Basic Hematology overview

Julia Fonseca BS, MT(ASCP), SCYM(ASCP), SH(ASCP)
Fonseca.julia56@gmail.com
Objectives

- Provide an overview of the clinical features of the most common hematologic disorders.
- Review of Erythrocytes
  - Rbc count and morphology, RBC indices
  - Haemoglobin, haematocrit
  - Retic count and ESR
- Diagnosis of anemias
- Review WBC
  - WBC count, morphology and differentials
  - Acute leukemias and cytochemical stains
  - Chronic leukemias
  - Reactive disorders of granulocytes, monocytes and lymphocytes
- Review haemostasis: PTT, APTT, Fibrinogen, FDP, D-dimer
- Review of haemoglobinopathies
- Review of fluids
Hematology

- Study of blood and blood forming tissues including the diagnosis, treatment, and prevention of diseases of the blood, bone marrow, and immunologic, hemostatic, and vascular systems

- Key components of hematologic system are:
  - Blood
  - Blood forming tissues
    - Bone marrow
    - Spleen
    - Lymph system
Blood components and function

- Delivery of nutrients
- Oxygen
- Food
- Vitamins
- Removal of wastes
- Carbon dioxide
- Nitrogenous wastes
- Cellular toxins
- Repair of its conduit
- Protection versus invading microorganisms
- Multiple cellular & acellular elements
Components

- Plasma 55%
- Blood Cells 45%
  - Erythrocytes/RBCs
  - Leukocytes/WBCs
  - Thrombocytes/Platelets
- Red Blood Cells (Oxygen & CO2 transport)
- Coagulation/platelets (Maintenance of vascular integrity)
- White Blood Cells (Protection versus pathogens)
Hematopoiesis

- In humans, occurs in bone marrow exclusively
- All cellular elements derived from pluripotent stem cell (PPSC)
- PPSC retains ability to both replicate itself and differentiate
- Types of differentiation determined by the influence of various cytokines
- **Cytokines** are a group of proteins secreted by cells of the immune system that act as chemical messengers. Cytokines released from one cell affect the actions of other cells by binding to receptors on their surface. Through this process, cytokines help regulate the immune response.
# Adult Reference Ranges for Red Blood Cells

<table>
<thead>
<tr>
<th>Measurement (units)</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (gm/dL)</td>
<td>13.6–17.2</td>
<td>12.0–15.0</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>39–49</td>
<td>33–43</td>
</tr>
<tr>
<td>Red cell count (10⁶/µL)</td>
<td>4.3–5.9</td>
<td>3.5–5.0</td>
</tr>
<tr>
<td>Reticulocyte count (%)</td>
<td></td>
<td>0.5–1.5</td>
</tr>
<tr>
<td>Mean cell volume (µm³)</td>
<td>82–96</td>
<td></td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (pg)</td>
<td>27–33</td>
<td></td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (gm/dL)</td>
<td>33–37</td>
<td></td>
</tr>
<tr>
<td>RBC distribution width</td>
<td>11.5–14.5</td>
<td></td>
</tr>
</tbody>
</table>
RED BLOOD CELLS

Normal - Anucleate, highly flexible biconcave discs, 80-100 femtoliters in volume

Flexibility essential for passage through capillaries

Major roles - Carriers of oxygen to & carbon dioxide away from cells
ERYTHROPOIETIN

- Cytokine - Produced in the kidney
- Necessary for erythroid proliferation and differentiation
- Absence results in apoptosis of erythroid committed cells
- Anemia of renal failure 2° to lack of EPO
- Nuclear signal sent to activate production of proteins leading to proliferation and differentiation
- Signal also sent to block apoptosis
Retic (% Reticulocytes) are immature red blood cells.

A reticulocyte count is a test used to measure the level of reticulocytes in your blood.

More accurate way to assess body’s response to anemia.

Normal result for healthy adults who are not anemic is around 0.5% (0.005 × 10^-3) to 2.5% (0.025 × 10^-3).
Because the reticulocyte count is expressed as a percentage of total RBCs, it must be corrected according to the extent of anemia with the following formula:

Count a minimum of 1000 erythrocytes.

\[
\frac{\text{# of retics}}{1000 \times 100} = \text{uncorrected retics} \%
\]

**absolutely retics** = # or uncorrected retics \( \times \) RBC count

**Corrected retics** = retics\% \( \times \) hct patient/ hct mean normal

**RPI** = corrected retics\%/ maturation time: >3 compensated bone marrow
RBC Assessment

- **Number** - Generally done by automated counters, using impedance measures

- **Size** - Large, normal size, or small; all same size versus variable sizes (anisocytosis). (Microcyte, Macrocyte, Normocyte)

- **Shape** - Normal biconcave disc, versus spherocytes, versus oddly shaped cells (poikilocytosis)

- **Polychromasia** is a lavender-bluish color to RBC's due to RNA retained in larger, immature cells (macrocytes). They are associated with:
  - acute and chronic hemorrhage
  - hemolysis
  - neonates
  - treatment for anemia
- **Inclusion bodies** are nuclear or cytoplasmic aggregates of stainable substance, usually proteins.

- The inclusion bodies in red blood cells are almost always indicative of some sort of pathology, and thus it is useful to understand each inclusion body that can occur within a red blood cell.

- **Dimorphic** is a term used to describe two circulating red cell populations and the RDW will be >14%. These are associated with:
  - transfusion
  - myelodysplastic syndrome
  - vitamin B12, folate or iron deficiency
<table>
<thead>
<tr>
<th>Common RBC Inclusions</th>
<th>Cartoon Image</th>
<th>Inclusion</th>
<th>May be associated with</th>
</tr>
</thead>
<tbody>
<tr>
<td>Howell Jolly Bodies</td>
<td>DNA</td>
<td></td>
<td>Hyposplenism, Asplenism, Severe hemolytic anemia</td>
</tr>
<tr>
<td>Heinz Bodies</td>
<td>Hemoglobin</td>
<td></td>
<td>G6PD deficiency, Oxidant drugs, Unstable hemoglobin</td>
</tr>
<tr>
<td>Pappenheimer Bodies</td>
<td>Iron deposits</td>
<td></td>
<td>Thalassemia, Sideroblastic anemia, Hemolytic anemia, Post-splenectomy</td>
</tr>
<tr>
<td>Hemoglobin H Inclusion</td>
<td>Hemoglobin</td>
<td></td>
<td>Hemoglobin H disease</td>
</tr>
<tr>
<td>Basophilic Stippling</td>
<td>Ribosomes</td>
<td></td>
<td>Lead poisoning, Thalassemia, Sickle cell anemia, MDS</td>
</tr>
</tbody>
</table>

Medical Laboratory Technologist – our proud profession
<table>
<thead>
<tr>
<th>Size variation</th>
<th>Hemoglobin distribution</th>
<th>Shape variation</th>
<th>Inclusions</th>
<th>Red cell distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Hypochromia 1+</td>
<td>Target cell</td>
<td>Pappenheimer bodies (siderotic granules)</td>
<td>Agglutination</td>
</tr>
<tr>
<td>Microcyte</td>
<td>Hypochromia 2+</td>
<td>Spherocyte</td>
<td>Cabot’s ring</td>
<td></td>
</tr>
<tr>
<td>Macrocyte</td>
<td>Hypochromia 3+</td>
<td>Helmet cell</td>
<td>Basophilic stippling (coarse)</td>
<td>Rouleaux</td>
</tr>
<tr>
<td>Oval macrocyte</td>
<td>Hypochromia 4+</td>
<td>Ovalocyte</td>
<td>Howell-Jolly</td>
<td></td>
</tr>
<tr>
<td>Hypochromic macrocyte</td>
<td>Polychromasia (Reticiuloocyte)</td>
<td>Stomatocyte</td>
<td>Howell-Jolly</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tear drop</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Crystal formation

- HbSC
- HbC
Observing and Recording Nucleated Red Blood Cells (nRBCs)

- If 10 or more nucleated RBC's (NRBC) are seen, correct the White Count using this formula:

  \[
  \text{Corrected WBC count (mm}^3\text{)} = \frac{\text{uncorrected WBC count} \times 100}{\text{number of nRBC’s per 100 WBC’s} + 100}
  \]

**Example:** If WBC = 5000 and 10 NRBCs have been counted

Then \(5,000 \times \frac{100}{110} = 4545.50\)

The corrected white count is 4545.50.
Red blood cell (RBC) indices are part of the complete blood count (CBC) test. They are used to help diagnose the cause of anemia. Mean corpuscular volume (MCV) is the average volume of a red blood cell and is calculated by dividing the hematocrit (Hct) by the concentration of red blood cell count. A normal range for MCV is between 80 and 96 femtoliters per cell. A low MCV indicates that the red blood cells are small, or microcytic. Hemoglobin is the protein in your red blood cells that transports oxygen to the tissues of your body. MCH value is related to two other values, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). Together, MCH, MCV, and MCHC are sometimes referred to as red blood cell indices.
- High RDW and low MCV. This suggests iron deficiency or microcytic anemia.

- High RDW and high MCV indicates a lack of B-12 or folate. It can also suggest macrocytic anemia or chronic liver disease.

- High MCH scores are commonly a sign of macrocytic anemia. This condition occurs when the blood cells are too big, which can be a result of not having enough vitamin B12 or folic acid in the body.

- High MCH scores may also be the result of liver diseases.

- A low mean corpuscular hemoglobin concentration (MCHC) shows that someone's red blood cells do not have enough hemoglobin.
Given the following values, which set of red blood cell indices suggests spherocytosis?

- a) MCV 76 um $^3$ MCH 19.9pg MCHC 28.5%
- b) MCV 90 um $^3$ MCH 30.5pg MCHC 32.5%
- c) MCV 80 um $^3$ MCH 36.5pg MCHC 39.0%
- d) MCV 81 um $^3$ MCH 29.0pg MCHC 34.8%

The correct answer is C: Spherocytes have a decreased cell diameter and volume, which results in loss of central pallor and discoid shape. The index most affected is the MCHC, usually being in excess of 36%.
SIZE REALLY DOES MATTER!

