CLS 291 Clinical Hematology 1

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Lecture 7

Classification and Identification of Anemia from Peripheral Blood Smear

Outlines

I. Normal blood film.

II. Iron Deficiency Anemia.

III. Hemoglobinopathies:

III.I Thalassemia: alpha and beta.

III.II Sickle cell anemia.

IV. Megaloblastic anemia.

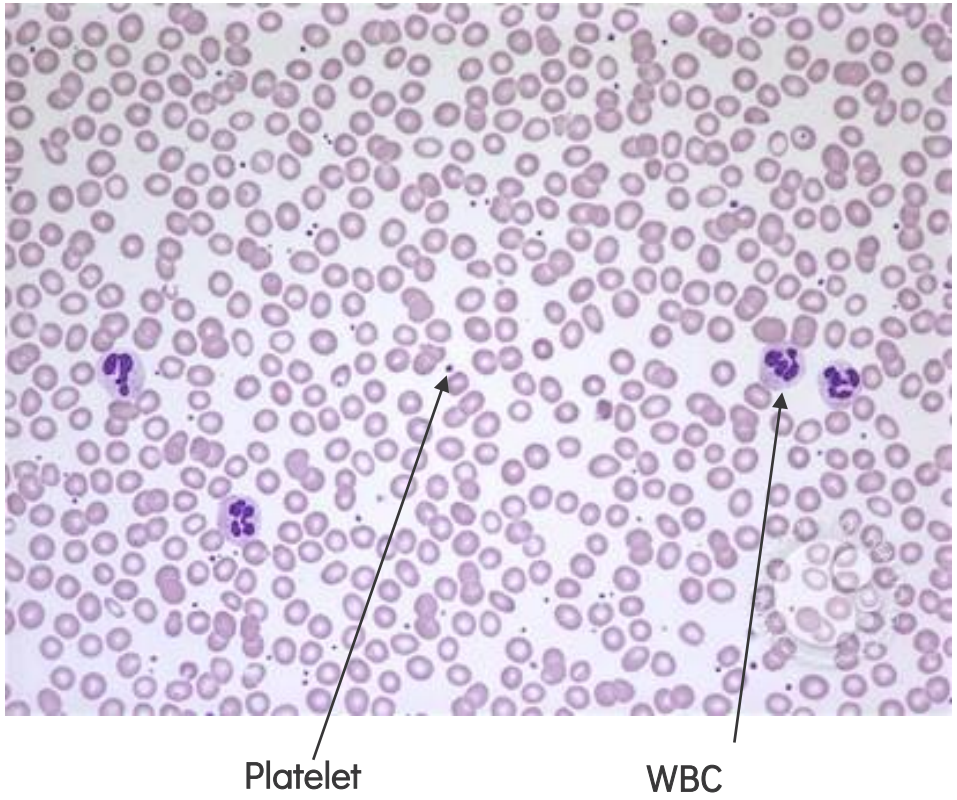
V. Hereditary hemolytic anemia:

V.I Hereditary spherocytosis.

V.II Hereditary elliptocytosis.

I. Normal blood film

- **Normal red blood cell:**
  - Normal RBCs are **biconcave** in shape.
  - Color: normochromic (the color is normal).
  - Size: normocytic (size is normal).
- **Normal platelets**
- **Normal white blood cells** (shape and percentage)



Classification of Anemia

- Red blood cell indices (**MCV and MCH**) may be used to classify anemia.

Table 2.4 Classification of anaemia.

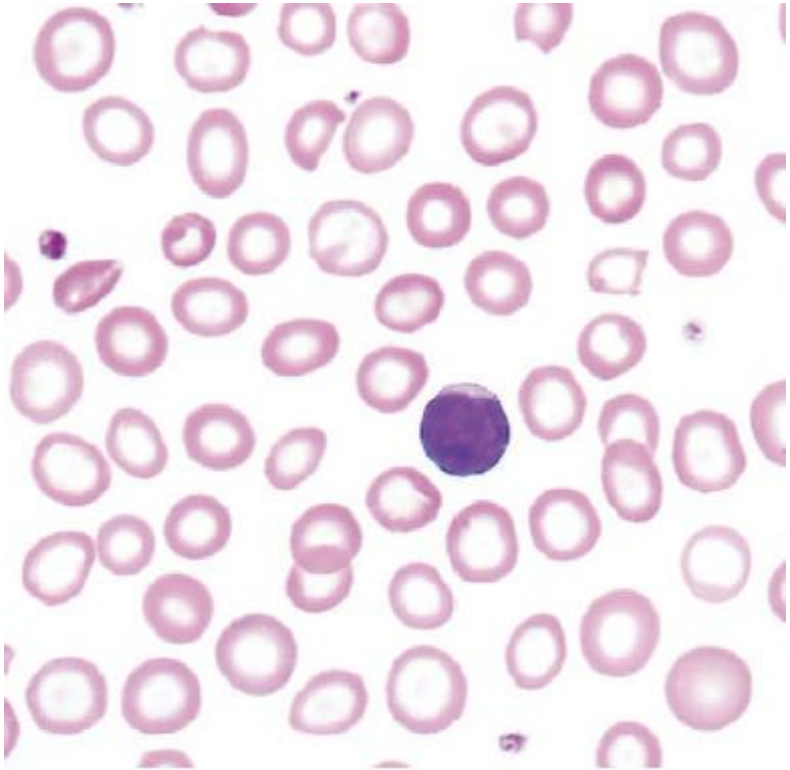
Microcytic, hypochromic	Normocytic, normochromic	Macrocytic
MCV <80 fL MCH <27 pg	MCV 80–95 fL MCH ≥27 pg	MCV >95 fL
Iron deficiency Thalassaemia Anaemia of chronic disease (some cases) Lead poisoning Sideroblastic anaemia (some cases)	Many haemolytic anaemias Anaemia of chronic disease (some cases) After acute blood loss Renal disease Mixed deficiencies Bone marrow failure (e.g. post-chemotherapy, infiltration by carcinoma, etc.)	Megaloblastic: vitamin B_{12} or folate deficiency Non-megaloblastic: alcohol, liver disease, myelodysplasia, aplastic anaemia, etc. (see Table 5.10)

MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

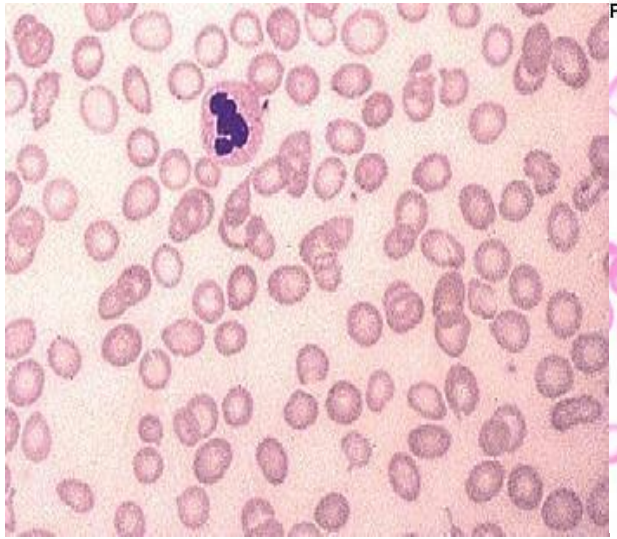
II. Iron deficiency anemia (IDA)

Blood film morphology:

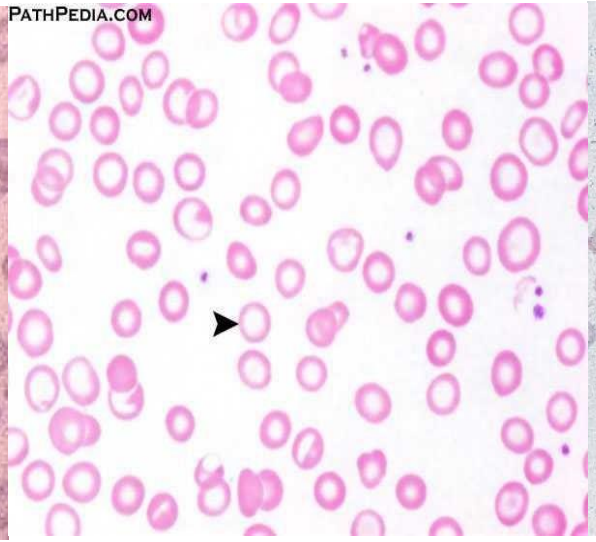
- The red cells are characteristically hypochromic and microcytic.
- There are a number of elliptocytes and teardrop cells.
- **Poikilocytes** have been observed to correlate with the severity of iron deficiency anemia.



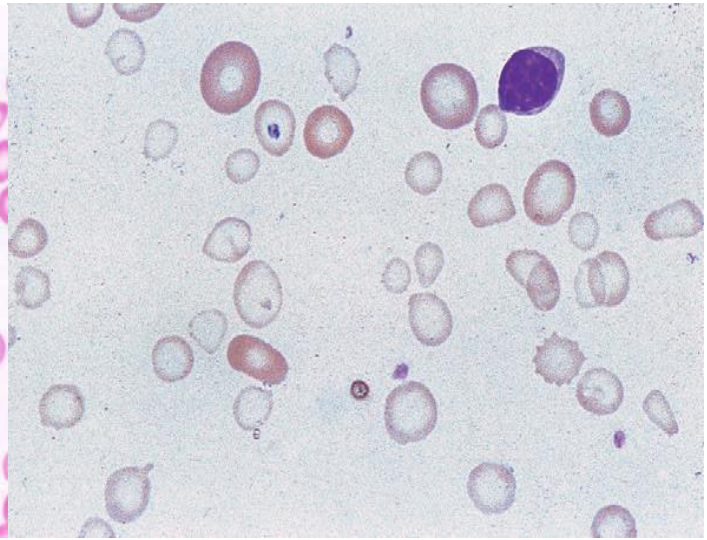
II. Iron deficiency anemia (IDA)



Iron deficiency anemia.
Shows hypochromia, microcytosis.



Iron deficiency anemia.
Shows a mild degree of hypochromia (ring shape) and microcytosis.



Iron deficiency anaemia.
Shows a marked degree of hypochromia, microcytosis, marked anisocytosis, and mild poikilocytosis; there are some normally haemoglobinized cells.

II. Iron deficiency anemia (IDA)

- **Screening tests:**
  - Hb estimation (low).
  - Hct (or PCV) (low).
  - MCV and MCH (low).
  - Reticulocyte count (low).
  - Blood film.

- **Specific tests:**
  - Serum iron (low).
  - Serum ferritin (reduced).
  - Serum Transferrin Receptor (raised).
  - Bone marrow iron stores (absent).
  - Hemoglobin electrophoresis (normal).

III. Genetic disorders of hemoglobin

- Hb is composed of **haem (iron)** and **globin (protein).**
- **Hemoglobin abnormalities result from either**

1. The production of <u>abnormal</u> Hb (e.g., Hb S).
2. <u>Reduction in the synthesis of</u> normal Hb proteins (α or β- globin) (e.g., α and β-thalassemia).

Table 2.3 Normal haemoglobins in adult blood.

