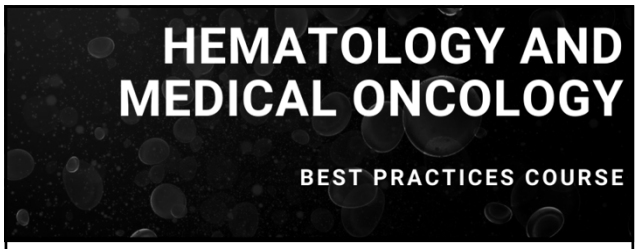

2 - Iron Deficiency and Iron Overload

Victor R. Gordeuk, MD

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Disclosures

Disclosures of Financial Relationships with Relevant Commercial Interests

- Grant support - CSL Behring, Global Blood Therapeutics, Imara, Ironwood, Novartis
- Consulting- CSL Behring, Global Blood Therapeutics, Novartis, Forma

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Outline

1. **Review of iron metabolism**
2. **Iron deficiency**
3. **Iron overload: hereditary, environmental, transfusional**

3

Iron

- **Essential nutrient for all living organisms.**
  - **Reversible binding of O_2: Hb, myoglobin**
  - **Enzyme systems:**
    - **heme (cytochromes, catalase, glutathione peroxidase, NO synthase)**
    - **non-heme (RNR, aconitase)**
  - **Immunity: free radicals to destroy microbes**
- **Highly reactive with O_2; can cause toxicity**

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Iron Metabolism: Broad Themes

- **Deficiency of iron**
  - **most common nutritional problem world wide**
- **Iron overload**
  - **less common**
  - **important health problem**
- **Absorption of iron**
  - **highly regulated to prevent excess Fe from being absorbed**
- **Excretion of iron**
  - **There is no physiologic pathway for excreting excess iron**

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Iron Requirements

	Men	Women
Obligatory losses	**1.0 mg/d**	**1.0 mg/d**
Menstruation	**0.0 mg/d**	**0.5 mg/d**
Total losses	**1.0 mg/d**	**1.5 mg/d**
Iron absorbed	**1.0 mg/d**	**1.5 mg/d**

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Body Iron Compartments

	65 kg F	75 kg M
Functional compounds		
Hemoglobin	1900 mg	2500 mg
Myoglobin	310 mg	340 mg
Enzymes	170 mg	190 mg
Transferrin	2.7 mg	3.2 mg
Storage compounds		
Ferritin & hemosiderin	300 mg	800 mg
Total	**~2700 mg**	**~3800 mg**

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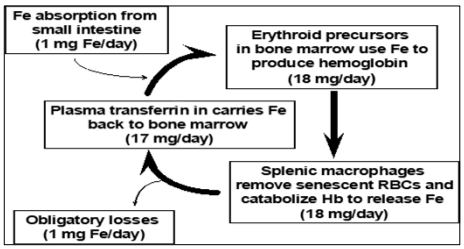
Simplified Diagram of Iron Movement in the Body



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Dietary Iron

- Typical diet men
  - Mean of 18 mg/day
  - SD range of 4 - 30 mg/day
- Typical diet women
  - Mean of 13 mg/day
  - SD range of 7 - 19 mg/day
- ~1/3 of Fe from fortification of flour

What We Eat in America, NHANES 2007-2008

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Iron absorption- proximal small bowel

Hepcidin- 25 aa peptide produced by liver that suppresses iron absorption

Enhanced absorption
- Low hepcidin:
  - Iron deficiency
  - ↑ erythropoiesis
- Dietary factors:
  - ascorbic acid (Fe^{+2} valance absorbed)
  - Heme vs non-heme Fe

Inhibited absorption
- High hepcidin
  - ↑ iron stores
  - Inflammation
- Dietary factors:
  - tannins (tea)
  - phytates (bran)

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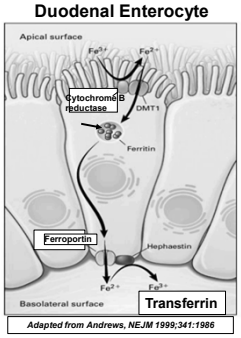
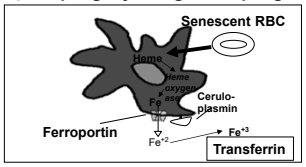
Duodenal Enterocyte



