

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

Andress, Benjamin, PhD; Miller, Sandra; Maus, Anthony, PhD; Kemp, Jennifer; Ness, Karl; Villont, Jarrod; Grebe, Stefan, MD PhD; Bornhorst, Joshua, PhD; Algeciras-Schimmich, Alicia, PhD  
Division of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN

## INTRODUCTION

### BACKGROUND

- Serum thyroglobulin (Tg) measurement is used in the post-operative monitoring for differentiated thyroid cancer (DTC) recurrence. Following total thyroidectomy, serum Tg should remain undetectable, and any measurable Tg indicates residual or recurrent disease<sup>1,2</sup>.
- Tg measurement in fine-needle aspirate biopsy (FNAB) saline washings is used for the evaluation of potential DTC metastases to lymph nodes<sup>1,2</sup>.
- Despite the development of highly sensitive Tg assays, serum Tg monitoring does not successfully detect all DTC recurrence. Known limitations in the measurement of Tg include:
  - Antibodies to Tg (TgAb), which develop in up to 30% of carcinoma patients, are likely to falsely suppress thyroglobulin results by immunoassay<sup>3</sup>.
  - Heterophile antibodies can cause both false positive and false negative results in immunoassays for Tg.
- Mass spectrometry (MS) assays were developed to address antibody interference and have been reported as not subject to TgAb or heterophile antibody interference<sup>4</sup>.
- Despite the availability of sensitive Tg-MS assays, recurrent DTC with unexplained undetectable serum Tg is a persistent occurrence.

### OBJECTIVE

- To determine whether post-thyroidectomy patients with undetectable serum Tg and cytology-confirmed lymph-node DTC metastases have detectable Tg in FNAB washings and whether the failure to detect serum Tg in this patient population is a result of TgAb interference.

## METHODS

- FNAB washings (n=30) from patients with cytology-confirmed DTC lymph node metastases but with an undetectable serum Tg by immunoassay were collected and assayed for Tg and TgAb. Serum Tg and TgAb concentrations were obtained within three months (mean: 14 days) prior to FNAB.
- Tg was measured on serum and FNAB washings using the Beckman Access Thyroglobulin (Tg2) immunoassay (Tg-IA) on a Dxl analyzer, and an in-house developed LC-MS/MS Tg assay (Tg-MS).
- TgAb in serum was measured using the Beckman Access Thyroglobulin Antibody II assay on a Dxl analyzer.
- Spike-recovery experiments were performed in a subset of patients (n=8) with residual serum by adding the patient's Tg-positive FNAB washings or certified reference material (BCR 457) to matched patient serum. Spiked serum was assayed using Tg-IA and percent recovery calculated as (observed concentration/expected concentration)\*100.
- Recovery (percent difference) between immunoassay and MS methods was calculated for FNAB washing samples, and mean recovery for antibody positive versus antibody negative patients was compared by Student's t-test.

## RESULTS

TABLE 1: FNAB Tg by TgIA and TgMS

	(+) FNA TgMS	(-) FNA TgMS	Total
(+) FNA TgIA	25	1	26
(-) FNA TgIA	0	4	4

- Of 30 samples with positive cytology and negative serum Tg, 26 (87%) had detectable Tg in the FNAB washings.
- One "discrepant" sample (TgIA positive, TgMS negative) was between the limit of detection of assays (0.1 ng/mL for TgIA, 0.2 ng/mL for TgMS).

TABLE 2: TgAb in FNAB and Serum

	(+) TgAb FNA	(-) TgAb FNA	Total
(+) TgAb (serum)	3	12	15
(-) TgAb (serum)	0	11	11

- Of the 26 FNAB-Tg(+)/serum-Tg(-) cases, 15 had detectable TgAb in serum, while 11 did not.
- TgAb were detected in FNAB washings for 3 of the 12 patients with TgAb present in serum. It cannot be determined whether the source of TgAb in these cases is biopsy material or serum contamination.

## CONCLUSIONS

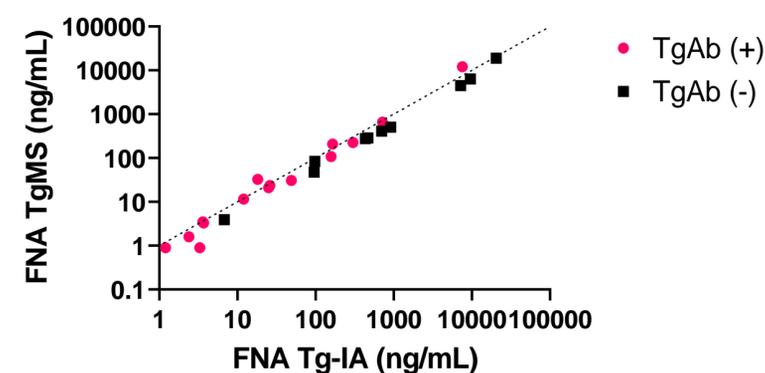
- Tg was detectable by both MS and immunoassay methods in the majority of FNAB washing samples from patients with cytology-confirmed DTC lymph node metastases and Tg-negative serum.
- The absence of detectable serum Tg in these patients does not appear to be completely explained by the presence of TgAb.
  - 11/26 (42%) of FNAB Tg (+), serum Tg (-) patients were TgAb negative.
  - TgAb (-) serum exhibited no evidence of interference with TgIA detection on spike-recovery experiments.
  - Of 8 TgIA (-) serum samples tested by TgMS, only one was positive by TgMS and not TgIA; interestingly, this patient was TgAb (-).
- No evidence of TgAb interference was observed in measurement of FNAB washing Tg; results of TgMS and TgIA measurements on FNAB washings were comparable in both TgAb positive and negative patients.
- Additional studies and testing in a larger number of patients are needed to further prove this concept.

TABLE 3: TG Recovery in TgAb (+) or (-) Serum

	Recovery >90%	Recovery <90%
(+) TgAb (serum)	0	4
(-) TgAb (serum)	4	0

- TgAb (+) serum exhibits TgIA interference, with 4/4 patients exhibiting <90% recovery (range: 27-87%) of Tg-positive FNA or Tg reference material when spiked into TgAb(+) serum.
- TgAb negative serum exhibited no signs of interference, with recovery ranging between 94-111% for Tg-positive FNAB washings or Tg spiked into TgAb (-) serum.

FIGURE 1: FNAB Tg by TgIA vs. TgMS



- No significant difference was observed in recovery between antibody positive and antibody negative patients' Tg in FNAB washings measured by Tg-MS versus Tg-IA.

### SIGNIFICANCE

**These findings imply that in a subset of patients with DTC lymph node metastases, Tg may not be secreted into the circulation, and the lack of measurable serum Tg is not related to the analytical limitations of the assays.**

## REFERENCES

- Algeciras-Schimmich, A. (2018). "Thyroglobulin measurement in the management of patients with differentiated thyroid cancer." *Crit Rev Clin Lab Sci* 55(3): 205-218.
- Li, S., et al. (2022). "The Role of Thyroglobulin in Preoperative and Postoperative Evaluation of Patients With Differentiated Thyroid Cancer." *Front Endocrinol (Lausanne)* 13: 872527.
- van Kinschot, C. M. J., et al. (2022). "Thyroglobulin and thyroglobulin antibodies: assay-dependent management consequences in patients with differentiated thyroid carcinoma." *Clin Chem Lab Med* 60(5): 756-765.
- Hoofnagle, A. N. and M. Y. Roth (2013). "Clinical review: improving the measurement of serum thyroglobulin with mass spectrometry." *J Clin Endocrinol Metab* 98(4): 1343-1352.