Fine Needle Aspiration: Role of Molecular Testing

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Key Points

- The malignant potential of thyroid nodules is determined through consideration of clinical risk, clinical findings, ultrasonographic features, and cytopathologic results, as classified by the Bethesda System of Reporting Thyroid Cytopathology.
- Cytologically indeterminate lesions yield a wide range of malignant outcomes on histopathology, which can be more precisely predicted using molecular information obtained by FNA.
- Clinically available methods of assessing the genetic profile of indeterminate thyroid nodules have been developed and include immunochemical staining for markers of thyroid cancer, identification of specific molecular

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mutations and microRNAs (markers) of thyroid malignancy, as well as a system which primarly was designed to identify molecular markers of benign lesions.
- Genetic mutation panels currently perform well at identifying indeterminate nodules that are most likely malignant, indicating the need for surgical intervention. As the number of molecular markers is so comprehensive, the absence of these markers is a good negative predictor of malignancy.
- The identification of benign pathology among BSRTC indeterminate nodules is accomplished through a system which provides a high level of accuracy to rule out malignancy, avoiding the need for surgery, while the ability to predict the presence of malignancy is more modest.

Introduction

Fine needle aspiration (FNA) findings are interpreted considering the pretest probability of malignancy. The Bethesda System of Reporting Thyroid Cytopathology (BSRTC [Bethesda will be referred to as B in this chapter]) was introduced to standardize reporting of results and associate an evidence-based risk of malignancy (ROM) for each category. Clinical action is taken based on ROM projections in 70–80% of BSRTC results. Surgical intervention is indicated for B VI lesions and follow-up is generally suggested for B II nodules; however, management is less certain for the 20–30% of cases that are B III (atypia of unknown significance [AUS] or follicular lesion of unknown significance [FLUS], with an estimated ROM 5–47%) and B IV lesions (follicular/Hürthle cell neoplasm [FN] or suspicious for follicular/Hürthle cell neoplasm [SFN], with an estimated ROM of 15–40%). Surgery has usually been carried out in B V lesions (suspicious for malignancy), due to the high ROM (60–75%). When originally

published, repeat FNA was recommended for B III lesions and hemithyroidectomy for B IV nodules. However, up to 70 + % of surgical procedures on B III and IV nodules result in benign pathology, and whereas surgery was previously deemed necessary to detect cancers that could not otherwise be diagnosed, concerns regarding "unnecessary surgery" have arisen, particularly in view of the risk of post-operative complications.

Investigation of the Indeterminate Thyroid Nodule

The pretest probably of malignancy is established by considering individual clinical risk factors, ultrasound and cytology findings (which may lead to adjustment of pretest malignancy risk rates from those originally reported in the BSRTC). To better define the ROM posed by a cytologically indeterminate thyroid nodule, a good test should be accurate, accessible, affordable, and have impact on patient management to "rule out" or "rule in" the presence of thyroid malignancy. A useful additional investigation would then predict the ROM to below 5% or well above the 30 + % range represented by the B III or B IV finding when risk factors are otherwise reassuring. In this context, a "rule-in" test would provide a positive predictive value (PPV) similar to a B VI diagnosis (≅ 96%) and an ideal "rule-out" test would generate a negative predictive value (NPV) of about 3–5%. The American Thyroid Association (ATA) recommends that patients be counseled regarding the potential benefits and limitations of molecular marker testing (no test will be 100%). Current ATA guidelines also recommend that these tests be performed in established, CLIA-certified molecular laboratories for the most consistent clinical reliability.

Several approaches have been investigated to further define the ROM of indeterminate nodules. One technique has used immunochemistry staining of prepared cytopathology specimens using markers of thyroid malignancy ("rule-in" tests with high PPV) such as galectin-3, cytokeratin-19, Hector Battifora mesothelial-1(HBME1), and trophoblast cell surface antigen 2 (TROP2).

