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Thyroglobulin (Tg) measurement used to monitor patients with differentiated thyroid carcinomas (DTC)

C.A. SPENCER

Serum Tg measurements are primarily used as a tumor-marker for patients with DTC. It is critical that circulating Tg concentrations be interpreted relative to the pathology, surgical history and TSH status of the patient. Biases between methods preclude the use of different Tg or TgAb assays for the serial monitoring of patients. Recovery tests often fail to detect interfering TgAb. In the future, the use of more sensitive Tg assays (functional sensitivity <0.1 µg/L) should obviate the need for expensive rhTSH stimulation testing. Because the specificity of using an "undetectable" Tg as a risk factor for disease is inversely related to Tg assay sensitivity, in the future serial sensitive basal Tg monitoring without rhTSH stimulation will be used in conjunction with ultrasound. Both TgAb and HAMA interferences remain problems with IMA methodology. Because RIA methods appear resistant to these interferences, discordance between the IMA and RIA measurements made on a specimen is a useful way to detect interference.

Thyroglobulin (Tg) is the 660,000 Da precursor protein backbone for thyroid hormone biosynthesis and is co-secreted with thyroid hormones in response to TSH stimulation. Because Tg is derived uniquely from thyroid follicular cells, serum Tg measurement is primarily used as a tumor-marker for patients with differentiated thyroid cancers (DTC). Most thyroid tumors have the capability to synthesize and secrete Tg, although there may be considerable heterogeneity in the circulating Tg isoforms arising from neoplasms (1). Tg measurement is primarily made in serum, however Tg measured in the washout from fine-needle aspiration of suspicious lymph nodes is becoming an important adjunctive test to cytology (2).

Correspondence: Carole A. Spencer, Ph.D., FACB, Department of Medicine, Division of Endocrinology, Keck School of Medicine, University of Southern California, 1840 North Soto Street, Los Angeles, CA 90032
E-mail: cspencer@usc.edu

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Variables influencing the interpretation of serum-Tg concentrations in DTC

A serum Tg elevation cannot be used to diagnose DTC because an elevated Tg is merely a non-specific indicator of the presence of thyroid pathology. It is only after a cytological/histological diagnosis has been made that serum Tg becomes a useful tumor marker for DTC. As summarized in figure 1, the Tg measured in the circulation reflects: 1) The mass of thyroid tissue present (the combined contribution from normal remnant tissue plus any tumor); 2) any thyroid injury, secondary to fine needle aspiration, surgery, radioiodine therapy or thyroiditis; and 3) the degree of TSH-receptor stimulation by endogenous or recombinant TSH, hCG (pregnancy) or TSH-receptor antibodies (TSAb) (present in Graves' hyperthyroidism).
As shown in figure 2, the Tg assay characteristics together with the patient's surgical history and TSH status can be used as benchmarks to interpret post-operative serum Tg concentrations (3). For example, using a Tg assay with functional sensitivity of 0.1

µg/L and a reference range of 3 to 40 µg/L, the Tg reference range needs to be adjusted by 50 percent (range 1.5 to 20 µg/L) to match the low TSH (<0.1 mIU/L) typical of TSH-suppressed DTC patients (4). It follows that after lobectomy, serum Tg should be below 10 µg/L, and if higher would suggest the presence of multicentric disease. After near-total thyroidectomy 1-2 grams of normal thyroid remnant typically remain, this would be expected to give rise to no more than 2 µg/L Tg in the circulation. In contrast, patients rendered completely athyreotic would be expected to have a serum Tg below 0.01 µg/L, irrespective of their TSH status.

