Iron—Too Much, Too Little, Too Late

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Sr. Associate Dean
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Case 1.

A 30 year old woman with menorrhagia is referred for persistent iron deficiency anemia despite reported compliance with oral ferrous sulfate 325 mg TID. Her current ferritin is 6 ng/mL. Her Hgb is 7.2 g/dL.
Iron Critical Functions

• Hemoglobin
• Myoglobin
• Electron transport
• Immune function
Dietary Iron Sources

• Heme iron—red meats
• Non-heme iron:
  – Iron fortified cereals  18 mg/cup
  – Soybeans  8.8 mg/cup
  – Lentils  6.6 mg/cup
  – Tofu  6.8 mg/cup
  – Spinach 6.4 mg/cup
Popeye Paradox

• 1870 Dr. von Wolf published a work claiming spinach had 10X iron of other vegetables
• 1929 Popeye appears in *Thimble*
• 1933 Popeye spun off
• 1937 corrected iron content published
• Most iron in spinach is not absorbed due to oxalate
Iron Balance

- Average total body iron content 35-45 mg/kg
- Most iron present as hemoglobin
- 1 cc of RBCs = 1 mg iron
- 1-2 mg iron required daily to compensate for losses
- 15-25 mg iron consumed daily (5-10% absorbed)
Adapted from Brittenham GM. Hematology: Basic Principles and Practice. Saunders, 6th ed., 2013
Which of the following is the major regulator of iron balance?

A. Ferroportin
B. HFE
C. Transferrin receptor 2
D. Ferritin
E. Hepcidin
Hepcidin

• 25 amino acid protein made in hepatocyte
• THE MAJOR REGULATOR OF IRON ABSORPTION FROM THE GUT
Hepcidin control of iron entry into plasma

Adapted from Brittenham GM. Hematology: Basic Principles and Practice. Saunders, 6th ed., 2013
Figure 1 - Mechanism of hepcidin-mediated cellular iron regulation

Low hepcidin

- Iron uptake
- Iron-exporting cells (duodenal enterocytes, macrophages, hepatocytes)
- Ferritin
- Fpn
- Fe
- Iron release into plasma

High hepcidin

- Iron uptake
- Ferritin
- Fpn
- Fe
- Hepcidin

Fpn = ferroportina (adaptado de Ganz).
4 Regulators of Hepcidin Expression

• Iron Regulator—high TF sat leads to increased hepcidin
• Erythroid Regulator—Anemia leads to decreased hepcidin
• Inflammatory Regulator—IL-6 leads to increased hepcidin
• Hypoxia Regulator—hypoxia leads to decreased hepcidin
Adapted from Brittenham GM. Hematology: Basic Principles and Practice. Saunders, 6th ed., 2013
Cellular iron homeostasis

Iron-deficient cells

- IRP1/2
- Ferritin
- Ferroportin
- eALAS
- m-Aconitase
- HIF-2α

5’ Translational repression 3’

mRNA stabilization

5’ TfR1 DMT1 3’

Iron-replete cells

- IRP1
- 4Fe-4S
- IRP2

5’ Translational activation 3’

mRNA degradation

5’ RNase 3’

Adapted from Brittenham GM. Hematology: Basic Principles and Practice. Saunders, 6th ed., 2013
Back to the case
Treatment of iron deficiency

• Oral iron is the treatment of choice, if tolerated
• Start with iron sulfate, 325 mg (65 mg elemental iron) cheapest
• Side effects are mainly GI
  – Nausea, constipation, dark stools, diarrhea
• Enhancing absorption
  – Take with vitamin C
  – Take on an empty stomach (may not be possible)
  – Avoid agents that block absorption
    • Tea
    • Whole grains
    • Tetracycline
    • Antacids and PPIs
What is the best way to dose oral iron replacement?

• Iron supplements increase hepcidin
• Blood study
• Based on hepcidin kinetics, providing lower doses (40-80 mg Fe daily) and spacing to every other day may improve absorption
• Maybe 325 mg FeSO4 every other day is the best schedule!

Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women.

Moretti D¹, Goede JS², Zeder C¹, Jiskra M¹, Chatzinakou V¹, Tjalsma H³, Melse-Boonstra A⁴, Brittenham G⁵, Swinkels DW³, Zimmermann MB¹.
Case 2.

A 44 year old woman with Crohn colitis presents with severe iron deficiency anemia. Her hgb is 6.8 g/dL with an MCV of 56 fL. Her iron studies reveal a ferritin of 2 ng/mL. She reports intolerance to oral iron.
How to calculate iron deficit

mg iron deficit = 2.21 \[\text{desired hemoglobin-observed hemoglobin}\] \times \text{lean body weight in kg} + (0.26 \times \text{lean body wt}).

