Seattle Cancer Care Alliance

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Iron metabolism disorders and hemolytic anemias

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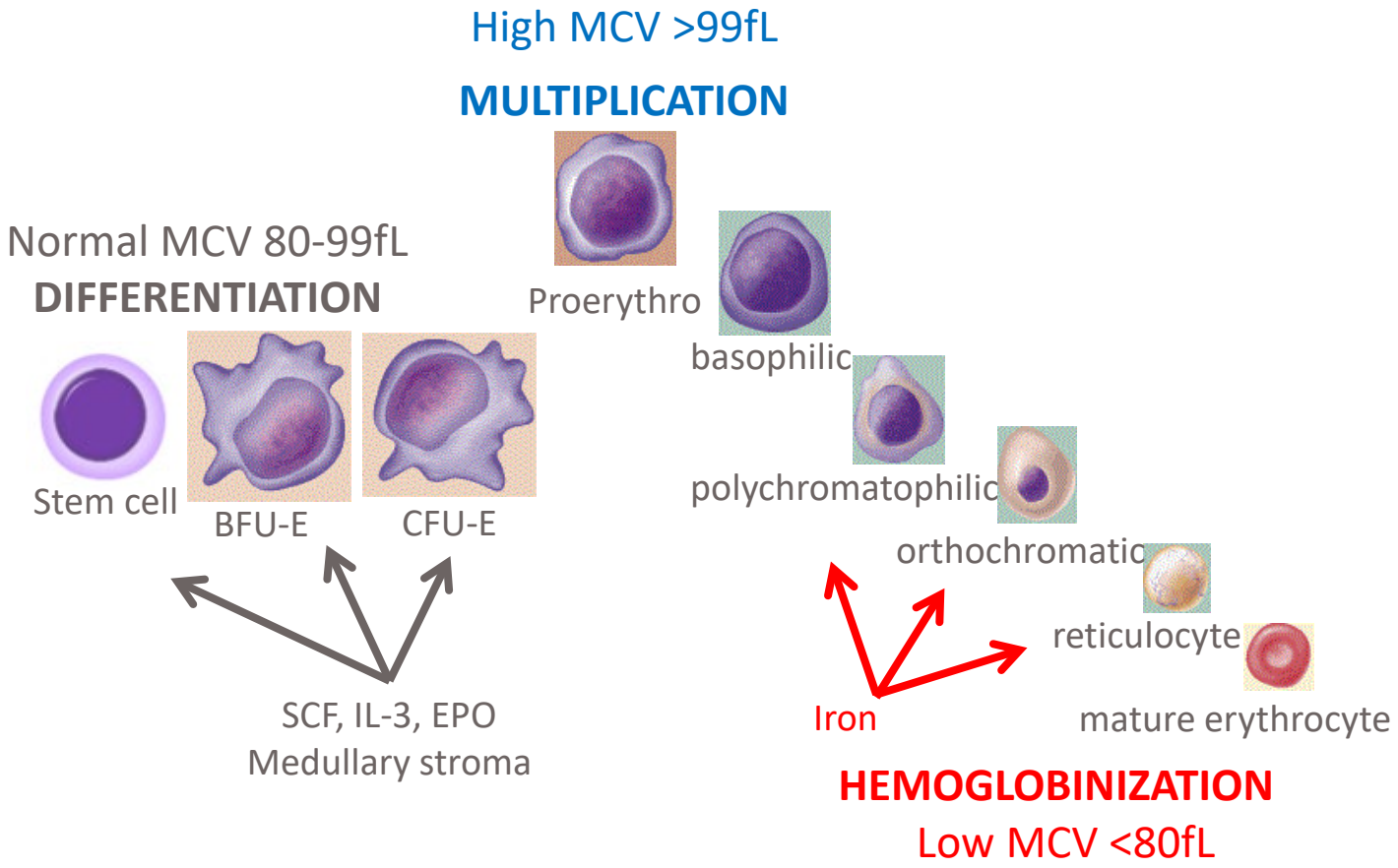
ABIM Hematology exam blueprint

		Diagnosis	Testing	Treatment/ Care Decisions	Risk Assessment/ Prognosis/ Epidemiology	Pathophysiology/ Basic Science
Red blood cell production disorders *(4% of exam)*						
Nutritional deficiencies		✓	✓	✓	/	/
Anemia of chronic inflammation		✓	✓	✓	/	✓
Red blood cell destruction disorders *continued...*						
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	/*	/*	✗*	✗*	✗*

