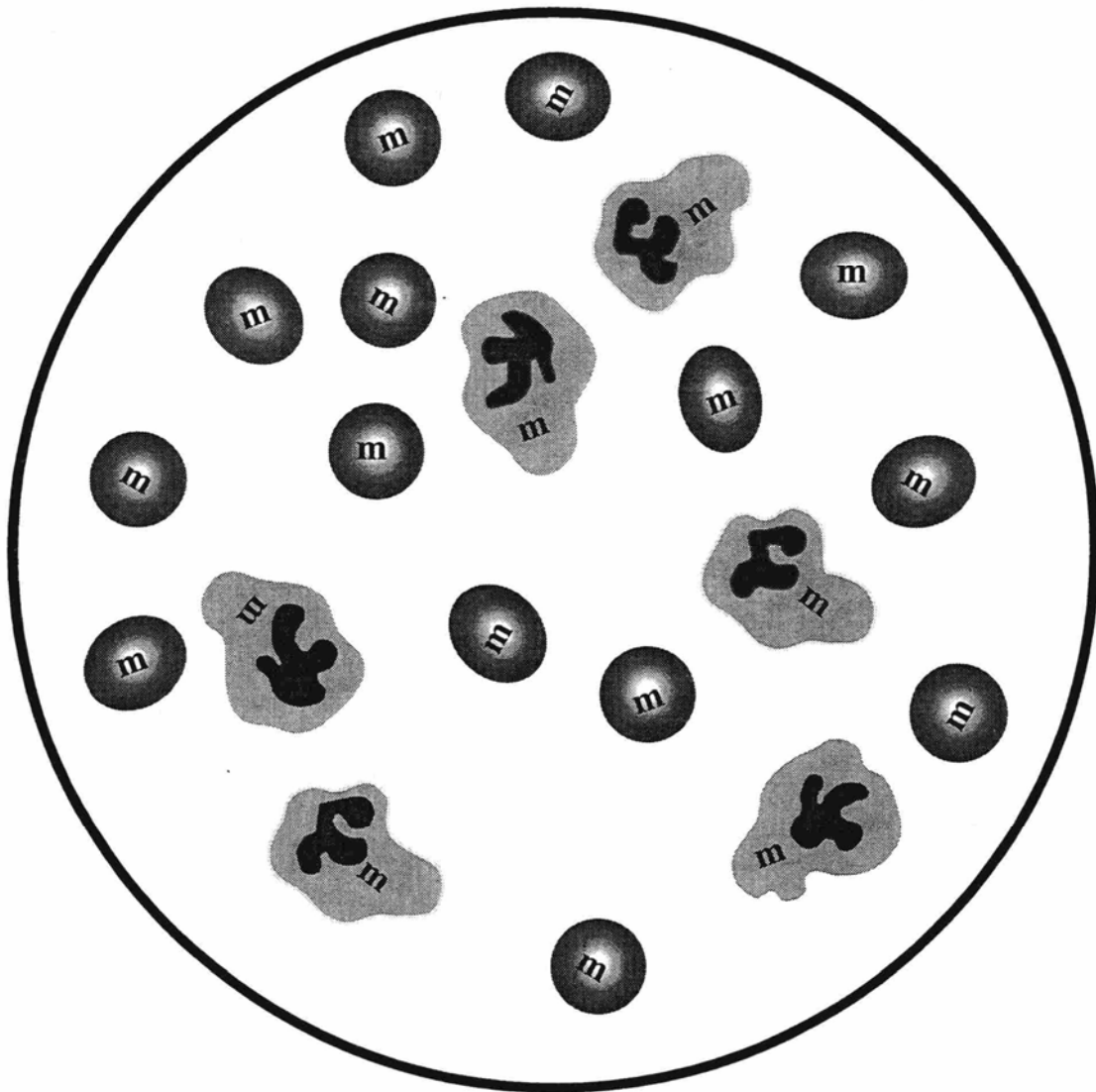


**HEMATOLOGY M+Ms**  
*The Maturation, Morphology  
and Mystery of Leukocytes*



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**Program Description:**

This presentation will review core concepts in the evaluation of leukocytes on Wright-stained peripheral blood and bone marrow smears. Topics to be discussed include maturation stages of leukocyte subtypes, selected hereditary and reactive WBC conditions, and a review of the FAB (French-American-British) subgroups of acute myeloblastic leukemia. Correlation of abnormal WBC morphologic variations will be presented in a case study format.

**Presentation Objectives:**

At the conclusion of this presentation, participants will be able to accurately:

- Describe the morphologic basis for differentiating normal leukocytes, benign WBC disorders, hereditary leukocyte abnormalities, and the subgroups of acute myeloblastic leukemia.
- List key characteristics of the World Health Organization (WHO) classification of myeloid and lymphoid neoplasms.
- Identify pitfalls and problems with the morphologic evaluation of peripheral blood cells.
- Correlate abnormal WBC morphologic variations with selected case studies.

