PERIPHERAL BLOOD SMEAR REVIEW

Daniel Baiyee, MD
Complete Blood Count

- Automated cell counting
- Peripheral blood morphology
Lecture Outline

- COMMON RED BLOOD CELL CHANGES
- NORMAL SMEAR, ARTIFACTS, & REPORTING
- ANEMIA DUE TO ABNORMAL/IMPAIRED HB SYNTHESIS
- ANEMIA DUE TO ABNORMAL/IMPAIRED DNA SYNTHESIS
- HEMOLYTIC ANEMIA
- INFECTIOUS DISEASES
- PLATELET MORPHOLOGY
- WHITE BLOOD CELLS >> NEXT SESSION
Peripheral Blood

- About 55% of blood is liquid
- Liquid portion of blood (before clotting) = plasma
- Liquid portion of blood (after clotting) = serum
- Cellular part of blood (45% of volume) is made up of a variety of different cell types
- Hematocrit (Hct) measures the volume occupied by the cells in the blood

Figure 12-1
Normal Peripheral Smear

“More information can be gained from examining the blood smear than from any single hematologic procedure”
Hematology Analyzer: Limitations

- Abnormalities and inclusions in WBC
- RBC shape abnormalities
- RBC inclusions
- Platelet abnormalities and clumping
Cellular Components of Blood

• **Erythrocytes**
  – Red blood cells (RBCs)

• **Leukocytes**
  – Also called white blood cells (WBCs)
  – 5 majors types:
    • Neutrophils (60%)
    • Lymphocytes (30%)
    • Monocytes (7%)
    • Eosinophils (2%)
    • Basophils (1%)

• **Platelets**
  – Also called thrombocytes
Indications for Examination of a Blood Smear

- Anemia, unexplained
- Thrombocytopenia or neutropenia
- Lymphoproliferative disorder
- Myeloproliferative disorder
- DIC
- Acute renal failure
- Infectious diseases that can be diagnosed on a smear
- Non-hematopoietic cancer (weight loss, bone pain)
- General ill health (malaise, fever)
Normal Peripheral Blood

Best area of slide to look at
Smear review

Observations Under $\times 10$, Then $\times 100$ or $\times 50$

- Check to see if there are good counting areas available free of ragged edges and cell clumps.
- Check the WBC distribution over the smear.
- Check that the slide is properly stained.
- Check for the presence of large platelets, platelet clumps, and fibrin strands.
Observing direction:

Observe one field and record the number of WBC according to the different type then turn to another field in the snake-like direction.

*avoid repeat or miss some cells*
Normal blood smear
RBCs
Erythroid maturation

- Early Pronormoblast
- Pronormoblast
- Basophilic normoblast
- Polychromatophilic normoblast
- Early orthochromic normoblast
- Orthochromic normoblast
- Reticulocyte
- Normal RBC
Term neonatal blood smear
Premature neonatal blood smear
Neonatal blood smear
Neonatal blood smear
FEATURES OF NEONATAL BLOOD SMEARS
MORPHOLOGIC CHANGES DUE TO AREA OF SMEAR

- **Thin area** - Spherocytes which are really "spheroidocytes" or flattened red cells. True spherocytes will be found in other (Good) areas of smear.

- **Thick area** - Rouleaux, which is normal in such areas. Confirm by examining thin areas. If true rouleaux, two-three RBC's will stick together in a "stack of coins" fashion.
Common causes of a poor blood smear

1. Drop of blood too large or too small.
2. Spreader slide pushed across the slide in a jerky manner.
3. Failure to keep the entire edge of the spreader slide against the slide while making the smear.
4. Failure to keep the spreader slide at a 30° angle with the slide.
5. Failure to push the spreader slide completely across the slide.
6. Irregular spread with ridges and long tail: Edge of spreader dirty or chipped; dusty slide
7. Holes in film: Slide contaminated with fat or grease
8. Cellular degenerative changes: delay in fixing, inadequate fixing time or methanol contaminated with water.
Biologic causes of a poor smear

1. **Cold agglutinin** - RBCs will clump together. Warm the blood at 37° C for 5 minutes, and then remake the smear.

