Reading Material for

Medical Lab. Technician

(Hematology & Blood Banking)

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PREFACE

A two years post matric teaching program of Medical Laboratory Technician for the students of Allied Health Sciences. The purpose of this reading material is to provide basic education to the paramedics about hematology and blood transfusion. This reading material attempts to cover almost all the basic theoretical knowledge required by students about hematology and blood transfusion so that they can perform their work better in Pathology laboratory and blood bank.

Table of Content:

SECTION 1

HAEMATOLOGY

Chapter 1: Introduction

Objective: By the end of this chapter students will be able to learn basic concepts of blood including definition of Hematology and blood banking, function and composition of blood.

1.1 Definition of Hematology and Blood banking:

Hematology: The study of blood and blood-forming tissues, including the physiology and pathology of blood cells. It covers a wide range of topics, from the production of blood cells in the bone marrow to the functions of different blood components.

Blood banking: This field specifically deals with the collection, processing, testing, storage, and transfusion of blood and its components. It ensures the availability of safe and compatible blood products for medical procedures, surgeries, and the treatment of various conditions.

Subjects to be taught

1. Basic hematology: Understanding the anatomy and physiology of blood cells, including red blood cells, white blood cells, and platelets.
2. Hematologic diseases: Studying disorders such as anemias, leukemia's, and clotting disorders.
3. Blood transfusion science: Covering the principles of blood donation, compatibility testing, and the administration of blood products.
4. Immunohematology: Exploring the immune aspects of blood compatibility and transfusion reactions.

Relationship with other branches of Pathology:

- Clinical Pathology: Hematology is a major component of clinical pathology, providing insights into overall health through blood tests.

- Immunology: Immunohematology involves understanding immune responses related to blood compatibility.
- Microbiology: Hematology works closely with microbiology to diagnose different blood infections and related conditions.

1.2 Blood Circulation:

The heart has actually two separate pumps:

- The right heart that pumps blood through the lungs
- The left heart that pumps blood through the peripheral organs.

Heart is composed of four chambers two atria and two ventricles . Each atrium is a weak primer pump for the ventricle, helping to move blood into the ventricle. The ventricles then supply the main pumping force that propels the blood either (1) through the pulmonary circulation by the right ventricle or (2) through the peripheral circulation by the left ventricle.(figure:1)

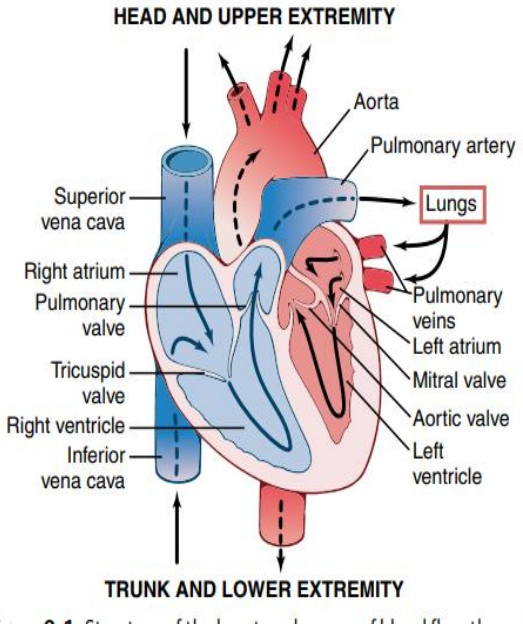


Figure:1 Structure of the heart, and course of blood flow through the heart chambers and heart valve

1.3Function of the blood: Blood has important transport, distribution, regulatory, and protective functions in the body.

Transportation and distribution:

- Oxygen is carried from the lungs to the tissues. This function is performed by hemoglobin which is present in large amounts in mature red cells.
- Nutrients absorbed from the digestive tract, e.g. monosaccharides (especially glucose), amino acids, fatty acids, glycerol, and vitamins, are transported to the cells of the body for use or storage.
- Waste products of metabolism are transported from the tissues to site of excretion, e.g. carbon dioxide produced from cellular activity is carried to the lungs for excretion, and the waste products of protein metabolism (urea, creatinine, uric acid) are transported to the kidneys for excretion.
- Hormones are carried from endocrine glands to the organs where they are needed.

Regulatory:

- Buffer systems in the plasma maintain the pH of the blood between pH 7.35–7.45.
- Proteins (particularly albumin) and salts (particularly sodium chloride) regulate plasma osmotic pressure, preventing excessive loss of fluid from the blood into tissues spaces.
- Blood assists in regulating the temperature of the body by absorbing and distributing heat throughout the body and to the skin surfacewhere heat which is not required is dissipated.

Protective:

- When a blood vessel is damaged, platelets and blood coagulation factors interact to control blood loss. Platelets adhere to the damaged tissue and to one another and activated coagulation factors lead to the formation of fibrin and a thrombus clot which reinforce the platelet plug.

- Leukocytes are involved in the body's protection against infections by producing antibodies in response to infection.

1.4Functional Parts of the Circulation:

- **Arteries:** The function of the arteries is to transport oxygenated blood under high pressure to the tissues. For this reason, the arteries have strong vascular walls, and blood flows at a high velocity in the arteries.
- **Arterioles:** The arterioles are the last small branches of the arterial system; they act as control through which blood is released into the capillaries.
- **Capillaries:** The function of the capillaries is to exchange fluid, nutrients, electrolytes, hormones, and other substances between the blood and the interstitial fluid.
- **Venules:** The venules collect blood from the capillaries and gradually coalesce into progressively larger veins.
- **Veins:** The veins function is to transport deoxygenated blood from the venules back to the heart.
- About 84 percent of the entire blood volume of the body is in the systemic circulation and 16 percent is in the heart and lungs. Of the 84 percent in the systemic circulation, 64 percent is in the veins, 13 percent in the arteries, and 7 percent in the systemic arterioles and capillaries. The heart contains 7 percent of the blood, and the pulmonary vessels, 9 percent. (figure:2)

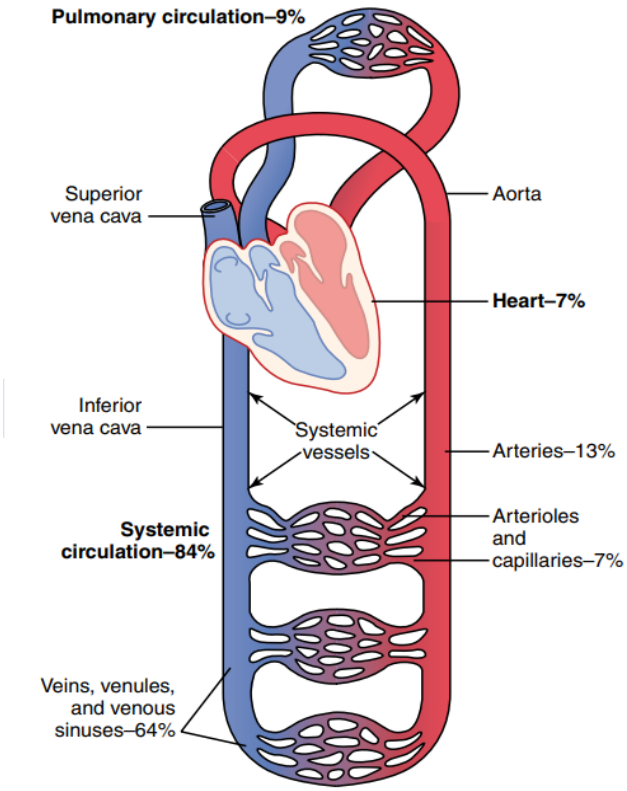


Figure:2 Distribution of blood (in percentage of total blood) in the different parts of the circulatory system

1.5Composition of Blood:

The average blood volume of adults is about 7 percent of body weight, or about 5 liters.

- 55 percent of the blood is plasma
- 45 percent is blood cells

These percentages can vary considerably in different people, depending on gender, weight, and other factors

a) Plasma: is a straw-colored (Pale Yellow), viscous fluid of the blood, in which the red blood cells, white blood cells, and platelets are suspended. It contains:

- Water (90-92%)

- Dissolved salts (electrolytes) and proteins (6-8%).

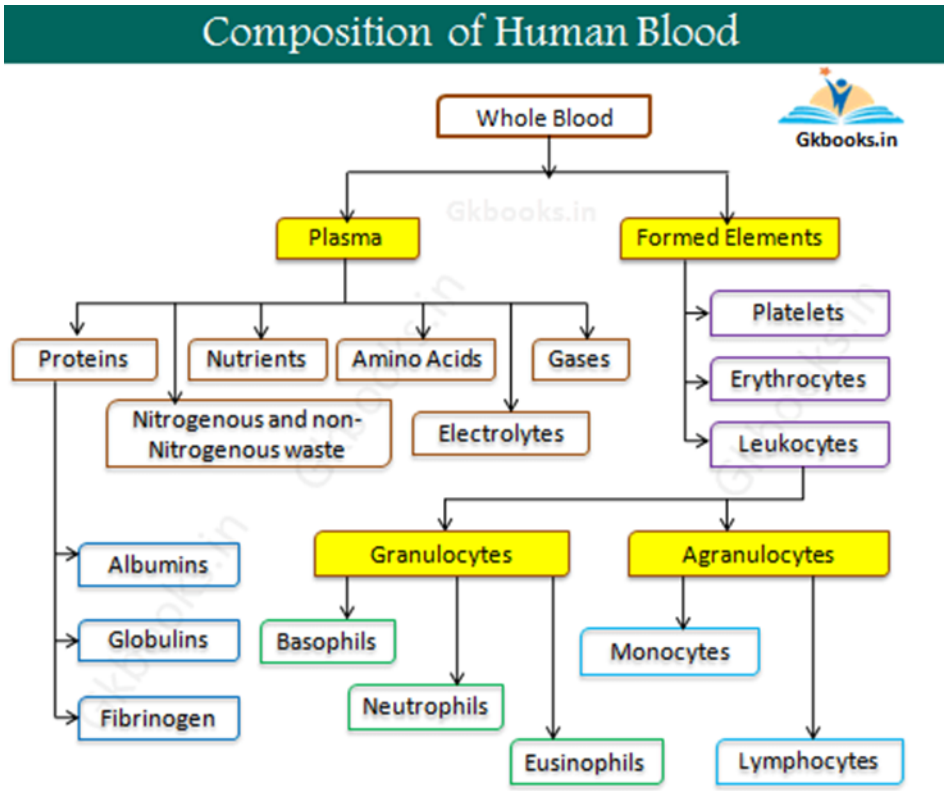
  b. Cells:

- Red blood cells 5,200,000 (±300,000) cells/mm3 in men, 4,700,000 (±300,000) in women.
- Platelets: 150000-350000 platelets/mm3.
- White blood cells: 4000-11000 cells/mm3

  The normal percentages of the different types OF WHITE BLOOD CELLS are approximately the following:

  - neutrophils 62. %
  - eosinophils 2.3%
  - basophils 0.4%
  - Monocytes 5.3%
  - Lymphocytes 30.0%

Question: Define hematology and blood banking? Write down composition of blood?


Composition of Human Blood
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Whole Blood
Plasma
Formed Elements
Proteins
Nutrients
Amino Acids
Gases
Nitrogenous and non-Nitrogenous waste
Electrolytes
Platelets
Erythrocytes
Leukocytes
Albumins
Globulins
Fibrinogen
Granulocytes
Agranulocytes
Basophils
Neutrophils
Eusinophils
Monocytes
Lymphocytes

Chapter 2:

Hematopoiesis

Objective: By the end of this chapter students will be able to learn what is hematopoiesis, its different sites in different stages of development of human beings, steps of formation of blood cells, normal values and morphological characters of different blood cells.

2.1Definition:

The physiologic process of formation of blood cells is known as hematopoiesis.

All blood cells are derived from pluripotent hematopoietic stem cells, which are present in small numbers in the bone marrow. The hematopoietic stem cell is the most primitive cell in the bone marrow. It has the ability of proliferation, self-renewal, and differentiation. The capacity of self-renewal permits life-long continuation of the process. The myeloid and lymphoid stem cells originate from the pleuripotent haematopoietic stem cell. From myeloid and lymphoid stem cells progressively more committed progenitors arise having progressively restricted potential to generate different types of blood cells.

2.2 Site of Haemopoiesis/Hematopoiesis:

- In the first few weeks of gestation, the embryonic yolk sac is a transient site of haemopoiesis called ‘primitive haemopoiesis’.
- From 6 weeks until 6–7 months of fetal life, the liver and spleen are the major haemopoietic organs and continue to produce blood cells until about 2 weeks after birth. (Table:1)
- The placenta also contributes to fetal haemopoiesis.
- The bone marrow is the most important site from 6–7 months of fetal life.

  During normal childhood and adult life, the marrow is the only source of new blood cells. In younger age, whole of the skeletal marrow participates in blood cell production. By late childhood, haematopoiesis becomes restricted to the flat

bones such as sternum, ribs, iliac bones and vertebrae and proximal ends of long bones. At other skeletal sites haematopoietic areas are replaced by fat cells.

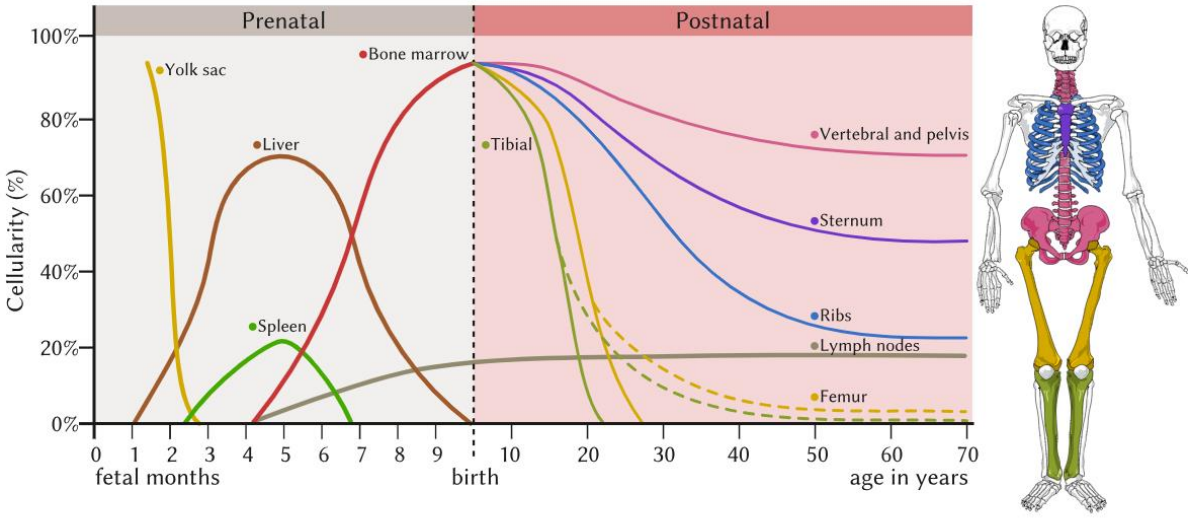

Figure:3 Sites of hematopoiesis

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However, when there is increased demand for blood cell production, conversion of yellow fatty inactive marrow to red active marrow can occur. Moreover, in certain disease states the liver and spleen can resume their fetal haemopoietic role (‘extramedullary haemopoiesis). (figure:3)

Fetus	0–2 months (yolk sac)
	2–7 months (liver, spleen)
	5–9 months (bone marrow)
Infants	Bone marrow (practically all bones); dwindling post-parturition contribution from liver/spleen that ceases in the first few months of life
Adults	Vertebrae, ribs, sternum, skull, sacrum and pelvis, proximal ends of femur

Table:1 Sites of haemopoiesis at different stages of development

2.3Origin/ Development of blood cells: (Steps of Hematopoiesis):

The blood cells begin their lives in the bone marrow from a single type of cell called the **pluripotential hematopoietic stem cell,** from which all the cells of the circulating blood are eventually derived.

The haematopoietic stem cell is the most primitive cell in the bone marrow. It has the ability of proliferation, self-renewal, and differentiation along several lineages. The capacity of self-renewal permits life-long continuation of the process. The myeloid and lymphoid stem cells originate from the pleuripotent haematopoietic stem cell. (figure:4)

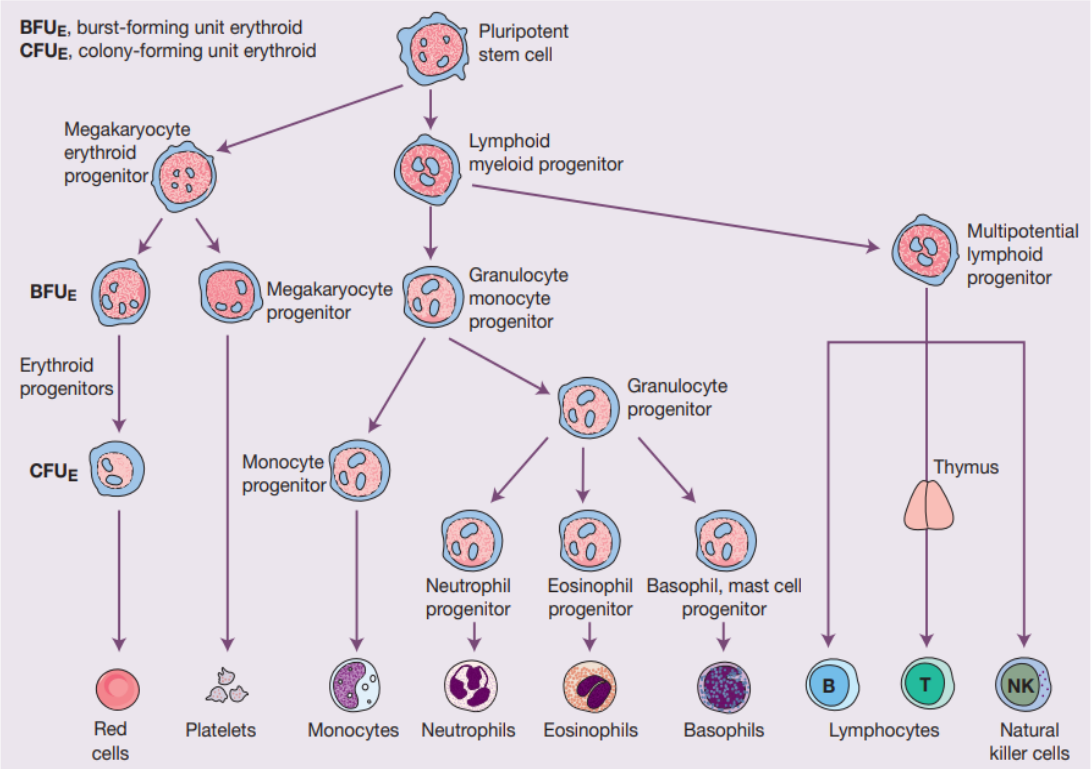


Figure 1.2 Diagrammatic representation of the bone marrow pluripotent stem cells (haemopoietic stem cells, HSC) and the cell lines that arise from them. A megakaryocytic/erythroid progenitor (MkEP) and a mixed lymphoid/myeloid progenitor are formed from the pluripotent stem cells. Each gives rise to more differentiated progenitors. The MkEP divides into erythroid and megakaryocyte progenitors. The mixed lymphoid progenitor gives rise to B and T lymphocytes and to natural killer cells. A granulocyte/monocyte progenitor gives rise to progenitors for monocytes, neutrophils, eosinophils, basophils and mast cells. The erythroid progenitors are also termed BFU-E and CFU-E. BFU-E, burst-forming unit erythroid; CFU-E, colony-forming unit erythroid.

Figure:4 Bone marrow pluripotent stem cells and the cell lines that arise from them

2.4 Normal values of Blood cells:

Table 2.4 Normal values for blood cells and haematinics.

	Males	Females
Haemoglobin (g/L)	135.0–175.0	115.0–155.0
Red cells (erythrocytes) ($\times 10^{12}$/L)	4.5–6.5	3.9–5.6
PCV (haematocrit) (%)	40–52	36–48
Mean cell volume (MCV) (fL)	80–95	
Mean cell haemoglobin (MCH) (pg)	27–34	
Reticulocyte count ($\times 10^9$/L)	50–150	
White cells (leucocytes)		
Total ($\times 10^9$/L)	4.0–11.0	
Neutrophils ($\times 10^9$/L)	1.8–7.5	
Lymphocytes ($\times 10^9$/L)	1.5–3.5	
Monocytes ($\times 10^9$/L)	0.2–0.8	
Eosinophils ($\times 10^9$/L)	0.04–0.44	
Basophils ($\times 10^9$/L)	0.01–0.1	
Platelets ($\times 10^9$/L)	150–400	

2.5 Morphological characters of blood cells:

All the circulating blood cells derive from pluripotential stem cells in the marrow. They divide into three main types.(table:2)

1. **Red cells:** Mature red cells are biconcave in shape. They carry oxygen from the lungs to the tissues and carbon dioxide from tissues to the lungs. They have a life span of 4-months (approximately 120 days)

2. **White cells:** which includes:

1) Phagocyte: which includes:
    a) Granulocytes : neutrophils, eosinophils, basophils
    b) Agarnulocytes: monocytes

Their function is to protect against bacterial and fungal infections.

2) Lymphocytes: which include B cells, involved in antibody production, T cells concerned with the immune response and in protection against viruses and other foreign cells. White cells have a wide range of lifespan.

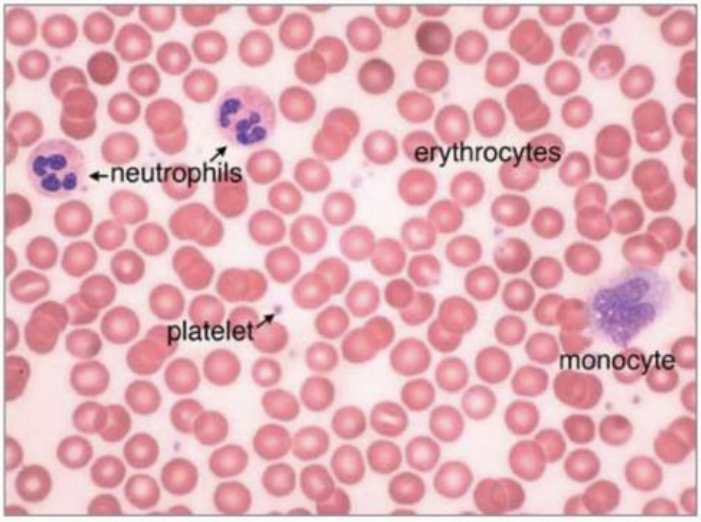
3. **Platelets:** They are the smallest blood cells and play an important role in hemostasis. They have life span of 10 days in vivo.

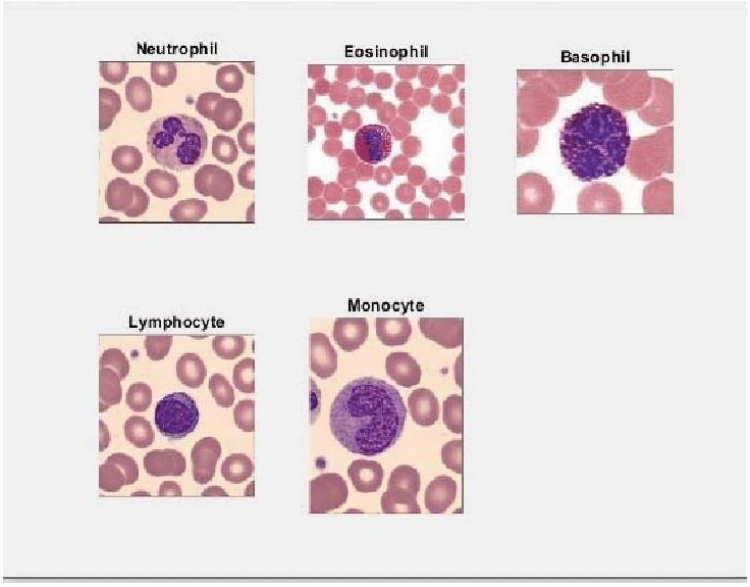
Table 2.1 The blood cells.

