Indeterminate Thyroid Nodules
Evolving Treatment and Management Strategies

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Disclosures

• Genzyme Advisory Board
• Research Funding Veracyte
Outline

• Epidemiology
• Risk of malignancy with indeterminate cytology
• Clinical and imaging considerations of nodules with indeterminate cytology
• Molecular markers in FNA
Epidemiology – thyroid nodules

Mazzaferri 1993 NEJM 328:553-9
Epidemiology—thyroid cancer

Prevalence of microcarcinoma of the thyroid

24 autopsy series with 7,156 cases

Adapted from: Pazaitou-Panayiotou, et al. 2007 Thyroid 17 (11): 1085-92
Changing mode of diagnosis in PTMC

1945-1979
N=378

1980-2004
N=552

- FNA for thyroid nodules has more than doubled from 2006-2011
- Thyroid FNA grew as a percentage of all FNA from 49% to 65%

Sosa et al 2013 Surgery epub

Hay et al 2008 Surgery 144: 980-7
Incidence rates of PTC by tumor size

- <1cm
- 1.1-2
- 2.1-5
- >5cm

Cramer et al 2010 Surgery 148: 1147-52
Papillary microcarcinoma
Likelihood of disease progression with observation

Ito et al 2014 Thyroid 24: 27-34

Multivariate analysis:
- **Age <40y**  RR 4.348 (2.3-8.2) \( p<0.0001 \)
- **T≥9mm**  RR 4.717 (1.9-11.4) \( p=0.0005 \)

Cumulative % progression to clinical disease

Follow-up times (years)

Patients at risk

Ito et al 2014 Thyroid 24: 27-34
### Postoperative malignancy rates for each cytology subtype

<table>
<thead>
<tr>
<th>NCI Classification</th>
<th>Frequency of diagnosis</th>
<th>% Malignant (ideally)</th>
<th>% Malignant (actually)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>32-76%</td>
<td>&lt;1%</td>
<td>2-18%</td>
</tr>
<tr>
<td>Follicular lesion of undetermined significance</td>
<td>3-15%</td>
<td>5-10%</td>
<td>0-48%</td>
</tr>
<tr>
<td>Atypia of undetermined significance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular neoplasm</td>
<td>5-20%</td>
<td>20-30%</td>
<td>14-49%</td>
</tr>
<tr>
<td>Hurthle cell neoplasm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspicious for malignancy</td>
<td>1-8%</td>
<td>50-75%</td>
<td>53-87%</td>
</tr>
<tr>
<td>Malignant</td>
<td>4-18%</td>
<td>98-100%</td>
<td>96-100%</td>
</tr>
<tr>
<td>Non-diagnostic</td>
<td>4-13%</td>
<td>0-50%</td>
<td></td>
</tr>
</tbody>
</table>

Wang et al 2011 Thyroid 21(3): 243-51
Baier et al. AJR 2009; 193: 1175-9
Interobserver concordance of thyroid FNA

Local vs Expert Cytopathologists

Cibas et al 2013 Ann Int Med 159: 325-332
Reproducibility of FNA results

Intraobserver Variability

Proportion of identical cytopathology diagnoses (%)

Cytologist 1 (n=96) 83%
Cytologist 2 (n=82) 78%
Cytologist 3 (n=75) 60%

Cibas et al 2013 Ann Int Med 159: 325-332
Impact of Pathologist case volume on cytologic interpretation


n=790 FNAs
Subtypes of AUS/FLUS

- Architectural atypia
- Nuclear atypia
- Oncocytic pattern
- Preparation artifact
- Lymphoid atypia
Repeat Atypia/FLUS FNAs Still Have a Significant Risk of Malignancy

<table>
<thead>
<tr>
<th>Cytology Diagnosis</th>
<th>Operated Risk of Malignancy</th>
<th>No. Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Initial AUS FNA</td>
<td>AUS</td>
<td>46%</td>
</tr>
<tr>
<td>First FNA</td>
<td>AUS</td>
<td>41%</td>
</tr>
<tr>
<td>Operated After Repeat FNA</td>
<td>AUS</td>
<td>43%</td>
</tr>
<tr>
<td></td>
<td>Benign</td>
<td>29%</td>
</tr>
<tr>
<td></td>
<td>Follicular Neoplasm</td>
<td>41%</td>
</tr>
<tr>
<td></td>
<td>Suspicious for Malignancy or Malignant</td>
<td>78%</td>
</tr>
<tr>
<td></td>
<td>Non-diagnostic</td>
<td>0%</td>
</tr>
<tr>
<td>All Repeats after Initial AUS FNA</td>
<td></td>
<td>50%</td>
</tr>
</tbody>
</table>

Mechanisms of neoplastic transformation

Cell growth, proliferation, survival
Cytologic atypia is a better predictor of malignancy in FNA than BRAF