2. **Lipemia** - holes will appear in the smear. There is nothing you can do to correct this.

3. **Rouleaux** - RBC’s will form into stacks resembling coins. There is nothing you can do to correct this.
Common Artifacts

- Fine stippling in reticulocytes (slow air-drying)
- Crenated cells: Common artifact (Aged Blood, Elevated PH, contact with glass and exposure to moisture)
- EDTA effects on blood cells
- OLD Blood
- Artifactual Changes in RBC, WBC and Platelets
Age-related changes

**RBC:**
-- Crenation (echinocyte formation), lysis, hemoglobin crystallization.

**WBC:**
-- Swelling and smoothing of the nuclear chromatin (mimicking band neutrophil formation), pyknosis and karyorrhexis of nuclei, cell smudging, and prominence of Dohle bodies (mimicking toxic change).

**Platelets:**
-- Clumping and, degranulation.
EDTA-related changes

**RBC:**

Extreme crenation of the erythrocytes
-- Masking or rendering suspect significant pathologic shape abnormalities.

**WBC:**

Pyknosis and karyorrhexis
-- Making certain cell identification impossible

**Platelet:**

Platelet clumps, Satellitosis
FRESH  EDTA 48h at RT  Overnight in Fridge
Fine stippling in reticulocytes
Bacterial contamination
ABNORMAL DIFFERENTIALS

1. 200 Cell diff:
   a. WBC > 15.0 (>20.0 for babies under 1 month and labor unit)
   b. Three or more basophils seen.
2. If more than five immature WBC's are seen (or any blasts) let someone else diff slide and average results.
3. Correct WBC for NRBC's if you seen ten or more NRBCs/100 WBC.
4. Always indicate number of cells counted on diff.
5. If any cell type is extremely elevated (such as bands, monos, or eos > 20) indicate that you are aware of the abnormality by circling or checking on the card next to the results.
Reporting results

• Where possible use macrocytic and microcytic, rather than simply anisocytosis alone, when describing red cell morphology.

• Use specific cell morphology when possible, rather than simply reporting poikilocytosis.

• When red cells are normocytic, normochromic, report out as NORMAL. When abnormal morphology has been noted, DO NOT indicate normal on the report form.

• EXAMPLE: 7-10 microcytic RBC's/OIF is reported out as: 2+ microcytosis or Moderate microcytosis.
# RBC Quantification

## Table 1: Qualitative Grading of RBC Morphology

<table>
<thead>
<tr>
<th>Grade Degree of Abnormalities</th>
<th>1 to 5 cells/10 fields</th>
<th>6 to 15 cells/10 fields</th>
<th>&gt;15 cells/10 fields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marked</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Peripheral Blood Morphology
RBC

Manual differential And Morphology
Red Blood Cells

Features:

1. Size and shape.
2. Relative hemoglobin content.
3. Polychromasia.
4. Inclusions.
5. Rouleaux formation or agglutination.
Normal Peripheral Smear
Common Red Blood Cell Changes
<table>
<thead>
<tr>
<th>Inclusion</th>
<th>Composition</th>
<th>Appearance</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basophilic stippling</td>
<td>Precipitated ribosomes</td>
<td>Evenly dispersed fine or coarse granules</td>
<td>Lead poisoning, thalassemia, other anemias</td>
</tr>
<tr>
<td>Howell-Jolly bodies</td>
<td>Nuclear fragment</td>
<td>Dense, round blue granule</td>
<td>Postsplenectomy</td>
</tr>
<tr>
<td>Pappenheimer bodies</td>
<td>Iron-containing granules</td>
<td>Small blue granules in clusters</td>
<td>Anemias</td>
</tr>
<tr>
<td>Organism</td>
<td></td>
<td>Small blue inclusion</td>
<td>Malaria, Babesiosis</td>
</tr>
</tbody>
</table>
Basophilic Stippling
Malaria
RBC Inclusions: Composite
RBC Distribution Abnormalities

