Thyroid Cancer and Molecular Testing: What the Surgeon should Know in 2012

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Nothing to Disclose

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Molecular Biology and Genetics
- Diagnostics
- Management
- Prognostication
Why is this important to the surgeon in 2012?

• BRAF and RET/PTC positive testing results provide a specificity of 99% for papillary thyroid cancer when the FNA result otherwise is cytologically indeterminate (atypia or follicular neoplasm).
• A therapeutic oncologically optimal procedure rather than a diagnostic thyroid procedure can be performed potentially reducing the rate of completion thyroidectomy.

Why is this important to the surgeon in 2012?

• 80% of diagnostic thyroid lobectomies for follicular neoplasm and atypia (indeterminate nodules) are ultimately benign.
• Molecular Classifier testing has a negative predictive value of 95% for atypia and 94% for follicular neoplasm.
• This testing may have a broad effect on reducing the number of unnecessary thyroid lobectomies.

Why is this important to the surgeon in 2012?

• BRAF positive patients have a higher rate of central neck recurrence and poorer prognosis.
• Tailored surgical management and surveillance strategies can be considered.

Why is this important to the surgeon in 2012?

Diagnosis and Management of Differentiated Thyroid Cancer using Molecular Markers

In Press: The Laryngoscope
2009 ATA Cytology Guidelines

- (1) Benign (negative predictive value is 95%)
- (2) Malignant
- (3) Suspicious for PTC (50-75% risk of CA)
- (4) Inadequate
- *(5) Indeterminate (Hurthle Cell or Follicular Neoplasm or Atypia)*-25% of nodules

Fine needle aspiration (FNA) cytology cannot make a definitive diagnosis in 25% of thyroid nodules—i.e., Follicular Neoplasm and FLUS (Gharib H. 2004)

- Only 20% of Follicular Neoplasm and Follicular Lesion of Undetermined Significance (FLUS) are malignant.
- Approximately 80% of thyroid lobectomies performed solely for diagnostic purposes are benign.

Genetics

- The most common and clinically useful molecular markers are:
  - BRAF and RAS point mutations are identified through DNA extraction
  - RET/PTC and the PAX8/PPARγ rearrangement mutations which require more complex RNA methodology.
3 Mutations occur in 75% of PTC (Bhaije et al 2011)

- BRAF-45%
- RET/PTC-15%
- RAS-15%

BRAF point mutation-Specific for PTC, not found in FC or FA

RET-PTC
Somatic Intra-Chromosomal Alteration in PTC
- Structural rearrangement of the chromosomal 10q11.2 locus (RET gene) is the most common chromosomal alteration in PTC 15% of cases. (Zhu Z et al. 2006)

RET/PTC rearrangement
- Specific for PTC, not found in FC or FA
RAS point mutation

- RAS mutation are not specific for malignancy and found in 10-20% of PTC, 40-50% of FTC and third of follicular adenomas (Rivera M 2010)

PAX8/PPARγ rearrangement

- Chimeric fusion rearrangement is found in about a third of conventional type FTC.
- Not specific for FTC as it is found in a small percentage of follicular adenomas and in follicular variants of PTC.

Genetic Alterations: Prevalence

<table>
<thead>
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<th>PTC (%)</th>
<th>FTC (%)</th>
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<tbody>
<tr>
<td>RET/PTC</td>
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<td>RAS</td>
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<tr>
<td>Pax8/PPARγ</td>
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Diagnostics

Molecular Biology
Sensitivity

Sensitivity = \[\frac{\text{True Positive}}{\text{True Positive} + \text{False Negative}}\]

The proportion of patients who truly have a disease that tests positive for that disease. If the sensitivity is high, a negative test is very likely to represent a true lack of disease.

Specificity

Specificity = \[\frac{\text{True Negative}}{\text{True Negative} + \text{False Positive}}\]

The proportion of patients who truly are disease free that tests negative for that disease. If the specificity is high, a positive test is very likely to represent the presence of true disease.

Positive and Negative Predictive Value

PPV = \[\frac{(\text{Sensitivity})(\text{Prevalence})}{(\text{Sensitivity})(\text{Prevalence})+(1-\text{Prevalence})(1-\text{Specificity})}\]

PPV will increase if prevalence increases, sensitivity increases and overall probability of a positive result decreases.

