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Iron Metabolism Disorders & Hemolytic Anemias

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Objectives

Understand iron physiology and the diagnosis and management of iron disorders.

Evaluate hemolytic anemias and apply management strategies including novel therapeutics.

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ABIM Hematology Exam Blueprint

HEMATOPOIETIC SYSTEM *(25% of exam)*	Diagnosis	Testing	Treatment/ Care Decisions	Risk Assessment/ Prognosis/ Epidemiology	Pathophysiology/ Basic Science
NORMAL HEMATOPOIESIS *(<2% of exam)*					
Normal hematopoiesis	✓	✓	✓	⊘	⊘
DISORDERS OF RED BLOOD CELLS OR IRON *(21% of exam)*					
Red blood cell production disorders *(4% of exam)*					
Nutritional deficiencies					
*Iron deficiency**	✓	✓	✓	✓	⊘
*Nutritional anemia, non–iron deficiency**	✓	✓	✓	⊘	⊘
Anemia of chronic inflammation	✓	✓	✓	⊘	✓
Red cell aplasia and hypoplasia LF	⊘	⊘	⊘	⊘	⊘
Sideroblastic anemia LF	⊘	⊘	⊘	⊘	⊘
Hemochromatosis	✓	✓	✓	⊘	⊘

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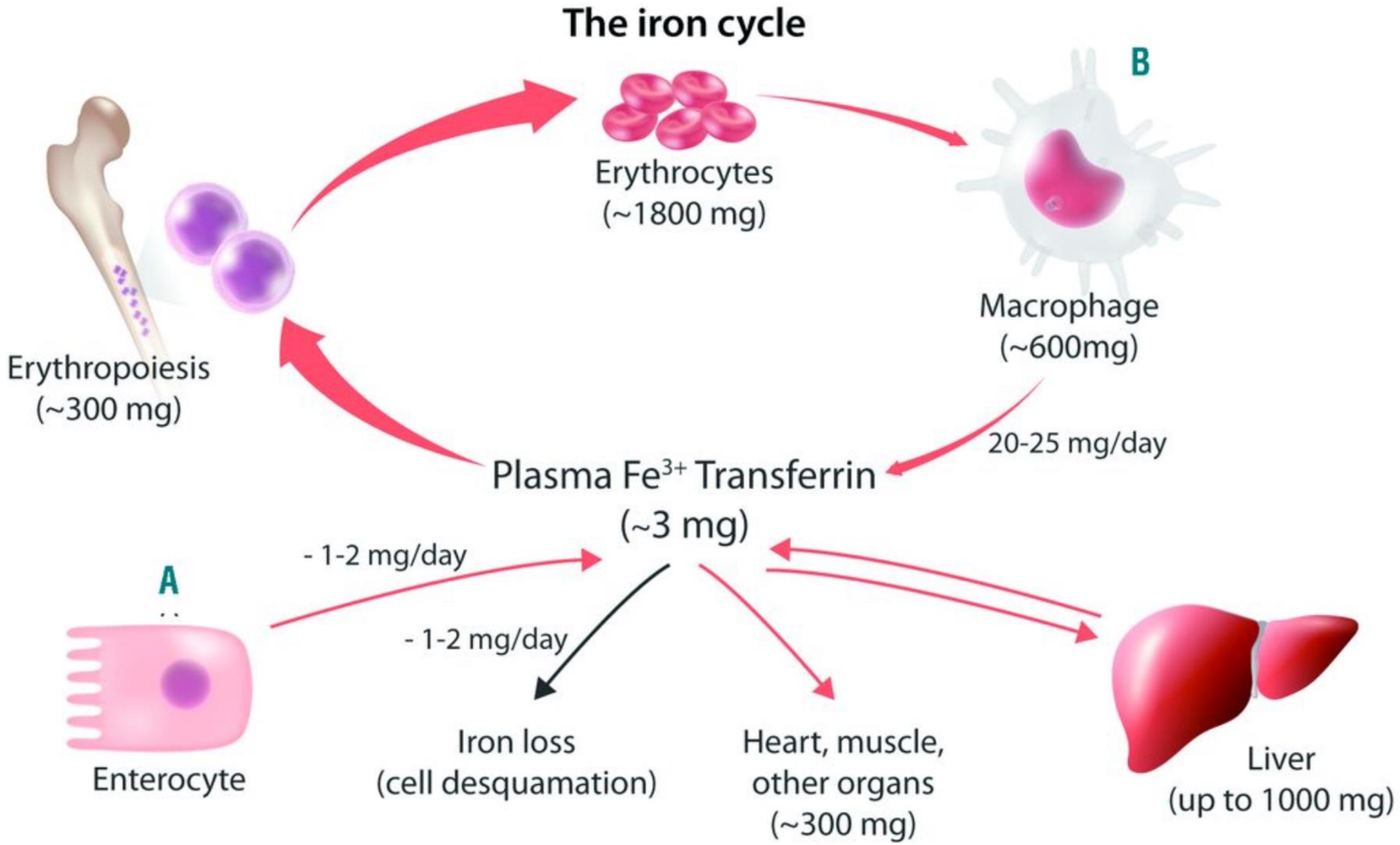
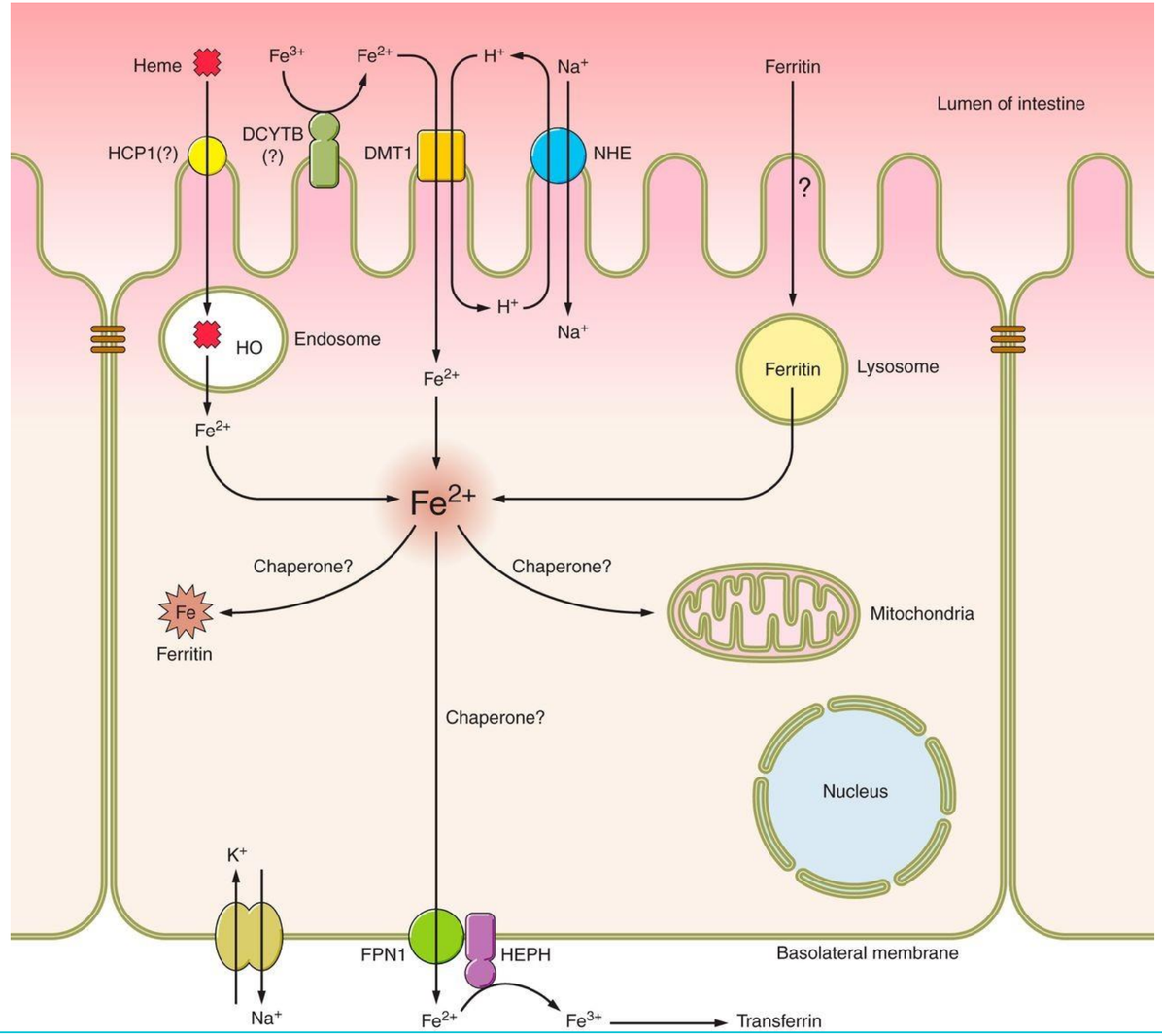
		Diagnosis	Testing	Treatment/ Care Decisions	Risk Assessment/ Prognosis/ Epidemiology	Pathophysiology/ Basic Science
Autoimmune hemolytic anemias (AIHA)						
Warm antibody-mediated autoimmune hemolytic anemia		✓	✓	✓	✓	⊘
Cold antibody-mediated autoimmune hemolytic anemia	LF	✓	⊘	✓	⊘	⊘
Drug-induced hemolysis	LF	⊘	⊘	⊘	⊘	⊘
Metabolic abnormalities and enzyme deficiency hemolytic anemias						
Oxidant hemolysis, including glucose-6-phosphate dehydrogenase (G6PD) deficiency	LF	⊘*	⊘*	⊘*	⊘*	⊘*
Pyruvate kinase deficiency and other metabolic deficiencies	LF	⊘*	⊘*	✗*	✗*	✗*
Paroxysmal nocturnal hemoglobinuria	LF	✓	⊘	⊘	⊘	⊘
Red blood cell membrane disorders	LF	⊘	⊘	⊘	⊘	✗
Microangiopathic hemolytic anemias (other than TTP, HUS, or DIC)		✓	✓	✓	✓	⊘
Non-autoimmune, acquired hemolytic anemias	LF	⊘	⊘	⊘	⊘	⊘



