UNIT 1

INVESTIGATION OF RED CELL DISORDERS

LEARNING OBJECTIVES

At the end of this chapter, students are expected to:

1. Ask for the usual investigations needed to diagnose anemia
2. Interpret the red cell changes on peripheral blood smear
3. Request and interpret the special laboratory tests in various types of anemias
4. Discuss the indications and the pathological findings for bone marrow aspiration

The most frequently encountered red cell disorder in clinical practice is anemia. Anemia is defined as the lowering of the red cell mass below the established normal levels. In practice we diagnose anemia when there is a:

- decrease in the amount of hemoglobin in the blood
- decrease in the number of circulating red cells
- hematocrit below the normal range.

Classification of ANEMIAS:

I. MORPHOLOGICAL
II. FUNCTIONAL
III. ETIOPATHOGENIC

The laboratory workup of anemias starts with:

1. Peripheral blood tests

1.1 Red blood cell count (RBC)

Normal: Male: $4.4 - 5.9 \times 10^{12} / l$
Female: $3.8 - 5.2 \times 10^{12} / l$

1.2 Hematocrit (Hct) or the packed cell volume (PCV)

Hematocrit is the proportion of the blood occupied by red cells that is expressed as a percentage (%).

\[
\text{Hct (PCV)} = \frac{\text{Packed red cells volume}}{\text{Plasma volume}}
\]

Normal values: Male: $40 - 52 \%$
Female: $35 - 47 \%$

1.3 Hemoglobin concentration (Hb)

Manual or automated measurements of cyanmethemoglobin concentration - a stable derivative of hemoglobin - provide comparable estimates of hemoglobin concentration.

Normal values: Male: $15.0 \pm 2.0 \ g/dL$
Female: $14.0 \pm 2.0 \ g/dL$

Values differ between men and women since androgens drive RBC production and hence adult men have higher Hb, PCV and RBC than adult women.

Hb is the main parameter used to assess the severity of anemia. Together with RBC, Htc, and the analysis of the peripheral blood smear allows the MORPHOLOGICAL classification of anemias.

Observation:

- After a major bleeding (acute blood loss), anemia may not be apparent for several days until plasma volume returns to normal (that is, only Hct will be decreased with a normal of Hb).
- Alterations in hematocrit and hemoglobin may occur as a result of changes in plasma volume, generating the so-called ‘relative’ changes:
  - 'Relative' anemia (spuriously low Hb level) is due to hypervolemia (from excessive fluid replacement, pregnancy, fluid retention in: heart failure, cirrhosis).
  - 'Relative' polycythemia (spuriously high Hb level) is due to hypovolemia (from severe vomiting or diarrhea).
WHO (World Health Organisation) classification of anemias, according to the level of Hb:
- mild: Hb = 11 – 9 g/dL
- moderate: Hb = 9 – 7,5 g/dL
- severe: Hb = 7,5 – 5 g/dL
- very severe: Hb < 5 g/dL

1.4 Red cells indices

Are measurements that indicate the size and hemoglobin content of red cells. These values can be calculated quantitatively from the hemoglobin concentration, red cells counts and packed red cell volume.

1.4.1 Mean corpuscular volume (MCV) refers to the average volume of the individual cells. The MCV is expressed in cubic micrometers (\( \mu m^3 \)) per red cell or femtoliters (fl) and is calculated as follows:

\[
MCV = \frac{Hematocrit (\%) \times 100}{Red \ cell \ count \ (millions/mm^3)}
\]

Normal value: 80 - 100 fl (femtolitre, \( 10^{-15} \)l).

