Molecular markers in thyroid cancer: current role in clinical practice

Chairs: P. Vitti - L. Hegedus (DK)

1. In which grey areas molecular diagnosis may be helpful?
   (A. Frasoldati RE, A. Crescenzi, RM)

2. BRAF in the diagnostic evaluation of thyroid nodules (L. Fugazzola, MI)

2. BRAF as a prognostic marker in papillary thyroid cancer (R. Elisei, PI)

3. Take Home Messages (L. Hegedus, DK)
Molecular markers in everyday’s clinical practice

THROUGH THE GREY ZONE

The indeterminate (Thy-3) nodules

Nature 453, 840-842 2008
Indeterminate (Thy-3) nodules are a moving target

- The cytological definition of Thy-3 lesions is challenging
- Thy-3 lesions are histologically heterogeneous
- Thy-3 series are heterogeneous
- Accuracy of molecular tests may vary across different Thy-3 series
- Reproducibility of molecular testing performance is not 100% across different centres
The “indeterminate” nodule

Basic data
10.1% frequency
28.2% Risk of malignancy

Bongiovanni et al., Cytopathology 2012

Recommended Management
Surgery or close surveillance
The cytological definition of Thy-3 lesions is challenging

<table>
<thead>
<tr>
<th>UK RCPath</th>
<th>Italian Consensus 2013</th>
<th>USA BETHESDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnostic category</td>
<td></td>
<td>Terminology</td>
</tr>
<tr>
<td>Thy 3a</td>
<td>Neoplasm possible – atypia/non-diagnostic</td>
<td>TIR 3A</td>
</tr>
<tr>
<td>Thy 3f</td>
<td>Neoplasm possible - suggesting follicular neoplasm</td>
<td>TIR 3B</td>
</tr>
</tbody>
</table>
The cytological definition of Thy-3 lesions is challenging.

Which of the five categories is most difficult to use?

- THY-3
- THY-4
- Don't know
- THY-1

Courtesy of F. Nardi
The cytological definition of Thy-3 lesions is challenging

Eliminating the “Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance” Category From the Bethesda System for Reporting Thyroid Cytopathology

Remmi S. Singh, MD, and Helen H. Wang, MD, DrPH

Usefulness of Diagnostic Qualifiers for Thyroid Fine-Needle Aspirations With Atypia of Undetermined Significance

Paul A. VanderLaan, MD, PhD,¹ Ellen Marqsee, MD,² and Jeffrey F. Krane, MD, PhD¹
So far, molecular testing for somatic mutations is most promising (e.g., BRAF), especially in FNAB samples.

The application of molecular markers will significantly improve thyroid tumor diagnosis and thus it will help to prevent unnecessary surgeries.

It will help to better characterize tumors with histologically uncertain biological behavior and it will help to guide mutation-specific targeted therapies.

Molecular fine needle aspiration biopsy diagnosis of thyroid nodules by tumor specific mutations and gene expression patterns. M. Eszlinger, R Paschke. Mol Cell Endocrinol 2010
Thy-3 lesions are histologically heterogeneous...

<table>
<thead>
<tr>
<th>Condition</th>
<th>Rago et al., 2007</th>
<th>Yang et al., 2007</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroiditis</td>
<td>4 (0.8%)</td>
<td>11 (3.4%)</td>
<td>1.8%</td>
</tr>
<tr>
<td>Goiter</td>
<td>52 (10.3%)</td>
<td>53 (16.3%)</td>
<td>12.6%</td>
</tr>
<tr>
<td>Foll/HC Adenoma</td>
<td>290 (57.4%)</td>
<td>157 (48.2%)</td>
<td>53.8%</td>
</tr>
<tr>
<td>PTC</td>
<td>138 (27.3%)</td>
<td>71 (21.8%)</td>
<td>25.1%</td>
</tr>
<tr>
<td>Foll/HC Carcinoma</td>
<td>21 (4.1%)</td>
<td>29 (8.9%)</td>
<td>6.0%</td>
</tr>
<tr>
<td>Other malignancy</td>
<td>-</td>
<td>5 (1.5%)</td>
<td>0.6%</td>
</tr>
<tr>
<td>Total</td>
<td>505</td>
<td>326</td>
<td></td>
</tr>
</tbody>
</table>
Thy-3 series are also heterogeneous!

