Even if you do not signout cytopathology, a working knowledge of basic thyroid cytology is valuable (e.g. frozen section lab intraop smears, interpreting cytology reports)

Thyroid FNA

"FNA is the most accurate and cost effective method for evaluating thyroid nodules."

Each year over 450,000 thyroid FNAs are performed in the U.S. !!!

**THYROID FNA: THE GOOD NEWS…**
- Reduced the number of surgeries by 50% [benign result in 60-70% of FNAs]
- Increased the yield of malignancies by 2-3X
- Decreased the costs of management by over 25%
- But with Bethesda and advances in molecular testing, we can do better!

**THYROID FNA RATIONALE**

**RATIONALE:**
- High prevalence of thyroid nodules (4-7%)
- Low incidence of malignancy (5%)
- Surgery for all nodules is not practical

Some examples of challenges in thyroid FNA
Thyroid FNA is often a critical test for the diagnosis of undifferentiated thyroid carcinoma.

**Undifferentiated Thyroid Carcinoma:**
Patterns that are easily recognized
- Bizarre tumor giant cells
- Multinucleated tumor cells

**Undifferentiated Thyroid Carcinoma:**
Pitfall: Predominance of spindled cells – a subset of these are keratin negative!
**Undifferentiated Thyroid Carcinoma**

**How to distinguish from other thyroid and non-thyroid lesions:**

- **Immunocytochemistry – often not helpful:**
  - LMW keratin +
  - P53 +
  - Thyroglobulin – often NEGATIVE
  - TTF-1 – often NEGATIVE
  - Pax 8 +
  - B-catenin +
  - Calcitonin & CEA -
- **EM:**
  - Demonstrates epithelial features

**Clinical:**

- Radiologic evidence of thyroid origin
- Clinical history of prior well differentiated thyroid carcinoma

---

**Medullary carcinoma presents challenges for FNA:**

*Important to recognize due to impact on management*

**Medullary Carcinoma:**

*Key to diagnosis is single cell pattern*

- Salt & Pepper Chromatin
- Focal Amyloid

**MTC – Oncocytic Variant**

*Can be mistaken for a Hurthle cell tumor*
Suspicious for a Hurthle cell neoplasm?  
Lobectomy vs Total Thyroidectomy & LN Dissection

Medullary Thyroid Carcinoma

- Immunocytochemistry for calcitonin is recommended before making a definitive FNA diagnosis.
- Immunoprofile:
  - Keratin +
  - Calcitonin +
  - Chromogranin/synaptophysin +
  - TTF-1 ±
  - CEA +

Anytime that the FNA diagnosis describes single cells or an unusual pattern, consider medullary thyroid carcinoma…and consider getting a serum calcitonin.

Thyroid FNA and Follicular-Patterned Lesions
**The Bethesda System for Reporting Thyroid Cytopathology**

**The Bethesda System for Reporting Thyroid Cytopathology: 6 Diagnostic Categories**

1. **NONDIAGNOSTIC** or **UNSATISFACTORY**
2. **BENIGN**
3. **ATYPIA OF UNDETERMINED SIGNIFICANCE** or **FOLLICULAR LESION OF UNDETERMINED SIGNIFICANCE**
4. **FOLLICULAR NEOPLASM** or **SUSPICIOUS FOR A FOLLICULAR NEOPLASM**
   - specify if Hurthle cell (oncocytic) type
5. **SUSPICIOUS FOR MALIGNANCY**
6. **MALIGNANT**

**Bethesda Terminology: Relationship to Clinical Algorithms**

<table>
<thead>
<tr>
<th>Category</th>
<th>Management</th>
<th>Implied Risk of Malignancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic</td>
<td>Repeat FNA</td>
<td>1-4%</td>
</tr>
<tr>
<td>Benign</td>
<td>Follow</td>
<td>&lt;1-3%</td>
</tr>
<tr>
<td>AUS/FLUS</td>
<td>Repeat FNA</td>
<td>~5-15%</td>
</tr>
<tr>
<td>Susp for Follicular Neoplasm</td>
<td>Lobectomy</td>
<td>20-30%</td>
</tr>
<tr>
<td>Susp for Hurthle Cell Neoplasm</td>
<td>Lobectomy</td>
<td>20-30%</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>Lobectomy/Total Thyroidectomy</td>
<td>60-75%</td>
</tr>
<tr>
<td>Malignant</td>
<td>Total Thyroidectomy</td>
<td>97-99%</td>
</tr>
</tbody>
</table>

**Announcements**

October 22nd - 23rd, 2007

Bethesda, Maryland

In September there were 7,235 visits to the website with 17,300 page views.

