Dr. Yasir Adil Alabdali

Blood and components and diseses

A single whole blood donation can be separated into different components to provide treatment to more than one patient.

There are two methods for collection of blood for preparation of blood components:

1. *Single whole blood donation*: Preparation of blood components has been greatly facilitated by the introduction of double and triple bags having closed integral tubing. After their separation, various components can be transferred from one bag to another in a closed circuit thus avoiding exposure to external environment and maintaining the sterility. Blood should be processed for component separation within 6 hours of collection (Figs 39.1 and 39.2).
2. *Apheresis*: This is a procedure in which a suitable donor is connected to an automated cell separator machine (that is essentially designed as a centrifuge) through which whole blood is withdrawn, the desired blood component is retained, and the remainder of the blood is returned back to the donor. Depending on the component that is separated and removed, the procedure is called as plateletpheresis, leukapheresis, or plasmapheresis.

Table 39.1: Definitions used in transfusion therapy

Blood product A therapeutic substance prepared from human blood
Whole blood One unit of non-separated donor blood collected in an appropriate container containing anticoagulant-preservative solution
Blood component A constituent separated from whole blood by differential centrifugation or that is obtained directly from donor by apheresis
Plasma derivative Human plasma proteins obtained from multiple donor units of plasma under pharmaceutical manufacturing conditions. These products are heat-treated or chemical-treated to inactivate lipid-enveloped viruses.
Plasma derivatives like factor concentrates and immunoglobulins can also be prepared by recombinant DNA technology

- **Components of blood plasma**

Blood plasma consists of elements following:

Water is form 95% of plasma volume its importance from its ability to transport organic materials and inorganic as well as maintaining body temperature, and a volume of plasma remaining 15% solid material and material non-solid, which inclued 9% organic material and 1% inorganic materials.

1-Organic material in plasma:

A- Proteins materials form for 6-8g/100cm^3 plasma and include:

1- Albumin: the proportion 3.8-501g/100cm^3 of plasma and its importance comes organization osmotic of blood, organization pH of blood, transmission of many hormones and minerals.

2- Globulins: the proportion 3g /100 cm^3 of plasma is divided to secondary species of (α1, α 2, β1, β2) in addition to Kama globulines У or immunoglobulines or antibodies that interact with pathogens or with the antigens (Antigens).

3- Fibrinogen: the proportion 200-400 mg/100cm^3 of plasma importance responsible for blood clotting and thus help to stop bleeding.

B- The material non protein is divided in to:
A-Nutrients are:
1-Glucose rate 80-120 mg/100cm^3
2-Lipids proportion 600-800 mg/100 cm^3.
Importance of food material is used to produce energy (ATP) necessary for growth and proliferation of cells.

B- Materials excretion such as:
1- Urine rate11-43 mg / 100 cm^3
2- Creatinine rate 0.8 - 1.2 mg / 100 cm^3
3- Uric acid and of 0.3 - 0.7 mg / 100 cm^3

2- Non organic materials: It includes:
1- Potassium rate 3.5 to 5.5 m Equivalent m Eq / L.
2- Sodium rate 135-153 m Eq / liter.
3- Calcium rate 8.8 to 10.2 mg /100 cm^3 of plasma.
4- Manganese rate 1.6 to 2.5 mg/100cm^3 of plasma.
5- Iron rate 100-150 mg /100 cm^3 of plasma.
6- Chlorine rate 38-110 m Eq / liter.
7- Bicarbonates
8-Phosphate
The importance of inorganic materials in maintaining osmotic of blood and keep on pH blood.

A complete blood count (CBC) is normally include:

Hemoglobin carries oxygen from the lungs to the tissues and carbon dioxide from tissues to the lungs. It is composed of heme (iron + protoporphyrin) and globin polypeptide chains. Hemoglobin is not homogeneous and normally different variants and derivatives exist. Normal hemoglobin variants are embryonic hemo- globins (Gower I, Gower II, and Portland), fetal hemo- globin (Hb F), adult hemoglobin (Hb A), and Hb A2. They differ from each other in the type of polypeptide chains (Box 18.1).

