

Use of molecular biomarkers in FNA specimens to personalize treatment for thyroid surgery

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ABSTRACT: *Background.* Accurate preoperative assessment of thyroid nodules with fine-needle aspiration biopsy (FNAB) continues to be a challenge, often resulting in unnecessary diagnostic surgical intervention. The detection of several novel gene mutations in differentiated thyroid cancer (DTC) over the last decade has led to the diagnostic use of these oncogenic alterations to improve FNAB sensitivity and specificity.

Methods and Results. Thyroid oncogene mutations including BRAF, RAS, and RET/PTC are reviewed. The potential benefit of using this

panel on fine-needle aspiration (FNA) cytology samples will be described.

Conclusion. Our use of "reflexive" molecular testing demonstrates its clinical value in conjunction with FNAB cytology, representing an application of personalized molecular medicine to guide appropriate surgical therapy. © 2012 Wiley Periodicals, Inc. *Head Neck* 35: 1499–1506, 2013

KEY WORDS: thyroid, diagnosis, biomarkers, molecular testing, prognosis

INTRODUCTION

Accurate assessment of thyroid nodules prior to surgical removal continues to challenge physicians who regularly treat these patients. Prior to the routine use of fine-needle aspiration biopsy (FNAB) of thyroid nodules, malignancy was found in only 14% of resected thyroid glands.¹ Although ultrasound guidance (USG) has improved the diagnostic accuracy of fine-needle aspiration (FNA) in low-risk populations, the specificity and positive predictive value remain 47% and 52%, respectively.² In a meta-analysis of 20 large patient series published from 2001 to 2006, approximately 24% of thyroid FNAs had an indeterminate result.³ Using current American Thyroid Association (ATA) guidelines to guide which of these indeterminate nodules are surgically excised, the majority of them will be found to have benign pathology. Thus, many patients undergo surgical resection of benign disease, resulting in a large amount of potentially avoidable, iatrogenic morbidity.⁴ More accurate FNAB cytology could therefore avoid "diagnostic" surgery and guide the extent of resection preoperatively.

The National Cancer Institute (NCI) has defined results of thyroid FNAB to fall in 1 of 4 categories based on a 2007 National Institutes of Health consensus: benign, malignant, nondiagnostic, and indeterminate. The last 2 results represent the limitations of FNA cytologic diagno-

sis. When the aspirate contains scant cellularity or the lesion is largely cystic, the result can be difficult to analyze, requiring the patient to undergo repeated aspirations or definitive surgery. The "indeterminate" results have been further subdivided by the NCI in the widely accepted 2007 Bethesda Classification (Table 1): (1) suspicious for malignancy, (2) follicular neoplasm, (3) follicular lesion of undetermined significance (FLUS)/atypia of undetermined significance (AUS). Each of these carries differing risks of malignancy, thereby requiring the surgeon to further guide the patient into making an informed decision about the need for surgery. The "suspicious for malignancy" category represents 3% to 9% of all thyroid FNA results, and 60% to 77% of these cases prove to be malignant. The "follicular neoplasm" result can either be a follicular adenoma (FA), follicular thyroid carcinoma (FTC), or a follicular variant of papillary thyroid carcinoma (fvPTC). Despite a lower malignancy rate of 14% to 32%, this result currently requires the patient to undergo at least a thyroid lobectomy to examine the lesion for vascular or capsular invasion for a definitive diagnosis to be made. Finally, FLUS/atypia of undetermined significance represents those lesions that do not fit in any of the other categories and carry a potential risk of at least 5% to 10%. The current recommended guidelines suggest observing these tumors with serial exams and repeat FNA because a large fraction may actually harbor a malignancy.⁵ Depending on the wishes of the patient, contralateral nodularity and need for thyroid hormone, however, these lesions may go on to be surgically extirpated for diagnostic purposes.

Improved pathologic accuracy with the use of molecular biomarkers has been demonstrated for a variety of

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TABLE 1. The National Cancer Institute suggested fine-needle aspiration terminology and the risk of malignancy based on the cytopathologic result.

FNA result	Alternate accepted nomenclature	% risk of malignancy
Benign	–	<1%
Atypia of undetermined significance	Atypical lesion of undetermined significance; follicular lesion of undetermined significance; indeterminate follicular lesion; atypical follicular lesion	5–10%*
Neoplasm suspicious for malignancy	Suspicious for neoplasm; follicular neoplasm	20–30% 50–75%
Malignant	–	100%
Nondiagnostic	Unsatisfactory	–

Abbreviation: FNA, fine-needle aspiration.

