Clinical performance and utility of Afirma GEC in a community hospital practice

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Epidemiology of Thyroid Nodules/Cancer

- >525,000 endocrinology visits each year in the United States due to a new palpable thyroid nodule or nodule detected via imaging
- Increased usage of ultrasound and CT scan has accelerated the findings of thyroid incidentalomas
- Greatest worry for patients and physicians is possibility of malignancy
- Four to 6.5 percent of all thyroid nodules are cancerous
Diagnostic dilemmas in thyroid nodules

RNA results by classification in US endocrinology centers

Malignant
Indeterminate
Benign
Nondiagnostic

Management of Indeterminate thyroid nodules

- Indeterminate nodules encompass following Bethesda classifications:
  - Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance (AUS/FLUS)
  - Follicular
  - Hürthle Cell Neoplasm (FN/HCN)

- Previous ATA guidelines recommended diagnostic lobectomies for final diagnosis
  - Too much surgery for those 70-90% of patients who have benign final histopathology
  - Too little surgery for those 10-30% of patients with malignant histopathology

- Molecular markers increasingly employed as an adjunct to fine needle aspiration (FNA) cytology in such nodules
Afirma GEC

- Aims to improve diagnostic yield in thyroid FNA
- Measures the expression of 167 gene transcripts
- Offered at a single CLIA-certified laboratory and classifies nodules as either benign or suspicious
- Sample report on right from Afirma:

Validation of Afirma GEC

- Alexander et al. 2012
  - 328 samples with successful classifier processing
    - 16 samples did not have histological results available
    - 47 samples were excluded for protocol violations
    - 265 samples included in analysis
  - Malignancy rate of 32% in analyzed nodules
  - Performance of Afirma GEC
    - Sensitivity 92% (95% CI 84%-97%)
    - Specificity 52% (95% CI 44%-59%)
    - Positive predictive value 47% (95% CI 40%-55%)
    - Negative predictive value 93% (95% CI 86%-97%)
Clinical Relevance and Performance of Afirma GEC

- NCCN recommends ultrasound observation for follow up in cytologically indeterminate-Afirma benign nodules based upon a single study with a sample of 81 FN/HCN and 120 AUS/FLUS nodules
  - Known pre-test probability of malignancy
  - Predictive value (PPV) of 47% (95% C.I. 40-55%)
  - Negative predictive value (NPV) of 93% (95% C.I. of 86-97%)\(^10\)
- Potential cost and harm savings based on 1 in 2 Afirma GECs classified as benign\(^12\)
- Three subsequent studies in clinical settings with disparate performance results of Afirma GEC
  - Varied positive predictive
  - Lower implied negative predictive values
  - McIver et al. reported only 28% of samples receiving benign GEC results
  - Harrell and Bimston (34% of nodules that were AUS/FLUS and SFN/SHCN were Afirma benign)

Purpose and Aim of Study

- Previous clinical studies of the Afirma GEC have had:
  - Small sample sizes
  - Disparate clinical results
  - Primarily involved patients presenting to academic medical centers
- Aim:
  - To assess the performance of the Veracyte’s Afirma Gene Expression Classifier (GEC) in a large community hospital setting
  - Correlate the histopathology of surgical specimens from both benign and suspicious test results with histologic diagnosis following surgery
  - Contribute to larger sample sizes to lend power to the studies and help to further inform current clinical practice
Methods

- Based on the study design utilized in McIver et al.13
- Study type: Retrospective medical record review
- Cohort: Patients who underwent fine needle aspiration of thyroid nodules at Sanford Medical Center in Fargo between April 1st, 2012 and September 30th, 2014.
- Inclusionary criteria:
  - GEC offered to patients with Indeterminate nodules
  - Those patients with GEC results available were included in the analysis

Methods

- Data obtained:
  - Age
  - Gender
  - Ultrasound size by largest nodule measurement, broken into categories 1 (0-2 cm) 2 (2-4 cm) and 4+ cm based on current staging criteria.
  - TSH levels
  - FNA results by nodule
  - FNA actionable results (that dictated treatment)
  - GEC results (if applicable)
  - Surgical pathology results (if applicable)
Methods

- GEC results compared to final surgical (histo)pathology
- Rationale for operating/not operating recorded
- SPSS 21.0 for Windows was used to analyze demographic and clinical characteristics of patients.
- Chi-square tests or Fisher’s exact tests determined which categories were significantly different from one another
- t-test and/or ANOVA used to compare continuous variables
- All p-values are two-sided, p-value < 0.05 considered to be significant
- Missing data was excluded from analysis.

