HEMOLYTIC ANEMIAS

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LEARNING OBJECTIVES

- To be able to define haemolysis and hemolytic anemia.
- To be able to classify hemolytic anemias into congenital and acquired types, and to know the etiological factors in each division.
- To understand the difference between intravascular and extravascular haemolysis, and to recognize the laboratory features of each.
- To appreciate some major examples of congenital disorders resulting in hemolysis like HS and G6PD deficiency.
- To understand the role of autoantibodies in the production of hemolytic anemias and to know the types of disease with which they are associated
- To understand some causes of non-immune acquired hemolytic anemias.

HEMOLYTIC ANAEMIAS

Hemolysis: is a state with a short of the lifespan of a mature red blood cell.

- IF hemolysis is not marked and can be compensated;

- increased red cell output from the marrow.
- stimulated by erythropoietin.
- will be sufficient.

- IF hemolysis is marked and can not be compensated;

- more marked reductions in red cell lifespan.
- say to 5-10 days from the usual 120 days.
- will result in ***hemolytic anemia.***

Hemolytic anemia: is a shorten in life span of RBC that can not be overcome by ability of bone marrow production.

INTRA vs. EXTRAVASCULAR HEMOLYSIS

- In the majority of hemolytic anemias, the macrophages in the spleen, liver and bone marrow remove red cells from the circulation by phagocytosis. This is termed **extravascular haemolysis**.

- By contrast, in **intravascular haemolysis**, the red cells are caused to rupture and release their hemoglobin (Hb) directly into the circulation.

- The intra/extravascular site of red cell destruction may give clues to the underlying etiology of the haemolysis.

Laboratory Evidence of Hemolysis

- A rise in the **unconjugated bilirubin** concentration in the plasma.
- **Lactate dehydrogenase**, an enzyme present in red cells, more with intra,.
- Reduction of serum **haptoglobin,** molecule binds to free Hb, intra,.
- Free hem can bind to albumin to form **methemalbumin**.
- Free Hb in the urine: **hemoglobinuria,** intra, (note the difference from hematuria, which describes the presence of intact red cells in the urine).
- Increased **reticulocyte count**. The number of reticulocytes in the blood is expressed either as a **percentage** of the total number of red cells **or as an absolute number** per liter of blood; in normal adults, the percentage is in the range of **0.5-3.0%** and the absolute count is **20-100x10^9/L**. Increase in the absolute reticulocyte count is an indication of increased **erythropoietic activity**.

Laboratory Evidence of Hemolysis; *cont'd…*

- As haemolysis will also increase the marrow's demand for folic acid, **macrocytosis,** high MCV**,** may also develop secondary to folate deficiency.
- **Polychromasia, a** bluish discoloration of RBC, due to reticulocytosis.
- Generally **extravascular haemolysis** is associated with spherocytosis on the peripheral blood film. **Intravascular haemolysis** is characterized by red cell fragmentation (**schistocytes**).
- If examination of the bone marrow is undertaken, there will be evidence of increased **erythropoiesis**. Marrows showing **erythroid hyperplasia** are also hypercellular, due to the replacement of fat cells by **erythroid precursors**. (Figure).

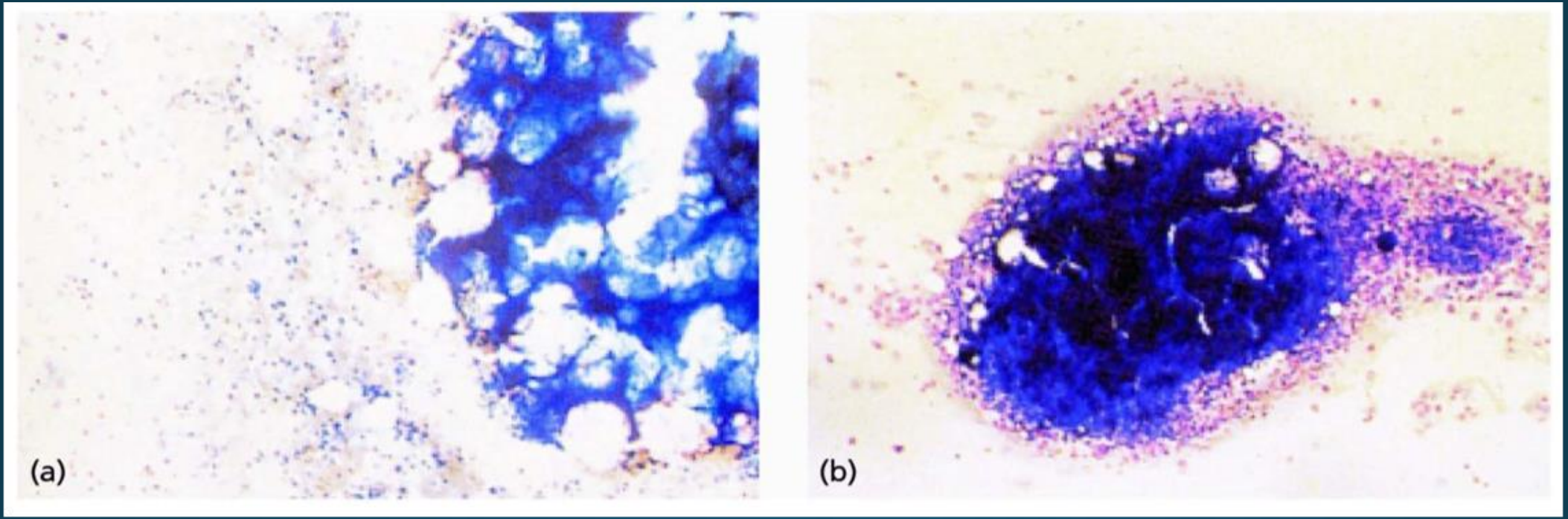

Figure 3.1 (a) A normocellular marrow fragment: about half its volume consists of haemopoietic cells (staining blue) and the remainder of unstained rounded fat cells. (b) A markedly hypercellular marrow fragment, as might be seen in the response to haemolysis: virtually all the fat cells are replaced by haemopoietic cells.

Table 3.1 Laboratory findings indicative of haemolysis.

Extravascular haemolysis	Intravascular haemolysis
Hyperbilirubinaemia (unconjugated)	Hyperbilirubinaemia (unconjugated)
	Reduced or absent serum haptoglobin
	Haemoglobinaemia, haemoglobinuria, haemosiderinuria
	Methaemalbuminaemia*
Increased serum lactate dehydrogenase (LDH)	Markedly increased serum LDH
Reticulocytosis	Reticulocytosis
Spherocytes	Red cell fragments (schistocytes)

Note: ***Now rarely used in investigating a patient.**

Clinical features of hemolysis

- **Pallor**, and **jaundice** secondary to the elevated **bilirubin** levels.
- **Splenomegaly** may be seen.

- Long-term complications of chronic hemolysis; expansion of erythropoiesis in the marrow cavities, thinning of cortical bone, **bone deformities** (e.g. frontal and parietal bossing) and, very occasionally, pathological fractures.

- **Pigment gallstones** are seen commonly.
- Risk of episodes of **pure red cell aplasia** especially with **parvovirus B19.**

Classification of Hemolytic Anemias

- Classified simply as either I] **congenital or II] acquired**.
- With the **congenital causes**, the underlying defect is typically intrinsic to the red cell itself, affecting the red cell
  - **A) membrane,**
  - **B) its enzymes or,**
  - **C) its hemoglobin.**
- **Acquired causes**, are typically due to defects extrinsic, outside the red cell (except PNH), and can be divided into those with an **immune basis** and those **without**.

