Thyroglobulin

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Abbreviations

CRM-457	Certified reference material
CV	Coefficient of variation
DTC	Differentiated thyroid cancer
FNA	Fine needle aspiration
FNAB	Fine needle aspiration biopsy
FS	Functional sensitivity
HAb	Heterophile antibody
HAMA	Human anti-mouse antibody
hCG	Human chorionic gonadotropin
IMA	Immunometric assay
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LOQ	Limit of quantitation
L-T4	Levothyroxine
Lx	Lobectomy
MAb	Monoclonal antibody
MCO	Manufacturer cutoff
NPV	Negative predictive value
PAb	Polyclonal antibody
PPV	Positive predictive value
PTC	Papillary thyroid cancer
PTH	Parathyroid hormone
RAI	Radioiodine
RF	Rheumatoid factor
rhTSH	Recombinant human TSH
RIA	Radioimmunoassay
Tg	Thyroglobulin
TgAb	Thyroglobulin autoantibodies
TRAb	TSH receptor antibodies
TSH	Thyroid stimulating hormone

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Thyroglobulin (Tg) Biosynthesis and Clearance

The Tg gene has been mapped to human chromosome 8q24.2–8q24.3 [1]. Tg genetic variants are not uncommon and likely play a role in the pathogenesis of autoimmune thyroid diseases [2–6], and some may be associated with increased risk for DTC [2, 3, 7–9]. Translation of the 8.7 kb mRNA transcript to form the initial 330 kDa monomeric protein is regulated by TSH, as well as the thyroid-specific transcription factors, TTF-1, TTF-2 and Pax8 [10–13]. Post-translational processing of the Tg transcript is complex, involving homodimerization and site-directed glycosylation, sulfation and folding in the Golgi complex to produce the mature 660 kDa dimeric Tg protein which undergoes chaperone-controlled transportation to the follicular lumen where hormonogenetic iodination of tyrosine residues occurs [13, 14]. Only mature Tg molecules that have an appropriate conformation can be

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trafficked to the apical membrane for iodination [10, 12–16]. TSH stimulates the endocytosis of iodinated Tg from the follicular lumen before lysozomal proteolysis that releases thyroid hormones along with some undigested Tg protein into the circulation [13] (Fig. 15.1).

Critical steps involved in the post-translational modifications necessary to form mature Tg molecules may become dysregulated in thyroid tumors, resulting in the secretion of Tg molecules with abnormal carbohydrate [17, 18], sulfate [19–21] and/or iodine [22–24] composition. Since Tg epitopes are conformational [25, 26], any abnormalities in Tg sequence, tertiary structure, carbohydrate, iodine or sulfate composition, have the potential to alter the immunoreactivity of the molecule [27–34]. This Tg molecular heterogeneity also has the potential to prevent the generation of the proteotypic Tg tryptic peptide(s) necessary for LC-MS/MS detection [13, 35–39]. Heterogeneity of circulating Tg [27–32, 34], as well as differences between circulating Tg and

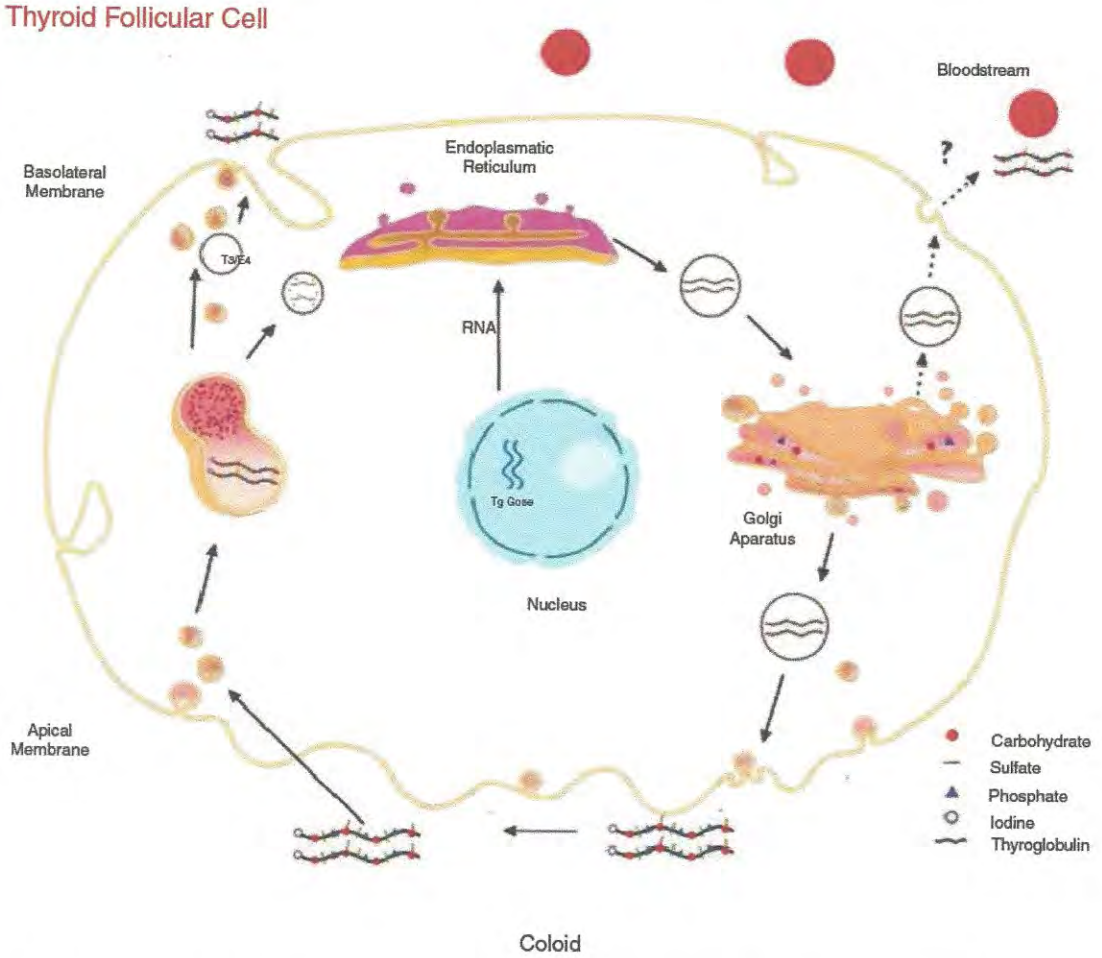

Fig. 15.1 Schematic model of synthesis and posttranslational modifications of thyroglobulin (Tg) in the follicular cell. The two 330-kDa polypeptide chains, linked by disulfide bonds, are synthesized in the endoplasmic reticulum from mRNA transcribed from the Tg gene, located on chromosome 8. Posttranslational modifications (glycation, sulfation, and phosphorylation) take place in the Golgi apparatus. Tg is then secreted into the colloid, where iodination occurs to form the thyroid hormone precursors MIT and DIT. Iodinated Tg enters the follicular cell cytoplasm by pinocytosis and combines with lysosomal vesicles containing proteolytic enzymes which lyse Tg and release the thyroid hormones into the bloodstream. Part of the remaining material is re-used by the cell from [13] with permission

the glandular Tg preparation (CRM-457) used for assay standardization [40], are reasons why up to a twofold difference in serum Tg values may be reported for the same specimen measured by different methods, even when TgAb is absent (Fig. 15.2) [30, 31, 41, 42].

As with other glycoproteins, Tg is primarily cleared from the circulation by the hepatic asialoglycoprotein receptor (ASGPR) [43–46] with a half-life approximating three days [47, 48]. Receptor-mediated clearance may be influenced by both the iodine and sialic acid composition of the Tg molecule [43, 46]. Since both iodine and sialic acid content tends to be low in the papillary morphotype (PTC), Tg molecules secreted by papillary tumors may have accelerated metabolic clearance that could lead to a disproportionally lower serum Tg relative to tumor burden [17, 20, 22, 48–54]. Indeed, preoperative serum Tg concentrations tend to be lower in papillary thyroid cancers (PTC), compared with Follicular or Hurthle Cell neoplasms [55, 56]. Tg metabolic clearance may also be altered by the presence of TgAb. Specifically, both animal and human

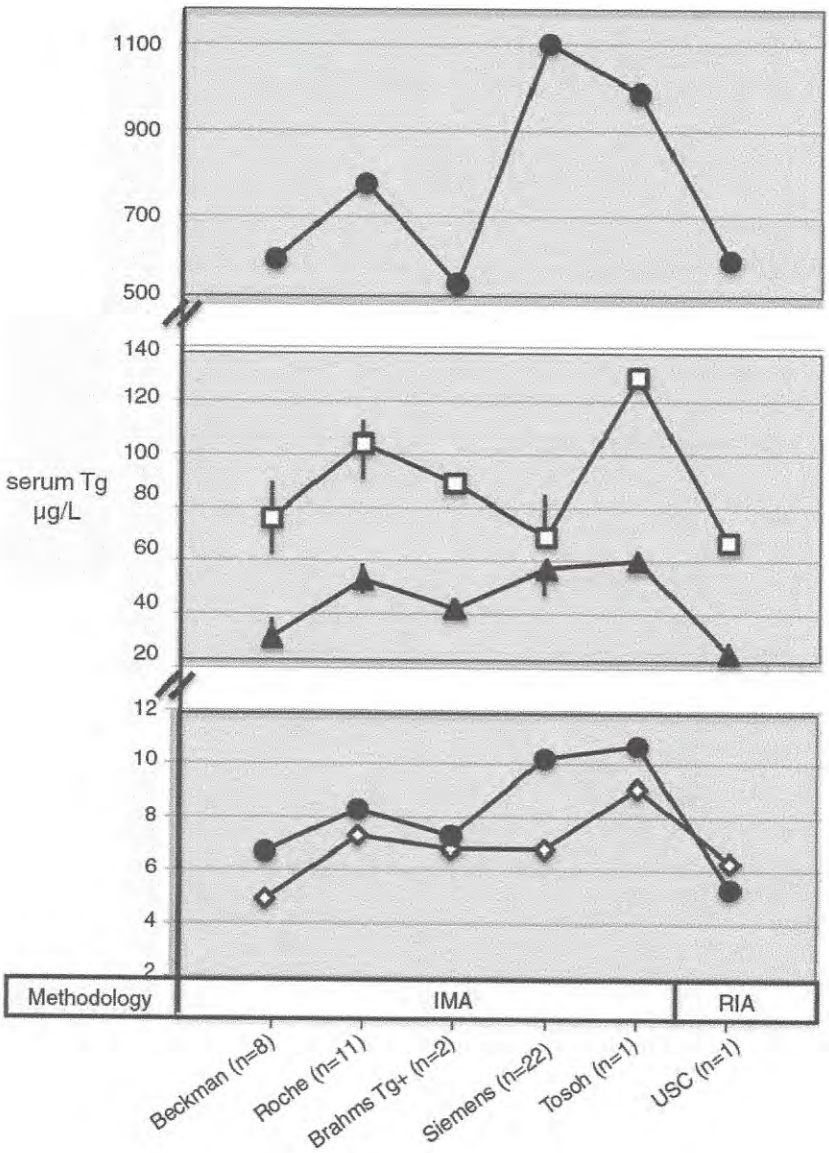

Fig. 15.2 2015–2016 Tg method comparison data from the United Kingdom National External Quality Assessment Service for Thyroglobulin Surveys. Five different TgAb-negative sera were measured by ~50 different laboratories. The percent confidence limits are shown for the three methods with sufficient participants. The data is shown with permission

studies suggest that TgAb complexing of Tg may enhance Tg metabolic clearance, perhaps because TgAb acts as a "sweeping antibody" for the hepatic ASGPR receptor to remove Tg-TgAb complexes from the circulation [46, 49, 57, 58]. Consistent with enhanced TgAb-mediated Tg clearance is the observation that TgAb-positive DTC patients with structural disease have lower serum Tg (irrespective of whether IMA, RIA or LC-MS/MS methodology is used), as compared with a comparable group of patients with structural disease and absent TgAb (Fig. 15.3b) [59]. TgAb concentrations are known to rise as disease progresses, such that the trend in TgAb can be used as a surrogate tumor-marker [25, 60–76]. It is important to investigate whether TgAb facilitates the clearance of Tg-TgAb complexes because as disease progresses and TgAb rises, a faster clearance of Tg-TgAb complexes would result in a paradoxical fall in serum Tg concentration, rendering serum Tg a misleading tumor-marker irrespective of the class of Tg method used.

Tg Measurement: Technical Issues

Tg methodology has evolved over four decades from RIA (1970s–present) to IMA (1980s–present) and most recently LC-MS/MS (2008–present). Methodological developments have been spurred by a quest for higher Tg assay sensitivity and freedom from TgAb interference. A comparison of the different classes of Tg method is shown

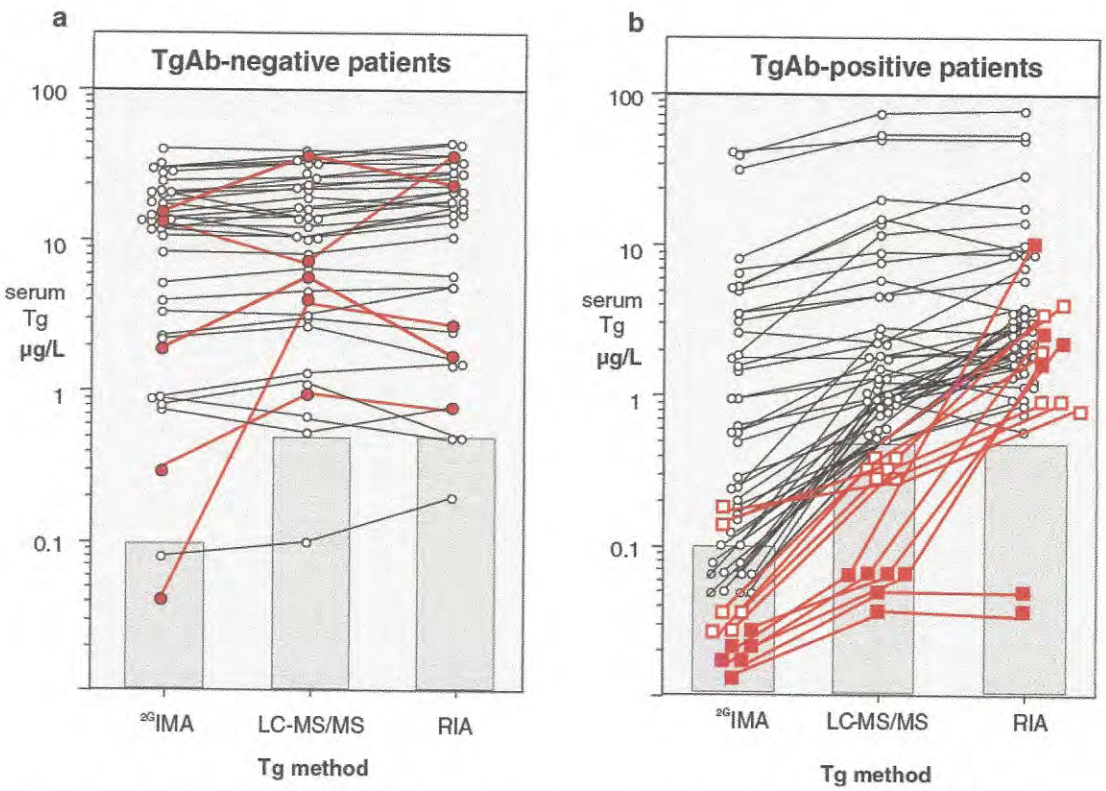

Fig. 15.3 Serum Tg was measured by the ^{2G}Tg-IMA (Beckman), Tg-LC-MS/MS (Mayo Medical Labs) and Tg-RIA (University of Southern California) methods. The functional sensitivities of the methods are shown by dark bars. (a) 37 sera from TgAb-negative DTC patients with structural disease. The sera shown in red had a >30% between-method differences. (b) Shows the method comparison for 52 TgAb-positive DTC patients with structural disease. Sera with unequivocally undetectable Tg-LC-MS/MS values (no peak) are shown by solid red squares, whereas sera with marginally detectable Tg-LC-MS/MS values (0.3–0.5 µg/L range) are shown by open red squares. Data from [59]

Table 15.1 Classes of Tg method

Method class	Principle	Turn-around time	• Functional sensitivity (FS) • Strengths/pitfalls
Radioimmunoassay (RIA) 1973–present	Competitive/ isotopic-serum Tg and ^{125}I-labeled Tg compete for a limited quantity of polyclonal (rabbit) antibody (PAb)	Up to 6 days (cannot be fully automated)	• FS ~0.5 μg/L • PAbs have broad epitope specificities for detecting heterogeneous tumor Tgs • No HAb/HAMA[a] interferences • Resists TgAb interference
Immunometric assay (IMA) 1990–present	Non-competitive/ non-isotopic-serum Tg is first "captured" by a solid-phase monoclonal Ab (MAb) before detection by a different liquid-phase labeled MAb	Hours (can be automated)	• FS ~0.1 μg/L • MAbs have limited epitope specificities to detect heterogeneous tumor Tgs • HAb/HAMA[a] interferes (false highs) • TgAb interferes (false lows)
Liquid chromatography Tandem mass spectrometry (LC-MS/MS) 2009–present	Specimens may be concentrated and/or reduced, alkylated and digested with trypsin before target peptides are immunoaffinity enriched prior to detection by LC/MS/MS	? 1–2 days (specimen preparation difficult to automate)	• FS ~0.5 μg/L • No HAb/HAMA[a] interference • Clinically insensitive when TgAb is present • Polymorphic tumor Tg may fail to yield target peptides

[a]*HAb/HAMA* heterophile antibodies/human anti-mouse antibodies

in Table 15.1. The three classes of method have intrinsic differences in functional sensitivity (FS), specificity for detecting Tg heterogeneous serum isoforms and propensity for interference by both heterophile antibodies (HAb) and Tg autoantibodies (TgAb). Currently, most Tg testing is made using automated, IMA methods. However, because the IMA class of method is especially prone to TgAb interference, some laboratories first establish the TgAb status of the specimen (negative or positive) in order to reflex TgAb-positive sera for Tg testing by RIA or LC-MS/MS—methodologies believed to be less affected by TgAb.

Functional Sensitivity (FS)

Functional Sensitivity represents the lowest analyte concentration that can be reliably detected under conditions used in clinical practice. For Tg assays, FS is defined as the lowest Tg concentration that can be measured in human serum with 20% coefficient of variation (CV) in runs made over a 6–12 month period, using at least two different lots of reagents and two instrument calibrations [41, 65, 76–78]. Such stipulations are necessary because assay precision erodes over the long clinical intervals (6–12 months) typical for DTC monitoring, due to a myriad of variables that include changes in reagent lots [79–81]. Functional Sensitivity is a more clinically relevant indicator of Tg assay sensitivity than a limit of quantitation calculation (LOQ = 20% CV), because LOQ does not stipulate a DTC-relevant time-span for assessing precision [65, 82–85]. Another stipulation of the FS protocol [65] is that because instruments and methods are matrix-sensitive [83] precision should be assessed in the relevant test matrix (human serum) in preference to a commercial quality control material. Thus, since Tg-IMA testing is typically restricted to TgAb-negative sera, IMA precision estimates should be made in TgAb-negative human serum pools [83]. In contrast, Tg-RIA and Tg-LC-MS/MS testing is typically reserved for sera containing

TgAb, necessitating precision assessment in TgAb-positive human serum pools. Improvements in the FS of Tg methods over time has led to the adoption of a generational approach to Tg assay nomenclature, analogous to TSH [86, 87]. Tg assays with first-generation functional sensitivity (FS = 0.5–1.0 μg/L) include some IMAs, all RIAs and all LC-MS/MS methods [41, 74, 88–90]. Because first-generation assays are too insensitive to distinguish a subnormal post-thyroidectomy Tg level from the serum Tg typical of patients with an intact thyroid gland (~2–40 μg/L), first-generation assays have been typically used in conjunction with recombinant human TSH (rhTSH) stimulation [91–96]. Over the last 10 years second-generation Tg IMAs (^{2G}Tg-IMA), characterized by an order of magnitude greater functional sensitivity (FS = ≤0.10 μg/L) have become available and are now the standard of care [76]. This is because in the absence of TgAb, ^{2G}Tg-IMA has sufficient FS to monitor post-thyroidectomy subnormal basal Tg concentrations without the need for rhTSH stimulation [41, 59, 74, 76, 89, 90, 93, 97–111].

