Application of Molecular Diagnosis: Present and Future

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May 2nd 2015, SICEM Thyroid Symposium 2
Today’s Talk

1) Present diagnosis of thyroid nodule & its pitfalls (FNA)

2) Molecular pathogenesis of thyroid cancer

3) Rule-in & rule-out molecular test for surgery – advantages & disadvantages

4) Expansion of molecular markers (DNA mutation or rearrangements)

5) Positioning of molecular test & future directions
The Bethesda System for Reporting Thyroid Cytopathology: Recommended Diagnostic Categories in Thyroid Nodule

1 Non-diagnostic or unsatisfactory
   Cyst Fluid Only
   Virtually acellular specimen, No follicular cells present
   Other (obscuring blood, clotting artifact, poor fixation, poor cell preservation etc)

2 Benign
   Consistent with a benign follicular nodule (adenomatoid nodules, colloid nodule, etc.)
   Consistent with lymphocytic (Hashimoto) thyroiditis in the proper clinical context
   Consistent with granulomatous (subacute) thyroiditis
   Other

3 Atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS)

4 Follicular neoplasm or suspicious for a follicular neoplasm
   Specify if Hürthle cell (oncocytic) type

5 Suspicious for malignancy
   Suspicious for papillary carcinoma
   Suspicious for medullary carcinoma
   Suspicious for metastatic carcinoma
   Suspicious for lymphoma
   Other

6 Malignant

2009 The Bethesda System for Reporting Thyroid Cytopathology
# The Bethesda System for Reporting Thyroid Cytopathology: Implied Risk of Malignancy and Recommended Clinical Management

<table>
<thead>
<tr>
<th>Diagnostic Category</th>
<th>Risk of Malignancy (%)</th>
<th>Usual Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nondiagnostic or Unsatisfactory</td>
<td>1-4</td>
<td>Repeat FNA</td>
</tr>
<tr>
<td>Benign</td>
<td>0-3</td>
<td>Clinical F/U</td>
</tr>
<tr>
<td>Atypia of Undetermined Significance (AUS) or Follicular Lesion of Undetermined Significance (FLUS)</td>
<td>5-15</td>
<td>Repeat FNA</td>
</tr>
<tr>
<td>Follicular Neoplasm or Suspicious for a Follicular Neoplasm</td>
<td>15-30</td>
<td>Surgical lobectomy</td>
</tr>
<tr>
<td>Suspicious for Malignancy</td>
<td>60-75</td>
<td>Total thyroidectomy or surgical lobectomy</td>
</tr>
<tr>
<td>Malignant</td>
<td>97-99</td>
<td>Total thyroidectomy</td>
</tr>
</tbody>
</table>

2009 The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC)
Postoperative Malignancy Rates for Each Cytology Subtype in Updated Meta-Review

A Large Multicenter Correlation Study of Thyroid Nodule Cytopathology and Histopathology

<table>
<thead>
<tr>
<th>Study</th>
<th>No. resected in series</th>
<th>% malignant postoperatively by cytopathology diagnosis</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>Blansfield et al. (20) (Abington Hospital, PA)</td>
<td>183</td>
<td>18</td>
</tr>
<tr>
<td>Sclabas et al. (21) (MD Anderson)</td>
<td>240</td>
<td>4</td>
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<td>N/A</td>
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<td>8</td>
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ATYP, FN/HN, and SUSP M combined are Indeterminate.

**ATYP, atypia of undetermined significance; FoN/HN, follicular/Hurthle cell neoplasm; SUSP M, suspicious for malignancy; I, indeterminate; M, malignant; ND, nondiagnostic; N/A, not applicable.**
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Differences of FNA Diagnostic Performance based on ‘2011 Wang et al. Thyroid‘ Study – 11 Major Hospitals

≈ Diagnostic Differences of Expert Pathologists

<table>
<thead>
<tr>
<th>Preop. cytology</th>
<th>Postop. malignancy rate (%) between hospitals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>2 ~ 18</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>14 ~ 48</td>
</tr>
<tr>
<td>AUS</td>
<td>0 ~ 48</td>
</tr>
<tr>
<td>FoN/HN</td>
<td>14 ~ 49</td>
</tr>
<tr>
<td>SUSP M</td>
<td>42 ~ 87</td>
</tr>
<tr>
<td>Malignant</td>
<td>93 ~ 100</td>
</tr>
<tr>
<td>Nondiagnostic</td>
<td>9 ~ 50</td>
</tr>
</tbody>
</table>

Need Molecular testing in Cytology Sample!

