8 Classifying Your Thyroid FNA Specimens Using Bethesda Terminology: Use of Adjunct Molecular Reflex Testing

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8 Classifying Your Thyroid FNA Specimens Using Bethesda Terminology: Use of Adjunct Molecular Reflex Testing

The Bethesda Thyroid Classification System is a novel method of Thyroid Nodule diagnostics that has yet to gain wide acceptance in the pathology community. The utilization of reflex molecular testing as an adjunct technique to the classification system has also yet to be described. This session will focus on the presenter's experience using the Bethesda Thyroid FNA system, including evidence of its clinical utility, guidance in its use, and its incorporation into practice as a diagnostic modality. The presenter's experience using BRAF V600E reflex molecular testing in conjunction with the Bethesda Classification System will also be covered, including evidence of its clinical utility, recommendations for various molecular techniques available and its incorporation into standard practice as both a diagnostic and prognostic modality.

- To apply the Bethesda Thyroid FNA Classification System in the evaluation of thyroid FNA.
- To utilize molecular testing as an adjunctive test in thyroid FNA.
- To advise clinicians on the implications of the each diagnostic category of the Bethesda Classification system and the results of molecular testing.

FACULTY:

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Practicing Pathologists
Cytopathology
Cytopathology (Non-Gynecologic)
2.0 CME/CMLE Credits

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Yale University School of Medicine
Department of Pathology

The Utilization of the Bethesda System & BRAF Molecular Adjunct Testing on Thyroid Cytologic Samples Part I of II

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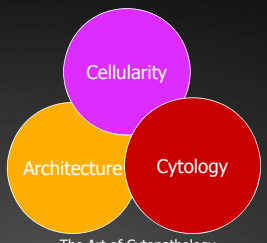
ASCP 2011

1

Objectives

- Review the Bethesda Thyroid FNA Classification System
- Describe its utilization at Yale & the questions it raises
- Posit molecular testing as prequel to part 2
- Challenge our view of the modern cytopathologist/surgical pathologist



The Art of Cytopathology

2

Background

- Thyroid Cancer
  - Most common endocrine malignancy
  - Incidence increasing
- Problematic clinically
  - Nodules
    - ~7% of the population
    - Only 5% are malignant
- Thyroid FNA (TFNA) =
  - the keystone modality
  - Diagnostic test
  - Screening test *

*more in a moment

- But TFNA is Compromised
  - 60% benign
  - 10% malignant
  - 20% equivocal
  - 10% non-diagnostic/unsatisfactory
- Lack of Uniformity
  - Terminology
  - Criteria
  - Clinical Implications
- Bethesda Classification
  - Resolutions?
- Molecular Diagnostics
  - Not expressly advocated in the Bethesda System

3

The Bethesda System

1. Nondiagnostic or Unsatisfactory
   - Cyst fluid only
   - Virtually acellular specimen
   - Obscuring factors
2. Benign
   - Benign follicular nodule e.g. adenomatoid nodule, colloid nodule
   - Lymphocytic thyroiditis
3. Atypia of undetermined significance
4. Follicular neoplasm or suspicious for a follicular neoplasm
   - Specify if Hürthle cell type
5. Suspicious for malignancy
6. Positive for malignancy
   - PTC
   - Medullary carcinoma
   - Anaplastic carcinoma
   - Lymphoma
   - Metastatic neoplasm
   - Other

Ali and Cibas (Ed): The Bethesda System for Reporting Thyroid Cytopathology. Springer. 2009

4

Audience Response

How many thyroid FNAs do you see at your institution?

- **Answer choice #1: 1-100 cases/year.**
- **Answer choice #2: 101-500 cases/year.**
- **Answer choice #3: 501-1000 cases/year.**
- **Answer choice #4: 1001-3000 cases/year.**
- **Answer choice #5: >3000 cases/year.**

Yale Endocrine Cytopathology and Surgical Pathology

- Endocrine Surgery Referral Center
- Over 3,200 Thyroid FNAs annually
- Representing 2,500 patients seen in clinic per year
- Approximately 400 operations performed annually

Yale Smilow Cancer Center

6

Audience Response

Do you utilize the Bethesda Thyroid FNA Classification at your institution?