## I. Introduction

## II. Maturation Sequences

### A. General Characteristics

1. Cells originate from committed stem cells in the bone marrow
2. Size decreases as cells mature
3. Nucleus becomes smaller and chromatin becomes more condensed. The nucleus is expelled in RBCs, but may become lobed in other cells, like neutrophils.
4. Cytoplasm becomes more abundant and less basophilic. Granules appear in some leukocytes.

### B. Granulocytes

1. Myeloblast  
Some hematologic disease states can be better defined by using modified definitions:
  - a. Type I blast  
no evidence of cytoplasmic differentiation, no granules in blue cytoplasm
  - b. Type II blast  
some evidence of differentiation, few primary granules in blue cytoplasm
  - c. loose chromatin with 1-5 nucleoli
  - d. prominent Golgi
  - e. represent 1-2% of cells in normal bone marrow
  - f. difficult to distinguish blasts using light microscopy
  - g. cytochemistry and immunologic cell markers used for leukemic blasts
  - h. a blast is a blast is a blast...
2. Promyelocyte
  - a. nucleoli may still be visible within a fairly immature-appearing nucleus

- b. primary granules present
  - granules become less visible as cell divides and matures
- c. represent 2-5% of cells in normal bone marrow
  - granules rich in peroxidase

### 3. Myelocyte

- a. nucleus round to oval and eccentrically located
- b. secondary granules form and give characteristic pinkish color to the cytoplasm
  - 1. sometimes a pink “arc” of specific granules is visible
  - 2. granules contain lysozyme and other proteins
- c. last stage capable of mitosis
- d. represent 10-20% of cells in normal bone marrow

### 4. Metamyelocyte (juvenile)

- a. indented (kidney-bean) nucleus with clumped chromatin
- b. full complement secondary granules present and no RNA remains in the cytoplasm
- c. represent 15-30% of cells in normal bone marrow

### 5. Band (stab)

- a. indented nucleus with clumped chromatin
- b. secondary granules in cytoplasm
- c. represent 18-40% of cells in normal bone marrow
- d. first stage that can be seen normally (2-6%) in the peripheral blood
- e. bands vs. metamyelocytes

- in the band, the indentation of the nucleus is more than half the width of the hypothetical round nucleus

6. Segmented neutrophil (polymorphonuclear neutrophil)

- nucleus has lobes
- mature neutrophils are about twice as large as a normal RBC
- represent 10-30% of cells in normal bone marrow
- normal relative reference range in adult peripheral blood is about 50-70%
- segs vs. bands
  - in the seg, the connection between lobes is by a strip so narrow that there is no visible nuclear chromatin between the two sides (and no parachromatin is visible)
  - any cell, in which the nucleus is twisted such that the entire outline of the nucleus is obscured because of superimposition of one part of the nucleus on another part, should also be classified as a seg
  - should a new cell name be used for those hard-to-classify situations?

7. Eosinophils

- same stages as neutrophils but usually are not counted and classified by stage
- bilobed nucleus
- distinctive orange granules in cytoplasm
- represent 0-4% of cells in normal peripheral blood

8. Basophils

- same stages as neutrophils but usually are not counted and classified by stage

- b. nucleus often obscured
- c. distinctive blue-black granules in cytoplasm
  - 1. granules sometimes seen outside of cell as an artifact of smear preparation
  - 2. granules are water soluble
- d. represent 0-2% of cells in normal peripheral blood

C. Monocytes

1. Early monocytic cells (monoblasts and promonocytes) are not easily identified in bone marrow or peripheral blood smears except in disorders in which there is a marked proliferation of monocytic cells. Blasts appear similar to blasts in other cell lines, but promonocytes may be distinguished by the following:
  - a. slightly indented or folded nuclei with brainlike convolutions
  - b. pseudopods
  - c. cytoplasm blue to blue-gray and moderately abundant
2. Mature monocytes
  - a. variable nuclear shape: round, horseshoe, lobulated – some folding or convoluting usually present
  - b. light purple chromatin arranged in loose strands
  - c. abundant gray-blue cytoplasm with fine, indistinct granules and often vacuoles are also present – cytoplasm has a “bumpy” appearance
  - d. irregular cell outline with occasional pseudopods
3. Key differentiating features in distinguishing immature and mature monocytes:
  - a. change from homogenous nuclear chromatin to pattern with creases or folds
  - b. change from darker, basophilic cytoplasm to pale gray-blue color with irregular margins

## D. Lymphocytes

### 1. Lymphoblast

- a. smaller than myeloblast but still has a large, round nucleus
- b. 1-2 nucleoli
- c. coarse chromatin that stains evenly
- d. cytoplasm is a deep blue with no granules

### 2. Prolymphocyte

- a. practical importance only in prolymphocytic leukemia
- b. round, oval nucleus
- c. chromatin has some clumps, but not as much as is seen in mature lymphs
- d. distinct nucleoli
- e. cytoplasm slightly more abundant than in the blast

### 3. Mature lymph

- variations in size possible
- all lymphs have a nucleolus that is usually obscured by chromatin but may sometimes be visible
- T lymphocytes and B lymphocytes are not morphologically distinguishable on Wright-stained smears

#### a. small

1. round nucleus with dense, clumped chromatin and scanty cytoplasm
2. parachromatin is distinct and appears lavender

