Working with your Cytopathologist to Improve Diagnostic Accuracy

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Disclosures

• None
Abu-al Qasim (936-1013 AD)

Kitab al-Tasrif

He described thyroid nodules/enlargements as “this tumor, which is called Elephant of the throat, is a large tumor which commonly occurs in women and is of congenital and acquired types. The congenital type is incurable, whereas, the acquired type is of two types: one resembles sebaceous cyst and other as an arterial aneurysm which is dangerous to incise, so never apply knife to it unless the tumor is small.

Reality Check

There is More to How Thyroid Nodules are Managed Then Just FNA and Cytologic Diagnosis
Let’s Make Sense of Present & Predict Future

In Light of Past

Thyroid Nodule Management Paradigms Aka Personalized Approach

Clinical Presentation

+ Ultrasound

+ FNA Diagnosis

+ Molecular Testing
A Diagnostic Thyroid FNA Specimen

Considerations

Specimen Adequacy
### Major Problems: Specimen Adequacy

- **Poor sampling and preparation**
  - Poor localization
  - Faulty technique
  - Inexperience
- **Cystic lesion**
- **Calcification and fibrosis**
- **Previous FNA**

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Non-Diagnostic

I did 12-passes

Look at the slides again
Problems: Cyto-preparations

Problems: Preparation *and* Fixation

- Poor smearing
- Fixation artifact
- Local anesthesia
- Air drying
Thyroid FNA Adequacy

• Abundant colloid and macrophages not adequate

• Representative, well preserved, follicular cells essential – *single most important factor*
  – 6 groups of cells with 10-20 cells each on two slides
  (Goellner 1987)

When Thyroid FNA Specimen is Adequate?

• A sample is adequate when:
  – It shows a pathologic process
  – But when the sample appears “benign”?

  • Is it safe to exclude malignancy?
Epithelial Quantitation

- Most commonly employed criterion:

  “at least 6 groups of benign follicular cells are required, each group composed of at least 10-20 cells.”

Thyroid Cysts

- True (pure) cysts are rare
  - 4% of thyroid “cysts” are true cysts

- Most thyroid nodules are complex
  - Mixed cystic and solid components
    - 30% of palpable thyroid masses
    - 50% of ultrasound detected nodules
  - Complex thyroid nodules:
    - Risk of malignancy 5 to 37% (estimated mean 15%)
      - Majority are papillary carcinomas

- FNA from thyroid “cysts” have a high rate of inadequacy
  - Often lack epithelial cells
Colloid and Adequacy

• Does the presence of colloid define an FNA as adequate?
    • Colloid without cells is “non-diagnostic”
  – The Thyroid Bethesda system
    • “Abundant colloid” lacking epithelial cells is benign
      – When is it abundant?
      – When is it colloid? - Problem with liquid based preparations
        » Loss of colloid through the filter
        » Less easily recognized

Colloid and Adequacy

**Personal Opinion**

• I do not accept abundant colloid lacking epithelial cells as benign – Unless no solid component on ultrasound
  – I know of no evidence to support this contention
    • It is in fact a rare situation
      – Usually you can find epithelial cells
Lesional Heterogeneity

All Thyroid Nodules are not Created Equal
Fact Well-known to Surgical Pathologist
Cytology Preparations

Cytomorphology vs. Cytopreps
Cytomorphology vs. Cytopreps

Clinical Information
Relevant Clinical Information

- **Demographics**: Age, sex
- **Nodule**: Single/multiple, size, growth, nature
- **History**: Head and neck irradiation
- **Family history**: Thyroid carcinoma
- **Non-palpable nodules**: $>1.5\text{cm}/<1.5\text{cm}$
- **Clinical features**: Hoarseness, dysphagia, dyspnea, regional lymphadenopathy
- ? **Graves’ and Hashimoto’s disease**

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Relevant Clinical Information

*Should be Provided to the Cytopathologist*

- **Why?** *Thyroid mass or even better Neck mass*

- Thyroid function tests
- Thyroid Scan
- History of previous FNA
Clinical History is as Important as your diagnosis

Thyroid FNA without history
Is this a test?

