

# Miami Thyroid Oncology Symposium

March 18-19, 2022

## Thyroglobulin: Biology, Physiology and Clinical Meaning

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# Learning Objectives

After this session, participants will be given the tools to:

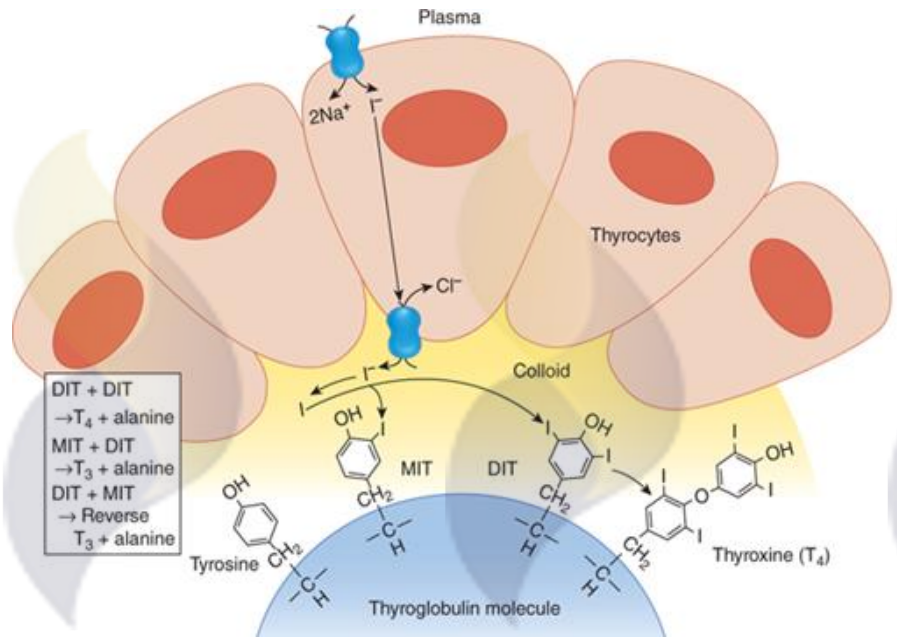
- **Thyroglobulin physiology and pathophysiology**
- **Pitfalls and caveats in clinical testing**
- **Diagnostic and prognostic value of thyroglobulin**

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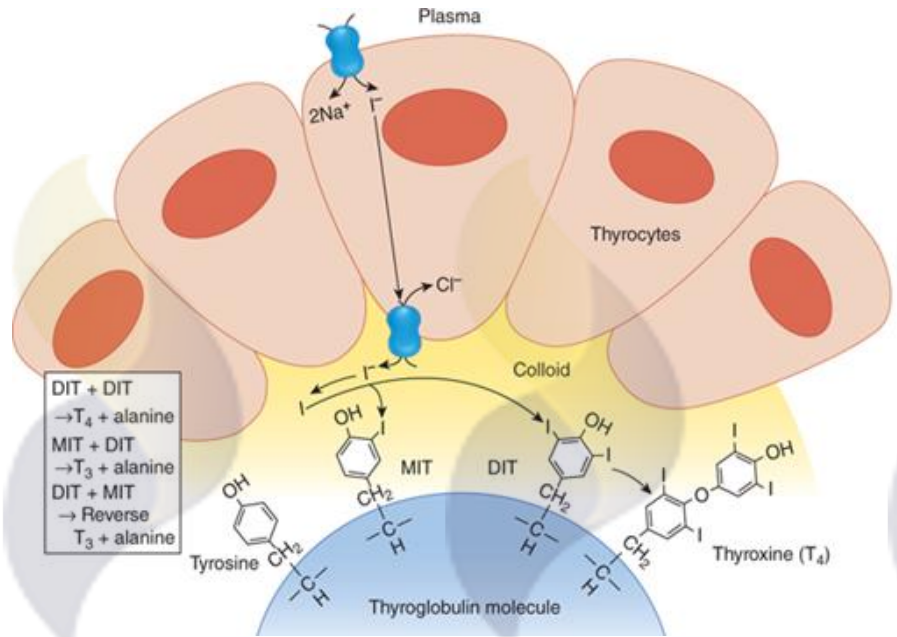
# Thyroglobulin



- 660kDa glycoprotein
- produced exclusively in the thyroid gland
- substrate for thyroid hormones production
- small amounts detected in the serum of healthy individuals  
(0.5-1 ug/L x g thyroid tissue)



# Thyroglobulin



## Undetectable serum thyroglobulin levels

- expected after removal of benign and malignant thyroid tissues

**Key point:** serum Tg is the primary biochemical tumor marker used to monitor differentiated thyroid cancer (DTC) after removal of thyroid tissues

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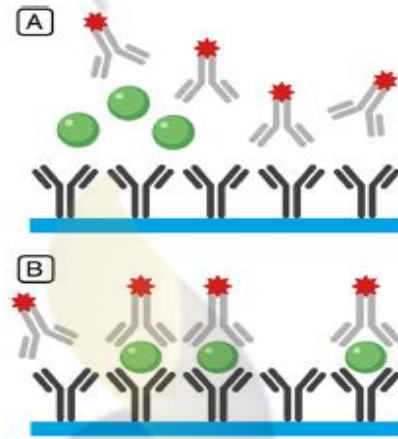
# Thyroglobulin testing

