Molecular Diagnostics in Thyroid Cancer

Current Practices & Future Trends

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Disclosure

• Nothing to disclose

Overview

• FNA Cytology
  – Bethesda Classification
  – Indications for Molecular testing
• Molecular Diagnostics
  – Gene Expression Classifier
  – Molecular Alteration Testing

Problem

• FNA Cytology
  • 525,000 FNAs annually
• Indeterminate FNA
  • 20-30% read as indeterminate (158,000)
  • 75% benign, 25% chance of malignancy
  • 30-40% → Diagnostic thyroidectomy
  • 75-80% of indeterminate FNAs taken to surgery for diagnostic thyroidectomy are benign on final path
  • 119,000 “unnecessary” surgeries
  • $6-$10K
  • Possible complications
  • Time lost from work, child care, etc
  • Anxiety, pain, recovery

Problem

• Molecular Testing of FNA Samples
  • Advances in molecular testing may increase diagnostic accuracy of FNA
    • Alexander EK et al. NEJM 2012.
      • Accurately predict whether a cytologically indeterminate nodule is benign in 93% of cases
      • Permits a more conservative approach to management
      • Avoid unnecessary thyroid lobectomy

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FNA Cytology
Bethesda Classification
- Category 1: Non-diagnostic
- Category 2: Benign
- Category 3: Indeterminate (FLUS and AUS)
- Category 4: Follicular neoplasm, SFN, HN, SHN
- Category 5: Suspicious for malignancy
- Category 6: Malignant

Improving the Indeterminate FNA
- Can indeterminate FNA samples be reclassified as benign using genetic testing?
  - Gene Expression Classifier → Rule-out cancer
- Can the risk of cancer in an FNA specimen be confirmed using mutational analysis?
  - Molecular Alteration Testing → Rule-in cancer

Gene Expression Classifier
Microarray Technology: Rule Out Cancer
- Multigene expression (mRNA) test
  - Total RNA is extracted from the FNA and whole-transcript amplification is prepared for hybridization onto a custom gene expression microarray.
  - Developed using 167 gene expression profiles
    - Result
      • Benign
      • Suspicious

GEC FNA Analysis
GEC benign: 93% chance of benign histology (<6% malignant)
GEC suspicious: 40% risk of malignancy

Affirma FNA Analysis
Affirma FNA Analysis: GEC Benign

- Multigene expression (mRNA) test
  - High sensitivity and NPV
  - NPV
    - 95% for FLUS
    - 94% for follicular neoplasm
  - Equivalent to a NPV of a benign FNA

Microarray Technology: Rule Out Cancer


- Microarray chip using 167 genes
- 49 clinical sites
- N = 3789 patients
- 4812 FNAs
- Final inclusion criteria
  \[\rightarrow\] 265 indeterminate FNAs

Gene Expression Classifier Multigene expression (mRNA) Pilot Study

Alexander EK, NEJM 2012

- Negative Predictive Value (NPV)
  - Bethesda 3: FLUS/AUS \( \rightarrow 95\% \)
  - Bethesda 4: FN, SFN \( \rightarrow 94\% \)
  - Bethesda 5: Suspicious \( \rightarrow 85\% \)

- Final Pathology (not cytopath): 85/265 indeterminate nodules were malignant (32%)
- 78/85 malignant nodules identified on cytopath GEC analysis as suspicious
- Sensitivity: 92%
- Specificity 52%, PPV 40%
- a positive test is less likely to represent cancer

- Summary
  - GEC is a multigene expression (mRNA) test used that can reliably reclassify indeterminate FNAs as benign and therefore allow conservative approach (observation)
    - High sensitivity: 92%
    - High NPV: 94-95% for FLUS and FN
  - Equivalent to NPV of a benign FNA
Gene Expression Classifier

Indeterminate FNA, GEC Negative: Risk of Malignancy

Gene Expression Classifier

Study Results: Duick et al, Thyroid 2012
- 368 patients treated by 51 physicians with benign gene expression classifier results
- 10-fold reduction in surgery rates for cytologically indeterminate nodules with benign GEC
  - From 74% (historical) to 7.6%
- Patients and physicians pursuing surveillance over surgery in a large majority of cases of indeterminate FNA, GEC negative ("benign")

The Thyroid Pathology Uncertainty Problem

- Prospective study of 776 nodules
  - Cytology and surgical histology
  - 90-91% pathologist concordance on histology
  - 64-75% pathologist concordance on cytology
  - Experienced cytopathologists less likely to call indeterminate
  - 41 vs 55%

Afirma Cost Effectiveness

FNA indeterminate
- Cost of test - $2000 to $3200
  - Patient out of pocket expense $200-$300
- Cost per patient for standard care
  - $12,172
- Cost per patient with Afirma test
  - $10,719
- Therefore covered by most insurance