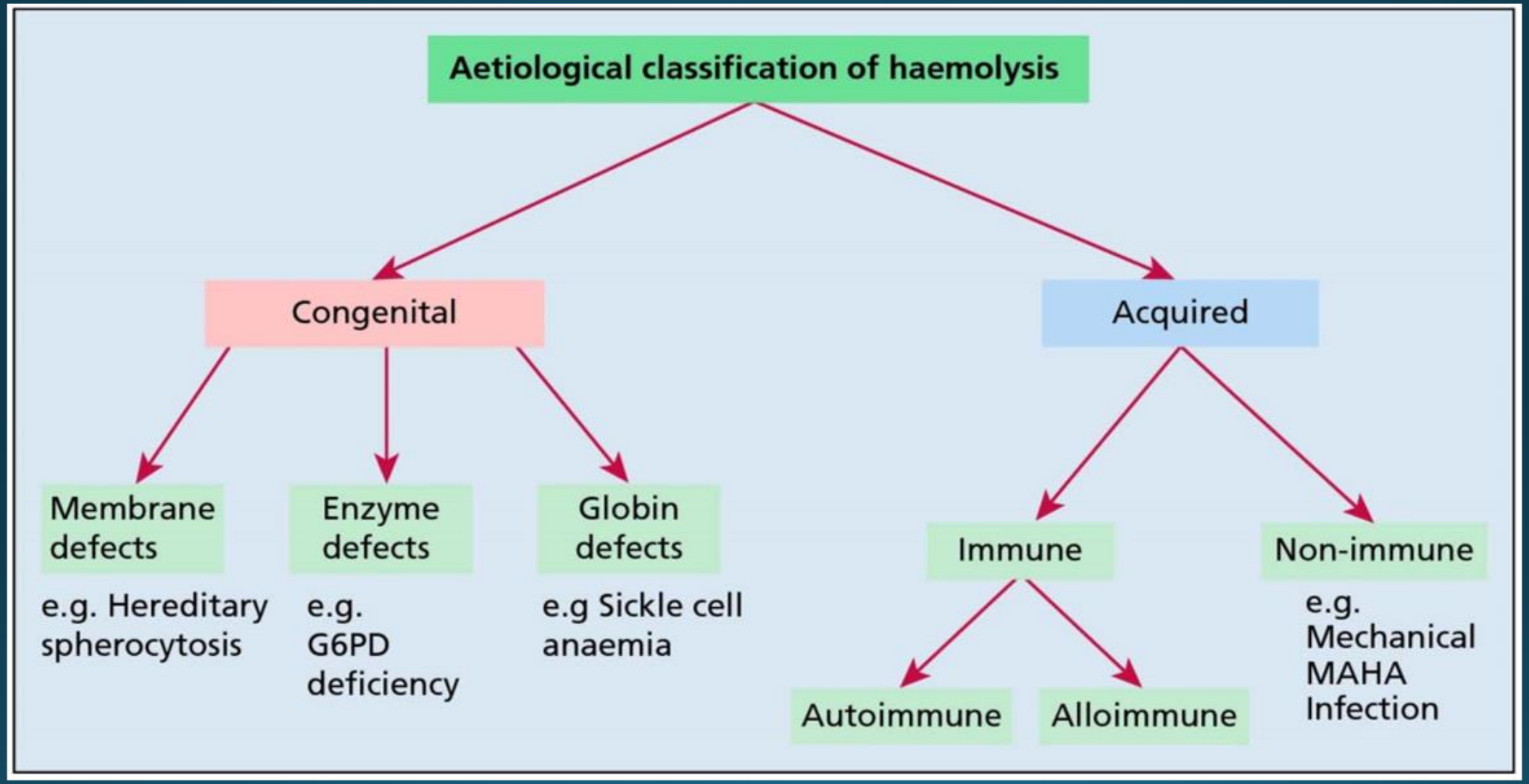

Figure 3.2 A classification of haemolytic anaemia by aetiology. Abbreviations: G6PD, glucose-6-phosphate dehydrogenase; MAHA, microangiopathic haemolytic anaemia.

1] Congenital Hemolytic Anemias

A) Red Cell Membrane "Membranopathy"

- The red cells undergo significant deformations while traversing the circulation. Thus, flexible red cell cytoskeleton is essential.

- Key components of the cytoskeleton include **α and β spectrins, actin and protein 4.1**, while connections linking the cytoskeleton to the overlying red cell phospholipid bilayer include **band 3**, Rh-associated glycoprotein and glycophorin C (see Figure).

- Defects in any of these proteins can jeopardize the **integrity of the red cell and shorten its lifespan.**

Hereditary spherocytosis (HS)

- The **most common** membranopathy is hereditary spherocytosis (HS) with ~ 60% related to **Ankyrin gene.** Loss of Ankyrin then leads to secondary reductions in **spectrin and protein 4.1** leading to **a spheroid shape, vertical.**

- Destroyed by splenic macrophages, **extravascular hemolysis,** with 20% of all HS have mild disease. The majority of patients have **moderate disease characterized by a Hb concentration of 8-11g/dl**, while a **small percentage have severe disease** requiring intermittent or even regular transfusions

- Complications of the chronic hemolysis in HS include the development of **pigment gallstones. Aplastic crises** may occur secondary **to parvovirus B19. Megaloblastic anemia is** occasionally found.

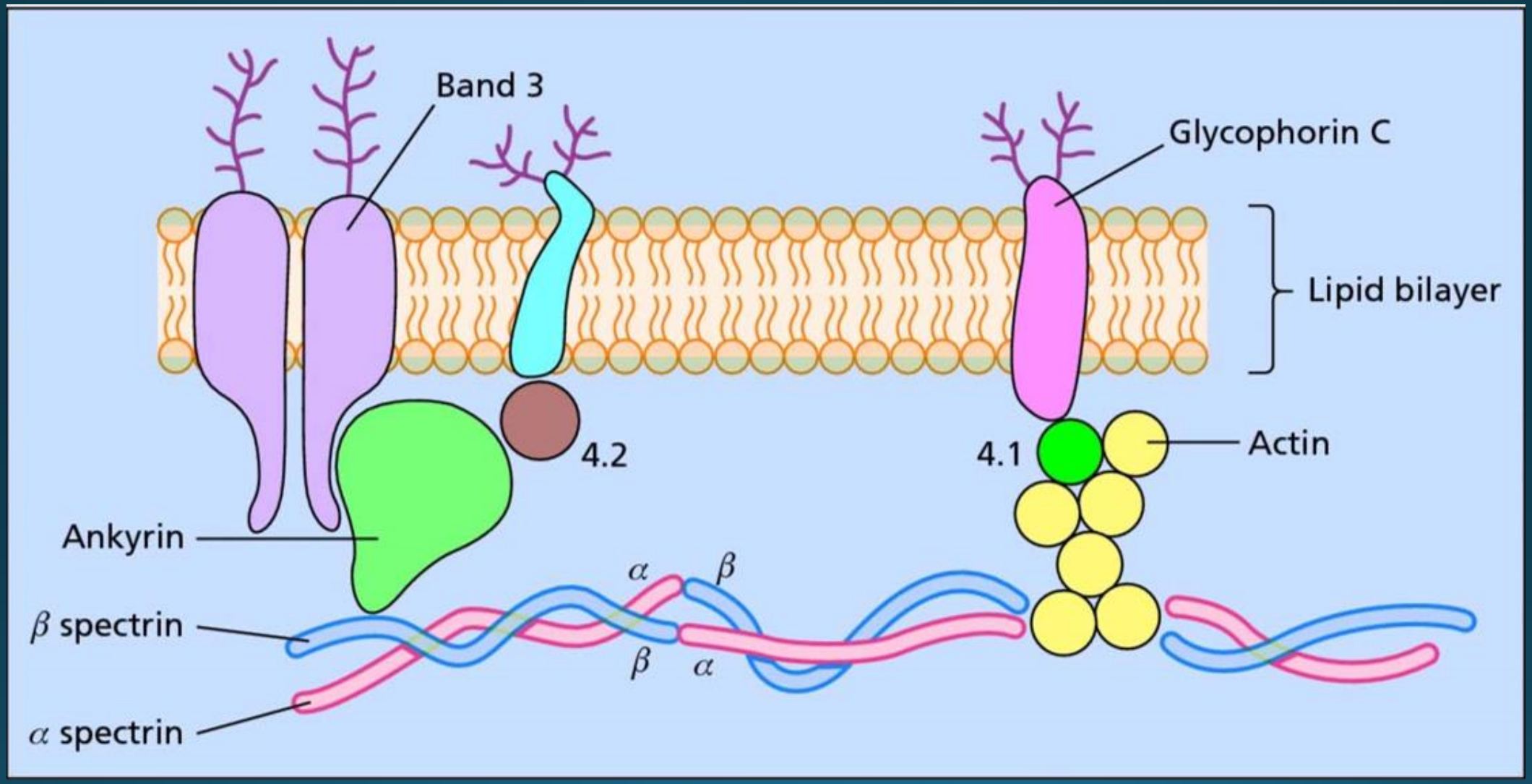

Figure 3.3 Schematic diagram of the red cell membrane cytoskeleton.

