National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Differentiated Epithelial Thyroid Carcinoma

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Abbreviations: rhTSH, recombinant human thyrotropin; RT-PCR, reverse transcription-polymerase chain reaction; Tg, thyroglobulin antigen; TgAb, anti-thyroglobulin autoantibodies; THST, levothyroxine-suppressed thyrotropin levels; THW, thyroid hormone withdrawal

INTRODUCTION

Thyroid carcinoma constitutes 1.6% of incident cancers of all ages and 3.8% of cancers for those less than 20 years of age. Women are affected more than men, in a ratio of 3 to 1. While the incidence of many cancers appear to be diminishing in North America, thyroid cancer shows the greatest increase in incidence of all cancers in American women as well as the most rapidly increasing rate of cancer mortality in American men (1, 2). Internationally, such statistics are variably accessible, however, notable accidents, such as with the nuclear reactor in Chernobyl, Belarus, in 1986, suggest significant environmental influences. Thyroid carcinomas, mostly derived from thyroid follicular cells, are frequently responsive to treatment with surgery and radioactive iodine. Some patients are at significant risk for persistent disease and subsequent mortality. Although this risk diminishes over time, life-long follow-up is necessary since a small number of patients have late recurrent disease or mortality despite apparent initial resolution of disease.

The most common presentation for thyroid cancer is as a painless nodule in the thyroid gland or an enlarged lymph node in the neck, although the cancer is sometimes first detected as a distant metastasis. The best diagnostic test to distinguish benign from malignant thyroid nodules or neck masses is the fine needle aspiration biopsy, although it is not uncommon for the surgical histological examination to be the first indicator of thyroid malignancy. There is no role for serum thyroglobulin assessment in distinguishing benign from malignant thyroid diagnoses. However, when the presenting tumor is distant from the neck, immunohistochemical assessment of thyroglobulin can often elicit the primary source of the tumor.

Both normal and malignant thyroid follicular cells usually produce thyroglobulin that can be measured in the peripheral circulation. Since no other tissues have been found to produce thyroglobulin, this protein serves as a specific marker for papillary or follicular thyroid cancers after complete surgical resection of the thyroid and radioiodine ablation of remnant thyroid tissue, removing all benign sources of thyroglobulin. Although nuclear scanning with radioactive iodine provides an effective means of detecting thyroid cancer after resection of the primary tumor and thyroid gland, thyroglobulin measurements often prove more sensitive for this purpose (3). Appreciation of the clinical utility of serum thyroglobulin in the follow-up of differentiated thyroid carcinomas has prompted consensus panels to report tumor assessment strategies based upon serum thyroglobulin measurements (4, 5). This discourse seeks to present guidelines on the use and interpretation of thyroglobulin measurements in the post-surgical detection of residual and recurrent thyroid cancer, with consideration of the practical

limitations of this methodology. There are evolving clinical strategies for the use of thyroglobulin, its associated autoantibody, and assessment of blood for circulating cells expressing its mRNA.

Since mortality and morbidity in thyroid cancer is often measured over decades, there is a paucity of prospective clinical studies that are capable of evaluating tests based on thyroglobulin-related parameters. Likewise, the recent evolution of these laboratory methods precludes use of most retrospective reviews. These circumstances place greater reliance upon the expertise of clinicians with special clinical experience in this rare cancer and are subject to future reassessments of this information.

CURRENTLY AVAILABLE MARKERS FOR THYROID CANCER

Three separate parameters, related to thyroglobulin, have been evaluated as clinical markers of thyroid cancer status. These include serum levels of thyroglobulin antigen (Tg), serum concentrations of anti-thyroglobulin autoantibodies (TgAb), and assessment for circulating thyroid cancer cells in peripheral blood using DNA amplification techniques that quantify thyroglobulin mRNA. Table 1 describes their phase of development and the levels of evidence (LOE) for evaluating their usefulness.

SERUM TUMOR MARKERS IN THYROID CANCER: NACB RECOMMENDATIONS

Table 2 summarizes recommendations from representative clinical management guidelines for thyroid cancer suggested by interested professional organizations, including those of the National Academy of Clinical Biochemistry (NACB). Supporting information on the clinical use of these parameters is presented in the following discussion.

