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Thyroglobulin Liquid Chromatography–Tandem Mass Spectrometry Has a Low Sensitivity for Detecting Structural Disease in Patients with Antithyroglobulin Antibodies

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Background: Thyroglobulin (Tg) measurement in patients with positive antithyroglobulin antibodies (anti-TgAbs) is not reliable. Tg measurement using liquid chromatography–tandem mass spectrometry (LC/MS) may be useful in this setting.
Methods: This is a retrospective study with the objective of determining the accuracy of Tg-LC/MS in patients with thyroid cancer with anti-TgAbs. All patients with follicular cell-derived thyroid cancer (TC) who had thyroglobulin measured using LC/MS assay from November 1, 2013, to November 7, 2014, were evaluated. The frequency of detectable Tg-LC/MS was evaluated, with a functional sensitivity (FS) of 0.5 ng/mL in patients with structural disease. Then performance of Tg-LC/MS versus Tg immunometric assay (IMA) was compared using either Immulite assay (Tg-1) with a FS of 0.9 ng/mL or Beckman assay (Tg-B) with a FS of 0.1 ng/mL in detecting structural disease in patients with positive anti-TgAbs.
Results: A total of 154 consecutive patients were included in this evaluation. Of these, 116 (75%) patients were positive for anti-TgAbs. In patients with structural disease and positive anti-TgAbs, Tg-LC/MS was undetectable in 43.7% of patients. Then the diagnostic accuracy for structural disease of Tg-LC/MS was compared with each Tg-IMA assay separately. In the 26 patients with positive anti-TgAbs where a Tg-I assay was used, the sensitivity and specificity for detecting structural disease were 33.3% and 88.2%, respectively, for the Tg-I assay, and 44.4% and 94.1%, respectively, for the Tg-LC/MS assay. In the 74 patients with positive anti-TgAbs where Tg-B was used, the sensitivity and specificity for detection of structural disease were 72.7% and 71.4%, respectively, for the Tg-B assay, and 62.6% and 93.7%, respectively, for the Tg-LC/MS assay.
Conclusion: In patients with thyroid cancer with positive anti-TgAbs, Tg-LC/MS was frequently undetectable and was less sensitive for detecting disease than a Tg assay was with a functional sensitivity of 0.1 ng/mL. For patients with detectable Tg-LC/MS and anti-TgAbs, use of the assay for monitoring requires further prospective studies.

Keywords: thyroid cancer, antithyroglobulin antibodies, thyroglobulin mass spectrometry

Introduction

Antithyroglobulin antibodies (anti-TgAbs) are detectable in about 20–25% of patients with thyroid cancer (TC) (1,2). These antibodies interfere with measurement of thyroglobulin (Tg), the key biomarker used to detect and monitor thyroid cancer (2). Tg is generally measured using immunometric assays (IMA) or radioimmunoassay (RIA), with the former being more commonly used in the United States. Anti-TgAbs can interfere with the measurement of Tg in both of these assays, with a tendency to underestimate Tg when IMA is used and either an under- or overestimation when RIA is used (1).

Measurement of Tg using peptide immunoaffinity enrichment with liquid chromatography–tandem mass spectrometry (Tg-LC/MS) has been proposed as a clinically accurate alternative method that is not influenced by the presence of anti-TgAbs (3–6). Indeed, the Tg-LC/MS assay is offered at a number of clinical laboratories as a test to be performed reflexively as a measure of Tg instead of Tg-IMA in the presence of anti-TgAbs. However, the usefulness of Tg-LC/MS measurement as an adjunctive test in thyroid cancer management

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in clinical practice has not been fully assessed. In a large series of patients using multiple assays, Netzel *et al*. reported that Tg-LC/MS was undetectable in 40% of patients with positive anti-TgAbs and structural disease, but that it incrementally increased the detection of circulating Tg in these patients in comparison to Tg-IMA, suggesting that it may be of benefit in some patients with anti-TgAbs (7). Spencer *et al*. reported 23% of patients with persistent disease and positive anti-TgAbs to have undetectable or marginally detectable Tg-LC/MS (8). However, more data are needed to study the utility of Tg-LC/MS as an adjunct to the management of thyroid cancer in clinical practice.

The aims of the present study were (i) to describe the accuracy of Tg-LC/MS for determining structural disease status in patients with thyroid cancer, and (ii) to compare the performance of Tg-LC/MS and Tg-IMA in detecting structural disease in those patients with circulating anti-TgAbs in a clinical practice setting.

