A Challenging Case of Falsely Elevated Free Thyroid Hormones

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CASE DESCRIPTION

The patient was a 73-year-old woman suffering from a right calcaneus (heel bone) fracture. She was treated with nebivolol, olmesartan, hydrochlorothiazide, and simvastatine for hypertension and hypercholesterolemia. The patient had no other relevant past history. At presentation in October 2018, concentrations of both free thyroxine [FT4; reference range (RR) 12.0–22.0 pmol/L] and free triiodothyronine [FT3; RR 3.1–6.8 pmol/L] were markedly increased (>100.0 and 13.3 pmol/L, respectively) but thyroid stimulating hormone (TSH) was not suppressed [1.12 mU/L (RR 0.27–4.20 mU/L)] and the patient was clinically euthyroid.

Such a pattern of normal TSH associated with markedly elevated FT4 and/or FT3 concentrations in a patient who is clinically euthyroid may be related to disorders of thyroid hormone transport, metabolism or action, acute L-thyroxine (over)administration, or interfering drugs (i.e., amiodarone, heparin) *(1)*. As the patient's condition was not suggestive of any of the above-mentioned conditions, an interference involving our assay was suspected and several procedures were undertaken to confirm and characterize this potential interference *(2)*. Most procedures were performed on 10 healthy volunteers to obtain specific post-test reference values.

In our laboratory, TSH is measured with a two-site electrochemiluminescent immunoassay (ECLIA) using a ruthenium (Ru) label, whereas FT4 and FT3 are measured by a competitive ECLIA also using a Ru label, both assays being run on a Cobas 8000 e602® module (Roche Diagnostics®). Sheep polyclonal antibodies are used for FT4 and FT3 measurement and mouse antibodies for TSH.

We first measured serum TSH, FT4, and FT3 concentrations of the patient in another laboratory using a different method, namely the Vitros 5600® (Ortho Diagnostics®). This assay uses chemiluminescent technology involving a luminol-derived molecule and peracid salt as luminescent materials. Results were within normal ranges for TSH and FT4 and slightly below the lower limit of the RR for FT3 (Table 1A). Furthermore, we also sent the patient's sample to a third laboratory using the Architect i2000® (Abbott®) for TSH, FT4, and FT3 testing. This assay uses chemiluminescent technology involving acridinium labeled conjugate

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Table 1. (A) Comparison of TSH, FT4 and FT3 values obtained with the Cobas e602, Vitros 5600, Architect i2000, and Centaur XP immunoassays. (B) Hormonal results obtained after serum treatment with heterophilic blocking tubes (HBT), streptavidin beads, and PEG precipitation procedure on the Cobas e602. The absolute bias from baseline (in %) along with reference ranges obtained in healthy volunteers (in % changes) are presented between round and square brackets, respectively.

(A)	Cobas e602	Vitros 5600	Architect i2000	Centaur XP
TSH, mU/L	1.12 (0.27–4.20)	1.84 (0.46–4.68)	1.63 (0.35–4.94)	1.50 (0.27–4.78)
FT4, pmol/L	**>100.0** (12.0–22.0)	17.8 (10.0–28.2)	14.1 (10.7–18.6)	18.7 (12.0–22.0)
FT3, pmol/L	**13.3** (3.1–6.8)	**4.1** (4.3–8.1)	3.2 (2.7–6.9)	**2.9** (3.1–6.8)
(B)	**TSH, mU/L (0.27–4.20)**	**FT4, pmol/L (12.0–22.0)**	**FT3, pmol/L (3.1–6.8)**	
First sample (October 2018)	1.12	**>100.0**	**13.3**	
After HBT	0.91 (−**18.8**%) [3.0 to 16.9%]	**>100.0** (0.0%) [−6.5 to 5.0%]	**18.1** (**26.5**%) [−6.8 to 7.0%]	
Streptavidin beads	1.02 (-8.9%) [−11.8 to 13.4%]	**>100.0** (0.0%) [−5.1 to 7.5%]	**14.3** (**-7.5**%) [−6.4 to −4.3%]	
Second sample (January 2019)	1.00	**>100.0**	**10.8**	
Streptavidin beads	0.94 (-6.0%) [−11.8 to 13.4%]	**>100.0** (0.0%) [−5.1 to 7.5%]	**10.9** (**-0.9**%) [−6.4 to −4.3%]	
Streptavidin beads (higher concentration)	0.98 (2.0%)	**>100.0** (0.0%)	**10.8** (0.0%)	
Post PEG[a]	1.13 (**13.0**%) [−43.8 to 2.0%]	**36.0** (−**64.0**%) [54.5 to 71.0%]	5.9 (−**45.4**%) [36.0 to 58.9%]	

[a] Multiplied by 2 to take the dilution titer into account.

and gave normal results for TSH, FT4, and FT3 (Table 1A). A biotin interference was first suspected given that the Vitros 5600 and the Architect i2000 assays do not use the streptavidin-biotin immobilization system for the measurement of FT4 and FT3. However, the patient had never taken biotin supplementation.

