

Contributors: (A), (C), (R)

Week 1 at-a-glance

Monday - CPD Patient w/ Anemia [mandatory] [white coat]

Monday - Hematology Overview & Introduction to Hematopoiesis

Tuesday - Venipuncture CBC Lab [mandatory] [in-class lab report worth 2%]

Wednesday - Hemoglobinopathies & Thalassemia

Wednesday - Nutritional Anemias/Hemochromatosis Lab [mandatory] [in-class lab report and quiz worth 4%]

Thursday - Hemolytic Anemia/Hemoglobinopathies & Thalassemia Lab [mandatory] [in-class lab report and quiz worth 4%]

Friday - Marrow Failure

Friday - Problem Set Approach to Anemia [in-class submission worth 5%]

Resources:

Practice Questions for Week 1

Anemia: Basic lab tests video (Kenadeed)

The Antiglobulin Tests (Kenadeed)

Pathology Thread - Online Handout

Comments (somevaluation@virginia.edu): Please use the [Google Doc for comments](#).

Hematology System

Curricular Week 69 - System Week 1: Red Blood Cells

Patient Presentation: Anemia

Peter Waldron, MD

Monday, November 28, 2011

8:10-9:20 – Learning Studio

Before class:

Required:

Read Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapters 1: Normal Erythropoiesis

Chapter 2: Clinical Approach to Anemia

Chapter 7: Hemoglobinopathies – ONLY section B. Sick Cell Anemia – I. Classic Symptoms and Signs (pg 85)

Learning objectives:

Patient: Anemia, Normal values for WBC/RBC/PLT

1. Recognize the signs and symptoms of anemia and distinguish between the symptoms of acute anemia with volume depletion and chronic anemia in the euvolemic state.

- Mild anemia: DOE, increased heart rate
- Anemia:
 - Rate of onset; ratio of RBC/plasma volume decrease; adequacy of pulmonary & CV compensation
 - **Increased heart rate**; cardiac output; stroke volume -> high output state -> palpitations, tinnitus, dizziness, syncope
 - Redistribution of flow -> visceral/cerebral (pallor of mucosa, nailbed, conjunctiva)
 - **Dyspnea on exertion**
 - Respiratory failure is frequent 2* to cardiac decompensation (pulm edema) [beware transfusion → volume overload]
- **Acute blood loss**, when severe, can be expected to produce a **hemorrhagic anemia** --> **tissue hypoxia and acute vascular collapse**
- Chronic blood loss will generally result in an iron deficiency anemia, but people are able to expand their total blood volume and compensate with an increase in cardiac stroke volume and changes in regional blood flow.

2. Identify the typical hemoglobin levels that define anemia in children/adolescents and post-pubertal men and women.

Normal range:

At birth 17

Childhood 12

Adolescence 13

Adult man 16 (±2)

Adult woman (menstruating) 13 (±2)

Adult woman (postmenopausal) 14 (±2)

During pregnancy 12 (±2)

3. Recognize normal/abnormal values for the WBC count and differential, RBC indices, and platelet count. See 'lab values' on exam

4. List and describe the laboratory examinations that can assist one in determining the **etiology of the anemia.**

Complete blood count

- Red blood cell number: Red blood cell count, hemoglobin, hematocrit
- Red blood cell indices:
 - Mean cell volume (MCV),
 - Mean cell hemoglobin (MCH),
 - Mean cell hemoglobin concentration (MCHC),
 - Red blood cell distribution width (RDW)
- White blood cell count, white blood cell differential
- Platelet count
- Reticulocyte count: reticulocyte production index

Marrow examination

- Marrow aspirate: **E/G ratio, cell morphology, iron stain**
 - the ratio of erythroid to granulocytic (myelocytic) precursors is normally about 1:3-4. With anemia and high levels of erythropoietin stimulation, the erythroid precursor number increases dramatically and can rise to a ratio of 1:1 or even greater

- Marrow biopsy: cellularity, morphology

Iron studies

- Iron transport: serum iron, total iron binding capacity
- Iron stores: serum ferritin, marrow iron stain, serum transferrin receptor

RBC morphology (blood smear) (details moved to CBC Lab LOs)

- **Blood film morphology:** cell size, hemoglobinization, anisocytosis, poikilocytosis, polychromasia

5. List factors that impair the normal reticulocyte response to anemia. **Feel free to add more plz. See CBC Lab LO4**

- erythropoietin causes reticulocytes to leave bone marrow earlier
- defects in erythroid precursor proliferation or maturation

TABLE 11-9. SUMMARY OF NORMOCYTIC ANEMIAS

ANEMIA	PATHOGENESIS	DISCUSSION
Reticulocytosis < 3%		
Acute blood loss	Loss of whole blood	Initial Hb and Hct normal Infusion of normal saline uncovers anemia Signs of volume depletion (e.g., absolute neutrophilic leukocytosis) commonly present; positive tilt test (refer to Chapter 4)
Early iron deficiency	Decreased iron stores	Normocytic <i>before</i> microcytic Iron studies abnormal (↓ serum ferritin)
Early ACD	Iron trapped in macrophages by hepcidin	Normocytic <i>before</i> microcytic Iron studies abnormal (↑ serum ferritin)
Aplastic anemia	Suppression or deficiency of myeloid stem cells	Pancytopenia Hypocellular marrow
Chronic renal failure	Deficiency of EPO	Presence of burr cells

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Hematology System

Curricular Week 69 - System Week 1: Red Blood Cells

[Hematology Overview/Introduction to Hematopoiesis](#)

[Donald Innes, MD](#)

Monday, November 28, 2011

9:30-10:00 – Learning Studio

10:10-12:00 - Auditorium

Before class:

Required:

1. Read the handout titled “[General Review of Hematopoiesis](#)”

2. Read Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapter 1: Normal Erythropoiesis

Chapter 2: Clinical Approach to Anemia

Chapter 4: Anemias Associated with a Reduced Erythropoietin Response p. 43-45

Chapter 10: Blood Loss Anemia p. 124-128

Chapter 13: Erythrocytosis and Polycythemia Vera p. 163-165, 168-171

Recommended:

Review the teaching materials from the following MCM sessions:

- [One-Carbon Metabolism](#) by Dr. Marty Mayo (09/22/10)

- [Porphyrin Synthesis and Degradation](#) by Dr. Bryce Paschal (09/23/10)

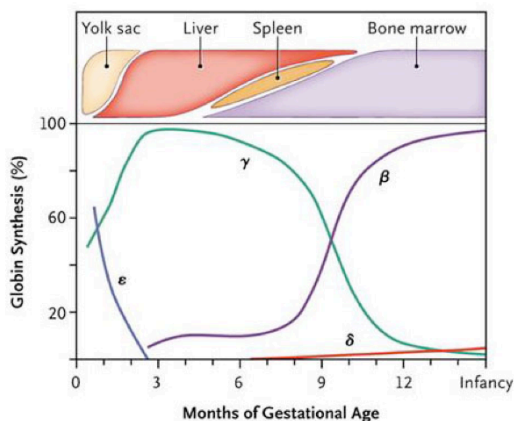
The [PowerPoint slides](#) used in this activity will be made available on the course website in advance of the session.

Learning objectives:

Basics of hematopoiesis, focus on RBCs,

1. Identify the different sites of hematopoiesis from fetal to adult life.

- Fetal: yolk sac (3-8wk), liver (6-30wks), spleen (9-28wk), bone marrow (>28wks) --from *First Aid*
 - Mnemonic: **Y**oung **L**iver **S**ynthesizes **B**lood.
- Adult
 - Bone Marrow (vertebra, sternum, pelvis, ribs, skull, scapula, proximal long bones (femur&humerus))



In this picture, the greek letters = the hemoglobin chains that are accompanying the two alpha chains

From MCM:

- Initially cells in the intraembryonic mesoderm lining the yolk sac
- 28 days: Mesoderm linking the dorsal aortae
- 6-8 weeks: Liver (ends shortly after birth)
- 12 weeks: Spleen
- 4 months: Bone marrow (principal source by month 6)

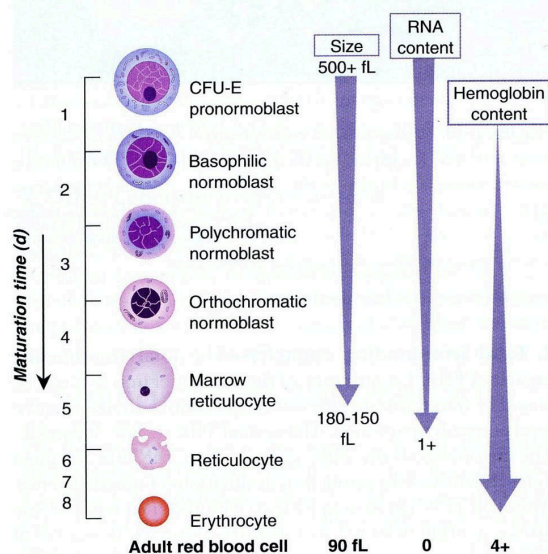
2. List the functional capabilities of hematopoietic growth factors by target cells.

Growth Factor	Source	Action in Vitro	Action in Vivo	Clinical Use
EPO (Erythropoietin)	• Kidney (peritubular interstitial cells)	• Increase survival and proliferation of BFU-E, CFU-E	Increase • RBC production • Hgb F	Anemia of • Renal failure • Chronic disease

	~10% made constitutively in liver		• marrow cellularity	• Malignancy
G-CSF (Granulocyte Colony Stimulating Factor)	• Monocytes, fibroblasts, endothelial cells	• Stimulates CFU-G to produce granulocytes • Granulocyte activator (superoxide, chemotaxis)	• Increase WBC count ~12 hrs • Increase granulocyte release from BM and stimulates CFU-G • ~3-5 days	Neutropenias • Acquired • Congenital Marrow transplantation
IL-5	Marrow stromal cells	• Eosinophilic growth factor • Stimulates lymphocytes		
SCF (Stem Cell Factor, Kit ligand) = a tyrosine kinase receptor ligand	• Fibroblasts, marrow stromal cells	• Enhances stem cell survival/differentiation • Mitogenic for most cells • Synergistic with IL-6, IL3, GM-CSF, G-CSF	• Deficient SCF leads to failed marrow microenvironment	Marrow failure • congenital • acquired Expand marrow stem cells

CFU-G - Colony Forming Unit - Granulocytic
CFU-E - Colony Forming Unit - Erythroid

3. Describe and recognize the maturation sequence in the development of erythroblasts to mature erythrocytes, including the temporal duration and the lifespan of these cells.



- The nucleus decreases in size and nucleoli are reduced in number or disappear.
- The nuclear shape may evolve from round/oval to a specific configuration and there is loss of mitotic competence.
- Fine, delicate nuclear chromatin mature into coarse, clumped, condensed chromatin
- Synchrony between nuclear and cytoplasmic maturation.
- Begin maturation in bone marrow. Completes maturation during 1st 24 hours in peripheral blood (reticulocyte, has increased RNA. Normally 0.5-2% reticulocytes in blood)
 - Make a lot of RBCs every day, so make mistakes. ~10% of RBCs will be defective in some way and die in the marrow -> normal
- Stays in peripheral blood for ~120 days

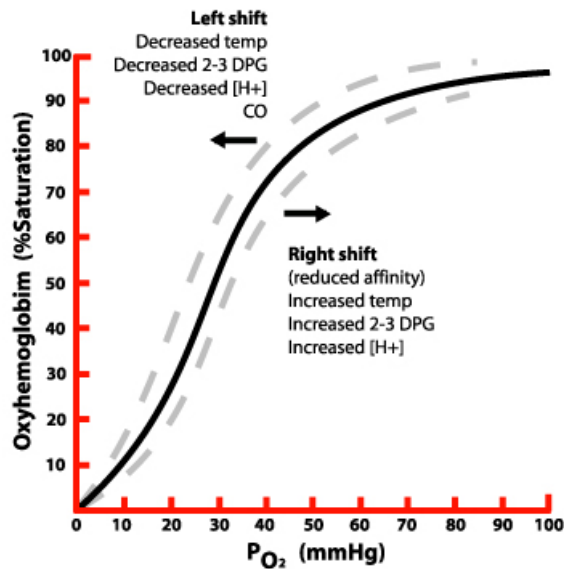
Myeloid:Erythroid ratio - normally 3:1

also called E/G ratio (erythroid precursors to granulocytic (myelocytic) precursors ratio), in which case the normal is 1:3-4

- Gives an idea of erythrocyte production in bone marrow
- Only count nucleated red blood cells

4. Describe the site of production for erythropoietin and the stimulus for its synthesis.

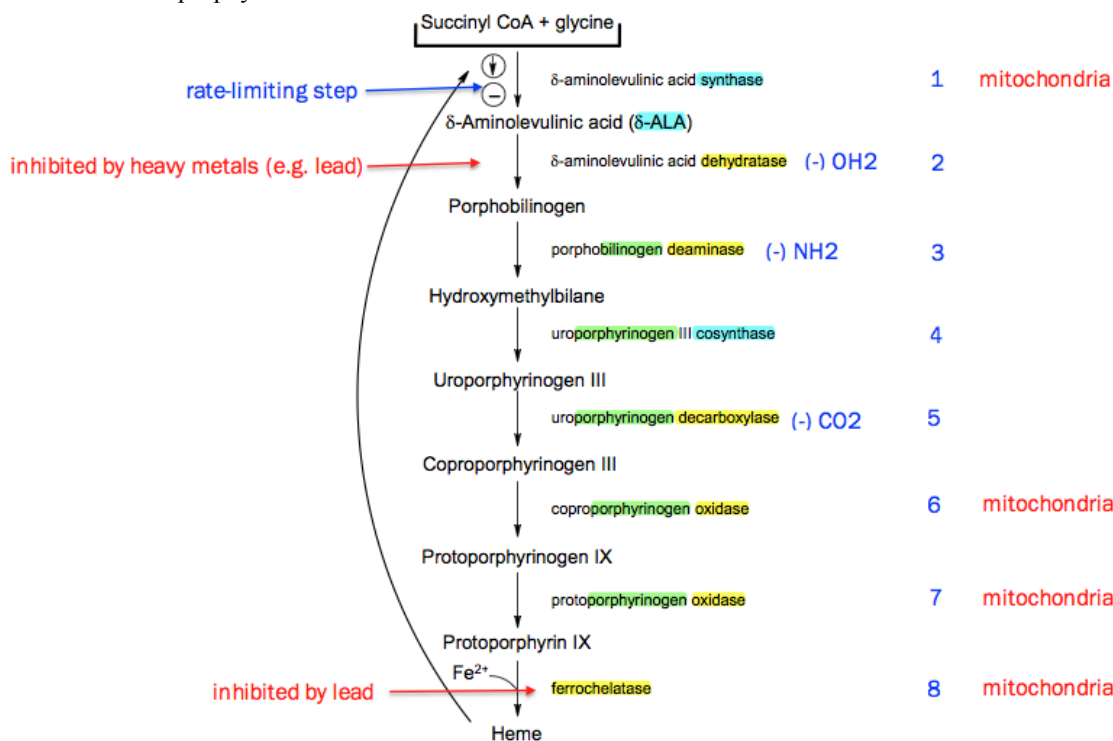
- Produced - **peritubular interstitial cells of the kidney**
- Stimulus - decreased pO₂ in the tissues
- Basal levels of [constitutive] erythropoietin are also produced by kidney and liver. Can also be stimulated by anemia (decreased hemoglobin) or a decrease in hemoglobin affinity for oxygen (shift in the oxygen dissociation curve)



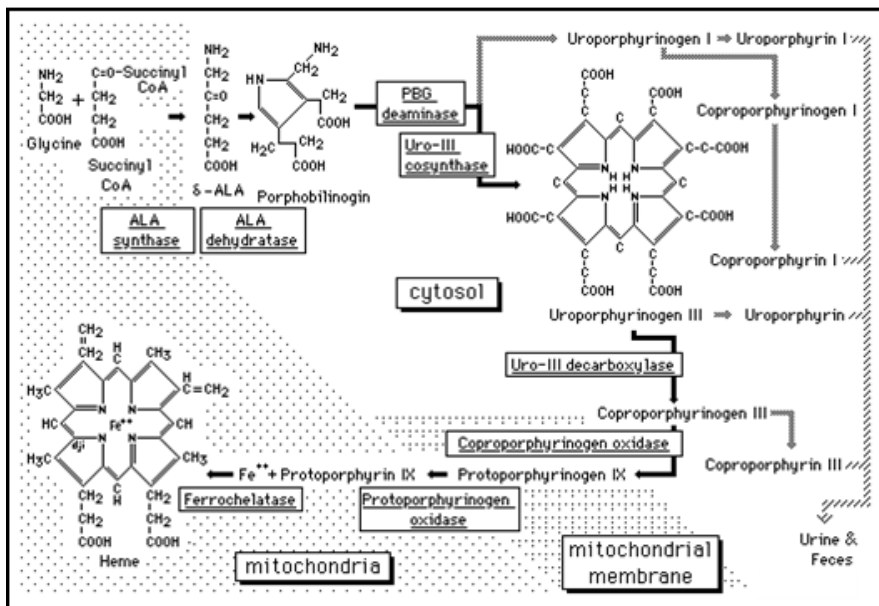
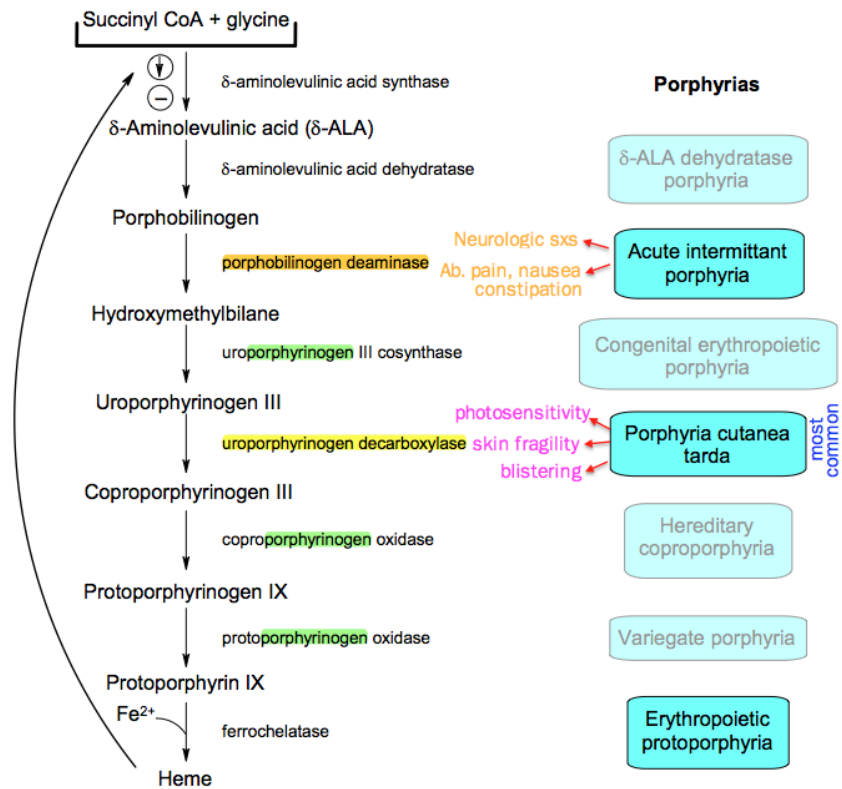
5. Describe the pathway and rate-limiting steps by which heme is synthesized. Including abnormalities, e.g. porphyrias, heavy metal toxicity.

