Case Report

Discordant TFT in Lupus Nephritis Patients: A Case Report and Comprehensive Literature Review

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Abstract

The thyroid function test (TFT) is usually easy to interpret, but only if the clinical assessment, thyroid-stimulating hormone (TSH), and free-T4 (fT4) levels are consistent with each other. However, there are cases where TFT results are inconsistent, which may be due to assay interference. In this regard, we presented a case study of a patient with discordant TFT results which was likely caused by assay interference. A 43-year-old woman with underlying lupus nephritis for 20 years presented with painless anterior neck swelling over three years duration. Clinically, she was euthyroid without any symptoms of thyroid gland compression. A series of TFT was requested to rule out hyperthyroidism, showing low normal fT4 ranging between 9.83 - 12.76 pmol/L (9 - 19.05 pmol/L) with suppressed TSH of 0.05 - 0.18 µIU/ml (0.35 - 4.94 µIU/ml), biochemically consistent with subclinical hyperthyroidism. The reflex free-triiodothyronine (fT3) test was normal at 3.85 pmol/L (2.63-5.7pmol/L) with negative anti-thyroglobulin and anti-thyroperoxidase antibodies. A neck ultrasound revealed four insignificant thyroid nodules. No hyperthyroid treatment was commenced except prolonged steroid therapy and azathioprine for the underlying autoimmune disease. An initial clinical assumption was made that the TFT result might be possibly due to assay interference. The TFT results led to several differential diagnoses: subclinical hyperthyroidism, nonthyroidal illness (NTI), recent hyperthyroid treatment, drug, or assay interference (biotin, anti streptavidin antibodies, macro-TSH, anti-ruthenium antibodies, thyroid hormone autoantibodies, and heterophilic antibodies) . The patient's normal fT3 ruled out NTI. Furthermore, the patient was not on any anti-hyperthyroid medications to explain it as the cause. Chronic glucocorticoid therapy reduces TSH secretion and inhibits thyroxine-binding globulin (TBG) synthesis, but steroids rarely result in thyroid dysfunction. Our laboratory method was a non-biotinylated chemiluminescent assay using the Abbott Allinity system, devoid of biotin-streptavidin interaction. The patient's results disagreed with other assay interference (macro-TSH, anti-ruthenium antibodies, thyroid hormone autoantibodies, and heterophilic antibodies), as they resulted in falsely high TSH and fT4 in most literature. Nonetheless, sample treatment with heterophile binding receptors can help to confirm further whether the patient's TFTs were consistent with true subclinical hyperthyroidism.

Keywords: Lupus nephritis; subclinical hyperthyroid; thyroid-stimulating hormone; thyroid dysfunction

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Introduction

The patterns of thyroid function tests are usually easy to understand and match the clinical assessment of the thyroid's condition. However, a noteworthy group of patients exhibit thyroid function test results that do not match the clinical presentation or are inconsistent with each other. For instance, some patients may have elevated thyroid hormones (TH) but with non-suppressed thyrotropin (TSH), while others may have

elevated TSH but with normal TH levels. We reported a patient with a known case of systemic lupus erythematosus (SLE) nephritis whose discordant TFT was thought to result from assay interference.

False positive, false negative or both types of results can be caused by interference, simulating a hormonal profile that appears coherent and may lead to needless investigations, improper therapies, or a missed diagnosis. The ultimate purpose of laboratory quality control initiatives (such as adhering to certification and regulatory requirements, maintaining instruments, and conducting internal and external review programmes) are quickly producing correct and dependable results. On the other hand, interferences in patient samples are repeatable within a specific analytical system and are not identifiable by typical quality control measures. Laboratory professionals must be skeptical of the data rather than putting their complete faith in the equipment. Understanding the physiology and normal fluctuations in biological markers is essential to determine whether a result that deviates from prior findings is acceptable. In addition, comprehensive understanding of analytical principles and assay design are crucial to interpreting laboratory results and identifying erroneous results correctly.

Type of hormone immunoassay interferences include pre-analytical factors, cross-reaction or assay specificity, hook effect, interfering antibodies, interference affecting immune complex separation, and interference affecting signal generation or quantification. Getting trustworthy lab results starts with pre-analytical conditions, such as the type of the tube (plasma vs serum, anticoagulant type for plasma, with or without a separating gel); the time of collection for hormones with time-dependent concentrations (e.g., testosterone and cortisol concentrations are higher in the morning, and estradiol concentrations depend on the day in the menstrual cycle); the temperature of storage and transportation (renin at 20–25°C, and adrenocorticotropic hormone (ACTH) at +4°C). Depending on the signal detection device and immunoassay design, lipids and bilirubin may also cause interference with immunoassays.

