Anemia 101

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Blood
• Blood has red cells (erythrocytes)
• White cells (leukocytes)
• Platelets (thrombocytes)

Blood Components
Plasma 54%
White cells and platelets 1%
Red Cells 45%

White Blood Cells
• Fight infections
• Are increased in infections
• Move inside and outside of blood vessels
• Are made in the bone marrow

White Blood Cells
WBC - White Blood Cells 4.5 - 11.0 K/uL
Low = Leukopenia High = Leukocytosis
WBC Differential
Neutrophils - Segs 54 - 62%
Neutrophils - Bands 3 - 5%
Lymphocytes - Lymphs 25 - 33%
Monocytes - Monos 3 - 7%
Eosinophils - Eos 1 - 3%
Basophils - Basos 0 - 0.75%
Atypical Lymphs 0

Platelets
• Plug holes in the body to stop bleeding
• Can help cause blood to clot
• Made in the bone marrow

Fibrin
Red Blood Cells

- Carry oxygen from the lungs
- Carry carbon dioxide back to the lungs
- Also carry CO and NO
- Normally live 120 days
- Contains the protein hemoglobin
- Made from iron, folic acid, vitamin B12
- Made in the bone marrow

Microscope View

Red Blood Cells

- Red Cell Flow

Blood Vessel

Red Blood Cells - Shape

Incidence of Sickle Cell Disease and Thalassemia Worldwide
http://www.who.int/genomics/public/geneticdiseases/en/index2.html#SCA
Red Blood Cells - Hemoglobin

Oxygen

Normal Hemoglobin A has 2 alpha and 2 beta globin chains with 4 iron binding sites.

Globin Synthesis in utero

Hemoglobin FA – Biochemistry – Normal Newborn

Chromosome 16
- alpha
- gamma
- delta
- beta

Chromosome 11
- alpha
- gamma
- delta
- beta

Mom: alpha alpha gamma gamma delta beta
Dad: alpha alpha gamma gamma delta beta

50 – 20 % Hemoglobin A

Hb F (newborn): 50% to 80%
(6 months): 8%
(over 6 months): 1% to 2%

2% Hemoglobin A2

Hemoglobin A – Biochemistry – Normal Adult

Chromosome 16
- alpha
- gamma
- delta
- beta

Chromosome 11
- alpha
- gamma
- delta
- beta

Mom: alpha alpha gamma gamma delta beta
Dad: alpha alpha gamma gamma delta beta

95 - 97% Hemoglobin A

1 – 2 % Hemoglobin F or Fetal

2 – 3% Hemoglobin A2

Red Blood Cells - Marrow

Red cells, white cells and platelets are made in the bone marrow.

Red Blood Cells - Retics

- Reticulocytes, or Retics are young red cells just released from the bone marrow. The Retic count is the best indicator about how the marrow factory is doing.
Red Blood Cells

- Red cells live 120 days in the circulation.
- Red cells are made in the bone marrow.

Food with iron and vitamins is digested.

Hemoglobin Recycled

- Bilirubin must be Directed through the Liver to be Conjugated.
- LDH - Lactate Dehydrogenase.
- Iron - Fe bound to Transferrin.
- Kidney - Hemoglobinuria.

Red Blood Cells - Recycled

- Red cells are recycled in the spleen and liver. The iron and protein are stored and bilirubin is released.
- Spleen
- Liver

Erythropoietin is made by the kidney as a signal to the bone marrow to make more red cells.

Increased levels blocks absorption of Iron and cell release - inflammation IL6.

Hepcidin

- Decreased levels increase iron absorption and release from cells - Erythropoetin, low iron.
- Increased levels increase iron absorption and release from cells - Erythropoetin, low iron.