Steps

- 1: Condensation of succinyl CoA and glycine by **ALA synthase** to ALA is the **rate-limiting step** in hepatic porphyrin synthesis
- 2: Dehydration of ALA by **ALA dehydratase** to Porphobilinogen
- 3: **Porphobilinogen deaminase** condenses 4 porphobilinogens together to form hydroxymethylbilane
- 4: Hydroxymethylbilane's ring is closed by **uroporphyrinogen synthase** to form uroporphyrinogen III
- 5: Uroporphyrinogen III is converted to coproporphyrinogen III by **uroporphyrinogen decarboxylase**, adding a vinyl bond
- 6: Coproporphyrinogen is III is methylated by **coproporphyrinogen oxidase** to form protoporphyrinogen
- 7: Protoporphyrinogen receives a propionyl group from **protoporphyrinogen oxidase** to form protoporphyrin IX
- 8: Protoporphyrin IX receives an Fe²⁺ from **ferrochelatase** to form heme



PNEUMONIC = Ala Placed in Urine Cup Produces Heme



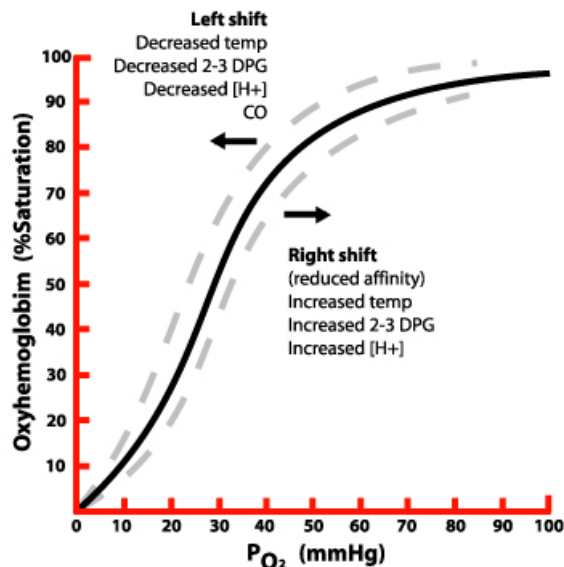
- Requires adequate supply of iron, amino acids, and pyroxidine (vitamin B6).
- ALA synthase is the rate limiting step for the entire process and also the committed step.
- **Acute Intermittent Porphyria**
 - Defect in porphobilinogen (PBG) deaminase
 - Sx - abd pain, neurologic deficits (motor paralysis)
- Variegate porphyria
 - decreased activity of protoporphyrinogen oxidase
 - Sx - abd pain, neurologic deficits, light sensitivity
- **Porphyria cutanea tarda**
 - partial defect in Uroporphyrinogen decarboxylase
 - *Dermal photo sensitivity
 - usually asymptomatic
 - alcohol and estrogen can trigger
- Protoporphyria

- Decreased heme synthetase
- dermal photosensitivity increased
- **Heavy metals (such as Pb)**
 - Down regulate ferrochetalase, ALA synthase, and ALA dehydratase, thereby inhibiting heme synthesis. Results in increased ALA in circulation.
 - decreased heme production → iron overload → sideroblastic anemia
 - Pb binds to sulfhydryl groups found in these enzymes and hinders their function.

6. List the normal hemoglobins found in fetal and adult blood.

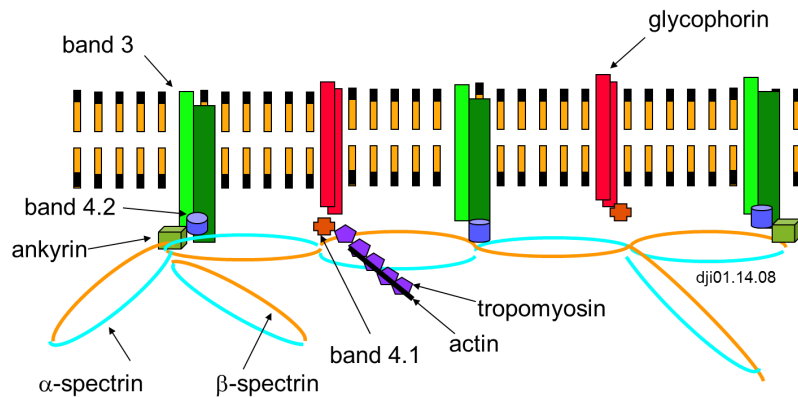
- Hemoglobin A = $2\alpha, 2\beta$ (two alpha chains and two beta chains)
 - 97% of adult hemoglobin
- Hemoglobin A2 = $2\alpha, 2\delta$ (two alpha chains and two delta chains)
 - 3% of adult hemoglobin
- Hemoglobin F = $2\alpha, 2\gamma$ (two alpha chains and two gamma chains)
 - 50% of fetal hemoglobin, disappears over the 1st 6 months of life (can be upregulated in hemoglobinopathies later in life)

7. Draw a normal hemoglobin oxygen dissociation curve, identify P50 on the curve, and show the direction of shift of the curve elicited by increases or decreases of pH, 2,3-DPG concentration, CO2 concentration, HbF, increased temperature, and HbS.



- Increased HbF - shifts to left
 - HbF has a greater affinity for O2 than does HbA and therefore releases it less easily
- Increased HbS - shifts to right
 - HbS has a lesser affinity for O2 than does HbA and therefore does not bind O2 as easily
- p50 = the pO2 at which hemoglobin is 50% saturated
 - here, at p50, pO2 roughly equals 30 mmHg.
- Decrease in pH → shifts curve to right, releasing O2 at tissues.
 - E.g. Lactic acid build-up during exercise (need more oxygen).
 - Chronic acidosis will result in a decrease in 2,3 DPG production over 12-36 hrs to normalize the dissociation curve.
- Increase in 2,3-DPG - shifts curve to right, stabilizes alpha1-beta2 interface, releasing O2 at tissues
 - recall that the relaxed form of hemoglobin has a higher affinity for O2 and therefore does not release O2 as easily. What does CO poisoning do? Puts heme into the relaxed form → doesn't release O2 as easily (treatment is with 100% O2 or hyperbaric O2)
- Increase in CO2 - shifts to the right (greater O2 demand)
- Increase in temperature - shifts to the right (greater O2 demand)

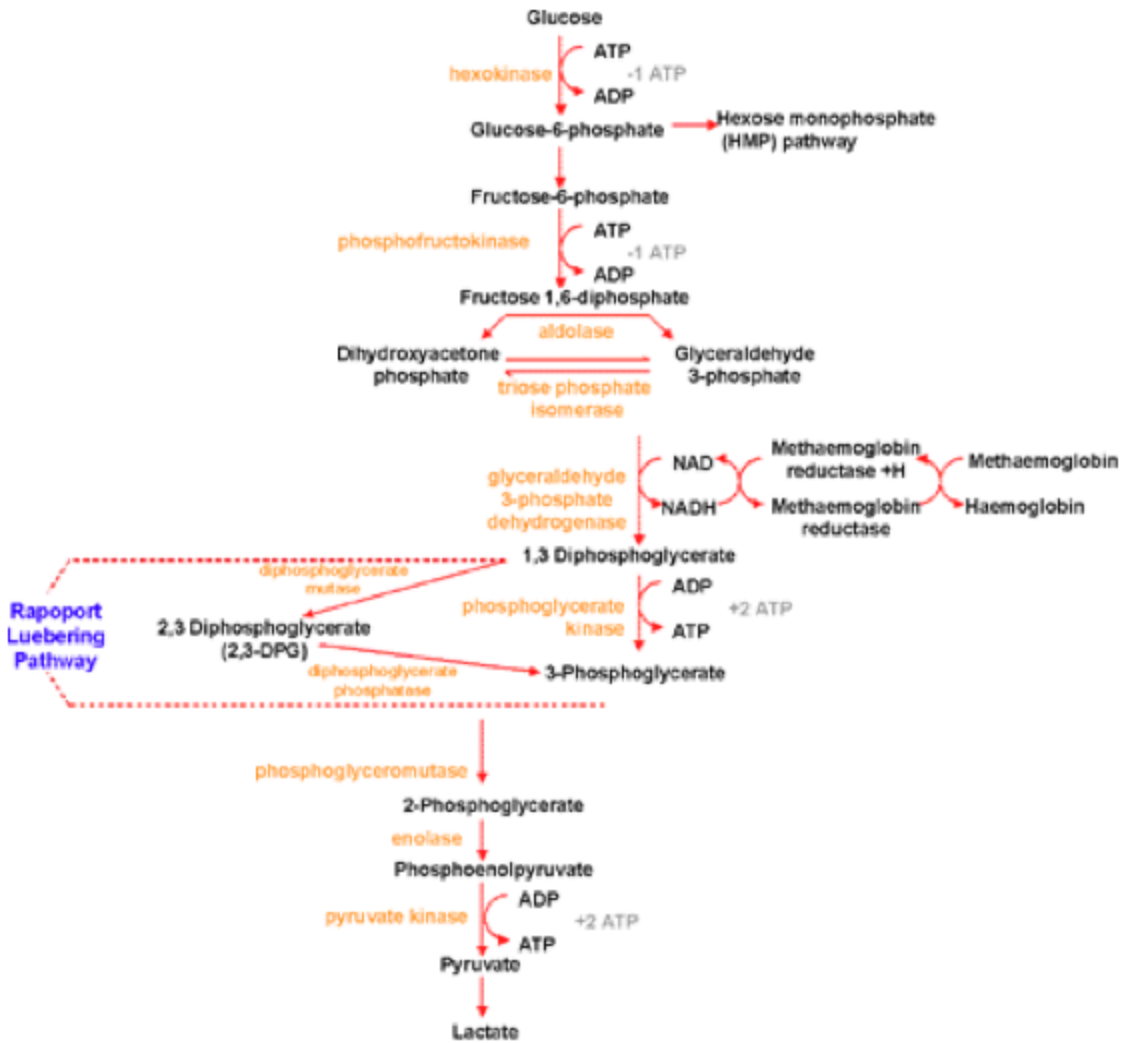
8. Using knowledge of the RBC membrane and RBC metabolism predict how defects in these structures and processes induce specific hematological disease states.



- ankyrin and band 4.1 help anchor the spectrin helices to the transmembrane proteins
- Outer lipid layer is rich in phosphatidyl choline, sphingomyelin, and glycolipid
 - recall that phosphatidylcholine (lecithin) and sphingomyelin are monitored prenatally to assess fetal lung maturation (the **lecithin:sphingomyelin ratio**)
- Inner lipid layer is rich in phosphatides of serine, ethanolamine, and inositol.
- Cholesterol makes up almost half of the lipid layer.
 - It is in equilibrium with unesterified cholesterol in plasma.
 - Abnormal LCAT (lecithin cholesterol acyltransferase) activity, abnormal cholesterol levels can cause abnormal RBC morphology (formation of target cells, reduced lifespan)
- Defects in vertical structure (deficiency in spectrin, ankyrin, or band 3) result in spherocyte formation.
- Defects in horizontal spectrin framework result in RBC fragmentation or mild elliptocytosis.

9. Recognize the Embden-Meyerhof (glycolysis) pathway and describe:

- Methemoglobin is when iron in heme group is in the Fe³⁺ (ferric) state. Cannot release bound O₂.
- The Embden-Meyerhof pathway is the process of glycolysis through which glucose is converted to pyruvate while producing ATP and NADH.



a. How the pathway helps regulate the reduction of methemoglobin back to hemoglobin.

- Supports the **methemoglobin reductase pathway**
- During glycolysis, NADH is formed (during the formation of 1,3 Biphosphoglycerate).
- Methemoglobin reductase acts to convert methemoglobin to hemoglobin using the energy stored in this NADH, and in the process converts NADH back to NAD⁺

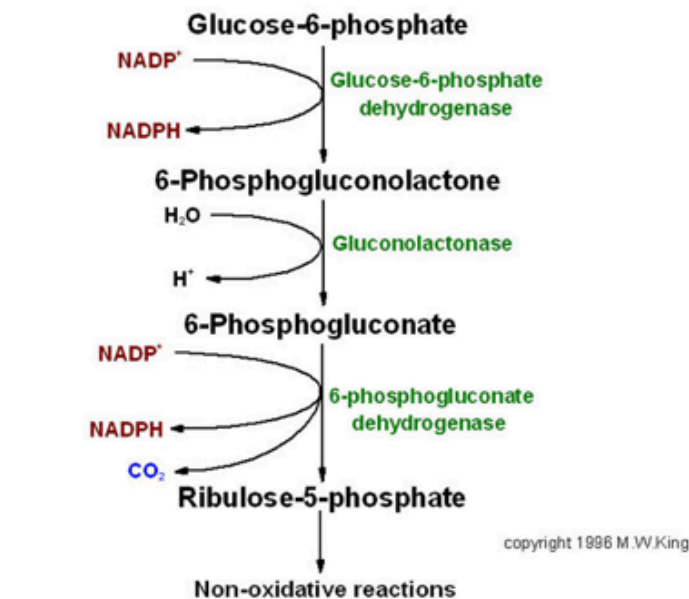
b. How the pathway relates to 2,3-BPG production.

- In glycolysis, 1,3 biphosphoglycerate is converted to 3 phosphoglycerate to form ATP.
- The Luebering-Rapaport Pathway acts as shunt from this step, forming 2,3 BPG.
- **The rate of this rxn is proportional to rate of glycolysis.**
 - As glycolysis increases, O₂ demand increases, and the increased 2,3 BPG stabilizes hemoglobin in its T-state, allowing for the release of more O₂ at the tissues.

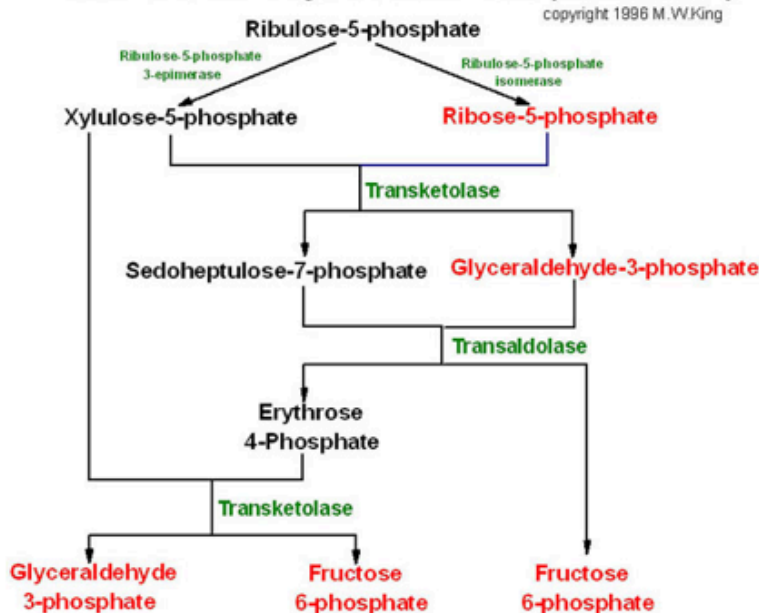
10. Describe the function of the pentose phosphate pathway (phosphogluconate pathway) and how this helps protect red cells from oxidant stress.

- Couples oxidative phosphorylation with NADP and **glutathione reduction**
 - Glutathione counteracts environmental oxidants and prevents globin denaturation
- The pentose phosphate pathway **results in the production of NADPH** through glucose-6-phosphate dehydrogenase (which can be used in fatty acid synthesis) and pentose sugars.
 - NADPH reduces GSSG to glutathione via glutathione reductase.
 - The oxidized form of glutathione is formed via glutathione peroxidase, which converts H₂O₂ to H₂O.
- Without this, H₂O₂ would form free radicals which could attack the cell → damage whenever oxidative agents are encountered
- When patients lack glucose-6-phosphate dehydrogenase or glutathione reductase, denatured hemoglobin precipitates on the inner surface of the RBC membrane, resulting in membrane damage and hemolysis

Oxidative Stage of Pentose Phosphate Pathway



Non-Oxidative Stage of Pentose Phosphate Pathway



- Note that **ribose-5-phosphate** is required for the synthesis of nucleotides and nucleic acids. All cells, including those G6PD deficient, can make ribose-5-phosphate from fructose 6-phosphate thanks to **transketolase** (this is why symptoms of R5P deficiency don't manifest in G6PD)

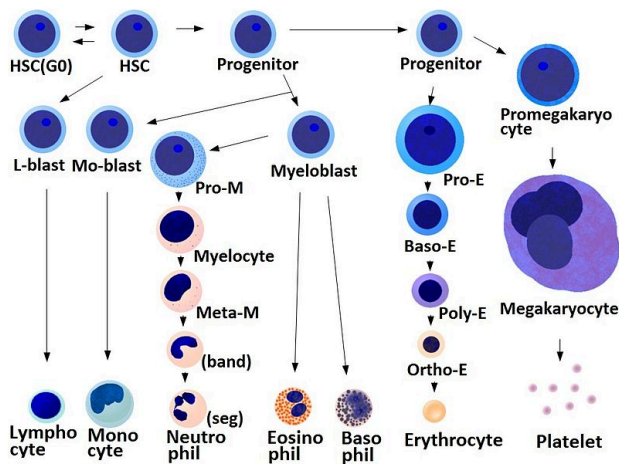
11. Identify sites of red blood cell destruction and the process by which this is accomplished. (pages 137-8)

- Aging RBCs
 - As RBCs become older, metabolic pathways decay, oxidized hemoglobin accumulates and oxidized phospholipids, especially phosphatidylserine, appear on the surface of the cell
 - A loss of flexibility interferes with their ability to move through the microvasculature
 - Both of these events initiate the process of removal by the **monocyte-macrophage system** via the CD36 receptor
- Spleen
 - Structure of the spleen is a testing ground for RBC flexibility and viability
 - Delivered by terminal arterioles to the splenic red blood cell pulp where the volume of plasma is reduced and the cell is subject to relatively hypoxic conditions
 - Tests the metabolic pathways and results in further reduced pliability in older cells
 - To escape and re-enter circulation, RBCs must squeeze through a 2-5 um opening in the **sinusoidal wall of the spleen**- traps rigid cells and leads to phagocytosis and destruction by reticuloendothelial cells lining the sinusoids
- Pathways of RBC Destruction
 - Effectively recover heme iron for new RBC production

- Intravascular: In circulation
 - Free hemoglobin dissociates into dimers that bind to **haptoglobin** or is oxidized to methemoglobin that dissociates to release heme for binding with albumin and hemopexin
 - Binding step prevents immediate loss of the heme group by glomerular filtration and allows clearance by hepatocytes
 - Liver breaks down the heme group to recover iron and produce bilirubin
- Reticuloendothelial Cell (Extravascular) Destruction: In the spleen
 - Used for most senescent cells
 - RBCs are phagocytosed by reticuloendothelial cells
 - Membrane is disrupted
 - Hemoglobin is broken down by lysozymal enzymes
 - Recovered iron is stored or transported back to marrow in the form of **hemosiderin** for RBC production
 - Amino acids are recovered
 - Protoporphyrin ring is metabolized to tetrapyrrol (**bilirubin**) with the release of CO
- Final Step for Both
 - Bilirubin is conjugated by hepatocytes
 - Conjugated bilirubin is excreted into bile
 - Converted by gut bacteria to urobilinogen and urobilin
 - Final products are excreted in stool (stercobilinogen) and urine (urobilinogen)

12. Compare and contrast the cause and pathophysiology of types of erythrocytosis – polycythemia vera, secondary erythrocytosis and relative erythrocytosis.

- Polycythemia = erythrocytosis
 - **Polycythemia vera** - absolute polycythemia due to **myeloproliferative disorder** which causes continual overproduction of erythrocytes.
 - **Stem cell disorder** (clonal myeloproliferative disorder) characterized by the production of an excessive number of mature hematopoietic cells, especially RBCs
 - Mutational event appears to involve pluripotent cells from the bone marrow, although some patients demonstrate clonality for all cell lines except T cells and natural killer lymphocytes
 - Progresses with time to various combinations of cell proliferation and cytopenia, myelofibrosis, distorted cell maturation, and even acute leukemia
 - Most prominent feature is **increased RBC mass**, associated with the appearance of a clone of erythroid precursors that grows in vitro in the absence/near absence of erythropoietin
 - Uncontrolled proliferation is believed to be due to somatic mutations in JAK2 V617F, which results in an increase in tyrosine kinase activity (present in 90-95% of patients); does not appear to be the sole event in the pathogenesis of the disease or outcome
 - Treated with **phlebotomy, chemo/radiation**, and complication management
- Since this is a stem cell disorder, all cell lines are increased. Recall:



- **Secondary erythrocytosis** - due to compensatory mechanism to stress placed on body. Can also be due to hemoglobin mutants, erythropoietin producing neoplasms, and inherited defects stabilizing HIF-1 alpha (hypoxia inducible factor).
 - **Increase in RBC mass without evidence of changes in other hematopoietic cell lines**
 - Normal physiologic response to hypoxia
 - Can result from increased autonomous production of erythropoietin by a diseased kidney or extrarenal tumor

- Causes
 - Hypoxic Erythrocytosis: Hypoxia increases erythropoietin levels, increases RBCs
 - High-Affinity Hemoglobinopathies: Hemoglobin doesn't want to unload, causes hypoxia but patients appear saturated because Hb is bound to O₂
 - Methemoglobinemias: Shifts the dissociation curve left, resulting in compensatory polycythemia
 - Increased erythropoietin production: Increased erythropoietin, increased RBCs
 - Erythropoietin receptor mutations: Failure to shut off signal transduction following erythropoietin binding
- **Relative erythrocytosis** - seemingly increased number of red blood cells due to a decreased plasma volume (e.g. dehydration).
 - Moderate elevation of hemoglobin (16-18 g/dL in men, 14-16 g/dL in women)
 - Caused by a decrease in plasma volume without a true increase in RBC mass
 - Acute Elevations
 - Severe dehydration secondary to salt and water deprivation, diarrhea, excessive vomiting
 - Overly aggressive use of diuretics
 - Sudden change in vascular permeability secondary to allergic reaction/anaphylaxis (can reach as high as 20 g/dL)
 - Chronic Elevations
 - Cushing Disease
 - Pheochromocytoma
 - "Stress Erythrocytosis:" Occurs in young to middle-aged males who smoke, are hypertensive, and live a high-pressure lifestyle (may be driven by a hormonal increase in vascular tone)
 - Treatment depends on the diagnosis

Polycythemia	RBC mass	Plasma volume	Sat. O ₂	EPO
Polycythemia vera	↑	↑	Normal	↓
Appropriate polycythemia (e.g. COPD, cyanotic congenital heart disease)	↑	Normal	↓	↑
Inappropriate polycythemia: ectopic EPO (e.g. renal cell carcinoma)	↑	Normal	Normal	↑
Relative polycythemia (e.g. volume depletion)	Normal	↓	Normal	Normal

13. Describe the mechanisms for cellular oxygen sensing and identify reasons for which increased oxygen delivery may be necessary.

