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IRON DEFICIENCY AND THE ANEMIA OF CHRONIC DISEASE

SIGNIFICANCE

Lack of iron and the anemia of chronic disease are the most common causes of anemia in the world. The majority of pre-menopausal women will have some element of iron deficiency. The first clue to many GI cancers and other diseases is iron loss. Finally, iron deficiency is one of the most treatable medical disorders of the elderly.

IRON METABOLISM

It is crucial to understand normal iron metabolism to understand iron deficiency and the anemia of chronic disease.

Iron in food is largely in ferric form (Fe^{+++}) which is reduced by stomach acid to the ferrous form (Fe^{++}). In the jejunum two receptors on the mucosal cells absorb iron. The one for heme-iron **(heme iron receptor**) is very avid for heme-bound iron (absorbs 30-40%). The other receptor - **divalent metal transporter (DMT1**) - takes up inorganic iron but is less efficient (1-10%). Iron is exported from the enterocyte via **ferroportin** and is then delivered to the **transferrin receptor (TfR)** and then to plasma **transferrin**. Transferrin is the main transport molecule for iron. Transferrin can deliver iron to the marrow for the use in RBC production or to the liver for storage in **ferritin**. Transferrin binds to the TfR on the cell and iron is delivered either for use in hemoglobin synthesis or storage. Iron that is contained in hemoglobin in senescent red cells is recycled by binding to ferritin in the macrophage and is transferred to transferrin for recycling. This system is extremely efficient and loses less than 5% of the iron contained in RBC. There are 1.7-2.4 grams of iron in an adult.

Transferrin receptor cycle: Transferrin carrying iron binds to the TfR. This complex of TfR-transferrin is then internalized in the cell by endocytosis. The lower pH of the endosome results in release of iron from the complex. Then the iron-less complex recycles to the cell surface, transferrin is released and the whole cycle starts over. It is estimated that in a growing red cell 80,000 transferrin molecules are popping on and off every second.

KEY PROTEINS:

Transferrin: Iron delivery protein with 2 iron binding sites
Ferritin: Iron storage - can store 24,000 iron molecules.
Transferrin Receptor: Cell surface protein that binds transferrin.
Ferroportin: Exports iron from cells
Hepcidin: Binds to ferroportin blocking export of iron

Iron response element binding protein (IRE-BP): Complex but worth understanding. Both the mRNA for transferrin and ferritin has sequences that bind this protein. When iron binds the

protein it cannot bind to the mRNA but without iron the protein binds the mRNA. This is how the cell senses when to make ferritin and the transferrin receptor. With **lack** of iron the IRE-BP **binds** mRNA. With iron IRE-BP floats around the cytoplasm of the cell. The binding of the IRE-BP to ferritin blocks protein production (with no iron why do you need a storage molecule?) but stabilizes the TfR mRNA resulting in increased TfR mRNA and more protein production. With iron the IRE-BP **pops off** of ferritin and TfR mRNA's, allowing ferritin protein synthesis but accelerating the degradation of TfR mRNA & with resultant decreased TfR protein production.

IRON DEFICIENCY

Kids become iron deficient due to dietary lack of iron and increased iron requirements for growth. The most common cause in men is bleeding-especially occult GI blood loss and bar fights. Etiologies in pre-menopausal women include menstrual loss and pregnancy. The GI tract is the most common site of blood loss in the elderly. Rarer causes include PNH and pulmonary hemosiderosis. Patients after gastrectomy may develop iron deficiency due to impaired iron absorption. The promiscuous use of antacids and protein pump inhibitors in modern society is leading to an increased incidence of iron deficiency.

ANEMIA OF CHRONIC DISEASE

This is the most common cause of anemia in hospitalized patients and occurs classically in infection, malignancies, and inflammatory disorders but is also seen with chronic obstructive lung disease, congestive heart failure, and diabetes. Although it used to be claimed that the hematocrit in anemia of chronic disease never gets below 30%, as many as 25% of patients with anemia of chronic disease will have hematocrits below 30%. The hematocrit falls in the first one to two months after the onset of inflammation but usually remains stable after that.

