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Serum Thyroglobulin Autoantibodies: Prevalence, Influence on Serum Thyroglobulin Measurement, and Prognostic Significance in Patients with Differentiated Thyroid Carcinoma*

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ABSTRACT

The prevalence of circulating thyroid autoantibodies (TgAb or antithyroid peroxidase) was increased nearly 3-fold in patients with differentiated thyroid cancers (DTC) compared with the general population (40% *vs.* 14%, respectively). Serum TgAb (with or without antithyroid peroxidase) was present in 25% of DTC patients and 10% of the general population. Serial postsurgical serum TgAb and serum Tg patterns correlated with the presence or absence of disease. Measurements of serum Tg were made in 87 TgAb-positive sera by a RIA and two immunometric assay (IMA) methods to study TgAb interference. TgAb interference, defined as a significant intermethod discordance (>41.7% coefficient of variation) between the Tg RIA and Tg IMA values relative to TgAb-negative sera, was found in 69% of the TgAb-positive sera. TgAb interference was characterized by higher Tg RIA *vs.* IMA values and was, in general, more frequent and severe in sera containing high TgAb concentrations. However, some sera displayed marked interference when serum TgAb was low (1–2 IU/mL), whereas other sera with very high TgAb values (>1000 IU/mL) displayed no interference. An agglutination method was found to be too insensitive to detect low TgAb concentrations (1–10 IU/mL) causing interference. Exogenous Tg recovery tests were an unreliable means for detecting TgAb interference. Specifically, the exogenous Tg recovered varied with the type and amount of Tg added and the duration of incubation employed. Further, recoveries of more than 80% were found for some sera displaying gross serum RIA/IMA discordances.

The measurement of serum Tg in DTC patients with circulating TgAb is currently problematic. It is important to use a Tg method that provides measurements that are concordant with tumor status. IMA methods are prone to underestimate serum when TgAb is present, increasing the risk that persistent or metastatic DTC will be missed. The RIA method used in this study provided more clinically appropriate serum Tg values in the group of TgAb-positive patients with metastatic DTC. Furthermore, as serial serum TgAb measurements paralleled serial serum Tg RIA measurements, TgAb concentrations may be an additional clinically useful tumor marker parameter for following TgAb-positive patients. Disparities between serial serum Tg and TgAb measurements might alert the physician to the possibility of TgAb interference with the serum Tg measurement and prompt a more cautious use of such data for clinical decision-making. (*J Clin Endocrinol Metab* **83:** 1121–1127, 1998)

SERUM thyroglobulin (Tg) is an established tumor marker used in the management of patients with a diagnosis of differentiated thyroid carcinoma (DTC) (1, 2). However, a number of technical problems impair the clinical utility of this test. These problems include a lack of method standardization, inadequate sensitivity, lack of interassay reproducibility, "hook" effects when measuring high concentrations, and Tg autoantibody (TgAb) interference (3). Recently, progress has been made in overcoming some of these limitations. For example, a collaborative effort has now produced an international Tg standard (CRM 457, BCR Brussels) (4, 5). RIAs are being replaced by more sensitive immunometric assay (IMA) methods with faster turn-around times, and recommendations for improving interassay precision and detecting hook effects have recently been published (3). Unfortunately, less progress has been made in detecting, quantifying, and eliminating the problem of TgAb interference, which may produce either under- or overestimation of serum Tg concentrations depending on the Tg method used (3, 6–8).

The present study investigated the prevalence of TgAb in a normal and DTC patient population and the influence of TgAb on serum Tg measurement. Two specific questions addressed were whether a threshold TgAb concentration existed that would predictably produce Tg assay interference and whether Tg recovery testing could be used to detect such interference. Attempts were also made to assess the clinical impact of TgAb interference on patient management by comparing different methods for measuring serum Tg and TgAb in TgAb-positive patients.

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Materials and Methods

Assays

All serum Tg and TgAb measurements were performed in duplicate. Results were reported as the average value.

Four different serum Tg assays methods, 1) RIA, 2) immunoradiometric assay 1 (IRMA-1); 3) IRMA-2, and 4) immunochemiluminometric assay (ICMA), were employed to measure serum Tg concentrations.

The Tg RIA was an in-house assay developed by the authors (University of Southern California Endocrine Services Laboratory, Los Angeles, CA). This method uses extensive ^{125}I-labeled Tg tracer (9, 10) together with a species-specific second antibody and a high affinity (1.16×10^{10} L/mol) rabbit polyclonal Tg antibody (11). This RIA has been reported to give clinically useful results in TgAb-positive patients (2, 11–14). Intraassay precision was 3.0% at 8.2 ng/mL. Interassay precisions across a 2-month period were 18.8%, 9.8%, and 4.7% at 1.5, 7.4, and 21.2 ng/mL, respectively.

