The clinical use of thyroid function tests

Utilização dos testes de função tireoidiana na prática clínica

Gisah Amaral de Carvalho[1], Camila Luhm Silva Perez[1], Laura Sterian Ward[2]

ABSTRACT

Laboratory tests are essential for the accurate diagnosis and cost-effective monitoring of thyroid dysfunction. Hormone levels can only confirm a diagnosis when there is a high level of clinical suspicion. For most patients, symptoms are nonspecific and subtle, so biochemical tests are necessary to identify the disorder. The purpose of this article is to critically analyze the use of standard thyroid function tests, which include measuring the levels of serum thyroid-stimulating hormone (TSH), thyroid hormones and antithyroid antibodies. The pitfalls of and impediments to the routine use of these tests are discussed based on a Medline database survey, and recommendations are presented to optimize the use of these diagnostic tools in clinical practice. Arq Bras Endocrinol Metab. 2013;57(3):193-204

Keywords

Thyroid function tests; thyrotropin; triiodothyronine; thyroxine; hypothyroidism; hyperthyroidism

[1] Department of Endocrinology and Metabolism, Hospital das Clínicas, Universidade Federal do Paraná, Serviço de Endocrinologia e Metabologia (HC-UFPR/SEMPR), Curitiba, PR, Brazil
[2] Faculdade de Ciências Médicas, Universidade Estadual de Campinas (FCM/Unicamp), Campinas, SP, Brazil

RESUMO

Exames laboratoriais são fundamentais para o diagnóstico acurado e o monitoramento custo-efetivo das disfunções tireoidianas. Quando há alta suspeita clínica, as dosagens hormonais apenas confirmam o diagnóstico. No entanto, na maioria dos pacientes, a sintomatologia é sutil e inespecífica, de forma que apenas testes bioquímicos podem detectar o transtorno. O objetivo deste artigo é fazer uma análise crítica do uso apropriado dos principais testes de função tireoidiana, entre eles a dosagem sérica do hormônio estimulante da tireoide (TSH), dos hormônios tireoidianos e dos anticorpos antitireoidianos. Mediante um levantamento na base de dados do Medline, são discutidas as principais armadilhas e interferências relacionadas ao uso cotidiano desses testes e são apresentadas recomendações para otimizar a utilização dessas ferramentas diagnósticas na prática clínica. Arq Bras Endocrinol Metab. 2013;57(3):193-204

Descritores

Testes de função tireoidiana; tireotropina; triiodotironina; tiroxina; hipotireoidismo; hipertireoidismo

Correspondence to:
Gisah Amaral de Carvalho
Rua Agostinho Leão Júnior, 285
80030-110 – Curitiba, PR, Brazil
carvalho.gisah@gmail.com

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INTRODUCTION

Thyroid dysfunction is prevalent in clinical practice and has significant consequences. Quality laboratory tests are essential for the accurate diagnosis of thyroid disorders because the signs and symptoms of thyroid disease are subtle or absent in most patients, making biochemical tests necessary to detect disease.

Therefore, all physicians must know when to request laboratory tests and how to interpret the results to correctly diagnose and cost-effectively manage thyroid dysfunction.

All of the articles in the Medline database (PubMed) were reviewed to provide an overview on the commonly utilized thyroid function tests and to enable a discussion of the strengths and limitations of each test. Recommendations emphasize key points for each topic and are categorized by the strength of the evidence upon which they are based (Table 1).

Table 1. Recommendation grade and strength of evidence

Recommendation	Strength of evidence
A	Systematic reviews and the most consistent experimental and observational studies
B	The least consistent experimental and observational studies
C	Case reports (uncontrolled studies)
D	Opinions without critical evaluation based on consensus, physiological studies or animal models

WHAT IS THE BEST SCREENING TEST FOR THYROID DYSFUNCTION?

The thyroid-stimulating hormone (TSH) assay is the most reliable test for diagnosing primary hypo- and hyperthyroidism, especially in outpatient settings (1,2) (**D,D**).

Pituitary TSH secretion regulates T4 (thyroxine) and T3 (triiodothyronine) secretion, which exert log-linear negative feedback on pituitary thyrotrophs (3,4) (**B,B**). Due to this relationship, small changes in the concentration of free thyroid hormone (TH) result in large changes in the serum concentration of TSH; therefore, TSH is the best indicator of subtle changes in thyroid production (5) (**D**).

