Kansas City

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Molecular Markers in Fine Needle Aspirates of the Thyroid

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Origination: 3/2013
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Next Review: 3/2015

Policy

Blue Cross and Blue Shield of Kansas City (Blue KC) will not provide coverage for molecular markers in fine needle aspirates of the thyroid. This is considered investigational.

When Policy Topic is covered

Not Applicable

When Policy Topic is not covered

Mutation analysis in fine-needle aspirates of the thyroid is considered to be **investigational.** (Note: This is a type of genetic testing that may be excluded in some contracts. Verify benefits prior to review.)

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 to be **investigational.**

Description of Procedure or Service

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 mutation 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) and a gene expression classifier to identify patients who do not need surgery and can be safely followed.

Mutations associated with thyroid cancer

Various mutations 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 [mutations] (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.

Rationale

This policy was created in January 2012. The most recent update includes a MEDLINE literature through December 2012.

Literature Review

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 and colleagues 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 and colleagues 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.

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 and colleagues prospectively tested FNA samples from 417 patients with 455 thyroid nodules for BRAF, NRAS, KRAS, and RET/PTC 1 and 3 and TRK1 mutations. (7) 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 and colleagues performed mutation screening in 117 FNA samples classified as a follicular lesion of indeterminate significance/atypia of indeterminate significance. (8) 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 and colleagues analyzed a panel of mutations in samples of 174 patients undergoing thyroid surgery for indeterminate/inadequate/benign FNA results. (9) 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 and colleagues 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. (10) 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 and colleagues 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 and colleagues investigated the utility of BRAF mutation testing of thyroid FNA specimens for preoperative risk stratification of PTC in 190 patients. (11) 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.

Molecular markers to predict benignancy (gene expression classifier)

Analytic validity

Walsh and colleagues verified the analytical performance of the Afirma gene expression classifier (GEC) in the classification of cytologically indeterminate fine-needle aspirates from thyroid nodules. (12) 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 and colleagues 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, NPV and specificity were estimated to be 96% and 84%, respectively.

Clinical validity

Alexander and colleagues 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. (13) 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 [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.

Clinical utility
Duick and colleagues reported on the impact of Afirma GEC test results on physician and patient decision making to operate on thyroid nodules with indeterminate cytology. (14) This retrospective, multicenter study included patients who were 21 years or older, and 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 was 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 these 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).

National Cancer Institute Clinical Trials
No Phase 3 trials analyzing mutation analysis in fine needle aspirates of the thyroid were identified.

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, and 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 and 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. However, only one retrospective study on the clinical utility of the GEC has been published, and long-term follow-up of the patients who avoided surgery based on a benign GEC test is not available; 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.

Practice Guidelines and Position Statements
The American Thyroid Association (ATA) guidelines suggest consideration of the use of molecular markers like BRAF, RAS, RET/PTC, PAX8-PPARγ or galectin-3 for patients with indeterminate cytology on FNA and that if detectable, these markers can help guide thyroid nodule management by assisting in estimating thyroid cancer risk. (15)

National Comprehensive Cancer Network (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”). (16)

Medicare National Coverage
There is no national coverage determination.

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Billing Coding/Physician Documentation Information

There is no specific CPT code for this testing. It would be reported using CPT code 81599 – unlisted multianalyte assay with algorithmic analysis.

Additional Policy Key Words

N/A

Policy Implementation/Update Information

3/1/13	New policy; considered investigational.
3/1/14	No policy statement changes.