ABIM Hematology exam blueprint

	Diagnosis	Testing	Treatment/ Care Decisions	Risk Assessment/ Prognosis/ Epidemiology	Pathophysiology/ Basic Science
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	⊘	⊘	⊘	⊘	⊘
Hemochromatosis	✓	✓	✓	⊘	⊘

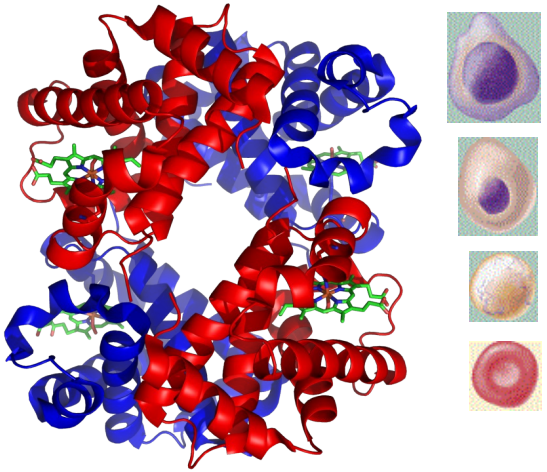
MCV signals what stage of erythropoiesis is affected



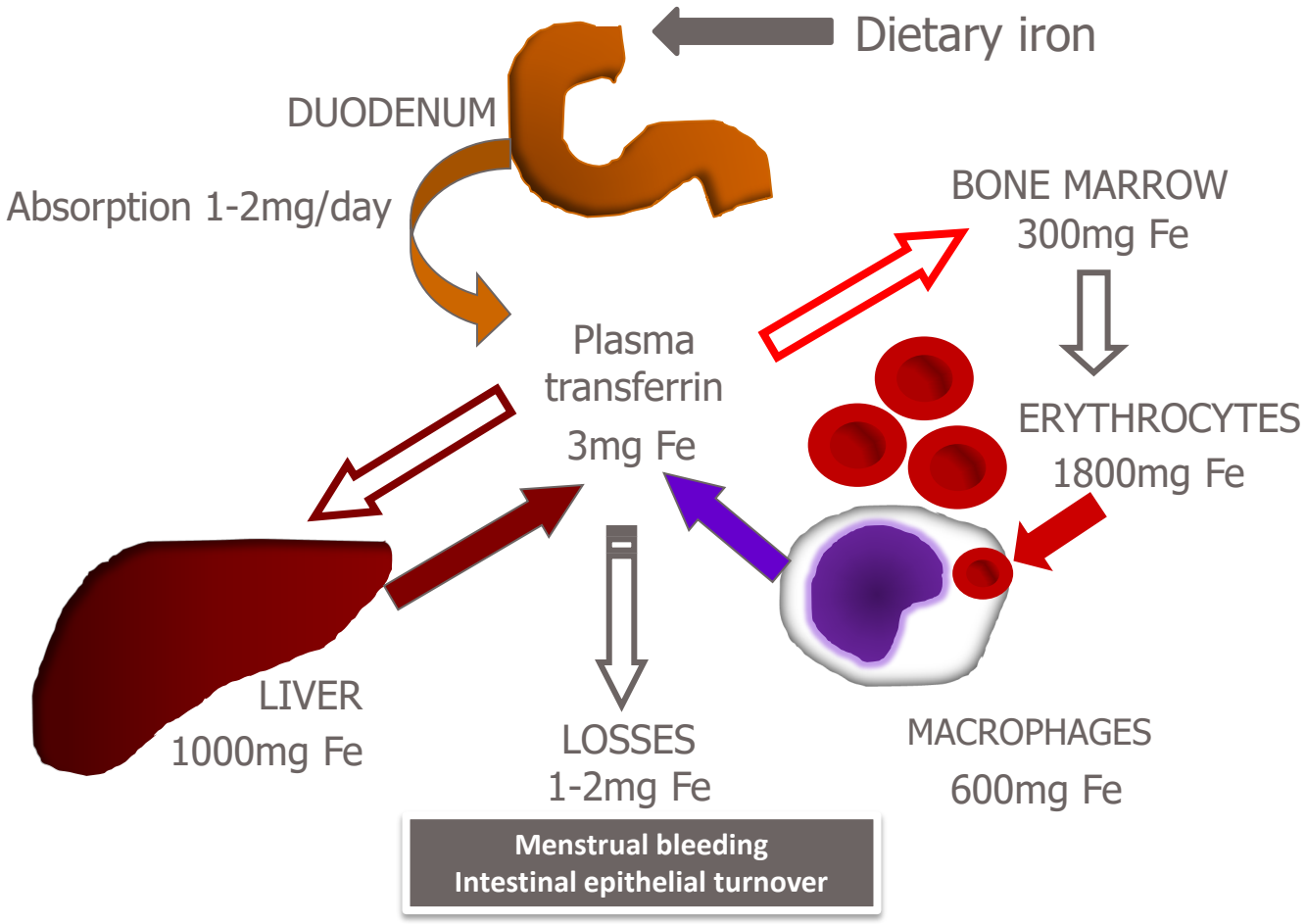
Anemias of the hemoglobinization stage (microcytic, MCV<80fL)

