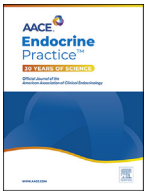
Endocrine Practice xxx (xxxx) xxx

Endocrine Practice™
www.endocrinepractice.org



Original Article

Undetectable Serum Thyroglobulin in Patients With Differentiated Thyroid Cancer: Antithyroglobulin Antibodies, Assay Limitation, or Other?

Benjamin Andress, PhD, Sandra A. Miller, BS, Anthony D. Maus, PhD, Jennifer V. Kemp, MHA, Joshua A. Bornhorst, PhD, Stefan K. Grebe, MD, PhD, Alicia Algeciras-Schimnich, PhD *

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota

ARTICLE INFO

Article history:
Received 5 February 2025
Received in revised form
21 May 2025
Accepted 4 June 2025
Available online xxx

Key words:
thyroglobulin
immunoassay
mass spectometry
differentiated thyroid cancer

ABSTRACT

Objective: To determine why some patients with differentiated thyroid cancer (DTC) lymph node (LN) metastases do not have detectable serum thyroglobulin (Tg).
Methods: Fine needle aspiration biopsy (FNAB) washout fluid from patients with DTC LN metastases and undetectable serum Tg measurement by immunoassay (Tg-IA) were evaluated for the presence of Tg and anti-Tg antibodies (TgAbs). Spike-recovery experiments in serum were performed to assess the reason for undetectable Tg.
Results: Of the 42 patients, 87% had detectable Tg in the FNAB washout fluid by Tg-IA, and 83% by Tg mass spectrometry (Tg-MS) measurement. Seventy-six percent of these patients had detectable serum TgAb, while 26% did not. Tg spike-recovery experiments performed on the TgAb+ (positive) serum samples showed decreased Tg recovery by Tg-IA but not by Tg-MS (Tg-IA mean, range: 50%, 12%-84%; Tg-MS 96%, 70%-117%). In TgAb- (negative) serum samples no interference was observed (>94% recoveries). No difference in FNAB washout fluid Tg recovery between TgAb- and TgAb+ patients was observed.
Conclusion: Tg was detected by both Tg-IA and Tg-MS methods in the majority of FNAB washout fluid from patients with DTC LN metastases who exhibited undetectable serum Tg by Tg-IA and Tg-MS. The absence of serum Tg could not be completely explained by the presence of TgAb. These results suggest that, for a subset of patients with DTC LN metastases, the absence of detectable Tg in serum does not appear to be due to analytical limitations of current Tg assays or the presence of TgAb interference.

Introduction

Serum thyroglobulin (Tg) measurements for posttreatment monitoring of differentiated thyroid carcinoma (DTC), is a key component of recurrence detection,[1] in particular for patients with near total or total thyroidectomy, with or without radioiodine ablation. The negative predictive value of a very low or undetectable posttreatment serum Tg measurement is reported as 98.6%[2]; however, for those patients that experience recurrence, sensitivity for detection of recurrent DTC is only around 80%, even with, highly sensitive serum Tg assays.[3] In fact, there are reports of metastatic DTC recurrence in the presence of undetectable serum Tg.[4-8] It remains unclear whether the lack of detectable serum Tg in these cases is due to assay interference from the presence of anti-Tg antibodies (TgAbs), a lack of Tg production by the recurrent tumor, insufficient sensitivity of current Tg assays, or other factors.

One of the limitations of most current Tg immunoassays (Tg-IAs) is potential analytical interference by TgAbs. TgAbs are detectable in

Abbreviations: DTC, differentiated thyroid cancer; FNAB, fine needle aspiration biopsy; LC, liquid chromatography; LN, lymph node; MS, mass spectrometry; Tg, Thyroglobulin; TgAb, anti-Tb antibody; Tg-IA, Tg immunoassay; Tg-MS, Tg mass spectrometry.

* Address correspondence to Dr Alicia Algeciras-Schimnich, Mayo Clinic, 200 1st Street SW, Rochester, MN 55905.
E-mail address: algeciras.alicia@mayo.edu (A. Algeciras-Schimnich).

https://doi.org/10.1016/j.eprac.2025.06.001
1530-891X/

Please cite this article as: B. Andress, S.A. Miller, A.D. Maus *et al.*, Undetectable Serum Thyroglobulin in Patients With Differentiated Thyroid Cancer: Antithyroglobulin Antibodies, Assay Limitation, or Other?, Endocrine Practice, https://doi.org/10.1016/j.eprac.2025.06.001

