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RESEARCH ARTICLE

Challenges in Thyroid Function Testing: Interferences and Clinical Implications

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ABSTRACT

In recent decades, significant advancements have been made in the methodologies used for evaluating thyroid dysfunctions. These advancements include the development of radioimmunoassays, immunometric assays, and liquid chromatography coupled with mass spectrometry.

The main thyroid function tests include thyroid-stimulating hormone (TSH) and the measurement of triiodothyronine (T3) and thyroxine (T4), including their total and free fractions.

Over time, different generations of TSH tests have been developed, starting from radioimmunoassays to immunometric assays, and more recently, tests utilizing fluorophores and chemiluminescent molecules. The third-generation tests are currently the most widely used due to their high sensitivity and specificity. Thyroid-stimulating hormone is preferred as the initial test for evaluating thyroid function since it has a log-linear relationship with free T4 levels, enabling the identification of subclinical hypothyroidism and subclinical hyperthyroidism.

The reference range for normal TSH levels is typically between 0.45 and 4.5 mIU/L. However, there can be variations in TSH levels based on factors such as sex, age, and ethnicity. Specific reference values may be required for certain populations, such as the elderly, pregnancy, and neonates. In the elderly, an increase in TSH levels is expected. In the neonatal period, TSH levels are high after birth and take a few weeks to normalize. During pregnancy, various physiological changes occur, leading to alterations in thyroid hormones to meet fetal demands.

Laboratory interferences in thyroid hormone assays must be considered to ensure accurate results. Biotin interference can lead to falsely low TSH and falsely high free T3 and T4 levels. Macro-thyrotropin can cause elevated TSH levels with normal thyroid hormone levels. Heterophilic antibodies can also cause false results. Additionally, the evaluation of autoantibodies and markers used in thyroid cancer follow-up needs special attention.

Patients experiencing conditions like trauma, particularly in severe cases, may undergo changes in thyroid hormone levels, even without having a specific thyroid disease, which is called low triiodothyronine syndrome.

In conclusion, it is essential to be aware of potential laboratory interferences and consider individual variations to ensure accurate interpretation and appropriate management of patients.

Keywords: thyroid function tests; hyperthyroidism; hypothyroidism; thyroid-stimulating hormone.

Introduction

A significant advancement has been observed in recent decades regarding the methodologies used for assessing thyroid dysfunctions. In the 1950s, only an indirect estimation of total thyroxine (T4) was possible using protein-bound iodine (PBI) analysis. Over time, new techniques have been developed and utilized, such as radioimmunoassays and immunoassays. More recently, the evaluation through liquid chromatography coupled with mass spectrometry has become possible, allowing for increased sensitivity and specificity.[1]

Despite the evolution of these methods, there may still be laboratory interferents that require special attention in the interpretation of tests. Similarly, certain clinical conditions can result in increased or decreased thyroid hormone levels.[1]

The objective of this article is to provide a comprehensive review of recent advancements in the methodologies used for assessing thyroid dysfunctions and to discuss the main clinical and laboratory considerations related to thyroid function tests. The article aims to cover different hormonal assessments of the thyroid, including thyroid-stimulating hormone (TSH), total and free triiodothyronine (T3), and thyroxine (T4).

Additionally, the article explores the key clinical situations that can impact thyroid hormone levels, such as pregnancy, aging, and the neonatal period. It discusses the expected hormonal changes during each life stage and emphasizes the importance of the interpretation to avoid misdiagnosis and inappropriate treatments.

Methods

We conducted a comprehensive literature search at PubMed and Google Scholar with the following MeSH terms and free text words: “hyperthyroidism”, “hypothyroidism”, “thyroid-stimulating hormone”, “triiodothyronine”, “thyroxine”, “anti-thyroid peroxidase antibody”, “anti-thyroglobulin antibody”, “anti-thyroid-stimulating hormone receptor antibody”, “calcitonin”, “biotin” and“macro-thyrotropin, heterophilic antibodies”. Were included articles in English and Portuguese language.

