

Connective Tissue

C.T. proper

Loose C.T.

Adipose C.T.

Reticular C.T.

Dense C.T.

Elastic C.T.

D Mucoid C.T.

Modified C.T.

Ground substance is modified

Solid nature = supporting C.T.

Cartilage (firm)

➢ Bone (hard)

Fluid nature (plasma)Blood

Blood Modified CT

- □ Adult has ~ **5.5 -6 L**
- Circulate in CVS
- Considered **modified** connective tissue :
- ≻Mesodermal in origin

➤ cells

liquid ground substance (called plasma)

dissolved protein fibers(fibrinogen) fibrin



CARDIOVASCULAR SYSTEM

BLOOD

Consists of liquid and cellular components by a machine called a centrifuge.

Plasma: 55%

- **Cells = Formed Blood elements 45%**
- Originate in the red bone marrow
- Blood formation = hematopoiesis

□ **No** aberrant fibers.





Withdraw blood into a syringe and place in a glass tube.

Place the tube into a centrifuge and spin for about 10 minutes.

centrifugation to reveal plasma,

buffy coat, and erythrocytes.



- 55% of blood volume:
- **Water 92%.**
- **Organic substances:7 %**
- plasma proteins
- (albumin, globulin, prothrombin and **fibrinogen**)
- ➢ Hormones & enzymes.
- □ Inorganic salts 1%
- (Na Cl, Bicarbonates, phosphates & calcium)



The Blood Film= Smear

Preparation of blood for laboratory study

- Why do we do a blood film ?
- 1.To study blood elements.
- 2.To make differential leukocytic count.

Steps :

- Put a small drop of blood
- Spread into a thin film
- Stain with Leishman or Giemsa stain

(methylene blue +eosin)







Blood film

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Steps:

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Giemsa's / Leishman's = **methylene blue + eosin**

- ► basophilic (violet)
- eosinophilic (pink)
- azurophilic (red purple)





Complete blood count (CBC)



Complete blood count (CBC)

1-Total count :

- It is the total number of blood elements (RBCs, WBCs, or Platelets) per cubic millimeter
- Measured by
- ➤ Hemocytometer
- > Or Automatic counter

2-Differential leukocytic count

• the percentage of each type of leucocytes to the total count





Blood cell count=CBC

Manual method= Conventional

=Hemocytometer= counting chamber.

Electronic method

= automated hematology analyzer.

<u>Total count</u>

- **<u>RBC count</u> 4.5-5** million/mm^{3 in female}
- **Total leukocytic count** 4,000-11,000/mm³
- □ <u>Platelet count</u> 250,000- 350,000/mm³

Differential leukocytic count





Blood cells

- 1. Total or Differential count
- 2. Shape & size
- 3. Structure (nucleus + granules)
- 4. Function
- 5. Life span
- 6. Abnormalities



Red Blood corpuscles

Normal RBCs total count:

- In <u>males</u> **†** 5- 5.5 millions / mm³ blood
- in <u>females</u> **‡** 4.5-5 millions / mm³ blood

• <u>LM of RBCs:</u>

- *Shape: Biconcave discs.
- Mature RBCs are membrane- bound corpuscle.

*<u>Size:</u>

- -Diameter 7.5 μm
- -Thickness 1 μm
- **♦ <u>Nucleus</u>**: Anucleate.
- Cytoplasm 33% of the corpuscular volume is Hemoglobin = heme "Fe"+ Globin 'protein'





EM picture of RBCs:

≻Glycocalyx

- ➢responsible for the ABO/ Rh blood group.
- ≻No nucleus, No typical organelles.
- ≻Only few mitochondria
- ➤subplasmalemmal cytoskeleton
- ➤(actin, spectrin & ankyrin) responsible for the flexibility of RBCs.
- ➢ Function of RBCs

Carry O2& CO2









life span:

- 100-120 days
- Then removed by <u>Macrophages</u> of spleen and liver sinusoids

Adaptation to function

- 1. Glycocalyx well developed
- **2.** ▲ surface area (Biconcave)
- 3. ▲ amount of HB

(no nucleus/ organelles)

- 4. \blacktriangle HB at the periphery
- 5. Selective permeability
- 6. Carbonic anhydrase
- 7. ▲ flexibility to squeeze without damage (cytoskeleton)



Abnormalities of RBCs

Abnormalities of RBCs in number Anaemia:

Decrease ??? in the total number of RBCs.

