Complementary Role of Cytology and Molecular Testing in Thyroid Nodule Management

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Objectives

- Illustrate the diagnostic evaluation of an identified thyroid nodule
- Understand the Bethesda Cytological Classification System for thyroid nodules
- Depict the role of molecular testing in thyroid nodule management

Thyroid Cancer in the United States

≈822,242 people in the US are living with thyroid cancer[1]

American Cancer Society®

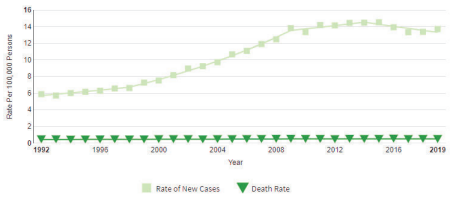
2022 American Cancer Society's data estimates:

≈43,800 new cases of thyroid cancer[2]
≈2,230 deaths from thyroid cancer[2]

5-year Relative Survival 98.4%

1. NIH. https://seer.cancer.gov/statfacts/html/thyro.html. Accessed September 9, 2019.
2. ACS. https://www.cancer.org/cancer/thyroid-cancer/about/key-statistics.html Accessed April 1, 2021.

Prevalence of Thyroid Cancer Over Time

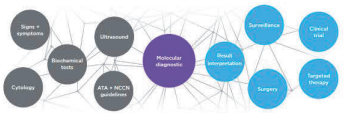


- "Oversurveillance and overdiagnosis" of small well-differentiated tumors due to improved imaging
- Small increase in true incidence

New cases come from SEER 12. Deaths come from U.S. Mortality.
NCI Surveillance, Epidemiology, and End Result Program

Thyroid Nodules: Overview of Patient Journey

- Initial Evaluation
- Ultrasound Imaging
- Fine Needle Aspiration (Cytology)
- Molecular Testing
- Surgery
- Post Operative Long-Term Care



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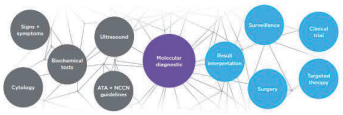
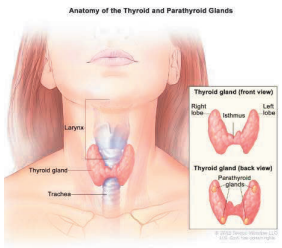
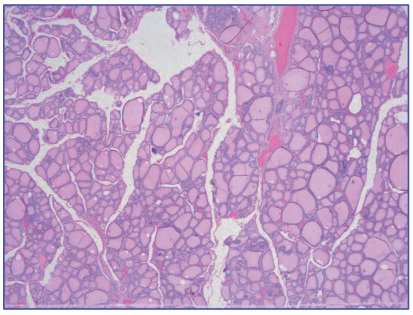


Thyroid Gland

- Located low in the neck just below the larynx
- Critical in the metabolic function of almost all our cells: including controlling heart, muscle, digestive function, brain development, and bone maintenance



Benign Histology



Detection of Thyroid Nodules

- Patient Detection
- History and Physical Exam
  - Patient history: Enlargement can cause compressive symptoms
  - Can be evaluated on physical exam with palpation during swallowing
- Incidentally detected on imaging done for other reasons
  - US, CT, Carotid Doppler, MRI, PET scan

Example patient case study #1- 56-year-old male

Clinical History

- Presents with a 2.2 cm thyroid nodule on chest CT

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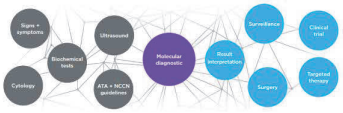
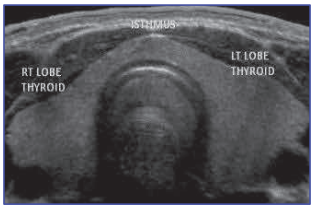
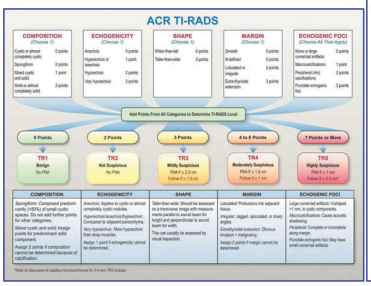


Ultrasound Imaging of the Thyroid



Ultrasound Guidelines

ACR-TIRADS



https://doi.org/10.1016/j.jacr.2017.01.046
American College of Radiology- Thyroid Image Reporting System

ATA

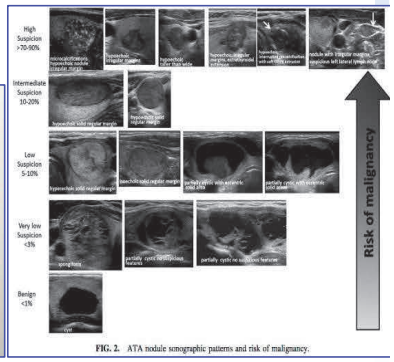


FIG. 2. ATA nodule sonographic patterns and risk of malignancy.

https://doi.org/10.1089/thy.2016.0555

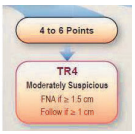
Example patient case study - 56-year-old male

