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Commentary on: Implications of Thyroglobulin Antibody Positivity in Patients with Differentiated Thyroid Cancer: A Clinical Position Statement

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Introduction

The position statement from Dr. Verburg and European colleagues provides a much-needed review of the current literature regarding thyroglobulin autoantibody (TgAb) interference with Tg measurement—a problem largely ignored by current guidelines but a problem that affects ~30% of differentiated thyroid cancer (DTC) patients at some time during their clinical course (1,2). The panel posed nine provocative questions and concluded with 26 graded consensus recommendations regarding TgAb and heterophilic antibody (HAb) interferences with Tg measurement (1). Because the prevalence of HAb interferences (primarily human anti-mouse antibodies [HAMA]) is low relative to TgAb (~0.5% vs. ~20% respectively), the review focused primarily on technical TgAb detection and clinical issues regarding TgAb interference broadly grouped in three main areas: (i) technical issues relating to TgAb detection, (ii) TgAb interference with Tg measurement, and (iii) TgAb used as a surrogate DTC tumor marker.

Technical Issues Relating to TgAb Detection

TgAb is detected either directly by immunoassay or indirectly by the exogenous Tg recovery approach, the latter widely used in Europe. It was recommended that a quantitative TgAb immunoassay be performed concurrently with every Tg test in preference to recovery. However, there were conflicting comments regarding the value of Tg recoveries. "Conventional" recoveries were not recommended because they frequently fail to detect interfering TgAb. Yet, a statement that "recoveries are an easy, low-cost alternative to detect TgAb or HAb" was made. The "mini-recovery" advocated by one of the authors and the assay manufacturer that provided support for the consensus process has yet to be validated. However, minimizing the Tg dose recovered is unlikely to overcome the fundamental limitations of recoveries—disequilibrium between the Tg and TgAb components and qualitative differences between the exogenous and endogenous Tg (2). Current TgAb assays use both noncompetitive (immunometric assay [IMA]) and competitive assay formats. Whereas IMA methodology was considered optimal for both TgAb and Tg methods, data suggest that assay performance (false-negative and false-positive TgAb) cannot be predicted from the format used (3,4). TgAb assays standardized against the International Reference Preparation (IRP) 65/93 were recommended. However, most current assays claim IRP standardization, but this does not overcome between-method sensitivity and specificity, differences that contribute to the 200-fold variations in numeric TgAb values reported for the same serum measured by different methods, necessitating the use of the same manufacturers method (in preferably the same laboratory). These between-method differences appear to arise because the methods recognize the Tg epitopes of the IRP differently from their proprietary internal standards, and this is compounded by patient-specific heterogeneity in serum TgAb binding of epitopes in the Tg reagent(s). Should a change in TgAb method become necessary, it is useful to note that the ratio between a specimen's old and new method values appear to provide a patient-specific parameter that can be used to re-baseline TgAb to a new method (2,5).

It was recommended that laboratories independently verify their TgAb and Tg assay performance parameters: limit of detection (LoD = within-run precision of the blank); limit of quantitation (LoQ = between-run 20% coefficient of variation [CV]); and assay reference range. It was surprising that LoQ and not functional sensitivity (FS) was recommended as the optimal cutoff for classifying a "positive" TgAb for DTC patients. Current guidelines (6) clearly state that FS, not LoQ, represents the lowest analyte concentration that should be reported in clinical practice. This is because LoQ is merely a precision target (20% CV), whereas FS encompasses the myriad of factors that erode low-end, between-run precision over the long clinical interval (6–12 months) typically used for monitoring TgAb and Tg for DTC. It follows that a rise or *de novo* appearance of TgAb or Tg in the range between the LoQ and FS limits has questionable clinical significance and could merely represent low-end imprecision. Because Tg and TgAb are measured concurrently, similar protocols are used to establish FS (6). Specifically, for TgAb and Tg methods, the FS limit is defined as the lowest analyte concentration that can be measured with 20% CV in runs made over 6 to 12 months,

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using the appropriate human serum matrix—TgAb-negative sera for IMA methods and TgAb-positive sera for the non-IMA methods (radioimmunoassay [RIA] and liquid chromatography/tandem mass spectrometry [LC-MS/MS]) used for TgAb-positive sera (Table 1). Two or more different lots of critical reagents should be used during the evaluation.

