Thyroid Nodules: Practical and Molecular Approaches to a Common Problem

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University of Colorado, School of Medicine
University of Colorado Cancer Center
Surgery Grand Rounds
May 9, 2011
Disclosure

Research support - Veracyte, Inc
Patient

A 52 yo male orthopedic surgeon was self-referred for thyroid nodule and indeterminate FNA biopsy

Member of scoliosis society (800-1000), lots of fluoroscopy, 3 members died of aggressive thyroid cancer

Ultrasound – 1.5 cm L thyroid nodule with microcalcifications

TSH 1.6 mU/L

Biopsy – FLUS/atypia

Recommended surgery
Thyroid Nodules

Mazzaferri, NEJM, 1993
Thyroid Nodules
what we think we know

• Thyroid nodules are common
• Thyroid cancer is not
• Biopsy the nodule
• Malignant = surgery
• Benign = leave it alone
• Indeterminate = Damn
What percentage of thyroid nodules are malignant?

A) 2%
B) 5%
C) 10%
D) 15%
E) 20%
F) 50%
What percentage of thyroid nodules are malignant?

“5-10%”??

<table>
<thead>
<tr>
<th>Paper</th>
<th>n</th>
<th>% malig</th>
<th>surgery</th>
<th>% malig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yassa Cancer, 2007</td>
<td>3589</td>
<td>14%</td>
<td>1242</td>
<td>56%</td>
</tr>
<tr>
<td>Yang Cancer, 2007</td>
<td>3207</td>
<td>15%</td>
<td>378</td>
<td>53%</td>
</tr>
<tr>
<td>Theoharis Thyroid, 2009</td>
<td>4703</td>
<td>16%</td>
<td>1052</td>
<td>46%</td>
</tr>
</tbody>
</table>
Definitions

‘gold standard’
(histopathology)

Positive  Negative

Positive  True positive  False positive  $\rightarrow$ PPV

Negative  False Negative  True negative  $\rightarrow$ NPV

Test result

PPV  Proportion of patients with a positive test result who are correctly diagnosed

NPV  Proportion of patients with a negative test result who are correctly diagnosed

Sensitivity  Specificity
Clinical Evaluation

Positive Predictive Value (PPV) - good (70-75%)

Negative Predictive Value (NPV) - unacceptable (85%)

High clinical suspicion
Rapid tumor growth
Very firm nodule (rock hard)
Fixation to adjacent structures
Vocal cord paresis
Enlarged regional lymph nodes
Family history of PTC or MEN 2
Distant metastases
Approach to the Patient with Thyroid Nodules

- TSH
- Free T4, Free T3
- Thyroid antibodies
- Thyroglobulin
- Calcitonin
- Ultrasound
- CT scan
- Nuclear Medicine ($^{123}$I, $^{99m}$Tc)
- $^{18}$FDG-PET
- Core biopsy
- Fine-Needle Aspiration Biopsy (FNAB)
**TSH and Thyroid Neoplasms**

<table>
<thead>
<tr>
<th>DTC stage</th>
<th>mean TSH (mU/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/II (204)</td>
<td>2.1 ± 0.24</td>
<td>0.002</td>
</tr>
<tr>
<td>III/IV (35)</td>
<td>4.9 ± 1.59</td>
<td></td>
</tr>
</tbody>
</table>

Haymart MR, JCEM 93:809, 2008

<table>
<thead>
<tr>
<th>Invasion</th>
<th>mean TSH (mU/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>yes</td>
<td>5.6 ± 2.9</td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>2.0 ± 0.2</td>
<td>0.004</td>
</tr>
</tbody>
</table>

p = 0.004 (multivariate)

Haymart MR, Clin Endo 71:434, 2009

**Graph:**
- X-axis: TSH (mU/L)
- Y-axis: Prevalence (%)
- Bars for different TSH ranges (0-0.4, 0.4-0.9, 1.0-1.7, 1.8-5.5, >5.5)

