Ministry of Higher Education and Scientific Research
The southern Technical University
Amara-Technical Institute

Hematology

Practical (2)

For Students of second stage

Medical Laboratory Department

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2024-202

Syllabus for Hematology practical (2) :

Weeks	Topics
1	Study the Clotting time
2	Hemoglobin electrophoresis
3	Study the Plasma fibrinogen
4	Examination
5	Study the total Count of the W.B.C
6	Differential Count of W.B.C
7	Count the eosinophil
8	L.E Cell
9	Examination
10	W.B.C Series
11	Study the Leukemia
12	Study the Myeloid . L
13	Study the Lymphatic . L
14	Study the monocytic . L
15	Use the Peroxides test for differential diagnosis

The general aim of study practical hematology (2):

1- Comprehend the four main steps of bleeding cessation, identify coagulation factors, and be able to perform and interpret basic tests.
2- Perform the total WBC Count and the differential WBC Count And differentiate between the five main types of white blood cells and calculate the percentage of each type.
3-Understand the principle of Electrophoresis and distinguish between normal hemoglobin types (HbA, HbA2, HbF).

4- Understand the Fundamentals of Leukemia and differentiate between its main types (acute and chronic, myeloid and lymphoid), and know the general risk factors and symptoms.

5- Understand the importance and use of the Myeloperoxidase (MPO) Stain as a vital tool to differentiate between Acute ,(Myeloid Leukemia (AML).

6- Develop the ability to integrate and interpret the results of all mentioned tests (coagulation, cell counts, electrophoresis, stains) to arrive at an accurate diagnosis and monitor hematological diseases.

Target group:

Second Year Students / Medical Laboratory Department

Educational Resources and Tools

1-Presentation and Interaction Technologies
- a-Data Show
- b-Interactive Whiteboard
- c-Laptop
- d-Blackboard and Pens

2-Laboratory Equipment
- a-Microscope
- b-Centrifuge
- c-Micro centrifuge
- d-Water Bath

3-Tools and Consumables
- a-Tourniquet
- b-Cotton and Alcohol
- c-Test Tubes
- d-Glass Slides
- e-Coverslips
- f-Pipettes

4-Laboratory Reagents and Solutions
- a-HCl con0.1
- b-PT reagent
- c-Ammonium oxalate 1 %
- d-Methylene Blue stain

Teaching and Learning Methods and Techniques

1-Enhanced Theoretical Lectures.
2-Practical Demonstrations.
3-Hands-on Laboratory Sessions.
4-Case-Based Learning.
5-Interactive and Collaborative Learning.
6-Self-Directed Learning.

Integrated Assessment Methodology

1-Assessment of Foundational Theoretical Knowledge
  a-MCQs
  b-Quiz
2-Assessment of Practical and Laboratory Skills
  a-Objective Structured Practical Examination
  b-Logbook Performance Assessment
3-Assessment of Integrated Professional Competency
  a-Laboratory Reports
  b-Presentations and Seminars

Lecture time : (Two hour)

Week 1
Lecture title:

Study the Clotting time

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define and differentiate between coagulation, clotting, and hemostasis .
2-Identify and list the thirteen primary coagulation (clotting) factors, recognizing their alternative names where applicable.
3- Explain the key factors that influence bleeding time .
4-Describe the three main components involved in the hemostatic mechanism .
5-Outline the four main steps of hemostasis .
6-Define clotting time and prothrombin time, differentiating between their clinical significance and measurement methods.
7-Identify common causes of abnormal prothrombin time.
8-List common methods for assessing hemostatic function.
9-Describe the principle of the PT test.
10-State the normal reference range.

Pre test

Q1-What is the primary role of coagulation factors ?
a) To transport oxygen
b) To fight infections
c) To form blood clots and stop bleeding .
d) To maintain blood pressure .

Q2-Which step is the first in the mechanism of hemostasis ?
a) Vasoconstriction b) Platelet plug formation
c) Blood coagulation d) Clot retraction.

Study the Clotting time

Blood Coagulation / Clotting

Coagulation or clotting (**Hemostasis**) is the process of forming clots in the walls of damaged blood vessels and preventing blood loss while maintaining blood in a fluid state within the vascular system.

occurs through a series of reaction due to activation of a group of substance, the substance necessary for clotting are called **(Coagulation factors).**

Coagulation factor :-

Are proteins in the blood They help form blood clots to stop bleeding when have an injury . These proteins are also called **(clotting factors).**

The blood have several different types of clotting factors that are all important for making blood clots, this factors is :

1- Fibrinogen (Factor 1).
2- Prothrombin (Factor II) .
3- Thromboplastin (Factor III).
4- Ionized Calcium (Factor IV).
5- Proaccelerin (Factor V).
6- Factor (VI).
7- Proconvertin (Factor VII).
8- Antihemophilic factor (Factor VIII).
9- Chrismats factor (Factor IX).
10- Stuart factor (Factor X).
11- Plasma thromboplastin antecedent (Factor XI).
12- Hageman's factor (Factor XII).
13- Fibrin-stabilizing factor (Factor XIII).

The factors which affect the bleeding time are:-

1- Size and nature of the injury.
2- Condition of the vessel wall.
3- Number of platelets.

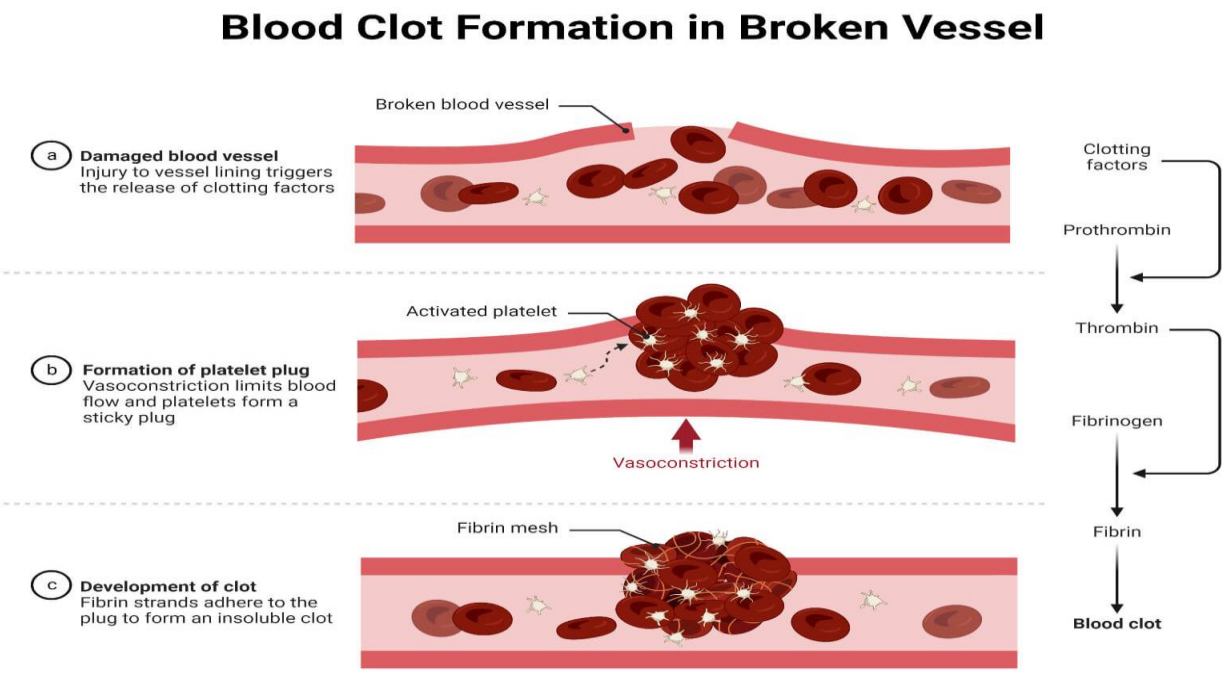
The hemostatic mechanism involves the following:-

1- Properties of the vessel wall.
2- The platelets.
3- The coagulation mechanism.

Mechanism of clotting blood

Hemostasis involves 4 main steps :-

1 -Vasoconstriction .
2 - Platelets plug formation .
3 - Blood coagulation or clotting
4 - Clot retraction.



Stages the blood clotting:-

- Formation of prothrombin activator .
- Conversion of **prothrombin** into **thrombin .**
- Conversion of **fibrinogen** into **fibrin** .

Methods of hemostatic function tests:-

1 - Bleeding time .
2 - Clotting time .
3 - Prothrombin time.

Bleeding time determination :-

Is the time taken from the onset of blood appears from of wound (puncture) until stopping of bleeding (Hemostasis).
Long time of bleeding are found in patients with disorder of **platelets function** and in some patient with **intrinsic vascular defects**.

Methods of bleeding time:-

There are two methods for measuring Bleeding time
1-Duke`s method.
2-Ivey`s method.

<u>1-Duke`s method</u>

Materials:-
1- Filter paper.
2- Timed watch.
3- Disposable lancet.
4- Cotton and 70% alcohol.

Procedure
1- Make the ear lobe warm either by rubbing or by hot water.
2- Clean and sterile the lobe of your subjects ear with 70% alcohol
3- Make a good puncture (by lancet) at the bottom of the ear lobe by inserting the whole pointed end of the lancet at the ear lobe .
4- Star the watch as soon as blood appears.
5- Remove the blood oozing from the wound every 30 second on a clean piece of filter paper , using a different area of the paper each time .
6- Continue until the bleeding stops.
7- Count the spots of the blood.
8- their number will divided by **two** ,the result will be the bleeding time in **minutes** by this method.

The normal bleeding time is :- (2 __ 5 MINUTES)

The bleeding time is prolonged in (purpura)
Is the spontaneous hemorrhages , usually beneath the skin from mucous membrane , and internal organs.

Clotting or coagulation time estimation

Clotting time:- The time taken from the onset of the wound until the formation of the clot without the addition any substance .
Clotting time estimation methods :-

1- Capillary tubes method .
2- Two slide method .

Capillary tubes method

Materials :-

1- Capillary tubes.
2- Timed watch .
3- Cotton , 70% alcohol.
4- Disposable lancet .

Procedure :-

1- Clean the finger with 70% alcohol.
2- Allow the finger to dry.
3- Make a puncture noting the time at which it is do done .
4- Fill the capillary tube with blood .
5- Break of a small pieces of the capillary tube every (30 second) until you notice that the blood has clotted.
6- When the blood clot you will see a thread of clotted blood to each end of the break.
7- Notice the time when clotting is first seen.

Reference range of clotting time: (4 _ 9 min)

Prothrombin time (PT)

Is a blood test that measures how long it takes blood to clot.
prothrombin time or (factor II) is one of clotting factors made by the liver.

A prothrombin time test can be used to check for:-
1- bleeding problems.
2-To check whether medicine to prevent blood clots is working.
3- Check for a low level of vitamin K ,its needed to make prothrombin and other clotting factors.
4- It checks to see if five different blood clotting factors (factors I, II, V, VII, and X) are present.

