The Bethesda System for Reporting Thyroid Cytopathology, 2017

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In Summary...

- No *major* changes for cytologists....

- The clinical team is faced with different risk of malignancies (ROM) associated with for Bethesda categories III to VI, especially III and IV (NIFTP constitutes a substantial proportion of the malignancies hidden in these categories).
I. Nondiagnostic or Unsatisfactory
   Cyst fluid only
   Virtually acellular specimen
   Other (obscuring blood, clotting artifact, etc)
II. Benign
   Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc)
   Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context
   Consistent with granulomatous (subacute) thyroiditis
   Other
III. Atypia of undetermined significance or follicular lesion of undetermined significance
IV. Follicular neoplasm or suspicious for a follicular neoplasm
   Specify if Hürthle cell (oncocytic) type
V. Suspicious for malignancy
   Suspicious for papillary carcinoma
   Suspicious for medullary carcinoma
   Suspicious for metastatic carcinoma
   Suspicious for lymphoma
   Other
VI. Malignant
   Papillary thyroid carcinoma
   Poorly differentiated carcinoma
   Medullary thyroid carcinoma
   Undifferentiated (anaplastic) carcinoma
   Squamous cell carcinoma
   Carcinoma with mixed features (specify)
   Metastatic carcinoma
   Non-Hodgkin lymphoma
   Other
<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Risk of malignancy if NIFTP ≠ CA (%)</th>
<th>Risk of malignancy if NIFTP = CA (%)</th>
<th>Usual management*³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic or Unsatisfactory</td>
<td>5-10</td>
<td>5-10</td>
<td>Repeat FNA with ultrasound guidance Clinical and sonographic follow-up</td>
</tr>
<tr>
<td>Benign</td>
<td>0-3</td>
<td>0-3</td>
<td>Repeat FNA, molecular testing, or lobectomy</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance</td>
<td>6-18</td>
<td>10-30</td>
<td>Molecular testing, lobectomy</td>
</tr>
<tr>
<td>Follicular Neoplasm or Suspicious for a Follicular Neoplasm</td>
<td>10-40</td>
<td>25-40</td>
<td>Near-total thyroidectomy or lobectomy*⁵,c</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>45-60</td>
<td>50-75</td>
<td>Near-total thyroidectomy or lobectomy*⁵,c</td>
</tr>
<tr>
<td>Malignant</td>
<td>94-96</td>
<td>97-99</td>
<td>Near-total thyroidectomy or lobectomy*⁵,c</td>
</tr>
</tbody>
</table>

Abbreviations: NIFTP, non-invasive follicular thyroid neoplasm with papillary-like nuclear features; CA, carcinoma; FNA, fine-needle aspiration.

*Actual management may depend on other factors (eg, clinical, sonographic) besides the FNA interpretation.

*bSome studies have recommended molecular analysis to assess the type of surgical procedure (lobectomy versus total thyroidectomy).

*cIn the case of “Suspicious for metastatic tumor” or a “Malignant” interpretation indicating metastatic tumor rather than a primary thyroid malignancy, surgery may not be indicated.

Adapted from Ali and Cibas with permission of Springer.
Non-diagnostic/Unsatisfactory

Bethesda Category I
• Any sample with significant cytologic atypia is adequate (a minimum number of follicular cells is not required).

• Any sample with abundant colloid is adequate and benign.

• Nodules in patients with lymphocytic thyroiditis, abscess, or granulomatous thyroiditis do not need a minimum number of follicular cells.

*Preliminary data suggest that requiring a smaller number of follicular cells would significantly reduce ND interpretations without significantly reducing the false-negative rate.*
Benign Follicular Nodule

Bethesda Category I
Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance

Bethesda Category III
• The provisional goal of limiting AUS/FLUS interpretations to 7% of all thyroid FNA interpretations is increased to 10%.

• The AUS/FLUS to malignant ratio may be a useful laboratory quality measure that should not exceed 3.0.

• Narrative comments are strongly recommended to further describe the findings, especially if it would potentially influence management.

