Introduction and Bethesda system classification

Diagnostic challenges and controversies

Specimen adequacy

Management options

Atypia and indeterminate thyroid FNAs

Molecular testing

Introduction

• FNA proven effective management tool in patients with thyroid nodules
• Thyroid FNA is diagnostic in many conditions
  – It is primarily a “screening test”
• Main purpose is to provide for a rational approach to management, and determine extent of surgery when needed
Introduction

• No standards existed for reporting thyroid FNAs
• Different classification schemes based on personal/institutional experiences and biases
• A survey of pathologists and clinicians on the perceptions of terminology used in reporting thyroid FNA showed a discord between pathologists and clinicians

Diagnostic Terminology and Reporting

• Surveyed 133 clinicians (Endocrinologists, Surgeons, Thyroid specialists)
• Implications of FNA DX on management options
  – Non-diagnostic → 98% repeat FNA
  – Suspicious for malig → 96% surgery
  – Indeterminate → 58% repeat FNA, 32% surgery
  – Atypical → 37% repeat FNA, 52% surgery
• “Indeterminate” was confused with ND in 58% of cases. “Atypical” was too ambiguous and treated as “Susp. malignant” in over ½ of cases

Bethesda System Classification

<table>
<thead>
<tr>
<th>Diagnostic Categories</th>
<th>Risk of Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>&lt; 2-3 %</td>
</tr>
<tr>
<td>Atypia (Follicular lesion) of US</td>
<td>5-10 %</td>
</tr>
<tr>
<td>Neoplasm (Follicular / Oncocytic)</td>
<td>20-30%</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>50-75%</td>
</tr>
<tr>
<td>Malignant</td>
<td>100 %</td>
</tr>
<tr>
<td>Non-diagnostic</td>
<td></td>
</tr>
</tbody>
</table>
**Justifications for Bethesda System Classification**

- FNA has become the standard of care for initial workup of thyroid nodules
- Most clinicians use FNA results in conjunction with clinical findings to guide treatment
- Clinicians generally utilize FNA to provide a relative risk of malignancy, from which they can base their management decisions
- The proposed diagnostic categories are important in providing a risk of malignancy to clinicians and patients → *Surgery vs. follow-up*

**Major Challenges and Controversies**

- Assessment of watery/thin colloid
  - Conventional Paps and ThinPrep
- Terminology
  - Two diagnostic categories (FLUS & FN) vs. one
- Criteria for diagnosis of FN
  - Proportion of microfollicles
  - Overall cellularity
- Suspicious for PTC
  - How much atypia is enough?
  - Minimal criteria for PTC

**Nodular Goiter/Hyperplastic Nodule**

- Abundant colloid
- Variable cellularity
- Oncocytic metaplasia
- Degenerative changes
• Flat sheets- honeycomb
  – Few microfollicles accepted
  – Occasional balls and micro-tissue fragments

Benign follicular cells
• Uniform nuclei:
  – Same size as RBC
  – Minimal nuclear overlapping
  – Finely granular chromatin
  – Rare nucleoli

Dense colloid
• Easy to recognize
• Dark blue-violet-magenta (DQ)
• Dark green-orange (Pap)
Watery/Thin Colloid
- Blue-violet (DQ), light green-orange (Pap)
- Cracks, folds, waves, "thin-membrane",
  "crazy pavement" appearance

Thin Colloid in Bloody Specimens
- Difficult to recognize
- Easily confused with serum in bloody specimens

(Stelow 2005)

Thin Colloid
- May disappear completely on LB preps
- Tissue paper like appearance on TP
• ThinPrep: 58 YOF, Right thyroid nodule

• Cytology Dx: Nodular goiter
• Histology follow-up: FVPC

• TP: Watery colloid has tissue paper appearance
Follicular Lesions

- Hyperplastic/adenomatoid nodule
- Follicular Neoplasm
  - Follicular adenoma
  - Follicular carcinoma
- Follicular variant of Papillary carcinoma

Differential Diagnosis of Follicular Lesions

- Overlapping cytologic features make it difficult, at times, to separate between HN & FN
- Indeterminate category accounts for 5-42% of FNA Dx's

- Abundant colloid = benign
- Marked cellularity = Neoplasm

Uniformity

- Permissiveness in applying strict criteria to DX of FN → significant reduction of malignancy rate on FU
Follicular Lesions and Terminology