Wcamaschella C. et al. Haematologica. 2020;105(2):260-272.

Intestinal Iron Absorption

- Nonheme iron in diet is in form of ferric iron
- Iron must be reduced to be absorbed (ferric to ferrous)
- Enterocyte iron must then be transported into circulation via ferroportin



Gulex S et al. Am J Physiol Gastrointest Liver Physiol. 2014;307(4):397-409.

Diet

- Typical Western diet contains ~15 to 25 mg of iron
- Inorganic iron (cereals, legumes) and heme iron (red meats, fish, poultry)
- Inorganic iron is less readily absorbed then heme iron (5-10% only)
- Calcium rich foods, tannins in tea, coffee, anti-acids decrease iron absorption
- Absorbic acid increases absorption

Table 1

Iron Sources in Food

Meats*	Size (oz.)	Iron (mg)
Veal liver	1	4-5
Beef	3	4-5
Lamb	4	4-5
Ham	2	1.5-2
Chicken	3-4	1.5-2
Bologna	3-4	1.5-2
Fruits, grains, vegetables†	**Quantity/Size**	**Iron (mg)**
Raisins	0.5 C	4-5
Peas, cooked	0.5 C	2-4
Beans, cooked	0.5 C	2-4
Figs	3 medium	2-4
Barley	0.5 C	1.5-2
Oatmeal	1 C	1.5-2
Beans, green	1 C	1.5-2
Rice	1 C	0.7-1.4
Potato	1 medium	0.7-1.4

**The body can absorb up to 40% of iron in these foods.*
†The body can absorb 10% or less of iron in these foods.
C: cup.
Source: Reference 12.

Saljoughian M. et al US Pharmacist. 2007;32(8):HS26-HS37.

Prevalence of Iron Deficiency Anemia (IDA)

Most common cause of anemia worldwide, affecting over one billion people, predominantly woman and children

US National Health and Nutrition Examination Survey (NHANES) from 2003-2010:

- 15% toddlers
- 11% adolescent girls
- 9% adult woman (age 20-49)

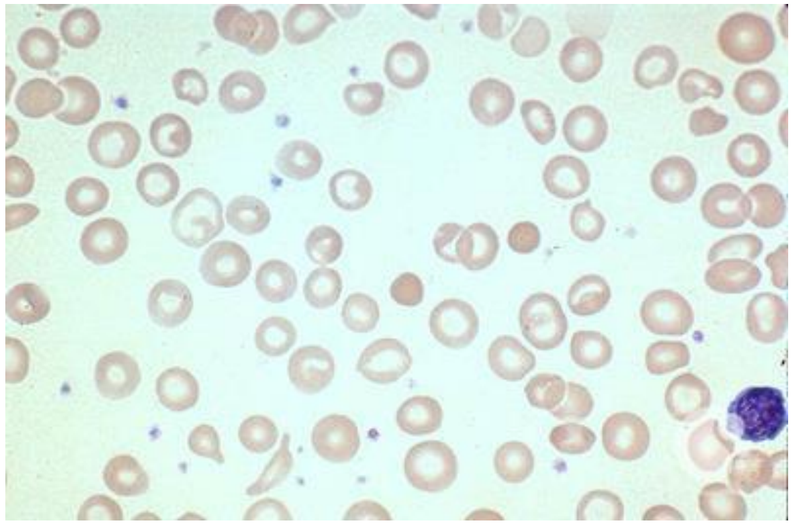
Nutrition Impact Model Study estimated global prevalence of anemia in pregnancy 38% in 2011

Stevens GA et al. Nutrition Impact Model Study. Lancet Glocal Health. 2013;1(1):e16-e25.

