3 Biochemical Diagnosis of Thyroid Dysfunctions

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3.1 Introduction

Thyroid disorders are commonly encountered in clinical practice and laboratory tests are integral to their diagnosis and management, including assessment of disease severity and response to therapy. This chapter details the pathophysiological background of thyroid function and the in vitro laboratory tests used in different thyroid diseases. Interpretation criteria, inappropriate or redundant testing, and relevant pitfalls are also reviewed, and guidance for rational test ordering and integration between clinical, laboratory, and imaging data is provided.

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3.2 Physiological Basis of Thyroid Function Laboratory Assessment

Thyroid hormone synthesis is finely tuned by the hypothalamus–pituitary–thyroid axis. In physiologic conditions, thyroid-stimulating hormone (TSH) regulates cellular activity, stimulating thyrocytes to express proteins necessary for thyroid hormones production and to increase thyroid hormones synthesis and secretion. Intracellular iodine transport across the follicular thyroid cell is generated by the Na^+/K^+ ATPase pump, which provides the transmembrane Na^+ gradient. The natrium iodide symporter transports one iodide ion together with two sodium ions, resulting in a significantly higher iodine concentration in the follicular cells (up to 500 times) compared with the bloodstream. Subsequently, through different membrane channels located at the apical membrane (e.g., pendrin, anoctamin, and chloride channel ClC5), iodine passes from the cytoplasm of the follicular cell into the lumen [1].

At the same time, the glycoprotein thyroglobulin moves from the apical membrane and enters into the follicular lumen (i.e., exocytosis). The thyroid follicular lumen consists of a colloidal suspension of thyroglobulin (concentration up to 750 mg/mL). Thyroglobulin serves as the backbone for thyroid hormones [2].

Iodine is then oxidated via action of the enzyme thyroid peroxidase (TPO): hydrogen peroxide, a

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substrate for TPO, is synthesized at the apical external surface of follicular thyroid cells. Oxidation is followed by organification (i.e., oxidized iodine links covalently to tyrosyl residues of thyroglobulin), enabling the biosynthesis of diiodotyrosines (DITs) and monoiodotyrosines (MITs), respectively. Under modulation by TPO, DITs and MITs are coupled to form triiodothyronine (T3), while two DITs form thyroxine (T4) (Fig. 3.1) [3].

The hormones T3 and T4 are phenolic rings coupled by an ether link and iodinated at three (3,5,3′-tri-iodo-L-thyronine, i.e., T3) or four (3,5,3′,5′-tetra-iodo-L-thyronine, i.e., T4) positions on the phenolic ring [4].

Thyroid hormones are stored in the lumen of follicular cells and, when required, the thyroglobulin–thyroid hormone complex internalizes to the cytoplasm and undergoes enzymatic disintegration, hydrolysis, and transport via the basolateral membrane across the monocarboxylate transporter 8.

Under normal conditions, the thyroid secretes ~90% T4, ~8–10% T3, and < 2% reverse T3. During intense TSH receptor stimulation, or in the case of iodine deficiency, the ratio of T3 formation increases [4]. More than 99% of circulating T4 and T3 molecules bind to carrier proteins (e.g., thyroxine-binding globulin, transthyretin, and albumin) and only small amounts circulate as free hormones (free thyroxine [FT4], free triiodothyronine [FT3]). These free hormones act on target tissues and bind thyroid receptors in the nuclei of target cells [5].

T3 is the bioactive thyroid hormone, with about 30 times higher affinity than T4 for the thyroid hormone receptor and is derived mostly from peripheral conversion of T4 via deiodinase activity [4]. Thyroid hormones also provide negative feedback to both the hypothalamus and the pituitary gland, closing the finely regulated homeostatic thyroid hormone biosynthesis loop. The relationship between TSH and FT4 is genetically determined and influenced by age and other

