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THYROID FUNCTION TESTS AND DIAGNOSTIC PROTOCOLS FOR INVESTIGATION OF THYROID DYSFUNCTION

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Summary. - *Since many tests to investigate thyroid function are currently available, appropriate selection is required to limit the number of assay needed to establish the correct diagnosis of thyroid dysfunction. The limitations inherent in the different tests, and the interferences caused by nonthyroidal factors, especially drugs, must, therefore, be taken into account. Serum total thyroid hormone (TT4 and TT3) determinations are largely affected by changes in the concentrations of thyroid hormone transport proteins (mainly T4-binding globulin). Thus, in many cases, serum TT4 and TT3 measurements do not reliably establish thyroid status. Serum free thyroid hormone (FT4 and FT3) concentrations are independent of transport proteins and more appropriately reflect thyroid status. Serum FT3 measurement is more appropriate for the diagnosis of hyperthyroidism and drug-overdosage in L-T4-treated patients. Conversely, serum FT4 measurement more correctly identifies hypothyroid patients. Serum TSH determination by the currently available sensitive (low detection limit) assays constitutes an indispensable complementary test in both conditions.*

KEY WORDS: thyroid, hyperthyroidsm, hypothyroidsm, TSH, T3, T4.

Riassunto (Test di funzione tiroidea e protocolli diagnostici delle tireopatie). - *E' attualmente disponibile un numero assai elevato di prove di funzione tiroidea. Questo rende indispensabile una scelta oculata per limitare il numero dei dosaggi richiesti per formulare una corretta diagnosi di tireopatia. E' per questo necessario tenere presente i limiti delle diverse prove funzionali tiroidee e l'interferenza di fattori non tiroidei, soprattutto dei farmaci. Il dosaggio degli ormoni tiroidei totali (TT4e TT3) è largamente influenzato dalla variazione delle proteine di trasporto degli ormoni tiroidei (soprattutto della TBG). Così, in molti casi il dosaggio della TT4 e della TT3 sieriche non permette di stabilire correttamente lo stato tiroideo. Le concentrazioni sieriche degli ormoni tiroidei liberi (FT4 e FT3) sono indipendenti dalle proteine di trasporto e quindi riflettono in maniera più appropriata lo stato tiroideo. Il dosaggio della FT3 è il test più indicato per la diagnosi di ipertiroidismo e di sovradosaggio farmacologico nei pazienti in trattamento con L-T4. E' invece la determinazione della concentrazione sierica di FT4 che permette di identificare correttamente i pazienti ipotiroidei. La determinazione dei livelli sierici di TSH, impiegando i metodi ultrasensibili di dosaggio attualmente disponibili, rappresenta l'indispensabile complemento ad entrambe le condizioni.*

PAROLE CHIAVE: tiroide, ipertiroidismo, ipotiroidismo, TSH, T3, T4.

Introduction

Evaluation of thyroid function is frequently carried out, in part because thyroid diseases constitute the second most common endocrine disorder after diabetes mellitus. Clinical investigations and recent commercial developments have made a plethora of laboratory tests available for assessment of thyroid dysfunction. Althoug technical innovations have increased the sensitivity and specificity of the tests, the physician should be aware also of their limitations, in order to select the most appropriate diagnostic assay(s) from the bewildering array of available thyroid function tests. In any case, as pointed out by the American Thyroid Association *ad hoc* Committee report, "... tests of thyroid function should not be part of multiphasic screening for the patients who are not suspected to have thyroid disease, except in certain high-risk populations" [1], such as newborns, individuals with strong family history of thyroid disorders, patients with other autoimmune disorders and women 4 to 8 weeks after delivery.

The aims of this presentation are to review thyroid function tests and to propose logical algorithms for the diagnosis of thyroid dysfunction.

Basics of thyroid physiology

Thyroxine (T4) and triiodothyronine (T3) represent the major and clinically important thyroid hormones. The thyroid gland is the only source of T4, whereas approximately 80% of T3 is derived from peripheral conversion of T4 rather than from direct secretion from the gland. T4 to T3 conversion occurs by monodeiodination mediated by 5'-deiodinase in several tissues, including muscle, liver, heart, kidney and brain. Reverse T3 (rT3) is an inactive metabolite of T4 produced by the removal of an iodine molecule from the inner ring of T4 catalysed by a second enzyme, 5-deiodinase. Several factors, including nonthyroidal illness, fasting and drugs, may affect peripheral deiodination [2].

