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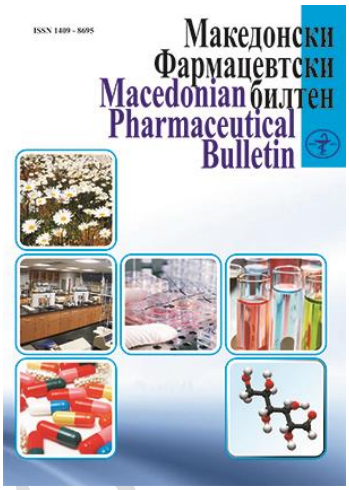

Title: Interference in thyroid function immunoassays: clinical consequences

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Interference in thyroid function immunoassays: clinical consequences

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Abstract

Thyroid function tests are prone to analytical interference, which can cause misleading results when performed on automated immunoassay analyzers. We present a case of a 68-years old woman diagnosed with primary hypothyroidism and chronically treated with levothyroxine. Her status has been followed-up in several different institutions and before readmission to our institute, she was diagnosed as T3 toxicosis according to the lab results of suppressed TSH, normal FT4 and highly elevated FT3 values. Due to lack of toxic symptoms, our clinician suspected FT3 test interference, which was confirmed in our lab by performing the test on a different immunoassay platform. In conclusion, every discrepancy between clinical presentation and laboratory test results has to be inspected by close communication between clinicians and laboratory specialists. Our goal was to raise the awareness within the healthcare community about the interference in immunoassays affecting different kit manufacturers and analytical platforms in order to avoid erroneous diagnosis and mistreatment of patients.

Key words: immunoassay, interference, free triiodothyronine, mistreatment

Introduction

Laboratory tests are integral part of clinical evaluation process, diagnostics decision making and patient follow-up. Concerning thyroid gland disorders, thyroid function tests (TFTs) are indicators of hypothalamic-pituitary-thyroid (HPT) axis consistency, which is negative feedback regulated. Thus, even minor changes of serum

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free-thyroid hormones (FT4 and FT3) concentration can increase or decrease thyroid stimulating hormone (TSH) secretion. In practice, clinicians rely on accurate measurements of TSH, as a first–line measurand, followed by free T4, performed by one-step or two-step assay principle (Beckett and Toft, 2003; Biondi et al., 2015; Daucourt et al., 2003; Schneider et al., 2018).

After established diagnosis of hypothyroidism and substitution therapy, the patients are followed-up periodically with measurement of serum TSH as a best biomarker for this status (Jonaklaas et al., 2014). When suspecting thyrotoxicosis, initial biochemical evaluation should include TSH, FT4 and total triiodothyronone (TT3) (Ross et al., 2016). Although measurements of total T4 and T3 are affected by binding proteins (Bartalena and Robbins, 1992), TT3 is preferred over FT3, due to the lack of robustness and validation of FT3 immunoassays, as predominant, routine biochemical techniques. From analytical perspective, immunoassays, involving indirect measurement principle, are prone to mild to substantial method variations, despite of long-lasting efforts of clinical chemistry community for standardization and harmonization of TFTs (Thienpont et al., 2010a; 2010b). Furthermore, different factors can interfere with test results (both in preanalytical and analytical phase), which can be falsely increased or decreased. As a consequence, these results may be inconsistent with the clinical presentation, or show confusing, non-physiological HPT pattern.

Analytical interference, as defined by Kroll and Elin (1994), is the effect of a substance present in the patient sample that alters the correct value of the result. Detection of analytical interferences is often a very challenging task. Usually clinicians do not provide sufficient data about diagnosis and patient's medication on request forms. It is well reported that heparin, phenytoin, furosemide, carbamazepine and salicylate affect the free thyroid hormone assessment (Surks and De Fesi, 1996; British Thyroid Association, 2006). Amiodarone, lithium and iodine can influence thyroid hormones secretion. Biotin may interfere with assay kit antibody components, resulting in falsely decreased or increased test results (Clerico and Plebani, 2017; Kwok et al., 2012; Wijeratne et al., 2012). In majority of cases, laboratory is informed about the discrepancy by clinicians, upon receipt of the results. Every misleading result has to be inspected according the laboratory protocol, and after exclusion of analytical error, the root-cause should be detected considering analytical interference.