October 4, 2007
Summary of the BSRTC: Our experience at MGH and BWH

<table>
<thead>
<tr>
<th>Study</th>
<th>ND</th>
<th>Benign</th>
<th>AUS</th>
<th>SusF</th>
<th>SUS</th>
<th>Malignant</th>
<th>TOTAL CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanderlaan et al BWH</td>
<td>587(12.5)</td>
<td>2941(62.7)</td>
<td>512(10.9)</td>
<td>198(4.2)</td>
<td>209(4.5)</td>
<td>244(5.2)</td>
<td>4691</td>
</tr>
<tr>
<td>Faquin MGH</td>
<td>762(13.9)</td>
<td>3658(66.9)</td>
<td>546(10.0)</td>
<td>111(2.0)</td>
<td>173(3.2)</td>
<td>214(3.9)</td>
<td>5464</td>
</tr>
</tbody>
</table>

Too much blood, and not enough follicular cells!

Satisfactory smears: At least six groups of follicular cells with at least 10 cells per group

- Approx. 5-15% of thyroid FNAs are non-diagnostic.

EXCEPTION TO ADEQUACY RULE: Colloid Nodule
Thyroid FNAs with abundant colloid only, can be placed into the BENIGN category.

EXCEPTION TO ADEQUACY RULE: Inflammation only in Inflammatory Conditions
Thyroid FNAs with abundant inflammatory cells only, can be placed into the BENIGN category.
Thyroid FNA Adequacy

Reducing your Non-Diagnostic rate:
• Ultrasound-guided FNA
• ROSE
• Use of liquid-based preparations
  • e.g. Thin Prep, Surepath
  • Concentrates cells into monolayer
  • Removes obscuring blood
  • Learning curve to interpret

Macrofollicular Pattern in Histology

BENIGN: 60-70% of Thyroid FNAs

Cytologic Reporting of Follicular Lesions

BENIGN
• Macrofollicles and colloid, consistent with a benign thyroid nodule.
When do we diagnose an FNA as “Suspicious for malignancy” or “Malignant” in the Bethesda System?

Papillary Thyroid Carcinoma is the Most Common Cause of a “Suspicious/Malignant” FNA Dx

- FNA is highly accurate:
  - >90% are diagnosed as Malignant or Suspicious by FNA

Papillary Thyroid Carcinoma

FNA is most useful as a diagnostic test for papillary thyroid carcinoma, probably better than frozen section!

What are the BASIC features that we use to diagnose PTC by FNA?
No single cytologic feature is **diagnostic** of papillary thyroid carcinoma!
Use a combination of features!
### Molecular Features of Papillary Thyroid Carcinoma: Useful in Molecular Panels to Identify PTC

- **BRAF** (esp. V600E) 40-50% Positive in aggressive forms of PTC
- **RET/PTC** (esp. types 1 and 3) 20-30% Non-specific; FVPTC
- **NRAS, HRAS, KRAS** 10% Non-specific; FVPTC
- **NTRK1 rearrangements** <5% Cribriform-morular
- **APC/b-catenin** <2% Cribriform-morular

### The Cancer Gene Atlas Project

- 71 gene expression profile
- Two broad categories:
  - BRAF-like: Tall cell variants and classic
  - RAS-like: FVPTC, resemble follicular neoplasms

### PAPILLARY THYROID CARCINOMA AND BRAF^{V600E}

**Is there a role for using BRAF^{V600E} testing?**

- **BRAF** point mutations (40-50% PTC)
  - Most PTC with papillary architecture have BRAF point mutations
  - PPV approaches 100%
  - Among aggressive PTCs BRAF mutation is most common
  - May have a role in the “suspicious for malignancy” category
  - BRAF antibody shows correlation with molecular testing