Case 1

• 52-year-old woman
• Ultrasound – Left thyroid lobe occupied by a predominantly ill-defined hypoechoic structure – suspicious for anaplastic carcinoma
Cytologic Diagnoses

Case 1:
• Original Diagnosis Suspicious for Anaplastic Carcinoma
  – More History
    • Transient symptomatic hyperthyroidism (TSH – 0.03) followed by hypothyroidism.
    • Current medication: Synthroid
  – Second opinion Dx: Suspect sub-acute thyroiditis
  – Surgical excision of left lobe
Lesson Learned

• History is as important as your diagnosis
  – Nodule characteristics
  – TFT’s
  – Prior FNA – Dx

Sampling

Fact well-known to surgical pathologist
Case of – Who Done It – The Sampling

Hemorrhagic 2.2 x 3.0 x 1.5 cm nodule
Adjacent 1.4 cm cystic nodule with papillary growth pattern.
Case of – Eager Patient or Clinician – Don’t tell the Pathologist of Prior FNA-3 days ago

Markedly Atypical Cells
Cytomorphology & Classification

Objectives of Thyroid FNA

• Recognize specific diagnostic entities
• Provide meaningful, management oriented diagnosis
• Potential utilization of ancillary techniques
Thyroid FNA
Bethesda Classification Scheme

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Risk of Malignancy (%)</th>
<th>Usual Management</th>
</tr>
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<tbody>
<tr>
<td>Non-diagnostic or Unsatisfactory</td>
<td></td>
<td>Repeat FNA with ultrasound guidance</td>
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<tr>
<td>Benign</td>
<td>0-3%</td>
<td>Clinical follow-up</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS)</td>
<td>~ 5-15%</td>
<td>Repeat FNA</td>
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<tr>
<td>Follicular Neoplasm or Suspicious for a Follicular Neoplasm (Specify if Hurthle type or Oncocytic)</td>
<td>15-30%</td>
<td>Surgical lobectomy</td>
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<tr>
<td>Suspicious for Malignancy</td>
<td>60-75%</td>
<td>Near-total thyroidectomy or surgical lobectomy</td>
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<tr>
<td>Malignant</td>
<td>97-99%</td>
<td>Near-total thyroidectomy</td>
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Easy-Breezy of Thyroid Cytopathology

Concordant Ultrasound Features, FNA cytomorphology & Histologic Follow-up
Nodular Goiter

Colloid: Generally abundant. Follicular cells Variable morphology Oncocytes, Macrophages
Degeneration/regeneration: Calcification, stromal proliferation, mitoses

Chronic Lymphocytic Thyroiditis

Oncocytes + Lymphocytes: In the background & infiltrating the cell groups
Papillary Thyroid Carcinoma

Nuclear features – Major Diagnostic Features
- Elongation, chromatin clearing,
- Nuclear membrane irregularities
- Intranuclear grooves, Inclusions
- Small peripheral nucleoli

Not so easy - Head Scratching
Everyday Thyroid Cytopathology

*Intdeterminate Lesions*

*Or*

*Indeterminate Pathologist?*
# Thyroid FNA
## Bethesda Classification Scheme

**The Bethesda System for Reporting Thyroid Cytopathology:**

*Implied Risk of Malignancy and Recommended Clinical Management*

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**Diagnosis Follicular Neoplasm**

**80% Benign on Surgical Excision**
Diagnosis
Follicular Lesion / Neoplasm

“Microfollicles” in FNA Specimens

Microfollicles = Neoplasm
Microfollicles – We Don’t Agree

- Inter-observer Agreement on Microfollicles
  - Renshaw AA et al. (Arch Pathol Lab Med 2006)
  - 12 cytopathologists were shown 45 small groups of follicular cells
    - 20 Microfollicles
    - 7 Macrofollicles
    - 18 Indeterminate
  - <15 cells arranged in circle that is at least two-thirds complete, should be classified as microfollicles.

