Thyroid Cytopathology: What’s New and What’s Old That We Don’t All Agree on?

RITU NAYAR, MD
PROFESSOR & VICE CHAIR OF PATHOLOGY
NORTHWESTERN UNIVERSITY FEINBERG SCHOOL OF MEDICINE
DIRECTOR OF CYTOPATHOLOGY, NORTHWESTERN MEMORIAL HOSPITAL, CHICAGO, ILLINOIS.
Disclosures

• Co-author AUS/FLUS. The Thyroid Bethesda Atlas For Reporting Thyroid Cytology. Ali SA and Cibas ES (eds), Springer, NY. 1st and 2nd editions
  – No royalties
Lecture Objectives

• Improve knowledge in areas of poor interobserver agreement in thyroid cytopathology to enable better cytologic classification of thyroid aspirates

• Discuss less reproducible areas in thyroid Bethesda reporting categories; emphasizing
  1. Benign – sampling/ pitfalls
  2. Neoplasms - NIFTP
  3. Malignant- Cystic PTC, MTC, Anaplastic pitfalls
  4. Atypia – 2018 Bethesda AUS/FLUS update
Prevalence of Thyroid Nodules

• Thyroid nodules are very common

• Fine needle aspiration (FNA) is the most important, first line diagnostic test for the management of nodular thyroid disease.

• Highest volume FNA type in most labs
Thyroid Cancer

- Approx 1% of all cancers
- Incidence recently increasing due to:
  - "Incidentalomas"
  - Overdiagnosis, esp. Foll Variant of PTC
  - Radiation exposure
- Death rate has remained stable

---

Source: National Cancer Institute
Graphic by: Ruben Luong
American University
The approach to a patient with a thyroid nodule is increasingly conservative, although treatment recommendations must be individualized.
Risk stratification by sonographic patterns

<table>
<thead>
<tr>
<th>Pure cyst</th>
<th>Mixed cystic/solid</th>
<th>Iso/hyperechoic, solid</th>
<th>Hypoechoic, solid</th>
<th>Microcalcifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spongiform</td>
<td>Partially cystic, w/ eccentric solid area</td>
<td>Irregular border, Taller&gt;wide shape</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**LOW**

**HIGH**

- Microcalcifications
- Irregular border
- Taller>wide shape

Metastatic LNs
Extrathyroidal invasion
### Table 1.2 The Bethesda System for Reporting Thyroid Cytopathology: implied risk of malignancy and recommended clinical management

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Risk of malignancy (%)</th>
<th>Usual managementa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic or Unsatisfactory</td>
<td>5–10(^b)</td>
<td>Repeat FNA with ultrasound guidance</td>
</tr>
<tr>
<td>Benign</td>
<td>0–3(^c)</td>
<td>Clinical and sonographic follow-up</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance</td>
<td>(~10–30(^d))</td>
<td>Repeat FNA, molecular testing, or lobectomy</td>
</tr>
<tr>
<td>Follicular Neoplasm or Suspicious for a Follicular Neoplasm(^e)</td>
<td>25–40(^f)</td>
<td>Molecular testing, lobectomy</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>50–75</td>
<td>Near-total thyroidectomy or lobectomy(^g,h)</td>
</tr>
<tr>
<td>Malignant</td>
<td>97–99</td>
<td>Near-total thyroidectomy or lobectomy(^h)</td>
</tr>
</tbody>
</table>

\(a\) Healthcare provider judgment for clinical management may vary. \(b\) Risk estimate may vary, depending on specific case. \(c\) Risk estimate may vary, depending on specific case. \(d\) Risk estimate may vary, depending on specific case. \(e\) Risk estimate may vary, depending on specific case. \(f\) Risk estimate may vary, depending on specific case. \(g\) Risk estimate may vary, depending on specific case. \(h\) Risk estimate may vary, depending on specific case.
Thyroid Bethesda: Benign