Adapted from Andrews, NEJM 1999;341:1986

Iron Transport into Plasma

Erythro-phagocytosing Macrophage



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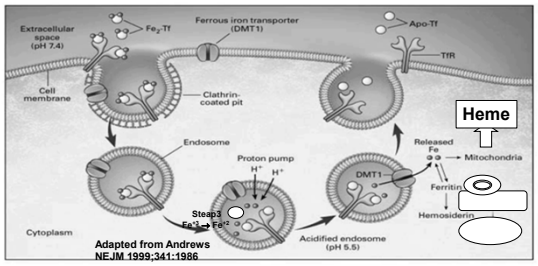
Iron Entry into Erythroid Precursors



Adapted from Andrews NEJM 1999;341:1986

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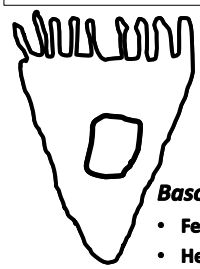
Molecules of Fe Metabolism

Duodenal Enterocyte: Fe Absorption



Brush Border

- **DMT-1 transports Fe^{+2} from lumen into enterocyte**
- **Cytochrome b ferri-reductase maintains Fe^{+2} valence**

Basolateral surface

- **Ferroportin exports Fe^{+2} to portal plasma**
- **Hephaestin converts Fe^{+2} released by fptn to Fe^{+3} for binding by Tf**

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Molecules of Fe Metabolism:

Iron Transport in Plasma

Transferrin

- **Dimer with two binding sites for Fe**
- **Normally ¼ to ⅓ of binding sites occupied by iron**
- **Delivers Fe released by macrophages and enterocytes to normoblasts in bone marrow**

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Molecules of Fe Metabolism:

Erythroid Progenitor: Hb production

Membrane

- **TfR1-mediated endocytosis of Tf-Fe**

Endosome

- **Acidification releases Fe from Tf**
- **Steap3 converts Fe^{+3} to Fe^{+2}**
- **DMT-1 exports Fe^{+2} to cytosol**

Cytosol

- **Ferritin stores excess Fe**

Mitochondrion

- **Mitoferrin-1 imports Fe^{+2}**
- **Ferrochelatase inserts Fe^{+2} into protoporphyrin to form heme**

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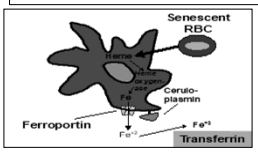
Molecules of Fe Metabolism:

Macrophage: phagocytosis; Fe processing



Cytosol

- **Heme oxygenase liberates Fe from heme**
- **Ferritin stores Fe for short-term**
- **Hemosiderin stores Fe for long-term**

Membrane

- **Ferroportin exports Fe^{+2} to plasma**
- **Ceruloplasmin- plasma and GPI-anchored ferrioxidase; converts Fe^{+2} (released by fpn) to Fe^{+3} (for binding by Tf)**

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Molecules of Fe Metabolism:

Hepatocyte: Systemic Iron Regulation

Hepcidin

- stimulated by ↑ Fe and inflammation
- suppressed by ↑ erythropoiesis
- binds ferroportin; ↓ Fe export (enterocyte, macrophage)

Up-regulation of hepcidin

- HFE, TfR2, Hemojuvelin, BMP6, BMPR, SMAD4

Down-regulation of hepcidin

- TMPRSS6 (matriptase-2)
- Erythroblast-derived: erythroferrone, GDF-15, GDF-11

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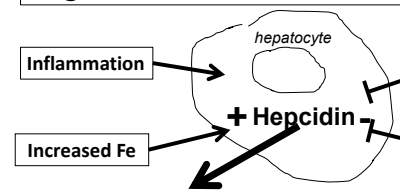
Regulation of Iron Metabolism



1. **Binds to ferroportin on enterocytes, macrophages**
2. **Hepcidin-ferroportin internalized and ferroportin degraded**

→

- **↓ Fe absorbed by enterocytes**
- **↓ Fe released by macrophages**
- **↑ Fe stored in macrophages**