- **Answer choice #1: No. We don't have a classification system per se.**
- **Answer choice #2: No. We have our own departmental system.**
- **Answer choice #3: Yes. We use TBS as it has been described in the literature.**
- **Answer choice #4: Yes, however, we've modified it somewhat to fit our needs.**

The Bethesda System (Yale Version)

1. Nondiagnostic/ Unsatisfactory
   - Insufficient cellularity
   - Poor preservation
   - Obscuring factors
2. Benign
   - Benign mixed macro/micro-follicular hyperplastic nodule i.e. goiter
   - Lymphocytic thyroiditis/ Hashimoto thyroiditis
   - Cyst Contents or Colloid Nodule-if USG matches
3. Indeterminate [AUS/FLUS]
   - Low cellularity with predominance of microfollicles and absence of colloid
   - Atypical nuclear features
4. Follicular neoplasm
   - Microfollicular pattern
   - S/O Follicular Variant of PTC
5. Hürthle cell neoplasm
6. Suspicious for malignancy
   - Papillary Thyroid Carcinoma
   - Medullary carcinoma
   - Anaplastic carcinoma
   - Lymphoma
   - Metastatic malignancy
7. Positive for malignancy
   - Papillary Thyroid Carcinoma
   - Medullary carcinoma
   - Anaplastic carcinoma
   - Lymphoma
   - Metastatic malignancy

Theoharis et al. Thyroid 2009;19:1215 8

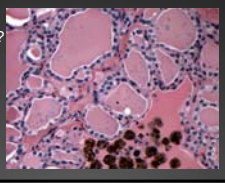
Is Thyroid FNA a Diagnostic or a Screening Test?

- Diagnostic
  - Positive for PTC/MTC/ATC
  - Suspicious for…
  - Negative for Malignancy
  - AUS/Indeterminate?
- Architecture
  - Group Cell Features
  - Single Cell Features
- Cytology
  - Nucleus v.s. Cytoplasm
- Screening
  - Follicular Neoplasm
  - Hürthle Cell Neoplasm
  - AUS/Indeterminate?
- Architecture
  - Group Cell Features
  - Single Cell Features
- Cytology
  - Nucleus v.s. Cytoplasm?

9

Cyto-Method Differs from Histology

- Cellularity
  - Follicular Cells
  - Adequacy
    - 6 groups
    - 10-15 cells/group
  - Colloid
    - Not needed
    - But useful
  - Lymphs /Macs/ Others?
- Architecture
  - Group Cell Features
  - Single Cell Features
- Cytology
  - Nucleus vs. Cytoplasm



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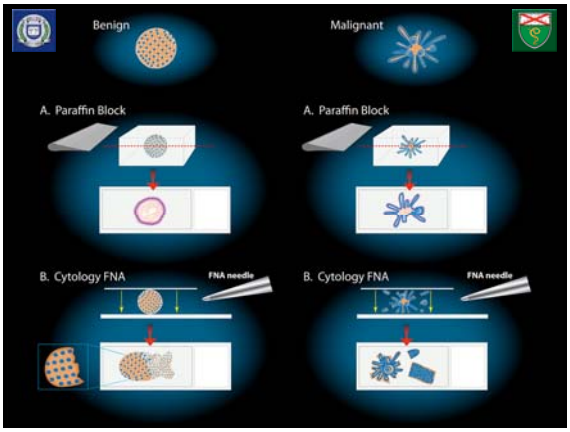
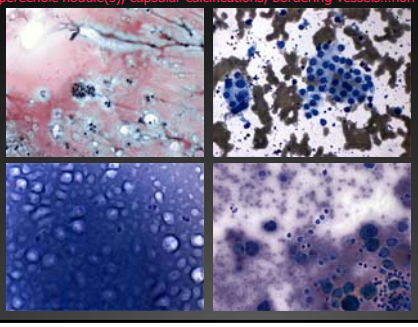


Negative

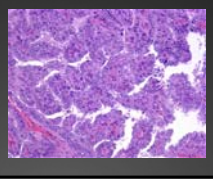
USG: Hyperechoic nodule(s), capsular calcifications, bordering vessels…non-specific



