Diagnosis of Recurrent Thyroid Cancer in Patients with Anti-thyroglobulin Antibodies

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Whole-body radioiodine scanning, measurement of serum thyroglobulin, and a number of different radiographic methods are used to monitor patients with thyroid cancer for recurrence of their tumors. Over the past decade, substantial improvements in thyroglobulin immunoassays and a greater recognition of the limitations of I-131 scanning, particularly regarding its relatively low sensitivity for neck recurrences, have led to recommendations that rely primarily on accurate thyroglobulin measurements and neck ultrasound [1, 2]. However, there are some limitations of current thyroglobulin immunoassays, including inadequate sensitivity during L-T4 therapy depending on the particular assay and the presence of interfering anti-thyroglobulin antibodies in approximately 20 % of patients. Because circulating anti-thyroglobulin antibodies interfere with accurate thyroglobulin measurement, there is increased reliance on radiographic testing to monitor patients with detectable anti-thyroglobulin antibodies. In this chapter, potential alternative approaches to blood-based monitoring methods in patients with circulating anti-thyroglobulin antibodies will be discussed. The use of imaging modalities in thyroid cancer is only briefly discussed in this chapter as they are unaffected by anti-thyroglobulin antibodies. Various imaging methods are part of a general approach to monitoring patients, however, and are discussed in detail in other sections of the book.

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Thyroglobulin Antibodies

Thyroglobulin is a thyroid-specific 660 KD protein that serves as a precursor and storage protein in thyroid hormone biosynthesis that is produced by normal thyroid cells and well-differentiated thyroid cancer cells. Its presence is therefore specific for thyroid cancer only in patients who are devoid of normal thyroid tissue. Patients who have a history of thyroid cancer and who are not completely athyreotic may have detectable levels that reflect the presence of remaining nonmalignant thyroid tissue. The quantitative amount of circulating thyroglobulin usually correlates with the extent of disease and the amount of residual or recurrent tissue, enhancing the clinical usefulness of the assay [3]. Similar to I-131 uptake, thyroglobulin production and release are regulated by thyrotropin (TSH). Consequently, TSH-stimulated thyroglobulin measurements are often performed in order to maximize sensitivity in recently diagnosed or high-risk patients. In this setting, the assay retains its specificity and becomes even more sensitive for disease detection. For these reasons, over the past 10–20 years, thyroglobulin monitoring has become an integral part of thyroid cancer monitoring.

Circulating anti-thyroglobulin antibodies are the major limitation of modern thyroglobulin immunoassays. Thyroglobulin is a large protein with numerous antigenic epitopes, many of which can induce antibody formation within an individual patient [4]. Major efforts have been made to circumvent the effects of these heterogenic antibodies on the thyroglobulin assays, but to date, none have proven successful for clinical practice. For this reason, it is recommended that anti-thyroglobulin antibodies are measured on the same serum sample as thyroglobulin to assess the validity of the thyroglobulin measurement. The accuracy of the anti-thyroglobulin antibody measurement is critical for clinical decision-making, a topic that is receiving increased scrutiny over the past few years and will be discussed below. Most laboratories now measure antibody presence using a sensitive

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immunoassay and a standard antibody preparation, rather than determining antibody titers [3]. Results between different anti-thyroglobulin antibodies are not always consistent, thus if possible the same assay system should be used over time for individual patients. Some authors have advocated the use of "recovery" assays to measure the degree of interference by anti-thyroglobulin antibodies, since not all antibodies may interfere with thyroglobulin measurement to the same degree. Another approach to identify non-interfering but detectable anti-Tg antibodies is to perform both RIA and IRMA assays on the same sample to identify concordant results. However, since the majority of anti-thyroglobulin antibodies appear to interfere with measurement [5], recovery assays and performing multiple types of thyroglobulin assays on patient samples are not routinely performed by most clinical laboratories.

