Medical Policy Manual Genetic Testing, Policy No. 49

Molecular Markers in Fine Needle Aspirates of the Thyroid

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Next Review: April 2019
Last Review: June 2018

IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

Molecular markers, gene expression tests and mutation analyses have been developed to help improve the diagnostic accuracy of indeterminate thyroid nodule cytology results and avoid unnecessary surgical resection.

MEDICAL POLICY CRITERIA

I. Gene expression classifiers, genetic variant analysis, and molecular marker testing in fine-needle aspirates of the thyroid may be considered **medically necessary** when any of the following criteria are met:

   A. Use of either the Afirma® Gene Expression Classifier (including *BRAF* and MTC reflex testing) or the ThyroSeq® Genomic Classifier when all of the following criteria (1-3) are met:

      1. Patients greater than or equal to 21 years of age; and
      2. Thyroid nodule greater than or equal to 1 cm; and
      3. Fine-needle aspirate samples from thyroid nodules that have indeterminate cytology as indicated by any of the following conditions:

a. Bethesda diagnostic category III, i.e., Atypia of undetermined significance/Follicular lesion of undetermined significance (AUS/FLUS); *or*

b. Bethesda diagnostic category IV, i.e., follicular neoplasm or suspicious for a follicular neoplasm; *or*

c. Follicular or Hürthle cell neoplasm.

B. Use of ThyroSeq®, ThyraMIR™, or ThyGenX® with or without ThyraMIR™, when all of the following criteria (1-3) are met:

1. Patients greater than or equal to 21 years of age; and
2. Thyroid nodule greater than or equal to 1 cm; and
3. Fine-needle aspirate samples from thyroid nodules that have indeterminate *or* suspicious cytology as indicated by any of the following conditions:

a. Bethesda diagnostic category III, i.e., Atypia of undetermined significance/Follicular lesion of undetermined significance (AUS/FLUS); or

b. Bethesda diagnostic category IV, i.e., follicular neoplasm or suspicious for a follicular neoplasm; or

c. Hürthle cell neoplasm; or

d. Bethesda diagnostic category V, i.e., suspicious for malignancy.

II. Gene expression classifiers, genetic variant analysis, and molecular marker testing in fine-needle aspirates of the thyroid, including but not limited to RosettaGX Reveal™ are considered **investigational** when the above criteria are not met.

NOTE: A summary of the supporting rationale for the policy criteria is at the end of the policy.

POLICY GUIDELINES

SUBMISSION OF DOCUMENTATION

In order to determine the clinical utility of gene test(s), all of the following information must be submitted for review. If any of these items are not submitted, it could impact our review and decision outcome:

- Name of the genetic test(s) or panel test
- Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
- The exact gene(s) and/or variant(s) being tested
- Relevant billing codes
- Brief description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing
- Medical records related to this genetic test:
    - History and physical/chart notes
    - Conventional testing and outcomes
    - Thyroid nodule size and cytology results

THYGENX® AND THYRAMIR™ COMBINATION TESTING

ThyGenX® is intended to be used in conjunction with the ThyraMIR™ microRNA expression test when the initial ThyGenX™ test is negative. Criterion I.B. is applicable to testing either test or both tests in combination.

CROSS REFERENCES

1. Genetic and Molecular Diagnostic Testing, Genetic Testing, Policy No. 20

BACKGROUND

BACKGROUND

Thyroid Nodules and Cancer

Thyroid nodules are solid or fluid-filled lumps that form within the thyroid gland. Thyroid nodules are common and present in 5% to 7% of the U.S. population. The vast majority of these nodules are benign, and most cases of thyroid cancer are curable by surgery if detected early; however, about 4% to 10% of thyroid nodules are deemed cytologically malignant.

Thyroid cancer accounts for 3.1% of all new cancer cases each year in the United States.[1] The most common type of thyroid cancers include well-differentiated papillary thyroid carcinoma (PTC), which accounts for nearly 80%, and follicular cell carcinoma, which accounts for about 10% of all thyroid cancers.[2] Poorly differentiated and anaplastic thyroid carcinomas are uncommon and can arise *de novo* or from preexisting well-differentiated papillary or follicular carcinomas. Medullary thyroid carcinoma originates from parafollicular or C cells and accounts for about 3% of all thyroid cancers.

Fine Needle Aspiration of the Thyroid

Fine needle aspiration (FNA) of the thyroid is used to obtain cells to distinguish between benign and malignant thyroid nodules. FNA uses a fine needle to biopsy the cells of the thyroid nodule for cytological examination. About 60-70% of thyroid nodules are classified cytologically as benign, and 4-10% of nodules are cytologically deemed malignant using The Bethesda System for Reporting Thyroid Cytopathology.[3,4] However, the remaining 20-30% have equivocal findings (inclusive, indeterminate, atypical, or suspicious), usually due to overlapping cytologic features between benign and malignant nodules. Historically, approximately 80% of patients with indeterminate cytology undergo surgical resection and postoperative evaluation most often revealed a malignancy rate ranging from 6-30%, making this clinical process one with very low specificity.[5]

Preoperative planning of optimal surgical management in patients with equivocal cytologic results is challenging, as different thyroid malignancies may require different surgical procedures (e.g. unilateral lobectomy versus total or sub-total thyroidectomy with or without lymph node dissection) depending on several factors, including histologic subtype and risk-stratification strategies (tumor size, patient age, etc.) If a diagnosis cannot be made intraoperatively, a lobectomy is typically performed, and if on postoperative histology the lesion is malignant, a second surgical intervention may be necessary for completion thyroidectomy.

If a case is indeterminate, surgical biopsy with intraoperative consultation is most often

diagnostic, although its efficacy and therefore use will vary between institutions, surgeons, and pathologists. For follicular carcinoma, the presence of invasion of the tumor capsule or of blood vessels is diagnostic and cannot be determined by cytology, as tissue sampling is necessary to observe these histologic characteristics. Intraoperative diagnosis of follicular carcinoma is challenging and often not feasible, as extensive sampling of the tumor and capsule is usually necessary and performed on postoperative permanent sections.

New approaches for improving the diagnostic accuracy of thyroid FNA include molecular analysis for somatic genetic alterations, in order to more accurately classify which patients need to proceed to surgery, and may include the extent of surgery necessary, versus those patients who do not need surgery and can be safely followed.

Molecular Markers Associated with Thyroid Cancer

Various molecular markers have been discovered in thyroid cancer. The four gene mutations that are the most common and carry the highest impact on tumor diagnosis and prognosis are *BRAF* and *RAS* point mutations and *RET*/*PTC* and *PAX8*/*PPARγ* rearrangements.

Papillary carcinomas carry point mutations of the *BRAF* and *RAS* genes as well as *RET/PTC* and *TRK* rearrangements, all of which are able to activate the mitogen-activated protein kinase (MAPK) pathway.[6] These mutually exclusive mutations are found in more than 70% of papillary carcinomas.[6] *BRAF* mutations are highly specific for *PTC*. Follicular carcinomas harbor either *RAS* mutations or *PAX8/PPARγ* rearrangement. These mutations are also mutually exclusive and identified in 70-75% of follicular carcinomas.[6] Genetic alterations involving the *PI3K/AKT* signaling pathway also occur in thyroid tumors, although they are rare in well-differentiated thyroid cancer and have higher prevalence in less differentiated thyroid carcinomas.[6] Additional mutations known to occur in poorly differentiated and anaplastic carcinomas involve the *TP53* and *CTNNB1* genes. Medullary carcinomas, which can be familial or sporadic, frequently possess point mutations located in the *RET* gene.