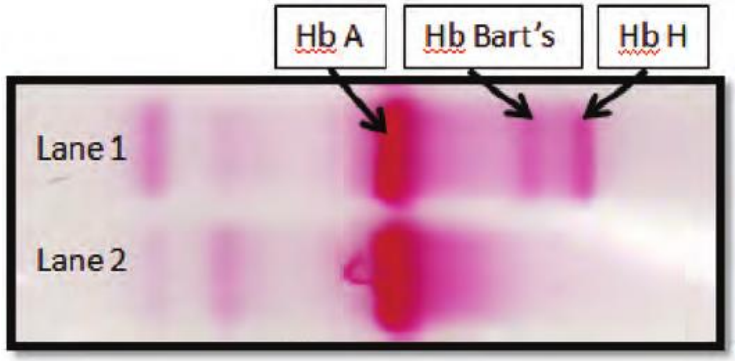
	Hb A	Hb F	Hb A_2
Structure	$\alpha_2\beta_2$	$\alpha_2\gamma_2$	$\alpha_2\delta_2$
Normal (%)	96–98	0.5–0.8	1.5–3.2

III.I Thalassemia

- **Cause**: reduced production rate of β or α- chain.
- **There are two types of Thalassemia:**
  - **α- Thalassemia** (defect in α globin chain)
  - **β -Thalassemia** (defect in β globin chain)

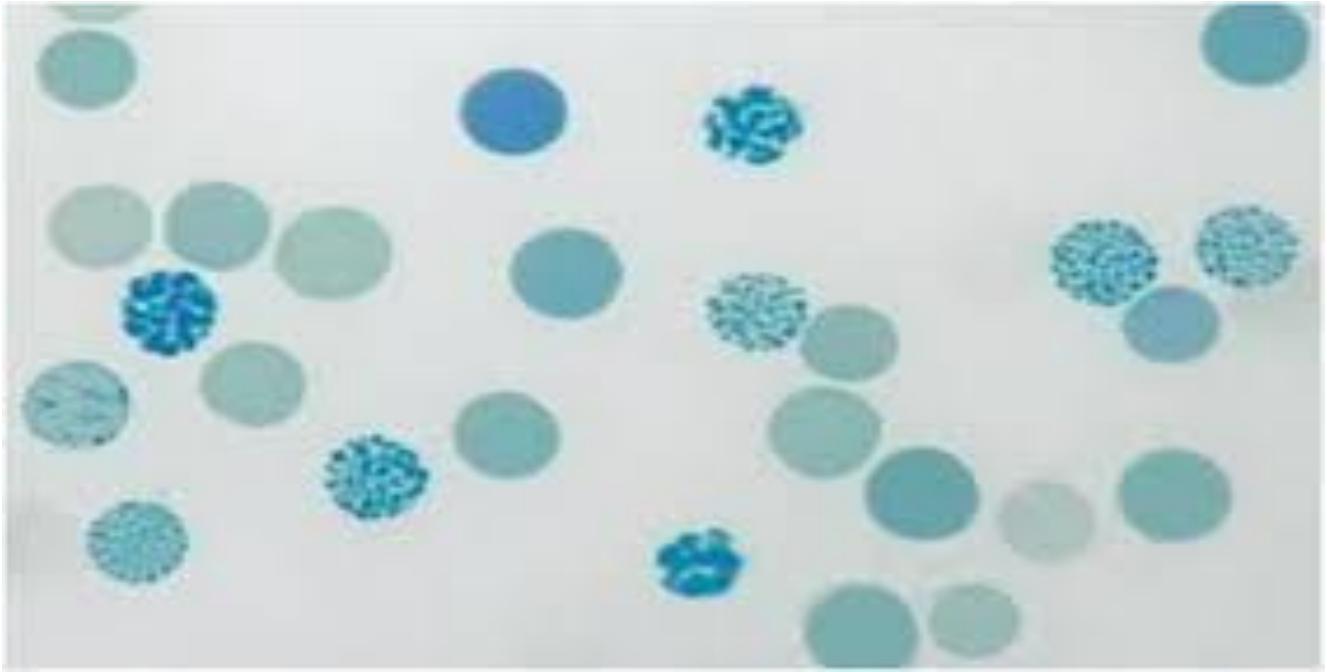
III.I Alpha Thalassemia

- α-thalassemia is caused by a deletion in α genes that leads to the loss of one or more α peptide chains.
- α-thalassemia type that is caused by a deletion in 3 α genes leads to Hb H disease. As a result, **Hb H** is composed mainly of 4 β globin chains.
- **Hb H Can be detected by:**
  - Staining by the supravital stain.
  - Electrophoresis (fast-moving band).



III.I Alpha Thalassemia

- α-Thalassemia: Hb H disease

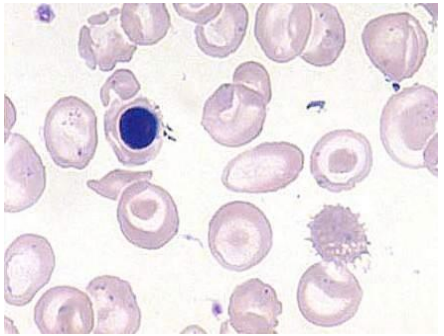


Multiple fine, deeply stained deposits (golf ball cells)

III.I Thalassemia (α or β)

- **Blood film morphology:**
  - Hypochromic, microcytic, target cell, nucleated RBCs

