Assessing Iron Deficiency in Adults

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Iron (Fe) deficiency remains as one of the major global public health problems for two reasons. It affects about one fourth of the world's population in both industrialized and developing countries, and its health consequences affect all sex and age groups to varying degrees. Even in spite of the efforts of governments and public health professional to stop anemia and Fe deficiency, both of them still remain endemic in many areas, and a complete elimination is far off reach.

In the elderly, the aging process is associated with changes in body composition and with physiological changes which are not due to any specific disease, but are just part of every one's cycle of life. Adequate dietary intake is fundamental for a good health and a good quality of life, while inadequate nutrition, neoplastic diseases, gastrointestinal problems and malabsorption will have a strong association with the elderly`s health. When studying the Fe status of this population, there are always two main issues that relate to them. 1- the presence of anemia chronic disease (ACD) and its confounding when measuring Fe status, and 2- a suddenly increase in the body Fe stores.

According to the Framingham study, " ACD is defined as a mild – to moderate, often microcytic, hypochronic anemia that accompanies most acute or chronic conditions of inflammation, infection, liver disease, and malignancy." Due to the fact that ACD mimics most of the Fe Deficiency Anemia (IDA) signs, it is a potential confounder for blood measures of Fe status when assessing the elderly population because they have a higher prevalence of disease.

Anemia in the elderly is more commonly associated with chronic inflammatory conditions than to Fe deficiency. Common cases of Fe deficiency anemia are usually caused by gastrointestinal blood loss from chronic use of drugs such as antiplatelets, anticoagulants and non-steroidal anti-inflammatory drugs (NSAIDS), such as aspirin, from lesions and tumors, and not just from an inadequate intake of the mineral.

The majority of the older population is likely to use NSAID quite frequent and this can cause some side effects because of the inverse association of aspirin intake and serum ferritin (SF).

Aspirin could be a confounder when measuring the Fe stores using SF. If Fe level is elevated, there is a high association to myocardial infarction (MI). The decrease in SF concentration due to aspirin intake may be done through occult blood loss and its anti-inflammatory action on cytokines, which induced ferritin production.

Fe depletion does not occur from one day to another, it is divided in three stages and each stage has its own biochemical test for assessment.

The three stages are:

Store Fe Depletion: it is a progressive reduction of the Fe stored in the liver. The level of transport of Fe and hemoglobin is normal. The biochemical test of serum ferritin level will show a depletion of stores.

Fe Deficiency without Anemia: there is a complete depletion of the Fe stores in the plasma. Fe supply of erythropoietic cells is reduced and a decrease in transferrin saturation occurs. Despite the decreases erythrocyte protoporphyrin increase. Hemoglobin levels decrease a little but it is still stable within the normal range. Adverse physiological consequences begin to occur in the individual.

Fe Deficiency Anemia: the Fe stores are empty and the level of Fe in circulation also declines. It is characterized by a decrease in serum ferritin, transferrin saturation, hemoglobin, and mean corpuscular volume (MCV), but there is an increase in erythrocyte protoporphyrin.

Since no single laboratory measure adequately characterizes an individual's Fe status, multiple measures are needed to determine the Fe reserves in the elderly. Serum ferritin concentration, trasnferritin saturation, free erythrocyte, protoporphyrin and MCV are among the tests used in addition to the two most widely used, which are hemoglobin and hematocrit. A discussion for each one with theirs pro and cons is essential to understand the reason of why one test is better than other, and why one test is not good enough to depict low levels of Fe in the elderly.

Hemoglobin (Hb)

Fe is an essential component of this O2 carrying protein to red blood cells. The measure of hemoglobin concentration in the body is the most used screening test for stage III of Fe deficiency anemia. A low Hb is associated with hypochromia. The use of Hb concentration in the elderly is complicated by changes that are associated with aging. The levels fall slightly as age increases and the sex differences seem early in life gradually decrease. This test is very insensitive due to the fact that the concentration only falls during extreme case of deficiency. It also has a low specificity because low levels can arise in chronic infections and inflammations, hemorrhage, protein energy malnutrition, B12 and folate deficiency and during other states such as overhydration.