For Red Blood Cells

- Normal Red Blood Cell: 77-95 fl
- Small Red Blood Cell = Microcytosis (less than 75 fl)
- Large Red Blood Cell = Macrocytosis (greater than 95 fl)

Red Blood Cell Measurements Are Reported on the MCV. It’s a Part of Most Routine Blood Tests!
RBC indices

- **MCV**: mean corpuscular volume
  - Hct (%) x 10 / RBC ct (x10^6/μl)

- **MCH**: mean corpuscular hemoglobin
  - Hgb (g/dL) x 10 / RBC ct (x10^6/μl)

- **MCHC**: mean corpuscular Hgb conc.
  - Hgb (g/dL) x 100 / Hct (%)

- **RDW**: rbc distribution width
## Affect of Hemolysis on CBC Parameters

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>AFFECT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>Probably unaffected</td>
</tr>
<tr>
<td>RBC</td>
<td>Falsely low due to RBC lysis and/or RBC fragments not being counted as RBCs</td>
</tr>
<tr>
<td>HGB</td>
<td>Reportable-accurate measurement relies on RBCs being completely lysed</td>
</tr>
<tr>
<td>HCT</td>
<td>Falsely low-calculated with an invalid MCV and falsely low RBC</td>
</tr>
<tr>
<td>MCV</td>
<td>Invalid, falsely low or high depending on the degree of hemolysis. May be falsely low if RBC fragments cause smaller pulses to be produced in the RBC aperture or falsely high if fragmented RBCs fall below the RBC threshold and are not counted.</td>
</tr>
<tr>
<td>MCH</td>
<td>Invalid-calculated with a falsely low RBC</td>
</tr>
<tr>
<td>MCHC</td>
<td>Invalid-calculated with a falsely low HCT</td>
</tr>
<tr>
<td>RDW</td>
<td>Falsely high due to RBC fragments increasing the CV of the RBC histogram</td>
</tr>
<tr>
<td>PLT</td>
<td>Falsely high due to RBC fragments being incorrectly counted as platelets</td>
</tr>
</tbody>
</table>
# MCV Parameter

## Interfering substances and Implications

<table>
<thead>
<tr>
<th>Test</th>
<th>Name</th>
<th>Interfering Agent</th>
<th>Clinical Implications</th>
</tr>
</thead>
</table>
| MCV        | Mean Corpuscular (Cell) Volume | • Very high WBC count  
• High concentration of very large platelets  
• Agglutinated RBCs  
• RBC fragments that fall below the 36 fl threshold  
• Rigid RBCs | **LOW:** <80 fl  
• Iron deficiency anemia  
• Thalassemia  

**HIGH:** >100 fl  
• B12  
• Folate Deficiency |
# HGB Parameter

**Interfering substances and Implications**

<table>
<thead>
<tr>
<th>Test</th>
<th>Name</th>
<th>Interfering Agent</th>
<th>Clinical Implications</th>
</tr>
</thead>
</table>
| HGB Or Hb | Hemoglobin   | • Very high WBC count  
• Severe lipemia  
• Heparin  
• Certain unusual RBC abnormalities that resist lysing  
• Anything that increases the turbidity of the sample such as elevated levels of triglycerides  
• High bilirubin | **LOW:**  
Male: <14 g/dL  
Female: <12.0 g/dL  
• Anemia  
**HIGH:**  
Male: > 17.4 g/dL  
Female: >16.0 g/dL  
• Polycythemia vera  
• fluid loss due to diarrhea, dehydration, burns |
# CBC

## Adult Reference Ranges

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adult Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>4.5-11.0 ( \times 10^3 ) ( /\mu L )</td>
</tr>
</tbody>
</table>
| RBC       | Male: 4.5-5.5 \( \times 10^6 \) \( /\mu L \)  
Female: 4.0-5.0 \( \times 10^6 \) \( /\mu L \) |
| HGB       | Male: 14-17.4 g/dL  
Female: 12.0-16.0 g/dL |
| HCT       | Male: 42-52%  
Female: 36-46% |
| MCV       | 80-100 fl |
| MCH       | 28-34 pg |
| MCHC      | 32-36 g/dL or % |
| RDW       | 12.0-14.6% |
| PLT       | 150-450 \( \times 10^3 \) \( /\mu L \) |
| MPV       | 6.8-10.2 fl |
Manual Cell Counting With Neubauer Chamber

- The ruled area is 3mm\(^2\) divided into **9 large squares** each with a 1 mm\(^2\) area. The large central square (which can be seen in its entirety with the 10X objective), is divided into **25 medium squares** with double or triple lines. Each of these 25 squares are is again divided into **16 small squares** with single lines, so that each of the smallest squares has an area of 1/400 mm\(^2\).

- The glass cover is a squared glass of width 22 mm. The glass cover is placed on the top of the Neubauer chamber, covering the central area. The ruled area is 0.1 mm lower than the rest of the chamber. So that when a cover slip is kept on the counting region, there is a gap of 0.1 mm (1/10mm) between the cover slip and the ruled area.
- **WBC Counting Area**
  The four large squares placed at the corners are used for white blood cell count. Since their concentration is lower than red blood cells a larger area is required to perform the cell count.

- **RBC Counting Area**
  The large center square is used for RBC counts. This area is subdivided into 25 medium squares, which in turn are each divided into 16 squares. Of the 25 medium squares, only the four corner squares and the center square within the large center square are used to perform RBC counts.

- **Platelet Counting Area**
  The large center square is used to count platelets. Platelets in all 25 squares within the large center square are counted.
The dilution factor used in the formula is determined by the blood dilution used in the cell count. The depth used in the formula is always 0.1. The area counted will vary for each type of cell count and is calculated using the dimensions of the ruled area.

Particles per µl volume = \( \frac{\text{Counted particles}}{\text{Counted surface (mm}^2\text{)} \cdot \text{Chamber depth (mm)} \cdot \text{Dilution}} \)
Example:

- calculate total WBC count by using Neubauer counting chamber.

  Number of cells counted = \( N = 150 \) (suppose)
  
  Area Counted = \( 1 \text{ mm}^2 \times 4 = 4 \text{ mm}^2 \) (area of four large corner squares)
  
  Depth = \( \frac{1}{10} \text{ mm} \)
  
  Dilution = \( 1:20 \)
  
  Hence WBC/Cubic mm of Whole Blood = \( N \times 50 = 150 \times 50 = 7,500 \)
Osmotic fragility test

- In the osmotic fragility test, whole blood is added to varying concentrations of NaCl and allowed to incubate at room temperature.
- The amount of hemolysis is then determined by examining the supernatant fluid either visually or with a spectrophotometer.
- If erythrocytes are placed in an isotonic solution (0.85%) of NaCl, water molecules will pass in and out of the membrane in equal amounts.
- In hypotonic solutions, erythrocytes will hemolyze because more water molecules enter into the cell than leave. This net influx of water molecules eventually ruptures the cell membrane.
- The main factor in this procedure is the shape of the erythrocyte, which is dependent on the volume, surface area and functional state of the erythrocyte membrane.
- A spherocyte erythrocyte ruptures much more quickly than normal erythrocytes, such as sickle cells and target cells.
- The clinical value of the procedure is in differentiating various types of anemia.
Osmotic fragility test
Decreased fragility:
- HbC disease
- Iron deficiency anemia
- Sickle cell anemia
- Thalassemia major
- Polycythemia Vera
- Hereditary spherocytosis

Increased fragility:
- Acquired autoimmune hemolytic anemia
- Burns
- Chemical poisons
- Hemolytic disease of the newborn
Sedimentation Rate

- Sed rate, or erythrocyte sedimentation rate (ESR), is a blood test that can reveal inflammatory activity in your body.
- A sed rate test isn't a stand-alone diagnostic tool, but it can help your doctor diagnose or monitor the progress of an inflammatory disease.
- The sed rate test measures the distance red blood cells fall in a test tube in one hour.
- The farther the red blood cells have descended, the greater the inflammatory response of your immune system.
- Sed rate tests might be useful when evaluating unexplained fever, some types of arthritis and symptoms that affect your muscles. Also, they can help confirm a diagnosis of certain conditions, including:
  - Giant cell arteritis
  - Polymyalgia rheumatica
  - Rheumatoid arthritis
The normal range is 0-22 mm/hr for men and 0-29 mm/hr for women.
ERYTHROCYTE SEDIMENTATION RATE (ESR)

- Specific weight of the RBC is higher than that of the plasma → in a stabilized blood, RBC slowly sink towards the bottom of the test tube - sedimentation

- Factors increasing ESR
  - ↓ Htc, ↓ blood viscosity
  - ↑ concentration of fibrinogen (i.e., pregnancy, vascular diseases, heart diseases), haptoglobin, lipoproteins, immunoglobulins
  - Macrocytic RBC
  - Extreme elevation of WBC count (leukemia)

- Factors decreasing ESR
  - ↑ Htc
  - Change in the RBC shape (i.e., sickle-cell anemia, poikilocytosis – nonuniformity of shape)
  - ↑ albumin concentration

Males – 3-6 mm/h
Females – 8-10 mm/h
Factors affecting ESR

**Physiological factors**
- Plasma factors
- Red cell factors
- Rouleaux formation
- Age
- Sex
- Pregnancy

**Laboratory factors**
- Temperature
- Time
- Anticoagulants
- Tube factor
- Tilting of tube
- Vibration
- Sunlight
ANEMIA

- Anemia is a condition with not enough red blood cells to carry adequate oxygen to the body's tissues. Having anemia may make you feel tired and weak.

- Causes:
  - Your body doesn't make enough red blood cells
  - Bleeding causes you to lose red blood cells more quickly than they can be replaced
  - Your body destroys red blood cells
- Blood loss

- Decreased production of red blood cells (Marrow failure)

- Hemolysis

- Distinguished by reticulocyte count

- Decreased in states of decreased production

- Increased in destruction of red blood cells
Pathogenic classification (Causes of anemia)

- Decreased RBC production
  - Decreased Hb production
  - Defective DNA synthesis
  - Stem cell defects
    - Pluripotent stem cell
    - Erythroid stem cell (progenitors)
  - Other less defined reasons

- Blood loss
  - Anemia due to acute bleeding

- Increased RBC destruction

- Relative (increased plasma volume)
RBC DESTRUCTION - EXTRAVASCULAR

- Heme metabolized to bilirubin in macrophage; globin metabolized intracellularly

- Unconjugated bilirubin excreted into plasma & carried to liver

- Bilirubin conjugated in liver & excreted into bile & then into upper GI tract

- Conjugated bilirubin passes to lower GI tract & metabolized to urobilinogen, which is excreted into stool & urine
RBC DESTRUCTION - INTRAVASCULAR

- Free Hemoglobin in circulation binds to haptoglobin, yielding low plasma haptoglobin

- Hemoglobin filtered by kidney and reabsorbed by tubules, leading to hemosideinuria

- Capacity of tubules to reabsorb protein exceeded, yielding hemoglobinuria
Classification of anemia

Decreased production vs. RBC loss (increased destruction or bleeding)

RBC Size: (Macrocytic vs. microcytic vs. normocytic)

Hemoglobin Content: (Hypochromic vs. normochromic)

Shape: Normal or abnormal
Laboratory tests needed for anemia diagnosis

- MCV, MCH, MCHC
- RDW
- Rbc count
- Hemoglobin
- Reticulocyte count
- Occult blood
- Direct antibody test
- Peripheral Smear
- Transferrin
- Fe & TIBC
- Ferritin
- Vitamin B12 & Folate
- Hemoglobin electrophoresis (Hgb A2 levels)
- Bone marrow aspirate
Classification of anemia based on red blood cell size

Anemia Differential Diagnosis

- **Microcytic** (MCV <80 fL)
  - Iron deficiency
  - Anemia of chronic disease
  - Thalassemias
  - Sideroblastic anemia
  - Lead poisoning

- **Normocytic** (MCV 80 - 100 fL)
  - **Hemolytic**
    1) Intrinsic
      - Hereditary spherocytosis
      - Paroxysmal nocturnal hemoglobinuria
      - G6PD deficiency
      - Pyruvate kinase deficiency
      - Sickle cell anemia
      - HbC disease
    2) Extrinsic
      - Microangiopathic hemolytic anemia
      - Macroangiopathic hemolytic anemia
      - Autoimmune hemolytic anemia
  - **Non-Hemolytic**
    - Iron deficiency
    - Anemia of chronic disease
    - Chronic kidney disease
    - Aplastic anemia

- **Macrocytic** (MCV >100 fL)
  - **Megaloblastic**
    - Folate (vitamin B9) deficiency
    - Cobalamin (vitamin B12) deficiency
    - Copper deficiency
    - Citric aciduria
    - Drug-induced (e.g., allopurinol)
  - **Non-Megaloblastic**
    - Alcohol-use disorder
    - Liver disease
    - Diamond-Blackfan anemia
CLASSIFICATION OF ANEMIAS