OR:

\[\text{Wt (lbs)} \times (\text{hgb desired} - \text{current hgb})\]

Add 600 mg for women and 1000 mg for men to replace stores
Parenteral Iron

• Should be the choice for patients intolerant of oral iron, or those with iron malabsorption.

• Four forms
  – Iron dextran (InFeD)
    • Can be given as total dose infusion
    • Risk of allergic reactions is up to 1%
    • Needs a test dose (0.5 ml = 25 mg)
  – Iron gluconate (Ferrlecit)
    • Maximum dose is 250 mg at one time—if reactions seen at this dose, decrease to 125 mg
  – Iron sucrose (Venofer)
    • Dose is 200 mg at a time
  – Ferumoxytol (ferraheme)
    • Each vial is 510 mg—may give up to 1020 mg over 15 minutes, then monitor checking vitals q 15 min x 60 min
    • Must have renal insufficiency
    • Only approved for outpatient use
Case 3

- 24 y.o. Caucasian woman with no PMHx presents with iron deficiency. She has heavy menses. Not on OCPs.
- Her Hgb is 9.3. MCV 72. Ferritin is 4
- She is started on iron sulfate 325 mg
- 3 mo later, hgb is 8.5. She swears she is taking her iron.
- What are the next steps?
Iron challenge test

• Check serum iron. Give 325 mg iron sulfate. Check serum iron 1 hour later. Should go up by 100.

• Pt’s iron level rose from 32 to 355. She does not have iron malabsorption. Admits to getting nausea with meds. Hasn’t been taking iron.
Case 4

• 45 y.o. man has had iron deficiency for 4 years. Hgb hovers around 10. He feels terrible.

• Colonoscopy has been negative. Ferritin is 10.

• Tissue transglutaminase IgA is positive

• Anti-endomysial antibody is positive

• EGD shows flattening of villi. Gluten free diet leads to remarkable improvement in sense of well-being.
Teaching points

• Iron malabsorption can occur at the level of the lips
• This can be proven with an iron absorption test
• Think of sprue
Case 4

- 55 yo man with neurofibromatosis presents for evaluation of iron deficiency anemia. Hgb is 12 g/dL. Ferritin is 10 ng/mL. MCV has fallen steadily for the past 5 years—now 10 fL.
- Colonoscopy and EGD are negative.
- What is going on?
- He is not a vegetarian.
Answers and teaching points

• He is a regular blood donor for the past 20 years.
• He only eats food out of vending machines—never eats vegetables—never eats meat.
• So a good dietary history is important.
• Not all blood loss is involuntary!!
Case 5

• A 32 y.o. woman is referred for iron deficiency anemia “refractory to iron”
• Hemoglobin is 10.8. Ferritin is 7.
• She is started on iron sulfate, 325 mg
• One month later, her hemoglobin is 12.2, ferritin is still 7
• One month later, her hemoglobin is 13.8. Ferritin is still 7.
• She is referred because her ferritin is “non-responsive to iron and she needs a bone marrow biopsy”
Case 5

• Huh?

• The response to iron repletion is
  – First, normalization of hemoglobin
  – Next, normalization of MCV
  – Only lastly does the ferritin normalize.

• And iron should be continued for 6 months after normalization of iron—this allows for repletion of stores
Case 6
A 45 year old man with a 20-year history of rheumatoid arthritis and HTN presents with a history of anemia of unknown cause. He is asymptomatic. There is no family history of blood diseases. His physical exam is significant for conjunctival pallor, a prominent S4 on cardiac exam and rheumatoid changes in his hands. His laboratory studies show:

<table>
<thead>
<tr>
<th>Test</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hgb</td>
<td>8.7 g/dl</td>
</tr>
<tr>
<td>HCT</td>
<td>26%</td>
</tr>
<tr>
<td>MCV</td>
<td>82 fL</td>
</tr>
<tr>
<td>Platelets</td>
<td>422,000/mm3</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.0 mg/dL</td>
</tr>
<tr>
<td>Fe</td>
<td>45 mcg/dL</td>
</tr>
<tr>
<td>TIBC</td>
<td>220 mcg/dL</td>
</tr>
<tr>
<td>Ferritin</td>
<td>565 ng/mL</td>
</tr>
</tbody>
</table>
What do you want to do next?

ALWAYS look at the peripheral smear
What is your diagnosis?