INDICATIONS FOR HEMOGLOBIN ESTIMATION

1. To determine presence and severity of anemia: Anemia refers to low hemoglobin concentration or oxygen-carrying capacity of blood. Clinical signs of anemia (pallor of skin, conjunctival vessels, or mucous membranes) are unreliable for diagnosis of anemia. Anemia is best assessed by estimation of hemoglobin or packed cell volume.
2. Screening for polycythemia: Polycythemia refers to increased hemoglobin level above the normal range. It may be primary, secondary, or relative (Box 18.2).
3. To assess response to specific therapy in anemia.

4. Estimation of red cell indices (along with packed cell volume and red cell count) i.e. mean cell hemoglobin and mean cell hemoglobin concentration.
5. Selection of blood donors

Box 18.2: Polycythemia

- **Primary:** Increase in total red cell mass with low erythropoietin level e.g. polycythemia vera
- **Secondary:** Increase in total red cell mass with increased erythropoietin level, e.g. hypoxia
- **Relative:** Decrease in plasma volume with red cell mass remaining normal, e.g. dehydration.

Box 18.1: Hemoglobin

- **Normal physiological hemoglobins:** Oxyhemoglobin, Reduced hemoglobin (i.e. iron of heme not associated with oxygen)
- **Hemoglobin derivatives:** Compounds formed from physiological hemoglobins through the action of acids, alkalis, oxidizing/reducing agents, etc. They are identified by their characteristic absorption spectra in a spectro- photometer.
- **Hemoglobin variants:** Different structural forms of hemoglobin that differ in structure of polypeptide chains. They are identified by hemoglobin electrophoresis.
- – **Normal**: HbA ($\alpha 2\beta 2$), HbF ($\alpha 2\gamma 2$), HbA_2 ($\alpha 2\delta 2$)
- – **Abnormal**: HbS, HbC, HbD, HbE, etc.

METHODS FOR ESTIMATION OF HEMOGLOBIN

There are various methods for estimation of hemoglobin. These are:

1. **Colorimetric methods**: In these methods, color comparison is made between the standard and the test sample, either visually or by colorimetric methods.
   A. Visual methods: Sahli's acid hematin method
   B. Photoelectric methods: Cyanmethemoglobin (hemiglobincyanide) method, oxyhemoglobin method, and alkaline hematin method.
2. Gasometric method: Oxygen-carrying capacity of blood is measured in a Van Slyke apparatus. The amount of hemoglobin is then derived from the formula that 1 gram of hemoglobin carries 1.34 ml of oxygen. However, this method measures only physiologically active hemoglobin, which can carry oxygen. It does not measure carboxyhemoglobin, sulfhemo- globin, and methemoglobin. Also, this method is time-consuming and expensive. The result is about 2% less than other methods.
3. Chemical method: Iron-content of hemoglobin is first estimated. Value of hemoglobin is then derived indirectly from the formula that 100 grams of hemoglobin contain 374 mg of iron. This method is tedious and time-consuming.
4. Specific gravity method: A rough estimate of hemoglobin is obtained from the specific gravity of blood as determined from copper sulphate technique. This method is useful in mass screening like selection of blood donors.

- Adult males: 13.0 - 17.0 gm/dl.
- Adult females (non-pregnant): 12.0 – 15.0 gm/dl.
- Adult females (pregnant): 11.0 - 14.0 gm/dl.
- Children, 6-12 years: 11.5 - 15.5 gm/dl.
- Children, 6 months to 6 years: 11.0 – 14.0 gm/dl.
- Children, 2 – 6 months: 9.5 – 14.0 gm/dl.
- At birth (full term): 13.6 – 19.6 gm/dl.

CRITICAL VALUES

- < 7 gm/dl (severe anemia)
- > 20 gm/dl (hyperviscosity)

Packed cell volume (PCV) is the volume occupied by the red cells when a sample of anticoagulated blood is centrifuged. It indicates relative proportion of red cells to plasma. PCV is also called as hematocrit or erythrocyte volume fraction. It is expressed either as a percentage of original volume of blood or as a decimal fraction.