ANEMIA
- Blood Loss
  - Acute
  - Chronic
- Impaired Production (Hypoproliferative)
  - Iron Deficiency
    - Megaloblastic (nuclear-cytoplasmic dysynchrony)
      - Vitamin B12 or follic acid
  - Anemia of Chronic Disease
  - Myelophthisic (infiltrative)
  - Aplastic Anemia: congenital or acquired
- Increase Destruction (Hemolytic)
  - Extrinsic to red blood cell:
    - Auto-immune or iso-immune
    - Infections
    - Physical or chemical agents
  - Intrinsic to red blood cell:
    - Membrane defects
    - Enzyme deficiencies (metabolic)
    - Hemoglobinopathies: chain synthesis defects, amino acid substitutions
<table>
<thead>
<tr>
<th>Microcytic Hypochromic Anemia</th>
<th>Macrocytic Normochromic Anemia</th>
<th>Microcytic hyperchromic Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Small RBCs" /> MCHC: &lt;32 g/dL</td>
<td><img src="image" alt="Big RBCs" /> MCHC: 32 – 36 g/dL</td>
<td><img src="image" alt="Small RBCs" /> MCHC: &gt;36 g/dL</td>
</tr>
<tr>
<td>Small sized RBCs with a large central pallor with concentration of hemoglobin decreased → Hence reduced MCHC</td>
<td>Big sized RBCs with a normal central pallor but concentration of hemoglobin remains → Hence MCHC is normal</td>
<td>Small sized RBCs with abnormal/without central pallor → increased hemoglobin concentration → hence increased MCHC</td>
</tr>
</tbody>
</table>

\[
\frac{\text{Hemoglobin}}{\text{Hematocrit}} = \text{MCH}
\]
Classification of Anemias (Morphologic)

- **Microcytic anemias**
  - Iron deficiency
  - Thalassemia
  - Sideroblastic anemia
  - Anemia of chronic diseases (severe cases)

- **Normocytic anemias**
  - Anemia of chronic diseases (most cases)
  - Iron deficiency (early)
  - Anemia of renal disease
  - Combined nutritional deficiencies (iron + folate or cobalamine)
  - Marrow failure
  - Hypothyroidism

- **Macrocytic anemias**
  - Megaloblastic anemia (folate or cobalamine deficiency)
  - Hemolytic anemia (reticulocytosis)
  - Liver disease
  - Hypothyroidism
  - Myelodysplasia
HEMOLYTIC ANEMIA

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulocyte Count</td>
<td>Increased</td>
</tr>
<tr>
<td>Unconjugated Bilirubin</td>
<td>Increased</td>
</tr>
<tr>
<td>Lactate Dehydrogenase</td>
<td>Increased</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>Decreased</td>
</tr>
<tr>
<td>Urine Hemoglobin</td>
<td>Present</td>
</tr>
<tr>
<td>Urine Hemosiderin</td>
<td>Present</td>
</tr>
</tbody>
</table>
Hemoglobinopathies/Thalassemia

- **Hemoglobinopathy** is a group of rare, inherited disorders involving inherited mutation of the globin genes leading to a qualitative or quantitative abnormality of globin synthesis.
## NORMAL HUMAN HAEMOGLOBINS

<table>
<thead>
<tr>
<th></th>
<th>Haemoglobin</th>
<th>Structural formula</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adult</strong></td>
<td>Hb-A</td>
<td>$\alpha_2 \beta_2$</td>
</tr>
<tr>
<td></td>
<td>Hb-A$_2$</td>
<td>$\alpha_2 \delta_2$</td>
</tr>
<tr>
<td><strong>Fetal</strong></td>
<td>Hb-F</td>
<td>$\alpha_2 \gamma_2$</td>
</tr>
<tr>
<td><strong>Embryonic</strong></td>
<td>Hb-Gower 1</td>
<td>$\zeta_2 \varepsilon_2$</td>
</tr>
<tr>
<td></td>
<td>Hb-Gower 2</td>
<td>$\alpha_2 \varepsilon_2$</td>
</tr>
<tr>
<td></td>
<td>Hb-Portland</td>
<td>$\zeta_2 \gamma_2$</td>
</tr>
</tbody>
</table>
δβ thal and HPFH carriers have normal HbA2 (<3.5%) and high HbF (10-25%) with variable RBC indices.
<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sickle cell syndromes</td>
<td>African, Greeks, Southern Italians, Turks, Arabs, Indians</td>
</tr>
<tr>
<td>Alpha thalassemia</td>
<td>Chinese, Taiwanese, South-East Asian, Cypriot, Greek, Turkish, Sardinian</td>
</tr>
<tr>
<td>Beta thalassemia</td>
<td>All ethnic groups except Northern Europeans</td>
</tr>
</tbody>
</table>
- **Structural hemoglobinopathy (qualitative)**
  - Amino acid substitution in the globin chain
  - 471 variants

- **The Thalassemias (quantitative)**
  - Syndromes in which the rate of synthesis of a globin chain is reduced.
    - Beta thalassemia reduced beta chain synthesis
    - Alpha thalassemia reduced alpha chain synthesis
CBC (complete blood count)
- the number, size and shape of the red blood cells present.
- how much hemoglobin is in RBC
- MCV (mean corpuscular volume) is a measurement of the size of the red blood cells.
- A low MCV is often the first indication of thalassemia.
- If the MCV is low and iron deficiency has been ruled out, the person may be a thalassemia trait carrier or have one of the hemoglobin variants that cause microcytosis
Blood smear of hemoglobinopathy

- Microcytic
- Hypochromic
- Varying in size (anisocytosis) and shape (poikilocytosis)
- Have uneven hemoglobin distribution (producing “target cells”)

**Detection of hemoglobin variants**

- These tests identify the type and measure the relative amount of hemoglobins.
  - hemoglobin electrophoresis
    - isoelectric focusing,
    - or high-performance liquid chromatography.
- These techniques separate different hemoglobins based on their charge.
- The relative amounts of any variant hemoglobin detected can help to diagnose combinations of hemoglobin variants and thalassemia.
DNA analysis

- This test is used to investigate deletions and mutations in the alpha and beta globin producing genes.
  Family studies can be done to evaluate carrier status and the types of mutations present in other family members.
  DNA testing is not routinely done but can be used to help diagnose hemoglobin variants, thalassemia, and to determine carrier status.

https://medquizzes.net/mcqs-hemoglobinopathies-and-thalassemias-quizzes/
Hemoglobin electrophoresis

Cellulose acetate strip (pH 8.0)
- Normal
- Sickle trait
- S.E. Disease
- C_{Harlem} trait
- Control

Citrate Agar Gel (pH 6.0-6.2)
- Normal
- Sickle trait
- S.E. Disease
- C_{Harlem} trait
- Control
**Hemoglobin E**
- 2nd most prevalent hemoglobin variant – 30,000,000 worldwide
- 80% in Southeast Asia
- Hb E trait: microcytosis (mean MCV=65fl).
- No anemia
- Hb E disease: MCV =55-65fl with minimal anemia
- On HPLC has similar migration pattern as Hb A2 H

**Hemoglobin C**
- Mutation in β-globin gene β(6glu->lys)
- Seen predominantly in blacks: Gene prevalence in US black population is 2 to 3%
- May confer malaria resistance
- Often asymptomatic, mild anemia, splenomegaly
- Blood smear shows many target cells, rare intracellular crystals
- Hb S/C disease causes moderate to severe anemia and hemolysis
Laboratory Testing

- Initial testing – CBC with peripheral smear
- Polychromasia, spherocytes, schistocytes, sickle cells, Heinz bodies, basophilic stippling; however, the lack of any of these cells does not rule out hemolytic anemia
- Many hemoglobinopathies can be diagnosed using electrophoretic or high-performance liquid chromatography (HPLC) techniques
- Genetic testing

Sickledex test (Screening test)

- Deoxygenated Hb-S is insoluble in a concentrated phosphate buffer solution and forms a turbid suspension
- Normal Hemoglobin A and other hemoglobins remain in solution
- It does not differentiate between Sickle Cell Disease (S/S) and Sickle Cell Trait (A/S)
- Alkaline hemoglobin electrophoresis migration (ph=8.4)
  C, A2, E, O
  S, D, G
  F alone
  A alone

- Acid hemoglobin electrophoresis migration (ph=6.0-6.2)
  A, D, G, E, O together
  S alone
  F alone
  C alone

- Isopropanol stability test - to detect unstable hemoglobin

- Alkali denaturation test for fetal hemoglobin - measures fetal hemoglobin

- Solubility test for sickle cell - screen for sickle cell trait, differentiate HgS from HgD, Hgb C Harlem

- Acid elution test (Kleihauer-Betke) - differentiate HPFH from other states with hgb F increased
Thalassemia

- A single deletion (α-thalassemia minor)
  silent carrier state – RBC morphology and hemoglobin concentrations are usually normal

- Two gene deletion (α-thalassemia minor)
  Mild microcytic anemia

- Three gene deletion (hemoglobin H disease)
  Patients have moderate anemia, marked microcytosis, splenomegaly, and bone marrow erythroid hyperplasia

- Four gene deletion (Hydrops fetalis)
  Not compatible with life. Hemoglobin is primarily comprised of γ4 (Bart’s), which has a very high affinity for O2 and is a poor oxygen transporter
Clinical Significance of Beta-Thalassemia

- Heterozygous asymptomatic •
- Homozygous β0 is a severe disorder associated with transfusion dependent hemolytic anemia
- Homozygous B+ is a heterogeneous disorder – severity depending on mutation and % of HbA
  
  Increased HbA = decreased severity

Sickle cell anemia

Single nucleotide base change codes for valine instead of glutamic acid at the 6th position from the N-terminus of the β-globin chain

Affects the shape and deformability of the red blood cell

Leads to veno-occlusive disease and hemolysis
Which of the following electrophoretic results is consistent with a diagnosis of sickle cell trait?

<table>
<thead>
<tr>
<th>Option</th>
<th>Hgb A</th>
<th>Hgb S</th>
<th>Hgb A2</th>
<th>Hgb F</th>
</tr>
</thead>
<tbody>
<tr>
<td>a)</td>
<td>40%</td>
<td>35%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>b)</td>
<td>60%</td>
<td>38%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>c)</td>
<td>0%</td>
<td>5%</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td>d)</td>
<td>80%</td>
<td>10%</td>
<td>10%</td>
<td></td>
</tr>
</tbody>
</table>

**The correct answer is: B:** electrophoresis at alkaline pH usually shows 50-70% Hgb A, 20-40% Hgb S, and normal levels of Hgb A2 in a patient with the sickle cell trait.
Normal peripheral blood cells
Normal mature lymphocytes
Normal eosinophil
Normal basophil
Normal mature platelets
Neutropenia vs Neutrophilia

- **Neutropenia** can be inherited or acquired.
- Neutropenia usually results from decreased production of neutrophil precursor cells in the marrow.
- **Neutrophilia** is an increase in the absolute neutrophil count to a concentration greater than two standard deviations above the normal population mean value.
# NEUTROPHILLS

<table>
<thead>
<tr>
<th>Neutrophilia</th>
<th>Neutropenia</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Infections, Inflammation, Metabolic disorders</td>
<td>- Decreased production</td>
</tr>
<tr>
<td>- Acute hemorrhage, corticosteroids</td>
<td>- Inherited/acquired stem cell disorder</td>
</tr>
<tr>
<td>- Stress, post-surgery, burns, HDN</td>
<td>- Benzene toxicity, cytotoxic drugs</td>
</tr>
<tr>
<td>- Lithium drugs, neoplasms</td>
<td>- Increased destruction</td>
</tr>
<tr>
<td></td>
<td>- Immune mechanism, sequestration</td>
</tr>
<tr>
<td></td>
<td>- BM depression, IM, varicella, Typhoid</td>
</tr>
<tr>
<td></td>
<td>- SLE, hepatitis or any viral infections</td>
</tr>
</tbody>
</table>
Monocytosis

- **Monocytosis** is an increase in the number of monocytes circulating in the blood.
- Monocytes are white blood cells that give rise to macrophages and dendritic cells in the immune system.
- High levels of monocytes may indicate the presence of chronic infection, an autoimmune or blood disorder, cancer, or other medical conditions.
- This condition is a normal immune response to an event, such as infection, injury, inflammation, some medications, and certain types of leukemia.
- Normal value of Monocytes - 0.2–1.0×10⁹/l (2–10%)
Lymphocytopenia vs Lymphocytosis

- **Lymphocytopenia** is the condition of having an abnormally low level of lymphocytes in the blood.