Specificity/Between-Method Tg Differences

Although most Tg methods claim standardization against the Certified Reference Preparation CRM-457 [80, 112, 113], Fig. 15.2 shows that there can be up to a twofold difference in the numeric Tg values reported for the same serum specimen when measured by different methods, even when methods claim CRM-457 standardization and TgAb is absent. The 95% confidence intervals of measurements made by laboratories using the same method shown in Fig. 15.2 indicate that this Tg variability reflects differences in method specificities for detecting heterogeneous serum Tg isoforms [30, 31, 37, 41, 42, 65, 74, 89, 114, 115]. These between-method biases far exceed the Tg biologic variability for euthyroid subjects (~16%) [79, 116]. Although some between-method variability arises from zero-matrix differences and differences between the secondary Tg standard and the CRM-457 reference, the major factor contributing to between-method variability is the heterogeneity of Tg in sera [31, 41, 89, 117–120]. This is especially the case for tumor-derived Tg that may be heterogeneous with respect to carbohydrate [17, 18], sulfate [19] and iodine [22, 23] composition, resulting in abnormal tertiary Tg molecular structures that may have altered immunoreactivity [31, 33, 41, 89, 117, 118]. Thus, because Tg epitopes are conformational [25, 26] abnormal Tgs may be detected by immunoassays differently [31, 89, 117, 118, 121]. IMA methods are especially sensitive to Tg molecular heterogeneity, because each method uses a different monoclonal antibody (MAb) pair to detect Tg in the serum sample, and in general MAbs have narrower epitope specificities for detecting abnormal Tgs than the polyclonal antibodies used for RIA methods [28, 29, 31, 80, 89, 118]. Tumor-related Tg heterogeneity is evident in Fig. 15.3a from the number of TgAb-negative patients with structural disease who displayed a greater than 30% difference in serum Tg when measured by ^{2G}IMA, LC-MS/MS and RIA [59]. These between-method biases contrast with the ~10% between-run precision (over 6–12 months) expected when using the same ^{2G}Tg-IMA consistently. The magnitude of between-method differences shown in Figs. 15.2 and 15.3 have the potential to disrupt serial Tg monitoring and negatively impact clinical management should the Tg method be changed without re-baselining the patient [41, 65, 84, 89]. Current guidelines recognize Tg between-method variability and recommend that the same Tg method (and preferably the same laboratory) be used for serial Tg monitoring of DTC patients [76]. Tg molecular heterogeneity may also impact the reliability of LC-MS/MS measurements, because tumors display a higher frequency of Tg polymorphisms than normal tissue [13, 39]. There can be a failure to generate the proteotypic Tg peptide necessary for LC-MS/MS detection either as a result of such Tg polymorphisms, or because post-translational modifications change the mass or charge of tryptic fragments [2, 8, 10, 13, 39]. Since TgAb interferes with different Tg methods to differing

extents, an additional cause of between-method variability can be a failure to detect interfering TgAb (Fig. 15.4) [37, 122, 123].

Interferences with Tg Measurement

Only the physician can suspect interference with a test result and request that the laboratory perform interference checks! This is because the hallmark of interference is discordance between the test result and the clinical presentation of the patient—information not usually available to the laboratory. Failure to recognize interferences can have adverse clinical consequences [124–130]. Laboratory checks for interference include showing discordance between different manufacturers methods [131–134], re-measurement of analyte after adding reagents to block heterophile antibody (HAb) interferences [93, 134–137], performing linearity studies or precipitating immunoglobulins with polyethylene glycol (PEG) [119, 125, 131, 132, 134, 135, 138, 139]. A change in analyte concentration in response to one of these maneuvers suggests interference, but a lack of effect does not rule out interference. Interferences with Tg measurements can be classified as either non-specific or Tg-specific [140, 141].

Non-Specific Interferences

Heterophile Antibodies (HAb)

HAbs, including Human Anti-Mouse Antibodies (HAMA) and Rheumatoid Factor (RF), are human poly-specific antibodies that target animal antigens [134, 141–143] and interfere with a broad range of tests that use non-competitive IMA methodology [131, 139, 141, 144–148]. HAbs have the potential to interfere with both Tg-IMAs [70, 93, 136, 148–153] and TgAb-IMAs [154]. Typically, HAb interference causes a falsely high Tg-IMA and/or TgAb-IMA, less commonly falsely low Tg-IMA values have been reported [151]. HAbs do not affect either RIA [93, 155] or Tg-LC-MS/MS methodologies

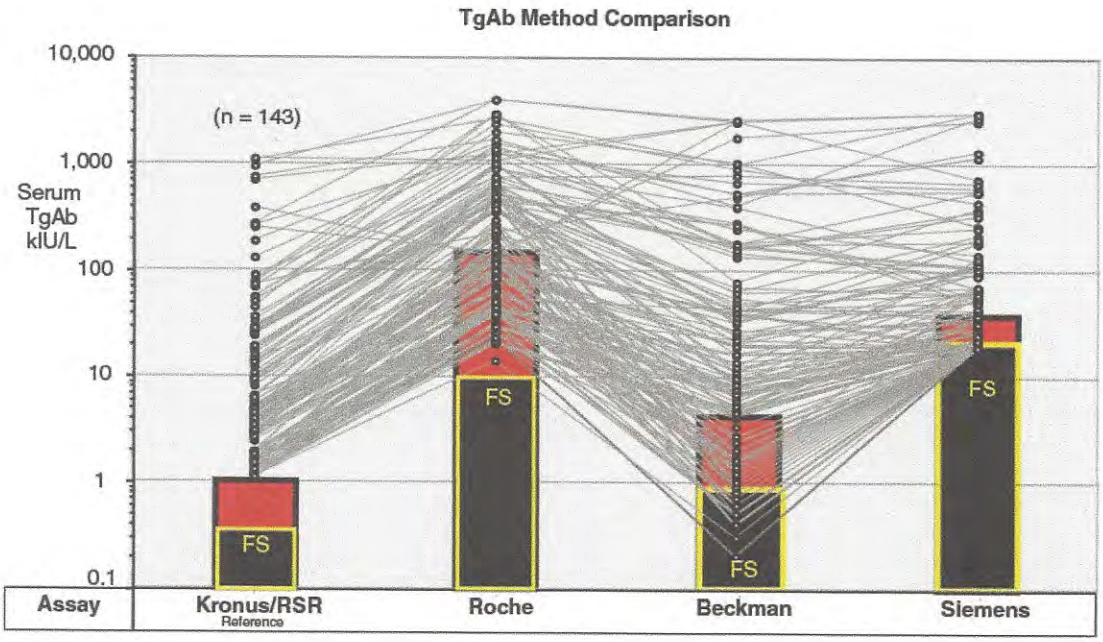

Fig. 15.4 Comparison of 143 DTC sera measured by four TgAb different methods—Kronus/RSR radioassay (reference method) versus Roche, Beckman and Siemens automated IMA tests. Red bars show the manufacturer recommended cutoffs (MCO) for TgAb-positivity of each method. Black bars denote the functional sensitivity limit of each method. All sera had TgAb above the MCO of the reference method and evidence of TgAb interference (a low ^{2G}Tg-IMA/Tg-RIA ratio). Data taken from [123]

[153]. Despite an overall HAb prevalence averaging 30–40% [155–157] manufacturers have reduced HAb interference to less than 1% by adding immunoglobulin blocker reagents to their IMA tests [93, 138, 146, 152]. However, the affinity and specificity of HAbs varies among patients and severe interference may be seen when using one manufacturer's test whereas a different manufacturer's test appears unaffected when measuring the same serum specimen. This is why the first step for investigating interference is re-measurement of the specimen with a different manufacturers method. It should be noted that patients receiving recent vaccines, blood transfusions or monoclonal antibodies (for treatment or scintigraphy), as well as veterinarians and those coming into contact with animals, are especially prone to interferences caused by induced HAbs [138, 158].

Reagent Interferences

Interference can result from antibodies targeting assay reagents. In the case of assays employing Streptavidin or Biotin methodology there can be interference from either Streptavidin [159] and/or Biotin antibodies [160]. Alternatively, exogenous high dose biotin ingestion can produce test interference in an analyte-specific, platform-specific manner [161–166].

Specific Interference

TgAb Interference

Approximately 25% of DTC patients have TgAb detected pre-operatively, or within 3 months of surgery [70, 123, 167–169]—a prevalence that is approximately double that of the general population [170]. TgAb prevalence is higher for papillary versus follicular tumors, and frequently associated with lymph node metastases [68, 70, 168, 171]. TgAb interference with Tg measurement remains the major problem limiting the clinical utility of Tg testing using any class of method. Either in-vitro mechanisms (epitope masking) [62, 89, 169, 172, 173] and/or in-vivo mechanisms (enhanced TgAb-mediated Tg clearance) could be responsible for these interferences [46, 49, 57, 58]. The propensity for TgAb interference differs between classes of Tg method (Table 15.1), as well as between methods from the same methodologic class. This in part reflects qualitative differences in the TgAb epitope specificities expressed by normal individuals versus patients with DTC, either associated with or without thyroid autoimmunity [171, 174–176]. It is clear that these patient-specific differences in TgAb specificities are maintained despite changing TgAb concentrations (Fig. 15.5). These specificity differences impact methodologic sensitivity as well as the propensity for that patient's TgAb to interfere with a Tg measurement [70, 171, 177]. These patient-related specificity differences are why no threshold TgAb concentration can preclude TgAb interference [59, 62, 65, 76, 80, 89, 122, 123, 171], and why high TgAb concentrations do not necessarily interfere, whereas low TgAb may profoundly interfere with a Tg measurement [25, 59, 62, 70, 80, 122, 123, 173, 178–181].

TgAb Methods

Tg autoantibodies predominantly belong to the IgG class of immunoglobulins, are not complement fixing, and are generally conformational [25]. Two approaches have been used to assess whether there is TgAb in the specimen causing interference with the Tg measurement. The older "Tg recovery" approach, whereby Tg is measured before and after the addition of a known amount of Tg standard, has mostly been replaced by direct quantitative TgAb tests. Current guidelines [76] mandate that a quantitative TgAb measurement be made with every Tg test, and stress that the Tg recovery approach is not a reliable method for detecting interfering TgAb [89, 173, 176]. Quantitative TgAb methods are based on RIA or non-isotopic IMA principles [89, 123, 167, 171, 174, 182–186]. Unfortunately, TgAb tests are highly variable with respect to sensitivity, specificity and the numeric values they report, despite using the same International Reference Preparation (MRC 65/93) (Fig. 15.4). In fact, there can be a 100-fold difference between the TgAb concentrations reported by different meth-

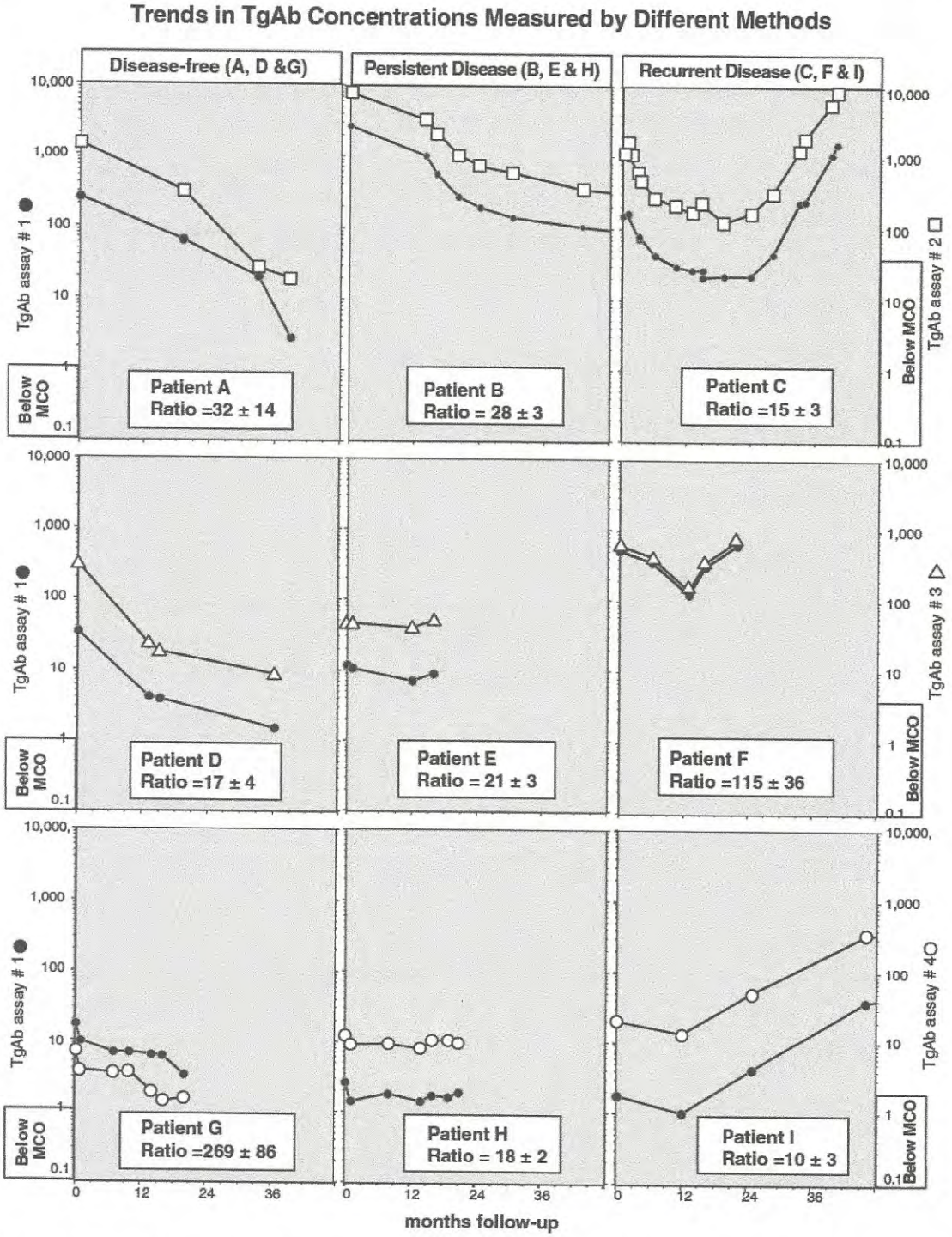

Fig. 15.5 Trends in serum TgAb measurements (ordinates) made for nine DTC patients (A–I) monitored over 1–4 year. Each specimen had TgAb measured by a reference assay (assay 1, Kronus/RSR, *solid circles*) and one of three test assays: (assay 2, Siemens Immulite, *open squares*) used for patients A–C; assay 3 (Beckman Access, *open triangles*) used for patients D–F; and (assay 4, Nichols Advantage, *open circles*) used for patients G–I. The *clear boxes* at the bottom of each patient's plot show the mean (±SD) of the ratios between test method TgAb values divided by the reference method values. Patients A, D, and G were judged disease-free after thyroidectomy (±RAI) and had a declining TgAb trend irrespective of the assay used. Patients B, E, and H had evidence of disease and maintained stable, detectable TgAb values. Patients C, F, and I had recurrences characterized by rising a TgAb trend. MCO = Manufacturer recommended cutoff for TgAb "positivity". The data were taken from [70, 212]

ods for the same serum (Figs. 15.4 and 15.5) [70, 123]. These differences decrease the reliability of TgAb detection [89, 123, 171, 183, 185–187], a problem exacerbated by manufacturer-recommended cutoffs (MCO) for TgAb "positivity" that are set for diagnosing thyroid autoimmunity and are too high to detect interfering TgAb concentrations [71, 123, 184–187]. It should be noted that current guidelines recommended that the assay functional sensitivity limit should be used as the cutoff for TgAb "positivity" [65, 71, 76, 123]. Some between-method variability relates to the purity and epitope specificity of the Tg reagent employed by the method, exacerbated by the fact that different patients produce TgAbs with different epitope specificities for the Tg reagent employed by the test [171, 176, 188]. This is evident in Fig. 15.5 that shows patient-specific differences in TgAb epitope specificity are maintained despite changes in the patient's TgAb concentration in response to treatment or progression of disease [42, 70]. Patient-specific TgAb epitope specificity results in a consistent patient-specific ratio between numeric TgAb values reported by two different methods that can be used to re-baseline the patient's TgAb trend should a change in TgAb method be necessary during TgAb monitoring [42, 70, 76].

TgAb Interference with Radioimmunoassay (Tg-RIA) Methodology

Tg-RIA methodology is based on the competition between Tg antigen (from serum or added ^{125}I-Tg tracer) and a low concentration of polyclonal (rabbit) Tg antibody (PAb). After incubation, the Tg-PAb complex is precipitated and the serum antigen concentration quantified as an inverse relationship to the ^{125}I-Tg in the precipitate. The use of a 48-h pre-incubation before adding a high specific activity ^{125}I-Tg tracer produces a maximal functional sensitivity of 0.5 μg/L for this class of method [189, 190]. The use of a high affinity PAb coupled with a species-specific second antibody can minimize the TgAb interference with the RIA method [191]. Resistance to TgAb interference is evidenced by appropriately normal Tg-RIA values for TgAb-positive euthyroid controls [89] and detectable Tg-RIA for TgAb-positive DTC patients with structural disease (Fig. 15.3b) [59]. This contrasts with IMA methods that report paradoxically undetectable serum Tg for some TgAb-positive normal euthyroid subjects [89] as well as TgAb-positive Graves' hyperthyroid patients [192] and TgAb-positive patients with structural disease (Fig. 15.3b) [89]. It should be noted that the propensity of TgAb to interfere with Tg-RIA determinations and cause underestimation [193] or overestimation [194, 195] depends not only on the assay formulation, but also patient-specific interactions between the endogenous serum Tg and TgAb and the exogenous RIA reagents [196].