The History

- Weakness
- Tiredness - Fatigue
- Dyspnea
- Dizzy – non vertigo
- Palpitations
- New angina
The History - 2

- History of melena, abdominal pain, Aspirin or non-steroidal anti-inflammatory agents (NSAIDs) use, past peptic ulcer disease, then consider GI bleeding, platelet dysfunction.
- In females the menstrual history quantifying the amount of bloodloss, or possible pregnancy should be obtained.
- History of pica or abnormal craving for ice, clay, starch...; dysphagia then consider iron deficiency.
- Poor diet, then consider iron or folate deficiency, and general malnutrition.
- History of gastric surgery, distal paresthesias, gait problems, then consider B12 deficiency.

The History - 3

- History of alcohol abuse - consider folate deficiency or liver disease. If moonshine use or lead paint/pipe exposure, consider lead toxicity.
- Family history of blood cell or bleeding disorder: consider Sickle Cell disease, G6PD, Thalassemia, Hemophilia, von Willebrand
- History of jaundice, transfusion, new medication, infection - consider hemolytic process
- History of weight loss, Cancer, HIV, rheumatoid arthritis, thyroid disease, renal disease - then consider secondary cause
- History of fever and chills, cough, dyspnea, then consider Infection.

Physical Exam

Sclera

Spoon Nails – Fe Def.

Glossitis and Chelosis – Fe and B12
Physical Exam

- **GENERAL INSPECTION** - clubbing in TB or lung cancer
- Skin - Hypothyroid, SLE, Bruises, lesions, petechiae or purpura.
- **Weight** - Loss in Cancer, HIV, Chronic disease, gain in hypothyroid
- **VITAL SIGNS** - Pulse: Tachycardia from increased cardiac output
- Respiration: Tachypnea from decreased oxygen transport
- Temp: fevers in infections and drug or transfusion reactions
- **HEENT** - Eye: Jaundice if hemolysis, pallor in palpebral conjunctiva
- Mouth: Glossitis and angular stomatitis in iron or B12 deficiency
- **NECK** - Thyroid enlargement or nodules, lymph nodes
- **HEART** - Increased output/murmur - consider high output failure
- **LUNG** - consider infection, lesion
- **ABDOMINAL** - Liver/spleen size, masses, tenderness, surgical scars
- **RECTAL** - Stool guaiac, prostate exam in men
- **PELVIC/BREAST** - Uterine abnormality, Pap smear, Breast nodule
- **LYMPHNODES** - consider lymphoma, leukemia, infection, connective tissue Disease
- **NEUROLOGIC** - Decreased vibratory and position sense in B12 deficiency

**LAB- INITIAL SCREENING TESTS**

- Urinalysis - Hematuria/proteinuria in renal disease hemoglobinuria in hemolysis.
- CBC, red cell morphology and white cell differential, Reticulocyte count
- Chemistry profile (LDH, Bilirubin- Direct and Indirect, BUN, Creatinine, GPT),
- Hemoglobin Electrophoresis if hereditary hemoglobinopathy is suspected
- IF BLEEDING - Platelet Count, PT, aPTT, PFA

**CBC values by age**

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>NORMAL ADULT</th>
<th>COMMENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HB - Hemoglobin</td>
<td>Male = 15.5 +/- 2 mg/dl</td>
<td>Low = Anemia</td>
</tr>
<tr>
<td></td>
<td>Female = 13.5 +/- 2</td>
<td>High = polycythemia</td>
</tr>
<tr>
<td>HCT - Hematocrit</td>
<td>Male = 46.6 +/- 6%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female = 41.0 +/- 6%</td>
<td></td>
</tr>
<tr>
<td>RBC - Red Blood Cell Count</td>
<td>Male = 4.3 - 5.9 Mill/mm3</td>
<td></td>
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<tr>
<td></td>
<td>Female = 4.0 - 5.2</td>
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</tbody>
</table>

**Red Cell Indices MCH, MCHC**

| MCH - Mean Corpuscular | 27 - 32 pg | Low = Hypochromic |
| MCHC - Mean Corpuscular | 30 - 36 gm/dl | Low = R/O Fe def. |

