

Chapter I.A.1: Thyroid Evaluation — Laboratory Testing

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THYROID FUNCTION TESTS

Overview

Thyroid-stimulating hormone (TSH) is produced by the anterior pituitary gland and is responsible for thyroid cell growth and hormone production by binding to the TSH receptor on the thyroid cell. TSH secretion is regulated by thyrotropin-releasing hormone (TRH) from the hypothalamus and serum levels of thyroid hormone (T3 and T4). TRH promotes the synthesis and release of TSH. TSH stimulates the production and release of T4 from the thyroid. T4 is converted to T3 in the tissues. High levels of T3 and — to a lesser extent — T4 negatively feed back to the hypothalamus and anterior pituitary to inhibit TRH and TSH secretion. Low levels of thyroid hormone stimulate TRH and TSH release (Fig. 1).

TSH (Normal Range 0.4–5.50 mU/L)

Serum TSH is the most common test used to screen for thyroid dysfunction, including hypothyroidism and hyperthyroidism. Mild degrees of thyroid dysfunction can be identified with TSH. A normal TSH excludes hyperthyroidism and primary hypothyroidism.

Advantages

The immunoassay has high sensitivity, wide availability, and low cost, making it a good screening test.

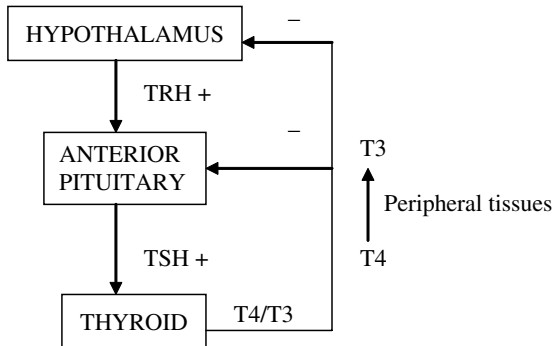


Fig. 1 Hypothalamic-pituitary–thyroid axis.

Limitations

Changes in serum TSH lag behind changes in T4/T3 levels. In the setting of primary hypothyroidism, TSH should not be checked unless it has been at least 6–8 weeks since the last levothyroxine dose change was made. It may take many weeks to correct the TSH after initiation of treatment for hyperthyroidism, so free thyroid hormone levels should be monitored instead for treatment adjustments. Measuring TSH alone is not appropriate when central hypothyroidism is suspected. Free hormone levels in addition to normal or low TSH are required for diagnosis and for monitoring treatment of central hypothyroidism.

Test interpretation (Table 1)

Causes of increased TSH:

- Primary hypothyroidism (high TSH, low T4)
- Subclinical hypothyroidism (TSH of 5–10 with normal T4)
- TSH-secreting tumor (rare) (high TSH, high T4)
- Isolated pituitary resistance to thyroid hormone (rare)

Causes of decreased TSH:

- Hyperthyroidism/thyrotoxicosis (low TSH, high T4/T3)
- Excess exogenous thyroid hormone (low TSH, normal/high T4)

Table 1 Interpretation of thyroid function tests.

Cause	TSH	Free T4	Free T3
Primary hypothyroidism	↑	↓	N or ↓
Subclinical hypothyroidism	↑	N	N
Central hypothyroidism	N or ↓	↓	N or ↓
Thyroid hormone resistance	N or ↑	↑	↑
TSH-secreting tumor	↑↑	↑	↑
Decreased thyroxine-binding globulin or Drugs: phenytoin, carbamazepine	N	↓	N or ↓
Increased thyroxine-binding globulin or Decreased conversion of T4 to T3 (amiodarone, radiocontrast agents, propranolol)	N	↑	N or ↓
Drugs: glucocorticoids, dopamine	↓	N	↓
Nonthyroidal illness (euthyroid sick syndrome)	↓	N or ↓	↓
Subclinical hyperthyroidism or Recently resolved/treated hyperthyroidism or First trimester of pregnancy or Early hyperthyroidism	↓	N	N
T3 thyrotoxicosis	↓	N	↑
Overt hyperthyroidism (Graves', toxic multinodular goiter, or thyroiditis)	↓	↑	↑