TgAb Interference with Immunometric Assay (Tg-IMA) Methodology

Non-competitive IMAs use a two-site reaction whereby Tg in serum is captured by a solid-phase MAb and quantified as a function of the binding of a different (labeled) MAb to Tg from serum that becomes bound to the solid support [197]. Recovery studies show that TgAb interferes with IMA methodology by steric inhibition of MAb binding to Tg epitopes. Specifically, when the Tg epitope(s) necessary for binding to the IMA MAbs are blocked by TgAb complexing, the two-site reaction cannot take place and Tg is reported as falsely low or undetectable. Tg-IMA underestimation caused by TgAb interference is evident from the paradoxically low/undetectable Tg-IMA seen for TgAb-positive normal controls with an intact thyroid [89], as well as patients with Graves' hyperthyroidism [192] and DTC patients with structural disease (Fig. 15.3b) [25, 71, 80, 89, 123, 175, 176, 198–200]. High Tg concentrations can overwhelm TgAb binding capacity rendering Tg-IMA concentrations detectable and lessening the degree of interference [59, 123]. It follows that as Tg concentrations rise with progression of disease, more Tg is free, the influence of TgAb lessens and the discordance between Tg-IMA and Tg-RIA disappears (Fig. 15.6a) [59, 123]. Although some IMA methods claim to overcome TgAb interference by using MAbs directed against specific epitopes not involved in thyroid autoimmunity [201, 202], this approach has not proved effective in clinical practice, possibly because less restricted TgAb

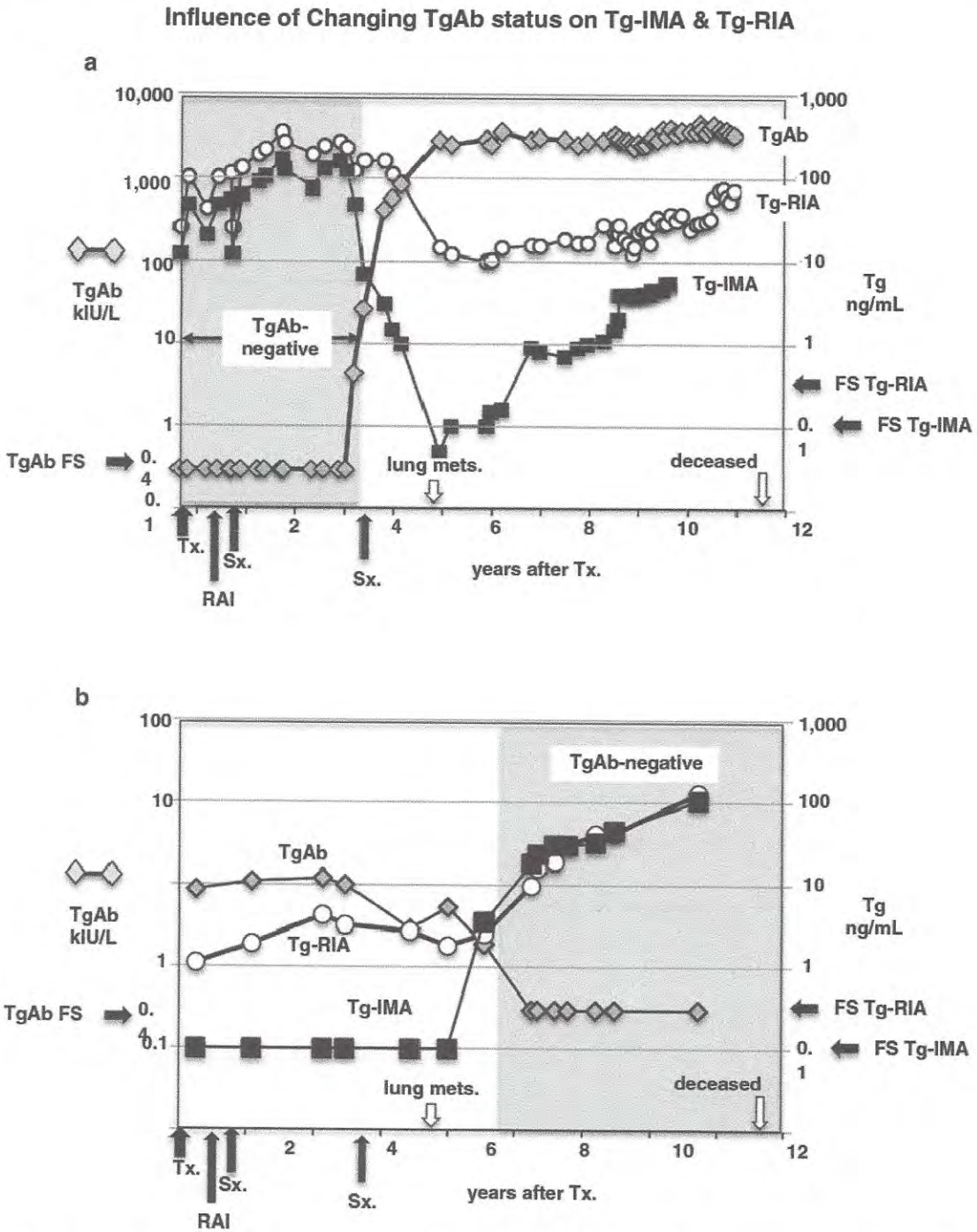

Fig. 15.6 Serial TgAb, Tg-RIA and Tg-IMA concentrations in two DTC patients with persistent/recurrent disease who underwent a change in TgAb status before death from disease-related complications. (**a**) A patient who converted from TgAb-negative to TgAb-positive. (**b**) A patient who converted from TgAb-positive to TgAb-negative

epitopes are more often associated with DTC than with autoimmune thyroid conditions [171, 175, 176, 199, 203].

TgAb Interference with Liquid Chromatography Tandem Mass Spectrometry (Tg-LC-MS/MS)

The new LC-MS/MS methods measure Tg as a Tg-specific peptide(s) generated after trypsinization of Tg-TgAb complexes in the serum specimen [39, 202, 204, 205]. Although LC-MS/MS methods currently only have first-generation functional sensitivity (FS ~0.5 μg/L) [37, 38, 114], they have the advantage of being free from HAb interferences [153]. Tg-LC-MS/MS methods were primarily developed to overcome TgAb interference and avoid falsely low/undetectable Tg-IMA results that can mask disease [37–39, 114, 204, 206, 207]. However, the diagnostic advantage of LC-MS/MS is currently questionable, given that a number of studies have now reported that over 40% of TgAb-positive patients with structural disease have paradoxically undetectable Tg-LC-MS/MS values [59, 114, 208, 209]. In fact, the most recent study concluded that Tg-LC-MS/MS offers no diagnostic advantage for detecting Tg in the presence of TgAb as compared with ^{2G}Tg-IMA [209], and confirmed another report that the higher the TgAb concentration the more likely that Tg-LC-MS/MS would be undetectable, despite disease [209, 210]. Note that an inverse relationship between TgAb concentration and Tg-LC-MS/MS detectability would be expected if TgAb enhanced in-vivo Tg clearance (see below).

In-Vitro Mechanisms for TgAb Interference

TgAb interferes with Tg measurements in a qualitative, quantitative and method-dependent manner [62, 70, 123, 172, 195, 196]. The potential for in-vitro interference is multifactorial and depends not only on the assay methodology (IMA, RIA or LC-MS/MS), but also the concentration and epitope specificity of the TgAb produced by the patient [70, 89, 180]. TgAb interference can be minimized using an RIA method that employs a PAb with broad epitope specificity to detect not only free Tg, but also Tg bound to TgAb where some epitopes may be masked by complexing. The selection of the PAb for maximal affinity for human Tg, and restricting the specificity of the second antibody reagent to precipitate selectively rabbit (not human) immunoglobulins, further minimizes TgAb interference [191]. In contrast, IMA methodology mainly detects the free Tg moiety—Tg molecules whose epitopes are not masked by TgAb complexing. Steric masking of Tg epitopes is the reason why TgAb interference with IMA methodology is always unidirectional (underestimation), and why a low Tg-IMA/Tg-RIA ratio has frequently been used to indicate TgAb interference [59, 120, 123, 167, 211, 212]. More studies are needed to determine why LC-MS/MS is undetectable in >40% of TgAb-positive patients with structural disease [59, 114, 208, 209]. Possibilities include tumor Tg polymorphisms that prevent the production of the Tg-specific tryptic peptide [2, 8, 13, 39], suboptimal trypsinization of Tg-TgAb complexes [35, 36], or Tg levels that are truly below detection because of TgAb-mediated increased clearance of Tg [46, 49, 57, 58].

In-Vivo Mechanisms for TgAb to Interference

Over past decades a number of studies have suggested that the presence of TgAb enhances Tg metabolic clearance. In 1967 Weigle et al showed increased clearance of endogenously ^{131}I-labeled Tg in rabbits after inducing TgAb by immunizing the animals with an immunogenic Tg preparation [57]. Human studies of the acute Tg and TgAb changes after sub-total thyroidectomy have also suggested that TgAb may increase Tg metabolic clearance [213]. TgAb-enhanced Tg clearance may result from TgAbs acting as "sweeper" antibodies to facilitate clearance of Tg antigen by the hepatic asialoglycoprotein receptor (ASGPR) [58, 167, 214–216]. Patients who undergo a permanent change in TgAb status (negative to positive or vice versa) that is discordant with their clinical disease status provide insights on the influence of TgAb on Tg-RIA and Tg-IMA mea-

surements and possible effects of TgAb on Tg metabolic clearance. The patient shown in Fig. 15.6a had a de novo appearance of TgAb 2.5 years after thyroidectomy (Tx) + RAI treatment for PTC. The appearance of TgAb was associated with a steep fall in ^{2G}-Tg-IMA to undetectability, consistent with masking of Tg epitopes by TgAb complexing. There was also a slower decline in Tg-RIA to levels that remained detectable but at levels that were tenfold lower than before the TgAb appearance. Thereafter as disease progressed, TgAb remained elevated and Tg-IMA rose to parallel Tg-RIA, but at a concentration fivefold lower than for Tg-RIA. Since Tg-RIA measurements are considered less prone to TgAb interference than Tg-IMA, the declining Tg-RIA trend seen after the appearance of TgAb would be consistent with a TgAb-mediated increase in Tg metabolic clearance [59, 74, 123, 167, 217].

Tg Measurement: Clinical Utility

Over the past decade, the incidence of DTC has risen, partly because small thyroid nodules and micropapillary cancers [76, 218–220] are increasingly being detected by anatomic imaging for non-thyroidal purposes [221–224]. Most DTC patients are rendered disease-free by their initial surgery, however, ~15% experience recurrences and ~5% die from disease-related complications [202, 225–228]. In most cases, persistent/recurrent disease is detected within the first five post-operative years, although recurrences can occur decades after initial surgery necessitating life-long monitoring for recurrence [226, 227]. Current guidelines recommend a risk-stratified approach to diagnosis and treatment of DTC [76, 228–230]. Since most patients have a low pre-test probability for disease recurrence, protocols for follow-up need a high negative predictive value (NPV) to eliminate unnecessary testing, as well as a high positive predictive value (PPV) for identifying patients with persistent/recurrent disease. Biochemical testing (serum Tg+TgAb) used in conjunction with periodic ultrasound is now recognized as more sensitive for detecting disease than diagnostic ^{131}I whole body scanning [76, 230–235]. However, close physician-laboratory cooperation is necessary when interpreting Tg and TgAb measurements given the persistent technical limitations affecting these tests discussed above.

Most (~75%) DTC patients have no TgAb detected [167]. When TgAb is absent, four factors influence the interpretation of serum Tg concentrations: (1) the mass of thyroid tissue present (normal tissue + tumor); (2) The intrinsic ability of the tumor to secrete Tg; (3) the presence of any inflammation of or injury to thyroid tissue, secondary to FNAB, surgery, RAI therapy or thyroiditis; and (4) the degree of TSH receptor stimulation by TSH, hCG or TRAb [65]. TgAb interference with Tg measurement remains problematic, irrespective of the class of Tg method used (RIA, IMA or LC-MS/MS). When TgAb is present, serum Tg is a less reliable tumor marker test making the serum TgAb concentration the primary (surrogate) tumor-marker. For patients either with or without TgAb, it is the *trend* in Tg and TgAb concentrations (measured by the same methods) that has the more prognostic value than the use of fixed cutoff values to assess risk for disease [74, 76, 97, 111, 229, 233, 236–242].

Preoperative Serum Tg Measurement

Current guidelines do not recommend routine preoperative Tg testing [76, 95]. However, some believe that serum Tg measured preoperatively may indicate the tumor's intrinsic ability to secrete Tg, and thus impact the interpretation of post-operative Tg changes [243, 244]. Approximately 50% of DTC patients have an elevated preoperative serum Tg—highest with Follicular > Hurthle > Papillary tumors [55, 56]. When small tumors give rise to an elevated preoperative Tg, that tumor may be an efficient Tg secretor. In other cases the relationship between serum Tg and tumor mass suggests that the tumor, especially BRAF-positive

tumors, may be a poor Tg secretor [56, 245]. When a tumor is known to be an inefficient Tg secretor, the clinical sensitivity of post-operative Tg monitoring may be decreased, necessitating an enhanced role for anatomic imaging [245, 246].

Serum Tg: First Post-Operative Year

The half-life of Tg in the circulation approximates 3 days [47, 48], such that the acute Tg release resulting from surgical injury and healing of tissue margins should largely resolve within the first six months, provided that thyroid hormone is initiated to prevent TSH stimulation [247]. However, when surgery is followed by RAI treatment there may be a slow Tg decline over subsequent years, presumably reflecting long-term radiolytic destruction of remnant tissue [248, 249]. A serum Tg measurement made as early as 6–8 weeks after thyroidectomy has been shown to have prognostic value, with the higher the serum Tg the greater the risk of persistent/recurrent disease [98, 241, 250–259]. After thyroidectomy, approximately one-gram of normal remnant tissue typically remains [202, 260]. This small thyroid remnant would be expected to produce a serum Tg approximating 1.0 μg/L, provided that TSH is not elevated [65]. Indeed, most disease-free PTC patients have ^{2G}-Tg-IMA measurements below 0.5 μg/L (with TSH below 0.5 mIU/L). In the absence of disease the low Tg concentration arising from the normal remnant remains remarkably stable during long-term monitoring (Fig. 15.7) [74, 261, 262]. This is in accord with studies reporting that a serum Tg below 1.0 μg/L, 6 weeks after thyroidectomy has a 98% NPV (PPV 43%) [241]. When L-T4 therapy maintains a stable TSH, a rising trend in ^{2G}-Tg-IMA concentrations that

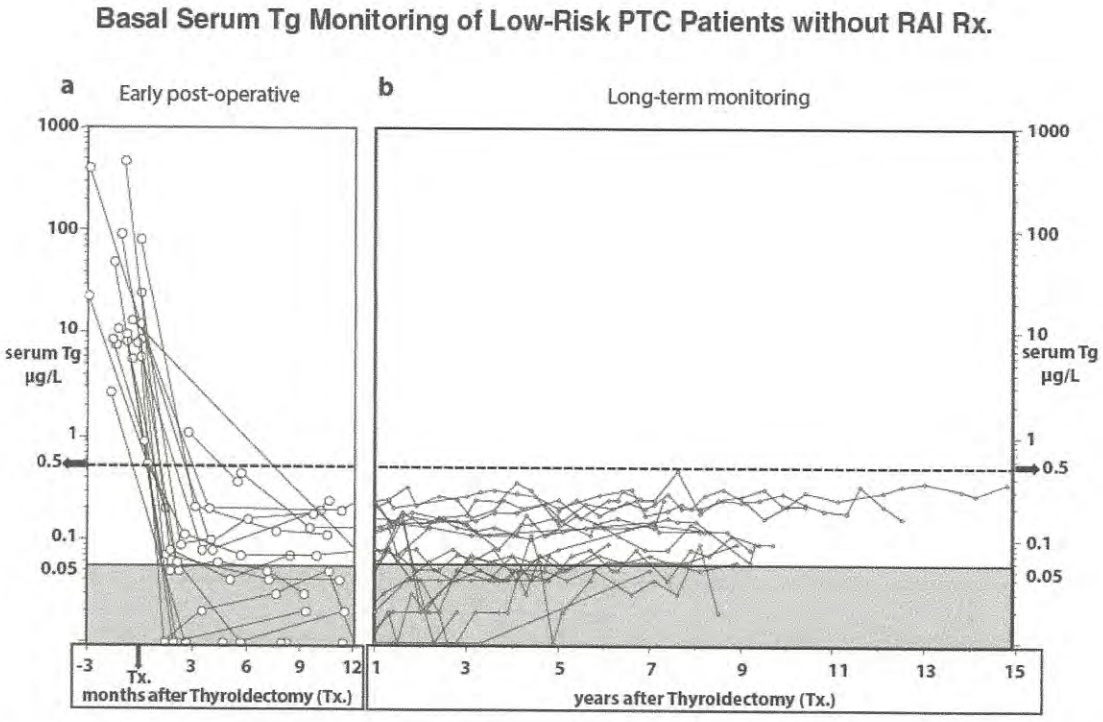

Fig. 15.7 Shows serum basal Tg^{2G}IMA measurements [Beckman Access analyses of frozen archived specimens {Spencer C, 2013 #8892}] made for 18 TgAb-negative PTC patients treated by thyroidectomy alone (no RAI treatment), maintained on long-term TSH suppression (<0.5 mIU/L), without evidence of recurrence at the end of >5 years of follow-up. (**a**) Shows that during the early post-operative phase, all patients achieved a basal serum Tg below 0.5 μg/L by 6–12 months after thyroidectomy (Tx). (**b**) Shows the stability of Tg secretion from normal remnant tissue when TSH is held constant. Data is taken from [74]

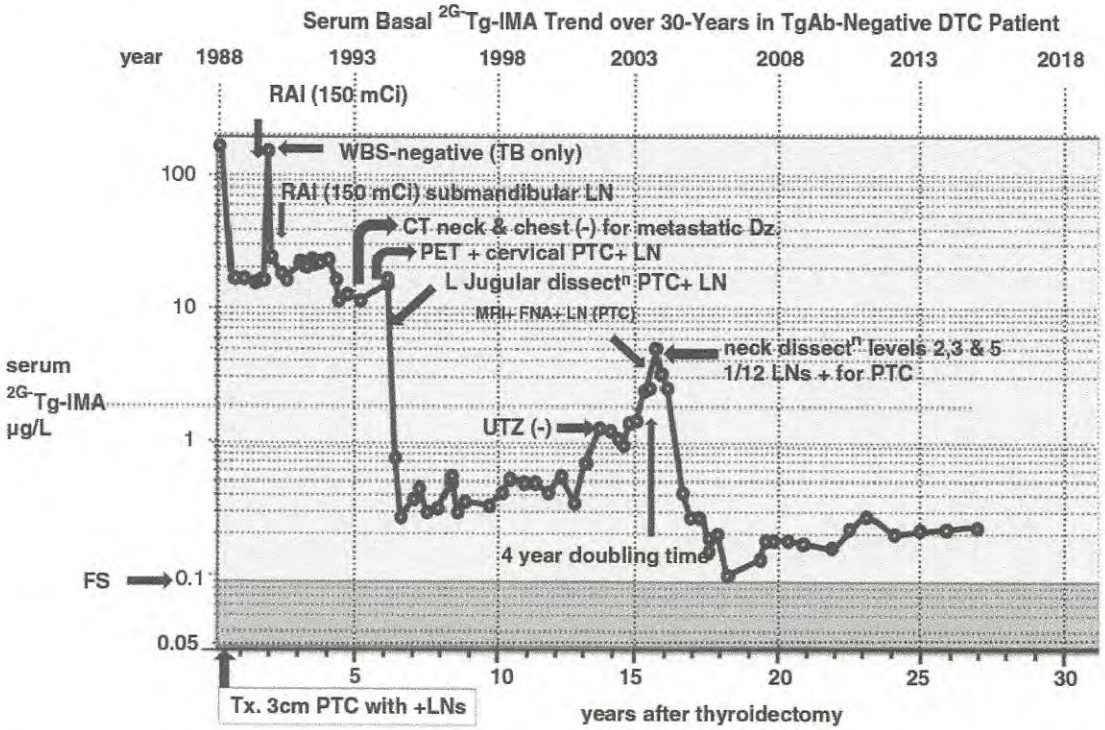

Fig. 15.8 A 30-year history of the basal Tg[2G]IMA trend (TSH <0.5 mIU/L) in a TgAb-negative patient treated for persistent/recurrent PTC. (The data was established using frozen archived specimens). Data taken from [74]

results in a doubling of serum Tg suggests recurrent disease (Fig. 15.8). Serum Tg can still be used as a tumor marker following lobectomy provided that a mass-adjusted reference range is employed. For example, the population reference range for TgAb-negative euthyroid subjects with an intact thyroid gland is broad, approximating 3–40 µg/L [34, 41, 65, 263, 264], whereas intra-individual serum Tg variability is relatively narrow (CV ~15%) [116, 117]. It follows that after a lobectomy, a mass-adjusted reference range of 1.5–20 µg/L would be appropriate, provided TSH is not elevated, but should be lowered an additional 50% to 0.75–10 µg/L should TSH be suppressed [65, 247].