Diagnosis of IDA

Stages

- Total body iron stores are reduced (low ferritin), even with normal CBC and MCV
- *Note retics can be increased initially if bleeding, but will decrease as iron stores depleted*



As iron stores are exhausted:

- Microcytic (MCV <80)
- Hypochromic (pale, MCHC)
- Anemic (Hgb decreased)

After iron replacement therapy:

- Retics increase ~7 days
- Hgb response ~2 weeks
- Ferritin stores correct once additional iron beyond that to correct Hgb

Confirming IDA

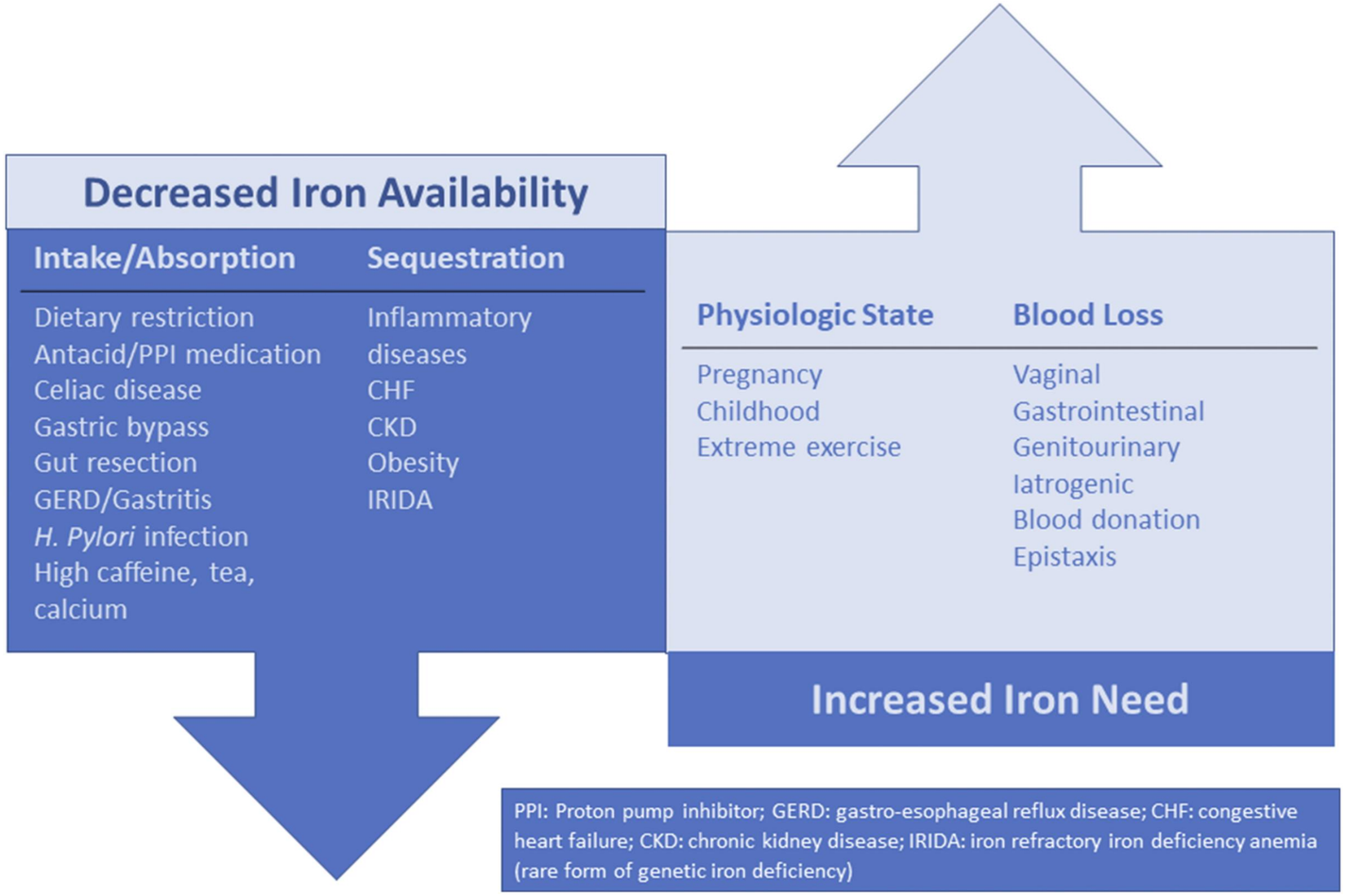
Iron studies

- Serum ferritin is the most reliable initial test *(correlates with the body's iron stores in the absence of inflammation)*
- Ferritin less than 30 ug/L achieves a high sensitivity (92%) and high specificity (98%) for diagnosis of IDA
- Ferritin 1 ug/L = ~8 to 10 mg tissue iron

- Inflammatory conditions may "normalize" ferritin *(acute phase reactant)*
- Low transferrin saturation (Tsat) less than 20% plus a higher ferritin threshold of less than 100 ug/L can be used for diagnosis of IDA in setting of inflammation

Test	Reference ranges
Serum ferritin (mcg/L)	20-200 (female) 30-300 (male)
Serum iron (mcg/dL)	60-180
Total iron binding capacity (TIBC) (mcg/dL)	270-535 (female) 250-460 (male)
Serum transferrin (mg/dL)	192-382 (female) 180-329 (male)
Transferrin saturation (TSAT) (Serum iron/TIBC) (%)	20-45 (female) 20-50 (male)

Causes of IDA



Shuoyan Ning,Michelle P. Zeller, Management of iron deficiency, Hematology Am Soc Hematol Educ Program, 2019, Figure 2.

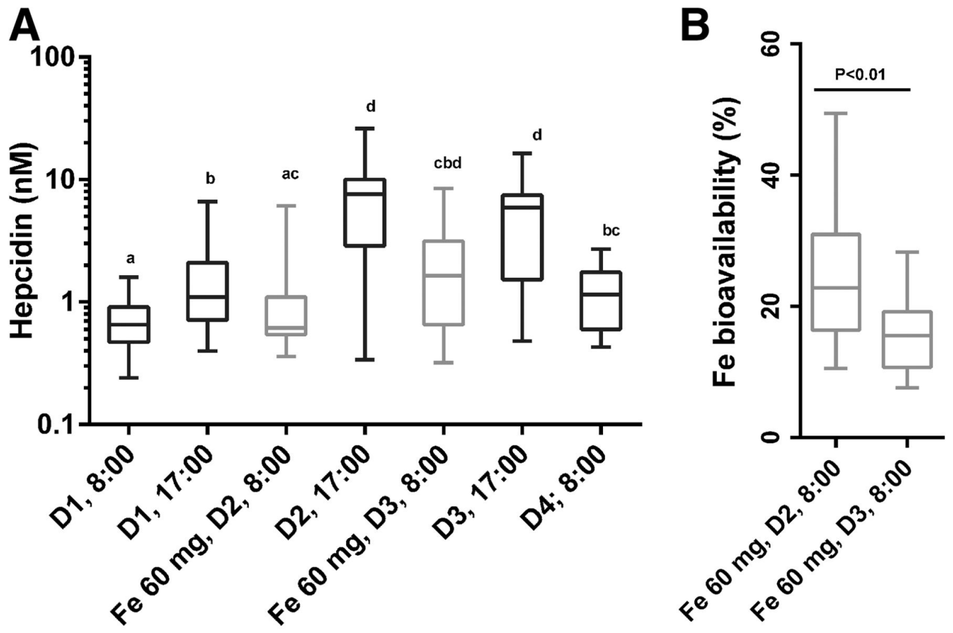
Treatment of IDA

Oral Iron

- Inexpensive, available in resource poor settings
- Effective in most patients w/ classic IDA, if taken
- *GI side effects common limiting factor, ~30 - 60%*
- *Requires 3 to 6 months of therapy (max oral absorption is ~25 mg/day)*

