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Review Article

Biochemistry Section

Diagnostic Challenges: A Review of Non Classic Thyroid Function Test Patterns

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ABSTRACT

Disorders of thyroid function are common. Aside from some obvious presenting features of thyroid disease, such as goitre, which can easily be identified, patients with thyroid conditions may present with non specific symptoms. Therefore, a high index of clinical suspicion is required, and confirmation of diagnosis usually depends on accurate measurement and interpretation of Thyroid Function Tests (TFTs). In most cases, the results of TFTs are straightforward and present a familiar pattern of hypo or hyperthyroidism that is easy to recognise. However, sometimes they can seem confusing. These are the discordant or non classic TFT reports. The extent of literature available on TFTs is enormous. The difficulty that arises when encountering a discordant TFT report lies in sifting through the vast amount of literature. A discordant TFT report implies situations where the thyroid hormone and Thyroid Stimulating Hormone (TSH) values do not fit into the pattern of either classic primary or secondary hypo or hyperthyroidism. The present article thus serves as a ready reckoner for those discordant TFT patterns. The way the present article may be utilised is as follows: when a TFT report appears discordant, the TFT pattern may be noted down. Then, the conditions in which the pattern may be seen could be searched in the present article. Next, by correlating with the clinical history, the best fit may be found among the conditions. If a reasonable explanation is not possible, we might need to look for any assay interference. Through a few case examples provided toward the end of the article, this process of deduction has been made evident. The deduction method moves from the more common to the less common causes for the unusual patterns of TFT. Interpretation of a discordant TFT report may be done by first considering a few common causes, such as pregnancy, Non Thyroidal Illness (NTI), and drug usage. Additionally, repeating the TFTs after a few weeks sometimes brings back the classic TFT pattern and helps us identify the factor that was leading to the discordant values. If neither of these conditions fits, assay interference or the less common causes for discrepant values might be considered and worked upon. These are the aspects that have been covered here to enable a successful interpretation of any TFT report.

Keywords: Deiodinase, Discordant thyroid function tests, Disequilibrium state, Drugs, Pregnancy

INTRODUCTION

Thyroid dysfunction can be broadly classified as either hypothyroidism or hyperthyroidism. Hypothyroidism can be further classified as primary (due to thyroid hormone deficiency), secondary (due to TSH deficiency), and tertiary (due to thyrotropin-releasing hormone deficiency). Secondary and tertiary hypothyroidism are together referred to as central hypothyroidism [1,2].

Causes of primary hypothyroidism include iodine deficiency, Hashimoto's disease, certain drugs (e.g., amiodarone, lithium), thyroid surgery and radioactive iodine therapy. Central hypothyroidism is rare (accounting for less than 1% of cases), with secondary hypothyroidism (due to pituitary dysfunction) being more common than tertiary hypothyroidism (due to hypothalamic dysfunction). Causes of central hypothyroidism include pituitary adenoma (the most common cause), pituitary apoplexy, Sheehan's syndrome, surgery or radiotherapy involving the pituitary, head injury and drugs affecting the hypothalamic-pituitary-adrenal axis.

Hyperthyroidism is most commonly caused by Graves' disease. Other causes of hyperthyroidism include multinodular toxic goitre, toxic nodules, iodide or drug-induced hyperthyroidism, and TSH-secreting pituitary tumours (which are rare) [1,2].

The classic TFT patterns are as follows [Table/Fig-1]:

i The concomitant finding of a high serum TSH concentration and a low free T4/thyroid hormone level confirms the diagnosis of primary hypothyroidism [1,2].

ii. Individuals with a high serum TSH concentration and a normal or low-normal serum free T4/thyroid hormone level have, by definition, subclinical hypothyroidism [1,2].

CONDITION	TSH	T4/free T4	T3/freeT3	CAUSES
HYPOTHYROID STATES				
PRIMARY HYPOTHYROIDISM	▲	▼	▼	iodine deficiency, Hashimoto's disease, drugs (e.g. amiodarone, lithium), thyroid surgery,
SECONDARY HYPOTHYROIDISM	▼	▼	▼	pituitary adenoma(commonest), pituitary apoplexy, Sheehan's syndrome, surgery/ radiotherapy involving the pituitary, head injury, drugs affecting the hypothalamic-pituitary-adrenal axis
SUBCLINICAL HYPOTHYROIDISM	▲	↔	↔	(Similar to primary hypothyroidism)
HYPERTHYROID STATES				
PRIMARY HYPERTHYROIDISM	▼	▲	▲	Graves' disease, multinodular toxic goitre, toxic nodule, iodide or drug-induced hyperthyroidism
SECONDARY HYPERTHYROIDISM	▲	▲	▲	TSH secreting pituitary tumour(rare)
SUBCLINICAL HYPERTHYROIDISM	▼	↔	↔	(Similar to primary hyperthyroidism)