### Thyroid FNA and Indeterminate Diagnoses
Bethesda Terminology: Indeterminate Thyroid Cytology

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Predicated Risk of Malignancy (%)</th>
<th>Actual Risk of Malignancy in Nodules Surgically Excised (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic/Unsatisfactory</td>
<td>1-4</td>
<td>20 (9-32)</td>
</tr>
<tr>
<td>Benign</td>
<td>0-3</td>
<td>2.5 (1-10)</td>
</tr>
<tr>
<td>AUS/FLUS</td>
<td>5-15</td>
<td>14 (6-68)</td>
</tr>
<tr>
<td>FN/SFN</td>
<td>15-30</td>
<td>25 (14-34)</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>60-75</td>
<td>70 (53-97)</td>
</tr>
<tr>
<td>Malignant</td>
<td>97-99</td>
<td>99 (94-100)</td>
</tr>
</tbody>
</table>

What features are used to diagnose an FNA as “Suspicious for a follicular neoplasm” in the Bethesda System?

FNA as a Screening Test for Follicular Carcinoma

The Riddle

If the criteria for classifying these lesions are purely histologic, what hope is there for fine-needle aspiration cytology?
FNA as a Screening Test for Follicular Carcinoma

- Multinodular goiter
- Adenomatous nodule
- Follicular adenoma
  - Macrofollicular
  - Microfollicular
- Trabecular
- Solid
- Follicular carcinoma

Cytologic Reporting of Follicular Lesions

- SUSPICIOUS FOR A FOLLICULAR NEOPLASM
  - Note: Distinction between a follicular adenoma and follicular carcinoma is not possible based upon cytologic material.

EVALUATING FOLLICULAR LESIONS

- All follicular lesions are a mixture of micro- and macrofollicles.
- Focus on the predominant pattern.
Molecular Features of Follicular Carcinoma

- NRAS, HRAS, KRAS 40-50% Non-specific (35%)
- PAX8/PPARg1 rearrangement 30-40% Solid growth/HBME+ gal+, Angioinvasion
- PIK3CA 6-13%
- PTEN 6-12%
- GRIM19 subset of Hurthle cell tumors

AUS/FLUS: The Problem

- The classic “indeterminate” category
- Cases that don’t fulfill criteria of other categories:
  - “The findings are not convincingly benign, yet the degree of cellular or architectural atypia is not sufficient for an interpretation of ‘follicular neoplasm’ or ‘suspicious for malignancy.’”
- 8 scenarios outlined in the Bethesda Atlas
- Heterogeneous category – WASTEBASKET
- Often a compromised specimen (obscuring blood, etc.)
  - Note: low cellularity, poor fixation, obscuring elements by themselves not sufficient for AUS/FLUS

AUS/FLUS- Scenario:
Hypocellular but Microfollicular

AUS/FLUS- Scenario:
Mixed Architectural Pattern
AUS/FLUS - Scenario: Scant Hurthle Cells Only

AUS/FLUS Scenario: "Benign" ...But Focal Features of Papillary Carcinoma

AUS/FLUS - Scenario: Artifact

AUS/FLUS - Scenario: Preparation Artifact and Mild Atypia
AUS/FLUS:

- Less than 7% of thyroid FNAs (range: 3-20% in lit.) – needs adjusting! …probably 10-12%
- Potential for overuse/abuse –
  - Role for intralab monitoring (QA metric)
- Recommended management: Repeat FNA or molecular
  - >50% of cases are reclassified as BENIGN on repeat FNA
- Surgery for “repeat atypicals”
  - 27% malignant with repeat AUS/FLUS FNA
  [Faquin and Baloch, 2009]
- Should AUS/FLUS be further subdivided?
  - Maybe!
    - Nuclear atypia = increased risk for PTC
    - Architectural atypia only = lower risk for PTC

Follicular Variant of PTC:
Common Cause of Difficult Thyroid FNA

A subset of these lesions will fall into the
“Suspicious for follicular neoplasm” “Suspicious
for malignancy” “AUS/FLUS” categories.