Cell	Diameter (μm)	Lifespan in blood	Number	Function
Red cells 	6–8	120 days	Male: $4.5–6.5 \times 10^{12}$/L Female: $3.9–5.6 \times 10^{12}$/L	Oxygen and carbon dioxide transport
Platelets 	0.5–3.0	10 days	$140–400 \times 10^{9}$/L	Haemostasis
Phagocytes				
Neutrophils 	12–15	6–10 h	$1.8–7.5 \times 10^{9}$/L	Protection from bacteria, fungi
Monocytes 	12–20	20-40 h	$0.2–0.8 \times 10^{9}$/L	Protection from bacteria, fungi
Eosinophils 	12–15	Days	$0.04–0.44 \times 10^{9}$/L	Protection against parasites
Basophils 	12–15	Days	$0.01–0.1 \times 10^{9}$/L	
Lymphocytes  B T	7–9 (resting) 12–20 (active)	Weeks or years	$1.5–3.5 \times 10^{9}$/L	B cells: immunoglobulin synthesis T cells: protection against viruses; immune functions
Natural killer cells 	10 (resting) 10–20 (active)	Hours or days	0.1–0.4	Protection against virus-infected and neoplastic cells

Table:2 blood cells size, lifespan in blood, number and functions.

Morphological charatertics of different blood cells





Question: Write down in detail steps of hematopoiesis/ hematopoiesis?

Chapter 3

RED BLOOD CELLS

Objective: At the end of the chapter students will be able to learn what is erythropioeisis, its different stages of developments, normal values, morphological characters of different types of RBCs.

3.1Introduction:

Red blood cells are biconcave cells and they are the most numerous cells found among the other blood cells. Their main function is to carry oxygen to the tissues and carbon dioxide from tissues back to the lungs. Their life span in the peripheral blood is 4 months (approximately 120 days).

3.2 Normal Values:

Males: 4.5- 6.5 $x10^{12}$/L

Females: 3.9- 5.6 x 10^{12}/L

3.3 Erythropoiesis:

The process of formation of mature red blood cells is called as erythropoiesis.

3.4Stages of Erythropoiesis:

1. **Proerythroblast:** The earliest morphologically identifiable erythroid cell in the bone marrow is the proerythroblast. It is a large (15-20 μm) cell with a fine, uniform chromatin pattern, one or more nucleoli and dark blue cytoplasm.
2. **Basophilic(early) Normoblast:** The next cell in the maturation process is the basophilic (early) normoblast. This cell is smaller in size (12-16 μm) and has a coarser nuclear chromatin with barely visible nucleoli. The cytoplasm is deeply basophilic.
3. **Polychromatic (intermediate) normoblast**: The more differentiated erythroid cell is the polychromatic (intermediate) normoblast (size 12-15 μm). The nuclear

size is smaller and the chromatin becomes clumped. This is the last erythroid precursor capable of mitotic division.

4. **Orthochromatic (late) normoblast:** The orthochromatic (late) normoblast is 8 to 12 μm in size. The nucleus is small, dense and pyknotic and commonly eccentrically-located. The cytoplasm stains mostly pink due to haemoglobinization. It is called as orthochromatic because cytoplasmic staining is largely similar to that of mature erythrocytes.

5. **Reticulocyte:** The nucleus is ultimately expelled from the orthochromatic normoblast with the formation of a reticulocyte. The reticulocyte still has remnants of ribosomal RNA in the form of a cytoplasmic reticulum.
6. **Mature Erythrocyte:** After 1 to 2 days in the bone marrow and 1-2 days in peripheral blood reticulocytes lose RNA and become mature pink-staining erythrocytes. Mature erythrocyte is a round biconcave disc about 7 to 8 μm in diameter. (figure:5)

With each stage, cell size and nuclear size become smaller, chromatin clumping increases and ultimately nucleus is extruded. Color of cytoplasm gradually changes from basophilic to orange-red.

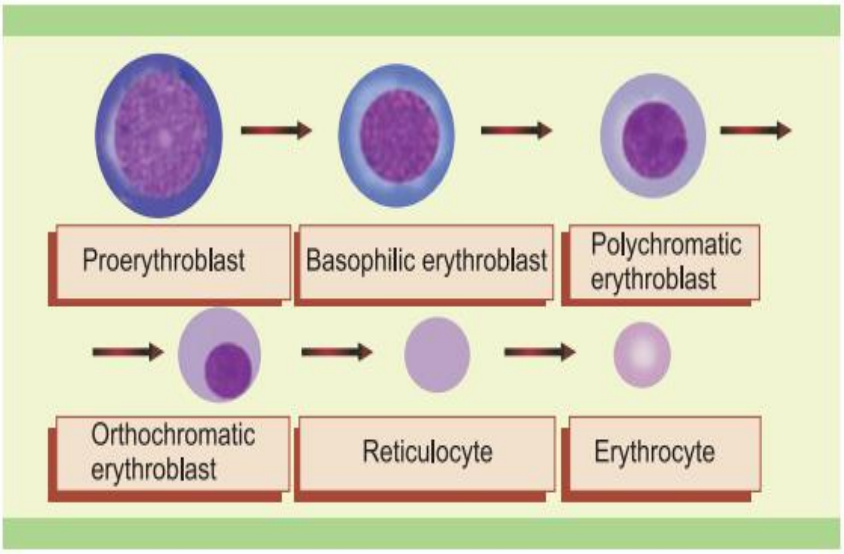


Figure:5 Stages in the formation of a mature red cell. With each stage,cell size and nuclear size become smaller, chromatin clumping increases, and ultimately nucleus is extruded. Colour of cytoplasm

gradually changes from basophilic to orange-red

3.5 Role of Erythropoietin in erythropioesis: It stimulates erythropoiesis by increasing the number of progenitor cells committed to erythropoietin. Normally, 90% of the hormone (erythropoietin) is produced in the kidney and 10% in the liver and elsewhere. The stimulus to erythropoietin production is the oxygen (O_2) tension in the tissues of the kidney . Erythropoietin production therefore increases in anemia, and also when hemoglobin for some metabolic or structural reason is unable to give up O_2 normally, when atmospheric O_2 is low or when defective cardiac or pulmonary function or damage to the renal circulation affects O_2 delivery to the kidney.

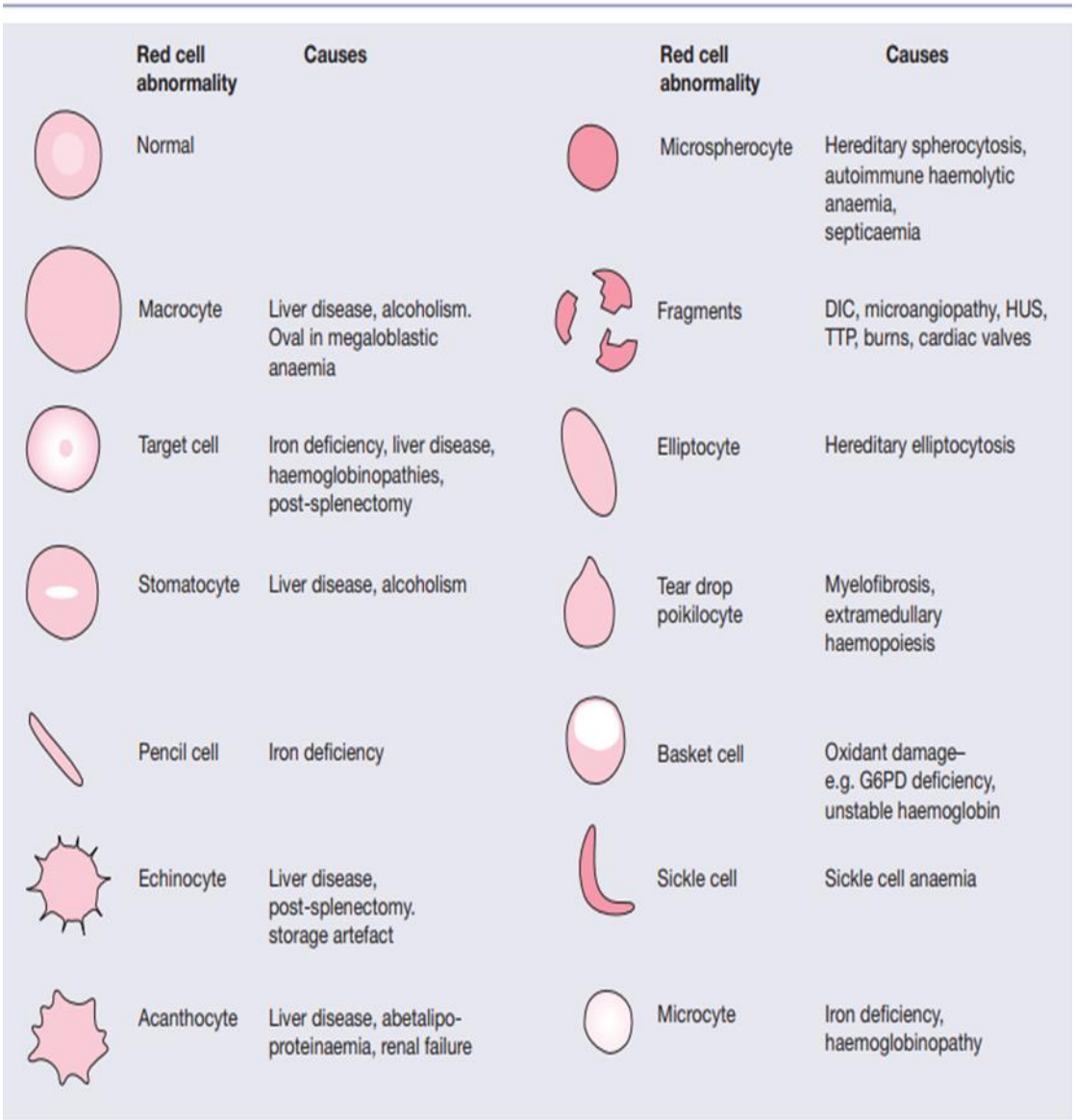
3.6 Morphological charactertics of red blood cells:

A detailed morphological study of RBCs is crucial in diagnosing and understanding various hematological disorders, providing valuable insights into patient's health and guiding regarding treatment of patient. The morphological study of RBCs involves examining their structure, shape, size and abnormalities.

Under the microscope, normal RBCs appear as biconcave discs without a nucleus. Morphological analysis of RBCs includes variation in size (anisocytosis), shape (pokilocytosis) and color (hypochromia or hyperchromia). The different terminology used in morphological study of RBCs are given in the table (table:3)

- *Normocytic normochromic:* Red cells with normal size and colour (i.e. normal haemoglobin content); 7-8 µ size; pink with small area of central pallor (1/3rd the diameter of red cell)
- *Anisocytosis:* Significant variation in size of red cells
- *Poikilocytosis:* Significant variation in shape of red cells; both aniso- and poikilocytosis are nonspecific features of a variety of anaemias
- *Microcytic hypochromic:* Red cells smaller than normal with increased area of central pallor due to deficiency of haemoglobin
- *Macrocytic:* Red cells larger in size than normal; may be round or oval
- *Sickle cells:* Elongated and narrow cells with one or both ends curved and pointed
- *Spherocytes:* Small and densely staining red cells without central area of pallor
- *Target cells:* Cells with accumulation of haemoglobin in centre and periphery with clear intervening area producing a bull's eye or target-like appearance
- *Schistocytes:* Irregular fragmented cells appearing as helmet-shaped and triangular
- *Burr cells:* Cells with many spiny, small, regularly spaced projections on surface
- *Tear drop red cells:* Cells with a tapering drop-like shape
- *Polychromatic red cells:* Slightly larger red cells with faint blue-grey tint due to presence of ribosomal RNA.
- *Basophilic stippling (punctate basophilia):* Presence of fine (megaloblastic anaemia) or coarse (lead poisoning) purple-blue granules (representing ribosomal aggregates) in red cells
- *Howell Jolly bodies:* Round, purple nuclear remnants in red cells
- *Rouleaux:* Arrangement of red cells like a stack of coins
- *Dimorphic red cells:* Presence of two different populations of red cells, e.g. macrocytic and hypochromic, normocytic and hypochromic, etc. Seen in sideroblastic anaemia, partially treated anaemia, myelodysplasia, and post-blood transfusion

Table 3:Red cell terminology



Red cell abnormality	Causes	Red cell abnormality	Causes
Normal		Microspherocyte	Hereditary spherocytosis, autoimmune haemolytic anaemia, septicaemia
Macrocyte	Liver disease, alcoholism. Oval in megaloblastic anaemia	Fragments	DIC, microangiopathy, HUS, TTP, burns, cardiac valves
Target cell	Iron deficiency, liver disease, haemoglobinopathies, post-splenectomy	Elliptocyte	Hereditary elliptocytosis
Stomatocyte	Liver disease, alcoholism	Tear drop poikilocyte	Myelofibrosis, extramedullary haemopoiesis
Pencil cell	Iron deficiency	Basket cell	Oxidant damage– e.g. G6PD deficiency, unstable haemoglobin
Echinocyte	Liver disease, post-splenectomy. storage artefact	Sickle cell	Sickle cell anaemia
Acanthocyte	Liver disease, abetalipo-proteinaemia, renal failure	Microcyte	Iron deficiency, haemoglobinopathy

Variation in shape and size of RBCs

Question: Write down steps of erythropoiesis?

Chapter 4

Hemoglobin

Objective: At the end of this chapter students will able to learn about heamoglobin, its normal values, composition, its structure and function.

Hemoglobin is present in red blood cells and is responsible for delivery of oxygen to the tissues.

A normal adult hemoglobin (Hb A) is composed of two alpha and two beta globin chains along with heam (iron) molecules.

4.1Synthesis of Hemoglobin:

Haem synthesis occurs largely in the mitochondria by a series of biochemical reactions, commencing with the condensation of glycine and succinyl coenzyme A under the action of the key rate-limiting enzyme δ-aminolaevulinic acid (ALA) synthase. Pyridoxal phosphate (vitamin B6) is a coenzyme for this reaction. The ultimately, protoporphyrin combines with iron in the ferrous (Fe2+) state to form haem. A tetramer of four globin chains, each with its own haem in a ‘pocket’, is then formed to make up a hemoglobin molecule.(figure:13).

4.2Normal value of Hemoglobin:

Male: 14-18 g/dL

Female: 12-16 g/dL

Normal range of hemoglobin in different age groups are given below (table:4)

Age	**Female range (g/dL)**	**Male range (g/dL)**
6–12 months	11.3–14.1	11.3–14.1
1–5 years	10.9–15.0	10.9–15.0

5–11 years	11.9–15.0	11.9–15.0
11–18 years	11.9–15.0	12.7–17.7

Table: 4 Normal range of Hb in children

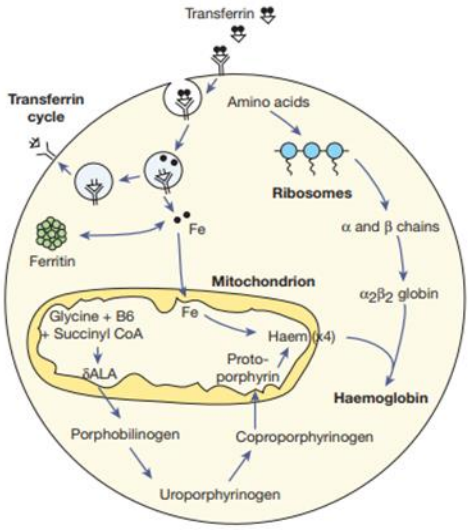


Figure: 13. Haemoglobin synthesis in the developing red cell. The mitochondria are the main sites of protoporphyrin synthesis, iron (Fe) is supplied from circulating transferrin and globin chains are synthesized on ribosomes.
δ-ALA, δ-aminolaevulinic acid; CoA, coenzyme

4.3Haemoglobin function:

The red cells in systemic arterial blood carry O2 from the lungs to the tissues and return in venous blood with CO2 to the lungs. As the haemoglobin molecule loads and unloads O2, the individual globin chains move on each other.

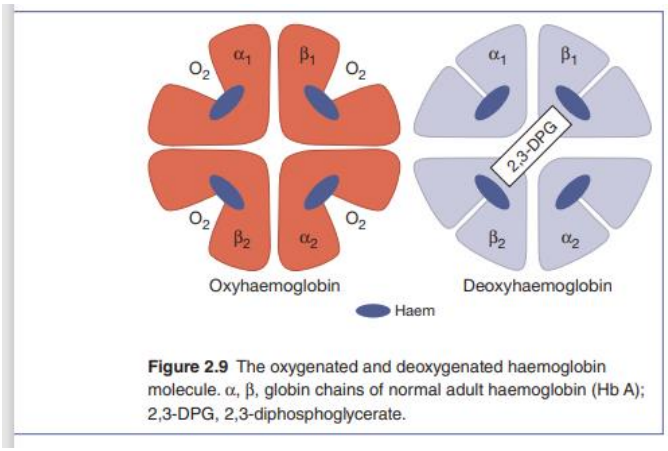


Figure 2.9 The oxygenated and deoxygenated haemoglobin molecule. α, β, globin chains of normal adult haemoglobin (Hb A); 2,3-DPG, 2,3-diphosphoglycerate.

Figure: 14 The oxygenated and deoxygenated Hb molecule

4.4Composition of various types of Hb:

The main function of red cells is to carry O2 to the tissues and to return carbon dioxide (CO2) from the tissues to the lungs. In order to achieve this gaseous exchange they contain the specialized protein haemoglobin. Each molecule of normal adult haemoglobin A (Hb A), the dominant haemoglobin in blood after the age of 3–6 months) consists of four polypeptide chains, α2 β2. each with its own haem group. Normal adult blood also contains small quantities of two other haemoglobins: Hb F and Hb A2.These also contain α chains, but with γ and δ chains, respectively, instead of β (table: 5)

	Hb A	Hb F	Hb A_2
Structure	$\alpha_2\beta_2$	$\alpha_2\gamma_2$	$\alpha_2\delta_2$
Normal (%)	96–98	0.5–0.8	1.5–3.2

Table: 5. types of normal hemoglobin in adults

Question: write down normal values of Hb in males and females? Also enumerate different types of Hb?

Chapter 5:

Red blood cells indices

5.1Definition: RBC indices are the part of complete blood count (CBC) that helps in morphological classification of different types of anemias.

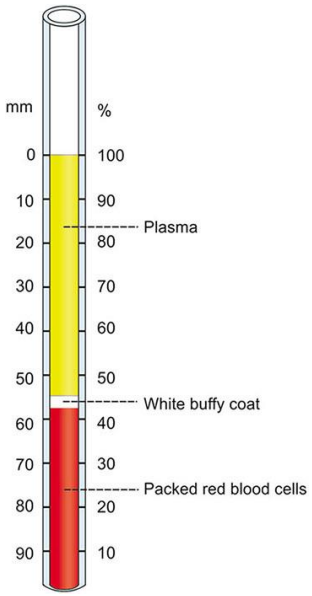
5.2Red cell indices includes:

1-Packed cell volume (PCV) or Hematocrit (HCT)
2-Mean corpuscular volume (MCV)
3- Mean corpuscular hemoglobin (MCH)
4-Mean corpuscular hemoglobin concentration (MCHC)

5.2.1. Determination of Packed Cell Volume or PCV (Hematocrit):

PCV is the volume of packed red cells obtained after centrifugation of a sample of anticoagulated venous or capillary blood. It is expressed either as a percentage of volume of whole blood or as a decimal fraction.

PCV is normally about three times the hemoglobin concentration when the latter is expressed in gm/dl There are two methods for determining PCV—Macro method (Wintrobe method) and Micro method.



5.2.2 Mean corpuscular volume (MCV): MCV represents the average volume of a single red cell. It is expressed in femtoliters or fl (1 fl = 10-15 litres). MCV is performed manually as follows:

$$\text{Mean cell volume (MCV) in femtolitres} = \frac{\text{Packed cell volume in \%}}{\text{Red cell count in millions per cmm}} \times 10$$

MCV measures average cell volume, it may be normal even though there is marked variation in size of red cells (anisocytosis).
Macrocytic anemias (MCV > 100 fl)
Microcytic anemias (MCV < 80 fl)
Normocytic anemias (MCV 80-100 fl)

5.2.3. Mean corpuscular haemoglobin (MCH): This is the average amount of haemoglobin in each red cell. It is expressed in picograms or pg (1 pg = 10-12 of a gram) and is derived manually from the following formula

$$\text{Mean cell haemoglobin (MCH) in picograms (pg)} = \frac{\text{Haemoglobin (gm/dl)} \times 10}{\text{Red cell count in millions/cmm}}$$

Low MCH is found in microcytic hypochromic anaemia, while high MCH in macrocytic anaemia.

5.2.4. Mean corpuscular haemoglobin concentration (MCHC): This represents the average concentration of haemoglobin in a given volume of packed red cells. It is expressed in grams/dl and calculated as follows:
Low MCHC occurs in microcytic hypochromic anaemia. An increase in MCHC occurs in hereditary spherocytosis.

$$\text{Mean cell haemoglobin concentration (MCHC) in gm/dl} = \frac{\text{Hb (gm/dl)}}{\text{PCV (\%)}} \times 100$$

Question: How will you determine PCV in a lab?

Chapter 6:

Reticulocyte count

Objective: By the end of this chapter students should able to know what are reticulocyte count, its normal count , its interpretation and different formulas

Reticulocytes are young red cells that contain RNA remnants. RNA stains with supravital dyes such as brilliant cresyl blue or new methylene blue with formation of blue precipitates of granules or filaments. (figure:11). Smears are made on a glass slide, and after staining reticulocytes are counted among 1,000 red cells, and the result is expressed as a percentage.

6.1Normal Count:

The reference ranges for the reticulocyte count are as follows:

- Adult/elderly/child: 0.5-2%
- Infant: 0.5-3.1%
- Newborn: 2.5-6.5%

6.2Causes of reticulocytosis(increase in reticulocyte count):

• Acute blood loss
• Haemolytic anaemia
• Response to specific therapy in nutritional anaemias.

6.3Causes of reticulocytopaenia(decrease in reticulocyte count):

• Deficient red cell production
• Iron deficiency anaemia
• Anaemia of chronic disease
• Aplastic anaemia
• Anaemia due to marrow infiltration (leukaemia, lymphoma, metastatic cancer).
• Ineffective erythropoiesis
• Megaloblastic anaemia.

6.4Interpretation of reticulocyte count:

In adults and children, the normal reticulocyte count is 0.5-2.5%. In newborns, reticulocyte count is 2-5%.

Reticulocyte count is performed to assess erythropoietic activity of the bone marrow in case of anaemia. A high reticulocyte count indicates increased RBC turnover, typically in response to conditions such as anemia, hemorrhage or certain medical treatments like chemotherapy. A low reticulocyte count shows bone marrow suppression, nutritional deficiency or chronic disease.

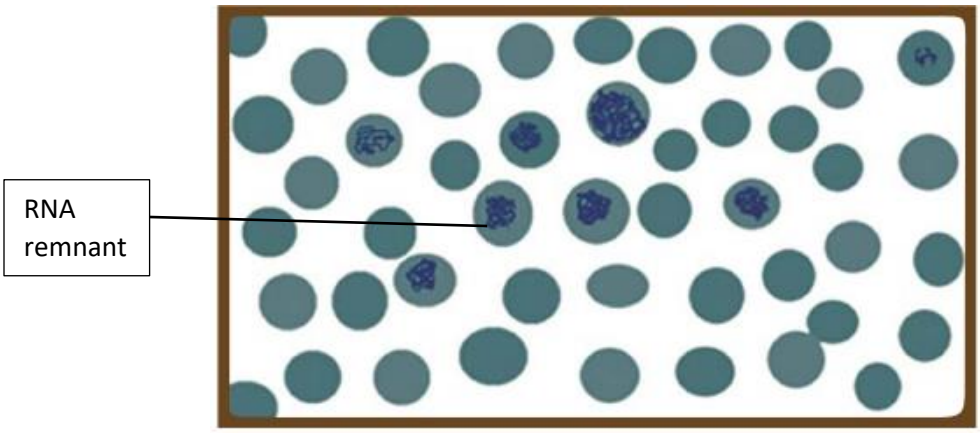


Figure: 11 Reticulocytes stained with a supravital stain

6.5 Measures of reticulocytes:

Reticulocyte count can be expressed in various ways as follows:

1. Reticulocyte count: This is the number of reticulocytes counted amongst 1000 red cells and expressed as a percentage.

$$\text{Reticulocyte count} = \frac{\text{Reticulocytes counted}}{\text{Number of red cells}} \times 100$$

2. Corrected reticulocyte count: This is the reticulocyte count corrected for the degree of anaemia.

$$\text{Corrected reticulocyte count} = \frac{\text{Reticulocyte count} \times \text{PCV of patient in \%}}{\text{Average PCV for age}}$$

3. Absolute reticulocyte count: This is the number of reticulocytes in 1 cmm of blood.

$$\text{Absolute reticulocyte count} = \frac{\text{Reticulocyte percentage} \times \text{Red cell count in million/cmm}}{}$$

Normal absolute reticulocyte count is 50,000-100,000/cmm.