- Do not give with food
- Gastric acidity and Vit C is helpful
- Ferrous best absorbed (Fe++)
- Every other day dosing results in greater absorption

- Ineffective if significant inflammatory block



Diego Moretti,Jeroen S. Goede,Christophe Zeder,Markus Jiskra,Vaiya Chatzinakou,Harold Tjalsma,Alida Melse-Boonstra,Gary Brittenham,Dorine W. Swinkels,Michael B. Zimmermann, Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women, Blood, 2015, Figure 3

Oral Iron

Drug class	Example	Dose per tablet (mg)	Elemental iron content per tablet (mg)	Dose	Special instructions
Iron salts	Ferrous gluconate	240	27	1-3 tablets, once per day or once every other day	Take on empty stomach; consider vitamin C; take at a different time of day than antacid or proton pump inhibitor. Acidic environment required.
		325	38		
	Ferrous sulfate	325	65	1-2 tablets, once per day or once every other day	
	Ferrous fumarate	325	106	1 tablet, once per day or once every other day	
Heme iron polypeptide	Proferrin	398	11	1-3 tablets per day	Can be taken with a meal. Acidic environment not required for absorption.
Polysaccharide iron complex	Feramax	150	150	1 tablet once per day	Can be taken with a meal. Acidic environment not required for absorption.
Ferric citrate	Auryxia	210	210	3-5 tablets once per day	Can be taken with a meal. Acidic environment not required for absorption.

Shuoyan Ning,Michelle P. Zeller, Management of iron deficiency, Hematology Am Soc Hematol Educ Program, 2019, Table 2.

IV Iron

- IV iron used for unresponsiveness to or intolerance of po iron
- IV iron used if rapid replacement is desired (severe anemia or preoperative)
- Choice of IV iron formulation guided by what is available at your institution, covered by insurance, etc

- Stability of the iron-carbohydrate complex (carbohydrate shell) that bind iron determines amount of iron safely delivered in single infusion
  - You can give higher single dose of iron dextran (InFed 1000 mg over 1to 4 hours) and ferric carboxymaltose (Injectafer 750 mg over 15 min) because they are more stable than ferric gluconate (Ferrlecit 125 mg-250 mg over 1-2 hours) and iron sucrose (Venofer 200-300 mg over 90 min)

- Excessive doses result in free iron release, which can result in the hypersensitivity (“pseudo-allergy”) reactions

- Dose Ganzoni equation (Ex: Hgb 8 g/dL, 70 kg = 2,200 mg deficit (FCM 750 mg x3 or iron sucrose 200 mg x10 or oral iron ~25 mg/d x90d).
- Do not check iron studies sooner than 4 weeks after IV iron.

IV Iron

Compound	Brand name	Recommended amount per dose	Infusion time	Availability	Reference
Low-molecular-weight iron dextran	INFeD	100 mg after uneventful 25-mg test dose	2-6 h (+ test dose)	United States, Europe	https://www.pdr.net/drug-summary/INFeD-iron-dextran-2087; https://www.allergan.com/assets/pdf/infed_pi
Ferrous gluconate	Ferrlecit	125 mg	12.5 mg/min	United States, Europe, Canada	http://products.sanofi.us/ferrlecit/ferrlecit.html
Iron sucrose	Venofer	200-300 mg	100 mg/30 min	United States, Europe, Canada	http://www.venofer.com/Indications_Dosage
Ferumoxytol	Feraheme	510 mg	15 min	United States, Europe	https://www.feraheme.com/dosing-and-administration/
Ferric carboxymaltose	Injectafer	750 mg	15 min	United States, Europe	https://injectaferhcp.com/iron-deficiency-anemia-dosing
	Ferinject	1000 mg	15 min	United States, Europe	https://www.ferinject.co.uk/simplified-dosing-for-all-patients/
Iron isomaltoside	Monofer	≤1000 mg	>15 min	United States, Europe	https://www.medicines.org.uk/emc/files/pil.5676.pdfinu

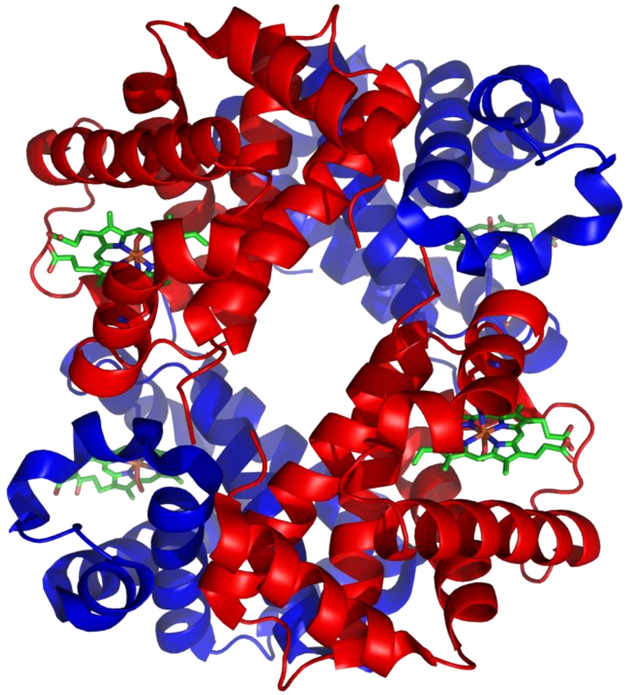
Shuoyan Ning,Michelle P. Zeller, Management of iron deficiency, Hematology Am Soc Hematol Educ Program, 2019, Table 3.

Microcytic Anemias (MCV <80fL)