approximately 20% to 30% of patients with thyroid cancer, while less than 10% of the general population have detectable levels of these antibodies.[9-11] TgAbs can cause either a false increase or false decrease in Tg-IAs, depending on the format.[11,12] Immunometric assays, which comprise most commercial Tg-IA utilized in current clinical practice (including the Beckman Access Thyroglobulin IA discussed in this report), exhibit a false decrease in Tg measurement in the presence of TgAbs, potentially yielding false-negative results when monitoring for disease recurrence. Therefore, it is recommended that TgAb always be measured in conjunction with Tg-IA measurement to assess the reliability of the Tg measurement.[1] In order to overcome TgAbs interference with Tg-IA, mass spectrometry (MS)–based assays incorporating trypsin digestion of all sample proteins (including TgAbs), followed by immuno-enrichment of Tg-specific peptides and detection by liquid chromatography (LC)-MS/MS methodology, have been developed. While early versions of these Tg mass spectrometry (Tg-MS) assays lacked sufficient analytical sensitivity for non-thyroid stimulating hormone–stimulated Tg monitoring postthyroidectomy,[13] current iterations of Tg-MS have enhanced analytical sensitivity comparable to high sensitivity Tg-IA methods (0.1-0.2 ng/mL). This is considered sufficient for non-thyroid stimulating hormone–stimulated surveillance and has been shown to effectively identify the presence of Tg in some patients with TgAbs and undetectable Tg by Tg-IA.[12] Since Tg-IA remains the most sensitive method for patients without TgAbs, testing algorithms starting with assessment of TgAbs status, followed by Tg-IA measurements if TgAb-negative and Tg-MS if TgAb-positive are commonly used for posttreatment surveillance.[14,15]

Measurement of Tg in fine needle aspiration biopsy (FNAB) washout fluid is also a well-established and useful method for investigating potential recurrence of DTC in suspicious lymph nodes (LNs) or nodules arising from the thyroid bed in post-thyroidectomy patients. FNAB washout Tg has been demonstrated to be much more sensitive than cytology alone,[16-19] with a reported sensitivity of greater than 90%. The combination of FNAB cytology and FNAB washout Tg can achieve greater than 95% sensitivity and 90% specificity for recurrent metastatic DTC.[16]

Despite advancements in Tg testing modalities, there are still patients with metastatic DTC to LNs, as confirmed by FNAB cytology, which appear to not have detectable serum Tg. This observation raises the question of whether these patients remain serum Tg-negative or undetectable due to (1) absence of clinically significant Tg production; (2) TgAbs interfering with Tg detection; or (3) nonsecretory Tg production. The aim of this study was to determine, if in patients with metastatic DTC to LNs and undetectable Tg in serum, Tg could be detected in the FNAB washout fluid, and if so, whether an explanation for the lack of detectable Tg in serum can be elucidated.

Highlights

- Some patients with cytology-confirmed differentiated thyroid cancer lymph node metastases have undetectable serum thyroglobulin (Tg)
- These patients have detectable Tg in the fine needle aspiration biopsy washout fluid
- Absence of serum Tg cannot be fully explained by the presence of anti-Tg antibodies in circulation

Clinical Relevance

The absence of serum thyroglobulin (Tg) in patients with differentiated thyroid cancer (DTC) lymph node metastases cannot be fully explained by assay interference from anti-Tg antibodies. Alternate hypothesized mechanisms for undetectable Tg may include decreased production of Tg in the DTC lymph node metastases, impaired Tg release into circulation, or rapid Tg clearance from circulation.

Methods

Study Cohort

FNAB washout fluid specimens and serum samples ($n = 42$) from patients with cytology-confirmed DTC LN metastases, with undetectable serum Tg were collected between May 14, 2019 and July 17, 2023. Serum samples were collected up to 90 days prior to FNAB (median: 6 days prior). Serum Tg negativity for this cohort was determined by Tg-IA, Tg-MS, or a combination of both. Of the 42 patients, 21 patients (10 TgAb+/11 TgAb-) had serum Tg measured by only Tg-IA and 7 patients (2 TgAb+/5 TgAb-) had serum Tg measured by only Tg-MS. The remainder ($n = 14$), which were all TgAb+, had serum Tg measured by both Tg-MS and Tg-IA; in all cases, both Tg-MS and Tg-IA were negative. Medical record review was performed to obtain additional demographics information.

In order to determine whether patients with detectable serum Tg had higher levels of Tg in FNAB washout fluid, a cohort of patients with FNAB washout fluid Tg reported up to 90 days prior of a positive serum Tg result ($n = 76$) were included. This control group was selected based on the following criteria: (1) a FNAB washout fluid collected within the same date range as the cohort of serum Tg-negative and cytology-confirmed DTC LN metastases patients; (2) the presence of cytology confirmed papillary thyroid cancer metastasis to LN; and (3) a detectable Tg in serum that was collected up to 90 days prior of the FNAB washout fluid collection (median: 2 days prior). Chart review was performed on this cohort to obtain additional demographic information.

The Institutional Review Board reviewed this study, and it was considered exempt as minimal risk research.