TSH-Stimulated Tg Measurements

When TSH is elevated, the degree of Tg stimulation depends on the chronicity of stimulation, with ~20-fold rise in Tg resulting from the endogenous TSH stimulation seen after thyroid hormone withdrawal, and ~tenfold Tg rise typically seen 5-days after short-term rhTSH stimulation [91, 94, 96, 265, 266]. Before sensitive [2G]-Tg-IMA methods became available, the rhTSH-stimulation test was adopted as a standardized approach for boosting the Tg level into the detectable range of the insensitive first-generation tests [91–96]. Specifically, a 72-h rhTSH-stimulated Tg value below an arbitrary cutoff of 2.0 µg/L was adopted as a "negative" rhTSH test and shown to have a high NPV [91, 94, 98, 233, 237, 238, 240, 241, 256, 266–269]. Recombinant human TSH testing had a number of limitations. First, a negative test did not guarantee the absence of tumor [91, 266, 267] and the biases between different Tg methods (Figs. 15.2 and 15.3) made the use of the fixed numeric cut-off value of 2.0 µg/L problematic [41, 89]. Furthermore, the rhTSH dose delivered from the injection site was influenced by absorption, surface area and age of the patient [270–273]. The TSH sensitivity of tumor tissue was also a factor, with poorly differentiated tumors having blunted Tg responses to TSH [245, 246, 274, 275]. Because

there is a strong correlation between the basal and rhTSH-stimulated Tg values [93, 106] it is not surprising that an undetectable (<0.10 μg/L) basal ^{2G}Tg-IMA has comparable NPV to a negative rhTSH test (<2.0 μg/L) [41, 93, 97, 101–103, 106, 108, 276, 277]. It follows that rhTSH-stimulated Tg testing provides little additional information above that of basal Tg measured by a ^{2G}Tg-IMA method [41, 76, 93, 101–103, 106, 108, 276]. Although a ^{2G}Tg-IMA below 0.1 μg/L predicts the absence of disease with a high degree of confidence [76, 93, 107, 108, 263], periodic cervical ultrasound is still recommended [76], because some lymph nodes metastases have inefficient Tg secretion associated with an undetectable ^{2G}Tg-IMA [101, 266, 269, 278, 279]. It should be noted that rhTSH stimulation testing can be useful when investigating interferences in patients who appear disease-free yet have paradoxically high basal ^{2G}Tg-IMA that appears clinically inappropriate [93]. Interference (usually from HAb/HAMA) is the most likely cause for an absent or blunted rhTSH-stimulated Tg response in a patient with a detectable basal ^{2G}Tg-IMA [93, 106, 152]. Alternatively, it should be noted that a blunted or absent rhTSH response is sometimes seen in the presence of TgAb, as would be expected if TgAb enhanced the clearance of Tg-TgAb complexes, as discussed above [57, 58, 192, 213].

TgAb-Negative Patients: Long-Term Follow-up of Basal Tg Trends

The higher the post-operative Tg the greater the risk for persistent/recurrent disease [98, 241, 251–258]. Now that ^{2G}Tg-IMA measurement has become the standard of care, subnormal basal (non-TSH stimulated) Tg can be monitored during L-T4 therapy without rhTSH stimulation [41, 76, 93, 101–103, 106, 108, 276]. The *trend* in basal Tg is now recognized as a better prognostic indicator than using a fixed Tg cutoff value or Tg "detectability" to determine disease risk [74, 76, 97, 111, 229, 233, 236–242], especially since Tg "detectability" is merely determined by assay functional sensitivity [41, 100, 101, 106, 262]. Thus, under non-elevated (≤0.5 mIU/L) TSH conditions [74, 262], the serum Tg trend reflects changes in tumor mass, with a declining Tg trend suggesting absence or regression of disease (Fig. 15.7) and a rising Tg trend being suspicious for tumor recurrence (Fig. 15.8) [74, 76, 84, 90, 97, 229, 233, 240, 248, 249, 280]. As with other tumor-markers such as calcitonin, the Tg doubling time, measured under stable, non-elevated TSH conditions, is now recognized as a prognostic marker for mortality [242, 261, 280–286]. However, between-method variability (Figs. 15.2 and 15.3) necessitates that the serum Tg trend be measured using the same method, and preferably the same laboratory [76]. One approach used to mitigate between-run imprecision and improve the reliability of assessing Tg trends has been to measure the current specimen concurrently (in the same run) with an archived specimen from that patient. Concurrent serum Tg measurement eliminates run-to-run variability and increases confidence for detecting small Tg changes [80, 82]. Figure 15.8 shows a 30-year history of serial Tg^{2G}IMA measurements made for a TgAb-negative PTC patient (T2N1M0) who had persistent/recurrent disease and in whom the post-operative serum Tg^{2G}IMA trend was monitored (during TSH suppression) using frozen archived specimens [42]. This case illustrates a number of points: (1) the high preoperative Tg (154 μg/L) suggested that serum Tg would be a sensitive post-operative tumor-marker; (2) the Tg stimulation in response to thyroid hormone withdrawal prior to the first RAI treatment suggested that the tumor was responsive to TSH and supported the efficacy for TSH suppression; (3) surgery was clearly a more effective treatment for metastatic PTC lymph nodes than RAI treatments; (4) combined imaging modalities were needed to detect disease; (5) persistent disease remained quiescent for many years during TSH suppression before an active recurrence manifested; (6) a rising trend in basal Tg^{2G}IMA (non-elevated TSH) suggested an increase in tumor mass [74, 262]; (7) the doubling of basal Tg^{2G}IMA during TSH suppression approximated 4-years—an interval indicating a good, long-term prognosis [242], and (8) the continued detection of serum Tg above the assay functional sensitivity limit 30 years after initial treatment and despite elimination of thyroid rem-

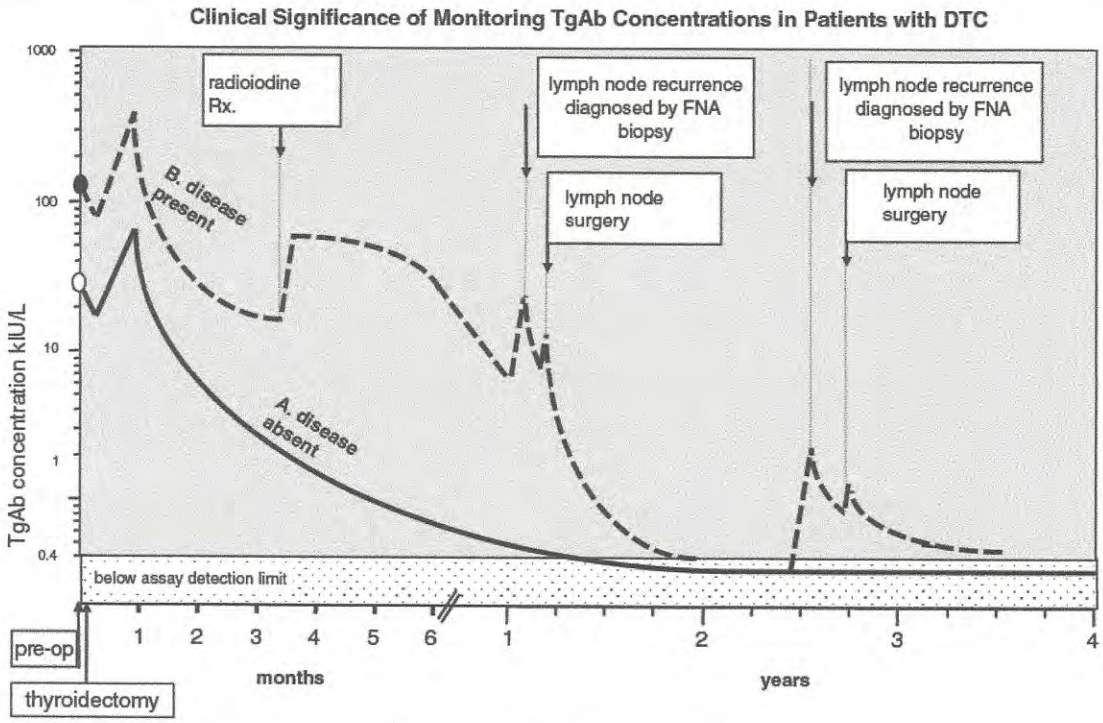

Fig. 15.9 Schematic representing changes in TgAb trends following thyroidectomy in patients rendered disease-free by surgery (pattern A) versus patients with persistent/recurrent disease (pattern B). TgAb concentrations may rise or become detectable de novo in response to an increase in Tg antigen following surgical injury, lymph node recurrence(s), lymph node resection(s) FNA biopsy of metastatic lymph nodes, or RAI treatment. Data taken from [70]

nant by two doses of RAI, suggests that disease may persist, warranting continued monitoring.

TgAb-Positive Patients: Monitor TgAb Trends as a Surrogate Tumor-Marker

The trend in serum TgAb concentrations can be used as a surrogate tumor-marker for TgAb-positive DTC patients, in whom Tg measurement may be unreliable (Figs. 15.9 and 15.10) [25, 60–76]. Because TgAb tests differ in sensitivity and specificity (Figs. 15.4 and 15.5), it is essential that the trend in TgAb concentrations be measured using the same manufacturers method and preferably the same laboratory [62, 71, 76, 80, 89, 123, 184–187, 314–317]. Studies have shown that after initial treatment (Tx ± RAI), serum TgAb progressively falls over time (months/years) when patients are disease-free. This is consistent with a decline in Tg antigen stimulation of the immune system [60, 61, 64, 67, 68, 70, 74, 76, 169, 318, 319]. The time needed for TgAb to become undetectable or fall below 10% of the initial value is related to the initial TgAb concentration, measured before or early in the post-operative period (Fig. 15.10a, b) [74]. Those patients exhibiting a TgAb decline of more than 50% by the end of the first post-operative year have been shown to have a low recurrence risk [42, 68, 75, 320, 321]. Approximately 3% of patients lose TgAb-positivity and yet still have persistent disease (Fig. 15.6b). Whereas most patients who undergo TgAb-positive to TgAb-negative conversions are disease-free and have low/undetectable Tg concentrations (<1.0 µg/L), those patients who lose TgAb-positivity despite disease typically have a detectable or rising serum Tg (Fig. 15.6b). The patient shown in Fig. 15.6b was TgAb-positive at the time of initial treatment at which time Tg-RIA was detectable and ^{2G}-Tg-IMA was undetectable. Despite extensive disease, TgAb became undetectable five years after initial treatment. The change in

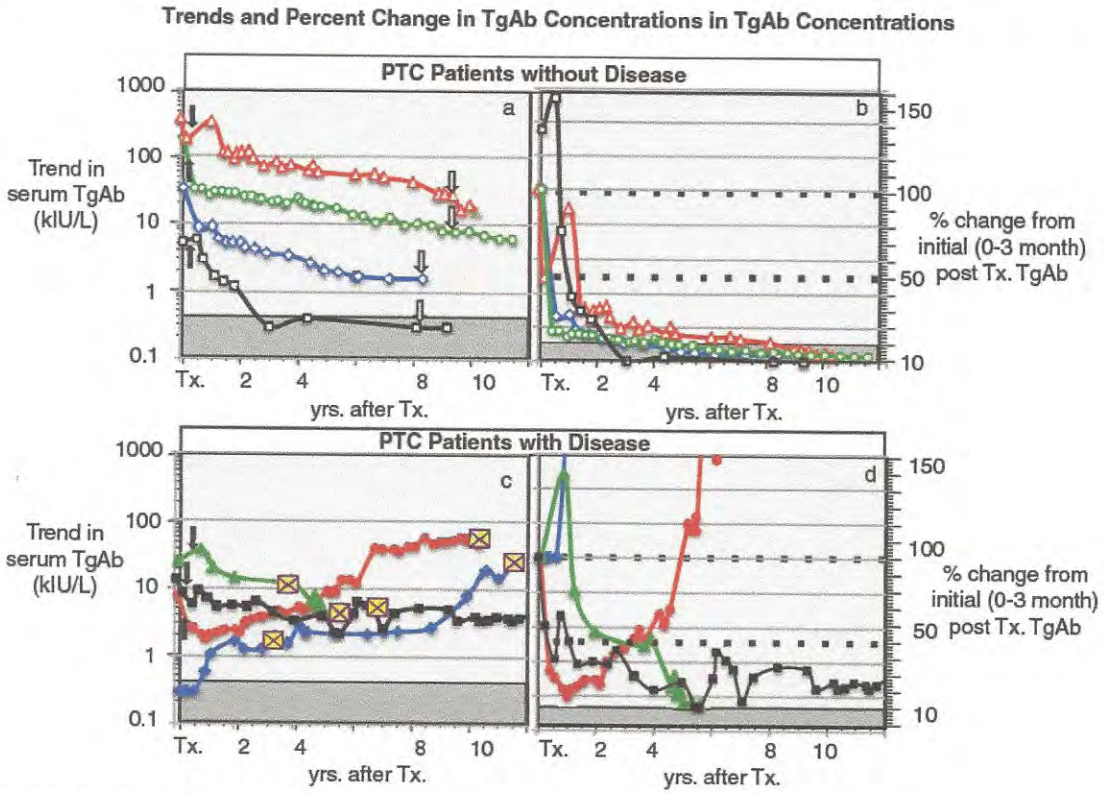

Fig. 15.10 Serial TgAb concentrations measured by the Kronus/RSR method showing how the TgAb trend (**a**, **c**) and the % change in TgAb concentrations from the initial (0–3 month) TgAb concentration (**b**, **d**) can be used as a surrogate tumor-marker. (**a**) Shows 4 PTC patients who were judged disease-free by ultrasound (open arrows). TgAb values progressively declined over time (years) to <10% of initial level. (**b**) Shows tht the higher the initial TgAb the longer it took for TgAb to fall below 10%. (**c**, **d**) Show comparative TgAb data for 4 PTC patients with persistent/recurrent disease detected during follow-up (indicated by crosses). A de novo TgAb appearance, a TgAb rise or a stable TgAb concentration that fails to fall below 10% of initial value were indicators of active disease. Data taken from [74]

TgAb status was associated with a rapid rise in Tg-IMA to parallel a steep increase in Tg-RIA with a doubling time of less than one year before death from disease-related complications [242, 261, 280–286]. This TgAb loss could reflect RAI destruction of the source (thyroid remnant tissue) of more antigenic, normally iodinated Tg, while the tumor was secreting poorly iodinated Tg molecules that were less antigenic [33] and did not stimulate the immune system. Most patients with persistent/recurrent disease exhibit only a marginal TgAb decline, or have a stable or rising TgAb that rarely falls below 10% of the initial value (Fig. 15.10c, d) [60–63, 68, 73–75, 80, 89, 123, 169, 185–187, 212, 322]. However, it should be noted that some patients maintain a low, stable TgAb concentration for many years without evidence of disease. This could reflect immune system sensitivity to Tg secreted by a small thyroid remnant or long-lived memory of plasma cells [323]. Approximately 10% of TgAb-negative patients convert to TgAb-positivity at some time in their course, emphasizing the need to measure TgAb with every Tg test [71, 76]. Because the immune system is sensitive to the Tg antigen concentration, there can be a *transient* rise or de novo appearance of TgAb in response to the acute release of Tg following thyroid surgery [324, 325], FNAB [326, 327] or more chronically (months) the radiolytic damage following RAI treatment [42, 70, 168, 247, 328–331]. However, the appearance of *permanent* TgAb-positivity after the first post-operative year typically indicates metastatic disease, as illustrated by the patient shown in Fig. 15.6a [68, 70, 167]. This appearance of permanent TgAb positiv-

ity likely reflects a change in in the heterogeneity of tumor-derived Tg (secretion of a more immunogenic Tg molecule), or a delayed recognition of tumor Tg by the immune system.

Tg Measurement in FNA Needle Washouts (FNA-Tg)

Because Tg protein is tissue-specific, the detection of Tg in non-thyroidal tissues or fluids (such as pleural fluid) indicates the presence of metastatic thyroid cancer [287]. Struma ovarii is the only (rare) condition in which the Tg in the circulation does not originate from the thyroid [288, 289]. A high concentration of Tg or parathyroid hormone (PTH) measured in the cyst fluid provides a reliable indicator of the tissue origin of a cyst (thyroid versus parathyroid, respectively), information that is critical for surgical decision-making [287, 290]. Although ultrasound characteristics are helpful for distinguishing benign reactive lymph nodes from those suspicious for malignancy, the finding of Tg in the needle washout of a lymph node biopsy has higher diagnostic accuracy than the ultrasound appearance [291–305]. The current protocol for obtaining FNA-Tg samples recommends rinsing the biopsy needle in 1.0 mL of saline and sending this specimen to the laboratory for Tg analysis. A common cutoff value used for a "positive" FNA-Tg in a thyroidectomized patient is 1.0 μg/L [295, 302, 306, 307], although this cutoff can vary by assay and institution [301, 308]. When investigating suspicious lymph nodes in patients with an intact thyroid, a higher FNA-Tg cutoff value (~35–40 μg/L) is recommended [292, 297, 307]. Although there is controversy whether TgAb interferes with FNA-Tg analyses [293, 309, 310], in a patient with a very high serum TgAb concentration there can be serum contamination of the FNA washout that may cause a falsely low/undetectable FNA-Tg due to TgAb interference when measured by an IMA method. This would occur if the expected serum dilution (~40-fold) in the wash fluid were insufficient to lower TgAb in the washout below detection. Note, the FNA needle washout procedure can also be used to detect calcitonin in neck masses of patients with primary and metastatic medullary thyroid cancer [290, 311–313], and FNA-PTH determinations may be useful for identifying parathyroid tissue [290].