Clinical History

- Presents with a 2.2 cm thyroid nodule on chest CT

Ultrasound

- Suspicious ultrasound characteristics: Hypoechoic nodule, >95% solid TiRads =4



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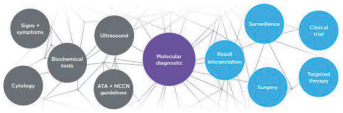
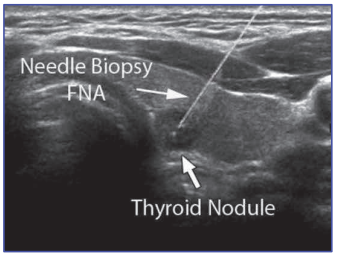
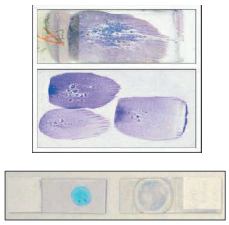


Thyroid Fine Needle Aspiration (FNA)



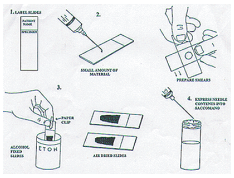
Thyroid FNA Slide Preparation

- Smears
  - Fixation: alcohol vs. air-dried
  - Stains: Pap vs. Diff-Quick
- Liquid Based Preparation (LBC)
  - CytoLyt (methanol based)
  - Saccomano (ethanol based)
  - SurePath (ethanol based)



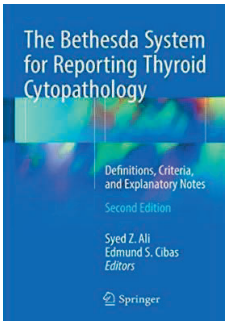
Cytopathology (Cytology) Review

- Subspecialty of pathology which makes diagnoses using single cells
- Used on samples of free cells or tiny tissue fragments, in contrast to histopathology, which is the study of intact tissue
- **Often utilizes smears** because the samples are predominantly liquid and must be spread across a microscope slide for subsequent staining and microscopic examination
- However, cytology samples may be prepared in other ways:
  - Liquid-based cytology
  - Cytocentrifugation
  - Cell block



Bethesda System

- First edition in 2010 with update in 2017
- Has 6 categories (I-VI)
- Standardize the nomenclature of diagnoses
- Ranges from Benign to Malignant with additional Non-Diagnostic category
- Each category has associated risk of malignancy and recommended follow-up treatment
- Standard way of reporting thyroid cytology in the US



Stratification of Thyroid Nodules by Cytology

Benign (60-70%)[1]

- Colloid Nodule
- Hyperplastic/Adenomatous Nodule -> Thyroid Follicular Nodular Disease
- Adenoma
- Lymphocytic Thyroiditis

Indeterminate (15-30%)[2]

- Can't tell if it's benign or malignant

Malignant (3-7%)[3]

- Primary Thyroid Carcinoma
  - Follicular cell derived
    - Papillary (80-85%)
    - Follicular/Oncocytic (10-15%)
    - Anaplastic
      - Squamous Cell
  - Medullary (C cell derived) <3%
- Lymphoma
- Metastatic Cancers from other sites

1. Ali S, Cibas E 2018 The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria, and Explanatory Notes. Second edition. Springer, New York, NY.

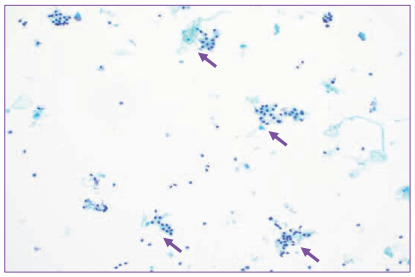
Bethesda System ROM & Management

Dx Category[1]	Cytologic Diagnosis[1]	Expected Rate of Dx[1]	Expected Risk of Malignancy[1]	Suggested Management[1]
I	Non-Diagnostic or Unsatisfactory	<10%	5-10%	Repeat FNA with U/S guidance
II	Benign	60-70%	0-3%	Clinical follow-up
III	Atypia of Unknown Significance (AUS)/ Follicular Lesion of Undetermined Significance (FLUS)	3-7%	10-30%	Repeat FNA, molecular testing, lobectomy
IV	Suspicious for Follicular Neoplasm	8-12%	25-40%	Molecular testing, Surgical lobectomy
V	Suspicious for Malignancy	~3%	50-75%	Lobectomy or total thyroidectomy
VI	Malignant	3-7%	97-99%	Lobectomy or total thyroidectomy

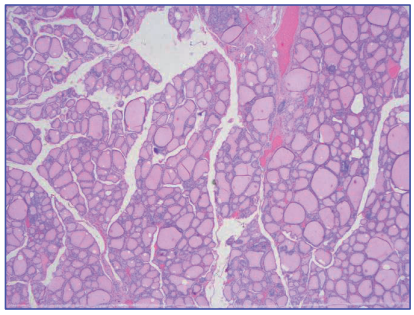
1. Ali S, Cibas E 2018 The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria, and Explanatory Notes. Second edition. Springer, New York, NY.

