Proto-oncogene mutation detection in fine needle aspiration is a useful adjunct to cytology for diagnosing malignancy

Cantara S, Capezzone M, Marchisotta S, Capuano S, Busonero G, Toti P, Di Santo A, Caruso G, Carli AF, Brilli L, Montanaro A, Pacini F. Impact of proto-oncogene mutation detection in cytological specimens from thyroid nodules improves the diagnostic accuracy of cytology. J Clin Endocrinol Metab 2010;95:1365-9. First published ahead of print on February 3, 2010 as doi:10.1210/jc.2009-2103

SUMMARY

BACKGROUND

Cytology is currently the gold-standard for the preoperative diagnosis of malignancy in thyroid nodules. There are important limitations to its use, however. False negative rates may be as high as 6%. Likewise, the malignant potential of an indeterminate or follicular neoplasm cannot be determined by cytology alone, necessitating surgery to obtain a diagnosis. Recent insights into the molecular pathogenesis of thyroid cancer have sparked interest in using oncogenes to aid in the preoperative diagnosis of malignancy. Analysis of tumor tissues has revealed the high frequency of BRAF and RAS mutations and RET/PTC rearrangements in thyroid cancers and their absence in benign lesions. This study by Cantara et al. seeks to evaluate the efficacy of using these oncogenes to predict malignancy when molecular analysis is performed on cytologic samples.

PATIENTS AND METHODS

In this prospective study, 174 consecutive patients scheduled to undergo thyroid surgery were evaluated. Included in this analysis were patients undergoing surgery for cytology suspicious (n = 22) or positive (n = 48) for malignancy (40.2%), for indeterminate lesions/follicular neoplasms (n = 50, 28.7%), and for compressive symptoms with benign or inadequate cytology (n = 54, 31.1%). Prior to surgery, all patients underwent repeat fine-needle aspiration (FNA) with cytologic evaluation and molecular analysis. At surgery, all nodules were again sampled to undergo molecular analysis.

All samples were screened for mutations in BRAF (V600E and K601E); H-,K-, N-RAS point mutations in codons 12, 13, and 61; and RET/PTC rearrangements. In the absence of any of these mutations, TRK and PAX8-PPARγ rearrangements were sought.

DNA extraction from FNA samples was performed using the QIAamp DNA microkit. RNA extraction was performed with the SV total RNA isolation system then reverse-transcribed into cDNA using the IScript cDNA synthesis kit. All samples were confirmed to the thyroid tissue through gene amplification for thyroglobulin.

Results All cytologic and tissue samples were able to be analyzed for BRAF and H-, K-, N-RAS mutations. The cytologic yield was too low to detect a RET/PTC rearrangement in 51% of the FNA samples. All tissue samples, however, were able to be analyzed for RET/PTC.

Mutations in Cytologic Samples (Figure 1)

In the 235 FNA specimens, BRAF (V600E and K601E) and N-, K-, and H-RAS mutations were possible in all FNA cytologic and tissue samples. Oncogene mutations were identified in 67 of 235 cytologic samples (28.5%). Of the 67 mutations, the most frequent was BRAF in 33 of 67 samples (49.3%), followed by RAS mutations in 23 (34.3%), and 11 RET/PTC rearrangements (16.4%). Four cases had two mutations present; RAS and BRAF coexisted in three nodules, and H- and N-RAS occurred simultaneously in one (Figure 1).

Mutations in Tissue Samples (Figure 2)

There were 76 mutations (32.3%) in the tissue samples, including BRAF (V600E) in 35 (14.9%), RAS in 25 (10.6%),

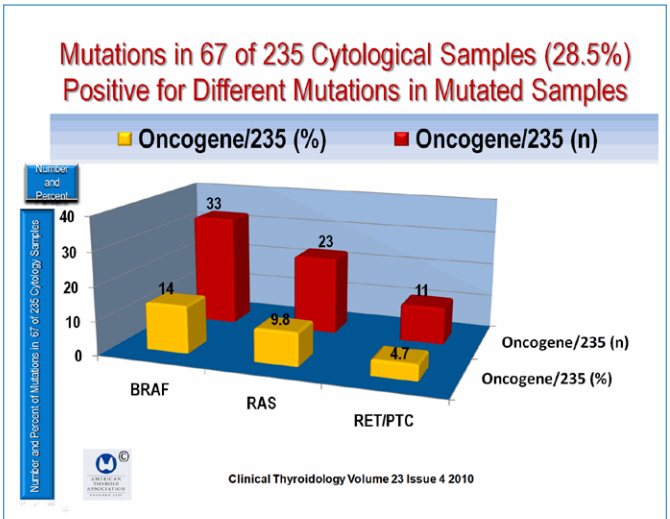

Figure 1. This figure depicts the number and percent of mutations in 235 cytologic samples and in 67 of the 235 samples that were positive for mutations (28.5%). Not shown are multiple mutations, BRAF + RAS found in 3 of 67 cytologic samples (4.5%), and H- and N-RAS in one sample (1.5%).

