The *De Novo* Detection of Anti-Thyroglobulin Antibodies and Differentiated Thyroid Cancer Recurrence

Ngwe Yin,¹ Steven I. Sherman,² Youngju Pak,³ Danielle R. Litofsky,² Andrew G. Gianoukakis^{3,4}; and for the National Thyroid Cancer Treatment Cooperative Study Group

Background: The prevalence and clinical significance of *de novo* detection of anti-thyroglobulin antibodies (TgAbs) during the follow-up of patients with differentiated thyroid cancer (DTC) is unknown.

Methods: We utilized the National Thyroid Cancer Treatment Cooperative Study registry (1987–2012). Patients registered after 1996 ($n=3318$) were analyzed. We identified 1545 subjects who had available TgAb status (TgAb cohort) between years 1996 and 2012, of whom 1325 were TgAb negative at first postoperative follow-up testing. From this initial TgAb-negative group, we excluded 513 patients: 423 patients who had less than 3 years of follow-up and/or fewer than three follow-up visits, 86 patients with persistent disease after initial treatment, and 4 patients with data entry errors. The remaining 812 patients were included for analysis, comprising the TgAb persistently negative group (defined as TgAb negative for at least 3 consecutive follow-up visits and at least 3 years of follow-up) ($n=772$) and the *de novo* TgAb-positive group in whom TgAbs became detectable ($n=40$). We then assessed whether *de novo* appearance of TgAb was associated with DTC structural recurrence by using the Kaplan–Meier method.

Results: The *de novo* detection of TgAb occurred in 5% of DTC patients. Recurrence of DTC in the TgAb persistently negative group compared with the *de novo* TgAb-positive group did not differ significantly (9.6% vs. 15.0%, $p=0.23$). Baseline characteristics, histology, history of radiation exposure, staging, and median duration of follow-up were similar between the two groups. Interestingly, in all six patients who suffered a recurrence in the *de novo* TgAb-positive group, the TgAbs were negative at the time of recurrence detection and became positive at a median of 2.1 (0.7–8.7) years after the structural recurrence.

Conclusions: Utilizing a large North American DTC registry, we found the prevalence of *de novo* TgAb detection to be 5% among initially TgAb-negative patients. We did not find a statistically significant association between *de novo* TgAb development and DTC structural recurrence. Larger prospective studies are required to confirm these findings and further assess the significance of *de novo* TgAb detection in the follow-up of DTC.

Keywords: *de novo*, anti-thyroglobulin antibody, differentiated thyroid cancer, recurrence

Introduction

THE INCIDENCE OF DIFFERENTIATED THYROID CANCER (DTC) has tripled over the past three decades in the United States, of which at least 85% are papillary carcinomas (1,2). Thyroglobulin (Tg), a necessary component of thyroid hormone biosynthesis, is produced by both normal thyroid follicular cells and DTC cells. As evidence of the presence of the cells from which it is produced, serum Tg is a key and sensitive biomarker utilized in the follow-up of DTC patients.

The serum Tg trend over time is used to assess the initial response to treatment (surgery and radioactive iodine [RAI]), as well as for long-term biochemical surveillance for detection of recurrent disease in DTC patients (3).

Autoantibodies to Tg (thyroglobulin antibody or TgAb) are present in ~25% of patients with DTC after initial surgery, and they are known to interfere with the measurements of Tg (by immunoassay) by yielding false negative results that would mask the presence of residual or recurrent disease (4). Simultaneous measurement of serum Tg and TgAb is

¹Division of Endocrinology, Diabetes and Metabolism, University of California, San Francisco (Fresno Medical Education Program), Fresno, California, USA.

²Department of Endocrine Neoplasia and Hormonal Disorders, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

³The Lundquist Institute, Torrance, California, USA.

⁴Division of Endocrinology, Diabetes and Metabolism, Harbor-UCLA Medical Center, Torrance, and David Geffen School of Medicine at UCLA, Los Angeles, California, USA.

The abstract was presented in part at the 2019 Endocrine Society Annual Meeting, New Orleans, LA, March 2019.

routinely performed so that clinicians can determine whether or not the result of the serum Tg immunoassay is reliable. Interestingly, when TgAbs are present from the time of DTC diagnosis, the TgAb trend over time (after the first postoperative year) correlates with disease prognosis (5). In addition, persistently positive TgAbs 6–14 months after initial treatment may portend a worse prognosis (6–8).