12

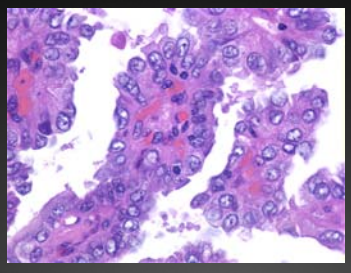
Papillary Thyroid Carcinoma

- Ultrasound
  - Hypoechoic
  - Microcalcifications
  - Increased Vascularity
- Cellularity
  - Follicular Cells!
- Architecture
  - +/- Papillae, sheets, caps
- Cytology
  - Irregular Nuclear Membranes & Grooves
  - Intra-Nuclear Cytoplasmic Invaginations (INCI's)



13

Papillary Thyroid Carcinoma - Papillae



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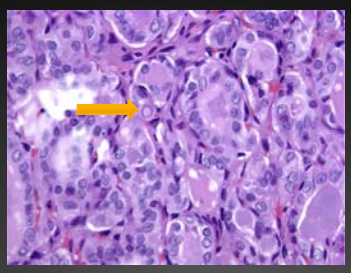
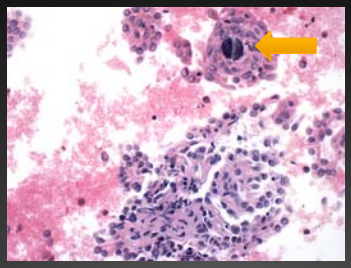
Papillary Thyroid Carcinoma - Inclusion (INCI)



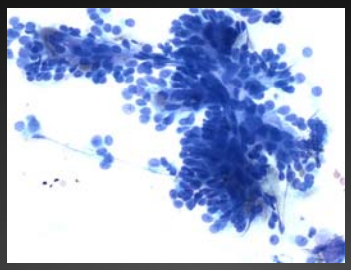
15

Psammoma Body (Calcospherite)



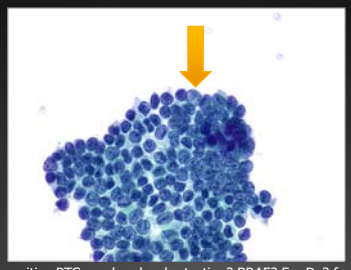
16

Papillary Thyroid Carcinoma- Papillae



17

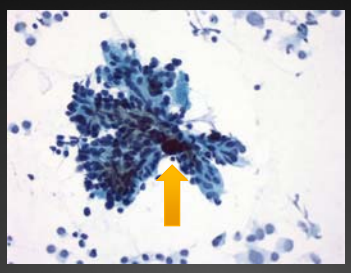
Papillary Thyroid Carcinoma – Inclusion (INCI)



Does a classic positive PTC need molecular testing? BRAF? For Dx? for Pgx?

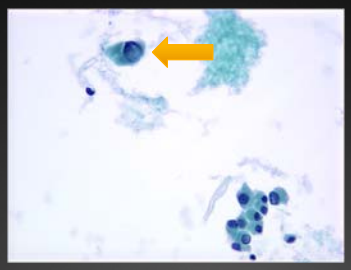
18

PTC Papillae & Psammoma



19

PTC with Oncocytic Features



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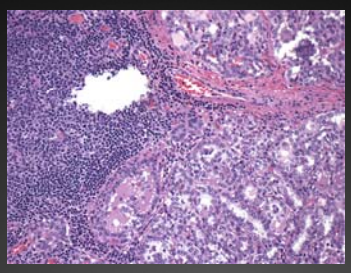
Papillary Thyroid Carcinoma to LN



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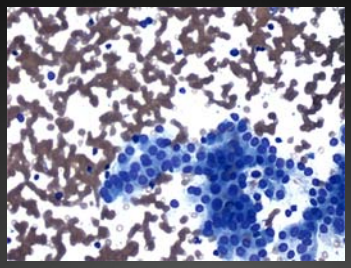
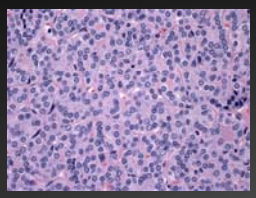
Papillary Thyroid Carcinoma to LN



