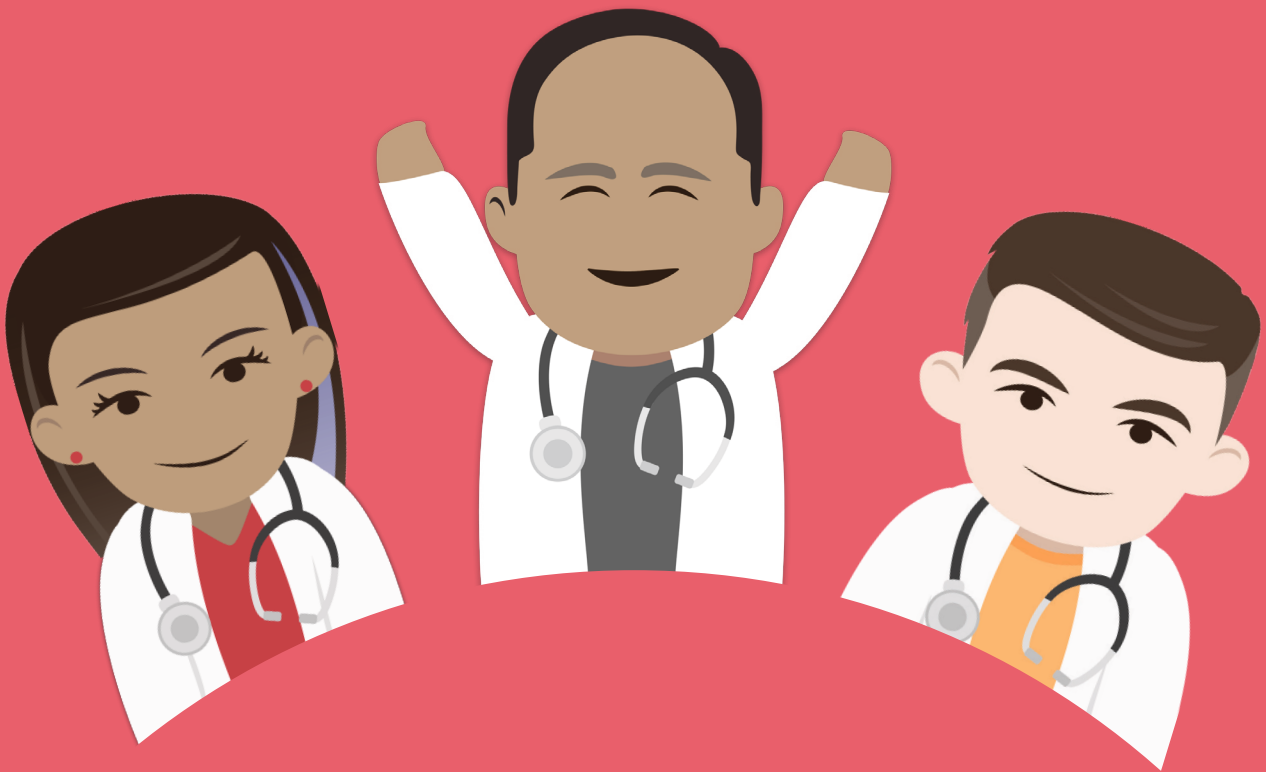




Hematology and Coagulation Essentials
Chapter 1

ANALYZING BLOOD SAMPLES



Amer Wahed

Analyzing blood samples

INTERPRETING COMPLETE BLOOD COUNT (CBC) RESULTS

A set of CBC values contains data related to red blood cells (RBCs), white blood cells (WBCs), and platelets.

RBC values

Hemoglobin level

- The amount of hemoglobin in a specific volume of blood
- Generally measured in g / dL

RBC count

- The number of RBCs in a given volume of blood
- Generally measured in cells / μ L

Hematocrit (Hct)

- The percentage of blood by volume represented by RBCs
- Measured as a percentage (vol%)

Mean corpuscular volume (MCV)

- The average RBC size

Mean corpuscular hemoglobin (MCH)

- The average amount of hemoglobin in each red cell

Mean corpuscular hemoglobin concentration (MCHC)

