Recoveries Cannot Be Used to Authenticate Thyroglobulin (Tg) Measurements When Sera Contain Tg Autoantibodies

Serum thyroglobulin (Tg) measurements are primarily used as a tumor marker for managing patients with differentiated thyroid carcinoma [1]. The serum Tg concentration reflects three factors: the mass of differentiated thyroid tissue present, any physical damage to or inflammation of thyroid tissue, and the magnitude of TSH-receptor stimulation [1]. Currently, Tg immunometric assay (IMA) techniques are gaining popularity over RIA methods [2, 3]. These new IMAs offer the practical advantages of shortened incubation times, a wider working range, a more-stable labeled antibody reagent that is less prone to labeling damage [4, 5], and more potential for automation. Most Tg IMAs are isotopic (IRMAs) [2, 6]; however, nonisotopic IMAs have been described, such as those used: recently in this issue, by Erali et al. [3].

Five technical problems currently impair the clinical value of serum Tg measurements, irrespective of which methodological type (RIA or IMA) is used: (a) differences in standardization, (b) inadequate sensitivity, (c) poor interassay precision over a typical clinical interval used for monitoring patients with differentiated thyroid cancers (6–12 months), (d) so-called "hook" problems when measuring very high Tg concentrations, and (e) thyroglobulin autoantibody (TgAb) interference. Recently, progress has been made in overcoming some of these problems. A new international reference preparation (CRM 457; BCR, Brussels, Belgium) has become available, recommendations have been improved to introduce interassay precision and detect hook effects [7]. Less progress has been made in detecting, quantifying, and eliminating the problem of TgAb interference, which appears to be the most difficult technical problem to overcome [6, 7, 9]. When sensitive TgAb immunoassays such as that described by Erali et al. [3] are used, TgAb is detected in ~20% of sera from patients with differentiated thyroid cancer [1]. Typically, patients that have persistent or recurrent disease postoperatively have TgAb that remains detectable throughout subsequent years of follow-up. In contrast, serum TgAb progressively decreases, typically becoming undetectable by the second postoperative year in patients who have undergone a successful tumor resection (10, Spencer et al., ms. in preparation).

Recovery studies have historically provided an important criterion for authenticating many biochemical tests, including serum Tg measurements made in TgAb-positive sera [2, 3, 6, 11]. Recoveries of Tg added to TgAb-positive sera suggest that TgAb can cause either over- or underestimation of the Tg measurement. RIA methods can exhibit bidirectional interference (over- or underestimation), the magnitude and direction of which relate to the affinity of the first antibody, the specificity of the second antibody, the volume of serum used, and the characteristics of the TgAb present [9]. In contrast, "sandwich" or IMA-type Tg methods, such as those recently reported by Marquet et al. [2] and Erali et al. [3], exhibit unidirectional interference (underestimation). In this issue, Erali et al. describe a well-designed study of the methodological performance of a new ELISA method that uses the recovery of a 12.5 μg/L Tg addition to detect interference from TgAb [3]. This method displayed good linearity and precision characteristics across the physiological range. The percent of exogenous Tg recovered ranged between 82% and 103% when sera were TgAb-negative, vs. 27% to 99% for sera containing TgAb; only 58% of these latter sera had recovery >80%. Interestingly, all TgAb-positive sera displaying a low recovery had an undetectable serum Tg measurement. These data led the authors to suggest that serum Tg measurements of TgAb-positive sera were reliable when the exogenous Tg recovered exceeded 80%. Marquet et al. have also used recovery as a criterion for authenticating serum Tg measurements [2]. Their Tg IRMA purportedly overcomes TgAb interference by using five monoclonal "capture" antibodies with specificity directed against antigenic domains on Tg that are not recognized by most human Tg autoantibodies [2, 12]. They concluded that their IRMA was minimally influenced by TgAb because they observed similar recoveries (range 50–150%) for TgAb-positive and TgAb-negative sera.

Recent studies raise serious questions as to whether recoveries of exogenous Tg from TgAb-positive sera can be used to detect TgAb interference and authenticate a serum Tg measurement (1, 7, Spencer et al., ms. in preparation). Recovery of an exogenous analyte from a serum is a valid test of that measurement only if the added analyte (in this case, Tg) is structurally identical to the substance being measured in samples submitted for analysis. For immunoassays, an additional criterion is that the added analyte should produce an antigen–antibody interaction identical to that of the endogenous analyte. Unfortunately, recovery of exogenous Tg from a TgAb-positive serum fails to meet either of these criteria. Not only are both Tg and TgAb in sera heterogeneous, but usually the recovery steps allow no time for the exogenous Tg to equilibrate with the endogenous Tg complexed with TgAb [13]. Recoveries are typically 10% higher when made under disequilibrium conditions (i.e., without pre-incubation; Spencer et al., ms. in preparation). These recoveries are higher presumably because immediately after addition the exogenous Tg is more accessible to the solid-phase "capture" antibody than is the Tg complexed with TgAb.

