Utility and Problems of Thyroglobulin (Tg) Assays for Detecting Recurrent Differentiated Thyroid Cancer (DTC)

Carole Spencer MT, Ph.D, FACB
Professor of Medicine
Department of Medicine
University of Southern California
email: cspencer@usc.edu
Faculty/Presenter Disclosure

• Faculty: Carole Spencer MT, Ph.D, FACB

• Relationships with commercial interests:
  – NONE
Thyroglobulin (Tg) – Biochemical DTC Tumor-Marker

- Pathophysiologic factors influencing serum Tg levels

- Technical Problems compromising Tg clinical utility:
  - Differences in Assay Specificity for Tg isoforms
  - Interferences
    - Tg autoantibodies (TgAb)
    - Human Anti-Mouse Antibodies (HAMA)
  - Assay Insensitivity

- Optimal Strategy for using Tg to detect recurrent DTC
Thyroglobulin (Tg) is tissue-specific not tumor-specific!

- Thyroid Tissue Mass (normal remnant + tumor)
- Thyroid Injury (FNAB/Surgery/RAI Rx.)
- Degree of TSH Receptor Stimulation
  - Specificity for abnormal tumor Tg isoforms
  - Propensity for interferences (TgAb & HAMA)
  - Functional Sensitivity of the assay

Serum Thyroglobulin (Tg) concentration

Assay characteristics influence the clinical utility of Tg testing for detecting disease
<table>
<thead>
<tr>
<th>Tg assay Classes</th>
<th>Principle</th>
<th>Turn-around Time</th>
<th>Strengths</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunometric Assay (IMA) (1990 - present)</td>
<td>Noncompetitive format uses monoclonal Abs (MAbs)</td>
<td>hours (can be automated)</td>
<td>Functional Sens. ~0.05 μg/L²</td>
<td>Prone to interference by TgAb (low) &amp; HAMA (high)</td>
</tr>
<tr>
<td>Use: TgAb- sera</td>
<td></td>
<td></td>
<td>MAb specificity limited epitope specificities to detect abnormal tumor TgS</td>
<td></td>
</tr>
<tr>
<td>Radioimmunoassay (RIA) (1973 - present)</td>
<td>Competitive format uses polyclonal Abs (PAbs)</td>
<td>~ 6 days (difficult to automate)</td>
<td>Functional Sens. ~0.5 μg/L²</td>
<td>Resists TgAb interference</td>
</tr>
<tr>
<td>Use: TgAb+ sera</td>
<td></td>
<td></td>
<td></td>
<td>No HAMA interference</td>
</tr>
<tr>
<td>Use: TgAb+ sera</td>
<td></td>
<td></td>
<td></td>
<td>PAb specificity broad epitope spec</td>
</tr>
<tr>
<td>Liquid Chromatog. Tandem Mass Spec. LC-MS/MS (2009 - present)</td>
<td>Preanalytic specimen preparation: reduction, alkylation trypsin digestion immunoaffinity concn. of target peptides before LC-MS/MS</td>
<td>? 1-2 day (extensive spec. prep. difficult to automate)</td>
<td>Functional Sens. ? 1-2 μg/L³</td>
<td>Should be no interferences from TgAb or HAMA (no clinical studies as yet)</td>
</tr>
<tr>
<td>Use: TgAb+ sera</td>
<td></td>
<td></td>
<td></td>
<td>? Polymorphic tumor Tg may not yield target peptides³</td>
</tr>
</tbody>
</table>

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Tg Immunometric Assays (IMA) May Detect Different Tg Isoforms

Monoclonal antibody specificity determines whether abnormal tumor-derived Tg isoforms are detected.
Tg Heterogeneity is One Reason why Methods Report 2-fold Differences in Tg
(51 Labs. using 10 methods measured the same 4 TgAb-negative sera A, B, C & D)
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- Technical Problems compromising Tg clinical utility:
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    - Tg autoantibodies (TgAb) (~30% DTC patients)
    - Human Anti-Mouse Antibodies (HAMA) (~0.5%)
  - Assay Insensitivity

- Optimal Strategy for using Tg to detect recurrent DTC
Risk of TgAb Interference Relates to the Class of Tg Method Used

Spencer et al. JCEM 96:1283, 2011
Clark et al. ACB 49:313, 2012
Clarke et al. JIM 60:1157, 2012
Currently a major problem is that TgAb assays do not reliably detect interfering TgAb!

Below analytical detection

<table>
<thead>
<tr>
<th>TgAb Assays</th>
<th>#1 Kronus Reference</th>
<th>#2 Roche</th>
<th>#3 Beckman</th>
<th>#4 Siemens</th>
</tr>
</thead>
<tbody>
<tr>
<td>% with Tg IMA &lt; 0.10 ng/L &amp; TgAb below MC cutoff</td>
<td>81</td>
<td>46</td>
<td>69</td>
<td></td>
</tr>
</tbody>
</table>

Spencer et al JCEM 96:1283, 2011
The TgAb trend can be used as a surrogate tumor marker because TgAb concentrations change in response to changes in Tg antigen sensed by the immune system.

Spencer JCEM 96:3615, 2011
Kim et al. JCEM 93:4683, 2008
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Optimal Strategy for using Tg to detect recurrent DTC
When it is important to detect low levels of analyte (i.e. TSH and Tg) assay sensitivity is often misrepresented for marketing purposes.

