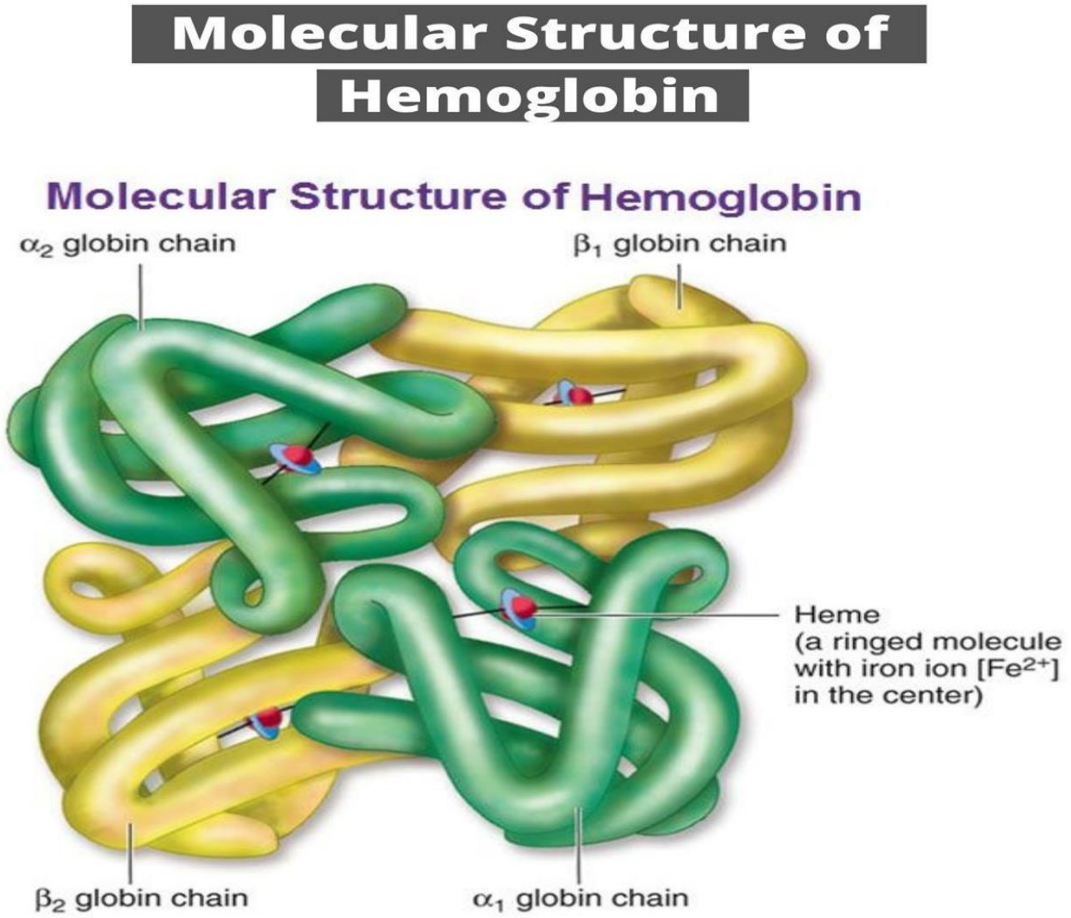
3rd Lecture Objectives (Hemoglobinopathies) (Sickle Cell Disease)

1. To review the normal structure of hemoglobin and its different types
2. To understand the definition of Hemoglobinopathies and recognize its different types according to their etiology
3. To be able to approaches to patient with sickle cell anemia (investigations and treatment)
4. To be able to recognize the complications of sickle cell anemia and treating it

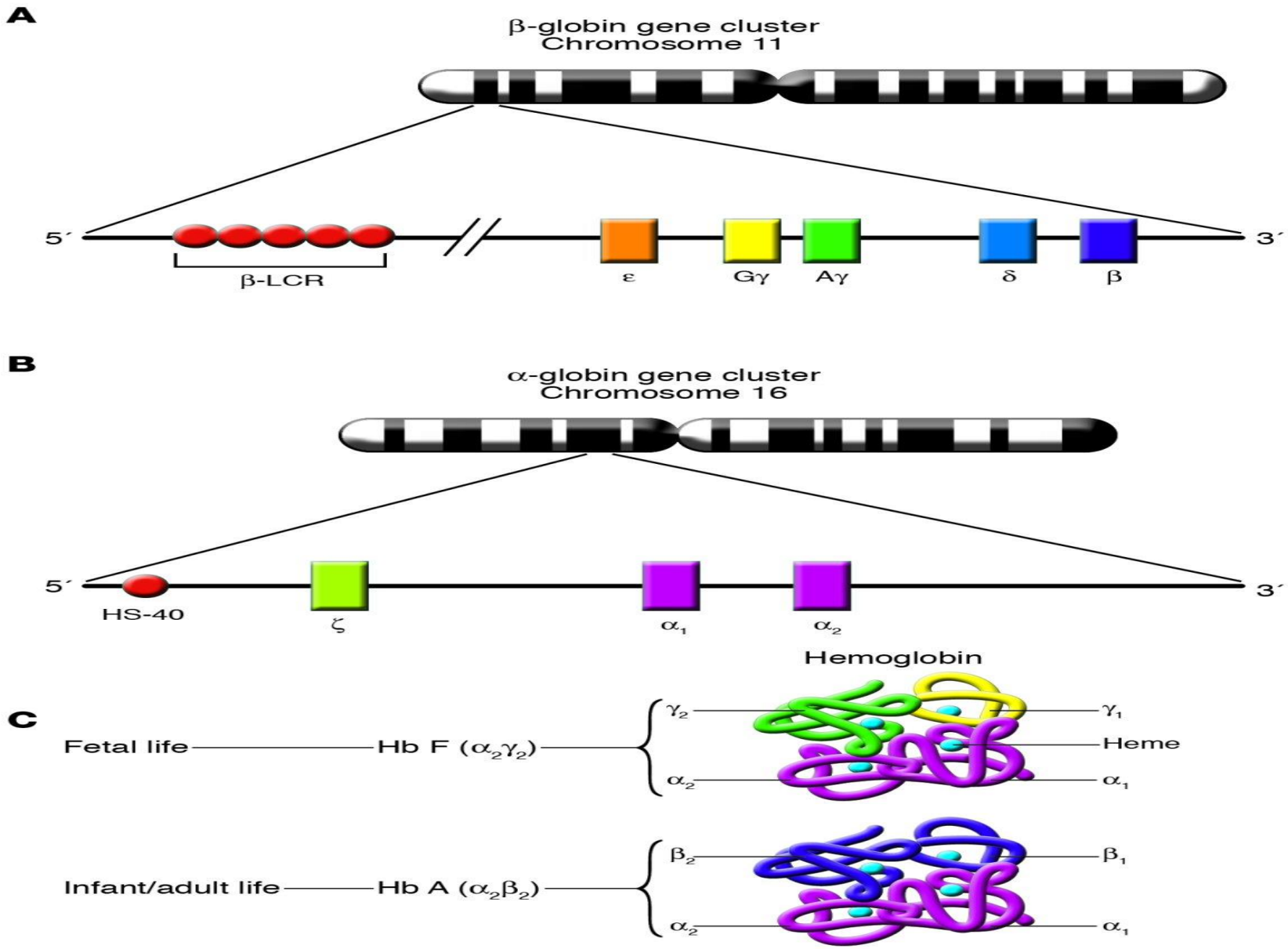
Hemoglobin is a tetramer consisting of 2 pairs of globin chains.
Abnormalities in these proteins are referred to as **Hemoglobinopathies**