- 65-75% of thyroid FNAs
- This category includes
  - Benign follicular nodule
    - Colloid/adenomatoid/hyperplastic nodule
  - Chronic lymphocytic (Hashimoto) thyroiditis
  - Other (subacute thyroiditis, amyloid goiter, etc.)
- False-negative rate <1 to 3%
- Patients with a benign nodule are followed by periodic clinical ± US examination.
Benign follicular nodule cytology

Follicular nodules undergo spectrum of hyperplastic/degenerative changes

Classic cytology

• Admixture of cells types
  – Variety of cell arrangements
  – Nuclear crowding absent
• Colloid is abundant to moderate
• Background with involutional changes
Involutional Changes in Hyperplastic Goiter

- Involut ed foll cells
- Cystic change, hemorrhage
- Fibrosis
- Calcium oxalate crystals
- Mesenchymal repair
- Broken balloon fibrosis
Follicular Patterned Lesions

- Macrofollicular #1
- Microfollicular #2
- Normofollicular #3

Sampling and numbering of passes.
Pitfall 1 - Normal architectural variations

**ISOLATED FOLLICULAR CELLS**
- Involuted SMALL follicular cells
- Often dissociated,
- “lymphocyte like”
- May be smaller than RBC/WBC
- No discernible cytoplasm
- Usually find small groups
- **NOT CLT**

Small groups similar to single cells
Pitfall 1- Normal architectural variations

- **Spherules**
  - Intact or non-neoplastic follicles
  - Need to differentiate from histiocytic giant cells
  - Colloid is not in backgrnd

- **Tissue fragments**
Pitfall 2 - Mesenchymal cells

- Singly scattered or sheets of spindle cells in a "repair-like pattern"
Pitfalls in Cystic Aspirates
Mesenchymal “cyst lining” cells

Nucleoli
Intranuclear inclusions
Cellular size and shape pleomorphism
Pitfall 3: Macrofollicular Neoplasms

- **Follicular Neoplasm**
  - Cytologically indistinguishable from hyperplastic nodule
  - Very low rate of malignancy
  - * Includes NIFTP

- **Includes NIFTP**
  - Micro/Macro architecture
  - Most useful criteria are nuclear features
    - Often present FOCALLY; Histologically PTC features often below capsule or peripheral
THYROID BETHESDA (Suspicious For) Follicular Neoplasm

• Criteria
  – Cases that show a **predominance** of microfollicles and/or crowded groups (architectural atypia)
  – No specific percentage

• “Neoplasm”
  – The distinction between follicular adenoma and carcinoma is not possible by FNA
  – Hurthle cell neoplasm is a subtype of follicular neoplasm
FOLLICULAR PATTERNED LESIONS

- Benign
- Indeterminate
- Neoplasm

GREY ZONE

Hyperplastic/colloid nodule

Hyperplasia vs neoplasia

Follicular/Hurthle cell neoplasm

Colloid

Epithelial cells

Practical Principles of Cytopathology, DeMay
Follicular Neoplasm

- Cellularity often high
- Repetitive pattern of microfollicles
- Single cell type
- Nuclei may be crowded and overlap
- Scant colloid, often inspissated in lumen
Pitfall 1: Follicular Neoplasm
Follicular Variant of Papillary Carcinoma (FVPCT)/ NIFTP
Papillary vs. Follicular Nuclear Features

- **Follicular lesions**
  - Round nuclei
  - Normochromatic
  - Even distributed chromatin
  - Nucleoli inconspicuous (except Hurthle cells)

- **Papillary carcinoma**
  - Oval/ elongated nuclei
  - Hypochromasemia
  - Small, eccentric dot like nucleolus
  - Intranuclear inclusions
  - Nuclear grooves
The term Non-Invasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Features” NIFTP was introduced in for tumors previously classified as noninvasive, encapsulated follicular variant of papillary thyroid carcinoma (EFVPTC).
- **NIFTP is a histologic diagnosis**
  - Well demarcated (radiology and path). Capsule +/-
  - Predominantly follicular architecture (mixed micro/macro); varying degrees of fibrosis; no papillae!
  - Histologic inclusion/exclusion criteria
  - Molecular profile similar to follicular neoplasm

