Endocrine Pathology (2019) 30:134–137
https://doi.org/10.1007/s12022-019-9571-x

Negative Results on Thyroid Molecular Testing Decrease Rates of Surgery for Indeterminate Thyroid Nodules

Rachel Jug[1] · Shobha Parajuli[2] · Sara Ahmadi[3] · Xiaoyin "Sara" Jiang[1]

Published online: 1 March 2019

Abstract
Molecular tests and mutational panels such as Afirma Gene Expression Classifier (GEC) and ThyroSeq, respectively, have been used to help risk stratify cytologically indeterminate thyroid nodules with the aim to reduce unnecessary surgeries. We studied the effect of molecular testing on the rate of surgical resection in these nodules. Thyroid nodules with indeterminate (Bethesda III/IV) cytology that underwent molecular testing (GEC or ThyroSeq) at our institution between June 2012 and August 2016 were retrospectively reviewed. We collected demographics, cytology diagnoses, molecular test results, and whether surgical resection was performed. Two hundred eighty-three nodules met inclusion criteria: 202 nodules tested with GEC and 81 tested with ThyroSeq. In the cohort of GEC-tested nodules, 99/202 (49%) yielded "suspicious" and 103/202 (51%) yielded "benign" results, with an overall resection rate of 70/99 (71%) in "suspicious" versus 13/103 (13%) in "benign" nodules. In the cohort of ThyroSeq-tested nodules, 13/81 (16%) of nodules yielded a "high-risk mutation" and 68/81 (84%) of nodules yielded "no high-risk mutation," with overall resection rates of 11/13 (85%) and 30/68 (44%), respectively. Rates of resection were higher for Bethesda IV than for III nodules, regardless of molecular results. For both GEC and ThyroSeq, molecular test results seemed to correlate with the rate of resection at our institution. Rates of resection for cytologically indeterminate nodules that were "benign" or "no high-risk mutation" appeared to differ from those that were "suspicious" or "high-risk mutation" on molecular panel testing by GEC and ThyroSeq, respectively. Our findings support that molecular test results are impacting management.

Keywords Afirma Gene Expression Classifier · ThyroSeq · Fine needle aspiration · Thyroid cytopathology · Molecular testing

Background

Thyroid nodule fine needle aspiration (FNA) biopsy results are cytologically indeterminate in 15–30% of cases [1]. Molecular tests such as the Afirma Gene Expression Classifier (GEC) and UPMC ThyroSeq mutation panel (ThyroSeq) have been developed with the aim to further risk stratify these nodules. Currently, the American Thyroid Association (ATA) recommends diagnostic lobectomy or molecular testing as the treatment of choice for indeterminate nodules; however, the guidelines suggest considering total thyroidectomy when cancer-specific mutations are detected through molecular studies [2].

The GEC measures the expression of 167 genes to identify a benign gene profile with high accuracy. This test classifies nodules as "benign" or "suspicious" with a negative predictive value (NPV) of 95% and a positive predictive value (PPV) of 38% for malignancy in AUS nodules. The accuracy of a benign GEC result is comparable with a benign cytopathology diagnosis, for which surveillance is generally recommended as opposed to a diagnostic thyroidectomy which is most often performed following a suspicious Afirma GEC result [2]. The ThyroSeq is a next-generation sequencing (NGS) mutational panel that examines 112 genes for a variety of genetic alterations implicated in the oncogenesis in thyroid cancer. This test classifies nodules as "no high-risk mutation" or "high-risk mutation," both of which have high positive and negative predictive values that potentially could both rule in and rule out malignancy. The University of Pittsburgh Medical Center (UPMC) published performance data on an FNA validation set using their latest platform, the

✉ Xiaoyin "Sara" Jiang
sara.jiang@duke.edu

[1] Department of Pathology, Duke University Medical Center, Box 3712 DUMC, Durham, NC 27710, USA

[2] Department of Pathology, University of Cincinnati Health, Cincinnati, OH, USA

[3] Division of Endocrinology, Department of Medicine, Duke University Medical Center, Durham, NC, USA

ThyroSeq v3, revealing a sensitivity of 98%, specificity of 81.8%, and accuracy of 90.9% with regard to classifying lesions as cancerous.

Real-world evidence for the accuracy and clinical utility of these molecular tests is beginning to emerge. In terms of the GEC, several institutions have reported a range of PPV between 18 and 39% [3–6]. Similarly, multiple studies outside of UPMC published performance data on the ThyroSeq with PPV between 22 and 57% [6–8]. Based on initial and these subsequent studies, these tests have become incorporated into many practices and are included in the 2015 American Thyroid Association (ATA) guidelines for the management of patients with indeterminate thyroid nodules on FNA cytology [2]. Some centers have demonstrated the potential influence of molecular test results on clinical practice. In cytologically indeterminate nodules, benign GEC results correlated with surgery rates of 7.6–13% at two different institutions. In this study, we will evaluate the effect of molecular testing on the management of these thyroid nodules.