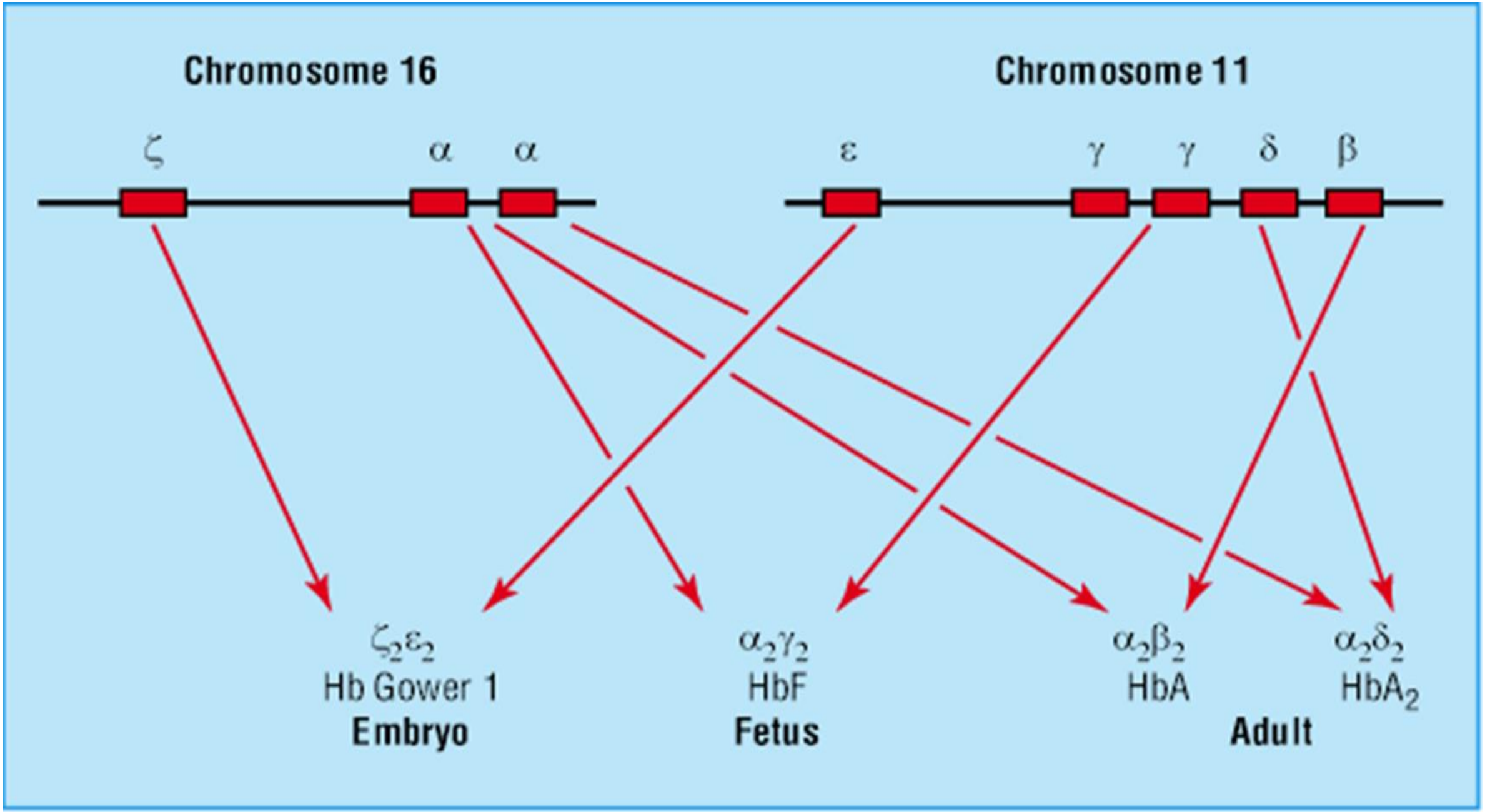


Two hemoglobin gene clusters are involved in the production of hemoglobin and are located at the end of the **short arm of chromosomes 16 and 11**

On chromosome 16, there are 3 genes within the alpha (α) gene cluster:
zeta (ζ) and 2 α genes (α_1, α_2).

On chromosome 11, there are 5 genes within the beta (β) gene cluster: epsilon (ε), delta (δ), beta (β), and 2 gamma (γ) genes.


A
β-globin gene cluster
Chromosome 11
5′
3′
β-LCR
ε
Gγ
Aγ
δ
β
B
α-globin gene cluster
Chromosome 16
5′
3′
HS-40
ζ
α1
α2
C
Hemoglobin
Fetal life
Hb F (α2γ2)
γ2
γ1
Heme
α2
α1
Infant/adult life
Hb A (α2β2)
β2
β1
α2
α1


Chromosome 16
Chromosome 11
ζ
α
α
ε
γ
γ
δ
β
ζ2ε2
Hb Gower 1
Embryo
α2γ2
HbF
Fetus
α2β2
HbA
α2δ2
HbA2
Adult

Hemoglobin Types

- **Embryonic hemoglobins(Gower-1 ($\zeta_2\varepsilon_2$), Gower-2 ($\alpha_2\varepsilon_2$), and Portland ($\zeta_2\gamma_2$))**
- **Hb F ($\alpha_2\gamma_2$)**
- **HbA ($\alpha_2\beta_2$)**
- **HbA_2 ($\alpha_2\delta_2$)**

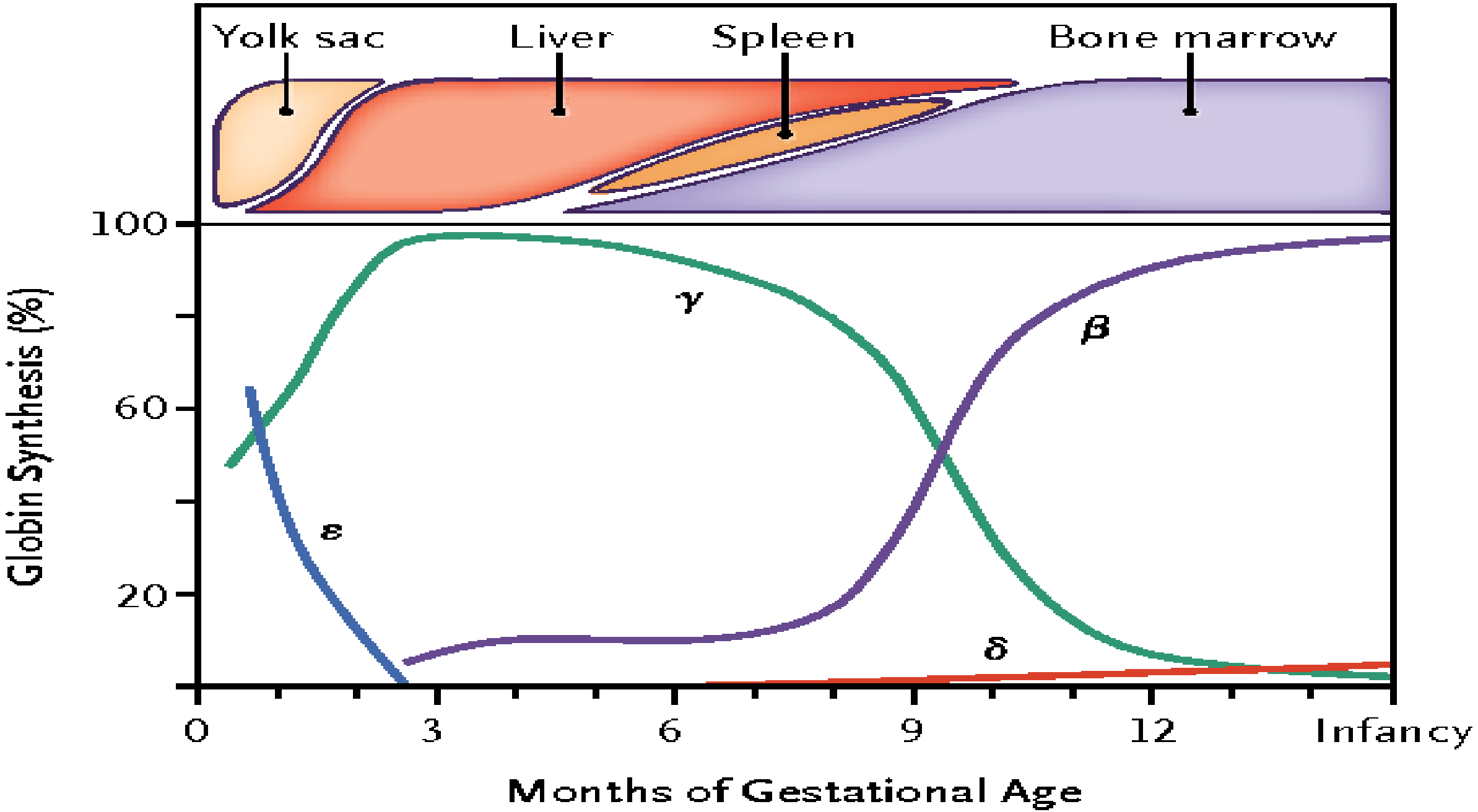
The final hemoglobin distribution pattern that occurs in childhood is not achieved until at least 6 mo of age, and sometimes later.