- Radioimmunoassays
- Immunometric assays
- Tandem Mass Spectrometry

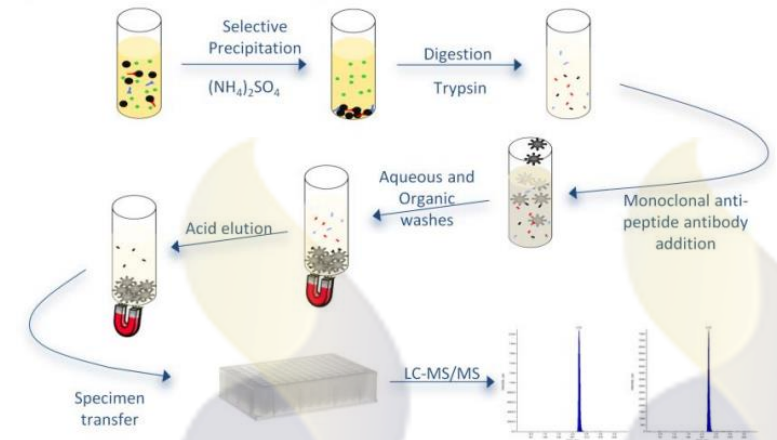


# Thyroglobulin testing

- Radioimmunoassays
- Immunometric assays
- Tandem Mass Spectrometry



Clin Diabet Endocrinol (2019) 5:12 <https://doi.org/10.1186/s40842-019-0086-7>



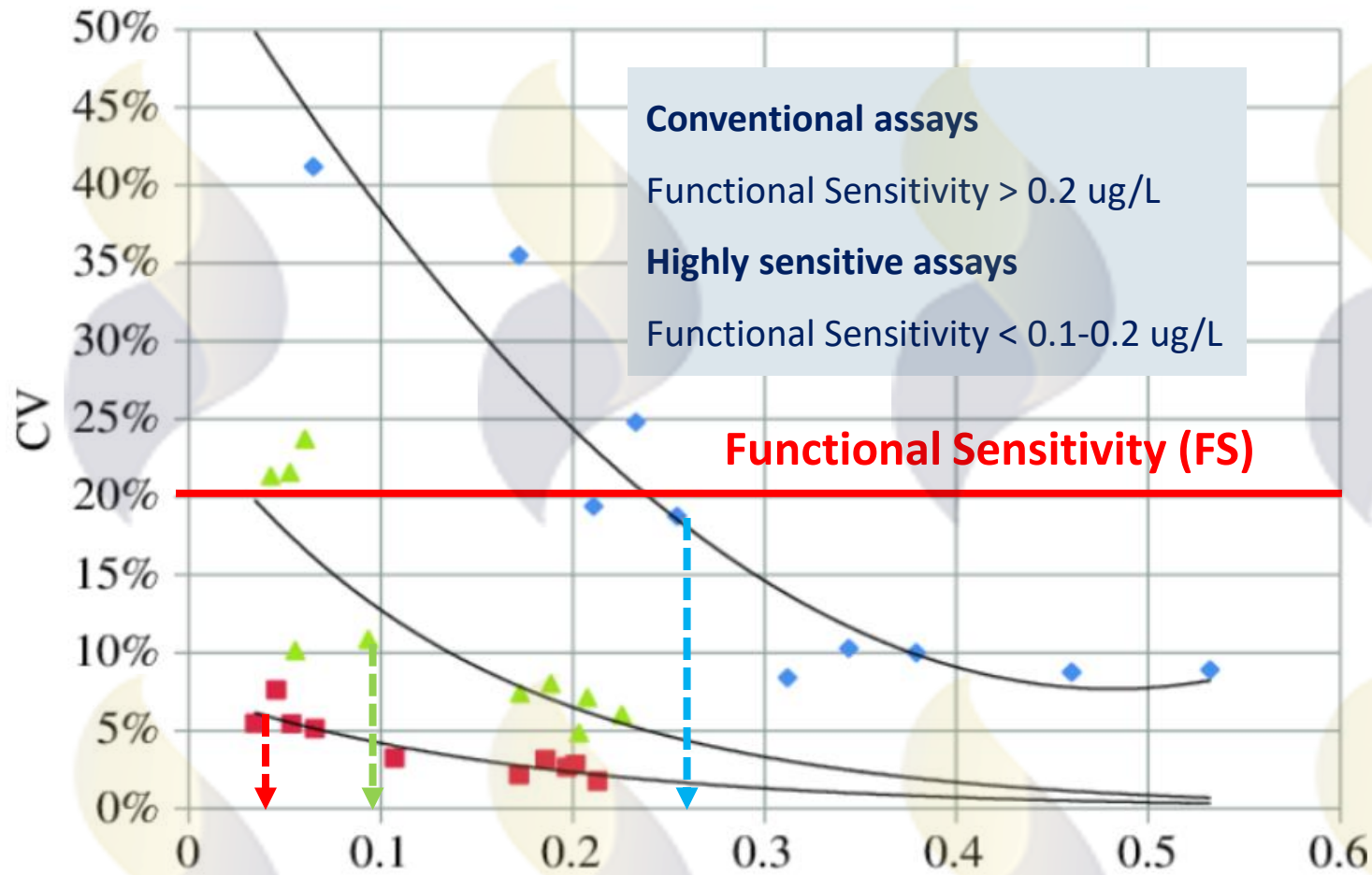
## Interferences

Assay	Source	Functional sensitivity ( $\mu\text{g/L}$ )	Interferences	
			Anti-Tg antibodies	Heterophile antibodies
RIA	LDT	0.5–5.0	Yes (falsely low or falsely high results)	No
IMAs	Commercial	0.1–0.9	Yes (falsely low results)	Yes (falsely high or falsely low results)
LC-MS/MS	LDT	0.5–1.0	No	No

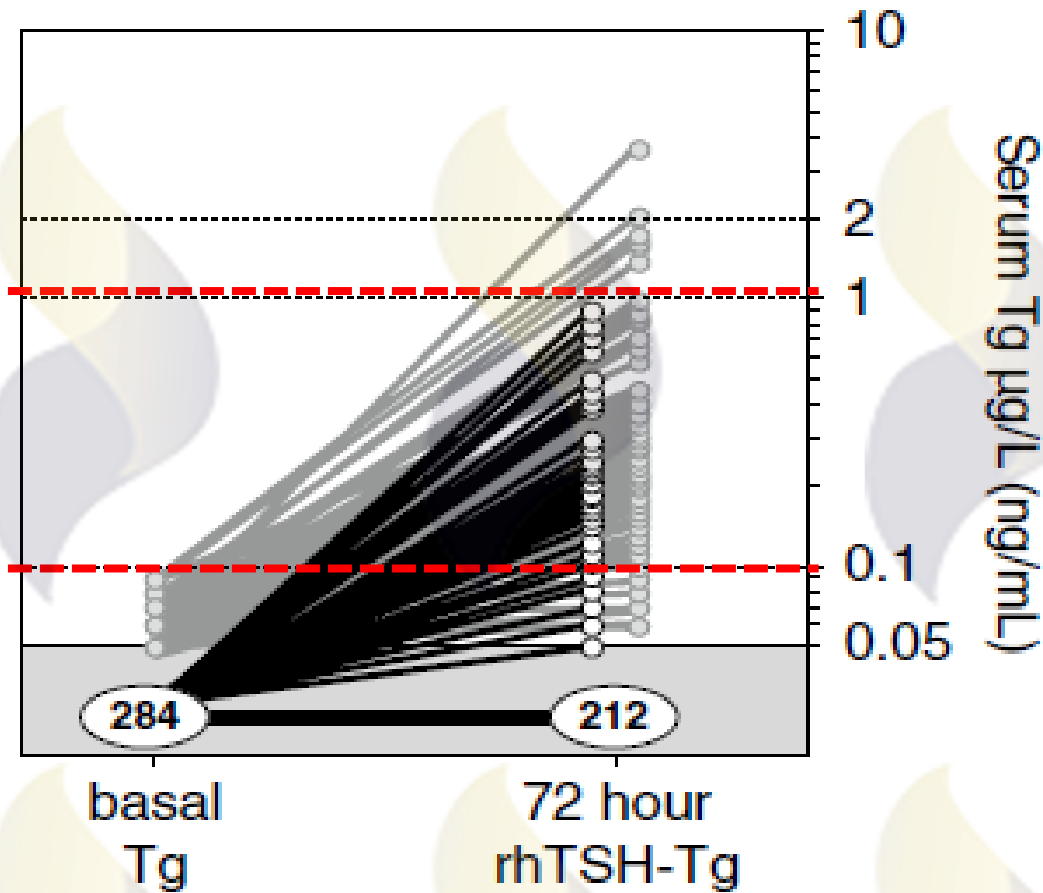
LDT: laboratory-developed test.

Algeciras-Schimnich A. Crit Rev Clin Lab Sci (2018)

# Thyroglobulin testing: the pivotal role of analytical sensitivity



# Thyroglobulin testing: the pivotal role of analytical sensitivity



Robust data suggest that an undetectable Tg value using a highly sensitive assay is associated with adequate sensitivity and NPV to obviate the need for measuring TSH-stimulated Tg concentrations in most cases

Giovanella L et al. *European Journal of Endocrinology* (2019) 181, R133–R145

Spencer et al. *Thyroid* 2010

***Key points:***

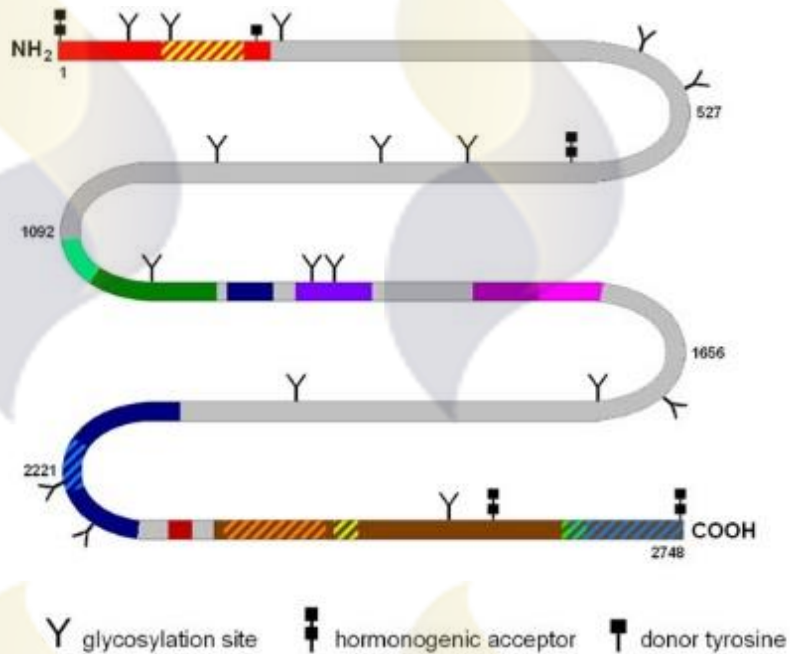
- 1. Thyroglobulin should not be measured routinely by RIA and MS methods in patients with DTC**
- 2. Thyroglobulin should be measured by immunometric assays, preferentially highly-sensitive ones**