- Hypoxia induces insulin-like growth factor-1 (IGF-1) binding protein
- IGF-1 may play an erythropoietin-like role
- Don't forget the chemoreceptors

14. Describe the mechanisms, specific causes and consequences of an elevated erythropoietin level

- Changes in oxygen delivery to tissue
 - Decrease in the oxygen content of hemoglobin (pulmonary dysfunction)
 - Decrease in the hemoglobin level (anemia)
 - Decrease in the hemoglobin affinity for oxygen (shift in the oxygen dissociation curve)

Normal mechanism:

Renal interstitial cells sense oxygen tension via a flavoheme protein that controls the level of hypoxia inducible factor (HIF-1). HIF-1 interacts with DNA to increase Epo production.

Effect: Epo travels to bone marrow, binds EPOR receptor on erythroid precursors. Increases proliferation and maturation of committed stem cells into new RBCs. Effect takes several days.

response to erythropoietin depends largely on iron supply for making hemoglobin

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Before class:

Required:

1. Review Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapter 2: Clinical Approach to Anemia

2. Watch the Hematology Laboratory Instructional Videos at:

<http://www.med-ed.virginia.edu/Courses/path/videos/index.cfm>

3. Review the [lab manual](#) and bring a copy to class.

Learning objectives:

1. Measure the hematocrit and calculate RBC indices; use them to identify and classify anemias by MCV and MCHC.

Measuring the Hematocrit (Hct): Hct is a measure of the volume of packed RBCs compared to total blood volume, and is thus given as a percent

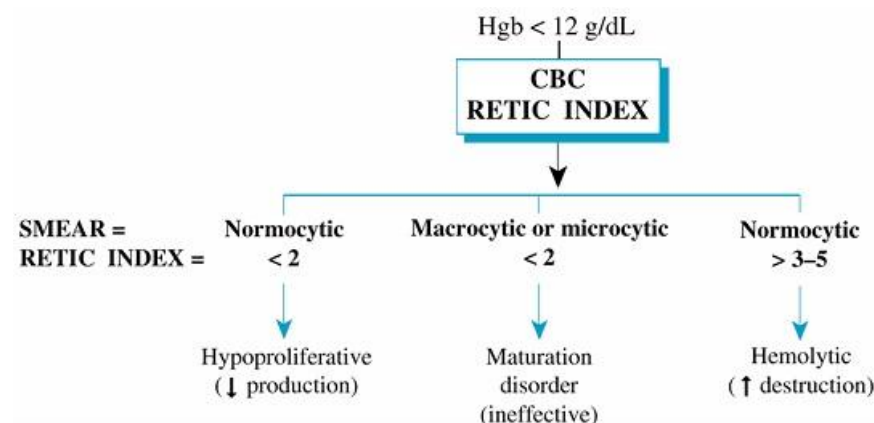
- Determine Hct reading from the top of the red cell column, using the reading device provided. Duplicate values should agree within 2 ml/100 ml.
- Traditional hematocrit methods employ centrifugation of anticoagulated whole blood to determine the volume of packed red cells.

RBC indices: MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular Hemoglobin), MCHC (Mean Corpuscular Hemoglobin Concentration)

- $MCV = Hct / \#RBCs$
- $MCH = Hgb / \#RBCs$
- $MCHC = Hgb / Hct$

Classification of anemias by MCV and MCHC:

- Normal MCV is 90 +/- 9 fL
 - Macrocytic anemia = $MCV > 100$ fL
 - Microcytic anemia = $MCV < 76$ fL
- Normal MCHC is 30 +/- 3%
 - Main use is to detect hereditary spherocytosis (very small, dense spherocytes in circulation, $MCHC > 36\%$)
 - **think this is wrong. Normal MCH is 30 +/- 4, normal MCHC is 320-360 g/L (or 32-36 g/dL)**



*Note here that the “Retic Index” (basically the reticulocyte count) is given as a percentage rather than an absolute count, as in LO#2 below.

Table 2.4 Classification of anemia.

Microcytic, hypochromic	Normocytic, normochromic	Macrocytic
MCV < 80 fL	MCV 80-95 fL	MCV >95 fL

MCH < 27 pg	MCH > 27 pg	
Iron deficiency Thalassemia Anemia of chronic disease (some cases) Lead poisoning Sideroblastic anemia (some cases)	Many hemolytic anemias Anemia of chronic disease (some cases) After acute blood loss Renal disease Mixed deficiencies Bone marrow failure (e.g. post-chemo, infiltration by carcinoma, etc.)	Megaloblastic: Vitamin B12 or Folate deficiency Non-megaloblastic: alcohol, liver disease, myelodysplasia, aplastic anemia, etc.

2. Classify anemias according to the reticulocyte count.

Red Cell Production

	Reticulocytes	M:E
Normal	25,000-75,000/uL	3:1
Hemolytic anemia	>100,000/uL	decreased
Hypoproliferative anemia	25,000-75,000/uL or lower	increased
Ineffective erythropoiesis	25,000-75,000/uL or lower	decreased

3. List and describe how other laboratory examinations can assist one in determining the etiology of the anemia.

- Elevations in serum bilirubin (indirect fraction) and lactic dehydrogenase (LDH) seen in patients with increased hemolysis and disorders of marrow precursor maturation with high levels of ineffective erythropoiesis (death of immature red cell precursors within the marrow).
- The indirect (unconjugated) serum bilirubin concentration correlates w/ rate of RBC turnover.
 - Normally, serum bilirubin = 0.4-1 mg/dL; most is indirect bilirubin (70-80%).
 - In hemolysis or severe ineffective erythropoiesis, indirect bilirubin increases to 0.8-3 mg/dL.
 - The direct or conjugated bilirubin level shows little change, as long as the patient's liver function is normal.
- Serum LDH is extremely sensitive to increased rates of red blood cell destruction, because red blood cells are rich in LDH, especially LDH 1.
 - Patients with hemolytic anemias show rises in their serum LDH concentrations in excess of 1000 IU/L (normal 300-600 IU/L), without abnormalities in other liver enzymes.
 - With severe hemolysis or marked ineffective erythropoiesis, the LDH level can reach several thousand units.

There's also a massive list of tests used to elicit the etiology of specific disorders in Table 2-3. Obviously we don't need to know all of these yet, but I will include for completeness:

"Laboratory assays in the diagnosis of specific red cell disorders."

Hypoproliferative anemias

- Cytometric assay of CD59/CD55 levels (Paroxysmal nocturnal hemoglobinuria)
- Chromosomal analysis (Leukemias)
- Marrow aspirate/biopsy special status
 - Trichrome stain (Myelofibrosis)
 - Silver stain for reticulin (Myelofibrosis)
 - Peroxidase, esterase, and PAS stains (Acute leukemia)

Maturation disorders

- Serum vitamin B12 level (Vitamin B12 deficiency)
- Serum/red blood cell folate levels (Folic acid deficiency)
- Urine/serum methylmalonic acid level (B12 and folate deficiency)
- DU suppression test (B12 deficiency)
- Hemoglobin electrophoresis (Thalassemia)
- Hemoglobin A2 level (Beta-thalassemia)
- Hemoglobin F level - alkali denaturation (Beta-thalassemia)
- Acid elution slide test for hemoglobin F (Thalassemia versus hereditary persistence of hemoglobin F)
- Brilliant cresyl blue stain (Hemoglobin H)
- Red blood cell protoporphyrin level (Iron deficiency - lead poisoning)

Hemolytic anemias

- Hemoglobin electrophoresis (Hemoglobinopathies)
- Coombs test (Autoimmune hemolytic anemia)
- Cold agglutinin titer (Autoimmune hemolytic anemia)

- Haptoglobin level (Hemolysis)
- Serum/urine hemosiderin (Intravascular hemolysis)
- Osmotic fragility (Hereditary spherocytosis)
- Incubated autohemolysis test (Congenital nonspherocytic hemolytic anemias)
- G6PD screen (G6PD deficiency)
- Heat/isopropanol denaturation tests (Unstable hemoglobins)

Polycythemia

- ^{51}Cr - red blood cell mass (Erythrocytosis)
- Red blood cell P50 (Abnormal hemoglobin)
- Red blood cell 2,3-DPG level (Abnormal hemoglobin)
- CO level (Carboxyhemoglobinemia)
- Serum erythropoietin level

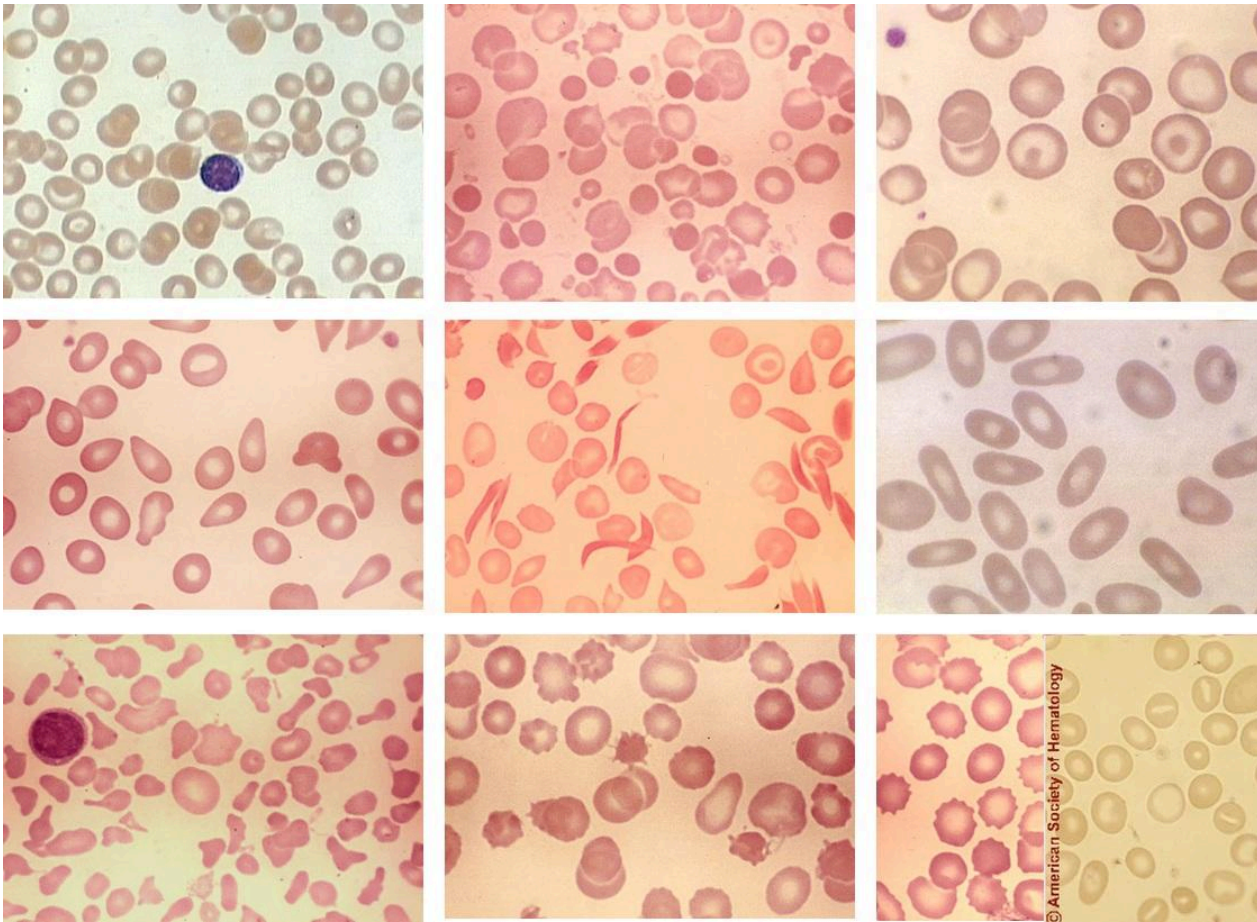
4. List factors that impair the normal reticulocyte response to anemia.

Table 2.5 Factors impairing the normal reticulocyte response to anemia.

- Marrow diseases, e.g. hypoplasia, infiltration by carcinoma, lymphoma, myeloma, acute leukaemia, tuberculosis
- Deficiency of iron, vitamin B12 or folate
- Lack of erythropoietin, e.g. renal disease
- Reduced tissue O₂ consumption, e.g. myxoedema, protein deficiency
- Ineffective erythropoiesis, e.g. thalassaemia major, megaloblastic anemia, myelodysplasia, myelofibrosis
- Chronic inflammatory or malignant disease

5. Identify structural red cell abnormalities on a peripheral blood smear and associate them with clinical conditions.

- **Spherocytes:** spherical RBC w/o central pallor; caused by severe burns, hereditary spherocytosis (caused by defects of spectrin, band 3 or ankyrin), autoimmune hemolysis
- **Target cells:** Hgb appears concentrated in the center of the cell in target or bull-eyes; caused by β hemolysis; seen in liver disease, thalassemia, hemoglobinopathies, iron deficiency, post-splenectomy
- **Dacryocytes:** teardrop shape; associated w/ **thalassemia**, myelofibrosis (abnormal vasculature of spleen/marrow), myelophthisic anemia (distorted marrow vasculature). Teardrop shaped RBCs are formed as RBCs are “pitted” in the spleen, removing denatured hemoglobin, DNA, RNA, and other cellular material. The RBC stretches and is distorted into a tear shape.
- **Elliptocytes:** oblong, ellipsoid shaped w/ nearly parallel sides; hereditary elliptocytosis caused by abnormal spectrin dimerization; hereditary pyropoikilocytosis caused by spectrin deficiency + abnormal dimerization
- **Ovalocytes:** egg shaped, megaloblastic anemia, marrow failure
- **Drepanocytes:** thin, curved, sickle-shaped cells; frequently have pointed ends; seen in sickle cell. Abn. β globulin chain: glutamine $\rightarrow$ valine forms Hgb S. The β -6 valine moves to the outside of the β tetramer allowing it to stick to the β -85 phenylalanine and the β -88 leucine on adjacent β tetramers. Also, in low oxygen situations, Hgb S forms a gel and polymerizes into fibrillary structures causing RBCs to become rigid and sickled.
- **Acanthocytes:** spiny spiked RBC w/o central pallor; occurs when anionic (-) compounds accumulate in outer leaflet of plasma membrane; associated w/ spur-cell anemia or liver disease
- **Stomatocytes:** mouth-like central pallor; occurs when cationic (+) compounds accumulating in inner leaflet of membrane; usually hereditary but can be seen in hyperlipidemia, “over-hydrated”, alterations to permeability leading to increased volume (looks like a flathead screw)
- **Burr cells (echinocytes):** Most commonly an artifact. Occasionally found in renal disease, reversible. Anionic (-) compounds may produce echinocytes.
- **Bite and blister cells:** due to incomplete repair of site of denatured Hb removal; “bites” associated w/ Heinz bodies, G6PD deficiency, oxidant drugs, unstable Hb
- **Anisocytosis:** variation in red cell size
- **Poikilocytosis:** variation in RBC shape
- **Schistocytes:** fragmented RBC; torn or broken by physical means; caused by hemolysis; associated w/ severe burns, uremia/HUS, microangiopathic anemia, TTP



Upper left: Anisocytosis (microcytosis, use the blue lymphocyte as a rough guide for appropriate size)

Upper middle: Spherocytes

Upper right: Target cells

Center left: Dacrocytes

Center middle: Sickle cells (Drepanocytes)

Center right: Elliptocytes

Lower left: Poikilocytosis and Schistocytes

Lower middle: Acanthocytes (recall no central pallor)

Lower right: Burr cells [Left], Stomatocytes [Right]

6. Estimate values for the WBC count and differential, RBC indices, and platelet count; distinguish normal from abnormal.

- WBC:
 - Leukocyte count: 4,500-11,000/mm³ or 4.5-11.0 x 10⁹/L
 - Segmented neutrophils: 54-62% or 0.54-0.62
 - Bands: 3-5% or 0.03-0.05
 - Eosinophils: 1-3% or 0.01-0.03
 - Basophils: 0-0.75% or 0-0.00750
 - Lymphocytes: 25-33% or 0.25-0.33
 - Monocytes: 3-7% or 0.03-0.07
- RBC Indices:
 - MCV: nml = 80-100 fL
 - MCH: nml = 26-34 pg
 - MCHC: nml = 32-36 g/dL
- Platelet count: 150,000-400,000/mm³ or 150-400 x 10⁹/L

[Lab Values](#)

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Before class:

Required:

Read Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapter 6: Thalassemia

Chapter 7: Hemoglobinopathies

The [PowerPoint slides](#) used in this activity will be made available on the course website in advance of the session.

Learning objectives:

1. Identify the normal chromosomes responsible for alpha-globin and beta-globin synthesis and list the three types of hemoglobin found in normal adult blood.

- The alpha globin chains are encoded by two closely linked genes (alpha-1 and alpha-2) on chromosome 16. Healthy individuals have 4 normal alpha genes, two on each chromosome 16.
 - The non-alpha globin chains include beta, delta, and gamma, and are all encoded by a cluster of genes on chromosome 11.
- The pairing of alpha and non-alpha chains makes the hemoglobin molecule.
 - Hemoglobin F is $\alpha_2\gamma_2$, hemoglobin A is $\alpha_2\beta_2$, and hemoglobin A2 is $\alpha_2\delta_2$. Only small amounts of hemoglobin A2 (3.5%) and hemoglobin F (<1%) are found in adult blood.

2. Describe the precipitating factors that influence the severity of the anemia and the pathophysiologic process by which hemoglobin S causes sickling.

- HbS = substitution of valine for glutamic acid at the 6th AA of the beta-chain
 - The oxygenated form of Hb cannot participate in polymer formation. With O₂ release there is a conformation or allosteric change in the Hb molecule. With HbS this conformation change exposes the mutated amino acid [#6 Glu>Val], which can bind to another HbS molecule → sickling
 - Thus, only the deoxygenated form of Hb S can participate in polymer formation (sickling)

Factors that increase sickling:

- acidosis
- increased temperature
- increased 2,3 BPG
- dehydration
- hypertonic environment (as in renal medulla)
- cool extremities (decreased rbc return to lungs = more deoxy form of HbS)

- hgb S polymerizes, forming long chains, deforming the cell membrane and causing sickling

3. Describe classic clinical features (symptoms, signs and complications) of the consequences of sickling.

- splenic infarction and sequestration esp at high altitude
 - leads to increased incidence of infections, especially with encapsulated organism like Strep pneumo, H. influenza, Neisseria, Salmonella (SHiNS)
- venous thromboembolism
- PE
- hyposthenuria (decreased renal concentrating)
- hematuria
- ESRD
- renal papillary necrosis
- pain attacks - due to ischemia/infarct; bone/marrow = common site
- acute chest syndrome: CXR infiltrate, fever, chest pain, tachypnea, cough (50% = infectious)
- increased risk of CVA
 - thrombolytic strokes in 1st decade of life; hemorrhagic in 3rd decade

4. Relate the pathophysiology of the sickling process to organ system involvement, specifically kidney and spleen and the complications of stroke and acute chest syndrome.

- Acute splenic sequestration
 - a large proportion of RBC mass is trapped in the spleen. The inciting factor is not known. It often occurs with other

complications such as infection, or vaso-occlusive events. In the worst cases hypotension (shock) and death occur. Splenic sequestration is a medical emergency. Due to early **splenic infarction and atrophy** this is most common among < 3 year olds.