Material and Methods

This was a retrospective cohort study conducted at the Ohio State University Wexner Medical Center and Arthur G. James Cancer Hospital and Solove Research Institute, the Ohio State Comprehensive Cancer Center, and was approved by the Ohio State University Institutional Review Board. Patients were identified through a query of the Ohio State University Endocrine Neoplasia Repository database. This repository is open to all thyroid cancer patients seen at any time during the course of treatment and follow-up for their disease. All patients were included who had a Tg-LC/MS (Mayo Medical Laboratories, Rochester, MN) for the one-year period from November 1, 2013, through November 7, 2014. Tg-LC/MS was performed at the discretion of the treating physician in a subspecialty thyroid cancer clinical practice. All patients with TC who had the test performed were included. Variables analyzed included patient age at thyroid cancer diagnosis, sex, thyroid cancer histological type, type of therapeutic surgical procedure, and the components of TNM staging. Other pathological features such as the presence or absence of vascular invasion and extrathyroidal extension were also included in addition to therapeutic radioactive iodine use, Tg and anti-TgAbs measurements and assays used, time since initial surgery and time since last intervention (such as surgery and radioactive iodine therapy), and the Tg-LC/MS measurement. A patient was considered to have positive anti-TgAbs if this was positive by any assay within six months of the measurement of Tg-LC/MS and if there were no interventions done between the measurement of Tg-LC/MS and the anti-TgAbs levels, if they were not done at the same time. All patients had Tg-LC/MS measured on levothyroxine therapy. Thirty-one patients also had levels measured with thyrotropin (TSH) stimulation. The disease status of the patients was defined by the assessment of the treating physician. Patients with structural disease required evidence of disease either by imaging or histology.

Description of the assays used for Tg and anti-TgAbs

During the course of the study, the laboratory test used for Tg and anti-TgAbs changed. Tg was measured by IMA (determined by Siemens Immulite or Beckman Coulter assays) or by Mayo Medical Laboratories LC/MS assay. The three assays used for Tg measurement were: (i) Immulite 2000 XPi Thyroglobulin assay (Tg-I; catalog no. PIL2KTY; Siemens, Inc., Deerfield, IL), which has a functional sensitivity defined by a CV <20% of 0.9 ng/mL; (ii) Beckman Access Tg (Tg-B; Beckman Coulter) performed at Mayo Medical Laboratories (Rochester, MN), which has a functional sensitivity of 0.1 ng/mL; and (iii) Tg-LC/MS performed at Mayo Medical Laboratories, which has a functional sensitivity of 0.5 ng/mL.

Anti-TgAbs were detected using three assay systems during the course of time of the study: (i) Immulite 2000 XPi Anti-Thyroglobulin Antibody assay (anti-TgAbs-I; catalog no. L2KTG2; Siemens, Inc), which is a two-step chemiluminescent immunoassay for quantitation of antibodies against Tg, with the manufacturer functional sensitivity listed as 20 IU/mL; (ii) Roche assay (anti-TgAbs-R) performed at the Mayo Medical Laboratories with a functional sensitivity of 20 IU/mL; and (iii) Access Thyroglobulin Antibody II assay (anti-TgAbs-B; Beckman Coulter) performed at the Mayo Medical Laboratories with a functional sensitivity of 1.8 IU/mL.

For this analysis, a detectable level on any one of the assays was reported as a positive result. One patient (without definitive evidence of structural disease) in the study was determined to have anti-TgAbs when detected using a radioimmunoassay with a sensitivity of 0.4 IU/mL (University of Southern California, Los Angeles, CA).

Statistics

Patient characteristics are summarized as median and range or number and percent as appropriate. The frequency of detectable Tg-LC/MS was measured in patients with structural disease. For the primary analysis, the diagnostic accuracy of Tg-LC/MS for detecting structural disease in patients who had positive anti-TgAbs was estimated by calculating sensitivity, specificity, PPV, and NPV. Then, this was compared to the accuracy of each Tg-IMA in detecting structural disease in those patients with positive anti-TgAbs. Spearman's correlation was calculated to investigate the relationship between anti-TgAbs levels and Tg-LC/MS.

Results

Patient characteristics

Table 1 describes the patient characteristics. Tg-LC/MS was performed on 154 patients during the time of the data collection. All but one patient underwent total thyroidectomy. Median age at diagnosis was at 40.5 years; 78% (120/154) were female, and 91% (140/154) had papillary thyroid cancer, including variants. Of the 154 patients, 116 (75%) had positive anti-TgAbs on at least one assay. This high percentage is consistent with the expected preferential use of the Tg-LC/MS assay in individuals with anti-TgAbs. The median time since initial surgery when Tg-LC/MS was performed was 97 months (range 1–432 months), and median time since last intervention (such as additional surgery or ^{131}I therapy) was 70.5 months (range 1–408 months).