Second, the cross-reactants may be native or drug metabolites, metabolites, or precursors of analytes. Pegvisomant, a growth hormone (GH) analogue and receptor antagonist, and fulvestrant (in estradiol immunoassays) are two classical instances of this problematic interference. Third, sandwich immunoassays are affected by the hook effect. Sample dilution will show whether an excess analyte is present; serial dilutions will raise the analyte concentration until two coherent subsequent dilutions are produced.

Next are heterophile antibodies (HA), which are endogenous, weakly poly-specific, mostly human-derived, and thought to arise naturally. They can react with Abs from different species. As opposed to this, human anti-animal antibodies (HAAAs) are high-affinity, monospecific antibodies that might develop in autoimmune clinical settings, following exposure to animals or animal products, or as a result of bacterial or viral infections. HAAAs have a stronger avidity and are potentially species-specific, which sets them apart from HA. Human anti-mouse antibodies (HAMA), anti-rabbit, anti-goat, anti-sheep, anti-cow, anti-pig, anti-rat, and anti-horse, as well as those with mixed specificity, are examples of anti-animal antibodies (IgG, IgM, IgA class, anti-isotype, and anti-idiotype specificity). It is crucial to know whether you have previously received diagnostic or therapeutic antibodies; in theory, you should only use the word "heterophile" in cases where there is no indication of animal exposure. Nonetheless, their mode of interference is comparable to that of HA. In most instances, false-positive interference arises from an HA forming a bridge (cross-linking the two antibodies) between capture and labelled antibodies in sandwich immunoassays. While sandwich immunoassays are most affected by this interference, competitive immunoassays can also have falsely elevated results due to the interfering ability of antibodies to block the capture of Ab. In competitive immunoassays, the interfering anti-analyte antibodies can bind to the labelled analyte in vitro. Thyroid hormone auto antibodies can cause erroneously high findings in one-step free thyroid hormone immunoassays.

Interference influencing the dissociation of immune complexes, either Antistreptavidin antibodies (ASA) or biotin, may also play a role in this pathway. Finally, anti-ruthenium antibodies or some other unknown materials may be the interfering components as they reduce the signal and cause falsely high or low analyte concentrations. Other diseases that impact immunoassay include monoclonal gammopathies, binding proteins, free hormone measures, and complement.

Case Report

A 43-year-old single nulliparous female was diagnosed with SLE Nephritis in 1998. She had completed intravenous (IV) Methylprednisolone, Plasmapheresis, Immunoglobulin (IG) and four courses of Cyclophosphamide. She had multiple

relapses, which last occurred in 2012. Previously, she was on Prednisolone 30 mg OD, Imuran 5 mg OD and Azathioprine 50 mg OD; however, she was still persistently nephrotic hypo-complement. Renal biopsy was consistent with Crescentic Lupus with underlying membranous glomerulonephritis (GN) Class 4-5 and immunofluorescent (IF) positive for IgG, IgM C3 and C4 IgA. ANA, anti-dsDNA, rheumatoid factor and anticardiolipin IgG were positive.

She presented with neck swelling, painless but slight discomfort, increasing in size. There was the presence of fine tremors and clubbing but no other significant clinical findings. There was no family history of thyroid disease as well. No recent treatment for hyperthyroidism had been initiated. Clinically, her blood pressure (BP) was slightly hypertensive, 127-148/88-100, tachycardic, and pulse rate (PR) ranged from 92-126bpm.

A series of TFT was requested to rule out hyperthyroidism, showing low normal fT4 ranging between 9.83 - 12.76 pmol/L (9 - 19.05 pmol/L) with suppressed TSH of 0.05 - 0.18 µIU/ml (0.35 - 4.94 µIU/ml), biochemically consistent with subclinical hyperthyroidism. The reflex free-triiodothyronine (fT3) test was normal at 3.85 pmol/L (2.63-5.7pmol/L) with negative anti-thyroglobulin and anti-thyroperoxidase antibodies.