Polycythaemia:

increase in the total number of RBCs.
Causes: (decreased oxygen tension)
Physiological: newborns ,high altitude
Pathological: chronic lung and heart
diseases.



Abnormalities of RBCs in size

□ <u>Microcytosis</u>:

diameter of RBCs is **less than 6µm**. (Microcytic anaemia)

□ <u>Macrocytosis</u>

diameter of RBCs is **more than 9µm**. (Macrocytic anaemia)

□ <u>Anisocytosis</u>

Variable in size



Abnormalities of RBCs in shape

- **1.** Rouleaux formation
- In slow circulation
- 2. Poikilocytosis
- Variable in shape
- 3. In hypertonic solution
 echinocytes(crenation)
 4.In hypotonic solution= swelling
 Ghosts
- 5. Sickle Cell Anemia
- (abnormal Hemoglobin









Reticulocytes = immature RBCs

Reticulocytes represent 1% of all RBCs in normal blood film.

 \succ Nucleated \implies No nucleus

differ than mature RBCs

- > slightly larger ($8\mu m$).
- Cytoplasm contains remnants of ribosomes.
- On staining with cresyl blue form a reticulate pattern.

<u>Clinical significance:</u>

An increase in this percentage indicates an

accelerated rate of erythropoiesis.

To compensate for anemia or severe hemorrhage.



BLOOD PLATELETS

<u>Origin</u>: from megakaryocyte in the bone

marrow.

- Cell fragments of megakaryocyte.
- Thrombocytes.
- Thromboplastids

*<u>Normal Platelet Count</u>

250,000-350,000/ mm³ (200,000-400,000)

Structure (L. M) :

- Shape: Anucleate, biconvex discs.
- **<u>Diameter</u>** :2-3 μm.

central granular zone (granulomere)

Granulomere, granular central region

& peripheral clear zone (hyalomere)

Hyalomere at the periphery, there is a pale basophilic zone



EM of the platelet :

≻<u>Shape:</u> Irregular, Pseudopodia.

- **≻<u>Diameter</u>** :2-3 µm.
- ➢ Shape: Anucleate, biconvex discs.

≻<u>Platelet membrane:</u>

- ▲ ▲ well developed cell coat glycoprotein for:
- Adhesion
- Aggregation
- > Hyalomere &granulomere



Granulomere

- ≻few mitochondria & ribosomes.
- ≻scattered glycogen particles.
- ≻3 types of granules:
- **Δ Alpha** (α)granules:
- Large, abundant, PD-GF, coagulation factors.
- Delta granules:
- Medium size, ATP, ADP, serotonin.
- **\Box** Lambda(λ) granules:



Hyalomere

- Electron-lucent.
- Lacks organelles.
- It contains:
- circumferential bundle of 10-15 microtubules
 <u>discoid</u> <u>shape</u>
- Actin & myosin
 motility + clot retraction
- Canalicular system =tubular system.



Functions of platelets

- Platelet aggregation-→white thrombus
- Local blood coagulation→ red thrombus
- Serotonin \rightarrow Vaso-constriction
- Clot retraction → by microfilaments
- Clot removal→ by **proteolytic** enzymes
- Life span: 10- 14 days in blood
- **Abnormality of the platelets :**
- □Thrombocytopenia ▼ ▼ ▼
- Thrombocytopenia (purpura)
- □▲ ▲ ▲ Thrombocythemia



	RBCs Red blood corpuscle Erythrocytes – Greek: "Red	Platelets Thrombocytes Thromboplastides
Number	<u>males</u> is 5 - 5.5 millions / mm ³ <u>females</u> it is 4.5-5 millions / mm ³ blood.	250,000-350,000/mm ³ 200,000- 400,000
Size	7. 5-8. 5 um Macrocytes > 9 μm, Microcytes < 6 μm Anisocytosis = variation in si	3μm 2-5 μm diameter
Shape	biconcave disc	Biconvex
Structure	no nuclei & other organelles only few mitochondria Bag of Haemoglobin	Fragments of megakaryocyte Not true cell (Non-nucleated) Granulomere & Hyalomere
Life span	100-120 days	10- 14 days in blood
Function	Carry O2 & Co2	 the process of thrombus formation (blood clotting) in response to any vascular endothelial injury to prevent excessive blood loss. clot retraction and removal of the blood clot after healing of the vessel wall to reestablish the flow of the blood.
Abnormality	Polycythaemia: i.e. increase in the total number of R.B.Cs. Anaemia: i.e. decrease in the total number of R.B.Cs. Sickle Cell Anemia	INCREASE Thrombosis Decrease Bleeding

Lecture 2 Granular leucocytes

The formed blood elements

Stains of blood film

- Giemsa's / Leishman's
- = methylene blue+ eosin
- ► basophilic (violet)
- eosinophilic (pink)
- ► azurophilic (red purple)

Blood cells = 45 % of blood volume

- **Red blood corpuscles = Erythrocytes (RBCs)**
- **Blood platelets = Thrombocytes**
- □ White blood cells =Leukocytes (WBCs):
- Granular leucocytes
- (neutrophils, eosinophils, basophils)
- Agranular leucocytes
- (lymphocytes, monocytes)





Normal total Count 4000-11,000 / mm³ blood.