A PT test may also be called an **INR test** . **(International Normalized Ratio)** stands for a way of standardizing the results of prothrombin time tests, no matter the testing method.
An abnormal prothrombin time is often caused by **liver disease or injury or by treatment with blood thinners .**

Prothrombin deficiency can be caused by :-
1- Lack of vitamin K (some babies are born with vitamin K deficiency) .
2- Liver disease .
3- Use of medicines that prevent clotting (anticoagulants such as Warfarin).

Principles

When reagent is add to normal anticoagulated plasma, the clotting mechanism initiated forming solid gel clot within a specified period of time.

Material:-

- Blood sample.
- Test tube contains anticoagulant (Sodium Citrate).
- PT reagent.
- Centrifuge.
- Water path.
- Stopwatch.

Procedure :-

1-Mix nine parts of freshly collected blood with one part of sodium citrate then centrifuge immediately for 10 min at 2500 rpm.

2-Add 0,1 ml of plasma In test tube and place tube in water bath for 3-5 min at37 c.

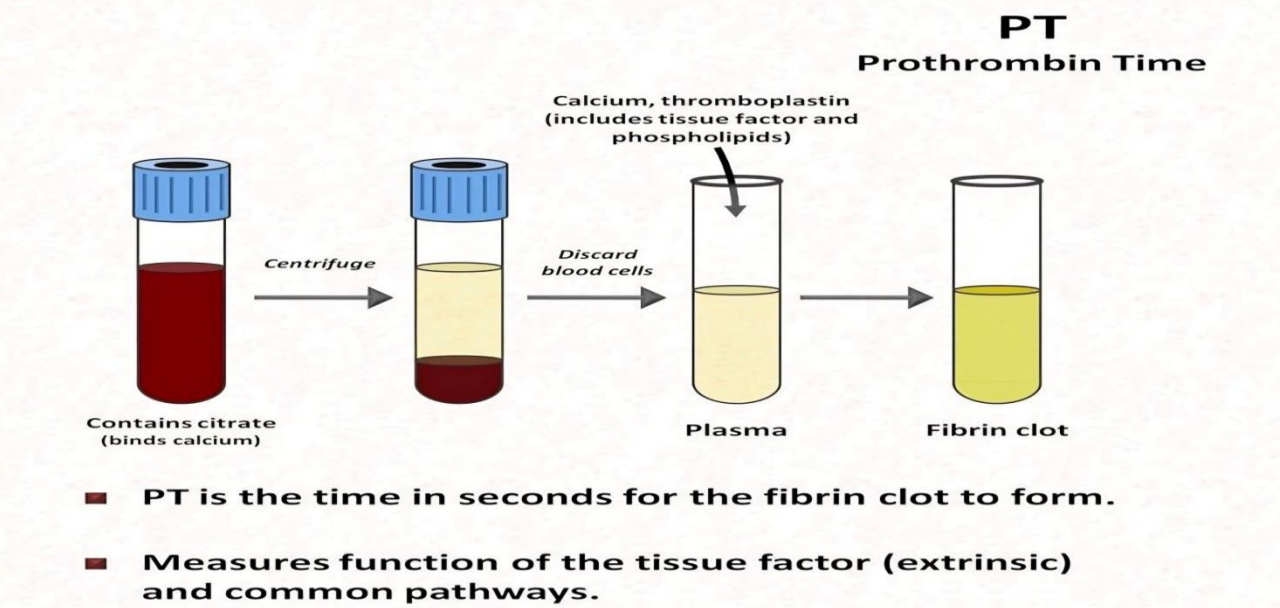
3-Add 0,2ml of reagent, simultaneously start stopwatch.

4-Gently tilt the tube back, forth and stop the stopwatch as soon as.

5-The first fibrin strand is visible and the gel clot formation begins.

6-Record the time in seconds.

The reference range for Prothrombin time is usually around **(11-16) seconds**.



Post test

Q1- Coagulation factors are primarily?
a) Carbohydrates.
b) Lipids .
c) Proteins.
d) Nucleic acids.

Q2-Which of these factors affects bleeding time?
a) Blood group.
b) Size and nature of the injury.
c) Age of the individual.
d) Body temperature.

Q3- A normal bleeding time using the Duke's method typically falls within the range of?
a) 0 - 1 minute.
b) 2 - 5 minutes.
c) 6 - 10 minutes.
d) 11 - 15 minutes.

Q4- Compare and contrast "clotting time" and "Prothrombin Time (PT)," highlighting their definitions, the factors they assess, and their primary clinical applications?

Q5- List at least three different conditions or situations that can lead to an abnormal Prothrombin Time (PT) result?

Week 2
Lecture title:

Hemoglobin Electrophoresis

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define electrophoresis and its application in separating biomolecules.
2-Explain the principle of hemoglobin electrophoresis, including how different hemoglobin types separate based on their charge.
3-Identify the major types of normal hemoglobin (HbA, HbA2, HbF) and their relative proportions in different developmental stages.
4-List the essential materials and equipment required to perform hemoglobin electrophoresis.
5-Outline the basic steps of the hemoglobin electrophoresis procedure.
6-distinguishing between normal patterns and those indicative of hemoglobinopathies.
7-Discuss the clinical significance and applications of hemoglobin electrophoresis in diagnosing and monitoring hemoglobin disorders.

Pre test

Q1- Hemoglobin is a tetramer composed of how many polypeptide chains?
a) One b) Two
c) Three d) Four

Q2- Which of the following is the most common form of hemoglobin in healthy adults?
a) Hemoglobin F (HbF) b) Hemoglobin A2 (HbA2)
c) Hemoglobin A (HbA) d) Hemoglobin S (HbS)

Hemoglobin Electrophoresis

Electrophoresis is a laboratory technique used to separate DNA, RNA or protein molecules based on their size and electrical charge. An electric current is used to move the molecules through a gel or other matrix.

Hemoglobin electrophoresis is a laboratory technique used to separate and identify different types of hemoglobin based on their electrical charge.

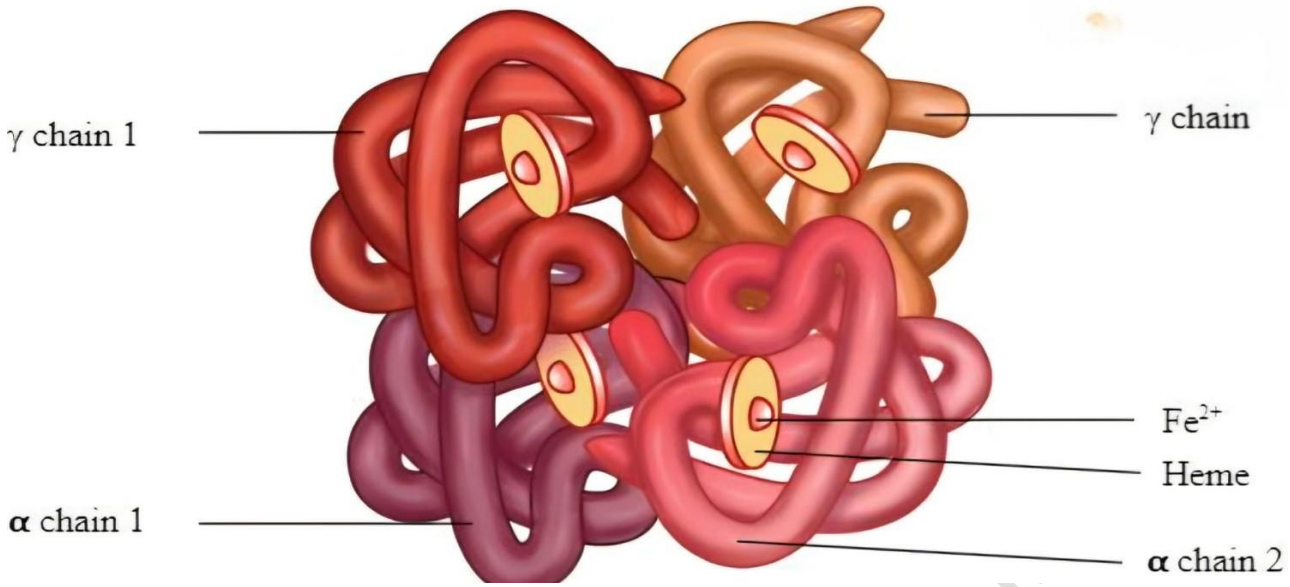
Hemoglobin is tetramer with four polypeptide chain **(two alpha α and two beta β)** each of the polypeptide chain has iron atom.

Types Of Hemoglobin

1- **Hemoglobin A**:-is the most common adult form of hemoglobin and exists as a tetramer containing two alpha subunits and two beta subunits (α2β2).

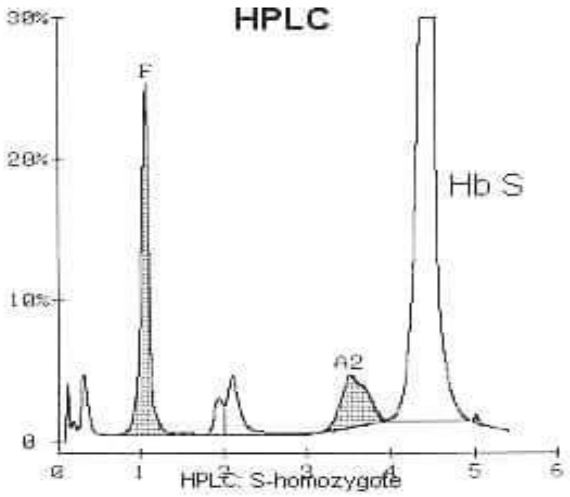
2- Hemoglobin A2 (HbA2):-is a less common adult form of hemoglobin and is composed of (two alpha and two delta-globin) subunits. This hemoglobin makes up 1-3% of hemoglobin in adults.

3- Hemoglobin F (alpha2gamma2):-is the major hemoglobin in fetal red blood cells (RBCs) during gestation and constitutes 60 to 80 percent of total hemoglobin in the full-term newborn. By approximately(6 to 12)months of age, Hb F is almost completely replaced by adult hemoglobin.



Principles

- Hemoglobin molecules have **different charges** based on their structure.
- The technique uses an electric field to separate them.
- Different hemoglobin variants migrate at different speeds.

