Abnormalities of Blood Count:
Pathophysiology and Laboratory Diagnosis of Anemias and other Blood Disorders

Anand Shreeram Lagoo, MD, PhD
Associate Professor of Pathology
Director, Clinical Flow Cytometry Laboratory

Phone: 668-0921, Pager 970-2903

May 18, 2010

you will see these tests everyday, so you need to be able to interpret them! -some of this material will be covered in the cpc due to short time during the lecture
Learning Objectives

- Recognize common quantitative abnormalities in complete blood count (CBC) and qualitative abnormalities on a peripheral blood smear (PBS)
- Interpret hematologic laboratory values to diagnose various types of anemias
- Define the terms used to identify hematologic abnormalities
- Understand the morphological and etiologic classification of anemias and the pathophysiological basis of anemias
- Perform a differential diagnosis in a case of anemia and select additional laboratory tests to define cause of anemia
- Recognize the common white cell and platelet abnormalities
Lecture Outline

- Basic mechanisms of hematological abnormalities
- Automated blood count (ABC) – Method and parts of a typical ABC
- Red blood cells
  - Classification of anemias (Note: Hereditary causes of anemia will be covered in CPC on May 23)
  - Case 1 – Iron deficiency anemia. Pathophysiology of iron metabolism. Additional tests.
  - Case 3 – Anemia of chronic inflammation. Hepcidin and related molecules controlling iron.
  - Case 4 – Autoimmune hemolytic anemia. Causes and mechanisms.
- White blood cells
  - Case 5 – CML. Philadelphia chromosome.
  - Case 6 – Polycythemia vera. Jak2 mutations and myeloproliferative neoplasms.
  - Case 7 – Aplastic anemia.
- Platelets
  - Case 8 – ITP. Causes of thrombocytopenia.
Would you mind putting that thing on vibrate?
## Peripheral Blood Cells: Basic Facts

<table>
<thead>
<tr>
<th></th>
<th>Number / cmm</th>
<th>Life Span in Days</th>
<th>Produced in</th>
<th>Destroyed in</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Red Cells</strong>*</td>
<td>5 x 10⁶</td>
<td>120</td>
<td>BM</td>
<td>Spleen</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td>5 x 10⁵</td>
<td>5-7</td>
<td>BM</td>
<td>Spleen</td>
</tr>
<tr>
<td><strong>White Cells</strong></td>
<td>5 x 10³</td>
<td>&lt;1 (PMN)</td>
<td>BM, lymph nodes</td>
<td>Tissues</td>
</tr>
</tbody>
</table>

*Reticulocytes: Without a nucleus, but contain RNA. Need 2 days in BM & 1 day in PB to mature to RBC. Normally 1% of RBC.
General Approach to Diagnosis of Hematological Abnormalities

- Is there an abnormality in the blood count?
- Which cell line(s) affected? (red cells, white cells, platelets)
- Morphology of affected cells –
  - Normal?
  - Abnormal?

NOTE: Calculated “indices” provide similar information

- What is the likely cause of the abnormality?
  - Additional tests (rational treatment needs to be directed towards cause)
Initial Division of Hematological Abnormalities

- Quantitative: one or more cell types may be involved
  - Reduced numbers of blood cells (=cytopenia)
  - Too many blood cells (=cytosis)
  - Complex: one cell type ↓, other ↑

- Qualitative
  - Presence of immature cells
  - Functionally abnormal cells
  - Presence of cells not belonging to blood

- Mixed
Quantitative Blood Cell Abnormalities - Basic mechanisms

- Causes of Cytopenias:
  - Decreased production
    - Lacks building blocks (nutritional, other)
    - Problems with production site (marrow pathology)
  - Excessive destruction
    - Intrinsic vs extrinsic abnormalities
  - Abnormal compartmentalization

- Causes of increased cell number:
  - Excessive production (reactive vs neoplastic)
  - Increased life-span (neoplastic)
  - Delayed exit from blood (steroids)
Basic Laboratory Tests in Hematology

- Automated blood count, with or without automated differential count
- Peripheral blood smear

blood tests you will see for many patients
- automated blood count = cbc
- peripheral blood smear

-CBC
Automated Blood Analyzer

Can analyze 110 – 150 samples / hour

XE-2100
Automated Blood Analyzer: The Coulter Principle

When particles are pulled through an orifice, through which an electric current is flowing, there is a change in impedance that is proportional to the size of the particle.

The Coulter principle was named for its inventor, Wallace H. Coulter.
Automated Blood Analyzer: Light Scatter

Light scatter is also used to analyze blood - laser points at single cells and angle of scatter tells you type of blood cell.
Peripheral Blood Smear