- The average concentration of hemoglobin in each red cell

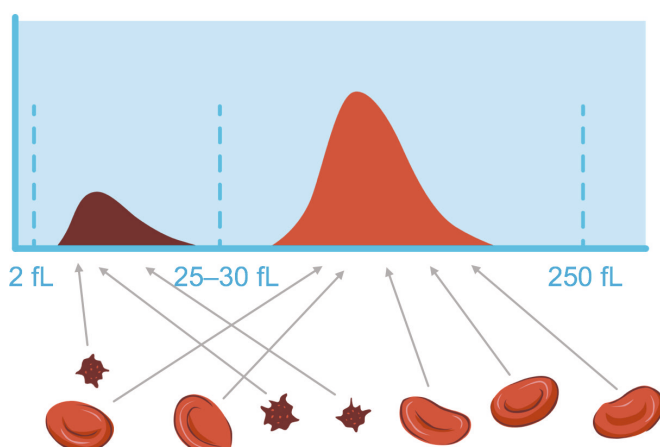
Red cell differential width (RDW)

- A measure of degree of variation in size of red cells or anisocytosis

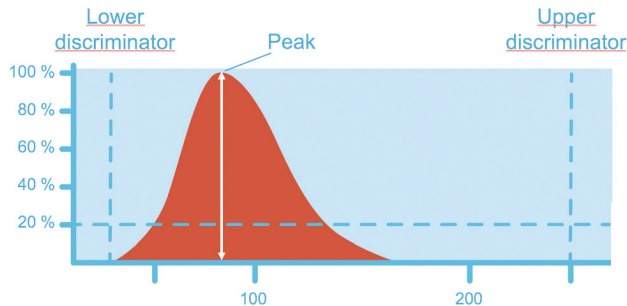
Hemoglobin, RBC count, MCV, and RDW are measured values, while hematocrit, MCH, and MCHC are calculated.

Measured values

The hemoglobin concentration is obtained after the red cells are lysed. The hemoglobin (along with methemoglobin and carboxyhemoglobin) are all converted to cyanmethemoglobin and light absorbance, at 540 nm, is measured. Smokers have high carboxyhemoglobin and thus, a falsely high hemoglobin level.



Red cells and platelets are counted using the same method, which is based on cell size. Cells which are smaller are designated as platelets and cells which are larger are designated as red cells. The analyzer is able to produce histograms representing platelets and red cells.



The MCV and mean platelet volume (MPV) values are obtained by drawing a vertical line from the peak of the histogram to the baseline. The RDW is obtained at 20% of the red cell population from the histogram.

Calculated values

RBC values

Hematocrit = MCV x RBC count

MCH = hemoglobin value / RBC count

MCHC = hemoglobin value / hematocrit

Platelet values

Platelet count

The number of platelets in a given volume of blood

Mean platelet volume (MPV)

The average platelet size

Sources of error

RBC agglutination

A clump of red cells will be counted as one red cell, thus lowering the red cell count. When the red cells are lysed the hemoglobin value will be accurate. Thus, there will be a discrepancy between the RBC count and the hemoglobin (Hb) value.

Sources of error

Platelet agglutination

Formation of platelet clumps will also artificially lower the platelet count.

Large platelets

Large platelets will be falsely counted as red cells. If there are too many large platelets, the platelet count may be artificially low.

Schistocytes

Conversely, fragmented red cells known as schistocytes may be counted as platelets, artificially raising the platelet count.

WBC values

WBC count

The number of WBCs in a given volume of blood

WBC differential

Percentage of total WBCs that are represented by each type of WBC (neutrophils, eosinophils, basophils, lymphocytes, and monocytes)

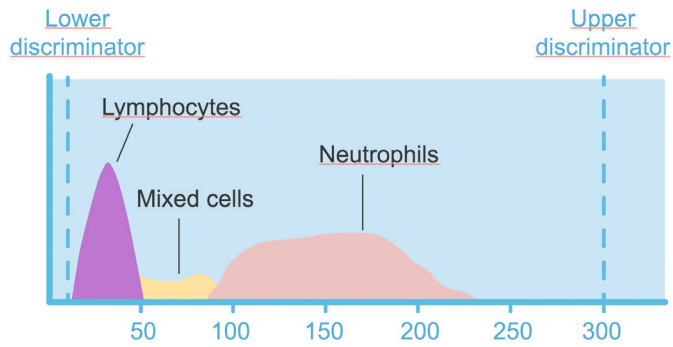
Individual WBC counts

Absolute numbers of each individual type of WBC in a given volume of blood

Sources of error

Nucleated red cells (NRBCs)

NRBCs may be counted as lymphocytes, thus artificially increasing the WBC count. An accurate WBC count can be obtained by repeating the count with the analyzer in the NRBC mode, where the red cells are lysed. This is referred to as the corrected WBC count.



