DEALING WITH 'INAPPROPRIATE' HIGH TSH

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Introduction

Thyroid function tests (TFTs) are among the most commonly requested laboratory investigations. Fortunately, in most patients the interpretation of TFTs is straightforward with the combination of thyroid hormone [TH – thyroxine (T4); triiodothyronine (T3)] and thyrotropin (TSH) measurements confirming euthyroidism, thyrotoxicosis or hypothyroidism consistent with their clinical status. However, in a small but significant group of contexts the laboratory results either do not 'fit' with the clinical picture and/or form an unusual, non-physiological pattern – so-called 'funny', 'perplexing' or 'weird' TFTs. Establishing the correct diagnosis in these cases is dependent on careful clinical assessment combined with focused laboratory, radiological and genetic testing – failure to adopt a structured approach may result in incorrect diagnosis/inappropriate management.

HPT axis physiology and TH action

The production of TH is stimulated by pituitary TSH whose synthesis is regulated by hypothalamic thyrotropin releasing hormone (TRH). In turn, T4 and T3 inhibit TRH and TSH production by negative feedback, thus establishing an equilibrium: this 'set point' of the HPT axis is tightly regulated within an individual, but varies between subjects, likely reflecting genetic and other factors. The actions of TH are mediated by two genes (*THRA*, *THRB*), which encode three nuclear receptor subtypes with differing tissue expression (TRα1: central nervous system, cardiac & skeletal muscle; TRβ1: liver and kidney; TRβ2: pituitary and hypothalamus). At a pre-receptor level, cellular deiodinases (DIO) mediate hormone metabolism: in the hypothalamus and pituitary, type 2 deiodinase (DIO2) converts T4 to T3; hepatic type 1 deiodinase (DIO1) mediates peripheral hormone conversion, contributing significantly to circulating T3 levels; in contrast, type 3 deiodinase (DIO3) converts T4 to inactive metabolites [reverse T3 (rT3) and T2], thus limiting TH action. Recently, membrane proteins [e.g. monocarboxylate transporter 8 (MCT8)] have been shown to mediate cellular influx/efflux of TH.

Thyroid function test patterns

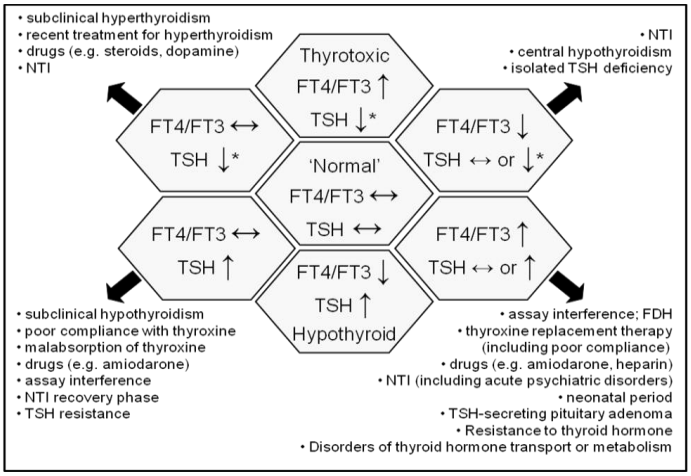

Fig. 1 *Schematic representation of different patterns of TFTs and their causes. Key: FDH, familial dysalbuminaemic hyperthyroxinaemia; NTI, non-thyroidal illness; *signifies that TSH may be either fully suppressed or partially suppressed.*

The typical thyroid function test (TFT) profiles that are seen in classical thyrotoxicosis and hypothyroidism are illustrated schematically in Fig. 1, together with various deviations from these patterns and possible causes. A detailed discussion of all these anomalous TFT profiles is beyond the scope of this session, and the reader is directed to other resources [e.g. *Association for Clinical Biochemistry/ British Thyroid Association/British Thyroid Foundation joint guidelines*; *Thyroid Disease Manager*] for a more general discussion of how to investigate/manage discrepant TFTs. Here, we focus on the differential diagnosis of conditions associated with elevated thyroid hormones (T4 and/or T3) together with non-suppressed or inappropriate TSH levels.