Description of patients with structural disease

Table 2 describes the characteristics of the 22 patients with structural disease. Sixteen patients in this group had positive anti-TgAbs. Tg-LC/MS was detectable in 15/22 (68.2%)

Table 1. Patient Characteristics

Age at diagnosis, years	40.5 (9–81)
Sex, female	120 (78%)
Type:	
PTC	140 (91%)
FTC	4 (3%)
HTC	4 (3%)
Mixed	3 (2%)
Poorly differentiated	2 (1%)
Anaplastic dedifferentiation	1 (1%)
Cell variant (in PTC patients)	
Usual	118 (83%)
fvPTC	17 (12%)
Tall cell	5 (4%)
Sclerosing	1 (1%)
Columnar	1 (1%)
Oncocytic variant	1 (1%)
Multifocal	86 (56%)
T stage	
1a	29 (19%)
1b	41 (27%)
2	33 (21%)
3	42 (27%)
4	4 (3%)
Unknown	2 (1%)
Size of largest focus, cm	2.4 (1.81)
Extrathyroidal extension	32 (21%)
Vascular invasion	39 (25%)
Hashimoto's thyroiditis	83 (54%)
N stage	
0	41 (27%)
1a	26 (17%)
1b	58 (38%)
Unknown	18 (12%)
M stage	
M1	6 (4%)
^{131}I administered	140 (91%)
Time since initial surgery, months	97.0 (1–432)
Time since last intervention, months	70.5 (1–408)
TgAbs positive by any assay	116 (75%)

Data are median (range) or *n* (%).
PTC, papillary thyroid carcinoma; FTC, follicular cell carcinoma; HTC, Hürthle cell carcinoma; fvPTC, follicular variant of PTC; TgAbs, antithyroglobulin antibodies.

patients (in one of these 15 patients, Tg-LC/MS was only detectable with TSH stimulation), while it was undetectable in 7/22 (32%). The seven patients with undetectable Tg-LC/MS included several individuals with metastatic disease in lungs (four patients), neck (five patients), mediastinum (two patients), and bone (one patient). Tg-IMA was detectable in 16/22 (72.7%) patients. In the 16/22 patients with structural disease who also had positive anti-TgAbs, Tg-LC/MS was detected in 9/16 (56.3%) patients (one was only detectable with TSH stimulation), which means that it was undetectable in 43.7%, and Tg-IMA was detectable in 10/16 (62.5%) patients. Eight of the nine patients with positive Tg-LC/MS also had detectable Tg by IMA. Conversely, seven of nine patients with positive anti-TgAbs and detectable Tg-IMA were also detected by Tg-LC/MS. The overall concordance between the two Tg assay systems in the presence of anti-TgAbs was 67%. Of the six patients with structural disease and undetectable anti-TgAbs, Tg-IMA and Tg-LC/MS were detectable in 6/6 (100%) of these patients.

Comparing the performance of Tg-LC/MS and Tg-IMA in predicting structural disease in patients with positive anti-TgAbs

Because the most clinically valuable use of the TG-LC/MS is in patients with anti-TgAbs, the accuracy of Tg-LC/MS for detecting structural disease in these patients was studied. Patients who were considered to be indeterminate for structural disease were excluded. In this analysis, the sensitivity and specificity were 56.3% and 93.9%, respectively, and the PPV and NPV were 69.2% and 89.9%, respectively, for the Tg-LC/MS (Table 3). Then the accuracy of Tg-IMA (whether done by Tg-I or Tg-B assays as one group) was analyzed in determining structural disease in these patients with positive anti-TgAbs. First, a cutoff of 0.1 ng/mL for Beckman and 0.9 ng/mL for Immulite for the functional sensitivity of the Tg-IMA assays was used. Here, the sensitivity and specificity were 62.5% and 73.8%, respectively, and the PPV and NPV were 37% and 88.9%, respectively (Table 3). Second, a cutoff equal to or greater than the Tg-LC/MS assay of 0.5 ng/mL (0.5 ng/mL for Beckman and 0.9 ng/mL for Immulite) was used. Here, the sensitivity and specificity were 43.8% and 95.4%, respectively, and the PPV and NPV were 70% and 87.3% respectively (Table 3). Thus, when using the same cutoff of 0.5 ng/mL, the accuracy of Tg-LC/MS and TG-IMA to detect disease were similar, except for a higher sensitivity using Tg-LC/MS (56.3% vs. 43.8%). The accuracy of Tg-LC/MS was then compared with each Tg-IMA assay separately as two different groups. In the 26 patients (Table 4) with positive anti-TgAbs where the presence or absence of structural disease could be determined, and where Tg-I assay was used, the sensitivity and specificity were 44.4% and 94.1%, respectively, and the PPV and NPV were 80% and 76.2%, respectively, for the Tg-LC/MS. The sensitivity and specificity were 33.3% and 88.2%, respectively, and the PPV and NPV were 60% and 71.4%, respectively, for the Tg-I assay. In the 74 patients with positive anti-TgAbs where the presence or absence of structural disease could be determined and where Tg-B was used (Table 5), the sensitivity and specificity for Tg-LC/MS were 63.6% and 93.7%, respectively, and the PPV and NPV were 63.6% and 93.7%, respectively. In these 74 patients, the sensitivity and specificity for Tg-B using the FS cut point of 0.1 ng/mL were 72.7% and 71.4%, respectively, and the PPV and NPV were 30.8% and 93.8%, respectively. In these 74 patients, the sensitivity and specificity of Tg-B using a FS cut point of 0.5 ng/mL (to match that of the Tg-LC/MS) were 45.5% and 96.8%, respectively, and the PPV and NPV were 71.4% and 91%, respectively.

