Addressing a Diagnostic Dilemma: Molecular testing for indeterminate thyroid FNA biopsy.

Fine Needle Aspiration biopsy and cytology diagnosis are the standard methods for the diagnosis of thyroid nodules. Palpable thyroid nodules occur in 5-7% of the adult population, 10-18 million in the U.S. FNA cytology provides answers for the majority of patients, 70-80%. However, in 20-30% of the cases, indeterminate results lead the patient to diagnostic surgery. Post-operative histopathology of thyroid tissue is benign in a majority of these cases, as high as 80% reported by Piana et al. For those with a malignant diagnosis, a second surgical procedure, complete thyroidectomy, is often required. These indeterminate FNA results create a medical dilemma as to how to obtain a definite diagnosis, given that diagnostic surgical procedures are invasive, with more risks, and increase significantly the healthcare costs.

Advancements in molecular testing offer adjunctive diagnostic tools when the cytopathological diagnosis remains indeterminate. Somatic mutations, gene expression, ICC protein analysis and miRNA analysis all offer advances and limitations. Only somatic mutation (Asuragen) and gene expression (Afirm) methodologies presently offer commercially available panels for patients.

The MiRInform™ Panel by Asuragen includes somatic mutations approved by the FDA: BRAF, RET-PTC, RAS and PAX8/PPAR-γ. This panel can help identify more malignant cases with reported specificity 97-100% from multiple studies. However its relatively low sensitivity (Nikiforov et al reported 62%) leaves some indeterminate cases with negative results still requiring further evaluation. Nikiforov et al, studied 1,056 consecutive thyroid FNA samples with indeterminate cytology and they recommended “total thyroidectomy in mutation-positive status. For mutation-negative patients, lobectomy was recommended as the first procedure.” Cost evaluation suggests this can be a cost effective part of the diagnostic algorithm, resulting in surgical efficiency and a decrease in diagnostic thyroid lobectomies.

The Veracyte Afirm Gene Classifier mutigene expression classifier uses mRNA extracted from FNAs and measures expression levels of 167 genes in order to distinguish benign from suspicious thyroid nodules. A study aimed to validate the test beyond the limits of the small sample studied by Veracyte, reported 92% sensitivity, 52% specificity, and negative predictive value of 94%. In contrast to Asuragen’s test, Afirm is a test most valuable when negative. With a high negative predictive value, a negative Afirm test is suggestive of postponing diagnostic surgery.

Although not commercially available at this time, the future should provide more tools for cytopathologists with immunocytochemistry (ICC) protein based thyroid assays. Literature has reported combining protein markers such as Galactin-3, HBME-1, CXCR4, CK19 yields sensitivity of 97% and 100% with combinations of galactin-3/ HBME-1 and HBME1/CK19 respectively. While the studies are indicating impressive results, variability in staining and interpretation of ICC represents limitations and one of the main reasons it has not made it to market. Small non-coding RNA segments, miRNA have been described as useful for cancer prognostication and classification with reported dysregulation in all human cancers including thyroid cancers. However, limited studies have looked at miRNA analysis as a diagnostic indicator in indeterminate FNA cases. In summary, molecular testing may be considered when the cytology diagnosis of thyroid nodules is indeterminate (Bethesda category III) or suspicious but not conclusive (category IV and V), and it can aid in the management of patients with cytologically indeterminate thyroid nodules. Independent studies have revealed diagnostic promise, but also limitations of each molecular test emerging as the next diagnostic tool. Of the commercially available options, Veracyte Afirm Gene Classifier offers a NPV of 94%. A negative result suggests triage of the indeterminate FNA with patient monitoring. In contrast, MiRInform by Asuragen reports high specificity 97-100%. A positive test results suggest early definitive surgical intervention thereby avoiding diagnostic thyroid lobectomy procedures.

Dr. Carla B. MacLeod is committed to serving the needs of our clients and can accommodate the send-out request of the clinician on a case by case basis. More information about each available test, requirements and pricing is available at http://asuragen.com/products-and-services/clinical-lab/mirinform-thyroid/ and http://www.veracyte.com/afirma. Dr. MacLeod performs ultrasound guided fine needle aspiration procedures in her office next to the laboratory in Gaithersburg, Maryland.

REFERENCES