

Laboratory Studies in the Diagnosis of Iron Deficiency, Latent Iron Deficiency and Iron Deficient Erythropoiesis

Summary

- Iron deficiency is often the most common cause of anemia.
 - Clinical suspicion is often raised by noting low hemoglobin value on a complete blood count (CBC).
 - Further workup often includes investigating additional serum chemistries, including a ferritin, transferrin saturation, and calculating a total iron binding capacity.
 - A low ferritin is almost always indicative of iron deficiency.
 - Additional studies (discussed in detail below) can provide information to further elucidate the etiology of anemia if this information cannot be easily gleaned from a CBC and iron studies.
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Introduction

The laboratory studies listed below are helpful in the diagnosis and management of iron deficiency. The first section provides guidance on interpreting the red cell portion of the complete blood count (CBC). The second section describes common tests used to confirm the diagnosis of iron deficiency.

Reference values are provided, but these may vary from laboratory to laboratory. Please consult your laboratory's reference values before making clinical decisions. Reference ranges or reference values are often referred to as "normal ranges". However, these are not the same. Reference ranges, sometimes referred to as reference limits, are based on population studies and represent, in most cases, the mean test result for a given parameter in a population, plus or minus two standard deviations. A patient's test result may be within the reference range and still indicate an abnormal result. For example, some laboratories report iron saturation as low as 10% as within the reference range. This is a result of having included many iron deficient patients in the population used to establish the reference range. The values provided in this summary reflect test results that should precipitate further evaluation or clinical action.

Interpretation of the Red Cell Portion of the Complete Blood Count (CBC)

ERYTHROCYTE COUNT

The erythrocyte count or red blood cell (RBC) count is measured directly on modern instruments using either an electrical impedance or laser light-scatter methodology. Very few clinical situations result in a false elevation or false decrease in the total red blood cell count (RBC). The RBC count is decreased in iron deficiency anemia. It may have value in helping to distinguish iron deficiency anemia from thalassemia in patients with an unknown microcytic anemia. **In iron deficiency anemia, the RBC decreases in proportion to the decrease in hemoglobin concentration while in thalassemia the RBC may be normal or increased relative to the degree of anemia as indicated by the hemoglobin concentration.** In iron deficiency anemia the RBC count is usually less than 5.0×10^6 /uL while in thalassemia trait the RBC count is usually greater than 5.0×10^6 /uL. A simple formula for separating likely thalassemia from likely iron deficiency is as follows: MCV divided by the RBC; a value greater than 13 favors iron deficiency while a value less than 13 favors thalassemia.

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Interpretation

Reference Range: $3.8-5.2 \times 10^{12}$ /liter (female); $4.4-5.9 \times 10^{12}$ /liter (male)

FYI

- Extreme microcytosis, autoagglutination, in vitro hemolysis and clotting may cause a spurious low RBC.
- Cryoglobulinemia, giant platelets, and white blood count $> 100,000$ /microliter may cause a spurious high RBC.
- RBC is usually decreased in anemia from any cause.
- RBC is increased in primary and secondary polycythemia.

HEMOGLOBIN

Hemoglobin constitutes over 90% of the red blood cell. It is measured directly, usually by a spectrophotometer method. In the United States, hemoglobin is reported in grams per deciliter. Improper sample collection or specimen abnormalities can result in falsely elevated or falsely decreased hemoglobin values. **While the reference range for hemoglobin is lower in females than in males, consistent with many published reference ranges, women with hemoglobin values within the reference range may still be iron deficient.**

Interpretation

Reference Range: 12-15 g/dL (female); 14-17 g/dL (male)

FYI

- Lipidemia or cryoglobulinemia may cause a spurious elevation in hemoglobin.
- Clotted sample may cause a spurious decrease in hemoglobin.
- Hemoglobin is decreased in anemia.
- Hemoglobin is increased in primary and secondary polycythemia.

MEAN CORPUSCULAR HEMOGLOBIN (MCH) AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC)

These are calculated values of little clinical significance and usually “track” the MCV in patients with iron deficiency. The MCH and MCHC are used primarily for quality control purposes in the

laboratory. A low MCH or MCHC may be an indicator to look carefully at all the red cell indices and results of iron studies to avoid missing a diagnosis of iron deficiency.