▲: High	▼: Low	↔: Normal

[Table/Fig-1]: The classic TFT patterns [2].

iii. The diagnosis of secondary hypothyroidism is based on the findings of a low serum free T4/thyroid hormone level and a serum TSH level that is normal or low [1,2].

iv. The diagnosis of primary hyperthyroidism is based on the findings of a high serum free T4/thyroid hormone level and a low serum TSH concentration [2].

Patients with a low serum TSH concentration and normal serum Free Thyroxine (FT4) and Free Triiodothyronine (FT3) levels have, by definition, subclinical hyperthyroidism [2].

A discordant TFT report implies a deviation from the classic TFT patterns and may pose diagnostic challenges. The first step in unravelling the complex TFT patterns is to understand that the thyroid gland is subject to regulation at multiple levels [3]. Discordant TFTs might result from any of these factors being affected. The factors are listed in [Table/Fig-2].

Factors responsible for TFT:
TSH secretion
TSH receptor
Thyroid hormone binding proteins
Free thyroid hormones
Deiodinases
Reverse T3 (rt3)
Thyroid hormone receptors
Specific membrane proteins (for transport of thyroid hormones)
Thyrotropin-releasing Hormone (TRH)
Test methodology and interfering factors

[Table/Fig-2]: List of factors that influence TFTs [4-8].

The present review was aimed to summarise the various conditions that might yield discordant TFT reports. The conditions and causes that result in classic hypothyroid and hyperthyroid states have not been covered in this review. There have only been a few published articles that comprehensively address the topic of discordant TFTs [4-6]. However, these articles do not exclusively focus on discordant TFTs; they utilise an algorithmic approach to interpret the various combinations of FT3/FT4 with TSH. While some topics are discussed extensively, not all causes of discordant TFTs have been included. In the present article, only the causes leading to discordant TFTs have been covered, and next to each condition, the TFT pattern is clearly stated, followed by a brief description. In writing this article, a significant amount of information has been sourced from the literature dealing with the subtopics to ensure that no cause of discordant TFTs is left out. In the end, some case examples have been provided to elaborate on practical scenarios. This article will therefore enable the laboratory consultant to resolve the diagnostic dilemma that follows a discordant pattern in TFT reports.

Stepwise Approach to A Discordant/Non Classic TFT Report

If a TFT report does not fit into either classic primary or secondary hypothyroidism or hyperthyroidism:

- The most common confounding factors include pregnancy, intercurrent non thyroidal illness, and drugs [4-7].
- An important strategy in diagnosis is the reassessment of thyroid status [8-10].
- Spurious values resulting from assay interference could mislead the diagnosis [11,12].

Effect of Pregnancy (normal physiology) on TFT:

Discordant pattern: ↑ T3, ↑ T4, ↔ TSH

The serum total T3 and T4 levels increase to approximately 1.5 times the non pregnant value [13]. This is a consequence of elevated Thyroid Binding Globulin (TBG) levels, as oestrogen induces increased hepatic synthesis of TBG. Therefore, measuring free T3 and T4 levels is advised during pregnancy.

Another finding noted in the Thyroid Function Tests (TFT) performed in the first trimester is that, compared to pre-pregnant values, the TFT pattern shows a lower TSH value and higher FT3 and FT4 values [13,14]. These effects are primarily mediated by the stimulatory effects of high Human Chorionic Gonadotropin (HCG) levels, as hCG shares the same α subunit with TSH but has a unique β subunit. It stimulates the thyroid gland in early pregnancy by binding to TSH receptors [5].

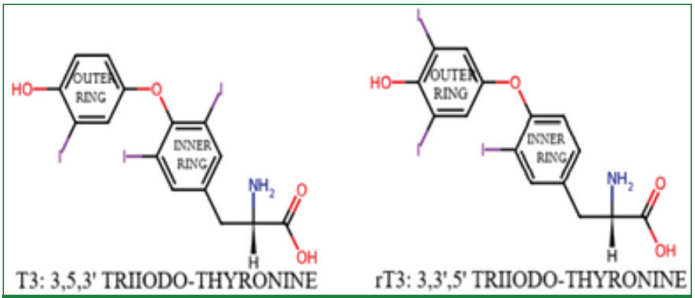
Consequently, the reference interval for TSH in pregnancy has both a lower upper limit and a lower limit compared to the TSH reference interval in non pregnant women, which is typically 0.4-4.0 mIU/L. This effect is most pronounced in the first trimester. For example, the first trimester range for TSH is 0.1-2.5 mIU/L [13].

