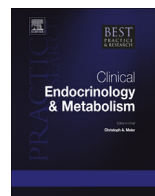




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Thyroglobulin antibody (TgAb) methods – Strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer



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Thyroglobulin autoantibodies (TgAb) are detected at diagnosis or during treatment in approximately 25% of patients with differentiated thyroid cancer (DTC). When present, TgAb interferes with thyroglobulin (Tg) measurement causing falsely low or undetectable Tg immunometric assay (IMA) values that can mask disease. Guidelines mandate that every Tg test have TgAb measured simultaneously and quantitatively by immunoassay and not a recovery test. The propensity and magnitude of TgAb–Tg interference relates to both Tg and TgAb concentrations and the class of Tg method used. Because the TgAb trend reflects changes in thyroid tissue mass, TgAb concentrations serve as a surrogate post-operative DTC tumor marker. A rising, or de novo appearance of TgAb may indicate recurrence, whereas a progressive decline suggests successful treatment. This review focuses on the technical limitations of current TgAb methods, characteristics of TgAb interference with different classes of Tg method, and the clinical value of monitoring TgAb trends as a surrogate DTC tumor marker.

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1. Introduction

Over the last five decades, the tissue-specific origin of Thyroglobulin (Tg) has established Tg measurement as the primary post-operative biochemical tumor-marker for differentiated thyroid cancers (DTC). During this period, the role of serum Tg autoantibody (TgAb) testing has evolved from being merely a test for thyroid autoimmunity, to being one ordered simultaneously with Tg to confirm the absence of TgAb interference. In recent years this qualitative TgAb role (positive or negative result) became expanded with the recognition that TgAb concentrations (measured in kIU/L) respond to changes in the mass of Tg-secreting thyroid tissue [1]. As a result, serum TgAb has evolved as a surrogate DTC tumor-marker test that can replace Tg immunometric assay (IMA) measurements that are compromised by TgAb interference [1–10]. Unfortunately, a number of methodologic factors negatively impact the clinical value of TgAb testing. These include insensitivity, the inappropriately high cut-off values that manufacturers set for diagnosing thyroid autoimmunity rather than detecting TgAb interference with Tg measurements, and interference from high endogenous Tg concentrations [1–5,7,10–17]. Current TgAb methods are primarily non-isotopic, automated and use either competitive or non-competitive IMA formats. Most methods are standardized against the International Reference Preparation (IRP MRC 65/93) and report TgAb values in kIU/L, yet between-method numeric serum TgAb values vary by factors of more than 100-fold that are patient-specific [16]. The cause for this variability is likely two-fold – differences in the methodologic specificity for recognizing the different Tg epitopes in endogenous Tg versus the Tg reagent(s), compounded by patient-specific serum TgAb heterogeneity. The practical consequences of these specificity differences is that serial TgAb monitoring of DTC patients can only be made by using the same method.

2. Strengths and pitfalls of current TgAb immunoassay methods

Serum TgAb measurement began five decades ago with insensitive qualitative techniques employing immunodiffusion, complement fixation, passive particle agglutination and indirect immunofluorescence tests. These early methods were phased out in the 1970s in favor of semi-quantitative passive hemagglutination tests reported in titers that are still used by a minority of laboratories [11]. Subsequently, more sensitive competitive radioassays that detected TgAb as a function of ¹²⁵I-Tg binding and reported results in kIU/L relative to the International Reference Preparation (IRP) MRC 65/93 became available, and are still used by some laboratories [4,8,10,16,18]. In the last ten years, most laboratories have adopted non-isotopic, non-competitive, automated TgAb methods in preference to isotopic radioassays or hemagglutination tests [8,12–17].