Available Molecular Diagnostic Testing

Variant Detection and Rearrangement Testing

Mutation analysis testing examines specific genes, which often include *BRAF*, *RAS*, and *RET*, and evaluates them for rearrangements which could be associated with thyroid cancers. Mutation analysis testing can be analyzed using Sanger sequencing, pyrosequencing, or by using real-time polymerase chain reaction (rtPCR). Panels of tests for mutations associated with thyroid cancer are also available. For example, Quest Diagnostics offers a Thyroid Cancer Mutation Panel, which includes *BRAF* and *RAS* mutation analysis and testing for *RET/PTC* and *PAX8/PPARγ* rearrangements. In addition to standard Sanger sequencing or rtPCR-based mutation testing for genes associated with thyroid cancer, next-generation sequencing (NGS) panels that simultaneously evaluate for point mutations or gene fusions in multiple genes have been developed. For example, the ThyroSeq® v.2 Next Generation Sequencing panel (CBLPath) includes sequencing of more than 60 genes.

According to the ThyroSeq®'s manufacturer's website, the test is indicated when FNA cytology indicates atypia of uncertain significance or follicular lesion of undetermined significance, follicular neoplasm or suspicious for follicular neoplasm, or suspicious for malignancy. In particular, it has been evaluated in patients with follicular neoplasm/suspicious for follicular neoplasm on FNA as a test to increase both sensitivity and specificity for cancer

diagnosis.

The ThyGenX™ Thyroid Oncogene Panel (formerly miRInform® Thyroid; Interpace Diagnostics; testing done at Asuragen Clinical Laboratory) is another NGS sequencing panel designed to be used in patients with indeterminate thyroid FNA results. It includes sequencing of eight genes associated with papillary thyroid carcinoma and follicular carcinomas (*KRAS, BRAF, HRAS, NRAS, RET/PTC1, RET/PTC3, PIK3CA, and PAX8/PPARγ*). ThyGenX™ is intended to be used in conjunction with the ThyraMIR™ microRNA expression test when the initial ThyGenX™ test is negative.

Gene Expression Classifier

Genetic alterations associated with thyroid cancer can be assessed through the use of gene expression profiling, which refers to analysis of messenger RNA (mRNA) expression levels of many genes simultaneously using microarray analysis. There are two gene expression profiling tests are now available to biologically stratify tissue from thyroid nodules: Afirma® and ThyraMIR™.

Afirma®

The Afirma® Gene Expression Classifier (Afirma® GEC; Veracyte) is a proprietary diagnostic test that analyzes the expression of 167-gene mRNA expressions to determine patterns associated with benign findings on surgical biopsy. It is designed to be used for thyroid nodules that have an “indeterminate” cytologic classification on FNA as a method to select patients who are at low risk for cancer (“rule out”).

Veracyte also markets two “malignancy classifiers” that use mRNA expression-based classification to evaluate for BRAF mutations or mutations associated with medullary thyroid carcinoma (Afirma® BRAF, and Afirma® MTC, respectively). In a description of the generation of the Afirma® BRAF test, the authors outline the following proposed benefits of the mRNA-based expression test for BRAF mutations: 1) PCR based methods may have low sensitivity, requiring that a large proportion of the nodule have a relevant mutation; 2) testing for only one mutation may not detect patients with low-frequency mutations that result in the same pattern of pathway activation; and 3) PCR-based approaches with high analytic sensitivity may require a large of among of DNA that is difficult to isolate from small FNA samples.[5] The Afirma® MTC is included when the Afirma® GEC is ordered for thyroid nodules with an “intermediate” classification on FNA, and can also be used for thyroid nodules with “malignant” or “suspicious” results on Afirma® GEC. The Afirma® BRAF is designed to be used for nodules with “suspicious” results on Afirma® GEC.

ThyraMIR™

ThyraMIR™ is a seven gene panel with a gene expression classifier involving 10 microRNAs: miR-29-b-1-5p, miR-31-5p, miR-138-1-3p, miR-139-5p, miR-146b-5p, miR-155, miR204-5p, miR-222-3p, miR-375, and miR-551b-3p. Similar in concept to the Afirma® GEC, the microRNA classifier provides a "positive" or "negative" results based on an algorithm trained on the microRNA expression profiles of histologically benign and malignant reference thyroid nodules. Interpace Diagnostics offers the ThyraMIR™ microRNA expression classifier as a reflex test on aspirates that are negative for the seven gene ThyGenX mutation analysis panel.

REGULATORY STATUS

Commercially available, laboratory-developed tests are regulated under the Clinical Laboratory Improvement Amendments (CLIA). Premarket approval from the U.S. Food and Drug Administration (FDA) is not required when the assay is performed in a laboratory that is licensed by CLIA for high-complexity testing.

EVIDENCE SUMMARY

Human Genome Variation Society (HGVS) nomenclature[7] is used to describe variants found in DNA and serves as an international standard. It is being implemented for genetic testing medical evidence review updates starting in 2017. According to this nomenclature, the term "variant" is used to describe a change in a DNA or protein sequence, replacing previously-used terms, such as "mutation." Pathogenic variants are variants associated with disease, while benign variants are not. The majority of genetic changes have unknown effects on human health, and these are referred to as variants of uncertain significance.

Validation of the clinical use of any genetic test focuses on three main principles:

1. The analytic validity of the test, which refers to the technical accuracy of the test in detecting a mutation that is present or in excluding a mutation that is absent;
2. The clinical validity of the test, which refers to the diagnostic performance of the test (sensitivity, specificity, positive and negative predictive values) in detecting clinical disease; and
3. The clinical utility of the test, i.e., how the results of the diagnostic test will be used to change management of the patient and whether these changes in management lead to clinically important improvements in health outcomes.

The literature on the use of molecular markers to characterize thyroid nodules diagnosed by fine needle aspiration (FNA) as indeterminate, atypical, or suspicious has been evaluated in a number of studies. Typically, FNA diagnoses are defined as either nondiagnostic, benign, atypia of undetermined significance (AUS/FLUS), or suspicious for follicular neoplasm (SFN), suspicious for malignancy (SFM), or malignant. Many studies have analyzed using the GEC or point mutation analysis in FNAs, and compared the preoperative cytologic diagnosis and mutation status to postoperative final histologic diagnosis to determine diagnostic accuracy of the presence of a mutation to predict the presence of malignancy. The remaining studies analyzed the clinical utility of the GEC (e.g. Afirma®), to determine how the results affected provider decision making about surgical resection thyroid.