- **Nuclear features in NIFTPs are often more subtle, patchy/focal compared to infiltrative FVPTC and classical PTC**
  - More obvious at periphery of neoplasm and in microfollicular areas
Noninvasive follicular thyroid neoplasm with papillary-like nuclear features: a review for pathologists

Raja R Seethala, Zubair W Baloch, Justine A Barletta, Elham Khanafseh, Peter M Sadow, Virginia A LiVolsi, Yuri E Nikiforov, Giovanni Tali, Lester DR Thompson

“How-to-guide” for the diagnosis of NIFTP.

Table 3 Summary of grossing and reporting guidelines

Grossing recommendations
- The entire tumor capsule or tumor normal interface is submitted for histologic evaluation
- For large lesions, stepwise submission of sections (ie, a limited number initially) until invasion is found or the lesion's border is entirely submitted is acceptable
- Multiple sections can be submitted per block, focusing on the tumor periphery and its junction to the parenchyma
- In the setting of multinodular disease gross identification of a fine needle tract may be beneficial to capture the lesion of interest
- For lesions with excessively overt nuclear features of papillary carcinoma but without exclusion criteria on initial sectioning, additional sections of the central portion should be submitted to exclude a conventional papillary thyroid carcinoma component

Reporting parameters
- NIFTP does not require a formal staging (ie, no AJCC or UICC stage)
- Certain oncologic parameters are redundant (ie, the absence of vascular invasion)
- A limited data set consisting of tumor size, laterality and margin status may be useful
- Ancillary immunohistochemical markers such as HBME-1 and Galectin-3 should be applied with caution
- During this current period of transition, a comment linking NIFTP to its prior designation as non-invasive/encapsulated/well-demarcated papillary thyroid carcinoma is recommended.

Table 1 An algorithm for the diagnosis of noninvasive follicular thyroid neoplasm with papillary-like nuclei (NIFTP)
NIFTP-Cytology

- **FNA cytology criteria**
  - **Follicular pattern**
  - **Nuclear features:**
    - Mild enlargement, elongation, pallor, overlap +/-
    - **Rare** pseudoinclusions, grooves
    - **Absent:** papillae and psammoma bodies

- Cytologic samples typically fall into one of the indeterminate categories:
  - Suspicious for PTC (25–35%)
  - FN/SFN (25–30%),
  - AUS/FLUS (10–20%)
NIFTP: Impact on Risk of Malignancy of Bethesda FNA Categories

Impact of reclassifying NI-FVPTC on ROM for Bethesda categories

Average ROM for all Bethesda categories 20-45% overall reduction

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Risk of malignancy with NIFTP (%)</th>
<th>Optional note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic or Unsatisfactory</td>
<td>No significant change</td>
<td>None</td>
</tr>
<tr>
<td>Benign</td>
<td>No significant change</td>
<td>None</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance</td>
<td>6–18</td>
<td>None</td>
</tr>
<tr>
<td>Follicular Neoplasm or Suspicious for a Follicular Neoplasm</td>
<td>10–40</td>
<td>The histopathologic follow-up of cases diagnosed as such includes follicular adenoma, follicular carcinoma, and follicular variant of papillary thyroid carcinoma, including its recently described indolent counterpart NIFTP.</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>45–60</td>
<td>The cytomorphologic features are suspicious for a follicular variant of papillary thyroid carcinoma and its recently described indolent counterpart NIFTP.</td>
</tr>
<tr>
<td>Malignant</td>
<td>94–96</td>
<td>A small proportion of cases (~3–4%) diagnosed as malignant – compatible with papillary thyroid carcinoma – may prove to be NIFTP on histopathologic examination.</td>
</tr>
</tbody>
</table>
• NIFTP diagnosis and ROM changes for cytology Bethesda categories will vary based on threshold of cytopathologists and surgical pathologists at individual institutions/groups.