Anti-thyroglobulin autoantibodies themselves are a marker of autoimmune thyroid disease in patients who are hypo- or hyperthyroid, but their specificity for predicting disease is not clear as they may also be seen in euthyroid adults. Their presence was recently reported in 4–10 % of unselected women and 1–3 % of unselected men in a large US population [6, 7]. For uncertain and highly debated reasons, patients with thyroid cancer have a much greater incidence of detectable anti-thyroglobulin antibodies with most studies reporting an incidence of approximately 20 % [1, 8]. It is uncertain whether the presence of thyroid autoantibodies is a cause or an effect of thyroid cancer. In some cases, the presence of circulating anti-thyroglobulin antibodies correlates with the presence of intrathyroidal autoimmunity as evidenced by the presence of chronic lymphocytic thyroiditis or a peritumoral lymphocytic infiltration on histopathology of the thyroid gland. The correlation between anti-thyroglobulin antibodies and thyroid autoimmunity has led to speculation that patients with detectable anti-thyroglobulin antibodies may have a better prognosis due to an enhanced anti-thyroid cancer cell immune response [9, 10]; however, correlation studies have reported inconsistent results [11–13]. The lack of consensus on this relationship may be, in part, due to a lack of distinction between patients with generalized lymphocytic infiltration associated with Hashimoto's thyroiditis and those with peritumoral lymphocytic infiltration. The latter is a well-recognized immune response to the presence of tumor and may be seen in various malignancies.

An additional confounding factor in the reliability of thyroglobulin measurement is heterophile antibodies; their presence may result in a false elevation of thyroglobulin levels [14]. Heterophile antibodies are those that can bind to animal antigens used in the immunoassay. One study reported the presence of interfering heterophilic anti-thyroglobulin antibodies in up to 3 % of thyroglobulin antibody-negative thyroid cancer serum samples [14]. More recent studies, however, have found the prevalence of such heterophile antibodies to be significantly lower [15, 16]. Testing for the presence of heterophile antibodies requires the use of heterophile-blocking tubes. Current assays may be less likely to be affected by such interfering substances as most laboratories routinely use additives to reduce heterophile interference. Nonetheless, their presence should be suspected when the serum thyroglobulin is elevated in the setting of low clinical suspicion of disease and the absence of radiographic abnormalities.

Alternative Laboratory Tests for Thyroid Cancer

Thyroglobulin Antibody Levels

Because thyroglobulin antibodies represent a response to a thyroid-specific antigenic stimulus, it has been speculated that the reduction and/or disappearance of anti-thyroglobulin antibodies in peripheral blood would correlate with a reduced antigenic burden, i.e., the remission and/or cure of thyroid cancer in patients with anti-thyroglobulin antibodies. In support of this concept, several studies have correlated the reduction and disappearance of anti-thyroglobulin antibodies with reduced tumor burden [5, 8, 17, 18]. One study found that the median time to disappearance of thyroglobulin antibodies after initial treatment was 3 years and that the coexistence of autoimmune thyroid disease did not modify the pattern of disappearance of thyroid antibody compared to those with focal lymphocytic infiltration [19]. Taken together, it appears that the loss of detectable anti-thyroglobulin antibodies in an individual with differentiated thyroid cancer who has a history of circulating anti-thyroglobulin antibodies reflects an improvement in disease burden and enhances the ability to accurately measure thyroglobulin. Conversely, the appearance of thyroglobulin antibodies in a previously negative anti-thyroglobulin antibody patient suggests the possibility of persistent or recurrent disease [5]. Therefore, it is reasonable to monitor thyroglobulin and anti-thyroglobulin antibodies in these patients [20].

Importantly, recent studies have suggested that there is variability in the sensitivity of various IMA assays to detect the presence of thyroglobulin antibodies [21]. Laboratories may adopt the manufacturers' cutoff for a "detectable" anti-thyroglobulin antibody which was created to detect thyroid autoimmunity and is insensitive for detecting interference with thyroglobulin measurements. Consequently, specimens may be incorrectly classified as anti-thyroglobulin antibody negative. Importantly, even a low anti-thyroglobulin antibody level has the potential to interfere with the measurement of thyroglobulin [21]. Such false results have the potential to influence patient management. It is imperative, therefore, that the clinician has a high index of suspicion for

the presence of interfering antibodies in any patient with clinically or radiographically detectable differentiated thyroid cancer but undetectable levels of thyroglobulin and anti-thyroglobulin antibodies.