Question: Write down the method/ procedure of making retic slide?

Chapter 7:

Erythrocyte Sedimentation Rate (ESR)

Objective: By the end of this chapter students should be able to know definition of ESR, its methods, its principle , normal ranges, its interpretation, factors that will effect ESR, significance of ESR.

The erythrocyte sedimentation rate (ESR) is the rate at which erythrocytes settle down at the bottom of the tube at the end of 1st hour. It is a commonly used non-specific test in clinical practice.

7.1Methods: Two methods:

1) Westergren methods
2) Wintrobes method

Westergren method is preferred.

7.2Principle of test: When citrated blood in a vertically positioned Westergren pipette is left undisturbed, red cells aggregate, stack together to form rouleaux, and sediment through the plasma. The ESR is the rate at which this sedimentation occurs in 1 hour as indicated by the length of the column of clear plasma above the red cells, measured in mm.

7.3Reference range

Men . . . . . Up to 10 mm/hour*

Women . . . . . Up to 15 mm/hour*

Elderly . . . . . Up to 20 mm/hour*

*These figures should be checked locally.

Note: Higher values are obtained during menstruation, pregnancy, puerperium.

7.4Causes of ESR

7.4.1Raised ESR

I. Anaemia due to any cause
II. Acute and chronic inflammatory conditions and infections including:
    a. HIV disease
    b. Tuberculosis*
    c. Acute viral hepatitis
    d. Arthritis
    e. Bacterial endocarditis
    f. Pelvic inflammatory disease
    g. Ruptured ectopic pregnancy
    h. Systemic lupus erythematosus
III. African trypanosomiasis (rises rapidly)
IV. Visceral leishmaniasis
V. Lymphoma, Hodgkins disease, some tumours
VI. Drugs, including oral contraceptives

Note: The ESR is not usually significantly raised in typhoid, brucellosis, malaria, infectious mononucleosis, uncomplicated viral diseases, renal failure

7.4.2 Reduced ESR:

I. Sedimentation is falsely low in:
II. Polycythaemia,
III. Dehydration,
IV. Dengue Haemorrhagic fever,
V. Other conditions associated with haemoconcentration.

7.5Factors affecting ESR:

- Conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and C-reactive protein.

- The ESR is also affected by many other factors including anaemia, pregnancy, haemoglobinopathies, haemoconcentration and treatment with anti-inflammatory drugs.

- Fibrinogen, immunoglobulins, and C reactive protein increase red cell aggregation.
- Sedimentation is increased when the ratio of red cells to plasma is altered, e.g. in anaemia. Sedimentation is reduced when the red cells are abnormally shaped, e.g. sickle cells. High temperatures (over 25 C) increase sedimentation.

7.6 Significance of ESR:

- It is a nonspecific test. When performed, test results must be interpreted in conjunction with clinical findings and the results of other laboratory tests.
- It is raised in a wide range of infectious, inflammatory, degenerative, and malignant conditions associated with changes in plasma proteins, particularly increases in fibrinogen, immunoglobulins, and C-reactive protein. The ESR is also affected by many other factors including anaemia, pregnancy, haemoglobinopathies, haemoconcentration and treatment with anti-inflammatory drugs.
- Moderately raised sedimentation rates can sometimes be found in healthy people, particularly those living in tropical countries and a ‘normal’ ESR cannot exclude disease.

Question: Write down normal values and significance of ESR?

Chapter 8:

Anemia

Objective: By the end of this chapter students should able to know what is anemia, its classification, lab findings, prevention of anemia.

What is iron deficiency anemia, its causes, clinical features and diagnosis of IDA.

8.1Definition:

It is defined as a reduction in the hemoglobin concentration of the blood below normal for age and sex.

Worldwide anemia is the commonest red cell disorder. It occurs when the concentration of haemoglobin falls below what is normal for a person's age, gender, and environment, resulting in reduced oxygen carrying capacity of the blood (table:6).

Haemoglobin values are lower in women than men, probably due to menstrual loss and the influence of hormones on erythropoiesis. Haemoglobin levels fall in normal pregnancy due to an increase in plasma volume. (table:6)

Normal lower limits for haemoglobin*

Anaemia is present when the haemoglobin concentration falls below:

Newborn infants	140 g/l (14.0 g/dl)
Child 6 months–4 years	110 g/l (11.0 g/dl)
Child 5–11 years	115 g/l (11.5 g/dl)
Child 12–14 years	120 g/l (12.0 g/dl)
Non-pregnant women	120 g/l (12.0 g/dl)
Pregnant women	110 g/l (11.0 g/dl)
Men and adolescent boys	130 g/l (13.0 g/dl)

*Values are for those living at sea level. Haemoglobin levels rise with altitude, e.g. by about 10 g/l at 2 000 m (6 500 feet) and about 20 g/l at 3 000 m (10 000 feet).

Anaemia is described as *mild* when the haemoglobin is between 100 g/l and the level in the above table, *moderate* when 70–100 g/l, and *severe* when below 70 g/l.

Note: Figures are taken from WHO publications *Iron deficiency: Indicators for assessment and strategies for prevention* (WHO NUT/96.12, 1996), and *Preventing and controlling iron deficiency anaemia through primary health care* (WHO, 1989).

Table:6 normal lower limits for Hb

8.2Classification of Anaemia:

The most useful classification is that based on red cell indices (morphological classification) This divides the anaemia into:

a. Microcytic, Hypochromic Anemia

b. Normocytic Normochromic Anemia

c. Macrocytic anemia

The normal ranges of red cell indices in adults are as follows

MCV = 80-100 fl

MCH = 27-32 pg

MCHC= 32-36 gm/dl

Microcytic, hypochromic	Normocytic, normochromic	Macrocytic
MCV <80 fL	MCV 80–95 fL	MCV >95 fL
MCH <27 pg	MCH ≥27 pg	Megaloblastic: vitamin B_{12} or folate deficiency Non-megaloblastic: alcohol, liver disease, myelodysplasia, aplastic anaemia, etc. (see Table 5.10)
Iron deficiency	Many haemolytic anaemias	
Thalassaemia Anaemia of chronic disease (some cases) Lead poisoning Sideroblastic anaemia (some cases)	Anaemia of chronic disease (some cases)	
	After acute blood loss	
	Renal disease	
	Mixed deficiencies	
	Bone marrow failure (e.g. post-chemotherapy, infiltration by carcinoma, etc.)	

MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

Table: classification of anemia

Red cell distribution width (RDW):

RDW is the degree of variation of red cell size and can be determined on some blood cell analysers. This parameter may sometimes be helpful for distinguishing iron deficiency anaemia from β thalassaemia minor (low MCV with high RDW: iron deficiency anaemia; low MCV with normal RDW: β thalassaemia minor).

8.3Laboratory findings in Anemia:

1-Although the **red cell indices** will indicate the type of anaemia, further useful information can be obtained from the initial blood sample.

2**- Leucocyte and platelet counts:** Measurement of these helps to distinguish 'pure' anaemia from 'pancytopenia' (subnormal levels of red cells, neutrophils and platelets), which suggests a more general marrow defect or destruction of cells (e.g. hypersplenism). In anaemias caused by haemolysis or haemorrhage, the neutrophil and platelet counts are often raised; in infections and leukaemias, the leucocyte count is also often raised and there may be abnormal leucocytes or neutrophil precursors present.

3**-Reticulocyte count**: The normal percentage is 0.5–2.5%, and the absolute count 50–150×109 /L This should rise in anaemia because of erythropoietin increase, and be higher the more severe the anaemia.

4**- Blood film** It is important to examine the blood film in all cases of anaemia. Abnormal red cell morphology (Figure:15) or red cell inclusions (Figure:16) may suggest a particular diagnosis. During the blood film examination, white cell abnormalities are sought, platelet number and morphology are assessed and the presence or absence of abnormal cells (e.g. normoblasts, granulocyte precursors or blast cells) is noted.(figure 15,16)

5-**Bone marrow examination** This is needed when the cause of anaemia or other abnormality of the blood cells cannot be diagnosed from the blood count, film and other blood tests alone.

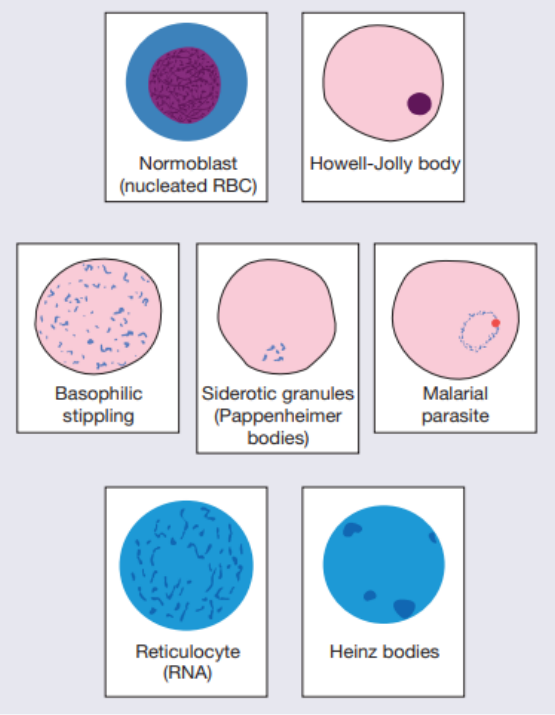


Figure:16 RBC inclusions

8.4Prevention of anemia:

Have adequate knowledge of anemia and anemia prevention and control measures.

1. ***Iron-rich foods:*** Include plenty of iron-rich foods in your diet, such as lean meats, poultry, fish, beans, lentils, tofu, and fortified cereals. Consuming vitamin C-rich foods alongside iron can enhance absorption.
2. ***Leafy greens***: Incorporate leafy green vegetables like spinach, kale, and broccoli into your meals. These are good sources of iron and other essential nutrients.
3. ***Vitamin B12:*** Consume foods rich in vitamin B12, such as eggs, dairy products, fish, and fortified cereals. For those following a vegetarian or vegan diet, consider B12 supplements.
4. ***Folic acid:*** Ensure an adequate intake of folic acid by including foods like lentils, beans, leafy greens, and fortified grains in your diet.

5. ***Regular health check-ups:*** Schedule regular check-ups with your healthcare provider to monitor your blood count and address any signs of anemia early on.
6. The need for presumptive treatment for parasitic infections;etc.
7. Preventing thalassemia involves a combination of genetic counseling, family screening.

Polycythemia, or Erythrocytosis:

it refers to an increase in the absolute red blood cell (RBC) mass in the body. In practice, this is reflected by an increase in hemoglobin levels, or hematocrit, over what is considered physiologic for the particular age and gender.

Thalassaemia:

It is the name for a group of inherited conditions that affect haemoglobin. People with thalassaemia produce either no or too little haemoglobin, which is used by red blood cells to carry oxygen around the body. This can make them very anaemic. There are two types of thalassemia

- Alpha thalassemia: Alpha thalassemia is caused by reduced or absent synthesis of alpha globin chains.
- Beta thalassemia: beta thalassemia is caused by reduced or absent synthesis of beta globin chains.

8.5 Iron deficiency anemia:

Iron deficiency is the major cause of a microcytic, hypochromic anaemia, in which the two red cell indices, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH), are reduced and the blood film shows small (microcytic) and pale (hypochromic) red cells.

Iron deficiency is a state of low total body iron content. Iron deficiency anaemia develops when body iron stores are depleted, level of circulating iron is reduced, and there is insufficient iron available for erythropoiesis.

8.5.1 Storage forms of Iron:

Iron is stored in the macrophages as ferritin and haemosiderin,

8.5.2 Source of iron:

Meat products, eggs, green leafy vegetables are rich sources of iron

8.5.3 Absorption of iron:

Iron absorption mostly occurs in duodenum and upper jejunum. Absorption is in ferrous form.

8.5.4Causes of Iron deficiency Anemia:

1. **Inadequate dietary intake of iron**
2. **Defective absorption of iron**—Subtotal gastrectomy, coeliac disease, *Helicobacter pylori* gastritis, antacids, proton pump inhibitors
3. **Excessive loss of iron**—Gastrointestinal bleeding (e.g. oesophageal varices, hiatus hernia, peptic ulcer, gastritis, Meckel's diverticulum, Crohn's disease, ulcerative colitis, hookworm infestation, various neoplasms especially carcinoma of colon, marathon runners); uterine bleeding (menorrhagia); urinary tract bleeding (haematuria, haemoglobinuria); respiratory tract bleeding (haemoptysis); bleeding disorders
4. **Increased requirements for iron**—Pregnancy, infancy, adolescents

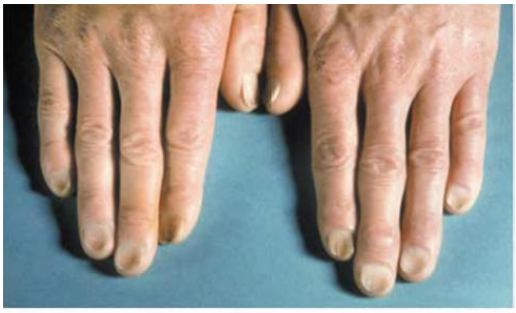
8.5.5Clinical features of Iron deficiency Anemia:

1-General Clinical Features of Anaemia:

Patients may present with non-specific symptoms and signs of anaemia such as weakness, easy fatiguability, breathlessness on exertion, tachycardia, and systolic heart murmur.

2- Clinical features related to Iron deficiency anemia:

-Unusual dietary cravings such as clay, paint, cardboard, coal----- called as Pica
-Painless glossitis,
-Angular stomatitis,
-Brittle, ridged spoon shaped nails (koilonychia)



Koilonychia (brittle, ridged spoon shaped nails)

Angular cheilosis (fissuring and ulceration of the corner of mouth)

8.5.6 Diagnosis of Iron Deficiency Anemia:

Proper history and examination findings are the key in diagnosing any anemia.

1-**Complete blood count:**
Shows low Heamoglobin levels , low RBC count and low red cell indices(Low MCV,MCH levels)

2- Peripheral smear:
The blood film shows hypochromic (pale), microcytic (small size) red cells with occasional pencil-shaped cells also seen.

The white cell count is normal

Platelets are often increased, especially in the presence of blood loss.

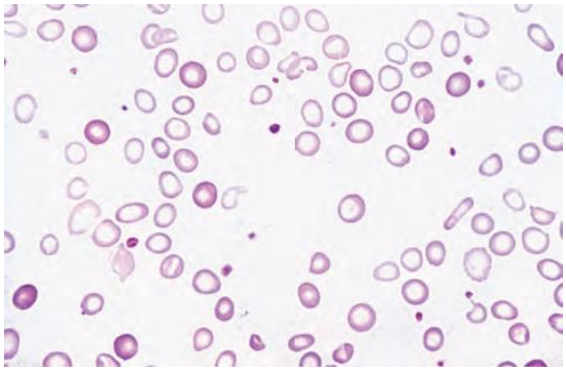
3- Reticulocyte count:
The reticulocyte count is low in relation to the degree of anaemia.

4- Serum Iron Profile:

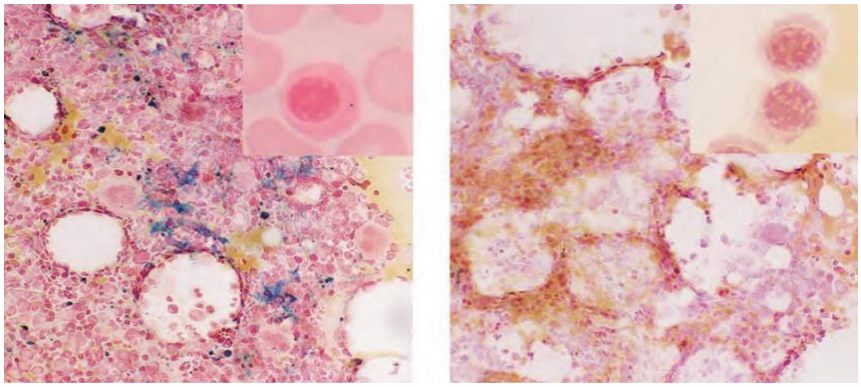
a-**Serum Iron**: low
b-**Serum total iron binding capacity (TIBC):** which reflects amount of transferrin in circulation, is increased in iron deficiency anaemia
c-**Serum Ferritin:** low

5- Bone marrow examination:

Absence of stainable iron in the bone marrow on Perl's Prussian blue reaction is a specific and a reliable test for diagnosis of iron deficiency anaemia.



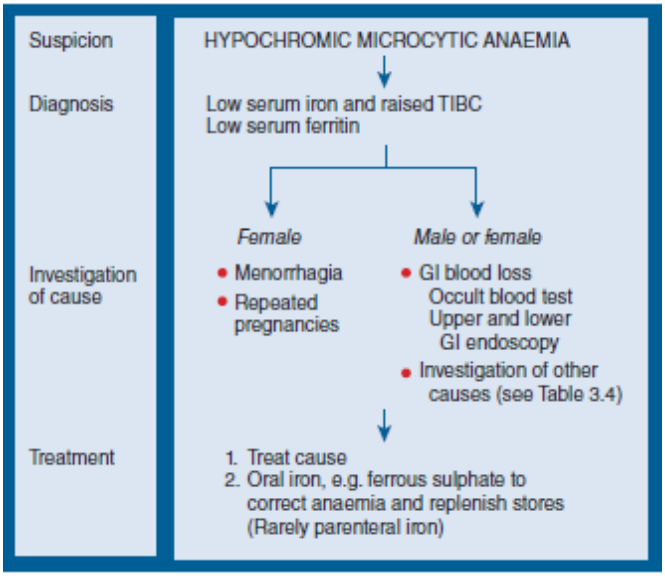
The peripheral blood film in severe iron deficiency anaemia. The cells are microcytic and hypochromic with pencil cells



Bone marrow iron assessed by Perls' stain. (a) Normal iron stores indicated by blue staining in the macrophages. (b) Absence of blue staining (absence of haemosiderin) in iron deficiency.

6- Investigations for underlying cause of Iron deficiency anemia:

This may be obvious (e.g. bleeding) or may require tests such as GIT work-up especially in adults (test for faecal occult blood, endoscopy or radiology), pelvic ultrasound in females (if menorrhagia is present), stool examination for hookworm, etc.



Flow chart showing Investigations and diagnosis of iron deficiency anemia

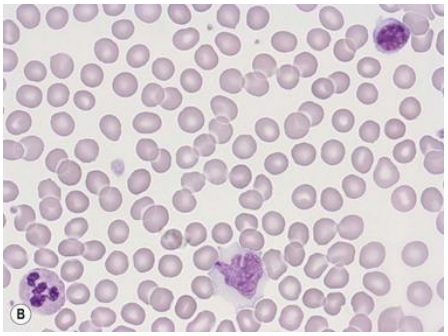
Differential Diagnosis of Hypochromic Microcytic Anemia

	Iron deficiency	Chronic inflammation or malignancy	Thalassaemia trait (α or β)	Sideroblastic anaemia
MCV/ MCH	Reduced in relation to severity of anaemia	Normal or mild reduction	Reduced; very low for degree of anaemia	Usually low in congenital type but MCV usually raised in acquired type
Serum iron	Reduced	Reduced	Normal	Raised
TIBC	Raised	Reduced	Normal	Normal
Serum ferritin	Reduced	Normal or raised	Normal	Raised
Bone marrow iron stores	Absent	Present	Present	Present
Erythroblast iron	Absent	Absent	Present	Ring forms
Haemoglobin electrophoresis	Normal	Normal	Hb A_2 raised in β form	Normal

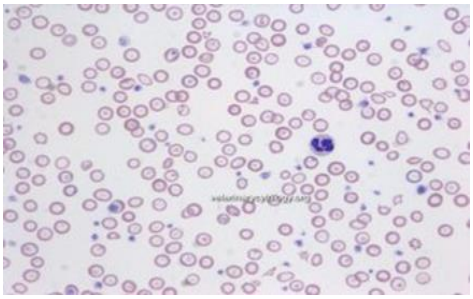
MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume; TIBC, total iron-binding capacity.

Question: Define Anemia? Write down its classification?

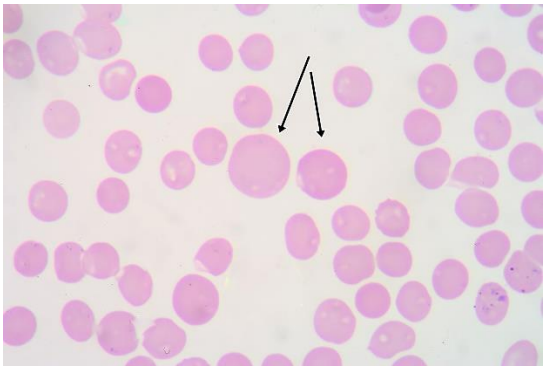
Red cell morphology in different types of Anemias



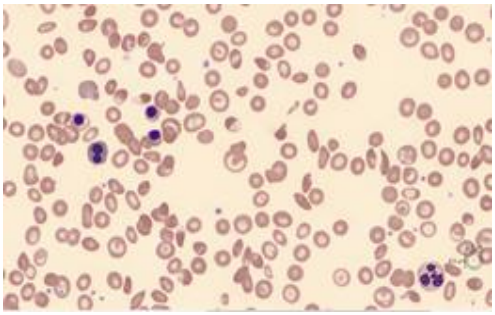
Normocytic Normochromic RBCs



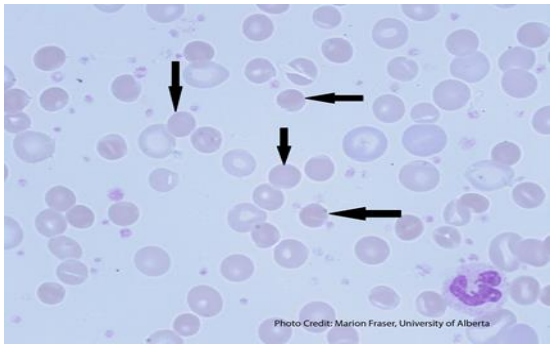
Microcytic Hypochromic RBCs



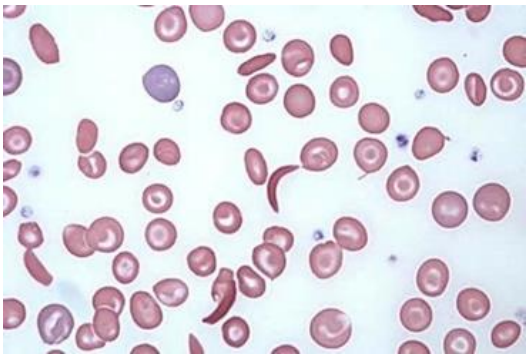
Macrocytic RBCs



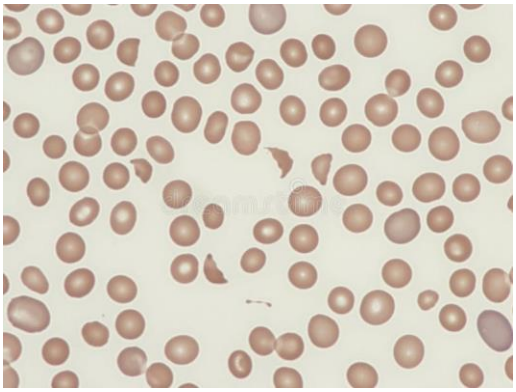
Beta Thalassemia



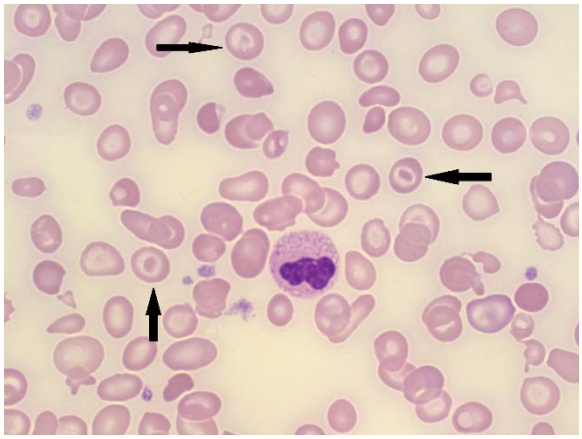
Spherocytes in Hereditary spherocytosis



Sickle shaped RBCs in Sickle cell Anemia



Fragmented RBCs



Target cells

Chapter9:

Haemoparasites

(Blood Parasites)

Objective: By the end of the chapter students will be able to know definition of hemoparasites, different haemoparasites, and their methods of detection in a Lab.