* Risk of malignancy may be greater than 10%.^{36,37}

malignancies.^{6,7} With any test, the benefit of identifying more cancers has to be weighed against false positivity and the increased morbidity of treatment. Additionally, the costs of the testing versus the potential economic benefits to the patient and society, in terms of years of life gained/lost, work productivity, quality of life, and health care expenditures need to be considered. As discussed above, there remains a large potential gain, both therapeutically and economically, for improved thyroid cancer detection with preoperative FNAB.

The detection of several novel gene mutations in differentiated thyroid cancer (DTC) over the last decade has led to the use of these oncogenes to improve FNAB sensitivity and specificity. The specific thyroid neoplasm gene mutations identified, as well as the potential benefit of using novel mutation detection strategies on FNA samples, will be the focus of this review. Our molecular testing algorithm at the University of Pittsburgh, implemented in 2007, demonstrates the clinical value of "reflexive" molecular testing in conjunction with FNAB, and represents the use of personalized molecular medicine to guide therapy.

MOLECULAR ETIOLOGIES OF THYROID NEOPLASMS

The identification of specific mutations in thyroid carcinomas has greatly improved the general understanding of how these tumors develop. Additionally, the identification of these markers has helped to identify the specific genetic alterations that occur during the progression from a follicular hyperplasia to a well-differentiated carcinoma. Although certain mutations have been identified for medullary and anaplastic carcinoma, the practical use of these mutations is less clinically applicable due to the ease at which these tumors are identified by current diagnostic methods. This section will focus on the 3 most commonly encountered neoplasms, which are difficult to differentiate between based on FNA alone: papillary thyroid carcinoma (PTC), FTC, and FA.

Papillary thyroid carcinoma

Several mutations comprising components of the mitogen-activated protein kinase (MAPK) pathway have been documented in the development of PTC (see Figure 1). The MAPK protein alters signaling pathways and induces cell cycle progression. Alterations in this growth factor pathway have been demonstrated in many different carcinomas and targeted therapies inhibiting

this pathway have been used for different tumor types (eg, Hodgkin's lymphoma, glioblastoma multiforme, squamous cell carcinoma, non-small cell lung cancer, and melanoma).

The most common genetic mutation for PTC is within the BRAF gene, occurring in approximately 45% of all PTC neoplasms.⁸ The vast majority of the alterations within this gene is a substitution of valine for glutamate at residue 600 (BRAF V600E), which has been implicated in other tumors including melanoma and colorectal carcinoma. The result of this mutation is a constitutive

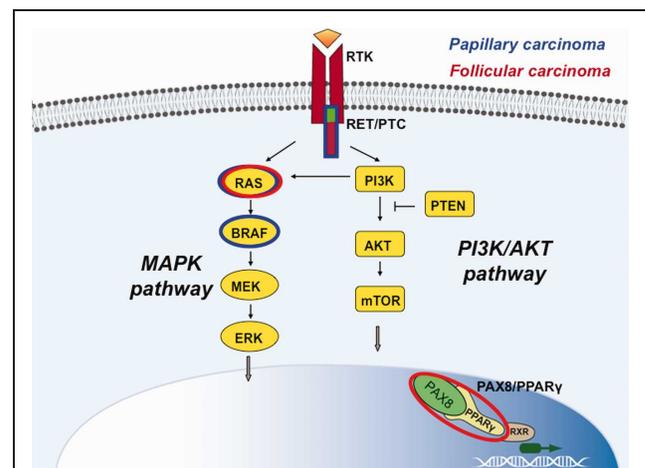


FIGURE 1. A schematic illustrating the mitogen-activated protein kinase (MAPK) pathway and the more commonly identified mutational sites in well-differentiated thyroid carcinoma: RET/PTC, RAS, BRAF, and PAX8/PPAR γ . The MAPK protein alters signaling pathways and induces cell cycle progression. The BRAF gene mutation is caused by substitution of valine for glutamate at residue 600 (BRAF V600E) and results in a constitutive activation of the BRAF kinase. The RAS gene encodes for G-proteins that are bound to cell membrane receptors, existing in an inactive state, where it is bound by guanosine diphosphate. Once it receives an extrinsic signal, it trades the diphosphate guanosine for guanosine triphosphate (GTP) and proceeds with the MAPK pathway. The more frequently encountered mutation is at codon 61, resulting in an inactivation of the GTPase function. RET/PTC mutations involve the 3' tyrosine kinase portion of the RET gene fusing with the 5' of a different gene, resulting in its interminable activity and stimulation of the MAPK pathway. The PAX8/PPAR γ results in an abundance of the fusion protein (PPF), but the carcinogenic mechanism of action for this pathway is unclear. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

activation of the BRAF kinase, which causes thyroid tumorigenicity via the MAPK pathway. There also appears to be an alteration within a sodium-iodide transporter in the thyroid cell membrane, which has implications for prognosis due to the decreased ability of the cell to uptake iodide.^{9,10} The mutation is more commonly seen in the classical version of PTC and the tall-cell variant, but can be found less commonly in the follicular-variant of PTC.¹¹ Anaplastic carcinoma and poorly differentiated thyroid cancers have also been shown to express BRAF V600E.⁸ Importantly, FTC, FA, and lesions of benign histology do not carry this mutation, thereby making it a unique diagnostic and prognostic marker for PTC, and its variants, among DTC.