(A more pronounced TSH suppression due to hCG is seen in gestational trophoblastic diseases) [15].

Intercurrent Non Thyroidal Illness (NTI)/euthyroid sick syndrome

Discordant pattern: ↓T3, ↓FT3, ↔T4, ↔TSH

The condition described is often seen in patients with critical illness, calorie deprivation, and those recovering from major surgeries or trauma [16]. Therefore, NTI may occur in cases of sepsis, starvation, major fractures (e.g., hip fractures), pneumonia, myocardial infarction, malignancies, burns, congestive heart failure, cirrhosis, renal failure and diabetic ketoacidosis [17]. The changes observed in TFTs are secondary adaptive changes, which is why this condition is referred to as euthyroid sick syndrome. These changes are most likely mediated by pro-inflammatory cytokines, endogenous glucocorticoids and leptin [16]. The beneficial effects of these changes remain uncertain. NTIs typically present with reduced total T3 and FT3 levels, along with an increase in reverse Triiodothyronine (rT3) formation [18]. Another name for this condition is low T3 syndrome, as total T3 estimation does not include reverse T3. Deiodinase D3 converts T4 to 3,3',5'-triiodothyronine (rT3) [Table/Fig-3] through inner ring deiodination of T4. FT3, or 3,5,3'-triiodothyronine, is formed by outer ring deiodination by either deiodinases D1 or D2 [5]. In NTI, FT4 and TSH levels may be normal or low. Severe suppression of thyroid hormone levels might predict illness mortality. Recovery from the illness typically leads to the normalisation of thyroid hormone levels, with elevated levels of TSH usually preceding the rise in T3 and T4. Therefore, to avoid inappropriate diagnosis and treatment, awareness of this condition is imperative [16].



[Table/Fig-3]: Structures of T3 and rT3 [5].

Effect of drugs on TFT: Various drugs and their effects are as follows:

Thyroxine: Discordant pattern: ↑FT4, ↑TSH, ↔FT3.

In patients receiving thyroid hormone replacement, a combination of slightly elevated FT4 with normal plasma TSH concentration is commonly observed. FT3 is usually normal, which is likely due to less efficient deiodination of FT4 to FT3 [19].

Drugs affecting TBG levels:

Discordant pattern: ↑T3, ↑T4, ↔TSH

Drugs that elevate TBG levels increase the total T3 and T4 levels without affecting the free hormone levels and TSH. Examples include estrogen, tamoxifen, raloxifene, and anticancer drugs like mitotane and 5-fluorouracil, as well as drugs of abuse like methadone and heroin [20-22]. A study by Arafah BM showed that women with no thyroid disease adapt quickly to estrogen-induced increases in serum thyroxine-binding globulin concentration [20]. In contrast, women with hypothyroidism who have high estrogen levels experience a decrease in serum free thyroxine concentration, which

is sufficient to increase serum thyrotropin concentration, resulting in an increased need for thyroxine [20].

Discordant pattern: ↓T3, ↓T4, ↔TSH

The drugs that reduce TBG levels decrease the total T3 and T4 levels without affecting the free hormone levels or TSH. These mainly include androgens, glucocorticoids and nicotinic acid [21].

Drugs that cause displacement of T3, T4 from thyroid hormone binding protein sites: Discordant pattern: ↑FT3, ↑FT4, ↔TSH (transient changes)

These drugs cause an initial transient increase in the levels of free hormones, while TSH remains normal. Continued administration results in reduced total hormone levels, with normal free thyroid hormone and TSH levels. Examples include furosemide (greater than 80 mg intravenously), aspirin, NSAIDs, phenytoin, carbamazepine, and heparin [21].

Drugs affecting TSH secretion: Discordant pattern: ↓TSH, ↔T3, ↔T4.

Dopamine and dopamine agonists, such as cabergoline and bromocriptine (which are used in the treatment of hyperprolactinemia), may suppress TSH secretion by acting through the dopamine D2 receptor. Glucocorticoids inhibit the synthesis and release of hypothalamic TRH. Somatostatin and its analogues (e.g., octreotide) suppress pituitary TSH secretion, while rexinoids (e.g., Bexarotene/ Targretin®, used to treat cutaneous T-cell lymphoma) inhibit TSHβ transcription [22]. The presentation in these cases is characterised by reduced TSH with normal thyroid hormone levels or resembles secondary (central) hypothyroidism (reduced TSH and thyroid hormones), and this condition is often transient.