Most current TgAb methods claim to be standardized against the IRP MRC 65/93 and report results in kIU/L [8,16,17]. However, despite IRP standardization, analytical sensitivity limits and the cut-off values that manufacturers recommend to define a “positive” TgAb result vary 200-fold [8,12,14–18]. It should be noted that this IRP is a 60-year old preparation made from blood products containing TgAb with Tg epitope specificities originally characteristic of thyroid autoimmunity that may have been changed by aging. Also of note is that each method is not directly standardized with the IRP but instead uses its own internal, proprietary TgAb standard. Tg epitope specificity differences between the IRP and these secondary standards likely contribute to the widely discrepant numeric TgAb values reported when the same serum specimen is measured by different methods. For example, one study of doubling dilutions of the IRP reported similar potency (in kIU/L) between two different methods yet a ten-fold difference between serum values [17]. Clearly the Tg epitope specificities of TgAb in sera differ when measuring the IRP versus the secondary assay standards. In general, serum TgAb concentrations appear to correlate with both the number of Tg epitopes recognized and the degree of restriction of the epitope specificities [19]. TgAb–Tg epitope heterogeneity in DTC sera is evident from studies showing that despite a broad ranking of values between different methods, individual sera display widely variable relationships across methods [4,10–12,14–17]. Glycosylation studies and epitope mapping using monoclonal antibodies or fragment antigen-binding (Fab) probes, suggest that TgAb associated with papillary thyroid cancers (PTC) may arise from two distinctly different pathologic mechanisms – either underlying autoimmune thyroid disease (histologic lymphocytic thyroiditis) or an immune response to the inflammation associated with tumorigenesis that may promote the release of post-translational

modified Tg antigens with enhanced immunogenicity [19–24]. This may in part explain the heterogeneous specificity of TgAb in patient sera and why TgAb detected by one method may not be detected by another [14–17]. It appears that each patient's TgAb has a characteristic IgG subclass and specificity for recognizing the Tg assay reagent(s) [25,24]. As a result, the same serum produces different TgAb numeric results with different methods [8,12,14–18]. The characteristics of any patient's TgAb appear to be fixed such that the ratios between the numeric values reported when comparing two different methods appear to be a patient-related constant, and independent of changes in TgAb concentration (measured by the same method), as shown in Fig. 1 [10]. This fixed ratio can be used to establish new baseline values for a patient without disrupting serial TgAb monitoring, should a change in TgAb method become necessary. This is provided that a specimen(s) is available for establishing the ratio between the new versus old TgAb method values, either because the laboratory gives physicians advanced notice of a method change (which generally does not occur), or there are archived frozen specimens available for measurement by the new method. Obviously, the stability of TgAb in frozen archived sera critically impacts the value of this re-baselining strategy. Short-term stability studies of serum Tg (IMA) and TgAb measurements have suggested that specimen stability relates to storage time and temperature, the number of freeze/thaw cycles, and whether there is sufficient specimen volume to minimize evaporation [26–29]. Two recent studies using the same Tg method (Roche) reported no loss of Tg or TgAb immunoactivity when specimens were refrigerated for up to 24 h, or stored for 2–3 weeks at -20°C , but a progressive loss of immunoactivity during longer-term frozen storage [29]. The practice of storing all specimen left after Tg + TgAb testing in a frozen (-20°C) archive allows physicians to request concurrent re-measurement of a past specimen alongside the patient's current specimen as a way to eliminate between-run variability and confirm a suspicious rising Tg and/or TgAb trend (see section 6 'TgAb trends during long-term follow-up monitoring'). Fig. 2 shows stability data using the same in-house Tg RIA, TgAb (Kronus/RSR) and Tg IMA (Beckman Access) methods [8,30] for concurrent re-measurement of frozen (-20°C) archived sera stored for either between 1 and 4 years or

