produce kidney infarctions (haematuria, papillary necrosis), priapism, atrophy of the spleen, bone necrosis (head of the femur, head of the humerus, metacarpals, vertebrae), cerebrovascular accident (CVA), including the rather rare Moyamoya syndrome (collateral circulation developing around blocked vessels, these collateral vessels are prone to bleeding aneurysm and thrombosis; moyamoya means “puff of smoke” in Japanese referring to the appearance of the collateral vessels on MRI), chronic skin wounds (mainly on the shins) and proliferative retinopathy. Hand-foot syndrome is sometimes the first clinical manifestation. The child then has acutely painfully swollen hands and feet. Chronic damage to the vertebrae leads to biconcave vertebrae (“fish vertebrae”; see Fig) with a typical appearance on X-ray.

Due to kidney damage, patients with sickle cell anaemia usually have difficulty in concentrating their

urine and are susceptible to dehydration. Hyposthenuria may become evident in childhood as enuresis.

Glomerular sclerosis, manifested by proteinuria, progresses as patients age. Chronic renal failure occurs in up to 5% of patients with sickle cell anaemia. Pulmonary infarctions contribute to acute chest syndrome, with pain, dyspnoea and a poor general condition. Small cerebral watershed infarcts may be clinically silent but produce cognitive defects shown by neuropsychiatric testing. Hemiplegia can result from cerebral infarction. Most patients with brain injury require long term transfusion therapy.

Eye problems

Occlusion of small retinal vessels with neovascularization is asymptomatic until haemorrhage occurs within the vitreous. Detachment of the retina, more common in late disease, is a feared complication, and an important cause of blindness, together with occlusion of the central retinal artery. The latter condition is a medical emergency, for which urgent transfusion is imperative.

Autosplenectomy



Splenic atrophy in sickle cell anaemia; photo Dr Van den Enden, ITM

Because of the repeated infarctions of the spleen, sickle cell anaemia patients over the age of 5 years no longer have a functioning spleen. Asplenic children are very susceptible to bacterial infections, including pneumococci (i.e. encapsulated bacteria, *Streptococcus pneumoniae*). Osteomyelitis caused by among others, *Salmonella* and staphylococci is common. Often it is difficult to distinguish between pulmonary infarction and pneumonia and between osteomyelitis and bone infarction.

Acute chest syndrome

This syndrome consists of a collection of problems, such as acute chest pain, dyspnoea, coughing, fever, hypoxemia, leukocytosis and pulmonary infiltrates, mainly in the inferior lobes. This can develop into a full-blown ARDS (Acute Respiratory Distress Syndrome). Bone marrow infarctions followed by fat and even bone marrow embolism play a part (beware of sickle patients with first pain in limbs, followed by chest problems). Atelectasis also contributes and often develops as a result of hypoventilation that accompanies rib pain and the use of opiates. At autopsy in 75% of fatal cases bone spicules are found in the lung. In 60% of patients with acute chest syndrome, fat-loaded

macrophages are found in the broncho-alveolar fluid. Because of the pain in the chest wall, patients are able to breathe less deeply (“splinting”) with hypoventilation, atelectasis and perhaps superinfection as a result. Hypoxemia increases the adhesion of red blood cells to the endothelium, via the increased expression of the adhesion molecule VCAM-1 on the endothelium. Breathing in regularly, as deeply as possible, is an important part of treatment (“incentive spirometry”). The patient is asked to breathe in deeply 10 times and to do this every two hours while awake. There is a clear role of opioids in promoting to control pain with careful monitoring to avoid over-sedation and hypoventilation. The administration of oxygen, antibiotics and standard or exchange transfusion completes the treatment. In a good hospital, the mortality of acute chest syndrome is 2% for children and 5% for adults.

Pulmonary hypertension

Pulmonary hypertension is a feared complication in chronic and severe haemolytic anaemias, such as thalassemia major, congenital spherocytosis and paroxysmal nocturnal haemoglobinuria. Pulmonary hypertension occurs in about one third of all patients with sickle cell disease. Asplenia increases the circulation of platelet-derived mediators, which promotes pulmonary microthromboses and adhesion of erythrocytes to the endothelium. Haemolysis results in the release of free haemoglobin, which scavenges nitric oxide, causing vasoconstriction.