Diurnal variations and cigarette smoking have been found to alter the Hb concentration. Methods applied for determination of the Hb levels include venous blood,

anticoagulated with EDTA, and an alternative is to use blood from heel, ear, or finger pricks. However, Hb is more precise using venous blood.

Hematocrit

Hematocrit is defined as the volume fraction of packed red cells and as well as hemoglobin measurement, it is not a suitable screening test for Fe deficiency. However, the difference between the two is that hematocrit levels will fall after Hb. So as a consequence of that, moderate Fe deficiency, a low Hb value may be associated with a near normal hematocrit and only in a severe deficiency both index will be reduced.

Some limitations for this test are insensitivity of the method and lack of specificity because it is also affected by all the factors that interfere with Hb, values are also dependent on age and sex (female having approx. 40% hematocrit between age 55-70 and male having approx. between 43-44% at the same age group). Overall, the method is not very precise particularly when capillary blood samples are used.

There is an association of lower white cells counts with bodies' hematocrit, which is seen as an evidence for a decreasing hemopoietic reserve in both sexes.

Some advantages of hematocrit are how easy to use it is and how fast one can get the results and it can also detect lower rates of anemia that previous Hb test could not identify because of either technical error.

Serum Iron

Serum Fe together with total iron binding capacity (TIBC) and transferrin saturation are the best indicators of nutritional deficiency of Fe and Fe deficiency arising from chronic infections, inflammations or chronic neoplastic diseases.

Serum Fe and TIBC indicates the Fe movement from reticulo endothelial system to bone marrow and it is a measure of the number of atoms of Fe bound to the protein trasnferrin. Generally, Serum Fe and TIBC are performed simultaneously and the % of transferrin saturation is the result of those two: **Serum Fe (**micromoles per liter) **/ TIBC** (micromoles per liter) x 100 = transferrin saturation (%)

Total Iron Binding Capacity

It has almost the same characteristics of serum Fe, but it is related to the total number of free Fe binding site on transferrin.

These three tests are influenced by several factors such as age, sex and oral contraceptives agents. Serum Fe and TIBC are age dependent, serum Fe rises in childhood and TIBC decreases at the same period of time. Despite this marked difference, both indices are at their highest in adults, but declines with the aging process. Sex does not affect either of them by much and sometimes there is no difference. However, women taking oral contraceptives were found to have an elevated level of TIBC, which is one of the characteristics for Fe deficiency.

Stages in Iron Deficiency Development and Changes in Biochemical Indices for Normal Individuals

Test	**1st Stage** Iron Depletion	**2nd Stage** Iron Deficient Erythropoiesis	**3rd Stage** Iron Deficiency Anemia
Reticuloendothelial Marrow Iron	↓	↓	↓
TIBC	↑	↑	↑
Ferritin	↓	↓	↓
Iron Absorption	↑	↑	↑
Serum iron	Normal	↓	↓
Transferrin saturation	**Normal**	↓	↓
Protoporphyrin	**Normal**	↑	↑
Erythrocyte Measures (MCV, etc.)	**Normal**	**Normal**	**Microcytic/ Hypochromic**

Differences in Lab Values for IDA vs. ACD

Test	**Iron Deficiency Anemia**	**Anemia of Chronic Disease**
Reticuloendothelial Marrow Iron	Low	Normal unless Iron deficient
Serum iron	Low	Low end of NL
TIBC	High	Low
Transferrin saturation	Low	Low
Iron Absorption	High	Normal
Ferritin	Low	Normal to High
Protoporphyrin	High	High
MCV	Low	Normal
MCHC	Low	Normal
MCH	Low	Normal
Tfr	High	Normal

****Note: High and Low mean Elevated (High) or Lower than Normal (Low)**

Erythrocyte protoporphyrin- Protoporphyrin IX, precursor of heme, found in low concentrations in erythrocytes. Protoporphyrin IX builds up in developing erythrocytes when iron supply is not sufficient to meet demands of heme synthesis.