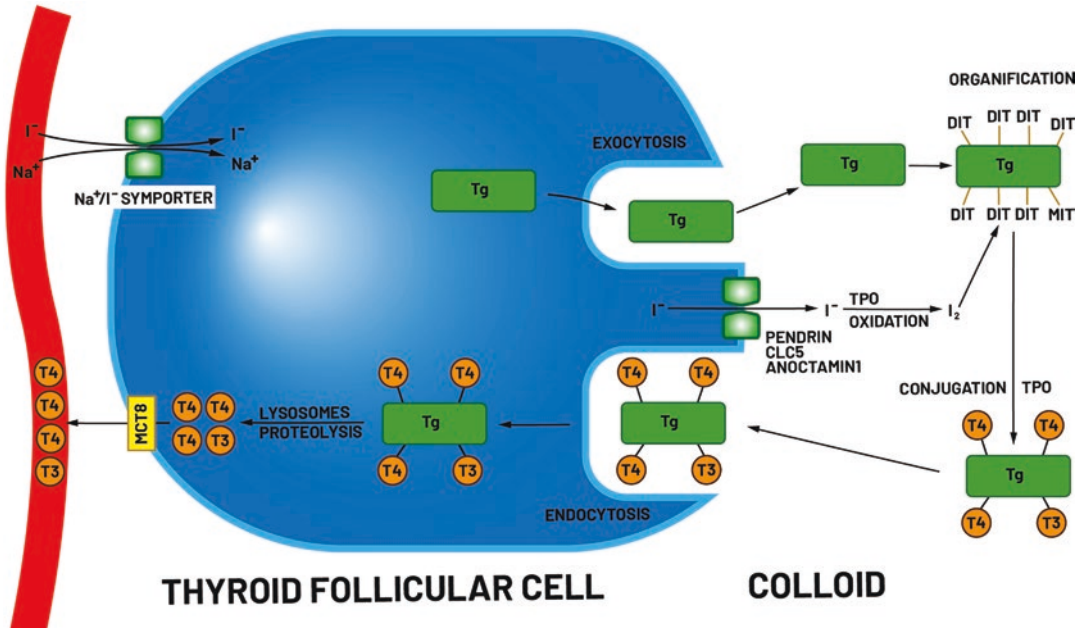

Fig. 3.1 Biosynthesis of thyroid hormones. *DIT* diiodotyrosine, *MCT8* monocarboxylate transporter 8, *MIT* monoiodotyrosine, *T3* triiodothyronine, *T4* thyroxine, *Tg* thyroglobulin, *TPO* thyroid peroxidase. Reproduced from D'Aurizio et al. Free thyroxine measurement in clinical practice: how to optimize indications, analytical procedures, and interpretation criteria while waiting for global standardization. Crit Rev. Clin Lab Sci 2022; doi: 10.1080/10408363.2022.2121960 [in press]

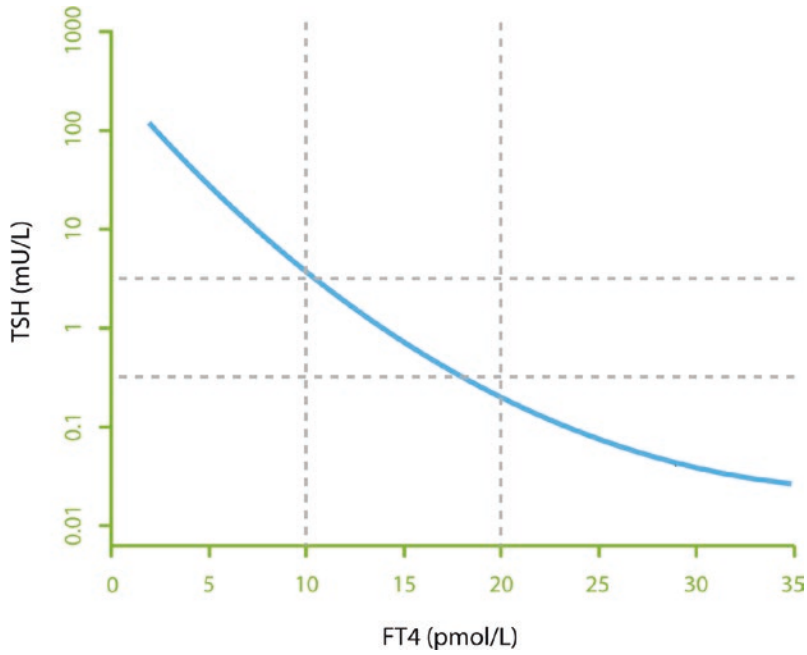

Fig. 3.2 The log–linear inverse relationship between TSH and FT4. The blue line represents an approximate relationship between TSH and FT4. The dotted gray lines represent the normal values for TSH (horizontal) and FT4 (vertical). *TSH* thyroid-stimulating hormone, *FT4* free thyroxine. Reproduced from D'Aurizio et al. Free thyroxine measurement in clinical practice: how to optimize indications, analytical procedures, and interpretation criteria while waiting for global standardization. Crit Rev. Clin Lab Sci 2022; doi: 10.1080/10408363.2022.2121960 [in press]

factors such as smoking status. With few exceptions, the TSH–free thyroid hormone relationship is largely inverse log-linear [6] (Fig. 3.2).