Pathological changes:
- low MCV is typical for microcytic, hypochromic anemias

1.4.2 Mean corpuscular hemoglobin (MCH) refers to the hemoglobin content per red cell. The MCH is expressed in picograms (pg) per red cell and is calculated as follows:

\[
MCH = \frac{Hemoglobin (g/l) \times 10}{Red \ cell \ count \ (millions/mm^3)}
\]

Normal value: 26 - 34 pg

Pathological changes: low MCH is typical for microcytic, hypochromic anemias

1.4.3 Mean corpuscular hemoglobin concentration (MCHC) refers to the hemoglobin concentration of the red cells. The MCHC is expressed in grams per deciliter of red cells and is calculated as follows:

\[
MCHC = \frac{Hemoglobin (g/l) \times 100}{Hematocrit (\%)}
\]

Normal value: 31 – 36 g/dl

Pathological changes: low MCHC is typical for microcytic, hypochromic anemias

1.4.4 Red cell distribution width (RDW) Measures the range of red cell size in a sample of blood, providing information about the degree of red cell anisocytosis, i.e. how much variation there is between the size of the red cells.

There are 3 major types of anemia, classified according to the appearance of red cells - morphological classification:

I. NORMOCYTIC NORMOCHROMIC
- Aplastic anemia
- Hemorrhagic anemia
- Hemolytic anemia
- Sickle-cell anemia
- Anemia of chronic disease

II. MICROCYTIC HYPOCHROMIC
- MCV < 80 fl
- MCH < 27 pg
- MCHC < 32 g/dl
- Iron-deficiency anemia
- Sideroblastic anemia
- Thalassemias

III. MACROCYTIC NORMOCHROMIC
- MCV > 96 fl, normal MCH, MCHC
- Vit. B12-deficiency anemia (Addison-Biermer or pernicious anemia)
- Folic acid – deficiency anemia
1.5. Reticulocyte count

Reticulocytes are 1-2 day-old red cells that still exhibit a network of purple strands, which are aggregates of ribosomes. **Normal values:** 0.5 – 1.5% of red blood cells (values are expressed as percentage of the RBC count).

**Observation!**
In the presence of anemia, the number of reticulocytes (Rt) must be corrected, because is falsely increased when is reported to the number of decreased erythrocytes:

\[
\text{Rt}_{\text{corr}} = \%\text{Rt} \times (\text{Ht patient}/45)
\]

- \( \geq 3\% \) \( \Rightarrow \) regenerative anemia
- \( < 3\% \) \( \Rightarrow \) aregenerative anemia

**Pathological changes**
- \( \text{Rt}_{\text{corr}}: \geq 3\%: \) elevate reticulocyte count (reticulocytosis) is a reflection of the release of an increased number of young cells from the bone marrow which occurs with:
  - hemolytic anemias (regardless the mechanism of hemolysis, reticulocytosis is the main difference between anemias due to hemolysis and anemia due to decreased red cell production)
  - anemias due to blood loss (in the first 5 -7 days after an acute hemorrhage)
  - pernicious anemias (7 -10 days later after receiving an injection of vitamin B12)
  - iron deficiency anemia (7 - 10 days after starting iron therapy)

- \( \text{Rt}_{\text{corr}}: < 3\%: \) low reticulocyte count (reticulopenia) reflects a failure to produce red cells encountered in:
  - aplastic anemias
  - anemia of chronic disease
  - bone marrow failure
  - renal disease

2. Blood smear examination
- Critical diagnostic information regarding the morphology of the cellular elements of the blood can be obtained through the examination of peripheral blood smear.
- Examination of a fixed peripheral blood smear stained with Giemsa stain allows identification of various changes in blood cell morphology (Table 1).
- Red cells should be examined for size, shape, hemoglobin content, staining properties, and inclusion bodies.

