Correlation of *Peripheral blood smears* and *CBC* in general hematopoietic abnormalities

Osvaldo Padilla, MD, MPH
*** What is your general area of specialty?

A. Internal medicine
B. Radiology
C. Psychiatry
D. OB/Gynecology
E. Pediatrics
F. Family practice
G. Surgery
H. Pathology
I. Other
*** How often do you see CBC studies?

A. All the time (>1 case / 3 month)
B. I see them frequently (about 1 case/ 3 month)
C. I do not see them frequently (1 case / year).
D. Never; only in board exams
E. Never have any exposure to them (not even for exams)
*** How often do you see peripheral blood smears studies?

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C. I do not see them frequently (1 case / year).
D. Never; only in board exams
E. Never have any exposure to them (not even for exams)
Conclusions........
White Sands National Park: 1.5 hours from El Paso, TX
Pre-test
*** Is MCV directly measured by the CBC machine?

A. Yes, the machine measures MCV directly
B. No, the machine does not measure MCV directly
C. Cannot be determined
D. I could answer, but I then I would be guessing

B. No, the machine does not measure MCV directly
*** What does this peripheral blood smear most say about the RDW?

A. It is increased

B. It is normal

C. It is decreased

D. Cannot be determined

A. It is increased
What does this peripheral blood smear most say about the patient?

A. Possible myeloma
B. Possible hemolysis
C. Possible carcinoma
D. Cannot be determined

A. Possible myeloma
What does this peripheral blood smear most say about the RBC number?

A. It is increased
B. It is normal
C. It is decreased
D. Cannot be determined

C. It is decreased
What does a CBC machine measure directly and what does it calculate?

• Leukocyte count
• Platelet count
• Percentage of different cell types
• Nuclear/granular complexity
• MCV (mean corpuscular volume)
• MCH (mean corpuscular hemoglobin)
• MCHC (mean corpuscular hemoglobin concentration)
• Hemoglobin amount
• RBC volume
• RDW (RBC distribution width)
• Hematocrit
What does a CBC machine measure directly and what does it calculate?

**Directly measure by CBC machine**
- Leukocyte count
- Platelet count
- Nuclear/granular complexity
- Hemoglobin amount
- RBC volume
- Hematocrit

**Calculated by CBC machine**
- Percentage of different cell types
- MCV (mean corpuscular volume)
- MCH (mean corpuscular hemoglobin)
- MCHC (mean corpuscular hemoglobin concentration)
- RDW (RBC distribution width)
General assessment - Complete Blood Count (CBC)

Results giving:
- RBCs (total number of RBCs)
- Hgb concentration
- Hct
- MCV
- MCH
- MCHC
- WBCs (total number of WBCs)
- Percent of each type of WBC
- Total number of platelets
How a CBC machine works?

Lyse RBCs for [Hb]
Detector at 180 degrees from incoming laser will “count” each cell and estimate its size as demonstrated in the picture above.

- Detector at 180 degrees from incoming laser will “count” each cell and estimate its size as demonstrated in the picture above.
Detector at 90 degrees from incoming laser will estimate the cell’s complexity from its granular and nuclear reflection of the laser as demonstrated in the picture above.
CBC machine approximates concentration of hemoglobin of the entire sample.

- Intensity of colored reaction indirectly measures amount of HGB within an established volume.
- Important to fill collecting tubes to established volume.
Complete Blood Count (CBC)

- Interpretation / Limitations:

  Each dot represents a cell.

  See blue box

<table>
<thead>
<tr>
<th>WBC</th>
<th>6.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE</td>
<td>52.6</td>
</tr>
<tr>
<td>LY</td>
<td>36.7</td>
</tr>
<tr>
<td>MO</td>
<td>7.8</td>
</tr>
<tr>
<td>EO</td>
<td>2.5</td>
</tr>
<tr>
<td>BA</td>
<td>0.4</td>
</tr>
<tr>
<td>RBC</td>
<td>5.29</td>
</tr>
<tr>
<td>HGB</td>
<td>16.2</td>
</tr>
<tr>
<td>HCT</td>
<td>47.0</td>
</tr>
<tr>
<td>MCV</td>
<td>88.8</td>
</tr>
<tr>
<td>MCH</td>
<td>30.7</td>
</tr>
<tr>
<td>MCHC</td>
<td>34.5</td>
</tr>
<tr>
<td>RDW</td>
<td>12.5</td>
</tr>
<tr>
<td>PLT</td>
<td>179</td>
</tr>
<tr>
<td>MPV</td>
<td>8.4</td>
</tr>
</tbody>
</table>
Complete Blood Count (CBC)

