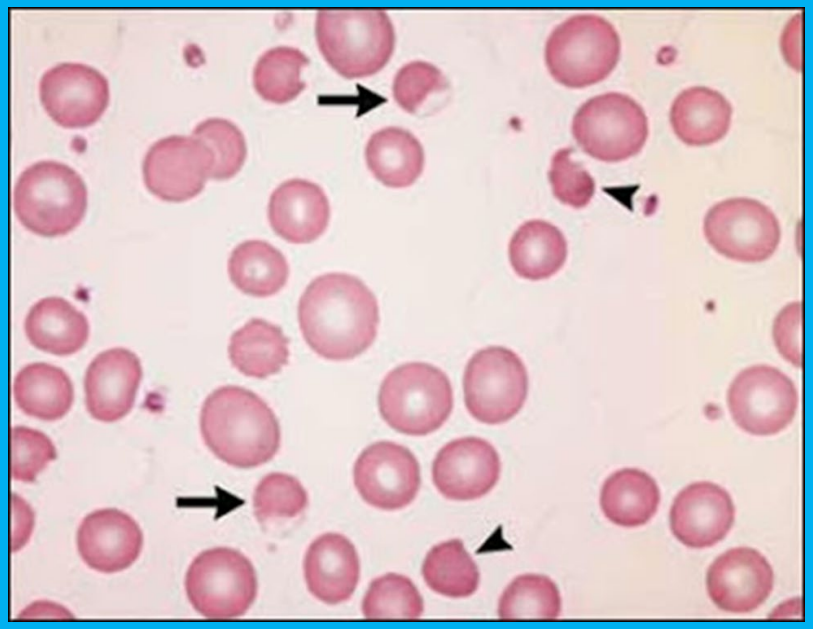
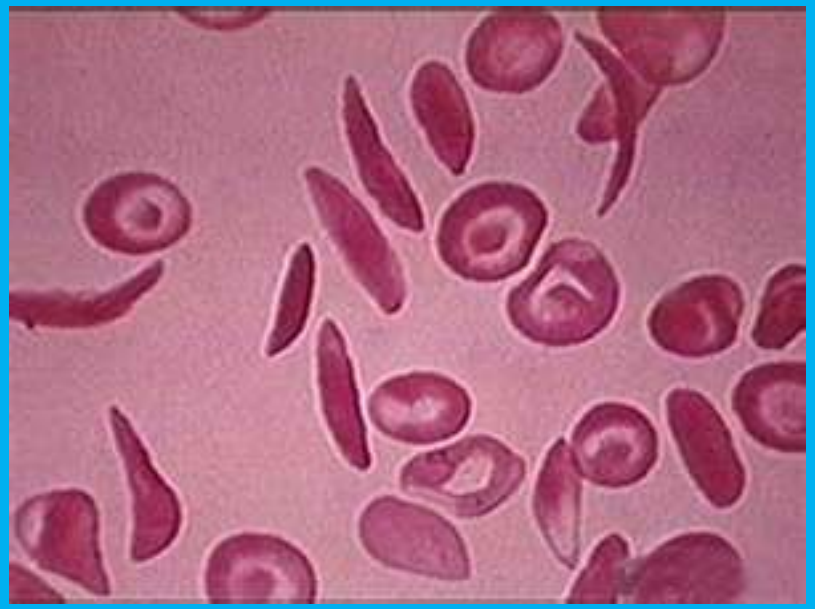
Sickle cell anaemia

Glucose-6-Phosphate dehydrogenase deficiency

ACQUIRED HAEMOLYTIC ANAEMIA

Prof. Dr. Mousa Qasim



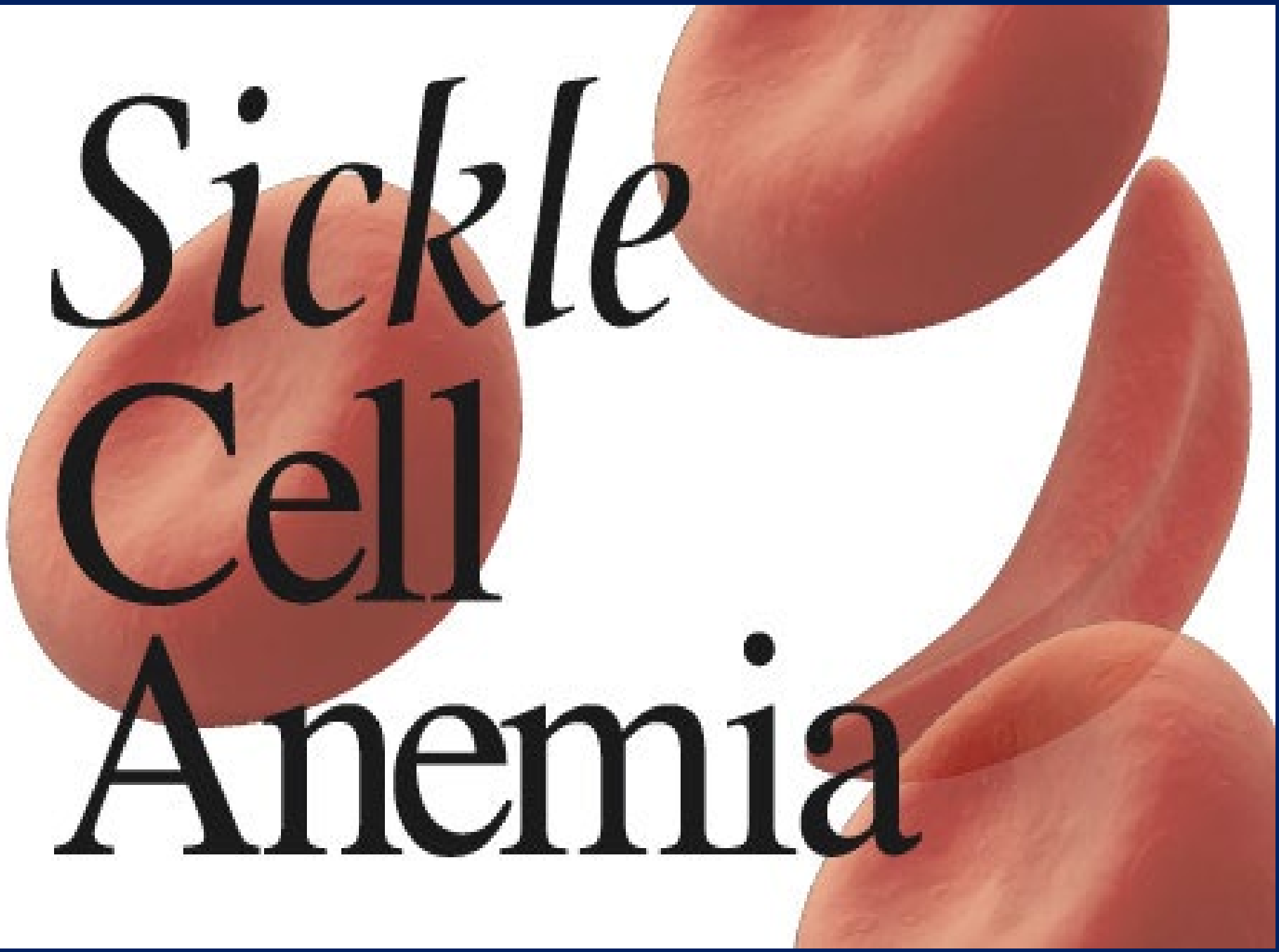


Qualitative abnormalities – abnormal haemoglobins

In qualitative abnormalities (called the abnormal haemoglobins),there is a functionally important alteration in the amino acid structure of the polypeptide chains of the globin chains. Several hundred such variants are known; they were originally designated by letters of the alphabet, e.g. S, C, D or E, but are now described by names usually taken from the town or district in which they were first described

These substitutions often change the charge of the globin chains, producing different electrophoretic mobility, and this forms the basis for the diagnostic use of haemoglobin electrophoresis to identify haemoglobinopathies.

Sickle Cell Anemia



SICKLE-CELL ANAEMIA

. Is type of Anemia results from single Glutamic acid to Valine substitution at position 6 of β globin p.p chain.

. Inherited as Autosomal recessive trait.

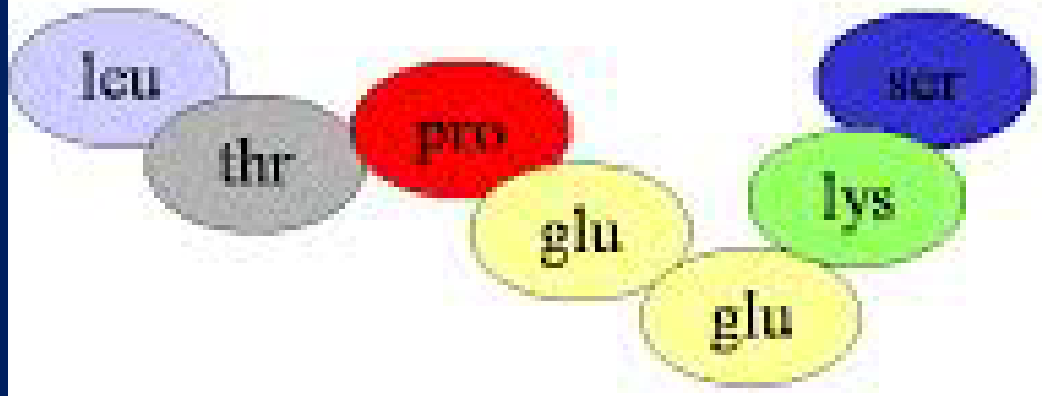
. Homozygotes only produce abnormal β chain that make HbS (termed SS) and it results in clinical syndrome of sickle cell disease.

. Heterozygote produces mixture Of normal and abnormal β chain makes normal HbA and HbS(termedAS) clinically asymptomatic sickle cell trait.