**Red Cell Indices MCV - RDW**

| MCV - Mean Corpuscular Volume | 80 - 94 fl | Low = Microcytosis |
| RDW - Red Cell Distribution Width | 11.5 - 14.5 | High = Macrocytosis |

| Ratio of 1:2 pallor | Hypochromic | Normal | Hyperchromic |

| Red Cell Indices |MCV - 80 - 94 fl| Normal | RDW - 11.5 - 14.5 | Variation in RBC size |
RBC Morphology

<table>
<thead>
<tr>
<th>Red Cell Morphology</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burr Cells</td>
<td>Uremia, Low K, artifact, Ca stomach, PUD</td>
</tr>
<tr>
<td>Spur Cell</td>
<td>Post-splenectomy, Alcoholic liver disease</td>
</tr>
<tr>
<td>Stomatocyte</td>
<td>Hereditary, Alcoholic liver disease, ( \text{Ca stomach, PUD} )</td>
</tr>
<tr>
<td>Spherocyte</td>
<td>Hereditary, Immune hemolytic anemia, water dilution, post-transfusion</td>
</tr>
<tr>
<td>Shistocyte - helmet</td>
<td>TTP, DIC, vasculitis, glomerulonephritis, heart valve, burns</td>
</tr>
<tr>
<td>Eliptocyte - Ovalocyte</td>
<td>Hereditary, Thalassemia, Fe Def., Myelophthistic, megaloblastic anemias</td>
</tr>
<tr>
<td>Sickle Cells</td>
<td>Sickle cell disease</td>
</tr>
<tr>
<td>Target Cells</td>
<td>Thalassemia, hemoglobinopathies</td>
</tr>
<tr>
<td>Microcytes</td>
<td>Thalassemia, Iron Def., Lead Toxic,</td>
</tr>
<tr>
<td>Macrocytes</td>
<td>B12 of Folate Def.</td>
</tr>
<tr>
<td>Parasites</td>
<td>Malaria, Babesiosis, Bartonellosis</td>
</tr>
</tbody>
</table>

Platelets

- **Platelet Count** 150 - 400 K cell/μL
  - Low = Thrombocytopenia
  - High = Thrombocytosis

Reticulocyte Production Index

- \(<2\): Decreased Production
- \(>2\): Increased Loss

Anemia Diagnosis

- Loosing red cells (high retic count)
  - Bleeding
  - Hemolysis (High indirect Bili and LDH)
- Not making enough — (low retic count)
  - Low materials — Fe, B12, Folate
  - Low epo (Kidney disease)
  - Marrow problem (replaced, toxin,...)

Correcting the Retic

- absolute reticulocyte count (measured)
- reticulocyte (%) = absolute number of reticulocytes + number of RBC \times 100
- reticulocyte index = % reticulocytes \times \text{actual hematocrit} + normal hematocrit
- corrected reticulocyte index (corrects for appropriate bone marrow release of reticulocytes) = reticulocyte index + maturation factor
- maturation factor = 3.25 – \left( \text{actual hematocrit} + 20 \right)
  - if hematocrit 35, maturation factor = 1.5
  - if hematocrit 25, maturation factor = 2
  - if hematocrit 15, maturation factor = 2.5

Diagnostic Pathway
Anemia – low Hb/Hct Lab work-up
BPH = Bleeding/Production/Hemolysis

Microcytic
- MICROCYTIC = "TICS"
- T-Thalassemias
- I-Iron Deficiency
- C-Chronic Inflammation
- S-Sideroblastic - lead, drug, or hereditary

Microcytic Tests
TESTS TO ORDER:
- Serum iron
- TIBC = Transferrin binding sites
- % Saturation = Transferrin saturation with Iron
- Ferritin = Storage iron
- HBELP = Hemoglobin Electrophoresis
- Lead level if exposed

Microcytic workup
TICS – Thalassemia, Iron Deficiency, Chronic inflammation, Sideroblastic (Lead)

