A Practical Approach Towards The Evaluation Of Aberrant Thyroid Function Tests

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Abstract
Thyroid function tests are amongst the most commonly requested laboratory investigations. Fortunately, based on the negative feedback principle, most thyroid function tests are straightforward interpretable and thus facilitate confirming the clinical impression of euthyroidism, hypothyroidism or hyperthyroidism. However, some thyroid function tests aberrantly deviate from this principle, making them potential diagnostic pitfalls.

Starting from a case report of a patient with non-suppressed serum TSH levels despite elevated levels of thyroid hormones, we present an overview of aberrant thyroid function tests and how to diagnose them.

Introduction
Endocrine practice is depending for a major part on laboratory analyses. Linking a clinical picture to biochemical findings can help the endocrinologist in making a correct diagnosis or deciding which further investigations are needed to make a diagnosis. The patient presented in this report was referred to us with a variety of complaints that were difficult to match with her aberrant thyroid function tests (TFT). This patient’s story shows the importance of knowing the limitations of laboratory tests that can lead to aberrant results. A first step is to confirm the aberrant results and to reveal the underlying cause of this aberration. Different classes of drugs can influence thyroid function tests (in vivo as well as in vitro). Certain clinical conditions can cause aberrant TFT, making comparison with historical lab results interesting. Analytical interference is another important cause of aberrant TFT that is frequently overlooked. This interference can be seen in thyroid hormone as well as in TSH assays, it can be directed against the analyte as well as against the antibody used in the assay and it can result in falsely low as well as falsely elevated tests results. Aberrant TFT can also be the result of a rare genetic or acquired disorder of the hypothalamic-pituitary-thyroid axis, such as the syndromes of reduced sensitivity to thyroid hormone or a TSH-secreting pituitary adenoma.
**Methodology**

The case report of a patient presenting with aberrant thyroid function tests was the foundation for this work. A systematic review of literature about thyroid hormone resistance was performed. Search items such as “resistance to thyroid hormone”, “RTH-β”, “Refetoff syndrome”, “impaired sensitivity to thyroid hormone”, “reduced sensitivity to thyroid hormone” were put into medical search robots such as PubMed, Web of Science, Access Medicine. This query has made it clear it was hard to talk about this subject without first focussing on the broader differential diagnosis of aberrant thyroid function tests, which includes thyroid hormone resistance. Search items such as “aberrant thyroid function tests”, “TSH-secreting pituitary adenoma”, “analytical interference AND thyroid function tests”, “heterophilic antibodies” were then added to the query. The search results were selected using publication date (only articles published after 2000 or if earlier, updated after 2000), international journals and English articles as selection criteria.

**Results & Discussion**

1. **Case report**

   In 12/2014, a 52-year-old woman was referred for evaluation of thyroid function. She reported various complaints including constipation, low energy level, cold feeling, fatigue, difficulty losing weight and anxiety.

   In 2009, she received 5 mCi radioactive iodine (I-131) because of an “enlarged thyroid with tendency to hyperthyroidism”. Post-radioiodine thyroxine replacement was initiated, though this was later discontinued for unknown reasons.

   This patient has several relatives with thyroid dysfunction (father, uncle, aunts and grandmother) but there is no further information about the underlying origin.

   She smokes 5 cigarettes a day and takes analgesics for back pain (up to 12 tablets of paracetamol codeine 500/30 mg a day). She was also being treated for arterial hypertension with amlodipine and for acid reflux with omeprazole.

   The clinical examination revealed an elevated blood pressure (150/100 mmHg), a BMI of 24.7 kg/m² and a somewhat enlarged thyroid.

   Laboratory results of 12/2014, presented in Table 1, show an elevated TSH as well as elevated peripheral thyroid hormone levels. In Table 2, historical lab results (3/2013
and 11/2014) of this patient are presented, showing similar TFT. There was no
On ultrasonography the thyroid appears enlarged and contains multiple nodules.
Thyroid scintigraphy shows an eager uptake of tracer (pertechnetate, TcO₄⁻-99m) in
both thyroid lobes: 1,8 % with reference values 0.5-2%.

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2. Thyroid hormone metabolism: general aspects

During infancy and childhood, thyroid hormones are critical determinants of brain and somatic development: untreated congenital hypothyroidism leads to permanent mental retardation and severe growth failure (1).

Adults need thyroid hormones for metabolic activity. The function of virtually every organ system is dependent on thyroid hormone exposure. The thyroid gland is responsible for production and storage of thyroid hormones (TH).

2.1. Regulation of thyroid hormone production

The production of thyroid hormones, triiodothyronine (T3) and thyroxine (T4), is stimulated by pituitary thyroid-stimulating hormone (TSH) whose synthesis is regulated by hypothalamic thyrotropin-releasing hormone (TRH). A negative feedback system with T3 and T4 inhibiting TRH and TSH production normally establishes an equilibrium (see Figure 1). Small alterations in thyroid hormone exposure are typically associated with a significant fall (in case of thyrotoxicosis) or rise (with hypothyroidism) in TSH levels, whereas the corresponding serum T3 and/or T4 levels may still be in the normal reference range. Diagnosis of thyroid dysfunction is based on this principle (see Figure 2) (2). For instance, a low (or even suppressed) TSH level in combination with normal TH levels most frequently indicates subclinical hyperthyroidism in patients with multinodular (not yet toxic) goitre. This pattern of TFT is often seen in elderly patients.

