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 [immunometric 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[a]/352)	29 (35[a]/120)	32 (35[a]/110)	Yes
Schlicke, 1960 (11)	1682	Tx	11 (103/1682)	7 (111[a]/1682)	8 (9[a]/111)	9 (9[a]/103)	Yes
Hirabayashi, 1965 (12)	9221	Tx	6 (752/9221)	4 (370[a]/9221)	46 (169[a]/370)	22 (169/752)	Yes
Ott, 1987 (13)	800	Sx/TN	33 (267/800)	13 (103/800)	38 (61[a]/161)	23 (61/267)	$P < 0.05$
Cipolla, 2005 (62)	178	Tx/DTC	15 (27/178)	39 (71/178)	27 (19/71)	28 (13/47)	$P < 0.02$
Kurukahvecioglu, 2007 (14)	922	Sx	11 (101/922)	22 (199/922)	19 (37/199)	37 (37/101)	$P < 0.006$
Larson, 2007 (22)	812	Tx	26 (214/812)	22 (179/812)	26 (46/179)	24 (52/214)	$P = 0.03$
Repplinger, 2008 (15)	1198	Sx	18 (217/1198)	24 (289/1198)	22 (63/289)	29 (63/217)	$P = 0.05$
Consorti, 2010 (17)	613	Tx/NG	15 (92/613)	28 (171/613)	23 (40/171)	33[a] (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)	$P < 0.001$

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.

[a] 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 $BRAF^{V600E}$ 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

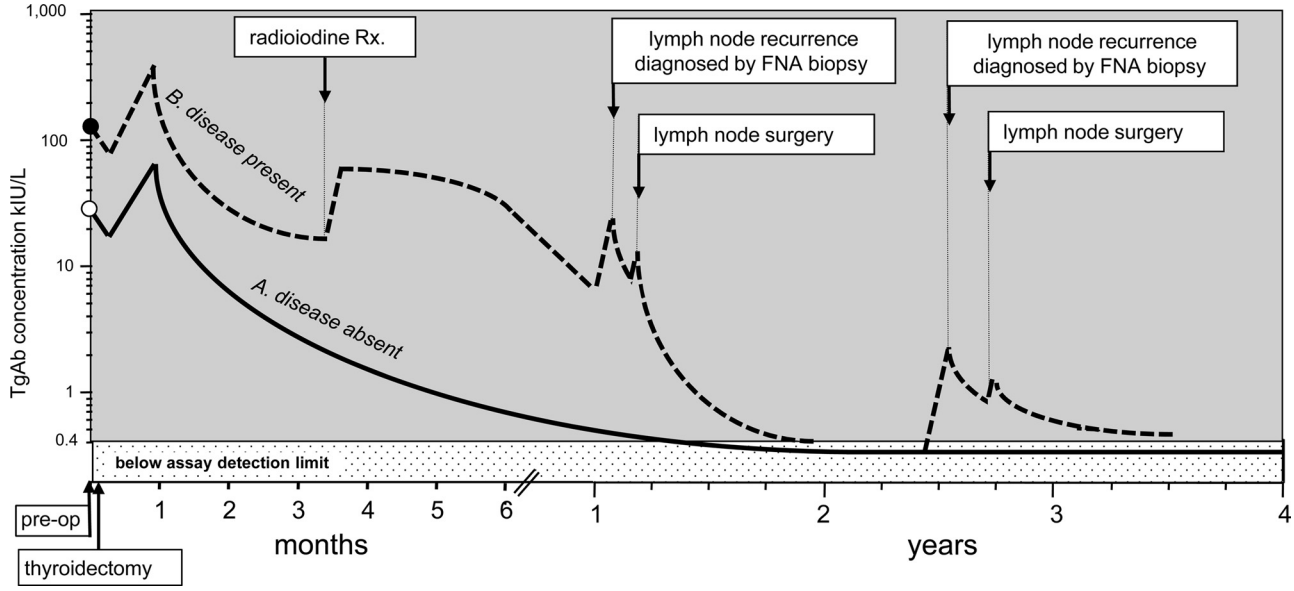

FIG. 1. Typical changes in TgAb trends after thyroidectomy in patients rendered disease-free by surgery (pattern A) *vs.* patients with persistent/recurrent disease (pattern B). TgAb concentrations may rise or become detectable *de novo* in response to increases in Tg antigen after thyroidectomy, lymph 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 (μg/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 (μ g/liter)			
Low (1–5)			
n	67	62	12
TgAb	2.5 ± 1.1 (1.0–5.0)	22 ± 19 (6–80)	535 ± 465 (162–1650)
Tg IMA	0.5 ± 0.7 (<0.1–2.7)	0.3 ± 0.6 (<0.1–3.6)	0.1 ± 0.2 (<0.1–0.6)
Tg RIA	1.8 ± 0.7 (1.0–3.8)	2.5 ± 1.2 (1.0–5.0)	2.7 ± 1.0 (1.6–4.5)
IMA/RIA % ratio	23 ± 35 (<1–200)	11 ± 20 (<1–96)	5 ± 7 (<1–21)
% with UD Tg IMA	52	63	100
Medium (5–20)			
n	20	31	37
TgAb	2.1 ± 1.7 (1.1–7.7)	36 ± 25 (6–83)	2231 ± 3057 (135–9900)
Tg IMA	6.5 ± 3.6 (<0.1–16.4)	1.8 ± 2.2 (<0.1–6.3)	1.7 ± 4.6 (<0.1–21.2)
Tg RIA	10.3 ± 4.5 (5.2–19.5)	10.6 ± 4.1 (5.1–17.6)	10.3 ± 4.3 (5.2–20.0)
IMA/RIA % ratio	68 ± 33 (<1–90)	16 ± 19 (<1–61)	14 ± 34 (<1–170)
% with UD Tg IMA	5	45	62
High (>20)			
n	27	19	20
TgAb	2.5 ± 1.2 (1.0–4.3)	24 ± 22 (6–76)	1091 ± 1257 (134–3300)
Tg IMA	400 ± 729 (4.3–3205)	232 ± 688 (22–3055)	135 ± 207 (<0.1–837)
Tg RIA	394 ± 607 (21.8–2450)	176 ± 350 (20–1580)	217 ± 321 (20.3–1450)
IMA/RIA % ratio	92 ± 32 (<1–149)	80 ± 38 (33–193)	45 ± 39 (<1–126)
% with UD Tg IMA	0	0	15

Data are expressed as mean ± 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 (μg/liter)], intermediate [5–20 ng/ml (μg/liter)], or high [>20 ng/ml (μg/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 (μg/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

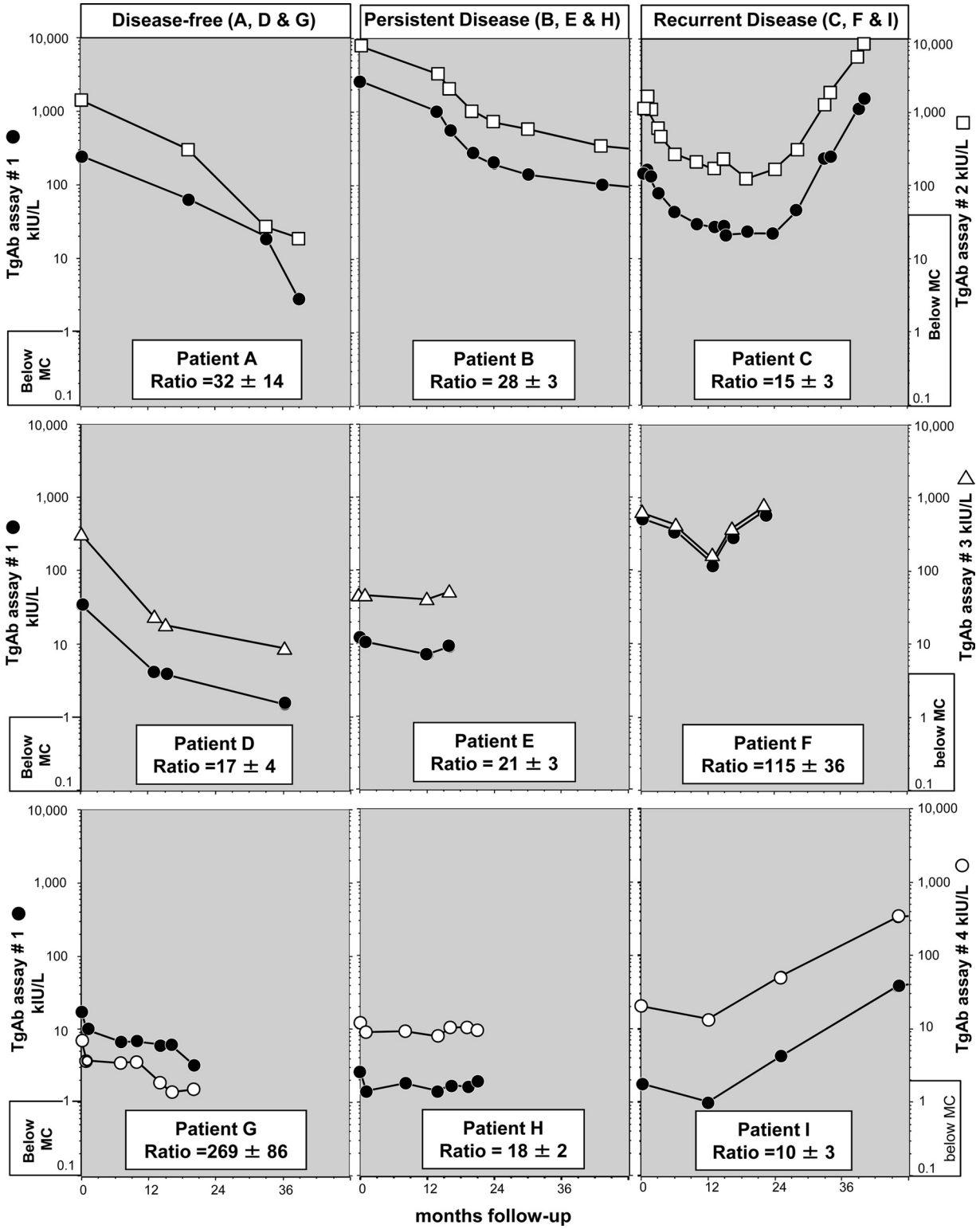

FIG. 2. The trend in serial serum TgAb measurements (on ordinates) made for nine DTC patients (A–I) monitored over 1–4 yr. Each specimen had TgAb measured by a reference assay (assay 1, Kronus/RSR, *solid circles*) and one of three test assays: (assay 2, Siemens Immulite, *open squares*) for patients 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 (± 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 (± 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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References

1. **Cooper DS, Doherty GM, Haugen BR, Hauger BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger**

M, Sherman SI, Steward DL, Tuttle RM 2009 Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 19:1167–1214
2. **Kumar A, Shah DH, Shrihari U, Dandekar SR, Vijayan U, Sharma SM** 1994 Significance of antithyroglobulin autoantibodies in differentiated thyroid carcinoma. Thyroid 4:199–202