Other biochemical disturbances not involving the thyroid hormones, such as a slightly low calcium level of 2.12 mmol/L (N: 2.14-2.58), were of no diagnostic value. Thyroid ultrasound revealed homogenous nodules 1-4 and no retrosternal extension with vascularity.

No hyperthyroid treatment was commenced except prolonged steroid therapy and azathioprine for the underlying autoimmune disease. An initial clinical assumption was made that the TFT result may be due to assay interference.

Discussion

Several differential diagnoses were made based on the TFT results, including assay interference (biotin, anti-streptavidin antibodies, macro-TSH, anti-ruthenium antibodies, thyroid hormone autoantibodies, and heterophilic antibodies), recent hyperthyroid treatment, nonthyroidal illness (NTI), and subclinical hyperthyroidism (1, 2)

NTI, also known as sick euthyroid syndrome, is a common occurrence after any illness, whether it is acute or chronic. It is characterised by the absence of inherent abnormalities in Hypothalamus-pituitary-thyroid (HPT) function and is considered a secondary adaptive change. Changes in TH (particularly T3) and TSH have been reported as early as 24 hours after the onset of a non-thyroidal illness, as per Kaptein (1996) (3). These changes have also been observed in patients with post-surgery, chronic liver and renal disease, burns, cancer, myocardial infarction, and inadequate nutrition or starvation. Low (or low-normal) FT4 and FT3 are commonly obtained from many commercial assays for free TH, along with normal or low (but rarely entirely suppressed) TSH. Reduced serum TH binding capacity in acute and chronically ill patients is likely due to a fall in TH binding protein concentrations and/or impaired T4/T3 binding. In mild NTI, reductions in total thyroxine (TT4), especially total triiodothyronine (TT3), are expected and usually more pronounced than the corresponding decreases in free hormone concentrations (4). In the context of biochemically subclinical hyperthyroidism, our patient's normal fT3 ruled out NTI.

Moreover, the patient's absence of anti-hyperthyroid medication did not support this diagnosis. Steroids seldom cause thyroid dysfunction. However, long-term glucocorticoid medication may further reduce pituitary TSH secretion and limit the synthesis of thyroxine-binding globulin (TBG).

Treatment with azathioprine did not significantly affect these measures, but it did cause a significant drop in thyroid microsomal antibodies and the immunoglobulin index that inhibits thyroid-stimulating hormone binding (5). Human serum TSH levels have long been recognised to be impacted by glucocorticoids (6,7). The diurnal variation in serum TSH levels seems to be influenced by physiological levels of hydrocortisone, which are more significant at night and decrease in the morning (7). Wilber and Utiger showed high doses of glucocorticoids to suppress serum TSH in both normal persons and hypothyroid patients (8). This effect involved TSH secretion and was regulated at the hypothalamic level. Although others have verified this effect, clinically noticeable central hypothyroidism necessitating thyroid hormone replacement does not seem to be caused by long-term high-dose glucocorticoids or Cushing's syndrome cortisol excess. (6,9). Serum TSH levels can be dramatically lowered by dexamethasone doses as low as 0.5 mg, but considerable TSH level alteration is probably dependent on 30 mg of prednisone (6). Through the protein annexin 1, glucocorticoids appear to inhibit the release of TSH from thyrotropes in a PKC-dependent manner (10). Glucocorticoids most likely suppress TRH in the hypothalamus, affecting TSH secretion. The

paraventricular nucleus (PVN)'s TRH neurons contain glucocorticoid receptors, and the TRH gene has a glucocorticoid response element identified in it. More recently, Alkemade and associates have demonstrated that high doses of glucocorticoids suppress human hypothalamic TRH mRNA levels, which is probably the primary mechanism underlying the pituitary's decreased secretion of TSH (11).

In conclusion, glucocorticoids directly affect TRH in the hypothalamus, which lower blood TSH levels and TSH secretion. Fortunately, even after chronic high-dose use, the commonly used glucocorticoids and somatostatin analogues do not cause clinically noticeable central hypothyroidism (12).

