Classification of anemias

What is anemia, how do you diagnose anemia, and how are the different anemias classified?
Definition of anemia

- In its broadest sense, anemia is a functional inability of the blood to supply the tissue with adequate O$_2$ for proper metabolic function.
- Anemia is not a disease, but rather the expression of an underlying disorder or disease.
  - A specific diagnosis is made by:
Definition of anemia

- Patient history
- Patient physical exam
- Signs and symptoms exhibited by the patient
- Hematologic lab findings
  - Identification of the cause of anemia is important so that appropriate therapy is used to treat the anemia.
- Anemia is usually associated with decreased levels of hemoglobin and/or a decreased packed cell volume (hematocrit), and/or a decreased RBC count.
Definition of anemia

- Occasionally there is an abnormal hemoglobin with an increased $O_2$ affinity resulting in an anemia with normal or raised hemoglobin levels, hematocrit, or RBC count.

- Before making a diagnosis of anemia, one must consider:
  - Age
Definition of anemia

- Sex
- Geographic location
- Presence or absence of lung disease

- Remember that the bone marrow has the capacity to increase RBC production 5-10 times the normal production.

- Thus, if all necessary raw products are available, the RBC life span can decrease to about 18 days before bone marrow compensation is inadequate and anemia develops.
Definition of anemia

- An increased production of RBCs in the bone marrow is seen in the peripheral smear as an increased *reticulocyte* count since new RBCs are released as reticulocytes.
- If the bone marrow production of RBCs remains the same or is decreased with RBCs that have a decreased survival time, anemia will rapidly develop.
Definition of anemia

- There is no mechanism for increasing RBC survival time when there is an inadequate bone marrow response, so anemia will develop rapidly.

- In summary, anemia may develop:
  - When RBC loss or destruction exceeds the maximal capacity of bone marrow RBC production or
  - When bone marrow production is impaired
Definition of anemia

- Various diseases and disorders are associated with decreased hemoglobin levels. These include:
  - Nutritional deficiencies
  - External or internal blood loss
  - Increased destruction of RBCs
  - Ineffective or decreased production of RBCs
Definition of anemia

- Abnormal hemoglobin synthesis
- Bone marrow suppression by toxins, chemicals, or radiation
- Infection
- Bone marrow replacement by malignant cells
Significance of anemia and compensatory mechanisms

- The signs and symptoms of anemia range from slight fatigue to life threatening reactions depending upon
  - Rate of onset
  - Severity
  - Ability of the body to adapt
Rate of onset and severity

- With rapid loss of blood:
  - Up to 20% may be lost without clinical signs at rest, but with mild exercise the patient may experience tachycardia (rapid heart beat).
  - Loss of 30-40% leads to circulatory collapse and shock
  - Loss of 50% means that death in imminent
Rate of onset and severity

- In slowly developing anemias, a very severe drop in hemoglobin of up to 50% may occur without the threat of shock or death.
  - This is because the body has adaptive or compensatory mechanisms to allow the organs to function at hemoglobin levels of 50% of normal. These include:
Adaptive or compensatory mechanisms

- An increased heart rate, increased circulation rate, and increased cardiac output.
- Preferential shunting of blood flow to the vital organs.
- Increased production of 2,3 DPG, resulting in a shift to the right in the O\textsubscript{2} dissociation curve, thus permitting tissues to extract more O\textsubscript{2} from the blood.
- Decreased O\textsubscript{2} in the tissues leads to anaerobic glycolysis, which leads to the production of lactic acid, which leads to a decreased pH and a shift to the right in the O\textsubscript{2} dissociation curve. Thus, more O\textsubscript{2} is delivered to the tissues per blood cell.
Diagnosis of anemia

How does one make a clinical diagnosis of anemia?

