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Large Discrepancy in the Results of Sensitive Measurements of Thyroglobulin Antibodies in the Follow-Up on Thyroid Cancer: A Diagnostic Dilemma

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Thyroid cancer · Thyroglobulin · Thyroglobulin antibodies

Abstract
During follow-up on patients treated for differentiated thyroid cancer, thyroglobulin (Tg) antibodies can interfere with the Tg assay, making the use of Tg less reliable as a tumor marker. ***Purpose:*** To compare Tg and Tg autoantibodies (Tg-Ab) methods used in Denmark, regarding the number of patient samples being accepted for evaluating the result of a serum thyroglobulin (s-Tg) measurement. ***Design:*** 95 consecutive blood samples drawn from patients in 2006 in one center were selected according to the following criteria: s-Tg <1μg/l and accepted BRAHMS Tg+ recovery test using 50 ng of Tg. Samples were retested with: (1) DPC IMMULITE 2000 Tg and Tg-Ab, (2) BRAHMS Tg and Tg-Ab on Kryptor, (3) BRAHMS Tg+ and Dynotest anti-Tg, (4) DELFIA hTg and recovery test using 25 ng of Tg, and (5) BRAHMS Tg+ with recovery test using 1 and 50 ng of Tg. ***Results:*** The number of patient samples that was not accepted for Tg evaluation varied from 2 to 26% when the reference values suggested by the manufacturers of the assay were used. When using the detection limit to the cutoff seen in epidemiological studies the number increased to 40%. ***Conclusion:*** We found large discrepancies in acceptance of patient samples for s-Tg evaluation, thus illustrating a diagnostic dilemma.

Introduction

The overall prognosis of differentiated thyroid cancer is good. Previous reports describe a 30 years' survival of approximately 75% [1, 2], and a tendency to even better survival is seen in more recent publications [3]. International guidelines [4, 5] suggest a differentiation of patients into low and high risk, low risk patients having a life expectancy close to that of the background population.

Serum thyroglobulin (s-Tg) is used as a tumor marker during follow-up after therapy [4, 5]. Stimulated s-Tg (ei-

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ther endogenous stimulation after withdrawal of L-T4 or L-T3 therapy, or exogenous stimulation after recombinant human TSH injection) and ^{131}I whole body scan (WBS) are recommended in the initial follow-up after surgery and radioiodine ablation. In the evaluation of recurrent or persistent cancer the sensitivity of stimulated s-Tg is approximately 85%, but only 20–34% when evaluated with WBS [6, 7]. The sensitivity of nonstimulated s-Tg is lower than the sensitivity of stimulated s-Tg. However, it is sufficient and recommended to use a nonstimulated s-Tg during follow-up on patients with a negative stimulated s-Tg as well as negative ultrasound scan and WBS 6–12 months after radioiodine ablation [4]. This recommendation is based on a low risk of recurrence combined with high economical cost of stimulated s-Tg measurements, when using recombinant human TSH, and a high physical and psychological cost for the patient if withdrawal of L-T4 or L-T3 is used.

A technical problem is the presence of circulating Tg autoantibodies (Tg-Ab). This may influence the serum Tg assay using modern immunometric Tg assays, leading to a false negative result, which may lead to overlooking persistent or recurrent thyroid cancer. The presence of Tg-Ab is seen in more than 10% of the general population [8], and it may be even more common in patients with thyroid cancer [9]. Even low values of Tg-Ab are taken as a sign of thyroid autoimmunity [10].

A study comparing 12 different methods of Tg-Ab measurements showed only a 65% concordance between assays [11] and handling of problems with Tg-Ab interference in clinical practice remains controversial.

The purpose of the present study was to evaluate the s-Tg and s-Tg-Ab methods used in centers treating thyroid cancer in Denmark, and to compare between centers the number of patient samples accepted for evaluation of s-Tg in a group of low risk thyroid cancer patients.

Material and Methods

For this retrospective study, we used 95 consecutive blood samples drawn from patients (69 females and 26 males – median age 53 years, range 20–85) with differentiated thyroid cancer in 2006 in one center (index center). The selection of sera was blinded regarding to the tumor stage. The sera were selected as consecutive Tg samples from our analyze-log using the following criteria: DTC, S-Tg ≤11 μg/l and the sample had been accepted as usable for evaluating s-Tg with no sign of antibody interferences evaluated by a recovery test (which was the test used at that period of time).

The test used in this index center was BRAHMS Tg+ and the method for assessment of the usability of a given blood sample was a recovery test using 50 ng of Tg. The sera were collected from patients at one center and sent to the other centers to be reanalyzed.

Samples were retested with the following methods for evaluation of acceptability of the Tg value:

- Tg-Ab quantitative measurements

DPC IMMULITE 2000 Tg and Anti-Tg (normal Tg-Ab <40 U/ml) (Diagnostic Products Corporation, UK);

BRAHMS Tg and Tg antibodies (Tg-Ab) on Kryptor (normal Tg-Ab <60 U/ml) (BRAHMS Diagnostic, Berlin, Germany);

BRAHMS Dynotest anti-Tg (normal Tg-Ab <60 U/ml).

