

## Clinical Utility of Thyroglobulin Antibody (TgAb) Measurements for Patients with Differentiated Thyroid Cancers (DTC)

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**Context:** Thyroglobulin autoantibodies (TgAb) are primarily measured in serum in conjunction with thyroglobulin (Tg)—the primary tumor marker used to monitor patients with differentiated thyroid cancers (DTC). Every specimen needs TgAb testing to authenticate that the Tg measurement is not compromised by TgAb interference. When present, TgAb concentrations *per se* can be monitored as a surrogate tumor marker.

**Objectives:** The aims of the study were: 1) to review published reports concerning whether there are associations between DTC, thyroid autoimmunity (Hashimoto's thyroiditis), and the presence of TgAb; and 2) to evaluate the methodological factors that influence TgAb interference with serum Tg testing.

**Data Sources:** PubMed was used to identify studies published over the last 55 yr that focused on DTC relationships with thyroid autoimmunity and the presence of thyroid autoantibodies.

**Results:** Many studies have reported significant associations between papillary thyroid cancer and Hashimoto's thyroiditis that may have a favorable prognostic significance. TgAb is detected in approximately 20% of DTC patients and may be a more specific thyroid tumor marker than thyroid peroxidase antibodies. TgAb interferes with Tg immunometric assay (IMA) measurements, causing falsely low/undetectable Tg values, especially when TgAb concentrations are high and serum Tg concentrations (measured by RIA) are low. TgAb concentrations respond to changes in Tg-secreting thyroid tissue such that the TgAb trend can be used as a more reliable surrogate DTC tumor marker than Tg IMA. Current TgAb assays may not always detect interfering TgAb because of insensitivity and specificity differences. It is critical to retain the same method for long-term TgAb monitoring.

**Conclusions:** Patients with Hashimoto's thyroiditis frequently have TgAb detected and may have a higher risk for papillary thyroid cancer. Although TgAb interferes with Tg IMA measurements, TgAb trends can be used as a surrogate DTC tumor marker in preference to Tg IMA, provided that the same method is used. (*J Clin Endocrinol Metab* 96: 3615–3627, 2011)

Differentiated thyroid cancers (DTC) account for approximately 1% of all malignancies but are the most common endocrine malignancy. The majority of DTC cases have papillary thyroid cancer (PTC) histology (~75%), whereas follicular thyroid cancers (FTC), including Hurthle cell cancers, are less common (~15%) but have higher mortality (1). Both PTC and FTC retain many characteristics of thyroid follicular cells, including

the expression of thyroid-specific proteins such as TSH receptor, thyroid peroxidase (TPO), and thyroglobulin (Tg) that serve as targets for thyroid autoimmunity. The incidence of both Tg autoantibodies (TgAb) and TPO antibodies (TPOAb) is approximately 2-fold higher in DTC [especially PTC (2, 3)], compared with the general population (~20 *vs.* ~10%, respectively), suggesting an association between autoimmune thyroid disease (AITD)

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Abbreviations: AITD, Autoimmune thyroid disease; DTC, differentiated thyroid cancer; FNA, fine-needle aspiration; FTC, follicular thyroid cancer; HT, Hashimoto's thyroiditis; IMA, immunometric assay; PTC, papillary thyroid cancer; Tg, thyroglobulin; TgAb, Tg autoantibodies; TPO, thyroid peroxidase; TPOAb, TPO antibodies.

and DTC (4–9). Indeed, many studies have reported an association between Hashimoto's thyroiditis (HT) and DTC. This association is more frequent for PTC than other DTC histotypes or benign thyroid conditions (2, 10–17). The mechanism(s) responsible for this association is currently unclear but may be multifactorial because HT and PTC share a number of morphological, immunohistochemical, and molecular features (9, 18–25). Moreover, the higher TSH often associated with HT may increase neoplastic risk, as suggested by the reports that TSH levels, even across the population reference range, correlate with a higher risk for nodular malignancy (26–30). Added to this, the inflammation that is characteristic of lymphocytic thyroiditis, together with TPOAb cytotoxicity, may increase cancer risk (31–36).

TgAb interference with serum Tg measurements severely compromises the clinical utility of Tg monitoring of DTC patients for recurrence (7, 32, 37–42). Current guidelines mandate that all specimens sent for Tg testing should have a TgAb measurement added, because the qualitative TgAb status (positive or negative) determines risk for Tg assay interference, and the quantitative TgAb concentration can serve as a surrogate tumor marker (1, 4, 5, 7, 8, 32, 38, 40, 41, 43–48). The potential for TgAb interference relates to the class of Tg method used [immunochemical assay (IMA) or RIA], with IMA methodology being more prone to interference than RIA (5, 39, 42, 49, 50). TgAb interference with IMA is always unidirectional (underestimation), which is especially problematic given that the persistence of TgAb after thyroidectomy appears to be a risk factor for disease (7, 32, 37–42). Although RIA methodology appears resistant to TgAb interference, RIA may under- or overestimate serum Tg in some patients, depending on the patient-specific characteristics of the TgAb and its interactions with the RIA reagents (5, 39, 42, 49, 50–55). The problem of TgAb interference necessitates the reliable detection of TgAb in the specimen before a Tg measurement can be authenticated. This is currently problematic because TgAb assays vary widely in sensitivity, specificity, and the numeric values they report, and the exogenous Tg recovery approach has been shown unreliable for detecting interfering TgAb (8, 38, 39, 42, 45, 53, 54, 56, 57). However, because TgAb concentrations appear to respond to changes in Tg-secreting tissue mass, it is becoming customary to monitor the TgAb trend as a surrogate DTC tumor marker in preference to Tg IMA measurements (1, 4, 5, 7, 32, 38, 40, 41, 43–48). This review will focus on whether there is a pathological link between AITD and DTC; whether the presence of AITD influences the outcome of DTC; how to interpret TgAb concentrations during different phases of managing patients with DTC; factors that influence TgAb interference

with serum Tg measurements; and the methodological problems with current TgAb assays that compromise the reliable detection of interfering TgAb.