• **Interpretation / Limitations:**

  - **#RBC** is total number of RBCs (red arrow) counted by the CBC machine.
  - **HGB** (hemoglobin or orange arrow) is total hemoglobin concentration in a sample, which is estimated by dividing total HGB divided by total volume of blood in that sample (making it a concentration).
  - **HCT** (yellow arrow) is total RBC volume in blood, which is estimated by adding all volumes (or sizes) of RBCs analyzed by the CBC machine.
  - **MCV** (mean corpuscular volume or green arrow) is average volume of each RBC, which is estimated by dividing the total RBC volume (or HCT) by total number of RBCs. So, $$MCV = \frac{HCT}{#RBC}$$
Complete Blood Count (CBC)

• Interpretation / Limitations:
  
  - **MCH** (or mean corpuscular hemoglobin – dark green arrow) is average hemoglobin within each RBC, which is estimated by \( \text{MCH} = \frac{\text{HGB}}{\# \text{RBC}} \)
  
  - **MCHC** (or mean corpuscular hemoglobin concentration – blue arrow) is the average HGB concentration in each RBC, which is estimated by \( \text{MCHC} = \frac{\text{MCH}}{\text{MCV}} \)
  
  - **RDW** (or red blood cell distribution width – purple arrow) is standard deviation size from MCV. In other words, how spread out are RBC sizes from the average MCV.
Complete Blood Count (CBC)

- Interpretation / Limitations:

  - PLT (or platelets – red box) are the number of platelets counted by the CBC machine.
  - MPV (or mean platelet volume – blue box) is the average of all the platelet sizes or volumes measured from the CBC machine.
How can the CBC machine “screw up”? How can you detect these mistakes?

Directly measure by CBC machine
- Leukocyte count
- Platelet count
- Nuclear/granular complexity
- Hemoglobin amount
- Individual RBC volumes
- Hematocrit

Calculated by CBC machine
- Percentage of different cell types
- MCV (mean corpuscular volume)
- MCH (mean corpuscular hemoglobin)
- MCHC (mean corpuscular hemoglobin concentration)
- RDW (RBC distribution width)
Why do you utilize Peripheral blood smear?

- **To analyze:**
  - RBCs
  - Platelets
  - WBCs
    - Lymphocytes
    - Monocytes
    - Granulocytes
      - Neutrophils
      - Eosinophils
      - Basophils

- Represents mature product of bone marrow
Technique for making a peripheral blood smear

Fig. 7 - How to prepare a blood smear
Staining peripheral blood smears

• May-Grůnwald Technique
  – 1. Fix the slide using pure methanol in a coplin jar for 2 minutes and air dry.
  – 2. Mix 1 part May-Grůnwald stain, 1 part Wright-Giemsa stain, and 1 part 6.8pH buffered water (distilled or deionized).
  – 3. Place the slide on the rack over the sink, and flood it with the above mixture.
  – 4. Add additional buffered water until a green sheen appears. Blow on the slide to mix. Do not allow the stain to dry on the slide.
  – 5. Leave the stain on the slide for 6 minutes, being careful not to let the stain dry on the slide by adding more stain mixture if needed, and then rinse the slide with buffer solution briefly. Air-dry or blot with bibulous paper.
Where to scan in a peripheral blood smear

Too thin

Just right

Too thick
How to scan peripheral blood smears
Peripheral blood smear costs

- Cost of performing PBS: < $2
- Technical component (manual count): $14
- Pathologist interpretation: $26
Hueco Tanks State Park: 45 minutes from El Paso, TX
Case #1 – What would you expect to see in the PBS?

<table>
<thead>
<tr>
<th>Complete blood count (CBC)</th>
<th>Patient value</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count (WBC)</td>
<td>5.7</td>
<td>4.5-11 (X10³/UL)</td>
</tr>
<tr>
<td>RBC number (RBC#)</td>
<td>3.6L</td>
<td>4.2-5.9 (X10⁶/UL)</td>
</tr>
<tr>
<td>Hemoglobin (Hgb)</td>
<td>10.5L</td>
<td>12-16 (g/dL)</td>
</tr>
<tr>
<td>Hematocrit (Hct)</td>
<td>32L</td>
<td>38-47 %</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td>75L</td>
<td>82-98 fL</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH)</td>
<td>26L</td>
<td>27-31 pg</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (MCHC)</td>
<td>35</td>
<td>31-36 g/dL</td>
</tr>
<tr>
<td>Red Blood Cell Distribution Width (RDW)</td>
<td>55H</td>
<td>33-51 fL</td>
</tr>
<tr>
<td>Platelet Count (Plt count)</td>
<td>255</td>
<td>150-450 (X10³/UL)</td>
</tr>
<tr>
<td>Mean platelet volume (MPV)</td>
<td>8.0</td>
<td>7.4-10 fL</td>
</tr>
</tbody>
</table>
PBS & BMBx IDA