# Thyroglobulin testing - *pitfalls and caveats*

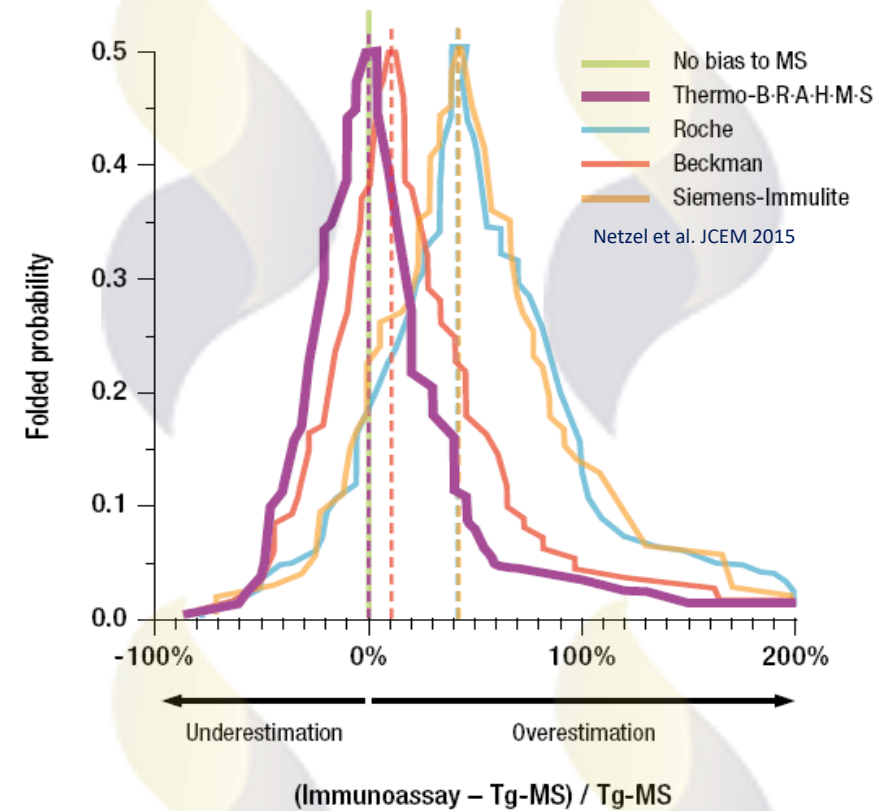
Manufacturer	Tg Assay	Procedure to assess the analytical sensitivity
<b>Abbott</b>	Architect Tg Alinity i Tg	LoQ determined from $n \geq 60$ replicates of low-analyte level samples and defined as the lowest concentration at which a maximum allowable precision of 20 % CV is met.
<b>Beckman Coulter</b>	Access Tg	AS determined as the lowest detectable level of Tg distinguishable from zero with 95% confidence (LoD).
<b>BRAHMS Thermofisher</b>	BRAHMS h-Tg Sensitive KRYPTOR	FS determined as inter-assay precision of 20% according to the CLSI EP5-A3 guidelines. LoQ determined as the lowest concentration with 40% total allowable error according to the CLSI EP5-A3 guidelines.
<b>Diasorin</b>	Liaison <sup>®</sup> Tg II Gen	FS defined as the lowest measureable analyte concentration with an inter-assay CV < 20%.
<b>Roche Diagnostics AG</b>	Elecsys Tg II	LoQ determined as the lowest concentration with 30% total allowable error according to the CLSI EP17-A2 guidelines.
<b>Siemens Healthineers</b>	Atellica <sup>®</sup> IM	LoQ defined as the lowest measurable concentration with intra-laboratory LoQ $\leq 20\%$ .
<b>Siemens Healthineers</b>	Immulite 2000 Tg	FS procedure unreported

# Thyroglobulin testing - *pitfalls and caveats*

## □ Molecular heterogeneity

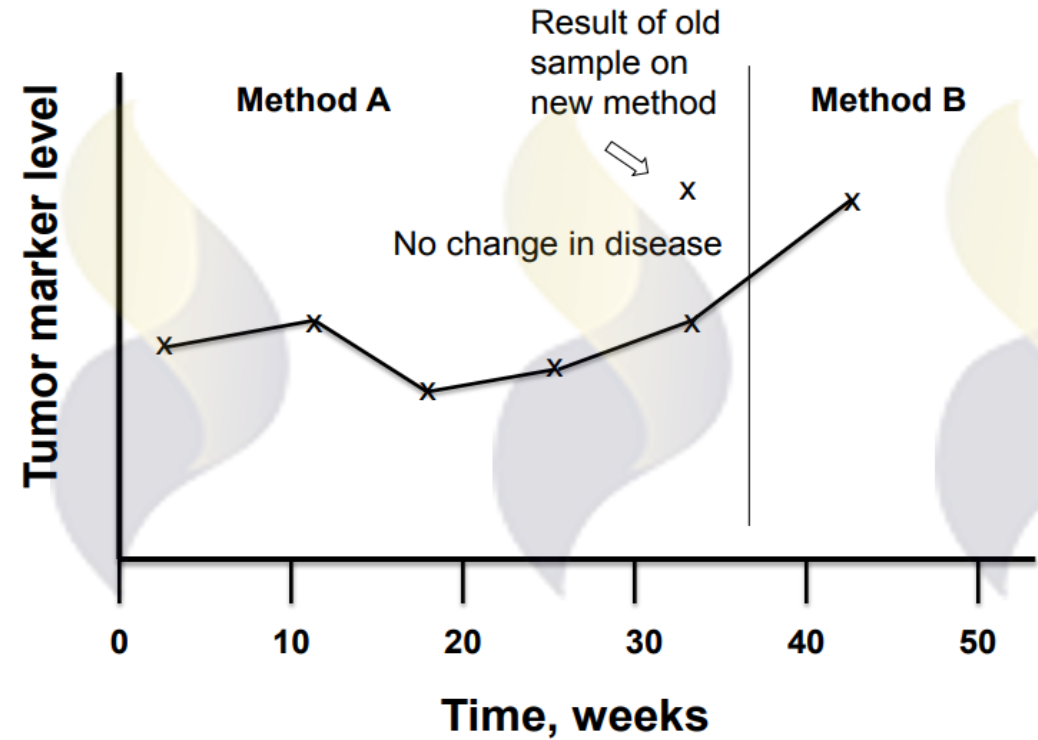


## □ Different antibodies in different assays



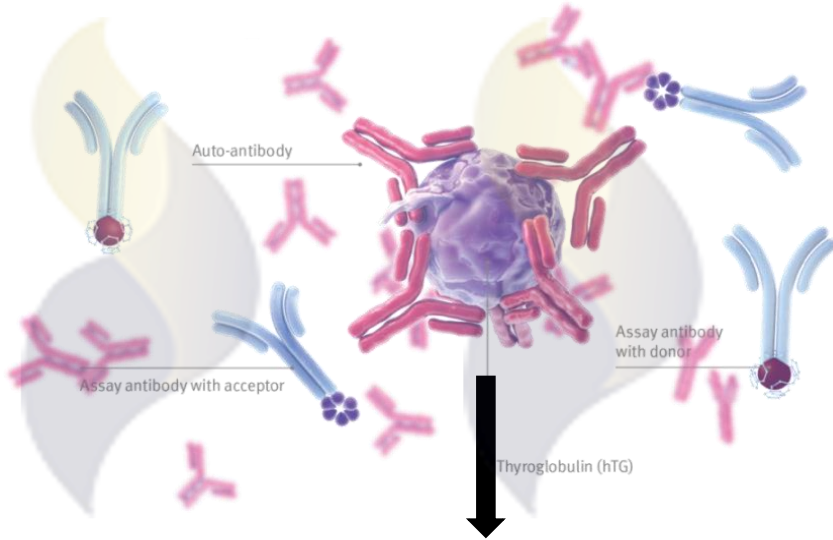
# Thyroglobulin testing - *pitfalls and caveats*

- ❑ Certified Reference Material (BCR® 457)
- ❑ Using different assays may disrupt serial monitoring
  - ⇒ Use the same assay during the patient's follow-up.
  - ⇒ If change unavoidable rebaseline is needed.



# Thyroglobulin testing - *pitfalls and caveats*

## Thyroglobulin autoantibodies (TgAb)



## Falsely reduced Tg levels

- TgAb prevalence: 15-30%

## TgAb assays: *limited agreement*

⇒ New TgAb assay: rebaseline!