The Tg IRMA-1 was DYNOtest TgS (Henning, Berlin, Germany). Intra- and interassay precisions for this method were 7.5% and 14% at 3.4 and 3.8 ng/mL, respectively.

The Tg IRMA-2 was OptiQuant Tg (Kronus, San Clemente, CA). Intra- and interassay precisions for this method were 2.7% and 9.9% at 5.5 and 5.8 ng/mL, respectively.

The Tg ICMA was a chemiluminescent ICMA (Nichols Institute Diagnostics, San Juan Capistrano, CA). Intraassay precision was 2.7% at 5.5 ng/mL. Interassay precisions were 8.9% and 6.3% at 2.0 and 20.6 ng/mL, respectively. The commercial Tg methods (IRMA-1, IRMA-2, and ICMA) were performed according to the manufacturer's instructions. Each assay included the CRM reference preparation diluted in the appropriate assay matrix so that intermethod variability could be minimized by reporting CRM-standardized serum Tg values (4, 5). Assay sensitivities, as determined from the 20% interassay coefficient of variation (CV; RIA) or as recommended by the manufacturer (IRMA-1, IRMA-2, and ICMA methods), were 0.5, 0.3, 0.3, and 0.5 ng/mL, respectively, when using CRM standardization.

The recovery of serum Tg (~10 ng/mL) from a TgAb-negative pool of DTC patient sera was made with the RIA, IRMA-1, and IRMA-2 methods. Other recovery studies with the IRMA-1 and IRMA-2 methods employed either the recovery preparation provided by the manufacturer (IRMA-1) or the assay standard diluted in the zero matrix (IRMA-2). In addition, recoveries were made with IRMA-1 and IRMA-2 and a low iodide Tg extract of an endemic goiter diluted in the appropriate zero standard.

Serum thyroid autoantibody assays

Three different commercial TgAb methods were tested: 1) agglutination (Sera-Tek, Miles, Elkhart, IN), 2) chemiluminescent immunoassay (Chemiluminescent ICMA, Nichols Institute Diagnostics; method 1), and 3) RIA (Kronus, San Clemente, CA; method 2). The intra- and interassay precisions were 8.7% and 5.9% at 2.0 and 40 IU/mL for method 1 and 8.7% and 14.2% at 3.1 and 1.9 IU/mL for method 2, respectively. Both methods 1 and 2 were calibrated against the WHO First International Reference Preparation (IRP) 65/93 and had an analytical detection limit of 1.0 IU/mL. Antithyroid peroxidase (anti-TPO) autoantibodies were measured using a commercial RIA method calibrated against the WHO First IRP 66/387, which had an analytical detection limit of 0.5 IU/mL (Kronus). All thyroid autoantibody measurements were made according to the manufacturer's recommended procedure.

Statistical methods

Student's *t* tests were used to analyze the data from the recovery studies. TgAb concentrations were analyzed by Wilcoxon scores, and relative risks were assessed by the Fisher exact test.

Study population and design

Prevalence of serum TgAb. The prevalence of thyroid autoantibodies in the general population was established using TgAb (method 2) and anti-TPO measurements of 4453 sera from ambulatory healthy subjects (mean age, 45.3 yr; range, 12–99 yr; male/female ratio, 0.69) undergoing routine multiphasic health examinations. The thyroid autoantibody prevalence in this population was compared with that of a group of 213 sera from patients with an established diagnosis of DTC in whom serial serum Tg measurements had been performed by our laboratory (mean age, 51 yr; range, 1–84 yr; male/female ratio, 0.29).

Serum Tg measurements in TgAb-negative and TgAb-positive sera. Serum Tg and TgAb concentrations were measured in 15 TgAb-negative sera using four Tg methods (RIA, IRMA-1, IRMA-2, and ICMA) and three TgAb methods (agglutination, method 1, and method 2). Serum TgAb was measured by these same methods in 97 TgAb-positive sera, of which a subset of 87 TgAb-positive sera with sufficient volume had serum Tg measured by the RIA, IRMA-2, and ICMA methods.

