Each year over 550,000 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 there are still many challenges....

The Bethesda System for Reporting Thyroid Cytopathology
The Indeterminate Thyroid FNA Comprises 15-30% of All Thyroid FNAs and Continues to Present a Challenge for Clinical Management

<table>
<thead>
<tr>
<th>Category Management Implied Risk of Malignancy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic Repeat FNA</td>
</tr>
<tr>
<td>Benign Follow</td>
</tr>
<tr>
<td>AUS/FLUS Repeat FNA</td>
</tr>
<tr>
<td>Susp for Follicular Neoplasm Lobectomy</td>
</tr>
<tr>
<td>Susp for Hurthle Cell Neoplasm Lobectomy</td>
</tr>
<tr>
<td>Suspicious for Malignancy Lobectomy/Total Thyroid</td>
</tr>
<tr>
<td>Malignant Total Thyroid</td>
</tr>
</tbody>
</table>

Bethesda Terminology: Relationship to Clinical Algorithms

TBSRTC: AUS/FLUS

Birth of a new indeterminate entity

A challenge for diagnosis, management, molecular testing

Benign

FN/SFN or SFM

Ancillary Molecular Testing in Thyroid Nodules

- Insights into molecular biology of thyroid cancer and aggressive thyroid disease
- NIFTP and its impact on diagnosis and management
- Expanded options for molecular testing of thyroid FNAs

The Overdiagnosis of Thyroid Carcinoma

Thyroid-Cancer incidence and Related Mortality in South Korea, 1993–2011

More Focus on Aggressive Thyroid Cancer

- Less focus on malignant vs benign
- More focus on identifying aggressive forms of thyroid cancer in the FNA and in the resection
- How to define aggressive thyroid carcinoma?
  - Microscopic analysis is mixed
  - Need for more effective molecular indicators:
    - BRAF
    - TERT
    - ALK
    - TP53
    - miR-150, miR-183-3p, miR-221 and miR-222

TERT Mutations... One example

- TERT: Telomerase reverse transcriptase
- TERT promoter activating mutations
  - Seen in any type of follicular-derived carcinoma
    - PTC-7.5%
    - Follicular -17.1%
    - Poorly differentiated -29.9%
    - Anaplastic-33.3%
  - Often associated with aggressive behavior
What is the role for ancillary testing of thyroid FNAs?

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

**Revised 2015 ATA Guidelines- Includes Molecular Testing Option**

<table>
<thead>
<tr>
<th>Category</th>
<th>Management</th>
<th>ROM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic</td>
<td>Repeat with US</td>
<td>0-4%</td>
</tr>
<tr>
<td>Benign</td>
<td>Clin + US F/U</td>
<td>0-3%</td>
</tr>
<tr>
<td>AUS/FLUS</td>
<td>Repeat FNA, Molecular, Lobectomy</td>
<td>5-15%</td>
</tr>
<tr>
<td>Susp for Follicular Neoplasm</td>
<td>Molecular, Lobectomy</td>
<td>15-30%</td>
</tr>
<tr>
<td>Susp for Hurthle Cell Neoplasm</td>
<td>Molecular, Lobectomy</td>
<td>15-30%</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>Lobectomy, Total thyroid, Molecular*</td>
<td>60-75%</td>
</tr>
<tr>
<td>Malignant</td>
<td>Total thyroidectomy; Lobectomy</td>
<td>97.99%</td>
</tr>
</tbody>
</table>

**The Cancer Gene Atlas Project**
- Implications for molecular testing
- 71 gene expression profile
- Two broad categories:
  - BRAF-like: Classical PTC and tall cell variants - diagnostic of carcinoma
  - RAS-like: FVPTC, NIFTP, resemble follicular neoplasms - indeterminate

**Molecular Tests for Thyroid FNA**

<table>
<thead>
<tr>
<th>Test</th>
<th>Company</th>
<th>Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afirma</td>
<td>Veracyte</td>
<td>Gene expression classifier (mRNA-based) with high NPV</td>
</tr>
<tr>
<td>ThyroSeq v.2</td>
<td>CBL Path</td>
<td>NGS for point mutations and fusions</td>
</tr>
<tr>
<td>ThyGenX-ThyraMIR</td>
<td>Interpace Diagnostics</td>
<td>NGS for point mutations and fusions (ThyGenX) plus microRNA panel (ThyraMIR)</td>
</tr>
<tr>
<td>RosettaGX Reveal</td>
<td>Rosetta Genomics</td>
<td>MicroRNA platform with high NPV; uses stained slides</td>
</tr>
</tbody>
</table>
Molecular Tests for Thyroid FNA: $$$$$$$$$$$