Summary: Key Points

1. The tissue-specific (thyroid) origin of Tg in the circulation is why serum Tg measurement is the primary biochemical tumor-marker test for monitoring patients with DTC.
2. The biosynthetic processes necessary to make a mature 660 kDa Tg molecule are complex, and may become dysregulated in thyroid tumors leading to serum Tg heterogeneity.
3. Tg molecular abnormalities and polymorphisms may alter serum Tg detection by either immunoassay or LC-MS/MS methods, necessitating monitoring the serum Tg trend using the same method and preferably the same laboratory.
4. Second-generation Tg IMA methods (^{2G}Tg-IMA), characterized by an assay functional sensitivity ≤0.1 μg/L, have now become the standard of care, because ^{2G}Tg-IMA has sufficient FS to monitor the low basal serum Tg concentrations typically seen after thyroidectomy without the need for rhTSH stimulation.
5. Tg-IMA methodology is most prone to interference by the TgAb that is present in ~25% of DTC patients. TgAb causes falsely low/undetectable Tg-IMA values that can mask disease. In-vitro and/or in-vivo TgAb interferences with the RIA and/or LC-MS/MS classes of Tg method are also possible.
6. It is currently unclear why Tg-LC-MS/MS methods fail to detect Tg in >40% of TgAb-positive patients with structural disease. The problem could relate to tumor Tg polymorphisms, post-translational Tg modifications that change the mass or charge of tryptic fragments, or exceedingly low Tg concentrations secondary to TgAb-enhanced clearance of Tg-TgAb complexes.
7. Both serum Tg and TgAb trends should be monitored as DTC tumor-marker tests (maintaining the continuity of methods), because the TgAb status of the patient can change and may become discordant with disease status.

8. When TgAb is present and Tg measurement is unreliable, the trend in TgAb concentrations should be used as the primary tumor-marker and the trend in Tg concentrations becomes the secondary tumor-marker.

References

1. Baas FBH, Van Geurts KA, Melsert R, Pearson PL, de Vijlder JJM, Van Ommen GJB. The human thyroglobulin gene: a ploymorphic marker localized distal to c-myc on chromosome 8 band q 24. Hum Genet. 1985;69:138–43.
2. Matakidou A, Hamel N, Popat S, Henderson K, Kantemiroff T, Harmer C, et al. Risk of non-medullary thyroid cancer influenced by polymorphic variation in the thyroglobulin gene. Carcinogenesis. 2004;25(3):369–73.
3. Hishinuma A, Fukata S, Kakudo K, Murata Y, Ieiri T. High incidence of thyroid cancer in long-standing goiters with thyroglobulin mutations. Thyroid. 2005;15:1079–84.
4. Vono-Toniolo J, Rivolta CM, Targovnik HM, Medeiros-Neto G, Kopp P. Naturally occurring mutations in the thyroglobulin gene. Thyroid. 2005;15:1021–33.
5. Caputo M, Rivolta CM, Mories T, Corrales JJ, Galindo P, Gonzalez-Sarmiento R, et al. Analysis of thyroglobulin gene polymorphisms in patients with autoimmune thyroiditis. Endocrine. 2010;37(3):389–95.
6. Citterio CE, Machiavelli GA, Miras MB, Gruñeiro-Papendieck L, Lachlan K, Sobrero G, et al. New insights into thyroglobulin gene: molecular analysis of seven novel mutations associated with goiter and hypothyroidism. Mol Cell Endocrinol. 2013;365:277–91.
7. Rubio IG, Medeiros-Neto G. Mutations of the thyroglobulin gene and its relevance to thyroid disorders. Curr Opin Endocrinol Diabetes Obes. 2009;16:373–8.
8. Akdi A, Pérez G, Pastor S, Castell J, Biarnés J, Marcos R, et al. Common variants of the thyroglobulin gene are associated with differentiated thyroid cancer risk. Thyroid. 2011;21:519–25.
9. Siraj AK, Masoodi T, Bu R, Beg S, Al-Sobhi SS, Al-Dayel F, et al. Genomic profiling of thyroid cancer reveals a role for thyroglobulin in metastasis. Am J Hum Genet. 2016;98(6):1170–80.
10. van de Graaf SA, Ris-Stalpers C, Pauws E, Mendive FM, Targovnik HM, de Vijlder JJ. Up to date with human thyroglobulin. J Endocrinol. 2001;170:307–21.
11. De Felice M, Di Lauro R. Thyroid development and its disorders: genetics and molecular mechanisms. Endocr Rev. 2004;25:722–46.
12. Lin JD. Thyroglobulin and human thyroid cancer. Clin Chim Acta. 2008;388(1-2):15–21.
13. Xavier AC, Maciel RM, Vieira JG, Dias-da-Silva MR, Martins JR. Insights into the posttranslational structural heterogeneity of thyroglobulin and its role in the development, diagnosis, and management of benign and malignant thyroid diseases. Arch endocrinol metabol. 2016;60(1):66–75.
14. Arvan P, Kim PS, Kuliawat R, Prabakaran D, Muresan Z, Yoo SE, et al. Intracellular protein transport to the thyrocyte plasma membrane: potential implications for thyroid physiology. Thyroid. 1997;7:89–105.
15. Lee J, Di Jeso B, Arvan P. The cholinesterase-like domain of thyroglobulin functions as an intramolecular chaperone. J Clin Invest. 2008; 118:2950–8.
16. Di Jeso B, Ulianich L, Pacifico F, Leonardi A, Vito P, Consiglio E, et al. Folding of thyroglobulin in the calnexin/calreticulin pathway and its alteration by loss of Ca2+ from the endoplasmic reticulum. Biochem J. 2003;370:449–58.
17. Shimizu K, Nakamura K, Kobatake S, Satomura S, Maruyama M, Kameko F, et al. The clinical utility of Lens culinaris agglutinin-reactive thyroglobulin ratio in serum for distinguishing benign from malignant conditions of the thyroid. Clin Chim Acta. 2007;379:101–4.
18. Kanai T, Amakawa M, Kato R, Shimizu K, Nakamura K, Ito K, et al. Evaluation of a new method for the diagnosis of alterations of lens culinaris agglutinin binding of thyroglobulin molecules in thyroid carcinoma. Clin Chem Lab Med. 2009;47(10):1285–90.
19. Schneider AB, Dudlak D. Differential incorporation of sulfate into the chondroitin chain and complex carbohydrate chains of human thyroglobulin: studies in normal and neoplastic thyroid tissue. Endocrinology. 1989;124(1):356–62.
20. Magro G, Perissinotto D, Schiappacassi M, Goletz S, Otto A, Muller EC, et al. Proteomic and postproteomic characterization of keratan sulfate-glycanated isoforms of thyroglobulin and transferrin uniquely elaborated by papillary thyroid carcinomas. Am J Pathol. 2003;163:183–96.
21. Emoto N, Kunii YK, Ashizawa M, Oikawa S, Shimizu K, Shimonaka M, et al. Reduced sulfation of chondroitin sulfate in thyroglobulin derived from human papillary thyroid carcinomas. Cancer Sci. 2007;98:1577–81.
22. Schneider AB, Ikekubo K, Kuma K. Iodine content of serum thyroglobulin in normal individuals and patients with thyroid tumors. J Clin Endocrinol Metab. 1983;57:1251–6.
23. Saboori AM, Rose NR, Bresler HS, Vladut-Talor M, Burek CL. Iodination of human thyroglobulin (Tg) alters its immunoreactivity. 1. Iodination alters muliple epitopes of human Tg. Clin Exp Immunol. 1998;113:297–302.

24. Gerard AC, Daumerie C, Mestdagh C, Gohy S, De Burbure C, Costagliola S, et al. Correlation between the loss of thyroglobulin iodination and the expression of thyroid-specific proteins involved in iodine metabolism in thyroid carcinomas. J Clin Endocrinol Metab. 2003;88:4977–83.
25. McLachlan SM, Rapoport B. Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies. Thyroid. 2004;14:510–20.
26. Prentice L, Kiso Y, Fukuma N, Horimoto M, Petersen V, Grennan F, et al. Monoclonal thyroglobulin autoantibodies: variable region analysis and epitope recognition. J Clin Endocrinol Metab. 1995;80:977–86.
27. Kohno Y, Tarutani O, Sakata S, Nakajima H. Monoclonal antibodies to thyroglobulin elucidate differences in protein structure of thyroglobulin in healthy individuals and those with papillary adenocarcinoma. J Clin Endocrinol Metab. 1985;61:343–50.
28. de Micco C, Ruf J, Carayon P, Chrestian MA, Henry JF, Toga M. Immunohistochemical study of thyroglobulin in thyroid carcinomas with monoclonal antibodies. Cancer. 1987;59:471–6.
29. Kim PS, Dunn AD, Dunn JT. Altered immunoreactivity of thyroglobulin in thyroid disease. J Clin Endocrinol Metab. 1988;67:161–8.
30. Hufner M, Pfahl H, Bethauser H, Heilig B, Georgi P. Comparative plasma thyroglobulin measurements with three non-cross-reactive monoclonal antibodies in metastatic thyroid cancer patients. Acta Endocrinol. 1988;118:528–32.
31. Schulz R, Bethauser H, Stempka L, Heilig B, Moll A, Hufner M. Evidence for immunological differences between circulating and tissue-derived thyroglobulin in men. Eur J Clin Investig. 1989;19:459–63.
32. Saboori AM, Rose NR, Kuppers RC, Butscher WG, Bresler HS, Burek CL. Immunoreactivity of multiple molecular forms of human thyroglobulin. Clin Immunol Immunopathol. 1994;72(1):121–8.
33. Rose NR, Saboori AM, Rasooly L, Burek CL. The role of iodine in autoimmune thyroiditis. Crit Rev Immunol. 1997;17:511–7.
34. Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, LoPresti JS. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. J Clin Endocrinol Metab. 2005;90(10):5566–75.
35. Saboori AM, Burek CL, Rose NR, Bresler HS, Talor M, Kuppers RC. Tryptic peptides of human thyroglobulin: I. Immunoreactivity with murine monoclonal antibodies. Clin Exp Immunol. 1994;98(3):454–8.
36. Saboori AM, Caturegli P, Rose NR, Mariotti S, Pinchera A, Burek CL. Tryptic peptides of human thyroglobulin: II. Immunoreactivity with sera from patients with thyroid diseases. Clin Exp Immunol. 1994;98(3):459–63.
37. Clarke NJ, Zhang Y, Reitz RE. A novel mass spectrometry-based assay for the accurate measurement of thyroglobulin from patient samples containing antithyroglobulin autoantibodies. J Investig Med. 2012;60(8):1157–63.
38. Kushnir MM, Rockwood AL, Roberts WL, Abraham D, Hoofnagle AN, Meikle AW. Measurement of thyroglobulin by liquid chromatography-tandem mass spectrometry in serum and plasma in the presence of antithyroglobulin autoantibodies. Clin Chem. 2013;59(6):982–90.
39. Hoofnagle AN, Roth MY. Clinical review: improving the measurement of serum thyroglobulin with mass spectrometry. J Clin Endocrinol Metab. 2013;98(4):1343–52.
40. Fenouillet E, Fayet G, Hovsepian S, Bahraoui EM, Ronin C. Immunochemical evidence for a role of complex carbohydrate chains in thyroglobulin antigenicity. J Biol Chem. 1986;261(32):15153–8.
41. Schlumberger M, Hitzel A, Toubert ME, Corone C, Troalen F, Schlageter MH, et al. Comparison of seven serum thyroglobulin assays in the follow-up of papillary and follicular thyroid cancer patients. J Clin Endocrinol Metab. 2007;92(7):2487–95.
42. Spencer C, Fatemi S. Thyroglobulin antibody (TgAb) methods - strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer. Best Pract Res Clin Endocrinol Metab. 2013;27:701–12.
43. Tatumi K, Suzuki Y, Sinohara H. Clearance of circulating desialylated thyroglobulins in the rat. Biochim Biophys Acta. 1979;583(4):504–11.
44. Brix K, Wirtz R, Herzog V. Paracrine interaction between hepatocytes and macrophages after extrathyroidal proteolysis of thyroglobulin. Hepatology. 1997;26:1232–40.
45. Whitley RJ, Ain KB. Thyroglobulin: a specific serum marker for the management of thyroid carcinoma. Clin Lab Med. 2004;24(1):29–47.
46. Sellitti DF, Suzuki K. Intrinsic regulation of thyroid function by thyroglobulin. Thyroid. 2014;24(4):625–38.
47. Feldt-Rasmussen U, Petersen PH, Nielsen H, Date J. Thyroglobulin of varying molecular sizes with different disappearence rates in plasma following subtotal thyroidectomy. Clin Endocrinol. 1978;9:205–14.
48. Hocevar M, Auersperg M, Stanovnik L. The dynamics of serum thyroglobulin elimination from the body after thyroid surgery. Eur J Surg Oncol. 1997;23:208–10.
49. Feldt-Rasmussen U. Serum thyroglobulin and thyroglobulin autoantibodies in thyroid disease. Allergy. 1983;38:369–87.
50. Jeevanram RK, Shah DH, Sharma SM, Ganatra RD. Disappearance rate of endogenously radioiodinated thyroglobulin and thyroxine after radioiodine treatment. Cancer. 1982;49:2281–4.
51. Ikekubo K, Pervos R, Schneider AB. Clearance of normal and tumor-related thyroglobulin from the circulation of rats: role of the terminal sialic acid residues. Metabolism. 1980;29:673–81.

52. Morell AG, Gregoriadis G, Scheinberg IH. The role of sialic acid in determining the survival of glycoproteins in the circulation. J Biol Chem. 1971;246:1461–7.
53. Sinadinovic J, Cvejic D, Savin S, Jancic-Zugurica M, Micic JV. Altered terminal glycosylation of thyroglobulin in papillary thyroid carcinoma. Exp Clin Endocrinol. 1992;100:124–8.
54. Bastiani P, Papandreou J, Blanck O, Fenouillet E, Thibault V, Miquelis R. On the relationship between completion of N-acetllactosamine oligosaccharide units and iodine content of thyroglobulin; a reinvestigation. Endocrinology. 1995;136:4204–9.
55. Hocevar M, Auersperg M. Role of serum thyroglobulin in the pre-operative evaluation of follicular thyroid tumours. Eur J Surg Oncol. 1998;24:553–7.
56. Ericsson UB, Tegler L, Lennquist S, Christensen SB, Ståhl E, Thorell JI. Serum thyroglobulin in differentiated thyroid carcinoma. Acta Chir Scand. 1984;150:367–75.
57. Weigle WO, High GJ. The behaviour of autologous thyroglobulin in the circulation of rabbits immunized with either heterologous or altered homologous thyroglobulin. J Immunol. 1967;98:1105–14.
58. Igawa T, Haraya K, Hattori K. Sweeping antibody as a novel therapeutic antibody modality capable of eliminating soluble antigens from circulation. Immunol Rev. 2016;270(1):132–51.
59. Spencer C, Petrovic I, Fatemi S, LoPresti J. Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer using sensitive (second-generation) immunometric assays can be disrupted by false-negative and false-positive serum thyroglobulin autoantibody misclassifications. J Clin Endocrinol Metab. 2014;99(12):4589–99.
60. Pacini F, Mariotti S, Formica N, Elisei R. Thyroid autoantibodies in thyroid cancer: incidence and relationship with tumor outcome. Acta Endocrinol. 1988;119:373–80.
61. Rubello D, Casara D, Girelli ME, Piccolo M, Busnardo B. Clinical meaning of circulating antithyroglobulin antibodies in differentiated thyroid cancer: a prospective study. J Nucl Med. 1992;33:1478–80.
62. Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, et al. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab. 1998;83(4):1121–7.
63. Chung JK, Park YJ, Kim TY, So Y, Kim SK, Park DJ, et al. Clinical significance of elevated level of serum antithyroglobulin antibody in patients with differentiated thyroid cancer after thyroid ablation. Clin Endocrinol. 2002;57:215–21.
64. Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, et al. Disappearance of humoral thyroid autoimmunity after complete removal of thyroid antigens. Ann Intern Med. 2003;139:346–51.
65. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, et al. Laboratory medicine practice guidelines. Laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid. 2003;13(1):3–126.
66. Küçük ON, Aras G, Kulak HA, Ibiş E. Clinical importance of anti-thyroglobulin auto-antibodies in patients with differentiated thyroid carcinoma: comparison with 99mTc-MIBI scans. Nucl Med Commun. 2006;27:873–6.
67. Thomas D, Liakos V, Vassiliou E, Hatzimarkou F, Tsatsoulis A, Kaldrimides P. 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 Investig. 2007;30:173–80.
68. Kim WG, Yoon JH, Kim WB, Kim TY, Kim EY, Kim JM, et al. 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. 2008;93(12):4683–9.
69. Feldt-Rasmussen U, Rasmussen AK. Autoimmunity in differentiated thyroid cancer: significance and related clinical problems. Hormones. 2010;9:109–17.
70. Spencer CA. Clinical utility of thyroglobulin antibody (TgAb) measurements for patients with differentiated thyroid cancers (DTC). J Clin Endocrinol Metab. 2011;96(12):3615–27.
71. Verburg FA, Luster M, Cupini C, Chiovato L, Duntas L, Elisei R, et al. Implications of thyroglobulin antibody positivity in patients with differentiated thyroid cancer: a clinical position statement. Thyroid. 2013;23(10):1211–25.
72. Hsieh CJ, Wang PW. Sequential changes of serum antithyroglobulin antibody levels are a good predictor of disease activity in thyroglobulin-negative patients with papillary thyroid carcinoma. Thyroid. 2014;24(3):488–93.
73. Feldt-Rasmussen U, Verburg FA, Luster M, Cupini C, Chiovato L, Duntas L, et al. Thyroglobulin autoantibodies as surrogate biomarkers in the management of patients with differentiated thyroid carcinoma. Curr Med Chem. 2014;21(32):3687–92.
74. Spencer C, LoPresti J, Fatemi S. How sensitive (second-generation) thyroglobulin measurement is changing paradigms for monitoring patients with differentiated thyroid cancer, in the absence or presence of thyroglobulin autoantibodies. Curr Opin Endocrinol Diabetes Obes. 2014;21(5):394–404.
75. Rosario PW, Carvalho M, Mourao GF, Calsolari MR. Comparison of antithyroglobulin antibody concentrations before and after ablation with 131I as a predictor of structural disease in differentiated thyroid carcinoma patients with undetectable basal thyroglobulin and negative neck ultrasonography. Thyroid. 2016;26(4):525–31.

76. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American thyroid association guidelines task force on thyroid nodules and differentiated thyroid cancer. Thyroid. 2016;26(1):1–133.
77. Giovanella L, Feldt-Rasmussen U, Verburg FA, Grebe SK, Plebani M, Clark PM. Thyroglobulin measurement by highly sensitive assays: focus on laboratory challenges. Clin Chem Lab Med. 2015;53(9):1301–14.
78. Rosario PW, Mourao GF, Calsolari MR. Low postoperative nonstimulated thyroglobulin as a criterion for the indication of low radioiodine activity in patients with papillary thyroid cancer of intermediate risk 'with higher risk features'. Clin Endocrinol. 2016;85(3):453–8.
79. Spencer CA, Wang CC. Thyroglobulin measurement. Techniques, clinical benefits, and pitfalls. Endocrinol Metab Clin N Am. 1995;24(4):841–63.
80. Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyroglobulin assays. Clin Chem. 1996;42:164–73.
81. Algeciras-Schimnich A, Bruns DE, Boyd JC, Bryant SC, La Fortune KA, Grebe SK. Failure of current laboratory protocols to detect lot-to-lot reagent differences: findings and possible solutions. Clin Chem. 2013;59:1187–94.
82. Spencer CA, Wang CC. Thyroglobulin measurement: techniques, clinical benefits and pitfalls. Endocrinol Metab Clin N Am. 1995;24:841–63.
83. Ross HA, Netea-Maier RT, Schakenraad E, Bravenboer B, Hermus AR, Sweep FC. Assay bias may invalidate decision limits and affect comparability of serum thyroglobulin assay methods: an approach to reduce interpretation differences. Clin Chim Acta. 2008;394:104–9.
84. Zucchelli G, Iervasi A, Ferdeghini M, Iervasi G. Serum thyroglobulin measurement in the follow-up of patients treated for differentiated thyroid cancer. Q J Nucl Med Mol Imaging. 2009;53:482–9.
85. Reix N, Massart C, Gasser F, Heurtault B, Agin A. Should functional sensitivity of a new thyroid stimulating hormone immunoassay be monitored routinely? The ADVIA Centaur TSH3-UL assay experience. Clin Biochem. 2012;45:1260–2.
86. Nicoloff JSC. Use and misuse of the sensitive thyrotropin assays. J Clin Endocrinol Metab. 1990;71:553–8.
87. Spencer CA, LoPresti JS, Patel A, Guttler RB, Eigen A, Shen D, et al. Applications of a new chemiluninometric thyrotropin assay to subnormal measurement. J Clin Endocrinol Metab. 1990;70:453–60.
88. Van Herle AJ, Uller RP, Matthews NL, Brown J. Radioimmunoassay for measurement of thyroglobulin in human serum. J Clin Invest. 1973;52:1320–7.
89. Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, Lopresti JS. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. J Clin Endocrinol Metab. 2005;90(10):5566–75.
90. Giovanella L, Clark P, Chiovato L, Duntas LH, Elisei R, Feldt-Rasmussen U, et al. Thyroglobulin measurement using highly sensitive assays in patients with differentiated thyroid cancer: a clinical position paper. Eur J Endocrinol. 2014;171:R33–46.
91. Haugen BR, Ladenson PW, Cooper DS, Pacini F, Reiners C, Luster M, et al. A comparison of recombinant human thyrotropin and thyroid hormone withdrawal for the detection of thyroid remnant or cancer. J Clin Endocrinol Metab. 1999;84:3877–85.
92. Pacini F, Castagna MG. Diagnostic and therapeutic use of recombinant human TSH (rhTSH) in differentiated thyroid cancer. Best Pract Res Clin Endocrinol Metab. 2008;22:1009–21.
93. Spencer CA, Fatemi S, Singer P, Nicoloff JT, LoPresti JS. Serum basal thyroglobulin measured by a 2nd generation assay correlates with the recombinant human TSH-Stimulated thyroglobulin response in patients treated for differentiated thyroid cancer. Thyroid. 2010;20:587–95.
94. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. The American Thyroid Association Guidelines Taskforce. Thyroid. 2006;16:109–42.
95. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol. 2006;154:787–803.
96. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid. 2009;19:1167–24.
97. Zophel K, Wunderlich G, Smith BR. Serum thyroglobulin measurements with a high sensitivity enzyme-linked immunosorbent assay: is there a clinical benefit in patients with differentiated thyroid carcinoma? Thyroid. 2003;13:861–5.
98. Kloos RT, Mazzaferri EL. A single recombinant human thyrotropin-stimulated serum thyroglobulin measurement predicts differentiated thyroid carcinoma metastases three to five years later. J Clin Endocrinol Metab. 2005;90:5047–57.
99. Rosario PW, Borges MA, Fagundes TA, Franco AC, Purisch S. Is stimulation of thyroglobulin (Tg) useful in low-risk patients with thyroid carcinoma and undetectable Tg on thyroxin and negative neck ultrasound? Clin Endocrinol. 2005;62:121–5.
100. Iervasi A, Iervasi G, Carpi A, Zucchelli GC. Serum thyroglobulin measurement: clinical background and main methodological aspects with clinical impact. Biomed Pharmacother. 2006;60:414–24.
101. Iervasi A, Iervasi G, Ferdeghini M, Solimeo C, Bottoni A, Rossi L, et al. Clinical relevance of highly sensitive Tg assay in monitoring patients treated

for differentiated thyroid cancer. Clin Endocrinol. 2007;67(6):434–41.
102. Smallridge RC, Meek SE, Morgan MA, Gates GS, Fox TP, Grebe S, et al. Monitoring thyroglobulin in a sensitive immunoassay has comparable sensitivity to recombinant human TSH-stimulated thyroglobulin in follow-up of thyroid cancer patients. J Clin Endocrinol Metab. 2007;92:82–7.
103. Mazzaferri EL. Will highly sensitive thyroglobulin assays change the management of thyroid cancer? Clin Endocrinol. 2007;67:321–3.
104. Persoon AC, Jager PL, Sluiter WJ, Plukker JT, Wolffenbuttel BH, Links TP. A sensitive Tg assay or rhTSH stimulated Tg: what's the best in the long-term follow-up of patients with differentiated thyroid carcinoma. PLoS ONE. 2007;2:e816.
105. Giovanella L. Highly sensitive thyroglobulin measurements in differentiated thyroid carcinoma management. Clin Chem Lab Med. 2008;46(8):1067–73.
106. Spencer CA, Lopresti JS. Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Nat Clin Pract Endocrinol Metab. 2008;4:223–33.
107. Malandrino P, Latina A, Marescalco S, Spadaro A, Regalbuto C, Fulco RA, et al. Risk-adapted management of differentiated thyroid cancer assessed by a sensitive measurement of basal serum thyroglobulin. J Clin Endocrinol Metab. 2011;96:1703–9.
108. Chindris AM, Diehl NN, Crook JE, Fatourechi V, Smallridge RC. Undetectable sensitive serum thyroglobulin (<0.1 ng/ml) in 163 patients with follicular cell-derived thyroid cancer: results of rhTSH stimulation and neck ultrasonography and long-term biochemical and clinical follow-up. J Clin Endocrinol Metab. 2012;97(8):2714–23.
109. Trimboli P, La Torre D, Ceriani L, Condorelli E, Laurenti O, Romanelli F, et al. High sensitive thyroglobulin assay on thyroxine therapy: can it avoid stimulation test in low and high risk differentiated thyroid carcinoma patients? Horm Metab Res. 2013;45(9):664–8.
110. Giovanella L, Treglia G, Sadeghi R, Trimboli P, Ceriani L, Verburg FA. Unstimulated high-sensitive thyroglobulin in follow-up of differentiated thyroid cancer patients: a meta-analysis. J Clin Endocrinol Metab. 2014;99:440–7.
111. Giovanella L, Clark PM, Chiovato L, Duntas L, Elisei R, Feldt-Rasmussen U, et al. Thyroglobulin measurement using highly sensitive assays in patients with differentiated thyroid cancer: a clinical position paper. Eur J Endocrinol. 2014;171(2):R33–46.
112. Feldt-Rasmussen U, Profilis C, Colinet E, Black E, Bornet H, Bourdoux P, et al. Human thyroglobulin reference material (CRM 457) 1st part: assessment of homogeneity, stability and immunoreactivity. Ann Biol Clin. 1996;54:337–42.
113. Feldt-Rasmussen U, Profilis C, Colinet E, Black E, Bornet H, Bourdoux P, et al. Human thyroglobulin reference material (CRM 457) 2nd part: physicochemical characterization and certification. Ann Biol Clin. 1996;54:343–8.
114. Netzel BC, Grebe SK, Carranza Leon BG, Castro MR, Clark PM, Hoofnagle AN, et al. Thyroglobulin (Tg) testing revisited: Tg Assays, TgAb assays, and correlation of results with clinical outcomes. J Clin Endocrinol Metab. 2015;100(8):E1074–83.
115. Kim M, Jeon MJ, Kim WG, Lee JJ, Ryu JS, Cho EJ, et al. Comparison of thyroglobulin measurements using three different immunoassay kits: a BRAMHS Tg-Plus RIA Kit, a BRAMHS hTg sensitive kryptor kit, and a Beckman Coulter ACCESS immunoassay kit. Endocrinol Metab. 2016;31:462–8.
116. Jensen E, Petersen PH, Blaabjerg O, Hegedüs L. Biological variation of thyroid autoantibodies and thyroglobulin. Clin Chem Lab Med. 2007;45:1058–64.
117. Feldt-Rasmussen U, Petersen PH, Blaabjerg O, Horder M. Long-term variability in serum thyroglobulin and thyroid related hormones in healthy subjects. Acta Endocrinol. 1980;95:328–34.
118. Heilig B, Hufner M, Dorken B, Schmidt-Gayk H. Increased heterogeneity of serum thyroglobulin in thyroid cancer patients as determined by monoclonal antibodies. Klin Wochenschr. 1986;64:776–80.
119. Ross HA, Menheere PP, Thomas CM, Mudde AH, Kouwenberg M, Wolffenbuttel BH. Interference from heterophilic antibodies in seven current TSH assays. Ann Clin Biochem. 2008;45:616–8.
120. Clark P, Franklyn J. Can we interpret serum thyroglobulin results? Ann Clin Biochem. 2012;49:313–22.
121. Fenouillet E, Fayet G, Hovsepian S, Bahraoui EM, Ronin C. Immunochemical evidence for a role of complex carbohydrate chains in thyroglobulin antigenicity. J Biol Chem. 1986;261:15153–8.
122. Cubero JM, Rodríguez-Espinosa J, Gelpi C, Estorch M, Corcoy R. Thyroglobulin autoantibody levels below the cut-off for positivity can interfere with thyroglobulin measurement. Thyroid. 2003;13:659–61.
123. Spencer C, Petrovic I, Fatemi S. 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. 2011;96(5):1283–91.
124. Rotmensch S, Cole LA. False diagnosis and needless therapy of presumed malignant disease in women with false-positive human chorionic gonadotropin concentrations. Lancet. 2000;355:712–5.
125. Jones AM, Honour JW. Unusual results from immunoassays and the role of the clinical endocrinologist. Clin Endocrinol. 2006;64(3):234–44.
126. Ballieux BE, Weijl NI, Gelderblom H, van Pelt J, Osanto S. False-positive serum human chorionic gonadotropin (HCG) in a male patient with a malignant germ cell tumor of the testis: a case report and review of the literature. Oncologist. 2008;13(11):1149–54.

127. Henry N, Sebe P, Cussenot O. Inappropriate treatment of prostate cancer caused by heterophilic antibody interference. Nat Clin Pract Urol. 2009;6(3):164–7.
128. Georges A, Charrie A, Raynaud S, Lombard C, Corcuff JB. Thyroxin overdose due to rheumatoid factor interferences in thyroid-stimulating hormone assays. Clin Chem Lab Med. 2011;49(5):873–5.
129. Bjerner J, Bolstad N, Piehler A. Belief is only half the truth--or why screening for heterophilic antibody interference in certain assays makes double sense. Ann Clin Biochem. 2012;49(Pt 4):381–6.
130. Pishdad GR, Pishdad P, Pishdad R. The effect of glucocorticoid therapy on a falsely raised thyrotropin due to heterophilic antibodies. Thyroid. 2013;23(12): 1657–8.
131. Marks V. False-positive immunoassay results: a multicenter survey of erroneous immunoassay results from assays of 74 analytes in 10 donors from 66 laboratories in seven countries. Clin Chem. 2002;48:2008–16.
132. Ellis MJ, Livesey JH. Techniques for identifying heterophile antibody interference are assay specific: study of seven analytes on two automated immunoassay analyzers. Clin Chem. 2005;51: 639–41.
133. Lewandowski KC, Dabrowska K, Lewinski A. Case report: When measured free T4 and free T3 may be misleading. Interference with free thyroid hormones measurements on Roche(R) and Siemens(R) platforms. Thyroid Res. 2012;5(1):11.
134. Bolstad N, Warren DJ, Nustad K. Heterophilic antibody interference in immunometric assays. Best Pract Res Clin Endocrinol Metab. 2013;27(5):647–61.
135. Emerson JF, Ngo G, Emerson SS. Screening for interference in immunoassays. Clin Chem. 2003;49(7):1163–9.
136. Massart C, Corcuff JB, Bordenave L. False-positive results corrected by the use of heterophilic antibody-blocking reagent in thyroglobulin immunoassays. Clin Chim Acta. 2008;388:211–3.
137. Koshida S, Asanuma K, Kuribayashi K, Goto M, Tsuji N, Kobayashi D, et al. Prevalence of human anti-mouse antibodies (HAMAs) in routine examinations. Clin Chim Acta. 2010;411:391–4.
138. Ismail AA. On detecting interference from endogenous antibodies in immunoassays by doubling dilutions test. Clin Chem Lab Med. 2007;45(7):851–4.
139. Gulbahar O, Konca Degertekin C, Akturk M, Yalcin MM, Kalan I, Atikeler GF, et al. A case with immunoassay interferences in the measurement of multiple hormones. J Clin Endocrinol Metab. 2015;100(6):2147–53.
140. Klee GG. Interferences in hormone immunoassays. Clin Lab Med. 2004;24(1):1–18.
141. Sturgeon CM, Viljoen A. Analytical error and interference in immunoassay: minimizing risk. Ann Clin Biochem. 2011;48:418–32.
142. Kricka LJ. Human anti-animal antibody interference in immunological assays. Clin Chem. 1999;45:942–56.
143. Levinson SS, Miller JJ. Towards a better understanding of heterophile (and the like) antibody interference with modern immunoassays. Clin Chim Acta. 2002;325:1–15.
144. Covinsky M, Laterza O, Pfeifer JD, Farkas-Szallasi T, Scott MG. Lamda antibody to Esherichia coli produces false-positive results in multiple immunometric assays. Clin Chem. 2000;46:1157–61.
145. Bjerner J, Olsen KH, Bormer OP, Nustad K. Human heterophilic antibodies display specificity for murine IgG subclasses. Clin Biochem. 2005;38(5):465–72.
146. Preissner CM, Dodge LA, O'Kane DJ, Singh RJ, Grebe SK. Prevalence of heterophilic antibody interference in eight automated tumor marker immunoassays. Clin Chem. 2005;51:208–10.
147. Despres N, Grant AM. Antibody interference in thyroid assays: a potential for clinical misinformation. Clin Chem. 1998;44:440–54.
148. Astarita G, Gutierrez S, Kogovsek N, Mormandi E, Otero P, Calabrese C, et al. False positive in the measurement of thyroglobulin induced by rheumatoid factor. Clin Chim Acta. 2015;447:43–6.
149. Preissner CM, O'Kane DJ, Singh RJ, Morris JC, Grebe SK. Phantoms in the assay tube: heterophile antibody interferences in serum thyroglobulin assays. J Clin Endocrinol Metab. 2003;88(7):3069–74.
150. Giovanella L, Ghelfo A. Undetectable serum thyroglobulin due to negative interference of heterophile antibodies in relapsing thyroid carcinoma. Clin Chem. 2007;53:1871–2.
151. Giovanella L, Keller F, Ceriani L, Tozzoli R. Heterophile antibodies may falsely increase or decrease thyroglobulin measurement in patients with differentiated thyroid carcinoma. Clin Chem Lab Med. 2009;47:952–4.
152. Verburg FA, Wäschle K, Reiners C, Giovanella L, Lentjes EG. Heterophile antibodies rarely influence the measurement of thyroglobulin and thyroglobulin antibodies in differentiated thyroid cancer patients. Horm Metab Res. 2010;42:736–9.
153. Netzel BC, Grebe SK, Algeciras-Schimnich A. Usefulness of a thyroglobulin liquid chromatography-tandem mass spectrometry assay for evaluation of suspected heterophile interference. Clin Chem. 2014;60:1016–8.
154. Petrovic I, Mandel S, Fatemi S, LoPresti J, Spencer C. Heterophile antibodies (HAb/HAMA) interfere with automated TgAb IMA tests. Thyroid. 2016;26(S1):A120.
155. Weber TH, Käpyaho KI, Tanner P. Endogenous interference in immunoassays in clinical chemistry. A review. Scand J Clin Lab Invest. 1990;201:77–82.
156. Bjerner J, Nustad K, Norum LF, Olsen KH, Børmer OP. Immunometric assay interference: incidence and prevention. Clin Chem. 2002;48:613–21.
157. Ghosh S, Howlett M, Boag D, Malik I, Collier A. Interference in free thyroxine immunoassay. Eur J Intern Med. 2008;19:221–2.

158. Nakano K, Yasuda K, Shibuya H, Moriyama T, Kahata K, Shimizu C. Transient human anti-mouse antibody generated with immune enhancement in a carbohydrate antigen 19-9 immunoassay after surgical resection of recurrent cancer. Ann Clin Biochem. 2016;53(Pt 4):511–5.
159. Rulander NJ, Cardamone D, Senior M, Snyder PJ, Master SR. Interference from anti-streptavidin antibody. Arch Pathol Lab Med. 2013;137(8):1141–6.
160. Vos MJ, Rondeel JM, Mijnhout GS, Endert E. Immunoassay interference caused by heterophilic antibodies interacting with biotin. Clin Chem Lab Med. 2016;55:e122–6.
161. Kwok JS, Chan IH, Chan MH. Biotin interference on TSH and free thyroid hormone measurement. Pathology. 2012;44(3):278–80.
162. Wijeratne NG, Doery JC, Lu ZX. Positive and negative interference in immunoassays following biotin ingestion: a pharmacokinetic study. Pathology. 2012;44(7):674–5.
163. Elston MS, Sehgal S, Du Toit S, Yarndley T, Conaglen JV. Factitious Graves' disease due to biotin immunoassay interference-A case and review of the literature. J Clin Endocrinol Metab. 2016;101(9):3251–5.
164. Pedersen IB, Laurberg P. Biochemical hyperthyroidism in a newborn baby caused by assay interaction from biotin intake. Eur Thyroid J. 2016;5:212–5.
165. Barbesino G. Misdiagnosis of Graves' disease with apparent severe hyperthyroidism in a patient taking biotin megadoses. Thyroid. 2016;26(6):860–3.
166. Chun KY. Biotin interference in diagnostic tests. Clin Chem. 2017;63(2):619–20.
167. Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, et al. Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab. 1998;83:1121–7.
168. Kumar A, Shah DH, Shrihari U, Dandekar SR, Vijayan U, Sharma SM. Significance of antithyroglobulin autoantibodies in differentiated thyroid carcinoma. Thyroid. 1994;4:199–202.
169. Gorges R, Maniecki M, Jentzen W, Sheu SN, Mann K, Bockisch A, et al. Development and clinical impact of thyroglobulin antibodies in patients with differentiated thyroid carcinoma during the first 3 years after thyroidectomy. Eur J Endocrinol. 2005;153(1):49–55.
170. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab. 2002;87(2):489–99.
171. Latrofa F, Ricci D, Montanelli L, Rocchi R, Piaggi P, Sisti E, et al. Thyroglobulin autoantibodies in patients with papillary thyroid carcinoma: comparison of different assays and evaluation of causes of discrepancies. J Clin Endocrinol Metab. 2012;97:3974–82.
172. Schaadt B, Feldt-Rasmussen U, Rasmussen B, Torring H, Foder B, Jorgensen K, et al. 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. 1995;5:165–70.
173. Spencer CA. Recoveries cannot be used to authenticate thyroglobulin (Tg) measurements when sera contain Tg autoantibodies. Clin Chem. 1996;42(5):661–3.
174. Ericsson UB, Christensen SB, Thorell JI. A high prevalence of thyroglobulin autoantibodies in adults with and without thyroid disease as measured with a sensitive solid-phase immunosorbent radioassay. Clin Immunol Immunopathol. 1985;37:154–62.
175. Ruf J, Carayon P, Lissitzky S. Various expressions of a unique anti-human thyroglobulin antibody repertoire in normal state and autoimmune disease. Eur J Immunol. 1985;15:268–72.
176. Okosieme OE, Evans C, Moss L, Parkes AB, Premawardhana LD, Lazarus JH. Thyroglobulin antibodies in serum of patients with differentiated thyroid cancer: relationship between epitope specificities and thyroglobulin recovery. Clin Chem. 2005;51(4):729–34.
177. Latrofa F, Ricci D, Montanelli L, Piaggi P, Mazzi B, Bianchi F, et al. Thyroglobulin autoantibodies switch to immunoglobulin (Ig)G1 and IgG3 subclasses and preserve their restricted epitope pattern after 131I treatment for Graves' hyperthyroidism: the activity of autoimmune disease influences subclass distribution but not epitope pattern of autoantibodies. Clin Exp Immunol. 2014;178(3):438–46.
178. Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyroglobulin assays. Clin Chem. 1996;42(1):164–73.
179. Tozzoli R, Bizzaro N, Tonutti E, Pradella M, Manoni F, Vilalta D, et al. Immunoassay of antithyroid autoantibodies: high analytical variability in second generation methods. Clin Chem Lab Med. 2002;40:568–73.
180. Benvenga S, Burek CL, Talor M, Rose NR, Trimarchi F. Heterogeneity of the thyroglobulin epitopes associated with circulating thyroid hormone autoantibodies in hashimoto's thyroiditis and non-autoimmune thyroid diseases. J Endocrinol Investig. 2002;25:977–82.
181. Rosário PW, Maia FF, Fagundes TA, Vasconcelos FP, Cardoso LD, Purisch S. Antithyroglobulin antibodies in patients with differentiated thyroid carcinoma: methods of detection, interference with serum thyroglobulin measurement and clinical significance. Arq Bras Endocrinol Metabol. 2004;48:487–92.
182. Beever K, Bradbury J, Phillips D, McLachlan SM, Pegg C, Goral A, et al. Highly sensitive assays of autoantibodies to thyroglobulin and to thyroid peroxidase. Clin Chem. 1989;35:1949–54.
183. Feldt-Rasmussen U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin and thyrotropin receptor. Clin Chem. 1996;42:160–3.

