

## Case-Report

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# An overlooked situation in the interpretation of serum thyroglobulin level in a papillary thyroid cancer patient

## Papiller tiroid kanseri hastasında serum tiroglobulin düzeyinin yorumlanmasında gözden kaçan bir durum



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**Abstract:** In the present study we report a case of thyroglobulin (TGB) measurement interference in a total thyroidectomized and radio-ablated 61-year old woman with papillary thyroid cancer. We investigated possible interference in the measurement of TGB due to discordant TGB in relation to clinical condition during the follow-up period. Serum TGB was measured with the chemiluminescence method using Beckman Coulter Unicel DxI 800 instrument. To investigate possible interference in TGB measurement serial dilutions, polyethylene glycol precipitation (PEG), treatment with heterophile blocking tube (HBT), rheumatoid factor level determination and retesting of TGB with an alternative method were performed. Serial dilutions of the serum sample revealed linearity but a remarkable decrease in TGB in the patient's serum samples post PEG and post HBT treatments. Also, TGB results under functional sensitivity level obtained with a different method suggested that TGB interference developed due to heterophile antibody presence in the serum sample. The patient had unnecessarily undergone expensive imaging techniques, and invasive procedures such as lymph node fine needle aspiration biopsy, before

the analytical interference was suspected by the clinician. This report illustrates the importance of early communication and close collaboration between clinicians and laboratorians in order to avoid unnecessary clinical intervention.

**Keywords:** heterophile antibodies; immunoassay; interference; thyroglobulin; thyroid carcinoma.

**Öz:** Papiller tiroid kanseri tanısı almış total tiroidektomi ve radyoaktif iyot ablasyon tedavisi uygulanmış 61 yaşındaki kadın hastada tiroglobulin (TGB) ölçüm interferansı olgusunu sunmaktayız. Takipte olan hastanın klinik durumu ile uyumsuz serum TGB sonucu nedeniyle olası TGB interferansı incelendi. Serum TGB ölçümü Beckman Coulter Unicel DxI 800 cihazı kullanılarak kemilüminesans yöntemi ile gerçekleştirildi. İnterferans varlığını araştırmak amacıyla seri dilüsyon, polietilen glikol (PEG) ile çöktürme, heterofil antikor bloke eden tüp (HBT) ile muamele yapılarak TGB ölçüldü. Ayrıca romatoid faktör (RF) seviyesi ve alternatif bir yöntemle TGB ölçümü gerçekleştirildi. Seri dilüsyon yapılarak elde edilen değerler doğrusallık gösterirken PEG sonrası ve HBT ile muamele sonrası ölçülen TGB değerlerinde belirgin düşüş gözlemlendi. Aynı numuneden farklı bir cihazda yapılan TGB ölçümünde de TGB değerinin fonksiyonel duyarlılık seviyesinin altında olması serum örneğinde heterofil antikorlar varlığı kaynaklı TGB interferansı geliştiğini düşündürdü. Klinisyen tarafından hastanın serum TGB ölçümünde analitik interferans varlığından şüphelenilmeden önce; hastaya gereksiz pahalı görüntüleme yöntemleri uygulanmış ve ek olarak lenf nodu ince iğne aspirasyon biyopsisi gibi girişimsel işlemler yapılmıştı. Bu olgu sunumu gereksiz klinik müdahalelerin

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önlenmesinde klinisyen ve laboratuvar arasında erken iletişimin ve yakın iş birliğinin önemini göstermektedir.

**Anahtar kelimeler:** heterofil antikorlar; interferans; immünassay; tiroglobulin; tiroid kanseri.

## Introduction

Thyroglobulin (TGB) is an approximately 660 kDa, tissue-specific glycoprotein produced by thyroid follicular cells as a precursor of thyroid hormones (thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>)) and is released into blood circulation along with those hormones. Small amounts of TGB in the range of up to about 30 µg/L are detectable in the serum of healthy individuals [1]. The main use of TGB measurement is as a tumour marker; it provides important information about the presence or absence of residual, recurrent, or metastatic disease in patients with differentiated thyroid cancer (DTC) [1–2]. Neck ultrasound (US) and serum TGB measurement are useful for standard long-term follow-up of patients with DTC after total thyroidectomy and radioactive iodine <sup>131</sup>I (RAI) ablation therapy. After total thyroidectomy and RAI ablation therapy, most patients reach disease-free status, and serum TGB concentrations should be undetectable or very low [3].

Nowadays, the immunometric second generation high-sensitive TGB (hs-TGB) assay was developed to accurately measure TGB at low concentrations with a functional sensitivity of 0.1 µg/L. These hs-TGB assays replaced the costly and uncomfortable TSH-stimulated TGB testing during follow-up of low-risk patients. TGB levels below 0.1 µg/L in athyrotic patients on suppressive levothyroxine therapy indicate a minimal risk (<1–2%) of clinically detectable recurrent papillary/follicular thyroid cancer and a high chance of being free of disease [3–5].

Here, we report a case with high level of TGB that is incompatible with her clinical condition due to interference of heterophile antibodies. We discuss the present case with the findings in the literature up to now.