I-Granular leukocytes:

- > Neutrophils. 60-70-%
- Eosinophils. 1-4%
- ➤ Basophils. 1/2-1%

Agranular leukocytes:

- Iymphocytes.20-30%
- Monocytes. 3-8%



Difference between RBCs & WBCs

<u>RBCs</u>

WBCs

- ➤ 4,5- 5million / mm3
- > Biconcave
- ➢ No nuclei. / no organelles
- ➢ Bag filled with hemoglobin
- ➤ Life span=120 days
- ➢ No ameboid movement
- ➢ Function : carry O2&CO2

- ➤ 4000-11000/ mm3
- ➤ Rounded
- Contain (nuclei+ organelles)
- No hemoglobin
- Life span= from days to years
- Amoeboid movement
- Defense & immunity

Neutrophils= Microphage (polymorphnuclear leukocytes

- Differential count 60-70%
- Size =10-12 microns
- Shape: rounded LM:
- <u>Nucleus</u> : multilobulated = 2-8 lobes Barr body ?? Condensed chromatin inactive X- Chromosome in females
- **Cytoplasm:** contains
- □ Specific granules
- (neutral & small)
- □ Non specific:
- azurophilic granules (few
- & large ,stained by **azure**)







EM of Neutrophils

- > Shape: irregular. When active
- Cytoplasm : Few organelles.
- ➤ Granules:.
- specific granules
- Small , Numerous , <u>Rice grain</u> appearance , **functional enzymes e.g. Collagenase**
- Non specific (Azurophilic)
- Large, few, dense
- **Contain lysosomal hydrolytic enzymes.**





Functions

□ The first line of defense.

≻Micro-organisms in the C.T.

Attraction of monocytes to the site of infection. Macrophages



Chemotaxis \rightarrow migration \rightarrow



Phagocytosis \rightarrow killing of bacteria by phagocytins (specific secondary granules) \rightarrow Secretion of cytokines



Nature Reviews | Immunology

• Stimulation of bone marrow to form new neutrophils



digestion by lysosomal enzymes (1ry, azurophilic granules)

Life span: 1-4 days in blood

- destruction of invader & CT byCollagenase
- death of neutrophils Production of pus (**pus cells**)

Neutrophil and macrophage activation and migration at the site of inflammation.

The main phagocytic cells are macrophages in the tissues and neutrophils in the bloodstream. Initially, macrophages in the tissue sense the presence of invaders act on the endothelial cells (ECs) and cause the release of interleukin binds to receptors on neutrophil surface causing neutrophil activation. Activated neutrophil helps in transmigration. Chemokine receptors help neutrophils to move towards chemotactic gradients and after reaching its target, they attach, ingest and kill the foreign agent by various mechanisms



Abnormality of neutrophil count



- in acute pyogenic infection =
 acute inflammations e.g.:
- Appendicitis
- Tonsillitis





- Chronic infection e.g. TB
- Severe viral infection e.g.
 Influenza , Measles



Infección tuberculosa inicial en el lóbulo superior derecho

Placa incicial activa que progresa hacia una cavitación

Numerosas cavidades tuberculosas y erosión bronquial

Eosinophils

- Differential count : 1- 4%
 Size : 12-15 microns.
 Shape: rounded
 L.M :
- □ Nucleus: bilobed C- shape
- **Cytoplasm** contains:

 \triangleright

- large specific acidophilic granules.
 - Few azurophilic granules





- > Bilobed C- shaped nucleus
- Cytoplasm contains
- Few organelles mitochondria, rER, & sER & glycogen
- Specific granules (Large, ovoid ,crystalloid core contain many hydrolytic enzymes histaminase, eosinophil peroxidase)
- Few non specific granulesazurophilic granules
- Small, sphericalLysosomal hydrolytic enzymes





Function of Eosinophils

- Migrate to mucosa of GIT, respiratory, genito-urinary& skin.
- regulation of allergic reactions.
- Parasitic infection. (Not phagocytic)

Life span: several days up to week

Abnormal Eosinophil Count

- **Eosinophilia** = increase
- Allergic reactions e.g. bronchial asthma, allergy, parasitic infections e.g. Bilharziasis.

