Regence Oregon and Utah | Regence Idaho and select counties of Washington

Medical Policy Manual

Topic: Molecular Markers in Fine Needle Aspirates of the Thyroid

Date of Origin: April 2013

Section: Genetic Testing

Last Reviewed Date: April 2014

Policy No: 49

Effective Date: July 1, 2014

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

Fine needle aspiration of a thyroid lesion to identify which patients need to undergo surgery has diagnostic limitations and has led to the development of molecular markers in an attempt to improve the accuracy.

Background

Fine Needle Aspiration (FNA) of the Thyroid

Thyroid nodules are common, present in 5-7% of the U.S. adult population. The vast majority are benign, and most cases of thyroid cancer are curable by surgery if detected early. Fine needle aspiration (FNA) of the thyroid is currently the most accurate procedure to distinguish benign thyroid lesions and malignant ones, reducing the rate of unnecessary thyroid surgery for patients with benign nodules and triaging patients with thyroid cancer to appropriate surgery.

About 60-70% of thyroid nodules are classified cytologically as benign, and 4-10% of nodules are cytologically deemed malignant.[1] However, the remaining 20-30% have equivocal findings (inclusive, indeterminate, atypical or suspicious), usually due to overlapping cytologic features between benign and malignant nodules; these nodules usually require surgery for a final diagnosis.

The current guidelines recommend repeat FNA for patients with a diagnosis of "atypia of undetermined significance" and lobectomy with or without intraoperative pathology consultation for those with a suspicious diagnosis.[2]

Approximately 80% of patients with indeterminate cytology undergo surgical resection, postoperative evaluation reveals a malignancy rate ranging from 6-30%, making this clinical process one with very low specificity.[3]

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.

Thyroid Cancer

Most thyroid cancers originate from thyroid follicular cells and include well-differentiated papillary thyroid carcinoma (PTC) (80% of all thyroid cancers) and follicular carcinoma (15%). 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 ~3% of all thyroid cancers.

The diagnosis of malignancy in the case of PTC is primarily based on cytologic features. If a FNA in a case of PTC is indeterminate, 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 4 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.[4] These mutually exclusive mutations are found in more than 70% of papillary carcinomas.[4] 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.[4] 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.[4] 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.

Regulatory Status

Commercially available panels of molecular markers utilizing FNA specimens from the thyroid include miRInform™ (Asuragen) and Veracyte® (Afirma).

miRInform is a panel of 7 analytically validated molecular markers (KRAS, BRAF, HRAS, NRAS, RET/PTC 1, RET/PTC3 and PAX8/PPARγ).

The Afirma "gene expression classifier" (GEC) is a proprietary diagnostic test offered by Veracyte, which claims to classify a thyroid nodule with indeterminate cytology as benign (with greater than 95% negative predictive value) or as suspicious for malignancy (>50% risk of malignancy). The GEC measures the gene expression of 142 genes and applies a multi-dimensional algorithm to classify whether a nodule with an indeterminate cytologic diagnosis is benign or suspicious.

These 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.

MEDICAL POLICY CRITERIA

I. Mutation analysis in fine-needle aspirates of the thyroid is considered **investigational.**

II. The use of a gene expression classifier in fine-needle aspirates of the thyroid that are cytologically considered to be indeterminate, atypical or suspicious for malignancy is considered **investigational.**

Validation of the clinical use of any genetic test focuses on 3 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.

SCIENTIFIC EVIDENCE

Literature Appraisal

The literature on the use of molecular markers for thyroid nodules diagnosed by fine needle aspiration (FNA) as indeterminate, atypical, or suspicious consists of approximately 20 publications. These studies have analyzed either panels of mutations or a single mutation in these fine needle aspirates 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. Some authors have also reported that the presence of certain mutations may predict more aggressive behavior in a malignant thyroid lesion. A gene expression classifier has been developed to predict the likelihood that a thyroid lesion with indeterminate cytology is benign, allowing a patient to avoid surgical excision if that action is deemed to be clinically appropriate. However, neither prospective nor comparative studies to determine how the preoperative result of the presence of a mutation in a thyroid nodule with equivocal cytology results would impact patient management have been performed.