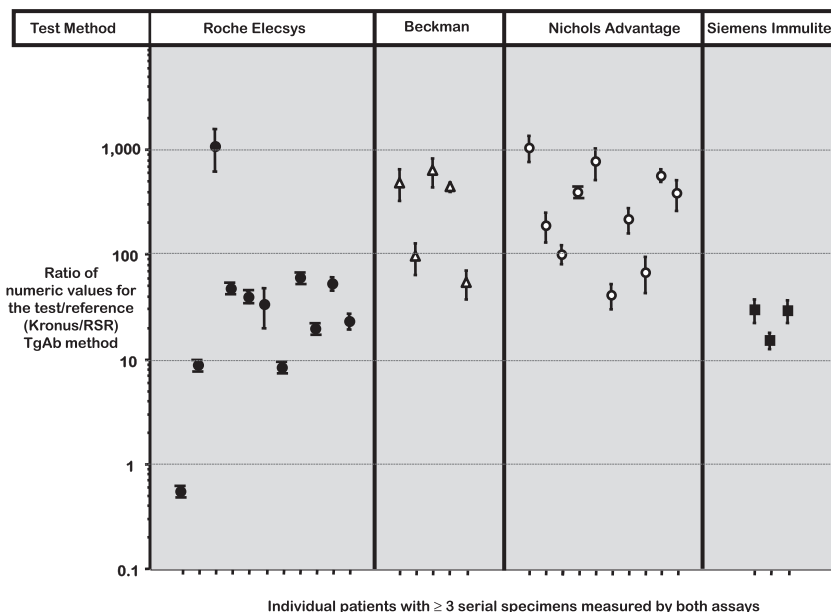


Fig. 1. Mean \pm sd of the ratios between the numeric values (kIU/L) reported by a test method, Roche (solid circles); Beckman (open triangles); Nichols Advantage (open circles) or Siemens Immulite (closed squares), divided by the numeric values of the reference method (Kronus/RSR) for individual DTC patients with 3 or more serial serum specimens sent for TgAb testing as a component of serial serum Tg monitoring over a period of 1–4 years.

for over five years. No change in TgAb, Tg RIA or Tg IMA values was evident provided that sufficient specimen volume was stored to minimize evaporation and that sera were subjected to no more than two freeze/thaw cycles (Fig. 2).

3. Tg recovery tests are unreliable for detecting interfering TgAb

For the last three decades, the presence of interfering TgAb has been determined either directly by TgAb immunoassay (Section 2) or indirectly by employing a Tg recovery test. Recovery tests typically involve measuring the Tg concentration before and after a known amount of Tg has been added to an aliquot of the test serum. Recoveries greater than 80% are typically considered to indicate the absence of interferences, thereby validating the Tg measurement [6,7,26,31–35]. In order for the recovery of an exogenous Tg preparation to mimic interactions between the endogenous Tg and TgAb components, the added and endogenous Tg should be immunologically identical, the amount of Tg added should not exceed the concentration of the endogenous Tg and there should be sufficient time allowed for the added Tg to equilibrate with the endogenous Tg and TgAb serum constituents before initiating the Tg IMA test. These criteria are not usually met. First, there can be three different types of Tg antigen present in the recovery test tube that can differ in structure and thereby the epitopes available for TgAb binding, these would be (1): the exogenous (added) Tg which is usually a thyroid gland extract (2), normal Tg secreted by normal remnant tissue, and (3) abnormal tumor-derived Tg isoforms. Second, because Tg IMA tests preferentially measure free Tg, any exogenous Tg added in excess of the endogenous (serum) Tg would tend to saturate TgAb binding sites and cause an excess of free exogenous Tg that would falsely elevate the recovery. Some manufacturers have recognized this potential problem and have reduced the mass of exogenous Tg [36]. However, it appears that reducing the amount of Tg added is less important than allowing time for constituents to equilibrate before initiating the IMA test. In one study that used a low concentration of exogenous Tg ($\sim 10 \mu\text{g/L}$) and an 18 h incubation to allow the added Tg to equilibrate with the endogenous Tg and TgAb constituents, the recovery of exogenous Tg was reduced by as much as 20% compared with initiating the IMA reaction without allowing time for equilibration [32]. This problem of disequilibrium may well