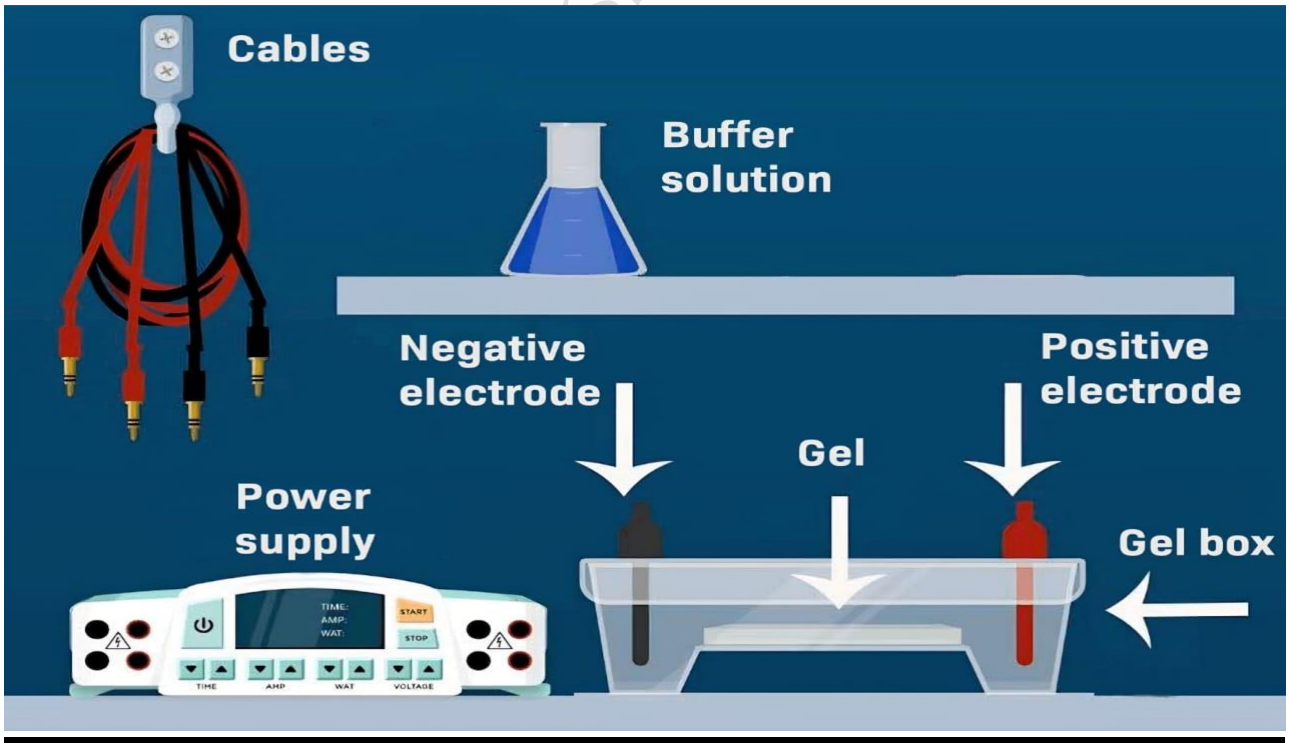


Materials and Equipment

- Hemolysate sample .
- Electrophoresis chamber .
- Buffer solution .
- Agarose or cellulose acetate gel .
- Staining solution .
- Power supply .

Procedure

- Prepare the hemolysate from a blood sample.
- Load the sample onto the gel.
- Place the gel in the electrophoresis chamber.
- Apply the electric current.
- Stain and analyze the bands.



Interpretation of Results

- Normal hemoglobin pattern includes HbA , HbA2, and HbF.
- Abnormal bands indicate hemoglobin opathies such as sickle cell disease (HbS) or thalassemia (HbH, HbE).

Clinical Applications

1- Diagnosis of sickle cell anemia and thalassemia.
2- Identification of hemoglobin variants.
3- Monitoring of hemoglobin disorder

Post test :

Q1-Hemoglobin electrophoresis separates different types of hemoglobin based on their?
a) Molecular weight
b) Antigenicity
c) Electrical charge
d) Oxygen-carrying capacity

Week **3**
Lecture title:

Study the plasma fibrinogen

Learning objective:

By the end of this lecture, university students should be able to:
1-Define fibrinogen (Factor I) and explain its primary role as a soluble plasma glycoprotein in the coagulation cascade.
2-Identify the primary site of fibrinogen synthesis and explain its regulation as an acute phase reactant.
3-Differentiate between quantitative fibrinogen disorders.
4-Explain the nature of dysfibrinogenemia as a qualitative fibrinogen disorder, outlining its genetic basis and variable clinical presentations (bleeding and/or thrombosis).
5-Compare and contrast the principles, advantages, and limitations of quantitative fibrinogen assays.

Pre test

Q1- Primarily synthesized of fibrinogen is ?

a) Bone marrow b) Liver
c) Kidney d) Skin

Study the plasma fibrinogen

Plasma fibrinogen is a key protein in blood coagulation, inflammation, and disease risk.it is a major plasma protein produced primarily in the liver and encoded by three genes (**FGA**, **FGB**, **FGG**). It is secreted glycoprotein and serves as the precursor to fibrin, forming the structural basis of blood clots after vascular injury. Its expression is up regulated by inflammatory cytokines, and levels can rise significantly during acute inflammation.

Clinical Significance

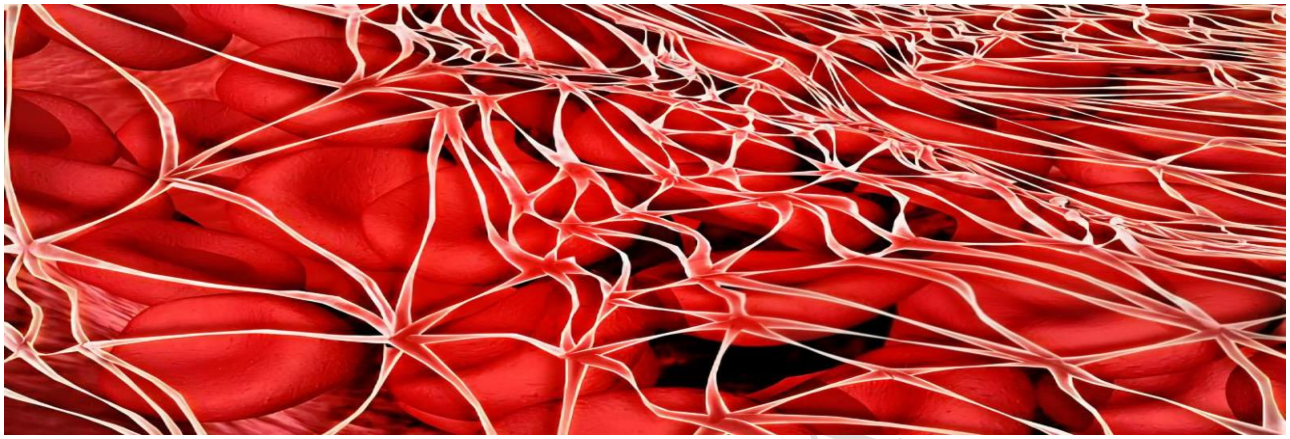
1-Hemostasis and Bleeding: Low plasma fibrinogen increases bleeding risk due to impaired clot formation. In trauma, fibrinogen levels drop earlier and more frequently than other coagulation factors, predicting massive bleeding and mortality .

2-Cardiovascular Disease: Elevated plasma fibrinogen is an independent risk factor for cardiovascular events, High levels are associated with atherosclerosis and thrombosis.

3-Other Diseases: Plasma fibrinogen is a qualified biomarker for prognosis in chronic obstructive pulmonary disease (COPD) and is linked to cancer prognosis, especially in digestive cancers.

Synthesis and Regulation

Primarily synthesized in the liver by hepatocytes , Acute phase reactant: upregulation of synthesis during inflammation, infection, and tissue injury.



Red blood cells with fibrin

Quantitative Disorders

A- **Hypofibrinogenemia**: Reduced levels of functionally normal fibrinogen .
Causes:
1- Liver disease.
2- DIC (consumption).
3- Severe hemorrhage.
4- Congenital forms.

Clinical Manifestations:
Bleeding diathesis (usually mild unless severe deficiency)

B- A fibrinogenemia: Complete absence of fibrinogen.
Causes:
Genetics: Autosomal recessive inheritance (mutations) .

Clinical Manifestations:
Severe bleeding from birth, omphalorrhagia, intracranial hemorrhage, recurrent hemarthroses. Paradoxically, some thrombotic episodes can occur .

C- Hyperfibrinogenemia: Elevated levels of fibrinogen.

Causes:
Acute phase response (inflammation, infection, trauma, surgery), cardiovascular disease (risk factor), malignancy, smoking, obesity

Clinical Significance:
Independent risk factor for thrombotic events (venous and arterial thrombosis.

Qualitative Disorders

-Dysfibrinogenemia:

Production of functionally abnormal fibrinogen molecules with normal or near-normal antigenic levels.

Genetics: Often autosomal dominant inheritance.

Clinical Manifestations:
Bleeding tendency: Impaired fibrin polymerization, weak clot.

A. Quantitative Assays :

Clauss Clotting Method (Gold Standard)

Principle: Measures the time it takes for a dilute plasma sample to clot after the addition of a high concentration of thrombin. The clotting time is inversely proportional to the fibrinogen concentration.

Advantages: Widely available, sensitive to fibrinogen functional.

Immunological Assays
Principle: Measures the antigenic concentration of fibrinogen using anti-fibrinogen antibodies.
Advantages: Not affected by functional abnormalities .

B. Qualitative Assays (for Dysfibrinogenemia)

Thrombin Time (TT):
Prolonged in dysfibrinogenemia (and hypofibrinogenemia, heparin).

Fibrin Polymerization Curve Analysis:
Research tool to assess the kinetics and structure of fibrin clot formation.

Genetic Testing:
Gold standard for definitive diagnosis of congenital afibrinogenemia and specific dysfibrinogenemias .

D-Dimer: Useful for assessing fibrin formation and degradation (fibrinolysis). Elevated in thrombotic states.

Post test

Q1- A complete absence of fibrinogen from birth, often leading to severe bleeding, is characteristic of ?
a) Hypofibrinogenemia
b) Afibrinogenemia
c) Hyperfibrinogenemia
d) Dysfibrinogenemia

Week 4 Examination
Week 5
Lecture title:

The Total Count Of The W.B.C

Learning objective:

Upon completion of this lecture, students will be able to:

accurately describe the process of total white blood cell (WBC) counting and differential leucocyte counting, including the underlying principles, necessary materials, procedural steps, and clinical significance of normal and abnormal ranges.

Pre test

Q1- What is the primary function of white blood cells ?

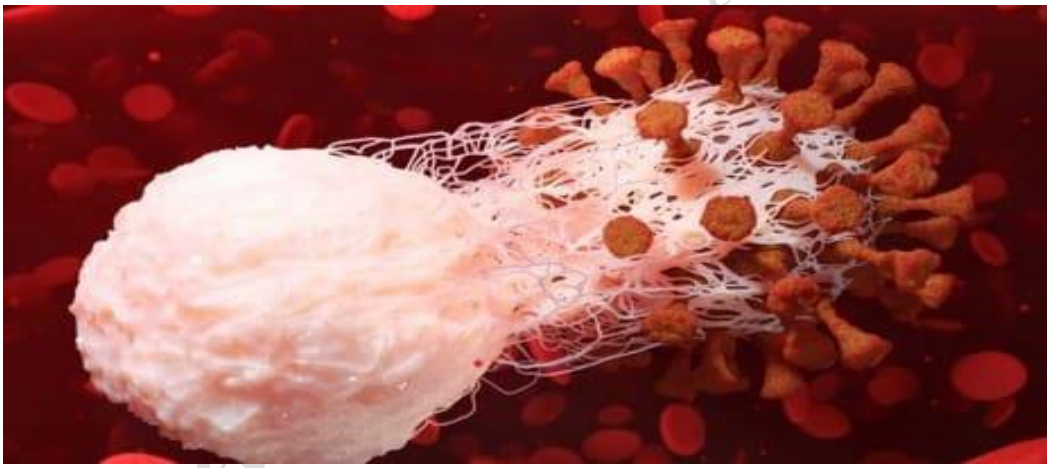
Q2- What is "phagocytosis ?