3. **Souza SL, Montalli Da Assumpção LV, Ward LS** 2003 Impact of previous thyroid autoimmune diseases on prognosis of patients with well-differentiated thyroid cancer. Thyroid 13:491–495
4. **Pacini F, Mariotti S, Formica N, Elisei R, Anelli S, Capotorti E, Pinchera A** 1988 Thyroid autoantibodies in thyroid cancer: incidence and relationship with tumor outcome. Acta Endocrinol 119: 373–380
5. **Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS, Nicoloff JT** 1998 Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 83: 1121–1127
6. **Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, Braverman LE** 2002 Serum thyrotropin, thyroxine, and thyroid antibodies in the United States population (1988 to 1994): NHANES III. J Clin Endocrinol Metab 87:489–499
7. **Chung JK, Park YJ, Kim TY, So Y, Kim SK, Park DJ, Lee DS, Lee MC, Cho BY** 2002 Clinical significance of elevated level of serum antithyroglobulin antibody in patients with differentiated thyroid cancer after thyroid ablation. Clin Endocrinol (Oxf) 57:215–221
8. **Görges R, Maniecki M, Jentzen W, Sheu SN, Mann K, Bockisch A, Janssen OE** 2005 Development and clinical impact of thyroglobulin antibodies in patients with differentiated thyroid carcinoma during the first 3 years after thyroidectomy. Eur J Endocrinol 153: 49–55
9. **Feldt-Rasmussen U, Rasmussen AK** 2010 Autoimmunity in differentiated thyroid cancer: significance and related clinical problems. Hormones (Athens) 9:109–117
10. **Dailey ME, Lindsay S, Skahen R** 1955 Relation of thyroid neoplasms to Hashimoto disease of the thyroid gland. AMA Arch Surg 70:291–297
11. **Schlicke CP, Hill JE, Schultz GF** 1960 Carcinoma in chronic thyroiditis. Surg Gynecol Obstet 111:552–556
12. **Hirabayashi RN, Lindsay S** 1965 The relation of thyroid carcinoma and chronic thyroiditis. Surg Gynecol Obstet 121:243–252
13. **Ott RA, McCall AR, McHenry C, Jarosz H, Armin A, Lawrence AM, Paloyan E** 1987 The incidence of thyroid carcinoma in Hashimoto's thyroiditis. Am Surg 53:442–445
14. **Kurukahvecioglu O, Taneri F, Yüksel O, Aydin A, Tezel E, Onuk E** 2007 Total thyroidectomy for the treatment of Hashimoto's thyroiditis coexisting with papillary thyroid carcinoma. Adv Ther 24: 510–516
15. **Repplinger D, Bargren A, Zhang YW, Adler JT, Haymart M, Chen H** 2008 Is Hashimoto's thyroiditis a risk factor for papillary thyroid cancer. J Surg Res 150:49–52
16. **Gul K, Dirikoc A, Kiyak G, Ersoy PE, Ugras NS, Ersoy R, Cakir B** 2010 The association between thyroid carcinoma and Hashimoto's thyroiditis: the ultrasonographic and histopathologic characteristics of malignant nodules. Thyroid 20:873–878
17. **Consorti F, Loponte M, Milazzo F, Potasso L, Antonaci A** 2010 Risk of malignancy from thyroid nodular disease as an element of clinical management of patients with Hashimoto's thyroiditis. Eur Surg Res 45:333–337
18. **Wirtschafter A, Schmidt R, Rosen D, Kundu N, Santoro M, Fusco A, Multhaupt H, Atkins JP, Rosen MR, Keane WM, Rothstein JL** 1997 Expression of the RET/PTC fusion gene as a marker for papillary carcinoma in Hashimoto's thyroiditis. Laryngoscope 107: 95–100
19. **Arif S, Blanes A, Diaz-Cano SJ** 2002 Hashimoto's thyroiditis shares features with early papillary thyroid carcinoma. Histopathology 41:357–362
20. **Unger P, Ewart M, Wang BY, Gan L, Kohtz DS, Burstein DE** 2003 Expression of p63 in papillary thyroid carcinoma and in Hashimoto's thyroiditis: a pathobiologic link. Hum Pathol 34:764–769
21. **Rhoden KJ, Unger K, Salvatore G, Yilmaz Y, Vovk V, Chiappetta G, Qumsiyeh MB, Rothstein JL, Fusco A, Santoro M, Zitzelsberger H, Tallini G** 2006 RET/papillary thyroid cancer rearrangement in nonneoplastic thyrocytes: follicular cells of Hashimoto's thyroiditis share low-level recombination events with a subset of papillary carcinoma. J Clin Endocrinol Metab 91:2414–2423
