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6th International Conference on Biomarkers & Clinical Research

Efficacy of TSHR mRNA in the follow-up of differentiated thyroid cancer

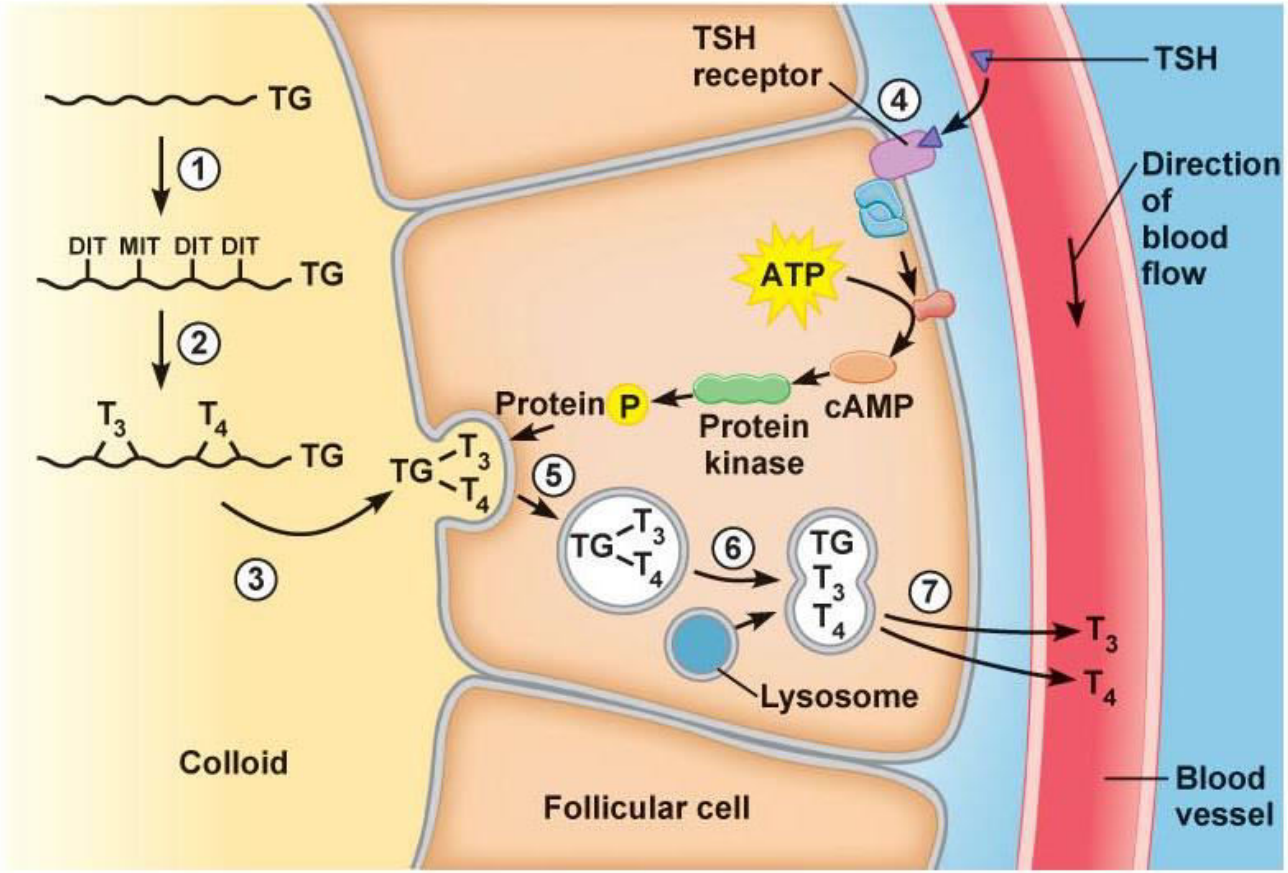
Surasawadee Ausavarat Ph.D.

