DIAGNOSIS OF ENDOCRINE DISEASE

How reliable are free thyroid and total T₃ hormone assays?

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Hypothyroidism is a very common disorder worldwide, for which the usual treatment is monotherapy with levothyroxine (L-T₄). However, a number of patients treated with L-T₄ continue to report symptoms of hypothyroidism despite seemingly normal levels of thyroid-stimulating hormone (TSH), free-T₃ (FT₃) and free-T₄ (FT₄) measured by immunoassay. This review summarizes the limitations of the immunoassays commonly used to measure thyroid hormone levels and emphasizes the advantages of the role of liquid chromatography-tandem mass spectrometry (LC-MS/MS). Immunoassays for free thyroid hormone are affected by alterations in serum binding proteins that occur in many physiological and disease states. Multiple studies show falsely normal values for T₃, FT₃ and FT₄ by immunoassay that are below the reference interval when measured by (ultrafiltration) LC-MS/MS, a reference method. We suggest evaluation of thyroid hormone levels by ultrafiltration LC-MS/MS for patients who continue to experience hypothyroid symptoms on LT-4. This may help identify the approximately 20% subset of patients who would benefit from addition of T₃ to their treatment regimen (combination therapy).

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(2016) **175**, R255–R263**Introduction**

Hypothyroidism is a common disorder. Overt hypothyroidism occurs in approximately 5% of the population in developed countries, whereas subclinical hypothyroidism (defined by elevated thyroid-stimulating hormone (TSH) with normal immunoassay-free thyroxine (FT₄)) occurs in 4–10% of people (1, 2). Measurement of those samples for FT₄ and free 3,5,3'-triiodothyronine (FT₃) by ultrafiltration LC-MS/MS demonstrates that 2 out

of 3 individuals classified as subclinical by immunoassay have FT₄ or FT₃s below the 2.5th percentiles and are therefore now classified as clinically hypothyroid (3). Hypothyroidism is more commonly found with increasing age and female gender, with a female-to-male ratio of approximately 9:1 (4). The signs and symptoms of hypothyroidism are dependent on the degree of thyroid hormone deficiency, the acuity of disease development,

Invited Author's profile

Dr Steven Soldin currently oversees the mass spectrometry section of the clinical chemistry service and research programs at the NIH Clinical Center, Bethesda, MD, USA. He is also involved in the routine laboratory work and directs the postdoctoral training of PhD and MD clinical chemistry fellows. He has served as President of several National organizations including the National Academy of Clinical Biochemistry, the American Board of Clinical Chemistry and the International Association of Therapeutic Drug Monitoring and Clinical Toxicology. His research interests focus on the role of specificity in improving patient diagnosis and treatment. Current foci include steroid, and thyroid hormone profiles; his group are also assessing the role of T₃ and choline during pregnancy regarding fetal maturation. He has to date published 269 papers in peer-reviewed journals.

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and the duration of the disease. The consequences of untreated or wrongly treated hypothyroidism can be significant, ranging from nonspecific symptoms to life-threatening manifestations. Even the syndrome 'subclinical hypothyroidism' may have clinical consequences and has been linked to infertility, pregnancy complications, psychiatric illness, neuromuscular symptoms, cardiac dysfunction and mortality (5, 6). Thus, accurate and timely diagnosis of hypothyroidism is essential. Extra-thyroidal diseases are also important conditions requiring accurate measurement of thyroid hormones. For example, euthyroid sick syndrome occurs in critically ill patients and is characterized by low FT₃, normal to low TSH, frequently elevated reverse T₃, and low FT₄ if of prolonged duration; an accurate FT₃ is especially important to the diagnosis (7). Other important conditions for measurement of free thyroid hormones include those associated with drugs that affect thyroid function, peripheral metabolism of thyroid hormones such as amiodarone (8), or binding of thyroid hormones to plasma proteins.