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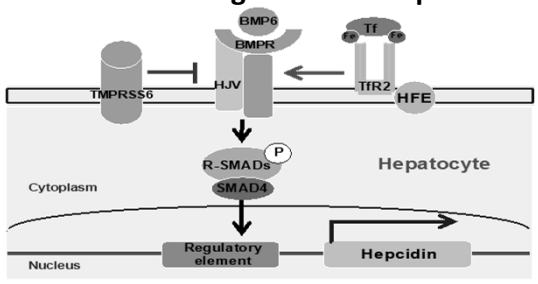
Iron-Mediated Regulation of Hepcidin



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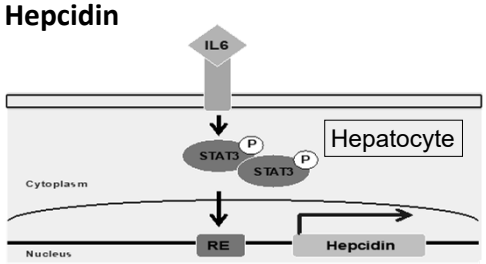
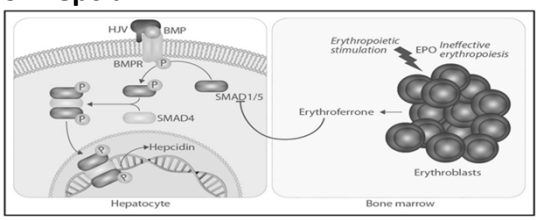
Inflammation-Mediated Regulation of Hepcidin



20

Erythropoiesis-Mediated Regulation of Hepcidin



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Indirect Indicators of Fe Status

- Daily clinical use
  - Serum iron concentration
  - Serum transferrin conc. or TIBC
  - Transferrin saturation [(Fe/TIBC) X 100]
  - Serum ferritin concentration
- Others
  - Serum transferrin receptor conc.
  - RBC protoporphyrin concentration
  - MRI of liver

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Direct Indicators of Fe Stores

- Bone marrow aspirate
  - Prussian blue or Perls stain
- Liver biopsy
  - Prussian blue stain
  - Chemical iron concentration
- Magnetic susceptibility measurement of liver

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Changes in Indirect Indicators of Fe Status

↑ Serum Fe & Tf Sat	↑ Serum Ferritin
• Iron overload • Hepatocellular damage • BM suppression • Recent ingestion of Fe preparation	• Iron overload • Hepatocellular damage • Inflammation • Hyperferritinemia/cataract syndrome – autosomal dominant – ↑ftn but no Fe-loading – mutation IRE L-ferritin mRNA

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Changes in Indirect Indicators of Fe Status

↓ Serum Fe & Tf Sat	↓ Serum Ferritin
• Iron deficiency • Inflammation • Diurnal variation – blood drawn afternoon or evening	• Iron deficiency

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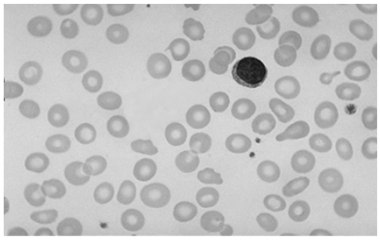
Fe deficiency anemia

- microcytosis
- central pallor



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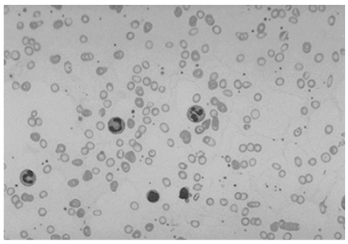
Fe deficiency anemia

- Marked microcytosis
- Marked central pallor



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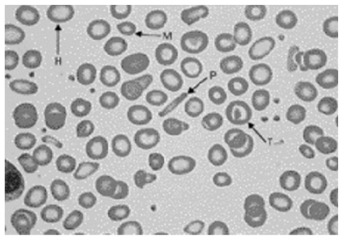
Fe deficiency anemia:

H- hypochromia
P- pencil RBC
T- targeting;
M- microcytosis
Lancet 2000;355:1260



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Fe Deficiency: Causes

1. Chronic blood loss
   - GI bleeding: ulcer, tumor, cancer, diverticuli, etc.
   - Heavy menses, menorrahgia
   - Frequent blood donation; hemodialysis
   - Hook worm infestation
2. Increased physiologic requirements
   - Women (menstruation, pregnancy, lactation),
   - Infants & adolescents (growth spurt)