Hereditary spherocytosis (HS): Diagnosis and management

The Diagnosis of HS:

- **Family history**, mild jaundice, pallor and splenomegaly.
- Laboratory findings (anemia, reticulocytosis and elevated plasma bilirubin).
- Presence of **spherocytes** on the peripheral blood film.
- Special Tests: The eosin-5-maleamide (**EMA**) binding test (definitive evidence) by **flow cytometry**. The red cell membrane proteins' genes, by **molecular testing**. **Protein electrophoresis** on a denaturing polyacrylamide gel.

Some Treatment of Significant HS:

- **Folic acid** supplementation.
- **Splenectomy** (children with severe disease), which increases the risk of significant infection, encapsulated organisms. This risk is especially marked in children under the age of 5. Administration of pneumococcal and meningococcal vaccine and *Haemophilus influenzae* type b vaccine (splenectomy preoperative preparation). Prophylactic penicillin V is advised lifelong (post splenectomy).

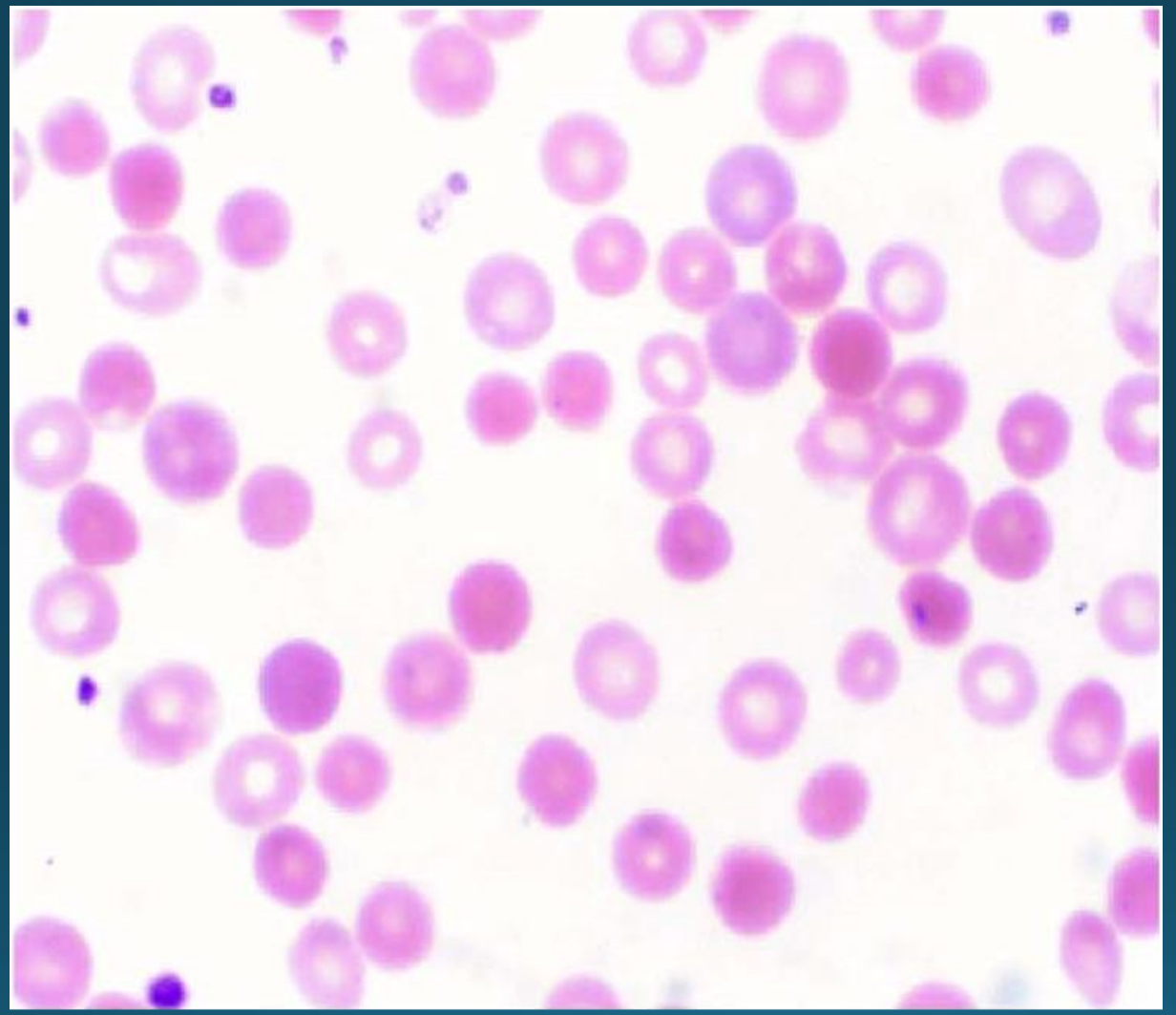

Figure 3.4 **A blood film from a patient with HS showing many spherocytes.**

Hereditary Elliptocytosis (HE) and Hereditary Pyropoikilocytosis (HPP)

- Hereditary elliptocytosis (HE) is also a relatively common condition, with many cases showing defects in α spectrin, **horizontal interaction**.
- Most patients are clinically asymptomatic, some will have a chronic symptomatic hemolytic anemia.
- All show the very characteristic elongated red cell shape on peripheral blood films (Figure).

- Severe disturbance of the multimerization of spectrin with a severe hemolytic anemia from infancy and a bizarre peripheral blood morphology, including microspherocytes and poikilocytes. Such patients are described as having **hereditary pyropoikilocytosis**.

