Days 10 and 11

Lecture: Introduction to Malignant Leukocyte Disorders, Acute and Chronic Leukemias, Chronic Myeloproliferative and Lymphoproliferative Disorders

Lab:
1. Begin abnormal differentials.
2. Perform a manual sedimentation rate.
3. Perform a fingerstick.

Upon completion of the day’s learning activities, the Clinical Laboratory Science student will:

Lecture Objectives:
1. Differentiate between reactive and malignant leukocyte disorders, including main cell type affected and/or morphologic changes in cell appearance
2. Contrast leukemia and lymphoma (e.g., site of origin).
3. Discuss theories for the cause of leukemia and lymphoma:
   a. Viral (e.g., EBV)
   b. Bone marrow damage
   c. Genetic and environmental factors
   d. Immune function
4. Contrast myeloproliferative and lymphoproliferative disorders (e.g., stem cell defect).
5. Discuss the criteria used to classify leukemias as acute or chronic, including:
   a. Duration
   b. Predominant cell type, based on cell maturity and malignant cell type
   c. Clinical manifestations
6. Identify the bone marrow blast percent required for a diagnosis of acute leukemia (FAB criteria) and the characteristic triad of blood findings.
7. Discuss the two most common causes of death in acute leukemia.
8. Discuss the use of the following to aid in the diagnosis of hematologic disorders:
   a. Evaluation of cell morphologic features
   b. Cytogenetic and molecular testing, including the significance of t(9;22)
   c. CD surface marker ‘panels’
   d. Cytochemical stain ‘panels’ (acute leukemias)
9. Correlate cell lineage and/or maturation stage with the following cluster designation (CD) markers:
   a. CD34 - stem/progenitor cells…early myeloid or lymphoid precursors
   b. CD33 – myeloid cells
   c. CD2/CD3 - lymphoid, T cells
   d. CD19/CD20 - lymphoid, B cells
10. Discuss the basic principle of the following cytochemical stains:
    a. Peroxidase
    b. Sudan Black B (SBB)
    c. Periodic Acid Schiff (PAS)
    d. Leukocyte Alkaline Phosphatase (LAP)
    e. Leukocyte Acid Phosphatase with or without tartrate (TRAP)
11. Correlate the staining pattern (i.e., positive or negative) of the following cytochemical stains with WBC disorders for which they have diagnostic value:
    a. Peroxidase
    b. Sudan Black B (SBB)
    c. Periodic Acid Schiff (PAS)
    d. Leukocyte Acid Phosphatase with or without tartrate (TRAP)
12. Using FAB criteria, evaluate the following chronic myeloproliferative disorders based on blood and bone marrow findings, clinical findings, age group affected, tests used to aid diagnosis, treatment and prognosis:
   a. Primary polycythemia vera (PV)
   b. Idiopathic myelofibrosis with myeloid metaplasia (MMM)
   c. Primary/Essential thrombocythemia (ET)
   d. *Chronic myelocytic/myelogeneous leukemia (CML)

13. Contrast primary, secondary and pseudo polythemias, including causes.

14. Contrast chronic myelocytic leukemia (CML) to a neutrophilic leukemoid reaction (NLR) caused by a severe bacterial infection, including results of the LAP stain.

15. Using FAB criteria, evaluate the following acute myeloproliferative disorders based on blood and bone marrow findings, clinical findings, age group affected, tests used to aid diagnosis, treatment and prognosis:
   a. *Acute myelocytic/myeloblastic leukemia (AML)
   b. Acute promyelocytic leukemia (AProl)

16. Designate the acute myelogenous leukemias as M₁-M₇ using the FAB classification. **BONUS**

17. Using FAB criteria, evaluate the following lymphoproliferative disorders based on blood and bone marrow findings, clinical findings, age group affected, tests used to aid diagnosis, treatment and prognosis:
   a. *Acute lymphocytic (lymphoblastic) leukemia (ALL)
   b. *Chronic lymphocytic leukemia (CLL)
   c. Hairy cell leukemia (HCL)
   d. Hodgkin's lymphoma (HL) - Reed-Sternberg cell
   e. Non-Hodgkin's lymphomas (NHL) - Rieder (clefted) cell, Burkitt cell
   f. Multiple myeloma (MM)
   g. Waldenstrom's macroglobulinemia

**NOTE:** You are responsible for information as designated in lecture or information covered on discussion questions, worksheets, or cases with an emphasis on AML, ALL, CML, and CLL.

Lab Objectives:
1. Perform abnormal differentials, identifying and quantitating abnormal RBC and/or WBC morphology.
2. Perform a manual sedimentation rate (ESR) following established procedure. **Day 10**
3. Interpret the test for infectious mononucleosis, including controls. **Day 10**
4. Complete lab cases of the non-malignant WBC disorders. **Day 10**
5. Complete lab cases of the malignant WBC disorders. **Day 11**
6. Perform a capillary puncture (fingerstick) following established procedure to collect 500 uL EDTA blood in a microtainer without clots. **Day 11**
Classification of Leukocyte Disorders

I. Non-Malignant Leukocyte Disorders

A. Reactive leukocyte disorders
   1. Neutrophilia (shift and pathologic including neutrophilic leukemoid reaction)
   2. Neutropenia
   3. Eosinophilia
   4. Basophilia
   5. Monocytosis
   6. Lymphocytosis (including infectious mononucleosis)
   7. Lymphopenia

B. Hereditary leukocyte disorders
   1. Pelger-Huet anomaly
   2. May-Hegglin anomaly
   3. Alder-Reilly anomaly
   4. Chediak-Higashi anomaly
   [5. Chronic Granulomatous disease]

II. Malignant Leukocyte Disorders (using FAB criteria)

A. Chronic myeloproliferative disorders (Chronic MPD)
   1. Primary polycythemia vera (PV)
   2. Idiopathic myelofibrosis with myeloid metaplasia (MMM)
   3. Primary/Essential thrombocythemia (ET)
   4. *Chronic myelocytic/myelogeneous leukemia (CML)

B. Acute myeloproliferative disorders (Acute MPD) or acute myeloid/myelogeneous leukemias
   1. *Acute myelocytic/myeloblastic leukemia (AML)
   2. Acute promyelocytic leukemia (AProL)
   3. Acute myelomonocytic leukemia (AMML)
   4. Acute monocytic leukemia (AMonoL)
   5. Acute erythroleukemia (EL)
   6. Acute megakaryocytic leukemia (AMegaL)

C. Myelodysplastic syndromes (MDS) – “preleukemia”]
   1. Refractory anemia (RA)
   2. Refractory anemia with ringed sideroblasts (RARS)
   3. Refractory anemia with excess blasts (RAEB)
   4. Refractory anemia with excess blasts in transition (RAEBIT)
   5. Chronic myelomonocytic leukemia (CMML)

D. Lymphoproliferative/lymphoid disorders (LPD)
   1. *Acute lymphocytic/lymphoblastic leukemia (ALL)
   2. *Chronic lymphocytic leukemia (CLL)
   3. Hairy cell leukemia (HCL)
   4. Hodgkin's lymphoma (HL)
   5. Non-Hodgkin's lymphoma (NHL)
   6. Multiple myeloma (MM)
   7. Waldenstrom's macroglobulinemia
MYELOPROLIFERATIVE DISORDERS (MPD)
- Defect of myeloid stem cell (NOT lymphs)

Myeloid Component

Myeloproliferative Disorders

Myelodysplastic Syndromes
(“preleukemia”)

Acute Myeloid Leukemias
Using FAB criteria

- M-0 AML (minimal differentiation)
- M-1 AML (acute myelocytic without maturation)
- M-2 AML (acute myelocytic with maturation)
- M-3 APoL (promyelocytic)
- M-4 AMML (myelomonocytic)
- M-5 AMonaL (monocytic)
- M-6 EL (erythrocytic)
- M-7 AMegaL (megakaryocytic)

Chronic Diseases

- CML Chronic myelocytic Leukemia
- PV 1* Polycythemia rubra vera
- MMM Myelofibrosis with myeloid metaplasia
- ET 1* Essential thrombocythemia

LYMPHOPROLIFERATIVE DISORDERS (LPD)
- Defect of lymphoid stem cell (ONLY lymphs)

Lymphoid Component

Acute Lymphoid Leukemias
Using FAB criteria

- ALL - Acute Lymphocytic leukemias
  - L-1 (monomorphic)
  - L-2 (pleomorphic)
  - L-3 (Burkitt type)

Chronic Diseases

- CLL Chronic lymphocytic leukemia
- HCL Hairy cell leukemia
- HL Hodgkin’s lymphoma
- NHL Non-Hodgkin’s lymphoma
- MM Multiple myeloma
- WM Waldenstrom’s macroglobulinemia
I. Malignant Disorders of the Leukocytes

Group of disorders in which cell proliferation is uncontrolled and not a response to tissue damage; stimulus is generally unknown. The malignant cells cease to respond to normal regulatory mechanisms, may produce substances that inhibit the growth of normal cells and/or may involve unregulated apoptosis.