Thyroid-Stimulating Hormone

Thyroid-stimulating hormone, a hormone produced in the anterior pituitary gland, stimulates the thyroid gland to produce other two hormones: T4 and T3. This glycoprotein consists of two subunits: alpha and beta. The alpha subunit is common to other hormones such as human chorionic gonadotropin (hCG), luteinizing hormone (LH), and follicle-stimulating hormone (FSH). The beta subunit, on the other hand, provides specificity.[2]

With advancements in TSH testing, it became possible to increase the sensitivity of detecting patients with hyperthyroidism compared to those with euthyroidism.[1] The first-generation tests, using radioimmunoassay, could only identify patients with hypothyroidism, as they had a lower limit of detection of approximately 1.0 mIU/L. To diagnose hyperthyroidism, a thyrotropin-releasing hormone (TRH) stimulation test was required.[1] Another disadvantage was the susceptibility of the method to cross-reactivity with hormones similar in structure to TSH, such as hCG, LH, and FSH.[2]

The second-generation tests introduced immunometric assays using a "sandwich" methodology with antibodies against TSH epitopes. This improved sensitivity and reduced the lower limit of detection to 0.1 mIU/L.[1]

The third generation allowed the detection of TSH values as low as 0.01 mIU/L. These tests utilize different signals, such as fluorophores and chemiluminescent molecules. Depending on the signal, the method may be referred to as immunofluorometric when fluorophores are used, or chemiluminescent when chemiluminescent molecules are used. This generation includes the most commonly used tests today, with a sensitivity of 97% and specificity of 93%, providing results in just a few minutes.[3] Fourth-generation tests have also been developed, but since they do not increase diagnostic accuracy, they are not routinely used.[4]

Thyroid-stimulating hormone is the preferred initial test for evaluating thyroid function in cases of suspected primary dysfunction. It exhibits a log/linear relationship with free thyroxine (T4) levels, as even small variations in free T4 can result in significant changes in TSH levels. Thus, it is possible to observe situations where TSH is elevated or decreased with normal free T4 levels, which are referred to as subclinical hypothyroidism and subclinical hyperthyroidism, respectively.[5]

Under normal conditions, TSH variations within an individual are considered small, around 0.5 mIU/L. Genetic factors appear to play a role in establishing a TSH set-point in the thyroid axis, as observed in studies involving identical twins.[6]

The reference range considered normal for TSH is between 0.45 and 4.5 mIU/L. Although TSH

secretion peaks at midnight and reaches its nadir around noon, the small amplitude does not significantly affect the test results.[1,7,8]

It is also known that TSH levels can vary depending on certain individual characteristics. In the NHANES III study (Third National Health and Nutrition Examination Survey), thyroid function was measured in 13,344 individuals over the age of 12 with no thyroid disorders and negative antithyroid peroxidase and antithyroglobulin antibodies. The study found an average TSH value of 1.50 mIU/L, but with variations according to sex, age, and ethnicity.[9]Therefore, it is suggested to use specific reference values for the elderly and neonates, which will be discussed further.[1]

Measurement of Total Triiodothyronine and Thyroxine Fractions

In the mid-1950s, only an indirect estimation of total T4 was possible. In the 1970s, the methodology was replaced by radioimmunoassays, and currently, most results are obtained through automated platforms using competitive assays.[5]

It is well established that thyroid hormones, for the most part, circulate bound to carrier proteins. Approximately 99.97% of T4 and 99.7% of T3 circulate bound to these proteins. The main carrier protein is thyroxine-binding globulin (TBG). Binding to transthyretin and albumin also occurs, although with lower affinity. Thyroid hormones also circulate in their free form, but to a lesser extent.[1,5]

The measurement of total T3 poses a greater challenge than that of total T4 because T3 concentrations are ten times lower than those of T4. Nonetheless, considering that circulating levels of total fractions are higher than free fractions, the measurement of these hormones is relatively straightforward.[1,5]

The main consideration is that, due to their binding to carrier proteins, total fractions can be influenced by situations that alter the levels of TBG, transthyretin, and albumin, making them sometimes unreliable. Various conditions that can lead to increased or decreased TBG levels are described, including medications, infections, liver and kidney diseases, alcoholism, malnutrition, and congenital conditions.[1]