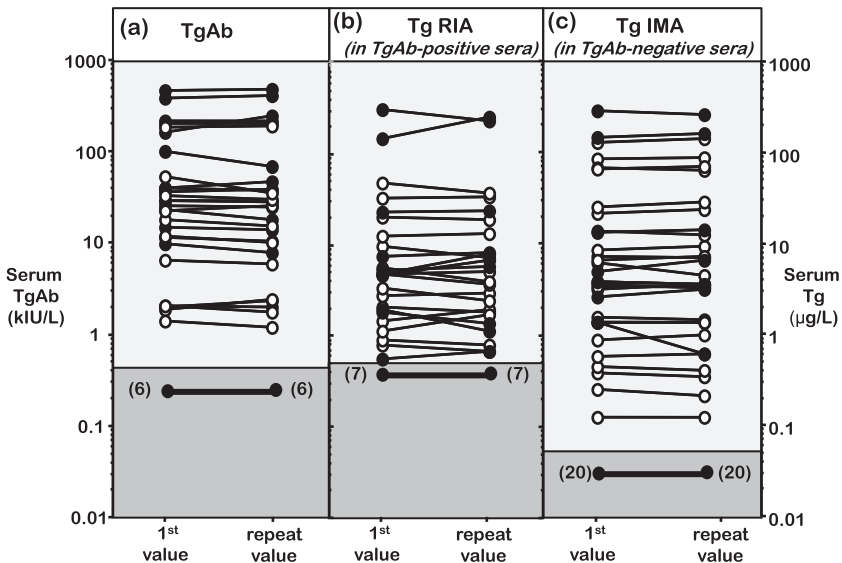


Fig. 2. The stability of (a) TgAb (Kronus/RSR), (b) an in-house Tg RIA and (c) Tg IMA (Beckman Access) concurrent re-measurements made in frozen (-20°C) archived sera stored for either between 1 and 4 years (open circles) or over five years (closed circles) [8,30].

explain why many studies report normal Tg recoveries for sera that clearly contain interfering TgAb [4,6–8,15,32,35]. In addition to the many technical limitations of using a recovery approach for detecting interfering TgAb, a further disadvantage is that recoveries cannot be used to “correct” the Tg result for TgAb interference, or quantitatively measure TgAb as a surrogate tumor marker test (see Section 6).

4. TgAb interference with Tg measurement

As shown in Table 1, there are three classes of Tg method having different propensities for TgAb interference: Radioimmunoassays (RIA), Immunometric assays (IMA) and Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) tests.

- **Tg Radioimmunoassays (RIA):** Tg RIAs were first developed in the 1970s and quantify Tg as a function of the competition between endogenous Tg and a ^{125}I -Tg reagent for a limited amount of polyclonal (rabbit) Tg antibody [37]. Because RIAs suffer from the dual disadvantages of being isotopic and requiring long incubation times (days) to maximize functional sensitivity (FS) [38] they have been mostly replaced by IMA methods that require shorter incubations (hours), are mostly non-isotopic, and can be automated [8].
- **Tg Immunometric assays (IMA):** Despite the greater FS potential of IMA versus RIA methodology, the first generation of IMA tests only achieved comparable FS to RIA (0.5–1.0 $\mu\text{g/L}$) [8,30,38]. More recently, a second generation of Tg IMAs, characterized by an order of magnitude better FS (0.05–0.10 $\mu\text{g/L}$) are rapidly becoming the standard of care in the wake of studies showing that the clinical utility of Tg testing is enhanced by assay sensitivity, and that second generation FS obviates the need for expensive recombinant human Thyroid Stimulating Hormone (TSH) TSH-stimulated Tg testing [30,39,40].
- **Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Tests:** In response to the burgeoning field of proteomics a new class of Tg method, i.e., liquid chromatography-tandem mass

Table 1
Classes of Tg method.

Assay class	Principle	Turn-around time (ease of automation)	<ul style="list-style-type: none"> • Functional sensitivity (FS) [38] • Strengths/pitfalls
Radioimmunoassay (RIA) 1973 – present	Competitive – uses a limited quantity of polyclonal antibody (PAb)	~6 days (difficult to automate)	<ul style="list-style-type: none"> • FS ~ 0.50 $\mu\text{g/L}$ [8,16] • PABs – broad epitope specificity for detecting abnormal tumor Tg isoforms • no HAMA^a interference • resistant to TgAb interference
Immunometric Assay (IMA) 1990 – present	Non-competitive – uses capture and signal monoclonal antibodies (MAbs)	Hours (easy to automate)	<ul style="list-style-type: none"> • FS range = 0.05–1.0 $\mu\text{g/L}$ [8,30] • prone to HAMA^a & TgAb interferences • MAbs – limited Tg epitope specificities for detecting abnormal tumor Tg isoforms
Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) 2009 – present	Specimens may be concentrated and/or reduced, alkylated and digested with Trypsin before target peptides are immunoaffinity enriched prior to detection by LC-MS/MS [44,48,49]	1–2 days (specimen preparation difficult to automate)	<ul style="list-style-type: none"> • FS 1.0–2.0 $\mu\text{g/L}$ [49] • may avoid interference from TgAb or HAMA^a [49] • polymorphic tumor Tg may fail to yield target peptides • no clinical studies as yet

^a HAMA = human anti-mouse antibody.

spectrometry (LC-MS/MS) has recently become available [44,48,49]. These methods are expected to overcome TgAb (and other interferences) by using Trypsin to digest Tg bound to TgAb and release a target Tg peptide that can be immunoaffinity enriched before being detected by LC-MS/MS. These methods involve extensive specimen preparation before the trypsin treatment(s). They currently only achieve first generation functional sensitivity [44,48,49].

