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Molecular Testing for Thyroid Nodules: Why, What and When

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Outline

- Incidence of Thyroid Cancer Rising due to Detection
- ATA Guidelines and Bethesda
- Molecular Testing Overview
- Incorporation of Ultrasound Data

Poll Questions 1

Outline

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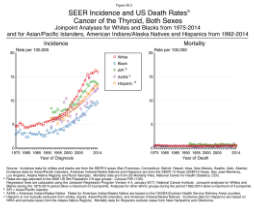
Thyroid Cancer

Incidence:

- Tripled from 1975 to 2009:
  - 4.9 to 14.3 per 100,000
  - 37,200 new cases of thyroid cancer in 2009
  - 52,000 new cases of thyroid cancer 2019



Thyroid Cancer

Increased Detection:

- Thyroid Cancers <1cm:
  - 25% of new thyroid cancers 1988-1989
  - 39% of new thyroid cancers 2008-2009
- Prevalence of thyroid cancer in autopsy specimens approx 11%
  - Stable over time in studies over last 60 years

Thyroid Nodules

Prevalence:

- Palpable Nodules:
  - 5% in women, 1% in men
    - iodine-sufficient parts of world
- Sonographic Nodules:
  - 19-68% of randomly selected individuals

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Thyroid Nodules and PET

- Retrospective study of 45,000 patients
  - 500 thyroid nodules identified (1%)
  - 36% malignant
  - 180 of the patients died, but only 1 died of thyroid cancer (medullary). All else died from primary cancer

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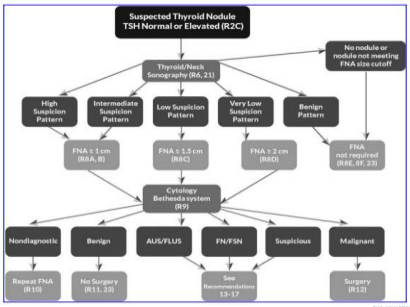
2015 ATA Guidelines: Thyroid



2015 ATA Guidelines: Thyroid

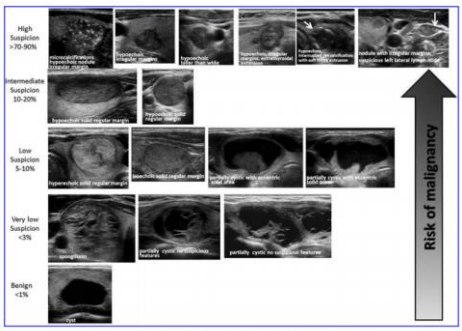
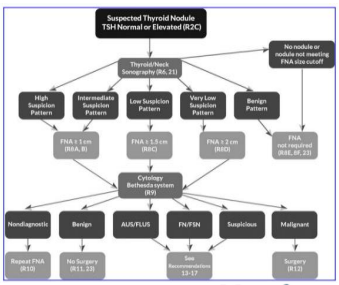


FIG. 2. ATA nodule sonographic patterns and risk of malignancy.

2015 ATA Guidelines: Thyroid



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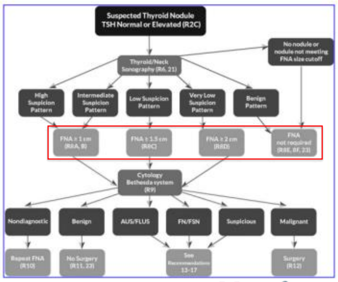

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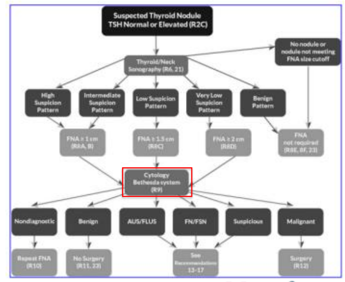
2015 ATA Guidelines: Thyroid



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Bethesda Classification

Bethesda diagnostic category			Risk of malignancy	Usual management
I	Nondiagnostic or unsatisfactory	Cyst fluid only Virtually acellular specimen Other (obscuring blood, clotting artifact, etc.)	1% to 4%	Repeat FNA with ultrasound guidance
II	Benign	Consistent with a benign follicular nodule (includes adenomatoid nodule, colloid nodule, etc.) Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context Consistent with granulomatous (subacute) thyroiditis Other	0% to 3%	Clinical follow-up
III	Atypia of undetermined significance or follicular lesion of undetermined significance		5% to 15%	Repeat FNA
IV	Follicular neoplasm or suspicious for a follicular neoplasm	Specify if Hurthle cell (oncocytic) type	15% to 30%	Surgical lobectomy
V	Suspicious for malignancy	Suspicious for papillary carcinoma Suspicious for medullary carcinoma Suspicious for metastatic carcinoma Suspicious for lymphoma Other	60% to 75%	Near-total thyroidectomy or surgical lobectomy
VI	Malignant	Papillary thyroid carcinoma Poorly differentiated carcinoma Medullary thyroid carcinoma Undifferentiated (anaplastic) carcinoma Squamous cell carcinoma Carcinoma with mixed features (specify) Metastatic carcinoma Non-Hodgkin lymphoma Other	97% to 99%	Near-total thyroidectomy