Several causes have been postulated for the anemia of chronic disease. Patients with anemia of chronic disease have **shortened RBC half-lives** which suggest mild hemolysis. Also the RBC precursors have a **decreased sensitivity to erythropoietin**. The serum erythropoietin levels in patients with anemia of chronic disease levels **are not elevated** commensurate with the severity of the anemia. For example, if I bleed down to a hematocrit of 25%, my erythropoietin level would be 4,000 but if I had the anemia of chronic disease my erythropoietin level may only rise to 40. The most consistent defect is a **failure of the RES to deliver iron to the developing RBC**. Thus at the level of the RBC anemia of chronic disease is the same as iron deficiency ("iron deficient erythropoiesis"). This has profound implications for diagnosis of iron deficiency.

The protein **hepcidin** is the key regulator of anemia of chronic disease. Inflammation leads to increase synthesis of hepcidin. Hepcidin then binds to ferroportin blocking iron export from both liver stores and the GI track. This leads to lower levels of iron in the plasma and less iron delivery to the developing red cell.

Philosophical Aside

In an evolutionary sense anemia of chronic disease may result from the body's attempt to sequester iron from invading organisms. All organisms need iron to grow and iron is a great growth supplement for bacterial. The body's uniform response to any stress is to rapidly decrease the levels of serum iron. Iron has also been implicated in redox reactions that promote tissue damage and this may be another evolutionary drive for the hypoferremia and anemia seen in inflammatory states.

DIFFERENTIAL DIAGNOSIS OF MICROCYTIC ANEMIAS

1. **Iron Deficiency.**
2. **Anemia of Chronic Disease**. (anemia of defective iron utilization).
3. **Thalassemia**. In this disorder it is the defective production of hemoglobin that leads to microcytosis. The main types are the beta-thalassemia, alpha-thalassemia and Hemoglobin E.

   Patients who are heterozygotes for **beta-thalassemia** have microcytic indices with mild (30ish) anemias. Homozygotes have very severe anemia. Peripheral smear in heterozygotes reveals microcytes and target cells. Diagnosis is established in by hemoglobin electrophoresis which shows an increased HbA_2. One should check iron stores since an elevated HbA_2 will not be present in patients with both thalassemia and iron deficiency. Beta-thalassemia occurs in a geographic belt ranging through Mediterranean countries, the Middle East, India, Pakistan and Southeast Asia. Patients with beta-thalassemia trait who are of child bearing age need to have their spouse screened for beta-thalassemia and Hemoglobin E.

   Alpha-thalassemia also presents with microcytosis. Patients with alpha-thalassemia will have normal hemoglobin electrophoresis. The diagnosis of alpha-thalassemia is made by excluding other causes of microcytosis, a positive family history of microcytic anemia, and a life-long history of a microcytic anemia. Exact diagnosis requires DNA analysis. Alpha-thalassemia is distributed is a similar pattern to beta-thalassemia except for a very high frequency in Africa (up to 40%).

   Hemoglobin E is actually an unstable beta-hemoglobin chain that presents in a similar fashion to the thalassemia. It is believed to be the most common hemoglobinopathy in the world. Hemoglobin E occurs in Southeast Asia, especially in Cambodia, Laos and Thailand. Patients who are heterozygotes are not anemic but are microcytic. Patients who are homozygous are mildly anemic with microcytosis and target cells. The importance of Hemoglobin E lies in the fact that patients with genes for Hemoglobin E and beta-thalassemia have severe anemia and behave in a similar fashion to patients with homozygote beta-thalassemia.