Alternatively, one can seek specific molecular markers of thyroid malignancy such as genetic mutations and rearrangements, for example, those that result in activation of the MAPK or PI3K/AKT pathways of malignancy. These include mutations of B-type RAF kinase (BRAF) and retrovirus-associated DNA sequences (RAS), as well as rearrangements of the RET proto-oncogene (a marker of medullary thyroid cancer) with regions of unrelated genes (e.g. RET/PTC), and fusion of the promoter and 5′-coding portion of the thyroid transcription factor PAX8 gene with the gene of the peroxisome proliferator-activated receptor γ (PAX8/PPARγ).

An alternative approach uses molecular techniques designed to identify benign thyroid tissue and provides a high NPV. Such a technique analyzes the mRNA expression of 167 genes in a gene expression classifier (GEC) and its current form, the gene sequencing classifier (GSC).

Immunohistochemical Staining

Immunohistochemical stains for galectin-3 and HBME-1 done on cell block specimens from material obtained by FNA using various methodologies have been reported. Using reverse transcriptase-PCR (RT-PCR), a small study of FNA samples classified as indeterminate was analyzed for the expression of galectin-3 and/or CD44v6 and resulted in a 100% sensitivity and 60% specificity for thyroid cancer. While potentially representing a good "rule-out test," the low specificity, technical difficulty in performing these tests, lack of widespread availability, and small series represent limitations of the clinical utility of this type of testing. Cytokeratin-19 and HBME1 (usually negative in normal thyroid cells) were assessed in a series of 150 FNAs from indeterminate nodules and were reported to be 100% sensitive and 85% specific for the presence of thyroid cancer. Overall, the accuracy of immunohistochemical staining has been limited in follicular cell lesions, particularly for distinguishing benign follicular adenomas from follicular thyroid cancer (FTC) and the follicular variant of papillary thyroid cancer (FVPTC).

Genetic Mutations in Thyroid Nodule Diagnosis

The discovery of molecular pathways driving carcinogenesis of thyroid follicular cell tumors has provided an opportunity to identify thyroid cancer at the molecular level, in order to differentiate benign from malignant B III and B IV nodules. Specific single mutations such as the BRAFV600E mutation have an estimated specificity of about 99% (high PPV) for papillary thyroid cancer (PTC) and the prevalence of this mutation is generally greater than 50% in PTC, though this estimate is population-dependent. In the past, BRAF positivity was viewed as an indication to perform a total thyroidectomy, as it confirms the presence of PTC. This mutation is not or is infrequently found in FTC, FVPTC, and Hürthle cell carcinoma (HCC). The prevalence of this mutation in cytologically indeterminate cases ranges from 0 to 48% (lower rates in Western countries). The MAPK pathway drives several human cancers and is strongly activated by the B-type RAF kinase (BRAF). The sensitivity of the BRAFV600E point mutation is too low, however, to reliably exclude the presence of thyroid malignancy in the assessment of indeterminate thyroid nodules.

Retrovirus-associated DNA sequences (RAS) point mutations constitute the second most frequently encountered (0–36%) finding in thyroid malignancy discovered among indeterminate BSRTC results. The 3 RAS subtypes are associated with follicular-patterned histology such as follicular adenoma (FA), FTC, FVPTC, and non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Gene mutations identified in the oncogenic Harvey rat sarcoma (HRAS) predict the presence of malignancy in 56% of cases, while mutations of the Kirsten murine sarcoma virus (KRAS) and neuroblastoma cells (NRAS) have been reported to be 100% and 74% predictive, respectively. Each encodes for RAS proteins that are involved in signaling in the MAPK/ERK pathway. Mutations in the RAS genes result in overactive RAS signaling, inducing malignant growth. In general, the presence of RAS mutations predicts thyroid cancer in more than 80% of cases, though generally with favorable clinical features, such as encapsulation and a paucity of lymph node metastases, for which hemithyroidectomy may be

considered as a therapeutic intervention. The remaining 16 + % represent benign lesions such as FA (which has been considered pre-malignant by some). Conversely, poor cell differentiation may be present in some, more aggressive cancers. Because the RAS mutations are not exclusive to thyroid malignancy, RAS positivity (unlike BRAFV600E) does not predict malignancy with high accuracy. Approximately 1/3 of malignancies in subjects with indeterminate cytopathology findings (FVPTC, FTC) are RAS-mutated.

