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Biochemical Testing in Thyroid Disorders

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INTRODUCTION

This review summarizes the main principles for the appropriate use of laboratory testing in the diagnosis and management of thyroid disorders, as well as controversies that have arisen in association with some of these biochemical tests. To place a test in perspective, the sensitivity and accuracy of the test should be taken into account. Ordering the correct laboratory tests facilitates the early diagnosis of a thyroid disorder and institutes timely and appropriate treatment. This review will focus on a comprehensive update regarding thyroid-stimulating hormone (TSH), thyroxine (T4)/triiodothyronine (T3), thyroid autoantibodies, thyroglobulin (Tg), and calcitonin. The clinical uses of these biochemical tests are outlined in Table 1.

THYROID-STIMULATING HORMONE

Overview

TSH or thyrotropin is a glycoprotein secreted by the anterior pituitary gland and is regulated by negative feedback from the serum free thyroid hormones (T4 and T3). TSH exhibits diurnal variation, with the lowest value in the late afternoon and highest value between midnight and 4 AM.^{1–3} Therefore, variations of serum TSH values within the normal range of up to 50% do not necessarily reflect a change in thyroid status.³ TSH secretion is extremely sensitive to minor changes in serum free T4, and abnormal TSH levels occur while developing hypothyroidism and hyperthyroidism before free T4 abnormalities are detectable.⁴

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Available Assays and Functional Sensitivity

Several advances have been made in the last few decades in the development of sensitive assays for TSH measurement. The first-generation of TSH assays were based on radioimmunoassay methodology that had limited functional sensitivity (~1.0 mIU/L).^{5–7} Second-generation assays were developed in the 1970s by using modified radioimmunoassay procedures and had a functional sensitivity of 0.1 mIU/L.^{8–11}

Currently, the most widely used assays are third-generation immunometric assays (also called “sandwich” or “noncompetitive” assays), which became available in the mid 1980s.⁴ Mechanistically, these assays use an excess of TSH monoclonal antibody bound to a solid support (“capture antibody”) that captures TSH from the serum specimen during an incubation period. Different polyclonal or monoclonal TSH antibodies, targeted at different TSH epitopes, and labeled with a signal (most recently chemiluminescent and fluorescent) are then added and, after further incubation, the unbound constituents are removed by washing. The signal bound to the solid support is quantified as being directly proportional to the serum TSH concentration in the test sample. More recent modifications to this concept include the use of chimeric monoclonal antibodies to reduce interference by heterophilic antibodies (defined as human antibodies with a broad reactivity with antibodies of other animal species), and the use of avidin–biotin and magnetic particle separation techniques.^{12–14} These assays have resulted in inherently better sensitivity and specificity, with a functional sensitivity at 0.01 mIU/L.

Reference Range

There continues to be ongoing debate regarding the upper limit of normal for serum TSH. According to the National Health and Nutrition Examination Survey III survey, the upper limit of normal for serum TSH level was found to be 4.5 mIU/L based on a disease-free population, excluding those on thyroid medications.¹⁵ When looking at a “reference population” taken from this disease-free population composed of nonpregnant adults, without laboratory evidence of thyroid dysfunction, undetectable thyroid autoantibodies and not on estrogens, androgens, or lithium, the upper normal TSH value of 4.12 mIU/L was found. The Hanford Thyroid Disease Study further supported this upper limit.¹⁶ However, the National Academy of Clinical Biochemists proposed that 95% of individuals without evidence of thyroid disease have TSH concentrations of less than 2.5 mIU/L, and it has been advocated by some investigators that the upper limit of the TSH reference range be lowered to 2.5 mIU/L.^{17, 18}

The National Health and Nutrition Examination Survey III reference population was also further analyzed to determine normal TSH ranges based on age, race and ethnicity, and sex.¹⁹ This study showed that the 97.5th percentile TSH values were as low as 3.24 mIU/L for African-Americans between the ages of 30 and 39 years, and as high as 7.84 mIU/L for Mexican Americans 80 years of age or older. For every 10-year age increase after 30 to 39 years, the 97.5th percentile of serum TSH was shown to increase by 0.3 mIU/L.¹⁹ Additionally, in adults without evidence of thyroid autoantibodies, TSH values higher than 3.0 mIU/L occur with increasing frequency with age, with individuals greater than 80 years of age having a 24% prevalence of TSH values ranging between 2.5 and 4.5 mIU/L and a

