Peripheral Smear

- Inexpensive, rapid, reliable
- Window into the functional status of the bone marrow
  - especially cytopenic states
Vs. automated machines

• Able to weigh importance of observed findings and assess their importance within context of clinical data

• Machines tend to generalize a wide array of morphologic abnormalities
Systematic approach

- Slide preparation
- Optimal area of review
- RBCs
  - Size
  - Shape
  - Pallor
  - Premature forms
  - Inclusions
- White cells
  - Lymphocytes
  - Neutrophils
  - Eosinophils
  - Basophils
  - Monocytes
  - Blasts
  - Organisms
- Platelets
Slide preparation

Precipitated Stain
Optimal area of review

Ideal thickness
RBCs

SIZE
RBC Size

• Normal RBC should be the size of a mature lymphocyte nucleus
• Measured by the MCV
Elevated MCV = Macrocytosis
MCV > 100\textmu m^3

- B12/Folate deficiency, aplastic anemia
- Autoimmune hemolytic anemia
- Liver and thyroid disease, alcoholism
- Cold agglutinin disease
Decreased MCV = Microcytosis
MCV < 80\text{um}^3

- Iron deficiency
- Thalassemias
- Anemia of chronic disease
- Hemoglobinopathies
  - C, E, S
SHAPE
<table>
<thead>
<tr>
<th>Type of Cell</th>
<th>Underlying Change</th>
<th>Disease States</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthocyte (spur cell)</td>
<td>Altered cell membrane lipids</td>
<td>Abetalipoproteinemia, liver disease, postsplenectomy</td>
</tr>
<tr>
<td>Bite Cell (degmacyte)</td>
<td>Heinz body pitting by spleen</td>
<td>G6PD deficiency, drug-induced oxidant hemolysis</td>
</tr>
<tr>
<td>Ovalocyte (elliptocyte)</td>
<td>Abnormal cytoskeletal proteins</td>
<td>Hereditary elliptocytosis</td>
</tr>
<tr>
<td>Rouleaux</td>
<td>Circulating paraprotein</td>
<td>Paraproteinemia</td>
</tr>
<tr>
<td>Schistocyte (helmet cell)</td>
<td>Mechanical destruction in microvasculature</td>
<td>TTP, HUS, prosthetic heart valves</td>
</tr>
<tr>
<td>Spherocyte</td>
<td>Decreased membrane redundancy</td>
<td>Hereditary spherocytosis, immunohemolytic anemia</td>
</tr>
<tr>
<td>Stomatocyte</td>
<td>Membrane defect with abnormal cation perm</td>
<td>Hereditary stomatocytosis, immunohemolytic anemia</td>
</tr>
<tr>
<td>Target Cell (codocyte)</td>
<td>Increased redundancy of cell membrane</td>
<td>Liver disease, thalassemia postsplenectomy, HgbC dz, Fe deficiency</td>
</tr>
<tr>
<td>Burr Cell (echinocyte)</td>
<td>Altered membrane lipids</td>
<td>Usually artifactual but maybe uremia</td>
</tr>
<tr>
<td>Tear Drop Cell (dacrocyte)</td>
<td></td>
<td>Myelofibrosis</td>
</tr>
</tbody>
</table>
Acanthocytes (Spur cells)

- Irregular, long, sharply pointed and bent spicules
- Absence of central pallor
- Most commonly seen in liver disease
Absence of apolipoprotein B results in inability to transport triglycerides in the blood.
McLeod phenotype/syndrome

- Absence of erythrocyte surface Kx antigen (part of Kell antigen group)
- Acanthocytes with chronic but well compensated hemolytic anemia
- Disease processes include muscular dystrophy, cardiomyopathy, and “neuroacanthosis”
- May be associated with chronic granulomatous disease
Tear Drop Cells (Dacrocytes)

Myelofibrosis or bone marrow infiltrate
Bite Cells

G6PD deficiency
STOMATOCYTES
RBC with slit-like or rectangular area of central pallor, a mouth

Most often seen in liver disease
Burr Cells (Echinocytes)

Projections - smaller and more regular than acanthocytes

Often Artifactual but seen in UREMIA
Spherocytes
Hereditary spherocytosis

- Northern European ancestry
- Spectrin, ankyrin or band 3 or 4.1 deficiency
Defects in vertical stabilization of the phospholipid bilayer of the RBC membrane cause separation of the spectrin - phospholipid bilayer.
Hereditary spherocytosis

