

PEARLS OF LABORATORY MEDICINE

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TITLE: Complete Blood Count (CBC) Basics

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Slide 1:

Good afternoon, my name is Dr. Michael Linden. I am the Director of Hematopathology at the University of Minnesota in Minneapolis, MN. With me is Dr. Sarah Drawz, who is a Hematopathology Fellow, also at the University of Minnesota.

I would like to introduce Dr. Sarah Drawz, who will be beginning our presentation as part of this AACC Pearl of Laboratory Medicine on “Complete Blood Count (CBC) Basics.”

Slide 2:

The complete blood count (CBC) is an important initial test in the evaluation of a patient’s hematologic function. The CBC is performed on whole blood, which is composed of two primary components: plasma and cells. The plasma fraction contains mostly water, numerous proteins, electrolytes, and clotting factors. The cellular component of blood is comprised of the three major categories of blood cells: red blood cells (RBCs), white blood cells (WBCs), and platelets. The CBC test yields numbers of these main types of cells, but also additional information, such as the volume of red blood cells, or hematocrit, and quantity of different types of white blood cells, the differential.

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CBCs are collected from patients in both inpatient and outpatient settings. Generally, 3 to 10 ml of whole blood are drawn from a peripheral vein directly into a tube. The tube must contain anticoagulant to prevent clots from obscuring the CBC analysis. Various types of anticoagulants are used including ethylenediaminetetraacetic acid (EDTA), heparin, and citrate. The most common anticoagulant used for the CBC is EDTA. These tubes are often described as “purple top” tubes as the cap is colored for easy identification.

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An accurate cell count is dependent on many factors. One of the most essential is dilution of the sample to achieve an even distribution of all cell types. Different cell types require different diluent solutions. For example, when the red blood cell count of the CBC is performed, an isotonic solution preserves red cell integrity. In contrast, for white blood cell and platelet counts, a lysis diluent is used to remove the numerous red blood cells.

CBCs can be performed either by manual counts or, more commonly, by automated analyzers.

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Manual counts are rarely used anymore for absolute counts, in large part due to imprecision, but also because of the technical time required. However, manual counts are still a useful tool for very low WBC or platelets counts and differentials for abnormal cell populations.

Hemocytometers are specially designed counting chambers of known area and volume - two views of this device are shown in the figure. The blood sample is diluted in the appropriate fluid and added to the counting chamber. Counting the cells with a microscope then allows for a calculation of concentrations.

Slide 6

Automated analyzers are by far the most common CBC counting method and offer improved speed and accuracy over manual counts. A variety of analyzers are available that differ by workload volume, testing capabilities, and processing technology. Modern instruments use cell counting technologies including electrical impedance, optical flow cytometry, and cytochemical staining. Electrical impedance-based analyzers, shown in the schematic figure, detect changes in voltage resistance when cells pass through an aperture disrupting a flow current. Electrical impedance and flow cytometry are the most commonly used technologies in large, high volume laboratories.

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Flow cytometric-based analyzers feed cells, in a single file stream, through a chamber that interacts with a laser light beam. The angle at which the cell scatters the light generates signals yielding information about cell size, structure, and granularity.

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We will next review the specific RBC, WBC, and platelet parameters included in a standard automated CBC.

RBCs are the most numerous cellular component and are responsible for oxygen delivery achieved by binding to the protein hemoglobin. RBCs are non-nucleated when mature. WBCs are primarily responsible for immune function and are subclassified into specific types typically enumerated in a CBC. Platelets help form clots and are actually cellular fragments.

Examples of each of these major cell types are shown in the pictures of stained blood smears.

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Before we cover the analysis and interpretation for each of these CBC cellular components in detail, it is important to discuss management of error on automated instruments. Accurate interpretation of any lab value requires keeping in mind the common sources of error and these will be mentioned in the following slides. Minimizing these errors is an extremely important step in laboratory automation. Modern automated CBC analyzers are programmed to communicate data to computers for analysis, monitoring, display, and quality control programs. These computers can run algorithms to identify data that do not meet preset criteria and create possible error “flags” to alert operators prior to release of results, allowing for intervention.

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There are three quantitative values included in the RBC parameters of a CBC. These are hemoglobin, hematocrit, and RBC count. You can see the specific units listed and we will define each of these on the next slide. The qualitative RBC measurements are calculated averages and include the mean corpuscular volume, hemoglobin and hemoglobin concentration, as well as the red cell distribution width. Depending on the type of analyzer, a count of the reticulocytes, or immature red blood cells, will sometimes be included in the RBC parameters.

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Hemoglobin is expressed in a concentration of grams per deciliter. Because hemoglobin is a colored protein, it can be measured by spectrophotometry, and specifically, it is determined by the absorbance of cyanhemoglobin at 540 nanometers. This parameter gives an estimate of the total oxygen-carrying capacity of the blood.