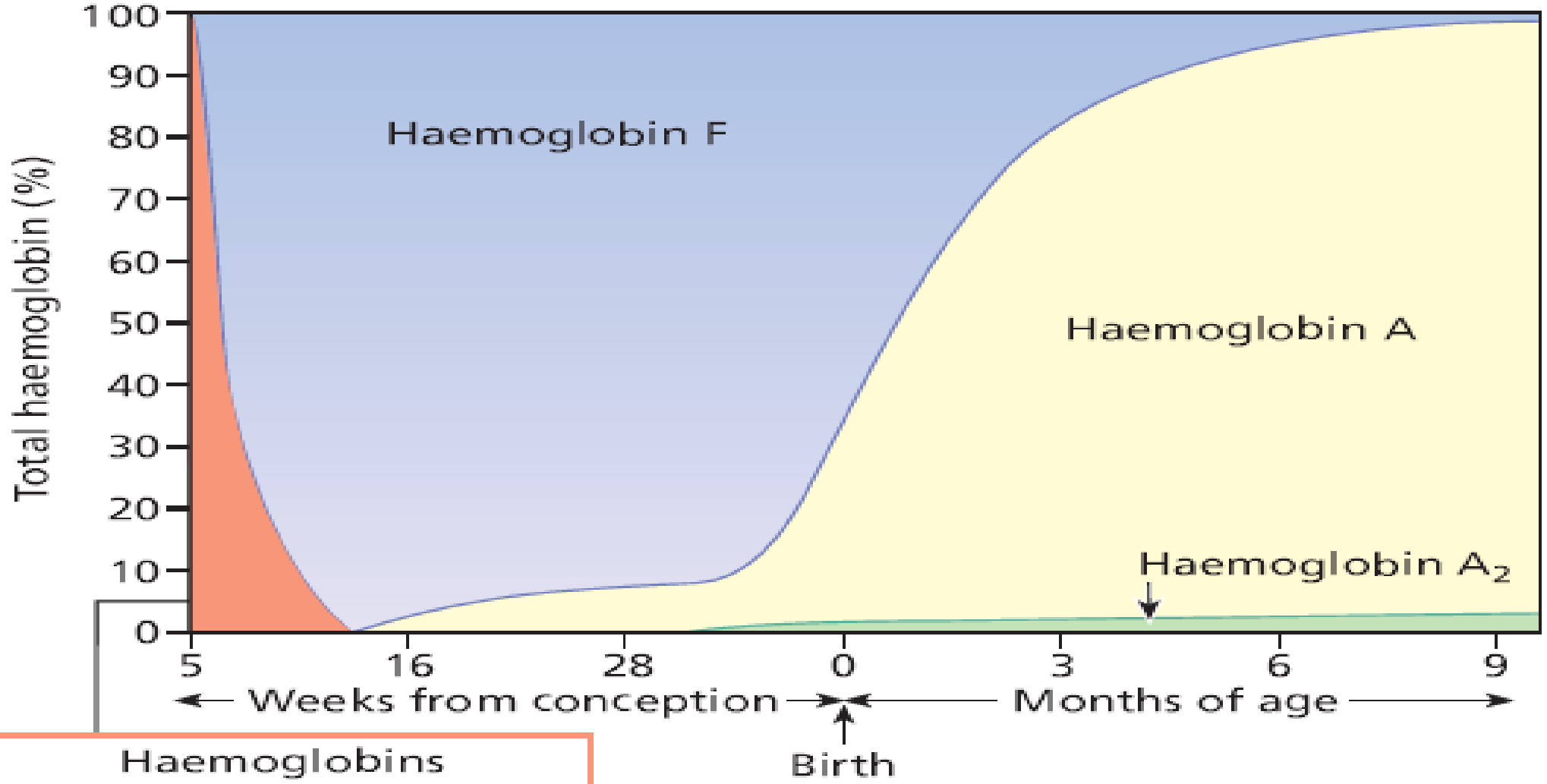
The normal hemoglobin pattern is

>95% Hb A

≤3.5 Hb A_2

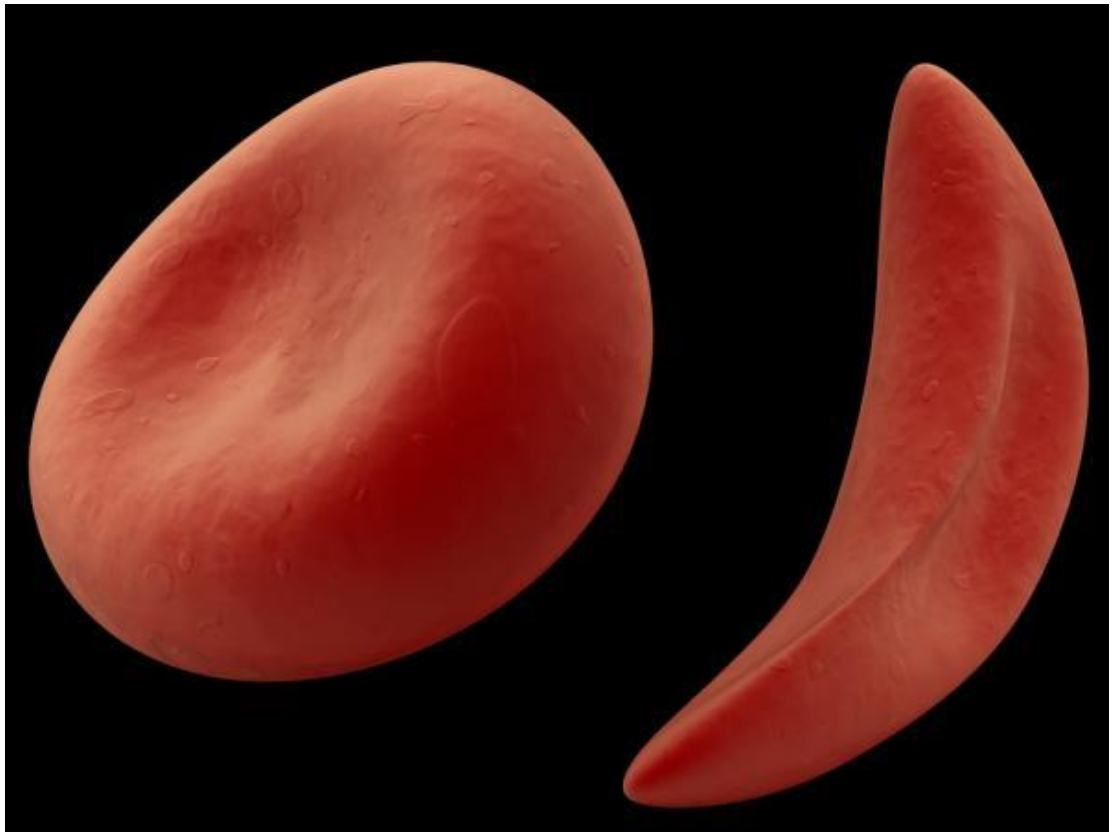
<2.5% Hb F


Yolk sac
Liver
Spleen
Bone marrow
100
60
20
Globin Synthesis (%)
γ
β
ε
δ
0
3
6
9
12
Infancy
Months of Gestational Age