Trimboli et al., Endocrine (in press)
Concordance on the histopathologic distinction between benign and malignant diagnoses was 91% comparing local with central histopathologists and 90% comparing 2 central histopathologists.

Central cytopathologists made fewer indeterminate diagnoses than local pathologists (41.2% vs. 55.0%).
Molecular pathways in thyroid tumors

Tumors harboring PAX8-PPARY rearrangement directly develop as follicular carcinomas without a benign adenoma stage. Acquisition of RAS mutations leads to the development of follicular adenomas, whereas additional and still unknown genetic alterations require for malignant transformation and progression to a follicular carcinoma stage.
Several immunohistochemical markers using various antibodies have been reported to be useful in differentiating benign from malignant thyroid lesions.

- Cell adhesion molecules (Gal-3)
- Cytokeratins (CK19)
- Cell cycle proteins (cyclin D1, p27, p53)
- Oncoprotein (RET, PPAR-γ, β-catenin)
- Others (HBME-1, CD10, CD57, TPO)
Panels of immunochemical markers that best support the diagnosis of malignancy

- PTC and FVPTC: Gal-3, HBME-1, CK19 (RET, β-catenina)

- FTC: Gal-3, HBME1, PPAR-γ

- Progression to poorly differentiated: CyclinD1, p53, Ki67

• VE1 antibody can be used in core thyroid specimens to detect BRAF(V600E) mutated PTC at the preoperative time.
• VE1 immunohistochemistry performed in thyroid CNB samples perfectly matches with genetic analysis of BRAF(V600E) status.
• These data should greatly improve the widespread diffusion of BRAF test
Genetic alteration in thyroid cancer

- Activating mutation BRAF (V600E) is found in about 45% (29% to 80%) of papillary carcinoma.

- RET/PTC rearrangement is found in approximately 20% of adult sporadic papillary carcinomas (50-70% in young/children and in patients with radiation exposure).

- PAX8/PPARγ rearrangement is found in 30-40% of conventional type follicular carcinomas.

- Point mutation of the RAS genes are not restricted to a specific thyroid tumor (in 40% of follicular carcinomas, in 10% of papillary carcinomas, 20-40% of follicular adenomas).
Which kind of prognostic marker?

- **BRAF**: BRAF mutation has been associated with clinical progression, recurrence, extra thyroidal invasion, lymph nodes metastasis and non response to iodine treatment.

- **RAS**: Several studies support a significant correlation between RAS mutation and metastatic behavior of follicular carcinomas.

- **RET/PTC**: RET/PTC1 rearrangement has been associated with a more favorable behavior of PTCs.
Prognostic significance of BRAFV600E

- BRAF V600E is a prominent oncogene in papillary thyroid cancer but oncogenesis is a multifactorial process.
- HT can be considered a shield against tumour progression, even in the presence of a negative prognostic factor such as the BRAF mutation.
MicroRNA: endogenous non-coding RNAs that negatively regulate the expression of protein coding genes.

PTC miRNAs: 187, 221, 222, 146b
PTC with RET/PTC: miRNA 187
FC miRNA 187
OC miRNA: 187, 221, 222, 197

Nikiforova et al. MicroRNA Expression Profiling of Thyroid Tumors: Biological Significance and Diagnostic Utility J Clin Endocrinol Metab 93: 1600–1608, 2008
Tumor gene expression profiling by DNA microarrays

• Gene expression studies in thyroid tumors also contribute to the growing experience on molecular differences between various types of thyroid disease.

• In the first study of papillary thyroid cancer (PTC) by high density DNA microarrays, published by Huang et al (2001) its gene expression profile was highly consistent.

• Some of these have already been tested as clinical markers.
Selected differentially expressed genes in PTC tumors compared with paired normal thyroid tissues

Huang Y et al. PNAS 2001;98:15044-15049
Veracyte’s Afiirma Thyroid FNA Analysis


Compared with current practice based on cytological findings alone, use of this test may result in lower overall costs and modestly improved quality of life for patients with indeterminate thyroid nodules.
METHODS
Prospective, multicenter double-blind validation study. 49 clinical sites, 3789 patients, and 4812 FNAs.