- Patient history
  - Dietary habits
  - Medication
  - Possible exposure to chemicals and/or toxins
  - Description and duration of symptoms
Diagnosis of anemia

- Tiredness
- Muscle fatigue and weakness
- Headache and vertigo (dizziness)
- Dyspnia (difficult or labored breathing) from exertion
- G I problems
- Overt signs of blood loss such as hematuria (blood in urine) or black stools
Diagnosis of anemia

- Physical exam
  - General findings might include
    - Hepato or splenomegaly
    - Heart abnormalities
    - Skin pallor
  - Specific findings may help to establish the underlying cause:
    - In vitamin B\(_12\) deficiency there may be signs of malnutrition and neurological changes
    - In iron deficiency there may be severe pallor, a smooth tongue, and esophageal webs
    - In hemolytic anemias there may be jaundice due to the increased levels of bilirubin from increased RBC destruction
Diagnosis of anemia

- Lab investigation. A complete blood count, CBC, will include:
  - An RBC count:
    - At birth the normal range is $3.9-5.9 \times 10^6/\text{ul}$
    - The normal range for males is $4.5-5.9 \times 10^6/\text{ul}$
    - The normal range for females is $3.8-5.2 \times 10^6/\text{ul}$
    - Note that the normal ranges may vary slightly depending upon the patient population.
  - Hematocrit (Hct) or packed cell volume in % or (L/L)
    - At birth the normal range is $42-60\% (.42-.60)$
    - The normal range for males is $41-53\% (.41-.53)$
    - The normal range for females is $38-46\% (.38-.46)$
    - Note that the normal ranges may vary slightly depending upon the patient population.
Diagnosis of anemia

- Hemoglobin concentration in grams/deciliter - the RBCs are lysed and the hemoglobin is measured spectrophotometrically
  - At birth the normal range is 13.5-20 g/dl
  - The normal range for males is 13.5-17.5 g/dl
  - The normal range for females is 12-16 g/dl
  - Note that the normal ranges may vary slightly depending upon the patient population.

- RBC indices – these utilize results of the RBC count, hematocrit, and hemoglobin to calculate 4 parameters:
  - Mean corpuscular volume (MCV) – is the average volume/RBC in femtoliters (10^{-15} L)
    - Hct (in %)/RBC (x 10^{12}/L) x 10
    - At birth the normal range is 98-123
    - In adults the normal range is 80-100
Diagnosis of anemia

- The MCV is used to classify RBCs as:
  - Normocytic (80-100)
  - Microcytic (<80)
  - Macrocytic (>100)

- Mean corpuscular hemoglobin concentration (MCHC) – is the average concentration of hemoglobin in g/dl (or %)
  - \[ \text{Hgb (in g/dl)/Hct (in \%)} \times 100 \]
  - At birth the normal range is 30-36
  - In adults the normal range is 31-37
  - The MVHC is used to classify RBCs as:
    - Normochromic (31-37)
    - Hypochromic (<31)
    - Some RBCs are called hyperchromic, but they don’t really have a higher than normal hgb concentration.
### Normocytic cell

**Description:**
Normal size and volume, mean cell volume (MCV) 80-100 fl.

**Shape:**
Flexible biconcave, discoid shape. Non-nucleated cell, no Golgi's complex, centriole or lysosomes.

**Significance:**
Healthy state.

**Staining method:**
May-Grünwald/Giemsa

**Microscope:**
Light

**Magnification:**
1.1000

**Size:**
7-8.5 μm
Microcytic cell
Macrocytic cell
## Normochromic Erythrocyte

### Description

**Description:**

Normally colored.

### Shape

**Flexible biconcave, discoid shape. Non-nucleated cell, no Golgi’s complex, centriole or lysosomes.**

### Staining method

- May-Grünwald/Giemsa
- Microscope: Light
- Magnification: 1:1000

### Size

- 7 - 8.5 μm
Hypochromic cell
Hyperchromic cell
Diagnosis of anemia

- Mean corpuscular hemoglobin (MCH) – is the average weight of hemoglobin/cell in picograms (pg = 10^-12 g)
  - Hgb (in g/dl)/RBC(x 10^{12}/L) x 10
  - At birth the normal range is 31-37
  - In adults the normal range is 26-34
  - This is not used much anymore because it does not take into account the size of the cell.
- Red cell distribution width (RDW) – is a measurement of the variation in RBC cell size
  - Standard deviation/mean MCV x 100
  - The range for normal values is 11.5-14.5%
  - A value > 14.5 means that there is increased variation in cell size above the normal amount (anisocytosis)
  - A value < 11.5 means that the RBC population is more uniform in size than normal.
Anisocytosis