MOLECULAR MARKERS TO PREDICT MALIGNANCY

Analytic Validity

Variant Detection and Rearrangement Testing

Point mutations in specific genes associated with thyroid cancer, such as the BRAF V600E gene, and the detection of genetic rearrangements associated with thyroid cancer, such as the RET/PTC rearrangement, are typically detected with real-time PCR (rtPCR) sequencing methods. In the case of mutation testing for genes associated with thyroid cancer malignancy, analytic validity refers to a test's technical accuracy in detecting a mutation that is present or in excluding a mutation that is absent. Generally, rtPCR-based methods are considered to have high accuracy. For example, Smith reported technical performance characteristics for

BRAF mutation detection by qualitative PCR in thyroid FNA samples with high within- and between-run reproducibility.[8]

Next-generation sequencing (NGS) is expected to have high accuracy for detecting a mutation that is present. However, with increasing numbers of tested mutations, there is increased risk of detection of variants of uncertain significance (VUS). The VUS rate for currently-available NGS panels for thyroid cancer is not well-characterized. Nikiforova described the development and validation of a multigene NGS panel for thyroid cancer, the ThyroSeq® panel.[9] The authors developed a custom library of gene sequence variants based on mutations previously reported in the literature. The assay demonstrated 100% accuracy in evaluating samples of 15 thyroid tumors and three cell lines with known genetic alterations and 15 DNA samples with no mutations. In analysis of 229 DNA samples from frozen tissues, formalin-fixed, paraffin-embedded tissues, and FNAs (n=105, 72, and 52, respectively), the panel identified mutations in 19 of 27 (70%) of classic papillary thyroid carcinomas (PTCs), 25 of 30 (83%) follicular variant PTCs, 14 of 18 (78%) conventional and 7 of 18 (39%) Hürthle cell carcinomas, three of 10 (30%) poorly differentiated carcinomas, 20 of 27 (74%) anaplastic thyroid carcinomas, and 11 of 15 (73%) medullary thyroid carcinomas. Of 83 benign nodules, five (6%) were positive for mutations.

Beaudenon-Huibregtset reported the results of a prospective evaluation of an NGS panel that evaluated for 14 single nucleotide substitutions in the *BRAF*, *HRAS*, *KRAS*, or *NRAS* genes and three fusion transcripts, *PAX8-PPARG*, *RET-PTC1*, and *RET-PTC3* (ThyGenX panel) in 806 nodule aspirates from 618 subjects.[10] A single genetic alteration was detected in 80% of cytology malignant cases, 21% of indeterminate, 7.8% of nondiagnostic, and 3.5% of benign cases

Gene Expression Classifier

In 2015, Diggans described the development and validation Afirma® BRAF malignancy classifier.[5] The study included FNA biopsies from 716 thyroid nodules. Biopsies were evaluated with quantitative PCR (qPCR) for the *BRAF* V600E mutation, with 181 used as a training sample and 535 used as a validation sample. The Afirma® BRAF malignancy classifier was generated using robust multichip average-normalized gene expression summaries, and the classifiers were evaluated for positive percent agreement (PPA) and negative percent agreement (NPA) with the PCR-derived gene classification. The highest scoring classification method and gene set were then used in a final round of model building. The maximum PPA and NPA for all cytology categories was observed when the threshold for *BRAF*-positive status was 5% or more BRAF mutations. At 5% analytic sensitivity, Afirma® BRAF demonstrates a PPA with PCR results of 90.4% (95% exact binomial confidence interval [CI], 83.5% to 95.1%) and an NPA of 99% (95% CI, 97.6% to 99.7%). There were two samples in the training set and four samples in the validation set that were Afirma® BRAF positive but negative (0% mutation) on PCR, which the authors attribute to either technical variability in either assay or variants other than *BRAF* V600E that cause similar gene expression changes.

Intra- and inter-run reproducibility of the classifier was evaluated using nine FNA biopsies (FNABs) and three tissue controls selected from among training samples with high (BRAF-positive) or low (BRAF-negative) classifier scores and scores near the classifier decision boundary. Each FNAB and tissue was processed from total RNA in triplicate in each of three different runs across days, operators and reagent lots. The intra-assay standard deviation

(SD) of Afirma® BRAF scores was 0.171 (95% CI, 0.146 to 0.204). Of the 106 Afirma® BRAF calls produced (two arrays failed quality control requirements), 106 resulted in concordant calls across all three runs (100% concordance). The interassay SD of scores was 0.204 (95% CI, 0.178 to 0.237) for scores measured on a six-point scale. These results suggest low intra- and inter-run variability. No studies describing the analytic validity of the Afirma® MTC test were identified.

Clinical Validity

Variant Detection and Rearrangement Testing

A number of studies have evaluated whether testing for point mutations or gene fusions (either single mutation or panels of mutations) can be used to improve the sensitivity and specificity for diagnosing indeterminate FNA of the thyroid, with the goal of identifying mutations that predict malignancy in FNA samples.

Variant Panel Testing

In 2016, Nishino published a review of methodology and test performance of molecular cytopathology for thyroid nodules that included mutation analysis testing for ThyGenX®.[11] This test uses nucleic acid preservative solution to assay for the most common oncogenic mutations in *BRAF, KRAS, HRAS, NRAS,* and chromosomal translocations results in *RET/PTC1, RET/PTC3,* and *PAX8/PPARy* fusions. The initial 7-gene panel has been examined in seven studies, summarized in Table 1 below.[10,12-17] Although this test identifies which oncogenic mutations and/or gene fusions are present or absent, this test, and the studies included in this review have significant limitations. The limitations noted in the test include, but are not limited to, the ability to customize the panels, which decreases the ability to compare testing, a low sensitivity and PPV, and a potential lack of applicability to every practice setting due to the differences in the pretest probability of malignancy of tested populations, as in cases where the pretest probability of malignancy is not known, or is much higher, a negative mutation panel may not be sufficient to forgo surgical resection. Finally, the studies included in the review have significant limitations, which include but are not limited to, the majority of samples collected were from a single institution, samples that were re-analyzed had been previously reported in prior studies, and repeated FNA procedures performed on the same nodule part of routine clinical care were not included in this study. The authors noted that, repeated FNA can yield a different cytological diagnosis, which may refine clinical management in the absence of molecular testing.

Table 1: Diagnostic Performance of ThyGenX®

	Nikiforov, 2009		Cantara, 2010	Nikiforov, 2011		Beaudenon-Huibregtse, 2014		Eszlinger, 2014	Eszlinger, 2015	Labourier, 2015
Cytologic category	**AUS/ FLUS**	**FN/ SFN**	**Indeterm**	**AUS/ FLUS**	**FN/ SFN**	**AUS/ FLUS**	**FN/ SFN**	**Indeterm**	**FN/ SFN**	**AUS/FLUS and FN/SFN**
N	21	23	41	247	214	22	19	141	163	109

	Nikiforov, 2009		Cantara, 2010	Nikiforov, 2011		Beaudenon-Huibregtse, 2014		Eszlinger, 2014	Eszlinger, 2015	Labourier, 2015
Prev. of malignancy on cytology	14%	52%	17%	14%	27%	50%	32%	16%	28%	32%
Sensitivity	100%	75%	86%	63%	57%	36%	67%	18%	49%	69%
PPV	100%	100%	86%	88%	87%	67%	80%	19%	71%	71%
Specificity	100%	100%	97%	99%	97%	82%	92%	86%	92%	86%
NPV	100%	79%	97%	94%	86%	56%	86%	85%	82%	85%

Indeterm: Indeterminate; NPV: negative predictive value; PPV: positive predictive value.