- Acute Chest Syndrome

- a new infiltrate on Chest X-ray of a person with sickle cell disease. This is usually accompanied by some combination of chest pain, respiratory distress, fever, cough and abnormal lung auscultation. The precipitating cause may be infectious, but other etiologies are identified in about half of the cases. **Acute and chronic pulmonary disease is the leading cause of death in sickle cell disease.**

- Cerebrovascular events

- present as neurological deficits which are sometimes transient. Stroke risk starts at age two. Children ages two to ten have predominantly thrombotic strokes; 7% of children with Hb SS will have stroke by age 10, and 11% will have a stroke by age 20. The risk of CNS infarct among two to fifteen year olds is associated with increased velocity in cerebral arteries. This fact was used to develop a screening test [transcranial Doppler] for stroke. Application of this test, and institution of a chronic transfusion regimen for children identified as high risk, has resulted in a marked decrease in the frequency of stroke in children with Hb SS. Adults 20 and older have mostly hemorrhagic strokes. Thrombotic stroke of small vessels detectable by MRI, but without stroke symptoms [“silent infarct”], occurs in an additional 20% of children.

- Renal

- Isosthenuria (inability to concentrate urine) occurs consistently and hematuria may occur. There is also an increased frequency of nephrotic syndrome and nephritis as well as the development of chronic renal failure in the third decade of life.

5. Describe how abnormal cell adhesion and abnormal nitric oxide metabolism can contribute to sickle cell disease complications.

- **Hemolysis causes NO deficiency** through binding of NO by the cell-free, plasma hemoglobin and by the liberation of arginase from the red cell to the plasma where it decreases availability of arginine the primary substrate for NO production.
 - decreased NO availability is associated with vasoconstriction, pulmonary HTN, and other SCD complications
- decreased NO availability are strongly associated with pulmonary hypertension in patients with Hb SS = less flexible and stickier rbc are moving through inappropriately constricted arterioles [due to less NO] lined with stickier endothelial cells.

6. Use the epidemiology and relative severity of the sickle cell genotypes to predict outcome; assess pain frequency as an indicator of disease severity.

- pain is a symptom and an indicator of disease severity

HbSS

- 0.14% or 1:700 African-Americans are affected
- A to T nucleotide change and Glu to Val amino acid change in β globin
- HbSS is the most severe form of SCD
- ½ of those with HbSS will have symptoms by age 2
- Complications are due to infection, anemia, and ischemia/infarction

Sickle cell trait: heterozygous HbS, largely asymptomatic

- protected against *Plasmodium falciparum* malaria

HbC: same codon is mutated as in HbS (β -chain, codon 6), except lysine (not valine) is substituted for the glutamic acid

- Homozygotes have milder disease than HbS homozygotes
- HbSC patients (heterozygous HbS and HbC) also have milder disease vs. HbS homozygotes

7. Explain the rationale for the following sickle cell disease therapies: antibiotics, folic acid, hydroxyurea, acetaminophen, narcotics and blood transfusion.

Increased Risk of Infection:

- due to gradual, complete, splenic infarction (SI) by sickle RBC's
 - recall: spleen is important for combating encapsulated bacteria, so with a poorly functioning spleen, you are particularly susceptible to SHiN (pneumonic): *Strep pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*
- The spleen is particularly vulnerable to infarction by Hb SS cells due to prolonged RBC transit time, decreased pH of the spleen, and possibly differences in the spleen's endothelium
- spleen makes component of complement cascade = without spleen there is less effective complement activation and more infections

Therapy for Increased Risk of Infection:

- Infection Prophylaxis: Penicillin age 3 mo. to 5 y.o.; immunizations against *S. pneumoniae*, *H. flu.*, meningococcus and yearly

Influenza, in addition to the standard immunizations

Nutritional Deficiency:

- **Folate deficiency** may occur due to increased demand of high RBC turnover and decreased dietary intake.

Therapy:

- Folic acid supplementation is indicated for individuals if there is any doubt about the adequacy of dietary intake. Due to folate supplementation of many processed foods in the USA, folate deficiency has become rare.

Hydroxyurea

- results in an increase in HbF (mechanism = unknown)
- reduces the frequency of painful events, acute chest syndrome, and frequency of transfusion in adults with sickle cell anemia.

Narcotics/NSAIDs/Acetaminophen

- treatment of pain associated with vaso-occlusive events, avascular necrosis of femoral head, and ischemia/infarction in bone marrow (deep pain in bones)

Blood Transfusion:

- indicated in the acute events of worsening anemia, severe acute chest syndrome, splenic sequestration, persistent priapism, any new neurologic deficit and prior to general anesthesia.
- It is indicated on a chronic basis among people who have had a cerebrovascular event.
- Note: carries the risk of infection, alloimmunization and if used chronically iron overload.
 - Iron overload can cause liver, heart, and endocrine dysfunction.
 - frequent transfusion carries risk of transmitted hepatitis and fibrosis from iron overload if no chelators are used

8. Describe the basic genetic differences between alpha-thalassemia and beta-thalassemia.

- Alpha-thalassemia → mutation or loss of alpha-1 or alpha-2 globin genes (or both)
 - very severe if both (incompatible with extrauterine life)
- Beta-thalassemia → mutation or loss of beta-globin genes (4)
 - Beta-thalassemia minor = 1 or 2 mutations
 - Beta-thalassemia major = 3 mutations

9. Describe the genetic, hematologic, and clinical differences between alpha-thalassemia trait, hemoglobin H disease, and hydrops fetalis.

Alpha-thalassemia trait, hemoglobin H disease, and hydrops fetalis exist on a continuum based on the number of mutations in the alpha1- and alpha2-globin genes.

Alpha-thalassemia trait

- Two functional alpha globin genes (aa/-- or a-/a-)
- Clinically normal, but have minimal anemia and reduced MCV and MCHC
- RBC count usually increased, typically exceeding $5.5 \times 10^{12}/L$

Hemoglobin H disease

- Single functional alpha globin gene
- Abundant formation of hemoglobin H
 - Hemoglobin H has a high affinity for oxygen and no Bohr effect or heme-heme interaction
 - **Ineffective supplier of oxygen** to the tissues under physiologic conditions
 - RBCs with HbH are sensitive to oxidative stress → more prone to hemolysis when oxidants (sulfonamides, etc) are administered
 - RBCs removed from circulation prematurely due to precipitation of HbH
- High ratio of beta globin to alpha globin (2-5 fold increase in beta globin production)
- Excess beta globin aggregates into tetramers, which accounts for 5-30% of hemoglobin in these patients
- Primarily characterized as a **hemolytic disease**

Hydrops fetalis

- Most severe; complete loss of both pairs of alpha globin gene products
- Cannot make functional alpha globin → unable to form functional hemoglobin A, F, or A2
- Incompatible with extrauterine life; fetuses die in utero or shortly after birth from severe anemia

Genotype	α genes	Name	
$\alpha\alpha / \alpha\alpha$	4	Normal	
$\alpha\alpha / \alpha-$	3	Silent Carrier	
$-- / \alpha\alpha$	2	Thalassemia trait	minor
$\alpha- / \alpha-$	2		
$-- / \alpha-$	1	Hgb H disease	intermedia
$-- / \alpha^{CS}\alpha$	1	Hgb H-Constant Spring	
$-- / --$	0	Hydrops fetalis	major

10. Describe the hematologic findings and pathophysiological changes and epidemiology, that are associated with beta-thalassemia major and with beta-thalassemia minor; hemoglobin E disorders.

- **β -Thalassemia Minor:**
 - Hematologic Findings: Hemoglobin 3-6 g/dL
 - Pathophysiological Changes: Mild, asymptomatic anemia
 - Epidemiology: Heterozygotes for β globin gene deletion
- **β -Thalassemia Major:**
 - Hematologic Findings: Microcytosis, hypochromia, severe anemia
 - Pathophysiological Changes: Severe, life-threatening anemia during the first 2 years of life; high serum iron; high saturation of total iron-binding capacity; marked tendency for tissue iron loading; failure to transfuse results in continued anemia, high levels of **ineffective erythropoiesis**, death during childhood, skeletal changes, growth retardation, and failure to reach sexual maturity
 - Epidemiology: Usually homozygotes (β globin gene deletion) or double heterozygotes
- **Hemoglobin E**
 - Hematologic Findings: Microcytosis, hypochromatosis
 - Pathophysiological Changes: Anemia in homozygotes
 - Epidemiology: Southeast Asians

Genotype	Description
β / β	Normal
β / β^0	Beta thalassemia trait (minor)
β / β^+	
β^+ / β^+	Beta thalassemia intermedia
β^+ / β^0	
β^E / β^+	
β^E / β^0	
β^0 / β^0	Beta thalassemia major (Cooley's Anemia)

Production of β -globin:
 β^+ = decreased
 β^0 = absent

11. List the mechanisms and consequences of iron overload and infections associated with beta-thalassemia major.

Iron overload

- High levels of erythropoietin (+inefficient erythropoiesis) → increased iron absorption
- Chronic transfusions
- Leads to iron accumulation and end organ failure. Can cause sideroblastic anemia
- Treatment: iron chelation therapy (deferrioxamine) + close monitoring of iron levels

Infections

- Yearly transfusion exceeds 250-300 mL/kg → hypersplenism → therapeutic splenectomy
- Lack of spleen → infection with encapsulated organisms (*S. pneumoniae* and *H. influenzae*)

12. Identify common abnormal hemoglobins, e.g. C, D and E, and their clinical-pathologic implications.

- Hemoglobin C: Results from abnormal substitution of lysine for glutamic acid at the sixth position of the β -globin chain
 - Forms intracellular blocklike crystals, which increase the rigidity of the RBC, shorten its lifespan, and cause

- fragmentation and formation of spherocytes in circulation
- Results in moderate hemolytic anemia, low hemoglobin, and splenomegaly
- RBCs = targets
- Heterozygotes are asymptomatic, have a normal erythropoietic profile
- Hemoglobin D: Results from substitution of glutamine for lysine at position 121 on the β -globin chain
 - Most prevalent in India
 - Homozygotes demonstrate mild hemolytic anemia
 - Heterozygotes are not anemic
- Hemoglobin E: Substitution of lysine for glutamic acid at position 26 on the β -globin chain
 - Unstable leading to microcytosis and hypochromatosis for hetero- and homozygotes
 - Homozygotes are anemic, but heterozygotes are not
 - Important in Southeast Asians

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Before class:

Required:

1. Read Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapter 4: Anemias Associated with a Reduced Erythropoietin Response

Chapter 5: Iron-Deficiency Anemia

Chapter 8: Macrocytic Anemia

Chapter 15: Hemochromatosis – Pages 182-189 ONLY

2. Read the provided handouts titled: “[Iron and the Erythron](#)”, “[Macrocytic and Megaloblastic Anemia](#)” and “[Iron Overload](#)”

The [PowerPoint slides](#) used in this activity will be made available on the course website immediately after the session.

Learning objectives:

Microcytosis – Iron Deficiency Anemia

1. Describe the route by which iron from the diet becomes incorporated into hemoglobin (including the absorption, transport, delivery, storage and loss of iron in humans).

- Absorption
 - Ferric iron is reduced to ferrous iron by a membrane-bound reductase. Then ferrous iron is transported across the apical membrane of the enterocyte by a **divalent metal transport (DMT1)**.
 - Dietary Heme: Heme iron enters intestinal mucosal cells directly, where it is metabolized to release ferric iron; mucin-bound iron forms a ferrous iron compound known as paraferitin
 - Hemoglobin Heme: Heme is broken from hemoglobin in the gut, then transferred across the absorptive surface of the enterocyte by heme carrier protein 1 (HCP1) and iron is separated from heme by heme oxygenase.
- Transport
 - Iron is bound to cytosolic metal-binding proteins and can accumulate in mucosal cells as **ferritin** or be transported into the plasma
 - Once absorbed, iron binds to **transferrin** (a plasma protein) which transports it to tissues.
 - Transport systems involved in the export of enterocyte iron to plasma include hephaestin, a copper-dependent ferroxidase, and **ferroportin**.
- Delivery
 - Maturing erythroid precursors express **transferrin receptors (TfR)** on the cell surface, which bind diferric iron-laden transferrin.
 - Aggregate iron-TfR complexes are then incorporated into the erythroid cell in intracytoplasmic endosomes.
 - The iron is then released and the transferrin-TfR complex returns to the cell surface where the transferrin molecule reenters the plasma. Acidic pH allows for iron dissociation within the lysosome
 - The free iron within the endosomes is then reduced by a **ferrireductase** to Fe²⁺ and exported into the cytosol for incorporation into **heme** or storage as **ferritin**. Heme synthesis also requires transport of the free iron into the mitochondria.
- Storage
 - Iron is primarily stored in RE (reticulo-endothelial) cells as ferritin and hemosiderin
 - A small but proportional amount is glycosylated and released into circulation.
 - Failure to regulate the absorption of iron leads to increased storage (hemochromatosis)
 - The greatest regulator of blood iron levels? The liver, through its production of **hepcidin**
- Iron Loss
 - Iron is highly conserved
 - There is no significant physiologic mechanism for the excretion of iron since excess iron is stored; only 1-4 mg are absorbed/day (presumably = to the amount lost)
 - There is either minimal or inducible excretion in the urine and feces
 - Major sources of iron loss (Avg: 1-2mg/ day):
 - menstruation (reason why women present with hemochromatosis later)
 - desquamation
 - sloughed mucosal cells
 - other blood loss

2. Describe the hematological changes associated with the development of iron deficiency and the timeline by which they occur.

From Goljan: page 200

- Stages of Iron Deficiency
 - Absent iron stores
 - Decreased serum ferritin (using up your storage)
 - Decreased serum iron (running out)
 - Increased TIBC (total binding iron capacity) (trying to salvage everything you can)
 - Decreased iron saturation (of the transferrin)
 - Normochromic anemia
 - Microcytic hypochromic anemia
- Low SI, ferritin, and ferritin saturation
- Increased TIBC and RDW

- decreased synthesis of heme

- Sequence of iron depletion = marrow (forced release of immature RBCs with increasing microcytosis and hypochromicity as the anemia worsens); then RBCs (increasing anisocytosis, poikilocytes; ***cigar-shaped cells are typical**); then other tissues

3. List the causes of iron deficiency and select appropriate investigational studies to evaluate for them.

- In its mildest form, iron store depletion results simply from an imbalance between normal physiologic demands, such as rapid growth and menstrual blood loss vs dietary iron intake.
- More severe iron deficiency with limitation of RBC production is usually the result of protracted blood loss from the GI tract or uterus.
- Less frequently, the cause is malabsorption secondary to small intestine disease or gastric surgery.
- Accurate diagnosis of iron-deficiency state requires several laboratory tests. Measurements of the serum iron, transferrin iron-binding capacity, and the serum ferritin level are of greatest importance and often are enough to make the diagnosis.

4. Describe the signs and laboratory findings associated with iron deficiency anemia

Signs and Symptoms:

Fatigue, malaise, shortness of breath, dizziness, and cold sensitivity are frequent symptoms of iron deficiency, but are common to all types of anemia.

- Fatigue and exercise intolerance with signs of cardiac decompensation
- Prolonged iron deficiency is associated with a sore mouth, difficulty swallowing, or a tendency for nails to soften/curl (can cause spooning- koilonychia)
- Children with iron deficiency may experience learning and behavioral problems

Laboratory findings:

Serum Iron ↓
TIBC ↑
Ferritin ↓
% saturation ↓↓

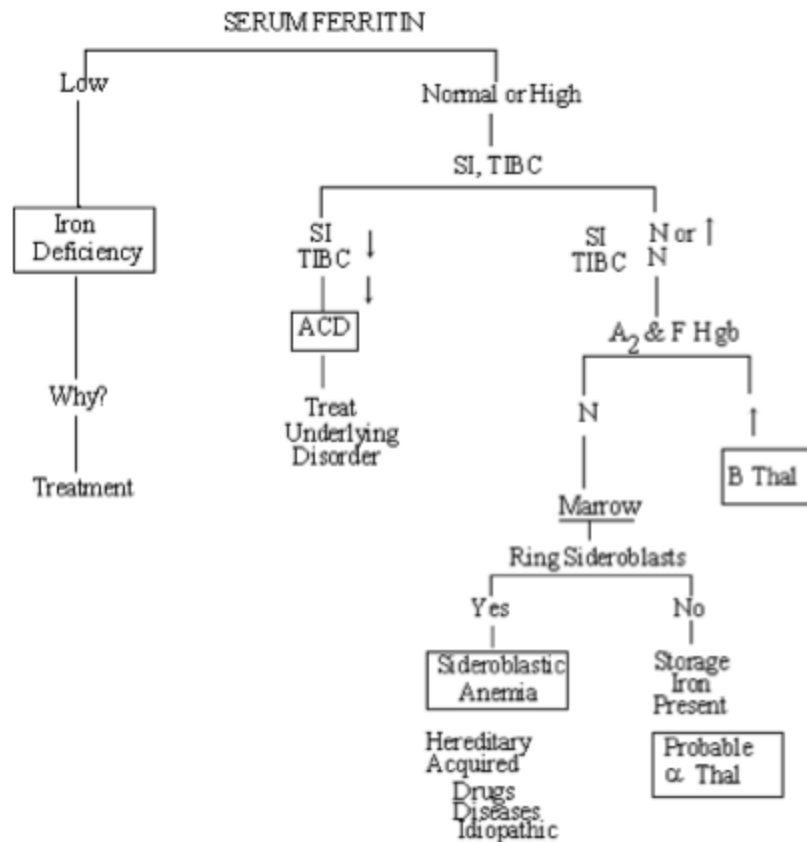
5. Differentiate iron deficiency anemia from other microcytic hypochromic anemias using **symptoms, signs, and laboratory findings.**

UWorld claims that [Plummer-Vinson syndrome](#) and brittle or curved fingernails are specific for iron deficiency anemia. Most other symptoms are common to all anemias.

	Thalassemia	Iron Deficiency	Chronic Inflammation
SI	Normal/Increased	Low	Low
TIBC	Normal	High	Low
Percent saturation	> 20%	< 10%	10-20%
Serum transferrin-receptor (sTfR)	Increased	Increased	Normal
Ferritin (ug/L)	> 50	< 12	30-200

Log (sTfR/ferritin ratio)	> 4	> 2.5	< 2.5
Iron stores	3-4+	0	1-4

DECISION DIAGRAM FOR HYPOCHROMIC AND/OR MICROCYTIC ANEMIA



6. Describe the role of hepcidin in the anemia of chronic inflammation and the resulting effects on serum iron, ferritin, and red cell size.

- Hepcidin, manufactured in the liver, is the primary regulator for the absorption of dietary iron and for release of iron from macrophages.
- Produced in response to changes in iron balance, oxygen supply, and the level of erythropoiesis (erythropoietin)
- Sinusoidal endothelial and Kupffer cells exposed to iron-saturated transferrin, bacteria, or inflammatory cytokines induce hepatocyte synthesis and secretion of hepcidin.
- Elevated levels are seen in patients with inflammatory anemias, resulting in a block in both iron absorption and iron release from reticuloendothelial cells
- Low plasma iron levels decrease synthesis of hepcidin, which allows increased release of iron (recirculation of storage iron) and increased absorption .
- High plasma iron levels increase synthesis of hepcidin, which decreases release of iron from enterocytes and macrophages.

Hillman Text: For anemia of chronic disease states:

- **low serum iron**
- low TIBC
- **increased serum ferritin**
- smear becomes **mildly microcytic**

* not sure what of this is directly related to hepcidin...

Hepcidin is increased in anemia of chronic inflammation;

7. Given a peripheral blood smear or marrow aspirate, identify structural red cell abnormalities and relate them to their

clinical associations - iron deficiency, thalassemia, anemia of chronic disease, sideroblastic anemia.