N = normal

- Central hypothyroidism (low TSH, low T4)
- Subclinical hyperthyroidism (low TSH, normal T4)
- Drugs which inhibit TSH secretion (corticosteroids, dopamine)

T4

Serum levels of T4 are inversely related to serum TSH. Obtaining a serum T4 level in addition to TSH is usually sufficient to distinguish primary from central hypothyroidism, overt (abnormal TSH and T4) from subclinical (abnormal TSH, normal T4) thyroid disease, or determine the severity of hyperthyroidism. 99.96% of all serum T4 is bound to thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA),

or albumin. Concentrations of total and free (unbound) T4 are measurable by a variety of assays.

Total T4 (Normal Range 5.6–13.7 mcg/dL)

This measures both bound and unbound hormones by a radioimmunoassay (RIA), chemiluminometric, or other immunometric technique.

Advantages

It is widely available and accurate.

Limitations

Drugs or illness can alter the concentrations of TBG or the binding of TBG with T4, leading to increases or decreases in the total hormone levels, but relatively normal free hormone levels. This can sometimes be the explanation for abnormal thyroid function tests in patients who do not have thyroid dysfunction.

Test interpretation

Causes of low serum TBG (falsely low total T4):

- Androgens, glucocorticoids, niacin, inherited deficiency of TBG, nephrotic syndrome, cirrhosis

Causes of high serum TBG (falsely elevated total T4):

- Estrogen, tamoxifen, 5FU, methadone, heroin, inherited excess of TBG, pregnancy, hepatitis

Free T4 (Normal Range 0.8–2.7 ng/ml)

Several methods are available for measuring free hormone: equilibrium dialysis, free T4 immunoassays, or calculating the free T4 index. With the advent of improved technology, free T4 immunoassays are the most common method used to measure the amount of unbound T4 present.

Equilibrium dialysis is considered the gold standard for free T4 determination, but it is cumbersome, expensive, and not widely available. Thus, it is best reserved for circumstances in which the diagnosis is not clear.

The free T4 index entails measuring the total T4 by immunoassay and estimating the uptake of radiolabeled T3 by plasma proteins and matrix added to the sample. It is calculated as the product of the total T4 multiplied by the percentage of the T3 tracer taken up by the matrix. This “T3 uptake” is unrelated to the T3 level in the serum.

Advantages

Immunoassays are sufficiently accurate in most settings, automatable, inexpensive, and the most widely used method for free T4 determination. They avoid the confusion related to binding protein abnormalities.

Limitations

No assay is available to correct for all possible binding protein abnormalities. When there is any doubt about the validity of the value, an equilibrium dialysis measurement should be performed.

Test interpretations

Free T4 levels can be abnormal in euthyroid patients (normal TSH). Elevated free T4 levels can be seen in patients with unusual plasma-binding protein abnormalities and patients on medications that block T4-to-T3 conversion (IV contrast, amiodarone, glucocorticoids, propranolol). These situations are distinguished from hyperthyroidism by a normal TSH. Decreased levels of free T4 but normal TSH can be seen in patients on antiepileptics like phenytoin and carbamazepine.

T3

Serum levels of T3 are also inversely related to serum TSH. They can be useful for recognizing T3 thyrotoxicosis (milder hyperthyroidism with elevated T3 but normal T4), to fully define the severity of hyperthyroidism

and monitor therapy response. Serum T3 concentrations alone are not accurate for diagnosis of hypothyroidism, because T3 levels are often normal in mild-to-moderate primary hypothyroidism.

Most of serum T3 is also bound to TBG, TBPA, and albumin. Concentrations of total and free (unbound) T3 are measurable by similar assays used to measure T4.

Total T3 (Normal Range 60–181 ng/dL)

This measures both bound and unbound hormones by RIA, chemiluminometric, or other immunometric assay.

Advantages

It is widely available and accurate.

Limitations

Drugs or illness altering the concentrations of TBG or the binding of TBG with T3 can lead to increases or decreases in the total hormone levels, but relatively normal free hormone levels.