Thyroid hormone synthesis and secretion are under intrathyroidal (autoregulatory) and extrathyroidal (pituitary and hypothalamus) control mechanisms, the most important being represented by thyrotropin (TSH). TSH stimulates every step of thyroid hormone synthesis and secretion. TSH secretion, in turn, is controlled by either stimulatory (TSH-releasing hormone, TRH) or inhibitory (e.g. thyroid hormones) mechanisms. Many factors may affect this homeostatic balance, thus influencing thyroid function tests [3].

T4 and T3 circulate in the bloodstream bound in the main to a set of plasma proteins which widely differ in their concentration and affinity for the hormones [4]. The three major transport proteins are T4-binding globulin (TBG), which carries approximately 70% of circulating T4, transthyretin (TTR), formerly named T4-binding prealbumin (TBPA), and albumin [5]. Thyroid hormones bound to transport proteins are in a constant reversible equilibrium with the small unbound or free fraction (0.03% of T4, 0.3% of T3) [5]. Free thyroid hormones are currently believed to be immediately available for the entry into the cell, thus representing the metabolically active fraction [6]. Binding of T4 and T3 to plasma proteins is very important because several factors may influence this, causing results of thyroid function tests to lie outside reference limits.

Thyroid function tests

Thyroid function tests may measure serum thyroid hormone concentrations, serum thyroid hormone binding, or evaluate thyroid regulation, as shown in Table 1. In this article, the focus will be on serum total and free thyroid hormone and TSH measurements.

Measurement of serum total thyroid hormone concentrations

Serum total T4 (TT4) and T3 (TT3) concentrations are usually measured by radioimmunoassay, reference values being 4-11 µg/dl for T4 and 100-200 ng/dl for T3. Measurement of serum total thyroid hormone concentrations usually provides useful information on thyroid status, in that increased concentrations are observed in hyperthyroid patients, and decreased concentrations in hypothyroid patients. However, inappropriately low or high concentrations of total thyroid hormones are found in a number of physiological and pathological conditions associated with changes in thyroid hormone-binding protein concentrations, the presence of circulating anti-iodothyronine autoantibodies or altered peripheral metabolism of T4. [7].

Serum TT4 and TT3 concentrations almost exclusively reflect circulating hormones bound to proteins. Therefore, an increase in serum TBG concentration determines an increase in serum TT4 and TT3 concentrations, whereas the reverse occurs when serum TBG concentration is diminished. An increase in serum TBG concentration may be genetically determined (X-linked) [8], occur physiologically during pregnancy, due to estrogen excess [5] or be caused by a variety of drugs and nonthyroidal illness [5], as illustrated in Tables 2 and 3. Low serum TBG concentrations may also be due to an X-linked inherited defect [8] or result from reduced hepatic synthesis of the protein, which may be drug-induced or disease-related (Tables 2 and 3).

Table 1. - *Classification of common thyroid function tests*

Measurement of serum thyroid hormone concentration
Serum total thyroxine (TT4)
Serum total triiodothyronine (TT3)
Serum free thyroxine (FT4)
Serum free triiodothyronine (FT3)
Serum reverse triiodothyronine (rT3)
Tests of thyroid regulation
Serum thyrotropin (TSH)
TRH test
Measurements of thyroid hormone binding
Serum T4-binding globulin (TBG)
Serum transthyretin (TTR)
Tests for autoimmune thyroid disease
Thyroid peroxidase antibody
Thyroglobulin antibody
TSH receptor antibody
Markers of differentiated thyroid function
Serum thyroglobulin
Serum calcitonin

Table 2. - *Drugs influencing serum TBG levels*

Drug	Effect of serum TBG
Thyroxine	Decrease
Estrogens	Increase
Androgens	Decrease
Glucocorticoids	Decrease
Anabolic steroids	Decrease
Heroin, methadone	Increase
5-fluorouracil	Increase
L-asparaginase	Decrease
Perphenazine	Increase
Clofibrate	Increase

TT4 and TT3 concentrations may also be affected by drugs competing with the hormones for their binding sites on TBG and/or TTR, generally leading to a reduction in serum TT4 and TT3 concentrations.