The Atypical Category

The Dreaded AUS/FLUS
Reasons for AUS/FLUS

- History
  - TFT’s, H/O prior FNA
- Ultrasound features
  - Cystic vs. solid
- Operator – sampling
- Adequacy
- Cytology Preparation
- Interpretation
- Surgical follow-up – ? Gold standard

How to Relay
The AUS/FLUS Diagnosis

*Explain, Explain & Explain*
### HUP Experience

**AUS/FLUS cases are further sub-classified into Following subcategories (SC):**

- **SC1** - favor benign, however, a follicular neoplasm (FN) could not be excluded due to increased cellularity
- **SC2** - specimens with focal nuclear overlapping and crowding
- **SC3** - scant specimens with focal nuclear overlapping and crowding
- **SC4** - specimens with focal nuclear overlapping and crowding in a background of lymphocytic thyroiditis
- **SC5** - few cells with features suspicious for papillary thyroid cancer (PTC)
- **SC6** - specimens in which a FN cannot be excluded (with miscellaneous morphologic descriptors)

<table>
<thead>
<tr>
<th>AUS/FLUS Subclasses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subclass</strong></td>
</tr>
<tr>
<td>SC1</td>
</tr>
<tr>
<td>SC2</td>
</tr>
<tr>
<td>SC3</td>
</tr>
<tr>
<td>SC4</td>
</tr>
<tr>
<td>SC5</td>
</tr>
<tr>
<td>SC6</td>
</tr>
</tbody>
</table>

**Malignancy rate for each subcategory**

- **SC1** - 0%
- **SC2** - 36%
- **SC3** - 33%
- **SC4** - 22%
- **SC5** - 7%
- **SC6** - 2%

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**HUP Experience**

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Malignancy rate for each subcategory
SC1 - 0%, SC2 - 36%, SC3 - 33%, SC4 - 22%, SC5 - 7% and SC6 - 2%

Molecular Profiling of Thyroid Tumors + Molecular Diagnosis of Thyroid Nodules

Diagnostic Tests with High Negative and Positive Predictive Value

Mutational Analysis  Gene Expression Classifier  Next Gene Sequencing
Increase rate of Suspicious GEC - Afierna Results in Oncocytic Nodules

<table>
<thead>
<tr>
<th>Researcher</th>
<th>Suspicious nodules w surgery</th>
<th>Benign</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harell et al. Endo Pract 2014</td>
<td>30</td>
<td>13 (43%)</td>
<td>17 (57%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 9 (69%) oncocytic lesions</td>
<td></td>
</tr>
<tr>
<td>McIver et al. JCEM 2014</td>
<td>32</td>
<td>27 (84%)</td>
<td>5 (16%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 12 (44%) oncocytic lesions</td>
<td></td>
</tr>
<tr>
<td>Brauner et al. Thyroid 2015</td>
<td>43*</td>
<td>37 (84%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>Lastra et al. Cancer Cytopath 2014</td>
<td>48</td>
<td>26 (54%)</td>
<td>22 (46%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 15 (58%) oncocytic lesions</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>153</td>
<td>103 (67%)</td>
<td>50 (33%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- 73 (71%) oncocytic lesions</td>
<td></td>
</tr>
</tbody>
</table>

Next-Generation Sequencing Assay
Nikiforov et al. Cancer 2014,120:3627-34

![Diagram of next-generation sequencing assay](image-url)
Change in the Gold Standard of Thyroid Cytology

Changes in Surgical Pathology Diagnosis / Classification of “Low Risk Tumor(s)”
The Endocrine Society Working Group for Re-evaluation of the Encapsulated Follicular Variant of Papillary Thyroid Carcinoma

**Project Goals**
- **Review** a cohort of cases by experts in the field of endocrine pathology
- **Establish** a consensus on diagnostic histologic criteria
- **Define** the risk of adverse events based on long follow-up
- **Recommend** new terminology that reflects tumor biology and patient outcome

**Naming**
**Non-Invasive Follicular Variant of PTC**
*as anything but “Not Carcinoma”*

**New Terminology Recommendation**
“Non-invasive follicular thyroid neoplasm with papillary-like nuclear features” (NIFTP)

*Adequate sampling of entire tumor capsule is required to establish this diagnosis*

- Molecular profile - RAS and RAS-like mutations
- Non-invasive FVPTC – Negligible risk of recurrence
- Invasive EFVPTC - Increased risk of distant metastases
### Integrated Genomic Characterization of Papillary Thyroid Carcinoma. *Cell* (2014)