On the other hand, a high TSH level combined with normal TH levels usually indicates subclinical hypothyroidism, a state that reflects mild thyroidal failure. It is a common condition in the population, affecting 5-10 % of all women. Mostly, autoimmune thyroiditis is underlying.

In more extreme clinical situations of hypothyroidism or thyrotoxicosis, both TSH and TH levels will be affected but typically in opposite directions.

Due to this tightly regulated hypothalamic-pituitary-thyroid (HPT) axis, appropriate levels of TH are delivered to target tissues.
2.2. Thyroid hormone synthesis

T3 and T4 are iodothyronines: they are the result of two coupled iodotyrosines. T3 and T4 are the only iodide-containing hormones in vertebrates. Without iodine (I) there is no biosynthesis of thyroid hormones. Thyroid hormone synthesis is dependent on the nutritional availability of iodine and is predominantly regulated by TSH. In response to feedback from circulating thyroid hormone, TSH binds to its receptor on the basolateral surface of the thyroid follicular cell. Transport of iodine into the thyroid follicular cell is mediated by a Na⁺/I⁻ symporter (NIS), an intrinsic plasma membrane transport protein that couples the inward “downhill” translocation of Na⁺ to the inward “uphill” translocation of iodide (I⁻).
driving force of this process is an inwardly directed Na+-gradient generated by the Na⁺/K⁺-ATPase. Once in the cytosol of the follicular cell, iodide is passively translocated across the apical membrane into the colloid in the follicular lumen (4).

There, iodide is oxidized by TPO (thyroid peroxidase) on the thyrosyl residues of the protein thyroglobulin, a process called “organification” or “iodination”. This results in mono- and diiodotyrosines (MIT, DIT). The subsequent “coupling reaction”, also catalyzed by TPO, couples two iodotyrosines to form T3 (MIT+DIT) or T4 (DIT+DIT). In response to demand for thyroid hormone secretion, thyroglobulin is internalized into the follicular cell and digested in lysosomes. Finally, the thyroid hormones are released into the bloodstream (5).

Once released in the circulation, TH are mostly bound to transport or binding proteins such as thyroxine-binding globuline (TBG), transthyretin (TTR) and albumin (HSA, human serum albumin). When the effects of the various binding proteins are combined, approximately 99,97 % of T4 and 99,70 % of T3 are protein-bound (6).

Only the free fraction of TH is supposed to be biochemically active.

Figure 3: Thyroid hormone biosynthesis (adapted from (5))
2.3. Thyroid hormone metabolism, transport and action

T3 is the primary mediator of the biological effects of TH. T4 can be seen as the prohormone for the bioactive T3. Approximately 20% of circulating T3 is released from the thyroid gland (4). The remainder is produced by conversion of T4 to T3, accomplished by selenoprotein iodothyronine deiodinases, a class of enzymes that operate in the circulation as well as intracellular (especially in the liver although iodothyronine deiodinases are expressed in virtually every organ). Three groups of deiodinase can be distinguished: type I deiodinase (D1) and type II deiodinase (D2) are 5’-iodothyronine deiodinases that catalyse TH activation by converting T4 to T3. Type III deiodinase (D3), a 5-iodothyronine deiodinase, is the main TH inactivator through conversion of T4 to rT3 and T3 to T2 (7). D1 is predominantly active in serum, while D2 and D3 act intracellular. The action of the deiodinases is illustrated in Figure 4.

![Figure 4. Action of deiodinases](adapted from (8))
Proper TH action requires more than just an intact TH and adequate TH serum levels. Because action of thyroid hormone takes place intracellular, transport of TH across the plasma membrane is required. This transport occurs in part by diffusion but in some tissues (in particular the brain) active transport using a transmembrane transporter (e.g. MCT8, monocarboxylase transporter 8) is required. In addition, deiodinases can adjust the intracellular thyroid hormone concentration conform the need in that tissue at that moment.

Thyroid hormone action takes place in the nucleus, therefore T3 needs to interact with a specific nuclear thyroid hormone receptor (TR). The T3-TR complex then binds to regulatory regions contained in the genes that are responsive to thyroid hormone. When T3 binds to a TR, a coactivator is recruited to make transcription of a target gene possible. In the absence of T3, a corepressor is recruited, inhibiting transcription of that target gene. Gene transcription is thus modified in the presence of T3, altering rates of protein synthesis and substrate turnover.

3. **TSH and thyroid hormone assays**

Knowledge of the method (or assay) that is used to measure hormones such as TSH, T4 and T3 is important in interpreting (aberrant) TFT.

The majority of TSH assays use an immunometric non-competitive or “sandwich” format with two antibodies: a capture antibody and a (labelled) detection antibody. Those antibodies are directed against different epitopes of TSH, with TSH acting as a bridge between the two antibodies. The capture antibody is immobilized to a solid phase to ensure good separation between bound and unbound label (2).

![Figure 5. Non-competitive “sandwich” assay for TSH measurement](adapted from (9))
In Belgium, TSH is mostly measured by immunometric non-competitive assays produced by Roche (Elecsys, Moderat E, Cobas E) or Siemens (Dimension Vista, Advia Centaur).

