Molecular Testing in the Evaluation of Thyroid Nodules

WCTC Advanced Thyroid Ultrasound Course
July 10, 2013

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Outline

• Thyroid Nodules
  – Strengths and Limitations of Cytopathology
  – Sensitivity, Specificity, NPV, PPV
  – Molecular Markers

Disclosure

Research support – Veracyte, Inc
The Problem

PTC incidence

Clinical Evaluation

What is the chance that he has cancer?
Specificity, PPV

What is the chance that a patient with low clinical suspicion does not have cancer?
Sensitivity, NPV
Approach to the Patient with Thyroid Nodules

TSH

Ultrasound

Fine-Needle Aspiration Biopsy (FNAB)
Thyroid Nodule Biopsy/Cytology

Benign

Malignant

Indeterminate

AUS/FLUS

Neoplasm (FN/SFN)

Suspicious
## Cytopathology Classification

<table>
<thead>
<tr>
<th>FNA</th>
<th>Risk of malignancy</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>2-10%</td>
<td>Monitor for growth</td>
</tr>
<tr>
<td>Malignant</td>
<td>93-100%</td>
<td>Surgery (neck US)</td>
</tr>
<tr>
<td>ND</td>
<td>5-50%</td>
<td>Repeat biopsy</td>
</tr>
</tbody>
</table>

**Indeterminate** 6-85%

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Baloch ZW, Diag Cytopath 36:425, 2008  
Yang J, Cancer, 2007  
Theoharis CGA, Thyroid, 2009  
Williams MD, Ann Surg Oncol, 2009  
Lanman R, Thyroid, 2011
Bethesda Cytopathology Classification

**FNA** | **Risk of malignancy** | **Action**
--- | --- | ---
Benign | 2-10% | Monitor for growth
Malignant | 93-100% | Surgery (neck US)
ND | 5-50% | Repeat biopsy

**Indeterminate**

**Suspicious** | 50-80% | Surgery (neck US)
**Neoplasmin** | 15-30% | Surgery vs scintigraphy
**AUS/FLUS** | 6-48% | Repeat biopsy vs surgery

Baloch ZW, Diag Cytopath 36:425, 2008
Yang J, Cancer, 2007
Theoharis CGA, Thyroid, 2009
Williams MD, Ann Surg Oncol, 2009
Lanman R, Thyroid, 2011
How reproducible are the Bethesda Classification Categories?

Interobserver variability

Cibas ES, Ann Int Med, 2013
How reproducible are the Bethesda Classification Categories?

Intraobserver variability

![Bar chart showing the proportion of identical cytopathology diagnoses for three cytopathology panel members.]

- CP1: 83.3% with p = 0.002
- CP2: 78.0%
- CP3: 60.0%

Cibas ES, Ann Int Med, 2013
Which patient(s) should I send for surgery?

Which patient(s) don’t need surgery?
Definitions

‘gold standard’
(histopathology)

Positive

Negative

Test result

Positive

True positive

False positive
(type I error)

Negative

False Negative
(type II error)

True negative

Sensitivity

Specificity

Cytology benign: 93-98% sensitivity, 90-98% NPV

Cytology malignant: 97-100% specificity, 99-100% PPV

Sangalli, Cytopath, 2006; Alexander, Cancer 2007;
Theoharis, Thyroid 2009; Renshaw, Cancer Cytopath, 2010
Which patient(s) with indeterminate cytology should I send for surgery and what is the extent of the surgery?

**BRAFV600E:** High specificity (100%) and PPV (100%)

*Cytology malignant:* 97-100% specificity, 99-100% PPV

Which patient(s) with indeterminate cytology can avoid surgery?