The Total Count Of The W.B.C

It is counting the number of different type of leucocyte in one cubic millimeter of blood .

Function of the white blood cells

Fight bacterial infection , they have the ability to ingest small particles , virus,bacteria and cell debris .this power is called **(Phagocytosis).**



White blood cell immune phagocytosis coronavirus

The principle

The blood is diluted 20 timed with the W.B.C diluting fluid causing lysis of the red blood cells and leaving the W.B.C with deep violet – black color of the nucleus.
The white blood cells (Leukocytes) are larger than the red blood cells ,measuring about 10 Micron in diameter, and they are less numerous , and there are **(4000 – 11000)**cells per cubic millimeter (mm) of blood.

The diluting solution called (TURK`S)fluid consist from :

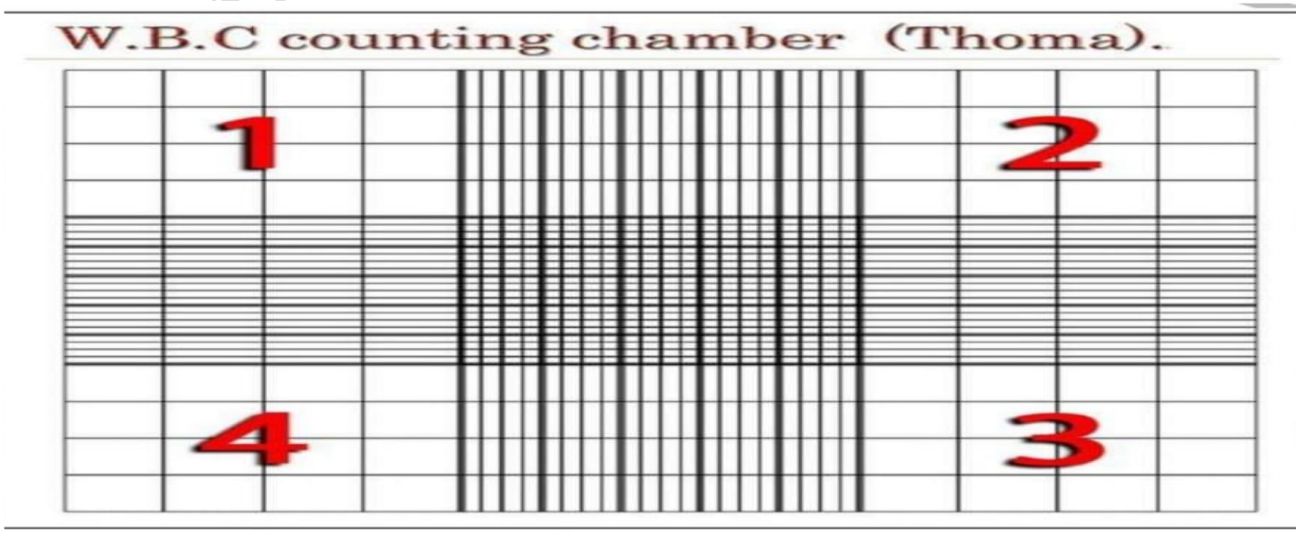
1- Glacial Acetic acid 1.5 Ml.
2- Methyl alcohol 1% (or gentian violet) in water 1 mL.
3- D.W (Distilled water) to 100 mL Material Required .

Material Required

1- Blood sample (0.02) mL
2- Haemocytometer
3- Micropipette 0.02 mL
4- Test tube
5- Test tube rack
6- Microscope

Procedure

1- Measure 0.4 mL of W.B.C diluting fluid and transferee it of test tube.
2- Measure 0.02 mL of blood by micropipette and dilute in the test tube which contain 0.4 mL of diluting fluid.
3-Mix the solution for 2 min.
4- Put one drop of the solution on the chamber. And leave cell or settle not more than 1 – 2 min .
5- Count the cell in the 4 corner square.



Calculation

1- Number of W.B.C one square x **200**
2- Number of W.B.C two square x **100**
3- Number of W.B.C four square x **50**

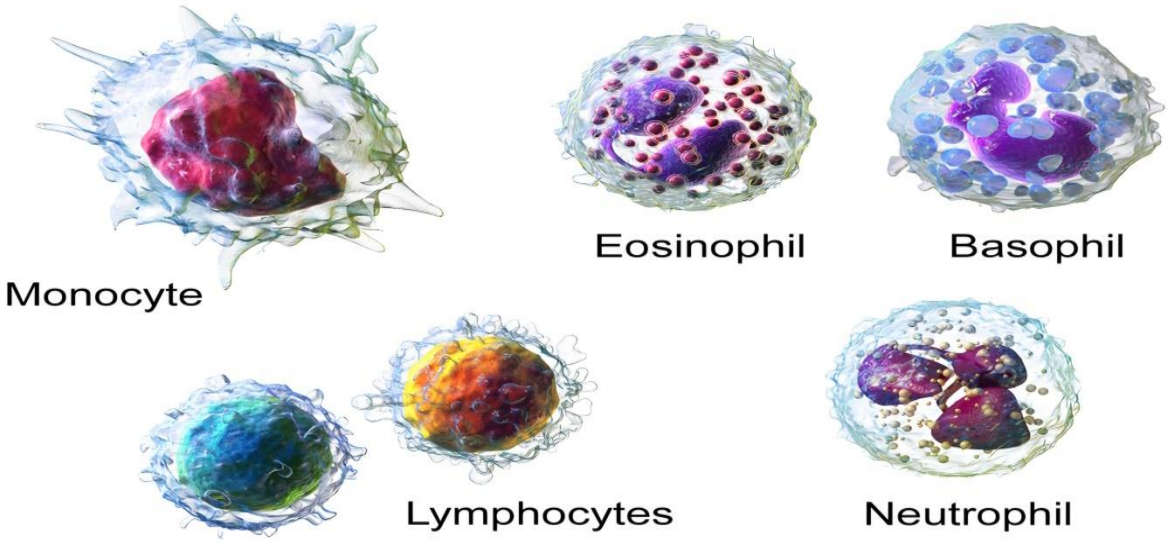
Normal range

- Adult (male ,female) 4000-11000 cells/cu.mm
- Children (1-10) 4500-13000 cells/cu mm
- Infant (at birth) 10000-25000 cells /cu mm

The white blood cell (Leucocytes)are divide in to two group
1- **Granulocytes** :- (10 – 14) micron in diameter
Characterized by the presence granules in the cytoplasm and a lobed nucleus.
Using Leishman`s stain , three types of cells can be recognizes by the character of their granules:-
a- **Neutrophil** :- include (65%) from the total count of white blood
cells with the fine red brown granules.
b- **Eosinophil** :- include (4%) from the total count of white blood cells, with large red granules.
c- **Basophil** :- include (1%) from the total count of white blood cells containing purple – blue granules.
so the granulocytes include (70 %) from the total white blood cells count.

2- **A granulocytes**
a- **Lymphocytes** :- divided in to
1-.small lymphocytes (7 – 10) micron
2-.Large lymphocytes (10 – 14) micron
These are round non – granular cells with large round nuclei which fill the cell substance.
The lymphocytes include (25 %)of white blood cells count.

b- **Monocytes** :- the largest type (10 - 18) micron with round or kidney shaped nucleus ,and non granular cells include (5 %) of white blood cells count.



White Blood Cells

Post test

Q1- Turk's fluid is used in WBC counting primarily to?
a) Stain red blood cells
b) Cause lysis of red blood cells
c) Enhance WBC visibility by making them colorless
d) Increase the number of WBCs

Q2- Which type of white blood cell constitutes approximately 65% of the total WBC count and has fine red-brown granules?
a) Eosinophil
b) Basophil
c) Neutrophil
d) Lymphocyte

Week 6
Lecture title:

Differential Count Of W.B.C

Learning objective:

Upon completion of this lecture, students will be able to:
1-Define the differential white blood cell (WBC) count and explain its clinical significance.
2-Identify and describe the five main types of white blood cells.
3-Correlate abnormal differential WBC counts with potential underlying conditions such as infections, inflammatory responses, autoimmune diseases, and various types of cancer
4-Outline the step-by-step procedure for performing a manual differential leukocyte count using a stained blood film and the Leishman staining technique.
5-Interpret differential WBC count results, including understanding normal reference ranges and identifying deviations that may indicate pathology .
6-Discuss the clinical implications of both abnormally high (leukocytosis) and abnormally low (leukopenia) total WBC counts and their associated causes .

Pre test

Q1- What is the primary purpose of a differential white blood cell (WBC) count?
a) To measure the total number of red blood cells .
b) To determine the percentage of each type of WBC.
c) To assess the clotting ability of blood .
d) To identify different types of platelets .

Q2- Name the five main types of white blood cells ?

Differential Count Of W.B.C

The differential W B C count is the percentage of each type of W B C which can indicate the presence of infection, disease, or an allergic reaction .

It is relative of each type of WBC present in blood

(**Neutrophil, Eosinophil, Basophil, Lymphocyte, Monocyte**).

It is also determine if there is abnormal or primitive type of these cells in the blood .

white blood cells are made in the bone marrow, which is the soft, spongy tissue . Sometimes the bone marrow stops making enough white blood cells.

In other cases, destroying the white blood cells faster than can make them. If the white blood cell count is too low, have a higher chance of getting an infection because it doesn't have enough immune cells to help fight off invaders. This often happens with diseases like HIV, or chemotherapy and radiation treatment for cancer.

Other diseases that affect blood cell levels include:

- Blood cancer .
- Infection .
- Autoimmune diseases .
- Allergic reaction .
- Liver disease .
- Spleen disease .

A high white blood cell count is also an abnormal result and might be due to :-

- High stress .
- Thyroid disease .
- Rheumatoid arthritis .
- Gout .
- Injury .
- Smoking .
- Viral infection .
- Bacterial infection .
- Leukemia .
- Parasite infection .
- Allergic reaction .

A low white blood cell count can be caused by different things, including:

- Anemia .
- Chemotherapy treatment .
- Flu .
- Radiation treatment or exposure .
- Severe bacterial infection .
- Viral infection .
- HIV .
- Leukemia .
- Steroid use .

The procedure of Differential leucocyte count is done stained blood film on ordinary thin microscope slide which done by the following step :-

1- Finger puncture ,blood sample .
2- Leishman stain and staining rack .
3- Slide& spreader with smooth edge .
4- Distal water .

Preparation of leishman stain :

- 0.2 gm of leishman powder
- 100 ml methanol
- Mix with warm for 15 minute with occasional shaking .
- The solution then is filtered

Procedure:

1-Spread drop of patient blood on the slide by the spreader of an angle about 45° to the slide, and then moved back to contact with the drop &spread quickly along the line of contact of the spreader with the slide .

The film must be(**not be too thin and not too thick)** and the tail of the film should be smooth .

