8 Classifying Your Thyroid FNA Specimens Using Bethesda Terminology: Use of Adjunct Molecular Reflex Testing

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The Bethesda Thyroid Classification System is a novel method of Thyroid Nodule diagnostics that has yet to gain wide acceptance in the pathology community. The utilization of reflex molecular testing as an adjunct technique to the classification system has also yet to be described. This session will focus on the presenter's experience using the Bethesda Thyroid FNA system, including evidence of its clinical utility, guidance in its use, and its incorporation into practice as a diagnostic modality. The presenter's experience using BRAF V600E reflex molecular testing in conjunction with the Bethesda Classification System will also be covered, including evidence of its clinical utility, recommendations for various molecular techniques available and its incorporation into standard practice as both a diagnostic and prognostic modality.

- To apply the Bethesda Thyroid FNA Classification System in the evaluation of thyroid FNA.
- To utilize molecular testing as an adjunctive test in thyroid FNA.
- To advise clinicians on the implications of the each diagnostic category of the Bethesda Classification system and the results of molecular testing.

FACULTY:

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Practicing Pathologists
Cytopathology
Cytopathology (Non-Gynecologic)
2.0 CME/CMLE Credits

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The Utilization of the Bethesda System & BRAF Molecular Adjunct Testing on Thyroid Cytologic Samples Part I of II

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ASCP 2011

Objectives
- Review the Bethesda Thyroid FNA Classification System
- Describe its utilization at Yale & the questions it raises
- Post molecular testing as prequel to part 2
- Challenge our view of the modern cytopathologist/surgical pathologist

Background
- Thyroid Cancer
  - Most common endocrine malignancy
  - Incidence increasing
- Problematic clinically
  - Nodules
    - ~7% of the population
    - Only 5% are malignant
- Thyroid FNA (FNA) =
  - The keystone modality
  - Diagnostic test
  - Screening test
- But FNA is Compromised
  - 60% benign
  - 10% malignant
  - 20% equivocal
  - 10% non-diagnostic/unsatisfactory
- Lack of Uniformity
  - Terminology
  - Criteria
  - Clinical Implications
- Bethesda Classification
  - Resolutions?
- Molecular Diagnostics
  - Not expressly advocated in the Bethesda System
The Bethesda System

1. Nondiagnostic or Unsatisfactory
   - Cyst fluid only
   - Virtually acellular specimen
   - Obscuring factors
2. Benign
   - Benign follicular nodule e.g. adenomatoid nodule, colloid nodule
   - Lymphocytic thyroiditis
3. Atypia of undetermined significance
4. Follicular neoplasm or suspicious for a follicular neoplasm
   - Specify if Hürthle cell type
5. Suspicious for malignancy
6. Positive for malignancy
   - PTC
   - Medullary carcinoma
   - Anaplastic carcinoma
   - Lymphoma
   - Metastatic neoplasm
   - Other

Audience Response
How many thyroid FNAs do you see at your institution?

- Answer choice #1: 1-100 cases/year.
- Answer choice #2: 101-500 cases/year.
- Answer choice #3: 501-1000 cases/year.
- Answer choice #4: 1001-3000 cases/year.
- Answer choice #5: >3000 cases/year.

Yale Endocrine Cytopathology and Surgical Pathology

- Endocrine Surgery Referral Center
- Over 3,200 Thyroid FNAs annually
- Representing 2,500 patients seen in clinic per year
- Approximately 400 operations performed annually

Yale Smilow Cancer Center
### Audience Response

**Do you utilize the Bethesda Thyroid FNA Classification at your institution?**

- **Answer choice #1:** No. We don’t have a classification system per se.
- **Answer choice #2:** No. We have our own departmental system.
- **Answer choice #3:** Yes. We use TBS as it has been described in the literature.
- **Answer choice #4:** Yes, however, we’ve modified it somewhat to fit our needs.

