

## Thyroid Function Testing: Why, What and When

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### ARTICLE INFO

Received Date: February 01, 2019

Accepted Date: March 04, 2019

Published Date: March 08, 2019

### KEYWORDS

Thyroid function tests  
Subclinical thyroid disease  
Hyperthyroidism  
Hypothyroidism  
Thyroid nodules  
Thyroid cancer  
Non-thyroidal illness  
Drugs

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**Citation for this article:** Chin-Shern LAU and Tar-Choon AW. Thyroid Function Testing: Why, What and When. Journal of Otolaryngology: Research. 2019; 2(1):128

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### ABSTRACT

Thyroid disorders are common. It is important to understand why thyroid function tests are performed, what tests are available, and when the tests are useful. This article summarizes the interpretation of laboratory tests of thyroid function and their use in managing thyroid disease. There are several pitfalls and caveats in the analysis of thyroid function tests that are noteworthy. Thyroid function is tightly controlled by the hypothalamic-pituitary-thyroid axis in the body, with the unbound free thyroid hormones being the metabolically active moieties. The thyroid function tests commonly used include thyroid stimulating hormone (TSH), free thyroxine, TSH receptor antibodies, thyroid peroxidase antibodies and biomarkers of thyroid cancer such as thyroglobulin and calcitonin. The use of these tests in the diagnosis of thyroid disorders and the factors that can affect them are reviewed. Their use in the different clinical scenarios is considered from screening for thyroid disease, subclinical thyroid dysfunction, overt hyperthyroidism and hypothyroidism, to thyroid nodules and cancer. Especially pertinent is the recent data on subclinical thyroid dysfunction where management is needed only for the severe cases where the thyroid stimulating hormone is below 0.1mIU/L or over 10mIU/L and associated with positive antibodies (thyroid peroxidase and TSH receptor antibodies). Also included are sections exploring special precautions in the interpretation of thyroid function testing in certain clinical situations such as non-thyroidal illness syndrome in hospitalized subjects, pregnancy and drugs that can affect results of thyroid function tests. An astute clinician must be aware of the tests available and their utility in the screening, diagnosis and management of thyroid disorders.

**Abbreviations:** TFT: Thyroid function test; TSH: Thyroid stimulating hormone; TRH: Thyrotropin releasing hormone; T4: Thyroxine; T3: Tri-iodothyronine; TBG: Thyroid binding globulin; FT4: Free thyroxine; FT3: Free tri-iodothyronine; NHANES: National Health and Nutrition Examination Survey; TPOAb: Thyroid peroxidase antibodies; Tg: Thyroglobulin; TgAb: Thyroglobulin antibodies; TRAb: TSH receptor antibodies; ATA: American Thyroid Association; DTC: Differentiated thyroid cancer; LC-MSMS: Liquid chromatography-tandem mass spectrometry; FNA: Fine needle aspiration; AACE: American Association of Clinical Endocrinologists; MTC: Medullary thyroid cancer; USPSTF: US Preventive Service Task Force; GD: Graves' disease; MNG: Multinodular goitre; hCG: Human chorionic gonadotropin; NTIS: Non-thyroidal illness syndrome; HPT: Hypothalamus-pituitary-thyroid; HG: Hyperemesis gravidarum

## INTRODUCTION

Thyroid disorders are common in clinical practice. A sound appreciation of thyroid physiology is necessary when interpreting Thyroid Function Tests (TFT). Understanding TFT is important for effective diagnosis and management of thyroid disorders. In this article, we provide a contemporary update and review the salient points in the evaluation and interpretation of TFTs. The hypothalamic Thyrotropin Releasing Hormone (TRH) acts on the anterior pituitary gland to produce Thyroid Stimulating Hormone (TSH). TSH stimulates the thyroid gland to produce thyroid hormones - 85-90% thyroxine (T4) and 10-15% tri-iodothyronine (T3). The bulk of T3 is produced from the conversion of T4 by T4-5'-deiodinases in peripheral tissues. More than 99% of the thyroid hormones (both T4 and T3) are bound to thyroid hormone binding proteins - Thyroid Binding Globulin (TBG), albumin, and transthyretin - making them unavailable to tissues. Only a small percentage of the biologically active hormones exist as free thyroid hormones - fT4 0.05% and fT3 0.5%. fT3 is the major bioactive thyroid hormone. The free hormones exert a negative feedback on both the hypothalamus and pituitary. TSH is exquisitely sensitive to fT4/fT3 levels. In response to a 2-fold change in fT4, the pituitary exhibits a much larger inverse variation (100 fold) in TSH secretion. This relationship renders TSH as a very sensitive indicator of thyroid status. However, TSH may not always reflect thyroid status accurately. When treatment is started or when medication dosages are changed pituitary TSH secretion is reset to a new steady state, a process that may take weeks or months. The fT4-TSH relationship gives rise to the entity of subclinical thyroid disorders - subclinical hypothyroidism when thyroid hormone levels are within the normal reference range but TSH levels elevated, and subclinical hyperthyroidism where TSH levels are decreased in the face of normal thyroid hormones (see Table 1).

