Blood Cells and the CBC

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This is a document in a five-part series on blood cells and anemia:

1. Blood cells and the CBC

2. Anemia: Pathophysiologic Consequences, Classification, and Clinical Investigation

3. Nutritional Anemias and Anemia of Chronic Disease

4. Hemolytic Anemias

5. Hemoglobinopathies and Thalassemias

Introduction

Hematopathology is not only the study of disease of the blood and bone marrow, but also of the organs and tissues which employ blood cells as principal effectors of their physiologic functions. Such would include the lymph nodes, spleen, thymus, and the many foci of lymphoid tissue found along the aerodigestive tract. Generally two types of medical subspecialists intensively practice in this area, the **hematologist** and the **hematopathologist**. The hematologist usually is a Board-certified internist who has completed additional years of training in hematology, usually as part of a combined fellowship in hematology and oncology. The thrust of this individual's work is toward the diagnosis and medical management of patients with hematologic disease, especially neoplasms, and medical management of other nonhematologic cancer. The hematopathologist, on the other hand, is usually Board-certified in anatomic and clinical pathology and has taken additional years of training in hematopathology. His or her principal activity is the morphologic diagnosis of conditions of the hematopoietic and lymphocyte-rich tissues and in the performance of laboratory testing that assists such diagnosis.

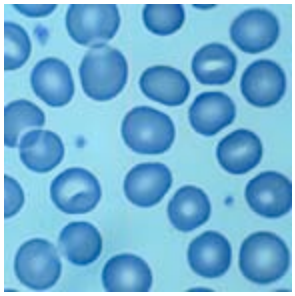
Hematopathology is somewhat unique in its approach to the patient and the disease, in that 1) many diseases are understood at the molecular level, 2) the patient's tissue is easily obtainable in large quantities (in the case of peripheral blood, at least) and easily kept viable for special studies, and 3) the function of the blood (or at least the erythroid component) is relatively simple when compared to that of other organ systems. Because it is a scientifically integrated discipline hematology/hematopathology is an area which is intellectually gratifying to the eclectic individual who is well-rounded in various biomedical endeavors, including biochemistry, physiology, pharmacology, microanatomy, morphologic diagnosis, and patient care.

The Blood

A few nights working in a trauma center would tend to convince one that the body is just a huge bag of blood. In fact, an "average" 70 liter human body contains only about 5 liters of blood, or 7% by volume. In the normal state, blood has no business anywhere except in the confines of the heart and blood vessels and in the sinusoids of the marrow, liver, and spleen. Of the average 5 L of blood, only 2.25 L, or 45%, consists of cells. The rest is plasma, which itself consists of 93% water (by weight) and 7%

solids (mostly proteins, the greatest proportion of which is albumin). Of the 2.25 L of cells, only 0.037 L (1.6%) are leukocytes. The entire circulating leukocyte population, if purified, would fit in a bartender's jigger. The total circulating platelet volume is even less -- about 0.0065 L -- or a little over one teaspoonful.

Erythrocytes



Structurally the simplest cell in the body, volumes have been written about the lowly red blood cell. The basic function of the rbc is the creation and maintenance of an environment salutary to the physical integrity and functionality of hemoglobin. In the normal state, erythrocytes are produced only in the skeleton (in adults only in the axial skeleton), but in pathologic states (especially myelofibrosis, which will be covered subsequently) almost any organ can become the site of erythropoiesis. Numerous substances are necessary for creation of erythrocytes, including metals (iron, cobalt, manganese), vitamins (B_{12}, B_6, C, E, folate, riboflavin, pantothenic acid, thiamin), and amino acids. Regulatory substances necessary for normal erythropoiesis include erythropoietin, thyroid hormones, and androgens. Erythrocytes progress from blast precursors in the marrow over a period of five days. Then they are released into the blood as reticulocytes, distinguishable from regular erythrocytes only with special supravital stains. The reticulocyte changes to an erythrocyte in one day and circulates for 120 days before being destroyed in the reticuloendothelial system.

Clinical laboratories measure several important parameters that reflect rbc structure and function. These measurements are used to 1) evaluate the adequacy of oxygen delivery to the tissues, at least as is related to hematologic (as opposed to cardiopulmonary) factors, and 2) detect abnormalities in rbc size and shape that may provide clues to the diagnosis of a variety of hematologic conditions. Most of these tests are performed using automated equipment to analyze a simple venipuncture sample collected in a universal lavender- (or purple-) top tube containing EDTA as an anticoagulant. Let us consider each of these tests.