- Normal biconcave red cell loses membrane fragments and adopts a spherical shape
- Inflexible cells are trapped in the splenic cords, phagocytosed by macrophages
Elliptocytes
Hereditary elliptocytosis

- Autosomal dominant trait
- Spectrin abnormality or deficiency of protein 4.1
- Asymptomatic without anemia and usually with no splenomegaly and only mild hemolysis
- RBC hemolysis occurs in the spleen, thus splenectomy corrects the hemolysis, but not the RBC membrane defect.
Target Cells

Characteristic of:

- Liver disease
- Post-splenectomy
- Hemoglobinopathies
  - Thalassemia
  - Hb C, D and E
Canoe cells (aka Taco cells, folded cells)
HgbSC disease with canoe and sickle cells

“Washington Monument crystals”
Schistocytes

Microangiopathic hemolytic anemia

DIC, TTP, HUS
Rouleaux and Clumping

Rouleaux

RBC Clumping
Quiz!

1. 60 yo woman with a history of treated breast cancer now presents with anemia
Most Likely Diagnosis?

- Tear drop cells 2\textsuperscript{o} to bone marrow infiltration with tumor
2. 45 yo man with macrocytic anemia
Most Likely Diagnosis?

• Liver disease
PALLOR
Hypochromic anemia
MCH < 27 pg

- Disorders of globin synthesis
  - Thalassemic syndromes
    - α-Thalassemia
    - B-Thalassemia
- Disorders of heme synthesis
  - Sideroblastic anemias
    - Hereditary (X-linked auto. Dominant)
    - Acquired idiopathic
    - Acquired toxic
- Disorders of Fe metabolism
  - Fe deficiency
  - Chronic disease
  - Neoplasia
Iron Deficiency
Iron deficiency s/p transfusion
PREMATURE RBCs
Reticulocytes

- Decreased cell survival
  - Blood loss
  - Autoimmune HA
  - Nonimmune HA
    - TTP, HUS, DIC
    - Spherocytes
    - G6PD
    - PNH
    - Hemoglobinopathy
    - Thalassemia
Nucleated RBCs

- Not normally present in adult patients PS
- Present in:
  - Severe hemolysis
  - Profound stress or hypoxemia
  - Myelophthisic condition
  - Leukoerythroblastic smear
INCLUSIONS
Basophilic Stippling

Precipitated ribosomes (RNA)

Fine – variety of anemias Sideroblastic, sickle cell, megaloblastic

Coarse – Pb intoxication, thalassemia
Heinz Bodies

- Precipitated denatured Hgb
- Seen in G6PD deficiency
- Seen with supravital staining
  - Crystal violet
  - Brilliant cresyl blue
Howell Jolly Bodies

Dense, usually single

Nuclear remnant

Seen in:
- Postsplenectomy
- Hemolytic anemia
- Megaloblastic anemia
Pappenheimer bodies

Small, dense basophilic granules
Fe-containing mitochondrial remnant or siderosome

Seen in:
- Sideroblastic anemia (Hereditary, idiopathic or secondary)
- Post-splenectomy
LEUKOCYTES
WBC

- Neutrophil
- Eosinophil
- Basophil
- Lymphocyte
- Monocyte
Polymorhponuclear Neutrophils (PMNs)
• Normal range ANC: 1.5-7.0 $10^3$/mm$^3$
• Reflects only the CIRCULATING PMNs
• Does not include marginated PMNs or stored PMNs in the bone marrow
• Mechanisms for neutrophilia:
  – Demargination
  – Release of bone marrow component
  – Increased production
# Mechanisms Causing Nonneoplastic Neutrophilia

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Time Course</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demargination</td>
<td>Minutes</td>
<td>Epinephrine release, acute stress, exercise</td>
</tr>
<tr>
<td>Mobilization of bone marrow</td>
<td>Hours</td>
<td>Corticosteroids, infections, inflammation</td>
</tr>
<tr>
<td>Increased production</td>
<td>Days</td>
<td>Sustained infection, chronic inflammation, CSF-producing tumors, CSF therapy</td>
</tr>
</tbody>
</table>
## Distinguishing Between Reactive Changes and Neoplasia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Reactive</th>
<th>Neoplastic</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>$&lt;30 \times 10^3/\text{mm}^3$</td>
<td>$&gt;50 \times 10^3/\text{mm}^3$</td>
</tr>
<tr>
<td>Toxic Neutrophils</td>
<td>Present</td>
<td>Absent*</td>
</tr>
<tr>
<td>Left Shift</td>
<td>Includes myelocytes</td>
<td>Includes blasts</td>
</tr>
<tr>
<td>Basophilia</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Variable, decreased in sepsis</td>
<td>Increased</td>
</tr>
<tr>
<td>Platelet Morphology</td>
<td>Unremarkable</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Nucleated RBC</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>

