How sensitive (second-generation) thyroglobulin measurement is changing paradigms for monitoring patients with differentiated thyroid cancer, in the absence or presence of thyroglobulin autoantibodies

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Purpose of review
To discuss new insights regarding how sensitive (second-generation) thyroglobulin immunometric assays (Tg^{2G}IMAs), (functional sensitivities $\leq 0.10\,\mu g/L$) necessitate different approaches for postoperative thyroglobulin monitoring of patients with differentiated thyroid cancer (DTC), depending on the presence of thyroglobulin autoantibodies (TgAbs).

Recent findings
Reliable low-range serum thyroglobulin measurement has both enhanced clinical utility and economic advantages, provided TgAb is absent ($\sim$75% DTC patients). Basal [nonthyroid-stimulating hormone (TSH) stimulated] Tg^{2G}IMA measurement obviates the need for recombinant human TSH stimulation because basal Tg^{2G}IMA below $0.20\,\mu g/L$ has comparable negative predictive value ($>$95%) to recombinant human TSH-stimulated thyroglobulin values below the cutoff of $2\,\mu g/L$. Now that radioiodine remnant ablation is no longer considered necessary to treat low-risk DTC, the trend and doubling time of low basal thyroglobulin values arising from postsurgical thyroid remnants have recognized prognostic significance. The major limitation of Tg^{2G}IMA testing is interference by TgAb ($\sim$25% DTC patients), causing Tg^{2G}IMA underestimation that can mask disease. When TgAb is present, the trend in TgAb concentrations (measured by the same method) can serve as the primary (surrogate) tumor-marker and be augmented by thyroglobulin measured by a TgAb-resistant class of method (radioimmunoassay or liquid chromatography-tandem mass spectrometry).

Summary
The growing use of Tg^{2G}IMA measurement is changing paradigms for postoperative DTC monitoring. When TgAb is absent, it is optimal to monitor the basal Tg^{2G}IMA trend and doubling time (using the same method) in preference to recombinant human TSH-stimulated thyroglobulin testing. When TgAb is present, interference renders Tg^{2G}IMA testing unreliable and the trend in serum TgAb concentrations *per se* (same method) can serve as a (surrogate) tumor-marker.

Keywords
differentiated thyroid cancer, thyroglobulin autoantibody interferences, thyroglobulin measurement

INTRODUCTION

Serum thyroglobulin is the primary biochemical tumor-marker used to monitor differentiated thyroid cancer (DTC) [1,2,3■■]. A global rise in the prevalence of DTC [4,5■,6] is increasing the number of thyroidectomized patients needing lifelong monitoring for persistent or recurrent disease, typically involving periodic (6–12 months) serum thyroglobulin measurements augmented by anatomic imaging, as appropriate for recurrence risk. For decades, standard DTC treatment has involved total thyroidectomy followed by one or more doses

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KEY POINTS

- The enhanced functional sensitivity of Tg^{2G}IMA (functional sensitivity ≤ 0.10 μg/l) obviates the need for most rhTSH-stimulated thyroglobulin testing.

- Now that RAI remnant ablation is no longer considered necessary to treat low-risk DTC, Tg^{2G}IMA measurements are needed for monitoring the low thyroglobulin concentrations (typically < 0.5 μg/l) arising from the normal thyroid remnant.

- Different paradigms for optimal postoperative DTC monitoring are needed for TgAb-negative (~75%) versus TgAb-positive (~25%) patients.

- When TgAb is absent, the basal (non-TSH stimulated) Tg^{2G}IMA trend and doubling time provide important prognostic information.

- When TgAb is present, interference renders Tg^{2G}IMA measurement unreliable and the trend in TgAb concentrations (measured using the same method) can serve as the primary (surrogate) tumor-marker test.

of radioiodine (RAI) before long-term thyroid hormone suppression of thyroid-stimulating hormone (TSH), without regard to recurrence risk [1,2,7,8]. Yet most thyroid tumors are small (≤1.0 cm), belong to the papillary histotype (PTC) and have a low recurrence risk even when treated by surgery alone [9–11]. Increasingly, a more individualized, risk-stratified, approach to DTC diagnosis and management is being adopted [5■,7,8,12] that limits RAI treatment to high-risk DTC (the minority of patients) [13,14■,15]. As a consequence, more sensitive (second-generation) thyroglobulin immunometric assay (Tg^{2G}IMA) measurements, which have an order of magnitude higher functional sensitivity (≤0.10 μg/l) than older (first-generation) tests (functional sensitivity ~1.0 μg/l), are rapidly becoming the standard of care [3■■,16■■]. Over recent years, it has become clear that thyroglobulin assays need second-generation functional sensitivity in order to monitor the low basal (non-TSH stimulated) thyroglobulin concentrations arising from the intact surgical remnant of low-risk DTC patients who no longer receive routine RAI remnant ablation. Also that a basal Tg^{2G}IMA below 0.20 μg/l has comparable negative predictive value to a recombinant human TSH (rhTSH)-stimulated thyroglobulin below the consensus cutoff of 2.0 μg/l [3■■,17–20]. Further, as with other biochemical tumor-marker tests such as calcitonin and carcinoembryonic antigen, the basal Tg^{2G}IMA trend and doubling time (measured using the same thyroglobulin method, preferably in the same laboratory) have been shown to be important prognostic parameters [21–25,26■].