⇒ to more accurately refine FNA diagnosis in the cytologically indeterminate group where the majority of cases prove to be benign
⇒ surgery could be avoided
Molecular Markers in Determining Malignancy: Where?

Benign on FNAC

Indeterminate cytology on FNAC

- Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance
- Follicular Neoplasm or Suspicious for a Follicular Neoplasm
- Suspicious cytology

Molecular Markers

- Benign - Observation
- Malignant - Surgery

Malignant on FNAC
# Summary of Studies of Diagnostic Molecular Markers on Thyroid FNAB Specimens with Indeterminate Cytology

<table>
<thead>
<tr>
<th>Study</th>
<th>n*</th>
<th>Malignant (%)†</th>
<th>Markers</th>
<th>Prospective</th>
<th>Multicentre</th>
<th>Blinded</th>
<th>Sensitivity</th>
<th>NPV</th>
<th>Specificity</th>
<th>PPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faroux et al, 1997&lt;sup&gt;26&lt;/sup&gt;</td>
<td>69</td>
<td>13%</td>
<td>A</td>
<td>NA</td>
<td>No</td>
<td>NA</td>
<td>89%</td>
<td>97%</td>
<td>58%</td>
<td>24%</td>
</tr>
<tr>
<td>Umbricht et al, 2004&lt;sup&gt;29&lt;/sup&gt;</td>
<td>100</td>
<td>48%</td>
<td>B</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
<td>90%</td>
<td>87%</td>
<td>65%</td>
<td>70%</td>
</tr>
<tr>
<td>Saggiorato et al, 2005&lt;sup&gt;30&lt;/sup&gt;</td>
<td>125</td>
<td>60%</td>
<td>C</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>100%</td>
<td>100%</td>
<td>82%</td>
<td>78%</td>
</tr>
<tr>
<td>Bartolazzi et al, 2008&lt;sup&gt;33&lt;/sup&gt;</td>
<td>432</td>
<td>30%</td>
<td>D</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>78%</td>
<td>91%</td>
<td>93%</td>
<td>82%</td>
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<tr>
<td>Franco et al, 2009&lt;sup&gt;31&lt;/sup&gt;</td>
<td>138</td>
<td>51%</td>
<td>E</td>
<td>No</td>
<td>Yes</td>
<td>NA</td>
<td>95%</td>
<td>92%</td>
<td>76%</td>
<td>83%</td>
</tr>
<tr>
<td>Nikiforov et al, 2009&lt;sup&gt;29&lt;/sup&gt;</td>
<td>52</td>
<td>40%</td>
<td>F</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>71%</td>
<td>84%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Moses et al, 2010&lt;sup&gt;27&lt;/sup&gt;</td>
<td>137</td>
<td>31%</td>
<td>F</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>48%</td>
<td>80%</td>
<td>94%</td>
<td>78%</td>
</tr>
<tr>
<td>Milas et al, 2010&lt;sup&gt;34&lt;/sup&gt;</td>
<td>61</td>
<td>75%</td>
<td>G</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>59%</td>
<td>80%</td>
<td>90%</td>
<td>39%</td>
</tr>
<tr>
<td>Samijia et al, 2011&lt;sup&gt;32&lt;/sup&gt;</td>
<td>142</td>
<td>20%</td>
<td>H</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>79%</td>
<td>91%</td>
<td>53%</td>
<td>28%</td>
</tr>
<tr>
<td>Fadda et al, 2011&lt;sup&gt;31&lt;/sup&gt;</td>
<td>119</td>
<td>45%</td>
<td>I</td>
<td>NA</td>
<td>No</td>
<td>NA</td>
<td>89%</td>
<td>85%</td>
<td>64%</td>
<td>71%</td>
</tr>
<tr>
<td>Nikiforov et al, 2011&lt;sup&gt;28&lt;/sup&gt;</td>
<td>513</td>
<td>24%</td>
<td>J</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>61%</td>
<td>89%</td>
<td>98%</td>
<td>89%</td>
</tr>
<tr>
<td>Shen et al, 2012&lt;sup&gt;34&lt;/sup&gt;</td>
<td>68</td>
<td>65%</td>
<td>K</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>89%</td>
<td>79%</td>
<td>79%</td>
<td>89%</td>
</tr>
<tr>
<td>Keutgen et al, 2012&lt;sup&gt;25&lt;/sup&gt;</td>
<td>72</td>
<td>31%</td>
<td>L</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>100%</td>
<td>100%</td>
<td>86%</td>
<td>73%</td>
</tr>
<tr>
<td>Agretti et al, 2012&lt;sup&gt;36&lt;/sup&gt;</td>
<td>53</td>
<td>28%</td>
<td>M</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>60%</td>
<td>78%</td>
<td>58%</td>
<td>39%</td>
</tr>
<tr>
<td>Rossi et al, 2012&lt;sup&gt;25&lt;/sup&gt;</td>
<td>123</td>
<td>36%</td>
<td>N</td>
<td>Yes</td>
<td>No</td>
<td>NA</td>
<td>32%</td>
<td>73%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Alexander et al, 2012&lt;sup&gt;26&lt;/sup&gt;</td>
<td>265</td>
<td>32%</td>
<td>O</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>92%</td>
<td>93%</td>
<td>52%</td>
<td>47%</td>
</tr>
</tbody>
</table>