A study published by Ferraz in 2011 evaluated 20 publications that reported on the type and number of mutations in cases of FNA of the thyroid diagnosed as indeterminate and compared the results to final histology after surgical resection.[18] Sixteen studies analyzed one mutation (e.g., *BRAF* or *RET/PTC*) and four studies analyzed a panel of several mutations (*BRAF*, *RAS*, *RET/PTC*, and *PAX8/PPARγ*). The detection of a mutation in a histologically (surgically resected) benign thyroid lesion was categorized as a false positive (FP) case; detecting no mutation in an FNA sample from a histologically benign surgical sample was considered a true negative (TN); and finding no mutation in a histologically malignant lesion was categorized as a false negative (FN). Based on four studies that examined a panel of mutations, there was a broad sensitivity range of 38-85.7% (mean 63.7%), a mean specificity of 98% (range 95-100%), mean false positive rate of 1.25% (0-4%) and mean false negative rate of 9% (1-21%). Based on two studies that examined *RET/PTC* rearrangements, mean sensitivity was 55% (50-60%), specificity 100%, false positive rate of 0% and mean false negative rate 3.5% (91-6%). Based on three studies that examined *BRAF* mutations, mean sensitivity was 13% (0-37.5%), mean specificity 92.3% (75-100%), mean false positive rate 0.5% (0-1%) and mean false negative rate of 6% (3-12%). The authors concluded that testing for a panel of mutations leads to an improvement in the sensitivity and specificity for indeterminate FNA of the thyroid but that further standardizations and further molecular markers are needed before broad application of molecular FNA cytology for the diagnosis of thyroid nodules.

In 2016, Nishino published a review on the methodology and test performance of molecular cytopathology for thyroid nodules that included NGS mutation analysis testing and the ThyroSeq® test. The ThyroSeq® test examines 7-gene mutations including *PIK3CA*, *PTEN*, *TP53*, *TSHR*, *CTNNB1*, *RET*, *AKT1*, and *TERT*. Gene fusions involving *RET*, *BRAF*, *NTRKI*, *NTRK3*, *AKT*, *PPARy*, and *THADA*. Three studies were included in the review, which are summaries below in Table 2,[16,19,20] along with the results of a 2015 study by Nikiforov that was not in the review.[21] The results of the review show a low sensitivity and PPV, and a variable specificity and NPV. Additionally, the studies included in this review have significant limitations, including, small sample sizes and testing completed in single institutions.

Since the review by Nishino was published, a study by Shrestha evaluated the performance of the ThyroSeq® test in patients who underwent FNA at a single center between January, 2011 and July, 2013.[22] Of the 261 patients who underwent surgery, FNA results were nondiagnostic for 2%, benign for 23%, AUS/FLUS for 28%, SFN for 11%, SFM for 9%, malignant for 27%. By histopathology, 48% were malignant, including 30% of those classified as AUS/FLUS. ThyroSeq® testing was performed in 44 AUS/FLUS samples, and demonstrated a sensitivity of 85% and specificity of 65%, with a PPV of 50% and an NPV of 91%.

Table 2: Diagnostic Performance of ThyroSeq®

	Nikforov, 2014	Nikforov, 2015	Labourier, 2015			La Mercier, 2015
N of indeterminate FNA with molecular test	143	98 (with known outcome)	109			34
Prev. of malignancy on cytology	27%	22%	32%			21%
Results negative	101 (71%)	72	83 (76%) miRNA classifier	75 (69%) 7-gene panel	67 (61%) both tests combined	26 (76%)
Results positive	42 (29%)	26	26 (24%)	34 (31%)	42 (39%)	8 (24%)
Sensitivity	90%	91%	57%	69%	89%	71%
PPV	83%	77%	77%	71%	74%	63%
Specificity	93%	92%	92%	86%	85%	89%
NPV	96%	97%	82%	85%	94%	92%

NPV: negative predictive value; PPV: positive predictive value.

BRAF

In 2015, Fnais reported on a systematic review and meta-analysis of studies reporting on the test accuracy of *BRAF* mutation testing in the diagnosis of PTC.[23] The review included 47 studies with 9924 FNA samples. For all cytologically indeterminate nodules, the pooled sensitivity estimate for *BRAF* mutation testing was 31% (95% CI, 6% to 56%). Among nodules suspicious for malignancy on FNA, the pooled sensitivity estimate for *BRAF* mutation testing was 52% (95% CI, 39% to 64%; I2=77%).

A similar systematic review by Jinih included 32 studies testing for *BRAF* V600E in indeterminate nodules.[24] The authors reported an overall sensitivity of 0.40 (95% CI: 0.32-0.48) and specificity of 1.00 (95% CI: 0.98-1.00), and concluded that despite the high

specificity, testing for the *BRAF* V600E mutation lacked diagnostic value due to the low sensitivity.

Su (2016) conducted a meta-analysis on the *BRAF* V600E in thyroid FNA, particularly indeterminate cases, which included 88 studies. The authors found that adding *BRAF* V600E to cytological analysis testing increased sensitivity from 81.4% to 87.4% and reduced the false-negative rate from 8% to 5.2%. The *BRAF* V600E mutation proportion was 23% in the indeterminate group, and that the sensitivity was higher in cases suspicious for malignant cells (SMC) than in cases of AUS/FLUS. This pattern was reversed for the test's specificity.

Adeniran conducted a study of 157 cases with equivocal thyroid FNA readings (indeterminate and suspicious for papillary thyroid carcinoma [PTC]) or a positive diagnosis for PTC and concomitant *BRAF* mutation analysis.[25] The results of histopathologic follow-up were correlated with the cytologic interpretations and *BRAF* status. Based on the follow-up diagnosis after surgical resection, the sensitivity for diagnosing PTC was 63.3% with cytology alone and 80.0% with the combination of cytology and *BRAF* testing. No false positives were noted with either cytology or *BRAF* mutation analysis. All PTCs with extrathyroidal extension or aggressive histologic features were positive for *BRAF* mutation. The authors concluded that patients with an equivocal cytologic diagnosis and *BRAF* V600E mutation could be candidates for total thyroidectomy and central lymph node dissection.

Xing investigated the utility of *BRAF* mutation testing of thyroid FNA specimens for preoperative risk stratification of PTC in 190 patients.[26] A *BRAF* mutation in preoperative FNA specimens was associated with poorer clinicopathologic outcomes of PTC. In comparison with the wild-type allele, a *BRAF* mutation strongly predicted extrathyroidal extension (23% vs. 11%; P=0.039), thyroid capsular invasion (29% vs. 16%; P=0.045), and lymph node metastasis (38% vs. 18%; P=0.002). During a median follow-up of 3 years (range, 0.6 to 10 years), PTC persistence/recurrence was seen in 36% of *BRAF* mutation-positive patients versus 12% of *BRAF* mutation-negative patients, with an odds ratio of 4.16 (95% confidence interval [CI]: 1.70 to 10.17; P=0.002). The positive and negative predictive values for preoperative FNA-detected *BRAF* mutation to predict PTC persistence/recurrence were 36% and 88%, respectively, for all histologic subtypes of PTC. The authors concluded that preoperative *BRAF* mutation testing of FNA specimens may provide a novel tool to preoperatively identify PTC patients at higher risk for extensive disease (extrathyroidal extension and lymph node metastases) and those who are more likely to manifest disease persistence/recurrence.

Jara retrospectively evaluated the utility of *BRAF* mutation testing in 66 thyroid nodules with "suspicious for PTC" on FNA and available histopathologic samples from thyroid biopsy.[27] Forty-two subjects (62.6%) had PTC diagnosed on final histopathology. A positive *BRAF* mutation test was associated with a sensitivity and specificity for PTC of 45.5% and 87.5%, respectively, and a PPV and NPV of 88.2% and 43.8%, respectively.