Molecular Markers to Predict Malignancy (Mutation Analysis)

Ferraz et al. 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.[5] Sixteen studies analyzed one mutation (e.g., BRAF or RET/PTC) and 4 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 4 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 2 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 3 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.

Nikiforov et al. prospectively tested a panel of mutations (BRAF, RAS, RET/PTC, and PAX8/PPARγ) in 470 FNA samples of thyroid nodules from 328 consecutive patients.[6] Mutational status was correlated with cytology and either surgical pathology diagnosis or follow-up (mean 34 months). A total of 40 patients were excluded for poor quality of specimen or loss to follow-up. Sixty-nine patients (with 86 thyroid FNA samples) underwent surgery soon after completion of the cytologic evaluation; preoperative cytologic diagnosis was: positive for malignancy in 22 samples, indeterminate (including atypical and suspicious for malignancy) in 52 samples, and negative for malignancy in 12 samples. By FNA, 32 mutations were found (18 BRAF, 8 RAS, 5 RET/PTC, and 1 PAX8/PPARγ); after surgery, 31 mutation positive nodules (97%) were diagnosed as malignant on pathological examination, and one was a benign tumor (3%). Thirteen of the 32 mutation-positive FNA samples had a definitive cytologic diagnosis of malignancy, whereas the rest were either indeterminate or negative for malignancy.

In an update to the study described above, Nikiforov et al. reported results of a prospective study to assess the clinical utility of a panel of mutations to predict the likelihood of malignancy in thyroid

nodules that were indeterminate on FNA.[7] The authors included 1056 consecutive FNA samples with indeterminate cytology on FNA that underwent mutation testing, with 967 of those adequate for molecular analysis (653 follicular lesion of undetermined significance/atypia of undetermined significance; 247 follicular or Hurthle cell neoplasm or suspicious for follicular neoplasm; 67 suspicious for malignant cells). All samples from the Ohori et al. analysis described above were included in this cohort. Eighty-seven BRAF, RAS, RET/PTC, or PAX8/PPARγ mutations were detected. At the time of analysis, 479 patients had undergone thyroidectomy for further evaluation, providing a histopathological diagnosis for 513 samples. The presence of a mutation had low sensitivity for predicting malignant histology (63%, 57%, 68% for samples with follicular lesion of undetermined significance/atypia of undetermined significance, follicular or Hurthle cell neoplasm/suspicious for follicular neoplasm, and suspicious for malignant cells on cytology, respectively), but high specificity (99%, 97%, and 96%, respectively). The negative predictive value for the mutation analysis results was 94%, 86%, and 72% for samples with follicular lesion of undetermined significance/atypia of undetermined significance, follicular or Hurthle cell neoplasm/suspicious for follicular neoplasm, and suspicious for malignant cells on cytology, respectively. The authors conclude that mutation analysis may be useful in surgical planning, such as determining whether patients should undergo a thyroid lobectomy or a total thyroidectomy as a first surgery. There were several limitations of this study. This prospective retrospective analysis was limited to samples collected from a single institution. There were samples that were re-analyzed as part of this study that had been previously reported in prior studies. Further, repeated FNA procedures performed on the same nodule part of routine clinical care were not included in this study. The authors note that, repeated FNA can yield a different cytological diagnosis, which may refine clinical management in the absence of molecular testing.

Of the remaining 219 patients, 147 (229 FNAs) who did not undergo surgery were followed by serial ultrasound with no change in the nodule status (124 patients) or by repeated FNA with cytology negative for malignancy (23 patients) and no mutation found in the FNA material. These nodules were considered as negative for malignancy.

The remaining 72 patients that were initially in the follow-up group underwent subsequent surgery.

Combining all 3 groups, the specificity for malignancy was high (99.7%), but the sensitivity of the molecular test alone was 62%.

Moses et al. prospectively tested FNA samples from 417 patients with 455 thyroid nodules for BRAF, NRAS, KRAS, and RET/PTC 1 and 3 and TRK1 mutations.[8] Overall, 50 mutations (23 BRAF V600E, 21NRAS and 4 RET/PTC1 and 2 RET/PTC3 rearrangements) were detected. There were significantly more mutations detected in malignant nodules than in benign (p=0.0001). For thyroid FNA biopsies that were indeterminate or suspicious (n=137), genetic testing had a sensitivity of 12%, specificity of 98%, positive predictive value of 38% and negative predictive value of 65%.