FNAB—fine needle aspiration biopsy. NPV—negative predictive value. PPV—positive predictive value. NA—not available. A—TPO immunocytochemistry (positive for malignancy <80% cells). B—human telomerase mRNA. C—galectin 3 plus KRT19 plus HBME-1 immunocytochemistry. D—galectin 3 immunohistochemistry (cell blocks). E—galectin 3 plus HBME-1 immunohistochemistry (cell blocks), either marker positive (>10% cells staining). F—BRAF and RAS mutations, RET-PTC and PAX8-PPAR rearrangements. G—peripheral blood (non-serum, non-erythrocyte) thyroid-stimulating hormone mRNA (>1 ng/µg RNA is positive). H—galectin 3 mRNA RT-PCR (visual band on gel). I—galectin 3 plus HBME-1 immunocytochemistry (>50% cell staining, either marker positive). J—BRAF and RAS mutations, RET-PTC and PAX8-PPAR rearrangements. K—four microRNA set (miR-30d, miR-146b, miR-187, miR-221) linear discrimination analysis. L—four microRNA set (miR-21, miR-197, miR-222, miR-328) support vector machine. M—three microRNA set (miR-146b, miR-155, miR-221) decision-tree analysis. N—BRAF<sup>missense</sup> mutation. O—gene expression classifier (mRNA expression levels of 142 genes). †Number of indeterminate FNAB with histopathology correlation. ‡Percentage malignancy among indeterminate FNAB nodules.

*Table 1: Summary of studies of diagnostic molecular markers on thyroid FNAB specimens with indeterminate cytology*
Molecular Markers to Evaluate Thyroid Nodules

Immunohistochemistry
- Galectin-3
- HBME-1
- CK19

Mutations and Gene Rearrangements
- BRAF (V600E)
- RAS (HRAS, KRAS, NRAS)
- RET/PTC (RET/PTC1, RET/PTC3)
- PAX8/PPARG

Multiple mutation testing
- Academic Laboratories and Asurgen, miRInform Thyroid Panel

Gene Expression and Microarray Analysis
- MicroRNA expression
- Afirma Gene Expression Classifier

2012 Kim MI et al Endocrine Prac
Multivariate Analysis of Lymph Node Involvement with Galectin-3-Negative (G3N) and Clinicopathologic Parameters in Papillary Thyroid Carcinoma

⇒ 5~10% of PTC showed negative Galectin-3 staining: a kind of pitfall of G3 staining

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted OR [CI]</th>
<th>p</th>
<th>Adjusted OR [CI]</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.98 [0.96–0.99]</td>
<td>&lt;0.001</td>
<td>0.96 [0.95–0.98]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, male</td>
<td>2.08 [1.34–3.23]</td>
<td>0.001</td>
<td>1.89 [1.14–3.13]</td>
<td>0.014</td>
</tr>
<tr>
<td>Tumor size (mm)</td>
<td>3.10 [2.26–4.25]</td>
<td>&lt;0.001</td>
<td>2.16 [1.57–2.98]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Multifocality</td>
<td>2.10 [1.52–2.91]</td>
<td>&lt;0.001</td>
<td>2.00 [1.38–2.90]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thyroid capsular invasion</td>
<td>3.87 [2.67–5.61]</td>
<td>&lt;0.001</td>
<td>1.60 [0.93–2.76]</td>
<td>0.089</td>
</tr>
<tr>
<td>Extrathyroidal extension</td>
<td>4.26 [3.04–5.97]</td>
<td>&lt;.001</td>
<td>2.66 [1.62–4.34]</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td>3.52 [0.70–17.57]</td>
<td>0.125</td>
<td>0.79 [0.12–5.25]</td>
<td>0.809</td>
</tr>
<tr>
<td>G3N</td>
<td>0.30 [0.16–0.58]</td>
<td>&lt;0.001</td>
<td>0.37 [0.18–0.78]</td>
<td>0.009</td>
</tr>
</tbody>
</table>

