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Avoiding Misdiagnosis Due to Antibody Interference with Serum Free Thyroxin

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Abstract

Introduction: Interfering antibodies are capable of causing potentially misleading results in automated thyroid hormone immunoassays.
Case Presentation: We report the case of a 46- year-old female patient with autoimmune hypothyroidism in chronic replacement treatment with levothyroxine who was presented 8 years after diagnosis with a thyroid function test showing an increased level of TSH and a very high level of FT4. Interference in the laboratory serum free thyroxin (FT4) test was suspected, due to the lack of symptoms of hyperthyroidism and a different immunoassay platform confirmed a low FT4 result. The discrepancy between the two results was explained by the presence of antiT4-autoantibodies.
Conclusions: Antibody interference with serum free thyroxine must be considered when clinical findings and laboratory results show discrepancies.

Keywords: Free Thyroxine, Antibody, Immunoassay, Interference

1. Introduction

Interferences in thyroid hormones assays have been previously reported on numerous occasions (1-3). Clinicians should be aware of their occurrence and should suspect them because they can result in abnormal concentrations of thyroid hormones and can be inconsistent with patient clinical status. Prompt communication between clinicians and laboratory professionals can avoid unnecessary diagnostic procedures and treatments.

2. Case Presentation

We report the case of a 46- year-old woman with falsely elevated FT4. She had no family history of thyroid disease and suffered from hypertension and dyslipidaemia. She was an active smoker and was having peri-menopausal symptoms. No other relevant medical conditions were present. She was first presented with a serum thyrotropin (TSH) level of 20.95 μUI/mL (0.5 - 5) and a serum free thyroxin (FT4) level of 7.7 pg/mL (8 - 18). The analysis was performed on Siemens Healthcare Reagents Kit in Advia centaur platform. Thyroid peroxidase and thyroglobulin auto-antibodies levels were positive; a thyroid ultrasound showed no goiter. The patient was diagnosed with clinical autoimmune hypothyroidism and was treated with 75 micrograms of levothyroxine daily. She was discharged from the endocrinologist to her primary care physician. Annual thyroid function tests were carried out and the results were within normal range, so the dose of levothyroxine remained unchanged.

Eight years after she was first diagnosed with hypothyroidism, she showed a FT4 level of 51.7 pg/mL (8 - 18), a free triiodothyronine (FT3) level of 2.8 pg/mL (2.3 - 4.2) and a TSH level of 7.393 μUI/mL (0.5 - 5) in one of the annual routine thyroid tests performed by her primary care physician. The results were confirmed by a repeated test three weeks later. On the basis of these results, her primary care physician recommended a reduction in the dose of levothyroxine from 75 micrograms to 50 micrograms and referred the patient to the endocrinologist for further assessment. On examination, the patient had a body mass index of 35 kg/m^2, no palpable goiter, a blood pressure of 129/88 mmHg and a heart rate of 98 bpm. She had recently increased her weight, to the extent of 7 kilograms in 6 month and she did not indicate tremor, palpitations, sweating, insomnia, diarrhoea or any other features of hyperthyroidism, in spite of the very high FT4 levels. She also denied having headaches or visual field problems that could have been caused by a TSH-producing pituitary adenoma. The test results in the past and the absence of family history of thyroid problems made the diagnosis of thyroid hormone resistance unlikely. Furthermore, the normal level of FT3 in the presence of high levels of FT4 pointed to the possibility of interference in the FT4 quantification.