*Except in patients with infection
Reactive Neutrophil

3 Features:
Toxic granulations
Dohle bodies
Cytoplasmic vacuoles
Immature Granulocyte Suggesting Neoplasia

Myeloblast

Promyelocyte
The Malignant Mimicker: Leukemoid Reaction

- All precursor granulocytes in the PBS
- WBC in the range up to 100K
- Response to severe stress or infection
- Other signs of malignancy not present
Neutrophil Disorders with Abnormal Morphology

- **Pelger-Huet anomaly**
  - Bilobed or nonsegmented nucleus
  - Asymptomatic

- **May-Hegglin anomaly**
  - Cytoplasmic inclusions resembling Dohle bodies
  - Many asymptomatic

- **Chediak-Higashi syndrome**
  - Giant cytoplasmic granules in all granulocytes
  - Immunodeficiency

- **Hypersegmentation**
  - B12/Folate deficiency, myelodysplasia, myeloid leukemia, chemotherapy, or renal failure
Pelger-Huet Anomaly

- Inherited, AD or acquired
- Acquired = “pseudo” Pelger-Huet as in MDS
Chediak-Higashi Syndrome

- Autosomal recessive
- Giant granules
- Severe immunodeficiency
Hypersegmentation

- Normal to have 4-5 lobes
- Seen most commonly in B12/folate def
- Uremia
- Chemotherapy
- Also seen in MDS and other myeloid neoplasms
- Can be inherited
Eosinophilia

- Allergic/hypersensitivity reactions
- Drug allergies
- Parasitic infections
- Connective tissue/collagen vascular disease
- Neoplasms
  - T-cell lymphoma
  - Hodgkin lymphoma
- Sarcoidosis
- Hypereosinophilic syndrome/Chronic eosinophilic leukemia
Basophilia

- Much more common in malignancies like CML vs. reactive
Reactive Lymphocytosis

• Diseases with nonreactive morphology
  – Infectious lymphocytosis (Whooping cough)
  – Transient stress lymphocytosis

• Diseases with reactive morphology
  – EBV, IM, CMV, Toxo, adenovirus, HHV-6, viral hepatitis
Atypical/reactive lymphocytes

Plasmacytoid lymphocyte

Atypical/reactive lymphocytes
Features of Leukemias/Lymphomas

- T-cell large granular lymphocyte leukemia/NK Cell Leukemia
- Blasts with and without Auer rods
- Hairy cells
- Cleaved cells
- Smudge cells
- "Clover cells"
T-cell LGL Leukemia/NK Cell Leukemia
Small Cleaved Cells

Follicular lymphoma
Smudge Cells

CLL
Hairy Cell Leukemia
Blasts with Auer Rods (Myeloblasts)

Auer Rod = Fused lysosomal granules

AML
Blasts without Auer Rods

Lymphoblasts (ALL) vs. Atypical lymphs
Clover Cells

Adult T-Cell Leukemia/Lymphoma
ORGANISMS
Ehrlichiosis

- Found in the SE and S. Central US
- Transmitted by ticks
- Rickettsial organism
Histoplasma
Babesiosis

- Protozoa
- Endemic in the NE US
- Transmitted by the Ixodidae tick
- Similar to Malaria
- Tetrad form is pathognomonic
- Risk Factors:
  - Post-splenectomy
  - Immunocompromised
Malaria

- Ringed stage (trophozoite)
- Can see other stages within RBCs
PLATELETS
Estimate platelet count on PBS

- 100x oil immersion
- Minimum of 5 fields
- Average # platelets, then X by 20,000
### Platelet Count

<table>
<thead>
<tr>
<th>Increased</th>
<th>Normal</th>
<th>Decreased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Over 20 platelets per HPF</td>
<td>7 – 20 platelets per HPF</td>
<td>Under 7 platelets per HPF</td>
</tr>
<tr>
<td>&gt; 400,000</td>
<td></td>
<td>&lt; 140,000</td>
</tr>
</tbody>
</table>
**Essential thrombocythemia** Peripheral smear from a patient with essential thrombocythemia shows an increased platelet number and clumps of large, abnormal platelets. Courtesy of Carola von Kapff, SH (ASCP).
Giant Platelets

- Size of an RBC
- Usually indicates a hyperreactive bone marrow 2\textsuperscript{o} to underlying condition
  - ITP, TTP, DIC
- Can be inherited in the form of Bernard-Soulier syndrome, platelet dysfunction
Platelet Clumping and Satellitosis

- Causes artificially low platelet counts
- 2° EDTA used in collection tubes

Solution is to use sodium citrate instead of EDTA
Quiz!