Normal PBS

PBS with IDA

Normal BMBx

BMBx with IDA
IDA physical exam findings

- Cheilitis
- Normal conjunctivae
- Pale conjunctivae
- Smooth tongue & Glossitis
- Cheilitis
- koilonychia (nail spooning)

[Images of normal and pale conjunctivae, smooth tongue and glossitis, cheilitis, and koilonychia]
Franklin Mountains State Park:
24 minutes from El Paso, TX
Case #2 – What would you expect to see in the PBS?

<table>
<thead>
<tr>
<th>Complete blood count (CBC)</th>
<th>Patient value</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count (WBC)</td>
<td>12H</td>
<td>4.5-11 (X10³/UL)</td>
</tr>
<tr>
<td>RBC number (RBC#)</td>
<td>3.9L</td>
<td>4.2-5.9 (X10⁶/UL)</td>
</tr>
<tr>
<td>Hemoglobin (Hgb)</td>
<td>11L</td>
<td>12-16 (g/dL)</td>
</tr>
<tr>
<td>Hematocrit (Hct)</td>
<td>33L</td>
<td>38-47 %</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td>81</td>
<td>82-98 fL</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (MCH)</td>
<td>26L</td>
<td>27-31 pg</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (MCHC)</td>
<td>33</td>
<td>31-36 g/dL</td>
</tr>
<tr>
<td>Red Blood Cell Distribution Width (RDW)</td>
<td>52H</td>
<td>33-51 fL</td>
</tr>
<tr>
<td>Platelet Count (Plt count)</td>
<td>255</td>
<td>150-450 (X10³/UL)</td>
</tr>
<tr>
<td>Mean platelet volume (MPV)</td>
<td>8.0</td>
<td>7.4-10 fL</td>
</tr>
</tbody>
</table>
PBS & BMBx of ACD

Normal PBS

PBS with ACD

Normal BMBx

Iron

BMBx with ACD
Tunnel leading to Cloudcraft, NM:
1 hours 52 minutes from El Paso, TX
Case #3 – What would you expect to see in the PBS?

<table>
<thead>
<tr>
<th>Complete blood count (CBC)</th>
<th>Patient value</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte count (WBC)</td>
<td>1.9L</td>
<td>4.5-11 (X10³/UL)</td>
</tr>
<tr>
<td>RBC number (RBC#)</td>
<td>2.9L</td>
<td>4.2-5.9 (X10⁶/UL)</td>
</tr>
<tr>
<td>Hemoglobin (Hgb)</td>
<td>8.0L</td>
<td>12-16 (g/dL)</td>
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<td>28</td>
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<td>52H</td>
<td>33-51 fL</td>
</tr>
<tr>
<td>Platelet Count (Plt count)</td>
<td>80L</td>
<td>150-450 (X10³/UL)</td>
</tr>
<tr>
<td>Mean platelet volume (MPV)</td>
<td>11H</td>
<td>7.4-10 fL</td>
</tr>
</tbody>
</table>
PBS and BMBx of AA & RCA

Normal PBS

PBS of pancytopenia

Normal BMBx

BMBx of RCA

BMBx of AA
Mesilla, NM:
30 minutes from El Paso, TX
Case #4 – What would you expect to see in the PBS?

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<tr>
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<th>Reference value</th>
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</thead>
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<tr>
<td>Leukocyte count (WBC)</td>
<td><strong>1.9L</strong></td>
<td>4.5-11 (X10^3/UL)</td>
</tr>
<tr>
<td>RBC number (RBC#)</td>
<td><strong>2.9L</strong></td>
<td>4.2-5.9 (X10^6/UL)</td>
</tr>
<tr>
<td>Hemoglobin (Hgb)</td>
<td><strong>8.0L</strong></td>
<td>12-16 (g/dL)</td>
</tr>
<tr>
<td>Hematocrit (Hct)</td>
<td><strong>24L</strong></td>
<td>38-47 %</td>
</tr>
<tr>
<td>Mean corpuscular volume (MCV)</td>
<td><strong>120H</strong></td>
<td>82-98 fL</td>
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<tr>
<td>Mean corpuscular hemoglobin (MCH)</td>
<td>28</td>
<td>27-31 pg</td>
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<td>33</td>
<td>31-36 g/dL</td>
</tr>
<tr>
<td>Red Blood Cell Distribution Width (RDW)</td>
<td><strong>53H</strong></td>
<td>33-51 fL</td>
</tr>
<tr>
<td>Platelet Count (Plt count)</td>
<td><strong>110L</strong></td>
<td>150-450 (X10^3/UL)</td>
</tr>
<tr>
<td>Mean platelet volume (MPV)</td>
<td><strong>12H</strong></td>
<td>7.4-10 fL</td>
</tr>
</tbody>
</table>
Ski Apache, Ruidoso, NM:
2 hours 27 minutes from El Paso, TX
Case #5 - How would the CBC look like?
Rouleaux