A. The "defect" in these neoplastic disorders causes an arrest at a normal maturation stage...a malignant clone of cells accumulate.

B. The malignant leukocyte disorders cause mortality by:
   1. progressive replacement of normal cells by malignant cells.
   2. invasion and impairment of vital organs/tissue function by malignant cells.

C. The etiology of malignant transformation remains unclear. It is likely that more than one factor or interacting factors are involved which increase the incidence of leukemia/lymphoma.

- Leukemia is an uncontrolled proliferation of malignant cells in the bone marrow; initially systemic.
- Lymphoma is an uncontrolled proliferation of malignant cells in lymphoid tissue; initially localized.

1. Viral theory
   a. HTLV-1 causes adult T cell leukemia/lymphoma in Japan.
   b. EBV can cause Burkitt's lymphoma and Hodgkin’s lymphoma.
   c. Viruses cause leukemia in animals...cats.
   d. Viruses may suppress immune function or ‘turn on’ oncogenes...HIV (AIDS).

2. Bone marrow damage
   a. Radiation - atomic bomb, radiologists.
   b. Chemicals - chloramphenicol, benzene, pesticides.

3. Chromosomal and genetic factors
   a. Philadelphia chromosome, t(9;22), is present in over 95% of chronic myelocytic leukemia cases; acquired mutation produces BCR/ABL fusion gene with uncontrolled growth-promoting activity.
   b. Presence of t(15;17) is diagnostic of acute promyelocytic leukemia; mutation causes PML/RARA fusion gene that arrests cell differentiation.
   c. Increased incidence in Down's, Fanconi's, families, twins.

4. Geographical/environmental hotspots
   a. Acute lymphocytic leukemia in kids in U.S.
   b. Burkitt's lymphoma in Africa.
   c. Lymphoma belt along Platte river valley.

5. Immune function
   a. Hereditary and acquired defects of immune function.
   b. Elderly and males have higher incidence.
The malignant leukocyte disorders include the **Myeloproliferative** and **Lymphoproliferative disorders**.

II. **Myeloproliferative Disorders (MPD)**

Myeloproliferative disorders are characterized by an uncontrolled proliferation of one or generally more of the bone marrow cell lines...granulocytic, erythrocytic, monocytic and megakaryocytic lines. **NOT** Lymphocytic Cells.

A. The basic defect in these conditions is believed to be found in the myeloid stem cell. The diseases represented in this group include some that are frankly malignant (acute) and others which are less clearly malignant (chronic); transitions between disorders are characteristic.

B. **Acute** myeloproliferative disorders.
   - Acute Myelogenous (or myeloid) leukemias

B. **Chronic** myeloproliferative disorders.
   - Polycythemia vera (PV)
   - Essential Thrombocythemia (ET)
   - Myelofibrosis with myeloid metaplasia (MMM)
   - Chronic Myelocytic leukemia (CML)

III. **Lymphoproliferative Disorders (LPD)**

Lymphoproliferative disorders are malignant disorders that **involve organs/tissues which produce lymphoid cells**...spleen, liver, thymus, lymph nodes, bone marrow, Peyer's patches.

A. The basic defect is believed to be in the **lymphoid stem cell**. Blocks occur in the normal maturation sequence of lymphoid cells **ONLY**.

B. The diseases represented in this group are:

   1. **Acute** lymphoproliferative disorders
      - Acute Lymphocytic (or lymphoid) leukemias (ALL)

   2. **Chronic** lymphoproliferative disorders
      - Chronic Lymphocytic leukemia (CLL)
      - Hairy cell leukemia (HCL)
      - Hodgkin's lymphoma (HL)
      - Non-Hodgkin's lymphomas (NHL)
      - Multiple myeloma (MM)
      - Waldenstrom's macroglobulinemia

C. Immune deficiency is often seen in the lymphoproliferative disorders – the specific defect depends on the type of lymphoid cell affected.
I. Leukemias

A. Definition:
A neoplastic proliferation of cells in the bone marrow, with or without involvement of the peripheral blood. The uncontrolled proliferation and accumulation leads to replacement of normal marrow elements. The leukemic cells commonly infiltrate the RES (liver, spleen, lymph nodes) and CNS or skin. Initially a systemic disease...abnormal blood count (CBC/Diff) at presentation.

B. Traditional classification criteria...Acute versus Chronic:

1. ● Duration
   b. Chronic - longer clinical course.

2. ● Predominant cell type based on cell maturity and malignant cell type.
   a. Cell maturity
      1) Acute - predominance of immature, undifferentiated BLAST cells. The malignant cell has ability to divide but loses the ability to mature.
      2) Chronic - predominance of differentiated cells, i.e., predominant cells are NOT BLASTS. Accumulation of cells which are able to divide and mature into end-stage cells.
   b. Malignant cell type
      1) Myeloid cells - defect of myeloid stem cell. AML (acute myelocytic/myeloblastic leukemia), myeloblasts CML (chronic myelocytic/myelogeneous leukemia), granulocytes
      2) Lymphoid origin - defect of lymphoid stem cell. ALL (acute lymphocytic/lymphoblastic leukemia), lymphoblasts CLL (chronic lymphocytic leukemia), lymphocytes

3. ● Clinical manifestations/findings
   a. Acute - sudden onset; symptoms and lab findings are the result of bone marrow failure.
      1) weakness and fatigue (due to anemia which may be severe)
      2) petechiae, bruising or bleeding gums (due to low platelet count)
      3) fever or infection (due to neutropenia)
      4) WBC count is variable (low/normal or high)
   b. Chronic - often asymptomatic initially.
      1) no anemia or may have mild anemia
      2) normal or high platelet count
      3) WBC count is usually high
   c. Patients with acute or chronic leukemia may present with:
      1) unexplained weight loss or night sweats (hypermetabolic rate)
      2) splenomegaly, hepatomegaly, lymphadenopathy, bone tenderness, headaches (due to organ and bone marrow invasion).
C. **Diagnosis** – Requires a bone marrow exam [or tissue biopsy/spinal tap]. Goal is identification of malignant cell type...must determine if cell lineage is myeloid or lymphoid for effective response to therapy. Diagnostic methods include:

1. Morphologic features of bone marrow cells – not definitive but important.

2. Cytochemical stains to identify cell enzymes/objects present in certain cell lines.

3. Immunologic analysis to detect CD markers characteristic of myeloid or lymphoid cells.

4. Chromosome analysis to detect abnormal karyotypes diagnostic of certain leukemias.

5. Molecular studies to identify gene mutations (oncogenes) undetectable by karyotyping. Cytogenetic and molecular tests are used to monitor and/or predict response to therapy.

D. **Treatment** - therapy goal is eradication of malignant cells (so that normal cells can grow again):

1. Cytotoxic chemotherapy - directed at specific cell type...myeloid or lymphoid.

2. Radiotherapy - directed at involved organs/tissues.

3. Bone marrow/stem cell transplant - requires donor (allogeneic BMT/SCT) because the bone marrow is involved.

4. Targeted therapies
   a. Act at genetic level to inhibit enzyme activity that causes uncontrolled cell proliferation (Gleevec).
   b. Use monoclonal antibodies to target antigens on malignant cells (Rituximab).

5. Supportive care - RBC/PLT transfusions, antibiotics; rescue with recombinant growth factors (G-CSF, EPO).

E. **Incidence and age distribution**:

1. About 9-10 cases/100,000 population/year.
   a. ½ acute leukemia and ½ chronic leukemia.
   b. males > females

2. Age has influence on leukemia types:
   a. ALL - children 2-10 yr  
   b. AML - adults/infants  
   c. CML - 30 to 50 yr  
   d. CLL - 50 yr or >
F. Comparison of acute and chronic leukemias (using FAB criteria):

<table>
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<tr>
<td>Bone marrow blasts</td>
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<td>Bone marrow cellularity</td>
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II. Acute Leukemias

A. Malignant disease of the blood forming organs where there is progressive infiltration and replacement of the normal bone marrow elements by immature cells...blasts. The malignant clone is arrested at a dividing stage and doesn't terminate division.

B. Acute leukemia is characterized by four findings:

1. An elevated number of blasts in the bone marrow...there must be >30% blasts in the bone marrow to make a diagnosis of acute leukemia (using FAB criteria). The bone marrow is usually hypercellular (>70%). The normal blast % in the marrow is ≤5%.

   Blood findings:
   2. Neutropenia
      Triad of blood findings are a consequence of bone marrow replacement of normal precursors by the blasts.
   3. Anemia
   4. Thrombocytopenia

   Example: WBC = 59,500/ul; HGB = 7.2 g/dl; PLT = 20,000/ul; Diff: 1-1-13-1-0-1-83 blasts

   NOTE: The total WBC/ul is variable in acute leukemia, i.e., the WBC count may be very high with many leukemic cells in the blood or can be normal. The bone marrow blast burden determines the degree of blood involvement. In some cases, blast cells are confined to the marrow (no leukemic cells in the blood).