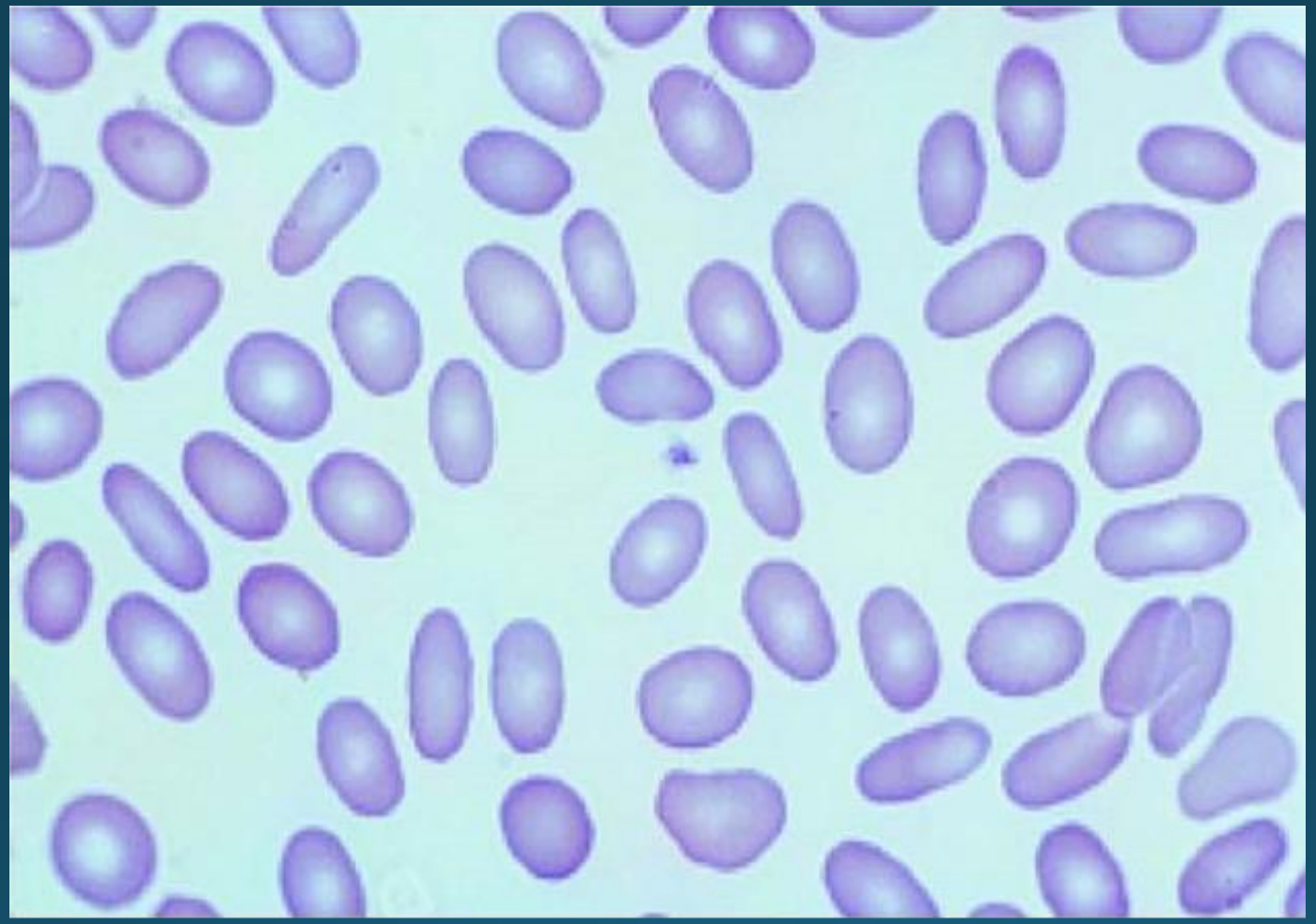

Figure 3.5 **A blood film from a patient with hereditary elliptocytosis showing a high proportion of elliptical red cells.**

I] Congenital Hemolytic Anemias

2) Red Cell Enzyme "Enzymopathy"

- Hemolytic anemias may also result from congenital abnormalities of the **enzymes required for energy** transfer in glucose **metabolism**.

- The red cell needs a continuous supply of energy for the maintenance of membrane flexibility and cell shape, the regulation of sodium and potassium pumps, and the maintenance of Hb in the reduced ferrous form which protects from an **oxidative stress**.

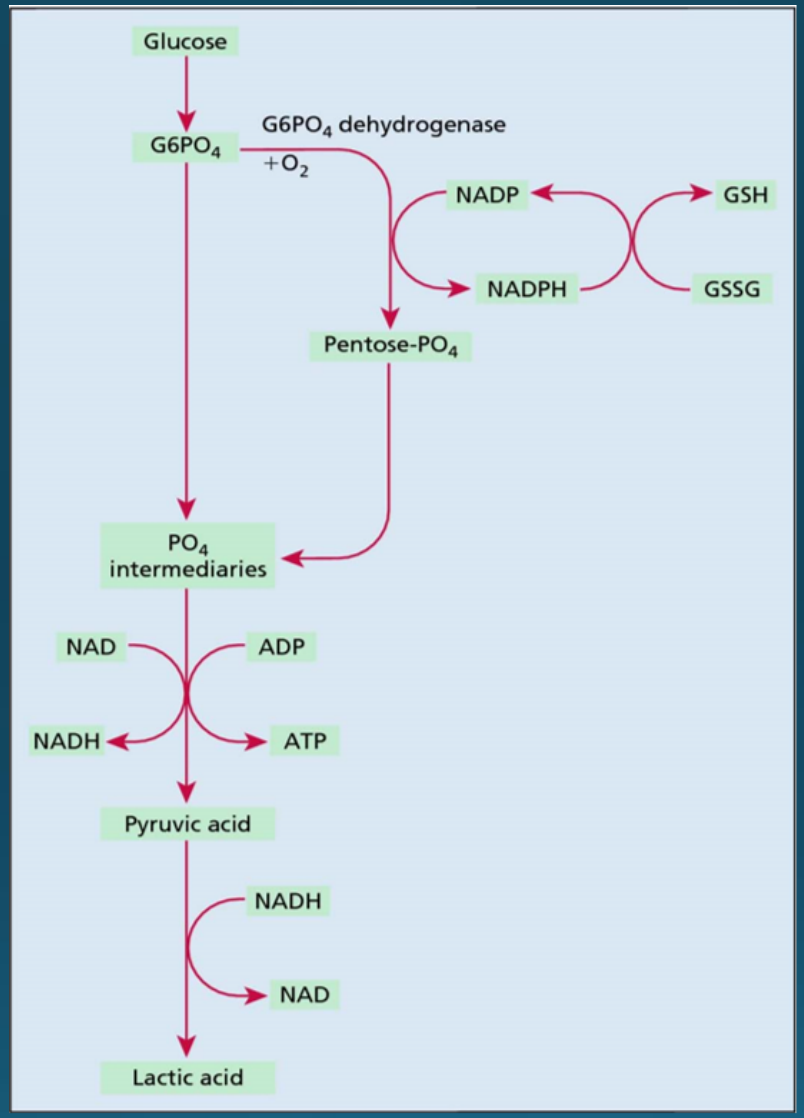

Figure 3.6 **A schematic diagram of the pathway of glucose metabolism in the red cell, to show the important role of G6PD. A decreased activity of the enzyme leads to a deficiency of the reducing compounds NADPH and GSH.**

Glucose-6-Phosphate Dehydrogenase Deficiency

- ✓Deficiency of **glucose-6-phosphate dehydrogenase (G6PD)**, the first enzyme of the **hexose monophosphate/pentose-phosphate shunt**, will prevent the normal generation of **NADPH**, with subsequent erythrocyte sensitivity to oxidative stress. Various mutations in the G6PD gene on **the X chromosome** results in this disorders with **a male predominance**.

- ✓When the red cell is exposed to **oxidants, e.g. some medications**, Hb is converted to methemoglobin and denatured. Denatured Hb then precipitates forming inclusions in the red cell (termed **Heinz bodies** and detected by supravital staining, as in Figure). Splenic macrophages, extra, remove Heinz bodies; the resulting inclusion-free cells display unstained areas at their periphery (‘**bite’ cells**, seen in Figure). **Screening tests and assays** for detecting G6PD deficiency are available.

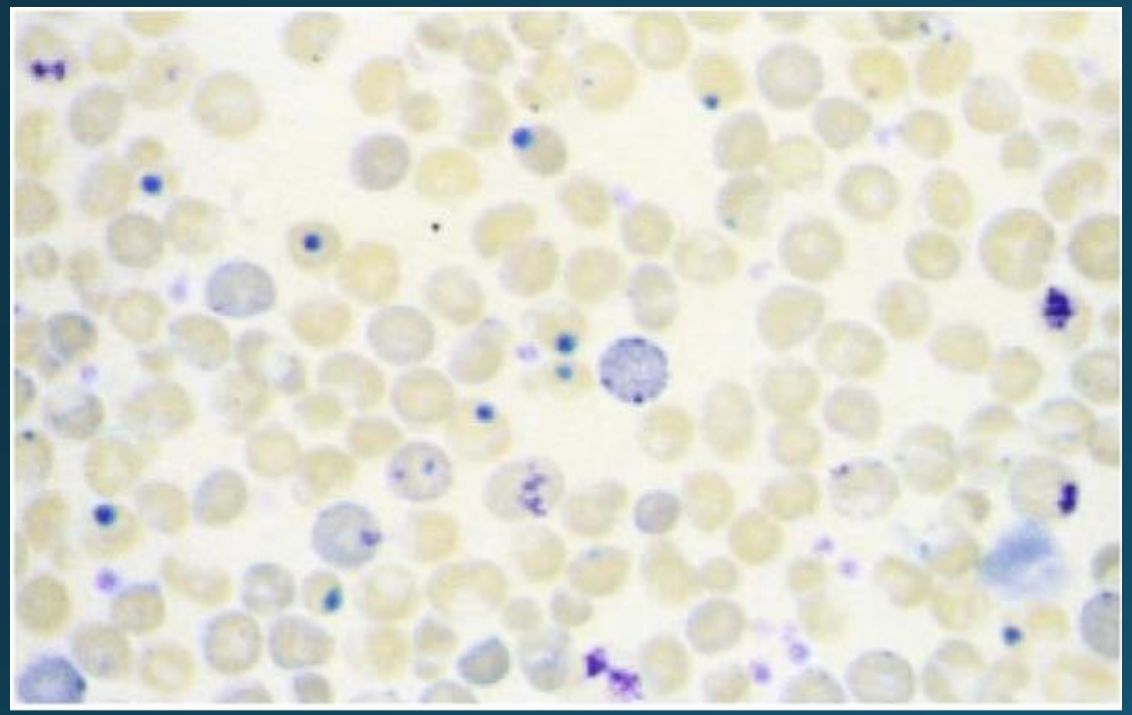

Figure 3.7 Membrane-bound Heinz bodies consisting of denatured haemoglobin (supravital staining with methyl violet).