### The Bethesda System (Yale Version)

<table>
<thead>
<tr>
<th>Category</th>
<th>Subcategories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic/Unsatisfactory</td>
<td>Insufficient cellularity, Poor preservation, Obscuring factors</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign mixed macro/microfollicular hyperplastic nodule, Lymphocytic Thyroiditis, Cyst Contents or Gross Nodular (GO) matches</td>
</tr>
<tr>
<td>Indeterminate [AUS/FLUS]</td>
<td>Low cellularity with predominance of small round lymphocytes and absence of colloid, Atypical nuclear features</td>
</tr>
</tbody>
</table>

### Is Thyroid FNA a Diagnostic or a Screening Test?

<table>
<thead>
<tr>
<th>Diagnostic</th>
<th>Screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive for PTC/MTC/ATC</td>
<td>Follicular Neoplasm</td>
</tr>
<tr>
<td>Suspicious for...</td>
<td>Hürthle Cell Neoplasm</td>
</tr>
<tr>
<td>Negative for Malignancy</td>
<td>AUS/Indeterminate?</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Architecture</th>
<th>Cytology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group Cell Features</td>
<td>Nucleus v.s. Cytoplasm</td>
</tr>
<tr>
<td>Single Cell Features</td>
<td></td>
</tr>
</tbody>
</table>

Theoharis et al. Thyroid 2009;19:1215
Cyto-Method Differs from Histology

- Cellularity
- Follicular Cells
- Adequacy
  - 6 groups
  - 10-15 cells/group
- Colloid
  - Not needed
  - But useful
- Lymphs/Macs/Others

Architecture
- Group Cell Features
- Single Cell Features

Cytology
- Nucleus vs. Cytoplasm

Negative

USG: Hypoechoic nodule(s), capsular calcifications, bordering vessels... non-specific
Papillary Thyroid Carcinoma

- Ultrasound
  - Hypoechoic
  - Microcalcifications
  - Increased vascularity
- Cellularity
  - Follicular cells
- Architecture
  - +/- Papillae, sheets, caps
- Cytology
  - Irregular nuclear membranes & grooves
  - intra-nuclear cytoplasmic invaginations (INCI’s)

**Papillary Thyroid Carcinoma - Papillae**

**Papillary Thyroid Carcinoma - Inclusion (INCI)**
Psammoma Body (Calciospherite)

Papillary Thyroid Carcinoma - Papillae

Papillary Thyroid Carcinoma - Inclusion (INCI)

Does a classic positive PTC need molecular testing? BRAF? For Dx? for Pgx?
Papillary Thyroid Carcinoma to LN

Often, few if any lymphocytes are present.

Follicular & Hürthle Cell Thyroid Neoplasms

- Architecture
  - Microfollicles
  - No Papillae
  - Capsule & Vessel?
- Cytoplasm
  - Amount v.s. Nucleus
  - Nuclei
    - Smooth
    - Enlarged
    - +/- Nucleoli
    - +/- Scant Colloid
    - +/- INC?

USG: Isoechoic nodule, no calcifications, no central vascularity, non-specific findings.

Follicular Adenoma Capsule

...
Follicular Thyroid Carcinoma

- Capsular invasion
- Vascular invasion
- Architecture
- Microfollicles
- No papillae
- Nuclei
- Smooth
- Enlarged
- +/- Nucleoli
- +/- Scant Colloid
- No INCs

Follicular Thyroid Carcinoma

FTC Capsular Mushrooming

Follicular Thyroid Carcinoma
Follicular Neoplasm > Follicular Adenoma

Follicular Neoplasm > Follicular Carcinoma

Hürthle Cell Carcinoma

Vascular Invasion
Hürthle Cell Neoplasm

Hürthle Cell Neoplasm > Hürthle Cell Carcinoma

Indeterminate/FLUS/AUS

- For specimens that contain cells (follicular, lymphoid, or other) with architectural and/or nuclear atypia insufficient to be classified as suspicious for a follicular neoplasm, suspicious for malignancy, or malignancy