Six primary forms of interference can affect measurements of thyroid stimulating hormone (TSH), free thyroxine (T4), and free triiodothyronine (T3), including biotin, anti-streptavidin antibodies, macro-TSH, anti-ruthenium antibodies, thyroid hormone autoantibodies, and heterophilic antibodies. Using the Abbott Allinity system, we performed a non-biotinylated chemiluminescent experiment in our lab. The sample, paramagnetic microparticles coated with anti-β TSH antibody, and TSH assay diluent were mixed and incubated for TSH. The anti-TSH antibody-coated microparticles binded to the TSH in the sample, and the mixture was washed. Conjugate labelled with anti-α TSH acridinium was added to make a reaction mixture and was incubated. Pre-Trigger and Trigger Solutions were added after a wash cycle. Relative light units (RLUs) measure the chemiluminescent reaction that resulted the reading. The system optics picked up RLUs in direct proportion to the sample's TSH quantity.

For fT4, paramagnetic microparticles coated with anti-T4 and the fT4 sample were mixed and incubated where the anti-T4-coated microparticles bonded to the free T4 in the sample. The blend was cleaned. To make a reaction mixture, T3 acridinium-labelled conjugate was added and then incubated. Pre-trigger and Trigger Solutions were added after a wash cycle. Relative light units (RLUs) measured the chemiluminescent reaction that resulted the reading. The quantity of free T4 in the sample and the RLUs picked up by the system optics were inversely correlated.

The two-phase conjugate acridinium labelling method:

1. Step 1: Combine the sample and paramagnetic microparticles coated with an antibody; incubate. The sample's analyte attaches itself to the microparticles coated with antibodies. The blend is cleaned.
2. Step 2: Add the conjugate labelled with acridinium to form a reaction mixture, then incubate. Pre-Trigger and Trigger Solutions are added after a wash cycle. Relative light units (RLUs) measure the chemiluminescent reaction that results.

It's worth noting that biotin has been observed to cause interference on several immunoassay systems. In TSH sandwich assays, excess biotin dislodges biotinylated antibody-antigen complexes from streptavidin-coated microparticles, which leads to artificially low TSH levels. Conversely, in competitive assays of FT4 and FT3, excess biotin causes an overestimation of both hormones because the signal is inversely proportional to hormone concentrations. It is important to remember that the effects of biotin depend on the platform being used. High biotin levels on Roche platforms may affect TSH, FT4, and FT3, while only TSH can be lowered on Ortho Clinical Diagnostics platforms (Raritan, NJ) because FT4 and FT3 do not use the biotin-streptavidin interaction.

On the other hand, TSH is unaffected by increased FT4 and FT4 on Beckman Coulter Diagnostics platforms (Brea, CA). Interestingly, Siemens Healthcare, Erlangen, Germany's Centaur FT4 platform, employs a premade streptavidin-biotin complex insensitive to biotin. Since TSH, FT4, and FT3 measurements do not use the biotin-streptavidin immobilisation technique, biotin does not affect Abbott or DiaSorin (Saluggia, Italy) immunoassays either. Consequently, selecting one of these final three platforms could be the best approach for determining biotin interference indirectly. Our lab used the Abbott Allinity system for a non-biotinylated chemiluminescent test, which includes no biotin-streptavidin interaction. This interference has been identified by method comparison, Heterophilic blocking tube (HBT), dilution test, drug anamnesis, and biotin withdrawal.

Anti-streptavidin interference is similar to biotin interference, causing an increase in FT4 and FT3 levels and low TSH levels. This interference can last up to 18 to 24 months, making washout periods ineffective for TSH immunoassays. A different platform such as DiaSorin or Abbott can be used to determine interference. The PEG precipitation process and dilution test have been used. Incubating the serum with streptavidin-linked agarose is another approach, but it is rarely used (11). Therefore, in ordinary practice, using the streptavidin beads provided by the manufacturer may be preferable. The HBT, aliquot to Roche Diagnostics, dilution test, and HAMA blockers helped identify this interference.

The patient's results disagreed with other assay interference (macro-TSH, anti-ruthenium antibodies, thyroid hormone autoantibodies, and heterophilic antibodies), as they resulted in falsely high TSH and/or fT4 fT3 in most literature. Macro-TSH is a large molecule that can accumulate in the blood and cause false readings of elevated TSH levels. This is in contrast to TSH, a small bioactive hormone that is easily filtered by the kidney. Macro-TSH is essentially an inactive version of TSH, comprising autoimmune anti-TSH antibody complexes with monomeric TSH. It is mostly made up of IgG-bound TSH, and can be identified through gel filtration chromatography (GFC). The prevalence of macro-TSH ranges from 0.6% to 1.6%.