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BRAF mut (n=13)</th>
<th>BRAF WT (n=297)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any atypia</td>
<td>11 (85%)</td>
<td>134 (45%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Nuclear grooves</td>
<td>5 (38%)</td>
<td>41 (14%)</td>
<td>0.03</td>
</tr>
<tr>
<td>Pseudoinclusions</td>
<td>5 (38%)</td>
<td>13 (4%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oval nuclei</td>
<td>2 (15%)</td>
<td>4 (1%)</td>
<td>0.022</td>
</tr>
<tr>
<td>Enlarged nuclei</td>
<td>2 (15%)</td>
<td>42 (14%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Clear chromatin</td>
<td>3 (23%)</td>
<td>26 (9%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Pleiomorphic nuclei</td>
<td>0</td>
<td>5 (2%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Kleiman et al 2012 Cancer epub

Patient and Nodule Characteristics Grouped by BRAF status

Multivariate Logistic Regression Analysis for the Presence of the BRAF mutation

<table>
<thead>
<tr>
<th>Cellular feature</th>
<th>Odds Ratio</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear grooves</td>
<td>2.3</td>
<td>0.211</td>
</tr>
<tr>
<td>Pseudoinclusions</td>
<td>11.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oval nuclei</td>
<td>10.0</td>
<td>0.022</td>
</tr>
</tbody>
</table>
Indeterminate FNA

Mutations tested:
BRAF, NRAS, KRAS, HRAS
RET/PTC1, RET/PTC3
PAX8/PPARγ

<table>
<thead>
<tr>
<th></th>
<th>Histology Malignant (n=93)</th>
<th>Histology Benign (n=368)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation Positive (n=63)</td>
<td>3 RAS FTC</td>
<td>8 (RAS+ Follicular adenomas)</td>
</tr>
<tr>
<td></td>
<td>42 RAS-PTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 BRAF-PTC</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3 PAX8/PPARγ –PTC</td>
<td></td>
</tr>
<tr>
<td>Mutation Negative (n=395)</td>
<td>38 (32 PTC, 6 FTC)</td>
<td>360</td>
</tr>
</tbody>
</table>

Sensitivity 59%
PPV 87.3%
Rate of malignancy=25.2%
Specificity 97.8%
NPV 90.4%

Nikiforov et al 2011 JCEM 96: 3390-97
### Genes included in ThyroSeq panel

<table>
<thead>
<tr>
<th>Chromosomes</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>chr 1</td>
<td>NRAS</td>
</tr>
<tr>
<td>chr 3</td>
<td>CTNNB1</td>
</tr>
<tr>
<td></td>
<td>PIK3CA</td>
</tr>
<tr>
<td>chr 7</td>
<td>BRAF</td>
</tr>
<tr>
<td>chr 10</td>
<td>RET</td>
</tr>
<tr>
<td></td>
<td>PTEN</td>
</tr>
<tr>
<td>chr 11</td>
<td>HRAS</td>
</tr>
<tr>
<td>chr 12</td>
<td>KRAS</td>
</tr>
<tr>
<td>chr 14</td>
<td>TSHR</td>
</tr>
<tr>
<td>chr 17</td>
<td>TP53</td>
</tr>
<tr>
<td>chr 20</td>
<td>GNAS</td>
</tr>
</tbody>
</table>

Nikiforova et al 2013 JCEM 98: E1852-60
Thyroseq V 2.0

- **Point mutations**
  - NRAS
  - KRAS
  - HRAS
  - TERT
  - BRAF
  - TP53
  - PIK3CA

- **Gene Fusions**
  - THADA
  - PPARG
  - NTRK3

**DISCUSSION**

In this study, we validated the performance of a novel genetic test based on a comprehensive panel of point mutations and gene fusions occurring in thyroid cancer in a large series of thyroid nodules with FN/SFN cytology and demonstrated that it allows accurate cancer risk assessment in these nodules, opening the possibility for improved management of these patients. On the basis of The Bethesda System, the cytologic diagnosis of FN/SFN is established in those aspirates that: 1) have follicular cells arranged in an architectural pattern characterized by cell crowding and/or microfollicle formation and lacking nuclear features of papillary carcinoma, or 2) are comprised almost exclusively of oncocytic (Hurthle) cells. Such cytologic patterns are observed in follicular and oncocytic carcinomas and in the follicular variant of papillary carcinoma, but they also are common in FAs and cellular hyperplastic nodules. Because such benign lesions are common, they determine a high false-positive rate on FN/SFN cytology, because only approximately 25% of nodules (range, 14%-34% of nodules) with FN/SFN cytology are identified as malignant after surgery.