• The possibility of a compromised sample with artifactual changes should be acknowledged in the report.
Atypia

Cytologic
Architectural
Cytologic and architectural
Hurthle cell aspirates
Atypia, NOS
Cytologic Atypia
Cytologic Atypia
Architectural Atypia
Cytologic & Architectural atypia
Hurthle-cell “Aspirates”
Atypia, NOS
• AUS/FLUS aspirates with **cytologic** (nuclear) atypia have an approximately twofold higher risk of malignancy (ROM) compared with cases with architectural atypia.

• Rare pseudoinclusions by themselves may prompt an AUS/FLUS diagnosis, but if accompanied by other compelling features of PTC, the case should be considered “suspicious for malignancy”.
• Hurthle cell type AUS/FLUS has a lower ROM than other atypical patterns.

• Considering the rarity of Hurthle cell carcinoma in a background of lymphocytic thyroiditis, cases with obvious Hashimoto thyroiditis and an atypical collection of Hurthle cells should typically be diagnosed as benign.

• It is acceptable to diagnose a moderately to markedly cellular sample composed exclusively of Hurthle cells yet the clinical setting suggests a benign Hurthle cell nodule, such as lymphocytic thyroiditis or a multinodular goiter, as AUS/FLUS.
• With the introduction of NIFTP entity, early data suggests that the ROM for this category may be reduced by as much as 45%.

• Aspirates with a pattern highly associated with the follicular variant of PTC/NIFTP (diffuse but subtle nuclear enlargement, focal nuclear irregularity, only occasional nuclear grooves, and a microfollicular architecture) are better classified as “suspicious for malignancy” when nuclear alterations are prominent, or “suspicious for a follicular neoplasm” when microfollicular architecture is more pronounced.
Follicular Neoplasm/Suspicious for a Follicular Neoplasm

Bethesda Category IV
IV

• Each lab should choose the name it prefers and use it exclusively for that category.

• The goal of this category is to identify all potential follicular carcinomas.

• Follicular-patterned aspirates with mild nuclear changes can be classified as FN/SFN so long as true papillae and intra-nuclear pseudoinclusions are absent.
IV

- Although FNA is highly sensitive for detecting oncocytic carcinomas, its specificity is low; most nodules diagnosed as FNHCT/SFNHCT are benign (ROM: 10-40%).

- It is advisable to use the guidelines of the WHO, which consider only those follicular neoplasms that are composed of >75% Hurthle cells to be a Hurthle-cell neoplasm.

- With lower percentages, a practical solution is to diagnose those aspirates as FN/SFN, with a comment that “there is some Hurthle cell differentiation, and therefore, a Hurthle cell neoplasm can not be ruled out”.
• The criteria for FNHCT/SFNHCT have lower predictive value for malignancy when a patient has *lymphocytic thyroiditis* or *multinodular goiter*.

• It is acceptable to interpret as AUS/FLUS, with a note explaining that “benign Hurthle-cell hyperplasia is favored”.

The goal is to provide the clinical team with the opportunity to avoid an unnecessary lobectomy in some of these patients....
Suspicious for Malignancy

Bethesda Category V
• Most FVPTCs/NIFTPs are diagnosed cytologically as either **SFM** (25-35%), **FN/SFN** (25-30%), or **AUS/FLUS** (10-20%).

• The malignancy risk of the SFM category falls to approximately 50% (range 45-60%) when NIFTPs are not counted as malignant.

• It remains to be seen whether any collection of cytologic features is sufficiently reliable to allow prospective identification of NIFTP and its distinction from an invasive FVPTC by FNA alone.
• ATA initiatives to reduce the extent of surgery for many low-risk thyroid cancers (4 cm or smaller, without extra-thyroidal extension and lacking regional nodal metastasis) and decreased routine use of postoperative radioactive iodine treatment increasingly raise the possibility for lobectomy as initial surgical management.

