THYROID CYTOLOGY

Basic Facts

- Thyroid nodules are common – any thyroid disease can present as a nodule – most are predominantly benign.
- Inability to distinguish malignant from benign thyroid nodules with non invasive techniques.
- FNA has proven to be the single most accurate diagnostic test for patients with thyroid nodules: 50% decrease in thyroid surgeries and doubling of % of thyroid cancer detected at surgery.

THYROID CYTOLOGY

How to Perform the FNA: Palpation-guided vs. US-guided

- Either method is acceptable
- An increasing number of FNAs are being performed using US guidance
- Benefits of palpation guidance
  - Reduced cost
  - Logistical efficiency
- Benefits of US evaluation and US guidance:
  - Reduces rate of unsatisfactory specimens
  - Reduces false-negatives

THYROID FNA: Technique

- The technique is simple in concept but the “devil” is as usual, in getting the “details” right.
- The majority of diagnostic problems are due to suboptimal sampling.
- There is a common misconception that FNA technique is simple/easy and requires little or no training beyond reading a description of the technique.
- Conventional smears are superior to thin layer techniques and cell blocks when used independently.

The Benefits of a Uniform Reporting System for Thyroid Cytopathology

- Improve communication
- Facilitate cytological-histological correlation
- Facilitate research into the epidemiology, molecular biology, pathology, and diagnosis of thyroid diseases
- Allow easy and reliable sharing of data from different laboratories for collaborative studies
Thy1/Thy1c
Non-diagnostic for cytological diagnosis
Unsatisfactory

Thy2/Thy2c
Non-neoplastic / Benign

Thy3a
Neoplasm possible – atypia/non-diagnostic

Thy3f
Neoplasm possible - suggesting follicular

Thy4
Suspicious of malignancy

Thy5
Malignant

General Principles
• Every interpretation should begin with a primary category.
• Unless “Insufficient”, the sample is presumed adequate.
• This is a flexible framework and can be modified by the lab to suit the needs of the referring physicians and their patients.
• The rationale for these categories is predicated on different risk associations for malignancy.

TBSRTC – DIAGNOSTIC CATEGORIES

• NONDIAGNOSTIC or UNSATISFACTORY

• BENIGN

• ATYPIA OF UNDETERMINED SIGNIFICANCE or FOLLICULAR LESION OF UNDETERMINED SIGNIFICANCE

• FOLLICULAR NEOPLASM or SUSPICIOUS FOR A FOLLICULAR NEOPLASM
  - specify if Hurthle cell (oncocytic) type

• SUSPICIOUS FOR MALIGNANCY

• MALIGNANT

TBSRTC- Probabilistic approach and Relationship to Clinical Algorithms

<table>
<thead>
<tr>
<th>Category</th>
<th>Risk of Malignancy (%)</th>
<th>Usual Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insufficient for Diagnosis</td>
<td>-</td>
<td>Repeat FNA w/ U/S</td>
</tr>
<tr>
<td>Benign</td>
<td>0-3</td>
<td>Follow</td>
</tr>
<tr>
<td>AUS or FLUS</td>
<td>~5-15</td>
<td>Repeat FNA or molecular testing</td>
</tr>
<tr>
<td>Follicular Neo or suspicious for a Follicular Neoplasm</td>
<td>15-30</td>
<td>Lobectomy or molecular testing</td>
</tr>
<tr>
<td>Suspicious for Malignancy (usually papillary CA)</td>
<td>60-75</td>
<td>Lobectomy or total thyroidectomy</td>
</tr>
<tr>
<td>Malignant</td>
<td>97-99</td>
<td>Total thyroidectomy</td>
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THE BETHESDA SYSTEM Impact of Molecular Testing

<table>
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<tr>
<th>Category</th>
<th>Risk of Malignancy (%)</th>
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TBSRTC – NON DIAGNOSTIC PPV 1-4%

• INCIDENCE: 2-20% (<10%)

• SPECIMEN PROCESSED AND EXAMINED

• ADEQUACY CRITERION
  ✓ At least 6 groups, each with at least 10 benign-appearing, well-visualized follicular cells.