C. Clinical manifestations and prognosis:

   ● Two main causes of death in acute leukemia are infection and bleeding...poor prognosis.
   1. Infection - due to neutropenia.
   2. Hemorrhage - due to thrombocytopenia.
   3. Tissue infiltration and organ failure...liver, lung, cardiac, renal.

D. Diagnosis:

   1. Presence of >30% blasts in the bone marrow (FAB criteria).
   2. Must identify the malignant cell type as myeloid or lymphoid (using morphology of cells in blood and marrow, cytochemical stains, CD markers, cytogenetics) so that appropriate therapy can be initiated.

E. The goal of treatment is remission...achieved when there are <5% blasts in the bone marrow with normal hematopoietic precursors present; the 1st remission is longest. Patient survival has improved...over 75% of children and one third of adults with acute leukemia survive 5 years without evident disease (influenced by age, specific type of leukemia, etc.).
Proliferating Cell Type in Acute Leukemia

Stem Cell Pool
- Erythrocytic Lineage
  - Rubriblast
  - Proerythroblast
  - Rubocyte
  - Metarubocyte
  - Reticulocyte
- Megakaryocytic Lineage
  - Megakaryoblast
  - Early Megakaryocyte
  - Rubocyte
  - Metarubocyte
  - Reticulocyte
- Monocytic Lineage
  - Monoblast
  - Promonocyte
  - Monocyte
- Granulocytic Lineage
  - Myeloblast
  - Promyelocyte
  - Myelocyte
  - Neutrophil
  - Basophil
  - Eosinophil

Bone Marrow
- Marrow
- Thymus

Blood
- Anemia
- Thrombocytopenia

Maturation Arrest

‘Out of control’
AML
ALL

Neutropenia

PATIENT D
AGE: 16 Y  SEX: M

CBC & DIFFERENTIAL

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CLS416 Clinical Hematology
III. Chronic Leukemias

Proliferation of initially more mature (differentiated) cells of a particular cell line which easily escape the bone marrow so there is better preservation of normal marrow elements

A. Chronic leukemia is characterized by:
   1. Hypercellular bone marrow but less than 30% blast cells (FAB criteria).
   2. Blood findings:
      a. Normal hemoglobin or mild anemia at presentation.
      b. Normal or increased platelets.
      c. An elevated WBC count is commonly present. A high degree of blood involvement is usual since the more mature cells can leave the bone marrow easier than blast cells.

B. Clinical manifestations
   1. Insidious onset with few symptoms initially.
   2. More involvement of spleen, lymph nodes and liver.
## PATIENT C
**AGE:** 82Y  **SEX:** M

### CBC & DIFFERENTIAL

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## PATIENT E
**AGE:** 53Y  **SEX:** M

### CBC & DIFFERENTIAL

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Lab Investigation of WBC Disorders
Infection versus Leukemia (FAB criteria)

Differential Diagnosis (Based on predominant cell type)
- Viral infection
- Bacterial infection (WBC <50.0 K/ul) or neutrophilic leukemoid reaction - NLR (WBC 50.0 -100.0 K/ul)
- Acute leukemia (AML, ALL)
- Chronic leukemia (CML, CLL)
THE USE OF CYTOCHEMICAL STAINS TO DIFFERENTIATE THE ACUTE LEUKEMIAS

Acute Leukemia (Blasts)

+ Peroxidase, Sudan Black B

Acute Myeloid Leukemia  →  Acute Myelocytic Leukemia (AML)

Specific, Chloroacetate esterase (CLE)
Non-Specific esterase (NSE)

CLE +  CLE +  CLE -
NSE -  NSE +  NSE +

Acute Myelocytic Leukemia (AML)
Acute Myelomonocytic Leukemia (AMML)
Acute Monocytic Leukemia (AMonoL)

Acute Promyelocytic Leukemia (AProL)

Erythroleukemia (EL)
Malignant erythroblasts are also PAS +

Acute leukemia ‘panel’ to ID cell line origin

<table>
<thead>
<tr>
<th>Stain</th>
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<th>Myeloblasts (AML)</th>
<th>Lymphoblasts (ALL)</th>
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<tr>
<td>Peroxidase</td>
<td>Enzyme</td>
<td>+</td>
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<tr>
<td>Sudan Black B</td>
<td>Lipid</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PAS</td>
<td>Glycogen</td>
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CD marker

CD33 Surface antigen + -
CD19/20 Surface antigen - +, B cell
CD2/3 Surface antigen - +, T cell
**Cytochemical Stains**
Since it is difficult to differentiate immature cells (blasts) on a Wright's stained smear, cytochemical stains are used to help make a diagnosis through identification of granule or cytoplasmic contents. Very primitive cells may only show positivity at the golgi area.

- Cytochemical stains are done on blood smears (EDTA, heparin, capillary blood) and bone marrow or tissue slides.
- MP, SBB, PAS and esterase stains are most often performed on bone marrow smears to help identify blast cells. Staining results are interpreted in conjunction with surface marker findings to determine the type of acute leukemia.
- LAP stain is performed on blood smears, not bone marrow; TRAP stain is done on marrow/tissue slides or blood. Staining results are mainly used for chronic leukemias.

A. **Peroxidase/Myeloperoxidase (MP) - Kaplow**
   1. Principle: *Cells of the granulocytic series and to a lesser degree the monocytic series contain the enzyme peroxidase in the granules (primary) which is detected by this stain. In the test reaction, peroxidase activity of the cell transfers hydrogen from the substrate to H2O2. Oxygen is liberated which oxidizes the substrate to a blue (color varies) compound at the site of enzyme activity.*
   2. Interpretation: *Positive cells include all stages of neutrophils and monocytes including the blast stage...granules or golgi area. Lymphocytic, erythrocytic, and megakaryocytic cells stain negative.*
      - Used to differentiate blasts of acute myelogenous leukemias (+) from blasts of acute lymphocytic leukemia (ALL) (-).

B. **Sudan Black B (SBB)**
   2. Interpretation:
      *Neutrophilic cells are positive with coarse black granules and monocytic cells are positive with scattered black granules. Lymphocytes, erythrocytic, and megakaryocytic cells stain negatively.*
      - Used to differentiate blasts of acute myelogenous leukemias (+) from blasts of ALL (-).

C. **Periodic Acid Schiff (PAS)**
   1. Principle: *PAS stains intracellular glycogen.
      Glycols + periodic acid oxidizes aldehydes + Schiff's reagent → fuschin that stains glycogen*
   2. Interpretation:
      *Immature lymphoid cells stain positively showing coarse clumps of PAS positive red/pink material. Mature granulocytes are positive. Myeloblasts are usually negative or show fine pink granules. Megakaryocytes may show faint rxn with fine granules. Megakaryocytic cells stain positively. Normal erythrocytic cells stain negatively.*
      - Performed to differentiate blasts of acute myelogenous leukemia (-) from blasts of ALL (+).
      - Also performed to differentiate malignant erythroblasts of EL (+) from normal erythrocytic cells (-).

D. **Esterases**
   1. Principle: *Detects esterase enzyme. Various substrates are incubated with a diazonium salt. Esterase activity will liberate compounds which couple with the salt and cause precipitation at the sites of enzyme activity; positivity and precipitate color will depend on substrate used.*
   2. Interpretation:
      Granulocytic cells and their precursors stain specific esterase (+); monocytic cells and their precursors stain non-specific esterase (+).
      - Performed to differ types of acute myelogenous leukemia...AML, AMML, and AMonoL.
E. **Leukocyte Alkaline Phosphatase (LAP)**

1. **Principle:**
   - Detects activity of the *enzyme alkaline phosphatase* in the cytoplasm of neutrophils…the secondary granules/membrane associated.

   Napthol AS-MX Phosphate (pH 8.6) hydrolyzed by LAP $\rightarrow$ $P_4$ + aryl naptholamide

   Aryl naptholamide + fast red violet (salt) $\rightarrow$ insoluble red dye precipitate (color varies)

2. **Specimen used:** Fingerstick or heparinized blood smears; **NOT bone marrow smears.**

3. **Interpretation:**
   - Neutrophils (bands and segs) are the only cells counted and they are rated 0 - 4+ on the basis of the quantity and intensity of the precipitate within the cytoplasm. The amount of precipitate is proportional to the amount of enzyme present. ● *Generally, high WBC counts with an increased number of neutrophils = high alkaline phosphatase enzyme activity.*

   The sum of the ratings of 100 cells = the LAP score. **Normal LAP score is 13-130.**

4. **Significance:**
   - Mainly performed to differentiate between Chronic myelocytic leukemia (malignant) and neutrophilic leukemoid reactions, most often due to a severe bacterial infection (benign).

   Also performed to differentiate the chronic myeloproliferative disorders (PV, ET, MMM, CML), to differentiate the polycythemias (primary vs secondary) and to monitor remissions.

   ► The LAP stain is **NOT** useful for acute leukemias with predominantly blast cells.