- Platelet
- Band
- Eosinophil
- Neutrophil
- Lymphocyte
- Basophil
- Monocyte

stained to show different types of white cells - should be able to identify different types when they are normal.
### Automated Blood Count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>15.5</td>
<td>g/dL</td>
<td>[13.7-17.3]</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.46</td>
<td>L/L</td>
<td>[0.39-0.49]</td>
</tr>
<tr>
<td>Red Blood Cell Count</td>
<td>4.95</td>
<td>X10^12</td>
<td>[4.37-5.74]</td>
</tr>
<tr>
<td>MCH</td>
<td>31.3</td>
<td>pg</td>
<td>[26.5-34.0]</td>
</tr>
<tr>
<td>MCHC</td>
<td>33.4</td>
<td>%</td>
<td>[31.5-36.3]</td>
</tr>
<tr>
<td>RDW-CV</td>
<td>13.4</td>
<td>%</td>
<td>[11.5-14.5]</td>
</tr>
<tr>
<td>MCV</td>
<td>94</td>
<td>fL</td>
<td>[80-98]</td>
</tr>
<tr>
<td>Nucleated RBC %</td>
<td>0.0</td>
<td>/100WC</td>
<td></td>
</tr>
<tr>
<td>Nucleated RBC Count</td>
<td>0.00</td>
<td>X10^9</td>
<td>[0.00-0.00]</td>
</tr>
<tr>
<td>Platelet Count /L</td>
<td>171</td>
<td>X10^9</td>
<td>[150-450]</td>
</tr>
<tr>
<td>White Blood Cell Count</td>
<td>4.5</td>
<td>X10^9</td>
<td>[3.2-9.8]</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>60.2</td>
<td>%</td>
<td>[37.0-80.0]</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>26.8</td>
<td>%</td>
<td>[10.0-50.0]</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>10.1</td>
<td>%</td>
<td>[0.0-12.0]</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>2.7</td>
<td>%</td>
<td>[0.0-7.0]</td>
</tr>
<tr>
<td>Basophil %</td>
<td>0.2</td>
<td>%</td>
<td>[0.0-2.0]</td>
</tr>
<tr>
<td>Neutrophil Count</td>
<td>2.7</td>
<td>X10^9</td>
<td>[2.0-8.6]</td>
</tr>
<tr>
<td>Lymphocyte Count</td>
<td>1.2</td>
<td>X10^9</td>
<td>[0.6-4.2]</td>
</tr>
<tr>
<td>Monocyte Count</td>
<td>0.5</td>
<td>X10^9</td>
<td>[0.0-0.9]</td>
</tr>
<tr>
<td>Eosinophil Count</td>
<td>0.12</td>
<td>X10^9</td>
<td>[0.00-0.70]</td>
</tr>
<tr>
<td>Basophil Count</td>
<td>0.01</td>
<td>X10^9</td>
<td>[0.00-0.20]</td>
</tr>
</tbody>
</table>
## Automated Blood Count

**Auto Blood CT with Auto Diff**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>15.5</td>
<td>g/dL</td>
<td>[13.7-17.3]</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.46</td>
<td>L/L</td>
<td>[0.39-0.49]</td>
</tr>
<tr>
<td>Red Blood Cell Count</td>
<td>4.95</td>
<td>X10^12</td>
<td>[4.37-5.74]</td>
</tr>
<tr>
<td>Mean Cell Hb</td>
<td>31.3</td>
<td>pg</td>
<td>[26.5-34.0]</td>
</tr>
<tr>
<td>Mean Cell Hb Concentration</td>
<td>33.4</td>
<td>%</td>
<td>[31.5-36.3]</td>
</tr>
<tr>
<td>Red Cell Distribution Width</td>
<td>13.4</td>
<td>%</td>
<td>[11.5-14.5]</td>
</tr>
<tr>
<td>Mean Cell Volume</td>
<td>94</td>
<td>fL</td>
<td>[80-98]</td>
</tr>
<tr>
<td>Nucleated RBC %</td>
<td>0.0</td>
<td>/100WC</td>
<td></td>
</tr>
<tr>
<td>Nucleated RBC Count</td>
<td>0.00</td>
<td>X10^9</td>
<td>[0.00-0.00]</td>
</tr>
<tr>
<td>Platelet Count /L</td>
<td>171</td>
<td>X10^9</td>
<td>[150-450]</td>
</tr>
</tbody>
</table>

### White Blood Count

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Units</th>
<th>Reference Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Blood Cell Count</td>
<td>4.5</td>
<td>X10^9</td>
<td>[3.2-9.8]</td>
</tr>
<tr>
<td>Neutrophil %</td>
<td>60.2</td>
<td>%</td>
<td>[37.0-80.0]</td>
</tr>
<tr>
<td>Lymphocyte %</td>
<td>26.8</td>
<td>%</td>
<td>[10.0-50.0]</td>
</tr>
<tr>
<td>Monocyte %</td>
<td>10.1</td>
<td>%</td>
<td>[0.0-12.0]</td>
</tr>
<tr>
<td>Eosinophil %</td>
<td>2.7</td>
<td>%</td>
<td>[0.0-7.0]</td>
</tr>
<tr>
<td>Basophil %</td>
<td>0.2</td>
<td>%</td>
<td>[0.0-2.0]</td>
</tr>
<tr>
<td>Neutrophil Count</td>
<td>2.7</td>
<td>X10^9</td>
<td>[2.0-8.6]</td>
</tr>
<tr>
<td>Lymphocyte Count</td>
<td>1.2</td>
<td>X10^9</td>
<td>[0.6-4.2]</td>
</tr>
<tr>
<td>Monocyte Count</td>
<td>0.5</td>
<td>X10^9</td>
<td>[0.0-0.9]</td>
</tr>
<tr>
<td>Eosinophil Count</td>
<td>0.12</td>
<td>X10^9</td>
<td>[0.00-0.70]</td>
</tr>
<tr>
<td>Basophil Count</td>
<td>0.01</td>
<td>X10^9</td>
<td>[0.00-0.20]</td>
</tr>
</tbody>
</table>
# Automated Blood Count