- **Rouleaux formation**: Stacking of RBCs due to increased plasma proteins coating RBCs

- **Agglutination**: Antibody-mediated clumping; temperature dependent
Agglutination Reaction
Variations in RBC Size and Shape

- **Anisocytosis** Variations in size (e.g. microcytes)
- **Poikilocytosis** Variations in shape (e.g. target cell)
- **Hypochromia** Increased central pallor due to decrease in hemoglobin
Hypochromic Microcytic RBC
Severe Hypochromia: Iron Deficiency Anemia
Microcytic Hypochromia: Beta Thalassemia Major
Macrocytic Anemia: Macro-Ovalocytes
<table>
<thead>
<tr>
<th>Terminology</th>
<th>Description</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target cells</strong></td>
<td>Central hemoglobin; target-shaped</td>
<td>Liver disease; thalassemia; Abnormal Hgb; iron deficiency</td>
</tr>
<tr>
<td><strong>Echinocyte</strong></td>
<td>Short spicules, equally-spaced</td>
<td>Uremia, hypokalemia, artifact</td>
</tr>
<tr>
<td><strong>Acanthocyte</strong></td>
<td>Spiculated, irregular</td>
<td>Liver disease (alcohol), Post-splenectomy</td>
</tr>
<tr>
<td><strong>Spherocyte</strong></td>
<td>Spherical, no central pallor</td>
<td>HS, Immune hemolytic anemia</td>
</tr>
<tr>
<td><strong>Schistocyte</strong></td>
<td>Fragmented RBC, helmet cells</td>
<td>MAHA, burns</td>
</tr>
<tr>
<td><strong>Ovalocyte</strong></td>
<td>Oval/elliptical shaped</td>
<td>Hereditary elliptocytosis, Megaloblastic anemia</td>
</tr>
<tr>
<td><strong>Sickle cell</strong></td>
<td>bipolar spiculated shape</td>
<td>Hgb S-containing hemoglobinopathy</td>
</tr>
<tr>
<td><strong>Teardrop cell</strong></td>
<td>single elongated extremity</td>
<td>Myelophthistic changes</td>
</tr>
<tr>
<td><strong>Bite cells</strong></td>
<td>Irregular gap in membrane</td>
<td>G6PD deficiency</td>
</tr>
</tbody>
</table>
Echinocytes (Burr Cells)
Burr + Spur Cells: Hepatorenal Syndrome
Burr cells (echinocytes).
Echinocytes
(“Crenated cells, burl cells”)

- Uremia
- HUS
- Crenated cells: Common artifact (Aged Blood, Elevated PH, contact with glass and exposure to moisture)
- Post-splenectomy
- Hepatitis of the newborn
- Malabsorption states
- After administration of heparin
- Pyruvate kinase deficiency
- Phosphoglycerate kinase deficiency
Spherocytes
Spherocytes: Autoimmune Hemolytic Anemia
RBC = 2 Helmet Cells

Point of fold

RBCs = 1 Schistocyte
1 Helmet Cell

= Fibrin

micro thrombi, narrow vessels, attachment

RBCs in circulation

RBCs in circulation
Elliptocytes: Hereditary Elliptocytosis
Sickle Cells
Target cells in Hemoglobin SC Disease
Hemoglobin S-Beta Thalassemia
Teardrop Cells
Bite Cells
Heinz Bodies
Stomatocyte

- Hereditary or acquired hemolysis
- Hereditary stomatocytosis
- Alcoholic cirrhosis & acute alcoholism
- Obstructive liver disease
- Malignancy,
- Severe infection
- Treated acute leukemia
- Artifact.
Polychromatophilic red cells
Echinocytes
(“Crenated cells, burr cells")

- Uremia
- HUS
- Crenated cells: Common artifact (Aged Blood, Elevated PH, contact with glass and exposure to moisture)
- Post-splenectomy
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RBC = 2 Helmet Cells

micro thrombi, narrow vessels, attachment

= Fibrin

Point of fold

RBCs = 1 Schistocyte 1 Helmet Cell

RBCs in circulation
Schistocytes and Helmet cells ('Fragmented cells')