The RIA class of Tg method appears more resistant to TgAb interference than the IMA class of method, although falsely low or high Tg RIA values can occur, depending on the interactions between the endogenous Tg and TgAb in the serum and the specificity of the RIA first and second antibody reagents [8,16,41–44]. The major disadvantage of the IMA class of method is their propensity for interference from even very low concentrations of TgAb [4,6–8,45]. This interference is characterized by falsely low/undetectable serum Tg values and is thought to result when free Tg is complexed by endogenous TgAb and is sterically hindered from binding to the capture and/or signal monoclonal antibody reagents and/or alternatively, endogenous TgAb binding to Tg masks the epitopes needed for recognition by the monoclonal antibody reagents. Evidence that TgAb interference causes underestimated Tg IMA values is seen by *in vitro* serum mixing studies [7,26,45] and supported by clinical data showing paradoxically undetectable serum Tg IMA values for some TgAb-positive euthyroid subjects with functioning thyroid glands [8], TgAb-positive untreated Graves' hyperthyroid patients [34,46] and TgAb-positive DTC patients with persistent/recurrent disease [4–6,9,47]. Early Tg LC-MS/MS studies of TgAb-positive sera report higher Tg LC-MS/MS and Tg RIA values as compared with Tg IMA, suggesting that Tg is indeed present in many sera with detectable Tg RIA yet undetectable Tg IMA values [16,44]. Whereas these early data affirm that TgAb interference causes Tg IMA underestimation, even were reliable Tg measurements available the significance of serum Tg changes in the presence of TgAb may be different if there is accelerated clearance of Tg complexed with TgAb [50,51]. The preliminary LC-MS/MS data also supports the use of Tg IMA/Tg RIA discordance as an independent indicator for TgAb interference [4,6,16,52,53]. Studies using a low Tg IMA/Tg RIA ratio to indicate the presence of TgAb interference find that the propensity for TgAb interference with IMA is related to the TgAb concentration, although high TgAb levels do not necessarily produce interference and TgAb below the level of detection by some methods may profoundly interfere [4,10,16,32,45,53]. In some cases discordance is seen in the absence of a detectable TgAb as a result of TgAb method insensitivity (discussed in Section 2) or because the TgAb lacks the specificity to interact with the Tg assay reagent(s). Likewise, the absence of Tg IMA/Tg RIA discordances at high TgAb concentrations could reflect either limited TgAb specificity, or result from a high endogenous Tg saturating TgAb binding sites and leaving more free Tg for the IMA to detect. Consequently, the frequency of Tg IMA/Tg RIA discordance (Fig. 3a) and the propensity for reporting falsely undetectable Tg IMA results (Fig. 3b) would be reduced at high Tg concentrations, especially when TgAb is low. Conversely, when the endogenous Tg is low, the Tg would primarily be bound to TgAb and would be rendered inaccessible for binding to the IMA monoclonal antibodies. Under these circumstances the frequencies of both Tg IMA/Tg RIA discordance (Fig. 3a) and falsely undetectable Tg IMA values (Fig. 3b) would be high, especially when TgAb is high.