We tested the cutoff level recommended by the manufacturers of the assays as well as a lower level <20 U/ml, as suggested in epidemiological studies [8].

- Tg-Ab interference evaluated from recovery test

DELFIA hTg with recovery test using 25 ng of Tg (acceptable recovery 80–120%) (PerkinElmer, Turku, Finland);

BRAHMS Tg+ with recovery test using 50 ng of Tg (acceptable recovery 70–130%);

BRAHMS Tg+ with recovery test using 1 ng of Tg (acceptable recovery 70–130%).

Follow-up regarding recurrent/persistent thyroid cancer was performed in June 2011 via the Danish Thyroid Cancer Registry (DATHYRCA) combined with data from medical records.

23 patients had been treated for follicular and 72 for papillary thyroid cancer. Regional lymph node metastases were found in 22 patients, and three patients had lung metastases at the time of diagnosis. All patients were treated with total thyroidectomy and radioiodine ablation. All patients had TSH-stimulated Tg measurements and WBS approximately 6 months after radioiodine ablation.

Registrations. The study was approved in the Danish Ethical Committee journal No. H-32009-121 and in the Danish Data Protection Agency journal No. HEH.afd.O.819.

Results

Tg-Ab measurements and Tg recovery tests that may suggest interference with Tg measurements were seen in many samples. A positive result in at least one of the participating laboratories was seen in 48 of the 95 samples tested.

Measurements of Tg-Ab

The percentage of patient samples characterized as failing to meet the criteria for being usable for evaluating the value of s-Tg varied from 7.4% (DPC IMMULITE using a cutoff <40 U/ml) to 40% (BRAHMS Kryptor using a cutoff <20 U/ml) (table 1).

Recovery Test

Abnormal recovery test varied from zero (BRAHMS Tg+ with recovery test using 50 ng of Tg – the primary

Table 1. Percentage of patient samples failing the criteria for being usable for evaluating the Tg concentration

	DPC IMMULITE 2000	*DPC IMMULITE 2000**	BRAHMS Tg Kryptor	*BRAHMS Tg Kryptor**	BRAHMS Dyno test anti-Tg	*BRAHMS Dyno test anti-Tg**	DELFIA hTg recovery test 25 ng	BRAHMS Tg+ recovery test 50 ng	BRAHMS Tg+ recovery test 1 ng
Method	Tg-Ab	*Tg-Ab*	Tg-Ab	Tg-Ab	Tg-Ab	Tg-Ab	recovery test	recovery test	recovery test
Limits of acceptance	<40 U/ml	*<20 U/ml*	<60 U/ml	*<20 U/ml*	<60 U/ml	*<20 U/ml*	80–120%	70–130%	70–130%
Not acceptable for Tg evaluation§	7.4%	*10.5%*	10.5%	*40.0%*	9.5%	*24.0%*	26.3%	0%	5.0%

* The reference range of <20 U/ml is suggested in epidemiological studies; § the percent of the 95 patient samples evaluated that were not acceptable for Tg evaluation.

selection of samples) to 26.3% (DELFIA hTg with recovery test using 25 ng of Tg) (table 1).

Nine patients had measurable Tg-Ab by all 3 autoantibody assays and in addition abnormal recovery test in one assay. Only 2 patient samples were found 'non-measurable' for s-Tg evaluation by all laboratories (excluding the recovery test 50 ng, used for the primary selection of samples).

Measurements of Tg

The 95 samples selected for the study had serum Tg ≤1μg/l as measured by BRAHMS Tg+ in 2006, and they were accepted for evaluation based on a Tg recovery test.

In 13 of these samples, an s-Tg value >1 μg /l was found in at least one of the other assays (1–2 μg/l in 7 samples). In 5 samples, the value were 2–3 μg/l by DPC IMMULITE 2000, and in one sample an s-Tg level of 5 μg/l was measured by DELFIA hTg. In none of these 6 patients were Tg-Ab detected with any of the tests used.

In 2 of the 7 samples with an s-Tg value of 1–2 μg/l, Tg-Ab were measurable by 2 assays.

Clinical Follow-Up

During the 5 years of clinical follow-up (2006–2011) no recurrence of cancer was observed in 92 of the 95 patients. Three patients deserve special attention: 2 patients had persistent disease and lung metastases evaluated on WBS, although no detectable TSH-stimulated s-Tg values were found in 2006 and nor were there signs of antibody interference in any of the tests.

Samples from a third patient who developed recurrence and metastasis showed a concomitant rise in s-Tg in 2007 and the patient had a negative Tg-Ab test.

Discussion

A recent publication described a follow-up of 944 patients treated for differentiated thyroid cancer with surgery and radioiodine ablation [3]. During a mean follow-up period of 28 months, persistent disease or recurrence were demonstrated in only 30 patients (<3%). This indicates that the modern way of treating differentiated thyroid cancer seems successful.