Genetic Translocations

Genetic translocation products, such as the Ret proto-oncogene rearrangements (RET/PTC), activate the MAPK and PI3K/AKT pathways through BRAF signaling, resulting in malignant transformation. Of the 12+ fusion variants identified, RET/PTC1 and RET/PTC3 are the most common. RET/PTC3 occurs in PTC in children and those exposed to irradiation and is associated with lymph node metastases. Benign adenomas that are positive for RET/PTC and occur after irradiation are considered pre-cancerous. RET/PTC rearrangements are seldom reported in most series of indeterminate nodules; however, one Italian series reported a 36% incidence in this setting. As such RET/PTC has low utility as a stand-alone test, but is most useful when included in a panel of molecular tests.

Rearrangements of the PAX8 and PPARγ (PAX8/PPARγ) genetic material have been detected in up to 45% of FTC, 33% of FA's, and up to 38% of FVPTC, but have not been routinely observed in Hürthle cell lesions. Some have reported that the presence of this fusion is associated with malignancy. In general, the PAX8/PPARγ fusion predicts encapsulated, indolent lesions and does not activate the MAPK pathway. PAX8/PPARγ does not occur frequently in cytologically indeterminate nodules. Approximately 2/3 of PAX8/PPARγ-positive nodules are malignant (FVPTC, FTC). Benign lesions known to be PAX8/PPARγ positive are also considered pre-malignant.

Other Genetic Anomalies of Interest

Special attention should be paid to another genetic alteration that has the potential to enhance prognostication of thyroid nodules. Telomerase enzymatically maintains healthy chromosomal telomeres. Telomerase reverse transcriptase (hTERT) is inactive in normal cells, but when hTERT promoter mutations are present, reactivation results in malignant behavior of thyroid cells. TERT promoter mutations have been described in PTC, FTC, and HCC. TERT promoter mutations are seen with higher positivity in more aggressive variants of thyroid cancer, such as poorly differentiated and anaplastic (70%) cancers. The presence of the TERT promoter mutation is highly correlated with mortality in differentiated thyroid cancer, especially when present in conjunction with BRAFV600E. TERT is potentially useful in the preoperative identification of differentiated thyroid cancer (DTC), with reported sensitivity and specificity rates of 57–88% and 75–85%, respectively.

Other genetic alterations observed in thyroid cancers include the eukaryotic translation initiation factor 1A, X chromosomal (EIF1AX), found in low percentages of PTC and FTC. The presence of this mutation in poorly differentiated thyroid cancers, however, is associated with poorer clinical outcomes. A gene normally encoding endoribonucleases (DICER1) has been found to be mutated in germline and somatic pediatric thyroid cancers (FTC and PTC). This mutation is usually reported in the context of a hereditary pediatric cancer predisposition syndrome, which includes pleuro-pulmonary blastoma, ovarian sex cord-stromal tumors, lung cysts, cystic nephroma, renal sarcoma and Wilms' tumor, and other lesions. In adults, the presence of a germline DICER1 mutation seems to be a risk factor for renal sarcoma, Wilms tumor, and other lesions and is associated with having had a thyroidectomy for reasons other than thyroid cancer or goiter.

Alterations in a gene encoding adenylate cyclase-stimulating G α-protein at codon 201 (GNAS) may result in activation of the cyclic AMP process affecting cell proliferation and function. GNAS alterations are more frequently found in hyperfunctioning adenomas of the thyroid, but may also be present in FTC. Long

known mutations in RET (RET proto-oncogene) may be detected by FNA specimens in medullary thyroid cancer.