Lack of a component of hemoglobin

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



Physiology of iron metabolism



Common “iron studies”

Test	Common reference ranges
Serum ferritin (mcg/L)	20-200 (female) 30-300 (male)
Serum iron (mcg/dL)	60-180
Total iron binding capacity (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)

Diagnosis of iron deficiency anemia

- **Anemia = low RBC production:** low Hb, Hct, and RBCs
  - Beta thal trait has normal or elevated RBCs
- **Hypoproliferative: normal or low** retics
  - Reticulocytosis (>100k): think acute bleeding or hemolysis!
- Biochemical evidence of **iron deficiency**
  - **Ferritin <30mcg/L (WHO 2020)**
    - Ferritin < 70mcg/L in inflammatory states (see later)
  - Low serum iron with high TIBC = low transferrin saturation <20% (typically <16%)
- **Work up for causes: blood loss or low iron intake/absorption**

Causes of iron deficiency

- Always investigate **bleeding** (GI, Gyn, epistaxis, hematuria)
- **Malabsorption**
  - Surgical (gastric bypass, resections…)
  - Inflammatory bowel disease
  - Parasites (hookworm)
  - Atrophic gastritis
  - Prolonged use of medications (e.g. **PPI**)
- Vegetarian/vegan diets DO NOT cause iron deficiency on their own!

→ Treat/control the underlying cause!

Treatment of iron deficiency anemia - 1

Goals of iron supplementation:

1. First phase: **Normalize CBC** (4-6 months)
   - Hb>12g/dL women, Hb>13g/dL men **AND**
   - Normal MCV (>80fL) **AND** MCH (>28pg)
2. Second phase: **Normalize iron stores** (3-4 more extra months)
   - Ferritin >30ug/L **AND**
   - Transferrin saturation > 20%

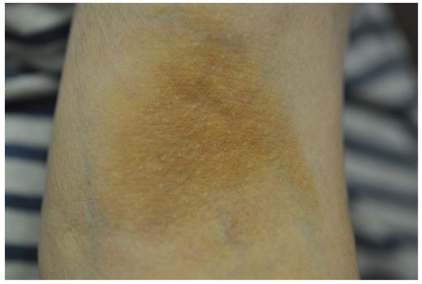
Oral iron: several salts - ferrous sulfate, fumarate, gluconate

- **First line of therapy**
- **Single dose, alternate days** (100-150mg elemental iron, e.g. ferrous sulfate 325mg 2 tab qod)
- Side effects: GI symptoms (>50%), dark stools