Definition:

Haemoparasites are pathogens that inhabit the bloodstream of the host and includes microorganisms such as plasmodium, leishmania etc.

1. Plasmodium species (cause malaria)
2. Trypanosoma species (cause African sleeping sickness and Chagas disease)
3. Babesia species (cause babesiosis)
4. Leishmania species (cause various forms of leishmaniasis)
5. Filariasis worms (e.g., Wuchereria bancrofti)
6. Toxoplasma gondii (can infect blood cells)
7. Microfilariae (larval forms of filarial worms)
8. Schistosoma species (cause schistosomiasis)

Plasmodium:

Malaria is one of the most widespread parasitic diseases of the world. It mainly occurs in tropical and subtropical areas but cases are found all over the world. Causative agent of malaria is called as Plasmodium. Four species are involved--namely, P.vivax, P.ovale, P.malariae and P.falciparum, P. knowlesi.All species differ in morphology, life cycle and the type of disease they cause. The parasite invades and destroys red blood cells. It is transmitted from one person to another through bites of a mosquito of the genus anopheles.

Characteristics of malarial parasite: Morphological characteristics of different species of malarial parasite(plasmodium) are describe as follows:

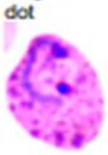
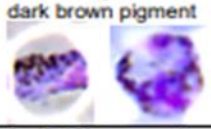
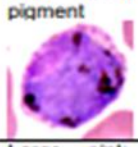
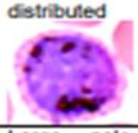
Form	*P. vivax*	*P. ovale*	*P.falciparum*	*P. malariae*
Ring form	1/3 of cell diameter, single, heavy, chromatin dot 	Similar to vivax but larger and more amoeboid 	Delicate, small, 1-2 dots, more than one in a cell, at the edge of Cell (applique) or drawn into a filament (accole form) 	Ring often smaller than *P.vivax* occupying 1/6 of cell heavy chromatin dot; pigment forms early. 
Trophozoites	Amoeboid, small vacuoles, fill the cell, fine brown pigment, stream of cytoplasm close to large chromatin dot 	Ring usually maintained until late 	Usually not seen 	Non-amoeboid, rounded or band shaped, solid forms; chromatin may be hidden by the coarse dark brown pigment 
Mature Schizonts	16 (12-24) merozoites, fill, entire RBC. Each has cytoplasm and chromatin dot 	¾ of cell occupied by 8 (8-12) merozoites, in rosette or irregular clusters, brown pigment in centre 	Rarely seen in peripheral blood 	8 (6-12) merozoites in rosettes or irregular clusters filling normal sized cells, central green-brown pigment 
Macrogamet-ocytes	Rounded or oval homogenous cytoplasm, with diffuse delicate light brown pigment. Large pink chromatin mass surrounded by colourless halo, evenly distributed pigment 	Similar to *P.vivax* 	Sex differentiation difficult; crescent or sausage shaped; may appear in showers; black pigment near chromatin dot, which is often central 	Similar to *P.vivax* but less in number, pigment darker and coarser 
Microgame-tocytes	Large pink to purple chromatin mass surrounded by pale or colourless halo; evenly distributed 	Similar but smaller than *P.vivax* 	Like macrogametocytes 	Similar to *P.vivax* but less in number, pigment darker and coarser 
Main differential Criteria	Large pale red cell; trophozoites irregular; pigment usually present; Schuffner's dots not always present; several phases of growth seen in one smear; gametocytes appear early.	Red cell enlarged, oval with fimbriated edges; Schuffner's dots seen all stages.	Develop in blood vessels in internal organs; delicate ring forms and crescent shaped gametocytes seen in peripheral blood.	Red cell normal in size and colour; trophozoites compact, stain usually intense, band form not always seen; coarse pigment; no stippling of red cells; gametocytes appear late

Table: species characteristics of malarial parasite

Procedure for detection malarial parasite:

The diagnosis depends upon demonstration of the parasite in blood.

Thick and thin blood smear are golden standard for diagnosis of plasmodium species. Thick smear is used as a screening test, whereas the thin smear is to identify the species.

The best time for collecting a blood sample is 6- 12 hours after the onset of a chill/ fever as the blood at this time will contain a larger number of trophozoites. It should be repeated 8 hours later to see mature trophozoites that are species specific. It is best to use fresh, non-coagulated capillary blood, obtained by a prick. EDTA is preferred but heparin can also be used.

i-Blood Film/ Smear:

Thick blood film

Principle:

A large amount of blood can be examined for parasitic forms by lysing the red cells and staining for parasite.

Procedure:

Touch a large drop of blood from the pulp of a finger with a glass slide and rotate it to spread blood in an area equal to a two-rupee coin. The film should be such that newsprint can be seen through it. Alternatively, place a drop of blood in the centre of a glass slide and spread it with a corner of another glass slide. Dry the blood film ,stain it and examine it under the microscope under an oil immersion lense.

Thin blood film

Principle:

By spreading the blood cells in a thin layer, the size of the red cells, inclusions and extracellular forms can be more easily visualised.

Procedure:
Slides are prepared in the usual manner and stained in the same way as for differential leukocyte count and red blood cell morphology More time should be spent on the examination of the edges and head-end of the slide.

Thick and thin unstained blood film for malarial parasite

ii-*Rapid diagnostic tests (RDT)* The tests aid in the diagnosis of malaria by detecting malaria parasite antigens in human blood.
iii-Molecular diagnosis PCR on blood.

Babesia

Babesia are sporozoan parasites that morphologically resemble P. falciparum. It is a tick-borne parasite. Most human cases were reported from the USA. In human, the parasites are found in the erythrocytes.

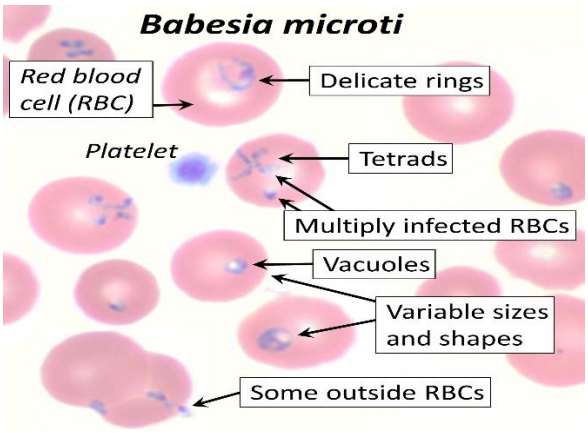
Characteristics of parasite:

Trophozoites are 2–5 μm in diameter found inside the red cells. The shape may be pyriform, amoeboid, or spindle-like, usually in pairs and are often mistaken as ring form

of Plasmodium.The definitive host is ticks. Humans and rodents are the intermediate hosts. Modes of transmission to human are through bite of ticks and via blood transfusion

Procedure for detection:

1. Microscopic examination Absence of schizonts and gametocytes and presence of tetrads in peripheral blood smear.
2. Molecular diagnosis PCR on blood.



Blood film under microscope showing tetrads

Trypanosoma brucei gambiense

It is endemic in scattered foci in West and Central Africa. The principal vectors are riverine tsetse flies. Trypanosomes live in human and other vertebrate hosts. From the blood, they invade regional lymph nodes and finally CNS.

Characteristics of parasite:

- In vertebrate host Trypomastigote form, which is highly pleomorphic (long slender form, stumpy short broad form and an intermediate form), is about 15–40 μm long and 1.5–3.5 μm broad. In fresh blood films, trypomastigotes are extracellular, colourless, spindle shaped bodies moving rapidly and spinning around the red cells.

- In vector (tsetse fly) Occurs in 2 forms: (a) Epimastigotes (b) Metacyclic trypomastigotes. Trypanosoma brucei gambiense completes its life cycle in 2 hosts. Vertebrate hosts are humans. Game and other domestic animals can also be infected.

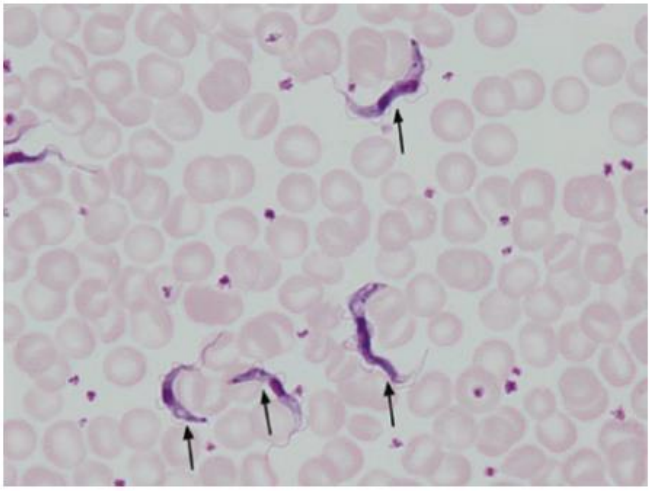


Figure:17 Trypanosoma brucei gambiense

Procedure for detection:

1. Microscopic examination: Wet mount preparation of lymph node aspirates, CSF, and chancre fluid are used for demonstration of trypomastigotes. These specimens can be fixed and stained with Giemsa.

.2. Specific antibodies or antigens can be detected in serum and CSF.

3. Molecular diagnosis PCR

Trypanosoma cruzi

It is limited to South and Central America and it causes Chagas' disease, which is a zoonotic disease. In human, trypomastigotes are in the blood and amastigotes are in tissue.

Characteristics of parasite:

In humans, Trypanosoma cruzi exists in 2 forms, **amastigote and trypomastigote**. Amastigotes are intracellular, oval bodies measuring 2–4 μm in diameter having a nucleus and kinetoplast. Multiplication of the parasite occurs in this stage. This form is found in muscles, nerve cells and reticuloendothelial systems.Trypomastigote is a non-multiplying form found in the peripheral blood of human and other mammalian hosts. In stained blood smears, they are 'C' or 'U' shaped, having a free flagellum of about one third the length of the body and a big kinetoplast.

Procedure for detection:

i. trypomastigotes are seen in thick and thin Giemsa-stained peripheral blood smears under the microscope in acute infection.
ii. Culture medium is used for growing T. cruzi.
iii. Histopathology Biopsy specimens may reveal amastigotes..

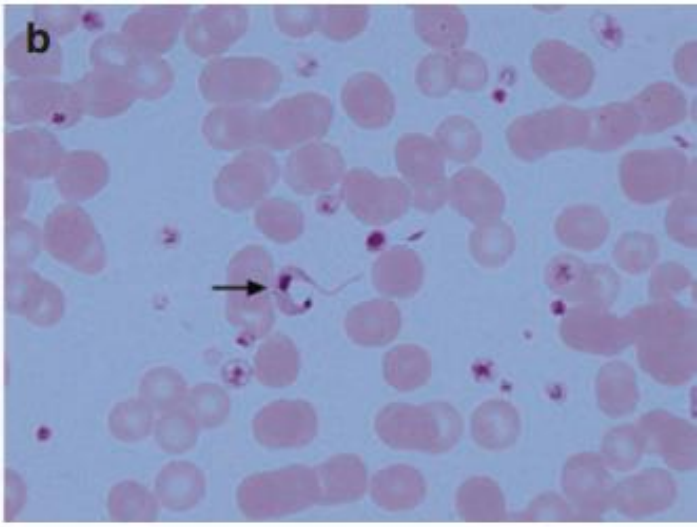


Figure: 18 Trypanosoma cruzi (Trypomastigote in blood)

Microfilariae

Microfilariae are the larvae of nematodes. The filarial worms are long and thin and inhabit the lymphatic system and deep, subcutaneous connective tissues. Most species produce microfilariae, which can be found in the peripheral blood. Microfilariae can cause serious diseases like elephantiasis and blindness. Only filariasis (Elephantiasis) caused by Wuchereria bancrofti occurs in Pakistan

Characteristics of microfilariae:

Microfilariae measure 250–300 μm in length. It has a body sheath. When stained with Giemsa, morphological details can be made out. Body nuclei are seen and they are discrete and countable. The sheath does not take up stain with Giemsa The microfilariae show nocturnal periodicity in peripheral circulation and are present in peripheral blood only at night (between 10 pm and 2 am).

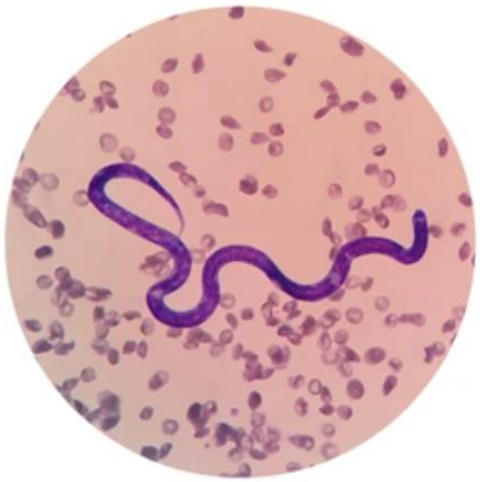


Figure: 19 microfilariae W. bancrofti

Procedure for detection:
Diagnosis of filarial infections is often based on clinical grounds, but demonstration of the parasite is the only accurate means of confirming the diagnosis. Blood should be collected around midnight, and blood film to be made so as to detect microfilariae.

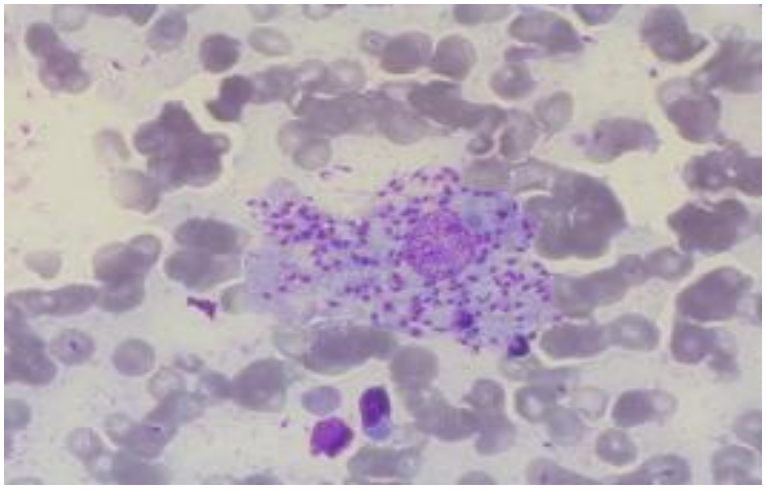
Leishmania donovani

It causes visceral leishmaniasis or kala azar which is a major public health problem in many parts of the world. Habitat In human, the amastigotes are found in the reticuloendothelial system.

The parasite exists in 2 forms. 1. Amastigote form is found in humans and other mammals. The amastigote form of the parasite seen in human samples is called Leishman Donovan (LD) body and it is intracellular. 2. Promastigote form is found in the sandfly. Humans acquire infection by bite of an infected female sandfly.

Procedure for detection:

i. Microscopic examination Demonstration of amastigotes in blood smears and tissue aspirates (bone marrow, spleen, lymph nodes) is the gold standard for diagnosis. Fixed smears can be stained with Giemsa to demonstrate amastigotes
ii. Culture Tissue specimens or blood are cultured in NNN medium.
iii. Specific antibodies or antigens can be detected.
iv. Molecular diagnosis PCR on clinical specimens.
v. Skin test Leishmanin skin test



Macrophage containing Leishman-Donovan (LD) bodies

Toxoplasma gondii

Toxoplasma gondii is an obligate intracellular parasite and it occurs in 3 forms: 1. Oocyst 2. Tachyzoite 3. Tissue cyst .All three forms occur in cat which is the definitive

host. Tachyzoite and tissue cysts are present in the intermediate hosts (other animals including humans). All the three forms are infectious to human.

Schistosoma haematobium

It is endemic in most parts of Africa and West Asia. It is a species of trematode belonging to a group of blood flukes. The adult worms live in the vesical and pelvic venous plexuses of humans.

Charactertics :The adult male worm is 10–15 mm long by 1 mm thick and is covered by a finely tuberculated cuticle. The adult female is 20 mm by 0.25 mm with the cuticular tubercles confined to the 2 ends. The eggs contain ciliated miracidium and are laid in the small veins of urinary bladder and produce eggs that are passed in the urine.

Procedure for detection:

1. Microscopic examination Detection of eggs with characteristic terminal spines in centrifuged urine sample. Eggs which are deposited in rectum may be occasionally found in faeces.
2. Biopsy Bladder mucosa or rectal biopsies to demonstrate eggs.
3. Molecular diagnosis PCR on clinical sample

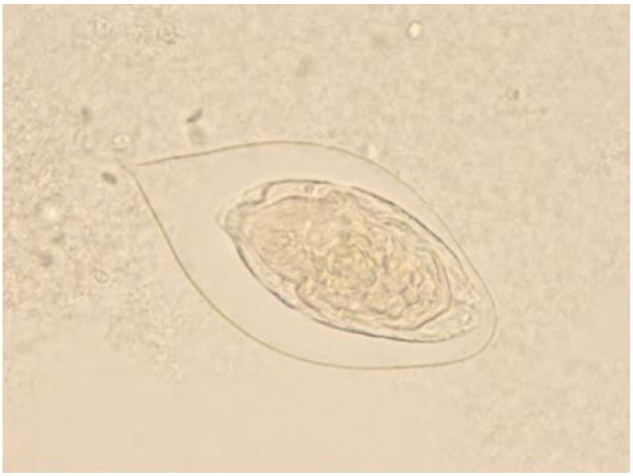


Figure: Schistosoma haematobium eggs

Question: Enumerate different heamoparasite in blood?

Chapter 10:

WHITE BLOOD CELLS

Objective: By the end of this chapter students will be able to know about WBCs ,its different types, normal values.

10.1 Introduction to WBCs:

WBCs are the most important blood cells as they help in defense of our body whenever any foreign substance (bacteria, virus,etc) enters in our body.

10.2 Types of white cells: includes:

1) Phagocyte: which includes:
   a) Granulocytes : neutrophils, eosinophils, basophils
   b) Agarnulocytes: monocytes
      Their function is to protect against bacterial and fungal infections.

2)Lymphocytes: which include B cells, involved in antibody production, T cells concerned with the immune response and in protection against viruses and other foreign cells. White cells have a wide range of lifespan.

10.3 Absolute normal values of WBCs:

Table 8.1 White cells: normal blood counts.

Adults	Blood count	Children	Blood count
Total leucocytes	$4.00–11.0 \times 10^9$/L*	*Total leucocytes*	
Neutrophils	$1.8–7.5 \times 10^9$/L*	Neonates	$10.0–25.0 \times 10^9$/L
Eosinophils	$0.04–0.4 \times 10^9$/L	1 year	$6.0–18.0 \times 10^9$/L
Monocytes	$0.2–0.8 \times 10^9$/L	4–7 years	$6.0–15.0 \times 10^9$/L
Basophils	$0.01–0.1 \times 10^9$/L	8–12 years	$4.5–13.5 \times 10^9$/L
Lymphocytes	$1.5–3.5 \times 10^9$/L		

* Normal black and Middle Eastern subjects may have lower counts. In normal pregnancy the upper limits are: total leucocytes 14.5×10^9/L, neutrophils 11×10^9/L.

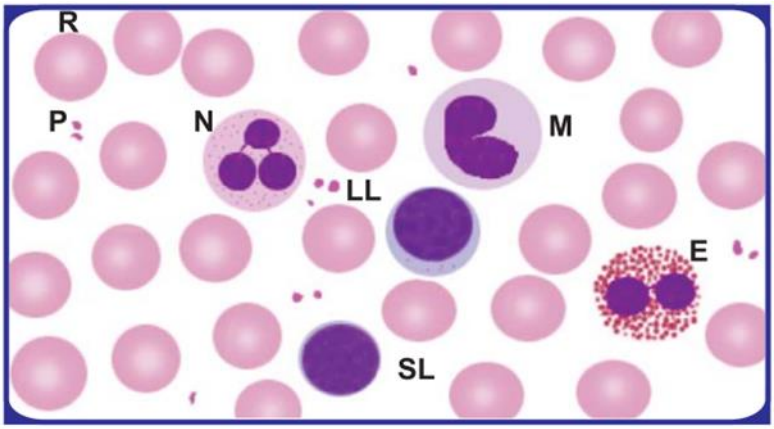


Figure:6 Normal peripheral blood smear showing normocytic normochromic red cells—(R),neutrophil (N),eosinophil (E), monocyte (M), small lymphocyte (SL),large lymphocyte (LL), and platelets (P)

Question: Classify WBCs along with their normal values?

Chapter 11:

STAGES OF DEVELOPMENT OF GRANULOCYTES
(Granulopioesis or Leucopoiesis)

Objective: By the end of this chapter students should be able to understand steps of development of WBCs.

11.1 Stages of Granulopoiesis:

The maturation sequence in granulopoiesis is—myeloblast, promyelocyte, myelocyte, metamyelocyte, band cell, and segmented granulocyte . This process occurs within the marrow.(figure:7,8)

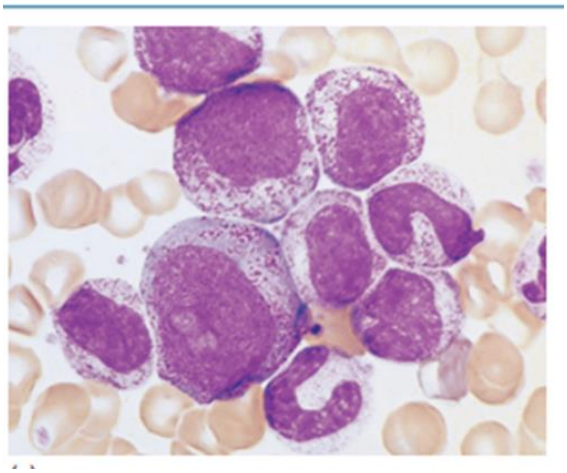


Figure:7 Granulopoiesis. A promyelocyte, myelocytes, and metamyelocytes. (b) The formation of the neutrophil and monocyte . Eosinophils and basophils are also formed in the marrow in a process similar to that for neutrophils

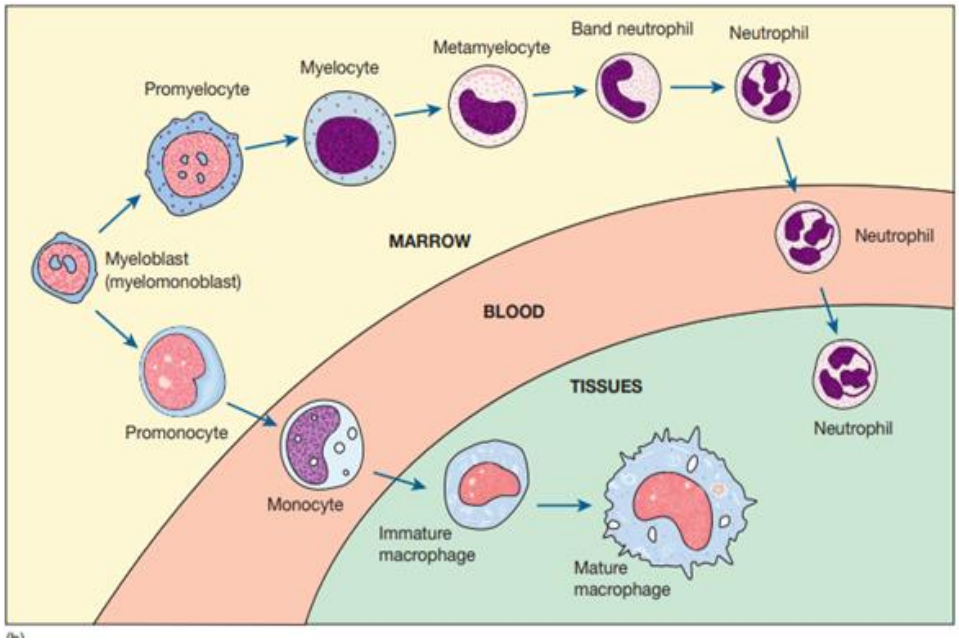
11.2 Steps of development of neutrophils:

After their formation, neutrophils remain in marrow for 5 more days as a reserve pool. Neutrophils have a life span of only 1 to 2 days in circulation

1. Myeloblast: Myeloblast is the earliest recognizable cell in the granulocytic maturation process. It is about 15 to 20 μm in diameter, with a large round to oval nucleus, and small amount of basophilic cytoplasm. The nucleus contains 2 to 5 nucleoli and nuclear chromatin is fine and reticular.
2. Promyelocyte: The next stage in the maturation is promyelocyte which is slightly larger in size than myeloblast. Primary or azurophil granules appear at the promyelocyte stage. The nucleus contains nucleoli as in myeloblast stage, but nuclear chromatin shows slight condensation.
3. Myelocyte: Myelocyte stage is characterized by the appearance of secondary or specific granules (neutrophilic, eosinophilic, or basophilic). Myelocyte is a smaller cell with round to oval eccentrically placed nucleus, more condensation of chromatin than in promyelocyte stage, and absence of nucleoli. Cytoplasm is relatively greater in amount than in promyelocyte stage and contains both primary andsecondary granules. Myelocyte is the last cell capable of mitotic division.
4. Metamyelocyte: In the metamyelocyte stage, the nucleus becomes indented and kidney shaped, and the nuclear chromatin becomes moderately coarse. Cytoplasm contains both primary and secondary granules.
5. Band stage (stab form): This is characterized by band-like shape of the nucleus with constant diameter throughout and condensed nuclear chromatin.
6. Segmented neutrophil (polymorphonuclear neutrophil): With Leishman's stain, nucleus appears deep purple with 2 to 5 lobes which are joined by thin filamentous strands. Nuclear chromatin pattern is coarse. The cytoplasm stains light pink and has small, specific granules.

