Hematology Workshop
Red Blood Cells (Erythrocytes)

- Most abundant blood cells (99.9% of formed elements)
  - In ♂, 1µL of blood contains 4.5-6.3 million RBCs
  - In ♀, 1µL of blood contains 4.2-5.5 million RBCs
- Contains the red pigment hemoglobin which binds and transports O₂ and CO₂
- Each RBC is a biconcave disc
  - Diameter → 7.5µm
  - Thickness → 2.0µm
Hemoglobin

- The $O_2$ binding and transporting protein found in extreme abundance in RBCs
- Hb levels are reported in grams of Hb per 100mL of whole blood (g/dL).
  - 14-18g/dL in adult ♂
  - 12-16g/dL in adult ♀
  - 14-20g/dL in infants
Hemoglobin

- Large protein consisting of 4 polypeptides
  - 2 α chains and 2 β chains
- Each chain contains a single molecule of heme, an iron-containing pigment
  - The iron ion in heme is able to reversibly bind an oxygen molecule.
  - Meaning, O₂ can bind to Hb at the lungs and then be released at the tissues

Note the 2 α chains and 2 β chains. Notice how each has an associated heme molecule with an iron atom.
Complete Blood Count (CBC)

- Determination of the number of red and white blood cells per mL of blood
- One of the most routinely performed clinical tests and a very valuable screening and diagnostic technique
- Can be performed manually or via an electric counter
- Different disorders can have a dramatic effect on the total number or relative proportion of blood cells.
Interpreting Blood Counts
The Blood Count (CBC)

- Hemoglobin (Hb g/L)
- Number of red blood cells (RBC x $10^{12}$/L)
- Size of the red blood cells (MCV fL)
- Number of white blood cells (WBC x $10^9$/L)
- Number of platelets (platelets x $10^9$/L)
- The differential and the blood film
White Blood Cells

- Leukocytes (*leuko*=white, *cyte*=cell)
- All contain nuclei and organelles unlike…

- Help defend the body against invasion by pathogens, and they remove toxins, wastes, and abnormal/damaged cells
- A typical µL of blood contains 6000-9000 WBCs. *Compare that to RBCs*
- Most of the WBCs in the body at a given moment are in the connective tissue proper or in organs of the lymphatic system
- Circulating WBCs are just a fraction of the total
Types of WBCs

- Can be classified based on the appearance of granules when viewed under the light microscope.

1. Granulocytes
   - Contain visible granules. Includes:
     - Basophils
     - Eosinophils
     - Neutrophils

2. Agranulocytes
   - Do not contain visible granules. Includes:
     - Lymphocytes
     - Monocytes
Granulocytes - Neutrophils

- 50-70% of circulating WBCs
- Cytoplasm is packed with pale (“neutral colored”) granules that contain bactericidal compounds
- Mature neutrophils have a segmented nucleus. They are polymorphonuclear leukocytes.
- About 12µm in diameter.
- Highly mobile and generally the first WBCs to arrive at an injury site.
- Specialize in attaching and digesting bacteria that have been “marked” for destruction.
- Survive in the bloodstream for only about 10hrs
Fig. 2 - Neutrophil
Granulocytes - Eosinophils

- 2-4% of circulating WBCs
- Similar in size to neutrophils but have reddish-orange staining granules (Eos is the Greek goddess of dawn) and a bilobed nucleus.
- They will phagocytize antibody-coated bacteria, protozoa, and cellular debris.
- Important defenders against large, multacellular parasites such as flukes or parasitic worms. They ↑ in dramatically during a parasitic infection.
- Also sensitive to allergens and ↑ in # during allergic reactions as well.
Granulocytes - Basophils

- >1% of circulating leukocytes
- Smaller than neutrophils and eosinophils, only about 8-10µm in diameter.
- Contain granules that appear deep purple or blue
- Basophils migrate to injury sites and discharge the contents of their granules – histamine (a vasodilator and increaser of capillary permeability) and heparin (an anticoagulant). This enhances the local inflammation initiated by mast cells and attracts other WBCs
Fig. 4 - Basophil
Agranulocytes - Lymphocytes

- 20-30% of circulating leukocytes
- Slightly larger than RBCs. In blood smears, you typically only see a thin halo of cytoplasm around a relatively large nucleus.
- Continuously migrate from the bloodstream thru peripheral tissues and back into the bloodstream.

Circulating blood contains 3 classes:
- **T cells**: defend against foreign cells and tissues and coordinate the immune response
- **B cells**: produce and distribute antibodies that attack foreign materials
Fig. 5 - Lymphocyte
Agranulocytes - Monocytes

- 2-8% of circulating WBCs
- Almost twice as big as an RBC
- Nucleus is large and tends to be oval or kidney-shaped
- Individual monocytes use the bloodstream as a highway, staying in circulation for only about 24hrs before entering peripheral tissues to become a tissue macrophage.
Fig. 6 - Monocyte
WBCs in order of abundance:

Never (neutrophils)
Let (lymphocytes)
Monkeys (monocytes)
Eat (eosinophils)
Bananas (basophils)

How do I remember the relative percentages?