Conversely, RAS mutations, found in approximately 10% to 20% of PTC tumors, are more commonly encountered in the follicular variant of PTC, as well as follicular hyperplasia, adenomas, and carcinomas.^{8,12} The RAS gene encodes for G-proteins that are bound to cell membrane receptors, which when stimulated by extracellular signals, result in cell propagation via the MAPK pathway. Since the RAS proteins are involved in communication of the cell nucleus with the outside environment, perpetually activated RAS proteins can induce uninhibited cell division in the absence of external signals. Mutations in RAS activation have been demonstrated in 20% to 25% of all human tumors.¹³ The protein exists in an inactive state where it is bound by guanosine diphosphate. Once it receives an extrinsic signal, it trades the diphosphate guanosine for guanosine triphosphate (GTP) and proceeds with the MAPK pathway. The RAS protein quickly gets turned off by its intrinsic GTPase activity by removing a phosphate from the GTP. Mutations within the RAS gene result in either an inactivation of the GTPase function or an increased binding affinity for GTP. The more frequently encountered mutation in PTC is at codon 61, resulting in an inactivation of the GTPase function. Of the 3 RAS genes, NRAS, KRAS, and HRAS, NRAS and HRAS mutations are more commonly implicated in thyroid carcinoma.

The RET proto-oncogene is classically known for its involvement in medullary thyroid carcinoma and the multiple endocrine neoplasia syndromes. However, its role in PTC has been well documented, with more than 15 different types of rearrangements described.¹⁴ The common theme among all of these chimeric proteins is the 3' tyrosine kinase portion of the RET gene fuses with the 5' of a different gene, resulting in its interminable activity. This protein hybrid then activates tumor proliferation via the MAPK-RAS pathway. The 2 most common versions of the RET proto-oncogene are RET/PTC1 (60% to 70%) and RET/PTC3 (20% to 30%), which are paracentric versions with the 5' domain of 2 different genes on chromosome 10: H4 and NCOA4, respectively.¹⁴ RET mutations are found in 13% to 43% of PTC neoplasms.¹⁵ However, in PTC patients with a previous history of radiation exposure (50% to 80%) or younger patients (40% to 70%) have a much higher incidence of the RET proto-oncogene.¹⁶⁻¹⁸ The RET/PTC1 tumors demonstrate either classic papillary architecture or diffuse sclerosing features, whereas the RET/PTC3 is associated more with the solid variant. All of the RET/PTC tumor subtypes possess a higher rate of lymph node metastases.¹⁹

Follicular thyroid carcinoma and follicular adenoma

As mentioned above, RAS mutations are found in the various follicular neoplastic processes: FTC, fvPTC, and FA. Follicular thyroid carcinoma harbors the RAS mutation in 40% to 50% of patients,¹⁵ whereas 20% to 40% follicular adenomas carry the mutation.⁸ Although the benign follicular lesions carrying the RAS mutations may actually be precancerous lesions, the biomarker remains a nonspecific marker for all follicular neoplasms.

A gene rearrangement leading to the fusion of the thyroid-specific paired domain transcription factor, PAX8, and the peroxisome proliferator-activated receptor gene, PPAR γ , which plays an important role in lipid metabolism, was discovered in follicular thyroid carcinoma in 2000.²⁰ The PAX8/PPAR γ results in an abundance of the fusion protein (PPFP), but the carcinogenic mechanism of action for this pathway is unclear. PAX8 plays an essential role in thyrocyte development, as well as being involved in gene expression of the sodium-iodide symporter, thyroglobulin and the TSH receptor.²¹ The PPFP gene also antagonizes the action of PPAR γ , which has been shown to be a causative agent in FTC in vitro and in vivo tumorigenesis.²¹ The proposed consequence of an overabundance of the PAX8/PPAR γ fusion protein remains theoretical.