The hematocrit is the proportion, or percent, of the blood volume that is occupied by RBCs. Manual measurement involves centrifugation of whole blood at a standardized time and speed. Hematocrit is then calculated by dividing the height of the red blood cells by the total blood volume. A picture of a capillary tube is shown.

Manual methods are rarely performed anymore but do provide relatively simple and accurate measurements. More commonly, automated analyzers determine hematocrit by directly measuring the red cell number divided by the red cell volume. These automated hematocrit values closely reflect manual measurements.

Automated analyzers determine red blood cell counts via electrical impedance and/or light scatter. RBCs and WBCs are counted together, and because RBCs so far outnumber WBCs, the error from including WBCs in the count is negligible. This can, however, lead to inaccurate RBC counts when a large number of WBCs are present.

Slide 12:

We now move to the qualitative, or calculated average, parameters. Mean corpuscular volume (MCV) reflects the average volume of each red blood cell and is expressed in femtoliters. Automated analyzers determine MCV by dividing the total red cell volumes by the RBC count.

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Mean corpuscular hemoglobin (MCH) reflects the average hemoglobin mass of each RBC and is calculated in picograms by dividing the hemoglobin by the red cell count. Mean corpuscular hemoglobin concentration (MCHC) is similar to MCH but gives the average hemoglobin concentration by dividing the hemoglobin by the hematocrit. This value, expressed in grams of hemoglobin per deciliter, is useful for determining the volume of an RBC taken up by hemoglobin and thus, includes information on density.

When interpreting the MCV, MCH, and MCHC, it is important to remember that these are average calculated values. Thus, if red cells of significantly different size or hemoglobin content are present, these parameters may not accurately reflect the mixed population. In such cases, manual review of smears and careful examination of additional size indices is essential.

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Red cell distribution width (RDW) is a useful tool for determining red cell size heterogeneity, as it describes the range of cell sizes in a percent. Reticulocytes are immature red blood cells and their presence and quantity reflects the bone marrow's ability to generate new red blood cells. These young RBCs are often larger than mature RBCs, as shown in the picture. While reticulocytes are anucleate, they still contain RNA, and thus, can be distinguished from mature RBCs using RNA-specific stains. Manual counts often use methylene blue-based "supravital" stains while automated analyzers employ fluorescent dyes.

Slide 14:

Integration of the RBC parameters provides useful information on the hematologic status of the patient. For example, a decrease in the hemoglobin, hematocrit, or RBC count reflects an anemia, illustrated in the picture where red blood cells are decreased in number and pale in color, reflecting low hemoglobin content. Anemia can have many etiologies, including iron deficiency. Iron is essential to the composition of hemoglobin and when a patient does not have access to adequate iron stores, a primary anemia can develop. Acute blood loss can lead to anemia of a secondary cause.

On the opposite end of the spectrum, an increase in hemoglobin, hematocrit, or RBC count indicates a polycythemia. A primary cause of polycythemia is a proliferative disease of the bone marrow, which synthesizes all the cells of the hematopoietic system. Patients may also develop a secondary cause of polycythemia as compensation for low oxygen states, such as those resulting from tobacco smoking, sleep apnea, or living at high altitudes.

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This slide lists just some examples of error in the RBC parameters, primarily those encountered on automated analyzers. For the total RBC count, we already discussed how a high WBC count could falsely increase the RBC count as the red and white cells are counted together because of the normally minimal count contribution from white cells. RBC counts can be falsely decreased if the red cells are lysed as part of, or following, the collection process. An example of a non-hemolyzed and a hemolyzed blood specimen is shown. Even grossly, one can appreciate that the color properties change significantly with RBC lysis. In addition to hemolysis, clotting of the sample after collection can lead to inaccurately low RBC counts.

Hematocrit can be erroneously increased by the presence of giant platelets, as these can be falsely counted as red cells. A giant platelet is pictured. In contrast, hematocrit can be falsely decreased by the same processes that falsely decrease the total RBC count.

The MCV shows false elevation when cells are clumped, whether this is due to inadequate anticoagulant in collection tubes or reflective of a true physiologic *in vivo* cell clumping. Giant platelets can lead to falsely decreased MCVs as these platelets would again be counted as RBCs, but their relatively smaller size would bring down the average cell volume calculation.

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Next, we move to a discussion of WBC parameters, of which a standard CBC includes just two: the quantitative cell count and the differential count.

Slide 17:

White blood cell counts require diluting a sample aliquot in buffers that lyse red blood cells, typically containing acids or detergents. Lysis leaves behind the nucleated white blood cells, also called leukocytes. Manual counts are performed with the hemocytometer. Analyzers most often obtain counts using flow cytometry or electrical impedance. The total WBC count is expressed as cells per microliter.

