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PEARLS OF LABORATORY MEDICINE

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TITLE: Iron Overload Disorders

PRESENTER: Qian Sun

Slide 1: Introduction

Hello, my name is Qian Sun. I am a clinical chemistry fellow at the National Institutes of Health. Welcome to this Pearl of Laboratory Medicine on "**Iron Overload Disorders**."

Slide 2: Outline

In this presentation, we will first review how iron is metabolized in the body and how dysregulation of the metabolic process can lead to iron overload disorders. Then we will go over typical clinical presentation, screening tests as well as the diagnostic workup of iron overload disorders. And finally we will briefly discuss some common therapies of iron overload disorders, which include therapeutic phlebotomy and chelation.

Slide 3: Iron Metabolism

Each day 1 to 2 mg of iron is absorbed by enterocytes of the small bowel. Absorbed iron is bound to transferrin in circulation and is primarily used for the synthesis of heme. When the red blood cells age, they are taken up by macrophages, which release iron from heme. After release, the iron can either be stored as ferritin or exported into the circulation. The liver, and hepatocytes in particular, is another site of iron storage, and therefore is a major organ affected in iron overload disorders.

Slide 4: Iron Overload Disorders

When some of these major steps in iron metabolism go wrong, iron overload disorders can occur. Some common mechanisms include increased iron absorption, decreased iron utilization, and the combination of the two. In the next few slides, we will briefly go over three major categories of iron overload disorders, namely hereditary hemochromatosis, disorders of erythroid maturation, and defects of iron transport.

Slide 5: Hereditary Hemochromatosis

Hereditary hemochromatosis is also known as genetic iron overload disease. Most subtypes of hemochromatosis are caused by an abnormal production of hepcidin, which is a liver protein regulated by the gene HFE. Hepcidin decreases the expression of ferroportin, which is an iron transport protein that mediates the release of iron from enterocytes and macrophages. Therefore in the presence of hepcidin, iron absorption from the small bowel and release from macrophages are tightly regulated.

Slide 6: Hereditary Hemochromatosis (continued)

However, in patients with hereditary hemochromatosis, mutation of HFE leads to decreased hepcidin expression. Inadequate hepcidin production will result in excessive iron absorption and release, subsequently causing iron overload.

Slide 7: Hereditary Hemochromatosis (continued)

As I just mentioned, most subtypes of hereditary hemochromatosis are caused by HFE gene mutation, which regulates hepcidin expression. These forms are also called HFE-associated hemochromatosis. Here H stands for hemochromatosis, and Fe is the abbreviation for iron. Over 80% of HFE-associated hemochromatosis cases are due to a patient having two copies of a gene mutation called C282Y, or C282Y homozygote. These patients are almost always of northern European ancestry, with the mutation occurring in about 1 out of every 200 people with this heritage. However, only a small proportion of C282Y homozygote develop clinical features of iron overload, and most patients do not present until middle age. In women, the presentation is even further delayed until after menopause. Other forms of hemochromatosis include mutations in transferrin receptor 2, or TFR2 gene; mutations in ferroportin, which cause excessive ferroportin-mediated iron export; as well as juvenile hemochromatosis, which has an earlier presentation compared with the classical hereditary hemochromatosis.

Slide 8: Disorders of Erythroid Maturation

In addition to hemochromatosis, which is a primary iron overload disorder, a major class of secondary iron overload is disorders of erythroid maturation. Some common diseases in this category include thalassemias, congenital sideroblastic anemias and aplastic anemias. Iron overload in these conditions is caused by a number of factors, including reduced utilization of iron due to defective incorporation of iron into heme; down-regulation of hepcidin, which persists despite iron overload; and finally frequent transfusion. It is estimated that transfused blood contains 200-250 mg of iron per unit. So for a patient receiving 2-4 units of blood per month, he or she will have an annual intake of 5-10g of iron. However, the body has no mechanism for excreting this excess iron.

Slide 9: Defects of Iron Transport

The last but not least, we will talk about the third class of iron overload, which is caused by defects of iron transport. Hypotransferrinemia is a rare autosomal recessive condition where functional transferrin concentrations are severely reduced. Iron overload in this condition is caused by insufficient delivery of transferrin-bound iron for the synthesis of heme and therefore increased iron storage in tissues. Another disease that causes defective iron transport is called aceruloplasminemia. In these patients, loss of ceruloplasmin ferroxidase activity decreases loading of iron onto transferrin, which leads to increased iron storage in cells.