5. Clinical significance of TgAb detected during management of DTC

TgAb prevalence

Over the last two decades, the prevalence of TgAb reported for patients diagnosed with DTC has varied between 8 and 36% and is approximately two-fold higher in DTC compared with the general population (24.9 versus 10.1%, respectively) [2–5,7,54,57]. The differences in TgAb prevalence in DTC reflect differences in methodologic sensitivities, specificities and the cut-offs used for a “positive” TgAb (see Section 2), as well as factors relating to the ascertainment biases of the different study cohorts. Specifically, TgAb prevalence increases with age and/or the proportion of females in the cohort and is higher in papillary versus follicular thyroid cancers [3]. TgAb prevalence rises with iodine supplementation of iodine deficient populations along with an increase in prevalence of thyroid

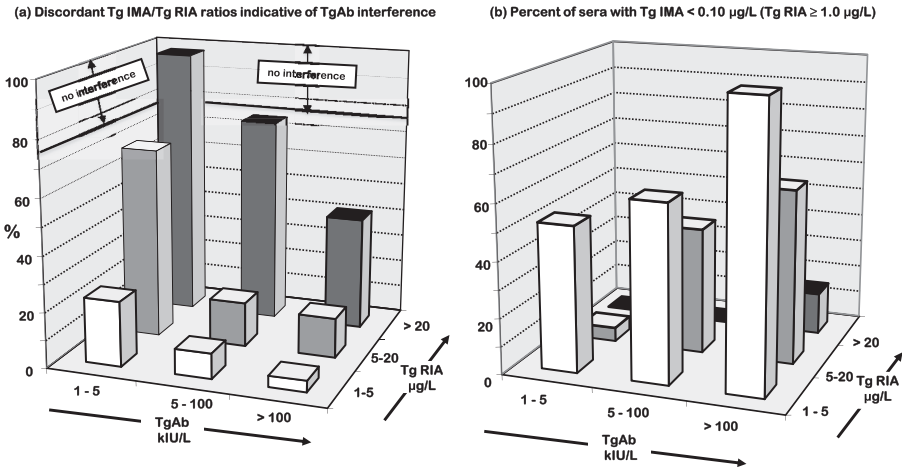


Fig. 3. How the relationship between serum Tg and TgAb concentrations relate to the propensity for TgAb interference, as judged by a low (<75%) Tg IMA/Tg RIA ratio (panel a) and an undetectable Tg IMA (<0.10 µg/L) associated with an unequivocally detectable Tg RIA (≥1.0 µg/L) [21]. The Tg RIA and Tg IMA data is shown for nine groups of DTC patients with Tg RIA values in the low (1–5 µg/L), intermediate (5–20 µg/L), or high (>20 µg/L) range, and TgAb concentrations in the low (1–5 kIU/L), intermediate (5–100 kIU/L) or high (>100 kIU/L) range. The data, was taken from reference [10].

autoimmunity (Hashimoto's thyroiditis) and an increased prevalence of papillary relative to follicular thyroid cancers [55,56]. Given the strong association between the presence of TgAb and lymphocytic thyroiditis, an association between Hashimoto's thyroiditis and thyroid cancer (primarily PTC) has been proposed and recently confirmed by meta-analysis [13,58–61]. This association appears to be supported by surgical studies made over the last 25 years that find on average 27.6% of patients receiving a thyroidectomy for PTC have histologic evidence of Hashimoto's thyroiditis (relative risk 1.59, 1.15–4.16) [60]. However, contrary to this, Hashimoto's thyroiditis is not generally considered a risk factor for DTC. Indeed, fine-needle aspiration biopsy studies find no association between cytologic lymphocytic thyroiditis and PTC (relative risk 0.69, 0.39–1.00), suggesting that Hashimoto's thyroiditis does not predispose to DTC [60]. It thus appears that any association between lymphocytic thyroiditis and PTC may not relate to Hashimoto's thyroiditis per se, but be secondary to chronic TSH stimulation, biomolecular commonalities linking the two conditions, or a process relating to chronic inflammation [10,23,59,60,62].

Prognostic significance of detecting TgAb at time of diagnosis

When evaluating patients with a thyroid nodule, the presence of circulating TgAb may be a risk factor for malignancy independent of thyroid autoimmunity or a detectable serum Thyroid peroxidase antibody [58,63]. Furthermore, studies find that patients with TgAb detected shortly after thyroidectomy have a higher risk for persistent/recurrent disease during long-term follow-up [5,64–66], and the higher the TgAb, the higher the risk [9]. However, thyroid antibodies are strongly associated with the histologic presence of lymphocytic thyroiditis [5,7,13,47,67,68]. The higher frequency of recurrences in TgAb-positive patients appears at odds with studies suggesting a more favorable outcome when PTC is associated with lymphocytic thyroiditis [58,59,67–73]. However, such outcome studies have not always achieved significance using multivariate analysis or distinguished between the generalized lymphocytic infiltration characteristic of Hashimoto's thyroiditis and non-specific tumor-infiltrating lymphocytes that could represent an immune response to tumor that could favorably influence prognosis [67,68,71,74]. Also, because PTC typically afflicts younger female patients with smaller and lower grade tumors, factors other than the lymphocytic thyroiditis per se might favorably influence outcome [10,70,72,75]. Offsetting this are reports that PTC associated with lymphocytic thyroiditis may