Division of Nuclear Medicine, Department of Radiology,
Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Outline Presentation

- Thyroglobulin (Tg) as a tumor marker of DTC
- Thyroid-specific mRNA as a surrogate or adjunctive marker
- Efficacy of TSHR mRNA in the follow-up of DTC
- Further studies

Thyroglobulin



(b) Synthesis and secretion of thyroid hormones

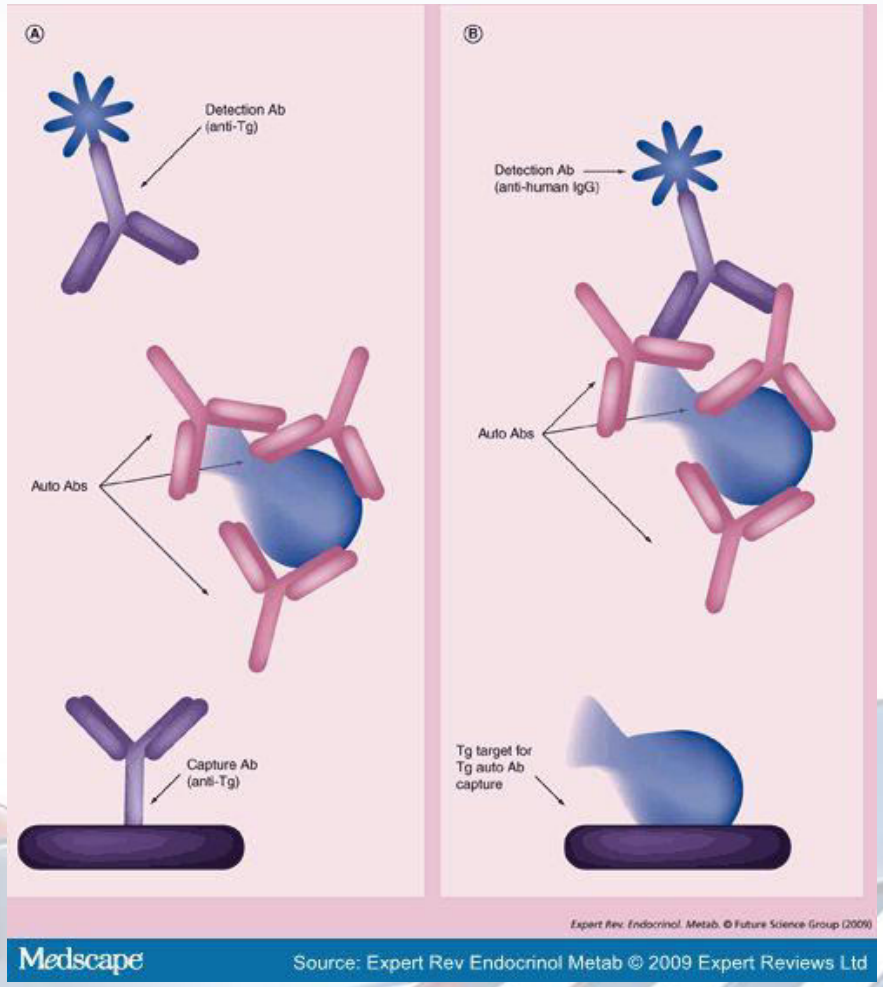
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Thyroglobulin