Dosage of free Triiodothyronine and Thyroxine fractions

Free fractions represent a small portion of thyroid hormones, around 0.02% of T4 and 0.2% of T3. Their measurements pose a challenge as they are present in picomolar concentrations.[5] The measurement can be performed using direct or indirect methods. In the direct analysis, equilibrium dialysis of serum followed by measurement of the dialysate using liquid chromatography coupled with tandem mass spectrometry is the reference method. However, this method is technically difficult and expensive, and is primarily reserved for research purposes. Interference from factors such as dilution, pH, membrane defects, fatty acids, and temperature can affect the evaluation.[10] In most cases, indirect methods, such as automated immunoassays, are used. Although these immunoassays have been improved over the years, they can still be influenced by changes in albumin, heterophilic antibodies, and reagents such as biotin.[5]

Special Clinical Situations

NEONATAL PERIOD

In the first trimester of gestation, the fetus is exclusively dependent on maternal thyroid hormones. These hormones are crucial for the development of the fetus, particularly the neurological system.[11] The fetal thyroid begins producing hormones around 18-20 weeks, leading to increased levels of circulating T4 and T3. However, due to the immaturity of the hypothalamic-pituitary-thyroid axis, minimal inhibition of TSH by negative feedback is observed.[12] It is also known that even though fetal thyroid hormone production starts in the second trimester of gestation, maternal hormones remain essential until the time of delivery. It is estimated that 30-50% of the measured T4 in the umbilical cord is of maternal origin.[13] After birth, there is a peak in TSH, which can reach values of 60-80 mIU/L, most notably within the first 30 minutes after delivery. Among the factors contributing to this increase, exposure of the newborn to a cold environment is believed to be one of the main triggers.[11,12] Thyroid-stimulating hormone levels decrease and reach normal values within 3 to 5 days, while total and free T4, stimulated by the TSH peak, take a few weeks to normalize.[12] The implementation of neonatal screening programs has significantly reduced the complications associated with congenital hypothyroidism, such as delayed neurological development and growth abnormalities.[11,14]

PREGNANCY

As discussed earlier, maternal thyroid hormones play a crucial role in fetal development. During pregnancy, various physiological changes occur that result in alterations in these hormones. It is of utmost importance to recognize and correctly

interpret the results of serum thyroid hormone and TSH measurements.[15]

Several factors contribute to the increase in thyroid hormones during this period: the need to meet the demands of the fetus, an increase in TBG due to elevated estrogen levels, increased iodine clearance, and increased degradation of hormones by type 3 deiodinase, which is present in the placenta. In the first trimester, there is an increase in hCG which shares similarities with the TSH molecule, stimulating the production of thyroid hormones. This leads to a temporary increase in total T4 and a subsequent reduction in TSH levels. These thyroid function changes that occur in the first trimester are referred to as "physiological hyperthyroidism of pregnancy" (Figure 1).[15]