- **A. Hemoglobin concentration in whole blood**

  Referred to simply as "hemoglobin," this test involves lysing the erythrocytes, thus producing an evenly distributed solution of hemoglobin in the sample. The hemoglobin is chemically converted mole-for-mole to the more stable and easily measured cyanmethemoglobin, which is a colored compound that can be measured colorimetrically, its concentration being calculated from its amount of light absorption using Beer's Law. The normal range for hemoglobin is highly age- and sex-dependent, with men having higher values than women, and adults having higher values than children (except neonates, which have the highest values of all). For a typical clinical lab, the young adult female normal range is 12 - 16 g/dL; for adult males it is 14 - 18 g/dL.

  This is an easy test to perform, as hemoglobin is present in the blood in higher concentration than that of any other measured substance in laboratory medicine. The result is traditionally expressed as unit mass per volume, specifically grams per deciliter (g/dL). Ideologues in lab medicine have been maintaining for years that this unit will be replaced by Système Internationale (SI) units of moles per liter, but this has not gained any significant acceptance in clinical medicine except in the most nerdly circles.

- **B. Erythrocyte count**

  Also referred to as just "rbc," this simply involves counting the number of rbcs per unit volume of whole blood. Manual methods using the hated hemocytometer have been universally replaced by automated counting. The major source of error in the rbc count is an artificially reduced result that occurs in some conditions where rbcs stick together in the sample tube, with two or more

cells being counted as one. The result of the test is expressed as number of cells per unit volume, specifically cells/μL. A typical lab's normal range is 4.2 - 5.4 x 10^6/μL for females; for adult males it is 4.7 - 6.1 x 10^6 /μL.

- **C. Hematocrit**

  This is also called the packed cell volume or PCV. It is a measure of the total volume of the erythrocytes relative to the total volume of whole blood in a sample. The result is expressed as a proportion, either unitless (e.g., 0.42) or with volume units (e.g., 0.42 L/L, or 42 cL/L [centiliters/liter]). An archaic way of expressing hematocrit is "volumes per cent" or just "percent" (42%, in the above illustration). Small office labs and stat labs measure hematocrit simply by spinning down a whole blood sample in a capillary tube and measuring the length of the column of rbcs relative to the length of the column of the whole specimen. Larger labs use automated methods that actually measure the volume individually of each of thousands of red cells in a measured volume of whole blood and add them up. The volume of individual erythrocytes can be electronically determined by measurement of their electrical impedance or their light-scattering properties. The normal range is 0.37 - 0.47 L/L for females, and 0.42 - 0.52 L/L for males.

- **D. Erythrocyte indices**

  The three cardinal rbc measurements described above (hemoglobin, hematocrit, and rbc count) are used to arithmetically derive the erythrocyte indices - mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration. As much as we all hate memorization, it is important to know how to calculate these indices and have some idea of the normal ranges. We will consider these individually.

  - **1. Mean corpuscular volume (MCV)**

    This is the mean volume of all the erythrocytes counted in the sample. The value is expressed in volume units, in this case very small ones - femtoliters (fL, 10-15 liter). The normal range is 80 - 94 fL. The formula for the calculation in general terms is

    MCV = hematocrit ÷ rbc count

    When using specific units, decimal fudge factors are required; for example,

    MCV (in fL) = (hematocrit [in L/L] x 1000) ÷ (rbc count [in millions/μL])

    I think that it is easier to forget the fudge factors, use the first formula, multiply out the values while ignoring the bothersome decimal, and reposition the decimal in the final result so as to approximate the order of magnitude of the normal range. This is safe, since you will not see an MCV of 8 fL, or one of 800 fL.

    When the MCV is low, the blood is said to be (strong>microcytic<, when high, **macrocytic**. **Normocytic** refers to blood with a normal MCV. Keep in mind that the MCV measures only average cell volume. The MCV can be normal while the individual red cells of the population vary wildly in volume from one to the next. Such an abnormal variation in cell volume is called **anisocytosis**. Some machines can measure the degree of anisocytosis by use of a parameter called the **red cell distribution width (RDW)**. This is simply a standardized parameter (similar to the standard deviation) for mathematically expressing magnitude of dispersion of a population about a mean. The normal range for RDW is 11.5 - 14.5 %.