The two major thyroid hormones produced by the thyroid gland are 3,5,3'-triiodothyronine (T₃) and thyroxine (T₄). The thyroid gland exclusively synthesizes T₄ and produces approximately 20% of T₃. The majority of T₃ is generated by 5'-deiodination catalyzed by type I and type II T₄-5'-deiodinases in peripheral tissue. Specifically, approximately 65% of peripheral-derived T₃ is produced by type II deiodinase, a microsomal enzyme with activity in the brain, muscle, pituitary, and placenta; the remaining 35% is derived from type I deiodinase activity, a plasma membrane enzyme located in the thyroid, liver, and kidney (9). A type III deiodinase inactivates T₃ and T₄ to produce reverse T₃ and diiodothyronine (10). The majority of circulating thyroid hormones are bound to serum proteins, allowing for their transport and extension of half-life; however, carrier protein binding makes thyroid hormones unavailable to tissues and thus protein-bound T₃ and T₄ are considered inactive. Thyroxine-binding globulin (TBG) is a high-affinity, but low-concentration, binding protein that binds approximately 80% of T₃ and 75% of T₄ (11). The remainder of circulating T₃ and T₄ are bound to the low-affinity proteins albumin and transthyretin. Changes in binding protein concentrations occur in a number of conditions and can significantly affect the total thyroid hormone concentrations. Only approximately 0.04% of total T₃ and 0.02% of T₄ are available in circulation as free hormones and are considered the biologically active forms because the free hormone is accessible to peripheral tissue. According to the free hormone hypothesis, only

the free thyroid hormone is available for cellular uptake, whereas the bound form is not immediately available (12). Recently, it has been shown that T₄ has a very poor affinity for the thyroid hormone nuclear receptor, whereas T₃ is avidly bound to the latter and responsible for the clinical effects, indicating that T₄ is the prehormone and needs to be converted to the active hormone T₃ (13, 14).

The laboratory assays for assessment of thyroid function include thyroid-stimulating hormone (TSH), total triiodothyronine (TT₃), total thyroxine (TT₄), FT₃, and FT₄. The measurement of FT₃ and FT₄ is the most clinically relevant for the evaluation of thyroid disorders, with total thyroid hormones being affected by variations in binding protein concentrations. It is essential that free thyroid hormone measurement accurately assess hormone concentration even in the presence of significant variation in the concentration of binding proteins that occur in a variety of physiological and disease states. This is clearly achievable using equilibrium dialysis or ultrafiltration to effect the separation and then measure the free thyroid concentrations in the dialysate or ultrafiltrate.

Increasing evidence by our group and others indicates that inaccurate values are being generated for thyroid hormones by the commonly used immunoassay platforms. SJS directed the chemistry laboratory at Children's National Medical Center from 1988 to 2008. Shortly after arriving, he met with Dr Wellington Hung, the head of endocrinology and a thyroid expert. Dr Hung emphasized that FT₄ tests run in the laboratory by immunoassay often did not agree with TSH, especially true when the TSHs were elevated. Repeating the immunoassay, FT₄s and FT₃s gave results that were very similar; i.e., immunoassays gave very precisely the wrong result. To establish why this happened, we sent these samples to Nichols laboratory for measurement of FT₄ by equilibrium dialysis followed by immunoassay. Almost all these results were found to agree with the high TSHs, thereby demonstrating conclusively that a distinct problem existed with currently used immunoassay platforms for the measurement of FT₄ and FT₃. Work by our team over the past two decades has verified that immunoassays for FT₄, FT₃, and TT₃ frequently overestimate values for all three tests especially at low concentrations (3, 14, 15, 16, 17, 18). Furthermore, these papers have shown a poor correlation of immunoassay values for FT₄, FT₃, and TT₃ with log TSH or TSH as well as with the patient's clinical condition. The Food and Drug Administration (FDA) is partially responsible for this as they have approved immunoassay platforms for the measurement of all three analytes and did not check whether the results generated by these assays agreed with TSH or the patient's clinical condition (19).

An important distinction between the immunoassay and LC-MSMS methods is that the latter removes the thyroid-binding proteins before measurement of FT₄ and FT₃ levels. This review discusses the assays available for total and free thyroid hormone measurement. Specific limitations of commonly used assays are discussed, and issues relevant to treatment are highlighted.

Limitations of current free thyroid hormone immunoassays

The majority of laboratories use one-step direct analog immunoassay for determination of free thyroid hormone. A complete review of assay methodology for total and free thyroid hormone measurement has been published elsewhere (14). A number of disease and physiological states have been shown to affect free thyroid hormone measurement. Furthermore, free thyroid measurements show poor agreement between various assays.