The primary limitation of Tg^{2G}IMA measurement is its high propensity for interference from both thyroglobulin autoantibodies (TgAb) [27–29,30■■] and heterophile antibodies, primarily human anti-mouse antibodies (HAMA) [31–33]. This review will focus on technical issues relating to current thyroglobulin methodology that impact the clinical utility of thyroglobulin testing and discuss how paradigms for postoperative DTC monitoring differ depending on the TgAb status of the patient.

REVIEW OF FOUR DECADES OF THYROGLOBULIN METHODOLOGY

During the last four decades, thyroglobulin has been measured by three different classes of methodology [34]: Radioimmunoassay (RIA) used since the 1970s [29,35,36,37■■], Immunometric assay (IMA) used since the 1980s [29,36,37■■,38] and liquid chromatography-tandem mass spectrometry (LC-MS/MS) developed in 2008 [39–41,42■]. These classes of thyroglobulin method differ fundamentally in functional sensitivity potential and propensity for interference from HAMA and TgAb [34].

Most thyroglobulin testing is made using IMA methods that can display ten-fold differences in functional sensitivity. A generational nomenclature system, similar to that used for TSH assays, has been adopted to distinguish between different assays because assay functional sensitivity is such a critical determinant of the clinical utility of thyroglobulin testing [3■■,19,43,44]. First-generation assays have functional sensitivity approximating 1.0 μg/L, whereas second-generation assays have functional sensitivity 0.10 μg/L or less. Both the RIA and LC-MS/MS class of method can achieve only first-generation functional sensitivity. Increasingly, laboratories are replacing first-generation tests with Tg^{2G}IMA measurement because superior functional sensitivity allows basal (non-TSH stimulated) Tg^{2G}IMA monitoring to replace the need for rhTSH stimulation [16■■,17–20,45–52] and facilitate the detection of the low thyroglobulin concentrations arising from postoperative thyroid remnants [53■■] and lymph node metastases secreting low levels of thyroglobulin [54,55]. Unfortunately, as with all IMA-class methods, Tg^{2G}IMAs are highly prone to interference from both HAMA and TgAb, potentially limiting their clinical value [3■■,30■■]. Some RIA methods are still in use because this competitive methodology appears to convey more resistance to TgAb interference than IMA-class tests, although some interfering TgAbs undoubtedly cause some falsely high or low serum RIA values [29,56]. The new LC-MS/MS class of method should be free from TgAb and HAMA interferences. These methods

employ extensive laboratory-specific specimen preparation using trypsin to break up serum thyroglobulin-TgAb complexes to release a conserved thyroglobulin peptide(s) for LC-MS/MS measurement [40,41,42■]. However, as yet these methods have only been validated by correlation studies that have confirmed the underestimation typical of TgAb interference with $Tg^{2G}IMA$ and that thyroglobulin RIA values appear resistant, although the cause of undetectable LC-MS/MS and detectable thyroglobulin RIA values in some TgAb-positive and TgAb-negative sera needs further study [40,41]. Currently, LC-MS/MS clinical utility is compromised by suboptimal functional sensitivity (only first-generation), longer turnaround times than $Tg^{2G}IMA$, limited availability and high instrumentation costs [42■].

TECHNICAL LIMITATIONS OF THYROGLOBULIN METHODOLOGY

Three principle technical limitations currently negatively impact the clinical utility of thyroglobulin methods: first, suboptimal functional sensitivity; second, between-method discordances and third, interferences, primarily from TgAb, but also heterophilic antibodies, the most common being HAMA.

Functional sensitivity

Manufacturers and laboratories are unsure which parameter best describes a thyroglobulin assay's detection limit [34]. Immunoassay precision profiles are typically U-shaped, with low range precision and matrix bias determining the reliability for detecting low analyte concentrations [46,57]. This low range precision erodes as measurements are made over longer periods of time as a consequence of changes in reagent lots and calibrators, instrumentation factors and other intangible variables [58]. This deterioration in precision is particularly problematic for serial thyroglobulin measurements because postoperative DTC monitoring necessitates monitoring low thyroglobulin concentrations across long clinical intervals (6–12 months) between determinations. Functional sensitivity is the only parameter that represents the imprecision of low-range thyroglobulin measurement made under conditions representative of clinical practice. Specifically, functional sensitivity is defined as 'the thyroglobulin concentration that can be measured in human serum with 20% between-run coefficient of variation over a 6–12 months period, during which time more than two reagent lots and more than two instrument calibrations should be used' [3■■,34,44]. Functional sensitivity contrasts with the limit of quantitation calculation favored by many manufacturers and laboratories that is defined

by the 20% coefficient of variation of runs made over short periods of time (days or weeks) without stipulations regarding the use of the clinically relevant matrix (human serum) or capturing any lot-to-lot variability [34]. It should be noted that because both thyroglobulin and TgAb are measured concurrently, the protocol for determining the functional sensitivity of thyroglobulin and TgAb assays is the same [44].