Differential Dx

- **Lack of a component of hemoglobin**

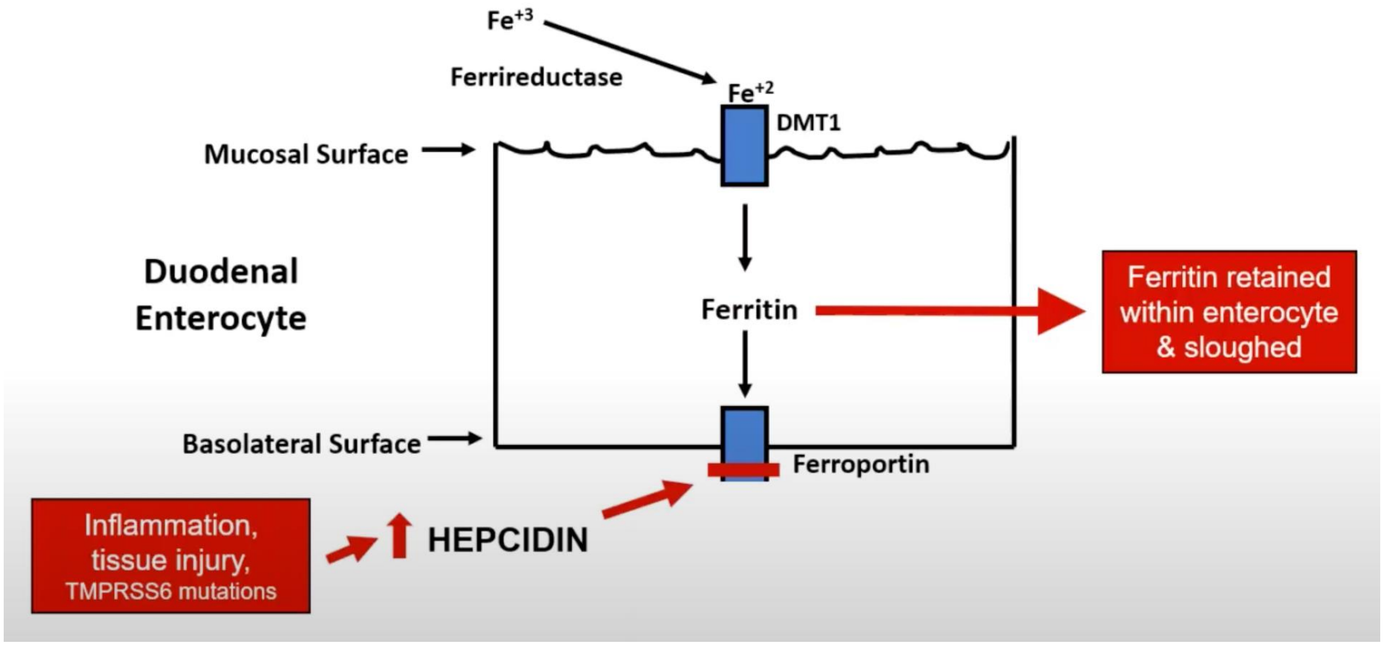
1. **Iron** deficiency
   - Absolute: iron deficiency anemia
   - Functional: anemia of inflammation /chronic disease
2. **Globin** deficiency
   - Thalassemia's (see lecture on Hgb disorders)
3. **Heme** deficiency
   - Hereditary sideroblastic anemia
     - ALA synthase mutation (*ALAS2* gene)
   - Chronic lead poisoning
     - ALA synthase inhibition



Anemia of Inflammation

- Inflammation induces hepcidin
- Immunoprotective response to minimize iron availability for pathogens

- Hepcidin internalizes and degrades ferroportin
- Iron gets trapped inside cells and not able to be utilized



Fertrin, Hematology Am Soc Hematol Educ Program 2020 Dec 4;2020(1):478-486.

Differential Diagnosis of IDA

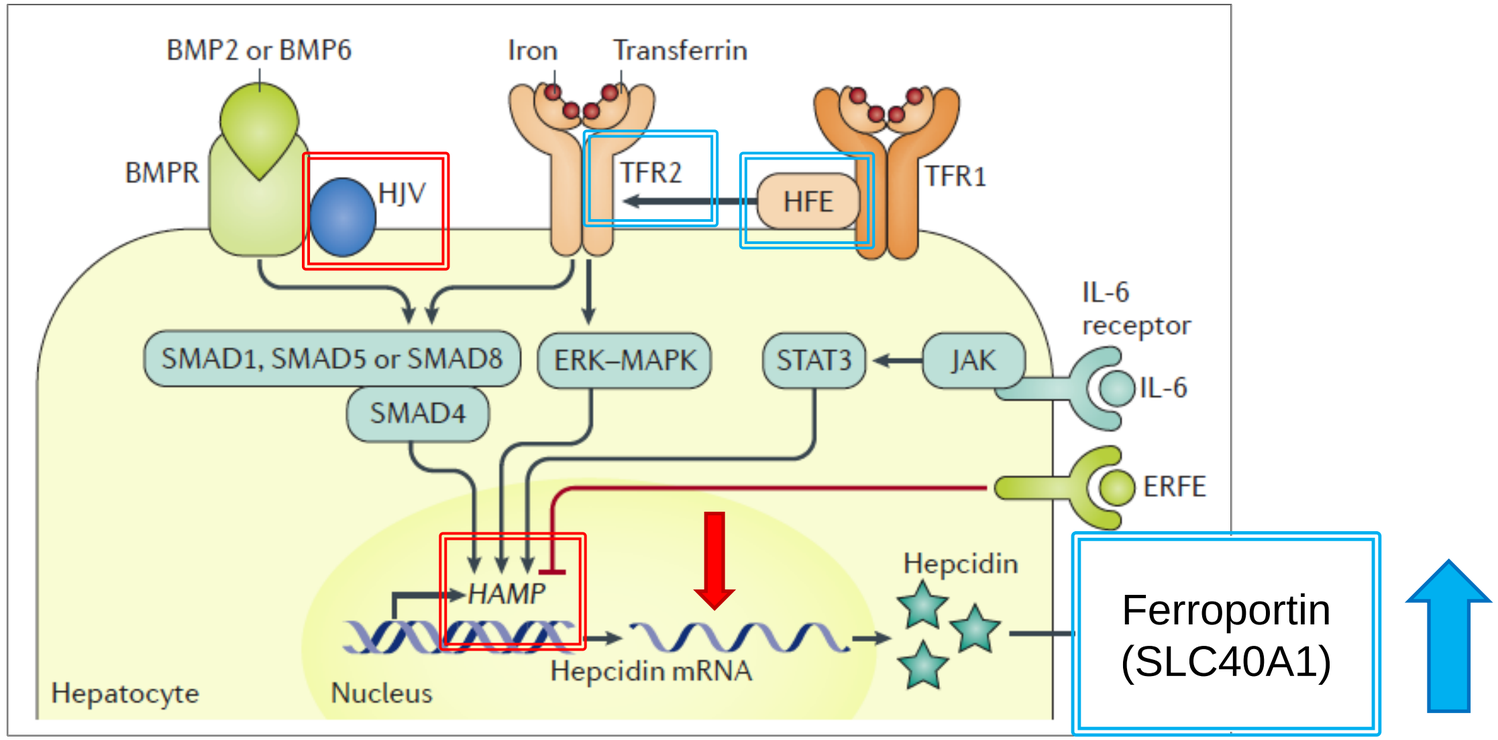
	Iron deficiency	Thalassemia trait	Inflammation
MCV	Low in proportion to anemia (may be nl in early stage)	Low even in absence of anemia	Normal or slightly low
Serum iron	Low	Normal	Low
TIBC	High	Normal	Normal or low
Serum ferritin	Low	Normal	Normal or high
Marrow iron	Absent	Present	Present

Anemia of Inflammation

- **Treatment of the underlying disorder** is usually best;
- **Iron supplementation**: usually NOT indicated unless combined iron deficiency exists (e.g. if ferritin <100ug/L), or if patient on ESA for CKD;
- **Erythropoiesis-stimulating agents**: consider if CKD-associated, or in some patients undergoing therapy for incurable malignancy
- **Transfusions**: only if symptomatic, significant anemia
- Investigational: hepcidin blockers

Iron Overload – Hereditary Hemochromatosis

Uncontrolled iron absorption due to hyperactivity of ferroportin (mostly due to hepcidin deficiency)