<table>
<thead>
<tr>
<th>Test</th>
<th>Company</th>
<th>Estimated Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afirma</td>
<td>Veracyte</td>
<td>$4875 (GEC + MTC)</td>
</tr>
<tr>
<td>ThyroSeq v.2</td>
<td>CBL Path</td>
<td>$3200</td>
</tr>
<tr>
<td>ThyGenX-ThyraMIR</td>
<td>Interpace Diagnostics</td>
<td>$1675/$3300</td>
</tr>
<tr>
<td>RosettaGX Reveal</td>
<td>Rosetta Genomics</td>
<td>$3700</td>
</tr>
</tbody>
</table>

Tests have $300-500 maximum out of network costs for patient.

**Despite cost-benefits, molecular testing represents a large expense for the healthcare system.**

**With competition and technological advances, it is hoped that prices will decrease.**

Molecular Testing and Thyroid FNA

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

**CONS:**
- Expensive if inappropriately applied
- Some tests require 1-2 extra FNA passes
- Reflex testing
- Takes clinician out of picture
- Potential loss of cyto-histo correlation

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
Cytologic Diagnosis

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

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)

The patient had a right thyroid lobectomy.

Histologic Diagnosis

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

The Afirm Test
Ancillary Molecular Testing in Thyroid Nodules

The Afirma Test
- Benign fingerprint (high NPV) – “rule out” test
  - Microarray data from 167 genes
  - “Benign” vs “Suspicious” Classification
  - $4875 cost
  - Requires 2 additional FNA passes
  - Also includes BRAF and RET mutation tests
  - Validated on unknown cases (Blinded study)
  - Overall NPV = 93%
  - False negative rate of 8.2%
  - Possibly 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 Afirma 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%)

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

Case Example Using ThyroSeq v.2
- BRAF mutation positive (g.1799A>T, v.1844A>G)
- Ret promoter mutation positive (g.1758G>T, C2287T)

Sample Afirma Report
- Report Status: FINAL
- Results Summary
- VEGF-A overexpression significant
- Gene expression analysis shows a gene expression pattern of unknown significance
- No BRAF V600E mutations detected
- No RET fusion detected

Next Generation Sequencing Panel for Thyroid Cancer (ThyroSeq)
- Specimen: Fine Needle Aspiration (FNA)
- Results:
  - BRAF mutation positive (g.1799A>T, v.1844A>G)
  - Ret promoter mutation positive (g.1758G>T, C2287T)

- Interpretation:
  - VEGF-A overexpression significant
  - Gene expression analysis shows a gene expression pattern of unknown significance
  - No BRAF V600E mutations detected
  - No RET fusion detected

- Clinical Implications:
  - The presence of BRAF and Ret mutations may indicate a thyroid cancer with a particular genetic profile.
  - Further genetic testing may be needed to confirm the diagnosis and guide therapy.
Case Example Using ThyroSeq v.2

- Instead of lobectomy, patient had a total thyroidectomy performed.
- Final Histologic Diagnosis: POORLY DIFFERENTIATED THYROID CARCINOMA
- 1.5 years later: Patient developed liver metastases

ThyroSeq v.2

- Next generation sequencing gene mutation panels
  - Mutations in 14 genes
  - 48 gene fusions
  - Single institution study
  - Histologists not blinded to molecular test results

Gene List for Mutations:
- AKT1, BRAF, CTNNB1, GNAS, HRAS, KRAS, NRAS, PIK3CA, PTEN, RET, TPS3, TBHR, TERT, EIF1AX

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

ThyroSeq v.2

- Sensitivity: 90%
- Specificity: 93%
- NPV: 97% AUS/FLUS, 96% FN, 72% Susp Mal
- PPV: 77% AUS/FLUS, 83% FN, 95% Susp Mal

ThyGenX-ThyraMIR

- BEST when both tests used together
- Validation studies involved multiple institutions
- ThyGenX:
  - NGS 8-gene panel with high PPV
  - PPV: 88% (AUS/FLUS), 87% (FN), 95% (Susp M)
- ThyraMIR to complement ThyGenX:
  - Micro-RNA based GEC
  - Recommended for ThyGenX negative cases

- COMBINATION:
  - Sensitivity: 89%
  - Specificity: 85%
  - NPV: 94%
  - PPV: 74%

RosettaGX Reveal

**Scraped slides can be up to 10 years old
**Destroyed slide is pre-scanned

RosettaGX Reveal:
Blinded multi-institutional study
NIFTP: How Does it Impact Thyroid FNA?

