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Utility of Diagnostic Molecular Markers for Evaluation of Indeterminate Thyroid Nodules

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An emerging philosophy of medicine in the 21st century is personalization of care. Critical factors in accomplishing this are to understand the genetic profile of the patient as well as the genetic profile and the clinicopathologic features of the disease affecting the patient. Progress has been made in defining the genetic alterations associated with differentiated thyroid cancers (DTCs). Unfortunately, the genetic profile of a DTC currently does not play a role in determining the extent of therapy for an individual patient with such a cancer.

Although some patients with DTC have a clinical presentation that establishes the diagnosis, most present with an asymptomatic thyroid nodule. The primary evaluation of such a patient includes fine-needle aspiration biopsy and ultrasonography to determine the risk of the patient's lesion being malignant. With use of the 6-tiered Bethesda classification, the accuracy of a benign interpretation (<5% false-negative result) as well as of a malignant interpretation (<3% false-positive rate) are excellent. Unfortunately, indeterminate results occur in 15% to 30% of cases of asymptomatic thyroid nodules and are associated with institutional variability in rates of confirmed malignancy. These indeterminate categories include the following: (1) atypia of undetermined significance or follicular lesion of undetermined significance (Bethesda category III); (2) follicular neoplasm or suspicious for follicular neoplasm (Bethesda category IV); and (3) suspicious for malignant cells (Bethesda category V). Currently, diagnostic lobectomy is the most common intervention for excluding malignancy in indeterminate lesions. This results in over-treatment in 60% to 70% of patients, with up to 30% of these patients requiring thyroid hormone replacement. The incorporation of molecular marker (MM) assays on the fine-needle aspiration biopsy of cytologically indeterminate nodules can personalize care and allow observation or total thyroidectomy as the initial intervention in lieu of diagnostic lobectomy in patients with such nodules.

The Cancer Genome Atlas sequencing project has substantially expanded the knowledge of genetic alterations in more than 90% of DTCs, with this knowledge being clinically

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integrated as diagnostic MM assays.¹ Currently, 3 MM panels are available for patients with cytologically indeterminate thyroid nodules on thyroid fine-needle aspiration biopsy. The first panel, Afirma (Veracyte), is a 167-gene expression classifier that functions primarily as a “rule-out” test, with a negative result being associated with a less than 5% risk of malignancy² (Table). Unfortunately, a positive result with this panel has a low predictive value for the presence of malignancy. A 7-gene panel was the initial alternative to Afirma and acted as a “rule-in” test. This panel has been replaced by 2 alternatives, which function as both rule-in and rule-out tests for indeterminate nodules. Both of these panels, ThyroSeq v2.1 (CBLPath) and ThyGenX/ThyraMir (Interpace Diagnostics), use a combination of genetic alterations as well as gene expressions to define the risk of malignancy. These panels have a negative predictive value similar to that of the Afirma panel, but a much improved positive predictive value^{3–5} (Table).

One must be thoughtful when incorporating MMs into the evaluation of thyroid nodules. These tests add costs when used inappropriately or unnecessarily. It is critical that this technology be used only for those patients with indeterminate nodules. Noureldine et al⁶ noted that 17 of 273 patients (6.2%) underwent gene expression classifier testing as part of the diagnostic evaluation of thyroid nodules when the cytologic diagnosis was not indeterminate. In addition, MM testing should be used only after considering all of the clinical information available for a particular patient. Frequently, the results of the clinical evaluation define the need and extent of surgery independently of any MM testing. Noureldine et al also noted that 53.5% of patients with cytologically indeterminate nodules underwent gene expression classifier testing even when the clinical evaluation alone provided an indication for surgery.⁶ Another important consideration is the rate of malignancy at one's institution for each Bethesda subclassification of thyroid cytopathology because this affects the accuracy of MM testing in predicting the presence or absence of malignancy. Last, patient preference can substantially influence the benefit that MM testing may offer. If a patient is unwilling to have an indeterminate thyroid monitored for change, there is no benefit to obtaining an MM evaluation as a rule-out test.

The development of more sophisticated technology and the ongoing modification of clinical guidelines will affect the role of MM testing in patients with thyroid nodules. Within the past 5 years alone, substantial changes have occurred in the management of thyroid nodules. With the release of the 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer, a more conservative approach of thyroid lobectomy for lesions larger than 1 cm but less than 4 cm and without other high-risk features is considered to be adequate treatment.⁷ Similarly, the technology of MM testing has progressed in the past 5 years from 2 options (one rule-in and one rule-out test) to 3 options, with 2 of these having both rule-in and rule-out functionality.

It is useful to consider how these changes affect the management of a patient with a 3-cm solitary thyroid nodule that is follicular neoplasm by fine-needle aspiration biopsy. In 2010, a total thyroidectomy would have been recommended if this lesion was malignant. Using a rule-out panel, a negative result would allow observation, whereas a positive result would require diagnostic lobectomy and the possibility of a staged completion thyroidectomy. Conversely, a positive result with a rule-in panel would allow one to proceed with total

thyroidectomy, whereas a negative result would still require a diagnostic lobectomy and the possibility of a staged completion thyroidectomy.

For this same patient in 2016, the American Thyroid Association Management guidelines strongly encourage adjusting the extent of surgery, and new MM panels are available for diagnostic evaluation. If the patient's physician considers thyroid lobectomy to be diagnostically and therapeutically adequate, a rule-in MM panel provides no advantage because it would not affect the extent of surgery. Conversely, a negative result on a rule-out MM panel would allow observation, whereas a positive result would support therapeutic lobectomy. If the physician believes that total thyroidectomy is appropriate therapy, then an MM panel that acts as both a rule-in and rule-out test should be used. A negative result would allow observation, whereas a positive result supports proceeding with total thyroidectomy.

Before considering MM testing in the evaluation of indeterminate thyroid nodules, it is essential that surgeons define their individual treatment philosophies for patients with DTC. After defining these philosophies, the surgeon can selectively determine which patients may benefit from MM testing. For any given surgeon, the potential benefit of an MM assay in a specific clinical situation will vary according to the surgeon's treatment philosophy, and must incorporate the patient's preferences. In addition, a surgeon's treatment philosophy may change over time, especially as new treatment guidelines and MM panels are developed.

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Table**Performance of Molecular Markers in Thyroid Cytopathology of Bethesda Categories III and IV**

Test	AUS/FLUS %	FN %
Afirma	PPV-38	PPV-37
	NPV-95 (Based on 24% cancer prevalence)	NPV-94 (Based on 25% cancer prevalence)
ThyroSeq v2.1	PPV-77	PPV-83
	NPV-97 (Based on 23% cancer prevalence)	NPV-96 (Based on 27% cancer prevalence)
ThyGenX/ThyraMir	PPV-68	PPV-82
	NPV-97 (Based on an overall 32% cancer prevalence)	NPV-91 (Based on an overall 32% cancer prevalence)

Abbreviations: AUS, atypia of undetermined significance; FLUS, follicular lesion of undetermined significance; FN, follicular neoplasm; NPV, negative predictive value; PPV, positive predictive value.