Bethesda System: Adequacy for thyroid cytology

- 6 groups of thyroid follicular cells
- Each group must consist of at least 10 cells
- Each group must be well-visualized
- If only cyst fluid > Non-diagnostic



Benign Histology revisited



Benign (Bethesda II) – Cytology

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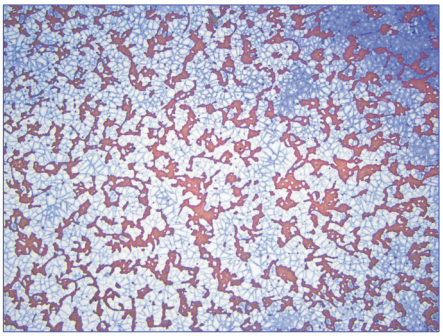

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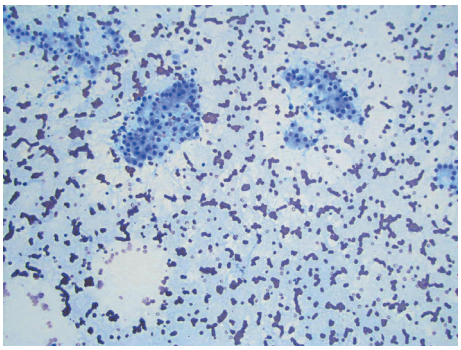
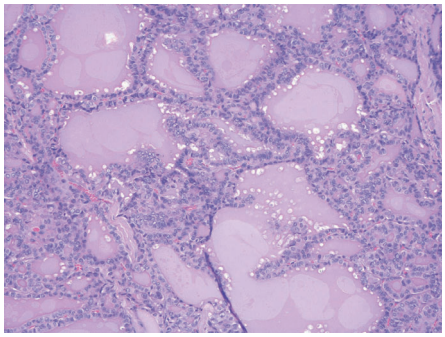


Papillary Thyroid Carcinoma (PTC) Histology



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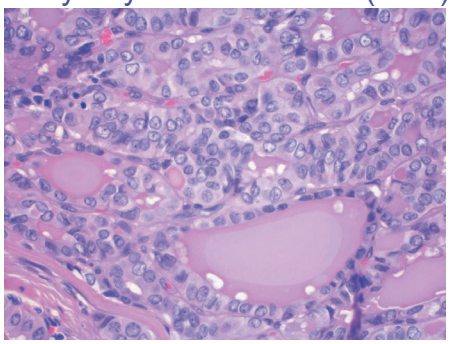

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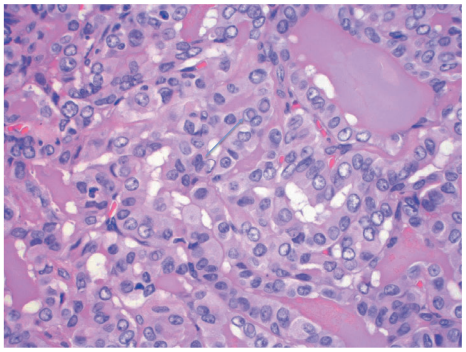

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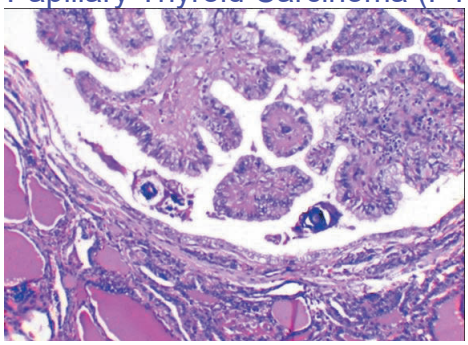

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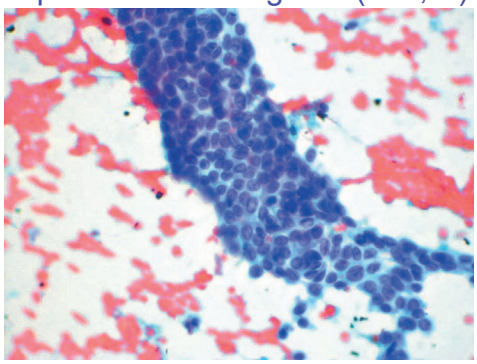
Suspicious and Malignant (B-V,VI) Cytology



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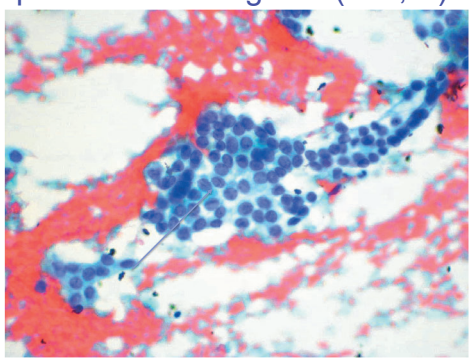

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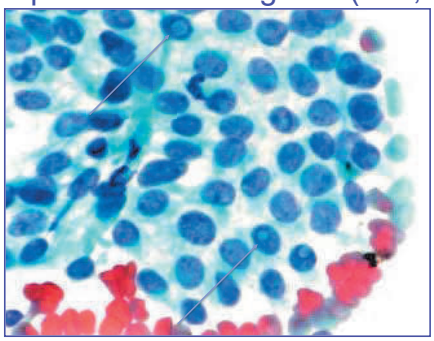

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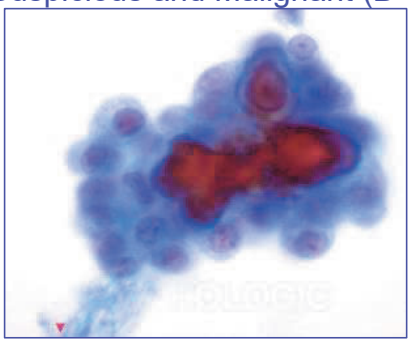


Summary of cytologic features of PTC

- Overlapping groups
- Enlarged nuclei
- Nuclear grooves
- Nuclear membrane irregularities
- Dispersed nuclear chromatin/ pallor
- Nuclear pseudoinclusions