**AUTO BLOOD CT WITH AUTO DIFF**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEMOGLOBIN</td>
<td>9.7 g/dL</td>
<td>[13.7-17.3]</td>
</tr>
<tr>
<td>HEMATOCRIT</td>
<td>0.26 L/L</td>
<td>[0.39-0.49]</td>
</tr>
<tr>
<td>RED BLOOD CELL COUNT</td>
<td>2.95 X10^12</td>
<td>[4.37-5.74]</td>
</tr>
<tr>
<td>MCH</td>
<td>32.9 pg</td>
<td>[26.5-34.0]</td>
</tr>
<tr>
<td>MCHC</td>
<td>37.3 %</td>
<td>[31.5-36.3]</td>
</tr>
<tr>
<td>RDW-CV</td>
<td>15.3 %</td>
<td>[11.5-14.5]</td>
</tr>
<tr>
<td>MCV</td>
<td>88 fL</td>
<td>[80-98]</td>
</tr>
<tr>
<td>NUCLEATED RBC %</td>
<td>0.0 /100wc</td>
<td>[0.00-0.00]</td>
</tr>
<tr>
<td>NUCLEATED RBC COUNT</td>
<td>0.00 X10^9</td>
<td>[150-450]</td>
</tr>
<tr>
<td>PLATELET COUNT /L</td>
<td>9 X10^9</td>
<td>[150-450]</td>
</tr>
</tbody>
</table>

This ALERT result has been called to BEAVEN by CYNTHIA KING on 06-07-16 at 13:39 and has been read back.

**WHITE BLOOD CELL COUNT**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEUTROPHIL %</td>
<td>19.0 %</td>
<td>[3.2-9.8]</td>
</tr>
<tr>
<td>LYMPHOCYTE %</td>
<td>0.0 %</td>
<td>[0.0-50.0]</td>
</tr>
<tr>
<td>MONOCYTE %</td>
<td>0.0 %</td>
<td>[0.0-12.0]</td>
</tr>
<tr>
<td>EOSINOPHIL %</td>
<td>0.0 %</td>
<td>[0.0-7.0]</td>
</tr>
<tr>
<td>BASOPHIL %</td>
<td>0.0 %</td>
<td>[0.0-2.0]</td>
</tr>
<tr>
<td>NEUTROPHIL COUNT</td>
<td>2.0 X10^9</td>
<td>[2.0-8.6]</td>
</tr>
<tr>
<td>LYMPHOCYTE COUNT</td>
<td>0.6 X10^9</td>
<td>[0.6-4.2]</td>
</tr>
<tr>
<td>MONOCYTE COUNT</td>
<td>0.0 X10^9</td>
<td>[0.0-0.9]</td>
</tr>
<tr>
<td>EOSINOPHIL COUNT</td>
<td>0.00-0.70 X10^9</td>
<td>[0.00-0.70]</td>
</tr>
<tr>
<td>BASOPHIL COUNT</td>
<td>0.00-0.20 X10^9</td>
<td>[0.00-0.20]</td>
</tr>
</tbody>
</table>

**BLOOD FILM REVIEWED**

---

*when values are abnormal, they are marked in RED by the laboratory. Alert value is seriously abnormal and maybe life threatening. Lab must call a nurse or doctor with the result. In this case platelets are dangerously low. Dr. H*
<table>
<thead>
<tr>
<th></th>
<th>Normal Range</th>
<th>Decreased below lower limit =</th>
<th>Increased above upper limit =</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hgb g/dL</strong></td>
<td>M 14 - 18</td>
<td>Anemia</td>
<td>Polycythemia</td>
</tr>
<tr>
<td></td>
<td>F 12 - 16</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MCV in fL</strong></td>
<td>80 - 98</td>
<td>Microcytic</td>
<td>Macrocytic</td>
</tr>
<tr>
<td><strong>MCH in pg</strong></td>
<td>27 - 34</td>
<td>Hypochromic</td>
<td>Hyperchromic</td>
</tr>
<tr>
<td><strong>Reticulocyte:</strong> %</td>
<td>0.5 – 1.5</td>
<td>Decreased production or</td>
<td>Increased production or</td>
</tr>
<tr>
<td></td>
<td>Abs /c mm</td>
<td>destruction in BM</td>
<td>early release from BM</td>
</tr>
<tr>
<td></td>
<td>20k – 100k</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Hb low - anemia
- Hb high - polycythemia
- MCV low - microcytic anemia
- MCV high - macrocytic anemia
- MCH low - hypochromic anemia
- MCH high - hyperchromic anemia
- reticulocytes low - decreased production of increased destruction in bone marrow
- reticulocytes high - increased production or early release from bone marrow
Anemia: A Major Health Problem Worldwide

Worldwide:
- Anemia affects 42% children <5 years old and 53% children 5–14 years old
- Anemia is 3rd leading cause of lost productivity in adult females
- Over 1 billion people have iron deficiency (Am J Trop Med Hyg. 2007 Jul;77(1):44-51)

In the US
- 3.5% of all persons enrolled in one health insurance plan in 2000 were found to be anemic
- Average annual cost for anemic patients was $14,535 compared to $9,451 in non-anemic patients (J Manag Care Pharm. 2005 Sep;11(7):565-74.)
I could help you, but I’m more into people with newer, more challenging diseases right now.
Classifications of Anemias

- **Morphological classification** - Based on size of RBC and their hemoglobin content
  - Normocytic vs Microcytic vs Macrocytic
  - Normochromic vs Hypochromic

  **NOTE:** The morphological classification suggests an etiologic differential which is confirmed by additional tests

- **Etiological Classification**
  - Decreased Hgb and/or RBC production
    - Deficiency of essential ingredients – Iron, Folate, B12, etc
    - Thalassemias
    - Decreased or defective progenitor cells
  - Defects of red cell survival
    - Hemoglobinopathies
    - Red cell membrane abnormalities
    - Red cell enzyme abnormalites
    - Immune destruction of RBC
    - Vascular and other extrinsic causes
    - Infections – Malaria
Case 1

