Laboratory Studies for Diagnosing Iron Deficiency

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SUMMARY

- Iron deficiency is defined by the WHO as under 13g/dL for males and 12g/dL for women.
- 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.

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 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.

Interpretation

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

HEMOGLOBIN (HGB)

Hemoglobin constitutes over 90% of the red blood cell. 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)

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.

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 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.

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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 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)

RED CELL DISTRIBUTION WIDTH (RDW)

The RDW quantifies the variation in red cell size.

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.

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

An elevated RDW and decreased MCV is associated with iron deficiency anemia.

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.

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.

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.

Absolute reticulocyte count requires no adjustment and is the preferred value.

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)

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

CHr and RET-He/RET-Hgb 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.

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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)

Iron Studies

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 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.

Interpretation
Reference Range: 45-340 ng/dL

IRON, SERUM

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.

- Maal 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

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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.

TfR 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

TRANSFERRIN, SERUM

Transferrin is the principal 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.

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

TRANSFERRIN SATURATION (TSAT)

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 UBIC 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 x serum iron/TIBC or transferrin).

Interpretation

Reference Range: 20-45%

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Anemia Lab Values

Lab Value	Reference Range		Anemia		Iron Deficiency	
	Female	Male	Female	Male	Female	Male
Hemoglobin (Hgb)	12-15 g/dL	14-17 g/dL			< 12 g/dL*	< 13 g/dL
Mean Corpuscular Volume (MCV)	80-100 femtoliters (fL)		MCV > 100 is associated with B12 or folate deficiency or myelodysplasia (MDS) MCV < 80 is associated with iron deficiency and thalassemia		< 80fL MCV > 80 fL can also been seen in patients with iron deficiency MCV < 80 fL may be seen in thalassemia	
Red Cell Distribution Width (RDW)	CV: 11.6 – 14.6 %; SD: 39-46 fl		> 14 in nutritional deficiency anemias		>14 fl	
Reticulocyte Count	25,000 – 85,000/microliter 0.5-1.5 % (must be adjusted for degree of anemia)		Reticulocyte count < 75,000 with anemia indicates RBC loss with inadequate marrow response to correct anemia Reticulocyte count > 85,000 with anemia indicates RBC loss with an increased compensatory production of reticulocytes to replace the lost red blood cells			
Reticulocyte Hemoglobin Content (CHr)	27 -32 picograms (pg)				CHr < 28 pg	
Soluble Transferrin Receptor (sTfR), (serum)	1.8 – 4.6 mg/L		Usually normal in absence of iron deficiency		>5.0 mg/L	
Ferritin (serum)	45-340 ng/dL		Increased in inflammation independent of iron status		< 100 ng/dL	
Transferrin Saturation (TSAT)	20-45%				<20%	
C –Reactive Protein (CRP), (serum)	≤ 0.8 mg/L		> 0.8 mg/L = inflammatory state		Independent of iron status	

*WHO defines anemia in women as Hgb < 12 g/dL but there are many women who are iron deficient, have a Hgb > 12 g/dL and whose Hgb increases by 1 g/dL or more when treated with iron.

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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