**Table 1. Red Cell Changes Observed on Blood Smear Examination**

<table>
<thead>
<tr>
<th>Red Cell Change</th>
<th>Description</th>
<th>Associated Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Abnormal size (anisocytosis)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcytes</td>
<td>MCV &lt; 80 fl</td>
<td>Iron deficiency anemias, sideroblastic anemias, thalassemias, lead poisoning.</td>
</tr>
<tr>
<td>Macrocyes</td>
<td>MCV &gt; 96 fl</td>
<td>Megaloblastic anemias due to vitamin B₁₂ deficiency (pernicious anemia) and folate deficiency, macrocytosis from alcoholic chronic liver disease or hemolytic anemias with reticulocytosis.</td>
</tr>
<tr>
<td>Megalocytes</td>
<td>MCV &gt; 120 fl</td>
<td></td>
</tr>
<tr>
<td><strong>Abnormal shape (poikilocytosis)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acanthocytes</td>
<td>Few large spicules</td>
<td>Advanced cirrhosis and renal disease with uremia</td>
</tr>
<tr>
<td>Echinocytes (&quot;burr cells&quot;)</td>
<td>Many tiny spicules</td>
<td>Severe liver disease, thalassemia, postsplenectomy.</td>
</tr>
<tr>
<td>Leptocytes (target cells)</td>
<td>Target forms</td>
<td>Hemolytic anemias</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Drepanocytes</td>
<td>Sickle shape</td>
<td>Sickle cell disease (Hb S), DIC, TTP</td>
</tr>
<tr>
<td>Schizocytes (&quot;schistocytes; spur cells&quot;)</td>
<td>Helmet forms, red cell fragmentation</td>
<td>Hemolytic anemias</td>
</tr>
<tr>
<td>Spherocytes</td>
<td>Spheroid shape</td>
<td>Hereditary spherocytosis</td>
</tr>
</tbody>
</table>

**Abnormal coloration**

<table>
<thead>
<tr>
<th>Hypochromic eritrocytes</th>
<th>MCH &lt; 27 pg and MCHC &lt; 32 g/dl</th>
<th>Iron deficiency anemias, sideroblastic anemias, thalassemias, lead poisoning.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anisochromic smear (hypochromic and normochromic cells)</td>
<td>Iron deficiency anemias, sideroblastic anemias, thalassemias, lead poisoning</td>
<td></td>
</tr>
</tbody>
</table>

**Inclusion bodies**

<table>
<thead>
<tr>
<th>Basophilic stippling</th>
<th>Punctate nucleus</th>
<th>Lead intoxication, sideroblastic anemia, severe hemolysis.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Howell-Jolly bodies</td>
<td>Small round bodies</td>
<td>Megaloblastic anemia, asplenia, severe hemolysis.</td>
</tr>
<tr>
<td>Cabot’s ring body</td>
<td>Ring of figures of eight</td>
<td>Severe hemolysis.</td>
</tr>
<tr>
<td>Heinz bodies</td>
<td>Precipitated and denaturated Hb (decreased solubility)</td>
<td>Unstable hemoglobin variants, methemoglobinemia, glucose-6-phosphate dehydrogenase deficiency.</td>
</tr>
</tbody>
</table>

DIC = disseminated intravascular coagulation; Hb = hemoglobin; TTP = thrombotic thrombocytopenic purpura. Synonymous terms are noted in parentheses.

### 4. Special laboratory tests in various types of anemias

#### 4.1 Iron metabolism

**4.1.1 Serum iron**

- **Normal values:**
  - Male: 59-158 μg/dL
  - Female: 37-145 μg/dL

**Pathological changes:**

<table>
<thead>
<tr>
<th>Decreased</th>
<th>Increased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron-deficiency anemia</td>
<td>Sideroblastic anemias</td>
</tr>
<tr>
<td>Acute and chronic infection</td>
<td>Transfusions (repeated)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>Thalassemia</td>
</tr>
</tbody>
</table>

#### 4.1.2 Total iron binding capacity (TIBC)

- **Normal values:** 250 - 450 μg/dL
- **Pathological changes:**
  - **increased values:** iron deficiency anemia, acute liver disease
  - **decreased values:** in: chronic infections, cirrhosis, hemochromatosis, hemolytic anemias, neoplastic diseases, renal disease, sideroblastic anemias, thalassemia.