**Table 1: TSH and fT4 in Different Categories of Thyroid Dysfunction**

THYROID STATUS	TSH (0.4-4.0 mU/L)	fT4 (10-20 pmol/L)
Overt Hyperthyroidism	Very low (<0.01)	Increased (>20)
Subclinical Hyperthyroidism -Severe -Mild	Suppressed (<0.1) Decreased (0.1-0.4)	Normal Normal
Euthyroid	Normal (0.4-4.0)	Normal (10-20)
Subclinical Hypothyroidism -Mild -Severe	Increased (4.0-10.0) Elevated (>10.0)	Normal Normal
Overt Hypothyroidism	Very High (>10.0)	Decreased (<10)

### Why test?

Thyroid disorders are common, tests are readily available and treatment is effective. In the US National Health and Nutrition Examination Survey (NHANES) 2007-2012, thyroid dysfunction was found in 7.1% of the US population [1] - 0.5% overt and 6.6% subclinical. However, the clinical assessment of thyroid disease is notoriously inaccurate. In a primary care clinic [2], most of the patients suspected of thyroid disease based on physical examination and history had normal thyroid function when tested biochemically. In another study [3], the original clinical diagnosis of thyroid dysfunction was revised in one third of patients after TFT results were known. While history and physical examination are necessary in thyroid evaluation, clinical assessment alone is insufficient. Thyroid Function Tests (TFTs) are needed.

### What tests?

Tests available include thyroid stimulating hormone (TSH), free thyroxine (fT4), free triiodothyronine (fT3), thyroglobulin (Tg), thyroglobulin antibodies (TgAb), thyroid peroxidase antibodies (TPO-Ab), TSH receptor antibodies (TRAb) and calcitonin. These tests assess function, etiology and cancer.

### Tests of function

**TSH:** The typical reference range for serum TSH is 0.4-4.0mIU/L. The 2016 American Thyroid Association (ATA) guidelines recommend TSH as the initial screening test for cases of suspected hyperthyroidism [4]. To improve diagnostic accuracy, fT4 should be analysed in conjunction with TSH [4]. Ordering both TSH and fT4 in every case is excessive and costly. A useful approach would be for the laboratory to test TSH first with reflex fT4 testing in the same sample when TSH is abnormal. This avoids unnecessary delays and a second visit for re-testing. Case finding is also not compromised even when fT4 is only tested for samples outside a wider TSH range of 0.2-6.0mIU/L [5].

TSH increases progressively in the elderly [6]; elevated TSH levels were more common in those aged above 50 years (11.3%) than younger subjects (4.7%). The NHANES III study (1988-1994) found raised concentrations of serum TSH (4.5-10mIU/L) in older patients [7]. The percentage of TSH values >4.5mIU/L increased from 2.5-14.5% with age [8]. In a follow-up NHANES III study [9], the 2.5th, 50th and 97.5th TSH

centiles increased with age. The age-related TSH increase may be due to higher concentrations of biologically inactive TSH isoforms in the elderly [10].

**FT4 and FT3:** Thyroid hormones can be measured either as total or free hormones. Total thyroid hormone levels are affected by variations in binding protein concentrations (e.g. pregnancy, acute illness, medication) and are more often abnormal due to these binding protein fluctuations than to thyroid dysfunction [11]. Total thyroid hormones also have a lower sensitivity for the detection of early thyroid dysfunction [12]. Another point of variation is the avidity of the binding proteins to T4. When different amounts of thyroxine were added to solutions with varying concentrations of T4 binding proteins, all measurements underestimated the added T4 concentration [13]. The prevalence of both hypo- and hyperthyroxinemia in euthyroid individuals without a thyroid disorder underscores the unreliability of total T4 as an index of thyroid status. Free thyroid hormones are less prone to effects of binding protein concentrations and are thus more accurate than total hormones. Modern automated immunoassay platforms have improved the measurement of FT4/FT3 [14]. Thus free thyroid hormones have largely replaced total hormones. Newer developments in liquid chromatography-tandem mass spectrometry (LC-MSMS) measurements show greater accuracy for FT4/FT3 [15] over immunoassays but LC-MSMS has a low throughput, is expensive and time-consuming.