⇒ Preoperative evaluation of G3 expression help predict cancer invasiveness in addition to cytological diagnosis of malignancy in thyroid nodule
Molecular Markers to Evaluate Thyroid Nodules

Immunohistochemistry (IHC) Markers

- Galectin-3
- HBME-1
- CK19
- Fibronectin-1
- CITED-1

- Immunostaining the cells from FNAC sample, used either individually or as panels

- Major problem: A lack of sensitivity and specificity to differentiate the AUS/FLUS from FN/HCN or from suspicious for malignancy cases

- Considerable overlap of IHC markers between indeterminate nodules and DTC

2012 Kim MI et al Endocrine Prac,
2012 Duick DS et al Endocrine Prac
# Types of Thyroid Cancer and Mutational Profiles

<table>
<thead>
<tr>
<th></th>
<th>Papillary Ca</th>
<th>Follicular Ca</th>
<th>Poorly differentiated Ca</th>
<th>Anaplastic Ca (undifferentiated Ca)</th>
<th>Medullary Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell type</strong></td>
<td>Follicular</td>
<td>Follicular</td>
<td>Follicular</td>
<td>Follicular</td>
<td>C cell</td>
</tr>
<tr>
<td><strong>Main histopathologic variants</strong></td>
<td>Classic papillary, Microcarcinoma, Follicular variant, Tall-cell variant,</td>
<td>Conventional Oncocytic (Hurthle cell) type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prevalence (%)</strong></td>
<td>80–85</td>
<td>10–15</td>
<td>&lt;2</td>
<td>1–2</td>
<td>3–5</td>
</tr>
<tr>
<td><strong>Frequency of familial forms (%)</strong></td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>15–30</td>
</tr>
<tr>
<td><strong>Typical route of spread</strong></td>
<td>Local LN metastasis</td>
<td>Hematogenous metastasis, typically to bones and Lungs</td>
<td>Invasive local growth, LN and hemat metastases</td>
<td>Invasive local growth, LN and hemat metastases</td>
<td>LN and hemat metastases</td>
</tr>
<tr>
<td><strong>10-year survival (%)</strong></td>
<td>95–98</td>
<td>90–95</td>
<td>~50</td>
<td>&lt;10</td>
<td>60–80</td>
</tr>
</tbody>
</table>
Somatic Mutation Frequencies Observed In Exomes From 3,083 Tumor–Normal Pairs

⇒ 0.41 non-silent mutations per Mb

2013 Lawrence et al. Nature
MAPK and PI3K-AKT-mTOR Pathways—Genetic Alterations and Therapeutic Targets in Thyroid Cancer

2013 Xing M et al. Lancet

- RET/PTC rearrangement
- NRAS, HRAS, KRAS mutation
- BRAF<sup>V600E</sup>
- PTEN, PI3KCA mutation

Aberrant cell signaling and activation
In an indeterminate FNA cytology

* A negative molecular-marker test - defer surgery – sensitivity & NPV
  Gene expression classifier (Afirma®)

* A positive molecular-marker test - pursue surgery – specificity & PPV
  Mutation-based oncogene panel (including BRAF)
BRAF V600E Mutation and Its Signal Pathway in PTC

BRAF structure

RAS-GTP binding domain
CR1
CR2
CR3
Kinase Domain

B-Raf^{V600E} in papillary thyroid cancer

Wild-type

Single Base Change

Single AA change
Glutamate to Valine

Oncogenic BRAF pathway

BRAF^{V600E} Pathway

2014 Parangi et al. Surg Clin N Am
FNAB Result

Nondiagnostic
- Repeat FNAB
  - Insufficient
    - Clinical Judgement (Size, US)
      - Benign
        - Follow up and repeat FNAB if their size increase
      - Solid nodules with size >2cm or any size with Malignant US features
    - Surgery
  - ACUS
    - BRAF (+)
    - BRAF (-)

Benign

Cytology
- ACUS (141)
  - Positive (45)
  - Negative (96)

BRAFV600E
- Benign (1): NH (1)
- Malignant (21): PTC (21)

Histology
- Benign (8): NH (7), FA (1)
- Malignant (3): PTC (2), FTC (1)