Haemoglobins
Gower 1, Gower 2 and
Portland 1

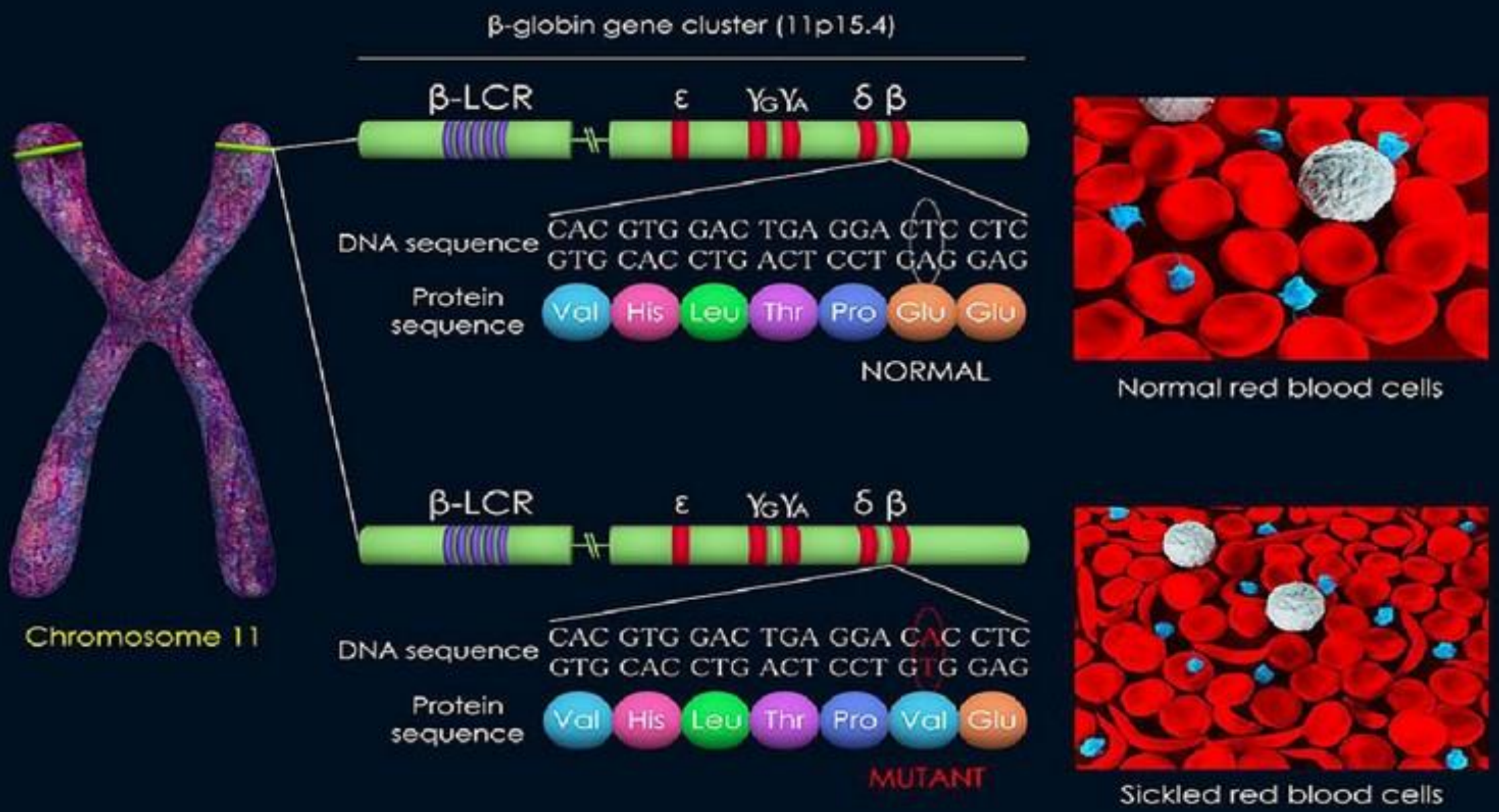
Sickle Cell Disease

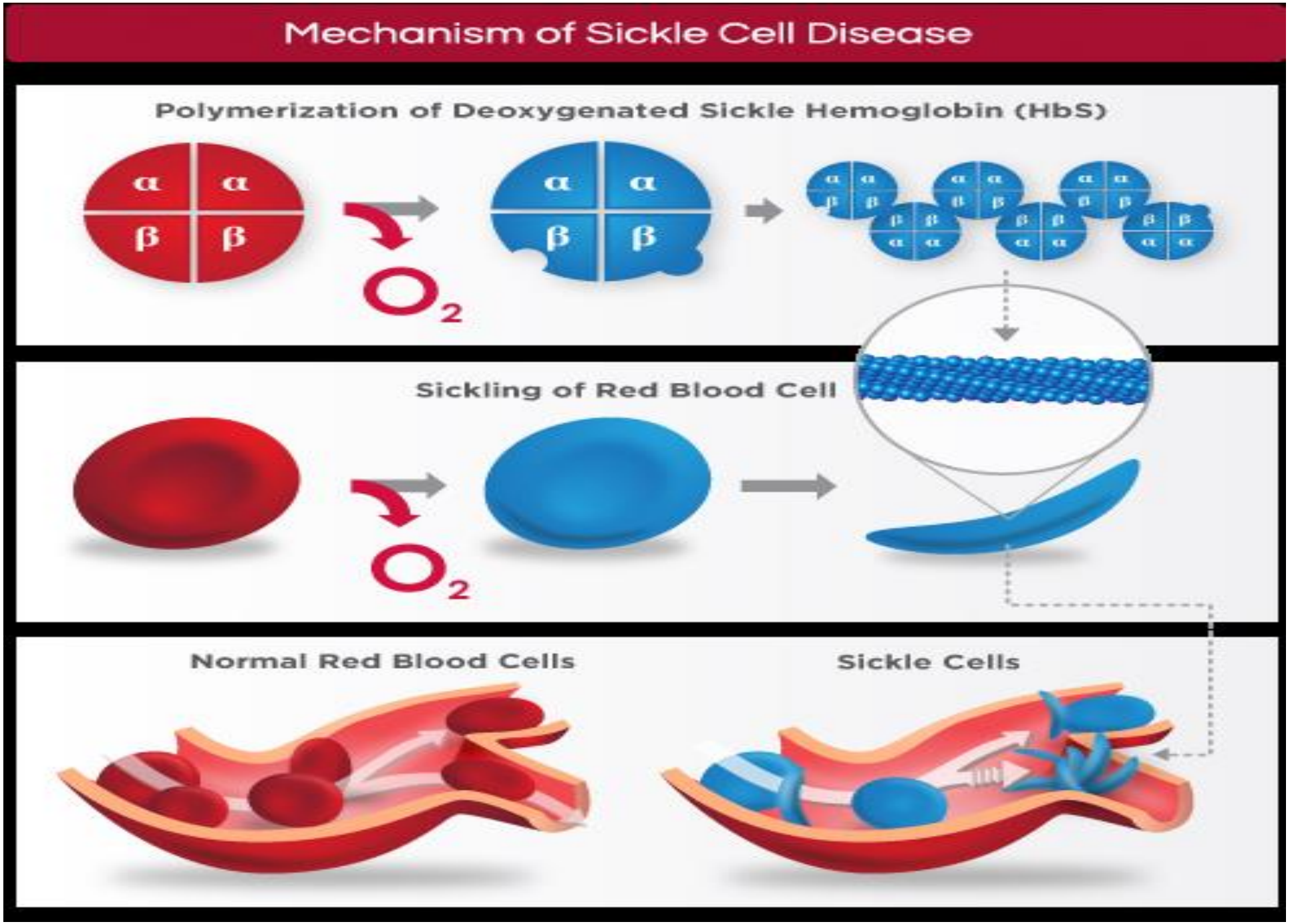


Pathophysiology

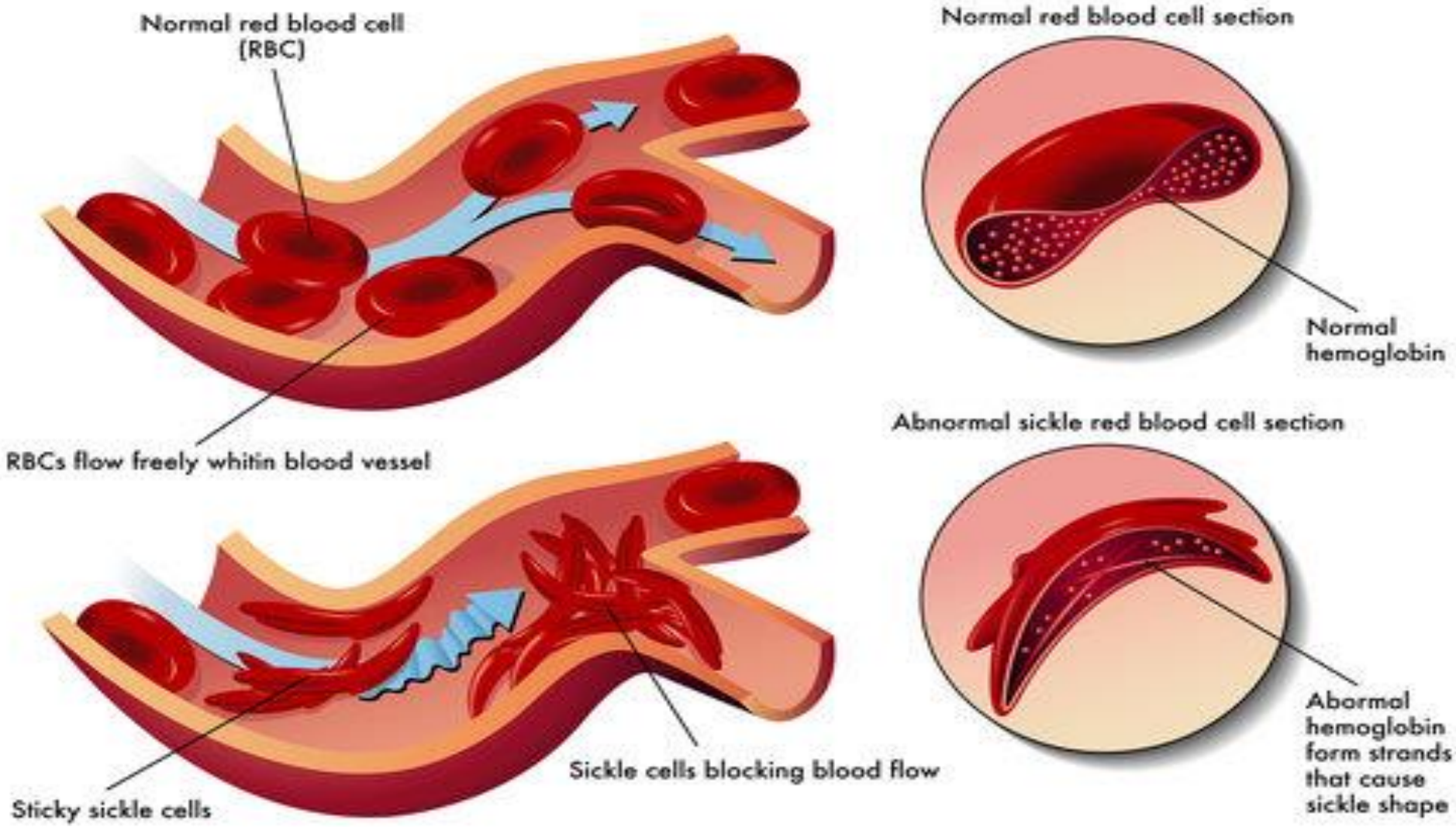
HbS arises as a result of a point mutation (A-T) in the sixth codon of the β-globin gene on chromosome 11,which causes a single amino acid substitution (glutamic acid to valine at position 6 of the β-globin chain). HbS is more positively charged than HbA and hence has a different electrophoretic mobility. Deoxygenated