Strengths and weaknesses of current Tg assays

Assay-to-assay biases

It is important to know the characteristics of the Tg assay used, because there are considerable biases as well as sensitivity differences among different methods. Most laboratories use immunometric assays (IMA) that require shorter incubation times than radioimmunoassays (RIA) and can be automated. The biases between different methods are more than twice (~37% CV) the biologic variability (~15% CV) of Tg in the circulation (1). This is evident from differences in the reference ranges reported for euthyroid controls (1). Assay-to-assay variability is higher among IMA methods as compared with RIA, probably because IMAs use monoclonal antibodies that differ in specificity for tumor-derived Tg isoforms (1). Furthermore, the class of assay, IMA or RIA, determines the propensity for interferences (1). Specifically, IMA methodology is more prone to both Tg autoantibody (TgAb) and heterophilic antibody (HAMA) interferences as compared with RIA (1,5).

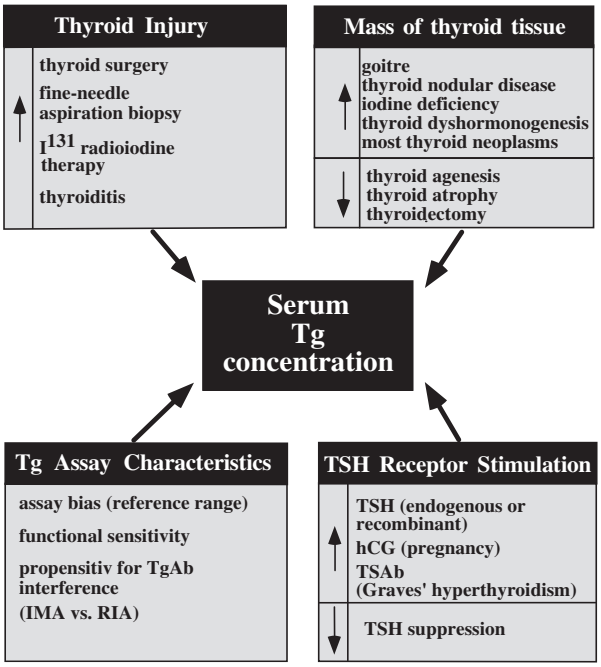

Figure 1. Four factors influence the interpretation of serum Tg concentrations.

Functional sensitivity

Most manufacturers have now recognized the importance of using a clinically relevant method to determine the lower reporting limit (functional sensitivity, FS) of an assay. Guidelines now define functional sensitivity as the lowest Tg concentration that can be measured in human sera with 20% CV, over a minimum of six months, using at least two different reagent lots and two different calibrator lots (3). Current assays exhibit ten-fold differences in functional sensitivity (<0.1-2.0 µg/L) (1).