#### b. large

1. round nucleus with chromatin slightly more loose and more abundant, blue cytoplasm

2. parachromatin only slightly stained or even unstained and indistinct
  3. a few azurophilic granules may be seen in the cytoplasm
- c. large lymphs vs. monos
1. chromatin
    - a. “blocked” in lymphs
    - b. lacy and linear in monos
  2. nuclear shape
    - a. round, oval, kidney-shaped in lymph
    - b. folded, U-shaped in mono
  3. nuclear staining
    - a. more intense in lymph
    - b. “lighter” in mono
  4. cytoplasm
    - a. clear, smooth blue in lymphs
    - b. appears blue-gray and grainy in monos; vacuoles often present
4. Plasma cell
- a. end stage of B cell maturation
  - b. oval cell with oval or round nucleus eccentrically located in cell
  - c. intensely blue cytoplasm with prominent unstained Golgi area
  - d. produce immunoglobulin
  - e. normally not seen in the peripheral blood
  - f. represent about 1% of cells in normal bone marrow

### III. Hereditary and Reactive WBC Disorders

#### A. Inherited

1. Pelger-Huet anomaly

- a. important to recognize so that it is not confused with a neutrophilic left shift
- b. defined as hyposegmentation of the granulocyte nucleus
- c. cells are cytochemically and functionally normal
- d. in heterozygotes, nucleus either consists of two symmetric, rounded lobes connected by a fine filament (“spectacles”) or fails to segment and resembles a dumbbell
- e. in rare homozygous state nuclei remain round

2. Pseudo-Pelger-Huet is an acquired condition

- a. associated with malignant myeloproliferative disorders, myelodysplastic syndromes, certain drugs and in some patients with infections or tumors that have metastasized to the bone marrow
- b. nuclei appear round
- c. cytoplasm often hypogranular
- d. distinguish inherited Pelger-Huet from acquired condition based on family history and the morphologic indication of any diseases associated with acquired Pelger-Huet

B. Reactive conditions

– The morphology of leukocytes may be altered by infection or by recombinant growth factor therapy

1. neutrophils

– several cytoplasmic, transient changes can be seen

a. Döhle bodies

- 1. light blue inclusions in cytoplasm
- 2. aggregates of rough endoplasmic reticulum (RER)
- 3. most often associated with bacterial infection, but can also be seen during

pregnancy and in cases of burns, cancer or aplastic anemia

4. often seen in combination with other cytoplasmic, reactive changes

b. toxic granulation

1. large, blue-black granules in cytoplasm
2. primary granules that have retained basophilia, possibly because of a lack of maturation
3. seen in same conditions as Döhle bodies
4. artifacts from staining process (increased staining time or decreased pH of the staining buffer)

c. vacuoles

1. clear, unstained areas in cytoplasm
2. end stage of phagocytosis or fat storage
3. seen in same conditions as Döhle bodies and toxic gran
4. artifacts in old EDTA blood

2. Reactive Lymphocytes (also called atypical and variant)

– Reactive lymphs can be seen normally in peripheral blood as they represent cells responding to a stimulus  
– However, numbers greater than about 20% in the peripheral blood are considered significant

a. morphologic characteristics

– no “typical” atypical lymph!  
– and not all characteristics seen in every cell

1. nuclear

- a. irregular shapes common: lobulated, oval, elongated, notched
- b. nucleoli may or may not be visible  
– sometimes prominent
- c. chromatin more fine with more evident parachromatin than in a normal lymph  
– parachromatin more white than lavender  
– sometimes chromatin has linear streaks or appears smeary or rope-like
- d. variable size

2. cytoplasmic
  - a. increased basophilia which can appear patchy
    - variable coloration from light gray to light blue to a deep blue is possible
  - b. skirting, sprawling of cytoplasm
    - cells embrace rounded contours of nearby RBCs
  - c. abundant cytoplasm
  - d. foamy or vacuolated cytoplasm
  - e. prominent granulation

b. reactive vs. malignant lymphs

1. reactive
  - a. heterogeneity within a single specimen
    1. large and small cells
    2. basophilic and pale cells
    3. loose and clumped chromatin
  - b. common in viral disorders
2. malignant
  - a. homogeneity: all abnormal cells appear very similar to one another
  - b. associated with lymphoproliferative disorders
    1. cutaneous T-cell lymphoma (mycosis fungoides) and Sezary cells
      - a. nuclei prominently folded, convoluted and often cerebriform in appearance
      - b. chromatin is hyperchromatic
    2. malignant lymphoma cells
      - often see prominent clefting of nuclei
    3. leukemia