  - Criteria
    - Borderline cellularity with predominance of follicular cells and absence/scant colloid
    - Borderline cellularity with predominance of Hürthle cells
    - Focal nuclear atypia s/o PTC (nuclear enlargement with pale chromatin, nuclear grooves) particularly in patients with lymphocytic thyroiditis or cystic changes
    - Atypical lymphoid population
    - Atypia with obscuring factors and/or air drying artifacts
AUS/FLUS/Indeterminate (Yale Version)

- Architectural Atypia
- Low Cellularity
- Microfollicles
- Absent/Scant Colloid
- Incipient Changes of Neoplasia?

Follicular Adenoma with intact capsule on resection

AUS/FLUS/Indeterminate (Yale Version)

- Nuclear Atypia
- Bongation/Enlargement
- Nuclear Membrane Irregularities/Grooves
- Rare possible (?) pseudoinclusions
- Incipient Changes of Neoplasia?
Mixed Classic and Follicular variant PTC on resection

Indeterminate/FLUS/AUS

- ‘At our institution, the term ‘indeterminate,’ corresponds to the NCI 2007 guidelines category ‘Follicular cells of undetermined significance.’ Lesions designated as such may benefit from re-aspiration in the appropriate clinical context.
- Repeat FNA in 3-6 months
- <20% of nodules are repeatedly diagnosed as "indeterminate"
- Surgery indicated if worrisome clinical and/or US findings
- Can we do better?
- Reflex BRAF mutational analysis?

GOAL:
- Maintain high PPV of malignancy on f/u but w/o compromising sensitivity

USUAL SUSPECTS:
- Suspicious for PTC
- Most common
- Lobectomy ± frozen section completion subsequently
- Suspicious MTC, ATC, NHL

Compromised:
- Quantity
- Quality
- Can we do better?
- Reflex BRAF mutational analysis?

The Need for Compromise
Suspicious for malignancy

- Papillary group
- Nuclear atypia

Overstained, obscuring blood, low cellularity, compromise sample

What's the difference?

- Is it Atypical?
  - Rule of Thumb:
    - "I'm not certain it's negative"
  - You the cytopathologist are communicating:
    - "Don't lose this patient to follow up"
  - Malignant risk should be low: <30%

- Is it Suspicious?
  - Rule of Thumb:
    - "I'm not certain it's positive"
  - You the cytopathologist are communicating:
    - "Consider resection based upon this sample"
  - Malignant risk should be high: >60%

Medullary Thyroid Carcinoma

- Cellularity
- Cellular
- Architecture
- SINGLE Cells
- Cytology
- Spindled
- Plasmacytid
- Nuclei
- Smooth
- Salt & Pepper
- Small nucleoli
- +/- INI1
- Ancillary Test?
- Calcitonin
Medullary Thyroid Carcinoma

Anaplastic Thyroid Carcinoma

**History**
- Older patient
- Rapid, recent growth

**Gross**
- Hemorrhage
- Necrosis

**Architecture**
- Single/Cellular
- Giant/Sarcoled
- Pleomorphic
- PMN’s

**Nuclei**
- Dark/irregular
- Mitoses
- INCi’s
ATC Immunostains

Anaplastic Thyroid Carcinoma

Anaplastic Thyroid Carcinoma
Non-Hodgkin Lymphoma

- Large B-Cell
- Hashimoto association
- Architecture
  - Monotypic/Cellular
  - Cytoplasmic vacuoles
- Nuclei
  - Immature chromatin
  - Multi-Nucleoli