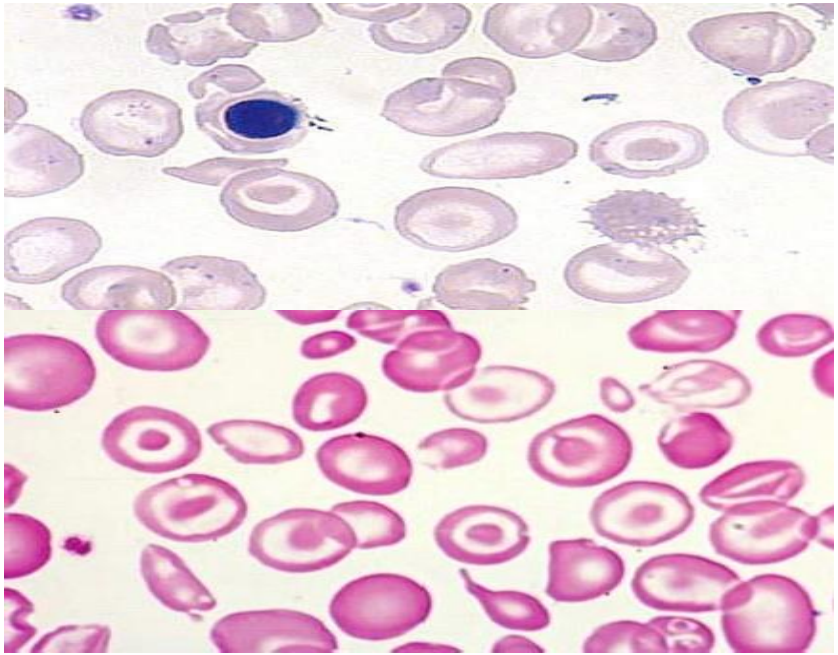
  (NRBCs not seen in IDA), poikilocytosis.

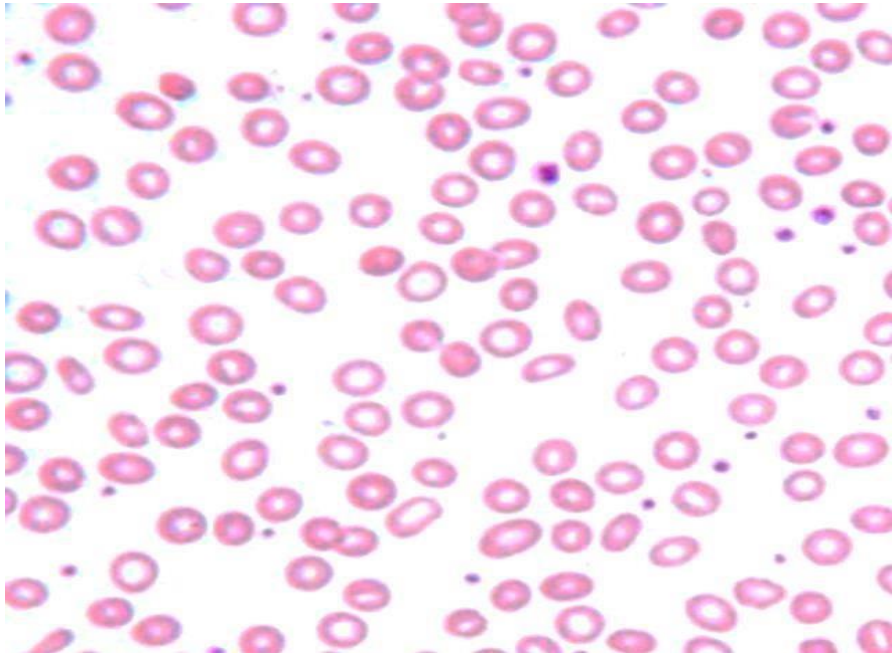


- **Screening test:**
  - Hb (low), MCV (low), MCH (low), PCV (low), reticulocyte (high), blood film.
- **Specific test:**
  1. Electrophoresis: shows an increase in Hb F.
  2. High-performance liquid chromatography (HPLC): shows an increase in Hb F.
  3. DNA analysis: shows a mutation in the related gene.

III.I Thalassemia

- Thalassemia (α or β) smear

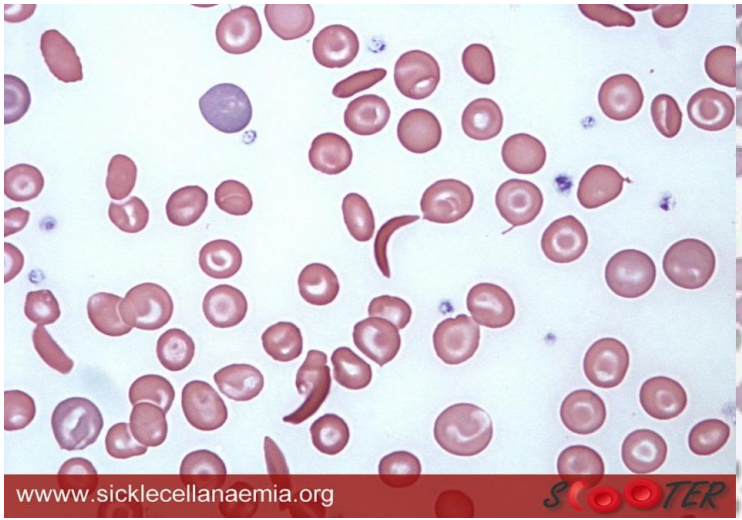


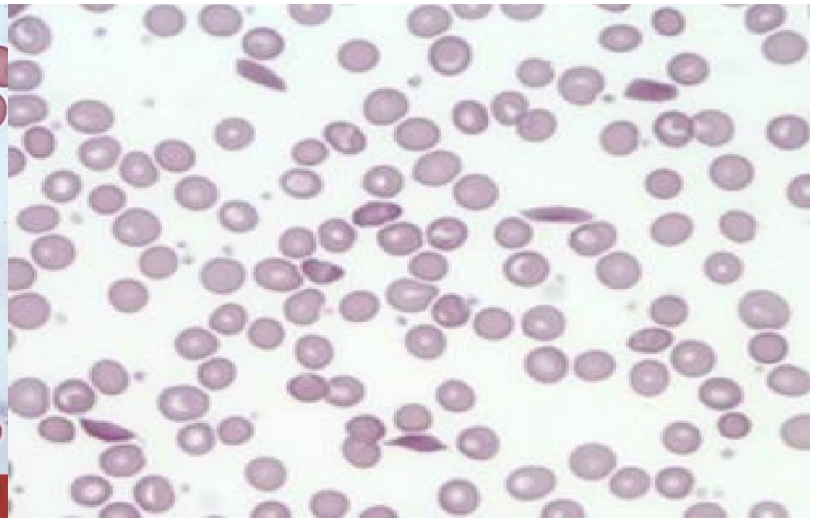


Hypochromic, microcytic, target cell, nucleated RBCs **(NRBCs not seen in IDA)**, poikilocytosis.