Data on patients who had TSH-stimulated Tg-LC/MS

There were 31 patients who had TSH-stimulated Tg-LC/MS levels. Nine patients had a detectable TSH-stimulated Tg-LC/MS level with a mean of 9.5 (range 0.6–70). Three of these nine patients had structural disease, two of whom also had available unstimulated Tg-LC/MS levels, one of which was detectable. Three of the nine patients had indeterminate structural disease, and three were considered free of structural disease. Twenty-two patients had undetectable Tg-LC/MS level with TSH stimulation, including 17 considered free

Table 2. Description of Patients with Structural Disease

Patient ID	*Cancer type*	*Location of metastases*	*Anti-TgAbs positive*	*Tg-IMA Beckman (ng/mL)*	*Tg-IMA Immulite (ng/mL)*	*Unstimulated Tg-LC/MS value (ng/mL)*	*Stimulated Tg-LC/MS value if done*
10	HTC	Neck	No	2	ND	3.4	
15	PTC	Neck	Yes	351	ND	115[a]	
19	PTC (tall cell)	Neck	No	2.8	ND	2.6	
20	PTC (fv)	Lungs, mediastinum	No	9.6	ND	6.8	
27	PTC	Lung	Yes	ND	<0.9	<0.5	
42	Poorly differentiated insular	Lungs	No	24	39	19	
44	PTC	Neck	Yes	2.6	27	30	
47	HTC	Neck	Yes	1.8	ND	2.3	
63	PTC	Neck	Yes	0.9	ND	1.1	
68	PTC	Neck	Yes	<0.1	ND	<0.5	
77	PTC	Neck	Yes	0.1	ND	<0.5	
93	HTC	Neck	No	4.7	6.3	ND	3.8
94	PTC	Neck	Yes	0.1	ND	<0.5	
97	HTC	Lungs	Yes	1.1	ND	6.8	
106	PTC	Neck, lungs	No	8.2	ND	8.3	
107	PTC (fv)	Lungs, mediastinum	Yes	ND	<0.9	<0.5	
108	PTC	Neck, lungs	Yes	ND	<0.9	<0.5	
134	PTC	Neck, lung, mediastinum, bone	Yes	<0.1	ND	<0.5	
139	PTC	Neck, lungs, bone	Yes	6810	ND	4250	
145	PTC (tall cell)	Neck, lungs, mediastinum	Yes	<0.1	<0.9	0.6	
146	PTC	Neck	Yes	0.4	ND	<0.5	0.9
152	PTC	Neck, lungs, mediastinum	Yes	ND	6	7.9	70

Median unstimulated Tg-LC/MS when detectable: 6.8 ng/mL (range 0.6–4250 ng/mL).
[a]TSH was at 15 when this value was obtained.
ND, not done; TSH, thyrotropin.

Table 3. Diagnostic Accuracy of Tg-LC/MS, Immunometric Tg (Cufoff 0.1 ng/mL), and Immunometric Tg (Cutoff 0.5 ng/mL) for Detecting the Presence of Structural Disease in Patients with Positive Anti-TgAbs

	Structural disease	*No structural disease*
Tg-LC/MS		
Tg detectable	9	4
Tg not detectable	7	62
Immunometric Tg (cutoff 0.1 ng/mL)		
Tg detectable	10	17
Tg not detectable	6	48
Immunometric Tg (cutoff 0.5 ng/mL)		
Tg detectable	7	3
Tg not detectable	9	62

