WHITE CELL (LEUKOCYTE) DIFFERENTIAL COUNT

Leukocytes are divided into:

I. Granulocytes which contain granulations in the cytoplasm named incorrectly in the past polymorph nuclear leukocytes (PMNs) for the reason that they should be provided with more nuclei; in fact they have only one polylobated nucleus. According to the affinity for acid or basic dyes, the granulocytes are grouped in: neutrophils, eosinophils, basophils.

II. Mononuclear leukocytes: monocytes, lymphocytes.

The differential count of white cells on stained smears represents the percentage corresponding to each class of white cells from the total number of white cells counted. Observation: Since the differential count concerns only the leukocyte series, the number of white cells is recorded separately from other nucleated forms. In recording results, the number of each class of leukocytes is also expressed as a percentage of the total number of leukocytes counted.

To recognize the different leukocytes, first blood smear have to be stained using the Giemsa's method. The Giemsa's stain is a neutral one because it is a mixture of two dyes, an acidic and a basic one (eosin is acid and methylene blue is basic).

Materials.
Methyl alcohol, Giemsa solution, cotton, alcohol, gauze, glass slides, sterile needles, distilled water, microscope, immersion oil

Work Technique.
1) With usual aseptic precautions, fingertip is pricked.
2) First blood drop is discarded by wiping it with sterile dry gauze.
3) A drop of blood is placed on a slide, and using a second slide, is spread evenly over the surface of the glass in a thin film
4) The smear prepared is allowed to dry.
5) The smear is fixed for 5 minutes with methanol.
6) Diluted Giemsa stain is put over the blood smear to cover it fully. Stain is kept for 20-30 minutes.
7) The blood smear is washed with distilled water.
8) Slide is allowed to dry.
9) The smear is visualized under oil immersion.

The different WBCs are identified from their characters such as size, nucleus, presence or absence of granules in the cytoplasm. Size of WBC is compared to that of RBC which is 7-8.5µm. There are counted between 200 and 400 WBCs and then the count is converted as percentage. Differential WBC count can be expressed by relative (%) and absolute values (/mm³).

Figure no. Leukocyte types on blood smear

1. RBCs (erythrocytes) are pink, almost round, with the edges well outlined, being a bit light-colored in the middle due to the depression in the central zone. The diameter 7-8.5 µm.

2. Neutrophils have a diameter of about 12 to 15 mm, polilobed nucleus, cytoplasm has granulations turns violet (affinity for acid and basic dyes). Granulations are lysosomes containing over 30 types of enzymes able to degrade foreign substances embedded. Because of this content play an essential role in the destruction of microbes. The lobes number depends on the age of the cell. According to Arneth, the number of lobes increases in the same time with the
age of the cell. Band neutrophils or unsegmented neutrophils are called the young neutrophils having a nucleus with a single or maximum 2 lobes and they are less than 4% of circulating neutrophils. Segmented neutrophils are called the cells with 3 or more lobes of the nucleus. If young blood elements predominate is considered deviation on left white blood cell counts, if are highly mature elements, the deviation is to the right.

3. **Eosinophils** have a diameter around 13 to 15 mm, contain coarse shining red granules in the cytoplasm, the nucleus is usually bilobated. The cytoplasm is basophil, but it is not seen because of the multitude of the granulations. They destroy parasites and detoxify foreign proteins in the body arrived. They are stationed in large numbers in the intestinal mucosa and lung (input).

4. **Basophils** have a diameter of 10 ÷ 12 mm, three-lobed nucleus. Granulations unequal, often conglomerated and intensely blue colored. Their function is mainly secretory: histamine, chemo tactic factors for eosinophils.

5. **Monocytes** have a diameter between 15 ÷ 24 mm. The nucleus has a deep depression on one side, which gives it a kidney shape (bean shape). The cytoplasm is bluish-gray and contains fine red granules contain numerous enzymes that gives it a ground glass appearance. Due to high enzyme content they have a strong phagocytic activity: germs, viruses, antigen-antibody complex, cellular debris, tumor cells, and mineral powders.

6. **There are small lymphocytes** (diameter about 8 µm) and about 10% are **large** lymphocytes with a diameter between 12-14 m. Their nucleus is large, round, almost completely filling the cell. The thin rim of blue cytoplasm surrounding the nucleus has no granules.

**INTERPRETATION**

Normal values are:
Neutrophils 60-65%
Eosinophils 1-3%
Basophils 0.15-0.5%
Monocytes 4-8%
Lymphocytes 25-35%
Band neutrophils = maximum 10% from segmented neutrophils = 2-6%

**Variations** are:
1. **Neutrophils:**
   a) **neutrophilia** — increased neutrophils in:
   - bacterial infections
   - tissue breakdown: burns, trauma, myocardial infarct, tumors
   - blood diseases: leukemia, hemorrhage
b) Neutropenia — low neutrophil number
   - viral infections: hepatitis, measles, mumps
   - bacterial infections: typhoid fever, brucellosis, septicemia
   - parasitic infections: malaria
   - bone marrow insufficiency
   - radiation

2) Eosinophils:
   a) eosinophilia = increased eosinophils
      - parasitic infections: trichinellosis
      - allergic diseases
   b) Eosinopenia — reduced neutrophils
      - ACTH, glycocorticoids administration
      - stress

3) Basophils: increased basophils number occurs in granulocytic and basophilic leukemias

4) Monocytes: the monocytes are increased in
   - viral infections: infectious mononucleosis, chickenpox, mumps
   - bacterial infections: tuberculosis, sub acute endocarditis
   - other diseases: malignant tumors, monocytic leukemia, liver cirrhosis

5) Lymphocytes:
   a) Lymphocytosis:
      - normal in children up 5 years old, there is a physiological lymphocytosis up 50%
      - viral infections: virus pneumonia, hepatitis, measles mumps
      - bacterial infections: tuberculosis, syphilis, healing infections
      - lymphatic leukemia
   b) Lymphopenia:
      - lymphatic system and blood diseases: Hodgkin disease, lupus
      - ACTH, glycocorticoids administration.