Ohori et al. performed mutation screening in 117 FNA samples classified as a follicular lesion of indeterminate significance/atypia of indeterminate significance.[9] BRAF, RAS, RET/PTC, or PAX8/PPARγ mutations were detected in 10% of this category. They demonstrated that the probability of having a malignancy in this cytology category together with a detection of one of the somatic mutations investigated was 100%, whereas the probability of having a thyroid malignancy without a mutation detected was 7.6%.

Cantara et al. analyzed a panel of mutations in samples of 174 patients undergoing thyroid surgery for indeterminate/inadequate/benign FNA results.[10] The most prevalent mutation was BRAF (49.3% of the positive samples), followed by RAS (34.3%) and RET/PTC (16.4%). The combination of cytology and mutation analysis improved the accuracy for diagnosing cancer from 83% to 93.2% when compared to cytologic analysis alone. Molecular analysis detected 8 thyroid cancers that were missed on cytology from a total of 32 cancers that were diagnosed as indeterminate/inadequate/benign. When the FNA mutation analysis was compared with the mutation analysis of the corresponding histologic material from the surgical sample, in 88.2% of cases, the mutation found in the FNA material was also detected in the histologic samples. The 11.8% discrepant results were due to the presence of a mutation in the tissue sample that was not found in the cytology sample.

Mathur et al. collected thyroid FNA samples, thyroid tissue, clinical and histopathology data, and tumor genotyping for mutations BRAF V600E, NRAS, KRAS, RET/PTC1, RET/PTC3, and NTRK1 for 341 patients with 423 dominant thyroid nodules.[11] A cytologic examination of the samples showed that 51% were benign (one-quarter of these were surgically resected), 21% were malignant, 11% were atypical lesions, 12% were follicular or Hurthle cell neoplasms, and 4% were suspicious for malignancy. On final analysis, 165 nodules were benign and 123 were malignant. Of the 423 FNA samples, 24 BRAF V600E mutations, 7 KRAS, 21 NRAS 4 PAX8-PPARγ rearrangements, 3 RET/PTC1, and 2 RET/PTC3 rearrangements were detected. In all, 17 of 165 (10.3%) benign thyroid nodules had a mutation compared with 26% (32 of 123) malignant tumors ($p<0.05$).

BRAF

Adeniran et al. 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.[1] 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 et al. investigated the utility of BRAF mutation testing of thyroid FNA specimens for preoperative risk stratification of PTC in 190 patients.[12] 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.

<u>Molecular markers to predict benignancy (gene expression classifier)</u>

Analytic Validity

Walsh et al. verified the analytical performance of the Afirma gene expression classifier (GEC) in the classification of cytologically indeterminate fine-needle aspirates from thyroid nodules.[13] The analytical performance studies were designed to characterize the stability of the RNA in the aspirates during collection and shipment, analytical sensitivity and specificity, and assay performance studies including intra-nodule, intra-assay, inter-assay, and inter-laboratory reproducibility. The authors concluded that the analytical sensitivity and specificity, robustness and quality control of the GEC were successfully verified.

Chudova et al. 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

Alexander et al. reported on a 19 month, prospective, multicenter validation study of the Afirma GEC, which involved 49 clinical sites (both academic and community centers), 3,789 patients and 4,812 FNAs from thyroid nodules that were at least 1 cm in size.[14] Local pathology reports of the cytologic diagnosis were collected for all patients, and reports without a definitive benign or malignant diagnosis at the local site were reviewed by 3 expert cytopathologists, who reclassified them as atypical, follicular neoplasm or suspicious for a follicular neoplasm, or suspicious for malignancy. Corresponding histopathologic diagnoses from excised specimens were available (excisions were performed without knowledge of the results of the GEC). After inclusion criteria were met, 265 FNA samples deemed to be cytologically indeterminate were successfully tested with the GEC assay at Veracyte Laboratory. Of the 265, 85 were malignant; the GEC correctly identified 78 of the 85 as suspicious (92% sensitivity; 95% CI: 84-97%), with a specificity of 52% (95% CI: 44-59%). NPV ranged from 85% for “suspicious cytologic findings” to 95% for “atypia of undetermined clinical significance”. There were 7 FNAs with false negative results, 6 of which were thought to be due to hypocellular aspirate specimens.