• *Description*: Cell aggregates resembling stack of coins.

*Underlying Change*: Cell clumping by circulating paraprotein.

*Diseases*: **Paraproteinemia**, artifact.
Blue Hole, Santa Rosa, NM:
4 hours 18 minutes from El Paso, TX
Case #6 - How would the CBC look like?
RBCs agglutination

• **Description:** Aggregate groups of RBCs

**Underlying change:** Antibodies attaches to RBCs together to form clumps of RBCs

**Diseases:** *Cold agglutinins (IgM -mediated)* or *Warm agglutinins (IgG - mediated).*
Comparison of Red Blood Cell Agglutination and Rouleaux Formation

The negative charge of red cells is produced by the high static acid content of membrane.

Positive charges (cations) form an "ionic cloud" around the negatively charged red cells.

The difference in electrical potential between negative and positive charges is the zeta potential. This normally keeps red cells 20 nm apart.

Abnormal or Increased Plasma Proteins

IgM Antibodies

Non-Specific Aggregation

Proteins change surface charge of red cells, reducing zeta potential and allowing cells to loosely join together in rows.

Specific Agglutination

IgM antibodies are large enough to span the zeta potential gap, forming a latticework that links the red blood cells.
## Warm vs Cold AIHA

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>Warm type (70%)</th>
<th>Cold type (30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Onset</strong></td>
<td>Abrupt with mostly extravascular hemolysis</td>
<td>Insidious with possible progression to intravascular hemolysis</td>
</tr>
<tr>
<td><strong>Jaundice</strong></td>
<td>Usually present</td>
<td>Often absent</td>
</tr>
<tr>
<td><strong>Splenomegaly</strong></td>
<td>Yes</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>All ages</td>
<td>All ages</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Slight more in women</td>
<td>Women predominate</td>
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</table>

### Origin of AIHA

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<th>Warm type (70%)</th>
<th>Cold type (30%)</th>
</tr>
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<tr>
<td><strong>Idiopathic</strong></td>
<td>50-60%</td>
<td>30-40%</td>
</tr>
<tr>
<td><strong>Drug-induced</strong></td>
<td>25-30%</td>
<td>1-5%</td>
</tr>
<tr>
<td><strong>Lymphomas</strong></td>
<td>10-15%</td>
<td>15-20%</td>
</tr>
<tr>
<td><strong>Viral or mycoplasma</strong></td>
<td>0%</td>
<td>25-35%</td>
</tr>
<tr>
<td><strong>Other (neoplasms, inflammatory disorders, etc.)</strong></td>
<td>5-10%</td>
<td>5-10%</td>
</tr>
</tbody>
</table>

Cold agglutinins (intravascular hemolysis)
Guadalupe Mountains National Park:  
1 hours 43 minutes from El Paso, TX
Sky Dive El Paso
Case #7 - How would the CBC look like?
Spherocytes

- Spherical cell with dense appearance, absent central pallor, and usually decreased in diameter

**Underlying changes:** Decreased membrane redundancy

**Diseases:** Hereditary spherocytosis, immunohemolytic anemia, transfusion, burn patients, artifact
### Warm vs Cold AIHA

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Carlsbad Caverns National Park:
2 hours 17 minutes from El Paso, TX
Case #8 - How would the CBC look like?
Types of Membrane Loss Leading to Spherocyte Formation

**Fibrin Strands**
- Red cell impaled on fibrin strand in microcirculation
- Pieces reseal and form schistocyte and spherocyte
- Helmet cell
- Microspherocyte

**Thermal Injury**
- Largest fragment reseals, forming spherocyte
- Membrane buds out from heat-damaged regions of spectrin
- Microspherocyte

**Intrinsic Abnormalities**
- Deficiency of spectrin, ankyrin or band 3
- Uncoupling of lipid bilayer and skeleton
- Microvesicle formation leading to membrane loss
- Surface area deficiency leading to spherocytosis
- Hereditary spherocytosis