This term is used to denote variations in cell size. Erythrocytes of various sizes are present in the same peripheral blood smear.
Diagnosis of anemia

- Reticulocyte count gives an indication of the level of the bone marrow activity.
  - Done by staining a peripheral blood smear with new methylene blue to help visualize remaining ribosomes and ER. The number of reticulocytes/1000 RBC is counted and reported as a %.
    - At birth the normal range is 1.8-8%
    - The normal range in an adult (i.e. in an individual with no anemia) is .5-1.5%. **Note that this % is not normal for anemia where the bone marrow should be working harder and throwing out more reticulocytes per day. In anemia the reticulocyte count should be elevated above the normal values.**
Reticulocytes

Description:
The development begins with the extrusion of the normoblast nucleus and ends when the reticulocyte has lost its organelles.

Cytoplasm:
In panoptic stains the cytoplasm is blue-pink. When stained by brilliant cresyl blue, the cytoplasm shows characteristic blue-purple fibers, remnants of the endoplasmatic reticulum.

Nucleus:
Absent.

Nucleoli:
Not visible.

Significance:
By determining the number of reticulocytes in the peripheral blood, the erythropoietic activity of the bone marrow can be estimated.

Size:
7 - 9 μm
Diagnosis of anemia

- The numbers reported above are only relative values. To get a better indication of what is really going on, a corrected reticulocyte count (patients Hct/.45 (a normal Hct) x the reticulocyte count) or an absolute count (% reticulocytes x RBC count) should be done.

- As an anemia gets more severe, younger cells that take longer than 24 hours to mature, are thrown out into the peripheral blood (shift reticulocyte). This may also be corrected for to give the reticulocyte production index (RPI) which is a truer indication of the real bone marrow activity.

- Blood smear examination using a Wright’s or Giemsa stain. The smear should be evaluated for the following:
  - Poikilocytosis – describes a variation in the shape of the RBCs. It is normal to have some variation in shape, but some shapes are characteristic of a hematologic disorder or malignancy.
Poikilocytosis (poikilocytemia) is the presence of poikilocytes in the peripheral blood. Poikilocytosis describes variations in the shape of erythrocytes (rocket, pear, club, comma, etc). Erythrocytes of different shapes are present in the same blood smear. Poikilocytosis is always accompanied by anisocytosis. Poikilocytosis is usually reported as slight, moderate, or marked, depending on the number of abnormal forms seen.
# Spherocytes

## Description:
Small erythrocytes containing an increased concentration of hemoglobin, resulting from a loss of the red cell membrane. Their lifespan in the circulation is extremely short, and osmotic resistance is decreased to hypotonic solution.

## Shape:
Spherical cells with very increased thickness (about 3 \( \mu m \)) and reduced diameter (about 6 \( \mu m \)), normal volume and biconcave shape.

## Significance:
Spheroid cells occur in hereditary spherocytosis and hemolytic anemia.

**Staining method:**
- May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** about 6 \( \mu m \)
Ovalocytes (elliptocytes)

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>VARIATIONS</th>
<th>CONFUSIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Results from a defect in the red cell membrane. The life span in the circulation is shortened, and the osmotic resistance is decreased in most cases. Hemolysis is not as marked as in spherocytosis.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shape:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elliptic shape is apparent after the reticulocyte stage.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Significance:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Found in hereditary elliptocytosis. Can also be found in iron deficiency anemia, sickle diseases, thalassemia, and myelofibrosis.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** 7.85 μm
**Leptocyte**

<table>
<thead>
<tr>
<th>Description</th>
<th>Variations</th>
<th>Confusions</th>
</tr>
</thead>
</table>

**Description:**
Erythrocytes of normal volume and very reduced thickness. In the smears they appear like anulocytes or "target" cells. Anulocyte has a more or less large, pale area in the center due to the lowered hemoglobin content. "Target" cells ("Mexican-hat cell") have ring zones on the periphery as well, but there is an increase of hemoglobin in the central part. An area of pallor between peripheral and central parts is visible.