Genetic Mutation Panels

A functional strategy toward the search for molecular markers to include multiple mutations/translocations including BRAFV600E, BRAFK601E, NRAS codon 61, HRAS codon 61, KRAS codon 12–13 point mutations, along with RET/PTC1, RET/PTC3, and PAX/PPARγ gene rearrangements, is designed to assess the malignant potential of indeterminate nodules more comprehensively (including newly described pre-malignant lesions such as NIFTP [see below]). This approach enhances the power of detection, given that these mutations are generally mutually exclusive. Usually, the presence of one characteristic mutation among those in the panel is a significant indicator of malignancy. In B III cases that are positive for BRAF, RET/PTC, or PAX8/PPARγ, a malignancy has been reported in all cases, while some RAS mutations may point to cancer in 84% of cases in this same series. In studies of B IV lesions, where a 15–30% risk of malignancy is assumed to exist, this seven-gene mutational panel was demonstrated to have a 57–75% sensitivity for malignancy, a specificity of 97–100%, PPV of 87–100% (highlighting its usefulness as a "rule-in" test), and a NPV of 79–86%. However, nodules lacking all 7 of these markers still harbored a significant risk of malignancy, limiting its utility as a "rule-out" test. In one study of these panels, only 1 of 18 nodules with Hürthle cell cytology tested positive, likely providing early evidence that the genetic basis of Hürthle cell lesions differs from that of other types of DTC. Additionally, the PPV of these panels is unlikely to ever exceed 90%, as RAS mutations trigger positive results and the prevalence of malignancy of populations studied has been 15–40%. A limitation to the accuracy of PPV and NPV calculations is the fact that histology data is not available for all nodules characterized as "not malignant" in these panels, rendering a precise calculation of false negatives impossible. Extending a 7-gene panel to include additional markers observed in thyroid cancer

and processed through next-generation sequencing (NGS) allows targeted testing of large panels with multiple mutations.

ThyroSeq®, now in version 3 (v3), analyzes 112 genes for point mutations, insertions, deletions, copy number alterations, fusions, and gene expression alterations, accounting for about 95% of genetic alterations known to occur in PTC, as well as including TERT promoter variants (See Table 5.1). Evaluation of B IV lesions yields predictably higher sensitivity (90%), but lower specificity (83%) and PPV (83%) while reportedly increasing the power to rule out the presence of cancer (NPV 96%). ThyroSeq v3 was assessed in a multi-institutional study of 286 indeterminate nodules: 72% were classified as benign, and a total of 28% (including NIFTP: 14% of the total, which likely impacted clinical management and calculation of PPV) were classified as malignant. A sensitivity of 94%, specificity of 82%, and NPV of 97% were observed, while the PPV for malignancy was 66%. ROM of positive tests ranged from 50 to 100% depending on the pattern of genetic abnormalities identified. An independent assessment of ThyroSeq v3 performance in 415 B III and B IV qualifying nodules resulted in a total of 121 positive (29%) and 294 negative (71%) results. Of ThyroSeq v3-positive cases with histopatho-

Table 5.1 Methods employed in molecular testing of indeterminate thyroid nodules

Method	Diagnostic utility	Markers of malignancy
Immunocytochemistry	"Rule-in" tests with high PPV	Proteins detected—Galectin-3, CytoKeratin, HBME1, TROP2
Molecular marker (mutation panel) testing	"Rule-in" tests with high PPV and in the absence of markers, good NPV	Mutations—BRAF, EIF1AX, DICER1, GNAS, RAS, RET proto-oncogene, hTERT Rearrangements—RET/PTC, PAX8/PPARγ
GEC (no longer available), GSC	Identification of benign thyroid tissue	Additional gene mutation/rearrangment expression available

See text for detail. *NPV* negative predictive value, *PPV* positive predictive value, *GEC* gene expression classifier, *GSC* gene sequencing classifier

logic confirmation, 32% were benign and 51% were malignant (NIFTP: 17% of malignant nodules). Assuming that those with negative ThyroSeq v3 results were indeed true-negatives (a common assumption among studies of this type), an overall sensitivity of 92.9% and specificity of 90.3% were observed. The NPV was 98.3% and PPV 67.7%, validating the "rule-out test" characteristics necessary to reassure patients. The performance of ThyroSeq has recently been assessed in practice in cases for which surgical pathology results were available and was found to perform better in larger thyroid nodules (<2 cm: PPV 25%; NPV 79% versus >4 cm: PPV 50%; NPV 89%).