This descriptive nomenclature has necessitated establishing a scientific definition for assay “Functional Sensitivity” - designed to represent the lowest analyte concentration that can be reliably detected under conditions used for clinical practice.
Assay Functional Sensitivity (FS) – needs to be calculated according to strict protocol guidelines\textsuperscript{1},

For Tg assays, Functional Sensitivity is defined as the lowest Tg value that can be measured:

- with 20% between-run coefficient of variation (CV)
- in runs made over 6-12 months (typical clinical interval)
- using direct CRM-457 standardization
- using appropr. matrix (TgAb+ sera for RIA or LC-MS/MS)
- involving $\geq 2$ lots of all critical reagents and calibrators

\textsuperscript{1}Baloch et al Thyroid 13:60, 2003
Serum Tg μg/L

1st generation functional sensitivity

2nd generation functional sensitivity

3rd generation functional sensitivity

Euthyroid reference range

Mean ~ 13.0

RIA

Most IMAs

LC-MS/MS

Some IMAs

Some future assays

Spencer. Thyroid International 4:7, 2003
Why a Minimum of 2\textsuperscript{nd} Generation Functional Sensitivity 
(\(FS = 0.05 - 0.10 \mu g/L\)) 
is Optimal for 
Detecting Recurrent DTC
Post-Thyroidectomy Tg nadir (low TSH) is typically below 1.0 μg/L

Tg half-life ~ 3 days

intact thyroid reference range

Tg nadir after Tx. depends on:
- size of surgical remnant
- presence of residual tumor
- degree of TSH suppression

Whether Tg remains “detectable” post-op depends on the functional sensitivity of the assay!

- 21 low-risk PTC
- 2 months post Tx
- negative surg. path
- TSH < 0.05 mIU/L
- disease-free @ 5 yrs

Angell et al. Thyroid 19: S76, 2009
Not all Thyroid Tumors are Efficient Tg Producers

Differentiated Gene Expression Reduced in Papillary Tumors

Relative to normal tissue

Reference normal tissue

BRAF-wt

BRAF-V600E

Durante et al. JCEM 92:2840, 2007
A Pre-operative Serum Tg Measurement May Be Useful to Indicate the Efficiency of Tumor Tg Secretion

Tumor pathology vs. serum Tg

PTC nodule size vs. preop. serum Tg

Guarino et al Thyroid 15:1041, 2005
A Serum Tg Below 0.20 μg/L Does Not Eliminate the Risk of Lymph Node Metastases

Before percutaneous ethanol injection

End of follow-up (mean 38.4 months)

Serum Tg μg/L (suppressed TSH)

Functional sensitivity

Before percutaneous ethanol injection

End of follow-up

25%

92%

Hello et al JCEM 96:2750, 2011
Can recombinant human TSH (rhTSH) stimulation adequately compensate for 1\textsuperscript{st} generation Tg assay insensitivity?
2nd gen. Tg assays reveal the strong correlation between basal Tg & rhTSH-Tg

\[
\text{rhTSH-Tg} = 17.6 \times \text{basal Tg} - 12.9 \quad r = 0.72, p < 0.0001
\]

The rhTSH-stimulated Tg consensus cutoff of 2.0 ng/mL has a high NPV but only a low PPV for disease.

Between-method differences preclude applying a fixed Tg cutoff (ie. 2.0 ng/mL) to all Tg assays.

RhTSH-stimulation merely amplifies basal Tg approximately 10 x (analogous to TRH-stimulated TSH response).

rhTSH-Tg fold responses (rhTSH / basal Tg) are patient-specific and reflect the TSH sensitivity of the Tg-secreting tissue – which may be inversely related to the degree of tumor differentiation.

Blunted rhTSH-stim. Tg responses (rhTSH-Tg/basal Tg = < 2 fold) suggest interference by TgAb or HAMA.
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Optimal Patient-Specific Tg Parameter for Long-term Monitoring for Recurrences

Serum Basal Thyroglobulin (Tg) measured by 2\textsuperscript{nd} generation IMA without TSH stimulation (i.e. fixed L-T4 dose)

Changes in Tumor Mass

Efficiency of Tg secretion by that individual patient’s tumor

Baudin et al JCEM 88;1107, 2003
Tuttle et al EMCNA 37:419, 2008
Basal Tg Doubling-Time (Tg-DT) (TSH < 0.1 mIU/L) has Prognostic Value

Miyahuchi et al. Thyroid 21:707, 2011

Tg-DT = < 1 yr
Tg-DT = 1-3 yrs
Tg-DT = ≥ 3 yrs
Tg-DT = -21.6 yrs

Thyroglobulin (μg/L)

Survival (%)
There are three classes of Tg method – IMA, RIA and LC-MS/MS. Most laboratories use IMA methods which have the highest functional sensitivity potential, but most prone to interferences.

Tg IMA methods display 2-fold differences in numeric values because they detect different Tg isoforms. This necessitates that the same method be used for monitoring patients.

IMA measurements should be restricted to TgAb-negative sera to avoid TgAb interference causing falsely low values. RIA and LC-MS/MS have suboptimal Funct. Sens. – use only TgAb+ sera.

The quality of the TgAb test is critical. Inaccurate triaging of sera to IMA vs. an RIA or LC-MS/MS method misses disease:
- If TgAb is false +: Tg is measured by a less sensitive method
- If TgAb is false neg: false low/undetectable Tg IMA reported

Monitoring the basal Tg trend by 2nd gen. assay (FS < 0.10 μg/L) is more reliable for detecting recurrent DTC than a rhTSH-Tg.