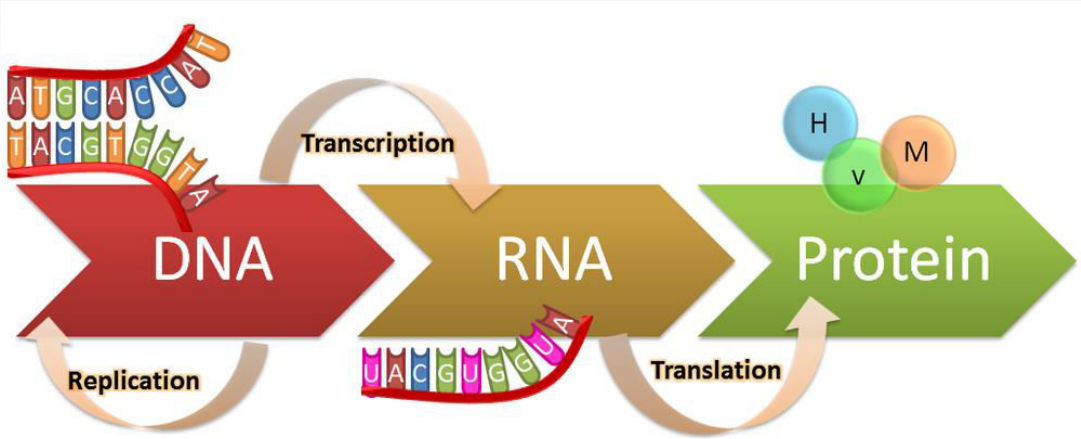
- Thyroglobulin levels in the blood are mainly used as a **tumor marker** for differentiated thyroid carcinoma.
- Following thyroidectomy and radioactive iodine ablation thyroglobulin levels can be **serially monitored**.
- A subsequent elevation of the thyroglobulin levels is an indication of **recurrence or metastatic disease.**

Limitations of Serum Thyroglobulin

- Endogenous anti-thyroglobulin antibodies (TgAb) can interfere with thyroglobulin measurement.
- 15–25% of patients with DTC are TgAb-positive
- Low sensitivity during thyroid hormone suppression therapy
- Different methods of detection:
  - RIA / IMAs / ECLIA



Detection of Thyroid-specific mRNA



Qualitative
Molecular detection

Quantitative
Molecular detection

Quantitative
Ag-Ab interaction detection

Picture from URL: http://genius.com/Biology-genius-the-central-dogma-annotated

Detection of Thyroid-specific mRNA

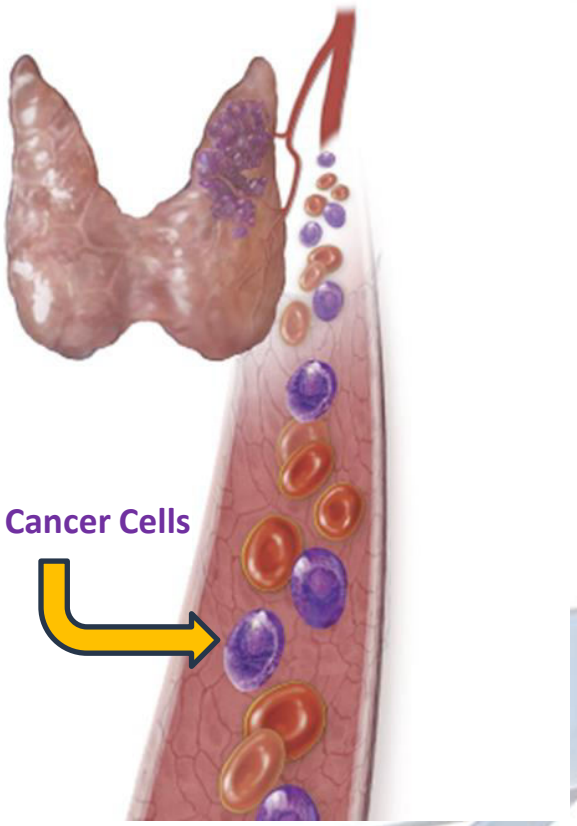
Detection of circulating thyroid cells in peripheral blood

Beth Ann Ditkoff, MD, Michael R. Marvin, MD, Shrishailam Yemul, PhD, Y. J. Shi, MD, John Chabot, MD, Carl Feind, MD, *and* **Paul Lo Gerfo, MD,** *New York, N.Y.*

Conclusions. *These data indicate that RT-PCR can be used to detect thyroglobulin mRNA in peripheral blood. The presence of these transcripts correlates with the existence of extrathyroidal disease. (Surgery 1996;120:959-65.)*

From the College of Physicians & Surgeons, Columbia University, New York, N.Y.