- The opposite is **lymphocytosis**, which refers to an excessive level of lymphocytes.

- **Lymphocytosis** is a feature of certain infections, particularly infections in children. It may be especially marked in pertussis, infectious mononucleosis, cytomegalovirus infection, infectious hepatitis, tuberculosis and brucellosis.

- Elderly patients with lymphoproliferative disorders, including chronic lymphocytic leukaemia and lymphomas, often present with lymphadenopathy and a lymphocytosis.
Normal bone marrow cells

- Blood cells are made in the bone marrow.
- Bone marrow is the soft inner part of some bones, like the skull, shoulder blades, ribs, pelvis, and backbones.
- Bone marrow is made up of:
  - A small number of blood stem cells
  - More mature blood-forming cells
  - Fat cells
  - Supporting tissues that help cells grow
- Inside the bone marrow, blood stem cells divide and mature to make new blood cells.
- During this process, the cells become either lymphocytes (a kind of white blood cell) or other blood-forming cells. These other blood-forming cells mature into red blood cells, white blood cells (other than lymphocytes), or platelets.
Normal bone marrow cells

- Red blood cells carry oxygen from the lungs to all other tissues in the body, and take carbon dioxide back to the lungs to be removed.

- Platelets are actually cell pieces made by a type of bone marrow cell called the megakaryocyte. Platelets are important in plugging up holes in blood vessels caused by cuts or bruises.

- White blood cells help the body fight infections. Having too few white blood cells (neutropenia) lowers your immune system.

- The percentage of blast cells. Blasts are normally 1 to 5 percent of marrow cells. Having at least 20 percent blasts is generally required for a diagnosis of AML.
Lymphocytes are mature, infection-fighting cells that develop from lymphoblasts, a type of blood stem cell in the bone marrow.

Lymphocytes are the main cells that make up lymphoid tissue, a major part of the immune system. Lymphoid tissue is found in lymph nodes, the thymus gland, the spleen, the tonsils, and adenoids. It's also scattered throughout the digestive and respiratory systems and the bone marrow.

The 2 main types of lymphocytes are:

- **B lymphocytes** (B cells) protect the body from invading germs by developing (maturing) into plasma cells, which make proteins called antibodies. The antibodies attach to the germs (bacteria, viruses, and fungi), which helps other white blood cells called granulocytes recognize and destroy them. B lymphocytes are the cells that most often develop into chronic lymphocytic leukemia (CLL) cells.

- **T lymphocytes** (T cells) can recognize cells infected by viruses and directly destroy these cells. They also help regulate the immune system.
Normal bone marrow:

- Granulocytes are mature, infection-fighting cells that develop from myeloblasts. Granulocytes have granules in them that contain enzymes and other substances that can destroy germs, such as bacteria (neutrophils, basophils, eosinophils).

- Monocytes develop from blood-forming monoblasts in the bone marrow and are related to granulocytes. After circulating in the bloodstream for about a day, monocytes enter body tissues to become macrophages. Macrophages also help lymphocytes recognize germs and start making antibodies to fight them.
Normal megakaryocyte
Normal mature Plasma cell
Myeloid maturation

- myeloblast
- promyelocyte
- myelocyte
- metamyelocyte
- band
- neutrophil
ERYTHROCYTE MATURATION

The overall trend in RBC maturation is large, pale nucleus to darker, smaller nucleus to loss of nucleus; increase in cytoplasm; gradual decrease in size; cytoplasm from intensely blue (full of RNA) to grayish (mixture of RNA and hemoglobin) to reddish (full of hemoglobin, no RNA). Identify the following cells.
# Morphological evaluation of monocytes

<table>
<thead>
<tr>
<th>Monocyte</th>
<th>Immature</th>
<th>Promonocyte</th>
<th>Monoblast</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Monocyte" /></td>
<td><img src="image2" alt="Immature" /></td>
<td><img src="image3" alt="Promonocyte" /></td>
<td><img src="image4" alt="Monoblast" /></td>
</tr>
</tbody>
</table>
Congenital immunodeficiency diseases are often caused by blocks at different stages of lymphocyte maturation.
Leukemia/Lymphoma

Definition of leukaemia:

- Cancer that starts in blood-forming tissue, such as the bone marrow and the lymphatic system and causes large numbers of abnormal blood cells to be produced and enter the bloodstream.

![Pie chart with estimated new cases of leukemia, lymphoma, and myeloma, 2019](image)

Figure 1. Source: Cancer Facts & Figures, 2019. American Cancer Society; 2019.
Five-Year Relative Survival Rates by Year of Diagnosis

Figure 2. Source: SEER (Surveillance, Epidemiology, and End Results) Cancer Statistics Review, 1975-2015. National Cancer Institute; 2018.

*The difference in rates between 1975-1977 and 2008-2014 is statistically significant (p<.05).

*Survival rate among whites.
Mature and immature leukemia classification
  - Myeloid and Lymphoid leukemias
  - Mature B and T lymphoid neoplasm
Leukemia is cancer of the body's blood-forming tissues, including the bone marrow and the lymphatic system.

Many types of leukemia exist. Some forms of leukemia are more common in children. Other forms of leukemia occur mostly in adults.

Leukemia usually involves the white blood cells. Your white blood cells are potent infection fighters — they normally grow and divide in an orderly way, as your body needs them. But in people with leukemia, the bone marrow produces abnormal white blood cells, which don't function properly.

Treatment for leukemia can be complex — depending on the type of leukemia and other factors. But there are strategies and resources that can help to make your treatment successful.
Leukemia symptoms

- Leukemia symptoms vary, depending on the type of leukemia.
- Common leukemia signs and symptoms include:
  - Fever or chills
  - Persistent fatigue, weakness
  - Frequent or severe infections
  - Losing weight without trying
  - Swollen lymph nodes, enlarged liver or spleen
    - Easy bleeding or bruising
    - Recurrent nosebleeds
  - Tiny red spots in your skin (petechiae)
  - Excessive sweating, especially at night
    - Bone pain or tenderness
How leukemia forms

- In general, leukemia is thought to occur when some blood cells acquire mutations in their DNA.
- Certain abnormalities cause the cell to grow and divide more rapidly and to continue living when normal cells would die.
- Over time, these abnormal cells can crowd out healthy blood cells in the bone marrow, leading to fewer healthy white blood cells, red blood cells and platelets, causing the signs and symptoms of leukemia.
Doctors classify leukemia based on its speed of progression and the type of cells involved:

**Acute leukemia:** In acute leukemia, the abnormal blood cells are immature blood cells *(blasts)*.

**Chronic leukemia:** Chronic leukemia involves more mature blood cells.

The second type of classification is by type of white blood cell affected:

**Lymphocytic leukemia.** This type of leukemia affects the lymphoid cells.

**Myelogenous leukemia.** This type of leukemia affects the myeloid cells. Myeloid cells give rise to red blood cells, white blood cells and platelet-producing cells.
The major types of leukemia are:

- **Acute lymphocytic leukemia (ALL).** This is the most common type of leukemia in young children. ALL can also occur in adults.

- **Acute myelogenous leukemia (AML).** AML is a common type of leukemia. It occurs in children and adults. AML is the most common type of acute leukemia in adults.

- **Chronic lymphocytic leukemia (CLL).** With CLL, the most common chronic adult leukemia, you may feel well for years without needing treatment.

- **Chronic myelogenous leukemia (CML).** This type of leukemia mainly affects adults. A person with CML may have few or no symptoms for months or years before entering a phase in which the leukemia cells grow more quickly.

- **Other types.** Other, rarer types of leukemia exist, including hairy cell leukemia, myelodysplastic syndromes and myeloproliferative disorders.
<table>
<thead>
<tr>
<th></th>
<th>FAB Classification: AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>M0</td>
<td>AML with no Romanowsky or cytochemical evidence of differentiation</td>
</tr>
<tr>
<td>M1</td>
<td>Myeloblastic leukemia with little maturation</td>
</tr>
<tr>
<td>M2</td>
<td>Myeloblastic leukemia with maturation</td>
</tr>
<tr>
<td>M3</td>
<td>Acute promyelocytic leukemia (APL)</td>
</tr>
<tr>
<td>M3h</td>
<td>APL, hypergranular variant</td>
</tr>
<tr>
<td>M3v</td>
<td>APL, microgranular variant</td>
</tr>
<tr>
<td>M4</td>
<td>Acute myelomonocytic leukemia (AMML)</td>
</tr>
<tr>
<td>M4eo</td>
<td>AMML with dysplastic marrow eosinophils</td>
</tr>
<tr>
<td>M5</td>
<td>Acute monoblastic leukemia (AMoL)</td>
</tr>
<tr>
<td>M5a</td>
<td>AMoL, poorly differentiated</td>
</tr>
<tr>
<td>M5b</td>
<td>AMoL, differentiated</td>
</tr>
<tr>
<td>M6</td>
<td>“Erythroleukemia”</td>
</tr>
<tr>
<td>M6a</td>
<td>AML with erythroid dysplasia</td>
</tr>
<tr>
<td>M6b</td>
<td>Erythroleukemia</td>
</tr>
<tr>
<td>M7</td>
<td>Acute megakaryoblastic leukemia (AMkL)</td>
</tr>
</tbody>
</table>
Acute Myeloid Leukemia

2008 WHO Classification of AML

- AML with recurrent genetic abnormalities
  - AML with t(8;21) (q22;q22) (RUNXI-RUNX1)
  - AML with inv(16)(p13.1;q22) or t(16,16) (p13.1;q22) (CBFB-MYH11)
  - Acute promyelocytic leukemia with t(15;17)(q24.1;q21.1) (PML-RARA)
  - AML with t(9;11)(p22;q23) (MLLT3-MLL)
  - AML with t(6;9)(p23;q34) (DEK-NUP214)
  - AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) (RPNI-EVI1)
  - AML (megakaryoblastic) with t(1;22)(p13;q13) (RBM15-MKL1)
  - Provisional entity: AML with mutated NPM1
  - Provisional entity: AML with mutated CEBPA

- AML with myelodysplasia-related changes
  - Therapy-related myeloid neoplasms
  - AML, not otherwise specified
    - AML minimally differentiated
    - AML without maturation
    - AML with maturation
    - Acute myelomonocytic leukemia
    - Acute monoblastic and monocytic leukemia
    - Acute erythroid leukemia
      - Acute megakaryocytic leukemia
      - Acute basophilic leukemia
      - Acute panmyelosis with myelofibrosis
  - Myeloid sarcoma
  - Myeloid proliferations related to Down syndrome
  - Blastic plasmacytoid dendritic cell neoplasm
Immature cells in leukemia

- In healthy bone marrow, blood-forming cells known as **hematopoietic stem cells** develop into red blood cells, white blood cells, and platelets through a process called **hematopoiesis**.

