Microcytic Hypochromic Anemia
An Approach to Diagnosis
- Decreased hemoglobin synthesis gives rise to microcytic hypochromic anemias.
- Hypochromic anemias are characterized by normal cellular proliferation and DNA synthesis but decreased RBC hemoglobin production.
Causes of hypochromic anemia

- Disorders of iron metabolism
  - Iron deficiency
  - Chronic disease
- Disorders of heme synthesis
  - Sideroblastic anemias
- Disorders of globin synthesis
  - Thalassemic syndromes
Iron deficiency anemia
Common causes of iron deficiency by age

- Infants and children
  - Inadequate intake
  - Growth spurts with increased iron requirements
- Premenopausal women
  - Menstrual blood loss
  - Pregnancy
- Adult men and postmenopausal women
  - Blood loss due to tumor, peptic ulcer, GI or GU bleeding
  - Malabsorption
  - Inadequate intake
Sideroblastic anemia
Group of disorders characterized by impaired utilization of iron resulting in diminished heme synthesis.

The diminished heme synthesis resulting from impaired utilization of iron therefore results in a continued stimulus for iron absorption despite an adequate or increased level of intracellular iron.

Excess iron is deposited in the mitochondria forming ringed-sideroblasts.

Usually due to disturbance in the heme biosynthetic pathway
- Hereditary (either X linked or autosomal)
- Idiopathic (usually as a part of myelodysplastic syndrome)
- Secondary to toxic insult (drugs, lead, alcohol)
Anemia of chronic disease
Conditions associated with anemia of chronic disease

- Chronic infections
  - Tuberculosis, Subacute bacterial endocarditis, Osteomyelitis, Pyelonephritis, PID etc

- Chronic inflammatory disorders
  - Rheumatoid arthritis, SLE, Sarcoidosis, Rheumatic fever

- Neoplasms
  - Carcinoma, Malignant Lymphoma
- Anemia of impaired iron utilization in the presence of adequate or increased iron stores indicating sequestration of iron in the reticuloendothelial system.

- Diminished erythrocyte survival time.

- Inability of the bone marrow to compensate by increasing the rate of erythropoiesis.

- Thought to be due to release of cytokines such as IL-1 and TNF during chronic inflammation and neoplasia.
Thalassemia
• Thalassemic syndromes arise from an impairment in the synthesis of globin chains leading to quantitative decrease in the amount of hemoglobin within the cell.

• Thalassemia minor/trait can give rise to a microcytic hypochromic blood picture.
Diagnostic approach

- Examination of RBC morphology, indices and RBC size distribution
Hematologic findings in Iron deficiency anemia

- Iron deficiency anemia usually shows microcytic hypochromic picture with decreased MCV, MCH and MCHC.
- Decreased RBC number.
- Characteristic increase in RDW (moderate to marked anisopoikilocytosis).
- Pencil cells.
Hematologic findings in Thalassemia

- Thalassemia minor shows microcytic hypochromic picture with reduction in MCV and MCHC that is generally greater than those observed in the same level of iron deficiency anemia.
- Normal or increased RBC number.
- Normal RDW (no or mild anisopoikilocytosis).
- Target cells and basophilic stippling in peripheral smear.
Hematologic findings in Sideroblastic anemia

- MCV may be high in many sideroblastic anemias although microcytosis is more common in the hereditary types. Hypochromia is often present but is not a universal finding.
- RBC number is decreased.
- RDW is variable but a characteristic dimorphic population of normocytic or microcytic cells and macrocytes is seen in acquired forms.
- Basophilic stippling and occasionally dysplastic features may be noted in the WBCs in the idiopathic cases.
Anemia of chronic disease

- Anemia usually mild, Hgb ranging between 7-11 g/dl. MCV, MCH and MCHC may be normal or mildly decreased.
- RBC count is decreased.
- RDW is normal or near normal. RBCs vary very little in size.
- No distinct features on peripheral smear.
<table>
<thead>
<tr>
<th>Cause of Anemia</th>
<th>RBC Number</th>
<th>Red Cell Distribution Width</th>
<th>Anisopoikilocytosis</th>
<th>Basophilic Stippling</th>
<th>Bone Marrow Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>Decreased</td>
<td>Increased</td>
<td>Yes</td>
<td>No</td>
<td>Decreased</td>
</tr>
<tr>
<td>Thalassemia minor</td>
<td>Normal or increased</td>
<td>Normal</td>
<td>No</td>
<td>Yes</td>
<td>Increased</td>
</tr>
<tr>
<td>Sideroblastic anemias</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hereditary</td>
<td>Decreased</td>
<td>Variable</td>
<td>Variable</td>
<td>Yes</td>
<td>Increased ringed sideroblasts</td>
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<tr>
<td>Acquired</td>
<td>Decreased</td>
<td>Dimorphic population</td>
<td>Yes</td>
<td>Yes</td>
<td>Increased ringed sideroblasts</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>Decreased</td>
<td>Variable</td>
<td>Variable</td>
<td>No</td>
<td>Decreased in siderocytes; increased in RE cells</td>
</tr>
</tbody>
</table>

RE = reticuloendothelial
Other lab tests

- Serum iron
- Total iron binding capacity (TIBC)
- Percentage transferrin saturation
- Serum ferritin
- Serum soluble transferrin receptor
- Free erythrocyte protoporphyrin
- Hb electrophoresis / HbA2 levels
Serum Iron Quantitation and TIBC
Helpful in distinguishing between iron deficiency anemia and other types of hypochromic microcytic anemias.

In mild iron deficiency decreased serum iron levels usually precede changes in RBC morphology or in RBC indices.
Principle

- Serum iron measures transferrin bound iron.