Drugs stimulating metabolism of thyroid hormones:

Discordant pattern: ↓T4, ↓FT4, ↔T3, ↔/↑TSH

Typically, 20% of the T4 produced is excreted in the faeces, mostly through glucuronide conjugation. Antiepileptic drugs such as phenytoin, phenobarbital and carbamazepine, as well as, the antitubercular drug rifampicin, can markedly decrease plasma thyroid hormone concentrations by inducing the liver enzymes involved in thyroid hormone excretion [23]. Total and free T4 levels are lower, T3 is usually normal, and TSH may increase slightly. Several environmental pollutants also produce similar effects [24].

Amiodarone: Discordant pattern: ↑T4, ↓T3, ↑TSH

Amiodarone is an iodine-rich antiarrhythmic agent used to treat ventricular and atrial tachyarrhythmias. It contains 39% iodine by weight and bears structural similarities to thyroid hormones. During the metabolism of amiodarone, a large amount of iodine is released into the plasma. A pattern that may be observed throughout therapy is elevated T4 (due to inhibition of cellular thyroid hormone uptake) and low T3 (resulting from the suppression of deiodinase D1), along with increased TSH [25]. Other thyroid conditions associated with amiodarone therapy include thyrotoxicosis or hypothyroidism, depending on the iodine status [25].

Heparin: Discordant pattern: ↑FT3, ↑FT4, ↔TSH {artifactual}

The in vitro artifact effect of enoxaparin and heparin is the elevation of free thyroid hormones in the presence of normal TSH [26]. This occurs because heparin induces the production of non esterified fatty acids by lipoprotein lipase, which is released under the influence of heparin. The released free fatty acids bind to albumin and displace the thyroid hormones, leading to an artificial increase in free thyroid hormone levels [27].

Drugs that affect the functioning of the thyroid gland at multiple levels are presented in [Table/Fig-4] [21-27].

Reassessment of thyroid status: A TFT report that initially appears discordant may align with the expected pattern when repeated after a few weeks [28]. One of the causes of such a finding has been discussed previously under the heading of NTI. Some drug effects may also be transient. Other situations include:

Drug	Cause	Effect (discordant pattern)
Thyroxine	Less efficient deiodination of T4 to T3	↑FT4, ↔TSH, ↔FT3
Oestrogen, tamoxifen, raloxifene, mitotane, 5-fluorouracil, methadone, heroin	Elevate the TBG levels	↑T3, ↑T4, ↔TSH
Androgens, glucocorticoids, nicotinic acid	Reduce the TBG levels	↓T3, ↓T4, ↔TSH
Furosemide (>80 mg i.v.), aspirin, NSAIDs, phenytoin, carbamazepine, heparin	Displacement of T3, T4 from thyroid hormone binding protein sites	↑FT3, ↑FT4, ↔TSH {transient changes}
Dopamine, dopamine agonists like cabergoline and bromocriptine, glucocorticoids, somatostatin and its analogues (e.g., octreotide)	Suppress TSH secretion	↓TSH, ↔T3, ↔T4
Phenytoin, phenobarbital, carbamazepine, rifampicin	Induce the liver enzymes involved in thyroid hormone excretion	↓T4, ↓FT4, ↔T3, ↔/↑TSH
Amiodarone	Elevated T4 is because of inhibition of cellular thyroid hormone uptake, low T3 is due to suppression of deiodinase D1. Low T3 leads to increased TSH	↑T4, ↓T3, ↑TSH (Note: there are multiple effects on thyroid due to amiodarone therapy)
Heparin	Lipoprotein lipase that is released under the influence of heparin releases free fatty acids that bind to albumin and displace the thyroid hormones leading to an artificial increase of free thyroid hormones	↑FT3, ↑FT4, ↔TSH (artifactual)

[Table/Fig-4]: Effect of drugs on TFT [21-27].
NSAIDs: Non steroidal anti-inflammatory drugs

Recent treatment of thyrotoxicosis:

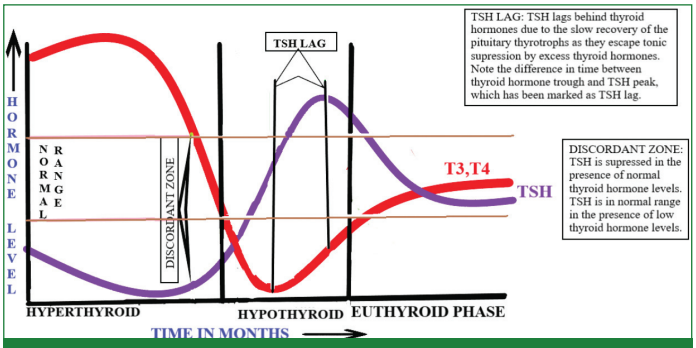
Discordant pattern: ↔FT4, ↓TSH

The TSH levels may remain suppressed even when thyroid hormone concentrations return to normal [29]. TSH recovery can lag behind FT4 recovery by several months. Therefore, for drug dose adjustment, FT4 levels should be considered during the initial phases of therapy.

Triphasic pattern seen in autoimmune thyroiditis:

Discordant pattern: ↓FT4, ↔/↓TSH

Painless thyroiditis (silent thyroiditis), postpartum thyroiditis [30], and painful subacute thyroiditis (De Quervain thyroiditis) may present with a clinical course characterised by an initial phase of hyperthyroidism. In this phase, inflammatory destruction of the thyroid gland results in the release of preformed thyroid hormones, with T4 being released more than T3, unlike in Graves' disease. Later, depletion of thyroid hormones leads to hypothyroidism. During this phase, TSH levels remain low or normal, even though the free thyroid hormone levels are also low [31]. This period is commonly referred to as the 'disequilibrium state' [Table/Fig-5]. This state occurs due to the slow recovery of the pituitary thyrotrophs as they escape tonic suppression by excess thyroid hormones. Finally, recovery leads to the return of the euthyroid state [32].



[Table/Fig-5]: Triphasic pattern seen in autoimmune thyroiditis [32].

Disorders of thyroid hormone transport or metabolism:

Discordant pattern: ↑FT3, ↓FT4, ↔TSH

Thyroid hormones are imported into cells through several transporter proteins. A mutation in the Monocarboxylate Transporter 8 (MCT8) gene causes Allan-Herndon-Dudley syndrome [33]. The estimated prevalence of this condition is 1 in 70,000 males [34]. In these individuals, thyroid hormone is unable to enter the brain cells due to a defect in the thyroid hormone transporter MCT8. This is an X-linked disorder that presents with severe psychomotor retardation. Excess thyroid hormone, specifically T3, enters tissues in the rest of the body, leading to life-threatening consequences. The TFT pattern observed is elevated FT3, low FT4 and normal TSH [34].

T3-toxicosis: Discordant pattern: ↑T3, ↔T4, ↓TSH

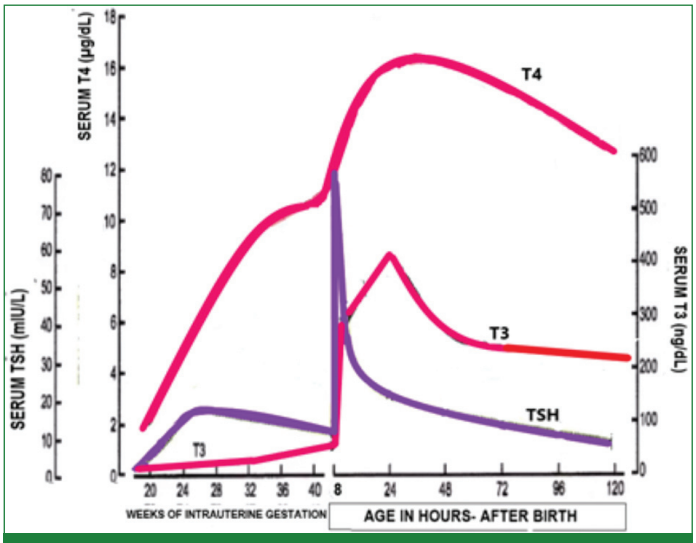
Serum T4 is normal, with elevated serum T3 and low or undetectable serum TSH. This condition is referred to as 'T3 toxicosis' [35]. This may be the presenting feature in the earliest stages of Graves' disease or in an autonomous thyroid nodule. In Graves' disease, Thyroid-stimulating hormone Receptor Antibodies (TRAb) bind to and activate the TSH receptor, stimulating follicular hypertrophy and hyperplasia, as well as, increasing the synthesis of thyroid hormones and the proportion of T3 relative to T4 [36]. In contrast, in destructive forms of thyroiditis, serum T3 concentration is not disproportionately elevated as it is in Graves' disease [37], since the mechanism of thyroid hormone elevation is due to a higher release from the gland rather than secondary to the activity of thyroid deiodinase.