MicroRNA (miRNA) Analysis

MicroRNA (miRNA) markers have been evaluated in nodules with indeterminate cytology. MicroRNAs are small, endogenous non-coding RNAs about 22 nucleotides in length that play a role in the regulation of posttranscriptional protein synthesis. Human cancers are associated with dysregulation of miRNA expression and these miRNAs appear as a pre-malignant event before deregulating tumor suppressor and oncogenes. These miRNAs are more stable than mRNA, may be detected in the circulation, and maintain their expression in formalin-fixed tissue and FNA samples, making retrieval from cytopathology slides possible. Studies have found that different miRNAs and levels of expression are of clinical utility in the identification of malignancy. For example, in PTC, there is a reported up-regulation of miR-146b, miR-221, miR-222, and miR-187 and a down-regulation of miR-1 and miR-138. Additionally, FTC, poorly differentiated, and anaplastic thyroid cancers are also associated with up-regulation of miR-221, miR-222, and miR-187. The degree of overexpression may also be of utility, as FVPTC appears to have twice the expression of miR-221 and miR-222 than PTC and FTC. Based on these and further characteristic variances in expression, diagnostic panels of miRNAs have been combined to evaluate the malignant potential of thyroid nodules with variable degrees of success. A commercial product evaluating 24 up- and down-regulated miRNAs

(RosettaGX Reveal®) using routinely stained cytology slide samples was reported to have a 90% PPV but only a 39% NPV, limiting its clinical utility.

Mutation and miRNA (Multiplatform) Analysis Combined

Combined sequential analysis of a gene mutational panel with an miRNA classifier if no mutations are identified has demonstrated promising results for both "rule in" and "rule out" purposes. A commercial product combining the sequencing of 8 genes (ThyGenX®) and 10 miRNAs (ThyraMIR®) reported a sensitivity of 94%, specificity of 85%, PPV of 68%, and a NPV of 97% in B III lesions. The most recent configuration includes an expanded gene panel (ThyGeNEXT®) with the ThyraMIR panel and has been reported to have a PPV of 75% and NPV of 97%.

Identification of Benign Nodule Characteristics

When first introduced, the 167 Afirma® gene expression classifier (GEC [a historical term, as this version is no longer in clinical use]) used quantification of messenger RNA (mRNA) run through a proprietary algorithm to differentiate benign from potentially malignant ("suspicious") thyroid nodules. The GEC was processed on material obtained by 2 additional FNA passes and was introduced as a "rule-out" test based on an initial reported 92% sensitivity and NPV of 93%. In one study, this resulted in a reduction of malignancy risk from 24% in AUS/FLUS lesions to 5%. In cases where surgical pathology was available, the relatively low specificity for predicting the presence of malignancy in indeterminate nodules (48–53%), however, was acknowledged to be a limitation to use as a "rule-in" test. The reporting protocol indicated that the results were either "likely benign," as reflected by the high NPV or designated as "suspicious." Some misinterpreted these reports to equate to the 7-gene mutation panel reporting nomenclature, which was reported as "positive" (for malignancy), an indication for total