Numerous protein-binding variations can affect free thyroid hormone measurement (14). Briefly, pregnancy is a well-established cause of increased TBG and lowered albumin levels, resulting in inaccurate free thyroid hormone by immunoassay (20). Genetic variations in binding proteins occur such as congenital TBG deficiency or excess or mutations in transthyretin and albumin that confound free hormone measurement by immunoassay (21). Many commonly used medications disrupt thyroid hormone binding to serum proteins; dilution used by immunoassay methods can open more free thyroid hormone-binding spots on proteins by reducing the competing drugs with potential alteration of free hormone measurement (14). For example, heparin administration in both fractionated and unfractionated forms causes displacement of T₃ and T₄ from binding proteins causing apparent elevations in measured FT₃ and FT₄ values, possibly due to heparin activation of lipoprotein lipase (22). Other common medications that displace T₃ and T₄ from serum binding proteins with implications on thyroid testing include furosemide, salicylate, and anti-epileptics (23, 24). Patients with certain medical conditions such as end-stage renal disease, major cardiac surgery, and critical illness have free thyroid hormones levels that are inaccurate by analog immunoassay methods (25, 26). The dependence of FT₄ and FT₃ on thyroid hormone-binding proteins thus makes most of these immunoassays unsuitable for a variety of populations. Indeed, several studies have shown the dependence of FT₄ measurement on serum binding protein conditions (27, 28, 29).

A number of studies have shown inconsistencies between the results of various free thyroid hormone immunoassays. An investigation by Giovannini *et al.* (30) evaluated 54 control samples representing a range of values for FT₃ and FT₄ measurement by 1000 laboratories. Substantial differences, of over 20%, were found for both FT₃ and FT₄ immunoassay methods. Furthermore, low concentrations of FT₃ and FT₄ showed higher imprecision compared with normal range samples (30). A College of American Pathologist study of fresh frozen serum sent to 3900 clinical laboratories reported significant analytic bias for nine of 11 free T₃ and 11 of 13 for free T₄ immunoassays (31). A number of other studies report significant differences in free thyroid hormone results according to the immunoassay method performed (32, 33, 34, 35, 36). The differences in free thyroid hormone values by the various immunoassay methods may reflect differential assay susceptibility to the various alterations in serum binding proteins. Regardless, the often times substantial differences in free thyroid hormone values according to the method performed have significant clinical implications.

Another indicator of the suboptimal performance of free thyroid hormone immunoassays is the poor correlation of their results on the inverse log-linear relationship between TSH and FT₄. The levels of thyroid hormone are tightly controlled by negative regulation of pituitary secretion of TSH such that increased thyroid hormone results in decreased production of TSH, whereas low thyroid hormone levels induce TSH secretion. The inverse log-linear association between TSH and FT₄ has been well documented (14). This relationship is shown graphically in Fig. 1 (37). An evaluation of three

Figure 1

Relationship between the log TSH and FT₄. Adapted from Spencer *et al.* (37) with permission.

commercially available immunoassays found relatively poor correlation coefficients between log-transformed TSH and FT₄ ranging from 0.72 to 0.76 in a euthyroid population (38). Worse correlation coefficients (0.08–0.58) are reported when the population includes patients with thyroid disease (16, 17, 18, 39). A representative image of the log TSH and FT₄ performed by immunoassay is shown in Fig. 2A. In contrast, substantially better correlation coefficients (0.77–0.90) are reported for the log-transformed TSH and FT₄ measured by ultrafiltration LC-MSMS (Fig. 2B) (16, 18, 39).

Emerging role of LC-MSMS

LC-MSMS identifies analytes by column retention time in combination with the mass-to-charge ratio (*m/z*) of parent and fragmentation product ions. Quantitation is accomplished by comparing the ratio of measured analyte to a labeled internal standard (isotope dilution tandem mass spectrometry). LC-MSMS assays are highly specific, sensitive, precise, and can detect hormones found in low concentrations. Isotope dilution LC-MSMS has been successfully applied to the measurement of a number of hormones including gonadal and adrenal steroids, thyroid hormones, and 25-hydroxyvitamin D (40, 41, 42, 43). Methodology to measure FT₃ and FT₄ simultaneously by isotope dilution tandem mass spectrometry has also been described (44).