The presence of abnormal thyroid hormone binding proteins may then be responsible for changes in serum total thyroid hormone concentrations. The condition known as familial dysalbuminemic hyperthyroxinemia (FDH) is an inherited autosomal dominant syndrome characterized by the presence in the serum of albumin molecules with an abnormally high affinity for T4 (and less frequently for T3) [9]. Another rare syndrome is characterized by an abnormal T4 binding to TTR [10]. Both these conditions are associated with increased serum TT4 and, sometimes, TT3 concentrations; as discusseed below, free thyroid hormone concentrations are normal in these syndromes, establishing euthyroidism (Table 4).

A large number of physiological and pathological conditions, including moderate to severe nonthyroidal illness, are associated with an impaired T4 to T3 monodeiodination in the absence of any abnormality of the thyroid gland (Low-T3 syndrome) [2, 11]. These conditions share a reduced 5'-deiodinase activity leading to decreased serum TT3, normal or increased serum TT4, and increased serum rT3 concentrations. In euthyroid patients with severe nonthyroidal illness, serum TT4 concentration may also be reduced [12].

Anti-iodothyronine autoantibodies, which are a relatively frequent finding in patients with thyroid autoimmune disorders [13], may interfere in TT4 and TT3 immunoassays and cause either falsely low or falsely high results depending on the assay procedure (see [13] for a review of methodological aspects).

Table 3. - *Effects of nonthyroidal illness on serum TBG concentrations*

Disease	Effect on serum TBG
Acute viral hepatitis	Increase
Liver cirrhosis	Increase, decrease
Acute intermittent porphyria	Increase
Hypercortisolism	Decrease
Diabetic ketoacidosis	Decrease
Chronic renal failure	Decrease
Nephrotic syndrome	Decrease
Malnutrition	Decrease

Table 4. - *Thyroid function tests in different forms of euthyroid hyperthyroxinemia*

	TT4	TT3	FT4	FT3	TSH
TBG excess	In	In	N	N	N
FDH	In	N[1]	N	N	N
Increased affinity for TTR	In	N	N	N	N

In: increase; N: normal; N[1]: increased in some instances.

All the above considerations underscore the limitations inherent in the measurement of total thyroid hormone concentration in the serum. Therefore, although in most cases serum TT4 and TT3 values can provide information which correctly support the clinical judgement of thyroid status, in view of the existence of several conditions leading to inappropriately abnormal results, serum total thyroid hormone measurement can be recommended no longer as the initial screening test of thyroid function.

Measurement of serum free thyroid hormone concentration

As mentioned above, it is widely accepted that free thyroid hormones represent the metabolically active fraction available to tissues [6]. For this reason and because of the fact that circulating free thyroid hormones are not affected by changes in the concentration of transport proteins, serum free T4 (FT4) and free T3 (FT3) measurements must be considered very relevant variables in the initial evaluation of thyroid function. Serum concentrations are 6-16 pg/ml for FT4, 2.5-5.5 pg/ml for FT3.

A number of methods are available for free thyroid hormone assay (see [7] for a detailed review of available methods). Free hormones cannot be determined directly in native serum, because, as mentioned earlier, they are in continuous and rapid equilibrium with protein-bound hormone. Thus, FT4 and FT3 must be separated from the serum by dialysis, ultrafiltration, column adsorption chromatography or immunoadsorption prior to measurement by immunoassay.

Several physiological and pathological conditions may interfere with free thyroid hormone determination by many of the available methods, especially those based on the use of labeled analogues of thyroid hormones. The analogue, in theory, should not react with serum proteins while maintaining its reactivity with the antibody to the native hormone. This approach would obviate the above mentioned need of the two-step procedure required to remove the serum. Unfortunately, this type of assay has been shown to have a major drawback, in that the analogue interacts with endogenous serum proteins, such as albumin [14] and anti-iodothyronine autoantibodies [15]. This interaction is responsible for the inappropriately low concentrations found in pregnancy, nonthyroidal illness and during heparin treatment, and for the inappropriately high concentrations observed in FDH and in the presence of anti-iodothyronine autoantibodies [7].