HbS polymerizes,leading to cellular alterations that distort the red cell into a rigid, sickled form. Vaso-occlusion with ischemia-reperfusion injury is the central event.

Sickle cell disease (SCD)




Mechanism of Sickle Cell Disease
Polymerization of Deoxygenated Sickle Hemoglobin (HbS)
α
α
β
β
O_2
Sickling of Red Blood Cell
O_2
Normal Red Blood Cells
Sickle Cells

Sickle-Cell Anemia



TYPES

1. Sickle cell anemia

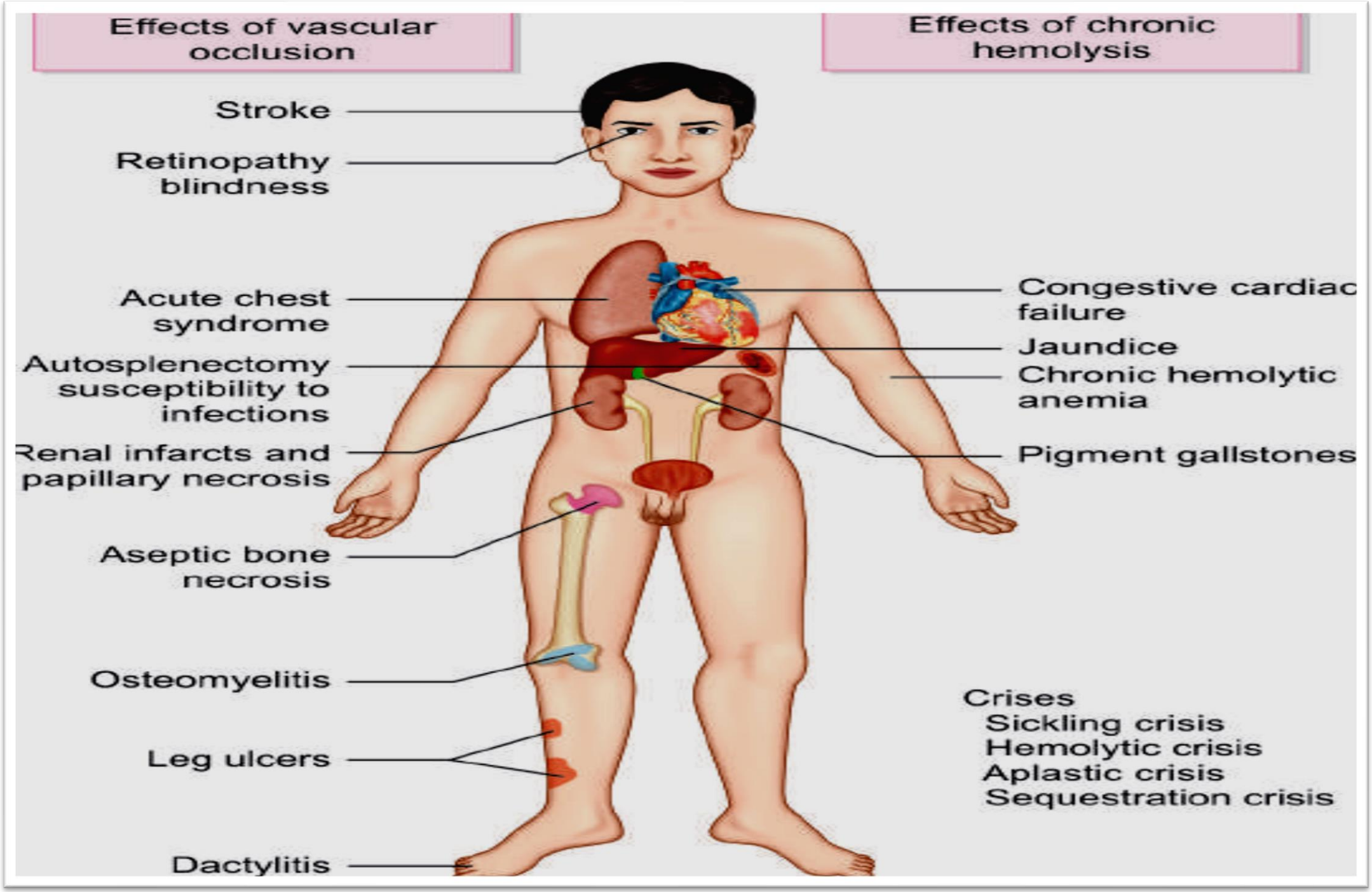
Homozygous state for HbS (βs βs)
>70% Hb is HbS

