Molecular Markers in Fine Needle Aspirates of the Thyroid

Description

To determine which patients need thyroid resection, many physicians will perform a cytologic examination of fine needle aspirate (FNA) samples from a thyroid lesion; however, this method has diagnostic limitations. As a result, assays using molecular markers have been developed to improve the accuracy of thyroid FNA biopsies.

OBJECTIVE

The objective of this evidence review is to evaluate whether testing for molecular markers in fine needle aspirates of the thyroid improves the net health outcome in individuals with thyroid nodule(s) with an indeterminate finding on the fine needle aspirate.
POLICY STATEMENT

The use of either Afirma Genomic Sequencing Classifier or ThyroSeq in fine needle aspirates of thyroid nodules with indeterminate cytologic findings (ie, Bethesda diagnostic category III [atypia/follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm/suspicion for a follicular neoplasm]) may be considered medically necessary in patients who have the following characteristics:

- Thyroid nodules without strong clinical or radiologic findings suggestive of malignancy
- In whom surgical decision making would be affected by test results.

The use of any of the following types of molecular marker testing or gene variant analysis in fine needle aspirates of thyroid nodules with indeterminate findings (Bethesda diagnostic category III [atypia/follicular lesion of undetermined significance] or Bethesda diagnostic category IV [follicular neoplasm/suspicion for a follicular neoplasm]) or suspicious findings (Bethesda diagnostic category V [suspicious for malignancy]) to rule in malignancy to guide surgical planning for initial resection rather than a 2-stage surgical biopsy followed by definitive surgery may be considered medically necessary:

- ThyroSeq;
- ThyraMIR microRNA/ThyGenX;
- Afirma BRAF after Afirma Genomic Sequencing Classifier; or
- Afirma MTC after Afirma Genomic Sequencing Classifier.

Gene expression classifiers, genetic variant analysis, and molecular marker testing in fine needle aspirates of the thyroid not meeting criteria outlined above, including but not limited to use of RosettaGX Reveal and single-gene TERT testing, are considered investigational.

POLICY GUIDELINES

In patients who do not undergo surgical biopsy or thyroidectomy on the basis of gene expression classifier or molecular marker results, regular active surveillance is indicated.

Use of molecular marker testing based on fine needle aspirate of a thyroid nodule to rule in malignancy prior to surgical biopsy may guide surgical planning, particularly factors such as choice of surgical facility provider to ensure that the capability is available to conduct a frozen section pathologic reading during surgical biopsy so that surgical approach may be adjusted accordingly in 1 surgery.

Genetics Nomenclature Update

The Human Genome Variation Society nomenclature is used to report information on variants found in DNA and serves as an international standard in DNA diagnostics. It is being implemented for genetic testing medical evidence review updates starting in 2017 (see Table PG1). The Society’s nomenclature is recommended by the Human Variome Project, the Human Genome Organization, and by the Human Genome Variation Society itself.

The American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for interpretation of sequence variants represent expert opinion from both organizations, in addition to the College of American Pathologists. These recommendations primarily apply to genetic tests used in clinical laboratories, including genotyping, single genes, panels, exomes, and genomes. Table PG2 shows the recommended standard terminology—"pathogenic," "likely pathogenic," "uncertain significance," "likely benign," and "benign"—to describe variants identified that cause Mendelian disorders.

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Table PG1. Nomenclature to Report on Variants Found in DNA

<table>
<thead>
<tr>
<th>Previous</th>
<th>Updated</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Disease-associated</td>
<td>Disease-associated change in the DNA sequence</td>
</tr>
<tr>
<td></td>
<td>variant</td>
<td></td>
</tr>
<tr>
<td>Variant</td>
<td>Change in the DNA</td>
<td>sequence</td>
</tr>
<tr>
<td></td>
<td>sequence</td>
<td></td>
</tr>
<tr>
<td>Familial</td>
<td>Disease-associated</td>
<td>variant identified in a proband for use in subsequent targeted genetic</td>
</tr>
<tr>
<td></td>
<td>variant</td>
<td>testing in first-degree relatives</td>
</tr>
</tbody>
</table>

