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TITLE: Iron Deficiency Anemia

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Slide 1
Hello, my name is Shannon Haymond. I am the director of Clinical Chemistry and Mass Spectrometry Laboratories at the Ann & Robert H. Lurie Children's Hospital of Chicago and an Assistant Professor of Pathology at Northwestern University Feinberg School of Medicine. Welcome to this Pearl of Laboratory Medicine on "Iron Deficiency Anemia."

Slide 2:
Iron is a highly abundant element and is a critical component of many biological processes. It is required for oxygen transport as part of hemoglobin and is also a necessary component of other heme proteins, such as myoglobin and the cytochromes. Additionally, iron is contained in several metalloproteins and enzymes.

Deficiency of iron, therefore, has significant clinical impact, adversely affecting cognitive ability, behavior, and physical growth in infants and children. In all ages, iron deficiency impairs immune status and increases morbidity from infections. Insufficient iron also results in defective utilization of energy sources in muscle. Although supplementation for iron deficiency is effective, prevention is key, as the effects of iron deficiency on children's cognitive performance, behavior, and growth are not reversible.

Slide 3:
Iron deficiency and iron deficiency anemia are often used interchangeably; however, it is important to understand their relationship. Iron deficiency is a continuum that begins as iron depletion from bone marrow stores and if untreated, progresses to severe anemia. Therefore, individuals may be iron deficient but not anemic or may be anemic because of reasons other than iron deficiency, although iron deficiency is the leading cause of anemia.

Slide 4:
Infants, toddlers, and women are at highest risk for iron deficiency and prevalence rates in these populations are relatively high. Infants and toddlers are at risk due to rapid growth, resulting in higher iron needs. On average, neonatal iron stores are sufficient for approximately 4 months without supplementation. Infant iron stores are created during the last trimester of pregnancy, so infants born prematurely are at increased risk for deficiency due to lower native iron stores.

In the US, infant formulas are fortified with iron, as is a large number of solid food items. Toddlers are at risk due to changes in diet during the transition from infancy to early childhood, particularly those that are picky eaters or those not consuming enough iron-rich foods. Excessive milk intake (i.e., greater than 16-24 oz per day) is another common cause for iron deficiency in this age group, as this leads to insufficient intake of other foods that are higher in iron.

Females that are pregnant or menstruating are at increased risk due to higher iron needs and blood loss, respectively. The American Academy of Family Physicians (AAFP), US Preventive Services Task Force (USPSTF), and Centers for Disease Control and Prevention (CDC) recommend routine screening of asymptomatic pregnant women for iron deficiency anemia. The American College of Obstetricians and Gynecologists (ACOG) recommends screening for anemia and implementing iron therapy if iron deficiency anemia is confirmed. Universal screening recommendations in children are more controversial, with the American Academy of Pediatricians (AAP) recommending screening in all children at 1 y, but the AAFP and USPSTF have found insufficient evidence to recommend such screening.

Individuals at higher risk due to presence of risk factors, including some described here, may be screened at any age. CDC has made additional screening recommendations for infants with risk factors. Measurement of hemoglobin is typically the first-line screening method.

Slide 5:

In the US and worldwide, iron deficiency is the most common nutritional deficiency in women and children and is the leading cause of anemia. In the US, approximately 2.4 million children, primarily those 0-3 years old, are affected. Worldwide, approximately 1.6 billion people have anemia and since iron deficiency is a leading cause, many of those have iron deficiency anemia. Prevalence is highest throughout Africa and Asia where rates are reported as high as 40-60%. The prevalence of anemia in North America is about 3.4%.

Slide 6:

Iron is tightly regulated with very little daily loss and no physiological means of excretion. Each day, ~20 mg iron is recycled between circulating transferrin and erythrocytes. There are four components to this pathway. The master regulator of iron metabolism is hepcidin. Hepcidin is an inhibitor of the export channel protein, ferroportin, which is located on the basal surface of gut enterocytes and in the plasma membrane of macrophages. Ferroportin enables iron to enter circulation. Iron is absorbed in the duodenum and is transported in circulation by transferrin, depicted in the figure as a Fe-Tf complex. Excess iron is stored in and released from hepatocytes via unknown mechanisms. Iron is transported to the bone marrow for incorporation into hemoglobin during erythropoiesis. Erythrocytes (RBCs) circulate for ~120 days and then undergo phagocytosis in the spleen, which releases iron back into the circulation.

Conditions of increased requirements, decreased intake, increased loss, or decreased absorption result in iron deficiency. Iron deficiency anemia arises when this balance of intake, stores and loss is disrupted to the point where the amount of available iron becomes insufficient to fully support production of erythrocytes.