12% prevalence of TSH values that are greater than 4.5 mIU/L.²⁰ These data suggest an age-related shift toward higher TSH concentrations in older patients and may indicate that mild increases in TSH in the elderly may not reflect thyroid dysfunction but rather be a normal manifestation of aging.²¹ Despite current guidelines not advocating for specific TSH goals for different age groups with hypothyroidism, clinical practice patterns reflect an impact of age in the management of hypothyroidism.²²

Clinical Usefulness and Test Interpretation

Serum TSH remains the primary screening test for thyroid dysfunction. Current guidelines recommend that serum TSH is used as the first-line test for detecting both overt and subclinical thyroid dysfunction in ambulatory patients who have intact hypothalamic and pituitary function.^{3,23} Furthermore, TSH is used to evaluate thyroid hormone replacement therapy in patients with primary hypothyroidism, and suppressive therapy in patients with follicular cell–derived thyroid cancer.³

Challenges in the interpretation of serum thyroid-stimulating hormone

Nonthyroidal illness: Nonthyroidal illness can often alter thyroid hormone peripheral metabolism and hypothalamic/pituitary function, and can lead to a range of thyroid test abnormalities, including both decreased and increased serum TSH levels.^{24–26} In hospitalized patients with acute illness, serum TSH level may be suppressed to less than 0.1 mIU/L, in combination with a subnormal free T4. This can especially be seen in patients receiving dopamine infusions²⁷ or high doses of glucocorticoids.²⁸ In addition, during the recovery phase from nonthyroidal illness, TSH levels may increase above normal, but usually are less than 20 mIU/L.²⁹ Therefore, in critically ill or hospitalized patients, a serum TSH measurement should only be obtained if there is high suspicion for thyroid dysfunction.^{3, 30}

Pregnancy: Variations in serum TSH can occur physiologically in pregnancy. During the first trimester, serum TSH usually becomes lower, but rarely decreases to less than 0.1 mIU/L, owing to the stimulatory effects of human chorionic gonadotropin on the thyroid. Serum TSH subsequently returns to normal in the second trimester.^{3,31} Trimester-specific ranges for serum TSH as set by each different laboratory should be used in pregnancy. If not available, the following upper limits of normal range are recommended: TSH 2.5 mIU/L for the first trimester, 3.0 mIU/L for the second trimester, and 3.5 mIU/L for the third trimester.³

Medication interference: Several medications may interfere with the measurement of serum TSH via a variety of mechanisms and therefore impact its interpretation. These mechanisms include interference with T4 absorption (eg, calcium, iron supplements), interference with thyroid gland hormone production and secretion (eg, amiodarone, lithium, tyrosine kinase inhibitors), direct and indirect effects on the hypothalamic–pituitary–thyroid axis (eg, bexarotene, dopamine, octreotide, ipilimumab), increased clearance (eg, phenytoin, carbamazepine), and interference with peripheral metabolism (eg, glucocorticoids, beta-blockers).³