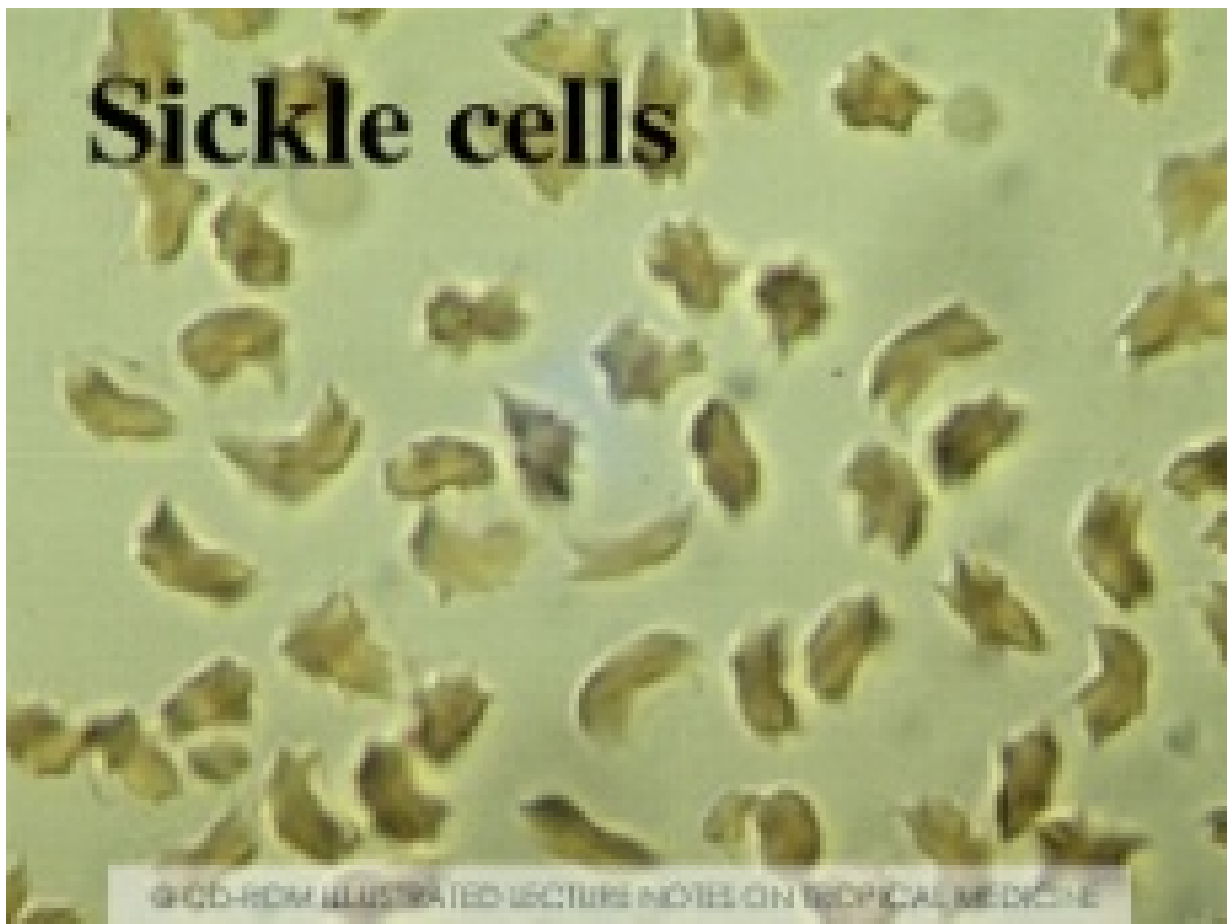
Priapism

Priapism is a persistent and painful erection [Lat. priapus, God of procreation]. It is not associated with sexual stimulation. It is an important complication of sickle cell disease. By adulthood, 90% of males with sickle cell anaemia will have had a least one episode of priapism. The blood that flows into the corpora cavernosa of the penis has difficulty leaving the organ due to venous thrombosis. Because of acidification and hypoxia, sickling of red blood cells increases still further. If priapism persists longer than 4 hours, surgery is definitely required. Persistent priapism (>24 hours) results in fibrosis and impotence. As an initial treatment the patient can be made to go up and down stairs in order to divert blood flow to the leg muscles (the “steal mechanism” principle) or have external compression of the perineum applied, perhaps with ice. General measures such as hydration, (exchange) transfusion and analgesics are necessary. Aspiration and irrigation of the corpus cavernosum with or without saline irrigation is necessary in an episode of priapism lasting more than four hours. The alpha-adrenergic agonist phenylephrine can be injected in the corpora cavernosa, causing blood to leave the corpora cavernosa due to smooth muscle contraction in the penile arteries.

Diagnosis

Laboratory

A sickling test can be carried out in field laboratories (Emmel's test). In this, a drop of blood is placed on a glass slide. This is covered with a coverslip and the edges are sealed with some vaseline (to prevent contact with the air). As time goes by and the oxygen in the blood falls further (due to the metabolism of the cells) the red blood cells will sickle. This test can be accelerated by adding a drop of sodium metabisulphite to the blood.



Red blood cells of a homozygote sickle cell anaemia patient undergo dramatic change in shape when oxygen is excluded from their environment (Emmel test). Copyright ITM

Heterozygotes

Since normal and mutated beta chains are produced equally rapidly, it may be expected that heterozygotes would have $\pm 50\%$ Hb S and $\pm 50\%$ of Hb A. However, because alpha chains bind more easily to the normal beta chains than the mutated forms, there is a relative excess of mutated beta chains in the tetramers. The excess mutated chains are then destroyed. As a result, most heterozygotes have about 35% Hb S rather than 50%, and about 65% Hb A. The diagnosis of sickle cell trait is established by haemoglobin electrophoresis. If a non-transfused patient with sickle cell disease would have e.g. 65% HbS and about 30% HbA, especially if Hb A2 would be elevated, the suspicion of Hb S/beta⁺thalassemia would be strong.

Homozygotes

There is severe anaemia (usually Hb 6-9 g%) with considerable reticulocytosis. A blood smear of a homozygote shows many sickle cells, in contrast to that of a heterozygote. The diagnosis can be confirmed by haemoglobin electrophoresis. On electrophoresis it can be seen that most of the haemoglobin consists of Hb S (often more than 80%); the remainder consists of Hb F and Hb A2. Of course, no Hb A can be found. There is often thrombocytosis and leucocytosis.

Treatment

General

Apart from bone marrow transplantation, there is no curative therapy. Hematopoietic stem cell therapy and gene therapy remain possibilities for the future. The suffering of children can be reduced.

It is possible to stimulate the induction of haemoglobin F by medication. In contrast with haemoglobin A2 (alpha2 delta2), a minor haemoglobin which is uniformly distributed in all adult red cells, haemoglobin F is found (in normal people) in 0,2 to 7 percent of the adult red cells, and in those cells, it constitutes 14 to 28 percent of the total haemoglobin. They are called "F"cells. Hb F contains gamma chains instead of beta chains (structure alpha2 gamma2). Hb F has a greater affinity for oxygen than Hb A. This helps the fetus to draw oxygen from the mother's blood. Hb F inhibits the polymerization of deoxy-Hb S. This inhibits the sickling of red blood cells. After birth, the neonate still has more than 50% Hb F in his blood.

This explains why very young children are free of sickling crises. After birth the genes for the gamma

chains are less active because they become methylated. This is reversible however.

Hydroxyurea is a mainstay in the treatment of sickle cell anaemia. Hydroxyurea (Hydrea®) is a cytostatic drug which was long used in patients with polycythemia vera or chronic myeloid leukaemia, to counter hyperleukocytosis. Another important effect of hydroxyurea is production of nitric oxide (NO), a vasodilator. The anti-sickling activity results from induction of haemoglobin F through activation of a specific promoter for the haemoglobin gamma-chain gene. There is also a reduced expression of adhesion molecules (e.g. VCAM-1, L-selectin), as a result of which red blood cells and neutrophils adhere less easily to the vascular endothelium. Hydroxyurea can be used in prevention (not in an acute crisis). Hydroxyurea reduces the frequency and the severity of the attacks (up to 40% decrease in mortality).

Maintenance treatment

1. **Folic acid.** The patients have a greater need for this vitamin due to the high demands of the red bone marrow. It is important to check vitamin B12 status, in order not to mask a cyanocobalamin deficiency.
2. **Penicillin prophylaxis** is given to reduce the number of infectious episodes. Today vaccination with **pneumococcal vaccine** lessens the importance, but prophylactic penicillin is still recommended to all children with sickle cell diseases till the age of 5 years. Penicillin V 125 mg orally twice daily till the age of 3 years is increased to 250 mg twice daily until the age of 5.
3. **Zinc.** Up to 20% of sickle cell anaemia patients have persistent leg wounds. Many patients tend to be zinc deficient, possible via excessive renal excretion due renal damage secondary to repeated infarctions in the hypertonic renal medulla. Zinc is a trace element needed for certain enzymes, including some metalloproteases which are important in wound healing. Zinc deficiency makes wounds heal more slowly. Zinc sulphate or zinc acetate per mouth (e.g. 30 mg per day) can help here, but is often given as part of multivitamin supplements (without iron). It is important to mention that prolonged treatment with hydroxyurea can lead to slower healing of leg ulcers.

Additional important measures

1. **Immunizations** are a cornerstone to prevent infections in sickle cell disease: routine childhood vaccinations are recommended including vaccination against *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae B* and hepatitis B. Yearly influenza vaccination is advised.
2. **Antibiotics.** Every patient must have at home a stand-by broad-spectrum antibiotic such as co-amoxiclav. Azitromycin can be used in case of penicillin-allergy. They should take this at the first

signs of infection. The reason for having the antibiotic at home is that patients often live a long way from a hospital and might lose lots of precious time before they are seen by a medical doctor.

3. **Malaria prevention** is absolutely indicated in an endemic area as infection with this parasite can be fatal.
4. **Preventive transfusions** are a double-edged sword and are not given routinely. With transfusions the haemoglobin level can be kept at a higher level, which reduces the consequences of anaemia. This also reduces the concentration of Hb S in the blood, thus reducing the risk of complications. However repeated transfusions gradually cause severe transfusion reactions. It is best always to give blood that is low in leukocytes. Since in the long term there will be sensitization to the minor blood groups, the red blood cells in later transfusions will be destroyed very quickly. Slow iron poisoning also occurs, damaging the heart, the liver and some endocrine organs (pancreas, testis). Iron chelation therapy is indicated. In the case of acute aplastic crisis (triggered by infection with parvovirus B19) and splenic sequestration crisis, transfusions are essential. They are also important in acute chest syndrome. Exchange transfusions are also a therapeutic option.