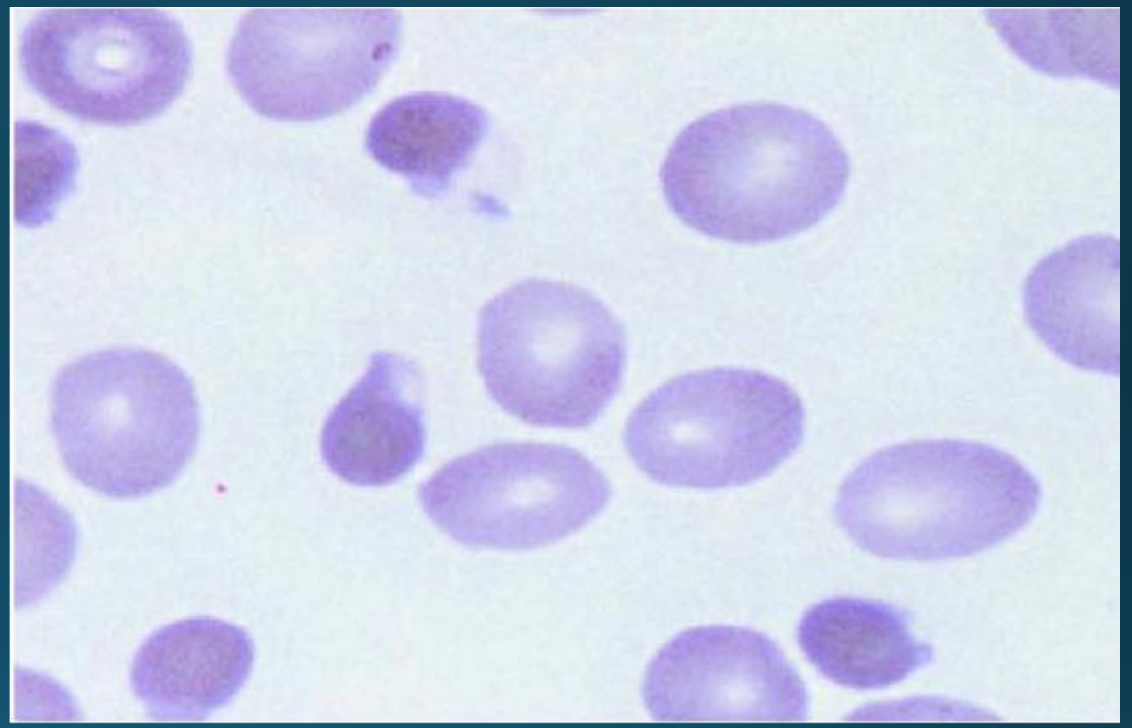

Figure 3.8 'Bite' cells in the blood film of a patient with G6PD deficiency who had received primaquine. These red cells are irregular in shape, are abnormally dense and show a poorly staining area just beneath part of the cell membrane (MGG stain).

Glucose-6-Phosphate Dehydrogenase Deficiency

- ✓Hemolysis begins 1-3 days post exposure to the oxidative stressor, with anemia being maximal about 7-10 days after exposure. Patient may report dark urine due to **hemoglobinuria**, **intravascular**.
- ✓***Favism:*** a syndrome in which an acute hemolytic anemia occurs after the ingestion of the broad **bean (*Vicia fava)*** in individuals with a deficiency of G6PD (commonly of the **Mediterranean type**), usually affects children; severe anemia develops rapidly and is often accompanied by hemoglobinuria.

- ➢Treatment generally focuses on the **avoidance of oxidative precipitants** to hemolysis. In many cases, hemolysis is **self limiting**. In children, **rehydration** is needed to avoid acute kidney injury. Packed **red cell transfusion** may be required in cases of severe hemolysis.

I] Congenital Hemolytic Anemias

2) Red Cell Enzyme "Enzymopathy"

- **Pyruvate kinase deficiency** is another relatively common example.
- There is usually a **chronic hemolytic anemia** and some patients may benefit from **splenectomy**.

I] Congenital Hemolytic Anemias

3) Red Cell Hemoglobin "Hemoglobinopathy"

- Defects in the structure of Hb. Structural variants of the globin chains may affect the lifespan of the red cell, with sickle cell anemia being the best-described example.

- A tendency of the HbS variant to polymerize under conditions of low oxygen tension leads to distortion of the erythrocyte in the well-recognized sickle shape.

II] Acquired Hemolytic Anemias

- In the acquired hemolytic anemias, red cells may be destroyed either by immunological or by non-immunological mechanisms.

II] Acquired Hemolytic Anemias
A) Immunological Causes

- Antigens on the surface of red cells react with antibodies and might complement activation.
- IgG-coated red cells interact with the Fc receptors on macrophages in the spleen, and are then either completely or partially phagocytosed (extra). When the phagocytosis is partial, the damaged cell will return to the circulation as a **spherocyte**. Sometimes, membrane attack complex (C5-C9), complement, leading to intravascular hemolysis.
- The immune hemolytic anemias may be due to **autoantibodies**; that is, antibodies formed against one or more antigenic constituents of the individual's own tissues. These include **autoimmune hemolytic anemia** (AIHA) and some **drug-related** hemolytic anemias.
- It is also possible to develop **alloimmune hemolytic anemia**, consequent on the production of antibodies against red cells from another individual, as in **hemolytic transfusion reactions** and **hemolytic disease of the newborn**.

II] Acquired Hemolytic Anemias
A) Immunological Causes
Autoimmune Hemolytic Anemias

'Warm' autoantibodies react best with the red cell antigen at **37°C** and are usually of **IgG** subtype.

'Cold' antibodies react best at temperatures **below 32°C** (usually below 15°C) and, since they are usually of **IgM** subtype, are capable of agglutinating red cells.

Table 3.2 Classification of AIHAs.

Caused by warm-reactive antibodies

Idiopathic

Secondary (chronic lymphocytic leukaemia, Lymphoma, systemic lupus erythematosus (SLE), some drugs)

Caused by cold-reactive antibodies

Cold haemagglutinin disease

Idiopathic

Secondary (*Mycoplasma pneumoniae* infection, infectious mononucleosis, lymphomas)

Paroxysmal cold haemoglobinuria

Idiopathic

Secondary (some viral infections, congenital and tertiary syphilis)

Warm AIHA

- In **idiopathic warm AIHA**, hemolysis dominates the clinical picture and no evidence can be found of any other disease. In **secondary AIHA**, the hemolysis linked with a primary disease like; chronic lymphocytic leukemia (CLL) or systemic lupus erythematosus (SLE).

- The antibody-coated red cells undergo partial or complete phagocytosis in the spleen and by the Kupffer cells of the liver. There may be partial activation of the complement cascade.

- Findings like; anemia, spherocytosis, reticulocytosis and rare nucleated red cells in the peripheral blood. The critical diagnostic investigation is the direct antiglobulin test (DAT).