thyroidectomy, or "negative," where a hemithyroidectomy was suggested as initial management due to the low NPV of the limited mutation panel. Performance of the GEC in those with B IV lesions in general showed a higher NPV (94%) but much more modest PPV (37%). Studies subsequently documented a limitation of the GEC in Hürthle cell lesions, where a "suspicious" result was often associated with benign final histopathology, although the NPV of the GEC was retained. Since 2018 the Afirma® system has been based upon next-generation sequencing (NGS), analyzing nuclear and mitochondrial RNA transcriptome gene expression, RNA sequencing, and genomic copy number analysis. This version is designated as the gene sequencing classifier (GSC) and incorporates BRAFV600E, RET/PTC fusion, parathyroid tissue, and medullary thyroid cancer (MCT) markers. The GSC was validated on a subset of the original pivotal GEC data set and demonstrated a sensitivity of 91% and specificity of 68% in a population with a 24% cancer prevalence. The NPV was 96% and PPV was 47%. Independent, real world evaluation from several institutions comparing the GSC performance with that of the original GEC configuration has led to a decrease in suspicious results by 21–54%, classification of more Hürthle cell lesions as benign, and a higher PPV of 57.1–61.5% (with a higher PPV in B IV vs. B III). Currently, the Afirma® system also offers optional testing for a limited number of specific driver mutations (as included in the mutation panels described above) when overall results are designated as suspicious. Use of this optional marker panel has enhanced preoperative PPV and surgical planning when specific markers are present. Meta-analysis of 4 independent studies evaluating the clinical performance of the GSC indicates an overall sensitivity of 95%, specificity of 51%, PPV of 60%, and NPV of 91%.

Genetic Testing and NIFTP Lesions

The performance of genetic testing in the identification of lesions diagnosed as NIFTP, an entity considered to have low malignant potential, is limited. Although the initial series of 109 cases of NIFTP reported no adverse clinical outcomes, subsequent studies have

reported lymph node (5%) and lung metastases (1%) in the follow-up of patients who had histologically classified NIFTP. Cytopathology findings are typically indeterminate or suggestive of PTC. As the definition of these lesions is based on presence or absence of invasion, only surgical histology is currently sufficient to establish this diagnosis. The BSRTC acknowledges that B III, IV, and V categories may all be seen in cases diagnosed as NIFTP. In the calculation of PPV, NIFTP lesions are considered by some as "malignant" in preoperative testing. As such, the PPV of malignancy in various systems is enhanced when NIFTP is present. Some consider such a designation a false positive. Although surgical removal of NIFTP lesions is appropriate due to NIFTP's potential pre-malignant nature, some have (retrospectively) opined that surgery may be overly aggressive for this final diagnosis. In the past, a total rather than limited (hemi) thyroidectomy was recommended for confirmed malignancy. Currently, as aggressive surgery or I-131 therapy is not indicated for NIFTP, excessive surgical intervention is generally to be avoided, though given the limitations of preoperative diagnosis and some challenges in making this pathology diagnosis, for example, in the distinction from FVPTC, an individualized management approach is needed, particularly in the case of larger nodules (>4 cm).

Genetic alterations detected in the evaluation of the indeterminate thyroid nodule differ depending on the approach taken. The presence of BRAF or RET/PTC would not generally support the diagnosis of NIFTP preoperatively (in fact the presence of BRAFV600E mutation is an exclusion criterion for the diagnosis of NIFTP). Other "positive" indicators of cancer, such as RAS mutations, occur more frequently in NIFTP. In addition, PAX8-PPARγ, THADA fusions and EIF1AX mutations may be found in NIFTP. In a similar way, a proportion of indeterminate nodules labeled as "suspicious" in the Afirma® GEC as well as GSC system have been found to be NIFTP.

Given these findings, repeat FNA of cytologically indeterminate nodules prior to molecular testing and following a conservative pathway to initial surgical intervention or, alternatively, clinical observation, seems prudent.

Table 5.2 summarizes the currently available commercial panels, their performance and method of collection.

