EDUCATIONAL COMMENTARY – ASSESSMENT OF IRON STATUS

Educational commentary is provided through our affiliation with the American Society for Clinical Pathology (ASCP). To obtain FREE CME/CMLE credits see the Continuing Education menu on the left side of the screen.

LEARNING OUTCOMES

Upon completion of this exercise, the participant will be able to

- list and discuss the symptoms of iron deficiency anemia.
- list and discuss laboratory tests used for the assessment of iron status.
- interpret results of laboratory tests used for the assessment of iron status.

Iron

Iron exists in the body in the ferrous (Fe++) or ferric (Fe+++) states and serves as an oxygen carrier and as a participant in oxidation and reduction reactions. Approximately 2.5 g of the 3 to 5 g of iron in the body is present in hemoglobin, but it is also present in myoglobin and several enzymes. Iron is converted to the ferric state and is transported by the plasma protein transferrin. Typically only approximately 3 mg of iron is found in plasma attached to protein. Ferritin, a protein found in nearly all cells of the body, is the major iron storage compound.

Deficiency

Iron deficiency may result from decreased iron intake, increased blood loss, or decreased release from ferritin. Anemia is the primary manifestation of iron deficiency, and approximately 500 million people are affected worldwide. Infants, children, and pregnant women have an increased risk of iron deficiency anemia (IDA) due to increased requirements. In women of reproductive age, blood loss during the menstrual cycle increases the risk of IDA. Symptoms of IDA and other anemias include chronic fatigue, weakness, dizziness, headaches, shortness of breath, tinnitus, drowsiness, and irritability. In severe cases chest pains and even heart failure may occur. IDA-specific symptoms include pica (cravings for substances such as licorice, chalk, dirt, or clay), smooth tongue or a burning sensation in the tongue, sores at the corners of the mouth, and brittle and/or spoon-shaped fingernails and toenails.

Toxicity

Iron overload with tissue damage is classified as hemochromatosis and without tissue injury is considered hemosiderosis, but in practice the term “hemochromatosis" is used as a synonym for all types of iron overload. The classic iron overload disorder is hereditary hemochromatosis due to an inborn error of iron metabolism. Symptoms of hemochromatosis include fatigue, weakness, joint pain, loss of sex drive, and abdominal pain. Clinical features of hemochromatosis include diabetes mellitus, arthritis, cardiac arrhythmia or heart failure, hepatic cirrhosis, hypothyroidism, hepatic cancer, or hyperpigmentation. Treatment includes therapeutic phlebotomy or administration of drugs to bind the iron (chelators) such as deferoxamine.
EDUCATIONAL COMMENTARY – ASSESSMENT OF IRON STATUS (cont.)

Laboratory Detection
Complete blood cell count parameters observed in IDA include a decreased hemoglobin, hematocrit, and red blood cell count and observation of hypochromic, microcytic red cells. Biochemical indicators of iron status include serum or plasma iron, ferritin, iron-binding capacity (total or unsaturated), and transferrin saturation.

Serum or Plasma Iron
Serum or heparinized plasma may be used for the measurement of iron, which is almost exclusively the Fe+++ bound transferrin. Anticoagulants such as oxalate, citrate, and EDTA that bind iron are unacceptable. The most common methods for measurement of serum/plasma iron are spectrophotometric. Typical steps include initial lowering of the pH to release iron from transferrin, reduction of released iron from the ferric to the ferrous state, and reaction with a chromogen to form a colored complex that can be measured spectrophotometrically. Chromogens that have been used include bathophenanthroline, ferrozine, tripyridyl triazine, ferene, and chromazurol B. Measurement of serum iron is usually performed in conjunction with determination of the iron-binding capacity using a modification of the method used for iron determination.