Non-Invasive Follicular Thyroid (NIFT) Neoplasm with
Papillary-Like Nuclear Features

- Reclassify non-invasive FVPTC as NIFT
- The prospects of NIFT will create some issues
  for thyroid cytopathology:
  - The ROM for certain diagnostic categories of the
    Bethesda System will decrease
  - The PPV/NPV of molecular testing panels will
    change
- Future modifications in our approach to the
  indeterminate thyroid FNA may be
  warranted

INDETERMINATE THYROID FNAS

What is the role for ancillary
testing of thyroid FNAs?
IHC, Afirma, MiRInform, others???
INDETERMINATE THYROID FNAS (15-30%)

- Clinical management for the “benign” and “malignant” categories is clear
- Most patients with “suspicious for malignancy” will have surgery (often total thyroidectomy)
- Management options for the AUS/FLUS and FN/SFN categories are more complex – molecular testing offers a solution

Immunocytochemistry
- Used primarily in Europe (Fadda et al., EJE 2011)
- Stratify indeterminate thyroid FNAs into low and high risk groups
- Liquid-based and smears
- Inexpensive and fast TAT
- HBME-1 and Galectin-3 are most popular
- Difficulties in reproducibility, specificity, and interpretation

INDETERMINATE THYROID FNAS

Molecular Testing Options:
- Afirma GEC (Veracyte)
- MiRInform (Asuragen)
- ThyroSeq V2

Molecular Testing and Thyroid FNA

**PROS:**
- Convenient
- Objective result
- Avoids waiting for repeat FNA
- Defines management and save dollars

**CONS:**
- Expensive if inappropriately applied
- Reflex testing
  - Takes clinician out of picture
  - Can add to overall expense (unnecessary testing)
- Loss of cyto-histo correlation
Molecular Testing and Thyroid FNA

The Afirma Test

CASE

A 47-year-old euthyroid woman presented to the endocrinology clinic with a 2.0 cm right thyroid nodule. A previous FNA on this patient’s thyroid nodule at an outside hospital was reported to have been diagnosed as AUS/FLUS. An FNA was performed.

Mixed Macro- and Microfollicles

Increased Proportion of Microfollicles
CASE
Cytologic Diagnosis

Satisfactory for evaluation
AUS/FLUS
Mixed pattern of fragmented macro- and microfollicles, and focal mild nuclear atypia.

CASE

In view of this repeat indeterminate diagnosis, the patient and her clinician decided to have Afirma testing performed on the FNA.

The result of the Afirma Test was: SUSPICIOUS (indeterminate)

CASE

The patient had a right thyroid lobectomy.
Histologic Diagnosis

Encapsulated follicular variant of papillary thyroid carcinoma (2.0 cm).
{aka NIFT}

The Afirma Test

- Benign fingerprint (high NPV) – "rule out" test
  - Microarray data from 167 genes
  - "Benign" vs "Suspicious" Classification
  - $3350 cost
  - Requires 2 additional FNA passes
  - Also includes BRAF and RET mutation tests
- Overall NPV = 93%
- Sensitivity=92%; specificity=52%
- False negative rate of 8.2%
  - Possibility due to inadequate sample RNA

The Afirma Test

- For AUS/FLUS (n=129; 24% malignant):
  - NPV=95%
  - 43% reclassified as “Benign”
  - Sensitivity: 90%, Specificity: 53%
  - 9.7% FN rate
The Afiroma Test

- FN/SFN (n=81; 25% malignant):
  - NPV=94%
  - 40% reclassified as “Benign” – avoids surgery!
  - Sensitivity: 90%, Specificity: 53%
  - 10% FN rate
- Not useful for “Suspicious for Malignancy” category (NPV=85%)

An example of a customized approach:

Molecular Testing and Thyroid FNA

The MiRInform Test

- Malignant fingerprint {high PPV} – “rule in” test
  - Testing for 7 genes:
    - BRAF V600E
    - RAS mutations (NRAS, KRAS, HRAS)
    - RET/PTC (RET/PTC1, RET/PTC3)
    - PAX8/PPARγ
MiRInform Test

• Nikiforov et al. J Clin Endocrinol Metab 2011
  – 479 indeterminate FNAs; 18% had mutations
  – 75% of PTC; 70% of FC
  – Overall specificity = 98%; sensitivity = 60%
  – $ 2250 cost

MiRInform Test:
Nikiforov et al. J Clin Endocrinol Metab 2011

• Probably most useful for “susp. for malignancy” group
• Does not decrease need for surgery, but can allow for TT in 56% of patients with a malignancy
• PPV:
  – AUS/FLUS 88%
  – FN/SFN 87%
  – Susp Mal. 95%
• All FPs due to RAS+ follicular adenomas