- 59 yo caucasian man
- Presents with fatigue and headache for 4 months
- He has noted some upper abdominal distress
- Physical examination is normal
- Lab data:
  - Hct: 27%
  - Hgb: 8.9 gm/dL
  - MCV: 67 fL
  - MCH: 22.6 pg
  - Platelets: 600,000
  - WBC: 4,900/cu mm

Anemia

- Microcytic
- Hypochromic
- Thrombocytosis

The normal values you need to know for this case:
- Hct - 0.39-0.49
- Hb for a man - 14-18
- MCV - 80-98
- MCH - 27-34
- platelets - 150,000 - 450,000
- WBC count - 3200 - 9800

Severe anemia - should be 14 or above for a man
- 12-14 - mild anemia
- 10-12 - moderate anemia
- Below 10 - severe anemia
- Everything lowered by 2 in women
Case 1: Microcytic, hypochromic anemia
(continued)

- 59 yo caucasian man with Microcytic anemia and thrombocytosis
  - Hct: 27 %
  - Hgb: 8.9 gm/dL
  - MCV: 67 fL
  - MCH: 22.6 pG
  - Platelets: 600,000
  - WBC: 4,900/cu mm

- Reticulocyte: 30,000/mm³

- Peripheral Blood Film-
  - WBC differential:
    - Neutrophils 65%
    - Lymphocytes 33%
    - Monocytes 2%
  - Abnormal RBC morphology

- Inappropriately low reticulocyte count

The normal values you need to know for this case:
- Reticulocyte count: 20-100 k/cumm

Normal differential white blood cell counts:
- Neutrophils 65%
- Lymphocytes 33%
- Monocytes 2%

Abnormal red cell morphology
Case 1 - Peripheral Blood Film
Microcytic hypochromic anemia

- Hypochromia
  - central pallor in center of red cells is increased

- Anisocytosis
  - large variation in diameter of red cells
  - RDW=19.6

- Poikilocytosis
  - difference in shape of cells - instead of biconcave disc, some are more pencil shaped
Microcytic hypochromic anemia: Etiological differential diagnosis

- Iron deficiency anemia
- Anemia of chronic inflammation
- Thalassemias
- Sideroblastic anemia
- Lead poisoning
Understanding iron metabolism:

- The body has **no mechanism to excrete excess iron**
- Absorption of dietary iron is strictly controlled to maintain total iron in the body
- **Free iron is toxic, therefore it is bound to proteins** –
  - Specific binding to transferrin and apoferritin
  - Non-specific binding to albumin

- Body does not have a good mechanism for excreting iron so the intake of iron must be tightly regulated
- Free iron is toxic so it must be bound to proteins - transferrin is primary transport protein
Understanding iron metabolism:

- Transferrin is the primary transport molecule for iron.
  - Blood transferrin level is referred to as “Total Iron Binding Capacity”
  - Proportion of transferrin molecules bound to iron = % saturation of iron binding capacity
  - This iron is most readily available for Hgb synthesis

- Some iron binds to another protein called apoferritin to form a water soluble molecule called ferritin
  - Ferritin is present in blood and ferritin iron can be easily delivered for Hgb synthesis.

- Excess iron is stored in bone marrow as water insoluble Hemosiderin

- transferrin bound iron is cash in your pocket that is the most readily available
- ferritin is like your atm card where you can go get cash if you need it
- hemosiderin is like your certificate of deposit that may be harder to access but may contain a lot of money
Additional Laboratory Tests In Microcytic, Hypochromic Anemia:

- Serum Iron level:
- Iron binding capacity = Transferrin level
- Transferrin saturation = % transferrin bound to iron
- Serum ferritin

any time you suspect problem with iron metabolism, do these 4 tests
Abnormally Low blood Ferritin = Low/ Absent storage iron*

Ferritin levels increase due to inflammation, even when iron stores are low. Therefore, normal or high Ferritin does NOT guarantee normal storage iron.

*Abnormally Low blood Ferritin = Low/ Absent storage iron*

- normally, iron is absorbed and binds to transferrin and most is used to make hemoglobin
- about 10% binds to ferritin and goes into the circulation
- when iron decreases, transferrin levels increase to try to absorb as much iron as possible so as soon as iron comes through intestine, it is bound up by high levels of transferrin
- when iron decreases, percent saturation of iron is low because there is little iron to bind to the transferrin
- ferritin is either high or low - inflammation can increase ferritin levels - low ferritin level suggests iron deficiency but normal ferritin level does not rule out iron deficiency

Trasferrin levels increase when iron stores decline

Total Iron Binding Capacity (TIBC) increases but it is less saturated

- normally, iron is absorbed and binds to transferrin and most is used to make hemoglobin
- about 10% binds to ferritin and goes into the circulation
- when iron decreases, transferrin levels increase to try to absorb as much iron as possible so as soon as iron comes through intestine, it is bound up by high levels of transferrin
- when iron decreases, percent saturation of iron is low because there is little iron to bind to the transferrin
- ferritin is either high or low - inflammation can increase ferritin levels - low ferritin level suggests iron deficiency but normal ferritin level does not rule out iron deficiency
Case 1  continued

- Additional laboratory tests:
  - Serum Iron: 10 (low)
  - Iron binding capacity: 450 (high)
  - Transferrin saturation: 2% (low)
  - Serum ferritin: 10 ng/mL (low)

- Diagnosis: Iron deficiency anemia

Must investigate causes of chronic blood loss in iron deficiency anemia in older adults. Dietary iron deficiency more common in children and reproductive age females.