Table 5.2 Comparison of commercial panels, their performance and the method of collection

Commercial panel	Sensitivity	Specificity	PPV	NPV	Reference	Collection
ThyroSeq v3™ (genomic classifier)	94%	82%	66%	97%	[1]	Rinsing residual FNA
NGS, 112 genes, point mutations	93%	90%	68%	98%	[2][a]	1 dedicated pass
Gene fusions, copy # alterations	99%	64%	78%	96%	[3][b]	
Gene expression alterations						
ThyGenX/ThyraMIR®	57%	92%	74%	94%	[4, 5]	Fixed, stained slides
NGS, 7 gene mutations	Due to limited data no meta-analysis estimation				[3][b]	
3 Gene fusions and 10 miRNAs						
Afirma GSC™	91%	68%	47%	96%	[6]	2 separate passes
NGS, RNA transcriptome	94%	61%	33%	98%	[7][a]	
Expression, specific gene	100%	73.7	62%	NR	[8][a]	
Mutations available	95%	51%	60%	91%	[3][b]	

[1] Steward DL, Carty SE, Sippel RS, Yang SP, Sosa JA, Sipos JA, et al. Performance of a multigene genomic classifier in thyroid nodules with indeterminate cytology: a prospective blinded multicenter study. JAMA Oncol. 2019;5(2):204–12.
[2] Desai D, Lepe M, Baloch ZW, Mandel SJ. ThyroSeq v3 for Bethesda III and IV: an institutional experience. Cancer Cytopathol. 2021;129(2):164–70.

[3] Silaghi CA, Lozovanu V, Silaghi H, Georgescu RD, Pop C, Dobrean A, et al. The Prognostic value of microRNAs in thyroid cancers—a systematic review and meta-analysis. Cancers (Basel). 2020;12(9).
[4] Ullmann TM, Gray KD, Moore MD, Zarnegar R, Fahey TJ 3rd. Current controversies and future directions in the diagnosis and management of differentiated thyroid cancers. Gland Surg. 2018;7(5):473–86.
[5] de Koster EJ, de Geus-Oei LF, Dekkers OM, van Engen-van Grunsven I, Hamming J, Corssmit EPM, et al. Diagnostic utility of molecular and imaging biomarkers in cytological indeterminate thyroid nodules. Endocr Rev 2018;39(2):154–191.
[6] Patel KN, Angell TE, Babiarz J, Barth NM, Blevins T, Duh QY, et al. Performance of a genomic sequencing classifier for the preoperative diagnosis of cytologically indeterminate thyroid nodules. JAMA Surg. 2018;153(9):817–24.
[7] Endo M, Nabhan F, Porter K, Roll K, Shirley LA, Azaryan I, et al. Afirma gene sequencing classifier compared with gene expression classifier in indeterminate thyroid nodules. Thyroid. 2019;29(8):1115–24.
[8] Gortakowski M, Feghali K, Osakwe IN. Single institution experience with Afirma and Thyroseq testing in indeterminate thyroid nodules. Thyroid. 2021;31:1376.
[a]Real world confirmation
[b]Meta-analysis
NGS next generation sequencing, *GSC* gene sequencing classifier

Initial Intervention Based on Molecular Results

The high PPV of mutation panels with a positive test result has been used to identify thyroid nodules with a high risk of malignancy. At the time of the original BSRTC report, total thyroidectomy was recommended by the ATA for all thyroid malignancies greater than 1 cm. As such, positive results were used to plan total thyroidectomy for >1 cm nodules that were identified as malignant. More recent guidelines and current practice, however, recommend hemithyroidectomy for unifocal lesions that are less than 4 cm with no clinical or ultrasound evidence of local invasion or metastases that are suspected of being malignant. This is based on the observation that specific tumor types (including minimally invasive FTC, FVPTC, and NIFTP, which may be molecularly identified as positive by these methods) have an observed clinical course that does not justify aggressive surgery and post-operative 131-I ablation, given a less favorable risk-benefit profile for total thyroidectomy. Any decision to recommend surgery, including hemithyroidectomy, however, is not without long-term consequences, as up to 43% of patients may develop hypothyroidism post-operatively. Ongoing investigation into how molecular information may guide preoperative planning and modify the subsequent clinical course of DTC is expected to further enhance the management of patients with indeterminate thyroid nodules (with the potential exception of finding a clinically unexpected medullary thyroid cancer). Figure 5.1 shows a suggested management approach for the incorporation of molecular testing in the evaluation of indeterminate thyroid nodules.