## TgAb assays: *different thresholds*

⇒ Adopt method-specific LOQ/FS

## Example

**DTC patient: TgAb 88 IU/mL**

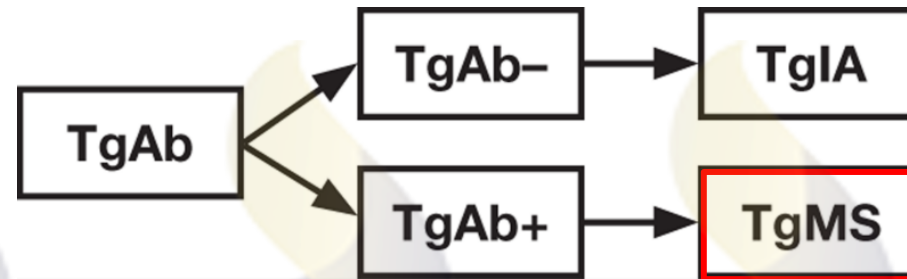
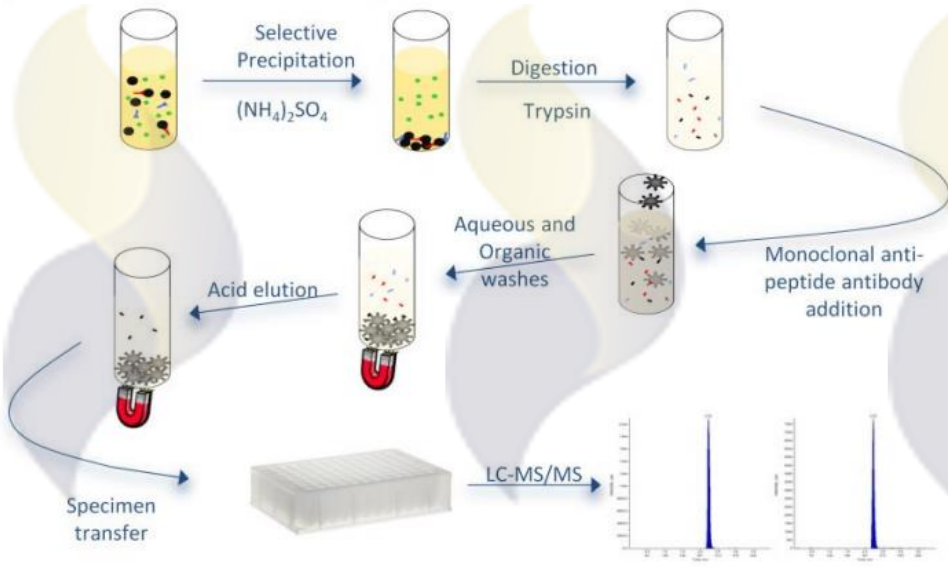
Cutoff 115 IU/mL

LOQ 40 IU/mL

⇒ **TgAb-positive**



# Thyroglobulin testing - *pitfalls and caveats*



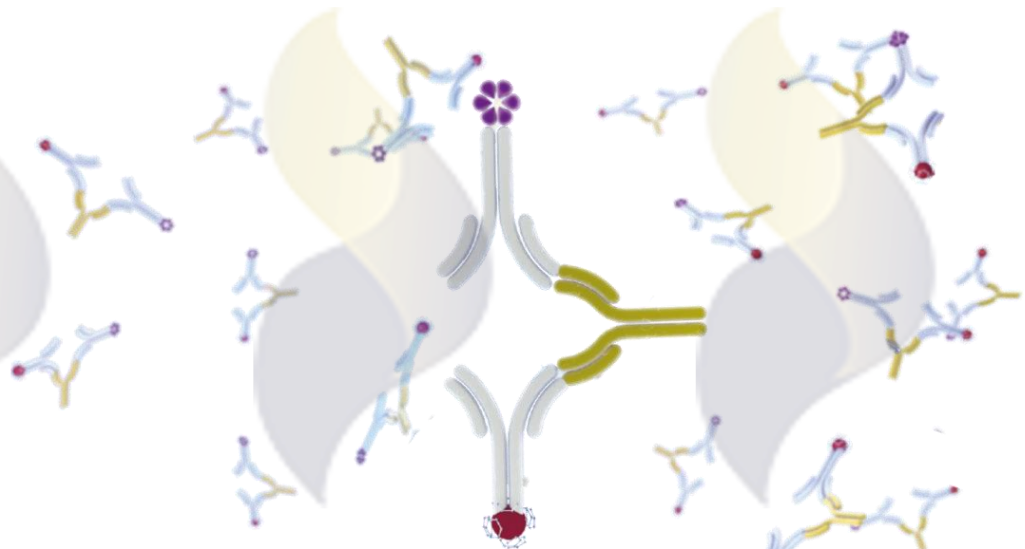
**Suboptimal FS [ $\sim 0.5 \text{ ug/L}$ ]**

Undetectable TgMS  $\sim 40\%$  of TgAb-pos. pts.

with structural DTC recurrence

Netzel BC et al. JCEM 2015

# Thyroglobulin testing - *pitfalls and caveats*



- HAb prevalence: 0.4-1%

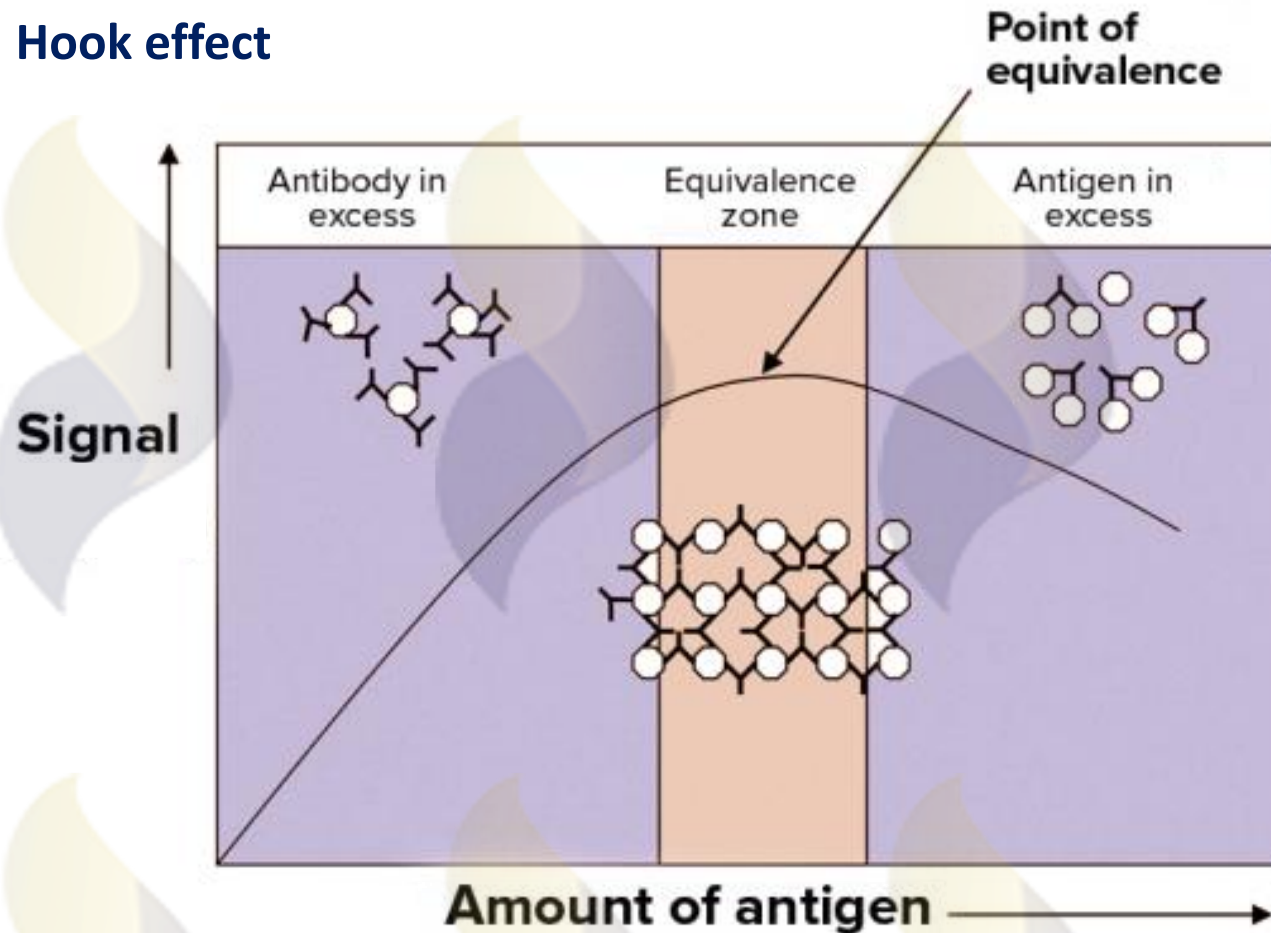
**Falsely increased Tg levels**

## Detection of HAb interferences

- test repetition with an alternative assay
- recovery test (i.e. over-recovery)
- measurement of serial dilutions of suspected samples
- precipitation polyethylene glycol.
- serum treatment with HAb-blocking reagents (i.e. HBT)