III.II Sickle cell anemia

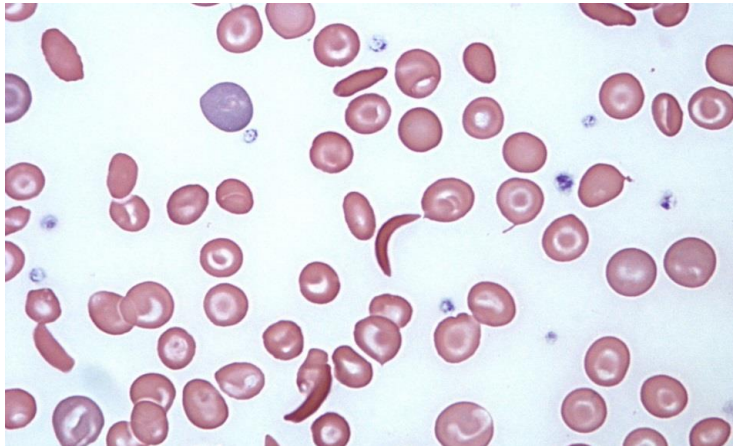
- **Cause**: mutation in the beta chain gene (HBB) that results in the production of abnormal Hb called **HB S**.
- **Blood film morphology:**
  - Sickle cells, target cells, polychromasia, Howell- Jolly bodies may be present.





III.II Sickle cell anemia

- **Screening test:** Hb (low), blood film (sickle cell).
- **Special sickle cell anemia screening test:**
  1. Sickling test (+ve).
  2. Solubility test (+ve).
- **Specific test:**

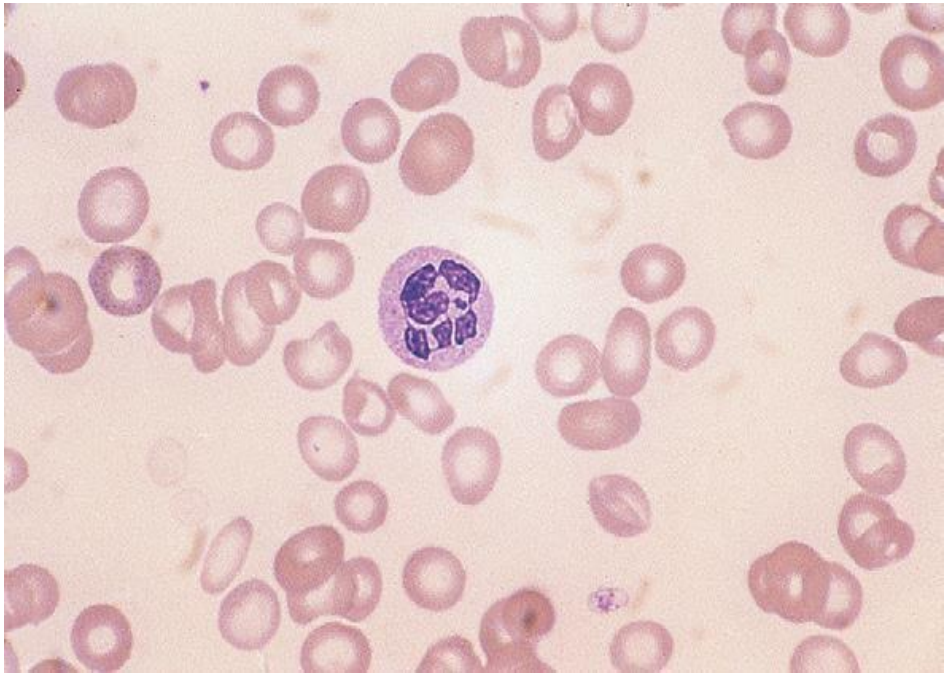


  1. **Electrophoresis**: shows the presence of Hb S and variation in the percentage of Hb A and Hb F.
  2. **High-performance liquid chromatography (HPLC):** shows the presence of Hb S and variation in the percentage of Hb A and Hb F
  3. **DNA analysis:** shows a mutation in the beta globulin (HBB) gene.

IV. Megaloblastic anemia

- **Macrocytic** anemia

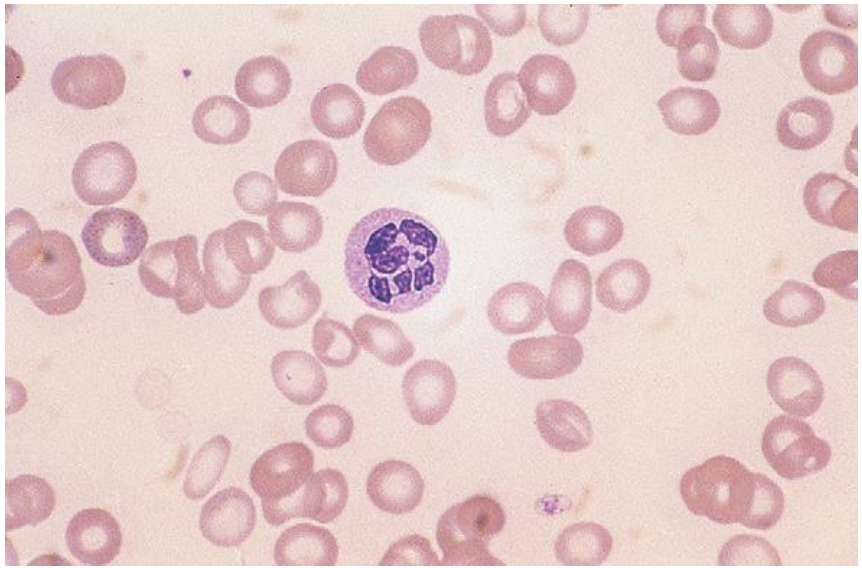
- **Blood film morphology:**
  - Macrocytic (oval).
  - **Hyper-segmented neutrophil nuclei (6 or more lobes).**



Photomicrograph of a blood film.
Megaloblastic anemia.
Shows macrocytes, oval macrocytes, and a hyper-segmented neutrophil.