### Does AITD predispose to DTC?

Since the first report by Dailey in 1955 (10), a number of surgical studies, including a meta-analysis, have investigated the coexistence of AITD and DTC (recently reviewed in Refs. 9 and 58). These studies have reported that HT (assessed by the presence of lymphocytic thyroiditis and/or thyroid autoantibodies) is associated with DTC approximately three times more frequently than with benign thyroid conditions, and that this association is 2.0 times higher for PTC as compared with the other DTC histotypes, especially when nodules are present (3, 10–17, 22, 28–30, 59–63). Currently, it remains unclear whether HT is a cause or an effect of PTC, or whether lymphocytic thyroiditis and/or the presence of TgAb has prognostic significance (9, 41, 64). If the coexistence of PTC and lymphocytic thyroiditis was not a random event, a relationship between PTC and lymphocytic thyroiditis could arise if preexistent lymphocytic thyroiditis was a risk factor for the development of PTC or alternatively arose secondarily as a result of preexistent carcinoma. HT and PTC share biomolecular characteristics that might predispose to carcinogenesis, such as RET/PTC rearrangements and the expression of P63 and Akt proteins that are thought to be involved in neoplastic transformation (9, 18–25, 58). In addition, chronic thyroidal stimulation secondary to the high TSH often associated with HT could be a factor that might promote tumorigenesis (26–28). Furthermore, the chronic inflammation that is associated with lymphocytic thyroiditis has the potential to activate cytokines and growth factors and create a mutagenic environment for multiple foci of carcinoma to develop (22, 31, 34, 35, 58).

The coincidence of PTC and lymphocytic thyroiditis varies widely among studies, ranging between 8 and 46% (Table 1) (9, 58, 62, 65). This variability undoubtedly reflects ascertainment biases, the size of study, differences in population ethnicity, iodine intake, and whether individuals with past radiation treatments were included (9, 22, 58, 65). Studies also differ regarding the mode used to diagnose HT—histological criteria (lymphocytic thyroiditis) *vs.* the serological presence of thyroid autoantibodies, criteria that are sometimes discordant (16, 66, 67). Unfortunately, some studies of thyroid autoantibodies have failed to differentiate between TgAb and TPOAb (4, 33, 63, 67). Additional complicating variables relate to differences in the sensitivity, specificity, and cutoff values of the TgAb methods used that can result in specimens being

**TABLE 1.** Associations between HT and PTC

First author, year (Ref.)	No. of patients	Type of surgery	% with LI of total	% with PTC	% (n/n) of LI in PTC	% (n/n) PTC in LI	LI in PTC relationship
Dailey, 1955 (10)	352	Tx	31 (110/352)	34 (120 <sup>a</sup> /352)	29 (35 <sup>a</sup> /120)	32 (35 <sup>a</sup> /110)	Yes
Schlicke, 1960 (11)	1682	Tx	11 (103/1682)	7 (111 <sup>a</sup> /1682)	8 (9 <sup>a</sup> /111)	9 (9 <sup>a</sup> /103)	Yes
Hirabayashi, 1965 (12)	9221	Tx	6 (752/9221)	4 (370 <sup>a</sup> /9221)	46 (169 <sup>a</sup> /370)	22 (169/752)	Yes
Ott, 1987 (13)	800	Sx/TN	33 (267/800)	13 (103/800)	38 (61 <sup>a</sup> /161)	23 (61/267)	<i>P</i> < 0.05
Cipolla, 2005 (62)	178	Tx/DTC	15 (27/178)	39 (71/178)	27 (19/71)	28 (13/47)	<i>P</i> < 0.02
Kurukahvecioglu, 2007 (14)	922	Sx	11 (101/922)	22 (199/922)	19 (37/199)	37 (37/101)	<i>P</i> < 0.006
Larson, 2007 (22)	812	Tx	26 (214/812)	22 (179/812)	26 (46/179)	24 (52/214)	<i>P</i> = 0.03
Reppinger, 2008 (15)	1198	Sx	18 (217/1198)	24 (289/1198)	22 (63/289)	29 (63/217)	<i>P</i> = 0.05
Consorti, 2010 (17)	613	Tx/NG	15 (92/613)	28 (171/613)	23 (40/171)	33 <sup>a</sup> (30/92)	Yes
Gul, 2010 (16)	404	Tx	17 (69/404)	25 (101/404)	30 (34/101)	23 (40/171)	RR = 1.6 (1.21–1.94)
Mazokopakis, 2010 (65)	140	Tx	30 (42/140)	23 (32/140)	38 (12/32)	29 (12/42)	No
Kim, 2011 (30)	1329	Sx	25 (336/1329)	77 (1028/1329)	30 (307/1028)	91 (307/336)	<i>P</i> < 0.001

This table references surgical studies that have investigated associations between HT (judged from histological LI) and PTC. LI, Lymphocytic infiltration; Tx, unselected thyroidectomies; Sx, unselected surgeries; Tx/NG, Tx for nodular goiter; Tx/DTC, Tx for DTC; Sx/TN, surgery for thyroid nodules without radiation treatment.