Between-method discordances

Restandardization against the Certified Reference Material, CRM-457, has greatly reduced differences between thyroglobulin assays over the last decade [59–61]. However, despite universal adoption of this standard, different methods can display a two-fold difference in the numeric thyroglobulin values reported for the same serum [29,45,46,57,62,63]. This is evident in Fig. 1 showing UK National External Quality Assessment Service for Thyroglobulin surveys for four TgAb-negative sera (A–D) that had thyroglobulin measured by each of 51 laboratories using 10 different methods. Thyroglobulin molecular heterogeneity is the likely cause of this method variability. Thyroglobulin is a large (660 kDa) dimeric glycoprotein that is heterogeneous with respect to differential thyroglobulin mRNA splicing, glycosylation and degree of iodination, also, the processes involved in thyroglobulin maturation, dimerization and molecular folding are complex and may become dysregulated in tumor tissue [64]. Given the conformational nature of thyroglobulin epitopes [65,66], it is not surprising that different immunoassays, employing different thyroglobulin antibody reagents, detect serum thyroglobulin isoforms with variable potency. The severe between-method discordances seen for tumor-derived thyroglobulins are reflected in abnormal ratios between the values reported by different methods that suggest different epitopes may be masked or exposed when the thyroglobulin structure is abnormal [62]. The magnitude of the between-method variability seen in Fig. 1 far exceeds the expected within-person thyroglobulin variability that approximates 15% [67]. In clinical practice, these between-method biases necessitate that postoperative thyroglobulin monitoring be made using the same manufacturers method and preferably the same laboratory [1,2,3■■,29,44,45].

Interferences

TgAb interference is the most serious problem currently limiting the clinical utility of thyroglobulin testing [27,30■■], whereas interference caused by HAMA is less common [19,30■■,33]. Both types of interference primarily affect the IMA class of
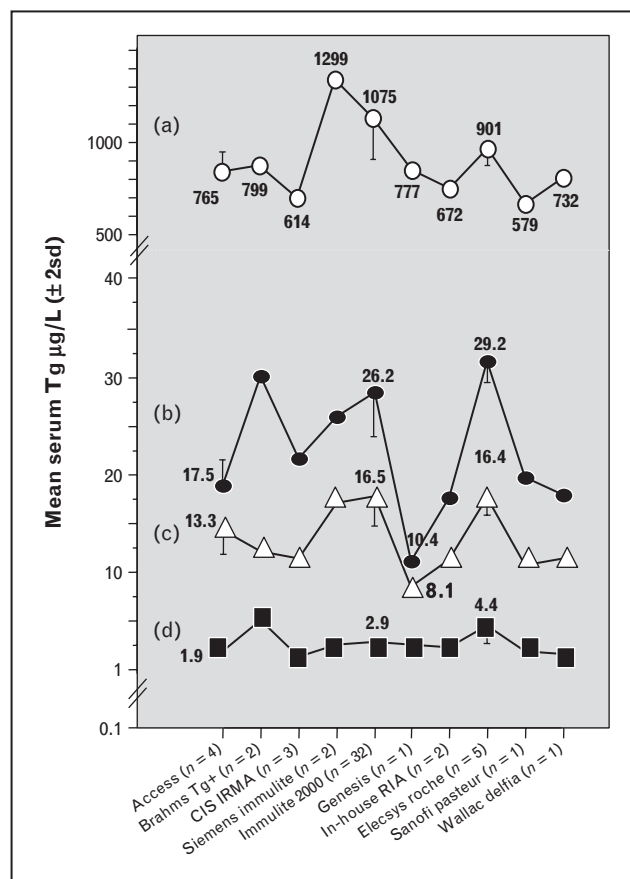
FIGURE 1. Between-method variability of current thyroglobulin methods. This figure shows the between-method variability of serum thyroglobulin values reported for four different sera (A–D) without TgAb, measured by 51 laboratories using 10 different methods. The +/−2 standard deviation limits are shown for methods with sufficient participating laboratories. The data were taken from the United Kingdom National External Quality Assessment Service for Thyroglobulin surveys with permission.

thyroglobulin method. TgAb causes underestimation that could mask the presence of disease [30■■,68], whereas HAMA typically causes thyroglobulin IMA overestimation [27,30■■]. The RIA class of thyroglobulin method is not affected by HAMA and appears resistant to TgAb interference, [27,36,37■■,56,69–73], whereas the new LC-MS/MS class of method should be free from both TgAb and HAMA interferences by virtue of trypsin digestion of antibody complexes and generation of a thyroglobulin-specific peptide that is the measurement parameter [40,41,42■]. However, clinical studies are needed to show that abnormal polymorphic tumor thyroglobulins always generate the tryptic peptide necessary for LC-MS/MS detection [42■].

Because the TgAb status of a patient can change (positive to negative or vice versa), current guidelines mandate that every thyroglobulin test have a TgAb measurement made directly by an immunoassay and not the indirect thyroglobulin recovery approach that often fails to detect interfering TgAb [2,3■■,29,30■■,34,44]. Unfortunately, TgAb detection by immunoassay is also problematic because current methods differ in sensitivity, specificity and the numeric values they report, despite claiming standardization against the same reference preparation (Medical Research Council 65/93). Many examples of the same serum being reported as 'TgAb-positive' by one method but 'TgAb-negative' by another have been reported [36,74–76]. Laboratories compound these problems by adopting different cutoff values to define a 'positive' TgAb result, even when they use the same manufacturer's test [29,30■■,36,37■■,48,75–79]. The selection of the TgAb cutoff impacts the reliability of TgAb detection, which is critical when a 'negative' TgAb result is used to authenticate a $Tg^{2G}IMA$ measurement [36]. The clinical consequences of a false-negative TgAb test would be that low or undetectable $Tg^{2G}IMA$ values might be reported that could mask disease [30■■,68]. In contrast, a false-positive TgAb test would unnecessarily decrease confidence in $Tg^{2G}IMA$ measurement and could prompt reflex testing by a less sensitive, but TgAb-resistant, class of thyroglobulin method (RIA or LC-MS/MS). One caution is that when laboratories use a 'positive' TgAb result to reflex thyroglobulin testing to different methods, the imprecision surrounding the TgAb cutoff value during DTC monitoring could lead to inappropriate changes in a patient's TgAb status (positive to negative or vice versa) that could prompt unnecessary reflexing between $Tg^{2G}IMA$ and RIA or LC-MS/MS testing.