Assays

FNAB washout material and serum samples were assayed for Tg using the Beckman Access Thyroglobulin IA on a DxI analyzer and an in-house developed LC-MS/MS Tg assay.[12,20] Briefly, the MS method uses 375 μL of patient serum. Large proteins including Tg are precipitated with saturated ammonium sulfate and the supernatant is discarded. The pellet is resuspended, and the proteins are reduced with dithiothreitol, alkylated with iodoacetamide, and then digested for 8 to 16 hours with trypsin. The digestion is stopped with N-alpha-Tosyl-L-lysyl-chloromethylketone, and immuno purification of the target peptide (FSPDDSAGASALLR) is performed using an antibody purchased from SISCAPA Assay Technologies, Inc (Washington, DC) immobilized on protein G PhyTips (PTH-93-05-02). The resulting enriched peptide is then measured using a Sciex M3-6500+ LC-MS/MS system.

Tg was considered positive if equal or greater than the lowest clinically reportable concentration of each assay (0.1 ng/mL for Tg-IA, 0.2 ng/mL for Tg-MS). Serum TgAb was measured using the Beckman Access Thyroglobulin Antibody II assay on a DxI analyzer and considered positive if ≥1.8 IU/mL.

Spike-Recovery Experiments

Spike-recovery experiments were performed in a subset of patients ($n = 20$; $n = 16$ TgAb+ and $n = 4$ TgAb-). Briefly, 0.1 mL of each patient's Tg-positive FNAB washout fluid was added to 0.9 mL

of matched patient serum or Access Thyroglobulin sample diluent (catalog # 252191). This mixture was incubated overnight at 4 °C to allow for any Tg-TgAb interaction to occur. Thereafter, Tg was measured by Tg-IA. A subset of the matched patient serum spiked samples was also measured by Tg-MS ($n = 9$), along with controls spiked at the same ratio into pooled Tg-negative serum (Tg-MS method assay diluent). Percent recovery was calculated as (observed concentration/expected concentration)*100.

Statistical Analysis

Statistical analysis was performed using Analyse-it version 6.15.4 (Analyse-it Software, Ltd) for Microsoft Excel and Prism version 10.4.1 (GraphPad Software). The proportion of FNAB samples with detectable Tg were compared between relevant groups by Fisher exact test. The median Tg and percent recovery was compared by the Kruskal-Wallis test, followed by Dunn test for multiple comparisons. Multiplicity adjusted *P* values were determined.

Results

A total of 42 serum Tg-negative patients with metastatic recurrent DTC were identified during the study period. All patients and controls had cytology-confirmed DTC LN metastases. Table 1 describes the demographics and clinical characteristics of both groups. For the cohort of patients with a negative serum Tg and metastatic recurrent DTC, the median age was 45 years, 81% were females, and 93% had undergone total thyroidectomy (Table 1). Of these 42, 9 (21%) were classified as high-risk papillary thyroid cancer. The majority of cases (81%) were treated with ethanol ablation using prior published protocols.[21,22] Of the 21 cases that were noted to have a second or third recurrence, 16 (76%) had surgical intervention to remove DTC LN metastases for the prior recurrence.

Table 1
Demographics and Clinical Characteristics of Cases and Controls

Characteristics	Cases ($n = 42$)	Controls ($n = 76$)
Age, years		
Median (range)	45 (17-86)	48 (18-85)
Sex		
Female	34 (81%)	42 (55%)
Male	8 (19%)	34 (45%)
Thyroidectomy		
Total	39 (93%)	71 (93%)
Near total	3 (7%)	5 (7%)
Radioiodine ablation		
Yes	34 (81%)	58 (76%)
No	8 (19%)	18 (24%)
Recurrence		
First	21 (50%)	38 (50%)
Second	11 (26%)	23 (30%)
Third or greater	10 (24%)	15 (20%)
TSH, serum (mIU/L)		
Median (range)	0.20 (<0.01-10.90)	0.40 (<0.01-7.50)
TgAb, serum		
Negative	11 (26%)	70 (92%)
Positive	31 (74%)	6 (8%)
Tg-IA, serum (ng/mL)		
Median (range)	<0.1 (<0.1-<0.1)	1.1 (0.2-103)
Tg-IA, FNAB washings (ng/mL)		
Median (range)	139 (0.19-9586)	1884 (0.20-85840)
Biopsied Nodule Size (mm)		
Median (range)	11 (3-35)	11 (3-31)

Abbreviations: FNAB = fine needle aspiration biopsy; TgAb = anti-Tg antibody; Tg-IA = Tg measurement, immunoassay; TSH = thyroid stimulating hormone.