- Hematopoiesis occurs throughout your entire lifespan. The stem cell chooses its path of development into one of two cell lines, the **lymphoid** cell line or the **myeloid** cell line.

- In the myeloid cell line, the term "**blast cell**" refers to myeloblasts or myeloid blasts. These are the very earliest and most immature cells of the myeloid cell line.

- Myeloblasts give rise to **white blood cells**. This family of white blood cells includes neutrophils, eosinophils, **basophils** and monocytes, and macrophages.
Blast cell

Blasts are precursors to the mature, circulating blood cells such as neutrophils, monocytes, lymphocytes and erythrocytes. Blasts are usually found in low numbers in the bone marrow. They are not usually found in significant numbers in the blood.
Common site for the bone marrow aspiration and biopsy in an adult
Blast morphology

- **Blasts** are cells that have a large nucleus, **immature chromatin**, a prominent **nucleolus**, scant cytoplasm and few or no cytoplasmic granules.

- **Cell size** - blasts are often medium to large cells. They are usually larger than a lymphocyte and at least the size of a monocyte.

- **Large nucleus** - most of the cell is taken up by the nucleus (a high nuclear to cytoplasmic ratio).

- **Immature chromatin** - the nuclear chromatin looks as if it composed of fine dots. One can visualize this chromatin as many tiny points made by the tip of a sharp pencil on a piece of paper. Monocyte chromatin is more linear and dark, looking like smudged pencil lines. Lymphocyte chromatin looks to be colored in heavy crayon.

- **Prominent nucleolus**.

- **Conformable nuclear membrane** - the nuclear membrane often conforms to the shape of the cytoplasmic membrane. The nucleus appears squishy. A lymphocyte nucleus appears rigid.

- **Few to no cytoplasmic granules**

- **Auer rods** - orange-pink, needle-like cytoplasmic structures in blasts of myeloid lineage. These may be numerous in acute promyelocytic leukemia. 20% of more blasts in the peripheral blood or bone marrow
Acute Myeloid Leukemia

- Diagnosis in bone marrow evaluation: 20% or more of blasts
- Hypercellular
- Predominance of blasts: medium size to large with moderate to abundant variably basophilic cytoplasm that may contain few azurophilic granules
- Auer rods present in 60-70% of cases
- Nuclear shape varies: round to irregular or convoluted
- Chromatin: dispersed with one or more nucleoli
- Retarded maturation
- Dysplastic changes in granulocytic, erythroid and megakaryocytic precursors
Normal maturation of WBC: granulopoiesis
Auer rods (see arrow in image) are cytoplasmic inclusions which result from an abnormal fusion of the primary (azurophilic) granules.

Auer rods may be seen in myeloblasts, promyelocytes and monoblasts, but are not seen characteristically in lymphoblasts.
Undifferentiated acute myeloblastic leukemia
AML without maturation (M0)

- 10% of cases of AML
- The sum of myeloblasts must be 90% or more of the non-erythroid cells in the bone marrow.
- Blasts may be MPO or SBB positive.
- Immunophenotyping studies recommended (Flow Cytometry)
- Blood: leukopenia with occasional myeloblasts or marked leukocytosis with 100% of blasts.
- There is anemia, neutropenia and thrombocytopenia (blood)
- In bone marrow is hypercellular with predominance of blasts; erythroid and megakaryocyte precursors are markedly decreased.
- The blast nucleus is round but may show irregularity in some cases; nucleoli are variably prominent.
- Auer rods may be found in 50% of cases.
- Evidence of maturation to promyelocytes is absent.
- Diagnose at any age with a median of 45-50 years.
Acute Myelocytic Leukemia (FAB M1)
AML with maturation(M1)

- The most common of AML; 30-45% of the cases.
- It has maturation beyond myeloblasts.
- The sum of myeloblasts is from 20-89% of non-erythroid cells; granulocytes to mature neutrophils are more than 10% of cells and monocytes less than 20%.
- Auer rods are present.
- Dysplastic mature neutrophils, erythroid and megakaryocyte precursors.
- MPO positive; chromosome abnormalities found: example: t (6;9)(p23;q34);DEK/CAN(maturation with a basophil component)
- Occurs at any age: median 45-50 years.
- Prognosis variable.
M2: Acute myeloblastic leukemia with maturation

- Presenting symptoms for M2 AML are similar to those of the M1 type

- 30% to 45% of cases of AML

- Blasts show azurophilic granules and Auer rods

- Evidence of maturation is present, with >10% of the marrow cells being promyelocytes, myelocytes, and mature neutrophils and <20% being monocytes
Acute promyelocytic leukemia
Acute promyelocytic leukemia M3 T15;17

- Leukocyte count decreased in typical hyper granular APL
- Has numerous red to purple cytoplasmic granules, they obscure the nuclear borders
- Associated to DIC; MPO positive, non-specific esterase weakly positive
- Sometimes has deeply basophilic cytoplasm; Auer rods present
- Nucleus: kidney shape or bilobed; cytoplasm with coalescent large granules(bright pink)
- Myeloblasts are minor component
- In microgranular variant: leukocyte count is elevated, fine granulation and markedly irregular nuclei; confused with acute monocytic leukemia
- Increased side scatter pattern, lack expression of Hla-Dr and Cd 34, weak Cd 117
- Breakpoint on band q22 of chromosome 15 and on band 12 at the first intron of the retinoic acid receptor alpha (RARalpha) gene on chromosome 17.
- Remission with ATRA
APL hypogranular variant
Acute Myelomonocytic Leukemia (M4)

- Accounts for 15-25% of AML.
- The sum of myeloblasts, monoblasts and promonocytes is 20% or more; 20-79% of bone marrow cells are monocyte lineage.
- Non-specific esterase is positive.
- The diagnosis of AMML is if the blood monocyte count exceeds 5 x 10^3/mm^3.
- Auer rods present; there are no specific cytogenetic abnormalities, some cases are inv(16) or 11q23.
- Occurs in both children and adults (50 years).
- Wbc elevated, organomegaly, lymphadenopathy and other tissue infiltration are common.
- Treatment response and survival varies.
Acute Monoblastic and Monocytic leukemia (M5)

- Account for 8% of AML.
- 80% or more of the non erythroid cells in the bone marrow are monoblasts, promonocytes and monocytes.
- In acute monoblastic leukemia 80% or more of the monocytic cells are monoblasts.
- In acute monocytic leukemia less than 80% of the monocytic cells are monoblasts.
- In acute monoblastic leukemia, blasts are large with abundant basophilic cytoplasm with fine peroxidase negative granules; \textit{auer rods are not observed}; the nucleus is round with reticular chromatin and one or more nucleoli.
- Monoblasts are \textit{non-specific esterase} positive and MPO negative.
M5-monocytic leukemia
Acute erythroid leukemia (M6)

- 5% of AML
- 2 subtypes: erythroid/myeloid and pure erythroid
  - In erythroid/myeloid, erythroblasts are 50% or more; 20% or more are myeloblasts. Dyserythropoiesis is prominent. It presents with pancytopenia and NRBC in blood. There is erythroid hyperplasia, dyserythropoiesis, megaloblastoid changes, karyorrhexis. Multiple nuclei are common. Vacuoles may be present. PAS positive. Auer rods are present in myeloblasts.
  - In pure erythroid leukemia, blasts are all erythroid.
- Primarily in adults of advance age. Evolve from MDS. Prognosis is poor.
M6 Erythroid Leukemia
M7 leukemia

- AML showing maturation in the megakaryocyte lineage and small and large megakaryoblasts.
- Most commonly in infants without Down Syndrome with a female predominance.
- De novo, restricted to infants and young children (3 years or less).
- Organomegaly, patient with anemia, thrombocytopenia and elevated WBC cell count.
- Morphology and cytochemistry similar to megakaryoblastic leukemia of AML(M7).
- SBB and MPO are negative.
- MPO negative; Cd 41, Cd61 positive expression.
FAB /WHO classification of acute lymphoblastic leukemia

- Acute lymphoblastic leukemia (L1/L2)
  - Precursor B cell
  - Precursor T cell
- Acute lymphoblastic leukemia, B-cell (L3) (equivalent to Burkitt lymphoma in leukemic phase)
- Biphenotypic

**FAB classification**

- ALL-L1: small uniform cells
- ALL-L2: large varied cells
- ALL-L3: large varied cells with vacuoles (bubble-like features
Flash cards to study leukemia

https://www.cram.com/flashcards/acute-leukemias-6033326
Cytochemical stains for leukaemia

- MPO
- SBB
- Specific Esterase: Naphthol As-D Chloroacetate esterase
- Non-specific esterase: Alpha naphthyl butyrate esterase; alpha naphthyl acetate
- Oil O-Red
- Toluidine Blue
- PAS
- Trap
Cytochemical stains

- **Myeloperoxidase (MPO):** Peroxidase is present in primary granules of neutrophils and in granules of eosinophils and monocytes. The identification of this enzyme in the cytoplasm of leukocytes is used to distinguish acute myelogeneous leukemia (AML) from acute lymphocytic leukemia (ALL).

- **Sudan Black B staining:** Stains a variety of lipids, including neutral fat, phospholipids, and sterols.

- **Periodic Acid Schiff (PAS):** Oxidizes carbohydrates, glycogen, and similar compounds to aldehydes. In ALL lymphoblasts show variability: 50% of cases contain coarse granules or large blocks of PAS positive material. A coarse diffuse satin is characteristic of ALL. L1 and L2 are positive but not L3. In M6, the reaction of erythrocytic precursors is intense. In M7, the blast cells usually show a diffuse and granular positive reaction.
- **Esterase staining:** represents a series of different enzymes acting upon select substrates. Distinguish granulocytes from monocytes. Naphthol As-D Chloroacetate esterase: specific for cells of granulocytic lineage. Alpha-naphtyl acetate esterase: detected primarily in monocytes, macrophages and histocytes. Alpha-naphtyl acetate esterase with fluoride inhibition: all monoblasts will be negative for enzyme activity.

- **Non specific esterase:** Alpha naphthyl butyrate esterase: this stain is positive in cells from the monocyte and megakaryocyte series. This stain is most commonly used to confirm a diagnosis of acute myelogenous leukemias with monocytic differentiation.

- **Oil O red stain** used for staining of neutral triglycerides and lipids on frozen sections and some lipoproteins on paraffin sections. Neutral lipids may be present in some lymphomas, especially in Burkitt’s lymphoma and large cell lymphoma. Leukemic lymphoblasts contain ORO positive inclusions. Myeloblasts are negative.

- **Toluidine Blue Stain** reacts with the acid mucopolysaccharides in human blood cells. Marker for basophilic leukemia and systemic mast cell disease.