A. Anemia of chronic disease
B. Iron deficiency
C. Thalassemia trait
D. Hereditary spherocytosis
E. Sideroblastic anemia
Anemia of chronic disease

- Modulated by hepcidin
- Small peptide
- Increased in response to inflammation
- Causes internalization and proteolysis of membrane channel ferroportin
- No commercially available assay for hepcidin
hepcidin

Reduced iron absorption

increased iron accumulation

enterocytes

macrophages
### Table 1: Differentiation Between Anemia of Chronic Disease and Iron-Deficiency Anemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Anemia of Chronic Disease</th>
<th>Iron Deficiency Anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Iron</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Transferrin</td>
<td>Reduced to normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Transferrin saturation</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Normal to increased</td>
<td>Reduced</td>
</tr>
<tr>
<td>Soluble transferrin receptor</td>
<td>Normal</td>
<td>Increased</td>
</tr>
<tr>
<td>Ratio of soluble transferrin receptor to log ferritin</td>
<td>Low (&lt;1)</td>
<td>High (&gt;2)</td>
</tr>
<tr>
<td>Cytokine levels</td>
<td>Increased</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Case 7

A 78 year old man presents with unexplained elevation in his transaminases. His uncle and father both died of alcoholic hepatitis. He has developed glucose intolerance. His iron studies are significant for a ferritin of 775 ng/mL, iron sat of 55%.
Hereditary Hemochromatosis

• First described in mid 19th century
• Iron overload, bronzing of skin, endocrinopathies, arthropathies
• HFE mutations most commonly associated with hemochromatosis
• HFE described in 1996 but mechanisms not elucidated until more recently
• Original investigations focused on HFE interactions with gut
Skin “bronzing”
What are hepcidin levels in patients with hereditary hemochromatosis?

A. High
B. Low
C. Normal
D. Don’t know
HFE

- Class I-like membrane associated protein
- Associates with TFR1, TFR2, HJV, etc
- Mutated HFE most common cause of hereditary hemochromatosis
- AR
# HFE Genotypes

<table>
<thead>
<tr>
<th>GENOTYPE</th>
<th>Prevalence among hemochromatosis pts</th>
</tr>
</thead>
<tbody>
<tr>
<td>C282Y/C282Y</td>
<td>60-90%</td>
</tr>
<tr>
<td>C282Y/H63D</td>
<td>0-10%</td>
</tr>
<tr>
<td>C282Y/WT</td>
<td>rare</td>
</tr>
<tr>
<td>H63D/H63D</td>
<td>0 to 4%</td>
</tr>
<tr>
<td>H63D/WT</td>
<td>rare</td>
</tr>
<tr>
<td>WT/WT</td>
<td>15-30%</td>
</tr>
</tbody>
</table>
Other Types of Hemochromatosis

- Juvenile HJV
- TFR2
- FPN
Hemosiderosis

- Secondary iron overload
- Hemolysis, thalassemia, transfusion, etc
- May be related to erythroferrone
ALL Associated with LOW Hepcidin
Diagnosis of HH

• ~70% of adults C282Y homozygotes have elevated ferritin and only a fraction of those have symptomatic iron overload
• Family history
• Serum ferritin > 200 ng/ml women, 300 ng/ml men, TF sat >45%
• Liver biopsy or MRI R2 or R2* to confirm hepatic iron
FDA Approves Blood Donation for HH

Guidance for Industry: Variances for Blood Collection from Individuals with Hereditary Hemochromatosis

This guidance document represents FDA's current thinking on the distribution of blood and blood components from individuals with hereditary hemochromatosis without disease labeling, and collecting blood more frequently than every eight weeks without a physical examination on the day of donation. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statutes and regulations.
Treatment of Iron Overload

- Hemochromatosis—phlebotomy
- Hemosiderosis—chelation
- Historically used parenteral chelator deferoxamine
- Chelates in 1:1 ratio
Oral Iron Chelators

• Deferiprone (ferriprox®)—introduced in 1980’s
• Side effects: agranulocytosis (up to 5%), GI symptoms (33%), arthralgias and arthritis (30-40%),
Deferasirox (Exjade®)

• Well tolerated (GI symptoms most common)
• Well-studied

• COST: deferasirox > deferiprone > deferoxamine

• Can combine for added chelation
New Targets for Iron Overload
Ineffective Erythropoiesis

• In mice, thalassemia is associated with high levels of erythroferone (Erfe)
• Suppresses hepcidin
• ? Improving IE as a target
• Activin receptor agonists
Modified activin receptor IIB ligand trap mitigates ineffective erythropoiesis and disease complications in murine β-thalassemia.

Suragani RN¹, Cawley SM¹, Li R¹, Wallner S¹, Alexander MJ¹, Mulivor AW¹, Gardenghi S², Rivella S³, Grinberg AV¹, Pearsall RS¹, Kumar R¹.