Slide 7:
Iron deficiency anemia is a microcytic, hypochromic anemia. The anemia is defined by hemoglobin below a cutoff, typically less than 2SD below the appropriate age/gender mean for an individual. The microcytosis is reflected as a low mean corpuscular volume (MCV). A peripheral blood smear shows characteristics of small red blood cells of varied shape and size with increased light center. The red cell distribution width (RDW) is elevated due to the high variance in the cell shapes. Iron stores measured by bone marrow or tissue staining or through iron biomarkers will be low. Symptoms of iron deficiency anemia are related to the many roles of iron in the body. These include, weakness, fatigue, pallor, irritability, poor appetite, and pica (consumption of substances with no significant nutritional value such as soil).

Slide 8:
As noted before, iron deficiency is a continuum that begins as iron depletion from bone marrow stores and if untreated, progresses to severe anemia. It is commonly described in several stages that correlate to the physical changes with the laboratory results. The gold standard of iron depletion is the reduced or absent staining of tissue or bone marrow iron. It is important to note that due to differences in physiology, it is normal that children under 3-4 years of age will not have detectable bone marrow iron.

When compared to stainable iron, serum ferritin is the most sensitive laboratory indicator of mild iron deficiency. The transferrin saturation does not become abnormal until tissue stores are depleted and erythropoiesis is impaired. As the iron deficiency progresses, a decrease in the hemoglobin concentration occurs because iron is unavailable for heme synthesis. Red blood cell indices, such as MCV, do not become abnormal for several months after tissue stores are depleted of iron, at which point iron deficiency anemia may be severe and symptomatic.

Slide 9:
Ferritin is recognized as the single best serum marker for iron deficiency. Its diagnostic performance is affected by the cutoff used and the population under evaluation. Ferritin is the primary iron storage protein so its measurement reflects iron stores. A ferritin less than 30 ng/mL has the highest positive predictive value for iron deficiency anemia. Measurement of ferritin by automated immunoassay is widely available. A ferritin less than 12-15 ng/mL has the highest specificity for early stage iron deficiency. The main limitation of ferritin is that it is a positive acute phase reactant so its concentration increases during infection and inflammation. This limits its discretionary ability in cases of iron deficiency anemia versus anemia of chronic disease (or inflammation).

Serum iron is also readily available from clinical laboratories and is frequently measured in the evaluation of iron deficiency or anemia. It is a measure of the total amount of iron in blood. Measurement of serum iron is limited by its wide within-day and day-to-day variation in individuals. The relationship of serum iron to transferrin (%Sat) is also used in the evaluation of iron deficiency anemia.

Slide 10:
Transferrin and TIBC are the other components typically found in routinely ordered ‘iron panels’. Transferrin is the primary iron transport protein so its measurement is a reflection of the amount of iron in circulation. Transferrin is normal until stages of latent iron deficiency. It is measured by

immunoassay and the percent (transferrin) saturation is calculated as the serum iron divided by TIBC, converted to a percentage. Transferrin is a negative acute phase reactant so its concentration decreases during stages of inflammation.

Total iron binding capacity (TIBC) is an indirect measurement of transferrin. It measures the amount of iron that can be bound by proteins. TIBC is calculated in one of two ways, which are equivalent. TIBC is the sum of the measured unsaturated iron binding capacity (UIBC) and serum iron or is the product of transferrin concentration and a conversion factor. The conversion factor is dependent upon the specific assay used and is derived from the type of transferrin standard used by each assay manufacturer.

Slide 11:

The evaluation of microcytic anemia begins with a thorough history and physical examination. The differential diagnosis includes iron deficiency anemia, thalassemia trait, anemia of chronic disease, and lead poisoning. Red blood cell indices and serum iron markers are used in combination to elucidate an etiology for the anemia. The characteristic features of all microcytic anemias is a low MCV. Iron deficiency anemia shows elevated RDW and low RBC count, results that may also be seen in lead poisoning. Ferritin, serum iron, and percent transferrin saturation will be low and TIBC will be high. A hallmark that is used often in pediatric primary care practice, in the absence of any testing, is the response to iron supplementation when hemoglobin indicates anemia. Iron deficiency anemia shows effective response when therapy is tolerated.

One can see from the table that a high degree of overlap exists among these markers for these different causes of anemia. Other lab tests may be used to assist in the diagnosis. Elevated blood lead is a specific marker for lead poisoning and many patients with thalassemia trait will show elevated Hb A_2 on hemoglobin electrophoresis. A diagnostic challenge exists in distinguishing iron deficiency anemia from that of anemia of chronic disease.

Slide 12:

This diagnostic quandary has led to interest in ‘other’ biomarkers of iron status. These include hepcidin, soluble transferrin receptor, and reticulocyte hemoglobin content. As previously described, hepcidin is the main regulator of iron in humans. Low or absent concentrations of hepcidin are touted as a potential early indicator of iron deficiency. However, assays are not standardized or widely available at this time.