2011 Kim SK et al, JCEM
Means from studies that analyzed BRAF mutations

<table>
<thead>
<tr>
<th></th>
<th>Indeterminate (24, 29, 30, 35)</th>
<th>Suspicious, follicular-neoplasm/suspicious for follicular neoplasm, Hurthle cell neoplasm, suspicious for malignancy (26, 29, 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>0.5 (0–1)</td>
<td>0</td>
</tr>
<tr>
<td>FN</td>
<td>6 (3–12)</td>
<td>13.3 (8–12)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>12.95% (0–37.5%)</td>
<td>62% (55–71%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.3% (75–100%)</td>
<td>100%</td>
</tr>
</tbody>
</table>

⇒ High specificity of BRAF mutation test leads to surgery
Gene Expression Classifier (GEC, Afirma® test)

The Afirma Solution
A Comprehensive Assessment for Thyroid Nodules

- Cytopathology
  - Benign
  - Indeterminate*
  - Suspicious or Malignant

- Afirma Gene Expression Classifier
  - Benign
  - Suspicious

- Afirma Malignancy Classifiers
  - MTC
  - BRAF

~50%
### Gene Expression Classifier (GEC, Afirma® test)

#### Performance across the Primary Data Set of Indeterminate Nodules (N=265)

<table>
<thead>
<tr>
<th>GEC result</th>
<th>Malignant reference standard (N=85)</th>
<th>Benign reference standard (N=180)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious</td>
<td>78</td>
<td>87</td>
</tr>
<tr>
<td>Benign</td>
<td>7</td>
<td>93</td>
</tr>
</tbody>
</table>

Sensitivity, 92% (84–97); specificity, 52% (44–59); PPV, 47% (40–55); NPV, 93% (86–97); prevalence of malignant lesions, 32%

#### Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (N=129, 48.7%)

<table>
<thead>
<tr>
<th>GEC result</th>
<th>Malignant reference standard (N=31)</th>
<th>Benign reference standard (N=98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious</td>
<td>28</td>
<td>46</td>
</tr>
<tr>
<td>Benign</td>
<td>3</td>
<td>52</td>
</tr>
</tbody>
</table>

Sensitivity, 90% (74–98); specificity, 53% (43–63); PPV, 38% (27–50); NPV, 95% (85–99); prevalence of malignant lesions, 24%
## Gene Expression Classifier (GEC, Afirma® test)

### Follicular or Hürthle-Cell Neoplasm or Suspicious for Follicular Neoplasm (N=81, 30.6%)

<table>
<thead>
<tr>
<th>GEC result</th>
<th>Malignant reference standard (N=20)</th>
<th>Benign reference standard (N=61)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious</td>
<td>18</td>
<td>31</td>
</tr>
<tr>
<td>Benign</td>
<td>2</td>
<td>30</td>
</tr>
</tbody>
</table>

Sensitivity, 90% (68–99); specificity, 49% (36–62); PPV, 37% (23–52); NPV, 94% (79–99); prevalence of malignant lesions, 25%

### Suspicious for Malignancy (N=55, 20.8%)

<table>
<thead>
<tr>
<th>GEC result</th>
<th>Malignant reference standard (N=34)</th>
<th>Benign reference standard (N=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td>Benign</td>
<td>2</td>
<td>11</td>
</tr>
</tbody>
</table>

Sensitivity, 94% (80–99); specificity, 52% (30–74); PPV, 76% (61–88); NPV, 85% (55–98); prevalence of malignant lesions, 62%

More suitable for AUS/FLUS & FN/HN than Suspicious for malignancy

2012 Alexander et al. NEJM
Gene Expression Classifier (GEC, Afirma® test)

### Performance on Cytopathologically Benign Samples (N=47)

<table>
<thead>
<tr>
<th>GEC result</th>
<th>Malignant reference standard (N=3)</th>
<th>Benign reference standard (N=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Benign</td>
<td>0</td>
<td>31</td>
</tr>
</tbody>
</table>

Sensitivity, 100% (29–100); specificity, 70% (55–83); prevalence of malignant lesions, 6%

### Performance on Cytopathologically Malignant Samples (N=55)

<table>
<thead>
<tr>
<th>GEC result</th>
<th>Malignant reference standard (N=55)</th>
<th>Benign reference standard (N=0)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspicious</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td>Benign</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Sensitivity, 100% (93–100); prevalence of malignant lesions, 100%