- **Acid Phosphatase resistant leukocytes TRAP stain** most of the acid phosphatase isoenzyme is inhibited by L-tartaric acid. The cells of hairy cell leukemia, Sezary syndrome and some T cell acute lymphoblastic leukemia are tartrate resistant (positive reaction).
## Cytochemical Reactions in Acute Leukemia

<table>
<thead>
<tr>
<th>Cytochemical Reaction</th>
<th>Cellular Element Stained</th>
<th>Blasts Identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloperoxidase (MPO)</td>
<td>Neutrophil primary granules</td>
<td>Myeloblasts strong positive; monoblasts faint positive</td>
</tr>
<tr>
<td>Sudan Black B (SBB)</td>
<td>Phospholipids</td>
<td>Myeloblasts strong positive; monoblasts faint positive</td>
</tr>
<tr>
<td>Specific esterase</td>
<td>Cellular enzyme</td>
<td>Myeloblasts strong positive</td>
</tr>
<tr>
<td>Nonspecific esterase (NSE)</td>
<td>Cellular enzyme</td>
<td>Monoblasts strong positive</td>
</tr>
<tr>
<td>Periodic acid-Schiff</td>
<td>Glycogen and related substances</td>
<td>Variable, coarse or block-like positivity often seen in lymphoblasts and pronormoblasts, myeloblasts usually negative although faint diffuse reaction may occasionally be seen</td>
</tr>
</tbody>
</table>
Cytochemical stains: con’t

- Leukocyte Alkaline Phosphatase (LAP)
  - Enzyme within the 2° or specific granules of maturing granulocytes
  - Distinguishes leukemoid reactions (†) from chronic myelogenous leukemia (↓)

- Acid Phosphatase
  - Present in lysosomes in normal leukocytes
  - Helpful in diagnosing hairy cell leukemia because they are NOT inhibited by TRAP
Identification of leukemias by Flow Cytometry

- Flow cytometry is a technique used to detect and measure physical and chemical characteristics of a population of cells or particles.
- The basic principle of flow cytometry is the passage of cells in single file in front of a laser so they can be detected, counted and sorted. Cell components are fluorescently labelled and then excited by the laser to emit light at varying wavelengths.
Flow Cytometry
CD45/SSC gating strategy is more sensitive than FSC/SSC gating and it delineates the blasts well.
<table>
<thead>
<tr>
<th>FAB</th>
<th>Immunological marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML with minimally differentiated</td>
<td>CD13, <strong>CD34</strong>, HLA-DR, CD33, CD117, CD2, CD7, TdT</td>
</tr>
<tr>
<td>AML without maturation</td>
<td>CD13, CD14, CD33, <strong>CD34</strong></td>
</tr>
<tr>
<td>AML with maturation and with t(8;21)</td>
<td><strong>CD34, CD56</strong></td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
<td>CD13, CD33, HLA-DR absent, <strong>CD34 negative</strong></td>
</tr>
<tr>
<td>Acute myelomonocytic leukemia with abnormal eosinophils and inversion 16</td>
<td>CD13, <strong>CD34</strong>, CD11b, CD11c, CD14, CD33</td>
</tr>
<tr>
<td>Acute monocytic leukemia and 11q23 abnormalities</td>
<td>CD14, CD4, CD36, CD64</td>
</tr>
<tr>
<td>Erythroleukemia</td>
<td>Glycophorin 7, Transferrin receptor CD71</td>
</tr>
<tr>
<td>Acute Megakaryocytic leukemia</td>
<td>cCD41, cCD42b, cCD61</td>
</tr>
</tbody>
</table>
### How to define the lineage of leukaemia

#### Antigens Used for Lineage Assignment in Acute Leukemia

<table>
<thead>
<tr>
<th></th>
<th>AML</th>
<th>ALL-B cell</th>
<th>ALL-T cell</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definitive</strong></td>
<td>Cytoplasmic MPO</td>
<td>None</td>
<td>Cytoplasmic CD3</td>
</tr>
<tr>
<td><strong>Strongly associated</strong></td>
<td>CD117</td>
<td>Cytoplasmic CD79a</td>
<td>Surface CD3 T cell receptor</td>
</tr>
<tr>
<td><strong>Moderately associated</strong></td>
<td>CD13</td>
<td>TdT—-moderate to bright</td>
<td>CD7—bright</td>
</tr>
<tr>
<td></td>
<td>CD33</td>
<td></td>
<td>CD5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD2</td>
</tr>
</tbody>
</table>

**The Flow Cytometric Evaluation of Hematopoietic Neoplasia** Brent L. Wood, Michael J. Borowitz. Henry’s, 22nd edition, Chapter 34
Antibodies used in immunotyping/Cytometry

Blasts antibodies used in immunotyping
  myeloid—CD 34, MPO, Cd235a, Cd117
  lymphoid—CD 10, TDT, kappa or Lambda light chain
    monoclonality, Cd34+/− Cyto cd3 or Cd79a

Myeloid (neutrophils & monos)-normal mature
  Cd13,33,14,15,64

Lymphoid- normal mature
  Lymph T- Cd2,3,4,5,7,8
  Lymph B- Cd19,20,22,25 Kappa & Lambda light chain
  NK lymphs-Cd3+/Cd56+/Cd16+

Plasma cell-
  Cd138+/38+/56+, Cd19/cd20, Kappa or Lambda
CD 56+ & CD 19- & CD 3-

CD 2+ &/or CD 7+ &/or CD 3+ &/or CD 5+
(T-cell lineage)

See Algorithm 4

CD 19+ &/or CD 20+ &/or CD 79a+
(B-cell lineage)

K/λ clonal
Follicular or Burkitt’s or Burkitt’s like Lymphoma

CD 103+ & CD 103 +
&/or CD 25 + &/or CD 11c ± K/λ clonal

Other lymphoma
- Burkitt’s
- Large cell
- Follicular
- Marginal

*

Acute lymphoblastic lymphoma (B-ALL)*

TdT +

CD 5 +

CD 5-

CD 10 +

Mantle cell lymphoma

Chronic/Small Lymphocytic lymphoma (CLL)

Hairy cell leukemia

Marginal Zone lymphoma

*It can also be CD 34 and CD 10 positive.
CD3+ &/or (CD 2+ & CD 7+)
(T-cell lineage)*

CD4+ →
- Mycosis fungoides/
  Sézary syndrome
- Adult T-cell
  lymphoma
- Post thymic
  lymphoproliferative
disease

TdT + &
CD 34 ± →
- Acute
  lymphoblastic
  leukemia
  (T-cell
  precursor)*

CD 56- & CD 4-
& CD8+ →
- Large granular
  lymphocytic
  leukemia
  - T lymphoproliferative
  disease, post thymic

CD 3+ & CD 56+ →
- Large granular
  lymphocytic
  proliferation-
  Natural killer-cell
  type

CD 16 + & CD 57±

*T-lymphoproliferative disorders can be diagnosed when there is lack of the expression of one or more T-cell markers. CD3 positivity could be only cytoplasmic and not surface.
Morphology
Blasts $> 20\%$

Cytochemistry

Negative
TdT

Positive MPO

Flow Cytometry

CD19 CD19
CD22 CD22
CD79 CD79

B markers

CD19 CD19
CD22 CD22
CD79 CD79

T markers

CD10 CD10
CD2 CD2
CD3 CD3
CD5 CD5
CD7 CD7
CD8 CD8

Mature B
(5%)

Pre B
(15%)

Common B
(45%)

Pre-Pre B
(15%)

T ALL
(25%)

Cytogenetic/Molecular Abnormalities

t(8;14)
c-myc

t(8;22)
t(2;8)

t(1;19)/E2A-PBX1

t(12;21)/TEL-AML1

t(9;22)
BCR-ABL

t(4;11)
MLL-AF4

t(10;24)/HOX11

clg; cytoplasmic immunoglobulin; MPO, myeloperoxidase; sig, secreted immunoglobulin; TdT, terminal deoxynucleotidyl transferase
Ancillary tests

- **Cytogenetics**: used to look for changes in chromosomes.

- Fish molecular testing method uses fluorescent probes to evaluate genes and/or DNA sequences on chromosomes.

- PCR is a laboratory method used for making a very large number of copies of short sections of DNA from a very small sample of genetic material. This process is called "amplifying" the DNA and it enables specific genes of interest to be detected or measured.
Chronic Leukemias

- In CML, a genetic change takes place in an early (immature) version of myeloid cells -- the cells that make red blood cells, platelets, and most types of white blood cells (except lymphocytes).
- This change forms an abnormal gene called BCR-ABL, which turns the cell into a CML cell.
- The leukemia cells grow and divide, building up in the bone marrow and spilling over into the blood.
- In time, the cells can also settle in other parts of the body, including the spleen.
- CML is a fairly slow growing leukemia, but it can change into a fast-growing acute leukemia that’s hard to treat.
- In chronic leukemia, the cells mature partly but not completely.
- They generally do not fight infection as well as normal white blood cells do.
- Chronic leukemias can take a long time before they cause problems, and most people can live for many years.
- Chronic leukemias are generally harder to cure than acute leukemias.
Chronic Leukemias

- **Myeloproliferative neoplasm:**
  - Chronic myelogeneous leukemia (BCR/ABL1)
  - Chronic neutrophilic leukemia
  - Chronic eosinophilic leukemia, nos
  - Chronic basophilic leukemia

- **Myelodysplastic/myeloproliferative**
  - Chronic myelomonocytic leukemia
  - Juvenile myelomonocytic leukemia
Chronic lymphoid leukemia

- Chronic lymphocytic leukemia: B or T cell origin
- B-cell prolymphocytic leukemia
- Hairy Cell leukemia
- T-cell prolymphocytic leukemia
- T-cell large granular lymphocytic leukemia
- Aggressive NK cell leukemia
- Adult T-cell Leukemia/Lymphoma
- Sezary Syndrome
Haemostasis mechanism

Maintains blood in the fluid state within the blood vessels
Prevents excessive blood loss after vascular injury

Vascular damage: blood clots to seal vessel: includes vascular constriction, platelet aggregation, and fibrin formation

Clot has formed, tissue repaired- fibrinolysis began(digestion of the clot) initiated by trauma to vessel : vasoconstriction(reduce blood flow), platelets adhere to subendothelial collagen fibers and micro fibrils, tissue factor(in the vessel) initiate coagulation.

Generation of thrombin converts fibrinogen to fibrin incorporated in the platelet plug
Stable clot formed(cross-linked fibrin strands by factor XIIIa and contraction of platelet mass.

Note: **white thrombus**: formed in arterial system, primarily of platelets
**red thrombus**: found in venous circulation erythrocytes and platelets
Platelets are exposed to collagen fibers at the vascular access site.

Attached platelets release serotonin, a vasoconstrictor, ADP and Thromboxane A2 which promote platelet aggregation, this response leads to rapid platelet plug formation. Activated platelets also stimulate the Clotting Cascades Contact ("Intrinsic") Pathway.

Clotting Cascades stimulation allows fibrin to form around the platelet plug. This fibrin mesh strengthens the platelet plug and traps red blood cells completing hemostasis.
Platelets

anucleated disc-shaped cytoplasmic fragments (2-4um)
blue gray cytoplasm and purplish granules
formed in bone marrow by megakaryocytes
9-10 days in circulation, some sequestered in the splenic pool
contains 3 types of secretory granules: lysosomes containing acid hydrolases; alpha granules containing platelet factor 4, and beta-thromboglobulin, platelet derived growth factor and coagulation proteins found in plasma (fibrinogen and von Willebrand factor); and delta granules containing ADP, ATP, Ca, serotonin.
Platelet MORPHOLOGY

Normal platelets

Platelet satellitosis, an in vitro phenomenon, in a peripheral blood film made from EDTA-blood

Tiny platelets
(microthrombocytes)
in a thrombocytopenic patient with Wiskott-Aldrich syndrome

Giant bizarre platelet with cytoplasmic vacuolization in a patient with MDS

Large platelet in a patient with Bernard-Soulier syndrome

Giant adendritic platelet exceeding the size of background RBCs in a patient with May-Hegglin anomaly.

Spectrum of platelet morphology in a myeloproliferative neoplasm, including giant, bizarre, and hypogranular forms

Large platelet with normal granularity in a patient with ITP
Primary hemostasis begins with adhesion of platelets.

Adhesion begins when von Willebrand factor binds to subendothelial receptors and to glycoprotein 1b on platelets).

