Test Specific Guidelines

Afirma Thyroid Cancer Classifier Tests

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Introduction

Afirma thyroid cancer classifier tests are addressed by this guideline.

Procedures Addressed

The inclusion of any procedure code in this table is provided for informational purposes and is not a guarantee of coverage nor an indication that prior authorization is required.

Procedures addressed by this guideline	Procedure codes
Afirma Genomic Sequencing Classifier	81546
Afirma Xpression Atlas	0204U
BRAF V600 Targeted Mutation Analysis	81210

What Are Thyroid Nodules?

Definition

Thyroid nodules are relatively common; however, only approximately 15% of nodules are malignant.[1] Fine-needle aspiration (FNA) biopsy with accompanying cytology examination is the standard method for distinguishing between benign and malignant nodules and subsequent removal of tumors. However, approximately 15 to 30% of thyroid nodules examined using FNA and traditional cytology examination are classified in one of the cytologically indeterminate categories of the Bethesda System for Reporting Thyroid Cytopathology. Due to the low to moderate cancer risks associated with these indeterminate categories, clinicians are faced with difficult management decisions.[1-3]

Molecular testing technologies have been developed to help further classify indeterminate nodules as either benign or malignant to guide management appropriately. These technologies usually involve assessment of known genetic point mutations and gene fusions, or through the expression of messenger RNA and/or microRNA.[2,3]

Test Information

Introduction

Afirma testing may include a combination of cytopathology and molecular testing.[4] This guideline addresses only the molecular testing components.

The Afirma Genomic Sequencing Classifier (GSC) is intended for:[4]

- cytologically indeterminate FNA biopsy samples including atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), and
- follicular or Hürthle Cell Neoplasms.[4]

The Afirma tests should be performed in conjunction with cytopathology, ultrasound assessment, and other clinical factors to determine an individual's risk of thyroid cancer and the necessity and extent of thyroid surgery.[5]

When Afirma Testing Is Performed

A FNA sample can be submitted for cytopathology assessment.

If the cytopathology assessment is ...	Then ...
benign or malignant	the analysis is complete.
indeterminate	the GSC is performed.

Afirma GSC

The Afirma Genomic Sequencing Classifier (GSC) is a second-generation test that has replaced the original Gene Expression Classifier (GEC).

The Afirma Genomic Sequencing Classifier (GSC) was developed and clinically validated to utilize genomic material obtained during the FNA to accurately identify benign nodules among those deemed cytologically indeterminate so that diagnostic surgery can be avoided.[6]

The GSC test is a next generation RNA sequencing analysis that assesses expression levels as well as analysis of copy number and loss of heterozygosity.[1,6] The purpose of the GSC is to further differentiate indeterminate FNA. The positive predictive value of the GSC is 47.1%.[1]

Results

Afirma GSC results may help guide surgical decision making in patients with thyroid nodules. [4,6]

In addition to the benign versus malignant classifier, the Afirma GSC suite includes three other genomic classifiers that may be requested or performed: a

parathyroid (PTA) classifier, a medullary thyroid cancer (MTC) classifier, and a BRAF V600E classifier.[6]

Afirma Malignancy Classifiers

The Afirma Malignancy Classifiers are intended to help guide surgical decisions when the cytopathology or Afirma GSC result suggests the individual should be considered for surgery.[4,6,7]

Afirma Xpression Atlas

The Afirma Xpression Atlas is an RNA sequencing-based test. The test is designed to analyze 905 variants and 235 fusions in 593 genes that have been linked to thyroid cancer. This testing is performed on nodules that are suspicious for malignancy.[4]

Guidelines and Evidence

Introduction

This section includes relevant guidelines and evidence pertaining to Afirma GSC testing.

American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi (AACE/ACE/AME) Guidelines

The AACE/ACE/AME 2016 Clinical Practice Guidelines for the Diagnosis and Management of Thyroid Nodules state the following:[8]

- In nodules with indeterminate cytologic results, no single cytochemical or genetic marker is specific or sensitive enough to rule out malignancy with certainty. However, the use of immunohistochemical and molecular markers may be considered together with the cytologic subcategories and data from US (ultrasound), elastography, or other imaging techniques to obtain additional information for management of these patients.
- When molecular testing should be considered:
  - To complement not replace cytologic evaluation (BEL 2, GRADE A)
  - The results are expected to influence 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)
- Molecular testing for cytologically indeterminate nodules
  - Cytopathology expertise, patient characteristics, and prevalence of malignancy within the population being tested impact the NPV and PPV for molecular testing (BEL 3, GRADE B)
  - Consider detection of BRAF and RET/PTC and, possibly PAX8/PPARG and RAS mutations if such detection is available (BEL 2, GRADE B)
  - Because of the insufficient evidence and limited follow-up, we do not recommend either in favor of or against the use of gene expression

classifiers (GECs) for cytologically indeterminate modules (BEL 2, GRADE B)

- Role of molecular testing for deciding the extent of surgery
  - Currently, with the exception of mutations such as BRAFV600E that have a PPV approaching 100% for papillary thyroid carcinoma (PTC), the 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)
- How should patient with nodules that are negative at mutation testing be monitored?
  - Since 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 GEC are still insufficient, close follow-up is recommended (BEL 3, GRADE B)