Slide 10: Clinical Presentation of Iron Overload

Now let's talk about the clinical symptoms of iron overload disorders. A variety of symptoms and signs can be caused by iron deposition in different organs. As the primary organ of iron storage, the liver is frequently involved in iron overload. It is estimated that 10-25% of patients develop hepatic fibrosis whereas 4-6% progress to cirrhosis. Other common organs involved include the pancreas, and specifically beta cells, which can lead to diabetes; the heart, which can lead to arrhythmias and heart failure.

Slide 11: Diagnostic Tests of Iron Overload

As you can see, the symptoms of iron overload are rather nonspecific. Therefore, laboratory tests are important for the early diagnosis of iron overload. Transferrin saturation and serum

ferritin are two major screening tests for iron overload. After patients at high risk are identified with these tests, final diagnosis is made by definitive tests including liver biopsy, HFE genetic testing and imaging technique such as the MRI for the quantification of hepatic and cardiac iron deposition.

Slide 12: Screening Tests of Iron Overload

As screening tests of iron overload, transferrin saturation and ferritin are frequently ordered together, and both have pros and cons to quantify iron burden in patients. Transferrin saturation, for example, is not directly measured, but calculated based on serum iron and transferrin concentrations. Compared with ferritin, it is a more sensitive test for hereditary hemochromatosis. However, since serum iron shows diurnal variation, and its concentration is affected by recent dietary intake, repeated testing or overnight fasting is often recommended. On the other hand, serum ferritin reflects tissue iron storage and body iron content, so it identifies clinically significant iron overload in all patients. In addition, it is a useful test to monitor the effect of iron overload treatment. The disadvantage of ferritin is that it is not a specific indicator of iron; because ferritin is an acute phase protein, its concentration increases with inflammation as well as liver diseases. As a result, if ferritin level is high but transferrin saturation is normal or low, the first thing physicians should consider is to rule out inflammation, and alcohol abuse. The cutoff for transferrin saturation is generally set as 45%, 200ug/L for ferritin in women, and 300ug/L in men.

Slide 13: Treatment of Iron Overload

And finally, I will briefly talk about the two major therapies of iron overload: phlebotomy and iron chelation. In the absence of anemia, the treatment of choice is iron removal by therapeutic phlebotomy. It is applicable in most forms of hereditary hemochromatosis, and it is associated with reduced incidence of liver cirrhosis. However, phlebotomy might perpetuate the underlying low hepcidin state and therefore leads to excessive iron absorption. On the other hand, for patients with iron loading anemias, phlebotomy is impossible because patients are anemic. In these conditions the best option is iron chelation therapy. There are three FDA-approved iron chelators, deferoxamine, deferiprone and deferasirox. They are designed to bind iron in the bloodstream or tissue, and enhance its elimination through urine or feces.

Slide 14: Points to Remember

Some take home messages are:

- There are three major categories of iron overload disorders: hereditary hemochromatosis, disorders of erythroid maturation, and defects of iron transport.
- Major organs affected by iron overload include liver, pancreas, and heart.
- Laboratory testing is important for the early diagnosis of iron overload. Two screening tests include transferrin saturation and serum ferritin.
- Patients are treated with therapeutic phlebotomy in the absence of anemia and with iron chelation if they have iron loading anemias.

Slide 15: References

Slide 16: Disclosures

None.

Slide 17: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**Iron Overload Disorders**."

QUESTION BANK TEMPLATE

Field	Instructions	
Stem	Write one question *Refer to Guide for Presenters for guidance (Page 5)*	Iron overload occurs when the absorption of iron exceeds the demand of the body, or when iron transport in the circulation is impaired. Which of the following conditions is not associated with iron overload?
Responses	Provide 5 responses *Refer to Guide for Presenters for guidance (Page 5)*	a. Hypotransferrinemia b. Aceruloplasminemia c. Hereditary hemochromatosis d. Neonatal bilirubinemia e. Beta-thalassemia
Answer	Indicate one correct response	d
Discussion	Provide a discussion of the correct response with main points explaining why it is the best choice	In all other above described conditions, iron overload occurs because of impaired hepcidin-ferroportin axis (Hereditary hemochromatosis), impaired iron transport (aceruloplasminemia and hypotransferrinemia), or ineffective erythropoiesis (thalassemia).
Source(s)	Provide the source(s) of information for further study *Refer to Guide for Presenters for full citation formatting (Page 3)*	Fleming RE, Ponka P. Iron overload in human disease. New Engl J Med. 2012;366:348-59.
Difficulty	Select one level of difficulty: *Easy, intermediate, advanced*	intermediate
Category	Select one category (*Refer to list in Guide for Presenters - Page 6)*	Hematology
Sub-category	Select one sub-category (*Refer to list in Guide for Presenters - Page 6)*	Hematology-Hemotopathology