Biological Features of Thyroglobulin

Human thyroglobulin is a large and complex glycoprotein, consisting of a 670 kDa dimer of two identical subunits. It is the unique product of thyroid follicular cells and their malignantly transformed progeny. Thyroglobulin has 20 asparagine-linked glycosylation sites that bear highly branched oligosaccharide chains. These oligosaccharides, as well as underlying peptide residues, are variably phosphorylated and sulphated, adding to the changeable complexity. Besides this, there are variable degrees of iodination of tyrosine residues in the protein, reliant upon independent processes of dietary iodine availability, iodide transport into the follicular cell, catalytic effects of thyroperoxidase and, likely, other enzymes involved in iodide organification (6-9). Thyroid follicular cells are stimulated to secrete thyroglobulin by thyrotropin (thyroid stimulating hormone, TSH), produced by the pituitary in response to hypothyroidism or provided

exogenously by injection of recombinant human thyrotropin (rhTSH). There is evidence that this stimulation may affect thyroglobulin structure, particularly in the makeup of its complex carbohydrate chains (10). In this way, the thyroglobulin molecule can be highly heterogeneous in its composition, significantly changing antigenicity and altering the effectiveness of immunological methods for quantification and assessment.

Thyroid carcinomas, specifically those derived from thyroid follicular cells, produce thyroglobulin with additional antigenic complexity. Thyroglobulin from thyroid cancer cells is often poorly iodinated compared to thyroglobulin from benign follicular cells (11, 12). Malignant transformation is known to alter the composition of secreted glycoproteins, often increasing the complexity of oligosaccharide branching (13). Similar changes have been seen in thyroid cancer cells (14). Considering that such moieties provide antigenic sites used for immunoassays, there is ample reason for a wide variation of assay results between different assays in different clinical conditions (15). Since the typical international thyroglobulin reference preparation (CRM-457, BCR Brussels) used to calibrate thyroglobulin immunoassays is derived from normal thyroid tissue (16, 17), the antigenic differences between malignancy-derived thyroglobulin and this reference standard are even more important in its use for measurements of thyroglobulin as a tumor marker.

Thyroglobulin gene transcription is stimulated by activation of the thyrotropin receptor, although a basal level of thyroglobulin secretion is often evident in the presence of residual thyroid cancer, even when thyrotropin levels are suppressed to undetectable levels by exogenous levothyroxine administration. In this way, assessment of thyroglobulin levels is most sensitive when thyrotropin levels are elevated (>30 mU/L) by levothyroxine withdrawal (18) or recombinant human thyrotropin (rhTSH) administration. Circulating thyroglobulin is cleared from the bloodstream with a mean half-life of 65 hours, requiring nearly one month for complete removal after a total thyroidectomy (19). These factors set the stage for clinical use of thyroglobulin assays to detect evidence of persistent or recurrent thyroid cancers following total thyroidectomy and radioiodine ablation of the post-surgical thyroid remnant. Such guidelines are predicated on the follow-up of patients so prepared. They are not applicable to patients with incomplete thyroid gland resections and to those who have not received appropriate post-operative radioiodine therapy.

Measurement of Thyroglobulin in the Serum

The two common methods for determining thyroglobulin levels in the circulation are the radioimmunoassay (RIA) and the immunometric assay (IMA) techniques. Each of these

methods has different attributes: RIAs may be less susceptible to interference by anti-thyroglobulin autoantibodies (TgAb) (20-22) while IMA methods have greater sensitivity and shorter incubation times, and are easier to automate (3). Each method may differ in accuracy of defining clinically useful information (23). Types of IMA assays using non-isotopic formats, such as enzyme-linked immunosorbent methods (ELISA) also have useful sensitivities (24, 25). Newer assays, particularly those using chemiluminescent technologies, have enhanced sensitivities below 0.1 µg/L and may provide additional clinical benefits (26, 27). All these methods need to be evaluated in terms of their sensitivity, precision, suitability for automation, standardization, vulnerability to high-dose "hook" effects, and susceptibility to TgAb interference.

The high-dose "hook" effect refers to the underestimation of the thyroglobulin level that occurs in the presence of extremely high concentrations of thyroglobulin, which exceed the binding capacity of the capture and signal antibodies of immunoassays (28). In practice, there is little concern regarding this phenomenon. Patients with thyroglobulin levels high enough to invoke this effect (>1000 µg/L) invariably have large tumor burdens presenting no questions regarding the presence of disease. Whereas changes in thyroglobulin levels might correspond to clinical responses to therapy, tumor burdens corresponding to such high thyroglobulin levels are best followed by radiographic techniques assessing tumor volume rather than surrogate tumor markers such as thyroglobulin (29). Additionally, many newer assays are much less likely to be affected by "hooking".