Measuring T4 and T3 is more challenging as the levels of free (active) hormone are usually very low compared to the vast excess of protein-bound analyte (< 0.5%). Since T4 and T3 are relatively small molecules, a “sandwich assay” as used for TSH, is not possible. Therefore, competition assays are commonly used for measuring free T3 and T4. The tracer in this assay is labelled T4 (or T3), which competes with serum T4 for a fixed number of anti-T4 antibody binding sites. The equilibrium between bound and unbound T4 is conserved, so that the amount of tracer displaced reflects the free rather than total hormone concentration (2, 9).

The most used assays for measuring fT3 and fT4 in Belgium are produced by Roche. Laboratories still measuring total rather than free T3 or T4 levels are scarce, amongst them is the laboratory of the University Hospital in Leuven.

4. Thyroid dysfunction

4.1. Hypothyroidism

Hypothyroidism is a state of deficiency of thyroid hormone. Worldwide the most common cause is iodine deficiency. In iodine sufficient areas, the most frequent causes for hypothyroidism are chronic autoimmune thyroiditis and iatrogenic causes of hypothyroidism. Other causes of hypothyroidism are acute thyroiditis (a hypothyroid phase is usually seen before reaching euthyroidism) or drugs (e.g. lithium and amiodarone). The clinical picture of hypothyroidism is often rather non-specific with fatigue, decreased appetite, weight gain, decreased energy level, depression, constipation, and cold intolerance being reported frequently.

The prevalence of overt hypothyroidism in the general European population varies from 1 to 5%, affecting more women than men (10). As thyroid hormone levels are insufficient in hypothyroidism, the negative feedback system of the HPT axis causes a rise in TSH to maximally stimulate the thyroid to produce thyroid hormones. Apart from an elevated TSH with low thyroid hormone levels, certain metabolic markers can also raise suspicion for hypothyroidism in patients. Due to decreased clearance of lipids, total and low-density lipoprotein (LDL) cholesterol is elevated in hypothyroid patients. Another metabolic marker for thyroid function is sex hormone binding
globulin (SHBG). A low SHBG is seen in hypothyroid patients, while thyrotoxic patients have higher SHBG (11).

4.2. Hyperthyroidism/thyrotoxicosis

In hyperthyroidism the thyroid gland is hyperactive resulting in inappropriately high synthesis and secretion of thyroid hormones. The main causes for hyperthyroidism are Graves’ disease, multinodular (toxic) goitre and toxic nodule. The clinical picture of hyperthyroidism consists of weight loss, tremor, anxiety, palpitations, heat intolerance, shortness of breath and increased frequency of bowel movements. In Europe the prevalence of hyperthyroidism is 0,3 - 1,3% (10).

Differential diagnosis between hyperthyroidism and thyrotoxicosis from other causes than hyperthyroidism is important. Thyrotoxicosis is a state where inappropriately high tissue thyroid hormones lead to high thyroid hormone action in tissues. Thyrotoxicosis without hyperthyroidism is mostly iatrogenic or caused by acute inflammation of the thyroid gland that leads to excessive release of preformed thyroid hormone, resulting from a form of thyroiditis (painless, subacute or postpartum thyroiditis).

Other (more rare) forms of thyrotoxicosis include factitia, ectopic thyroid tissue (struma ovarii) and thyrotoxicosis after excess iodine ingestion (Jod-Basedow effect, typically seen in patients with multinodular goitre). In gestational thyrotoxicosis a high concentration of HCG, biochemically similar to TSH, is responsible for excessive thyroid stimulation and thus hyperthyroidism. The drug amiodarone induces thyrotoxicosis in 10% of the individuals treated. Another drug that can cause hyperthyroidism is lithium, although hypothyroidism is seen more often.

5. Aberrant thyroid function tests

In most clinical situations, interpretation of thyroid function tests (TFT) is rather straightforward.

Based on the principles of a functioning HPT-axis, a clinician can mostly rely on TSH measurement alone for initial screening for thyroid disease. Only if TSH is abnormal, free T4 (and T3) should be measured to establish the diagnosis. An exception to this rule is pituitary dysfunction, where e.g. a normal TSH can make the clinician assume his patient is euthyroid, while measurement of free TH levels would be low, reflecting secondary hypothyroidism (12).
Apart from pituitary dysfunction, there are some unusual conditions that can lead to an aberrant pattern of TFT, which make it hard to distinguish between euthyroidism and hypo- or hyperthyroidism. Moreover, most of these aberrant patterns of TFT are not necessarily explained by an underlying medical condition but by analytical interference, resulting in “false positive” or “false negative” levels for either TSH, free T4 or free T3. For that reason, results that are clinically inconsistent or deviate from the negative feedback principle should always be confirmed with a further sample (rather than a repeat assay of the same sample) and/or by using another analytical assay (13). Diluting the sample can also be useful, especially in samples with extremely elevated analyte concentrations. Non-linearity of the results upon dilution usually indicates interference. Alternatively, adding heterophilic blocking agents (animal immunoglobulins or commercially available blocking tubes) to the serum can neutralize interfering antibodies (15).

An overview of aberrant patterns of TFT with possible causes is given in the following text.

5.1. TSH normal or elevated, fT3 and/or fT4 elevated

The pattern of normal or elevated TSH with elevated fT3 and/or fT4 is often artefactual.