MEAN CORPUSCULAR VOLUME (MCV)

MCV is the most useful of the red cell indices. **Anemia is classified as macrocytic (increased MCV), normocytic (normal MCV), or microcytic (decreased MCV) on the basis of the MCV.** The MCV is determined from the histogram of the red cell size distribution, based on electrical impedance, generated by the automated cell counter. The mean of the red blood cell distribution histogram is the MCV. (The coefficient of variation or the standard deviation of this size distribution is the Red Cell Distribution Width (RDW); see below).

The most common microcytic anemia is iron deficiency anemia. Less common causes of microcytosis include thalassemic syndromes, and some disorders of heme synthesis including hereditary sideroblastic anemia and acquired disorders of heme synthesis such as lead poisoning. On occasion, anemia of inflammation can be microcytic.

More frequently, anemia of inflammation or anemia of chronic disease presents as a normocytic anemia. Other common causes of normocytic anemia include some patients with iron deficiency, patients with hypothyroidism, and anemia associated with chronic renal failure. Anemia due to vitamin B12 deficiency or folate deficiency is typically macrocytic. Macrocytic anemia, especially with concomitant ovalocytosis, leukopenia and/or thrombocytopenia may signal the presence of a myelodysplastic process..

The MCV is a valuable tool in the initial classification of anemia and helps determine the most cost effective strategy for evaluating the etiology of a patient’s anemia. For example, the presence of microcytosis should lead to iron studies as part of the initial evaluation since vitamin B12 deficiency is uncommon in the setting of microcytosis. However, it is important to keep in mind that a patient’s anemia may be multifactorial and that the MCV may not follow the typical pattern

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in all patients with anemia. A patient may be normocytic and have either Vitamin B12 deficiency, iron deficiency, or both.

Interpretation

Reference Range: 80-100 femtoliters (fl)

FYI

- Macrocytosis (MCV > 100 fl) is associated with vitamin B12 deficiency and/or folate deficiency. Anemia secondary to myelodysplasia is usually macrocytic and associated with ovalocytosis, thrombocytopenia and/or leukopenia. Rarely, hemolytic anemia is associated with macrocytosis due to an increase in reticulocytes which are larger than mature red cells.
- Normocytosis (MCV between 80-100 fl) is most commonly associated with anemia of chronic disease or inflammation, but may be seen in hypothyroidism, chronic kidney disease, iron deficiency, and even vitamin B12 and folate deficiency.
- Microcytosis (MCV < 80 fl) is most commonly associated with iron deficiency or thalassemic syndromes.
- MCV should always be interpreted in the context of the other red cell indices, including the red cell distribution width and red cell shape.

RED CELL DISTRIBUTION WIDTH (RDW)

The RDW quantifies the variation in red cell size or (“anisocytosis”). It is derived from the red cell size distribution histogram, representing a value derived from the coefficient of variation (expressed in %) or, in some cases, the standard deviation (expressed in femtoliters). **In general, an elevated RDW has been associated with anemia from nutritional deficiencies such B12, folate or iron.** An increase in the RDW may be an early indicator of a deficiency in iron, B12 or folate, even before anemia appears. Myelodysplasia may also be associated with an increased RDW. Anemias secondary to thalassemia, chronic disease or inflammation more typically have a normal RDW. As with many laboratory parameters, there are several exceptions to these general guidelines and the RDW must be interpreted in the context of the remainder of the complete blood count (CBC).

Interpretation

Reference Range: CV: 11.6 – 14.6 %; SD: 39-46 fL

FYI

When anemia is present...

- A normal RDW and decreased MCV is associated with thalassemia.
- A normal RDW and normal MCV is associated with anemia of inflammation or chronic disease.
- A normal RDW and increased MCV is associated with aplastic anemia or liver disease.
- An elevated RDW and decreased MCV is associated with iron deficiency, or microangiopathic anemia.
- An elevated RDW and normal MCV is associated with transfusion, early iron replacement therapy, B12, or folate deficiency, some myelodysplastic and myeloproliferative disorders.
- An elevated RDW and increased MCV is associated with folate deficiency, B12 deficiency, hemolytic anemia, chemotherapy and many myelodysplastic syndromes.