Functional deiodinase deficiency: Discordant pattern: ↑FT4, ↓FT3, ↔TSH

This rare condition results from a defect in the incorporation of selenocysteine into the deiodinase enzymes [38,39]. It is characterised by a multisystem phenotype due to deficiencies of antioxidant and tissue-specific selenoproteins, along with abnormal thyroid hormone levels. Selenocysteine is critical for deiodinase function. Inadequate function of deiodinases leads to a significant deficiency of the T3 hormone, as the conversion of T4 to T3 is impaired [38]. The systemic features of this disorder include growth retardation in childhood, male infertility, skeletal myopathy, photosensitivity and hearing loss [4].

Reference range in newborns: Discordant pattern: ↑T3, ↑T4, ↑TSH (physiological pattern, not hyperthyroidism).

At first glance, if one is unaware of the newborn ranges for TSH and thyroid hormones, and if the reference range specific to newborns has not been provided in the report, the values might appear to indicate secondary hyperthyroidism [Table/Fig-6]. Thyroid hormone levels and TSH are higher in the first days of life and usually fall within the first two to four weeks [39-41]. Therefore, values should be compared with appropriate age-dependent reference intervals.



[Table/Fig-6]: Changes in TSH, T3, T4 in newborns [40].

Newborn screening for congenital hypothyroidism should ideally be performed between 2 to 4 days after birth. Typically, the TSH cut-off is 20-40 mU/L, as a range of 20-40 is considered instead of a cut-off value of 40 mU/L, since 10% of infants with confirmed congenital hypothyroidism have TSH values between 20 mU/L and 40 mU/L [41]. The status of prematurity and the clinical state of the newborn may mask the true state of thyroid function. Therefore, repeat screening is recommended for preterm and sick infants [41].

Circadian rhythm: Discordant pattern: There is intraindividual variability in TSH values. TSH levels exhibit a clear daily rhythm. Plasma concentrations begin to rise in the late afternoon or early evening before sleep onset and reach maximal levels during the early part of the night. TSH concentration then declines during the rest of the sleep period, reaching low daytime levels [42]. Morning TSH levels may be roughly twice as high following a sleepless night when compared to a night of normal sleep [43].

Hypothalamic/tertiary hypothyroidism:

Discordant pattern: Central hypothyroidism may present with elevated TSH. Slight elevations of serum TSH concentrations can also be found in some patients with central hypothyroidism who have a predominant hypothalamic defect. In this subgroup of patients, TSH levels are similar to those generally found in subclinical or mild primary hypothyroidism. Here, TSH is devoid of full biological activity, and FT4 is in the hypothyroid range [44]. TRH secreted from the pituitary regulates not only the secretion of thyrotropin but also its specific molecular and conformational features required for hormone action. Therefore, this rare form of hypothalamic hypothyroidism, characterised by deficient TRH, might present with high TSH and low FT4 [45].

Assay interferences [46,47] [Table/Fig:7]:

Macro TSH:

Discordant pattern: ↑TSH (markedly elevated-false high value), ↔T3, ↔T4

Macro-TSH is composed of monomeric TSH complexed with autoimmune anti-TSH antibodies. Currently, none of the available immunoassays for TSH can completely discriminate between macro-TSH and free TSH [46].

Biotin: Discordant pattern: ↓TSH (false low), ↑FT3 and ↑FT4 (false high)

This interference is observed in immunoassay platforms that utilise biotin-streptavidin complex formation for analysis. High doses of biotin are given in rare metabolic disorders like biotidinase deficiency, as well as in a few other conditions [46].

Heterophilic antibody interference:

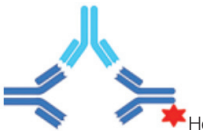
Discordant pattern: ↑TSH (false high-more common)

The term 'heterophilic antibody interference' refers to a patient's sample that contains antibodies which cause false results by binding to the assay antibodies. Since TSH is measured using a sandwich-type immunoassay, if the heterophilic antibody cross-links the capture and detection antibodies, it will result in a positive assay interference. Conversely, if it binds only to one of the antibodies, it will result in a negative interference. Manufacturers have developed strategies to eliminate these interferences, such as adding non specific animal immunoglobulins to the reagent [46].