Often, few if any lymphocytes are present

22

Follicular & Hürthle Cell Thyroid Neoplasms

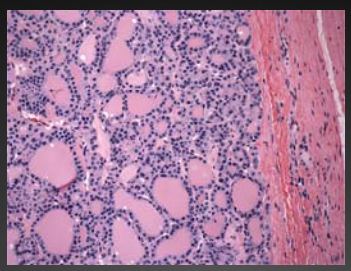


USG: Isoechoic nodule, no calcifications, no central vascularity…non-specific findings

- Architecture
  - Microfollicles
  - NO Papillae
  - Capsule & Vessel?
- Cytoplasm
  - Amount v.s. Nucleus
- Nuclei
  - Smooth
  - Enlarged
  - +/- Nucleoli
  - +/- Scant Colloid
  - NO INCI's

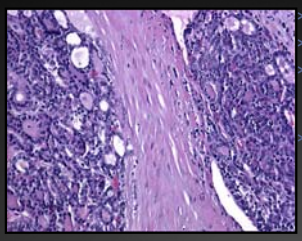
23

Follicular Adenoma Capsule



24

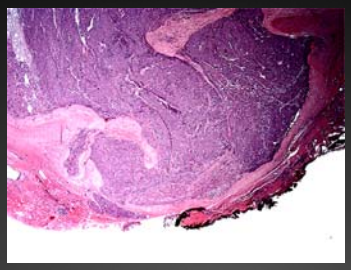
Follicular Thyroid Carcinoma



- CAPSULAR INVASION
- VASCULAR INVASION
- Architecture
  - Microfollicles
  - NO Papillae
- Nuclei
  - Smooth
  - Enlarged
  - +/- Nucleoli
  - +/- Scant Colloid
  - NO INCI's