Molecular Testing and Thyroid FNA

The ThyroSeq Test v.2

ThyroSeq v.2

– Next generation sequencing gene mutation panels
  – Mutations in 14 genes
  – 42 gene fusions

• Indications:
  – Thyroid FNA diagnosed as indeterminate by cytology
  – Thyroid FNA diagnosed as malignant, when molecular testing is expected to affect the decision to perform surgery or extent of surgery
  – Thyroid FNA diagnosed as benign by cytology, when strong clinical suspicion for cancer exists based on imaging and clinical studies.
  – Diagnosis of cancer is established in FNA or surgically excised thyroid tissue, when molecular profiling of cancer will affect administration of radioactive iodine, intensity of follow up, or targeted therapies for advanced cancer.
ThyroSeq v.2

Gene List for Mutations:
- AKT1, BRAF, CTNNB1, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TP53, TSHR, TERT, EIF1AX

Gene List for Gene Fusions and Gene Expression:
- RET, PPARG, NTRK1, NTRK3, ALK, IGF2BP3, BRAF, MET, CALCA, PTH, SLC5A5, TG, TTF1, KRT7, KRT20

ThyroSeq v.2

- Sensitivity: 90%
- Specificity: 93%
- PPV:
  - 88% AUS/FLUS
  - 93% FN
  - 95% Susp Mal
- NPV:
  - 97% AUS/FLUS
  - 96% FN
  - 72% Susp Mal

Molecular Analysis at MGH

NGS-SNaPshot Panel
- Anchored Multiplex PCR (AMP)
- ~190 target amplicons across 39 genes
- High-quality sequence:
  - Staggered start sites
  - >100X target coverage
  - Molecular indexing
  - Bi-template coverage
  - ~2% analytical sensitivity
- Fast turn-around (~2 weeks)
- Cost-effective (<$500)
- Small tissue amounts (5-10 ng)
**TERT Mutations**

- **TERT**: Telomerase reverse transcriptase
- **TERT** promoter activating mutations
  - Seen in any type of follicular-derived carcinoma
    - PTC-7.5%
    - Follicular -17.1%
    - Poorly differentiated -29.0%
    - Anaplastic-33.3%
  - Associated with aggressive behavior


**TERT and BRAF:**

Act synergistically to predict aggressive behavior

_Xing et al J Clin Oncol (2014)_

---

**Case Example Using ThyroSeq v.2**

- 55 yo male with 3.0 cm right thyroid nodule
- FNA diagnosis: Susp for FN
- ThyroSeq v.2 testing

**RESULTS**

B. TERT promoter mutation POSITIVE (c.1-1244T>C228T): see interpretation below.

**INTERPRETATION**

Two mutations were identified in this sample, NRAS and TERT. The finding of RAS mutation alone in the FNA sample is associated with 74-85% risk of cancer in a given nodule (1-3). The most common type of cancer associated with this mutation is the follicular variant of papillary carcinoma, typically the encapsulated follicular variant, followed by follicular carcinoma (4). Mutations in the promoter region of the TERT gene, most frequently C228T, have been reported in 7-22% of well-differentiated thyroid papillary and follicular carcinomas and in 29-52% of dedifferentiated thyroid cancers (4-8). TERT mutations were not found in any of 210 benign thyroid tumors studied (7-9). Recent studies showed that the presence of TERT mutations was associated with more invasive tumor phenotype at presentation (6,8) and with higher risk of distant metastases and disease persistence (6). The finding of both of these mutations confers >95% risk of cancer in this nodule.
Case Example Using ThyroSeq v.2

- Instead of lobectomy, patient had a total thyroidectomy performed.
- Final Histologic Diagnosis:
  - POORLY DIFFERENTIATED THYROID CARCINOMA

SUMMARY

- FNA has emerged as an essential initial test for evaluating thyroid nodules
- The new Bethesda System is highly recommended...It provides a risk of malignancy for each diagnostic category
- Molecular testing options are many: Likely to be an integral part of thyroid FNAs in future
- More to come on the impact of NIFT!

Thank You!