In recent years, the effect of biotin on TSH measurement has received considerable attention. Biotin (vitamin B₇) is a cofactor for carboxylases involved in fatty acid synthesis, gluconeogenesis, and energy production. Biotin is a common component of multivitamins with the daily recommended dose ranging from 30 to 70 µg.³² It has also been shown that biotin improves clinical outcomes and quality of life in patients with progressive multiple sclerosis at very high doses.³³ However, in moderate doses, biotin can cause interference in some TSH immunoassays, resulting in abnormal thyroid function tests.^{34,35} Many immunoassays use the biotin–streptavidin interaction as an immobilizing system. Streptavidin binds biotin with high affinity and high specificity, making it useful as a general bridge system.³⁶ Ingestion of high doses of biotin may cause spurious results in these assays. Mechanisms to remove biotin, such as a streptavidin agarose column in nonbiotinated assays, minimize the impact of assay interference and improve the accurate measurement of TSH. In immunometric assays, excess biotin displaces the biotinylated antibodies and causes spuriously low results, whereas in competitive assays, excess biotin competes with biotinylated analogue and results in falsely high results.^{34,37} There have been cases of factitious Graves' disease reported in the literature owing to high doses of biotin.^{37,38} Physicians must be aware of immunoassay interference by biotin to avoid misdiagnosis and unnecessary treatment. If patients taking high doses of biotin are found to have suppressed TSH and elevated T₄, they should stop taking biotin and have repeat measurements at least 2 days later before making the diagnosis of hyperthyroidism.²³

Other considerations: Patients with anorexia nervosa may have low TSH levels in combination with low levels of free T₄,³⁹ mimicking laboratory results seen in critically ill patients and in patients with central hypothyroidism owing to pituitary and hypothalamic disorders. Patients with central hypothyroidism, for example, owing to nonfunctioning pituitary adenomas, may have mildly elevated serum TSH levels that are explained by the secretion of bioinactive isoforms of TSH.⁴⁰ Increased TSH levels with elevated serum thyroid hormone levels are seen in patients with resistance to thyroid hormone.⁴¹ Adrenal insufficiency may also be associated with TSH elevations that are corrected with glucocorticoid replacement.^{42,43}

Heterophilic or interfering antibodies including human anti-animal (most commonly mouse) antibodies, rheumatoid factor, and autoimmune anti-TSH antibodies may cause falsely elevated serum TSH values by interfering with the assays.^{44,45}

THYROXINE AND TRIIODOTHYRONINE

Overview and Available Assays

Approximately 99.97% of serum T₄ and 99.7% of T₃ are bound to T₄-binding globulin, transthyretin, prealbumin, or albumin.^{3,46,47} Therefore, only a small amount of T₄ and T₃ are unbound and act on the hypothalamus–pituitary–thyroid axis as the metabolically available moieties. Assessment of serum free T₄ has now largely replaced serum total T₄ as a measure of thyroid status. Methods for assessing serum free T₄ include a direct immunoassay of free T₄ after ultrafiltration or equilibrium dialysis of serum or after addition of anti-T₄ antibody to serum.^{48,49} Additionally, measurement of the serum free T₄ index can

be derived as the product of total T4 and a thyroid hormone binding ratio.⁴⁸ Methods for assessing free T3 concentration by direct immunoassay have also been developed and are currently used.⁴⁹ However, assays for estimating free T3 are not as widely validated as those for free T4, and measurement of total T3 may be preferred in clinical practice.

Clinical Usefulness and Test Interpretation

A low serum free T4 indicates hypothyroidism, either primary when TSH is elevated, or central, when TSH is normal or low.^{6,40} Serum free T4 is also the test of choice for detecting hypothyroidism in patients with treated hyperthyroidism (either by antithyroid drugs, radioiodine ablation, or surgery), because serum TSH may remain low for many weeks to months. In pregnancy, serum total T4 measurement is recommended instead of serum free T4 measurement.³¹ This is recommended because changes in serum proteins in pregnancy may lead to lower values of free T4 by direct immunoassay based on reference ranges that were established with normal nonpregnant sera. Total T4 increases during the first trimester of pregnancy and the reference range is approximately 1.5-fold that of the nonpregnant range throughout the pregnancy.^{50,51}

Serum T3 measurement, whether total or free, has limited usefulness in hypothyroidism because levels are often normal owing to hyperstimulation of the remaining functioning thyroid tissue by elevated TSH, and also because of upregulation of type 2 iodothyronine deiodinase.⁵² Additionally, T3 levels are low in the absence of thyroid disease in patients with severe illness because of reduced peripheral conversion of T4 to T3 and increased inactivation of thyroid hormone.^{3,30,53} In contrast, free or total T3 should be measured in patients who are suspected to have hyperthyroidism.²³

THYROID AUTOANTIBODIES

Overview

Thyroid autoantibodies are circulating antibodies against several thyroid antigens, which are present in most patients with autoimmune thyroid disorders, such as Hashimoto's thyroiditis and Graves' disease.⁵⁴ The thyroid autoantibodies discussed here are widely available in clinical diagnostic laboratories and commonly used, and these include antibodies to thyroid peroxidase (TPOAb), antibodies to Tg (TgAb) and antibodies directed against the TSH receptor (TRAb).