- TIBC is the iron concentration necessary to saturate the iron binding sites of transferrin - is a measure of the transferrin concentration.

- \( \% \text{ Transferrin Saturation} = \left( \frac{\text{Serum iron}}{\text{TIBC}} \right) \times 100 \)
Specimen

Blood should be drawn in the morning owing to diurnal variations in serum iron levels. Serum is used for the determination.
Normal range of values

- Serum iron- 60-180 ug/dl
- TIBC- 250-410 ug/dl
- % transferrin saturation- 20-50%
## Interpretation

<table>
<thead>
<tr>
<th>Cause of anemia</th>
<th>Serum iron</th>
<th>TIBC</th>
<th>Percent saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Thalassemias</td>
<td>↑ / N</td>
<td>↓ / N</td>
<td>↑ / N</td>
</tr>
<tr>
<td>Sideroblastic anemia</td>
<td>↑</td>
<td>↓ / N</td>
<td>↑</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
</tbody>
</table>
Precautions in interpretation

- Serum iron concentrations show wide diurnal variations, with highest levels in the morning.
- A normal plasma iron level and TIBC do not rule out the diagnosis of iron deficiency when the hemoglobin level of the blood is above 9 g/dl in women and 11 g/dl in men.
Serum Ferritin Quantitation
Ferritin is a storage complex of the protein apoferritin and iron. The largest quantities of ferritin are found in the liver and reticuloendothelial cells. Serum ferritin concentration reflects the amount of stored iron.
Specimen

- Serum is used for testing
Normal concentration

- Serum ferritin - 10-500 ng/ml
Interpretation

- Serum ferritin levels are markedly decreased in iron deficiency anemia.
- Serum ferritin levels may be low in iron deficiency that is not associated with overt anemia.
- Serum ferritin is usually normal or increased in patients with anemia of chronic disease, reflecting their abundant storage iron.
Interpretation

- Elevated ferritin levels are common in iron overload states such as hemochromatosis and sideroblastic anemia.
- Serum ferritin levels are elevated in patients with inflammatory diseases.
Precautions in interpretation

- When iron deficiency and inflammatory disease coexist, serum ferritin levels may be in the normal range
Serum soluble transferrin receptor
Principle

- The transferrin receptor is a transmembrane protein that transfers iron from plasma transferrin into cell. A truncated form of the tissue receptor that is complexed with transferrin is found soluble in the serum. Transferrin receptor levels reflect iron status, with receptor synthesis being rapidly induced by decreased iron levels.
Specimen

- Serum is used
Interpretation

- Levels of serum soluble transferrin receptors greater than 3.1 mg/L have been used as an indicator of iron deficiency in most studies. This test is best used in combination with other tests of iron status (ferritin, TIBC and serum iron).

- Levels are not altered by inflammatory states and may provide a sensitive means to quantitate iron stores when borderline values for iron deficiency are obtained by other testing. This is useful in distinguishing between iron deficiency anemia and anemia of chronic disease.
Precautions in interpretation

- Elevated serum soluble transferrin receptor levels have been noted irrespective of patient status in patients with hematologic malignancies or conditions with increased effective or ineffective hematopoiesis (i.e., hemolytic anemias, hemoglobinopathies, or deficiencies of vitamin B12 or folate).

- Normal ranges for pregnant women and pediatric patients are not well established.

- Black patients and those living at high altitudes may have normal serum soluble transferrin levels 6% higher than upper normal limits.
<table>
<thead>
<tr>
<th></th>
<th>Serum iron</th>
<th>Serum ferritin</th>
<th>Soluble transferrin receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IDA</strong></td>
<td>decreased</td>
<td>decreased</td>
<td>increased</td>
</tr>
<tr>
<td><strong>ACD</strong></td>
<td>decreased</td>
<td>increased</td>
<td>normal</td>
</tr>
<tr>
<td><strong>IDA+ACD</strong></td>
<td>decreased</td>
<td>Increased/normal</td>
<td>increased</td>
</tr>
</tbody>
</table>
Free Erythrocyte Protoporphyrin (FEP)
Principle

- When insufficient iron is available for developing erythroblasts, excess protoporphyrin that was destined to be converted to heme accumulates as FEP. This accumulates both in iron deficiency and in conditions associated with an internal block in iron utilization, such as ACD, lead poisoning and sideroblastic anemias.
Specimen

- Whole anticoagulated blood is collected. There is also a spot test for blood specimens collected on filter paper.
Normal values

- FEP - <100 ug/dl
Interpretation

- Elevated levels are seen in patients with iron deficiency, chronic disease states associated with decreased transferrin saturation and acquired idiopathic sideroblastic anemia.

- Marked elevation in patients with sideroblastic anemia secondary to lead intoxication (>1000 ug/dl).

- In patients with microcytic anemias associated with abnormal globin synthesis rather than abnormal heme synthesis (such as thalassemia minor), FEP levels are normal.
Measurement of FEP may be useful as a screening test to distinguish between thalassemia and other causes of microcytic hypochromic anemias.
HbA2 levels
Principle

- Levels of HbA2 are elevated in thalassemia minor and are decreased in iron deficiency anemia
- HbA2 is measured using chromatography or HPLC
Specimen

- Anticoagulated whole blood
Normal values

- Normal range for HbA2 – 1.6% to 3.5%
- In beta-thalassemia, the range is – 3.5 to 8%
Precautions in interpretation

- A number of hemoglobin variants are co-purified under the usual test conditions (C, E, O, D).
- HbA2 levels may not be elevated in the presence of coexisting iron deficiency.