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Hereby we present a case of thyroid hormone interference observed in patient with long-term hypothyroid history, admitted to our institution for second opinion investigation. The report from the previous clinician, according to the presented TFTs results, suggested that the patient developed T3 toxicosis, and he changed the medication treatment. Our primary goal was to raise the awareness among clinicians and laboratory specialists about possible immunoassay analytical interference in order to prevent the erroneous treatment and health consequences in patients.

Case presentation

A 68-year-old woman was diagnosed with primary hypothyroidism, one month after the delivery of her first child, when she was 26 years old. Replacement therapy was proscribed with levothyroxine (150 μg/day six days and 200 μg on the seventh day). She was admitted for the first time at the Institute of Pathophysiology and Nuclear Medicine (IPNM) aged 41, and had been followed-up until the end of 2010. Thyroid autoantibodies (anti-thyroid peroxidase and anti- thyroglobulin antibodies) were assessed as well, and revealed no autoimmune etiology of the disease. Ultrasonography of thyroid gland showed reduced contour volume, with no evident change over the whole follow-up period. She remained clinically and laboratory euthyroid (TSH serum concentrations within reference interval of 0.4-4.5 mIU/L) until aged 56, when she enters menopause. For the first time, despite being clinically euthyroid, TSH levels were suppressed and the medication dose was adjusted to 100 μg/day. Three months later, TSH levels normalized and the patient was last examined after 4 months, remaining euthyroid. During the next 8 years her thyroid status was not followed-up in our institution.

She reappeared at the IPMN for second-opinion investigation in December 2018, with diagnosis of T3 toxicosis, according the endocrinologist from a private healthcare institution and a huge documentation of reports from different laboratories, as presented on Table 1. From June 2018 until October 2018 TFTs were assessed by Lab A. In June, with TSH result of 0.01 μIU/mL and high FT3 (15.8 pg/mL), the patient was advised to decrease the dose of levothyroxine until total withdrawal and to start the therapy with antithyroid drug tiamazole (20 μg/day). The first control afterwards

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evidenced TSH increase up to 5.87 μIU/mL, FT3 remaining high and FT4 in reference interval.

Table 1

The patient was than advised to take both - substitutional, levotiroxine therapy (50 μg/day six days and 75 μg on the seventh day) and suppression therapy with propilthyouracil 100 mg/day. Three months later, laboratory results from Lab A showed no improvement of patient's thyroid status. A month later, TFTs were performed by Lab B on Immulite 2000 autoanalyzer and revealed TSH and FT4 results within reference range, with again, highly increased FT3.

When admitted to our Institute in December 2018, according to anamnestic data, she was taking 100 μg/day levothyroxine and 150 mg/day propilthyouracil. Clinically, she appeared euthyroid and our laboratory results for TSH and FT4 confirmed this status, but FT3 was significantly elevated. She complained fatigue and weight gain. Anti-thiroidperoxidase antibodies were negative (<10 kIU/L). The results were obtained on Immulite 2000 analyzer (Siemens Healthiners, USA) with CLIA method and were in agreement with those reported one month before by Lab B, performed on the same immunoassay platform. As FT3 result was inconsistent with the clinical presentation, we suspected analytical interference in FT3 immunoassay test. Therefore, we measured TFTs from the same specimen on another immunoassay platform – Maglumi 800 with Snibe reagents (Snibe Co. Ltd., China). Results obtained are depicted on Table 2. TSH and FT4 results were in agreement on both platforms and within reference ranges. FT3 value measured on Maglumi 800 platform was 4.0 pmol/L (ref. 2.8-6.5 pmol/L) indicating euthyroid status. This result confirmed the analytical interference which affected Immulite 2000 FT3 assay, resulting in high FT3 measurements. This falsely elevated FT3 value was obtained by Lab A either, performed with EACLIA method.