Whereas the FS limit is an appropriate cutoff for defining a "positive" TgAb for DTC, the manufacturer-recommended cutoff, which is inappropriately high to use for DTC, could be adopted as a separate cutoff for diagnosing autoimmune thyroid disease, as suggested by the panel.

TgAb Interference with Tg Measurement

Table 1 contrasts the performance characteristics of the three classes of Tg method currently available: RIA (2,3), IMA (2,3), and LC-MS/MS (7). The panel considered that only second-generation Tg IMA methods (Tg ^{2G}IMA, FS $\leq 0.10\ \mu g/L$) had sufficient sensitivity to distinguish disease-free patients from those with persistent disease. Unfortunately, IMA is also the class of method most prone to TgAb interferences causing Tg underestimation with the potential to mask disease. The TgAb-resistant methodologies (RIA and LC-MS/MS) were considered inferior to Tg ^{2G}IMA because of an order of magnitude less functional sensitivity (RIA, FS = 0.5 $\mu g/L$; LC-MS/MS, FS = 1–2 $\mu g/L$). The common practice of triaging TgAb-positive specimens to RIA methodology was considered suboptimal because of limited RIA availability and inferior sensitivity compared with Tg ^{2G}IMA. In the United States, some laboratories have begun to use LC-MS/MS methods to measure Tg in specimens with a "positive" TgAb status, reserving Tg ^{2G}IMA methodology for TgAb-negative specimens. TgAb test sensitivity, specificity, and cutoff adopted to define a "positive" TgAb critically impact the frequency of false-positive and false-negative TgAb classifications that can mask disease. Specifically, false-positive tests prompt the unnecessarily triaging of specimens to less sensitive Tg methodology, whereas false-negative tests prompt inappropriate use of Tg ^{2G}IMA leading to Tg underestimation.

It was recommended to "select a TgAb assay most likely to recognize TgAb interference with the particular Tg assay used." Data failed to support the assumption that it is optimal to select both TgAb and Tg methods from the same manufacturer. It follows that the selection of a clinically optimal TgAb and Tg method may necessitate the use of different manufacturers' platforms—something impractical for most routine laboratories. The reliable detection of TgAb remains a problem, given that TgAb is qualitatively heterogeneous and the same serum may be reported as TgAb-negative by one method but not by another. The panel discussed the problem of patients who are known to have thyroid tissue but have an undetectable Tg ^{2G}IMA and yet a "negative" TgAb. Clinical management could be impacted if it was known that the cause of discordance was technical (TgAb assay insensitivity or an abnormal tumor Tg not detected by the Tg ^{2G}IMA), as opposed to a reflection of a poorly differentiated tumor with impaired Tg secretion. Because TgAb interacts with Tg IMA and RIA methodologies differently, a low ^{2G}IMA/Tg RIA ratio has been used as an independent indicator for TgAb interference (2,5). Although the panel questioned the merits of this approach, it is unlikely that anything other than TgAb would cause a lowering of ^{2G}IMA/Tg RIA ratios in proportion to increasing TgAb concentrations (2,5). The only alternative independent parameter of TgAb interference

Table 1. Thyroglobulin Methods: Strengths and Limitations

	Tg assay classes		
	IMA	*RIA*	*LC-MS/MS*
Dates of use	1990–present	1973–present	2009–present
Used for	TgAb− sera	TgAb+ sera	TgAb+ sera
Principle	Noncompetitive format; uses MAbs	Competitive format; uses PAbs	Extensive preanalytical specimen preparation; +/− immunoaffinity concentration reduction, alkylation, and trypsin digestion before immunoaffinity concentration of target peptide(s)
Turn-around time	<12 hours	~6 days	~1–2 days
Automation	Can be automated	Difficult to automate	Extensive specimen preparation; cannot be automated
Strengths and limitations	• FS range: ~0.05–1.0 $\mu g/L$ (2) • Prone to interference by TgAb (low) and HAMA (high) • MAbs: limited epitope specificities to detect abnormal tumor Tg isoforms	• FS range: 0.5–1.0 $\mu g/L$ (2) • Resistant to TgAb interference • No HAMA interference • PAbs: broad epitope specificity to detect abnormal tumor Tgs	• FS range: 1–2 $\mu g/L$ (7) • Should be no interference from TgAb or HAMA (no clinical studies as yet) • Polymorphic tumor Tg may not yield target peptide(s) (7)

FS = 20% between-run CV in human sera measured for 6–12 months with ≥2 lots of reagents (6).