If a method not affected by these methodological problems is selected, free thyroid hormone determination provides more reliable information on thyroid status than total thyroid hormone measurement. The problem of whether serum FT4 or FT3 should be assayed is discussed later.

Measurement of serum TSH concentration

Circulating TSH concentrations mainly reflect the feed-back inhibition of thyroid hormones at the cellular level. Thus, serum TSH values are increased in hypothy-

roid patients and decreased or undetectable in hyperthyroid patients.

Serum TSH concentrations have been measured for almost twenty years by conventional radioimmunoassays using polyclonal anti-TSH antibodies. The major drawback of these assays was their limited sensitivity. For this reason, a substantial proportion of euthyroid subjects had undetectable serum TSH concentrations, although their TSH secretion was not truly suppressed as in hyperthyroid patients. Recently, immunoradiometric or immunoenzymometric TSH assays with low detection limit have been developed which employ monoclonal anti-human TSH antibodies [16]. These assays are about tenfold more sensitive than conventional radioimmunoassays; the low threshold of measurement of TSH (0.1 mU/l or less) has allowed a nearly complete discrimination of euthyroid subjects having low-normal TSH values from hyperthyroid patients whose TSH concentration is subnormal or really undetectable [17]. This has obviated the need for the TRH test in most cases [18]. Serum TSH concentrations are 0.4-4.5 mU/l in healthy subjects.

Serum TSH concentrations are affected by many nonthyroidal factors. They may be decreased, with the loss of the nocturnal surge, in euthyroid patients with nonthyroidal illness [19-21], or following the administration of drugs, such as glucocorticoids or dopamine. Conversely, increased serum concentrations may be found in euthyroid individuals in the recovery phase of nonthyroidal illness or following the administration of drugs, such as dopamine antagonists, domperidone, chlorpromazine or haloperidol [22]. Possible methodological interference may derive from circulating anti-human TSH antibodies or from high concentrations of serum chorionic gonadotropin [23]: both conditions usually are responsible for an underestimation of serum TSH concentrations.

Although the measurement of serum TSH concentrations has been proposed as the first-line test in the assessment of thyroid function, a TSH-based testing strategy still needs to prove to be cost-effective in the different pathophysiological situations [24].

Diagnostic protocols for thyroid dysfunction

Hyperthyroidism

In the overt forms of hyperthyroidism, diagnosis is easily made on clinical grounds and laboratory determinations are complementary and substantiate clinical judgement. In these cases TT4 and TT3 provide adequate information. However, as shown in Fig. 1, the finding of increased serum TT4 and TT3 concentrations *per se* does not allow the diagnosis, since, as mentioned above, this might simply reflect an increased thyroid hormone binding capacity, most frequently related to an increase in serum TBG concentrations [4]. Conversely, normal TT4 and TT3 concentrations might be found in the presence of reduced serum TBG concentrations (Fig. 1). Thus, serum

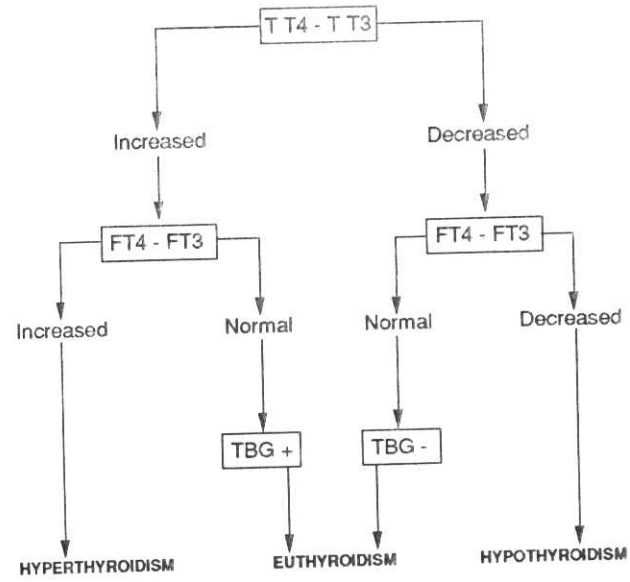

Fig. 1. - Diagnostic algorithm for investigation of thyroid dysfunction based on serum total thyroid hormone measurement as the first test.

free thyroid hormone determination is usually required to confirm the diagnosis (Fig. 1). This is especially true for subtle or initial forms of thyroid hyperfunction, which may be associated with still normal serum total thyroid hormone concentrations even in the absence of thyroid hormone binding abnormalities [25].