Table 2

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Taking into consideration our laboratory findings, the clinician decided to change the therapy, by withdrawal of suppressant proscribed by the endocrinologist and to treat the patient with 100 μg/day levothyroxine only. Further on, the patient status was stabilized, her well-being restored and she was regularly followed-up by TSH assessment only.

Discussion

When facing confusing TFTs results, the first step according our laboratory protocol is to exclude random analytical error, while having acceptable internal quality control assured. As we obtained similar results after the reassessment on Immulite 2000 autoanalyzer and we discuss it with the clinician, possible analytical interference was suspected. Numerous authors have reported FT4 (or both FT4 and FT3) assay – dependent test interference involving Immulite platforms (Beato-Vibora and Alejo Gonzalez, 2017; Revet et al., 2016; Srichomkwun et al., 2017), but FT3 interference alone is by our knowledge very rarely presented in recent literature. We found several published algorithms regarding assay interference detection and management of the obtained results (Favresse et al., 2018; Sturgeon and Viljoen, 2011).

Firstly, we excluded possible medication interference, because the patient denied taking any of the known interfering drugs. Biotin is reported to interfere with FT3 assessment in the FT3 Immulite 2000 kit package insert, but the patient was not taking any biotin-containing supplements either. Another cause of discrepancy could be the presence of interfering antibodies in patient sera from exogenous or endogenous origin (heterophile antibodies, human anti-animal antibodies, autoanalyte antibodies) (Tate and Ward, 2004). While exogenous antibody interference origin was excluded (no antibodies containing medication reported), the focus of possible root-cause for interference was on endogenous antibodies. Considering the fact that heterophile and human anti-animal antibodies would interfere with both free thyroid hormones, but in our case FT4 has not been affected, we presumed the presence of auto triiodothyronine antibodies. Although the prevalence of thyroid hormone auto antibodies (THAAbs) is higher in patients with autoimmune thyroid diseases, they may be present in general population in lesser extent (Sakata et al., 1994). The impact of THAAbs on the

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measured analyte concentrations is influenced by the assay design: the nature of tracer, types of antibodies, immunoassay principle (one-step or two- step). In general, one-step immunoassays are more susceptible to THAAbs interference. Therefore, one of the possible approaches in detection of interference problems is reassessment of TFTs on different assay platform, based on distinct principle (Favresse et al., 2018). We were able to assess the FT3 on Maglumi 800 analyzer, which utilize different labelling technique and analyte based reagents. Short description of two assay principles is given below.

Immulite 2000 Free T3 is competitive, one-step, analog-based chemiluminescent immunoassay. In brief, FT3 from patient's sera and T3 analogue, enzyme labeled with alkaline phosphatase, compete for limited amount of monoclonal murine anti-T3 antibodies, coated on solid phase beads. The unbound fractions are washed away from reaction tubes and a luminogenic substrate (adamantyldioxetane phosphate) is added to bound immunocomplex to produce enzymatic reaction, yielding chemiluminiscence. Maglumi 800 FT3 is solid phase, one-step, competitive immunoassay. The reaction occurs in micro wells after addition of serum, ABEI (N-(4-aminobutyl)-N-ethylisoluminol) labeled anti-T3 monoclonal antibody and T3 antigen coated magnetic microbeads. After the formation of immune-complex on the labeled antibody and precipitation in magnetic field, the unbound liquid fractions are aspirated. Signaling reagents are introduced in the well to initiate chemiluminescent reaction and the photo-multiplied light is detected as relative light units.

Anti-T3 auto antibodies may bind to both the measured analyte and labeled tracer, thereby altering the true concentration of FT3 (Despr´es and Grant, 1998). In one-step immunoassays, the patient's serum and labeled hormone analog are added to the reaction unit at the same time and compete for the solid phase antibody. The unbound fraction is afterwards washed away, with only the bound analog measured. THAAbs bind to analogs because they are less available for competition. As a consequence, the signal is reduced, and falsely elevated hormone value is obtained, due to the inverse relationship between signal and analyte concentration. We assume that this might be the root-cause of the interference we have observed in the results of our patient measured on Immulite 2000 analyzer, using analogue FT3 label.

