MOLECULAR ANALYSIS FOR MUTATIONS IN THYROID FNA IMPROVES THE DIAGNOSIS OF MALIGNANCY FOR ALL CATEGORIES OF INDETERMINATE CYTOLOGY

Nikiforov YE, Ohori NP, Hodak SP, Carty SE, Lebeau SO, Ferris RL, Yip L, Seethala RR, Tublin ME, Stang MT, Coyne C, Johnson JT, Stewart AF, Nikiforova MN. **Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples.** J Clin Endocrinol Metab 2011;96:3390-7. Epub August 31 2011.

BACKGROUND

Nodules classified as indeterminate make up about one-fourth of those tested by thyroid fineneedle aspiration biopsy (FNAB). In the Bethesda classification, these are further classified into one of three categories that have increasing likelihood of thyroid cancer: (1) follicular lesion of undetermined significance (FLUS), (2) follicular or oncocytic (Hürthle)-cell neoplasm or suspicious for follicular or oncocytic-cell neoplasm (FN), and (3) suspicious for malignant cells (SMC). Studies of oncogene mutations in thyroid FNAB have improved the diagnosis of thyroid cancer. The current report is a large prospective study that defines the diagnostic utility of analysis for oncogene mutations in patients with indeterminate cytology on FNAB.

METHODS

From April 2007 through April 2009, a total of 1056 consecutive FNA samples from thyroid nodules with indeterminate cytologic diagnoses were tested prospectively for mutations at the University of Pittsburgh Medical Center. Samples were from 762 patients, including 294 who contributed multiple FNAB samples from the same or different nodules. Each sample was considered independently.

The sample was placed into a preservative solution; the adequacy of the quantity and quality of DNA was assessed by polymerase-chain-reaction (PCR) amplification of RAS and BRAF genes and glyceraldehyde-3phosphate dehydrogenase (GAPDH) cDNA. The sample was considered satisfactory when the amplification cycle threshold was less than 35 cycles. The proportion of epithelial cells within the FNAB sample was assessed by performing PCR measurement of cytokeratin gene KRT7, which is found in thyroid epithelial cells, and comparing it to the housekeeping gene GAPDH. The difference in amplification between KRT7 and GAPDH had to be more than 3.5 cycles for an adequate sample.

Clinical

THYROIDOLOGY

BRAF V600E, NRAS codon 61, HRAS codon 61, and KRAS codons 12 and 13 point mutations were detected using real-time PCR and fluorescence melting curve analysis. RET/PTC1, RET/PTC3, and PAX8/PPARg rearrangements were detected by real-time reverse transcriptase–PCR.

RESULTS

Of 1056 consecutive FNAB samples with indeterminate cytology, 50 had an insufficient amount of isolated nucleic acids and another 39 had insufficient epithelial cells for mutational analysis. The remaining 967 samples collected from 729 patients were subjected to mutational analysis. Among these samples, a cytologic diagnosis of FLUS was established in 653, FN in 247, and SMC in 67. Molecular analysis revealed 87 mutations, including 19 BRAF V600E, 47NRAS, 12HRAS, and three KRAS, as well as one RET/ PTC1 and five PAX8/PPARg rearrangements. A total of 479 patients underwent thyroidectomy that provided histopathological diagnosis for 513 FNAB samples. The results of the mutational analysis were correlated with the pathological diagnosis.

In the group of 247 FLUS samples in patients who had surgery, mutations were found in 22 of 38 cancers; RAS was the most common mutation; 3 RAS mutations were found in follicular adenomas. The risk of cancer in nodules in the FLUS category that were negative for mutations was only 5.9%.

There were 247 surgical samples in the FN group; 33 of 58 cancers were found to have mutations in the FNAB sample. Of 34 FNABs positive for RAS *continued on next page*

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mutations, 29 were in malignant nodules, mainly the follicular variant of papillary thyroid carcinoma, and 5 occurred in follicular adenomas.

Of the 52 FNABs classified as SMC for which surgical material was obtained, malignancy was found in 28 nodules, and 19 had mutations; 10 were BRAF, 7 RAS, 1 PAX8/PPARg, and1 RET/PTC1; 9 were negative for mutations.

For the three cytologic categories, FLUS, FN, and SMC,

the cancer risk by cytology alone was 14%, 27%, and 52%, respectively; based on finding any mutation, this increased to 88%, 87%, and 95%. Correlation of mutation testing in surgical samples and FNAB showed that the FNAB study detected 95% of the mutations found in the surgical samples.

CONCLUSIONS

Molecular analysis for a panel of mutations has significant diagnostic value for all categories of indeterminate cytology.

This group has taken the leading role in diagnosis of thyroid cancer using oncogene biomarkers in FNAB material. The present paper validates an earlier study on a smaller number of samples (1). Using the molecular markers for the indeterminate group is probably the most cost-effective approach, even though the oncogene may be positive in some samples deemed to be inadequate for cytologic diagnosis or in some diagnosed as hyperplastic colloid goiters; however, this may be so uncommon that it is not justified economically.

The high frequency of RAS mutations in this study is probably related to the selection of samples in the indeterminate category. If samples that were positive for papillary thyroid carcinoma had been tested, then the BRAF mutation would have been more frequent than RAS. Although RAS mutations were found in follicular adenomas, the finding of a RAS mutation in an indeterminate sample increases the likelihood of

References

 Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopper JP, Zhu Z, Fagin JA, Falciglia M, Weber K, Nikiforova MN. Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab 2009;94:2092–8. thyroid cancer to 80%. This is certainly a justification for sending a patient for thyroidectomy. The authors propose that when the tested mutations are negative, patients should be recommended for lobectomy rather than total thyroidectomy because of the reduced possibility of cancer. The frequency of the three RAS mutations was not reported in this paper.

The follicular variant of papillary thyroid cancer (FV/ PTC) made up 72% of the 93 cancers found in the FLUS and FN categories. Recently, Daniels wrote a scholarly analysis of FV/PTC proposing that it be subdivided into three categories based on histology and molecular markers: (1) papillary thyroid carcinoma with follicular architecture, (2) follicular thyroid carcinoma with nuclear atypia, and (3) follicular adenoma with nuclear atypia (2). A discussion of the consequences of this classification is beyond the scope of my commentary, but this new classification provides food for thought in this controversial area.

— Jerome M. Hershman, MD

 Daniels GH. What if many follicular variant papillary thyroid carcinomas are not malignant? A review of follicular variant papillary thyroid carcinoma and a proposal for a new classification. Endocr Pract 2011;17:768-87.