Hydroxyurea (= hydroxycarbamide, Hydrea®). Usually 500 mg three times daily is given in order to raise the level of haemoglobin F to above 15%. Regular checking of the number of white blood cells is indicated (it is a cytostatic drug). Another side-effect is slower healing of leg ulcers. Pregnancy is a contra-indication. Since the introduction of hydroxyurea in treatment, the quality of life for many patients has improved dramatically. Since the drug is cheap, it is not outside the means of many third-world families and hospitals.

Heredity should be explained to the parents so that they have the correct information in order to decide whether or not to have another child. If both parents are carriers, the probability of a normal child (Hb A) is 25%, the probability of a healthy heterozygous child is 50% and the probability of a homozygous Hb S child is 25%.

Management of ARDS in sickle cell crisis

The mainstay of treatment for patients with ARDS is supportive care and mechanical ventilation.

Although the ventilator can be lifesaving, it can be a source of further lung injury. A crucial intervention in the acute chest syndrome is reduction of the percentage of haemoglobin S in the patient's blood. One can use "normal" transfusions, but this also increases blood volume and viscosity. Red-cell exchange transfusion avoids this complication. The aim is to reduce the percentage haemoglobin S to well below 30%. The final (desperate) measure is the use of extracorporeal membrane oxygenation (ECMO).

What should be done in the event of a sickle cell crisis?

Antibiotics, transfusions (normal or exchange transfusion), oxygen, pain control with paracetamol-codeine, ibuprofen or morphine analogues are all part of sickle cell crisis management. Sufficient fluid should be administered because the kidneys have difficulty in producing concentrated urine. Often 3 to 4 litres a day are given (adults) if possible orally, otherwise IV. Severe acidosis is best corrected quickly with bicarbonate, although no spectacular results can be expected. In the case of rib or tissue infarctions, and also in chest disorders, it is important that the patient is urged to breathe deeply (10 maximum inspirations) at regular intervals e.g. every two hours. This prevents atelectasis. The polymerizing of Hb S is promoted strongly by dehydration. The higher the salt concentration in the blood, the more quickly the cells sickle. Patients with a sickle cell crisis often have a hypercoagulable state, thus thromboembolism prophylaxis is essential during hospitalization. This can be done with LMWH's or unfractionated heparin.

What happens if an operation is carried out?

Many homozygous sickle cell patients have to undergo surgery due to complications of their illness (mainly cholecystectomy or orthopaedic surgery) or for other reasons. Perioperative complications are common in patients with sickle cell anaemia. During anaesthesia, the operation itself and in the post-operative phase hypoxia must be avoided. Perioperative hypoxia, tissue hypoperfusion and acidosis can trigger vaso-occlusive crises and cause organ dysfunction (mainly acute chest syndrome and pain crises). Pre-operatively (exchange-)transfusion can be given.

Pregnancy and prenatal care

Pregnant homozygous sickle cell anaemia patients are rare in Africa. In the absence of medical care, mortality for mother and neonate can be as high as 20% and 50% respectively. The most common complications during pregnancy for women with sickle cell disease are hypertension and preeclampsia (14%). It has been suggested that maternal anaemia and placental ischemia may play a role, as slow placental circulation and a high degree of oxygen-extraction promote sickling. A high percentage of the pregnancies result in preterm deliveries (27%) and infants small for gestational age (21%). It is best to keep the mother's haemoglobin level above 10 gram %, although there is controversy about the use of prophylactic transfusions. It seems logical to reserve transfusions for complications, rather than use them routinely. Hydroxyurea is contra-indicated in pregnancy as it is teratogenic. However, in a small number of cases where hydroxyurea was taken throughout pregnancy, no fetal malformations occurred. During labour and delivery the mother should receive

oxygen and should be well hydrated.

Bone marrow transplantation

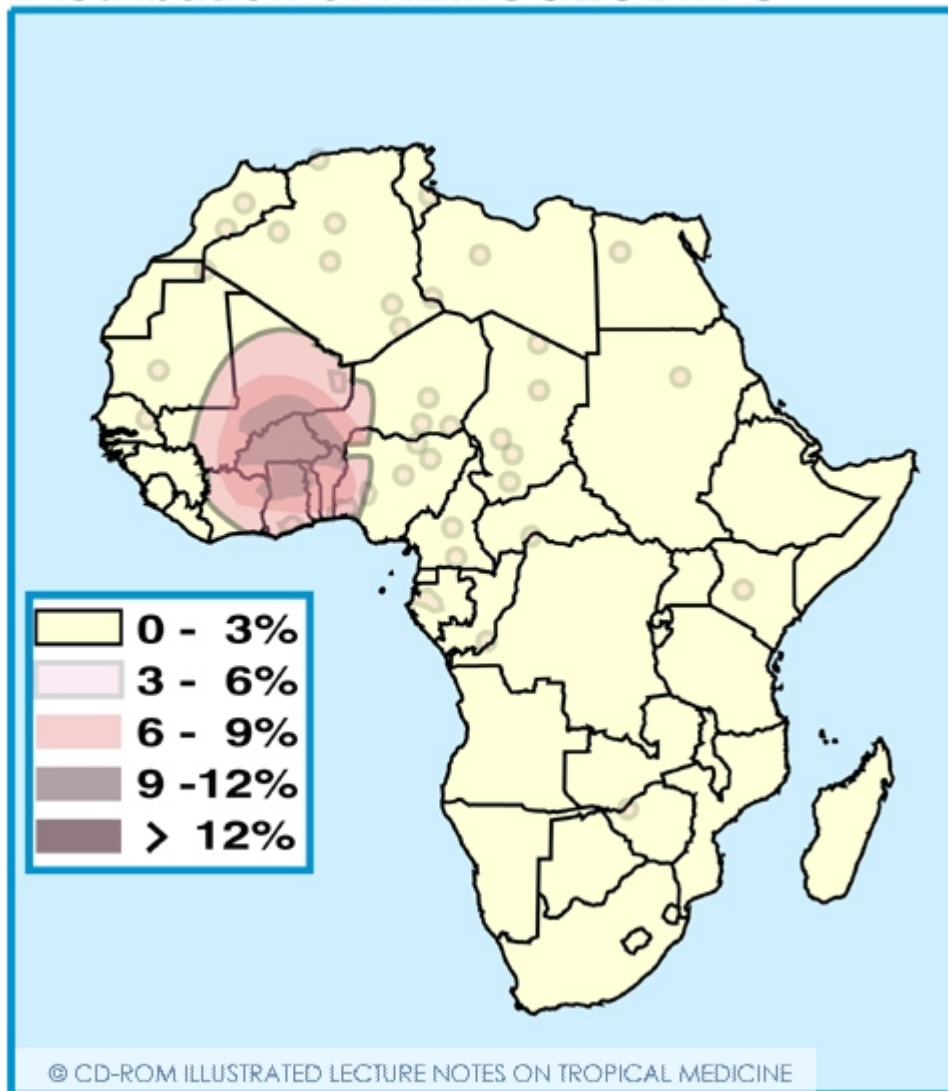
For patients with severe symptoms, especially severe neurological symptoms or complications, an argument could be made for early bone marrow transplantation if a HLA-identical sibling donor were available. The principal complication of allogeneic stem-cell transplantation (the transplantation of grafts from genetically different donors) is graft-versus-host disease (GVHD), which can occur despite aggressive immunosuppressive prophylaxis, even when the donor is a so-called “perfectly matched” (syn. HLA-identical) sibling. The few patients, mostly children, with sickle cell disease who have undergone bone marrow transplantation after a myeloablative conditioning regimen have become asymptomatic despite incomplete replacement of their marrow with donor cells (mixed chimerism).

