Thyroid Cancer: New Diagnostic Approaches

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Molecular testing of thyroid nodules for genetic mutations is being used to augment fine-needle aspiration (FNA) cytology (FNAC) to improve the accuracy of diagnosing thyroid cancer in the ~25% of thyroid nodules with indeterminate cytology [Nikiforov YE et al. *J Clin Endocrinol Metab* 2011].

UNDERSTANDING COMMERCIALLY AVAILABLE MOLECULAR DIAGNOSTIC METHODS

The attributes of the commercially available molecular tests and open questions regarding their validation were reviewed by Steven P. Hodak, MD, New York University Langone Medical Center, New York, New York, USA.

Three important caveats must be kept in mind about the available molecular tests. One, the methods for each test differ and this may affect the clinical outcomes; tests with high sensitivity require additional validation. Two, the tests for the same markers are not actually the same; the specific mutations in the gene panel vary between the tests. Three, more investigation is required to determine whether low-frequency mutations in a tumor sample is a true positive.

Dr. Hodak noted that the characteristics of each must be understood, including their limitations. The molecular tests have been shown to be reproducible and to accurately detect mutations in laboratory specimens. However, clinical validation of the molecular tests against the clinical outcomes, including the correlation with histology, has not been done for all of the available tests.

A 7-gene panel test is available from Quest Diagnostics and Asuragen, and an enhanced multigene panel called ThyroSeq is available from the University of Pittsburgh and CBLPath. Three studies showing that molecular testing improved diagnosis and that molecular markers were highly specific have served as the foundation against which the tests from Quest Diagnostics and Asuragen have been validated [Nikiforov YE et al. *J Clin Endocrinol Metab* 2011; Cantara S et al. *J Clin Endocrinol Metab* 2010; Nikiforov YE et al. *J Clin Endocrinol Metab* 2009]. A comparison of the methods and sensitivities between the published studies and the tests is provided in Table 1. In this comparison, Sanger sequencing had an analytic threshold of ~20% of mutation-bearing cells, and Pyro sequencing had a threshold of ~5% of mutation-bearing cells.

The ThyroSeq molecular test has shown promise as one with high sensitivity and specificity, with a high negative predictive value and positive predictive value, stated Dr. Hodak. The peer-reviewed data about the diagnostic sensitivity and specificity have not been published.

The sensitivity of the molecular tests is important because they define a threshold below which any mutation that may be present cannot be detected. Dr. Hodak stated that the methods used by Asuragen and Quest Diagnostics are markedly more sensitive than the methods used in the validation studies, and they will identify tumors with a lower prevalence of mutations, but that different analytical methods may produce different results in the same specimen. Clinical validation is needed to understand whether this greater discrimination is clinically relevant.

DIAGNOSIS OF THYROID NODULAR DISEASE

The diagnosis of thyroid nodular disease (TND) by FNAC may be improved by measuring calcitonin (CT), which is secreted in medullary thyroid cancer (MTC). Rossella Elisei, MD, University of Pisa, Pisa, Italy, stated that CT measurement allows for early diagnosis and the opportunity to plan appropriate surgical treatment and improve outcomes. However, there are some limitations: CT measurement has a low specificity, especially in patients with low CT levels; a stimulation test must be conducted to distinguish between true and false positive serum CT results; and interpretation of CT test results requires skill.

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Table 1. Comparison of Methods and Sensitivity of Molecular Testing in the Literature and Commercially Available Tests

Moledular Marker	Published Studies	Quest	Asuragen
BRAF	RT-PCR Sanger Confirmation 20%	RT-PCR 0.1%	Luminex 2.5-5%
RAS		Pyrosequencing 5%	
RET/PTC	RT-PCR 2%	RT-PCR 2.5%	
PAX8/PPARγ			
Type of Validation			
Analytical	✓	✓	✓
Clinical	✓✓	×	✓

RT-PCR=reverse transcription polymerase chain reaction; RET/PTC=RET proto-oncogene; PAX8/PPARγ=paired box gene 8/peroxisome proliferator-activated receptor gamma fusion protein

An elevation in serum CT in the low-to-medium range alone is not diagnostic of MTC. Further testing should include FNAC, CT measurement in the wash-out, an ultrasound of the neck lymph nodes, and a stimulation test. If the diagnosis still remains unclear, Prof. Elisei recommends waiting for 6 to 12 months and repeating the basal CT measurement and neck ultrasound, especially if the nodule is < 1 cm.

A clinical review has shown that FNAC was accurate in detecting MTC in 151 of 192 patients (79%) prior to surgery [Ahmed SR, Ball DW. *J Clin Endocrinol Metab* 2011]. Problems associated with FNAC to diagnose MTC include: overlooking typical features, such as amyloid content, with routine methods; difficulty in detecting occult MTC with nodules that are too small or located posteriorly in the thyroid lobe that cannot be reached; or inconsistency in the cytology pattern of MTC, which can mimic that of other cancers.

A stimulation test can be conducted using calcium gluconate 25 mg/kg (or 2.5 mg/kg of calcium element) diluted in a final volume of 50 mL of a sodium chloride solution administered intravenously over 5 to 10 minutes. The 2 essential guidelines used to interpret the result of a stimulation test are, first, the peak CT must be >100 pg/mL, and second, the delta for the increase in CT should be ≥ 4 times that of the basal value. Prof. Elisei noted that CT values between 60 pg/mL and 100 pg/mL are in a gray zone in regard to how they are interpreted. Two other approaches are the pentagastrin stimulation test and the basal ultrasensitive CT measurement [Pina G et al. *Clin Endocrinol (Oxf)* 2013].

A consensus statement recommended the addition of CT measurement to screen patients with TND to improve the early diagnosis of MTC [Pacini F et al. *Eur J Endocrinol* 2006]. Another consensus statement could not recommend for or against routine CT measurement because of the unresolved issues related to sensitivity, specificity, assay performance, and cost-effectiveness [Cooper DS et al. *Thyroid* 2009]. A high level of serum CT is usually diagnostic of MTC, yet a complete work-up of the suspected nodule with FNAC and measurement of CT in the wash-out is indicated. If there are discrepancies in the results of these tests, a stimulation test should be conducted.