Exogenous Tg recovery studies. Ten TgAb-negative (all methods) sera from clinically disease-free DTC patients and 11 sera from TgAb-positive patients with documented recurrent or metastatic DTC were used for recovery studies employing the RIA, IRMA-1, and IRMA-2 methods. Serum Tg recoveries were made by preincubating (18 h at ambient temperature to allow the Tg and Tg-TgAb serum complexes to equilibrate before assay) an equal quantity (1:1 mixture) of test serum with either a TgAb-negative DTC serum pool containing about 10 ng/mL Tg or the zero matrix. Tg recoveries were calculated from the observed/expected Tg concentrations. In other experiments, IRMA-1 and IRMA-2 recoveries were made without preincubation using the procedure recommended for IRMA-1 and employing different quantities of different Tg preparations. IRMA-1 was evaluated with the manufacturer's recovery preparation (47 ng/mL), the internal standard (93 ng/mL), and a low iodine Tg preparation at two doses (7 and 66 ng/mL). IRMA-2 was evaluated with two different doses of its internal standard (10 and 96 ng/mL) and the low iodine preparation (7 and 66 ng/mL). These various Tg preparations were diluted in the appropriate zero standard, as needed. The normal range of Tg recovery was determined from the 95% confidence limits established from the 10 TgAb-negative sera.

Assessment of long term serial serum TgAb measurements. A retrospective review of laboratory records was used to identify 15 patients (3 men and 12 women) who met 3 criteria: 1) TgAb positive before or shortly after the initial surgical treatment for DTC, 2) 3 or more serial serum specimens covering follow-up periods averaging 59 months (range, 10–161) available in −20 C storage for batchwise reanalysis of TgAb (method 2), and 3) sufficient information from morphological testing (computed tomography and/or magnetic resonance imaging), imaging studies (radioiodine and/or thallium-201) and physician examination taken at the most recent follow-up visit for a clinical classification as either disease free or persistent/recurrent disease.

Results

Prevalence of TgAb in DTC and gender differences

The prevalence of detectable TgAb was higher in DTC patients than in the general population (24.9% and 10.1%, respectively), whether TgAb was present alone (8.0% *vs.* 3.1%, respectively) or in combination with anti-TPO (16.9% *vs.* 7.0%, respectively), as shown in Fig. 1. The prevalence of anti-TPO alone was also increased in the DTC patient group (15.0% *vs.* 4.0%, respectively). When gender was considered, the prevalence of TgAb was increased in both women (2-fold) and men (3.3-fold) compared with that in the general population. Anti-TPO prevalence was similarly increased in DTC female (2.4-fold) and male (3.7-fold) patients.

Comparison of TgAb methods

Figure 2 displays correlations between the serum TgAb concentrations of 97 sera measured by 3 different methods. Panels a and b show correlations between each of the immunoassay methods (methods 1 and 2) and agglutination, respectively, and panel c shows the correlation between

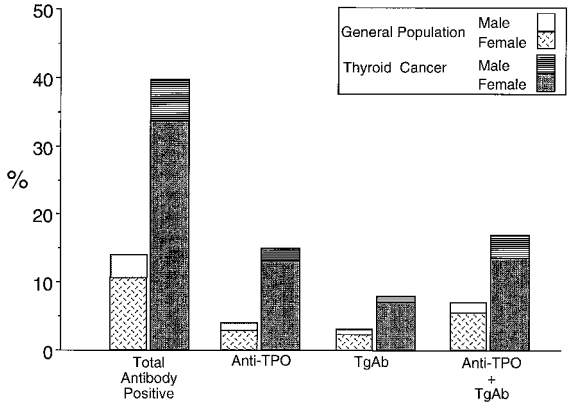

FIG. 1. Prevalence of thyroid autoantibodies (anti-TPO or TgAb alone or in combination) in the general population (multiphasic health evaluations) compared with that in patients with DTC. The percentage of total antibody-positive patients includes all patients with either antibody (anti-TPO or TgAb) detected.

immunoassay methods 1 and 2. Both methods 1 and 2 correlated with the agglutination titers (r = 0.91 *vs.* 0.88, respectively); however, the lowest agglutination titers (1:100 and 1:400) correlated with method 1, but not method 2. Overall, there was a weaker correlation between the two immunoassay methods (panel c; r = 0.67; $P < 0.001$) than between either immunoassay and agglutination. In addition, 6 sera had TgAb detected by method 1, but not by method 2. In 48 sera (49.5%), serum TgAb was detected by both immunoassay methods 1 and 2, but not by agglutination. In fact, most sera with TgAb below 10 IU/mL by immunoassay were agglutination negative (93.8% and 79.2% for methods 1 and 2, respectively). Furthermore, despite the use of the same IRP standard, method 1 produced systematically higher values than method 2, as shown in c.