**Shape:**
Center and the periphery are colored and separated by a clear ring. So, it has the appearance of a target (bull's-eye).

**Significance:**
Found most frequently in iron deficiency - sideropenic anemia, as a result of disturbances in the hemoglobin synthesis. They can be found in hypochromic anemia, homozygous and heterozygous forms of C, D, E hemoglobinopathies. The presence of leptocytes in β-thalassemia major is a pathognomonic.
Acanthocyte

**ACANTHOCYTE (SPUR CELL)**

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>VARIATIONS</th>
<th>CONFUSIONS</th>
</tr>
</thead>
</table>

**Description:**
Erythrocytes that have lost their discoid shape and which have 8-10 spicules of uneven length irregularly distributed over the red cell surface. They have a star-like shape. Lack a central area of pallor.

**Shape:**
Acanthocytes appear smaller than discoid cells because they have spheroidal shape. Characteristically, the spicules are distributed irregularly over the surface, and individual spicules differ from each other. It resembles head burdock.

**Significance:**
They can be found in patients with an inherited decrease or complete deficiency of β-lipoproteins (abetalipoproteinemia) and in patients with some neurological diseases. They can sometimes be found in azotemia, stomach cancer, bleeding stomach ulcer, and vitamin E deficiency.

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** Changeable μm
**Stomatocyte**

<table>
<thead>
<tr>
<th>Description</th>
<th>Variations</th>
<th>Confusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes with a linear central zone, mouth-shaped area of pallor as a result of the disturbance of erythrocyte membrane.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Shape</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>May have a form of fish mouth.</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Significance</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Present in a rare type of congenital hemolytic anemia, when intracellular potassium is decreased and sodium increased (hereditary stomatocytosis), and in alcoholism.</td>
<td></td>
</tr>
</tbody>
</table>

Staining method: May-Grünwald/Giemsa
Microscope: Light
Magnification: 1:1000

Size: 7 - 8,5 μm
**Schistocyte**

**Description:**
Erythrocyte fragment, 2-4 µm in diameter, due to localized membrane damage.

**Shape:**
Fragmented or irregularly contracted cells, varying in size from small triangular cells to cells of near normal size with markedly irregular outlines.

**Significance:**
Found in patients with congenital hemolytic anemia and megaloblastic anemias. Schistocytes are also formed in hemolytic anemias due to mechanical stress (micro-angiopathy, heart valve prosthesis, severe burns), cancer and myelofibrosis.

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** 2 - 4 µm
## Dacrocyte

**DACRYOCYTE (TEAR DROP, TENNIS RACQUET CELL)**

<table>
<thead>
<tr>
<th>DESCRIPTION</th>
<th>VARIATIONS</th>
<th>CONFUSIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dacrocytes or &quot;tear drop&quot; cells are in fact discocytes drawn into a spicule on one end. They can be of normal, reduced or increased size.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shape:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pear shaped with a more or less extended tail that sometimes ends in a swelling.</td>
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<td></td>
</tr>
<tr>
<td><strong>Significance:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>They can be found in patients with myelofibrosis with myeloid metaplasia and metastatic cancer to the bone marrow.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** Changeable \( \mu m \)
Sickle cells (drepanocytes)

Drepanocytosis is the presence of drepanocytes in the circulating blood. These erythrocytes have a sickle shape, i.e., the shape of a half-moon. They are characteristic for cases of hereditary hemolytic anemia-drepanocytosis (sickle-cell anemia). Drepanocytosis is characterized by crises with great pain (vaso-exclusive crises) caused by the obstruction of blood vessels with rigid sickled red cells that contain HbS.
Macroovalocyte