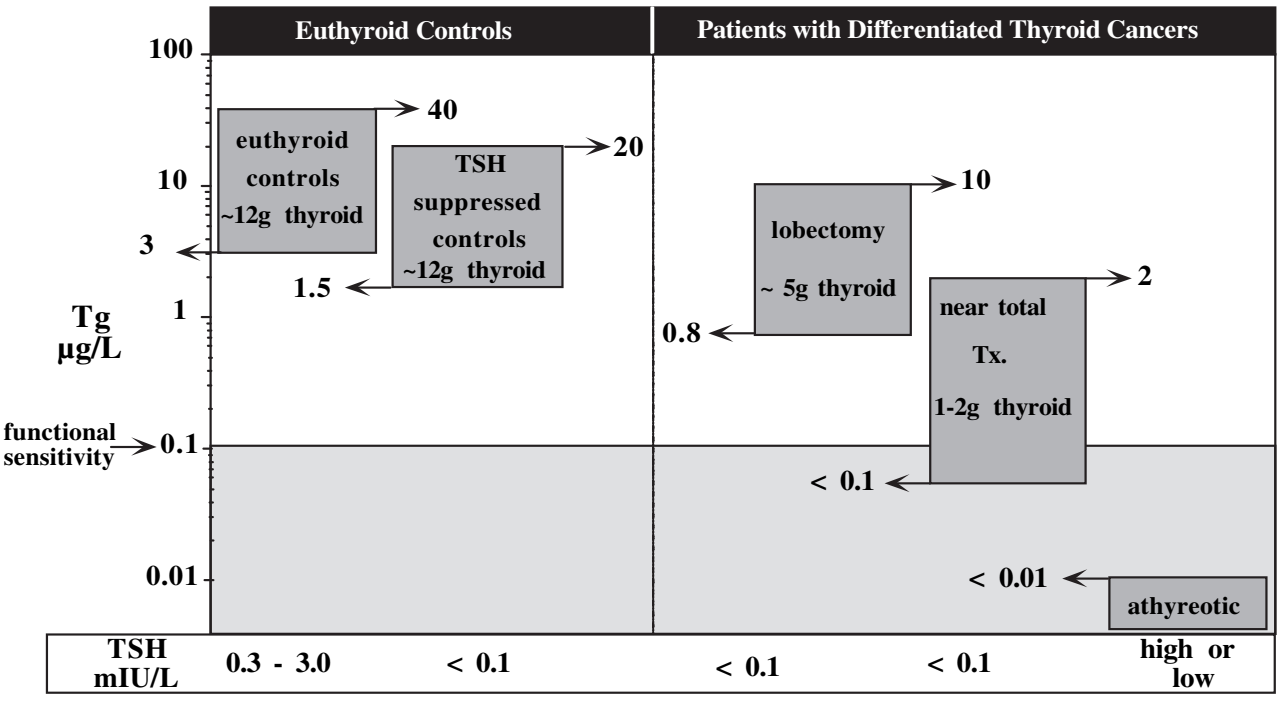

Figure 2. An example of how to use the assay benchmarks (functional sensitivity and reference range) in conjunction with surgical history and TSH status to interpret serum Tg concentrations (adapted from reference #3).

Recombinant human TSH (rhTSH)-stimulated Tg measurements

Since 1999, recombinant human TSH (rhTSH) stimulation has been employed to overcome Tg assay insensitivity. When using conventional assays (FS ~1.0 µg/L) three types of Tg response are seen 72-hours after rhTSH administration. Patients having a detectable (above 1.0 µg/L) basal Tg display an approximate 10-fold rhTSH-stimulation of Tg above basal. Such patients have a high risk (~45%) of having persistent/recurrent disease (6). Most (80%) of the patients with undetectable basal TSH by conventional assay (FS ~ 1.0 µg/L) still have no Tg detected after rhTSH and have a low (~1%) risk of having persistent/recurrent disease (7). The efficacy of rhTSH-stimulation testing is greatest in the 20% of patients with undetectable basal Tg in whom rhTSH stimulates Tg into the detectable range (>1.0 µg/L) because 6% of these patients have persistent/recurrent disease (6,8). It should be noted that when TgAb is present the expected ~10-fold rhTSH-stimulated Tg response is blunted or absent, irrespective to the class of assay (IMA or RIA) used to measure Tg (9). This blunting may reflect an enhanced metabolic clearance of Tg-TgAb complexes.

Following the recent development of more sensitive Tg assays (FS < 0.1 µg/L), many patients who were thought to have "undetectable" rhTSH-stimulated Tg values by conventional assays were found to have 'detectable' rhTSH-stimulated Tg in the 0.1 to 1.0 µg/L range (10,11). It has become apparent (figure 3) that there is a strong relationship between the basal (TSH-suppressed) Tg measured before rhTSH administration and the rhTSH-stimulated Tg response 72-hours after rhTSH (Figure 3) (12). Because few patients (<1%) with Tg below 0.1 µg/L have rhTSH-stimulated Tg values above the cut-off of 1.0 µg/L, and because rhTSH-stimulated Tg responses are predictable (~10-fold stimulation) rhTSH stimulation becomes unnecessary when using a Tg assay with a functional sensitivity <0.1 µg/L.