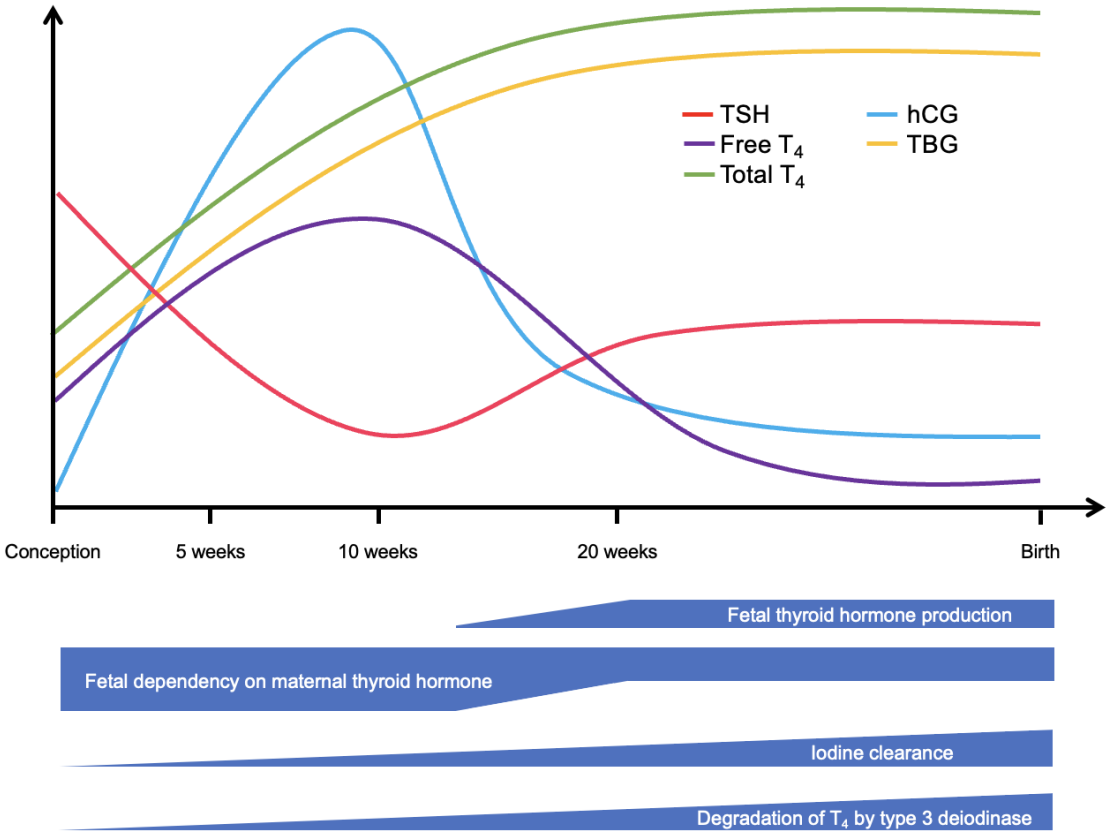

Figure 1: Physiological changes during pregnancy. Adapted from Korevaar TIM, et al., 2017.[16]

Universal screening is a point of debate because, in addition to identifying patients with overt hypothyroidism (OH), it also leads to an increase in the diagnosis of subclinical hypothyroidism (SCH). The benefit of treating SCH has been proven in relation to obstetric complications, but its impact on the fetus is not well established. We must consider that universal screening could potentially result in overtreatment, exposing both the mother and the fetus to complications associated with excessive thyroid hormone.[17]

In a longitudinal Dutch study evaluating 2198 pregnant women with no history of thyroid disease, the prevalence of subclinical hypothyroidism was 3.2%. Fifteen patients required treatment after screening, 11 with overt hypothyroidism and 4 with TSH > 10 mIU/L and normal free T4, with only one of them reporting significant thyroid-related symptoms.[18]

The American Thyroid Association (ATA), in its latest guideline, concluded that there is insufficient evidence to recommend or discourage universal TSH screening. However, in women with risk factors for thyroid disease who are confirmed to be pregnant, an initial evaluation of thyroid function should be performed, with TSH being the preferred test.[17]

During pregnancy, different reference ranges should be used compared to those for non-pregnant individuals. Ideally, specific TSH reference values should be established for each local population, as there are differences between races, ethnicities, and iodine intake.[17,19] However,

considering the impracticality of conducting studies in all regions, the ATA suggests that a lower limit for TSH can be established in the first trimester, i.e., 0.4 mIU/L below the lower limit in the general population. For the upper limit of TSH, a value of 4.0 mIU/L is accepted for most pregnant women, as a reduction of 0.5 mIU/L from the upper reference value of the general population is recommended.[17]

For patients with known thyroid dysfunction who are taking antithyroid drugs or levothyroxine, the evaluation of thyroid hormones in addition to TSH measurement is necessary. However, special care is required in this situation. Most assays for free T4 (FT4) use indirect methods, which can be affected by higher concentrations of TBG and non-esterified fatty acids in pregnant women, as well as the relatively lower concentration of albumin, especially in the second and third trimesters. Since it is difficult to establish specific values for each population and trimester of pregnancy, laboratories often rely on values provided by manufacturers.[17]

Regarding the evaluation of total T4 (TT4) during pregnancy, some studies suggest that it represents a reliable measure, especially towards the end of pregnancy, as long as the values are adjusted for the increase in TBG. However, it can be challenging to adjust these values in the early phase when a 5% increase in TT4 is presumed each week starting from the seventh week. After the 16th week, an approximately 50% increase in TT4 is estimated, which remains until the end of pregnancy.[17]

Aging

It is expected that as the years go by, there is a reduction in the clearance of T3 and T4, resulting in a longer half-life of these hormones in the elderly population and, consequently, a lower production by the thyroid gland.[20]

Studies have shown that TSH levels increase with age, without a reduction in measured levels of free T4.[21-23] This increase in TSH is observed even in patients without thyroid diseases and negative antibodies (Figure 2).[23] Therefore, a significant portion of elderly patients falls into a scenario of subclinical hypothyroidism, which actually confers a protective effect on the cardiovascular system.[21,22,24]