The patient's sample was sent to a final laboratory using another different platform (Centaur XP®, Siemens Healthcare) for TSH, FT4, and FT3. This assay uses chemiluminescent technology, use the avidin-biotin immobilization system, and involves an acridinium ester for detection. Results were within normal ranges for TSH and FT4 and slightly below the lower limit of the RR for FT3 (Table 1A).

An additional procedure recently published by Piketty et al. *(3)* was also performed. Briefly, streptavidin beads (0.72 mg/mL in HEPES–bovine serum albumin buffer, pH 7.4; reagent included in the Cobas® assays kits supplied by Roche) were incubated for 1 h at room temperature with the serum of the patient. The treated serum was then retested on the Cobas e602 after removal of beads by centrifugation. This method was designed to identify biotin interference (high affinity to streptavidin) but also appeared to be useful to identify anti-streptavidin antibodies in the past

(4, 5). However, we did not observe significant changes in the TSH and FT4 levels, and the FT3 level was still elevated following the treatment (Table 1B). The presence of biotin interference or anti-streptavidin antibodies was therefore unlikely.

We next treated the serum of the patient with heterophilic blocking tubes (HBT, Scantibodies Laboratory, Inc.), which contain binders that can inactivate heterophilic antibodies. After this procedure, no significant change was observed in FT4 value whereas small changes were found for TSH and FT3 levels that were significantly different to those observed in healthy volunteers. Nevertheless, TSH and FT3 concentrations after the HBT test were still normal and elevated, respectively, and although the presence of some heterophilic antibodies could therefore not be ruled out completely, it did not seem to be clinically relevant (Table 1B).

In January 2019, a follow-up sample from the same patient was again sent to our laboratory. The same interfering pattern was observed: normal TSH associated to an increase in FT4 and FT3 (Table 1B). The polyethylene glycol (PEG) precipitation procedure was used with this sample. Briefly, 200 μL of PEG solution was added to 200 μL of serum sample, briefly vortexed-mixed, and centrifuged 2 min at 19,744*g* (6). The PEG reagent consisted of 25% (w/w) PEG 6000 (Sigma-Aldrich). As compared to healthy volunteers, the results obtained after the procedure in our patient revealed a clear decrease in FT4 and FT3 levels suggesting that the interference was related to the presence of antibodies (Table 1B).

A treatment with streptavidin beads was also performed in the follow-up sample. First, the same procedure as described above was realized (3). Next, an increased quantity of streptavidin beads (1 volume of serum sample + 20 volumes of streptavidin beads solution) was used in a similar procedure, based on the hypothesis of Berth et al. (7) that a higher ratio might be useful to search for anti-streptavidin antibodies. Hormone results were unchanged after both procedures and this confirmed that the probability of an interference with biotin or anti-streptavidin antibodies was very low (Table 1B).

We also sent an aliquot to Roche Diagnostics. However, they were not able to confirm the presence of anti-Ru antibodies.

DISCUSSION

Interferences affecting both FT4 and FT3 have been previously related to biotin, anti-streptavidin antibodies, heterophilic antibodies, thyroid autoantibodies (THAAbs), and anti-Ru antibodies (5, 8, 9). In our study, the interference was likely due to anti-Ru antibodies given that (1) the Cobas FT3 and FT4 assays are not known to be sensitive toward THAAbs, (2) that we excluded the presence of biotin, anti-streptavidin antibodies, and heterophilic antibodies (at least in significant amounts), and (3) that normal of near-normal hormone results were obtained with other platforms tested (Centaur XP, Vitros 5600, Architect i2000) not using the Ru label (9). The possibility of an interference involving human anti-sheep antibodies is also unlikely given that the Vitros 5600 and the Architect i2000 also use anti-sheep antibodies for the measurement of free thyroid hormones.

The prevalence of anti-Ru interference has been estimated to range from <0.1 to 0.24% (9). Ru (^{44}Ru) is a chemical element and rare transition metal belonging to the platinum group. Ru is mainly used as a chemical catalyst in electrical contacts, thick-film chip resistors, and platinum alloys. Ru can also be found in clothing residues and in the food chain (9). Roche Diagnostics has extensively used Ru as a label in its immunoassays based on ECLIA technology.