Primary and secondary granules: The neutrophil granules are of two types: primary or azurophilic granules and secondary or specific granules. Azurophil granules contain myeloperoxidase, lysozyme, acid phosphatase, elastases, collagenases, and acid hydrolases. Specific granules contain lysozyme, lactoferrin, alkaline phosphatases, vitamin B12-binding protein and other substances.

Stages of maturation



11.3 Eosinophils

Eosinophil forms via same stages as the neutrophil and the specific granules first become evident at the myelocyte stage. The size of the eosinophil is slightly greater than that of neutrophil. The nucleus is often bilobed and the cytoplasm contains numerous, large, bright orange-red granules. Maturation time for eosinophils in bone marrow is 2 to 6 days and half-life in blood is less than 8 hrs. In tissues, they reside in skin, lungs, and GIT.

11.4 Basophils

These are only occasionally seen in normal peripheral blood. Basophils are small (5-7 µm); nucleus of the basophils are multilobed, round to oval cells which contain very large, coarse, deep purple granules. The nucleus has condensed chromatin and is covered by granules.

11.5 Monocytes

These are usually larger than other peripheral blood leucocytes and possess a large central oval or indented nucleus with clumped chromatin.

- Monoblast: The initial cell in development is monoblast, which is indistinguishable from myeloblast.
- Promonocyte: The next cell is promonocyte which has an oval or clefted nucleus with fine chromatin pattern and 2 to 5 nucleoli.
- Monoccyte: The monocyte is a large cell (15-20 μm), with irregular shape, oval or clefted (often kidney-shaped) nucleus and fine, delicate chromatin. Cytoplasm is abundant, blue-grey with ground glass appearance and often contains fine azurophil granules and vacuoles

Monocytes circulate in blood for about 1 day and then enter and settle in tissues where they are called as macrophages or histiocytes.

11.6 Lymphocytes:

In postnatal life, the bone marrow and thymus are the primary lymphoid organs in which lymphocytes develop (Fig. 9.2). The secondary lymphoid organs in which specific immune responses are generated are the lymph nodes, spleen and lymphoid tissues of the alimentary and respiratory tracts. In the bone marrow lymphocytes derive from haemopoietic stem cells through a common myeloid lymphoid progenitor.

Lymphocytes are of two types:

- small (75%)
- large(15%)

Most of the lymphocytes in peripheral blood are small (7-10 μm). The nucleus is round or slightly clefted with coarse chromatin and occupies most of the cell.The cytoplasm is basophilic, slight and is visible as a thin border around the nucleus. Around 10-15% of lymphocytes in peripheral blood are large (10-15 μm). Their nucleus is similar to that of small lymphocytes but their cytoplasm is relatively more and contains few azurophilic

(dark red) granules. On immunophenotyping, there are two major types of lymphocytes in peripheral blood:B lymphocytes (10-20%) and T lymphocytes (60-70%).

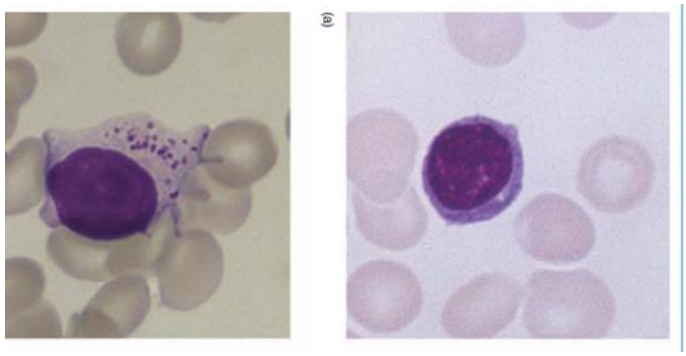


Figure:9 Lymphocytes: (a) small lymphocyte
(b) large granular lymphocyte;

Question: Write down steps of maturation of monocytes?

Chapter12:

Leukemia

Objective: By the end of this chapter students should be able to understand briefly about what is leukemia, its brief classification and diagnosis.

12.1 Definition:

The leukaemias are a group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood. These abnormal cells cause symptoms because of:

(i) Bone marrow failure (e.g. anaemia, neutropenia, thrombocytopenia); and, less commonly,

(ii) Infiltration of organs (e.g. liver, spleen, lymph nodes, meninges, brain, skin or testes).

12.2 Classification of leukaemia :

They are classified broadly into four types:

- Acute Leukemia
  - Acute Lymphoid Leukemia
  - Acute Myeloid Leukemia
- Chronic Leukemia
  - Chronic Lymphoid leukemia
  - Chronic Myeloid leukemia

Acute leukaemia s:

There are two major forms of acute leukaemias: (table:12)

- Acute lymphoblastic leukaemia (ALL)
- Acute myeloid leukaemia (AML).

Table 5.2: The French-American-British (FAB) Co-operative Group classification of acute leukaemias

Acute lymphoblastic leukaemias (ALL)

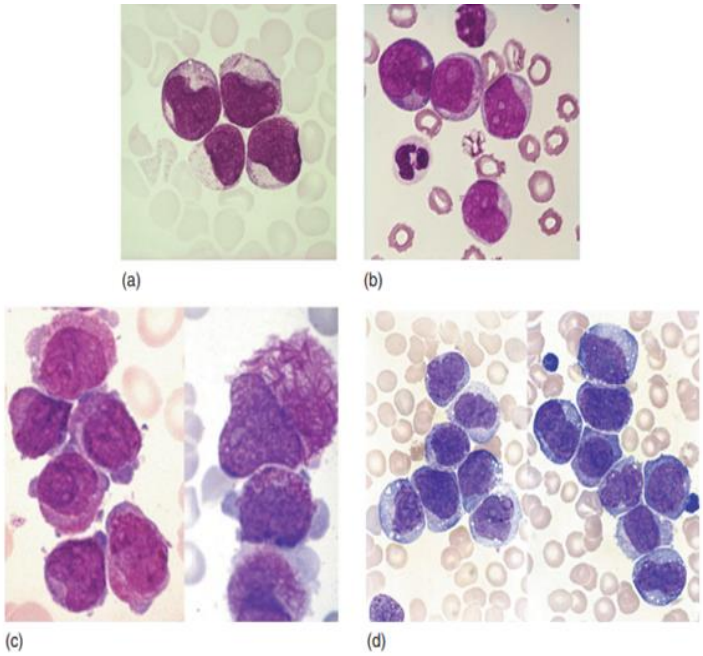
- ALL-L1 type
- ALL- L2 type
- ALL- L3 type

Acute myeloid leukaemia (AML)

- Acute myeloblastic leukaemia-minimally differentiated (M0)
- Acute myeloblastic leukaemia without maturation (M1)
- Acute myeloblastic leukaemia with maturation (M2)
- Hypergranular promyelocytic leukaemia (M3)
  - Hypo-or micro-granular promyelocytic leukaemia (M3 variant)
- Acute myelomonocytic leukaemia (M4)
  - Acute myelomonocytic leukaemia with bone marrow eosinophilia. (M4 Eo)
- Acute monocytic leukaemia (M5)
  - Undifferentiated (monoblastic) (M5a)
  - Well-differentiated (promonocytic-monocytic) (M5b)
- Acute erythroleukaemia (M6)
- Acute megakaryocytic leukaemia (M7)

Table : 12 Classification of acute leukemia

Acute leukemias are usually aggressive diseases in which malignant transformation occurs in a haemopoietic stem cell or early progenitor. Acquired genetic damage results in an increased rate of proliferation, reduced apoptosis and a block in cellular differentiation. Together these events cause accumulation in the bone marrow of early haemopoietic cells known as blast cells.



12.3 Diagnosis of acute leukaemia:

Acute leukaemia is normally defined as the presence of at least 20% of blast cells in the bone marrow or blood at clinical presentation. However, it can be diagnosed with less than 20% blasts if certain leukaemia-specific cytogenetic (chromosomal abnormalities) or molecular genetic abnormalities are present.

The investigations required for patients of acute leukemia are as follows:

Investigations of acute Leukemia

Table 13.3 The initial evaluation of a new patient with suspected acute myeloid leukaemia.
Assessment of medical history, examination and performance status; analysis for co-morbidities (see Chapter 12)
Full blood count and differential
Bone marrow aspirate and trephine biopsy
Immunophenotyping of bone marrow (and/or blood if blast cells present)
Cytogenetic analysis by karyotype
Mutation analysis
Cytochemical analysis (performed in some countries instead of immunophenotyping)
Biochemistry (liver, renal, uric acid, calcium, LDH)
Coagulation
Pregnancy test
Information on oocyte or sperm storage
Assessment of eligibility for stem cell transplantation
Hepatitis B, C and HIV test
CXR with ECG and ECHO
CXR, chest X-ray; ECG, electrocardiography; ECHO, echocardiography; HIV, human immunodeficiency virus; LDH, lactate dehydrogenase.

Chronic Leukemia:

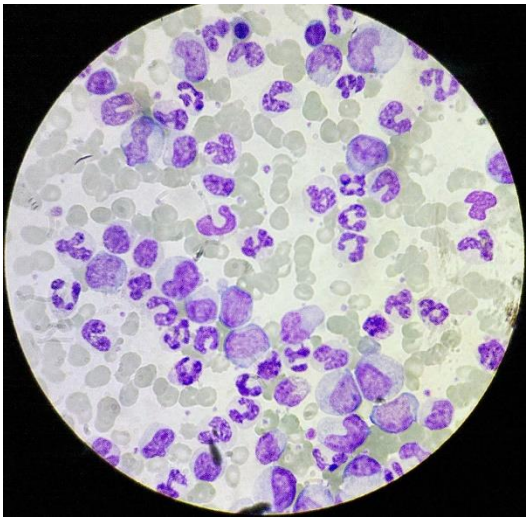
The chronic leukaemias are distinguished from acute leukaemias by their slower progression; with currently available treatments, most patients with chronic leukaemias will live many years.

Chronic leukaemias can be broadly subdivided into :

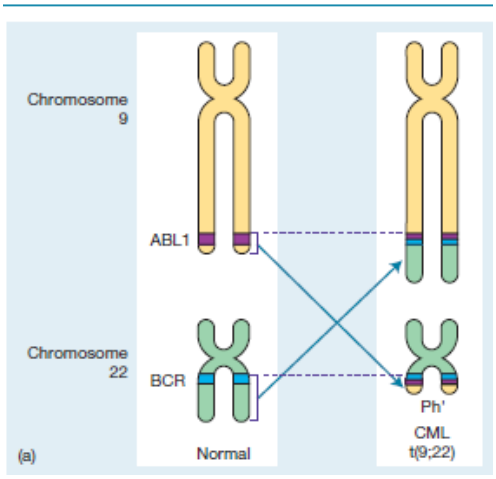
Chronic myeloid Leukemia (CML)

Chronic Lymphoid Leukemia (CLL)

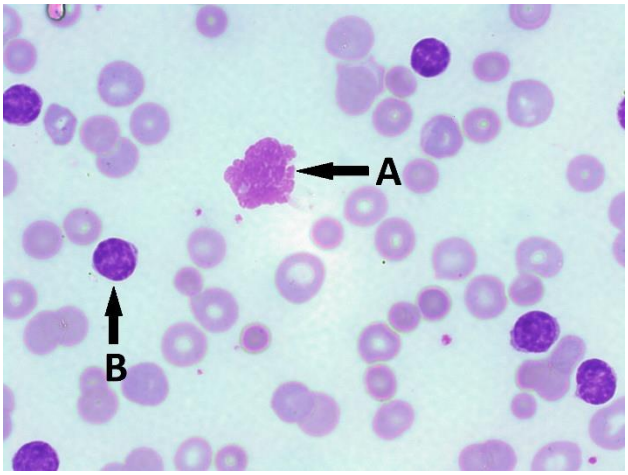
The disease accounts for around 15% of leukaemias and may occur at any age.



CML morphology showing neutrophils and myelocytes peak



Philadelphia Chromosome: Translocation between chromosome (9:22) in CML



Morphology of CLL a) smudge cells b) mature Lymphocytes

Question: Define Leukemia? Briefly classify leukemia?

Chapter 13:

INTRODUCTION TO COAGULATION AND PLATELETS

Objective: By the end of this chapter students will be able to learn briefly about what are platelets, its normal count and its production.

13.1 Introduction to platelets:

Platelets or thrombocytes are tiny particles in blood that helps in blood clotting along with clotting factors, leading to stoppage of bleeding.

13.2 Normal count: In adult normal range of platelets are 150-400x10^9/L

13.3 Production of platelets (Megakaryopioesis) :

1-Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body.

2-The precursor of the megakaryocyte – the megakaryoblast – arises by a process of differentiation from the haemopoietic stem cell.

3- The megakaryocyte matures by endomitotic synchronous replication (i.e. DNA replication in the absence of nuclear or cytoplasmic division) enlarging the cytoplasmic volume as the number of nuclear lobes increases in multiples of two.

4-At a variable stage in development the cytoplasm becomes granular.

Mature megakaryocytes are extremely large, with an eccentrically placed single lobulated nucleus and a low nuclear: cytoplasmic ratio.

5- Platelets form by fragmentation from the tips of cytoplasmic extensions of megakaryocyte cytoplasm, each megakaryocyte giving rise approximately to 1000–5000 platelets.

6-The time interval from differentiation of the human stem cell to the production of platelets average 10 days.

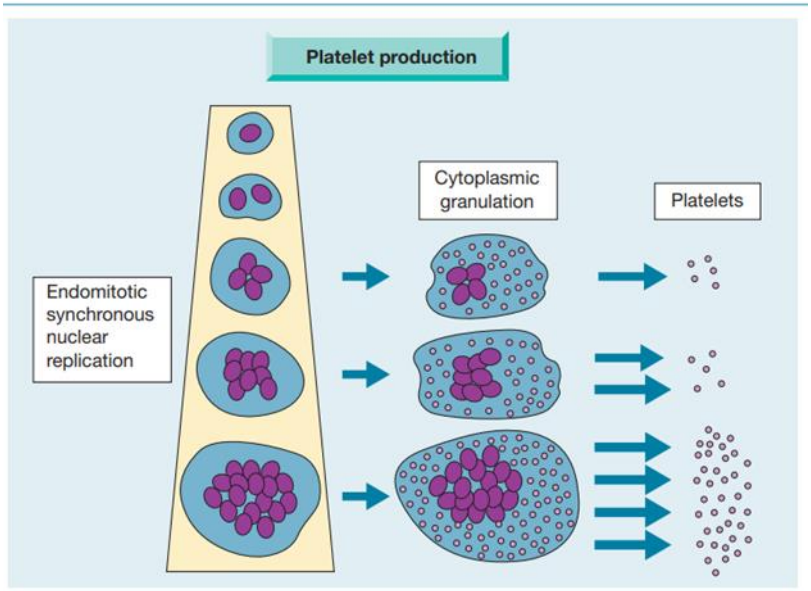
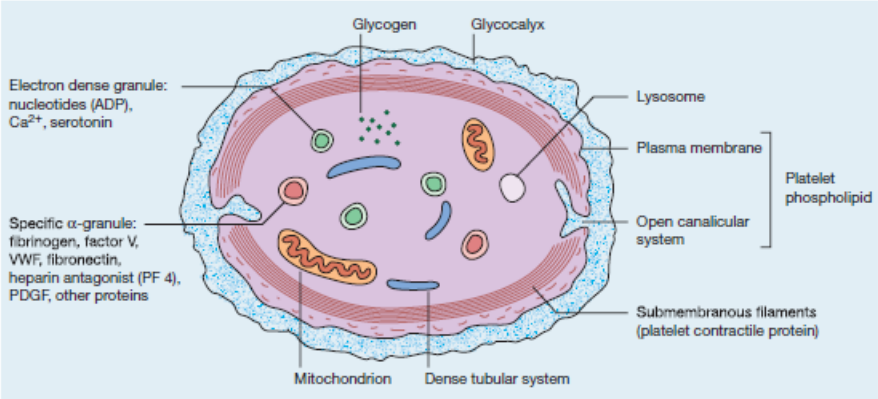


Figure: Simplified diagram to illustrate platelet production from megakaryocytes



Ultrastructure of platelet

Question: Write down normal values of platelet in an adult male? Draw the ultrastructure of a platelet?

Chapter 14:

MECHANISM OF BLOOD COAGULATION

Objective: By the end of this chapter students will be able to understand components of blood clotting,its mechanism, different clotting factors and clotting pathways.

The normal haemostatic response to vascular damage depends on a closely linked interaction between the **blood vessel wall, circulating platelets and blood coagulation factors**

An efficient and rapid mechanism for stopping bleeding from sites of blood vessel injury is clearly essential for survival.

The haemostatic system thus represents a delicate balance between procoagulant and anticoagulant mechanisms allied to a process for fibrinolysis.

14.1 Components of blood clotting:

The five major components involved are platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels.

14.2 Mechanism of Haemostasis:

Following vessel injury, haemostasis can be considered as occurring in two stages: primary and secondary.

- **Primary haemostasis**: is the initial stage during which vascular wall and platelets interact to limit the blood loss from damaged. Another vascular factor promoting haemostasis is vasoconstriction of small vessels following injury.
- **During secondary haemostasis**: a stable fibrin clot is formed from coagulation factors by enzymatic reactions.

Although formation of blood clot is necessary to arrest blood loss, ultimately blood clot needs to be dissolved to resume the normal blood flow. The process of dissolution of blood clot is called **as fibrinolysis**.

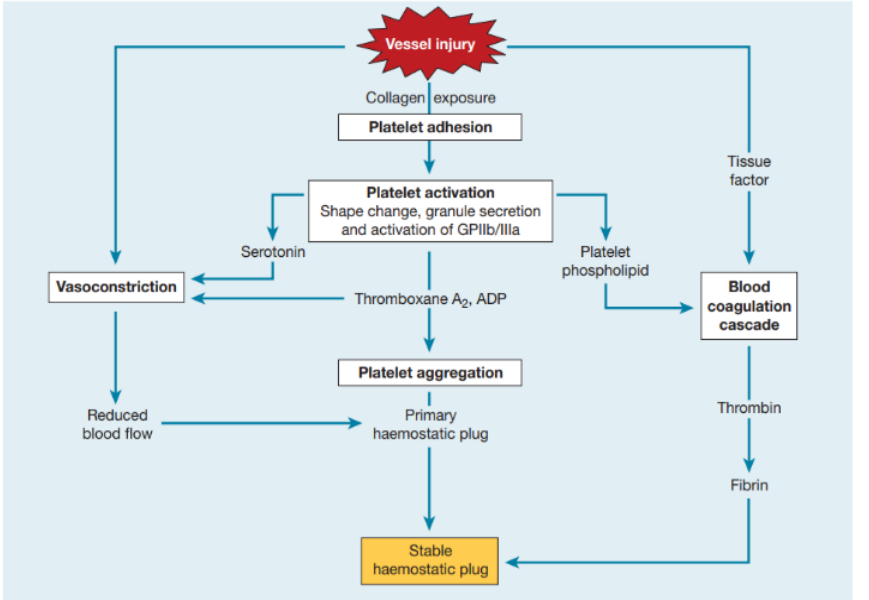


Figure: The involvement of blood vessels, platelets and blood coagulation in haemostasis. ADP, adenosine diphosphate

14.3 Coagulation factors and pathways:

A number of coagulation proteins (factors) participate in coagulation reactions, which ultimately lead to the formation of a fibrin clot. According to the International System of Nomenclature, coagulation factors are designated by Roman numerals (I to XIII).

Table 24.1 The coagulation factors.

Factor number	Descriptive name	Active form
I	Fibrinogen	Fibrin subunit
II	Prothrombin	Serine protease
III	Tissue factor	Receptor/cofactor*
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart–Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman (contact) factor	Serine protease
XIII	Fibrin-stabilizing factor	Transglutaminase
	Prekallikrein (Fletcher factor)	serine protease
	HMWK (Fitzgerald factor)	Cofactor*

*Active without proteolytic modification.
HMWK, high molecular weight kininogen.

Blood coagulation is divided into

- Extrinsic pathway
- Intrinsic pathway
- Common pathway .

Extrinsic pathway: The extrinsic pathway is initiated by tissue injury with release of tissue thromboplastin which causes activation of F VII; the enzyme which is formed activates FX. F VII complexes with tissue factor released after tissue injury in the presence of calcium ions and activates F X and F IX. F Xa and thrombin.

Intrinsic pathway: This pathway is initiated by contact activation and consists of interaction of contact factors (FXII, F XI, prekallikrein, and high molecular weight kininogen), FIX, F VIII, phospholipid, and calcium; these reactions generate a complex which causes activation of F X to F Xa.(figure:23)

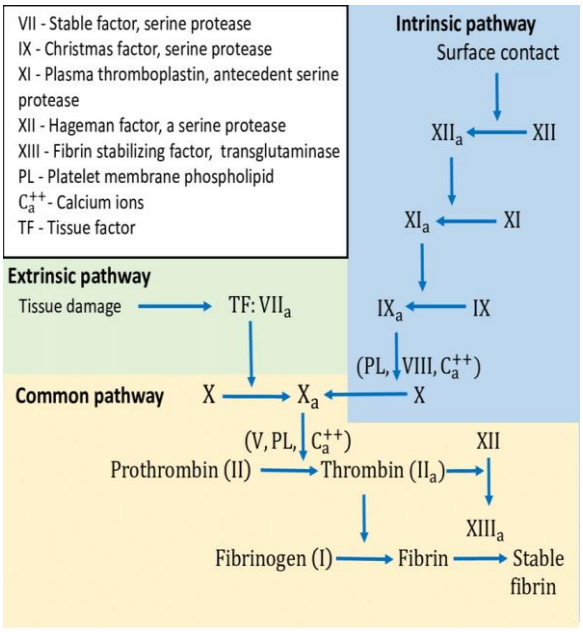
Initiation of intrinsic pathway occurs when plasma comes in contact with a negatively charged surface such as glass, kaolin, celite, or ellagic acid in vitro. In vivo, this surface is probably provided by subendothelium of a damaged vessel. Following contact with a negatively charged surface, a conformational change in FXII with exposure of enzymatically active site probably occurs and in this way a small amount of FXIIa is

formed. FXIIa converts prekallikrein to kallikrein and F XI to FXIa in the presence of high molecular weight kininogen. Kallikrein. This in turn activates more F XII. F XIa activates F IX to yield F IXa; this reaction requires the presence of phospholipid and calcium. F IXa complexes with activated F VIII, phospholipid, and calcium and activates F X to F Xa. F VIII is activated by thrombin and also by F Xa. F VIII does not possess enzymatic activity but functions as a cofactor; in its presence the reaction rate is enhanced several thousand times.