Raised T4/T3 with non-suppressed TSH

1. Altered serum binding proteins

Quantitative: Thyroid hormones circulate bound to carrier proteins [thyroxine binding globulin (TBG), albumin, transthyretin (prealbumin)] and increased concentrations of these can result in elevated Total T4 or T3 measurements. Pregnancy, oestrogens (oral contraceptive, HRT, tamoxifen) and hepatic disorders, or rarely hereditary TBG excess, can raise TBG levels. However, the advent of assays which measure non-protein bound or free TH (FT4, FT3) has largely eliminated this problem, although there are still situations in which misleading results can arise (see below).

Qualitative: Dominantly-inherited genetic variants of albumin, e.g. familial dysalbuminaemic hyperthyroxinaemia (FDH) [or transthyretin: transthyretin-associated hyperthyroxinaemia (TTR-AH)], which alter its affinity for iodothyronines, can cause FT4 (and less frequently FT3) to be overestimated, particularly in 'one-step' analogue hormone assays; even some 'two-step' assays are susceptible to interference. Hormone measurement by equilibrium dialysis can circumvent this (see below). Genetic diagnosis of FDH is facilitated by its common association with a restricted repertoire (e.g R218H/P, L66P) of albumin gene mutations.

2. Assay interference

TSH measurement: the majority of commercially available TSH assays use a non-competitive or 'sandwich' format with two antibodies – capture and (labeled) detection – directed against different epitopes on TSH, with the TSH moiety essentially acting as a bridge between the two (Fig. 2a). The capture antibody is typically immobilised to a solid phase to ensure good separation between bound and unbound label, thus increasing sensitivity; several different detection antibodies may also be employed to further improve assay sensitivity.

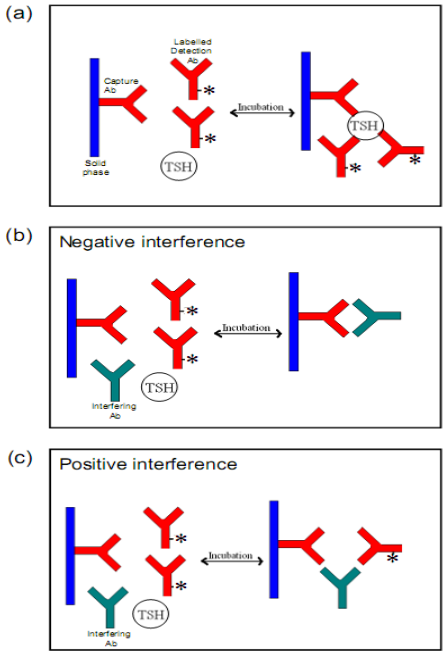

Fig. 2 *Potential mechanisms of TSH assay interference.*

The presence of human anti-animal antibodies (HAAs) in a patient's serum can interfere with TSH measurement if directed against the same species as the assay antibodies: thus, a HAA that blocks TSH binding to either capture or detection antibodies will result in 'negative interference', causing a falsely low TSH readout (Fig. 2b); conversely, a HAA that is capable of cross-linking the capture and detection antibodies may cause 'positive interference', leading to a falsely high TSH (Fig. 2c).

Many manufacturers include panels of antigens or pre-immune serum from source animals to 'mop up' HAAs. However, heterophile antibodies (which are weak polyspecific antibodies that are similarly capable of causing negative or positive interference) can prove more difficult to remove. Such interference in the TSH assay can be seen in cases of Graves' Disease and in patients with positive rheumatoid factor (RhF).

Interfering antibodies may also bind the analyte (TSH) rather than the assay antibodies. An extreme example of this type of interference is the 'macro hormone' complex, in which a specific anti-TSH immunoglobulin binds TSH and neutralizes its biological activity, but leaves epitopes exposed for interaction with the assay antibodies. The consequence is analogous to the high prolactin levels seen in patients with macroprolactinaemia.