Available Assays

Modern assays for thyroid autoantibodies depend on direct measurement of the interaction between the autoantibody (patient's serum) and the labeled thyroid antigen. Despite improvement of these assays in recent years, specificity remains an issue, because many euthyroid individuals exhibit low levels of these autoantibodies. The higher the concentration of the autoantibody, the greater is its clinical specificity.¹⁵ Attempts have been made to standardize these assays to allow for comparisons of thyroid autoantibody concentrations from one office visit to the next, among different patients, and among laboratories. However, owing to autoantibodies differing considerably in their affinity and

epitope recognition of antigen, results from different commercial assays may still vary significantly.⁵⁵

Clinical Usefulness and Test Interpretation

Autoantibodies to thyroid peroxidase and to thyroglobulin—Both TPO and Tg autoantibodies are polyclonal antibodies and are thought to occur owing to a secondary response to thyroid injury, and may contribute to the development and chronicity of disease. Almost 100% of patients with Hashimoto's thyroiditis have elevated TgAb and TPOAb, but TPOAb have higher affinity and occur in higher concentrations. TgAb and TPOAb are also detectable in 50% to 90% of patients with Graves' disease. These antibodies are also frequently seen in the general population and are 5-fold more common in women than in men.¹⁵ However, their significance in individuals with normal thyroid function remains uncertain, except that they confer a risk factor in families with autoimmune thyroid disorders.⁵⁶

In patients with known overt hypothyroidism, measurement of these antibodies is not required, because it does not alter management. However, current guidelines recommend measurement of TPOAb when evaluating patients with subclinical hypothyroidism, because their presence may influence the decision to treat.³ If positive, progression to overt hypothyroidism occurs at a rate of 4.3% per year versus 2.6% per year when TPOAb are negative. Additionally, measurement of TPOAb should be considered when evaluating patients with recurrent miscarriage, with or without infertility.³ This is because women with positive TPOAb may have an increased risk of miscarriage in the first trimester,⁵⁷ for preterm delivery,⁵⁸ and for offspring with impaired cognitive development.⁵⁹ It is hypothesized that these increased risks may be owing to decreased thyroid functional reserve from chronic autoimmune thyroiditis leading to subtle hypothyroidism.⁶⁰

Autoantibodies to the thyroid-stimulating hormone receptor—TRAb are directed against the TSH receptor. In hyperthyroid patients with Graves' disease, these autoantibodies behave as thyroid-stimulating antibodies (thyroid-stimulating immunoglobulin), because they compete with TSH for binding to its specific receptor site in the cell membrane.⁶¹ This stimulation induces thyroid growth, increases gland vascularity, and leads to an increased rate of thyroid hormone production and secretion. Other types of TRAbs exist, including antibodies that act as TSH antagonists and are referred to as blocking TRAbs (thyrotropin-binding inhibitor immunoglobulin) and neutral antibodies, which do not influence TSH binding but may act as weak agonists.⁶¹ Blocking TRAbs can be found in 15% of patients with autoimmune thyroiditis, especially in patients without a goiter.⁶² However, TRAbs are not detectable in the normal population with the use of currently available assays, and thus are disease specific.