Common pathway: Both intrinsic and extrinsic pathways proceed to common pathway which begins with the activation of F X. F Xa generated by intrinsic or extrinsic pathway complexes with Factor V, phospholipid and calcium. This is called as prothrombinase complex, which activates prothrombin to thrombin. FV is modified by thrombin or F Xa to form activated F V which functions as a cofactor in the above reaction. Thrombin activates fibrinogen molecule to form fibrin monomer. Free fibrin monomers spontaneously polymerize to form fibrin polymer.

Thrombin activates Factor XIII to Factor XIIIa in the presence of calcium, mediates the formation of covalent bonds between adjacent polypeptide chains. This crosslinking of fibrin monomers imparts structural stability to the clot.

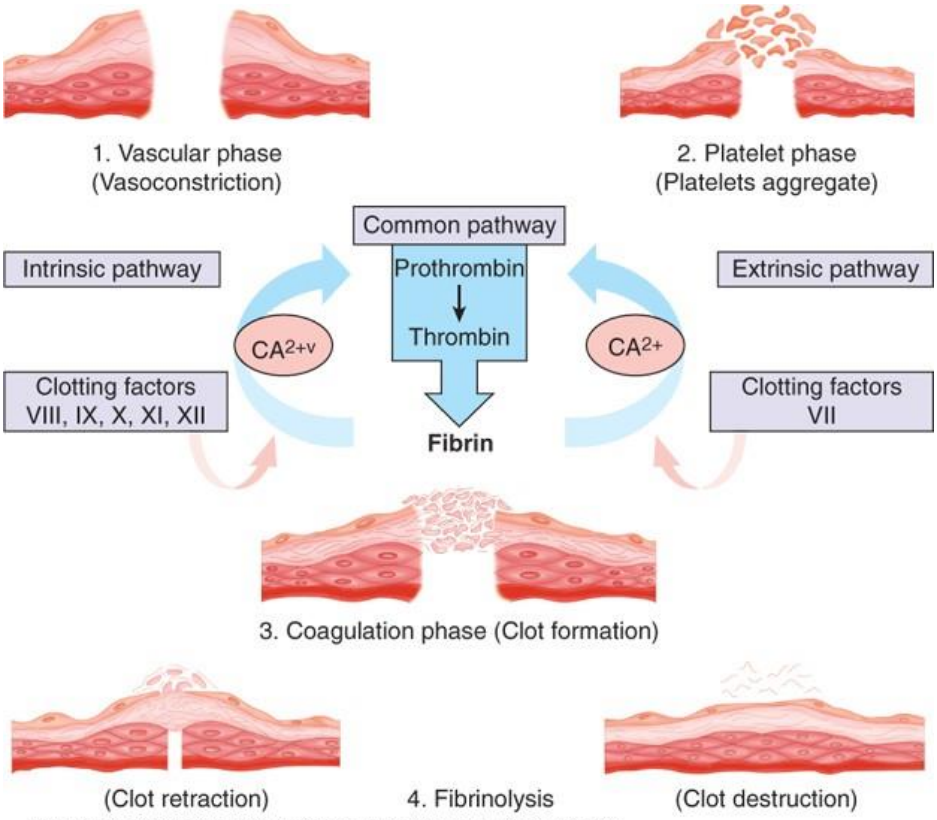
Blood coagulation pathway



14.4 Fibrinolytic System:

Fibrinolysis is the process of dissolution of blood clots which is necessary to maintain the free flow of blood in the vascular system. The major enzyme of the fibrinolytic system is plasmin, which is generated from proteolytic cleavage of plasminogen. Plasmin can cause cleavage of both fibrinogen as well as fibrin. Plasmin digests insoluble or cross-linked fibrin to release fibrin degradation products or FDPs which are then cleared from the circulation by macrophages of the mononuclear phagocytic system.

Summary of blood clotting



Question: Write down steps of blood clotting?

Chapter 15:

TESTS OF HAEMOSTATIC FUNCTION

Objective: By the end of this chapter students should be able to know which blood tests are to be performed when abnormal bleeding patients comes in a Lab.

Defective haemostasis with abnormal bleeding may result from:

- A blood vessel disorder
- Platelet disorders Which may be due to
  1)Thrombocytopenia (low platelet count) or
  2) A disorder of platelet function
- Defective blood coagulation.

A number of simple tests are employed to assess the platelet, vessel wall and coagulation components of haemostasis.(table:11)

1.Blood count (CBC) including platelet count

2.Blood film examination: for confirmation of platelet count and any other abnormality in blood (like leukemia) can be observed.

3.The prothrombin time (PT)

It measures extrinsic pathway factors that is factor VII, X, V, prothrombin and fibrinogen. The normal prothrombin time is 10–14s. It may be expressed as the international normalized ratio (INR)

4.The activated partial thromboplastin time (APTT)

It measures intrinsic pathway factors that is factor VIII, IX, XI and XII in addition to factors X, V, prothrombin and fibrinogen. The normal time for APTT is approximately 30–40s.

5. Mixing studies:

Prolonged clotting times in the PT and APTT because of factor deficiency are corrected by the addition of normal plasma to the test plasma (50 :50 mix). If there is no correction

or incomplete correction with normal plasma, the presence of an inhibitor of coagulation is suspected.

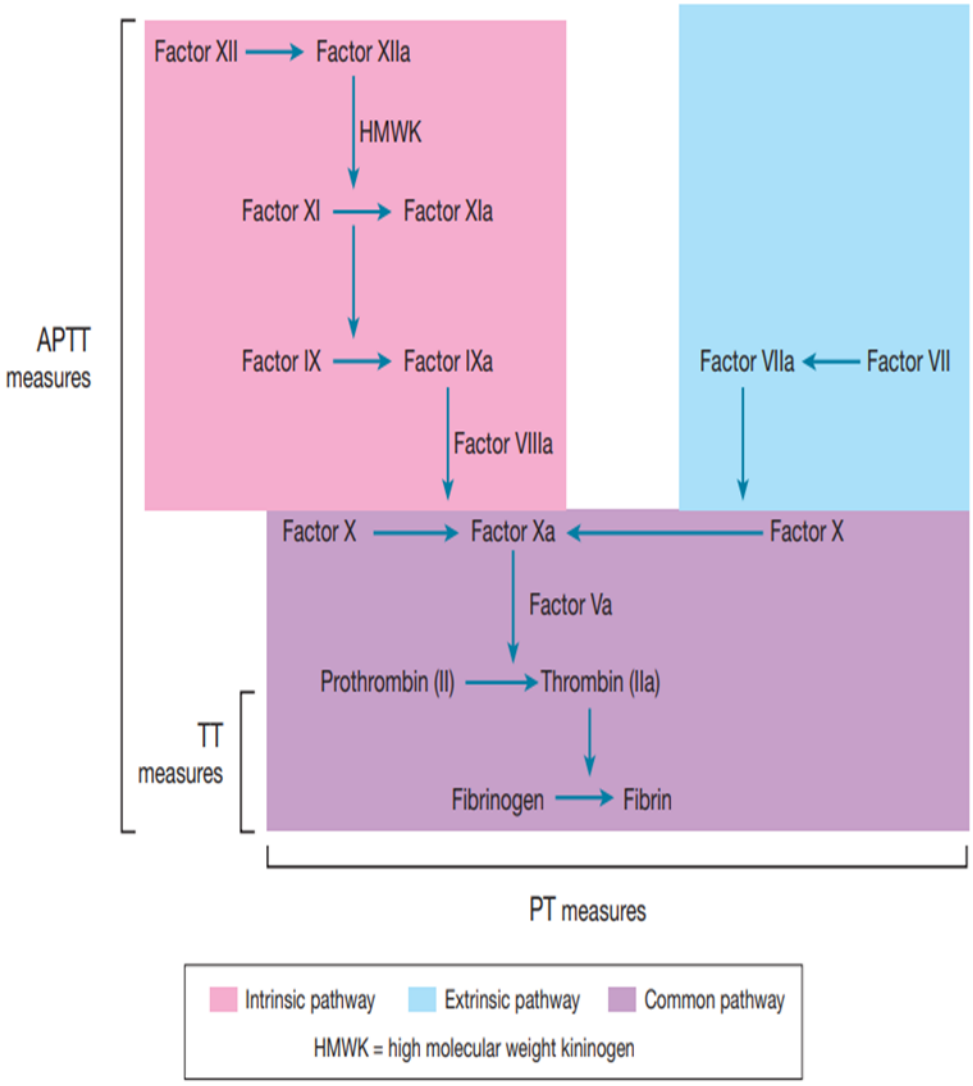


Figure: The intrinsic (contact), extrinsic and common pathways of blood coagulation. The APPT tests the intrinsic and common pathways, the PT the extrinsic and common pathways and the TT tests for thrombin inhibitors and deficiency or abnormality of fibrinogen APTT, activated partial thromboplastin time; HMWK, high molecula weight kininogen; PT, prothrombin time; TT, thrombin time

6.The thrombin (clotting) time (TT)

Thrombin time is sensitive to a deficiency of fibrinogen or inhibition of thrombin. Diluted bovine thrombin is added to citrated plasma at a concentration giving a clotting time of 14–16 s with normal subjects

Table: Screening tests for coagulation disorders

Table 24.3 Screening tests used in the diagnosis of coagulation disorders (see also Fig. 24.12).

Screening tests	Abnormalities indicated by prolongation	Most common cause of coagulation disorder
Thrombin time (TT)	Deficiency or abnormality of fibrinogen or inhibition of thrombin by heparin or FDPs	DIC, heparin therapy
Prothrombin time (PT)	Deficiency or inhibition of one or more of the following coagulation factors: VII, X, V, II, fibrinogen	Liver disease, warfarin therapy, DIC
Activated partial thromboplastin time (APTT or PTTK)	Deficiency or inhibition of one or more of the following coagulation factors: XII, XI, IX (Christmas disease), VIII (haemophilia), X, V, II, fibrinogen	Haemophilia, Christmas disease (+ conditions above)
Fibrinogen quantitation	Fibrinogen deficiency	DIC, liver disease

N.B. Platelet count and the tests of platelet function are also used in screening patients with a bleeding disorder (p. 322).
DIC, disseminated intravascular coagulation; FDPs, fibrin degradation products.

Question: Enumerate first line of tests that you will perform in a bleeding patient?

Chapter 16:

BLEEDING DISORDERS

Objective: By the end of this chapter students should be able to understand the basic concept of congenital and acquired bleeding disorders and how do we investigate them in a Lab.

Abnormal bleeding may result from:

1 Vascular disorders
2 Platelet defect which may be reduced platelet count and defective platelet function
3 Defective coagulation

16.1Vascular bleeding disorders:

The vascular disorders are characterized by easy bruising and spontaneous bleeding from the small vessels. The underlying abnormallarity is either in the vessels themselves or in the perivascular connective tissues. They are either inherited vascular disorders or acquired vascular disorders.

16.2 Platelet defect:

Abnormal bleeding associated with thrombocytopenia (low platelet count) or abnormal platelet function is characterized by spontaneous skin purpura and mucosal haemorrhage and prolonged bleeding after trauma:

Thrombocytopenia: low platelet count

The main causes of thrombocytopenia :

a.Failure of platelet production

b.Increased consumption of platelet

c.Abnormal distribution of platelet

d. Dilutional loss

Causes of thrombocytopenia

Table 25.3 Causes of thrombocytopenia.

Failure of platelet production
Selective megakaryocyte depression
- rare congenital defects (see text)
- drugs, chemicals, viral infections

Part of general bone marrow failure
- cytotoxic drugs
- radiotherapy
- aplastic anaemia
- leukaemia
- myelodysplastic syndromes
- myelofibrosis
- marrow infiltration (e.g. carcinoma, lymphoma, Gaucher's disease)
- multiple myeloma
- megaloblastic anaemia
- HIV infection

Increased consumption of platelets
Immune
- autoimmune
- idiopathic
- associated with systemic lupus erythematosus, chronic lymphocytic leukaemia or lymphoma;
- infections: *Helicobacter pylori*, HIV, other viruses, malaria
- drug-induced, e.g. heparin
- post-transfusional purpura
- feto-maternal alloimmune thrombocytopenia

Disseminated intravascular coagulation
Thrombotic thrombocytopenic purpura

Abnormal distribution of platelets
Splenomegaly (e.g. liver disease)

Dilutional loss
Massive transfusion of stored blood to bleeding patients

HIV, human immunodeficiency virus.

Disorders of platelet function:

Disorders of platelet function are suspected in patients who show skin and mucosal haemorrhage despite a normal platelet count and normal levels of VWF. These disorders may be hereditary or acquired.

Tests performed for platelet functional disorders are

- Platelet function analysis (PFA-100)
- Platelet aggregation studies
- VWF assays may be needed to diagnose platelet functional defects.

Hereditary disorders:

- Thrombasthenia (Glanzmann's disease):

It usually presents in the neonatal period and, characteristically, platelets fail to aggregate in vitro to any agonist except ristocetin.

This autosomal recessive disorder resulting from failure of platelet aggregation.

It is caused by a variety of different mutations in the genes coding for GPIIb or IIIa (glycoprotein IIb or IIIa).

** Platelet aggregation: is defined as binding of platelet to platelet.*

- Bernard–Soulier syndrome:

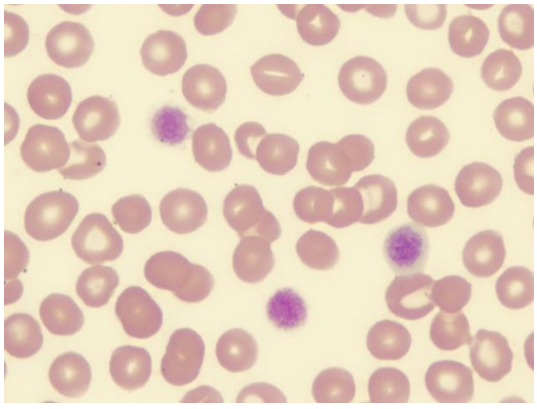
In this autosomal recessive disease due to mutations in the GPIb gene, the platelets are larger than normal.

There is defective binding to VWF, defective adherence to exposed sub - endothelial connective tissues and platelets do not aggregate with ristocetin.

The platelets are larger than normal.

**Platelet adhesion: is defined as binding of platelet to exposed sub endothelial collagen of injured vessel wall.*

Peripheral smear showing Giant platelet in Bernard soulier syndrome

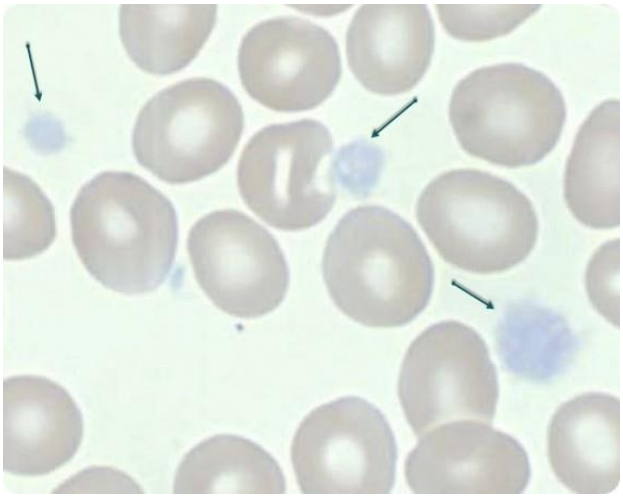


- *Storage pool disorders:*

There is absence of alpha granule content of platelet (grey platelet syndrome).Platelets are large and grey in colour.

There is absence of dense granule content of the platelets (delta storage disorders).

Grey platelet syndrome



Acquired disorders
Antiplatelet drugs

Aspirin therapy is the most common cause of defective platelet function. The other drugs which can cause platelet dysfunction are dipridamole, Clopidogrel etc

16.3 Bleeding due to defective coagulation:

It may be hereditary or acquired disorders that can lead to abnormal bleeding**.**

Hereditary disorders:

Hemophilia A:

It is caused by Factor VIII deficiency (low or absent factor VIII)

Clinical features:

- Infants may develop profuse post-circumcision haemorrhage
- joint and soft tissue bleeds.
- Recurrent painful haemarthroses (joint bleed) and muscle haematomas.

Lab investigations:
The following tests are abnormal:

1 Activated partial thromboplastin time (APTT).

2 Factor VIII clotting assay.

3 The platelet function analysis ‑ 100 (PFA ‑ 100) and prothrombin time is normal.

Hemophilia B:

It is caused by Factor IX deficiency (low or absent factor IX)

Clinical features:

Similar to hemophilia A.

Lab investigations:

The following tests are abnormal:

1 Activated partial thromboplastin time (APTT).

2 Factor IX clotting assay.

3 The platelet function analysis ‑ 100 (PFA ‑ 100) and prothrombin time is normal.

Von Willibrand disease:
In this disorder there is either a reduced level or abnormal function of von Willebrand factor (VWF). Normal function of VWF is that it is a carrier molecule protein for factor VIII.
Clinical features: bleeding from mucous membranes, skin cuts, post trauma and post-operative bleed.

Lab investigations:

Platelet count: normal

PFA 100: prolonged

PT: normal

APTT: prolonged

Factor VIII: may be moderately reduced

VWFactor: low or abnormal function.

Acquired disorders:

Liver disease

Disseminated intravascular coagulation

Circulating anticoagulants

Question: Define thrombocytopenia? Enumerate the causes of thrombocytopenia?

Chapter 17:

BONE MARROW STUDY

Objective: By the end of the chapter students should be able to learn about procedure of bone marrow, its common sites, its indication and contraindications and complications of the procedure.

Biopsy of the bone marrow is an indispensable adjunct to the study of diseases of the blood and may be the only way in which a correct diagnosis can be made.

Marrow can be obtained by

- Needle aspiration,
- Percutaneous trephine biopsy,

Bone marrow aspiration is simple and safe procedure if performed correctly. It seems to be safe in almost all circumstances, even when platelets are reduced. However, when there is a major disorder of coagulation, such as in haemophilia, this procedure should be avoided or performed under the cover of factor concentrates.

Trephine biopsy is a little less simple, but it too can be performed on outpatients. The trephine biopsy can provide information about the structure of relatively large pieces of marrow.

17.1 Indications of bone marrow examination:

- Anemia
- Blood cell conditions in which too few or too many of certain types of blood cells are produced, such as leukopenia, leukocytosis, thrombocytopenia, thrombocytosis, pancytopenia and polycythemia
- Cancers of the blood or bone marrow, including leukemias, lymphomas and multiple myeloma
- Cancers that have spread from another area (metastasis), such as the breast, into the bone marrow.
- Fevers of unknown origins (PUO)

17.2 Contraindications:

- Hemorrhagic disorders such as congenital coagulation factor deficiencies (eg, hemophilia), disseminated intravascular coagulation and concomitant use of anticoagulants.
- Skin infection or recent radiation therapy at the sampling site.
- Bone disorders such as osteomyelitis or osteogenesis imperfecta.

17.3 Sites of bone marrow aspiration:

Bone marrow sample can usually be aspirated from the

- sternum
- anterior or posterior iliac spines
- Tibial tuberosity (in case of children's less than one year)

17.4 Procedure of aspirate:

1-To perform a marrow aspiration, clean the skin in the area with 70% alcohol (e.g., ethanol) or 0.5% chlorhexidine (5% diluted 1 in 10 in ethanol).

2-Infiltrate the skin, subcutaneous tissue, and periosteum overlying the selected site with a local anaesthetic such as 2-5 ml 2% lignocaine.

3-Wait until anaesthesia has been achieved.

4- Pass the needle perpendicularly into the cavity of the ilium at the centre of the oval posterior superior iliac spine or 2 cm posterior and 2 cm inferior to the anterior superior iliac spine.

5-When the bone has been penetrated, remove the stilette, attach a 1 or 2 ml syringe, and suck up marrow contents for making films.

6- Failure to aspirate marrow—a "dry tap"—suggests bone marrow fibrosis or infiltration.

7-Because bone marrow clots faster than peripheral blood, films should be made from the aspirated material without delay at the bedside.

8-Make films, 3-5 cm in length, of the aspirated marrow using a smooth-edged glass spreader of not more than 2 cm in width.

9-Fix some of the films in absolute methanol as soon as they are thoroughly dry for subsequent staining by a Romanowsky stain/giemsa stain and Perl's' stain for iron. These films are also suitable for cytochemical staining like sudan black stain.

Percutaneous Trephine Biopsy of the Bone Marrow:

Trephine biopsies of the bone marrow are invaluable in the diagnosis of conditions that yield a “dry tap” on bone marrow aspiration (e.g., myelofibrosis, infiltrations) or when disrupted architecture of the marrow is an important diagnostic feature (e.g., Hodgkin's disease, lymphoma).

- Like marrow aspirations, they can be carried out at the bedside or in outpatient departments.
- The posterior iliac spine is the usual site, although the anterior iliac spine can also be used. The posterior iliac spine is said to provide samples that are longer and larger, and the aspiration is less uncomfortable for the patient.
- The trephine specimen is obtained by inserting the biopsy needle into the bone and using a to-and-fro rotation to obtain a core of tissue. The main problems with this method are that the specimen may be crushed, thereby distorting the architecture, and it is difficult to detach the core of bone from inside the marrow space.

Figure: 24 Jamshidi trephine needle

Figure25: Disposable bone marrow needle

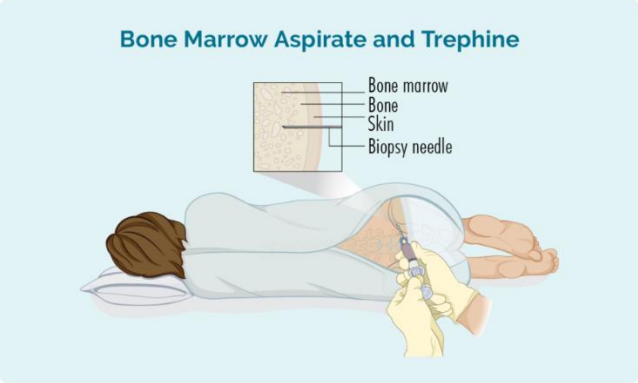
17.5 Complications of Bone Marrow Biopsy:

Bone marrow biopsy is generally a safe procedure, and serious adverse events occur in less than 0.05% of procedures. The most common complication is bleeding, heamatoma formation at procedure site.

17.6 Video Link for bone marrow biopsy procedure:

https://www.youtube.com/watch?v=EYd7OnCt7ug

Bone marrow aspirate and trephine biopsy



Question: Write down the site and procedure of bone marrow biopsy?

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SECTION II

Blood Banking

Chapter 18:

INTRODUCTION AND IMPORTANCE OF BLOOD BANK

Objective: By the end of the chapter students should know the importance of IBTS.

Blood transfusion services are an integral part of the healthcare system. Millions of lives are saved each year through blood transfusions. The provision of safe blood and blood components is the responsibility of the national healthcare system. According to the World Health Organization (WHO), safe blood transfusion is a universal human right. Safe blood means blood that will not cause harm to the recipient; and that has been fully screened; and is not contaminated by any blood-borne disease such as HIV/AIDS, hepatitis, malaria or syphilis; is free of infections or contamination due to drugs or other chemical substances; is used within the specified time period and stored in the right conditions, correctly labeled, and properly sealed.

It is estimated that 2.7 million blood donations are collected in Pakistan annually from approximately 650 blood centers of varying workloads. IBTS has shifted the blood screening from ICT Method to CLIA to get 99.9 % accurate results.

. **Functions of IBTS (institute of blood transfusion services):**

- To maintain quality in the blood banks, standardize equipment, reagent, techniques and personnel.
- To investigate and perform research on the hematological and serological problems such as blood group discrepancies, transfusion reactions etc.
- To conduct diploma courses in blood transfusion.
- Providing facilities to Thalassemia patients.
- To make provision of equipment and consumables to blood units under administrative control of IBTS.
- To make provision of screening kits to blood units under the administrative control of District Governments.
- To prepare blood components like packed cells, fresh frozen plasma, platelet concentrates.
- To arrange blood donation camps to meet the demand of blood and its products.

