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# Practical Diagnosis of Iron Deficiency Anemia

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Both iron deficiency (ID) and iron deficiency anemia (IDA) are major global problems that are more prevalent in certain parts of Africa and Asia. Furthermore, they disproportionately affect children, women of child-bearing age and pregnant women [1–5]. Adverse effects in children include cognitive impairment, in pregnant women serious adverse outcomes for both mother and baby, and impaired physical capacity in adults. In this Editorial we briefly discuss iron physiology and metabolism, the clinical presentation including causes and rationale and practical laboratory testing.

Previously, we have detailed iron biology in a review article [6], here we briefly discuss physiology and metabolism. The normal circuit of iron begins in utero with the trans-placental passage of iron. By one week of embryonic life, hematopoiesis can begin. Postnatally, dietary iron is intestinally absorbed predominantly from the duodenum. During childhood and adolescence, the body must be in positive iron balance to provide iron for growth with an expanding red blood cell mass. Once growth is complete, iron absorption must match iron loss to prevent iron deficiency. Even without visible hemorrhage, iron is lost from the gastrointestinal tract as enterocytes are normally shed. Additionally, during their reproductive years, women need to absorb more iron than men because of menstrual blood loss. Excess iron is toxic so maintaining iron balance is important. There is no mechanism to excrete excess iron. Total body iron is controlled by absorption.

In the intestine dietary non-heme iron [i.e., ferric iron ($Fe^{+++}$)] is reduced to ferrous iron ($Fe^{++}$) by duodenal cytochrome b (Dcytb). However, the failure of a Dcytb-knock-out mouse to manifest iron malabsorption has questioned the role of Dcytb in iron absorption [7]. Ferrous iron is then transported into enterocytes via the dimetal ion transporter 1 (DMT-1). Iron as part of heme is more efficiently absorbed than elemental iron. Heme is absorbed by the transmembrane protein heme carrier protein-1. Iron absorption is facilitated by the complete digestion of food, low duodenal pH, and the absence of substances that can bind iron (e.g., clay or starch).

Once within the cytoplasm of the enterocyte, ferrous iron can be oxidized back to ferric iron to be incorporated into ferritin. Thus, the formation of iron-loaded ferritin will protect cells from the oxidative injury of excess free iron. In the circulation, there is very little free iron which would also be toxic.

As an alternative to iron being bound to ferritin, ferrous iron can be transported outside the enterocyte via the iron transporter ferroportin. Ferroportin is the sole transporter exporting cytoplasmic iron to the interstitium. Ferritin is also exported from the tissues to enter the circulation but circulating ferritin is relatively iron poor.

Ferroportin is regulated by hepcidin, a 25 amino acid polypeptide which is produced by the liver. Hepcidin inhibits iron export from enterocytes by binding to and internalizing ferroportin for degradation. Hepcidin release is increased when iron stores are high or inflammation is present as mediated by increased interleukin-6 (IL-6) or the BMP/SMAD/hemojuvalin pathways [8]. In contrast, iron deficient states with low transferrin saturation, increased bone marrow erythropoietic activity (mediated by bone-marrow produced erythroferrone and possibly growth differentiation factor 11), and states of low tissue oxygen tension reduce hepcidin levels.

Once in the interstitium, ferrous iron is oxidized to ferric iron for systemic transport on transferrin. In the gut, the membrane protein hephaestin oxidizes ferrous to ferric iron. At this point, transferrin-transported iron can supply iron to the tissues, liver and bone marrow.

Concerning tissues, hepatocytes and the bone marrow, transferrin binds to the transferrin receptor-1 of cells and the transferrin-transferrin receptor complex is internalized. In bone marrow developing erythroblasts, the cytoplasmic iron is used for heme synthesis that contributes to hemoglobin production.