In this cohort, 36 out of 42 (86%) had detectable Tg in FNAB washout fluid (Table 2). The six patients with undetectable Tg in both the serum and the FNAB washout fluid all had cytology-confirmed metastatic papillary thyroid cancer in the biopsied LNs. Tg concentrations in the FNAB washout fluid as measured by either Tg-IA or Tg-MS were highly concordant (41/42; 98%) with the only discordant result between methods being a sample with a Tg concentration of 0.19 ng/mL as measured by Tg-IA, which is below the limit of quantification of Tg-MS (0.2 ng/mL) but within range for Tg-IA (0.1 ng/mL). The presence of serum TgAb was not associated with FNAB washout fluid Tg positivity; 26/31 (84%) of TgAb+ patients had detectable Tg in FNAB washout fluid, while 10/11 (91%) of serum TgAb patients were positive for Tg in the FNAB washout fluid (Table 1, $P = 1$). TgAb concentrations in the FNAB washout fluid were detectable in 3 out of the 42 (7%) patients with concentrations ranging from 32 to 75 IU/mL. All 3 of these patients had detectable Tg in the FNAB washout fluid (1 TgAb 32 IU/mL: Tg-IA FNAB 3.3 ng/mL; 2 TgAb 63 IU/mL: Tg-IA FNAB 720 ng/mL; 3 TgAb 75 IU/mL: Tg FNAB 7566 ng/mL), demonstrating that the presence of TgAb did not prevent Tg detection in the FNAB washout fluid. Concentrations of Tg in the FNAB washout fluid in this cohort ($n = 36$) ranged over several orders of magnitude and did not vary substantially whether measured by Tg-IA (median: 139 ng/mL, minimum: 0.19, maximum: 9586) or Tg-MS (median: 84 ng/mL, minimum: 0.88, maximum: 12 100, $P > .99$; Fig. 1). Concentrations of Tg in FNAB washout fluid from patients with negative serum Tg were significantly different from those measured in FNAB washout fluid from patients who were serum Tg-positive within 90 days prior to FNAB collection (median: 1884 ng/mL, minimum: 0.2, maximum: 85 840, $P = .002$).

Spiking Tg + FNAB washout fluid into matched patient serum demonstrated clear differences in recovery for TgAb+ patients when measured by Tg-IA (percent recovery median, min-max: 55%, 12%-84%) versus TgAb patients (96%, 94%-111%) ($P = .005$) (Fig. 2). Recovery for TgAb+ patients was significantly improved when Tg + FNAB washout fluid was spiked into assay diluent (median, min-max recovery: 91%, 80%-131%) ($P < .0001$) indicating that the underrecovery in the patient's serum is likely due to the presence of TgAb. There was no difference in recovery for patients with TgAbs, regardless of whether Tg + FNAB washout fluid was spiked into their own serum or assay diluent (86%, 79%-108%) ($P > .99$) (Fig. 2). In a subset of TgAb+ patients ($n = 9$), with sufficient residual sample volume, spike-recovery studies were also performed using the Tg-MS assay. There was no significant difference in recovery observed for FNAB washout fluid spiked into matched patient serum when measured by Tg-MS (99%, 70%-116%) versus control Tg serum pool (87.5%, 81% to 114%) ($P > .99$) (Fig. 2) in the TgAb+ patients indicating that, as previously published, the Tg-MS assay is able to overcome interference due to the presence of TgAb. Despite the absence of TgAb interference, all these patients had an undetectable Tg (<0.2 ng/mL) in serum when measured in the Tg-MS assay.

Discussion

In this study, we found that the majority of serum Tg-negative patients with recurrent DTC metastases, as indicated by cytology examination, had detectable concentrations of Tg in FNAB washout fluid, indicating that the failure to detect serum Tg was not due to a lack of Tg production by the metastatic tissue. However, median Tg concentrations in FNAB washout fluid in these patients were significantly lower than median Tg concentrations in FNAB washout fluid from a cohort of patients who were serum Tg-positive, suggesting that the recurrent DTC associated with undetectable Tg in serum may generally produce less Tg levels than those who were serum Tg-positive. Many of the serum Tg patients had circulating

Table 2
Tg Concentrations by IA and MS for FNAB Washout Samples From Patients With Recurrent Differentiated Thyroid Cancer and an Undetectable Serum Tg at the Time of the FNAB Collection

All FNAB washout samples ($n = 42$)		
	Tg-IA (+)	Tg-IA (−)
Tg-MS (+)	35	0
Tg-MS (−)	1[a]	6
FNAB washout samples with a positive serum TgAb ($n = 31$)		
	Tg-IA (+)	Tg-IA (−)
Tg-MS (+)	26	0
Tg-MS (−)	0	5
FNAB washout samples with a negative serum TgAb ($n = 11$)		
	Tg-IA (+)	Tg-IA (−)
Tg-MS (+)	9	0
Tg-MS (−)	1[a]	1

Abbreviations: FNAB = fine needle aspiration biopsy; Tg = thyroglobulin; Tg-IA = Tg measurement, immunoassay; Tg-MS = Tg measurement, MS.
[a] Between lower reportable limit for assays (0.1 ng/mL for Tg-IA, 0.2 ng/mL for Tg-MS).

TgAb in serum; however, Tg was still undetectable when measured by Tg-MS, despite the lack of TgAb interference in this assay. Furthermore, there was no indication of other analytical assays interferences resulting in the lack of detection of serum Tg based on the Tg spike-recovery experiments.