<sup>a</sup> Type of DTC not specified.

classified as “TgAb-positive” using one method but not others (39, 42, 68, 69).

Although the majority of studies have reported that HT is a risk factor for PTC, this remains controversial. Also, it is currently unclear whether the mechanism(s) for this association might relate to biomolecular commonalities, chronic TSH stimulation, or inflammation. In addition, these surgical series are limited by their inability to assess the length of time from the diagnosis of HT to the development of cancer and are clearly skewed by ascertainment biases because most patients with HT are not treated with surgical resection (22). Thus, it is unclear whether any apparent increased risk for PTC in patients with HT, especially those with nodularity, should influence management.

### Prognostic Implications of Lymphocytic Thyroiditis Associated with PTC

A number of PTC outcome studies have evaluated whether the histological presence of lymphocytic thyroiditis or the serological detection of TgAb has prognostic significance (12, 59, 60, 66, 70, 71). The data from such studies is equivocal, probably reflecting ascertainment and treatment biases as well as the inherent limitations of a retrospective design. In general, lymphocytic thyroiditis is considered a favorable prognostic factor for PTC (longer disease-free interval, decreased mortality), although this has not always achieved significance using multivariate analysis (3, 59, 60, 66, 70, 72–77). Study variability could

in part relate to a failure to distinguish between the generalized lymphocytic infiltration characteristic of HT and nonspecific tumor-infiltrating lymphocytes that could represent an immune response to tumor (17, 34, 59, 60, 66, 72, 73, 76). This distinction is important because in humans the presence of lymphocytic infiltration in or around a tumor is commonly viewed as representing the host’s immune response to that tumor that may favorably influence prognosis (34, 61, 66). Other confounding factors have been that PTC patients with lymphocytic thyroiditis tend to be younger and female (14, 15, 28, 59); have smaller, lower grade tumors (10, 14, 15, 30, 59, 75, 77); and have a lower frequency of BRAF<sup>V600E</sup> mutations (25, 78–81)—all factors that would be expected to favorably influence outcome. In contrast, other studies have reported that PTC tumors displaying lymphocytic thyroiditis are more likely to be bilateral and multifocal (17, 28, 30, 66, 82), to have higher stage disease (17), and to have a higher frequency of lymph node metastases (2, 28)—all considered unfavorable prognostic factors.

Generally, lymphocytic thyroiditis is highly correlated with the serological presence of thyroid antibodies; however, whether the presence of TgAb *per se* has prognostic significance remains controversial (8, 17, 28, 29, 41, 73). Both the presence and the concentration of TgAb appears to be associated with adverse clinicopathological factors such as bilateral disease, multicentricity, extrathyroidal extension, increased stage of disease, higher frequency of lymph node metastases, as well as increased risk of recurrence (2, 7, 40, 41, 43, 48, 83). However, such studies have

often been compromised by a failure to distinguish between the two different pathogenetic mechanisms likely responsible for any association between TgAb and PTC. Specifically, whereas TPOAb are believed to fix complement and be more diagnostic for HT and hypothyroidism than TgAb, TgAb may be more tumor-specific than TPOAb (6, 32, 84). Evidence for this is 2-fold. First, it appears that when evaluating thyroid nodules, the presence of TgAb may be a risk factor for PTC, independent of preexisting AITD or the presence of TPOAb (29, 41, 64). Second, the changes in TgAb and TPOAb appear independent and more rapid for TgAb than TPOAb (32, 44, 47, 85). The view that TgAb arises in PTC from two distinctly different pathogenetic mechanisms (AITD *vs.* tumor-related) is supported by epitope mapping studies that have found different epitope specificities for TgAb associated with PTC in the presence *vs.* the absence of histological evidence of lymphocytic thyroiditis (86).

Further studies are needed to clarify whether the histological presence of lymphocytic thyroiditis is a favorable prognostic factor, and/or whether the histological distinction between generalized and tumor-infiltrating lymphocytes in the surgical specimen might have prognostic significance. Additionally, the relationship between TgAb and PTC needs further study. Such studies should focus on distinguishing between TgAb arising as a result of underlying AITD, *vs.* as a result of an immune response to the inflammation associated with tumorigenesis that may have the potential to release posttranslational modified Tg antigens with enhanced immunogenicity (22, 31, 34, 35, 41, 56, 86–88).

### TgAb Measurements Made during Different Stages of Managing DTC

All specimens sent for Tg measurement require adjunctive TgAb testing because TgAb status can change over time (positive to negative or negative to positive) (38). This testing is imperative because even very low TgAb concentrations can interfere with Tg measurements (5, 7, 8, 39, 42, 89). Although TgAb interference severely limits the reliability of Tg testing, TgAb secretion appears to be quite sensitive to changes in the mass of Tg-producing tissue, such that serial TgAb measurements can be monitored as a surrogate DTC tumor marker (1, 4, 5, 7, 8, 32, 40, 41, 43–48). It is generally believed that in autoimmune diseases thyroid antibody production primarily arises in intrathyroidal lymphocytes. It is therefore surprising that after thyroidectomy, TgAb may persist for many years without clear evidence of persistent disease. This may be because cervical lymph nodes initiate and disseminate the

autoimmune response or reflect the persistence of Tg in antigen-presenting cells (44, 90).

### Preoperative serum TgAb measurements

Preoperative serum TgAb measurement is not recommended by current guidelines, despite a recent report that the presence and concentration of TgAb is a risk factor for malignancy in thyroid nodules (1, 41). Often, however, the preoperative TgAb status of the patient would be known as part of a preoperative Tg test made to gauge tumor Tg secretory capability (1). This could be viewed as a useful baseline for assessing postoperative TgAb trends (48). However, depending on the time of sampling, a preoperative TgAb measurement could be falsely elevated in response to the rise in Tg antigen secondary to fine-needle aspiration (FNA) biopsy injury of thyroid tissue (91, 92).