Kumar H, Thyroid 9:1105, 1999
Boelaert K, JCEM 91:4295, 2006
PET and Thyroid Nodules

Systematic Review

18 studies - 55,160 patients
571 (1%) focal uptake in thyroid

33% malignant

NPV high (90-95%)
PPV poor (0-50%)

Shie P, Nuc Med Comm 30:742, 2009
Approach to the Patient with Thyroid Nodules

TSH

Ultrasound

Fine-Needle Aspiration Biopsy (FNAB)
Thyroid Ultrasound

R2 Thyroid sonography should be performed in all patients with known or suspected thyroid nodules (A)

ATA guidelines, Cooper DS, Thyroid, 2009

- Is the palpable abnormality a thyroid nodule?
- Are other nodules present?
- Size(s)?
- Suspicious features?
- > 50% cystic?
- Posterior?
- Associated abnormal lymph nodes?
US features

benign

Pseudonodules

benign

Colloid nodules

Spongiform ('leave me alone lesion')
US features
indeterminate

Hypoechoic
Sharp margins

Isoechoic
Internal vascularity

Mixed echogenicity
Taller than wide
US features
malignant

Irreg margins
Internal echoes

Abnormal LN

Marked hypoechoic
Irregular margins

Marked hypoechoic
Irregular margins

Taller-than-wide

Marked hypoechoic
Irregular margins

Taller-than-wide
52 yo orthopedic surgeon
Outside US - L 1.5 cm nodule, microcalcifications
Which nodule(s) should I biopsy?

Which nodule(s) don’t need a biopsy?
## Predictive Value of US features

### Features of malignancy (PPV)

<table>
<thead>
<tr>
<th>Feature</th>
<th>PPV</th>
<th>Source</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcalcifications</td>
<td>78%</td>
<td>Moon, 2008</td>
<td>(n=849)</td>
</tr>
<tr>
<td>Irregular margins</td>
<td>81%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Taller than wide</td>
<td>77%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoechoicogenicity</td>
<td>50%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Malignancy rate</strong></td>
<td>42%</td>
<td>Frates, 2006</td>
<td>(n=865)</td>
</tr>
</tbody>
</table>

### Features of benignity (NPV)

<table>
<thead>
<tr>
<th>Feature</th>
<th>PPV</th>
<th>NPV</th>
<th>Source</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spongiform</td>
<td>98%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cystic</td>
<td>91%</td>
<td>98%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoechoic</td>
<td>87%</td>
<td>91%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biopsy</td>
<td>Indication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------------</td>
<td>----------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>High risk (FH, radiation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Abnormal LN (Bx LN)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\geq 1) cm</td>
<td>Microcalcifications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Solid nodule (esp hypoechoic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\geq 1.5) cm</td>
<td>Solid (iso or hyperechoic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixed cystic solid (suspicious features)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\geq 2) cm</td>
<td>Mixed (no suspicious features)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spongiform</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No biopsy</td>
<td>Pure cyst</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(r/o malignancy)</td>
<td>Spongiform?</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Revised ATA guidelines, Thyroid 2009
Thyroid Nodule Biopsy
Thyroid FNA Cytology

Benign  Malignant  Indeterminate
<table>
<thead>
<tr>
<th>NCI classification</th>
<th>Alternate classification</th>
<th>% malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td></td>
<td>&lt;1%</td>
</tr>
<tr>
<td>FLUS (indeterminate)</td>
<td>Atypia</td>
<td>5-10%</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>r/o neoplasm</td>
<td>5-10%</td>
</tr>
<tr>
<td></td>
<td>Follicular neoplasm</td>
<td>20-30%</td>
</tr>
<tr>
<td></td>
<td>Hurthle neoplasm</td>
<td>20-30%</td>
</tr>
<tr>
<td>Suspicious</td>
<td></td>
<td>50-75%</td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
<td>98-100%</td>
</tr>
<tr>
<td>Nondiagnostic</td>
<td>Unsatisfactory</td>
<td></td>
</tr>
</tbody>
</table>