Thalassemia	MCV	Hgb	Electrophoresis	Other Features
Beta-Thalassemia				
Major	50-75	< 7	Raised HbA2	Severe anemia,
Intermedia	50-75	< 9	Raised HbA2	Target cells on smear
Trait	65-75	9-10	Raised HbA2	Target cells on smear
Alpha Thalassemia				
Trait-1 (α α/ α-)	80-85	Nl	Normal	
Trait-2 (α -/ α-) or (α α/ --)	65-75	12-13	Normal	
Hemoglobin H (α -/ --)	60's	9-8	HgbH	Hemolysis, splenomegaly
Hemoglobin Barts(- -/ --)	-	-	HgbH, Hbg Barts	Hydrops fetalis
Hemoglobin E				
Heterozygous	80-85	12	HgbE present	Rare target cells on smear
Homozygous	70's	11-12	HgbE predominant	Target cells on smear

4. **Sideroblastic Anemia.** Defective production of the heme molecule is the basis of this

disorder. The deficit of heme leads to the underhemoglobinization of the erythroid precursors and microcytosis. Sideroblastic anemia can be congenital, can be due to toxins such as alcohol, lead, INH, or can be an acquired bone marrow disorder. The peripheral smear may show basophilic stippling in lead poisoned patients, a dimorphic (macrocytic and intensely microcytic red cells) in patient with acquired sideroblastic anemia, or stigmata of a myelodysplastic syndrome. Diagnosis is made by the finding of ringed sideroblasts on the bone marrow iron stain. Iron studies in patients with sideroblastic anemia usually show sign of iron-overload.

APPROACH TO DIAGNOSIS OF IRON DEFICIENCY ANEMIA

RDW: Based on faulty assumption. Has been shown in multiple trials to be of **NO** value in the diagnosis of iron deficiency, anemia of chronic disease, myelodysplasia, or thalassemia or any other hematological disease.

MCV: Decreased due to under-hemoglobinization of the developing RBC. Since at the level of the RBC both iron deficiency and anemia of chronic disease are the same it is not surprising that 25% of patients with anemia of chronic disease will be microcytic. MCV's under 70 fl are very suggestive of iron deficiency but this only happens 10-20% of the time. Many patients with iron deficiency will have normal or even high MCV.

Free Erythrocyte Protoporphyrin: The key step in the production of heme is the insertion of the iron molecule into protoporphyrin. If iron is lacking this protoporphyrin is released. Thus FEP is elevated in both anemia of chronic disease and iron deficiency and is of no diagnostic use.

Serum Iron: Although the serum iron is decreased in iron deficiency it is also decreased in any stressful situation. Serum iron plummets within minutes of fever. Serum iron varies from day-to-day and hour-to-hour. The minute amount of iron in a Fred Flintstone multi-vitamin can falsely raise the serum iron for over 24 hours.

Total Iron Binding Capacity (Transferrin): In iron deficiency serum transferrin rises to above normal. This is the only situation in which an above normal TIBC occurs. Therefore, an above normal TIBC is specific for iron deficiency. Unfortunately transferrin is a negative acute phase reactant so it falls in any inflammatory state even in the presence of iron deficiency. In most cases of concurrent inflammation/iron deficiency the TIBC is in the normal range but can be below normal thus greatly limiting the usefulness of this test.

Iron Saturation: This is one of the most misunderstood tests of iron deficiency. Due to the fact that serum iron decreases in both anemia of chronic disease and iron deficiency the saturation is decreased below 15% in **BOTH**. Very low saturations (below 5%) may be more specific for iron deficiency but this is an unusual finding. The saturation does not add any information to the TIBC and suffers from all the liabilities of the serum iron.

Serum Ferritin: The test that gets no respect. The amount of ferritin that is produced by the liver is dependent on two factors. Transcription of the ferritin mRNA can be dramatically increased by inflammation but iron is an **absolute** requirement for the mRNA to be translated to protein. Thus, in states of iron deficiency no ferritin is produced even in florid inflammatory states. Realistically since minute amounts of iron are present in iron deficiency more ferritin is made in inflammatory states for a given amount of iron stores than in non-inflammatory states. However, it is most unusual for the ferritin to be over **100 ng/dl** in

patient with iron deficiency even in the most florid inflammatory states. Thus the serum ferritin is the best non-invasive means of detecting iron deficiency.

In normal individuals the serum ferritin is proportional to the body's iron stores. In men the body iron stores increase steadily after age twenty while in women iron stores do not increase until after menopause. In men the serum ferritin rises in conjunction with increasing iron stores and is often over 100 ng/dl by age 50. In women the ranges of normal are more difficult to state with certainty since many of the "normal" women used in the studies to establish the standard were iron deficient. In the uncomplicated patient a ferritin under 35 ng/dl in a male is indicative of iron deficiency. In young women 12 ng/dl is the level suggestive of deficiency but a ferritin between 12-35 may also be indicative of depleted iron stores. In patients over 65 a ferritin less than 50 ng/dl is indicative of depleted iron stores.