• EXCEPTIONS
  ✓ Chronic lymphocytic thyroiditis
  ✓ Abundant colloid
  ✓ Any atypia
  ✓ REASPIRATE 3 mo (with U/S)
TBSRTC – NON DIAGNOSTIC

Recommendations

• Pure acellular heavy colloid
  - Aspirates composed only of pure heavy colloid may be followed without reaspiration (some conference participants considered these to be benign).

• Cystic aspirates with watery colloid, blood and histiocytes require correlation with ultrasound findings.

• If US has “concerning features”, a repeat FNA under US guidance should be performed at least 3 months later

• If repeat FNA is “Non-diagnostic”, correlation with family history and close clinical and US follow-up is appropriate.

TBSRTC – BENIGN

• INCIDENCE: 60-65%

• THIS CATEGORY INCLUDES
  - Hyperplastic/adenomatoid nodule.
  - Colloid nodule
  - Chronic lymphocytic thyroiditis
  - Graves’s disease

• F/U BY CLINICAL AND POSSIBLY US EXAMINATION

Putting an Eye on Cytological Specimens: An Audit of the Clinical Impact of Thyroid Fine-Needle Aspiration in Different Health Care Settings

Bernardo Dias Feres, M.D.¹, René Gehrke, M.D.²,³,⁴
and Fernando Schnirch, M.D.²,³,⁴

Table II. Bethesda Categories of 2005 Thyroid FNA Samples, Distributed by Type of Care

<table>
<thead>
<tr>
<th>Bethesda Category (%)</th>
<th>GP⁶</th>
<th>E²</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non diagnostic⁵</td>
<td>115/11.6</td>
<td>76/7.5</td>
<td>191/9.5</td>
</tr>
<tr>
<td>Benign¹</td>
<td>74/7.5</td>
<td>83/8.1</td>
<td>157/7.85</td>
</tr>
<tr>
<td>Atyia of undetermined significance</td>
<td>69/6.9</td>
<td>55/5.6</td>
<td>85/4.2</td>
</tr>
<tr>
<td>Follicular tumour</td>
<td>66/6.5</td>
<td>57/5.5</td>
<td>123/6.3</td>
</tr>
<tr>
<td>Suspicious for malignancy</td>
<td>60/6.0</td>
<td>80/8.8</td>
<td>140/7.0</td>
</tr>
<tr>
<td>Malignant</td>
<td>90/9</td>
<td>70/7</td>
<td>160/8</td>
</tr>
<tr>
<td>Total</td>
<td>991/100</td>
<td>1014/100</td>
<td>2005/100</td>
</tr>
</tbody>
</table>

*General Practitioners (GP) and Endocrinologists (E).
²Non-diagnostic (p² = 0.002) and “Benign” categories (p² < 0.001).

Analysis of Non-diagnostic Results in a Large Series of Thyroid Fine-Needle Aspiration Cytology

Table 2. Comparison of nondiagnostic results between the first and second repeated FNAC

<table>
<thead>
<tr>
<th></th>
<th>Nondiagnostic</th>
<th>Diagnostic</th>
<th>Total</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st repeat</td>
<td>98 (43.6%)</td>
<td>127 (56.4%)</td>
<td>225 (100.0%)</td>
<td>0.653</td>
</tr>
<tr>
<td>2nd repeat</td>
<td>8 (36.4%)</td>
<td>14 (63.6%)</td>
<td>22 (100.0%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>106 (42.9%)</td>
<td>141 (57.1%)</td>
<td>247 (100.0%)</td>
<td></td>
</tr>
</tbody>
</table>
**COLLOID NODULE**
- Sparsely to moderately cellular
- Abundant colloid
- Benign follicular cells (nuclear features of papillary CA absent)
- Predominantly macrofollicles