184. La'ulu SL, Slev PR, Roberts WL. Performance characteristics of 5 automated thyroglobulin autoantibody and thyroid peroxidase autoantibody assays. Clin Chim Acta. 2007;376:88–95.
185. Krahn J, Dembinski T. Thyroglobulin and antithyroglobulin assays in thyroid cancer monitoring. Clin Biochem. 2009;42:416–9.
186. Pickett AJ, Jones M, Evans C. Causes of discordance between thyroglobulin antibody assays. Ann Clin Biochem. 2012;49:463–7.
187. Taylor KP, Parkington D, Bradbury S, Simpson HL, Jefferies SJ, Halsall DJ. Concordance between thyroglobulin antibody assays. Ann Clin Biochem. 2011;48(Pt 4):367–9.
188. Ruf J, Henry M, DeMicco C, Carayon P. Characterization of monoclonal and autoimmune antibodies to thyroglobulin: application to clinical investigation. In: Hufner M, Reiners C, editors. Thyroglobulin and thyrolobulin antibodies in the follow-up of thyroid cancer and endemic goiter. Stuttgart: G Thieme; 1987. p. 21–30.
189. Spencer CA, Platler BW, Nicoloff JT. The effect of 125-I thyroglobulin tracer heterogeneity on serum Tg RIA measurement. Clin Chim Acta. 1985;153:105–15.
190. Spencer CA, Platler B, Guttler RB, Nicoloff JT. Heterogeneity of 125-I labelled thyroglobulin preparations. Clin Chim Acta. 1985;151:121–32.
191. Black EG, Hoffenberg R. Should one measure serum thyroglobulin in the presence of antithyroglobulin antibodies? Clin Endocrinol. 1983;19:597–601.
192. Mariotti S, Barbesino G, Caturegli P, Marino M, Manetti L, Pacini F, et al. Assay of thyroglobulin in serum with thyroglobulin autoantibodies: an unobtainable goal? J Clin Endocrinol Metab. 1995;80:468–72.
193. Weightman DR, Mallick UK, Fenwick JD, Perros P. 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. 2003;98(1):41–7.
194. Jahagirdar VR, Strouhal P, Holder G, Gama R, Singh BM. Thyrotoxicosis factitia masquerading as recurrent Graves' disease: endogenous antibody immunoassay interference, a pitfall for the unwary. Ann Clin Biochem. 2008;45(Pt 3):325–7.
195. Schneider AB, Pervos R. Radioimmunoassay of human thyroglobulin: effect of antithyroglobulin autoantibodies. J Clin Endocrinol Metab. 1978;47:126–37.
196. Feldt-Rasmussen U, Rasmussen AK. Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb in vivo and in vitro. J Endocrinol Investig. 1985;8:571–6.
197. Miles LEM, Hales CN. Labeled antibodies and immunological assay systems. Nature. 1968;219:186–9.
198. Bayer MF, Kriss JP. Immunoradiometric assay for serum thyroglobulin: semiquantitative measurement of thyroglobulin in antithyroglobulin-positive sera. J Clin Endocrinol Metab. 1979;49:557–64.
199. Latrofa F, Ricci D, Grasso L, Vitti P, Masserini L, Basolo F, et al. Characterization of thyroglobulin epitopes in patients with autoimmune and non-autoimmune thyroid diseases using recombinant human monoclonal thyroglobulin autoantibodies. J Clin Endocrinol Metab. 2008;93:591–6.
200. Rahmoun MN, Bendahmane I. Anti-thyroglobulin antibodies in differentiated thyroid carcinoma patients: study of the clinical and biological parameters. Ann Endocrinol (Paris). 2014;75(1):15–8.
201. Marquet PY, Daver A, Sapin R, Bridgi B, Muratet JP, Hartmann DJ, et al. Highly sensitive immunoradiometric assay for serum thyroglobulin with minimal interference from autoantibodies. Clin Chem. 1996;42:258–62.
202. Grebe SKG. Diagnosis and management of thyroid carcinoma: a focus on serum thyroglobulin. Expert Rev Endocrinol Metab. 2009;4:25–43.
203. Haapala AM, Soppi E, Morsky P, et al. Thyroid antibodies in association with thyroid malignancy II: qualitative properties of thyroglobulin antibodies. Scand J Clin Lab Invest. 1995;55:317–22.
204. Hoofnagle AN, Becker JO, Wener MH, Heinecke JW. Quantification of thyroglobulin, a low-abundance serum protein, by immunoaffinity peptide enrichment and tandem mass spectrometry. Clin Chem. 2008;54(11):1796–804.
205. Netzel BC, Grant RP, Hoofnagle AN, Rockwood AL, Shuford CM, Grebe SK. First steps toward harmonization of LC-MS/MS thyroglobulin assays. Clin Chem. 2016;62(1):297–9.
206. Hoofnagle AN, Wener MH. The fundamental flaws of immunoassays and potential solutions using tandem mass spectrometry. J Immunol Methods. 2009;347(1-2):3–11.
207. Grebe SK. Soluble thyroid tumor markers – old and new challenges and potential solutions. N Z J Med Lab Sci. 2013;67:76–87.
208. Jindal A, Khan U. Is thyroglobulin level by liquid chromatography tandem-mass spectrometry always reliable for follow-up of DTC after thyroidectomy: a report on two patients. Thyroid. 2016;26(9):1334–5.
209. Azmat U, Porter K, Senter L, Ringel MD, Nabhan F. Thyroglobulin liquid chromatography-tandem mass spectrometry has a low sensitivity for detecting structural disease in patients with antithyroglobulin antibodies. Thyroid. 2016;27(1):74–80.
210. Petrovic I, Fatemi S, LoPresti J, Grebe SK, Algeciras-Schimnich A, Netzel BC, et al. SerumTg is frequently undetectable by mass spectrometry (Tg-MS) IN TgAb-positive differentiated thyroid cancer (DTC) patients with structural disease. Thyroid. 2015;25(S1):A251.
211. Crane MS, Strachan MW, Toft AD, Beckett GJ. Discordance in thyroglobulin measurements by radioimmunoassay and immunometric assay: a use-

ful means of identifying thyroglobulin assay interference. Ann Clin Biochem. 2013;50:421–32.
212. Spencer C, Fatemi S. Thyroglobulin antibody (TgAb) methods - strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer. Best Pract Res Clin Endocrinol Metab. 2013;27(5):701–12.
213. Feldt-Rasmussen U, Petersen PH, Date J, Madsen CM. Sequential changes in serum thyroglobulin (Tg) and its autoantibodies (TgAb) following subtotal thyroidectomy of patients with preoperatively detectable TgAb. Clin Endocrinol. 1980;12:29–38.
214. Fleck RA, Rapaport SI, Rao LV. Anti-prothrombin antibodies and the lupus anticoagulant. Blood. 1988;72(2):512–9.
215. van der Laken CJ, Voskuyl AE, Roos JC, Stigter van Walsum M, de Groot ER, Wolbink G, et al. Imaging and serum analysis of immune complex formation of radiolabelled infliximab and anti-infliximab in responders and non-responders to therapy for rheumatoid arthritis. Ann Rheum Dis. 2007;66(2):253–6.
216. Richards DB, Cookson LM, Berges AC, Barton SV, Lane T, Ritter JM, et al. Therapeutic clearance of amyloid by antibodies to serum amyloid P component. N Engl J Med. 2015;373(12):1106–14.
217. Latrofa F, Ricci D, Sisti E, Piaggi P, Nencetti C, Marino M, et al. Significance of low levels of thyroglobulin autoantibodies associated with undetectable thyroglobulin after thyroidectomy for differentiated thyroid carcinoma. Thyroid. 2016;26(6):798–806.
218. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. J Cancer Epidemiol. 2013;2013:965212.
219. Ahn HS, Kim HJ, Welch HG. Korea's thyroid-cancer "epidemic"--screening and overdiagnosis. N Engl J Med. 2014;371(19):1765–7.
220. Oda H, Miyauchi A, Ito Y, Yoshioka K, Nakayama A, Sasai H, et al. Incidences of unfavorable events in the management of low-risk papillary microcarcinoma of the thyroid by active surveillance versus immediate surgery. Thyroid. 2016;26(1):150–5.
221. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA. 2006;295:2164–7.
222. Zhu C, Zheng T, Kilfoy BA, Han X, Ma S, Ba Y, et al. A birth cohort analysis of the incidence of papillary thyroid cancer in the United States, 1973–2004. Thyroid. 2009;19:1061–6.
223. Udelsman R, Zhang Y. The epidemic of thyroid cancer in the United States: the role of endocrinologists and ultrasounds. Thyroid. 2014;24(3):472–9.
224. Vaccarella S, Franceschi S, Bray F, Wild CP, Plummer M, Dal Maso L. Worldwide thyroid-cancer epidemic? The increasing impact of overdiagnosis. N Engl J Med. 2016;375(7):614–7.
225. Hundahl SA, Cady B, Cunningham MP, Mazzaferri E, McKee RF, Rosai J, et al. Initial results from a prospective cohort study of 5583 cases of thyroid carcinoma treated in the United States during 1996. U.S. and German Thyroid Cancer Study Group. An American College of Surgeons Commission on cancer patient care evaluation study. Cancer. 2000;89(1):202–17.
226. Mazzaferri EL, Kloos RT. Current approaches to primary therapy for papillary and follicular thyroid cancer. J Clin Endocrinol Metab. 2001;86:1447–63.
227. Hay ID, Thompson GB, Grant CS, Bergstralh EJ, Dvorak CE, Gorman CA, et al. Papillary thyroid carcinoma managed at the Mayo Clinic during six decades (1940-1999): temporal trends in initial therapy and long-term outcome in 2444 consecutively treated patients. World J Surg. 2002;26:879–85.
228. Pitoia F, Bueno F, Urciuoli C, Abelleira E, Cross G, Tuttle RM. Outcomes of patients with differentiated thyroid cancer risk-stratified according to the american thyroid association and latin american thyroid society risk of recurrence classification systems. Thyroid. 2013;23:1401–7.
229. Tuttle RM, Leboeuf R. Follow up approaches in thyroid cancer: a risk adapted paradigm. Endocrinol Metab Clin N Am. 2008;37:419–35.
230. Pitoia F, Ward L, Wohllk N, Friguglietti C, Tomimori E, Gauna A, et al. Recommendations of the Latin American Thyroid Society on diagnosis and management of differentiated thyroid cancer. Arq Bras Endocrinol Metabol. 2009;53(7):884.
231. Ashcraft MW, Van Herle AJ. The comparative value of serum thyroglobulin measurements and iodine 131 total body scans in the follow-up of patients with treated differentiated thyroid cancer. Am J Med. 1981;71:806–14.
232. Pineda JD, Lee T, Ain K, Reynolds JC, Robbins J. Iodine-131 therapy for thyroid cancer patients with elevated thyroglobulin and negative diagnostic scan. J Clin Endocrinol Metab. 1995;80:1488–92.
233. Baudin E, Do Cao C, Cailleux AF, Leboulleux S, Travagli JP, Schlumberger M. Positive predictive value of serum thyroglobulin levels, measured during the first year of follow-up after thyroid hormone withdrawal, in thyroid cancer patients. J Clin Endocrinol Metab. 2003;88:1107–11.
234. Smallridge RC, Diehl N, Bernet V. Practice trends in patients with persistent detectable thyroglobulin and negative diagnostic radioiodine whole body scans: a survey of American Thyroid Association members. Thyroid. 2014;24(10):1501–7.
235. Lamartina L, Durante C, Filetti S, Cooper DS. Low-risk differentiated thyroid cancer and radioiodine remnant ablation: a systematic review of the literature. J Clin Endocrinol Metab. 2015;100(5):1748–61.
236. Pacini F, Agate L, Elisei R, Capezzone M, Ceccarelli C, Lippi F, et al. Outcome of differentiated thyroid cancer with detectable serum Tg and negative diagnostic (131)I whole body scan: comparison of patients treated with high (131)I activities versus untreated patients. J Clin Endocrinol Metab. 2001;86:4092–7.

237. Schaap J, Eustatia-Rutten CF, Stokkel M, Links TP, Diamant M, van der Velde EA, et al. Does radioiodine therapy have disadvantageous effects in non-iodine accumulating differentiated thyroid carcinoma. Clin Endocrinol. 2002;57:117–24.
238. Valadão MM, Rosário PW, Borges MA, Costa GB, Rezende LL, Padrão EL, et al. Positive predictive value of detectable stimulated tg during the first year after therapy of thyroid cancer and the value of comparison with Tg-ablation and Tg measured after 24 months. Thyroid. 2006;16:1145–9.
239. Rosario P, Borges M, Reis J, Alves MF. Effect of suppressive therapy with levothyroxine on the reduction of serum thyroglobulin after total thyroidectomy. Thyroid. 2006;16:199–200.
240. Huang SH, Wang PW, Huang YE, Chou FF, Liu RT, Tung SC, et al. Sequential follow-up of serum thyroglobulin and whole body scan in thyroid cancer patients without initial metastasis. Thyroid. 2006;16:1273–8.
241. Giovanella L, Ceriani L, Suriano S, Ghelfo A, Maffioli M. Thyroglobulin measurement before rhTSH-aided (131)I ablation in detecting metastases from differentiated thyroid carcinoma. Clin Endocrinol. 2008;68:659–63.
242. Miyauchi A, Kudo T, Miya A, Kobayashi K, Ito Y, Takamura Y, et al. Prognostic impact of serum thyroglobulin doubling-time under thyrotropin suppression in patients with papillary thyroid carcinoma who underwent total thyroidectomy. Thyroid. 2011;21(7):707–16.
243. Gibelli B, Tredici P, De Cicco C, Bodei L, Sandri MT, Renne G, et al. Preoperative determination of serum thyroglobulin to identify patients with differentiated thyroid cancer who may present recurrence without increased thyroglobulin. Acta Otorhinolaryngol Ital. 2005;25:94–9.
244. Giovanella L, Ceriani L, Ghelfo A, Maffioli M, Keller F. Preoperative undetectable serum thyroglobulin in differentiated thyroid carcinoma: incidence, causes and management strategy. Clin Endocrinol. 2007;67:547–51.
245. Durante C, Puxeddu E, Ferretti E, Morisi R, Moretti S, Bruno R, et al. BRAF mutations in papillary thyroid carcinomas inhibit genes involved in iodine metabolism. J Clin Endocrinol Metab. 2007;92:2840–3.
246. Lazar V, Bidart JM, Caillou B, Mahe C, Lacroix L, Filetti S, et al. Expression of the Na+/I- symporter gene in human thyroid tumors: a comparison study with other thyroid-specific genes. J Clin Endocrinol Metab. 1999;84:3228–34.
247. Gardner DF, Rothman J, Utiger RD. Serum thyroglobulin in normal subjects and patients with hyperthyroidism due to Graves' disease: effects of T3, iodide, 131I and antithyroid drugs. Clin Endocrinol. 1979;11:585–94.
248. Padovani RP, Robenshtok E, Brokhin M, Tuttle RM. Even without additional therapy, serum thyroglobulin concentrations often decline for years after total thyroidectomy and radioactive remnant ablation in patients with differentiated thyroid cancer. Thyroid. 2012;22(8):778–83.
249. Durante C, Montesano T, Attard M, Torlontano M, Monzani F, Costante G, et al. Long-term surveillance of papillary thyroid cancer patients who do not undergo postoperative radioiodine remnant ablation: is there a role for serum thyroglobulin measurement? J Clin Endocrinol Metab. 2012;97:2748–53.
250. Hjiyiannakis P, Mundy J, Harmer C. Thyroglobulin antibodies in differentiated thyroid cancer. Clin Oncol. 1999;11:240–4.
251. Ronga G, Filesi M, Ventroni G, Vestri AR, Signore A. Value of the first serum thyroglobulin level after total thyroidectomy for the diagnosis of metastases from differentiated thyroid carcinoma. Eur J Nucl Med. 1999;26:1448–52.
252. Lima N, Cavaliere E, Tomimori E, Knobel M, Medeieros-Neto G. Prognostic value of serial serum thyroglobulin determinations after total thyroidectomy for differentiated thyroid cancer. J Endocrinol Investig. 2002;25:110–5.
253. Lin JD, Huang MJ, Hsu BR, Chao TC, Hsueh C, Liu FH, et al. Significance of postoperative serum thyroglobulin levels in patients with papillary and follicular thyroid carcinomas. J Surg Oncol. 2002;80:45–51.
254. Hall FT, Beasley NJ, Eski SJ, Witterick IJ, Walfish PG, Freeman JL. Predictive value of serum thyroglobulin after surgery for thyroid carcinoma. Laryngoscope. 2003;113:77–81.
255. Toubeau M, Touzery C, Arveux P, Chaplain G, Vaillant G, Berriolo A, et al. Predictive value for disease progression of serum thyroglobulin levels measured in the postoperative period and after (131) I ablation therapy in patients with differentiated thyroid cancer. J Nucl Med. 2004;45:988–94.
256. Kim TY, Kim WB, Kim ES, Ryu JS, Yeo JS, Kim SC, et al. Serum thyroglobulin levels at the time of 131I remnant ablation just after thyroidectomy are useful for early prediction of clinical recurrence in low-risk patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2005;90:1440–5.
257. Makarewicz J, Adamczewski Z, Knapska-Kucharska M, Lewiński A. Evaluation of the diagnostic value of the first thyroglobulin determination in detecting metastases after differentiated thyroid carcinoma surgery. Exp Clin Endocrinol Diabetes. 2006;114:485–9.
258. Heemstra KA, Liu YY, Stokkel M, Kievit J, Corssmit E, Pereira AM, et al. Serum thyroglobulin concentrations predict disease-free remission and death in differentiated thyroid carcinoma. Clin Endocrinol. 2007;66:58–64.
259. Karatzas T, Vasileiadis I, Zapanti E, Charitoudis G, Karakostas E, Boutzios G. Thyroglobulin antibodies as a potential predictive marker of papillary thyroid carcinoma in patients with indeterminate cytology. Am J Surg. 2016;212:946–52.
260. Shih ML, Lee JA, Hsieh CB, Yu JC, Liu HD, Kebebew E, et al. Thyroidectomy for Hashimoto's