2012 Alexander et al. NEJM
### Gene Expression Classifier (GEC, Afirma® test)

<table>
<thead>
<tr>
<th>Histopathological Subtype</th>
<th>No. of Nodules (%)</th>
<th>Result with Gene-Expression Classifier</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benign</strong></td>
<td></td>
<td>no. benign/no. suspicious</td>
</tr>
<tr>
<td>Total</td>
<td>180 (100)</td>
<td>41/30</td>
</tr>
<tr>
<td>Benign follicular nodule*</td>
<td>71 (39.4)</td>
<td>41/30</td>
</tr>
<tr>
<td>Follicular adenoma</td>
<td>64 (35.6)</td>
<td>37/27</td>
</tr>
<tr>
<td>Follicular tumor of uncertain malignant potential</td>
<td>11 (6.1)</td>
<td>5/6</td>
</tr>
<tr>
<td>Well-differentiated tumor of uncertain malignant potential</td>
<td>9 (5.0)</td>
<td>4/5</td>
</tr>
<tr>
<td>Hürthle-cell adenoma</td>
<td>21 (11.7)</td>
<td>4/17</td>
</tr>
<tr>
<td>Chronic lymphocytic thyroiditis</td>
<td>2 (1.1)</td>
<td>0/2</td>
</tr>
<tr>
<td>Hyalinizing trabecular adenoma</td>
<td>2 (1.1)</td>
<td>2/0</td>
</tr>
<tr>
<td><strong>Malignant</strong></td>
<td></td>
<td>no. benign/no. suspicious</td>
</tr>
<tr>
<td>Total</td>
<td>85 (100)</td>
<td>4/38</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma†</td>
<td>42 (49.4)</td>
<td>4/38</td>
</tr>
<tr>
<td>Papillary thyroid carcinoma, follicular variant</td>
<td>19 (22.4)</td>
<td>2/17</td>
</tr>
<tr>
<td>Hürthle-cell carcinoma‡</td>
<td>10 (11.8)</td>
<td>1/9</td>
</tr>
<tr>
<td>Follicular carcinoma‡</td>
<td>10 (11.8)</td>
<td>0/10</td>
</tr>
<tr>
<td>Medullary thyroid cancer</td>
<td>2 (2.4)</td>
<td>0/2</td>
</tr>
<tr>
<td>Malignant lymphoma</td>
<td>2 (2.4)</td>
<td>0/2</td>
</tr>
</tbody>
</table>

**GEC Should not be applied to benign cytology!**
Gene Expression Classifier - Disadvantage

- High false-positive rate (50~60%), repeated FNA

- No benefit to the AUS/FLUS category with a low prevalence of malignancy

- Nearly same rate of malignancy in NEJM 2012 study: 24% vs. 25% (AUS/FLUS vs. FN/SFN) - exaggerated value?

- High cost: $4200
Molecular Markers to Evaluate Thyroid Nodules

Immunohistochemistry
Galectin-3
HBME-1
CK19

Mutations and Gene Rearrangements
  \textbf{BRAF (V600E)}
  \textbf{RAS (HRAS, KRAS, NRAS)}
  \textbf{RET/PTC (RET/PTC1, RET/PTC3)}
  \textbf{PAX8/PPARG}

Multiple mutation testing
  Academic Laboratories and Asurgen
  miRInform Thyroid Panel

Gene Expression and Microarray Analysis
  MicroRNA expression
  Afirma Gene Expression Classifier