TSH secretion is highly sensitive to small fluctuations in thyroid hormone levels, and abnormal TSH levels indicate early thyroid dysfunction, well before clear actual hormone abnormalities emerge.

Notably, currently used FT4 (and FT3) immunoassays are binding protein-dependent and their performance may be suboptimal at low or high thyroid hormone concentrations. Liquid chromatography-tandem mass spectrometry (LC-MS/MS), for the most part, avoids the inherent shortcomings of competitive immunoassays, as demonstrated by the stronger TSH–FT4 relationship when FT4 is measured by LC-MS/MS versus conventional immunoassays [7]. However, LC-MS/MS remains largely unavailable in clinical laboratories, and selective and appropriate use of FT4 and FT3 testing based on TSH results remains pivotal to avoiding diagnostic pitfalls.

3.3 Thyroid Function Tests

Thyroid function is assessed by measuring TSH and free thyroid hormones. As previously discussed, TSH and FT4 have a complex, nonlinear relationship, and small changes in FT4 result in relatively large changes in TSH [8]. With some rare exceptions (i.e., central hypothyroidism, resistance to thyroid hormones, TSH-secreting pituitary adenoma [TSH-oma], treated hyperthyroidism, and nonthyroidal illness syndrome), TSH measurement is a sensitive first-line test for thyroid dysfunction. Guidelines from the American Thyroid Association [9], the American Association of Clinical Endocrinologists [10], and the National Academy of Clinical Biochemistry [11] have endorsed TSH measure-

ment as the best first-line strategy for detecting thyroid dysfunction in most clinical settings. However, TSH levels are relatively inadequate in evaluating the severity of thyroid dysfunction, and FT4 (with or without FT3) should be tested when abnormal TSH levels are found. To reduce the overuse of FT4 testing without compromising the detection of overt thyroid dysfunction, FT4 may be added to existing requests, either automatically on the basis of algorithms (i.e., reflex testing) or by laboratory professionals (i.e., reflective testing). These strategies have proven to be clinically appropriate and cost-effective in the first-line assessment of thyroid function [12, 13].

3.3.1 Performance Characteristics of Thyroid Function Assays

Most thyroid function tests are performed by immunoassays on automated platforms. Immunoassays for FT4 and FT3 are competitive, as the small size of thyroid hormones precludes the use of sandwich immunometric assays [13]. However, competitive thyroid hormone assays are occasionally affected by cross-reactivities, and the dynamic range limitations create a difficult task for manufacturers of producing assays that are very accurate at both low and high concentrations [14] (Tables 3.1 and 3.2).

Conversely, immunoassays used to measure TSH are largely sandwich immunometric assays. This assay architecture offers advantages over competitive assays, such as reduced cross-reactivity, better detection sensitivity, and a wider dynamic measurement range. Currently available TSH assays have detection sensitivities of <0.01–0.02 mIU/L, a 4 Log^{10}, and a more dynamic range, respectively [13] (Table 3.3). Finally, they also have no cross-reactivity with pituitary glycoproteins luteinizing hormone and follicle-stimulating hormone, and the human chorionic gonadotropin, respectively.

Table 3.1 Main analytical characteristics of the most frequently used FT4 immunoassays, as quoted by manufacturers