**CHARACTERISTICS OF LYMPHOCYTES AND MONOCYTES**

	<i>Small Lymph</i>	<i>Reactive Lymph</i>	<i>Monocyte</i>
<b>Cell Size</b>	<b>8-12 µm</b>	<b>Variable, can be large (9-30 µm)</b>	<b>Large (15-18 µm)</b>
<b>Nucleus</b>			
<b>Shape</b>	<b>Round</b>	<b>Irregular (lobulated, oval, notched)</b>	<b>Horseshoe, round, folded</b>
<b>Chromatin</b>	<b>Clumped; parachromatin not evident and more lavender</b>	<b>More fine (but not like a blast); parachromatin more evident and white</b>	<b>Lacy, loose strands, brain-like</b>
<b>Nucleoli</b>	<b>Usually absent</b>	<b>May be prominent</b>	<b>Absent</b>
<b>Cytoplasm</b>			
<b>Amount</b>	<b>Scant</b>	<b>Abundant</b>	<b>Abundant</b>
<b>Color</b>	<b>Blue</b>	<b>Often deep, intense blue with darker edges at contact points with other cells</b>	<b>Light bluish gray</b>

**IV. Acute Myeloid (Myeloblastic) Leukemia (AML)**

This leukemia is the most predominant type found in adults, but it is not uncommon at any age. Most patients are often very anemic (normocytic-normochromic) and thrombocytopenic. The leukocyte count is moderately to markedly elevated. Many blasts (or blast ‘equivalents’) can be seen on a peripheral blood smear. Auer rods may sometimes be visible in the cytoplasm of these cells and when noted, are almost diagnostic for AML.

The French-American-British (FAB) subclassification of AML is based on morphologic and cytochemical criteria. The following descriptions briefly identify noteworthy characteristics (from bone marrow smears) of each subgroup.

**A. AML - MO**

Acute myeloid leukemia without morphological or cytochemical maturation

The blast cells are large and lack granules and morphologically may resemble lymphoblasts. This classification must be defined using immunophenotyping and molecular tests.

- B. AML - M1  
Acute myeloid leukemia with minimal morphologic and cytochemical maturation  
Greater than 90% of marrow cells are blasts that are nongranular or contain only a few azurophilic granules and/or Auer rods.
- C. AML - M2  
Acute myeloid leukemia with maturation  
More than 30-90% of marrow cells are blasts. Greater than 10% of cells are granulocytes, with maturation from promyelocytes to segmented neutrophils. Fewer than 20% of cells are monocytic.
- D. AML - M3  
Acute promyelocytic leukemia  
The majority of cells are abnormal promyelocytes with numerous bizarre granules and Auer rods. This subgroup is associated with a high incidence of disseminated intravascular coagulation (DIC). A microgranular variant (M3m) is characterized by clefted nuclei and indistinct granules.
- E. AML - M4  
Acute myelomonocytic leukemia  
Granulocytic and monocytic components coexist. More than 30% of marrow cells are blasts. Monocytes and promonocytes exceed 20%, but are less than 80%. Evidence of tissue infiltration (into gingivae, skin, and meninges) is common at diagnosis. The M4e variant is associated with an increase in bone marrow eosinophils that often appear bizarre and may have bluish granules.
- F. AML - M5  
Acute monocytic leukemia  
Two groups of AML-M5 are recognized depending on the degree of differentiation noted: (a) poorly differentiated (greater than 80% monoblasts) and (b) well-differentiated (more than 80% monocytic cells, but predominantly promonocytes and monocytes).
- G. AML - M6  
Erythroleukemia  
More than 50% of the cells in the bone marrow are nucleated red blood cells. Erythroblasts often show bizarre changes, with multinucleation, giant forms, and megaloblastic features. Over 20% (WHO system) of nonerythroid cells are blasts. This classic stage may also be called erythroleukemia.

H. AML - M7  
 Acute megakaryocytic (megakaryoblastic) leukemia  
 This leukemia subgroup is a systemic and rapidly progressive proliferation of atypical and primitive megakaryocytes. The blasts are variable in size, but generally smaller than normal megakaryocytes, have a high nuclear to cytoplasmic ratio, and sometimes may demonstrate cytoplasmic vacuoles or blebs.

V. Acute Lymphocytic (Lymphoblastic) Leukemia (ALL)

ALL is the predominant leukemia seen in children, although it may also be seen in adults. The peak incidence in childhood occurs during the ages of 2-10. The leukocyte count may be low, normal, or high. However, the WBC count is usually greater than 10,000/ $\mu$ l. A severe normocytic-normochromic anemia is present as is thrombocytopenia. Blasts can usually be seen on a peripheral blood smear along with immature lymphocytes. The blasts often have lymphoid characteristics, but there are no easily recognizable intracellular markers like Auer rods associated with AML.

**Lymphoblasts vs. myeloblasts**

	<u>ALL</u>	<u>AML</u>
Blast Size	Small	Large
Cytoplasm	Scant	Moderate
Chromatin	Dense	Fine, Lacy
Nucleoli	Indistinct	Prominent
Auer Rods	Never Present	Present in about 50%

The FAB classification divides ALL into three subgroups based on cell size, nuclear chromatin pattern, amount of cytoplasm, amount of basophilia in the cytoplasm, nuclear shape, nucleoli, and cytoplasmic vacuolization.

In addition to categorizing ALL using morphologic criteria, an immunologic classification is important in determining the correct diagnosis and prognosis.