Measurement of TRAbs can be used to diagnose Graves' disease. Most TRAb assays are specific for Graves' disease, but thyroid-stimulating immunoglobulin and first-generation thyrotropin-binding inhibitor immunoglobulin assays are less sensitive.^{63–65} Measurement of TRAb levels before stopping antithyroid drug therapy is recommended, because it helps in predicting which patients can be weaned from the medication, with normal levels

indicating a greater chance for remission.⁶³ Persistently high levels of TRAb along with high thyroid blood flow identified by color Doppler ultrasound imaging are associated with higher relapse rates,^{66–69} and these patients should be assessed more frequently and at shorter intervals after antithyroid drugs are discontinued. In contrast, patients with mild disease, small goiters, and negative TRAb have remission rates of greater than 50%, making the use of antithyroid medications potentially more favorable in this group of patients.⁷⁰

TRAb levels should be measured in pregnant women with hyperthyroidism when the etiology is unclear. If Graves' disease is confirmed with elevated TRAbs, then these antibodies should be measured again at 22 to 26 weeks of gestation. In hypothyroid pregnant patients who were treated for Graves' disease with radioactive iodine or thyroidectomy before pregnancy, TRAb levels should be measured using a sensitive assay either initially at 20 to 26 weeks of gestation, or initially during the first trimester, and if elevated, again at 22 to 26 weeks of gestation.⁶³ This recommendation is based on the strong correlation between a high titer of TRAbs and the development of fetal or neonatal Graves' disease, because TRAbs can cross the placenta and affect the fetal thyroid gland. Thus, TRAb levels measured at 22 to 26 weeks of gestation should be used to guide decisions regarding neonatal monitoring.⁶³

THYROGLOBULIN

Overview

Tg is a large, homodimeric glycoprotein (660 KDa) that is produced by thyroid follicular cells. It contains 8% to 10% carbohydrates and iodine. T4 and T3 are synthesized on Tg within the lumen of thyroid follicles. Most Tg is reabsorbed into thyrocytes and proteolytically degraded during T4 and T3 secretion. However, small amounts of intact Tg are secreted with T4 and T3 and are detectable in the serum of healthy individuals, with levels roughly paralleling thyroid gland size (0.5–1.0 ng/mL Tg per gram of thyroid tissue, depending on the TSH level).⁷¹ TgAb are present in approximately 10% of the general population and an estimated 25% of patients with differentiated thyroid cancer.^{15,72,73}

Available Assays

Several assays are available for Tg measurement. Even though significant improvements have been made in standardizing Tg assays, marked variability still exists between some assays.^{74,75} Because of this, the current recommendation is that measurements in individual patients be performed with the same method for reliable interpretation.⁷² If a change in the assay method is necessary, it is recommended that a baseline level is reestablished, which can be used to interpret change over time.⁷⁶

Immunometric assays

Immunometric assays are the most commonly used assays to measure serum Tg in clinical laboratories. Guidelines recommend that these assays be calibrated against the CRM-457 international standard.⁷² In addition to their limited dynamic range, immunometric assays are prone to interference by TgAb, which often cause falsely low serum Tg measurements.

Additionally, heterophile antibodies, if present, can interact with the antibodies used in immunoassays, usually resulting in erroneously high Tg measurements.

Radioimmunoassays

Tg measurement by radioimmunoassay has been traditionally used for TgAb-positive patients, because it is less prone to antibody interference. Nowadays, these assays are not as widely available as immunometric assays because they require handling and disposal of radioactive materials, often necessitate prolonged incubation times, and may need organic extraction and chromatography before the actual assay procedure to minimize nonspecific and specific interferences (cross-reactivity).⁷⁷ Additionally, they may be less sensitive than immunometric assays in detecting small amounts of Tg.⁷²

Liquid chromatography/tandem mass spectrometry

This new methodology has been recently introduced by some laboratories for quantitative Tg measurement in patients with positive TgAb (or heterophile antibodies) and validated as a cost-effective method with acceptable performance characteristics for use in clinical diagnostic applications.⁷⁸ This method overcomes the issue of TgAb interference by using tryptic digestion of patient serum with subsequent measurement of Tg-proteotypic peptides by liquid chromatography/tandem mass spectrometry.^{78–80} Limitations include the high complexity of the instrumentation's operation and maintenance, as well as sample throughput limits.⁷⁷