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Fe Deficiency: Causes

3. Decreased absorption
   - Celiac disease (gluten enteropathy)
   - Autoimmune (atrophic) gastritis; *H. pylori*
   - Inflammatory bowel disease
   - Gastrectomy- loss of gastric acid
   - Duodenal bypass surgery

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Fe Deficiency: Causes

4. Hemoglobinuria (intravascular hemolysis)

- PNH
- Runners
- Damaged heart valves
- Microangiopathic hemolysis

5. Sequestration

- pulmonary alveolar bleeding in idiopathic pulmonary hemoiderosis or Goodpasture's syndrome

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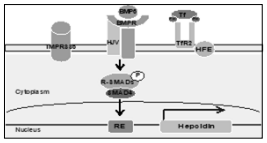
Fe Deficiency: Causes

6. Genetic predisposition

- *TMPRSS6* mutations (Iron-refractory IDA- excessive production of hepcidin)



TMPRSS6 down-regulates hepcidin

- Inactivating mutations lead to ↑ hepcidin

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Iron-Refractory Iron Deficiency Anemia

- Severe autosomal recessive anemia
- Lack of response to oral Fe
- Partial response to IV Fe
- Several *TMPRSS6* mutations implicated

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Fe Deficiency: Clinical Manifestations

- Impaired psychomotor development
- Fatigue, irritability, ↓ work productivity
- Pica
- Koilonychiae, glossitis, angular stomatitis
- Dysphagia 2° to esophageal web (Plummer-Vinson or Patterson-Kelly Sx)
- Increased risk of thrombosis

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Fe Deficiency: Lab Findings

1. Peripheral blood cells

- ↑ RDW, platelets
- ↓ MCV, MCH, MCHC, RBC, Hb, Hct
- Retics not increased
- ↑ RBC protoporphyrin

2. Serum tests

- ↓ Fe , Tf Sat, ferritin (< 12 µg/L)
- ↑TIBC, transferrin, transferrin receptor

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Fe Deficiency: Lab Findings

3. Bone marrow aspirate

- absent macrophage Fe; ↓ sideroblasts
- erythroid hyperplasia

4. Other tests if blood loss not found

- autoimmune gastritis: ↑ gastrin, antiparietal cell ab +
- gluten enteropathy: anti-endomyoseal ab +
- *H. pylori*: ab +, urease breath test +

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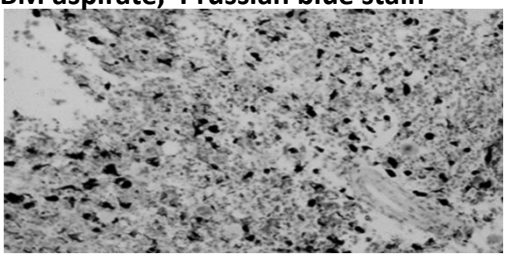
BM aspirate, Prussian blue stain



↑ Fe stores (sideroblastic anemia)

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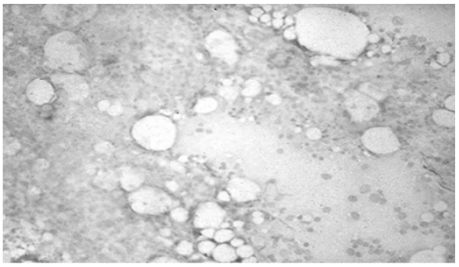
BM aspirate, Prussian blue stain



Absent Fe stores (Fe deficiency)

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Fe Deficiency: Therapy

1. Oral
   - Ferrous sulfate: standard approach
     - 200 mg elemental Fe/d (3- 325mg tabs/d)
     - 5.0 mg elemental Fe/kg per day in children
     - risk of iron poisoning
   - Once a day or alternate day dosing may be preferable

Lancet Haematol 2017; 4:524-33

Iron absorption from oral iron supplements given on consecutive versus alternate days and as single morning doses versus twice-daily split dosing in iron-depleted women: two open-label, randomised controlled trials