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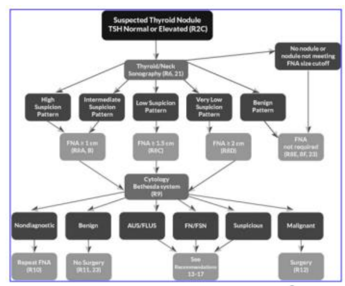
2015 ATA Guidelines: Thyroid



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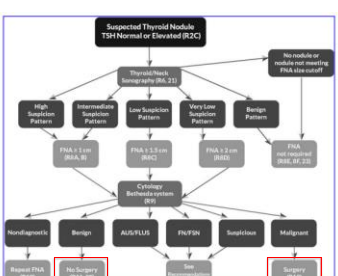
2015 ATA Guidelines: Thyroid



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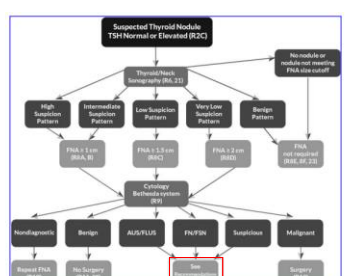

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Summary Point #1: Why?

- Thyroid cancer incidence is rising almost exclusively due to increased detection
- Majority of nodules with Indeterminate cytology (approx 80%) are benign and thus could potentially avoid surgery (morbidity, cost, resource utilization etc.)

Poll Questions 2

Outline

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2015 ATA Guidelines

Bethesda 3:

– Atypia of Undetermined Significance

– *[A17] AUS/FLUS cytology*

■ **RECOMMENDATION 15**

(A) For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making.

(Weak recommendation, Moderate-quality evidence)

(B) If repeat FNA cytology, molecular testing, or both are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference.

(Strong recommendation, Low-quality evidence)

2015 ATA Guidelines

Bethesda 4:

– Follicular Neoplasm

– *[A18] Follicular neoplasm/suspicious for follicular neoplasm cytology*

■ **RECOMMENDATION 16**

(A) Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making.

(Weak recommendation, Moderate-quality evidence)

(B) If molecular testing is either not performed or inconclusive, surgical excision may be considered for removal and definitive diagnosis of an FN/SFN thyroid nodule.

(Strong recommendation, Low-quality evidence)

2015 ATA Guidelines

Bethesda 5:

– *[A19] Suspicious for malignancy cytology*

■ **RECOMMENDATION 17**

(A) If the cytology is reported as suspicious for papillary carcinoma (SUSP), surgical management should be similar to that of malignant cytology, depending on clinical risk factors, sonographic features, patient preference, and possibly results of mutational testing (if performed).

(Strong recommendation, Low-quality evidence)

(B) After consideration of clinical and sonographic features, mutational testing for *BRAF* or the seven-gene mutation marker panel (*BRAF*, *RAS*, *RET/PTC*, *PAX8/PPARγ*) may be considered in nodules with SUSP cytology if such data would be expected to alter surgical decision-making.

(Weak recommendation, Moderate-quality evidence)

2015 ATA Guidelines

Patient Counseling:

- **RECOMMENDATION 13**
  If molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing and about the possible uncertainties in the therapeutic and long-term clinical implications of results.
  (Strong recommendation, Low-quality evidence)

Molecular Testing Questions

Depending on the question you would like answered about your patient – the testing can be different:

- Rule out cancer?
- Confirm diagnosis of cancer?