- 2 European studies found no malignancies on F/U of FNAs diagnosed as FL and FN
- Authors advocated a less aggressive approach to management, i.e. clinical follow-up
- **FN was defined as:**
  - Hypercellular smear
  - Scant colloid
  - Microfollicles present

(Forpiani 2003, Piromalli 1992)

NCI Thyroid Conference Approach to Grey Zone and Terminology

- Although “Follicular Lesion” & “Follicular Neoplasm” are used interchangeably by some authors
  - We do not consider them synonymous
- “Indeterminate” cytologic category included FN, FL, Susp. for malignancy, Atypia NOS

Bethesda System Diagnostic Categories

1. Benign
2. FLUS/AUS
3. Follicular Neoplasm
4. Suspicious for Malignancy
5. Malignant
6. Non-diagnostic
Bethesda System Diagnostic Categories

1. Benign
2. FLUS/AUS
3. Follicular Neoplasm
4. Suspicious for Malignancy
5. Malignant
6. Non-diagnostic

Follicular Neoplasm

- Cytologic DDx:
  - Follicular adenoma
  - Follicular carcinoma
  - FVPC
- Need histologic confirmation
- Follow-up:
  - 70% neoplasm
  - 30% cancer (FC, FVPC)

Follicular Neoplasm

Cytologic Criteria

- Cytology can not distinguish between FA and FC
- High cellularity
- Scant colloid
- Prominent microfollicles and/or syncytial fragments (> 50-75% of cells)
- Significant nuclear overlapping and crowding
- Monotonous cell population
Microfollicles

- <15 cells arranged in circle that is at least 2/3 complete
- Nuclear crowding/overlap
- Microfollicles + no atypia: low cancer risk (6%)
- Microfollicles + abundant colloid + absence of nuclear overlap: 0% cancer

Follicular Neoplasm

*Cytologic Criteria*

- Uniform enlargement >2X RBC
- Coarse and clumped chromatin
- ± Prominent nucleoli
- ± Severe nuclear pleomorphism


Case study

L thyroid nodule (1.3 cm) from a 32 year old man

NG

A,B: Benign
C,D: AUS/FLUS
Did We Really Need an “Atypical” Category?

Follicular lesions:
- Hyperplastic nodule/ NG
- Atypia (Follicular lesion) of US
- Follicular Neoplasm

Diagnostic Challenges in Hyperplastic/Adenomatoid nodule

- Most difficult problem is distinguishing HN with little colloid from FN with some colloid
- Microfollicles may be focally seen in HN (5-10% of cases)
- High cellularity → up to 30% of HN
- Scant colloid → 15-20% of HN
- Should not make DX of HN in absence of colloid

Diagnostic Challenges in Follicular Neoplasm

- Degenerative changes → up to 30% of neoplasms
- Low cellularity due to:
  - Poor biopsy technique
  - Macrofollicular architecture

Atypia (Follicular Lesion) of US

*Cytologic Features*

- Major differential diagnoses are
  - HN vs. FN
  - Reactive changes vs. PTC
- High cellularity, scant colloid
- Smears from different passes show a spectrum ranging from “benign” to “possible FN”
  - Admixture of flat sheets and microfollicles/syncytia
- Minimal nuclear overlapping and crowding
- Low cellularity, but prominent microfollicles and nuclear overlap (highly vascular lesions)

AUS/FLUS
• Specimen consisted predominately of blood
• Rare groups of follicular cells
  – Clue: abundant blood with rare microfollicles or syncytia

(Yang 2003, Lowhagen & Oertel)

R thyroid nodule, 45 yoF
• FU: FVPC

• Nuclear grooves in NG, mimicking PTC
Nuclear irregularities and grooves may be associated with LT, NG, and repair.

**AUS/FLUS**

- Cytology not convincingly benign, yet degree of nuclear or architectural atypia is not sufficient for diagnosis of “FN” or “susp. for malignancy”
- Some cases are due to a compromised specimen, i.e. low cellularity, poor fixation, obscuring blood
- Avoid overuse of this category
  - Ideally < 12% of thyroid FNAs (BTS <7%)

**Follicular Variant of PTC**

- Second to sampling error as most common cause of false negative Dx's
  
  *(Wu 2006)*
Branching monolayered sheets: most significant low power discriminator from FN (Fulcinii 2001)