Several studies have compared immunoassays on a variety of platforms to ultrafiltration and equilibrium dialysis LC-MSMS. Reasonable correlation of

immunoassay to LC-MSMS is often noted in the euthyroid range; however, the assays frequently disagree at the low and high values for concentrations of thyroid hormone, which are the most critical because these values represent the points that clinical decisions are made. Jonklaas *et al.* (15) reported lower TT₃ values by ultrafiltration LC-MS/MS compared with a chemiluminescent immunoassay platform in patients with TSH over 4.5 mIU/L. A different investigation with 63 outpatients and 37 inpatients noted discrepant values at the high and low values of the reference interval, especially for TT₃, FT₃ and FT₄, with low values more frequently shown by LC-MS/MS. Furthermore, patients with high concentrations for TSH often had thyroid hormones below the reference interval for LC-MS/MS that were misclassified as normal by immunoassay (45). A study of 40 patients classified as subclinical hypothyroidism by elevated TSH with normal FT₄ levels by conventional immunoassay found that 65% of such patients actually had FT₃ or FT₄ levels below the reference range by ultrafiltration LC-MSMS (3). Laboratory assays must have acceptable performance throughout the entire analytical range. The assay performance is especially important at the decision thresholds, specifically high and low concentrations of free hormones. Inaccurate values at the high and low range of free hormone can have significant clinical implications by delaying appropriate diagnosis and treatment of thyroid disorders.

Another indicator of the superiority of LC-MSMS is the improved correlation coefficient for free thyroid hormone and the log-transformed TSH compared with immunoassay. An evaluation of 38 pediatric samples, 200 euthyroid adults, and 128 pregnant women reported an improved log TSH correlation with free thyroid hormones when measured by LC-MSMS compared with immunoassay (16). Other studies have also reported improved correlation coefficients, ranging from 0.86 to 0.90 (15, 18). The inverse relationship between TSH and T₃ is also superior when evaluated by LC-MSMS compared with immunoassay (Fig. 3) (15, 18, 46). A recent investigation found significantly lower TT₃ values when measured by isotope dilution LC-MSMS compared with immunoassay in patients with high TSH values, confirming the superior correlation with TSH (19).

Figure 2

Relationship of FT₄ with the log TSH. (A) TSH and FT₄ performed on the Siemens Immulite 2500 analyzer show a poor correlation. (B) Improved correlation of the inverse log-linear relationship between TSH and FT₄ when FT₄ is measured by ultrafiltration followed by LC-MSMS. Adapted from (18) with permission from the American Association for Clinical Chemistry.

Clinical implications

The normal thyroid gland produces both T₃ and T₄; however, only levothyroxine (L-T₄) is the usual

Figure 3

TT₃ by immunoassay and LC-MSMS at TSHs above the 95th percentile (3.70 mIU/L). Immunoassay TT₃ and TSH was measured on the Roche Cobas 6000. Low values of TT₃ are considered <80 ng/dL. More patients were classified as having low TT₃ level when measured by LC-MSMS, indicating a superior correlation with TSH compared with immunoassay.

replacement treatment used for patients with hypothyroidism in the USA. L-T₄ is one of the most commonly given drugs worldwide and overall has an excellent efficacy and safety record. The majority of patients respond well to L-T₄ monotherapy, and patients administered L-T₄ can have adequate levels of T₃ formed by monodeiodination of T₄ (47). However, a subset of patients treated with L-T₄ monotherapy continues to report symptoms suggestive of hypothyroidism (48, 49); such patients may account for as many as 20% of hypothyroid patients. These patients usually have within-range immunoassay values for TSH, total and free T₃, and FT₄, giving the suggestion of appropriate L-T₄ monotherapy.

The numerous studies reviewed in the above sections demonstrate that the immunoassays used by most laboratories are inaccurate for thyroid hormones, particularly at the low end of the reference interval. Specifically, many patients are classified as having normal levels of thyroid hormones by the commonly used immunoassay methods that are actually below the reference interval by the reference method LC-MSMS. This overestimation by immunoassay can be significant; for example, a study of euthyroid inpatients placed on approximately six to eight medications found that 48% of T₃ values were below the 2.5th percentile by LC-MSMS compared with only 11% by immunoassay (45).

We hypothesize that many patients who still experience hypothyroid symptoms despite treatment with L-T₄ may actually have low levels of T₃ that are incorrectly classified by the common immunoassay methods. We recommend patients who continue to experience symptoms of hypothyroidism despite treatment with L-T₄ be evaluated for T₃, FT₃, and FT₄ measurement by LC-MSMS. This may identify patients who would benefit by addition of T₃ to their treatment regimen. Thyroid hormone measurement by LC-MSMS is available through most large commercial laboratories.