  - **2. Mean corpuscular hemoglobin (MCH)**

The MCH represents the mean mass of hemoglobin in the RBC and is expressed in the mass unit, picograms (pg, 10^{-12} gram). The value is determined by the formula,

$$\text{MCH (in pg)} = (\text{hemoglobin [in g/dL]} \times 10 \div (\text{rbc count [in millions/}\mu\text{L]})$$

Again, a fudge factor is required in this equation, so it helps to get some feel for the normal range (27 - 31 pg) and gestalt the decimal point, as described for MCV, above. Since small cells have less hemoglobin than large cells, variation in the MCH tends to track along with that of the MCV. The MCH is something of a minor leaguer among the indices in that it adds little information independent of the MCV.

- **3. Mean corpuscular hemoglobin concentration (MCHC)**

  This is the mean concentration of hemoglobin in the red cell. Since whole blood is about one-half cells by volume, and all of the hemoglobin is confined to the cells, you would correctly expect the MCHC to be roughly twice the value for hemoglobin in whole blood and to be expressed in the same units; the normal range is 32 - 36 g/dL. The value is calculated using the formula,

$$\text{MCHC [in g/dL]} = \text{hemoglobin [in g/dL]} \div \text{hematocrit [in L/L]}$$

  Cells with normal, high, and low MCHC are referred to as **normochromic, hyperchromic, and hypochromic**, respectively. Again, these terms will have importance in anemia classification.

Further reading on red cell disease
Anemia: Pathophysiologic Consequences, Classification, and Clinical Investigation is an introduction to anemia **Nutritional Anemias and Anemia of Chronic Disease** deals with anemias caused by iron, folate, and vitamin B_{12} deficiencies. **Hemolytic Anemias** is concerned with anemias caused by red cells being destroyed faster than a healthy marrow can replace them. **Hemoglobinopathies and Thalassemias** covers sickle cell disease, hemoglobins C and E, and alpha- and beta-thalassemias. **Understanding Anemia**, my first book, is now available in hardback and paper. The publisher has kindly allowed me to post the full text of Chapter 1 online. You can access it through the book outline at this link. There is also a link to buy the book from online bookstores at a substantial discount. This book is aimed at general readers and presumes a knowledge of biology at the high school level, then builds from there.

Leukocytes and the leukocyte differential count

To consider the leukocytes together as a group is something of a granfalloon, because each type of leukocyte has its own function and ontogeny semi-independent of the others. To measure the total leukocyte count and allow this term to mean anything to the doctor is a travesty, yet the "wbc" count has traditionally been considered a cardinal measurement in a routine laboratory workup for just about any condition. I cannot emphasize too much that to evaluate critically the hematologic status of a patient, one must consider the individual absolute counts of each of the leukocyte types rather than the total wbc count. For such a critical evaluation, the first step is to order a wbc count with differential. In many labs,

the result will be reported as a relative differential, something like this:

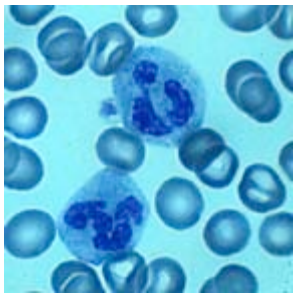
WBC	6000/μL
segmented neutrophils	60%
band neutrophils	2%
lymphocytes	25%
monocytes	8%
eosinophils	3%
basophils	2%

Your first task is to multiply the wbc count by each of the percentages given for the cell types; this gives you an **absolute differential**. Now you're in business to get some idea as to the pathophysiologic status of the patient's blood and marrow. Thus, the illustration above becomes:

WBC	6000/μL
segmented neutrophils	3600/μL
band neutrophils	120/μL
lymphocytes	1500/μL
monocytes	480/μL
eosinophils	180/μL
basophils	120/μL

The total wbc count is invariably done using an automated method. Routinely, the differential count is done "by hand" (i.e., through the microscope) in smaller labs, and by automated methods in larger facilities. The automated methods are amazingly accurate, considering the fine distinctions that must often be made in discerning one type of leukocyte from the other. One manufacturer's machine can quite reliably pick out one leukemic blast cell in eight hundred or more leukocytes. Now we shall consider each of the leukocyte types individually.

- **A. Neutrophils**



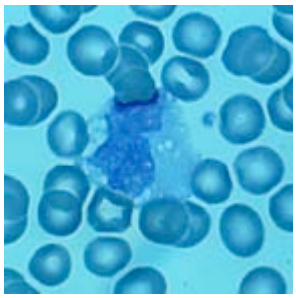
The most populous of the circulating white cells, they are also the most short lived in circulation. After production and release by the marrow, they only circulate for about eight hours before proceeding to the tissues (via diapedesis), where they live for about a week, if all goes well. They are produced as a response to acute body stress, whether from infection, infarction, trauma, emotional distress, or other noxious stimuli. When called to a site of injury, they phagocytose invaders and other undesirable substances and usually kill themselves in the act of doing in the bad guys.