- **No interference of TgAb by molecular method**
- **High sensitivity during thyroid hormone therapy**
- **Identify postoperative patients at risk for metastatic thyroid cancer**



Picture from Gutnick J, Soldes O, Gupta M, Milas M. Circulating thyrotropin receptor messenger RNA for evaluation of thyroid nodules and surveillance of thyroid cancer in children.J Pediatr Surg. 2012 Jan;47(1):171-6.

Example of Thyroid-specific mRNA

- Thyroglobulin (Tg)
- Thyroid stimulating hormone receptor (TSHR)
- Thyroid peroxidase (TPO)
- Sodium/iodide symporter (NIS)

Review of Tg mRNA

British Journal of Cancer (2004) 91, 200–204
© 2004 Cancer Research UK All rights reserved 0007 – 0920/04 $30.00
www.bjcancer.com

Minireview

Detection of circulating Tg-mRNA in the follow-up of papillary and follicular thyroid cancer: how useful is it?

FA Verburg*,[1], CJM Lips[2], EGWM Lentjes[3] and JMH de Klerk[1]

[1]Department of Nuclear Medicine, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; [2]Department of Endocrinology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands; [3]Department of Laboratory Medicine, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

Table 1 Summary of results of various studies into the usefulness of Tg-mRNA detection

Study	Quantitative?	False positive	False negative	True positive	True negative	Positive controls	Negative controls
Ditkoff	No	7/78	0/9	9/9	71/78	0/15	15/15
Biscolla	No	3/19	5/14	10/15	16/19	—	—
Ringel (1999)	Yes	13/33	13/74	61/74	20/33	—	—
Savagner	Yes	3/15	6/25	19/25	12/15	—	—
Bojunga, method 1	No	63/137	4/13	9/13	74/137	30/135	105/135
Bojunga, method 2	No	111/137	2/13	11/13	26/137	102/135	33/135
Elisei	Yes	22/29	2/17	15/17	7/29	17/20	3/20
Takano	Yes	All patients and controls are positive					
Bugalho	Semi	No difference in expression levels between individuals with and without thyroid glands					
Bellantone	No	Varies according to histology					
Eszlinger	Yes	No difference between patients with and without metastases or other thyroid diseases					
Span	Yes	No difference between patients and controls					

ATA Guidelines 2009

- Tg mRNA may be a sensitive marker for persistent thyroid cells
- RNA extraction is not well standardized
- Question about its specificity
- Future studies optimizing the measurement are needed

TSH-receptor mRNA

TSHR mRNA Arrives as Thyroid Cancer Marker

BY PATRICE WENDLING

FROM THE ANNUAL MEETING OF THE AMERICAN SURGICAL ASSOCIATION

CHICAGO — Thyroid-stimulating hormone receptor messenger RNA is a clinically useful blood test in the pre- and postoperative management of thyroid cancer, based on results from a prospective validation study in 1,095 consecutive patients.

If detectable preoperatively, when fine-needle aspiration suggests follicular neoplasm, thyroid-stimulating hormone receptor (TSHR) messenger RNA (mRNA) accurately predicts thyroid cancer and guides the extent of surgery, reported Dr. Mira Milas, principal investigator.

"It has a practical role to predict thyroid cancers where other modalities may fall short," she said. In addition, high levels on the first postoperative day alert physicians to persistent disease, while its presence during long-term surveillance aids in identifying recurrence.

"We are encouraged by this performance and the fact that it is a convenient blood test to perform," said Dr. Milas, director of the thyroid center at the Cleveland Clinic. "Our next steps are to search for multicenter trials and [Food and Drug Administration] approval for some of these indications."

No new blood tests for differentiated thyroid cancer have been introduced into routine clinical practice since thyroglobulin. Thyroglobulin is used for cancer follow-up, but not initial diagnosis.

The TSHR mRNA assay was developed to distinguish benign from malignant thyroid diseases.

A recent report suggests that TSHR mRNA is detectable even in thyroid microcarcinomas, and may characterize those with potentially more aggressive histology (Surgery 2009;146:1081-9).

The aim of the current analysis was to validate the clinical use of the marker 1 year after its introduction at the Cleveland Clinic, where the test is now used daily in all patients scheduled for thyroid surgery and those undergoing consultation for thyroid disease in the office.

