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Serum Transferrin Receptor Assay in the Diagnosis of Iron Deficiency

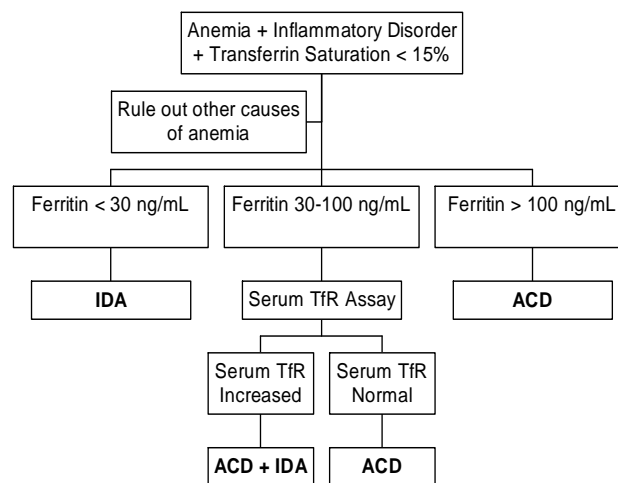
Transferrin receptor (TfR) is present on the surface of all cells, with the highest concentration found on erythroid precursors. Soluble TfR (sTfR) is detectable in the plasma, and its concentration increases in iron deficiency. Other causes of increased sTfR include increased erythropoiesis, such as occurs in hemolytic anemia, megaloblastic anemia and myelodysplastic syndrome. Causes of decreased sTfR include iron overload and decreased erythropoiesis, such as occurs in aplastic anemia and bone marrow ablation.

Serum ferritin and sTfR levels reflect different stages of iron deficiency. In early iron deficiency serum ferritin levels decline as iron stores decrease. Only when iron stores are fully depleted do sTfR levels begin to rise, reflecting tissue iron deficiency and iron-deficient erythropoiesis. Only at this late stage will transferrin saturation, MCV and hemoglobin begin to decrease.

The distinction between iron deficiency anemia (IDA) and anemia of chronic disease (ACD) is of great clinical importance, since unexplained IDA warrants extensive evaluation of the GI tract for a possible source of chronic blood loss. ACD is the second most prevalent cause of anemia, after IDA. It occurs in patients with acute or chronic immune activation, and is associated with increased uptake and retention of iron by cells of the reticuloendothelial system, with subsequent decreased availability of iron for erythropoiesis. Standard measures of iron status (specifically serum transferrin and ferritin levels) are often adequate for this differentiation, as shown below.

Lab Test	IDA	ACD
Serum iron	↓	↓
Transferrin	↑	↓ / N
Transferrin sat. %	↓	↓
Ferritin	↓	↑ / N

In patients who have a known chronic inflammatory disorder standard measures of iron status may not be able to clearly identify iron deficiency, especially when it coexists with ACD, due to a misleading acute phase elevation of serum ferritin. A bone marrow examination may be required to evaluate iron stores. In this situation sTfR is very useful, because the level increases when iron deficiency is present, even when it coexists with ACD. sTfR is normal in ACD alone. There is evidence that a serum ferritin <30 ug/L in patients with anemia and concurrent inflammatory disease is a reliable indicator of iron deficiency (92-98% PPV). On the other hand, iron deficiency is unlikely if the ferritin level is >100 ug/L. The following algorithm illustrates the recommended approach to the differential diagnosis of anemia in patients with inflammatory disorders. With this approach, the need for a bone marrow examination to evaluate iron stores may be avoided in many cases.



Recently, there has arisen an interest in computing a ratio of sTfR to log ferritin, termed the sTfR/ferritin index. This value is reportedly <1.0 in ACD, and >2.0 in IDA (alone or coexistent with ACD). This index may become widely adopted in the future, following validation by each laboratory.

Metanephrines for Pheochromocytoma

Pheochromocytomas occur in about one of every 1000 hypertensive patients; about half of those affected have paroxysmal hypertension. These tumors secrete the catecholamines, epinephrine and norepinephrine. Most tumors secrete more norepinephrine than epinephrine. Both compounds are inactivated by catechol-O-methyl transferase to form normetanephrine and metanephrine. Plasma metanephrines exist as free (<7%) and conjugated (93%) forms. Some normetanephrine and metanephrine is converted to vanillylmandelic acid (VMA). Urine contains small amounts of catecholamines and much larger amounts of metanephrine, normetanephrine, and VMA.

The biochemical diagnosis of pheochromocytoma depends on the demonstration of excessive production of these hormones. Measurement of metanephrines is more sensitive than measurement of catecholamines because they are more stable. The most reliable screening tests for diagnosis of pheochromocytoma is measurement of plasma free fractionated metanephrines and urinary fractionated metanephrines, in which normetanephrine and metanephrine are quantitated separately. In patients that are highly suspect for pheochromocytoma the best strategy is to initially test for plasma free fractionated metanephrines because it is the most sensitive assay. The 24-hour urinary fractionated metanephrines, which is a more specific assay, should be used as the first test for lower suspicion cases and also as a confirmatory study in patients with <2-fold elevation in plasma free fractionated metanephrines.

Test	Sensitivity	Specificity
Plasma free metanephrines	99%	89%
Urine fractionated metanephrines	97%	69%
Urine catecholamines	86%	88%
Plasma catecholamines	84%	81%
Urinary total metanephrines	77%	93%
VMA	64%	95%

The high sensitivity of plasma free or urinary fractionated metanephrines means that normal test results almost always exclude the presence of

pheochromocytoma. Exceptions include asymptomatic small tumors that produce negligible amounts of norepinephrine or epinephrine and patients who have already been treated with metyrosine.

Elevated metanephrine levels do not always indicate pheochromocytoma because they may be caused by physical stress or medications. Many of the patients being tested for pheochromocytoma have underlying clinical conditions that are associated with increased sympathetic activity such as hypertension, heart failure, stroke, baroreflex failure and cardiogenic shock.

Drugs that increase endogenous catecholamine levels include:

- Monoamine oxidase inhibitors
- TCA, levodopa and beta blockers
- Catecholamine reuptake inhibitors including cocaine & local anesthetics
- Some anesthetic gases, especially halothane
- Withdrawal from alcohol, benzodiazepines, opioids and central acting anti-hypertensives

Usually these conditions can be distinguished from pheochromocytoma by the magnitude of the metanephrine level. Hypertensive patients without pheochromocytoma may have total urinary metanephrine levels up to 1300 ug per 24 hours, while patients with pheochromocytoma usually have higher levels. Further clinical investigation and radiographic studies are warranted for patients with total metanephrine values >1300 ug/24 hours. Pheochromocytoma is likely if the plasma free normetanephrine level is >1.40 nmol/L (reference range is <0.9) and if the plasma free metanephrine level is >0.5 (reference range <0.5 nmol/L).

For a patient with episodic hypertension, specimen collection should ideally begin with the onset of symptoms. If possible, medications associated with elevated catecholamines should be discontinued at least one week prior to specimen collection. Specimen requirement for plasma fractionated free metanephrines is one lavender top (EDTA) tube of blood. Specimen requirement for urinary fractionated metanephrines is 10 mL of urine from a 24-hour urine specimen collected in a container with 10 g of boric acid or 25mL of 50% acetic acid. Urine specimen should be kept cold during collection.