Ruthenylated anti-T4/T3 antibodies and the Ru crosslinker complex have been reported and may therefore be targets of anti-Ru antibodies (10, 11). The suggested mechanism of interference consists of a decreased signal due to prevention of [FT4*–

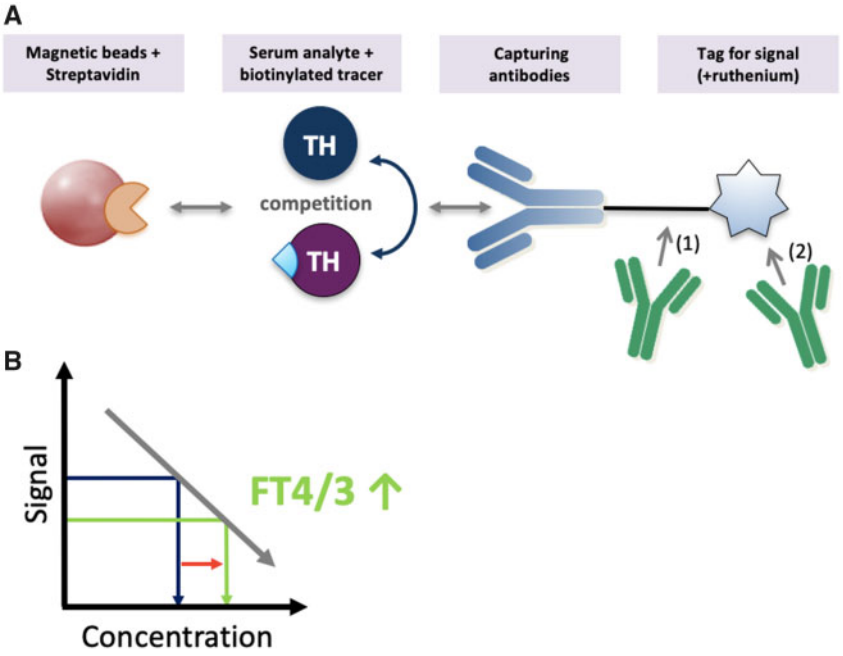

Fig. 1. (A) Schematic principle of competition for the measurement of FT4 and FT3. Interfering antibodies (anti-ruthenium antibodies represented in *green*) may target the ruthenylated anti-T4/T3 antibodies *(1)* and/or the Ru crosslinker complex *(2)*. Interfering antibodies prevent the binding of the labeled tracer. (B) Relationship between the signal and the concentration of FT4/3 in absence of the interference (*blue*) and in presence of the interference (*green*). The gray line represents the calibration curve.

anti-FT4] and [FT3*–anti-FT3] binding, resulting in falsely elevated free hormone concentrations *(9)* (Fig. 1). Even if most published cases reported increased levels of FT4 and FT3, falsely low levels of TSH or FT4 and FT3 as well as falsely high TSH levels have also occasionally reported (*8*, *12*–*14*). The impact of Ru interference could therefore be considered as heterogeneous *(9)*. The method comparison with another manufacturer not using the Ru label (i.e., Abbott and Ortho Diagnostics) is a valuable option when there is suspicion for anti-Ru antibody interference. We still do not know why this patient developed this interference and no particular exposure to Ru-containing devices was noticed.

Fortunately, clinicians were quickly warned about the presence of interference and further investigations related to thyroid dysfunction were immediately stopped. An alert comment is now present in the patient's clinical file to avoid future potential harmful consequences.

The occurrence of analytical errors has drastically decreased over the past 50 years *(15)*. Nevertheless, it is important to remember that all immunoassays are still subject to interference that cannot be detected by quality control procedures. The detection of thyroid function test interferences is critically important because of the clinical impact of this phenomenon: at least 50% of 150 cases of thyroid test interferences reported between 1981 and 2017 led to misdiagnosis and inappropriate management by clinicians (i.e., prescription of L-thyroxine, TRH stimulation tests,

thyroid scans (mostly radioactive), and the prescription of antithyroid drugs) (9). The continuous discussion between clinicians, biologists, and manufacturers is therefore paramount to avoid unnecessary investigations, inappropriate treatments, and contribute to the improvement of in vitro diagnostic reagents.

TAKEAWAYS

- Discrepancy between clinical findings and biochemical parameters raises suspicion for the presence of assay interferent.
- The prevalence of anti-Ru interference has been estimated to range from <0.1% to 0.24%
- A single test is rarely sufficient to identify interferences
- The reporting of interferences is the responsibility of the clinical laboratory
- Ongoing communication among biologists, clinicians, and manufacturers is essential to identify and prevent such interferences

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