# Thyroglobulin testing - *pitfalls and caveats*

Hook effect



**Very rare in modern Tg IMAs**

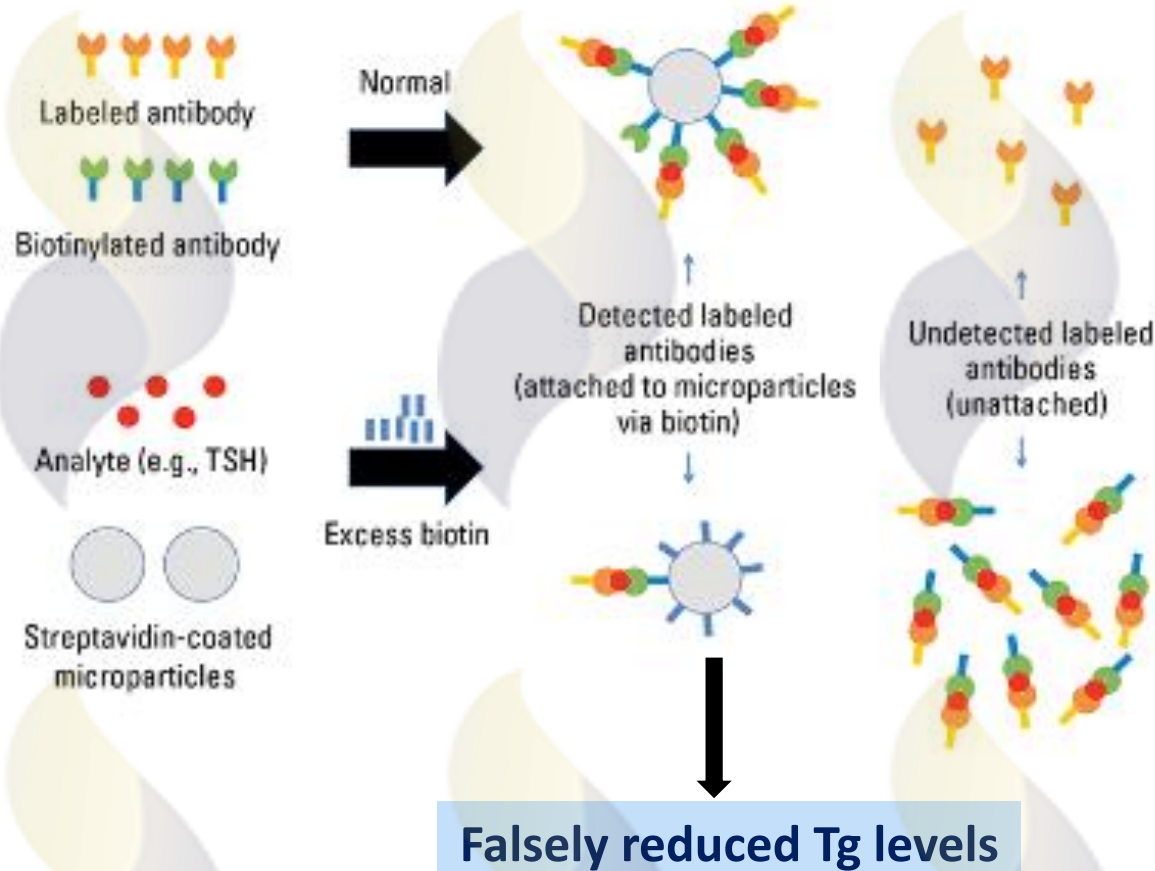
- advanced metastatic disease
- Tg measurement on FNAC washouts

**Detection**

- Tg increases in serially diluted samples

# Thyroglobulin testing - *pitfalls and caveats*

## Biotin



## Strategies

- test repetition after biotin discontinuation (>48 hours)
- test repetition with a non-(streptavidin/biotin)-based IMA
- use new biotin-protected immunoassay

- Prevalence: no cases reported so far

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## Current clinical settings

**Total thyroid ablation (TTx + I-131):** removal of benign and malignant thyroid tissues

**Total thyroidectomy w/o I-131:** post-op circulating Tg produced by thyroid remnants

**Lobectomy:** post-op circulating Tg produced by contralateral lobe

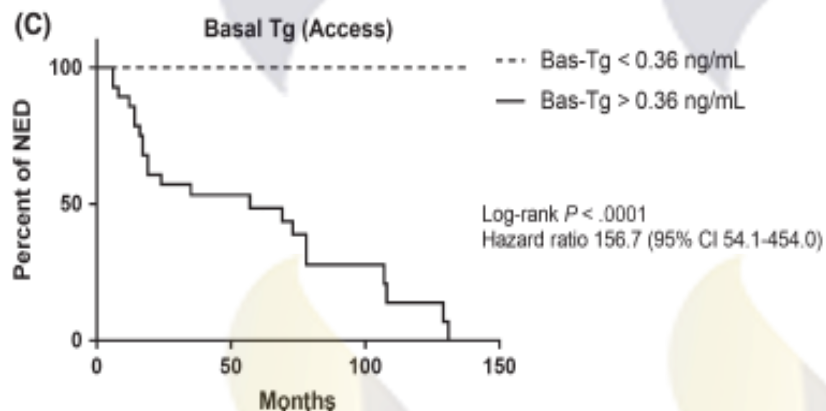
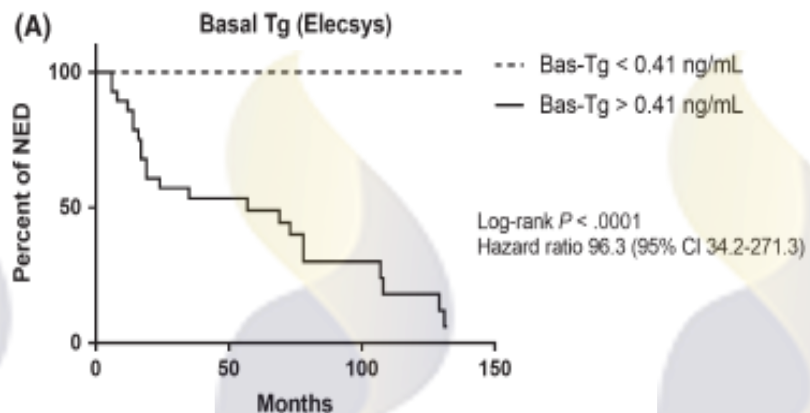
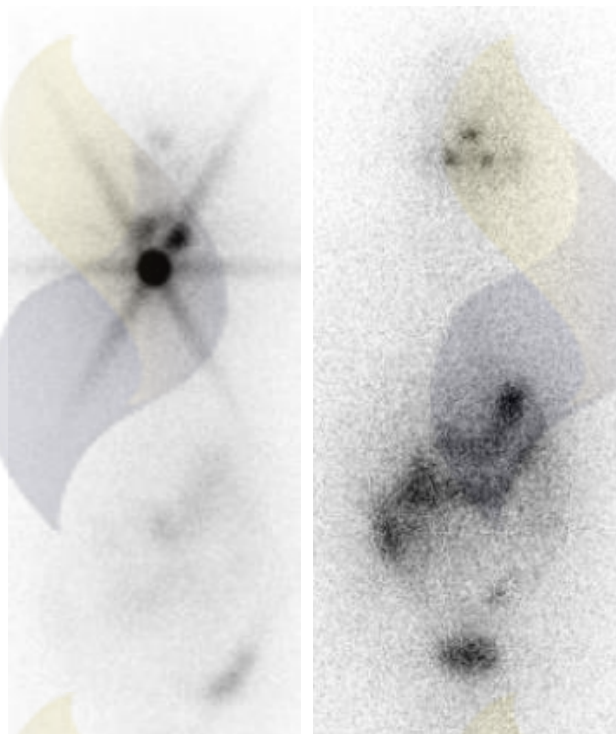
# Total thyroid ablation



**Table 5.** Response assessment after total thyroid ablation [2].

Response	Criteria	Imaging	Thyroglobulin (ng/mL)
Excellent	No evidence of disease (clinical, biochemical, or structural)	Negative	Basal Tg < 0.2 ng/mL <i>OR</i> stimulated-Tg < 1.0 ng/mL
Incomplete biochemical	Abnormal Tg <i>OR</i> increasing TgAb No evidence of structural disease	Negative	Basal Tg > 1.0 ng/mL <i>OR</i> stimulated-Tg > 10 ng/mL <i>OR</i> Rising TgAb
Incomplete structural Indeterminate	Evidence of structural disease Aspecific findings	Positive Indeterminate	Any Tg and TgAb value Basal Tg 0.2–1.0 ng/mL Stimulated-Tg 1–10 ng/mL

# Total thyroid ablation



	Hazard Ratio	95% CI	P
Univariate analysis			
Age >55	3.54	1.3-9.65	.013
High risk according to ATA	3.24	1.23-8.49	.017
TNM stage IV	4.45	1.79-11.03	.001
Basal Tg (Elecsys <sup>®</sup> )	102.19	13.48-774.39	<.001
Basal Tg (Access <sup>®</sup> )	108.27	14.36-816.29	<.001
Stimulated Tg (Elecsys <sup>®</sup> and Access <sup>®</sup> )	94.07	12.44-711.5	<.001
Multivariate analysis (with Tg Elecsys <sup>®</sup> )			
Age >55	2.43	0.88-6.71	.087
High risk according to ATA	1.33	0.5-3.56	.566
TNM stage IV	2.03	0.77-5.35	.153
Basal Tg	67.94	8.68-531.87	<.001
Multivariate analysis (with Tg Access <sup>®</sup> )			
Age >55	1.29	0.42-3.93	.655
High risk according to ATA	2.53	0.83-7.72	.103
TNM stage IV	1.58	0.58-4.28	.368
Basal Tg	81.61	10.26-648	<.001