Manufacturer/Assay	Assay principle	LOD (pmol/L)	LOQ (pmol/L)	Assay range (pmol/L)	Imprecision (CV %) (intra-assay; inter-assay; total)	Reference interval[a] (pmol/L)
Abbott Alinity i Free T4	CMIA, competitive	3.60	5.41	5.41–64.5	1.7–3; 2–3.1; ND	9.0–19.1
Beckman Coulter Access Free T4	CLIA, competitive	3.22	ND	3.22–77.20	1.8–4.4; 3.3–8.1; 4.3–9.2	7.86–14.41
DiaSorin Free T4	CLIA, competitive	1.29	ND	1.29–28.70	2.0–3.3; 2.0–4.4, ND	10.29–21.88
Mindray FT4	CLIA, competitive	3.86	ND	3.86–77.23	2.05–3.17; 1.58–1.98; 4.38–4.64	7.72–15.45
Ortho Vitros FT4	CLIA, competitive	0.88	ND	0.88–90.0	1.6–2.8; 2.4–5.8; 2.5–6.2	10.0–28.2
Roche cobas Elecsys FT4 IV	ECLIA, competitive	0.5	1.3	0.5–100.0	1.6–5.0; 1.9–6.3; ND	11.9–21.6
Siemens Healthineers Atellica IM FT4	CLIA, competitive	1.3	ND	1.3–154.8	1.2–4.7; 2.2–6.8; ND	11.5–22.7
SNIBE Maglumi FT4	CLIA, competitive	1.9	ND	1.9–154.5	2.76–4.99; 1.51–6.17; 3.15–7.94	11.5–22.1

[a]Reference intervals were calculated in a population of apparently healthy adults. Information updated to August 2022
CLIA chemiluminescent assay, *CMIA* chemiluminescent microparticle immunoassay, *CV* coefficient of variation, *ECLIA* electrochemiluminescence assay, *FT4* free thyroxine, *LOD* limit of detection, *LOQ* limit of quantitation, *ND* not disclosed

Table 3.2 Main analytical characteristics of the most frequently used FT3 immunoassays, as quoted by manufacturers

Manufacturer/Assay	Assay principle	LOD (pmol/L)	LOQ (pmol/L)	Assay range (pmol/L)	Imprecision (CV %) (intra-assay; inter-assay; total)	Reference interval[a] (pmol/L)
Abbott Alinity i Free T3	CMIA, competitive	1.46	1.92	2.30–30.72	2.4–3.8; ND; 3.6–4.8	2.43–6.00
Beckman Coulter Access Free T3	CLIA, competitive	1.40	ND	1.40–46.00	2.6–6.6; 1.3–8.0; 5.3–10.4	3.8–6.0
DiaSorin Free T3	CLIA, competitive	0.46	1.54	0.46–46.2	2.6–4.7; 2.4–4.7, ND	3.39–6.47
Mindray FT3	CLIA, competitive	1.35	ND	1.35–46.20	1.89–2.65; 2.16–2.41; 2.62–3.31	3.54–6.16
Ortho Vitros FT3	CLIA, competitive	0.77	ND	0.77–35.00	1.1–4.0; 2.0–11.3; 2.3–14.7	4.26–8.10
Roche cobas Elecsys FT3 III	ECLIA, competitive	0.6	1.5	0.6–50.0	1.4–7.6; 1.6–8.3; ND	3.1–6.8
Siemens Healthineers Atellica IM FT3	CLIA, competitive	0.31	ND	0.31–30.80	0.67–7.59; ND; 1.07–9.14	3.5–6.5
SNIBE Maglumi FT3	CLIA, competitive	0.62	ND	0.62–77.00	2.64–4.63; 1.77–6.12; 3.68–7.67	3.10–6.47

[a]Reference intervals were calculated in a population of apparently healthy adult males and females. Information updated to August 2022

CLIA chemiluminescent assay, *CMIA* chemiluminescent microparticle immunoassay, *CV* coefficient of variation, *ECLIA* electrochemiluminescence assay, *FT3* free triiodothyronine, *LOD* limit of detection, *LOQ* limit of quantitation, *ND* not disclosed

Table 3.3 Main analytical characteristics of the most frequently used TSH immunoassays, as quoted by manufacturers