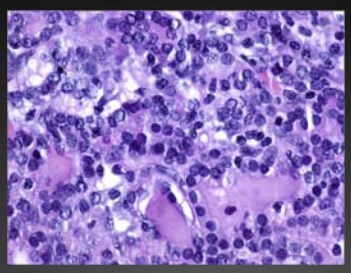
25

FTC Capsular Mushrooming



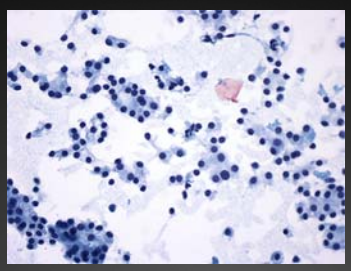
26

Follicular Thyroid Carcinoma



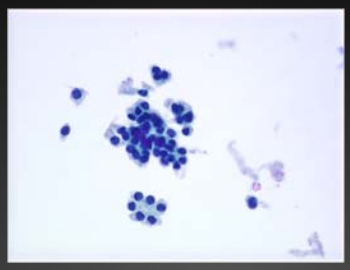
27

Follicular Neoplasm > Follicular Adenoma



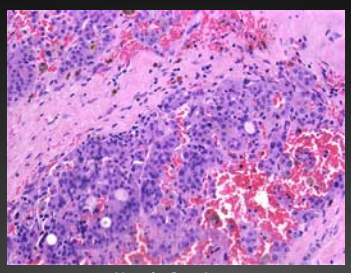
28

Follicular Neoplasm > Follicular Carcinoma



29

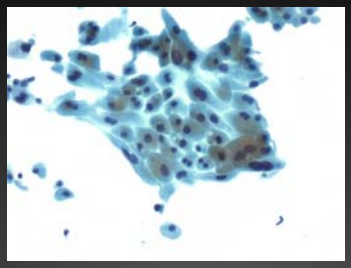
Hürthle Cell Carcinoma



Vascular Invasion

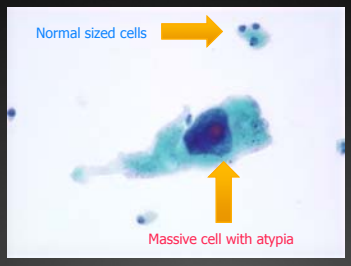
30

Hürthle Cell Neoplasm



31

Hürthle Cell Neoplasm > Hürthle Cell Carcinoma



Rarely, the degree of atypia is so great…

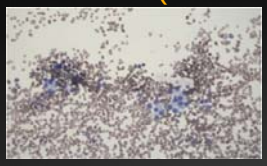
32

Indeterminate/FLUS/AUS

- For specimens that contain cells (follicular, lymphoid, or other) with architectural and/or nuclear atypia insufficient to be classified as suspicious for a follicular neoplasm, suspicious for malignancy, or malignancy
- Criteria
  - Borderline cellularity with predominance of follicular cells and absence/scant colloid
  - Borderline cellularity with predominance of Hürthle cells
  - Focal nuclear atypia s/o PTC (nuclear enlargement with pale chromatin, nuclear grooves) particularly in patients with lymphocytic thyroiditis or cystic changes
  - Atypical lymphoid population
  - Atypia with obscuring factors and/or air drying artifacts

33

AUS/FLUS/Indeterminate (Yale Version)

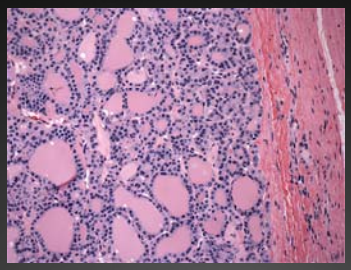


Indeterminate-Architectural Atypia

- Architectural Atypia
  - Low Cellularity
  - Microfollicles
  - Absent/Scant Colloid
- Incipient Changes of Neoplasia?

34

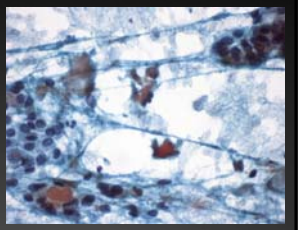
Follicular Adenoma with intact capsule on resection



35

AUS/FLUS/Indeterminate (Yale Version)

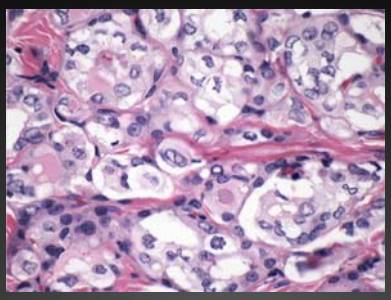
- Nuclear Atypia
  - Elongation/Enlargement
  - Nuclear Membrane Irregularities/Grooves
  - Rare possible(?) pseudoinclusions
- Incipient Changes of Neoplasia?



Indeterminate-Nuclear Atypia

36

Mixed Classic and Follicular variant PTC on resecton



37

Indeterminate/FLUS/AUS

- "At our institution, the term 'indeterminate,' corresponds to the NCI 2007 guidelines category "Follicular cells of undetermined significance." Lesions designated as such my benefit from re-aspiration in the appropriate clinical context."
- Repeat FNA in 3- 6 months
- ~20% of nodules are repeatedly diagnosed as "indeterminate"
- Surgery indicated if worrisome clinical and/or US findings
- Can we do better?
- Reflex BRAF mutational analysis?*

*more on this coming in part 2!

38

Suspicious for malignancy

The Need for Compromise

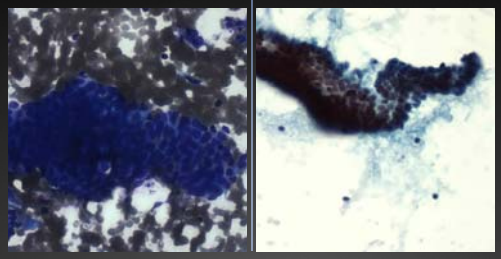
- GOAL:
  - Maintain high PPV of malignancy on f/u but w/o compromising sensitivity
- USUAL SUSPECTS:
  - Suspicious for PTC
    - Most common
    - Lobectomy ± frozen section (completion subsequently
  - Suspicious MTC, ATC, NHL
- Compromised:
  - Quantity
  - Quality
  - Can we do better?
- Reflex BRAF mutational analysis?*

*more on this coming in part 2!