Table PG2. ACMG-AMP Standards and Guidelines for Variant Classification

<table>
<thead>
<tr>
<th>Variant Classification</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathogenic</td>
<td>Disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Likely pathogenic</td>
<td>Likely disease-causing change in the DNA sequence</td>
</tr>
<tr>
<td>Variant of uncertain</td>
<td>Change in DNA sequence with uncertain effects</td>
</tr>
<tr>
<td>significance</td>
<td>on disease</td>
</tr>
<tr>
<td>Likely benign</td>
<td>Likely benign change in the DNA sequence</td>
</tr>
<tr>
<td>Benign</td>
<td>Benign change in the DNA sequence</td>
</tr>
</tbody>
</table>

ACMG: American College of Medical Genetics and Genomics; AMP: Association for Molecular Pathology.

Genetic Counseling

Experts recommend formal genetic counseling for patients who are at risk for inherited disorders and who wish to undergo genetic testing. Interpreting the results of genetic tests and understanding risk factors can be difficult for some patients; genetic counseling helps individuals understand the impact of genetic testing, including the possible effects the test results could have on the individual or their family members. It should be noted that genetic counseling may alter the utilization of genetic testing substantially and may reduce inappropriate testing; further, genetic counseling should be performed by an individual with experience and expertise in genetic medicine and genetic testing methods.
BENEFIT APPLICATION

Screening (other than the preventive services listed in the brochure) is not covered. Please see Section 6 General exclusions.

Benefits are available for specialized diagnostic genetic testing when it is medically necessary to diagnose and/or manage a patient's existing medical condition. Benefits are not provided for genetic panels when some or all of the tests included in the panel are not covered, are experimental or investigational, or are not medically necessary.

Experimental or investigational procedures, treatments, drugs, or devices are not covered (See General Exclusion Section of brochure).

FDA REGULATORY STATUS

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests must meet the general regulatory standards of the Clinical Laboratory Improvement Amendments. Thyroid variant testing and gene expression classifiers are available under the auspices of the Clinical Laboratory Improvement Amendments. Laboratories that offer laboratory-developed tests must be licensed by the Clinical Laboratory Improvement Amendments for high-complexity testing. To date, the U.S. Food and Drug Administration has chosen not to require any regulatory review of this test.

In 2013, the THxID™-BRAF kit (bioMérieux), an in vitro diagnostic device, was approved by the Food and Drug Administration through the premarket approval process to assess specific BRAF variants in melanoma tissue via real-time PCR. However, there are currently no diagnostic tests for thyroid cancer mutation analysis with approval from the Food and Drug Administration.

Table 1 provides a summary of commercially available molecular diagnostic tests for indeterminate thyroid pathology.

<table>
<thead>
<tr>
<th>Test</th>
<th>Predicate</th>
<th>Methodology</th>
<th>Analyte(s)</th>
<th>Report</th>
</tr>
</thead>
<tbody>
<tr>
<td>Afirma GSC</td>
<td>AfirmaGEC</td>
<td>mRNA gene expression</td>
<td>1,115 genes</td>
<td>Benign/suspicious</td>
</tr>
<tr>
<td>Afirma BRAF</td>
<td></td>
<td>mRNA gene expression</td>
<td>1 gene</td>
<td>Negative/positive</td>
</tr>
<tr>
<td>Afirma MTC</td>
<td></td>
<td>mRNA gene expression</td>
<td></td>
<td>Negative/positive</td>
</tr>
<tr>
<td>ThyroSeq v3</td>
<td>ThyroSeq v2</td>
<td>Next-generation sequencing</td>
<td>112 genes</td>
<td>Specific gene variant/translocation</td>
</tr>
<tr>
<td>ThyGeNEXT</td>
<td>ThyGenX³, miRInform³</td>
<td>Next-generation sequencing</td>
<td>10 genes and 32 gene fusions</td>
<td>Specific gene variant/translocation</td>
</tr>
<tr>
<td>ThyraMIR™</td>
<td></td>
<td>microRNA expression</td>
<td>10 microRNAs</td>
<td>Negative/positive</td>
</tr>
</tbody>
</table>