If interference is suspected it is best to seek the advice of the laboratory as there are several ways to confirm this such as showing:

- discordant TSH results in an assay that utilizes different antibody pairs
- altered TSH result following immunosubtraction [using polyethylene glycol (PEG) or Protein G/A]
- non-linear TSH measurement following sample dilution: if either TSH or the assay reagents are weakly bound by interfering antibodies this interaction may be disrupted by dilution and a non-linear dilution series will result.

T4 and T3 measurement: determination of FT4 (and FT3) is particularly challenging as the assay must detect very low levels of 'free' hormone relative to a vast excess of protein-bound analyte (>99.5%). The relatively small size of T4 (and T3) precludes use of a 'sandwich' assay format, so 'competition assays' are commonly used; here, labeled T4 (the tracer) competes with serum T4 for a fixed number of anti-T4 antibody binding sites. Free hormone assays are designed such that the equilibrium between T4 and it's binding proteins is conserved during measurement, so that the amount of tracer displaced reflects the 'free' rather than 'total' hormone concentration. Clearly the presence of factors in serum which affect this equilibrium will confound hormone measurement. Examples include:

- both fractionated and unfractionated heparin can cause an artifactual elevation in measured concentrations of FT4/FT3 by displacement of T4 and T3 from their carrier proteins. The mechanism is poorly understood, but is likely to involve generation of free fatty acids (FFAs) via heparin-mediated activation of endothelial lipoprotein lipase (LPL), with FFAs displacing T4 from albumin. The extent to which FFAs rise is variable and, as displacement continues *in vitro*, pre-analytical delay can compound the situation. Thyroid function testing prior to administration of heparin or by

measurement of total rather than free T4 (as the artifact is secondary to hormone displacement) can circumvent the problem

- antiiodothyronine antibodies which can bind the tracer
- HAAs or heterophile antibodies that block the assay antibody.
- variant thyroid hormone binding proteins (e.g. albumin in FDH) with altered affinity for T4.

The use of a 'two-step' ('back titration') assay method, with a wash step prior to tracer addition, may reduce but not completely eliminate such interference. If erroneous results are suspected with a 'one-step' assay, then re-measurement using a 'two-step' assay would be a logical step. If the problem persists, hormone measurement following equilibrium dialysis (ED) remains the gold-standard for eliminating FT4 assay interference.

3. Thyroxine therapy and poor compliance

Optimised physiological thyroxine replacement therapy in primary hypothyroidism is sometimes associated with slightly elevated FT4 and normal TSH, but FT3 levels which are usually normal. This abnormal pattern has been ascribed to the differential action of central (DIO2) versus peripheral (DIO1) deiodinases in mediating T4 to T3 conversion.

Poor compliance with thyroxine replacement is a relatively common cause of anomalous TFTs: hormone ingestion shortly before testing may yield normal or even elevated TH levels, but is insufficient to restore TSH to normal. A high TSH–normal FT4 pattern then prompts an escalation in thyroxine dose. Eventually, intermittent compliance with thyroxine in supraphysiologic dosage can normalize TSH levels, but raises the possibility of Resistance to Thyroid Hormone (see below). Other causes of supraphysiologic thyroxine requirement to normalise TSH in hypothyroidism include malabsorption (e.g. occult coeliac disease, cholestyramine, iron) and accelerated hormone metabolism (e.g. phenytoin, carbamazepine).

4. Drug treatment

***Amiodarone*:** the pleiotropic effects of amiodarone on thyroid function include significant inhibition of DIO1 and hepatic T4 to T3 conversion. Patients on amiodarone alone, or in combination with exogenous thyroxine, can exhibit elevated FT4 with normal TSH, but FT3 levels are usually normal.