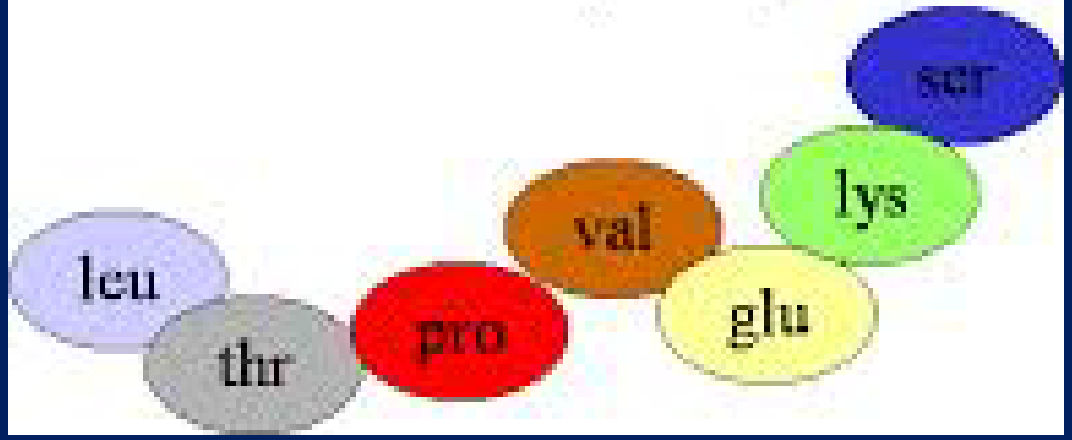
sickle-cell trait; although this was previously thought of as asymptomatic, it may be associated with an increased risk of sudden and cardiovascular death.

Amino Acid Sequence

Normal hemoglobin (HbA)



Sickle cell hemoglobin (HbS)



PATHOGENESIS:

. When HbS is deoxygenated, the molecule of Hb polymerizes to form Pseudo crystalline structures Called TACTOIDS, these distort red cell membrane and produce Characteristic sickle-shaped cells.

This polymerization is reversible when reoxygenation occurs otherwise the distorted RBC membrane Is irreversibly sickled.

The greater concentration of sickled cell Hb in individual cell, the more easily tactoids are formed, But this process may be enhanced by presence of other Hb.

HbC participates in polymerization more readily than HbA.

HbF strongly inhibits polymerization

CLINICAL FEATURES:

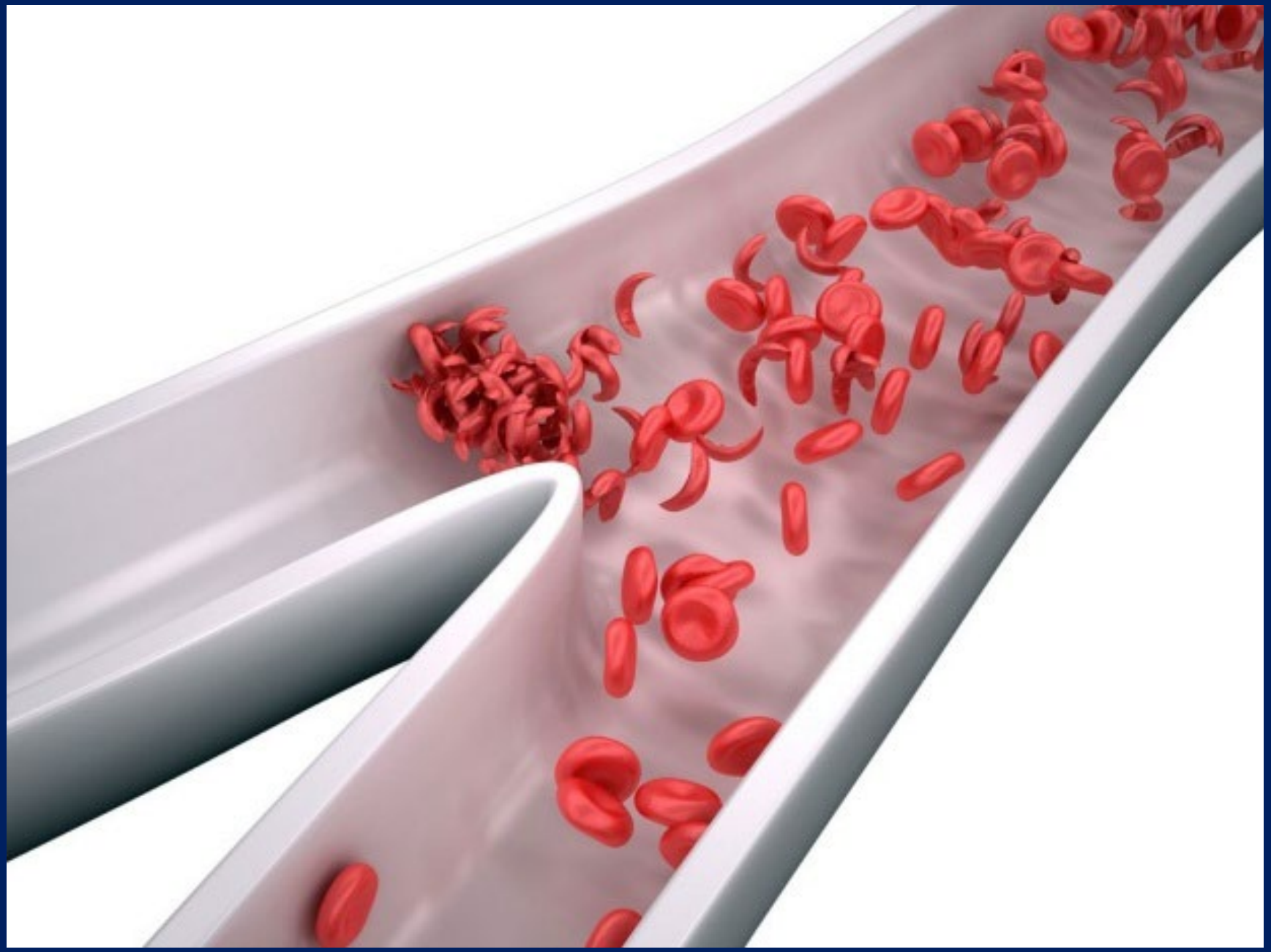
Sickling is precipitated by:

1.Hypoxia
2.Acidosis
3.Dehydration
4.Infection

Irreversible sickled cells have short survival and plug vessels in microcirculation.This results in a no. of acute syndromes (Crises) and chronic organic damage.

1.Vaso-Occlusive Crises: (PAINFULL)

Plugging of small vessels in bone produces acute sever bone pain. This affects areas of active marrow, the hands and feet in children so called (Dactylitis) or femora, humeri, ribs, pelvis and vertebrae in adult. Patients usually have a systemic response that includes tachycardia, sweating, fever and this type is the most common crisis.



2-Stroke.

The single most devastating consequence of sickle-cell disease is stroke. Stroke or silent stroke occurs in 10–15% of children with sickle-cell disease. Children at risk of stroke can be identified by screening with transcranial Doppler ultrasound, with fast flow associated with increased stroke risk. These children may be offered strategies such as transfusion or treatment with hydroxycarbamide to reduce the risk of stroke.

3.Sickle chest syndrome:

May follow on from a vaso-occlusive crises and it is the most common cause of death in adult sickled disease. Bone marrow infarction results in fat emboli to the lungs which cause sickling and infarction leading to Ventilatory failure if not treated.

4.Sequestration Crises:

Thrombosis of venous outflow causes loss of function and acute painful enlargement. Spleen is most common site in children.

massive splenic enlargement may result in sever anemia and circulatory collapse and death.

Recurrent splenic sickling in childhood result in infarction and adults may have no functional spleen and the liver may undergo sequestration and sever pain will occur due to capsular stretching priapism May occur in affected individuals.

5.Aplastic Crises:

Infection of adult with human erythrovirus 19 results in sever but self-limiting RBC aplasia. This produce a very low Hb that may cause Heart Failure. unlike all other sickle crises the reticulocytes count is low.

6-Pregnancy:

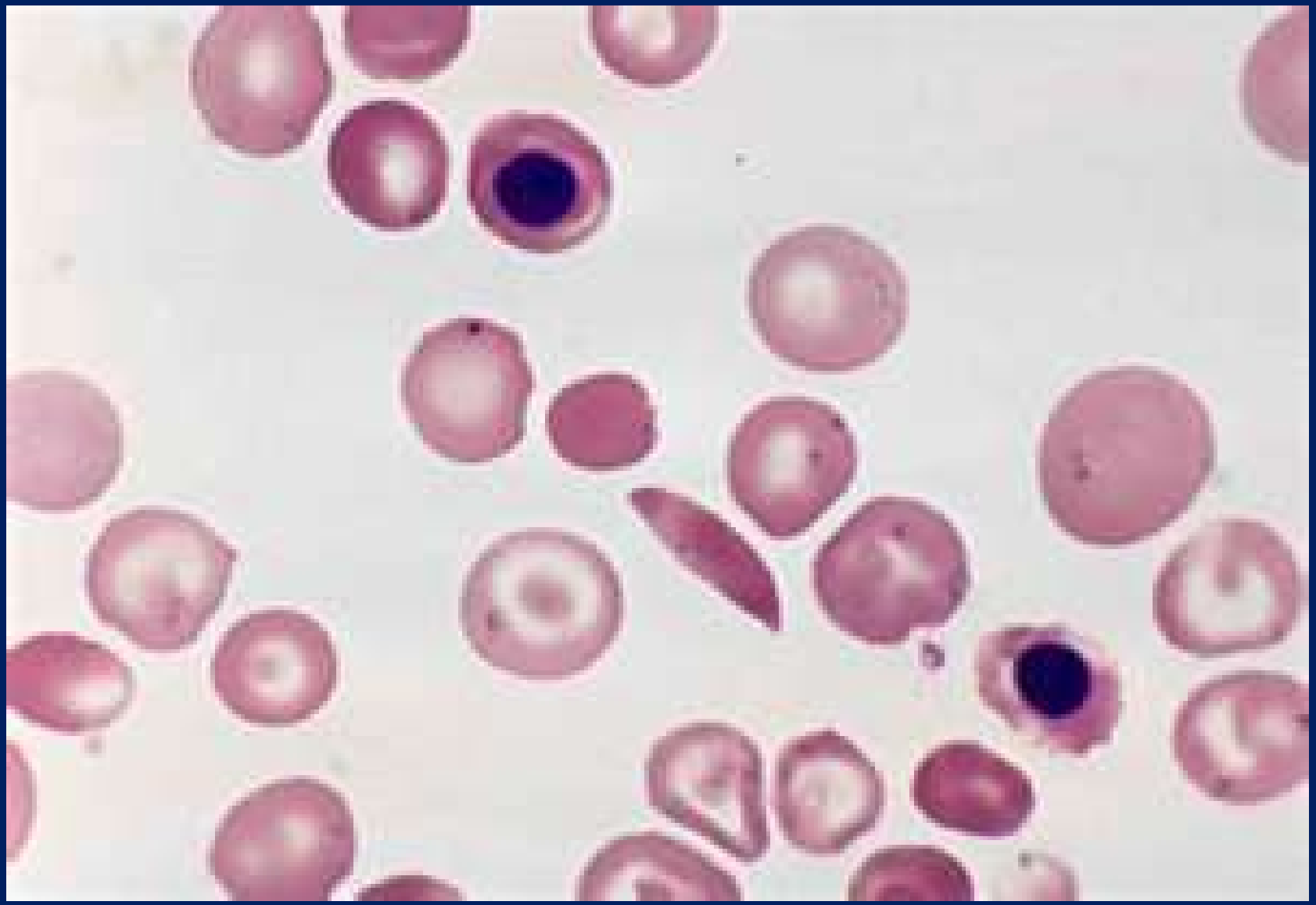
Pregnancy in sickle-cell disease requires planning and multidisciplinary management. Women with sickle-cell disease have increased pregnancy-related morbidity, which includes painful crisis, placental failure and thrombosis .

INVESTIGATIONS:

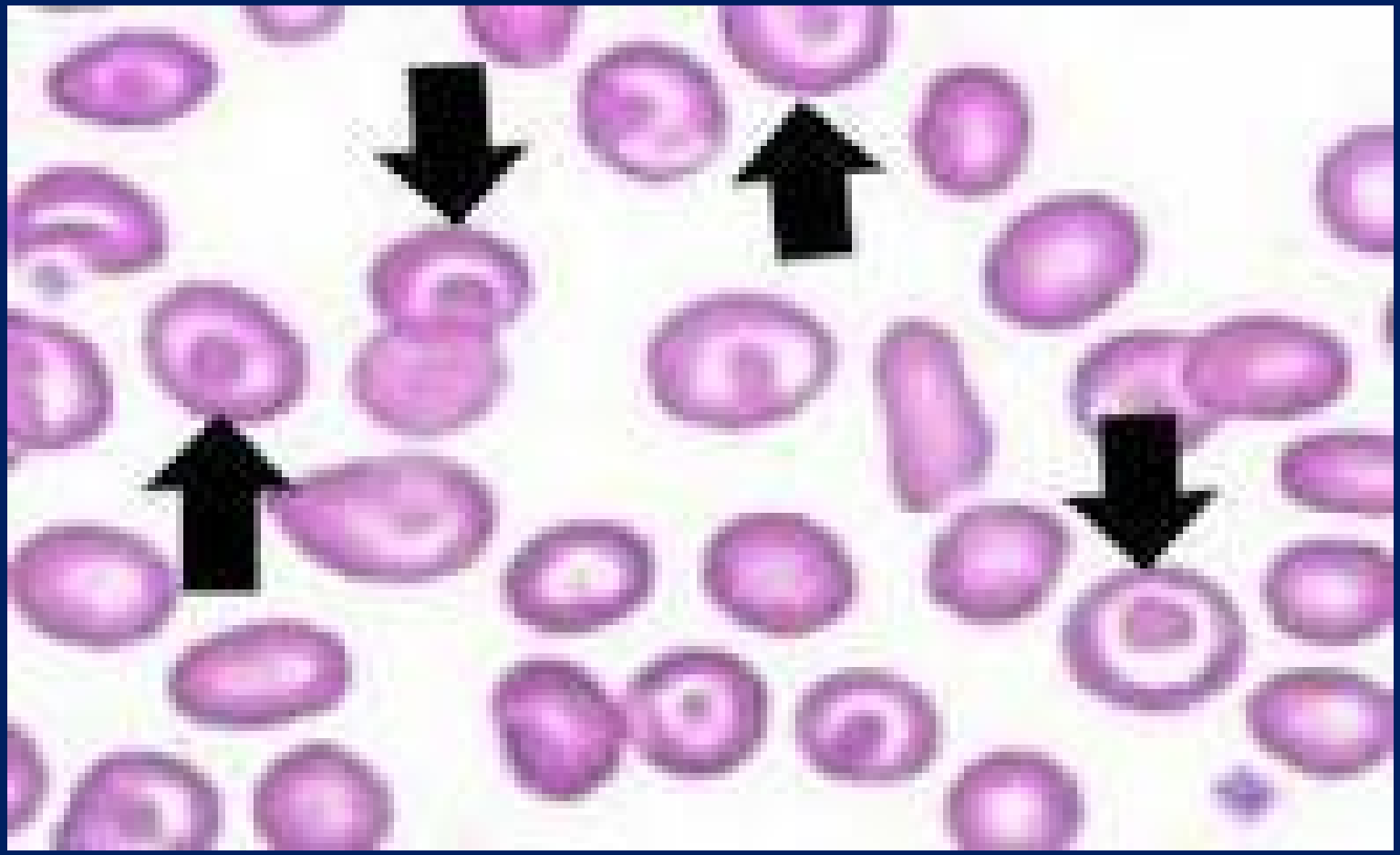
1. Compensated anemia: Hb% 6-8 g/dl
2. Blood film: sickled cells, target cells, features of hyposplenism include **Howell-Jolly bodies, Pappenheimer bodies, target cells and irregular contracted red blood cells**.
3. Reticulocytosis
4. Sickling test: exposing red cells to reducing agent (Na dithionite):

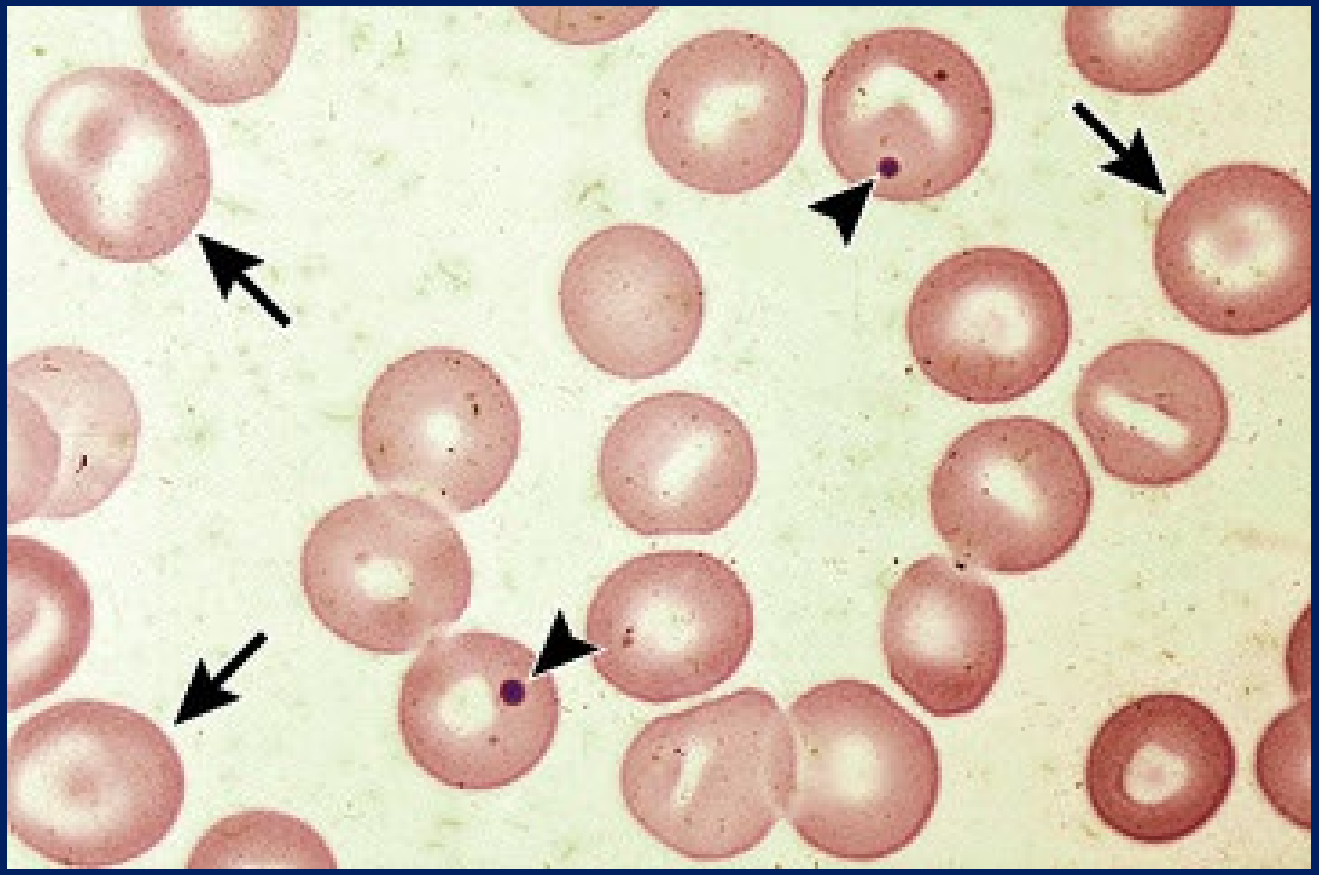
-HbA clear solution

-HbS turbid solution due to polymerization. We can't distinguish between sickle trait and disease.