Functional Sensitivity

Although most data arise from studies using methods with a functional sensitivity of 1 ng/mL, many contemporary assays have a functional sensitivity as low as 0.1 ng/mL or less. These sensitive assays may reduce the need to perform TSH-stimulated Tg measurements during the initial and long-term follow-up of follicular cell-derived thyroid cancer patients and allow Tg surveillance measurements without interrupting thyroid hormone therapy instead.⁷² In general, the highest degrees of sensitivity for serum Tg occur after thyroid hormone withdrawal or stimulation using recombinant human TSH (rhTSH),⁸¹ and basal serum Tg increases by 5- to 10-fold with TSH stimulation. Patients with an unstimulated serum Tg of greater than 0.2 ng/mL using a highly sensitive assay during T4 suppression therapy are likely to have a TSH-stimulated Tg of greater than 1 ng/mL using a less sensitive assay.⁷²

Clinical Usefulness and Test Interpretation

Tg is primarily used as a tumor marker to evaluate the effectiveness of treatment for differentiated thyroid cancer and to monitor for residual or recurrent disease.^{72,82,83} Because it is only produced by thyroid follicular cells, Tg is expected to be undetectable in patients who underwent total or near-total thyroidectomy and 131-I remnant ablation.⁸¹ This is defined as a serum Tg level less than 0.2 ng/mL during TSH suppression or a serum Tg of less than 1 ng/mL after stimulation in the absence of interfering antibodies.⁷² However, serum Tg levels should always be interpreted in view of the pretest probability of clinically significant residual disease. Serum Tg measurements obtained during thyroid hormone

suppression should be interpreted cautiously because they may fail to identify patients with small amounts of residual disease.^{84–87} A neck ultrasound examination is thus invaluable to identify possible residual cancer even when serum Tg is undetectable.^{88–90} Additionally, even TSH-stimulated Tg measurement may fail to identify patients with clinically significant thyroid cancer because of either anti-Tg antibodies (which should always be quantitatively assessed with every measurement of serum Tg) or because of decreased or absent production and secretion of immunoreactive Tg by tumor cells, as seen in poorly differentiated thyroid cancers.^{72,85,86}

During the initial follow-up of patients with differentiated thyroid cancer who have a low or intermediate risk for recurrence, serum Tg on levothyroxine therapy should be measured every 6 to 12 months. For those patients who achieve an excellent response to therapy, the usefulness of subsequent Tg measurements has not been established, but current recommendations advocate that the time interval between serum Tg measurements can be increased to 12 to 14 months.⁷² For patients with differentiated thyroid cancer at high risk for recurrence regardless of response to therapy, and for patients who have biochemical incomplete, structural incomplete, or indeterminate response to therapy, Tg levels should be checked at least every 6 to 12 months for several years.⁷² Additionally, all patients should undergo a serum Tg on levothyroxine therapy with a sensitive Tg assay (<0.2 ng/mL) or after rhTSH stimulation at 6 to 18 months to verify excellent response and absence of neoplastic disease. A single rhTSH-stimulated serum Tg of less than 0.5 to 1.0 ng/mL in the absence of interfering antibodies has a 98% to 99.5% likelihood of identifying patients completely free of thyroid cancer on follow-up, indicating excellent response to treatment.^{91–95} If excellent response to treatment is not confirmed, subsequent rhTSH-stimulated Tg testing may be considered to monitor and reassess response to additional therapies.⁷²

Tg levels can also be increased in patients with goiter (degree of elevation correlates with thyroid size), hyperthyroidism, or inflammatory or physical injury to the thyroid gland.⁵⁵ Subnormal or undetectable Tg concentrations are seen in patients with an intact thyroid gland who have thyrotoxicosis factitia owing to suppression of endogenous thyroid function. This aids in differentiating thyrotoxicosis factitia from other causes of thyrotoxicosis with a low thyroid radioiodine uptake.⁹⁶

CALCITONIN

Overview

Calcitonin is a polypeptide produced almost exclusively by neuroendocrine C cells of the thyroid gland. It results from cleavage and posttranslational processing of procalcitonin, a precursor peptide derived from pre-procalcitonin.⁹⁷