Bubble Gum Colloid and PTC

- Squamoid cytoplasm
- Oval nuclei, powdery chromatin
- Grooves/irregular nuclear membranes
- Marginated nucleoli
- Intranuclear holes
Case Study

- 2.5 cm R thyroid nodule in a 56 year old woman

FVPC, false negative

- FVPC may show:
  - Paucity of nuclear features of PTC
  - Abundant colloid
  - Misdiagnosed as B9 or FN
  - Should have been Dx’d as “susp. for PTC”
Suspicious for PTC

– Strong suspicion for malignancy

Cytologic criteria:

1. Quantitative:
   • PTC features present but very sparse cellularity
   • Patchy/focal nuclear changes of PTC

2. Qualitative
   • Diffuse but incomplete nuclear changes of PTC
     – i.e. generalized nuclear enlargement and pallor, but rare grooves or inclusions
   • Hypervacuolated and atypical histiocyteid cells

Suspicious for PTC

• Sensitive cytologic criteria for detecting FVPC
  – Flat syncytial sheets
  – Nuclear enlargement
  – Fine chromatin
  – Nuclear grooves
  • < ½ FVPC showed intra-nuclear holes
  • Important NOT to lump these cases (70-75% cancer risk) with other “indeterminate” Dx’s:
    • AUS (5-10% cancer risk)
    • FN (20-30% cancer risk)

Suspicious for PTC

• Focal grooves, nuclear enlargement and powdery chromatin

COMBINED in same nuclei

Most sensitive (Wu 2003)

Most specific
• Generalized nuclear enlargement & pallor, but rare grooves

• Rare cells with distinct mild focal nuclear atypia
  • More commonly associated with LT and cyst
  • Occasionally with FVPC

• Hypervacuolated and atypical histiocytoid cells
Clinical Implications and Management

- **Benign**
  - < 3% cancer risk
  - Clinical/periodic US exams @ 6-18 month intervals, for at least 3-5 years
  - Repeat FNA if significant increase in nodule size

- **Follicular neoplasm**
  - 20-30% cancer risk
  - Lobectomy

Clinical Implications and Management

- **AUS/FLUS**
  - Approximately 10% cancer risk
  - Repeat FNA in 3-6 months, correlate with clinical and radiologic findings
  - If repeat FNA is “Atypical” or worse → consider surgery
  - NOT equivalent to “Susp. for malignancy”


Clinical Implications and Management

- **Suspicious for PTC**
  - 50-75% cancer risk

  *Options:*
  1. Lobectomy
  2. Lobectomy + intra-operative consult
     - Helpful in additional 30% of cases
       *(Baloch 2002)*
  3. Total thyroidectomy

Bethesda System Diagnostic Categories

1. Benign
2. Atypia (Follicular lesion) of US
3. Follicular/Oncocytic Neoplasm
4. Suspicious for Malignancy
5. Malignant
6. Non-diagnostic
Specimen Adequacy Criteria

**General Principles**

- Main purpose is to minimize # of false negative diagnoses
- Provide a meaningful interpretation that is clinically useful
  - A diagnosis of “rare/few benign follicular cells” without qualification, is not considered meaningful
- An adequate sample should be representative of the lesion (appropriate cellularity) and technically well prepared, i.e. good fixation, thin smear, adequate staining

Adequacy Criteria

**NCI Thyroid Conference Conclusions**

- Specimen processed and examined, but “Non-diagnostic” due to:
  - No follicular cells or limited cellularity
  - Poor fixation and preservation
- Optimal # of passes: 2-5
- Any significant cytologic atypia precludes the interpretation of “ND”
- **Solid nodules**: minimum of 5-6 groups (at least 10 cells/group), preferably on a single slide
- A repeat FNA can be recommended

Exceptions to Minimal Number Criteria

- Inflammatory process such as thyroiditis
- Abundant colloid → C/W Colloid nodule
- Cyst fluid with rare benign follicular cells → C/W benign cyst

**CONTROVERSY:**

Cyst fluid only → Diagnostic vs. Non-diagnostic?
Thyroid Cysts and Malignancy

- Cysts most commonly due to cystic degeneration in NG
- Any residual mass after aspiration should be sampled
- Risk of malignancy
  - Of all aspirated cysts, as low as 1% are malignant
  - Simple, non-complex cysts = 1-4% cancer risk
  - Mixed solid-cystic nodules, large cysts (>3cm) and recurring cysts = Up to 14% cancer risk