2. Sickle cell trait

Heterozygous carrier state for HbS (βs β)
25-40% of Hb is Hbs

3. Sickle cell – β thalessemia

Double heterozygote in which sickle cell gene is inherited from one parent and beta thalessemia gene from other parent (βs β0) or (βs β+)


Effects of vascular occlusion
Effects of chronic hemolysis
Stroke
Retinopathy blindness
Acute chest syndrome
Autosplenectomy susceptibility to infections
Renal infarcts and papillary necrosis
Aseptic bone necrosis
Osteomyelitis
Leg ulcers
Dactylitis
Congestive cardiac failure
Jaundice
Chronic hemolytic anemia
Pigment gallstones
Crises
Sickling crisis
Hemolytic crisis
Aplastic crisis
Sequestration crisis

NO SYMPTOMS IN INFANTS ???

It is able to block the sickling action of the RBCs so infants who have inherited the disease do not develop symptoms.

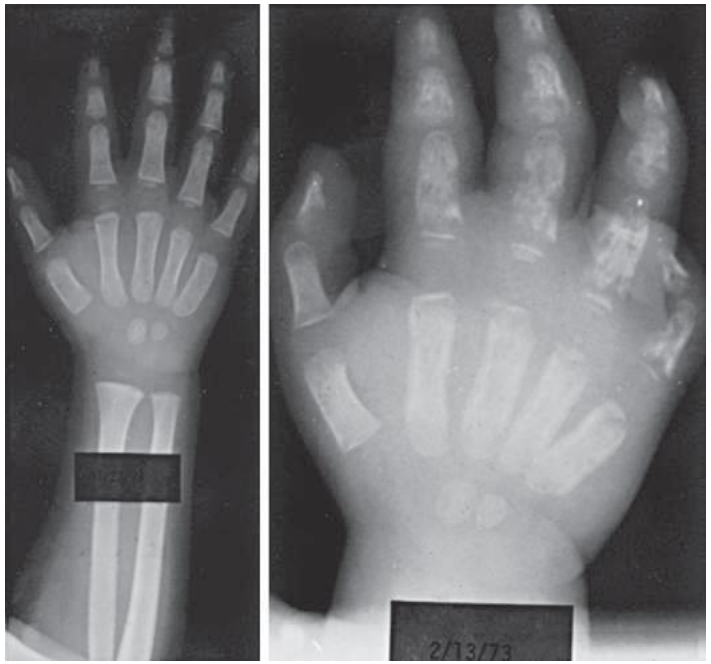
People with sickle cell gene who continue to carry some HbF are better protected from severe form of the disease.

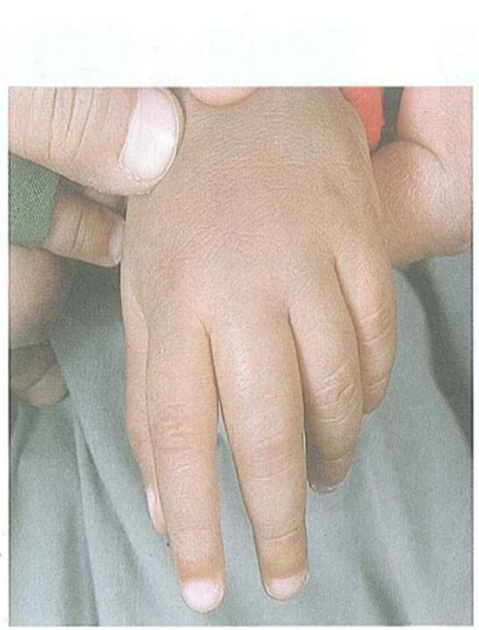
SICKLE CELL ANEMIA (HOMOZYGOUS HEMOGLOBIN S)

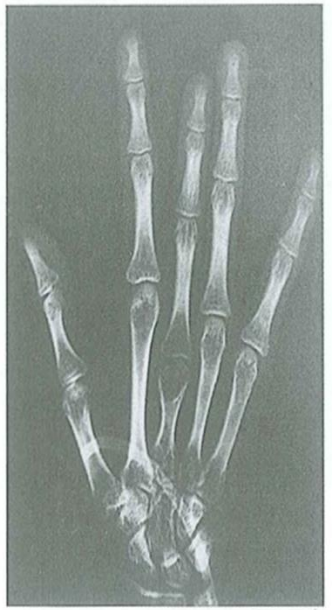
CLINICAL MANIFESTATIONS AND TREATMENT

vaso-occlusive episode is the cardinal clinical feature of sickle cell anemia , The pain may occur in any part of the body, but most often occurs in the chest, abdomen, or extremities. These painful episodes are often abrupt .
The pathogenesis of pain is disruption of blood flow in the microvasculature by sickle cells, resulting in tissue ischemia. Precipitating causes of painful episodes can include **physical stress, infection, dehydration, hypoxia, systemic acidosis, exposure to cold, and swimming in non-heated water for prolonged periods**.
Successful treatment of painful episodes include the use of acetaminophen or a nonsteroidal agent early in the course of pain, followed by acetaminophen with codeine and short- or long-acting oral opioids, or hospitalization with IV administration of morphine or morphine derivatives .
Hydroxyurea, a myelosuppressive agent, is the only effective drug proven to reduce the frequency of painful episodes. Hydroxyurea raises the level of Hb F and the hemoglobin level.

Dactylitis, often referred to as *hand-foot syndrome,* is frequently the 1st manifestation of pain in children with sickle cell anemia, presents with symmetric swelling of the hands and/or feet. Unilateral dactylitis can be confused with osteomyelitis and requires careful consideration because treatment of the former requires palliation with pain medication, often acetaminophen with codeine, whereas osteomyelitis requires at least a 4–6 wk course of IV antibiotics .







If *Salmonella* or *Staphylococcus* bacteremia occurs, strong consideration should be given to evaluation of osteomyelitis with a bone scan or an MRI, given the higher risk of osteomyelitis in children with sickle cell anemia compared with the general population.