NIFTP has created some challenges for cytology!

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

- The prospects of NIFTP for thyroid cytology:
  - The ROM for indeterminate diagnostic categories of TBSRTC will change
  - The PPV/NPV for molecular testing panels will change
  - Management issues for follicular-patterned lesions
  - Medicolegal issues for FP diagnosis of PTC

Effect of NIFTP reclassification on ROM for different Bethesda categories – Primarily affects indeterminate categories

Revised Bethesda ROM Based on NIFTP

<table>
<thead>
<tr>
<th>Category</th>
<th>ROM with NIFTP</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic</td>
<td>No Change</td>
<td>None</td>
</tr>
<tr>
<td>Benign</td>
<td>No Change</td>
<td>None</td>
</tr>
<tr>
<td>AUS/FLUS</td>
<td>6-18%</td>
<td>None</td>
</tr>
<tr>
<td>Susp for Follicular Neoplasm</td>
<td>10-40%</td>
<td>Optional Note</td>
</tr>
<tr>
<td>Susp for Hurthle Cell Neoplasm</td>
<td>10-40%</td>
<td>Optional Note</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>40-60%</td>
<td>Optional Note</td>
</tr>
<tr>
<td>Malignant</td>
<td>94-96%</td>
<td>Optional Note</td>
</tr>
</tbody>
</table>

How should FNA classification & clinical management change based upon expected impacts on the ROM for thyroid FNA reporting categories?

- Modify the cytologic criteria for classifying follicular patterned FNA:
  - FN with atypia vs Susp Malignancy
- Avoid diagnosing follicular-patterned PTC as Malignant
- Put an optional note about possible NIFTP on selected cases
Is there a potential role for molecular testing?

**NIFTP: Molecular Profile**

Most common molecular changes:
- RAS mutations
- BRAF K601E mutation
- PPARgamma fusion
- THADA fusion
- BRAF V600E is essentially absent

**NIFTP: RAS+ Cases**

- 79% of RAS+ thyroid cancers had an indeterminate thyroid FNA
- Among indeterminate thyroid FNAs, 63% were NIFTP
- Suggests that lobectomy should be considered as the treatment of choice for indeterminate thyroid FNAs that are RAS+.
- Potential role for mutational molecular testing

**NIFTP Accounts for Over Half of “Carcinomas” Harboring RAS Mutations.**

Paulson VA1, Shivdasani P2, Angell TE3, Alexander EK4, Cibas E5, Krane JF6,7, Lindeman NI8, Barletta J9.

**Thyroid, 2017.**

**What about NIFTP and Afirma?**

- The Afirma GEC appears to detect nearly all NIFTP that are tested
- Suggests that lobectomy should be favored for cases with AUS/FLUS or SFN and Suspicious Afirma result

**Noninvasive Follicular Variant of PTC and the Afirma Gene-Expression Classifier**

Wong KS, Angell TE, Strickland RC, Alexander EK, Cibas ES, Krane JF, Barletta J.

**Thyroid, 2016.**

**NIFTP – Summary**

- Most NIFTP are detected as abnormal by FNA +/- molecular testing
- Most NIFTP are triaged for surgery
- NIFTP is considered a potential precursor to carcinoma...
- Lobectomy is an appropriate treatment for NIFTP

**SUMMARY**

- Indeterminate thyroid FNAs continue to pose a problem for thyroid cytology
- Several molecular testing options are available: Becoming an integral part of thyroid FNAs
- Proper application is needed
- Await lower prices with competition and technical improvements
- NIFTP has brought changes to thyroid ROM and to how we diagnose thyroid FNAs
- It primarily impacts the indeterminate categories
- There is an evolving role for molecular testing and NIFTP
Thank You!