Li H et al JCEM 2011

FNA Cytology

Reducing Unnecessary Diagnostic Procedures
- Improving the Indeterminate FNA
  - Can indeterminate FNA samples be re-classified as benign using genetic testing?
    - Gene Expression Classifier → Rule-out cancer
- Can the risk of cancer in an FNA specimen be confirmed using mutational analysis?
  - Molecular Alteration Testing → Rule-in cancer

Molecular Alteration Testing

Thyroid Cancer Genetics: MAPK Cascade Activation
- Activation of RET protein at the cell membrane (ligand binding at the extra-cell)
- Activation of RAS protein
- BRAF effector protein binding
- Downstream intermediates: MEK and ERK
  - Mitogen-activated protein kinase
  - Extracellular signal regulated kinase → Differentiation, proliferation, and survival
Molecular Alteration Testing

DTC Mutations: MAPK Cascade

- Most common and clinically useful molecular markers
- **BRAF & RAS**
  - Point mutations
  - DNA extraction
- **RET/PTC & PAX8/PPARγ**
  - Rearrangement mutations
  - Complex RNA technology

DTC Mutations: BRAF

- **BRAF**
  - **BRAF V600E**: Point mutation
    - Thymidine to adenine transversion (chrom 7, ex 15)
    - Specific for PTC, not found in FA or FC
  - **BRAF** → High Specificity & PPV
    - High Specificity
      - Nikiforov 2009: 99.3% specificity for PTC
    - High PPV
      - Positive test very likely represents presence of true disease
  - **BRAF** → Low Sensitivity
    - Other mutations involved in PTC
    - Many PTCs (55%) are negative for BRAF mutation
    - Diagnostic thyroid lobectomy is still necessary in many indeterminate FNAs that are BRAF-

Molecular Alteration Testing

DTC Mutations: RET/PTC

- **75% of PTCs → 3 mutations**
  - BRAF – 45%
  - RET/PTC – 15%
  - RAS – 15%

DTC Mutation Prevalence

<table>
<thead>
<tr>
<th>Mutation</th>
<th>PTC</th>
<th>FTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>RET/PTC</td>
<td>15%</td>
<td>0%</td>
</tr>
<tr>
<td>BRAF</td>
<td>45%</td>
<td>0%</td>
</tr>
<tr>
<td>RAS</td>
<td>15%</td>
<td>40%</td>
</tr>
<tr>
<td>PAX8/PPARγ</td>
<td>1%</td>
<td>40%</td>
</tr>
</tbody>
</table>
**Molecular Alteration Testing**

**DTC Mutations: RET/PTC**
- All of the RET/PTC tumor subtypes possess a higher rate of lymph node metastases
  - RET/PTC1+ → classic papillary architecture or diffuse sclerosing features
  - RET/PTC3 → associated with radiation exposure and solid variant PTC
  - Lower probability of tumor dedifferentiation

**Molecular Alteration Testing**

**DTC Mutations: RAS**
- Most frequent mutations detected in cytologically indeterminate FNA results
- RAS mutation are not specific for malignancy
  - Found in f/fPTC, FTC and one third of follicular adenomas

**Molecular Alteration Testing**

**DTC Mutations: PAX6 & PPARy**
- Chimeric fusion rearrangement is found in about a third of conventional type FTC
- PAX/PPARy translocation is found in classic FTC, f/fPTC, and 2-10% of FA
- Not specific for FTC

**Molecular Alteration Testing**

**Mutation Panel: Rule-In Cancer**
- Nikiforov 2013:
  - BRAF, RAS, RET/PTC, PAX6/PPARy (Asuragen)
  - High specificity, high PPV
  - Asuragen positive → cancer in 91% of cases
  - Primary benefit of panel was to improve PPV of preoperative testing
  - Allows for Oncologic optimization
  - Total thyroidectomy may be considered as the initial surgical procedure where indicated using clinical factors

**Summary**

**Molecular Management in Indeterminate Cytology**
- **Gene Expression Classifier**
  - Highly sensitive test to rule out malignancy with 95% NPV
  - May reduce the number of diagnostic lobectomies and allow for a conservative approach
- **Molecular Alteration Testing**
  - Highly specific test to rule-in malignancy with 91% specificity
  - May reduce the number of completion thyroidectomies by leading to up-front total thyroidectomy

**Mutation Panel: Study Results**
- Yip 2013
  - Series of indeterminate FNA results (AUS/FLUS/FN)
  - N=471
  - Prospective molecular testing using mutation panel associated with 2.5-fold reduction in 2-stage thyroidectomy for histologic clinically significant thyroid cancer