Before investigating possible analytical interference, confounding drug therapy should always be considered.

- Certain drugs, when administered in high dose, displace T4 or T3 in vitro from its binding protein, resulting in spuriously high results for free T3 or T4. Examples are heparin (both fractionated and unfractionated), furosemide (especially when given intravenously), aspirin, certain NSAID (diclofenac, fenclofenac) and certain anti-epileptic drugs (phenytoin, carbamazepine) (2, 9, 13).

- Amiodarone has pleiotropic effects on thyroid function and TH metabolism. Significant inhibition of type I deiodinase (D1) is an important effect. In the majority of patients, amiodarone causes a transient rise in TSH and a subsequent rise in T4 during the first 3 months of treatment while fT3 is low. Later, TSH returns to normal values (in the absence of amiodarone-induced hypo- or
hyperthyroidism), fT4 decreases but often stays slightly above the reference value. Free T3 remains in normal or low-normal ranges.

- Patients on **thyroxin replacement therapy** after thyroablation (surgery or radioiodine) or because of hypothyroidism can have normal TSH with slightly elevated fT4 and normal fT3 (2).

In these patients however, TSH can be elevated as well, indicating poor compliance: because of different half-lives between TSH and TH, intermittent thyroid hormone ingestion may result in normal or even elevated TH levels, but fails to normalize longstanding elevated TSH (2).

Analytical interference is defined as the effect of a substance present in the sample that alters the correct value of the result (14). Examples of analytical interference leading to normal/elevated TSH with elevated fT4 and/or fT3 are:

- **Heterophilic antibodies** are endogenous antibodies that can bind the assay antibody and interfere with the measurement of the analyte. They should be considered in immunoassays with aberrant TFT since these assays use animal antibodies to bind the analyte. Heterophilic antibodies are weak polyspecific antibodies, they can be divided in three groups: human anti-animal antibodies (HAAA), rheumatoid factors and heterophilic antibodies with unknown exposure to antigen (15).

HAAA are produced as a response to injection of animal antibodies for diagnostic or therapeutic purposes; so called “biologials” using chimeric or humanized anti-animal monoclonal antibodies. Human-anti mouse antibodies (HAMA) are of particular concern since most antibodies in both clinical medicine and immunoassays are derived from mice (15).

Rheumatoid factor is present in 5-10% of the general population and in 70% of patients with rheumatoid arthritis. It is an IgM antibody that can cross-react to assay antibodies (15).

Heterophilic antibodies with unknown animal exposure are present in 40% of the general population, however only 0,05-0,5 % will create interference in immunoassays.
In immunometric “sandwich” assays (e.g. TSH assay) heterophilic antibodies can cross-link the assay antibodies, forming a bridge between the capture and the detection antibody in the absence of the analyte, generating a false assay signal.

- **Presence of streptavidin-antibodies** and ingestion of biotin can result in spuriously high TH levels (as well as low TSH levels), see section 5.2 for further details.

- **Anti-iodothyronine auto-antibodies** (anti-T3 and/or anti-T4 antibodies). The presence of these auto-antibodies that bind the tracer used in the assay for measuring T3 or T4 results in spuriously high or spuriously low results of free T3 or T4, depending on the type of assay used. In this situation, the free T3 and T4 assay are widely discordant with each other or with the clinical status. Interestingly, anti-TPO and/or anti-thyroglobulin antibodies are almost invariably present in these patients, which could be of some diagnostic value (12). The occurrence of anti-iodothyronine auto-antibodies is estimated to be 1.8% in healthy subjects and 5% in patients with thyroid disease (16).

- **Macro-TSH** can lead to spuriously high values for TSH due to autoantibodies that create TSH-anti-TSH IgG complexes. PEG precipitation can reveal this problem.

- **Anti-TSH antibodies** are rare.

After exclusion of both confounding drugs and analytical interference (e.g. by using another platform, performing linear dilution or use of heterophilic blocking agents to exclude heterophilic antibodies), some important pathologic causes for normal or elevated TSH with elevated TH should be considered:

- Patients with **familial dysalbuminaemic hyperthyroxinaemia** (FDH) have a dominantly inherited genetic variant of albumin with altered affinity for iodothyronines. They are clinically euthyroid but their total T4 is elevated due to increased binding of T4. The labelled tracer used in a competition assay for T4 is also more bound with higher affinity for albumin, resulting in less tracer available for competition with non-albumin bound T4 resulting in spuriously high free T4 levels. To overcome this problem, equilibrium dialysis is recommended. FDH can be confirmed by genetic analysis.
• Central hyperthyroidism secondary to a TSH-secreting pituitary tumour (TSHoma) is a rare disorder with an incidence of 1 per million (2). Clinical evaluation looking for possible presence of neurological signs and symptoms of expanding tumour mass (visual defects, headache) or clinical features of concomitant hypersecretion of other pituitary hormones (acromegaly, galactorrhea, amenorrhea) can give an important clue. Biochemically, besides elevated free T3 and T4 with a non-suppressed TSH, an elevated glycoprotein hormone α-subunit/TSH molar ratio can be seen, however this ratio can also be elevated in non-functioning and GH-secreting pituitary tumours as well as in postmenopausal women. Imaging of the pituitary (preferably MRI) can reveal a lesion responsible for TSH hypersecretion. TSH response to TRH administration will be blunted or even absent, as TSH secretion is autonomous. This autonomous secretion will even remain after T3 administration following a protocol for T3 dosing during a period of ten days (12, 17). As mentioned above, SHBG is a valuable tissue marker of thyroid hormone exposure. This protein will be elevated in TSHoma, reflecting too high peripheral tissue thyroid hormone exposure. An exception is patients who take synthetic oestrogen therapy (SHBG will be high apart from thyroid dysfunction) or in patients with liver disease. Other markers such as ferritin, serum total cholesterol, LDL and creatine kinase are of limited help (12, 17).