FACTORS INFLUENCING SERUM THYROGLOBULIN CONCENTRATIONS

Because the source of thyroglobulin protein is thyroid tissue-specific and not tumor-specific, a number of factors will influence the interpretation of serum thyroglobulin concentrations [44]. These factors include the following: first, the mass of thyroid tissue present (normal thyroid remnant plus any tumor); second, the tissue's efficiency or inefficiency for thyroglobulin secretion; third, injury secondary to surgery, fine-needle aspiration biopsy, or RAI therapy can cause thyroglobulin release from damaged tissue that elevates serum thyroglobulin concentrations; fourth, the degree and chronicity of TSH stimulation that has a profound effect on serum thyroglobulin and fifth, the possibility of increased metabolic clearance of thyroglobulin-TgAb complexes that would alter the interpretation of the

serum thyroglobulin concentrations in the presence of TgAb [80–83].

POSTOPERATIVE SERUM THYROGLOBULIN MONITORING

Serum thyroglobulin measurement only becomes a reliable tumor-marker after total thyroidectomy. Preoperative thyroglobulin measurement is considered to have limited value, although a number of studies report that an elevated preoperative serum thyroglobulin is a risk factor for nodular malignancy [84–87]. In addition, the relationship between the preoperative serum thyroglobulin and tumor burden may give some indication of the tumor's efficiency for thyroglobulin secretion and set a threshold for determining the significance of postoperative serum thyroglobulin changes [2].

Preoperative TgAb measurement may also have clinical utility. One report found nodular malignancy more likely when TgAb, but not thyroid peroxidase antibodies, were detected [88]. Most DTC patients with circulating TgAb have evidence of thyroid autoimmunity [66,89,90], although any causal relationship between Hashimoto's thyroiditis and PTC is unclear [90–92]. However, TgAb-positive PTC patients may be more likely to have extrathyroidal extension and a higher risk for persistent or recurrent disease than TgAb-negative PTC patients [14■,93–95]. It follows that a preoperative TgAb measurement, and/or histologic evidence of lymphocytic infiltration in the surgical specimen, may be a risk factor and early indicator that postoperative $Tg^{2G}IMA$ measurement may be unreliable because of TgAb interference [90,96,97].

Optimal monitoring of differentiated thyroid cancers patients without thyroglobulin autoantibody

The majority ($\sim$75%) of DTC patients either have no TgAb detected throughout their course or become TgAb-negative in the years following successful surgery [14■,27,37■■,89,92,98–100]. With RAI treatment now being limited to high-risk DTC patients [13,14■,15], most postoperative thyroglobulin testing will be made for low-risk PTC patients with low thyroglobulin concentrations arising from postsurgical thyroid remnants. This more conservative use of RAI, coupled with the growing use of $Tg^{2G}IMA$ measurement, is changing paradigms for postoperative serum thyroglobulin monitoring [5■]. No longer can an 'undetectable' serum thyroglobulin be considered a useful criterion for the absence of disease because thyroglobulin that is 'undetectable' using an insensitive first-generation test may be

'detectable' when using more sensitive $Tg^{2G}IMA$ measurement [3■■,29,45,46,62]. Further, rhTSH stimulation should no longer be needed now that reliable basal $Tg^{2G}IMA$ measurement is available [16■■]. Studies have found that rhTSH typically stimulates basal thyroglobulin approximately tenfold [19,46], so that the negative predictive value of a rhTSH-stimulated thyroglobulin value below the fixed cutoff of 1 to 2 µg/l is comparable to a basal $Tg^{2G}IMA$ value below 0.10–0.20 µg/L [16■■,49]. Although the low frequency of DTC recurrences impacts the ability to study positive predictive values (PPVs) [45], the PPV of an rhTSH-stimulated thyroglobulin above 1 to 2 µg/l appears comparable to a basal $Tg^{2G}IMA$ above 0.10–0.20 µg/l [20,49, 101]. By considering the principle factors influencing serum thyroglobulin concentrations (thyroid tissue mass, injury and TSH), it is evident that the trend in basal $Tg^{2G}IMA$, measured when TSH is suppressed, should reflect changes in thyroid tissue mass and thus provide a more sensitive parameter for disease than rhTSH-stimulated thyroglobulin testing. This is supported by a growing number of studies showing the prognostic utility of monitoring the basal $Tg^{2G}IMA$ trend and thyroglobulin doubling time [22,25,102], as is customary for other tumor-marker such as carcinoembryonic antigen and calcitonin [21].