Methods

Following Institutional Review Board approval, a retrospective search for thyroid FNAs that were sent for molecular testing using either the Afirma GEC or Thyroseq mutational testing was conducted using our institutional laboratory information system (LIS) from June 2012 to August 2016. For each case, the cytopathology accession number, patient demographics, FNA diagnosis, surgical procedure (if applicable), final histopathology diagnosis, and molecular test results were recorded. Both pediatric and adult patients were included in this study. Patients without molecular testing or with molecular test results consistent with parathyroid tissue were excluded. Clinical notes generated from visits with endocrinology, endocrine surgery, or oncology were reviewed for treatment decision outcome following molecular test result issuance. Patients who were lost to follow-up following molecular testing were also excluded.

Thyroid FNA at our institution is predominantly performed under ultrasound guidance by radiologists, endocrinologists, surgeons, and cytopathologists, followed by cytopathologic evaluation by six board-certified cytopathologists using The Bethesda System for Reporting Thyroid Cytopathology (BSRTC) [1]. Six surgeons at our institution performed thyroidectomies or diagnostic lobectomies on patients included in this series. Molecular testing of indeterminate thyroid nodules at our institution is ordered by the submitting clinician if the repeat FNA is indeterminate in an effort to reduce the cost and potential false-positive rate associated with molecular testing. The choice of which molecular test to be performed was made at the discretion of the ordering clinician based on the clinical scenario.

Over the course of the study period, the mutation panel performed by the University of Pittsburgh Medical Center (UPMC) shifted from a seven-gene (BRAF, NRAS, HRAS, KRAS, RET/PTC1, RET/PTC3, and PAX8/PPARg) LightCycler polymerase chain reaction assay (Roche Applied Science, Penzberg, Germany) to the targeted NGS ThyroSeq assays V1 and V2. Therefore, our study included all these models of the UPMC mutational panel.

Our study population was divided into two cohorts based on the molecular test performed (GEC versus ThyroSeq). These groups were further divided based on results of molecular testing and subsequent surgical management. Data collection and analyses were performed using Microsoft Excel 2010 (Microsoft Corporation, Redmond, WA). In addition, we performed a review of the literature by searching related articles in PubMed from November 2012 to January 2018 to compare the impact of molecular testing on surgical decision-making at our institution with other reported experiences.

Results

Two hundred eighty-three nodules from 283 patients met inclusion criteria; 202 nodules tested with GEC and 81 tested with ThyroSeq. In the cohort of GEC-tested nodules (Table 1, Fig. 1), 99/202 (49%) yielded “suspicious” and 103/202 (51%) yielded “benign” results, with an overall resection rate of 80/99 (81%)in “suspicious” versus 13/103 (13%) in “benign” nodules. In the cohort of ThyroSeq-tested nodules (Table 2, Fig. 1), 13/81 (16%) nodules yielded a “high-risk mutation” and 68/81 (84%) nodules yielded “no high-risk mutation,” with overall resection rates of 11/13 (85%) and 30/68 (44%), respectively.

Follow-up data was available for ten of the patients with indeterminate nodules by FNA and suspicious GEC results who did not undergo thyroidectomies. Seven patients refused surgery following discussions with the clinical team and three were poor surgical candidates. Clinical notes were reviewed to explore possible reasons for opting against surgery. One patient initially refused surgery expressed concerns related to the impact surgery could have on her voice because she was a professional singer. For two patients, corroborative clinical data led patients and their physicians to choose surveillance over surgery, including nodule size < 1 cm for one and imaging favoring a benign nodule for the other.

Table 1 Comparison of Afirma GEC results with histological diagnoses

Afirma GEC result ($n = 202$)	Not resected	Resected		Total
		Benign	Malignant	
Benign	90	13	–	103
Suspicious	19	57	23	99

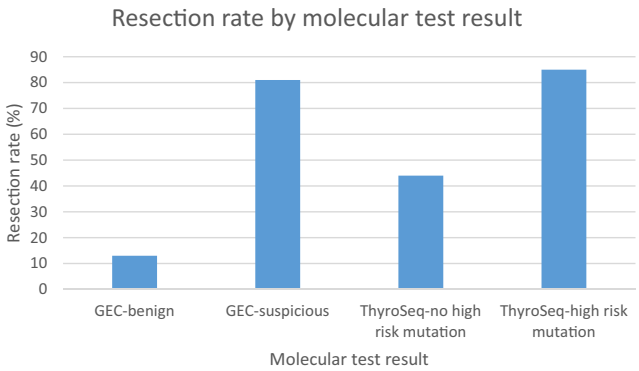

Fig. 1 Resection rates by GEC and ThyroSeq molecular test results

Two patients with indeterminate nodules by FNA and high-risk mutations detected by UPMC mutation panel testing did not undergo thyroidectomies. One patient (with a low level HRAS mutation) opted for surveillance after discussion with clinician who cited an 8% risk of malignancy at our institution for patients with FLUS lesions by FNA and the other patient (with NRAS and TERT mutation) opted against surgery due to their age and comorbid advanced stage lung cancer.