• Resistance to thyroid hormone (RTH) is another rare disorder with an incidence of 1 per 40 to 50 000 living births. The nomenclature can be confusing since this disorder is linked to a genetic mutation in one of the genes for thyroid hormone receptor (THR): THR-α or THR-β gene. The term “RTH” is often used referring to the clinical and biochemical picture of a THR-β mutation with elevated TSH and elevated TH, although “RTH-β” would be a more correct term (See section 7 for further details). Seventy five percent of RTH-β cases are dominantly inherited; so similar TFT in first-degree relatives is highly suggestive for this condition. Normally, imaging of the pituitary reveals no lesions in RTH-β. However, as in the general population (prevalence 10,6 % (18)), a pituitary incidentaloma can
occur in a patient with RTH-β. Furthermore, diagnostic confusion can occur since thyrotrophic hyperplasia and pituitary enlargement is possible after chronic noncompliance in concomitant primary hypothyroidism or following inappropriate thyroid ablation in RTH-β (2).

A normal glycoprotein hormone α-subunit/TSH molar ratio is seen in RTH-β. Patients with RTH-β have a normal or exaggerated TSH response to TRH administration; they show TSH suppression after T3 administration following a protocol for T3 dose during a period of ten days (12, 17).

SHBG should be normal in patients with RTH-β since there is no noted high thyroid hormone exposure in THR-β expressing tissues.

• **Defects in the transmembrane transporter** responsible for transporting TH into the cell can cause a similar aberrant pattern of TFT. A genetic mutation in the gene for monocarboxylate transporter 8 (MCT8) causes a recessive X-linked defect that affects males while females are carriers. As a consequence of this defect, affected males have a complex and severe neurodevelopmental phenotype with pathognomonic thyroid tests including high serum T3, low rT3, reduced or low normal T4 and normal or slightly elevated TSH (7). This condition belongs to the “syndrome of reduced sensitivity to thyroid hormone” and will be treated more detailed in section 7.1.

• **A thyroid hormone metabolism defect** caused by a genetic mutation in the protein that is responsible for inserting selenocystein into selenoproteins such as iodothyronin deiodinases can cause a similar pattern of TFT as well. Patients with this mutation have a complex clinical phenotype (delayed growth and puberty are mostly present), associated with high T4, low T3, high rT3 and slightly elevated TSH (7). This condition also belongs to the “syndrome of reduced sensitivity to thyroid hormone” and will be treated more detailed in section 7.2.

• **Acute psychiatric illness.** Being a form of non-thyroidal illness, one would expect low TH levels, though a specific pattern of raised TSH with raised TH can be seen in certain acute psychiatric illnesses: schizophrenia, affective psychosis and amphetamine abuse. This effect rarely persists beyond day 14 (12).

• Finally, in the **neonatal period**, an elevated TSH with elevated TH can also be seen. In a healthy term baby, secondary to the exposure to a colder environment
and clamping of the umbilical cord, TSH rises quickly after delivery. A subsequent rise in T3 and T4 is seen in the first 24-36 hours. The level of TSH, T3 and T4 then gradually decreases in the first four weeks of life, to remain at a higher level than adults during the first two years of life.

5.2. **TSH normal or low, fT3 and/or fT4 low**

In contrast to the previous section, this pattern of TFT is rarely a consequence of lab interference.

- **Certain drugs** can cause central hypothyroidism through inhibition of TSH secretion. This is the case for glucocorticoids (at a dose of ≥ 20 mg prednisone or equivalent), dopamine agonists, somatostatin analogues and bexarotene (a retinoid used in the treatment of cutaneous T-cell lymphoma). TSH as well as TH levels can be normal or low.

- **Patients who had recent treatment for hyperthyroidism** (e.g. methimazole for Graves’ disease) can have a TSH that is still supressed while thyroid hormones are already normal or even low. An unsuspecting clinician could assume the patient is still thyrotoxic, when in fact the patient is already hypothyroid. This TSH can remain supressed up to 2 to 3 months after start of treatment (12).

- **Secondary (or central) hypothyroidism due to pituitary disease** is an important diagnosis since concomitant hypoadrenalism could be life threatening. Local pressure effects of a pituitary tumour should also be considered in these cases. Note that measuring only TSH in these patients can lead to misdiagnosis of euthyroidism.

- **Non-thyroidal illness** is the finding of abnormal TFT in people who are seriously ill (mostly patients on intensive care unit). The most common combination in this setting is a low free T3 with a TSH in the normal range. Since a conclusive statement about thyroid function in these patients cannot be made, TFT should be repeated after recovery from the acute illness.