Infections By 5 yr of age, most children have functional asplenia. Regardless of age, all patients with sickle cell anemia are at increased risk for infection and death as a result of bacterial infection, particularly with encapsulated organisms, such as *Streptococcus pneumoniae* and *Haemophilusinfluenzae* type B. Children with sickle cell anemia should receive prophylactic oral penicillin V (125 mg twice daily up to age 3 yr, then 250 mg twice daily). An alternative for children who are allergic to penicillin is erythromycin 10 mg/kg twice daily. In addition to penicillin prophylaxis, routine childhood immunizations and annual administration of influenza vaccine are highly recommended.

management of fever in a child with sickle cell anemia is a medical emergency that requires prompt medical evaluation and antibiotics because of the high risk of bacterial infection ,parenteral long-acting 3rd-generation cephalosporin. For patients with a positive blood culture, pathogen-specific therapy (1st empirically, then based on sensitivities) must be initiated.

Acute splenic sequestration is a life-threatening complication occurring primarily in infants, and may occur as early as 5 wk of age.
The **etiology** of splenic sequestration episodes is unknown. **Clinically,** these events are associated with engorgement of the spleen, with a subsequent increase in spleen size, evidence of hypovolemia, and a decline in hemoglobin.
These events can be accompanied by upper respiratory tract infections, bacteremia, or viral infection.
Treatment maintenance of hemodynamic stability with either isotonic fluid or blood transfusions.
Repeated episodes of splenic sequestration are common, occurring in approximately **50%** of patients. **Prophylactic splenectomy** performed after the acute episode has resolved is the only effective strategy for prevention of future life-threatening episodes.

Acute chest syndrome (ACS)

fever, respiratory distress, and pain that often occurs in the chest, but may include only the back and/or the abdomen.
Even when no respiratory symptoms are present, all patients with fever should receive a chest radiograph to identify ACS.
Radiographs may show single-lobe involvement, most often the left lower lobe, and when multiple lobes are involved, usually both lower lobes are affected. Pleural effusions, either unilateral or bilateral, may not be present initially (or may be minimal in size), but may progress rapidly to a total whiteout.

Treatment

-oxygen administration, Oxygen should be administered when the oxygen saturation is <90%.

-simple or exchange blood transfusion therapy .

-opioids.

-As a result of the clinical overlap between pneumonia and ACS, all episodes should be treated promptly with antimicrobial therapy that includes at least a macrolide and a 3rd-generation cephalosporin to treat the most common pathogens associated with ACS

S. pneumoniae

Mycoplasma pneumoniae

Chlamydia pneumoniae

Neurologic complications

Approximately 11% and 20% will have either **overt** or **silent** strokes, respectively, before their 18th birthday.

Treatment of stroke includes

- oxygen administration to maintain oxygen saturation at >96%
- simple blood transfusion therapy to increase the hemoglobin to a maximum of 11.0 g/dL, exceeding this hemoglobin threshold may limit oxygen delivery to the brain because hyperviscosity of the blood may decrease oxygen delivery.

Human parvovirus B19 Any child with reticulocytopenia should be considered as having parvovirus B19 until proven otherwise. Acute infection with parvovirus B19 is usually associated with red cell aplasia (**aplastic episode**). **Treatment** requires packed red blood cell transfusions for hemodynamic instability.

Priapism, a common problem in sickle cell anemia, is an involuntary penile erection lasting longer than 30 min. **Treatment** of priapism is supportive therapy, such as a sitz bath or pain medication. If the priapism lasts longer than **4 hr**, then aspiration of blood from the corpora cavernosa, followed by irrigation with dilute epinephrine. For the prevention of recurrent priapism, **hydroxyurea** is effective.

Renal disease **Seven** sickle cell anemia nephropathies have been identified:
(1) gross hematuria
(2) papillary necrosis,
(3) nephrotic syndrome,
(4) renal infarction,
(5) hyposthenuria,
(6) pyelonephritis, and
(7) renal medullary carcinoma.
The presentation of these entities may include hematuria, proteinuria, renal insufficiency, concentrating defects, or hypertension.
other significant complications include
.Sickle cell retinopathy
.Delayed onset of puberty
.Avascular necrosis of the femoral head and humerus
.Leg ulcers

DIAGNOSIS OF SICKLE CELL DISEASE

1. In utero: Sickle cell disease can be diagnosed accurately in utero by mutation analysis of DNA by **polymerase chain reaction (PCR)** prepared from chorionic villus biopsy or fetal fibroblasts (obtained by amniocentesis).These techniques should be employed before **10 weeks' gestation**. The desire to avoid the small risk of fetal loss associated with these invasive techniques has led to investigation of noninvasive prenatal diagnosis by testing fetal cells or free fetal DNA known to circulate in the plasma of pregnant women.

2. Newborns

The newborn with SCD is generally not anemic or symptomatic until toward the end of the first year of life because of the protective effects of fetal Hb.

Recognition of the disease in a newborn can lead to prevention of mortality and morbidity.

The methods used for initial screening **are**

- **high-performance liquid chromatography (HPLC)**
- **capillary electrophoresis (CE)**
- **isoelectric focusing (IEF)**

These tests can be performed on cord blood or on dried blood specimen blotted on filter paper.

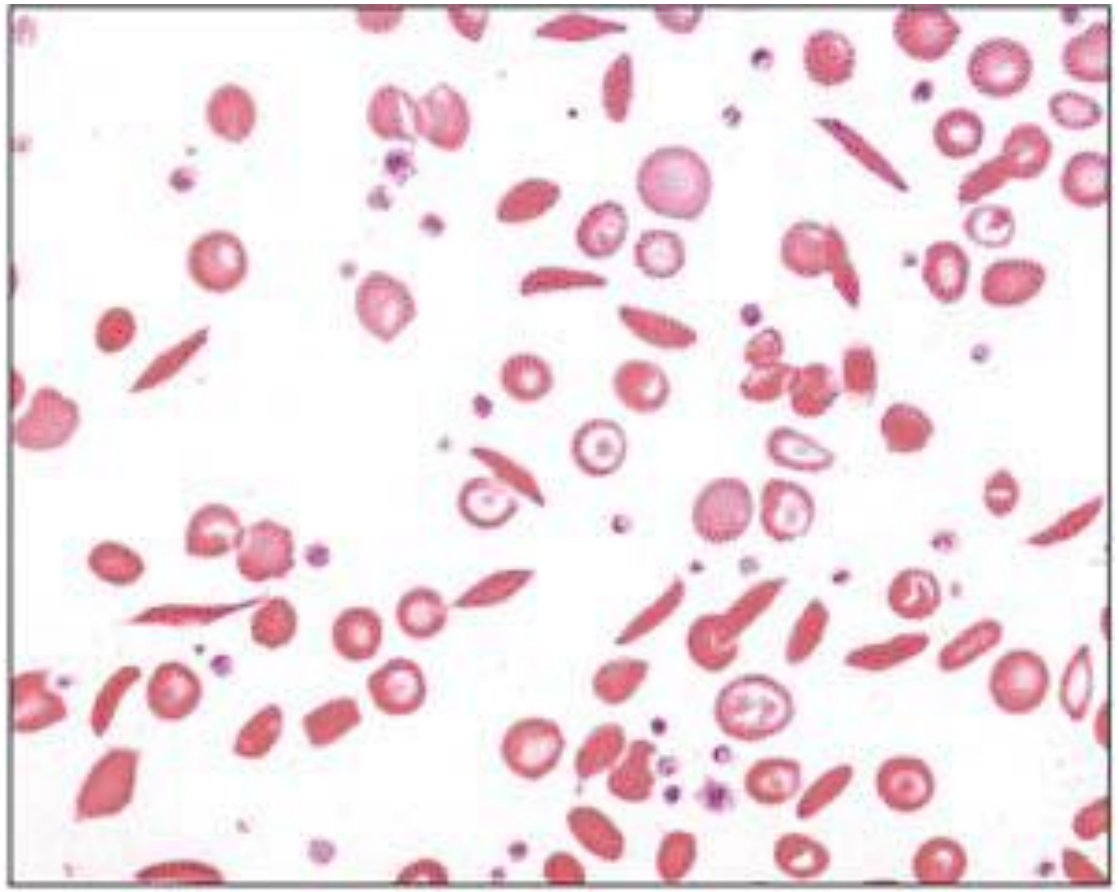
False negative screens using these methods have been reported in infants who received perinatal transfusions before screening.