The PAX8/PPAR γ translocation is found in 30% to 40% of classic follicular thyroid carcinoma, 2% to 10% of follicular adenomas, and rarely in fvPTC.²² The follicular adenomas with the mutation tend to have features more consistent with a carcinoma, including immunohistochemical staining for markers more consistent with FTC. These adenomas, much like the RAS-positive FAs, may actually represent a carcinoma in situ or were misclassified by the pathologist rather than simply a benign neoplasm. The PPRP-positive FTCs tend to occur in younger patients, those with solid patterns and tumors with vascular invasion.²²

BIOMARKERS USED FOR TUMOR DETECTION AND PROGNOSTICATION

Several prospective and retrospective studies have demonstrated that using modern molecular detection techniques to search for genetic alterations, the accuracy of fine-needle aspiration samples is significantly improved. Additionally, whereas most patients with DTC fare well, biomarkers have also been shown to provide prognostic information that could be used to guide further management. The most commonly used techniques and biomarkers will be discussed in the following text, with special attention to the reflexive testing performed routinely at the University of Pittsburgh molecular anatomical pathology laboratory (Y. N., director).

Molecular testing of FNA samples

BRAF. The majority of clinical studies looking at biomarker detection and clinical usage in FNA thyroid specimens have been focused on BRAF mutation. In a 2009 review,⁸ Nikiforov reported on testing for BRAF in 2766 samples, which included 9 prospective FNA studies, 7 retrospective FNA studies, and 2 studies of research FNA performed on postoperative thyroid specimens (Table 2).

TABLE 2. Review of all thyroid FNA studies using the BRAF mutation prior to 2009.

Thyroid FNA studies	No. of samples	BRAF positive	Final diagnosis in BRAF-positive samples (%)
Prospective studies	1814	159	PTC = 159 (100%)
Retrospective studies	685	291	PTC = 291 (100%)
FNA on thyroid specimens	267	131	PTC = 130 (99.2%) Hyperplasia = 1 (0.8%)
Total	2766	581	PTC = 580 (99.8%)

Abbreviations: FNA, fine-needle aspiration; PTC, papillary thyroid carcinoma.

Note: Results of the prospective, retrospective, and FNA on surgically removed thyroid specimens are shown.

BRAF positivity shows an almost universal correlation with a final pathologic result of PTC.

Adapted from Nikiforova MN, Nikiforov YE. Molecular diagnostics and predictors in thyroid cancer. *Thyroid* 2009;19:1351–1361. doi: 10.1089/thy.2009.0240, with permission.

In the review of this body of literature, it was noted that all 450 BRAF-positive clinical FNA samples studied prospectively and retrospectively were positive for papillary carcinoma, and only 1 reported a BRAF-positive sample, obtained by research aspiration of the nodule in a surgically removed thyroid gland, which appeared to be benign.²³ The nodule was reported as atypical nodular hyperplasia, but did not include specifics in the appearance of the pathologic specimen. Additionally, it was reported 5 years ago and did not use the use of modern technology and immunohistochemical staining, which can further differentiate difficult to diagnose lesions. If this single case were to be accepted as a false positive, it would imply that in the reported literature, 580 of the 581 BRAF-positive nodules tested in various types of FNA samples are PTC, resulting in a false-positive rate of 0.2%. The more important clinical question, however, is the ability of the biomarker to accurately differentiate malignant and benign histology in those lesions that are indeterminate or nondiagnostic by FNA cytology. In the 16 studies reviewed,⁸ 15% to 39% of BRAF-positive FNA samples fell into the nondiagnostic or "indeterminate" categories: (1) suspicion for malignancy, (2) suspicion for neoplasm, and (3) follicular lesion of undetermined significance (FLUS)/atypia of undetermined significance. Given the extremely low false-positive rate of the BRAF biomarker, these tumors could then be treated as PTC by convention. Also, several patients with preoperative benign FNA results were found to be positive for BRAF mutation, and then confirmed as PTC after surgical removal of the thyroid gland. As mentioned above, 5% to 7% of all tumors reported as benign on preoperative FNA will actually harbor malignancy. The routine use of BRAF testing would further decrease this false-negative rate.