   **High Score (>130)**
   - Neutrophilic leukemoid reaction due to severe bacterial infection
   - Primary polycythemia vera
   - Myelofibrosis (or may be normal)
   - Active Hodgkin's lymphoma
   - Pregnancy (3rd trimester or immediately post-partum) – control

   **Normal score (13-130)** – normal individual
   - Chronic myelocytic leukemia and Hodgkin's disease in remission.
   - Secondary polycythemia
   - Essential Thrombocythemia (or may be ↑)

   **Low Score (<13)**
   - Chronic myelocytic leukemia (diagnostic)
   - Paroxysmal nocturnal hemoglobinuria

F. **Leukocyte Acid Phosphatase** - Tartrate Resistant Acid Phosphatase (TRAP)

1. **Principle:**
   - Acid phosphatase is an enzyme that has been demonstrated in RBCs, WBCs and various tissues. The reaction is the same as the leukocyte alkaline phosphatase except the pH is 5.2 with maroon/red deposits at the sites of acid phosphatase enzyme activity.

2. **Interpretation:**
   - All cells demonstrate positivity but the addition of tartrate (tartaric acid) will inhibit the staining of all cells except those containing tartrate resistant acid phosphatase $\rightarrow$ **Hairy cells are TRAP (+).**

   ● Used primarily to diagnose Hairy cell leukemia in which cells are acid phosphatase positive and resistant to tartrate inhibition.
ACUTE LEUKEMIAS

Proliferative of immature/primitive cells of a particular cell line with replacement of normal bone marrow hematopoietic cells.

● Characterized by acute onset (symptoms related to marrow failure), neutropenia, anemia, and thrombocytopenia. Total WBC/cmm is variable...depends on degree of blood involvement. (May present with elevated WBC and high #'s of circulating blasts in the blood or with a normal WBC and fewer circulating blasts)

A. Acute Myelogenous leukemias - (FAB types M1-M7)

These are acute myeloproliferative disorders - defect of the myeloid stem cell. 90% of cases are adults.

Note: the term myelogenous refers to the bone marrow, and in the context of leukemias, it is used to describe malignancies that myeloid in origin (rather than lymphoid).

1. Acute Myelocytic leukemia (AML) - Also called acute myeloblastic leukemia. 50% of cases. Includes FAB type M₁ (little maturation beyond blast) and FAB M₂ (with maturation beyond blast).

   a. *Middle-aged and older mainly; also <1 year. May present with fatigue, bruises, petechiae, fever, weight loss, mild hepatosplenomegaly.

   b. Bone marrow findings:
      ● Hypercellular with >30% myeloblasts...predominant cell type. (Maturation arrest occurs at myeloblast). Decreased RBC and PLT precursors. Lack of maturing neutrophils = leukemic hiatus.

   c. Auer rods possible in blasts.

   d. Blood findings:
      ● Severe anemia, thrombocytopenia, neutropenia; few nucRBCs. WBC variable, up to 100,000/cmm with myeloblasts.

      Example: WBC = 59.5 K/ul; HGB = 7.2 g/dl; PLT = 20,000/ul; Diff: 1-1-13-1-0-1-83 blasts

   e. Diagnosis:
      >30% myeloblasts in bone marrow (FAB). [20% myeloblasts using WHO]
      Perox (+), SBB (+), PAS (-), CD33 (+), [CLE (+), NSE (-), CD13 (+)]
f. Treatment (to achieve remission):
Chemotherapy (AraC), BMT/SCT, transfusion support, antibiotics, growth factors during aplasia.

g. Prognosis:
Up to 2 years with treatment if remission achieved; better for young adults vs >70yo.
Relapse - die of infection and bleeding.

h. AML is frequently the terminal event for other myeloproliferative disorders (*CML, MMM, PV, EL) and can follow such conditions as PNH, Fanconi's aplastic anemia and the Myelodysplastic syndromes.

2. Acute Promyelocytic leukemia (AProL) - M3. 5% of cases.

a. Adults - rare; bleeding at diagnosis is frequent.

b. Bone marrow findings:
● Hypercellular with >30% promyelocytes (progranulocytes)...predominant cell type.
Decreased RBC and PLT precursors. Lack of maturing neutrophils.

c. Multiple Auer rods may be present in promyelocytes.

d. Blood findings:
Anemia, thrombocytopenia, neutropenia; WBC count is commonly low rather than high.

e. Diagnosis:
>30% promyelocytes in the bone marrow (FAB). [20% promyelocytes using WHO]
Perox (+), SBB (+), PAS (-), CD33 (+), [CLE (+), CD13 (+), CD2(+)]

\textbf{t(15;17) is virtually diagnostic for AProL.}

f. Treatment and prognosis:
● High incidence of **DIC** (Disseminated Intravascular Coagulation) at diagnosis. The widespread clotting is thought to be initiated by abnormal primary granules of promyelocytes. Must control DIC; high risk of cerebral bleeds.

AProL patients with the PML/RARA fusion gene (retinoic acid receptor) respond well to retinoic acid therapy...causes maturation of the malignant promyelocytes.
3. **Acute Myelomonocytic leukemia** (AMML) - M4. 30% of cases.

   a. Young adults and adults greater than 50 years; may present with gum/skin infiltration; headaches due to CNS invasion.

   b. Bone marrow findings:
      Hypercellular with **>30% primitive cells with granulocytic and monocytic characteristics** due to proliferation of the common unipotential stem cell (CFU-GM) that gives rise to both granulocytes and monocytes. Decreased RBC and PLT precursors. Lack of maturing neutrophils.

   c. Auer rods possibly in blasts.

   d. Blood findings:
      Anemia, thrombocytopenia, neutropenia, few nucRBC's. Total WBC up to 100,000/cmm with blasts, cells with granulocytic/monocytic features, and immature monocytes.

   e. Increased serum lysozyme = muramidase - reflects the proliferation of monocytes.

   f. Diagnosis:
      >30% blasts in bone marrow (FAB). [20% using WHO]
      Perox (+), SBB (+), PAS (-), CLE (+), NSE (+), CD33 (+), CD13 (+), CD14 (+).

   g. Prognosis:
      About 2 years with treatment.
      Die of infection and bleeding.

4. **Acute monocytic/monoblastic leukemia** (AMonoL) - M5. 10% of cases.

   a. Older adults; gum and skin infiltration by malignant cells is a common finding; may have CNS involvement.

   b. Bone marrow findings:
      Hypercellular with **>30% monoblasts**...predominant cell type. Decreased RBC and PLT precursors. Lack of maturing neutrophils.

   c. Blood findings:
      Anemia, thrombocytopenia, neutropenia.
      WBC variable, up to 100,000 with large, atypical monocytes and monoblasts.

   d. Increased serum lysozyme = muramidase.

   e. Diagnosis:
      >30% monoblasts in bone marrow (FAB). [20% using WHO]
      Perox (+/-), SBB (+/-), PAS (-), CLE (-), NSE (+), CD33 (+), CD13 (+), CD14 (+).

   f. Prognosis:
      1-2 years with treatment.
      Die of infection and bleeding; DIC may be a problem.
5. **Acute Erythroleukemia** (EL) - M6. 5% of cases.
   a. 40 years or older; splenomegaly common.
   b. Bone marrow findings:
      Shows erythroid hyperplasia with >50% **giant** bizarre-shaped and multinucleated RBC precursors called **megaloblastoid** RBCs; >30% myeloblasts, and decreased normal precursor cells.
   c. Blood findings:
      Anemia with numerous bizarre/giant nucleated red cells, thrombocytopenia, neutropenia WBC rises as disease progresses with increased #s myeloblasts that may have Auer rods.
   d. Phases:
      (1) Erythroleukemia with **increased erythroblasts and myeloblasts**....predominance of abnormal erythroblasts.
      (2) Progresses to acute myelocytic leukemia with predominance of myeloblasts.
   e. Diagnosis:
      >50% erythroid cells in bone marrow and >30% myeloblasts.
      *Malignant erythroblasts are PAS (+); normal erythrocytes are PAS (-). Myeloblasts are Perox (+), SBB (+).
      This disorder may mimic Megaloblastic anemia (see giant red cell precursors) but EL has normal folate and B12 levels…the red cells in EL are termed megaloblastoid.
   f. Prognosis:
      2-23 months from diagnosis. Always terminates in **blast crisis** (AML). Die of infection and bleeding.

6. **Acute megakaryocytic leukemia** (AMegaL) - M7. Rare.
   a. Occurs in any age group; bleeding at presentation.
   b. Bone marrow findings:
      Proliferation of **megakaryoblasts** and atypical megakaryocytes in bone marrow, dry tap Marrow aspirate. Decreased normal precursors.
   c. Blood findings:
      Pancytopenia common.
   d. Difficult to diagnose; PAS (+); CD markers may be helpful (CD61).
   e. Poor prognosis, 2-90 days.