Like macro-prolactin (macro-PRL), macro-TSH is considered inactive due to its high molecular weight, which restricts it to the intravascular compartment. Autoantibodies linked to TSH may also inhibit TSH receptor activation. Therefore, it is advisable to screen for macro-TSH using a TSH concentration of >10 mUI/L in conjunction with normal thyroid hormones. This can help identify interference in patients without symptoms or signs of thyroid dysfunction, who have an isolated rise in TSH (usually significantly raised) and THs in the upper half of the normal range.

While some authors have suggested a limit of <20% or <25% for macro-TSH, others have indicated a 40% cutoff for macro-PRL. GFC is still the recommended technique for diagnosing macro-TSH, and limited recovery following PEG treatment should always be verified by GFC.

Anti-Ru interferences have been found to cause lowered TSH and/or high FT4 or FT3 levels more frequently (20 of the 22 cases reported), although they can also cause elevated TSH and decreased FT4 or FT3 levels. According to the manufacturer, in a competitive assay, the amount of light released during electrochemiluminescence is inversely proportional to the FT4 or FT3 concentration in the sample; in a one-step sandwich test, it is directly proportional to the TSH level. Following the launch of Roche Diagnostics' FT3 assay, multiple observations of high FT3 concentrations without the anticipated TSH suppression were documented by Sapin et al. (2007) (14). One possible explanation for the fact that only FT3 was affected by this interference is that FT3 experiments utilised fewer Ru-labelled antibodies. Roche Diagnostics introduced free Ru crosslinkers, a new blocking protein, to FT3 tests in 2006 in response to this interference, and they discovered that this new formulation reduced the amount of false-positive results in most samples. In these patients, PEG precipitation helped to reduce (or return to normal) the signal, indicating that immunoglobulins could be the form of the interfering agent.

Additionally, the FT3 results were lower when using a different non-Ru technique. Although not consistently, using next-generation assays significantly decreased vulnerability to anti-Ru interference. There are reports of precipitation working, though only sometimes. This interference was also identified by method comparison, dilution test, mouse serum incubation, streptavidin beads, HBT, and aliquot forwarded to Roche.

Antibodies against microsomal thyroid peroxidase, TSH receptor, thyroglobulin, and THAAbs (mostly against T4 and T3) have also been reported. IgG isotypes known as THAAbs, which exhibit a polyclonal autoreactive reaction, are more common in individuals suffering from autoimmune diseases. Thyroid peroxidase or thyroglobulin antibodies have been detected in THAAbs-positive samples in as much as 80% to 100% of cases. Therefore, if any interference is seen in individuals with autoimmune illnesses, screening for THAAbs should be done. Theoretically, only one-step immunoassays (e.g., Siemens Healthcare's Immulite 2000 and 2500, Advia Centaur, Tosoh AIA 1800, Tokyo, Japan) are likely to be impacted by THAAb interference; comparing these results to a two-step immunoassay (e.g. Abbott AxSYM or Architect, Beckman DXI 800 or Access (Beckman Coulter), Immunotech radioimmunoassays, RIA-gnost (Cisbio Bioassays, Codolet, France)) is most likely the first worthwhile course of action. Since THAAbs are mostly made up of IgG subclasses, treating serum with protein G (or protein A), Sepharose beads may also be helpful. The dilution test may also be employed in certain situations, although it should not be done so exclusively. The more intricate and focused technique of radioimmuno-precipitation is used to identify THAAbs. This approach is challenging to implement since it relies on radioactivity and its detection. Because of this, several writers have suggested using the considerably simpler PEG precipitation method to measure any hormone level drops that may occur after treatment. TSH testing is the most accurate thyroid function test in people with THAAbs. Ignorance about THAAbs may result in incorrect Graves' disease diagnosis and treatment.