Free T3 (Normal Range 2.3–4.2 pg/mL)

This measures only free hormone by immunoassay.

Advantages

It is sufficiently accurate in most settings, automatable, and inexpensive. It avoids confusion related to binding protein abnormalities.

Limitations

It is not as widely available as total T3. No assay is available to correct for all possible binding protein abnormalities.

Reverse T3 (Normal Range 0.19–0.46 ng/mL)

Reverse T3 (rT3) is measured by radioimmunoassay. Its concentration is about one-third of the total T3 concentration. There is little or no clinical indication for rT3 measurement. It was previously used to help distinguish thyroid function tests due to nonthyroidal illness from true hypothyroidism, but the assay is not accurate enough.

Clinical Applications

- Screening for thyroid dysfunction = check TSH.
- Working up a low TSH = check free T4, total T3.
- Working up a high TSH = check free T4.
- If on medications or have medical conditions that interfere with TBG, then check free instead of total hormone levels.

THYROID ANTIBODIES

Anti-TPO Antibodies (Normal is Negative)

Thyroid peroxidase (TPO), formerly known as the microsomal antigen, is an enzyme which catalyzes the iodination and coupling of tyrosine residues within thyroglobulin. The presence of autoantibodies to this antigen suggests the diagnosis of autoimmune thyroid disease, particularly Hashimoto's thyroiditis. RIA is the most sensitive assay and is generally preferred.

Prevalence

- General population 8–27%
- Graves' disease 50–80%
- Autoimmune thyroiditis (Hashimoto's) 90–100%
- Relatives of patients with Hashimoto's 30–50%
- Type 1 diabetes 30–40%
- Pregnant women ~14%

Test interpretation

A positive titer suggests thyroid autoimmunity and the propensity for thyroid dysfunction to develop. Patients can have positive anti-TPO antibodies and have normal thyroid function.

TSH Receptor Antibodies (Normal < 125% of Basal Activity)

TSH receptor antibodies can be stimulating, binding, and inhibiting. Thyroid-stimulating immunoglobulin (TSI) is the most often measured and is present in most patients (90%) with Graves' disease. Unlike anti-TPO, it is rarely detected in patients with other autoimmune thyroid diseases. Usually Graves' can be diagnosed clinically without measuring TSI. However, in pregnant women with hyperthyroidism TSI is useful for determining the risk of neonatal hyperthyroidism from TSI crossing the placenta. TSI can help differentiate Graves' from thyroiditis in someone who is unable to receive radioactive iodine, like a breastfeeding mother. TSI can also establish the diagnosis of Graves' in someone who is euthyroid but has signs of orbitopathy.

Prevalence

- General population 0%
- Graves' disease 80–95%
- Autoimmune thyroiditis (Hashimoto's) 10–20%
- Relatives of patients with Hashimoto's 0%
- Type 1 diabetes 0%
- Pregnant women 0%

Test interpretation

The test involves isolating the immunoglobulins, exposing cultured thyroid cells to them, and then measuring their cAMP response compared to reference TSI and TSH standards.

- Basal activity is <110%: normal.
- Basal activity is >125%: positive for Graves'.
- Basal activity is 110–125%: indeterminate; further studies are indicated.

Antithyroglobulin Antibodies (Normal <20 IU/mL)

Antithyroglobulin antibodies are a general marker of thyroid autoimmunity. Their presence poses a problem for thyroid cancer patients who rely on thyroglobulin measurements to monitor for recurrence. They interfere with the thyroglobulin assay by binding free thyroglobulin in the serum, decreasing the amount available for detection and negating the value of the serum thyroglobulin determination.