The key to a simple follow-up is measurement of the tumor marker s-Tg, and methods with high sensitivity are recommended [3]. However, it is crucial to be able to discriminate which Tg values are usable for evaluation of tumor recurrence, and which are not due to potential Tg-Ab interference with the assay. Such discrimination is useful to differentiate which patients should undergo further diagnostic tests or even treatment, and which should just be followed. Tg-Ab may interfere with the Tg assay, but the extent of the problem differs between assays.

A prevalence of approximately 20% [9] of measurable Tg-Ab has been reported in patients with differentiated thyroid cancer, and there is a growing acceptance that an undetectable s-Tg value combined with the presence of Tg-Ab does not exclude the presence of cancer. In patients where s-Tg is not accepted as a tumor marker, WBS may be used as the diagnostic procedure, but it has a low sensitivity. Ultrasound can be used for the detection of recurrence in the neck region, but not for detection of distant metastases. PET-CT is useful for detection of distant metastases; however, the sensitivity is low, probably due to the slow growth of most thyroid cancer.

This nationwide study involving all centers treating thyroid cancer in Denmark demonstrates large discrep-

ancies between assays in their validation of s-Tg values in the follow-up on patients treated for thyroid cancer.

Our results emphasise the dilemma, on how to optimally exclude antibody interference with the Tg measurement. The recommendation [4] is always to measure Tg-Ab when measuring s-Tg. However, in a recent study comparing 12 different Tg-Ab assays in 42 patients showing detectable Tg-Ab in at least one method, only 4 patient samples were positive in all tests and a concordance of only 65% was found between assays [10]. In another study from the same group, 2 of 4 Tg-Ab assays failed to detect interfering Tg-Ab in 20–30% of cases [11]. Another question is: What level of measured antibodies is of clinical relevance? Several studies have found that the propensity for Tg-Ab to interfere was only weakly related to the Tg-Ab concentration. Still direct Tg-Ab measurement was more reliable than the recovery approach for detecting interfering Tg-Ab [10, 12, 13].

The recommended clinical cutoff for BRAHMS Tg-Ab is <60 U/ml. However in epidemiologic studies [9] Tg have been measurable down to <20 U/ml using this assay and some authors advocate a low cutoff due to a possible interference even at low levels of Tg-Ab. By using the clinical cutoff level recommended by the company (<60 U/ml for the BRAHMS assay and <40 U/ml for DPC IMMULITE) we found that samples from 12 patients were positive in at least one of the assays. If, however, the cutoff was reduced to <20 U/ml the number increased to 38.

Another approach to screening samples for antibody interference with the s-Tg analysis is to perform a Tg recovery test, i.e. to measure the recovery of a known amount of Tg added to the sample. In a study from 1995 by Mariotti et al. [14], they questioned if it is an unobtainable goal to measure thyroglobulin in serum with TgAb and described recovery as unreliable in some patients with positive TgAb, undetectable serum Tg and metastatic DTC or autoimmune thyroid diseases (Graves' disease and Hashimoto's thyroiditis) [13]. In accordance with this, a study using BRAHMS Dynotest Tg+ found that 72 of 153 patients with Graves' disease had measurable Tg-Ab and that 34 of these had normal recovery test, indicating that Tg-Ab did not interact with the s-Tg assay.

Serial Tg-Ab measurements per se may provide a clinically valuable, surrogate tumor marker, because values of Tg-Ab seem to respond to changes in the presence of Tg antigens [10]. However, as mentioned, sensitivity of Tg-Ab assays is highly variable and cannot be interchanged [11]. Another way of detecting the presence of Tg-producing cells is to measure circulating mRNA of Tg, and recent studies have showed promising results using this approach [15, 16].

Most studies performed in this field evaluated technical/methodological details of assays. Our intention was to evaluate the quality of the Tg assays in the follow-up of patients with thyroid cancer seen from a clinician's perspective.

None of the assays tested would have allowed a better classification than the method originally applied in 2006. Some of the assays would have excluded many more samples from the use of Tg evaluation. The very low recurrence rate seen during the 5 years follow-up speaks in favor of a correct detection of recurrent disease. However, the patients were not all followed by additional stimulated Tg or 131-I WBS and our material is too small and the follow-up period too short to rule out false-negative test results. Nevertheless, our study was not performed to evaluate thyroid cancer recurrence. Our focus was the dilemma how to optimally exclude antibody interference with the Tg measurement.

A recent editorial [17] dealt with the problem of interfering Tg antibodies and discussed whether Tg measurements are flawed for use in monitoring patients treated for differentiated thyroid cancer. The diagnostic dilemma is whether a more intensive evaluation, leading to higher costs, and an increased psychological burden for the patient should be performed in patients with positive Tg-Ab, having in mind the overall good prognosis of differentiated thyroid cancer and the low sensitivity of WBS, ultrasound and PET-CT.

Conclusion

Among the centers in Denmark treating thyroid cancer, large discrepancies were demonstrated in acceptances of s-Tg values for the follow-up of treated thyroid cancer. Our results illustrate a diagnostic dilemma when using low-level cutoff values of Tg-Ab.

Disclosure Statement

Dr. Nygaard has received free Tg and Tg-Ab assays from BRAHMS Diagnostic for this study. Otherwise the authors declare that no financial or other conflicts of interest exist in relation to the content of this article.

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