Megalocytosis (macrocythemia) is the occurrence of unusually large numbers of macrocytes. Megalocytes have increased longitudinal diameter (oval), thickness, and volume. They can be observed in vitamin $B_{12}$ and folic acid deficiencies. Also called macroovalocytes.
Target cells
Summary of variations in RBC shape (poikilocytosis)

<table>
<thead>
<tr>
<th>Abnormalities in the Shape of Erythrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPHEROCYTE</td>
</tr>
<tr>
<td>OVALOCYTE (ELLIPTOCYTE)</td>
</tr>
<tr>
<td>LEPTOCYTE (PLATICYTE)</td>
</tr>
<tr>
<td>ACANTHOCYTE</td>
</tr>
<tr>
<td>MEGALOCYTE (MACROOVALOCYTE)</td>
</tr>
<tr>
<td>STOMATOCYTE</td>
</tr>
<tr>
<td>SCHIZOCYTE</td>
</tr>
<tr>
<td>POIKILOCYTE</td>
</tr>
<tr>
<td>DACRYOCYTE</td>
</tr>
<tr>
<td>ROULEAUX FORMATION</td>
</tr>
</tbody>
</table>
Diagnosis of anemia

- Erythrocyte inclusions – the RBCs in the peripheral smear should also be examined for the presence of inclusions:
| **Description** | | | **Variations** | | | **Confusions** |
| --- | --- | --- | --- | --- | --- |
| Basophilic rings or loops, remnants of the nuclear membranes. | | | | | |
| **Shape:** | | | | | |
| Purplish rings, figures-of-eight, incomplete rings or similar configurations of a reddish-violet fine filament appearing in the center or near the periphery of erythrocytes. | | | | | |
| **Significance:** | | | | | |
| Cabot's rings have been described in pernicious anemia, lead poisoning, leukemia, alcoholic jaundice, congenital dyserythropoietic anemia and in some forms of severe anemia. They can be seen in patients taking cytostatics. | | | | | |

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** Changeable μm
## Howell-Jolly bodies

### Description:
Howell-Jolly bodies are nuclear remnants, usually located eccentrically in reticulocytes and erythrocytes. They are generally derived from chromosomes during abnormal divisions. Similar small nuclear fragments may be produced by pathologic fragmentation of the nucleus (karyorrhexis).

### Shape:
Round body inclusions that stain homogenously dark purple by Romanowsky stains.

### Significance:
Very rare in normal people but common after the removal of the spleen, in some hemolytic anemias and in megaloblastic anemia. They may occur in very severe anemias, especially megaloblastic anemia.

### Staining method:
May-Grünwald/Giemsa

### Microscope:
Light

### Magnification:
1:1000

### Size:
1 - 1.5 μm
**Nuclear dust**

## Nuclear Dust in Erythrocytes

### Description:
Fine, small, reddish-blue granules spread in erythrocytes; residues of nuclear material. They must be differentiated from basophilic granules. They occur exclusively in non-nucleated erythrocytes.

### Shape:
Smaller than Howell-Jolly bodies and usually only just visible.

### Significance:
They reflect severe anemias.

---

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** Changeable \( \mu m \)
Basophilic stippling

**Description:**
Erythrocytes with basophilic stippling are cells with bluish-black granular inclusions and usually contain 10-20 blue colored granules. Basophilic punctuations are partially or completely made of RNA. They can be seen in smears stained with a Romanowsky stain. They can be counted after staining with methylazur after Manson-Schwartz method.

**Shape:**
Basophilic punctations in erythrocytes are round or irregularly shaped granules of variable size which are stained blue by Giemsa.

**Significance:**
They are frequent in anemias as a result of lead or other heavy metals poisoning.

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:**
Changeable $\mu$m
Heinz bodies

**Description:**
Heinz bodies are composed of denatured proteins, primarily hemoglobin. They are formed by intra-erythrocytic precipitation of hemoglobin.