- Stool samples positive for occult blood

do not miss the CAUSE of iron deficiency - in this case it was a GI malignancy
dietary causes are more common in younger patients whereas malignancies increase in likelihood in older patients

what is the cause of iron deficiency? -adult male or post menopausal females --> GI tract malignancy?

need to do colonoscopy
Case 2

54 yo man
- Presents with nausea, poor appetite, mild diarrhea
- PE: Normal

CBC:
- Hct: 35 %
- Hgb: 12 gm/dl (Anemia)
- MCV: 115 fl (Macrocytosis)
- Retic: 65,000/ cu mm (not elevated, relatively low)
- Platelets: 200,000
- WBC: 4,000

Blood film: Macrocytosis, WBC differential is normal

Normal upper and lower GI studies
Macrocytic Anemias with low Retics: Megaloblastic or Normoblastic?

- **Megaloblastic** (specific morphological change in red cell precursors in bone marrow)
  - Vit B12 deficiency
  - Folate deficiency
  - Myelodysplastic syndromes
  - Drug-induced

- **Normoblastic**
  - Hypothyroidism
  - Liver disease
  - Alcohol

Macrocytic anemias can be either:
- megaloblastic - abnormal erythropoiesis in bone marrow
- normoblastic - normal erythropoiesis
Case 2 - Peripheral Blood Film

Hypersegmented neutrophils are commonly seen with megaloblastic anemias, particularly vit b12 and folate deficiency.
Case 2 continued

- Several months later -
  - Paresthesias of hands and feet
  - Difficulty using the clutch and gas pedals while driving

- PE:
  - Mild scleral icterus
  - Absent position and vibratory sensation
  - Diminished two-point discrimination
Case 2 continued

- Diagnostic laboratory evaluation:
  - Serum B12 level: 30 (normal > 180)
  - Anti-intrinsic factor antibodies positive

- Diagnosis: **B12 deficiency**
  - **Pernicious anemia**

  - Macrocytic anemia + neurological symptoms --> typical for vit b12 deficiency

  - Most common cause of b12 deficiency in adults
  - Autoimmune process in which absorption of vit b12 is impaired
Back to the Basics…

**B12**

Cobalamin

**Folate**

Pteroyl glutamic acid

Both cause macrocytic megaloblastic anemias, but folate deficiency doesn't cause neurological deficits.
Dietary B12 (cobalamin, Cbl)

Intrinsic Factor (IF) - Secrepted by gastric parietal cells - Required for absorption of B12

Autoantibodies disrupt B12 absorption in pernicious anemia

Autoantibodies against IF, against parietal cells, or against receptor in small intestine all can cause malabsorption of vit b12 - takes a long time to develop bc vit b12 is stored in the body - in the stomach, dietary vit b12 binds to intrinsic factor - intrinsic factor + vit b12 bind to a specific receptor in the small intestine and is absorbed
Actions of B12 and Folate:

- Folate is directly required for Purine (DNA) synthesis, B12 is indirectly involved through folate metabolism
  - Only tetra-hydro folate (THF) can participate in purine synthesis
  - Dietary folate is converted to THF and then to methyl-THF
  - Methyl-THF can be converted back to THF if B12 is present
    - Only B12 can transfer the methyl group from Methyl-THF to homocysteine
  - In the absence of B12, most folate is “trapped” as methyl-THF, levels of THF decline, and DNA synthesis suffers

- Treatment with large doses of folate will form “new” THF, bypassing requirement for B12
- Treatment with folate will correct anemia due to folate deficiency or B-12 deficiency

Folate independent vit b12 actions are responsible for the neurological symptoms in vit b12 deficiency that not seen in folate deficiency.
Anemia due to B12 or Folate Deficiency

- Treatment with folate will correct anemia due to folate deficiency or B-12 deficiency
- Mitochondrial action of B12: (Folate independent)
  - Adenosyl-Cbl acts as coenzyme for conversion of methylmalonyl-CoA to succinyl-CoA
  - ? Associated with myelin formation and etiology of neuropathy observed in B12 deficiency
- Neuropathy of B12 deficiency may be aggravated by folate administration
- B12 administration will not correct anemia due to folate deficiency
Case 3

[Image of a woman with a malar rash]

- **23 yo woman**
  - Fatigue, arthralgias, skin rash for several months
  - PE: Malar rash

- **Lab data:**
  - Hct: 29 %
  - Hgb: 9.2 gm/dl (anemia)
  - MCV: 82 fl (borderline microcytic)
  - Platelets: 150,000
  - WBC: 4,900

- **Blood film:**
  - Normochromic, normocytic RBCs
  - WBC diff: Normal

- **Retic:** 60,000/cu mm (inappropriately low)

The normal values you need to know for this case:
- Hct - 0.39 - 0.49
- Hb for a woman - 12-14
- MCV - 80-98
- MCH - 27-34
- Platelets - 150,000 - 450,000
- WBC count - 3200 - 9800
- Reticulocytes - 20-100 K / cumm
Case 3 - Peripheral Blood Film

Normochromic, normocytic RBCs
Normocytic - Normochromic Anemia and Low Retic Count: differential diagnosis

- **Primary BM (stem cell) disorders**
  - Aplastic anemia
  - Pure Red Cell aplasia
  - Infiltrative disorders

- **Secondary to systemic illness**
  - Anemia of chronic inflammation
  - Renal insufficiency
  - Endocrine disorders

Primary causes of normal red blood cells + anemia + low reticulocyte count: remember that when there is anemia, the reticulocyte count should be higher to compensate, so something is wrong if the reticulocyte count is also low.