Tg is known to be structurally heterogeneous with respect to size, iodination, and glycosylation [14–18]. Immunoreactivity differences are also seen, not only between serum Tg isoforms and the Tg in the thyroid gland extracts typically used to assess recovery, but also between serum Tg isoforms arising from neoplastic and nonneoplastic thyroid tissues [19, 20]. The discovery of different Tg preparations from TgAb-positive sera has been shown to vary by as much as 20% (7, Spencer et al., ms. in preparation)—most likely reflecting the heterogeneous antibody–antigen reactions arising from differences in epitope specificity among serum Tg isoforms, the thyroid gland-derived added Tg, the Tg antibody used in the assay, and the polyclonal TgAbs encountered in different patients' sera. This latter source of heterogeneity (TgAb heterogeneity) is evident from the dissociation between the TgAb concentration and the recovery found both by Erali et al. [3] and others (6, 7, Spencer et al., ms. in preparation). Recovery is also influenced by the amount of Tg added. Erali et al. found that recovery was greater when larger amounts of Tg (50–100 μg) were used [3]; other studies have found the opposite effect [7]. It follows that there are several pathophysiological and technical reasons why the per-
percentage of exogenous Tg recovered from a TgAb-positive serum does not necessarily reflect the amount of endogenous Tg that would be recovered from that serum.

Studies that have used recovery to authenticate Tg measurements of TgAb-positive sera have rarely correlated the serum Tg results with the clinical status of the patients. In this issue, Erali et al. [3] provide no clinical data to justify that measurements that display >80% recovery and parallelism on dilution are clinically valid. Surprisingly, the study by Marquet et al. [2], in which a major conclusion was that the IRMA displayed minimal TgAb interference, failed to include any TgAb-positive sera in their data showing assay sensitivity and specificity and did not correlate serum Tg measurements of TgAb-positive sera with disease status. In an earlier study, Mariotti et al. [6] found that this IRMA offered no clinical advantages in comparison with another IRMA that did not use epitope selection. Specifically, Mariotti et al. reported that inappropriately undetectable serum Tg concentrations were seen in some TgAb-positive patients with metastatic thyroid cancer, despite the epitope selection design of the method, and despite studying the patients when Tg was maximally stimulated by a high TSH concentration [6].

Recently, my colleagues and I studied the clinical concordance between Tg measurements of TgAb-positive sera (using three different Tg methods: two IRMAs and a TgAb-resistant RIA [1, 21–23] and the recovery of various types of exogenous Tg [7]). All of the TgAb-positive sera were drawn from patients with unequivocal recurrent or metastatic thyroid cancer, in whom detectable serum Tg was expected. Our data supported the contention that recovery studies are not a reliable way to detect TgAb interference and should not be used to authenticate serum Tg measurements [7, Spencer et al., ms. in preparation]. Specifically, we found a significant discordance between serum Tg concentrations measured by IMA (both methods) and the RIA values: i.e., IMA values were undetectable or very low, whereas RIA values averaged 33.9 μg/L (range 1.2–92). Because the patients studied all had unequivocal evidence of disease, the IMA values were judged to be inappropriately low relative to the patients' clinical status, presumably reflecting underestimation attributable to TgAb interference. Some TgAb-positive sera with undetectable Tg IMA values have been shown to display a paradoxical rise in the Tg IMA measurement when rerun at a dilution, suggesting that TgAb interference causing underestimation can sometimes in part be diluted out [7, 11]. Importantly, this interference was not always evident from the recovery estimate, which was "appropriate" (>80%) by all three Tg methods in 4 of 11 of the TgAb-positive sera that displayed gross discrepancies between the IMA and RIA measurements [7].

When serum TgAb remains detectable in the postoperative period, patients usually have persistent disease and require long-term monitoring to detect recurrence [1, 10], Spencer et al., ms. in preparation). For such patients, interference causing underestimation of serum Tg concentrations is more clinically misleading than interference causing overestimation because an inappropriately low serum Tg may delay the diagnosis and treatment of recurrent or metastatic disease. This clinical perspective is not reflected by the statement of Erali et al. that "the development of IRMA and EIA methods has reduced and limited the autoantibody interference to causing falsely lowered values" [3]. To date, there is no reliable way to ensure that Tg measurements made in TgAb-positive sera are valid. RIA using high-affinity polyclonal antibodies and species-specific second antibodies, which reportedly provide good concordance between serum Tg and the clinical status of TgAb-positive patients, are not widely available [1, 7, 21–23]. Current commercially available Tg methods are primarily based on IMA methodology, which is prone to the problem of underestimation. It is unlikely that the approach of capture antibody selection [2, 6, 24] will overcome TgAb interference sufficiently that Tg measurements of TgAb-positive sera will be reliable enough for clinical decision-making. Although the epitopes selected are minimally expressed in TgAb arising from autoimmune thyroid diseases, patients with differentiated thyroid cancer typically have TgAb with broader-based epitope specificity than do patients with autoimmune thyroid conditions [27].

A laboratory's responsibility to the physician, and ultimately to the patient, is to report biochemical values (in this case, serum Tg concentrations) that prompt an appropriate clinical response. Given that the underestimation typical of TgAb interference with IMA measurements may have serious clinical consequences, identification of sera containing TgAb is imperative. Only TgAb immunoassays are sensitive enough for this; many sera with TgAb concentrations below the limit of detection of insensitive agglutination techniques have been found to display interference, as judged by an IMA/RIA discordance (Spencer et al., ms. in preparation). Even when TgAb is detected, apparently the qualitative characteristics of the TgAb and not the serum TgAb concentration per se determine whether interference occurs (Spencer et al., ms. in preparation). If recovery is not a reliable indicator of interference, a new test(s)—perhaps something like the IMA/RIA discordance test described [7, Spencer et al., ms. in preparation] is needed to identify which TgAb-positive sera cause interference. Until such a test is developed, clinical considerations dictate that laboratories using Tg IMA methods should carefully screen all sera by a sensitive TgAb immunoassay and refrain from reporting any Tg result for sera containing TgAb.

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References
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