2012 Kim MI et al Endocrine Prac
### Summary of four studies with a panel of mutation analyses

<table>
<thead>
<tr>
<th>First author, year (Ref.)</th>
<th>Nikiforov et al., 2009 (40)</th>
<th>Moses et al., 2010 (20)</th>
<th>Ohari et al., 2010 (41)</th>
<th>Cantara et al., 2010 (39)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytology categories investigated:</strong> sample number</td>
<td>Indeterminate: 52; cancer positive: 22; cancer negative: 12</td>
<td>Indeterminate: 110; malignant: 57; suspicious: 27; nondiagnostic: 2; benign: 257</td>
<td>Follicular lesion of indeterminate significance/atypia of indeterminate significance: 117</td>
<td>Indeterminate: 41; suspicious: 54; inadequate: 53; benign: 87</td>
</tr>
<tr>
<td><strong>Material</strong></td>
<td>FNAB (4 needle passes)—a small portion—Roche nucleic acid preservative solution for cytology and remaining material from needle washing for molecular analysis</td>
<td>FNAB—cytological analysis and remaining material for molecular analysis</td>
<td>FNAB (3 or 4 needle passes)—direct smear and monolayered slides by ThinPrep processing. Residual FNA material—nucleic acid preservative solution for molecular analysis</td>
<td>FNAB (1 or 2 needle passes) 2/3 of the material for cytology and 1/3 for molecular analysis. Tissue collected at surgery with 1.5 ml microcentrifuge tube containing Allprotect tissue reagent</td>
</tr>
<tr>
<td><strong>Mutation identified (indeterminate samples)</strong></td>
<td>Indeterminate: N-, H-, KRAS: 5; BRAF: 7; RET/PTC1-3: 2; PAX8/PPARγ: 1</td>
<td>Indeterminate: BRAF: 3; RET/PTC1,3: 1; NRAS: 8; KRAS: 0</td>
<td>Follicular lesion of indeterminate significance/atypia of indeterminate significance: BRAF: 3; NRAS: 7; HRAS: 1; PAX8/PPARγ: 0</td>
<td>Indeterminate: N-, H-, KRAS: 3; BRAF: 2; RET/PTC1,3: 2; PAX8/PPARγ: 0</td>
</tr>
<tr>
<td><strong>Histology for indeterminate samples</strong></td>
<td>PTC: 17; FTC: 4; FA: 4; HN: 27</td>
<td>PTC: 19; FA: 29; FTC: 8; Hurthle cell adenoma: 6; hyperplastic nodule: 40; Hurthle cell carcinoma: 2; lymphocytic thyroiditis: 6</td>
<td>PTC: 20; nonneoplastic: 79; adenoma: 18</td>
<td>PTC: 7; FA: 26; HN: 8</td>
</tr>
<tr>
<td><strong>Indeterminate</strong></td>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>Follicular lesion of indeterminate significance</td>
<td>Indeterminate; suspicious</td>
</tr>
<tr>
<td><strong>FP/FN (n)</strong></td>
<td>0/6</td>
<td>4/21a</td>
<td>0/8</td>
<td>1/1; 0/9</td>
</tr>
<tr>
<td><strong>Sensitivity/Specificity (%)</strong></td>
<td>71/100</td>
<td>38/95a</td>
<td>60/100</td>
<td>85.7/97; 80.4/100</td>
</tr>
</tbody>
</table>

---

Suitable for processing therapeutic surgery

---

2011 Ferraz C et al. JCEM
Summary of four studies with a panel of mutation analyses

<table>
<thead>
<tr>
<th></th>
<th>Several mutations analysis&lt;sup&gt;a&lt;/sup&gt;</th>
<th>RET/PTC rearrangements analysis&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>FP</td>
<td>1.25 (0–4)</td>
<td>0</td>
</tr>
<tr>
<td>FN</td>
<td>9 (1–21)</td>
<td>3.5 (1–6)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>63.7% (38–85.7%)</td>
<td>55% (50–60%)</td>
</tr>
<tr>
<td>Specificity</td>
<td>98% (95–100%)</td>
<td>100%</td>
</tr>
</tbody>
</table>

Suitable for processing therapeutic surgery
Added Value of Molecular Methods for Follicular Proliferation/indeterminate FNABs

"Follicular proliferation/indeterminate"

- 80% benign samples
- 20% malignant samples

50% mutation negative

FUTURE: identification of miRNA markers for benign tumors and mutation negative malignant tumors

50% mutation positive (BRAF, RAS, RET/PTC, PAX8/PPARγ)

Molecular diagnosis
Progress in Identifying Mutational Markers in Thyroid cancer

⇒ Next generation whole genome/exome sequencing-based diagnosis

Hsiao SJ et al. 2014 Endoc Relat Cancer
Integrated Genomic Characterization of PTC

496 Papillary thyroid carcinomas

Mutations
Copy number alterations
mRNA expression
miR expression
Protein expression
DNA methylation

Braf\(^{V600E}\) v. Ras signaling

Thyroid differentiation

BRAF\(^{V600E}\) - RAS score

Thyroid differentiation score

Molecular classification of papillary carcinoma

RAS-like papillary carcinoma

BRAF\(^{V600E}\) -like papillary carcinoma

Subtypes of BRAF\(^{V600E}\) -like papillary carcinoma

2014 Cell, Cancer Genome Atlas Research Network
Downstream Signaling of BRAF^{600E}-like and RAS-like PTCs

TCGA project - Extended the set of known PTC driver alterations to include EIF1AX, PPM1D, and CHEK2 and diverse gene fusions.