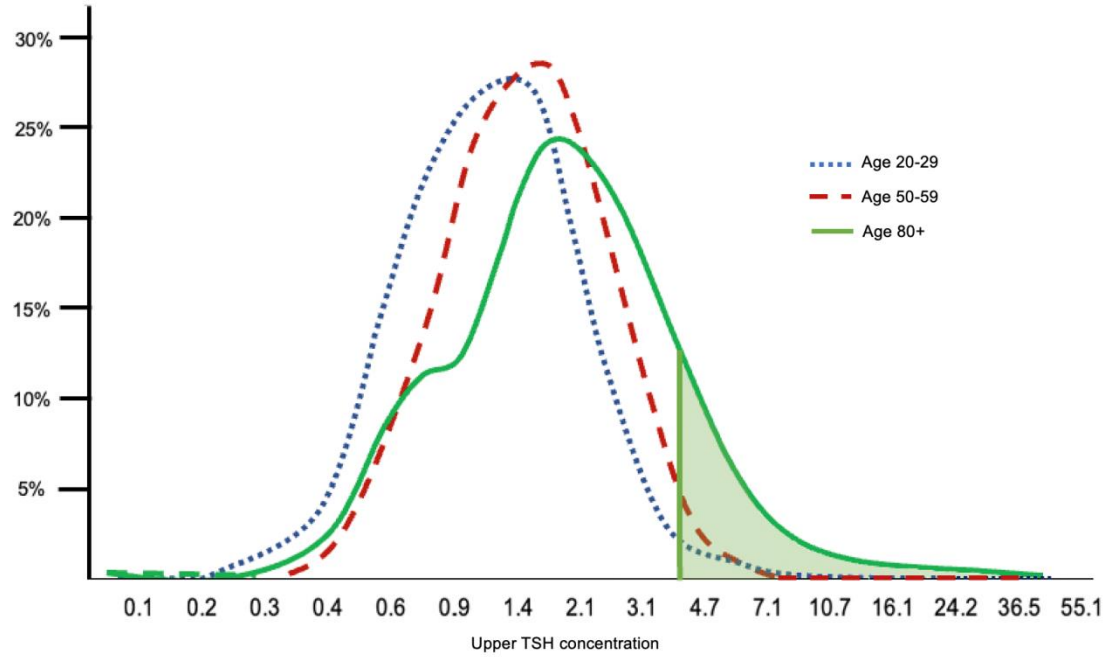

Figure 2. Distribution of TSH by age groups in the United States, in patients without known thyroid diseases and with undetectable thyroid antibodies. The green area represents the population (12%) in the age group above 80 years, without thyroid disease, with TSH > 4.5 mIU/L. Adapted from Surks MI, et al., 2011.[23]

The indication for treating subclinical hypothyroidism varies according to age group, and careful evaluation of thyroid function should be considered, as well as complications related to overtreatment, especially from a cardiovascular perspective. It is known that in subclinical hypothyroidism, when TSH is below 7 mIU/L, there is no association with an increased risk of

cardiovascular disease, heart failure, or dementia.[24-27] TSH levels up to 10 mIU/L, with normal free T4, have also not been correlated with an increased risk of ischemic stroke.[28]

In the elderly population, there is also a subset of individuals with subclinical hyperthyroidism, mainly due to autonomous production by nodules. In this situation, even without TSH suppression, studies have shown that when TSH levels are between 0.1 and 0.44 mIU/L, there is an association with a higher risk of atrial fibrillation and hip fractures. For this reason, there is a higher tendency for treatment compared to younger individuals with the same hormone levels.[29,30]

Low Triiodothyronine Syndrome

Patients under conditions such as trauma, infections, and surgeries, especially in more severe situations, may experience alterations in thyroid hormone levels, even if they do not have a specific thyroid disease.[31] This is partly due to a reduction in the carrier proteins of T3 and T4, as there is a decrease in their synthesis and an increase in their degradation. Additionally, there is impaired binding of these proteins with thyroid hormones due to conformational changes and altered affinity between the carrier proteins and thyroid hormones.[32]