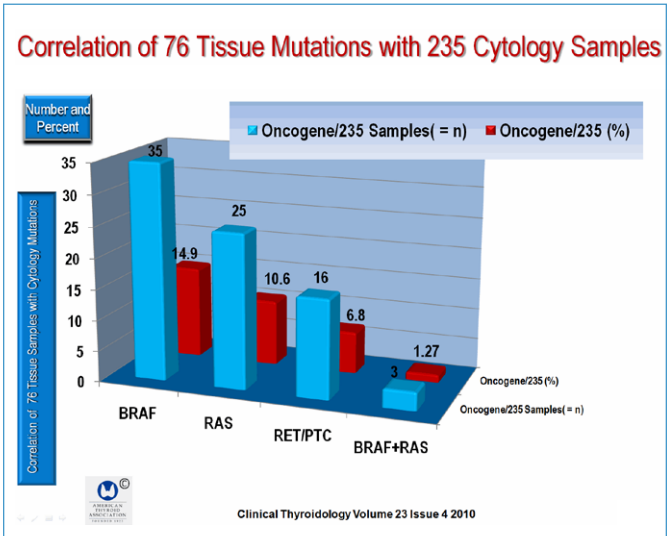

Figure 2. This figure shows the correlation of cytologic mutations with 76 tissue samples in 235 cytologic samples

and RET/PTC in 16 (6.8%). Four cases were noted to have two mutations, all of which corresponded to those seen on cytologic examination. There was an 88.2% concordance of the mutations between FNA and tissue samples. The 11.8% of discrepant results were attributed to mutations found in tissues but not identified in cytologic material. RET/PTC rearrangements were the most frequent mutations missed on cytology. Several cases of RAS mutations also went undetected on cytology. There were no TRK or PAX8-PPARγ mutations found in both the cytologic and surgical specimens.

Correlation of Benign Cytologic Mutations with Tissue Mutations (Figure 3)

Analysis of the 87 nodules with benign cytology revealed a mutation in 9 (10.3%) (Figure 2). There were two nodules with BRAF mutations, two with RET/PTC rearrangements, and five with RAS mutations. Final histology on the nodules with mutations revealed PTC in six and follicular adenomas in three. The 78 nodules with no mutations had two papillary thyroid carcinomas (PTCs) and one follicular thyroid carcinoma (FTC). The remainder were follicular adenomas and hyperplastic nodules.

Correlation between Cytologic Results and Final Histologic Diagnosis (Figure 4)

The mutations in cytologic samples correlated with the cytologic diagnosis and are shown in Figure 4. Of the nodules with a cytologic diagnosis suspicious for thyroid cancer (n = 54), 37 (68.5%) were found to have a mutation (21 BRAF, 6 RET/PTC, 10 RAS) and all were confirmed to be PTC on tissue histology. Of the remaining 17 patients with suspicious cytology but no mutations, 9 were diagnosed with PTC, 4 with follicular adenomas, and 4 with hyperplastic nodules.

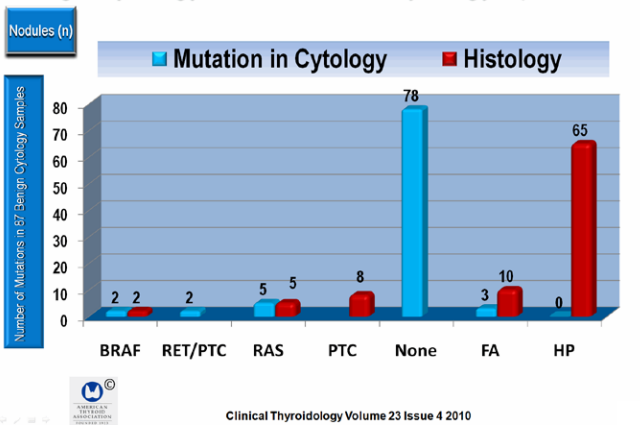

Figure 3. This figure shows the mutations in 87 benign cytologic specimens and diagnoses of follicular adenoma (FA) and hyperplastic nodule (HP)