39

Suspicious for malignancy

Papillary group | **Nuclear atypia**



Overstained, obscuring blood, low cellularity, compromise dsample

40

What's the difference?

Is it Atypical?

- Rule of Thumb:
- ***"I'm not certain it's negative"***
- You the cytopathologist are communicating:
  - "Don't lose this patient to follow-up"
- Malignant risk should be low: <30%

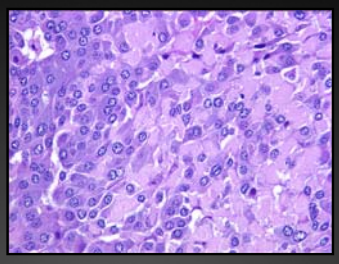
Is it Suspicious

- Rule of Thumb:
- ***"I'm not certain it's positive"***
- You the cytopathologist are communicating:
  - "Consider lobectomy based upon this sample"
- Malignant risk should be high: >60%

41

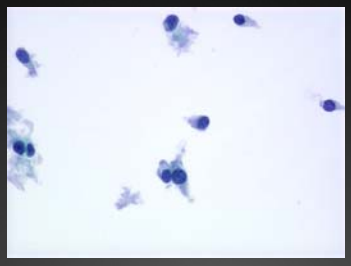
Medullary Thyroid Carcinoma

- Cellularity
  - Cellular
- Architecture
  - SINGLE Cells
- Cytology
  - Spindled
  - Plasmacytoid
  - Nuclei
  - Smooth
  - Salt & Pepper
  - Small nucleoli
  - +/- INCI's
  - Ancillary Test?
  - Calcitonin



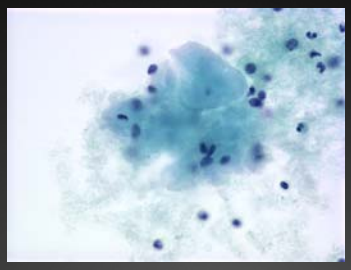
42

Medullary Thyroid Carcinoma



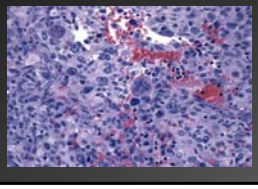
43

Medullary Thyroid Carcinoma



44

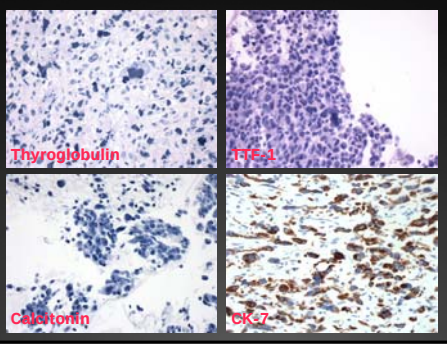
Anaplastic Thyroid noma



- History
  - Older patient
  - Rapid, recent growth
- Gross
  - Hemorrhage
  - Necrosis
- Architecture
  - SINGLE/Cellular
  - Giant/Spindled
  - Pleomorphic
  - PMN's
- Nuclei
  - Dark/Irregular
  - Mitoses
  - INCI's