American Thyroid Association

The American Thyroid Association (ATA, 2016) makes the following statement regarding molecular testing and FNA-indeterminate thyroid nodules:[9]

- "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)"
- "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. (Strong recommendation, Low-quality evidence)"

National Comprehensive Cancer Network

The National Comprehensive Cancer Network (NCCN, 2021) Thyroid Carcinoma Guidelines state the following:[10]

- "The diagnosis of follicular carcinoma or Hürthle cell carcinoma requires evidence of either vascular or capsular invasion, which cannot be determined by FNA. Molecular diagnostics may be useful to allow reclassification of follicular lesions (i.e. follicular neoplasm, AUS, FLUS) as either more or less likely to be benign or malignant based on the genetic profile. If molecular testing suggests papillary thyroid carcinoma, especially in the case of BRAF V600E, see (PAP-1). If molecular testing, in conjunction with clinical and ultrasound features, predicts a risk of malignancy comparable to the risk of

malignancy seen with a benign FNA cytology (approximately 5% or less), consider active surveillance. Molecular markers should be interpreted with caution and in the context of clinical, radiographic, and cytologic features of each individual patient."

- "Because the published studies have focused primarily on adult patients with thyroid nodules, the diagnostic utility of molecular diagnostics in pediatric patients remains to be defined. Therefore proper implementation of molecular diagnostics into clinical care requires an understanding of both the performance characteristics of the specific molecular tests and its clinical meaning across a range of pre-test disease probabilities."
- "While molecular diagnostic testing may be useful for diagnosing NIFTP [noninvasive follicular thyroid neoplasms with papillary-like nuclear features] in the future, currently available tests were not validated using NIFTP samples. … However, multiple studies investigating the performance of molecular diagnostics for this subtype have reported that most thyroid nodules histologically diagnosed as NIFTP are classified as "suspicious" by GEC, possibly leading to a more aggressive surgical treatment than is necessary. Therefore the validation of molecular diagnostics with NIFTP samples will be necessary to ensure that the tests are accurately classifying these."

Selected Relevant Publications

Endo et al (2019) compared the performance of the Afirma GSC test (146 nodules) with that of the GEC test (343 nodules). They found the GSC test to have higher positive predictive value (60% vs. 30%) and sensitivity (94% vs 61%) in Bethesda III and IV nodules.[11]

A single peer-reviewed study evaluated the analytical and clinical validity of Xpression Atlas testing.[12] This study evaluated Xpression Atlas against targeted DNA and RNA panels in thyroid FNA samples. No confidence intervals were provided in this study for sensitivity, specificity, PPV, or NPV. The authors did provide confidence intervals for performance estimates but these were wide, suggesting low precision, high uncertainty, and/or too small of a sample size. Thus, the clinical usefulness of Xpression Atlas remains uncertain. Additionally, the training and test sets were data used from previous validation studies of other Afirma tests. No clinical utility studies were identified evaluating the use of Xpression Atlas.

Criteria

Introduction

Requests for Afirma GSC testing are reviewed using these criteria.

Afirma Genomic Sequencing Classifier (GSC)

- Testing Multiple Samples:
  - The Afirma GSC is reimbursed only once per date of service regardless of the number of nodules submitted for testing, and
  - The Afirma GSC is indicated only once per thyroid nodule per lifetime.

- **Required Clinical Characteristics:**
  - **Afirma GSC is indicated for thyroid nodules with indeterminate FNA results that are included in the following cytopathology categories:**
    - **Atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), or**
    - **Follicular or Hürthle cell neoplasm, and**
  - **The patient is not undergoing thyroid surgery for diagnostic confirmation.**
- **Required Testing Process:**
  - **If FNA of a nodule is indicated to evaluate for malignancy, and the sample is sent to Veracyte for cytopathology, the classifier is only indicated when the result is indeterminate, and**
  - **Supporting documentation of an appropriate indeterminate cytology result will be required for reimbursement.**

Afirma BRAF V600E

Afirma BRAF testing may be considered for either GSC or FNA suspicious or malignant results. For information on BRAF testing, please refer to the guideline *Somatic Mutation Testing - Solid Tumors*.

Afirma Xpression Atlas

This test is considered investigational and/or experimental.

- **Investigational and experimental (I&E) molecular and genomic (MolGen) tests refer to assays involving chromosomes, DNA, RNA, or gene products that have insufficient data to determine the net health impact, which typically means there is insufficient data to support that a test accurately assesses the outcome of interest (analytical and clinical validity), significantly improves health outcomes (clinical utility), and/or performs better than an existing standard of care medical management option. Such tests are also not generally accepted as standard of care in the evaluation or management of a particular condition.**
- **In the case of MolGen testing, FDA clearance is not a reliable standard given the number of laboratory developed tests that currently fall outside of FDA oversight and FDA clearance often does not assess clinical utility.**

Billing and Reimbursement

Afirma BRAF testing in conjunction with a GSC indeterminate result will not be reimbursed.

Afirma MTC may not be billed separately using an additional unit or procedure code.

References

Introduction

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