• The exact clinical and surgical impact of re-classification of NIFTP is yet to be determined, but clearly will help push the pendulum toward consideration of a more conservative initial surgical procedure in many circumstances.
• The ultimate goal of separating a “suspicious” from “malignant” category is to preserve the very high PPV of the malignant category without compromising the overall sensitivity of FNA.
Malignant

Bethesda Category VI
VI

- NIFTP comprises approximately 20-25% of all thyroid tumors previously classified as malignant.

- Taking into consideration the reclassification of some PTCs as NIFTP, when a definitive diagnosis of PTC is made by FNA, 94-96% prove to be PTC on histologic follow-up (drop from previous 99% PPV).

- It is desirable to eliminate tumors likely to represent NIFTP.
• Preliminary data suggest that a definitive ("malignant") diagnosis of PTC should be reserved for cases that have, in addition to other characteristic features, at least one of the following: papillary architecture, psammoma bodies, and INCIs.

• A suspected PTC with an exclusively follicular architecture, especially one that lacks such features (e.g. many follicular variants of PTC), is best interpreted as "suspicious for malignancy" rather than malignant.
“Nevertheless, given the histologic criteria for NIFTP, it is unlikely that NIFTPs can be completely eliminated from the malignant category”....
Overview of Commonly Used Molecular Tests
### Gene mutations in thyroid tumours

<table>
<thead>
<tr>
<th>Mutations</th>
<th>Types of thyroid tumours</th>
<th>Approximate prevalence (%)</th>
<th>Primary signalling pathways affected</th>
<th>Functional impact on the protein and tumour</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRAF</strong> V600E</td>
<td>CPTC</td>
<td>45</td>
<td>MAPK</td>
<td>Activating; promoting tumorigenesis, invasion, metastasis, recurrence and mortality</td>
</tr>
<tr>
<td></td>
<td>FVPTC</td>
<td>15</td>
<td>MAPK</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TCPTC</td>
<td>80–100</td>
<td>MAPK</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATC</td>
<td>25</td>
<td>MAPK</td>
<td></td>
</tr>
<tr>
<td><strong>BRAF</strong> V600E</td>
<td>FVPTC</td>
<td>5</td>
<td>MAPK</td>
<td>Activating; probably similar to <strong>BRAF</strong> V600E</td>
</tr>
<tr>
<td><strong>HRAS, KRAS, NRAS</strong></td>
<td>FTA</td>
<td>20–25</td>
<td>MAPK and PI3K–AKT</td>
<td>Activating; promoting tumorigenesis, invasion and metastasis of PDTC and FTC</td>
</tr>
<tr>
<td></td>
<td>FTC</td>
<td>30–45</td>
<td>MAPK and PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>FVPTC</td>
<td>30–45</td>
<td>MAPK and PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PDTC</td>
<td>20–40</td>
<td>MAPK and PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATC</td>
<td>20–30</td>
<td>MAPK and PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td><strong>PTEN</strong>(mutation)</td>
<td>FTA</td>
<td>0</td>
<td>PI3K–AKT</td>
<td>Inactivating the gene but activating the PI3K pathway; promoting tumorigenesis and invasiveness</td>
</tr>
<tr>
<td></td>
<td>FTC</td>
<td>10–15</td>
<td>PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATC</td>
<td>10–20</td>
<td>PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTC</td>
<td>1–2</td>
<td>PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td><strong>PTEN</strong>(deletion)</td>
<td>FTC</td>
<td>30</td>
<td>PI3K–AKT</td>
<td>Inactivating the gene but activating the PI3K pathway; promoting tumorigenesis and invasiveness</td>
</tr>
<tr>
<td><strong>PIK3CA</strong></td>
<td>FTA</td>
<td>0–5</td>
<td>PI3K–AKT</td>
<td>Activating; promoting tumorigenesis and invasiveness</td>
</tr>
<tr>
<td></td>
<td>FTC</td>
<td>5–15</td>
<td>PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATC</td>
<td>15–25</td>
<td>PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PTC</td>
<td>1–2</td>
<td>PI3K–AKT</td>
<td></td>
</tr>
<tr>
<td><strong>AKT1</strong></td>
<td>Metastatic cancer</td>
<td>15</td>
<td>PI3K–AKT</td>
<td>Unclear; seems to favour metastasis</td>
</tr>
<tr>
<td><strong>CTNNB1</strong></td>
<td>PDTC</td>
<td>25</td>
<td>WNT–β-catenin</td>
<td>Activating; promoting tumour progression</td>
</tr>
<tr>
<td></td>
<td>ATC</td>
<td>60–65</td>
<td>WNT–β-catenin</td>
<td></td>
</tr>
<tr>
<td><strong>TP53</strong></td>
<td>PDTC</td>
<td>25</td>
<td>p53-coupled pathways</td>
<td>Inactivating; promoting tumour progression</td>
</tr>
<tr>
<td></td>
<td>ATC</td>
<td>70–80</td>
<td>p53-coupled pathways</td>
<td></td>
</tr>
</tbody>
</table>
AFIRMA Gene Expression Classifier