Serum Levels of Other Cancer-Related Proteins

There has been interest in the development of new markers for recurrent thyroid cancer because of antibody interference, TSH dependency, lack of cancer specificity, and the loss of thyroglobulin production in some poorly differentiated cancers. A number of growth factors are crucial to thyroid cancer growth and progression, including basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). Expression of bFGF and VEGF has been described in thyroid cancer and is associated with tumor aggressiveness [22–24]. For this reason, several groups have evaluated the utility of serum levels of these two proteins in peripheral blood of patients with thyroid cancer. Serum VEGF levels appear to be elevated in patients with progressive and metastatic thyroid cancer in comparison to those free of clinically detectable disease [25–29]. In these studies, the response of serum VEGF levels to recombinant human TSH administration was reported to be either unchanged or reduced. Similar results have been reported for serum bFGF levels and also levels of matrix metalloproteinase-9 [26], intracellular adhesion molecule-1 [30–32], and oncofetal fibronectin [33].

Another approach to serum markers is to identify global changes in serum protein expression in patients with metastatic cancer, due either to tumor cells or to responses to metastatic cancer. Indeed, it has been recently reported that the patterns of protein expression among patients with benign thyroid nodules and patients with papillary thyroid cancer differ significantly. This technology even identified a pattern that distinguished different stages of the cancer [34]. Further analysis has identified additional biomarkers, improving the sensitivity and specificity (95 % and 94 %, respectively) of this promising field [35]. Additional studies are needed in larger cohorts of patients to determine if protein expression profiles can be used to predict remission or recurrence of disease.

DNA- and RNA-Based Assays

Because of antibody interference, and the potential for enhanced assay sensitivity, there has been an interest in developing DNA- and RNA-based assays for thyroid cancer monitoring using polymerase chain reaction (PCR, for DNA) or reverse transcription PCR (RT-PCR, for RNA). These types of assays do not rely on antibodies to detect protein, and thus are unaltered by anti-thyroglobulin antibodies and are more sensitive than immunoassays. The sensitivity of these assays is due to the inherent nature of PCR which logarithmically amplifies the target DNA (or cDNA if starting with RNA). More recently, the ability to accurately quantify RT-PCR reactions using probes that are specific for the gene that is being detected (real-time PCR) has generated even greater interest in these techniques. The potential for circulating DNA- and RNA-based detection for thyroid cancer has been mirrored for many other hematologic and solid malignancies for similar reasons. For DNA assays, detection of a somatically mutated gene (e.g., BRAF V600E) in peripheral blood has been thought to imply the presence of circulating thyroid cancer cells [36]. Thus, this approach would be applicable for those patients with tumors in which a mutation can be defined. Specificity may be limited by the presence of another occult cancer that might harbor the same mutation. For RNA assays, the principle has been used to detect thyroid-specific RNAs in the circulation similar to use of serum Tg protein assays. Because of the high sensitivity of RT-PCR, the principal issues in developing accurate thyroid-specific or thyroid cancer-specific RNA-based assays relate to specificity and also to the relative instability of isolated RNA.

Studies from a number of groups have been reported using several thyroid-specific markers, such as thyroglobulin, thyroid peroxidase, and TSH receptor mRNA, and also thyroid cancer-specific markers such as BRAF V600E or RET/PTC. Using qualitative methods, initial studies suggested that the presence of circulating thyroglobulin mRNA correlated with the presence of thyroid cancer and was absent in the setting of a normal thyroid [37, 38]. Follow-up studies demonstrated that thyroglobulin mRNA could be detected in most patients with thyroid tissue (benign or malignant) but rarely in patients who were athyreotic [39]. The subsequent results from multiple investigators have been highly varied, some supporting a relationship with thyroid cancer and others demonstrating no relationship [40–44]. The differences in results are likely due to enhanced assay sensitivity, alternative PCR primer design, or other methodologic differences as reviewed in detail elsewhere [45]. More recently, one group has reported utility in the preoperative diagnosis of thyroid cancer using their TSH receptor and thyroglobulin mRNA method [41, 46, 47].