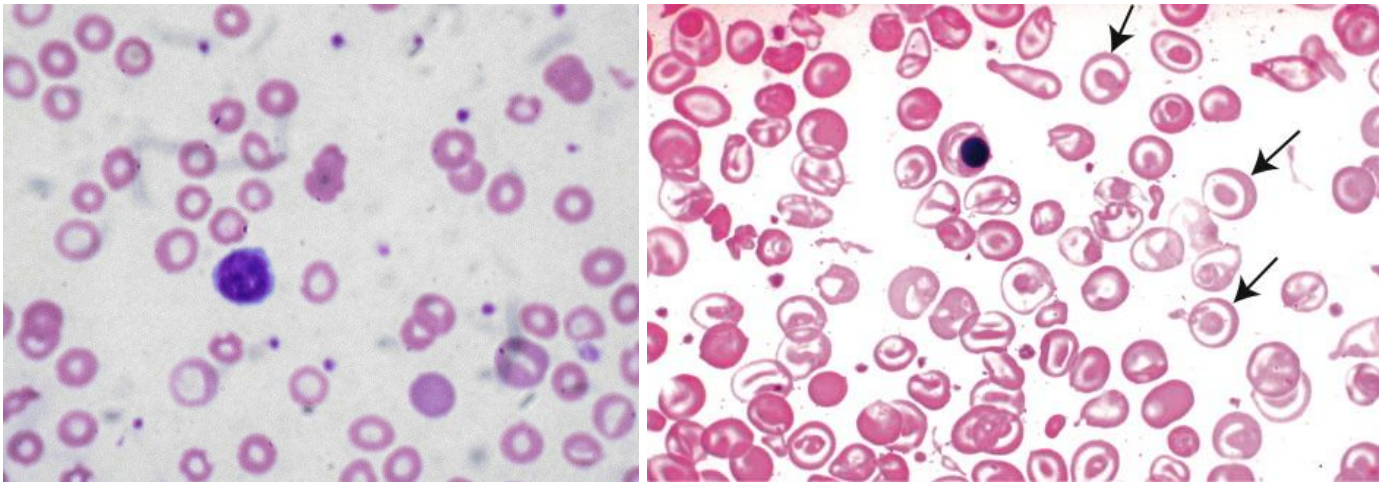
(Source = First Aid)

Iron deficiency - microcytosis and hypochromia, basophilic stippling

Thalassemia - Basophilic stippling and target cells

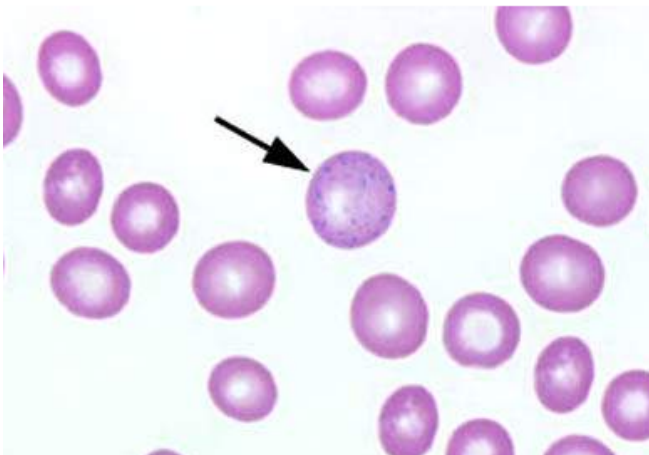
Anemia of chronic disease - basophilic stippling

Sideroblastic anemia - See ringed sideroblasts (and possibly basophilic stippling if due to lead)



Left: Iron deficiency anemia w/ microcytic, hypochromic RBCs

Right: Thalassemia. The peripheral blood erythrocytes are hypochromic and microcytic and show anisocytosis, poikilocytosis, and target cells (arrows)



Cell with arrow: basophilic stippling

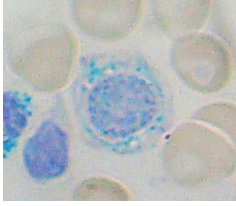
8. Compare the advantages and disadvantages of diet, oral iron and intravenous iron therapy.

- Summary
 - An oral iron preparation is the recommended 1st line therapy, unless the patient is elderly with cardiopulmonary disease or needs immediate surgery, when RBC transfusions are indicated.
 - For those situations where GI malabsorption or severe intolerance prevents effective oral iron therapy, parenteral iron can be administered.
- Dietary Iron
 - Advantages: Normal administration
 - Disadvantages: Clearly insufficient for avoiding anemia in cases of malabsorption, may benefit patients with very, very mild anemia who are open to eating more iron-rich foods?
- Oral Preparations
 - Advantages: First choice, at-home administration, supports marrow production levels up to 3 times normal in patients with moderate to severe anemia
 - Disadvantages: GI intolerance or malabsorption
- IV Iron
 - Advantages: Bypasses GI tract
 - Disadvantages: Requires professional administration, risk of anaphylaxis, risk of hypotension, serum sickness-like

reaction with malaise, fever, arthralgias, skin rash, and lymphadenopathy

9. Recognize the key morphologic findings of sideroblastic anemia and the clinical implications.

In sideroblastic anemias, the incorporation of iron into heme is defective. This defective pathology takes place in the mitochondria which leads to build up of iron granules around the nucleus and the appearance of the pathognomonic 'ringed-sideroblast' cell.



Symptoms: Dependent on the severity of the anemia- weakness, fatiguability
primarily a disease of the elderly (fifth-seventh decade)

Hereditary: x-linked defect in δ -aminolevulinic acid synthase

Etiology: May result from myeloproliferative syndromes/hematologic malignancies- important to check

Reversible etiology: alcohol, lead, INH (INH leads to Vit B6 deficiency; Vit B6 = co-factor for ALA synthase)

Tx: B6 (pyridoxine)

Morphology (pg 112)

- RBC Morphology: Mixed macro-, normo-, microcytic; moderate aniso- and poikilocytosis; excess cytoplasmic iron granules in a perinuclear distribution (mitochondrial iron encrustation)
- Reticulocyte Index: <2
- Marrow E/G Ratio: $>1:1$ to hypoplastic
- Marrow Morphology: Megaloblastic to normoblastic, ringed sideroblasts
- SI/TIBC: Increased-normal
- Serum ferritin: Normal

Sideroblastic Anemias – See [Bone Marrow Failure](#) lecture

Macrocytic Anemia

10. Define and list common causes of macrocytosis.

CAUSES OF MACROCYTOSIS:

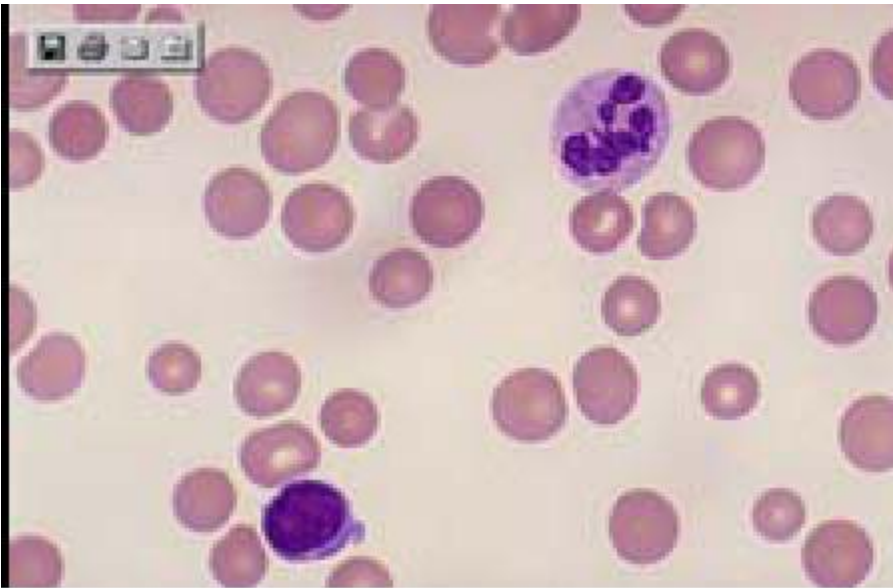
- -Defective DNA synthesis (megaloblastic anemias, myelodysplastic states).
- -Accelerated erythropoiesis - erythropoietin stimulated. Increased percentage of young erythrocytes (reticulocytes). E.g. Hemolytic anemia, decreased marrow erythroid mass (aplastic anemia, myelodysplastic syndrome), physiologic (newborn, pregnancy)
- -Increased membrane surface area (hepatic disease, obstructive jaundice, postsplenectomy)
- -Nonhematologic. Hypothyroidism, alcohol ingestion, liver disease
- -Drugs. Hydroxyurea, antifolates, cytotoxins (antipurines and antiprimidines)

11. Define and list common causes of macrocytic anemia.

- **Macrocytic Anemia**: Large RBCs associated with an insufficient number of cells and insufficient hemoglobin content/cell
 - Megaloblastic: Folate or B12 deficiency
 - Non-Megaloblastic: All others
- Causes
 - Folic acid and vitamin B12 deficiency are primary causes of macrocytic anemia in adults
 - Chronic alcoholism can result in macrocytic anemia without vitamin deficiency
 - Drugs: antiviral (AZT), chemotherapies (5-FU), hydroxyurea, phenytoin, trimethoprim, oral contraceptive, alcohol
 - Rapid RBC Turnover/Reticulocytosis: COPD, Trauma
 - Hepatic Disease/Obstructive Jaundice
 - Reticulocytosis
 - Metabolic Disorder: Orotic aciduria, congenital deficiencies of purine/pyrimidine synthesis

12. Given a PBS or BM identify the morphologic hallmarks of megaloblastic erythropoiesis and granulopoiesis in the blood and bone marrow.

PBS:



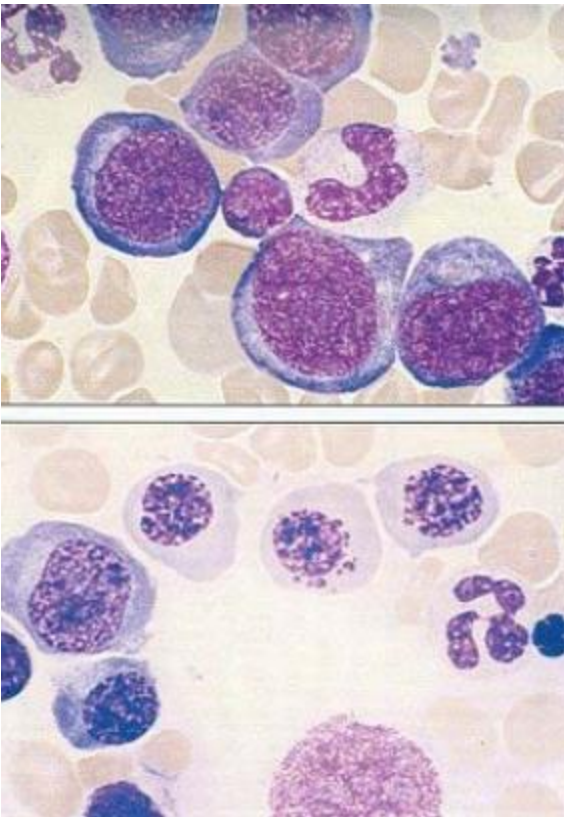
<http://pathwiki.pbworks.com/w/page/14674011/HemaSlide6>

Erythrocytes are larger than normal, typically reported as mean cell volume (MCV) greater than 100 fL. The amount of hemoglobin in the cell increases proportionately, so the mean cell hemoglobin concentration (MCHC) remains within normal limits.

BLOOD- Depending on the etiology of the macrocytosis, peripheral smear may reveal nucleated RBCs, target cells, RBC fragments, hypersegmentation of neutrophils, immature WBCs, large platelets, or pancytopenia.

MARROW- Depending on the etiology of the macrocytosis, marrow may reveal hypercellularity, megaloblastic changes, fibrosis, infiltration by tumor or granulomatous disease, leukemic changes, or erythroid hyperplasia.

BMB:



<http://pathwiki.pbworks.com/w/page/14674016/HemaSlides9-10>

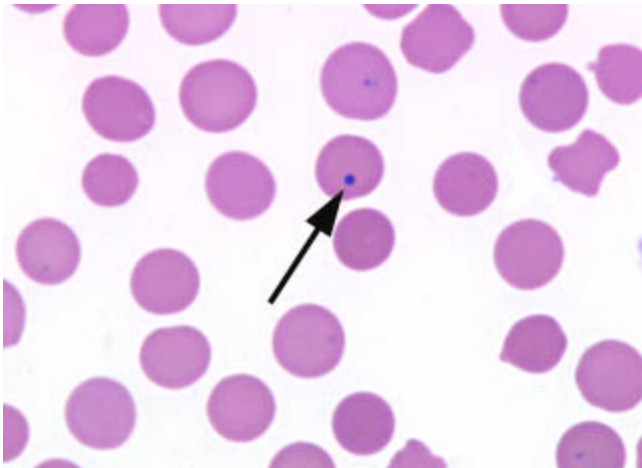
The bone marrow is markedly hypercellular due to increased numbers of megaloblasts. These cells are **larger than normal** and have a **finely reticulated nuclear chromatin** (suggests nuclear immaturity) and an abundant, strikingly **basophilic cytoplasm**. As the megaloblasts differentiate and begin to acquire Hb, the nucleus retains its finely distributed

chromatin and fails to undergo the chromatin clumping typical of a normal RBC. Macrocytes will also appear oval, a characteristic of megaloblastic anemia.

From Medscape article on megaloblastic anemia

The bone marrow is hypercellular with erythroid hyperplasia. Erythroid precursors have megaloblastic features in that they are larger than normoblastic cells and they have immature nuclear development. Megaloblastic changes are most prominent in more mature RBC precursors. Cytoplasmic maturation is normal, but nuclear remnants, Howell-Jolly bodies, may be present in the cytoplasm. Giant bands (neutrophils) can be present. Megakaryocytes may be large and hyperlobulated.

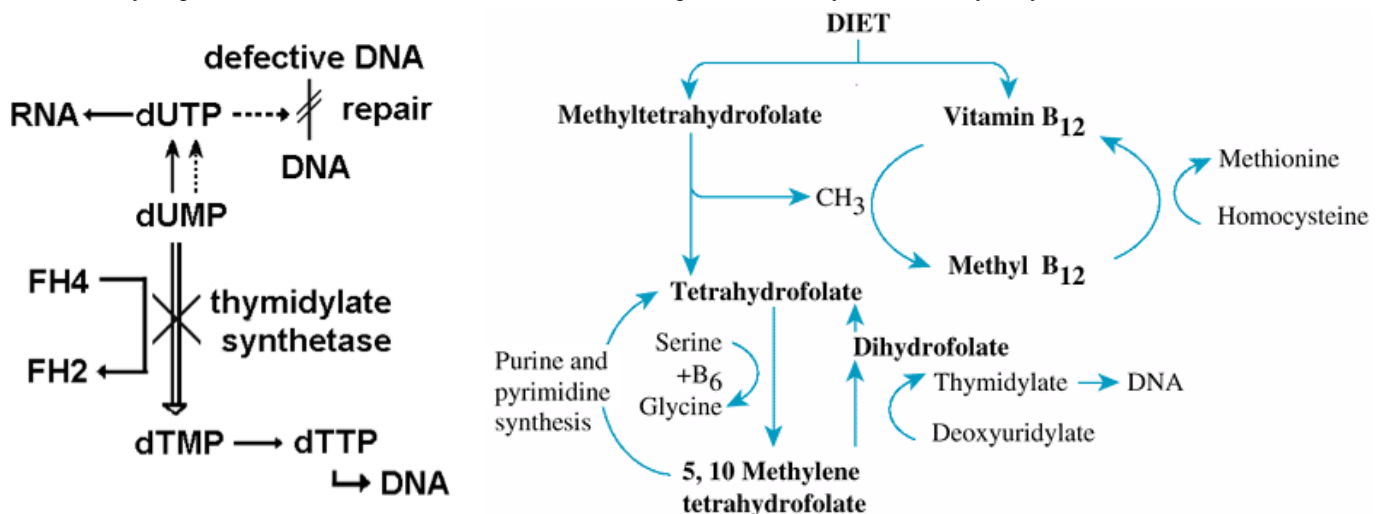
With peripheral smears, macroovalocytes and hypersegmented neutrophils are present. Megaloblasts may be present. RBCs may have multiple Howell-Jolly bodies in their cytoplasm. Pancytopenia is a common finding.



Howell-Jolly body

13. Diagram the biochemical pathway which explains how folate and vitamin B12 deficiency ultimately impair thymidylate synthesis, and methionine and fatty acid metabolism.

Folate is necessary for conversion of deoxyuridine to thymidine. In folate deficiency, dUTP accumulates replacing dTTP in newly synthesized DNA. The faulty sequences are excised but repair is not possible because dTTP is lacking resulting in defective DNA. B12 is indirectly required for the conversion of folate to a form required for the synthesis of thymidylate.



- Methyltetrahydrofolate donated the methyl group to vitamin B12, to become Tetrahydrofolate. The conversion of vitamin B12 to Methyl B12 supports methionine metabolism. Tetrahydrofolate is necessary for the synthesis of various folate coenzymes needed for purine and pyrimidine synthesis and for converting deoxyuridylate to thymidylate.
- Limited availability of dietary folic acid directly affects the levels of methyltetrahydrofolate while lack of vitamin B12 results in a “methylfolate trap” as there is no acceptor for the methyltetrahydrofolate methyl group and therefore cells are unable to generate tetrahydrofolate to support DNA synthesis.

14. Identify the dietary sources of vitamin B12 and folate and describe their respective sites and mechanisms of absorption,

means of transport, and duration and location of storage.

- Folates
 - Source: Readily available in green leafy vegetables, fruit, dairy products, yeast and animal liver.
 - Absorption
 - Intact mucosa is needed to digest and absorb folate.
 - Enterohepatic circulation maintains plasma level - alcohol blocks hepatic release of folate into bile.
 - Polyglutamate forms are converted to monoglutamates by the intestinal mucosal deconjugase pteroylpolyglutamate hydrolase.
 - Monoglutamates are absorbed in the **jejunum**
 - After being reduced and methylated folates enter the circulation as N5- methyltetrahydrofolate (N5-methyl-FH4).
 - Transport
 - Circulates as methyltetrahydrofolate
 - Storage
 - Stored in the liver for 3 to 4 months
- Vitamin B12
 - Source: The sole source of cobalamin is dietary intake of certain bacteria and animal muscle/organs (meat, eggs, dairy)
 - Absorption:
 - The gastric phase involves B12 dissociation from food and binding to R protein (from saliva).
 - In the duodenum, B12 is released from the R- binding protein and bound to IF produced by gastric parietal cells.
 - The ileal phase involves binding of the B12-IF complex to receptors located on the **ileal brush border**
 - Transport
 - B12 transported in the plasma bound to transcobalamin II (TCII) produced by liver, macrophages and endothelial cells.
 - B12 delivery to cells occurs through receptor-mediated endocytosis of the TCII-B12 complex.
 - Following uptake, B12 (cobalamin) is reduced and converted to the coenzymatically active forms methylcobalamin (cytosolic) and adenosyl cobalamin (mitochondrial).
 - Storage
 - Stored in the liver for 6-9 years

	Cobalamins	Folates
Dietary source	Animal	Vegetable + animal
Absorption	Stomach + ileum	Jejunum
Transport	Transcobalamin II	Not Known
Stores	Large (years)	Small (months)
Daily requirements	1 ug	100 ug

15. Compare and contrast vitamin B12 deficiency and folate deficiency with respect to:

* their most common causes

Several disorders of the gastrointestinal tract can result in vitamin B12 deficiency. The most common causes in the United States include pernicious anemia associated with antibodies to parietal cells, intrinsic factor, or the cobalamin-IF complex; gastrectomy, gastric bypass, or bowel resection; bacterial overgrowth of the small intestine; and pancreatic insufficiency.

Decreased intake	
Impaired absorption Intrinsic factor deficiency Pernicious anemia; Gastrectomy; Destruction of gastric mucosa; Anti-IF antibody in gastric juice	Most common ≈85-95%
Intestinal disease Selective malabsorption; Ileal resection, ileitis; Sprue, celiac disease; Intrinsic intestinal disease; Para-aminosalicylic therapy;	≈must be screened
Competitive parasites Fish tapeworm; Bacteria in diverticula; “Blind loop” syndrome	
Poor diet	Rarely if ever
Increased requirements	
Pregnancy; Nonneoplastic disease; Hyperthyroidism	Very rare

Folate deficiency:

Decreased intake	
Poor Diet: Alcoholism; Infancy; Dialysis	Most common #1
Impaired absorption: Intestinal short circuits; Steatorrhea; Sprue, celiac disease; Anticonvulsants, oral contraceptives	Must be screened if #1 or #2 is not present
Increased requirements: Alcohol; Dialysis; Pregnancy; Hyperactive hematopoiesis; Neoplastic disease	#2
Blocked activation: Folic acid antagonists	

*** time to development of the clinical deficiency state**

Presumably folate deficiency will manifest sooner than B12 deficiency given that B12 is stored longer than folate

*** presence of neurologic and neuropsychiatric abnormalities**

Vitamin B12 deficiency has an impact on the central nervous system. Patients develop a demyelinating lesion of the neurons of the spinal column and cerebral cortex. This condition results in paresthesias of the hands and feet, unsteadiness of gait, and eventually memory loss and personality changes. The most distinctive characteristics of the neuropathy are the dorsal column and corticospinal tract lesions (subacute combined degeneration of the spinal cord), which result in a loss of vibration and position sense and the development of an unsteady gait and a positive Romberg test. Peripheral neuropathies and neuropsychiatric disorders (dementia, psychosis, and depression) in adult patients who are folate deficient and have normal vitamin B12 levels are not observed.