- Sepsis
- DIC
- microangiopathic hemolytic anemia
- thrombotic thrombocytopenic purpura
- Hemolytic uremic syndrome
- Mechanical (Prosthetic heart valves, severe valvular stenosis)
- Malignant hypertension
- Drugs (e.g. cyclosporin)
- Normal newborns
- Eclampsia (HELLP syndrome)

- pyruvate kinase deficiency
- Glomerulonephritis
- Renal graft rejection
- severe burns
- Severe iron deficiency,
- Thalassemias
- Myelofibrosis with myeloid metaplasia
- Hypersplenism
- Vascular (cavernous hemangiomas and some vasculitis)
Macrocyte

Oval macrocyte
- Accelerated erythrocytosis.
- Myelodysplastic syndrome
- Megaloblastic anemia
- Fanconi anemia
- Congenital dyserythropoietic anemia (CDA types I and III)

Round macrocyte
- Liver disease
- Alcoholism
- Aplastic anemia
- Hypothyroidism
Rouleaux Formation
Acanthocytes (Spur Cells)
Acanthocyte ("Spur cells")

- Abetalipoproteinemia,
- Asplenia (postsplenectomy)
- Alcoholic cirrhosis
- Anorexia nervosa
- Alimentation (hyperalimentation)
- Liver failure
- Kidney failure
  - Hemolytic anemia (HA) – microangiopathic anemia, autoimmune hemolytic anemia
  - Sideroblastic anemia
  - Thalassemia
  - Severe burns
  - Pyruvate kinase deficiency
  - McLeod phenotype
  - Infantile pyknocytosis
Target and Spur cells: Changes in Liver Disease

Target Cells

Spur Cells
Howell-Jolly bodies

- Post-splenectomy
- Functional asplenia (hyposplenism)
- Acute hemolysis
- Megaloblastic anemia
- Myelophthistic anemia.
Anemia Due to Abnormal/Impaired Hb Synthesis
Microcyte

- Iron deficiency
- Thalassemias
- Anemia of chronic disease
- Lead poisoning
- Sideroblastic anemia
Basophilic stippling

- Heavy metal poisoning (e.g. lead and arsenic)
- Hemoglobinopathies
- Thalassemias
- sideroblastic anemias
- pyrimidine-5’-nucleotidase deficiency
Target cells

- Splenectomy
- Thalassemia
- Hemoglobinopathies (hemoglobin SS, SC, CC, EE, AE, sickle cell-thalassemia)
- iron deficiency anemia
- liver disease
- Postsplectomy
- familial lecithin-cholesterol acyltransferase (LCAT) deficiency.
Nucleated red blood cells ("NRBCs")

- Acute bleeding
- Severe hemolysis
- Myelofibrosis
- Leukemia
- Myelophthysis
- Asplenia
- Newborn
Target cells

- Splenectomy
- Thalassemia
- Hemoglobinopathies (hemoglobin SS, SC, CC, EE, AE, sickle cell-thalassemia)
- iron deficiency anemia
- liver disease
- Postsplectomy
- familial lecithin-cholesterol acyltransferase (LCAT) deficiency.
Sickle cells

- **Hb S hemoglobinopathies:**
  - sickle cell anemia
  - hemoglobin SC disease
  - hemoglobin S-beta-thalassemia
  - hemoglobin SD disease
  - hemoglobin Memphis/S disease

Other hemoglobinopathies
  - Hb I, Hb CHarlem, HbCCapetown
Anemia Due to Abnormal/Impaired DNA Synthesis
Hemolytic Anemia
Autoagglutination

- Anti-RBC antibody
- Paraprotein
- Cold agglutinin disease
- Autoimmune hemolytic anemia
- Macroglobulinemia
- Hypergammaglobulinemia
Rouleaux Formation
Rouleaux