Baloch ZW, Diag Cytopath 36:425, 2008
# NCI Thyroid FNA

## State of the Science Conference

<table>
<thead>
<tr>
<th>NCI classification</th>
<th>Alternate classification</th>
<th>% malignant</th>
<th>% malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>&lt;1%</td>
<td>0.3-10%</td>
<td></td>
</tr>
<tr>
<td>FLUS (indeterminate)</td>
<td>Atypia</td>
<td>5-10%</td>
<td>7-48%</td>
</tr>
<tr>
<td></td>
<td>r/o neoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neoplasm</td>
<td>Follicular neoplasm</td>
<td>20-30%</td>
<td>21-34%</td>
</tr>
<tr>
<td></td>
<td>Hurthle neoplasm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspicious</td>
<td>50-75%</td>
<td>52-80%</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>98-100%</td>
<td>96-98%</td>
<td></td>
</tr>
<tr>
<td>Nondiagnostic</td>
<td>Unsatisfactory</td>
<td>5-25%</td>
<td></td>
</tr>
</tbody>
</table>

Baloch ZW, Diag Cytopath 36:425, 2008  
Theoharis CGA, Thyroid 19:1215, 2009  
Williams MD, Ann Surg Oncol 16:3146, 2009  
## What to do with a biopsy report?

<table>
<thead>
<tr>
<th>Classification</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>Monitor for growth</td>
</tr>
<tr>
<td>Malignant</td>
<td>Surgery (neck US)</td>
</tr>
<tr>
<td>Suspicious</td>
<td>Surgery (neck US)</td>
</tr>
<tr>
<td>Nondiagnostic</td>
<td>Repeat biopsy (cystic vs solid)</td>
</tr>
</tbody>
</table>

**FLUS/indeterminate**

- reassuring features: Repeat biopsy
- concerning features: Surgery

**Neoplasm**

- consider nuclear imaging
52 yo male orthopedic surgeon

Outside FNA - FLUS/atypia
Dr Raab (UCH/UCD) - probably benign, suboptimal
UCD US-guided biopsy
On-site cytopathology
## Prospective, Multicenter Study

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Specialty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erik Alexander, PI</td>
<td>Harvard / Brigham &amp; Women’s</td>
<td>Endocrinology</td>
</tr>
<tr>
<td>Bryan Haugen, PI</td>
<td>University Colorado</td>
<td>Endocrinology</td>
</tr>
<tr>
<td>Edmund Cibas</td>
<td>Harvard / Brigham &amp; Women’s</td>
<td>Cytopathology</td>
</tr>
<tr>
<td>Richard Kloos</td>
<td>Ohio State University</td>
<td>Endocrinology</td>
</tr>
<tr>
<td>Susan Mandel</td>
<td>University Pennsylvania</td>
<td>Endocrinology</td>
</tr>
<tr>
<td>Martha Zeiger</td>
<td>Johns Hopkins University</td>
<td>Endocrine Surgery</td>
</tr>
<tr>
<td>Electron Kebebew</td>
<td>UC San Francisco</td>
<td>Endocrine Surgery</td>
</tr>
<tr>
<td>Virginia LiVolsi</td>
<td>University Pennsylvania</td>
<td>Anatomic Pathology</td>
</tr>
<tr>
<td>Stephen Raab</td>
<td>University Colorado</td>
<td>Cytopathology</td>
</tr>
<tr>
<td>Juan Rosai</td>
<td>Centro Diagnostico Italiano</td>
<td>Anatomic Pathology</td>
</tr>
</tbody>
</table>

4,000 patients/49 sites  
Kaiser (Bill Georgitis)  
Full accrual May-June 2010  
142 gene set (RNA/DNA-based)  

Supported by Veracyte, Inc
Local Histopathology for Each Local Cytology Category (N=376)

- 30% Malignant by Local Histopathology
- 11% Benign (n=81)
- 30% Indeterminant* (n=223)
- 97% Malignant (n=64)
- 13% Nondiagnostic (n=8)

*Indeterminate=FLUS/Atypia, Follicular or Hürthle Cell Neoplasm, and Suspicious for Malignancy.
Concordance Between Local and Central Cytopathology Diagnoses

4 category (B/I/M/ND):
- Agree: 67%
- Disagree: 33%

6 category (Bethesda):
- Agree: 52%
- Disagree: 48%
Indeterminate biopsy?