<table>
<thead>
<tr>
<th>Chronic blood loss (gastrointestinal, uterine)</th>
<th>Acute liver disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothyroidism</td>
<td></td>
</tr>
<tr>
<td>Postoperative state</td>
<td></td>
</tr>
</tbody>
</table>

| Hemochromatosis |
| Hemolytic anemia |
| Lead toxicity |
4.1.3 Transferrin saturation (i.e. serum iron divided by total iron-binding capacity)

Normal values: 16 - 45 %

Pathological changes:
- increased values: in sideroblastic anemia
- decreased values: in iron deficiency anemia.

4.1.4 Serum ferritin

Iron is stored in the tissues as ferritin and haemosiderin. Assessment of serum ferritin correlates closely with total-body iron stores.

Normal values: Male: 15 – 400 ng/ml
Female: 10 – 200 ng/ml.

Pathological changes:
- increased values: iron overload in sideroblastic anemia or increased release of ferritin from extensive damaged tissues in patients with inflammation, liver diseases and certain malignancies
- decreased values: iron deficiency (which obviates the need to perform bone marrow aspiration in order to assess Prussian blue-stainable iron stores).

4.2 Vitamin B₁₂ and folate metabolism

4.2.1 Serum B₁₂: 200 – 600 pg/ml.

4.2.2 Serum folate: 3,6 – 15 ng/ml.

4.2.3 Schilling test

The absorption of B₁₂ can be measured using the Schilling test. A radioactive dose of B₁₂ is given orally and the total body activity is measured.

Part I
- Give 1 µg ^58^Co-B₁₂ orally to fasting patient.
- Give 1000 µg B₁₂ (non - radioactive) by intramuscular injection to saturate B₁₂ – bindings proteins and to flush out ^58^Co-B₁₂.
- Collect urine for 24 h.
- Normal subjects secrete more than 8 - 10% of the radioactive dose.

If abnormal (excretion less than 8 %) it follows:

Part II
- Repeat part I after giving oral intrinsic factor capsules.

Results:
- If excretion now normal, diagnosis is pernicious anemia.
- If excretion still abnormal, lesion is in the terminal ileum or there is bacterial overgrowth.

If abnormal (excretion less than 8 %) it follows:

Part III
- Repeat part II after giving antibiotics or anti-inflammatory drugs.
- If excretion normalizes after antibiotics, there is bacterial overgrowth; if it does after anti-inflammatory therapy, B₁₂ malabsobtion is due to inflammatory bowel disease (especially, Crohn’s disease which affects primarily the terminal ileum).

4.3 Assessment of hemolysis

Hemolytic anemias are characterized by increased rate of destruction of red cells with shortened survival in the circulation. The hallmark of hemolysis is an increased number of reticulocytes (reticulocytosis) in the peripheral blood. Two types of hemolysis may occur:

- **Extravascular hemolysis** occurs in the macrophages of the reticuloendothelial system and has the following characteristics:
  - Increased level of indirect (unconjugated) bilirubin with jaundice
  - Increased urobilinogen in urine and in feces
  - Increased serum lactate dehydrogenase from lysed erythrocytes
  - Compensatory erythroid hyperplasia of the bone marrow

- **Intravascular hemolysis** is characterised by few additional features:
  - Decreased level of serum haptoglobin (globulin synthetized by liver that binds free Hb)
  - Free hemoglobin in the plasma (hemoglobinemia)
  - Free hemoglobin in the urine (hemoglobinuria) in chronic hemolysis

4.3.1 Coombs antiglobulin test

Represents the major tool for diagnosing immunohemolytic anemias (hemolysis due to red cells antibodies).
This test relies on the ability of an *antiglobulin serum* (that is, antibodies prepared in rabbits by injecting human globulin and directed against that globulin) to agglutinate red blood cells, if these globulins are present on the erythrocytes surface. There are two technical variants:

**Direct Coombs test:**
- Refers to the ability of antiIgG antiserum to agglutinate the patient's red blood cells, if coated with IgG antibodies - that is identifies *fixed red cells antibodies*.
- Detect autoimmune hemolytic anemia or hemolytic disease of the newborn
- Evaluate suspected drug-induced hemolytic anemia
- Evaluate transfusion reaction

**Indirect Coombs test:**
- Identifies *free antibodies in the serum* reactive against red cells.
- It is performed by incubating ABO- and Rh-compatible red blood cells with the patient's serum and subsequently performing a direct Coombs test on these incubated red cells.

### 4.3.2 Hemoglobin electrophoresis

Represents the main tool for diagnosing abnormal hemoglobins in disorders of hemoglobin structure and function (hemoglobinopathies), e.g. increased HbS in sickle cell anemia, increased Hb F and A2 in thalassemias.

**Normal values:** % Hemoglobin: Hb A >95% Hb A₂ 1.5 – 3.7 % and Hb F <2%

### 5. Bone marrow evaluation

**Aspiration and biopsy** from the sternum or posterior iliac crest are two techniques through which bone marrow can be examined.

**Indications for bone marrow aspiration:**
- **Confirm a diagnosis made from peripheral blood count**
- **Determine the cellularity of the marrow and the proportion of various cell lines** (normal, cells of the myeloid series exceed erytroid cells by a ratio of 3-4 :1)
- **Determine the type of erytropoiesis**, e.g. normoblastic or megaloblastic
- **Evaluate the size of the iron stores and abnormal presence of iron in erytroid precursors** in patients with conditions such as: anemia of chronic diseases, sideroblastic anemias (unless the iron deficiency is obvious)
- **Diagnosis of tumor involvement of the bone marrow** for assessment of the patient's tumor stage (lymphomas, metastatic lung carcinoma) and in evaluation for chemotherapy.
- **Investigate an immunologic disorder**, such as monoclonal increase of immunoglobulins in multiple myeloma, as well as investigation of lymphomas.

**Pathological findings:**
- **Hypercellularity** of the marrow is recognized as an increased cellularity and it represents either hyperplasia or neoplasia
  a. **Hyperplasia** of the marrow occurs when there is an increased demand for hematopoietic cells:
  - in conditions of increased red cell need (chronic hypoxia) or red cell destruction (hemolytic anemia) there is erytroid hyperplasia
  - in severe acute inflammation there is hyperplasia of the neutrophil series
  - in peripheral destruction of the platelets there is megakaryocyte hyperplasia
  b. **Neoplasms** of bone marrow include leukemias, malignant lymphoma, plasma cell myeloma and metastasis from a malignant tumor with other location.
- **Hypocellularity** of the marrow (hypoplastic or aplastic anemia):
  - is caused by failure, suppression or destruction of stem cells
  - it results in decreased production of one/all cell lines in periphery (pancytopenia)
  - the etiology of hypocellularity is as follows:
    - drugs (most common cause): chloramphenicol, anti-cancer drugs, phenothiazines, phenylbutazone
    - radiation
    - chemical agents: benzene, insecticides
    - idiopathic (in 50% of cases of aplastic anemia the cause is not found)
# Laboratory tests used to diagnose different types of anemia