Keywords	Include at least 1-2 keywords *Keywords should describe a subtopic to the sub-category selected. Examples include, thyroid, electrolytes, diabetes, pregnancy, etc.*	Iron overload
Field	**Instructions**	
Stem	Write one question *Refer to Guide for Presenters for guidance (Page 5)*	Duodenal enterocytes absorb iron every day to offset losses. How much iron is absorbed per day?
Responses	Provide 5 responses *Refer to Guide for Presenters for guidance (Page 5)*	a. 1-2mg/day b. 5-8mg/day c. 25-30mg/day d. 1-2µg/day e. 5-8µg/day
Answer	Indicate one correct response	a
Discussion	Provide a discussion of the correct response with main points explaining why it is the best choice	Maintaining iron homeostasis requires 1 to 2 mg of absorbed iron per day to offset losses from desquamated cells.
Source(s)	Provide the source(s) of information for further study *Refer to Guide for Presenters for full citation formatting (Page 3)*	Fleming RE, Ponka P. Iron overload in human disease. New Engl J Med. 2012;366:348-59.
Difficulty	Select one level of difficulty: *Easy, intermediate, advanced*	Easy
Category	Select one category (*Refer to list in Guide for Presenters - Page 6)*	Chemistry
Sub-category	Select one sub-category (*Refer to list in Guide for Presenters - Page 6)*	Chemistry-General Clinical Chemistry

Keywords	Include at least 1-2 keywords *Keywords should describe a subtopic to the sub-category selected. Examples include, thyroid, electrolytes, diabetes, pregnancy, etc.*	iron

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PEARLS OF LABORATORY MEDICINE

Iron Overload Disorders

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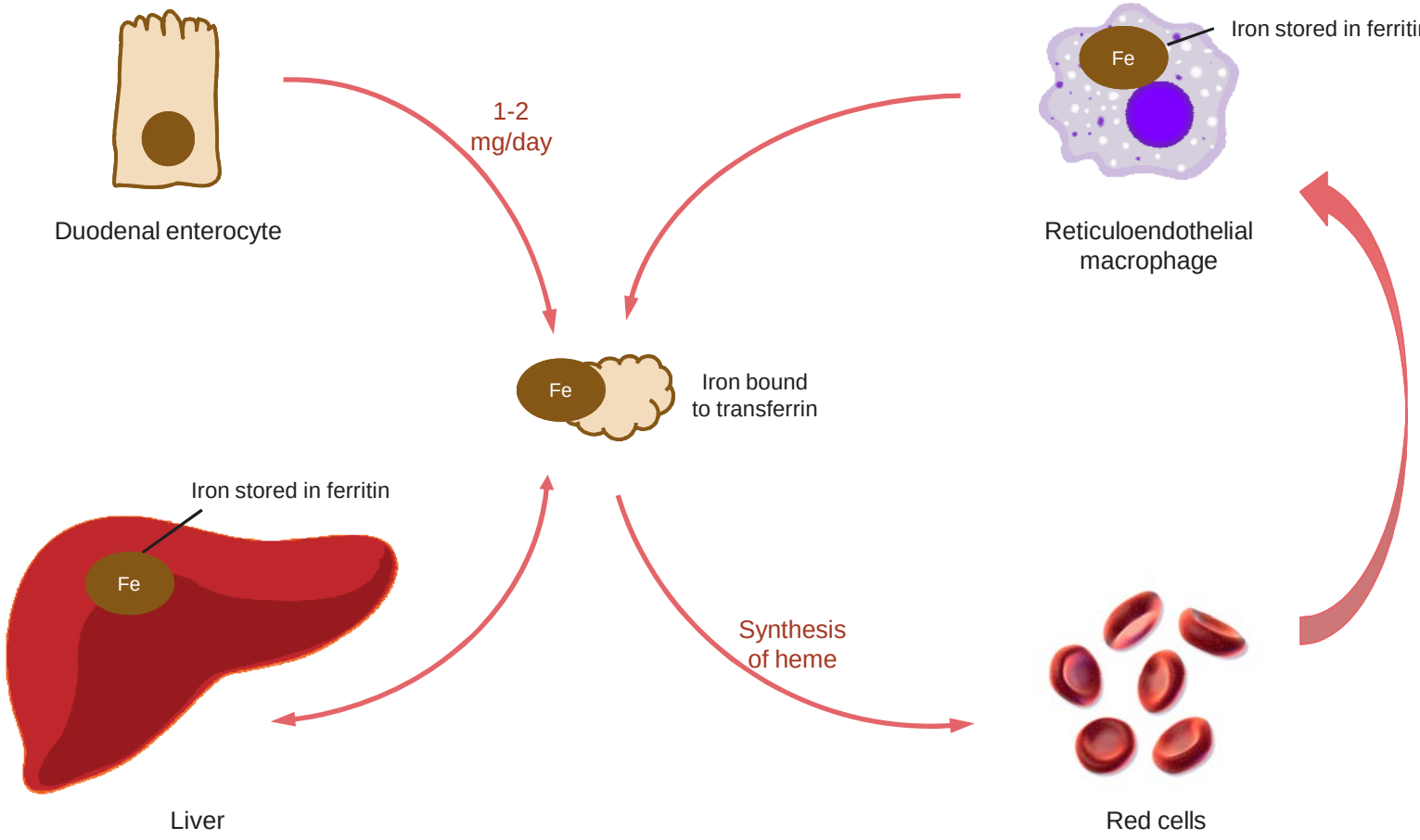
Outline