Harrell and Bimston reported a single center’s results for the diagnostic accuracy of the Afirma GEC.[15] Out of a total sample of 645 FNA results, 58 were classified as indeterminate on cytology (either follicular lesion of undetermined significance/atypia of undetermined significance or follicular neoplasm). Of these, 36 (62%) were classified as suspicious on the Afirma GEC, 20 (34%) were classified as benign, and 2 were inadequate due to low mRNA content. Thirty patients with suspicious GEC findings underwent thyroidectomy, of whom 21 had malignancy on pathology. Five patients with benign GEC findings underwent thyroidectomy, of whom 2 had malignancy on pathology. Based on an assumption about the cancer prevalence in the patient population, the authors report an NPV of 89.6%. Given that a significant proportion of the samples were not assessed with the gold standard for diagnosis, this study does not provide meaningful information about the validity of the Afirma GEC.

Clinical Utility

Duick et al. reported on the impact of Afirma GEC test results on physician and patient decision making to operate on thyroid nodules with indeterminate cytology.[16] This retrospective, multicenter study included patients who were 21 years or older, had one or more thyroid nodules 1 cm or greater by ultrasound, and had an indeterminate diagnosis by cytology and a GEC from the same nodule that was reported as benign. A total of 51 endocrinologists at 21 endocrinology practices in 11 states participated. Data were collected on 368 patients with 395 nodules. The data collection period was September 2011 through March 2012. Surgery was performed in 7.6% of the patients with indeterminate cytology and a benign GEC. (Surgery was primarily performed on those patients with indeterminate cytology and a benign GEC because of large or symptomatic nodules, rapidly growing nodules or a second suspicious or malignant nodule in the same patient, the same reasons typically given for operation on cytologically benign nodules). The authors compared this surgical excision rate of the study population (7.6%) to a historical rate of surgical excision of 74% previously reported for patients with an indeterminate cytologic diagnosis (but no GEC test).

In 2014, Alexander et al. reported results from a retrospective analysis of 339 thyroid nodules which underwent Afirma GEC testing for indeterminate cytology on FNA (follicular lesion of undetermined significance/atypia of undetermined significance, follicular neoplasm, or suspicious for malignancy) at 5 academic medical centers.[17] The majority of nodules sent for GEC testing were follicular lesions of undetermined significance/atypia of undetermined significance or follicular neoplasm. A subset of patients whose nodules underwent GEC testing underwent a subsequent thyroid resection. Among 148 cases with suspicious Afirma GEC findings, surgery (thyroid resection) was recommended for 141 (95%). For the 174 cases with benign Afirma GEC findings, surgery was recommended for 4 (2%; $p<0.01$). Using the assumption that, in the absence of the GEC results, thyroid surgery would be recommended for patients with cytologically indeterminate FNA results, the authors report that the GEC results altered management in 50% of patients. Seventeen patients who had indeterminate cytology, benign Afirma GEC results, and did not undergo surgery had follow up beyond 1 year. Of those, 3 patients underwent surgical removal of the nodule because of compressive symptoms (N=2) or nodule growth (N=1); all nodules were benign on final histology. The remaining 14 patients had ongoing follow up with ultrasound with no ongoing evidence of malignancy. The study demonstrated site-to-site variation in the proportion of samples that were GEC benign. This study suggests that the Afirma GEC may alter clinical management of patients with indeterminate thyroid nodules. While the treating physicians presumably elected to obtain the GEC testing with the intent of altering management recommendations, the magnitude of the difference in surgical recommendations for patients with GEC suspicious or benign results was large. A limitation of this study is its retrospective, unblinded nature; thus, factors other than GEC testing may have contributed to either the recommendation for surgery or patients' decisions to undergo surgery. A benign GEC result did not completely rule out malignant pathology. Long-term follow up was available for only a small proportion of patients with benign GEC findings who did not undergo surgery.