Lymphoid cells have unique antigens and receptor sites on their surface or in their cytoplasm (and sometimes in both locations) that can be identified and used as markers. Cell marker studies can be performed on cell suspensions made from peripheral blood, bone marrow aspirates, and body fluids.

The FAB classification of ALL into three subgroups based on morphology lacks the specificity and relevance of immunophenotyping and is not retained in subsequent classification systems, such as the World Health Organization (WHO) scheme. However, ALL-L3 in the FAB classification is equivalent to Burkitt cell leukemia in the WHO system.

## **VI. World Health Organization (WHO) Classification**

- A. A new classification system for AML and ALL has been proposed by the WHO.
- B. This system incorporates cytogenetic, molecular and immunophenotypic features into the classification of acute leukemias.
- C. This proposal emphasizes these changes because they provide more relevant clinical, diagnostic and prognostic information than morphology and cytochemistry alone
- D. A major change in this proposal is the reduction in the percentage of blasts needed for a diagnosis of acute leukemia from 30% to 20%.
- E. The myelodysplastic syndrome (MDS) category of refractory anemia with excess blasts in transformation (RAEB-T) is therefore eliminated.
- F. This classification system recognizes specific cytogenetic defects that affect prognosis, that some cases of AML develop multilineage dysplasia, and that AML can evolve after therapy
- G. In addition, three new subgroups of AML are defined (Acute basophilic leukemia, Myeloid sarcoma and Acute panmyelosis with myelofibrosis).
- H. Cytogenetics is an especially useful technique in defining prognosis (remission and survival time) in ALL.

## **WHO CLASSIFICATION OF MYELOID NEOPLASMS**

- **MYELOPROLIFERATIVE DISEASES**
- **MYELOYDYSPLASTIC/MYELOPROLIFERATIVE DISEASES**
- **MYELOYDYSPLASTIC SYNDROMES (MDSs)**
- **ACUTE MYELOID LEUKEMIA**
- **ACUTE BIPHENOTYPIC LEUKEMIAS**

## **WHO CLASSIFICATION OF LYMPHOID NEOPLASMS**

- **B-CELL NEOPLASMS**
- **T-CELL AND NK\*-CELL NEOPLASMS**
- **HODGKIN'S LYMPHOMA (HODGKIN'S DISEASE)**

**\*NK = NATURAL KILLER**

### WHO Classification of AML

- I. AML with recurrent cytogenetic translocations  
AML with t(8:21)  
AML with inv(16) or t(16:16)  
Promyelocytic leukemia with t(15:17)  
Promyelocytic leukemia with t(V;17)  
AML with 11q23 abnormalities
- II. AML with multilineage dysplasia (classify using subgroups of category III)  
De novo AML with multilineage dysplasia  
AML following an MDS
- III. AML, NOS (not otherwise specified)  
M0-M7 (FAB groups)  
Acute basophilic leukemia  
Myeloid sarcoma  
Acute panmyelosis with myelofibrosis
- IV. Therapy-related AML
- V. Acute biphenotypic leukemia

### WHO Classification of ALL

Acute lymphoblastic leukemia/lymphoma

Synonyms: Former FAB L1/L2

Precursor B acute lymphoblastic leukemia/lymphoma

Cytogenetic subtypes:

t(12;21) (p12; q22) TEL-AML1\*

t(1;19)(q23; p13) PBX-E2A\*

t(9;22) (q34; q11) ABL-BCR\*

t(v;11) (v; q23) V-MLL\*

Burkitt's leukemia/lymphoma (Synonym: Former FAB L3)

Precursor T acute lymphoblastic leukemia/lymphoma

\*These abbreviations refer to specific genes or regions where abnormalities occur:

TEL-AML1	Translocation, Ets (E-twenty six), Leukemia – Acute Myeloblastic Leukemia (Ets refers to a group of transcription factors containing an E-box motif)
PBX-E2A	PBX is also called PRL and refers to the Pre-B-cell leukemia gene; “X” in PBX indicates the presence of a “homeobox” domain – E2A is located on chromosome 19 in a region that encodes at least 2 transcription factors. These proteins are helix-loop-helix containing proteins that bind to the “E-box element” in the kappa light chain DNA enhancer region.
ABL-BCR	Abelson-breakpoint cluster region
V-MLL	Variant-mixed lineage leukemia gene

## ANALYSES USEFUL IN SUPPORTING A DIAGNOSIS OF LEUKEMIA

- I. Cytochemistry
  - A. To differentiate AML from ALL:  
Peroxidase (or Sudan Black B – SBB), and Terminal deoxynucleotidyl transferase (TdT)  
AML: Peroxidase/SBB positive  
TdT negative  
  
ALL: Peroxidase/SBB negative  
TdT positive
  - B. To support a diagnosis of AML-M1, AML-M2, or AML-M3:  
Peroxidase, SBB and Nonspecific esterase (NSE) with acetate or butyrate substrates  
AML-M1, AML-M2, AML-M3: Peroxidase/SBB positive  
NSE negative
  - C. To differentiate AML-M4 from AML-M5:  
Peroxidase, SBB and Nonspecific esterase (NSE) with acetate or butyrate substrates  
AML-M4: Peroxidase/SBB positive  
NSE positive  
  
