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Rethinking Malignancy Risk in Indeterminate Thyroid Nodules with Positive Molecular Studies: Southern California Permanente Experience

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Abstract

Objectives. To recognize that thyroid nodules with atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS; Bethesda III) have different risks of malignancy based on genetic mutation and to consider molecular testing of nodules with AUS/FLUS to help avoid unnecessary morbidity or cost.

Study Design. Retrospective cohort study.

Setting. Multiple locations within Southern California Permanente Medical Group.

Subjects and Methods. Patients included those with indeterminate thyroid nodules and AUS/FLUS on 2 separate fine-needle aspirations with positive ThyGenX testing from 2014 to 2017 who underwent thyroid surgery. Patients were classified as having benign or malignant disease. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features was considered benign.

Results. A total of 231 patients had repeat AUS/FLUS with positive molecular testing and surgery. The most frequent type of malignancy was papillary carcinoma, followed by follicular carcinoma. The overall prevalence of malignancy in nodules with mutations was 74.0%, although there was considerable variation: *BRAF* = 100%, *RET* = 100%, *PAX8-PPARγ* = 84.6%, *HRAS* = 70.7%, *HRAS* = 63.4%, and *KRAS* = 33%—a statistically significant finding ($P < .001$).

Conclusions. Not all molecular mutations in thyroid nodules with AUS/FLUS have a high risk of malignancy. Of note, patients with *BRAF* and *RET* mutations in our population had a 100% risk of malignancy. Patients with *PAX, HRAS*, or *NRAS* mutations had a high risk of malignancy, while patients with *KRAS* mutations had a lower risk of malignancy. Further studies are needed to determine if the presence of certain molecular mutations can help personalize care and aid in the decision for thyroid surgery.

Keywords

thyroid nodule, biopsy, fine needle, thyroid neoplasms, mutation, prevalence, retrospective studies, BRAF, genes, RAS, PPAR gamma

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Over the recent years, molecular testing of fine-needle aspiration (FNA) samples from indeterminate thyroid nodules has become a useful tool with incredible potential to aid in the detection of thyroid malignancies.[1] The routine use of molecular testing and detection of mutations in FNA material has led to significant diagnostic improvements and a "rule in" approach, especially in the evaluation of indeterminate thyroid nodules.[2] Indeterminate nodules—those with either atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS; Bethesda

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category III) or follicular neoplasm (Bethesda category IV)—carry a wide risk of malignancy, thought to be between 5% and 45%.[3] Additionally, many studies identified risk factors that may place patients or certain nodule types at higher malignancy rates, including clinical, cytologic, radiologic, and molecular data.[4] Despite this, the rate of malignancy seen in this population varies significantly among studies.

Significant focus has been placed on ultrasonographic features and use of surgeon-performed ultrasound in improving the ability to predict malignancy in indeterminate thyroid nodules.[5] Classical suspicious features, including microcalcifications, increased vascular flow, and hypoechogenicity, often herald a malignant process, even among patients with AUS/FLUS.[6] Additional studies showed that incorporating these features into preoperative evaluation was helpful in differentiating between benign and malignant processes in Bethesda III thyroid nodules, with suspicious features found more commonly in malignant neoplasms.[7] Additionally, certain cytopathologic features, such as nuclear atypia, architectural atypia, and other atypical features, can effectively stratify the risk of malignancy of thyroid nodules with indeterminate cytology and thereby improve cytology-histology correlation.[8] Consequently, some pathology studies called for a standardization of the use of the word *atypia* in terms of FNA findings, as it may be a significant indicator of malignancy in indeterminate nodules.[9] Other studies cited external factors, such as institutional practices or geographic location, as further confounders to the rate of malignancy in indeterminate nodules and advocated for an institutional-specific approach, recognizing that malignancy rates vary frequently in indeterminate nodules.[10]