Abstract

In β-thalassemia, unequal production of α- and β-globin chains in erythroid precursors causes apoptosis and inhibition of late-stage erythroid differentiation, leading to anemia, ineffective erythropoiesis (IE), and dysregulated iron homeostasis. Here we used a murine model of β-thalassemia intermedia (Hbb(th1/th1) mice) to investigate effects of a modified activin receptor type IIB (ActRIIB) ligand trap (RAP-536) that inhibits Smad2/3 signaling. In Hbb(th1/th1) mice, treatment with RAP-536 reduced overactivation of Smad2/3 in splenic erythroid precursors. In addition, treatment of Hbb(th1/th1) mice with RAP-536 reduced α-globin aggregates in peripheral red cells, decreased the elevated reactive oxygen species present in erythroid precursors and peripheral red cells, and alleviated anemia by promoting differentiation of late-stage erythroid precursors and reducing hemolysis. Notably, RAP-536 treatment mitigated disease complications of IE, including iron overload, splenomegaly, and bone pathology, while reducing erythropoietin levels, improving erythrocyte morphology, and extending erythrocyte life span. These results implicate signaling by the transforming growth factor-β superfamily in late-stage erythropoiesis and reveal potential of a modified ActRIIB ligand trap as a novel therapeutic agent for thalassemia syndrome and other red cell disorders characterized by IE.
TMPRSS6
An RNAi therapeutic targeting Tmprss6 decreases iron overload in Hfe(-/-) mice and ameliorates anemia and iron overload in murine β-thalassemia intermedia.

Schmidt PJ¹, Toudjarska I, Sendamarai AK, Racie T, Milstein S, Bettencourt BR, Hettinger J, Bumcrot D, Fleming MD.

Abstract
Mutations in HFE lead to hereditary hemochromatosis (HH) because of inappropriately high iron uptake from the diet resulting from decreased hepatic expression of the iron-regulatory hormone hepcidin. β-thalassemia is a congenital anemia caused by partial or complete loss of β-globin synthesis causing ineffective erythropoiesis, anemia, decreased hepcidin production, and secondary iron overload. Tmprss6 is postulated to regulate hepcidin production by cleaving Hemojuvelin (Hjv), a key modulator of hepcidin expression, from the hepatocyte surface. On this basis, we hypothesized that treatment of mouse models of HH (Hfe(-/-)) and β-thalassemia intermedia (Hbb(th3/+)) with Tmprss6 siRNA formulated in lipid nanoparticles (LNPs) that are preferentially taken up by the liver would increase hepcidin expression and lessen the iron loading in both models. In the present study, we demonstrate that LNP-Tmprss6 siRNA treatment of Hfe(-/-) and Hbb(th3/+) mice induces hepcidin and diminishes tissue and serum iron levels. Furthermore, LNP-Tmprss6 siRNA treatment of Hbb(th3/+) mice substantially improved the anemia by altering RBC survival and ineffective erythropoiesis. Our results indicate that pharmacologic manipulation of Tmprss6 with RNAi therapeutics is a practical approach to treating iron overload diseases associated with diminished hepcidin expression and may have efficacy in modifying disease-associated morbidities of β-thalassemia intermedia.
Hepcidin

- Mini hepcidins being developed
Minihepcidins prevent iron overload in a hepcidin-deficient mouse model of severe hemochromatosis.

Ramos E\(^1\), Ruchala P, Goodnough JB, Kautz L, Preza GC, Nemeth E, Ganz T.

**Abstract**

The deficiency of hepcidin, the hormone that controls iron absorption and its tissue distribution, is the cause of iron overload in nearly all forms of hereditary hemochromatosis and in untransfused iron-loading anemias. In a recent study, we reported the development of minihepcidins, small drug-like hepcidin agonists. Here we explore the feasibility of using minihepcidins for the prevention and treatment of iron overload in hepcidin-deficient mice. An optimized minihepcidin (PR65) was developed that had superior potency and duration of action compared with natural hepcidin or other minihepcidins, and favorable cost of synthesis. PR65 was administered by subcutaneous injection daily for 2 weeks to iron-depleted or iron-loaded hepcidin knockout mice. PR65 administration to iron-depleted mice prevented liver iron loading, decreased heart iron levels, and caused the expected iron retention in the spleen and duodenum. At high doses, PR65 treatment also caused anemia because of profound iron restriction. PR65 administration to hepcidin knockout mice with pre-existing iron overload had a more moderate effect and caused partial redistribution of iron from the liver to the spleen. Our study demonstrates that minihepcidins could be beneficial in iron overload disorders either used alone for prevention or possibly as adjunctive therapy with phlebotomy or chelation.
Pearls

- Best iron replacement may be 325 mg FeSO4 every other day
- Iron replacement: wt (kg) X Δ Hgb
- Iron challenge test can identify true non-absorbers
- Ferritin can take weeks to normalize
- Hemochromatosis is associated with low hepcidin