60 + 30 + 6 + 3 + 1

(i.e., 60% neutrophils, 30% lymphocytes, 6% monocytes, 3% eosinophils & 1% basophils)
In the above picture, compare normal and leukemic blood.
Fig. 7 - How to prepare a blood smear
Case 1

- 32 y/o lady who comes on with fever, yellowish discoloration of skin and sclera since 3 days ago.

She reports that doctors said to her previously she had mild anemia and after delivery she was received pack cell
• P/E: T:37, jaundice, hepatosplenomegally,
Lab data's

- HB: 9.5 mg/dl, platelet: 150,000
- WBC: 95,000, ESR: 32
- LFT: ALT: 54, AST: 43, AP: 245
- D.B: 0.3, T.B: 4.0

Abdominal sonography: hepatosplenomegally
What is your diagnosis?
Case 2

• A 37-year-old woman was seen in the clinic because of numbness in the arms and legs. She noticed the onset of numbness and tingling that extended from the fingertips to the upper arms; subsequently, numbness and tingling developed in the legs as well. She also noticed weakness in her legs, to the extent that she could no longer run a mile and was losing her balance.
At the time of the patient's first visit to the neurologist, four months after the initial onset of tingling and numbness, the blood pressure was 110/80 mm Hg, and the weight 68.8 kg. She appeared well. Her mental status was normal, as were her cranial-nerve and motor functions. There was a slight delay in the relaxation of the deep-tendon reflexes; the plantar responses were flexor. Vibratory sensation was reduced in the feet, as was proprioception; other sensory responses were intact. The result of Romberg's test was normal. Cortical sensation (including graphesthesia, stereognosis, and tactile extinction) was normal.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>35.7</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.6</td>
</tr>
<tr>
<td>Red cells (per mm$^3$)</td>
<td>$3.61 \times 10^6$</td>
</tr>
<tr>
<td>Mean corpuscular volume ($\mu$m$^3$)</td>
<td>99</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (pg/red cell)</td>
<td>34.9</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (g/dl)</td>
<td>35.2</td>
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<tr>
<td>White cells (per mm$^3$)</td>
<td>5,900</td>
</tr>
<tr>
<td>Differential count (%)</td>
<td></td>
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<tr>
<td>Neutrophils</td>
<td></td>
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<tr>
<td>Lymphocytes</td>
<td></td>
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<tr>
<td>Monocytes</td>
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<tr>
<td>White-cell morphology</td>
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<tr>
<td>Red-cell morphology</td>
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<tr>
<td>Anisocytosis</td>
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<tr>
<td>Macrocytosis</td>
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<tr>
<td>Schistocytosis</td>
<td></td>
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<tr>
<td>Tear-drop forms</td>
<td></td>
</tr>
<tr>
<td>Platelets (per mm$^3$)</td>
<td>342,000</td>
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</tbody>
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Any Questions?
Thrombocytopenia: Case

A previously healthy 23 year old female competitive player presents to your office with a three day history of increased bruising and petechiae. Her only medications are naproxen and an oral contraceptive. Physical exam shows petechiae on the legs and several small bruises on the extensor surfaces.

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocytes (x 10⁹/L)</td>
<td>6.8</td>
<td>[4.0 - 11.0]</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>130</td>
<td>[120-160]</td>
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<tr>
<td>MCV (fL)</td>
<td>87</td>
<td>[80 - 100]</td>
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<tr>
<td>Platelet count (x 10⁹/L)</td>
<td>11</td>
<td>[150 - 450]</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>12.5</td>
<td>[7.4 -10.4]</td>
</tr>
</tbody>
</table>
Thrombocytopenia: Case 1

How do you approach this problem diagnostically?

How you manage this patient and what do you advise her about her activities and medications?
Approach to thrombocytopenia

THROMBOCYTOPENIA

rule out pseudothrombocytopenia

SEQUESTRATION

look for splenomegaly

Causes of splenomegaly
- infection
- inflammation
- congestion
- malignancy
- red cell disorders
- storage diseases

PRODUCTION

bone marrow investigation

- aplasia
- infiltration
- ineffective megakaryopoiesis
  eg. MDS
- selective impairment of platelet production

DESTRUCTION

look for underlying disorders

- immune
  auto-immune (ITP, SLE
  drugs
  infections
  allo-immune
- non-immune
  sepsis
  DIC, TTP, HUS
  hypertensive disorders of pregnancy

review meds

look for underlying disorders
review meds
Any Questions?