RETICULOCYTE COUNT

The reticulocyte is a “young” peripheral red blood cell, having been released from the bone marrow, ejected its nucleus but still containing residual ribosomes. The reticulocyte percentage, adjusted for the degree of anemia, and especially the absolute reticulocyte count, may be used as a marker of erythropoiesis in the bone marrow. Anemia can be broadly categorized as one in which the bone marrow is able to regenerate red cells (increased reticulocyte count) it, or one in which the bone marrow is ineffective at regenerating red cells (hypoproliferative anemia, no increase in reticulocyte count).

An increase in reticulocytes is seen in acute blood loss or hemolysis when the patient is nutritionally replete with adequate iron stores, B12 and folate and there is an adequate erythropoietin response and marrow reserve. Lack of peripheral blood reticulocytosis is seen when the bone marrow is deficient in any of these factors or when the bone marrow itself is abnormal as in when it is replaced by tumor or the cells are

abnormal as in myelodysplastic or myeloproliferative states. Iron deficiency anemia is a hypoproliferative anemia.

The reticulocyte count expressed as a percentage must be corrected for the degree of anemia since the value is the number of reticulocytes per unit volume of blood divided by the total number of erythrocytes. In an anemic patient with no increase in marrow production of red cells in response to the anemia, the relative percentage of reticulocytes increases as anemia worsens. Absolute reticulocyte count requires no adjustment and is the preferred value. Reticulocyte percentage is usually reported when the laboratory provides a manual reticulocyte count while an absolute reticulocyte count is reported by the more accurate automated methods. Importantly, reticulocyte count can be used as an early indicator that the patient has the marrow reserves to respond to hemorrhage and as an early indicator of response to therapy such as intravenous iron replacement.

Interpretation

Reference Range: 25,000 – 85,000/microliter; 0.5-1.5 % (must be adjusted for degree of anemia)

FYI

- No increase in iron, B12, or folate deficiency anemia.
- No increase in anemia of chronic kidney disease.
- No increase in anemia of inflammation.
- No increase in anemia secondary to infiltrative marrow processes such as metastatic tumor.
- No increase in anemia secondary to myelodysplasia or chemotherapy.
- Increased in response to acute or chronic anemia when there is adequate available iron, B12, folate, nutritional reserves and erythropoietin response.
- Increased in response to effective treatment with intravenous iron, erythropoietic stimulating agents, or both.

RETICULOCYTE HEMOGLOBIN CONTENT (CHR) / RETICULOCYTE HEMOGLOBIN EQUIVALENT (RET-HE/RET-HB)

CHr and RET-He/RET-Hb are measures of the hemoglobin content in reticulocytes. The method used is flow cytometry. The hemoglobin content is measured on a per cell basis by dual angle light scatter. Measurement of CHr provides a real time snapshot of the iron directly available for hemoglobin synthesis, since reticulocytes circulate in the peripheral blood as reticulocytes for only 24-48 hours after release from the bone marrow.

Patients may have adequate storage iron and still have iron deficient erythropoiesis (functional iron deficiency). Traditional measures of iron status including ferritin, iron, and transferrin saturation may be difficult to interpret in the setting of acute inflammation. CHr is affected to a lesser degree by inflammation.

Interpretation

Reference Range: 27 -32 picograms (pg)

FYI

- CHr < 27 pg is consistent with iron deficient erythropoiesis.
- CHr is reported to have better sensitivity and specificity for diagnosing functional iron deficiency than traditional iron measurements of ferritin, iron, iron binding capacity and transferrin saturation.
- CHr may show a response to administration of erythropoietic stimulating agents or intravenous iron within 48-96 hours (usually a decrease with ESAs and an increase with IV iron).
- Inflammation may influence CHr results.

Chemistry Studies Useful in Evaluating Patients for Iron Deficiency States

FERRITIN, SERUM

Ferritin is a high molecular weight protein that consists of approximately 20% iron. It is found in all cells, but especially in hepatocytes and reticuloendothelial cells, where it serves as an iron reserve. A small amount is present in plasma and serum and reflects the adequacy of iron stores in

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normal individuals. Iron is released from ferritin and binds to transferrin for transport to developing red blood cells in the bone marrow. Inadequate iron stores results in a decrease in ferritin (in the absence of inflammation) and may result in iron deficient erythropoiesis.