22. **Larson SD, Jackson LN, Riall TS, Uchida T, Thomas RP, Qiu S, Evers BM** 2007 Increased incidence of well-differentiated thyroid cancer associated with Hashimoto thyroiditis and the role of the PI3k/Akt pathway. J Am Coll Surg 204:764–775
23. **Kang DY, Kim KH, Kim JM, Kim SH, Kim JY, Baik HW, Kim YS** 2007 High prevalence of RET, RAS, and ERK expression in Hashimoto's thyroiditis and in papillary thyroid carcinoma in the Korean population. Thyroid 17:1031–1038
24. **Antonaci A, Consorti F, Mardente S, Giovannone G** 2009 Clinical and biological relationship between chronic lymphocytic thyroiditis and papillary thyroid carcinoma. Oncol Res 17:495–503
25. **Muzza M, Degl'Innocenti D, Colombo C, Perrino M, Ravasi E, Rossi S, Cirello V, Beck-Peccoz P, Borrello MG, Fugazzola L** 2010 The tight relationship between papillary thyroid cancer, autoimmunity and inflammation: clinical and molecular studies. Clin Endocrinol (Oxf) 72:702–708
26. **Boelaert K, Horacek J, Holder RL, Watkinson JC, Sheppard MC, Franklyn JA** 2006 Serum thyrotropin concentration as a novel predictor of malignancy in thyroid nodules investigated by fine-needle aspiration. J Clin Endocrinol Metab 91:4295–4301
27. **Haymart MR, Repplinger DJ, Leverson GE, Elson DF, Sippel RS, Jaume JC, Chen H** 2008 Higher serum thyroid stimulating hormone level in thyroid nodule patients is associated with greater risks of differentiated thyroid cancer and advanced tumor stage. J Clin Endocrinol Metab 93:809–814
28. **Kim HS, Choi YJ, Yun JS** 2010 Features of papillary thyroid microcarcinoma in the presence and absence of lymphocytic thyroiditis. Endocr Pathol 21:149–153
29. **Fiore E, Rago T, Latrofa F, Provenzale MA, Piaggi P, Delitala A, Scutari M, Basolo F, Di Coscio G, Grasso L, Pinchera A, Vitti P** 2011 Hashimoto's thyroiditis is associated with papillary thyroid carcinoma: role of TSH and of treatment with L-thyroxine. Endocr Relat Cancer 18:429–437
30. **Kim KW, Park YJ, Kim EH, Park SY, Park do J, Ahn SH, Park do J, Jang HC, Cho BY** 2011 Elevated risk of papillary thyroid cancer in Korean patients with Hashimoto's thyroiditis. Head Neck 33: 691–695
31. **Balkwill F, Mantovani A** 2001 Inflammation and cancer: back to Virchow? Lancet 357:539–545
32. **McLachlan SM, Rapoport B** 2004 Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies. Thyroid 14:510–520
33. **Boi F, Lai ML, Marziani B, Minerba L, Faa G, Mariotti S** 2005 High prevalence of suspicious cytology in thyroid nodules associated with positive thyroid autoantibodies. Eur J Endocrinol 153: 637–642
34. **de Visser KE, Eichten A, Coussens LM** 2006 Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 6:24–37
35. **Guarino V, Castellone MD, Avilla E, Melillo RM** 2010 Thyroid cancer and inflammation. Mol Cell Endocrinol 321:94–102
36. **Rebuffat SA, Morin M, Nguyen B, Castex F, Robert B, Péraldi-Roux S** 2010 Human recombinant anti-thyroperoxidase autoantibodies: in vitro cytotoxic activity on papillary thyroid cancer expressing TPO. Br J Cancer 102:852–861
37. **Mariotti S, Barbesino G, Caturegli P, Marinó M, Manetti L, Pacini F, Centoni R, Pinchera A** 1995 Assay of thyroglobulin in serum

with thyroglobulin autoantibodies: an unobtainable goal? J Clin Endocrinol Metab 80:468–472
38. **Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, LiVosli VA, Niccoli-Sire P, John R, Ruf J, Smyth PP, Spencer CA, Stockigt JR** 2003 Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 13:57–67
39. **Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, LoPresti JS** 2005 Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. J Clin Endocrinol Metab 90:5566–5575
40. **Seo JH, Lee SW, Ahn BC, Lee J** 2010 Recurrence detection in differentiated thyroid cancer patients with elevated serum level of antithyroglobulin antibody: special emphasis on using (18)F-FDG PET/CT. Clin Endocrinol (Oxf) 72:558–563