Discussion

Overall, our results suggest that the mutational panel results impact clinical decision-making on indeterminate thyroid nodules at our institution. The majority of patients with a suspicious GEC result proceeded to surgical resection (81% underwent surgery) whereas the bulk of patients with a benign result were not treated surgically (13% underwent surgery).

Surgical decision-making influenced by GEC results faired similarly at other institutions. Hang et al. found that 177/384 (46%) nodules diagnosed as AUS or SFN underwent surgical resection; of these nodules, 202/384 (53%) yielded a positive GEC result [9]. Samulski et al. found that 107/136 (79%) of "suspicious" nodules and 23/158 (15%) of "benign" or "quantity insufficient" nodules underwent surgical resection [10]. Wong et al. found that 63/249 (25%) of nodules sent for GEC testing were classified as "suspicious" and underwent surgical resection [11].

At our institution, a higher proportion of patients with high-risk mutations detected by ThyroSeq underwent surgery (85%) compared with those without high-risk mutations (44%). The preponderance of patients with positive mutation panels proceeding to surgery is similar to trends observed and reported by other institutions [9–12]. Surgical decision-making was influenced by ThyroSeq in a series by Valderrabano et al. who found that 33/45 (71%) of "high-risk mutation" nodules and 64/137 (47%) of "no high-risk mutations" nodules underwent surgical resection [12].

In contrast, the finding of non-"high-risk" mutations using the ThyroSeq panel did not significantly impact the decision to undergo surgical resection of the lesion/thyroid as a little less than half underwent surgical treatment (41%) whereas the remainder (59%) opted for surveillance.

Table 2 Comparison of ThyroSeq results with histological diagnoses

ThyroSeq result ($n = 81$)	Not resected	Resected		Total
		Benign	Malignant	
No high-risk mutations	38	29	1	68
High-risk mutations	2	7	4	13

Conclusions

While no test is perfect, thyroid molecular tests have become important tools to help manage the cytologically indeterminate thyroid nodule. As the scientific community works to better characterize the real-world performance of these tests, there remains some variability in how differing practices utilize these tests to guide patient care.

At our institution, indeterminate thyroid nodules with benign/no high-risk mutations detected by either molecular test were less likely to be treated surgically than those classified as suspicious/with high-risk mutations. Despite the low positive predictive value of each test at our institution, we found molecular panel testing results influenced the rate of resection of indeterminate thyroid nodules. The rates of surgical resection as opposed to surveillance are higher when molecular tests, both GEC and ThyroSeq, yield "positive" ("suspicious" and "high-risk mutation") results. As our experience with these tests grow—and particularly information on long-term clinical follow-up for these patients, we will continue to hone our ability to care for the patient with the indeterminate thyroid nodule.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Cibas, E.S. & Ali, S.Z. The Bethesda System for Reporting Thyroid Cytopathology. Thyroid 19, 1159–1165 (2009).
2. Haugen, B.R., et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 26, 1-133 (2016).
3. Harrison, G., Sosa, J.A. & Jiang, X. Evaluation of the Afirma Gene Expression Classifier in Repeat Indeterminate Thyroid Nodules. Arch Pathol Lab Med 141, 985–989 (2017).

4. Roychoudhury, S., et al. How "suspicious" is that nodule? Review of "suspicious" Afirma gene expression classifier in high risk thyroid nodules. Diagn Cytopathol 45, 308–311 (2017).
5. Chaudhary, S., Hou, Y., Shen, R., Hooda, S. & Li, Z. Impact of the Afirma Gene Expression Classifier Result on the Surgical Management of Thyroid Nodules with Category III/IV Cytology and Its Correlation with Surgical Outcome. Acta Cytol 60, 205–210 (2016).
6. Livhits, M.J., et al. Gene Expression Classifier vs Targeted Next-Generation Sequencing in the Management of Indeterminate Thyroid Nodules. J Clin Endocrinol Metab 103, 2261–2268 (2018).
7. Valderrabano, P., et al. Evaluation of ThyroSeq v2 performance in thyroid nodules with indeterminate cytology. Endocr Relat Cancer 24, 127–136 (2017).
8. Taye, A., et al. Clinical performance of a next-generation sequencing assay (ThyroSeq v2) in the evaluation of indeterminate thyroid nodules. Surgery 163, 97–103 (2018).
9. Hang, J.F., Westra, W.H., Cooper, D.S. & Ali, S.Z. The impact of noninvasive follicular thyroid neoplasm with papillary-like nuclear features on the performance of the Afirma gene expression classifier. Cancer Cytopathol 125, 683–691 (2017).
10. Samulski, T.D., LiVolsi, V.A., Wong, L.Q. & Baloch, Z. Usage trends and performance characteristics of a "gene expression classifier" in the management of thyroid nodules: An institutional experience. Diagn Cytopathol 44, 867–873 (2016).
11. Wong, K.S., et al. Noninvasive Follicular Variant of Papillary Thyroid Carcinoma and the Afirma Gene-Expression Classifier. Thyroid 26, 911–915 (2016).
12. Valderrabano, P., et al. Evaluation of ThyroSeq v2 performance in thyroid nodules with indeterminate cytology. Endocrine-Related Cancer (2017).