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**RATIONALE**

**Summary of Evidence**

To determine which patients need thyroid resection, many physicians will perform a cytologic examination of FNA samples from a thyroid lesion; however, this method has diagnostic limitations. As a result, assays using molecular markers have been developed to improve the accuracy of thyroid FNA biopsies.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular tests to rule out malignancy and to avoid surgical biopsy or resection, the evidence includes a prospective clinical validity study with the Afirma GEC and a chain of evidence to support clinical utility. The relevant outcomes are disease-specific survival, test accuracy and validity, morbidity events, and resource utilization. In a multicenter validation study, the Afirma GSC was reported to have a high (NPV 96%; 95% CI, 90.9%-99%). These results are consistent with an earlier study on the Afirma GEC in the same study population. In other multicenter and single-center studies, there is suggestive evidence that rates of malignancy are low in Afirma patients who are classified as benign, but the exact NPV is unknown. The available evidence suggests that the decisions a physician makes regarding surgery are altered by Afirma GEC/GSC results; however, it should be noted that long-term follow-up of patients with thyroid nodules who avoided surgery based on GEC results is limited. A chain of evidence can be constructed to establish the potential for clinical utility with GEC testing in cytologically indeterminate lesions, but there is only a single study of the marketed test reporting a true NPV. Clinical input, obtained in 2017, supported the use of the previous version of the Afirma test in FNA of thyroid nodules with indeterminate cytologic findings to rule out malignancy and avoid surgical biopsy with an acceptably low trade-off in missed malignancy. The evidence is sufficient to determine that the technology improves the net health outcome.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular tests to rule out malignancy and to guide surgical planning, the evidence includes prospective and retrospective studies of clinical validity. The relevant outcomes are disease-specific survival, test accuracy and validity, morbidity events, and resource utilization. Variant 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. Single-center studies have suggested that testing for a panel of genetic variants associated with thyroid cancer may allow for the appropriate selection of patients for surgical management for the initial resection. Prospective studies in additional populations are needed to validate these results. Although the presence of certain variants may predict more aggressive malignancies, the management changes that would occur as a result of identifying higher risk tumors, are not well-established. Clinical input, obtained in 2017, considered ThyraMIR microRNA/ThyGenX, Afirma BRAF after Afirma GEC, and Afirma MTC after Afirma GEC to provide a clinically meaningful improvement for patients with cytologic findings suspicious for malignancy to guide surgical planning for the initial resection. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

For individuals with thyroid nodule(s) and indeterminate findings on FNA who receive FNA sample testing with molecular tests to rule out malignancy and avoid surgical biopsy or to rule in malignancy for surgical planning, the evidence includes multiple retrospective and prospective clinical validation studies for the ThyroSeq test and 2 retrospective clinical validation studies that used a predicate test 17-variant panel (miRInform) test to the current ThyGenX and ThyraMIR. The relevant outcomes are disease-specific survival, test

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accuracy and validity, morbid events, and resource utilization. In a retrospective validation study on FNA samples, the 17-variant panel (miRInform) test and ThyraMIR had a sensitivity of 89%, and an NPV of 94%. A prospective clinical validation study of ThyroSeq v3 reported an NPV of 97% and PPV of 68%. No studies were identified demonstrating the diagnostic characteristics of the marketed ThyGenX. No studies were identified demonstrating evidence of direct outcome improvements. A chain of evidence for the ThyroSeq v3 test and combined ThyGenX and ThyraMIR testing would rely on establishing clinical validity. Clinical input, obtained in 2017, considered ThyroSeq v2 to provide a clinically meaningful improvement for patients with indeterminate cytologic findings to rule out malignancy and avoid surgical biopsy and in patients with cytologic findings suspicious for malignancy to guide surgical planning for the initial resection. The evidence is sufficient to determine that the technology results in a meaningful improvement in the net health outcome.

SUPPLEMENTAL INFORMATION

Practice Guidelines and Position Statements

American Association of Clinical Endocrinologists et al

The American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazone Medici Endocrinologi (2016) updated their joint guidelines on molecular testing for cytologically indeterminate thyroid nodules, stating:59:

- "Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the negative predictive values (NPVs) and positive predictive values (PPVs) for molecular testing."
- "Consider the detection of BRAF and RET/PTC and, possibly, PAX8/PPARG and RAS mutations if such detection is available."
- "TERT mutational analysis on FNA, when available, may improve the diagnostic sensitivity of molecular testing on cytologic samples."
- "Because of the insufficient evidence and the limited follow-up, we do not recommend either in favor of or against the use of gene expression classifiers (GECs) for cytologically indeterminate nodules."

