Dr. Muhsin S. Ghalib

Clinical Hematology

Second lactures

4-Determination of Hematocrit (Hct) (Packed Cell Volume; PCV)

Measurement of hematocrit (Hct) or packed cell volume (PCV) is the most accurate and simplest of all tests in clinical hematology for detecting the presence and degree of anemia or polycythemia. In comparison, hemoglobin estimation is less accurate, and RBC count far less accurate.
Also, if Hb, RBC count, and PCV are determined at the same time, various absolute corpuscular values (e.g., volume and Hb content of a single red cell) of a person can be determined. These values help in the laboratory diagnosis of the type of anemia in a person.

Normal values. The average value of PCV is 42% when the RBC count is 5 million/mm3 and their size and shape are normal.

- Males: 44 percent (38–50 percent)
- Females: 42 percent (36–45 percent)

The PCV for newborns is about 50 percent.
Q what is the importance of determining hematocrit?
It is a simple but accurate test for determining the presence of anemia or polycythemia, as it is more accurate than the red cell count or Hb. It is also employed for determining various absolute, corpuscular values. Sometimes, it is used for screening for anemia.
Q which cells make up the buffy layer? How thick is it and when can it increase in thickness?

The buffy layer consists of packed platelets and leukocytes. Platelets being less dense, settle in a separate layer above the leukocytes. The buffy layer is about 1 mm thick but the thickness increases in cases of severe leukocytosis, leukemia, and thrombocytosis, especially primary thrombocytosis where the count may exceed 800,000/mm3.

Q Name the conditions where the PCV is in**creased and those where it is decreased?**
Increased PCV is seen in:

i. All cases of polycythemia (newborns, high altitude), hypoxia due to lung and heart diseases, etc.

ii. Congestive heart failure, burns (loss of plasma), dehydration, after severe exercise, and emotional stress. In all these cases, there is a change in the plasma volume, or redistribution of red cells. (The spleen is not responsible for these changes in man because there is no smooth muscle in this organ).

Decreased PCV is seen in

i. All types of anemia.

ii. Pregnancy (due to hemodilution), and ingestion of large amounts of water.

5-Normal blood standards (Absolute Corpuscular values indices)

The basic values of Hb, RBC count, and PCV (Hct) do not give any information about the condition of an average red cell, such as its volume, Hb content, or its percentage saturation with Hb. Neither can this information, which is important in diagnosing the type of anemia in a patient, be obtained directly from any experimental method.

However, this information, in the form of absolute corpuscular values, especially if these are done electronically, can be calculated from 3 basic values of Hb, RBC count, and PCV.

Further, the basic values found in a patient/subject can be compared with arbitrarily set "normal" values. This information, the red cell indices, have been discarded in favor of absolute corpuscular values.

PROCEDURES

1. Use your own values of Hb, RBC count and the value of PCV obtained during the demonstration

experiment on a volunteer. This value of PCV, however, will not be strictly applicable to any person other than the volunteer.

Your teacher may also provide each one of you with an arbitrary value of PCV from your Hb and RBC counts.

2. Calculate your absolute values for MCV, MCH, MCHC, and color index as shown below:

I. Mean Corpuscular Volume (MCV)

The MCV is the average or mean volume of a single red blood cell expressed in cubic micrometers (μm3 or femtoliters). It is calculated from the following two basic values:

i. Red cell count in million/mm3

ii. Packed cell volume (PCV) in 100 ml blood.

Normal range = 74 – 95 μm3

II. Mean Corpuscular Hemoglobin (MCH)

The MCH, which is also determined indirectly, is the average hemoglobin content (weight of Hb) in a single red blood cell expressed in picograms (micro-microgram, μμg).

basic values:

- RBC count in million/mm3.
- Hb in g percent.
- *Normal range* = 27–32 pg
-

III. Mean Corpuscular Hemoglobin Concentration (MCHC)

The MCHC represents the relationship between the red cell volume and its degree or percentage saturation with hemoglobin, that is, how many parts or volumes of a red cell are occupied by Hb.
If the MCHC is within the normal range, the cell is normochromic, if it is below the range, the cell is hypochromic. However, it cannot be hyperchromic for the reason mentioned above. A large cell may contain more Hb, but its percentage saturation will not be more than 36%.

IV. Mean Corpuscular Diameter (MCD)

The MCD is determined by direct micrometric measurements of the red cells in a stained film. The range is 6.9 to 8 micrometers, with an average of 7.5 μm. MCD can be used for measuring the mean corpuscular average thickness (MCAT).

V. Mean Corpuscular Diameter (MCD)

The MCD is determined by direct micrometric measurements of the red cells in a stained film. The range is 6.9 to 8 micrometers, with an average of 7.5 μm. MCD can be used for measuring the mean corpuscular average thickness (MCAT).