Manufacturer/Assay	Assay principle	IRP	LOD (mIU/L)	LOQ (mIU/L)	Assay range (mIU/L)	Imprecision (CV %) (intra-assay; inter-assay; total)	Reference interval[a] (mIU/L)
Abbott Alinity i TSH	CMIA, non-competitive	ND	0.0036	0.0083	0.0083–100	1.3–1.6; ND; 1.5–2.1	0.35–4.94
Beckman Coulter Access TSH 3rd IS	CLIA, non-competitive	81/565	0.005	0.01	0.005–50	2–4; 0.2–2; 3–6	0.38–5.33
DiaSorin Liaison TSH	CLIA, non-competitive	80/558	0.004	0.02	0.004–100	0.7–1.9; 1.6–5.1; ND	0.3–3.6
Mindray TSH	CLIA, non-competitive	81/565	0.005	0.02	0.005–100	1.75–2.39; 1.40–1.65; 2.32–3.13	0.35–5.1
Ortho Vitros TSH	CLIA, non-competitive	80/558	0.014	0.097	0.014–150	0.9–5.3; 1.7–7.4; 2.0–8.8	0.47–4.68
Roche cobas Elecsys TSH	ECLIA, non-competitive	80/558	0.005	0.005	0.005–100	0.7–3.4; 1.5–11.2; ND	0.27–4.20
Siemens Healthineers Atellica IM TSH3-UL	CLIA, non-competitive	81/565	0.008	0.008	0.008–150	1.5–3.6; 2.9–4.5; ND	0.55–4.78
SNIBE Maglumi TSH	CLIA, non-competitive	81/565	0.006	ND	0.006–100	1.76–2.53; 1.40–2.04; 2.25–3.71	0.3–4.5

[a]Reference intervals were calculated in a population of apparently healthy adult males and females. Information updated to August 2022

CLIA chemiluminescent assay, *CMIA* chemiluminescent microparticle immunoassay, *CV* coefficient of variation, *ECLIA* electrochemiluminescence assay, *IRP* international reference preparation, *LOD* limit of detection, *LOQ* limit of quantitation, *ND* not disclosed, *TSH* thyroid-stimulating hormone

3.3.2 Thyroid-Stimulating Hormone

As previously discussed, Thyroid-Stimulating Hormone (TSH) is the single most useful test of thyroid function in most patients. Generally, no further testing is indicated when TSH appears within the normal range. Nevertheless, several issues should be considered when interpreting a TSH value, the importance of which requires that a clinical decision not be made based on a single TSH value when it is within or close to the normal range [13].

3.3.2.1 Normal Range

Considerable literature exists regarding the "normal" range for TSH, which is generally quoted to be between 0.40 and 4.00 mIU/mL [15, 16]. However, the accuracy of any given immunoassay can strongly affect TSH cutoffs, as inter-method differences of about 1 mU/L at concentrations of 4–5 mU/L have been reported [17]. The lack of interchangeability of laboratory results and cutoffs (due to poor harmonization of TSH assays) does not allow standardized cutoffs to be used in clinical practice. Therefore, each clinical laboratory must establish reliable cutoffs based on their adopted method. Adopted cutoffs may differ based on population and clinical context. In the general population, the approach is to adopt cutoffs that facilitate the greatest reduction in the frequency of FT4 testing; in other clinical settings, a different cutoff may be preferable to avoid missing hypo- and/or hyperthyroidism diagnoses.

3.3.2.2 Circadian Variability

Though not usually accounted for in clinical practice TSH secretion follows a circadian rhythm, with maximal levels in the early morning and a nadir in the late afternoon to mid-evening. Generally, TSH levels remain within the normal range, but variation in TSH by a mean of 0.95–2.00 mIU/mL can be observed and may affect clinical decisions [18].

3.3.2.3 Individual Variation

Individual variation in TSH levels may occur without an obvious cause. In a study assessing TSH values monthly for 1 year in healthy men, random variations occurred, with a mean TSH of 0.75 mIU/mL and a range of 0.2–1.6 mIU/mL [19]. Thus, variation in TSH of up to 40–50% within the normal range does not necessarily indicate a change in thyroid function or status [10, 19].

Overall, the "reflex TSH" strategy highlights an opportunity to improve appropriateness in test requests and save unjustified costs for healthcare systems. However, caution must be exercised when using solely TSH tests for subclinical thyroid dysfunction and for secondary hypothyroidism, and during the initial phases of medical therapy for hyper- and hypothyroidism [20].

3.3.3 Free Thyroid Hormones

Measurement of FT4 levels is integral in both the diagnosis and management of relevant central dysfunctions, as well as therapy monitoring in hyperthyroid patients treated with antithyroid drugs or radioiodine. FT3 measurement may also add useful information in patients with suppressed TSH and normal FT4 levels to distinguish subclinical hyperthyroidism (i.e., normal FT3) from T3-thyrotoxicosis (i.e., high FT3). The accuracy of FT4 and FT3 tests depends greatly on the assay used. Unfortunately, the assays used in the vast majority of clinical laboratories are still hindered by some limitations and pitfalls. Although considerable progress has been made in the standardization of FT4 procedures, some challenges remain, including establishing clinical decision limits in varying patient populations and education of stakeholders [21]. As such, different assays and reference values cannot be interchanged at present. Two-way communication between laboratory and clinical specialists is vital in choosing a reliable FT4 assay, establishing local reference ranges, investigating discordant results, and monitoring the analytical and clinical performance of the assay over time.