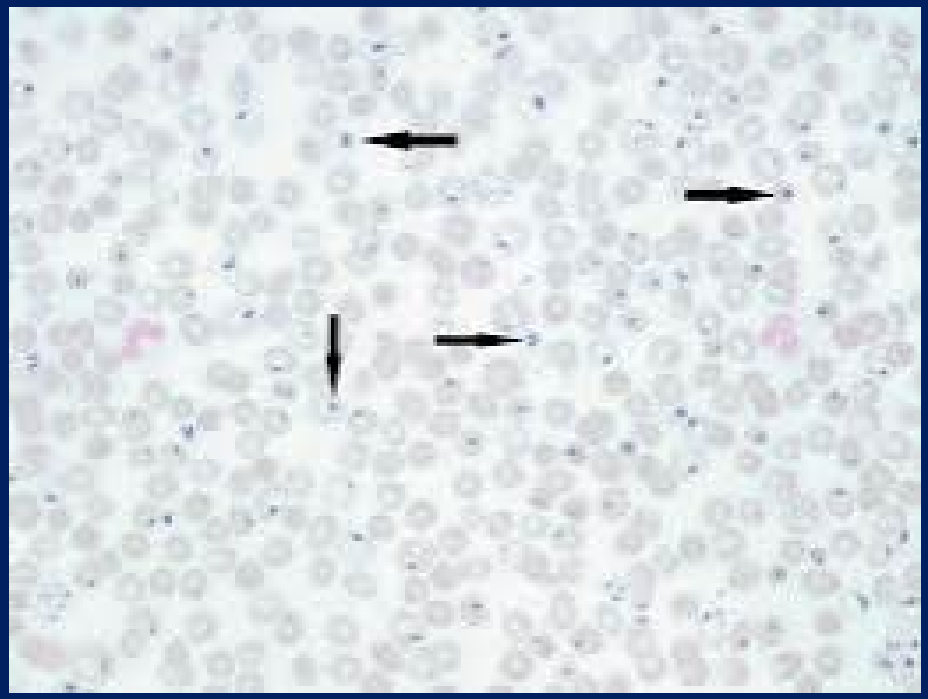


If you are going to look at it using an optical microscope, you will notice that the target cells have a dark center (filled with hemoglobin) and surrounded by a white ring with a dark outer second ring (contains a band of hemoglobin).



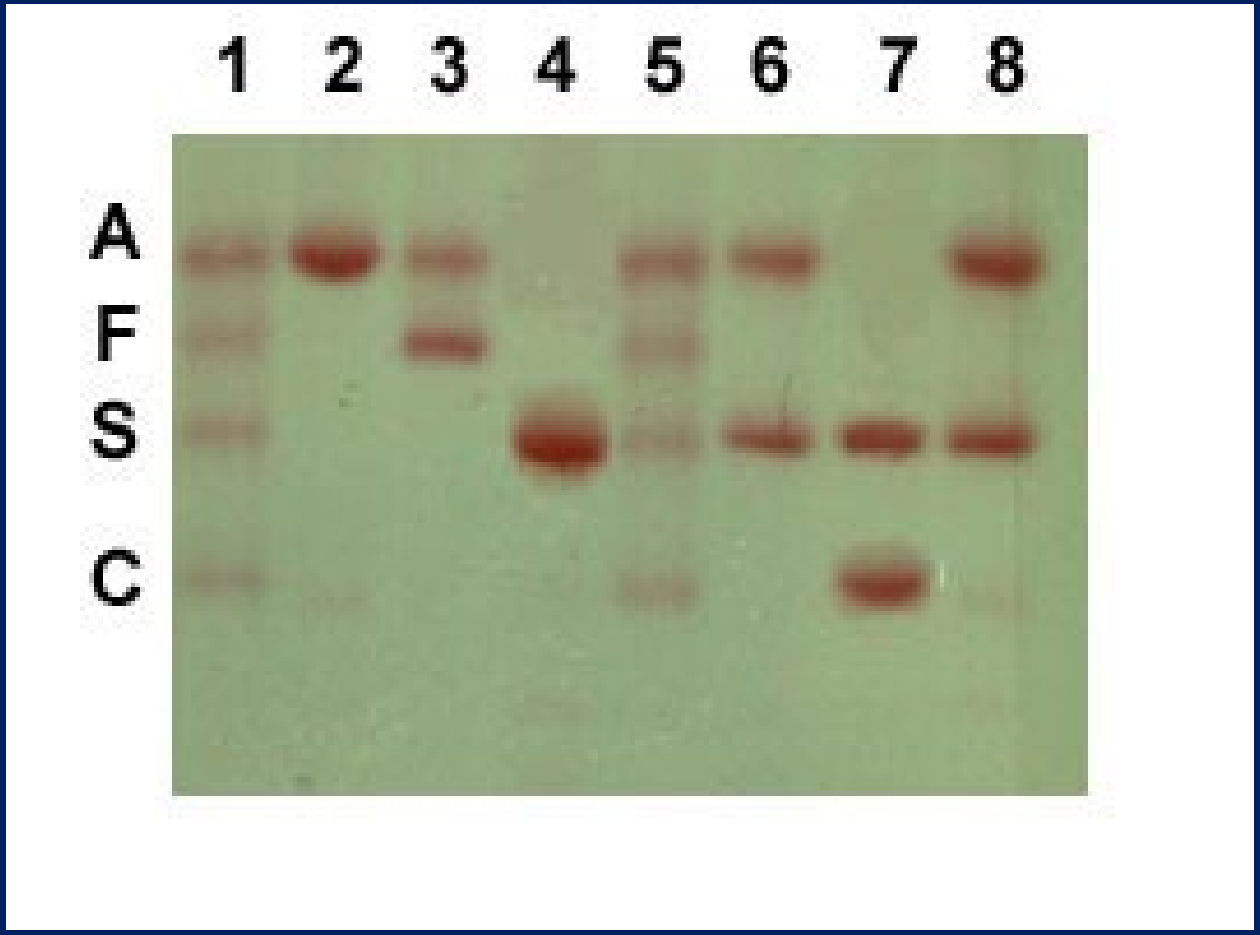


Howell-Jolly bodies are red cell inclusions which are residual nuclear fragments. They may be seen in hemolysis, megaloblastic anemia, or post-splenectomy.



Pappenheimer bodies are **small debris containing iron that can be found in red blood cells**. This debris is normally eliminated by the spleen. Pappenheimer bodies are found in patients with no spleen (surgical splenectomy).

5. Definitive diagnosis requires Hb electrophoresis that demonstrates:
1-no HbA.
2- 2-20 % Hbf .
3- predominance of HbS.
4-Both parents of affected individual will be sickle trait



Pattern of hemoglobin electrophoresis from several different individuals. Lanes 1 and 5 are hemoglobin standards. Lane 2 is a normal adult. Lane 3 is a normal neonate. Lane 4 is a homozygous HbS individual. Lanes 6 and 8 are heterozygous sickle individuals. Lane 7 is a SC disease individual.

Management

All patients with sickle-cell disease should receive prophylaxis with daily folic acid, and appropriate management of the hyposplenic state that is uniformly found in these patients from an early age .

Seasonal vaccination against influenza is also advised.

Management of the splenectomised patient

-Vaccinate with pneumococcal, Haemophilus influenzae type B, meningococcal group C and influenza vaccines at least 2–3 weeks before elective splenectomy.

Vaccination should be given after emergency surgery but may be less effective.

- Pneumococcal re-immunisation should be given at least 5-yearly and influenza annually. Vaccination status must be documented

- Life-long prophylactic penicillin V (500 mg twice daily) is recommended. In penicillin-allergic patients, consider a macrolide.

- Patients should be educated regarding the risks of infection and methods of prophylaxis

- A card or bracelet should be carried to alert health professionals to the risk of overwhelming sepsis
- In sepsis, patients should be resuscitated and given intravenous antibiotics to cover pneumococcus, Haemophilus and meningococcus, according to local resistance patterns
- The risk of cerebral malaria is increased in the event of infection
-Animal bites should be promptly treated with local disinfection and antibiotics to prevent serious soft tissue infection and sepsis

Vaso-occlusive crises are managed by aggressive rehydration, oxygen therapy, adequate analgesia (which often requires opiates) and antibiotics.

Transfusion should be with fully genotyped blood wherever possible.

Simple top-up transfusion may be used in a sequestration or aplastic crisis. A regular transfusion programme to suppress HbS production and maintain the HbS level below 30% may be indicated in patients with recurrent severe complications, such as cerebrovascular accidents in children or chest syndromes in adults. Exchange transfusion, in which a patient is simultaneously venesected and transfused to replace HbS with HbA, may be used in life-threatening crises or to prepare patients for surgery.

A high HbF level inhibits polymerisation of HbS and reduces sickling. Patients with sickle-cell disease and high HbF levels have a mild clinical course with few crises. Some agents are able to increase synthesis of HbF and this has been used to reduce the frequency of severe crises.

The oral cytotoxic agent hydroxycarbamide has been shown to have clinical benefit with acceptable side-effects in children and adults who have recurrent severe crises.

The P-selectin inhibitor, crizanlizumab, is currently under review as an agent to reduce vasoocclusive crisis.

Relatively few allogeneic stem cell transplants from HLA-matched siblings have been performed, but this procedure appears to be potentially curative .

Prognosis

In Africa, few children with sickle-cell anaemia survive to adult life without medical attention. Even with standard medical care, approximately 15% die by the age of 20 years and 50% by the age of 40 years.