6. The Total Leukocyte Count(TLC)
White Cell Count (WCC)

The white blood corpuscles (WBCs; leukocytes) constitute the major defense system of the body against invasion by bacteria, viruses, fungi, toxins and other foreign invaders. Their number is kept remarkably constant in health, but it increases or decreases in many diseases, particularly acute and chronic infections. A clinician generally wants this test done, along with differential count, Hb, etc. as part of “full blood count” (FBC or complete blood count, CBC) in cases of fever.

Sources and Degrees of Error

The sources of errors are include: pipette error, chamber error, field error, and experimental error. The degree of error, which may be 30% or more in RBC counting, is much less in TLC (about 5–10%) because of the low dilution employed (1 in 20) in this case.

What is the normal total leukocyte count?

The normal count in adults ranges between 4000/mm3 and 11,000/mm3, with an average of 7000/mm3. The count after birth may be as high as 18,000 to 20,000/mm3, the normal levels being reached in a few years. In the adults, about 55 to 75% of the WBCs are granulocytes, while in young children, lymphocytes dominate. The count may be high in some physiological conditions (see below) such as heavy exercise, stress, etc.

Q **What is the difference between differential leukocyte count and absolute leukocyte count?**

In differential leukocyte count (DLC), the percentages of various types of WBCs are determined, while in absolute leukocyte count, the number of different WBCs per mm3 are calculated. (This is done from TLC and DLC).

Q What are the various types of leukocytes **and what are their functions?**

The WBCs, unlike red cells, contain nuclei but no hemoglobin. Depending on the presence or absence of clearly visible and conspicuous, chemical filled granules (vesicles) in their cytoplasm (that are made visible by staining), they are grouped into 2 types: ***granular*** and ***agranular***.

a. Granulocytes.

There are 3 types of granulocytes that can be recognized under the compound microscope according to the coloration of their cytoplasmic granules: ***neutrophils, eosinophils*** (eosin loving) ***and basophils*** (basic loving).

b. Agranulocytes (mononuclear cells).

In contrast to granulocytes whose nuclei are lobed, the nuclei of agranulocytes are not lobed but appear as a single mass. Although the cytoplasm contains chemical filled granules, these are not visible under the light microscope due to their small size and poor staining with the usual dyes. The agranulocytes include: ***monocytes,*** and ***lymphocytes.***

Q What is meant by the terms leukocytosis **and granulocytosis? Name the physiological and pathological conditions which cause leukocytosis.**

Leukocytosis: The term refers to an increase in the number of WBCs beyond 11000/mm3 irrespective of the type of cells (granulocytes, monocytes, lymphocytes, etc.) that are involved in raising TLC.

Leukocytosis is a normal, protective response of the body to various types of stresses, such as infections, severe exercise, surgery, tissue injury, etc.

Physiological Leukocytosis

About 95% of the people have a TLC within the normal range. Physiological leukocytosis (i.e., in the absence of infection or tissue injury) has no clinical significance. There is no decrease or absence of eosinophils (eosinopenia), which is a feature of leukocytosis due to infection. Physiological leukocytosis is due to mobilization of WBCs from the marginal pool or bone marrow reserve ("Shift" leukocytosis).
The count may be as high as 18–20,000/mm3 but it returns to normal level within 1–2 years.

Pathological Leukocytosis

A rise in TLC in disease is seen in:

1. **Acute infection with pyogenic (pus forming) bacteria:** The infection (due to cocci bacteria— streptococcus, staphylococcus) may be:

a. Localized, such as boils, abscess, tonsillitis, appendicitis, etc.

b. Generalized, such as in septicemia and pyemia, bronchitis, pneumonia, peritonitis, meningitis, etc.

2. **Myocardial infarction:** The rise in TLC due to tissue injury is not seen immediately after a heart attack but only after 4–5 days.
3. **Acute hemorrhage:** Maximum response occurs in 8–10 hours, the count returning to normal in 5–6 days.
4. **Burns:** Maximum response occurs in 5–15 hours, the count returning to normal in 2–3 days.
5. **Amebic hepatitis.**
6. **Malignancies:** High counts are seen in half the cases; secondary infection enhances the count.
7. **Surgical operations:** A postoperative rise is seen in all cases.

Q What is leukopenia? Name the physi**ological and pathological conditions causing it.**

Physiological Leukopenia

A decrease in TLC under normal physiological conditions is unusual and rare. Exposure to extreme cold, even under arctic conditions and in spite of acclimitization, may reduce the count to only slightly below the 4000/mm3 level.

Pathological Leukopenia

Leukopenia due to disease, where TLC is abnormally low, is never beneficial to the body. In fact, it may endanger the life of the patient. The condition is almost always due to a decrease in neutrophils (neutropenia) and may be caused by various drugs used in treatment, radiation, or certain infections as described below.