As the concentration of FT3 in the serum is much lower than FT4, its measurement will be less precise and accurate than FT4. In a recent study [16], the correlation of FT4 in patients with hyperthyroidism, hypothyroidism and healthy patients was better than that of FT3 in all patient groups. There is no role for FT3/T3 in the diagnosis of thyroid disorders, unless T3-toxicosis is suspected, or if the patient is on combination T3/T4 therapy. In fact in hypothyroidism FT3/T3 may remain normal in the face of abnormal TSH and fT4. In addition, hospitalised sick euthyroid individuals often have low fT3/T3.

#### Tests of etiology

**TRAb:** There are three different kinds of TRAb (stimulating, blocking or neutral); stimulating TRAb is the most common [17]. Automated TRAb immunoassays are widely available [18]; TRAb levels >1.5-2.0 IU/L are regarded as positive. However, most TRAb assays detect inhibition of TSH binding to the TSH

receptor and are unable to differentiate between the types of TRAb present. TRAb is the diagnostic marker for Graves' Disease (GD) with a sensitivity and specificity of over 98% [19]. TRAb levels decline with treatment, especially with surgery followed by drugs and radioiodine the least [19]. Measuring TRAb at presentation and cessation of therapy can guide management. High TRAb at diagnosis (>12 IU/L) and/or positivity at cessation of therapy (>3.85 IU/L) suggests a higher chance of relapse within the first 2 years and thus identify patients that need closer monitoring [20]. TRAb are risk factors for and parallels the course of Graves' ophthalmopathy [21]. Advances in immunoassay methods now allow the detection of stimulating TRAb in GD [22], which may further improve the diagnosis and follow up of progress/remission.

**TPO-antibodies:** TPO-Ab is present in patients with autoimmune thyroid disease (90-95%), GD (80%) and non-autoimmune thyroid disease (10-15%) [23]. TPO-Ab does not have an established role in GD, but is implicated in Hashimoto's thyroiditis. Even without overt hypothyroidism, the presence of TPO-Ab predicts the development of subsequent thyroid disease. In the Busselton Thyroid study [6], carried out over a 13-year period (1981-1994), overt biochemical hypothyroidism and hyperthyroidism were found in 0.54% and 0.30% of subjects, and subclinical hypothyroidism and hyperthyroidism occurred in 5.10% and 0.34% of the population. In those without any history of thyroid disease, 12.4% had elevated thyroid antibodies (TPO-Ab or TgAb), and 6.9% were positive for TPO-Ab only. At the end of the 13-year period [24] TPO-Ab positivity with TSH>4.0mIU/L predicted long-term risk for hypothyroidism. Thus, TPO-Ab positive patients should be monitored more closely. .

#### Tumour biomarkers

**Thyroglobulin (Tg) and Thyroglobulin antibodies (TgAb):** Tg is the tumour marker in the management of patients with DTC [25]. Current Tg immunoassays have improved detection limits of 0.1ug/L obviating the need for recombinant TSH stimulated Tg testing [26]. After thyroidectomy periodic Tg measurements (3-6 months) in conjunction with imaging is needed. In low-risk DTC radioiodine ablation to the normal thyroid remnant may not be needed. Thus reliable measurement of the low Tg levels (<0.5ug/L) is required. An undetectable Tg argues against tumor recurrence while measurable or increasing Tg should

prompt investigation for relapse. However, in the presence of TgAb, immunoassays underestimate the true Tg concentration [26]. In fact many patients with DTC (25%) have circulating TgAb. Both Tg and TgAb are required to correctly interpret Tg values. In thyroid nodule, persistence of TgAb after thyroidectomy is a risk factor for thyroid cancer [27]. In some centers Tg is measured in the Fine Needle Aspiration (FNA) washout from cervical lymph nodes suspected of metastatic DTC. Tg, as an adjunct to FNA cytology, has a pooled sensitivity of 95% and specificity of 94% [28] and its use is endorsed by the American Association of Clinical Endocrinologists (AACE) [29].

**Calcitonin:** Calcitonin is the traditional tumour marker for medullary thyroid carcinoma (MTC) [30,31]. However, calcitonin is raised in a plethora of medical conditions (e.g: autoimmune thyroiditis, hypercalcemia, chronic renal failure, bacterial infection) and is thus not specific [32]. It requires an overnight fast before sampling because food intake stimulates its release. It is temperature sensitive and requires cold transport of samples. It has an extremely short half-life of <30 minutes. All these factors contribute to the poor reliability of calcitonin measurements. Indeed, AACE guidelines for thyroid nodules still cannot recommend either for or against routine calcitonin testing [29]. AACE only recommends calcitonin testing in subjects with a family history of MTC, patients with cytology suspicious for MTC, and patients undergoing surgery for goitre to avoid incomplete treatment of detected MTC.