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Other haemoglobinopathies

Haemoglobin C

Distribution of HEMOGLOBIN C

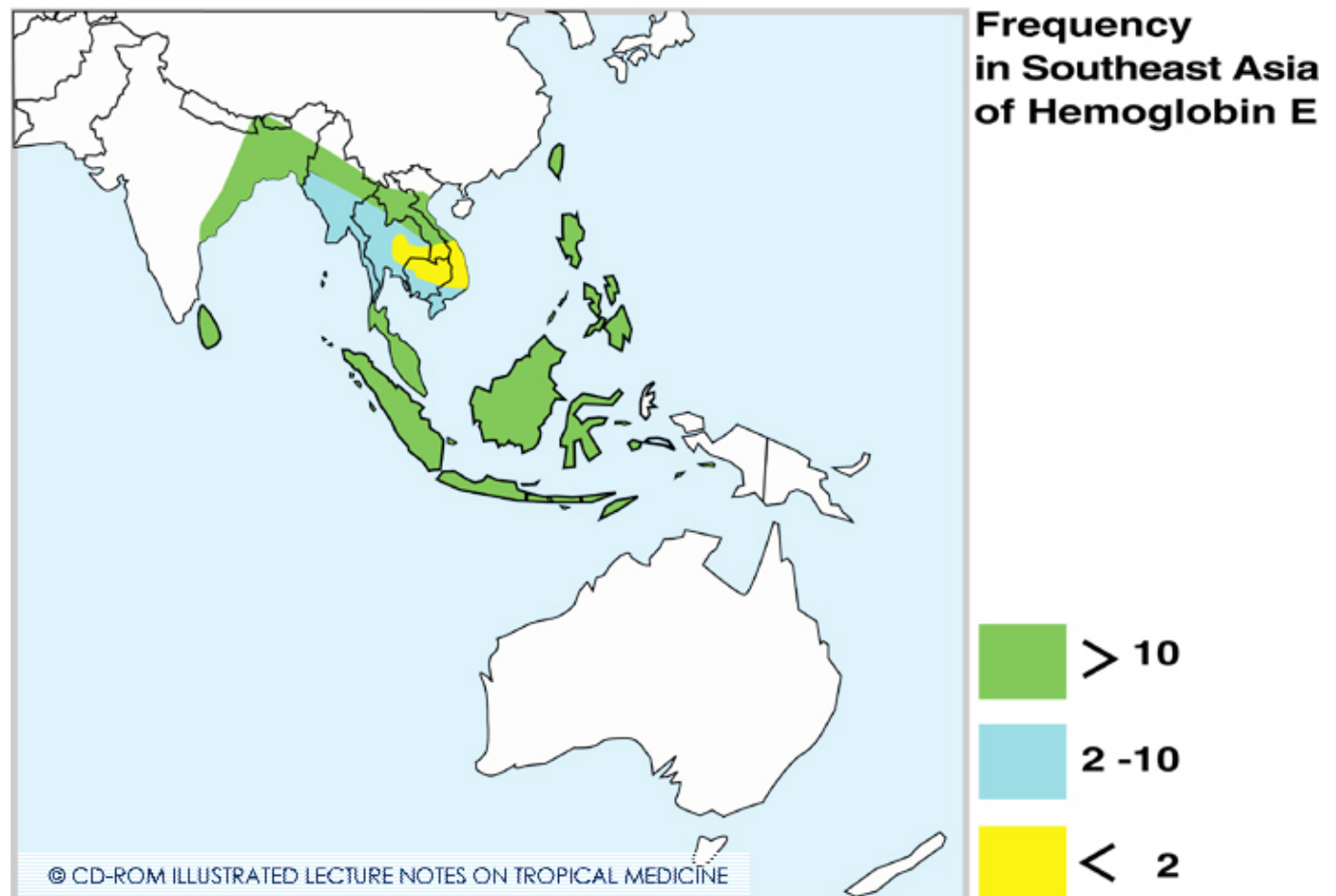


Map, distribution of haemoglobin C. Copyright ITM

Hb C is another haemoglobin variant that is common in West Africa. Here the 6th amino acid of the beta chain (glutamic acid, negative charge) is not replaced by a valine but by a lysine (positive charge, basic amino acid), i.e.: (beta6 Glu -> Lys) mutation. Sickling does not occur. Haemoglobin C has no protective effect on *P. falciparum* infection. The heterozygote state for Hb C is clinically silent. By electrophoretic analysis, 30-40% of the haemoglobin is Hb C and 50-60% is Hb A. People who are homozygous for Hb C (Hb CC) display mild chronic haemolysis, mild to moderate anaemia and mild splenomegaly. On electrophoresis Hb A is absent. There is often microcytosis and there are many target cells and some spherocytes. Cholelithiasis is common. There are rarely major complications.

Treatment is not necessary.

Haemoglobin E



Above: Map, distribution of haemoglobin E in Southeast Asia. Copyright ITM

Three splice site mutations are known to occur in exon 1 of the beta globin gene. These mutations result in three different abnormal haemoglobins: Malay, E, and Knossos. Haemoglobin E is a very common abnormal haemoglobin in Southeast Asia and India. The mutation GAG to AAG which leads to haemoglobin E, creates an alternate splice site competing with the normal splice site. This results in abnormal haemoglobin production and mild thalassemia in the homozygous state, with a mild microcytic anaemia with a haemoglobin usually above 10 g%. Clinically the affected persons are not ill, although a mild splenomegaly can develop. Electrophoresis reveals approximately 90% Hb E with varying amounts of Hb F.

The heterozygote has a haemoglobin of about 12 g% with microcytosis and an electrophoretic pattern showing Hb E plus Hb A₂ of 20 to 30%. On standard alkaline electrophoresis haemoglobin E co-migrates with Hb A₂.

When Hb E trait combines with a beta⁰ thalassemia mutation, a severe transfusion-dependent (EBeta⁰) anaemia will ensue. EBeta⁰ thalassemia patients who undergo splenectomy may stop being dependent on transfusions.

Glucose-6-phosphate dehydrogenase deficiency

General

In 1926 some people who had been given primaquine (an antimalarial) developed dark urine and haemolytic anaemia. The mechanism was not understood until 30 years later. Adult red blood cells have neither mitochondria nor a nucleus. The cells have no Krebs cycle and meet their energy requirements by glycolysis, an anaerobic process (Embden-Meyerhof chain). This is a very inefficient way of producing ATP, but in this way the erythrocytes do not use the oxygen they transport and the cells are effective carriers of oxygen. By glycolysis a molecule of glucose supplies two ATP molecules and two NADH molecules. By a side-reaction, 2,3 diphosphoglycerate is also produced, a substance that has an important effect on the release of oxygen (see oxygen dissociation curve).

Another metabolic pathway in the cytosol of the red blood cell is the hexose monophosphate shunt (also called the pentose phosphate shunt). The first enzyme in this latter chain is G6PD. The hexose monophosphate chain provides two molecules of NADPH per molecule of glucose. It is the only source of NADPH in the red blood cell.