***Goljan says the symptoms of folate deficiency are the same as those for B12 deficiency EXCEPT for the neurologic disease = neurological symptoms in B12 deficiency only; folate deficiency can cause neural tube defects in the fetuses of mothers deficient prior to conception**

16. Recognize the clinical, laboratory and autoimmune findings associated with pernicious anemia.

Autoantibodies against parietal cells, intrinsic factor, and cobalamin- intrinsic factor complex can be detected in the sera of most patients with pernicious anemia

Pernicious Anemia is an autoimmune disease in which parietal cell antibodies against gastric H⁺/K⁺- ATPase result in the destruction of gastric parietal cell. This results in a chronic atrophic gastritis (Type A-immune form) with achlorhydria and decreased or absent IF secretion. More common in women and the elderly. There may be other associated autoimmune disease (e.g. thyroid).

17. List the appropriate therapies for B12 deficiency and folate deficiency.

B12: B12 via intramuscular injection, deep subcutaneous injection, nutritional supplements

Folate: PO monoglutamic folic acid

Hemochromatosis

18. Recognize the signs and symptoms of iron overload.

- liver: cirrhosis; Fe in lysosomes
- skin: pigmented (bronze)
- pancreas: diabetes
- heart: CHF → death
- endocrine: decreased libido, amenorrhea, testicular atrophy, impotence
- joints: arthropy/arthritis
- metal detectors: set them off
 - might also be susceptible to Magneto breaking out of jail lol
 - <http://www.youtube.com/watch?v=cNbXq2RNYC4> one of the best scenes in the movie
 - <http://www.youtube.com/watch?v=ECf2CEusUFU> one of the best lines
- Labs
 - Plasma Fe is 2x normal
 - Transferrin is 100% saturated
 - Ferritin is elevated

19. Relate the accumulation of excess iron in tissue to the clinical features.

see above

20. Differentiate between primary and secondary iron overload disorders.

- Primary:
 - If hereditary hemochromatosis, see Fe deposited in **parenchymal** cells of liver, heart, etc; normal reticuloendothelial amounts of Fe
- Secondary:
 - If increase in Fe from multiple transfusions or hemolytic process, see Fe in macrophages (and only later in parenchyma); high levels of Fe in **reticuloendothelial** cells

21. Identify four major genes/gene products implicated in HH.

Hemochromatosis Type 1: HFE gene HFE protein - essential for hepcidin production by liver

Hemochromatosis Type 2: HJV gene - hemojuvelin protein - help regulate hepcidin production

HAMP gene - make hepcidin, which is the master regulator of Fe metabolism

Hemochromatosis Type 3: TFR2 gene - transferrin receptor 2 - regulation of hepcidin production

Hemochromatosis Type 4: SLC40A1 gene - makes ferroportin, a cell membrane Fe transporter

22. Describe the role of the hepcidin/ferroportin regulatory axis in the pathogenesis of HH. from wiki:

- ferroportin = cell membrane transporter of Fe; in both gut enterocytes and reticuloendothelial/ macrophages

hepcidin = master regulator for Fe metabolism; inhibits ferroportin, so enterocytes cannot put Fe into circulation & traps Fe inside the reticuloendothelial cells (basically decreases amount of Fe in circulation); elevated in pts w/ renal failure; partially responsible for iron sequestration seen in anemia of chronic disease

23. Select basic diagnostic studies for an individual suspected of iron overload.

- 1) Serum Iron - nml = 50-150; from primary hemo: 1000+; from secondary hemo: 150-300; high sensitivity, low specificity
- 2) Marrow Fe test: lots of Fe in marrow for secondary hemo; nml in primary
- 3) liver biopsy: primary hemo: Fe in hepatocyte lysosomes; secondary hemo: Fe in kupffer cells
- 4) genetic tests

Disease	Iron	TIBC/Transferrin	%Transferrin Saturation	Ferritin
Iron Deficiency	Low	High	Low	Low
Hemochromatosis	High	Low	High	High
Chronic illness	Low	Low	Low	Normal/High

Hemolytic anemia	High	Normal/Low	High	High
Sideroblastic anemia	Normal/High	Normal/Low	High	High
Iron poisoning	High	Normal	High	Normal

[Link](#)

24. Design a simple treatment plan explaining available options for an individual with iron overload.

bleed 'em. weekly for 2-3 years then every 2-3 months...so blood letting DOES cure something! BRING ON THE LEECHES

<http://espn.go.com/video/clip?id=7236121>

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Hematology System

Curricular Week 69 - System Week 1: Red Blood Cells

[Hemolytic Anemia / Hemoglobinopathies & Thalassemia](#)

[Donald Innes MD](#), [Pamela Clark MD](#), [Michael Douvas MD](#), [Colleen Druzgal MD](#)

Thursday, December 1, 2011

Time: 9:10-12:00 – Learning Studio

Before class:

Required:

1. Review Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapter 6: Thalassemia

Chapter 7: Hemoglobinopathies

2. Read Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapter 10: Blood Loss Anemia

Chapter 11: Hemolytic Anemias

3. Watch the two Camtasia lectures for Hemolytic Anemia (available in the [Presentations](#) section for this session on the Hematology System website) and review the [accompanying handouts](#).

The  Powerpoint slides used in this activity will be made available on the course website immediately after the session.

Learning objectives:

Hemolytic anemias - General and Immune-mediated

Red Cell Degradation in the Normal State and Disease

1. Define the terms "intravascular hemolysis" and "extravascular hemolysis" and identify which mechanism predominates in normal red cell destruction.

Extravascular Hemolysis – phagocytosis of red blood cells by reticuloendothelial cells. **90% of senescent red blood cells** are removed by extravascular hemolysis.

1. Senescent or abnormal RBCs ingested by macrophages in the spleen and liver. Increased extravascular hemolysis leads to hypersplenism
2. Requires surface membrane abnormalities such as bound immunoglobulin or physical factors that restrict RBC deformability (senescent red cells are less deformable) preventing exit from the spleen.
3. Increased extravascular hemolysis generally can be identified by spherocytes in the peripheral smear.

Intravascular Hemolysis – RBCs lyse in the intravascular space and release their contents into the plasma. About **10% of senescent RBCs** removed by intravascular hemolysis

1. Usual causes of increased intravascular hemolysis are trauma to rbc's, complement fixation or exogenous toxic factors.
2. Generally, intravascular hemolysis caused by trauma to rbc, can be identified on peripheral smear by the presence of schistocytes. Schistocytes are simply rbc fragments
 - a. Schistocyte formation is most pronounced in microangiopathic processes but not where intravascular hemolysis due to toxins or to complement fixation.

2. Describe the fate of free hemoglobin following red blood cell destruction.

Catabolism in extravascular hemolysis:

- a. RBC's are sequestered in the liver and spleen where they are destroyed by macrophages. Rbc's coated with IgG and/or C3b adhere to corresponding receptors on macrophage membrane. Macrophages can either phagocytose the entire rbc or merely a piece of the rbc membrane resulting in a spherocyte
- b. Globin is split from heme and its component amino acids are recycled
- c. Iron is released from heme and reutilized
- d. Heme is metabolized to form bilirubin

Bilirubin, a tetrapyrrole produced by the oxidative metabolism of heme, is present in two forms: unconjugated (indirect) and conjugated (direct); the unconjugated form is transiently present in the plasma bound to albumin before conjugation in the liver.

- Unconjugated bilirubin is elevated in patients with hemolysis because the pathways for uptake of bilirubin into hepatocytes are overwhelmed.
 - Conjugated bilirubin is usually normal unless a hepatic or biliary dysfunction exists.
- a. Excess unconjugated bilirubin remains in the plasma.
 - b. Bilirubin is conjugated with glucuronide in the liver and is excreted into the gut where it is oxidized to urobilinogen and excreted as stercobilinogen in feces
 - c. Some urobilinogen is reabsorbed and excreted by the kidney into the urine

- d. Haptoglobin levels do not decline much with extravascular hemolysis because only a small fraction of hemoglobin escapes into the plasma

Catabolism of rbc in intravascular hemolysis:

- Hemoglobin is released and immediately bound to haptoglobin. Haptoglobin levels are often undetectable when intravascular hemolysis is severe
- The hemoglobin-haptoglobin complex is cleared from the plasma within minutes by hepatocytes and reticuloendothelial system
- Once the binding capacity of haptoglobin is saturated excess free hemoglobin is filtered by the renal glomeruli and reabsorbed by the proximal tubules where it is catabolized and the heme iron is converted into ferritin and hemosiderin. Hemosiderin in urine is a sensitive indicator of intravascular hemolysis and persists in the urine much longer than hemoglobin.
- Hemoglobinuria occurs once the absorptive capacity of the tubular cells is exceeded.
- Any hemoglobin that is not bound to haptoglobin or excreted by the kidneys is oxidized to methemoglobin, which quickly dissociates to ferriheme. Ferriheme is carried to the liver by hemopexin and albumin where it is metabolized to bilirubin
- LDH is released into the plasma

3. Explain why and in what direction the following laboratory measurements are altered from normal in hemolytic anemias: serum indirect bilirubin concentration, serum LDH level, reticulocyte count, serum haptoglobin concentration, and red blood cell survival.

Serum indirect bilirubin concentration (unconjugated bilirubin): Increases in hemolytic anemia because it remains in the plasma after the metabolism of heme and the liver's pathways for uptake are overwhelmed.

Serum LDH level: Increases because RBCs are rich in LDH, so it is released when they get cut up.

Reticulocyte count: Increases as a compensatory mechanism for the decreased RBC count.

Serum haptoglobin concentration: Decreases as the newly-formed hemoglobin-haptoglobin complexes are cleared from the plasma.

Red blood cell survival: Decreases because it's freaking anemia.

→ My personal answers. CONFIRM PLZZZ; i like

Think it depends on the type of hemolysis a bit more. My version (influenced by HO and Goljan) <- included your work in table cool

	Intravascular	Extravascular
Indirect (unconjugated) bilirubin	normal	increased (heme converted to bilirubin)
LDH	increased, due to lysis of RBC	increased <i>Camtasia 1 explains that "macrophages are messy eaters" in the spleen, and that LDH "can be" increased (haptocytes also "can be" decreased- thus I think both can happen just to a lesser level than intravascular)</i>
Reticulocyte count	increased	increased
Serum Haptoglobin	decreased (binds to free Hb, complexes are removed by hepatocytes and RE cells)	normal, since Hgb doesn't escape into the blood
RBC Survival	decreased	decreased

Classification of Hemolytic Anemias

4. List hereditary and acquired non-immune causes of hemolytic anemia. For the hereditary conditions, be able to describe the mode of inheritance.

Hereditary (intrinsic) causes of hemolytic anemia:

- Membrane abnormalities:
 - Hereditary Spherocytosis: decreased spectrin [Autosomal Dominant]
 - Hereditary elliptocytosis: defective formation of spectrin tetramers; [Autosomal Dominant]
 - Hereditary pyropoikilocytosis: defective formation of spectrin tetramers [Autosomal Recessive]
 - Hereditary stomatocytosis: defect in Na permeability, [Autosomal Dominant]
- Enzyme Deficiency

- G6PD [X-linked]
 - Pyruvate Kinase [Autosomal Recessive]
- Hemoglobinopathies and Thalassemia
 - Auto R, no? Yes

First Aid also lists Sick Cell, HbC defect, and Paroxysmal nocturnal hemoglobinuria as intrinsic.

Acquired (extrinsic) non-immune causes of hemolytic anemia:

Mechanical Hemolysis-produces characteristic rbc morphology (schistocytes) -- all are DAT (Direct Coombs Test) negative

1. Microangiopathic processes
 - a. thrombotic thrombocytopenic purpura
 - b. hemolytic uremic syndrome
 - c. disseminated intravascular coagulation
 - d. HELLP
2. Traumatic Hemolysis due to turbulent flow
 - a. cardiac hemolysis
 - b. malignant hypertension
 - c. malignant tumors
 - d. march hemoglobinuria
3. Miscellaneous causes
 - a. hypersplenism
 - b. hypophosphatemia
 - c. alterations in plasma lipids
 - d. infectious agents--e.g. malaria, babesia
 - e. toxins--snake venom, clostridial exotoxin
 - f. paroxysmal nocturnal hemoglobinuria This one is listed as intrinsic in First Aid, although it doesn't say what the mode of inheritance is. UPDATE: Several other sources say it's acquired.

Immune-Mediated Hemolytic Anemias

5. Describe the pathophysiology and site of red blood cell destruction of immune-mediated hemolysis due to IgG, IgM, and complement. I think this answer is in the 1st camastasia

IgG: extravascular, destroyed by splenic macrophages (weak complement activation since two IgGs are needed),

IgM: mostly intravascular, activates complement mediated destruction. Also extravascular destruction by Kupffer cells of liver

Goljan:

- IgG
 - RBCs coated by IgG are phagocytosed by splenic macrophages
 - Extravascular hemolysis
- Complement-Mediated Hemolysis
 - RBCs coated by C3b alone are phagocytosed by liver macrophages (Kupffer cells)
 - Extravascular hemolysis
 - RBCs coated by C5-C9 (MAC)
 - Intravascular hemolysis
 - RBCs coated by IgG and C3b are phagocytosed by liver and splenic macrophages (SLE)
 - Extravascular hemolysis
- IgM
 - Mostly intravascular
 - Activates complement
 - Agglutination

6. Describe the mechanism of the procedures involved in performing a direct antiglobulin test (direct Coombs test) and an indirect antiglobulin test (indirect Coombs test).

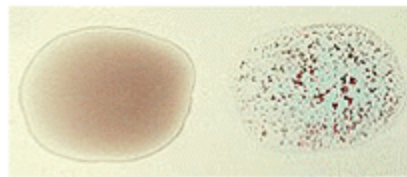
Direct Antiglobulin Test (Direct Coombs Test)-detects the presence of IgG antibody or complement on the patient's RBC. The test is very important for detecting immune mediated causes of hemolytic anemia.

The direct antiglobulin test (DAT) or direct Coombs test detects abnormal antibodies attached to the surface of the patient's RBCs.

The patient's RBCs are incubated with anti-human IgG antiserum. If the patient's RBCs are coated with IgG, the RBCs will agglutinate.

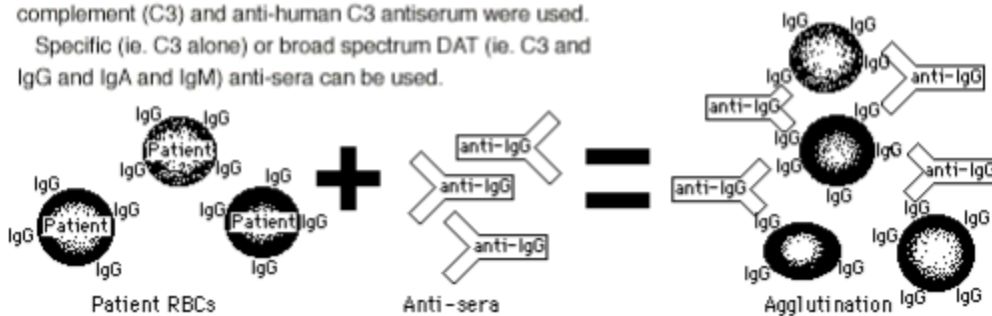
The same would be true if the RBCs were covered with complement (C3) and anti-human C3 antiserum were used.

Specific (ie. C3 alone) or broad spectrum DAT (ie. C3 and IgG and IgA and IgM) anti-sera can be used.



DAT negative

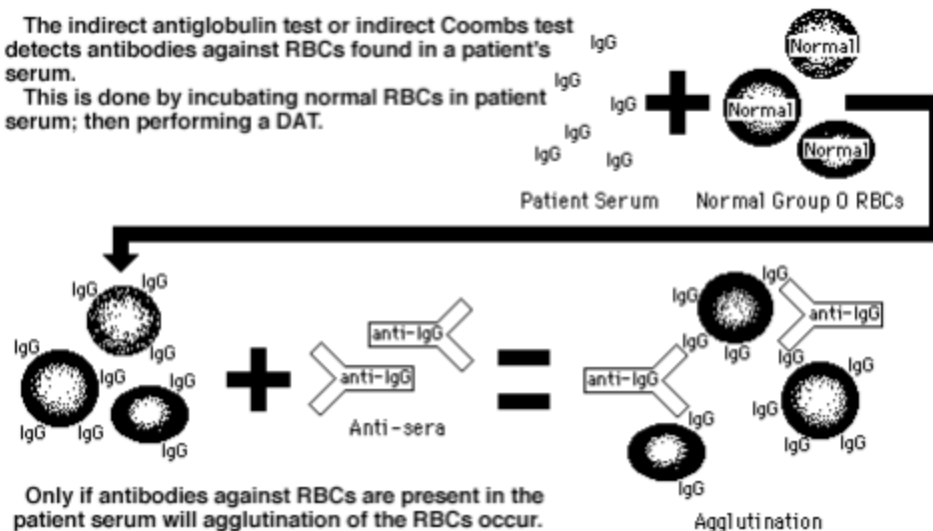
positive



Indirect Antiglobulin Test-(Indirect Coombs Test) detects antibodies to red cells in the patient's serum. The Indirect test is usually positive in autoimmune hemolytic anemias and in patients who have been transfused and made antibodies to foreign red cell antigens.

The indirect antiglobulin test or indirect Coombs test detects antibodies against RBCs found in a patient's serum.

This is done by incubating normal RBCs in patient serum; then performing a DAT.



Only if antibodies against RBCs are present in the patient serum will agglutination of the RBCs occur.

7. Describe the pathophysiology of Warm Autoimmune Hemolytic Anemia and understand its association with other disease processes

Warm Autoimmune Hemolytic Anemia

Pathophysiology of WAIHA--This is the quintessential disease causing extravascular hemolysis

1. This disorder is caused by IgG autoantibody capable of reacting with the RBC membrane at an optimum temperature of 37 degrees
2. The autoantibody is directed against red cell antigens present on human rbc.
3. The autoantibody often has specificity for basic structural components of the Rh system. (Note that so-called Rh-negative cells will react with the autoantibody)
4. Autoantibody coated rbc's bind to splenic macrophages via Fc receptors with subsequent extravascular hemolysis, and spherocyte formation.
5. If sufficient amounts of IgG are present on rbc, complement fixation can occur. This rarely causes formation of the attack complex (i.e. intravascular hemolysis does not occur) but can result in C3b deposition on the rbc membrane. RBC's with both C3b and IgG on them are removed more rapidly. Less commonly IgM or IgA are also present

Warm Autoimmune Hemolytic Anemia indicates the presence of underlying disease in up to 50% of cases and requires that the clinician investigate for underlying illness.

1. frequent complication of autoimmune disorders or hematologic malignancies, especially SLE and CLL
2. Occasionally caused by other diseases that adversely affect the immune system
3. after use of drugs that cause autoantibody formation

8. Describe the pathophysiology of Cold Agglutinin Disease and understand how this relates to the symptoms seen with this illness

Pathophysiology--quintessential disease causing intravascular hemolysis (usually mild and chronic)

1. IgM complement-fixing autoantibody binds to rbc's at pathologically high- I think this should say "low" (mistake in the handout) temperature (28-31 C). I think high is correct -- for a cold agglutinin it's a very warm temperature. oh, ok. Isn't it that it's at a low temperature, it's just "high" relative to your normal cold IgM Abs?
2. autoantibody binds to rbc's in acral parts of body if patient exposed to cold temperatures.
3. IgM autoantibody binds complement
4. As RBC return to core portion of body bound IgM is released but complement activation progresses. Three possible fates of rbc's:
 - a. intravascular hemolysis occurs if complement activation progresses all the way to the attack complex
 - b. extravascular hemolysis occurs if complement activation stops with c3b deposition. c3b covered rbc undergo phagocytosis by macrophages
 - c. normal survival if c3b on rbc surface is cleaved to c3d

Most people have cold IgM antibodies, which will bind to their own RBC's. The difference is that in Cold Agglutinin Disease:

1. the antibody has a much higher thermal amplitude (28-31 C);
2. the antibody has a much higher titer than normal cold antibody
3. the antibody is a monoclonal IgM, and often the patient has a underlying lymphoreticular disorder

9. List infectious agents associated with Cold Agglutinin Disease

Acute Postinfectious Cold Agglutinin-Induced Hemolysis

Pathogenesis--also causes intravascular hemolysis that can be acute and severe.

- Certain infections can acutely stimulate cold agglutinin production--most notably mycoplasma pneumoniae and Epstein Barr Virus (rarer)

10. Understand the application of the Donath Landsteiner test in diagnosing PCH

Paroxysmal Cold Hemoglobinuria (PCH)-- RARE

Pathogenesis--caused by the Donath Landsteiner antibody

- a. IgG antibody that is a biphasic hemolysin. The antibody binds to the P antigen on rbc membrane at cold temperature and activates complement at warmer temperature causing intravascular hemolysis
- b. Usual causes are:
 1. idiopathic, after an acute viral infection
 2. as a complication of tertiary syphilis.
- c. Symptom: sudden onset of hemoglobinuria after cold exposure
- d. Definitive lab test is the Donath-Landsteiner test--A serum from the patient is added to test tubes containing rbc's. The tubes are chilled to 4 degrees C and then warmed to 37 degrees. Significant hemolysis indicates a positive test.