Immunometric Tg done by either Beckman or Immulite assay.
Tg-LC/MS: sensitivity = 9/16 (56.3%); specificity = 62/66 (93.9%); PPV = 9/13 (69.2%); NPV = 62/69 (89.9%).
Tg-IMA (cutoff 0.1 ng/mL): sensitivity = 10/16 (62.5%); specificity = 48/65 (73.8%); PPV = 10/27 (37.0%); NPV = 48/54 (88.9%).
Tg-IMA (cutoff 0.5 ng/mL): sensitivity = 7/16 (43.8%); specificity = 62/65 (95.4%); PPV = 7/10 (70%); NPV = 62/71 (87.3%).

of structural disease and five considered to have indeterminate structural disease. In this group of patients, there were only six patients who could be defined as free of disease (no structural disease, undetectable Tg, and anti-TgAbs) and six patients who had structural disease and/or detectable Tg in the absence of anti-TgAbs. If the latter group was considered as having evidence of disease structurally and/or biochemically,

Table 4. Diagnostic Accuracy of Tg-LC/MS and Immulite Immunometric Tg (Cutoff 0.9 ng/mL) for Detecting the Presence of Structural Disease in Patients with Positive Anti-TgAbs and Having Tg Immulite Measured ($N = 26$)

	Structural disease	*No structural disease*
LC/MS		
Tg detectable	4	1
Tg not detectable	5	16
Immulite assay		
Tg detectable	3	2
Tg not detectable	6	15

Tg-LC/MS: sensitivity = 4/9 (44.4%); specificity = 16/17 (94.1%); PPV = 4/5 (80.0%); NPV = 16/21 (76.2%).
Immulite Tg-IMA: sensitivity = 3/9 (33.3%); specificity = 15/17 (88.2%); PPV = 3/5 (60.0%); NPV = 15/21 (71.4%).

Table 5. Diagnostic Accuracy of Tg-LC/MS, Immunometric Tg (Cutoff 0.1 ng/mL), and Immunometric Tg (Cutoff 0.5 ng/mL) for Detecting Presence of Structural Disease in Patients with Positive Anti-TgAbs and Having Tg Beckman Measured ($N = 74$)

	Structural disease	*No structural disease*
Tg-LC/MS		
Tg detectable	7	4
Tg not detectable	4	59
Tg-IMA (cutoff 0.1 ng/mL)		
Tg detectable	8	18
Tg not detectable	3	45
Tg-IMA (cutoff 0.5 ng/mL)		
Tg detectable	5	2
Tg not detectable	6	61

Tg-LC/MS: sensitivity = 7/11 (63.6%); specificity = 59/63 (93.7%); PPV = 7/11 (63.6%); NPV = 59/63 (93.7%).

Tg-IMA (cutoff 0.1 ng/mL): sensitivity = 8/11 (72.7%); specificity = 45/63 (71.4%); PPV = 8/26 (30.8%); NPV = 45/48 (93.8%).

Tg-IMA (cutoff 0.5 ng/mL): sensitivity = 5/11 (45.5%); specificity = 61/63 (96.8%); PPV = 5/7 (71.4%); NPV = 61/67 (91.0%).

then the TSH-stimulated Tg-LC/MS sensitivity and specificity were 83.3% and 83.3%, respectively, and the PPV and NPV were 83.3% and 83.3%, respectively.

Correlation between anti-TgAbs and Tg-LC/MS levels

Anti-TgAbs and Tg-LC/MS were negatively correlated, but the correlation was statistically significant only for the Roche assay with Spearman's correlation of −0.31 ($\rho = 0.001$). In patients with structural disease, anti-TgAb levels and Tg-LC/MS were negatively correlated in all assays, but this was statistically significant only for anti-TgAb Immulite and Roche assays values with Spearman's correlation of −0.69 ($\rho = 0.04$) and −0.61 ($\rho = 0.03$), respectively. The one patient who had positive anti-TgAbs by RIA assay was excluded from these correlation analyses.

Discussion

Anti-TgAbs pose a challenge in the management of thyroid cancer, as they interfere with the measurement of Tg. The trend of anti-TgAb levels over time (9–12) and serial imaging based on the extent of disease (13,14) are generally recommended for monitoring these patients. Measurement of Tg-LC/MS has been developed as a method to provide an accurate level of Tg, avoiding the interference of these antibodies, and over the past few years, it has been adopted by several commercial laboratories. Methodologically, there should be no interference with anti-TgAbs on the LC/MS method, making it an attractive option for clinical development (3–6). The current study presents how this assay performed when applied to a clinical practice environment.