3.4 Diagnosis of Thyroid Dysfunctions

Symptoms of thyroid dysfunction may be nonspecific with minimal signs; thus, the use of thyroid function tests in patient evaluation is vital. While testing TSH alone is sufficient for general screening, both FT4 and TSH assays are needed for diagnosing subclinical thyroid dysfunction, central hypothyroidism, drug effects, and hospitalized patients, as well as for accurate assessment of treatment effects. Close communication between the bedside and bench-side is crucial for the successful interpretation of thyroid function test results, particularly when inconsistent results are rendered. An algorithm for thyroid function test interpretation is presented in Fig. 3.3.

3.4.1 Subclinical Thyroid Dysfunctions

Increased frequency of screening and routine blood tests have resulted in more patients being diagnosed with subclinical thyroid dysfunction. In subclinical hyperthyroidism, TSH level is low/suppressed and FT4 level is within the normal range. FT3 should be measured to rule out T3 toxicosis in these cases. Patients with subclinical hyperthyroidism should be further screened and considered for treatment, especially if they are elderly and/or at risk for atrial fibrillation or osteoporosis [22].

In subclinical hypothyroidism, TSH level is elevated and the FT4 level is within the normal range. Levothyroxine should be started if the TSH level exceeds 10 mIU/L. Treatment may also be considered in other situations, such as in patients with consistent symptoms, poorly controlled hypercholesterolemia, or subfertility [9].

3.4.2 Overt Thyroid Dysfunctions

3.4.2.1 Hyperthyroidism

Hyperthyroid patients may present with symptoms such as palpitations, tremors, anxiety, weight loss, and heat intolerance. Clinicians should in the first instance confirm biochemical thyrotoxicosis by testing both TSH and FT4 [23].

Most patients with thyrotoxicosis have primary hyperthyroidism, giving a typical constellation of elevated FT4 and suppressed TSH. The most frequent cause of thyroid hyperfunction is Graves' disease, the diagnosis of which is typically obvious upon clinical examination in many cases. In other cases, however, it may be challenging to distinguish Graves' disease from other

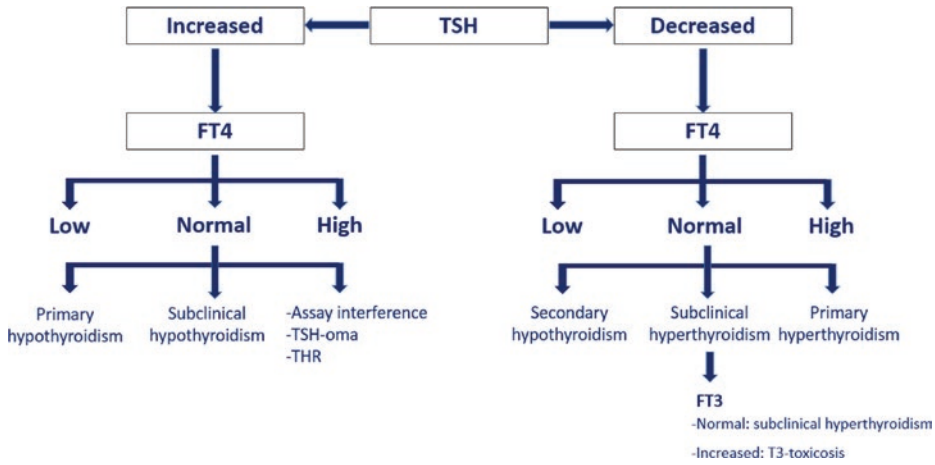

Fig. 3.3 Algorithm for thyroid function test interpretation. *FT3* free triiodothyronine, *FT4* free thyroxine, *T3* triiodothyronine, *THR* thyroid hormone receptor, *TSH* thyroid-stimulating hormone, TSH-oma, TSH-secreting pituitary adenoma

forms of thyrotoxicosis, and several etiologies should be considered before making a definitive diagnosis and starting treatment [24, 25] (Table 3.4). In unclear cases, thyroid scintigraphy, TSH receptor antibody (TRAb) measurement, or ultrasound with Doppler analysis of thyroid vascularity are recommended, depending on local availability and clinical preferences [23]. In addition to aiding diagnosis of Graves' disease, the magnitude of TRAb elevation can serve as a prognostic indicator of remission during medical treatment. In pregnant women with current or previous Graves' disease, TRAb should be tested during the later stages of gestation to assess the risk of fetal/neonatal thyrotoxicosis [24].