The normal enzyme is called “type B”.

About 20% of Black people in Africa have “type A+”. This variant is functionally normal, but has a different electrophoretic pattern. “Type A-” has the same electrophoretic characteristics as “type A+”, but has lesser activity. This form is common in Central Africa. People with “type A-” are normally not anaemic. Enzymes with little or moderate activity rarely cause clinically serious problems.

Another important variant is the “Mediterranean type” and is virtually totally inactive. The less active the enzyme, the easier it is for the red blood cell to be damaged by certain chemical substances.

Enzymes with very little or no activity are common in people in the Mediterranean basin.

Clinical aspects

People with a very low G6PD activity can lead a normal life. In some situations, problems can arise. A crisis begins acutely and symptoms worsen in the course of a week. Jaundice, renal pain, haemoglobinuria and mild splenomegaly occur. Newborn children with G6PD deficiency are at greater risk of kernicterus and phototherapy is sometimes necessary. In many people the haemolysis is self-limiting, even if primaquine, for example, is continued to be administered. Circumstances that can trigger symptoms include:

The neonatal period (neonatal jaundice). Severe kernicterus due to G6PD-deficiency-related haemolysis is an avoidable cause of mental retardation. It is possible that the icterus is due to haemolysis combined with impairment of the liver function in these neonates.

A short list of drugs and chemicals that should be avoided by persons with G6PD deficiency includes: primaquine, methylene blue, niridazole, nitrofurantoin, sulfacetamide, sulfamethoxazole, sulfanilamide, sulfapyridine, phenylhydrazine, uropyrine. The administration of such medication is followed, after a 1- or 2-day delay, by falling haemoglobin concentration. Heinz bodies (denatured haemoglobin adherent to the RBC membrane), appear in the early stages of drug administration and disappear as haemolysis progresses.

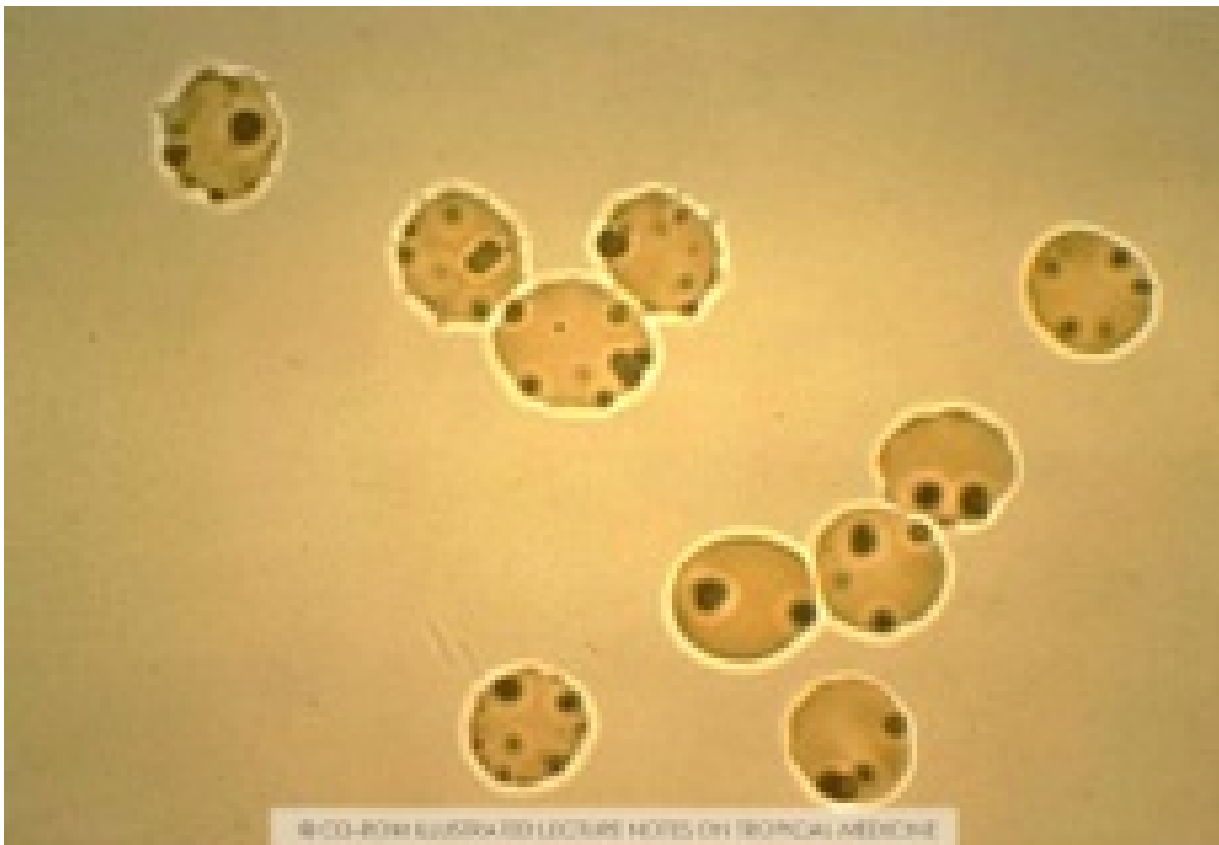
In sub-Saharan Africa there are few clinically relevant problems, but in the Mediterranean basin severe, even life threatening reactions are more common. Serious infections involving acidosis can cause acute haemolysis. The mechanism by which this occurs is not clear, but leukocytes might damage erythrocytes in their environment by releasing active oxygen species during phagocytosis (cfr production of H₂O₂ by neutrophils and macrophages).

Favism. In the case of severe deficiency, serious haemolysis can occur if fava beans [*Vicia fava*, favism] are eaten or the pollen of the plants are inhaled. The symptoms occur quite quickly after aerogenic exposure but only develop after 5 to 24 hours after eating fava beans. Divicine and isouramil are oxidants present in this plant and they are normally reduced and inactivated by reduced glutathione. Our knowledge of favism is however incomplete. There is no absolute correlation between G6PD activity and the clinical symptoms. Other factors undoubtedly also play a part. People with "type A-" do not suffer from favism.

Transfusions. Normal red blood cells keep their G6PD activity if they are stored for transfusion. The

small amount of activity that G6PD-deficient cells still have, will decrease as time goes by. If this type of blood is transfused into a person who is already ill and who may be receiving potentially haemolysing medication, haemolysis of the transfused blood can occur quickly.

Diagnosis



G6PD-deficiency. Heinz bodies are visible in the erythrocytes and consist of denatured haemoglobin.

The morphology of the red blood cells is normal between crises. During a crisis inclusions can be detected in red blood cells (Heinz bodies) by means of a supravital dye such as crystal violet. Heinz bodies are formed by denatured, damaged haemoglobin. Cells with these inclusions are quickly removed via the spleen. Detection of Heinz bodies is an insensitive test for G6PD deficiency. G6PD activity can be measured directly in a well-equipped laboratory. The simplest quantitative assay measures the reduction of NADP to NADPH in the presence of glucose-6-P and haemolysate. It is important to know that a test might give misleading too high results if performed in less than 2 weeks after a haemolytic episode. Older erythrocytes have less enzyme activity and will be eliminated first after a haemolytic crisis. If the test is then performed on the remaining younger red cells (which have

a higher enzyme activity), the activity of the enzyme is overestimated. In a blood smear stained with May-Grünwald Giemsa; Heinz bodies cannot be detected, but one can recognise 'bite cells' (keratocytes, blister cells) and dense erythrocytes with irregular outline. In normal people, the activity of G6PD is reduced by half over 120 days (the normal life span of an erythrocyte). It is therefore mainly the older cell population that is affected. This also explains why most clinical episodes are self-limiting (usually about 25% of the cells are haemolysed). It is precisely because of this limited haemolysis that people with, for example, leprosy, can often continue to take dapsone. Reticulocytosis increases after a few days.