Several studies have demonstrated that some patients with recurrent or residual DTC have undetectable Tg as measured by Tg-IA and Tg-MS assays.[11-13,23] Lack of Tg detection by Tg-IA has been attributed to potential interference from TgAb in these patients.[11,12] In contrast, the lack of Tg detection by Tg-MS assays cannot be explained by the presence of TgAb as these assays are not susceptible to TgAb interference.[12,24] Other reasons to explain an undetectable serum Tg in patients with recurrent or residual DTC have been proposed. Molecular heterogeneity and conformational abnormalities that can affect the tertiary structure of Tg have been reported.[25-27] These changes have the potential to disrupt the conformational epitope targets of Tg-IA, but it would not explain the lack of detection by Tg-MS assays. Tg-MS assay can be affected by polymorphisms and posttranslational modifications that may

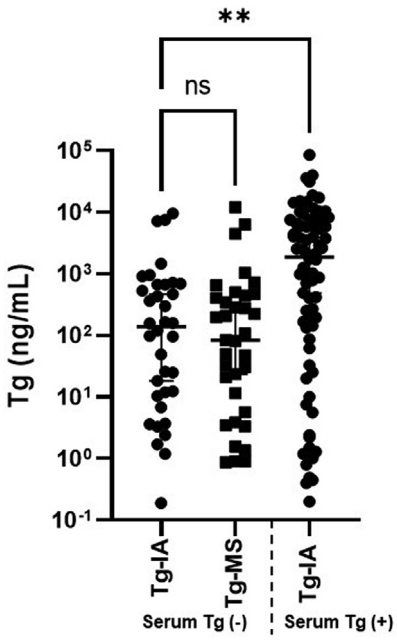


Fig. 1. Tg concentrations in FNAB washout fluid specimens from individuals with recurrent DTC and a negative serum Tg. Tg was measured in the FNAB washout fluid by IA and MS. Tg measured by Tg-IA in FNAB washout fluid specimens from individuals with recurrent DTC and a positive serum Tg is shown for comparison. Median Tg concentrations were not significantly different between the Tg-IA and Tg-MS in patients with a negative serum Tg ($P > .99$); however, median Tg concentrations in FNAB washout fluid were significantly different between the negative serum Tg patients and the control group with positive serum Tg ($P = .002$). DTC = differentiated thryoid cancer; *FNAB* = fine needle aspiration biopsy; ns = not significant; *Tg* = thyroglobulin; *Tg-IA* = Tg measurement, immunoassay; *Tg-MS* = Tg measurement, MS. **$P < .01$.

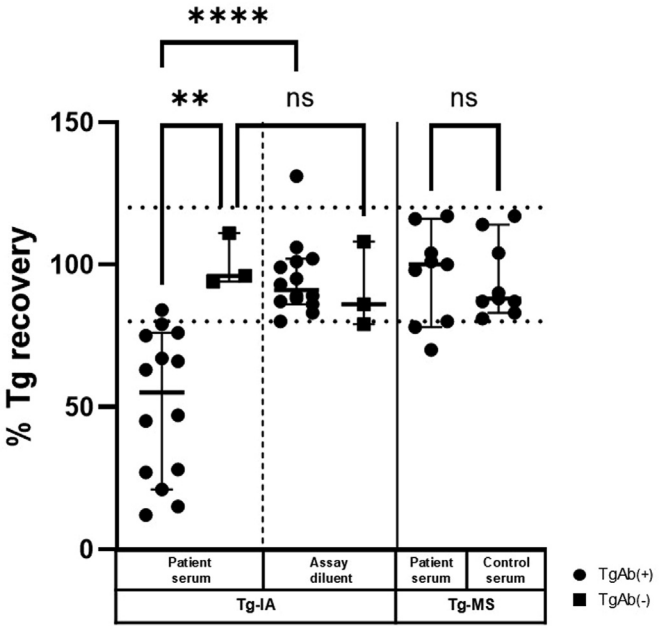


Fig. 2. Recovery of Tg in serum samples from individuals with recurrent DTC and a negative serum Tg. Tg spike recovery using Tg-positive FNAB washout material was performed in patient serum or assay diluent for Tg-IA (Tg-IA) or patient serum or control serum (Tg and TgAb negative serum) for Tg-MS assay. Patients were classified based on TgAb status. When measured by Tg-IA, TgAb+ patients had significantly reduced recovery when diluted in their own serum compared to assay diluent ($P < .001$). TgAb patients had significantly higher recovery than TgAb+ patients when spiked into their own serum ($P < .01$) and no significant difference was observed between dilution in their own serum versus assay diluent ($P > .99$). When measured by Tg-MS, there was no significant difference in recovery whether diluted in patient serum versus control (TgAb-) serum for TgAb+ patients ($P > .99$). *FNAB* = fine needle aspiration biopsy; ns = not significant; *Tg* = thyroglobulin; *TgAb* = anti-Tg antibody; *Tg-IA* = Tg measurement, immunoassay; *Tg-MS* = Tg measurement, MS. **$P < .01$; ****$P < .0001$.

prevent efficient digestion of the Tg peptide being monitored in the Tg-MS assays or a change in peptide mass and can potentially result in false-negative results.[28] Given that in this study we were able to detect Tg in the FNAB washout fluid by both IA and MS assays, it would seem unlikely that changes in Tg structure or conformation would be responsible to the lack of Tg detection in the serum.