Molecular Testing Goals

Depending on the question you would like answered about your patient – the testing can be different:

- Rule out cancer?
  - Avoid unnecessary surgery
- Confirm diagnosis of cancer?
  - Properly address the disease with the correct extent of surgery at first operation

Molecular Testing Goals

Depending on the question you would like answered about your patient – the testing can be different:

- Rule out cancer?
  - Avoid unnecessary surgery
- Confirm diagnosis of cancer?
  - Properly address the disease with the correct extent of surgery at first operation
    - Total versus Hemithyroidectomy
    - Central Neck Dissection

Molecular Testing Goals

Depending on the question you would like answered about your patient – the testing can be different:

- Rule out cancer?
  - Avoid unnecessary surgery
- Confirm diagnosis of cancer?
  - Properly address the disease with the correct extent of surgery at first operation
    - Total versus Hemithyroidectomy
      - Avoid completion thyroidectomy
    - Central Neck Dissection
      - Avoid dissecting a previously operated neck

2015 ATA Guidelines

Operative Approach:

[B7] Operative approach for a biopsy diagnostic for follicular cell–derived malignancy

- **RECOMMENDATION 35**

(A) For patients with thyroid cancer ≥4 cm, or with gross extrathyroidal extension (clinical T4), or clinically apparent metastatic disease to nodes (clinical N1) or distant sites (clinical M1), the initial surgical procedure should include a near-total or total thyroidectomy and gross removal of all primary tumor unless there are contraindications to this procedure.

(Strong recommendation, Moderate-quality evidence)

(B) For patients with thyroid cancer ≥1 cm and <4 cm, without extrathyroidal extension, and without clinical evidence of any lymph node metastases (cN0), the initial surgical procedure can be either a bilateral procedure (near-total or total thyroidectomy) or a unilateral procedure (lobectomy). Thyroid lobectomy alone may be sufficient initial treatment for low-risk papillary and follicular carcinomas; however, the treatment team may choose total thyroidectomy to enable RAI therapy or to enhance follow-up based upon disease features and/or patient preferences.

(Strong recommendation, Moderate-quality evidence)

(C) If surgery is chosen for patients with thyroid cancer <1 cm without extrathyroidal extension and cN0, the initial surgical procedure should be a thyroid lobectomy unless there are clear indications to remove the contralateral lobe. Thyroid lobectomy alone is sufficient treatment for small, unifocal, intrathyroidal carcinomas in the absence of prior head and neck radiation, familial thyroid carcinoma, or clinically detectable cervical nodal metastases.

(Strong recommendation, Moderate-quality evidence)

Bethesda Classification

Bethesda diagnostic category			Risk of malignancy	Usual management
I	Nondiagnostic or unsatisfactory	Cyst fluid only Virtually acellular specimen Other (obscuring blood, clotting artifact, etc.)	1% to 4%	Repeat FNA with ultrasound guidance
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ThyroSeq

ThyroSeq v3:

Multi-gene Next Generation Sequencing Panel:

– University of Pittsburgh

- 112 genes
  - – >12,000 single nucleotide variations, insertions, deletions
  - – >120 gene fusion alterations
  - – > 90 abnormal gene expression alterations
  - – copy number alterations in 10 genomic regions

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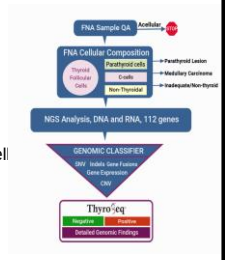
ThyroSeq

What is it?

Quantity and Composition Verification:

- gene panel
  - –Parathyroid tissue markers
  - –C-cell/medullary thyroid carcinoma cell markers
  - –Thyroid follicular cell markers
    - Cutoff level ensures adequacy for analysis
    - Needs 12% of sample to be the neoplastic cells (down to 2.5 ng)



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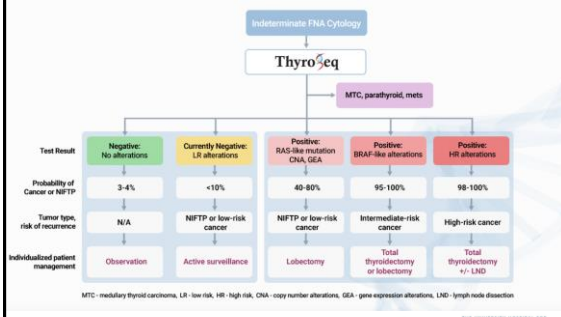
ThyroSeq v3



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ThyroSeq v3

Steward., 2019

JAMA Oncology | Original Investigation

Performance of a Multigene Genomic Classifier in Thyroid Nodules With Indeterminate Cytology