A relevant question is whether FT4 or FT3 represent the most appropriate assay for the diagnosis of hyperthyroidism. Recently, the American Thyroid Association has indicated that FT4 is the test to perform (in conjunction with serum TSH measurement) to diagnose thyroid hyperfunction [1]. This approach is correct in most cases. There are, however, relevant exceptions to consider. Euthyroid subjects chronically treated with the iodine-rich drug, amiodarone, may frequenly have increased serum FT4 concentrations, due to the inhibition of peripheral conversion of T4 to T3 [26]. Increased serum FT4 concentrations may also be found in acute psychosis, hypermesis gravidarum, FDH, in subjects living at high altitude and in patients receiving heparin treatment [24]. In hospitalized patients with nonthyroidal illness, serum FT4 may be increased, in the absence of hyperthyroidism, because of the impairment of T4 clearance or inhibition of T4 to T3 monodeiodination (e.g. related to pharmacological use of glucocorticoids or dopamine or to the exposure to iodinated contrast media) [24]. Conversely, hyperthyroid patients with severe nonthyroidal illness may have normal TT4 and FT4 concentrations [27]. Furthermore, at least 40% of patients with so-called T3-toxicosis have normal TT4 and FT4 concentrations [28].

On the basis of the above considerations, a diagnostic strategy for thyroid hyperfunction may be proposed, which is based on serum FT3 measurement. As illustrated in Fig. 2, increased FT3 concentrations are sufficient to substantiate the diagnosis of thyroid hyperfunction, whereas

concentrations within reference limits reasonably rule out hyperthyroidism. In most instances, and particulary when serum FT3 concentrations are high-normal or slightly increased, serum TSH determination is required to confirm the diagnosis and also to rule out the possible central origin of thyroid hyperfunction (inappropriate TSH secretion) (Fig. 2). Should the diagnostic strategy be TSH-based, as shown in Fig. 3, serum FT3 determination would in any case be needed to confirm the diagnosis.

Further testing may be required to clarify the etiology of hyperthyroidism, although the presence of diffuse goiter and exophthalmos easily establish the diagnosis of Graves' disease. Thyroidal radioiodine uptake (RAIU) is an essential test (Fig. 4). RAIU is generally high in Graves' disease, associated with diffuse uptake on the thyroid scan and with the presence of circulating thyroid-directed autoantibodies. The presence of localized uptake in one or more nodules is consistent with the diagnosis of toxic adenoma or multinodular goiter. Low RAIU values may be due to iodine load, as in iodine-induced thyrotoxicosis, but are also encountered in thyrotoxicosis factitia and in the hyperthyroid phase of subacute thyroiditis. Measurement of serum thyroglobulin concentration which is high in subacute thyroiditis and low or undetectable in thyrotoxicosis factitia, allows the differential diagnosis between the latter two conditions [29].

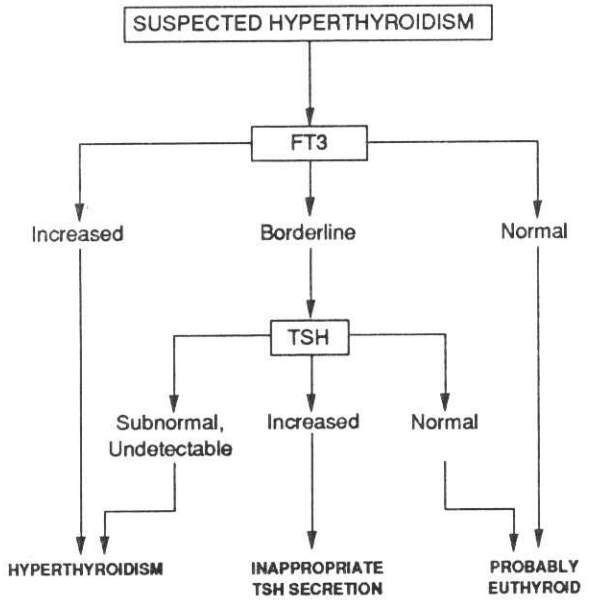

Fig. 2. - Diagnostic algorithm for suspected hyperthyroidism.