11. List mechanisms by which drugs induce immune hemolytic anemia.

1. Hapten Mechanism (Drug Absorption Mechanism) [Prototype drug is Penicillin]
 - a. drug binds to rbc. RBC acts as the hapten and drug as antigen
 - b. antibody made is IgG and no complement is activated
 - c. DAT: IgG only on RBC
 - d. Extravascular hemolysis.
2. Immune Complex Mechanism (Innocent Bystander Mechanism) [Prototype drug is Quinidine]
 - a. IgM antibody directed against drug circulating in plasma
 - b. drug antibody interactions create circulating immune complexes that bind to rbc via complement receptor causing rbc lysis
 - c. DAT: complement only on RBC
 - d. Intravascular hemolysis
3. Membrane Modification Mechanism
 - a. drug damages rbc membrane resulting in nonspecific binding of plasma proteins and a positive DAT
4. Autoimmune Mechanism [Prototype drug is Methyl dopa]
 - a. drug does not participate as an antigen but induces warm autoimmune hemolytic anemia
 - b. autoantibody directed against an RBC antigen, often Rh group causes positive ;
 - c. The DAT will remain positive for several months after the drug is discontinued
5. Unified theory of drug induced hemolytic anemia
 - a. New theories propose that drugs cause an antibody response based on their ability to interact with specific cell membrane components, altering them so that a neoantigen is produced consisting of drug and cell component. The location of the neoantigen explains why three different clinical syndromes are seen with drug-induced antibodies.

Drug Induced Hemolytic Anemia Serologic Findings

Mechanism	Antibody	DAT	Drug Type
Drug Adsorption	IgG	IgG	penicillins cephalosporins
Immune Complex	IgM	C3	cephalosporins quinidine
Autoantibody	IgG	IgG/ occ C3	Aldomet procainamide
Nonspecific Adsorption	IgG/IgM/C3 etc	IgG/ C3	cephalosporins

12. Distinguish warm antibody-induced autoimmune hemolytic anemia from cold antibody-induced autoimmune hemolytic anemia on the basis of:

a. Immunoglobulin class of the antibody

warm: IgG: Extravascular hemolysis

- Inefficient activation of complement- requires 2 IgG molecules
- No agglutination
- Removed by splenic macrophages

cold: IgM: Intravascular hemolysis

- Activation of complement
- Agglutination
- Removed by Kupffer cells of the liver

b. Presence of red blood cell agglutination

warm: none

cold: agglutinates (because IgM)

c. Direct antiglobulin test results

Warm: DAT positive for IgG

Cold: DAT positive for complement

Both are DAT positive, but warm is IDAT negative

Warm can be IDAT positive or negative

So is warm IDAT positive or negative? These two lines say two different things.

d. Clinical manifestations

Clinical manifestations of warm antibody-induced autoimmune hemolytic anemia

1. symptoms and signs—variable and depend upon rapidity of hemolysis and degree of anemia
2. laboratory evidence of:
 - a. increased rbc destruction characterized by anemia with spherocytes seen on peripheral smear,
 - b. increased red cell production; reticulocytosis, polychromatophilic macrocytes and (usually) nucleated rbc's on peripheral smear.
 - c. POSITIVE DAT with or without positive IDAT (rarely DAT is negative--about 4% of patients) Remember that patients can have positive DAT with IgG only or both IgG and C3b.
 - d. elevated serum bilirubin (mild) due to increase in indirect reacting bilirubin. Jaundice will occur if bilirubin exceeds 2.5 mg/dL.

Clinical manifestations of cold antibody-induced autoimmune hemolytic anemia

1. symptoms and signs
 - a. hemolysis is usually ongoing and mild because complete complement attack complex not formed.
 - b. episodes of Hemoglobinuria and intravascular hemolysis after cold exposure.
 - c. acrocyanosis and Raynaud's phenomenon can occur upon cold exposure due to agglutination of rbc's in small vessels.
 - d. If an underlying malignancy is present, the patient may demonstrate lymphadenopathy and splenomegaly.
2. laboratory evidence of:

- a. mild to moderate anemia; blood smear may demonstrate agglutinated rbc's; occasional spherocytes may be seen.
- b. positive DAT with broad-spectrum Coomb's reagent and complement specific Coomb's reagent
- c. elevated cold agglutinin titer with high thermal amplitude
- d. monoclonal IgM on immunoelectrophoresis

Non-immune hemolytic anemias

Hereditary Spherocytosis (HS)

13. Determine whether a patient may have hereditary spherocytosis (HS), given the history, physical examination, hemogram, peripheral blood smear findings, reticulocyte count, and direct antiglobulin test (direct Coomb's test) results.

Clinical Features

1. Chronic ongoing hemolysis (primarily extravascular)
2. moderate normocytic anemia and splenomegaly
3. spherocytes on peripheral smear
4. Increased osmotic fragility; most sensitive test to confirm diagnosis
5. autosomal dominant inheritance is most common; recessive forms of inheritance occur
6. most common hereditary hemolytic disorder in Caucasians; one in 5000
7. may have aplastic crisis with parvovirus B19 infection
8. high incidence of gallstones
9. splenectomy effective if symptomatic

Diagnosis

- Spherocytes with negative DAT
- Increase in osmotic fragility
- mildly elevated indirect bilirubin
- elevated reticulocyte count

14. Define the molecular basis of hereditary spherocytosis and describe the resultant structural changes to red blood cells.

Pathogenesis

1. decreased amounts of spectrin in rbc membrane. Can be caused by deficiencies in spectrin or in other membrane proteins; including deficiencies of ankyrin, or band 3
2. Decreased density of spectrin causes decreased attachment of protein lattice to lipid bilayer
3. Lipid bilayer not adequately anchored to protein lattice is lost resulting in spherocyte formation
4. Decrease in the amount of membrane results in increased osmotic fragility
5. Poorly deformable spherocytes are trapped in spleen and removed

Enzyme Deficiency

15. Describe the pathway by which G6PD normally protects the red blood cell from oxidant stresses.

- Glucose 6-phosphate dehydrogenase normally helps generate enough NADPH to maintain glutathione and prevent hemoglobin oxidation (inadequate glutathione → H₂O₂ accumulation)

16. Describe the morphologic and clinical effects of G6PD and pyruvate kinase deficiency on red blood cell survival.

- G6PD Deficiency
 - Oxidative damage to hemoglobin by excess H₂O₂ causes Heinz body formation
 - **Heinz Body**: Aggregate of denatured HgB
 - H₂O₂ also damages the RBC membrane directly
 - Extravascular and intravascular hemolysis can occur
 - Bite cells may be seen where spleen has removed portions of RBC membrane
 - Most common RBC enzyme deficiency; affects 10% of African American males
 - A more serious form occurs in Mediterraneans; enzyme is absent and minor oxidative stress causes life-threatening hemolysis (fava beans can cause severe or fatal hemolysis)
 - Signs/Symptoms: Anemia, intravascular hemolysis during/after exposure to oxidant drugs, weakness, dizziness, diaphoresis, pallor, icterus, jaundice, dark urine, tachycardia
 - Histologic Findings: Intravascular and extravascular hemolysis- increased reticulocytes, ghost cells, blister cells, falling hematocrit, increased serum bilirubin, and increased urobilinogen
- Pyruvate Kinase Deficiency
 - Failure to make adequate amounts of ATP to support metabolic functions with consequent hemolysis
 - Rare
 - Variable presentation due to many mutant forms of enzyme

- Severe hemolysis responds to splenectomy
- Increased reticulocyte count

17. Describe the inheritance patterns and prognosis of G6PD and pyruvate kinase deficiencies.

- G6PD Deficiency: X-linked; prognosis depends on the form
 - Type A: Normal activity
 - Type A-: Unstable with RBC deterioration with age; 10% of normal G6PD activity, moderate anemia
 - Type B: Most common
 - Mediterranean: More serious
- Pyruvate Kinase: Autosomal recessive; severe hemolysis but responds to splenectomy

Fragmentation Hemolysis

18. List the causes of fragmentation hemolysis. Fragmentation hemolysis=mechanical hemolysis

- Thrombotic Thrombocytopenic Purpura: TTP
- Hemolytic Uremic Syndrome (HUS): *E. coli* O157:H7 infection or hereditary deficiency of Factor H in alternative complement pathway
- Turbulent flow (mechanical heart valve, malignant hypertension, etc).

19. Describe the clinical findings and pathophysiologic process associated with TTP.

- Clinical Findings
 - Hemolysis: Schistocytes and elevated LDH; HCT < 30
 - Thrombocytopenia: Usually <80,000; normal PT and PTT
 - Fluctuating neurologic deficits: HA, confusion, stroke, coma, visual disturbance, seizure
 - Mild renal dysfunction (elevated BUN)
 - Fever- Minimal or absent
- Pathophysiology
 - Deficiency of ADAMTS13 caused by autoAb formation
 - Accumulation of ultralarge von Willebrand multimers in plasma
 - Intravascular platelet aggregation
 - Microthrombi plug microvasculature
 - Fibrin deposited on microthrombi
 - Microangiopathic hemolytic anemia and ischemia occur followed by multiorgan failure

20. Understand the relationship of ADAMTS13 to the pathophysiology of TTP

- Most cases of TTP are caused by decreased levels of ADAMTS13
 - Hereditary: Upshaw Schulman Syndrome
 - Idiopathic
 - Severe (<5%)
 - With Inhibitor: AutoAb to ADAMTS13
 - Without Inhibitor
 - Without severe deficiency
- Secondary (non-idiopathic) TTP- ADAMTS13 is NORMAL
 - HIV infection
 - Post bone marrow transplant
 - Drug-induced ticlopidine, clopidine, clopiogrel, quinine, mitomycin, cyclosporin, pentostatin, tacrolimus, D-pencillamine
 - Cancer-associated
 - Pregnancy
 - In association with other autoimmune disorders

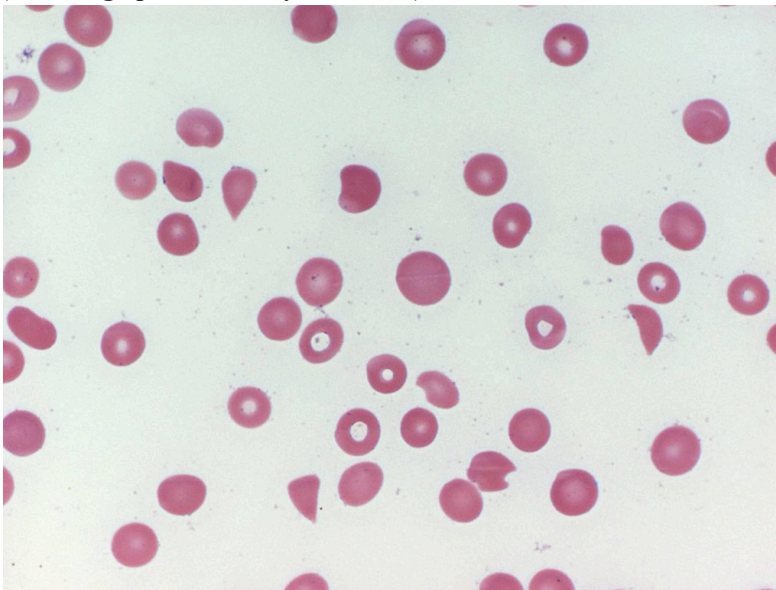
21. Know the major treatment modality for TTP

- Medical emergency- mortality is 100% without treatment
- Therapy: Plasma exchange until platelet count normalizes
 - Works because it adds metalloproteinase while removing autoantibody
 - platelet transfusions are contraindicated because they may exacerbate the underlying condition (should only be given for life-threatening bleeding)
- Relapse is not uncommon- up to 40%, some patients have a chronic relapsing course
- Other therapies can be added including glucocorticoids, vincristine, staph protein A columns, splenectomy

22. Describe the clinical findings and pathophysiologic processes associated with D+ and D- HUS

- Associated with *E. coli* O157:H7 infection or hereditary deficiency of Factor H in alternative complement pathway
- D+ Diarrhea Associated: Shiga toxin associated
 - Most common cause of acute renal failure in kids
 - Hemorrhagic colitis followed rapidly by renal failure
 - Predisposing factors for renal failure
 - WBC >13,000
 - Treatment with anti-motility agents
 - Treatment with antibiotics
 - Genetic factors
 - Probable pathogenesis- toxins carried to renal endothelium by leukocytes, renal endothelial cells internalize toxins via a glycosphingolipid on endothelial cell membrane
 - Microthrombi formed on damaged endothelium
 - Endothelial swelling and luminal narrowing
 - Increased sheer stress within narrow vessel lumina causes microangiopathic hemolytic anemia
 - Renal Abnormalities
 - Acute renal failure
 - Proteinuria
 - Hematuria
 - Ancillary hypertension
- D-: Heterogenous Causes, often deficiency of factors in the alternative complement pathway
 - No prodromal phase
 - Leads to endothelial damage, mostly in the kidneys
 - Poor prognosis- relapsing, often requires combined liver-kidney transplant
 - Liver: Makes normal complement
 - Kidney: Functions normally (no damage from HUS)
- Usually does not require treatment, just support with hemodialysis

23. Given a peripheral smear identify changes in red cell morphology consistent with fragmentation hemolysis (microangiopathic hemolytic anemia).



Hemoglobinopathies & Thalassemia [These are the same as the LOs for [Wednesday's session](#)].

24. Identify the normal chromosomes responsible for alpha-globin and beta-globin synthesis and list the three types of hemoglobin found in normal adult blood.
25. Describe the precipitating factors that influence the severity of the anemia and the pathophysiologic process by which hemoglobin S causes sickling.
26. Describe classic clinical features (symptoms, signs and complications) of the consequences of sickling.
27. Relate the pathophysiology of the sickling process to organ system involvement, specifically kidney and spleen; the complications of stroke and acute chest syndrome.
28. Describe how abnormal cell adhesion and abnormal nitric oxide metabolism can contribute to sickle cell disease complications.
29. Use the epidemiology and relative severity of the sickle cell genotypes to predict outcome; assess pain frequency as an indicator of disease severity.
30. Explain the rationale for the following sickle cell disease therapies: antibiotics, folic acid, hydroxyurea, acetaminophen, narcotics and blood transfusion.
31. Describe the basic genetic differences between alpha-thalassemia and beta-thalassemia.
32. Describe the genetic, hematologic, and clinical differences between alpha-thalassemia trait, hemoglobin H disease, and hydrops fetalis.

33. Describe the hematologic findings and pathophysiological changes that are associated with beta-thalassemia major and with beta-thalassemia minor.
 34. List the mechanisms and consequences of iron overload and infections associated with beta-thalassemia major.
 35. Identify common abnormal hemoglobins, e.g. C,D and E, and their clinical-pathologic implications.

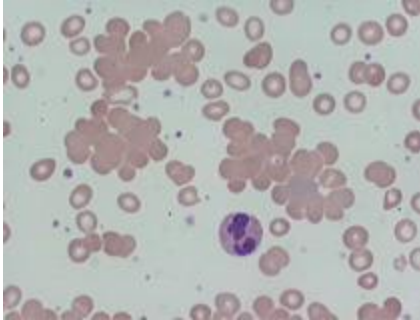
General

36. Given a peripheral smear identify changes in red cell morphology consistent with immune-mediated hemolytic anemia, non-immune-mediated hemolytic anemia, e.g. hereditary spherocytosis, hereditary elliptocytosis, microangiopathic hemolytic anemia, sickle cell anemia, or thalassemia.

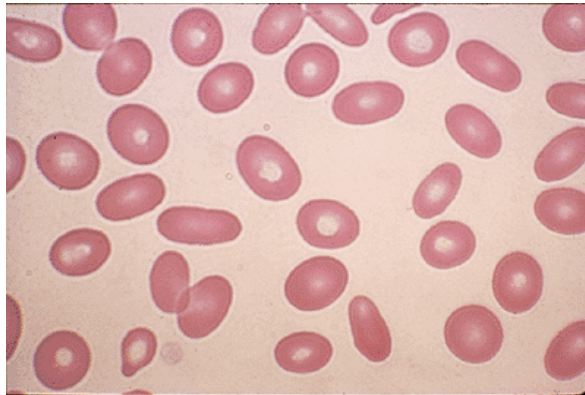
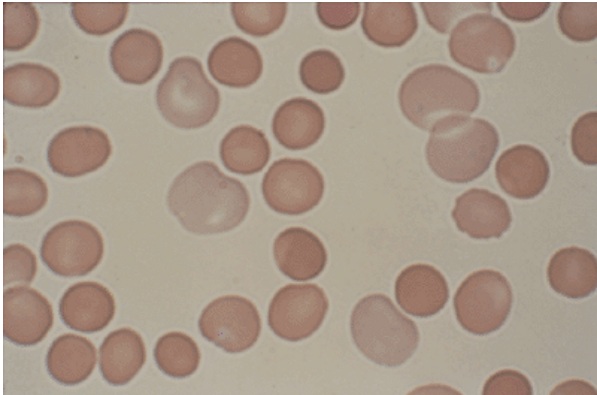
Immune-Mediated Hemolytic Anemia

-Warm: Spherocytes

-Cold: Agglutination w/ occasional spherocytes (see below)

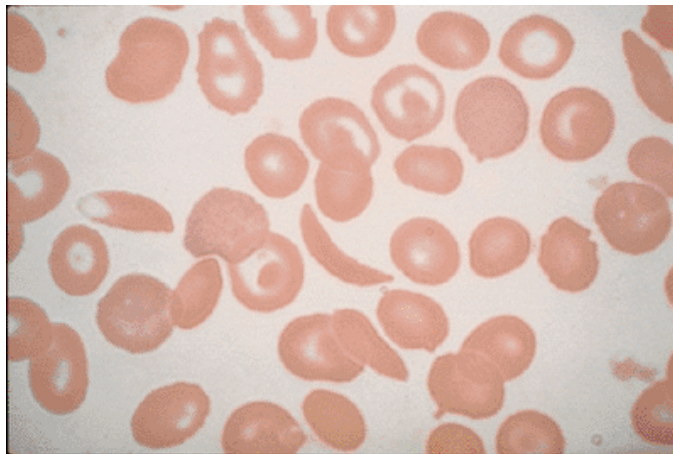
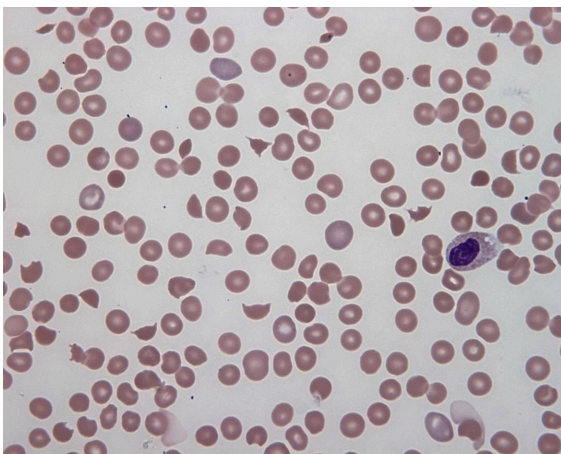


Non-Immune Mediated Hemolytic Anemia



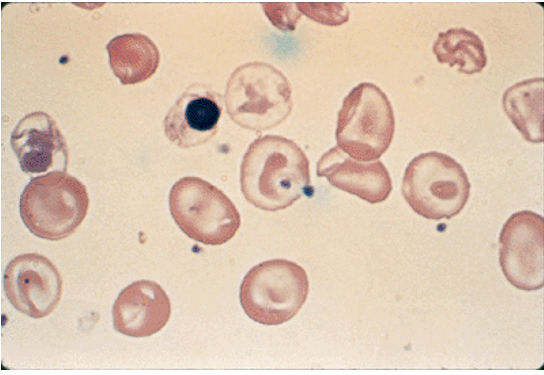
Left: Hereditary Spherocytosis

Right: Hereditary Elliptocytosis



Left: Microangiopathic Hemolytic Anemia

Right: Sickle Cell Anemia



Thalassemia

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Before class:

Required:

Read Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapter 3: Marrow-Damage Anemia

Chapter 9: The Dysplastic and Sideroblastic Anemias

Learning objectives:

1. Describe the mechanisms (production, destruction, and sequestration) and consequences of pancytopenia.

Mechanisms

- Lack of Production = bone marrow failure
- Immune Destruction
 - Immune thrombocytopenia
 - Autoimmune hemolytic anemia
 - Evans syndrome (combination of autoimmune cytopenias)
- Sequestration - Predominantly hypersplenism
- Bleeding/sepsis/consumption

Consequences of pancytopenia:

- Anemia
 - Fatigability, headaches, tissue damage (CVA, MI, ARF) – threshold varies by patient (age, co-morbid illnesses, acuity/chronicity of anemia)
- Leukopenia
 - Infections (usually not until ANC < 0.3)
- Thrombocytopenia
 - Bleeding (risk increases mainly below 10k)

2. Recognize the major forms of marrow failure - congenital and **acquired**

Table 3-2. Causes of marrow-damage anemias.