The study comprised 154 patients with TC who had measurements of Tg-LC/MS available. The first key finding was that 32% of the entire subgroup with structural disease had undetectable Tg on the LC/MS method, and 43.7% of those with structural disease and positive TgAbs had undetectable Tg-LC/MS. The second key finding was that in patients with positive anti-TgAbs, the sensitivity of detecting structural disease was higher with Tg-B using a cutoff of 0.1 ng/mL compared with Tg-LC/MS, indicating no clear additional benefit in disease detection by measuring Tg-LC/MS compared to Tg-B in these patients. In fact, only 1/16 patients who had structural disease and who was positive for anti-TgAbs had undetectable Tg-IMA but detectable Tg-LC/MS. These similarities between Tg-LC/MS and Tg-IMA in detecting structural disease in the presence of anti-TgAbs was unexpected and suggest no major additional clinical benefit in measuring Tg-LC/MS to detect disease in patients with positive anti-TgAbs. While Tg-LC/MS had better sensitivity, specificity, PPV, and NPV than Tg-I did when the two were compared independently of Tg-B, it is noted that the FS of Tg-I used was 0.9 ng/mL, while for Tg-LC/MS it was 0.5 ng/mL, and this could explain the better performance of Tg-LC/MS compared with Tg-I. On the other hand, if Tg-LC/MS is compared with Tg-B alone, independently of Tg-I, then the sensitivity is still better in Tg-LC/MS than it is in Tg-B, but only if using the same FS of 0.5 ng/mL (63.6% vs. 45.5%), but if using the actual FS of Tg-B at 0.1 ng/m:, the sensitivity of Tg-B was higher than Tg-LC/MS at 72.7% compared with at 63.6%. These results demonstrate that at the current FS of the Tg-B assay of 0.1 ng/mL, there was no incremental benefit in measuring Tg-LC/MS. However, if Tg-LC/MS had an improved FS, it is possible that its performance would also improve.

Netzel *et al.* (7) reported undetectable Tg-LC/MS in 40% of patients with positive anti-TgAbs who had structural disease, which is very similar to the results of the present study (43.7%). In addition, the PPV and NPV in patients with structural disease were similar to the data of Netzel *et al*. In that study, however, 8% of additional samples were detected by Tg-LC/MS compared with Tg-B, but it is not certain how many of these patients had structural disease. Differences between the studies include a smaller population and the use of a single Tg-LC/MS assay in the present study. A strength of the present study is that it was limited to patients followed in a dedicated thyroid cancer clinic with clinical management and ultrasounds performed by experienced thyroid cancer clinicians rather than a more broad clinical practice pattern.

There are several limitations to this study. First, it was a retrospective cohort study assessing patients who had Tg-LC/MS performed, and while it was the practice to check Tg-LC/MS on patients with positive anti-TgAbs in the period when the data were collected, this was not prospectively performed to confirm that all patients with positive antibodies had measurement of Tg by LC/MS. Also, the reasons for ordering Tg-LC/MS in patients who were negative for anti-TgAbs varied between different endocrinologists and were not always clearly stated, but they were usually related to suspicion of interference due to mismatch between clinical data and Tg levels or lack of rise of Tg following TSH stimulation in the past. Thus, there may be a selection bias, but this seems limited, as it would need to be uniform for all of the clinicians in the practice. The second limitation is that the anti-TgAb levels were usually, but not always, measured in the same sample as the Tg-LC/MS. Levels were accepted that were performed within six months prior to Tg-LC/MS and in whom there were no interventions between laboratory tests. From a clinical perspective, the latter approach is reasonable due to the small number of patients likely to convert from a positive to a negative level over six months. This same

limitation applies to the fact that Tg levels by different assays (Tg-I or Tg-B) were not performed on the same phlebotomy sample. Third, as a retrospective study, the status of disease determination was not uniform. In patients with localized or no evidence of disease, neck ultrasound was performed in all patients. However, additional cross-sectional imaging was performed as deemed appropriate by the treating clinician based on the status of the patient and the tumor characteristics. Thus, it is possible that some of the ''structural negative'' group was incorrectly classified according to the extent of imaging performed in each patient. Fourth, the anti-TgAbs were not measured by all assays on every patient, and it is possible that some patients may have tested positive for anti-TgAbs if the measurement had been done using a different assay. This is evident in the fact that there was some discordance between the assays when they were done on the same patient, a result that is supported by data in previous studies but also reflecting common clinical practice (15,16). Finally, it is uncertain how many of the false-negative results would have become positive on Tg-LC/MS if it was performed following TSH stimulation, as this was not done routinely in this cohort. This is significant, since structural disease can be identified with low unstimulated Tg levels. As an example, Heilo *et al*. (17) reported a median Tg level of 0.7 μg/L (range <0.2–26μg/L) in patients with metastatic cervical nodes. This emphasizes the importance of assay sensitivity for detecting residual thyroid cancer. However, in patients with positive anti-TgAbs, a blunted response of Tg to stimulation has also been reported (18). In the present study, among the subgroup of individuals who had stimulated Tg-LC/MS following TSH stimulation, the PPV and NPV were 83.3% and 83.3%, respectively, compared with a PPV and NPV of 84% and 65.4%, respectively, for Tg-LC/MS in the whole cohort. However, the number of patients that could be defined as true positive and true negative in this subgroup of patients who had stimulated Tg-LC/MS was small. Thus, the observation of possible improved accuracy requires confirmation in larger and, optimally, prospective studies.