While a low serum ferritin is widely viewed as the best single laboratory indicator of iron depletion, the result must be interpreted with caution in any patient with an underlying inflammatory process, as ferritin is an acute phase reactant, and is increased when an acute or chronic inflammatory process is present.

Virtually all patients with low serum iron and low serum ferritin have iron deficiency. Patients may have a low normal serum iron level and normal hemoglobin in the presence of decreased ferritin, indicating an iron-depletion state, before anemia develops. Ferritin may be useful in helping distinguish between iron deficiency anemia and functional iron deficiency (anemia of inflammation). While transferrin saturation is low in both iron deficiency and functional iron deficiency, ferritin is decreased only in iron deficiency and is normal or even increased in functional iron deficiency.

Ferritin is also a useful screening test to distinguish iron deficiency from thalassemia minor in patients with anemia and erythrocyte microcytosis; ferritin is decreased in iron deficiency and normal or increased in thalassemia.

Ferritin is increased in iron overload states and inflammation including hereditary hemochromatosis, acute hepatitis, Gaucher's disease, malignancy, etc.

Interpretation

Reference Range: 45-340 ng/dL

FYI

- Values less than 100 ng/dL usually indicate depleted iron stores.
- Ferritin is decreased in patients with an iron-depletion state.
- Ferritin may be normal to mildly increased in functional iron deficiency (anemia of inflammation) and in anemia secondary to thalassemia minor.
- Ferritin is increased in inflammation.

- Ferritin may be markedly increased in hereditary hemochromatosis and other iron overload states, acute hepatitis, and many malignancies and in Gaucher's disease.

C-REACTIVE PROTEIN (CRP), SERUM

C-reactive protein (CRP) is an acute phase reactant and a very sensitive indicator for inflammation. CRP is synthesized by the liver and consists of five identical polypeptide chains that form a ring with a molecular weight of 120,000 daltons. CRP can increase dramatically (100 –fold or more) in trauma, bacterial infection, rheumatoid arthritis and related diseases, inflammation, surgery, and neoplastic disease. It can be used to assess and monitor activity of inflammatory disease.

In the evaluation of patients with suspected iron depletion states, an elevated CRP helps with interpreting ferritin results; a normal ferritin is unreliable as an indicator of adequate iron stores in patients with an elevated CRP, since ferritin itself is an acute phase reactant. An elevated CRP may indicate an increase in serum hepcidin and functional iron deficiency in the presence of adequate serum iron or reticuloendothelial iron.

Serum CRP should not be confused with high sensitivity CRP (hs-CRP). The hs-CRP assay has been modified from the usual serum CRP assay to increase its low-end sensitivity and detect very minor elevations in serum CRP. Elevated hs-CRP has been associated with an increased risk of atherosclerotic cardiovascular disease and is used in cardiovascular risk assessment. It should not be used in the evaluation of iron deficiency states.

Interpretation

Reference Range: \leq 0.8 mg/L

FYI

- Elevated values are consistent with an inflammatory state.
- Oral contraceptives have been shown to increase CRP.
- CRP will increase within 2 hours of an acute insult (surgery, infection) and will begin to decrease within 48 hours if no other inflammatory event occurs.
- Chronic inflammation will result in a sustained elevation in CRP.

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Ingested iron is absorbed primarily from the intestinal tract, temporarily stored as ferritin in intestinal mucosal cells, and then released into the blood as Fe^{3+} - transferrin in equilibrium with a very small amount of free Fe^{3+} . Serum iron can be used as one test to evaluate patients for iron deficiency, especially in combination with iron binding capacity (transferrin and transferrin saturation). Serum iron alone is unreliable due to considerable physiologic variation in the results.

Many normal subjects demonstrate a predictable diurnal variation with highest values in the morning and lowest values in the evening. Values in an individual may vary 10-40% within a single day or day-to-day due to changes in iron absorption, timing of sample collection relative to meals or ingestion of iron supplements, marrow iron uptake, or storage iron outflow. Therefore, serum iron results should always be interpreted in the context of other studies.

Interpretation

Reference Range: 50-150 ug/dL

FYI

- Useful for diagnosis of iron depletion states especially when used in combination with transferrin and transferrin saturation.
- Can be used for evaluation of chronic iron overload states.
- Subject to physiologic variation including diurnal variation, and variation in response to iron therapy.
- Fasting morning serum iron may be more reliable and consistent than random samples without regard to meals, medication or time of day.