When rhTSH was initially released in 1999, patients with a serum Tg above a cut-off value of 2 µg/L 72-hours after rhTSH, were considered at risk for disease (13). Not surprisingly, the specificity for detecting disease was shown to be inversely proportional to the Tg cut-off value employed (13). Subsequently, the Tg cut-off value for risk of disease has been lowered to the functional sensitivity of the conventional assays (~1 µg/L) (7,8). One problem is that fixed Tg cut-offs are assay dependent. For example, the cut-off of 1.0 µg/L originally established (13) is equivalent to a value in a range of 0.5 to 2.0 µg/L, depending on the Tg method employed (12). Just as there is an inverse relationship between the Tg cut-off used and its specificity for detecting disease (13) there will be an inverse relationship between specificity for detecting disease and the functional sensitivity of the assay if an "undetectable" rhTSH-stimulated Tg is adopted as the cut-off. The clinical value of improving Tg assay sensitivity has been questioned because many patients thought to be disease-free exhibit a low level (0.1-1.0 µg/L) of Tg following rhTSH-stimulation (14). This small response is likely to represent the stimulation of normal remnant that can persist even after a large dose of radioactive iodine (RAI) (15). The specificity of a detectable rhTSH-stimulated Tg is also reduced if normal thyroid remnant is not reduced with radioactive iodine (RAI) treatment (16). However, a recent meta-analysis and new guidelines recommend that RAI treatment be restricted to higher risk patients (14,17,18). It follows that many patients

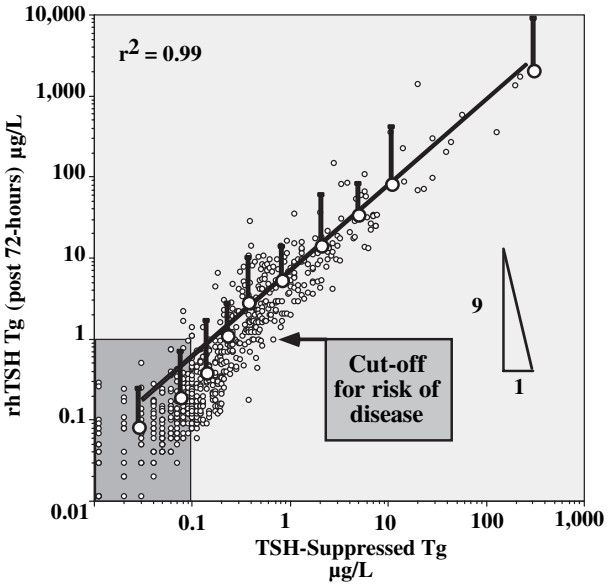

Figure 3. Correlation between basal (TSH-suppressed) Tg and the 72-hour (4-day) post rhTSH-stimulated Tg concentration. Recombinant TSH tests were grouped (mean ± 2sd) according to the basal Tg: <0.05; 0.05-0.1; 0.11-0.2; 0.21-0.3; 0.31-0.5; 0.51-1.5; 1.6-3.0; 3.1-5; 5.1-20 and >20 µg/L. The rhTSH-stimulated Tg response averaged 9-times the basal Tg (adapted from reference #12).

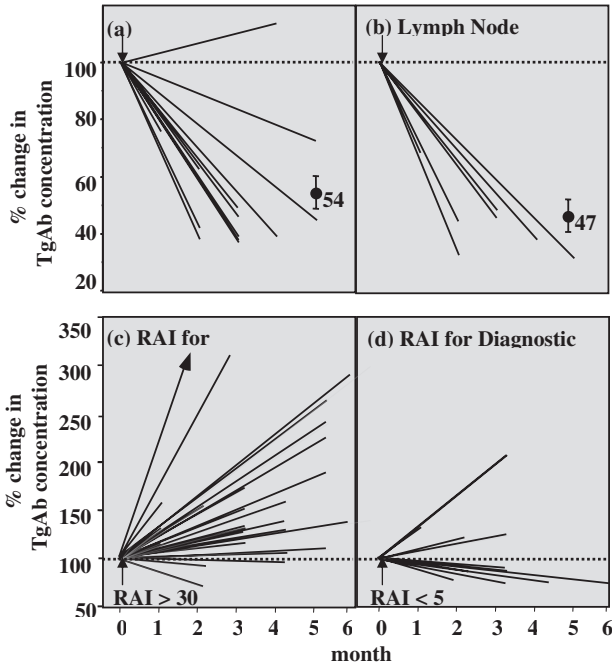

Figure 4. Percent change in TgAb concentrations over six months following either (a) thyroidectomy, (b) lymph node resection, (c) >30 mCi radioactive iodine (RAI) treatment or >5 mCi RAI for diagnostic scan.