• Pathology practices should track statistics for both thyroid cytology and surgical pathology resection diagnosis and perform cytologic-histologic correlation
Pitfall 2: Intrathyroidal Parathyroid Adenoma

- Can mimic follicular neoplasm
- Hypercellular tissue
- Can show chief, oncocytic or clear cells
- Vascular stroma
- Naked nuclei +
- No true colloid or paravacuolar granules

- PTH assay on FNA
- Immunostains- PTH/TTF
- Afirma (Veracyte, Inc.) and Thyroseq recognize the gene expression profile of parathyroid neoplasms
Hurthle Cell (Oncocytic) Lesions

- **Non-Neoplastic (Metaplastic)**
  - Adenomatous goiter
  - Hashimoto’s thyroiditis
  - Graves disease
  - Radiation
  - Partial thyroidectomy

- **Neoplastic**
  - Hurthle cell adenoma/carcinoma
  - Papillary carcinoma: oncocytic variant
Hurthle cells in Hashimoto’s

- In florid chronic lymphocytic thyroiditis
  - Abundant lymphoid infiltrate with germinal center fragments
  - Hurthle cell metaplasia
  - **Hurthle cells can be very pleomorphic**
Chronic lymphocytic thyroiditis - CLUES

- Colloid is typically scant
- Plasma cells, smudge cells, lymphoid tangles
- Giant cells in 30% of cases, muscle, epithelioid Hurthle cells
Hurthle Cell Neoplasm

- Monotonous Hurthle cell population
- Patterns: single cell, sheets, microfollicles
- Scant colloid
- No lymphocytes
- Useful if present
  - Transgressing vessels
  - Cytoplasm well defined
  - Binucleation
1. Hurthle cell hyperplasia/ metaplasia
   - Chronic lymphocytic thyroiditis
   - Adenomatoid nodule

2. Papillary carcinoma
   - Pseudopapillary pattern
   - Intranuclear inclusions
   - See under PTC pitfalls
Hurthle Cell Neoplasm - Pitfalls

3. Metastatic RCC
   - Admixture of granular, clear and frilly cytoplasm
   - Hyaline globules

4. Medullary carcinoma
   - Pleomorphism of shape and size
   - Nuclear chromatin salt and pepper
   - Cytoplasmic NE granules
5. Parathyroid adenoma
   - Oncocytic variant
Thyroid Bethesda: Malignant

- 5-10% of thyroid FNA’s
- Rate of malignancy at surgery 97-99%

Includes
  - Papillary carcinoma - 85% of all malignancies
  - Medullary carcinoma
  - Poorly differentiated carcinoma
  - Undifferentiated (anaplastic) carcinoma
  - Lymphoma
  - Metastatic tumors
The Many Faces Of Papillary Carcinoma

Papillae

Microfollicles

Syncytial sheets

Abortive papillae

Macrofollicular Flat sheets

Cystic-Clusters
Papillary Carcinoma

- Diagnosis is dependant on characteristic nuclear features:
  - ovoid nucleus
  - hypochromasia
  - nuclear grooves
  - intranuclear cytoplasmic inclusions
  - micronucleolus
PAPILLARY CARCINOMA
“Minor” Diagnostic Features

- Cellular swirls
- Well defined edges
- Giant cells
PAPILLARY CARCINOMA
“Minor” Diagnostic Features

- Bubble gum colloid
- Psammoma bodies
- Squamoid cytoplasm
- Histiocytoid cells
Pitfall 1 - Cystic Papillary Carcinoma

• Important cause of false negatives
  – PTC most common and easiest to miss

• Look carefully for “histiocytoid” cells

• Adequate aspiration of cyst wall / solid area crucial to obtain neoplastic cells
Clues For Cystic Papillary Carcinoma