RAS and TIMP1. RAS mutations are most commonly found in follicular thyroid neoplasms, both benign and malignant. A retrospective study by Mathur et al,²⁴ looking at tumors from 341 patients and using a combination of FNA cytology and molecular diagnostic techniques, demonstrated statistical significance with an association between malignancy and the FNA cytologic result ($p \leq .001$), NRAS mutation (0.016) and TIMP1 (0.067). The overall diagnostic accuracy was 91%, with a specificity of 97% and a sensitivity of 76% when putting to use all 3 techniques to the entire cohort. The benefit in "indeter-

minate" lesions was not as readily appreciated. The accuracy with molecular testing dropped to 61% for "atypical lesions" or "suspicious for neoplasm" FNA samples with a specificity of 81% and a low sensitivity of 27% for the atypical lesions alone. In the tumors with "suspicious for neoplasm" FNA cytology, the scoring model with all 3 techniques had a specificity of 100% and a sensitivity of 14%. Finally, with the FNA subgroup suspicious for malignancy, the model was 77% accurate with a 100% sensitivity. The use of NRAS in combination with TIMP1 and traditional FNA analysis therefore shows benefit when deciding surgical options for the FLUS and "suspicious for neoplasm" patients, but has limited utility in the suspicious for malignancy FNA results. Based on the result for the FLUS and "suspicious for neoplasm," the clinician could then decide preoperatively whether to proceed with a total thyroidectomy versus performing a diagnostic lobectomy. These results are in concordance with the associations of both, so these mutations were associated with benign and malignant thyroid neoplasms.

RET/PTC. Among the DTC, the RET proto-oncogene is typically associated with PTC. In a retrospective analysis comparing patient-matched FNA and postthyroidectomy specimens, the presence of a RET/PTC fusion transcript was in 50% of the FNA samples, all of which were histologically proven PTCs in the surgically removed thyroids.²⁵ No false-positive results were reported in this study. The results confirmed that RET/PTC is a highly specific biomarker for the diagnosis of papillary thyroid carcinoma. Additionally, the data suggested that molecular investigation was most informative for aspirates that would otherwise have been nondiagnostic. In 2 of the 6 histologically proven PTCs, which had insufficient aspirate for diagnosis by cytologic examination, the correct diagnosis was made by screening for RET/PTC on the FNA samples. Of the 15 "indeterminate" FNA biopsies that were eventually diagnosed as PTC postoperatively, 9 were positive for RET/PTC. However, if looking at RET/PTC alone as the diagnostic biomarker, only 50% of the PTCs were identified based on the use of molecular diagnostics with RET/PTC as the biomarker. Finally, in this series, when both cytologic analysis and RET/PTC detection were used, it allowed an increased diagnostic yield from 12 cases definitively diagnosed by cytologic examination alone to 23 cases diagnosed by cytology and molecular marker amplification.

TABLE 3. Results of prospective, mutation-panel testing on 1056 thyroid patient samples.

Cytologic result	Molecular analysis result	Cancer risk, %
AUS/FLUS	Positive	88%
	Negative	5.9%
Follicular Neoplasm	Positive	87%
	Negative	14%
Suspicious for Malignancy	Positive	95%
	Negative	28%

Abbreviations: AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; FNA, fine-needle aspiration.

Note: The probability of malignancy is shown based on positivity of the cytologic result with and without the molecular analysis result. As shown, the molecular panel testing greatly improves the utility of the thyroid FNA.

Adapted from Nikiforov YE, Ohori NP, Hodak SP, et al. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. *J Clin Endocrinol Metab* 2011;96:3390–3397. ©2011, The Endocrine Society, with permission.

Panel testing. After accomplishing routine molecular testing of a single biomarker with FNA cytologic analysis, a panel of markers was assembled to improve identification of DTC and was implemented at the University of Pittsburgh in 2007. The largest, prospective study done thus far used molecular techniques to test for a panel of mutations: BRAF, RAS, RET/PTC, and PAX8/PPAR γ .²⁶ The investigators tested 1056 consecutive FNA samples from thyroid nodules (Table 3). In all, 479 of the patients underwent a thyroidectomy, providing histopathologic information on 513 FNA samples. In the patients with a FLUS result on FNA, mutations were identified in 25 FNA samples (19 RAS, 5 BRAF, and 1 PAX8/PPAR γ). Malignancy was found on final pathology in 22 of the 25 nodules (88%). The 3 false-positive nodules, all with RAS mutations, were follicular adenomas. For the mutation-negative group, 13 nodules (5.9%) were found to be malignant after surgery, providing a negative predictive value of 94% and 99% specificity. The "suspicious for neoplasm" group had 33 of 38 (87%) mutation-positive nodules test positive for carcinoma on histopathologic analysis. Of the 176 mutation-negative samples, 151 (86%) were benign and 25 (14%) were malignant. Nineteen of 20 (95%) of the mutation-positive "suspicious for malignancy" nodules were diagnosed as carcinoma after resection. In the 32 mutation-negative nodules with "suspicious for malignancy" cytology, 23 (72%) were benign and 9 (28%) were found to be malignant after surgery. Overall for the lesions with indeterminate cytology, atypia of undetermined significance/follicular lesion of undetermined significance, follicular neoplasm/suspicious for a follicular neoplasm, and suspicious for malignancy, the detection of any mutation translated into a risk for malignancy of 88%, 87%, and 95%, and 6%, 14%, and 28% in mutation-negative lesions, respectively. If all 4 biomarkers and cytology were negative, the false-negative rate decreased from 2.1% to 0.9%.