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Fe Deficiency: Therapy

2. Intravenous
   - Iron dextran (INFeD)
   - Ferric gluconate complex in sucrose (FERRLECIT)
   - Iron sucrose (VENOFER)
   - Ferumoxytol- Fe oxide (FERAHEME)
   - Ferric carboxymaltose (INJECTAFER)

- Risk of hypersensitivity rxns: premedicate with steroid and antihistamine in patients at risk
- Used successfully and safely during pregnancy

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Other conditions with microcytosis (approximate Hb and MCV values)

	Hb	MCV
Anemia of inflammation	10	83
Thalassemia minor	12	68
Thalassemia major	2-7	48-72
Hb H disease	9	70
Hb E trait	14	73
Hb CC	10	74
Hb SC	11	78
Hereditary sideroblastic anemia	6	77

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Ftn cutoffs of absent BM Fe in anemia associated with inflammation

Ftn (ng/ml)	Sensitivity	Specificity	Positive predictive value
<100	88-100%	46-64%	50%
<60	82-86%	84-88%	84%
<50	71-82%	84-97%	88%
<40	71%	98%	92%
<12	24%	100%	100%

Punonnen et al, Blood 1997;89:1052
Kis and Carnes, J Gen Int Med 1998;13:455

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What is Iron Overload?

- **Excessive amounts of storage iron**
  - **parenchymal cells liver, heart, pancreas (HH)**
  - **macrophages spleen, BM, liver (Txs)**
  - **mixed pattern**
- **Quantitative considerations**
  - **mild: 2-4 g; moderate: > 4-10 g**
  - **marked: 10-20 g; severe: >20 g**

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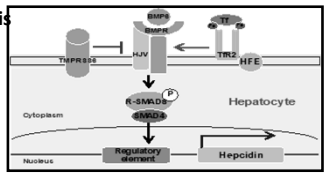
Iron Overload: Classification

1. **Hereditary conditions causing ↓ hepcidin**
   - ***HFE* hemochromatosis**
   - ***TFR2* hemochromatosis**
   - **Hemojuvelin hemochromatosis**
   - **Hepcidin hemochromatosis**



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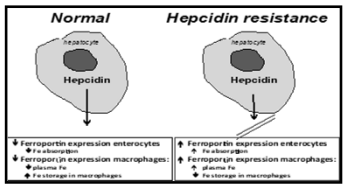
Iron Overload: Classification

2. **Hereditary conditions causing resistance of ferroportin to hepcidin**
   - **Ferroportin disease**



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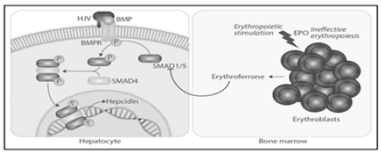
Iron Overload: Classification

3. **Ineffective erythropoiesis: erythroblast-derived erythroferrone suppresses hepcidin:**
   - **β-thal major & intermedia; Hb E/β-thal; Hb H**
   - **congenital dyserythropoietic and sideroblastic anemias**



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Iron Overload: Classification

4. **Multiple blood transfusions**
   - **Diamond Blackfan anemia**
   - **aplastic anemia**
   - **thalassemia major**
   - **sickle cell anemia**
   - **myelodysplasia**

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Iron Overload: Classification

5. **Other hereditary conditions**
   - **Aceruloplasminemia**
   - **Atransferrinemia**
   - **African dietary iron overload**

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Fe Overload: Clinical Manifestations

1. Fatigue, abd. pain
2. Liver
   - ↑ ALT
   - fibrosis and cirrhosis
   - Hepatoma
3. Heart
   - CHF (restrictive or dilated)
   - arrhythmias
4. Endocrine
   - diabetes mellitus
   - secondary amenorrhea
   - Impotence
5. Hyperpigmentation
6. Certain infections
7. Arthritis
   - esp. 1st and 2nd MP joints (HFE hemochromatosis)

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Screening for hemochromatosis or increased iron stores

1. Screen pts
   - with family hx or compatible clinical picture
   - without inflamm., infection, trauma, surgery
2. Serum ferritin conc.
   - ↑ raises possibility (esp. if Tf Sat ↑ or upper nl)
   - Ferritin >1000 ug/L esp. of concern
3. PCR for *HFE* mutations, esp. Caucasians