41. **Kim ES, Lim DJ, Baek KH, Lee JM, Kim MK, Kwon HS, Song KH, Kang MI, Cha BY, Lee KW, Son HY** 2010 Thyroglobulin antibody is associated with increased cancer risk in thyroid nodules. Thyroid 20:885–891
42. **Spencer C, Petrovic I, Fatemi S** 2011 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
43. **Adil A, Jafri RA, Waqar A, Abbasi SA, Matiul-Haq, Asghar AH, Jilani A, Naz I** 2003 Frequency and clinical importance of anti-Tg auto-antibodies (ATG). J Coll Physicians Surg Pak 13:504–506
44. **Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, Grasso L, Pinchera A** 2003 Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. Ann Intern Med 139:346–351
45. **Rosário PW, Maia FF, Fagundes TA, Vasconcelos FP, Cardoso LD, Purisch S** 2004 Antithyroglobulin antibodies in patients with differentiated thyroid carcinoma: methods of detection, interference with serum thyroglobulin measurement and clinical significance. Arq Bras Endocrinol Metabol 48:487–492
46. **Küçük ON, Aras G, Kulak HA, Ibiş E** 2006 Clinical importance of anti-thyroglobulin auto-antibodies in patients with differentiated thyroid carcinoma: comparison with 99mTc-MIBI scans. Nucl Med Commun 27:873–876
47. **Thomas D, Liakos V, Vassiliou E, Hatzimarkou F, Tsatsoulis A, Kaldrimides P** 2007 Possible reasons for different pattern disappearance of thyroglobulin and thyroid peroxidase autoantibodies in patients with differentiated thyroid carcinoma following total thyroidectomy and iodine-131 ablation. J Endocrinol Invest 30:173–180
48. **Kim WG, Yoon JH, Kim WB, Kim TY, Kim EY, Kim JM, Ryu JS, Gong G, Hong SJ, Shong YK** 2008 Change of serum antithyroglobulin antibody levels is useful for prediction of clinical recurrence in thyroglobulin-negative patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 93:4683–4689
49. **Black EG, Hoffenberg R** 1983 Should one measure serum thyroglobulin in the presence of anti-thyroglobulin antibodies? Clin Endocrinol (Oxf) 19:597–601
50. **Stanojevic M, Savin S, Cvejic D, Djukic A, Jeremic M, Zivanceviæ Simonovic S** 2009 Comparison of the influence of thyroglobulin antibodies on serum thyroglobulin values from two different immunoassays in post surgical differentiated thyroid carcinoma patients. J Clin Lab Anal 23:341–346
51. **Schneider AB, Pervos R** 1978 Radioimmunoassay of human thyroglobulin: effect of antithyroglobulin autoantibodies. J Clin Endocrinol Metab 47:126–137
52. **Feldt-Rasmussen U, Rasmussen AK** 1985 Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb in vitro and in vivo. J Endocrinol Invest 8:571–576
53. **Schaadt B, Feldt-Rasmussen U, Rasmusson B, Tørring H, Foder B, Jørgensen K, Hansen HS** 1995 Assessment of the influence of thyroglobulin (Tg) autoantibodies and other interfering factors on the use of serum Tg as tumor marker in differentiated thyroid carcinoma. Thyroid 5:165–170
54. **Spencer CA, Takeuchi M, Kazarosyan M** 1996 Current status and performance goals for serum thyroglobulin assays. Clin Chem 42:164–173
55. **Weightman DR, Mallick UK, Fenwick JD, Perros P** 2003 Discordant serum thyroglobulin results generated by two classes of assay in patients with thyroid carcinoma: correlation with clinical outcome after 3 years of follow-up. Cancer 98:41–47
56. **Okosieme OE, Evans C, Moss L, Parkes AB, Premawardhana LD, Lazarus JH** 2005 Thyroglobulin antibodies in serum of patients with differentiated thyroid cancer: relationship between epitope specificities and thyroglobulin recovery. Clin Chem 51:729–734
57. **La'ulu SL, Slev PR, Roberts WL** 2007 Performance characteristics of 5 automated thyroglobulin autoantibody and thyroid peroxidase autoantibody assays. Clin Chim Acta 376:88–95
