Editorial: Challenges of Serum Thyroglobulin (Tg) Measurement in the Presence of Tg Autoantibodies

The study of Gupta and colleagues (1) reports that the presence of TSH receptor (TSHR) mRNA and/or thyroglobulin (Tg) mRNA, detected by RT-PCR in the peripheral blood of patients with differentiated thyroid carcinomas (DTCs), is a highly sensitive and specific marker for disease in both the pre- and postoperative periods. The authors assert that rigorous primer selection was the reason that their Tg mRNA analyses had a higher specificity for disease than reported by other studies.

Several limitations of the Gupta study (1) include a relatively small number of patients, the modality used to detect recurrent disease, and the Tg cut-off values used to assess the presence of disease. This study primarily used low-dose radiiodine (RAI) imaging to detect disease—a modality that is now considered less sensitive than serum Tg measurement, especially when Tg testing is made under the influence of TSH stimulation (2). In fact, false-negative low-dose RAI scans occur in approximately 20% of cases after thyroid hormone withdrawal and approximately 30% of cases after recombinant human TSH (rhTSH) (3). As the authors acknowledge, the qualitative detection of Tg mRNA is unlikely to replace quantitation of serum Tg protein as a DTC tumor marker test. The Gupta study (1) assessed the sensitivity and specificity of Tg protein measurements using serum Tg cut-off values of greater or equal to 1 μg/liter during thyroid hormone suppression of TSH (THST) and/or greater or equal to 2 μg/liter after rhTSH stimulation. There is growing recognition that the clinical sensitivity of Tg testing is compromised by the use of such high cut-off values. Specifically, serum Tg concentrations in the 1–2 μg/liter range are close to the lower reference limit for healthy euthyroid subjects with intact thyroid glands, thus making it increasingly difficult to discern between true disease and normal remnant tissue (2–4). Despite the use of these high Tg cut-off values, there was only a minor difference in clinical sensitivity between the detection of TSHR and/or Tg mRNAs vs. the detection of serum Tg protein. In the future, as more sensitive Tg assays become available, it is likely that measurement of serum Tg protein during THST will prove superior to mRNA detection and that rhTSH stimulation will become unnecessary (5). Indeed, the authors acknowledge that mRNA determinations are unlikely to replace serum Tg measurements as postoperative tumor marker tests in patients without Tg autoantibodies (TgAbs), but they suggest that Tg and/or TSHR mRNA testing may prove useful disease markers when the clinical utility of serum Tg testing is compromised by TgAb interference.

It has been recognized for more than 30 yr that endogenous TgAb has the potential to interfere with measurements of Tg protein made by either immunometric assay (IMA) or RIA methodology (4, 6–8). TgAb interference with serum Tg measurement is undoubtedly the most serious technical problem that currently compromises the use of serum Tg as a tumor marker test for patients with DTC. Approximately 25% of DTC patients have TgAb detected in their circulation as compared with 11% in the general population (4, 9, 10). When TgAb is present, Tg protein molecules circulate as free Tg or complexed with the endogenous TgAb. The magnitude and direction of TgAb interference with a serum Tg measurement depends both on the affinity and capacity of the endogenous TgAb and the class of Tg method used (IMA or RIA) (9, 11). There is growing recognition that RIA methodology is generally less prone to TgAb interference than IMA methodology (4, 8, 9, 12, 13). Although bidirectional TgAb interference is possible with RIA methods, the direction and magnitude of any interference primarily depends on the affinity and specificity of the first and second antibody reagents (6, 14). Overestimation of a Tg RIA measurement would occur if the endogenous TgAb sequestered Tg-125I tracer and prevented it from participating in the competitive reaction. Conversely, underestimation would result if the second antibody reagent lacked species specificity and precipitated tracer bound to the endogenous (human) TgAb (6, 14). RIAs constructed with a high affinity first antibody and a species-specific second antibody generally report clinically appropriate total Tg (free Tg plus Tg complexed with TgAb) concentrations for TgAb-positive subjects (9, 12, 13). In contrast, TgAb interference with IMA methodology is always unidirectional (underestimation). This is evident from studies showing that serum Tg is often paradoxically undetectable in TgAb-positive DTC patients with disease, TgAb-positive normal euthyroid subjects, and TgAb-positive patients with Graves’ thyrotoxicosis (8, 9, 13, 15). Physicians should be aware that any IMA method has the potential to underestimate serum Tg, even when TgAb levels are very low or below the cut-off for positivity (9, 16). In contrast, as with other IMA tests, the presence of heterophilic antibodies (HAMA) in the circulation can cause overestimation of serum Tg measured by IMA methodology (17, 18). RIA measurements are less prone to TgAb interference and not affected by the presence of HAMA (4).