**Hashimoto thyroiditis**

**Subacute thyroiditis**  
(de Quervain)
- viral etiology, heredity (antigen HLA-B35)
- follows acute respiratory infection
- may be unilateral (single lobe)
- solitary nodule
- moderate cellularity
- cellular debris, small amounts of colloid, regressive changes of follicular cells
- lymphocytes, PMNs and macrophages
- multinucleated giant cells (reaction to colloid)
FOLLICULAR LESION OF UNDETERMINED SIGNIFICANCE
DO NOT FIT EASILY INTO BENIGN OR SUSPICIOUS CATEGORIES
RECOMMENDED MANAGEMENT: REPEAT FNA
AVOID OVERUSE OF THIS CATEGORY (7-10% BENCHMARK)
A SINGLE DIAGNOSIS CARRIES A LOW RISK
SURGERY CONSIDERED FOR REPEAT AUS CASES

TBSRTC – ATYPIA OF UNDETERMINED SIGNIFICANCE (AUS)

• FOLLICULAR LESION OF UNDETERMINED SIGNIFICANCE
• DO NOT FIT EASILY INTO BENIGN OR SUSPICIOUS CATEGORIES
• RECOMMENDED MANAGEMENT: REPEAT FNA
• AVOID OVERUSE OF THIS CATEGORY (7-10% BENCHMARK)
• A SINGLE DIAGNOSIS CARRIES A LOW RISK
• SURGERY CONSIDERED FOR REPEAT AUS CASES

AUS – Proposed Performance Measure

AUS:M

• Reviewed 8 published series
• AUS: 3-18% (MEDIAN -9.9%)
• AUS: M – 0.5-4.9% (MEDIAN – 2.0)
• RECOMMENDATION – MEDIAN RATIO OF 1.0-3.0
• AUS-M>3.0
  ✔ Overdiagnosis of AUS
  ✔ Under diagnosis of M

Why some specimens are classified as AUS/FLUS?

• Something is wrong with the specimen-poor preservation, drying, etc.
• There are disturbing features in the cells (nuclear enlargement, irregularity, clearing, rare grooves) but the amount of material is insufficient for diagnosis.
• Diffuse but mild nuclear clearing with incipient irregularities of the nuclear membrane, some crowding and pseudostratification.
TBSRTC – SUSPICIOUS FOR A FOLLICULAR NEOPLASM OR FOLLICULAR NEOPLASM

PPV 15-30%

- INCIDENCE: 7-18%
- SIGNIFICANT ARCHITECTURAL ATYPIA
  - A predominance of microfollicles and/or trabecula
- RAISING THE POSSIBILITY OF FOLLICULAR CARCINOMA
- DISTINCTION BETWEEN FOLLICULAR ADENOMA AND CARCINOMA
- SURGERY (usually lobectomy) IS NEEDED FOR DEFINITIVE DIAGNOSIS

TBSRTC – SUSPICIOUS FOR A HURTHLE CELL NEOPLASM

PPV 15-45%

- COMPOSED EXCLUSIVELY OF HURTHLE CELLS
- DIFFERENTIAL DIAGNOSIS IS DIFFERENT (MEDULLARY CA)
- DISTINCTION BETWEEN HCA AND HCC
- SURGERY (usually lobectomy) IS NEEDED FOR DEFINITIVE DIAGNOSIS
A category bearing high probability of malignancy with lack of quantity and/or quality/spectrum of diagnostic features of a particular malignancy in question. This classification indicates the possibility of a slightly protracted and less aggressive solution. (hemithyroidectomy, intraoperative frozen section).

Most primary thyroid malignancies possess diagnostic features allowing FNA diagnosis in an optimal sample or suspicious in a suboptimal one. Exceptions are represented by follicular and oncocytic lesions.