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In patients with existing THAAbs, the most reliable TFT is TSH measurement (Zouwail et al., 2008). This approach was implemented by our clinician when FT3 interference was confirmed. For adequate treatment of such cases, it is important for the patient to be informed about the risk of getting false results depending of the lab method.

Conclusion

In conclusion, for the management of every discordant test result, it is essential to have close communication between laboratory and clinical staff and awareness of possible analytical interference. The clinician should provide sufficient information about diagnosis, as well as the medications and supplements taken by patient. Investigation of the interference is primary task of the laboratory staff, who should be well informed about assay performance of different immunoassay analyzers, the influence of interfering agents and procedures for detection of interference. This practice will prevent the cases of misdiagnose, mistreatment and possible serious health consequences in patients.

References

Bartalena, L., Robbins, J., 1992. Variations in thyroid-hormone transport proteins and their clinical implications. Thyroid 2, 237–245. Available at: https://doi.org/10.1089/thy.1992.2.237.

Beato-Vibora, P.I., Alejo Gonzalez, S., 2017. Avoiding misdiagnosis of antibody interference with serum free hyroxin. Int. J. Endocrinol. Metab. 15(1), e37792. Available at: https://doi.org/10.5812/ijem.37792.

Beckett, G.J., Toft, A.D., 2003. First-line thyroid function tests – TSH alone is not enough. Clinical Endocrinology 58, 20–21. Available at: https://doi.org/10.1046/j.1365-2265.2003.01690.x.

Biondi, B., Bartalena, L., Cooper, D.S., Hegedus, L., Laurberg, P., Kahaly, G.J., 2015. The 2015 European Thyroid Association Guidelines on Diagnosis and

*Corresponding author email: skuzmanovska@gmail.com

Treatment of Endogenous Subclinical Hyperthyroidism. Eur. Thyroid J. 4(3), 149-163. Available at: https://doi.org/10.1159/000438750.

British Thyroid Association, 2006. UK Guidelines for the use of thyroid function tests. Available at: http://www.british-thyroidassociation.org/info-forpatients/DocsTFTguideline_final_version_July_2006.

Clerico, A., Plebani, M., 2017. Biotin interference on immunoassay methods: sporadic cases or hidden epidemic? Clin. Chem. Lab. Med. 55, 777-779. Available at: https://doi.org/10.1515/cclm-2017-0070.

Daucourt, V., Saillour-Glenisson, F., Michel, P., Jutland, M.A., Abouelfath, A., 2013. A multicenter cluster randomized controlled trial of strategies to improve thyroid function testing. Medicalcare 41(3), 432–441. Available at: https://doi.org/10.1097/01.MLR.0000053216.33277.A4.

Despr´es, N., Grant, A.M., 1998. Antibody interference in thyroid assays: a potential for clinical misinformation. Clin. Chem. 44(3), 440–454.

Favresse, J., Burlacu, M.C., Maiter, D., Gruson, D., 2018. Interference with thyroid function immmunoassays: clinical implications and detection algorithm. Endocrine Review 39, 830-850. Available at: https://doi.org/10.1210/er.2018-00119.

Jonaklaas, J., Bianco, A.C., Bauer, A.J., Burman, K.D., Cappola, A.R., Celi, F.S., Cooper, D.S., Kim, B.W., Peeters, R.P., Rosenthal, M.S., Sawa, A.M., 2014. Guidelines for the treatment of hypothyroidism. Thyroid 24(12), 1670-1751. Available at: https://doi.org/10.1089/thy.2014.0028.

Kroll, M.H., Elin, R.J., 1994. Interference with clinical laboratory analyses. Clin. Chem. 40, 1996-2005. Available at: https://doi.org/10.1093/clinchem/40.11.1996.