1. Histiocytoid cells

2. Hemosiderin in tumor cells

3. Bubble gum colloid
Clues For Cystic Papillary Carcinoma

Cell block

Psammoma bodies
Pitfall 2: Papillary Hyperplasia versus Carcinoma

- **Pap. Hyperplasia**
  - Similar architecture
  - Lack of diagnostic nuclear features
  - Seen in
    - Adenomatous glands
    - Follicular neoplasms
    - Graves disease
Pitfall 2: Pseudopapillary Pattern

- Can occur in any vascular lesion
  - Hurthle cell neoplasm
  - Metastatic renal cell carcinoma

- Normal tissue fragments
  - Seen in good aspirations and touch preparations of core biopsies
Pitfall 3: Hurthle Cell Neoplasm vs Papillary Ca

- **Features in common with papillary carcinoma**
  - Intranuclear inclusions
  - Nuclear grooves
  - Calcifications

- **Unique to HCN**
  - Nuclei are round
  - Prominent nucleoli
  - Cytoplasmic blush or granules versus dense cytoplasm in papillary carcinoma
  - Low N/C ratio consistent
Pitfall 4: Hyalinizing trabecular adenoma

- HTA is a rare tumor of follicular cell origin with trabecular growth, marked hyalinization, and the nuclear changes of PTC

- Hypercellular smear
- Epithelioid/ few spindled cells.
- Pale metachromatic cytoplasm
- Numerous well-formed intranuclear inclusions
- Colloid is absent.
- No other nuclear features of PTC
MEDULLARY CARCINOMA

- Malignant neuroendocrine neoplasm derived from the parafollicular (C) cells of the thyroid

- Cellular aspirates
  - large proportion of single cells, few cell clusters

- Architecture
  - Marked pleomorphism of size and shape
  - Classic is plasmacytoid/spindle cells
MEDULLARY CARCINOMA: CLUES

**Nuclei**
- Anisonucleosis
- Eccentric nuclei
- Stippled or salt-pepper chromatin
- Bi- and multinucleation
- Intranuclear inclusions

**Cytoplasm**
- Red granules in cytoplasm on Diff Quik in 20%
Medullary Carcinoma

• Amyloid
  – Can mimic colloid
  – Look at epithelial cells

• Stains
  – Calcitonin/CEA/ NE markers and Congo red
MTC Pitfalls: “melanoma of the thyroid”
Many morphologies of medullary carcinoma

- Plasmacytoid
- Follicular-like
- Syncytial, PD ca-like
- Small cell-like
- Spindle cell
MTC Pitfalls

Melanin pigment  Intracytoplasmic vacuoles  Intranuclear Inclusions

Bethesda atlas Fig. 9.15 Medullary carcinoma (Lt) versus undifferentiated/anaplastic thyroid carcinoma (rt).
(a) The giant-cell variant of medullary thyroid carcinoma exhibits markedly enlarged, epithelioid tumor cells with pleomorphic nuclei, often admixed with more conventional-appearing tumor cells (ThinPrep, Papanicolaou stain).
(b) Note the resemblance to undifferentiated (anaplastic) thyroid carcinoma, which can also exhibit an epithelioid cytomorphology and nuclear pleomorphism.
This rapidly growing primary thyroid tumor was positive for PAX8 and negative for TTF1, calcitonin, synaptophysin, and chromogranin.
<table>
<thead>
<tr>
<th>MTC variant</th>
<th>Differential diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphlcrine (mucin and calcitonin-producing cells)</td>
<td>Secretory carcinoma, metastatic adenocarcinoma</td>
</tr>
<tr>
<td>Clear cell</td>
<td>Renal cell carcinoma, follicular neoplasm with clear cells</td>
</tr>
<tr>
<td>Follicular/tubular</td>
<td>Follicular neoplasm</td>
</tr>
<tr>
<td>Giant cell</td>
<td>Undifferentiated (anaplastic) thyroid carcinoma (UTC)</td>
</tr>
<tr>
<td>Melanin-producing/pigmented</td>
<td>Melanoma</td>
</tr>
<tr>
<td>Mixed follicular and medullary</td>
<td>Follicular neoplasm</td>
</tr>
<tr>
<td>Oncocytic (oxyphilic)</td>
<td>Oncocytic variants of follicular neoplasm and PTC</td>
</tr>
<tr>
<td>Papillary/pseudopapillary</td>
<td>Papillary thyroid carcinoma (PTC)</td>
</tr>
<tr>
<td>Parangangioma-like</td>
<td>Parangangioma, hyalinizing trabecular tumor</td>
</tr>
<tr>
<td>Small cell/neuroblastoma-like</td>
<td>Small-cell carcinoma of the lung, lymphoma</td>
</tr>
<tr>
<td>Spindle cell</td>
<td>Sarcoma, UTC</td>
</tr>
<tr>
<td>Squamous</td>
<td>Squamous-cell carcinoma, UTC, PTC with squamous differentiation/metaplasia</td>
</tr>
</tbody>
</table>