FNAB washout fluid is a different matrix than serum; however, it is unlikely that an analytical matrix effect explains the discrepancy in FNAB washout fluid and serum Tg observed in our cohort. The Tg assay used on this study have been validated and deemed to have acceptable performance of the measurement of Tg in FNAB washout fluid (which at our institution is normal saline).[29] In addition, the spike-recovery experiments demonstrate that the Tg present in FNAB washout fluid was equally measurable in serum. While the effect of TgAb on Tg measurement in FNAB washout fluid remains unclear and results have been inconclusive,[30,31] it is known that TgAb presence does not significantly affect diagnostic accuracy of Tg in FNAB washout fluid, though it may cause slight underestimation.[30] TgAb interference depends on serum TgAb concentration, TgAb to Tg ratio in FNAB washout fluid, and dilution effects of varying washout volumes. In this study, we did detect TgAb in FNAB washout fluid of 3 patients; however, it is possible that this was due to blood contamination of the FNAB, as the majority of patients with serum TgAb did not have TgAb detected in FNAB washout fluid.

Increased metabolic clearance of Tg in TgAb-positive patients could result in undetectable Tg in patients with residual or recurrent disease. This mechanism has been proposed in the context of animal models and in patients with DTC.[32,33] While TgAb mediated accelerated clearance of Tg-TgAb complexes may partially explain the study findings, there was a subset of TgAb-negative patients with DTC recurrence and lack of measurable Tg in serum.

Another explanation to our findings is that some patients with DTC produced less Tg or do not effectively release Tg into circulation, despite having well-differentiated pathology. This would result in undetectable serum Tg in both Tg-IA and Tg-MS assays. Additionally, while the mechanisms of Tg secretion in the setting of recurrent metastatic DTC have not been completely studied, it has been shown that extracellular secretion of Tg requires proper protein folding and processing by the endoplasmic reticulum.[34,35] Recent studies have demonstrated that specific point mutations could disrupt Tg protein folding, preventing extracellular secretion and resulting in intracellular accumulation of Tg.[36] It is possible that point mutations in the recurrent DTC tissue preventing proper protein folding or otherwise interfering with Tg secretion may play a role in the phenomenon of undetectable serum Tg in some patients with recurrent DTC.

There are some limitations to this study. First, only one specific Tg-IA and one Tg-MS assay were included. Therefore, the findings are specific to these Tg assays and may differ for other combinations of Tg-IA or Tg-MS assays. Additionally, including a Tg radioimmunoassay as a third methodology would have been valuable, but due to the retrospective nature of the study, there was insufficient residual sample volume for an additional testing. Second, the Tg-MS assay is based on a single peptide, which contains a polymorphism resulting in a 1:100 chance of missing half of the Tg in heterozygous individuals with the polymorphism, leading to a 50% false low bias. If the individual is homozygous (a probability of 1:10 000), the Tg by MS would be false negative. However, the likelihood of a false negative is extremely low.

In conclusion, these results suggest that, for a subset of patients with DTC LN metastases, the absence of detectable Tg in serum is not due to the analytical limitations of current Tg assays. Alternate hypothesized mechanisms may include decreased production of Tg in the DTC LN metastases, impaired Tg release into circulation, or rapid Tg clearance from circulation. This study underscores the importance of using multiple modalities for the surveillance of patients with a history of thyroid cancer. In this study cohort, relying solely on serum Tg concentrations would have missed recurrent disease that was detected by ultrasound followed by cytopathology and Tg measurement in the FNAB washout fluid of suspicious nodules.

Disclosure

The authors have no conflicts of interest to disclose.