**Immune Hemolysis**
- Damaged red cell reseals and forms microspherocyte
- Antibodies attach to red cell
- Fc receptor on splenic macrophage binds to antibody
- Splenic macrophage pits antigen-antibody complex, fragmenting red cell membrane
- Microspherocyte
Schistocytes

- Distorted, fragmented cells with 2 or 3 pointed ends

**Underlying Change:** Mechanical distortion in microvascular by fibrin strands, disruption or prosthetic heart valve

**Disease States:** Microangiopathic hemolytic anemia (DIC, TTP), prosthetic heart valves
How Fragmented Cells Form

1. red cell is impaled on fibrin strand in microcirculation
2. the cell is ripped in two
3. larger half rescales to become a helmet cell
4. smaller half becomes a microspherocyte or a misshapen fragmented cell

How Prekeratocytes Form

1. red cell is transiently draped over fibrin strand in microcirculation, much like saddlebag
2. cell is damaged but not ripped into pieces
3. damaged membrane fuses and produces a clear area lacking any interposed hemoglobin
4. opposing membranes fuse, forming a prekeratocyte

How Keratocytes Form

1. prekeratocyte (blister cell)
2. fused membranes rupture in circulation
3. the ruptured ends give rise to two "horns"
4. keratocyte (horn cell)
El Paso Mission Trail

Ysleta Mission ★ Socorro Mission ★ San Elizario Chapel

The nine-mile historic Mission Trail corridor in El Paso County’s Mission Valley is worthy of a trip, for a look at the three oldest churches in Texas, originally built during the 1600-1700s. See the first El Paso County Jail, where Billy the Kid broke into, in 1876. See the sites of the Salt War of 1877. See the Tigua Pueblo Cultural Center. See over 17 sites of the Presidio de San Elizario (the fort). Visit the only Art District in El Paso County, with over 30 Artist Studio/Galleries and over 100 Artists represented. Enjoy various award winning restaurants. See what everyone is talking about in El Paso County, ‘See it! Feel it! Believe it!’ Visit the El Paso Mission Trail.
Case #9 - How would the CBC look like?
Sickle Cell (drepanocyte)

• *Description*: bipolar, spiculated forms of sickle-shaped RBCs that are pointed at both ends.

*Underlying change*: molecular aggregation of hemoglobin S distorting RBC morphology.

*Disease*: **Sickle cell disease** (not including S trait).
More Specific RBC morphologic changes

- Acanthocyte (spur cell)
- Bite cell (degmacyte)
- Echinocyte (Burr or crenated cell)
- Hypochromic cell
- Macrocyte
- Microcyte
- Nucleated RBC
- Ovalocyte (elliptocyte)
- Polychromatophilia
- RBC Agglutination
- Rouleux

- RBC Inclusion
  - Howell-Jolly bodies
  - Pappenheimaer bodies
  - Basophilic stippling
  - Heinz bodies
- Schistocyte
- Sickle cell (drepanocyte)
- Spherocyte
- Stomatocyte
- Target Cell (codocyte)
- Tear Drop cell (dacrocyte)
Cattleman’s steak house, Faben, TX: 40 minutes from El Paso, TX
Post test
*** Is MCV directly measured by the CBC machine?

A. Yes, the machine measures MCV directly
B. No, the machine does not measure MCV directly
C. Cannot be determined
D. I could answer, but I then I would be guessing

Correct answer: B. No, the machine does not measure MCV directly
** Is MCV directly measured by the CBC machine?

- Yes, the machine...
- No, the machine...
- Cannot be determined...
- I could answer...
What does this peripheral blood smear most say about the RDW?

A. It is increased
B. It is normal
C. It is decreased
D. Cannot be determined

A. It is increased
*** What does this peripheral blood smear most say about the RDW?

- It is increased
- It is normal
- It is decreased
- Cannot be determined
What does this peripheral blood smear most say about this patient?

A. Possible myeloma
B. Possible hemolysis
C. Possible carcinoma
D. Cannot be determined

A. Possible myeloma

25% 25% 25% 25%
It is increased  It is normal  It is decreased  Cannot be determined
*** What does this peripheral blood smear most say about the MCV?

- It is increased
- It is normal
- It is decreased
- Cannot be determined

First Slide: 100%
Second Slide: 100%
*** What does this peripheral blood smear most say about the RBC number?

A. It is increased
B. It is normal
C. It is decreased
D. Cannot be determined

A. It is increased
*** What does this peripheral blood smear most say about the RBC number?

- It is increased
- It is normal
- It is decreased
- Cannot be determined

First Slide: 100
Second Slide: 100

Legend:  
First Slide  Second Slide