Procalcitonin, the stable precursor of calcitonin, is gaining greater usage as more studies [33] show it has greater sensitivity with much less tedious sample handling. In a recent study [34], procalcitonin accurately detected MTC with 100% sensitivity and 100% negative predictive value, indicating that it can be used to exclude MTC in patients with thyroid nodules and increased calcitonin levels. Procalcitonin may be a more appropriate marker for MTC.

#### When to test?

**Screening?** Thyroid disease is prevalent and screening can identify patients with subclinical or overt thyroid disease. But there is insufficient evidence for the benefits or harms of TFT screening. The US Preventive Service Task Force (USPSTF) [35] is unable to recommend for or against the routine screening of thyroid disease in adults, except for high-risk patients with

diffuse or nodular goiters, concurrent autoimmune disease (e.g. type I diabetes, pernicious anemia), osteoporosis, supraventricular tachycardia, atrial fibrillation, medications (e.g. amiodarone, alpha-interferon, interleukin-2, lithium), prior neck radiation/surgery or family history. The USPSTF highlighted the negative effects of screening [36], particularly false-positive results. TSH is sensitive to several factors unrelated to thyroid disease e.g. diurnal variation, age, and non-thyroidal illness. From predictive value theory [37], at a 0.5% prevalence of overt thyroid disease, the predictive value of a negative TFT (rule-out) is excellent. However, the predictive value of a positive TFT (rule-in) is only 9% for a test sensitivity/specificity of 95%. Even with superb test sensitivity/specificity of 99%, the positive predictive value of TFT only improves to 33%. A consensus on TFT screening for the general population is unlikely as it will depend on local factors and resources. Physicians will thus have to settle for case finding in those clinically suspected of thyroid disease. .

**Subclinical thyroid disease:** Patients with subclinical thyroid disease are typically asymptomatic and can be divided into mild or severe forms (see Table 1). Mild subclinical disease is often transient [38]; 37% of initial subclinical hypothyroidism and 29% of subclinical hyperthyroidism reverted to euthyroidism on follow up.

Subclinical hyperthyroidism may be due to endogenous or exogenous causes. Common endogenous causes include early GD, toxic Multinodular Goitre (MNG) and solitary toxic adenomas. Common exogenous causes include excessive thyroxine therapy, thyroiditis or even thyroid carcinomas. Subclinical hyperthyroidism is usually mild and reverts to normal spontaneously; 11.8% progress to overt hyperthyroidism [39]. Progression is significantly higher in patients with TSH<0.1mIU/L. In GD, the natural history of subclinical hyperthyroidism follows the rule of thirds [40] - one third return to normal, a third persist, and another third progress to overt hyperthyroidism. Treatment for mild subclinical hyperthyroidism is unnecessary. Studies regarding the efficacy of treatment in reducing complications are lacking [41]. TFT should be re-assessed 2-3 months later. The risks of mild subclinical hyperthyroidism are minimal. There is no correlation between mild subclinical thyroid dysfunction and hip fractures in older men [42]. Severe subclinical hyperthyroidism

(TSH<0.1mIU/L) should be treated as it predisposes to atrial fibrillation, osteoporosis, coronary heart disease mortality, heart failure, fractures, and excess mortality [43-47]. Patients with GD are more likely to progress to overt hyperthyroidism [43]. Treatment may be indicated in patients older than 65 years who are symptomatic to avoid potential adverse events and disease progression, especially if they have other risk factors, co-morbidities or are TRAb positive.

Subclinical hypothyroidism may be transient or persistent. Transient subclinical hypothyroidism is mild and include recovery phase of non-thyroidal illness, recovery from thyroiditis or non-compliance with levothyroxine treatment. Persistent causes of subclinical hypothyroidism include chronic autoimmune thyroiditis, partial thyroidectomy, post-radioactive iodine therapy, drugs (e.g. amiodarone and lithium), or external radiotherapy for hyperthyroidism [47]. The risk of progression to overt hypothyroidism depends on the initial TSH elevation, thyroid reserve, and presence of TPO-Ab. Subclinical hypothyroidism resolved after 2 years in 46% in those with baseline TSH of 4.5–6.9mIU/L compared to <10% in those with higher baseline TSH [48]. In contrast, progression to overt hypothyroidism was 10% in those with TSH>10mIU/L versus 1% in TSH<10mIU/L. In supposedly hypothyroid individuals thyroxine was withheld [49]; 39.2% had true hypothyroidism while 60.8% remained euthyroid especially and those with mildly elevated TSH. The clinical benefit of treating mild subclinical hypothyroidism is questionable; only 1% of middle-aged and older individuals with subclinical hypothyroidism had improved quality of life with levothyroxine treatment [50].