From a clinical perspective, it is important to draw attention to the patients with structural disease who had undetectable Tg-LC/MS. As shown in Table 2, this included several individuals with large-volume distant metastases in whom a measurable Tg by IMA in the absence of antibodies would be expected. These individuals raise concern that the use of non-TSH-stimulated Tg-LC/MS as a replacement test for Tg-IMA and TgAb levels may lead clinicians to assume there is a very low likelihood of anatomic disease. It is not clear why some patients with high-volume structural disease had undetectable Tg-LC/MS. It is possible that some patients have variants in the secreted Tg protein that alter trypsin-binding sites such that LC/MS will not detect the secreted Tg, or that there are several variants secreted and the sensitivity is reduced when the measured peaks are lower per amount of secreted Tg protein. It is also possible that these particular tumors do not secrete much Tg, despite having well-differentiated pathology, or that the metastatic disease has dedifferentiated. As a retrospective study, surgical biopsies were not taken in patients with large-volume distant metastases. While this could be a factor, the frequency of such tumors is likely to be rare, as the study cohort was nearly exclusively comprised of patients with well-differentiated thyroid cancer. Another possibility is increased metabolic clearance of Tg in patients with positive anti-TgAbs, which has been postulated before (19,20). This latter theory could be supported by the finding of statistically significant negative correlation between anti-TgAb levels in Immulite and Roche assays and Tg-LC/MS levels.

It should be emphasized that in addition to the role of Tg in predicting detection of disease at a given time, Tg measurement also has an important role in monitoring disease progression over time. The present study only analyzed the ability of Tg-LC/MS to *detect* disease at the time that the data were collected. Its role in *monitoring* disease in patients with a measurable Tg-LC/MS was not addressed. Although there is evidence that reducing or rising anti-TgAb levels correlate with the likelihood of detecting disease, the results are not always consistent in individual patients and can take months or years to change (9). Thus, Tg-LC/MS levels may play an important role, if positive, in monitoring these patients with thyroid cancer who have anti-TgAbs. Defining the role of Tg-LC/MS for this purpose will require prospective studies beyond the scope of the present study.

Conclusion

In conclusion, in the present study, Tg-LC/MS and Tg-IMA displayed similar detection characteristics in patients with positive anti-TgAbs. Together with other published data, the results raise caution regarding the use of Tg-LC/MS alone to detect thyroid cancer in patients with anti-TgAbs and the current use of this assay as a reflex test when antibodies are detected by some clinical laboratories. While the data do not confirm the prior results of an incremental benefit of Tg-LC/MS compared with Tg-IMA in thyroid cancer detection in patients with positive anti-TgAbs, others have shown benefit that may be magnified depending on the specific antibody and Tg immunoassay being used. Further prospective studies are needed to determine the role of Tg-LC/MS, including following TSH stimulation, in monitoring disease progression, regression, or response to therapy in patients with detectable levels.

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Author Disclosure Statement

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References

1. Spencer CA, Takeuchi M, Kazarosyan M, Wang CC, Guttler RB, Singer PA, Fatemi S, LoPresti JS, Nicoloff JT 1998 Serum thyroglobulin autoantibodies: prevalence, influence on serum thyroglobulin measurement, and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab **83:**1121–1127.
2. Pacini F, Mariotti S, Formica N, Elisei R, Anelli S, Capotorti E, Pinchera A 1988 Thyroid autoantibodies in thyroid cancer: incidence and relationship with tumour outcome. Acta Endocrinol (Copenh) **119:**373–380.
3. Hoofnagle AN, Becker JO, Wener MH, Heinecke JW 2008 Quantification of thyroglobulin, a low-abundance serum