- Hemolysis can be limited by treatment with **prednisolone**. If reduction in hemolysis is not maintained when the dose of steroids is lowered, **splenectomy** or alternative immunosuppressive therapy should be considered. The **anti-CD20** monoclonal antibody **rituximab**, as well as immunosuppressants such as **azathioprine** or **cyclophosphamide**.

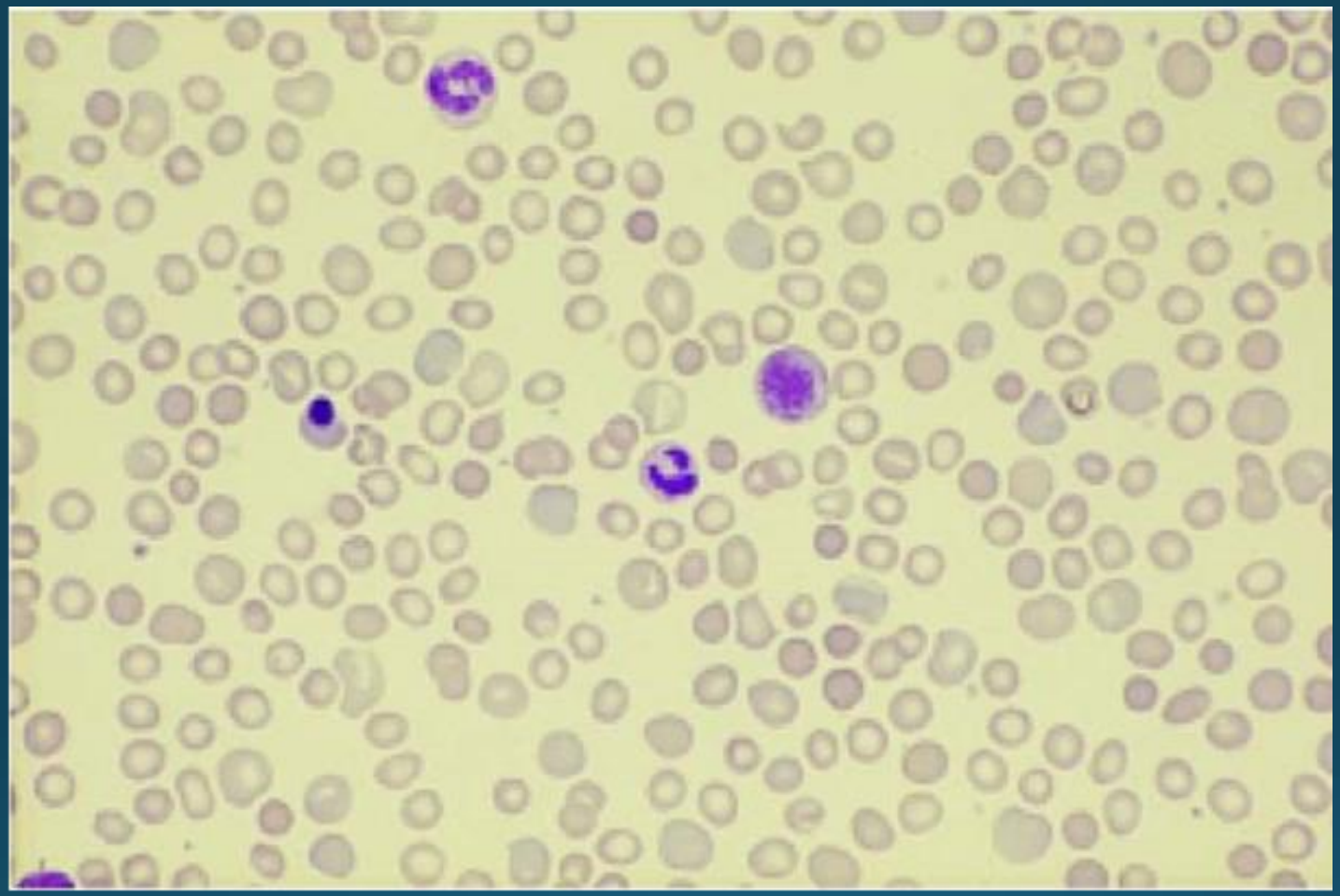

Figure 3.9 **Blood film from a patient with idiopathic AIHA (warm-reactive antibody) showing prominent spherocytosis and polychromasia.**

Cold Hemagglutinin Disease (CHAD)

- Cold antibodies bind to the red cell surface in the cooler superficial blood vessels of the peripheries. IgM subtype, **pentameric** structure, permits direct agglutination of red cells coated with antibody; they are therefore sometimes termed **cold agglutinins**.
- Symptoms due to cold AIHA are worse during cold weather. Exposure to cold provokes acrocyanosis. The direct activation of the complement system leads to red cells lysis and, consequently, to hemoglobinemia and hemoglobinuria (intra).
- Chronic idiopathic CHAD is managed initially simply by **keeping the patient warm**. Treatment with **rituximab** may be effective.

- **Other causes of hemolytic anemia with an immune** element include:
  - ✓1) Paroxysmal nocturnal hemoglobinuria (PNH);
  - ✓2) Paroxysmal cold hemoglobinuria;
  - ✓3) Drug-related hemolytic anemias.

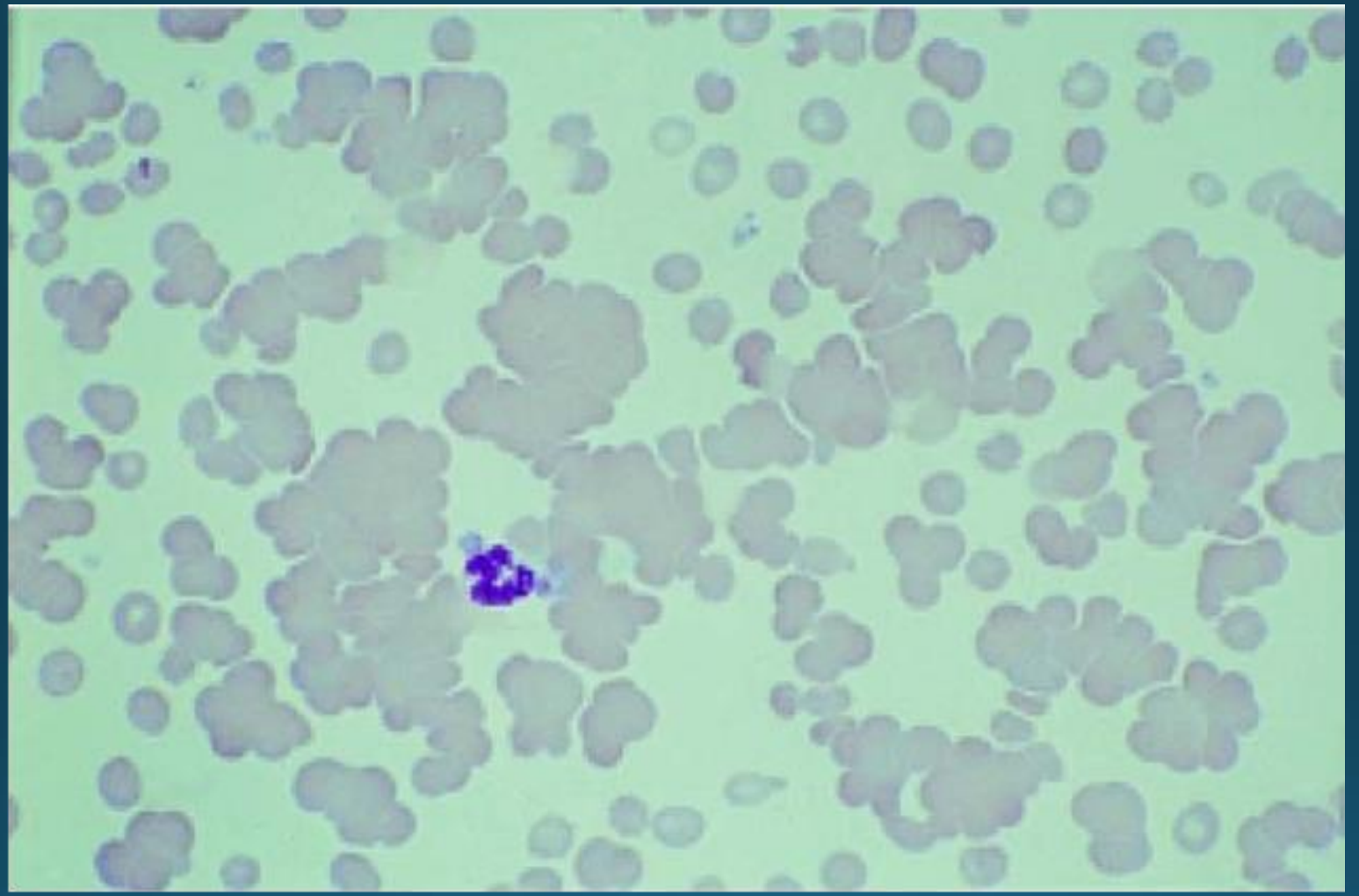

Figure 3.10 Numerous red cell agglutinates on a blood film from a patient with idiopathic CHAD.