Red cell enzymopathies

The mature red cell must produce energy via ATP to maintain a normal internal environment and cell volume while protecting itself from the oxidative stress presented by oxygen carriage. ATP is generated by glycolysis, while the hexose monophosphate shunt produces nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione to protect against oxidative stress. The impact of functional or quantitative defects in the enzymes in these pathways depends on the importance of the steps affected and the presence of alternative pathways.

In general, defects in the hexose monophosphate shunt pathway result in periodic haemolysis precipitated by episodic oxidative stress, while those in the glycolysis pathway result in shortened red cell survival and chronic haemolysis

Glucose-6-Phosphate dehydrogenase deficiency

This enzyme is pivotal in the hexose monophosphate shunt pathway. Deficiencies result in the most common human enzymopathy, affecting 10% of the world's population. The enzyme is a heteromeric structure made of catalytic subunits which are encoded by a gene on the X chromosome.

The deficiency therefore affects males and rare homozygotic females , but it is carried by females. Carrier heterozygous females are usually only affected in the neonatal period or in the presence of skewed X- inactivation.

CLINICAL FEATURES:

1. Acute drug induced Hemolysis. This occurs with many drugs:

Analgesics: aspirin and Phenactin

Antimalarial: primaquine, quinine, chloroquin and pyrimethamine

Antibiotics: Sulphanamide, Nitrofurantoin, Ciprofloxacin, miscellaneous(Quinidine, Probencid, vitamin K, Dapsone)

2. Chronic compensated Hemolysis
3. Infection or acute illness
4. Neonatal jaundice (feature of B-enzyme)
5. Favism or acute Hemolysis after ingestion of Broad beans.

- **The onset can be extremely abrupt, especially with favism in children.**
- **The anemia is from moderate to extremely severe.**
- **It is usually normocytic and normochromic.**

LAB DIAGNOSIS:

1. During the attack, there will be evidence of non-Spherocytic intravascular Hemolysis.

Blood film will show :

*Bite Cell —» red cell with a bite of membrane missing.

*Blister Cell —» red cell with membrane surface blistered.

*Irregular small shaped cells .

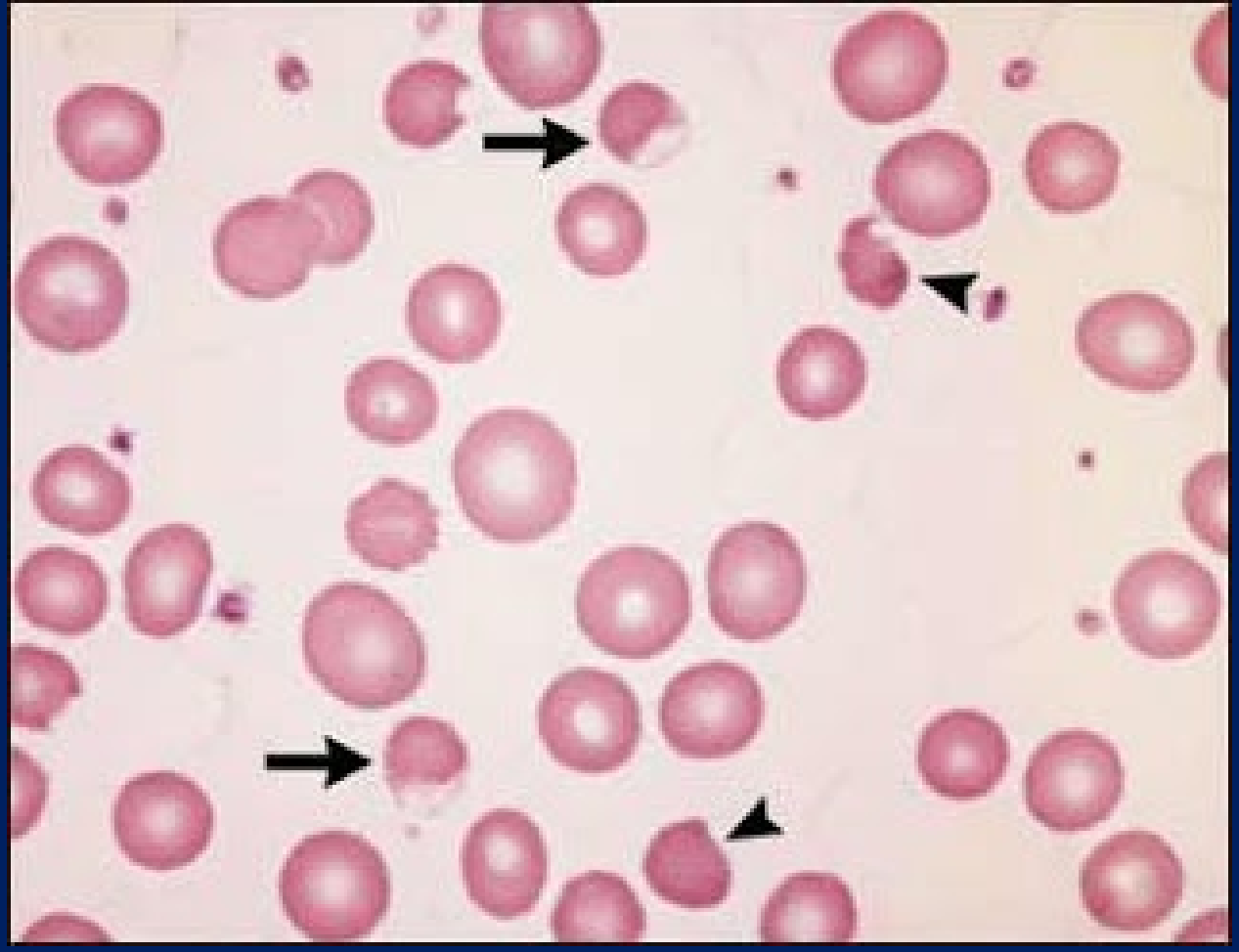
* polychromasia which reflect Reticulocytosis.

*Denatured Hb visible as Heinz body within the red cell cytoplasm,if stained with a supravital stain such as methyle violet.

2.* The level of G6PD can be indirectly assessed by screening method, which usually depends on decreased ability to reduce dyes.

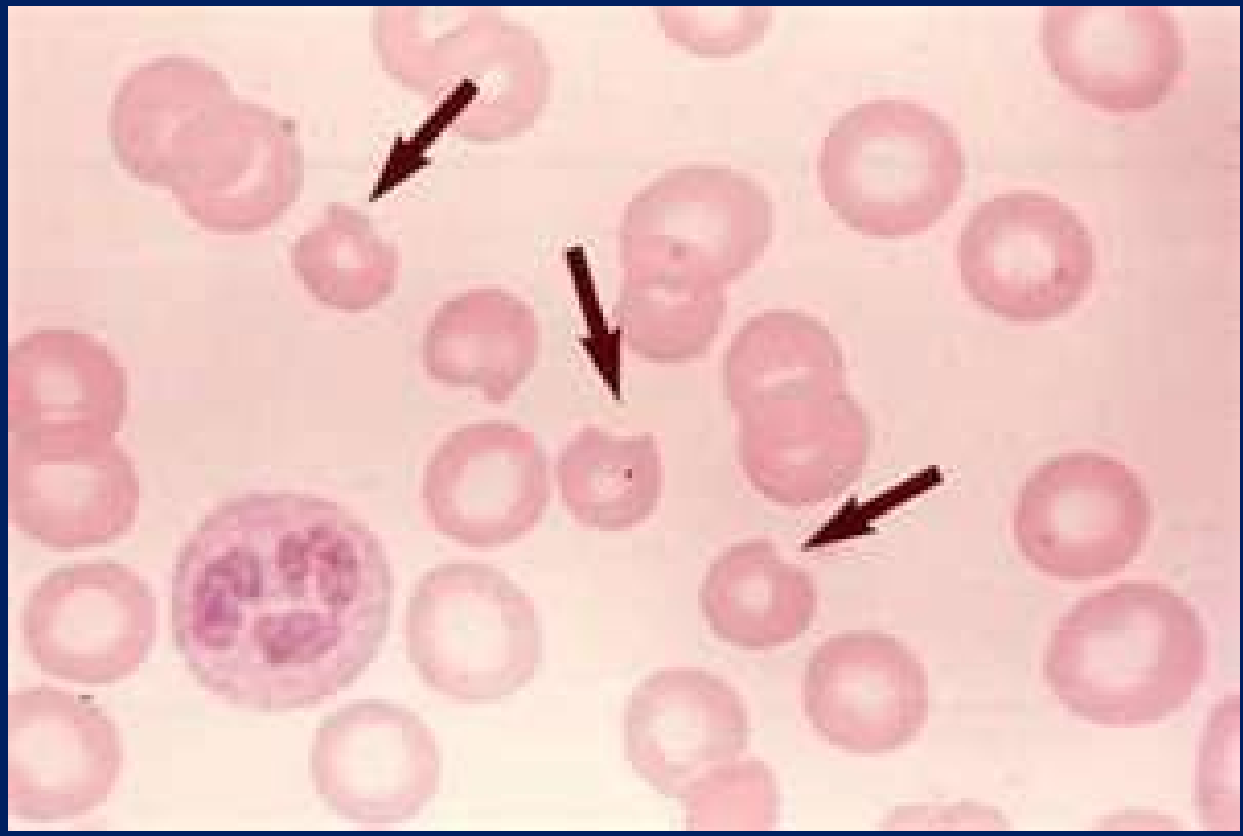
*** Direct assessment is made in those with low screening values.**