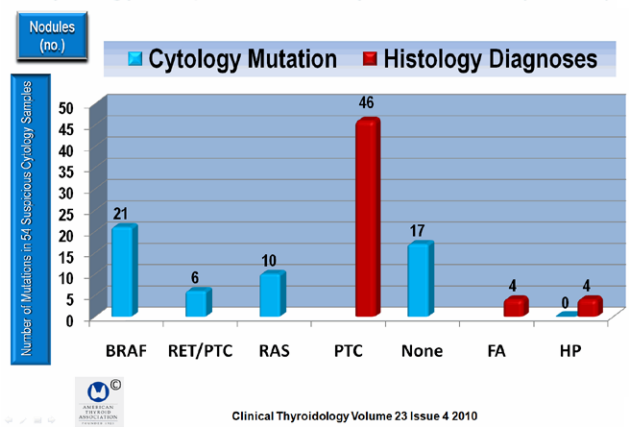

Figure 5. This figure shows the correlation of 54 suspicious cytologic specimens with molecular findings. FA = follicular adenoma; HP = hyperplastic nodule.

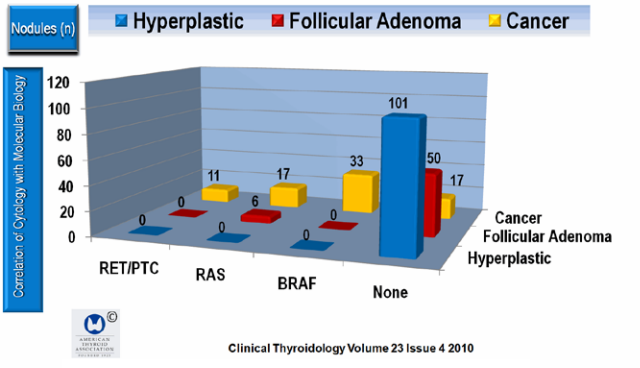

Figure 4. This figure shows the correlation of cytologic and molecular mutations in 87 benign cytologic specimens and diagnoses of follicular adenoma (FA) and hyperplastic nodule (HP) and the correlation of cytologic and molecular findings and the number of benign cytologic samples that were positive for BRAF, RET, and RAS oncogenes. Adapted from Figure 1 of Cantara et al.

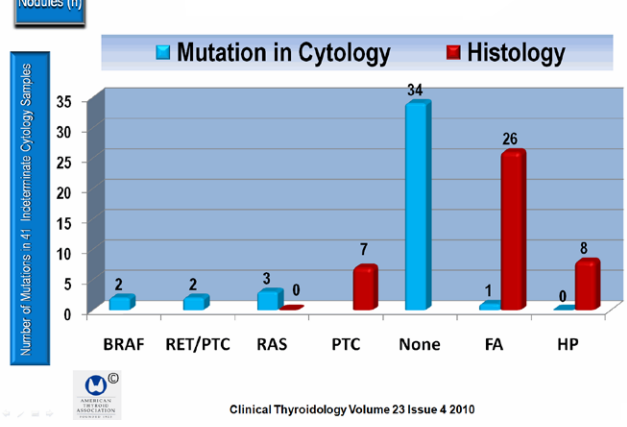

Figure 6. This figure shows the correlation of indeterminate cytology (n = 41) with tumor histology. FA = follicular adenoma; HP = hyperplastic nodule.

Correlation between Suspicious Cytologic Samples and Histologic Diagnosis (Figure 5)

Of the 235 nodules evaluated, there were 78 cancers found on final histology. The molecular analysis of FNA specimens correctly identified 61 (78.2%) malignancies, as compared with cytology, which diagnosed 46 (58.9%). Mutations (all RAS) were detected in 10.7% of follicular adenomas; no hyperplastic nodules were associated with a mutation. The presence of a mutation was associated with a diagnosis of cancer in 91.1% and follicular adenomas in 8.9%.