- **Heterophilic antibodies** also occur in competitive assays (e.g. free T4 or free T3 assay), although this is less common than in immunometric sandwich assays. They can result in spuriously low free T4 and/or free T3 by binding of the label antibody.
• Ingestion of biotin (vitamin B7) can also cause an aberrant pattern of TFT. Because of the high affinity of streptavidin for biotin, many immunoassays use streptavidin-biotin complexes (e.g. streptavidin-coated particles as capture antibody and biotinylated labelled detection antibody). In patients taking supplements of biotin, binding of biotin with the streptavidin-coated antibody can occur, this means the analyte will not bind with the detection antibody, leading to spuriously low values for the analyte in sandwich immunoassays (e.g. TSH) and spuriously high values in competitive assays (e.g free T3, free T4). This effect can already occur from daily doses of biotin starting from 5 mg a day (19).

• Anti-streptavidin antibodies are directed against the streptavidin-coated particles used in different immunoassays. As in biotin use, they can result in spuriously low values for TSH in sandwich immunoassays. However they seem to have a more pronounced effect in in competitive immunoassays, where they induce spuriously high values for free T4 or free T3 (19).

• Congenital TRH- and TSH-deficiency are both very rare thyroid disorders, the diagnosis is usually made in infancy.

Figure 6: Differential diagnosis of abnormal TFT
6. Case Report: continued
We performed thyroid function tests of our patient on another analytical platform, but these results were similarly aberrant. Theoretically, we could have considered dilution of the sample and blocking of heterophilic antibodies, but this was not performed.
A TRH-test showed increased response of TSH after administration of thyrotropin releasing hormone, this response was congruent with the elevated basal TSH, making central hyperthyroidism due to a TSH-secreting pituitary tumour unlikely. At this point, suspicion for resistance to thyroid hormone (RTH-β) was raised.

7. Reduced sensitivity to thyroid hormone
In 1967, dr. Samuel Refetoff described a clinical syndrome in three siblings with high peripheral TH levels without clinical symptoms of TH excess or even with symptoms of TH deficiency. In 1989, he discovered this syndrome was due to inactivating mutations in THR-β, the gene encoding for one of the thyroid hormone receptors. The syndrome was called “resistance to thyroid hormone” (also: “Refetoff syndrome”). Between 2004 and 2012, three more genetic defects causing reduced sensitivity to thyroid hormone were discovered, involving thyroid hormone transport, metabolism or action. Since then, the term “resistance to thyroid hormone” was broadened to “reduced sensitivity to thyroid hormone” since defects in TH metabolism or transport reduce the effectiveness of thyroid hormone rather than causing any resistance. The coexistence of cell-specific thyroid hormone deprivation and excess is characteristic in these syndromes (20).
To understand the nature of these syndromes, it is important to remember that normal TH action is not only dependent on adequate serum TH concentration, regulated by the hypothalamic-pituitary-thyroid axis, but also on adequate signalling at the cellular level.
7.1. **Thyroid hormone cell membrane transport defect**

The prototype for transmembrane thyroid hormone transport is monocarboxylate transporter 8 (MCT8).

SCL16A2 is the gene for MCT8; it is located on the X chromosome. Mutations in this gene cause severe psychomotor retardation and typically disturbed TFT in affected males. Female carriers are asymptomatic and have TFT that are intermediate between unaffected family members and affected males.

After the clinical picture of MCT8 mutations was described in 2004, it was clear that this mutation is the genetic basis of Allan-Herndon-Dudley syndrome, the first X-linked mental retardation syndrome described in 1944 (1).

The psychomotor retardation in affected males is characterized by severe cognitive impairment, poor head control and failure to thrive. Diagnosis is usually made during childhood. Truncal hypotonia and feeding problems become apparent in the first 6 months of life. Flaccidity progresses to limb rigidity and later to spastic quadriplegia. Most patients are unable to walk, stand or sit independently and do not develop speech (20).

Affected males are infertile, however MCT8 mutations are maintained in the population due to carrier females who are asymptomatic and can reproduce.
TFT in these patients show typically elevated serum total and free T3, rT3 is reduced. T4 is reduced in most cases and TSH can be slightly elevated but rarely above 6 mU/L (20).

This syndrome is rare, with only over 300 individuals in 90 families that have been reported with pathogenic mutations in MCT8 (1, 7, 20).

7.2. Thyroid hormone metabolism defect

Metabolism of thyroid hormone is controlled by deiodinases, a group of selenoproteins containing the rare amino acid selenocysteine (Sec) in their catalytic domain. Incorporation of Sec into deiodinase is a process requiring selenocysteine insertion sequence-binding protein 2 (SBP2).

A mutation in the SECISBP2 gene leads to a defective SBP2, resulting in defective synthesis of deiodinase. In 2005, a syndrome with growth retardation, (mild) mental and motor retardation, muscle weakness, hypoglycemia, impaired hearing, infertility and typically disturbed TFT was ascribed to mutations in SBP2 (1).

Elevated serum total and free T4 and rT3 levels are found, together with low to low-normal serum T3 levels and normal to slightly elevated serum TSH levels.

With only 11 patients of 8 families, this syndrome is even more rare than the Allan-Herndon-Dudley syndrome. Since most of the patients are children, the natural course of this syndrome is still unknown (1, 7).

7.3. Thyroid hormone action defect (“resistance to thyroid hormone”)

The principal and best-studied effect of T3 is its genomic effect. After translocation in the nucleus, T3 interacts with nuclear T3 receptors (TR) to activate or repress transcription of target genes.