First-generation TSH assays can only diagnose hypothyroidism. Hyperthyroidism can be detected using second- (functional sensitivity: 0.1-0.2 mIU/L) and third (functional sensitivity: 0.01-0.02 mIU/L)-generation TSH assays (6) (**D**). First-generation TSH assays have a sensitivity of 75% and a specificity of 90%, resulting in a 7.50 positive likelihood ratio (LR+, 95% CI: 4.12-13.65) (7) (**B**). Second-generation assays increase the diagnostic certainty because they have a sensitivity and specificity of 96% and 93%, respectively, with an increased LR+ of 13.71 (95% CI: 6.71-28.05) (8) (**B**). The third-generation assay slightly increases the diagnostic certainty, with 97% sensitivity and 93% specificity, and maintains a similar LR+ value (13.86, 95% CI: 6.78-28.33) but provides results in 18 minutes (9) (**B**). Second- and third-generation assays increase the diagnostic certainty from 1% to 12% and from 4% to 36%-37%, respectively, in populations with prevalent disease. Conversely, first-generation assays increase the diagnostic certainty from 7% to 24%. Fourth-generation TSH assays are currently available, since they do not increase the diagnostic certainty the third generation assays are routinely.

Recommendation

The TSH assay is the best for initially evaluating thyroid function (**B**). There are no significant differences between the second- (**B**) and third-generation (**B**) assays; however, first-generation TSH assays should be avoided (**B**) because they offer less diagnostic certainty than the other two previously described methods. Third-generation assay should be performed with sensibility ≤ 0.002 μIU/ml.

WHAT TSH VALUES ARE CONSIDERED NORMAL?

TSH secretion is pulsatile and follows a circadian rhythm. Major secretory pulses occur between 10 p.m. and 4 a.m., and the mean levels are approximately 1.3-1.4 mIU/L, with a lower limit of 0.3-0.5 mIU/L and an upper limit of 3.9-5.5 mIU/L (10) (**B**). Changes in serum TSH levels can be attributed to its pulsatile and nocturnal secretion (11) (**B**).

Currently, the normal reference range of serum TSH for adults is 0.4-4.5 mIU/L (12,13) (**D,D**). However, it is difficult to establish a universal reference range because variations occur depending on the specific tests utilized, and serum TSH is heterogeneous in terms of glycosylation and biological activity (12) (**D**).

The upper limit of the normal range for TSH (approximately 4.5 mIU/L) has recently been questioned, and some researchers advocate reducing this limit to 2.5 mIU/L (14) (**D**). This position is supported by the higher rate of progression to overt hypothyroidism and the increased presence of anti-thyroid antibodies in patients with TSH levels above 2.5 mIU/L compared with subjects whose TSH levels are between 0.5 and 2.5 mIU/L (15) (**B**). This change is supported by studies with strict selection criteria (patients with thyroid disease were excluded) that were based on clinical data, the presence of auto-antibodies, and changes detected by ultrasound; these studies established that mean TSH levels are approximately 1.5 mIU/L in the healthy population and that more than 95% of the normal population has TSH levels ≤ 2.5 mIU/L (16,17) (**B**).

However, not all researchers agree (18,19) (**D,D**) because some patients in the initial stages of hypothyroidism and those with suspected Hashimoto's thyroiditis exhibit TSH serum levels between 2.5 and 4.5 mIU/L. Evidence for a treatment benefit is limited to certain age groups and patients with TSH levels above 7 mIU/L (20) (**A**). Some studies suggest that

increased TSH levels may be normal in the elderly, particularly those over 80 years old, and in certain ethnic groups (21,22) (**A,B**). The risk of changing the normal range is as follows: in large population groups, including the NHANES study, a normal range of 0.4-4.5 mIU/L results in a hypothyroidism diagnosis in 9.7%-12.9% of the subjects over 65, but if the upper limit of normal is lowered to 2.5 mIU/L, hypothyroidism would be diagnosed in 35% of the same population (23) (**A**).

Consistent studies confirm that serum TSH levels are age-dependent and that adult reference values do not correspond to those observed in pediatric patients; TSH levels continuously decrease from the neonatal period until the end of adolescence (24-28) (**B**).

Recommendation

The normal adult reference range for serum TSH is 0.4-4.5 mIU/L (**D**). A convincing demonstration of the positive impact of identifying and treating patients with high normal TSH is required before potentially lowering the cutoff to 2.5 mIU/L. Monitoring asymptomatic patients with TSH levels between 3.0 and 4.5 mIU/L is recommended, particularly those with positive autoantibodies (**B**). Patients with TSH levels between 4.5 and 10 mIU/L and normal serum free T4 levels (subclinical hypothyroidism) should be carefully evaluated and monitored by following changes in their hormone levels (**A**). Pediatric patients must be evaluated according to the normal ranges proposed for each age group (**B**).

IN WHAT SITUATIONS SHOULD TSH TESTING BE PERFORMED?