- Iron metabolism and common iron overload disorders
- Clinical presentation of iron overload disorders
- Diagnostic tests
- Treatment

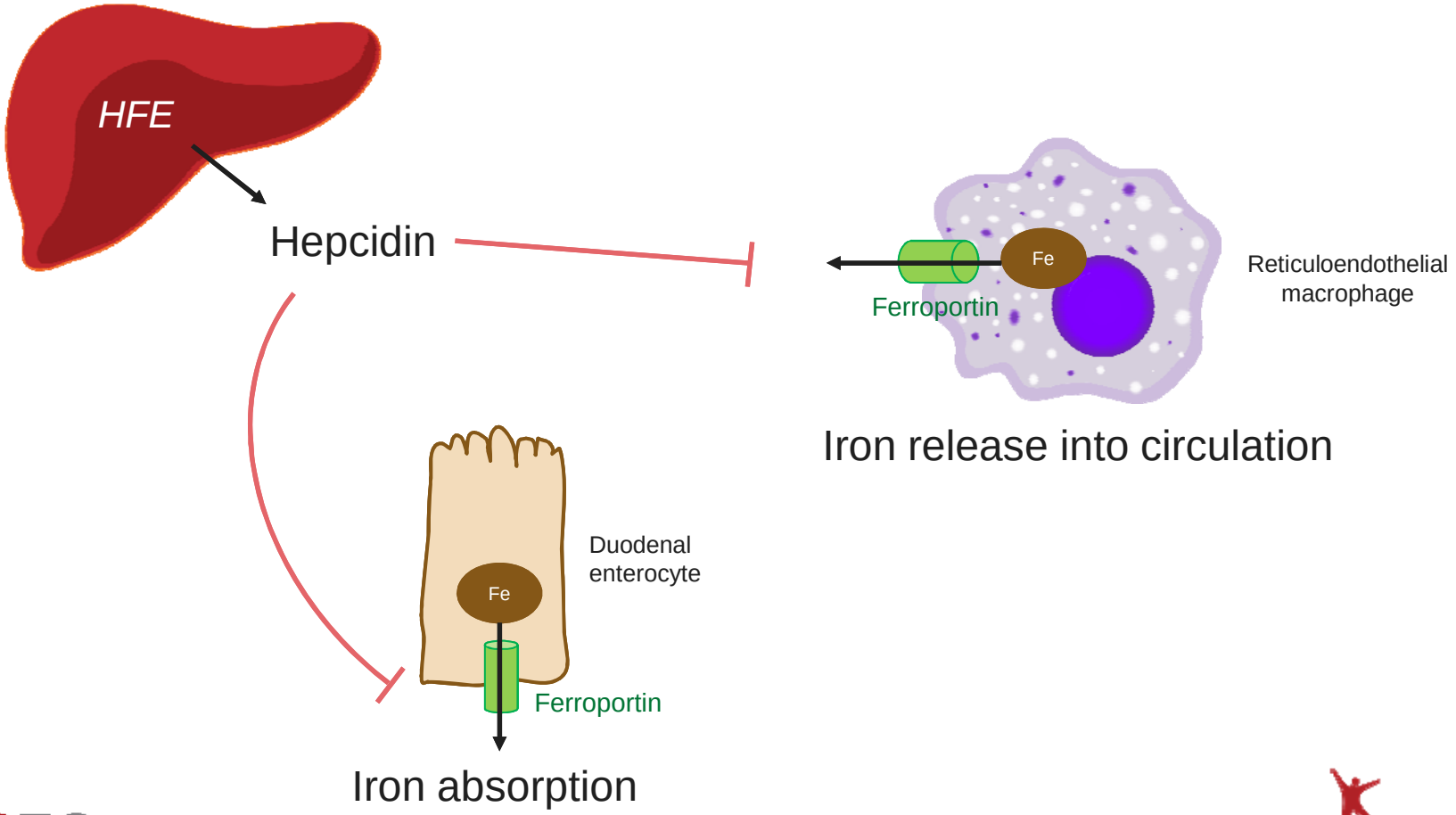
Iron Metabolism



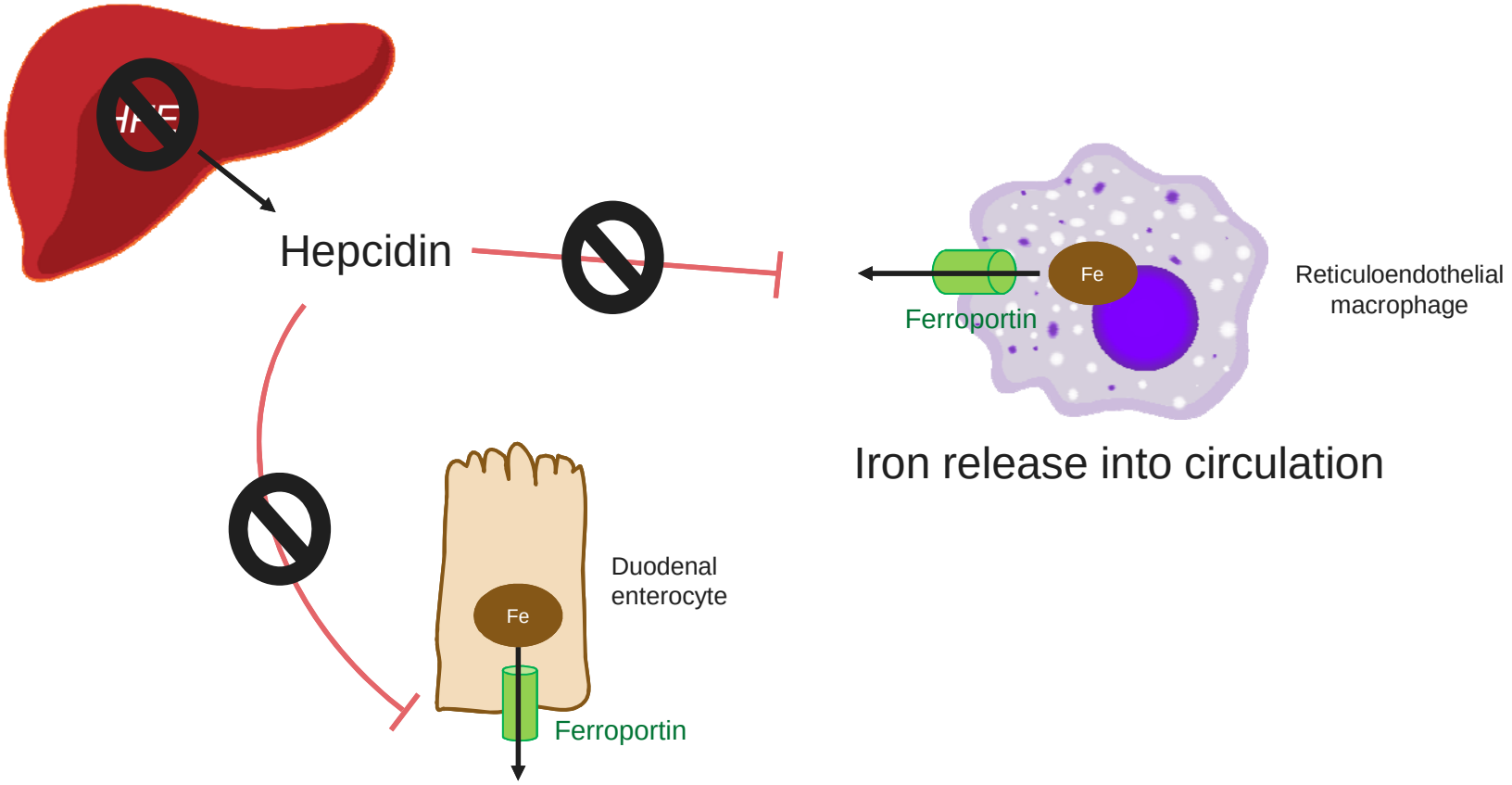
Iron Overload Disorders

- Hereditary hemochromatosis (HH)
- Disorders of erythroid maturation
- Defects of iron transport

Hereditary Hemochromatosis



Hereditary Hemochromatosis



Hereditary Hemochromatosis

- Most common form: HFE-associated hereditary hemochromatosis
  - H: hemochromatosis
  - Fe: Iron
- 80-90% of patients are C282Y homozygotes
- Prevalence of C282Y homozygosity
  - 1 in 200 persons of northern European ancestry
- Only a small portion of C282Y homozygous subjects develop clinical disease.
- Other forms of hemochromatosis:
  - Mutations in transferrin receptor 2 (*TFR2*)
  - Mutations in ferroportin
  - Juvenile hemochromatosis

Disorders of Erythroid Maturation

- Thalassemias, congenital sideroblastic anemias, aplastic anemias

- Secondary iron overload
  - Reduced utilization of iron
  - Down-regulation of hepcidin
  - Frequent transfusion (200-250mg iron per unit of blood)

Defects of Iron Transport

- Hypotransferrinemia
  - Autosomal recessive condition
  - Reduced transferrin concentration