While heterophilic antibodies are weak, polyspecific antibodies that are generated early in the immune response before affinity maturation, HAAAs are high-affinity, monospecific antibodies targeted against animal epitopes from mice, goats, rabbits, sheep, horses, or other animals. Usually, they react to

immunoglobulins that come from two or more different species. This also includes rheumatoid factor, which exhibits cross-reactivity with animal antibodies by reacting with the Fc region of human immunoglobulins. The phrase "heterophilic antibody" is commonly used in laboratory practice to describe any suspicion that an assay antibody in a patient's sample is causing misleading results. Whereas RFs are primarily of the IgM isotype, heterophile Abs might be of the IgG, IgM, or IgA isotype. Depending on the interference site within the process, heterophilic antibody-induced interference might result in falsely high or low analyte levels in one or more test methods. The literature more frequently reports mistakenly raised readings than falsely low ones, while few cases of falsely low values due to heterophilic antibody interference have been recorded. While FT3 and FT4 assays are less susceptible to these interfering substances, two-site immunoassays (usually TSH assays) are more sensitive to heterophilic antibodies. In 20 out of the 38 investigated cases, a comparison against an assay that used a different antibody species was beneficial, whereas, in 30 out of 32 cases, the dilution test revealed interference. You can alternatively utilise the heterophilic blocking tube (HBT) test to get around this interference. Heterophilic antibodies are rendered inactive by a blocking reagent in HBTs comprising certain binders. In addition to using F(ab')2 fragments for the solid phase, manufacturers have developed strategies to eliminate these interferences, such as the addition of heat-aggregated, nonspecific murine monoclonal anti-bodies, trace amounts of animal serum of the same species used in assay reagents, and nonspecific animal immunoglobulins. Even these methods work well in most situations, some sera have extremely high concentrations of interfering antibodies, which could still cause problems for the experiment.

Other interferences include variants of TH transport proteins, such as T4-binding globulin (TBG), transthyretin (TTR), and human serum albumin (HSA), are additional sources of interference. The equilibrium between T4 or T3 and their binding proteins is also impacted by medications like aspirin, furosemide, carbamazepine, phenobarbital, phenytoin, nonsteroidal anti-inflammatory drugs, phenylbutazone, and heparin (fractionated or unfractionated). Paraproteins and TSH variations can also cause immunoassay interference.

As new treatments are developed, newer interferences are also becoming apparent. Positive or negative bias can result from interference, and the interfering material can be either endogenous (such as antibodies made by the patient) or exogenous (such as a medicine or other substance taken by the patient). When doctors use inaccurate test data to diagnose, interference can have negative effects by pushing for pointless investigations or unsuitable therapies. Manufacturers and clinical laboratories are still researching ways to identify, remove, and avoid interferences. But there will always be these kinds of instances in any system. While a positive test result is likely suggestive of an interference, a negative test does not rule out one. In this text, we will discuss the common tests that are used to screen for interferences in current immunoassays. Furthermore, we will propose an algorithm to help with this screening process.

Frequently employed procedures to identify interferences in the current immunoassays and suggested algorithms (Fig. 1) include size-exclusion, doubling serial dilution, adding blocking agents and depleting interfering antibodies through polyethylene glycol (PEG) or ammonium sulfate precipitation, affinity extraction using protein G or A columns (e.g., Sepharose linked), and repeating the analysis using the same and another assay method. Several other tests, including treatment with streptavidin beads, immunofixation and electrophoresis, ammonium sulfate precipitation, incubation with a sample from a patient with hypothyroidism (high TSH), measurement of T4 and TBG to suspect FDH, THAAb, or the heparin artefact, molecular genetic testing for FDH, and heating to 70°C to 90°C (for heat-stable analytes only), have also been successfully employed.

In order to identify interferences in laboratory samples, additional testing is usually only required for samples that show signs of interference. There are two methods that can be used together to detect interferences. The first method involves performing a set of tests (such as method comparison, dilution test, and HBTs) recommended by Ismail et al. (2022) (15). The second method involves screening for macro-TSH when the TSH levels are increased, based on the understanding of the interference pattern (e.g., by using PEG precipitation). While some tests, such as HBTs, dilution tests, and PEG precipitation, are simple and easy to perform, other tests, such as affinity extraction and size-exclusion chromatography, require specialised laboratories. When used together, the comparison method, dilution test, and blocking agents can identify antibody interference in approximately 90% of suspected samples.