Correlation of Indeterminate Cytologic Mutations with Tumor Histology (Figure 6)

There were 7 mutations (17%) in the nodules with indeterminate cytology (Figure 3). On final histologic sectioning, the diagnosis was PTC in all but one, which was follicular adenoma. Among the 34 indeterminate nodules without mutations, only 1 was PTC; the remainder were hyperplastic nodules and follicular adenomas.

Correlation of Mutations in Inadequate Cytology with Tumor Histology (Figure 7)

The nodules with inadequate cytology had 14 (26.4%) mutations (8 BRAF, 1 RET/PTC, 5 RAS); histology revealed that all but two were malignant (Figure 7). The two benign lesions were follicular adenomas with RAS mutations. The inadequate samples with no mutations had 4 cancers (2 PTCs and 2 FTCs), 11 follicular adenomas, and 24 hyperplastic nodules. RAS mutations were associated with malignancy rate 74% and follicular adenomas 26% of the time. In contrast, BRAF and RET/PTC were always associated with malignancy. Approximately 10% of malignancies had no associated malignancy.

Diagnostic Performance of Cytology, Molecular Analysis, or Both (Figure 8)

The specificity of cytology was strong at 94.9%, but was encumbered by a low sensitivity at 59%. The positive predictive value was 85.2% and the negative predictive value (NPV) 82.3%, giving a total accuracy of 83%. In contrast, molecular analysis had a specificity of 96.2% and improved sensitivity at 78.2%. The positive and negative predictive values were 91.1% and 89.9%, respectively, and total accuracy of 90.2%. Combination of these two techniques further improved the sensitivity and NPV, at 89.7% and 94.9%, respectively. Accuracy improved to 93.2% by combining the two techniques.

CONCLUSIONS

Molecular analysis of FNA samples in conjunction with cytology offers increased sensitivity and negative predictive value as compared with either method alone.

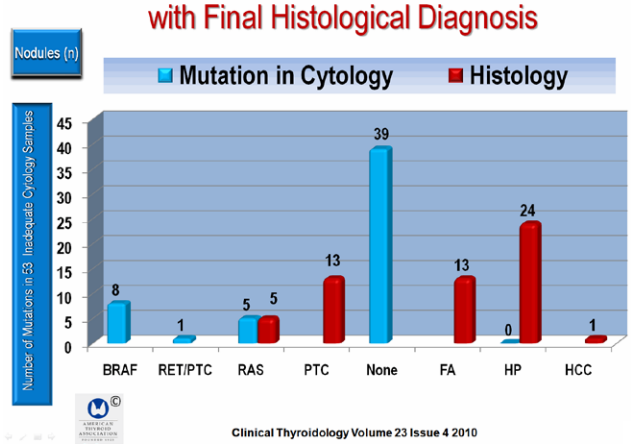

Figure 7. This figure shows the correlation of inadequate cytology (n = 53) with final histologic tissue diagnosis. FA = follicular adenoma; FTC = follicular thyroid cancer; HCC = Hürthle-cell carcinoma; HP = hyperplastic nodule.

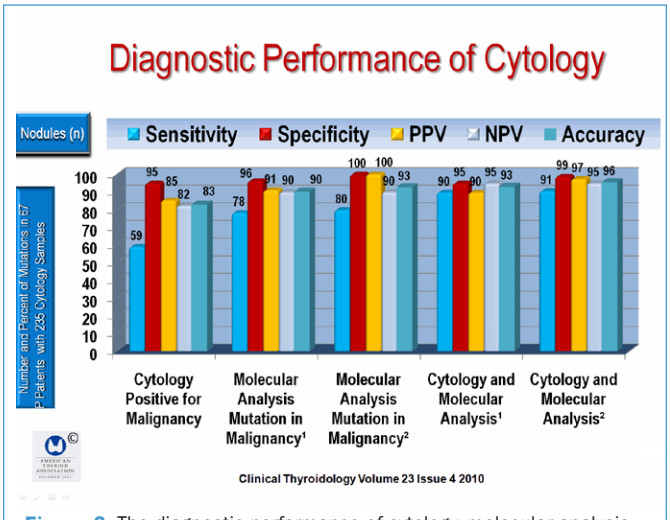

Figure 8. The diagnostic performance of cytology, molecular analysis, or a combination of both. Two methods for molecular analysis mutation in malignancy were used, one in which follicular adenomas were computed as false positive[1] and the other for mutated follicular adenomas computed as true positive.[2]