Other agents: propylthiouracil, glucocorticoids, propranolol and some iodinated contrast media or iodine-containing supplements/non-prescription medications can also diminish T4 to T3 conversion via a similar mechanism to amiodarone.

5. Non-thyroidal illness

Raised TH levels with non-suppressed TSH are a recognized pattern during non-thyroidal illness (NTI) including acute psychiatric states, but the abnormalities (which reflect a secondary adaptive response rather than primary hypothalamic-pituitary thyroid dysfunction) usually revert with recovery. Where available, measurement of TH levels on a sample taken just prior to/at the onset of NTI may confirm previously normal thyroid status, strongly suggesting this diagnosis.

6. Resistance to Thyroid Hormone versus TSH-secreting pituitary tumour

Clinical features: RTH and TSHoma occur in patients of a similar age range and either gender. A subset of patients with predominant central/pituitary resistance to thyroid hormone (PRTH) exhibit thyrotoxic symptoms and signs, such that these features are not discriminatory.

***Imaging*:** An obvious lesion (macroadenoma) on magnetic resonance imaging (MRI) or computed tomography (CT) can be diagnostic; however, potential 'pitfalls' include: the rising incidence of TSH-secreting microadenomas (perhaps due to earlier diagnosis of this disorder) and dynamic MRI and/or octreotide scintigraphy may be required to visualize these; conversely, as in other contexts, patients with RTH do harbour 'incidental' abnormalities on imaging, causing diagnostic confusion; finally, persistently elevated TSH levels, either in the context of chronic non-compliance in hypothyroidism or following thyroid ablation in RTH, results in thyrotroph hyperplasia and pituitary enlargement which is reversible.

***Serum α-subunit*:** Excess serum levels of pituitary glycoprotein α-subunit (α-SU) are associated with TSHomas, but also found in non-functioning and GH-secreting pituitary tumours. Furthermore, normal α-SU levels, but an elevated α-SU/TSH molar ratio (>1.0), are a recognized finding in TSH-secreting microadenomas; however, the latter needs careful interpretation with clinical context – elevated molar ratios are seen in some normal subjects, especially postmenopausal women.

Tissue markers of TH action: Amongst various peripheral markers of TH action, basal levels of serum sex hormone binding globulin [(SHBG) – analysed using age and gender-specific reference ranges] are the most discriminatory, being elevated in TSHoma and normal in RTH. Falsely low SHBG levels can occur in mixed GH/TSHoma due to inhibition of its synthesis by growth hormone; conversely, synthetic oestrogen therapy in RTH can falsely elevate SHBG. Basal levels of other biochemical markers (e.g. serum cholesterol, creatine kinase) are less useful, but comparison of changes in these parameters following T3 administration (see below) with reference data from normal subjects can discriminate refractoriness or resistance to hormone action.

***Dynamic testing*:** 80 to 90% of patients with TSHoma show a blunted or absent TSH response (TSH increment <150% of baseline) following TRH stimulation; conversely, in RTH the TSH response is either preserved or even exaggerated. Protocols of T3 administration to suppress TSH secretion involve giving either a fixed (100 mcg/day) or graded doses (50 then 100 then

200 mcg/day each for 3 days) of liothyronine (L-T3) over a ten day period, with TRH testing and measurement of other biochemical markers of thyroid hormone action. TSH secretion in pituitary tumour patients remains autonomous, while in RTH there is qualitatively normal inhibition of TSH secretion, but the degree of suppression is not complete. An acute octreotide test (100 mcg s.c.) reduces TSH, FT4 & FT3 levels in both RTH and TSHoma patients, but chronic administration of long-acting somatostatin analogue maintains reduced TH levels in TSHoma, whereas subjects with RTH are refractory.

***TFTs in relatives*:** 75% of RTH cases are dominantly-inherited, with the remainder being caused by sporadic, 'de novo' gene defects. Thus, a similar pattern of abnormal TFTs in first-degree relatives strongly suggests RTH.