Little, however, is known about the prevalence and clinical significance of the *de novo* appearance of TgAbs in patients who previously did not have detectable TgAbs. In a study by Kim *et al.* (7) among the 20 cases exhibiting persistent or recurrent disease, 3 patients were noted to have a change in TgAb status (negative to positive), 4 patients remained TgAb negative, and the rest of the patients remained TgAb positive. Chung *et al.* (8) noted a change in TgAb status (negative to positive) in 2 patients after RAI ablation, and no recurrence was found during the follow-up period in these 2 patients. Gorges *et al.* (9) measured post-RAI ablation TgAb in 42 patients. Among 29 TgAb-negative patients, 3 patients were noted to have a change in TgAb status (negative to positive) over a 3-year follow-up period. Unfortunately, the disease status of these patients was not reported. In a case report, the sustained *de novo* appearance of TgAb was suggested by the authors to signal recurrent disease (10). A recent review article by Verburg and others comments that there is insufficient evidence to follow the TgAb trend in patients who were never positive (11). Laurberg *et al.* (12) demonstrated that there is a transient exacerbation of autoimmunity against the thyrotropin receptor immediately after RAI treatment in Graves' disease. It may be speculated that a similar transient TgAb rise after RAI or surgery may occur but may not have prognostic value. The prevalence and significance, therefore, of the *de novo* appearance of TgAb in TgAb-negative DTC patients is largely unknown.

The aim of this retrospective study was to establish the prevalence of *de novo* appearance of TgAb in a real-world setting, and to investigate their clinical significance in initially TgAb-negative DTC patients.

Methods

Registry protocol and data collection

The data collection and analytical methods of the National Thyroid Cancer Treatment Cooperative Study (NTCTCS) have been described elsewhere (13–20). Briefly, 11 North American centers contributed patient data, with registration beginning in January 1987 and continuing through to 2012 (this data analysis captures patients registered from 1996 through to 2012 as TgAb status began to be recorded in 2000 and any TgAb data before the year 2000 were recorded retrospectively if available). New patients were registered within 3 months of their initial surgery. Institutional Review Boards (IRB) of contributing centers approved the study, and ongoing oversight of the project occurs through the University of Texas MD Anderson Cancer Center IRB, where the central database is currently maintained. Management of patients was non-randomized and was solely at the discretion of their treating physicians on the basis of perceived best practice and clinical need at that period of time at their institution, independent of registry participation. Pre-specified baseline demographic, clinical, histologic, and radiologic data were entered into a personal computer-based clinical

data management system locally (Medlog v2000-2, Incline Village, NV) and transmitted to the central registry database. Clinical status, investigations, and treatments were updated on a yearly basis.

Patient selection

Of the 3318 eligible registered DTC subjects, we identified 1545 subjects who had available TgAb status (TgAb cohort) between years 1996 and 2012, of whom 1325 were TgAb negative at first postoperative follow-up testing. From this initial TgAb-negative group, we excluded 513 patients: 423 patients who had <3 years of follow-up and/or fewer than three follow-up visits, 86 patients with persistent disease after initial treatment, and 4 patients with data entry errors. The remaining 812 patients were included for analysis, comprising the TgAb persistently negative group ($n = 772$) (defined as TgAb negative for at least 3 consecutive follow-up visits and at least 3 years of follow-up) and a *de novo* TgAb-positive group ($n = 40$) in whom TgAbs became detectable (Fig. 1). Patients with at least one *de novo* TgAb were included.

Clinical data were collected, including age, sex, race/ethnicity, and history of prior radiation exposure, histology, NTCTCS staging, RAI for remnant ablation, additional RAI, and/or surgical therapy for recurrence. For the purposes of the analyses performed in this study, the results of each TgAb assay were recorded as “positive” or “negative” based on a titer above or below the functional sensitivity cut-off of the specific assay used in each center where the patient was being followed. Information regarding institutional methodologic changes for TgAb quantitation during the study period were not recorded. Structural recurrence was defined as structural evidence of disease determined either radiographically or by pathology.

Statistical analysis

Continuous variables were compared by using the Student's *t*-test and categorical variables with a Chi-squared test or the Fisher's exact test, wherever appropriate. Time from the date of initial surgery after diagnosis of DTC to the date of recurrence or the date of last lab was collected, the latter being a censored event. Kaplan–Meier survival curves were generated for the TgAb persistently negative group and the *de novo* TgAb-positive group with the log rank test (Fig. 2). All tests were two-sided, and α was set to 0.05 for the level of significance. All analyses were performed by using SPSS software (IBM SPSS Statistics 24; SPSS, Inc., Chicago, IL).