58. **Cunha LL, Ferreira RC, Marcello MA, Vassallo J, Ward LS** 2011 Clinical and pathological implications of concurrent autoimmune thyroid disorders and papillary thyroid cancer. J Thyroid Res 2011:387062
59. **Loh KC, Greenspan FS, Dong F, Miller TR, Yeo PP** 1999 Influence of lymphocytic thyroiditis on the prognostic outcome of patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 84:458–463
60. **Singh B, Shaha AR, Trivedi H, Carew JF, Poluri A, Shah JP** 1999 Coexistent Hashimoto's thyroiditis with papillary thyroid carcinoma: impact on presentation, management, and outcome. Surgery 126:1070–1076; discussion 1076–1077
61. **Tamimi DM** 2002 The association between chronic lymphocytic thyroiditis and thyroid tumors. Int J Surg Pathol 10:141–146
62. **Cipolla C, Sandonato L, Graceffa G, Fricano S, Torcivia A, Vieni S, Latteri S, Latteri MA** 2005 Hashimoto thyroiditis coexistent with papillary thyroid carcinoma. Am Surg 71:874–878
63. **Anil C, Goksel S, Gursoy A** 2010 Hashimoto's thyroiditis is not associated with increased risk of thyroid cancer in patients with thyroid nodules: a single-center prospective study. Thyroid 20:601–606
64. **Grebe SK** 2010 Thyroglobulin autoantibodies, thyroid nodules, and new insights into some old questions. Thyroid 20:841–842
65. **Mazokopakis EE, Tzortzinis AA, Dalieraki-Ott EI, Tsartsalis AN, Syros PK, Karefilakis CM, Papadomanolaki MG, Starakis IK** 2010 Coexistence of Hashimoto's thyroiditis with papillary thyroid carcinoma. A retrospective study. Hormones (Athens) 9:312–317
66. **Kebebew E, Treseler PA, Ituarte PH, Clark OH** 2001 Coexisting chronic lymphocytic thyroiditis and papillary thyroid cancer revisited. World J Surg 25:632–637
67. **Fiore E, Rago T, Scutari M, Ugolini C, Proietti A, Di Coscio G, Provenzale MA, Berti P, Grasso L, Mariotti S, Pinchera A, Vitti P** 2009 Papillary thyroid cancer, although strongly associated with lymphocytic infiltration on histology, is only weakly predicted by serum thyroid auto-antibodies in patients with nodular thyroid diseases. J Endocrinol Invest 32:344–351
68. **Tozzoli R, Bizzaro N, Tonutti E, Pradella M, Manoni F, Vilalta D, Bassetti D, Piazza A, Rizzotti P** 2002 Immunoassay of anti-thyroid autoantibodies: high analytical variability in second generation methods. Clin Chem Lab Med 40:568–573
69. **Krahn J, Dembinski T** 2009 Thyroglobulin and anti-thyroglobulin assays in thyroid cancer monitoring. Clin Biochem 42:416–419
70. **Kashima K, Yokoyama S, Noguchi S, Murakami N, Yamashita H, Watanabe S, Uchino S, Toda M, Sasaki A, Daa T, Nakayama I** 1998 Chronic thyroiditis as a favorable prognostic factor in papillary thyroid carcinoma. Thyroid 8:197–202
71. **Ugolini C, Basolo F, Proietti A, Vitti P, Elisei R, Miccoli P, Toniolo A** 2007 Lymphocyte and immature dendritic cell infiltrates in differentiated, poorly differentiated, and undifferentiated thyroid carcinoma. Thyroid 17:389–393

72. **Segal K, Ben-Bassat M, Avraham A, Har-El G, Sidi J** 1985 Hashimoto's thyroiditis and carcinoma of the thyroid gland. Int Surg 70:205–209
73. **Matsubayashi S, Kawai K, Matsumoto Y, Mukuta T, Morita T, Hirai K, Matsuzuka F, Kakudoh K, Kuma K, Tamai H** 1995 The correlation between papillary thyroid carcinoma and lymphocytic infiltration in the thyroid gland. J Clin Endocrinol Metab 80:3421–3424
74. **Ozaki O, Ito K, Mimura T, Sugino K, Hosoda Y** 1996 Papillary carcinoma of the thyroid. Tall-cell variant with extensive lymphocyte infiltration. Am J Surg Pathol 20:695–698
75. **Schäffler A, Palitzsch KD, Seiffarth C, Höhne HM, Riedhammer FJ, Hofstädter F, Schölmerich J, Rüschoff J** 1998 Coexistent thyroiditis is associated with lower tumour stage in thyroid carcinoma. Eur J Clin Invest 28:838–844