Collagen induces platelets to aggregate by stimulating them to secrete ADP and to synthesize thromboxane A2.

ADP amplifies aggregation.

Thrombin formed by the soluble coagulation system also activates platelets.

Vasoconstriction is enhanced by the release of serotonin and thromboxane A2.

Platelet activation induces expression of binding sites for coagulation proteins (platelet factor 3).

Adhesion and aggregation of platelets occur mediated by fibrinogen which links 2 platelets by the fibrinogen receptor (glycoprotein IIb-IIIa).

The platelet plug is provisional unless a firm fibrin clot forms around it. Platelet actomyosin provides for clot retraction and consolidation.
2nd phase of hemostasis

-soluble plasma fibrinogen is converted to an insoluble fibrin clot

-enzymatic pathways (zymogen, enzyme precursor) initiated by 2 mechanism of vitamin k-dependent proteins:
  procoagulant (factor II, VII, IX, X)
  anticoagulant (protein C and protein S)
Clots requires plasma coagulation proteins (4 categories):

1) **serine endopeptidases** (proteases): Factors II, VII, IX, X, XII and pre-kallikrein circulate in the zymogen form. Factors VII, IX, X requires vitamin K to synthesize active coagulation proteins that binds with Calcium ions.

2) **cofactors**: required for activation of some of the procoagulant proteins: High Molecular Weight kininogen, factors V, VIII

3) **fibrinogen**: Factor I (soluble protein) becomes insoluble fibrin clot following cleavage by thrombin

4) **factor XIII**: is a plasma transglutaminase that activated stabilizes the fibrin clot
Activation of coagulation: 2 mechanism

**Extrinsic (PT):**

Tissue factor initiates the pathway (skin, brain, lung, placenta, monocytes, in the vessels) forming a complex with factor VIIa.

This complex activates factor X; factor Xa in the presence of cofactor (Va), activates prothrombin to form thrombin.

Excessive activity is regulated by tissue factor pathway inhibitor.

Prothrombin activation occurs on cellular surface of platelets, endothelial cells, smooth muscle cells, and monocytes, and requires Ca and factor Va.

Once thrombin is formed, clotting occurs. Polymerization of fibrin monomers and cross-linking of fibrin by thrombin-activated factor XIIIa lead to an insoluble fibrin clot.
Intrinsic pathway: APTT

Exposition to collagen (subendothelial connective tissue) activate factor XII.

Factor XIIa converts prekallikrein to kallikrein, which convert more factor XII to factor XIIa which in turn activates factor XI.

This requires the cofactor protein HMW kininogen.

Factor XIa converts factor IX to factor IXa.

Factor XII, prekallikrein, and HMW kininogen are the contact proteins (glass or kaolin).

IXa factor activate X to Xa, Xa activate prothrombin to thrombin, thrombin convert fibrinogen into fibrin.
Activated Partial Thromboplastin Time

- Used to detect inherited and acquired factor deficiency of the intrinsic pathway, to screen for the lupus anticoagulant, and to monitor heparin therapy.
- Is an assay of the intrinsic and common pathway
- A platelet substitute (phospholipid) and a surface-activating agent such as micronized silica (to activate factor XII) are added to platelet poor plasma (contact activation). Calcium is added, and the clotting time is recorded.
- Measures all the factors except VII and XIII
- Reference values: 25-45 seconds
APTT
Blood Coagulation And Fibrinolysis

**The fast extrinsic clotting system (Injured cells)**
- Factor III
- Factor VII
- Factor IV (Ca\(^{2+}\))

Cascade in seconds

**Coagulation**
- Factor X $\rightarrow$ Xa
- Factor V $\rightarrow$ Va
- Ca\(^{2+}\)
- Phospholipid

**The slow intrinsic clotting system (Injured platelets)**
- Factor XII $\rightarrow$ XIIa
- Factor XI $\rightarrow$ XIa
- Factor IX $\rightarrow$ IXa
- Factor VIII $\rightarrow$ VIIIa

Cascade in min

- THROMBIN
  - Ca\(^{2+}\)
  - XIIIa

**Fibrinogen (I)**

- Prothrombin (II)
- Fibrinogen (I)

**Fibrin monomer**

**Fibrin polymer**

**Fibrinolysis:**
- Plasminogen
- Plasmin
- Fibrin fragments
- Antiplasmin

Fig. 8-7
PT:

- Used to screen for inherited or acquired abnormalities in the extrinsic (factor VII) and common (factors V and X, prothrombin, and fibrinogen) pathways.
- Clotting is initiated by commercial tissue factor reagent (thromboplastin that contains phospholipids), which is mixed with Calcium and the clotting time is determined.
- Useful in determining Vitamin K dependent factors (prothrombin, factor VII, factor X)
- Normal ranges: 10-16 seconds
- Increased PT usually indicates decreased synthesis of Vitamin K dependent factors, factor V and fibrinogen concentrations or antibodies against prothrombin and factor V or disfibrinogenemia.
- Decrease PT result may be due to poor quality venipuncture
FIBRINOLYSIS

- Lysis of the fibrin clot
- Thrombin stimulates secretion of vascular endothelial cell tissue plasminogen activator (t-PA).
- Plasminogen and t-PA diffuse within the thrombus
- t-PA activates plasminogen to plasmin,(protease capable of degrading fibrin) restricted to the clot
- inhibitors to t-PA, plasmin are present in blood (plasminogen activator inhibitor, alpha2-antiplasmin)
Fluid Phase

Plasminogen → Activation → Plasmin → Degrades

Fibrinogen Factor V Factor VIII

Nonfibrin-Specific or Less Fibrin-Specific Plasminogen Activators

Fibrin-Specific Plasminogen Activators

PAI-1

α2-Antiplasmin

Plasminogen → Activation → Plasmin

Fibrin strands network

Degradation → Fibrin degradation products

Fibrin Surface
Coagulation Factors measured by aPTT and PT assays

<table>
<thead>
<tr>
<th>aPTT</th>
<th>PT</th>
<th>Both Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>XII</td>
<td>VII</td>
<td>X</td>
</tr>
<tr>
<td>HMW-K</td>
<td>X</td>
<td>V</td>
</tr>
<tr>
<td>Prekakirein</td>
<td>V</td>
<td>Prothrombin</td>
</tr>
<tr>
<td>XI</td>
<td>Prothrombin</td>
<td>Fibrinogen</td>
</tr>
<tr>
<td>IX</td>
<td>Fibrinogen</td>
<td></td>
</tr>
<tr>
<td>VIII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrinogen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prolonged PT Normal aPTT</td>
<td>Prolonged aPTT Normal PT</td>
<td>Prolonged Prolonged PT aPTT</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Decreased Factor VII</td>
<td>Decreased Factor VIII</td>
<td>Decreased Factor X</td>
</tr>
<tr>
<td>Decreased Vitamin K</td>
<td>Decreased Factor IX</td>
<td>Decreased Factor V</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decreased Factor XI</td>
<td>Decreased Factor II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Decreased Factor XII</td>
<td>Decreased Fibrinogen (I)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased Vitamin K</td>
</tr>
<tr>
<td>Warfarin therapy</td>
<td>Heparin therapy</td>
<td>Warfarin therapy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lupus anticoagulant</td>
<td>Elevated Hematocrit</td>
</tr>
</tbody>
</table>
Criteria for rejection of coagulation testing specimens

- Specimen not labelled properly
- Specimen missing patient identification
- Sample spilled or the tube broken
- Illegible test request form
- Haemolysed sample
- A clot in the sample
- Inadequately filled tube
- Specimens received more than 4 hours after collection
- Hematocrit over 55% and not adjusted for a high hematocrit
- Frozen whole blood sample
- Overfilled tube
Common collection and handling problems that affect coagulation test results

<table>
<thead>
<tr>
<th>Short draw</th>
<th>Prolonged PT and aPTT</th>
<th>Anticoagulant to blood ratio exceeds 1:9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Failure to mix specimen gently</td>
<td>Prolonged PT and aPTT</td>
<td>Even small blood clots will affect the assay</td>
</tr>
<tr>
<td>Excessive mixing</td>
<td>Shortened PT and aPTT</td>
<td>Hemolysis and platelet activation affect the assay</td>
</tr>
<tr>
<td>Hemolysis caused by slow collection</td>
<td>Shortened PT and aPTT</td>
<td></td>
</tr>
<tr>
<td>Improper storage; wrong temperature</td>
<td>Prolonged PT and aPTT</td>
<td></td>
</tr>
<tr>
<td>Undue chilling or placing the sample on ice</td>
<td>Shortened PT and aPTT</td>
<td>Activation of factor VII on chilling</td>
</tr>
<tr>
<td>Prolonged tourniquet application</td>
<td>False elevation of factor VIII and von Willebrand factor</td>
<td>Due to venous stasis, which elevates the concentration of large molecules</td>
</tr>
<tr>
<td>Inadequate centrifugation</td>
<td>Inaccurate factor assay results; false negative lupus anticoagulant results</td>
<td>Plasma is not platelet poor</td>
</tr>
</tbody>
</table>
### Reference intervals

<table>
<thead>
<tr>
<th>Test</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>11.8-15.0 secs</td>
</tr>
<tr>
<td>aPTT</td>
<td>22.4-38.3 secs</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>220-500mg/dl</td>
</tr>
<tr>
<td>Thrombin</td>
<td>&lt;21 secs</td>
</tr>
</tbody>
</table>
### Critical values

<table>
<thead>
<tr>
<th>PT $&gt;$ 55 secs</th>
<th>INR $&gt;$ 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>aPTT $&gt;$ 137 secs</td>
<td>INR $&gt;$ 6 (patients in oral anticoagulant)</td>
</tr>
<tr>
<td>Fibrinogen $&lt;$ 88 mg/dl $&gt;$ 775 mg/dl</td>
<td>Thrombin time $&gt;$ 60 secs</td>
</tr>
</tbody>
</table>
Principle:
This assay detects the presence of circulating fragments of fibrin degradation products (FDPs) such as fibrinogen and soluble (non-cross linked) fibrin that are produced by the action of plasmin.

Plasmin acts on these 2 substrates similarly, producing an initial cleavage product called fragment X.

Plasmin then acts on fragment X, cleaving it into a transient fragment Y, and fragment D.

Further cleavage of fragment Y, produces the terminal fibrin(ogen) degradation products, fragment D and E.

Thus from one molecule of fibrinogen or soluble fibrin, 2 terminal FDP fragments D and one terminal fragment E is produced.