Hepatocytes also monitor iron stores. The degree of transferrin saturation regulates hepcidin secretion. Transferrin saturation is assessed via several cell-surface proteins: the transferrin receptor-1, the transferrin receptor-2 and the protein HFE (the homeostatic iron regulator) whereas liver sinusoidal endothelial cells assess tissue iron. In other words, if iron is abundant, hepcidin levels rise reducing intestinal iron absorption. However, if iron is deficient, hepcidin levels decline allowing increased intestinal iron absorption.

The majority of total body iron is located in the circulating red blood cell (RBC) pool. With the "death" of RBCs in the spleen, the iron is recycled back to transferrin for recirculation to the tissues, liver and bone marrow. Splenic macrophages recycle all of the components of the RBC except for the protoporphyrin IX moiety which is degraded to bilirubin.

Just as the transport of iron to the outside of enterocytes is controlled by the ferroportin-hepcidin axis, so to is iron export from splenic macrophages so controlled. The oxidation of newly exported iron in the spleen is accomplished by the ceruloplasmin.

Clinically patients with IDA can present with the non-specific symptomatology of anemia including pallor, fatigue, poor concentrating ability, dyspnea and headache. Other less common symptoms include atrophic glossitis, dry skin, tachycardia, angina pectoris and neuro-cognitive impairment [2,4,5]. Unique to IDA are koilonychia (flattening of the nails) and Plummer-Vinson or Kelly-Paterson syndrome, which appears to be more common in women, comprising IDA, dysphagia and esophageal webs and predisposes to squamous cell carcinoma. Also iron deficiency can predispose to infection and precipitate heart failure.

There are multiple causes of iron deficiency anemia as has been well detailed in recent reviews [1–5]. The causes of IDA are different in the developing world countries compared to developed countries and multiple factors can cause iron deficiency. Physiological causes include pregnancy and lactation, infancy, and menstruation. The most common cause of IDA in the western world is blood loss from the gastro-intestinal tract or less commonly the genitourinary system (colon cancer, peptic ulcers, hematuria, hemodialysis, menorrhagia, chronic aspirin or corticosteroid use, intravascular hemolysis, etc.). Also iron malabsorption from celiac disease, *Helicobacter pylori* infection, and proton pump inhibitor therapy can cause IDA. In developing countries dietary deficiency coupled with parasitic infections (e.g., hookworm infestation) can result in IDA. Also vegetarianism can result in low iron intake and ID. Obesity may also be associated with iron deficiency due to impaired absorption, inflammation, and elevated hepcidin levels [4]. Once the diagnosis of IDA is made, it is both imperative and mandatory to elucidate the cause(s). Treating IDA but missing the diagnosis of colon cancer will lead to a poor outcome for the patient.

Finally whilst rare there are genetic causes such as iron refractory iron deficiency anemia (IRIDA). The gene, transmembrane protease 6 (TMPRSS6), downregulates hepcidin. IRIDA, is an autosomal recessive disorder with loss of function mutations in TMPRSS6 resulting in increased levels of hepcidin which inhibits intestinal export of iron resulting in ID which is resistant to oral iron therapy. These patients have decreased TSAT with normal or low ferritin and microcytosis. In other rare genetic disorders there is defective iron absorption (e.g., ferroportin loss-of-function mutations and DMT-1 deficiency.), defective iron transport (e.g., atransferrinemia), defective iron loading onto transferrin (e.g., aceruloplasminemia) [9]

There is a large repertoire of laboratory tests that are available for the diagnosis of ID and IDA [2,4,10,11].

In this editorial we provide a practical and cost-effective approach to IDA.

Serum Ferritin is the best indicator of iron stores and 1.0 ug/L is equivalent to 8 mg of stored iron [4,5,10,11]. In uncomplicated ID, levels are usually <15 ug/L. However, a cut point of 30 ug/L improves both sensitivity and specificity to >90% for the diagnosis of ID. Both hypothyroidism and ascorbate deficiency can also result in lower ferritin levels [12]. Also ferritin levels are increased with acute and chronic inflammation, chronic renal failure, liver disease and malignancies. In these situations a level <100 ug/L is consistent with ID. This cut point in combination with other tests discussed below can help differentiate IDA from anemia of chronic disease (ACD) since hepcidin levels which are elevated or normal with ACD and low with IDA are not available as a standardized routine test in the majority of clinical laboratories globally.