- To register blood donors and maintain blood units under the administrative control.
- To make arrangements to screen the blood for HIV 1 and 2, Hepatitis B, Hepatitis C, Malaria and Syphilis in order to achieve the objectives of safe blood
- To carry out monitoring and surveillance of blood units.

Services of IBTS:

- Blood Banks
- Thalassemia Centers
- Blood Camps/Blood Donation Drives
- Monitoring and Evaluation
- Store

Facilities :

1. Immunohematology laboratory
2. Screening laboratory
3. Component laboratory

1-Immunohematology laboratory

This laboratory performs the following tests/procedures/activities:

- Blood Grouping and Cross Match
- Complete Blood Count and peripheral smear
- Forward and Reverse blood grouping (Slide and Tube Method)
- ABO and Rh factor Discrepancies
- Direct and indirect Coombs Test
- Training of Doctors and Paramedical Staff
- Quality control of instruments, procedures, reagents used in blood banks.

2-Screening laboratory:

The screening procedures and other activities performed by this laboratory are mentioned in the following:

- Screening of Donor HCV, HBsAg, HIV, Malaria Parasite and Treponema pallidum.
- Evaluation of Screening Kits
- Screening of Random Samples (Bags) from Different Blood Banks
- Training of Doctors and Paramedical staff.
- Quality control of procedure, reagents.

3-Component laboratory: This laboratory is responsible for forming different components from the whole blood like:

- PCV (packed cell volume)
- Washed Red Cells
- FFPs (fresh frozen plasma)
- Platelet Rich Plasma (PRP)
- Platelet Concentrates
- Buffy Coat
- Cryoprecipitate

All the blood banks are providing services round the clock. The services are being provided free of cost for all the admitted patients in public sector hospitals. The staff of blood banks performs the duty efficiently in three shifts round the clock.

Question: Write down the importance of IBTS?

Chapter 19:

BLOOD GROUP SYSTEM

Objective: By the end of the chapter students should know different types of blood groups, ABO antigens, inheritance, formation and antibodies

There are many blood group systems including ABO, Rh, Kell, Kidd, Duffy ,Lewis, Lutheran, MNS. However the ABO and Rhesus blood group systems are clinically the most important. Blood donors and patients must be correctly ABO grouped because transfusing ABO incompatible blood may result in the death of a patient.

19.1 ABO blood group system:

In ABO system, there are four main types of blood groups- A, B, AB, and O. Identification of these four blood groups is based on presence or absence of A and/or B antigens on red cells.

19.2Inheritance of ABO blood group system:

A person's ABO blood group depends on the *A*, *B*, or *O* gene (located on chromosome 9) inherited from each parent as follows:

Genes inherited (genotype)	*Blood group* (phenotype)
A and *A* *A* and *O**	*Group A*
B and *B* *B* and *O**	Group B
A and *B*	Group AB
O and *O*	Group O

**A* and *B* genes are dominant. The recessive *O* gene is expressed only when *A* and *B* dominant genes are absent. A person who is group O must be of the genotype *OO*.

19.3ABO antigen:

● A person who inherits *A* gene (*AA* and *AO*) belongs to Group A and expresses A antigen on their red cells.
● A person who inherits *B* gene (*BB* or *BO*) belongs to Group B and expresses B antigen on their red cells.
● A person who inherits *A* and *B* genes belongs to Group AB and expresses both A and B antigens on their red cells.

● A person who inherits *O* genes belongs to Group O and does not express A or B antigens on their red cells.
Antigens A and B are formed from H substance.

19.3.1Formation of red cell antigens A and B from H substance:

- The dominant *H* gene (located on chromosome 19) encodes for an enzyme fucosyltransferase or H transferase which converts a carbohydrate precursor substance in red cells into H substance (H antigen).
- *A* and *B* genes encode for specific transferase enzymes which convert H substance into A and B red cell antigens by adding sugar molecule to H antigen or H substance.
- O gene encodes for an inactive transferase enzyme which results in no conversion of the H substance in group O red cells.

Some persons do not inherit the H gene (genotype hh) and thus cannot synthesize H substance. Such persons may inherit the A or B gene but cannot express it, as they are unable to produce the H substance. Such individuals are said to have **Bombay phenotype or Bombay blood group (Oh).** Their red cells type as group O; however, unlike group O individuals, Oh persons have no H antigen on their red cells and their plasma contains strong anti-H in addition to anti-A and anti-B. Therefore Bombay group persons should be transfused only with Oh blood.

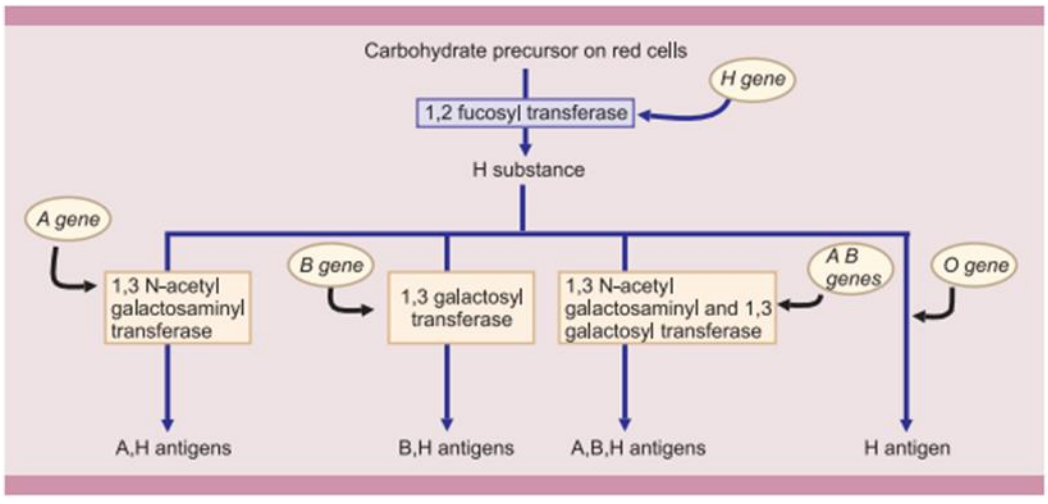


Figure:28 Formation of antigens of ABO blood group system

19.3.2 ABO Secretors and Non Secretors:

Up to 80% or more of people inherit the secretor gene *Se* and secrete water soluble H, A, and B antigens in their saliva, plasma, and other body fluids in addition to expressing the antigens on their red cells. Remaining 20% are non-secretors.

19.4 Subgroups of ABO blood group:

- Antigen A exists as strongly reacting antigen A1 and a weakly reacting antigen A2.
- Most people who are group A or Group AB possess A1 antigen, i.e. they are group A1 or group A1B.
- Fewer people (up to 20%) belong to the subgroups A2 or A2B.
- Antibodies anti A and anti A1
- Anti A1 is a weakly reacting antibody.

Group	*Antibody in serum*
A1	*Anti B*
A2	anti-B, (anti-A1* in up to 2%)
A1B	None
A2B	anti-A1* in up to 25%

19.5 ABO Antibodies:

- ABO blood group antigens are present on RBC and antibodies are present in serum in the absence of the corresponding red cell antigen.
- A person who is group A has anti-B antibody in their serum
- A person who is group B has anti-A antibody in their serum.
- A person who is group AB has neither anti-A nor anti-B antibody in their serum.
- A person who is group O has both anti-A and anti-B antibody in their serum.

Antibodies of the ABO blood group system are naturally occurring IgM antibodies which are produced in the first year of life by sensitization to environmental substances, like food , bacteria, viruses.

They are not detectable at birth but start developing at about 3-6 months of age following exposure to A and B like substances in the environment.

Rarely ABO blood group antibodies are IgG type.

Phenotype	Genotype	Antigens	Naturally occurring antibodies	Frequency (UK, %)
O	OO	O	Anti-A, anti-B	46
A	AA or AO	A	Anti-B	42
B	BB or BO	B	Anti-A	9
AB	AB	AB	None	3

Table:14 The ABO blood group system

Question: Write down antigen and antibodies of ABO blood group?

Chapter 20

Rh BLOOD GROUP SYSTEM

Objective: By the end of this chapter students should be able to understand Rh antigens and antibodies along with their clinical significance

20.1Introduction:

When Rhesus monkey red cells were injected into rabbits and guinea pigs, antibody, which was raised, was found to react with Rhesus monkey red cells as well as with 85% of human red cells. The antigen involved was called as Rh .

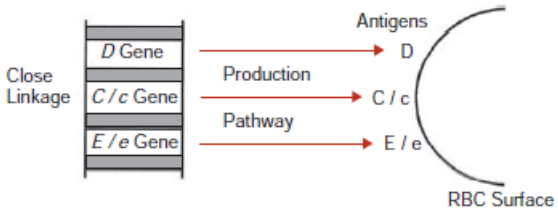
According to the recent nomenclature by international society of blood transfusion (ISBT), the system has been named as RH. The Rh system is only next in importance to ABO system in transfusion practice. The importance of this system lies in the high immunogenicity of Rh D antigen, which readily induces formation anti-D antibodies in Rh D-negative individuals. Anti-D antibodies can cause haemolytic transfusion reaction or, in pregnant women, Rh haemolytic disease of newborn.

20.2 Antigens of the Rh System:

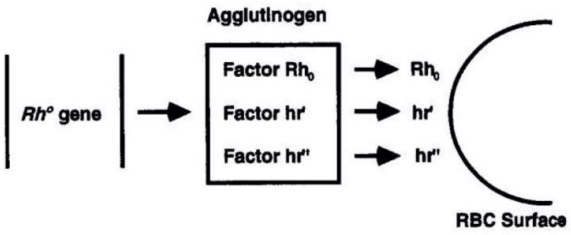
The important antigens of the Rh system are C, D, E, c, and e. D antigen is the most Immunogenic.

There are various nomenclature systems for Rh antigens inheritance:

- **Fisher-Race or CDE Theory:** is simpler and is outlined below. According to Fisher and Race, three closely linked genes are inherited together on one chromosome (haplotype) from each parent and are responsible for formation of different Rh antigens. Possible combinations of Rh antigens includes: Cde/cde, Cde/cDe, CDE/cde, etc.



- **Weiner Rh Theory:**
  According to Wiener theory there was one gene responsible for defining/ forming Rh antigen.



Weiner Rh Theory

Rh antigens are expressed only on red cells and not on any other tissues. They are also not secreted in body fluids.

In contrast to ABO antigens, Rh antigens are fully expressed on red cells before birth and also on red cells of early fetuses.

Depending on the presence or absence of antigen D on red cells, a person is grouped either as Rh positive (when red cells express antigen D) or Rh negative (when D antigen is absent on red cells).

Complete absence of all Rh antigens on red cells (Rh null cells) is associated with stomatocytosis (red cells have a slot-like area of central pallor, reminiscent of mouth) and compensated haemolysis.

Significance of Rh system:

A person who is Rh positive will not make anti-Rh antibodies. Those with Rh negative will produce the antibodies. Therefore, someone with Rh+ blood can receive both Rh+ and Rh- transfusions but those with Rh- can receive only Rh- blood.

20.3 Rh antibodies:

They are not naturally occurring.

Rh antibodies are immune mediated that results from previous transfusion or pregnancy.

These antibodies are usually IgG in nature

Reacts at 37C

Do not bind to complement system.

Significance of Rh antibodies:

Rh antibodies can cause hemolytic transfusion reaction or hemolytic disease of newborn.

Due to high immunogenicity of D antigen, Rh-negative persons (esp. women of child bearing age) should be transfused only with Rh-negative blood. During pregnancy, IgG anti-D can cross the placenta and induce haemolytic disease of newborn by causing immune haemolysis of foetal red cells.

Question: Write down the significance of Rh antibodies?

Chapter 21

COMPATIBILITY TEST

(CROSS MATCH)

Cross-matching is one of the most important serological procedure pertaining to blood group serology for safe blood transfusion. By cross matching we are able to detect the atypical and clinically significant antibody mostly IgM and IgG present in donor serum or recipient serum.

21.1 Purpose:

Purpose of compatibility testing
The purpose of compatibility testing (cross-matching) is to prevent a transfusing reaction by ensuring:

- The ABO group of the blood to be transfused is compatible with the patient's ABO group.
- There are no detectable irregular antibodies in the patient's serum that will react with the donor's red cells, causing their destruction or reducing their normal survival.

In many transfusion centres, the cross-match procedure has been replaced with 'type and screen' policy, according to which both the donor and the recipient are typed for ABO & Rh 'D' groups and screened for atypical antibodies. However, in centers in which antibody screening is not done, the following tests should be included as part of the compatibility testing:

1. ABO & Rh 'D' grouping of the donor's unit.

2. ABO & Rh D' grouping of the patient/ recipient.

3. An Indirect Antiglobulin Test (IAT) using the patient's serum and the donor's red cells. The incubation time can be reduced to 10 minutes if LISS is used as the potentiating agent.

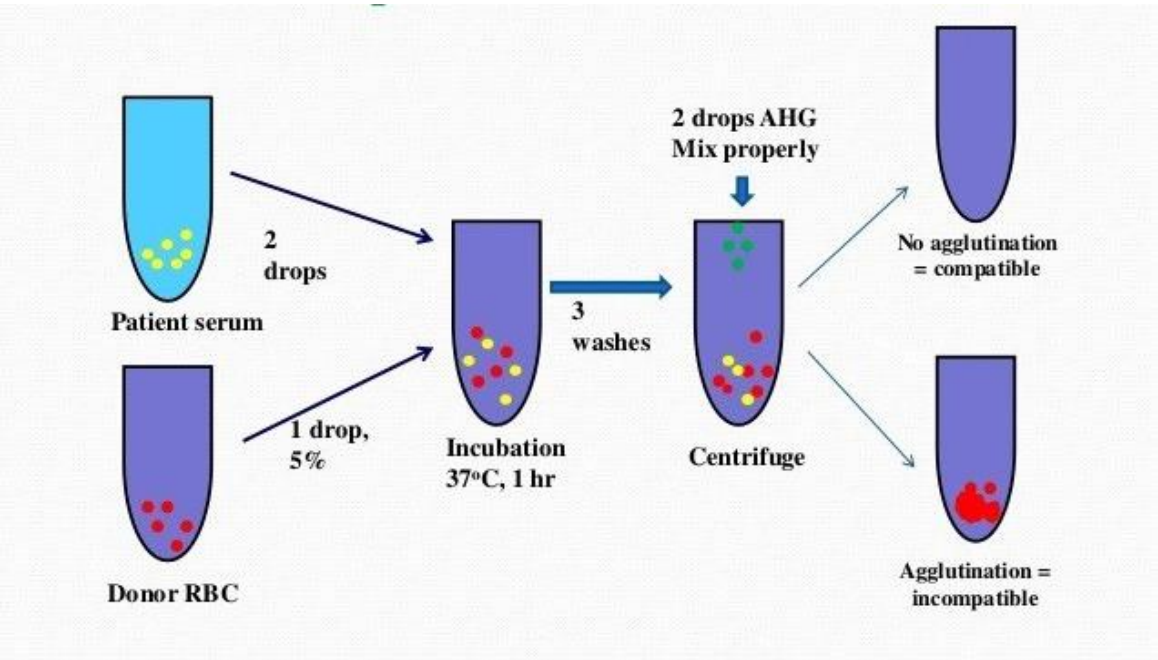
21.2 Phases of cross match:

The cross-match should include

Testing at room temperature,

LISS phase (low ionic strength solution) at 37C, and

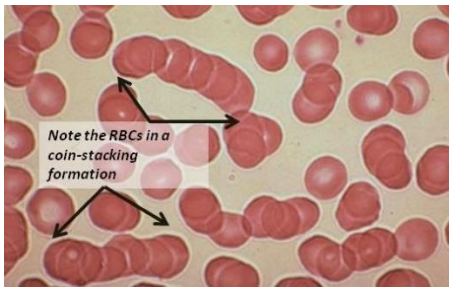
The Indirect Antiglobulin Test at 37C.



Cross match procedure

21.3 Problems in Cross matching:

- Incorrect ABO grouping of patient or donor results in difficulty in cross match.
- Rh blood typing errors cannot be detected by cross-matching.
- An alloantibody in the patient's serum reacting with the corresponding antigen on donor RBCs
- An auto-antibody in patients serum reacting with corresponding antigen on donor red cells. This can be solved by putting auto control which will be positive.
- Problems in patients serum like Imbalance of the normal ratio of albumin and gamma globulin (A/G ratio), as in diseases such as multiple myeloma may cause RBCs to stick together on their flat sides, giving the appearance of stacks of coins when viewed microscopically. This is called rouleaux formation . This property of the serum will affect all tests.
- Dirty glass ware, bacterial contamination of donor serum.



Rouleux formation

Question: Write down the significance of cross match?

Chapter 22:

THE ANTIGLOBULIN (COOMBS') TEST

Objective: By the end of this chapter students should be able to understand the concept of coombs test, its types, procedure, and source of errors and the interpretation of the test.

It is based on the agglutination (clumping) of red blood cells.

It is widely used test in both blood group serology and general immunology. Antihuman globulin (AHG) is produced in animals following the injection of human globulin, purified complement or specific immunoglobulin (e.g. IgG, IgA or IgM). Monoclonal preparations are also now available. When AHG is added to human red cells coated with immunoglobulin or complement components, agglutination of the red cells indicates a positive test.

Antihuman globulin (Coombs) reagent

22.1 Principle of Coombs test

Red cells coated with complement or IgG antibodies do not agglutinate directly when centrifuged. These cells are said to be sensitized with IgG or complement. In order for agglutination to occur an additional antibody, which reacts with the Fc portion of the IgG antibody, or with the C3b or C3d component of complement, must be added to the

system. This will form a “bridge” between the antibodies or complement coating the red cells, causing agglutination.

22.2 Types of antiglobulin (coombs) test:

The antiglobulin test may be either direct or indirect.

22.2.1 Direct antiglobulin test (DAT):

Principle of direct antiglobulin (coombs) test:

The direct antiglobulin test (DAT) is used for detecting antibody or complement already on the red cell surface where sensitization has occurred in vivo.

Procedure of Direct Coombs Test:

1. Prepare a 5 % suspension in isotonic saline of the red blood cells to be tested.
2. With clean pipette add one drop of the prepared cell suspension to a small tube.
3. Wash three times with normal saline to remove all the traces of serum.
4. Decant completely after the last washing.
5. Add two drops of Anti-human serum.
6. Mix well and centrifuge for one minute at 1500 RPM.
7. Resuspend the cells by gentle agitation and examine macroscopically and microscopically for agglutination.(figure:29)

Interpretation of results:

Negative Result:

No clumping of cells (no agglutination). This means you have no antibodies to red blood cells.

Positive Result:

Clumping (agglutination) of the blood cells during a direct Coombs test means that you have antibodies on the red blood cells and that you may have a condition that causes

the destruction of red blood cells by your immune system (hemolysis). This may be due to

- Autoimmune Hemolytic anemia,
- Hemolytic disease of the newborn
- Drug induced immune heamolytic anemia
- Transfusion reactions

22.2.2 Indirect antiglobulin test (IAT)

Principle:

The indirect antiglobulin test (IAT) is used to detect antibodies that have coated the red cells in vitro.

It is a two-stage procedure: the first step involves the incubation of test red cells with serum; in the second step, the red cells are washed and the AHG reagent is added. Agglutination implies that the original serum contained antibody which has coated the red cells in vitro.

Procedure of indirect antiglobulin test: (IAT):

1. Label three test tubes as T (test serum) PC (Positive control) and NC (negative control).
2. In the tube labeled as T (Test), take 2 drops of test serum.
3. In the test tube labeled as PC (Positive control), take 1 drop of anti D serum.
4. In the test tube labeled as NC (Negative control), take 1 drop of normal saline.
5. Add one drop of 5 % saline suspension of the pooled ‘O’ Rho (D) positive cells in each tube.
6. Incubate all the three tubes for one hour at 37°C.
7. Wash the cells three times in normal saline to remove excess serum with no free antibodies, (in the case of inadequate washings of the red cells, negative results may be obtained).

8. Add two drops of Coombs serum (anti human serum) to each tube.
9. Keep for 5 minutes and then centrifuge at 1,500 RPM for one minute.
10. Resuspend the cells and examine macroscopically as well as microscopically. (figure:30)

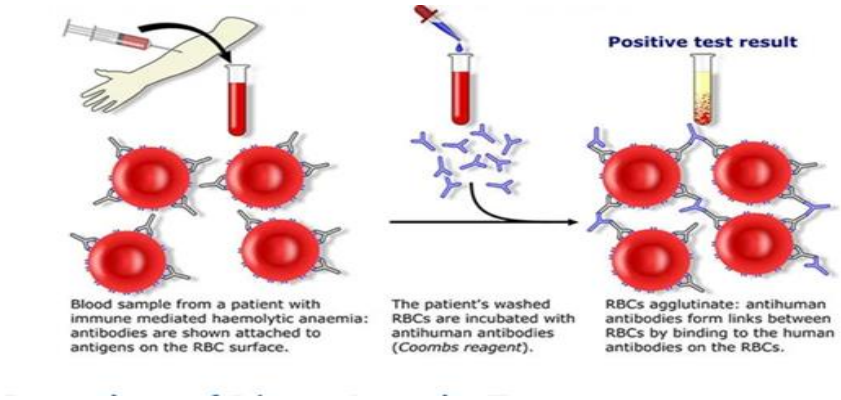


Figure:29 Procedure of direct coombs test

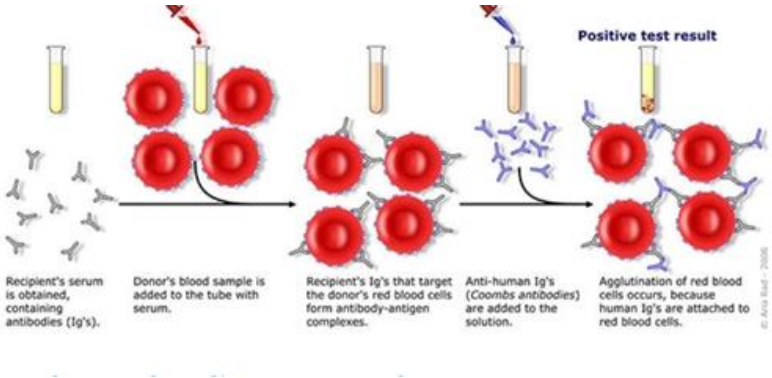


Figure:30 Procedure of Indirect coombs test

22.3 Sources of error in Antiglobulin tests: (false negative tests)

I. The test tube or pipettes may be dirty.
II. The red cells may have been inadequately washed.

III. Proteins on the fingertip may neutralizes AHG and thus a false negative result may be obtained.
IV. The incubation time was too long long/too short.
V. The incubation was at the temperature that did not activate antibody.
VI. There was delay in reading the test or in performing the test, thus allowing the antibody to be eluted off the red cells.
VII. The test cells were stored improperly, causing them to loose activity.

Question: Write down the principle and procedure of IAT?

Chapter 23:

BLOOD DONATION PROCESS

Objective: By the end of this chapter student should be able to describe steps of blood donation

1-Blood donor selection

In most countries blood donors contribute on a voluntary basis and this is generally preferable in terms of product safety. Prospective blood donors are asked a series of specific, direct questions about risk factors for infection with blood-transmissible diseases, and this screening is estimated to eliminate more than 90% of unsuitable donor.