Results

The *de novo* appearance of TgAbs occurred in 40 out of 812 patients who were initially TgAb negative (5%). *De novo* appearance of TgAbs occurred across institutions and throughout the study period, with 18 patients converting before 2005 and 22 patients converting after 2005 (data not shown). Table 1 shows the characteristics of the TgAb persistently negative group ($n = 772$) and the *de novo* TgAb-positive group ($n = 40$). Baseline characteristics (age, sex, race/ethnicity), histology, history of radiation exposure, NTCTCS staging, RAI for remnant ablation (Table 1), as well as additional therapy (RAI and/or surgery) for recurrence (Table 2) were compared and found not to differ significantly between the TgAb persistently negative group and the *de*

FIG. 1. Flow chart showing selection of study population. Persistent disease group (patients with metastatic disease at entry were categorized as persistent disease group). DTC, differentiated thyroid cancer; TgAb, thyroglobulin antibody.

novo TgAb-positive group. Similarly, there was no difference in the median duration of follow-up (time from initial surgery to last lab result) between the TgAb persistently negative group (7.5 [3.0–24.4] years) and the *de novo* TgAb-positive group (8.3 [1.8–13.0] years; $p=0.51$).

Recurrence of DTC in the TgAb persistently negative group (74/772=9.6%) and the *de novo* TgAb-positive group (6/40=15.0%) was compared by the Kaplan–Meier survival curves and did not differ significantly ($p=0.23$) (Fig. 2); the median time to structural recurrence was 1.6 years (0.6–12.1) for the TgAb persistently negative group and 1.2 years

(0.3–2.9) for the *de novo* TgAb-positive group ($p=0.20$). Five year recurrence rates after initial surgery were calculated by the Chi-squared test and found to be 9% and 15% for the TgAb persistently negative group and the *de novo* TgAb-positive group, respectively, and the difference was not statistically significant ($p=0.13$). Interestingly, in all six patients who suffered a recurrence in the *de novo* TgAb-positive group, the TgAbs were negative at the time of recurrence detection and became positive at a median of 2.1 (0.7–8.7) years after the recurrence. Patients were then followed for a median of 2.9 years (range 0–10.7 years) after the

FIG. 2. Recurrence rate over time using the Kaplan–Meier method by TgAb status. Kaplan–Meier curve demonstrating time to recurrence of the *de novo* TgAb-positive group and the TgAb persistently negative group ($p=0.23$).

TABLE 1. BASELINE CHARACTERISTICS OF THYROGLOBULIN ANTIBODY PERSISTENTLY NEGATIVE GROUP AND *DE NOVO* TgAb POSITIVE GROUP

Characteristic	TgAb persistently negative group (n=772), n (%)	De novo TgAb-positive group (n=40), n (%)	p
Age, years (mean $\pm$ SD)	46 $\pm$ 14	46 $\pm$ 16	0.20
Sex			
Female	560 (73)	32 (80)	0.60
Male	208 (27)	8 (20)	
Race/ethnicity			
Caucasian	625 (81)	29 (72)	0.20
Non-Caucasian	147 (19)	11 (28)	
Histology			
Papillary	681 (88)	36 (90)	0.80
Follicular	57 (7)	3 (8)	
Hürthle cell	34 (5)	1 (2)	
History of prior radiation exposure			
No	691 (97)	35 (95)	0.50
Yes	24 (3)	2 (5)	
NTCTCS stage			
I	342 (44)	12 (30)	0.40
II	225 (29)	14 (35)	
III	189 (25)	12 (30)	
IV	16 (2)	1 (3)	
RAI remnant ablation	648 (84)	29 (73)	0.06

NTCTCS, National Thyroid Cancer Treatment Cooperative Study; RAI, radioactive iodine; SD, standard deviation; TgAb, thyroglobulin antibody.

TgAbs became positive. Between detection of recurrence and the development of *de novo* TgAb, 3 out of 6 patients received no additional therapy, 2 out of 6 underwent a neck dissection, and 1 out of 6 received RAI therapy. All 6 patients survived, and 1 out of 6 patients underwent an additional lymph node dissection.

