LABORATORY TESTS OF THYROID FUNCTION: PITFALLS IN INTERPRETATION

Many factors can compromise results of laboratory tests of thyroid function, leading to possible misinterpretation and misdiagnosis.

CAROLYN FEDLER

BSc (Hons), MB BCh, FCPath (SA), MMed (Chem Path)

Senior Pathologist and Consultant

Department of Chemical Pathology

National Health Laboratory Services and University of the Witwatersrand

Johannesburg

Carolyn Fedler qualified at the University of the Witwatersrand and is a consultant in the Department of Chemical Pathology, National Health Laboratory Services and University of the Witwatersrand School of Pathology. She is extensively involved the training of undergraduate students. Her interests lie in the field of obesity, endocrinology and lipid disorders.

Clinicians rely on the laboratory for quality testing in order to make decisions concerning diagnoses and cost-effective management of thyroid disorders. Because of the diverse clinical presentations of thyroid dysfunction (the majority of thyroid disease symptoms are often subtle and nonspecific in presentation), initial requests for assessing thyroid function are often made.

When the clinical suspicion is strong (e.g. in a patient who exhibits overt symptoms and signs of hyperthyroidism), thyroid function tests are helpful in confirming the diagnosis. However, situations may occur where clinicians receive 'abnormal' test results that appear to be discordant with the clinical findings, and which also occur in the absence of a clearly definable thyroid disease. Such test results may be misinterpreted, the unfortunate outcome being that inappropriate management may be instituted.

Thus, while clinicians may be alert to the possibility of thyroid dysfunction, they may not be familiar with the interpretation of laboratory tests, or with the various factors that can compromise the laboratory tests. Good communication between the requesting clinician and the laboratory is therefore essential for correct interpretation.

Much of the difficulty in diagnosing thyroid dysfunction arises from a lack of awareness regarding the complexities in the biology of thyroid physiology and pathophysiology, limitations of the test methods in terms of diagnostic accuracy, and misunderstanding the meaning of thyroid function results in the clinical context.

This article aims to address these issues by discussing the variables (pre-analytical and analytical – see Table I) that affect the interpretation of test results, in the hope that clinicians will have a better understanding of how to interpret discrepant test results in the clinical context.

Table I. **Pitfalls in thyroid interpretation**

Pre-analytical

- Anomalous binding of thyroid hormones (T4 & T3) to serum proteins
  - Genetic
  - Drug-induced
  - Disease-induced
  - Pregnancy
- Altered reference intervals for thyroid hormones or TSH
  - Childhood
  - Pregnancy
  - Old age
- Disrupted set point of the hypothalamic-pituitary-thyroid axis
  - Non-thyroidal illness (NTI), including acute psychiatric illness
  - Drugs
  - Unusual thyroid conditions, including thyroid hormone resistance
- Specimens
  - Free fatty acid (FFA)
  - Heparin artifact

Analytical

- Heterophilic antibodies
- Auto-antibodies

PRE-ANALYTICAL FACTORS

Physiological variables

TSH/FT4 relationship

Familiarity with the normal physiology and pathophysiology is required for the selection and accurate interpretation of test results.

When the hypothalamic-pituitary-thyroid function is normal, the relationship between the thyroid-stimulating hormone (TSH) and the biologically active thyroid hormone, free thyroxine (FT4), is inverse, which is produced by the negative feedback inhibition of pituitary TSH secretion by thyroid hormones (thyroxine T4 and triidothyronine T3) secreted from the thyroid gland.

It therefore follows that thyroid function can be determined directly (by measuring the thyroid gland product, free thyroxine FT4 and/or free triidothyronine FT3) or indirectly (by measuring the TSH level, which inversely reflects the thyroid hormone concentration that is sensed by the pituitary). In straightforward clinical situations, a high TSH and low FT4 result would indicate a hypothyroid condition, whereas a low TSH and high FT4 result would indicate a hyperthyroid condition.

The indirect approach (TSH testing) is today recognised as a better test for detecting thyroid dysfunction than FT4 testing, and the reasons for this TSH-based strategy are:

- Current laboratory TSH tests offer better sensitivity and specificity than FT4 assays.
- Subtle alterations in FT4 levels produce an amplified, inverse response in blood TSH levels. This is particularly useful in the detection of sub-clinical thyroid disorders, where an abnormal TSH result occurs in the presence of normal FT4 levels.
- In the early stages of developing thyroid dysfunction, a blood TSH abnormality often precedes the development of an abnormal FT4 level, which is, however, within the normal population reference limits. The FT4 population reference limits are broad due to individual variations in genetically determined FT4 set-points.