2-Marked the slide of each patient on beginning of smear (thick area).
3-Leave to dry on the air .
4-Put the slide on the rack.
5-Put (10) drops of the leishman stain on the blood film & leave
6-Add for (30 second -1 minute).
equal or double from fresh distal water ,observe the appearance of a violet mirror on top of the stain.
7-Leave 10-15 minute .
8-Wash with fresh D.W .
9-Clean the back of the slide by water.
10-Leave to dry in the air.
11-Examine under the microscope using oil immersion lens.

Normal range :

Neutrophil (N): 2500-7500 cells/cu.mm
Eosinophil (E): 40-440 cells/cu.mm
Basophil (B) : 20-100 cells/cu.mm
Lymphocyte(L) :1500-4500cells/cu.mm
Monocyte (M) : 200-800 cells/cu.mm

Post test

Q1- Explain the difference between a total white blood cell count and a differential white blood cell count?

Q2- For each of the following WBC types, state its primary function in the immune system ?

a- Neutrophil
b- Eosinophil
c- Basophil
d- Lymphocyte
e- Monocyte

Week 7
Lecture title :

Count the Eosinophil

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define eosinophil's, describing their characteristic morphology and primary components
2-Explain the key physiological roles of Eosinophil in the human body.
3-Recall the normal reference range for absolute eosinophil count (AEC) and categorize different levels of eosinophilia (mild, moderate, severe).
4-Identify and list the common causes of elevated eosinophil counts.
5-Describe the principles behind manual eosinophil counting, including the function of specialized diluting fluids and the use of a hemocytometer .
6-Outline the step-by-step procedure for performing a manual eosinophil count.
7-Apply the correct formula to calculate the absolute eosinophil count from manual counting data.
8-Recognize and list potential sources of error in manual eosinophil counting.

Pre test

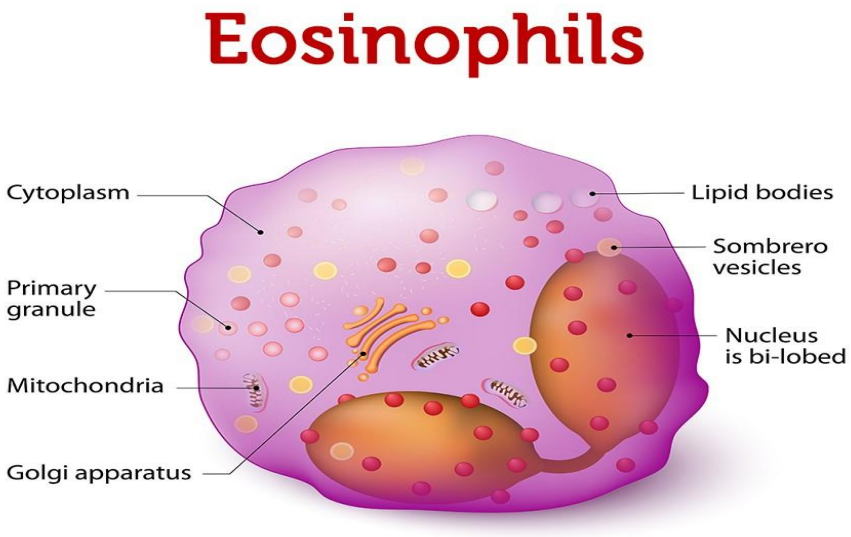
Q1- Eosinophil's are most well-known for their primary role in fighting which type of infection?
a) Bacterial infections b) Viral infections
c) Fungal infections d) Helminth (worm) infections

Q2- A patient with an absolute eosinophil count of 600 cells/µL would be classified as having?
a) Normal eosinophil count b) Mild eosinophilia
c) Moderate eosinophilia d) Severe eosinophilia

Count the Eosinophil

Eosinophil is a type of white blood cell (leukocyte) characterized by their distinctive bilobed nucleus and large, bright red-orange granules when stained with eosin (an acidic dye). These granules contain a variety of proteins, including :

1- Major Basic Protein (**MBP**)
2- Eosinophil Cationic Protein (**ECP**)
3- Eosinophil Derived Neurotoxin (**EDN**)
4- Eosinophil Peroxidase (**EPO**) which play crucial roles in their functions .



functions of Eosinophil

1-Allergic Reactions: They are key effector cells in allergic diseases like asthma, allergic rhinitis, and atopic dermatitis. They release pro-inflammatory mediators that contribute to tissue damage and symptoms.

2-Parasitic Infections: They are particularly effective against helminthes (worm) infections. Their granules contain proteins that are toxic to parasites.

3-Immune Modulation:
They can also participate in modulating immune responses, including antigen presentation and cytokine production.

Normal Reference Range:
Absolute Eosinophil Count (AEC):Typically **50-500** cells/mu L

Elevated Eosinophil Count: An increase in Eosinophil's above the normal range.

-Mild Eosinophilia: 500-1500 cells/mu L
-Moderate Eosinophilia: 1500-5000 cells/mu L
-Severe Eosinophilia (Hypereosinophilia): >5000 cells/mu L

Common causes:

1-**Allergies**: Asthma, hay fever, drug reactions, eczema .
2-**Parasitic Infections**: Especially helminthes (e.g., Ascaris, Strongyloides, Trichinella).
3-**Autoimmune Diseases**: Certain types of vacuities, rheumatoid arthritis .
4-**Cancers**: Lymphomas (especially Hodgkin lymphoma), certain leukemias (e.g., chronic myeloid leukemia, acute myeloid eosinophilia), hypereosinophilic syndromes leukemia with.
5-**Adrenal Insufficiency** (Addison's disease).

Eosinopenia : Decreased Eosinophil Count, A decrease in eosinophil's below the normal range

Common Causes :

1-Stress and Acute Infections: Release of corticosteroids can suppress eosinophil production.

2-**Cushing's Syndrome**: Excess cortisol.

3-**Corticosteroid Therapy**: Exogenous corticosteroids.

Eosinophil counts

Eosinophil counts can be performed using various methods, ranging from manual techniques to automated analyzers.

I. Manual Eosinophil Count (Direct Method)

This method involves using a specialized diluting fluid that stains eosinophil's and lyses other cells, followed by counting in a hemocytometer. While largely replaced by automated methods, it's important for understanding the principles and for use in resource-limited settings or for confirmation.

A specific diluting fluid

e.g., Pilot's solution, Randolph's solution, or a simpler Eosin-) Acetone solution) is used to selectively stain eosinophil's red while lysing red blood cells and other white blood cells (or rendering them invisible). The stained eosinophil's are then counted in a counting chamber.

Material Required

1- Neubauer improved hemocytometer with cover slip.

2- Eosinophil diluting fluid (e.g., Pilot's solution: 1% eosin in 50% propylene glycol, with a small amount of lithium carbonate to lyse red cells).

3- micropipette with tips.

4-Microscope.

5- Blood sample (EDTA anticoagulated blood).

6- Lint-free wipes Procedure (using Thoma pipette and standard dilution).

Procedure:

1- Draw blood to the 0.5 mark in the WBC pipette .
2- Wipe excess blood from the outside of the pipette.
3- Draw eosinophil diluting fluid to the 11 mark, creating a 1:20 dilution.
4- Mix thoroughly for 2-3 minutes using a mechanical mixer or by hand.
5- Discard the first few drops (2-3 drops) to ensure only the mixed sample is used.
6- Load the hemocytometer: Carefully load both chambers of the hemocytometer, avoiding overfilling or air bubbles.
7- Allow to settle for 5-10 minutes in a moist chamber to prevent drying and allow cells to settle.
8- Place the hemocytometer on the microscope stage.
9- Start with 10 objective to locate the counting area.
Switch to 40 objective for counting.
10- Count the Eosinophils in all nine large squares of the hemocytometer.

Sources of Error in Manual Counting:

1- Improper mixing of blood and diluting fluid.
2- Inaccurate pipetting
3- Uneven filling of the hemocytometer
4- Presence of air bubbles
5-Failure to allow cells to settle
6- Clumping of cells
7- Identification errors (e.g., confusing Eosinophils with other stained cells or debris).

II. Automated Eosinophil Count (Indirect Method)

This is the most common method in modern laboratories, performed by automated hematology analyzers as part of a CBC with differential.

Post test

Q1- Which characteristic best describes the nucleus of an eosinophil?
a) Multi-lobed (3-5 lobes)
b) Kidney-shaped
c) Bilobed
d) Round

Q2- In manual eosinophil counting, the diluting fluid is designed to?
a) Stain all white blood cells equally
b) Lyse red blood cells and selectively stain Eosinophils
c) Only lyse Eosinophils
d) Prevent staining of any cells

Q3- Automated hematology analyzers typically determine Eosinophil counts as part of?
a) A blood culture
b) A complete blood count (CBC) with differential
c) A coagulation panel
d) A urine analysis

Week 8

Lecture title:

The L.E CELL (Lupus Erythematous)

Learning objective:

By the end of this practical lecture, students will be able to :

1-Identify what this cell is and its components.

2-Comprehend how LE cells are formed and the immunological basis behind it.

3-Know the tools and steps required to perform the LE cell test in the laboratory.

4-Understand how to read and interpret test results positive/negative.

5-Understand the role of the LE cell test in diagnosing Systemic, Lupus Erythematous (SLE) and its position compared to more sensitive modern tests.

6-Understand the importance of applying safety and accuracy procedures to ensure reliable results.

Pre test

Q1- What is a Lupus Erythematous (LE) cell?

a) A damaged red blood cell

b) A plasma cell that produces antibodies

c) A neutrophil or macrophage that has engulfed denatured nuclear material

d) A lymphocyte that activates against self-tissues

Q2- What is the primary role of the LE cell test?

a) To detect immature white blood cells

b) To diagnose anemia

c) To assist in the diagnosis of Systemic Lupus Erythematous (SLE).

d) To detect bacterial infections

The LE CELL (Lupus Erythematous)

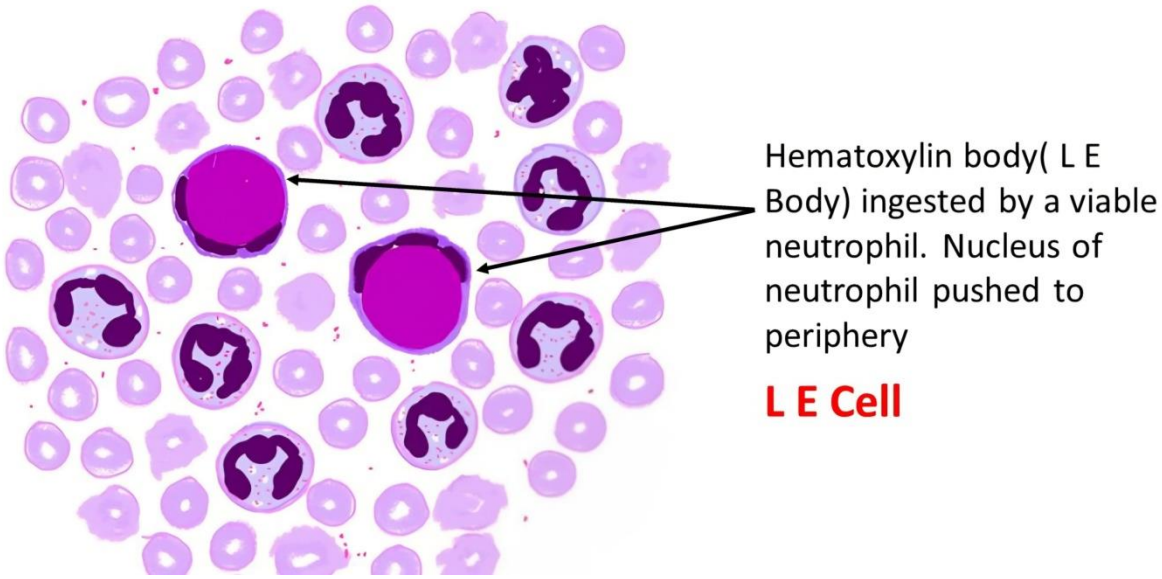
A lupus erythematous cell (LE cell), also known as Hargraves cell, is a neutrophil or macrophage that has phagocytized (engulfed) the denatured nuclear material of another cell.