The following case reports are of complicated patients seen at the NIH that highlight the ability of ultrafiltration LC-MSMS to correctly classify their thyroid status. The first case highlights a patient with a protein-binding abnormality and the inability of immunoassays to correctly classify these patients (50). The second and third cases demonstrate patients who clinically benefited from measurement of thyroid hormones by ultrafiltration LC-MSMS.

Case report 1

The patient is a 30-year-old male with acquired immunodeficiency syndrome and Kaposi's sarcoma. The patient was clinically euthyroid with a TSH level within the reference interval (Table 1). However, free and total thyroid hormone levels measured by immunoassay were decreased. Laboratory medicine was consulted to provide an explanation for the decreased free and total thyroid hormones in the presence of a normal TSH. A TBG

Table 1 Laboratory values by immunoassay and LC-MSMS on the same sample for a 30-year-old euthyroid male with AIDS-related Kaposi's sarcoma.

	Value	Reference range
Immunoassay results		
FT4	0.66 ng/dL	0.8–1.5 ng/dL
FT3	1.41 pg/mL	1.8–4.2 pg/mL
TT3	42 ng/dL	90–215 ng/dL
TT4	3.1 µg/dL	4.5–12.5 µg/dL
TSH	3.52 µIU/mL	0.36–4.0 µIU/mL
TBG	4.3 µg/mL	13–39 µg/mL
LC-MSMS results		
FT4	1.8 ng/dL	0.8–2.0 ng/dL
FT3	1.7 pg/mL	1.5–6.0 pg/mL
TT3	25.2 ng/dL	80–187 ng/dL
TT4	2.4 µg/dL	4.9–10.5 µg/dL
rT3	10.7 ng/dL	9–21 ng/dL

AIDS, acquired immunodeficiency syndrome.

T₃ ng/dL × 0.0154 = T₃ nmol/L; T₄ µg/dL × 12.87 = T₄ nmol/L.

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level was ordered by the senior clinical chemistry staff. TBG was decreased at 4.3 µg/mL, indicating a protein-binding abnormality as the likely cause of the discrepant thyroid hormone values. Thyroid hormone measurement was repeated on the same sample by LC-MSMS, which showed FT₃ and FT₄ values within the reference interval, which correlates with the patient's clinical symptoms. This case example highlights the utility of LC-MSMS to accurately classify thyroid hormone status in the presence of severe protein-binding abnormalities. Also important is the inability of thyroid data generated by routine immunoassay platforms to correctly classify these patients.

Case report 2

A 68-year-old woman with a medical history of hypothyroidism reports feeling lethargic despite treatment with L-T₄. Her laboratory studies are shown in Table 2. Immunoassay results show thyroid function tests within normal limits. However, LC-MSMS showed TT₃ and FT₃ values below the reference interval. T₃ was added to the patient's regimen, with improvement of symptoms and normalized LC-MSMS measurements. The patient's cholesterol also decreased from 220 to 160 mg/dL after addition of T₃. Of note, the patient was found to be heterozygous for the D2 Thr92Ala polymorphism.

Case report 3

A 70-year-old woman with a history of Hashimoto's thyroiditis is taking LT-4, but reports feeling sluggish and

unwell. Immunoassay results for TSH, TT₃, TT₄, FT₃, and FT₄ were within normal limits, and thus, the patient's primary care physician was reluctant to change the LT-4 regimen. Ultrafiltration LC-MSMS performed at our institution revealed decreased TT₃ of 74 ng/dL (reference interval 80–187 ng/dL) and low-normal FT₃ of 1.8 pg/mL (1.5–6.0 pg/mL). T₃ was added to the patient's regimen, after which the FT₃ increased to 3.3 pg/mL and the patient's symptoms resolved.