**Shape:**
They can be visualized by special staining procedures, e.g., acetyl-phenyl-hydrazine.

**Significance:**
They may be found in the following conditions: anemias caused by G6PD deficiency, phenylhydrazine therapy, sulphanilamide therapy, α-thalassemia.
Heinz bodies (new methylene blue stain)
Siderocytes

**Description:**
Erythrocytes containing one or more iron-containing granules of non-hemoglobin iron, giving a positive Prussian blue reaction. Granules appear as basophilic granules. In the bone marrow smears in healthy people, the number of sideroblasts (normoblasts containing iron granules) is 20-90%. The granule size is about 2 μm.

**Shape:**
Granules appear as basophilic granules.

**Significance:**
In healthy people, siderotic granules are not normally found in peripheral blood erythrocytes. They may be found in the peripheral blood in disorders associated with impaired hemoglobin synthesis, e.g., sideroblastic anemia, thalassemia and lead poisoning. They are also present in blood after splenectomy.

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** Changeable μm
Plasmodium (malarial parasite)

Three species of plasmodia, a parasite, infect human erythrocytes:

- *Plasmodium falciparum*,
- *Plasmodium malariae*, and
- *Plasmodium vivax*. 
Diagnosis of anemia

- A variation in erythrocyte distribution such as rouleaux formation or agglutination

**ROULEAUX FORMATION**

**Description:**
Larger or smaller aggregation of erythrocytes.

**Shape:**
Erythrocytes in freshly taken blood aggregate on each other like stacked coins.

**Significance:**
Present in thick portions of smears, especially in patients with increased erythrocyte sedimentation rate. Can also be found pathologically in multiple myeloma.

Staining method:
May-Grünwald/Giemsa
Microscope: Light
Magnification: 1:1000

Size:
Changeable μm
Agglutination of RBCs
Diagnosis of anemia

- A variation in size should be noted (anisocytosis) and cells should be classified as
  - Normocytic
  - Microcytic
  - Macrocytic
- A variation in hemoglobin concentration (color) should be noted and the cells should be classified as
  - Normochromic
  - Hypochromic
  - Hyperchromic
- Polychromasia (pinkish-blue color due to an increased % of reticulocytes) should be noted
**Normocytic RBC**

<table>
<thead>
<tr>
<th>NORMOCYTE</th>
<th>DESCRIPTION</th>
<th>VARIATIONS</th>
<th>CONFUSIONS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Description:</strong></td>
<td>Normal size and volume, mean cell volume (MCV) 80-100 fl.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Shape:</strong></td>
<td>Flexible biconcave, discoid shape. Non-nucleated cell, no Golgi’s complex, centriole or lysosomes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Significance:</strong></td>
<td>Healthy state.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Staining method:**
May-Grünwald/Giemsa

**Microscope:**
Light

**Magnification:**
1:1000

**Size:**
7 - 8.5 μm
Microcytic RBC

**Description:**
Smaller diameter and volume than normal, round or slightly oval red cells, normal thickness (MCV < 70 fl).

**Shape:**
Flexible biconcave, discoid shape.

**Significance:**
Iron deficiency, thalassemias, sideroblastic anemia.
Macrocytic RBC

**Description:**
Larger volume than normal (MCV >100 fl).

**Shape:**
Discoid shape, a complete mature cell filled with hemoglobin.

**Significance:**
Liver disease, drug induced anemia, Vitamin B₁₂ deficiency, folic acid deficiency.

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:** 9 - 10 μm
Normochromic RBC

**Description:**
Normally colored.

**Shape:**
Flexible biconcave, discoid shape. Non-nucleated cell, no Golgi's complex, centriole or lysosomes.

**Staining method:**
May-Grünwald/Giemsa

**Microscope:** Light

**Magnification:** 1:1000

**Size:**
7 - 8.5 μm
Hypochromic RBC

**Description:**
Increased area of pallor to >1/3 diameter of cell.

**Shape:**
Flexible biconcave, discoid shape. Non-nucleated cell, no Golgi's complex, centriole or lysosomes.
### Hyperchromic RBC

#### Description:
Intensive coloration, "stuck-on" appearance. No central area of pallor.

#### Shape:
Flexible biconcave, discoid shape.

Staining method:
- May-Grünwald/Giemsa

Microscope:
- Light

Magnification:
- 1:1000

Size:
- 7 - 8.5 μm
Erythrocytes stain bluish. This is the result of the persistence of RNA and is a sign of cell immaturity.