# Total thyroid ablation

# Thyroidectomy

## I-131 therapy

(no extra-thyroid uptake on post-treatment whole body scan)

6-12 months

onT4-hsTg / Neck US

- onT4-hsTg < 0.2 ug/L
- Negative US

Excellent response  
*every 12-24 months*

- Clinical examination
- onT4-hsTg

- onT4-hsTg ≥ 0.2 ug/L
- Negative US

- onT4-hsTg 0.2-1 ug/L
- *Monitor Tg trend*

- onT4-hsTg > 1 ug/L
- *Work-up, ev treatment*

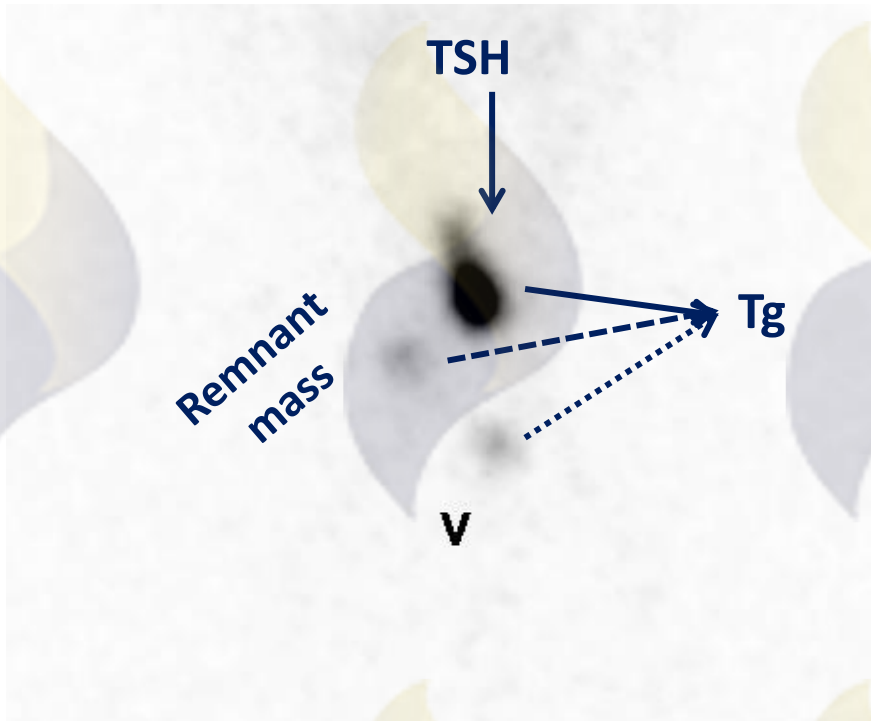
- Positive US
- Any Tg value

Incomplete structural response

- *Work-up, ev treatment*

## TgAb-negative

# Thyroidectomy w/o I-131

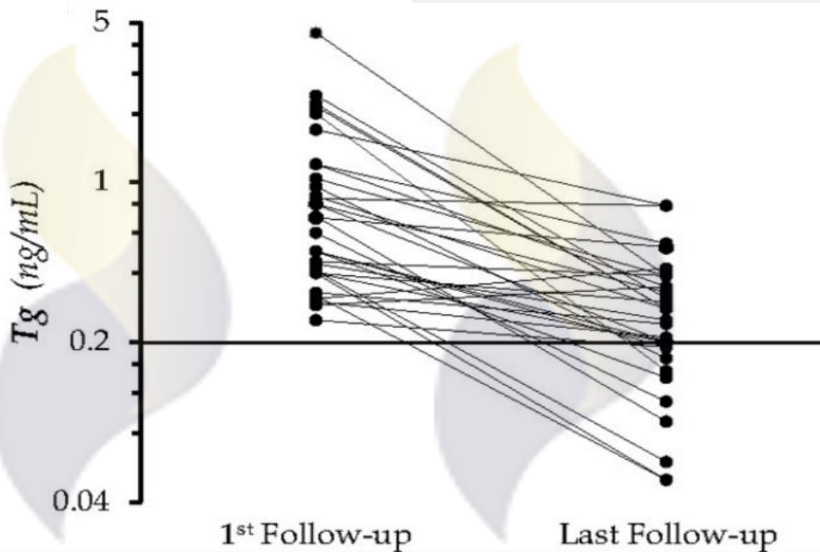
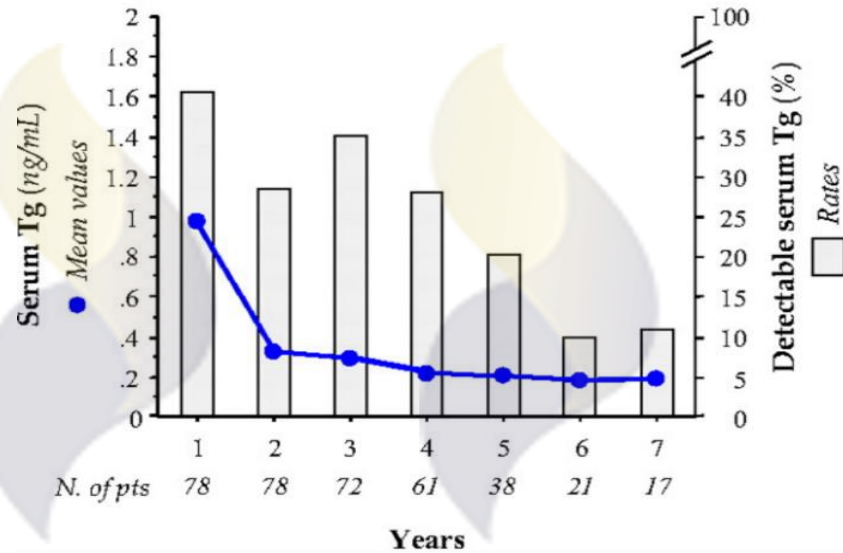


Tg will become a significantly less-useful marker in this scenario, and more sophisticated Tg reference intervals, mathematically normalized to TSH level and residual thyroid tissue tailored to individual patients, will have to be established. *Grebe SKG. Expert Rev Endocrinol Metab 2010*

# Thyroidectomy w/o I-131

Retrospective (selection bias)

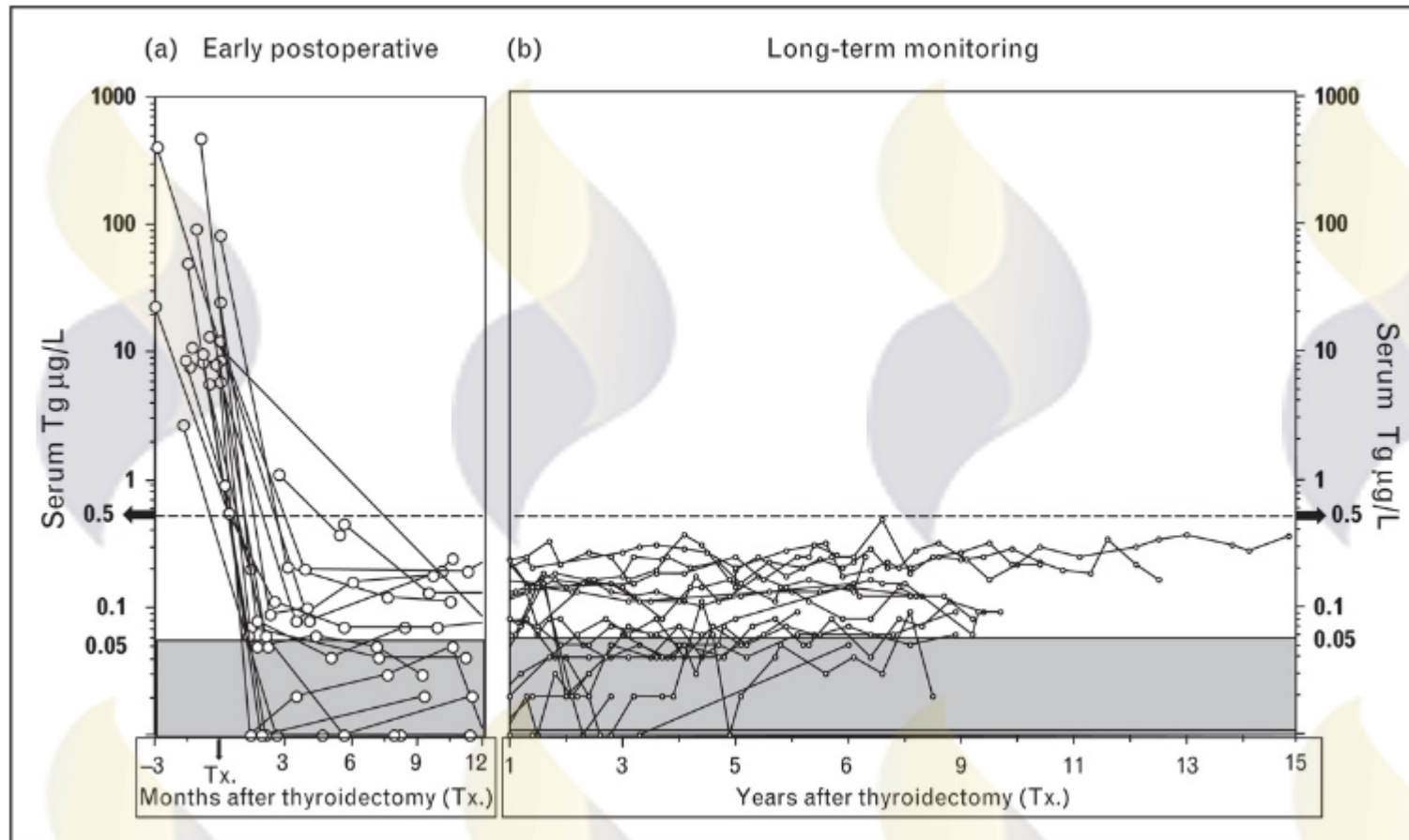
Tumor size (mm) RAI- **4** (0.5–25) vs RAI+ **12** (0.5–40)  $p < 0.0001$