Eosinopenia = decrease

Steroid therapy. Bone marrow depression.





Basophils Mast cell of the blood

- Differential count : 1/2 1%
- Size : 10 microns
- Shape : Rounded
- LM:
- > Nucleus: Bilobed, (S-shaped)
- * obscured by large granules
- ➢ Cytoplasm:
- * abundant deep blue granules.
- * Metachromasia.









<u>E.M.</u>

Nucleus : Bilobed S shape nucleus Cytoplasm: mitochondria, ribosomes, glycogen

Granules :

specific granules

• Large, contain histamine, heparin

Non specific (azurophilic granules)

• Contain lysosomal hydrolytic enzymes.



Functions

=Mast cell of blood:=

- heparin: anticoagulant
- bistamine: (anaphylaxis)
- Secretion of histamine which initiates allergic reactions.
- Secretion of heparin which is a natural anti-coagulant.
- Secretion of eosinophil chemotactic factor to limit allergic reaction.
- hypersensitivity reaction



Life span: 1-2 week

<u>Abnormal count</u> Basophilia:increase ▲ ▲ ▲ > viral infections e.g. <u>small pox</u> and chicken pox. > Systemic allergy



	Neutrophils	Eosinophils	Basophils mast cell of the blood .
Number	60-70% of leukocytic count	1-4% of leukocytic count	0-1% of leukocytic count
Size	10-1 2 μm in diameter	larger than neutrophils (12-15 µm in diameter,	(10 mm) in diameter,
Shape	spherical in shape + Neutral granules	spherical in shape + Acidophilic granules	spherical in shape (basophilic) specific granules with heparin and histamine
Structure	multi-lobed nucleus human females may have inactivated second X chromosome (Barr body drum stick	bi-lobed nucleus C-shape or Blood Smear - Leishman B1	S-shape lobed nucleus, obscured by basophilic granules
Life span	lifespan 1-4 days in circulation;	several days Up to week	1-2 weeks
Function	first line of defense against any invading micro-organism	Kill parasites,associated with allergic reactions	Basophils are 1 release of Hist allergic reactio
Abnormality	Neutrophilia: i.e. abnormal increase in the number of neutrophils. This is observed in acute inflammations e.g. appendicitis, tonsillitis. Neutropenia: i.e. abnormal decrease in the number of neutrophils e.g. in influenza, typhoid fever.	<u>1-Eosinophilia:</u> i.e. abnormal increase in the number - Allergic reactions e.g. asthma, urticaria -Parasitic infections e.g. Bilharziasis. <u>2-Eosinopenia:</u> i.e. 1 decrease in the number prolonged corticosteroid therapy .	Basophilia in systemic allergic reaction

Agranular leukocytes Monocyte

- Differential count: 3 8%
- Size: 20 microns = Largest in blood film
- Shape : rounded

LM:

> Nucleus:

Large, eccentric , Kidney- shaped (Indented)

> Cytoplasm:

 Finely granular, abundant pale basophilic non specific granules
 =Azurophilic granules



EM:

□ Irregular = Pseudopodia

- Nucleus: Large, eccentric kidneyshaped (Indented)
- □ The cytoplasm contains
- ➤ a moderate amount of organelles.
- Non specific (Azurophilic granules) few small dense granules containing lysosomal hydrolytic enzymes.



Function :

- Trans- migration & differentiation to tissue MACROPHAGE
- Immunologic function:
- Phagocytosis and intracellular digestion of bacteria, virus
- Ag- presenting cell

Life span : 1-2 days circulation in the blood, then enter the CT and trasform into macrophages





 Circulate from region to another & Function in CT=

Immunological function

Mononuclear phagocytic cells



Abnormal Monocyte count

Increase number = Monocytosis

Causes:

- 1- Malaria
- 2- Chronic infections (glandular fever, syphilis, T.B.)
- 3-Lymphomas & Leukemia.



- Bone marrow depression
- > drugs
- > Irradiation
- Severe chronic diseases

Lymphocytes

- <u>Differential count:</u> 20-30%
- Size : 9-12 microns
- According to the sizes:
- large lymphocytes.
- Medium-sized lymphocytes.
 - Small lymphocytes:
- ***Diameter**=**RBC**.
- Most numerous.
- Functionally mature.