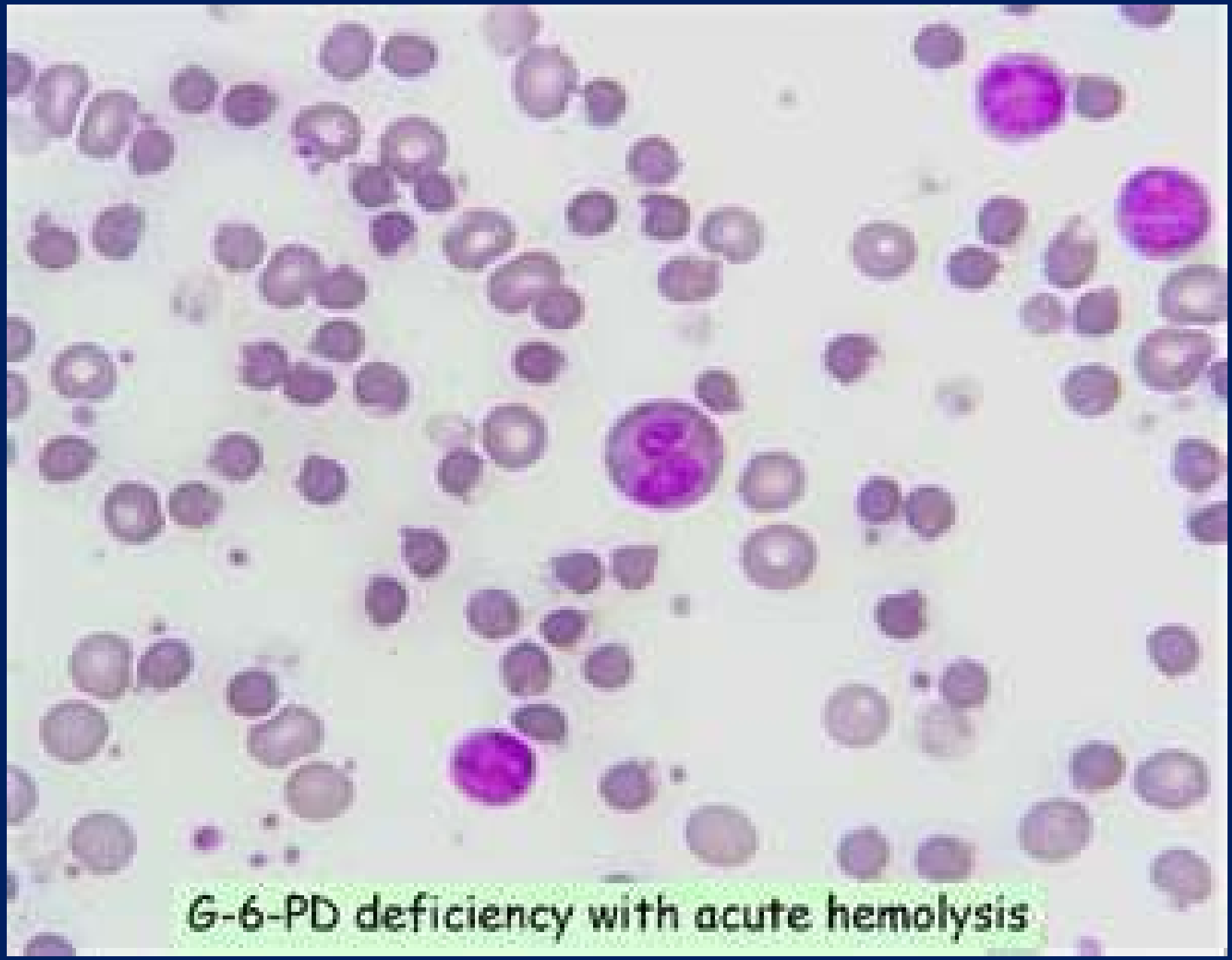
***care must be taken close to an acute haemolytic episode because reticulocytes may have normal enzyme level and give rise to a false Normal result.**



Acute hemolysis in G6PD deficiency
(arrows indicate “blister cells,” and arrowheads irregularly contracted cells)

Heinz bodies, bite cells




G-6-PD deficiency with acute hemolysis

G6PD level

- Can be indirectly assessed by screening methods that usually depend on the decreased ability to reduce dyes
- Direct assessment of G6PD is made in those with low screening values
- Care must be taken close to an acute haemolytic episode because reticulocytes may have higher enzyme levels and give rise to a false normal result

Management aims to:

1- stop the intake of any precipitant drugs or foods .

2-treat any underlying infection.

Favism due to the consumption of fava beans is the classically described precipitant of haemolysis in patients with G6PD deficiency.

Acute transfusionsupport may be life-saving.

ACQUIRED HAEMOLYTIC ANAEMIA

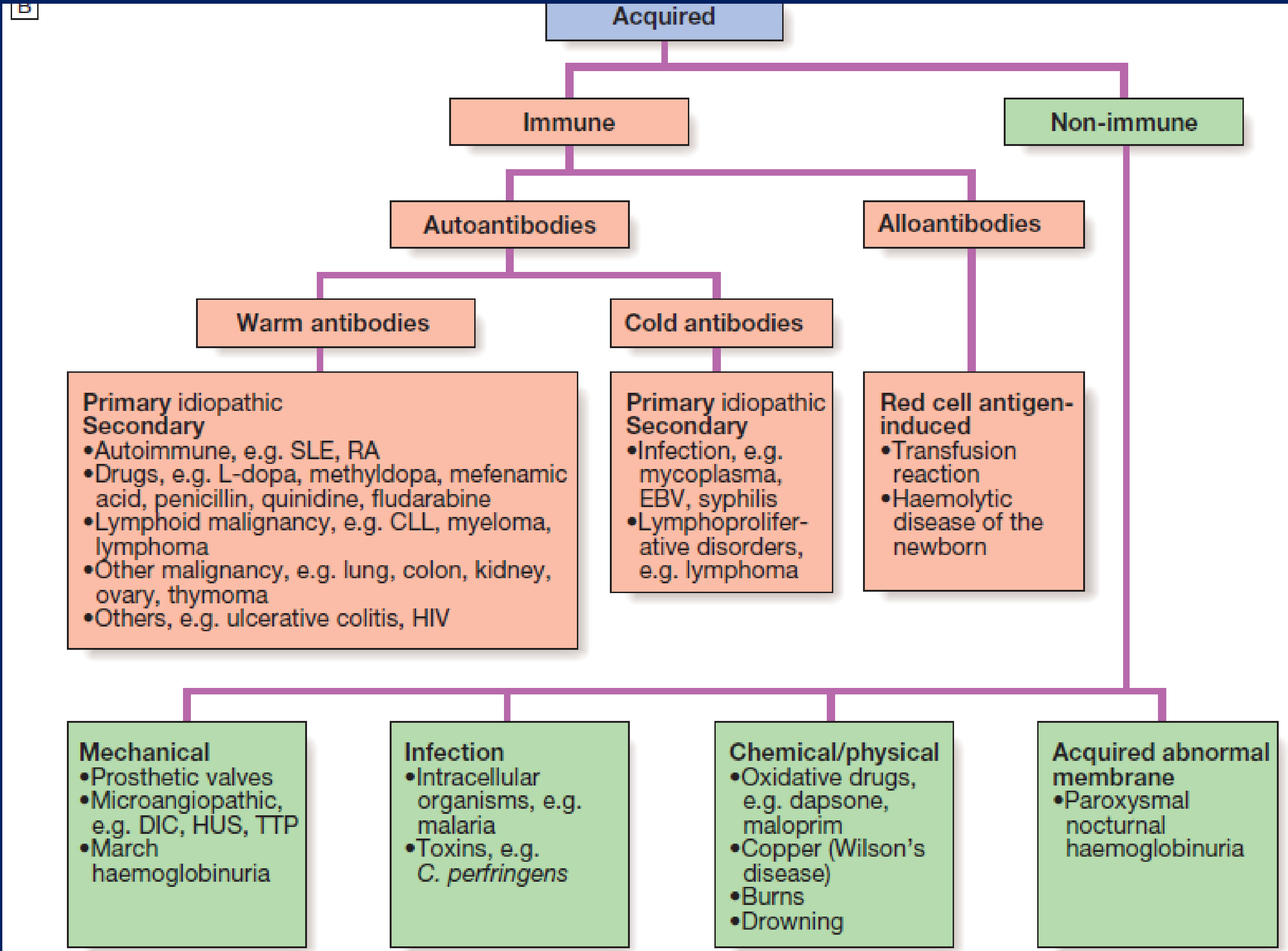


Fig. 24.21 Causes of haemolysis. [A] Inherited causes. [B] Acquired causes. (CLL = chronic lymphatic leukaemia; DIC = disseminated intravascular coagulation; EBV = Epstein–Barr virus; G6PD = glucose-6-phosphate dehydrogenase; HUS = haemolytic uraemic syndrome; PK = pyruvate kinase; RA = rheumatoid arthritis; SLE = systemic lupus erythematosus; TTP = thrombotic thrombocytopenic purpura)

mechanical trauma (heart valves, DIC) or oxidative damage (e.g. drugs such as dapsone and maloprim). When intravascular red cell destruction occurs, free hae-

degrade it and store the iron as haemosiderin. When the tubular cells are subsequently sloughed into the urine, they give rise to haemosiderinuria, which is always

ACQUIRED HAEMOLYTIC ANAEMIA:

Autoimmune heamolytic anemia:

This results from increased red cell destruction due to red cell autoantibodies. The antibodies may be IgG or M, or more rarely IgE or A.

If an antibody avidly fixes complement, it will result in intravascular haemolysis,

but if complement activation is weak, the haemolysis will be extravascular. Antibody-coated red cells lose membrane to macrophages in the spleen and hence spherocytes are present in the blood

The optimum temperature at which the antibody is active (thermal specificity) is used to classify immune haemolysis:

1.Warm antibodies bind best at 37°C and account for 80% of cases. The majority are IgG and usually react against Rhesus antigens.

2.Cold antibodies bind best at 4°C but can bind up to 37°C in some cases. They are usually IgM and bind complement. They account for the other 20% of cases.

Warm autoimmune haemolysis

The incidence of warm autoimmune haemolysis is approximately 1/100 000 population per annum; it occurs at all ages but is more common in middle age and there is a female excess.
No underlying cause is identified in up to 50% of cases.