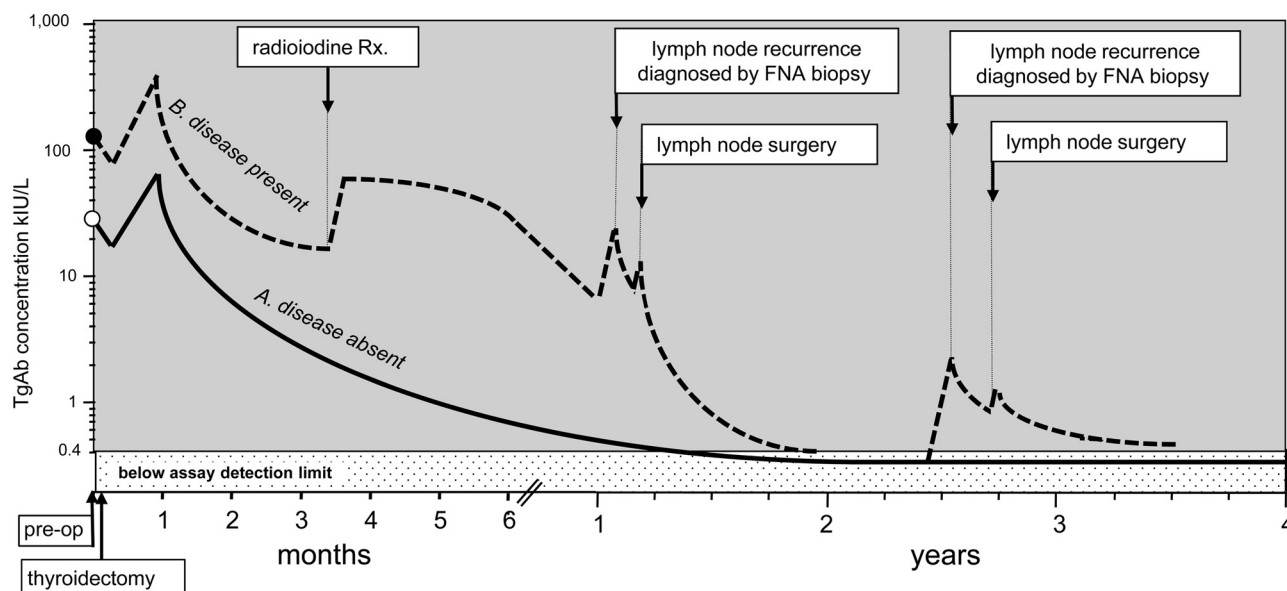
### Early (first year) postoperative serum TgAb responses

As shown in Fig. 1, TgAb concentrations may fall transiently during the first few days after thyroidectomy (93–95). Given that the median half-life of TgAb after treatment for DTC approximates 10 wk (8), this early transient TgAb decline likely results from increased formation and rapid metabolic clearance of Tg-TgAb complexes formed in response to the rise in Tg after surgical injury (93, 96). Thereafter, during the first few weeks after surgery, this Tg release may initiate a rise, or *de novo* appearance, of TgAb concentrations that decline over several months (94, 97). In the longer term, TgAb concentrations fall over subsequent months and years as thyroid tissue mass and Tg antigen levels decline (8, 44). Patients may not achieve a negative TgAb status during the first postoperative year and may even exhibit a rise in (or *de novo* appearance of) TgAb during the 6 months after a radioiodine treatment when there is release of Tg antigen secondary to radiolytic damage of thyroid tissue (2, 8, 98–101). Clearly, it is the *trend* in TgAb concentrations that is more significant than any single TgAb concentration *per se*. This was apparent from the 2008 study by Kim *et al.* (48) that found that less than 1% of patients who became TgAb-negative or displayed more than a 50% decline in TgAb over the 6 to 12 months after radioiodine treatment had a recurrence detected during follow-up. In contrast, 19% of patients in whom TgAb declined less than 50%, as well as the 37% in whom TgAb concentrations rose, were diagnosed with recurrences (48).

### Long-term serial TgAb monitoring

It is now clear that removing the Tg antigenic stimulus by thyroidectomy and radioiodine remnant ablation results in the eventual disappearance of TgAb over a median





**FIG. 1.** Typical changes in TgAb trends after thyroidectomy in patients rendered disease-free by surgery (pattern A) vs. patients with persistent/recurrent disease (pattern B). TgAb concentrations may rise or become detectable *de novo* in response to increases in Tg antigen after thyroidectomy, lymph node recurrence(s), lymph node resection(s) FNA biopsy of metastatic lymph nodes, or radioiodine therapy.

time of 3 yr (8, 44, 47). Conversely, TgAb concentrations rise, or may become detectable *de novo*, in response to an acute increase in Tg antigen after either the initial or secondary thyroid surgeries (97), FNA biopsy (92, 102), or radioiodine therapy (2, 98–101). Importantly, most studies have reported that the *de novo* appearance, persistence, or a rising trend in TgAb concentrations in the postoperative period is a significant risk factor for persistent/recurrent disease (5, 7, 8, 40, 43, 48). It is likely that the time needed for TgAb to become undetectable after thyroidectomy relates to the size of the surgical remnant, which may still be detected after radioiodine treatment (103). The persistence of a low TgAb concentration years after the initial surgery does not necessarily indicate the presence of disease, especially when TgAb concentrations display a declining trend (48). For effective long-term TgAb monitoring, it is critical to use a sensitive method with excellent between-run precision (<15%) established over the typical clinical interval used to monitor DTC patients (6–12 months) (38).