- Microarray technology used to analyze the mRNA expression of 167 genes (the selected gene profile is based on the gene expression identified from FNAs of surgically proven benign and malignant thyroid nodules).
- Only AUS/FLUS and FN/SFN- SFNTCT/FNHCT cases are accepted.
- Needs two dedicated passes, in a nucleic acid preservative solution.
- Reported as *benign* or *suspicious*.
- Can pickup parathyroid origin.
AMCs

• Later on, Veracyte introduced the *Afirm*na Malignancy Classifiers (AMCs) to further enhance the test as a comprehensive diagnostic tool to assess the risk of malignancy including medullary thyroid carcinoma.

• Include an mRNA profile for medullary carcinoma and/or BRAF V600E gene mutation.

• Only performed on FNA samples carrying a suspicious and malignant cytomorphologic diagnosis, or a suspicious AFIRMA GEC result.
AFIRMA

- High negative predictive value (NPV) for nodules in the AUS/FLUS and FN/SFN categories (almost equal to the NPV of an FNA morphologically diagnosed as benign); “rule out” test.

- Then NPV in FNAs morphologically diagnosed as “suspicious for malignancy” is much lower.

- Low positive predictive value (PPV) for nodules in the AUS/FLUS and FN/SFN categories.

- Tendency to report a high percentage of benign Hurthle-cell nodules as suspicious.
Thyroseq Test

- Next generation sequencing (NGC) based mutation and fusion panel initially designed to target 12 cancer genes and 284 mutational hot spots. The enhanced version (Thyroseq v2) includes a more extensive panel of DNA alterations (14 genes, including >1000 mutations) and RNA alterations (42 fusions, 16 genes for expression).

- Only AUS/FLUS and FN/SFN- SFNTCT/FNHCT are accepted.

- Needs 1-2 drops from the first pass if adequate cellularity on, stored at -20.

- Reported as specific gene mutation/translocation.

- Can pickup parathyroid origin.
Thyroseq

• High positive predictive value (PPV); “rule in” test.
• Low negative predictive value (NPV).

The new version (v2) has been claimed to have improved NPV, enough to potentially function as a “rule out” test as well (the exception would be a population with a very high pre-test probability of malignancy).

Potential increased chance of detecting “false-positive” molecular abnormalities associated with the expanded NGS-based mutational profile; i.e. lower PPV compared to the original version.
Important To Remember

- While sensitivity and specificity are intrinsic characteristics, the PPV and NPV of any test depends on the prevalence of disease in the tested population/practice setting.

- The PPVs are expected to decrease for all currently available molecular tests, as NIFTP was considered a malignant neoplasm in the validation studies and subsequent studies.
Current ATA Guidelines

• The molecular testing should be performed in a CLIA/CAP-certified molecular laboratory, or international equivalent.

• There is currently no single optimal molecular test that can definitely rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data providing clinical utility are required.
Current ATA Guidelines

• For nodules with AUS/FLUS cytology, after consideration of clinical and sonographic features, investigations such as repeat FNA or molecular testing *may* be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery.

• For nodules with FN/SFN cytology, after consideration of clinical and sonographic features, molecular testing *may* be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery.
Thank You