Therefore, with panel testing, a positive result almost definitively indicates the presence of malignancy. Patients with these mutations would be candidates for total thyroidectomy irrespective of the cytologic diagnosis. Additionally, the need for intraoperative, frozen-section analysis, or a possible completion thyroidectomy would be

eliminated, reducing overall costs and morbidity to the patient. When the molecular testing and the FNA are negative, the patient is much more likely to have a benign tumor. With the indeterminate result, the physician and patient can both make a much more informed decision with a negative mutational testing result due to the improved sensitivity.

Prognostication using biomarkers

Although most well-differentiated thyroid carcinomas have indolent behavior and are easily cured by surgical removal, a minority of tumors can be highly aggressive and difficult to eradicate. The current classifications rely on clinical characteristics (ie, age, extrathyroidal extension, size, and tumors grade) and molecular biomarkers have also shown some promise in this regard.

The BRAF V600E mutation has been well studied as a prognostic biomarker for PTC. In the review by Xing et al,¹¹ the authors discuss that BRAF mutations were correlated with aggressive tumor characteristics (ie, extrathyroidal extension, advanced tumor stage at presentation, and lymph node or distant metastases). The mutation was further shown to be a poor prognostic indicator when positive in FNA samples. The BRAF mutation has also demonstrated itself as an independent predictor of tumor recurrence, even in patients with early disease. Elisei et al²⁷ examined 102 PTC patients over a median of 15 years with the BRAF V600E mutation as an independent risk factor for tumor-related death. As mentioned earlier, BRAF activation via BRAF V600E mutation has been shown to alter the function of the sodium-iodide symporter and other proteins involved in iodide metabolism. Also, the BRAF mutation predisposes the progression of the PTC to poorly differentiated and anaplastic carcinoma. Finally, several studies have discovered that the BRAF V600E alteration in micro papillary carcinomas correlates with both higher rates of extrathyroidal tumor extension and cervical lymph node metastasis.^{28–30} The majority of the micro PTCs are indolent tumors that are incidentally discovered during the removal of presumed large, benign neoplasms. They are almost universally cured by surgical resection. However, certain micro papillary carcinomas can behave aggressively, leading to significant morbidity and death. In a recent study of 25 aggressive and 26 nonaggressive micro PTCs, those with recurrent disease and/or lymph node metastases, BRAF V600E mutations were detected in 20 aggressive TPMCs (77%) compared with 8 nonaggressive TPMCs (32%; $p = .001$).³¹ Having a possible prognostic biomarker (BRAF V600E) along with histopathologic features can further guide the treatment algorithm into a more aggressive approach for those carcinomas that test positive for the mutation.

The role of RAS mutation as a prognostic biomarker has not been as clearly delineated. As previously mentioned, this tumor is found in both benign and malignant follicular processes, making it difficult to use it for prognosis. However, there is evidence to suggest that a follicular adenoma that is RAS positive is more likely to be a carcinoma precursor or carcinoma in situ. Some studies have suggested a significant correlation between the RAS mutation and metastatic potential of FTCs, especially to