### TgAb Interference with Tg Measurements

TgAb interferes with Tg measurements in a qualitative, quantitative, and method-dependent manner (5, 51–53). Interference with Tg IMA methodology is always unidirectional (underestimation), whereas interference with RIA has the potential to cause either under- or overestimation of Tg, depending on the characteristics of the interactions between the patient-specific TgAb and the RIA reagents (1, 5, 37, 39, 49–55). Because laboratories favor

the speed and automation of IMA methodology, there have been attempts to overcome interference by developing multisite IMA using selected monoclonal antibodies with specificities for Tg epitope domains different from those commonly involved in thyroid autoimmunity (37, 104). Whereas this approach is conceptually attractive, it has not eliminated TgAb interference, possibly because of differences in the epitope specificities of TgAb arising in DTC as compared with thyroid autoimmunity (32, 56, 86, 105). Furthermore, the epitope-targeting approach would not overcome the problem of steric inhibition of the Tg complexed with TgAb from participating in the noncompetitive IMA reaction (106).

A number of clinical studies support the contention that IMA methodology underestimates serum Tg in the presence of TgAb. First, TgAb-positive DTC patients with unequivocal evidence of disease frequently have inappropriately low or undetectable Tg IMA values (5, 7, 8, 40, 48). Second, TgAb-positive euthyroid control subjects with intact thyroid glands often have low or paradoxically undetectable Tg IMA values compared with subjects without TgAb, who have serum Tg concentrations that are typically higher than 2.0 ng/ml ( $\mu\text{g/liter}$ ) (39). Even more striking are the reports that Tg IMA values can be paradoxically low or even undetectable in Graves' hyperthyroid patients, the majority of whom have circulating TgAb (37, 107). These reports contrast with the clinically more appropriate serum Tg RIA values seen with these same conditions, suggesting that RIA methodology is resistant to TgAb interference (5, 37, 39, 49, 99, 108, 109). Even if RIA provides an accurate measurement of total (TgAb-

**TABLE 2.** TgAb interference: relationships between TgAb and Tg concentrations

	TgAb (kIU/liter)		
	Low (1–5)	Medium (5–100)	High >100
Tg RIA, ng/ml ( $\mu$ g/liter)			
Low (1–5)			
n	67	62	12
TgAb	2.5 $\pm$ 1.1 (1.0–5.0)	22 $\pm$ 19 (6–80)	535 $\pm$ 465 (162–1650)
Tg IMA	0.5 $\pm$ 0.7 (<0.1–2.7)	0.3 $\pm$ 0.6 (<0.1–3.6)	0.1 $\pm$ 0.2 (<0.1–0.6)
Tg RIA	1.8 $\pm$ 0.7 (1.0–3.8)	2.5 $\pm$ 1.2 (1.0–5.0)	2.7 $\pm$ 1.0 (1.6–4.5)
IMA/RIA % ratio	23 $\pm$ 35 (<1–200)	11 $\pm$ 20 (<1–96)	5 $\pm$ 7 (<1–21)
% with UD Tg IMA	52	63	100
Medium (5–20)			
n	20	31	37
TgAb	2.1 $\pm$ 1.7 (1.1–7.7)	36 $\pm$ 25 (6–83)	2231 $\pm$ 3057 (135–9900)
Tg IMA	6.5 $\pm$ 3.6 (<0.1–16.4)	1.8 $\pm$ 2.2 (<0.1–6.3)	1.7 $\pm$ 4.6 (<0.1–21.2)
Tg RIA	10.3 $\pm$ 4.5 (5.2–19.5)	10.6 $\pm$ 4.1 (5.1–17.6)	10.3 $\pm$ 4.3 (5.2–20.0)
IMA/RIA % ratio	68 $\pm$ 33 (<1–90)	16 $\pm$ 19 (<1–61)	14 $\pm$ 34 (<1–170)
% with UD Tg IMA	5	45	62
High (>20)			
n	27	19	20
TgAb	2.5 $\pm$ 1.2 (1.0–4.3)	24 $\pm$ 22 (6–76)	1091 $\pm$ 1257 (134–3300)
Tg IMA	400 $\pm$ 729 (4.3–3205)	232 $\pm$ 688 (22–3055)	135 $\pm$ 207 (<0.1–837)
Tg RIA	394 $\pm$ 607 (21.8–2450)	176 $\pm$ 350 (20–1580)	217 $\pm$ 321 (20.3–1450)
IMA/RIA % ratio	92 $\pm$ 32 (<1–149)	80 $\pm$ 38 (33–193)	45 $\pm$ 39 (<1–126)
% with UD Tg IMA	0	0	15

Data are expressed as mean  $\pm$  SD (range). Table shows how relationships between serum Tg and TgAb concentrations influence the propensity for TgAb interference, as judged from a low (<75%) Tg IMA/Tg RIA ratio as previously described (42). Tg RIA and Tg IMA data are shown for nine groups of DTC patients with Tg RIA values in the low [1–5 ng/ml ( $\mu$ g/liter)], intermediate [5–20 ng/ml ( $\mu$ g/liter)], or high [>20 ng/ml ( $\mu$ g/liter)] range, and TgAb concentrations in the low (1–5 kIU/liter), intermediate (5–100 kIU/liter), or high (>100 kIU/liter) range. UD, Below assay functional sensitivity [ $<0.1$  ng/ml ( $\mu$ g/liter)]. [Data were taken from C. Spencer *et al.*: Current thyroglobulin autoantibody (TgAb) assays often fail to detect interfering TgAb that can result in the reporting of falsely low/undetectable serum Tg IMA values for patients with differentiated thyroid cancer. *J Clin Endocrinol Metab* 96:1283–1291, 2011 (42), with permission. © The Endocrine Society.]