**BRAFV600E:** Low sensitivity (32%) and NPV (73%)

*Cytology benign:* 93-98% sensitivity, 90-98% NPV

Rossi M, J Clin Endo Metab, 2012
Molecular Markers

- Diagnosis
- Prognosis
- Therapeutic Targets
- Pathogenesis

- Genetics
- Genomics
- Proteomics
- Metabolomics
- Candidate
- Discovery
- Nodule
- Peripheral
Candidate Approach
Proteomics
Genetics
**Galectin-3**

**Immunocytochemistry - Prospective studies**

**Indeterminate biopsies**

<table>
<thead>
<tr>
<th>Final pathology</th>
<th>Galectin-3 positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>3/60 (5%)</td>
</tr>
<tr>
<td>Malignant</td>
<td>13/13 (100%)</td>
</tr>
</tbody>
</table>

(sensitivity 100%, NPV 100%, specificity 90%, PPV 81%)


**Multicenter, prospective, blinded trial**

432 nodules

Sensitivity 78%, NPV 91%, Specificity 93%, PPV 82%

Thyroid Tumor Signaling MAPK

Papillary Thyroid Cancer
- RET/PTC 15%
- Ras 10-15%

Follicular Thyroid Cancer
- Pax8-PPARγ 20-40%
- BRAF 40-60%

Tumor growth, invasion, dedifferentiation
FNA Prospective, Blinded Molecular Analysis

328 patients, 470 nodules

Univ Cincinnati, Univ Colorado

Ret/PTC1, Ret/PTC3, Ras, BRAF, Pax8/PPARγ

52 indeterminate cytology (40% malignant)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>71%</td>
</tr>
<tr>
<td>NPV</td>
<td>84%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
</tr>
<tr>
<td>PPV</td>
<td>100%</td>
</tr>
</tbody>
</table>

FNA Genetic Analysis

479 patients, 513 indeterminate biopsies (247 AUS/FLUS, 214 FN/SFN, 52 suspicious)

<table>
<thead>
<tr>
<th>Malignant (121)</th>
<th>Benign (392)</th>
<th>24% malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ras (50)</td>
<td>Negative (383)</td>
<td>Sensitivity 61%</td>
</tr>
<tr>
<td>Negative (47)</td>
<td>Ras (9)</td>
<td>NPV 89%</td>
</tr>
<tr>
<td>BRAF (17)</td>
<td></td>
<td>Specificity 98%</td>
</tr>
<tr>
<td>Pax8-PPAR (4)</td>
<td></td>
<td>PPV 89%</td>
</tr>
<tr>
<td>RET/PTC1 (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Discovery Approach
Genomic

Chudova D, JCEM, 2010
Alexander EK, NEJM, 2012
Hypothesis
A molecular classifier can be developed to categorize indeterminate nodules with a high sensitivity and negative predictive value (NPV)

Methods
Train and validate a gene expression classifier (GEC) against the ‘gold standard’ of histopathology by experts (Virginia LiVolsi and Juan Rosai)
Complex Biology of Thyroid Neoplasm Subtypes Requires High-dimensionality Genomic Data

A multidimensional algorithm required to separate complex data sets

Whole Transcriptome approach using microarray technology

Molecular Classifier Trained and Validated to Distinguish Benign vs. Suspicious Nodules

Methods
Gene Signature Cassettes
167 genes

Benign vs Suspicious
142 genes

Parathyroid
7 genes

Hurthle Cell
27 genes

Renal Cell Carcinoma
7 genes

Medullary Thyroid Ca
5 genes
Calcitonin-related polypeptide
CEA-related 5
Secretogranin III
Sodium channel 9
Synaptotagmin 4

Breast Carcinoma
5 genes

Melanoma
6 genes
Results

Training the final Molecular Classifier

Validation Set (n=265)

AUC = 0.95

Training

Final Locked Molecular Classifier

n = 426
Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology


Prospective, Blinded, Multicenter Study

3,789 patients - 4,812 nodules
49 sites (70% community)
265 cytologically indeterminate nodules
<table>
<thead>
<tr>
<th>Test Type</th>
<th>Sensitivity</th>
<th>NPV</th>
<th>Group</th>
<th>Sens</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>92%</td>
<td></td>
<td>AUS/FLUS (129)</td>
<td>90%</td>
<td>95%</td>
</tr>
<tr>
<td>Specificity</td>
<td>52%</td>
<td></td>
<td>Neoplasm (81)</td>
<td>90%</td>
<td>94%</td>
</tr>
<tr>
<td>PPV</td>
<td>47%</td>
<td></td>
<td>Suspicious (55)</td>
<td>94%</td>
<td>85%</td>
</tr>
</tbody>
</table>

7 false negatives (6 low follicular content)

Which patient(s) should I send for surgery and what is the extent of the surgery?

