

ASC Companion Meeting at the 2017 USCAP: Ancillary Molecular Testing in "Indeterminate" Thyroid Nodules: How Far Have We Come?

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The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) includes 3 diagnostic categories that are considered indeterminate or the so-called "gray zone." Various molecular testing panels have been introduced to help resolve management issues for thyroid aspirates classified into one of the indeterminate categories. These panels differ in their negative and positive predictive values, effectiveness for different cytologic patterns, accuracy, and cost. Despite progress in the application of various molecular tests, recent introduction of non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) creates new challenges for the way that we use molecular testing for thyroid FNAs within the 3 indeterminate categories.

Objective: The participant will be familiar with molecular testing options and limitations for thyroid FNAs within the indeterminate categories.

OVERVIEW:

Molecular testing has proven useful when applied to indeterminate thyroid FNAs, and its use has been endorsed by the American Thyroid Association Guidelines. Recent application of molecular testing to the subset of indeterminate thyroid FNAs has shown that the presence or absence of specific somatic mutations, gene rearrangements, or miRNA expression profiles have high predictive values for benign and malignant thyroid disease. As a consequence, ancillary molecular testing has developed in order to improve the FNA performance for those patients with an indeterminate thyroid FNA. The Afirma Gene Expression Classifier (GEC) has a high negative predictive value (NPV) and can be used to "rule out" malignancy for the AUS/FLUS and SFN/FN categories, whereas ThyroSeq v.2 and multipanel testing with ThyGenX/ThyraMIR are marketed as having high positive predictive values (PPV) and NPVs that could potentially both "rule in" and "rule out" malignancy. In addition, the RosettaGX Reveal (Rosetta Genomics, Inc, Philadelphia, USA) which has been introduced recently, is a microRNA based rule out test that works directly on stained smears.

Like any other diagnostic test, performance of molecular tests in an individual center is closely related to the ‘pretest probability of malignancy’ (malignancy rate of an FNA category which is not conferred by molecular testing) for lesions diagnosed within a particular diagnostic category. Before utilizing a particular molecular test, the probable negative and positive predictive values can be modelled using Bayes’ theorem which is based on the pretest malignancy rate of an individual institution and the sensitivity/specificity of the test that is reported in the validation studies. All three commercially available molecular testing panels require dedicated FNA passes (two for GEC, one for Thyroseq v.2 or ThyGenX/ThyraMIR). The Reveal test uses existing smears. FNA samples are collected in vials of specific nucleic acid preservative solutions provided by the companies, and should be stored and shipped strictly according to the individual testing instructions in order to ensure an accurate molecular testing result.

The Afirma GEC from Veracyte (South San Francisco, Calif) uses microarray technology to investigate mRNA expression profiles of 167 genes. Afirma GEC does not provide information about the specific genetic alterations. However, in 2014 Afirma BRAF and Afirma MTC tests were released, which can be used with or without the Afirma GEC to assess the BRAF^{V600E} status or to screen for mRNA of 5 genes related to MTC. Performance analyses from the key validation study demonstrated a NPV of 95% for thyroid nodules classified as AUS/FLUS, and 94% for those classified as SFN/FN with associated malignancy rates of 24% and 25%, respectively. GEC reduced the risk of malignancy to ≤6% for the AUS/FLUS and SFN/FN categories, which is similar to a cytologically "benign" thyroid nodule. For thyroid FNAs classified as SFM, the NPV was 85% and PPV was 76%.

ThyGenX (Interpace Diagnostics, Parsippany, NJ), formerly known as miRInform (Asuragen, Austin, Tex) is the current commercially available 7-gene panel which uses next generation sequencing (NGS) to detect genetic alterations. Studies show that detection of any of these genetic alterations have a PPV of 88%, 87% and 95% for the AUS/FLUS, SFN/FN and SFM categories, respectively. The detection of either BRAF^{V600E} or RET/PTC was associated with a near 100% risk of malignancy, whereas a lower and wider range for malignancy was associated with the detection of RAS (12-87.5%) and PAX8/PPAR γ (50-100%) alterations. The ThyraMIR test screens for miR-29-b-1-5p, miR-31-5p, miR-138-1-3p, miR-139-5p, miR-146b-5p, miR-155, miR-204-5p, miR-222-3p, and miR-551b-3p. Analogous to the Afirma GEC test, ThyraMIR gives a qualitative “positive” or “negative” result for the sample. When combined, the ThyGenX/ThyraMIR testing algorithm has

demonstrated a high sensitivity (94% for AUS/FLUS and 82% for SFN/FN) and specificity (80% for AUS/FLUS and 91% for SFN/FN) for the AUS/FLUS and SFN/FN.

Nikiforova et al. developed a NGS mutational panel (ThyroSeq v1) consisting of 12 genes that include AKT1, BRAF^{V600E}, NRAS, HRAS, KRAS, PTEN, TP53, TSHR, CTNNB1, RET, PIK3CA, and PAX8; GNAS was also included as a marker of benign disease. The latest version, Thyroseq v2 incorporates an even larger mutational panel using point mutations of TERT and EIF1AX, as well as 42 new fusions involving RET, PPAR, NTRK1, NTRK3, ALK, BRAF and IGF2BP3. Published performance analyses indicate that ThyroSeq v2 has both a high sensitivity (> 90%) and specificity (> 92%) when applied to thyroid FNAs classified as AUS/FLUS or SFN/FN, respectively.

Studies indicate that the molecular profile of NIFTP is generally distinct from cPTC. NIFTP is characterized by alterations in either RAS, PAX8/PPAR γ , or BRAF^{K601E}, in contrast to the frequent BRAF^{V600E} and RET/PTC alterations found in cPTC. Molecular testing with ThyroSeq v2 or ThyGenX could therefore be used to help guide the surgical management (total versus hemithyroidectomy) of patients with thyroid aspirates where NIFTP is in the differential diagnosis.

Table: Comparison of molecular testing for thyroid FNA

	Afirma	ThyGenX	ThyGenX/Thyra MIR	ThyroSeq
Method	GEC (mRNA expression profile)	NGS	NGS/GEC (miRNA expression profile)	NGS
Sensitivity	83-100%	68.6%	89%	90-91%
Specificity	7-52%	86.5%	85%	92-93%
NPV	75-100%	85.3%	94%	96-97.2%
PPV	14-57%	70.6%	74%	76.9-83%

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