- 75 yo asymptomatic man with WBC 60,000
Diagnosis?

- CLL (chronic lymphocytic leukemia)
• 35 yo man with WBC 33,000 and thrombocytopenia
Diagnosis

- Reactive neutrophilia, sepsis
Conclusion

• Systematic approach to reviewing a peripheral blood smear
• All cell lines are evaluated
• MUST take into account the clinical history
• Integrate the information to make a differential diagnosis
• Most peripheral smears are non-specific and have a constellation of findings
White Blood Cell Morphologic Abnormalities Related to Hereditary Disorders

(Part 2)

CP Talk
Jacqueline Nguyen, DO
April 2, 2007 8am
White Blood Cells

- Qualitative abnormalities
  - Hereditary disorders
    - Morphologically abnormal
      - Neutrophil inclusions
      - Abnormal neutrophil nuclei
      - Macrophage/histiocytic abnormalities
Neutrophil Inclusions

• Neutrophil inclusions-inherited
  – Alder Reilley anomaly
  – May Hegglin anomaly
  – Chediak-Higashi Syndrome
Alder Reilley Anomaly

- Resemble the large primary granules of promyelocytes
- Large, purple to purplish-black, coarse azurophilic granules
- No impaired PMN function
- Inclusion is a mucopolysaccharide (PAS+)
Alder Reilley Anomaly

- Vacuolated/abnormally granulated lymphocytes in some case
- Eosinophils and basophils contain large basophilic granules

www.academic.marist.edu/.../HematologyI/7-24.jpg
Alder Reilley Anomaly

- Autosomal recessive
- Associated with several different types of genetic mucopolysaccharide disorders (Hurler, Hunter, San Fillipo, Maroteaux-Lamy, but not Moriquo)

www.academic.marist.edu/.../tn_bloodsmears28.jpg
Alder Reilley Anomaly

- Not specific for one of the mucopolysaccharidoses
- First discovered in Hurler's syndrome
- May be seen following bone marrow transplants and chemotherapy

www.med-ed.virginia.edu/.../wcd/qualitative.cfm
May Hegglin Anomaly

- Thrombocytopenia
- Enlarged platelets
- Variable neutropenia
- Inclusions also seen in eosinophils, basophils, and monocytes
May Hegglin Anomaly

- Autosomal dominant
- Many patients are asymptomatic
- Non-muscle myosin heavy chain A (MYH9) mutation
- No impairment on PMN function

www.bekkoame.ne.jp/.../WBC/photo/MayHeggrin3.jpg
Chédiak-Higashi Syndrome

- Large, well-defined, round to irregular, blue to green-gray cytoplasmic granules (MPO+)
- All granulated cells and even lymphocytes/natural killer cells affected

hsc.unm.edu/pathology/MedLab/images/chediak.jpg
Chédiak-Higashi Syndrome

- Autosomal recessive
- Cytopenias
- Platelet and NK-cell dysfunction

www.pathology.ucla.edu/.../case6/image6.gif
Chédiak-Higashi Syndrome

- Affects many granule-containing cells
  - Melanosomes - partial oculocutaneous albinism
  - Neurons - neurological abnormalities
  - Impaired PMN function - severe, recurrent pyogenic infections (decrease killing and chemotaxis)

http://www.medscape.com/content/2003/00/46/65/466530/art-adnc466530.fig8.jpg
Chédiak-Higashi Syndrome
Quiz

• Match the picture:
  – May Hegglin?
  – Chediak-Higashi?
  – Alder Reilley?
Answer

Alder Reilley

Chediak-Higashi

May Hegglin
Abnormal Neutrophil Nuclei

- Pelger-Huët anomaly
- Hereditary hypersegmentation of neutrophils
Pelger-Huët Anomaly

- Bilobed (pince nez) or non-segmented neutrophil nuclei seen in most PMNs
- Coarse clumping of the nuclear chromatin in neutrophils, lymphocytes, and monocytes
Pelger-Huët Anomaly