Approximately two hours after a significant stress event, decreases in T3 levels and increases in reverse T3 (rT3) are observed. These findings are mainly a result of reduced activity of type 1 and type 2 deiodinases, which are enzymes present in peripheral tissues responsible for converting T4 to T3 in locations such as the liver, kidneys, muscles, and brain. Furthermore, more T4 becomes available for conversion into rT3, an inactive form of the hormone, by type 3 deiodinase.[32]

Individuals who undergo prolonged intensive treatment also experience reductions in T4 levels, with T3 levels remaining very low or even undetectable. Despite these significant reductions, paradoxically, TSH often remains normal in many patients, indicating potential hypothalamic dysfunction in the pathogenesis of more chronic conditions.[32,33]

More notable reductions in T3 and T4 levels have been correlated with higher mortality in critically ill patients.[34] Similarly, reduced T3 levels represent a condition associated with unfavorable outcomes in non-critically ill patients admitted to general hospital wards.[35]

Despite increasing studies evaluating thyroid hormones as potential prognostic predictors, it is known that numerous medications commonly used in hospital settings, such as corticosteroids, vasoactive drugs, antiarrhythmics, and anticonvulsants, interfere with the evaluation of thyroid hormones. Therefore, routine measurement of these hormones is not recommended, except in cases of suspected thyroid disease.[4,36,37]

Evaluation of Autoimmunity and Markers Used in Thyroid Cancer Follow-up

ANTI-THYROID PEROXIDASE ANTIBODY

Anti-thyroid peroxidase antibody (TPOAb) is present in the majority of patients with Hashimoto's thyroiditis, the main cause of hypothyroidism. It is estimated that over 90% of individuals with Hashimoto's have this antibody detected, making it the most sensitive antibody in this scenario.[4] In patients with Graves' disease, the prevalence is lower, around 74%, according to data from an Italian study in the 1980s.[38]In approximately 11% of the general population, TPOAb can be identified even in the absence of thyroid dysfunction.[9] Individuals without hypothyroidism but with positive antibodies have a higher risk for the development of thyroid hormonal dysfunction.[38]

The main indication for performing this test is suspicion of autoimmune thyroid disease. Patients with subclinical hypothyroidism may also benefit from evaluation, as the presence of antibodies is associated with a higher risk of progression to overt hypothyroidism.[4]

Euthyroid women with a history of infertility or recurrent miscarriages, but with positive TPOAb, should be evaluated for the indication of levothyroxine treatment in the preconception period or during pregnancy. However, there is no clear evidence regarding the benefit of this approach.[39]

ANTI-THYROGLOBULIN ANTIBODY

Anti-thyroglobulin antibody (TgAb) is less sensitive than TPOAb in autoimmune thyroid diseases. It is present in approximately 70% of patients with autoimmune-related hypothyroidism. Therefore, if there are limitations in financial resources, TPOAb should be the preferred test when Hashimoto's is suspected. Anti-thyroglobulin antibody can also be found in up to 40% of individuals with Graves' disease and in 10 to 15% of those without thyroid disease.[4]

It is a widely used test in the follow-up of differentiated thyroid carcinoma (DTC). In this context, its presence, occurring in up to 1/4 of patients, can interfere with thyroglobulin (Tg) results and lead to false-negative or false-positive findings, depending on the methodology used. When TgAb is positive, it is essential for appropriate risk stratification in DTC patients and renders Tg values less reliable.[40]

ANTI-THYROID-STIMULATING HORMONE RECEPTOR ANTIBODY

Anti-thyroid-stimulating hormone receptor antibody (TRAb) is the most specific antibody in Graves' disease, present in 9 out of 10 patients affected by this condition. When a case of moderate to severe hyperthyroidism associated with diffuse goiter and ophthalmopathy is identified, TRAb testing is unnecessary for the diagnosis of Graves' disease. In other patients with autoimmune hyperthyroidism, TRAb evaluation is often necessary as an auxiliary method for differential diagnosis. Additionally, this antibody plays an important role in considering the discontinuation of anti-thyroid drugs, as normalization of TRAb is associated with lower recurrence rates.[41]

Due to its ability to pass through the placenta, TRAb levels should be measured in pregnant women with Graves' disease. If TRAb levels are elevated, it should be repeated between weeks 18 and 22 of gestation to assess the risk of thyroid dysfunction in the fetus. Levels three times above the upper limit of normal have been mainly correlated with fetal hyperthyroidism.[42]