- In most patients, serum Tg values spontaneously drop to undetectable levels within 5-7 yr after thyroidectomy.
- Thus, in later phases, Tg may be a valuable tool for follow-up.

Durante C et al. J Clin Endocrinol Metab 2012

# Thyroidectomy w/o I-131

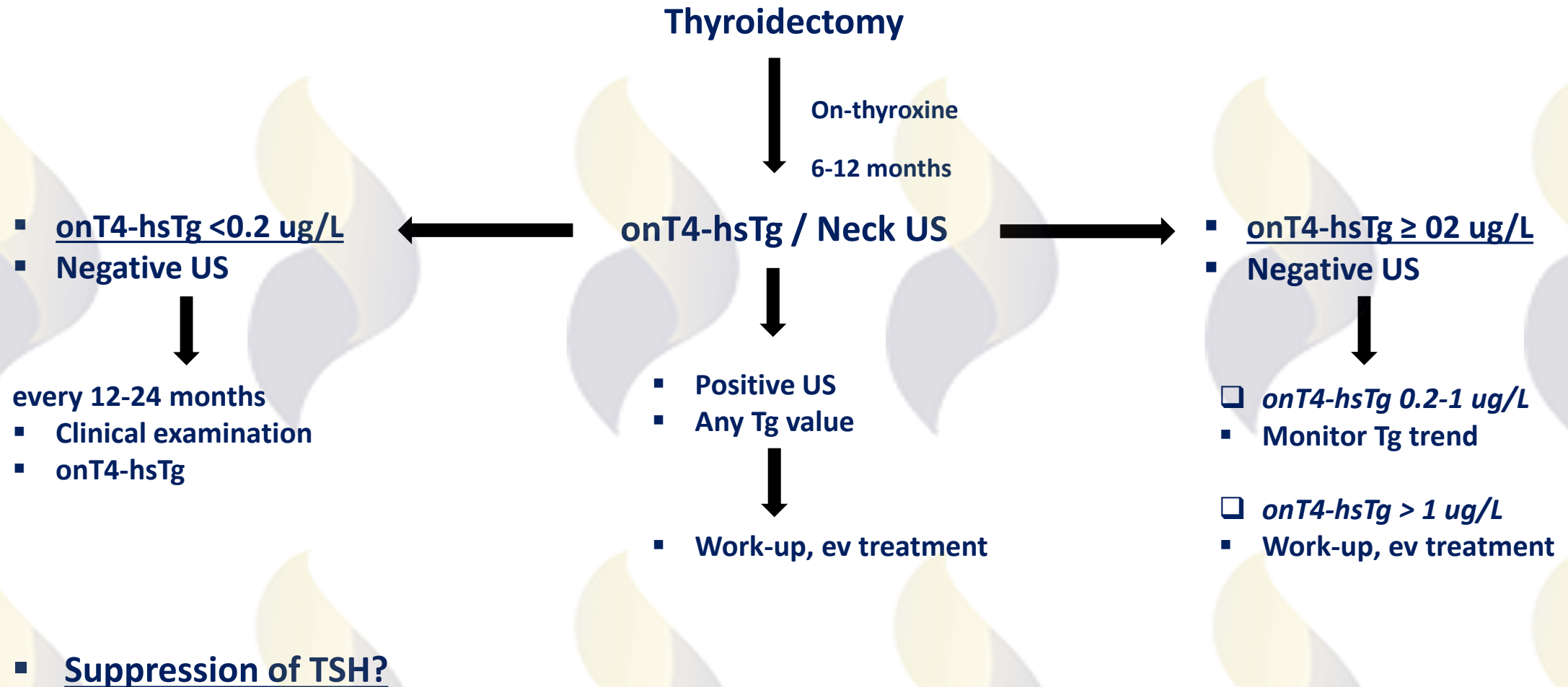


Spencer 2014

# Confounders

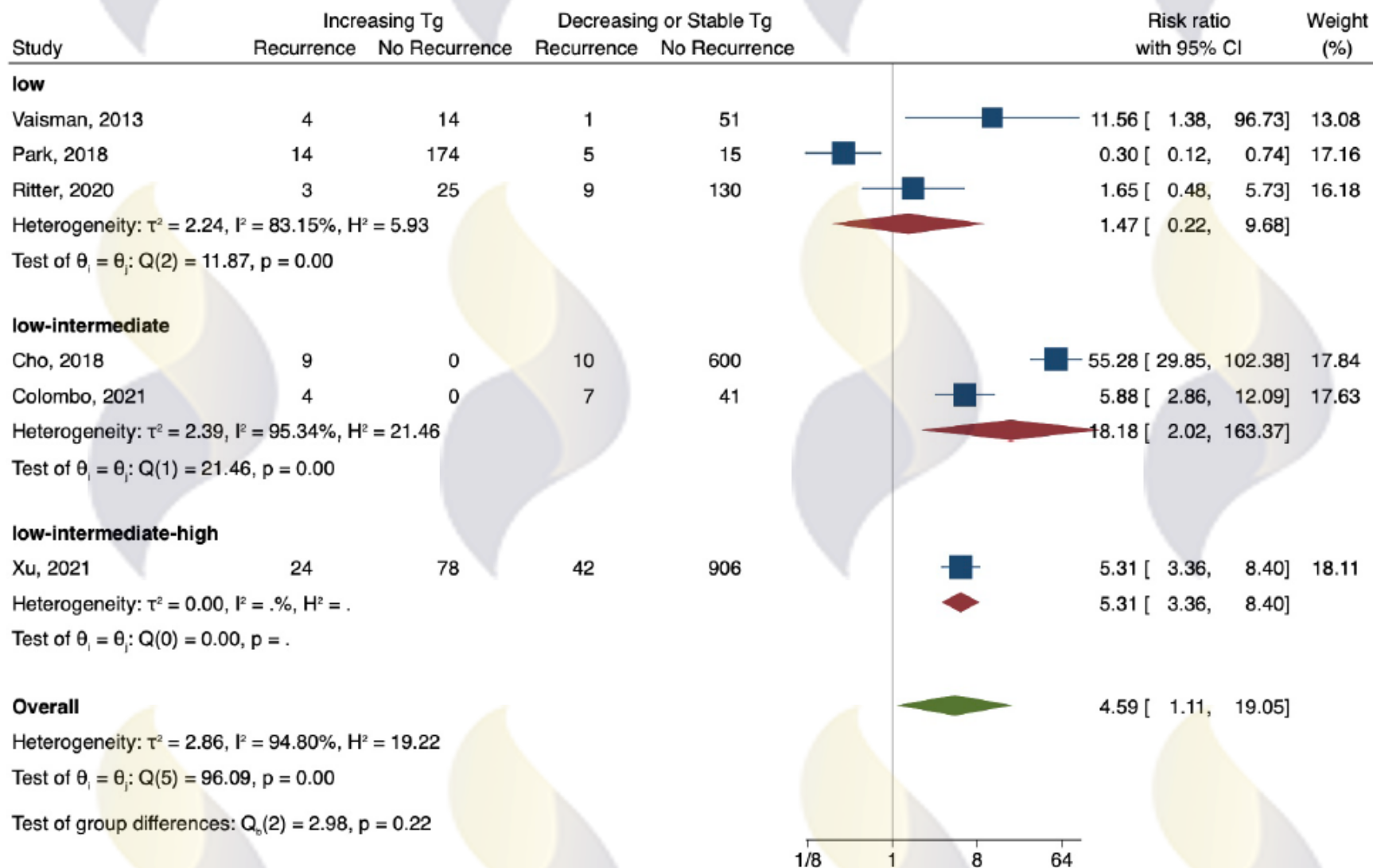
- the time elapsed since total thyroidectomy
- the amount of thyroid remnant
- the individual's risk of having metastasis
- the Tg cutoff used for analysis
- the TSH level at the time of Tg measurement.