1. **Infection with non-pyogenic organisms:** Typhoid and paratyphoid fevers, and sometimes in protozoal infection like malaria.
2. **Viral infections:** Influenza, mumps, smallpox, AIDS (Acquired immunodeficiency syndrome).
3. **Drugs:** Chloramphenicol, sulphonamides, aspirin, penicillins, cyclosporins, phenytoin, etc. Cytotoxic drugs used in treating malignancies may also cause leukopenis by depressing the bone marrow (other blood cells may also decrease).
4. **Repeated exposures to X-rays and radium:** These are used as radiotherapy in cancers, and cause bone marrow depression.
5. **Chemical poisons that depress bone marrow:** Arsenic, dinitrophenol, antimony and others.
6. **Malnutrition:** Deficiency of vitamin B_{12} and folate, general malnutrition, starvation, extreme weakness and debility.
7. **Hypoplasia and aplasia:** Partial or complete depression of bone marrow, i.e., failure of stem cells, may occur as a result of autoimmunity, and other factors.
8. **Preleukemic stage of leukemias** may show leukopenia.

Q What is leukemia and what are its major types?

Leukemias is a group of malignant (dangerous) neoplasms (new growths) of WBC forming organs— bone marrow and lymphoid tissue. There is an uncontrolled production and release of mature and immature WBCs into the circulation. The leukemias (commonly called blood cancers) may be **myeloid** (usually involving neutrophils) or **lymphatic** (involving lymphocytes), and acute or chronic.

In acute leukemia, there is accumulation of immature cells in the blood. (Acute lymphatic leukemia is the most common malignancy in children while acute myeloid leukemia is common in adults.) Chronic leukemia begins more slowly and may remain undetected for months. Mature cells accumulate in blood, because they do not die at the end of their normal life-span.

In most cases the cause is not known. However, genetic factors, viruses (e.g., human T cell leukemia, lymphoma virus-1; HTLV-1) chemical factors, and ionizing radiations (accidents in atomic power plants,

Q What is the difference between leukocytosis, leukostasis, leukemoid reaction and leukemia?

Leukocytosis is an increase in TLC count above 11,000/mm3, irrespective of the types of cells involved. It may be physiological or pathological. The pathological causes include infection and tissue injury. The count usually does not exceed 20–25,000/mm3 and there are no immature cells in the circulation.

Leukostasis: If the count is more than 100,000/mm3, white cell thrombi may form in the brain, lung, and heart—a condition called leukostasis. Transfusion of blood before TLC is reduced, increases blood viscosity, thus increasing the risk of leukostasis.

Leukemoid reaction: It is an extreme elevation of TLC above 50,000/mm3 as a result of the presence of mature and or immature neutrophils. The causes include: severe chronic infections, especially in children, severe hemolysis, malignant growths (cancer of breast, lung, kidney). It is not leukemia, and can be distinguished from chronic myelogenous leukemia (CML) by estimating the leukocyte alkaline phosphatase (LAP level which is elevated in leukemoid reaction, but depressed in CML.

Leukoerythroblastic reaction is similar to leukemoid reaction but with the addition of nucleated red cells (normoblasts) on blood smear. The causes include: marrow infiltration by malignancy, hypoxia, and severe anemia.

Leukemia: As described above, leukemia is a cancerous growth of blood forming organs (bone marrow or lymphatic tissues). Due to uncontrolled production, both immature and mature WBCs are released into circulation. The TLC is generally above 40–50,000/mm3 or even a few lacs. Even when the count is moderately high, it is not called leukocytosis. Most cells are, however, functionally incompetent.

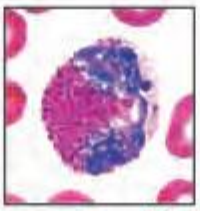
The term aleukemic leukemia is sometimes used for the preleukemic stage when blood picture is normal but the bone marrow study points to leukemia.

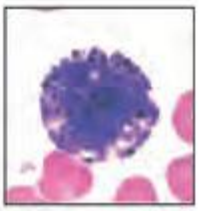
7. **Staining a Peripheral Blood Film .The Differential Leukocyte Count (DLC)**

Many hematological and other disorders can be diagnosed by a careful examination of a stained blood film. A physician may order a differential leukocyte count (always along with TLC) to detect infection or inflammation, determine the effects of possible poisoning by chemicals, drugs, chemotherapy, radiation, etc. DLC is also done to monitor blood diseases like leukemia, or to detect allergic and parasitic infections.