**Figure 1.** This is a schematic representation of the study cohorts, test results, and overall performance of the targeted next-generation sequencing panel of thyroid cancer-related genetic markers (ThyroSeq v2). FN/FSN indicates follicular (or oncocytic) neoplasm/suspicious for a follicular (or oncocytic) neoplasm; PPV, positive predictive value; NPV, negative predictive value; CI, confidence interval.

Rate of malignancy 27.2%
ThyroSeq Testing of Follicular Neoplasms

• Prospective analysis of 62 consecutive nodules with follicular neoplasm by cytology

<table>
<thead>
<tr>
<th>ThyroSeq malignant</th>
<th>Histologically malignant</th>
<th>Histologically benign</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 BRAF (PTC)</td>
<td>1 NRAS (FA)</td>
</tr>
<tr>
<td></td>
<td>6 KRAS (PTC)</td>
<td>1 PTEN (FA)</td>
</tr>
<tr>
<td></td>
<td>4 NRAS (PTC)</td>
<td></td>
</tr>
<tr>
<td>ThyroSeq benign</td>
<td>2 FVPTC</td>
<td>47 hyperplastic nodules</td>
</tr>
</tbody>
</table>

NPV 95.7%
Prevalence of malignancy 21.3%

Nikiforova et al 2013 American Thyroid Association annual meeting. Oral Abstract 13. San Juan, Puerto Rico
Thyroseq v. 2.0

The knowledge of cancer risk associated with specific mutations, together with prognostic associations conferred by any gene fusion conferred a 100% risk of cancer in these situations identified using this broad panel of molecular markers. As a consequence, the majority of patients with this cytologic diagnosis who are classified as GEC suspicious but ultimately have been identified as cancer after surgery; therefore, the GEC test, commercially known as Afirma (Veracyte, South San Francisco, Calif), offers a high NPV, but its sensitivity is very low (ie, approaching 30%). In contrast, testing for a 7-oncogene panel offers a high PPV but a low NPV, which helps to select patients with a higher cancer risk for the appropriate therapeutic surgery (ie, total thyroidectomy) but does not prevent surgery (ie, diagnostic lobectomy) in those patients with a history of thyroid cancer, or a clinical setting with an unusually high pretest probability of cancer. Indeed, for many of those cancer risk factors, such as prior irradiation, a strong family history of thyroid cancer, or a clinical setting with an unusually high pretest probability of cancer, the PPV of ThyroSeq was 83%, and the risk of cancer was different from the average cancer rate reported in many studies. Possible exceptions would include high-risk patients with a clinical history of intrathyroidal tumors with no histologic features of usual histologic types, such as wel differentiated adenocarcinoma, medullary carcinoma, or medullary thyroid carcinoma. Possible exceptions would include high-risk patients with a clinical history of other invasive thyroid carcinomas, such as papillary thyroid carcinoma (PTC) with anaplasia, classic PTC, or medullary thyroid carcinoma. Possible exceptions would include high-risk patients with a clinical history of medullary carcinoma. Possible exceptions would include high-risk patients with a clinical history of medullary thyroid carcinoma. Possible exceptions would include high-risk patients with a clinical history of medullary thyroid carcinoma.
AUS/FLUS malignancy risk
Role of US features

• Prospective study of 150 pts at a single center in Brazil between 2009-2013
• FNA with 22 gauge needle
• All AUS nodules—9.3% of cytologic dx
• Clinical and US data collected
• FNA repeated after 3 months
• Surgery if cytology is: unsat, AUS/FLUS, follicular neoplasm, SFM, malignancy.
• Benign cytology with suspicious US also had surgery
Study protocol

- **FNA → FLUS/AUS**
  - (n = 162)
  - Excluded (n = 12)
  - 2nd FNA (n = 150)
    - Non-benign (n = 96)
      - Surgery (n = 96)
    - Benign (n = 54)
      - Suspicious US (n = 7)
      - Non-suspicious US (n = 47)
        - Surgery (n = 7)
        - Surgery (n = 32)
        - Follow-up (n = 15)

64% vs. 36%
Cytologic dx on 2nd FNA

- Benign: 36
- AUS/FLUS: 48.6
- Follicular neoplasm: 6.6
- SFM: 7.3
- Nondiagnostic: 1.3

N=150

Rosario 2014 Thyroid 24: 1-6
Malignancy rates by 2\textsuperscript{nd} cytologic dx

TABLE 2. RATE OF MALIGNANCY CONSIDERING THE RESULTS OF THE FIRST AND SECOND FINE-NEEDLE ASPIRATION

<table>
<thead>
<tr>
<th>First FNA</th>
<th>ND</th>
<th>Benign</th>
<th>FLUS/AUS</th>
<th>FN</th>
<th>Suspicion of malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLUS</td>
<td>0/1</td>
<td>1/42 (2.4%)</td>
<td>6/38 (15.8%)</td>
<td>3/11 (27.3%)</td>
<td>0</td>
</tr>
<tr>
<td>AUS</td>
<td>0/1</td>
<td>1/12 (8.3%)</td>
<td>15/35 (42.8%)</td>
<td>0</td>
<td>8/10 (80%)</td>
</tr>
<tr>
<td>FLUS/AUS</td>
<td>0/2</td>
<td>2/54 (3.7%)</td>
<td>21/73 (28.7%)</td>
<td>3/11 (27.3%)</td>
<td>8/10 (80%)</td>
</tr>
</tbody>
</table>

AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; FN, follicular neoplasm; FNA, fine-needle aspiration; ND, nondiagnostic.