bound + free) Tg, the metabolic clearance of Tg-TgAb complexes may be enhanced relative to the metabolic clearance of free Tg that has a half-life approximating 3 d (93, 95, 96). Tg antibodies are typically conformational with high affinities ( $10^{-10}$ ) for the intact Tg molecular structure (32, 110). If Tg-TgAb complexes were cleared faster than free Tg, a change in the total Tg would reflect not only changes in Tg secretion but also the TgAb concentration and epitope specificity for binding not only the endogenous serum Tg isoforms in the specimen, but also the thyroid glandular Tg preparations used as assay standards (52, 111, 112). In the case of some tumors, abnormal posttranslational modification of Tg could result in the secretion of Tg isoforms with altered immunoactivity, size, and/or glycosylation that might lack the appropriate conformation to bind normally to endogenous TgAb or the Tg assay reagents (56, 86–88, 95, 113, 114). Thus, patient-specific interactions between TgAb and Tg influence Tg-TgAb complex formation and the propensity for TgAb interference with the serum Tg measurements made for any given patient.

It is difficult to predict the influence of any individual patient's TgAb on Tg measurement. Because IMA methodology preferentially measures free Tg, interference

should be minimal when the Tg concentration is in excess of that needed to saturate TgAb binding sites, leaving high levels of free Tg for IMA detection (5, 42, 45, 93, 115). This is evident from the Table 2 data showing that the propensity for TgAb interference [as judged by a Tg IMA/Tg RIA ratio below 75% (42)] is lowest when Tg (RIA) concentrations are high and TgAb is low, and highest when Tg is low and TgAb is high. Although the frequency of TgAb interference clearly relates to the quantitative TgAb level, different patients with the same TgAb concentration display qualitative TgAb differences as evident from the variability in Tg IMA/Tg RIA ratios. In individual cases, low TgAb concentrations clearly produced profound interference that was not overcome by a high Tg concentration, whereas in other cases high levels of TgAb displayed very little interference despite a low Tg concentration. It follows that for any specimen, the potential for TgAb interference is multifactorial and relates to the format of the Tg assay (IMA or RIA), the TgAb concentration, and the TgAb epitope specificity for both the Tg isoforms secreted by the patient and the Tg assay reagent(s) (39, 116, 117). The continuing problem of TgAb interference with Tg IMA measurements would suggest that laboratories adopt a dual strategy for serum Tg

testing. This would involve first establishing the TgAb status of the specimen using a sensitive TgAb immunoassay method to restrict Tg IMA measurements to TgAb-negative sera while using a Tg RIA method for TgAb-positive sera. However, this approach is impractical for routine clinical laboratories, and it is only available in laboratories with strong research interests. In the routine laboratory, TgAb trends should be monitored as a preferable surrogate tumor marker to Tg measured by IMA (1, 4, 5, 7, 8, 32, 40, 41, 43–48).

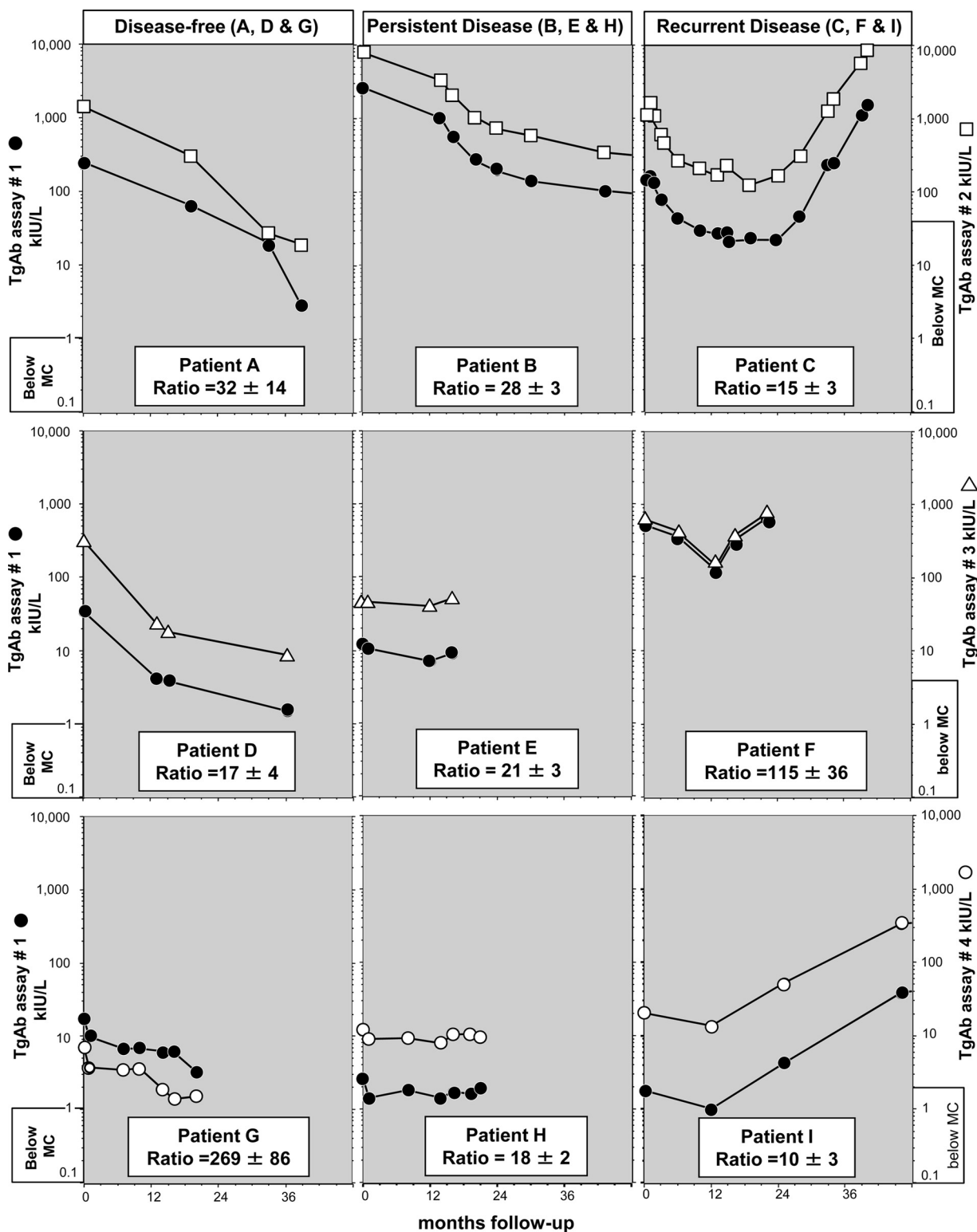
Non-immunoassay techniques such as liquid chromatography-tandem mass spectrometry are increasingly being employed to measure proteins (118). However, at present it is questionable whether liquid chromatography-tandem mass spectrometry of tryptic digests of Tg protein might allow the reliable quantitation of serum Tg in the presence of TgAb (118). Studies to date using purified glandular Tg protein preparations have reported inferior sensitivity as compared with current immunoassays (118). Furthermore, it remains to be determined whether the heterogeneity characteristic of tumor-derived Tg protein would prevent the generation of characteristic tryptic peptides and/or their isolation using monoclonal antibody-based affinity chromatography (119).