Comparison of Tg methods for measuring TgAb-negative and TgAb-positive sera

The mean ± SD intermethod (RIA, IRMA-1, IRMA-2, and ICMA) CV of 15 TgAb-negative sera was 29.0 ± 8.8 (range, 13.9–47.8% (Fig. 3). Eighty-seven of the 97 TgAb-positive sera shown in Fig. 2 had serum Tg measured by the RIA, IRMA-2, and ICMA methods. Intermethod discordance was judged to be present when the intermethod CV of a TgAb-positive serum exceeded the 97.5% confidence limit of the TgAb-negative group (41.7%). Sixty of the 87 (69%) TgAb-positive sera displayed such a discordance (CV = 120.2 ± 35.2; range, 52.9–168.8%), whereas 26 TgAb-positive sera did not (CV, 25.7 ±2.0; range, 8.2–41.4%). Discordance was always characterized by higher serum Tg RIA compared with one or more Tg IMA values.

TgAb interference and serum TgAb concentration

The 60 TgAb-positive sera displaying discordance had higher serum TgAb concentrations [method 1; median, 35.3 (range, 1.3–239,000) *vs.* 3.5 (range, 1.0–12,200 IU/mL), discordant *vs.* concordant, respectively], as shown in Fig. 4. However, in some cases discordance was seen in sera with very low (1–2 IU/mL) serum TgAb concentrations, whereas discordance was absent in other sera with very high TgAb (>1,000 IU/mL).

Exogenous Tg recovery studies

Table 1 shows mean recoveries for a serum Tg source (Tg, ~10 ng/mL) added to each of 10 TgAb-negative and 11 TgAb-positive (TgAb concentration mean, 220; range, 2–1720 IU/mL; by method 2) sera, as measured by 3 Tg assays (RIA, IRMA-1, and IRMA-2). The expected recovery, calculated from the 95% confidence limits of the TgAb-negative sera, was 99 ± 8% (±SD; range, 84–114%) for the RIA, 95 ± 8% (range, 82–108%) for IRMA-1, and 95 ± 6% (range, 82–107%) for IRMA-2. Although all of the Tg methods produced lower mean recoveries with TgAb-positive sera, the recoveries were systematically higher using the RIA method (81.1 ± 19.7%; range, 48–106%) than using the IMA methods [73.1 ± 30.3% (range, 44–106%) *vs.* 73.3 ± 37.7% (range, 0–100%); IRMA-1 *vs.* IRMA-2, respectively]. The mean recovery for the TgAb-positive group was lower for all methods compared with the corresponding mean recovery for the TgAb-negative group ($P < 0.001$). More importantly, 4 of 11 (36%) of the TgAb-positive sera with Tg recoveries in excess of 80% (in all Tg methods) had grossly discordant serum Tg values (15, 17, 35, and 92 ng/mL by RIA *vs.* undetectable using both IRMA-1 and IRMA-2).

Influence of Tg source and recovery procedure

As shown in Table 2, the Tg source and the amount of Tg added as well as the recovery procedure used influenced the efficiency of Tg recoveries from TgAb-positive sera. Specifically, recoveries varied up to 20% according to the Tg source used. Also, the addition of the larger amounts of Tg produced up to about 10% lower recoveries compared with smaller amounts of the same Tg source. Further, when the added Tg was allowed to equilibrate with the test serum before assay (18 h at ambient temperature), recoveries were about 10% lower using both the IRMA-1 and IRMA-2 methods compared with recoveries made without prior equilibration of the exogenous Tg with the serum ($P < 0.04$).

Long-term serial serum TgAb and Tg measurements in DTC

Serial serum TgAb (method 2) and Tg RIA measurements were performed in a selected group of 15 TgAb-positive DTC patients, as shown in Fig. 5. Six patients were considered to be clinically free of cancer after an average follow-up of 37.2 months (range, 10–75), whereas 9 patients had clinical or radiographic evidence of persistent or recurrent disease after follow-up averaging 68.9 months (range, 26–161). The serum Tg RIA was selected for this comparison because it produces clinically concordant values in the presence of TgAb (2, 11–14). Serum TgAb and Tg concentrations declined to low or undetectable levels on or before the second postoperative year in the disease-free group. In contrast, all patients with persistent or recurrent disease retained detectable serum TgAb and Tg concentrations throughout the follow-up period. Serum TgAb and Tg RIA values tended, in general, to parallel each other. Further, serum TgAb measurements ap-