Treatment of iron deficiency anemia - 2

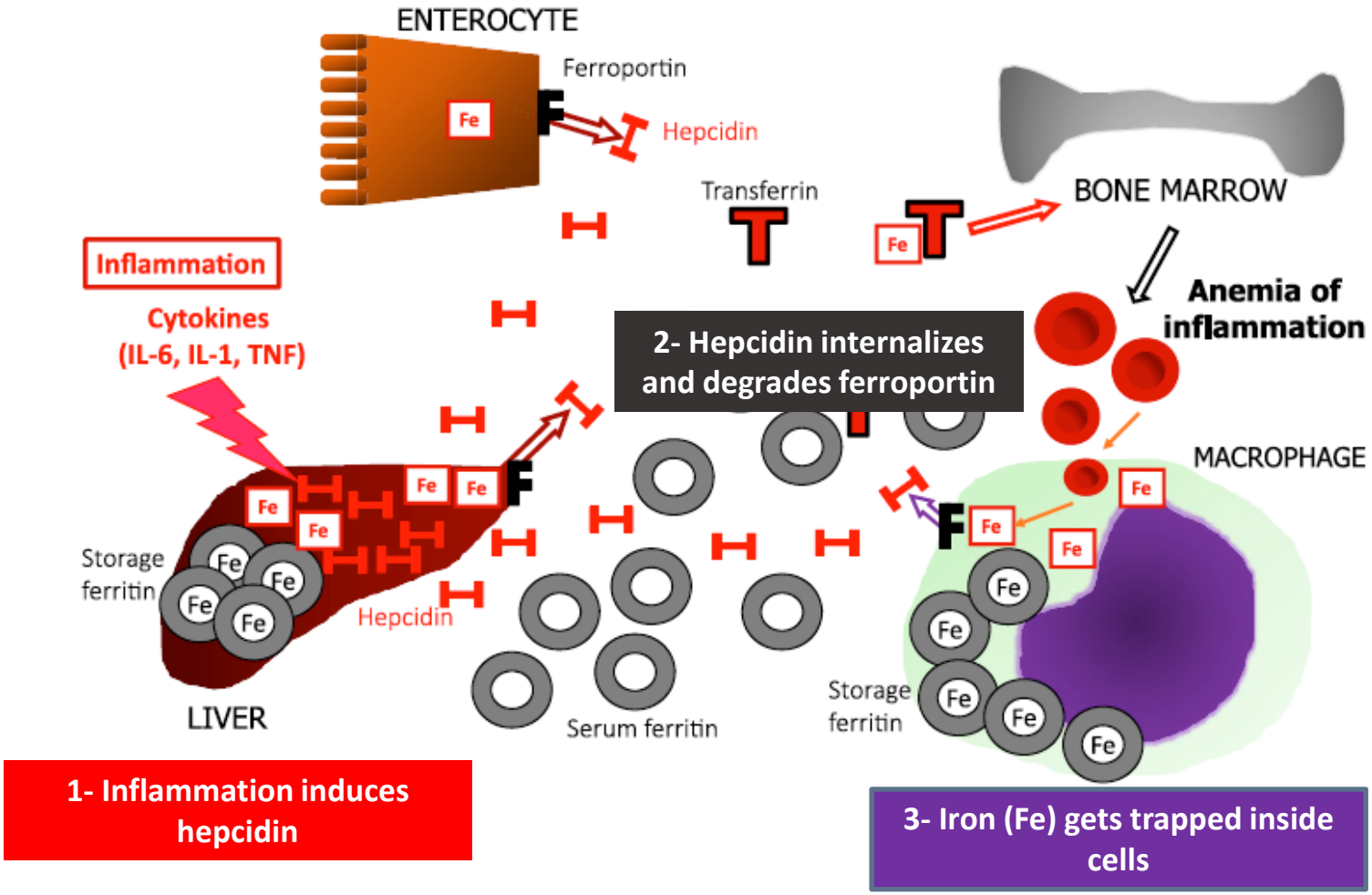
Intravenous iron

- Formulations: Iron sucrose, low molecular weight iron dextran, iron gluconate, ferric carboxymaltose, ferumoxytol, iron isomaltoside
- Consider if :
  - Intolerance/failure to oral iron
  - Malabsorption (e.g gastric bypass, IBD)
  - CKD
- Side effects:
  - **Anaphylaxis**: RARE these days, mostly associated with HIGH-molecular weight dextran (discontinued);
  - **Skin hyperpigmentation**
  - **Hypophosphatemia** (usually asymptomatic, more frequent in ferric carboxymaltose, rarely in isomaltoside)



Gan & Orringer, Dermatol Surg 2015

Pathophysiology of anemia of inflammation



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

Diagnosis of anemia of inflammation

- History of **underlying chronic disease**:
  - Inflammatory: RA, SLE, IBD, Castleman's disease
  - Infections: Tb, osteomyelitis, endocarditis
  - Malignancy: lymphoma and other hematologic
  - Other chronic conditions: CHF, COPD