76. **Gupta S, Patel A, Folstad A, Fenton C, Dinauer CA, Tuttle RM, Conran R, Francis GL** 2001 Infiltration of differentiated thyroid carcinoma by proliferating lymphocytes is associated with improved disease-free survival for children and young adults. J Clin Endocrinol Metab 86:1346–1354
77. **Kim EY, Kim WG, Kim WB, Kim TY, Kim JM, Ryu JS, Hong SJ, Gong G, Shong YK** 2009 Coexistence of chronic lymphocytic thyroiditis is associated with lower recurrence rates in patients with papillary thyroid carcinoma. Clin Endocrinol (Oxf) 71:581–586
78. **Kim KH, Suh KS, Kang DW, Kang DY** 2005 Mutations of the BRAF gene in papillary thyroid carcinoma and in Hashimoto's thyroiditis. Pathol Int 55:540–545
79. **Sargent R, LiVolsi V, Murphy J, Mantha G, Hunt JL** 2006 BRAF mutation is unusual in chronic lymphocytic thyroiditis-associated papillary thyroid carcinomas and absent in non-neoplastic nuclear atypia of thyroiditis. Endocr Pathol 17:235–241
80. **Kim SK, Song KH, Lim SD, Lim YC, Yoo YB, Kim JS, Hwang TS** 2009 Clinical and pathological features and the $BRAF^{(V600E)}$ mutation in patients with papillary thyroid carcinoma with and without concurrent Hashimoto thyroiditis. Thyroid 19:137–141
81. **Basolo F, Torregrossa L, Giannini R, Miccoli M, Lupi C, Sensi E, Berti P, Elisei R, Vitti P, Baggiani A, Miccoli P** 2010 Correlation between the $BRAF^{V600E}$ mutation and tumor invasiveness in papillary thyroid carcinomas smaller than 20 millimeters: analysis of 1060 cases. J Clin Endocrinol Metab 95:4197–4205
82. **Asanuma K, Sugenoya A, Kasuga Y, Itoh N, Kobayashi S, Amano J** 1998 The relationship between multiple intrathyroidal involvement in papillary thyroid carcinoma and chronic non-specific thyroiditis. Cancer Lett 122:177–180
83. **Hjiyiannakis P, Mundy J, Harmer C** 1999 Thyroglobulin antibodies in differentiated thyroid cancer. Clin Oncol (R Coll Radiol) 11:240–244
84. **Nordyke RA, Gilbert Jr FI, Miyamoto LA, Fleury KA** 1993 The superiority of antimicrosomal over antithyroglobulin antibodies for detecting Hashimoto's thyroiditis. Arch Intern Med 153:862–865
85. **Chen CR, Hamidi S, Braley-Mullen H, Nagayama Y, Bresee C, Aliesky HA, Rapoport B, McLachlan SM** 2010 Antibodies to thyroid peroxidase arise spontaneously with age in NOD.H-2h4 mice and appear after thyroglobulin antibodies. Endocrinology 151:4583–4593
86. **Latrofa F, Ricci D, Grasso L, Vitti P, Masserini L, Basolo F, Ugolini C, Mascia G, Lucacchini A, Pinchera A** 2008 Characterization of thyroglobulin epitopes in patients with autoimmune and non-autoimmune thyroid diseases using recombinant human monoclonal thyroglobulin autoantibodies. J Clin Endocrinol Metab 93:591–596
87. **Heilig B, Hüfner M, Dörken B, Schmidt-Gayk H** 1986 Increased heterogeneity of serum thyroglobulin in thyroid cancer patients as determined by monoclonal antibodies. Klin Wochenschr 64:776–780
88. **Magro G, Perissinotto D, Schiappacassi M, Goletz S, Otto A, Müller EC, Bisceglia M, Brown G, Ellis T, Grasso S, Colombatti A, Perris R** 2003 Proteomic and postproteomic characterization of keratan sulfate-glycanated isoforms of thyroglobulin and transferrin uniquely elaborated by papillary thyroid carcinomas. Am J Pathol 163:183–196
89. **Cubero JM, Rodríguez-Espinosa J, Gelpi C, Estorch M, Corcoy R** 2003 Thyroglobulin autoantibody levels below the cut-off for positivity can interfere with thyroglobulin measurement. Thyroid 13:659–661
90. **Weetman AP, McGregor AM** 1994 Autoimmune thyroid disease: further developments in our understanding. Endocr Rev 15:788–830
91. **Luboshitzky R, Lavi I, Ishay A** 2006 Serum thyroglobulin levels after fine-needle aspiration of thyroid nodules. Endocr Pract 12:264–269
92. **Polyzos SA, Anastasilakis AD** 2010 Alterations in serum thyroid-related constituents after thyroid fine-needle biopsy: a systematic review. Thyroid 20:265–271