Even though measurement of serum TSH levels is currently considered to give the better first-line discrimination, this strategy can, in reality, only be applied for ambulatory patients who are assumed to have intact and normal hypothalamic-pituitary-thyroid function and who have not previously been treated for thyroid diseases. This is based on the assumption that primary thyroid disorders (particularly primary hypothyroidism) is much more common than central or secondary hypothyroidism. The sensitivity of TSH-based strategy is seriously impaired when the blood TSH levels may be normal in the presence of clinically important thyroid dysfunction.

There are, in reality, many clinical situations where the TSH/FT4 relationship is disrupted, leading to discordant results. Examples include abnormalities in hypothalamic or pituitary function (such as TSH-secreting pituitary tumours, central hypothyroidism etc.); postpartum thyroiditis; non-thyroidal illness (NTI); during the early phases of treating overt hyper- or hypothyroidism; or when changing the dose of thyroid hormone replacement drugs. In the latter 2 situations, it takes 6 - 12 weeks for pituitary TSH secretion to adjust to the new thyroid status, resulting in misleading TSH blood levels. During such transitional stages FT4 is the more reliable parameter of thyroid function.

Effects of age on thyroid function test population reference ranges

Awareness that age has an effect on TSH/FT4 levels is important in that failure to recognise this may lead to missed or undertreated thyroid disorders. This may happen when one reference range is quoted for all age groups in the laboratory reports.

TSH levels tend to increase in older people. However, despite this well-recognised fact, the current guideline recommendation is that a single adult reference range be quoted for all adult age groups.

In children, the hypothalamic-pituitary-thyroid axis matures throughout infancy and childhood until puberty is reached. As a consequence of this maturation process, TSH and FT4 results in children are higher than those of adults, particularly in the first week of life and throughout the first year. This fact is important to recognise because missed cases of congenital hypothyroidism may occur if the age-adjusted reference range is not taken into account.

Before making any decisions, clinicians should therefore be aware of those laboratory reports that do not quote age-related reference limits.

Pregnancy

Thyroid disease is common in women of childbearing age. Furthermore, hypothyroidism during pregnancy has been shown to have a detrimental effect on fetal outcome. It is therefore crucial that the thyroid status of pregnant women be established during the gestation period.

In pregnancy, laboratories should not be reporting test results using assays that measure total thyroid hormones (e.g. TT4, TT3). Total thyroid hormones are bound to proteins (unlike the free thyroxine FT4 which exists in the unbound form), and during pregnancy the concentration of these thyroid hormone-binding proteins increases due to the effect of increased oestrogen production. The consequence is that of falsely elevated total thyroid hormone concentrations. Fortunately most, if not all, laboratories have circumvented this problem by utilising free unbound thyroid hormone assays.

Physiological changes occur during pregnancy that may affect blood TSH and FT4 levels in a certain proportion of normal pregnancies. In the first trimester, a decrease in blood TSH levels occurs (and hence 'subnormal'

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TSH results). This is attributed to thyroid gland-stimulating activity of human chorionic gonadotrophin (hCG) that is secreted by the growing placenta. hCG is genetically very similar to pituitary TSH and thus mimics TSH action. The fall in TSH is associated with a mild elevation in blood FT4 levels. In a very small proportion of cases, the blood FT4 may reach very high levels and when prolonged can lead to 'gestational transient thyrotoxicosis'. This clinically similar overt hyperthyroid condition is frequently associated with hyperemesis (vomiting) during the first trimester.

In the second trimester, the free thyroid hormone blood levels may decrease to 20 - 40% below the normal reference range mean limit, with some cases showing levels below the lowest reference limit for non-pregnant adults. This decrease in free thyroid hormone concentration may be further exacerbated if the woman is deficient in iodine.

Based on the above, the current recommendation is that the thyroid status of pregnant women be checked during each trimester, preferably using trimester-specific reference limits.

Pathological variables

Effects of medications

Clinicians need to know if the patient is on medications that affect the interpretation of thyroid function tests. Medications do not interfere chemically with the tests (with the exception of heparin) – they interfere with the metabolism of thyroid hormone production and release, and affect TSH secretion. Table II illustrates common examples, although the list is by no means comprehensive.

Heparin artifact

Heparin causes an increase in blood free thyroid hormone (FT4) levels. This is an important phenomenon to recognise as it can lead to spurious FT4 results.

Intravenous heparin administration induces lipoprotein lipase activity *in vitro,* resulting in the liberation of free fatty acids, which are known to displace thyroid hormones from the thyroid-binding proteins, leading to falsely elevated free thyroid hormone (FT4, FT3) blood levels. This effect is accentuated by incubation of blood at 37°C and by increased blood triglyceride or low serum albumin concentrations.

Medical conditions

Non-thyroidal illness (NTI)

A spectrum of thyroid function test abnormalities is often encountered in patients with both acute and chronic critical illnesses (physical and psychiatric) who usually do not have underlying thyroid dysfunction. The terms non-thyroidal illness and euthyroid sick syndrome are used interchangeably to describe such cases. Examples of illness include the following: sepsis, starvation, myocardial infarction, burns, trauma, surgery, malignancy, and psychiatric illness.