For didactic examples of the more unusual blood smears (including G6PD-pathology), see: <http://content.nejm.org/cgi/content/full/353/5/498>

G6PD-deficiency, methaemoglobinemia and methylene blue

In haemoglobin, iron in haem is present as Fe^{2+} . If iron in haem becomes oxidised to Fe^{3+} it is called methemoglobin. This cannot carry oxygen. Normally, the unpronounceable enzyme NADH-dependent cytochrome b_5 methemoglobin reductase will reduce methemoglobin to haemoglobin. This is a rather slow process. When methemoglobinemia occurs, one would usually administer methylene blue. However, after administration methylene blue first has to be reduced in the body to its active metabolite leukomethylene blue. It is the leukomethylene blue which will convert Fe^{3+} in haem into Fe^{2+} . The conversion of methylene blue to leukomethylene blue is catalysed by NADPH methemoglobin reductase, a reaction requiring NADPH. Because there is an important NADPH-deficit in G6PD-deficient red blood cells, this conversion will not take place, and treatment with methylene blue will not work. What is more, administration of methylene blue in case of important methemoglobinemia is dangerous in case of G6PD-deficiency, because it will increase haemolysis. Methylene blue is an oxidant which will increase the anaemia and the hypoxemia. If one cannot wait for spontaneous improvement, blood transfusion and oxygen administration are warranted.

G6PD deficiency, hereditary transmission

The activity level of the G6PD enzyme is genetically determined. The G6PD gene is located on the **X** chromosome (a man has XY and a woman has XX). A man with a defective gene (hemizygote) and a woman with 2 defective genes (homozygote) are affected. A woman with just 1 mutant gene (heterozygote) is a carrier, but normally does not display any symptoms. She may well pass the defective gene on to her child. Because in women 1 of the 2 X chromosomes is inactivated in each nucleated cell (Lyons hypothesis), a heterozygous woman has 2 populations of erythroblasts and

therefore also 2 populations of red blood cells: a normal population and a deficient population.

Heterozygous women with a high percentage of deficient cells may become symptomatic. Normal women are therefore genetic chimaeras: some cells contain an active paternal X-chromosome and others contain an active maternal X-chromosome. There are no cells in which both chromosomes are active. Note of course that early precursor cells contain DNA but erythrocytes themselves have no nucleus, and therefore contain no chromosomes or even DNA.

Oxidative stress

Oxidative stress is defined as an imbalance between free-radical production and antioxidant protection. There are many varieties, but important ones include the hydroxyl radical ($^{\circ}\text{OH}$), hydrogen peroxide (H_2O_2) and the superoxide radical $\text{O}_2^{\circ-}$, with the $^{\circ}$ symbol indicating an unpaired electron. To give a ballpark idea, it is estimated that an average adult human forms 1.7 kilograms of superoxide each year. Each cell in our body produces about 50 hydroxyl radicals each second, one of the most reactive species which exists. It basically reacts instantly with any other molecule, be it fat, protein or DNA which it encounters, thereby damaging it. If there would be no defence against free-radicals, cellular damage would advance at a very fast pace. The cellular defence against free-radicals include antioxidants such as vitamin C and E, glutathione and catalase.

Hexose monophosphate shunt

In order to have enough reduced glutathione, a supply of NADPH is needed. The first reaction in the hexose monophosphate shunt produces NADPH. This reaction is catalyzed by the enzyme G6PD. In very general terms it can be said that the hexose monophosphate shunt (= pentose phosphate chain), has two main functions:

- the production of ribose, a component of nucleotides, for e.g. DNA and ATP. In summary, this pathway transforms glucose-6-phosphate into ribose-5-phosphate. However the erythrocyte has no nucleus nor ribosomes, there is no need for ribose synthesis in these cells.
- the generation of reducing power in the form of NADPH. The pentose phosphate chain reduces NADP^+ to NADPH. By oxidising NADPH to NADP^+ again, other substances are reduced via a redox reaction. NADPH is an important electron donor (= reducing capacity). The main function of NADPH is to reduce oxidised substances such as glutathione and to allow reductive biosyntheses to take place.

The NADPH/NADP ratio controls the rate of reaction in an autoregulatory manner. In a quiescent

state, this ratio is very high and G6PD is nearly completely inhibited. When NADPH is oxidized, as when glutathione is reduced in the glutathione reductase reaction, NADPH is converted to NADP⁺ and G6PD becomes active, reconverts NADP⁺ to NADPH.

Red blood cells and NADPH

Why does G6PD deficiency seem to affect red blood cells especially? Erythrocytes do not have mitochondria therefore red blood cells do not have a back-up system for NADPH production. They have no alternative source of NADPH, as opposed to other cells which have mitochondria. Acetyl-CoA in the mitochondria (entry point for the Krebs cycle) cannot pass through the mitochondrial membrane by itself. If it is bound to citrate it can pass through the membrane. In cells with mitochondria some citrate bound to acetyl-CoA shifts from the mitochondrial matrix to the cytosol, after which the compound is divided again. The citrate therefore acts as a carrier. Citrate is then converted to oxaloacetate and then to malate. Afterwards (malate + NADP⁺) is converted to (pyruvate + CO₂ + NADPH). As a result, even if the hexose monophosphate shunt is functioning poorly, cells with mitochondria can still produce NADPH. The effects of G6PD deficiency are therefore most apparent in the red blood cells (cells without mitochondria).

Glutathione

Why do we need glutathione? Haemoglobin and many other biological molecules contain many sulphur groups (SH groups =sulfhydryl groups). These are necessary for the molecule to function properly. If these are oxidized, haemoglobin can no longer function as it should. Glutathione is a tripeptide containing cysteine as the second amino acid. This amino acid has a SH group. The reduced glutathione (i.e. with a SH group), converts non-functional, oxidized cysteine disulphide groups (S-S) in other molecules such as haemoglobin into functional SH groups via the enzyme glutathione peroxidase. In this process glutathione itself is oxidized (two glutathione molecules are then bound by a disulphide bridge). Glutathione also reacts with hydrogen peroxide (H₂O₂) and corrosive organic peroxides. In this way it has an important protective role as an anti-oxidant. If the G6PD enzyme is deficient, no NADPH is formed, neither is any protective reducing glutathione formed and haemoglobin molecules and red blood cell membrane molecules that contain SH groups may be permanently damaged by oxidizing substances. The non-functional, denatured haemoglobin is precipitated in the form of Heinz bodies and the resulting damage to the membrane then leads to haemolysis resulting in moderate, but acute anaemia.

Note on *P. vivax* eradication: In countries attempting to eliminate *P. vivax* infection, the existence of G6PD deficiency is driving the development of a simple, user-friendly point-of-care test for its detection. Today, primaquine and tafenoquine are the only drugs capable to eliminate the hypnozoites in *P. vivax* infections. However, both drugs can provoke a severe haemolytic crisis in a person with G6PD deficiency. Therefore, testing for G6PD deficiency is imperative before these drugs can be administered safely.