Discussion

The prevalence of *de novo* TgAb development in DTC patients was previously unknown. We have shown that this phenomenon occurs uncommonly, in only about 5% of patients with a median follow-up of about 8 years. The con-

version occurred evenly throughout the study period as well as across institutions, so any individual institutional changes in TgAb assay methodology are unlikely to be a contributing factor. Second, additional therapy (RAI and/or surgery) is unlikely to be a significant factor as similar rates of additional therapy were noted between the persistently TgAb negative and *de novo* TgAb-positive groups. We did not detect a statistically significant association between *de novo* TgAb development and DTC structural recurrence in this dataset.

Interestingly, in all cases, the *de novo* TgAb developed a median time of 2.1 (0.7–8.7) years after structural recurrence was noted. Why would TgAbs develop after the structural recurrence? We hypothesize that recurrent tumor Tg may be structurally different and consequently more immunogenic, leading to an increased likelihood of *de novo* TgAb after recurrence. Tg is synthesized in the endoplasmic reticulum of follicular cells and undergoes post-translational modification, including glycosylation, sulfation, phosphorylation, and iodination (21). Changes to any one of these processes may alter the immunogenicity of Tg. It has previously been demonstrated that iodine content of tumor Tg is lower when compared with normal human Tg (22,23). In addition, Druetta *et al.* (24) demonstrated that tumor Tg has distinctive biochemical properties when compared with the serum Tg of patients with Graves' disease or subacute thyroiditis. In their study, tumor Tg was found to be homogenous, comprising dimers that were dissociable into uncleaved monomers. In contrast, serum Tg from patients with Graves' disease or subacute thyroiditis was heterogeneous with respect to its sedimentation properties and/or the structural integrity of its polypeptide chains. In addition, Magro *et al.* (25) illustrated that papillary thyroid carcinoma (PTC) cells synthesize unique, post-translationally modified Tg, which contains high levels of keratan sulfate. Human Tg contains a chondroitin sulfate chain, and Emoto *et al.* (26) showed that PTC-derived Tg has a less sulfated chondroitin sulfate side chain and is less negatively charged compared with normal human Tg. These biochemical differences, if associated with Tg derived from progressing tumors, may account for enhanced immunogenicity of recurrent disease.

To our knowledge, no investigator has compared patterns of Tg epitope recognition between *de novo* TgAbs and TgAbs present at diagnosis. However, Latrofa *et al.* (27) found high variability in the Tg epitope patterns recognized by TgAb from PTC patients. In addition, Lupoli and colleagues (28) examined Tg binding epitope patterns of TgAbs in 61 DTC patients and found a restrictive epitope pattern to be associated with higher recurrence/persistence (81% vs. 17%, $p < 0.001$) when compared with an unrestrictive pattern. In our study, the recurrence of DTC in the TgAb persistently negative group compared with the *de novo* TgAb-positive group did not differ significantly (9.6% vs. 15%, $p = 0.23$). This, however, could be due to the small number of actual recurrences, limiting the power of the analysis to identify an association.

There are several limitations of our study, including its retrospective nature, the institutional and longitudinal differences in TgAb assays, and the exclusion of a large group of subjects due to lack of TgAb data or inadequate follow-up. Further studies using larger and prospective cohorts evaluated with a common TgAb assay will be needed to confirm the prevalence of *de novo* TgAb and to examine any potential

TABLE 2. TREATMENT FOR RECURRENCE BETWEEN STUDY GROUPS

	TgAb persistently negative group (n=74), n (%)	De novo TgAb-positive group (n=6), n (%)	p
Surgery for recurrence	37/74 (50)	3/6 (50)	1.00
Surgery + RAI for recurrence	4/74 (5.4)	0/6 (0)	0.60
RAI for remnant ablation	62/74 (84)	4/6 (67)	0.30
RAI for recurrence	7/74 (9.5)	1/6 (16.6)	0.60

clinical significance in the follow-up of DTC. However, these data do not suggest that patients in whom *de novo* TgAbs are detected are at high risk for subsequent recurrence.

Utilizing a large North American DTC registry, we found the prevalence of *de novo* TgAb appearance to be 5% among initially TgAb-negative patients. We did not find a statistically significant association between *de novo* TgAb development and DTC structural recurrence. Larger prospective studies are required to confirm these findings and further assess the significance of *de novo* TgAb development in the follow-up of DTC.