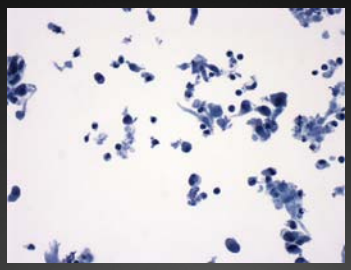
45

ATC Immunostains



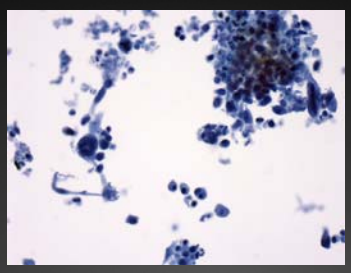
46

Anaplastic Thyroid Carcinoma



47

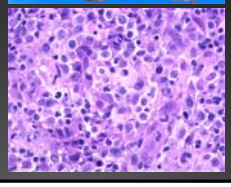
Anaplastic Thyroid Carcinoma



48

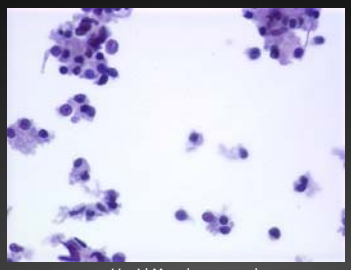
Non-Hodgkin Lymphoma

- Large B-Cell
- Hashimoto association
- Architecture
  - Monotypic/Cellular
  - Cytoplasmic vacuoles
- Nuclei
  - Immature chromatin
  - Multi-Nucleoli



49

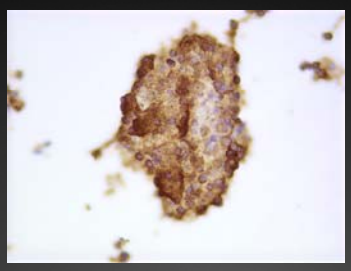
Thyroid NHL



Liquid Monolayer pseudo-groups

50

Thyroid NHL +CD45



51

Distribution of cytologic categories

Cytologic Category	By Nodules 2008	By Patients 2008	Expected frequency
Unsatisfactory	357 (11.7%)	230 (9.0%)	10% to 15%
Benign/Negative for Malignancy	2368 (78.0%)	1799 (72.8%)	70% to 80%
Indeterminate/Atypia of Undetermined Significance	95 (3.0%)	89 (3.6%)	3% to 18%
Follicular /Hürthle Cell Neoplasm	176 (5.8%)	166 (6.7%)	5% to 8%
Suspicious for Malignancy*	43 (1.4%)	39 (1.6%)	2.5% to 8%
Malignancy*	168 (5.5%)	145 (5.9%)	4% to 8%
Total	3207	2468	

* Majority of them were PTC

Modified from Theoharis et al. Thyroid 2009;19:1215

52

Comparison before and after TBS

Cytologic Category	By Nodules 2008	By Patients 2008	By Nodules 2007	By Patients 2007
Unsatisfactory*	357 (11.7%)	230 (9.0%)	293 (14.4%)	197 (12%)
Benign/Negative for Malignancy*	2368 (78.0%)	1799 (72.8%)	1361 (66.9%)	1053 (65.9%)
Indeterminate/AUS	95 (3.0%)	89 (3.6%)	*Susp FN 48 (2.3%)*	*Susp FN 45 (2.8%)*
Follicular /Hürthle Cell Neoplasm*	176 (5.8%)	166 (6.7%)	174 (8.3%)	156 (9.8%)
Suspicious for Malignancy	43 (1.4%)	39 (1.6%)	29 (1.4%)	26 (2%)
Malignancy	168 (5.5%)	145 (5.9%)	119 (6%)	112 (7%)
Total	3207	2468	2035	1596

* Differences were statistically significant

Theoharis et al. USCAP 2010

53

Cytologic-Histologic Correlation

Cytologic category (% surgery)	MNG/HT	FA	CA	Total
Unsatisfactory (11%)	9	8	8	25
Benign/Negative for Malignancy (0.3%)	61	13	8*	82
Indeterminate (30%)	7	7	13	27
Follicular / Hürthle Cell Neoplasm (61%)	33	34	35**	102
Suspicious for Malignancy (77%)	2	2	26	30
Malignancy (77%)	0	0	112	112
Total	112	64	202	378

*The false negatives were micro PTC (≤ 1cm), not initially sampled by FNA
** included both follicular CA and FV PTC

54

Operating Characteristic

	As a Screening test for NEOPLASM	As a Diagnostic test for MALIGANCY
Sensitivity	NA	NA
Specificity	68%	93%
Positive predictive value	NA	NA
Negative predictive value	83%	91%

Theoharis et al. Thyroid 2009;19:1215

55

Risk of malignancy per Dx

Diagnostic Category	Incidence of malignancy at Yale	NCI recommended rate of malignancy
Benign/Negative for Malignancy	10%* (0.3%)	0%-3%
Indeterminate	30%* (14%)	5%-15%
~~Follicular /Hürthle Cell~~ Neoplasm	~~33%~~	~~20%-30%~~
Suspicious for Malignancy	87%	60%-75%
Malignancy	100%	97%-99%