The underlying pathophysiology which results in patients with NTIs presenting with abnormal thyroid functions is unclear. It is thought to arise from a maladjusted hypothalamic-pituitary-thyroid axis.

The spectrum of changes in the thyroid tests is related to both the severity and stage of the illness (not to mention the effects of medications that these patients may be on) and is complex. This is beyond the scope of this article and the reader is advised to refer to recommended literature for more details. Clinicians should be aware that thyroid function test results may be non-interpretable in the

Table II. **Effects of some drugs on thyroid function tests**

Cause	Drugs	Effects
Inhibit thyroid hormone synthesis or release from the thyroid gland	Lithium, sulfonylureas	↓ FT4, ↓ FT3, ↑ TSH
Decreases triiodothyronine hormone production by inhibiting peripheral conversion of FT4 to FT3	Glucorticoids, propranolol, amiodarone, propylthiouracil	↓ FT3 leads to ↑ FT4
Stimulate TSH secretion	Iodine, lithium, dopamine antagonists, cimetidine	↑ TSH
Inhibit TSH secretion	Glucocorticoids, dopamine agonists, somatostatin	↓ TSH
Inhibit T4 and T3 binding to transport proteins	Phenytoin, sulfonylureas, diazepam, furosemide, salicylates	↑ FT4, ↑ FT3
Inhibit gastrointestinal absorption of ingested thyroid hormones for those on thyroid treatment	Cholestyramine, ferrous sulfate, aluminum hydroxide, and sucralfate	↓ FT4, ↑ TSH

severely ill, and more especially when patients are being treated with multiple medications. Whenever possible, it is preferable to defer diagnostic testing until the illness has resolved, except when there is a strong index of clinical suspicion of thyroid dysfunction.

TSH, in the absence of dopamine or glucocorticoid administration (both of which decrease TSH secretion), is considered to be more reliable than FT4 and FT3 testing in NTI patients. The blood TSH levels are affected in variable degrees but may be mildly low during the acute phase of the non-thyroidal illness. TSH assessment in severe NTI depends on the sensitivity of the particular method that is used by the laboratory. The 'third-generation' assays which have a functional sensitivity below 0.01 mU/l are considered sensitive enough to differentiate the very low levels in hyperthyroid patients from the subnormal, but somewhat higher TSH levels in patients with NTI. However, cases of NTI have been described where TSH levels are below the functional sensitivity of the third-generation assay. Thus, in principle, it is recommended to defer diagnosis until the illness has resolved.

Unusual thyroid disorders

Thyroid function tests can result in misdiagnoses in the following situations, especially if the TSH first-line testing is adopted: hypothalamic/pituitary disease leading to hypothyroidism (paradoxically normal or mildly elevated TSH, low FT4 blood levels); in cases of thyrotoxicosis caused by TSH-secreting pituitary tumours (rare, and represents < 1% of inappropriate TSH secretion and is characterised by non-suppressed TSH levels with no response to TRH stimulation, and MRI evidence of a pituitary mass); or in patients who are thyroid hormone-resistant, characterised by normal/slightly elevated TSH that responds to TRH stimulation with elevated FT4/FT3 levels. Such cases are often misdiagnosed as being hyperthyroid and subjected to inappropriate thyroid gland ablation. These cases can be diagnosed by non-suppressed TSH levels in response to TRH stimulation. This TRH response is appropriate despite elevated thyroid hormone levels, as the tissue response to thyroid hormone is reduced, requiring higher hormone levels to maintain the normal metabolic state.

Thyroid hormone-binding protein abnormalities

The impetus for the development of free thyroid hormone tests has been due to the high occurrence of binding protein abnormalities that resulted in discordant total thyroid hormone tests. However, it needs to be remembered that interpretation of free T4 assays can also be misleading, in that there are pre-analytical artefacts that exist in many situations associated with binding protein abnormalities. Such examples include the following: genetic abnormalities in thyroid-binding proteins and medications that displace T4 from thyroid-binding proteins (both of which may result in spuriously high FT4 blood levels); during the critical phases of NTI; and in pregnancy. The point that needs to be made here is that it is far more common to encounter misleading total and free thyroid hormone results than misleading TSH results. Given the improved sensitivity and specificity of TSH assays, the indirect approach (TSH testing) offers better sensitivity for detecting thyroid dysfunction than does thyroid hormone testing.

So what test to order and when?

To conclude from all the above, it can be seen that requesting a single thyroid parameter alone as a first-line test may lead to misinterpretation, with TSH testing offering better sensitivity than FT4 testing. The TSH or the TSH/FT4 relationship is a more reliable parameter to use than FT4 alone.