Normally, the circulating neutrophil series consists only of band neutrophils and segmented neutrophils, the latter being the most mature type. In stress situations (i.e., the "acute phase reaction"), earlier forms (usually no earlier than myelocytes) can be seen in the blood. This picture is called a "left shift." The band count has been used as an indicator of acute stress. In practice, band counts tend to be less than reliable due to tremendous interobserver variability, even among seasoned medical technologists, in discriminating bands from segs by microscopy. Other morphologic clues to acute stress may be more

helpful: in the acute phase reaction, any of the neutrophil forms may develop deep blue cytoplasmic granules, vacuoles, and vague blue cytoplasmic inclusions called Döhle bodies, which consist of aggregates of ribosomes and endoplasmic reticulum. All of these features are easily seen (except possibly the Döhle bodies), even by neophytes.

The normal range for neutrophil (band + seg) count is 1160 - 8300 /μL for blacks, and 1700 - 8100 /μL for other groups. Keeping in mind the lower expected low-end value for blacks will save you much time (and patients much expense and pain) over the course of your career. Obesity and cigarette smoking are associated an increased neutrophil count. It is said that for each pack per day of cigarettes smoked, the granulocyte count may be expected to rise by 1000 /μL.

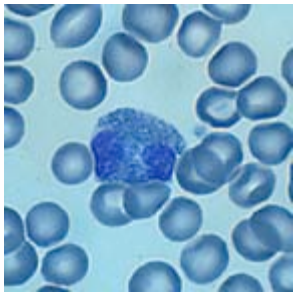
- **B. Monocytes**



These large cells are actually more closely related to neutrophils than are the other "granulocytes," the basophil and eosinophil. Monocytes and neutrophils share the same stem cell. Monocytes are to histiocytes (or macrophages) what Bruce Wayne is to Batman. They are produced by the marrow, circulate for five to eight days, and then enter the tissues where they are mysteriously transformed into histiocytes. Here they serve as the welcome wagon for any outside invaders and are capable of "processing" foreign antigens and "presenting" them to the immunocompetent lymphocytes. They are also capable of the more brutal activity of phagocytosis. Unlike neutrophils, histiocytes can usually survive the phagocytosis of microbes. What they trade off is killing power. For instance, mycobacteria can live in histiocytes (following phagocytosis) for years.

The normal range for the monocyte count is 200 - 950 /μL.

- **C. Eosinophils**

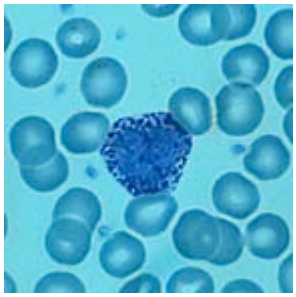


These comely cells are traditionally grouped with the neutrophils and basophils as "granulocytes," another granfalloon. Current thinking is that eosinophils and neutrophils are derived from different stem cells, which are not distinguishable from each other by currently available techniques of examination. Although the hallmark of the eosinophil is the presence of bright orange, large, refractile granules, another feature helpful in identifying them (especially on H&E-stained routine histologic sections) is that they rarely have more than two nuclear lobes (unlike the neutrophil, which usually has three or four). The normal range of the absolute eosinophil count is 0 - 450 /μL.

Eosinophils are capable of ameboid motion (in response to chemotactic substances released by bacteria and components of the complement system) and phagocytosis. They are often seen at the site of invasive parasitic infestations and allergic (immediate hypersensitivity) responses. Individuals with chronic allergic conditions (such as atopic rhinitis or extrinsic asthma) typically have elevated circulating eosinophil counts. The eos may serve a critical function in mitigating allergic responses, since they can 1) inactivate slow reacting substance of anaphylaxis (SRS-A), 2) neutralize histamine, and 3) inhibit mast cell degranulation.

The life span of eos in the peripheral blood is about the same as that of neutrophils. Following a classic acute phase reaction, as the granulocyte count in the peripheral blood drops, the eosinophil count temporarily rises.