### TgAb Measurement—Technical Challenges

The prevalence of TgAb in patients with DTC appears to approximate twice that of the general population (~20% *vs.* ~10%, respectively, depending on age, gender and methodology) (2, 4–8, 42, 43, 45). This high prevalence, together with studies showing that even low levels of TgAb can interfere and cause falsely low Tg IMA values that can mask persistent/recurrent disease, is why all specimens need sensitive TgAb screening before authenticating a Tg measurement (1, 5, 7, 8, 38–40, 42, 45, 48, 89, 120). Over the last 50 yr, the sensitivity and specificity of TgAb methodology has dramatically improved as qualitative techniques such as immunodiffusion, complement fixation, passive hemagglutination, particle agglutination, and indirect immunofluorescence have been replaced by quantitative competitive and noncompetitive immunoassay methods (32, 39, 42, 52, 57, 68, 69, 120). Currently, most immunoassay platforms include an automated nonisotopic TgAb test. The manufacturers of these methods claim that the secondary standards included in their kit are calibrated against an International Reference Preparation (First IRP 65/93)—a reference serum pool that is more than 50 yr old and is made from patients with thyroid autoimmunity, not DTC. The age of this primary standard, together with the use of different secondary serum

pool standards, is one reason why different methods report disparate numeric values that vary 100-fold to an unpredictable, specimen-dependent degree that precludes establishing between-method conversion factors (39, 42, 57, 68, 69, 121). This methodological variability reflects not only suboptimal sensitivity and specificity but also different interactions between the patient-specific entities in the specimen (Tg, TgAb, and Tg-TgAb immune complexes) and the assay reagents (Tg standards/tracer and antibody), as discussed above. These variables are the reason why high TgAb concentrations do not always interfere, yet in other cases very low TgAb concentrations exhibit profound interference (5, 32, 39, 41, 42, 45, 47, 56, 57, 68, 69, 86, 88, 89, 117, 121, 122). Whatever the factors responsible for different methods reporting different numeric values, it is clear that TgAb method variability and reliability are the most serious technical problems currently limiting the use of Tg as a DTC tumor marker. This problem is exacerbated when laboratories adopt the manufacturers' cutoff for a "detectable" TgAb. Such cutoffs are optimized for diagnosing thyroid autoimmunity and not for detecting TgAb interference with Tg measurements and, as a result, are set too high (39, 42, 57, 68, 69, 121). Inappropriately high cutoffs can lead to falsely classifying specimens with interfering TgAb as "TgAb-negative" that may prompt the reporting of falsely low Tg IMA measurements (1, 4, 5, 7, 8, 32, 38, 40, 41, 43–48).