Systemic inflammatory causes

Usually microcytic anemia
Case 3: Additional Tests

- ESR: 80 mm/hr
- BUN: 42
- Creatinine: 2.0
- Anti Nuclear Antibody: 1:1256
- Complement C3/C4: Low
- Anti-ds DNA: Positive

Diagnosis

- Systemic Lupus Erythematosus (SLE)
- Renal insufficiency
- Anemia of Chronic Disease (= anemia of inflammation)
  - Possibly worsened by low erythropoietin
Hepcidin: The inflammation-anemia connection

- Macrophages make IL6 in response to inflammation
- IL6 causes hepatocytes to make hepcidin
- Hepcidin reduces intestinal absorption of iron and inhibits macrophages from releasing iron that they are storing

Reduced availability of iron >> Anemia

Anemia of inflammation: the cytokine-hepcidin link
Nancy C. Andrews
Hepcidin

Duodenum

Enterocyte brush border

Luminal or apical side

Basolateral side

Plasma

Transferrin

Fe³⁺ Fe³⁺

Transferrin

Fe³⁺

normally, iron is transported through in intestine by ferroportin

if hepcidin is present, it does not allow iron to be transported by ferroportin --> dietary iron is not absorbed
Other Molecules Involved In Iron Absorption

- These molecules are required for appropriate synthesis of Hepcidin
  - Mutations lead to reduced hepcidin and excess iron absorption = **HEMOCHROMATOSIS**

- Hemochromatosis (HFE) gene
  - Mutations cause adult hemochromatosis

- Hemojuvelin
  - Mutations cause a severe hemochromatosis in children

- Transferrin receptor 2
Case 4

55 yo man
One month history of fatigue and palpitations

PE:
- Pallor
- Palpable spleen tip (spleenomegaly)

Lab data:
- Hct: 20%
- Hgb: 6.9 gm/dl (severe anemia)
- MCV: 100 fl (slightly elevated)
- Platelets: Normal
- WBC: Normal
- Retic: 154,000/ cu mm (HIGH)

The normal values you need to know for this case:
- Hct: 0.39-0.49
- Hgb for a man: 14-18
- MCV: 80-98
- MCH: 27-34
- Platelets: 150,000-450,000
- WBC count: 3200-9800
- Reticulocytes: 20-100K / cumm

-if anemia develops slowly, cardiovascular/respiratory adaptation is beneficial
-if anemia develops acutely, cardiovascular/respiratory adaptation is deleterious
Anemia with Reticulocytosis: Differential Diagnosis

- **Bleeding** Rule out first

- **Hemolytic Anemias**
  - Immune- Autoimmune, alloimmune, drug induced
  - Inherited- Hemoglobinopathies, RBC membrane/enzyme disorders
  - Mechanical Prosthetic valves, Microangiopathic (MAHA)
  - Infections- Malaria, babesia

- **Hypersplenism**
Case 4 - Peripheral Blood Film

Microspherocytes

spherocytic cells in one type of autoimmune hemolytic anemia
Case 4 continued

- Diagnostic laboratory evaluation
  - Direct Coombs test: Positive, 4+, IgG
  - Warm autoantibody eluted from RBCs

- Diagnosis: Autoimmune hemolytic anemia

Coombs test: looking for presence of antibodies bound to red cells
- take another antibody to recognize human antibodies (Coombs reagent), and these antibodies bind to that autoantibodies on the red cells and cause agglutination that can be seen directly
Myeloid Differentiation

Bone marrow

Blast → Promyelocyte → Myelocyte → Metamyelocyte

Peripheral blood

Band → PMN
Case 5

- 42 yo dentist
  - Turned down as a blood donor because of Hgb of 11.5
- PE Splenomegaly 4cm below left costal margin
- Further testing revealed:
  - WBC: 47,000/ cu mm
  - WBC diff: Neutrophils 40%
  - Bands: 20%
  - Metamyelocytes: 16%
  - Myelocytes: 8%
  - Promyelocytes: 6%
  - Blasts: 2%
  - Eos: 2%
  - Basos: 4%
  - Monos: 2%
  - Platelets: 680,000/ cu mm

The normal values you need to know for this case:
- Hb for a man - 14-18
- platelets - 150,000 - 450,000
- wbc count - 3200 - 9800
- Neutrophils - 37-80%
- lymphocytes - 10-50%
- monocytes - 0-12%
- basophils - 0-7%
- eosinophils - 0-2%

Immature myeloid cells

Moderate anemia

High white cell count with immature myeloid cells
- basophils were increased
- platelets were increased
- basically diagnostic for CML but need a blood smear and test for Philadelphia chromosome
Case 5 - Peripheral Blood Film

Leukocytosis with left shift

Metamyelocyte
Myelocyte
Blast

lots of immature myeloid cells
elevated basophils and platelets
Case 5  continued

- Diagnostic evaluation

  - Cytogenetics: Philadelphia chromosome +
  
  (due to translocation between chromosomes 9 and 22, producing an abnormal product by splicing ABL and BCR genes)

- Diagnosis: Chronic myelogenous leukemia (CML)
  - A type of chronic myeloproliferative neoplasm

Philadelphia chromosome - presence is diagnostic of CML.
- Translocation of chromosomes 9 and 22 producing a fusion product BCR ABL.
- Gleevec specifically binds to tyrosine kinase site on BCR ABL and is revolutionary drug for treating CML.
Chronic Myeloproliferative Neoplasms (MPN)