protein, by immunoaffinity peptide enrichment and tandem mass spectrometry. Clin Chem **54:**1796–1804.
4. Clarke NJ, Zhang Y, Reitz RE 2012 A novel mass spectrometry-based assay for the accurate measurement of thyroglobulin from patient samples containing antithyroglobulin autoantibodies. J Investig Med **60:**1157–1163.
5. Kushnir MM, Rockwood AL, Roberts WL, Abraham D, Hoofnagle AN, Meikle AW 2013 Measurement of thyroglobulin by liquid chromatography-tandem mass spectrometry in serum and plasma in the presence of antithyroglobulin autoantibodies. Clin Chem **59:**982–990.
6. Hoofnagle AN, Roth MY 2013 Clinical review: improving the measurement of serum thyroglobulin with mass spectrometry. J Clin Endocrinol Metab **98:**1343–1352.
7. Netzel BC, Grebe SK, Carranza Leon BG, Castro MR, Clark PM, Hoofnagle AN, Spencer CA, Turcu AF, Algeciras-Schimnich A 2015 Thyroglobulin (Tg) testing revisited: Tg assays, TgAb assays, and correlation of results with clinical outcomes. J Clin Endocrinol Metab **100:** E1074–1083.
8. Spencer C, Petrovic I, Fatemi S, LoPresti J 2014 Serum thyroglobulin (Tg) monitoring of patients with differentiated thyroid cancer using sensitive (second-generation) immunometric assays can be disrupted by false-negative and false-positive serum thyroglobulin autoantibody misclassifications. J Clin Endocrinol Metab **99:**4589–4599.
9. Kim WG, Yoon JH, Kim WB, Kim TY, Kim EY, Kim JM, Ryu JS, Gong G, Hong SJ, Shong YK 2008 Change of serum antithyroglobulin antibody levels is useful for prediction of clinical recurrence in thyroglobulin-negative patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab **93:**4683–4689.
10. Durante C, Tognini S, Montesano T, Orlandi F, Torlontano M, Puxeddu E, Attard M, Costante G, Tumino S, Meringolo D, Bruno R, Trulli F, Toteda M, Redler A, Ronga G, Filetti S, Monzani F, Group PTCS 2014 Clinical aggressiveness and long-term outcome in patients with papillary thyroid cancer and circulating anti-thyroglobulin autoantibodies. Thyroid **24:**1139–1145.
11. Rubello D, Casara D, Girelli ME, Piccolo M, Busnardo B 1992 Clinical meaning of circulating antithyroglobulin antibodies in differentiated thyroid cancer: a prospective study. J Nucl Med **33:**1478–1480.
12. Chung JK, Park YJ, Kim TY, So Y, Kim SK, Park DJ, Lee DS, Lee MC, Cho BY 2002 Clinical significance of elevated level of serum antithyroglobulin antibody in patients with differentiated thyroid cancer after thyroid ablation. Clin Endocrinol (Oxf) **57:**215–221.
13. Verburg FA, Luster M, Cupini C, Chiovato L, Duntas L, Elisei R, Feldt-Rasmussen U, Rimmele H, Seregni E, Smit JW, Theimer C, Giovanella L 2013 Implications of thyroglobulin antibody positivity in patients with differentiated thyroid cancer: a clinical position statement. Thyroid **23:** 1211–1225.
14. Ringel MD, Nabhan F 2013 Approach to follow-up of the patient with differentiated thyroid cancer and positive anti-thyroglobulin antibodies. J Clin Endocrinol Metab **98:** 3104–3110.
15. Latrofa F, Ricci D, Montanelli L, Rocchi R, Piaggi P, Sisti E, Grasso L, Basolo F, Ugolini C, Pinchera A, Vitti P 2012 Thyroglobulin autoantibodies in patients with papillary thyroid carcinoma: comparison of different assays and evaluation of causes of discrepancies. J Clin Endocrinol Metab **97:**3974–3982.
16. Spencer C, Petrovic I, Fatemi S 2011 Current thyroglobulin autoantibody (TgAb) assays often fail to detect interfering TgAb that can result in the reporting of falsely low/undetectable serum Tg IMA values for patients with differentiated thyroid cancer. J Clin Endocrinol Metab **96:** 1283–1291.
17. Heilo A, Sigstad E, Fagerlid KH, Haskjold OI, Groholt KK, Berner A, Bjoro T, Jorgensen LH 2011 Efficacy of ultrasound-guided percutaneous ethanol injection treatment in patients with a limited number of metastatic cervical lymph nodes from papillary thyroid carcinoma. J Clin Endocrinol Metab **96:**2750–2755.
18. Spencer CA, Lopresti JS 2008 Measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Nat Clin Pract Endocrinol Metab **4:** 223–233.
19. Weigle WO, High GJ 1967 The behavior of autologous thyroglobulin in the circulation of rabbits immunized with either heterologous or altered homologous thyroglobulin. J Immunol **98:**1105–1114.
20. Feldt-Rasmussen U, Petersen PH, Date J, Madsen CM 1980 Sequential changes in serum thyroglobulin (Tg) and its autoantibodies (TgAb) following subtotal thyroidectomy of patients with preoperatively detectable TgAb. Clin Endocrinol (Oxf) **12:**29–38.

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