The clinical utility of monitoring $Tg^{2G}IMA$ trends is illustrated in Figs 2 and 3. Figure 2 shows postoperative serum $Tg^{2G}IMA$ monitoring (TSH < 0.10 mIU/l at all points) of 18, non-RAI treated, TgAb-negative, PTC patients who displayed no evidence of disease at the end of more than 5 years follow-up. As previously reported, serum $Tg^{2G}IMA$ fell to a nadir of less than 0.05 to 0.5 µg/l during the first postoperative year (Fig. 2a) – the same thyroglobulin range as seen following thyroidectomy for medullary carcinoma [103] and consistent with the thyroglobulin secretion expected from the approximately 1 g of normal thyroid remnant remaining after thyroidectomy [53■■,104–106]. It should be noted that at a median of 0.17 postoperative years, serum $Tg^{2G}IMA$ was below 0.10 µg/l in 12 out of 18 (67%) cases, and even below the functional sensitivity limit of 0.05 µg/l in three out of 18 (17%) cases, affirming that without RAI treatment the normal thyroid remnant typically secretes a very low thyroglobulin level in the face of TSH suppression [53■■,104,105]. Figure 2b shows that serum $Tg^{2G}IMA$ values remain remarkably constant in the less than 0.05–0.5 µg/l range when TSH suppression is maintained during long-term follow-up. In fact, the median within-person percentage coefficient of variation of serum $Tg^{2G}IMA$ measurements made throughout 2–15 years of postoperative
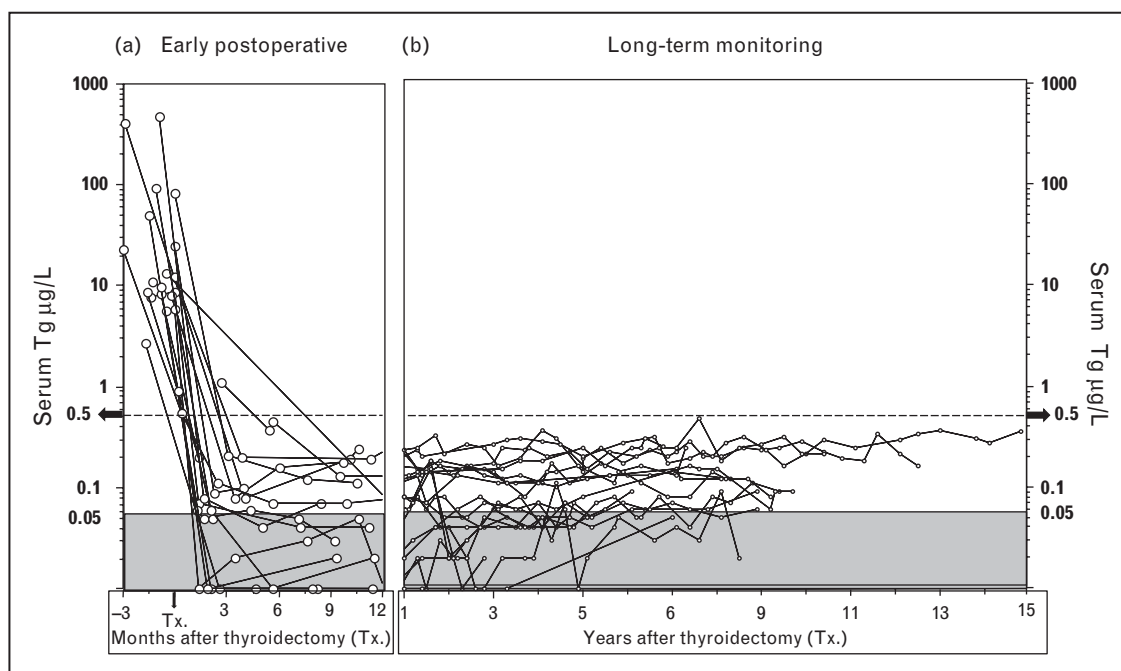
FIGURE 2. Basal serum (second-generation) thyroglobulin immunometric assay trends for disease-free, thyroglobulin autoantibody-negative papillary histotype (no radioiodine treatment). This figure shows serum basal $Tg^{2G}IMA$ measurements (Beckman Access analyses of frozen archived specimens [37■■]) made for 18 TgAb-negative PTC patients treated by thyroidectomy alone (no RAI treatment), maintained on long-term TSH suppression (<0.1 mIU/l), without evidence of recurrence at the end of follow-up. Panel (a) shows that during the early postoperative phase, all patients achieved a basal serum thyroglobulin below 0.5 µg/l by 6–12 months after thyroidectomy (Tx.). Panel (b) shows the stability of thyroglobulin secretion from normal remnant tissue when TSH is held constant (median within-person percentage coefficient of variation ~30%). PTC, papillary histotype; RAI, radioiodine; Tg, thyroglobulin; TgAb, thyroglobulin autoantibody; TSH, thyroid-stimulating hormone.

monitoring approximated 30%, illustrating the consistency of thyroglobulin secretion from normal thyroid remnant tissue. It is this consistency of remnant secretion that is why the basal $Tg^{2G}IMA$ doubling time (measured at a constant TSH level) is a sensitive parameter for detecting recurrence [22,25,102]. Figure 3 shows 25 years of postoperative $Tg^{2G}IMA$ monitoring (Beckman Access $Tg^{2G}IMA$ measurement of frozen archived specimens [37■■]) of a TgAb-negative PTC patient with persistent and recurrent disease. This case illustrates a number of points which are as follows: first, the high preoperative thyroglobulin (154 µg/L) suggested that serum thyroglobulin would be a sensitive postoperative tumor-marker; second, the ~ten-fold thyroglobulin stimulation in response to thyroid hormone withdrawal prior to RAI suggested the tumor was responsive to TSH and supported the efficacy for TSH suppression; third, surgery was clearly a more effective treatment for metastatic PTC lymph nodes than RAI treatments; fourth, combined imaging modalities were needed to detect disease; fifth, persistent disease remained quiescent during TSH suppression for many years before an active recurrence manifested; sixth, a rising trend in basal $Tg^{2G}IMA$

(measured at constant TSH) suggested an increase in tumor mass because thyroglobulin secretion from normal remnant tissue (Fig. 2) remains constant. It was also likely that this patient had little or no normal remnant left after two doses of RAI and seventh, the doubling of basal $Tg^{2G}IMA$ during TSH suppression approximated 4 years – an interval indicating a good, long-term prognosis [22].