Thyroglobulin Assay Sensitivity and Stimulation Methods

The minimum detection limit of an assay, calculated from the mean plus two standard deviations of replicate measurements of the zero calibrator within an assay run, defines the lowest concentration of thyroglobulin able to be distinguished, i.e. the analytical sensitivity. In contrast, the functional sensitivity limit, represented as the lowest thyroglobulin concentration with an inter-assay coefficient of variation of 20%, provides a better indication of an assay's actual performance over time. Clinically useful assays should at the minimum be able to detect serum thyroglobulin in euthyroid individuals with intact thyroid glands. Ultimately, the ideal assay would have sufficient sensitivity to detect thyroglobulin, i.e. to herald recurrent thyroid cancer, without thyrotropin stimulation (24). Unfortunately, in thyroid cancer patients with levothyroxine-suppressed thyrotropin levels (THST) and thyroglobulin levels below 1 µg/L, there is still a 3-8% risk of significant disease (4) making THST thyroglobulin assessment clinically insensitive. For this reason, thyrotropin stimulation, either by thyroid hormone withdrawal or administration of

rhTSH (30), augments the ability of each assay to detect thyroglobulin produced by residual or recurrent thyroid cancer, representing the best clinical sensitivity of the assay.

In evaluating the clinical sensitivity of thyrotropin-stimulated thyroglobulin assessment in monitoring thyroid cancer patients, it may be important to consider the adequacy of the method of thyrotropin elevation, by either thyroid hormone withdrawal (THW) or rhTSH administration, and the thyroglobulin threshold used. Using a rhTSH-stimulated thyroglobulin threshold of 1 µg/L, 2 of 78 patients (3%) beneath that threshold had macroscopic tumor detected with neck ultrasonography (31). In another study, with the threshold increased to 2 µg/L, 13% of patients revealed tumor on radioiodine scanning despite absence of a positive thyroglobulin response (32). An additional study with a rhTSH-stimulated thyroglobulin threshold of 2.5 µg/L (33), found over 23% of patients with evident tumor on their scans and negative thyroglobulin results. Likewise, THW-stimulated thyroglobulin assessments can be negative in the face of detectable disease, even with a threshold of 1 µg/L, as was evident in 13/18 patients (72%) undergoing radioiodine dosing and repeat neck surgery with an intra-operative gamma probe to detect tumor sites (34). Both methods of thyrotropin stimulation, using a thyroglobulin threshold of 2 µg/L, proved insensitive for 12.5% of patients found to have persistent tumor sites using positron emission tomography (PET) scans (35). A comparison of both methods of thyrotropin stimulation was undertaken in a prospective evaluation of six thyroid cancer patients with persistent disease and low thyroglobulin levels while on THST. Five of these patients had greater thyroglobulin stimulation with THW than using rhTSH (36). As would be expected, all of these studies excluded TgAb positive patients that would have added insurmountable complexity to their analyses. A recent meta-analysis also suggests that THW is preferable to rhTSH (37); however, both methods need to be considered in relation to risk stratification for recurrent disease.

Antibody Interference: Heterophile Antibodies and Autoantibodies

Human heterophile antibodies bind immunoglobulins of the species used to generate the antibody components of the assay, such as mouse. They are often unsuspected, are difficult to detect, and may occur in as many as 3% of thyroid cancer patients. The typical effect of these antibodies is to cause spurious elevations of thyroglobulin levels. Use of heterophile-blocking tubes to treat 1106 serum samples with thyroglobulin levels greater than 1 µg/L, prior to repeating the thyroglobulin assay, resulted in undetectable thyroglobulin levels (<0.1 µg/L) in 20 samples (38). Considering that a major value of thyroglobulin is to detect persistent or recurrent disease, often before there are clinical signs or positive radiographic or nuclear images,

artifactual elevations of this kind can result in significant and unwarranted anxiety and diagnostic or therapeutic efforts. If thyroglobulin results remain unsupported by all other diagnostic and clinical assessments, it is important to consider heterophile antibody effects.

Anti-thyroglobulin autoantibodies (TgAb) constitute the greatest impediment to clinical use of thyroglobulin assays for thyroid cancer patients. Such antibodies are far more frequent among thyroid cancer patients, affecting 25% of them compared to 10% of the general population (21). Most IMA methods provide spuriously low results in the presence of TgAb while RIA levels may be inappropriately high. Such discordant results, obtained using both assays for the same sample, may provide evidence of this interference. TgAb hinders thyroglobulin-directed detection of disease by sufficient degree to warrant mandatory TgAb testing of all serum samples submitted for thyroglobulin assessment (20, 22). Even when some TgAb assays do not detect antibodies, sufficient TgAb levels may be present to interfere with thyroglobulin quantification (39). Despite many approaches to this problem, including using RIA methods less vulnerable to interference from TgAb (40), assessing recovery of exogenous thyroglobulin (41), and selecting assay antibodies directed against non-autoimmune thyroglobulin epitopes (42), TgAb interference issues have not been solved (43).