Typically, when TgAb is present in the circulation, there is discordance between the serum Tg measurements made by RIA vs. IMA methodology. It is difficult to know which discordant Tg result (detectable Tg by RIA or lower/undetectable Tg by IMA) more appropriately reflects the disease.

Abbreviations: DTC, Differentiated thyroid carcinoma; HAMA, heterophilic antibodies; IMA, immunometric assay; RAI, radiiodine; rhTSH, recombinant human TSH; Tg, thyroglobulin; TgAb, Tg autoantibody; THST, thyroid hormone suppression of TSH; TSHR, TSH receptor.

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

doi: 10.1210/jc.2004-0986
status of a TgAb-positive DTC patient. For example, the undetectable Tg IMA result might appear appropriate for a thyroidectomized patient, yet the persistence of TgAb in the circulation indicates that the immune system is still sensing the presence of Tg antigen, suggesting that the detectable RIA result is more clinically appropriate (9, 19). An ongoing Tg method comparison study (13) is providing further support for the contention that the higher Tg RIA value more appropriately reflects the clinical status of TgAb-positive DTC patients with Tg RIA vs. Tg IMA discordances. Specifically, this study measured Tg concentrations in sera from both TgAb-negative and TgAb-positive normal euthyroid subjects, using 11 different IMA methods and four different RIA methods. The serum Tg concentrations of the TgAb-positive subjects measured by the RIA methods were similar to those of TgAb-negative euthyroid subjects, whereas all of the IMA methods reported generally lower values, some of which were paradoxically undetectable (13). It is clear that, given the propensity for IMA methods to underestimate serum Tg in the presence of TgAb, an undetectable serum Tg IMA value reported for a thyroidectomized TgAb-positive DTC patient has no clinical value (8, 9). This underscores the importance of the new guideline (no. 46) published by the DTC patient has no clinical value (8, 9). This underscores the importance of the new guideline (no. 46) published by the National Academy of Clinical Biochemistry (NACB) recommending that “laboratories should not report undetectable serum Tg values in the presence of TgAb if that method produces inappropriately low or undetectable serum Tg values for TgAb-positive DTC patients with documented disease” (4). Currently, most clinical laboratories use IMA methodology to measure serum Tg in both TgAb-negative and TgAb-positive sera. Most of these laboratories persist in reporting undetectable Tg IMA values for TgAb-positive DTC patients, despite acknowledging that the result might be unreliable and potentially mask disease, the risk of which is increased when TgAb is detected (8, 20). Other laboratories now limit the use of IMA methodology to TgAb-negative sera and use RIA methodology to measure serum Tg when TgAb is detected (9). Overestimation of serum Tg, either as a result of HAMA interference with an IMA measurement or TgAb interference with an RIA measurement, is less problematic than underestimation. Although a Tg overestimate raises patient and physician concerns for recurrence, imaging studies can be used to provide reassurance that there is no evidence of disease. However, it should be acknowledged that such patients may be given unnecessary empiric RAI treatment because it is difficult to know whether the detection of Tg in a RAI-scan-negative patient reflects the insensitivity of low-dose RAI scanning or Tg overestimation due to an interference (2, 18, 21, 22).