With the suspicion of interference in the FT4 quantification, the laboratory chemist was contacted and a second FT4 test, in a specimen collected 20 days after the previous one, was analyzed in parallel in two different platforms. Blood specimens were collected from the patient between 8 - 10 hours AM after overnight fasting and 24 hours after the last dose of levothyroxine. Sera of patient were left at room temperature 20 minutes, followed by centrifugation at 3000g for 15 minutes. The sera were then analyzed or immediately stored at -70°C for seven days. First, an Advia centaur platform with a Siemens Healthcare Reagents Kit (Method 1) was used with quality control material freeze-dried and human based "Immunoassay Plus Control" from Biorad and a within-run CV < 5% and inter-run CV < 8%. Second an alternative immunoassay platform Cobas e 411 with Roche Diagnostics Corporation (Indianapolis) Reagent kit (Method 2) was used with quality control material human based and freeze-dried Precinorm U from Roche Corporation and an intra-assay CV < 8.11% and an inter-assay CV < 11.2%. The TSH coefficient of variation intra-assay and inter-assay in Advia Centaur Platform were < 2.8% and < 4.28%, respectively. Both Roche Cobas "e" and Advia Centaur are competitive one-step immunoassay for FT4 quantification but differ in reagents labeling. In Advia Centaur, the labeling is an analogue of T4 hormone, labelled with acrydinium-ester, which compete with the patient's FT4 for a little quantity of polyclonal rabbit antibody bound to a solid phase. However, in Roche, the labeling is a polyclonal sheep antibody (immunometric one-step assay) labelled with sulfonyl-Rhutenium; the solid phase bound T4 analogue and the patient's FT4 patient hormone compete for a little quantity of polyclonal sheep antibody labeled with suffonyl-Rhutenium. The results obtained by both methods are shown in Table 1. After confirming an under-treated hypothyroidism, the dose of levothyroxine was increased to the initial of 75 micrograms.

Autoantibodies anti-T4 presence was confirmed using PEG precipitation and dilution methods, following an in house protocol based in the radioactivity measurement of the precipitant after addition of Polyethylene glycol solution 20% w/v in water to the serum patient and serum negative control previously incubated with I-125 radiolabeled -Thyroxine in the same run. Serum patient was found to be positive with a percentage of 77.4% of fixation (positive if higher than 10%). Control negative serum showed a percentage of 5% of fixation. Auto-antibodies anti-T3 were negative following the same in house protocol. The patient sample was PEG-precipitated in order to confirm the autoantibodies anti-T4 interference in FT4 Advia Centaur assay with a validated in-house protocol in an Advia centaur platform. We selected a patient control specimen with a very low FT4 concentration like Blank and another patient control specimen with very high FT4 concentration. To compare both, the matrix effect by PEG in Advia centaur acrydinium-ester label and the behaviour of a sample in the same patient range after PEG precipitation. The antibody interference gave a very discordant result before and after PEG precipitation in the patient's problem specimen. No relevant disagreement was found in the control specimens before and after PEG precipitation.

To further evaluate the antibody interference, we performed a double-dilution test in order to confirm the T4-autoantibodies interference in the Advia centaur platform. We did this by using a very low FT4 level pool from hypothyroid patients as a zero diluent and patient´s control specimens without FT4 test interference as well as a very high FT4 level. All of them were analyzed at the same run. The patient sample showed a non-linear increase in the FT4 level due to the decreasing title of high avidity interfering T4-autoantibodies after double-dilution test (Table 1). Finally, total T4 and T3 levels were also analyzed in Advia centaur platform in order to detect interference in total thyroid hormones. No relevant discordance was detected after the total T4 and total T3 quantification in Advia centaur platform respect the FT4 and FT3 hormones in the same platform and patient' specimen. The total T4 level was 67.9 μg/dL (4.5 - 10.9) and the total T3 was 1.43 ng/mL (0.6 - 1.81). PEG precipitation and dilution tests were performed (Table 1). Patient specimen showed a very low PEG precipitated recovery, which accounted for the PEG eliminated interference probably owing to the antiT4-autoantibodies. We speculate that the high title and high avidity patient index anti T4 autoantibodies were quenching the T4 coated paramagnetic solid phase beads, displacing the low title monoclonal mouse anti T4 from the binding site in the T4 molecule. After washing the monoclonal acrydinium labeled mouse, antiT4-antibodies were discarded and no signal was detected in the competitive assay, which explained the high Total T4 concentration observed in the patient sample in the Advia centaur platform total T4 assay.