Bethesda Conference Conclusions

- Cystic lesions, lacking follicular cells, that collapse completely following aspiration → "Cyst fluid only"
- Include under "Non-diagnostic" (majority agreement) or "Benign"
- Options:
  - Recommend correlation with cyst size, complexity and US features
  - Disclaimer that cystic CA cannot be entirely excluded

Atypia and Indeterminate Lesions
Defining Atypia

- Irregularities in cell morphology beyond what is typically seen in “normal or reactive” state
- Atypia is subjective and often varies among pathologists
  - One pathologist’s “atypical” is another’s “reactive” or “suspicious”

Atypia in Benign lesions

- Long standing hypothyroidism
- Toxic multinodular goiter
- Graves disease treated with radioactive iodine and/or Tapazole
- Reactive/reparative change associated with cystic degeneration or prior FNA
- Dyshormonogenetic goiter
- Atypical adenoma
- Air-Drying Artifact

- Atypia secondary to $^{131}$I therapy
Atypical Repair in cyst

Types of Atypia in Thyroid FNA

- **Architectural atypia**
  - Microfollicles and/or syncytia
  - Nuclear enlargement
  - Nuclear crowding
    - FN: > 50-75 % of cell groups
    - AUS: 25-50 % of cell groups
    - Benign: < 25% of cell groups

- **Nuclear atypia**
  - Nuclear enlargement
  - Powdery chromatin
  - Nuclear grooves
  - Pseudoinclusions
Case Examples

Susp. for PTC

Susp. for PTC
• 54 YOF, left thyroid nodule

• Cytology Dx: Susp. for PTC
  • Thyroidectomy: Lymphocytic thyroiditis
    - Atypia threshold should be increased in LT

• PTC associated with LT
Molecular Testing
## Bethesda System Diagnostic Categories

<table>
<thead>
<tr>
<th>Diagnostic Categories</th>
<th>Risk of Malignancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUS</td>
<td>Low (5-10%)</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>Intermediate (20-30%)</td>
</tr>
<tr>
<td>Susp. for Malignancy</td>
<td>High (50-75%)</td>
</tr>
</tbody>
</table>

### Molecular Testing

- Major purpose is to increase predictive power of thyroid FNA diagnosis
- Currently, there is no single molecular marker that is sensitive or specific enough to justify its use alone as a predictor of benign or malignant disease

### Best Available Commercial Tests

1. Asuragen- miRInform thyroid panel
   - Confirms malignant diagnosis
2. Veracyte- Afirma Gene expression classifier
   - Confirms benign diagnosis
1. miRInform thyroid (Asuragen)

- Panel of oncogene mutations
  - BRAF V600E mutation
  - RAS mutations
  - RET/PTC re-arrangements
  - PAX8/PPARγ fusion
- Identify lesions that are malignant, i.e. increase PPV of FNA
- Excellent specificity but up to 30% NPV

- BRAF: mostly classic and tall cell PTC
  - PPV approx 100%
  - associated with more aggressive tumor behavior
- RAS has no clear predictive role in tumor aggressiveness
  - found in benign adenomas and PD carcinomas

<table>
<thead>
<tr>
<th></th>
<th>PTC</th>
<th>FC</th>
<th>FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF</td>
<td>45%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAS</td>
<td>15% (FVPC)</td>
<td>40-50%</td>
<td>20-40%</td>
</tr>
<tr>
<td>RET/PTC</td>
<td>20% (Adults)</td>
<td>30-40%</td>
<td>2-10%</td>
</tr>
<tr>
<td>PAX8/PPARγ</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Largest prospective trial to date: 479 indeterminate FNA samples
- Yielded 87 positive mutations (18%)
  - 19 BRAF, 62 RAS, 1 RET/PTC, 5 PAX8/PPARγ
- All FP results (9) due to RAS+ follicular adenomas
- Suggest that the presence of any mutation, especially BRAF and RET/PTC, would be strong indication for total thyroidectomy

*Nikiforov 2011*
Proposed clinical algorithm for management of patients with cytologically indeterminate thyroid FNA applying the results of mutational analysis.