B. **Myelodysplastic syndromes (MDS)** – “Preleukemia” [marrow-abnormal-growth]

1. Occurs in elderly; disorders with high risk of progression to AML. Patients with MDS have up to 30% blasts (FAB) in the bone marrow and are classified into 5 FAB types based on blood and marrow findings. These patients may develop AML with >30% blasts but not always so.
2. The "preleukemic phase" is characterized by blood cytopenias with features of abnormal growth....**dyspoiesis or dysplasia**....in one or more cell lines such as pelgeroid or hypogranular neutrophils, megaloblastoid red cells, elevated # monocytes, bizarre platelets. See classification of MDS on page 178.
C. **Acute Lymphocytic/Lymphoblastic leukemia/ALL** – (FAB types L₁, L₂, L₃)

**Acute lymphoproliferative disorder - defect of lymphoid stem cell**

1. *Occurred primarily in children, 2-10 years is peak age. Splenomegaly, lymphadenopathy, CNS involvement, and bone pain are more commonly seen in ALL than in AML.*

2. Bone marrow findings:
   ● Hypercellular with >30% lymphoblasts...predominant cell type. Decreased RBC and PLT precursors. Lack of maturing neutrophils.

3. Blood findings:
   ● Severe anemia, thrombocytopenia, neutropenia. WBC variable, up to 100,000/cmm with lymphoblasts. **NO** Auer rods.

   Example: WBC = 59.5 K/ul; HGB = 7.2 g/dl; PLT = 20,000/cmm; Diff: 1-13-1-0-1-83 blasts

4. Diagnosis:
   >30% lymphoblasts in bone marrow (FAB). [20% WHO]

   Perox (-), SBB (-), PAS (+/-), CD2/3 (+) or CD19/20 (+), [TdT (+)].

5. Types of ALL: **CD surface markers are very important for the diagnosis of ALL types.**
   a. Early pre-B ALL - 60-75% of childhood cases, CD19/20 positive and CD10 positive. CD10 = common ALL antigen, also known as CALLA (+) type.
   b. T ALL - 15-25%; CD2/3 positive
   c. B ALL - 1-5%; CD19/20 positive, L₃ type = Burkitt’s type.

6. Prognosis:
   **Good in kids with early pre-B, CALLA(+) type, (usually L₁) - high remission rate and cure. Poor prognosis in T or B cell types or types with certain chromosomal translocations; poor in adults (usually L₂). Die of infection and bleeding. ALL can relapse in the CNS; spinal taps are routinely done to monitor during remission.**

7. Treatment:
   Chemotherapy (vincristine), monoclonal antibodies, BMT, transfusions, antibiotics, GF's.

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FAB classification of Acute Leukemias (Traditional classification - acute vs chronic)

Based on the predominate cell type that has proliferated abnormally...myeloid (M) or lymphoid (L)....using the FAB (French-American-British) classification of acute leukemias.

A. Acute Myelogenous/Myeloid leukemias – acute myeloproliferative disorders

1. Characterized by granulocytic cells.
   a. Acute Myelocytic (myeloblastic) leukemia - AML - M
   b. Acute Promyelocytic leukemia - AProL - M

2. Show significant monocytic features.
   a. Acute Myelomonocytic leukemia – AMML - M
   b. Acute Monocytic (monoblastic) leukemia – AMonoL - M

3. Show erythrocytic features.
   a. Acute Erythroleukemia - EL - M

4. Show megakaryocytic features.
   a. Acute Megakaryocytic leukemia – AMegaL - M

B. Acute Lymphocytic (lymphoblastic) leukemias – ALL - acute lymphoproliferative disorders

1. L1 - small, homogeneous lymphoid cells, kids.
2. L2 - large, heterogeneous lymphoid cells, adults.
3. L3 - Burkitt's type, B cell type.

The World Health Organization (WHO) has reclassified all malignant WBC disorders (myeloid and lymphoid). One significant change is the bone marrow blast percent required to make a diagnosis of acute leukemia has been changed to 20% (rather than >30% using FAB criteria).

The FAB classification was originally based on cell morphology and cytochemical stains. The WHO classification incorporates the FAB classification and is based on cellular morphology, cytochemistry, immunophenotyping, genetic karyotype, molecular mutations and clinical findings.
CHRONIC LEUKEMIAS

Proliferation of initially more mature, differentiated cells of a particular cell line which easily escape the bone marrow so there is better preservation of normal marrow elements. Chronic leukemias have greater involvement of the spleen, nodes and liver.

● Characterized by insidious onset, mild anemia if present, normal or high platelets, and an elevated WBC count.....a high degree of blood involvement is usual since the more mature cells can leave the marrow easier.

A. Chronic Myelocytic/Myelogeneous leukemia (CML) [formerly chronic granulocytic leukemia/CGL]

Chronic myeloproliferative disorder - defect of myeloid stem cell.

1. 25-60 years old.

2. Bone marrow findings:
   Hypercellular but <30% blasts. ● Marked increase in granulocytic precursors...predominant cell type, increased M:E ratio. RBC and PLT precursors are present; megakaryocytes often ↑.

3. Blood findings:
   a. WBC = 50-300.0 K/uL - all stages of neutrophils (myelocyte peak); ↑ basos and ↑ eos; a few blasts in blood but <10% in chronic phase; predominant cell type is NOT a blast.
   b. Platelets normal or increased initially; bizarre/giant forms.
   c. Mild anemia if present; may see a few nucRBCs.

Example: WBC = 400,000/cmm; HGB = 11.0 g/dl PLT = 550,000/cmm Diff: 25-25-3-5-4-6 5 metas, 25 myelos, 1 promyelo, 1 blast

4. Hepatosplenomegaly common; weight loss, bone tenderness, leukostasis (WBCs plug vessels). Increased uric acid - reflects granulocyte breakdown.

5. Philadelphia chromosome is a 9/22 translocation that is present in ~95% of CML cases (or higher, depends on detection method). This acquired mutation results in the BCR/ABL fusion protein which has growth promoting activity.

6. Phases:
   a. Chronic phase - 1 to 5 years = CML.
      1) ↑↑ WBC count with granulocyte proliferation.
      2) Bone marrow has <30% blasts; blood has ≤10% blasts.
   b. Accelerated/acute phase - 3 to 6 months = CML in blast crisis → acute leukemia.
      1) >30% blasts in bone marrow with decreased normal precursors and severe anemia, thrombocytopenia, neutropenia and and >10% blasts in blood.
      2) CML always terminated in ‘blast crisis’ unless a transplant was done in chronic phase UNTIL the drug Gleevec. [70% of cases → AML; 30% → ALL]

7. Diagnosis of CML:
   a. Bone marrow shows granulocytic hyperplasia (increased M:E ratio, e.g., 10:1).
   b. Chromosome analysis to detect the Philadelphia chromosome and molecular detection of BCR/ABL mutation.
   c. Low LAP score ( <13)

   ► CML must be differentiated from a neutrophilic leukemoid reaction caused by a severe bacterial infection. Both are characterized by an elevated total WBC count > 50,000/ul and a neutrophilia with immature neutrophils (left shift). The LAP score is high (>130) in NLR.

8. Treatment:
Chemo (bisulfan), irradiate spleen, transplant was done in early chronic phase until ‘smart’ molecular targeted drugs. Gleevec acts to correct the genetic defect…it inhibits tyrosine kinase activity of the BCR/ABL fusion protein that causes cells to proliferate wildly. Gleevec appears to be a lifelong requirement; some patients become resistant.
<table>
<thead>
<tr>
<th>Neutrophilic Leukemoid Reaction (NLR) versus Chronic Myelocytic Leukemia (CML)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NLR (benign)</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>Cause</strong></td>
</tr>
<tr>
<td><strong>LAP score</strong></td>
</tr>
<tr>
<td><strong>Philadelphia chromosome</strong></td>
</tr>
<tr>
<td><strong>Splenomegaly</strong></td>
</tr>
<tr>
<td><strong>Basophilia and Eosinophilia</strong></td>
</tr>
<tr>
<td><strong>Toxic granules, Dohle bodies, Vacuoles</strong></td>
</tr>
<tr>
<td><strong>WBC count &gt;50,000/uL</strong></td>
</tr>
<tr>
<td><strong>Neutrophilia with left shift</strong></td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
</tr>
<tr>
<td><strong>Blasts in peripheral blood</strong></td>
</tr>
<tr>
<td><strong>WBC count &gt;100,000/uL</strong></td>
</tr>
<tr>
<td><strong>Bone marrow cellularity</strong></td>
</tr>
</tbody>
</table>

9. **Prognosis:**
   a. CML to blast crisis (i.e., AML) is about 3 years without Gleevec; die of infection and bleeding. May also have a basophil crisis or develop fibrosis.
   b. The higher the number of circulating blasts or # of basos, the more accelerated the disease...CML (chronic phase) is changing to acute leukemia.