Table III illustrates situations in which TSH alone can give a false or uncertain indication of thyroid status. Thus, testing for both thyroid hormones and TSH should ideally be requested, particularly in difficult cases, and the factors that may affect such results need to be understood. FT3 should be requested as a second-line test when the TSH and FT4 are equivocal, e.g. low TSH and normal FT4 results in a clinically hyperthyroid patient may suggest the presence of a FT3 thyrotoxicosis.

ANALYTICAL FACTORS

This article would not be complete without mention of the interfering factors involved in the analysis of thyroid hormones. An ideal assay method would demonstrate no interference with any compound, be it a drug or endogenous compound, in any specimen and at any given concentration. We have already seen that this is not always possible given the pre-analytical issues mentioned above. To compound this further, there are assay technical factors that can interfere with the interpretation of test results.

Heterophile antibodies

Some patients have certain types of antibodies in their serum that have been induced by infections or exposure to therapeutic agents containing specific animal antigens, or by unintentional immunisation through exposure to animals in the workplace (e.g. pet handlers, sheep herdsmen, etc.). They may also be polyreactive antibodies such as IgM rheumatoid factor. These heterophile antibodies may cross-react with any of the thyroid function test assay methods (immunoassay-based, utilising animal antigens), leading to false results, which are more often inappropriately high. The inappropriate result may not necessarily be abnormal, but in fact inappropriately normal.

Should such a problem be encountered or suspected, it is advisable to request a retest in another laboratory that performs a different immunoassay method containing a different animal antibody source (e.g. from mouse to sheep in the case of TSH; from rabbit to goat in the case of FT4).

Endogenous thyroid hormone autoantibodies

Autoantibodies to thyroid hormones thyroxine (T4) and triiodothyronine

Table III. **Situations in which serum TSH alone can give a false or uncertain indication of thyroid status**

Condition	TSH	FT4	FT3
Primary abnormality of TSH secretion			
Pituitary-hypothalamic abnormality	L-N	L	
Premature infants	L-N	L	L
Central TSH excess	N-H	H	H
Thyrotoxicosis			
T3 toxicosis	U	N	H
Subclinical	U	N	N
Early treatment	U	H-N-L	H-N-L
TSH assay artefact	L-N-H	H	H
Hypothyroidism			
Subclinical	H	N	
Early treatment	H	L-N	
Thyroid hormone resistance	N-H	H	H
Medications			
Dopamine	L	N	N
Glucocorticoids	L	N	L-N
Amiodarone (acute)	H	N-H	L

N = normal; L = low; H = high; U = undetectable.

(T3) are frequently found in the serum of patients with auto-immune (both thyroidal and non-thyroidal) disorders. These endogenous compounds may result in methodological artifacts in both total and free thyroid hormone analyses. These interferences are often method-dependent and another assay method may need to be used in another laboratory.

Further reading

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IN A NUTSHELL

The majority of thyroid disease symptoms are often subtle and nonspecific in presentation.

While clinicians may be alert to the possibility of thyroid dysfunction, they may not always be familiar with the interpretation of laboratory tests, or with the various pre-analytical and analytical factors that can compromise the laboratory tests.

Discordant thyroid test results are often encountered, and an understanding of the TSH/FT4 relationship is required for the selection and accurate interpretation of test results.

The indirect approach (TSH testing) has better diagnostic accuracy in detecting thyroid dysfunction than FT4 testing.

It is more common to encounter misleading FT4 results than misleading serum TSH levels in clinical practice.

The sensitivity of TSH-based strategy is seriously impaired when the blood TSH levels may be normal in the presence of clinically important thyroid disorders, e.g. central hypothyroidism.

Altered thyroid hormone and TSH levels occur in early childhood, in old age and in pregnancy, which may lead to misinterpretation when compared with adult reference range values.

Non-thyroidal factors, such as medications and non-thyroidal illness, can lead to confounding thyroid function test results. In the case of a non-thyroidal illness, it is preferable to defer testing until the acute illness has resolved, unless there is a strong index of clinical suspicion.

An understanding of the pitfalls of the use of both total (TT4, TT3) and free thyroid hormone (FT4, FT3) use is required for adequate interpretation in certain clinical situations, e.g. pregnancy, and in patients on medications which cause displacement of thyroid hormones from binding proteins.

In addition to the pre-analytical factors, potential analytical factors that interfere with the thyroid function tests assays such as heterophilic antibodies and autoantibodies, may lead to discordant thyroid function test results.

The optimal use of thyroid function tests should be patient-specific and depends on the patient's specific thyroid disease, the stage of the disease and co-existing medical conditions.

Results should be interpreted in the appropriate clinical context of the individual patient with good communication between clinicians and the requesting test laboratory.