*MTC* medullary thyroid carcinoma (Adapted from [9]. With permission from Wolters Kluwer Health, Inc.)

Anaplastic Carcinoma

- **PRESENTATION**
- Variety of patterns
- Variable cellularity
- Extreme cellular and nuclear pleomorphism in majority
- Infiltration by PMN’s common
- Necrosis can be seen

- Mimics primary/ metastatic tumors
- Clinical history
- IHC (vimentin +, TSH -, CK +/-, PAX8+)
Pitfalls for Anaplastic Carcinoma

1. Benign cells misinterpreted as anaplastic ca.
   - Fibroblasts (granulation, stromal, Riedel’s thyroiditis), mesenchymal repair
   - Atypical follicular cells secondary to therapy

2. Spindle cell anaplastic carcinoma
   - Associated with tall cell PTC called benign

3. Other malignant neoplasms misinterpreted as anaplastic ca.
   - Medullary carcinoma
   - Metastatic poorly differentiated carcinoma, sarcoma, melanoma, Lymphoma
Anaplastic Spindle Cell carcinoma
ANAPLASTIC CARCINOMA

Anaplastic spindle cell carcinoma arising in association with tall-cell papillary thyroid cancer: (Saunders C, Nayar R. Diagn Cytopathol 1999; 21:413-418)

- Association of tall cell type of papillary carcinoma and anaplastic spindle cell squamous carcinoma (Bonner, LiVolsi, 1991)
- Few cases described
- Transition can be abrupt
- Prognosis variable
- Think about anaplastic carcinoma when “tall” papillary carcinoma cells are seen
Suspicious for Malignancy

- Criteria fall short of a definitive diagnosis of malignancy (*but more than atypical*)
- Comprises <2% of all FNA diagnosis
- Rate of malignancy at surgery ~60-75%

- Details of what is suspected and guidance to clinician is helpful
  - Repeat FNA with/without ancillary studies (Flow, PTH, TG, cell block for IHC, etc)
  - Clinical workup (serum calcitonin/ CEA)
  - Surgery
Thyroglobulin (TG) Assay as an Adjunct to Cytology

- Small, focally involved or cystic neck LN with metastasis
- Postoperative Thyroid bed “SOL”
  - have a higher possibility of having non diagnostic or “indeterminate” cytology

- TG measurements on FNA aspirates (FNA-Tg)
  - Elevated levels highly predictive of metastatic disease
  - TG +ve even if patient has anti-TG antibodies.
THYROID BETHESDA
Atypia of Undetermined Significance (AUS)
OR Follicular Lesion of Undetermined Significance (FLUS)

- Use only one term in one laboratory (AUS or FLUS)

- Criteria
  - Cases that contain cells (follicular, lymphoid, or other) with nuclear and/or architectural atypia
    - Atypia not sufficient to be classified as follicular/Hürthle cell neoplasm, suspicious for malignancy, or malignant.
    - On the other hand, the atypia is more marked than can be ascribed confidently to benign changes.
Atypia of Undetermined Significance (AUS) OR Follicular Lesion of Undetermined Significance (FLUS)

• Literature on AUS/FLUS and ROM
  – Aspirates with cytologic (nuclear) atypia have an approximately two fold higher ROM compared with AUS/FLUS cases with architectural atypia.
  – Hürthle cell-type AUS/FLUS has a lower ROM than other AUS/FLUS patterns.