## Patients and methods

The patient was a 61-year old woman, with diagnosis of papillary thyroid carcinoma.

She was total thyroidectomized and radio-ablated with 100 mci I<sup>131</sup> in 2010. Since that time, she was followed by suppressive treatment with levothyroxine (LT<sub>4</sub>). She was admitted to Endocrinology and Metabolism Outpatient Clinic for her follow-up visits, which consisted of clinical examination with neck US and serum TGB determination between February 2014 and August 2015. In that period, serum TGB

levels of the patient were found to be <0.1 µg/L and neck US was normal, but the patient interrupted her follow-up visits for three years. In October 2018 when she was admitted to our hospital three years later, thyroglobulin antibody (anti-TGB) value was 1.4 kIU/mL (near to limit of detection; 0.9 kIU/mL) and TGB value was 40.17 µg/L. This relatively high TGB value was confirmed by three subsequent tests on the same platform (36.54, 44.92 and 32.32 µg/L) on different days. During this process, thyroid stimulating hormone levels were (TSH) all below 0.5 mIU/L and anti-TGB were all negative. On neck US, no residual thyroid tissue was visualised but bilateral cervical lymphadenopathy was present. Due to the TGB positivity and suspicious US findings, the patient underwent fine needle aspiration biopsy (FNAB) and TGB was measured in the needle washout (FNAB-TGB). Also other imaging modalities such as thyroid scintigraphy (technetium 99m pertechnetate), whole body scanning using radioactive iodine (I<sup>131</sup>), neck and thorax computed tomography (CT), and imaging with positron emission tomography (PET) were performed in order to eliminate local recurrence in cervical chain lymph nodes or distant metastases. All the investigations, other than the high serum TGB levels, ruled out recurrence and metastases of the disease. After all these tests were performed, communication between the endocrinologist and laboratory occurred on March 2019, and then interference studies were performed in the biochemistry laboratory.

After a minimum 8 h of fasting, venous blood samples were collected in a serum separator clot activator blood collection tube (BD Vacutainer® SST™ Tubes, 5 mL, 13 × 100 mm, NJ, USA). Serum specimens were separated after centrifugation at 3000 rpm for 10 min. A Beckman Coulter Unicel Dxl 800 instrument (Beckman Coulter, Brea, CA, USA) was used with Access chemiluminescent immunoassays (Beckman Coulter, Brea, CA, USA) for *in vitro* quantitative determination of thyrotropin (TSH, HYPERSensitive hTSH assay third generation, reference number; B63284), free thyroxine (fT<sub>4</sub>, reference number; 33880), thyroglobulin (second generation TGB; reference number; 33860) and thyroglobulin antibody (anti-TGB, reference number; A32898) levels in human serum. Access TGB assay' functional sensitivity was 0.1 µg/L and included 'mouse monoclonal antibodies'.

For TGB interference studies, serial dilution, polyethylene glycol (PEG) precipitation, treatment with heterophile blocking tubes (HBT), RF measurement, and also analysis with a different manufacturer's instrument were performed.

## Results

Firstly, serial dilutions (1/2, 1/4, 1/8) were performed on the suspicious serum sample using the manufacturer's diluent, which revealed linearity with a recovery of 95–105% and suggested no assay interference but we continued the investigation.

Secondly, PEG treatment was performed by mixing equals parts serum with a 25% (w/v) buffered phosphate solution of PEG 6000 (Merck, Darmstadt, Germany). Samples were mixed and incubated at 4 °C for 10 min and centrifuged at 14,000 g for 5 min, and then TGB measurement was performed with the supernatant obtained from this PEG-treated serum [6].

Thirdly, treatment of serum with HBT (Scantibodies Laboratory, Santee, CA, USA) was performed according to the manufacturer's instructions. Serum TGB levels were measured on the Beckman Coulter DXI 800 before and after treating serum samples with HBT.

Fourthly, due to the positivity for HAb, we performed an additional rheumatoid factor (RF) measurement using the immunoturbidimetric method, (Roche Cobas 6000, Roche Diagnostics, Mannheim, Germany). RF result was found to be 9.5 IU/mL and within the reference range (<14 IU/mL).

Lastly, the sample was sent to an external laboratory for determination of TGB on a different platform (Roche Cobas e601, Roche Diagnostics, Mannheim, Germany) using Elecsys TGBII reagent with electrochemiluminescence immunoassay method. Functional sensitivity for Elecsys TGBII of 0.040 µg/L. The TGB interference study results are summarised in Table 1.

## Discussion

This report is about a rare case of a patient with a diagnosis of papillary thyroid carcinoma that showed false positive TGB levels during the follow-up associated with no clinical evidence of disease relapse.

Up to now, three possible causes of interference were identified in the measurement of TGB. The presence of TGB antibody may result in falsely low TGB measurement; therefore, it is recommended to analyse anti-TGB with TGB [1, 3, 4]. In our case, anti-TGB was negative in all the samples investigated. RF positivity was also shown to be a cause of false positivity in TGB measurement [7] but our patient had low RF levels (9.5 IU/mL) as well. The presence of HAb, a rare type of TGB interference [8], mostly causes a falsely high TGB result but also may cause a false negative result as well [8, 9].