- SUSPICIOUS FOR PTC
- SUSPICIOUS FOR MEDULLARY CARCINOMA
  - Serum calcitonin level
- SUSPICIOUS FOR MALIGNANT LYMPHOMA
  - Recommendation to repeat FNA with flow cytometry
- SUSPICIOUS FOR METASTATIC CANCER

TBSRTC – SUSPICIOUS FOR MALIGNANCY
PPV 60-65% (up to 77%)

- PAPILLARY CARCINOMA (including variants)
- MEDULLARY CARCINOMA
- POORLY DIFFERENTIATED CARCINOMA
- ANAPLASTIC CARCINOMA
- LYMPHOMA
- METASTATIC CANCERS
- OTHERS
PAPILLARY CARCINOMA

“A malignant epithelial tumour showing evidence of follicular cell differentiation and characterized by distinctive nuclear features”

WHO 2004

Endocrine Pathology Society Recommends Re-naming the Non-invasive FVPTC as...

“Non-invasive follicular thyroid neoplasm with papillary-like nuclear features” (NIFT)

Note: This is a neoplasm of very low malignant potential. Studies indicate that no further surgery after complete excision and no RAI therapy is required for the majority of these lesions based on poor studies of non-invasive encapsulated follicular variant of papillary thyroid carcinoma.

ANAPLASTIC CARCINOMA

- elderly patients
- large and rapidly growing mass
- extrathyroidal spread frequent
- bad prognosis

MEDULLARY CARCINOMA

- moderately to highly cellular smears
- poorly cohesive cells, absence of colloid
- fragments of amyloid (1/4 cases)
- round, oval, triangular, spindle cells

ANAPLASTIC CARCINOMA

- high cellularity
- absence of colloid
- necrotic debris and neutrophils
- poorly cohesive cell groups
- large polygonal cells
- spindle cells
- bi- or multinucleated cells
- Pan-cytokeratin +
- PS1 +
- Tg, TTF1 -
POORELY DIFFERENTIATED CARCINOMA

- High cellularity
- Poorly cohesive cell groups
- Microfollicles or papillary clusters
- Presence of necrosis
- Absence of bizarre cells

Schmitt et al. Cytopathology 1996

METASTASIS TO THYROID GLAND

- rare (24% in autopsies)
- direct growth (larynx, hypopharynx, esophagus)
- hematogenous – tumor generalization
- solitary metastasis
- renal, lung, breast, GI ca, melanoma

PARATHYROID LESIONS - FNA

Multidisciplinary approach:
- US
- FNA: “water liquid”
- Biochemistry: PTH

Hashimoto Thyroiditis vs. Lymphoma

- relatively rare
- 2% of extranodal lymphomas
- 5% of all thyroid malignancies
- thyroid enlargement, growth of nodule
- virtually always in the background of HT
- non-Hodgkin ML, 98% B cells
- high-grade transformation from MALT-lymphoma
- Immunophenotyping and flow cytometry

TBSRTC – DIAGNOSTIC CATEGORIES

- NONDIAGNOSTIC or UNSATISFACTORY
- BENIGN
  - ATYPIA OF UNDETERMINED SIGNIFICANCE or FOLLICULAR LESION OF UNDETERMINED SIGNIFICANCE
  - FOLLICULAR NEOPLASM or SUSPICIOUS FOR A FOLLICULAR NEOPLASM
    - specify if Hurthle cell (oncocytic) type
  - SUSPICIOUS FOR MALIGNANCY
  - MALIGNANT

- Problems not solvable by cytology
  - Patients with indeterminate cytology typically undergo a lobectomy.
  - After malignancy is established by histopathology these patients require to complete the thyroidectomy with additional costs and morbidity.
  - In addition, 1-3% of nodules diagnosed as benign by FNA are later found to be malignant.
  - Therefore, additional methods to improve the sensitivity and specificity of FNA diagnosis are highly desirable.
**THE IDEAL TEST**

- **TEST +**  → HIGH PROBABILITY OF MALIGNANCY
  - BRAF
  - RET/PTC
  - RAS
  - PAX8-PPAR GAMMA mRNA (miRInform)
  - Avoid SURGERY

- **TEST +**  → LOW PROBABILITY OF MALIGNANCY
  - AFIRMA GEC
  - BRAF mutation are not randomly distributed by PTC, it is especially observed in the classic variant (up to 69% vs 20% of follicular variant).