B. **Chronic Lymphocytic leukemia** (CLL)

**Chronic** lymphoproliferative disorder - defect of **lymphoid stem cell**.

1. • 50 years old or greater; males 2:1; often found accidentally. Enlarged lymph nodes and spleen are common findings.

2. Bone marrow findings:
   Initially - clusters of **hyperclumped lymphs...predominant** cell type.
   Later - diffuse infiltration by lymphocytes; bone marrow is hypercellular with increased lymphocytes (but <30% blasts).

3. Blood findings:
   a. WBC = 20,000-200,000/cmm with 80-90% small, **hyperclumped lymphocytes** (very coarse/clumpy nuclear chromatin pattern) and a high number of smudge cells. This lymphocytosis is NOT accompanied by a predominance of reactive lymphs.
   b. No anemia and normal platelets initially; late - anemia develops and platelets decrease.

   **Example:** WBC = 150.0 K/uL; HGB and PLT normal
   **Diff:** 3-2-90-1-1-1  **Hyperclumped lymphs noted**

4. CLL is almost always a **B cell** malignancy – lymphoid cells are CD19/20(+)
   Patients may develop autoantibodies → warm autoimmune hemolytic anemia (WAIHA) and/or hypogammaglobulinemia.

5. Diagnosis of CLL:
   Elevated WBC count due to an absolute lymphocytosis with hyperclumped lymphocytes.
   *Must differ CLL from a reactive lymphocytosis → lymphs are in different stages of activation.*

6. **Prognosis:**
   5 to 10 years; die of infection or unrelated disease. No cure. CLL does NOT terminate in acute leukemia/blast crisis.

7. **Treatment:**
   None for some patients; depends on disease course - control symptoms; prednisone.
C. **Hairy Cell leukemia (HCL)**

**Chronic** lymphoproliferative disorder - **defect of lymphoid stem cell**.

1. Older adults, 50 to 60 or >; males 5:1.

2. Bone marrow findings:
   - **Presence of hairy cells** ➔ lymph-like cells with hairy projections...predominant cell type ("special B cells"). Decreased normal precursors. Bone marrow aspirate often ‘dry tap’ due to marrow fibrosis.

3. Blood findings:
   - Anemia, thrombocytopenia, and leukopenia.....pancytopenia usual. May see circulating hairy cells that indent the red cells. **The KEY to seeing “hairs” is looking in thick part of blood smear.**

4. Present with **massive splenomegaly** - hairy cells infiltrate spleen, also liver and lymph nodes; hypersplenism removes normal cells and worsens pancytopenia.

5. Diagnosis:
   - Hairy cells contain tartrate resistant acid phosphatase....stain TRAP (+). Performed on bone marrow or spleen biopsy slides; blood may be used.

6. Treatment:
   - Long-term remission (90%) with interferon, other drugs; can monitor # of hairy cells with TRAP.

7. Prognosis:
   - >5-10 years but increased infection risk. (Splenectomy and chemotherapy no longer used).
CASE 1
A 43 year old woman presented with a three month history of fatigue, easy bruising and a 15 pound weight loss. She currently has a tooth abscess. She was admitted to the hospital with the following lab findings:

- WBC 39,200/uL
- HGB 6.5 g/dl
- MCV 91.1 fl
- PLT 64 K/cmm

Diff: 2 segs-1 band-5 lymphs-92 blasts
Ab# neutrophils = 1,176/cmm (1800-7500)
Ab# lymphocytes = 1,960/cmm (1000-3400)

Normal RBC and WBC morphology.

What is the most likely disorder?

What would the bone marrow reveal?

Type if the predominant cell type is a myeloblast: _________________

Type if the predominant cell type is a lymphoblast: _________________

What additional testing should be done?

CASE 2
A 80 year old male was being seen in the clinic prior to minor elective surgery. Physical examination revealed lymphadenopathy. Lab data showed:

- WBC 122,000/cmm
- HGB 13.5 gms%
- MCV 85.8 fl
- PLT 200,000/cmm

Diff: 2 segs-2 bands-95 lymphs-1 monos
Ab# neutrophils = 4,880/cmm (1800-7500)
Ab# lymphocytes = 115,900/cmm (1000-3400)

Normal RBC morph; small, hyperclumped lymphocytes were noted; many smudge cells were present. (Reactive lymphs were NOT noted.)

What is the most likely disorder?

What is the predominant cell type?

What would the bone marrow reveal?

CASE 3
A 54 year old male saw his physician because he was experiencing pain in his shoulders and wrists. He also complained of abdominal discomfort and night sweats for the past two weeks. Physical examination revealed a palpable spleen. Lab tests:

- WBC 78.0 x 10^3/cmm
- HGB 12.0 g/dl
- MCV 90.0 fl
- PLT 500 K/uL

Diff: 22 segs-30 bands-14 lymphs-2 monos-4 eos-5 basos-5 metas-17 myelos-1 promyelo

RBC and WBC morphology appeared normal. (Toxic inclusions in the neutrophils were NOT noted.)

What is the most likely disorder?

What is the predominant cell type?

What would the bone marrow reveal?
CHRONIC MYELOPROLIFERATIVE DISORDERS (FAB)

Includes:  
- Primary polycythemia vera (PV)  
- Idiopathic myelofibrosis with myeloid metaplasia (MMM)  
- Primary/Essential thrombocythemia (ET)  
- Chronic myelocytic leukemia (CML) - granulocytic cells are predominant.

Characterized by:  
- Defect of the **myeloid stem cell**.
- Proliferation of all bone marrow cell lines (not lymphoid) but **one line predominant**.
- Hypercellular bone marrow.
- Extramedullary hematopoiesis = *myeloid metaplasia*.
- Giant and bizarre platelets.
- Increased basophils – **a noted finding in CML**

JAK2 kinase mutation is present in almost all polycythemia vera cases and about half of those with essential thrombocythemia & myelofibrosis; BCR/ABL mutation is present in 100% of CML cases.

Transitions between disorders and to AML

---

A. **Polycythemia vera (PV)** - Primary polycythemia, Polycythemia rubra vera, malignant erythrocytosis

1. **Definition:**  
   Chronic myeloproliferative disorder characterized by proliferation of erythrocytic, granulocytic, monocyctic, and megakaryocytic elements in the marrow and extramedullary sites. ●The erythroid line is predominant.

2. **Etiology:**  
   Purposeless malignant hyperplasia of the multipotential myeloid stem cell causing pancytosis,...all cells ↑ (opposite of aplastic anemia). ●This is the only malignant disorder with ↑ RBC/HGB values.

3. **Occurs in middle-aged >50 years. Affects males more frequently than females.**

4. **Clinical features:**  
   - Splenomegaly; hepatomegaly; uncontrollable itching.
   - High blood viscosity (due to ↑↑ RBC mass) and high blood pressure.
   - Ruddy cyanosis - enlarged capillaries.
   - Thrombotic and bleeding episodes - due to abnormal function of malignant platelets.

5. **Blood findings:**  
   **Pancytosis** (only disorder)
   - RBC 7-10 M/ul  
   - WBC 12-30,000/cmm  
   - PLT 400,000 to 1 million/cmm  
   - Hgb 18-24 g/dl  
   - Immature neutrophils  
   - Giant/bizarre platelets  
   - RBC mass ↑  
   - Basos ↑  
   - Normal plasma volume  
   - LAP ↑
   - **EPO level decr^**  
   - ESR ↓↓ (e.g., 0 mm/hr); normocytic rbc, occ NRBCs

^Malignant proliferation of red cells that do not need erythropoietin stimulation.
6. Bone marrow findings: Hypercellular (>70%); M:E ratio usually 3:1....relative % of all cell types in normal range because all cell lines are increased (RBC's are predominant).

7. Treatment: Phlebotomy to reduce RBC mass and blood viscosity. Alkylating agents (leukaran, bisulfan) and radiation with P23.

8. Prognosis: Chronic disease, survive 10 - 20 years. Spent polycythemia - bone marrow wears out; become anemic with hypo/micro rbc's. May die of bleeding/thrombosis. A high percentage of patients develop myelofibrosis or may develop AML.