* Only a selected subset of patients underwent surgery

Modified from Theoharis et al. Thyroid 2009;19:1215

56

Indeterminate/FLUS/AUS

- 171 nodules diagnosed as indeterminate/FLUS/AUS between Jan 2008 to Jun 2009;
- Accounting for 2.8% of all cases

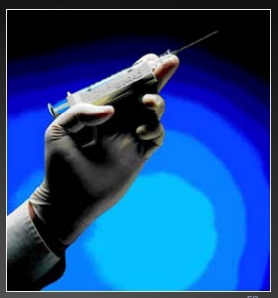
Category	Number of cases	Case with Follow-Up (Surgery/Repeat FNA)	Malignant Follow-Up
Low cellularity/ microfollicular pattern	104 (61%)	59(59%/41%)	7%
Nuclear atypia	67 (39%)	45(73%/27%)	56%
Total	171	104 (65%/35%)	20%

Adeniran et al USCAP 2010

57

Problems with equivocation

- On Re-FNA
  - <10% of Indeterminates were re-dx'd as Indeterminate
- Majority are PTC; most classified as having "nuclear atypia" cytologically
- Re-FNA benign?
  - Hyperplastic nodules in both groups with Hashimoto thyroiditis more prevalent in the 2nd group
- Suspicious Category less problematic (87% CA risk)
- Adjunct testing?
  - Immunostaining?
  - Molecular testing?



58

Molecular Diagnostics?

*primum non nocere**

MAPK SIGNALING PATHWAY

* From the Greek: ὠφελέειν, ἢ μὴ βλάπτειν

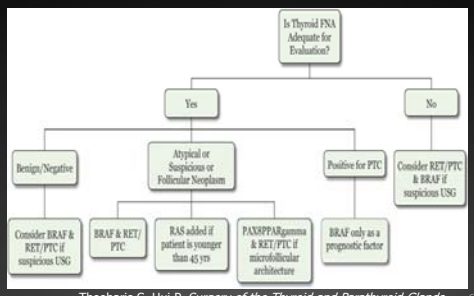
59

Audience Response

Do you utilize molecular testing on thyroid FNAs at your institution?

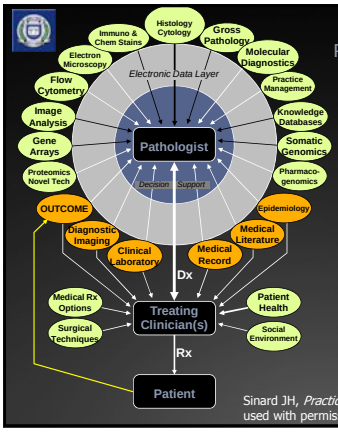
- **Answer choice #1: No.**
- **Answer choice #2: BRAF only.**
- **Answer choice #3: BRAF, RET only.**
- **Answer choice #4: BRAF, RAS only.**
- **Answer choice #5: Panel of BRAF, RET, RAS, PAX etc.**

A Possible Guideline to Molecular Thyroid FNA Testing



Theoharis C, Hui P. *Surgery of the Thyroid and Parathyroid Glands 2nd Ed.* (in press)

61



Pathologist/Cytopathologist as Diagnostic Specialist

- Pathology develops and adopts tools to leverage data from emerging technologies
- We become diagnostic consultants, providing outcomes based treatment recommendations to treating clinicians
- We become the Department of Diagnostic Medicine

Sinard JH, *Practical Pathology Informatics,* used with permission

62

Summary

- The Bethesda 6-tier classification system
  - Conveys different levels of risk of malignancy
  - Excellent screening test for follicular/Hürthle cell neoplasm
  - Superb diagnostic test for identifying PTC with a specificity of 93%
- Sub-classifying indeterminate category into 2 descriptive groups conveys different levels of risk
- Molecular Testing may have a role especially in equivocal cases
  - Part 2 follows shortly…
  - Thank you!

63

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64