Unknown oncogenic drivers → 3.5%
Extended Molecular Marker Panel

- PIK3CA & AKT1 mutations
- TP53, CTNNB1 mutations
- TSH-R, GNAS mutations – mostly benign autonomous functioning but TSH-R mutation in thyroid cancer

⇒ Targeted NGS-based panel is being evaluated
Emerging Novel Molecular Markers

- Fusion of BRAF with a kinase anchor protein (AKAP9)
  - rarely found in sporadic thyroid cancer
  - higher frequencies (~11%) in radiation exposure

- NTRK1 rearrangement – 1~5% of PTC

- ETV6-NTRK3 rearrangement
  - 2% in sporadic thyroid cancer
  - higher frequencies (14.5%) in radiation exposure

- STRN-ALK gene rearrangements
  - 9% in PDC, 4% in ATC

- TERT promoter mutation
  - not in benign, only in aggressive cancer (with metastasis)

- Over 60 genes oncogene panel (point mutation & gene fusions)

⇒ Over 95% NPV in thyroid nodule with AUS/FLUS & FN

2014 Hsiao SJ and Nikiforov YE et al. Endor relat Cancer
Suggested Algorithm for management of thyroid nodules on the basis of FNAB and molecular marker tests

- **mRNA gene expression classifier**
- **Oncogene panel including BRAF mutation**

2013 Mingzhao Xing et al. Lancet
Ultrasound Elastography

- Measures the deformation in response to an applied force and displays the stiffness or strain of a tissue.

- Previous studies demonstrated the potential of using US elastography for noninvasively differentiating malignant nodules from benign nodules.

- Sensitivity and specificity as high as 97% and 100%.
- Sensitivity of 88% and specificity of 90%.

Rago et al., J Clin Endocrinol Metab 2007
Hong et al., J Ultrasound Med 2009
Core Needle Biopsy in Non-diagnostic Results

17,045 thyroid FNACs
- ND 1,636 (9.6%)
- 140 Excluded

1,496 Nondiagnostic on 1st FNAC
- 89 (5.9%) Thyroidectomy
  - Due to clinical suspicion for a malignancy (63)
  - Due to other suspicious thyroid lesion (26)
- 514 (34.4%) Repeat testing
- 893 (59.7%) Clinically benign

389 (75.7%) 2nd FNACs
- ND, 33.2%

125 (24.3%) CNBs
- ND, 2.4%

357 final results Refer to Table 1
121 final results Refer to Table 1

Lee SH et al Ann Surg Oncol 2014, Seoul St. Mary's Hospital Data
Core Needle Biopsy in Non-diagnostic Results

- 17,045 thyroid FNACs
  - ND: 1,636 (9.6%)
  - Excluded: 140

1,496 Nondiagnostic on 1st FNAC

- 89 (5.9%) Thyroidectomy
  - Due to clinical suspicion for a malignancy (63)
  - Due to other suspicious thyroid lesion (26)

- 514 (34.4%) Repeat testing
  - 893 (59.7%) Clinically benign

389 (75.7%) 2nd FNACs

- AUS: 4.9%
- FN/SFN: 0.3%
- SM: 1.8%
- M: 3.9%
- ND: 33.2%
- Benign: 56.0%

125 (24.3%) CNBs

- AUS: 4.0%
- FN/SFN: 8.8%
- SM: 0.0%
- M: 5.6%
- ND: 2.4%
- Benign: 79.2%

357 final results
Refer to Table 1

121 final results
Refer to Table 1

Still need molecular testing for the indeterminates!
Future Directions

- NGS-based molecular tests: smaller sample, more efficient & cutting costs
  ⇒ predict the risk of cancer with very high accuracy
  ⇒ eliminate the uncertainty of indeterminate FNA cytology

- Several specific molecular signatures:
  Driver mutations like BRAF and RAS
  TP53 mutations
  TERT promoter mutations
  ⇒ optimal surgical and post-surgical management of patients

- Determination of a personalized cancer genome
  ⇒ offer truly individualized medicine for patients with thyroid nodules and cancer

_Hsiao SJ et al. 2014 Endoc Relat Cancer_
Present & Future Perspectives of Diagnosis in Thyroid Nodule in Korea

Non-invasive Methods

- FNAC
- Core biopsy
- Molecular Marker Test

Real Benign
Real Malignant
TAKE HOME MESSAGE

- New categorization in Bethesda System
  ⇒ interobserver variability in cytology interpretation
  ⇒ Needs for molecular marker testing

- Molecular marker tests
  Rule-in test – oncogene mutation panel – limited but more promising
  Rule-out test – gene expression classifier – several limitations

- Recent WGCTCGA study recognized nearly all mutation of driver genes
  - New oncogene panels will improve diagnostic accuracy

- Competition with other diagnostic utilities - active investigation
  US elastography, optical probes – non-invasive tool
  Core needle biopsy revisited – more definite tool but invasive
  Serum markers