**Primary polycythemia must be differentiated from other types of polycythemia** (also pgs 134-135)

<table>
<thead>
<tr>
<th>RBC mass</th>
<th>Primary Polycythemia (pancytosis)</th>
<th>Secondary Polycythemia (2⁰ to hypoxia)</th>
<th>Pseudo Polycythemia (hemocentration)</th>
<th>Pseudo Anemia (hemodilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inc</td>
<td>Inc</td>
<td>Dec</td>
<td>Nor</td>
<td>Nor</td>
</tr>
<tr>
<td>Plasma volume</td>
<td>Nor</td>
<td>Nor</td>
<td>Dec</td>
<td>Inc</td>
</tr>
<tr>
<td>Inc</td>
<td>Inc</td>
<td>Inc</td>
<td>Dec</td>
<td>Nor</td>
</tr>
<tr>
<td>WBC count</td>
<td>Inc</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
</tr>
<tr>
<td>PLT count</td>
<td>Dec</td>
<td>Inc</td>
<td>Nor</td>
<td>Nor</td>
</tr>
<tr>
<td>EPO level</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Basos</td>
<td>Inc</td>
<td>Nor</td>
<td>Not done</td>
<td>Not done</td>
</tr>
<tr>
<td>LAP score</td>
<td>Inc</td>
<td>Nor</td>
<td>Not done</td>
<td>Not done</td>
</tr>
</tbody>
</table>

B. **Idiopathic Myelofibrosis with Myeloid Metaplasia (MMM)** - Agnogenic = Idiopathic Myelofibrosis

1. **Definition:** Chronic progressive myeloproliferative disorder characterized by:
   - proliferation of erythroid, granulocytic, monocytic, and megakaryocytic precursors.
   - **leukoerythroblastic blood picture** → nucleated RBC's and immature neutrophils that escape due to:
   - progressive bone **marrow fibrosis**...predominant feature.
   - **myeloid metaplasia** in the spleen and liver = extramedullary hematopoiesis

2. **Etiology:** Cause unknown; malignant disorder of the myeloid stem cell. Thought that megakaryocytes stimulate fibroblasts to produce increased fibrotic tissue in the bone marrow (replacement).

3. Occurs 50-60 years old; 1:1 ratio.

4. Clinical findings:
   *Massive splenomegaly common* - due to myeloid metaplasia/extramedullary hematopoiesis; liver may be enlarged. Bleeding (due to abnormal platelet function) and infection problems can be life-threatening.

5. Blood findings:
   **Leukoerythroblastic blood picture** - presence of immature neutrophils and immature/nucleated red cells is classic finding.

   **Example:** Diff: 14-30-21-5 5 metas, 7 myelos, 10 nucRBC's

   | Early - mild anemia | WBC 12 to 30,000/cmm | PLT #s vary |
   | Late - severe anemia | Immature neutrophils | Giant/bizarre |
   | Nucleated RBC's Basos ↑ | Basos ↑ | Abn plt function |
   | Small teardrops LAP ↑ or N | LAP ↑ or N | |
6. Bone marrow findings:
Bone marrow aspirate frequently ‘dry tap’ (fibrotic bone marrow with increased reticulin and residual islands of precursor cells).

7. Treatment:
Little influence by therapy. Splenectomy controversial. Transplant??

8. Prognosis:
Median 5 years - up to 10 years.
Problems with infection, bleeding, or organ failure. Myelofibrosis may be the terminal phase for polycythemia vera. About 20% terminate in AML.

C. Essential/Primary Thrombocythemia (ET) - Hemorrhagic thrombocythemia

1. Definition:
Chronic myeloproliferative disorder characterized by hyperplasia of the megakaryocytic element…● platelets are predominant and to a lesser degree the other marrow cell lines.

2. Etiology:
Cause unknown; malignant disorder of the myeloid stem cell.

3. Average age 50 years; affects sexes equally.

4. Clinical findings:
Spontaneous excessive bleeding (GI) and/or thrombosis due to malignant platelets with abnormal function. May have splenomegaly due to extramedullary thrombopoiesis.

5. Blood findings:
Thrombocythemia - PLT >1 million/cm3 usual with abnormal platelet function (abnormal bleeding time test and platelet aggregation studies). Platelets are often giant and bizarre; micromegakaryocytes may be found on the blood smear.

   WBC 12-30 K/ul; LAP normal or may be increased; basos may be ↑.
Normocytic anemia during bleeding episodes, occ NRBCs.

   ▶ Must distinguish primary thrombocythemia with a PLT count >1 million/cm3 from reactive thrombocythosis and primary polycythemia:
   - Reactive thrombocythosis can occur following surgery or infection…the PLT count is increased but usually <1 million/ul and the platelets are normal in function.
   - Primary polycythemia…may have a PLT count ≥1 million/cm3 but has pancytosis.

6. Bone marrow findings:
Hypercellular with predominant increase in megakaryocytes (giant).

7. Prognosis:
Good; no treatment if asymptomatic; chemotherapy used to reduce platelet mass. Must control bleeding episodes. May develop AML.
## Findings and Significant Features of Chronic Myeloproliferative Disorders (FAB)

Defect of the myeloid stem cell with proliferation of all cell lines (except lymphs) but one line predominant

<table>
<thead>
<tr>
<th>Predominant Cell Type Affected</th>
<th>Granulocytes</th>
<th>Erythrocytes</th>
<th>Platelets</th>
<th>Fibroblasts</th>
<th>Neutrophils only</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>WBC count/μL</strong></td>
<td><strong>50-100,000 or &gt;</strong></td>
<td>12-30,000</td>
<td>12-30,000</td>
<td>12-30,000</td>
<td><strong>50,000-100,000</strong></td>
</tr>
<tr>
<td><strong>RBC count/μL &amp; Hgb g/dL</strong></td>
<td>Normal or mildly anemic</td>
<td><strong>Increased (Hgb &gt;18)</strong></td>
<td>Normal or anemic</td>
<td>Mild to severe anemia</td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Platelet count/μL</strong></td>
<td>Normal or increased</td>
<td>Increased, up to 1 million</td>
<td><strong>&gt;1,000,000</strong></td>
<td>Variable</td>
<td>Normal, can vary</td>
</tr>
<tr>
<td><strong>Toxic Inclusions</strong></td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td><strong>Present</strong></td>
</tr>
<tr>
<td><strong>Basophilia &amp; Eosinophilia</strong></td>
<td><strong>Prominent</strong></td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>LAP score</strong></td>
<td><strong>Decreased &lt;13</strong></td>
<td>Increased</td>
<td>Increased or normal</td>
<td>Increased or normal</td>
<td><strong>Increased &gt;130</strong></td>
</tr>
<tr>
<td><strong>Philadelphia chromosome</strong></td>
<td><strong>Present (~95%)</strong></td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Gene mutation</strong></td>
<td>BCR/ABL (100%)</td>
<td>JAK2 (100%)</td>
<td>JAK2 (~50%)</td>
<td>JAK2 (~50%)</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Splenomegaly</strong></td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td><strong>Prominent</strong></td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Giant/bizarre platelets</strong></td>
<td>Present</td>
<td>Present</td>
<td><strong>Present</strong></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Marrow Fibrosis</strong></td>
<td>Late in disease</td>
<td>Late in disease</td>
<td>+/-</td>
<td><strong>Prominent</strong></td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Leukoerythroblastic Reaction</strong></td>
<td>Immature granulocytes</td>
<td>+/-</td>
<td>+/-</td>
<td><strong>Prominent</strong></td>
<td>Immature neutrophils</td>
</tr>
<tr>
<td><strong>RBC Morphology</strong></td>
<td>Normal</td>
<td>Normal or microcytic</td>
<td>Normal</td>
<td><strong>Small teardrops</strong></td>
<td>Normal</td>
</tr>
<tr>
<td><strong>Hypercellular marrow</strong></td>
<td>Present</td>
<td>Present</td>
<td>Present</td>
<td>Variable</td>
<td>Variable</td>
</tr>
</tbody>
</table>

CML = Chronic myelocytic leukemia  
PV = Primary Polycythemia vera  
ET = Essential/Primary thrombocythemia  
MMM = Myelofibrosis with myeloid metaplasia

^Leukoerythroblastic reaction = presence of immature neutrophils & nucleated red cells  
^Myeloid metaplasia = extramedullary hematopoiesis

NLR = Neutrophilic leukemoid reaction
LYMPHOMAS [lymph-tumor]

These are lymphoproliferative disorders - **defect of the lymphoid stem cell**.

Definition:
Proliferation of malignant lymphoid cells in solid lymphatic tissues; the lymphoid cells may be primitive, blast-like or differentiated, mature. Lymphoid cells are dispersed through all tissues...especially lymph nodes, spleen, and GI tract. **Lymphomas often begin in the lymph nodes**, with enlarged lymph nodes being the major clinical presentation.

Lymphoma versus leukemia:

**Lymphoma** - initially a localized tumor (-oma means tumor) of malignant cells in **lymphatic tissue** that may spread. The blood count (CBC/Diff) is often normal at presentation. As the disease progresses, tumor proliferation can spread to the bone marrow (and involves the blood).

**Leukemia** - proliferation of malignant cells in **bone marrow**; initially **systemic**, usually involves the blood so the CBC/Diff is often abnormal at presentation.

TWO TYPES of lymphoma:  **HL and NHL**

● **Hodgkin's lymphoma** may spread to the bone marrow in advanced disease but **does not have blood involvement**.

● **Non-Hodgkin's lymphomas** may spread to the bone marrow and the cells then enter the peripheral blood resulting in a **peripheralized** lymphoma with circulating lymphoma cells.

Diagnosis of lymphoma is made by identification of **tissue biopsy/bone marrow cells** using CD surface markers, cytogenetics, molecular analysis. Treatment is chemotherapy, radiation, targeted therapies, autologous BMT/SCT (donor is not needed if marrow not involved), and RBC/PLT transfusion/antibiotic/growth factor support.