Although TRAb is frequently associated with the development of hyperthyroidism, cases of hypothyroidism caused by a blocking type of TRAb have been reported for over 40 years.[43,44]It should be noted that this phenomenon is rare but well-described. Furthermore, there can be an alternation between the stimulatory state, which is the most common, and an inhibitory state.[44]

In general, TRAb assays can be divided into two types: a) assays that detect TRAb by its competitive binding capacity with a substance known to bind to the TSH receptor; b) assays that detect the production of cyclic adenosine monophosphate (cAMP) in the serum of patients. Classically, the first methodology cannot differentiate between stimulatory and inhibitory TRAb, while the second method can only identify stimulatory TRAb.[45]

From a practical standpoint, however, the need for laboratory differentiation between these antibodies in Graves' disease is limited. If there is an increase in thyroid hormones, it will be considered a consequence of stimulatory TRAb. A different situation may occur in a pregnant woman who is euthyroid at the time of evaluation but has a history of previous thyroid dysfunction and still has detectable high levels of TRAb. In this scenario, TRAb could also represent an increased risk of fetal hypothyroidism if it has an inhibitory effect.[45]

THYROGLOBULIN

Thyroglobulin (Tg) is a high molecular weight protein expressed exclusively in thyroid follicular cells. Its main role in testing is in the follow-up of patients with differentiated thyroid carcinoma. Although widely used for monitoring this thyroid neoplasm, there is no indication for measuring it as an initial evaluation of a suspected thyroid nodule, as there is an overlap of values in benign and malignant diseases.[40]

Thyroglobulin measurement can aid in the differential diagnosis between painless subacute thyroiditis and factitious thyrotoxicosis, with high or low levels observed, respectively.[46]

Three methods can be used to measure Tg: immunometric assay, radioimmunoassay, and liquid chromatography coupled with tandem mass spectrometry. The immunometric assay is automated, has good sensitivity, and is commonly used in practice. The presence of TgAb can lead to falsely low or high Tg results. Other challenges include interference from heterophilic antibodies and the presence of different Tg isoforms. Evaluation by liquid chromatography is an option in case of these laboratory interferences, but as discussed above, it is a less available, more expensive, and more complex test.[47]

CALCITONIN

Calcitonin is a hormone produced by the thyroid parafollicular cells (C-cells). Calcitonin measurement is essentially used as a marker for medullary thyroid carcinoma, with importance in the diagnosis, monitoring, and prognosis of these patients. Very high baseline values, in the absence of interferents, are highly suggestive of metastasis, especially when values exceed 500 pg/mL.[48]

Several clinical conditions can cause alterations in serum calcitonin levels, such as pregnancy, lactation, chronic kidney disease, lung neoplasms, food and alcohol intake, and the use of proton pump inhibitors.[49] In rodent models, glucagon-like peptide-1 (GLP-1) agonists have been shown to

increase calcitonin levels, with positive regulation of its gene expression, and have been associated with C-cell hyperplasia.[50] In the post-hoc analysis of the LEADER trial (The Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results), with over 9,000 patients treated with liraglutide 1.8 mg per day or placebo, no difference in calcitonin levels was observed between the groups over a 3-year period.[51] However, long-term studies are still needed to understand the effect of this medication on C-cells in humans.[50]

Another important aspect in calcitonin measurement is the need for rapid centrifugation after sample collection and the low stability of the material at room temperature.[52]

MAIN LABORATORY INTERFERENCES IN THYROID HORMONE ASSAYS

The assay methods used for thyroid hormone measurement are vulnerable to various types of interference. Despite ongoing efforts to reduce these potential interferents, they remain factors that must be taken into consideration when interpreting results, especially when discrepancies between values or dissociations between results and the patient's clinical condition are observed. Recognizing these interferences can help avoid making incorrect decisions.[53]

BIOTIN

Biotin is a B-complex vitamin found abundantly in animal and plant sources. Biotin deficiency is quite rare.[54] Studies in patients with multiple sclerosis have shown positive results with the use of high doses of biotin.[55] It is frequently used as a vitamin supplement to reduce hair loss and improve the appearance of nails and skin.[53]