<table>
<thead>
<tr>
<th>MUTATIONS</th>
<th>Classic PTC</th>
<th>Encapsulated FVPTC</th>
<th>Foll Thyr CA</th>
<th>Poorly Dif Thy CA</th>
<th>Anap Thy CA</th>
<th>Foll Adenoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF V600E</td>
<td>+++</td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF K601E</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRAS</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>HRAS</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>KRAS</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>TSHR</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>GNAS</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GENE FUSIONS</th>
<th>RET/PTC</th>
<th>PAX8/PPARG</th>
<th>ALK fusions</th>
<th>BRAF fusions</th>
<th>ETV6/NTRK3</th>
<th>NTRK1 fusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
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**New Terminology Recommendation**

“Non-invasive follicular thyroid neoplasm with papillary-like nuclear features” (NIFTP)

*Adequate sampling of entire tumor capsule is required to establish this diagnosis*

**Changes in the Implied Risk of Malignancy for TBSRTC Categories**

**AUS/FLUS**

**Suspicious for Follicular Neoplasm**

**Suspicious for Malignancy – 50% decrease**

(Strickland et al. Thyroid 2015 & Faquin et al. Cancer Cytopathology 2015)
### Combined Institutional Data Showing TBSRTC Diagnostic Categories, Surgical Follow-Up, Risk Of Malignancy With and Without Cases of Non-Invasive Follicular Variant of Papillary Thyroid Carcinoma (NI-FVPTC)

<table>
<thead>
<tr>
<th>TBSRTC Diagnostic Categories</th>
<th>ND</th>
<th>Benign</th>
<th>AUS/FLUS</th>
<th>FN/SFN</th>
<th>SM</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of FNABs, n=6943</td>
<td>406 (5.8%)</td>
<td>4221 (60.8%)</td>
<td>1028 (14.8%)</td>
<td>463 (6.6%)</td>
<td>238 (3.4%)</td>
<td>587 (8.4%)</td>
</tr>
<tr>
<td>Total number of cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benign Surgical FU, n=949</td>
<td>52</td>
<td>386</td>
<td>273</td>
<td>203</td>
<td>31</td>
<td>4</td>
</tr>
<tr>
<td>Malignant Surgical FU, n=949</td>
<td>18</td>
<td>40</td>
<td>124</td>
<td>101</td>
<td>148</td>
<td>446</td>
</tr>
<tr>
<td>Total PTC, n=756</td>
<td>1</td>
<td>15</td>
<td>54</td>
<td>46</td>
<td>42</td>
<td>15</td>
</tr>
<tr>
<td>Total NI-FVPTC, n=173</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Risk of Malignancy

| ROM | 25.3% | 9.3% | 31.2% | 33.2% | 82.6% | 99.1% |
| OROM | 4.4% | 0.3% | 12.1% | 21.8% | 71.3% | |
| ROM excluding NI-FVPTC Cases | 23.9% | 9.8% | 17.6% | 18.0% | 59.2% | 95.7% |
| **p-value** | 0.19 | 0.04 | 0.03 | 0.03 | 0.01 | 0.1 |
| OROM excluding NI-FVPTC Cases | 4.1% | 0.5% | 6.8% | 11.8% | 44.5% | 73.4% |
| **p-value** | 0.18 | 0.05 | 0.02 | 0.02 | 0.1 | 0.1 |

#### % Decrease in Risk of Malignancy

| ROM excluding NI-FVPTC Cases | 1.4% | 1.0% | 13.6% | 15.1% | 23.4% | 3.3% |
| OROM excluding NI-FVPTC Cases | 0.2% | 0.3% | 5.2% | 9.9% | 17.6% | 2.5% |

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*Where Are We Heading to?*
Thyroid nodules are Common

- The Data from future thyroid FNA studies based on changes in surgical pathology diagnoses will be important for recommending potential changes in TBSRTC

- The Adjunct Molecular tests are here to stay
  - Never going to replace thyroid FNA cytology
  - Play a role in the current management paradigm of thyroid nodules


2012
450,000 FNAs estimated in USA
What I Struggle with Everyday?

When My Roots are Basic Cytomorphology & My Practice is Facing Many Practice Changers

What I Struggle with?

How to avoid loosing thyroid FNA specimens?

• Good relationship with the clinicians
  – History
  – Results discussion
  – All matters
• Good relationship with radiologist and knowledge of ultrasound
• Empowering the workforce of cytopathology