When adults older than 65 years (mean age 74.4, 53.7% women) with persistent subclinical hypothyroidism (368/737) received levothyroxine [51], baseline TSH (mean  $\pm$  SD: 6.40  $\pm$  2.01mIU/L) declined at 1 year to 5.48  $\pm$  2.48mIU/L in the placebo group compared to 3.63mIU/L in the levothyroxine group ( $P<0.001$ ). But there was no difference in hypothyroid symptom scores or secondary outcomes. Unfortunately, there were too few participants with TSH>10mIU/L to determine the benefit of thyroxine treatment. An algorithm for subclinical hypothyroidism has been proposed [52]. Wait for 2-3 months and re-test to see if the TFT changes are transient. If high TSH persists, stratify patients by their TSH. For TSH 4.0-7.0mIU/L, no treatment is needed unless symptomatic. For TSH 7.0-

10.0mIU/L, treat if the patient is <70 years old, have cardiac risk factors, or elevated TPO-Ab. For TSH>10.0mIU/L treatment is warranted, especially if symptomatic or TPO-Ab positive. Elevated TPO-Ab in severe subclinical hypothyroidism is an indicator of Hashimoto's thyroiditis. Severe subclinical hypothyroidism is associated with increased in all-cause mortality, cardiovascular events and mortality, osteoporosis, and cognitive decline [46,53].

**Overt hyperthyroidism:** Overt hyperthyroidism is a classic syndrome associated with weight loss, heat intolerance, anxiety, sweating and palpitations, with or without eye signs such as exophthalmos [54]. Symptoms and complications (e.g: atrial fibrillation) tend to be more common in older patients and thus they require additional attention [55]. In the elderly, an atypical presentation of thyrotoxicosis is apathetic thyrotoxicosis. The features are atypical (no goiter, no exophthalmos, diabetes, congestive cardiac failure and depression) but tachycardia and weight loss is prominent [56]. Remember this condition when investigating elderly patients with weight loss or functional decline. Osteoporosis is more prevalent in older patients with chronic hyperthyroidism. An unusual complication of hyperthyroidism in Asian males is thyrotoxic periodic paralysis [54], characterized by muscle paralysis and hypokalemia requiring treatment with potassium supplementation and beta blockers.

Overt hyperthyroidism is commonly due to GD and nodular goitre. In GD, thyroid antigen-specific T cells infiltrate the thyroid gland [55,57] and TRAb (predominantly IgG1 isotype) stimulates the thyroid gland. GD is often associated with thyroid-associated ophthalmopathy (proptosis, eyelid swelling and diplopia). About 30% of GD has a family history of GD or Hashimoto's thyroiditis. Usually euthyroid, hyperthyroidism develops occasionally in MNG. Thyroiditis, another cause of overt hyperthyroidism, can be distinguished from GD by the ratio of FT3/FT4; GD typically has a higher FT3/FT4 ratio >4.4 [58]. On radionuclide uptake scan, GD shows diffuse increased uptake while uptake is reduced in thyroiditis. Exogenous hyperthyroidism can occur from over-zealous thyroxine in hypothyroidism, under treatment of hyperthyroidism with anti-thyroid drugs or thyroid hormones found in certain weight loss products [59]. Radionuclide scans will show a thyroiditis picture because exogenous thyroid



hormone reduce thyroidal iodine uptake. Besides, exogenous thyroid hormones lower serum Tg in contrast to high Tg in GD. Rarely, thyroid cancer can be associated with hyperthyroidism; 1.65% of hyperthyroid patients have thyroid cancer [60], with papillary carcinoma being the most common. The incidence of papillary thyroid cancer in GD is higher in those with thyroid nodules than those without [61]. Patients with nodules and hyperthyroidism should be screened for malignancy. Occasionally, TSH mediated hyperthyroidism from TSH-omas may be encountered. These patients have an elevated FT4/FT3 and high/normal TSH. Tests should be done to rule out pituitary lesions [62]. Another rare condition is genetic thyroid hormone resistance, where patients appear euthyroid or slightly hyperthyroid but have elevated FT4 with low TSH. Some uncommon tumours e.g. struma ovarii [63] contain large amounts of thyroid tissue and can become autonomous. Gestational trophoblastic diseases (hydatidiform mole or choriocarcinoma) may secrete large amounts of hCG and cause hyperthyroidism [64].