Familial Dysalbuminemic Hyperthyroxinemia (FDH):

Discordant pattern: ↑T4 (true), ↑FT4 (false), ↔FT3, ↔TSH

Immunoassay methods for estimating free hormones fail when the FT4:T4 equilibrium is altered due to changes in the levels of T4 binding proteins, as observed in Familial Dysalbuminemic Hyperthyroxinemia (FDH). This condition is characterised by an increase in the affinity of albumin for T4 but not for T3. This occurs because the FT4:T4 ratio of the serum-based calibrator differs from that of the sample [5].

Interference due to macro-TSH: Macro-TSH refers to the presence of circulating, bioinactive TSH held in complex by immunoglobulins. Therefore, even though this TSH bound to immunoglobulins does not exert its effect on the thyroid gland, it is detected by the immunoassays leading to spuriously high TSH result. Present with clinical and biochemical (normal thyroid hormones) euthyroid state. Gel filtration chromatography is the confirmatory test.
Biotin interference: When biotin-streptavidin binding principle is used in immunoassay, the binding of biotinylated antibodies to the streptavidin coated microparticles is inhibited in the presence of excess free biotin. The free biotin binds to the streptavidin coated microparticles and does not allow the biotinylated antibodies to bind. This causes false low result in sandwich assays, and false high result in competitive assays. A non biotinylated immunoassay method may be used to mitigate this effect.
Heterophile antibody:  Heterophilic antibodies usually apply to polyreactive antibodies against poorly defined or unknown antigens. If the heterophilic antibody cross-links the capture and detection antibodies (as shown in the figure), it will result in a positive assay interference. If it binds only to either of the antibodies it will result in a negative interference. Interference can be minimised with the addition of normal sera or purified antibodies from the same species used in the assay. These normal antibodies saturate the binding sites of the heterophilic antibodies and prevent interference from occurring. This is how reagents avoid heterophilic antibody interference.
Familial Dysalbuminemic Hyperthyroxinemia (FDH): In FDH, enhanced interaction of the labelled T4 analogue with mutant albumin decreases its availability to compete with free T4 for capture antibody binding sites, resulting in spuriously high FT4 measurements. Also, FT4:T4 equilibrium is altered, making this ratio different from the serum-based calibrator. This effect is difficult to mitigate.
Autoantibodies against T4: In one step immunoassay techniques, sample and tracer (labelled T4 analogue) are added at the same time. Therefore, the tracer gets bound by the autoantibody and binds less to the immobilised anti-FT4 antibody. This generates a low signal and hence a spuriously high result for FT4. Two-step immunoassays eliminate this effect by washing off the autoantibodies before the addition of tracer in the second step.

[Table/Fig-7]: Mechanisms of assay interferences in thyroid immunoassays [46,47].

Autoantibodies against T4:

Discordant pattern: ↑FT4 (spurious elevation), ↔FT3, ↔TSH

This pattern is observed with one-step immunoassay techniques used in some immunoassay platforms. In this method, the sample and tracer are added simultaneously. As a result, the tracer binds to the autoantibody and binds less effectively to the Immobilised anti-FT4 antibody in the reagent. Consequently, a low signal is generated, leading to a spuriously high value of FT4 being reported (competitive immunoassay). Immunoassays employing a two-step assay technique are more resistant to this type of interference, as the autoantibodies are washed off before the addition of the tracer in the second step [5].

Equilibrium dialysis and ultrafiltration are considered the gold standard methods for the measurement of free hormones [47].

Summary

To reiterate, here are a few guiding principles [5]:

- Conditions where the TSH levels do not align with the hypothalamic-pituitary feedback axis include:

a) Recent treatment for thyrotoxicosis or hypothyroidism, or during the hypothyroid phase of autoimmune thyroiditis, indicating TSH lag.

b) Non Thyroidal Illness (NTI).

c) Medications such as glucocorticoids, which transiently depress TSH.

d) Central hypothyroidism.

e) TSHomas.

f) Resistance to thyroid hormone.

g) Disorders of thyroid hormone transport or metabolism.

If TSH is undetectable, free T3 aids in the diagnosis of T3 toxicosis. TSH Receptor Antibodies (TRAb) help confirm the diagnosis of Graves' disease, which may present with a T3 toxicosis pattern [5]. Normal plasma TSH with slightly elevated free T4 is commonly seen in patients on thyroid hormone replacement due to less effective deiodination of T4 to T3 [5]. FDH results in an assay artifact to which most current free T4 assays are susceptible, presenting with elevated FT4. In the most common form of FDH, free T3 is typically within the reference interval. Assay interferences should always be considered when the clinical history fails to explain the TFT pattern.