A Prospective Blinded Multicenter Study

David L. Steward, MD; Sally E. Carty, MD; Rebecca S. Sippel, MD; Samantha Peiling Yang, MBBS, MRCP, MMed; Julie A. Sosa, MD, MA; Jennifer A. Sipos, MD; James J. Figge, MD, MBA; Susan Mandel, MD, MPH; Bryan R. Haugen, MD; Kenneth D. Burman, MD; Zubair W. Baloch, MD, PhD; Ricardo V. Lloyd, MD, PhD; Raja R. Seethala, MD; William E. Gooding, MS; Simion I. Chiosea, MD; Cristiane Gomes-Lima, MD; Robert L. Ferris, MD, PhD; Jessica M. Folek, MD; Raheela A. Khawaja, MD; Priya Kundra, MD; Kwok Seng Loh, MBBS; Carrie B. Marshall, MD; Sarah Mayson, MD; Kelly L. McCoy, MD; Min En Nga, MBBS; Kee Yuan Ngiam, MBBS, MRCS, MMed; Marina N. Nikiforova, MD; Jennifer L. Poehls, MD; Matthew D. Ringel, MD; Huaitao Yang, Md, PhD; Linwah Yip, MD; Yuri E. Nikiforov, MD, PhD

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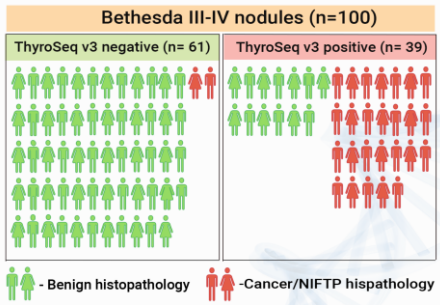
ThyroSeq v3



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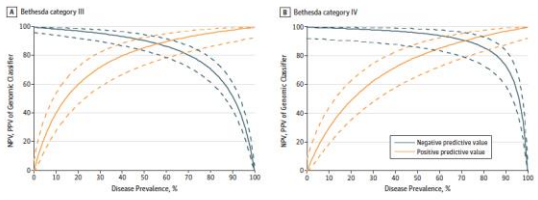

Predicted negative predictive value (NPV) (solid blue lines) and positive predictive value (PPV) (solid orange lines) with 95% CIs (dotted lines) based on sensitivity and specificity of the multigene GC test established in this study for

Bethesda III and IV cytology thyroid nodules. NIFTP indicates noninvasive follicular thyroid neoplasm with papillary-like nuclear features.

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Afirma

- Afirma GSC
  - >10,000 genes
  - RNA sequencing data
  - Other components
    - parathyroid
    - Medullary thyroid CA
    - BRAF V600E
    - RET/PTC fusion
    - Follicular content
    - Hurthle Cell

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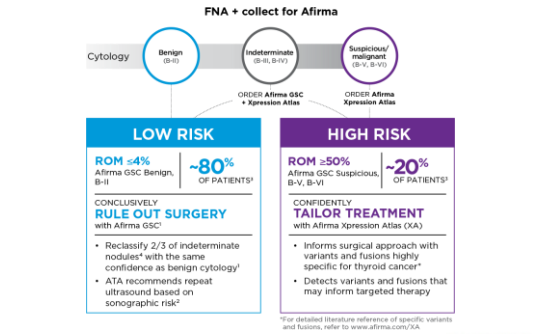
Afirma



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Afirma GSC

JAMA Network

JAMA Surg. 2018 Sep; 153(9): 817–824. PMCID: PMC6583881
Published online 2018 May 23. doi: 10.1001/jamasurg.2018.1153 PMID: 29799911

Performance of a Genomic Sequencing Classifier for the Preoperative Diagnosis of Cytologically Indeterminate Thyroid Nodules

Kepal N. Patel, MD,[1][2] Trevor E. Angell, MD,[2] Joshua Babiarz, PhD,[3] Neil M. Barth, MD,[4,5] Thomas Blevins, MD,[6] Quan-Yang Duh, MD,[7] Ronald A. Ghossein, MD,[8] R. Mack Harrell, MD,[9,10,11] Jing Huang, PhD,[3] Giulia C. Kennedy, PhD,[3] Su Yeon Kim, PhD,[3] Richard T. Kloos, MD,[4] Virginia A. LiVolsi, MD,[12] Gregory W. Randolph, MD,[13] Peter M. Sadow, MD, PhD,[14] Michael H. Shanik, MD,[15] Julie A. Sosa, MD,[16] S. Thomas Traweek, MD,[17] P. Sean Walsh, MPH,[3] Duncan Whitney, PhD,[3] Michael W. Yeh, MD,[18] and Paul W. Ladenson, MD[19]

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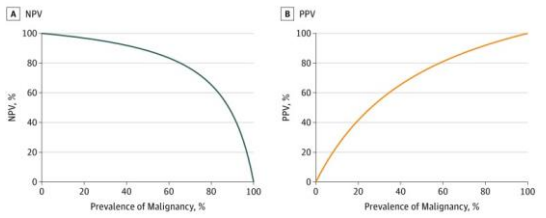
Afirma