Genetic testing: RTH is associated with *THRB* gene defects and their identification by gene sequencing can confirm the diagnosis; however, ~15% of RTH cases are not associated with *THRB* mutations, such that absence of an abnormality in this gene does not exclude the diagnosis.

7. Other genetic disorders of TH transport or metabolism

(i) MCT8 mutations: An X-linked disorder of childhood-onset, with psychomotor retardation including speech and developmental delay and spastic quadriplegia, is caused by defects in the *MCT8 (SLC16A2)* gene, encoding a membrane transporter. In addition to neurological abnormalities, male patients exhibit a characteristic pattern of abnormal TFTs with elevated FT3, low FT4 and normal TSH levels.

(ii) Functional deiodinase deficiency: The deiodinase enzymes are part of a larger family of 25 human proteins containing selenocysteine. Recently, a multisystem selenoprotein deficiency disorder, manifesting with growth retardation in childhood or other features (male infertility, skeletal myopathy, photosensitivity, hearing loss) in adults, has been associated with a thyroid signature – raised FT4, normal/low FT3 and normal TSH levels, due to functional DIO deficiencies.

An algorithm summarizing the approach to investigation and differential diagnosis of patients with hyperthyroxinaemia and non-suppressed TSH levels is shown below (Fig. 3), together with a summary of the key laboratory and clinical features of genetic disorders that may be associated with elevated TH levels and detectable TSH (Table 1).

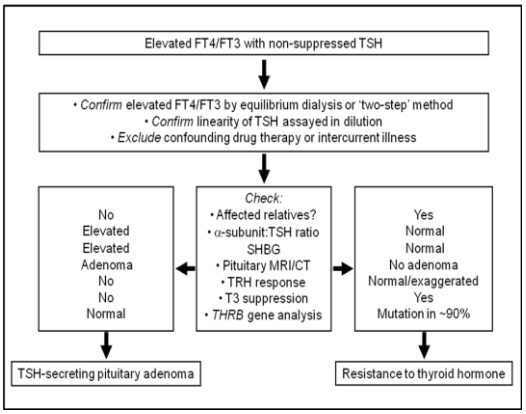

Fig. 3 *An algorithm to exclude assay artifact or other confounding causes and differentiate RTH from TSHoma.*

Disorder	Familial dysalbuminaemic hyperthyroxinaemia (FDH)	Resistance to thyroid hormone (RTH)	Allan Herndon Dudley syndrome	Selenoprotein Deficiency disorder
GENE	***ALB***	***THRB***	***SLC16A2 (MCT8)***	***SECISBP2***
FT4	Raised	Raised	Normal or Low	Raised
FT3	Normal*	Raised	Raised	Normal or Low
TSH	Normal	Normal or mildly raised	Normal or mildly raised	Normal
rT3	Raised	Raised	Low	Raised
SHBG	Normal	Normal	Raised	Normal
Clinical features	Euthyroid	Goitre; tissue-selective hyperthyroidism	Mental and psychomotor retardation	Growth retardation, male infertility, skeletal myopathy, hearing loss

Table 1. *Typical TFT patterns and clinical features in genetic disorders associated with raised thyroid hormones. Key: *FT3 is raised with a rare albumin gene variant (L66P) causing FDH.*

Key references

1. Beck-Peccoz P, Persani L, Mannavola D & Campi I (2009). TSH-secreting adenomas. *Best Practice & Research Clinical Endocrinology & Metabolism*, **23**, 597-606.
2. Gurnell M, Visser T, Beck-Peccoz P & Chatterjee VKK (2010). Resistance to Thyroid Hormone. In: J.L. Jameson & L.J. De Groot eds. *Endocrinology,* 6th edn. Saunders Elsevier, Philadelphia, PA. 1745-1759.
3. Gurnell M, Halsall DJ & Chatterjee VKK (2011). What should be done when thyroid function tests don't make sense? *Clinical Endocrinology – in press.*

Further advice

Either visit: http://www.sas-centre.org/centres/hormones/cambridge.html

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