• Subclassification is encouraged to
  – Enhance communication with other pathologists and clinicians and
  – Facilitate further refinement of the category as new information becomes available and new entities (like NIFTP) are defined.

Thyroid Bethesda Atlas 2nd Ed.
1. Cytologic Atypia

- **Focal cytologic atypia**
- **Extensive but mild cytologic atypia**

When such cells are few, an atypical interpretation is more appropriate than “suspicious for malignancy”
1. Cytologic Atypia

- Atypical cyst lining cells

- “Histiocytoid” cells

*Thyroid Bethesda Atlas 2nd Ed; Ali S, Cibas E. Springer 2018*
2. Architectural atypia

- A scantly cellular specimen with rare clusters of follicular cells, almost entirely in microfollicles or crowded 3-D groups and with scant colloid

- Focally prominent microfollicles with minimal nuclear atypia

Air dried smear, Giemsa

ThinPrep, Papanicolaou stain

*Thyroid Bethesda Atlas 2nd Ed; Ali S, Cibas E. Springer 2018*
3. Cytologic & Architectural Atypia

- The presence of both mild cytologic and architectural atypia may be more common with NIFTP but this has not been firmly established.
4. Hürthle cell aspirates

A sparsely cellular aspirate comprised exclusively (or almost exclusively) of Hürthle cells with minimal colloid

A moderately or markedly cellular sample composed exclusively (or almost exclusively) of Hürthle cells, yet the clinical setting suggests a benign Hürthle cell nodule, such as in lymphocytic (Hashimoto) thyroiditis or a multinodular goiter (MNG)
Atypia, not otherwise specified (NOS)

A minor population of follicular cells shows nuclear enlargement, often with prominent nucleoli

E.g. Specimens from patients with history of RAI, PTU

AUS/FLUS may be appropriate when the findings are particularly pronounced or there is uncertainty regarding the clinical history.
Psammomatous calcifications in the absence of nuclear features of PTC

Papillary hyperplasia without nuclear cytologic features

*Thyroid Bethesda Atlas 2nd Ed*
Ali S, Cibas E. Springer  In Press
A. Heterogeneous infiltrate of lymphoid cells, including occasional atypical forms. There is a tingible body macrophage in the center of the field. Clonality studies were not available in this case (ThinPrep, Papanicolaou stain).

B. Cell block with similar features (hematoxylin and eosin stain).

Suboptimal Specimens without Cytologic Atypia

TBSRTC 2- non diagnostic aspirates should not be called AUS

Papanicolaou stained smears

Thyroid Bethesda Atlas; Ali S, Cibas E. Springer 2010
AUS/FLUS Follow up

• Repeat FNA
  – More definitive cytologic interpretation in most; only about 10–30% remain AUS/FLUS

• Molecular testing

Update on Molecular Testing for Cytologically Indeterminate Thyroid Nodules

Mihayla Nikolaeva, MD, PhD, Marina Nikolaeva, MD

*Context.*—Approximately 15% to 30% of thyroid nodules that undergo fine-needle aspiration are classified as cytologically indeterminate, presenting management challenges for patients and clinicians alike. During the past several years, several molecular tests have been developed to reduce the diagnostic uncertainty of indeterminate thyroid fine-needle aspirations.

*Objective.*—To review the methodology, clinical validation, and recent peer-reviewed literature for 4 molecular tests that are currently marketed for cytologically indeterminate thyroid fine-needle aspiration specimens: Afirma, ThyroSeq v1.0, ThyroSeq v2, and RosettaGX Reveal.

