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A Gene-Expression Classifier on FNA Biopsy of a Thyroid Nodule May Be Helpful to Determine Whether an Indeterminate Nodule Is Benign

Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, Livolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR. Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med. June 25, 2012 [Epub ahead of print].

SUMMARY

Background

Fine-needle aspiration biopsies of thyroid nodules yield "indeterminate" cytologic diagnosis in 15 to 30% of biopsies. According to the Bethesda classification, the indeterminate category is further subdivided into three categories: (1) atypia of undetermined significance or follicular lesion of undetermined significance, (2) follicular neoplasm or suspicious for a follicular neoplasm, and (3) suspicious for malignancy, with estimated risks of malignancy of 5 to 15%, 15 to 30%, and 60 to 75%, respectively (1). Various molecular diagnostic procedures have been reported to clarify the diagnosis of malignant or benign in the indeterminate category in order to determine whether surgical thyroidectomy is appropriate therapy. The authors report a multicenter study that evaluates a commercial method that classifies indeterminate FNA biopsies into either a benign or a suspicious category.

Methods

Samples. The Veracyte company collected 4812 nodule aspirates from 3789 patients at 49 clinical sites in the United States; 577 were classified as indeterminate (12%). For 413 of the 577 samples, surgical resection of the nodule was performed. Based on quality-control procedures, 328 samples were processed to yield valid "classifier" results, but pathologic diagnosis was available for only 312 samples. Forty-seven samples were excluded for various reasons, leaving 265 samples for primary analysis; 129 were in the atypia category, 81 in the follicular neoplasm category, and 55 in the suspicious for malignancy category. In addition to the indeterminate samples, 47 cytologically benign and 55 cytologically malignant surgical samples were evaluated by the classifier.

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Gene-expression classifier. The method is a microarray assay to determine the expression of 167 genes. The assay includes 142 genes in the main classifier to determine benign versus malignant, and 25 genes in cassettes that are labeled parathyroid, medullary cancer, Hürthle, renal carcinoma, breast carcinoma, and melanoma. "A linear modeling approach was used for feature selection, and a support-vector machine was used for classification." To obtain sufficient RNA for the microarray, two needle insertions were performed after one needle insertion in the first part of the study was apparently unsatisfactory for producing sufficient cellular material. The data on 367 samples is available on the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus website.

Results

In the atypia category, 31 of 129 samples (24%) were malignant by pathology; 28 of 31 were classified as suspicious by the gene-expression classifier (GEC). Ninety-eight of the 129 in the atypia category were benign, but the GEC result was suspicious in 46 of them and benign in 52.

In the follicular neoplasm category, 20 of 81 samples (25%) were malignant by pathology, and the GEC was suspicious in 18 of 20 but benign in two. Sixty-one of the 81 were benign, but 31 of them were classified as suspicious by GEC.

In the suspicious for malignancy category, 34 of 55 samples (62%) were malignant by pathology, and the GEC classified 32 of them as suspicious; of the 21 that were benign, the GEC classified only 11 as benign and the other 10 as suspicious.

Of the seven false benign results, one was a Hürthle-cell carcinoma and the others were papillary thyroid cancers. The results were attributed to the low content of follicular cells in the samples used for microarray gene analysis.

All of the additional 55 pathologically malignant samples were categorized as suspicious. Of the 47 additional samples considered benign cytologically, 3 were malignant and 44 were benign by pathology; of these 44, the GEC considered 13 as suspicious.

Conclusions

The gene-expression classifier may be used to identify a subpopulation of patients with a low likelihood of thyroid cancer who might otherwise have been treated by thyroidectomy.

ANALYSIS AND COMMENTARY

This is an interesting report of a validation study that has long been awaited for a commercial method that has been recommended for patients who had a previous indeterminate FNA biopsy. A benign GEC would spare the patient from unnecessary surgery. The authors are outstanding authorities in the area of diagnosis and management of thyroid nodules, thyroid cancer, and thyroid pathology. However, there are weaknesses in the paper, the study, and the interpretation of results.

Most clinicians would recommend surgery for patients in the suspicious for malignancy category. The GEC missed 2 of 34 malignancies (6%) in patients who would otherwise have surgery. It categorized as suspicious 10 of 21 benign lesions in this category, so it was incorrect in 48% of these cases.

In the follicular neoplasm cytology category, the GEC again identified only about half of the benign nodules and missed 10% (2 of 20) that were malignant. In the atypia of undetermined significance category, the GEC misclassified almost 10% (3 of 31) of the malignant

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lesions and was correct for only 52% (52 of 98) that were benign.

In the atypia category, I use clinical parameters, such as size and ultrasound characteristics, for making the decision about surgery, rather than recommending surgery for all such patients. I am not convinced that the current GEC adds much to this approach. Experience suggests that about 90% of the samples in this category are likely to be benign (1); the GEC found that 57% (74 of 129) were suspicious. In this series the proportion of the atypia category that were malignant, 24%, is higher than expected; the GEC misclassified about 10% of the malignant nodules (3 of 31).

It should be noted that about 30% of the benign nodules in the additional group were classified as suspicious by GEC. Because of this, the authors recommend that the test not be used for nodules that are cytologically benign, as the suspicious categorization may be tantamount to a recommendation for surgery.

My additional concern is the method itself. The relative weight given to the expression of each of the 142 genes in the classifier is not described in the appendix; only the approach to validation is described. Apparently the details of the GEC are proprietary. My browsing of the National Center for Biotechnology Information website and a cursory examination of the data on several benign samples convinced me that analysis of this gene-expression data is exceedingly complicated. As with any test, the physician must accept the data based on faith in the veracity and integrity of the laboratory that produced it.

Currently the Veracyte Affirma GEC method "retails" for $3,350 plus $300 for cytopathology. I regard this as a substantial cost for its possible contribution to avoiding diagnostic surgery, in part because it also misclassifies lesions as suspicious about half the time. However, another interpretation is that the method can be used only to classify a nodule as benign and that the "suspicious" category by GEC should not be used.

The other approach to molecular diagnosis of thyroid cancer is the measurement of oncogenes, such as BRAF, on FNA to make a positive diagnosis of thyroid cancer in cytologically indeterminate FNA biopsies (2). This approach is being marketed by several laboratories and was reviewed in the December 2011 issue of Clinical Thyroidology. The oncogene molecular method misses cancers that do not express the oncogenes tested, but has the advantage of having a much lower rate of false positives as compared with the GEC method, assuming that "suspicious" is positive. In a world where there are unlimited financial resources, both the oncogene and the GEC methods could be applied to all indeterminate nodules, but this approach is not practical currently.

— Jerome M. Hershman, MD

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