Ferritin by age

<table>
<thead>
<tr>
<th>Ferritin</th>
<th>Ferritin by age</th>
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</thead>
<tbody>
<tr>
<td>Young</td>
<td>25–300ng/mL</td>
</tr>
<tr>
<td>1 y.o.</td>
<td>200–400ng/mL</td>
</tr>
<tr>
<td>3–6 mo</td>
<td>10–300ng/mL</td>
</tr>
<tr>
<td>1–5 yr</td>
<td>7–140ng/mL</td>
</tr>
<tr>
<td>Adult male</td>
<td>20–200ng/mL</td>
</tr>
<tr>
<td>Adult female</td>
<td>10–120ng/mL</td>
</tr>
</tbody>
</table>

- Too Low – Iron deficiency
- Too high >2,000 Iron overload (20 – 25 transfused units) Need to chelate iron
Thalassemia Syndromes.
- Hereditary – Alpha or Beta chain
- Decrease Hemoglobin A
- Hemoglobin ELP abnormal and normal Ferritin are diagnostic
- Hemolysis (increased indirect Bili and LDH)
- Target Cells
- Supportive therapy or BMT

Iron deficiency
- Low Serum iron, Low Ferritin, High TIBC
- Find out why – Gi bleed, menses, diet
- Treat FeSO4 300mg tid
- Add vitamin C to increase absorption
- Follow up Retic increase 1 week, Ferritin 1 month

Chronic Inflammation
- Block of normal iron stores transport to bone marrow factory
- Normal Ferritin, serum iron and TIBC are low with a low saturation
- 30% Microcytic, 70% Normocytic
- High Sed rate or c-reactive protein
- Treat inflammation – RA, SLE, HIV....

Sideroblastic
- Ring sideroblasts in bone marrow
- Serum iron is increased and TIBC normal resulting in a high saturation. Serum ferritin is increased
- Basophilic stippling
- Lead toxicity is suspect

Normocytic Anemia
- NORMOCYTIC = "NORMAL SIZE"
- N-Normal Pregnancy
- O-Over hydration, Drowning
- R-Renal Disease
- M-Myelophthistic – Marrow replaced
- A-Acute Blood Loss
- L-Liver Disease
- SI-Systemic Infection/Inflammation
- Z-Zero Production- Aplastic anemia
- E-Endocrine: Hypothyroid, hypoadrenal, decreased androgen

Normocytic Tests
- Blood Urea Nitrogen (BUN), Creatinine, SGOT, Alkaline Phosphatase, Bilirubin, Erythrocyte Sedimentation Rate (ESR), Urinalysis, and Thyroid profile
- Renal Function tests
- Pregnancy Test
- Bone Marrow Biopsy
**Normocytic workup**

**“NORMAL SIZE”**

- Check BUN/Creat, Liver, UA, West Sed Rate, Preg Test
- BUN/Creat elevated or abnormal UA
- Work up for Renal Disease and Low EPO
- TSH elevated = Hypothyroid
- AST/ALT/AlkP – Liver disease
- West Sed rate elevated – Inflammatory Block
- Pregnancy test + = Prenatal care
- Pancytopenia
  - No - Repeat CBC, Retic in 2 weeks
  - Refer to Hematologist for Bone Marrow Bx

**Normocytic - Renal Failure**

- Anemia caused by decrease erythropoetin production causing decreased bone marrow production
- Can monitor erythropoetin levels
- Treat with epoetin alfa injections weekly or darbepoetin alpha every other week or monthly

**Parvo – Fifth’s Disease**

- Red cell production shut down in the bone marrow for 5 – 10 days
- May cause hemolysis
- May cause severe anemia in patients with chronic hemolysis like Thalassemia and Sickle cell. Use supportive transfusions.