The denatured material is an absorbed hematoxylin body (also called an LE body).

The LE (Lupus Erythematous) cell test is a laboratory technique used in the diagnosis of systemic lupus erythematous (SLE).

It detects the presence of LE cells, which are neutrophils that have engulfed nuclear material from damaged cells.

L E Cell (Lupus Erythematosus cell)



Principle

LE cells are formed when antinuclear antibodies (ANA) bind to the nuclei of damaged cells, causing them to be engulfed by neutrophils. The LE cell test is performed using peripheral blood or bone marrow samples and involves staining techniques to visualize these cells under a microscope.

Materials Required

1- Patient's Blood Sample (Anti coagulated with Heparin).
2- Reagent.
    -Phosphate-buffered saline (PBS).
    -Hypotonic solution to lyse red blood cells.
    -Wright's or Giemsa stain.
3- Glass Slides & Coverslips.
4- Centrifuge.
5- Microscope.
6- Pipettes & Test Tubes.

Procedure

1-Blood Sample Preparation :
Collect 2–5 mL of blood in a heparinized tube to prevent clotting.
2- Induction of LE Cells :
Mix the blood sample with a hypotonic solution to lyse red blood cells.
Allow the sample to incubate at 37°C for 1–2 hours, promoting LE cell formation.
3- Centrifugation :
Centrifuge the sample at 2500 rpm for 10 minutes to separate cells.
4- Smear Preparation :
Prepare a thin blood smear from the pellet.

Air dry and fix the smear
5- Staining :
Stain the slide with Wright's or Giemsa stain for (10–15) minutes.
Wash with PBS and allow it to dry.
6- Microscopic Examination :
Examine under 100x oil immersion objective.
Identify LE cells, which appear as neutrophils engulfing homogenous nuclear material.

Interpretation of Results

-Positive Result :
Presence of LE cells, indicating a possible diagnosis of SLE.
-Negative Result :
Absence of LE cells (does not rule out SLE, as other tests like ANA and anti-ds DNA are more sensitive).

Clinical Significance

A positive LE cell test supports the diagnosis of SLE, but it has been largely replaced by ANA and anti-ds DNA antibody tests due to its lower sensitivity.
LE cells may also be seen in drug-induced lupus and some other autoimmune disorders.

Precautions

1- Use fresh blood samples for accurate results.
2- Avoid contamination and over-staining , which can obscure cell morphology.
3- Always compare findings with other laboratory tests to confirm the diagnosis.

Post test

Q1- What solutions are used to stain slides for the LE cell test?
a) Gram stain or Ziehl-Neelsen stain
b) Wright's stain or Giemsa stain
c) Methylene blue stain
d) Crystal violet stain

Q2- What is a positive result for the (LE) cell test?
a) Presence of clumped red blood cells
b) Absence of neutrophils
c) Presence of Lupus Erythematous (LE) cells
d) Presence of immature white blood cells

Week 9 : Examination
Week 10
Lecture title:

W.B.C Series

Learning objective:

By the end of this practical lecture, students will be able to :

1-Categorize white blood cells (WBCs) into their main groups (granulocytes and a granulocytes) and identify the specific cell types within each.

2-Describe the key morphological characteristics and primary functions of each type of white blood cell.

3-Recall the normal reference ranges for total WBC count and the differential percentages of each WBC type in adults.

4-Define common terminology related to WBC count abnormalities.

5-List the common causes associated with increased and decreased total WBC counts.

6-Explain the principles and procedures for performing a manual total WBC count .

7-Appreciate the role of automated hematology analyzers in modern laboratory diagnostics.

Pre test

Q1- Which of the following white blood cells is typically the first responder to bacterial infections?

a) Lymphocyte
b) Eosinophil
c) Neutrophil
d) Basophil

Q2- A normal total white blood cell count for an adult is generally in the range of ?

a) 1,000 - 3,000 cells/microliter
b) 4,000 - 11,000 cells/microliter
c) 15,000 - 20,000 cells/microliter
d) 50,000 - 100,000 cells/microliter

W.B.C Series

White blood cells (WBCs), or leukocytes, are essential components of the immune system, playing a vital role in defending the body against infection and other diseases.

White blood cells are divided into two main groups :

1- Granulocytes

-**Neutrophils**: the first line of defense against bacterial and fungal infections. Characterized by fine granules and a multi-lobed nucleus.

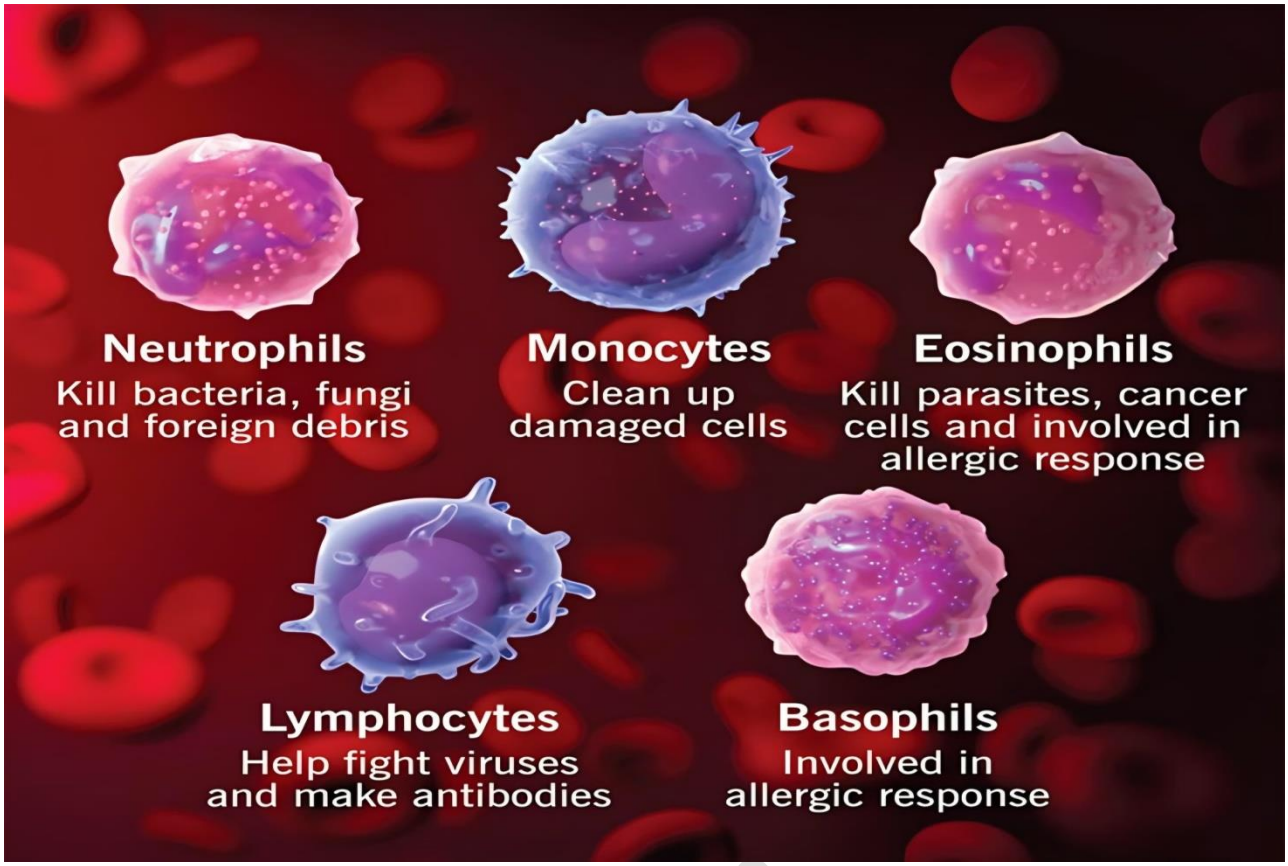
-**Eosinophil's**:Involved in allergic reactions and parasitic infections. Contain large, reddish-orange granules and often a bilobed nucleus.

-**Basophils**:Least common, release histamine and other substances in allergic responses. Contain large, dark granules that often obscure the nucleus.

2- A granulocytes

-**Lymphocytes**: Essential for adaptive immunity (T and B cells), fight viruses and cancer cells. Characterized by a large, round nucleus and scanty cytoplasm.

-**Monocytes**: Transform into macrophages in tissues, engulf microorganisms and cellular debris, and present antigens. The largest WBCs, with a kidney-shaped nucleus and abundant cytoplasm.



Normal Reference Ranges for WBCs (Adults)

Total WBC Count: 4,000 - 11,000 cells / microliter

Differential Count

- Neutrophils: 40-70%
- Lymphocytes: 20-40%
- Monocytes: 2-8%
- Eosinophil: 0-5%
- Basophils: 0-1%

Common Terminology

- **Leukocytosis**: An increase in the total WBC count above the normal range.
- **Leukopenia**: A decrease in the total WBC count below the normal range.
- **Neutrophilia**: Increased neutrophil count .
- **Neutropenia**: Decreased neutrophil count .
- **Lymphocytosis**: Increased lymphocyte count .
- **Lymphopenia**: Decreased lymphocyte count .
- **Eosinophilia**: Increased eosinophil count .
- **Monocytosis**: Increased monocyte count .
- **Basophilia**: Increased basophil count .

Leukocytosis (Increased WBC Count)

Causes :

1-**Neutrophilia**: Acute bacterial infections, inflammation, stress, tissue necrosis, some cancers (e.g., Chronic Myeloid Leukemia CML).

2-**Lymphocytosis**: Viral infections , Chronic Lymphocytic Leukemia (CLL).

3-**Monocytosis**: Chronic infections (tuberculosis), chronic inflammation.

4-**Eosinophilia**: Allergies (asthma, eczema), parasitic infections, some autoimmune diseases, certain malignancies.

5-**Basophilia**: Rare, may indicate severe allergic reactions or CML.

Leukopenia (Decreased WBC Count)

Causes:

1- **Neutropenia**: Severe viral infections, chemotherapy, certain medications, autoimmune diseases, aplastic anemia, myelodysplastic syndromes.

2- **Lymphopenia**: Severe stress, corticosteroid therapy, acquired immunodeficiency (AIDS), some cancers.