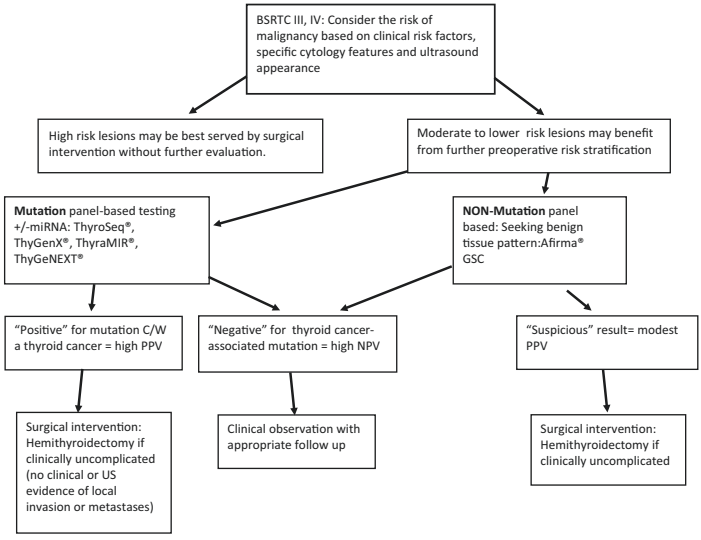

Fig. 5.1 Molecular testing of the indeterminate thyroid nodule

Further Reading

de Koster EJ, de Geus-Oei LF, Dekkers OM, van Grunsven IVE, Hamming J, Corssmit EPM, et al. Diagnostic utility of molecular and imaging biomarkers in cytological indeterminate thyroid nodules. Endocr Rev. 2018;39(2):154–91.

Desai D, Lepe M, Baloch ZW, Mandel SJ. ThyroSeq v3 for Bethesda III and IV: an institutional experience. Cancer Cytopathol. 2021;129(2):164–70.

Endo M, Nabhan F, Porter K, Roll K, Shirley LA, Azaryan I, et al. Afirma gene sequencing classifier compared with gene expression classifier in indeterminate thyroid nodules. Thyroid. 2019;29(8):1115–24.

Gortakowski M, Feghali K, Osakwe IN. Single institution experience with Afirma and Thyroseq testing in indeterminate thyroid nodules. Thyroid. 2021;31:1376.

Grani G, Sponziello M, Pecce V, Ramundo V, Durante C. Contemporary thyroid nodule evaluation and management. J Clin Endocrinol Metab. 2020;105(9):2869.

Kumar N, Gupta R, Gupta S. Molecular testing in diagnosis of indeterminate thyroid cytology: trends and drivers. Diagn Cytopathol. 2020;48(11):1144–51.

Patel KN, Angell TE, Babiarz J, Barth NM, Blevins T, Duh QY, et al. Performance of a genomic sequencing classifier for the preoperative diagnosis of Cytologically indeterminate thyroid nodules. JAMA Surg. 2018;153(9):817–24.

Sliaghi CA, Lozovanu V, Georgescu CE, Geogescu RD, Susman S, Nasui BA, et al. Thyroseq v3, Afirma GSC and microRNA panels versus previous molecular tests in the preoperative diagnosis of indeterminate thyroid nodules: a systematic review and meta-analysis. Front Endocrinol. 2021;12(649522):19.

Steward DL, Carty SE, Sippel RS, Yang SP, Sosa JA, Sipos JA, et al. Performance of a multigene genomic classifier in thyroid nodules with indeterminate cytology: a prospective blinded multicenter study. JAMA Oncol. 2019;5(2):204–12.

Ullmann TM, Gray KD, Moore MD, Zarnegar R, Fahey TJ 3rd. Current controversies and future directions in the diagnosis and management of differentiated thyroid cancers. Gland Surg. 2018;7(5):473–86.