Bone Marrow: The fastest and most reliable means of measuring iron stores is to perform a bone marrow aspirate and stain for iron. The aspirate smear is stained with Prussian Blue stain which stains iron blue and thus marrow storage of iron can be directly assessed. Furthermore one can make the morphologic diagnosis of anemia of chronic disease by finding adequate marrow iron but no iron in developing red cells. This test is considered the "gold standard" for iron deficiency.

Trial of Oral Iron: If a patient is anemic due to iron deficiency repletion of iron stores should increase their hemoglobin. This forms the basis for the "trial of iron". Unfortunately no standards have been set for what constitutes an adequate trial. Most papers states that after two weeks of iron the hemoglobin should rise by 1g/dl. Obviously patient compliance is key. Also fluctuation in any underlying inflammatory state will also affect the results. This test is of limited use but may be helpful in a select group of patients (e.g., young women with anemia).

SUMMARY:

RDW, FEP, serum iron, saturation-worthless.
TIBC-specific for iron deficiency if above normal but poor sensitivity.
Ferritin-best non-invasive test.
Bone marrow iron stain-"gold standard".
Trial of oral iron-simple but compliance and concurrent fluctuation in underlying inflammatory states a problem.

THERAPY

Treatment

Oral iron is the best treatment option. The gut can only absorb so much iron so there is no utility in taking more than one pill per day. Taking iron with some food can help with GI tolerance. Meals that contain meat will double iron absorption. Vitamin C 500 units can also help absorption. Tea and coffee within an hour of oral iron should be avoided as this will decreased iron absorption.

Some patients cannot replete their iron stores with oral iron and will benefit from intravenous iron. The most expedient is 1000mg of iron dextran over one hour. Other IV options include:

- Low Molecular Weight Iron Dextran: 1000mg over 1 hours

• Ferumoxytol: 510mg X 2 or 1020mg over 15 minutes
• Ferric Carboxymalate: 750 mg x 2
• Iron isomaltoside: 1000mg x 1
• Ferric Gluconate: 125mg X 8
• Iron Sucrose: 2-300mg x3-5

With effective iron therapy, the reticulocyte count should rise in one week and the hematocrit should increase by 3% in two weeks. Unless the cause of the blood loss is obvious, all patients with iron deficiency should undergo a gastrointestinal evaluation. In older patients studies show that 50% will have an identifiable source of blood loss and that 10-15% of iron deficiency patients will have colon cancer. In patients with iron deficiency that is resistant to iron therapy one should check anti-gliadin and anti-endomysial antibodies to check for celiac disease and for h. Pylori. Recently achlorhydria due to anti-parietal cell antibodies has been implicated in refractory iron deficiency.

One thing that absolutely, positively must be done in any patient with iron deficiency is to **explain** why the patient is iron deficiency. In America, nutritional deficiency is not a cause of iron deficiency and therefore in adults iron deficiency almost always results from external loss. The only site of bleeding that will not be noticed by patients is occult blood loss from the gastrointestinal tract. Therefore, in non-menstruating adults, the finding of iron deficiency mandates detailed studies of the GI tract to rule out significant pathology such as cancer, polyps or ulcers.

TEST	IRON DEFICIENCY	ANEMIA OF CHRONIC DISEASE
MCV	LOW	NORMAL (LOW IN 30% OF PATIENTS)
FEP	HIGH	HIGH
SERUM IRON	LOW	LOW
TIBC	HIGH/NORMAL	NORMAL/LOW
IRON SATURATION	LOW	LOW
FERRITIN	LOW	HIGH
MARROW SIDEROBLASTS	ABSENT	ABSENT
MARROW IRON	ABSENT	PRESENT
ERYTHROPOIETIN LEVEL	VERY HIGH	INAPPROPRIATE LOW FOR DEGREE OF ANEMIA