A number of studies report that serial TgAb measurements per se have clinical efficacy as a surrogate tumor marker test for TgAb-positive DTC patients, because TgAb concentrations respond to changes in circulating Tg antigen (9, 19, 23, 24). Specifically, there is a dramatic (~50%) fall in TgAb concentrations during the first 6 months after thyroidectomy or lymph node removal (9, 25). In fact, when TgAb-positive patients are rendered athyroidic, serum TgAb concentrations progressively decline and typically become undetectable within the first few postoperative years. In contrast, patients with persistent disease characteristically maintain detectable, or exhibit rising, serum TgAb concentrations (8, 9, 19, 23, 24). Whereas a rise in serum TgAb is often the first indication of recurrence, a transient rise in TgAb should be expected as a response to increased Tg antigen released by radiolytic destruction of tumor during the first few months after RAI treatment (25, 26). In fact, a rise in TgAb after a therapeutic dose of RAI may even be an indicator of the efficacy of RAI treatment of a TgAb-positive patient. Clearly TgAb is heterogeneous, and TgAb methods differ in sensitivity and specificity (9, 11). It is important to recognize that serial TgAb measurements can only be used as a surrogate tumor marker if the same manufacturer’s method is used, because the absolute TgAb values reported by different methods can differ by as much as 100-fold despite claims that all methods are standardized against the MRC 65/93 reference preparation (4, 9). It is not uncommon for a patient to be judged TgAb-negative by one manufacturer’s method and TgAb-positive by another (9). Low concentrations of TgAb that may not be detected by some methods can interfere with Tg measurement (9, 16). Failure to detect the presence of TgAb can lead to the reporting of falsely low or paradoxically undetectable serum Tg IMA values for patients with metastatic disease (8, 9).

All sera sent for Tg measurement require adjunctive TgAb testing (4). The potential for TgAb interference is only weakly related to the TgAb concentration, and even low levels have the potential to interfere. This means that it is critical to measure TgAb concentrations directly using a sensitive and precise immunoassay method and not a recovery test (4, 8, 16, 27–29). Recoveries are not only an unreliable means to detect interfering TgAb, but recoveries cannot be used as a serial quantitative tumor marker test as with direct TgAb measurement (4). In fact, NACB guideline no. 46 states that “recovery tests do not reliably detect TgAb and should be discouraged and eliminated” (4). Failure to detect TgAb in a specimen clearly has serious consequences, because a falsely undetectable serum value could cause a delay in the treatment of metastatic disease (4, 8, 9, 16).

There is growing use of rhTSH stimulation for unmasking occult disease in patients with undetectable serum Tg during THST (2). In fact, a recent metaanalysis reported that TgAb-negative patients with serum Tg less than 1 µg/liter during THST have a 3–8% risk of having significant disease (2). Unfortunately, the value of rhTSH stimulation testing is compromised by the presence of TgAb. Specifically, many TgAb-positive patients display blunted or absent TSH-stimulated serum Tg responses, regardless of the class of Tg method used (IMA or RIA) (13). Currently, the most likely explanation for paradoxical blunted rhTSH responses in the presence of TgAb is a difference in the metabolic clearance of free Tg, as compared with Tg complexed with TgAb. Specifically, the metabolic clearance of free Tg is estimated to approximate 3 to 4 d (30, 31). The rapid removal of TgAb seen after Tg release during thyroidectomy lends support to the contention that newly formed Tg-TgAb complexes may be rapidly cleared (32).

The clinical value of Tg and/or TSHR mRNA testing has to be unequivocally established before mRNA testing can be used to facilitate therapeutic decision-making for DTC patients. It is likely that as more sensitive serum Tg assays...
become available the clinical utility of serum Tg testing during THST will increase, obviating the need for either rTSH-stimulated serum Tg testing or mRNA analyses. However, TgAb interference with serum Tg measurements, especially when made by IMA methodology, is likely to remain a problem for the foreseeable future. Fortunately, serial TgAb concentrations are a useful surrogate tumor marker test for monitoring the disease status of TgAb-positive DTC patients. Because Tg and/or TSHR mRNA testing would be more expensive than serum Tg measurements, even if such tests were proved clinically useful, mRNA testing would likely be reserved for high-risk or TgAb-positive patients in whom the reliability of serum Tg and/or TgAb measurements were diagnostically questionable.

Carole A. Spencer
Department of Medicine
Keck School of Medicine
University of Southern California
Los Angeles, California 90033

Acknowledgments

Received May 25, 2004. Accepted June 9, 2004.
Address all correspondence and requests for reprints to: C. A. Spencer, Research Medicine, Department of Medicine CRC 18400, Los Angeles, California 90033. E-mail: cspencer@usc.edu.

References

11. Spencer CA. 2003 New insights for using serum thyroglobulin (Tg) measurement for managing patients with differentiated thyroid carcinoma. Thyroid International 4:1–14
12. Feldt-Rasmussen U, Rasmussen AK. 1985 Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb in vitro and in vivo. J Endocrinol Invest 8:57–64

JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.