Degrees:
- light polychromasia (+);
- polychromasia (++);
- marked polychromasia (+++).
Summary of variations in color and size

<table>
<thead>
<tr>
<th>VARIATIONS IN ERYTHROCYTE COLOR</th>
<th>ABNORMALITIES IN ERYTHROCYTES SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMOCHROMIC ERYTHROCYTE</td>
<td>NORMOCYTE</td>
</tr>
<tr>
<td>HYPOCHROMIC ERYTHROCYTE</td>
<td>MICROCYTE</td>
</tr>
<tr>
<td>HYPERCHROMIC ERYTHROCYTE</td>
<td>MACROCYTE</td>
</tr>
</tbody>
</table>
Diagnosis of anemia

- The peripheral smear should also be examined for abnormalities in leukocytes or platlets.
  - Some nutritional deficiencies, stem cell disorders, and bone marrow abnormalities will also effect production, function, and/or morphology of platlets and/or granulocytes.
  - Finding abnormalities in the leukocytes and/or platlets may provide clues as to the cause of the anemia.

- The lab investigation may also include:
  - A bone marrow smear and biopsy
    - Used when other tests are not conclusive
Diagnosis of anemia

- In a bone marrow sample, the following things should be noted:
  - Maturation of RBC and WBC series
  - Ratio of myeloid to erythroid series
  - Abundance of iron stores (ringed sideroblasts)
  - Presence or absence of granulomas or tumor cells
  - Red to yellow ratio
  - Presence of megakaryocytes

- Hemoglobin electrophoresis – can be used to identify the presence of an abnormal hemoglobin (called hemoglobinopathies). Different hgb's will move to different regions of the gel and the type of hemoglobin may be identified by its position on the gel after electrophoresis.
Hemoglobin electrophoresis

8. $\text{AA}_2\text{S, D or G}$
7. $\text{AA}_2\text{S, D or G}$
6. $\text{AA}_2$
5. $\text{AS, D or G}$
4. AFSC Control
3. AFSA$_2$ Control
2. Homozygous S, D or G
1. $\text{AA}_2\text{S, D or G}$

A F S A$_2$ E
D C

Application Point
Carbonic Anhydrase
Diagnosis of anemia

- Antiglobulin testing – tests for the presence of antibody or complement on the surface of the RBC and can be used to support a diagnosis of an autoimmune hemolytic anemia.

- Osmotic fragility test – measures the RBC sensitivity to a hypotonic solution of saline. Saline concentrations of 0 to .9% are incubated with RBCs at room temperature and the percent of hemolysis is measured. Patients with spherocytes (missing some membrane) have increased osmotic fragility. They have a limited ability to take up water in a hypotonic solution and will, therefore, lyse at a higher sodium concentration than will normal RBCs.
Osmotic fragility test
Normal osmotic fragility curve

**FIGURE 40-4** Normal osmotic fragility curve. The osmotic fragility curve of a normal individual would fall within the area defined by the two sigmoid curves. A curve to the left of normal indicates increased fragility, and a curve to the right decreased fragility.
Diagnosis of anemia

- Sucrose hemolysis test – sucrose provides a low ionic strength that permits binding of complement to RBCs. In paroxysmal nocturnal hemoglobinuria (PNH), the RBCs are abnormally sensitive to this complement mediated hemolysis. This is used in screening for PNH.