- Acute and chronic inflammatory disorders
- Plasma cell dyscrasia
  - Waldenstrom's macroglobulinemia
  - Multiple myeloma.
Spherocytes

- Congenital (hereditary) spherocytosis
- Immune hemolytic anemias
- Microangiopathic hemolytic anemia (MAHA)
- Hypersplenism and post-splenectomy
- Myelofibrosis with myeloid metaplasia
- ABO/RH incompatibility
- Normal in newborn
- Oxidative changes – some hemoglobinopathies
- Malaria,
- Liver disease,
- Older population of transfused cells
- Artifact.
- Microspherocytes
  - severe burns
  - hereditary pyropoikilocytosis.
Spherocytes result from rigid surface membrane causing Heinz Bodies to precipitate (not visible with Wright-Giemsa stain).

- Culling will cause loss of Surface membrane.

HbC must round up to form spherocyte however, membrane is rigid causing pressure points in the RBC leading to hemoglobin puddling.

To Spleen
(will selectively cull Heinz Bodies)
Bite cells

- Oxidant stress.
- Normal individuals receiving large quantities of aromatic drugs (or their metabolites) – e.g. dapsone, Quinines, Doxorubicin.
- Individuals with red-cell enzymopathies involving the pentose phosphate shunt (most notably G6PD deficiency).
The mechanism of spherocyte formation in Heinz body anemia.
Blister cells

- Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency.
- Other oxidant stress
Spherocytes

- Congenital (hereditary) spherocytosis
- Immune hemolytic anemias
- Microangiopathic hemolytic anemia (MAHA)
- Hypersplenism and post-splenectomy
- Myelofibrosis with myeloid metaplasia
- ABO/RH incompatibility
- Normal in newborn
- Oxidative changes – some hemoglobinopathies
- Malaria,
- Liver disease,
- Older population of transfused cells
- Artifact.
- Microspherocytes
  - severe burns
  - hereditary pyropoikilocytosis.
Tear drops

- Myelophthisic anemia
- Myelofibrosis (primary or secondary)
- Myelodysplastic syndrome in low number
- Thalassemia
- Megaloblastic anemia
  - Anemia of renal failure
  - Tuberculosis
  - Heinz body disease
  - Hemolytic anemias
- Hypersplenism.
Elliptocytes (ovalocytes)

- Hereditary elliptocytosis
- Southeast Asian ovalocytosis
- Hereditary pyropoikilocytosis
- Iron deficiency anemia (e.g. pencil cells)
- Megaloblastic anemia
- Myelophthisic anemia
- Myelodysplastic syndrome
  - Thalassemia,
  - Sickle cell trait
  - Hb C trait
  - Cirrhosis
  - Decreased erythrocyte glutathione
  - Glucose-6-phosphate deficiency
  - Hereditary hemorrhagic telangiectasia
  - Mechanical trauma
RBC, RBC INCLUSIONS AND THEIR MIMICS

- ACANTHOCYTE VS ECHINOCYTE
- SPHEROCYTE VERSUS PSEUDOSPEROCYTE
- HOWELL-JOLLY BODIES VS PAPPENHEIMER BODIES
- PLATELET OVERLYING A RED CELL VERSUS HOWELL-JOLLY BODIES
- PLATELET OVERLYING A RED CELL VERSUS INTRACELLULAR ORGANISMS (RING FOORM OF BABESIA OR PLASMODIUM SPECIES)
RBC, RBC INCLUSIONS AND THEIR MIMICS

- BASOPHILIC STIPPLING VERSUS CLUSTERS
  PAPPENHEIMER BODIES IN RBC
- ROULEAUX VERSUS AGGLUTINATION
- ELLIPTOCYTES VERSUS PARTIALLY SICKLED RED CELLS
- TEAR DROP VERSUS TEAR DROP-LIKE CELLS
- PARTIALLY SICKLED CELLS VERSUS SICKLE-LIKE CELLS
- BLISTER CELLS VERSUS BLISTER-LIKE CELLS