- **D. Basophils**



The most aesthetically pleasing of all the leukocytes, the basophils are also the least numerous, the normal range of their count in peripheral blood being 0 - 200/μL. They are easily recognized by their very large, deep purple cytoplasmic granules which overlie, as well as flank, the nucleus (eosinophil granules, by contrast, only flank the nucleus but do not overlie it). It is tempting to assume that the basophil and the mast cell are the blood and tissue versions, respectively, of the same cell type. Actually it is controversial as to whether this concept is true or whether these are two different cell types.

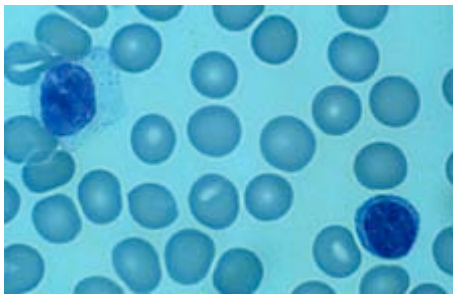
The table below presents some of the contrasts between mast cells and basophils.

Feature	Basophils	Mast cells
Nuclear morphology	segmented	round or ovoid
Mitotic potential	no	yes
Peroxidase content	+	-
Acid phosphatase	-	+
Alkaline phosphatase	-	+
PAS reaction	++++	+

In active allergic reactions, blood basophils decrease in number, while tissue mast cells increase. This reciprocal relationship suggests that they represent the same cell type (i.e., an allergen stimulates the passage of the cells from the blood to the site of the allergen in the tissues). Some experiments with animals have also shown that mast cells are marrow-derived and are capable of differentiating into cells that resemble basophils. Conversely, some recent evidence suggests that basophils (as well as eosinophils) can differentiate from metachromatic precursor cells that reside among epithelial cells in the nasal mucosa

Without invoking religion or Alexander Pope ("Whatever is, is right," *An Essay on Man*, 1732-34) it is hard to see any useful role of the basophil/mast cell in human physiology. The mast cell is the essential effector of immediate (Type 1) hypersensitivity reactions, which produce only misery, dysfunction, and occasionally death for the hapless host.

- **E. Lymphocytes**



In the immune/inflammatory response, if the neutrophils and monocytes are the brutes, the lymphocytes are the brains. It is possible to observe the horror of life without lymphocyte function by studying the unfortunate few with hereditary, X-linked, severe combined immune deficiency. Such individuals uniformly die of systemic infections at an early age (except for the "bubble boys" of yesteryear, who lived out their short lives in antiseptic prisons). The functions of lymphocytes are so diverse and complex that they are

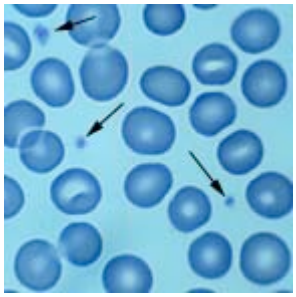
beyond the scope of this text (and the scope of the author, it must be admitted). What follows are a few general remarks concerning examination of lymphocytes in peripheral blood.

After neutrophils, lymphocytes are the most numerous of the circulating leukocytes. The normal range of the lymphocyte count is 1000 - 4800/μL. Their life span may vary from several days to a lifetime (as for memory lymphocytes). Unlike neutrophils, monocytes, and eosinophils, the lymphocytes 1) can move back and forth between the vessels and the extravascular tissues, 2) are capable of reverting to blast-like cells, and 3) when so transformed, can multiply as the immunologic need arises.

In normal people, most of lymphocytes are small, innocent-looking round cells with heavily "painted-on" nuclear chromatin, scant watery cytoplasm, and no granules. A small proportion of normal lymphs are larger and have more opaque, "busy-looking" cytoplasm and slightly irregular nuclei. Some of these have a few large, dark blue granules, the so called "azurophilic granules." It has been maintained that these granulated cells are T-gamma cells (i.e., T-cells that have a surface receptor for the IgG Fc region) or natural killer (NK) null-cells. Other phenotypes of lymphocytes are not recognizable as such on the routine, Wright-stained smear and require special techniques for identification.

When activated by whatever means, lymphocytes can become very large (approaching or exceeding the diameter of monocytes) and basophilic (reflecting the increased amount of synthesized cytoplasmic RNA and protein). The cytoplasm becomes finely granular (reflecting increased numbers of organelles), and the nuclear chromatin becomes less clumped (the better to transcribe you with, my dear!). Such cells are called "transformed lymphocytes," "atypical lymphocytes," or "viral lymphocytes" by various votaries of blood smears. Although such cells are classically associated with viral infection (particularly infectious mononucleosis), they may also be seen in bacterial and other infections and in allergic conditions. A morphologic pitfall is mistaking them for monocytes (a harmless mistake) or leukemic blasts (not so harmless).