NPV = \[\frac{(\text{Specificity})(1-\text{Prevalence})}{(\text{Specificity})(1-\text{Prevalence})+(\text{Prevalence})(1-\text{Sensitivity})}\]

NPV increases if the prevalence decreases and the specificity increases.

BRAF Point Mutation Specificity

- Specific for PTC, not found in FC or FA
- A meta-analysis, 22 studies, FNA samples tested for BRAF mutation found that of 1109/1117 (99.3%) were classified as PTC on final histopathology (Nikiforov MN et al 2009)
- BRAF has > 99% specificity (specificity is high, a positive test is very likely to represent the presence of true disease).
- Cost $200.00
**BRAF Point Mutation Sensitivity**

- The sensitivity for diagnosing PTC increased from 63.3% to 80.0% with cytology alone compared to cytology and BRAF testing (Adeniran AJ 2011)

**BRAF Point Mutation Sensitivity**

- BRAF mutation has limitations for FLUS diagnoses because many thyroid cancers are negative for the BRAF mutation (inadequate sensitivity).
- Diagnostic Thyroid lobectomy is still necessary in many of these patients

**A panel of mutations**

- BRAF and RAS point mutations and RET/PTC and PAX8/PPARγ rearrangements have been proposed to increase sensitivity over each alone (Nikiforov YE et al. 2009, Ohori et al. 2010)
- Testing for a panel of mutations resulted in a gain of sensitivity from 44 to 80% (Nikiforov YE et al. 2009)

**A Panel of Mutations**

- BRAF and RET/PTC mutations correlated with malignant outcome 100% of the time, whereas RAS mutations had a positive predictive value of 74-87% (Nikiforov YE et al. 2009)
- Diagnostic Thyroid lobectomy is still necessary in many of these patients.
A Panel of Mutations

- The risk of cancer in nodules that have molecular testing negative indeterminate cytology is between 7 and 11% (Nikiforov et al. 2009, Ohori et al. 2010, Cantara et al. 2010)
- A panel of mutations costs $650-2200.00.
- The 2009 American Thyroid Association guidelines recommend consideration for the use of a panel of molecular markers for indeterminate FNA cytology (Cooper DS. et al. 2009)

Management

BRAF+

- Risks and benefits of upfront therapeutic total thyroidectomy for BRAF+ molecular testing in the setting of FLUS should be discussed with the patient
- Potentially facilitating the optimal oncologic procedure being performed at the initial operation (therapeutic rather than diagnostic thyroidectomy).

BRAF mutation

- The cost of BRAF mutational analysis is covered by many third party payers and ranges from $150-300.
- It becomes cost-effective if one considers the decrease in rate of completion thyroidectomy (Musholt T. et al. 2010)
  - Cost thyroid lobectomy $3500
  - Total cost with completion thyroidectomy $7000
Prognostication

**BRAF+ Prognosis**

- *BRAF* mutation is associated with extrathyroidal extension, central and lateral lymph-node metastasis, more advanced stage of presentation, tumor recurrence, associated tall cell variant, tumor-related mortality and re-operation (Xing M et al. 2009, Lin et al. 2010)

- *BRAF* is found in classic papillary and tall cell thyroid cancer
- In a third of poorly differentiated and anaplastic thyroid carcinomas
- Dedifferentiated thyroid cancer can have areas of differentiation and both areas can contain mutated *BRAF*. This suggests progression from differentiated to dedifferentiated neoplasm (Nikiforova MN 2004)

**BRAF+ Prognosis**

- *BRAF+ PTC* is known to have a higher rate of central node compartment metastasis.
- **11/106** *BRAF+* mutated patients required re-operation compared to **3/106** patients without *BRAF* mutation (Yip et al. 2009)
**BRAF+ Central Neck**
(Laryngoscope 2012)

- In 197 cases requiring re-operative central compartment dissection
- 75% were BRAF+
- This may pending further validation have implications for tailored treatment (N0-central neck dissection).

**BRAF+ Prognosis**

- Particularly when they recur or undergo dedifferentiation BRAF+ PTC exhibit a decreased response to radio-iodine treatment

**BRAF+ Prognosis**

- 70-80% of BRAF+ tumors do not behave aggressively
- Prognostication remains to be fully defined.
- BRAF mutated tumors may benefit from more intensive follow up.