Despite their role as a “spoiler” of Tg assessment, TgAb titers have shown promise as independent markers for the presence of thyroid cancer (21, 44, 45). This is a reasonable expectation, considering the limitations of the immune system’s anamnestic response for antibody production in the absence of antigen. There are patients with a median time of TgAb clearance, after complete removal of thyroidal tissues, of three years (46). Persistence of TgAb for longer periods of time suggests the persistence of sources of Tg antigen, namely normal or malignant thyroid tissues. Rising or stable TgAb titers signal the presence of thyroid carcinoma in the follow-up of thyroidectomized patients even in the absence of discernible thyroglobulin antigen (46).

Measurement of Thyroglobulin mRNA in Circulating Cells

One approach towards accurate assessment of disease status in thyroid cancer patients, particularly in those with TgAb, is to assess peripheral blood samples for circulating thyroid cancer cells. Use of the reverse transcription-polymerase chain reaction (RT-PCR) to look for cells containing thyroglobulin mRNA suggests advantages of independence from effects of TgAb and the ability to detect cancer cells directly rather than rely upon a surrogate tumor marker. Its use is predicated on the presumption of absence of “illegitimate transcription” of thyroglobulin in non-thyroidal cells (47). Early studies, some using quantitative RT-PCR

techniques, suggested the value of this approach (48-50). Unfortunately, many investigators have not found this technique to have diagnostic utility (51-56). Clearly, there are numerous performance characteristics and techniques that must be optimized to make this approach more useful. Current testing methodologies have some diagnostic value in patients with TgAb, but cannot yet supplant the clinical information derived from thyrotropin-stimulated serum thyroglobulin assessment (57-60). At the current time, RT-PCR-based methods of assessment for circulating thyroid cancer cells should be considered research procedures with significant potential for future clinical applications.

Thyroglobulin Assessment in Clinical Practice

Serum thyroglobulin assessment is a critical component of thyroid cancer management. In view of the variety of assay methodologies, the complexity of the thyroglobulin molecule, the wide range of antibodies used in these assays, and the diversity of potential epitopes for binding, it seems reasonable to make certain that clinicians learn the performance parameters of their own particular assay. Such parameters include the name of the commercial assay kit, its functional sensitivity and precision, and whether samples are routinely screened for TgAb. In order to provide meaningful clinical continuity, it is advised that the same assay is used in the same patient longitudinally.

Patient Prerequisites

There are two prerequisites of patient preparation that are critical components of a diagnostic monitoring strategy that employs thyroglobulin testing. First, the patient should have undergone a total or near-total thyroidectomy, resecting macroscopic sources of thyroglobulin, both benign and malignant. Following this, radioiodine therapy sufficient to deliver at least 300 Gray to the post-thyroidectomy remnant (61) provides the best assurance that measurable thyroglobulin levels are consequent to residual malignant cells. Under these circumstances, aside from potential effects of heterophile antibodies and interference from TgAb, any measurable thyroglobulin constitutes evidence of residual or recurrent thyroid carcinoma.

In patients with interfering TgAb titers, serum levels of both thyroglobulin and TgAb should be ascertained at regular intervals, both on THST and with thyrotropin-stimulation. This is appropriate because there is evidence that TgAb titers may serve as surrogates for thyroglobulin levels (20, 62) and because serial thyroglobulin measurements may reveal changes reflecting disease status, even despite the presence of TgAb. For example, an

unmeasurable or low level of thyroglobulin, in the presence of TgAb, may become detectable or elevated if the TgAb level diminishes despite the same degree of tumor burden.

Relationship of Thyroglobulin to Disease Status

It is useful to review the relationship between the results of diagnostic radiological testing and concurrent thyroglobulin levels, also noting whether the thyroglobulin is obtained while on THST or thyrotropin stimulation. If, at any time thyroid carcinoma is detected, using physical examination, radioiodine scanning, ultrasound or other radiological methods (and verified by biopsy studies if findings are uncertain), the thyroglobulin level (in the absence of TgAb) should be seen to correspond to the mass of tumor discovered. Undetectable thyroglobulin in the face of known disease, particularly under thyrotropin stimulation (34), defines the loss of expression of thyroglobulin by the patient's thyroid cancer, much as it can lose the expression of other differentiated functions (63). If this is seen, thyroglobulin is no longer useful as a tumor marker in that particular patient.