Different types of TR exist: TRα and TRβ, encoded by genes THR-α and THR-β, located on chromosomes 17 and 3 respectively. Alternative splicing and promotor usage results in 2 major isoforms of TRα (TRα1 and TRα2) and TRβ (TRβ1 and TRβ2) (20).

Since TRα2 does not bind TH due to absence of a ligand-binding domain (LBD), only 3 thyroid hormone receptors play a role in binding T3: TRα1, TRβ1 and TRβ2. TRα1 is predominantly expressed in central nervous system, bone, GI tract and heart; TRβ1 in thyroid, liver and kidney; TRβ2 in pituitary, cochlea and retina. Genetic mutations
in each of those receptors lead to different syndromes with clinical symptoms dependent on the relative expression of these TH receptors in different tissues.

### 7.3.1. Mutations in THR-α: RTH-α

In 2012, a syndrome of delayed bone development, growth retardation and mild motor and mental retardation was linked to a mutation in THR-α, resulting in a mutant TRα1. Thyroid function tests in this syndrome show a normal TSH with high fT3 and low fT4 and rT3 levels. An elevated T3/T4 ratio and low serum rT3 are the hallmarks of diagnosis. Symptoms that occur in patients with THR-α mutations such as delayed bone age, motor or mental retardation and constipation can be explained by the fact that T3 activity in bone, brain and GI tract is reduced due to a mutant TRα1. Altered expression of deiodinases is also suspected in this syndrome, which results in increased D1 and decreased D3 activity, explaining high T3 and low rT3 levels in these patients.

![Figure 8: Overview of tissues and homeostatic functions affected in RTH-α](adapted from (21))
7.3.2. Mutations in THR-β: RTH-β

Mutations in THR-β have been recognized since 1986. They are the most prevalent causes for reduced sensitivity to thyroid hormone and have been described in more than 3000 patients belonging to more than 350 families (1, 7).

Characteristic of this syndrome is the relative sparseness of clinical symptoms. Diffuse goitre is the most common finding, reported in 66-95%. Sinus tachycardia is also reported often and together with goitre, this could lead to the erroneous diagnosis of autoimmune/toxic goitre. Learning disability can be seen but real mental retardation (IQ <60) is rare (20). Some patients also have affected vision and hearing.

Since TRβ2 is expressed in the pituitary, an impaired negative feedback of the HPT axis will lead to normal to slightly increased TSH, high T4 and high rT3 with normal or elevated T3 levels (21). Thyroglobulin levels also tend to be high as a result of the TSH-induced thyroid gland hyperactivity. Patients with RTH-β secrete a form of TSH rich in sialic acid with a higher bioactivity, leading to goitre despite normal (or only slightly elevated) TSH levels (1).

A pathogenic mutation in the THR-β gene confirms diagnosis of RTH-β. However, in 15% of patients with clinical and biochemical findings suggestive for RTH-β, no mutation in THRB can be detected, a phenomenon called non-thyroid hormone receptor RTH (NT-RTH).

The discrete clinical picture of RTH-β makes early diagnosis challenging. Severe resistance with growth or mental retardation, learning disability, attention deficit usually leads to medical attention early in life, while mild resistance with only goitre and TFT that are still more or less in the reference range does not and is often coincidentally discovered at adult age. As symptoms as hyperactivity tend to improve with age, most patients with RTH-β lead a normal life at the expense of high TH levels and a slight thyroid gland enlargement.

Awareness of this entity is however important as misdiagnosis can lead to inappropriate treatment such as radioiodine (I-131) and thyroidectomy (20, 22).

The combination of the higher prevalence compared to the other syndromes of reduced sensitivity to thyroid hormone and their discrete clinical picture, delaying the diagnosis until adult life, makes RTH-β an important player in the differential diagnosis of aberrant thyroid function tests, next to analytical interference, drugs, etc… (see section 5).
Figure 9: overview of tissues and homeostatic functions affected in RTH-β (adapted from (21))

Table 3: Comparison of TFT abnormalities in syndromes of reduced sensitivity to thyroid hormone

No = normal; sl = slightly

<table>
<thead>
<tr>
<th>Gene involved</th>
<th>fT4</th>
<th>fT3</th>
<th>rT3</th>
<th>TSH</th>
<th>Principal clinical manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCL16A2</td>
<td>↓</td>
<td>↑↑</td>
<td>↓</td>
<td>↑</td>
<td>No or sl ↑ Psychomotor retardation</td>
</tr>
<tr>
<td>SECISBP2</td>
<td>↑↑</td>
<td>↓</td>
<td>↑↑</td>
<td>↑↑</td>
<td>No or sl ↑ Growth retardation</td>
</tr>
<tr>
<td>THR-α</td>
<td>↓</td>
<td>No or ↑</td>
<td>↓</td>
<td>No</td>
<td>Growth retardation, delayed bone development</td>
</tr>
<tr>
<td>THR-β</td>
<td>↑↑</td>
<td>No or ↑</td>
<td>↑↑</td>
<td>No or sl ↑ Goiter, sinus tachycardia</td>
<td></td>
</tr>
</tbody>
</table>

In this patient with repeated measurement of elevated TSH and TH that could not be explained by confounding drugs or analytical interference, genetic analysis for RTH was performed. This analysis revealed a causative mutation in the thyroid hormone receptor β (THR- β) gene (c.959G>A; p.R320H), confirming the diagnosis of RTH-β.