The association between BRAF mutations and PTC is supported by a report by Park (2015) on 294 patients with thyroid nodules whose FNA samples were evaluated with BRAF mutation testing by two methods: real-time PCR with Taq-Man minor groove-binding probes and allele-specific PCR using dual-priming oligonucleotides.[28] The detection rate of PTC by BRAF mutation testing by real-time PCR and allele-specific PCR was 80.2% (95% CI, 71.9% to 86.9%) and 76.9% (95% CI, 68.3% to 84.0%), respectively.

A number of other studies have reported on the link between BRAF mutations and PTC, with

mixed findings.[29-32] Some studies have reported high specificity (100%) of *BRAF* in the detection of PTC in indeterminate thyroid nodules.[31,33,34]

Gene Expression Classifier

Although less evidence exists about validity of gene expression profiling, the GEC can be used to improve the sensitivity and specificity for diagnosing indeterminate FNA of the thyroid. One of the goals of the GEC is to identify mutations that predict malignancy in FNA samples.

In the Diggans study describing the development and validation of the Afirma® BRAF test, previously described, for a subset of 213 thyroid nodule FNA samples for which histopathology was available, the Afirma® BRAF test results were compared with pathologic findings.[5] The Afirma® BRAF classified all histopathologically benign samples as *BRAF* V600E–negative (specificity 100%; 95% CI, 97.4% to 100%). Of the 73 histopathologically malignant samples, the Afirma® BRAF test identified 32 as *BRAF*-positive (sensitivity, 43.8%; 95% CI, 32.2% to 55.9%).

In the Kloos study describing the development and validation of the Afirma® MTC classifier, the MTC classifier was evaluated in a sample of 10,488 thyroid nodule FNA samples referred for GEC testing (the Afirma® GEC described below).[35] In this sample, 43 cases were Afirma® MTC-positive, of which 42 were considered to be clinically consistent with medullary thyroid carcinoma on pathology or biochemical testing, for a PPV of 97.7% (95% CI, 86.2% to 99.9%).

The use of the Afirma® GEC to predict malignancy was evaluated by Roychoudhury (2017) in a retrospective community practice study that included patients tested between 2013 and 2015.[36] All Afirma® GEC “suspicious” results were compared to cytological evaluation. In this group, there were 69 patients with “suspicious” GEC results. Six of these patients had an FNA diagnosis of benign, 43 had a diagnosis of atypia of unknown significance (AUS), 18 had FNA findings suspicious for neoplasm (SFN). There were 60 patients that had surgical resection, and of these, 82% were found to be benign and 18% were malignant. FNA results were superior to GEC at predicting malignancy in this group, however the Afirma® GEC is recommended for prediction of benignancy and not malignancy.

Abeykoon (2016) studied the impact of implementing Afirma® GEC at a single center.[37] Surgical recommendations for patients with indeterminate thyroid nodules decreased from 81.5% pre-Afirma® GEC to 50% post-Afirma® GEC. The rate of malignant surgical pathology diagnosis increased from 20% pre-Afirma® GEC to 85.7% post-Afirma®. The implementation of Afirma® GEC decreased the number of surgical recommendations and increased the rate of malignancy detected for patients who received a surgical biopsy.

Chaudhary (2016) studied the impact on surgical outcomes pre- and postimplementation of Afirma® GEC.[38] A total of 158 FNAs were sent for Afirma GEC® with 73 suspicious and 8 benign Afirma cases going for surgeries. Compared with before implementation of Afirma® GEC, the rate for surgical biopsy decreased from 61% to 54% but was not statistically significant. In the SFN, the rate of surgical biopsy significantly decreased from 76% to 52%.

Dhingra (2016) studied the effects of a FNA protocol combining expert thyroid cytopathology and Afirma GEC® in a community practice.[39] Historical data were compared with data after implementation of the FNA protocol. Prior to protocol implementation, the rates of indeterminate cytology and diagnostic surgery were 26% and 24%. After protocol implementation, the rates of indeterminate cytology and diagnostic surgery decreased to 10%

and 6%. The effect of Afirma® GEC implementation could not be ascertained given the FNA protocol combining expert thyroid cytopathology and Afirma® GEC used in the study.

Villabona (2016) compared the Afirma® GEC to ultrasound for prediction of malignancy in retrospective single center study.[40] The study included 119 patients with FNA diagnosis of AUS, 48 of whom had GEC testing directly afterward, while the other 71 had a repeat FNA. Of those having a repeat FNA, 52 were again diagnosed as AUS, and these samples were sent for GEC testing. The PPV for GEC testing after the first AUS diagnosis was 66.6%, while the PPV for the GEC after the second FNA was 91.4%. The authors noted that among those having a second FNA, ultrasound detection of hypoechoic, solid nodules demonstrated a 92% PPV for malignancy.

Labourier (2015) reported on the sensitivity and specificity of a test algorithm combining micro-RNA measurements from 17 genes (miRInform; Asuragen Laboratory, Austin, TX) with a 10-gene GEC in 109 FNA samples with atypia of undetermined significance/follicular lesion of undetermined significance or follicular neoplasm/suspicious for follicular neoplasm on cytology evaluated at the Asuragen Laboratory with known final pathology.[16] Seventy-four nodules were diagnosed as benign and 35 as malignant. The performance of the combined test (micro-RNA measurements and the 10-gene GEC) is summarized in Table 1.

Clinical Utility

Variant Detection and Rearrangement Detection

Testing for specific mutations associated with thyroid cancer (e.g., *BRAF* V600E mutations, *RET* mutations, and *RET/PTC* and *PAX8/PPARγ* rearrangements) are generally designed to "rule in" cancer in nodules that have indeterminate cytology on FNA.[41] A potential area for clinical utility for this type of mutation testing would be in informing preoperative planning for thyroid surgery following initial thyroid FNA, such as planning for a hemi- versus a total thyroidectomy or performance of a central neck dissection.

In a 2014 retrospective analysis, Yip reported outcomes after implementation of an algorithm incorporating molecular testing of thyroid FNA samples to guide the extent initial thyroid resection.[42] The study included a cohort of patients treated at a single academic center at which molecular testing (*BRAF* V600E, *BRAF* K601E, *NRAS* codon 61, *HRAS* codon 61, and *KRAS* codon 12 and 13 point mutations; *RET/PTC1*, *RET/PTC3*, and *PAX8/PPARγ* rearrangements) was prospectively obtained for all FNAs with indeterminate cytology (follicular lesion of undetermined significance, follicular neoplasm, and suspicious for malignancy), and for selective FNAs at the request of the managing physician for selected nodules with either benign or nondiagnostic cytology. The study also included a second cohort of patients who did not have molecular testing results available. For the patients treated with molecular diagnosis, a positive molecular diagnostic test was considered to be an indication for an initial total thyroidectomy. Patients with follicular lesion of undetermined significance and negative molecular diagnostic results were followed with repeat FNA, followed by a lobectomy or total thyroidectomy if indeterminate pathology persisted. Patients with follicular neoplasm or suspicious for malignancy results on cytology and a negative molecular diagnostic result were managed with lobectomy or total thyroidectomy.