IV. Megaloblastic anemia

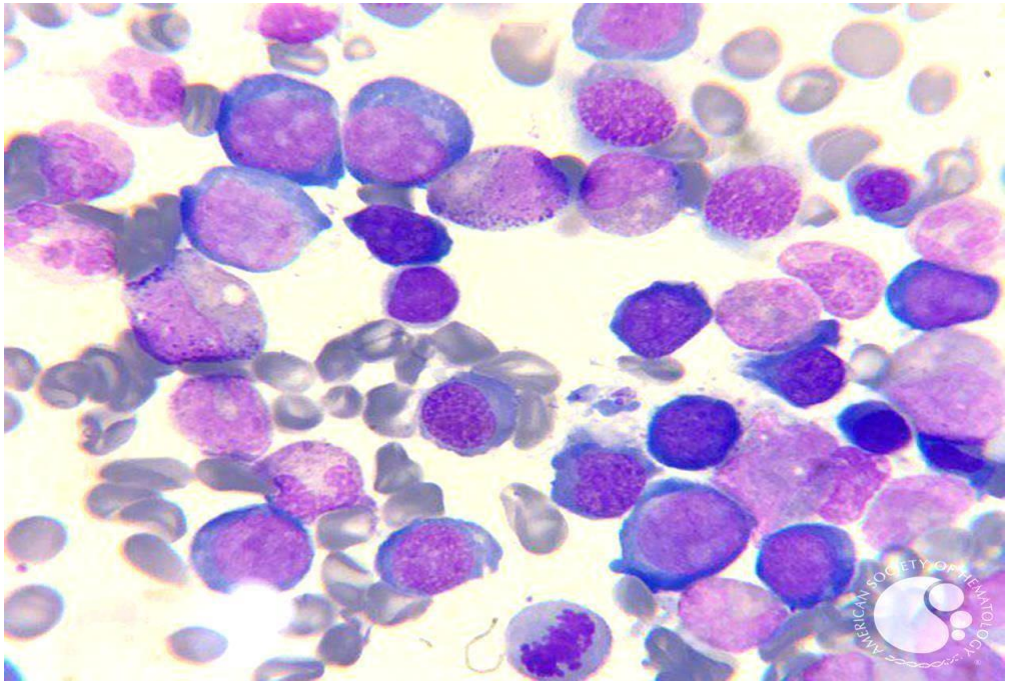
- Screening test:
  - MCV (high), reticulocyte count (low), blood film, Hb (low)
- Specific tests:
  1. Serum vitamin B12 (depend)
  2. Serum folate (depend)
  3. Red cell folate (low)
  4. BM examination



IV. Megaloblastic anemia

- **Bone Marrow Aspirate** from a patient with **megaloblastic anemia**.

- Erythroid hyperplasia (decreasing of myeloid to erythroid ratio) is seen in the bone marrow.



V. Hereditary hemolytic anemias

- **Hereditary hemolytic anemia** is increased RBC destruction due to intrinsic red cell defects, which include:
  1. **RBC enzyme defect.**
  2. **RBC membrane defect** in proteins involving vertical or horizontal interactions of the RBCs Cell membrane.

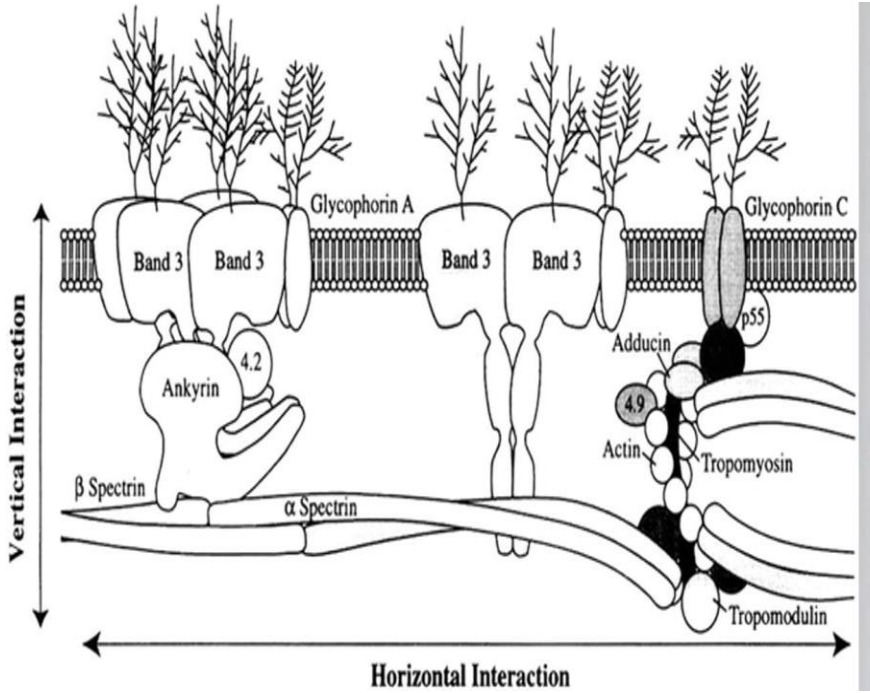
     Types include:

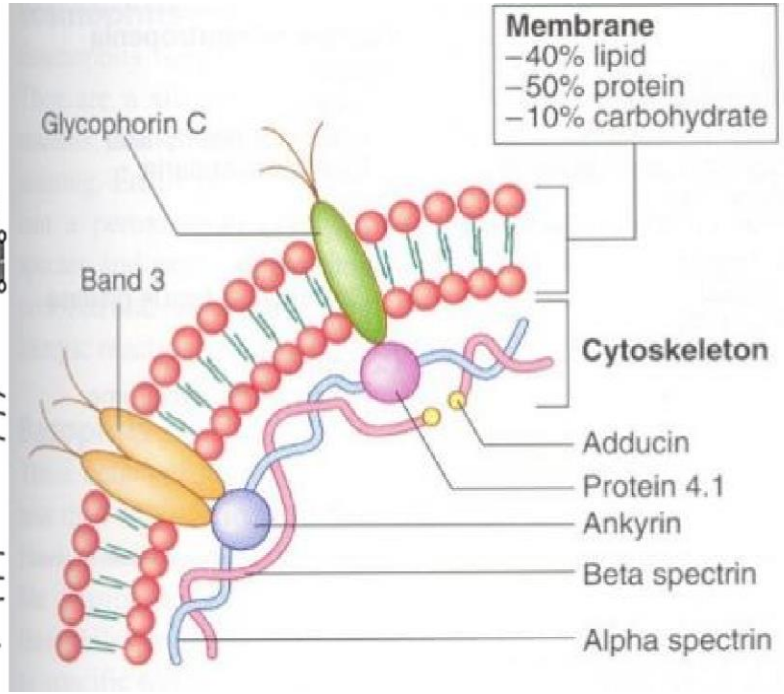
     a) Hereditary spherocytosis (HS)

     b) Hereditary elliptocytosis (HE)
  3. **Hb synthesis defect.**

Red cell membrane structure

- The red cell membrane comprises a lipid bilayer, integral membrane protein, and a membrane skeleton.





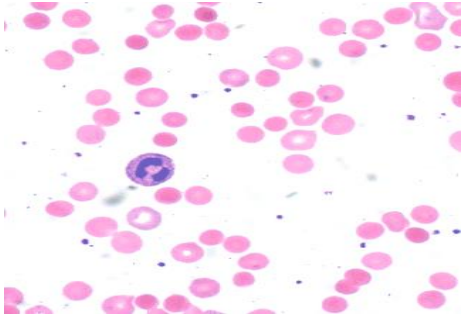
V. Hereditary spherocytosis and elliptocytosis

Hereditary spherocytosis

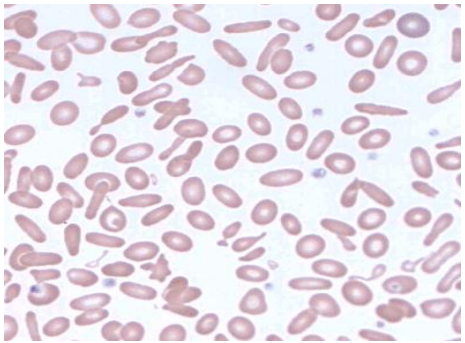
- Defects in the proteins involved in the vertical interactions between the membrane skeleton and the lipid bilayer of the red cells: (e.g. ankyrin, Band3, protein 4.2).

Hereditary elliptocytosis

- Defects in the proteins involved in the horizontal interactions.