References

1. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer: the American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2016;26(1):1–133. https://doi.org/10.1089/thy.2015.0020
2. Malandrino P, Latina A, Marescalco S, et al. Risk-adapted management of differentiated thyroid cancer assessed by a sensitive measurement of basal serum thyroglobulin. *J Clin Endocrinol Metab*. 2011;96(6):1703–1709. https://doi.org/10.1210/jc.2010-2695
3. Schlumberger M, Hitzel A, Toubert ME, et al. Comparison of seven serum thyroglobulin assays in the follow-up of papillary and follicular thyroid cancer patients. *J Clin Endocrinol Metab*. 2007;92(7):2487–2495. https://doi.org/10.1210/jc.2006-0723
4. Bachelot A, Leboulleux S, Baudin E, et al. Neck recurrence from thyroid carcinoma: serum thyroglobulin and high-dose total body scan are not reliable criteria for cure after radioiodine treatment. *Clin Endocrinol*. 2005;62(3):376–379. https://doi.org/10.1111/j.1365-2265.2005.02228.x
5. de Geus-Oei LF, Oei HY, Hennemann G, Krenning EP. Sensitivity of 123I whole-body scan and thyroglobulin in the detection of metastases or recurrent differentiated thyroid cancer. *Eur J Nucl Med Mol Imaging*. 2002;29(6):768–774. https://doi.org/10.1007/s00259-002-0781-x
6. Giovanella L, Suriano S, Ceriani L, Verburg FA. Undetectable thyroglobulin in patients with differentiated thyroid carcinoma and residual radioiodine uptake on a postablation whole-body scan. *Clin Nucl Med*. 2011;36(2):109–112. https://doi.org/10.1097/RLU.0b013e318203bb84
7. Mouaden A, Guerrouj H, Ghfir I, Aouad NB. Clinical benefit of radioiodine administration in a rare case of iodine avid thyroid carcinoma with No secretion of thyroglobulin. *World J Nucl Med*. 2023;22(4):300–305. https://doi.org/10.1055/s-0043-1777692
8. Thai JN, De Marchena IR, Nehru VM, et al. Low correlation between serum thyroglobulin and (131)I radioiodine whole body scintigraphy: implication for postoperative disease surveillance in differentiated thyroid cancer. *Clin Imaging*. 2022;87:1–4. https://doi.org/10.1016/j.clinimag.2022.04.003
9. Hollowell JG, Staehling NW, Flanders WD, et al. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): national health and nutrition examination survey (NHANES III). *J Clin Endocrinol Metab*. 2002;87(2):489–499. https://doi.org/10.1210/jcem.87.2.8182
10. Rosario PW, Côrtes MCS, Franco Mourão G. Follow-up of patients with thyroid cancer and antithyroglobulin antibodies: a review for clinicians. *Endocr Relat Cancer*. 2021;28(4):R111–r119. https://doi.org/10.1530/erc-21-0012
11. Spencer CA. Challenges of serum thyroglobulin (Tg) measurement in the presence of Tg autoantibodies. *J Clin Endocrinol Metab*. 2004;89(8):3702–3704. https://doi.org/10.1210/jc.2004-0986
12. Netzel BC, Grebe SKG, Carranza Leon BG, et al. Thyroglobulin (Tg) testing revisited: Tg assays, TgAb assays, and correlation of results with clinical outcomes. *J Clin Endocrinol Metab*. 2015;100(8):E1074–E1083. https://doi.org/10.1210/jc.2015-1967
13. Azmat U, Porter K, Senter L, Ringel MD, Nabhan F. Thyroglobulin liquid chromatography–tandem mass spectrometry has a low sensitivity for detecting structural disease in patients with antithyroglobulin antibodies. *Thyroid*. 2016;27(1):74–80. https://doi.org/10.1089/thy.2016.0210
14. Powers JL, Strathmann FG, Straseski JA. Thyroglobulin antibody screen prior to mass spectrometry provides measurable cost savings and optimal laboratory utilization. *Am J Clin Pathol*. 2017;147(3):309–314. https://doi.org/10.1093/ajcp/aqw228
15. Algeciras-Schimnich A. Thyroglobulin measurement in the management of patients with differentiated thyroid cancer. *Crit Rev Clin Lab Sci*. 2018;55(3):205–218. https://doi.org/10.1080/10408363.2018.1450830
16. Xu Y, Wu D, Wu W, et al. Diagnostic value of cytology, thyroglobulin, and combination of them in fine-needle aspiration of metastatic lymph nodes in patients with differentiated thyroid cancer: a systematic review and network meta-analysis. *Medicine (Baltimore)*. 2019;98(45):e17859. https://doi.org/10.1097/md.0000000000017859
17. Zhu XH, Zhou JN, Qian YY, et al. Diagnostic values of thyroglobulin in lymph node fine-needle aspiration washout: a systematic review and meta-analysis diagnostic values of FNA-Tg. *Endocr J*. 2020;67(2):113–123. https://doi.org/10.1507/endocrj.EJ18-0558

