

Heterophile antibodies may falsely increase or decrease thyroglobulin measurement in patients with differentiated thyroid carcinoma

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Abstract

Background: To examine the prevalence of significant interference from heterophile antibodies (HAb) in the measurement of serum thyroglobulin (Tg), we evaluated a large cohort of samples from patients with differentiated thyroid carcinoma (DTC).

Methods: Serum Tg measurements were performed in 406 serum samples before and after incubation of each serum sample in heterophile-blocking tubes (HBT) at room temperature for 1 h. We calculated the difference between the original Tg value and the value obtained after HBT treatment. We considered any sample showing an absolute percent difference >3 SD from the mean percent difference as being affected by HAb interference.

Results: We identified five patients (1%) as showing interference from HAb. Of these, three (60%) showed a false positive or falsely increased Tg concentration without any recurrence following clinical work-up; two (40%) showed a false negative or falsely reduced Tg levels, and metastases were detected in both cases by imaging procedures.

Conclusions: HAb may increase as well as reduce the measured Tg in a significant number of patients. A positive HAb interference should be suspected if Tg elevation does not fit the clinical pictures. A negative interference is a more challenging problem because increases in Tg generally occur as the first sign of recurrence of DTC. Therefore, treatment using HBT tubes of all sera referred for Tg measurement should be considered in order to prevent both unwarranted investigations or therapy, and delayed diagnosis of recurrence in patients affected by DTC.

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Introduction

Serum thyroglobulin (Tg) measurements are a major tool for detecting recurrence of differentiated thyroid carcinoma (DTC), provided that anti-Tg antibodies (TgAb) are absent (1–3). Heterophile antibodies (HAb) can bind to animal antigens or antibodies employed in immunometric assays (4). HAbs can form a bridge between the capture and detection antibody, leading to a false positive result in absence of analyte, or to a falsely higher result in presence of analyte (5). Preissner and co-workers reported 32 false positive or falsely increased serum Tg values from 1106 patients with serum Tg \geq 1.00 ng/mL (2%) (6). However, HAb may also bind to the capture antibody preventing the binding of analyte and/or detection antibody causing false negative or falsely low results (5). Accordingly, we observed a case of HAb-induced false negative Tg measurement in a patient with lymph-node metastases from DTC (7). Therefore, we decided to examine the prevalence of significant HAb interferences in our current Tg assay systematically by evaluating a large cohort of samples with all concentrations of Tg.

Materials and methods

We included all TgAb-negative samples that had been referred for Tg measurements to our laboratory by the Department of Nuclear Medicine or outside customers during October 2006–October 2008. For all samples, we determined the clinical indication for testing by review of the medical record. Following routine measurement of Tg, all samples were re-tested immediately for possible HAb interference as described below.

Tg and TgAb assay

All Tg and TgAb concentrations were measured using the Immulite 2000 immunoassay system with the manufacturer's (DPC, Los Angeles, CA, USA) reagent packs and procedures. The functional sensitivity (i.e., lower limit of detection) for our Tg assay is 0.36 ng/mL, as described previously (8). Samples with positive TgAb (i.e., >40 UI/mL) were excluded from the study.

Identification of HAb interferences

For all study samples, we repeated the initial Tg measurements after incubating 500 μ L of serum from each sample in heterophile-blocking tubes (HBT, Scantibodies, Santee,

CA, USA) at room temperature for 1 h. We calculated the differences between the original Tg value and the measurement obtained following HBT treatment, expressed as a percentage of the original result. We considered any sample showing an absolute difference percentage of >3 SD from the mean difference percentage as possibly being affected by heterophile interference. The ± 3 SD cut-off was chosen because, statistically, only 0.2% of measurements would be expected to fall outside these limits, making this cut-off not likely to be affected by random experimental error (i.e., sample manipulation).

Results

A total of 406 samples fulfilled the inclusion criteria, representing 88% of all Tg measurements performed during the study period. The remaining samples (12%) were TgAb positive. Of the 406 samples, 404 were performed for follow-up of thyroid cancer and two were performed in patients with neck lymph node metastasis with unknown primary carcinoma. For 67 patients, serum Tg was measured during both thyroxine treatment and following thyrotropin (TSH) stimulation [thyroxine (T4) withdrawal: 42; rhTSH: 25] (134 samples). The Tg values of the original samples ranged from <0.36 ng/mL to 2790 ng/mL, with a mean of 3.7 ng/mL and a median of 1.2 ng/mL. These were not significantly different from the corresponding values following HBT treatment, which resulted in a Tg measurement range of <0.36 –2820 ng/mL, with a mean of 4.1 ng/mL and a median of 1.1 ng/mL. The mean difference between the original Tg values and Tg values following HBT treatment was $0.47\% \pm 0.105\%$ (SD) [median 0%]. Thus, the mean ± 3 SD limits were 0.16% and 0.78%, respectively. The 0.47% mean difference represents a decrease in measured Tg following treatment with HBT. However, approximately equal numbers of samples showed a decrease or increase in Tg values following HBT treatment. In two samples, the increase in Tg values following HBT treatment exceeded the 0.78% limit. The pre-HBT treatment serum Tg values (during T4 in both cases) in these samples were <0.36 and 0.98 ng/mL, increasing to 4.10 ng/mL and 12.4 ng/mL, respectively. Following rhTSH administration, no significant changes occurred in pre-HBT treatment Tg measurements in both patients. However, a significant increase occurred after treatment with HBT (Table 1).