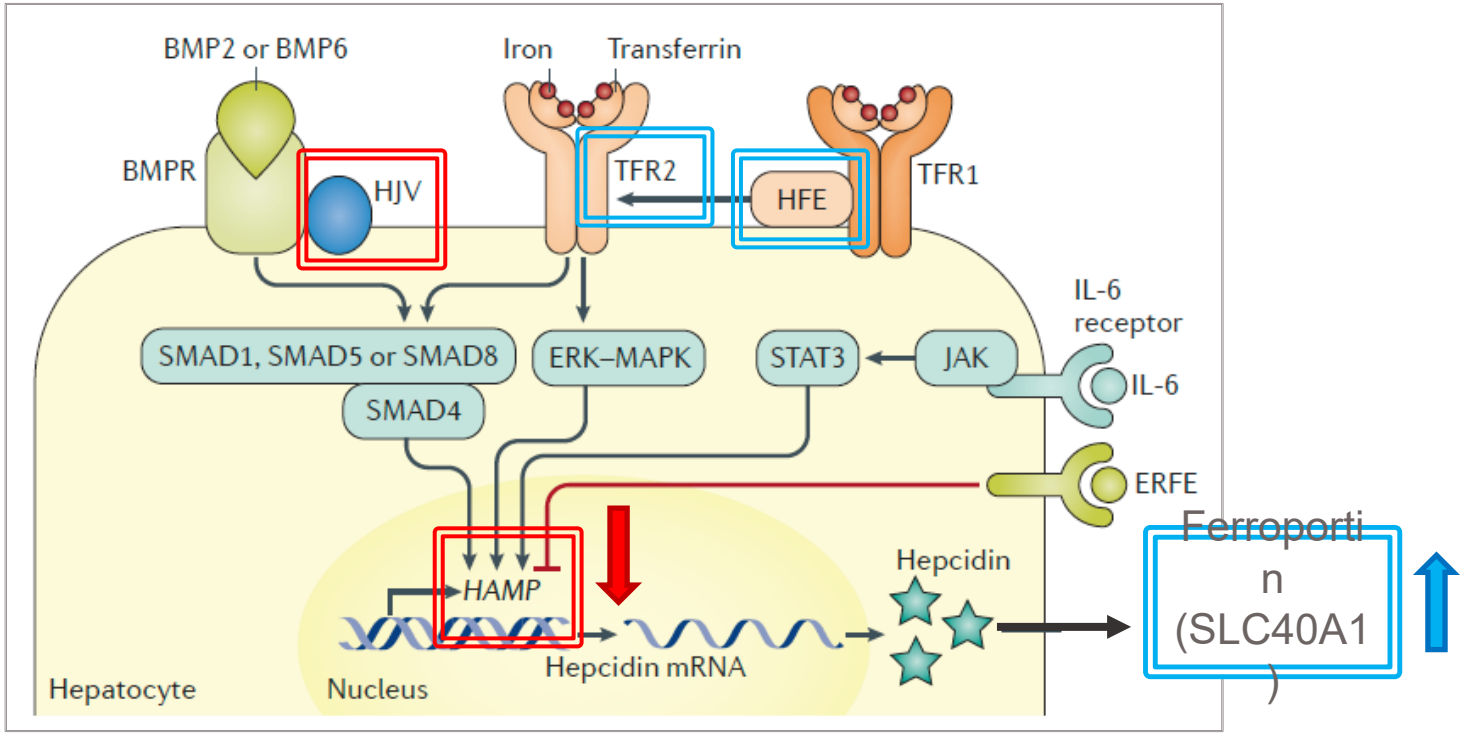
- Lab findings:
  - Mild to moderate **hypoproliferative N/N anemia** (occasionally microcytic)
  - Low serum iron with low TSAT <20%
  - Normal to increased serum ferritin (typically >100 mcg/L)
  - May have elevated CRP>5 mg/L but not required
  - Investigational: hepcidin levels

Management of 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 chemotherapy for malignancy
- **Transfusions**: only if symptomatic, life-threatening anemia
- Investigational: hepcidin blockers