From October 2008 through September 2009, TSHR mRNA was measured by quantitative real-time polymerase chain reaction from blood drawn in 403 patients undergoing thyroid surgery, postoperatively in 541 patients, and in 151 patients monitored for benign goiters.

Preoperative Use

Preoperative TSHR mRNA greater than 1 ng/mcg as a sole predictor of cancer had a positive predictive value (PPV) of 81% and specificity of 83% in 374 patients with surgically confirmed pathology, Dr. Milas said. Sensitivity was modest at 61%, as was negative predictive value at 64%. However, its PPV was 100% in patients with papillary thyroid cancers greater than 1 cm.

She observed that TSHR mRNA is particularly useful in detecting cancer in patients with follicular neoplasms on fine-needle aspiration. In 54 such patients, TSHR mRNA alone had a PPV of 96%, a specificity of 96%, and accuracy suggestive of cancer in follicular neoplasms, but none are independently diagnostic. The highest risk of a false positive occurs with Hashimoto's disease, Dr. Milas acknowledged.

Postoperative Use

Use of the blood test in 69 paired samples with thyroid cancer revealed that elevated TSHR mRNA levels became undetectable in all patients on postoperative day 1, except in seven who had persistent or recurrent cancer within the year and unfavorable histologic features.



TSHR mRNA had a positive predictive value of 100% in patients with papillary thyroid cancers greater than 1 cm.

DR. MILAS

In contrast, all 40 patients with benign disease had undetectable TSHR mRNA levels on day 1 after thyroidectomy. "This suggests the potential use of TSHR mRNA as an early marker of adequate surgical clearance of disease or future recurrence," Dr. Milas said in an interview.

Long-Term Surveillance

TSHR mRNA also showed merit during long-term follow-up of thyroid cancer, notably in two particularly challenging scenarios: when thyroglobulin antibodies in 60 patients with elevated thyroglobulin antibodies, detectable TSHR mRNA was the only blood test to confirm cancer in four patients. Negative TSHR mRNA reassured absence of disease in 54 of the 56 remaining patients whose imaging was also negative.

All 59 patients with residual thyroid tissue because of partial thyroidectomy for cancer had undetectable TSHR mRNA levels and no radiologic evidence of cancer recurrence at follow-up, she said.

"This assay has come of age," remarked Dr. Collin Weber, the invited discussant and chief of endocrine surgery at Emory University Hospital in Atlanta.

He questioned whether total thyroidectomy is performed at the Cleveland Clinic on all indeterminate lesions when the assay is positive and whether its accuracy is good enough to recommend observation of TSHR mRNA-negative follicular neoplasms greater than 2.5 cm in size.