- **Chronic myelogenous leukemia (CML)** - ↑Neutrophils, basophils
- **Polycythemia vera (PV)** - ↑RBC
- **Essential thrombocythemia (ET)** - ↑Plt
- **Idiopathic myelofibrosis (MF)** - ↑Fibrosis
Chronic Myeloproliferative Neoplasms: Clinical Features

- Enlarged spleen (except in Essential Thrombocythemia)
- Present with abnormal WBC, RBC, or platelet count
- Thrombosis and bleeding → ? Platelet dysfunction
- Must be distinguished from a reactive state, i.e.,
  - ↑ RBC → due to: Hypoxic stimulation Excess Erythropoietin
  - ↑ Plts → due to: infection, inflammation
  - ↑ WBC
- Natural history evolve over years. ie. not acute
- Usually NOT associated with fever, night sweats etc
Case 6

- 60 yo woman
  Presents with pruritus, headache and early satiety
- PE  Splenomegaly 5cm below left costal margin

CBC
- Hgb: 20 gm/dL
- MCV: 88 fl
- Platelets: 580,000 / cu mm
- WBC: 18,500
- WBC diff: Normal

Smear: No immature cells. Neutrophilia. Thrombocytosis

The normal values you need to know for this case:
- Hb for a woman - 12-14
- MCV - 80-98
- Platelets - 150,000 - 450,000
- WBC count - 3200 - 9800
Differential Diagnosis of Polycythemia

- Secondary
  - Smoking
  - Excessive erythropoietin
- Primary = Polycythemia vera

Diagnostic test:
- Mutation analysis of JAK2 gene - POSITIVE
- DIAGNOSIS - Polycythemia Vera
JAK-2 mutation results in activation of JAK-STAT pathway in absence of ligand – “cytokine independent constitutive activation”
Case 7

- 22 yo mechanic
  Admitted with fever, sore throat and numerous bruises
- PE - Purulent tonsillitis, petechiae and ecchymoses
- CBC:
  - Hgb: 6.1 gm/dl
  - MCV: 106 fl
  - Retic: 5,000/ cu mm
  - Platelets: 5,000 / cu mm
  - WBC: 1,900
  - WBC diff: Neutrophils 10%
    - Lymphs: 88% (relative lymphocytosis)
    - Monos: 2%
- Blood Smear: No immature cells. Severe neutropenia and thrombocytopenia confirmed. RBCs normal
Differential Diagnosis of Pancytopenia

- Reduced Production:
  - Hematologic malignancy – Acute leukemia
  - Myelodysplasia
  - Myelofibrosis
  - Aplastic anemia
  - Bone marrow suppression
    - Drugs, radiation, infections, toxins
  - Metastatic tumor in marrow
  - B12/folate/copper deficiency

- Increased destruction:
  - Paroxysmal nocturnal hemoglobinuria
  - Hemophagocytic syndrome
  - Hypersplenism
Case 7 - Bone marrow

Diagnosis: Aplastic Anemia
Case 8

- 29 yo woman, previously healthy
  Presents with heavy menstrual bleeding, numerous bruises
  PE: Petechiae and ecchymoses. No splenomegaly

Lab data:
- Hgb: 13.4 gm/dL
- MCV: 85 fl
- Platelets: 5,000 / cu mm
- WBC: 10,500
- WBC diff: Normal
- Smear: No immature cells. Thrombocytopenia. No schistocytes

DIAGNOSIS: Immune thrombocytopenic purpura (ITP)
Differential Diagnosis of Thrombocytopenia

- Impaired production
- Accelerated destruction
- Disorder of distribution (hypersplenism)
- Multifactorial

In this case it was autoimmune destruction of platelets - antibodies against platelets - underlying cause largely unknown - may also have alloantibodies from multiple blood transfusions - is production low? is there destruction?
Differential Diagnosis of Thrombocytopenia

- Impaired production
  - Drugs
  - Infections
  - Aplastic anemia
  - Hematologic malignancy
  - Myelophthysis
  - Myelodysplasia
  - B12/folate deficiency
Differential Diagnosis of Thrombocytopenia

- Impaired production
- Accelerated destruction
  - ITP
  - Drugs, including Heparin
  - Collagen vascular diseases
  - Infections including HIV
  - Disseminated intravascular coagulation (DIC)
  - TTP/HUS
  - Alcohol
  - Inherited platelet disorders
  - Post-transfusion purpura
  - Non-Hodgkin lymphomas

- Disorder of distribution (hypersplenism)
- Multifactorial
Microangiopathic hemolytic anemia

Hemolysis due to intravascular fragmentation of red blood cells; may be due to microcirculatory lesions or the insertion of cardiac or intravascular prosthetic devices.