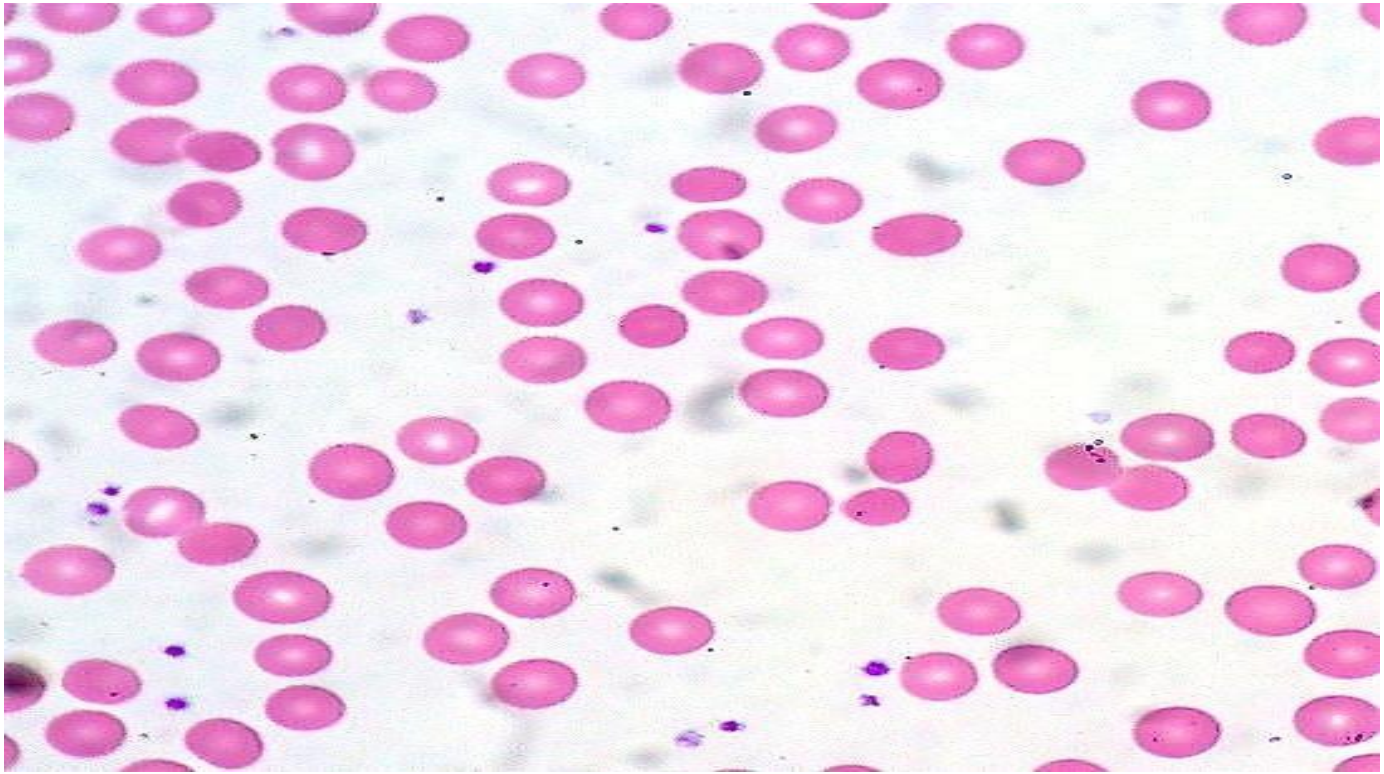


Hereditary spherocytosis

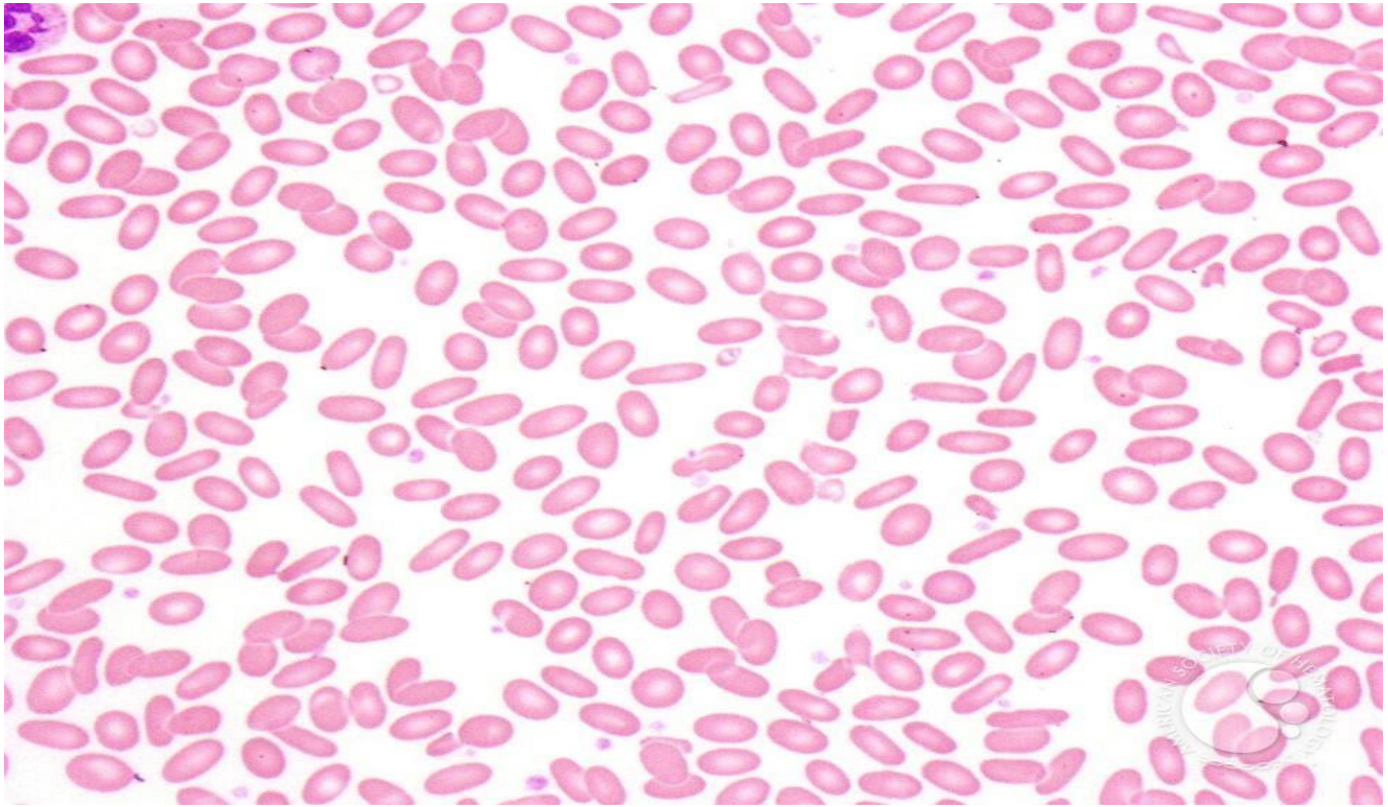


Hereditary elliptocytosis

Hereditary spherocytosis smear



Hereditary elliptocytosis smear



HS and HE lab finding

- **Screening test:**
  - Hb (low)
  - Reticulocytosis 5-20%
  - Blood film shows spherocytosis or elliptocytosis.
  - MCHC (Increased) - (characteristic feature)
  - MCV (Decrease).

- **Specific test:**
  - Osmotic fragility test (increase).

Microscopy check list

Practice identifying abnormalities found in the following conditions from the blood film:

1. IDA
2. Hemoglobinopathies:
    a. Thalassemia: alpha and beta
    b. Sickle cell anemia.
3. Megaloblastic anemia.
4. Hereditary hemolytic anemia:
    a. Hereditary spherocytosis.
    b. Hereditary elliptocytosis.