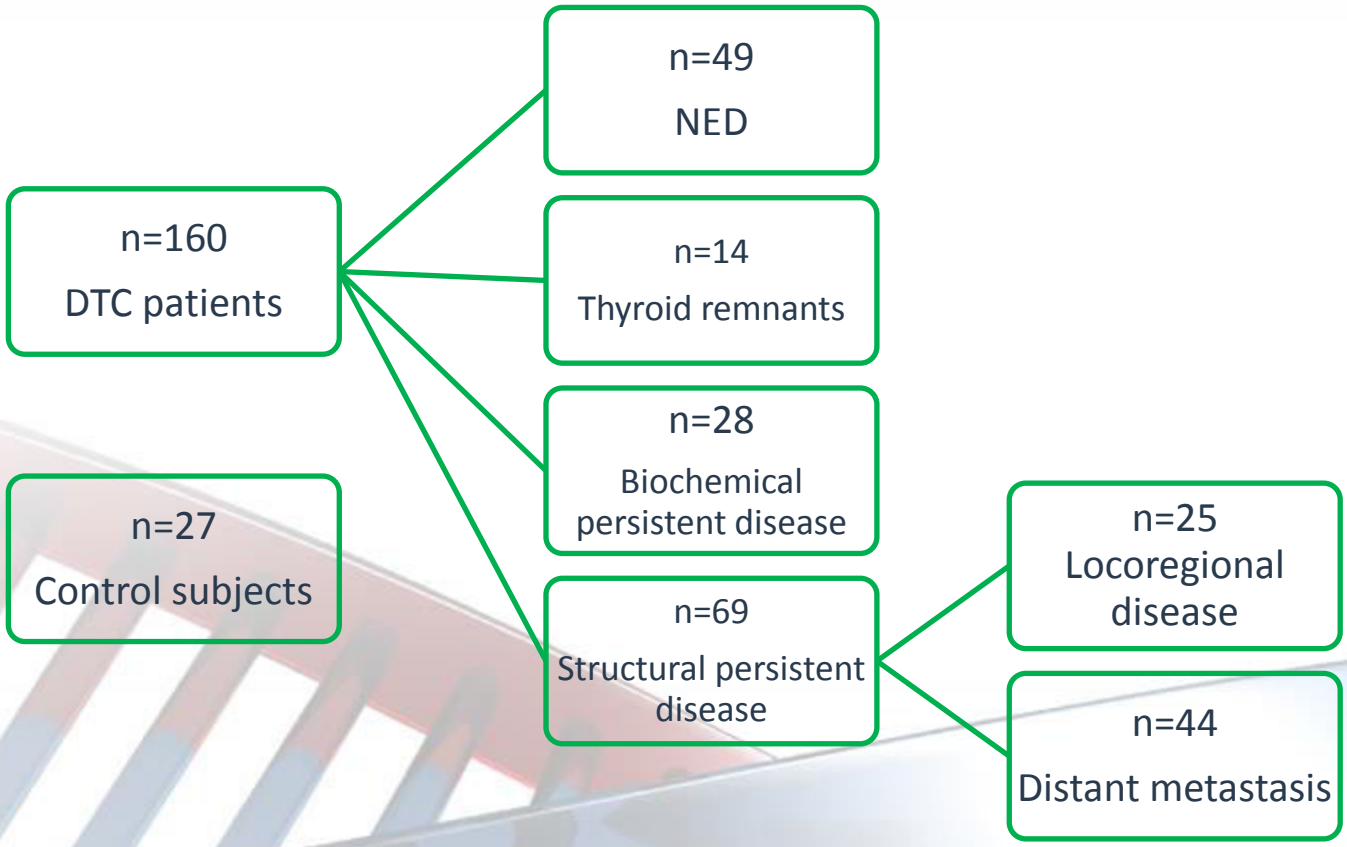
Dr. Milas responded that total thyroidectomy would be advised based on abnormal TSHR mRNA levels because a positive predictive value of 96% is reassuring and has held up in daily practice since the analysis was closed.

She said the assay is not accurate enough, however, to recommend observation in sonographically suspicious, 2.5-cm or greater tumors with undetectable TSHR mRNA levels, but "is possibly and cautiously" enough when combined with an informed discussion with the patient when smaller lesions lack concerning ul-

Objectives

- **Evaluate the efficacy of TSHR mRNA in the follow-up of DTC**
- **Efficacy of TSHR mRNA in TgAb-positive patients**
- **Comparative study of serum Tg, TSHR mRNA and total body scan**