Adapted from Brissot et al., Nat Rev Dis Primers 2018

Hereditary Hemochromatosis

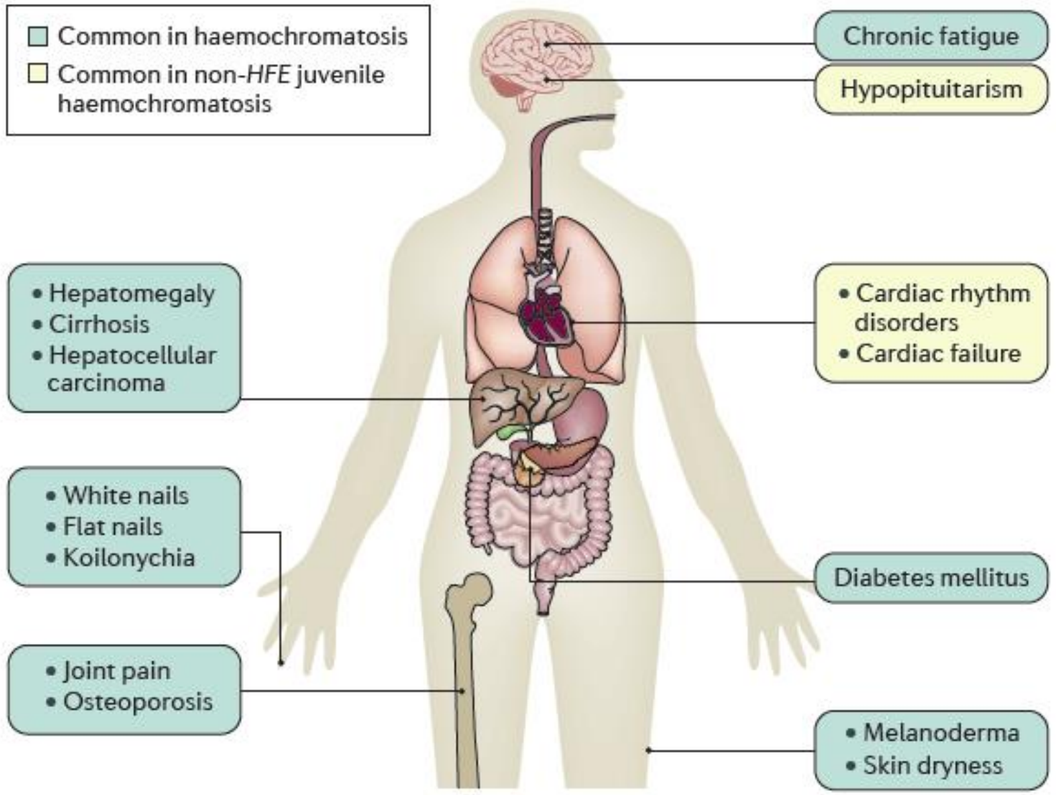
Clinical Features

Classical HH

- type 1, *HFE* mutation (Northern Europe origin)
- type 3, *TFR2* mutation (rare, may have earlier onset)
- type 4B, *SLC40A1* mutation (gain-of-function ferroportin)

Juvenile HH

- type 2A, HJV hemojuvelin mutation
- type 2B, hepcidin mutation (extremely rare)



Brissot et al., Nat Rev Dis Primers 2018

Hemochromatosis Diagnosis

- Labs:
- No anemia
- High ferritin AND TSAT>45%

- Northern European descent: start with ***HFE* testing**
  - *HFE* C282Y/C282Y or heterozygote C282Y/H63D: diagnosis of HH
  - *HFE* H63D/H63D: diagnosis is debatable; low penetrance
  - Other genotypes: non-diagnostic, pursue other causes
- No obvious Northern European ascent: **start with MRI T2*** to confirm iron overload; if positive for liver iron overload:
  - If age<30, consider testing for *HAMP, HJV, TFR2* genes
  - If age>30, consider testing for *HFE, TFR2, SLC40A1* genes

Hemochromatosis Treatment

- Avoid iron supplements and alcohol;
  - No need to follow iron-poor diet (as long as compliant with phlebotomy)
  - Avoid vitamin C supplements

- **Phlebotomy** – GOAL: ferritin 50-100mcg/L
  - Induction: 400-500mL weekly provided Hb>11g/dL
  - Maintenance: maximum interval to keep ferritin at goal
  - Blood donation: acceptable in some countries

- **Erythrocytapheresis**: allows faster iron removal; higher cost; side effects of procedure (hypocalcemia, longer procedure)
- **Iron chelation**: low dose deferasirox may be used for those intolerant to phlebotomy
- **Liver transplantation** may be required and is curative

Other Iron Overload

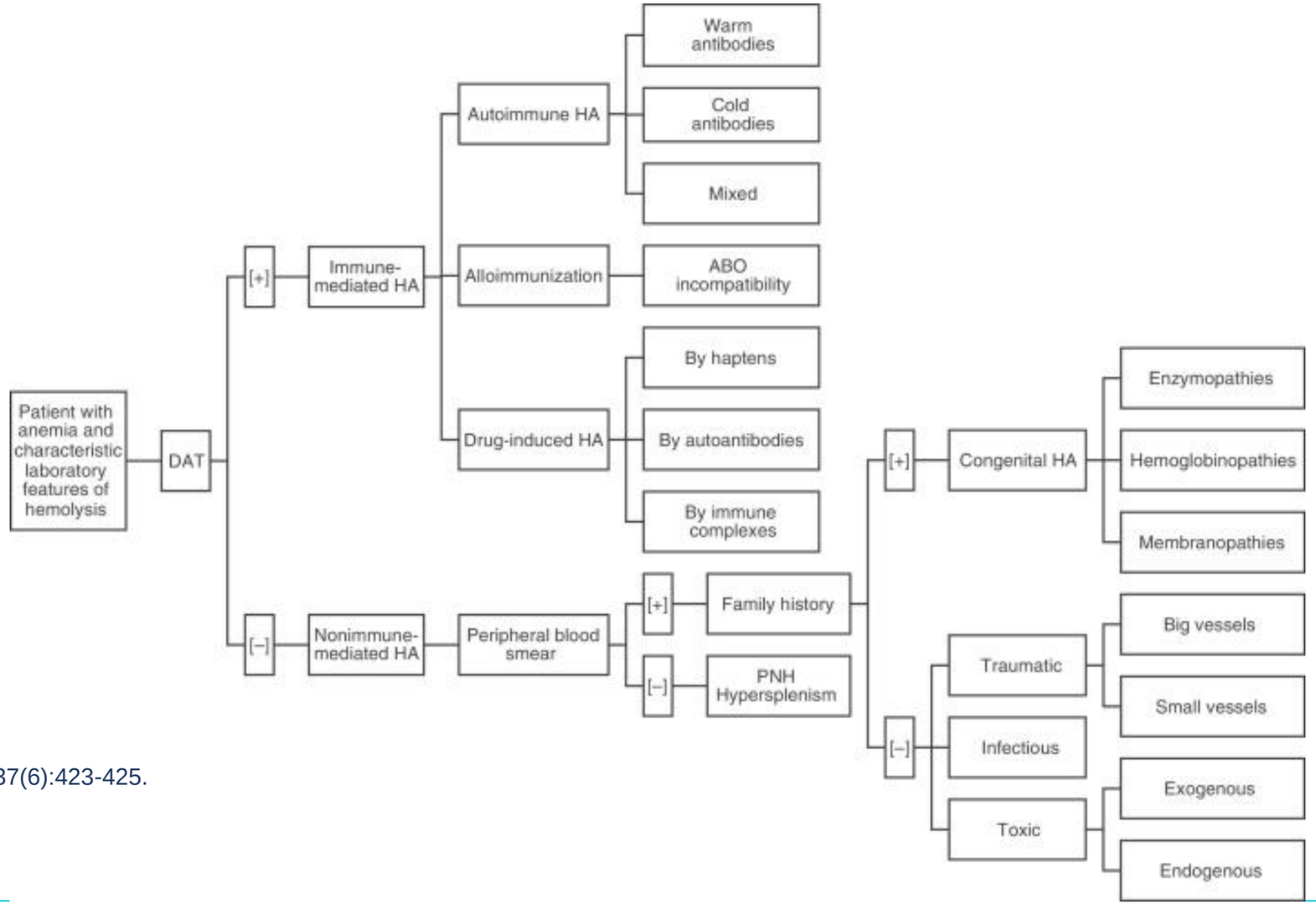
- **Ineffective Erythropoeisis**
  - Individuals with ineffective erythropoesis (thalassemia) have increased eryropoetic stimulation
  - EPO-> erythoblasts, which produce hormone erythroferronethat down-regulates hepcidin production -> more iron absorption
  - Higher risk for iron loading in pancreas and heart