be more likely to be bilateral and multicentric, have higher stage disease, a higher frequency of lymph node metastases and an increased risk of recurrences [3,5,10,63,66,68,72]. Clearly long-term prospective studies are needed to elucidate whether the association between PTC and lymphocytic thyroiditis is cause or effect, and whether lymphocytic thyroiditis per se has an independent influence on outcome. The association between lymphocytic thyroiditis and the presence of TgAb has some practical implications for patient management. When thyroid tissue is still present shortly after surgery for PTC, serum Tg tends to be lower (even undetectable) when histologic lymphocytic thyroiditis is seen, as compared with PTC without lymphocytic thyroiditis [7,76,77]. Given the strong association between lymphocytic thyroiditis and the presence of TgAb, this likely reflects TgAb interference with serum Tg measurements, even when TgAb is reported as “negative” given the TgAb assay detection problems discussed in Section 2. This suggests that it is important to review the pathology report for histologic evidence of lymphocytic thyroiditis, because when present, there is a higher risk of occult TgAb producing falsely low or undetectable Tg IMA values that have the potential to negatively impact post-operative management decisions.

6. Clinical utility of monitoring TgAb trends as a surrogate tumor marker test

With the recognition that TgAb concentrations respond to changes in the mass of Tg-secreting thyroid tissue [1], it is clear that monitoring changes in the TgAb concentration has more clinical value as surrogate DTC tumor-marker than measuring TgAb merely as a qualitative (positive versus negative) test for validating that a Tg measurement is free from TgAb interference [59]. When used qualitatively, TgAb test parameters such as sensitivity, specificity and the cut-off value used to define a “positive” TgAb result are of paramount importance. When used quantitatively as a surrogate tumor-marker, an added requirement is that the between-run precision (% coefficient of variation) of the TgAb test is below 10% across 12–18 month follow-up intervals typical for monitoring DTC patients after the first post-operative year.

TgAb changes during the early post-operative period (first post-operative year)

A transient, (~two-fold) rise, or de novo appearance of, TgAb is seen in approximately 40% of patients studied 4–8 weeks after thyroidectomy, before any radioiodine treatment [7]. This rise appears unrelated to the preoperative Tg or TgAb concentration or recurrence risk, and is likely the immune response to the acute release of a large amount of Tg antigen by the trauma of surgery. Thereafter, the magnitude of TgAb change has been shown to have prognostic significance [47]. Patients whose TgAb concentrations fall to less than 50% of their initial value in the first post-operative year have a low (<3%) risk of having recurrence detected during the subsequent five years of follow-up, compared with those displaying less than a 50% TgAb decline or a TgAb rise during the first post-operative year [47]. The favorable prognostic significance of a >50% post-operative TgAb decline is seen even though TgAb becomes undetectable within the first post-operative year in only a minority (~30%) of cases [7]. Shown in Fig. 4 is the clinical significance of TgAb trends analyzed over the first 3–5 post-operative years as; (a) a falling TgAb trend (to <50% of the initial value); (b) a stable TgAb (less than a 50% change from initial value); or (c) a rising TgAb trend (a >50% sustained rise at any time).