AML-M5: Peroxidase/SBB negative  
NSE positive
  - D. To support a diagnosis of AML-M6:  
NSE (with acetate and butyrate substrates), Periodic Acid Schiff (PAS), and Immunophenotyping  
AML-M6: NSE positive with acetate but not with butyrate  
PAS positive  
Immunophenotyping using monoclonal antibodies directed to erythroid glycoprotein A
  - E. To support a diagnosis of AML-M7:  
NSE (with acetate and butyrate substrates), PAS, Platelet peroxidase (PPO), and Immunophenotyping  
AML-M7: NSE positive with acetate but not with butyrate  
PAS positive  
PPO positive  
Immunophenotyping using anti-gpIIIa (antibody to platelet glycoprotein IIIa-CD 61) or anti-gpIIb/IIIa (antibody to the platelet fibrinogen receptor-CD 41)

## II. Cytogenetics

A. Many leukemias have some type of chromosomal abnormality

B. Example: t(15;17) (q22;q12) in AML-M3

## III. Immunophenotyping

A. Use of monoclonal antibodies to help subclassify leukemic cells and predict prognosis.

B. Example: Myeloid antigens (CD33 and CD13) to help distinguish AML from ALL (CD19, CD10 for B cells and CD2, CD3 for T cells)

## IV. Molecular Techniques

A. Used to detect antigen receptor gene rearrangements

B. Detect numeric and structural chromosomal abnormalities

## VII. Pitfalls and Problems with Morphologic Evaluations

### A. Smear preparation

A peripheral blood smear that has not been properly prepared will make any evaluation of cells difficult, even for an experienced observer. Common sources of poor quality smears arise from dirty slides, chipped spreader slides, drops of blood that are too big or too small, excessive pressure, or an angle of the pusher slide that is too steep. Cells may appear variously disrupted, contracted, or broken when smears are not properly prepared.

### B. Adequacy of staining

A wide variation in staining properties can be found between the same stain type provided by different suppliers and even between different batches of stain from the same supplier. Small variations in stain color and intensity may be tolerated by an experienced observer, but such distortions make examining the smear difficult for a novice reviewer.

Heavy deposits of precipitated stain can be a particular nuisance, but are readily recognizable. However, sparse, fine deposits may be mistaken for inclusions, especially in red blood cells; some erythrocytes may even appear to have parasites to the inexperienced observer. Precipitated stain deposits have an uncanny tendency to adhere to the surface of cells.

Water in the methanol or condensation of moisture on the blood smears before fixation also induces artifact formation and disfigures RBCs. Moisture or water vapor may sometimes decolorize the granules of unfixed eosinophils and basophils.

### C. Area for examination

Sources of confusion in evaluating stained peripheral blood smears can be encountered when the smear is reviewed in a less than optimum area. In thick areas of the smear, the RBC will be crowded and have no central pallor; the WBCs will appear contracted. In thin areas of the smear, the RBCs will be flattened with no central pallor and large gaps will be seen between clusters of erythrocytes; WBCs will appear distorted. An ideal area for examination is one in which the RBCs are barely touching or overlapping and the central pallor or “whites of their eyes” is readily visible.

D. Source of the specimen and type of anticoagulant (if any)

Smears made directly from finger punctures can yield unusual results if not collected properly. Heparin causes a blue background to appear on a smear.

Several changes to cells may occur when EDTA is used as the anticoagulant:

1. Erythrocytes tend to crenate after about 6 hours.
2. Additional vacuoles may be seen in monocytes after a few minutes.
3. Variant lymphocytes may become vacuolated.
4. Blasts may acquire very convoluted nuclei.

E. Observation of cells

1. Erythrocytes

The process of making a peripheral blood smear can naturally distort and disturb RBC morphology. Therefore, it is always possible to see some type of abnormal variations in red blood cells on a peripheral blood smear.

2. Leukocytes

a. Bands versus segmented neutrophils

This will always be a source of controversy even with guidelines that help to explain the differences. A careful observation of the marginations and shape of the nucleus is essential. The decision is not always clearly determined. If in doubt, call the cell the most mature form, or segmented cell. Caution is needed when a patient has the Pelger-Huet anomaly or an acquired pseudo-Pelger-Huet condition (commonly associated with malignant myeloproliferative disorders or myelodysplastic syndromes). It is important not to confuse the Pelger-Huet forms with a neutrophilic left shift in which bands, metamyelocytes, and possibly even myelocytes may be present. Careful observation of the nuclear shape and texture will indicate whether the cell is immature, as in a leukocytosis, or fully mature but an expression of a Pelger-Huet condition.

b. Blasts

Characteristics of immaturity are nonspecific – loose chromatin with nucleoli, deeply basophilic and scanty cytoplasm – and can describe many cells. It can be difficult at times to distinguish blast cells. Recognizing a cell as immature is important. Cytochemistry and immunohistochemistry can be used to designate a cell line.

c. Lymphocytes

It is not possible to distinguish T or B lymphocytes or their subsets using only morphology. Immunohistochemistry is needed to define these specific types of cells.