Acknowledgments

Investigators of the National Thyroid Cancer Treatment Cooperative Study are as follows: Kenneth B. Ain, S. Thomas Bigos, James D. Brierley, David S. Cooper, Henry G. Fein; Bryan R. Haugen, Jacqueline Jonklaas, Paul W. Ladenson, Harry R. Maxon, Donald S.A. McLeod, Douglas S. Ross, Monica C. Skarulis, and David L. Steward.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

The NTCTCS has been supported in part by a research grant from Genzyme, a Sanofi company, and by the University of Texas MD Anderson Cancer Center Support Grant (NCI Grant P30 CA016672).

References

- Davies L, Welch HG 2014 Current thyroid cancer trends in the United States. *JAMA Otolaryngol Head Neck Surg* **140**: 317–322.
- Fagin JA, Wells SA 2016 Biologic and clinical perspectives on thyroid cancer. *N Engl J Med* **375**:1054–1067.
- Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, Pacini F, Randolph GW, Sawka AM, Schlumberger M, Schuff KG, Sherman SI, Sosa JA, Steward DL, Tuttle RM, Wartofsky L 2016 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* **26**:1–133.
- Spencer CA, LoPresti JS, Fatemi S, Nicoloff JT 1999 Detection of residual and recurrent differentiated thyroid carcinoma by serum thyroglobulin measurement. *Thyroid* **9**:435–441.
- Spencer C, Fatemi S 2013 Thyroglobulin antibody (TgAb) methods—Strengths, pitfalls and clinical utility for monitoring TgAb-positive patients with differentiated thyroid cancer. *Best Pract Res Clin Endocrinol Metab* **27**:701–712.
- Durante C, Tognini S, Montesano T, Orlandi F, Torlontano M, Puxeddu E, Attard M, Costante G, Tumino S, Merigolo D, Bruno R, Trulli F, Totada M, Redler A, Ronga G, Filetti S, Monzani F 2014 Clinical aggressiveness and long-term outcome in patients with papillary thyroid cancer and circulating anti-thyroglobulin autoantibodies. *Thyroid* **24**: 1139–1145.
- 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.
- 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.
- Gorges R, Maniecki M, Jentzen W, Sheu SN, Mann K, Bockisch A, Janssen OE 2005 Development and clinical impact of thyroglobulin antibodies in patients with differentiated thyroid carcinoma during the first 3 years after thyroidectomy. *Eur J Endocrinol* **153**:49–55.
- Tumino S, Belfiore A 2000 Appearance of antithyroglobulin antibodies as the sole sign of metastatic lymph nodes in a patient operated on for papillary thyroid cancer: a case report. *Thyroid* **10**:431–433.
- 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.
- Laurberg P, Wallin G, Tallstedt L, Abraham-Nordling M, Lundell G, Tørring O 2008 TSH-receptor autoimmunity in Graves' disease after therapy with anti-thyroid drugs, surgery, or radioiodine: a 5-year prospective randomized study. *Eur J Endocrinol* **158**:69–75.
- Cooper DS, Specker B, Ho M, Sperling M, Ladenson PW, Ross DS, Ain KB, Bigos ST, Brierley JD, Haugen BR, Klein I, Robbins J, Sherman SI, Taylor T, Maxon HR, 3rd 1998 Thyrotropin suppression and disease progression in patients with differentiated thyroid cancer: results from the National Thyroid Cancer Treatment Cooperative Registry. *Thyroid* **8**:737–744.
- Sherman SI, Brierley JD, Sperling M, Ain KB, Bigos ST, Cooper DS, Haugen BR, Ho M, Klein I, Ladenson PW, Robbins J, Ross DS, Specker B, Taylor T, Maxon HR, 3rd 1998 Prospective multicenter study of thyroid carcinoma treatment: initial analysis of staging and outcome. National Thyroid Cancer Treatment Cooperative Study Registry Group. *Cancer* **83**:1012–1021.
- Taylor T, Specker B, Robbins J, Sperling M, Ho M, Ain K, Bigos ST, Brierley J, Cooper D, Haugen B, Hay I, Hertzberg V, Klein I, Klein H, Ladenson P, Nishiyama R, Ross D, Sherman S, Maxon HR 1998 Outcome after treatment of high-risk papillary and non-Hurthle-cell follicular thyroid carcinoma. *Ann Intern Med* **129**:622–627.
- Jonklaas J, Sarlis NJ, Litofsky D, Ain KB, Bigos ST, Brierley JD, Cooper DS, Haugen BR, Ladenson PW, Magner J, Robbins J, Ross DS, Skarulis M, Maxon HR, Sherman SI 2006 Outcomes of patients with differentiated thyroid carcinoma following initial therapy. *Thyroid* **16**: 1229–1242.
- Ross DS, Litofsky D, Ain KB, Bigos T, Brierley JD, Cooper DS, Haugen BR, Jonklaas J, Ladenson PW, Magner J, Robbins J, Skarulis MC, Steward DL, Maxon HR, Sherman SI 2009 Recurrence after treatment of micropapillary thyroid cancer. *Thyroid* **19**:1043–1048.
- McLeod DS, Cooper DS, Ladenson PW, Ain KB, Brierley JD, Fein HG, Haugen BR, Jonklaas J, Magner J, Ross DS,