- **Transfusion-related**
  - One unit of pRBCs is approx 250 mg of iron
  - One unit of pRBCs per month will provide 3-4 g/year
  - After a year, expect ferritin to rise ~1000 ng/mL

Monitor with T2* MRI noncontrast

Hemolytic Anemias

Check Coombs (DAT)



Ruiz EF, et al. Rev Bras Hematol. 2015;37(6):423-425.

Autoimmune Hemolytic Anemia

- Acquired hemolytic conditions with production of abnormal antibodies reacting against RBC antigens
- **Positive hemolytic markers** (increase in reticulocytes, LDH, indirect bilirubin, with low haptoglobin)
- **Direct antiglobulin test**: detects immunoglobulins and complement bound to red blood cells (“direct Coombs’ test”)
    - IgG alone: warm AIHA (typically with spherocytes in peripheral blood smear);
    - Complement (C3) and/or IgM: cold agglutinin disease, agglutination on p smear
    - IgG and C3: mixed AIHA
    - 10% of AIHA is Coombs negative

Warm Autoimmune Hemolytic Anemia

Management

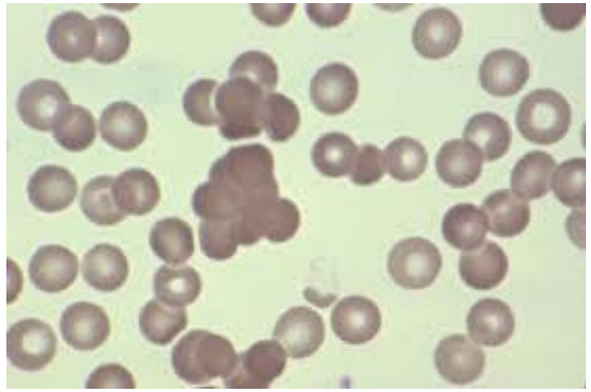
- **Transfusions**: if severe anemia (Hb<6), instability; beware of history of alloimmunization
- **First line of therapy is glucocorticosteroids** (e.g. prednisone 1-2mg/kg/day with taper after 2-3 weeks if response)
- Second line therapy:
  - **Rituximab** (may be used as first line)
  - Splenectomy (often third line)
  - Other immunosuppressants
    - MMF, cyclophosphamide, azathioprine, cyclosporine, sirolimus

Cold Agglutinin Disease

15-20% of AIHA

- Cold-induced symptoms
  - Acrocyanosis
  - Livedo reticularis / skin ulcers
  - Raynaud's phenomenon
  - Dysphagia or pain upon ingesting cold food

- Extravascular hemolytic anemia
  - May be precipitated by cold or infection
  - Spurious macrocytosis
  - *In vitro* agglutination
  - Draw samples warm



- Venous thromboembolism

Cold Agglutinin Disease

Diagnosis

- Evidence of hemolysis
- DAT positive for complement (C3d)
- Cold agglutinin titer 1:64 or higher at 4^{o}C
    - IgM with specificity anti-I (often linked to *Mycoplasma pneumoniae*) or anti-i (often linked to mononucleosis/EBV)

- Classification:
- **Primary CAD**: typically associated with a monoclonal IgM kappa not meeting criteria for a lymphoproliferative disorder (MGUS)
- **Secondary Cold Agglutinin Syndrome (= Secondary)**: infections, autoimmune disorder, or lymphoid malignancy

Cold Autoimmune Hemolytic Anemia

Management

- **Cold avoidance, warm clothing**
- **Folic acid**
- **Transfusions**: avoid cooling down patient's sample for crossmatch; use of blood warmers
- **Plasmapheresis (remove IgM)** can be used as temporizing measures in severe cases
- For secondary CAD, treatment of the underlying disorder is appropriate
- For primary CAD:
  - Consider first line with rituximab (50% ORR) or combo regimen (e.g. rituximab + bendamustine 70% ORR); bortezomib (ORR 30%).
  - **Anti-C1 complement therapies – sutimlimab SQ q2 weeks**
    - Not expected to improve clinical manifestations caused by agglutination
    - Rapid response in hemolytic parameters

Berensten S et al. Rituximab for primary cold agglutnin disease. Blood 2004.
Roth A et al. Sutimlimab in patients with cold agglutinin disease: results of phase 3 CADENZA. Blood.2022.

Drug-induced Hemolytic Anemia

- **Mechanisms:**
  - DAT-positive:
    - IgG alone: Hapten formation-drug adsorption: penicillin, piperacillin, oxaliplatin
    - IgG +/- C3: Autoantibody: alpha-methyldopa, diclofenac
    - C3 alone: Ternary-immune complex formation: 3rd gen cephalosporins, diclofenac
  - **Oxidative hemolysis:** primaquine, dapsone, phenazopyridine – worse if associated with G6PD deficiency
  - **Methemoglobinemia:** anesthetics, nitrites
  - Drug-induced thrombotic **microangiopathy:** quinine, Bactrim, oxaliplatin, gemcitabine, mitomycin, bevacizumab, sunitinib, proteasome inhibitors, quetiapine, cyclosporine, tacrolimus, sirolimus
  - Other mechanisms: ribavirin, artesunate (for malaria), interferon alpha

Paroxysmal Nocturnal Hemoglobinuria (PNH)

- Acquired clonal disorder with *PIGA* gene mutation → loss of GPI-anchored proteins → susceptibility to complement destruction

1. **Classical PNH**
    - **Pancytopenia**
    - **Non-immune hemolytic anemia**
        - Fatigue, jaundice, hemoglobinuria
        - Smooth muscle dystonia: dysphagia, erectile dysfunction
    - **Hemostasis activation:** venous thromboembolic events in unusual vessel beds
        - Abdominal VTE (Budd-Chiari syndrome portal vein thrombus))
        - Upper extremity
        - Venous sinus thrombosis

2. **PNH clone in the context of another hematologic disorders** (aplastic anemia, MDS, PMF)