USES OF PCV

- Detection of presence or absence of anemia or polycythemia
- Estimation of red cell indices (mean cell volume and mean corpuscular hemoglobin concentration)
- Checking accuracy of hemoglobin value (Hemoglobin in grams/dl × 3 = PCV).

There are two methods for estimation of PCV: macro method (Wintrobe method) and micro method (micro- hematocrit method). Micro method is preferred because it is rapid, convenient, requires only a small amount of blood, capillary blood from skin puncture can be used, and a large number of samples can be tested at one time. This method is also more accurate as plasma trapping in red cell column is less.

- Adult males: 40-50%
- Adult females (nonpregnant): 38-45%
- Adult females (pregnant): 36-42%
- Children 6 to 12 years: 37-46%
- Children 6 months to 6 years: 36-42%
- Infants 2 to 6 months: 32-42%
- Newborns: 44-60%

CRITICAL VALUES

- Packed cell volume: < 20% or > 60%

Reticulocytes are young red cells released from the bone marrow into the bloodstream and that contain remnants of ribonucleic acid (RNA) and ribosomes but no nucleus. After staining with a supravital dye such as new methylene blue, RNA appears as blue precipitating granules or filaments within the red cells. Reticulocyte count is performed to assess erythropoietic activity of the bone marrow

USES

1. As one of the baseline studies in anemia with no obvious cause

2. To diagnose anemia due to ineffective erythropoiesis (premature destruction of red cell precursors in bone marrow seen in megaloblastic anemia and thalassemia) or due to decreased production of red cells: In hypoplastic anemia or in ineffective erythropoiesis, reticulocyte count is low as compared to the degree of anemia. Increased erythropoiesis (e.g. in hemolytic anemia, blood loss, or specific treatment of nutritional anemia) is associated with increased reticulocyte count. Thus reticulocyte count is used to differentiate hypoproliferative anemia from hyperproliferative anemia.
3. To assess response to specific therapy in iron deficiency and megaloblastic anemias.
4. To assess response to erythropoietin therapy in anemia of chronic renal failure.
5. To follow the course of bone marrow transplantation for engraftment
6. To assess recovery from myelosuppressive therapy
7. To assess anemia in neonate

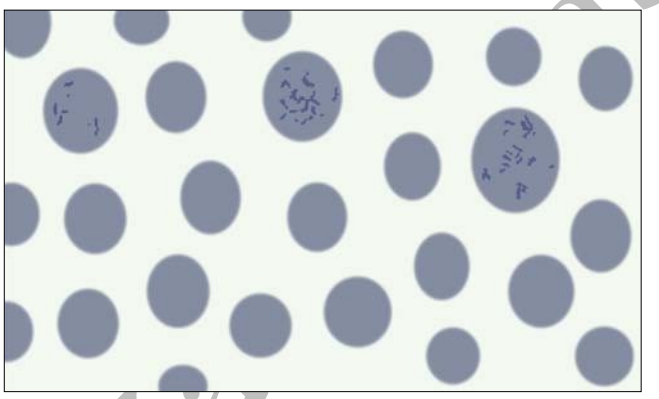

Fig. 21.1: Reticulocytes stained with a supravital stain

A blood smear or film is a specimen for microscopic examination prepared by spreading a drop of blood across a glass slide followed by staining with one of the Romanowsky's stains.

USES

1. Blood smear is helpful in suggesting the cause of anemia or thrombocytopenia, identifying and typing of leukemia, and in diagnosing hemoparasitic infections (malaria, filaria, and trypanosomiasis). It is also helpful in the management of these conditions.
2. To monitor the effect of chemotherapy and radio- therapy on bone marrow.
3. To provide direction for further investigations that will help in arriving at the correct diagnosis (e.g. in infections, drug toxicity, etc.).