Materials and Methods

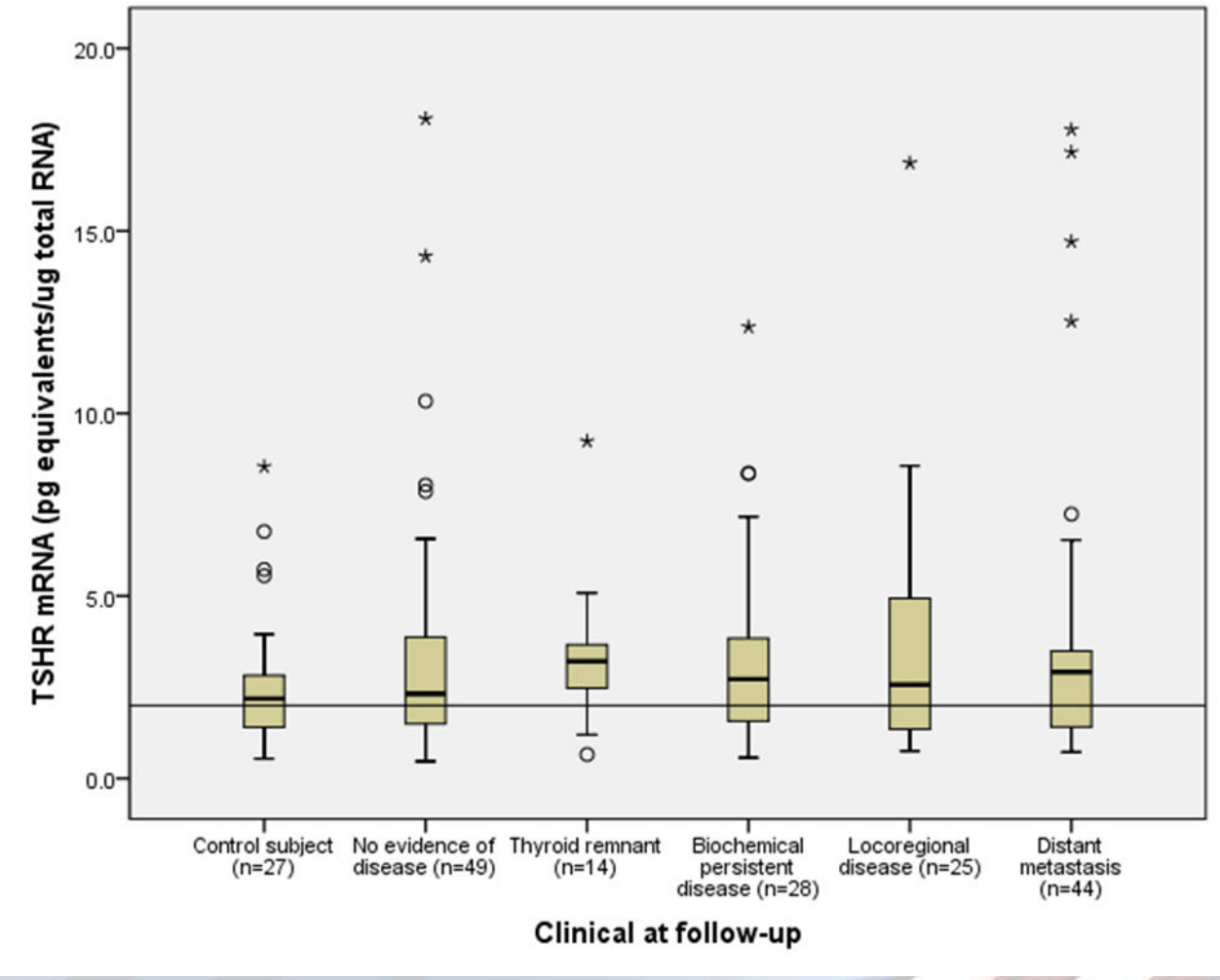


Materials and Methods

Table 1 Demographic of DTC patients

Characteristics		N (%)
Age (years)		
Mean ± SD	49.9 ± 14.3	
Gender		
Female		133 (83.1)
Male		27 (16.9)
Follow-up duration (years)		
Median (interquartile range)	4.0 (2.0–7.0)	
Histology		
Papillary		135 (84.4)
Follicular		17 (10.6)
Mixed		7 (4.4)
Hürthle cell		1 (0.6)
TgAb status during thyroid hormone therapy		
Positive TgAb (≥40 IU/mL)		27 (16.9)
Negative TgAb (<40 IU/mL)		133 (83.1)

Results


TSHR mRNA (pg equivalents/ug total RNA)
20.0
15.0
10.0
5.0
0.0
Control subject (n=27)
No evidence of disease (n=49)
Thyroid remnant (n=14)
Biochemical persistent disease (n=28)
Locoregional disease (n=25)
Distant metastasis (n=44)
Clinical at follow-up