Primary idiopathic
Secondary
-Autoimmune, e.g. SLE, RA
-Drugs, e.g. L-dopa, methyldopa, mefenamic acid, penicillin, quinidine, fludarabine
-Lymphoid malignancy, e.g. CLL, myeloma, lymphoma
-Other malignancy, e.g. lung, colon, kidney, ovary, thymoma
-Others, e.g. ulcerative colitis, HIV

Investigations:

1-There is evidence of haemolysis, polychromasia and spherocytes on the bloodfilm.

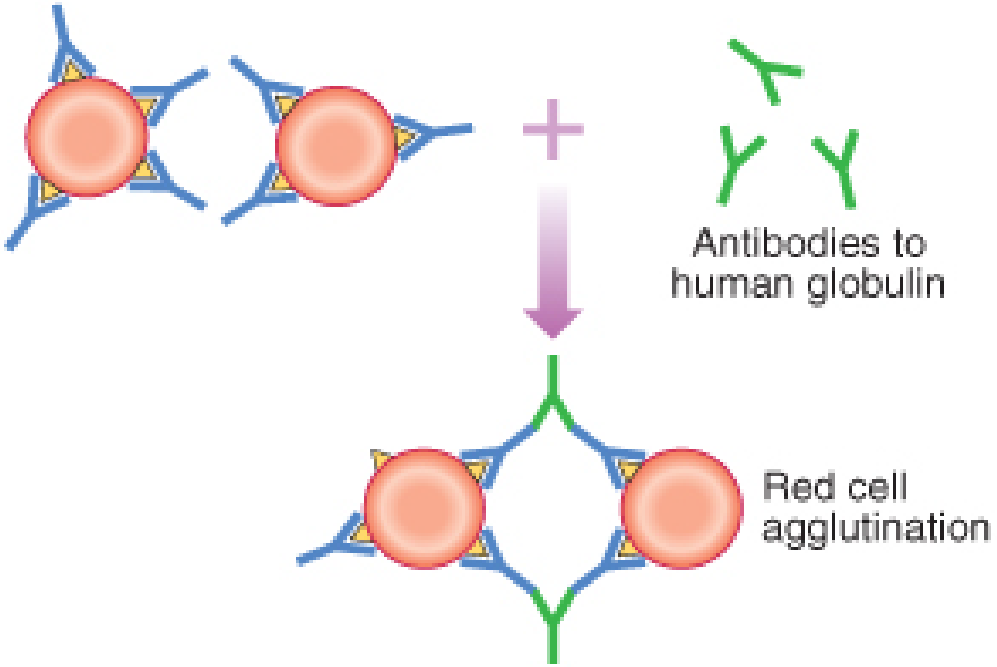
The diagnosis is confirmed by the direct Coombs test

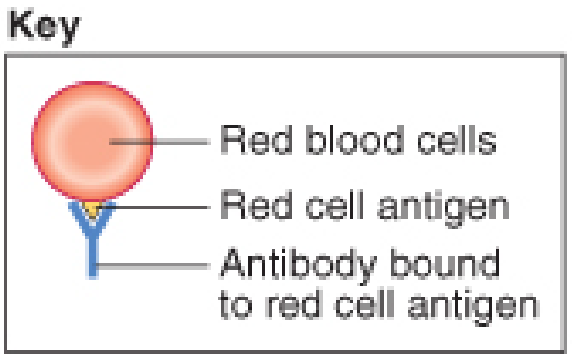
In this, red cells are mixed with Coombs reagent which contains . antibodies against human IgG/M/complement.

If the red cells have been coated by antibody in vivo, the Coombs reagent will induce their agglutination and this can be detected .visually.

A Direct antiglobulin test (DAT) (Coombs test)

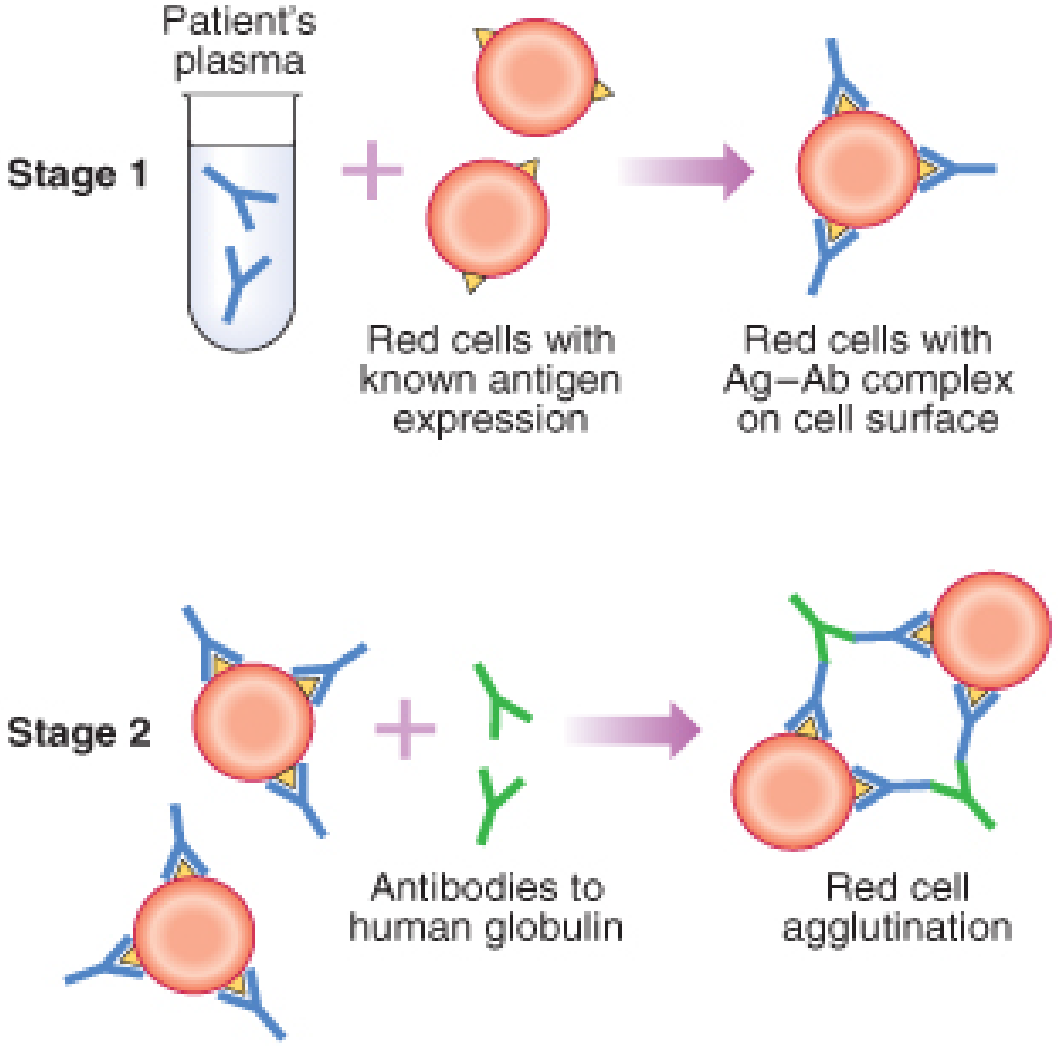
Detects the presence of antibody bound to the red cell surface, e.g.
1. autoimmune haemolytic anaemia
2. haemolytic disease of newborn (HDN)
3. transfusion reactions





B Indirect antiglobulin test (IAT) (indirect Coombs test)

Detects antibodies in the plasma, e.g. antibody screen in pre-transfusion testing



Management:

-If the haemolysis is secondary to an underlying cause, this must be treated and any offending drugs stopped

-It is usual to treat patients initially with prednisolone 1 mg/kg orally. A response is seen in 70-80% of cases but this may take up to 3 weeks; a rise in haemoglobin will be matched by a fall in bilirubin and LDH and Reticulocytes levels.

Once the haemoglobin has normalised And the reticujocytosis resolved the corticosteroid dose can be Reduced slowly over about several weeks

Steroids work by decreasing macrophage destruction of antibody-coated red cells and reducing antibody production

-Transfusion support may be required for life-threatening problems.

The least incompatible blood should be used but may still give rise to transfusion reactions or the development of further alloantibodies

If the haemolysis fails to respond to glucocorticoids or can only be stabilised by large doses, then second-line therapy with the anti-CD20 monoclonal antibody rituximab should be considered. Failure to respond to rituximab can be followed by consideration of treatment with a range of immunomodulatory/ immunosuppressive agents or splenectomy. Pharmacological agents include azathioprine, ciclosporin, danazol and mycophenolate mofetil

Splenectomy is associated with a good response in 50%–60% of cases. The operation can be performed laparoscopically with reduced morbidity. There are concerns about all modes of third-line therapy as long-term immunosuppression carries a risk of malignancy, while splenectomy is associated with an excess of severe infection due to the capsulate organisms pneumococcus and meningococcus

Cold agglutinin disease

This is due to antibodies, usually IgM, which bind to the red cells at low temperture and cause them to agglutinate. It may cause intravascular haemolysis if complement fixation occurs.

This can be chronic when the antibody is monoclonal, or

Acute or transient when the antibody is polyclonal.

Chronic cold agglutinin disease

This affects elderly patients and may be associated with an underlying low-grade B cell lymphoma. It causes a low-grade intravascular haemolysis with cold, painful and often blue fingers, toes, ears or nose (so-called acrocyanosis). The latter is due to red cell agglutination in the small vessels in these colder exposed areas. The blood film shows red cell agglutination and the MCV may be spuriously raised because the automated analysers count aggregates as single cells..

Treatment

is primarily by transfusion support but may also be directed at any underlying lymphoma. Patients must keep extremities warm, especially in winter. Some patients respond to glucocorticoid therapy and rituximab. Fludarabine can be added in if a clonal abnormality is detected.