577 indeterminate, 413 with histopathological diagnosis. Gene-expression classifier (GEC) used to test 265 indeterminate nodules.

RESULTS
GEC correctly identified 78/85 malignant nodules

92% sensitivity; 52% specificity
NPV = 93%  PPV = 47.3%

Conclusions: Analytical sensitivity, analytical specificity, robustness, and quality control of the GEC were successfully verified, indicating its suitability for clinical use.

Cost of the test about 3200 $
The alternative commercially available approach

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Result</th>
<th>Mutation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRAF p.V600E (GTG&gt;GAG)</td>
<td>+</td>
<td>KRAS p.G12S (GGT&gt;AGT)</td>
<td>-</td>
</tr>
<tr>
<td>NRAS p.Q61R (CAA&gt;CGA)</td>
<td>-</td>
<td>KRAS p.G12R (GGT&gt;CGT)</td>
<td>-</td>
</tr>
<tr>
<td>NRAS p.Q61K (CAA&gt;AAA)</td>
<td>-</td>
<td>KRAS p.G12V (GGT&gt;GTT)</td>
<td>-</td>
</tr>
<tr>
<td>NRAS p.Q61L (CAA&gt;CTA)</td>
<td>-</td>
<td>KRAS p.G13D (GGC&gt;GAC)</td>
<td>-</td>
</tr>
<tr>
<td>HRAS p.G12V (GGC&gt;GTC)</td>
<td>-</td>
<td>KRAS p.G12D (GGT&gt;GAT)</td>
<td>-</td>
</tr>
<tr>
<td>HRAS p.Q61L (CAG&gt;AAG)</td>
<td>-</td>
<td>RNA Translocations</td>
<td></td>
</tr>
<tr>
<td>HRAS p.Q61R (CAG&gt;CAG)</td>
<td>-</td>
<td>RET/PTC1 TRANSLOCATION</td>
<td>-</td>
</tr>
<tr>
<td>KRAS p.G12A (GGT&gt;GCT)</td>
<td>-</td>
<td>RET/PTC3 TRANSLOCATION</td>
<td>-</td>
</tr>
<tr>
<td>KRAS p.G12C (GGT&gt;TGT)</td>
<td>-</td>
<td>PAX8/PPARγ TRANSLOCATION</td>
<td>-</td>
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### Issue 3. Indeterminate nodules (Thy-3) Accuracy of Molecular Tests

<table>
<thead>
<tr>
<th>All 4 studies*</th>
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<tbody>
<tr>
<td>PTC</td>
<td>34</td>
</tr>
<tr>
<td>FTC</td>
<td>27</td>
</tr>
<tr>
<td>fvPTC</td>
<td>80</td>
</tr>
<tr>
<td>Adenoma</td>
<td>267</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>351</td>
</tr>
<tr>
<td>total</td>
<td>706</td>
</tr>
</tbody>
</table>

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sens</td>
<td>54.2%</td>
</tr>
<tr>
<td>Spec</td>
<td>95.8%</td>
</tr>
<tr>
<td>PPV</td>
<td>74.7%</td>
</tr>
<tr>
<td>NPV</td>
<td>90.1%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>88%</td>
</tr>
</tbody>
</table>

Cantara et al., JCEM 2010, 95: 1365-1369  
Ohori et al., Cancer Cytopathol 2010,  
Nikiforov Y E et al. JCEM 2011;96:3390-3397  
Eszlinger et al., Thyroid 2013 (in press)
This mutation panel is currently offered in the United States for $600 to $2,400, depending on whether the test is reimbursed privately, by insurance companies, or by a public entity, such as Medicare.

Mutation analysis for some genes, such as RAS and PAX-8/PPAR-g, is limited by a rate of 20% to 40% and 2% to 10% in benign lesions respectively. As our data show, the benefits of this panel are limited, and it is questionable if it should be applied routinely to the clinical practice.

Management of Indeterminate (Thy-3) nodule

In a hypothetical population of 1000 patients submitted to thyroid FNA

**Without Molecular analysis**

- Indeterminate Thy-3 results 10 (5-25) % of all FNAs
  - N=100
  - Repeat FNA
    - N=25
    - (Still) Indeterminate n = 85
  - N=60
    - Surgery (70%) Malignant n = 16
      - Benign n = 47
  - N=25
    - Follow-up (30%) Malignant left?