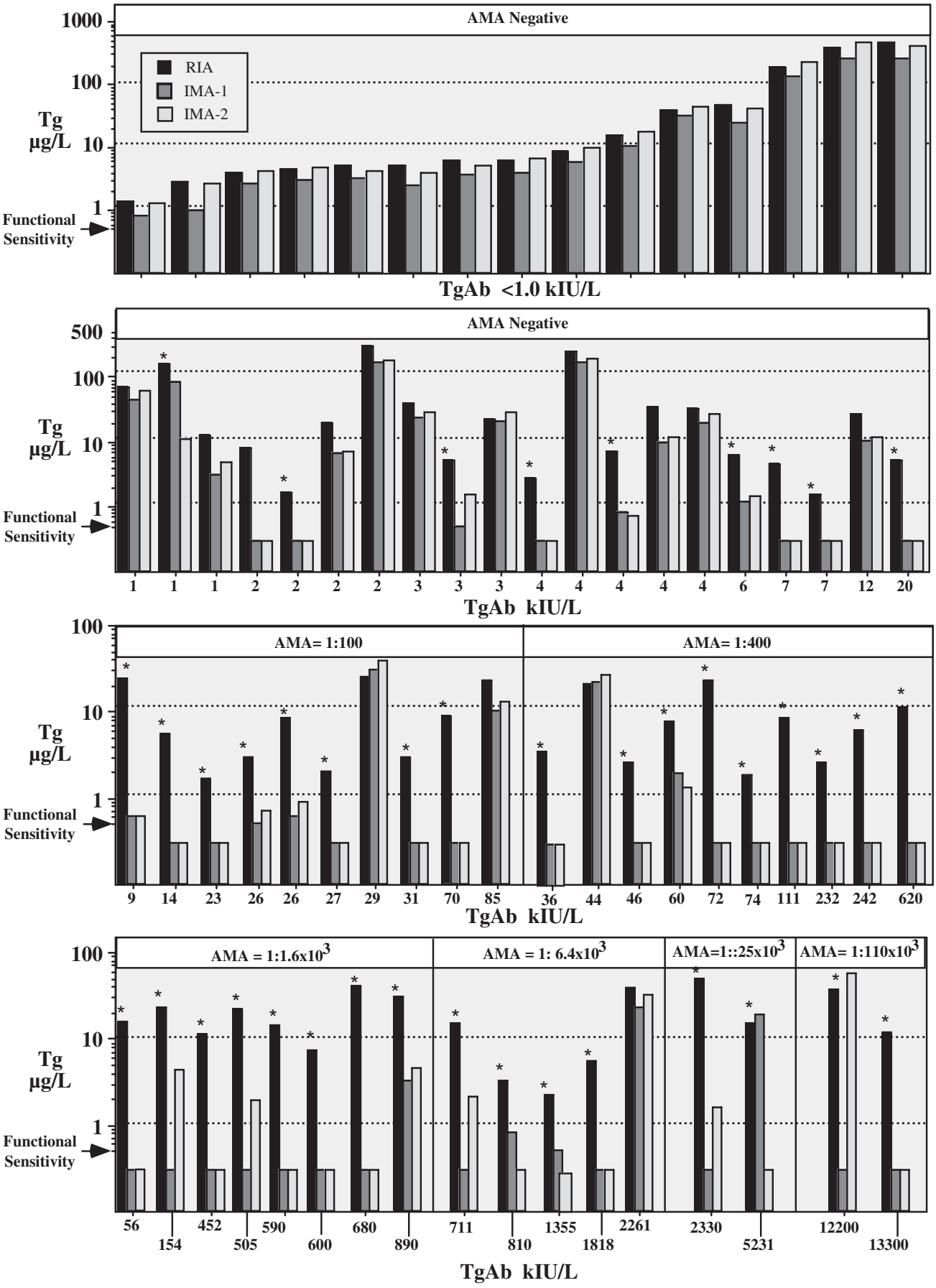

Figure 5. Discordances between Tg concentrations on the ordinate measured by an RIA versus two IMA methods. Specimens are ordered according to TgAb concentrations measured by a direct method (Kronus/RSL) and antimicrosomal antibody(AMA) titres measured by hemagglutination. Asterisks mark specimens the RIA is discordant with and one or both of the IMA results, indicating interference.