- Acidified serum test (Ham’s test) – is the definitive **diagnostic test for PNH**. In acidified serum, complement is activated by the alternate pathway, binds to RBCs, and lyases the abnormal RBCs found in PNH.
Acidified serum test

**TABLE 40-1**

Schematic Outline of Acidified Serum Test Procedure and Expected Results in PNH

<table>
<thead>
<tr>
<th>Tube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient serum (mL)</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Control serum (mL)</td>
<td></td>
<td></td>
<td>0.5*</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 N HCl (mL)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient RBC (mL)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control RBC (mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Results observed</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in PNH</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

PNH is suggested if hemolysis occurs in tubes #2 and #5. No hemolysis should be seen in tube #3. Key: * = Serum is heat inactivated; 0 = no hemolysis; + = hemolysis.
Diagnosis of anemia

- Evaluation of RBC enzymes and metabolic pathways – enzyme deficiencies in carbohydrate metabolic pathways are usually associated with a hemolytic anemia.
- Evaluation of erythropoietin levels – is used to determine if a proper bone marrow response is occurring.
  - Low levels of RBCs could be due to a bone marrow problem or to a lack of erythropoietin production.
- Serum iron, iron binding capacity and % saturation – used to diagnose iron deficiency anemias (more on this later)
- Bone marrow cultures – used to determine the viability of stem cells.
Anemias may be classified morphologically based on the average size of the cells and the hemoglobin concentration into:

- Macrocytic
- Normochromic, normocytic
- Hypochromic, microcytic
Morphological classification of anemias

![Diagram showing the morphological classification of anemias]

- **Macrocytic**
  - RPI > 2
    - Survival Defect
      - hemolysis
      - hemorrhage
    - Nuclear Maturation Defect (megablastic)
      - $B_12$ deficiency
      - folate deficiency
      - drug induced
      - congenital
      - myelodysplasia
  - RPI < 2
    - Non-Megaloblastic
      - liver disease
      - alcoholism
      - endocrinopathy
      - aplasia

- **Normocytic**
  - RPI > 2
    - Survival Defect
      - hemolysis
      - hemorrhage
  - RPI < 2
    - Proliferation Defect
      - marrow damage or replacement
      - stem cell defects
      - renal disease
      - endocrinopathies
      - chronic disease
      - liver disease

- **Microcytic**
  - Hypochromic
    - Serum iron normal
    - decreased
    - Cytoplasmic Maturation Defect
      - iron deficiency
      - chronic disease
    - Hemoglobinopathies
    - sideroblastic anemia
    - lead intoxication
    - porphyrias

*with marked reticulocytosis*
Macrocytic anemias

**MACROCYTIC ANEMIA**

Reticulocyte count

- **RPI > 2**
  - Smear for fragments/microspherocytes
    - Absent
      - Hemorrhage
      - Intravascular
        - Hemoglobin
        - Hemoglobinuria
        - Hemoglobinuria
    - Present
      - Hemolysis
      - Extravascular
        - Indirect bilirubin
        - Serum urobiilinogen

- **RPI < 2**
  - Serum B12 and folate
    - Decreased
      - Vitamin B12 or Folic acid deficiency
    - Normal/high
      - WBC and Platelet counts
        - Decreased
          - Bone marrow
            - Aplastic anemia
            - Myelodysplasia
        - Normal/slightly decreased
          - Drug inhibition
          - Liver function studies
            - Abnormal
              - Liver disease
              - Alcoholism
            - Normal
              - Endocrine studies
Normochromic, normocytic anemias

NORMOCYTIC NORMOCHROMIC ANEMIA

Reticulocyte count

RPI > 2

Blood Smear for schistocytes/microspherocytes

- Absent
  - Hemorrhage

- Present
  - Hemoysis
    - Intravascular
      - haptoglobin
      - hemoglobinemia
      - hemosidinurea
      - hemoglobinurea
    - Extravascular
      - indirect bilirubin
      - urine urobilinogen

RPI < 2

WBC and platelet count

- Decreased
  - Bone marrow
    - Marrow damage
    - Marrow replacement
    - Stem cell defect

- Normal
  - Serum iron/TIBC
    - Decreased
      - Chronic Disease
    - Normal
      - Renal Function Studies
        - Abnormal
          - Renal disease
        - Normal
          - Pure red cell aplasia
Hypochromic, microcytic anemias
Classification of anemias

- Anemias may also be classified functionally into:
  - Hypoproliferative (when there is a proliferation defect)
  - Ineffective (when there is a maturation defect)
  - Hemolytic (when there is a survival defect)
Functional classification of anemias