As previously discussed, thyrotropin stimulates thyroid carcinoma cells to secrete thyroglobulin, whereas THST decreases this secretion, sometimes to undetectable levels. Measurable thyroglobulin levels after thyroidectomy and radioiodine ablation denotes residual or recurrent disease, even in the absence of positive findings on other diagnostic studies (64-67). Thyrotropin-stimulation can uncover thyroglobulin-secreting thyroid cancer cells, even when patients with apparently low-risk prognostic factors have undetectable levels while on THST. In one study 22% of such patients had elevated thyroglobulin levels when thyrotropin levels were increased by THW (68).

The presence of detectable thyrotropin-stimulated thyroglobulin in the absence of positive diagnostic radioiodine whole body scans provides sufficient justification for a trial of administration of a therapeutic dosage of radioiodine. A reasonable portion of these patients will demonstrate either positive post-therapy whole body scans (at 2-7 days after the therapy dose) revealing the sites of disease, reductions in thyroglobulin levels on follow-up evaluation, or later resolution of metastatic sites after repeat therapeutic radioiodine administration (69-73). On the other hand, the absence of such a response documents the absence of iodine avidity in those patients, permitting the clinician to focus on alternative diagnostic techniques to identify tumor sites.

CONCLUSION

Serum thyroglobulin measurements provide a critical tumor marker for differentiated thyroid carcinomas, derived from thyroid follicular cells, after primary thyroidectomy and radioiodine ablation. Appropriate attention to features of the thyroglobulin assay, the method of patient preparation (either with THST or with thyrotropin stimulation), and potential interference from TgAb or heterophile antibodies, permits this assay to provide the greatest wealth of clinical information regarding disease status.

Table 1. Useful and potentially useful markers for well-differentiated epithelial thyroid cancer

Cancer Marker	Proposed Use/Uses	Phase of Development	LOE[1]	Refs
Thyroglobulin	Surveillance for recurrent/residual disease following thyroidectomy and radioiodine ablation	In clinical use; value validated by empiric clinical experience	I	4, 5
	Assess changes in tumor mass	As above	II	4, 5
	Predict post-thyroidectomy Tg-secretion ability of thyroid cancer in the presence of metastatic disease on radiological or nuclear scanning	Proposed, with some empiric evidence, but not yet sufficiently validated	IV	63
Thyroglobulin antibodies	Assess validity of serum Tg measurements following thyroid surgery and radioiodine ablation	In clinical use; value validated by empiric clinical experience	II	20, 22
	Surveillance of recurrent/residual disease	Proposed, with some empiric evidence, but not yet sufficiently validated	III	21, 44, 45
Thyroglobulin mRNA	Surveillance for recurrent/residual disease following thyroidectomy and radioiodine ablation in the presence of TgAb	Not in routine clinical use; undergoing development and prospective evaluation. Current studies are conflicting.	IV	48-56
	Higher sensitivity surveillance (compared to serum Tg) for residual disease following thyroidectomy and radioiodine ablation	Proposed and experimental, but not validated. Likely dependent upon future new methodologies	None	

[1] LOE, level of evidence (as described in Section 1); Tg, thyroglobulin

Table 2. Recommendations for use of markers in well-differentiated epithelial thyroid cancer

Marker(s)	Application	ATA[1]	Joint AACE/ AAES[2]	NCCN[3]	BTA[4]	NACB[5] 2005
Thyroglobulin	Surveillance for recurrent/residual disease following thyroidectomy and radioiodine ablation	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
	Assess changes in tumor mass	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
	Predict Tg-secretion ability of thyroid cancer	None published	None published	None published	None published	**Yes**
Thyroglobulin antibodies	Assess validity of serum Tg	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
	Surveillance following surgery and radioiodine ablation	None published	None published	None published	None published	**Yes**
Thyroglobulin mRNA	Surveillance for recurrent/residual disease following thyroidectomy and radioiodine ablation in the presence of TgAb	None published	None published	None published	None published	None published
	Higher sensitivity surveillance (compared to serum Tg) for residual disease following thyroidectomy and radioiodine ablation	None published	None published	None published	None published	None published

[1]ATA, American Thyroid Association (74); [2]AACE/AAES, American Association of Clinical Endocrinologists/American Association of Endocrine Surgeons (75); [3]NCCN, National Cancer Center Network (76); [4]BTA, British Thyroid Association (77); [5]NACB, National Academy of Clinical Biochemistry

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