In a single-center study, Aragon Han et al. reported surgical management decision-making outcomes among 114 patients with thyroid nodules who underwent molecular testing.[18] Of 114 patients, 87 underwent thyroid surgery. Testing included a combination of the Afirma gene-expression classifier (N=37), a DNA-based somatic mutation panel (N=21), and testing for BRAF mutations (N=29), BRAF/NRAS (N=1), and BRAF/RET/PTC (N=1). A surgical decision-making algorithm that did not include mutation testing was developed by consensus among four thyroid surgeons. If the surgeon performed the same surgery as anticipated by the management algorithm, then the molecular test was

considered to have no impact. If the surgeon performed a different surgery than anticipated by the management algorithm, the molecular test was considered to effect a change in management. The authors report that surgical management was not changed by molecular testing in 89.7% of cases. This study is limited by its use of multiple types of molecular testing, along with a non-standardized incorporation of molecular genetic testing results into the surgical decision-making. As such, the study has limited implications for the clinical utility of molecular diagnostics for thyroid cancer.

Clinical Practice Guidelines

American Thyroid Association (ATA)

The ATA states that the currently available mutation analysis panel and gene expression classifier have promising roles, but that at this time, experience with them remains limited. The ATA feels that until an expert consensus review of existing data (currently underway) can be completed, no evidence-based recommendation for or against the use of these methods can be made.[19] The most recent ATA guidelines on the management of thyroid nodules is from 2009, before the widespread clinical availability of mutation analysis or gene expression profiles for thyroid cancer.[2]

National Comprehensive Cancer Network (NCCN)

NCCN Guidelines on the treatment of thyroid cancer state that "molecular diagnostics to detect individual mutations in BRAF, RET, or RAS or pattern recognition approaches using molecular classifiers may be useful in the evaluation of FNA samples that are indeterminate (i.e., follicular lesion of undetermined significance") (evidence is category 2A).[20] In the guidelines' 2013 update, the NCCN added the guidelines to consider molecular diagnostics in cases where FNA results were (1) Follicular or Hurthle cell neoplasm or (2) Follicular lesion of undetermined significance (category 2A recommendation).

Summary

Mutation Analysis

Mutation analysis of fine needle aspirates (FNA) of the thyroid that are cytologically indeterminate has a high positive predictive value for malignancy. However, patients with an equivocal FNA result would likely proceed to surgery regardless of mutation status, with intraoperative consultation to guide the necessity and extent of surgery. Mutation analysis does not achieve a high enough negative predictive value to identify which patients can undergo watchful waiting over thyroid surgery. Although the presence of certain mutations may predict more aggressive malignancies, the clinical utility of identifying these mutations preoperatively has not been established. The incremental added value of mutation analysis to an equivocal FNA result is not known. Although mutation analysis has the potential to improve the accuracy of an equivocal FNA of the thyroid and may play a role in preoperative risk stratification and surgical planning, at this time, it is not clear how it will impact patient management or surgical decision making; therefore, it is considered investigational.

Gene Expression Classifier (GEC)

The reported negative predictive value of the GEC in predicting which thyroid nodules with indeterminate cytology are benign is high. It is not clear whether the reported diagnostic accuracy is high enough to allow for following these thyroid lesions clinically instead of surgically resecting them;

therefore, the use of a GEC to predict which thyroid nodules with indeterminate cytology are benign is considered investigational.