Results: Figure 1
No statistically significant correlation could be made between TSH levels and rate of malignancy.
Table 2
Results of GEC According to final histopathology for indeterminate nodules/GEC suspicious nodules that underwent surgery

### Suspicious for Follicular Neoplasm (SFN) n=5

<table>
<thead>
<tr>
<th>Path general</th>
<th>Benign</th>
<th>Malignant</th>
<th>Total</th>
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<tbody>
<tr>
<td>GEC benign</td>
<td>1</td>
<td>0</td>
<td>4</td>
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<tr>
<td>% within GEC</td>
<td>100.0%</td>
<td>0.0%</td>
<td>100.0%</td>
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<tr>
<td>GEC suspicious</td>
<td>4</td>
<td>0</td>
<td>4</td>
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<tr>
<td>% within GEC</td>
<td>100.0%</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>% within GEC</td>
<td>100.0%</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

### Suspicious For Hurthle Cell Neoplasm (SHCN) n=2

<table>
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<th>Path general</th>
<th>Benign</th>
<th>Malignant</th>
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<tbody>
<tr>
<td>GEC suspicious</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>% within GEC</td>
<td>50.0%</td>
<td>50.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>% within GEC</td>
<td>50.0%</td>
<td>50.0%</td>
<td>100.0%</td>
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### Suspicious for Neoplasm (SHCN & SFN) n=7

<table>
<thead>
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<th>Path general</th>
<th>Benign</th>
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<tr>
<td>GEC benign</td>
<td>3</td>
<td>0</td>
<td>3</td>
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<tr>
<td>% within GEC</td>
<td>100.0%</td>
<td>0.0%</td>
<td>100.0%</td>
</tr>
<tr>
<td>GEC suspicious</td>
<td>5</td>
<td>1</td>
<td>6</td>
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<tr>
<td>% within GEC</td>
<td>83.3%</td>
<td>16.7%</td>
<td>100.0%</td>
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<tr>
<td>Total</td>
<td>8</td>
<td>1</td>
<td>9</td>
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<tr>
<td>% within GEC</td>
<td>88.9%</td>
<td>11.1%</td>
<td>100.0%</td>
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### Atyplia/FLUS n=22

<table>
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<th>Benign</th>
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<tbody>
<tr>
<td>GEC benign</td>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>% within GEC</td>
<td>100.0%</td>
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<td>100.0%</td>
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<tr>
<td>GEC suspicious</td>
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<td>20</td>
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<tr>
<td>% within GEC</td>
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<td>50.0%</td>
<td>100.0%</td>
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<td>Total</td>
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<tr>
<td>% within GEC</td>
<td>54.5%</td>
<td>45.5%</td>
<td>100.0%</td>
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Discussion

- **Small sample sizes across studies**
  - Our study analyzed final 66 GEC results (n=66)
  - Sample size consistent with single-center studies

- **Rates of surgery avoidance inconsistent across studies**
  - Our data showed the rate of benign GEC profiles to be 56.1%
  - Largest study sample size from multicenter trial by Alexander et al.14 (showed 52% of nodules that were AUS/FLUS and SFN/SHCN were Afirma benign)
  - Approximately one in every two patients avoided surgery for every two tests run in our study

- **Varied PPV across studies**
  - Our study found 11 patients out of 26 (PPV=42.3%) with suspicious GEC results had confirmed malignancies within the nodules sampled.
  - Alexander et al. (original validation) had PPV=38%11
  - PPV varied across studies from 15.6%13 to 57%15

- **Variability in the cytology of nodules available for Afirma GEC**
  - Our study:
    - 20 suspicious for FN (SFN) (30.3%)
    - 11 suspicious for HCN (SHCN) (16.7%)
    - 35 Atypia/FLUS (53.0%)
  - Percentage of AUS/FLUS versus FN/HCN differed significantly when compared to other analogous studies12,13

- **Possible explanation for variability and effect on results**
  - Follicular lesions in particular have a high rate of discordance between inter- and Intra-observer comparisons across studies17,18
  - Smaller nodules (~1 cm) have low risk of malignancy
  - Our cytopathologists rate such nodules as FLUS
  - Ten of twenty patients (50%) with initial cytology of atypia/FLUS and suspicious GEC had malignant findings at final diagnosis—a higher rate of malignancy than previous studies, which could indicate that some of these nodules could be classified as SFN
Limitations

- **Retrospective nature of study**
  - Could lead to both referral and sample bias
  - Factors are controlled somewhat by the standardized NCCN guidelines regarding indeterminate thyroid nodules followed by study institution

- **Small sample size**
  - Expected due to novelty of GEC
  - In line with previous single-center clinical evaluations

- **Analysis does not account for all outcomes**
  - 8.1% of patients with benign GEC and 90% of patients with suspicious results have had final surgical diagnoses
  - Remaining patients have either been followed with serial ultrasound or managed conservatively

- **NPV cannot be directly measure in our study and all clinical studies since it is inadvisable to operate on all patients with Afirma-benign results**

Future Studies

- Prospective studies in larger sample sizes
  - Some utilizing the Afirma GEC in all nodules

- Comparisons between Afirma GEC and newer molecular markers

- Cost-benefit analyses to determine the benign-GEC rate that translates into patient and financial benefits
Conclusions

- FNA classification is variable across institutions, but doesn’t appear to affect Afirma’s efficacy
- Implementation of the Afirma GEC reduces unnecessary thyroid surgery when applied to a population at a large community medical center
  - One in every two patients avoided surgery in this study due to GEC analysis
- PPV and NPV reported are in line with previous studies that support the use of Afirma GEC
  - Variability in reported PPV and NPV likely reflects patient population characteristics limiting studies’ results (including our own) generalizability

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References