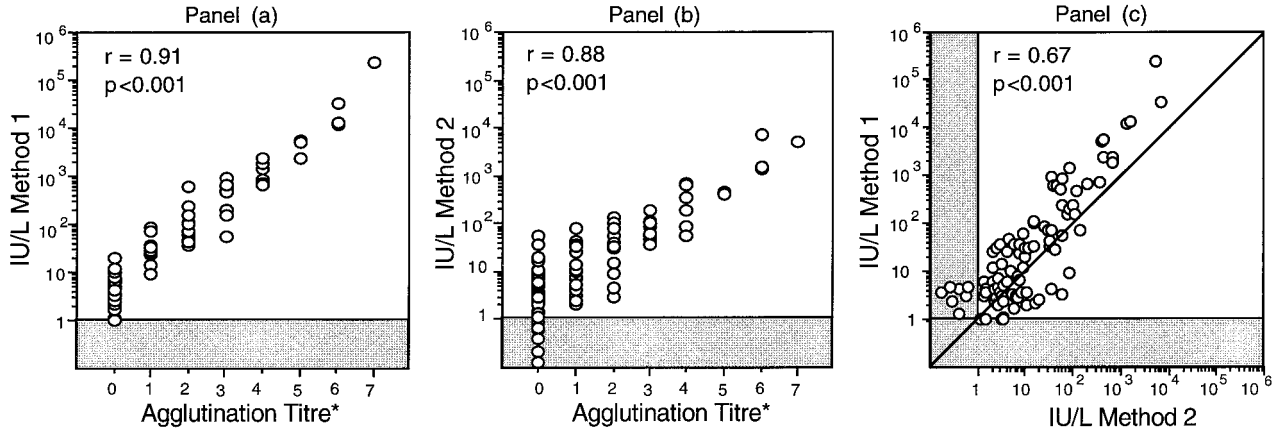

FIG. 2. Correlations between different TgAb methods. a, Serum TgAb concentrations measured by method 1 (Nichols Institute ICMA) *vs.* TgAb titers by agglutination (SeraTek). b, Serum TgAb concentrations measured by method 2 (Kronus RIA) *vs.* agglutination. c, Correlation between method 1 and method 2 values. *, Titers: 0 = negative; 1 = 1:100; 2 = 1:400; 3 = 1:1,600; 4 = 1:6,400; 5 = 1:25,000; 6 = 1:102,000; and 7 = 638,000. The *shaded area* represents undetectable values.

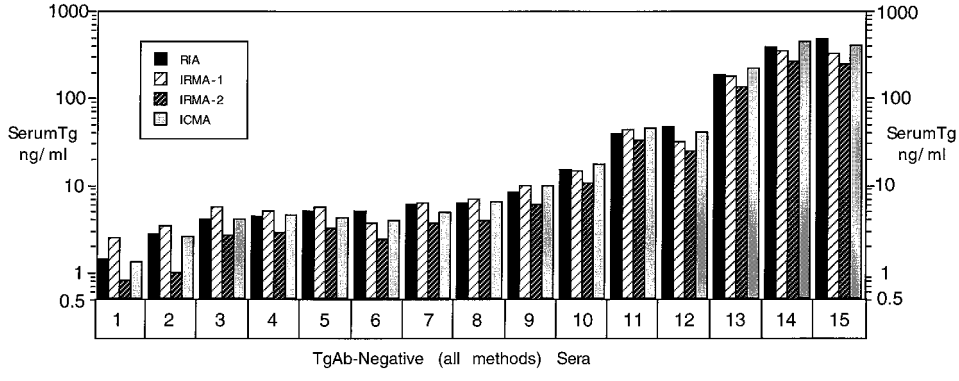

FIG. 3. Serum Tg values in 15 TgAb-negative (all TgAb methods) patients using RIA (University of California Endocrine Services Laboratory), IRMA-1 (DYNOtest Tg, Henning, 1995 formulation), IRMA-2 (OptiQuant Tg, Kronus), and ICMA Tg (Nichols Institute Diagnostics).