- Skarulis MC, Steward DL, Maxon HR, Sherman SI 2014 Prognosis of differentiated thyroid cancer in relation to serum thyrotropin and thyroglobulin antibody status at time of diagnosis. *Thyroid* **24**:35–42.
19. Jonklaas J, Nogueras-Gonzalez G, Munsell M, Litofsky D, Ain KB, Bigos ST, Brierley JD, Cooper DS, Haugen BR, Ladenson PW, Magner J, Robbins J, Ross DS, Skarulis MC, Steward DL, Maxon HR, Sherman SI 2012 The impact of age and gender on papillary thyroid cancer survival. *J Clin Endocrinol Metab* **97**:E878–E887.
20. Carhill AA, Litofsky DR, Ross DS, Jonklaas J, Cooper DS, Brierley JD, Ladenson PW, Ain KB, Fein HG, Haugen BR, Magner J, Skarulis MC, Steward DL, Xing M, Maxon HR, Sherman SI 2015 Long-term outcomes following therapy in differentiated thyroid carcinoma: NTCTCS registry analysis 1987–2012. *J Clin Endocrinol Metab* **100**:3270–3279.
21. Xavier AC, Maciel RM, Vieira JG, Dias-da-Silva MR, Martins JR 2016 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* **60**:66–75.
22. Dunn JT, Ray SC 1973 Changes in iodine metabolism and thyroglobulin structure in metastatic follicular carcinoma of the thyroid with hyperthyroidism. *J Clin Endocrinol Metab* **36**:1088–1096.
23. Schneider AB, Ikekubo K, Kuma K 1983 Iodine content of serum thyroglobulin in normal individuals and patients with thyroid tumors. *J Clin Endocrinol Metab* **57**:1251–1256.
24. Druetta L, Croizet K, Bornet H, Rousset B 1998 Analyses of the molecular forms of serum thyroglobulin from patients with Graves' disease, subacute thyroiditis or differentiated thyroid cancer by velocity sedimentation on sucrose gradient and Western blot. *Eur J Endocrinol* **139**:498–507.
25. Magro G, Perissinotto D, Schiappacassi M, Goletz S, Otto A, Muller EC, Bisceglia M, Brown G, Ellis T, Grasso S, Colombatti A, Perris R 2003 Proteomic and postproteomic characterization of keratan sulfate-glycanated isoforms of thyroglobulin and transferrin uniquely elaborated by papillary thyroid carcinomas. *Am J Pathol* **163**:183–196.
26. Emoto N, Kunii YK, Ashizawa M, Oikawa S, Shimizu K, Shimonaka M, Toyoda A, Toyoda H 2007 Reduced sulfation of chondroitin sulfate in thyroglobulin derived from human papillary thyroid carcinomas. *Cancer Sci* **98**:1577–1581.
27. Latrofa F, Ricci D, Grasso L, Vitti P, Masserini L, Basolo F, Ugolini C, Mascia G, Lucacchini A, Pinchera A 2008 Characterization of thyroglobulin epitopes in patients with autoimmune and non-autoimmune thyroid diseases using recombinant human monoclonal thyroglobulin autoantibodies. *J Clin Endocrinol Metab* **93**:591–596.
28. Lupoli GA, Okosieme OE, Evans C, Clark PM, Pickett AJ, Premawardhana LD, Lupoli G, Lazarus JH 2015 Prognostic significance of thyroglobulin antibody epitopes in differentiated thyroid cancer. *J Clin Endocrinol Metab* **100**:100–108.

Address correspondence to:

Andrew G. Gianoukakis, MD
Division of Endocrinology, Diabetes and Metabolism
The Lundquist Institute at Harbor-UCLA
Medical Center, Torrance, and David Geffen School
of Medicine at UCLA
1124 West Carson Street RB-1
Torrance, CA 90502
USA

E-mail: agianoukakis@mednet.ucla.edu