Optimal monitoring of patients with thyroglobulin autoantibody

A recent review of 1500 consecutive DTC patients with more than 2 years postoperative follow-up found that although 22.7% were currently TgAb-positive, an additional 12.7% had a past history of TgAb-positivity but had become TgAb-negative during the postoperative period [27,37■■,98,99]. Thus, approximately one-third of DTC patients have TgAb detected at some time in their course, and clearly the TgAb status of a patient can change over time necessitating TgAb measurement with every thyroglobulin test [1,2,14■,16■■,30■■,44]. Although the most common change in TgAb status is positive to negative in response to successful treatment
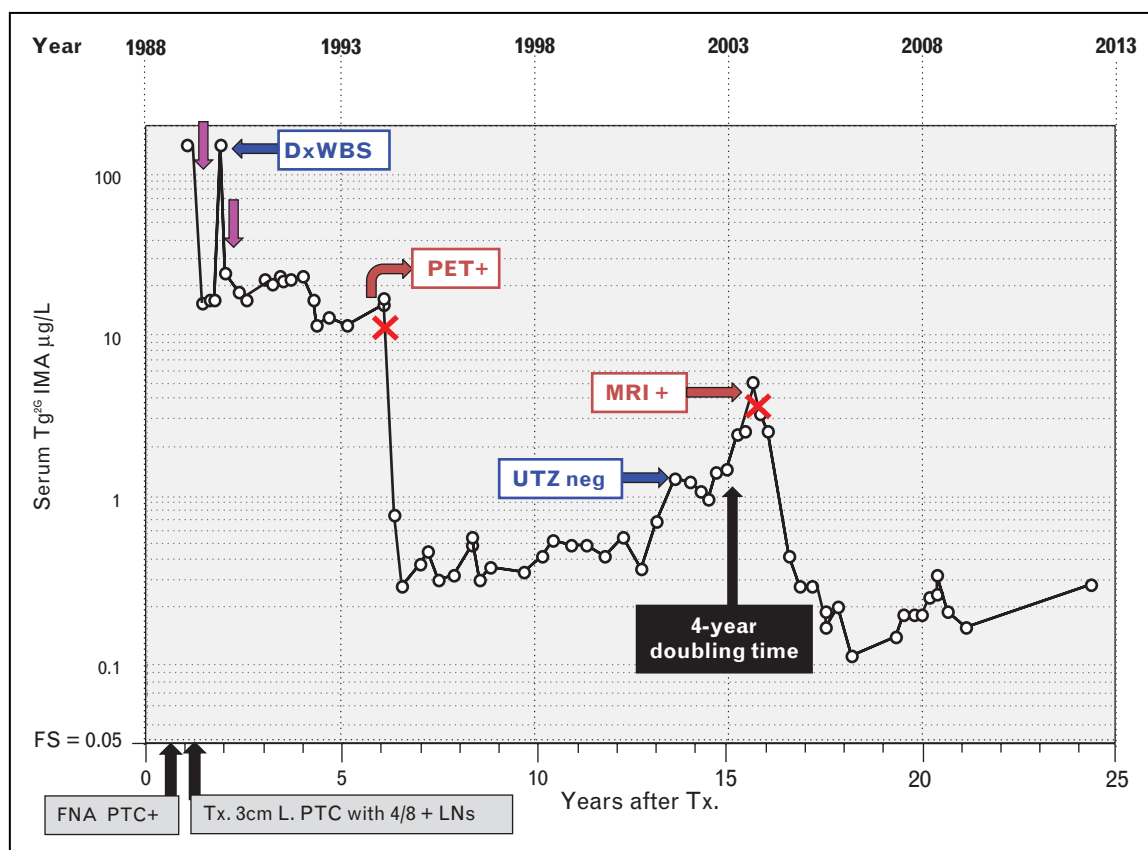
FIGURE 3. Serum (second-generation) thyroglobulin immunometric assay monitoring of a patient with persistent and recurrent papillary thyroid cancer (PTC) and negative thyroglobulin autoantibodies. This female patient presented in 1988 at age 42 with a 3-month history of an enlarging anterior neck mass. PTC was diagnosed by fine-needle aspiration biopsy (FNA) and treated by total thyroidectomy to remove a 3 cm tumor, together with 4 out of 8 PTC-positive lymph nodes. Preoperative serum thyroglobulin was elevated (154 μg/L) suggesting that postoperative serum thyroglobulin monitoring would be efficacious. Serum basal $Tg^{2G}IMA$ measurements made during 25 years of postoperative thyroglobulin monitoring are shown (Beckman Access $Tg^{2G}IMA$ measurement of frozen archived specimens [37■■]) during thyroid hormone therapy (TSH < 0.05 mIU/L). Following an initial RAI treatment (∼150 mCi) (purple arrows), persistent disease was suspected by inappropriately high thyroglobulin values (15–20 μg/L). Despite a negative diagnostic whole body scan (DxWBS), the elevated thyroglobulin prompted a second RAI treatment (150 mCi) that revealed foci of thyroid uptake and a submandibular lymph node on the post-treatment scan. However, the two RAI treatments had little effect on the elevated serum thyroglobulin, which remained persistently elevated for 5 years prompting computed tomography scans of the neck and chest (negative) followed by a PET scan that revealed a lymph node, confirmed by FNA as positive for PTC. During a left jugular dissection (surgeries indicated by red crosses), a 1.5 cm lymph node metastasis was removed producing a significant fall in serum $Tg^{2G}IMA$ into the 0.3– 0.5 μg/L range, a level that was sustained for approximately 5-years. Thereafter, despite continued TSH suppression, $Tg^{2G}IMA$ rose with an approximate 4-year doubling time prompting an ultrasound investigation (negative) and later an MRI that detected a single 1.0 cm lymph node metastasis that was removed during a level 2, 3 and 5 neck dissection. Thereafter, serum $Tg^{2G}IMA$ values fell to the 0.20–0.40 μg/L range but remained detectable. The persistence of the detectable serum $Tg^{2G}IMA$ despite two doses of RAI suggests that disease may still be present. FNA, fine-needle aspiration; FS, functional sensitivity; LNs, lymph nodes; PTC, papillary histotype; RAI, radioiodine; $Tg^{2G}IMA$, (second-generation) thyroglobulin immunometric assay; TSH, thyroid-stimulating hormone; Tx., thyroidectomy.