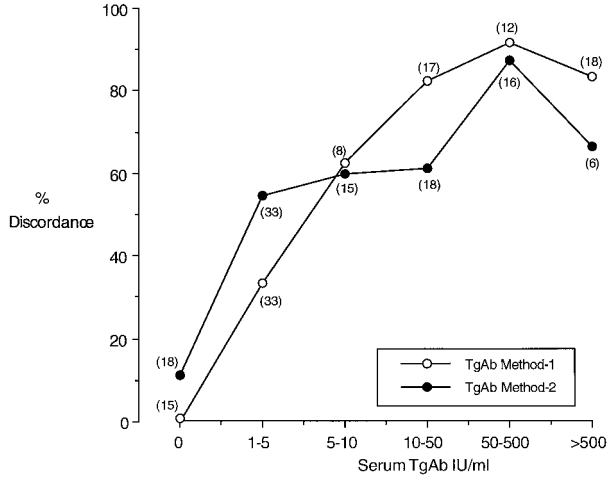

FIG. 4. Percentage of TgAb-positive sera displaying an RIA/IMA discordance *vs.* TgAb concentration measured by TgAb method 1 (*open symbols*) and TgAb method 2 (*closed symbols*). The numbers in *parentheses* indicate the number of determinations included in each group.

peared to produce more consistent and reproducible results than the serum Tg RIA in this group of patients. Decreases in both serum TgAb and serum Tg concentrations were noted in 2 patients who underwent additional surgeries (1 patient twice) to remove metastatic or recurrent tumor, as shown by the *arrows* in Fig. 5.

Discussion

The present study confirmed other reports showing an increased prevalence (40% *vs.* 14%) of thyroid autoantibodies (TgAb and/or anti-TPO) in patients with DTC compared with the general population, respectively (15, 16). In particular, TgAb (with or without anti-TPO) was detected in 25% of DTC patients compared with 10% of the general population. Thus, the relative risk of TgAb positivity in the DTC compared to the control group was 2.5, with a 95% confidence interval of 2.0–3.2. This high prevalence of autoantibodies is consistent with both the use of more sensitive immunoassay TgAb methods in preference to agglutination as well as the 2-fold greater female representation in the DTC group *vs.* the control population (15–18). However, even after accounting for gender differences, the increased autoantibody prevalence in DTC was still increased 2-fold relative to that in the controls. The increased prevalence of thyroid antibodies in DTC might reflect enhanced presentation of thyroid tumor antigens to the immune system, although contrary to this viewpoint are studies suggesting that tumor Tg has reduced antigenicity as a result of lower iodine content (19).

The clinical significance of TgAb positivity in DTC depends on the stage of disease. It is unclear whether the

TABLE 1. Recoveries of serum Tg (~10 ng/mL) from TgAb-negative and TgAb-positive sera

Tg method	Tg conc., mean ± SD (range)	Antibody status (n)	% Mean ± SD recovery of serum Tg[a]	Range
RIA	6.6 ± 1.3 (2.0–12.7)	TgAb-negative (10)	99 ± 8	89–109
	15.6 ± 3.7 (2.8–45.0)	TgAb-negative (11)	81 ± 19	48–106
IRMA-1	6.4 ± 1.2 (2.5–14.5)	TgAb-negative (10)	95 ± 8	82–108
	<0.3 (<0.3 to 1.1)	TgAb-positive (11)	73 ± 30	44–106
IRMA-2	5.8 ± 1.1 (2.1–13.1)	TgAb-negative (10)	95 ± 6	89–109
	<0.5 (<0.5 to 0.9)	TgAb-positive (11)	73 ± 38	0–100

[a] Using a 1:1 mixture with a TgAb-negative DTC serum pool containing about 10 ng/mL Tg.

TABLE 2. Analysis of exogenous Tg recovered from TgAb-positive sera (n = 11)

Tg method	Tg source (dose)	% Recovery, mean ± SE (range)	*18-h preincubation	Different Tg sources/comparable doses	Comparable Tg sources/different doses
IRMA-1	Recovery standard (47 ng/mL)*	80.9 ± 10.5 (10–131)	$P < 0.04$		
	Recovery standard (47 ng/mL)	89.3 ± 8.2 (30–123)			$P < 0.0001$
	Recovery standard (93 ng/mL)	75.1 ± 8.5 (17–117)		$P < 0.05$	
	Low iodine Tg (7 ng/mL)	86.8 ± 9.9 (18–117)			NS
	Low iodine Tg (66 ng/mL)	82.0 ± 10.0 (10–131)			
IRMA-2	Kit standard (10 ng/mL)*	54.8 ± 10.8 (0–101)	$P < 0.04$		
	Kit standard (10 ng/mL)	64.8 ± 10.9 (0–101)		$P < 0.005$	$P < 0.04$
	Kit standard (96 ng/mL)	58.5 ± 9.8 (0–97)			
	Low iodine Tg (9 ng/mL)	84.5 ± 13.6 (0–117)		$P<0.005$	$P < 0.04$
	Low iodine Tg (99 ng/mL)	73.5 ± 11.6 (0–113)			