**RET/PTC mutation**

- Very low probability of progression to poorly differentiated or anaplastic carcinoma (Giordano TJ. 2005)
- Classic papillary architecture
RAS point mutation

• The encapsulated follicular variant of PTC is associated with positive RAS testing.
• Infiltrative follicular variant of PTC is associated with positive BRAF (Rivera M et al 2010)

Micro array technology

• Improve understanding of the pathophysiology and molecular etiology of thyroid neoplasia
• Detect genetic markers that could improve the differential diagnosis of thyroid tumors.

RAS point mutation

• The potential implications are that RAS positive encapsulated follicular variants of PTC may be treated more conservatively like minimally invasive follicular thyroid carcinomas pending further validation.
Micro array technology

- A collection of microscopic DNA spots attached to a solid surface
- DNA microarrays measure the expression levels of large numbers of genes simultaneously to genotype multiple regions of a genome.

Fodor et al 2001 (Affymetrix, Santa Clara CA)

- Combined photolithography and chemical DNA synthesis
- Miniturization of the assay
- 1 million distinct oligonucleotide sequences

Micro array technology

- The core principle behind microarrays is hybridization between two DNA strands
- Fluorescently labeled target sequences that bind to a probe sequence generate a signal

Limitations of microarrays

- Quantitatively measuring RNA markers is more susceptible to potential limitations of FNA such as limited and variable numbers of follicular cells obtained in each biopsy (Ezlinger et al 2007 Endocrine Reviews)
Multigene expression (mRNA) test

- In contrast to the high specificity and high positive predictive value of tests for somatic point mutations such as **BRAF** and **RET/PTC**
- Gene expression microarrays demonstrate a greater sensitivity and negative predictive value.

Multigene expression (mRNA) Pilot Study

- In the 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.

Multigene expression (mRNA) test Pilot Study

- In 48 prospective FNA samples, 24 were indeterminate thyroid lesions. The test set had a negative predictive value, sensitivity and specificity of 96, 92 and 84%. (Chudova D et al. 2010)
- The pilot study was based on a limited number of samples and the validation set included only 2 Hürthle cell cancers and no FTC.

NEJM 6/2012 (Alexander et al)

- Micro array chip using 167 genes
- 49 clinical sites
- 3789 patients
- 4812 FNA’s
- 413 indeterminates with corresponding histopathological specimens
- Final inclusion criteria 265 indeterminates
- The Holm procedure was used to correct for testing association with multiple clinical variables.
NEJM 6/2012
Sensitivity and Specificity

- 142 genes in the main classifier
- 25 genes filter out rare neoplasms
- 265 indeterminates 85 (32%) were malignant
- Gene classifier identified 78/85 samples as suspicious; Sensitivity- 92%
- 93/180 nonmalignant correctly identified as benign; Specificity- 52% (Specificity is low, a positive test is less likely to represent cancer)

NEJM 6/2012-Negative Predictive Value

- FLUS (atypia)- 95%
- Follicular neoplasm or lesion suspected of being follicular lesion- 94%
- Suspicious cytological findings- 85%
- 7 aspirates with false negatives, 6 had paucity of thyroid follicular cells.

NEJM 6/2012

- Of the Cytologically benign (6%):
  that were malignant on pathology.
- Gene molecular classifier correctly identified all false negatives as suspicious.

Multigene expression (mRNA) test

- May have a profound impact on reducing the need for unnecessary diagnostic lobectomy.
Thyroid 2012 (Duick et al)

- 368 patients treated by 51 physicians with benign gene expression classifier results
- 10-fold reduction in surgery rates for cytologically indeterminate nodules from a previous historical rate of 74% to 7.6%
- Patients and physicians in this study substituted clinical and sonographic follow-up for diagnostic surgery in a large majority of cases with cytopathologically indeterminate FNA and benign gene expression classifier results

Conclusions

- Approximately 80% of thyroid lobectomies performed solely for diagnostic purposes are benign.
- Molecular alteration testing may reduce the number of unnecessary thyroid procedures.
- May reduce the number of completion thyroidectomies
- May lead to more individualized operative and post-operative management.

Molecular Biology Conclusions

- Molecular testing for \textit{BRAF}, \textit{RAS}, \textit{RET/PTC} and \textit{PAX8/PPARγ} for indeterminate thyroid nodules improves specificity analysis.
- Molecular classifiers add to sensitivity analysis and negative predictive value.
Totalitarian Regime

Profound Cultural Differences

Winds of Change are palpable with a markedly Pro-American Younger Generation
Woman make up more than half of University and Medical Students

Persepolis, Achaemenids; 500 B.C.