Beta thalassemia

General

In addition to the Mediterranean basin the disease also occurs in Africa, the Middle East, India and Myanmar, Southeast Asia including southern China, Malaysia and Indonesia. There are indications that the high frequency of heterozygous beta thalassemia carriers in the tropics can be explained by a relative protection against the fatal *P. falciparum* malaria (compare with sickle cell trait and G6PD deficiency), but this is controversial.

Embryonal Hb	Fetal Hb	Adult Hb
Hb Gower 1: $\zeta_2\epsilon_2$ Hb Gower 2: $\alpha_2\epsilon_2$ Hb Portland: $\zeta_2\gamma_2$	Hb F: $\alpha_2\gamma_2$	Hb A: $\alpha_2\beta_2$ Hb A ₂ : $\alpha_2\delta_2$

An average normal adult has Hb A 97%, Hb A₂ 2%, Hb F 1%.

About 150 different mutations have been reported in people with beta thalassemia. About 20 mutations are responsible for 80% of the beta thalassemias. Some are simple nucleotide substitutions, with missense or nonsense consequences, multiple substitutions, deletions with frameshifts or abnormalities in the promoter. Sometimes something goes wrong with the splicing of mRNA. Within each geographic population there are unique mutations. Individuals who have beta thalassemia major are usually homozygous for one of the common mutations, or heterozygous for one of the common mutations and one of the geographically-unique mutations. All result in reduced synthesis of beta globin chains (beta⁺-thalassemia) or the absence of synthesis of beta globin chains

(beta⁰-thalassaemia). Clinically mild forms of beta thalassemia are called thalassemia intermedia, whereas minor forms are non-symptomatic. If the production of both beta and delta chains is diminished, there is delta-beta-thalassemia (a consequence of gene fusion). The imbalance in globin chain synthesis (there are more alpha chains than beta chains) leads to precipitation of alpha chains in the red cell (= inclusion bodies or α -hemichromes), which leads to premature destruction of the cell in the bone marrow or the peripheral blood.

Clinical aspects

Beta thalassemia minor

The heterozygous condition is known as beta thalassemia minor. One beta gene is defective, the other is normal. Fewer beta globin chains than normal are produced but the healthy gene largely compensates for this. There is a typical microcytosis but rarely anaemia. This form is often found by chance and can wrongly be regarded as an iron deficiency.

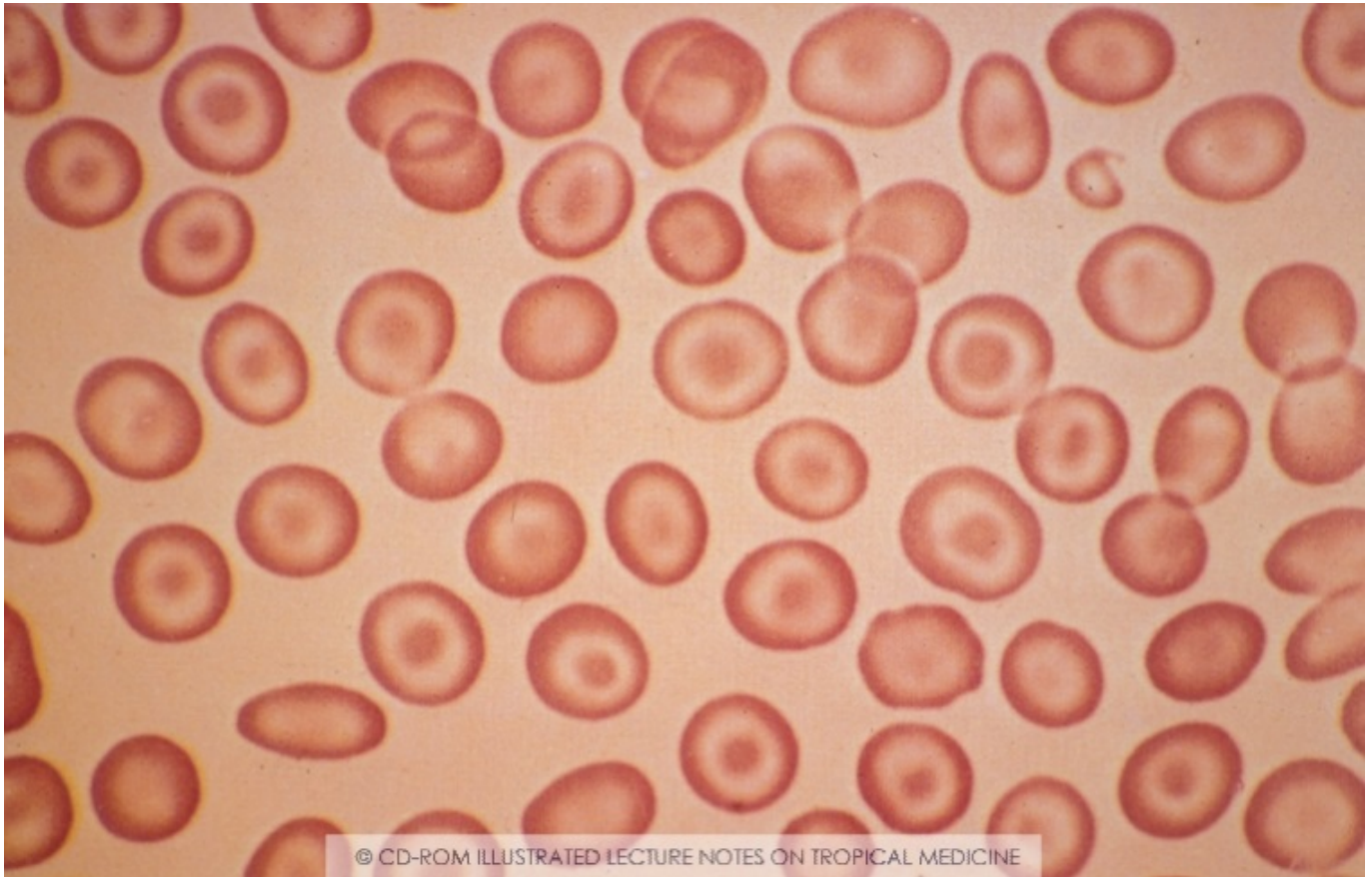
There is a diagnostic problem for patients suspected to be double heterozygous for Hb E and beta-thalassemia. Hb E and Hb A₂ cannot be distinguished in alkaline gel, but diffuse differently in an acid gel.

But Hb A and E cannot be distinguished in acid gel. This occurs mainly in people from Southeast Asian origin.

Beta thalassemia major



Beta-thalassemia major. Skull bossing due to expansion of the diploë (red bone marrow in skull). See also “hair-on-ends” aspect by Rx



Beta-thalassemia minor with microcytes and target cells

The homozygous or doubly heterozygous condition is much more serious. The severity depends on which mutation(s) causes or cause the disorder and how many beta globin chains can still be produced.