18. Liu RB, Zhou DL, Xu BH, et al. Comparison of the diagnostic performances of US-guided fine needle aspiration cytology and thyroglobulin measurement for lymph node metastases in patients with differentiated thyroid carcinoma: a meta-analysis. *Eur Radiol*. 2021;31(5):2903–2914. https://doi.org/10.1007/s00330-020-07400-9
19. Wang Y, Duan Y, Zhou M, et al. The diagnostic value of thyroglobulin in fine-needle aspiration of metastatic lymph nodes in patients with papillary thyroid cancer and its influential factors. *Surg Oncol*. 2021;39:101666. https://doi.org/10.1016/j.suronc.2021.101666
20. Netzel BC, Grebe SK, Algeciras-Schimnich A. Usefulness of a thyroglobulin liquid chromatography-tandem mass spectrometry assay for evaluation of suspected heterophile interference. *Clin Chem*. 2014;60(7):1016–1018. https://doi.org/10.1373/clinchem.2014.224816
21. Lewis BD, Hay ID, Charboneau JW, McIver B, Reading CC, Goellner JR. Percutaneous ethanol injection for treatment of cervical lymph node metastases in patients with papillary thyroid carcinoma. *Am J Roentgenol*. 2002;178(3):699–704. https://doi.org/10.2214/ajr.178.3.1780699
22. Hay ID, Lee RA, Davidge-Pitts C, Reading CC, Charboneau JW. Long-term outcome of ultrasound-guided percutaneous ethanol ablation of selected "recurrent" neck nodal metastases in 25 patients with TNM stages III or IVA papillary thyroid carcinoma previously treated by surgery and 131I therapy. *Surgery*. 2013;154(6):1448–1454. ; discussion 1454-5https://doi.org/10.1016/j.surg.2013.07.007
23. Petrovic I, LoPresti J, Fatemi S, et al. Influence of thyroglobulin autoantibodies on thyroglobulin levels measured by different methodologies: IMA, LC-MS/MS, and RIA. *J Clin Endocrinol Metab*. 2024;109(12):3254–3263. https://doi.org/10.1210/clinem/dgae286
24. Kushnir MM, Rockwood AL, Roberts WL, Abraham D, Hoofnagle AN, Meikle AW. Measurement of thyroglobulin by liquid chromatography-tandem mass spectrometry in serum and plasma in the presence of antithyroglobulin autoantibodies. *Clin Chem*. 2013;59(6):982–990. https://doi.org/10.1373/clinchem.2012.195594
25. Xavier AC, Maciel RM, Vieira JG, Dias-da-Silva MR, Martins JR. Insights into the posttranslational structural heterogeneity of thyroglobulin and its role in the development, diagnosis, and management of benign and malignant thyroid diseases. *Arch Endocrinol Metab*. 2016;60(1):66–75. https://doi.org/10.1590/2359-3997000000103
26. Berg G, Bjorkman U, Ekholm R. Conformational change of the thyroglobulin molecule induced by oxidation in vitro. *Mol Cell Endocrinol*. 1980;17(3):139–144. https://doi.org/10.1016/0303-7207(80)90108-2
27. Peixoto A, Relvas-Santos M, Azevedo R, Santos LL, Ferreira JA. Protein glycosylation and tumor microenvironment alterations driving cancer hallmarks. *Front Oncol*. 2019;9:380. https://doi.org/10.3389/fonc.2019.00380
28. Hoofnagle AN, Roth MY. Clinical review: improving the measurement of serum thyroglobulin with mass spectrometry. *J Clin Endocrinol Metab*. 2013;98(4):1343–1352. https://doi.org/10.1210/jc.2012-4172
29. Snozek CL, Chambers EP, Reading CC, et al. Serum thyroglobulin, high-resolution ultrasound, and lymph node thyroglobulin in diagnosis of differentiated thyroid carcinoma nodal metastases. *J Clin Endocrinol Metab*. 2007;92(11):4278–4281. https://doi.org/10.1210/jc.2007-1075
30. Boi F, Baghino G, Atzeni F, Lai ML, Faa G, Mariotti S. The diagnostic value for differentiated thyroid carcinoma metastases of thyroglobulin (Tg) measurement in washout fluid from fine-needle aspiration biopsy of neck lymph nodes is maintained in the presence of circulating anti-Tg antibodies. *J Clin Endocrinol Metab*. 2006;91(4):1364–1369. https://doi.org/10.1210/jc.2005-1705
31. Shin HJ, Lee HS, Kim EK, Moon HJ, Lee JH, Kwak JY. A study on serum antithyroglobulin antibodies interference in thyroglobulin measurement in fine-needle aspiration for diagnosing lymph node metastasis in postoperative patients. *PLoS One*. 2015;10(6):e0131096. https://doi.org/10.1371/journal.pone.0131096
32. Weigle WO, High GJ. The behavior of autologous thyroglobulin in the circulation of rabbits immunized with either heterologous or altered homologous thyroglobulin. *J Immunol*. 1967;98(6):1105–1114.
33. Feldt-Rasmussen U, Petersen PH, Date J, Madsen CM. Sequential changes in serum thyroglobulin (Tg) and its autoantibodies (TgAb) following subtotal thyroidectomy of patients with preoperatively detectable TgAb. *Clin Endocrinol (Oxf)*. 1980;12(1):29–38. https://doi.org/10.1111/j.1365-2265.1980.tb03129.x
34. Kim PS, Arvan P. Hormonal regulation of thyroglobulin export from the endoplasmic reticulum of cultured thyrocytes. *J Biol Chem*. 1993;268(7):4873–4879. https://doi.org/10.1016/S0021-9258(18)53477-3
35. Muresan Z, Arvan P. Enhanced binding to the molecular chaperone BiP slows thyroglobulin export from the endoplasmic reticulum. *Mol Endocrinol*. 1998;12(3):458–467. https://doi.org/10.1210/mend.12.3.0069
36. Citterio CE, Kim K, Rajesh B, Pena K, Clarke OB, Arvan P. Structural features of thyroglobulin linked to protein trafficking. *Protein Sci*. 2023;32(11):e4784. https://doi.org/10.1002/pro.4784