*Data Sources.*—Peer-reviewed literature retrieved from PubMed search, data provided by company websites and representatives, and authors’ personal experiences.

*Conclusions.*—The 4 commercially available molecular tests for thyroid cytology offer unique approaches to improve the risk stratification of thyroid nodules. Familiarity with data from the validation studies as well as the emerging literature about test performance in the post-validation setting can help users to select and interpret these tests in a clinically meaningful way. (Arch Pathol Lab Med. doi: 10.1001/jama.2018.000002)

Table 1. Comparison of Commercially Available Tests and Their Clinical Validation for Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance and Follicular Neoplasm Aspirates

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Methodology</th>
<th>Sample Collection</th>
<th>Sample Type Required</th>
<th>Sample Type Required for Molecular Testing</th>
<th>Methodology</th>
<th>DNA and RNA Targeting</th>
<th>Methodology</th>
<th>DNA and RNA Targeting</th>
<th>Methodology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afirma</td>
<td>Expression analysis of 167 mRNAs (25 genes in screening step) 142 genes in classifier by microarray</td>
<td>Yes</td>
<td>2 dedicated FNA passes collected in nucleic acid preservative</td>
<td>1-2 drops from first FNA pass on 1 dedicated pass collected into nucleic acid preservative; cell blocks and FFPE issues can also be used</td>
<td>Expression analysis of 24 mRNAs by qRT-PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ThyroSeq v1.0</td>
<td>DNA and RNA targeted next-generation sequencing (56 genes) for mutations, gene fusions, and gene expression</td>
<td>Yes</td>
<td>1 dedicated FNA pass collected into nucleic acid preservative</td>
<td>1 dedicated FNA pass collected into nucleic acid preservative</td>
<td>Targeted next-generation sequencing for mutations (5 genes) and 3 gene fusions expression analysis of 10 mRNA by qRT-PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ThyroSeq v2</td>
<td>ThyroSeq v1.0</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>1 direct smear or ThinPrep slide with adequate cellularity for cytologic diagnosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RosettaGX Reveal</td>
<td>Expression analysis of 24 mRNAs by qRT-PCR</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Expression analysis of 24 mRNAs by qRT-PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: FFPE, formalin-fixed paraffin-embedded; FNA, fine-needle aspiration; GEC, Gene Expression Classifier; mRNA, microRNA; MTC, medullary thyroid carcinoma; N/P, negative predictive value; PPV, positive predictive value; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction.
### Table 1.2 The Bethesda System for Reporting Thyroid Cytopathology: implied risk of malignancy and recommended clinical management

<table>
<thead>
<tr>
<th>Diagnostic category</th>
<th>Risk of malignancy (%)</th>
<th>Usual management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic or Unsatisfactory</td>
<td>5–10(^b)</td>
<td>Repeat FNA with ultrasound guidance</td>
</tr>
<tr>
<td>Benign</td>
<td>0–3(^c)</td>
<td>Clinical and sonographic follow-up</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance</td>
<td>~10–30(^d)</td>
<td>Repeat FNA, molecular testing, or lobectomy</td>
</tr>
<tr>
<td>Follicular Neoplasm or Suspicious for a Follicular Neoplasm(^e)</td>
<td>25–40(^f)</td>
<td>Molecular testing, lobectomy</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>50–75</td>
<td>Near-total thyroidectomy or lobectomy(^g)</td>
</tr>
<tr>
<td>Malignant</td>
<td>97–99</td>
<td>Near-total thyroidectomy or lobectomy(^h)</td>
</tr>
</tbody>
</table>
Quality Assurance and Outcomes

• Laboratory Thyroid Bethesda statistics
  – Directly proportional to FN/SFN and Suspicious for malignancy
  – AUS/FLUS rate- upper limit of 10% may be more realistic
  – AUS: malignant ratio may be a useful laboratory quality measure that should not exceed 3.0

• Surgical follow-up
  – Cytologic–histologic correlation