The TSH test has been used for triage in the diagnosis of thyroid dysfunction, especially in cases of minimal thyroid failure (subclinical hypothyroidism). TSH testing is recommended every five years in subjects over 35 years old (1) (**D**). Routine TSH testing in pregnant women is also recommended because undetected hypothyroidism during pregnancy can affect fetal neurodevelopment (29) (**B**) and survival (30) (**B**) and is associated with hypertension and toxemia (31) (**B**), although there is no consensus on this indication in pregnant women (33) (**D**).

Triage is also appropriate for patients with an increased risk of thyroid dysfunction, including the following (1) (**D**): previous history of thyroid dysfunction; incidence of goiter; previous history of thyroid surgery; previous history of cervical radiation therapy; other autoimmune diseases (for example, type 1 *diabetes mellitus*, vitiligo, pernicious anemia and primary adrenal insufficiency); use of particular medications: lithium, cytokines, amiodarone and contrast agents; family history of thyroid disease or other autoimmune disease; laboratory tests that suggest hypothyroidism: hypercholesterolemia, hyponatremia, anemia, increased creatine phosphokinase and lactate dehydrogenase and hyperprolactinemia; and the presence of comorbidities, including sleep apnea, depression and dementia.

Medical conditions that may reflect a risk for thyroid dysfunction and justify triage in pediatric patients include the following: children and adolescents with short stature and/or low growth rate (34,35) (**B,B**); children with pubertal development disorders (34,35) (**B,B**); and children and adolescents with suspected attention deficit hyperactivity disorder (ADHD) or a decline in school performance of unknown cause (36,37) (**C,C**).

In all situations, increased TSH should be confirmed, and testing should be repeated before starting replacement therapy with levothyroxine (1,12) (**D,D**).

TSH concentration accurately reflects T4 replacement in patients with primary hypothyroidism and is the best marker to evaluate and control the dose of T4 (38) (**D**).

Recommendation

Serum TSH levels should be determined in patients over 35 years old every 5 years, in pregnant women and in patients of all ages with risk factors for thyroid dysfunction (**D**). If the results indicate high TSH levels, a second confirmatory sample including TSH and free T4 levels should be tested prior to starting treatment (**D**).

WHAT SPECIAL SITUATIONS SHOULD BE CONSIDERED WHEN PERFORMING TSH TESTING?

In numerous situations, we cannot rely solely on TSH testing to evaluate thyroid function, which somewhat limits its utility.

TSH may remain abnormal despite normalizing free T4 levels in patients with chronic hypothyroidism or hyperthyroidism. In these situations, which can last for two months to a year after normalizing T3 and T4 levels, TSH testing may not accurately reflect the condi-

tion of the thyroid because of thyrotroph hypertrophy or prior suppression, respectively (4,39) (**B,B**).

We observe discordant TSH and free T4 (FT4) levels in patients with hypothyroidism who have poor treatment compliance and use T4 intermittently. Whereas TSH testing reflects the steady state achieved after 6 to 8 weeks of T4 treatment, FT4 testing reports the most recent adjustments in T4. In these patients, TSH can be elevated despite normal or elevated FT4 levels (4) (**B**).

Serum TSH confirms or excludes the diagnosis of all cases of primary hypothyroidism but is not a reliable test for secondary (central) hypothyroidism because TSH levels can be low, normal or slightly elevated in people with this disorder. When pituitary or hypothalamic disease is suspected, a diagnosis and replacement therapy with levothyroxine should be made based on the measurement of free hormones, not of serum TSH (1,2) (**D,D**).

It is essential to recognize that isolated abnormalities in serum TSH do not always correspond with thyroid dysfunction and may be caused by other conditions and medications. Causes of isolated TSH elevation include the following (1,2) (**D,D**): recovery from hypothyroxinemia resulting from nonthyroidal illness (40,41) (**B,D**); and medications, including lithium (42) (**D**) and amiodarone (43) (**D**), because inhibition of TH production by these drugs can cause both transient and reversible TSH elevations and true hypothyroidism.

With the exception of central hyperthyroidism and pituitary resistance to TH, hyperthyroidism observed in clinical practice is accompanied by decreased TSH, typically to below 0.1 mIU/L. T4 and T3 testing is indicated to evaluate patients with TSH levels below 0.1 mIU/L (1,2) (**D,D**).

TSH suppression occurs in situations other than thyroid disease, including the following: recovery from hyperthyroidism (39) (**B**); severe nonthyroidal illness that causes transient central hypothyroidism with low free T4 (44,45) (**B,B**); first trimester of pregnancy (46) (**C**); and the use of drugs, such as dopamine (47) (**C**) or glucocorticoids (48) (**C**), that suppress TSH secretion.