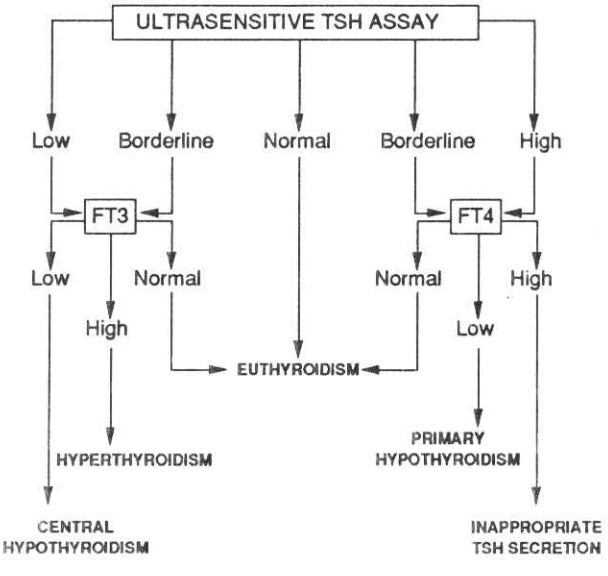

Fig. 3. - Diagnostic algorithm of thyroid dysfunction based on THS assay.

Hypothyroidism

Hypothyroidism encompasses a wide spectrum of situations, ranging from overt forms, in which clinical diagnosis is relatively easy and laboratory tests are only confirmatory, to subtle forms which require appropriate laboratory investigations to establish the correct diagnosis [30].

In a patient suspected to be hypothyroid, serum FT4 and TSH determinations represent the clue to the diagnosis (Fig. 5). Serum total thyroid hormone concentrations

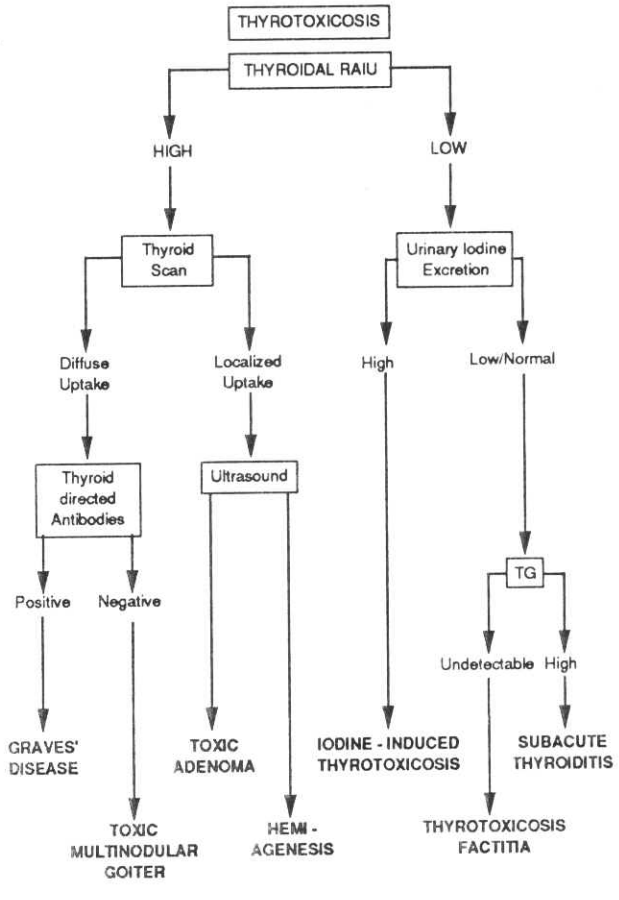

Fig. 4. - Thyroidal radioiodine uptake (RAIU) in the different forms of thyrotoxicosis.