Platelets



The main thing to remember about platelets is to look for them first! A typical tyro maneuver is to study a blood smear for an hour looking for some profound hematological abnormality, never to realize there is nary a platelet in sight. It is therefore necessary to discipline yourself to first check for a normal number of platelets when sitting down with a slide, before being seduced by the midnight beauty of the basophil's alluring granules or the monocyte's monolithic sovereignty. The normal platelet count is 133 - 333 x $10^3/\mu$L.

Platelets are counted by machine in most hospital labs and by direct phase microscopy in smaller facilities. Since platelets are easily mistaken for garbage (and vice versa) by both techniques, the platelet count is probably the most inaccurate of all the routinely measured hematologic parameters. Actually, you can estimate the platelet count fairly accurately (up to an absolute value of about 500 x $10^3/\mu$L) by multiplying the average number of platelets per oil immersion field by a factor of 20,000. For instance, an average of ten platelets per oil immersion field (derived from the counting of ten fields) would translate to 200,000/μL (10 x 20,000). Abnormal bleeding generally does not occur unless the platelet count is less than 30,000/μL, if the platelets are functioning properly. Screening for proper platelet function is accomplished by use of the bleeding time test.

Other cells in peripheral blood

Plasma cells sometimes appear in the peripheral blood in states characterized by reactivity of lymphocytes. Old time hematologists often maintain that the cells that look exactly like plasma cells on the smear are really "plasmacytoid lymphs," and it is usually nonproductive to argue this point with them. **Endothelial cells** occasionally get scooped up into the phlebotomy needle during blood collection and show up on the slide. They are huge and tend to be present in groups. **Histiocytes**, complete with pseudopodia and phagocytic vacuoles, may appear in states of extreme reactivity, especially in septic neonates. **Nucleated red cells** may also be seen in small numbers in the peripheral blood of newborns; however, in adults, even a single nucleated rbc on the slide is abnormal, indicating some sort of serious marrow stress, from hemolytic anemia to metastatic cancer. **Myeloblasts** are always abnormal and usually indicate leukemia or an allied neoplastic disease. Rarely they may be seen in non-neoplastic conditions, such as recovery from marrow shutdown (aplasia). Later stages of myeloid development (promyelocyte, myelocyte, metamyelocyte) may be represented in the peripheral blood in both reactive states and leukemias.

Bone marrow examination

This is one of the most common biopsy procedures performed on both outpatients and the hospitalized. Two types of specimens are generally obtained, the aspirate and the core biopsy. The site of biopsy is usually the posterior iliac crest (via the posterior superior iliac spine) in adults and the anterior tibia in children, although other sites are available. After local anesthesia is applied to the periosteum and overlying skin, a small needle (usually the "University of Illinois needle") is introduced (or crunched actually) into the medullary space through a small skin incision. About 0.5 mL of marrow material is aspirated and smeared onto several glass slides and stained with a stain identical or similar to the Wright stain used on peripheral blood. Some material usually remains in the syringe where it is allowed to clot. It is then fished out of the syringe, processed like all other biopsy tissue, embedded in paraffin, sectioned, and stained with hematoxylin/eosin and other selected stains. The core biopsy, generally performed after the aspirate is done, is taken with a larger, tapered needle, typically the "Jamshidi needle." This yields a core of bone (similar to a geologic core sample) which is fixed, decalcified, processed, and sectioned. The H&E-stained core biopsy and aspirate clot sections are best for assessment of marrow cellularity and the presence of metastatic neoplasms or granulomas. The

Wright-stained aspirate smears are best for studying the detailed cytology of hematopoietic cells.

The bone marrow biopsy procedure produces some pain for the patient, since it is impossible to anesthetize the inside of bone. The level of pain ranges from mild discomfort to agony, depending on the individual's pain threshold and level of apprehension. Some physicians elect to precede the biopsy with a benzodiazepine or other minor tranquilizer. Generally the aspiration action produces much more pain than the core biopsy.

For a procedure that involves invasion of bone, the marrow biopsy is remarkably free of complications. Bleeding and infection may occur but are rare, even in severely thrombocytopenic and immunosuppressed patients. It is highly recommended that med students learn how to perform this useful procedure during the clinical years of their training.

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