Pathophysiology of Hereditary Hemochromatoses

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



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

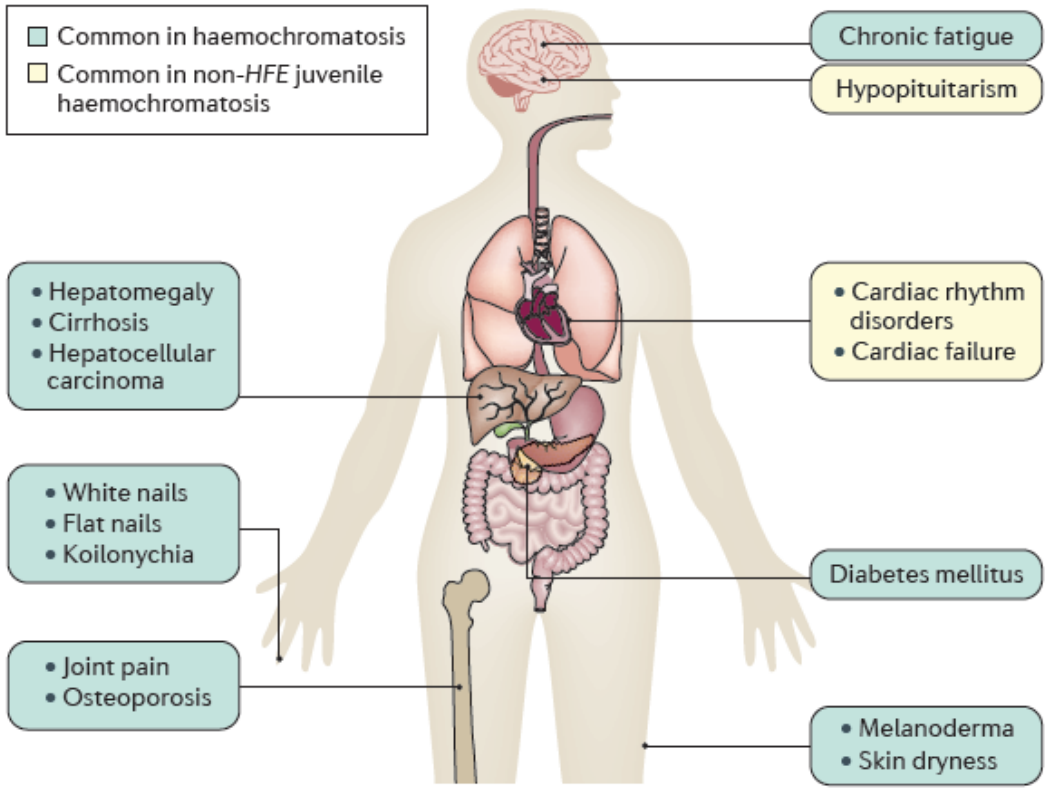
Hemochromatosis – Clinical features

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



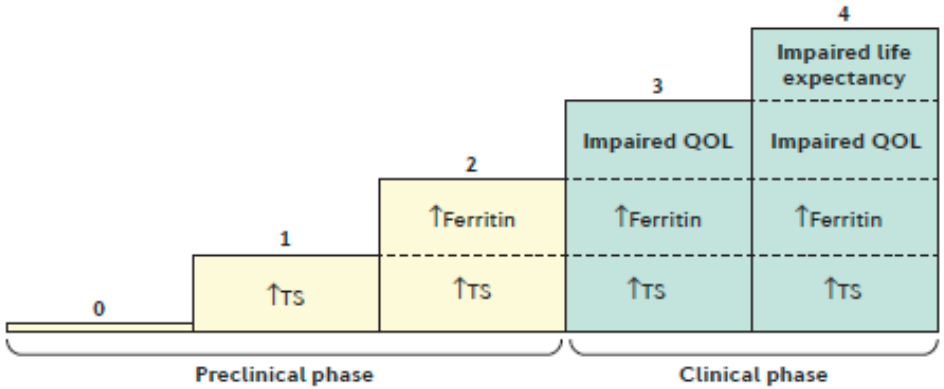
Brissot et al., Nat Rev Dis Primers 2018

Hemochromatosis - Diagnosis

Labs:

No anemia

high ferritin AND TSAT>45%



- Northern European ascent: 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;
  - Tea, coffee consumption and use of PPI can decrease absorption
  - No need to follow iron-poor diet
  - Avoid vitamin C supplements
- **Phlebotomy** – GOAL: ferritin 50-100mcg/L *Bacon et al; Hepatology*. 2011;54(1):328–343
  - 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