Diagnosing thyroid dysfunction in a critically ill patient with one or more comorbidities is challenging, and clinicians should not rely solely on TSH testing because stress and drug use can suppress or elevate TSH (40,41) (**B,D**). A medical history contains the most significant data for differentiating between changes in thyroid function that result from systemic disease and changes that result from thyroid disease. It is not uncommon for endocrinologists to treat a critically ill patient who has neither a previous history of pituitary disease nor clinical signs of hypothyroidism but does have normal or reduced serum levels of TSH and free T4 and low total and free T3. Therefore, thyroid function tests should not be routinely performed on critically ill patients except when there is a strong suspicion of thyroid dysfunction (for example, previous history of thyroid dysfunction, goiter, ophthalmopathy or unexplained bradycardia) (40) (**B**).

There are two rare types of TSH-mediated hyperthyroidism, TSH-secreting pituitary adenomas and selective pituitary resistance to thyroid hormone, that cannot be identified by testing for TSH alone. T4 and T3 testing should be requested when these conditions are suspected (1,2) (**D,D**).

Recommendation

In the first months of treatment for hypothyroidism or chronic, severe hyperthyroidism, TSH may remain abnormal despite the normalization of TH levels. In this case, free T4 is a more reliable measure of thyroid function than TSH (**B**).

In patients with hypothyroidism and poor levothyroxine treatment compliance, TSH and free T4 should be monitored because noncompliant patients exhibit discordant TSH and free T4 values (high TSH/low free T4) (**B**).

TSH should not be measured in isolation in patients with suspected hypothalamic-pituitary disease; the measurement of free T4 is essential (**D**).

Conditions other than thyroid disease should be considered when TSH levels are abnormal: physiological changes associated with pregnancy, severe nonthyroidal illness and medication use (including amiodarone, lithium, dopamine and glucocorticoids) (**D**).

HOW TO ASSESS IODOTHYRONINES (T4 AND T3)

T4 is the primary hormone secreted by the thyroid gland. Approximately 80% of serum T3 results from the peripheral conversion of T4 via 5'-mono-deiodination in various tissues. Nearly all THs circulate in the bloodstream bound to plasma proteins, and only 0.02% of T4 and 0.2% of T3 circulate in the free form (49) (**D**).

FT4 and free T3 (FT3) levels are more relevant than total hormone levels. The free hormone is the biologically active form of the hormone. Furthermore, various acquired or inherited changes in transporter proteins

alter T4 and total T3 (T3T) serum levels, regardless of thyroid status (50,51) (**B,B**).

TSH and FT4 are routinely used to assess thyroid function and to monitor hyper- and hypothyroidism treatment. FT4 is not susceptible to changes in the expression of TH transporters and has little intra-individual variability (52) (**D**). Total T4 (TT4) should be measured when discrepancies exist in the previously cited tests (53) (**D**).

T3 levels are not appropriate for diagnosing hypothyroidism because increased conversion of T4 to T3 maintains T3 serum levels within the normal range until hypothyroidism becomes severe (52) (**D**).

T3 testing, combined with an interpretation of FT4 levels, is useful for diagnosing and monitoring hyperthyroidism for the following reasons (2,54) (**D**,**B**): a high TT3/TT4 ratio (> 20) suggests Graves' disease (GD); high or paradoxically normal T3 levels may indicate hyperthyroidism in critical patients with nonthyroidal illness and suppressed TSH (< 0.01 mIU/L); high or inappropriately normal T3 may indicate amiodarone-induced hyperthyroidism; high T3 is common in patients with TSH-secreting pituitary tumors and those with resistance to TH syndrome without hyperthyroidism symptoms; T3 levels are useful for monitoring the acute response to thyrotoxicosis treatment in GD; elevated T3 is a common, early sign of GD relapse; and T3 can detect a thyrotoxicosis relapse early after an interruption of anti-thyroid drug treatment.

Total and free T3 and T4 serum levels are measured using competitive immunoassays (IMAs) (55) (**D**). The TT4 reference range for adults is 4.5-12.6 µg/dL (58-160 nmol/L), and that for TT3 is 80-180 ng/dL (1.2-2.7 nmol/L) (2,56) (**D**).

The routine methods for measuring free T3 and T4 are dependent on TH-binding proteins. Therefore, these methods are not entirely reliable in patients with nonthyroidal illness, changes in transporter proteins (changes in T4-binding globulin (TBG) affinity or abnormal transport proteins) or anti-T3 and anti-T4 antibodies (2,57) (**D**,**B**). The adult reference range of FT4 using comparative direct methods is 0.7-1.8 ng/dL (9-23 pmol/L) and that for FT3 is 2.3-5.0 pg/mL (35-77 pmol/L). The upper limit of normal for FT4 is 2.5 ng/dL using the absolute direct method of equilibrium dialysis, which is considered the gold standard method (58) (**B**).