The sample included 671 patients, 322 and 349 managed with and without molecular diagnostics, respectively. Positive molecular testing results were obtained in 56 patients (17% of those managed with molecular diagnostics), most commonly *RAS* mutations (42/56; 75%),

followed by *BRAF* V600E (10/56; 18%), *BRAF* K601E (2/56; 4%), and *PAX8/PPARγ* rearrangements (2/56; 4%). Compared with those managed without molecular diagnostics, patients managed with molecular diagnostics were nonsignificantly less likely to undergo total thyroidectomy as an initial procedure (63% vs 69%; p=0.08). However, they had nonsignificantly higher rates of central compartment lymph node dissection (21% vs 15%; p=0.06). Across both cohorts, 25% of patients (170/671) were found to have clinically significant thyroid cancer, with no difference in thyroid cancer rates based on the type of initial operation (26% for total thyroidectomy vs 22% for lobectomy; p=0.3). The incidence of clinically significant thyroid cancer after initial lobectomy (i.e., requiring a 2-stage surgery) was significantly lower for patients managed with molecular diagnostics (17% vs 43%; p<0.001). An indeterminate FNA result had sensitivity and specificity for the diagnostic of thyroid cancer of 89% and 27%, respectively, with PPV and NPV of 29% and 88%, respectively. The addition of molecular diagnostics to FNA results increased the specificity for a cancer diagnosis to 95% and the PPV to 82%.

In 2015, a task force from the American Thyroid Association (ATA) reported on a review with recommendations for the surgical management of FNA-indeterminate nodules with various molecular genetic tests.[43] This review reported on the estimated likelihood of malignancy in an FNA-indeterminate nodule depending on results of the Afirma® GEC and other panels designed to rule in malignancy. Depending on the estimated prebiopsy likelihood of malignancy, recommendations for surgery include observation, active surveillance, repeat FNA, diagnostic lobectomy, or oncologic thyroidectomy.

Section Summary

The available evidence suggests that the use of mutation analysis testing in cytological thyroid FNA samples is generally associated with a high sensitivity, and positive predicted value, but a lower specificity and negative predicted value for clinically significant thyroid cancer. More prospective validation of these study findings in additional settings is needed.

The available evidence also suggests that the use of the gene expression classifier testing in cytological thyroid FNA samples is generally associated with a higher sensitivity, positive predicted value, specificity, and non-predicted value for clinically significant thyroid cancer. Although there are few studies, they suggest that testing this test may assist in provider decision making about the appropriate selection of patients for an initial total thyroidectomy.

MOLECULAR MARKERS TO PREDICT BENIGNANCY; GENE EXPRESSION CLASSIFIERS

Analytic Validity

Afirma® GEC

Walsh verified the analytical performance of the Afirma® gene expression classifier (GEC) in the classification of cytologically indeterminate fine-needle aspirates from thyroid nodules.[44] RNA content within FNAs preserved in FNAProtect was determined stable for up to 6 days at room temperature with no changes in RNA yield (P = 0.58) or quality (P = 0.56). FNA storage and shipping temperatures were found to have no significant effect on GEC scores (P = 0.55) or calls (100% concordance). Analytical sensitivity studies demonstrated tolerance to variation in RNA input (5-25 ng) and to the dilution of malignant FNA material down to 20%. Analytical specificity studies using malignant samples mixed with blood (up to 83%) and

genomic DNA (up to 30%) demonstrated negligible assay interference with respect to false-negative calls, although benign FNA samples mixed with relatively high proportions of blood demonstrated a potential for false-positive calls.

Chudova developed a molecular test to distinguish between benign and malignant thyroid nodules using fine-needle aspirates.[3] The authors used mRNA analysis to measure >247,000 transcripts in 315 thyroid nodules. The data set consisted of 178 retrospective surgical specimens, representing the most common benign and malignant histologic subtypes, and 137 prospectively collected aspirate specimens. Two classifiers were trained separately on surgical samples and aspirates. The performance was evaluated using an independent test set of 48 prospective FNA samples which had known surgical pathology diagnoses, and included 50% with indeterminate cytopathology. The performance of the classifier was markedly lower in the FNAs than in tissue, likely due to differences in cellular heterogeneity between the two types of specimens. On the test set, negative predictive value (NPV) and specificity were estimated to be 96% and 84%, respectively.

Clinical Validity

Afirma® GEC

In 2016, Santhanam published results from a systematic review and meta-analysis to examine the performance of the Afirma® GEC in predicting benign and malignant nodules in patients with cytologically indeterminate nodules.[45] Seven studies were included in the analysis.[46-51] A QUADAS-2 report for all studies included in the final analysis was tabulated for risk of bias and applicability. The pooled sensitivity of the GEC was 95.7 % (95 % CI 92.2-97.9, I (2) value 45.4 %, p = 0.09), and the pooled specificity was 30.5 % (95 % CI 26.0-35.3, I (2) value 92.1 %, p < 0.01). The pooled positive LR was 1.20 (95% CI 0.996-1.44), and the pooled negative LR was 0.2 (95% CI 0.11-0.36). Overall, the diagnostic odds ratio was 7.9 (95 % CI 4.1-15.1). The overall false-negative rate (1-sensitivity) was 0.04 (0.02-0.08), and the overall false-positive rate (1-specificity) was 0.69 (0.65-0.74). Patients with benign GEC were not followed long enough to ascertain the actual false-negative rates of the index test. The evidence suggests that the Afirma® GEC is a useful diagnostic test to rule our malignancy and avoid thyroid surgery for patients with an indeterminate FNA.

Another systematic review published in 2016 by Nishino also reviewed the performance of the Afirma® GEC.[11] Eight studies were included in the review for the Afirma® GEC, which are summarized in table 3 below.[46-50,52,53] Overall, the studies show that the Afirma® GEC has a high sensitivity and NPV value, thereby reducing the number of required surgical sections, and instead triaging patients safety toward watchful waiting with close ultrasound monitoring of the nodule and re-aspirating any nodule that demonstrates significant growth or concerning changes.

Table 3: Diagnostic Performance of Afirma® GEC

	Veracyte Validation Study	Alexander, 2014	Harrell, 2014	McIver, 2014	Lastra, 2014	Marti, 2015 (MSK data)	Marti, 2015 (MSBI data)	Brauner, 2015
N	210	309	56	60	132	94	71	71
GEC	87 (41%)	170 (55%)	20 (36%)	16 (27%)	70 (53%)	24 (26%)	37 (52%)	26 (26%)

	Veracyte Validation Study	Alexander, 2014	Harrell, 2014	McIver, 2014	Lastra, 2014	Marti, 2015 (MSK data)	Marti, 2015 (MSBI data)	Brauner, 2015
result Benign								
GEC result suspicious	123 (59%)	139 (45%)	36 (64%)	44 (73%)	62 (47%)	70 (74%)	34 (48%)	45 (63%)
Surgically resected cases	210	123	35	36	50	44	26	46
Sensitivity	90%	98%	94%	83%	100%	100%	100%	100%
PPV	37%	42%	57%	16%	46%	57%	14%	14%
Specificity	52%	12%	24%	10%	7%	10%	22%	8%
NPV	94%	90%	80%	75%	100%	100%	100%	100%

NPV: negative predictive value; PPV: positive predictive value.