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Diagnosis of hemochromatosis or increased iron stores

1. Liver biopsy
   - Histology
   - Perl's stain
   - Chemical iron concentration
2. MRI measurement hepatic or cardiac iron
3. SQUID magnetic measurement hepatic iron
4. Quantitative phlebotomy

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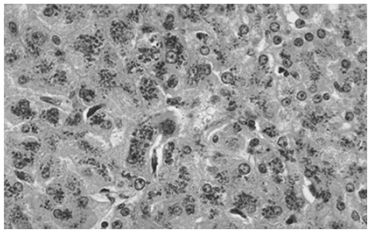
Liver Bx, *HFE* hemochromatosis (H&E):



Golden brown hemosiderin pigment predominantly in hepatocytes

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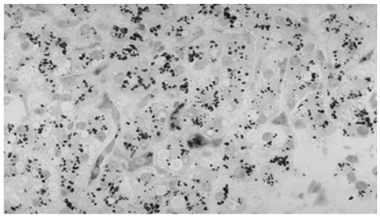
Liver Bx, *HFE* hemochromatosis Prussian blue stain:



Granules predominantly in hepatocytes

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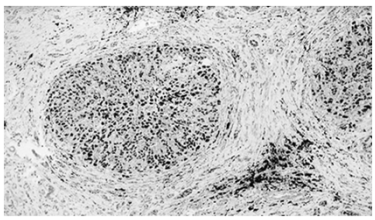
Liver Bx, *HFE* hemochromatosis Prussian blue stain:



Cirrhosis; hepatocytes in regenerating nodule heavily laden with iron

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HFE Hemochromatosis

1. Classic triad
   - Hepatomegaly
   - Diabetes
   - Hyperpigmentation
2. Classically death from cirrhosis, HCC or CHF
3. Goal now- make dx and rx in presymptomatic stage

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HFE Genetic Defect

1. C282Y mutation, *HFE* gene, 6p21.3
   - Autosomal recessive
   - Predominantly in N. Europeans: "Celtic Disease"
   - high prevalence in pop.
     - homozygotes: 3-5/1000
     - heterozygotes: 10-15%
2. *HFE* protein deficiency
   - ↓ hepcidin production
     - ↑ Fe absorption
     - ↑ serum Fe and transferrin saturation
     - ↓ storage of Fe in macrophages
     - Fe-loading of parenchymal cells

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HFE Genetic Defect

3. Incomplete C282Y/C282Y penetrance
   - Elevated SF
     - <50% F homozygotes
     - ≈75% M homozgotes
   - Organ damage <25%
   - Modifying factors
     - genetic
     - environmental
4. Second mutation *H63D*
   - Very little iron-loading tendency
   - ↑ Fe stores may rarely develop in
     - *H63D/H63D*
     - *C282Y/H63D*
     - *C282Y heterozygote*

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HFE C282Y in 99,711 1º Care Pts (HEIRS)

	N	C282Y/ C282Y	No. per 100,000
Whites	44,082	0.44%	440
African Americans	27,124	0.014%	14
Asians	12,772	0.0%	0
Hispanics	12,459	0.027%	27

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Management of *HFE* hemochromatosis

1. Nl life expectancy if phlebotomy Rx started before cirrhosis or diabetes
2. If ferritin <1000 µg/L and LFTs nl at dx
   - Proceed to phlebotomy therapy
3. If ferritin >1000 and/or LFTs abnl
   - Consider liver biopsy
   - ↑ risk HCC in cirrhotics even with phleb. Rx
4. Screen first degree family members

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Phlebotomy Therapy

1. <u>Initial program to remove excess body Fe</u>
   - Remove 1-2 units of blood weekly as long as Hct ≥ 35% (M) or 32% (F).
   - Continue until mild iron deficit: Pt. does not tolerate wkly phleb.; MCV declines; ftn < 50 ug/L

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Phlebotomy Therapy

2. Quantitative phlebotomy

- Maintain weekly schedule and tally ml of whole blood removed until Fe deficiency develops.
- Calculate Fe stores at beginning of program (1 mg Fe/2 ml whole blood); >4g Fe- "Fe overload"

3. Maintenance to prevent the Fe reacummulation

- Remove 1 unit each 2-6 mos. to maintain serum ftn around 50 µg/L.