A. **Hodgkin's Lymphoma/Disease** (HL, HD) - 40% of lymphomas; **EBV association**.

1. 15-30 years and >50 years; males predominant.
2. Patients usually present with painless enlarged lymph nodes; infiltration of lymph nodes by a variety of cells. The presence of B symptoms (night sweats, fever, itching) have worse prognosis.
3. The **diagnostic feature** of Hodgkin's disease is the presence of the **Reed-Sternberg cell**.
   a. The Reed-Sternberg cell is a malignant cell with two or more nuclei and each nuclei contains a prominent round nucleolus (of B cell lineage).
   b. The RS cell is **only** found in lymph node or lymphatic tissue biopsy. **NOT found circulating in the blood**.
4. Hodgkin's is classed histologically according to the predominant cell types present in the lymph nodes (Rye classification) and staged according to areas affected by the lymphoma (Ann Arbor staging system). Staging determines therapy used and prognosis; staging includes tissue biopsies and bone marrow evaluation.
5. Stages: I - disease is limited to nodes in 1 anatomical site.
   II - >2 anatomical sites on same side of the diaphragm.
   III - both sides of diaphragm involved but confined to lymph nodes or spleen.
   IV - disease involves liver, lungs, skin, kidneys, marrow...**prognosis worst in stage IV**.
6. Blood findings:
   a. Blood counts are often normal or mild anemia of chronic disease (ACD) may develop; increased eos and monocytes.
   b. ↑ LAP score and ↑ ESR result during active disease but both tests are normal during remission; ESR result may be monitored to detect relapse.

7. Prognosis - good if caught in early stage; treatment depends on stage of the disease.

B. **Non-Hodgkin's Lymphomas** (NHL's) - 60% of lymphomas.

   1. 50 to 60 years old; males predominant; very heterogeneous group with good to poor prognosis.

   2. Present with enlarged lymph nodes or abdominal pain due to gastrointestinal (GI) tumors. Frequently have normal blood counts at presentation. Unpredictable spread and certain types commonly spread to bone marrow and involve the peripheral blood = peripheralized lymphoma.

   3. Classed according to predominant cell type; biopsies are performed to determine B or T/NK cell lineage (85% are B cell). The malignant cells vary from small, mature cells to large, primitive lymphoid cells and from slow growing to very aggressive, fast growing types.

   4. Some types:
      a. **Small lymphocytic lymphoma** - SLL
         (1) Proliferation of small, hyperclumped B lymphocytes.
         (2) "Tissue CLL" with same prognosis as CLL.
      b. **Mixed** lymphocytic lymphoma  
         [Follicular cell, Mantle cell]
         (1) Proliferation of malignant lymphoid cells that spread from the tissues to the bone marrow and can involve the peripheral blood  →  **Rieder/clefted cells**.
         (2) Rieder cells are not reported; rather, the malignant lymphoma cells are described as to maturity, size, shape, e.g., clefted or convoluted nucleus.
      c. **Burkitt's lymphoma** associated with **EBV**
         (1) Proliferation of large, immature B cells  →  **Burkitt cells** with fat vacuoles in the nucleus and cytoplasm; high incidence of t(8;14).
         (2) African type - jaw tumor, 2 to 6 years old; rarely spreads, good prognosis.
         (3) American type - stomach tumor, commonly spreads to bone marrow and blood, poor prognosis; same disease as L3 type of ALL but begins in lymphoid tissues.
      d. **Mycosis fungoides** = cutaneous T cell lymphoma
         (1) T cell lymphoma which initially involves the skin; itching/erythroderma  →  plaques  →  ulcerative tumors.
         (2) Lymphoma spreads to the bone marrow = leukemic phase called **Sezary syndrome** with circulating Sezary cells in the blood.

   5. Prognosis for NHL's is good to poor. Some types are very resistant to therapy; problems with defective immunity.

   Many classifications have been used for the non-Hodgkin's lymphomas (International Working classification, REAL and WHO).

**NOTE:** The World Health Organization (WHO) has reclassified all malignant WBC disorders (myeloid and lymphoid). Lymphoid neoplasms are grouped into B cell, T/NK cell and Hodgkin's lymphoma; B and T cell neoplasms are further separated into conditions with precursor cells versus mature cells.
PLASMA CELL DYSCRASIAS

Definitions:
The proliferation of cells involving antibody (Ig) production...plasmacytoid lymphs or plasma cells → B cells.

• Polyclonal gammopathy - ↑ in several immunoglobulins due to antigenic stimulation.

• Monoclonal gammopathy - ↑ in one immunoglobulin type due to a malignant clone...monoclonal peak (M spike or protein) present on electrophoresis.

Multiple myeloma (↑ IgG or ↑ IgA) and Waldenstrom's (↑ IgM) are lymphoproliferative disorders - **defect of the lymphoid stem cell**.

A. **Multiple Myeloma** (MM) - NOT a lymphoma. [multiple-marrow-tumor]

1. Adults 40 or greater; African ancestry 2 times greater than Caucasian.

2. • Proliferation of malignant plasma cells, primarily of the bone marrow...**predominant** cell type. Begins as isolated tumors of plasma cells in the bone that produce excessive globulin of one type – increased production of IgG or IgA is most frequent.

3. Tumors cause bone lesions and bone destruction - causes bone pain and spontaneous fractures.

4. Bone marrow findings:
   a. Initially - **multiple** isolated tumors of enlarged plasma cells...patchy distribution so bone marrow aspirate may miss.
   b. There must be >20% plasma cells in the bone marrow for a diagnosis of MM.
   c. Late - diffuse ‘sheet of plasma cells’ which crowd out normal marrow precursor cells.
   d. May observe Russell bodies in plasma cells (sites of immunoglobulin/antibody), Mott cells (multiple Russell bodies) or Flame cells in IgA type (plasma cells with pink edges).

5. Lab findings:
   a. M spike on protein electrophoresis - ↑↑ amounts of immunoglobulin...half of cases are IgG type, half produce excessive IgA.

   b. Early - **rouleaux of red cells** on blood smear, occ nucRBC's and immature neutrophils; ↑↑ ESR due to spontaneous rouleaux. Clue is blue background on stained blood smear.

   c. Late - may see plasma cells on blood smear, severe anemia, low platelets, neutropenia.

   d. Excess light chains = Bence-Jones protein in urine – “myeloma kidney” causes renal damage; ↑ BUN & creatinine levels.

   e. ↑ calcium levels due to bone destruction by tumors.

6. Problems with infection and bleeding (platelets are coated with Ig); decreased normal antibody production.
7. Treatment:
   a. Chemotherapy/radiation; thalidomide.
   b. Plasmapheresis to reduce blood viscosity; replace bad plasma with normal plasma. Can monitor with serum viscosity test.

8. Prognosis:
   About 2-3 years but has improved with thalidomide therapy.
   Die of infection and bleeding. May terminate in plasma cell leukemia.

B. Waldenstrom's Macroglobulinemia

1. Proliferation of malignant plasmacytoid lymphs..predominant cell which produces excessive IgM globulin. *IgM is the MACRO globulin.

2. Enlarged spleen and liver are common due to infiltration by the malignant cells. This disorder has more tissue/organ involvement than multiple myeloma; may spread to bone marrow late in the disease. Waldenstrom’s acts like a lymphoma and may be termed lymphoplasmacytoid lymphoma.

3. NO bone destruction (as seen in multiple myeloma).

4. Lab findings:
   a. M spike on electrophoresis - ↑↑ amounts IgM.
   b. Marked rouleaux - hyperviscous blood. Blue background on blood smear.
   c. ↑↑ ESR due to spontaneous rouleaux.

5. Problems with bleeding due to platelet coating with IgM; problems with decreased normal antibody production.

6. Bone marrow findings:
   Late - ↑ # of lymphs with plasmacytoid features (aka Turk cells).

7. Treatment:
   a. Chemotherapy.
   b. Plasmapheresis to reduce blood viscosity, may be monitored by serum viscosity test.

8. Prognosis - about 5 years.

Comparison of Multiple Myeloma and Waldenstrom’s Macroglobulinemia

<table>
<thead>
<tr>
<th></th>
<th>Multiple Myeloma</th>
<th>Waldenstrom’s Macroglobulinemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoclonal (M) spike</td>
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<td>Rouleaux of red cells</td>
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<td>Abnormal/high ESR</td>
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<td>+</td>
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<tr>
<td>Lytic bone lesions</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Renal failure</td>
<td>++</td>
<td>-</td>
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<tr>
<td>Elevated calcium levels</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
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<td>++</td>
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<tr>
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<td>+</td>
<td>++</td>
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<tr>
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<td>+</td>
<td>++</td>
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<td>IgG or IgA</td>
<td>IgM</td>
</tr>
<tr>
<td>Malignant cell type</td>
<td>Plasma cell</td>
<td>Plasmacytoid lymph</td>
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</tbody>
</table>

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