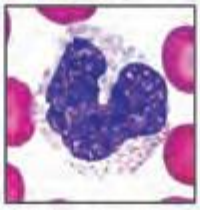
Type	Microscopic appearance	Diagram	Diameter µm	Pevcentage of TLC
Neutrophil			10–14	40–70
Eosinophil			1–6	10–15
Basophil			0–1	10–15
Lymphocyte			20–40 (S) 5–10 (L)	7–9 10–15
Monocyte			5–10	12–20


Neutrophil


Eosinophil


Basophil


Lymphocyte


Monocyte

Erythrocyte Sedimentation Rate (ESR)

The determination of the rate at which the red cells settle or sediment (ESR) is often required by a physician to rule out the presence of organic disease, or to follow the progress of a disease. ESR is generally done as part of complete blood tests.
In the circulating blood the red cells remain uniformly suspended in the plasma. However, when a sample of blood, to which an anticoagulant has been added, is allowed to stand in a narrow vertical tube, the red cells (specific gravity = 1.095) being heavier (denser) than the colloid plasma (specific gravity = 1.032), settle or sediment gradually towards the bottom of the tube. The rate, in mm, at which the red cells sediment, called ESR, is recorded at the end of one hour.

Normal values
Males : 3–9 mm 1st hour
Females : 5–12 mm 1st hour.

What are the factors on which the rate of **sedimentation of red cells depends?**
Rate of settling of red cells depends on:

1. A downward gravitational force acting on the red cells due to their weight (mass) and,
2. An upward force due to viscosity of plasma, and the area of surface of red cells where viscous retardation occurs, i.e. the plasma-red cell interface.

Name the physiological and pathological **variations in ESR.**

Tests for Hemostasis (Bleeding time; Coagulation time; Platelet count; and other tests)-------

Commonly used anticoagulants

Anticoagulants are substances employed to delay, suppress, or prevent clotting of blood. They are classified into 2 groups: the *in vitro* (outside the body) anticoagulants, and the in vivo (in the body) anticoagulants.
A. In vitro Anticoagulants
1. Ethylene Diamine Tetra -acetic Acid (EDTA). EDTA prevents clotting by removing ionic calcium

2. Trisodium Citrate (Na3 C6 H5 O2 . 2 H2O).
Trisodium citrate is the anticoagulant of choice in blood tests for disorders of coagulation. Any substance that deionizes the blood calcium will prevent clotting.
3. Double Oxalate mixture: **Oxalates prevent clotting** by forming insoluble calcium salts, thus removing ionic calcium.
4. Sodium Fluoride.
A mixture of 10 mg of sodium fluoride and 1 mg thymol is an anticoagulant as well as a preservative when a blood sample has to be stored for a few days. Since fluoride inhibits glycolytic enzymes (thus preventing loss of glucose), it is employed when plasma glucose is to be estimated.
5. Heparin. Mode of action and uses. Heparin by itself has no anticoagulant activity. However, when it combines with **antithrombin III**, the ability of the latter to remove thrombin (as soon as it is formed) increases hundreds of times. The complex of these two substances removes many other activated clotting factors- such as IX, X, XI, and XII.
B. In Vivo Anticoagulants and Their Clinical Use
The two in vivo anticoagulants are heparin and coumarins. Patients at increased risk of forming blood clots in their blood vessels, e.g. leg veins during prolonged confinement to bed, or during long flights, are sometimes put on these drugs (e.g. warfarin) to prevent thromboembolism. Their BT, CT, and PT are checked from time to time to adjust the dosage of the drug.
1. Dicoumarol and warfarin. The coumarin derivatives are vitamin K antagonists and thus inhibit the action of this vitamin that is essential as a cofactor for the synthesis of six glutamic acid- containing proteins—namely, factors II (prothrombin), VII, IX, and X, protein C, and protein S. The action of this anticoagulant is, however, slower than that of heparin.

2. Heparin is particularly used during open-heart surgery in which the blood has to be passed through a heart-lung machine; or the dialysis machine during hemodialysis in kidney failure, and then back into the patient.

For a Sample of Whole Blood or Plasma. (Plasma = Blood minus all the blood cells). Draw blood from a vein as described below and transfer it from the syringe to a container containing a suitable anticoagulant. Mix

the contents well without frothing. A sample of whole blood is now ready for tests.

If plasma is desired, centrifuge the anticoagulated blood for 20–30 minutes at 2500 rpm, as described later. Collect the supernatant plasma with a pipette and transfer it to another container. (The packed RBCs will be left behind).

For a Sample of Serum. (Serum = Plasma minus fibrinogen and all the clotting factors). Transfer the blood from the syringe to a container *without any anticoagulant* in it, and keep it undisturbed. After the blood has clotted in an hour or two and the clot shrunk in size, the serum will be expressed. Remove the supernatant serum with a pipette and transfer it to a centrifuge tube. Centrifuge it to remove whatever red cells may be present. Clear serum can now be collected with another pipette.