There are several potential mechanisms to account for why certain patients, such as cases 2 and 3 reported above, continue to experience symptoms of hypothyroidism while on L-T₄ therapy and have normal levels of TSH. First, treatment with L-T₄ monotherapy may not result in sufficient peripheral conversion to T₃ to provide adequate amounts of T₃ at the tissue level. Patients with certain deiodinase polymorphisms have been shown to have reduced T₃ levels (51, 52). For example, the Thr92Ala polymorphism of D2 occurs at a frequency of 0.35 and is associated with reduced D2 activity and a decreased rate of T₃ release from thyroid-releasing hormone stimulation (53, 54). Secondly, the normalization of TSH that occurs during L-T₄ therapy is likely due to the known effect of T₄ as an important regulator of TSH release; however, TSH is not an indicator of the tissue bioavailability of active T₃ (48). LC-MSMS is a potential tool for the laboratory evaluation of patients who may have issues converting L-T₄ to bioavailable T₃.

In the USA, L-T₃ and L-T₄ combination therapy remains a controversial issue. The guidelines of the American Thyroid Association suggest L-T₄ monotherapy for the treatment of hypothyroidism, whereas the European Thyroid Association states that LT-3/LT-4 combination therapy may be used in selected patients (49, 55). Regardless, LT-3 and LT-4 combination is still considered an experimental treatment modality in the USA; however, this is not the case in many countries such as South Africa and parts of Canada, where combination therapy is routinely employed in around 20% of patients. The use of LC-MSMS may provide objective laboratory evidence to assist in the identification of the subset of patients who may benefit from combination therapy. Both the American and the European Thyroid Associations provide a set of research goals for optimizing therapy for patients with hypothyroidism. We recommend that future clinical trials evaluating LT-3 treatment incorporate LC-MSMS as a tool for identification of patients with issues converting L-T₄ to T₃ who may benefit the most by combination therapy.

Table 2 Laboratory values by immunoassay and LC-MSMS on the same sample for a 68-year-old woman with persistent symptoms of hypothyroidism while on L-T₄ therapy.

	Value	Reference range
Immunoassay results		
FT ₄	1.2 ng/dL	0.8–1.5 ng/dL
FT ₃	2.5 pg/mL	1.8–4.2 pg/mL
TT ₃	99 ng/mL	90–215 ng/dL
T ₄	8.0 µg/dL	4.5–12.5 µg/dL
LC-MSMS results		
FT ₄	1.2 ng/dL	0.8–2.0 ng/dL
FT ₃	1.5 pg/mL	1.5–6.0 pg/mL
TT ₃	64 ng/dL	80–187 ng/dL
TT ₄	7.2 µg/dL	4.9–10.5 µg/dL
rT ₃	8.7 ng/dL	9–21 ng/dL

T₃ ng/dL × 0.0154 = T₃ nmol/L; T₄ µg/dL × 12.87 = T₄ nmol/L.

There are a number of important limitations to the routine incorporation of thyroid hormone measurement by LC-MS/MS. First, the technique requires use of specialized and very expensive equipment. Secondly, trained and experienced personnel are required. Although thyroid hormone measurement by LC-MS/MS is available at most major commercial reference laboratories and many teaching hospitals, the cost is higher than measurement by immunoassays performed on automated platforms. Despite this higher cost, it does not come close to the cost of misdiagnosing a patient requiring small doses of T_3 . Finally, LC-MS/MS does have a longer turnaround time even if performed in-house and cannot provide rapid results in emergency settings.

Conclusions

Measurement of T_3 , FT_3 , and FT_4 by the immunoassay methods commonly used by the majority of laboratories is problematic, especially for low levels of thyroid hormones. A significant number of patients are misclassified as having normal levels of thyroid hormones when they actually have levels below the reference range by LC-MS/MS. There are approximately 19 million patients with hypothyroidism in the USA alone. The misclassification of patients by a commonly used laboratory strategy thus has the potential to adversely affect a substantial number of patients. Laboratory assays are expected to be accurate over the entire analytical range, especially at the high or low end of the range where clinical decision points are often made. Assays for TT_3 , FT_3 , and FT_4 should correlate with both log TSH and the patient's clinical condition, which is not achieved on any of the commercially available FDA-approved immunoassay platforms. LC-MS/MS provides better correlation with these parameters over the entire analytical range. We suggest evaluation of patients employing LC-MS/MS measurement of thyroid hormones when they continue to report symptoms of hypothyroidism despite therapy with LT-4. This approach may aid in identification of patients who may benefit from combination treatment with LT-3 and LT-4.

Declaration of interest

The authors have no conflicts of interest that could be perceived as prejudicing the impartiality of the review.

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