thyroiditis: complications and associated cance. Thyroid. 2008;18:729–34.
261. Tomoda C, Miyauchi A. Undetectable serum thyroglobulin levels in patients with medullary thyroid carcinoma after total thyroidectomy without radioiodine ablation. Thyroid. 2012;22(7):680–2.
262. Angell TE, Spencer CA, Rubino BD, Nicoloff JT, LoPresti JS. In search of an unstimulated thyroglobulin baseline value in low-risk papillary thyroid carcinoma patients not receiving radioactive iodine ablation. Thyroid. 2014;24:1127–33.
263. Spencer CA, Lopresti JS. Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Nat Clin Pract Endocrinol Metab. 2008;4(4):223–33.
264. Giovanella L, Imperiali M, Ferrari A, Palumbo A, Furlani L, Graziani MS, et al. Serum thyroglobulin reference values according to NACB criteria in healthy subjects with normal thyroid ultrasound. Clin Chem Lab Med. 2012;50(5):891–3.
265. Spencer CA, LoPresti JS, Fatemi S, Nicoloff JT. Detection of residual and recurrent differentiated thyroid carcinoma by serum Thyroglobulin measurement. Thyroid. 1999;9:435–41.
266. Pacini F, Molinaro E, Castagna MG, Agate L, Elisei R, Ceccarelli C, et al. Recombinant human thyrotropin-stimulated serum thyroglobulin combined with neck ultrasonography has the highest sensitivity in monitoring differentiated thyroid carcinoma. J Clin Endocrinol Metab. 2003;88:3668–73.
267. Mazzaferri EL, Robbins RJ, Spencer CA, Braverman LE, Pacini F, Wartofsky L, et al. A consensus report of the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab. 2003;88(4):1433–41.
268. Mazzaferri EL, Kloos RT. Is diagnostic iodine-131 scanning with recombinant human TSH useful in the follow-up of differentiated thyroid cancer after thyroid ablation? J Clin Endocrinol Metab. 2002;87:1486–9.
269. Bachelot A, Cailleux AF, Klain M, Baudin E, Ricard M, Bellon N, et al. Relationship between tumor burden and serum thyroglobulin level in patients with papillary and follicular thyroid carcinoma. Thyroid. 2002;12:707–11.
270. Vitale G, Lupoli GA, Ciccarelli A, Lucariello A, Fittipaldi MR, Fonderico F, et al. Influence of body surface area on serum peak thyrotropin (TSH) levels after recombinant human TSH administration. J Clin Endocrinol Metab. 2003;88:1319–22.
271. Montesano T, Durante C, Attard M, Crocetti U, Meringolo D, Bruno R, et al. Age influences TSH serum levels after withdrawal of l-thyroxine or rhTSH stimulation in patients affected by differentiated thyroid cancer. Biomed Pharmacother. 2007;61:468–71.
272. Braverman L, Kloos RT, Law B Jr, Kipnes M, Dionne M, Magner J. Evaluation of various doses of recombinant human thyrotropin in patients with multinodular goiters. Endocr Pract. 2008;14:832–9.
273. Over R, Nsouli-Maktabi H, Burman KD, Jonklaas J. Age modifies the response to recombinant human thyrotropin. Thyroid. 2010;20:1377–84.
274. Schlumberger M, Charbord P, Fragu P, Lumbroso J, Parmentier C, Tubiana M. Circulating thyrotropin and thyroid hormones in patients with metastases of differentiated thyroid carcinoma: relationship to serum thyrotropin levels. J Clin Endocrinol Metab. 1980;51:513–9.
275. Robbins RJ, Srivastava S, Shaha A, Ghossein R, Larson SM, Fleisher M, et al. Factors influencing the basal and recombinant human thyrotropin-stimulated serum thyroglobulin in patients with metastatic thyroid carcinoma. J Clin Endocrinol Metab. 2004;89:6010–6.
276. Nakabashi CC, Kasamatsu TS, Crispim F, Yamazaki CA, Camacho CP, Andreoni DM, et al. Basal serum thyroglobulin measured by a second-generation assay is equivalent to stimulated thyroglobulin in identifying metastases in patients with differentiated thyroid cancer with low or intermediate risk of recurrence. Eur Thyroid J. 2014;3(1):43–50.
277. Groen AH, Klein Hesselink MS, Plukker JT, Sluiter WJ, van der Horst-Schrivers AN, Brouwers AH, et al. Additional value of a high sensitive thyroglobulin assay in the follow-up of patients with differentiated thyroid carcinoma. Clin Endocrinol. 2016;86(3):419–24.
278. Heilo A, Sigstad E, Fagerlid KH, Håskjold OI, Grøholt KK, Berner A, et al. Efficacy of ultrasound-guided percutaneous ethanol injection treatment in patients with a limited number of metastatic cervical lymph nodes from papillary thyroid carcinoma. J Clin Endocrinol Metab. 2011;96:2750–5.
279. Hay ID, Lee RA, Davidge-Pitts C, Reading CC, Charboneau JW. Long-term outcome of ultrasound-guided percutaneous ethanol ablation of selected "recurrent" neck nodal metastases in 25 patients with TNM stages III or IVA papillary thyroid carcinoma previously treated by surgery and 131I therapy. Surgery. 2013;154(6):1448–54. discussion 54-5
280. Yim JH, Kim EY, Bae Kim W, Kim WG, Kim TY, Ryu JS, et al. Long-term consequence of elevated thyroglobulin in differentiated thyroid cancer. Thyroid. 2013;23(1):58–63.
281. Pacini F, Sabra MM, Tuttle RM. Clinical relevance of thyroglobulin doubling time in the management of patients with differentiated thyroid cancer. Thyroid. 2011;21:691–2.
282. Giovanella L, Trimboli P, Verburg FA, Treglia G, Piccardo A, Foppiani L, et al. Thyroglobulin levels and thyroglobulin doubling time independently predict a positive 18F-FDG PET/CT scan in patients with biochemical recurrence of differentiated thyroid carcinoma. Eur J Nucl Med Mol Imaging. 2013;40:874–80.
283. Miyauchi A, Kudo T, Kihara M, Higashiyama T, Ito Y, Kobayashi K, et al. Relationship of biochemically persistent disease and thyroglobulin-doubling time to age at surgery in patients with papillary thyroid carcinoma. Endocr J. 2013;60(4):415–21.

284. Elisei R, Agate L, Viola D, Matrone A, Biagini A, Molinaro E. How to manage patients with differentiated thyroid cancer and a rising serum thyroglobulin level. Endocrinol Metab Clin N Am. 2014;43(2):331–44.
285. Kelders A, Kennes LN, Krohn T, Behrendt FF, Mottaghy FM, Verburg FA. Relationship between positive thyroglobulin doubling time and 18F-FDG PET/CT-positive, 131I-negative lesions. Nucl Med Commun. 2014;34:176–81.
286. Rossing RM, Jentzen W, Nagarajah J, Bockisch A, Gorges R. Serum thyroglobulin doubling time in progressive thyroid cancer. Thyroid. 2016;26(12):1712–8.
287. Pacini F, Pinchera A. Serum and tissue thyroglobulin measurement: clinical applications in thyroid disease. Biochimie. 1999;81:463–7.
288. Rotman-Pikielny P, Reynolds JC, Barker WC, Yen PM, Skarulis MC, Sarlis NJ. Recombinant human thyrotropin for the diagnosis and treatment of a highly functional metastatic struma ovarii. J Clin Endocrinol Metab. 2000;85:237–44.
289. Russo M, Marturano I, Masucci R, Caruso M, Fornito MC, Tumino D, et al. Metastatic malignant struma ovarii with coexistence of Hashimoto's thyroiditis. Endocrinol Diabetes Metabol Case Rep. 2016;2016:160030.
290. Trimboli P, D'Aurizio F, Tozzoli R, Giovanella L. Measurement of thyroglobulin, calcitonin, and PTH in FNA washout fluids. Clin Chem Lab Med. 2016;55:914–25.
291. Pacini F, Fugazzola L, Lippi F, Ceccarelli C, Centoni R, Miccoli P, et al. Detection of thyroglobulin in fine needle aspirates of nonthyroidal neck masses: a clue to the diagnosis of metastatic differentiated thyroid cancer. J Clin Endocrinol Metab. 1992;74:1401–4.
292. Uruno T, Miyauchi A, Shimizu K, Tomoda C, Takamura Y, Ito Y, et al. Usefulness of thyroglobulin measurement in fine-needle aspiration biopsy specimens for diagnosing cervical lymph node metastasis in patients with papillary thyroid cancer. World J Surg. 2005;29:483–5.
293. Boi F, Baghino G, Atzeni F, Lai ML, Faa G, Mariotti S. The diagnostic value for differentiated thyroid carcinoma metastases of thyroglobulin (Tg) measurement in washout fluid from fine-needle aspiration biopsy of neck lymph nodes is maintained in the presence of circulating anti-Tg antibodies. J Clin Endocrinol Metab. 2006;91:1364–9.
294. Rosario PW, de Faria S, Bicalho L, Alves MF, Borges MA, Purisch S, et al. Ultrasonographic differentiation between metastatic and benign lymph nodes in patients with papillary thyroid carcinoma. J Ultrasound Med. 2005;24:1385–9.
295. Snozek CL, Chambers EP, Reading CC, Sebo TJ, Sistrunk JW, Singh RJ, et al. Serum thyroglobulin, high-resolution ultrasound, and lymph node thyroglobulin in diagnosis of differentiated thyroid carcinoma nodal metastases. J Clin Endocrinol Metab. 2007;92:4278–81.
296. Bruno R, Giannasio P, Chiarella R, Capula C, Russo D, Filetti S, et al. Identification of a neck lump as a lymph node metastasis from an occult contralateral papillary microcarcinoma of the thyroid: key role of thyroglobulin assay in the fine-needle aspirate. Thyroid. 2009;19:531–3.
297. Jeon SJ, Kim E, Park JS, Son KR, Baek JH, Kim YS, et al. Diagnostic benefit of thyroglobulin measurement in fine-needle aspiration for diagnosing metastatic cervical lymph nodes from papillary thyroid cancer: correlations with US features. Korean J Radiol. 2009;10:106–11.
298. Cunha N, Rodrigues F, Curado F, Ilhéu O, Cruz C, Naidenov P, et al. Thyroglobulin detection in fine-needle aspirates of cervical lymph nodes: a technique for the diagnosis of metastatic differentiated thyroid cancer. Eur J Endocrinol. 2007;157:101–7.
299. Suh YJ, Son EJ, Moon HJ, Kim EK, Han KH, Kwak JY. Utility of thyroglobulin measurements in fine-needle aspirates of space occupying lesions in the thyroid bed after thyroid cancer operations. Thyroid. 2012;23(3):280–8.
300. Cappelli C, Pirola I, De Martino E, Gandossi E, Cimino E, Samoni F, et al. Thyroglobulin measurement in fine-needle aspiration biopsy of metastatic lymph nodes after rhTSH stimulation. Head Neck. 2013;35:E21–3.
301. Grani G, Fumarola A. Thyroglobulin in lymph node fine-needle aspiration washout: a systematic review and meta-analysis of diagnostic accuracy. J Clin Endocrinol Metab. 2014;99(6):1970–82.
302. Torres MR, Nóbrega Neto SH, Rosas RJ, Martins AL, Ramos AL, da Cruz TR. Thyroglobulin in the washout fluid of lymph-node biopsy: what is its role in the follow-up of differentiated thyroid carcinoma? Thyroid. 2014;24:7–18.
303. Chung J, Kim EK, Lim H, Son EJ, Yoon JH, Youk JH, et al. Optimal indication of thyroglobulin measurement in fine-needle aspiration for detecting lateral metastatic lymph nodes in patients with papillary thyroid carcinoma. Head Neck. 2014;36(6):795–801.
304. Shi JH, Xu YY, Pan QZ, Sui GQ, Zhou JP, Wang H. The value of combined application of ultrasound-guided fine needle aspiration cytology and thyroglobulin measurement for the diagnosis of cervical lymph node metastases from thyroid cancer. Pak J Med Sci. 2015;31(5):1152–5.
305. Tang S, Buck A, Jones C, Sara Jiang X. The utility of thyroglobulin washout studies in predicting cervical lymph node metastases: one academic medical center's experience. Diagn Cytopathol. 2016;44:964–8.
306. Jeon MJ, Kim WG, Jang EK, Choi YM, Lee YM, Sung TY, et al. Thyroglobulin level in fine-needle aspirates for preoperative diagnosis of cervical lymph node metastasis in patients with papillary thyroid carcinoma: two different cutoff values according to serum thyroglobulin level. Thyroid. 2015;25(4):410–6.
307. Pak K, Suh S, Hong H, Cheon GJ, Hahn SK, Kang KW, et al. Diagnostic values of thyroglobulin measurement in fine-needle aspiration of lymph

nodes in patients with thyroid cancer. Endocrine. 2015;49(1):70–7.
308. Zanella AB, Meyer EL, Balzan L, Silva AC, Camargo J, Migliavacca A, et al. Thyroglobulin measurements in washout of fine needle aspirates in cervical lymph nodes for detection of papillary thyroid cancer metastases. Arq Bras Endocrinol Metabol. 2010;54(6):550–4.
309. Baskin HJ. Detection of recurrent papillary thyroid carcinoma by thyroglobulin assessment in the needle washout after fine-needle aspiration of suspicious lymph nodes. Thyroid. 2004;14(11):959–63.
310. Shin HJ, Lee HS, Kim EK, Moon HJ, Lee JH, Kwak JY. A study on serum antithyroglobulin antibodies interference in thyroglobulin measurement in fine-needle aspiration for diagnosing lymph node metastasis in postoperative patients. PLoS One. 2015;10(6):e0131096.
311. Boi F, Maurelli I, Pinna G, Atzeni F, Piga M, Lai ML, et al. Calcitonin measurement in wash-out fluid from fine needle aspiration of neck masses in patients with primary and metastatic medullary thyroid carcinoma. J Clin Endocrinol Metab. 2007;92:2115–8.
312. Abraham D, Gault PM, Hunt J, Bentz J. Calcitonin estimation in neck lymph node fine-needle aspirate fluid prevents misinterpretation of cytology in patients with metastatic medullary thyroid cancer. Thyroid. 2009;19:1015–6.
313. Massaro F, Dolcino M, Degrandi R, Ferone D, Mussap M, Minuto F, et al. Calcitonin assay in wash-out fluid after fine-needle aspiration biopsy in patients with a thyroid nodule and borderline value of the hormone. J Endocrinol Investig. 2009;32:308–12.
314. Sapin R, d'Herbomez M, Gasser F, Meyer L, Schlienger JL. Increased sensitivity of a new assay for anti-thyroglobulin antibody detection in patients with autoimmune thyroid disease. Clin Biochem. 2003;36:611–6.
315. Donegan D, McIver B, Algeciras-Schimnich A. Clinical consequences of a change in anti-thyroglobulin antibody assays during the follow-up of patients with differentiated thyroid cancer. Endocr Pract. 2014;20:1032–6.
316. Gianoukakis AG. Thyroglobulin antibody status and differentiated thyroid cancer: what does it mean for prognosis and surveillance? Curr Opin Oncol. 2015;27(1):26–32.
317. Lupoli GA, Okosieme OE, Evans C, Clark PM, Pickett AJ, Premawardhana LD, et al. Prognostic significance of thyroglobulin antibody epitopes in differentiated thyroid cancer. J Clin Endocrinol Metab. 2015;100(1):100–8.
318. Spencer CA. New insights for using serum thyroglobulin (Tg) measurement for managing patients with differentiated thyroid carcinomas. Thyroid Int. 2003;4:1–14.
319. Nascimento C, Borget I, Troalen F, Al Ghuzlan A, Deandreis D, Hartl D, et al. Ultrasensitive serum thyroglobulin measurement is useful for the follow-up of patients treated with total thyroidectomy without radioactive iodine ablation. Eur J Endocrinol. 2013;169(5):689–93.
320. Yamada O, Miyauchi A, Ito Y, Nakayama A, Yabuta T, Masuoka H, et al. Changes in serum thyroglobulin antibody levels as a dynamic prognostic factor for early-phase recurrence of thyroglobulin antibody-positive papillary thyroid carcinoma after total thyroidectomy. Endocr J. 2014;61(10):961–5.
321. Tsushima Y, Miyauchi A, Ito Y, Kudo T, Masuoka H, Yabuta T, et al. Prognostic significance of changes in serum thyroglobulin antibody levels of pre- and post-total thyroidectomy in thyroglobulin antibody-positive papillary thyroid carcinoma patients. Endocr J. 2013;60:871–6.
322. Tumino S, Belfiore A. Appearance of antithyroglobulin antibodies as the sole sign of metastatic lymph nodes in a patient operated on for papillary thyroid cancer: a case report. Thyroid. 2000;10:431–3.
323. Slifka MK, Antia R, Whitmire JK, Ahmed R. Humoral immunity due to long-lived plasma cells. Immunity. 1998;8:363–72.
324. Uller RP, Van Herle AJ. Effect of therapy on serum thyroglobulin levels in patients with Graves' disease. J Clin Endocrinol Metab. 1978;46:747–55.
325. Feldt-Rasmussen U, Blichert-Toft M, Christiansen C, Date J. Serum thyroglobulin and its autoantibody following subtotal thyroid resection of Graves' disease. Eur J Clin Investig. 1982;12:203–8.
326. Benvenga S, Bartolone L, Squadrito S, Trimarchi F. Thyroid hormone autoantibodies elicited by diagnostic fine needle biopsy. J Clin Endocrinol Metab. 1997;82:4217–23.
327. Polyzos SA, Anastasilakis AD. Alterations in serum thyroid-related constituents after thyroid fine-needle biopsy: a systematic review. Thyroid. 2010;20:265–71.
328. Izumi M, Larsen PR. Correlation of sequential changes in serum thyroglobulin, triiodothyronine, and thyroxine in patients with Graves' disease and subacute thyroiditis. Metabolism. 1978;27:449–60.
329. Feldt-Rasmussen U, Bech K, Date J, Hyltoft Pedersen P, Johansen K, Nistrup Madsen S. Thyroid stimulating antibodies, thyroglobulin antibodies and serum proteins during treatment of Graves' disease with radioiodine or propylthiouracil. Allergy. 1982;37:161–7.
330. Feldt-Rasmussen U, Bech K, Date J, Petersen PH, Johansen K. 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. 1982;99:379–85.
331. Stevic I, Dembinski TC, Pathak KA, Leslie WD. Transient early increase in thyroglobulin levels post-radioiodine ablation in patients with differentiated thyroid cancer. Clin Biochem. 2015;48(10-11):658–61.