Blood smear examination is therefore indicated in clinically suspected cases of anemia, thrombocytopenia, hematological malignancies (leukemia, lymphoma, multiple myeloma), disseminated intravascular coagulation, parasitic infections (like

malaria or filaria), infectious mononucleosis, and various inflammatory, or malignant diseases.

STAINING OF BLOOD SMEAR

Blood smears are routinely stained by one of the Romanowsky stains (Box 22.1). Romanowsky stains consist of a combination of acidic and basic dyes and after staining various intermediate shades are obtained between the two polar (red and blue) stains. Romanow- sky stains include May-Grunwald-Giemsa, Jenner, Wright's, Leishman's, and Field's stains. Staining properties of the Romanowsky stains are dependent on two synthetic dyes: methylene blue and eosin.

Box 22.1: Romanowsky stains

Two main components of all Romanowsky stains are an acidic dye (eosin Y) and a basic dye (oxidized methylene blue).

- *Basic or cationic dye*: It is positively charged and binds to anionic sites and imparts blue-gray color to nucleic acids, nucleoproteins, and granules of basophils. Examples: methylene blue, azure B.
- *Acidic or anionic dye*: It is negatively charged and binds to cationic sites and imparts orange-red color to hemoglobin and eosinophil granules. Example: eosin Y.

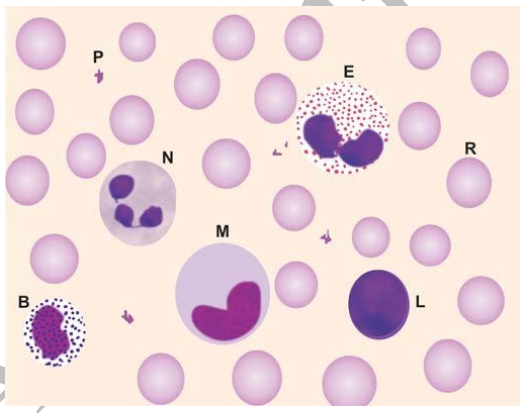

Fig. 22.3: Normal blood smear showing red cells (R), a neutrophil (N), a lymphocyte (L), eosinophil (E), basophil (B), monocyte (M), and platelets (P)

A well-stained smear shows following features (Fig. 22.3):

- *Red cells*: pink-red or deep pink
- *Polychromatic cells (Reticulocytes)*: Gray-blue
- *Neutrophils*: Pale pink cytoplasm; mauve-purple granules
- *Eosinophils*: Pale-pink cytoplasm; orange-red granules
- *Basophils*: Blue cytoplasm; dark blue-violet granules
- *Monocytes*: Gray-blue cytoplasm; fine reddish (azurophil) granules
- *Small lymphocytes*: Dark blue cytoplasm
- *Platelets*: Purple
- *Nuclei of all cells*: Purple-violet

EXAMINATION OF BLOOD SMEAR

A blood smear is examined for:

- *Red cells*: Morphology, immature forms, inclusion bodies, arrangement of cells.
- *White cells*: Differential count, abnormal or immature forms.
- *Platelets*: Adequacy, abnormal forms.
- *Parasites*: Malaria, filaria.

Red Cells

Red cells are best examined in an area where they are just touching one another (towards the tail of the film). Normal red cells are 7-8 µm in size, round with smooth contours, and stain deep pink at the periphery and paler in the center. Area of central pallor is about 1/3rd the diameter of the red cell. Size of a normal red cell corresponds roughly with the size of the nucleus of a small lymphocyte. Normal red cells are described as normocytic (of normal size) and normochromic (with normal staining intensity i.e. hemoglobin content).

Morphologic abnormalities of red cells in peripheral blood smear can be grouped as follows:

- Red cells with abnormal size (Fig. 22.5)
- Red cells with abnormal staining
- Red cells with abnormal shape (Fig. 22.5)
- Red cell inclusions (Fig. 22.6)
- Immature red cells (Fig. 22.7)
- Abnormal red cell arrangement (Fig. 22.8).