Results

Table 2 TSHR mRNA levels of the study groups

Group	TSHR mRNA median (range)[a]		
	Total	TgAb-ve	TgAb + ve
Control subject	2.19 (1.30–2.99)	2.19 (1.30–2.99)	-
	$n = 27$	$n = 27$	
No evidence of disease	2.32 (1.44–3.94)	2.32 (1.44–3.94)	-
	$n = 49$	$n = 49$	
Thyroid remnants	3.21 (2.22-3.88)	3.05 (1.71–3.63)	4.24 (3.39–5.08)
	$n = 14$	$n = 12$	$n = 2$
Biochemical persistent disease	2.72 (1.51–3.98)	2.42 (1.21–3.98)	3.59 ± 2.37
	$n = 28$	$n = 20$	$n = 8$
Structural persistent disease	2.75 (1.37–3.83)	2.22 (1.13–3.28)	3.47 (2.97–9.53)[c]
	$n = 69$	$n = 52$	$n = 17$
Locoregional disease	2.56 (1.19–5.03)	2.00 (1.03–4.93)	3.01 (1.89–8.16)
	$n = 25$	$n = 19$	$n = 6$
Distant metastasis	2.92 (1.40–3.49)	2.24 (1.17–3.19)	5.55 (3.28–12.52)[c]
	$n = 44$	$n = 33$	$n = 11$

[a]Unit of TSHR mRNA: pg equivalents/μg total RNA
[b]Unit of serum Tg: ng/mL
[c]Significance at $p < 0.05$ comparing to no evidence of disease

Results

Table 3 Diagnostic performance of TSHR mRNA, serum Tg, and ^{131}I uptake in identifying structural persistent disease

	Diagnostic Performance (%)								
	TSHR mRNA[a]			Serum Tg[b]			^{131}I whole body scan		
	Total	TgAb-ve	TgAb + ve	Total	TgAb-ve	TgAb + ve	Total	TgAb-ve	TgAb + ve
Sensitivity	62.3	53.8	88.2	79.7	90.4	47.1	46.4	42.3	58.8
Specificity	42.9	42.9	42.9	81.6	81.6	81.6	87.8	87.8	87.8
PPV[c]	60.6	50.0	34.9	85.9	83.9	47.1	84.2	78.6	62.5
NPV[d]	44.7	46.7	91.3	74.1	88.9	81.6	53.8	58.9	86.0
Accuracy	54.2	48.5	54.5	80.5	86.1	72.7	63.6	64.4	80.3

[a]Using a cutoff of at least 2.00 pg equivalents/μg total RNA on thyroid hormone therapy
[b]Using a cutoff of at least 0.3 ng/mL on thyroid hormone therapy
[c]PPV, Positive predictive value
[d]NPV, Negative predictive value

Conclusions

- **TSHR mRNA seems to be highly sensitive in TgAb-positive patients only.**
- **24 out of 27 TgAb+ve patients were positive for either TSHR mRNA with sensitivity 88.2% of and specificity of 42.9%.**
- **2 patients with distant metastasis who were positive for TSHR mRNA had undetectable serum Tg (<0.1 ng/mL) and higher than the upper limit of TgAb (>4,000 IU/mL).**

Conclusions

- This emphasizes that serum Tg remains the most efficient marker for thyroid carcinoma management.
- TSHR mRNA assay depends on molecular technique which is much more expensive and time-consuming.
- Second generation of Tg assay made it possible to be sensitive enough for detecting basal Tg without TSH stimulation.
- TSHR mRNA assay may be used as an adjunctive marker in patients whose serum Tg are not reliable due to the interference of TgAb.

Further Studies

- **Emphasize the cost-effectiveness of routine usage of the assay in clinical practice for TgAb+ve patients.**

- **Studies of other novel molecular marker such as circulating braf^{V600E} in the diagnosis and follow-up of DTC**

Thank you

Team Biomarkers welcomes you all to the next chapter – 7th International Conference on Biomarkers & Clinical Research scheduled for Nov 28-30, 2016 in Baltimore, USA

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