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A differential count, expressed in cells per microliter or as a percent, is the absolute or relative number, respectively, of each of the major leukocyte cell types. This slide shows a picture of each of these leukocytes, including immature neutrophils, also called “bands,” a mature neutrophil, basophil, eosinophil, lymphocyte, and monocyte. The pictures illustrate some of the unique features of the cells, such as cytoplasmic granularity, nucleus density, and shape.

Slide 19:

Automated analyzers use these cell features to determine differential counts. Most instruments use flow cytometry. Cells in suspension are passed, single file, through an optical flow cell where each interacts with a laser beam. Light is deflected differentially depending on cell size and complexity, or granularity. These signals can be plotted on a scattergram, as shown in the figure. Cell types are then assigned based on the expected profile. The colored circled populations on the pictured scattergram each represent a leukocyte type. For example, the neutrophil population, colored green, is found at intermediate forward scatter, reflecting size larger than most lymphocytes but typically smaller than monocytes, and far side scatter because of the complexity of the cytoplasmic granules. Monocytes are colored red and show large forward scatter with less side scatter than the neutrophil population.

Slide 20:

While analyzers are time- and cost-effective and generally quite accurate, instruments are not very good at identifying and typing abnormal or immature cells. These unclassifiable cell populations will be flagged for manual review of a blood film.

Blood films are prepared by placing a drop of blood onto a glass slide and smearing it evenly, usually by the edge of a second slide. Aniline dyes are used to stain the blood. Structures such as nuclei are stained by basic dyes, while other structures, including some granules, are stained by the acidic dyes. These dyes appear basophilic or blue and eosinophilic or pink-red, respectively. Adequate staining is essential to proper identification of different leukocytes.

Slide 21:

As with the RBC parameters, much clinical information can be gleaned from a patient's WBC count and differential. Peripheral blood with a decreased total WBC count, or leukopenia, may reflect a primary production process such as HIV, or bone marrow disease such as aplastic anemia. A relatively common secondary cause of leukopenia is the use of immunosuppressant drugs.

Increased total WBC counts, or leukocytosis, may be seen in association with primary causes such as acute leukemia. A secondary increase in neutrophils and/or lymphocytes, called neutrophilia and lymphocytosis, respectively, is often the result of a bacterial or viral infection. A neutrophilia is shown in the picture.

Slide 22:

Error can affect both the total WBC count as well as the differential count. For example, a falsely increased total WBC count may result from large number of nucleated immature RBCs being erroneously counted as WBCs by the automated analyzer. The top picture shows a nucleated RBC in the center of the field, illustrating the increase in size over anucleate mature RBCs. Additionally, a rise in certain types of plasma antibodies can lead to RBC clumping, and miscounting of these clumps as WBCs. An example of a smear showing RBC agglutination is shown in the lower picture.

Most errors in an automated differential count occur when the instrument is unable to identify correctly abnormal leukocyte populations. Similarly, manual counts may also be subject to cell identification errors, particularly if smear staining is poor. Another source of error encountered is the preferential distribution of large cells at the edge of glass slides, where they can be easily missed during a manual count.

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The final major cellular component in the CBC is the platelet count. Platelets are typically counted in a similar manner to total counts of RBCs, on an automated instrument by either electrical impedance or light scatter and manually with a hemocytometer.

Slide 24:

A decreased platelet count is called thrombocytopenia, and is commonly caused primarily by decreased bone marrow production. Secondary causes of thrombocytopenia include the destruction of circulating platelets by an immune process or sequestration of platelets, often in the spleen.

An elevated platelet count, or thrombocytosis, can also be caused primarily by a bone marrow process, although proliferative in this case. Increased platelets may also result from an acute response to physiologic stress, such as infection or inflammation.

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Platelet counts are subject to error when automated instruments are unable to differentiate between abnormal forms of both platelets and RBCs. A falsely increased platelet count may occur if a significant population of RBCs are small, called microcytes. Microcytes are shown in the picture on the left, and may be miscounted as platelets. Post-collection hemolysis of the blood sample may also lead to erroneously elevated counts as RBC fragments are mistaken for platelets.

False decreases in platelet counts result from both platelet clumping, often the result of insufficient anticoagulation in a collection tube, or so-called “giant platelets.” These larger than average platelets, shown circled in the picture on the right, can approximate the size of RBCs and thus, are counted in the RBC count.

Slide 26:

On this last slide, we review some of the more important CBC principles. The CBC is a valuable diagnostic tool and contains relevant clinical information on the patient. In today’s hematology laboratories, the vast majority of CBCs are run on automated analyzers which operate on principles of flow cytometry and electrical impedance. Manual smear examination, however, still holds an important place in the complete evaluation of a CBC, particularly in patients with abnormal circulating cells. Finally, as with any laboratory test, appreciation and awareness of the sources of error are essential to accurate interpretation of the CBC.

Slide 27: References

Slide 28: Disclosures

Slide 29: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on “Complete Blood Count (CBC) Basics.”