Pros:
- Sensitive indicator of inadequate iron supply
- Same measurement as transferrin saturation, but more stable and responds more gradually to changes in iron supply available to the marrow
- Seen in second stage of iron deficiency
- Differences b/t sexes small
- Can be measures rapidly and is relatively cheap involving a portable hematofluorometer
- Only requires a single drop of blood
- Corresponds to severity of progression of disease and iron supply

Cons:
- Nonspecific-cannot be used to differentiate b/t anemia of iron deficiency and that occurring in chronic inflammatory disorders (mucosal block defect)-both lead to elevated levels
- Response seen a week after transferrin saturation values fall below 15%
- Greatest range between values seen in children and the elderly
- Several readings are required until stable values reached
- Values fluctuate depending on method used
- Requires other methods to determine deficiency

Zinc Protoporphyrin:Heme Ratio-Protoporphyrin IX, which binds iron to form heme portion of hemoglobin, also binds zinc. As iron becomes depleted, an increase in zinc binding occurs in place of iron. Iron stores are considered depleted when zinc:heme ratio reaches 1 in 12,000 vs. 1 in 20,000 in normal individuals.

Pros:
- Only requires a single drop of blood
- Relatively cheap and can be done anywhere with portable machine (hematofluorometer)
- Not affected by recent blood loss or hydration status b/c not concentration based, but rather by a ratio
- Can be seen in second stage of iron deficiency
- Very stable in iron-replete individuals
- Depletion of storage pool can be monitored
- Minimally affected by factors that alter other iron assessment measures

Cons:
- Lead poisoning and chronic inflammation raise levels
- Cannot be used alone to diagnose deficiency

Serum Ferritin-iron storage protein that grabs iron that has not yet gathered in the liver, spleen, or marrow. It is used to store iron in cell and storage form found in cell. As iron supply increases, intracellular ferritin levels increase to accommodate iron storage. A small amount of ferritin leaks into circulation, by an unknown mechanism.

Pros:

- Parallels total amount of storage iron in most individuals-reflects severity until iron stores depleted
- Only index that can reflect deficient, excess, or normal iron status
- Correlates with bone marrow smears ("gold-standard")
- In healthy individuals, most sensitive (non-invasive) test of iron deficiency
- Fall in levels seen in first stage or iron deficiency and before changes seen in serum iron or TIBC
- Low concentration is characteristic **only** of iron deficiency (levels are only low when iron deficient)
- Levels less than 12 ug/L almost always indicative of depleted iron stores
- Iron overload associated with ferritin synthesis and serum levels
- Elevated levels useful in diagnosing iron overload disorders
- Diurnal variations small
- Sensitive ELISA methods now developed that require only small sample
- More precise methods being implemented

Cons:

- Significant inter-subject variation b/t ferritin and tissue iron stores
- Once iron stores depleted, severity of iron deficiency no longer reflected
- In more than ½ of elderly patients with IDA levels are high or w/in reference
- Increased ferritin synthesis occurs with infection, inflammation, or certain neoplastic diseases, therefore, in populations where mucosal block defect occurs with deficiency, ferritin should not be used. Values may be in the NL range despite deficiency
- In cases such as those above, protoporphyrin, and serum iron or TIBC must be used as well to determine deficiency
- Decreased erythropoiesis which occurs in other deficiencies or in presence of certain drugs, serum ferritin levels may remain NL from depressed Hgb utilization
- Increased erythropoiesis is associated with NL levels, which decline slowly as storage iron starts to be used up. Rapid changes occur to serum iron and TIBC comparable to those seen in iron deficiency. This requires all measurements to be used in diagnosis.
- Abnormally high levels seen in acute and chronic liver disease. This does not reflect a high intracellular concentration of ferritin.
- Raised levels seen in Hodgkin's disease and leukemia
- Not reliable test in the elderly b/c levels increase with age and lower reference range in this age group not well defined
- Levels increase in women after menopause and in men after adolescence
- Expensive-assayed using a 2-site immunoradiometric method that requires a gamma counter and trained person

Reticuloendothelial Bone Marrow Aspiration Sample-bone marrow sample stained with Prussian blue allows for definitive diagnosis of IDA. Less than 10% normoblasts signify deficiency.