---

Detection of BRAF mutation using ICC

- BRAF V63 monoclonal antibody is able to detect BRAF V600E mutation in malignant melanoma, lung carcinoma, GI carcinoma, PTC and gliomas with a sensitivity of 98% and specificity of 97%.

- BRAFV600E mutations in FNA specimens (cell blocks) from PTC can be reliably detected by ICC with sensitivity and specificity of 93.8%.

- A study on LBC specimens from PTC found 81.5% of sensitivity and 100% of specificity in cases with moderate to intense staining.

- Problems: quantification of immunostaining, other BRAF mutations, heterogeneity, still lack validation in large series.

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BRAF Mutations

- Most prevalent oncogenic mutation in PTC (V600E)
- BRAF mutation are not randomly distributed by PTC, it is especially observed in the classic variant (up to 69% vs 20% of follicular variant).

---

**BRAF and morphological features on Thyroid FNA**

- BRAF mutation is associated with some specific morphological features which can be observed on thyroid FNA.

- The presence of these morphological features provide important insights in selecting cases for BRAF molecular analysis.

- BRAF mutation can be used as a pre-operative risk stratification for PTC.
“INDETERMINATE” THYROID NODULE
Molecular Diagnostic Tests

• A robust molecular marker to distinguish benign from malignant would be a useful adjunct to cytology.

• Molecular testing of thyroid FNA is already impacting clinical practice in some countries.

• 2013 NCCN guidelines: “consider molecular testing” for AUS/FLUS and FN/Susp. For FN.

• Rule-in and rule-out tests: Thyroseq v.2 and Afirma

• Decision generally not in the hands of the pathologists!

ThyroSeq and Afirma

<table>
<thead>
<tr>
<th></th>
<th>ThyroSeq</th>
<th>Afirma</th>
</tr>
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<tbody>
<tr>
<td>Approach</td>
<td>Next generation sequencing (NGS) for mutations in 13 genes and 42 gene fusions (known thyroid cancer genes)</td>
<td>Gene expression classifier trained and optimized for high negative predictive value (NPV)</td>
</tr>
<tr>
<td>Study design</td>
<td>Single institution, retrospective</td>
<td>Multicenter, prospective</td>
</tr>
<tr>
<td>Adjudicated histopathology?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Histopathology reviewed without knowledge of molecular results?</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>NPV established for:</td>
<td>FN/SFN</td>
<td>AUS/FLUS and FN/SFN</td>
</tr>
<tr>
<td>Marketed by:</td>
<td>CRLPath (NY)</td>
<td>Veracyte (CA)</td>
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Can a Gene-Expression Classifier With High Negative Predictive Value Solve the Indeterminate Thyroid Fine-Needle Aspiration Dilemma?

William C. Fajardo, MD, FAC

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• Despite the limitations of all the ancillary methods it is likely that in the future the number of indeterminate cases on FNA of thyroid will decline.

• However, it is worth remembering that even the histological criteria used for the diagnosis of these lesions are not entirely accurate.

• The histological diagnosis of adenomatoid goiter, follicular adenomas and carcinomas and FVPC also have problems of reproducibility. With this in mind, it is not surprising that different institutions may have different histological diagnoses for the same cytological diagnosis.

SUCCESS IN THYROID CYTOLOGY

• Service minded – availability

• Sampling by training cytopathologist (preferentially)

• Quick staining to avoid insufficient material

• Application of ancillary techniques (when necessary)

• Close contact with clinicians

• Triple diagnosis