The patient was treated with L-thyroxine (LT4) in incremental doses. At a dose of 150 µg a normalization of TSH (0.97 mU/L) was seen whereas TH levels remained elevated. The clinical improvement was modest (less anxiety, less constipation).

9. Conclusion

This case report showed us the importance of being aware of aberrant patterns of TFT. Recognizing them and revealing the cause of the aberrant pattern is important to avoid misdiagnosis. Crucial in the workout of aberrant patterns of TFT is to find out whether the tests are falsely disrupted or can be explained by an underlying pathology.

Excluding confounding drugs, previous treatment and intercurrent illness is a first step. Next, analytical interference should always be considered. Finally, rare genetic and acquired disorders of the HPT-axis should be taken into account as a possible explanation.
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Nederlandstalige samenvatting
Schildkliertesten behoren tot de meest aangevraagde laboratoriumtesten. Als endocrinoloog zijn we grotendeels afhankelijk van de laboratoriumresultaten omdat deze ons vaak in een bepaalde richting doen denken die, in combinatie met het klinisch beeld waar de patiënt zich mee presenteert, leiden tot een diagnose.

De patiënt die we in deze tekst voorstellen presenteerde zich met verschillende klachten die niet duidelijk konden gelinkt worden aan haar afwijkende schildkliertesten. De zoektocht naar een verklaring voor deze testen leerde ons dat het belangrijk is om, naast een brede differentiaal diagnose van mogelijke pathologieën, de beperkingen van laboratoriumresultaten te kennen.

Deze beperkingen kunnen namelijk leiden tot een vals resultaat en zo de aanvragend arts op een verkeerd spoor zetten. In deze tekst wordt, vertrekkende vanuit het verloop van deze patiënt, een overzicht gegeven van mogelijke verklaringen voor afwijkende schildkliertesten. Belangrijk is te vermelden dat we het hierbij enkel hebben over schildkliertesten die buiten het spectrum van euthyroidie, hypothyroidie of hyperthyroidie vallen. De schildkliertesten bij eu-, hypo- of hyperthyreote patiënten zijn immers “eenvoudig” te verklaren aan de hand van de werking van de hypothalomo-hypofysaire-schildklieras, die berust op een negatief feedback systeem. Hierbij gaan kleine veranderingen in schildklierfunctie (T3, T4) gepaard met duidelijke wijzigingen in de TSH-concentratie.

Een verhoogd of normaal TSH met verhoogde perifere schildkliertesten (T3, T4) heeft vaak te maken met medicatie of labo-interferentie. Voorbeelden hiervan zijn macro-TSH, biotine innname, streptavidine-antilichamen, heterofiele antilichamen, antilichamen tegen iodotyronines (anti-T3 of anti-T4) of tegen TSH. Onderliggende pathologieën die deze schildkliertesten ook kunnen verklaren zijn familiale dysalbuminemische hyperthyroxinemie (FDH), een TSH-secreterend hypofysair adenoom of een vorm van “reduced sensitivity to thyroid hormone”, waarbij de zogenaamde schildklierhormoonresistentie (“resistance to thyroid hormone-β”, RTH- β) de meest voorkomende is. Ten slotte kan dit patroon van afwijkende schildkliertesten ook voorkomen bij psychiatrische ziekten (voornamelijk de eerste 14 dagen) en tijdens de neonatale periode.

Een laag of normaal TSH met lage perifere schildkliertesten kan verklaard worden door medicatie die inhibitie van TSH-secretie veroorzaakt (vb.
Recent na opstarten van een behandeling voor hyperthyroïdie kan TSH eveneens verlaagd blijven terwijl de perifere schildkliertesten reeds verlaagd zijn. Centrale hypothyroidie en “non-thyroidal illness” kunnen eveneens dit patroon van schildkliertesten veroorzaken. Labo-interferentie speelt hier minder vaak een rol, maar toch kunnen ook hier biotine inname, streptavidine-antilichamen, of heterofiele antilichamen de resultaten verstoren. TRH- en TSH-deficiëntie zijn zeldzame oorzaken van dit soort testen die meestal al van jongsaf aan gekend zijn.

De patiënt in onze casus nam geen interfererende medicatie en vertoonde gelijkaardig gestoorde schildkliertesten op andere platformen, zodat we labo-interferentie naar alle waarschijnlijkheid konden uitsluiten. Een TRH-test toonde toegenomen respons van TSH, zodat ook een TSH-producerend adenoom uitgesloten werd. De verdenking op RTH-β werd bevestigd door het uitvoeren van een genetische analyse die een pathogene mutatie in het THR-β gen aantoonde. Een behandeling met L-thyroxine werd opgestart, met voorzichtige verbetering van de klachten tot gevolg.

Deze casus en de uitwerking ervan toont aan dat afwijkende schildkliertesten altijd gecorreleerd moeten worden met het klinisch beeld en dat eerst aan labo-interferentie of interfererende medicatie dient gedacht te worden alvorens uit te werken voor een genetische of verworven aandoening van de hypothalamo-hypofysaire-schildklieras, zoals schildklierhormoonresistentie.