# Thyroidectomy w/o I-131





# Lobectomy

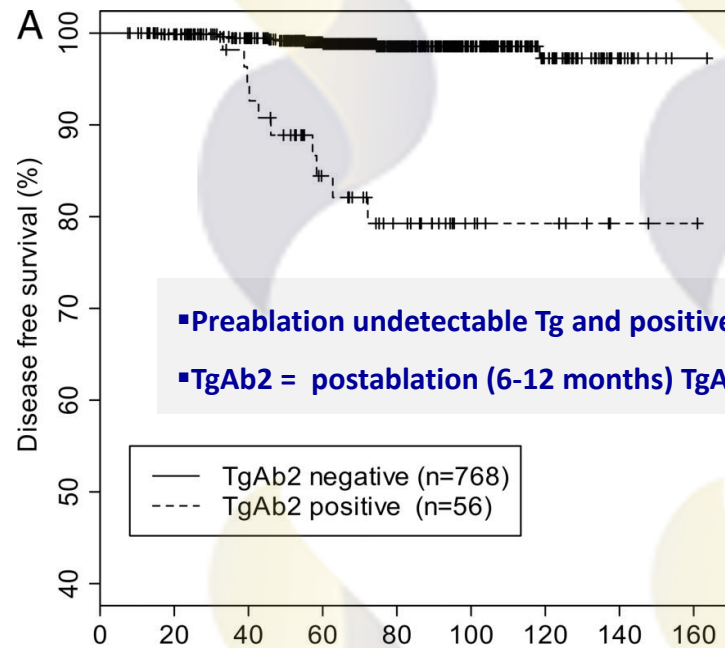




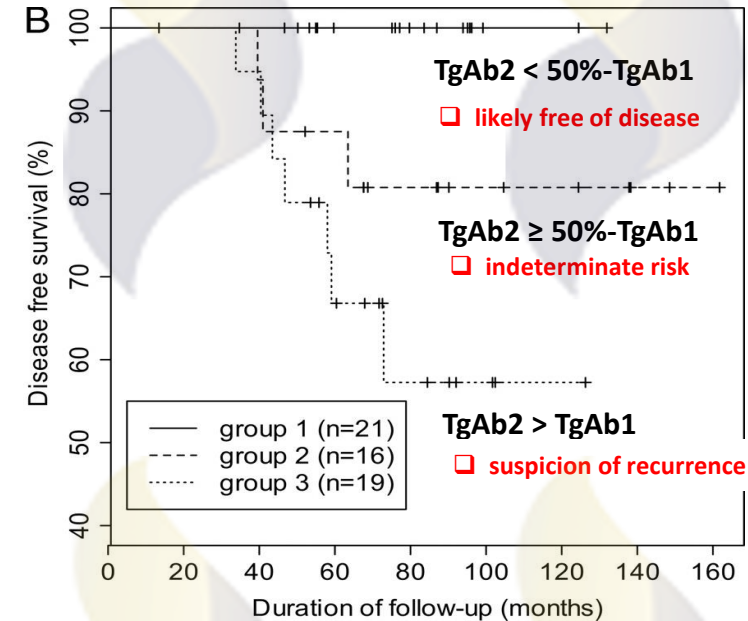
# TgAb-positive patients

- ❑ TgAb can be used as an imprecise surrogate marker of residual benign/malignant thyroid tissue.
- ❑ TgAb levels do not correlate with the tumor load: the trend is more important than the absolute level.

Verburg FA et al. Thyroid 2013



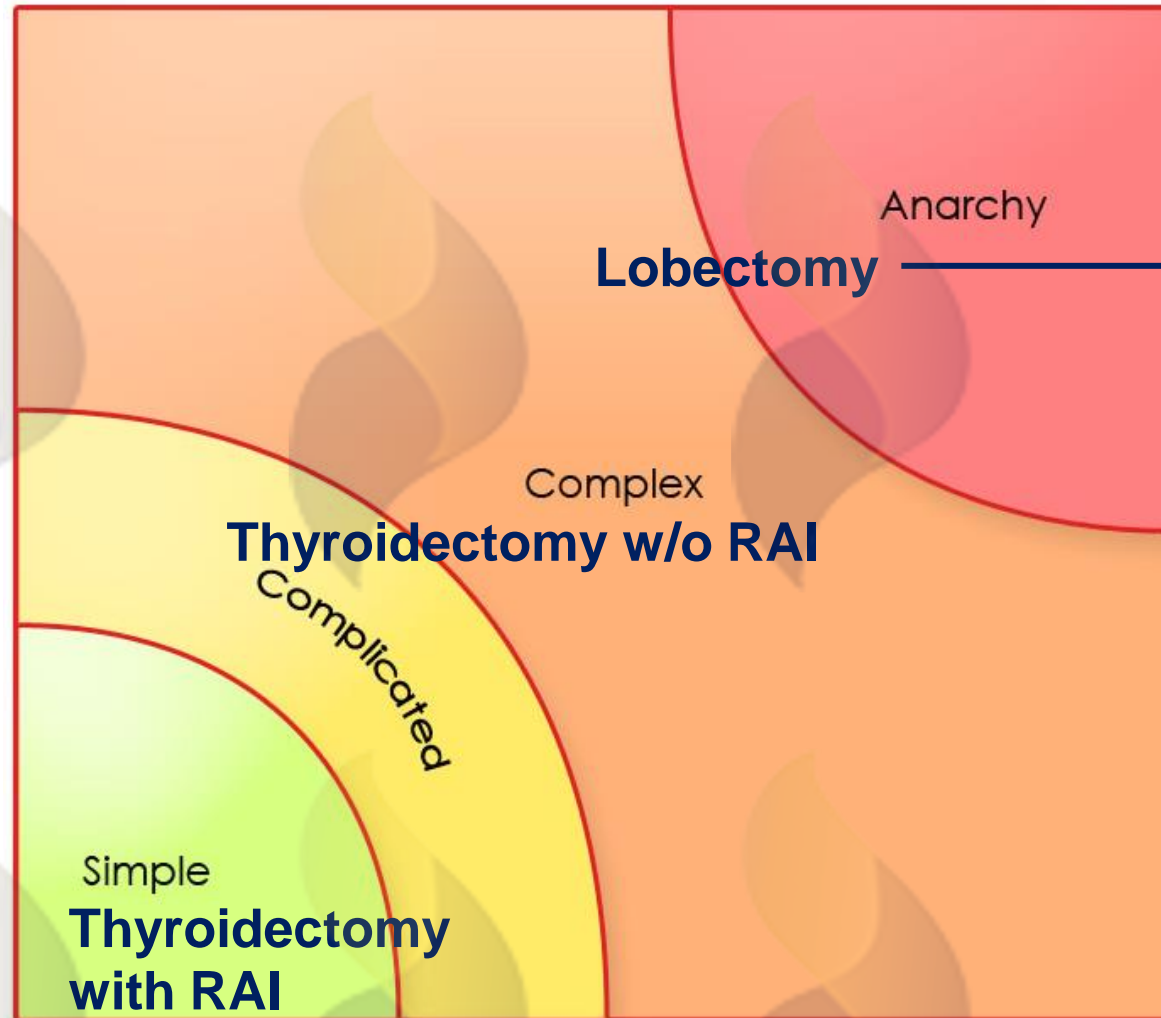
Kim et al. JCEM 2008



## Conclusions

- ✓ hsTg assays obviate the need for TSH-stimulated Tg testing in most DTC patients.
- ✓ Serum Tg measurement may be employed in patients treated with thyroidectomy without radioiodine, as decreasing Tg levels are reassuring. Accurate data are required, however, to better define the diagnostic performance, interpretation criteria, and pitfalls of both in these patients.
- ✓ The role of serum Tg (and TgAb) measurement is limited if any in patients treated with lobectomy alone.
- ✓ In TgAb-negative patients, the non-stimulated hsTg trend provides highly relevant prognostic information.
- ✓ In TgAb-positive patients, interferences preclude reliable Tg measurements. The kinetics of TgAb levels (measured with the same method over time) serves as a useful (surrogate) tumor marker.
- ✓ Future improvements in mass spectrometry Tg assays may solve the problem of TgAb interferences but currently Tg-MS should not be used in clinical practice due to suboptimal sensitivity..

## DTC follow-up and serum Tg measurement



- new markers?
- normalized thresholds?