[27,37■■,98,99], the persistence of TgAb, a rising TgAb trend, the failure of TgAb to fall or a de-novo TgAb appearance is highly suspicious for active disease [7,14■,37■■,89,92,100,107]. However, a rising TgAb concentration is not specific for recurrence but can also result from any thyroid injury causing thyroglobulin release (RAI treatment, lymph node biopsy or additional surgery) [90]. It is now apparent that TgAb concentrations respond to changes in the mass of thyroglobulin-secreting thyroid tissue so that the trend in TgAb concentrations can be used as a surrogate tumor-marker [7,14■,30■■,37■■,65,89, 90,98,100,107]. Thus, TgAb measurement is not merely a qualitative (positive versus negative) test

for validating that a Tg^{2G}IMA measurement is free from TgAb interference but acts as an indicator for thyroglobulin antigen sensed by the immune system. Unfortunately, given the difference in numeric values reported by different TgAb methods, and the time needed to establish the TgAb trend (TgAb half life ~10 weeks [99]), it is critical that postoperative TgAb monitoring be performed using the same manufacturer's method [29,30■■,36,90]. Alternatively,

when a change in TgAb method is necessary, the patient-specific ratio between the new versus the old TgAb test can be used to rebaseline TgAb testing to the new method, as previously described [37■■,90].

The clinical utility of monitoring TgAb trends as a surrogate tumor-marker is illustrated in Fig. 4, in which serum TgAb monitoring of four PTC patients without disease is shown in the upper panels, to contrast with four PTC patients with persistent or
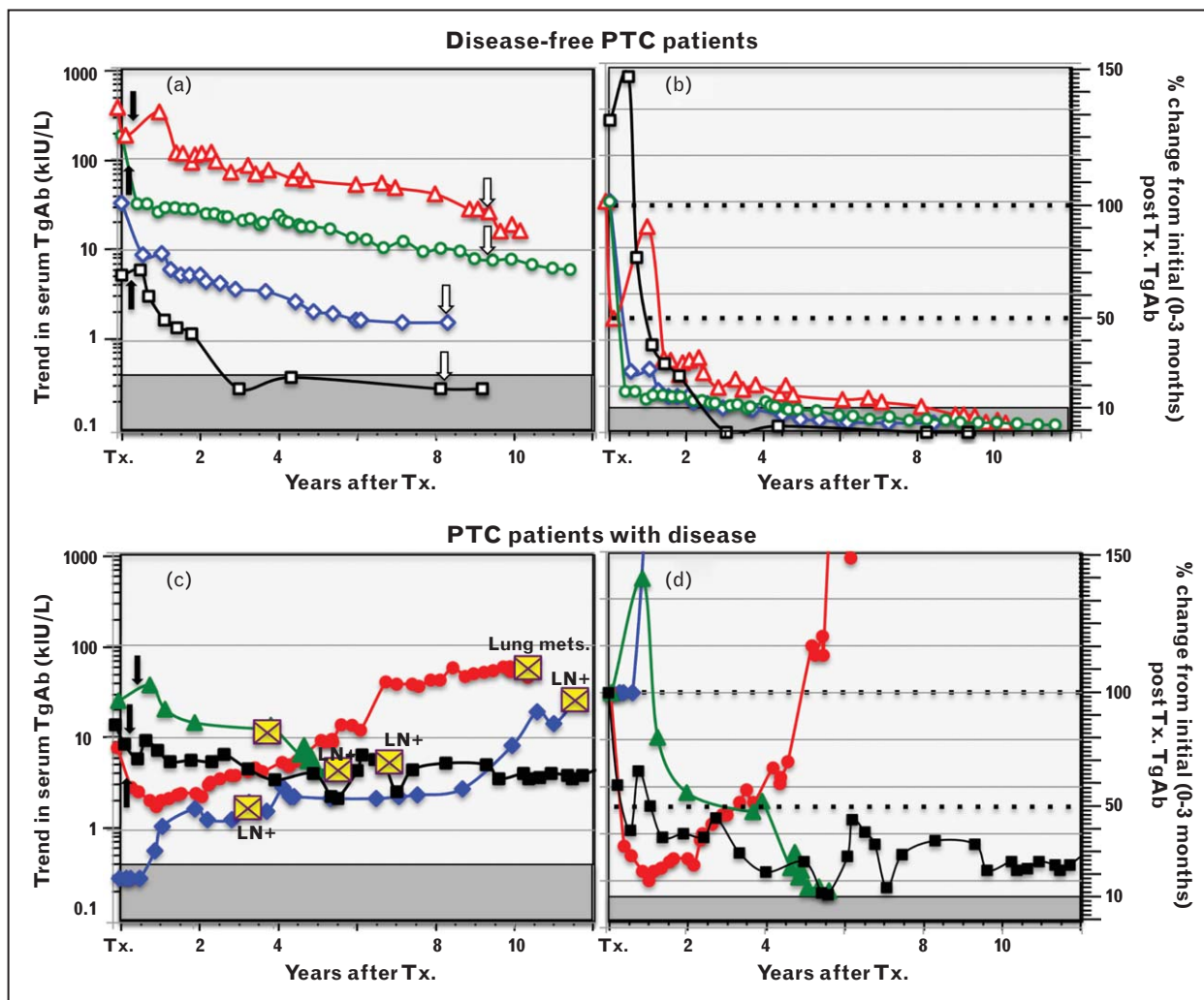