II] Acquired Hemolytic Anemias
B) Non-immune hemolytic anemias
Mechanical Damage to Red Cells

- Several of the mechanical causes of acquired non-immune hemolytic anemia are **summarized in Table**. Red cells are mechanically damaged when they impact upon abnormal surfaces.

- In disseminated intravascular coagulation (DIC) inappropriate activation of the coagulation cascade produces fibrin strands which are thought to cause mechanical destruction of red cells. Such damage usually results in the presence of **red cell fragments (schistocytes)** in the blood film.

Table 3.3 Causes of acquired non-immune haemolytic anaemias.

Mechanical trauma to red cells

Abnormalities in the heart and large blood vessels

Aortic valve prostheses (Figure 3.11), severe aortic valve disease

Microangiopathic haemolytic anaemia

Haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura, metastatic malignancy, malignant hypertension, disseminated intravascular coagulation

March haemoglobinuria

Burns

Infections

Clostridium perfringes (welchii), malaria (Figures 3.12 and 3.13), bartonellosis

Drugs, *chemicals and venoms

Oxidant drugs and chemicals, arsine, acute lead poisoning, copper toxicity, venoms of certain spiders and snakes

Hypersplenism

***Note:* *Some drugs cause haemolysis by immune mechanisms.**

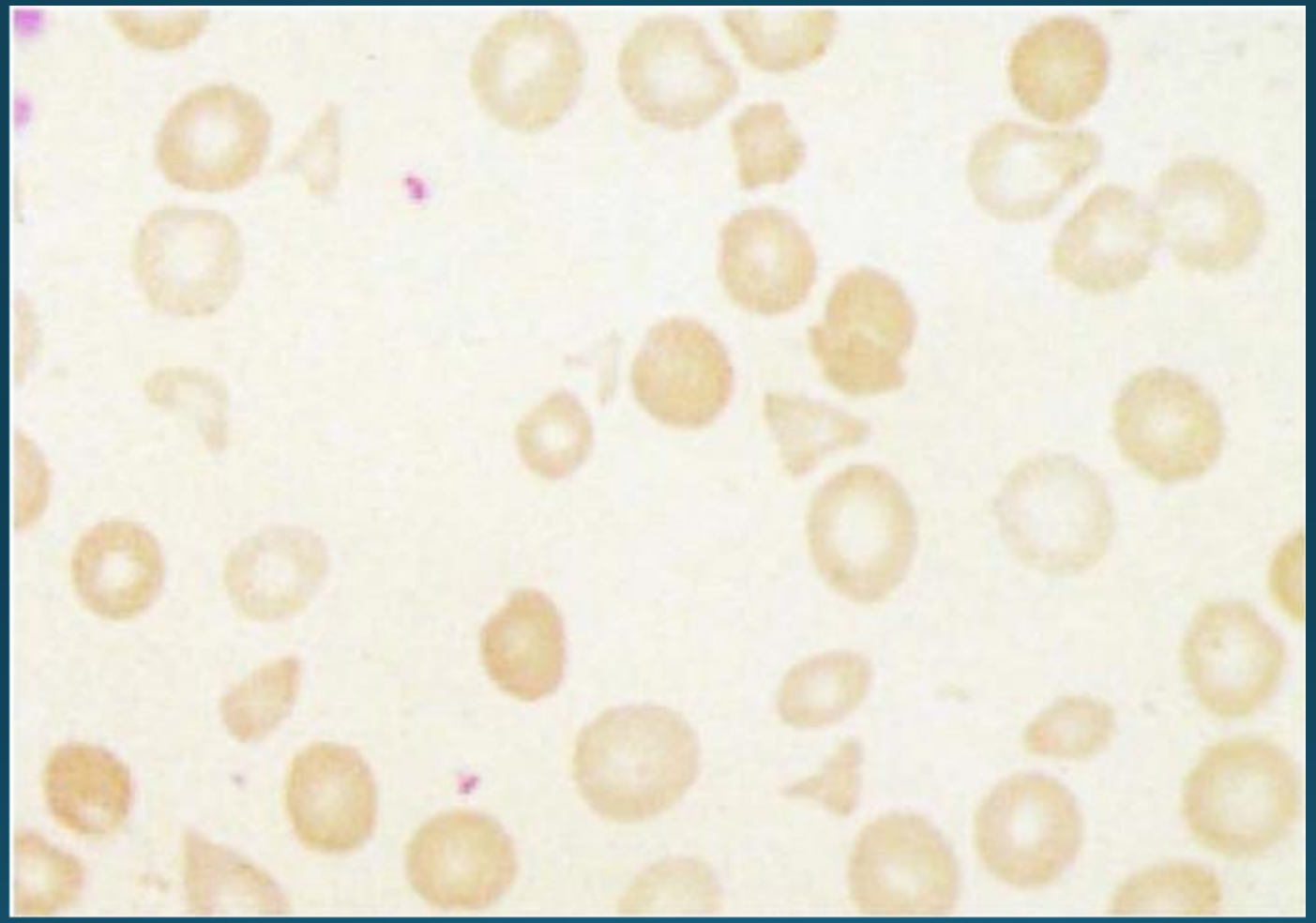

Figure 3.11 **Fragmented red cells (schistocytes) in the blood film of a patient with a malfunctioning aortic valve prosthesis.**