IRON BINDING CAPACITY, TOTAL

See Transferrin

PERCENT SATURATION

See Transferrin Saturation

SOLUBLE TRANSFERRIN RECEPTOR (STFR), SERUM

Transferrin receptors are present on the external surface of the plasma membrane. In order for iron to

be internalized into cells, the iron-transferrin complex binds to these receptors. It is then internalized through endosomes and the iron released into the cytoplasm. Proteolytic cleavage of the transferrin receptor releases a truncated version of the transferrin receptor as **soluble transferrin receptor** circulating in the blood.

Membrane expression of transferrin receptors (TfR) are regulated by iron status. There is increased expression of TfR in iron deficiency states and this results in an increase in serum soluble TfR as well. In iron repletion states, there is a decrease in membrane and soluble TfR.

TfR is not an acute phase reactant. While ferritin, which is an acute phase reactant, increases in response to inflammatory states, malignancy, infection, and chronic disease, soluble TfR is not affected by these confounding pathologies and may help determine the status of iron stores in patients with inflammation. sTfR should not be used routinely for evaluation of iron status as it is referral test for most hospital laboratories with a higher cost and slower turn-around time than ferritin, transferrin and transferrin saturation.

Interpretation

Reference Range: 1.8 – 4.6 mg/L

FYI

- Useful for evaluating iron status in patients with inflammation .
- sTfR concentration is inversely related to iron status; sTfR elevates in response to iron deficiency and decreases in response to iron repletion.
- Patients with hemolysis or recent blood loss may have falsely elevated sTfR levels.
- sTfR is elevated in patients with thalassemia and sickle cell disease. Caution should be exercised in interpreting sTfR in these patients
- African Americans may have a slightly higher reference range.

TRANSFERRIN, SERUM

Transferrin is the principle plasma protein for transport of iron. Its concentration correlates with the total iron-binding capacity of serum. For diagnosis of iron depletion states, transferrin and iron-binding capacity may be used interchangeably.

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Transferrin is synthesized primarily in the liver. In otherwise healthy individuals with iron depletion states, transferrin levels in serum increase due to an increase in synthesis. High levels can be seen in pregnancy and during estrogen administration. Decreased transferrin may be seen in chronic liver disease, malnutrition, and protein loss. It is important to note that transferrin is decreased in malignancy and in both acute and chronic inflammation.

Interpretation

Reference Range: 200-400 mg/dL

FYI

- Transferrin is significantly increased in iron depletion states.
- Transferrin is decreased in inflammatory states including anemia of chronic inflammation (functional iron deficiency).
- May be decreased in malnutrition, chronic liver disease, malignancy, and protein loss.
- High levels of transferrin may be seen in pregnancy (and may be an indicator of iron depletion) and during estrogen administration.

TRANSFERRIN SATURATION

Transferrin, the principal plasma protein for transport of iron, binds iron strongly at physiologic pH. Transferrin is generally 20-45% saturated with iron. The additional amount of iron that can be bound is the unsaturated iron-binding capacity (UIBC). The sum of the serum iron and UIBC represents the total iron-binding capacity (TIBC). TIBC is an indirect measure of transferrin concentration and the two terms are often used interchangeably. The transferrin saturation (TSat) is usually reported as percent saturation ($100 \times \text{serum iron}/\text{TIBC}$ or transferrin).

Interpretation

Reference Range: 20-45%

FYI

- Transferrin saturation less than 20% is indicative of an iron deficiency state, either, latent iron deficiency, functional iron deficiency (usually associated with a decrease in transferrin but a disproportionately larger decrease in iron resulting in a transferrin saturation < 20%) or true iron deficiency where a decrease in serum iron is associated with an increase in transferrin.
- Transferrin saturation > 45% may suggest hereditary hemochromatosis.
- Transferrin saturation > 45% may indicate an iron overload state (hemosiderosis) due to multiple transfusions or iron poisoning.
- A transient increase in transferrin saturation is seen after intravenous iron infusion. The duration is dependent on the type and dose of the iron infusion.

References

Greer JP, Arber DA, Glader BE, List AF Means RT and Rodgers G authors. *Wintrobe's Clinical Hematology*. 14th edition. Wolters Kluwer. Philadelphia, Pa; 2018.

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