TgAb trends during long-term follow-up monitoring

- Falling TgAb trends:** During long-term follow-up, approximately 75% of TgAb-positive patients display a falling TgAb trend in response to treatment (thyroidectomy ± radioiodine) [1,5,7]. Such patients have a lower risk of recurrent/persistent disease (<3%) and usually have no disease detected by anatomic imaging, despite only half of such patients achieving a TgAb-negative state [1,5,6,47,64]. It appears that the presence of Tg-secreting thyroid tissue is necessary to sustain continued TgAb production [1]. However, when TgAb persists at low levels for long periods of time it is often difficult to determine if the continued source of Tg antigen is a small amount of normal remnant tissue or a micro-foci of tumor, because even radioiodine treatment may not completely

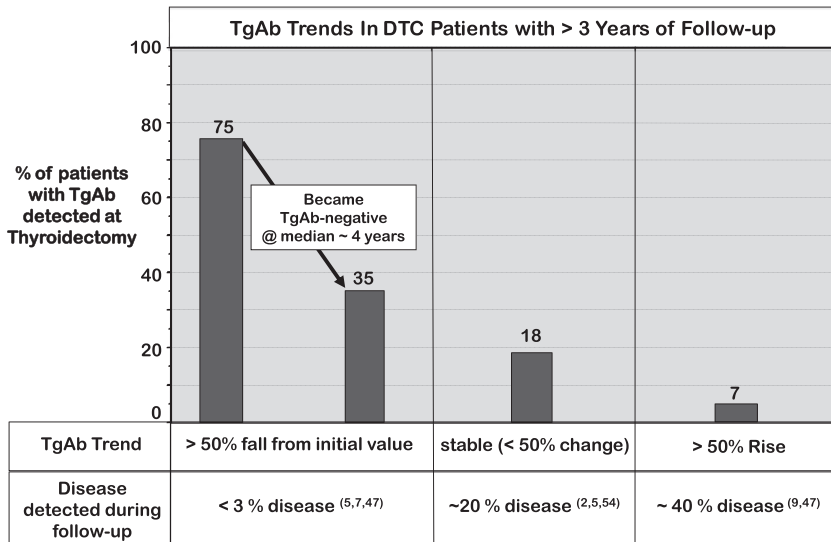


Fig. 4. Trends in TgAb concentrations versus the risk of having persistent or recurrent disease detected during follow-up of DTC patients with detectable TgAb at the time of initial treatment.

eradicate all normal remnant and takes time (years) to achieve its maximal effect [1,78,79]. Studies suggest that the likelihood of achieving a TgAb-negative state in response to treatment is inversely related to the initial TgAb concentration, so that TgAb is less likely to become undetectable in patients with a high TgAb concentration at the time of initial surgery [7].

- **Stable TgAb:** In approximately 20% of patients, TgAb declines but only to a minor degree in the year following thyroidectomy and then plateaus and persists without evidence of disease by anatomic imaging. The maintenance of TgAb could reflect continued Tg antigen secretion by small amounts of remnant tissue, micro-foci of tumor not detected by anatomic imaging [79], or reflect long-lived antibody-producing plasma cells [80]. Patients with stable but significantly elevated TgAb concentrations have a higher recurrence risk (~20%) and warrant closer follow-up than patients in whom TgAb progressively declines over time (years) [2,5,64,79].
- **Rising TgAb trends:** It is well documented that a rising TgAb trend [5,7,64], or a de novo appearance of TgAb [3,5,65,81], is commonly seen in the first few months following radioiodine therapy or fine-needle aspiration biopsy [82] in response to Tg antigen released by damaged cells. These responses tend to be transient (1–8 months) and should be distinguished from a sustained, progressive TgAb rise [4,6,7,9,65], or sustained de novo appearance of TgAb [5,47,81], that may be an early indicator of recurrence.

Practice points

1. For TgAb-positive patients, the TgAb trend is a more reliable tumor marker than Tg IMA.
2. Closer follow-up is warranted when TgAb persists for years without declining.
3. Lymphocytic thyroiditis on the pathology report suggests TgAb maybe occult and cause falsely low Tg IMA.
4. Ensure laboratories notify physicians before changing Tg or TgAb methods to facilitate re-baselining.
5. TgAb and Tg method functional sensitivities and between-run precisions should be established using human serum pools measured over 6–12 months according to current guidelines [38].

Research agenda

1. Studies of the pathologic significance of heterogeneous serum TgAb-Tg specificity and how this impacts TgAb and Tg measurements.
2. Long-term prospective studies are needed on whether the association between PTC and lymphocytic thyroiditis is cause or effect, and relationships between lymphocytic thyroiditis and outcome.
3. Studies are needed to assess whether the Tg complexed with TgAb has a different metabolic clearance rate than from free Tg.

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