Table 1 Effects of rhTSH-stimulation on Tg concentrations measured before and following HBT treatment in patients with interferences from heterophile antibodies (Tg expressed as ng/mL).

	onT4-Tg		rhTSH-Tg	
	pre-HBT	post-HBT	pre-HBT	post-HBT
1	<0.36	4.10	<0.36	10.7
2	0.98	12.4	1.2	26.2
3	4.1	<0.36	–	–
4	1.9	<0.36	–	–
5	5.7	0.8	–	–

HBT, heterophile-blocking tubes; Tg, thyroglobulin.

A small (8 mm) cervical lymph-node metastases was detected in the first patient by neck ultrasound and fine-needle cytology, which was subsequently confirmed by histology. Coexisting cervical and mediastinal metastases were found in the second patient, and subsequently treated by surgery and radioiodine administration. In three samples, the drop in Tg values following HBT treatment exceeded the 0.16% limit. In two patients, serum Tg concentrations, during treatment with T4, dropped from 4.1 and 1.9 ng/mL to <0.36 ng/mL following HBT treatment, and from 5.7 ng/mL to 0.8 ng/mL in one other patient (Table 1). No recurrences of DTC were detected by ultrasound of the neck and 18 Fluorodeoxyglucose-positron emission tomography/computed tomography (18 FDG-PET/CT). Further clinical follow-up at 14–26 months was negative for these patients.

Discussion

The follow-up of patients with DTC is based primarily on regular serum Tg measurements. The clinical consequences of an artifactual elevation or decrease in serum Tg concentrations can be considerable. In the first scenario, additional investigations will ensue, which may involve radiation exposure or even invasive procedures. Additionally, the clinical trend to occasionally treat thyroid cancer with radioiodine, solely on the basis of increased Tg, can also result in unnecessary therapy for patients without actual recurrence (9, 10). In the second scenario, a negative result may falsely reassure both patient and physician and effective treatments may be withheld. While TgAb interference with Tg measurements are well known, and still a challenge, few physicians are aware of the potential problems due to HAb interference on Tg measurements (11, 12). We remeasured serum Tg following treatment with HBT tubes for 406 samples, and identified five samples (1%) showing HAb interference. Of these, three (60%) sera showed false positive or falsely increased Tg, while two (40%) showed false negative or falsely decreased Tg levels. Following HBT treatment, a 0.47% mean decrease in measured Tg was found. Possible bias introduced by sample manipulation and HBT treatment itself could be hypothesized. However, about equal numbers of samples showed a decrease and an increase in Tg values following HBT treatment, as would be expected for repeated measurements. Our data are in contrast with those from Preissner and co-workers, that found 2% false positive or falsely increased Tg results, but no false negative or falsely decreased Tg which was measured using an automated chemiluminometric Tg assay. However, they excluded sera with Tg <1.00 ng/mL. Consequently false negative or falsely decreased results were unlikely to be detected in their series. Our results show that HAb may increase as well as reduce measured Tg in a significant number of patients. Positive HAb interference should be suspected if Tg elevation does not fit the clinical picture; in this case, the simplest approach is to repeat the

testing using a different assay. Samples that show interference in one particular assay may not show any problems using an assay from another manufacturer. On the contrary, a negative HAb interference is a challenging problem because a Tg increase generally occurs as the first sign of recurrence of DTC and false negative results are generally difficult to detect on the basis of clinical findings. In this instance, the role of imaging procedures, primarily ultrasound of the neck, still remains of pivotal importance in the clinical management of these patients. From the laboratory perspective, we propose that all sera referred for Tg measurement be treated using HBT tubes. This approach, in combination with appropriate imaging procedures and good clinical judgement, can help prevent unnecessary investigations or therapy and delayed diagnosis of recurrences in patients with DTC.

References

- Mazzaferri E, Robbins RJ, Spencer CA, Bravermann LE, Pacini F, Wartofsky L, et al. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. *J Clin Endocrinol Metab* 2003;88:1433–41.
- Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W, et al. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. *Eur J Endocrinol* 2006;154:787–803.
- Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, LoPresti JS. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. *J Clin Endocrinol Metab* 2005;90:5566–75.
- Kricka L. Human anti-animal antibody interferences in immunological assays. *Clin Chem* 1999;45:942–56.
- Levinson SS, Miller JJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. *Clin Chim Acta* 2002;325:1–15.
- 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 Metab* 2003;88:3069–74.
- Giovannella L, Ghelfo A. Undetectable serum thyroglobulin due to negative interference of heterophile antibodies in relapsing thyroid carcinoma. *Clin Chem* 2007; 53:1871–2.
- Giovannella L, Ceriani L, Ghelfo A. Redefining functional sensitivity of thyroglobulin assay on Immulite platform: implications in thyroid cancer management. *Clin Chem Lab Med* 2007;45:1523–4.
- Mazzaferri EL. Treating high thyroglobulin with radioiodine: a magic bullet or a shot in the dark? *J Clin Endocrinol Metab* 1995;80:1485–7.
- Fatourechi V, Hay ID, Javedan H, Wiseman GA, Mullan BP, Gorman CA. Lack of impact of radioiodine therapy in tg-positive, diagnostic whole-body scan-negative patients with follicular cell-derived thyroid cancer. *J Clin Endocrinol Metab* 2002;87:1521–6.
- Bjerner J, Nustad K, Norum LF, Olsen KH, Borner OP. Immunometric assay interference: incidence and prevention. *Clin Chem* 2002;48:613–21.
- Ward G, McKinnon L, Badrick T, Hickman PE. Heterophilic antibodies remain a problem for the immunoassay laboratory. *Am J Clin Path* 1997;108:417–21.