Subtypes of Papillary Thyroid Carcinoma

- Conventional
- Follicular variant
  Encapsulated *
  Infiltrative
- Tall cell
- Oncocytic
- Columnar cell
- Diffuse sclerosing
- Solid
- Clear cell

Non-invasive Follicular Thyroid Neoplasm with Papillary-Like Nuclei

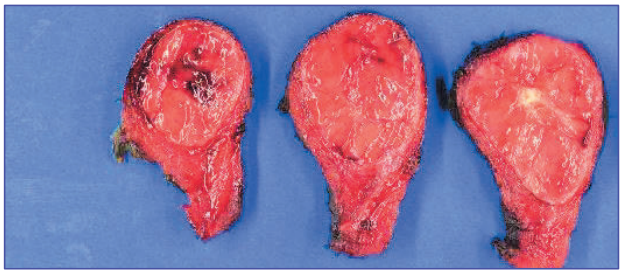
- Low-risk follicular thyroid neoplasm in new 2022 WHO
- Encapsulated tumor
- Papillary nuclei
- Must not have:
  - Necrosis
  - Papillae formation > 1%
  - Increased mitosis
  - Psammoma bodies
  - >30% solid/trabecular/insular growth pattern


The New York Times

It's Not Cancer: Doctors Reclassify a Thyroid Tumor

Give this article 217

Follicular Neoplasm Pathology/Histology



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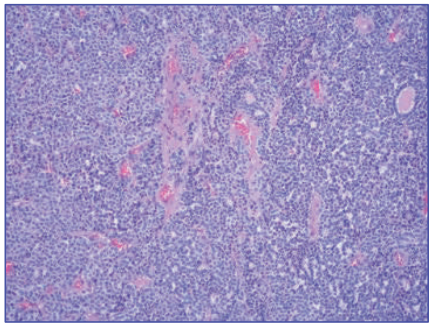

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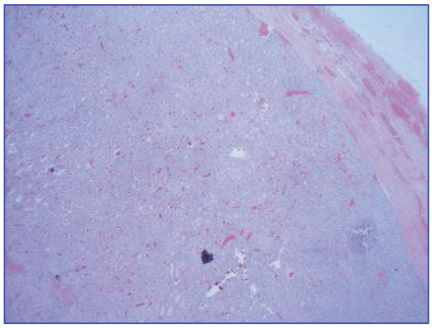
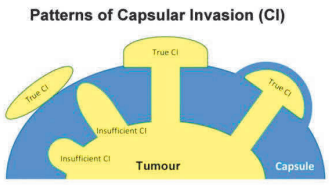


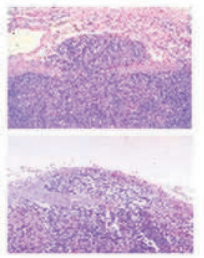
Differentiating follicular adenoma vs carcinoma

- Requires evaluation of entire capsule of the tumor BY HISTOLOGY!!!
- Look for:
  - Invasion of capsule by tumor cells
  - Invasion of capsular vessel by tumor cells

Differentiating adenoma vs carcinoma



Capsular invasion is defined by tumour penetration through the tumour capsule unassociaqted with the site of a previous fine needle aspiration biopsy. (WHO 2004)



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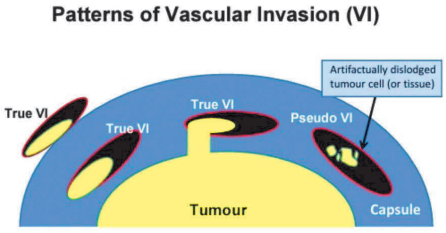
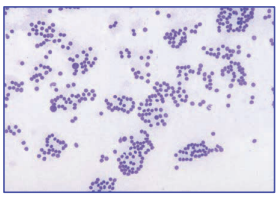
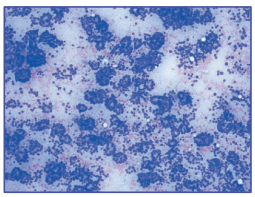


Capsular invasion is defined by the presence of intravascular tumour cells either covered by endothelium or associated with thrombus. (WHO 2004)

Follicular Neoplasm FN/SFN (B- IV)

- Risk of cancer between 25 to 40%[1]
- Traditionally patients would proceed to next steps including surgery





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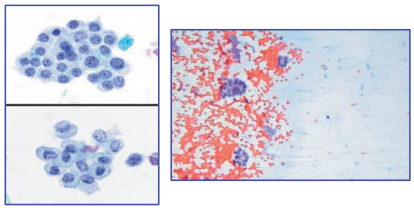
Indeterminate Cytology: Atypia of Undetermined Significance AUS/FLUS (B-III)

- Risk of cancer between 5 to 15%[1]
- Incomplete features of papillary carcinoma or follicular neoplasm
- Traditionally patients would proceed to next steps including surgery



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Example patient case study - 56-year-old male

Clinical History

- Presents with a 2.2 cm thyroid nodule on chest CT, patient asymptomatic

Ultrasound

- Suspicious ultrasound characteristics: Hypoechoic nodule, >95% solid

FNA

- Cytology: Bethesda IV, Suspicious for Follicular Neoplasm (SFN)

Microscopy

- Highly cellular and predominantly containing microfollicles or crowded groups without colloid