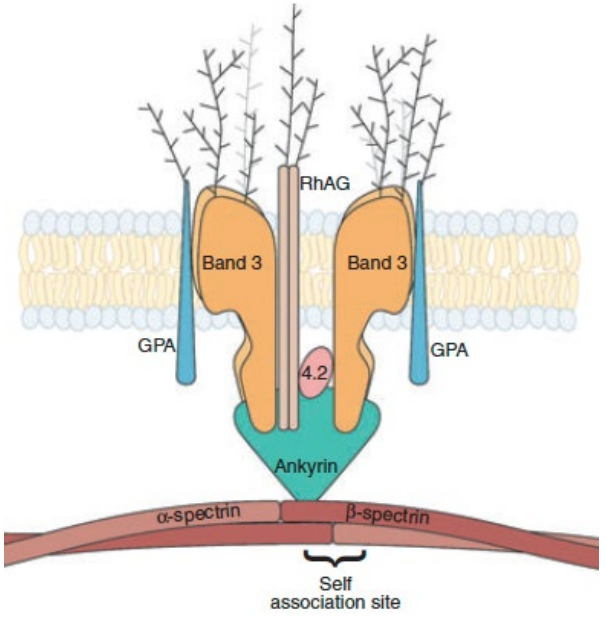
Hemolytic anemias

CAUSES

1. **Ma**laria (and other infections- *Clostridium, Babesia, Bartonella*, endocarditis, Gram-positive cocci, *Salmonella typhi*)
2. **Me**dications (drug-induced or oxidative)
3. **Mi**croangiopathic or fragmentation hemolysis
4. **Mo**therhood (think antibodies: hemolytic disease of the newborn; transfusion reactions; don't forget **autoimmune**)
5. **Mu**tations
   a) Acquired mutation → PIG-A: paroxysmal nocturnal hemoglobinuria
   b) Congenital (think COMPONENTS OF A RED CELL):
      - Hemoglobin (other lecture)
      - Membrane: HS, elliptocytosis, stomatocystosis, xerocytosis...
      - Enzyme: G6PDD, PKD

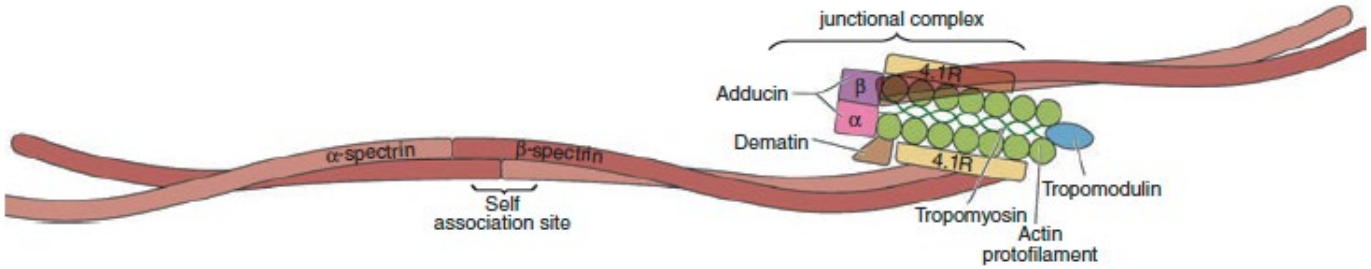
Red cell membranopathies - HS

- **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

Other red cell membranopathies



Brissot et al., Nat Rev Dis Primers 2018

1. **Hereditary elliptocytosis**
   - 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: 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 (dapsone, primaquine)

- **Pyruvate kinase deficiency (PKD)**
  - Most common defect of the glycolytic pathway; AR
  - Chronic non-spherocytic anemia with variable severity
  - Macrocytosis and extreme reticulocytosis (>50%) postsplenectomy
  - May develop spontaneous iron overload

Autoimmune hemolytic anemias

- Acquired hemolytic conditions with production of abnormal antibodies reacting against red cell epitopes
- **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, paroxysmal cold hemoglobinuria
    - IgG and C3: mixed AIHA

Warm autoimmune hemolytic anemia - management

- **Transfusions**: if severe anemia (Hb<6), instability; beware of history of alloimmunization; failure to respond may indicate IVIg.