For the role of molecular testing for deciding the extent of surgery the following recommendations were made:

- "Currently, with the exception of mutations such as BRAFV600E that have a PPV approaching 100% for papillary thyroid carcinoma (PTC), evidence is insufficient to recommend in favor of or against the use of mutation testing as a guide to determine the extent of surgery."

American Thyroid Association

The American Thyroid Association (2016) updated its guidelines on the management of thyroid nodules and differentiated thyroid cancer in adults.60 These guidelines made the following statements on molecular diagnostics in thyroid nodules that are atypia of undetermined significance or follicular lesion of undetermined significance on cytology and follicular neoplasm or suspicious for follicular neoplasm on cytology (see Table 2).

Table 2. Molecular Diagnostics in Thyroid Nodules That Are AUS or FLUS or FN or SFN on Cytology

<table>
<thead>
<tr>
<th>Recommendation</th>
<th>SOR</th>
<th>QOE</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUS or FLUS</td>
<td></td>
<td></td>
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</tbody>
</table>
"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."

| AUS: atypia of undetermined significance; FLUS: follicular lesion of undetermined significance; FN: follicular neoplasm; FNA: fine needle aspirate; QOE: quality of evidence; SFN: suspicious for follicular neoplasm; SOR: strength of evidence. |
|---|---|

"If repeat FNA cytology, molecular testing, or both are not performed or inconclusive, either surveillance or diagnostic surgical excision may be performed for an AUS/FLUS thyroid nodule, depending on clinical risk factors, sonographic pattern, and patient preference."

| "Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making." |

| FN or SFN |
|---|---|

"Diagnostic surgical excision is the long-established standard of care for the management of FN/SFN cytology nodules. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery. Informed patient preference and feasibility should be considered in clinical decision-making." |

The guidelines also stated: "there is currently no single optimal molecular test that can definitively rule in or rule out malignancy in all cases of indeterminate cytology, and long-term outcome data proving clinical utility are needed."

**National Comprehensive Cancer Network**

National Comprehensive Cancer Network (v.1.2019) guidelines on the treatment of thyroid cancer comment on the use of molecular diagnostics in thyroid cancer. For thyroid nodules evaluated with FNA, molecular diagnostics may be employed when lesions are suspicious for:

- Follicular or Hurthle cell neoplasms.
- Atypia of undetermined significance or follicular lesions of undetermined significance.

The guidelines state that molecular diagnostics may not perform well for Hurthle cell neoplasms.

**U.S. Preventive Services Task Force Recommendations**

Not applicable.

**Medicare National Coverage**

There is no national coverage determination. In the absence of a national coverage determination, coverage decisions are left to the discretion of local Medicare carriers.
MolDX Program contractors Palmetto GBA, Wisconsin Physicians Service Insurance Corp., and CGS Administrators determined that the Afirma Gene Expression Classifier test meets criteria for analytic and clinical validity and clinical utility as a reasonable and necessary Medicare benefit. Effective 2015, the MolDX Program contractors will reimburse Afirma Gene Expression Classifier services for patients with the following conditions:

- Patients with one or more thyroid nodules with a history or characteristics suggesting malignancy such as:
  - Nodule growth over time
  - Family history of thyroid cancer
  - Hoarseness, difficulty swallowing or breathing
  - History of exposure to ionizing radiation
  - Hard nodule compared with rest of gland consistency
  - Presence of cervical adenopathy
- Have an indeterminate follicular pathology on fine needle aspiration.

REFERENCES


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13. Song YS, Lim JA, Choi H, et al. Prognostic effects of TERT promoter mutations are enhanced by coexistence with BRAF or RAS mutations and strengthen the risk prediction by the ATA or TNM staging system in differentiated thyroid cancer patients. Cancer. May 1 2016;122(9):1370-1379. PMID 26969876.


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