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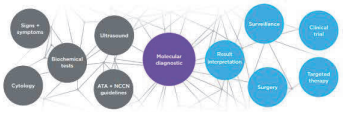
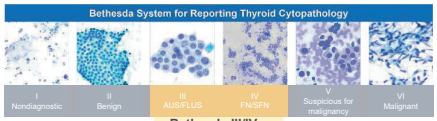


Complementary role of molecular testing



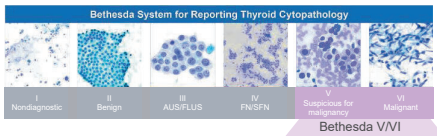
Bethesda III/IV
10–30% of thyroid cases[1,2]

Thyroid Cancer Guidelines

- **NCCN**: recommends **molecular diagnostic testing** for **indeterminates** to reclassify follicular lesions as more or less likely to be benign or malignant[3]
- **ATA**: the proposed use of molecular markers in indeterminate thyroid FNA specimens is diagnostic (to rule out malignancy)[4]

1. Sosa JA, et al. *Surgery* 2013. 2. Bongiovanni M, et al. *Acta Cytol* 2012. 3. NCCN Clinical Practice Guidelines In Oncology. Thyroid Carcinoma, v2.2019. National Comprehensive Cancer Network website. Published September 16, 2019. Accessed November 11, 2019. 4. Haugen BR, et al. *Thyroid* 2016. Photo credit: Papanicolaou Society.Bethesda System for Reporting Thyroid Cytopathology

Complementary role of molecular testing

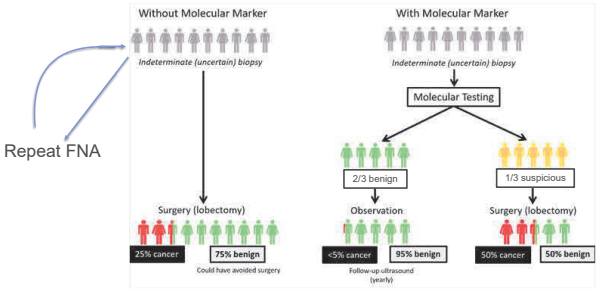


Thyroid Cancer Guidelines

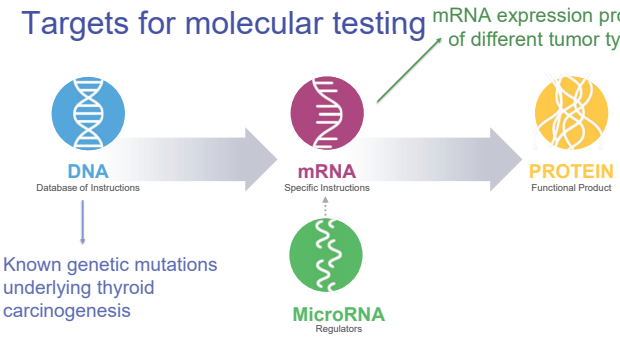
- **NCCN**: recommends molecular testing to help inform decisions regarding systemic therapy[1]
- **ATA**: recommends **considering mutational testing** to refine risk ahead of surgery and use to inform decision making for surgical treatment[2]

1. NCCN Clinical Practice Guidelines in Oncology. Thyroid Carcinoma, v2.2019. National Comprehensive Cancer Network website. Published September 16, 2019. Accessed November 11, 2019. 2. Haugen BR, et al. *Thyroid* 2016. Photo credit: Papanicolaou Society Bethesda System for Reporting Thyroid Cytopathology

Molecular Testing Overview



Targets for molecular testing



Clinical Utility of Molecular Testing

Rule-In Testing	Rule-Out Testing
Identifying nodules with a high probability of being malignant	Identifying low risk nodules that can be observed
Identify personalized treatment	*Saving benign nodules from unnecessary surgery*
~98%	~96%
PPV should be similar to cytology malignant[1]	**NPV** should be similar to cytology benign[1]

***The PPV and NPV, are influenced by the prevalence of cancer in that cytologic category and population

1. Haugen BR, et al. *Thyroid* 2016.

Current testing options

Afirma GENOMIC SEQUENCING CLASSIFIER

mRNA NGS test for rule out Thyroid Cancer and XA variant/fusion panel to inform treatment

Sensitivity: 91%
NPV: 96%[1]

ThyGeNEXT THYROID ONCOGENE PANEL **ThyraMIR** THYROID miRNA CLASSIFIER

ThyGeNEXT variant panel to rule-in and reflex to ThyraMIR microRNA classifier for Rule-Out

Sensitivity: 95%
NPV: 97%[2]

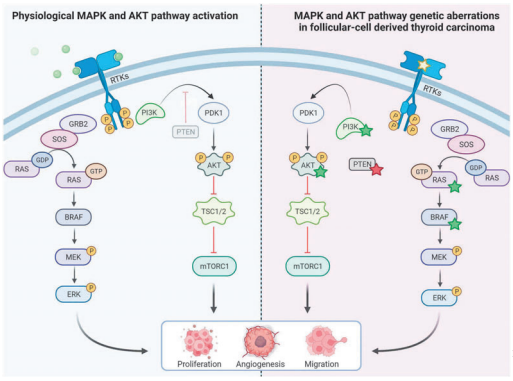
ThyroSeq® Thyroid Genomic Classifier

DNA/RNA NGS test for to rule-in/rule-out based on cancer risk of genomic & copy number alterations and variants/fusions

Sensitivity: 94%
NPV: 97%[3]