Diagnosis can also be performed using polymerase-chain reaction amplification of deoxyribonucleic acid (DNA) extracted from filter paper. Universal screening compared with targeted screening of newborns of parents at risk has been shown to identify more infants with disease, prevent more deaths.

3. The haemoglobin is usually 6-9 g/dL.

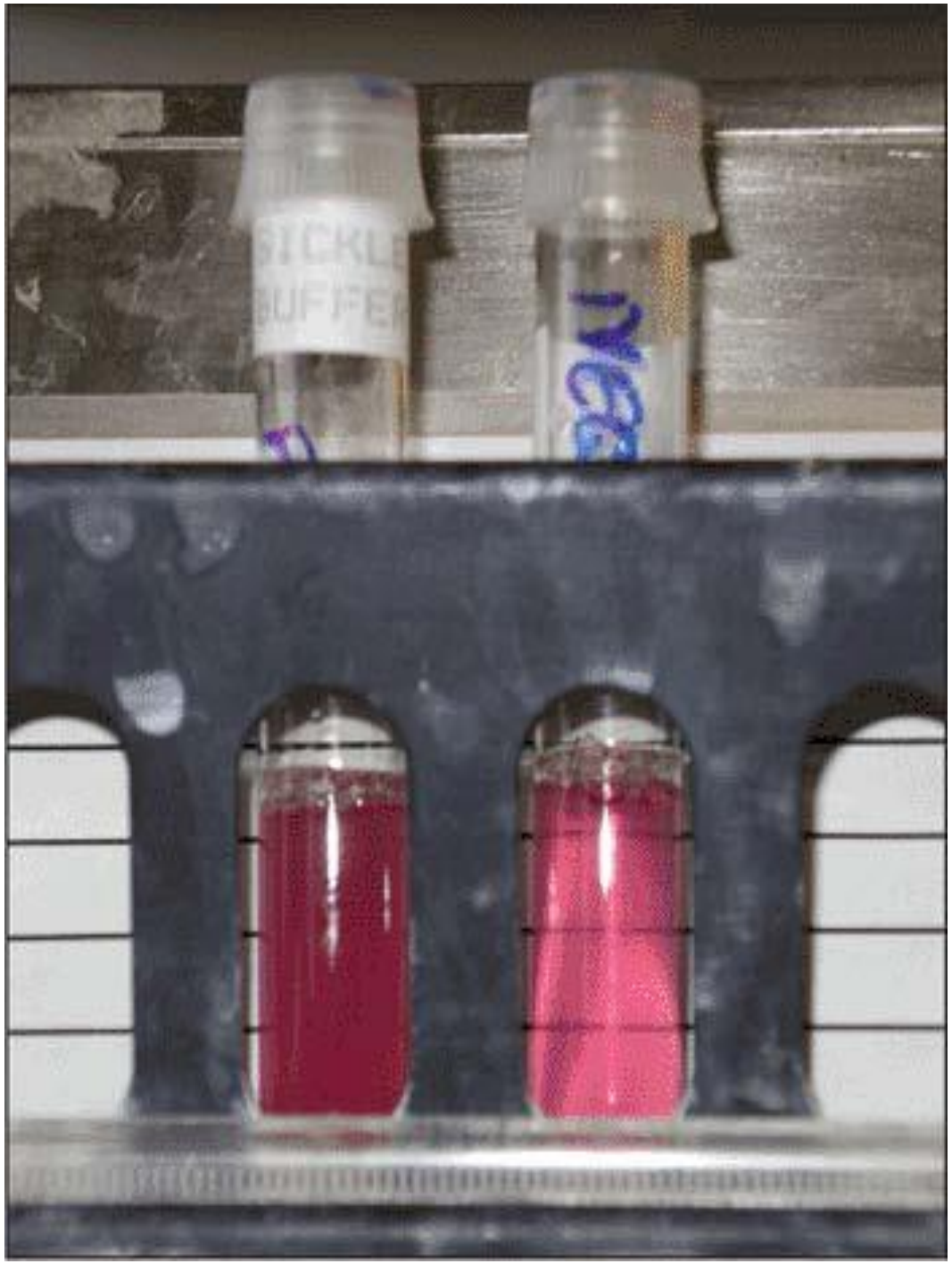
4. Sickle cells and target cells occur in the blood film after **3–6 months** of life.

5.A complete blood cell count as well as hemoglobin analysis is recommended on both parents to confirm the diagnosis and provide an opportunity for genetic counseling



6.Sickle solubility tests depend on the decreased solubility of deoxygenated hemoglobin S . **It is positive in patients with both sickle trait and sickle cell anemia and therefore cannot be used to distinguish homozygous from heterozygous hemoglobin S.**

➪ Fetal hemoglobin interferes with polymerization of hemoglobin S, and the sickle solubility test can give a false-negative result if hemoglobin F makes up more than ~10 to 20% of hemoglobin in the sample. Therefore, the sickle solubility test is not reliable in infants during the first few months of life.



Management

1. Infection:

Because of a marked incidence of bacterial sepsis and meningitis and fatal outcome under 5 years of age, the following management is recommended:

a. Prophylactic antibiotics:

- All children with SCD should receive oral penicillin prophylaxis starting by 3_4 months of age, with 125 mg bid under 3 years old and 250 mg bid for 3 years and older.
- In patients allergic to penicillin erythromycin ethyl succinate 10 mg/kg orally twice a day should be prescribed.

b. Vaccination:

- All children with SCD should receive routine childhood immunizations,
- The 23-valent pneumococcal vaccine (PPV-23) should be administered at 2 years of age with a booster administered 5 years later.
- Meningococcal vaccination should also be administered.
- Influenza virus vaccine should be given yearly, each fall.

2. Transfusion therapy:

- Chronic red cell transfusion therapy or repeated intermittent transfusions
- Exchange transfusion limits or prevents iron loading and should be utilized, when possible, for chronic transfusion therapy.

3. Induction of HbF:

Sustained elevations in HbF (>20%) are associated with reduced clinical severity in SCD, as HbF interferes with HbS polymerization and RBC sickling.

4. Newly approved therapies:

- L-Glutamine
- Voxelotor
- Crizanlizumab

5. HSCT (haematopoietic stem cell transplantation)

Currently HSCT [including umbilical cord blood (UCB)] is the only curative therapy.

6. Gene therapy

Sickle cell trait (heterozygous form, AS)

Pathophysiology

The concentration of HbS in red cells is low, and sickling does not occur under normal conditions.

Diagnosis

1. Indices—usually normal
2. Blood smears—normal with few target cells
3. Hemoglobin electrophoresis—AS pattern **HbA 55-60%**
   HbS 35-45%

Clinical features

1. Usually asymptomatic.
2. Hematuria rarely.
3. Increased propensity for renal medullary cancer.
4. Exertional rhabdomyolysis-/exercise-related sudden death. Ensure adequate hydration with sports activities.
5. Complicated hyphema—with secondary hemorrhage, increased intraocular pressure, and central retinal artery occlusion. This requires evaluation/treatment by an ophthalmologist.
6. Infarction rare, occurring during flights in unpressurized aircraft.