FIGURE 4. Thyroglobulin autoantibody trend and percentage change used as a surrogate differentiated thyroid cancer tumor-marker. This figure illustrates how the trend in TgAb concentrations (left panels a and c) and the percentage change in TgAb concentrations (Kronus/RSR method) relative to an initial (0–3 month) postoperative TgAb value (panels b and d) can be used as a surrogate DTC tumor-marker. Figure 4a shows TgAb trends for four PTC patients who were judged disease-free by ultrasound (open arrows) in the postoperative period. Figure 4b shows these data converted to percentage of the initial value. Disease-free patients may have a transient early TgAb rise in response to thyroglobulin released by surgical injury or RAI treatment (solid arrows), but thereafter TgAb values typically decline over time (years) to less than 10% of initial level. These disease-free patients were selected to have different initial TgAb concentrations to illustrate that declining TgAb concentrations do not necessarily become undetectable during follow-up, unless the initial TgAb was low. Figures 4c and 4d show comparative data for four PTC patients with persistent or recurrent disease detected during follow-up (indicated by yellow crosses). A de-novo TgAb appearance, a TgAb rise or a stable TgAb concentration that fails to fall below 10% of initial value are indications of active disease. DTC, differentiated thyroid cancer; LN, lymph node metastases; PTC, papillary histotype; RAI, radioiodine; TgAb, thyroglobulin autoantibody; Tx., thyroidectomy.

recurrent disease shown in the lower panels. Figure 4a shows that TgAb-positive patients rendered disease-free by thyroidectomy may display a transient early rise in TgAb in response to the thyroglobulin released by the surgery and/or RAI treatment, but thereafter TgAb progressively declines and may become undetectable during the early years of follow-up, especially if the initial TgAb concentration was low. Disease-free patients with high TgAb concentrations typically display a slow progressive TgAb decline over years but may not achieve full TgAb-negativity, possibly because of the long-lived memory of plasma cells [108]. The left-hand panels show the TgAb concentrations expressed as percentage of an initial (0–3 month) value. Figure 4b shows that when patients are disease-free, TgAb concentrations typically fall by more than 50% during the first postoperative year [26■,30■■,34,100] and thereafter continue to fall to less than 10% of initial value over subsequent years. These TgAb trends contrast with those of the PTC patients with persistent or recurrent disease shown in the lower panels. Figure 4c shows that when there is active disease, TgAb concentrations may show a decline in the early months following thyroidectomy that is not sustained, or may be followed by a progressive TgAb rise or even a de-novo TgAb appearance sometimes years after TgAb absence, necessitating concurrent TgAb measurement with every thyroglobulin test [30■■,44,107]. Figure 4d illustrates that although some patients with active disease have a 50% TgAb decline in the first postoperative year, TgAb rarely declines to less than 10% unless the disease is successfully treated.

CONCLUSION

Now that RAI treatment is no longer considered necessary to treat low-risk DTC, postoperative serum thyroglobulin monitoring will primarily be made for disease-free patients who have functioning thyroid remnants that typically give rise to serum basal $Tg^{2G}IMA$ concentrations in the 0.05–0.5 µg/l or less range when TSH is suppressed. When TgAb is absent (∼75% of DTC), the serum basal $Tg^{2G}IMA$ trend and doubling time are important prognostic parameters. However, when TgAb is present, $Tg^{2G}IMA$ measurement is unreliable because of interference causing $Tg^{2G}IMA$ underestimation and the trend in TgAb concentrations (measured by the same method) becomes the primary (surrogate) tumor-marker.

Acknowledgements

Conflicts of interest

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