1. Patel et al. JAMA Surgery 2018.
2. Lupo et al. Diagnostic Cytopathology 2020.
3. Stewart et al. JAMA Oncology 2019.

Basis of thyroid molecular testing



Endocrine Pathology (2022) 33:27–63

Molecular Expression Signatures

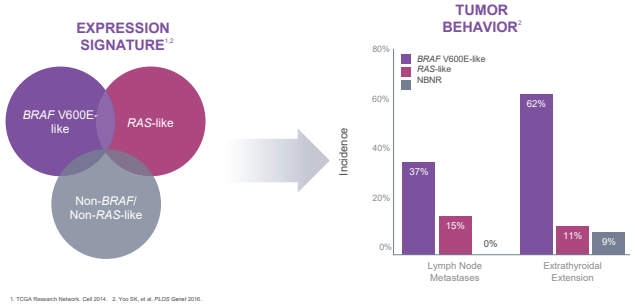
RAS-like mutations : result in tumors that have an expansile pattern of growth and subtle/less florid nuclear atypia

BRAF-like mutations: give rise to infiltrative tumors with florid nuclear atypia.

BRAF-like
- Classical Papillary carcinoma
- Infiltrative follicular variant of papillary carcinoma

RAS-like
- Encapsulated follicular variant of papillary carcinoma
- Non-invasive follicular thyroid neoplasms with papillary like nuclei
- Follicular adenoma

Molecular Expression Signatures



1. TCGA Research Network. Cell 2014. 2. Yoo SK, et al. PLOS Genet 2016.

Some of the important players in PTC

PTCs exhibit among the lowest mutational burden of all human cancers and are highly stable from a pan-genomic perspective

•BRAF V600E is the most common molecular alteration in classic PTC and its subtypes with papillary growth pattern and inflitrative tumors with follicular architecture.

• RET gene rearrangements
  •A strong association with radiation-induced PTC
  •Most common genetic change in pediatrics

•NTRK1/3 - 3–5% of PTC
•ALK
•MET

•Telomerase reverse transcriptase (TERT) promoter mutations
  •secondary pathogenic event in 10% of PTCs and are usually associated with an aggressive clinical course (BRAF + TERT > bad prognosis)

Some of the important RAS-like alterations

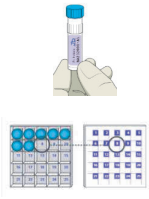
Mutations in:

- BRAF K601E
- DICER1
- EZH1
- EIF1AX
- PTEN

PPARG and THADA gene fusions

Incorporating molecular testing

- Additional sample is collected in special preservative media at the time of initial thyroid biopsy
- Can be stored in freezer for long periods of time
- If biopsy result is indeterminate, sample is sent for molecular testing
- Pre-stained slides may be used to perform testing if they have adequate cellularity on some platforms



Example patient case study #1 - 56-year-old male

Clinical History

- Presents with a 2.2 cm thyroid nodule on chest CT

Ultrasound

- Suspicious ultrasound characteristics: Hypoechoic nodule, >95% solid

FNA

- Cytology: Bethesda IV, Suspicious for Follicular Neoplasm (SFN)

Microscopy

- Highly cellular and predominantly containing micro follicles or crowded groups without colloid

Afirma Genomic Sequence Classifier Results: Benign

RESULTS

Nodule: A Thyroid, Middle Left, 2.2 cm

AFIRMA GENOMIC SEQUENCING CLASSIFIER			AFIRMA XPRESSION ATLAS
Benign (Risk of Malignancy ~4%)	**MTC:** Negative **Parathyroid:** Negative	***BRAF* p. V600E c. 1799T>A:** Negative ***RET/PTC1, RET/PTC3*:** Not Detected	N/A

RESULTS INTERPRETATION

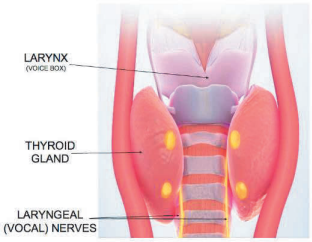
The result of this 2.2cm Bethesda IV nodule A is Afirma GSC Benign, which suggests a low risk of cancer at approximately 4%. Treatment like a cytologically benign nodule may be appropriate, including clinical correlation. Afirma XA is not performed on GSC Benign nodules[2].

Afirma GSC + XA Result:

- Afirma GSC Benign
- Afirma XA: N/A
- Treatment plan - clinical and radiologic follow-up. NO SURGERY!!!!

Potential Complications from Surgery[1]

- Permanent Hypothyroidism
- Hypoparathyroidism
- Vocal Nerve Injury leading to hoarseness
- Airway obstruction caused by bleeding



1. Gourin, CG, Johnson, JT, Randolph, G. "Postoperative complications". *Surgery of the Thyroid and Parathyroid Glands.* August 2012.

Example patient case study #2- 43-year-old female

Clinical History

- Presents with a 3.3 cm thyroid nodule on right side
- Asymptomatic and negative personal/family history

Ultrasound

- Hypoechoic nodule, >95% solid

FNA

- Cytology: Bethesda III, Atypia of Undetermined Significance (AUS)

Afirma GSC Results: Suspicious – BRAF V600E

Afirma GSC + XA Result:

- Afirma GSC Suspicious. Afirma XA: BRAF V600E

RESULTS

Nodule: A Thyroid, Middle Right, 3.3 cm

AFIRMA GENOMIC SEQUENCING CLASSIFIER	AFIRMA XPRESSION ATLAS			
Suspicious	*BRAF*p.V600E c. 1799T>A	***BRAF*p.V600E c. 1799T>A:** Positive ***RET/PTC1, RET/PTC3*:** Not Detected		
MTC: Negative **Parathyroid:** Negative	**Clinical Relevance**	**Risk of Malignancy**	**Associated Neoplasm Type**	**FDA Approved Therapy[4]**
	Evidence of clinical significance in thyroid cancer	>95%[5]	PTC	Only in Anaplastic Thyroid Carcinoma. See medication prescribing information for appropriate patient selection.