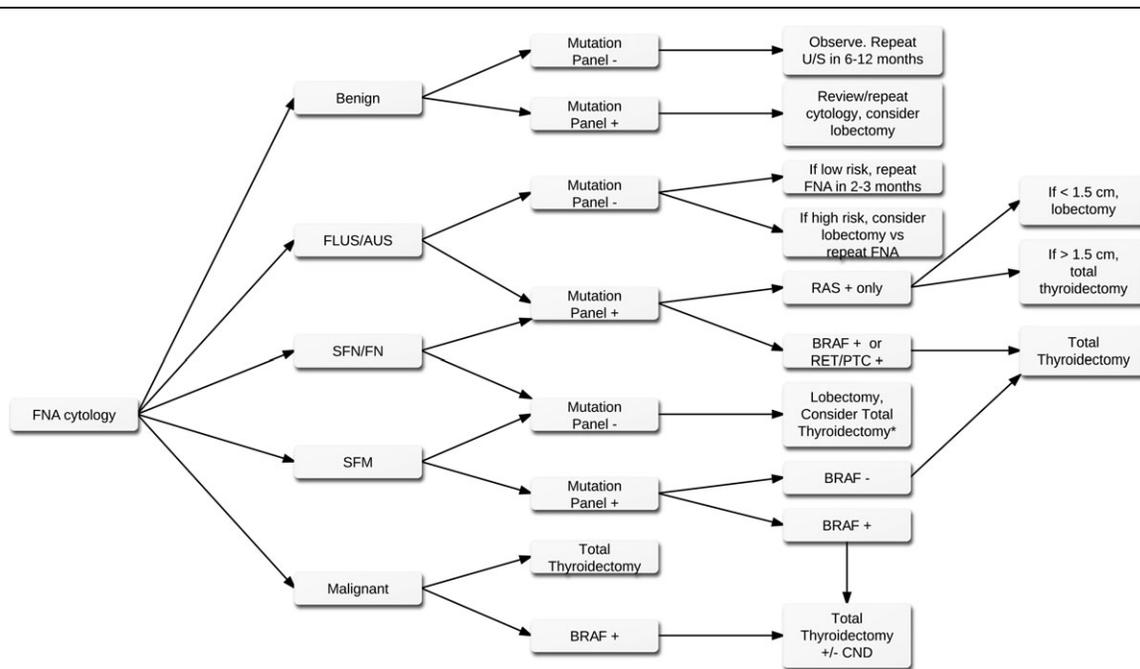


FIGURE 2. A diagnostic and therapeutic algorithm based on FNA cytology and molecular panel testing (BRAF, RET/PTC, and RAS). The “high-risk” factors are: age >45 years, male, or lesion >3–4 cm. Low risk is considered age <45 years, female, or lesion <3 cm. FLUS/AUS, follicular lesion of undetermined significance/atypia of undetermined significance; SFN/FN, suspicious for neoplasm, follicular neoplasm; SFM, suspicious for malignancy; CND, central neck dissection. *Indication for central and/or lateral neck dissection should be based on the current National Cancer Institute guidelines, which include suspicion on preoperative clinical exam/ultrasound and palpable lymphadenopathy intraoperatively. BRAF positivity may imply a more aggressive neoplasm with a propensity for nodal metastasis and may be considered for central neck dissection.

the patient’s bone. This may be due to the role of RAS mutation in decreased tumor differentiation and progression to anaplastic carcinoma.⁸ In a series of 91 tumors followed for a median of 14 years, the possession of a RAS mutation was correlated with distant metastasis and a significantly higher mortality rate.³² Overall, due to its presence in benign and largely indolent tumors, the RAS mutation has not been a consistently reliable, prognostic biomarker.

Unlike the PTCs with the BRAF and RAS mutations, those with the RET/PTC genetic rearrangement have shown a favorable prognosis when compared with their negative counterparts. They have been found to have a lower probability of tumor dedifferentiation and metastasis.³³ Since the mechanism by which RET/PTC leads to thyroid carcinogenesis is unclear, the reason for this improved outcome remains a mystery requiring further investigation.

CLINICAL APPLICATION

Based on clinical applications of FNA cytology and molecular testing, we propose a diagnostic and therapeutic algorithm (see Figure 2), which incorporates the current NCI guidelines for management of thyroid nodules. If the FNA cytologic result is benign and the mutational panel testing is negative, the patient can be safely observed with a 0.9% false-negative rate.²⁷ When the FNA indicates a benign lesion, but the mutation panel is positive, the patient should be closely observed and the cytology should be reexamined or repeated. The surgeon may also consider a lobectomy in this rare scenario. If

the lesion is >4 cm, based on the NCI guidelines, a lobectomy is performed due to the inaccuracy of FNA in nodules of that size. For an FNA cytologic result of malignancy, a total thyroidectomy should be performed, regardless of mutational status. A central neck dissection (CND) is indicated if lymphadenopathy is detected on preoperative ultrasound or intraoperatively by palpation. Additionally, if the lesion is BRAF positive, the clinician can consider a CND due to the potential aggressive nature of BRAF-positive PTC, although clear evidence for this recommendation has not been established and warrants further investigation.