Fragmented RBCs
Summary

- CBC and peripheral blood smear are the mainstays of diagnosing disorders of blood cells
- Anemia is very common worldwide and has many causes
- Anemias are classified based on red cell morphology followed by an etiological classification using special tests
- Leukocytosis is often reactive but various leukemias must be considered
- Immune destruction of platelets is a common cause of thrombocytopenia but decreased production due to bone marrow abnormalities must also be considered.
### EXAMPLES OF ANEMIA RESULTING FROM DECREASED RED CELL PRODUCTION

<table>
<thead>
<tr>
<th>Type</th>
<th>Mechanism</th>
<th>Diagnostic Features</th>
<th>Major Etiologic Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron Deficiency Anemia</td>
<td>Impaired heme synthesis</td>
<td>Hypochromia and microcytosis; decreased serum iron and increased total iron binding capacity; decreased serum ferritin</td>
<td>Dietary deficiency in infants and preadolescents; excess menstrual bleeding; chronic blood loss from the GI tract such as malignancy</td>
</tr>
<tr>
<td>Pernicious Anemia</td>
<td>Autoimmune gastritis leading to lack of gastric intrinsic factor and failure of vit B12 absorption; vit b12 deficiency delays DNA replication because it a cofactor in synthesis of THF</td>
<td>Pancytopenia, oval macrocytes, and hypersegmented neutrophils; megaloblastic hyperplasia; achlohydria; anti-intrinsic factor antibodies; hyperreflexia; absent position and vibration sensations; impaired vit b12 absorption corrected by intrinsic factor</td>
<td>Autoimmunity</td>
</tr>
<tr>
<td>Folate Deficiency</td>
<td>Delayed DNA replication</td>
<td>Pancytopenia, oval macrocytes, and hypersegmented neutrophils; megaloblastic hyperplasia</td>
<td>Dietary deficiency; malabsorption syndromes</td>
</tr>
<tr>
<td>Aplastic Anemia</td>
<td>Greatly diminished hematopoiesis</td>
<td>Pancytopenia, reticulocytopenia, marked hypocellularity of the bone marrow</td>
<td>Toxic drugs and chemicals; often idiopathic</td>
</tr>
<tr>
<td>Anemia of chronic disease</td>
<td>Diverse mechanisms; macrophages produce IL6, which causes hepatocytes to produce hepcidin and reduce iron absorption</td>
<td>Anemia most often normochromatic and normocytic or macrocytic; may be hypochromic and microcytic with decreased serum iron-binding capacity</td>
<td>Various chronic diseases, especially rheumatoid arthritis or SLE, renal disease and chronic infection</td>
</tr>
<tr>
<td>Myelophthisic</td>
<td>Bone marrow replacement; usually by a malignant tumor</td>
<td>Severe anemia; small numbers of nucleated red cells and immature granulocytes in the peripheral blood; tumor cells in the bone marrow</td>
<td>Malignancy</td>
</tr>
</tbody>
</table>
### Examples of Anemias Resulting from Increased Red Cell Production

<table>
<thead>
<tr>
<th>Type</th>
<th>Mechanism</th>
<th>Diagnostic Features</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warm antibody autoimmune hemolytic anemia (primary and secondary forms)</td>
<td>IgG autoantibodies combine with red cell surface antigens; Fc combining site of IgG antibody further reacts with Fc receptor of phagocytic cells</td>
<td>Anemia, spherocytosis, and reticulocytosis; unconjugated hyperbilirubinemia and acholic jaundice; positive direct Coombs test</td>
<td>Often secondary to lymphocytic neoplasms, Hodgkin's disease, or autoimmune disease; sometimes associated with methyldopa or penicillin therapy</td>
</tr>
<tr>
<td>Hemolytic disease of the newborn (erythroblastosis fetalis)</td>
<td>Maternal alloimmunization of fetal red cell antigens; classically of Rh system; can also be caused by alloimmunization to ABO blood groups</td>
<td>Rising titer of maternal anti-Rh antibodies during the later part of pregnancy; cord blood at delivery contains immature red cell precursors; direct Coombs test positive on cord blood; progressive increase in postnatal unconjugated bilirubin</td>
<td>Prevented by administration of anti-Rh antibody to mother at time of delivery of first and subsequent children</td>
</tr>
<tr>
<td>Hereditary spherocytosis</td>
<td>Red cell membrane skeletal protein abnormality</td>
<td>Autosomal dominant; anemia, spherocytosis, and reticulocytosis; increased mean corpuscular hemoglobin concentration; unconjugated hyperbilirubinemia and acholic jaundice; increase erythrocyte osmotic fragility in hypertonic saline; splenomegaly</td>
<td>Quantitative deficiency of spectrin due to diverse mechanisms</td>
</tr>
<tr>
<td>Glucose 6 phosphate dehydrogenase deficiency</td>
<td>Failure of erythrocyte hexose monophosphate shunt under oxidative stress</td>
<td>Self limited hemolytic anemia; reduced activity of erythrocyte G6PD</td>
<td>X linked inheritance</td>
</tr>
<tr>
<td>Sickle cell anemia</td>
<td>B globin hemoglobinopathy</td>
<td>Anemia and reticulocytosis; sickle shaped erythrocytes demonstrable on peripheral blood smear; homozygosity for hemoglobin S demonstrated with electrophoresis</td>
<td>Severe anemia, recurrent painful and asplastic crises, and nonhealing leg ulcers; recurrent splenic infarcts with progressive fibrosis result in autosplenectomy</td>
</tr>
<tr>
<td>B thalassemia major</td>
<td>Diverse mutations in B globin gene causing decreased synthesis of B globin chains, aggregation of alpha chains causes hemolytic anemia and ineffective erythrocytosis</td>
<td>Severe anemia; thalassemic red cell morphology; increase hemoglobin F</td>
<td>Occurs frequently in Mediterranean populations</td>
</tr>
<tr>
<td>Alpha thalassemia</td>
<td>Deletion of one or more of the four alpha globin genes</td>
<td>Differ according to the number of deletions</td>
<td>No clinical abnormalities with one gene deletion’ mild to moderate thalassemic state with 2 or 3 deletions; intrauterine death with 4 deletions- hemoglobin barts in fetal life and hemoglobin H in adult life</td>
</tr>
</tbody>
</table>