High Variability Between Local Indeterminate and Expert Panelist Cytology

Local Indeterminate Cytology
- Atypical, n=36

Expert Cytology
- Benign: 36%
- Atypical: 33%
- Follicular or Hürthle cell neoplasm: 25%
- Suspicious for malignancy: 16%
- Malignant: 41%
- Nondiagnostic: 8%

Haugen BR, et al, 14th International Thyroid Congress, 2010
Local vs Expert Cytopathology
Who is more confident?

- Local Cytology (NPV = 94%)
- Expert Cytology (NPV = 96%)
## Local vs Expert Cytopathology

### Who’s Right?

#### Expert cytopathologist

- Confident
- High NPV

#### Local Cytology (NPV = 94%)

<table>
<thead>
<tr>
<th>Category</th>
<th>Expert Cytology</th>
<th>Local Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>96%</td>
<td>81%</td>
</tr>
<tr>
<td>FLUS/Atypia</td>
<td>27%</td>
<td>19%</td>
</tr>
<tr>
<td>FN/HN</td>
<td>17%</td>
<td>24%</td>
</tr>
<tr>
<td>Suspicious</td>
<td>88%</td>
<td>73%</td>
</tr>
<tr>
<td>Malignant</td>
<td>0%</td>
<td>25%</td>
</tr>
<tr>
<td>ND</td>
<td>0%</td>
<td>4%</td>
</tr>
</tbody>
</table>

[Bar chart showing comparison between expert and local cytology results.

- **Local Cytology (NPV = 94%)**
- **Expert Cytology (NPV = 96%)**

---

Note: NPV: Negative Predictive Value

---

If you have any questions or need further clarification, feel free to ask!
Which patient(s) should I send for surgery?

Which patient(s) don't need surgery?
Thyroid Nodule Classification

Goals:
- Diagnose and treat significant disease
- Limit excessive testing and treatment
Thyroid Tumor Signaling MAPK

Molecular Markers

Papillary Thyroid Cancer

RET/PTC 15%

Ras 10-15%

BRAF 40-60%

MEK-ERK

Follicular Thyroid Cancer

Pax8-PPARγ 20-40%

Tumor growth, invasion, dedifferentiation
FNA Prospective Molecular Analysis

328 patients, 470 nodules

Univ Cincinnati, Univ Colorado Denver

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

<table>
<thead>
<tr>
<th>Cytology and Molecular Analysis</th>
<th>Cancer Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indeterminate Cytology</td>
<td>40%</td>
</tr>
<tr>
<td>Indeterminate Cytology, Pos Mutation</td>
<td>100%</td>
</tr>
<tr>
<td>Indeterminate Cytology, Neg Mutation</td>
<td>16%</td>
</tr>
<tr>
<td>Negative Cytology</td>
<td>2.1%</td>
</tr>
<tr>
<td>Negative Cytology, Neg Mutation</td>
<td>0.9%</td>
</tr>
</tbody>
</table>

Available Molecular Markers

Inform (Asuragen)
BRAF, Ras, RET/PTC, Pax8-PPARγ

Cleveland Clinic
Blood TSHR mRNA

Afirma (Veracyte)
Exon array
The Evolution of Molecular Analysis:

**Recent Studies:**

- **BRAF V600E**
- **PAX8: PPARγ Translocation**
- **Galectin-3**
- **SNP: 9q22.33, and 14q13.3**
- **RAS, RET/PTC Oncogenes**