3 functional types:

- T-lymphocytes:
- Start development in bone marrow.
- Differentiate in thymus.
- Cell-mediated IR.
- B-lymphocytes:
- Develop & differentiate in bone marrow.
- Humoral immune response.
- Natural killer cells = Null cells
- Develop in bone marrow.
- Lack CDs of B or T.
- Are null cells(non B, nonT).
- They don't enter the thymus to be competent.
- They act nonspecifically to kill virally infected cells &tumor cells



> Shape = rounded

- Large nucleus, thin cytoplasmic rim
- No stained granules in the cytoplasm (except small Azurophilic granules
- Small most common 90%
- **Types: B** and **T**-lymphocytes (morphologically not distinguishable)
- Null-cells (somewhat smaller size) Non B Non T

* <u>EM:</u>

- > Nucleus: dense clumps.
- **Cytoplasm** thin rim
- No specific granules Lysosomes= small & dense Azurophilic granules
- Many free ribosomes& few mitochondria + 2 centrioles
- \blacktriangle The cell coat = antigenic markers.



Antigenic markers of lymphocytes

The cell coat: Large no. of cell receptors.

- 1. Major histocompatibility complex (MHC) Glycoprotein + specific a.a. sequence.
- Tissue typing & antigenic recognition.
- 2 subclasses:
- MHC I & MHC II.

2- The cluster of differentiation antigens (CDs):

- Cell- surface glycoprotein + specific a.a. sequence.
- Expressed on different types of lymphocytes
- Marker proteins upon which

Functional types of lymphocytes.

Antigenic markers of lymphocytes Major histocompatibility complex (MHC)

* <u>MHC I:</u>

- > On all nucleated cells.
- Glycoprotein + specific a.a. sequence.
- Tissue typing.
- <u>Endogenous</u> antigenic recognition:
 - virus- infected cells.
 - > malignant cells.

* <u>MHC II:</u>

- Expressed on antigenpresenting cells.
- Glycoprotein + specific a.a. sequence.
- Tissue typing.
- <u>Exogenous</u> antigenic recognition:
 - Phagocytosed foreign Ags.

Function :

- After stimulation T-cells and B-cells become : Effector cells & Memory cells
- B cells form plasma cells, function in humoral immunity via immunoglobulins
- T cells function in cell-mediated immunity
- Effector T-cells: T helper cells, T suppressor cells, cytotoxic T cells
- Some T cells with "memory" of antigen exposure survive long periods; immunization
- Null Cells are composed of: Stem cells and Natural killer cells
- NK cells kill some foreign and virally alerted cells



months---- years

Functions of Lymphocytes



Abnormal lymphocyte count

1-Lymphocytosis:

Causes:

Physiological: in children Pathological:

1-chronic infections tuberculosis, syphilis,

2-leukemia, Lymphoma.

2-Lymphopenia:

Bone marrow depression.

- * drugs
- * Irradiation
- Severe chronic diseases





Abnormalities in leukocytic count



Acquired Causes of decrease in number

Decreased Production	Increased Destruction	Shift to Marginating Pool
Bone marrow	Peripheral circulation	Move from the circulating pool to attach along the vessel wall
Medication: Chemotherapy Antibiotics, etc	Autoimmune diseases (Rheumatoid arthritis, SLE, etc)	Severe infection Endotoxin release Hemodialysis Cardiopulmonary bypass

Key



Basophil



Eosinophil



Neutrophil



Monocyte



Lymphocyte

Monocyte

Subsets T, B, natural killer

Number	3-8% of WBCs	20-25 % of WBCs Next most common after neutrophils	
Size	12-20 μm diameter	9-11 µm diameter Small , medium , large	
Shape	Spherical	Spherical	
Structure	Spherical, Nucleus kidney-shaped No obvious granules	Spherical, Nucleus indented No obvious granules	
Life span	Circulate for 3-4 days before enter into tissues and organs	variable life spans Month – years (memory cell)	
Function	Precursor of <u>macrophages</u> in tissues Macro = "big"; phage = "eat" Phagocytic function	 B Cells involved in humoral immunity T Cells involved in cell-mediated immunity T helper cells, T suppressor cells, cytotoxic T c & memory cell 	
Abnormality	<i>Monocytosis:</i> is an abnormal increase in the number of blood monocytes. It occurs in diseases	<i>Lymphocytosis:</i> It is an abnormal increase in the number of lymphocytes as in:	