93. **Feldt-Rasmussen U, Petersen PH, Date J, Madsen CM** 1980 Sequential changes in serum thyroglobulin (Tg) and its autoantibodies (TgAb) following subtotal thyroidectomy of patients with preoperatively detectable TgAb. Clin Endocrinol (Oxf) 12:29–38
94. **Bech K, Feldt-Rasmussen U, Bliddal H, Date J, Blichert-Toft M** 1982 The acute changes in thyroid stimulating immunoglobulins, thyroglobulin and thyroglobulin antibodies following subtotal thyroidectomy. Clin Endocrinol (Oxf) 16:235–242
95. **Feldt-Rasmussen U** 1983 Serum thyroglobulin and thyroglobulin autoantibodies in thyroid diseases. Pathogenetic and diagnostic aspects. Allergy 38:369–387
96. **Weigle WO, High GJ** 1967 The behaviour of autologous thyroglobulin in the circulation of rabbits immunized with either heterologous or altered homologous thyroglobulin. J Immunol 98:1105–1114
97. **Feldt-Rasmussen U, Blichert-Toft M, Christiansen C, Date J** 1982 Serum thyroglobulin and its autoantibody following subtotal thyroid resection of Graves' disease. Eur J Clin Invest 12:203–208
98. **Izumi M, Larsen PR** 1978 Correlation of sequential changes in serum thyroglobulin, triiodothyronine, and thyroxine in patients with Graves' disease and subacute thyroiditis. Metabolism 27:449–460
99. **Uller RP, Van Herle AJ** 1978 Effect of therapy on serum thyroglobulin levels in patients with Graves' disease. J Clin Endocrinol Metab 46:747–755
100. **Feldt-Rasmussen U, Bech K, Date J, Petersen PH, Johansen K** 1982 A prospective study of the differential changes in serum thyroglobulin and its autoantibodies during propylthiouracil or radioiodine therapy of patients with Graves' disease. Acta Endocrinol (Copenh) 99:379–385
101. **Rubio IG, Silva MN, Knobel M, Romão R, Possato R, Gebrin EM, Buchpiguel C, Medeiros-Neto G** 2007 Peripheral blood levels of thyroglobulin mRNA and serum thyroglobulin concentrations after radioiodine ablation of multinodular goiter with or without pre-treatment with recombinant human thyrotropin. J Endocrinol Invest 30:535–540
102. **Benvenga S, Bartolone L, Squadrito S, Trimarchi F** 1997 Thyroid hormone autoantibodies elicited by diagnostic fine needle biopsy. J Clin Endocrinol Metab 82:4217–4223
103. **Pacini F, Schlumberger M, Harmer C, Berg GG, Cohen O, Duntas L, Jamar F, Jarzab B, Limbert E, Lind P, Reiners C, Sanchez Franco F, Smit J, Wiersinga W** 2005 Post-surgical use of radioiodine (131I) in patients with papillary and follicular thyroid cancer and the issue of remnant ablation: a consensus report. Eur J Endocrinol 153:651–659
104. **Marquet PY, Daver A, Sapin R, Bridgi B, Muratet JP, Hartmann DJ, Paolucci F, Pau B** 1996 Highly sensitive immunoradiometric assay for serum thyroglobulin with minimal interference from autoantibodies. Clin Chem 42:258–262
105. **Ruf J, Carayon P, Lissitzky S** 1985 Various expressions of a unique

anti-human thyroglobulin antibody repertoire in normal state and autoimmune disease. Eur J Immunol 15:268–272
106. **Bayer MF, Kriss JP** 1979 Immunoradiometric assay for serum thyroglobulin: semiquantitative measurement of thyroglobulin in antithyroglobulin-positive sera. J Clin Endocrinol Metab 49:557–564
107. **Henniker AJ, Luttrell BM, Ward P, Stiel JN, Wilmshurst EG** 1983 The measurement of antithyroglobulin autoantibodies in the presence of thyroglobulin. J Endocrinol Invest 6:277–285
108. **Feldt-Rasmussen U, Bech K, Date J** 1979 Serum thyroglobulin in patients with toxic and non-toxic goitres compared to sex- and age-matched control subjects. Acta Endocrinol (Copenh) 91:264–270
109. **Gardner DF, Rothman J, Utiger RD** 1979 Serum thyroglobulin in normal subjects and patients with hyperthyroidism due to Graves' disease: effects of T3, iodide, 131I and antithyroid drugs. Clin Endocrinol (Oxf) 11:585–594