Thyroid NHL

- Liquid Monolayer pseudo-groups

Thyroid NHL +CD45
### Distribution of cytologic categories

<table>
<thead>
<tr>
<th>Cytologic Category</th>
<th>By Nodules 2008 (%)</th>
<th>By Patients 2008 (%)</th>
<th>Expected Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>35 (11.7%)</td>
<td>230 (9.0%)</td>
<td>10% to 15%</td>
</tr>
<tr>
<td>Benign/Negative for Malignancy</td>
<td>238 (7.5%)</td>
<td>1701 (62.8%)</td>
<td>7% to 80%</td>
</tr>
<tr>
<td>Indeterminate/Atypia of</td>
<td>95 (3.0%)</td>
<td>89 (3.6%)</td>
<td>3% to 18%</td>
</tr>
<tr>
<td>Undetermined Significance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular/Hürthle Cell Neoplasm</td>
<td>176 (5.8%)</td>
<td>166 (6.7%)</td>
<td>5% to 8%</td>
</tr>
<tr>
<td>Suspicious for Malignancy*</td>
<td>41 (1.4%)</td>
<td>39 (1.6%)</td>
<td>2-5% to 8%</td>
</tr>
<tr>
<td>Malignancy*</td>
<td>148 (5.3%)</td>
<td>143 (5.9%)</td>
<td>4% to 8%</td>
</tr>
<tr>
<td>Total</td>
<td>3207</td>
<td>2468</td>
<td></td>
</tr>
</tbody>
</table>

* Majority of them were PTC. Modified from Theoharis et al. Thyroid 2009;19:1215

### Comparison before and after TBS

<table>
<thead>
<tr>
<th>Cytologic Category</th>
<th>By Nodules 2008 (%)</th>
<th>By Patients 2008 (%)</th>
<th>By Nodules 2007 (%)</th>
<th>By Patients 2007 (%)</th>
<th>Differences were statistically significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>577 (11.7%)</td>
<td>230 (9.0%)</td>
<td>577 (12.0%)</td>
<td>230 (9.0%)</td>
<td>Theoharis et al. USCAP 2010</td>
</tr>
<tr>
<td>Benign/Negative for Malignancy*</td>
<td>250 (10.0%)</td>
<td>179 (66.7%)</td>
<td>250 (10.0%)</td>
<td>179 (66.7%)</td>
<td></td>
</tr>
<tr>
<td>Indeterminate/Atypia of</td>
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<td>89 (3.6%)</td>
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<tr>
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<td>166 (6.7%)</td>
<td>176 (5.8%)</td>
<td>166 (6.7%)</td>
<td></td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>37 (1.4%)</td>
<td>31 (1.6%)</td>
<td>37 (1.4%)</td>
<td>31 (1.6%)</td>
<td></td>
</tr>
<tr>
<td>Malignancy</td>
<td>168 (5.5%)</td>
<td>145 (5.9%)</td>
<td>168 (5.5%)</td>
<td>145 (5.9%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3207</td>
<td>2468</td>
<td>2035</td>
<td>1596</td>
<td></td>
</tr>
</tbody>
</table>

* Differences were statistically significant

### Cytologic-Histologic Correlation

<table>
<thead>
<tr>
<th>Cytologic category (%) surgery</th>
<th>MNG/HT</th>
<th>FA</th>
<th>CA</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory (11%)</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>25</td>
</tr>
<tr>
<td>Benign/Negative for Malignancy (0.3%)</td>
<td>61</td>
<td>13</td>
<td>8*</td>
<td>82</td>
</tr>
<tr>
<td>Indeterminate (50%)</td>
<td>7</td>
<td>7</td>
<td>13</td>
<td>27</td>
</tr>
<tr>
<td>Follicular/Hürthle Cell Neoplasm (61%)</td>
<td>33</td>
<td>34</td>
<td>35**</td>
<td>102</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>2</td>
<td>2</td>
<td>26</td>
<td>30</td>
</tr>
<tr>
<td>Malignancy</td>
<td>12</td>
<td>0</td>
<td>112</td>
<td>112</td>
</tr>
<tr>
<td>Total</td>
<td>112</td>
<td>64</td>
<td>202</td>
<td>378</td>
</tr>
</tbody>
</table>