will have a low level of basal Tg detected by sensitive assay that will be clinically insignificant. In the future the hallmark of persistent/recurrent disease will not be an "undetectable" Tg but a rising Tg in the face of TSH suppression (10).

Tg autoantibodies (TgAb)

Use of TgAb measurements as a surrogate tumor marker

Thyroglobulin autoantibodies (TgAb) are detected in approximately 20 percent of patients with DTC - twice the prevalence of the general population (19,20). DTC patients who have TgAb detected at the time of their thyroidectomy have a ten-fold higher risk of developing recurrent disease than patients without detectable TgAb (21). In recent years it has become apparent that serial Tg measurement can be used as a surrogate tumor marker because TgAb concentrations fall acutely following thyroidectomy or lymph node resection, and decrease progressively to become undetectable approximately three years after a patient has been rendered disease-free (19, 22). In contrast, TgAb concentrations remain detectable when patients have persistent disease, and typically rise before a lymph node recurrence is detected. It is also important to note that in patients with a current or past history of detectable TgAb there is typically an acute rise in the TgAb concentration following a RAI treatment. This is likely the immune response to an acute release of antigen (Tg) from damaged cells, because TgAb concentrations usually do not rise following a diagnostic RAI scanning dose (figure 4). Qualitative TgAb differences among patients are likely responsible for the variability in absolute TgAb values reported by different methods, despite their use of the same IRP (MRC 65/93) for standardization (1). It follows that it is critical to use the same method when using TgAb measurements as a surrogate tumor marker.

Interferences with serum-Tg measurement

IMA methodology is more prone to interferences by both heterophilic antibody (HAMA) and TgAb, as compared with RIA methods (1,5,19). Whereas IMA kit manufacturers typically include "blockers" to inhibit HAMA interference, this does not eliminate HAMA effects in all cases (CA Spencer). TgAb interference remains a major technical limitation of serum Tg measurement, especially when Tg is measured by IMA methodology (1).

Circulating TgAb interferes with serum Tg measurements in a qualitative, quantitative and method-dependent manner, such that interfering TgAb may not be detected by all TgAb methods (1). It is clear that direct TgAb measurements detect interfering TgAb more reliably than antimicrosomal antibody (AMA) titers or the Tg recovery approach (1). In fact, current guidelines recommend that Tg recoveries be discouraged and eliminated (3). All Tg IMA methods are prone to underestimate Tg in the presence of TgAb and no current method is immune to this problem (1). It appears that the use monoclonal antibodies coupled with the "sandwich" format often fails to detect Tg complexed with TgAb. This is seen in Figure 5 as discordance between RIA and IMA measurements. The Tg underestimation characteristic of IMA measurements is a serious problem, because TgAb-positive patients are more at risk for persistent/recurrent disease and an inappropriately low or undetectable Tg can potentially mask the presence of disease (1,19,21). Although TgAb has the potential to cause either under- or overestimation with RIA methodology (depending on reagent characteristics), current RIA methods show no evidence of overestimation and appear to detect both free and TgAb-bound Tg (12). In fact, because RIA methodology is resistant to interference by either HAMA or TgAb, discordance between the Tg IMA and Tg RIA values reported for a specimen is a useful hallmark for interference (figure 5) (12). Some laboratories take advantage of the speed and automation inherent with IMA methodology yet avoid the problem of TgAb interference by triaging TgAb-positive specimens to RIA measurement and limiting the use of IMA to the majority of patients who have no TgAb detected.

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