The Bethesda Indeterminate Categories: An Update to Diagnosis and Molecular Testing

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Disclosure of Relevant Financial Relationships

No relevant disclosures

WC Faquin, MD, PhD
The Bethesda System for Reporting Thyroid Cytopathology

- Most widely used reporting system for thyroid cytopathology in the world
- Translated into 4 languages
- Has helped to revolutionize the practice of thyroid cytopathology and prepare it for the application of molecular diagnostics
## Bethesda Terminology: Relationship to Clinical Algorithms

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

<table>
<thead>
<tr>
<th>Bethesda Terminology</th>
<th>Clinical Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUS/FLUS</td>
<td>Repeat FNA</td>
</tr>
<tr>
<td>Susp for Follicular Neoplasm</td>
<td>Lobectomy</td>
</tr>
<tr>
<td>Susp for Hurthle Cell Neoplasm</td>
<td>Lobectomy</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>Lobectomy/ Total Thyroid</td>
</tr>
<tr>
<td>Malignant</td>
<td>Total Thyroid</td>
</tr>
</tbody>
</table>
Are adjustments needed as we prepare for the second edition of TBSRTC, and if so which ones???
IAC- Yokohama, Japan 2016
The Bethesda System for Reporting Thyroid Cytopathology: Proposed Modifications and Updates for the Second Edition from an International Panel

Marc Pusztaszeri, Esther Diana Rossi, Manon Auger, Zubair Baloch, Justin Bishop, Massimo Bongiovanni, Ashish Chandra, Beatrix Cochand-Priollet, Guido Fadda, Mitsuyoshi Hirokawa, SoonWon Hong, Kennichi Kakudo, Jeffrey F. Krane, Ritu Nayar, Sareh Parangi, Fernando Schmitt, William C. Faquin

Address for correspondence: Marc Pusztaszeri, M.D., Department of Pathology, Genoa University Hospitals, Genova, Switzerland, email: marc.pusztaszeri@unige.ch
What are the prospects for the second edition of TBSRTC Atlas?

• Many advances, large amount of published literature, and new questions for TBSRTC:
  • Maintain 6 diagnostic category designations
  • Diagnostic category names – continue with multiple options
  • Refinements to the ROM for each corresponding diagnostic category
  • 2015 ATA Guidelines – impact on clinical management
  • NIFTP and its impact on the indeterminate categories
  • Include newly described thyroid entities
  • Other minor adjustments within each category
AUS/FLUS Updates since 2009:

- AUS and FLUS are synonymous terms
- 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 testing
- Subclassification to help guide management
<table>
<thead>
<tr>
<th>Category</th>
<th>2009 ROM</th>
<th>Revised ROM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic</td>
<td>0-4%</td>
<td>5-10%</td>
</tr>
<tr>
<td>Benign</td>
<td>0-3%</td>
<td>0-3%</td>
</tr>
<tr>
<td>AUS/FLUS</td>
<td>5-15%</td>
<td>10-30%</td>
</tr>
<tr>
<td>Susp for Follicular Neoplasm</td>
<td>15-30%</td>
<td>25-40%</td>
</tr>
<tr>
<td>Susp for Hurthle Cell Neoplasm</td>
<td>15-30%</td>
<td>25-40%</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>60-75%</td>
<td>50-75%</td>
</tr>
<tr>
<td>Malignant</td>
<td>97-99%</td>
<td>97-99%</td>
</tr>
</tbody>
</table>

ROM UPDATES IN NEXT BETHESDA
<table>
<thead>
<tr>
<th>Category</th>
<th>Management</th>
<th>ROM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Diagnostic</td>
<td>Repeat with U/S</td>
<td>0-4%</td>
</tr>
<tr>
<td>Benign</td>
<td>Clin + U/S 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</td>
<td>97-99%</td>
</tr>
</tbody>
</table>
What is the role for ancillary testing of thyroid FNAs?
TBSRTC and Improved Diagnostic Accuracy Using Commercial Molecular Tests

- Afirma, Thyroseq V2, ThyGenX-ThyraMIR, RosettaGX
- Useful for “indeterminate” thyroid FNAs
  - 15-30% of FNAs
- Increase the pre-operative diagnostic accuracy of thyroid FNA
- May be useful to address NIFTP
## 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>
Molecular Testing and Thyroid FNA

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

**CONS:**
- Expensive if inappropriately applied
- Reflex testing
  - Takes clinician out of picture
  - Can add to overall expense (unnecessary testing)
  - Potential loss of cyto-histo correlation
Preoperative Diagnosis of Benign Thyroid Nodules with Indeterminate Cytology

The Afirm 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**
  - Prospective multicenter study (49 US cities)
- **Overall NPV = 93%**
- **False negative rate of 8.2%**
  - Possibly due to inadequate sample RNA
Sample Afirma Report

PATIENT: Jane Doe  
DOB: 25 Nov 1941  
Gender: F  
Lab ID:  
MRN: AA1234

REPORT INFORMATION:  
Collection Date: 05 May 2014  
Submitting Clinician: John Smith  
Treating Clinician:  
Received Date: 06 May 2014  
Phone #: 555.123.1111  
Fax #: 555.123.2222  
Report Date: 22 May 2014  
Clinic Name: Thyroid & Endocrine Center of Anytown  
Report CC:  
Affirma Req #: AA1234