Table 3.4 Causes of thyrotoxicosis: etiology and pathophysiology

Cause	Etiology	Pathophysiology
Graves' disease	Autoimmune	TSH-R stimulation
Thyroid functional autonomy	Somatic mutations	Overactive TSH-R and/or Gsα subunit
Subacute thyroiditis	Viral	Inflammatory destruction
Painless thyroiditis	Autoimmune	Immune-mediated destruction
Drug-induced	Iodine overload type 1	Pathologic escape from Wolff-Chaikoff effect
	Iodine overload type 2	Iodine-induced destructive thyroiditis
	TKI, ICPI	Destructive thyroiditis
	Factitious thyrotoxicosis	T4, T3, TH analogs
Tumor	Struma ovarii	Ovarian TH biosynthesis
	Thyroid cancer	Functioning metastasis
	Germinal tumors	βHCG overproduction
Central	Pituitary resistance	THR mutation (TRβ)
	Pituitary adenoma	TSH-secreting tumor

HCG human chorionic gonadotropin, *ICPI* immune checkpoint inhibitors, *T3* triiodothyronine, *T4* thyroxine, *TH* thyroid hormone, *THR* TH receptor, *TKI* tyrosine kinase inhibitors, *TRAb* TSH receptor antibody, *TSH* thyroid-stimulating hormone, *TSH-R* TSH-receptor

In some cases, patients may present with an elevated FT4 level and elevated or inappropriately normal TSH level. While laboratory assay imprecision and/or interferences may explain this abnormality when thyrotoxicosis symptoms are absent, two differential diagnoses should be considered: secondary hyperthyroidism from TSH-oma [prevalence 0.85/1 million] and resistance to thyroid hormone-β (RTHβ) [prevalence 1/40,000]. Patients with TSH-oma have elevated levels of alpha-subunit, a high alpha-subunit/TSH ratio, and a blunted response to thyrotropin-release hormone (TRH) stimulation. Magnetic resonance imaging of the pituitary gland reveals an adenoma (typically a macroadenoma) [26].

Resistance to thyroid hormone (RTH) is a rare genetic syndrome that affects the thyroid hormone receptor isoforms β and α. RTHβ must be considered in patients with unexplained elevated FT4 and unsuppressed TSH levels (inappropriately normal or elevated). Most patients have a positive family history (autosomal dominant inheritance) and show decreased serum FT4/T3 ratio and normal or exaggerated response to TRH stimulation [27].

Finally, it is important to keep in mind that FT4 responds faster to antithyroid therapy and radioiodine than TSH. Consequently, TSH recovery can lag behind FT4 recovery by several months. Clinical actions, such as antithyroid drug titration or introduction of thyroid substitution, should be undertaken according to improvement in FT4 levels in these cases (Table 3.5).

3.4.2.2 Hypothyroidism

Patients who present with overt symptoms of hypothyroidism have low FT4 and elevated TSH levels. The most common cause of primary hypothyroidism in iodine-replete regions is autoimmune thyroiditis (Hashimoto's thyroiditis). Antithyroid peroxidase antibodies (TPOAb) may be tested to confirm the diagnosis. Previous neck surgery, radioactive iodine therapy, and antithyroid drug therapy are also frequent causes of primary hypothyroidism. Patients with secondary hypothyroidism have

Table 3.5 Measurement of FT4: clinical indications

Indication	Aim
Suppressed TSH	To differentiate subclinical from overt hyperthyroidism To assess the degree of overt hyperthyroidism
Increased TSH	To differentiate subclinical from overt hypothyroidism
ATD therapy	To monitor response in the initial months of therapy
RAI therapy	To monitor response in the initial months after RAI therapy
Pituitary disease	To evaluate/monitor patients (TSH not reliable)