**Ongoing Studies:**

- High **POSITIVE** Predictive Value
- High **NEGATIVE** Predictive Value
Development of a novel molecular classifier to accurately identify benign thyroid nodules in patients with indeterminate FNA cytology


14th International Thyroid Congress
Sept 11-16, 2010
Hypothesis

A molecular classifier can be developed to categorize indeterminate nodules with a high negative predictive value (NPV)

Methods

Train and validate a molecular classifier against the ‘gold standard’ of histopathology by experts (Virginia LiVolsi and Juan Rosai)
Complex Biology of Thyroid Subtypes Required High-dimensionality Genomic Data

Whole Transcriptome approach using microarray technology

Complex algorithm required to separate classes in multi-dimensional space

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

Methods

LOCKED FINAL CLASSIFIER FOR VALIDATION

Chudova D, JCEM 2010 NPV 96% (n=24)

n = 426

Final Training

Train 2

Train 1

Concept

Test 1

Test 2

Test 3

Validation (n=43)
Molecular Classifier Utilizes 142 Genes in Multiple Biological Pathways

- Signaling: 31%
- Development: 19%
- Cell Cycle: 12%
- Adhesion: 10%
- Immune Response: 8%
- Transcription: 6%
- Apoptosis: 4%
- Migration: 3%
- Inflammation: 3%
Results

Training the final Molecular Classifier

**AUC = 0.95**

Validation Set (n=43)

n = 426
### Independent Validation of Molecular Classifier by Expert Consensus Histopathologic Diagnosis

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical pathology, indeterminate cytology (n=43)</td>
<td>95%</td>
<td>63%</td>
<td>57%</td>
<td>96%</td>
</tr>
<tr>
<td>Surgical pathology, all cytology (N=66)</td>
<td>97%</td>
<td>64%</td>
<td>58%</td>
<td>98%</td>
</tr>
</tbody>
</table>
# Thyroid FNA

## Cytopathology

<table>
<thead>
<tr>
<th>NCI classification</th>
<th>% malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>0.3-10%</td>
</tr>
<tr>
<td>FLUS</td>
<td>7-48%</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>21-34%</td>
</tr>
<tr>
<td>Suspicious</td>
<td>52-80%</td>
</tr>
<tr>
<td>Malignant</td>
<td>96-98%</td>
</tr>
<tr>
<td>Nondiagnostic</td>
<td>5-25%</td>
</tr>
</tbody>
</table>

- Theoharis CGA, *Thyroid* 19:1215, 2009
Thyroid FNA
Cytopathology

NCI classification

- Benign: 4% 0.3-10%
- FLUS: 7-48%
- Neoplasm: 21-34%
- Suspicious: 52-80%
- Malignant: 96-98%
- Nondiagnostic: 5-25%

Molecular classifier

Baloch ZW, Diag Cytopath 36:425, 2008
Theoharis CGA, Thyroid 19:1215, 2009
Williams MD, Ann Surg Oncol 16:3146, 2009
• Measure TSH (A)
• US (neck) in all patients with suspected nodule (A)
• Consider surgery for nondiagnostic solid nodules (B)
• Molecular markers may be considered (C)
• Benign nodule.......FU US 6-18 months (C)
• Routine LT4 not recommended for benign nodules (F)
• Children same approach as adults (A)
• Pregnancy – biopsy nodules (A), defer surgery for PTC unless growing (C), consider LT4 (C)
Thyroid Nodules 2011

Patient with a thyroid nodule → NPV 85-90%

PPV 70-75% ← Clinical evaluation → NPV 85-90%

PPV 30-35% ← TSH → NPV 85-90%

Select nodules to biopsy ← Ultrasound → Cyst, spongiform NPV 96-98%

malignant PPV 90-98% ← Cytology → benign NPV 93-97%

suspicious PPV 50-80% ← Indeterminate → FLUS, neoplasm NPV 80-85%

PPV 60% ← Molecular classifier → NPV 96%

BRAF?

Future: prognosis (not all DTC is ‘bad’) therapeutic targets

Cancer

No cancer