Available Assays and Functional Sensitivity

Commercial assays for measuring calcitonin have shifted over the past decade to the immunochemiluminometric assays that are highly sensitive and specific for monomeric calcitonin. With these assays, the cross-reactivity with procalcitonin and other calcitonin-

related peptides is essentially eliminated. This is important because inflammatory conditions, such as sepsis, can lead to significant elevations of procalcitonin in tissues.^{98,99}

Depending on the assay, 56% to 88% of normal subjects have calcitonin levels below the functional sensitivity, and up to 10% have calcitonin levels of greater than 10 pg/mL. Reference ranges for calcitonin are higher in men as compared with women; this is owing to the larger C-cell mass in men.^{100,101} However, the current revised medullary thyroid cancer guidelines do not specify reference ranges of basal or stimulated serum calcitonin levels; rather, individual laboratories may set their own criteria defining these reference ranges.¹⁰² Owing to variability in calcitonin measurements among different commercial assays, individual patient samples should be evaluated using the same assay whenever possible.

Heterophile antibodies can cause falsely elevated (and rarely falsely lower) serum calcitonin levels.¹⁰³ It is important to consider the “hook effect” in patients with a large tumor burden from medullary thyroid cancer and unexpectedly low serum calcitonin levels. This condition occurs when very high serum calcitonin levels saturate the binding capacity of the capture antibody, leading to the detection of falsely low analyte levels in the sample.¹⁰⁴ The “hook effect” is less likely to occur with current immunochemiluminometric assays than with some of the older assays, but physicians should still be aware of it.

Last, provocative testing with the use of potent secretagogues, such as intravenous calcium or pentagastrin, has been shown to increase the sensitivity of calcitonin testing.^{105–108} However, with the introduction of the sensitive immunochemiluminometric assays, the usefulness of provocative testing has become less widespread.

Clinical Usefulness and Test Interpretation

Calcitonin is the most specific and sensitive serum marker for medullary thyroid cancer both before and after total thyroidectomy.^{109,110} Basal serum calcitonin correlates well with tumor burden and also reflects tumor differentiation.¹¹¹ To detect the presence of residual disease, a calcitonin level should be checked 3 to 6 months after the initial operation.¹⁰² If the calcitonin level is undetectable, the patient is considered biochemically cured with excellent prognosis, with a 5-year recurrence rate of only 5%.¹¹² It is important to remember that calcitonin has a long half-life. Therefore, the rate of decrease in serum calcitonin can be slow in some patients.¹¹³ There has been controversy regarding the length of time needed to reach the nadir of the calcitonin level after total thyroidectomy. In some patients who are surgically cured, the calcitonin level declines rapidly within the first postoperative hour, achieving an undetectable level with the first few days after the operation.^{114–116} However, owing to differences in clearance, it has been proposed that 3 months postoperative is the optimal time to determine serum calcitonin levels.^{110,114} If calcitonin is undetectable at 3 to 6 months postoperative, it should be measured every 6 months for 1 year and then annually thereafter.¹⁰² Persistently elevated calcitonin levels at 6 months postoperative indicate persistent disease. Additionally, the calcitonin level can also indicate the site of recurrence. When serum calcitonin is less than 150 pg/mL, this usually indicates persistent locoregional disease in the neck.^{102,117} If the serum calcitonin is greater than 150 pg/mL, this may point to the possibility of distant metastases.¹⁰² However, many patients with distant metastases often have a serum calcitonin level of greater than 1000 pg/mL.¹⁰²

Persistent hypercalcitoninemia should lead to further workup with several different imaging studies to localize the recurrence or persistent disease. It should be noted that very few patients may develop tumor recurrence without elevated calcitonin.¹¹⁸

It is important to recognize that serum calcitonin can be falsely elevated in several conditions other than medullary thyroid cancer, including chronic renal failure, autoimmune thyroiditis, large cell lung cancers, prostate cancer, mastocytosis, gastrointestinal and pulmonary neuroendocrine tumors, and hyperparathyroidism.^{119–123}