- Limitations: Most pathologists were aware of molecular results before signing out histology → significant bias

**Ohori 2010**

- Focused on AUS FNA cases, using a mutation panel
- Detected 12 out of 20 (60%) PTC in the sample study:
  - Excellent specificity with no FP results, but
  - False neg in 7.6% of cases (mutations-/histology+)
- Repeat FNA (without molecular tests) found malignancies in 8/20 patients

**2. Afirma (Veracyte)**

- Gene expression profiling
- Measures expression of 167 RNA transcripts from “indeterminate” thyroid FNAs
- Identify lesions that are benign, i.e. increase NPV of FNA
- Generates a diagnosis of “benign” or “suspicious”
  - *Cytology must be interpreted by Veracyte*
    - Exceptions: Some academic centers
Veracyte Validation Study

- Prospective multicenter study tested 265 indeterminate FNAs
- Sensitivity 92%, Specificity 52%
- Identified 78 of 85 malignancies as “suspicious”
- NPV 93% among all indeterminate lesions

<table>
<thead>
<tr>
<th></th>
<th>NPV %</th>
<th>Cancer prevalence %</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUS</td>
<td>95</td>
<td>24</td>
</tr>
<tr>
<td>FN</td>
<td>94</td>
<td>25</td>
</tr>
<tr>
<td>Susp</td>
<td>85 (FN 15%)</td>
<td>62</td>
</tr>
</tbody>
</table>

Alexander 2012

Veracyte Validation Study

<table>
<thead>
<tr>
<th>GEC results</th>
<th>Malignant histology (n=85)</th>
<th>Benign histology (n=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susp</td>
<td>78</td>
<td>87</td>
</tr>
<tr>
<td>Benign</td>
<td>7</td>
<td>93</td>
</tr>
</tbody>
</table>

- Sens 92%
- Spec 52%
- PPV 47%
- NPV 93%
- Prevalence of cancer 32%
- Accuracy 65%

- Moving “tumors of UMP” from the “benign” to the “malignant” category significantly impacts NPV
Veracyte Validation Study- *Elsheikh modified*

<table>
<thead>
<tr>
<th>GEC results</th>
<th>Malignant histology (n=105)</th>
<th>Benign histology (n=160)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susp</td>
<td>28/89</td>
<td>22/76</td>
</tr>
<tr>
<td>Benign</td>
<td>2/16</td>
<td>2/84</td>
</tr>
</tbody>
</table>

- Sens: 92% 84%
- Spec: 52%
- PPV: 47% 57%
- NPV: 93% 84%
- Prevalence of cancer: 32% 39%
- Accuracy: 65%

- Decreased NPV most likely reflection of increased cancer prevalence, as accuracy of test remained the same

---

**How Can We Incorporate Molecular Tests Into Our Practice Today?**

- Consider only if results significantly change management
  - Observe vs. surgery or extent of surgery
- **AUS/FLUS (5-10% cancer risk)**
  - Only if considering surgery, i.e. if repeat FNA is AUS/FLUS
  - Veracyte performs best (high NPV) to rule in benign Dx
How Can We Incorporate Molecular Tests Into Our Practice Today?

- **Neoplasm** (20-30% cancer risk)
  - Veracyte if clinically low risk disease
  - Asuragen if clinically high risk disease
  - Perhaps combination of both. Cost?? ($2200-3000 per panel)

- **Susp. For malignancy** (60-75% cancer risk)
  - Limited to no value
  - Asuragen performs best (high PPV)
  - May help confirm extent of surgery, i.e. BRAF+

**micro RNA expression**

- Initial studies showed predictive diagnostic accuracies ranging from 76-90%
- No large scale prospective trial has been performed yet
- Not commercially available
- Available only through research protocols

**Future Trends in Molecular Testing**

- Ultimate goal is effectively differentiating benign from malignant thyroid lesions
- This may eventually be achieved by:
  1. Combination of cancer mutations and GEP
  2. Identification of new mutations and additional miRNA expression profiles
  3. Next-generation sequencing analysis of benign and malignant lesions
Summary

• FNA can provide a definitive Dx in most instances
• Thyroid FNA, however, is primarily a screening tool, therefore a conclusive Dx is not always required
• Pathologist’s role: minimize # of indeterminate diagnoses without yielding an unacceptably high false negative rate

Summary 2

• The use of the term “Atypical” or “Indeterminate” as a stand alone diagnosis is not recommended. Its meaning is not standardized and may be interpreted in different ways
• Dx should be qualified, when applicable, with appropriate differential diagnosis
• As cost falls and discriminant capability of molecular testing improves, it is anticipated that it will become standard practice
Thank You