**Macrocytic Anemia**

- MACROCYTIC = “BIG FAT RED CELLS” Or my “BF”
- B12 Malabsorption
  - I-Inherited
  - G-Gastrointestinal disease or surgery
- F-Folic Acid Deficiency
  - A-Alcoholism responsive
  - T-Thiamine responsive
  - R-Reticulocytes miscounted as large RBCs
  - E-Endocrine - hypothyroid
  - D-Dietary
  - C-Chemotherapeutic Drugs
  - E-ErythroLeukemia
  - L-Liver Disease
  - L-Lesch-Nyhan Syndrome
  - S-Splenectomy

**Macrocytic Tests**

- The peripheral blood changes include:
  - Anemia with decreased reticulocyte count, Increased MCV
  - Neutropenia with hypersegmented
  - Neutrophils
  - Thrombocytopenia with large platelets.

- LABS to order:
  - B12, Serum Folate, RBC Folate
  - if all normal, consider Methylmalonic Acid and Homocytene levels, TSH, and a Bone Marrow Bx.

**Macrocytic Work-up**

- B12 normal/Folate normal - Order Methylmalonic Acid and Homocytene levels
- B12 low/Folate low - B12 Deficiency and Folate Replacement with oral, nasal or IM B12 and Folate
B12 Cobalamin Deficiency

Physical signs include edema, pallor, jaundice, smooth tongue, decreased vibratory and position sensation

Hypersegmented polys

Low serum B12 level

Metformin, Gastric bypass, or PPI as cause?

Methylmalonic acid and homocysterine levels

Pernicious anemia - anti-intrinsic factor antibodies Schilling’s test

Rx - cobalamin 1000 mg I.M., oral, or Nasal Spray

Folate Deficiency

• Causes - liver disease, diet vitamin

B12 deficiency (needed as co-factor), and drugs such as methotrexate, ethanol, and dilantin.

• Lab – low serum and RBC Folate

• Rx – Folate 1mg po qD

Hemolysis (HIT)

• Hereditary (HEM)

— Hemoglobin (sickle cell, thalassemia)

— Enzyme (G6PD deficiency)

— Membrane (Spherocytosis, Eliptocytosis)

• Immune attack – Coombs positive (transfusion, IgM – cold antibody-infections, IgG warm antibody – Drug induced, PNH)

• Trauma – Microangiopathic (TTP, ITP, HUS, DIC, HIT, HELLP- Eclampsia, Malaria, Splenomegaly)

Hemolytic Anemia

• HEMOLYTIC = “HEMATOLOGIST”

• H-Hemoglobinopathy: sickle cell disease

• - Hemoglobinuria: Paroxysmal Nocturnal Hemoglobinuria

• E-Enzyme Deficiency

• M-Medication - drug induced: aldomet, INH

• A-Antibodies - Immune attack

• T-Trauma to the red cells: D.I.C., artificial heart valves

• O-Osmotic fragility in Hereditary spherocytosis

• G-G6PD Glucose-6-Phosphate Dehydrogenase Deficiency

• I-Infection: malaria, babesiosis

• S-Splenic destruction in hypersplenism

• T-Transfusion

• - Thalassemias

Hemolytic Signs

• 1. Elevated reticulocyte count, with stable or falling hemoglobin.

• 2. Elevated indirect bilirubin -

• 3. Elevated serum lactate dehydrogenase (LDH)-

• 4. Decreased Haptoglobin levels - Haptoglobin binds hemoglobin released in the plasma from red cell breakdown.

• 5. Hemoglobinemia and hemoglobinuria

• 6. Erythroid hyperplasia in bone marrow in chronic hereditary causes

• 7. Abnormal Hemoglobin Electrophoresis

Hemolytic Tests

• 1. The direct antiglobulin (Coombs’) test Direct Coombs test looks for antibody on the red cells. The Indirect Coombs looks for antibody in the serum.