In parallel with calcitonin, carcinoembryonic antigen (CEA) can be used as another tumor marker to detect persistent or recurrent medullary thyroid cancer, because neoplastic C cells also produce CEA. CEA is a nonspecific tumor marker for medullary thyroid cancer, but it does help to predict outcome.^{124–126} Owing to its prolonged half-life, serum levels of CEA may take even longer to reach a nadir. In addition, CEA level can be falsely elevated owing to heterophilic antibodies, smoking, gastrointestinal tract inflammatory disease, benign lung tumors, or several nonthyroid malignancies.¹⁰²

In patients with medullary thyroid cancer, simultaneously increasing serum CEA and calcitonin levels indicate disease progression. If these patients have increased CEA levels but stable or decreasing calcitonin levels, physicians should consider poorly differentiated medullary thyroid cancer.¹²⁷ Therefore, it is important that calcitonin and CEA levels are measured concurrently. Finally, assessment of calcitonin and CEA doubling times postoperatively provides a useful tool for assessing the progression and aggressiveness of medullary thyroid cancer.^{128,129} In patients with persistent and recurrent disease, serum calcitonin and CEA should be monitored every 6 months to determine doubling times.¹³⁰ If the doubling time is less than 6 months, the 5- and 10-year survival rates are 23% and 15%, respectively. If the doubling time is greater than 24 months, the 5- and 10-year survival rates are 100% and 100%, respectively.^{128,129} A calculator is available on the American Thyroid Association website to determine doubling times of serial serum calcitonin and CEA measurements.¹³¹

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KEY POINTS

- Serum thyroid-stimulating hormone (TSH) remains the primary screening test for thyroid dysfunction. Current guidelines recommend that serum TSH is used as the first-line test for detecting thyroid dysfunction.
- Thyroid autoantibodies are present in autoimmune thyroid disorders. Measurement is recommended in evaluating subclinical hypothyroidism; antibodies directed against the TSH receptor can be used in Graves' disease.
- Thyroglobulin (Tg) is primarily used as a tumor marker to evaluate the effectiveness of treatment and to monitor for recurrence of well-differentiated thyroid cancers.
- When measuring a Tg level, Tg antibodies should always be measured concurrently to allow for accurate interpretation of the Tg level.
- Calcitonin is mainly used as a tumor marker to monitor for the recurrence of medullary thyroid cancer.

Table 1

Clinical uses of biochemical tests for thyroid disorders

Biochemical Test	Clinical Uses
TSH	<ul style="list-style-type: none"> • Primary screening test for thyroid dysfunction • Evaluation of thyroid hormone replacement therapy in patients with primary hypothyroidism • Evaluation of suppressive therapy in patients with follicular cell-derived thyroid cancer
T4	<ul style="list-style-type: none"> • Detection of thyroid dysfunction in conjunction with TSH • Evaluation of thyroid hormone replacement therapy in patients with secondary hypothyroidism (free T4) • Evaluation of thyroid dysfunction in pregnancy (total T4)
T3	<ul style="list-style-type: none"> • Detection of hyperthyroidism • No usefulness in the management of hypothyroidism • May be useful in diagnosis of nonthyroidal illness
Thyroid autoantibodies	<ul style="list-style-type: none"> • Positive in autoimmune thyroid disease • TPOAb – evaluation of patients with subclinical hypothyroidism and women with recurrent miscarriages • TRAb – diagnosis of Graves' disease; help to predict which Graves' patients can be weaned from antithyroid medications
Thyroglobulin	<ul style="list-style-type: none"> • Evaluation of effectiveness of treatment for differentiated thyroid cancer and monitoring for residual or recurrent disease • Diagnosis of thyrotoxicosis factitia
Calcitonin	<ul style="list-style-type: none"> • To diagnose medullary thyroid cancer and monitor for recurrence, progression, and response to treatment

Abbreviations: T3, triiodothyronine; T4, thyroxine; TPOAb, antibodies to thyroid peroxidase; TRAb, antibodies directed against the thyroid-stimulating hormone receptor; TSH, thyroid-stimulating hormone.