A few examples of cases that had discordant TFTs are shared in [Table/Fig-8] [10,48,49]. The cases illustrate how, when a patient presents initially based on their complaints and basic thyroid screening, a diagnosis is reached and treatment is instituted. However, follow-up clinical presentations and TFTs do not match what was initially thought. Further work-up helps resolve the issue and successfully manage the condition.

Authors name	Place/year of the study	Case	Diagnosis	Discordant pattern	Follow-up value	Conclusion
Pantalone KM and Nasr C [10]	Cleveland Clinic/2010	A 34-year-old woman, 4 months postpartum. Complains of palpitation, heat intolerance, and difficulty sleeping from 2 months.	Postpartum thyroiditis TFT report: TSH: 0.005 µIU/mL (RR 0.4-5.5) FT4: 2.4 ng/dL (RR 0.7-1.8).	After 6 weeks: TSH: 0.085 µIU/mL FT4: 0.6 ng/dL. Pattern is discordant as FT4 is below the RR and TSH is still suppressed.	After further 2 weeks: TSH: 3.5 µIU/mL FT4: 0.8 ng/dL TFT looks normal.	The discordant pattern of low TSH in the setting of the low FT4 noted after 6 weeks reflects a disequilibrium state, which occurs during the hypothyroid phase of thyroiditis. The subsequent TFT report suggests a euthyroid state following resolution of thyroiditis.
Moran C et al., [48]	UK/2023	A 67-year-old man with a TSH: 9.7 mU/L (RR 0.4-5.5).	Hypothyroid Treated with 100 µg of T4 per day.	Follow-up: TFT: TSH: 13.7 mU/L (RR 0.4-5.5) FT4: 5.36 ng/dL (RR 0.7-1.8) This pattern is discordant as both FT4 and TSH are high. Consequent to this report, thyroxine supplementation was stopped.	Thyroid testing after few weeks: TSH: 45.6 mU/L Antithyroid peroxidase (anti TPO) antibody: >1300 IU/mL (RR 0.4-5.5). Treatment with thyroxine was restarted. Repeat TFT after a few weeks: TSH: 22.1 mU/L FT4: 4.74 ng/dL.	TSH and free T4 both were estimated by two different immunoassay methods. TSH values from the two methods were 43.8 mU/L and 45.6 mU/L (values are similar), whereas FT4 values from the two methods were: 4.66 ng/dL and 0.54 ng/dL. This pattern is highly suggestive of measurement interference due to autoantibodies against T4. The first FT4 (spuriously high) result is from a one-step immunoassay method, whereas the second FT4 result which is below the reference range, is from a two-step immunoassay technique which can overcome the interference due to anti-FT4 antibodies by washing them off before the addition of the tracer in the second step.

Bitton RN and Wexler C [49]	New York/1989	A 52-year-old woman was referred for evaluation of goiter. She complained of hair thinning and irregular menses. There was a history of facial and neck irradiation 30 years previously. 131I-thyroid scan showed uptake only in the right thyroid lobe.	Hypothyroid based on history and initial TFT: T4 level of 3.5 µg/dL (RR 4.5-12) T3 level of 63.9 ng/dL (RR 85-185) Started therapy with L-Thyroxine. Following the treatment, patient experienced weight loss and nervousness. Pulse-84/min and BP of 160/80 mm Hg. Slight tremor was present. Thyroxine was discontinued.	Repeat TFT: T4:3.7 µg/dL, T3:123 ng/dL, TSH <0.1 µIU/mL. TBG level <5.0 µg/mL (RR 10-50), free T3 level: 349 pg/dL (RR: 210-330), free T4 was normal.	Patient had concomitant TBG deficiency and T3 toxicosis. Decrease in TBG secondary to hyperthyroidism itself may sufficiently lower T3 into the normal range in some individuals with T3 toxicosis. A subgroup of patients with thyroid gland autonomy, defined by suppressed TSH levels and normal free T4 levels may have unrecognised free T3 toxicosis, as exemplified.

[Table/Fig-8]: Case reports demonstrating discordant TFTs [10,48,49].

CONCLUSION(S)

Evaluation of a discordant TFT report may be conducted through careful consideration of the patient's clinical history and an awareness of the various factors that may affect the levels of thyroid hormones and TSH. Therefore, with a solid understanding of laboratory science and an appreciation of the medical context, accurate and successful interpretation of these reports is not difficult.

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