110. **Prentice L, Kiso Y, Fukuma N, Horimoto M, Petersen V, Grennan F, Pegg C, Furmaniak J, Rees Smith B** 1995 Monoclonal thyroglobulin autoantibodies: variable region analysis and epitope recognition. J Clin Endocrinol Metab 80:977–986
111. **Schulz R, Bethäuser H, Stempka L, Heilig B, Moll A, Hüfner M** 1989 Evidence for immunological differences between circulating and tissue-derived thyroglobulin in men. Eur J Clin Invest 19:459–463
112. **Feldt-Rasmussen U, Profilis C, Colinet E, Black E, Bornet H, Bourdoux P, Carayon P, Ericsson UB, Koutras DA, Lamas de Leon L, DeNayer P, Pacini F, Palumbo G, Santos A, Schlumberger M, Seidel C, Van Herle AJ, DeVijlder JJ** 1996 Human thyroglobulin reference material (CRM 457) 2nd part: physicochemical characterization and certification. Ann Biol Clin (Paris) 54:343–348
113. **Ito N, Yokota M, Kawahara S, Nagaike C, Morimura Y, Hirota T, Matsunaga T** 1995 Histochemical demonstration of different types of poly-N-acetyllactosamine structures in human thyroid neoplasms using lectins and endo-β-galactosidase digestion. Histochem J 27:620–629
114. **Shimizu K, Nakamura K, Kobatake S, Satomura S, Maruyama M, Tajiri J, Kato R** 2007 Discrimination of thyroglobulin from thyroid carcinoma tissue and that from benign thyroid tissues with use of competitive assay between lectin and anti-thyroglobulin antibody. Rinsho Byori 55:428–433
115. **Spencer CA, LoPresti JS, Fatemi S, Nicoloff JT** 1999 Detection of residual and recurrent differentiated thyroid carcinoma (DTC) by serum thyroglobulin (Tg) measurement. Thyroid 9:435–441
116. **McLachlan SM, Feldt-Rasmussen U, Young ET, Middleton SL, Dlichert-Toft M, Siersboek-Nielsen K, Date J, Carr D, Clark F, Rees Smith B** 1987 IgG subclass distribution of thyroid autoantibodies: a 'fingerprint' of an individual's response to thyroglobulin and thyroid microsomal antigen. Clin Endocrinol (Oxf) 26:335–346
117. **Benvenga S, Burek CL, Talor M, Rose NR, Trimarchi F** 2002 Heterogeneity of the thyroglobulin epitopes associated with circulating thyroid hormone autoantibodies in Hashimoto's thyroiditis and non-autoimmune thyroid diseases. J Endocrinol Invest 25:977–982
118. **Hoofnagle AN, Becker JO, Wener MH, Heinecke JW** 2008 Quantification of thyroglobulin, a low-abundance serum protein, by immunoaffinity peptide enrichment and tandem mass spectrometry. Clin Chem 54:1796–1804
119. **Kohno Y, Tarutani O, Sakata S, Nakajima H** 1985 Monoclonal antibodies to thyroglobulin elucidate differences in protein structure of thyroglobulin in healthy individuals and those with papillary adenocarcinoma. J Clin Endocrinol Metab 61:343–350
120. **Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W** 2006 European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol 154:787–803
121. **Sapin R, d'Herbomez M, Gasser F, Meyer L, Schlienger JL** 2003 Increased sensitivity of a new assay for anti-thyroglobulin antibody detection in patients with autoimmune thyroid disease. Clin Biochem 36:611–616
122. **Haapala AM, Soppi E, Mörsky P, Salmi J, Laine S, Mattila J** 1995 Thyroid antibodies in association with thyroid malignancy II: qualitative properties of thyroglobulin antibodies. Scand J Clin Lab Invest 55:317–322
123. **Gao Y, Yuan Z, Yu Y, Lu H** 2007 Mutual interference between serum thyroglobulin and antithyroglobulin antibody in an automated chemiluminescent immunoassay. Clin Biochem 40:735–738
124. **de Micco C, Ruf J, Carayon P, Chrestian MA, Henry JF, Toga M** 1987 Immunohistochemical study of thyroglobulin in thyroid carcinomas with monoclonal antibodies. Cancer 59:471–476
125. **Kim PS, Dunn AD, Dunn JT** 1988 Altered immunoreactivity of thyroglobulin in thyroid disease. J Clin Endocrinol Metab 67:161–168
126. **Ratcliffe JG, Ayoub LA, Pearson D** 1981 The measurement of serum thyroglobulin in the presence of thyroglobulin antibodies. Clin Endocrinol (Oxf) 15:507–518