The use and utility of molecular testing to aid in the management of indeterminate nodules has advanced rapidly over the past several years, and many methods of molecular tests are readily available for commercial use.[11] Testing can be used as a ''rule in,'' a ''rule out,'' or a combined adjunct test depending on positive or negative predictive values, respectively. When used, molecular testing should include multiple mutations, as testing for a single mutation often has a low overall sensitivity and therefore limited diagnostic value.[12] Despite the potential, obstacles have limited the widespread adoption of molecular mutation testing in indeterminate thyroid nodules in the United States. Molecular tests often vary in their sensitivity, specificity, positive predictive value, and negative predictive value depending on patient populations, thereby complicating its clinical implementation and usefulness.[13] Additionally, the cost of molecular testing has been a significant hurdle in its implementation, and many centers or solo practitioners struggle to utilize testing due to a lack of availability or issues of reimbursement. Despite these limitations, the use of molecular markers is expected to improve the accuracy of cancer diagnosis in indeterminate thyroid nodules and allow for more individualized surgical and postsurgical treatment of patients with thyroid cancer.[14] This has led to a more tailored approach to dealing with patients' indeterminate nodules and could give patients and surgeons a more accurate picture regarding malignancy risk in discussions or advisement of surgery.[15]

While there is interest regarding the rate of malignancy in mutation-positive indeterminate thyroid gland nodules, certain mutations are much more thoroughly studied than others.[16] There is an abundance of data regarding malignancy in *BRAF* and *RET* mutations, but there are considerably fewer studies on other mutations, including *PAX-PPARγ, HRAS, NRAS*, and *KRAS* mutation groups. Additionally, some studies suggested that *KRAS* may have a significantly reduced rate of malignancy versus other molecular mutations.[17] The 2015 American Thyroid Association guidelines consider certain mutations a risk factor and prompt an upstaging of Bethesda category based on mutation type: cytology that is positive for *BRAF* or *RET* mutations is to be considered ''malignant'' (Bethesda VI) no matter their Bethesda category, and *RAS* mutations are to be considered ''suspicious for malignancy'' (Bethesda V).[18] In spite of this recommendation, the utility of positive molecular mutations in the preoperative evaluative period and its accuracy in predicting malignancy remain to be fully developed, especially among *RAS* classes (*HRAS, NRAS, KRAS*) and *PAX-PPARγ*.

In this study, we wish to evaluate the malignancy risk of patients with AUS/FLUS (Bethesda III) as seen on 2 separate FNA samples with a positive mutation detected on reflex molecular testing—the standard in our institutions. In this way, we wish to confirm the high risk of certain molecular mutations, such as *BRAF* and *RET*, and further illustrate the risk of *PAX-PPARγ, HRAS, NRAS*, and *KRAS* mutations. We also want to explore mutation subtypes to evaluate malignancy rate and determine if there is any significant difference in our cohort.

Methods

This clinical investigation was conducted in accordance and compliance with an Internal Review Board authorization (No. 5968) performed under the direction of Southern California Permanente Medical Group. Queries were made into the Southern California Permanente Medical Group cytopathology database to identify patients who had 3 qualifying conditions: (1) FNA of an indeterminate thyroid nodule showing AUS/FLUS, (2) repeat FNA within at least 6 months showing repeat AUS/FLUS, and (3) positive molecular testing for a genetic mutation as identified by reflex testing with ThyGenX (Interpace Diagnostics, Parsippany, New Jersey). Molecular testing is a reflex test after a second FNA demonstrates AUS/FLUS; thus, additional ethical approval was not required. FNA samples were cytologically categorized by experienced cytopathologists, and if there was any discrepancy, samples were further reviewed by an additional thyroid cytopathologist (J.E.T.I.). Inclusion criteria included all patients with the inclusion criteria who underwent thyroid gland surgery with available pathology material from 2014 to 2017. Nodules with multiple genetic mutations were excluded to avoid bias (n = 3), including a patient with *PIK3CA* (*E545K*) and *HRAS-Q61R* mutations, a patient with *PIK3CA* (*E545K*) and *NRASQ61R* mutations, and a patient with *BRAF V600E* and

HRAS-Q61K mutations. Of note, all 3 of these patients had malignant pathology.

Surgical pathology material was reviewed, and neoplasm type was categorized into benign or malignant tumors and further stratified per currently accepted diagnostic terminology. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (formerly, encapsulated follicular variant of papillary carcinoma) was considered benign, as is concordant with most recent guidelines.[19] Of note, microscopic carcinoma (<1 cm) of the thyroid gland was considered ''malignant'' if the microscopic tumor was identified as the subcentimeter nodule that originally prompted FNA. If the microscopic tumor was in the contralateral side from the nodule prompting FNA or was in the ipsilateral lobe but not the dominant nodule or reason for the FNA, it was interpreted to be incidental and thus not included in the evaluation (ie, ''benign''). All pathology reports were evaluated and then reviewed by an expert thyroid gland pathologist (L.D.R.T.) to further classify the neoplasms. Specifically, the expert pathologist was blinded from molecular results when re-reviewing pathology for classification purposes. A total of 231 patients met inclusion and exclusion criteria.