High specificity and PPV

Which patient(s) don't need surgery?

High sensitivity and NPV
### FNA Comparative Analyses

<table>
<thead>
<tr>
<th></th>
<th>Genomic (Afirma)</th>
<th>Proteomic (Galectin-3)</th>
<th>Genetic (miRInform and Quest)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>92%</td>
<td>78%</td>
<td>61%</td>
</tr>
<tr>
<td>NPV</td>
<td>93%</td>
<td>91%</td>
<td>89%</td>
</tr>
<tr>
<td>Specificity</td>
<td>52%</td>
<td>93%</td>
<td>98%</td>
</tr>
<tr>
<td>PPV</td>
<td>47%</td>
<td>82%</td>
<td>89%</td>
</tr>
</tbody>
</table>

**Nikiforov Y, et al**  
*JCEM, 2011*

**Genomic**  
*Afirma*

**Proteomic**  
*Galectin-3*

**Genetic**  
*miRInform and Quest*

**Diagnostic hemi- or total thyroidectomy?**

*Cytology benign*: 93-98% sensitivity, 90-98% NPV  
*Cytology malignant*: 97-100% specificity, 99-100% PPV

**Benign Afirma GEC**

- Monitor as benign

**Genetic mutation positive**

- Therapeutic thyroidectomy

**Alexander EK, et al**  
*NEJM, 2012*

**Bartolazzi A, et al**  
*Lancet Oncol, 2008*

**Nikiforov Y, et al**  
*JCEM, 2011*
Practical Clinical Experience

339 patients (Oct 2010-Jan 2013)
Indeterminate FNAB
Afirma Gene Expression Classifier

<table>
<thead>
<tr>
<th>Afirma GEC</th>
<th>Surgery recommended</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>174 (51%)</td>
<td>4 (2%)</td>
</tr>
<tr>
<td>Suspicious</td>
<td>148 (44%)</td>
<td>141 (95%)</td>
</tr>
<tr>
<td>No result</td>
<td>17 (5%)</td>
<td>4 (34%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/11* (9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53/121 (44%)</td>
</tr>
</tbody>
</table>

* - mean FU 8.5 mo, one microPTC in 3.2 cm nodule

163 benign GEC avoided surgery

Alexander EK, et al, Submitted
**Goals:** (1) Reduce unnecessary surgery  
(2) Use the least aggressive surgery to achieve therapeutic goals  
(3) Try to make first surgery the last surgery

![Diagram of thyroid nodule management](image)

Goals of Cancer Management

1) Identify and appropriately treat malignancy
2) Avoid excessive testing and therapy for benign disease

How do we define malignancy/cancer?

Histopathology (‘gold standard’)

Behavior

21st Century Diagnosis of Cancer
‘A tumor that will likely threaten one’s life or well-being if not treated’

Molecular Prognostics
Acknowledgements

University of Colorado
Joshua Klopper
Rebecca Schwegge
Jen Tryggestad
Katie Weber
Ken Shroyer
Sherif Said

University of Pittsburgh
Yuri Nikiforov

Erik Alexander
Ed Cibas
Rick Kloos
Susan Mandel
Martha Zeiger
Virginia LiVolso
Stephen Raab
Juan Rosai

Veracyte
Rick Lanman
Giulia Kennedy
Lyssa Friedman

Mary Rossick Kern and Jerome H Kern Endowment