CLINICAL HISTORY:  
Suspicious Ultrasound Characteristics: Nodule A: Hypoechoic, Microcalcifications  
Nodule B: Irregular Border, Microcalcifications, Intranodular vascular pattern

RESULTS SUMMARY

<table>
<thead>
<tr>
<th>Nodule</th>
<th>Size</th>
<th>Location</th>
<th>Cytopathology</th>
<th>Afirma Gene Expression Classifier</th>
<th>Afirma MTC</th>
<th>Afirma BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.4 cm</td>
<td>Lower Right</td>
<td>Benign</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>B</td>
<td>1.6 cm</td>
<td>Upper Left</td>
<td>Indeterminate</td>
<td>Benign</td>
<td>Neg.</td>
<td>N/A</td>
</tr>
</tbody>
</table>

RESULT DETAILS

NODULE A

Cytopathology Diagnosis: Benign (Bethesda Category II)  
Diagnostic Comments: The features are consistent with a benign hyperplastic/adenomatoid nodule  
Microscopic Description: The cytologic preparations are moderately cellular and show sheets and clusters of follicular cells, some macrophages and colloid

NODULE B

Cytopathology Diagnosis: Indeterminate - Atypia of Undetermined Significance (AUS - Bethesda Category III)  
Diagnostic Comments: These features are best classified as atypia of undetermined significance (Bethesda Category III)  
Microscopic Description: The cytologic preparations are sparsely cellular and show a few scattered clusters of enlarged follicular cells in crowded groups with some colloid

Afirma GEC Result: Benign  
Afirma MTC Result: Negative
The Afierna 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

Next Generation Sequencing Panel for Thyroid Cancer (ThyroSeq)

Specimen Type: FNA

RESULTS:

A. NRAS mutation POSITIVE (p.Q61R, c.182A>G), see interpretation below.
B. TERT promoter mutation POSITIVE (c.1-124C>T; 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 (6-8). 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 (6,9) and with higher risk of distant metastases and disease persistence (8). 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
ThyroSeq v.2

Next generation sequencing gene mutation panels
- Mutations in 14 genes
- 48 gene fusions
- Single institution study
- Cost: $3200
- Histologists not blinded to molecular test results

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%
- 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!
- ThyGenX:
  - NGS 8-gene panel with high PPV
  - PPV: 88% (AUS/FLUS), 87% (FN), 95% (Susp M)
  - NPV: 94% (AUS/FLUS), 86% (FN), 72% (Susp M)
- ThyraMIR to complement ThyGenX:
  - Micro-RNA based GEC
  - Recommended for ThyGenX negative cases
- NPV: 94%
- PPV: 74%
RosettaGX Reveal

Comparison:

- **Others**: Additional Biopsy Required
- **RosettaGX REVEAL**: Utilize Existing Smear, No Additional Biopsy Required
RosettaGX Reveal

Stained FNA Smear → RNA extraction → microRNA profiling → RosettaGX Reveal™ Algorithm

Suspicious for Malignancy
Positive for Medullary Marker
Benign

RosettaGX Reveal™ Validation Data

<table>
<thead>
<tr>
<th>Bethesda III, IV, V (Indeterminate Thyroid FNAs)</th>
<th>NPV</th>
<th>PPV</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Agreement Set (n=150)*</td>
<td>99%</td>
<td>62%</td>
<td>98%</td>
<td>78%</td>
</tr>
<tr>
<td>Entire Validation Set (n=189)*</td>
<td>91%</td>
<td>59%</td>
<td>85%</td>
<td>72%</td>
</tr>
</tbody>
</table>
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 ROM Based on NIFTP

Zubair W. Baloch M.D., Ph.D.\textsuperscript{a*}, David S. Cooper, M.D.\textsuperscript{b}, Hossein Gharib M.D.\textsuperscript{c} and Erik K. Alexander M.D.\textsuperscript{d}

<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>45-60%</td>
<td>Optional Note</td>
</tr>
<tr>
<td>Malignant</td>
<td>94-96%</td>
<td>Optional Note</td>
</tr>
</tbody>
</table>
An option is to add a NIFTP note on the cytology report for selected categories

**FN/SUSP FN:** “The histopathologic follow-up of cases diagnosed as such includes follicular adenoma, follicular carcinoma, and follicular variant of PTC, including the recently described indolent counterpart NIFTP.”

**SUSP MAL:** “The cytomorphologic features are suspicious for follicular variant of PTC and its recently described indolent counterpart NIFTP.”

**MALIGNANT:** “A small proportion of cases (~3-4%) diagnosed as malignant – compatible with PTC, may prove to be NIFTP on histopathologic examination.”
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 FNAs:
  - FN with atypia vs Susp Malignancy
  - Avoid diagnosing follicular-patterned PTC as Malignant
- Put an optional note about possible NIFTP on selected cases
- Rely more on pre-op molecular testing (BRAF vs RAS)
- Increase clinical threshold for performing TT
NIFTP: Molecular Profile

- RAS mutations
- BRAF K601E mutation
- PPARgamma fusion
- THADA fusion
- BRAF V600E is essentially absent
Molecular testing panels (e.g. high PPV or NPV) identify NIFTP cases as “abnormal”
Most NIFTP are detected 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
Most FP diagnoses of NIFTP can be avoided
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!