Kwok, J.S., Chan, I.H., Chan, M.H., 2012. Biotin interference on TSH and free thyroid hormone measurement. Pathology 44, 278-280. Available at: https://doi.org/10.1097/PAT.0b013e3283514002.

Revet, I., Boesten, L.S.M., Linthorst, J., Yildiz, E., Janssen, J.N., de Rijke, Y.B., Albersen, A., 2016. Misleading FT4 measurement: assay-dependent antibody interference. Biochemia Medica 2016(3), 436-443 Available at: http://dx.doi.org/10.11613/BM.2016.046.

*Corresponding author email: skuzmanovska@gmail.com

Ross, D.S., Burch, H.B., Cooper, D.S., Greenlee, M.C., Laurberg, P., Maia, A.L., Rivkees, S.A., Samuels, M., Sosa, J.A., Stan, M.N., Walter, M.A., 2016. American thyroid association guidelines for diagnosis and management of hyperthyroidism and otherc auses of thyrotoxicosis. Thyroid 26(10), 1343–1421. Available at: https://doi.org/10.1089/thy.2016.0229.

Sakata, S., Matsuda, M., Ogawa, T., Takuno, H., Matsui, I., Sarui, H., Yasuda, K., 1994. Prevalence of thyroid hormone autoantibodies in healthy subjects. Clin. Endocrinol. (Oxf).41(3), 365–370. Available at: https://doi.org/10.1111/j.1365-2265.1994.tb02558.x.

Schneider, C., Feller, M., Bauer, D.C., Collet, T.H., da Costa, B.R., Auer, R., Peeters, R.P., Brown, S.J., Bremner. A.P., O''Leary, P.C., Feddema, P., Leedman, P.J., Aujesku, D., Walsh, J.P., Rodondi, N., 2018. Initial evaluation of thyroid dysfunction – Are simultaneous TSH and FT4 testsnecessary? PLoSONE 13(4), 1-12. Available at: https://doi.org/10.1371/journal.pone.0196631.

Srichomkwun, P., Scherberg, N.H., Jakšić, Refetoff, S., 2017. Diagnostic dilemma in discordant thyroid function tests due to thyroid hormone autoantibodies. AACE Clinical Case Rep. 3, 22-25. Available at: https://doi.org/10.4158/EP151142.CR.

Sturgeon, C.M., Viljoen, A., 2011. Analytical error and interference in immunoassay: minimizing risk. Ann. Clin. Biochem. 48, 418–432. Available at: https://doi.org/10.1258/acb.2011.011073.

Surks, M.I., DeFesi, C.R., 1996. Normal serum free thyroid hormone concentrations in patients treated with phenytoin or carbamazepine. A paradox resolved. JAMA 275, 1495–1498. Available at: https://doi.org/10.1001/jama.1996.03530430039036.

Tate, J., Ward, G., 2004. Interferences in immunoassay. Clin. Biochem. Rev. 25, 105-120.

Thienpont, L.M., Van Uytfanghe, K., Beastall, G., Faix, J.D., Ieiri, T., Miller, W.G., Nelson, J.C., Ronin, C., Ross, H.A., Thissen, J.H., Toussaint, B., IFCC Working Group on Standardization of Thyroid Function Tests, 2010. Report of the IFCC working group for standardization of thyroid function tests - part 1: thyroid-stimulating hormone. Clin. Chem. 56, 902–911. Available at: https://doi.org/10.1373/clinchem.2009.140178.

*Corresponding author email: skuzmanovska@gmail.com

Thienpont, L.M., Van Uytfanghe, K., Beastall, G., Faix, J.D., Ieiri, T., Miller, W.G., Nelson, J.C., Ronin, C., Ross, H.A., Thissen, J.H., Toussaint, B., IFCC Working Group on Standardization of Thyroid Function Tests, 2010. Report of the IFCC Working Group for Standardization of Thyroid Function Tests - part 2: freet hyroxine and free triiodothyronine. Clin. Chem. 56, 912–920. Available at: https://doi.org/10.1373/clinchem.2009.140194.