CV, coefficient of variation; FS, functional sensitivity; HAMA, human anti-mouse antibodies; IMA, immunometric assay; LC-MS/MS, liquid chromatography/tandem mass spectrometry; MAbs, monoclonal antibodies; PAbs, polyclonal antibodies; RIA, radioimmunoassay; Tg, thyroglobulin; TgAb, thyroglobulin autoantibodies.

suggested was a preoperative Tg and TgAb measurement designed to detect a paradoxically undetectable Tg ^{2G}IMA, presumably indicating TgAb interference. One cautionary note was that even if a Tg methodology free from TgAb interference was developed, the presence of TgAb may increase Tg metabolic clearance and distort the relationship between tissue Tg secretion and the circulating Tg concentration, as previously suggested for both TgAb and other antigen–autoantibody relationships.

TgAb Used as a Surrogate DTC Tumor Marker

The panel concluded that only Tg or TgAb currently have sufficient sensitivity and specificity for clinical use as DTC tumor-marker tests. There is a growing body of evidence suggesting that when Tg ^{2G}IMA measurement is invalidated by the presence of TgAb, the *trend* in TgAb concentrations (measured using the same method preferably by the same laboratory) can serve as a surrogate DTC tumor marker. In fact, it was suggested that the follow-up of TgAb-positive patients should be stratified on the basis of the TgAb trend. The panel stressed that TgAb is not a direct tumor marker because TgAb concentrations are not directly related to tumor burden but merely reflect the immune system response to changes in Tg antigen arising from benign or malignant tissue. This response is typically quite slow (half-life $\sim$10 weeks) necessitating a six-month interval between TgAb measurements following treatments likely to increase TgAb transiently—immune system modulating drugs, ^{131}I therapy, FNA biopsies, or lymph-node surgeries. A formula based on TgAb analytical and biological variation was used to calculate that a relative TgAb change of $>$50% should be considered clinically significant. This was in accord with a clinical report that a $>$50% TgAb decline in the first postoperative year predicted an excellent prognosis, such that follow-up could be limited to cervical ultrasound and serial TgAb. Most patients ($\sim$75%) exhibit a declining TgAb trend after successful treatment, although only half of such patients become TgAb-negative after three or four years of follow-up (2). Patients with very high TgAb detected at diagnosis rarely become TgAb-negative, even when apparently disease free. Whether TgAb persistence is due to continued Tg antigen secretion by small amounts of remnant tissue, micro-foci of tumor not detected by anatomic imaging, or a reflection of long-lived antibody-producing plasma cells is unclear. The panel stressed that a persistent or progressively increasing TgAb should increase suspicion for recurrence and necessitate anatomic imaging (cervical ultrasound, dxWBS, rxWBS, FDG-PET/CT, as appropriate) at periodic intervals. Increasingly, FNA cytology of sonographically abnormal lymph nodes is augmented by Tg measurement made in a saline washout of the FNA needle. Studies were cited suggesting that TgAb interference with Tg ^{2G}IMA was not seen in these saline washes. However, it should be noted that most TgAb-positive patients have a serum TgAb that is too low to interfere after the $\sim$1/40 dilution in 1.0 mL saline. Only a serum TgAb more than 40 times higher than the FS limit of the test would be likely to interfere.

Conclusions

Over the last five years, there has been growing acceptance that the detection of recurrent/persistent DTC is enhanced by Tg assay sensitivity, and as a result, the basal Tg trend, measured by Tg ^{2G}IMA without recombinant human TSH stimulation, is becoming the standard of care. However, the superior functional sensitivity of the Tg ^{2G}IMAs are offset by profound sensitivity to TgAb interference causing falsely low or even undetectable Tg that can mask disease. The graded recommendations of Dr. Verburg *et al.*, based on a comprehensive review of the current literature, provide valuable technical and clinical insights that will hopefully prompt further studies leading to evidence-based guidelines for managing TgAb-positive DTC patients.

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