The mean normal serum FDP is 4.9 ± 2.8 µg/ml

The results should never be interpreted alone, without evaluation of clinical signs and results of other coagulation tests.
D-dimer

- The D-dimer test measures the amount of a protein called “fibrin D-dimer” in the blood.
- Fibrin D-dimer is produced whenever fibrin is being actively degraded somewhere within the vascular system.
- This second process, which limits the growth of a forming blood clot, is mediated by a protein called plasmin. Plasmin degrades the edges of the growing blood clot to make sure it stays just the right size.
- The amount of D-dimer found in the blood reflects the amount of active blood clot formation that is occurring in the body.
- An elevated blood level of D-dimer indicates that active blood clotting is taking place.
- A positive D-dimer result may indicate the presence of an abnormally high level of fibrin degradation products. It indicates that there may be significant blood clot (thrombus) formation and breakdown in the body.
Specific inhibitors of clotting factors may prolong aPTT (most common factor VIII)

Prolonged aPTT of unknown cases should be evaluated by mixing studies
<table>
<thead>
<tr>
<th>Immediate aPTT</th>
<th>Correction &lt;15%</th>
<th>Correction &gt;15%</th>
<th>No correction/partial correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours aPTT</td>
<td>Correction</td>
<td>No Correction</td>
<td>No correction/Partial correction</td>
</tr>
<tr>
<td>Control tube vs. patient</td>
<td>Similar results</td>
<td>Different (patient results longer) Inhibitor dependent on time or temperature</td>
<td>Similar results: Inhibitor not dependent on time or temperature</td>
</tr>
<tr>
<td>Defect</td>
<td>Factor deficiency</td>
<td>Factor VIII inhibitor</td>
<td>Lupus anticoagulant</td>
</tr>
</tbody>
</table>
Thrombin Time assay

- Used to screen for abnormalities in the conversion of fibrinogen to fibrin.
- The addition of thrombin to plasma converts fibrinogen to fibrin bypassing the intrinsic and extrinsic pathway. The time for fibrinogen to clot is a function of fibrinogen concentration.
- Thrombin is added to the patient's plasma and the clotting time measured.
- Reference range: 15-20 seconds
- Common causes of prolonged time: fibrinogen deficiency, heparin, elevated FDP, hyperfibrinogemia
- High concentrations of thrombin result in shorter clotting time.
Platelet count assay

- Part of the routine complete CBC
- Normal ranges: 150-440 x $10^3$/mm$^3$
- Bleeding disorders caused by thrombocytopenia or thrombocytosis should be evaluated by a bone marrow examination.
Bleeding Time assay

- Used to screen inherited platelet dysfunction
- Measures bleeding cessation from a small, superficial wound made under standardized conditions
- The Ivy bleeding time is the preferred method: a blood pressure cuff is placed around the patient’s upper arm and the pressure is raised to 40mmHg. Two small punctures are made along the volar surface of the patient’s forearm. The drops of blood issuing from the bleeding points are absorbed at intervals of 30 seconds into 2 filter paper disks - one for each puncture wound until bleeding ceases. The average of the times required for bleeding to stop is taken as the bleeding time.
- Normal value: <8 minutes
Ivy bleeding time vs. automated analyzers
Thrombocytopenia

**Causes:**

1. failure of bone marrow
   - reduced megakaryocytes, marrow infiltration with tumor, infection, or fibrosis, marrow aplasia,
   - congenital abnormalities, ineffective megakaryopoiesis, megaloblastic anemia,
   - myelodysplasia, alcohol suppression
2. increased platelet destruction
   - immune thrombocytopenia, autoantibody mediated
   - alloantibody mediated, non immune thrombocytopenia,
3. splenic sequestration
4. hemodilution
5. spurious
6. EDTA-pseudothrombocytopenia
Laboratory Tests for thrombocytopenia

- **Bleeding time**: should be prolonged
- **PT and aPTT**: normal results, exclude DIC
- **Platelet aggregation studies**: should not be performed routinely unless an inherited disorder is suspected
- **Heparin-induced thrombocytopenia**: may occur as a complication of heparin therapy; thrombocytopenia may occur after only 2 days in patients who have received heparin therapy previously; positive when there is more than 20% release at 0.1U/ml of heparin and less than 20% release at 100U/ml of heparin.
- **Platelet-associated immunoglobulin**: measures IgG and IgM bound to the patient’s platelets; the results are reported as the number of immunoglobulin molecules per platelet.
- **Tests for TTP**: is a disorder of vWF protease(ADAMTS-13); patients of TTP has levels of <5-10U/ml.
Platelet aggregation studies
Qualitative Platelet disorders

- Main groups: Bernard Soulier syndrome, Glanzmann’s thrombasthenia, thrombopathies.

- **Bernard Soulier syndrome**: failure of platelets to aggregate with ristocetin, aggregation is normal with ADP, epinephrine, collagen and thrombin. Similar to von Willebrand. Platelets are large; mild thrombocytopenia. Defect: abnormal membrane glycoprotein(GPIb-V-IX). Inherited, autosomal recessive, rare. Cd 42 deficiency is observed by flowcytometry.

- **Glanzmann’s thrombasthenia**: no aggregation of ADP, epinephrine, or collagen; however aggregation with ristocetin is normal. Defect in glycoprotein IIb-IIIa. Associated with severe bleeding, inherited, autosomal recessive. Deficiency of Cd41/Cd61.
**Thrombopathies**: abnormalities in the release reaction.

- Divided in 2 subgroups: *storage pool disease* (deficiency of the specialized pool of ADP) and *defects in the mechanism* responsible for the release of the storage pool contents

- Both are characterized by the absence of a secondary wave of aggregation with *epinephrine or ADP*, aggregation with *ristocetin* is normal.

- In *storage pool disease*, there is a decrease in dense granules.

- In the second group, the dense granules appear normal, but fail to release their constituents when platelets are exposed to ADP, epinephrine or collagen. This is the most frequently encountered type in aspirin ingestion.
## Defects of platelet function

<table>
<thead>
<tr>
<th>Defect</th>
<th>ADP Primary/secondary</th>
<th>Epinephrine Primary/secondary</th>
<th>Arachidonic Acid</th>
<th>Collagen</th>
<th>Thrombin time</th>
<th>Ristocetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bernard Soulier</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N or D</td>
<td>D</td>
</tr>
<tr>
<td>Von Willebrand</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>D</td>
</tr>
<tr>
<td>Glanzmann's Thrombasthenia</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>N</td>
</tr>
<tr>
<td>Storage Pool disorder</td>
<td>N or D</td>
<td>D</td>
<td>D</td>
<td>N or D</td>
<td>N or D</td>
<td>+/- N</td>
</tr>
<tr>
<td>Aspirin-like disorder</td>
<td>N or D</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>N or D</td>
<td>+/- N</td>
</tr>
</tbody>
</table>
Laboratory tests for common bleeding disorders

- Primarily on plasma (anticoagulated Na citrate 3.2-3.8%, 9:1 blood/sample ratio, plastic tubes)

<table>
<thead>
<tr>
<th>Disorder</th>
<th>PT</th>
<th>aPTT</th>
<th>Platelet count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Von Willebrand disease</td>
<td>Normal</td>
<td>Normal or Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Hemophilia A or B</td>
<td>Normal</td>
<td>Increased</td>
<td>Normal</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>Normal</td>
<td>Normal</td>
<td>Decreased</td>
</tr>
<tr>
<td>Vitamin K deficiency</td>
<td>Increased</td>
<td>Normal or increased</td>
<td>Normal</td>
</tr>
</tbody>
</table>
Acquired coagulation disorders

- **Causes**: decreased or abnormal synthesis of clotting factors caused by liver disease or DIC, vitamin K deficiency, lupus anticoagulant, antibodies to PT, factor V, VIII, XIII.

- **Liver disease and Vitamin K deficiency**: the liver is the major site of clotting factor synthesis. Hemorrhage occurs in hepatitis and cirrhosis. Vitamin K-dependent procoagulant factors and anticoagulant proteins (protein C, S), are reduced.

- Von Willebrand’s factor is elevated in liver disease. Fibrinolysis may be enhanced in liver disease.

- Coagulopathy of liver disease is complex and affects platelets, coagulation and fibrinolysis.
Inherited Thrombotic Disorders

- **Thrombosis**: formation of a blood clot in the circulatory system during life.
- **Arterial thrombi**: thrombi in the smallest vessels composed of platelets
- **Venous thrombi**: thrombi of fibrin in vessels
- Abnormalities of the protein C pathway, protein S, APC resistance, thrombomodulin constitute half of all cases of inherited thrombosis.
- Inherited thrombosis are transmitted in an autosomal dominant manner and venous thromboembolism is the most common.
- Homocysteinemia is found associated with thrombosis.
- Risk factor: elevated factor VIII, IX, XI
Body **fluids** are one of the unique specimens received in the lab that require multidisciplinary testing.

The types of **fluids** most commonly examined in **hematology** are cerebrospinal (CSF), serous (pleural and peritoneal) and synovial.
Cerebral Spinal Fluid

- CSF is normally a clear, colorless fluid which circulates around the brain and spinal cord, providing a protective cushion for the delicate tissues.

- This cushion is more chemical than physical in nature.

- The CSF protects the brain and central nervous system from sudden changes in pressure and pH, maintains a stable chemical environment, supplies nutrients and removes metabolic waste products.

- The total volume of CSF present is 90-150 mL in adults and 10-60 mL in neonates.

- CSF is a dynamic and constantly changing fluid.

- Laboratory findings can certainly vary accordingly in the presence of disease.

- There are four legitimate reasons, or categories of disease, which warrant the examination of CSF.
  - suspected meningitis
  - detection of subarachnoid hemorrhage
  - detection of central nervous system (CNS) malignancy/leukemia
  - diagnosis of demyelinating disease
Serous Fluids

- Serous fluids include both pleural and peritoneal fluids.
- The pleura and peritoneum are thin, double-layered membranes that surround the lungs and the abdominal/pelvic organs respectively.
- The space between these membranes forms the pleural and peritoneal cavities which are lined by a single layer of mesothelial cells.
- Normally, there is just enough fluid between the two membranes to provide lubrication.
- The normal adult has just 5-15 mL of pleural fluid and less than 50 mL of peritoneal fluid.
- Thus, these are potential cavities and become true cavities only in the presence of diseases which cause an accumulation of fluid.
- Common causes of a pleural effusion are congestive heart failure, infection and malignancy.
- Alcoholic cirrhosis is the most common cause of ascites.
- Pleural and peritoneal effusions are traditionally separated into transudates and exudates for diagnostic purposes.
## Fluids

- **Remind**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Transudate</th>
<th>Exudate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ph</td>
<td>7.4-7.5</td>
<td>7.35-7.45</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>&lt;1.016</td>
<td>&gt;1.016</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>Few</td>
<td>Variable</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>&lt;1,000</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>Glucose</td>
<td>Equal to serum</td>
<td>Decreased</td>
</tr>
<tr>
<td>Protein</td>
<td>&lt;3.0 g/dl</td>
<td>&gt;3.0 g/dl</td>
</tr>
<tr>
<td>Pleural fluid serum protein</td>
<td>&lt;0.5</td>
<td>&gt;0.5 g/dl</td>
</tr>
<tr>
<td>Ldh level</td>
<td>&lt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Pleural fluid LDH</td>
<td>&lt;2.3</td>
<td>&gt;2.3 g/dl</td>
</tr>
</tbody>
</table>

- Congestive heart failure
- Infectious disease
- Cirrhosis with ascites
- Lymphoma
- Rheumatoid arthritis
Synovial Fluid

- Synovial fluid is an ultrafiltrate of plasma combined with a hyaluronate-protein complex produced by the lining cells of the synovial membrane.
- The complex is responsible for the thick and gooey nature of synovial fluid and makes it an excellent lubricant.
- Synovial fluid not only lubricates the joint space, but also provides nutrients to the cartilage.
- Inflammatory joint diseases cause depolymerization of the hyaluronate-protein complex and a resulting decrease in lubricating ability.
- Examination of synovial fluid is usually performed to:
  - * detect sepsis
  - * detect a hemorrhage
  - * diagnose crystal induced inflammation
A thoracentesis was performed on a 64-year-old male with nephrotic syndrome. Identify the cell:

- A) Lymphocyte
- B) Malignant cell
- C) Mesothelial cell
- D) Monocyte

The correct answer is B
This is a pleural fluid, identify the cell type at the end of the arrow.

a) Lymphocyte
b) Malignant Cell
c) Mesothelial Cell
d) Monocyte

The correct answer is C
The special people that cross our path in this life make the journey more beautiful.

Thanks for making mine so beautiful!