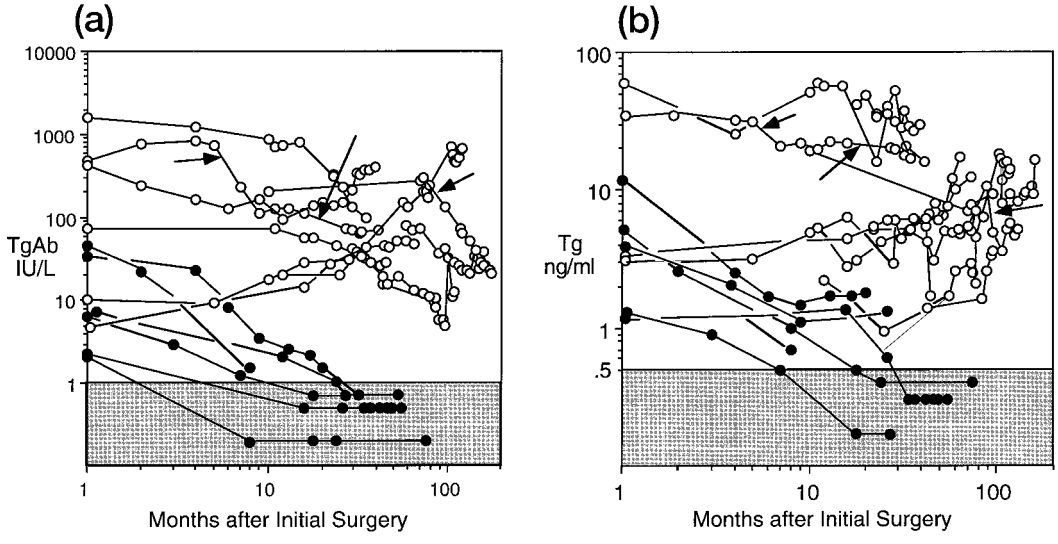

FIG. 5. Serial serum TgAb (panel a; method 2) and Tg RIA (panel b) measurements after initial thyroid surgery (month 0) for DTC. Patients were classified at their most recent follow-up visit as having persistent or recurrent disease (*open symbols*) or were judged to be disease free (*closed symbols*). The *shaded area* represents undetectable serum TgAb levels. *Arrows* indicate additional surgeries.

finding of thyroid antibodies at the time of DTC diagnosis and initial surgery has any clinical significance (17). One study reports a correlation among lymphocytic infiltration, serum thyroid autoantibodies, and a favorable long term outcome (18). Our data are in accord with those of other reports showing that the retention of TgAb positivity during long term follow-up indicates persistent disease, whereas the loss of TgAb positivity suggests a surgical cure (16, 20–22).

The three TgAb methods used in this study differed in sensitivity and specificity, as shown in Fig. 2. These differences were seen despite the use of the same IRP standard, suggesting that TgAbs with different epitope specificities were being recognized. The TgAb immunoassays had superior sensitivity compared with the agglutination test, as previously reported (15–18). Specifically, nearly 50% of sera with TgAb detected by immunoassay had no TgAb detected by agglutination. As most sera with TgAb below 10 IU/mL were agglutination negative, and 35% of these sera had evidence of TgAb interference as judged from an intermethod discordance, agglutination was judged to be too insensitive a screen for TgAb interference. Indeed, as two of the three sera with TgAb detected by method 1 but not method 2 displayed a Tg intermethod discordance, it appears critical that only the

most sensitive immunoassay methods should be used to screen for TgAb interference.