Overall malignancy rate = 22.6%
Utility of US features for predicting malignancy

Overall malignancy rate with cytology = 22.6%

<table>
<thead>
<tr>
<th>US result</th>
<th>ND</th>
<th>Benign</th>
<th>FLUS/AUS</th>
<th>FN</th>
<th>Suspicion of malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious</td>
<td>0</td>
<td>2/7</td>
<td>16/21</td>
<td>2/3</td>
<td>7/7</td>
</tr>
<tr>
<td>Nonsuspicious</td>
<td>0/2</td>
<td>0/47</td>
<td>5/52</td>
<td>1/8</td>
<td>1/3</td>
</tr>
</tbody>
</table>

US, ultrasonography.

Performance of US

- Sensitivity: 79.4%
- Specificity: 90.5%
- PPV: 71%
- NPV: 93.75%

Rosario 2014 Thyroid 24: 1-6
Gene Expression Classifier (GEC) to Identify *Benign* Nodules among those with Indeterminate Cytology

Identify genes and measure their expression using microarray technology

Multidimensional algorithm

22,000 genes → 3000 genes → 142 genes

Afirma GEC Identifies Cytologically Indeterminate Thyroid Nodules with a Low Risk of Malignancy

- **Pretest probability of malignancy**
- **Posttest probability of malignancy**

**Cytopathology Diagnosis (N)**

- Benign (47)
- AUS/FLUS (129)
- FN/HCN (81)
- SFM (55)

Performance of the GEC
NEJM 2012

Rate of malignancy of 24%


McIver 2013 *Oral Oncology* 49: 645-53
Multicenter clinical experience with Afirma Gene Expression Classifier\(^1\)

Distribution of Afirma ‘benign’ vs. ‘suspicious’

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>GEC Benign (%)</th>
<th>GEC Suspicious (%)</th>
<th>GEC Non-Diag (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Nodules:</strong></td>
<td>339</td>
<td>174 (51%)</td>
<td>148 (44%)</td>
<td>17 (5%)</td>
</tr>
<tr>
<td><strong>FNA Cytology:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUS/FLUS:</td>
<td>165</td>
<td>91 (55%)</td>
<td>66 (40%)</td>
<td>8 (5%)</td>
</tr>
<tr>
<td>Validation Trial(^2):</td>
<td></td>
<td>43%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>Follicular Neoplasm:</td>
<td>161</td>
<td>79 (49%)</td>
<td>73 (45%)</td>
<td>9 (6%)</td>
</tr>
<tr>
<td>Validation Trial(^2):</td>
<td></td>
<td>40%</td>
<td>60%</td>
<td></td>
</tr>
</tbody>
</table>

1 Alexander EK et al. 2014 JCEM 99: 119-25
Multicenter Afiirma Experience
Proportion of ‘Afiirma Suspicious’ Malignant

Cyto Indeterminate & Afiirma “Suspicious”  
Histological Dx Cancer

121  →  53 (44%)

Validation Trial\(^2\): 43%

- 46 (87%) Papillary Carcinoma
- 5 (9%) Follicular Carcinoma
- 1 (2%) Hurthle Cell Carcinoma
- 1 (2%) Other

1 Alexander EK et al 2014 JCEM 99: 119-25
Follow up of Afirma Benign Nodules

71 patients

- 60 stable nodules (84%)
- 11 surgical removal (15%)
  - 4 MD recommendation
  - 4 compressive sxs
  - 3 cosmetic
- 9 benign nodules
- 2 malignant nodules:
  - 6mm FVPTC (cyto SFM)
  - 3.5cm hyperplastic nodule with two foci of PTMC
Management algorithm for thyroid nodules

>1cm thyroid nodule

- Low TSH
  - Radionuclide scan
    - Hot nodule
      - Ablate, resect, or medically treat
    - Cold nodule
      - US-guided FNA
        - Benign
          - Monitor
        - AUS/FLUS
        - Follicular neoplasm
          - Gene Expression Classifier
            - Benign
              - Monitor
            - Suspicious
              - Surgery
          - SFM
            - Malignant
              - Surgery

Questions?