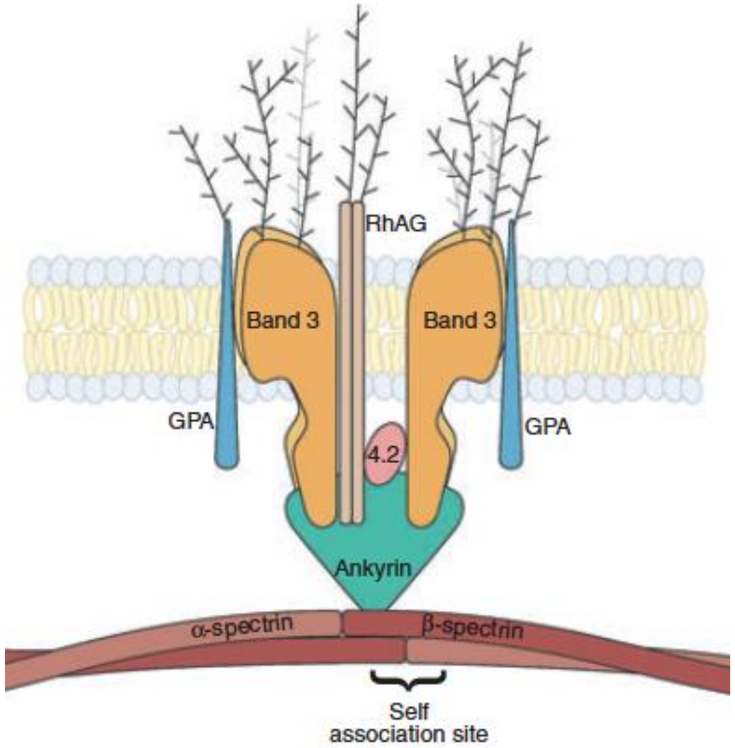
Paroxysmal Nocturnal Hemoglobinuria

- Diagnosis: **Peripheral blood flow cytometry**
  - lack of at least 2 GPI-anchored proteins in at least 2 different lineages

- Treatment:
  - **Support** for anemia: folic acid, transfusion, iron supplementation if iron deficient due to hemoglobinuria
  - Symptomatic disease: C5 **complement inhibitors** eculizumab or ravulizumab, C3i pegcetacoplan
    - prophylaxis for meningococcal infections
  - **Allogeneic hematopoietic cell transplant** for AA/MDS, refractory disease, or severe disease without access to anti-complement therapy
  - December 2023: **Iptacopan po** (Factor B inhibitor)

Red Cell Membranopathies

- **Hereditary spherocytosis** is the most common inherited hemolytic anemia due to membrane defects (1/3,000, all racial groups)
- AD in 75%; mutation in ankyrin, spectrin, band 3, protein 4.2 (VERTICAL linkages); may occur *de novo;*
- Family history of **gallstone and/or splenectomy**;
- Clinical features: hemolysis with **high MCHC**; negative DAT; may have hypersplenism
- Diagnosis:
  - osmotic fragility test with right shift of the curve; reduced fluorescence with **eosin-5'-maleimide (flow cytometry)**
  - Treatment: splenectomy is very effective



Na & Mohandas, Br J Haematol 2008;141(3):367-375op

Red Cell Membranopathies

1. **Hereditary elliptocytosis (HE):**
   - AD, more common in malaria endemic regions
   - Alpha spectrin (65%), beta spectrin or protein 4.1R mutations (HORIZONTAL linkages)
   - Hereditary pyropoikilocytosis – homozygous or compound heterozygous spectrin mutations causing severe HE (*pyros*, “fire”- thermal instability)

2. Southeast Asian Ovalocytosis (SAO): mild or no hemolysis with ovalocytes causes by unique 27bp deletion in band 3

3. Hereditary stomatocytoses: AD defects in volume control
   1. xerocytosis (compensated hemolysis, macrocytosis, <10% stomatocytes)
   2. overhydrated stomatocytosis (frank stomatocytosis with hemolytic anemia)

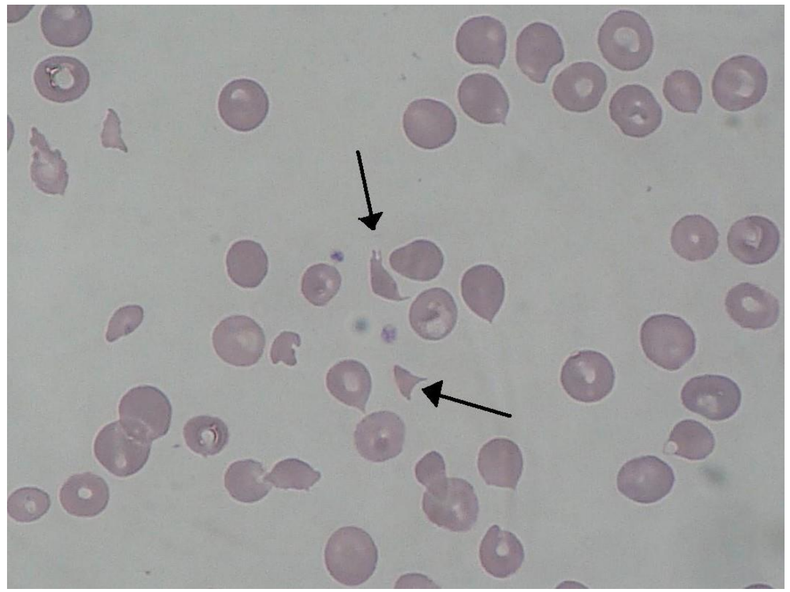
Red Cell Enzymopathies

- **Glucose-6-phosphate dehydrogenase (G6PD) deficiency**
  - Recessive X-linked inheritance
  - Variable phenotype: mostly episodic hemolytic crises; may present as chronic non-spherocytic hemolytic anemia
  - Diagnosis: Heinz bodies during hemolysis; low G6PD activity outside of hemolytic episode (false normal G6PD with reticulocytosis)
  - Triggers: infections, medications (sulfa, dapsone, primaquine)

- **Pyruvate kinase deficiency (PKD)**
  - Most common defect of the glycolytic pathway; AR
  - Chronic non-spherocytic anemia with variable severity
  - **Mitapivat** FDA approved therapy
  - May develop spontaneous iron overload

Fragmentation Hemolysis

1. Thrombotic microangiopathy: TTP, HUS
2. Systemic conditions:
   - DIC
   - Pre-eclampsia / HELLP syndrome
   - Malignancy
   - Scleroderma renal crisis
   - Malignant hypertension
   - Antiphospholipid syndrome
   - Trauma, Burns
3. Localized hemolysis:
   - Vascular Malformations
   - TIPS
   - Mechanical Valves
   - March hemoglobinuria



Fred Hutch
Cancer Center

Thank you – please fill out evaluation below.

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Q1. A 31 yo woman G1P0 at 29 weeks gestation is referred for evaluation of anemia. At obstetrician visit hemoglobin 9, ferritin 10, iron saturation 10%. Which of the following is the most appropriate therapy?

A. Administration of EPO stimulating agents
B. Administration of iron sucrose
C. Administration of oral ferrous sulfate
D. Treatment with packed red blood cells

Q2. A 19 yo man is evaluate for anemia after a recent urinary tract infection treated with trimethoprim/sulfamethoxazole. Laboratory evaluation showed Hgb 6.9 g/dL, retic 11%, negative Coombs, bilirubin 6. Which of the following is most likely cause of this patient's anemia?

A. PK deficiency
B. Hereditary spherocytosis
C. G6PD deficiency
D. B12 deficiency