- Autosomal dominant
- No other lineage abnormalities
- No functional abnormalities
- Mutations in lamin β-receptor (LBR gene on Chrom 1)

http://www.bphealthcare.com/healthcare/galleries/haem/case5-a1.jpg
Pelger-Huët Anomaly

- Heterozygous in good health, and their natural resistance to infection is unimpaired
- Homozygous PHA is associated with skeletal anomalies, developmental delay, and seizures
Hereditary Hypersegmentation

- AKA Undritz anomaly
- More than 3 cells having 5 lobes or a single cell with 6 lobes found in the course of a 100 cell differential (or 5% with 5 lobes)

http://www.med-ed.virginia.edu/courses/path/innes/images/wcdjpeg/wcd%20hyperseg.jpeg
Hereditary Hypersegmentation

- Autosomal dominant
- No other abnormalities
- No associated findings

Wright's x1000
Histiocyte/Macrophage Abnormalities (Bone Marrow Cells)

- Inherited abnormalities
  - Gaucher cell
  - Niemann Pick cell
Gaucher Cell

- Crumpled tissue-paper cytoplasm
- Caused by enlarged, elongated lysosomes filled with glucocerebroside
- Accumulation in BM, liver, spleen, and lungs leads to pancytopenia, hepatosplenomegaly, and pulmonary disease
- Infiltration in BM causes thinning of the cortex, pathologic fractures, bone pain, bony infarcts, and osteopenia

http://pathcuric1.swmed.edu/PathDemo/gen1/gen130.jpg
Gaucher Cell

- Seen in
  - Gaucher’s disease
  - Myeloproliferative syndromes (CML) = “pseudo-Gaucher” cells

[Image of Gaucher cell]

http://www.sfu.ca/biology/faculty/kermode/laboratory/gaucher-cells.jpg
Gaucher Disease

The enlarged spleen shows effacement of white pulp and massive expansion of pale red pulp.

pathology.catholic.ac.kr/.../genetic/ge13.jpg
Gaucher Cell

- Acid phosphatase +
- PAS+
- Labs
  - ↓ in WBC acid β-glucosidase
  - ↑ in serum acid phosphatase
  - ↑ in serum ACE

http://www.neuropathologyweb.org/chapter10/images10/10-GCl.jpg
Gaucher Cell

- Enzyme replacement therapy now available (imiglucerase [Cerezyme])

http://www.academic.marist.edu/~jzmz/HematologyI/MicroexamBM25.jpg
Niemann Pick Cell

- Foamy, vacuolated cytoplasm
- Accumulation of sphingomyelin
- Seen in Niemann-Pick disease (sphingomyelinase deficiency), Wolman disease, cholesterol ester storage disease, lipoprotein lipase deficiency, and, GM1 gangliosidosis type 2

http://www.thecrookstoncollection.com/Collections/medslides/Slides/Niemann-pick-cell.jpg
Niemann Pick Cell

- Weakly PAS+
- Birefringence on polarized light
- Yellow-green on UV

http://pathology.catholic.ac.kr/pathology/specimen/genetic/ge13.jpg
Niemann Pick Disease

• Systemic involvement
  – Progressive lung disease
  – Hepatosplenomegaly
  – Short stature
  – Pancytopenia

• Lab findings
  – ↓ WBC sphingomyelinase
  – May see vacuolation of PB lymphocytes and monocytes
Niemann Pick Disease

• No specific treatment available
• Generally more rapid clinical course than Gaucher’s disease
NORMAL CELL BREAKDOWN AND DIGESTION BY MACROPHAGES

Globoside or Ganglioside
- glycolipid and phospholipid debris released when PMNs and other cells die

Sphingomyelin
- Ceramide
  - Glucosidase
  - Sphingomyelinase
  - These two enzymes are needed for lysosomal degradation of glycolipid and phospholipid debris released from cells when they die. The enzymes cleave ceramide from the rest of the molecule. In Gaucher's disease, different enzymes cleave all but the ceramide-glucose portion. In Niemann-Pick disease, sphingomyelin remains intact. Undigested lipids accumulate in the lysosomes of macrophages.

ACCUMULATION OF UNDIGESTIBLE LIPIDS DUE TO ENZYME DEFICIENCY

Quiz

• Name that cell
  – Gaucher cell?
  – Niemann Pick cell?
Answer:

Gaucher cell

Niemann Pick cell
References

- Kjeldsberg, Practical Diagnosis of Hematologic Disorders
- Osler Notes
- Internet