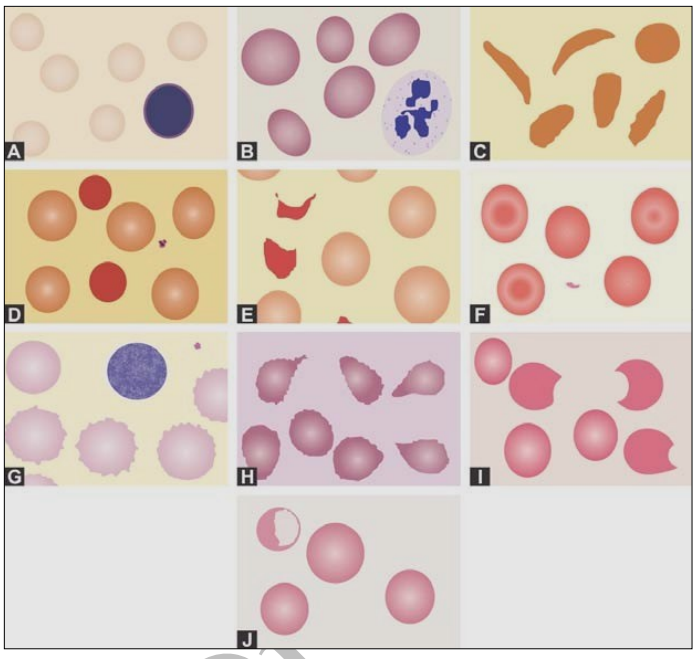

Fig. 22.5: Variations in size and shape of red cells:

(A) Microcytic hypochromic red cells in iron deficiency anemia; (B) Oval macrocytes and a hypersegmented neutrophil in megaloblastic anemia; (C) Sickle cells in sickle cell anemia; (D) Spherocytes in hereditary spherocytosis; (E) Fragmented red cells or schistocytes in microangiopathic hemolytic anemia; (F) Target cells in hemoglobinopathy; (G) Burr cells in chronic renal failure; (H) Tear drop red cells in myelofibrosis; (I) Bite cells and (J) Blister cell in glucose-6-phosphate dehydrogenase deficiency

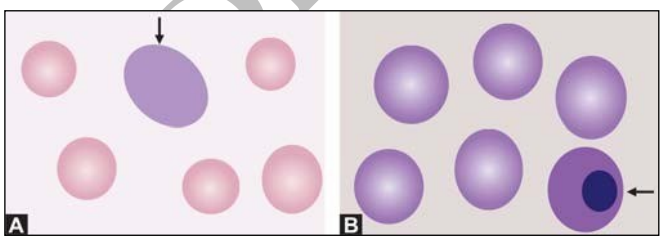

Fig. 22.7: Immature red cells: (A) Polychromatic red cell; (B) Nucleated red cell

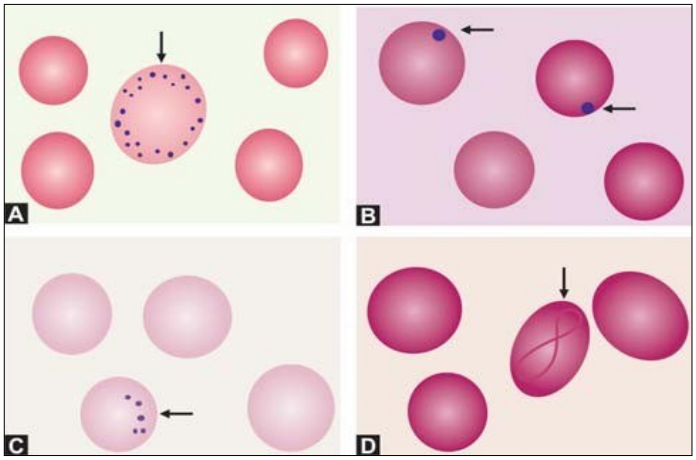

Fig. 22.6: Red cell inclusions: (A) Basophilic stippling; (B) Howell-Jolly bodies; (C)Pappenheimer bodies; (D)Cabot's ring