3. Discussion

Interfering antibodies capable of causing potentially misleading results in immunoassays have been reported (4, 5). There is no practical way to predict which samples are likely to have immunoassay interference; only a high suspicion index and a good communication between the clinician and the clinical chemist in presence of discordant results can avoid misinterpretation (6-8). Different modern automated thyroid hormone immunoassays can also be sporadically affected by interfering antibodies (1, 3, 9, 10). Thyroid hormone -autoantibodies directed against T3 and T4 are uncommon and have been reported

Table 1. Thyroid Hormone Levels

	TSH (μUI/mL), [0.5 - 5]	FT4 (pg/mL), [8 - 18] Before PEG	FT4 (pg/mL), [8 - 18] Double-Dilution Test	FT4 (pg/mL), [9.3 - 17]	FT4 (pg/mL), [8 - 18] After PEG	FT3 (pg/mL), [2.3 - 4.2]	TT4 (pg/mL), [4.5 - 10.9] Before PEG	TT4 (pg/mL), [4.5 - 10.9] Double-Dilution Test	TT4 (pg/mL), [4.5 - 10.9] After PEG
At presentation									
Method 1	20.95	7.7	-	-	-	-			
4 years after presentation									
Method 1	1.486	12.3	-	-	-	-			
6 years after presentation									
Method 1	2.903	14.5	-	-	-	-			
8 years after presentation									
Method 1	7.396	51.7	(2) 48; (4) 61; (8) 82	-	11	2.8	67.9	(2) > 30; (4) 93.6; (8) 93.6	3.8
Method 2	9.333	-	-	7.4	-	2.97			

Abbreviations: TSH, serum thyrotropin; FT4, free thyroxin; FT3, free triiodothyronine; TT4, total thyroxin; Method 1, Siemens Healthcare Reagents Kit, Advia centaur; Method 2, Roche Cobas "e", Roche Diagnostics Corporation; PEG, polyethylene glycol; Double serial dilution from the primary specimen were analyzed at the same run in an Advia centaur platform.

in patients with and without thyroid and autoimmune diseases (3, 9-13). Several cases of patients in which antiT3-autoantibodies and/or antiT4-autoantibodies interference led to a misinterpretation of FT3 and/or FT4 results have been reported (2, 14-18); some of them reported antiT4-autoantibodies interfering with FT4 measurement in modern immunoassay platforms (19). In our patient, the interference found in the FT4 measurement was attributed to circulating antiT4-autoantibodies.

In immunometrics one-step assays, physicochemical differences arising from the binding of labeled antibodies to the solid, support confer kinetic differences that result in decreased analogue affinity for endogenous binding proteins and thus produce more reliable free hormones results (20, 21). The antiT4-autoantibodies present in the patient's sample represent another endogenous binding protein. This seems likely and the increased protein binding capacity for thyroid hormones, coming from autoantibodies, could account for the decreased patient's FT4.

The thyroid immunoassay in our laboratory had not changed in the past 8 years, which the patient had been biochemically followed for levothyroxine replacement. We could hypothesize that the antiT4-autoantibodies have either appeared during the course of the disease or increased their title and/or affinity so they started interfering with our assay.

In one-step FT4 immunoassay, when the analogue label is an enzymatic label, there is a steric hindrance protecting antiT4-autoantibodies from interacting with the analogue. The acrydinium ester label from Advia centaur may not protect this unexpected interaction. The immunometric one-step FT4 immunoassay value (Roche platform) is the selected value to adjust the patient's dose of levothyroxine because it has been shown to accommodate the vast majority of high title and/or affinity antiT4-autoantibodies patients within the correct range for their underlying functional state (20). Laboratories should choose assays that are well protected and if possible, replace poorly protected methods (4). For assay manufacturers, the investment in interference protection is a case of priority (22).

In summary, we report a 46- year-old patient with long standing autoimmune hypothyroidism in chronic replacement treatment with levothyroxine who was presented 8 years after diagnosis with a thyroid function test showing an increased level of TSH and a very high level of FT4. The lack of symptoms of hyperthyroidism led us to suspect interference in the laboratory FT4 test. A different immunoassay platform confirmed a low FT4 result, which led us to the diagnosis of undertreated hypothyroidism and to an increase in the dose of levothyroxine. The discrepancy between the two results was explained by the presence of antiT4-autoantibodies. Antibody interference with thyroid assays must be considered when clinical findings and laboratory results show discrepancies, specifically when very high FT4 levels are found in absence of symptoms of hyperthyroidism. The communication between the clinician and the laboratory staff can avoid unnecessary diagnostic procedures and inappropriate treatments.

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