REFERENCES

1. Adeniran, AJ, Theoharis, C, Hui, P, et al. Reflex BRAF testing in thyroid fine-needle aspiration biopsy with equivocal and positive interpretation: a prospective study. *Thyroid*. 2011 Jul;21(7):717-23. PMID: 21568726
2. Cooper, DS, Doherty, GM, Haugen, BR, et al. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid*. 2009 Nov;19(11):1167-214. PMID: 19860577
3. Chudova, D, Wilde, JI, Wang, ET, et al. Molecular classification of thyroid nodules using high-dimensionality genomic data. *J Clin Endocrinol Metab*. 2010 Dec;95(12):5296-304. PMID: 20826580
4. Nikiforov, YE. Molecular diagnostics of thyroid tumors. *Arch Pathol Lab Med*. 2011 May;135(5):569-77. PMID: 21526955
5. Ferraz, C, Eszlinger, M, Paschke, R. Current state and future perspective of molecular diagnosis of fine-needle aspiration biopsy of thyroid nodules. *J Clin Endocrinol Metab*. 2011 Jul;96(7):2016-26. PMID: 21593119
6. Nikiforov, YE, Steward, DL, Robinson-Smith, TM, et al. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. *J Clin Endocrinol Metab*. 2009 Jun;94(6):2092-8. PMID: 19318445
7. Nikiforov, YE, Ohori, NP, Hodak, SP, et al. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. *J Clin Endocrinol Metab*. 2011;96:3390-7. PMID: 21880806
8. Moses, W, Weng, J, Sansano, I, et al. Molecular testing for somatic mutations improves the accuracy of thyroid fine-needle aspiration biopsy. *World J Surg*. 2010 Nov;34(11):2589-94. PMID: 20703476
9. Ohori, NP, Nikiforova, MN, Schoedel, KE, et al. Contribution of molecular testing to thyroid fine-needle aspiration cytology of "follicular lesion of undetermined significance/atypia of undetermined significance". *Cancer cytopathology*. 2010 Feb 25;118(1):17-23. PMID: 20099311
10. Cantara, S, Capezzone, M, Marchisotta, S, et al. Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. *J Clin Endocrinol Metab*. 2010 Mar;95(3):1365-9. PMID: 20130073
11. Mathur, A, Weng, J, Moses, W, et al. A prospective study evaluating the accuracy of using combined clinical factors and candidate diagnostic markers to refine the accuracy of thyroid fine needle aspiration biopsy. *Surgery*. 2010 Dec;148(6):1170-6; discussion 6-7. PMID: 21134548
12. Xing, M, Clark, D, Guan, H, et al. BRAF mutation testing of thyroid fine-needle aspiration biopsy specimens for preoperative risk stratification in papillary thyroid cancer. *J Clin Oncol*. 2009 Jun 20;27(18):2977-82. PMID: 19414674
13. Walsh, PS, Wilde, JI, Tom, EY, et al. Analytical performance verification of a molecular diagnostic for cytology-indeterminate thyroid nodules. *J Clin Endocrinol Metab*. 2012 Dec;97(12):E2297-306. PMID: 23087323
14. Alexander, EK, Kennedy, GC, Baloch, ZW, et al. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. *N Engl J Med*. 2012 Aug 23;367(8):705-15. PMID: 22731672

15. Harrell, RM, Bimston, DN. Surgical utility of afirma: effects of high cancer prevalence and oncocytic cell types in patients with indeterminate thyroid cytology. *Endocr Pract*. 2014;20:364-9. PMID: 24246351
16. Duick, DS, Klopper, JP, Diggans, JC, et al. The impact of benign gene expression classifier test results on the endocrinologist-patient decision to operate on patients with thyroid nodules with indeterminate fine-needle aspiration cytopathology. *Thyroid*. 2012 Oct;22(10):996-1001. PMID: 22873825
17. Alexander, EK, Schorr, M, Klopper, J, et al. Multicenter clinical experience with the Afirma gene expression classifier. *J Clin Endocrinol Metab*. 2014;99:119-25. PMID: 24152684
18. Aragon Han, P, Olson, MT, Fazeli, R, et al. The Impact of Molecular Testing on the Surgical Management of Patients with Thyroid Nodules. *Ann Surg Oncol*. 2014 Feb 13. PMID: 24522987
19. Hodak, SP, Rosenthal, DS. Information for clinicians: commercially available molecular diagnosis testing in the evaluation of thyroid nodule fine-needle aspiration specimens. *Thyroid*. 2013 Feb;23(2):131-4. PMID: 22984796
20. National Comprehensive Cancer Network (NCCN). Clinical Practice Guidelines in Oncology™. Thyroid Carcinoma. v.2.2013. [cited 03/2014]; Available from: http://www.nccn.org/professionals/physician_gls/pdf/thyroid.pdf

CROSS REFERENCES

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

CODES	NUMBER	DESCRIPTION
CPT	81210	BRAF (v-raf murine sarcoma viral oncogene homolog B1) (eg, colon cancer), gene analysis, V600E variant
	81401	Molecular pathology procedure, Tier 2, Level 2
	81404	Molecular pathology procedure, Tier 2, Level 5
	81405	Molecular pathology procedure, Tier 2, Level 6
	81406	Molecular pathology procedure, Tier 2, Level 7
	81599	Unlisted multianalyte assay with algorithmic analysis
HCPCS	None	