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Other Forms of Genetic Hemochromatosis

- *TFR2* hemochromatosis
  - autosomal recessive; intermediate phenotype
  - TfR2 mutation 7q22
- Juvenile hemochromatosis
  - autosomal recessive; severe phenotype
  - hemojuvelin mutation 1q21.1
  - *HAMP* (hepcidin) mutation 19q13.1

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Other Forms of Genetic Hemochromatosis

- Ferroportin disease
  - autosomal dominant mutations ferroportin 2q32
  - two phenotypes
    - reduced response to hepcidin: predominant parenchymal iron-loading
    - increased sensitivity to hepcidin: predominant macrophage iron-loading

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African Iron Overload

1. High dietary Fe in traditional beverage
2. Familial pattern
   - ? "gene by environment interaction"
   - *HFE* mutations not present
   - Ferroportin Q24H in some iron-loaded subjects
3. Fe-loading macrophages and hepatocytes
5. Clinical associations
   - hepatic fibrosis, cirrhosis, hepatoma
   - diabetes mellitus, cardiomyopathy
   - osteoporosis, scurvy
   - infections
   - esophageal carcinoma
6. Management
   - prevention
   - phlebotomy therapy

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Iron Overload in African Americans

- Condition present; may be under-dxed
- Sometimes related to African-specific ferroportin Q248H mutation
- Screen with serum ferritin
- Responds well to phlebotomy therapy
- Evaluate for hepatitis C- may play etiologic role by suppressing hepcidin

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Transfusional Iron Overload Quantitative Considerations

- One unit blood contains about 225 mg Fe as Hb
- Hepatic Fe concentration
  - normal <30 µmol/g dry wt. 0 units
  - worrisome 180 µmol/g dry wt. 50 units
  - Toxic 360 µmol/g dry wt. 100 units
  - highly toxic 720 µmol/g dry wt. 200 units
- At 2 units/month: 50 units in two yrs

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Transfusional Iron Overload Treatment

1. Institute iron chelation when
   - >20 units of blood transfused, or
   - hepatic iron conc. > 180 µmol/g dry wt.
2. Desferrioxamine, original parenteral chelator
   - 40-50 mg/kg per day
   - s.c. infusion over 8-12 hours
   - 5 days/week

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Transfusional Iron Overload Treatment

3. Deferasirox
   - Single oral dose per day; similar potency to DFO
   - Exjade: powder; starting dose 20 mg/kg/day; advance to 30 or 40 mg/kg/day if needed
   - Jadenu: tablet; doses slightly lower
   - Monitor CBC, creatinine, LFTs, gastritis sx's q2-4wks

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Transfusional Iron Overload Treatment

4. Deferiprone
   - Oral chelator
   - Risk of agranulocytosis
   - Approved in US for use in thalassemia patients with transfusional iron overload who did not respond to other chelators

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Monitoring Iron Chelation Rx

1. CBC; renal and liver function tests; GI Sxs monthly
2. Audiometry and ophthalmologic yearly
2. Serum ferritin every three months
3. Avoid use of phenothiazines
4. Vitamin C 100 mg daily may enhance Fe excretion; do not give to heavily iron-loaded subjects
5. Modify dose as body Fe burden ↓'s

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Other Iron Overload Conditions

1. Congenital atransferrinemia (3q22.1)
   - parenchymal Fe-loading; anemia
2. Congenital aceruloplasminemia (3q23-q25)
   - Fe-loading paremchyma, macrophages, brain
   - extrapyramidal sxs, cerebellar ataxia, DM

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Other Iron Overload Conditions

4. Neuroferritinopathy
   - autosomal dominant, late-onset
   - abnl aggregates ferritin & Fe in basal ganglia
   - Mut. ftn light gene (19q13.3-13.4); ser. ftn low
5. Hallovorden-Spatz disease
   - autosomal recessive; onset in childhood
   - iron deposits in the basal ganglia
   - mutation in pantothenate kinase 2 gene (20p13)

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