The problem of TgAb method variability necessitates the retention of the same TgAb method for long-term monitoring. This requirement is problematic for laboratories because manufacturers sometimes withdraw or change their methods without adequate notification. Furthermore, it is not unusual for patients to change physicians and/or insurance plans, and this often prompts a change in the TgAb method. The patient-specific qualitative differences in TgAb that result in different TgAb rankings among methods prevent the adoption of universal conversion factors to relate the values of different methods (42). However, the qualitative characteristics of the TgAb secreted by individual patients remains constant over time during long-term monitoring, independent of changes in TgAb concentration. This is evident from Fig. 2, which shows serial TgAb measurements made for nine patients, each patient having TgAb measured by two different methods. Although the mean ratios between the numeric values reported by the methods were both patient- and method-specific, the average ratio remained relatively constant over years of monitoring. TgAb trends reported by the individual methods were similar (declining when disease-free, constant for persistent disease, and rising with recurrences). These data suggest that when a change in TgAb method is unavoidable, an archived specimen



**FIG. 2.** The trend in serial serum TgAb measurements (on ordinates) made for nine DTC patients (A–I) monitored over 1–4 yr. Each specimen had TgAb measured by a reference assay (assay 1, Kronus/RSR, *solid circles*) and one of three test assays: (assay 2, Siemens Immulite, *open squares*) for patients A–C; assay 3 (Beckman Access, *open triangles*) for patients D–F; and (assay 4, Nichols Advantage, *open circles*) for patients G–I. The *clear boxes* at the bottom of each patient’s plot show the mean ( $\pm$  SD) of the ratios between the TgAb values reported by test divided by the reference method. These ratios were highly patient- and method-specific and were independent of both the TgAb trend and the TgAb concentration, illustrating the qualitative differences in TgAb secreted by different patients. Patients were selected from three clinical categories. Patients A, D, and G were judged disease-free after thyroidectomy ( $\pm$  radioiodine) and, irrespective of the assay used, showed a declining trend in TgAb values. Patients B, E, and H had evidence of disease and maintained stable, detectable TgAb values. Patients C, F, and I had recurrences detected characterized by rising TgAb values. MC, Manufacturer’s recommended cutoff for a detectable TgAb. The data were taken from previous studies (39, 42).



from the patient could be used to establish the ratio between the old and new method, thereby allowing re-baselining of TgAb and the continued monitoring of the TgAb trend.

The detection of TgAb is more reliable when made directly by immunoassay compared with a recovery test (1, 38). Typically, an 80% recovery of added Tg is considered to indicate the absence of interference. However, despite some correlation between TgAb concentrations and low Tg recoveries, most studies have now concluded that recovery tests are an unreliable way to detect interfering TgAb (5, 8, 39, 45, 53, 54, 56, 123). The failure of Tg recoveries to detect TgAb interference likely reflects the source and concentration of added Tg, as well as the recovery protocol employed (5, 54). Specifically, the epitope specificities of the added Tg (typically a thyroid gland extract) differ from those of the endogenous (serum) Tg isoforms, such that the formation of the Tg-TgAb complexes with the added Tg may not represent endogenous Tg-TgAb complex formation (5, 111, 124, 125). Besides these qualitative differences, recoveries are influenced by the relative concentrations of the added Tg *vs.* the Tg in the specimen. This problem is exacerbated when the Tg concentration added is greater than that of the Tg in the specimen, leading to an excess of free Tg that would cause an overestimate of recovery (5, 54). Lastly, it takes time (~24 h) for large molecules like Tg to form immune complexes that reach equilibrium (107, 126). When a quantity of Tg is added to the specimen immediately before adding the assay reagents, there is insufficient time for the added Tg to equilibrate with the Tg and TgAb in the specimen. This would result in a high concentration of free Tg that would result in an overestimate of recovery (5, 54). These reasons likely explain why Tg recoveries do not reliably detect interfering TgAb and why current guidelines recommend that the practice of performing recoveries should be discouraged and eliminated (1, 38).

## Conclusions

Many surgical studies have concluded that HT, characterized by histological lymphocytic infiltration and/or the serological detection of TgAb, appears to convey an approximately 3-fold increased risk for PTC, and that this association may have favorable prognostic significance. Whether the mechanism(s) for this association relates to biomolecular commonalities, chronic TSH stimulation, or inflammation remains unclear. However, because surgical treatment is not typically used for most cases of HT, selection bias clearly limits the value of these observations. It is currently unclear whether any apparent increased risk

for PTC in patients with HT, especially those with nodularity, should influence management.

Studies suggest that TgAb may be more tumor-specific than TPOAb and has a higher prevalence in DTC patients than the general population. This high prevalence exacerbates the problem of TgAb interference with serum Tg measured as a DTC tumor marker. All specimens sent for Tg measurement require adjunctive TgAb testing because TgAb status can change over time (positive to negative or negative to positive) and even very low TgAb concentrations can interfere. TgAb interference causes underestimation of serum Tg when measured by IMA, and either under- or overestimation when measured by RIA. Clinical correlation studies suggest that RIA measurements are less prone to TgAb interference than IMA and that the most sensitive indicator of TgAb interference is a low Tg IMA/Tg RIA ratio. Unfortunately, TgAb assays vary broadly in sensitivity, specificity, and the numeric values they report. Very low TgAb concentrations may interfere with Tg without being detected by some methods. In fact, the propensity for TgAb to interfere is multifactorial and relates to the class of Tg assay used (IMA *vs.* RIA) as well as both qualitative and quantitative relationships between the different entities in the assay milieu (Tg, TgAb, Tg-TgAb complexes). In general, interference appears to be minimal when Tg (RIA) in the specimen is high and TgAb is low, but severe when Tg (RIA) is low and TgAb is high.

It is now clear that TgAb concentrations respond to changes in the levels of circulating Tg antigen and thereby indirectly to changes in thyroid tissue mass. It follows that when present, TgAb can become the preferred surrogate tumor marker that can replace unreliable Tg IMA measurements. However, because current TgAb methods differ in sensitivity, epitope specificity, and the numeric values they report, it is critical to use the same method to monitor TgAb concentrations or have archived specimens available for re-baselining. Because TgAb can serve as a surrogate tumor marker test, a baseline preoperative TgAb measurement may be valuable.

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