There is therefore a spectrum of clinical severity: thalassemia major or thalassemia intermedia. Patients with thalassemia major are by definition transfusion dependent. The affected infants are normal at first. Newborns still have Hb F, which is not affected in this condition. By the age of 6 to 9 months, the children develop faulty erythropoiesis with anaemia and hypertrophy of the bone marrow, spleen and liver with hepatosplenomegaly. In severe beta thalassemia, erythropoiesis can increase up to 10-fold. The relative excess of alpha globin chains interferes with the normal maturation of the cells in the bone marrow. Ineffective erythropoiesis occurs. There is pronounced haemolysis with considerable splenomegaly. Enlargement of the liver always occurs. Sometimes there are gallstones (bilirubin stones due to the haemolysis). The red bone marrow increases in volume, with swelling of the diploke in the cranial bones, osteopenia and a lowering of the fracture threshold

and often microfractures around the main joints. The diploë is the central layer of spongy bone between the two layers of compact bone of the flat cranial bones. The face is often deformed somewhat due to cranial bossing and hypertrophy of the maxillae resulting in a mongoloid appearance. Bone marrow expansion can lead to compression of the spinal cord. Extramedullary haematopoiesis can occur, not only in spleen and liver, but also in the posterior mediastinum and even kidneys, leading to local masses which can resemble lymphoma.

There is haemolytic anaemia; microcytosis with normoblasts in the peripheral blood and an increase in the minor haemoglobins (Hb F, Hb A₂). Children can survive only with regular blood transfusions and folic acid supplements. Iron overload and infections due to the repeated transfusions are a very real risk. The abnormal accumulation of iron results in dilated cardiomyopathy, endocrine disorders (destruction of the pituitary gland and hypogonadism with impaired sexual development) diabetes, liver disease (often together with hepatitis B and C). Later, restrictive lung disease and pulmonary hypertension can occur (pulmonary hypertension tends to occur in all chronic severe haemolytic diseases).

Laboratory

Severe haemolytic anaemia is present, which is accompanied by microcytosis (low MCV), target cells, a high RBC count with a relatively low reticulocyte count considering the severity of anaemia. The high RBC count is a compensation for the low amount of normal Hb in each red blood cell (contrary in iron deficiency where the marrow cannot produce as many RBCs). The Mentzer index is helpful in differentiating iron deficiency anaemia from thalassemia: it is the quotient of the MCV (in fl) divided by the red blood cell count (in millions per µl). If the Mentzer index is less than 13, thalassemia is more likely, if the result is greater than 13, iron-deficiency is more plausible.

On hemoglobinophoresis, a higher level of HbA₂ (α₂δ₂) is usually found in beta thalassemia patients: the excess alpha globin chains bind to the delta globin chains. Diagnostic confirmation by globin gene testing will be rarely available in the tropics.

Prevention

A child born of two heterozygous parents has a 25% probability of being homozygous. There are screening programmes for detecting carriers in Italy, Sardinia, Cyprus and Greece. These are based on MCV and the concentration of Hb A₂. Prenatal diagnosis can be carried out with various techniques (e.g. villous chorion sampling carried out in weeks 9-13).

Therapy

Non-transfused thalassemia intermedia patients are encouraged to avoid high-iron and iron supplemented foods and are encouraged to drink tea with meals, which decreases iron absorption.

Folic acid is usually given. With beta thalassemia major there is a great need for transfusion. Because of the repeated transfusions, iron overload occurs after a number of years (the time varies). Iron chelation is carried out with deferoxamine (Desferal).

Bone marrow transplantation can be carried out as curative therapy and at present is the only definite treatment. Of course, the bone marrow of an identical twin cannot be used but that of a HLA-DR matched relative can be used.

Alpha thalassemia

Alpha genes can be lost through deletion or inactivated by point mutations. If insufficient alpha chains are produced, the condition is known as alpha thalassemia. This condition is very frequent in Asia (from India to China, including Southeast Asia). The disease also occurs in Africa. Since 4 genes code for alpha chains, there are a number of possibilities:

All 4 alpha genes functional: normal. Genetic $\alpha\alpha/\alpha\alpha$

Only 3 alpha genes functional: silent carrier with no symptoms or signs (thalassemia minima). Genetic $\alpha\alpha/\alpha\alpha^-$

Only 2 alpha genes functional: silent carrier, often microcytosis (alpha thalassemia minor or alpha thalassemia trait). Genetic $\alpha\alpha/\alpha\alpha^0$ (= $\alpha\alpha^0$ thalassemia) or $\alpha\alpha^-/\alpha\alpha^-$ (= $\alpha\alpha^+$ thalassemia). The two genes can either occur on the same chromosome (cis-type) or on each of the pairs (trans-type). Cis-type $\alpha\alpha^0$ thalassemia trait tends to be found in individuals of Asian descent, while trans-type $\alpha\alpha^+$ tends to run in individuals of African descent. Expert laboratory tests help to distinguish between these two conditions, which is important. If a mother is a carrier of alpha thalassemia, her pregnancy is at risk for Bart's hydrops fetalis syndrome (worst case scenario), while the worst possible outcome of a pregnancy of a mother with $\alpha\alpha^+$ thalassemia is a much milder condition, haemoglobin H disease.

Only 1 alpha gene functional: excess of beta globin chains. Genetic $\alpha\alpha^-/\alpha^-$. The excess beta chains form tetramers and are deposited: β_4 (haemoglobin H). Haemoglobin H is not stable and

thermally labile. It contains two reactive SH groups per beta chain. The beta chains in Hb A have only one SH group. This may explain the susceptibility of Hb H to oxidation. The red blood cell inclusions (Heinz bodies = β -hemichromes) can be seen readily with brilliant cresyl blue staining (the same dye as for reticulocytes). The patient is anaemic and there is splenomegaly.

No alpha genes functional: the excess of gamma chains leads to the depositing of tetramers composed of four gamma chains: γ_4 (Barts haemoglobin). Without the alpha globin chains, there can be no fetal or adult haemoglobin which means the red blood cells cannot carry oxygen efficiently throughout the body. Hydrops fetalis with stillbirth is the result. There is an increased risk of toxemia of pregnancy and of post-partum haemorrhage (hypertrophy of the placenta). The only haemoglobins found in these infants are: Hb Portland ($\delta_2\gamma_2$), Hb H (β_4), and Hb Bart's (γ_4), and no Hb A, Hb A₂ or Hb F. Electrophoresis of fetal haemoglobins shows about 80% Barts haemoglobin and about 20% Portland haemoglobin (normally only present in the embryo in the first trimester).

Onyalai

General

Onyalai is a rather mysterious disease, which only seems to occur in central southern Africa (southern Angola and northern Namibia; Kavango and Ovambo territories). Onyalai means “blood blister” in the language of the Kimbundu, an Angolan tribe. Onyalai is a disease of unknown aetiology. Defective nutrition may be the cause. One hypothesis is that a toxin, possibly acting as a hapten, is responsible for this form of thrombocytopenia. The possible etiological role of mycotoxins from contaminated millet, sorghum and/or maize requires further investigation.

Clinical aspects

The disease differs clinically, epidemiologically and immunologically from immune (previously idiopathic) thrombocytopenic purpura (ITP). It is an acute disease, characterized by the formation of haemorrhagic vesicles and blisters on the palatal and buccal mucous membranes, together with severe thrombocytopenia. This acquired form of thrombocytopenic purpura can lead to haematuria and melena. Epistaxis, petechiae and ecchymoses are common, as are subconjunctival bleeding and menorrhagia. Haemorrhage from ruptured bullae, epistaxis or gastrointestinal bleeding can be severe and may cause shock and even death.

Treatment

Transfusion of blood and of platelets can be lifesaving. High dose intravenous gammaglobulin may be followed by a rise in the platelet count and cessation of haemorrhage but in general this treatment is disappointing (and expensive). Splenectomy can be considered for patients with severe uncontrollable bleeding, although splenectomy does not always control the disease.