Total Iron-Binding Capacity (TIBC) and Unsaturated Iron-Binding Capacity (UIBC)
Normally only about one third of the iron-binding sites of transferrin are occupied by Fe+++, leaving a substantial capacity for further binding. This capacity can be measured and expressed as either total or unsaturated iron-binding capacity. Measurement of TIBC includes saturation of the binding sites by addition of Fe+++, removal of the excess iron by precipitation or absorbants such as alumina, and measurement of the iron bound to protein using spectrophotometric iron detection methods. The necessity of pretreatment and/or removal of excess iron make total automation of these procedures difficult. Automation is easier to achieve if the unbound or unsaturated iron-binding capacity (UIBC) is measured. UIBC methods typically include addition of a known concentration of iron to saturate the transferrin followed by measurement of the excess iron. The difference between the unbound iron and the known concentration added is the UIBC. Because TIBC equals serum iron plus UIBC, determination of serum iron plus either TIBC or UIBC allows calculation of the other binding capacity. Measurement of UIBC followed by calculation of TIBC allows automation but is dependent on accurate addition of a known concentration of iron solution. Development of direct TIBC methods allowed automation without the addition of the iron solutions of known concentrations. Direct TIBC methods utilize the pH-dependent difference in binding of iron to a dye (such as chromazurol B) and transferrin. Iron, released from transferrin in an acidic buffer containing ferric chloride and a dye, forms a colored complex with the dye. This complex includes both the serum iron and the excess iron in the reagent. The addition of a neutral buffer changes the pH and results in transferrin abstracting iron from the complex. The decreased absorbance of the dye-iron complex is directly proportional to the TIBC.
EDUCATIONAL COMMENTARY – ASSESSMENT OF IRON STATUS (cont.)

Transferrin Saturation
TIBC is an indirect measurement of transferrin. The ratio of serum iron to TIBC expressed as a percent is called the percent saturation or transferrin saturation. As the name suggests, this represents the percentage of transferrin that is saturated with iron.

Ferritin
The primary iron storage protein, ferritin, is measured by immunoassay methods. Although reference ranges for ferritin may be method and population specific, typically the range for adult males is higher than that for adult females.

Interpretation
The Table presents interpretive information for the tests discussed. The reference ranges listed are approximate, and each laboratory should determine ranges for its methodology and patient population. The most sensitive indicator of IDA is a decreased serum ferritin level, with concentrations <15 ng/mL (<15 µg/L) indicative of iron deficiency. The detection of IDA in the presence of conditions known to cause increased ferritin levels can be difficult. Conditions known to cause increased ferritin levels include chronic inflammatory disorders such as rheumatoid arthritis or renal disease, infections, malignancy, heart disease, and viral hepatitis. In the absence of a reliable ferritin level, a decreased serum iron and increased TIBC may be used for the detection of IDA, although these changes occur later than the decrease in ferritin levels. Measurement of serum iron and TIBC/UIBC/transferrin saturation are useful when screening for detection of early iron overload including hemochromatosis. In hemochromatosis, the iron, ferritin, and transferrin saturation are increased, and the TIBC/UIBC levels are decreased.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Iron, µg/dL (µmol/L)</th>
<th>TIBC, µg/dL (µmol/L)</th>
<th>UIBC, µg/dL (µmol/L)</th>
<th>% Transferrin Saturation</th>
<th>Ferritin, ng/mL (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal male</td>
<td>65-175 (11.6-31.3)</td>
<td>250-425 (44.8-76.1)</td>
<td>–</td>
<td>20-50</td>
<td>20-250 (20-250)</td>
</tr>
<tr>
<td>Normal female</td>
<td>50-170 (9.0-30.4)</td>
<td>250-425 (44.8-76.1)</td>
<td>–</td>
<td>15-50</td>
<td>10-120 (10-120)</td>
</tr>
<tr>
<td>Iron deficiency</td>
<td>Low</td>
<td>High</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Hemochromatosis</td>
<td>High</td>
<td>Low</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>Low</td>
<td>Low</td>
<td>Low / Normal</td>
<td>Low</td>
<td>Normal / High</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>High</td>
<td>Normal / Low</td>
<td>Low / Normal</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Sideroblastic anemia</td>
<td>Normal / High</td>
<td>Normal / Low</td>
<td>Low / Normal</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Iron poisoning</td>
<td>High</td>
<td>Normal</td>
<td>Low</td>
<td>High</td>
<td>Normal</td>
</tr>
</tbody>
</table>

EDUCATIONAL COMMENTARY – ASSESSMENT OF IRON STATUS (cont.)

SUGGESTED READING
Lab Tests Online. Iron tests; Anemia; Hemochromatosis. Lab Tests Online website: www.labtestsonline.org

© ASCP 2010