Results

The rate of malignancy based on molecular mutation group and subtype is summarized in **Table 1**. The overall rate of malignancy in our cohort was 74.0%. Patients with *BRAF* and *RET-PTC*/*CCD6-RET* mutations had a 100% rate of malignancy. Patients with *PAX-PPARγ* mutations also had a high rate of malignancy (84.6%), while patients with *HRAS* (70.7%) and *NRAS* (63.4%) had high rates but to a lesser extent. Of note, patients with *KRAS* mutations had a lower rate of malignancy (33.0%) when compared with the other mutations. Statistical significance was seen among mutation groups regarding rate of malignancy ($P < .001$). No statistical significance was seen among subtypes within group mutations, although mutations at the Q61K/R locale were somewhat different in *HRAS* (91.7% vs 64.3%) and *NRAS* (76.9% vs 60.9%).

Cancer morphology incidence among molecular mutation group and subtype is shown in **Table 2**. Well-differentiated papillary thyroid cancer was most common and accounted for all cancers in the *BRAF* and *RET-PTC*/*CCD6-RET* groups. In the *PAX-PPARγ* group, 63.6% of cancers were papillary carcinomas, while 36.4% were follicular carcinomas. Similar incidence of papillary and follicular carcinomas were seen in the *HRAS* (86.2% vs 10.3%), *NRAS* (88.5% vs 7.7%), and *KRAS* (87.5% vs 12.5%) groups. *HRAS* and *NRAS* groups had rare incidences of poorly differentiated carcinoma (3.5% and 3.8%, respectively), with these tumors progressing from papillary carcinoma.

Table 1. Malignancy Rate Based on Mutation Group and Subtype.

	Surgical Procedures		
Mutation Group: Subtype	Total	Malignant	Rate, %
BRAF	69	69	100
V600E	66	66	100
K601E	3	3	100
RET	2	2	100
RET-PTC	1	1	100
CCDC6-RET1	1	1	100
PAX-PPARγ	13	11	84.6
PAX-PPARγ-1	1	1	100
PAX-PPARγ-2	7	6	85.7
PAX-PPARγ-4	2	2	100
PAX-PPARγ unspecified	3	2	66.7
HRAS	41	29	70.7
HRAS-G13R	1	0	0
HRAS-Q61K	12	11	91.7
HRAS-Q61R	28	18	64.3
NRAS	82	52	63.4
NRAS-Q61K	13	10	76.9
NRAS-Q61R	69	42	60.9
KRAS	24	8	33.0
KRAS-G12A	1	0	0
KRAS-G12D	12	3	25
KRAS-G12V	3	1	33.3
KRAS-G13D	2	1	50
KRAS-Q61H	2	1	50
KRAS-Q61R	4	2	50
Totals	231	171	74.0

Discussion

The rate for malignancy among indeterminate thyroid nodules varies considerably, with molecular testing helping to identify an individual's malignancy risk. Despite the utility of molecular testing, its widespread incorporation remains to be universally implemented in the United States. Although the surgical treatment of thyroid cancer continues to be hotly debated and is beyond the scope of this project, the incorporation of molecular testing, especially as a reflex study, could conceivably be used in the future to help direct clinical decision making to optimize patient care by reducing morbidity and helping curb health care expenses. Despite this, many thyroid surgeons are still unfamiliar with the nuances of a positive molecular test and often equate positive mutations with malignancy. In our study, we wished to characterize the malignancy risk of patients with positive molecular mutations to better illustrate the variability among mutations.

In our cohort, patients with *BRAF* and *RET* mutations carried a 100% rate of malignancy, which is in agreement with prior studies and American Thyroid Association guidelines suggesting that these mutations be treated as a ''malignant'' FNA (Bethesda VI).[18] Additionally, our study confirmed that there are differences in the rate of malignancy seen among *RAS* mutations, with the *HRAS* and *NRAS* groups similar (70.7% and 63.4%, respectively) and the *KRAS* group substantially lower (33.0%). The relatively

Table 2. Cancer Morphology Based on Mutation Group and Subtype.