  - Insufficient delivery of transferrin-bound iron for the synthesis of heme

- Aceruloplasminemia
  - Loss of ceruloplasmin ferroxidase activity
  - Decreased loading of iron onto transferrin

Clinical Presentation of Iron Overload

- Liver disease
  - Liver is the primary organ of iron storage
  - 10-25% of patients develop hepatic fibrosis
  - 4-6% of patients develop cirrhosis
- Endocrine disease
  - Accumulation of iron in beta cells (pancreas)
- Cardiac involvement
  - Accumulation of iron in the heart

Diagnostic Tests of Iron Overload

- Screening tests:
  - Transferrin saturation
  - Serum ferritin

- Confirmatory/definitive tests:
  - Liver biopsy
  - *HFE* genetic testing
  - MRI

Screening Tests of Iron Overload

- **Transferrin saturation**
  - Calculated:

$$\frac{\text{Serum Fe}\left(\frac{\mu g}{dL}\right)}{\text{Transferrin}\left(\frac{mg}{dL}\right)} \text{x factor}(\sim 71)$$

  - Useful in patients with HH
  - Repeated testing or overnight fasting recommended due to high diurnal variation
  - Cutoff: 45%

- **Serum ferritin**
  - Reflects the body Fe content
  - Useful in all patients with iron overload
  - Monitor the effect of treatment
  - Not specific: Acute phase protein
  - Cutoff: 200µg/L women
    300µg/L men

Treatment of Iron Overload

- Phlebotomy
  - Treatment selected in the absence of anemia
  - Applicable in most forms of HH
  - May trigger excessive iron absorption
- Chelation
  - Treatment selected in iron-loading anemias
  - FDA-approved iron chelators: Deferoxamine, deferiprone, deferasirox
  - Excretion of tissue iron through urine or feces by forming complexes

Points to Remember

- There are three major categories of iron overload disorders: hereditary hemochromatosis, disorders of erythroid maturation, and defects of iron transport.
- Major organs affected by iron overload include liver, pancreas and heart.
- Laboratory testing is important for the early diagnosis of iron overload. Two screening tests include transferrin saturation and serum ferritin.
- Patients are treated with therapeutic phlebotomy in the absence of anemia and with iron chelation if they have iron loading anemias.

References

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Disclosures/Potential Conflicts of Interest

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- **Employment or Leadership**:
- **Consultant or Advisory Role:**
- **Stock Ownership:**
- **Honoraria:**
- **Research Funding:**
- **Expert Testimony:**
- **Patents:**

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