Nucleoli in small lymphocytes may be present and are evidence that these cells are capable of growth and replication. These nucleoli can be considered “pseudo nucleoli” as they do not represent an immature cell in such situations.

d. Monocytes versus large lymphocytes

When distinguishing monocytes from large lymphocytes, nuclear structure, character of the cytoplasm, and the overall shape of the cell can be useful criteria. The nucleus in monocytes tends to be lacy, whereas it is more clumped in the lymphocyte. Monocyte nuclei may also tend to be more open and brainlike. The cytoplasm in monocytes is grainy and a light blue. Lymphocytes have an agranular, smooth, blue cytoplasm. Monocytes are bossy cells and tend to extend pseudopods between cells and compress cells. Lymphocytes, on the other hand, are often indented by surrounding erythrocytes.

3. Platelets

Platelets, which have settled “above” red blood cells, may appear as if they are an actual inclusion within the red cell. This can be particularly distracting when the smear is being evaluated for malarial parasites. However, when the smear is focused up and down, the platelet will not focus with the cell. Any inclusion in a red cell will focus with the red cell.

Enlarged platelets may be confused with large, polychromatophilic erythrocytes. Platelets, however, are somewhat granular in appearance whereas RBCs will appear more smooth and homogeneous.

4. Miscellaneous

Cells that have died will often have pyknotic or featureless nuclei and will be difficult to evaluate. Other possibly unidentifiable forms may include smudge cells or basket cells. If the disrupted cell is clearly recognizable, perhaps an eosinophil, include it in the evaluation of the smear. Less than 2% of the white cells on a stained peripheral blood smear should be disrupted or nonidentifiable forms, except in certain abnormal states such as chronic lymphocytic leukemia with the associated increase in smudge cells. When an increase in nonidentifiable forms is detected, classify these “cells” as “other” and include a comment on the laboratory report. [Clinical and Laboratory Standards Institute, *Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods*; Approved Standard, 2<sup>nd</sup> edition, H20-A2, 2007.]

## VIII. Case Studies (the 'Mystery'!)

### Case W-1

This patient presented with acute respiratory failure with a superimposed history of emphysema. His physician discovered a high white blood cell count while evaluating the emphysema. As far as is known, previous counts had been normal. A CBC showed the following:

WBC	136.6 x 10 <sup>3</sup> /μl
RBC	4.17 x 10 <sup>6</sup> /μl
Hgb	13.4 g/dl
Hct	37.3%
MCV	89.4 fl
MCH	32.1 pg
MCHC	35.9 g/dl
RDW	17.1%
Plt	43 x 10 <sup>3</sup> /μl
Neutrophils	25%
Lymphs	5%
Monos	5%
Metamyelocytes	2%
Bands	5%
Other	56%

Cytochemistry tests performed on a bone marrow aspirate showed:

Leukocyte alkaline phosphatase: 113

Non-specific esterase: Strongly positive in majority of immature cells

Myeloperoxidase: Negative in immature cells

1. Describe the morphology of the prominent abnormal cells present on the picture.
2. What classification of acute leukemia is suggested by the cytochemical results?
3. How should this leukemia be subclassified?

### Case W-2

This female patient presented to her physician for a routine physical exam and laboratory evaluation. The results of her CBC are as follows:

WBC	9.2 x 10 <sup>3</sup> /μl
RBC	5.34 x 10 <sup>6</sup> /μl
Hgb	13.7 g/dl
Hct	41.0%
MCV	76.7 fl
MCH	25.7 pg
MCHC	33.4 g/dl
RDW	14.7%
Plt	248 x 10 <sup>3</sup> /μl
MPV	11.0
PMNs	31%
Bands	23%
Lymphs	43%
Monos	2%
Eos	1%
Atypical lymphs	2+
Hypochromia	1+
Microcytosis	1+

1. Describe the significant abnormality associated with the granulocytes.
2. What is the etiology of this finding?
3. What is the clinical significance of this finding?

### Case W-3

A three-year-old presented to her physician with arthritic symptoms. Preliminary laboratory results indicated a possible leukemia. She was referred for repeat peripheral blood and bone marrow exams as well as diagnosis. Her hematologic results on admission are as follows:

WBC	42.02 x 10 <sup>3</sup> /μl
RBC	3.30 x 10 <sup>6</sup> /μl
Hgb	10.3 g/dl
Hct	31.4%
MCV	94.9 fl
MCH	31.2 pg
MCHC	32.9 g/dl
RDW	16.6%
Plt	44.0 x 10 <sup>3</sup> /μl
Neutrophils	17.6%
Lymphs	18.6%
Monos	6.7%
Eos	0.2%
Basos	2.7%
“Other” cells	56.9%

Cytochemistry on bone marrow aspirate smears showed less than 5% of nucleated cells positive for peroxidase, Sudan black B and NSE; 80% of nucleated cells stained positive for TdT.

1. Is this a lymphocytic or non-lymphocytic leukemia?
2. Review the picture of the Wright-stained bone marrow aspirate smear and suggest a subgroup classification of this leukemia.