Repeating the analysis using the same methodology as the last option is common practice. In numerous instances, inaccurate results could have been produced by pipetting issues, insufficient washing, tracer particles, or bubbles. Heterophilic interference, a

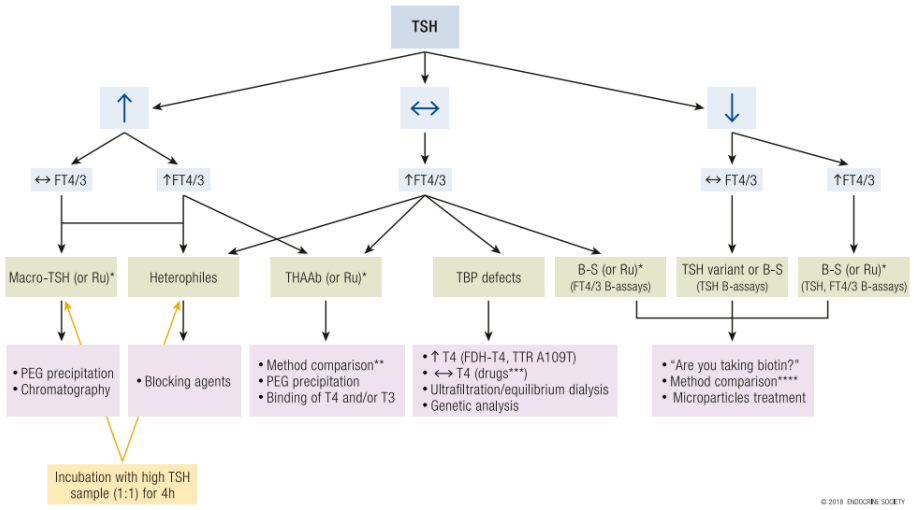

FIGURE 1: Proposed algorithm to screen for common thyroid interferences. B-S=biotin-streptavidin immobilization system (biotin or antistreptavidin interferences); Ru=ruthenium; TBP=thyroxine-binding proteins; *only Roche platforms are affected and that method comparison with another platform not using the ruthenium label is advised; **if available, a comparison against equilibrium dialysis represents the best choice; ***e.g., heparin (fractionated or unfractionated), furosemide, carbamazepine, or phenytoin; ****assays not affected by biotin or antistreptavidin antibodies should be preferred

distinct immobilising system for biotin or anti-streptavidin antibodies, a different detection system for anti-Ru antibodies, and variations between one- and two-step immunoassays for THAAbs are all suggested by a technique utilising antibodies from other animal species. In a doubling serial-dilution investigation, an interfering agent with concentrations at one-half, one-fourth, and one-eighth can skew linearity and impair parallelism.

Heterophilic antibody screening has been made easier using the commercially available HBT (Scandibodies Laboratories, Santee, CA), which uses a standardised methodology. In short, 500 µL of the sample is put into the blocking tube along with a blocking reagent pellet. After a gentle mixing, the tube sits at room temperature for an hour. Only a large departure from the first result should be interpreted as heterophilic interference after the sample is retested. Sometimes, HBT with smaller sample quantities (250 microL, for example) can help determine high heterophilic antibody titers.

Interfering antibodies can be reduced or eliminated via precipitation, affinity extraction, or size-exclusion techniques. Protein precipitation occurs when PEG, or the less common (NH4)2SO4, decreases the solubility of proteins in plasma or serum. Macro-PRL and macro-TSH have been successively screened for using the PEG precipitation technique. All antibody-related interferences, such as THAAbs, heterophilic anti-Ru, or anti-streptavidin antibodies, are also handled using this technique. With a high affinity, immunoglobulins can be bound by protein G or A columns (such as Sepharose linked). As a result, IgG may be extracted from serum or plasma, and a subsequent eluent test could be used to indirectly identify the interference caused by heterophilic, anti-streptavidin, or macro-TSH THAAbs. The screening of macro-PRL and macro-TSH has made considerable use of GFC. However, if the patient's TFTs are compatible with real subclinical hyperthyroidism, sample treatment with the mentioned tests can aid in additional confirmation.

Conclusion

In most cases, thyroid function test results are easy to understand and align with the clinical assessment of thyroid health. However, there is a small but significant minority of individuals who may have inconsistent or conflicting test results, which could lead to unnecessary or improper treatment and investigation. In such situations, it is important to take a systematic approach to further evaluation. One should exclude any confounding factors such as physiological changes related to aging, pregnancy, non-thyroidal illness, or medication use. One should also work closely with the clinical biochemistry laboratory to rule out any interference from thyroid

hormone and TSH assays. Only after these steps have been taken, then should consider researching uncommon acquired or genetic causes of abnormal or discordant TFTs.

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