The WBC histogram has three parts. The first peak is due to small cells, which represent lymphocytes. The third area represents neutrophils. All other WBCs are represented in the second area.

Reticulocyte count

Normally a reticulocyte count is not part of a CBC test, and therefore must be ordered specifically. It is measured as a percentage. To interpret the reticulocyte count it is important to take into account the patient's Hct to see whether the production of reticulocytes is appropriate for the degree of anemia. This is called corrected reticulocyte count. The formula used is reticulocyte count, multiplied by the patient's Hct, divided by normal Hct, which is 45%.

Analyzing blood samples

ORDERING A BONE MARROW EXAM

A bone marrow study should always include an analysis of the peripheral blood. This is done by examining the CBC values as well as reviewing the peripheral blood smear.

A typical bone marrow procedure has two components

- Aspirate
- Trephine biopsy

Aspirate

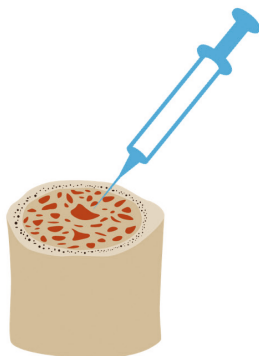
A needle is introduced into an appropriate area of bone and a syringe is used to aspirate a small amount of blood, which contains bone marrow particles. A drop or two of blood is used to make slides, which are stained and reviewed under the microscope to look at the various cell types. We call this the assessment of morphology.

The remaining portion of the blood is allowed to clot. The clot is then fixed in formalin and made into slides. This is called the clot section.

The clot section provides similar information to the biopsy. If the biopsy does not yield good results, then we can use the clot section slide as a substitute.

Just like the biopsy slides, we can perform immunohistochemistry (IHC) on the clot section.

Sometimes attempts to obtain an aspirate do not yield anything. This is referred to as a dry tap. Naturally, faulty technique is a common cause of dry tap. Other causes include packed marrow as seen in leukemias, fibrotic marrow as seen in myelofibrosis, and in hairy cell leukemia.



Trephine biopsy

After the aspirate, the needle is withdrawn and a second needle is introduced into the bone. The purpose is to obtain a small core biopsy. The small piece of bone is rolled onto a slide. Cells which are present on the surface of the bone will be transferred onto the slide. This is called a touch prep. The biopsy is then fixed in formalin and subsequent slides are made.

The biopsy is used to assess cellularity of the bone marrow. The overall architecture is also assessed. Evidence for any abnormal infiltrate (such as granulomas, lymphoma, metastases, fibrosis) are looked for. The megakaryocyte population is also assessed from the biopsy slide. Immunohistochemistry (IHC) can be performed on these slides.

Additional tests

In addition to morphological assessment, a number of laboratory tests can be used to examine bone marrow aspirate and / or samples, as well as peripheral blood samples.

Flow cytometry is a form of immunophenotyping that can identify various cell types based on the presence of specific cell-surface antigens. Flow cytometry is especially valuable for diagnosing most leukemias and lymphomas.

Polymerase chain reaction (PCR) can be performed on blood or bone marrow samples to test for the presence of specific genes that are characteristic of certain types of hematological disorders or malignancies.

Fluorescence in situ hybridization (FISH) is a cytogenetic test that can be used to detect specific gene sequences within a chromosome.

Cultures can also be performed from bone marrow aspirate.

When to order a bone marrow exam

A bone marrow exam is invaluable for diagnosing leukemias and for staging, but not for diagnosing lymphomas. This is important. If a patient has a lymphoma the bone marrow may or may not be involved. So, a bone marrow study is appropriate for staging but does not aid in the initial diagnosis. Since we assess over all cellularity from the biopsy, we can identify cases where the bone marrow cellularity is low, indicating hypoplastic anemia, or when it is significantly low, as in aplastic anemias.

Since we assess morphology of red cells, white cells, and megakaryocytes, we can detect disorders involving these cell types. In myelodysplastic syndrome the morphology of cells in the bone marrow is typically abnormal and the bone marrow is usually hypercellular.