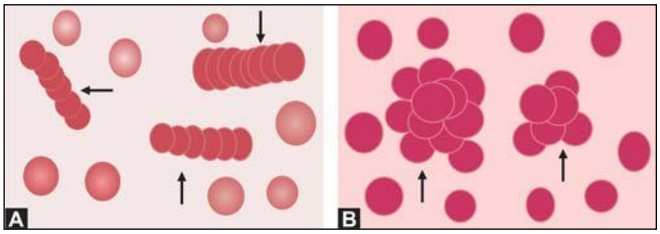

Fig. 22.8: Abnormal red cell arrangement: (A) Rouleaux formation; (B) Autoagglutination

1. **Red cells with abnormal size**:

Mild variation in red cell size is normal. Increased *variation* in red cell size is called as **anisocytosis**. This is a feature of most anemias and is non-specific. Anisocytosis is due to the presence of microcytes, macrocytes, or both in addition to red cells of normal size.

Microcytes are red cells smaller in size than normal. They are seen when hemoglobin synthesis is defective

i.e. in iron deficiency anemia, thalassemias, anemia of chronic disease, and sideroblastic anemia.

Macrocytes are red cells larger in size than normal. Oval macrocytes (macro-ovalocytes) are seen in megaloblastic anemia, myelodysplastic syndrome, and in patients being treated with cancer chemotherapy. Round macrocytes are seen in liver disease, alcoholism, and hypothyroidism.

2. **Red cells with abnormal staining (hemoglobin content):** Staining intensity of red cells depends on hemoglobin content. Red cells with increased area of central pallor (i.e. containing less hemoglobin) are called as hypochromic. They are seen when hemoglobin synthesis is defective, i.e. in iron deficiency, thalassemias, anaemia of chronic disease, and sideroblastic anemia. In dimorphic anemia, there are two distinct populations of red cells in the same smear. An example is presence of both normochromic and hypochromic red cells seen in **sideroblastic anemia, iron deficiency anemia** responding to treatment, and following blood transfusion in a patient of hypochromic anemia. In **myelodysplastic syndrome, dimorphic** picture results from admixture of **microcytic hypochromic cells and macrocytes**.

3. **Red cells with abnormal shape**: Increased variation in red cell shape is called as **poikilocytosis** and is a feature of many anemias. A red cell that is abnormal in shape is called as a poikilocyte.

Sickle cells are narrow and elongated red cells with one or both ends pointed. Sickle form is assumed when a red cell containing **hemoglobin S** is deprived of oxygen. Sickle cells are seen in sickle cell disorders, particularly sickle cell anemia. Sickle cells are not seen on blood smear in neonates with sickle cell disease because high percentage of fetal hemoglobin in red cells prevents sickling.

Spherocytes are red cells, which are slightly smaller in size than normal, round, stain intensely, and do not have central area of pallor. The surface area of spherocytes is less as compared to the volume. They are seen in hereditary spherocytosis, autoimmune hemolytic anemia (warm antibody type), and ABO hemolytic disease of newborn.

Schistocytes are fragmented red cells, which take various forms like helmet, crescent, triangle, etc. and usually have surface projections or spicules. They are seen in microangiopathic hemolytic anaemia, cardiac valve prosthesis, and severe burns.

Target cells are red cells with bull's eye appearance. These red cells show a central stained area and a peripheral stained rim with unstained cytoplasm in between.

They are seen in hemoglobinopathies (e.g. thalassemias, hemoglobin C disease, sickle cell disease), obstructive jaundice, and following splenectomy.

Burr cells or echinocytes are small red cells with regularly placed small projections on surface. They are seen in uremia.

Acanthocytes are red cells with irregularly spaced sharp projections of variable length on surface. They are seen in spur cell anemia of liver disease, McLeod phenotype, and following splenectomy.

Teardrop cells **or dacryocytes** have a tapering drop- like shape. Numerous teardrop red cells are seen in myelofibrosis and myelophthisic anemia.

Blister cells **or hemi ghost cells** are irregularly contracted cells in which hemoglobin is contracted and condensed away from the cell membrane. This is seen in glucose-6-phosphate dehydrogenase deficiency during acute hemolytic episode.