RESULTS INTERPRETATION

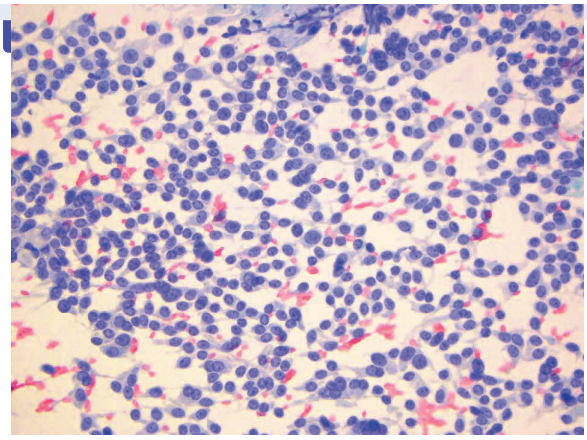
The result of this 3.3 cm nodule A is Afirma GSC Suspicious and *BRAF*p.V600E positive which suggests a risk of cancer of >95%.[5] This genomic alteration is associated with PTC and a *BRAF* V600E-like profile, which includes a higher rate of lymph node metastases and extrathyroidal extension than alterations that are *RAS*-like, or Non-*BRAF*-Non-*RAS*-like.[6,7] Clinical correlation and surgical resection should be considered.

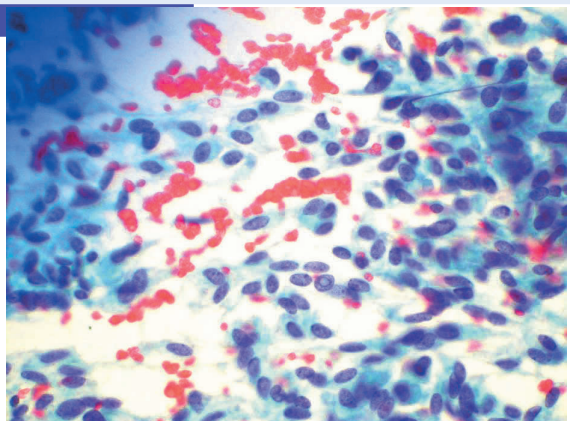
Post operative care

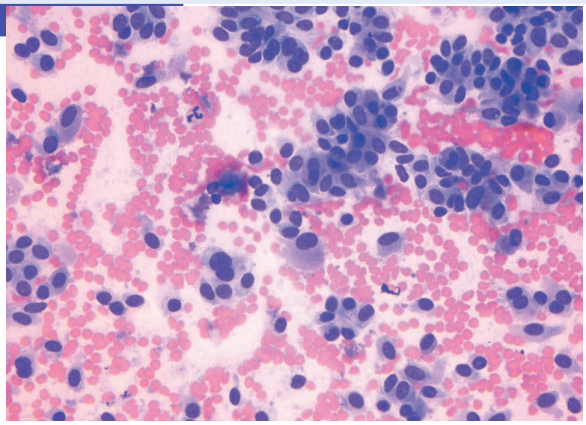
- Possible Radioactive Iodide Therapy
- Regular Monitoring of thyroglobulin levels
- Thyroid replacement to maintain low TSH levels
- Radiation therapy if highly invasive
- Consideration of molecular therapies in metastatic disease.

Bonus Case #1

- 24 year old woman presents with a thyroid nodule







MTC Positive Result

RESULTS

Nodule: A Thyroid, Right Side, 2.0 cm

AFIRMA GENOMIC SEQUENCING CLASSIFIER	AFIRMA XPRESSION ATLAS
Suspicious	*RET*:p.M918T c.2753T>C — ***BRAF*p:V600E c. 1799T>A:** TNP; ***RET/PTC1, RET/PTC3*:** TNP
MTC: Positive (Risk of Malignancy >99%)	
Parathyroid: Negative	

Clinical Relevance	Risk of Malignancy	Associated Neoplasm Type	FDA Approved Therapy[4]
Evidence of clinical significance in thyroid cancer	>99%[3]	MTC	Yes, a *RET* variant-specific therapy is currently approved. See medication prescribing information for appropriate patient selection.

RESULTS INTERPRETATION

The result of this 2.0 cm nodule A is Afirma GSC Suspicious and was MTC Classifier positive, which suggests a risk of medullary thyroid cancer of >99%.[3] *RET*:p.M918T may be germline or somatic. Consider genetic counseling/germline testing for MEN2 syndrome. Clinical correlation and surgical resection should be considered. *BRAF* V600E and *RET/PTC* testing not performed (TNP) due to the identification of an MTC gene expression signature.