In our investigation, firstly we discovered that TGB measurements were performed with a Roche instrument during the time period of the early follow up (between February 2014 and August 2015) but in 2018 TGB measurements were performed with a Beckman Coulter

instrument in our laboratory. Due to analysis with different assay and reagent antibodies, blood was sent to an external laboratory that used a Roche instrument. TGB negativity with the alternative method and the remarkable decrease in TGB in the patient's serum samples with post PEG and post HBT treatments suggest that HAb was the cause of discordant results. In our case only the serial dilution test did not support the presence of interfering antibodies, but it was reported that approximately 40% of samples containing interfering antibodies may fail to show a nonlinear relationship in the serial dilution test [10].

HABs are human-developed antibodies to animal antigens. In immunometric methods, two antibodies that capture and mark the antigen are used. HAb reacts with both antibodies even though there is no antigen so they lead to a false result as if there is a high antigen level. This type of interference occurs in more than 90% of cases with HAb interference. More rarely, in less than 10% of HAb interference cases, they cause low antigens to appear [8]. False positive TGB measurements due to the presence of HAb may cause unnecessary investigations and RAI ablation treatments in differentiated thyroid cancer.

In our case, TGB interference developed due to HAb presence in serum samples and the patient had unnecessarily undergone expensive imaging techniques, and also invasive procedures such as lymph node FNAB, before the analytical interference was suspected by the clinician. In order to solve this problem, Giovenalla et al. suggested repeating the TGB testing using a different assay as the simplest way to reveal positive HAb interference, and also the use of HBT tubes for all sera referred for TGB measurement should be considered in order to prevent both unnecessary investigations or therapy, and delayed diagnosis of recurrence in patients with DTC [9].

## Conclusion

Early communication between clinicians and laboratorians is enormously important before further investigations

**Table 1:** TGB interference studies.

	First measurement	Diluted			PEG (pre/after)	After HBT treatment
		1:2	1:4	1:8		
Beckman Coulter, µg/L	32.94	16.40	8.27	3.99	16.80/0.18	5.34
Recovery %	–	99.6 <sup>a</sup>	100.4 <sup>a</sup>	96.9 <sup>a</sup>	1.07 <sup>b</sup>	16.2 <sup>a</sup>
Roche, µg/L	<0.040			–	–	–

\*PEG, polyethylene glycol precipitation; HBT, heterophile blocking tube. <sup>a</sup>Diluted samples or after HBT vs. first measurement. <sup>b</sup>Pre vs. after PEG precipitation.

are started if the laboratory result does not correlate with the clinical presentation. If there is a suspicion of analytical interference, the laboratory can carry out extra confirmatory tests which are relatively simple, inexpensive and also don't take much time. The follow-up of DTC patients should be done using the same assay and the simplest approach to confirm the TGB interference suspicion is to repeat the assay with different methods.

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## References

- Guber HA, Farag AF. Evaluation of endocrine function. In: McPherson RA, Pincus MR, editors. *Henry's clinical diagnosis and management by laboratory methods*, 23rd ed. St Louis, Missouri: Elsevier; 2017:362–95.
- Evans C, Tennant S, Perros P. Serum thyroglobulin in the monitoring of differentiated thyroid cancer. *Scand J Clin Lab Invest* 2016;76:119–23.
- Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid* 2016;26:1–133.
- Giovanella L, Clark PM, Chiovato L, Duntas L, Elisei R, Feldt-Rasmussen U, et al. Thyroglobulin measurement using highly sensitive assays in patients with differentiated thyroid cancer: a clinical position paper. *Eur J Endocrinol* 2014;171:33–46.
- Giovanella L, Castellana M, Trimboli P. Unstimulated high-sensitive thyroglobulin is a powerful prognostic predictor in patients with thyroid cancer. *Clin Chem Lab Med* 2019;58:130–7.
- Sturgeon CM, Viljoen A. Analytical error and interference in immunoassay: minimizing risk. *Ann Clin Biochem* 2014;48:418–32.
- Astarita G, Gutierrez S, Kogovsek N, Mormandi E, Otero P, Calabrese C, et al. False positive in the measurement of thyroglobulin induced by rheumatoid factor. *Clin Chim Acta* 2015; 447:43–6.
- Preissner CM, O'Kane DJ, Singh RJ, Morris JC, Grebe SK. Phantoms in the assay tube: heterophile antibody interferences in serum thyroglobulin assays. *J Clin Endocrinol Metabol* 2003;88:3069–74.
- Giovanella L, Keller F, Ceriani L, Tozzoli R. Heterophile antibodies may falsely increase or decrease thyroglobulin measurement in patients with differentiated thyroid carcinoma. *Clin Chem Lab Med* 2009;47:952–4.
- Ismail AA. On detecting interference from endogenous antibodies in immunoassays by doubling dilutions test. *Clin Chem Lab Med* 2007;45:851–4.