TgAb interference can produce either under- or overestimation of serum Tg depending on the method (7, 8, 23). Tg IMAs typically underestimate serum Tg when sera contain TgAb, presumably because the endogenous Tg complexed with TgAb cannot participate in the reaction (8, 26). Typical double antibody RIA methods may either under- or overestimate serum Tg. The magnitude and direction of the interference are determined by the affinity of the first antibody, the species specificity of the second antibody, the volume of serum used, and the characteristics of the TgAb present (7). These factors influence the partitioning of the serum and tracer Tg moieties between the endogenous (human) and exogenous (animal) antibody-bound fractions during the separation step (7). The Tg RIA employed in this study was used as the reference method because it appears minimally affected by TgAb, as judged by studies showing concordance of Tg values with tumor status (2, 11–14). In this study, the serum Tg RIA measurements made in the TgAb-positive patients with metastatic or recurrent disease were all appropriately detectable (range, 1.2–90 ng/mL). In contrast, the Tg IMA values (IRMA-1 or IRMA-2) were all undetectable in these same patients. This RIA/IMA discordance was characteristic of many TgAb-positive sera and most likely represented TgAb interference (3, 8). There appeared to be no threshold TgAb level below which discordance did not occur. Some sera with very low (1–2 IU/mL) TgAb displayed a RIA/IMA discordance, whereas other sera with very high TgAb concentrations (>1000 IU/mL) did not. The weak correlation between the presence of discordance and the TgAb concentration is in accord with the results of other studies, suggesting that TgAb interference cannot be predicted from the TgAb concentration with any degree of certainty (17, 27).

Typically, the recovery of exogenous Tg from a TgAb-positive serum has been used to detect TgAb interference (7, 8, 25, 28–31). Table 1 contrasts the recoveries of serum Tg (~10 ng/mL) from TgAb-positive and TgAb-negative sera using the RIA, IRMA-1, and IRMA-2 methods. All of these TgAb-positive sera had a RIA/IMA discordance despite appropriate (>80%) recoveries in 73%, 54%, and 64% of the sera, measured by the RIA, IRMA-1, and IRMA-2 methods, respectively. Importantly, there was an RIA/IMA discordance (Tg RIA, 15, 35, 92, and 17 ng/mL *vs.* undetectable serum Tg IRMA-1 and IRMA-2 values) in four sera with recoveries that exceeded 80% by each method. The fact that TgAb-positive sera can exhibit appropriate recoveries and yet have grossly discordant serum Tg values depending on the Tg method is evidence that a recovery test cannot be used to validate a Tg measurement in serum containing TgAb (32). Any immunological difference between exogenous Tg and endogenous Tg would invalidate the recovery approach. Recoveries were shown to be influenced by the amount and type of exogenous Tg. This might reflect inherent Tg heterogeneity, as multiple Tg isoforms with differing epitope specificities have been found in both serum and the tissue-derived Tg preparations typically used for recovery (33). The heterogeneity inherent in serum TgAb is an additional factor that impacts and may explain the dissociation between the TgAb concentration and recovery (7, 27).

Currently, there is no reliable method for detecting and overcoming the problem of TgAb interference with serum Tg measurements. The strategy of using monoclonal capture antibodies with specificities for Tg epitopes not involved in the autoimmune response is conceptually attractive, but may be flawed by the broader based epitope specificity encountered in DTC *vs.* autoimmune thyroid disease (35). Such epitope selection approaches have not been successful in practice, as evidenced by the finding of undetectable serum Tg IMA values in patients with metastatic disease (8, 34). It is important to consider the clinical impact of the direction of TgAb interference. Underestimation of serum Tg in a TgAb-positive patient is more problematic for both the patient and physician than interference causing overestimation and concomitant unnecessary imaging studies. Specifically, this and other studies show that patients with persistent TgAb are more likely to have residual disease, such that a false negative result may cause a delay in detecting and treating recurrent or metastatic DTC. In this study the Tg RIA appeared less prone to producing false negatives and appeared to provide a more clinically conservative serum Tg estimate. It is important for physicians to know the direction of TgAb interference expected with their Tg assay and factor this into clinical decision-making.

No current Tg method (whether IMA or RIA) can claim freedom from TgAb interference in every patient. Even if the technical problems surrounding the measurement of total (free and TgAb-bound) Tg are solved, it is uncertain how a total Tg result in a TgAb-positive patient should be interpreted. It is likely that total Tg is influenced not only by the rate of secretion of Tg from the tumor and by the capacity and affinity of the TgAbs present, but also by changes in the rate of clearance of Tg bound to immune complexes (36). This study shows that it is imperative that sera sent for Tg measurement be screened for TgAb by sensitive immunoassays and not insensitive agglutination tests. As there is currently no reliable way to identify which TgAb-positive sera suffer from interference, all serum Tg measurements of TgAb-positive sera should be interpreted with caution. This study suggests that serial TgAb measurements have independent clinical value for following TgAb-positive DTC patients. A concordant serial Tg and TgAb pattern suggests fairly accurate tumor marker data. In contrast, the development of a disparity between these two parameters suggests that TgAb interference may be compromising the accuracy of the serum Tg measurement for clinical decision-making.

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