Prevalence

- General population 5–20%
- Graves' disease 50–70%
- Autoimmune thyroiditis (Hashimoto's) 80–90%
- Relatives of patients with Hashimoto's 30–50%
- Type 1 diabetes 30–40%
- Pregnant women ~14%

Test interpretation

If present in a nonthyroid cancer patient, they are an indicator of thyroid autoimmunity. If present in a thyroid cancer patient, they may interfere with the thyroglobulin assay and lead to difficulties with interpretation. Their persistence in a thyroid cancer patient without thyroid autoimmunity more than one year after thyroidectomy and radioiodine ablation may indicate residual thyroid tissue and increased risk of recurrence. However, in thyroid cancer patients with a prior history of thyroid autoimmunity, these autoantibodies may persist longer (median time to disappearance three years).

THYROID TUMOR MARKERS

Thyroglobulin (Normal 3.5–56 ng/mL; After Thyroidectomy <2 ng/mL)

Thyroglobulin is the precursor to thyroid hormones and is synthesized only by thyroid follicular cells, making it a good marker for papillary and

follicular thyroid cancer recurrence after thyroidectomy. It can be measured in the serum by immunoassay, which is limited by interference caused by the presence of circulating antithyroglobulin antibodies.

Thyroglobulin is mainly measured when monitoring for residual or recurrent papillary or follicular thyroid cancers after thyroidectomy. It can also be useful in the differentiation of thyrotoxicosis due to exogenous thyroid hormone from endogenous hyperthyroidism (Graves', thyroiditis).

Most thyroid cancer patients are on thyroid hormone suppression therapy after thyroidectomy and radioactive iodine ablation. Thyroglobulin can be measured while the patient is on thyroid hormone suppression, after recombinant TSH stimulation, or withholding thyroid hormone suppression.

Advantages

When thyroglobulin is measured after withholding thyroid hormone suppression or receiving recombinant TSH, it has a high degree of sensitivity and specificity to detect thyroid cancer after total thyroidectomy and remnant ablation.

Limitations

Thyroglobulin levels may be low in aggressive or poorly differentiated disease or elevated in patients at low risk for clinically significant morbidity, so they should be interpreted in the setting of pretest probability of clinically significant residual tumor.

Test interpretation

Ideally, thyroglobulin levels drawn while on thyroid hormone suppression should be <1 ng/mL. If they are >1 ng/mL, further testing with recombinant TSH or withholding thyroid hormone suppression should be performed. A recombinant-TSH-stimulated thyroglobulin level (or after withholding thyroid hormone) of >2 ng/mL is suggestive of recurrent disease.

In the differentiation of thyrotoxicosis, endogenous forms of hyperthyroidism cause elevations of thyroglobulin and exogenous thyroid hormone causes suppression of thyroglobulin.

Calcitonin (Normal Males <8 ng/l; Females <4 ng/l)

Calcitonin is the product of thyroid parafollicular or C cells. It is a useful tumor marker for medullary thyroid carcinoma (MTC). Calcitonin levels are usually many times higher than normal in disseminated MTC. Mild elevations of calcitonin are not specific for MTC and calcitonin can be normal in early tumors. In patients with high suspicion for MTC (family history of MTC or MEN2A or MEN2B), provocative testing with pentagastrin (0.5 mcg/kg IV over 5 s) or calcium gluconate (2 mg calcium/kg over 1 min) is recommended if the calcitonin is indeterminate (less than 100).

Test interpretation

Screening test:

- >100 is diagnostic for MTC.
- If the patient is high-risk and the calcitonin is <100 but >normal, the test is indeterminate and further provocative testing is indicated.

Patients with known MTC:

- 10–40 is suggestive of nodal disease.
- >150 is often seen in distant metastatic disease, and frequently the calcitonin is >1000 in this setting.
- >3000 is suggestive of extensive metastatic MTC.

SELECTED REFERENCES

- Cooper DS, Doherty GM, Haugen BR, *et al.* American Thyroid Association Guidelines Taskforce. Management Guidelines for Patients with Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* 2006;16(2):109–142.
- Gardner DG, Shoback D. *Greenspan's Basic and Clinical Endocrinology*, 8th Edn. McGraw-Hill, 2007.
- Henderson KE, Baranski TJ, Bickel PE, *et al.* *The Washington Manual Endocrinology Subspecialty Consult*, 2nd Edn. Wolters Kluwer/Lippencott Williams and Wilkins, 2009.