Practical Tests for the White Blood Cell Series

1- Manual Total WBC Count

Principle:

A whole blood sample is diluted with an acidic solution (e.g., Turk's Fluid) that lyses red blood cells while preserving white blood cells. WBCs are then counted in specific areas of a counting chamber under a microscope to calculate the total count.

Materials

1- Whole blood sample (mixed with EDTA anticoagulant
2- WBC diluting fluid (Turk's Fluid)
3- WBC pipette or micropipettes with tips
4- Improved Neubauer Counting Chamber with coverslip
5- microscope
6- Cotton, sterile alcohol

Procedure

1- Draw blood to the 0.5 mark on the pipette stem
2- Wipe any excess blood from the outside of the pipette
3- Draw Turk's fluid up to the 11 mark in the bulb,(1:20 dilution (0.5 parts blood + 10 parts fluid) avoiding air bubbles.
4- minutes to ensure complete mixing of blood with the fluid
5- Clean the counting chamber and coverslip thoroughly
6- Place the coverslip over the counting chamber
7- Place a small drop of the mixture at the edge of the coverslip and allow it to spread under the coverslip by capillary action, being careful to avoid air bubbles or overfilling
8- Allow the chamber to rest for 2-3 minutes for cells to settle
9-Count Under the Microscope
10-Count the white blood cells in the four large corner squares.

2- Peripheral Blood Smear Preparation and Differential WBC Count

Principle: A drop of blood is spread thinly on a glass slide to create a smear that allows for the examination of cell morphology. After staining with a Wright-Giemsa stain, the smear is examined under a microscope to determine the percentage of each WBC type and detect any morphological changes or immature cells.

3- Automated Hematology Analyzers

These instruments use principles such as electrical impedance and optical scattering with laser technology to determine the number, size, and classification of different blood cells with high accuracy and speed.

Post test

Q1- Which white blood cell type is primarily involved in allergic reactions and parasitic infections ?

a) Neutrophil
b) Basophil
c) Eosinophil
d) Monocyte

Q2- When performing a manual total WBC count using an Improved Neubauer Counting Chamber, which objective lens is typically used for clearer cell visualization after locating the corner squares ?

a) X4
b) X10
c) X40
d) X100 (oil immersion)

Week 11

Week 12
Week 13
Lecture title:

LEUKEMIA
Study the Myeloid . L
Study the Lymphatic. L

Learning objective:

By the end of this practical lecture, students will be able to :
1-Define leukemia as a cancer of the blood and bone marrow, characterized by abnormal white blood cell production.
2-Explain the impact of abnormal white blood cells in leukemia on the body's ability to fight infection and produce normal blood cells.
3-Identify potential risk factors associated with developing leukemia, including environmental, genetic, and medical exposures.
4-Distinguish between lymphoid and myeloid leukemia based on the type of cell lineage affected.
5-Differentiate between acute and chronic leukemia based on their progression speed and typical onset.
6-List common symptoms associated with leukemia across various types and stages.
7-Identify the four main types of leukemia and recognize their typical age groups affected.
8-Describe the diagnostic procedures used to identify leukemia.
9-Outline the primary treatment options for leukemia.

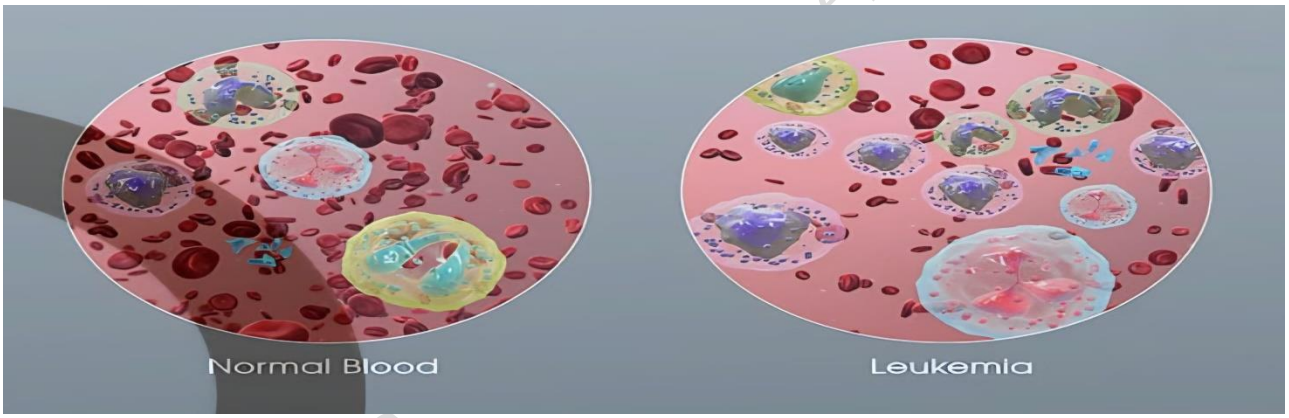
Pre test

Q1- What is leukemia primarily a cancer of ?
a) Red blood cells b) Platelets
c) White blood cells d) Bone cells
Q2- True or False: Leukemia is caused by the slow production of abnormal white blood cells?

LEUKEMIA

Leukemia is a type of cancers found in the blood and bone marrow and is caused by the rapid production of abnormal white blood cells .

In leukemia, the WBCs don't function like normal WBCs. They can also divide too quickly and eventually crowd out normal cells These abnormal white blood cells are not able to fight infection and impair the ability of the bone marrow to produce red blood cells and platelets .



Causes:

Scientists do not yet understand the exact causes of leukemia It seems to develop from a combination of genetic an environmental factors.

Risk factors :

The following factors may increase the risk of developing leukemia:

1- Exposure to high energy radiation .

2-Exposure to certain chemicals, such as benzene or formaldehyde.

3- Blood disorders.
4- Exposure to chemotherapy or radiation therapy.
5- Genetic disorders such as Down syndrome.
6- Some types of viruses can cause tumors, such as the hepatitis B virus and the HIV virus.
7- Family history of leukemia, but this is very rare.

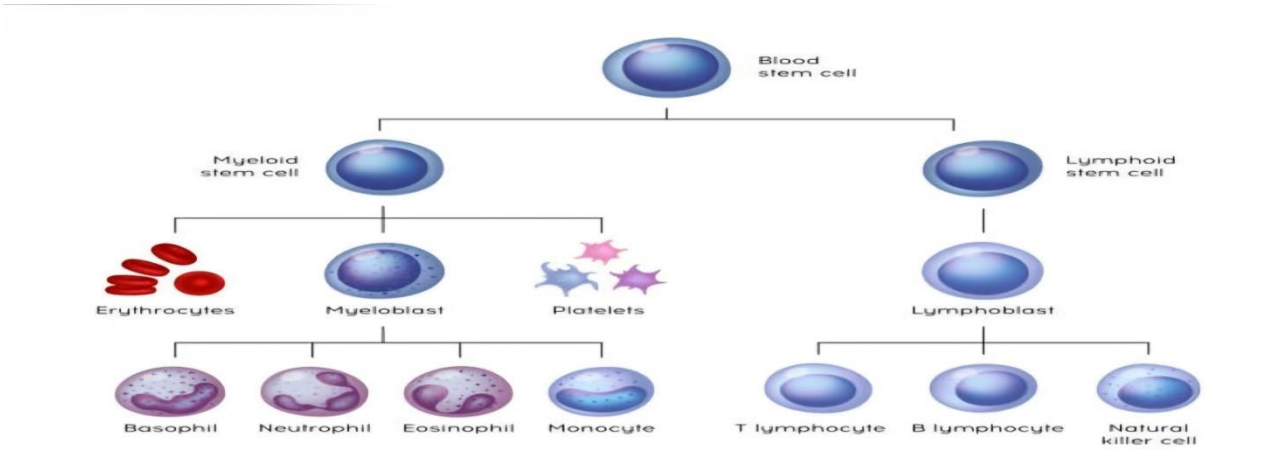
Leukemia can be either (chronic or acute) .

Chronic leukemia progresses more slowly than acute leukemia, which requires immediate treatment. and the disease progresses slowly and early symptoms may be very mild.
The onset of leukemia can be acute (sudden onset) or chronic (slow onset) .
Acute leukemia, cancer cells multiply quickly In chronic leukemia .

Leukemia is classified as :-

1 - Lymphoid
2 - Myeloid

When the cancer develops in the lymphocytes (lymphoid cells), it is called lymphocytic leukemia.
When the cancer develops in the granulocytes or monocytes (myeloid cells), it is called myelogenous leukemia .
Based on the predominant lineage of the malignant cells
Lymphocytic leukemia refers to abnormal cell growth in the marrow cells that become lymphocytes, a type of white blood cell that plays a role in the immune system .
In myelogenous leukemia, abnormal cell growth occurs in the marrow cells that mature into red blood cells, white blood cells, and platelets .



Symptoms

Symptoms vary depending on the type and stage of leukemia, but they can include the following :

1- Fever, chills, night sweats and other flu-like symptoms.
2- Weakness and fatigue.
3- Swollen or bleeding gums .
4- Headaches.
5- Enlarged liver and spleen.
6- Swollen tonsils.
7- Bone pain.
8- Paleness.
9- Weight loss.

There are four main types of leukemia:

1-Acute myelogenous leukemia (AML)
can occur in children and adults. According to National Cancer Institute (NCI). This is the most common form of leukemia .

2- Acute lymphocytic leukemia (ALL)
occurs mostly in children .

3- Chronic myelogenous leukemia (CML)
affects mostly adults .

4-Chronic lymphocytic leukemia (CLL)
is most likely to affect people over the age of 55. It's very rarely seen in children.

DIAGNOSIS

To diagnose leukemia, doctors use certain diagnostic tests and procedures, such as:

1-Physical examination: Doctors examine the lymph nodes, spleen and liver to check if there is any swelling .

2-Blood tests: Blood tests determine the number of blood cells and platelets. They are also used to identify any abnormalities in the blood cells .

3-Bone marrow biopsy: to check for cancer cells .

4-Genetic testing: to examine the genetic material in the bone marrow, blood cells and lymph nodes .

Is Leukemia Preventable ?

Because the cause of leukemia remains unknown, there is no certain way to prevent it. However, avoiding exposure to solvents, such as benzene and toluene, and unnecessary exposure to x-rays is generally good practice.

If you think you may be exhibiting signs of leukemia, being aware of the risk factors and symptoms and talking with your doctor are critical to early diagnosis and treatment .

It is especially important for people who have a family history of leukemia to be aware of symptoms and share their family medical history with their doctors .

What are the treatment options for leukemia?

Leukemia treatment methods depend on several basic factors, like age, overall health, type of leukemia and whether or not it has spread to other parts of the body.

Common treatments used to fight leukemia include:-

1- Chemotherapy .

2- Radiation therapy .

3- Stem cell transplant: which replaces the cells that were damaged during radiation therapy and chemotherapy .

4- Immunotherapy: this type of treatment is given to enhance the body's immunity .