The high affinity interaction between biotin and streptavidin has been widely employed in competitive and immunometric assays.[53,54]Excess biotin displaces the antibody-antigen complex from streptavidin. Therefore, in a "sandwich" type assay for TSH, where the signal is directly proportional to the concentration of TSH, biotin levels become falsely low. The opposite occurs in competitive assays for free T3 and T4 fractions, as their levels are overestimated due to the inversely proportional signal. However, it should be noted that not all platforms are affected by this interference since biotin-streptavidin interaction is not always used.[53]

The scenario of a possible decrease in TSH with an increase in free T3 and T4 fractions becomes even more interesting considering that TRAb levels can also be falsely elevated due to biotin interference, mimicking Graves' disease.[56]

Patients who obtain biotin exclusively through diet usually do not present the described interferences. However, the use of high doses in neurological diseases and supplementation with multivitamins can cause significant alterations.[53,54] To avoid misdiagnosis and unnecessary treatments, several authors have proposed discontinuing biotin supplementation for up to 72 hours before the sample collection.[54,55] The ATA guideline suggests discontinuing biotin supplementation two days before laboratory testing.[41]

MACRO-THYROTROPIN

Macro-thyrotropin (macro-TSH) is a circulating form of TSH, but with a high molecular weight, generally exceeding 150 kDa. The formation of this molecule occurs through the binding of TSH to autoantibodies, mainly of the immunoglobulin G (IgG) class. In this way, antigen-antibody complexes are formed, which can be recognized by commercially available assays but do not exert a biological effect.[53]

Laboratory findings in macro-TSH show elevated levels of TSH with normal T3 and T4. Most of the time, TSH levels are above 10 mIU/L, but more subtle alterations have also been described.[58,59]

Suspicion of macro-TSH arises when TSH is elevated in isolation, and thyroid hormone levels are normal. In addition, the patient does not exhibit symptoms consistent with hypothyroidism.[53]

To confirm the presence of macro-TSH, gel filtration chromatography is the most appropriate method, but it is not widely available and is costly.[60] The use of polyethylene glycol (PEG) precipitation procedure is well-documented for the detection of macroprolactin and has been extrapolated to the detection of macro-TSH. The presence of the high molecular weight interferent occurs when TSH recovery is low, with proposed values below 20 or 25%.[53,58]

HETEROPHILIC ANTIBODIES

Heterophilic antibodies are antibodies against animal immunoglobulins. They can be formed after an individual's exposure to an external antigen, such as through vaccines.[60] Human Anti-Mouse Antibody (HAMA) is one of the most common antibodies in this category, with a prevalence ranging close to 11% in the general population.[61]

In "sandwich" format assays, HAMA can lead to an increase in the analyzed signal, causing a false

elevation of TSH in some situations. False negative results have also been described, but to a lesser extent. Fortunately, manufacturers have improved their tests to reduce these interferences, and most assays now come with anti-HAMA antibodies.[60]

Table 1. The most frequent laboratory alterations in clinical practice discussed above and includes other causes of possible discrepancies between TSH and T4 levels. Adapted from Graf H et al. 2002.[62]

		Free T_4		
		Normal	**Low**	**High**
TSH	**Normal**	- Normal	- Central hypothyroidism with normal TSH	- Syndrome of resistance to thyroid hormone action - Drugs - Autoantibodies or heterophile antibody
	Low	- Levothyroxine overdose - Initial treatment of hyperthyroidism - Subclinical hyperthyroidism - Drugs	- T_3 thyrotoxicosis - Central hypothyroidism - T_3 therapy	- Hyperthyroidism - Factitious hyperthyroidism - Use of biotin
	High	- Irregular use of levothyroxine - Macro-TSH - Heterophile antibody	- Primary hypothyroidism - Central hypothyroidism with high TSH	- Syndrome of resistance to thyroid hormone action - Thyrotropin-secreting pituitary adenomas

Conclusion

As discussed, significant advancements have been made in recent years with the development of new methodologies and laboratory assays. However, it is important to note that falsely elevated or reduced results are still frequently encountered in various scenarios. Therefore, the interpretation of thyroid function tests can be challenging in clinical practice. Recognizing the main interferences in this process, as well as the expected hormonal changes throughout different stages of life, is crucial for the appropriate management of patients and to avoid unnecessary and potentially harmful treatments.

Conflicts of Interest Statement

The authors have no conflicts of interest to declare.

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