Several studies on the performance of the Afirma® GEC have been published recently and are not included in the systematic reviews above. In 2015, Angell compared outcomes between 95 cytologically indeterminate and GEC benign nodules and 1224 cytologically benign nodules.[54] Wong (2016) evaluated the proportion of patients with a suspicious GEC that were subsequently diagnosed with noninvasive follicular variant of papillary thyroid carcinoma, a particularly indolent variety.[55] Chaudhary (2016) correlated results of the GEC with surgical outcome in 158 patients, and found that of the 73 that were suspicious by GEC, 28 (38%) had carcinoma, while all of the 8 patients with benign GEC results that had surgery did not have carcinoma.[38] Baca (2017) assessed GEC and clinical outcome for patients with FNA AUS results indicating architectural atypia, cytological atypia, or both.[56] They found that the rate of benign GEC findings was higher in patients with architectural atypia compared to cytological atypia or both (65% vs. 59% and 38%, respectively), and the risk of cancer among those with suspicious GEC results who had surgery showed a similar pattern, with architectural atypia cases having the lowest risk (19%) compared with cytological atypia cases (45%, p=0.07)) or cases with both (57%, p=0.003). A 2016 study by Samulski. reported on their institution experience with the GEC, noting that the test had a relatively low specificity and PPV, but showed improved performance when coupled with a repeat FNA.[57]

ThyraMIR™

Wylie (2016) reported on the development of the ThyraMIR miRNA classifier, along with a 17-variant oncogene panel including *BRAF*, *RAS*, *RET*, or *PAX*.[58] An miRNA classifier was originally developed using rt-PCR methodology in a sample of 257 surgical specimens, and validated in an independent set of 42 nodules with indeterminate cytology. A 17-variant panel covering validated oncogenic gene alterations for *BRAF*, *RAS*, *RET*, or *PAX8* genes was

tested on preoperative FNA and surgical specimens. Optimization of miRNA classifiers A and B resulted in the commercial ThyraMIR™ Classifier. ThyraMIR™ was used on a subset of thyroid tissues negative by the targeted 17-variant panel and resulted in a sensitivity of 85% and specificity of 95%.

In 2016, Nishino published results from a systematic review that included the ThyraMIR™ gene expression classifier test.[11,16] The ThyraMIR™ test was validated in a cross-sectional cohort study of 109 cytologically indeterminate (AUS/FLUS and FN/SFN) thyroid nodules. The original study found that the ThyrMIR™ had a sensitivity of 57%, PPV of 77%, a specificity of 92%, and a NPV of 82%.[16]There is only one study that demonstrates the clinical validity for the ThyraMIR™ test. Therefore, more studies are needed to validate the clinical validity of the ThyraMIR™ gene expression classifier and determine the efficacy of the test on health outcomes.

Hadd (2013) reported on targeted NGS of cancer genes in 38 FFPE and 10 FNA tumor specimens.[59] The results showed an accuracy rate of 96.1% (95% CI, 96.1% to 99.3%) compared with Sanger sequencing; Sanger sequencing has an analytic sensitivity of approximately 15% to 20%. When NGS was compared with a multiplex detection system with a 1% variant detection rate, the accuracy was reported to be 99.6% (95% CI, 97.9% to 99.9%)

Clinical Utility

Afirma® GEC

Numerous single institution studies have been conducted demonstrating the clinical utility of the Afirma® GEC. These studies have shown the rate of surgical resection of the thyroid has decreased due to the ability of the Afirma® GEC to classify indeterminate FNA results.[51,52,60-63]

In 2016, Sipos published results from a retrospective study that assessed the operative rate in patients with a benign results from the Afirma® GEC in the longest follow up study to date.[64] Additionally, the study examined the physician's opinion regarding the safety of GEC use compared to the hypothetical situation of providing thyroid nodule management without the GEC. In total, 16 nonacademic medical facilitates participated, 16 endocrinologists and one radiologist's submitted data on 98 patients. The median follow-up time was 26 months (range 0-44 months). The results showed that thyroidectomy was performed in 17 patients (17.3%). Using Kaplan-Meier analysis, the authors found that the majority of surgeries (10 of 17, or 58.8%) occurred within the first year after obtaining the GEC 'benign' result. Within two years of receiving a 'benign' GEC result, 88% of all surgeries were performed. In the second and third years after a 'benign' GEC result was obtained, an additional seven patients underwent surgery. The most common indications for surgery were rapid nodule growth, and large nodule. No significant differences were identified between groups with regard to age or gender. Finally, when physicians were surveyed about the utility of the GEC testing, 78 of 91 (86%) physicians reported that patient safety was improved by using the GEC test compared to not using it. In comparison, 11 out of 91 physicians (12%) reported that using the GEC test had no impact on perceived patient safety. Two physicians indicated that patient safety was diminished using the GEC. This study has several limitations, including but not limited to, sponsorship, sample size, and physician feedback. Veracyte, the maker of Afirma®, the GEC used in this study, sponsored data collection and statistical analysis. Furthermore, there was a lack of description about how the medical facilities were chosen to be included in the sample

and about how physicians were surveyed for feedback.

In 2016, Singer published results from a retrospective cohort study that evaluated the long-term management patterns and thyroid surgery rates of Afirma® GEC benign patients compared to a control group of cytopathology benign patients.[65] This study used laboratory test results linked to payer medical claims data and examined patients who underwent FNA biopsy between January 2011 and July 2013. GEC benign patients were matched 1:3 to cytopathology benign patients on biopsy year, gender, nodule size, and age. Outcomes measured included thyroid-related follow-up clinic visits, ultrasound examinations, and surgeries. Out of 2059 patients, 804 were included in the study (201 GEC benign patients, 603 cytopathology benign patients) and were evaluated over an average follow-up of 20 months. The proportions of GEC benign and cytopathology benign patients that underwent thyroid surgery (11.4% versus 10.1%, p = 0.594), and received a follow-up ultrasound exam (60.2% versus 61.7%, p = 0.706), respectively, were not significantly different. This study had limitations, which include but are not limited to, the design and sample, as the cohort only included patients who test results and administrative claims data were uniquely identifiable and could be linked on multiple identifiers.

In a large, retrospective cohort analysis by Sacks (2016), cases from January, 2012 to July, 2013, before the Afirma® GEC was used (pre-Afirma®), were compared to cases from July, 2013 through December, 2014 (post-Afirma®).[66] A total of 4,292 FNAs were performed during these time frames. There was a significant increase in the proportion of Bethesda III and Bethesda IV FNA determinations (13.4% vs. 10.7%, p<.005. and 2.9% vs 1.8%, p<.01, respectively), and a reduction in Bethesda II determinations (68.8% vs. 74.6%, p<.001) in the post-Afirma® cohort compared to the pre-Afirma® group. The rates of surgery and malignancy were not significantly different between cohorts. The authors concluded that the availability of the Afirma® test may have the unintended effect of shifting the interpretation of FNAs toward Bethesda III and IV – cases in which the test may be used, without changing the overall rates of surgery.

ThyGenX® and ThyraMIR™

Direct evidence for the clinical utility for the ThyroSeq® and the combined ThyGenX® and ThyraMIR™ diagnostic testing algorithm is lacking. In the absence of direct evidence for the clinical utility of the combined testing, a chain of evidence may be constructed to infer potential clinical utility of the combined diagnostic testing algorithm. No studies using ThyGenX NGS panel in FNA samples were identified. However, available evidence has suggested that use of variant testing using NGS in thyroid FNA samples is generally associated with a high specificity and PPV for clinically significant thyroid cancer. There is potential clinical utility for identifying malignancy with higher certainty on FNA if such testing permits better preoperative planning at the time of thyroid biopsy, potentially avoiding the need for a separate surgery. However, variant analysis does not achieve an NPV sufficiently high enough to identify which patients can undergo active surveillance over thyroid surgery. In the diagnostic algorithm that reflexes to the ThyraMIR™ after a negative ThyGenX® result, patients receiving reflex testing could identify who may undergo active surveillance over thyroid surgery. A single study using a 17-variant panel with ThyraMIR™ showed a NPV of 94%. Therefore, the high NPV of ThyraMIR™ has the potential to accurately predict benignancy and triage patients to active surveillance.