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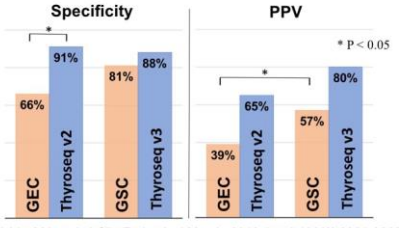
Thyroseq vs Afirma

Figure 2. Comparison of GSC and Thyroseq v3 to Gene Expression Classifier (GEC)a and Thyroseq v2a



aLivhits MJ et al. J Clin Endocrinol Metab. 2018 Jun 1;103(6):2261-2268

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Thyroseq v3 vs Afirma GSC

	ThyroSeq GC[1]	Afirma GSC[2]
Study type	Multicenter, prospective, double-blind	Multicenter, retrospective, double-blind
Total number, samples	247	191
Nodule size by ultrasound, median (range), cm	2.1 (0.5-7)	2.6 (1.0-9.1)
Disease prevalence	27.5%	23.7%
Sensitivity, (95%CI)	94.1% (86-98%)	91.1% (79-98%)
Specificity, (95%CI)	81.6% (75-87%)	68.3% (60-76%)
NPV	97.3% (93-99%)	96.1% (90-99%)
PPV	65.9% (56-75%)	47.1% (36-58%)
Benign call rate	61%	54%
Avoidable surgeries for histologically benign nodules with indeterminate cytology	82%	68%

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Summary Point #2: What?

- Thyroseq v3 and Afirma GSC are the two most widely used platforms
  - Others exist although not as extensively studied
- Both with good NPV and decent PPV
  - Depends on cancer prevalence
  - can potentially help guide extent of surgery as well

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Poll Questions 3

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What's the problem?

- NIFTP
- $$$
- How much does it change what we are really going to do???

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Ultrasound Risk Stratification

Table 5 | Summary of diagnostic performance and other clinical parameters for contemporary ultrasound stratification systems for thyroid nodules

System	Categories	Expected POM	Reported POM	Diagnostic performance	Unnecessary biopsy rate*
ATA, 2015	Benign	0%	0%	Sensitivity 75-98%; specificity 21-73%; PPV 9.6-87%; NPV 89-99%; accuracy 33-88	44-51%
	Very low suspicion	<3%	0-4%		
	Low suspicion	5-10%	2-6%		
	Intermediate suspicion	10-20%	6-34%		
	High suspicion	>70-90%	28-87%		
ACR-TIRADS, 2017	Benign	<2%	0-0.3%	Sensitivity 80-97%; specificity 53-75%; PPV 13-89%; NPV 69-98%; accuracy 71-81	17-28%
	Not suspicious	<2%	0-4%		
	Mildly suspicious	5%	1-9%		
	Moderately suspicious	5-20%	10-52%		
	Highly suspicious	>20%	35-88%		
K-TIRADS, 2016	Benign	<3%	0-3%	Sensitivity 71-100%; specificity 23-88%; PPV 16-80%; NPV 82-100%; accuracy 33-70	17-66%
	Low suspicion	3-15%	2-5%		
	Intermediate suspicion	15-50%	14-34%		
	High suspicion	>60%	61-80%		

ACR-TIRADS=American College of Radiology Thyroid Imaging Reporting and Data System; ATA=American Thyroid Association; K-TIRADS=Korean Thyroid Imaging Reporting and Data System; NPV=negative predictive value; PPV=positive predictive value; POM=prevalence of malignancy.
*Defined as number of benign nodules among nodules needing fine needle aspiration biopsy.

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Ultrasound Data vs Molecular Testing

- Study of 97 nodules with thyroseq data
- K-TIRADS correlated most strongly with end result
- After incorporation of U/S risk stratification, thyroseq changed management 19% of the time

AI and Ultrasound

- PPV and NPV range from 66-90% and 74-93% in two recent studies of >500 nodule images
- Comparable NPV but improved PPV to traditional ultrasound models

Summary Point #3

- Ultrasound data can help greatly with risk stratification
- Use molecular testing when it will actually change what you are going to do

Conclusions

- Thyroid cancer incidence is rising almost exclusively due to increased detection
- Molecular testing is now available that can provide additional information for a thyroid nodule with an indeterminate cytology result
- No test is perfect: NPV and PPV depend on cancer prevalence in individual area
- Only utilize if has potential to modify clinical treatment plan ($$)