Initial tests to evaluate overt hyperthyroidism are TSH and FT4 [4]. As GD is the most common cause of primary hyperthyroidism, TRAb should be ordered as well as TRAb are prognostic for GD remission [19]. Ultrasonography may help in the evaluation of hyperthyroidism; colour-flow Doppler show increased blood flow in GD and decreased flow in destructive thyrotoxicosis [54].

When hyperthyroidism is treated with anti-thyroid drugs, the ATA recommends [4] assessment of FT4 and TSH 1-3 weeks after initiating treatment and 4-6 weeks when drug dosages are adjusted. Serum TSH may remain suppressed for several months after starting therapy and is not a good parameter for disease monitoring in the early course of treatment. TRAb needs to be followed up in patients who are TRAb positive. Patients with persistently hyperthyroid TFTs or high TRAb despite treatment may need further radioactive iodine (RAI) treatment. Re-assess FT4 and TSH within the first 1-2 months after RAI and at 4-6 week intervals for the first 6 months or until the patient becomes stable. Following thyroid surgery TSH and FT4 levels should also be obtained at 4-6 weeks.

**Overt hypothyroidism:** Overt hypothyroidism is characterized by weight gain, lethargy, dry skin, cold intolerance and constipation [65]. Hashimoto's thyroiditis is the most common

cause of hypothyroidism and a positive TPO-Ab is diagnostic. Hypothyroidism is more frequent in patients with other autoimmune diseases, such as type 1 diabetes, autoimmune gastric atrophy and celiac disease [65]. Individuals with Downs' or Turners' syndrome are also at increased risk of Hashimoto's thyroiditis. Other causes of clinical hypothyroidism are iatrogenic (prior thyroidectomy, prior radio-iodine therapy or anti-thyroid medication). Secondary hypothyroidism is due to pituitary or hypothalamic pathology. Possible causes include prior pituitary instrumentation, pituitary irradiation, pituitary tumours, pituitary apoplexy, hypothalamic tumours or surgery. Macroadenomas (>1cm) are commonly associated with deficiencies in anterior pituitary hormones [66] and laboratory evaluation of pituitary function is warranted. In secondary hypothyroidism, TFTs show a low FT4 with low TSH, which differentiates it from primary hypothyroidism. Patients with non-functioning pituitary adenomas or central hypothyroidism have only mildly elevated TSH (generally not above 6-7mIU/L) due to secretion of bioinactive TSH isoforms [67].

Primary hypothyroidism with TSH>10mIU/L should be treated [68]. At follow up visits TSH and fT4 should be measured. When monitoring patients on L-thyroxine replacement, blood should be collected before thyroxine dosing as the FT4 level can increase transiently after dosing [69]. Physicians typically wait 6-8 weeks for thyroxine to impact TSH levels. Once thyroxine dosing is stable follow up TSH can be monitored 4-6 monthly.

**Thyroid nodules:** Palpable thyroid nodules occur in roughly 6% of the population [70]. On ultrasound, nodules are found in 19%-68% of randomly selected individuals, with higher frequencies in women and the elderly. However, only 7-15% of nodules are cancerous. MNG is one of the most common causes of thyroid nodules. Largely benign, MNG has a significantly higher rate of incidental cancers (18%) compared to GD (6%) [71]. In MNG, impairment of thyroid hormone synthesis and/or increased TSH levels leads to thyroid hypertrophy and hyperplasia. This may cause hyperthyroidism and contribute to a higher risk of malignancy. As such, it is important to do TFT and screen for malignancy as needed.

An excellent framework for managing thyroid nodules is available [72]. Red flags in the clinical history include DTC in at least one first-degree relative, radiation exposure as a child or

adolescent or prior diagnosis of thyroid cancer. Serum TSH should be performed routinely in the assessment of thyroid nodules; a low TSH indicates a hyperfunctioning nodule while higher TSH levels are associated with increased risk of malignancy [73,74]. All patients should undergo ultrasonography to characterize the nodule [72]. Based on ultrasonography, nodules are classified as high, intermediate, low, or very-low risk. Thereafter, FNA of the nodule is done if it is 1cm or larger. When FNA findings are suggestive of malignancy, thyroidectomy is carried out. If cytology is inconclusive, newer modalities such as gene-expression classification (e.g. for BRAF, RAS, RET/PTC mutations) have excellent predictive values. Those with benign cytology findings are followed up with repeat ultrasonography every 1-2 years.