*The false negatives were micro FTC (≤1cm), not initially sampled by FNA
** included both follicular CA and PTC FTC
**Operating Characteristic**

<table>
<thead>
<tr>
<th></th>
<th>As a Screening test for NEOPLASM</th>
<th>As a Diagnostic test for MALIGNANCY</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>68%</td>
<td>93%</td>
</tr>
<tr>
<td><strong>Positive predictive value</strong></td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Negative predictive value</strong></td>
<td>83%</td>
<td>91%</td>
</tr>
</tbody>
</table>

Theoharis et al. Thyroid 2009;19:1215

**Risk of malignancy per Dx**

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Incidence of malignancy at Yale</th>
<th>NCI recommended rate of malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign/Negative for Malignancy</td>
<td>10% (0.3%)</td>
<td>0%-3%</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>30% (14%)</td>
<td>5%-15%</td>
</tr>
<tr>
<td>Follicular/Hürthle Cell Neoplasm</td>
<td>33%</td>
<td>20%-30%</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>87%</td>
<td>60%-75%</td>
</tr>
<tr>
<td>Malignancy</td>
<td>100%</td>
<td>97%-99%</td>
</tr>
</tbody>
</table>

* Only a selected subset of patients underwent surgery

Modified from Theoharis et al. Thyroid 2009;19:1215

**Indeterminate/FLUS/AUS**

- 171 nodules diagnosed as indeterminate/FLUS/AUS between Jan 2008 to Jun 2009;
- Accounting for 2.8% of all cases

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of Cases</th>
<th>Case with Follow-Up (Surgery/Repeat FNA)</th>
<th>Malignant Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low cellularity/ microfollicular pattern</td>
<td>104 (61%)</td>
<td>59/59/41%</td>
<td>7%</td>
</tr>
<tr>
<td>Nuclear atypia</td>
<td>67 (39%)</td>
<td>45/73/27%</td>
<td>56%</td>
</tr>
<tr>
<td>Total</td>
<td>171</td>
<td>104 (65%/35%)</td>
<td>20%</td>
</tr>
</tbody>
</table>

Adeniran et al USCAP 2010
Problems with equivocation

- On re-FNA, a significant percentage of indeterminates were re-classified as indeterminate.
- Majority are PTC, most classified as having "nuclear atypia" cytologically.
- Re-FNA benign?
- Hyperplastic nodules in both groups with Hashimoto thyroiditis more prevalent in the 2nd group.
- Suspicious category less problematic (87% CA risk).
- Adjunct testing?
- Immunostaining?
- Molecular testing?

Molecular Diagnostics?

* primum non nocere*

* From the Greek: οὐφελέειν, ἢ μὴ βλάπτειν

Audience Response

Do you utilize molecular testing on thyroid FNAs at your institution?

- Answer choice #1: No.
- Answer choice #2: BRAF only.
- Answer choice #3: BRAF, RET only.
- Answer choice #4: BRAF, RAS only.
- Answer choice #5: Panel of BRAF, RET, RAS, PAX etc.
A Possible Guideline to Molecular Thyroid FNA Testing

Pathologist/Cytopathologist as Diagnostic Specialist
- Pathology develops and adopts tools to leverage data from emerging technologies
- We become diagnostic consultants, providing outcomes based treatment recommendations to treating clinicians
- We become the Department of Diagnostic Medicine

Summary
- The Bethesda 6-tier classification system
  - Conveys different levels of risk of malignancy
  - Excellent screening test for follicular/Hürthle cell neoplasms
  - Superb diagnostic test for identifying PTC with a specificity of 93%
  - Sub-classifying indeterminate category into 2 descriptive groups conveys different levels of risk
- Molecular Testing may have a role especially in equivocal cases
- Part 2 follows shortly...
- Thank you!
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Surgeons
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- Sanziana Roman
- Julie Ann Sega

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