<table>
<thead>
<tr>
<th>Lab test</th>
<th>Iron deficiency anemia</th>
<th>Sideroblastic anemia</th>
<th>B 12 deficiency anemia</th>
<th>Folic acid deficiency anemia</th>
<th>Aplastic anemia</th>
<th>Post hemorrhagic anemia</th>
<th>Hemolytic anemia</th>
<th>Anemia associated with chronic infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓/N</td>
<td>N/↓</td>
<td>↓/N</td>
<td>↓/N</td>
</tr>
<tr>
<td>Ht</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓/N</td>
<td>N/↓</td>
<td>↓/N</td>
<td>↓/N</td>
</tr>
<tr>
<td>Reticulocyte count</td>
<td>N/ slightly↑/↓</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>N/↑</td>
<td>N/↓</td>
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<tr>
<td>MCV</td>
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<td>↑</td>
<td>N/↑</td>
<td>N/↓</td>
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<tr>
<td>Serum iron</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>N</td>
<td>N/↑</td>
<td>↓/N</td>
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<tr>
<td>TIBC</td>
<td>↑</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N/↑</td>
<td>↓/N</td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>N</td>
<td>N/↑</td>
<td>N/↓</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>↓</td>
<td>↑</td>
<td>slightly↑</td>
<td>slightly↑</td>
<td>N/↑</td>
<td>N</td>
<td>N/↑</td>
<td>N/↓</td>
</tr>
<tr>
<td>Vit. B₁₂</td>
<td>N</td>
<td>N</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N/↑</td>
<td>N/↓</td>
</tr>
<tr>
<td>Folic acid</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↓</td>
<td>N</td>
<td>N</td>
<td>N/↑</td>
<td>N/↓</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>N</td>
<td>↑</td>
<td>slightly↑</td>
<td>slightly↑</td>
<td>N/↑</td>
<td>N</td>
<td>N/↑</td>
<td>N/↓</td>
</tr>
</tbody>
</table>
CHECKPOINT

1. A 42-year-old woman presented with a history of increasing lethargy, dizziness and breathlessness. She had brittle hair and nails. She complained of heart palpitation on exercise and reported particularly heavy periods.

**Biochemical investigations:**
- Serum iron = 20 μg/dL
- Transferrin saturation = 10%
- Ferritin = 5 μg/L

What is the suspected diagnosis?
A. Iron deficiency anemia  
B. Sideroblastic anemia  
C. Hemolytic anemia  
D. Hemochromatosis  
E. Iron poisoning.

2. 38 y old woman, vegetarian, presents fatigue and bilateral paresthesia. The symptoms have aggravated since previous year. Clinical examination revealed tachycardia and pale skin.

**Lab analysis:**
- Leukocyte count = 4.000 /mm$^3$
- Hb = 9 g/dl
- Ht = 27%
- MCV = 120 fL
- Platelets = 150,000 /mm$^3$

Which is the most probable diagnosis?
A. Aplastic anemia  
B. Thalassemia  
C. Megaloblastic anemia  
D. Biermer anemia  
E. Hemolytic anemia

3. 25 years old mediterranean woman, pregnant in 12 weeks, refers to her OBG for her first prenatal medical visit. The anamnesis has revealed as a familial medical condition a slight form of anemia, except for her brother who was transfused at the age of 10.

**Lab analysis:**
- Hb = 10,3 g/dl
- Ht = 34,9%
- VEM = 62 fL
- MCH = 18,4 pg
- MCHC = 29,5 g/dl
- Leukocyte = 8.500/mm$^3$
- Platelets = 298.000/mm$^3$

**ELFO Hb** – the presence of pathologic Hb

Which is the most probable diagnosis?
A. Aplastic anemia  
B. Thalassemia  
C. Megaloblastic anemia  
D. Biermer anemia  
E. Hemolytic anemia

4. The Schilling test is useful in diagnosis of which type of anemia?
A. Folic acid deficiency anemia  
B. Aplastic anemia  
C. Iron-deficiency anemia  
D. Biermer anemia  
E. Thalassemias

5. Which of the following represent normochromic anemias:
A. Aplastic anemia  
B. Folic acid deficiency anemia  
C. Post-hemorrhagic anemia  
D. Hemolytic anemia  
E. Sideroblastic anemia

6. In which type of anemia the electrophoresis of Hb should be recommended?
A. Pemicious anemia  
B. Thalassemias  
C. Aplastic anemia  
D. Anemia of chronic disease  
E. Sickle-cell anemia