Chronic lymphocytic leukemia (CLL)

CLL results from mutation (change) to the DNA of a single marrow cell that develops into a lymphocyte.
In 95 percent of people with CLL, the change occurs in a B lymphocyte.
In the other 5 percent of people with CLL, the change occurs in a T lymphocyte or a natural killer (NK) cell.
any of the three major types of lymphocytes (T cells, B cells or NK cells) can undergo a malignant transformation that causes diseases related to B-cell CLL.

Causes and Risk Factors

First-degree relatives of patients with CLL are three to four times more likely to develop CLL than people who do not have first-degree relatives with the disease.

Signs and Symptoms

1-Tired more easily, and or feel short of breath as a result of anemia .
2- Lose weight because of decreased appetite .
3- Have lymph nodes and a spleen enlarged .
4- Have infections of the skin, lungs and kidneys .
5- Low red blood cell counts, Low platelet counts.

Diagnosis

1- Blood Cell Count and Examination .
2- Bone Marrow Examination .
A bone marrow aspiration and biopsy .
3-Immunophenotyping (or flow cytometry)
4-Immunoglobulin Levels .

Chronic myeloid leukemia (CML)

GML is called by several other names including :-

1- Chronic myelogenous leukemia .
2- Chronic granulocytic leukemia .
3- Chronic myelocytic leukemia .

CML results from an acquired (not present at birth) or a genetic injury to the DNA of a single bone marrow cell.

Causes

No one is born with CML, It happens when there is an injury to the DNA of a single bone marrow cell.

Risk Factor

CML is caused by exposure to very high doses of radiation , also occurs in some individuals treated with high-dose radiation therapy for other cancers .

Signs and Symptoms

1- Being very tired or tiring easily .
2- Shortness of breath during basic, everyday activities .
3- Unexplained weight loss .
4- Enlarged spleen or pain or dragging feeling on upper left side of abdomen under the ribs .
5- Being pale from anemia .
6- Night sweats.

Diagnosis

1- Complete Blood Count (CBC)
People with CML often have :-
- Decreased hemoglobin concentration .
- Increased white blood cell count .
- Possible increase or decrease in the number of platelets depending on the severity of the person's CML.

2- Bone Marrow Aspiration and Biopsy .
3- Cytogenetic Analysis :
This test measures the number and structure of the chromosomes.
4- FISH (Fluorescence In Situ Hybridization) :
FISH is a more sensitive method for detecting CML than the standard cytogenetic tests, FISH is a quantitative test.
5- Polymerase Chain Reaction (PCR).

Post test

Q1- Which of the following is NOT a common sign or symptom of CLL?
a) Increased appetite
b) Enlarged spleen
c) Recurrent infections
d) Unexplained weight loss

Q2- In the context of CLL, a malignant transformation can occur in any of the following lymphocytes except?
a) T cells
b) B cells
c) Plasma cells
d) NK cells

Q3- Chronic Myeloid Leukemia (CML) is characterized by?
a) A congenital chromosomal abnormality
b) An acquired injury to the DNA of a single bone marrow cell
c) A viral infection of blood cells
d) An autoimmune response against bone marrow

Q4- A bone marrow aspiration and biopsy are crucial diagnostic tools for CML because they?
a) Directly measure the patient's radiation exposure
b) Allow for direct examination of bone marrow cells and their abnormalities
c) Quantify the number of red blood cells in circulation
d) Primarily detect viral infections

Week 14

Lecture title:

Study the Monocytic leukemia

Learning objective:

1-Define monocytic leukemia (AMoL) and describe its origin
Identify the key signs and symptoms associated with monocytic leukemia, relating them to underlying pathophysiological mechanisms.
2-Explain the role of various laboratory tests.
3-Interpret peripheral blood and bone marrow findings characteristic of monocytic leukemia.
4-Differentiate monocytic leukemia from other types of acute myeloid leukemia based on diagnostic features.

Pre test

Q1- Which of the following cell types is primarily proliferated in Acute Monocytic Leukemia (AMoL)?
a) Lymphoblasts
b) Myeloblasts
c) Monoblasts and promonocytes
d) Erythroid precursors

Q2- Acute Monocytic Leukemia (AMoL) originates from the malignant transformation of ?
a) Lymphoid stem cells
b) Myeloid stem cells committed to the monocytic lineage
c) Mature monocytes
d) Erythroid stem cells

Study the monocytic leukemia

Monocytic leukemia primarily refers to Acute Monocytic Leukemia (AMoL), It is a subtype of AML where there is a predominant proliferation of monoblasts and promonocytes.
The origin is Malignant transformation of myeloid stem cells committed to the monocytic lineage.
Bone marrow is typically hypercellular due to the proliferation of abnormal monocytic cell.

Signs and Symptoms

Patients with monocytic leukemia, like other acute leukemias, often present with symptoms related to bone marrow failure and extramedullary infiltration:
1-**Anemia**: Fatigue, pallor, shortness of breath, palpitations
2-**Thrombocytopenia**: Easy bruising, petechiae, epistaxis (nosebleeds), bleeding from gums.
3-**Neutropenia**: Recurrent infections, fever.
4-**Other common symptoms**: Hepatosplenomegaly, lymphadenopathy (less common than in ALL), gingival hypertrophy, skin lesions.

Laboratory Tests for Monocytic Leukemia Diagnosis

A. Peripheral Blood Analysis

1-Complete Blood Count (CBC) with Differential
White Blood Cell (WBC) Count: Often significantly elevated, but can be normal or even low.

- Differential Count: Crucially, a high percentage of monocytes, promonocytes, and monoblasts will be observed. The presence of

monoblasts (immature monocytic cells) in the peripheral blood is .highly suggestive of acute monocytic leukemia
- Red Blood Cell (RBC) Count and Hemoglobin: Typically reduced, indicating anemia.
- Platelet Count: Usually decreased (thrombocytopenia)

2-Peripheral Blood Smear Examination
This is a cornerstone of diagnosis
- Morphology: Examine for the presence and percentage of immature monocytic cells (monoblasts and promonocytes).

- Blast Percentage: According to WHO criteria, 20\% blasts (including monoblasts, promonocytes, and myeloblasts in combined forms) in the bone marrow or peripheral blood is (required for a diagnosis of acute leukemia.

B. Bone Marrow Examination
Essential for definitive diagnosis, blast quantification, and detailed cellular analysis.

C. Immunophenotyping (Flow Cytometry)
Principle: Identifies specific cell surface and intracellular antigens using fluorescently labeled antibodies. This is crucial for lineage assignment and identifying aberrant phenotypes .

D. Cytogenetic Analysis and Molecular Testing
Cytogenetic: Examines chromosomal abnormalities in leukemic cells.
Molecular Testing : Detects specific gene mutations or rearrangements that play a role in pathogenesis and prognosis.

E.Myeloperoxidase (MPO) Stain

MPO is an enzyme found in myeloid cells (granulocytes)and their precursors This helps differentiate monocytic leukemia from other myeloid leukemias ,which are strongly MPO positive.

Post test

Q1- Which of the following laboratory findings is most characteristic of peripheral blood analysis in monocytic leukemia?
a) Elevated Red Blood Cell (RBC) count
b) Predominance of mature neutrophils
c) High percentage of monocytes, promonocytes, and monoblasts
d) Markedly increased platelet count

Q2- Which diagnostic test is used to detect specific gene mutations or rearrangements in leukemic cells?
a) Peripheral Blood Smear
b) Cytogenetic Analysis
c) Molecular Testing
d) Complete Blood Count (CBC)

Week 15

Lecture title:

Use the Peroxides test for differential diagnosis

Learning objective:

By the end of this practical lecture, students will be able to :

1-Explain the principle behind the Peroxidase (Myeloperoxidase/MPO) stain test in hematology.

2-Outline the key steps involved in performing the MPO stain test on a blood sample.

3-Describe the expected microscopic findings for positive and negative reactions to the MPO stain.

4-Discuss the diagnostic significance of the MPO stain in differentiating Acute Myeloid Leukemia (AML) from Acute Lymphoblastic Leukemia (ALL).

5-Recognize the broader application of the Peroxidase test within hematology for white blood cell classification and leukemia diagnosis.

Pre test

Q1- What is the primary purpose of the Peroxidase test ?

a) To measure red blood cell count

b) To differentiate types of white blood cells, especially in leukemia

c) To assess platelet function

d) To detect blood clots

Q2- The MPO stain is particularly useful in distinguishing between which two types of leukemia?

a) Chronic Myeloid Leukemia and Chronic Lymphocytic Leukemia

b) Acute Myeloid Leukemia and Acute Lymphoblastic Leukemia

c) Multiple Myeloma and Hodgkin Lymphoma

d) Myelodysplastic Syndromes and Aplastic Anemia

Use the Peroxides test for differential diagnosis

The Peroxidase test, also known as Myeloperoxidase Stain (MPO Stain), is used in hematology to differentiate white blood cells, especially in cases of leukemia .

Steps for Performing the Myeloperoxidase (MPO) Stain Test:-

1. Blood Sample Preparation:
A blood sample is collected from the patient, and a blood smear is prepared on a glass slide.

2. Fixation of the Sample:
The smear is fixed using methanol to preserve the cells on the slide.

3. Adding the Stain:
The Myeloperoxidase stain is applied to the smear.
This stain reacts with the myeloperoxidase enzyme found in myeloid cells, causing them to take up color.

4. Washing and Drying:
The slide is washed to remove excess stain and then dried.

5. Microscopic Examination:
The slide is examined under a microscope.

Cells containing the myeloperoxidase enzyme will appear **brown or black**, indicating a positive reaction.

Importance of the Test in Differentiating White Blood Cells in Leukemia:

Acute Myeloid Leukemia (AML):
Myeloblasts (immature myeloid cells) show a positive reaction for MPO stain, helping in AML diagnosis.

Acute Lymphoblastic Leukemia (ALL):
Lymphoblasts (immature lymphoid cells) show a negative reaction for MPO stain, distinguishing them from AML.
This test is a crucial diagnostic tool for leukemia, as it helps determine the type of blast cells involved, guiding appropriate treatment strategies .

Application of the Peroxidase Test in Hematology

1- Classification of White Blood Cells:
Myeloperoxidase (MPO) Stain: Used to distinguish myeloid cells from lymphoid cells. Myeloid cells show a positive reaction to the stain, while lymphoid cells remain negative.

2. Diagnosis of Leukemia:
Acute Myeloid Leukemia (AML): Myeloid cells appear positive for MPO staining, aiding in diagnosis.
Acute Lymphoblastic Leukemia (ALL): Lymphoid cells appear negative, helping differentiate ALL from AML.

Additional Notes
The Peroxidase test is also used in other fields, such as detecting thyroid peroxidase antibodies (TPO) to diagnose thyroid disorders. However, this application is outside the scope of hematology.

Post test

Q1- When examining an MPO-stained slide under a microscope, what color indicates a positive reaction for the myeloperoxidase enzyme?

a) Red or pink
b) Blue or purple
c) Brown or black
d) Green or yellow

Q2- Why is the Peroxidase test considered a crucial diagnostic tool in leukemia?

a) It determines the patient's blood type
b) It helps identify the specific type of blast cells, guiding treatment
c) It measures the overall white blood cell count
d) It evaluates the bone marrow cellularity

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