In an effort to determine whether patients with greater tumor burden or extent of disease can be identified based upon their thyroid mRNA levels, several groups have attempted to quantify circulating thyroid mRNA transcripts [44, 48–53]. Overall, the results of these studies are also highly varied. In all published reports there is considerable overlap between patients with different stages of disease suggesting that none would be useful for individual patients on a routine basis. Further, all studies using the highly sensitive techniques of real-time RT-PCR have unequivocally

demonstrated the presence of low levels of thyroglobulin mRNA variants in patients without evidence of thyroid tissue, leading to important questions regarding the specificity of "thyroid-specific" transcripts when detected using this highly sensitive method.

As noted above, one of the key assumptions for either DNA- or RNA-based detection of thyroid cancer is that the circulating nucleic acids are contained and protected within circulating thyroid cells. However, over the past several years, it has become apparent that not all circulating DNA and RNAs are carried in circulating tumor cells. For example, cancer cells (and other cells) secrete exosomes and microvesicles into the circulation. Exosomes are lipid bilayer structures that contain and protect mRNA, microRNA, and DNA [54]. Assays in which DNA and RNA are isolated and analyzed from circulating exosomes are being developed and studied at present in different cancers [55]. The detection of exosome-derived tumor-specific DNA or RNA species may be a potential marker of residual or recurrent thyroid cancer that could be developed for patients with anti-Tg antibodies.

Circulating Tumor Cells

Direct detection of circulating tumor cells (CTCs) has been reported for a variety of cancers and has been FDA approved for breast cancer [56]. The approach that is approved identifies cells based on expression of epithelial cell marker in a "buffy coat" preparation. This approach, or the use of more specific thyroid cell surface markers, has not yet been systematically applied to a thyroid cancer population but holds promise as an alternative to detect the potential for distant metastases or monitoring patients with metastatic disease with anti-Tg antibodies.

New Approaches to Thyroglobulin Measurement

Although thyroglobulin is a large protein, there are now alternative approaches to measuring proteins that do not rely on antibodies. These approaches, used in laboratories for years, are now being developed for clinical assay systems. One approach is mass spectroscopy which has been explored for measuring circulating thyroglobulin [57]. Although there are technical issues [58], such a detection system may represent the most promising approach for thyroglobulin measurement in patients with anti-thyroglobulin antibodies as it directly identifies proteins in a sample and does not rely on antibody-based systems prone to interference by competing antibodies.

Approach to Disease Monitoring for Patients with Anti-thyroglobulin Antibodies

Because of the difficulties in reliable serum testing for thyroid cancer monitoring in patients with anti-thyroglobulin antibodies, there is a greater reliance on radiographic testing. This includes the use of I-131 scanning, neck ultrasound, and chest CT scanning. In addition, for patients with aggressive tumors, PET scanning with [18] FDG also plays a potentially important role. The utility of these studies is not different in patients with anti-thyroglobulin antibodies and is discussed in detail in other chapters in this text. However, it is important to recognize that current proposed algorithms that rely heavily on measurement of serum thyroglobulin exclude patients with circulating anti-thyroglobulin antibodies and therefore do not apply to this population. As a result, one approach is to monitor patients with both quantitative thyroglobulin and anti-thyroglobulin antibody measurements as tumor markers and to perform neck ultrasonography and I-131 scanning yearly for the first several years after diagnosis, depending on the initial extent of the tumor. Additional radiographic studies, such as chest CT scans and PET scans, may be needed in high-risk patients. Advances in the evaluation of alternative serum markers, global analysis of peripheral blood proteins, the development of non-antibody-dependent serum Tg assays, and molecular diagnostics are promising areas of future research to aid in the management of patients with anti-thyroglobulin antibodies.

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