Mutation Group: Subtype	Malignant Pathology, n	Morphology, % (n)		
		Papillary	Follicular	Poorly Differentiated
BRAF	69	100 (69)	0	0
V600E	66	100 (66)	0	0
K601E	3	100 (3)	0	0
RET	2	100 (2)	0	0
RET-PTC	1	100 (1)	0	0
CCDC6-RET1	1	100 (1)	0	0
PAX-PPARγ	11	63.6 (7)	36.4 (4)	0
PAX-PPARγ-1	1	100 (1)	0	0
PAX-PPARγ-2	6	50 (3)	50 (3)	0
PAX-PPARγ-4	2	50 (1)	50 (1)	0
PAX-PPARγ unspecified	2	100 (2)	0	0
HRAS	29	86.2 (25)	10.3 (3)	3.5 (1)
HRAS-Q61K	11	90.9 (10)	0	9.1 (1)
HRAS-Q61R	18	83.3 (15)	16.7 (3)	0
NRAS	52	88.5 (46)	7.7 (4)	3.8 (2)
NRAS-Q61K	10	100 (10)	0	0
NRAS-Q61R	42	85.7 (36)	9.5 (4)	4.8 (2)
KRAS	8	87.5 (7)	12.5 (1)	0
KRAS-G12D	3	100 (3)	0	0
KRAS-G12V	1	100 (1)	0	0
KRAS-G13D	1	100 (1)	0	0
KRAS-Q61H	1	0	100 (1)	0
KRAS-Q61R	2	100 (2)	0	0
Total	171	91.2 (156)	7.0 (12)	1.8 (3)

low incidence of malignancy seen in nodules with *KRAS* mutations was evaluated in other studies, with similar findings.[17] The variable rate of malignancy seen in *RAS* mutations is very important, as the American Thyroid Association guidelines suggest that these mutations should all be treated as ''suspicious for malignancy'' FNA (Bethesda V), carrying a 60% to 75% malignancy risk.[18] Although this may be accurate for *HRAS* and *NRAS* mutations, it appears that the *KRAS* mutation may not carry a similar risk of malignancy. Furthermore, patients with *PAX-PPARγ* mutations carried an 84.6% risk of malignancy in our cohort, which is higher than that described in other studies.[20] Additionally, we chose to exclude patients with multiple mutations in our study due to concern for confounding bias. This highlights the need for larger clinical studies to adequately evaluate each mutation or combination of mutations to better characterize the malignancy risk in each situation with the hope of guiding treatment to minimize morbidity. Since thyroid surgeons may be unfamiliar with the rate of malignancy for specific molecular mutations, we believe that the disparity among mutations and subtypes should be highlighted, reported, and further studied.

While many patients undergo surgery for diagnostic purposes, we see the utility of the diagnostic thyroid lobectomy coming to a close. Studies showed that molecular testing may reduce the number of completion thyroidectomies and may lead to more individualized operative and postoperative treatment of patients with indeterminate nodules.[21] Other reviews discussed the clinical utility of various molecular testing done on FNA indeterminate nodules to avoid diagnostic thyroidectomies.[22] Ultimately, we wish to more accurately identify preoperative cancer risk so that patients and surgeons can tailor treatment based on a variety of preoperative assessment tools, of which molecular mutation type or subtype should be a major consideration. Strengths of our study include adequate power and potential for long-term follow-up. Limitations include size of study and the exclusion of patients who had multiple mutations present—although this group all had malignancies present.

Certainly, there is great potential in terms of customizing treatment for patients with indeterminate thyroid nodules based on an individual's specific findings to minimize morbidity and optimize cost-effectiveness. The incorporation of molecular testing into clinical decision making, including decision for surgery and type of thyroid surgery, remains to be explored. Additionally, the risk of malignancy in mutation-negative AUS/FLUS nodules would be of additional benefit in the decision between which patients should be offered surgery and which may be observed. Furthermore, the long-term prognosis, morbidity, disease-free interval, and

mortality for patients with positive molecular testing warrant additional investigation.

Author Contributions

David S. Cohen, project conception, data review and analysis, manuscript main writing, editing and revision of manuscript, final approval/accountability; **Jane E. Tongson-Ignacio**, majority of project data collection, editing of manuscript, final approval/accountability; **Christopher M. Lolachi**, project conception, data interpretation, editing of manuscript, final approval/accountability; **Vanessa S. Ghaderi**, project conception, editing of manuscript, final approval/accountability; **Babek Jahan-Parwar**, project conception, data interpretation, editing of manuscript, final approval/accountability; **Lester D. R. Thompson**, project conception, re-review of pathologic specimens, data analysis and interpretation, editing of manuscript, final approval/accountability.

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