Pros:
- "Gold standard"
- Definitive test
- Stain is sensitive to iron we are interested in

Cons:
- Painful and invasive-not safe for the elderly
- Expensive
- Not useful in field studies

Future Methods

Serum Transferrin Receptor (sTfR) Test-Tfr is expressed on surface of all body cells, particularly rapidly dividing cells like erythroid (RBC precursors) and placenta. About 15-80% present on erythroid marrow. The receptor protein binds holotransferrin (transferrin + Fe(III)), during cellular iron uptake. The number of Tfr on cell surfaces controls the uptake of extracellular transferrin-bound iron by receptor mediated endocytosis. Tfr returns to the cell surface once iron is inside the cell. When reticulocytes mature into RBC's, Tfr is lost through exosomes and released into circulation. Soluble Tfr found in the circulation is broken extracellular part that was proteolytically cleaved. It can be detected in serum or plasma attached to transferrin. When iron levels are low, Tfr production increases in hopes of "latching" on to whatever iron is available.

Pros:

- Tfr are direct quantitative proportion to total iron tissue content
- Serum levels reflect the amount of membranous Tfr, which inversely correlates to iron storage levels
- Tfr not affected by inflammation, and can adequately assess iron status in the normal state and during overload as well
- Study shown to have high specificity (93%) and sensitivity (88%) for IDA diagnosis in elderly vs. low sensitivity (16%) seen in other lab measures used (serum iron, transferrin saturation, and ferritin)
- Assesses iron available for erythropoiesis
- Seen in 1st stage of iron deficiency (iron depletion) and exceeds all other tests in diagnosis

Cons:

- Value of serum Tfr measurement for IDA not precisely defined in elderly
- Most studies done on younger populations
- Level of cell expression and rate of proteolytic cleavage may be different in the elderly
- About 5 times more expensive than routine lab tests
- A NL finding with Tfr in elderly patients with inflammatory variables, does not exclude IDA, therefore marrow smear should also be conducted
- Not widely used and accepted yet

Hemoglobin

Pros

- Most use screening test for stage III of Fe deficiency anemia
- Several methods apply for determination of Hb, such as venous blood, anticoagulated with EDTA, and another alternative is to use blood from heel, ear or finger pricks.
- More precise is using venous blood
- Sex differences is very little

Cons

- Complicated to use with the elderly due to the changes of aging
- Levels fall as age increase
- Insensitive test because the concentration falls in extreme cases of deficiency
- Low specificity, levels can arise in chronic infections, inflammations, hemorrhage, proteins energy malnutrition, B12 and folate deficiency and overhydration
- Diurnal variations and cigarette smoking alter the levels.

Hematocrit

Pros

- Easy to use
- Fast results
- Ability to identify rates of anemia that Hb could not because of technical errors

Cons

- Not suitable screening test for Fe deficiency because levels fall after Hb levels
- In moderate Fe deficiency a low Hb level might be associated with a near normal hematocrit
- Only in severe deficiency hematocrit levels are reduced
- Very insensitive method
- Lack of specificity because it is also affected by the same factors that affect Hb.
- Values depend on age and sex (females have approx. 40% hematocrit levels at age 55-70 and males have 43-44% at the same age)
- Not a very precise method if blood capillaries are used.

Serum Iron

Pros

- Together with TIBC and trasferrin saturation are the best indicators of Fe nutritional deficiency and Fe deficiency from chronic disease infections, inflammation or/and chronic diseases.
- Measure the number of atoms bound to the protein transferrin
- Indicates the Fe movement from reticulo endothelial system to bone marrow

Cons

- Influenced by sex. Not by a whole lot but in some cases it can make a difference.
- Influenced by age, it rises in childhood but decreases in adulthood.
- Influenced by oral contraceptives agents

Total Iron Binding Capacity (TIBC)

Pros

- Together with serum Fe and transferrin saturation are the best indicators of Fe nutritional deficiency and Fe deficiency from chronic disease infections, inflammation or/and chronic diseases.
- It is related to the number of free Fe binding on trasferrin.

Cons

- Influenced by sex but it does not make a big difference.
- Women taking oral contraceptives have an elevated TIBC, indicating Fe deficiency.
- Influenced by age, TIBC decrease in childhood but it then catch up with Serum Fe.

Transferrin Saturation

Pros

- It depends on Serum Fe and TIBC

Cons

- Because it is a ratio of those two previously mentioned, any change can affect the result of the test