Blood Donors Selection Criteria:

The interval between blood donations should be no less than three months. The donor shall be in good health, mentally alert and physically fit. Accept only voluntary/replacement, non-remunerated blood donations if donors fulfill the following criteria/requirements:

- The donor shall be in the age group of 18 to 60 years
- The donor shall not weigh less than 45 kilograms
- Temperature and pulse of the donor shall be normal
- The systolic and diastolic blood pressures are within normal limits without medication
- Hemoglobin shall not be less than 12.5 g/dl
- The donor shall be free from acute respiratory diseases
- The donor shall be free from any skin disease at the site of phlebotomy
- The donor shall be free from any disease transmissible by blood transfusion so far as can be determined by history and examination indicated above
- The arms and forearms of the donor shall be free from skin punctures or scars indicative of professional blood donors or addiction of self-injected narcotics

:

Deferring the Donor (For a Certain Duration)

Defer the donor for the period as indicated in the following table on the basis of medical condition/diseases mentioned:

Medical History/Diseases	Duration
Abortion	6 Months
History of Blood Transfusion	6 Months
Surgery	12 Months
Typhoid Fever	12 Months after Recovery
History of Malaria (Duly Treated)	3 Months Endemic Area and 3 Y
Tattooing	6 Months
Breast Feeding	12 Months after Delivery
Immunization (Cholera, Typhoid, Diphtheria, Tetanus, Plague, Gamma Globulins)	15 Days
Rabies Vaccination	1 Year after Vaccination
Hepatitis in Family of Close Contacts	12 Months
Hepatitis Immune Globulin	12 Months

Deferring the Donor (Permanently)

Defer the donor **permanently** if suffering from any of the following diseases:

- Cancer
- Heart Disease
- Abnormal Bleeding Tendencies
- Unexplained Weight Loss
- Diabetes
- Hepatitis B Infection
- Chronic Nephritis
- Signs and Symptoms, Suggestive of AIDS
- Liver Diseases
- Tuberculosis
- Polycythemia Vera
- Asthma

- Epilepsy
- Leprosy
- Schizophrenia
- Endocrine Disorders
-

2- Donor Interview

A detailed history including sexual history should be taken. Positive history should be recorded on confidential notebook.

3-Informed Consent of donor

The informed consent of the donor is key that includes the following steps:

Provide information regarding

- Need for blood
- Need for voluntary donation
- Regarding transfusion transmissible infections
- Need for questionnaire and honest answer
- Safety of blood donation
- How and where the donated bleed is processed and used
- Tests carried out on donated blood

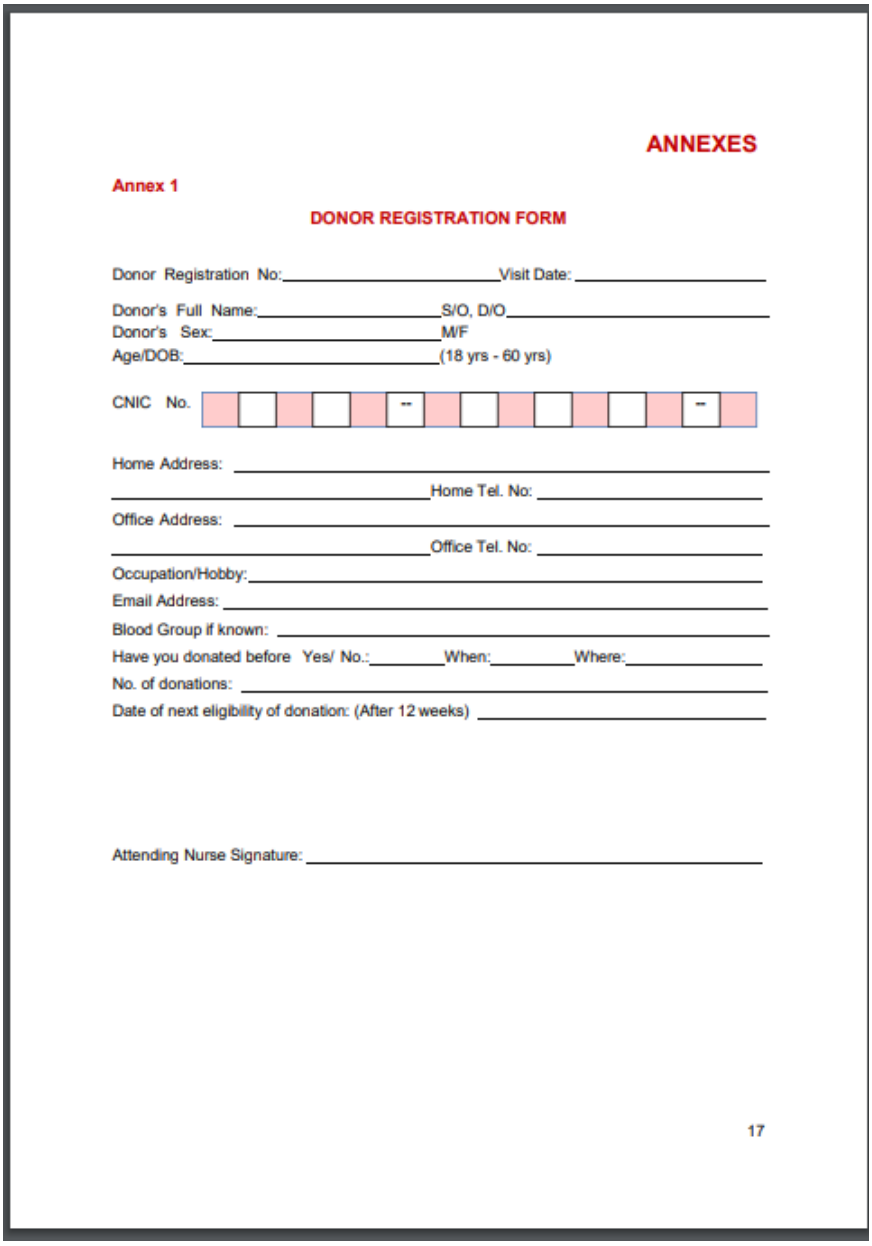
This gives the donor an opportunity to give his/her consent if they feel they are safe donors. Request the donors to sign on the donor card indication that he is donating voluntarily.

4-Donor registration

Enter all detail in the donor questionnaire form/card and computer as well. (form:1)

5-Donor past blood bank record if any.

6-Donor history questionnaire form should be filled (form:2)



ANNEXES

Annex 1

DONOR REGISTRATION FORM

Donor Registration No:_____ Visit Date: _____

Donor's Full Name:_____ S/O, D/O_____
Donor's Sex:_____ M/F
Age/DOB:_____ (18 yrs - 60 yrs)

CNIC No. | | | | | | -- | | | | | | | -- | |

Home Address: _____
_____ Home Tel. No: _____
Office Address: _____
_____ Office Tel. No: _____
Occupation/Hobby:_____
Email Address: _____
Blood Group if known: _____
Have you donated before Yes/ No.:_____ When:_____ Where:_____
No. of donations: _____
Date of next eligibility of donation: (After 12 weeks) _____

Attending Nurse Signature: _____

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Form:1 Donor registration form

DONOR HISTORY QUESTIONNAIRE FORM
For medical history and general health

	YES	NO
Are you		
1. Feeling healthy and well today?		
2. Currently taking an antibiotic or any other medication for an infection?		
3. Have you taken aspirin or anything that has aspirin in it?		
4. Currently taking or have you ever taken any medications mentioned on the Medication Deferral List? ***(Cf. annex)***		
5. Have you read and understood the educational material on donation process and information on AIDS (HIV infection) and Hepatitis?		
In the past 6 weeks		
6. Female donors: Have you been pregnant or are you pregnant now?		
In the past 8 weeks H/O		
7. Donated blood, platelets or plasma?		
8. Vaccinations or other shots?		
9. Dental treatment or tooth extraction?		
10. Allergies or rashes		
In the past 16 weeks		
11. Have you donated a double unit of red cells using an aphaeresis machine?		
12. Unexplained weight loss		
In the past 12 months have you had		
13. Blood Transfusion?		
14. Serious illness or an operation		
15. Transplant such as organ, tissue, or bone marrow?		
16. Graft such as bone or skin?		
17. An accidental needle-stick?		
18. Ear or body piercing?		
19. A tattoo?		
High risk behavior/unusual sexual practices		
20. Sexual contact with someone who		
• Is HIV positive or has hepatitis?		
• Receives or has received payment for sex in money or drugs?		
• Has injected drugs?		
21. For women: has any man with whom you have had sex in the past 12 months had sex with a man?		

22. For men: have you ever had sex with another man?		
23. Have you ever treated for syphilis or gonorrhea?		
Have you ever had		
24. A positive test for the HIV/AIDS virus?		
25. Used needles to take drugs, steroid, or anything not prescribed by your doctor?		
26. Hepatitis/Jaundice?		
27. Sexually transmitted disease e.g. Syphilis		
28. Tuberculosis?		
29. Typhoid fever?		
30. Rheumatic fever?		
31. Any heart disease/hypertension?		
32. Asthma?		
33. Persistent cough and chest pain?		
34. Any type of cancer, including Leukemia?		
35. Bleeding condition or a blood disease?		
36. Epilepsy?		
37. Diabetes?		
38. Malaria/Dengue?		
39. Leismaniasis?		
40. Any foreign visit in recent past?		
41. Any history of imprisonment?		

Form:2 history Questionnaire form

7-Basic physical examination:

Health check before donating blood.

- Check for swollen glands, skin rashes, signs of intravenous drug use or abnormal bleeding (purpura).
- Weight of the person: Persons weighing 45–50 kg or more can safely donate 450 ml of blood.
- Measurement of temperature and blood pressure: A donor should not have an abnormally low blood pressure nor a high blood pressure. The acceptable limits are a diastolic pressure of 100 mm Hg and systolic pressure of 180 mm Hg. The minimum acceptable blood pressure is 90/50 mm Hg.

- Pulse rate of the person: The pulse rate should be regular and less than 100 beats/minute (counting for at least 30 seconds).
- Test to check for anaemia: For example, measurement of haemoglobin or PCV.

8-Donor blood testing:

The following blood tests of donor are done (table:15)

-Blood group ABO/Rh status

-Test for red cell alloantibodies

-Screening blood for transfusion transmitted infections (Microbiological tests)

- Human immunodeficiency virus (HIV) 1 and 2
- Hepatitis B virus (HBV) ● Hepatitis C virus (HCV)
- Treponema pallidum (agent of syphilis)
- Plasmodium species (agents of malaria).
- Trypanosoma cruzi (agent of Chagas' disease)

1 Blood group, Rh status (D, C, E, c, e), K
2 Screen for red cell alloantibodies
3 *Microbiological tests* Human immunodeficiency virus (HIV) 1 and 2; antibody and RNA Hepatitis B virus (HBV) – antibody and RNA Hepatitis C virus (HCV) – antibody and RNA Human T-cell leukaemia viruses (HTLV) – antibody Cytomegalovirus (CMV) – antibody, for immunosuppressed recipients Malaria – antibody screening of potentially exposed donors Chagas' disease – antibody screening of potentially exposed donors Bacteria – all donations tested for antibody to syphilis (*Treponema pallidum*)

Table:15 Donor blood testing

9-Blood collection:

In the hospital, blood donors should be bled in a pleasant, light and airy place close to, but not in the laboratory and not in an open out-patient department without privacy. The donating area should be easily accessible to donors. When donating blood, a donor must not be left unattended

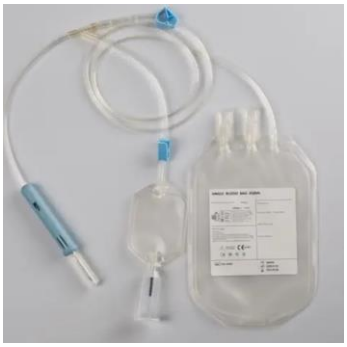
Blood bags Most of the hospitals and transfusion centres use plastic, single use, closed system blood packs to collect blood from donors. Several types of blood collection pack are available, including:

● Single bag collection pack for collecting 450 ml blood. When concentrated red cells are required, the plasma can be removed and discarded following sedimentation of the red cells

● Double bag collection pack (450 ml blood) which enables plasma to be saved following its transfer (in a closed system) to the attached sterile bag. A double bag pack costs twice the price of a single bag pack.

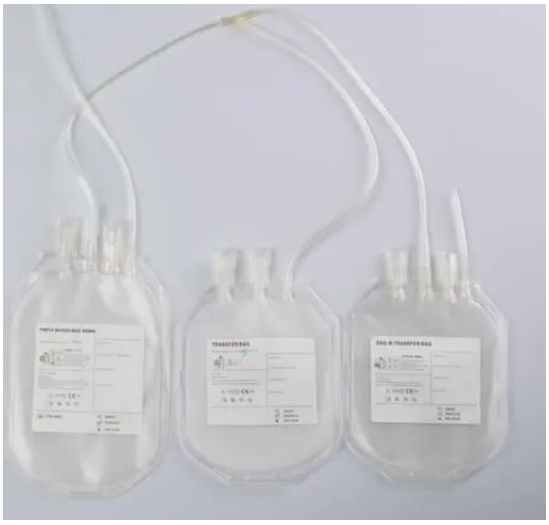
● Paediatric quadruple bag collection pack which consists of a blood collecting bag (usually for 250 ml blood) with three small sterile bags attached.

After blood collection donor should be looked for any side effects.



Single blood bag

Double blood bag



Triple bag

10.Donor record register:

All the required information should be entered in register.(Table:16)

Blood Bag No.	Donor's Name	Donor's Unique ID	Donor's Blood Group	Donor's Age/Sex	Donor's Hb/B.P	Date and Time of donation	Donor's address & Tel. No.	Donor's Screening			Post donation Adverse reaction	Remarks/ Signature
								HBs Ag	HCV Ab	HIV Ab		

Table:16 Donor record register

11-Storage of blood:

Ideally blood should be stored in an electric thermostatically controlled refrigerator of the compression type with fitted temperature record Maintains the correct temperature for storing blood, ideally 4–6 C.

- the storage temperature for red cells is between +2°C and +6°C
- the storage temperature of Platelets and leucocytes-between +20°C and +24°C
- the storage temperature for plasma products -below −18°C.

13-Transportion of donor blood:

To transport blood, always use a well-insulated cool box with sufficient freezer packs. The freezer packs should still be frozen when the blood arrives at its destination. Pack a thermometer in with the blood and ensure it is read immediately after opening the cool box. Always use a secure and reliable method of transporting blood.

Question: Write down steps of blood donation process?

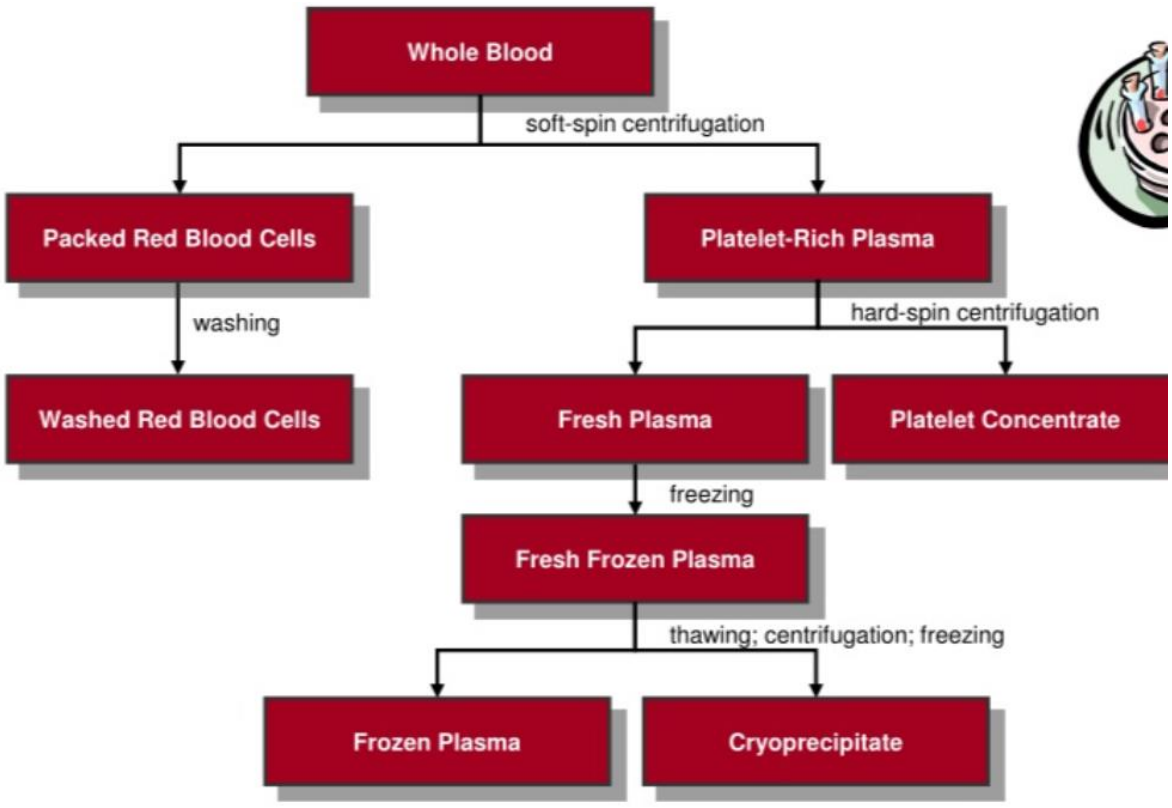
Chapter 24:

BLOOD TRANSFUSION AND ITS ADVERSE EFFECT

Objective: By the end of this chapter student should be able to know adverse reaction that can occur during a blood transfusion

In many tropical countries there is a high risk of transfusion transmitting infections in blood and blood products, when blood is not screened for infectious agents. Blood transfusion also carries the risk of immunological adverse reactions. Because of the risks involved, and high cost of blood collection, screening, and testing, it is important for clinicians to ensure blood is used only when it is absolutely necessary after a careful clinical assessment and measurement of a patient's haemoglobin (or PCV).

24.1 Blood components:



A blood donation is taken by an aseptic technique into plastic bags containing an appropriate amount of anticoagulant, usually citrate, phosphate, dextrose (CPD)or

CPDA. The citrate anticoagulated the blood by combining with the blood calcium. Three components are made by initial centrifugation of whole blood:

- Red cells
- Buffy coat (platelets and WBCS)
- Plasma (fresh frozen plasma)

1- **Red cells** are stored at 4–6°C for up to 35 days, depending on the preservative.

2-**Granulocyte concentrates:**These are prepared as buffy coats or on blood cell separators from normal healthy donors. They have been used in patients with severe neutropenia.

3-Platelet concentrates: These are made by cell separators or from individual donor units of blood. They are stored at room temperature (22-24C). Platelet transfusion is used in patients who are thrombocytopenic (low platelet count), or have disordered platelet function and who are actively bleeding (therapeutic use) or are at serious risk of bleeding (prophylactic use).

Preparations from human plasma:

These include fresh frozen plasma and cryoprecipitate.

Fresh frozen plasma: Rapidly frozen plasma separated from fresh blood is stored at less than –30°C . Frozen plasma is usually prepared from single donor units, although pooled products are also available.

Cryoprecipitate: This is obtained by thawing fresh frozen plasma at 4C and contains factor VIII and fibrinogen. It is stored at less than -30 C.

24.2<u>Adverse effects of blood transfusion (table:17)</u>

1- Haemolytic transfusion reactions:

Haemolytic transfusion reactions may be immediate or delayed. Immediate life-threatening reactions associated with massive intravascular haemolysis if wrong blood group of ABO or Rh system are transfused.

2-Febrile reactions

They occur because of white cell antibodies Human leucocyte antigen (HLA) antibodies are usually the result of sensitization by pregnancy or a previous transfusion. They produce rigors, pyrexia(fever) and, in severe cases, pulmonary infiltrates. They are minimized by giving leucocyte depleted (i.e. filtered) packed cells.

3-Post-transfusion acute circulatory overload (TACO):

The management is that of cardiac failure. These reactions are prevented by a slow transfusion of packed red cells or of the blood component required, accompanied by diuretic therapy.

4-Transfusion of bacterially contaminated blood:

This is very rare, but may be serious. It can present with circulatory collapse (hypotension, weak pulse, loss of consciousness) It is a particular problem with platelet packs that are stored at 20–24°C.

5-Graft-versus-host disease (GVHD):

This may occur when live lymphocytes are transfused to an immunocompromised patients (low immunity). It is prevented by irradiation of the blood products for susceptible recipients. It is uniformly fatal.

6-Transfusion related acute lung injury (TRALI):

This presents within 6 hours of an infusion with cough, breathlessness, fever and rigors, depending on severity.

7- Post-transfusion purpura:

This is a rare problem of severe thrombocytopenia 7–10 days after transfusion of a platelet-containing product, usually red cells.

8-Viral transmission:

Post-transfusion hepatitis may be caused by one of the hepatitis viruses, although cytomegalovirus (CMV) and Epstein–Barr virus (EBV) have also been implicated. Post-

transfusion viral hepatitis, Human T cell leukemia virus (HTLV) or HIV infection is very rarely seen because of routine screening of all blood donations.

9-Other infections:

Toxoplasmosis, malaria and syphilis may be transmitted by blood transfusion.

10-Post-transfusional iron overload: Repeated red cell transfusions over many years, in the absence of blood loss, cause deposition of iron initially in reticuloendothelial tissue(that is in spleen and liver) This becomes a major problem in thalassemia major and other severe chronic anaemia.

Video link for blood component preparation

https://www.youtube.com/watch?v=tCJBdWOA3Ok

Early (hours)	Late (days or years) Delayed transfusion reaction
Acute haemolytic reaction	Post-transfusion purpura
Reactions caused by infected blood	Immune sensitization, e.g. to red cells, platelets or Rh D antigen
Allergic reactions to white cells, platelets or proteins	Transfusion-associated graft-versus-host disease
Pyrogenic reactions (to plasma proteins or caused by HLA antibodies)	Transfusional iron overload (see Chapter 4)
Circulatory overload	
Hypothermia	
Bacterial contamination (acute sepsis, endotoxin shock)	
Air embolism	
Thrombophlebitis	
Citrate toxicity	
Hyperkalaemia	
Hypocalcaemia (infants, massive transfusion)	
Clotting abnormalities (after massive transfusion)	
Transfusion related acute lung injury (TRALI)	
Anaphylaxis (in IgA-deficient subjects)	

CMV, cytomegalovirus; HIV, human immunodeficiency virus; HLA, human leucocyte antigen; Ig, immunoglobulin.

Table:17 Adverse effect/ dangers of blood transfusion

Question: Which blood components can be made from whole blood?

SECTION III

Sample Questions

Q1. a) Define hematology and blood banking. What is the relationship of haematology and blood banking with other branches of Pathology? b) Write in detail about circulation of blood in human body. (4+4+12)

Q2. a) Write in detail about different stages of development of neutrophils with diagram b) Write the composition of blood. (15+5)

Q3. a) What are reticulocytes and their normal range in adults and children? How can you measure reticulocyte count and corrected reticulocyte in pathology laboratory?

(4+6+5+5)

Q4.a) Write the mode of formation of hemoglobin with normal range of hemoglobin in adults and children. b) Write in detail about osmotic fragility test with significance.

(6+6+8)

Q5. a) What are haemoparasites? Write the procedure for detection of malarial parasite in detail. (5+15)

Q5. a) Write the classification of acute leukemia with investigation required for the diagnosis of acute leukemia. b) Describe in detail about ESR with its significance.

(10+10)

Q6. a) Write in detail about mechanism of blood coagulation. b) Which screening tests can be done to check the clotting factors? (15+5)

Q7. a) Write in detail about ABO blood group system. b) write any five complications due to blood transfusion. (15+5)

Q 8 a) Describe in detail about Rh blood group system. b) Write the procedure of coombs test with its uses. c) What are the sources of error of coombs test? (10+5+5)

Q9 a) Write in detail about blood registration, donor blood collection, storage and transportation of blood in blood bank. b) Which screening tests of donor blood are done before collecting blood (15+5)

Q10 a) What is anemia with classification of anemia. b) define the following terms:

1-macrocytes2- microcytes 3normocytes 4-spherocytes 5-hypochlomia. (2+8+10)

References

1) Hoffbrand's Essential Haematology, A. Victor Hoffbrand, Eighth Edition
2) Essentials of Haematology, Shirish M Kawthalkar, First Edition
3) District Laboratory Practice in Tropical Countries Part 2, Monica Cheesbrough, Second Edition
4) Medical Parasitology, Rohela Mahmud Yvonne Ai Lian Lim • Amirah Amir, first edition
5) Website of Institute of Blood Transfusion Service Punjab.gov.pk. https://ibts.punjab.gov.pk
6) website Punjab Blood Transfusion Authority.https://pbta.punjab.gov.pk › SOP for Blood bank, 2014
7) Manual of Laboratory medicine 4th edition (AFIP) 2012
8) Modern blood banking and transfusion practices 6th edition Denise M. Harmening