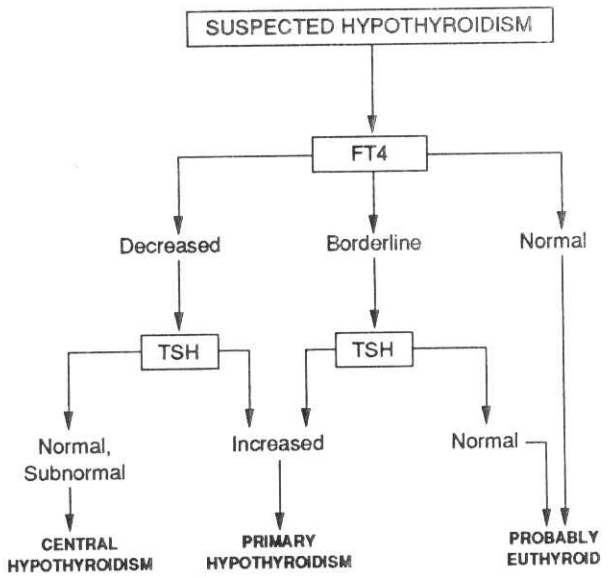

Fig. 5. - Diagnostic algorithm for investigation of suspected hypothyroidism.

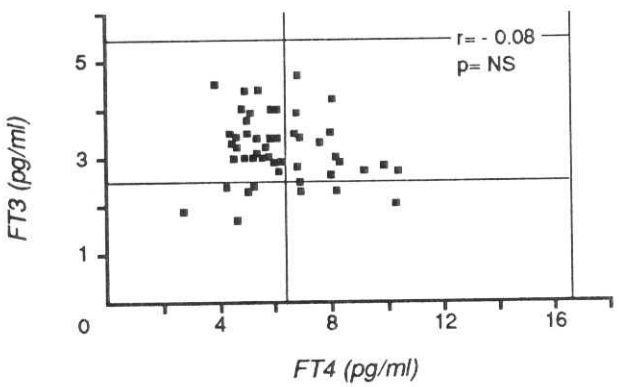

Fig. 6. - Serum free thyroid hormone concentrations in patients with subclinical hypothyroidism. Vertical lines identify FT4 reference limits, horizontal lines indicate FT3 reference limits. From [31].

may not establish the diagnosis, since, as already noted, reduced TT4 and TT3 concentrations are found in euthyroid patients whose serum TBG concentration is decreased (Fig. 1). The importance of a correct laboratory assessment is particularly evident in subclinical hypothyroidism. This condition is characterized by the absence of clinical signs and symptoms of thyroid hypofunction, and by normal serum total thyroid hormone concentrations in the presence of increased baseline or TRH-stimulated TSH concentrations [30]. The evaluation of serum free thyroid hormone concentrations in these patients does show that the majority of them have low FT4 but FT3 is normal in most cases (Fig. 6) [31]. Thus, serum FT4 determination, together with serum TSH measurement, permits assessment that most patients are indeed mild hypothyroid. This finding may be clinically relevant, because it has been shown by some, although not accepted by others, that L-T4 replacement therapy has beneficial effects also in these asymptomatic patients [32].

Another situation demonstrating the diagnostic usefulness of FT4 measurement is amiodarone iodine-induced hypothyroidism [33]. This conditions, which is more frequently encountered in iodine sufficient areas [34], is often associated with serum TT3 and FT3 concentrations within reference limits [33].

The diagnosis of hypothyroidism is far more complex in sick patients who are hospitalized for nonthyroidal illness, since these patients may undergo changes in thyroid hormone secretion, distribution and metabolism, as well as decreases in serum thyroid hormone binding protein concentrations [35]. Moreover, serum inhibitors of thyroid hormone binding to transport proteins have been described in nonthyroidal illness [36]. Serum FT4 is the key test for the diagnosis of primary hypothyroidism in these patients, since serum TSH concentrations may be normal or even decreased and because of the use of drugs suppressing TSH secretion, such as glucocorticoids and dopamine [35]. Some hypothyroid patients with nonthyroidal illness may have serum TSH concentrations which are inappropriately low or normal independently of drug administration [19-21]; this supports the case for a central defect of TSH secretion in sick patients. Central (pituitary or hypothalamic) hypothyroidism may occur independently of nonthyroidal illness, generally in association with deficient secretion of other pituitary hormones [37].

Thus, although the algorithm illustrated in Fig. 5 shows the use of TSH determination as a secondary test, when there is a strong suspicion of thyroid deficiency it is more cost-effective to obtain both FT4 and TSH values at the same time, which clarifies the primary or central origin of the hypothyroidism.