• 2. Hemoglobin electrophoresis

• 3. Heinz body stain

• 4. Osmotic fragility

• 5. Blood smear
Hemolysis
Retic Production Index > 2, high LDH High indirect Bilirubin

Coombs or DAT

No = + Heinz body

No = HbF>4% hematobgic 
50-80 Hb A for Fetal
2% Hb A2

Yes = G6PD Deficiency
Warm Antibody
Cold Antibody

No = No warm antibodies
Shistocytes and Low Platelets in DIC, HIT, TTP, HELLP

Genetic Hemoglobin Issues

• Thalassemia – Normal DNA sequence, Reduction in globin production
• Alpha - Not enough alpha globin production - Southeast Asian, Indian, southern Chinese, Middle Eastern and African ancestry
• Beta – Not enough Beta globin production Greek, Italian, Middle Eastern, Southeast Asian, southern Chinese and African descent

Medical Care for Thalassemia Patients

• If Symptomatic – Get expert care with Hematologist
• Transfusion support and/or BMT
• Watch Ferritin/Iron and chelate to prevent overload
• Give Folic Acid (Folate)1 mg
• Do not give supplemental iron unless proven low and monitor

Hemoglobinopathy

Sickle Cell Disease – SS, SC, SD, SE, SOarab, S beta Thal
Newborn Screening or HbELP
Daily Penicillin –birth -6yo
Hydroxyurea prolongs life, prevents complications
Hydration, Oxygen, Temperature, and Folate
Hemoglobin FSA – Biochemistry – SB+Thal disease

<table>
<thead>
<tr>
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<tr>
<td>alpha alpha</td>
<td>gamma gamma delta Beta-5</td>
</tr>
<tr>
<td>alpha alpha</td>
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</tr>
<tr>
<td>alpha alpha</td>
<td>beta beta</td>
</tr>
<tr>
<td>beta S beta S</td>
<td>gamma gamma</td>
</tr>
<tr>
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</tr>
<tr>
<td>alpha alpha</td>
<td>delta delta</td>
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65-90% Hemoglobin S
5-30% Hemoglobin A
2-10% Hemoglobin F or Fetal
3.5-6% Hemoglobin A2

Hemoglobin – Biochemistry – Sickle Trait

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</tr>
<tr>
<td>alpha alpha</td>
<td>beta beta</td>
</tr>
<tr>
<td>beta S beta S</td>
<td>gamma gamma</td>
</tr>
<tr>
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</tr>
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</table>

47% Hemoglobin S
50% Hemoglobin A
1% Hemoglobin F or Fetal
2% Hemoglobin A2

Resource
- World Wide Web Site - The Sickle Cell Information Center
  - http://www.SCInfo.org
  - Information for providers, patients, teachers, employers, administrators
  - Monthly E-mail Newsletter aplatt@emory.edu
  - Listing of Clinics
  - Guidelines

Enzyme - G6PD - Glucose - 6 - Phosphate Dehydrogenase Deficiency
X linked genetic
Precipitated by oxidant drugs
Heinz body stain show denatured Hb
Avoid medications such as antimalarials, aspirin, sulfa drugs, and avoid eating fava beans.

Immune Attack
- Coombs Test: IgG and Compliment +/-
- Transfusion reaction: immediate or delayed
- IgM – (IgG Neg Comp +) cold antibody-infections like mycoplasma, EBV, HIV
- IgG warm antibody – Drug induced – Antibiotics, Ibuprofen, Autoimmune diseases
- PNH Paroxysmal Nocturnal Hemoglobinuria – Red cells attacked by complement Lack of CD55 or CD59 on RBC surface

Membrane problems Spherocytosis and Ovalocytosis
Trauma To Red Cells

- Microangiopathic - Coagulation gone wild, fibrin shredding red cells - TTP, ITP, HUS, DIC, HELLP - Eclampsia
- Splenomegaly
- Infections within the red cell
  - Malaria – Mosquito parasite - Tropics
  - Babesiosis – Tick parasite - New England area
  - Bartonellosis – Bacteria - Cat Scratch

Parasites – Malaria, Babesiosis, Bartonellosis