For indeterminate lesions, mutational panel testing plays a more crucial role in clinical decision making. An AUS/FLUS result carries at least a 5% to 10% risk of malignancy, with some studies reporting up to 16% to 19%.^{34,35} However, with panel testing, the risk is stratified into an 88% chance of malignancy with a positive result and a 5.9% chance with a negative result.²⁷ A similar pattern is seen with a “suspicious for neoplasm” or “follicular neoplasm” finding on cytology: the risk for malignancy changes from 20% to 30% to 87% for mutation panel-positive lesions and 14% for mutation panel-negative lesions. Therefore, for mutation positive nodules with AUS/FLUS or follicular neoplasm cytology, a total thyroidectomy is recommended. If, however, only the RAS mutation is positive, the lesion still has a reasonable possibility of being benign. In these cases, patients with smaller lesions (<1.5 cm) or those with low risk factors (age <45 years, female) can be considered for a lobectomy alone. For mutation panel-negative AUS/FLUS, we observe with a repeat ultrasound in 2 to 3 months or

perform a lobectomy, based on patient preference. In high-risk patients with AUS/FLUS cytology (lesion >3–4 cm, age >45, male), surgical intervention is more strongly recommended by the ATA guidelines. For a follicular neoplasm/suspicious for neoplasm result that is mutation negative, a lobectomy is usually recommended due to a 14% risk of malignancy.

Finally, those patients with "suspicious for malignancy" on cytology and negative mutation panel testing, still carry a 28% risk of malignancy and warrant at least a lobectomy, with possibly a total thyroidectomy based on clinical or patient factors. Any mutation-positive "suspicious for malignancy" patient should receive a total thyroidectomy due to a 95% risk of malignancy. The proposed algorithm is meant to be an aid to guiding treatment. Due to the current estimated cost of the panel test, approximately \$650, the judicious use of the molecular testing may involve reserving it only for lesions where the FNA is indeterminate and the panel testing results most significantly impact management. As mentioned above, the "indeterminate result" occurs in 24% of patients. The additional cost of mutational panel testing in this smaller subset of patients may be warranted when compared with the cost and risks of unnecessary or delayed therapy. A cost/benefit study of the panel testing demonstrated an increase in the average per patient cost of nodule evaluation by \$104, to \$682 and a savings of approximately \$3000 when a total thyroidectomy was performed for a mutation-positive tumor compared with a diagnostic lobectomy followed by a completion.³⁶ Interestingly, the analysis also indicated that if the cost of molecular testing were to increase to more than \$870, the associated savings would no longer be observed. In another study looking at the cost effectiveness of a commercially available, molecular diagnostic test (Afirma Gene Expression Classifier; Veracyte, South San Francisco, CA), which examines mRNA expression of 142 genes, the authors concluded that this \$3200 test resulted in a cost savings of \$1453 per patient with an indeterminate nodule.³⁷

To most appropriately personalize the extent of surgery for each patient, the clinician should incorporate all clinical, cytologic, and "patient preference" information before proceeding with therapy.

CASE EXAMPLES

To further illustrate our current application of panel testing, we present 2 cases in which the results of mutation analysis helped to personalize therapy.

A 35-year-old woman with a 3-year history of bilateral thyroid nodules, the largest being 1.5 × 1.1 × 0.9 cm, was being followed by serial ultrasound and physical exam. The original FNA of the dominant nodule, 3 years prior to her presentation at our institution, was read as a FLUS (confirmed by our pathologists) and the patient elected for annual observation. Her repeat ultrasound showed minimal growth in the nodule over the 3-year period and a repeat FNA had a similar FLUS result as the initial specimen. However, since her repeat FNA was performed at our center, on panel testing, the lesion was noted to be BRAF V600E positive. Based on a discussion with the patient, including the increased risk of malig-

nancy from 5%–10% to 99%,²³ she opted for a total thyroidectomy. The surgical pathology report revealed 2 foci of papillary carcinoma (1.5 and 1.0 cm) with extracapsular extension. The advantage of mutation testing was the ability to establish a more definitive diagnosis and avoid further ultrasound and cytologic testing in this patient.

The second case was a 62-year-old man with a 3.5-cm solitary nodule. The histopathologic analysis of his FNA demonstrated a follicular neoplasm, which carried a 20% to 30% risk of malignancy. After panel testing, the NRAS61 mutation was identified. Given his high-risk demographic and RAS positivity, the patient elected for a total thyroidectomy due to an estimated 87% malignancy risk. On final surgical pathology, the lesion was found to be a fvPTC, partially encapsulated. Due to this patient's age, sex, and tumor size, the NCI guidelines would recommend that this patient have surgical intervention despite the panel testing results. However, the identification of the RAS mutation allowed more efficient treatment and avoided a second anesthetic by identifying a highly likely carcinoma preoperatively.

CONCLUSION

Although the evidence is limited to a relatively small number of institutions, the clinical application of molecular techniques to detect mutations in thyroid FNA samples has shown significant promise in improving detection and prognostication for well-differentiated thyroid carcinomas. Biomarker identification has been especially useful for those tumors that are "indeterminate" by traditional cytologic analysis. Extension of molecular panel testing to other sites in the future is warranted to assist in clinical decision making.

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