Serum iron which circulates in the ferric form bound to transferrin displays a circadian rhythm with highest values in the morning and has major day to day fluctuations [10,11]. It is also decreased with inflammation and other chronic diseases. Thus, on its own it is not an accurate test to diagnose ID. However, it is very useful in combination with transferrin in determining percent saturation of transferrin (TSAT).

Transferrin is a beta-1 globulin produced by the liver. It has 2 iron binding sites and transfers iron to tissues by the transferrin receptor which is recycled back to the cell surface. The total iron binding capacity (TIBC) is calculated as transferrin (g/L) × 1.3 = TIBC (mg/L). While the iron binding capacity of plasma (due to transferrin)

can be chemically measured, it is more accurate to immunologically measure the transferrin concentration and calculate the "total iron binding capacity" (TIBC) based upon the ability of each transferrin homodimer to carry two ferric iron ions.

With ID there is an increase in plasma transferrin levels to facilitate iron transport. Transferrin saturation (TSAT) is the percentage of transferrin saturated with iron and is calculated as follows; serum iron/TIBC × 100. TSAT in healthy individuals ranges from 20–55%. It is decreased in ID <16% since iron levels are low and transferrin levels high. Transferrin levels are decreased with inflammation (negative phase reactant) and also with liver disease and malignancy. Thus, in the presence of inflammation, a TSAT of <20% is recommended to diagnose ID.

Serum soluble transferrin receptors (sTfR) are derived by proteolytic cleavage of the membrane bound receptors and is a valuable predictor of erythropoiesis. Thus in IDA, sTfR levels are increased since there is increase in the synthesis of transferrin receptors due to the increased demand for iron [4,5,10,11]. However, sTfR levels are usually normal with ACD since it is not altered by inflammation. A major issue with sTfR is that the assay is not generally available in most clinical laboratories, especially in the developing world, and needs harmonization and standardization. Also, levels are increased with increased erythropoiesis such as hemolytic anemia. Since sTfR levels are increased and ferritin levels decreased in IDA the ratio of sTfR /log Ferritin has been proposed to be a superior test. Values >2.0 are consistent with IDA and values <1.0 are present with ACD [5,10].

An essential test in the work up of suspected ID is the complete blood count (CBC). However the findings present are seen late in the course of IDA [4,10,13]. Features include anemia, microcytosis (decreased MCV), hypochromia (decreased MCHC), anisocytosis (increase in RBC width) and thrombocytosis.

Reticulocyte hemoglobin content can be useful in certain cases and appears to be a sensitive indicator of ID erythropoiesis if decreased. However this assay is not widely available. Also an increase in the percentage of hypochromic cells has been suggested to be a useful adjunct by some investigators but requires further validation

Zinc-Protoporphyrin (ZPP) and free erythrocyte protoporphyrin have been suggested to be useful by some investigators. However elevated levels occur with both ID and lead poisoning and also ACD.

A bone marrow aspirate is considered the gold standard to confirm iron deficiency [10]. The marrow is stained with Prussian Blue to determine iron content. Also it is not affected by inflammation. It is invasive, requires sufficient sample due to uneven iron distribution and is semi-quantitative. However it remains the final arbiter in confirming iron deficiency when all the above tests are inconclusive.

In conclusion, as reviewed above the most cost effective tests to diagnose ID and IDA in the majority of patients include the following: ferritin, TSAT, CBC and sTfR levels (depending on availability). In IDA, ferritin levels are low, TSAT is decreased and sTfR levels are increased (best test to differentiate from ACD) and anemia is present. The other tests mentioned above, including hepcidin, need better standardization and greater general availability before they can become part of a practical cost effective panel.

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Both I.J. and W.E.W. generated the original version and edited multiple iterations. Both authors have read and agreed to the published version of the manuscript.

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