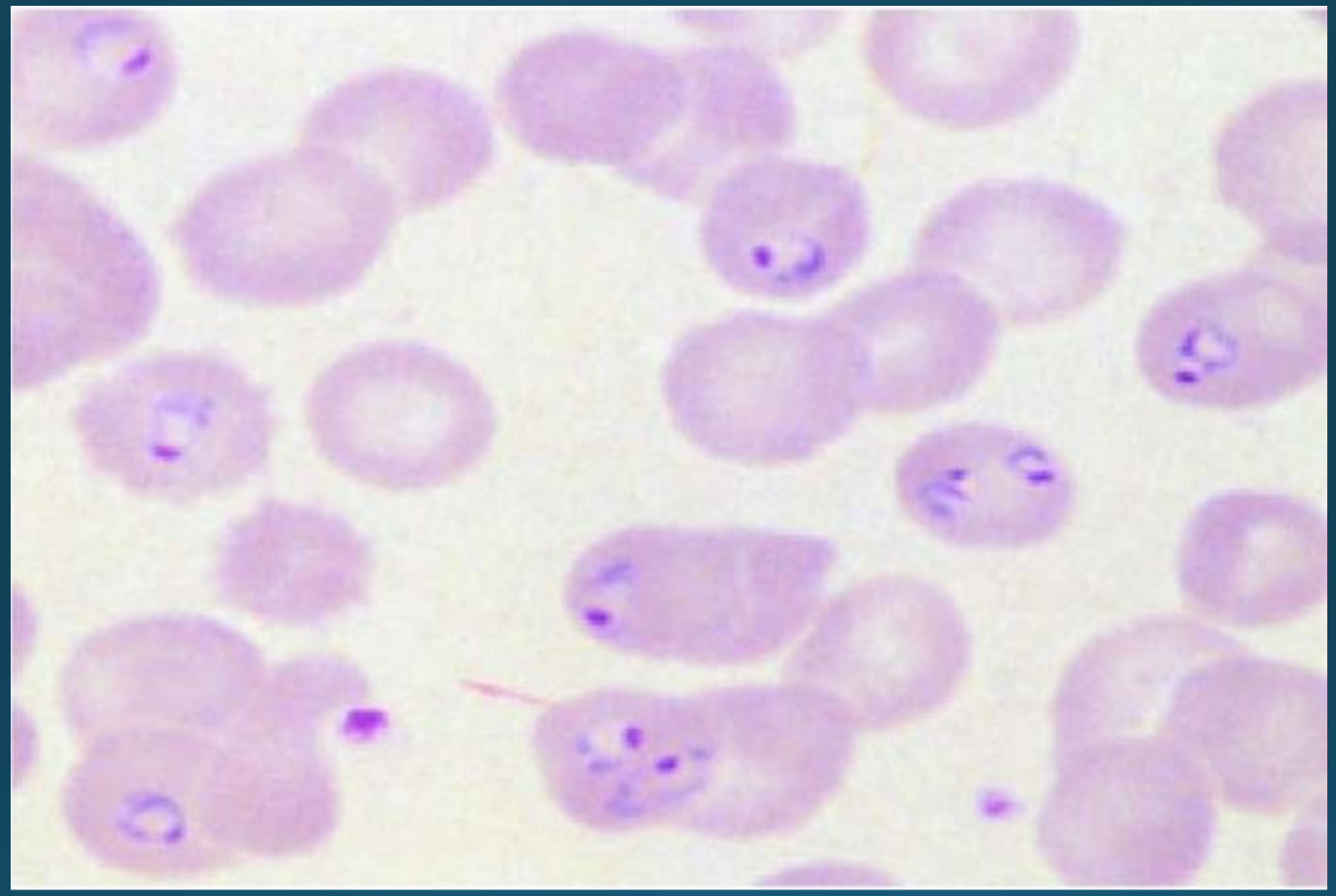

Figure 3.12 Blood film from a patient with *Plasmodium falciparum* malaria showing several parasitized red cells. Red cells heavily parasitized with malaria may be subject to intravascular lysis.

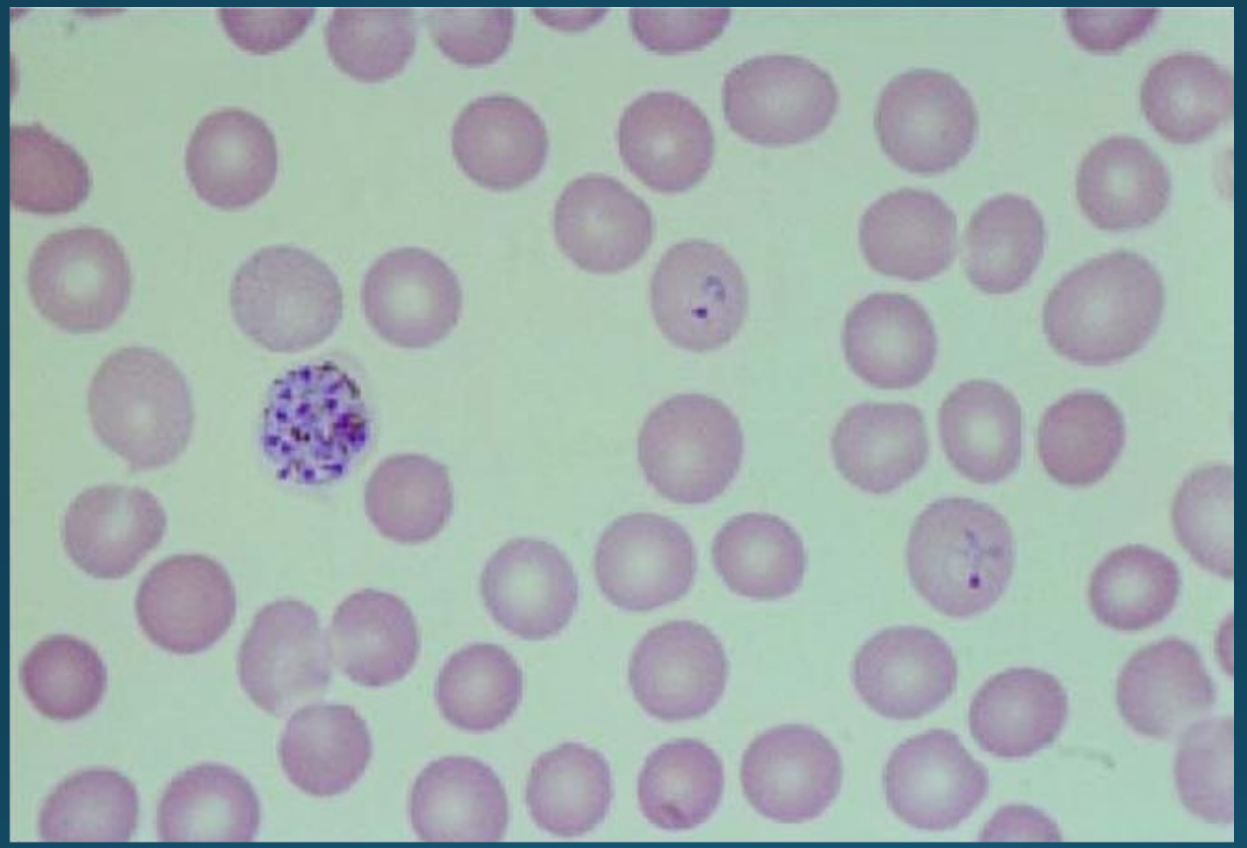

Figure 3.13 Blood film from a patient with *Plasmodium vivax* malaria showing two parasitized red cells, each containing a single parasite (ring form or early trophozoite and an ameboid late trophozoite). Another red cell contains a schizont. Some of the parasitized cells are slightly enlarged.

II] Acquired Hemolytic Anemias
B) Non-immune hemolytic anemias
Some Drugs

- While **immune** mechanisms of drug-induced haemolysis are well described, there are also **non-immune mechanisms** by which the red cell lifespan may be shortened. Chemicals, such as benzene, toluene and saponin, which are **fat solvents**, act on the red cell membrane directly and disrupt its lipid components, inducing hemolysis.

Hypersplenism

- **Hypersplenism** results in the reduction in the lifespan of red cells, granulocytes and platelets that may be found in patients with splenomegaly due to any cause.

- The cytopenias found in patients with enlarged spleens are also partly caused by **increased pooling** of blood cells within the spleen and might be treated with a **splenectomy**.

Q1) Which ONE of the following is TRUE about glucose-6-phosphate dehydrogenase (G6PD) deficiency?

- A) It is NOT a cause of neonatal jaundice.
- B) It protects against malaria.
- C) It commonly presents as a chronic hemolytic anemia.
- D) Carrier females have approximately 10% G6PD levels.

Q2) Spherocytosis in the blood film is a feature of which ONE of the following?

- A) Thalassemia major.
- B) Reticulocytosis.
- C) Glucose-6-phosphate dehydrogenase (G6PD) deficiency.
- D) Autoimmune hemolytic anemia.

Q3) Which ONE of the following is an only cause of intravascular hemolysis?

- A) Glucose-6-phosphate dehydrogenase (G6PD) deficiency.
- B) Rhesus incompatibility.
- C) Red cell fragmentation syndrome.
- D) Hereditary spherocytosis.

Q4) Which ONE of these statements is TRUE regarding hereditary spherocytosis?

- A) It is caused by an inherited defect in hemoglobin.
- B) It is more common in males.
- C) It can be treated by splenectomy.
- D) It is more frequent in southern Europe

Q5) Which ONE of the following is TRUE about autoimmune hemolytic anemia?

- A) It is associated with pernicious anemia.
- B) Hemolytic anemia is minimal.
- C) It may complicate B-cell chronic lymphocytic leukemia.
- D) It is associated with a positive indirect antiglobulin test.

Thank You!!!