#### Case W-4

A three-year-old female with Down's Syndrome presented to the emergency room with otitis media that did not respond to antibiotics. Her automated CBC results were as follows:

WBC	48.06 x 10 <sup>3</sup> /μl
RBC	2.03 x 10 <sup>6</sup> /μl
Hgb	6.0 g/dl
Hct	19.2%
MCV	94.6 fl
MCH	29.4 pg
MCHC	31.1 g/dl
RDW	19.5%
Plt	61 x 10 <sup>3</sup> /μl
Neutrophils	19.1%
Lymphs	18.1%
Monos	2.9%
Eos	0.2%
Basos	3.3%
“Other” cells	59.7%

Bone marrow aspirate revealed a population of blasts that comprised about 35% of the cells present. These cells were intermediate to large in size and had deeply basophilic cytoplasm; large, smooth-contoured nuclei with prominent nucleoli were observed. Myeloperoxidase and Sudan black B stains showed less than 10% positive cells. TdT staining indicated less than 5% positive. A NSE stain using alpha-naphthol butyrate as the substrate was negative. Another NSE stain using an acetate substrate was positive.

1. Suggest a classification for this leukemia. What is the most probable subgroup?
2. What additional tests could be performed to help confirm this diagnosis?

### Case W-5

The patient was a previously healthy 18-year-old white female who initially presented to the emergency room of a Montana hospital after a skiing accident in which she suffered abdominal trauma. An abdominal CT scan showed a large hemoperitoneum while resulting exploratory laparotomy indicated a laceration of the left ovary. The injury was repaired, but the patient received three units of autologous blood intraoperatively and two units packed red blood cells post-operatively. The patient was stable until four days later, when she developed postoperative bleeding, epistaxis, and vaginal bleeding.

She was subsequently transfused with significant amounts of packed red blood cells and fresh frozen plasma, but the hemorrhaging continued. Therefore, five days later, she was transferred to the University of Utah Medical Center for further evaluation and therapy.

	<u>Selected Lab Results</u> <u>Four Days postoperatively</u>	<u>Selected Lab Results</u> <u>Nine Days postoperatively</u>
WBC	9.7 x 10 <sup>9</sup> /L	17.3 x 10 <sup>9</sup> /L
Hgb	8.0 g/dl	12.2 g/dl
Hct	23.3%	35.1%
Platelets	<25 x 10 <sup>9</sup> /L	69 x 10 <sup>9</sup> /L
PT	18.2 sec. (10-13)	21.4 (12.3 – 15.2)
Fibrinogen	118 mg% (200-400)	74 mg% (150-350)
FDPs	>1.0 mg/dl (<0.5)	-
D-dimer	-	32.0 µg/ml (0-0.5)
Differential		
Segs	59	19
Bands	7	6
Lymphs	9	18
Monocytes	-	1
Eosinophils	1	2
Promyelocytes	6	51
Myelocytes	10	2
Metamyelocytes	8	1
NRBC	3	3
Cytochemistry:	Peroxidase positive	

1. What diseases should be considered in the differential diagnosis of this patient's condition?
2. What is the most likely diagnosis based on clinical features, routine hematology test results, and cytochemistry?
3. How do the cytochemical test results help confirm a diagnosis?
4. What is the etiology of this patient's DIC?

### Case W-8

A 25-year-old female complained of headache, fatigue and shortness of breath for several days. After developing a rash on her trunk and extremities, she was admitted to a local hospital. Three days after admission, she developed a sore throat, enlarged lymph nodes, fluctuating fever and enlarged spleen. Laboratory data are as follows:

WBC	8.1 x 10 <sup>3</sup> /μl
Hemoglobin	13.0 g/dl
Hematocrit	39.0%
Segs	30%
Lymphs	60%
Monos	5%
Eosinophils	5%

1. Is this case representative of a benign process or a malignant process? What is the most probable diagnosis of this patient's condition?
2. What additional tests should be performed to confirm a diagnosis?

## **BIBLIOGRAPHY AND REFERENCES**

1. Bick, R., *Hematology Clinical and Laboratory Practice*, Mosby, 1993.
2. Harmening, D., *Clinical Hematology and Fundamentals of Hemostasis*, fourth edition, F.A. Davis, 2002.
3. Kjeldsberg, C., ed., *Practical Diagnosis of Hematologic Disorders*, fourth edition, ASCP Press, 2006.
4. Kapff, C., and Jandl, J., *Blood*, second edition, Little, Brown, and Company, 1992.
5. Koepke, J., *Practical Laboratory Hematology*, Churchill Livingstone, 1991.
6. McKenzie, S., *Clinical Laboratory Hematology*, Prentice Hall, 2004.
7. Carr, JH and Rodak, BF. *Clinical Hematology Atlas*, second edition, W.B. Saunders, 2004.
8. Steine-Martin, E., Lotspeich-Steininger, C., and Koepke, J., *Clinical Hematology*, second edition, J.B. Lippincott, 1998.
9. Turgeon, M., *Clinical Hematology*, third edition, Lippincott Williams and Wilkins, 1999.
10. Williams, W., et. al., *Hematology*, fifth edition, McGraw-Hill, 1995.