Wijeratne, N.G., Doery, J.C., Lu, Z.X., 2012. Positive and negative interference in immunoassays following biotinin gestion: a pharmacokinetic study. Pathology 44, 674-675. Available at: https://doi.org/10.1097/PAT.0b013e32835a3c17.

Zouwail, S.A., O'Toole, A.M., Clark, P.M., Begley, J.P., 2008. Influence of thyroid hormone autoantibodies on 7 thyroid hormone assays. Clin. Chem. 54(5), 927–928. Available at: https://doi.org/10.1373/clinchem.2007.099770.

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Резиме

Интерференција кај имунолошките анализи за тироидна функција: клинички последици

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Клучни зборови: имунолошка анализа, интерференција, слободен тријодтиронин, погрешен третман

Тестовите за тироидна функција се подложни на интерференција, што може да предизвика погрешни резултати добиени од автоматските имунолошки анализатори. Презентираме случај на 68-годишна жена со дијагноза на примарен хипотироидизам, која е хронично третирана со левотироксин. Состојбата е следена во неколку различни институции и пред повторно да се појави на преглед во нашата институција, дијагностицирана е Т3 токсикоза врз основа на лабораториските резултати на супримирани вредности на TSH, нормални вредности на FT4 и високо покачени за FT3. Поради отсуство на клиничка слика на токсикоза, нашиот клиничар се посомнева на можна интерференција кај вредностите добиени за FT3, што се потврди во нашата лабораторија, со повторно изведување на тестот со други реагенси и на различен имунолошки анализатор.

Заклучуваме дека секоја неусогласеност помеѓу клиничката слика и резултатите од лабораториските тестови треба да се истражи преку блиска соработка помеѓу клиничарите и специјалистите од лабораторијата. Наша цел беше да ја зголемиме свесноста помеѓу здравствените работници за постоење на аналитичка интерференција кај имунолошките анализи, со која се засегнати различни производители на китови и аналитички платформи, со цел да се избегне погрешна дијагноза и третман на пациентите.

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Table 1. Laboratory results for TFTs from Lab A and Lab B

Date (Lab A)	TSH[1] (μIU/mL)	FT4[2] (ng/dL)	FT3[3] (pg/mL)
06.06.2018	0.012	1.45	15.8
25.06.2018	5.87	0.91	15.3
11.07.2018	19.7	0.67	17.4
22.8.2018	17.8	0.98	15.8
03.10.2018	9	1.29	16.8
Date (Lab B)	TSH[4] (mIU/L)	FT4[5] (pmol/L)	FT3[6] (pmol/L)
02.11.2018	2.89	18.5	30.9

Legend: [1]immunoassay method ELISA ref. (0.4-4.0 μIU/mL); [2]imunoassay method DCLIA ref. (0.89-1.76 ng/dL); [3]imminoassay method EACLIA ref. (1.5-4.1 pg/mL); [4]immunoassay method CLIA ref. (0.4-4.5 mIU/L); [5]immunoassay method CLIA ref. (9.5-25 pmol/L); [6]immunoassay method CLIA ref. (4.2-8.1 pmol/L)

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Table 2. Laboratory results for TFTs performed in IPNM lab

Analyzer/method	TSH (μIU/mL)	FT4 (pmol/L)	FT3 (pmol/L)
Immulite 2000/CLIA*	1.52	17.7	22.4
Maglumi 800/Flash CLIA**	1.45	19.8	4.0

Legend: IPNM – Institute of Pathophysiology and Nuclear Medicine; *TSH ref. (0.4-4.5 mIU/L); FT4 ref. (11-25 pmol/L); FT3 ref. (2.8-6.5 pmol/L); **TSH ref. (0.4-4.5 mIU/L); FT4 ref. (11-27 pmol/L); FT3 ref. (2.8-6.5 pmol/L)

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