The reference ranges for total T4 and T3 and free T4 and T3 for pediatric patients are age-dependent, similar to TSH, with increased serum levels in the neonatal period that continuously and gradually decrease until adolescence, when they reach adult values (24-28) (**B**).

Recommendations

Free T4 testing, combined with TSH testing, is recommended for the routine assessment and diagnosis of thyroid function and following hyperthyroidism treatment and may or may not be used in cases of hypothyroidism (**D**).

Abnormal levels of total T4 and T3 often result from changes in transport proteins, not thyroid function (**D**).

Total hormone testing should only be performed when there are discrepancies in the free hormone levels (**D**). Serum T3 testing has a low sensitivity and specificity for diagnosing hypothyroidism (**D**).

Serum T3 tests, interpreted in combination with free T4, are useful for diagnosing complex and unusual presentations of hyperthyroidism (**D**).

Total and free T4 and T3 values should be evaluated based on patient age in the pediatric population (**D**).

WHAT SPECIAL SITUATIONS SHOULD BE CONSIDERED WHEN PERFORMING IODOTHYRONINE TESTING (T4 AND T3)?

Malnutrition, starvation and fasting cause decreased free and total T3. Conversely, overeating increases free and total T3 (59,60) (**C**).

Stress, whether physical or emotional, causes increased adrenocortical activity and inhibits T3 production, consequently decreasing serum total and free T3 levels (61) (**C**).

Changes in absorption, which are most common in bowel surgery patients, should be considered in cases in which TSH remains high and T4 and T3 decrease after the onset of replacement therapy (62) (**C**).

Acquired or inherited variations in TH transporters alter serum total T3 and T4 levels. An increase or decrease in TBG, the main TH transporter, will increase or decrease TT3 and TT4, but serum levels of TSH, FT3 and FT4 will remain normal (63) (**C**).

Most changes in thyroid function associated with amiodarone use are similar to those observed with iodinated contrast agents, including a marked decrease in T3 and a modest increase in T4 resulting from the inhibition of 5'-deiodinase type 1 and 2 (64) (**B**).

Endogenous (pregnancy or hydatidiform mole) or exogenous hyperestrogenism increase serum TBG levels. Consequently, higher T3 and T4 levels and normal TSH levels are observed (65) (**C**). In contrast, androgen decreases TBG levels and therefore T3 and T4 levels without affecting TSH (66) (**B**).

Several drugs displace T3 and T4 from TBG, leading to increased FT3 and FT4. The principal drugs that compete with T4 for TBG binding are salicylates, phenytoin, carbamazepine and furosemide. Salicylates increase the fraction of FT4 up to 100%, and carbamazepine and furosemide increase it by approximately 30% (67,68) (**D**,**C**). Phenytoin, phenobarbital, carbamazepine, rifampicin and sertraline accelerate T4 and T3 hepatic metabolism, therefore decreasing the serum levels without affecting TSH (69,70) (**C**,**C**).

Heparin-induced increases in FT4 are an *in vitro* phenomenon. The storage or incubation of samples from heparin-treated patients induces lipoprotein lipase activity. This enzyme increases the concentration of non-esterified fatty acids, consequently elevating FT3 and FT4 levels (71) (**C**).

Most factors that interfere with total and free T4 and T3 tests cause inappropriately abnormal values in the presence of normal serum TSH levels (72) (**B**). Immunoassay disturbances can be attributed to cross-reactivity, drug interactions and the presence of antibodies (autoantibodies or heterophils). The prevalence of these complications ranges from approximately 0.1% to 2% in the general population and from approximately 1% to 10% in patients with thyroid disease (73) (**B**).

Recommendations

T4 and T3 serum levels may be altered by hereditary or acquired conditions that affect plasma protein levels, particularly those of TBG (**B**).

Patients taking certain medications, salicylates, phenytoin, carbamazepine, furosemide and exogenous estrogens may exhibit altered T4 and T3 serum levels (**D**). Sample collection and storage and possible disturbances in the immunoassays should be considered when analyzing thyroid function tests (**D**).

These complications occur in approximately 0.1% to 0.2% of the population, and this number increases to 1% to 10% in the presence of thyroid disease (**B**).

TSH and FT4 testing should be performed in combination, even under special circumstances when T4 and T3 serum levels have been assessed (**D**).

HOW SHOULD THYROID FUNCTION TESTS BE INTERPRETED DURING PREGNANCY?