**Thyroid cancer:** DTC is the most common thyroid cancer accounting for 95% of cases [75]. Of the DTC, papillary cancer is the most frequent (85%) [76], followed by medullary (4%) and follicular (2%) thyroid carcinomas. Encapsulated non-invasive follicular variants of papillary thyroid carcinoma have been reclassified as a benign entity called “non-invasive follicular thyroid neoplasm with papillary-like nuclear features” [76]. Anaplastic thyroid cancers (1%) often arise from and coexist with DTC. They have a poor prognosis and commonly metastasize to lungs, bone and brain [75]. FNA is required to diagnose thyroid cancers. When FNA is inconclusive, next-generation sequencing of a panel of oncogenes and tumour-suppressor genes may help e.g. gene mutation profiling panels have strong positive predictive value and the 167 gene expression classifiers have stronger negative predictive value. Radioiodine therapy, lobectomy or total thyroidectomy is the treatment of choice for thyroid cancer. However, several drugs have become available for the treatment of thyroid cancer. Selumetinib, a MEK inhibitor, sensitizes papillary thyroid cancers to radioiodine therapy in previously resistant cases [77]. Cabozantinib, a tyrosine kinase inhibitor of hepatocyte growth factor receptor (MET), has promising activity in patients with medullary thyroid cancer and DTC. Cabozantinib showed anti-tumor activity in patients with RAI-refractory DTC with a response rate of 53% [78]. Lenvatinib, another tyrosine kinase inhibitor, has been used in the treatment of anaplastic thyroid cancers. Immunotherapy with immune checkpoint inhibitors, such as Nivolumab, show promise [79]. However, tyrosine kinase

inhibitors can cause thyroid dysfunction - recurrence of hypothyroidism in pre-existing hypothyroidism or hypothyroidism in previously normal patients [80]. Four out of five patients on Lenvatinib developed clinical hypothyroidism [81]. TSH increased by  $>0.5\text{mIU/L}$  in 61.5% of patients treated with Lenvatinib [82]. In patients receiving these drugs, serum TSH and FT4 should be monitored. Other chemotherapy agents causing hypothyroidism include Bexarotene (a selective agonist of the retinoid X receptor), immunomodulators and iodine-based cancer therapies [80].

Since DTC responds to TSH stimulation it is important to monitor TSH and keep TSH levels suppressed after surgery [70]. In thyroid cancer, tumor markers are more useful in follow up and surveillance rather than diagnosis. Their initial levels should be noted and any decrease reflects effectiveness of surgery or treatment; doubling times can be used as a measure of tumor aggressiveness to aid prognosis.

### SPECIAL PRECAUTIONS

There are several scenarios where TSH alone may be misleading and caution should be exercised. These are non-thyroidal illness, pregnancy, and the effect of other drugs.

#### Hospitalization and non-thyroidal illness

Non-Thyroidal Illness Syndrome (NTIS) can be difficult to differentiate from hypothyroidism, particularly in the critically ill. Feedback regulation of the hypothalamus-pituitary-thyroid (HPT) axis is altered in illness. TSH remains normal or even decreases despite low FT4/FT3 [83]. Soon after acute stress (e.g. surgery), serum T3/T4 declines and decrease further as the disease progresses. Other changes include lower concentrations of thyroid hormone binding proteins/transporters and decreased expression and activity of thyroid hormone deiodinases/thyroid hormone receptors [84]. Diminished nutrition in critical illnesses further aggravates these changes. TSH is the most useful test in evaluating NTIS as it is high in primary hypothyroidism but normal or slightly low in NTIS. However, both conditions may co-exist; NTIS can blunt the TSH rise in critically ill patients with severe hypothyroidism. Whether NTIS per se should be treated remains unanswered, but the use of thyroid hormones have been negative [83].

#### In pregnancy

Pregnancy has a great impact on thyroid function [85]. Thyroid hormone and TBG production increase with a 50% increase in

the daily iodine requirement. T4 and TBG peak at week 16 and remain high until delivery. In early pregnancy serum TSH is low in response to the hCG peak. When hCG falls after the first trimester, free thyroid hormones decrease with an increase in TSH. The Endocrine Society and the ATA recommend using trimester specific TFT reference ranges [85]. In Asian women the TSH ranges for the first, second and third trimesters are 0.16-3.78, 0.34-3.52, and 0.34-4.32mIU/L respectively [86]. TPO-Ab or TgAb are present in 2-17% of pregnant women. Thus pregnant euthyroid women with positive thyroid antibodies should have their TSH checked every 4 weeks [85]. The thyrotropic effects of hCG may cause transient hyperthyroidism in the first trimester and be accompanied by hyperemesis gravidarum (HG). Anti-thyroid antibodies are higher in patients with HG. Thyroid antibodies can be a risk factor for postpartum thyroiditis, miscarriage and premature birth. Patients with TPO-Ab positivity might benefit from levothyroxine treatment [87]. However, in antibody negative subjects, the HG-associated thyroiditis is usually transient [88]. When gestational thyrotoxicosis persists it may herald the onset of new GD and is characterised by a positive TRAb. TRAbs can cross the placenta and cause neonatal thyrotoxicosis. Thus TRAb-positive mothers should be monitored closely.