ATD antithyroid drug, *FT4* free thyroxine, *TSH* thyroid-stimulating hormone, *RAI* radioactive iodine

low FT4 and low or inappropriately normal TSH levels. After exclusion of assay imprecision or interferences, when a normal TSH level is found alongside low/low-normal T4 but high/high-normal T3 levels, it is important to rule out hypopituitarism and consider resistance to thyroid hormone α-subtype (RTHα). Notably, during early treatment of secondary hypothyroidism with levothyroxine, FT4 levels improve while TSH levels remain low/low-normal, thus making TSH unsuitable for patient monitoring. In these cases, measurement of FT4 alone is recommended (Table 3.5). In pregnant women with hypothyroidism, the usual levothyroxine dose is increased by 30% due to physiological changes. Thyroid function must be monitored closely (every 4–6 weeks) during pregnancy, as maternal hypothyroidism is associated with suboptimal obstetric outcomes and poor fetal neurocognitive development [28].

3.5 Management of Inconsistent Results

Although thyroid function tests are routine examinations, the analytical procedure for determining TSH, FT3, and FT4 remains a major challenge due to multiple interference factors. Notably, most inconsistent thyroid test results are related to nonspecific fluctuations, assay imprecision, or inappropriate reference range rather than clinically relevant dysfunctions [29]. Indeed, falsely increased or decreased thyroid hormone measurements caused by interference factors in immunoassays may result in a considerable number of possible misinterpretations of laboratory findings [30, 31].

3.5.1 Analytical Interferences in Immunoassays

Numerous factors may interfere with immunoassay measurements of TSH, FT3, and FT4, such as macromolecules (frequency < 1:100) [32], interfering antibodies (frequency < 1.1:100) [33], and amino acids and/or glycosylation variants (frequency < 1:100,000), respectively [34, 35]. The use of high-dose biotin (100–300 mg/day) for multiple sclerosis and inherited metabolic disorders has attracted the attention of laboratorians and clinicians, as it can cause inexplicable thyroid test results. Biotin is also advertised and sold for healthy nails and hair and may be present in supplements for this purpose in doses of up to 10 mg per tablet. As most manufacturers have enhanced the biotin tolerance of their assays in recent years, this effect is mainly theoretical unless patients are taking very large doses of biotin or have concomitant renal failure [36, 37]. Nevertheless, it is important that endocrinologists understand the risks of potential interactions with exogenous biotin. Some drugs (e.g., aspirin, furosemide, and phenytoin) may displace the equilibrium between thyroid hormones and binding proteins; others may increase (e.g., estrogen, fluorouracil, and tamoxifen) or inhibit (e.g., androgens, glucocorticoids, and nicotinic acid) the synthesis of thyroxine-binding globulin, leading to dubious method-dependent results in the measurement of FT4 [8]. The administration of heparin may also cause an artificial elevation in FT4 by displacing thyroid hormones from binding proteins via rapidly generated non-esterified fatty acids, especially when FT4 is measured via equilibrium dialysis [38].

3.5.2 Nonthyroidal Illness

Interpretation of thyroid function tests can be confounded by several factors in critically ill patients, depending on the onset, severity, and duration of the critical illness [39]. During critical illness, FT3 levels are the first to decrease, typically within the first 24 h (i.e., low T3 syndrome). FT4 levels decrease subsequently, followed by a decrease in TSH. During the recovery phase, TSH increases early and can transiently exceed the normal range; however, normalization of free thyroid hormones will ensue. These changes in thyroid hormones in critical illness may be due to several factors, such as reduced deiodinase activity, reduced thyroid hormone-binding protein concentrations, increased circulating pro-inflammatory cytokines, and use of certain medications, such as dopamine and glucocorticoids. Whether these changes are a form of beneficial or maladaptive response remains unclear. From a practical point of view, thyroid function tests should be performed during critical illness only if strictly necessary and interpreted with great caution. Otherwise, it is recommended to postpone thyroid testing until the resolution of the acute illness phase.

3.6 Conclusion

Laboratory tests are integral in the management of thyroid dysfunction, and their rational use and proper interpretation may greatly simplify management of patients, and avoid inappropriate diagnostic procedures and clinical actions, including drug administration. In most clinical situations, a concrete understanding of thyroid physiology and the various thyroid tests suffices for the proper and accurate interpretation of the test results. However, unexpected and inconsistent results should be interpreted with caution; clinicians and laboratorians should consider laboratory assay interferences, concurrent medications, pregnancy, nonthyroidal illness, and older age, and interpret results according to the clinical setting. Close communication between all members of the care team is vital.

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