During pregnancy, estrogen production gradually increases, elevating TBG serum levels and therefore increasing serum T3 and total T4 levels (74,75) (**B**).

TSH levels fall during the first trimester of pregnancy; therefore, subnormal serum TSH values are observed in approximately 20% of normal pregnancies (75) (**B**). This decreased TSH results from the thyroid-stimulating activity of human chorionic gonadotropin (hCG), which is a structural homologue of TSH. The hCG peak and the TSH nadir occur simultaneously at approximately 10-12 weeks gestation (76) (**B**). The drop in TSH during the first trimester of pregnancy is associated with a modest increase in FT4, but supraphysiological increases can occur that lead to a spectrum of thyrotoxicosis symptoms (pregnancy hyperthyroidism) in 10% of cases (2% of all pregnancies) (75) (**B**).

Subsequently, T3 and FT4 levels decrease in the second and third trimesters to approximately 30% below the average normal value. Albumin-dependent methods produce lower values (down to 50%) because serum albumin decreases in pregnant women (74,75) (**B**,**B**).

Recommendations

Maternal hypothyroidism causes adverse effects on fetal psychomotor development, highlighting the significance of evaluating thyroid function during pregnancy (**B**).

Thyroid dysfunction triage should be performed pre-pregnancy or in the first trimester with TSH tests that can detect mild thyroid failure (TSH > 2.5 mIU/L) (**D**).

During pregnancy, the total levels of T3 and T4 are high because of increased TBG, and free T4 levels may slightly increase during the first trimester but will subsequently decline in the second and third trimesters (**B**).

HOW SHOULD THYROID FUNCTION TESTS BE INTERPRETED IN CRITICALLY ILL PATIENTS?

Critically ill patients often exhibit abnormal thyroid function in the absence of thyroid dysfunction (77) (**B**). The terms "nonthyroidal illness" (NTI), "euthyroid sick syndrome" and "low T3 syndrome" are used to define the incidence of abnormal thyroid function tests in patients with severe systemic disease (78) (**D**).

Decreased T3, increased reverse T3 (rT3), normal or low T4 and normal or low TSH are typical in the-

se situations (78) (**D**). These laboratory abnormalities occur in NTI through different mechanisms: inhibition of 5'-deiodinase, hypothalamic-pituitary axis suppression, iodine uptake disorders, decreased expression of TH transporters and the use of medications such as glucocorticoids, dopamine, amiodarone, furosemide, salicylates, phenytoin and beta-adrenergic agonists (79) (**D**).

The degree of abnormal thyroid function in patients with acute systemic disease correlates with the severity of symptoms, and low levels of T3 and T4 are associated with reduced survival rates (79) (**D**).

Considering all the aforementioned confounding factors, thyroid function tests should not be routinely performed in critically ill patients unless there is a strong suspicion of thyroid disease (80) (**D**).

When necessary, TSH should be measured using a sensitive assay (TSH < 0.02 mIU/L) that can differentiate hyperthyroid patients with suppressed TSH from those with reduced TSH due to NTI. A euthyroid patient with NTI presents with transiently reduced TSH and normal or low T3 and FT4. A patient with NTI and hyperthyroidism typically has suppressed TSH and normal or elevated T3 and FT4. A hypothyroid patient exhibits normal or elevated TSH and low T3 and FT4 (81) (**B**).

Recommendations

Given the poor specificity of thyroid function tests in NTI, they should not be routinely performed in these patients unless thyroid disease is strongly suspected (**B**).

TSH and T4 (total or free) levels in NTI should be interpreted cautiously, but they are the most reliable for distinguishing primary thyroid disease (concordant TSH and T4) from the transient abnormalities that are typical of NTI (discordant TSH and T4) (**B**).

High T3 (total or free) levels are indicative of hyperthyroidism in hospitalized patients, but normal or low levels do not exclude this diagnosis (**B**).

WHAT IS THE SIGNIFICANCE OF ANTITHYROID ANTIBODIES? IN WHICH CLINICAL CONDITIONS SHOULD ANTITHYROPEROXIDASE AND ANTITHYROGLOBULIN ANTIBODIES BE ASSESSED?

The three primary thyroid antigens involved in the pathogenesis of autoimmune thyroid diseases (ATD) are the following: thyroglobulin (Tg), thyroperoxidase (TPO) and TSH receptor (TSH-R).

High thyroid autoantibody concentrations are usually found in the serum of patients with ATD. However, anti-Tg antibodies (TgAb) and anti-TPO antibodies (TPOAb) are present in a significant percentage of healthy individuals (9%-25%) (15) (**B**).