Postpartum thyroiditis is the most common endocrine disorder associated with pregnancy [89]. It is defined as changes in TFT in the first year after pregnancy caused by an immune rebound from the partial immunosuppression in pregnancy. Factors that predispose to postpartum thyroiditis include previous type I diabetes, a positive family history or previous postpartum thyroiditis [85]. Postpartum thyroiditis is strongly associated with TPO-Ab positivity; 50% of TPO-Ab positive women develop postpartum thyroiditis [90]. TRAb might also be positive in up to 25% of postpartum thyroiditis [89]. This condition can be distinguished from GD. Postpartum thyroiditis is much more common than newly diagnosed postpartum GD. Hyperthyroid symptoms in postpartum thyroiditis appear much earlier (2-6 months postpartum) than GD (4-12 months postpartum). Postpartum GD is less common than postpartum thyroiditis, with the highest risk occurring at 7-9 months postpartum [54]. FT4 and TRAb should be measured if TSH falls below 0.1mIU/L postpartum. Women who develop postpartum thyroiditis have a 25–30% risk of developing permanent

hypothyroidism, especially if they have high TPO-Ab titres, TSH >20mIU/L or hypo-echogenicity on thyroid ultrasound. They need to be followed up accordingly.

### Drugs

Some drugs may affect TFT results or cause thyroid dysfunction with long-term use [91]. They act at the pituitary-thyroid axis or affect thyroid hormone absorption/metabolism. Some drugs (glucocorticoids, dopamine agonists, somatostatin analogs) suppress TSH at the level of the hypothalamus or pituitary. Lithium and amiodarone inhibit the secretion of T4/T3. Lithium is actively concentrated in thyroid follicular cells downregulating thyroid hormone secretion leading to hypothyroidism and even goiter. Amiodarone, routinely used in the treatment of arrhythmias, contains iodine in its molecular structure. It causes transiently elevated FT4 and TSH levels at initiation of therapy; hypothyroidism or thyrotoxicosis may occur in chronic users.

Drugs can interfere with levothyroxine absorption e.g. iron, calcium, aluminium and cholestyramine [92]. Thyroid hormones are actively oxidized and conjugated in the liver by cytochrome P450. Drugs that activate this system (e.g. rifampin, phenytoin and carbamazepine) can increase clearance of thyroid hormones. Metformin decreases hepatic metabolism of thyroid hormones.

Biotin [4,93] can interfere with the measurement of TFTs. In the laboratory, some immunoassay platforms employ biotin to label the capture antigen/antibody or the signal antibody. High doses of exogenous biotin prevent the formation of biotin-antigen-antibody complexes resulting in a low assay signal. Low signals are translated as high analyte concentration in competitive immunoassays (fT4/TRAb) but connotes low analyte concentration in sandwich assays (TSH). This combination of high fT4, high TRAb and low TSH is the erroneous biotin effect that has received much attention recently. However, biotin does not always lead to aberrant TFT results. Biotin levels in over-the-counter supplements are very low (30-300ug). Daily biotin intake of 3mg [94] and 10mg [95] did not affect fT4/TSH. At 30mg/d of biotin, the achieved serum biotin concentration of 56.8ng/mL affected fT4 and TSH slightly but results were still within the reference range [93]. Serum spiked with biotin to 15.6ng/mL and 31.2ng/mL, affected TSH and FT4 minimally [96]. Only when biotin concentrations approached 500ng/mL, a level expected with 100-300mg/d of biotin therapy for



multiple sclerosis patients, was there significant shifts in TSH and FT4. Such high concentrations are infrequent. In an emergency department population [97] only 7 samples (0.5%) had biotin concentrations of >30ng/mL despite nearly 42% reporting multi-vitamin or supplement use. There were no cases where biotin caused significant interference on test results. At biotin doses in over-the-counter supplements, TFT results are unaffected. Only supra-therapeutic doses of biotin (100-300mg/d) will cause significant derangements in TFT results.

## CONCLUSIONS

Clinicians must be aware of when to suspect thyroid dysfunction. Many tests are available to guide doctors in the diagnosis and management of thyroid disorders. The clinician must be cognizant of the caveats in thyroid function test interpretation. When spurious results are found discussion with the laboratory or endocrinologist is in order.

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