Soluble transferrin receptor increases when the demand for iron is increased. This marker is of interest due to its potential to circumvent the acute phase reactant limitation of ferritin. Soluble transferrin receptors in anemia of chronic disease are not significantly different from normal, because transferrin-receptor expression is negatively affected by inflammatory cytokines. Some studies have demonstrated superior performance of the sTfR index, calculated as the ratio of soluble transferrin receptor divided by the log of ferritin, in distinguishing iron deficiency from anemia of chronic disease. A ratio of less than 1 suggests anemia of chronic disease, whereas a ratio of more than 1 suggests absolute iron deficiency coexisting with anemia of chronic disease. An index greater than 2 suggests iron deficiency anemia. Disorders of erythropoiesis, including sickle cell disease, will affect soluble transferrin receptor. Assays are available at reference laboratories but are not widely used in hospital clinical laboratories.

Reticulocyte hemoglobin content is calculated as the product of reticulocyte hemoglobin and reticulocyte volume. It provides a 'snapshot' of the amount of iron that was recently (~3 d) available for erythropoiesis. It is of limited use in those with red cell disorders, including thalassemias. Automatic calculation of CHr is currently available on some hematology analyzers, but its use is not widespread.

Slide 13:

The promise of other markers lies in their potential ability to distinguish iron deficiency anemia from that of chronic disease. Soluble transferrin receptor is markedly elevated in iron deficiency anemia but is normal in anemia of chronic disease. It has a theoretical benefit, in these cases, over ferritin since it is not affected by inflammation. Complex cases that involve concomitant iron deficiency and anemia of chronic disease are particularly challenging and difficult for ferritin to accurately diagnose, given its elevations in chronic disease. Soluble transferrin receptor and its index may be of value in such cases.

Reticulocyte hemoglobin content has shown utility as an early indicator of decreased iron stores and of response to iron therapy. Studies have shown it has high specificity and sensitivity for early iron deficiency, particularly in children.

All markers of iron status should be interpreted in the context of the patient's erythrocyte physiology, including knowledge of recent transfusions, iron therapy, vitamin B12 or folate deficiency, and the results of hemoglobin electrophoresis.

Slide 14:

A proposed algorithm for the evaluation of iron deficiency anemia is presented here, as published in a recent review article. In patients with microcytic anemia that require additional investigation for iron deficiency, testing should begin with serum ferritin. If ferritin is less than or equal to 30 ng/mL, probability is high that the anemia is due to iron deficiency. Although ferritin <15 ng/mL is associated with absent iron stores, using a cutoff of 30 ng/mL provides the best positive predictive value for iron deficiency. Ferritin greater than or equal to 100 ng/mL generally excludes iron deficiency. When ferritin is between these cutoffs, additional testing is needed to determine iron status and will be reviewed on the next slide.

Slide 15:

Results of other iron markers that are consistent with iron deficiency, including increased TIBC, low serum iron, and low transferrin saturation, support a diagnosis of iron deficiency anemia. If TIBC is decreased, serum iron is normal or high, and transferrin saturation is high, the anemia is unlikely due to iron deficiency. In cases that have mixed or unclear iron panel results, a soluble transferrin receptor test may be helpful. If this is increased, it suggests iron deficiency. If decreased, iron deficiency may be ruled out. Normal soluble transferrin receptor, in this situtation, is inconclusive and leads to decisions about additional investigation by erythrocyte protoporphyrin or possibly bone marrow biopsy for iron staining.

Slide 16:
The underlying cause of iron deficiency should be determined and managed. Iron deficiency is treated through supplementation, typically using oral ferrous sulfate unless a patient has a known malabsorptive state or cannot tolerate oral iron. Hemoglobin may rise 2-3 g/dL over a 1 month period with effective supplementation.

Approximately 25% of patients experience side effects from oral iron replacement, including GI upset, constipation, or diarrhea, that prevents them from tolerating doses needed to replenish stores. A stool softener may be used to aid with constipation and vitamin C is recommended to increase iron absorption. When needed, intravenous iron can be prescribed.

Slide 17:
This presentation covered key aspects of iron deficiency anemia and the associated laboratory evaluation. Some highlights include that iron deficiency anemia is a major US and global health issue, particularly for women and children. Iron deficiency anemia arises when the balance of iron intake, iron stores, and the body's loss of iron are insufficient to fully support production of erythrocytes. Iron deficiency is a continuum starting as iron depletion that progresses to anemia. Ferritin is the earliest serum marker of depleted iron stores but it may be falsely elevated due to inflammation. Newer markers of iron status, including hepcidin, soluble transferrin receptor, and reticulocyte hemoglobin content are not widely available at this time but may have some advantages over ferritin in the diagnosis of iron deficiency anemia.

Slide 18: References

Slide 19: Disclosures

Slide 20: Thank You from www.TraineeCouncil.org
Thank you for joining me on this Pearl of Laboratory Medicine on “Iron Deficiency Anemia.”