TgAb are present in 70% to 80% of patients with autoimmune thyroiditis (AT), 30% to 40% of patients with GD and 10% to 15% of patients with non-thyroid autoimmune diseases. Radioimmunoassays (RIAs), immunoradiometric assays (IRMAs) and enzyme-linked immunosorbent assays (ELISAs) are recommended for detecting TgAb (82) (**B**). TgAb interferes with Tg tests, even the ultra-sensitive ones. This effect is relevant for patients with differentiated thyroid cancer, wherein Tg is a key laboratory marker after thyroidectomy. TgAb should always be tested in combination with Tg in thyroid cancer patients, and its ability to interfere with Tg should be considered (83) (**D**).

TPOAb are present in the serum of 90% to 95% of AT patients, approximately 80% of GD patients and 10% to 15% of patients with non-autoimmune thyroid disease. IRMAs are the most sensitive detection methods and should be used preferentially (84) (**B**).

TgAb and TPOAb testing can be performed when AT is suspected based on family history, primary hypothyroidism and/or diffuse goiter. However, the absence of TgAb and TPOAb does not exclude thyroiditis because these antibodies are undetectable in a small group of patients. Conversely, the presence of antibodies is insufficient to diagnose ATD because patients with non-autoimmune thyroid disease and a few normal individuals have detectable antibody levels. Therefore, other clinical tests are necessary to confirm an AT diagnosis, such as a thyroid ultrasound (85) (**B**). Regardless of the underlying thyroid disease, TPOAb are more prevalent than TgAb, and TPOAb testing is the most sensitive way to detect ATD. TPOAb testing is preferable when cost constraints exist.

Determining TgAb and TPOAb serum levels in GD may reveal the autoimmune nature of hyperthyroidism, although the anti-TSH receptor antibody (TRAb) is more specific (86) (**B**).

TPOAb testing in early pregnancy can predict the probability of postpartum thyroiditis, which occurs in 5% to 10% of women. Approximately 50% of pregnant women with positive TPOAb will develop postpartum thyroiditis, which is usually transient. Of the women

with postpartum thyroiditis, 67% have clinical symptoms, and 33% have subclinical symptoms, even when TSH is abnormal (87) (**B**). TPOAb testing may be useful to evaluate infertility because high TPOAb levels have been associated with an increased risk of miscarriage and unsuccessful *in vitro* fertilization therapy (88) (**C**).

TgAb and TPOAg testing is indicated during treatment with amiodarone, interferon and lithium because the presence of these autoantibodies increases the risk of treatment-induced alterations in thyroid function (89-91) (**B,B,C**).

There is evidence of decreased antibody levels during treatment for GD and autoimmune hypothyroidism (92) (**C**), but in most cases, autoantibody tests to monitor ATD treatment are not recommended (93) (**B**).

Recommendation

Serum TgAb and TPOAb levels reveal the autoimmune nature of thyroid dysfunction (**B**) and should be combined with TSH and FT4 testing (**D**).

TPOAb is more prevalent than TgAb, and TPOAb testing is the most sensitive way to detect ATD (**B**).

TgAb and TPOAb testing should be performed in the following scenarios: a) when ATD is suspected; b) in patients at risk for thyroid dysfunction; c) in patients using interferon, lithium or amiodarone; and d) in patients with a history of infertility or failed assisted reproductive therapy (**D**).

TPOAb testing can assess the risk of postpartum thyroiditis (increased aTPO (anti-TPO) antibody levels) (**B**).

TgAb and Tg should be quantitated in combination while monitoring patients with differentiated thyroid cancer because the presence of serum TgAb can cause deceptively low results (false-negatives) (**B**).

There is no indication for monitoring antithyroid antibodies during hypothyroidism treatment (**D**).

IN WHICH CLINICAL CONDITIONS SHOULD THE PRESENCE OF ANTI-TSH RECEPTOR ANTIBODIES BE DETERMINED?

The anti-TSH receptor antibody (TRAb) directly stimulates thyroid function and inhibits the biological activity of TSH (94) (**B**). The radioreceptor assay is robust and commercially available but fails to differentiate the stimulating antibody from the blocking antibody.

TRAb is present in the serum of more than 90% of GD patients, but its diagnostic utility is limited. In most patients, the clinical and diagnostic tests commonly used to determine thyroid status render TRAb unnecessary to confirm GD (95) (**B**). There are special circumstances in which the differential diagnosis of hyperthyroidism may justify a TRAb test: hyperemesis gravidarum with thyrotoxicosis, subclinical hyperthyroidism with diffuse goiter and euthyroid Graves' ophthalmopathy (96) (**D**).