- Stem cell/marrow structural damage
 - Chemo/radiotherapy
 - Gaucher disease
 - Myelofibrosis
- Autoimmune disease
 - Rheumatologic disorders
 - Viral infections
 - Graft-versus-host disease
 - Idiopathic aplastic anemia
 - Pure red cell aplasia
- Congenital disorders
 - Fanconi anemia
 - Diamond-Blackfan anemia
 - Dyskeratosis congenita

Congenital - e.g. Diamond-Blackfan anemia, Fanconi anemia, dyskeratosis congenita

Diamond-Blackfan anemia

- A congenital form of pure red blood cell aplasia that appears early in childhood
 - By 6 months of age, 80% of children are anemic; by 9 months, 90% are affected.
- Typical erythropoietic profile shows a somewhat macrocytic, normochromic anemia, reticulocytopenia, and an **absence of red blood cell progenitors** in a marrow with normal white blood cells and platelet production.
 - Serum levels of erythropoietin increased.
 - Many children have minor physical defects including strabismus and bony abnormalities of fingers and ribs.
- Disruption of the **ribosomal protein gene, RPS19**, localized to chromosome 19q13.2, has been implicated in a quarter or more of cases, with evidence suggesting two other loci in the remainder.

- The nature of the red blood cell defect in Diamond-Blackfan anemia is unclear.
 - Studies suggest that erythroid progenitors are intrinsically abnormal, responding poorly to erythropoietin or SCF, and exhibiting accelerated programmed death.
- Although the presence of a humoral or cellular inhibitor has not been detected, 70% or more of patients with Diamond-Blackfan anemia will respond clinically to relatively low doses of **prednisone**.
 - **Allogeneic bone marrow transplantation** has also proved effective in patients with matched donors. Transfusion is the mainstay for the others.
- Overall, life expectancy has improved significantly; patients now live well into their third or fourth decade.
- Diamond-Blackfan has been associated with an increased incidence of malignancy, especially acute myeloid leukemia (AML; 15:17 translocation).

Fanconi anemia a.k.a. Constitutional Aplastic Anemia

- Autosomal recessive
- Presents as **severe pancytopenia** during first 2 decades of life → progresses to acute leukemia in many cases
- Heterozygote frequency is ~1/200 in Western societies, as high as 1/80 in white South Africans
- When fully expressed (1/100,000 live births), disorder is characterized by:
 - Progressive marrow failure
 - Multiple physical defects
 - Chromosomal abnormalities (Chromosome 1 or 2 abnormalities or Monosomy 7)
 - Cancer predisposition
- Patients can present w/o the typical physical defects → consider in children and young adults w/ AML or non-hematologic tumors
- Risk of developing marrow failure and cancers increases dramatically w/ age
- Diagnosis is confirmed by demonstration of DNA hypersensitivity to crosslinking agents
 - Traditional test uses mitomycin C or diepoxybutane as provocative agents in lymphocyte/fibroblast culture to bring out chromosomal abnormalities
- Up to 11 different Fanconi genes have been reported
- Normally, the Fanconi proteins combine, enter the nucleus, and help mediate chromosomal repair/stabilization
 - Failure of the Fanconi genes interferes w/ binding and subsequent translocation of the protein complex to the nucleus → chromosomal instability
- Congenital abnormalities
 - Absent radius; absent thumbs
 - Microcephaly; short stature
 - Hypopigmentation or café au lait spots
 - Pancytopenia - childhood [macrocytic anemia, thrombocytopenia, neutropenia]
 - Abnormalities of chromosomes; monosomy 7; MDS
 - Predisposition to malignancy - 10% AML, SCC, GYN

Dyskeratosis congenita

- An inherited X-linked, recessive disorder characterized by:
 - Skin pigmentation
 - Oral leukoplakia
 - Nail dystrophy
- **Bone marrow failure - cytopenias, MDS, AML**
- Common somatic abnormalities: epiphora, developmental delay, pulmonary disease, short stature, dental caries, premature grey hair and hair loss
- Aplastic anemia is seen in at least 50% of these patients
- The mucocutaneous abnormalities generally appear well before puberty, whereas the aplastic anemia occurs late in the second or third decades.
- Since the clinical presentation can be quite variable, it should be considered in the differential of adult aplastic anemia.
- Responsible gene has been mapped to Xq28 locus.
- Like Fanconi anemia, the dyskeratosis congenita defect best fits a chromosome instability abnormality, although the patient's lymphocytes do not show sensitivity to mitomycin C or diepoxybutane.

Inherited Bone Marrow Failure Syndromes

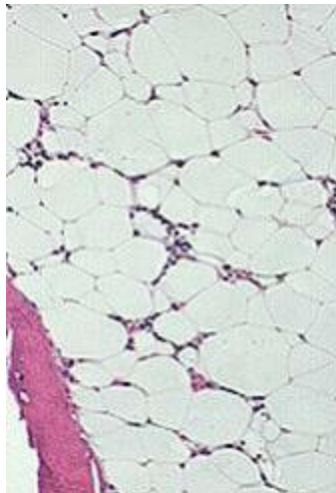
Syndrome	Age	Clinical Features	Pathology	Diagnostic Test	Genetics
Diamond-Blackfan anemia	0 → 50	Short stature, abnormal thumbs	Pure red cell aplasia, macrocytosis, anemia w/ reticulopenia, erythroid	Elevated red cell adenosine deaminase	Autosomal dominant small ribosomal protein (RPS19) at 19q13

			hypoplasia, MDS, leukemia, solid tumors		
Fanconi anemia	0 → 50	Skin hyperpigmentation and cafe-au-lait spots, short stature, triangular face, abnormal thumbs, radii, microcephaly, abnormal kidneys, decreased fertility	Pancytopenia, macrocytosis, anemia, hypocellular marrow, MDS, AML, solid tumors	Increased chromosome intrastrand cross-link breakage	Autosomal X-linked recessive (>12 FANC genes)
Dyskeratosis congenita	0 → 50	Dyskeratotic nails, lacey reticular rash, oral leukoplakia	Macrocytosis, anemia, thrombocytopenia, neutropenia, hypocellular marrow, MDS, leukemia, solid tumors	- None - Finding short telomeres?	- X-linked (DKC1) dyskerin (telomere maintenance) - Autosomal dominant telomerase RNA template (TERC)

Acquired - e.g. aplastic anemia, myelodysplasia, leukemia, replacement with tumor

Aplastic anemia

- Clinical presentation of aplastic anemia:
 - Any age - more common in young adults & elderly
 - Symptoms - weakness, fatigue, fever, recurrent infections, easy bruising, bleeding, purpura
 - Physical findings – pallor, petechiae, purpura, ecchymoses; usually no lymphadenopathy or splenomegaly
 - Laboratory – pancytopenia - granulocytopenia, usually normocytic RBCs and decreased reticulocytes, thrombocytopenia
 - Aplasia must be confirmed by bone marrow biopsy showing - sparse cellularity (<25%), small islands of predominantly erythroid cells, and other marrow elements are absent or sparsely distributed.



Myelodysplasia = conditions that involve ineffective production of the myeloid cells

Leukemia

Replacement with tumor

3. Identify the associated pathophysiologic mechanisms of bone marrow failure, e.g. defective DNA repair, defective telomerase, abnormal nucleolar ribosomal protein or transcription factors, radiation, viruses

Defective DNA repair - Fanconi Anemia

- FA genes (13) all function in a common pathway
- FANC-A,B,C,E,F,G,I,L&M form a nuclear protein complex which monoubiquitinates FANCD2
- Monoubiquitinated FANCD2 forms a complex with FANCD1 & non-FAN proteins, e.g. BRCA1 required for DNA repair foci
- Hematopoietic failure in FA may be independent of the defective FA complex.

Defective telomerase - Dyskeratosis congenita

- Dyskeratosis congenita - rare inherited bone marrow failure syndrome
 - Genetic & Pathophysiology
 - autosomal dominant - 10%
 - defective telomerase RNA gene
 - disease anticipation - onset of disease at increasingly earlier ages in successive generations
 - autosomal recessive – 10%
 - 60% BMF in 2nd decade
 - X-linked disease - 80%
 - defective nucleolar protein dyskerin (part of the telomerase complex)
 - 30% BMF in 2nd decade
 - Epithelial malignancies begin in 3rd decade
 - 80% of deaths due to infection (most before age 30)

Abnormal nucleolar ribosomal protein or transcription factors - Diamond-Blackfan

- “Single” lineage marrow failure – often present within first year of life
- Diamond-Blackfan anemia - erythropoietic failure
 - pure red cell aplasia
 - abnormalities of nucleolar protein(s)
 - decreased formation of 40s ribosomal units

Radiation

- Hematopoietic cells are particularly sensitive to high energy (gamma; X-ray) radiation. Total-body irradiation of 300-500 Grays can cause complete hematopoietic failure.
- Severe, but limited (4-6 weeks) anemia, leukopenia, and thrombocytopenia can result from sublethal radiation.
- Long term low energy radiation (alpha and beta particles) may damage hematopoietic cells if ingested.
- Radium watch dial painters in Waterbury, CT ingested radium by pointing their brushes on their lips.

Viruses

- Viral infection: common with viral hepatitis (5%)
 - Hepatitis B most common association; some non A, B or C
- Pathogenesis: probably immune mediated
- Parvovirus B19 infection
 - is a major cause of pure red cell aplasia
 - only the erythrocyte cell line is affected
 - may be acute or chronic, congenital or acquired
- Epstein Barr virus DNA can be found in some marrows of affected patients
- HIV
- CMV

4. Identify the molecular and pathophysiologic defects in paroxysmal nocturnal hemoglobinuria (PNH).

Deficiency of cell membrane glycoproteins

- Complement regulatory proteins
 - Decay Accelerating Factor (CD 55)
 - Membrane Inhibitor of Lysis (CD 59)
- Membrane enzymes
- Other membrane associated proteins
 - Lymphocyte Function Antigens, CD14, CD16

Decreased glycosylphosphatidylinositol (GPI)

- anchor linking proteins to the cell membrane

Gene defect

- PIGA gene* is mutated
- little or no GPI anchor made

*phosphatidylinositol glycan complementation class A gene

PNH is a clonal disorder of hematopoietic cells caused by a somatic mutation of the pig-A gene on the short arm of the X chromosome.

- More than 170 mutations in the pig-A gene have been reported. Some result in inactivation of the gene and a complete failure in the production of the GPI anchor protein glycosylphosphatidylinositol glycan (PNH III), while others result in a partial

deficiency of the GPI anchor protein (PNH II).

- In both cases, binding of several other proteins is disturbed, including the amount of protectin (CD59) on the cell membrane.
- The loss of CD59, which normally regulates the assembly of the C9 attack complex, appears to play a key role in both red cell hemolysis and the hypercoagulable state seen in PNH patients.
- The phenotype of the disease is in part influenced by the nature of the mutation, with PNH III cells being more susceptible to hemolysis.
 - However, it is also clear that both PNH II and PNH III clones can exist side by side in the same patient.

• **Relate the immunologic tests used to diagnose PNH to the underlying molecular defect.**

Historically, the sugar water test was used as a convenient screening test for PNH.

- It is performed by adding a small amount of the patient's red blood cells to an isotonic sucrose solution laced with fresh complement.
- More than 5% lysis of cells suggests PNH, whereas less than 5% lysis may be seen in patients with megaloblastic and autoimmune hemolytic anemias.

A somewhat more definitive test is the Ham-acid hemolysis test, which detects sensitivity of the PNH red blood cells to fresh complement and acidification.

However, now that flow cytometry is available in most laboratories, a cytometric assay of red cell CD59/CD55 levels is clearly preferred.

- It is more sensitive and specific and can identify small subpopulations of abnormal cells.
- Two other GPI-anchored proteins, CD16 and 66b, can also be measured on granulocytes in patients with equivocal red cell results to further increase the sensitivity of the test.

All cell lines, including granulocytes and platelets, are involved in PNH and are susceptible to lysis.

- Although this may be responsible in part for the pancytopenia, many patients present with a hypocellular, even aplastic, marrow.
 - In these patients, marrow culture studies demonstrate a reduced number of all progenitor lines, similar to the findings in marrow damage/aplastic anemia.
- The presence of GPI-negative cells is most often seen with T-cell mediated marrow failure.
- It has now been reported that 15-50% of patients with idiopathic aplasia or myelodysplasia will demonstrate a PNH clone.
 - Patients with marrow failure after chemotherapy, transplantation, immunotherapy, or secondary to large granular lymphocytosis (T-cell lymphoma) rarely have GPI-negative cells detected.

• **List complications of PNH.**

Presenting symptoms:

- acquired intravascular hemolytic anemia due to abnormal susceptibility of the RBC membrane to the hemolytic activity of complement
- thromboses in large vessels, such as hepatic, abdominal, and cerebral veins
- GPI-deficient platelets are more readily activated by complement than normal platelets
- variable bone marrow failure - often pancytopenia

PNH: Clinical Findings

- Presents most often between ages 25-35 years
- Hemolysis and hemoglobinuria occur continuously; may be exacerbated by infection, operations, transfusion
 - No characteristic peripheral blood smear findings
 - Anemia, reticulocytosis, decreased MCV
 - Iron deficiency, bleeding, thrombosis, leukopenia
 - Development of aplastic anemia ~10%
 - Variable marrow pathology
 - hypercellular - erythroid hyperplasia
 - hypocellular or aplastic
 - decreased storage iron
- Neurologic manifestations
 - Severe headaches, pain in the eyes (small venous occlusions)
- Renal manifestations
 - Tubular dysfunction causing acute or chronic renal failure

Patients with PNH present either with a hemolytic anemia with intravascular hemolysis and episodes of nocturnal hemoglobinuria or with a pancytopenia with a dysplastic or aplastic marrow morphology.

- Hemolytic disease is more often associated with a large, dominant clone of PNH cells, while aplastic and dysplastic individuals usually have few GPI-negative cells in circulation.
- One conclusion drawn from this observation is that marrow damage (bone marrow failure) somehow induces the PNH somatic mutation as a secondary manifestation of the disease, not that the mutation is the primary cause of the dysplastic or aplastic anemia.

Patients with PNH are also at increased risk for venous thrombosis, owing to complement-driven activation of platelets lacking CD59 and also, perhaps, impaired fibrinolysis.

- Thrombosis of hepatic veins (Budd-Chiari syndrome) or the portal, splenic, or mesenteric veins is a common manifestation of the hypercoagulable state and can lead to death or a clinical picture similar to that of disseminated intravascular coagulation.

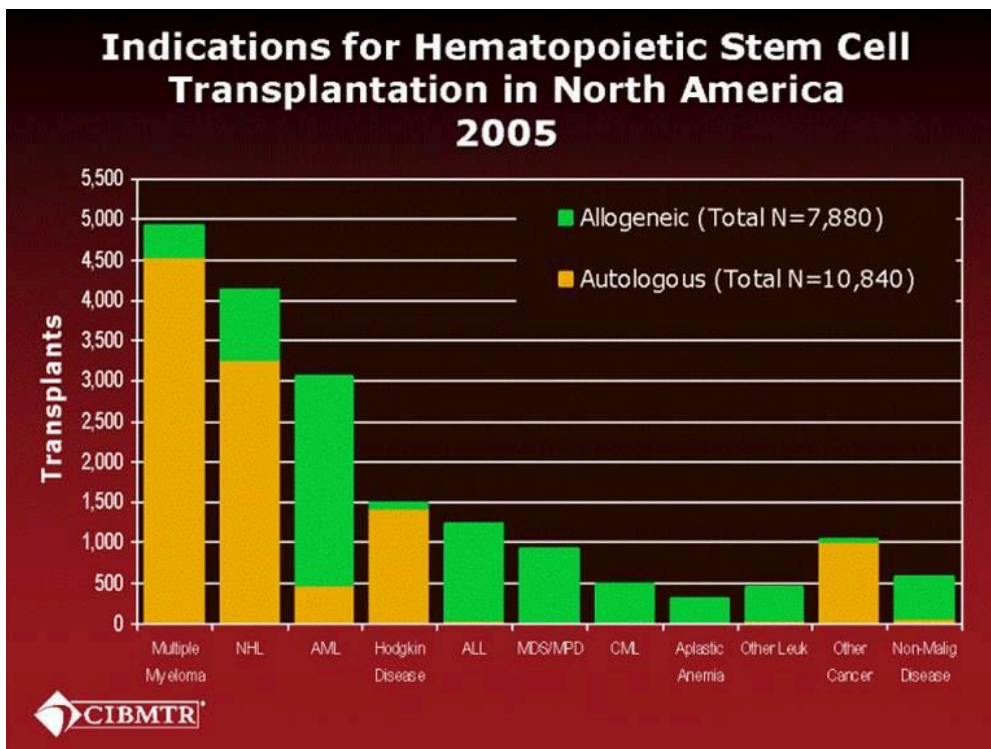
Patients with chronic hemolysis are at risk for developing iron deficiency secondary to urine iron loss.

Finally, severe thrombocytopenia and granulocytopenia can lead to major bleeding or infection.

Originally it was thought that the nocturnal hematuria was due to the decrease in pH that occurs naturally during sleep - it is now believed that the hemolysis occurs persistently regardless of the time of day and that the reason it was noticed in the early mornings was due to the urine accumulation and concentration during sleep.

5. List the indications for hematopoietic stem cell transplant and the rationale for this treatment choice.

Stem cell transplants are done for many indications, the most common malignancy. Bone marrow failure is another less common indication.



6. Describe the different types and sources of hematopoietic stem cells.

Types of Stem Cell Transplants

- Autologous vs. Allogeneic
- Rationales:
 - Replace damaged stem cells/immune system
 - High dose chemotherapy
 - Graft vs. cancer effects
- Related vs. Unrelated
- Conditioning regimen: chemotherapy, immunosuppressives (ATG), radiation
- Stem Cell Source:
 - Bone Marrow
 - Mobilized peripheral blood stem cells
 - Umbilical cord

7. Describe principles of pre-transplant conditioning and the role for post-transplant immunosuppression.

- Ablation of patient marrow
 - High dose chemotherapy
 - Whole body radiation

8. Compare graft versus host disease and graft versus tumor effect.

- graft vs tumor effect is essentially the same mechanistically as graft vs host disease
- usually most bone marrow transplants will be allogenic and often these transplants will contain immunocompetent cells from the donor
- if the histocompatibility isn't perfect (and even if it is), it is very likely these immunocompetent donor cells will attack the host (GVHD)
the bright side is - the donor cells will also attack any tumors the host has (graft vs tumor effect)

9. Describe the pathophysiologic basis for acute and chronic graft versus host disease. source: up to date

originally the difference was based on an arbitrary time cut-off of 100 days

acute - before 100 days

chronic - after 100 days

now there has been a shift in classifying acute vs chronic based on the symptoms

acute - commonly demonstrate a classic maculopapular rash, abdominal cramps with diarrhea, and a rising serum bilirubin concentration

chronic - have skin involvement resembling lichen planus or the cutaneous manifestations of scleroderma, dry oral mucosa with ulcerations and sclerosis of the gastrointestinal tract, and a rising serum bilirubin concentration

10. List changes in humoral and cellular immunity following stem cell transplant and relate the changes to infectious complications.

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Hematology System

Curricular Week 69 - System Week 1: Red Blood Cells

[Problem Set: Approach to Anemia](#)

[Pam Clark, MD](#) ; [Michael Douvas, MD](#) ; [Donald Innes, MD](#)

Friday, December 2, 2011 10:10-10:00 – Learning Studio

Before class:

Recommended:

1. Review materials from the week, as the problem set is intended to apply and integrate concepts introduced during this week's sessions.

2. Read Hillman RS, Ault KA, Leporrier M, Rinder HM: Hematology in Clinical Practice. 5th Edition.

Chapter 12: Anemia in the Elderly

The answers to the problem set will be made available on the course website immediately after the session.

Learning objectives:

Review LOs from the following sessions:

- [Introduction to Hematopoiesis](#) (Monday 11/28)

- [Nutritional Anemias/ Hemochromatosis Lab](#) (Wednesday 11/30)

- [Hemolytic Anemia/ Hemoglobinopathies and Thalassemia Lab](#) (Thursday 12/01)

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I'm on a blood buzz / Yes I am