Section Summary

There is are two commercially-available gene expression classifier (GEC) that are designed to exclude malignancy in individuals with indeterminate thyroid FNA results Afirma® and ThyraMIR™.

The Afirma® GEC has been reported to have a high NPV in a limited number of studies. While the available evidence suggests that physician decision making about surgery may be altered by GEC results, long-term follow-up of patients with thyroid nodules who avoided surgery based on GEC results is limited.

There is just one published clinical validation study for ThyraMIR™ gene expression classifier test. More studies are needed to efficacy of the test to determine benignancy in patients with indeterminate thyroid nodule cytology. However, there is potential clinical utility for identifying malignancy with higher certainty on FNA. Such testing potentially avoids the need for additional invasive procedures, which may improve overall health outcomes.

PRACTICE GUIDELINE SUMMARY

AMERICAN THYROID ASSOCIATION

In 2015, the American Thyroid Association (ATA) updated their recommendations on molecular markers in FNA of the thyroid:[67]

- Recommendation 13: If molecular testing is being considered, patients should be counseled regarding the potential benefits and limitations of testing about the possible uncertainties in the therapeutic and long-term clinical implications of results (strong recommendation, low-quality evidence).
- Recommendation 14: If intended for clinical use, molecular testing should be performed in a CLIA/CAP-certified molecular laboratories, or the international equivalent because reported quality assurance practices may be superior compared to other settings (strong recommendation, low-quality evidence).
- Recommendation 15: (A) For nodules with AUS/FLUS cytology, after consideration of worrisome clinical and sonographic features, investigations such as repeat FNA or molecular testing may be used to supplement malignancy risk assessment in lieu of proceeding directly with a strategy of either surveillance or diagnostic surgery. Informed patient preference and feasibility should be considered in clinical decision-making. (weak recommendation, Moderate-quality evidence)
- Recommendation 16: (A) Diagnostic surgical excision is the long-established standard of care for the management of follicular neoplasm/suspicious for follicular neoplasm (FNSFN) cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk data, in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making (weak recommendation, moderate-quality evidence.)

NATIONAL COMPREHENSIVE CANCER NETWORK

The National Comprehensive Cancer Network (NCCN) recommendations on molecular markers in FNA of the thyroid (v1.2018) include considering molecular diagnostic testing in certain clinical scenarios. The policy criteria are mostly consistent with these recommendations, including evaluating FNA results when cytology is indeterminate or suspicious. Recommendations are all Category 2A, which is based upon lower-level

evidence, though there is uniform NCCN consensus that the intervention is appropriate.

AMERICAN COLLEGE OF ENDOCRINOLOGY, AMERICAN ASSOCIATION OF CLINICAL ENDOCRINOLOGISTS, ASSOCIAZION MEDICI ENDOCRINOLOGI

In 2016, the American College of Endocrinology (ACE), American Association of Clinical Endocrinologists (AACE), and the Association Medici Endocrinology (AME) published medical guidelines for clinical practice for the diagnosis and management of thyroid nodules.[68] They made the following recommendations:

- 4.6.1 Molecular testing should be used to:
  - complement not replace cytologic evaluation [Bel 2, Grade A]
  - Are expected to influence the clinical management [Bel 2, Grade A]
  - As a general rule, not recommended in nodules with established benign or malignant cytologic characteristics. (Bel 2, Grade A]

- 4.6.2 Molecular testing for cytologically indeterminate nodules:
  - Consider the detection of *BRAF* and *RET/PTC* and possible *PAX8/PPARG* and *RAS* mutations if such detections are available [BEL2, Grade B]
  - Due to insufficient evidence and the limited-follow-up, we do not recommend either in favor of or against the use of gene expression classifiers for cytologically indeterminate nodules. [Bel 2, Grade B]

- 4.6.3 Molecular testing for deciding the extent of surgery:
  - With the exception of mutations such as *BRAF* V600E that have a PPV approaching 100% for papillary thyroid carcinoma, evidence is insufficient to recommend in favor of or against the use of mutation testing as a guide to determine the extent of surgery. [Bel 2, Grade A]

- 4.6.4 The false negative rate for indeterminate nodules is 5 to 6% and the experience and follow-up for mutation-negative nodules or nodules classified as benign by a gene expression classifier are still insufficient, close follow-up is recommended. [Bel 3, Grade B]

Bel 2: RCTs with limited body of data, well-conducted prospective cohort studies, and well-conducted meta-analyses of cohort studies.

Bel 3: Methodologically flawed RCTs, observational studies, case series or case reports.

Grade A: >1 Conclusive level 1 publications demonstrating benefit >> risk=Action based on strong evidence

Grade B: No conclusive level 1 publication, ≥1 Conclusive level 2 publications demonstrating benefit >> risk

SUMMARY

There is enough research to show that the Afirma® Gene Expression Classifier, ThyroSeq® Genomic Classifier, ThyGenX®, and ThyraMIR™ tests may help to predict whether certain thyroid nodules may be non-cancerous, or identify variants that are linked to thyroid cancer. Such tests may be useful in classifying risks before surgery, or avoid

surgery entirely. Therefore, the use of the Afirma® Gene Expression Classifier, ThyroSeq® Genomic Classifier, ThyGenX®, and ThyraMIR™ tests may be considered medically necessary when criteria are met.

There is not enough research to show health outcomes are improved by gene expression classifiers, genetic variant analysis, and molecular marker testing in fine-needle aspirates of the thyroid, other than those specified above. This includes, but is not limited to the RosettaGX Reveal™ test. Therefore, gene expression classifiers, genetic variant analysis, and molecular marker testing in fine-needle aspirates of the thyroid, other than those specified in criteria, are considered investigational.

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69. BlueCross BlueShield Association Medical Policy Reference Manual "Molecular Markers in Fine Needle Aspirates of the Thyroid." Policy No. 2.04.78

CODES

Codes	Number	Description
CPT	0018U	Oncology (thyroid), microRNA profiling by RT-PCR of 10 microRNA sequences, utilizing fine needle aspirate, algorithm reported as a positive or negative result for moderate to high risk of malignancy
	0026U	Oncology (thyroid), DNA and mRNA of 112 genes, next-generation sequencing, fine needle aspirate of thyroid nodule, algorithmic analysis reported as a categorical result ("Positive, high probability of malignancy" or "Negative, low probability of malignancy")
	81210	BRAF (B-Raf proto-oncogene, serine/threonine kinase) (eg, colon cancer, melanoma), gene analysis, V600 variants
	81401	Molecular pathology procedure, Level 2
	81404	Molecular pathology procedure, Level 5
	81405	Molecular pathology procedure, Level 6
	81406	Molecular pathology procedure, Level 7
	81479	Unlisted molecular pathology procedure
	81545	Oncology (thyroid), gene expression analysis of 142 genes, utilizing fine needle aspirate, algorithm reported as a categorical result (eg, benign or suspicious)
	81599	Unlisted multianalyte assay with algorithmic analysis
HCPCS	None	

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