- **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 – may be preferred in children/young adults with ALPS

Cold agglutinin disease – clinical features

- 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 infections)
  - Spurious macrocytosis
  - *In vitro* agglutination

- Venous thromboembolism

Cold agglutinin disease – diagnosis

- Evidence of hemolysis
- DAT positive for complement (C3d)
- Cold agglutinin titer 1:64 or higher at 4°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 CAD**: infections, autoimmune disorder, or lymphoid malignancy

Cold agglutinin disease – treatment

- **Cold avoidance**
- **Transfusions**: avoid cooling down patient's sample for crossmatch; use of blood warmers
- **Plasmapheresis and IVIg** 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 containing regimen (e.g. rituximab + bendamustine); may associated with fludarabine, prednisone, interferon, or monotherapy;
  - Alternative regimen: bortezomib.
  - Investigational: anti-complement therapies (sutimlimab)

Drug-induced hemolysis

- **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)

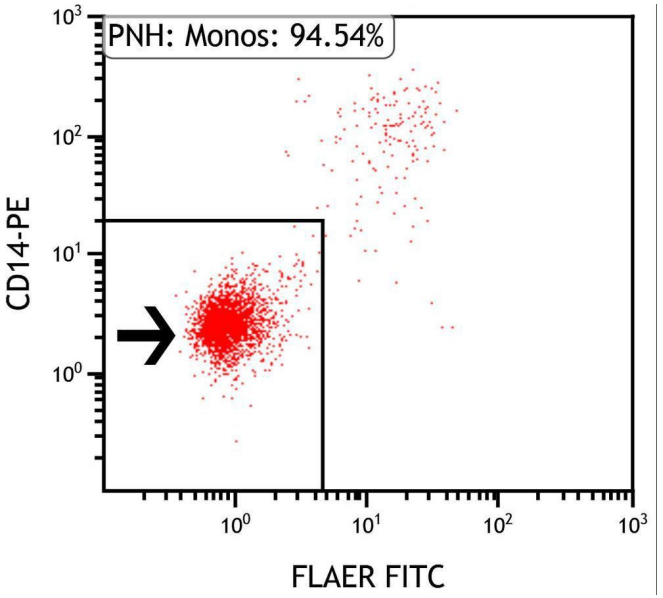
- Acquired clonal disorder with *PIGA* gene mutation → loss of GPI-anchored proteins → susceptibility to complement destruction

1. **Classical PNH**
   - **P**ancytopenia
   - **N**on-autoimmune hemolytic anemia
     - Fatigue, jaundice, hemoglobinuria
     - Smooth muscle dystonia: dysphagia, erectile dysfunction
   - **H**emostasis activation: venous thromboembolic events in unusual vessel beds
     - Abdominal VTE (Budd-Chiari syndrome)
     - Upper extremity
     - Venous sinuses

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

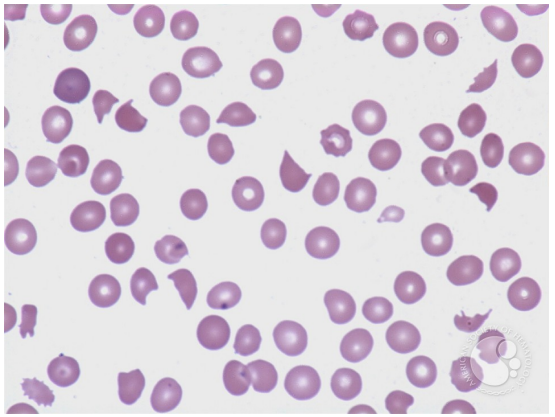
Paroxysmal nocturnal hemoglobinuria

- 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: **complement inhibitors** eculizumab or ravulizumab
    - prophylaxis for meningococcal infections
  - **Allogeneic hematopoietic cell transplant** for AA/MDS, refractory disease, or severe disease without access to anti-complement therapy



Fragmentation hemolysis

1. Thrombotic microangiopathy: TTP, HUS
2. Systemic conditions:
   - DIC
   - Pre-eclampsia / HELLP syndrome
   - Malignancy
   - Scleroderma renal crisis
   - Malignant hypertension
   - Antiphospholipid syndrome
3. Localized hemolysis:
   - Hemangioendothelioma (Kasabach-Merritt syndrome)
   - TIPS
   - Malfunctioning cardiac valve or assist device
   - March hemoglobinuria (includes extreme running, bongo drumming)



Educational resources

- American Society of Hematology Self-Assessment Program 7th Ed. (ASH SAP)
- ASH Pocket Guides (download from App store)
- Hematology/Oncology question bank http://hemeoncquestions.com/
- Hematology-Oncology board review questions www.turner-white.com/brm/bonco.htm

THANK YOU