Medullary thyroid carcinoma

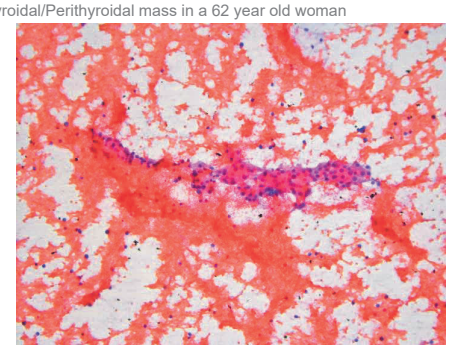
- 1-2 % of thyroid carcinomas
- Tumor cells are variable and can appear spindled, epithelial or plasmacytoid
  - Can have morphologic overlap with PTC
- Derived from C cells (neuroendocrine cells) rather than thyroid follicular cells
  - C cells make calcitonin - can measure levels clinically and can use for diagnosis via immunhistochemistry
- Sporadic: 70%, age 40 - 60, solitary
- Familial: 30%, younger patients (mean age 35)
  - Due to MEN 2A or 2B syndromes, familial medullary thyroid carcinoma syndrome, von Hippel-Lindau disease or neurofibromatosis
  - RET Mutation

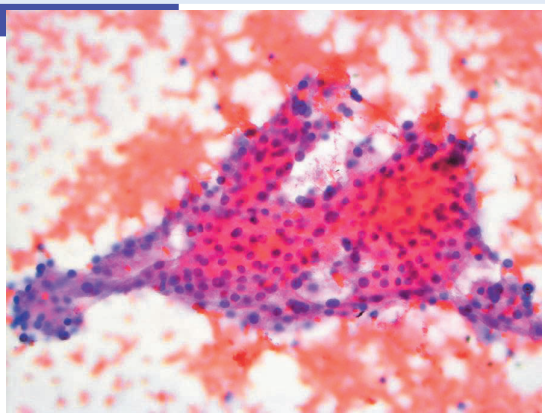
Medullary carcinoma

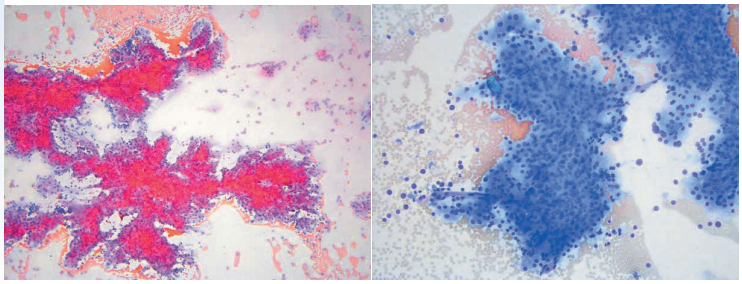
- Check serum calcitonin and CEA levels pre op.
- Genetic testing for germline RET mutation
  - Evaluate for pheochromocytoma and hyperparathyroidism
- Neck ultrasound and CT to evaluate for metastasis
- Patient underwent total thyroidectomy
- Continued measurement of calcitonin post op

Bonus case #2

- Thyroidal/Perithyroidal mass in a 62 year old woman







Parathyroid Positive Result

RESULTS

Nodule: A Thyroid, Lower Right, 2.1 cm

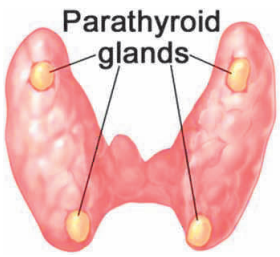
AFIRMA GENOMIC SEQUENCING CLASSIFIER	AFIRMA XPRESSION ATLAS			
Parathyroid Signature Detected **Parathyroid:** Positive	Test Not Performed (TNP)		***BRAF* p. V600E c. 1799T>A:** TNP ***RET/PTC1, RET/PTC3*:** TNP	
	Clinical Relevance	**Risk of Malignancy**	**Associated Neoplasm Type**	**FDA Approved Therapy[#]**
	N/A	N/A	Parathyroid Adenoma, Hyperplasia, or Carcinoma	N/A

RESULTS INTERPRETATION

A gene expression signature for parathyroid tissue has been identified by the parathyroid RNA Classifier®. Correlation with serum calcium and PTH levels along with ultrasound and other clinical findings is recommended. A parathyroid lesion should be ruled out before considering the possibility of a thyroid neoplasm. BRAF V600E, RET/PTC, and Afirma XA testing not performed (TNP) due to the identification of a parathyroid gene expression signature.

Parathyroid disease

- Secretes parathyroid hormone which is involved in maintaining calcium homeostasis.
- Parathyroid adenoma (85%)
- Parathyroid carcinomas (<1%)
- Parathyroid hyperplasia (15%)
- Hyperparathyroidism leads to hypercalcemia and associated symptoms
  - Kidney stones
  - Bone disease
  - Mood disturbances
  - GI issues



Bonus Case #2

- Patient's subsequent labs:
- Calcium 11.4 (8.5-10.4)
- PTH 128 (10-65)
- Patient underwent parathyroidectomy with resolution of hyperparathyroidism.

Conclusions

- Cytologic diagnosis of thyroid nodules utilizes the Bethesda system which has well-defined criteria and extensive data on clinical risks
- Years of research on thyroid cancer has provided a robust understanding of the molecular underpinnings and genetic aberrations in this disease.
- While many lesions are well defined by cytology alone, a few Bethesda categories that are "indeterminate" can be further risk stratified with the addition of molecular testing.
- Understanding the differing characteristics of RAS and BRAF-like tumors can help guide clinical management.
- Molecular testing can also provide additional information to help with diagnosis of medullary carcinoma and parathyroid lesions.