Two considerations for patients requiring blood transfusion is that the cross-match sample must be placed in a transport flask at a temperature of 37°C and blood administered via a blood-warmer. All patients should receive folic acid supplementation.

Alloimmune haemolytic anaemia

Alloimmune haemolytic anaemia is due to an antibody against non-self red cells. It has two main causes:
- an unmatched transfusion of red cells (a haemolytic transfusion reaction)
-maternal sensitisation to paternal antigens on fetal cells (haemolytic disease of the newborn)

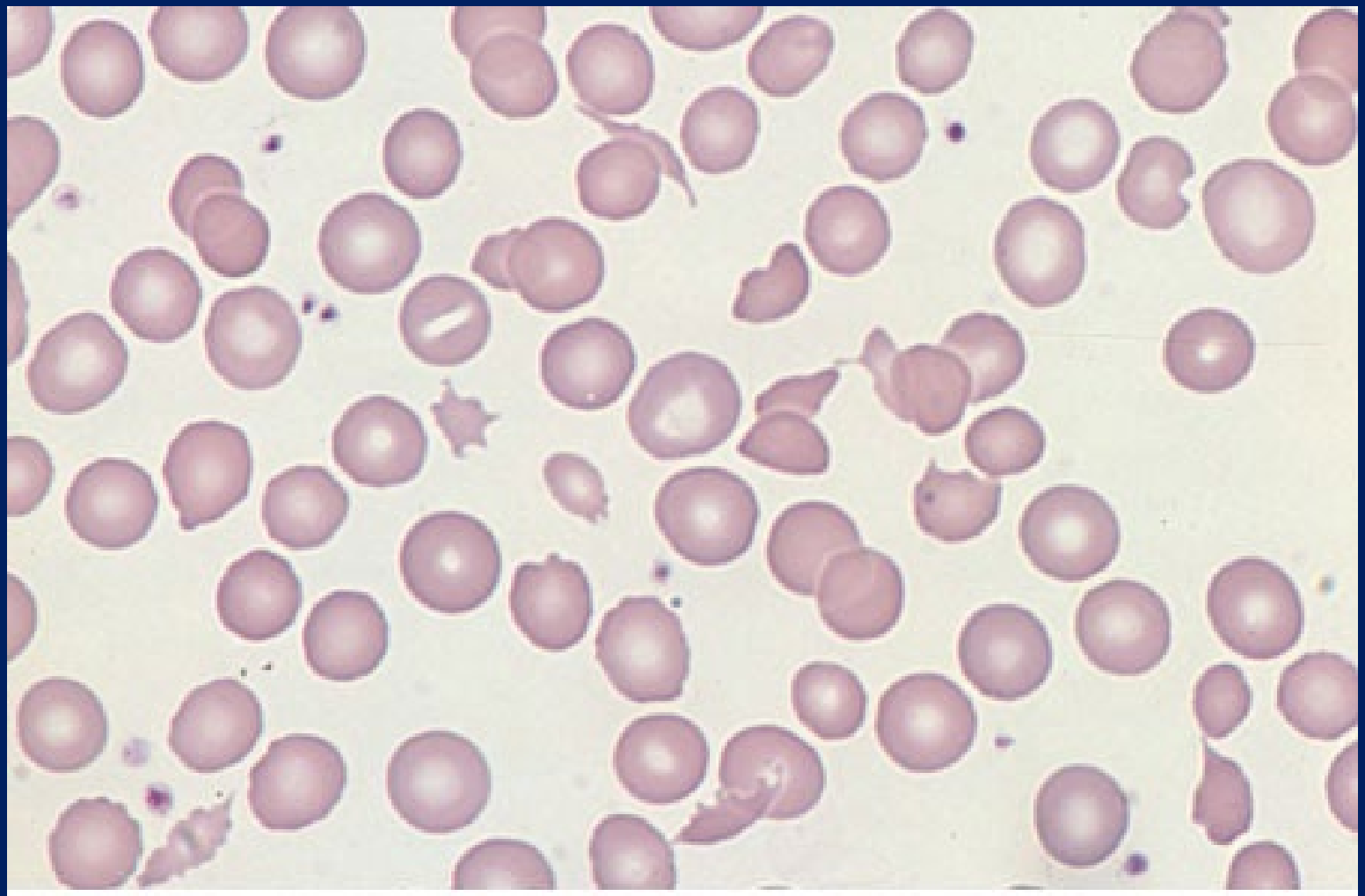
Non-immune haemolytic anaemia

1-Endothelial damage

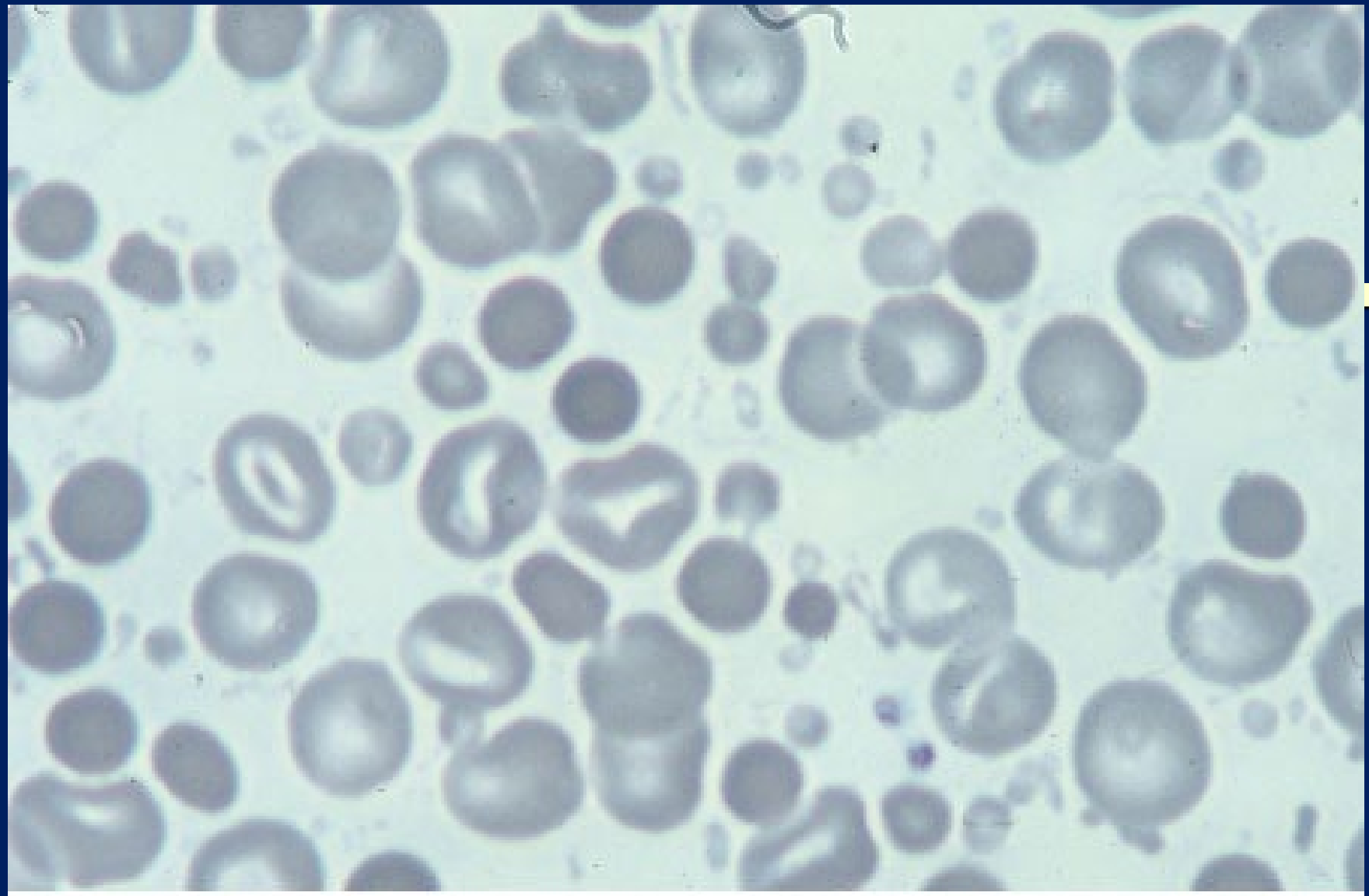
Disruption of red cell membrane may occur in a number of conditions and is characterised by the presence of red cell fragments on the blood film and markers of intravascular haemolysis:

- *Mechanical heart valves. High flow through incompetent* valves or periprosthetic leaks through the suture ring holding a valve in place result in shear stress damage.

- *March haemoglobinuria. Vigorous exercise, such as* prolonged marching or marathon running, can cause red cell damage in the capillaries in the feet.
- *Thermal injury. Severe burns cause thermal damage to red* cells, characterised by fragmentation and the presence of microspherocytes in the blood.
- *Microangiopathic haemolytic anaemia. Fibrin deposition in* capillaries can cause severe red cell disruption. It may occur in a wide variety of conditions: disseminated carcinomatosis, malignant or pregnancy-induced hypertension, haemolytic uraemic syndrome , thrombotic thrombocytopenic purpura and disseminated intravascular coagulation .



Source: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology*, 7th Edition: http://www.accessmedicine.com



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2-infections:

* falciprum malaria: may associate with intravascular haemolysis when sever is termed black water fever due to haemoglobinuria
*Clostridium perfringens septicemia: in the context of an ascending cholangitis it May cause sever intravascular haemolysis with Spherocytosis due to bacterial productions of lecithinase which destroys the RBC membrane

3-Chemical and drugs:

These agent lead to haemolysis by oxidant denaturations Of Hb, dapson, sulphsalazine can produce haemolysis . Arsenic gas, copper, chlorates, nitrites, nitrobenzene derivatives may all cause haemolysis

THANK YOU