Further testing may be required to ascertain the etiology of hypothyroidism. The positivity of circulating thyroid-directed autoantibodies indicates that the patient suffers from Hashimoto's thyroiditis or idiopathic myxoedema, whereas negative tests will suggest a dyshormonogenetic goiter, especially if there is a strong family history of goiter and hypothyroidism. Relevant information on the origin of hypothyroidism may also come from a history of radioiodine therapy, thyroidectomy or antithyroid drug therapy.

L-T4 therapy

L-T4 may be administered either at replacement or suppressive doses. The former are used to correct hypothyroidism, whereas suppressive doses are employed either to shrink goiter or suppress TSH-dependent growth of thyroid cancer.

Patients receiving L-T4 replacement therapy may have TT4 and FT4 concentrations above reference limits in the presence of detectable TSH concentrations [25]. This finding suggests that increased serum FT4 concentrations are not *per se* an indicator of drug overdosage. This is confirmed by the observation that unsuppressed TSH concentrations are found in a relevant proportion of athyreotic patients receiving suppressive doses of L-T4 and showing increased serum FT4 concentrations [38]. This

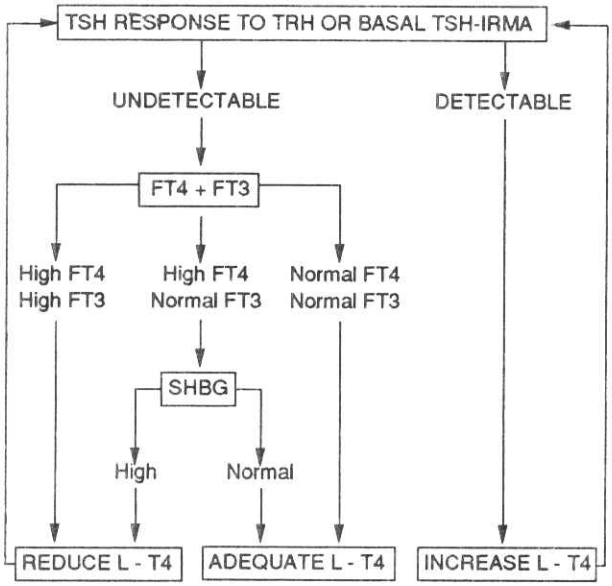

Fig. 7. - Diagnostic algorithm for evaluation of L-T4 therapy.

supports the concept that the adequacy of TSH suppression may only be established by serum TSH measurement. As shown in Fig. 7, however, the finding of undetectable serum TSH concentrations does not provide any information as to whether the dosage of the drug is excessive. In this regard, the most important assay, on the basis of the above considerations, is represented by FT3. Tests of peripheral effect of thyroid hormones, such as serum sex hormone-binding globulin (SHBG) measurement, may help identify patients receiving L-T4 overdosage (Fig. 7).

Effects of drugs on thyroid function tests

Physicians may be asked to ascertain whether a given patient suffers from hyper-or hypothyroidism while receiving drugs which interfere with thyroid function tests. Diphenylhydantoin administration is associated with a 30-40% decrease in serum TT4 and FT4 concentrations and with low-normal or slightly decreased serum TT3 and FT3 concentrations [39]. Similar changes may be caused by rifampicin [40]. Several other drugs, as already mentioned, inhibit peripheral monodeiodination of T4 to T3, thus decreasing TT3 (and sometimes FT3) and increasing TT4 and FT4 concentrations. These drugs include glucocorticoids, β-blocking agents (in pharmacological doses), iodinated contrast media and amiodarone [1]. Glucocorticoids, as well as dopamine, also inhibits TSH secretion. With the latter two exceptions, however, the finding of serum TSH concentrations within reference limits is sufficient to ascertain euthyroidism.

Concluding remarks

Although a large number of tests which explore thyroid function are currently available, the physician must select the most appropriate assay(s) in different clinical situations to establish a correct diagnosis and to fulfill cost/benefit criteria. Serum FT4 and TSH (by modern low detection limit assays) should be used for the diagnosis of hyperthyroidism and to ascertain the adequacy of L-T4 therapy. Serum FT4 and TSH are the key tests when hypothyroidism is suspected. Serum TSH is probably the best assays to rule out changes of thyroid status in patients receiving drugs known to alter thyroid function tests.

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