By themselves, TRAb values at diagnosis fail to predict the odds of a response to antithyroid drug treatment. However, by titrating TRAb and assessing other clinical indicators (age, gender, thyroid volume and severity of hyperthyroidism), subgroups of patients can be identified with a high or low risk of remission following drug treatment; female patients with mild disease, small goiters and negative TRAb have a remission rate above 50%, making antithyroid drug use a more favorable option in this group of patients (96,97) (**D,D**). Persistently elevated TRAb levels following drug treatment are associated with increased recurrence of GD (98,99) (**B,B**).

TRAb titration is useful for determining the extent of GD in pregnant women. In many patients, GD gradually diminishes during pregnancy. The disappearance of TRAb is an indicator that antithyroid drug treatment may no longer be necessary, and its continuity can place the fetus at risk for hypothyroidism (96,100) (**D,D**). TRAb testing may be used to ascertain the risk for neonatal thyrotoxicosis, which occurs in approximately 2% of pregnant women with GD. TRAb tests should be performed in the third trimester to predict this particular thyroid dysfunction when there is previous history of neonatal hyperthyroidism or when the mother has GD or has had GD in the past (96,101) (**D,B**).

TRAb is present in 10% to 75% of patients with atrophic thyroiditis and in 0% to 20% of patients with Hashimoto's thyroiditis (102) (**D**). High titers are observed in mothers of children with transient neonatal hypothyroidism. TRAb testing is recommended during pregnancy for mothers with autoimmune hypothyroidism to efficiently predict the potential for neonatal hypothyroidism (101) (**B**).

Recommendations

TRAb testing has good specificity for diagnosing GD but in most cases is not essential for the diagnosis. In some instances, it may facilitate the differential diagnosis of hyperthyroidism (**B**).

An initial assessment of TRAb is useful to ascertain disease severity and may, in combination with other clinical indicators, contribute to treatment decisions (**D**).

Quantitating TRAb levels prior to discontinuing antithyroid drug treatment may identify those patients who should initiate medication cessation; normal TRAb levels indicate higher odds of remission (**D**).

TRAb testing is recommended in pregnant women with GD at the beginning and in the third trimester of pregnancy (between gestational weeks 20 and 24) to determine the risk for fetal hyperthyroidism and transient neonatal thyrotoxicosis (**B**).

SUMMARY OF RECOMMENDATIONS

After reviewing and discussing the key topics related to thyroid function testing, the primary recommendations include the following:

- TSH testing is the best method for triaging thyroid dysfunction and for monitoring patients being treated for hypothyroidism. TSH is also a good marker for monitoring the replacement dose of levothyroxine.
- Abnormal TSH is not synonymous with thyroid disease, and other conditions should be considered.
- Free T4 is critical for evaluating patients with hypothalamic-pituitary disease. It is also useful for evaluating the response to levothyroxine in cases of poor compliance and in the first months of treating patients with chronic, severe hypothyroidism.
- Total T3/T4 abnormalities typically result from changes in transport proteins, not thyroid function. Therefore, total hormone testing should be performed in particular circumstances.
- There is no indication for routine serum T3 testing for the diagnosis and monitoring of patients with hypothyroidism.
- Serum T3 testing, interpreted in combination with FT4, is useful for evaluating hyperthyroidism symptoms.
- Thyroid function should be closely monitored during pregnancy because of the negative impact that maternal hypothyroidism can have on obstetric outcome and fetal development. TSH tests should be performed prior to pregnancy or in the first trimester; mild thyroid failure is indicated by TSH values > 2.5 mIU/L in the first trimester.
- Thyroid function tests should not be routinely performed in severely ill hospitalized patients (NTI).
- The concentrations of TPOAb and TgAb suggest the autoimmune etiology of thyroid dysfunction. There is no indication for monitoring antithyroid antibody levels during hypothyroidism treatment. TPOAb testing is common, is the most sensitive and is useful for determining the risk of postpartum thyroiditis.
- TgAb testing, in combination with Tg testing, is crucial for monitoring patients with differentiated thyroid cancer.
- TRAb testing enables the differential diagnosis of hyperthyroidism and has good specificity for GD but is not commonly essential for diagnosis. An initial evaluation of TRAb levels enables the determination of disease severity and may contribute to treatment decisions and estimating the odds of remission. TRAb testing should be performed in pregnant women with GD in the third trimester to assess the risk of transient neonatal thyrotoxicosis.

FINAL CONSIDERATIONS

This manuscript illustrates the relevance of adequate laboratory testing in the management of thyroid dysfunction, a group of disorders that are frequently seen by endocrinologists. For each test, the precise indications and the individual peculiarities must be considered. Guidelines help to schematically evaluate the available published data, but the clinician should always treat each patient individually, considering the clinical context in combination with additional tests.

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