COMMENTARY

The problem of indeterminate cytology has plagued clinicians for years, and the search for alternatives to surgery that will determine which nodules are malignant has been extensive. Ultrasound characteristics (1), in combination with patient characteristics (2), have been analyzed, and scoring systems have been created to predict the likelihood of malignancy in a given nodule. To date, studies of various combinations of these features have failed to provide sufficient sensitivity and specificity to routinely advocate their use. Likewise, fluorodeoxyglucose–positron-emission tomographic scanning has poor sensitivity and specificity for predicting malignancy in indeterminate nodules (3). One promising method, elastography, had a 100% positive predictive value to differentiate benign from malignant nodules in a pilot study (4). Its routine use, however, is limited by the need for expensive equipment and highly trained technicians. Thus, molecular analysis of FNA samples offers a glimmer of hope for a readily available test that can distinguish benign from malignant lesions. It is an attractive technique because molecular analysis can be performed on FNA samples; no additional testing of the patient is needed beyond the existing standard of care.

Numerous studies have paved the way for the current analysis by Cantara et al. (5;6). A great deal of emphasis has been placed on determining the presence of BRAF in FNA samples. Indeed, it is an exquisitely specific test; of the 2766 samples in the literature tested for its presence, there has only been one false positive result (7). Restriction of the molecular examination to BRAF mutations alone results in missing many tumors, however, as multiple mutations have been identified in differentiated thyroid cancers, these oncogenes are mutually exclusive (7). The authors of the present study sought to examine the feasibility of screening FNAs for multiple oncogenes. In addition, this article augments the existing literature in that all patients proceeded to surgery, removing the element of doubt about the true false negative rate of molecular analysis. The utility of molecular analysis on FNA samples was further validated in this study by comparing the results to those performed on the histologic samples; the concordance rate was very high.

Molecular analysis was very specific for BRAF mutations and RET/PTC rearrangements; in all cases, the presence of one of these mutations was associated with malignancy. In contrast, RAS was less specific, as 26% of the time it was associated with a diagnosis of a follicular adenoma. Although technically categorized as a false positive finding, it may provide clinically useful information. Such tumors may represent a precursor lesion to the RAS-positive follicular carcinomas (8). If a malignant transformation is made in an individual nodule, the presence of N-RAS mutations is associated with a more aggressive tumor phenotype (9). As such, some might justify the removal of these tumors to prevent progression to overt carcinoma (7).

It is important to note that molecular analysis of FNA samples cannot replace cytology. A significant number of patients had a false negative mutational analysis. Specifically, there were 9 nodules with suspicious cytology that had no mutations identified. In patients with suspicious cytology, molecular analysis adds little useful clinical information because the false positive rate is so low with cytologic examination. Rather, mutational analysis appears to be most beneficial in patients with benign, indeterminate, or insufficient diagnoses on cytology. When used in this manner, the sensitivity and specificity for diagnosing malignancy is significantly improved over cytology alone.

There were eight patients with negative cytology and negative molecular analysis who were identified on final histology as having thyroid cancer. This group of nodules with false negative findings represents an important area for further study. It may be beneficial to include other molecular markers to see whether the sensitivity of this technique can be improved. An attractive candidate is microRNAs (miRs), endogenous noncoding RNAs that regulate gene expression posttranscriptionally (7). Many miRs that are dysregulated in thyroid cancer have been identified (10). Preliminary studies have confirmed that the up-regulation of these specific miRs in thyroid cancer may be useful to predict malignancy in thyroid nodules on FNA (10). It is important to note that the use of miR expression profiling is in the early stages of investigation, however. Additional studies are needed to confirm its utility in distinguishing malignant thyroid nodules from FNA samples.

While the current study reduces the false negative rate of cytology alone, it may not be appropriate to routinely test all nodules with benign, indeterminate, or inadequate cytology. Studies of the most cost-effective use of molecular analysis in thyroid nodules are needed. Clinical risk factors and sonographic appearance may also play a role in deciding which nodules merit further investigation with molecular analysis. Nevertheless, this study provides confirmation that molecular analysis of FNAs is an accurate method to distinguish benign from malignant lesions and will provide valuable insight into which patients should proceed to thyroidectomy and which may safely avoid it. Further, its use can reduce the costs and morbidity associated with hemithyroidectomies performed on nodules with indeterminate cytology that ultimately are deemed benign on final histologic examination. This is an exciting new technology with broad-reaching benefits for patients and clinicians alike.

— Jennifer A. Sipos, MD

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