**With Molecular analysis**

- Diagnostic Upgrade (Thy4/Thy-5) n = 5
- Diagnostic Downgrade (Thy-2) n = 10
In a hypothetical population of 1000 patients submitted to thyroid FNA

Indeterminate Thy-3 results
10 (5-25) % of all FNAs

N=100
Cytological review
n = 64

+ GEC

Risk of malignancy
42%

n = 40
Surgery
Malignant  n = 17
Benign  n = 23
Follow-up
1-2 Malignant left
Long-term follow-up

---

Risk of malignancy
7.5 %
n = 24

No more indeterminate  n = 36

---

Indeterminate Thy-3 results
10 (5-25) % of all FNAs

N=100

+ BRAF

PAX8/PPARγ

RAS

RET/PTC

Risk of malignancy
75.7%
n = 13
Surgery
Malignant  n = 10
Benign  n = 3
Follow-up
8-9 malignant left
Long-term follow-up

---

Risk of malignancy
9.7%
n = 87

---
Clinical management of “positive” lesions

Cytologic Diagnosis

<table>
<thead>
<tr>
<th>AUS/FLUS</th>
<th>FN/SFN</th>
<th>SMC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer Risk Based on Cytology Only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14% ↓</td>
<td>27% ↓</td>
<td>54% ↓</td>
</tr>
</tbody>
</table>

Testing for Panel of Mutations (BRAF, RAS, RET/PTC, PAX8/PPARγ)

Mutational Status

<table>
<thead>
<tr>
<th>Positive</th>
<th>Negative</th>
<th>Positive</th>
<th>Negative</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>88%</td>
<td>5.9%</td>
<td>87%</td>
<td>14%</td>
<td>95%</td>
<td>28%</td>
</tr>
</tbody>
</table>

Cancer Risk

<table>
<thead>
<tr>
<th>Total thyroidectomy</th>
<th>Lobectomy vs. observation +/- repeat FNA</th>
<th>Total thyroidectomy</th>
<th>Lobectomy</th>
<th>Total thyroidectomy</th>
<th>Lobectomy</th>
</tr>
</thead>
</table>

Clinical Management

Nikiforov Y E et al. JCEM 2011;96:3390-3397
Issue 2. Benign (THy-2) samples

Basic data
- 80% Frequency
0-3% Risk of malignancy

Recommended Management
Clinical and US Follow-up
Repeat FNA in selected cases*

*Systematic repeat FNA suggested by some authors

Bongiovanni et al., Cytopathology 2012
Results of molecular analysis in Thy-2 samples

Cantara et al. 2010

<table>
<thead>
<tr>
<th>Benign (87)</th>
<th>BRAF 2</th>
<th>RET/PTC 2</th>
<th>RAS 5</th>
<th>None 78</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PTC 2</td>
<td>PTC 2</td>
<td>PTC 2 FA 3</td>
<td>PTC 2 FTC 1 FA 10 Hyperplastic 65</td>
</tr>
</tbody>
</table>

10.3 % Mutation + 
9 VP 65 VN 0 FP 13 FN
NPV = 83.3% PPV = 100%

Rossi et al. 2012

1.3% Mutation POS
19VP VN? 0 FP 17 (?) FN
NPV ? PPV 100%
Benign (Thy-2) nodules

In a hypothetical population of 1000 patients*

Benign Thy-2 results
80% of all FNAs

N=800, Repeat FNA

Confirmed Benign

- 85% n = 680

Follow-up 645

Surgery 35

Malignant 2

Diagnostic
68 Thy-2 12 Thy 3-4-5

Possible role of molecular analysis for avoiding repeat FNA and/or reducing life-long US monitoring
Conclusions

Recent studies focused on application of molecular markers to thyroid FNA have shown quite promising results.

These results need to be weighed against some limitations:
- different methodology/approaches
- different case series and selection of patients
- scant clinical/ultrasonographical data

Furthermore, an accurate cytological examination is the premise for a proficient use of molecular testing.

Nevertheless....
Conclusions

Accurate stratification of malignancy risk in indeterminate thyroid nodules now seems a low-hanging fruit!