Bite cells result from removal of Heinz bodies by the pitting action of the spleen (i.e. a part of red cell is bitten off by the splenic macrophages). They are seen in glucose-6-phosphate dehydrogenase deficiency and unstable hemoglobin disease.

4. **Red cell inclusions**: Those inclusions that can be visualized on Romanowsky-stained smears are basophilic stippling, Howell-Jolly bodies, Pappenheimer bodies, and Cabot's rings.

Basophilic stippling or punctate basophilia refers to the presence of numerous, irregular basophilic (purple-blue) granules which are uniformly distributed in the red cell. These granules represent aggregates of ribosomes. Their presence is indicative of impaired erythropoiesis and they are seen in thalassemias, megaloblastic anemia, heavy metal poisoning (e.g. lead), and liver disease.

Howell-Jolly bodies are small, round, purple-staining nuclear remnants located peripherally in red cells. They are seen in megaloblastic anemia, thalassemias, hemolytic anemia, and following splenectomy.

Pappenheimer bodies are basophilic, small, iron- containing granules in red cells. They give positive Perl's Prussian blue reaction. Unlike basophilic stippling, Pappenheimer bodies are few in number and are not distributed throughout the red cell. They are seen following splenectomy and in thalassemias and sideroblastic anemia.

Cabot's rings are fine, reddish-purple or red, ring-like structures. They appear like loops or figure of eight structures. They indicate impaired erythropoiesis and are seen in megaloblastic anemia and lead poisoning.

5. **Immature red cells:**

Polychromatic cells are young red cells containing remnants of ribonucleic acid. These cells are slightly larger than normal red cells and have a diffuse bluish- grey tint. (They represent reticulocytes when stained with a supravital stain like new methylene blue). Polychromasia is due to the uptake of acid stain by hemoglobin and basic stain by ribonucleic acid. Presence of polychromatic cells is indicative of active

erythropoiesis and are increased in hemolytic anemia, acute blood loss, and following specific therapy for nutritional anemia.

Nucleated red cells are red cell precursors (erythroblasts), which are released prematurely in peripheral blood from the bone marrow. They are a normal finding in cord blood of newborns. Large number of nucleated red cells in blood smear is seen in hemolytic disease of newborn, hemolytic anemia, leukemias, myelophthisic anemia, and myelofibrosis.

6. Abnormal red cell arrangement:

Rouleaux formation refers to alignment of red cells on top of each other like a stack of coins. It occurs in multiple myeloma, Waldenström's macroglobulinemia, hyper-gammaglobulinemia, and hyperfibrinogenemia.

Autoagglutination refers to the clumping of red cells in large, irregular groups on blood smear. It is seen in cold agglutinin disease.

Role of blood smear in anemia is shown in Box 22.2 and Figures 22.9 to 22.11.

Box 22.2: Role of blood smear in anemias

- *Macrocytic anemia*: Differential diagnosis between megaloblastic anemia (oval macrocytosis and hyper- segmented neutrophils), liver disease (round macrocytosis and target cells), hemolytic anemia (numerous poly- chromatic cells), and myelodysplastic syndrome (dimor- phic red cells, pseudo-Pelger-Huet neutrophils, giant platelets, occasional blast cell).
- *Microcytic anemias*: Differential diagnosis between iron deficiency anemia (microcytic hypochromic red cells, pencil cells), thalassemia minor (microcytic hypochromic red cells, basophilic stippling), and sideroblastic anemia (dimorphic anemia).
- *Sickle cell disease*: Differentaition between sickle cell trait (target cells with no sickle cells), sickle cell anemia (sickle cells), and sickle cell β-thalassemia (microcytic hypo-chromic red cells, sickle cells).
- *Hemolytic anemias*: Spherocytosis (hereditary sphero- cytosis, autoimmune hemolytic anemia), fragmented cells (microangiopathic haemolytic anemia), bite cells or blister cells (Glucose-6-phosphate dehydrogenase deficiency), and autoagglutination of red cells (cold hemagglutinin disease).