Office-Based Ultrasound-Guided FNA with Molecular Testing for Thyroid Nodules

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Abstract

Objective. Ultrasound-guided fine-needle aspiration (FNA) biopsy is the primary method of evaluating thyroid nodules. Up to one-third of FNA results are reported to be of “indeterminate” cytology, which carries a 25% malignancy risk. Most of these patients are referred for diagnostic surgery, which results in many unnecessary interventions. We implemented an FNA protocol combining expert thyroid cytopathology and molecular testing of indeterminate lesion in our community practice. This study is a report of the outcomes from this protocol as compared with historical data in the same setting over a similar period.

Study Design. Case series with planned data collections and retrospective chart reviews.

Setting. A large community-based practice with multiple satellite offices.

Subjects and Methods. A total of 264 thyroid nodules (196 patients) were evaluated under the new protocol from January to December 2014, and data were collected in a prospective manner. Historical data for a similar period (2012), obtained by chart review, included 164 nodules (134 patients) biopsied in a hospital setting by a number of radiologists, with cytologic interpretations completed by community-based pathologists. Statistical analyses included $\chi^2$ and Fischer’s exact tests.

Results. Based on the new protocol, the rate of indeterminate lesion diagnosis was reduced from 24% to 10% ($P = .006$) and the rate of diagnostic surgery from 24% to 6% ($P < .001$). Of the patients who underwent diagnostic surgery, 58% had evidence of malignancy, as compared with 12% in our previous experience ($P = .04$).

Conclusion. Expert cytopathologic analysis combined with molecular testing of indeterminate FNA samples significantly reduced unnecessary operations.

Keywords

thyroid, FNA, molecular diagnostics

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presented with thyroid nodules and met American Thyroid Association guidelines\(^2\,^3\) (ie, predominantly solid nodules >1 cm; or nodules of any size with history of radiation exposure or strong family history of thyroid cancer; or highly suspicious ultrasonographic features such as associated lymphadenopathy or microcalcification) underwent an ultrasound-guided FNA biopsy. Procedures were performed by radiologists in 5 community hospitals. The cytologic interpretation was performed by cytopathologists attached to these hospitals.

We retrospectively collected and analyzed data from all patients with thyroid nodules who underwent ultrasound-guided FNA based on the above protocol between January and December 2012. We analyzed the cytology data for the frequency of indeterminate results. Histologic data on indeterminate nodules from patients who underwent surgery were also analyzed.

In October 2013, we instituted a new protocol involving a centralized office-based ultrasound clinic. All patients referred to our practice for the evaluation of thyroid nodules who met the American Thyroid Association criteria for FNA were referred to the ultrasound clinic\(^2\,^3\). Ultrasound-guided FNAs were performed by a single surgeon using a capillary technique with a 25-gauge needle. Four needle passes were made for each nodule. Cytologic analysis and molecular studies were performed via the Afirma protocol\(^1\,^5\). Aspirate from 2 passes (the first and third) was placed in CytoLyt solution for cytologic analysis, whereas the other 2 aspirates (the second and fourth) were placed in a special transport medium for RNA analysis for the Afirma Gene Expression Classifier (GEC) test. Samples were transported on dry ice for cytologic processing and possible molecular analysis of indeterminate samples.

Cytology was interpreted by a single group of experienced thyroid cytopathologists, and the results were described according to the Bethesda system for reporting thyroid cytopathology\(^4\,^6\). GEC analysis was performed on all specimens with indeterminate cytology—AUS/FLUS (type 3) or FN/SFN (type 4).

Surgical intervention was recommended for indeterminate nodules that had suspicious GEC test results. Histologic data were collected on the nodules from patients who underwent surgical resection.

Statistical analyses were performed with the \(\chi^2\) and Fischer’s exact tests, and a \(P\) value < .05 was considered statistically significant.

### Results

In the 12-month period from January to December 2012, a total of 164 nodules from 134 patients underwent ultrasound-guided FNA biopsy. Of 164 nodules, 43 (26\%) from 36 patients were classified as indeterminate. Of 134 patients, 32 (24\%) underwent diagnostic surgery for indeterminate cytology results, of which 4 (12\%) were positive for malignancy. Therefore, unnecessary operations were carried out on approximately 88\% of patients with indeterminate cytology (Table 1).

| Table 1. Historical Data (2012) versus Data after Implementation of the Afirma Protocol\(^a\) (2014). |
|---|---|---|
| Data, n (%) | 2012 | 2014 |
| Patients | 134 | 196 |
| Nodules | 164 | 264 |
| Indeterminate cytology | 43 of 164 (26) | 27 of 264 (10) |
| Diagnostic surgery | 32 of 134 (24) | 12 of 196 (6) |
| Positive histopathology | 4 of 32 (12) | 7 of 12 (58) |

*Protocol for performing and interpreting cytology along with molecular testing.*

Under the new protocol for the performance and interpretation of ultrasound-guided FNA, a total of 264 nodules (from 196 patients) were studied in a comparable period (between January and December 2014), and indeterminate cytology was reported in only 27 of 264 (10\%) of those nodules (Figure 1).

All indeterminate nodules were subjected to GEC testing, with equal numbers being identified as suspicious (12) and benign (12) in the GEC results. Insufficient RNA led to no GEC result in 3 of 27 (10\%) of the indeterminate nodules (Figure 2).

Surgery was offered to all patients with suspicious GEC results, as well as to patients without GEC results (13 patients, 15 nodules). Twelve patients underwent surgical intervention, and 7 (60\%) were positive for malignancy (follicular variant of papillary carcinoma in 6 and microinvasive follicular carcinoma in 1).

By instituting the new protocol, which centralized the FNAs and the cytopathologic analysis, the rate of indeterminate lesions was significantly reduced from 24\% (32 of 164 nodules) to 10\% (27 of 264 nodules; \(P = .006\)). The rate of diagnostic surgery was reduced from 24\% (32 of 132 patients) to 6\% (12 of 196 patients; \(P < .001\)). Under the new protocol, of the patients who underwent diagnostic surgery, 58\% (7 of 12) showed evidence of malignancy, as compared with 12\% (4 of 32) in our previous experience (\(P = .04\)).

### Discussion

The Bethesda system for reporting thyroid cytopathology, adopted in 2009, laid out well-defined cytomorphologic criteria for the interpretation of thyroid FNA specimens\(^4\,^6\). According to this tiered classification scheme, thyroid nodules can be broadly categorized as nondiagnostic (type 1), benign (type 2), suspicious for malignancy/malignant (types 5 and 6), and indeterminate (types 3 and 4).\(^4\,^6\) The risk for malignancy in thyroid nodules that are diagnosed as “benign” is reported to be <5\%, and clinical and radiologic follow-up is recommended.\(^3\) Conversely, nodules diagnosed as “suspicious for malignancy” and “malignant” carry a malignancy risk of 75\% and >95\%, respectively, and require surgical intervention.\(^3\)
The indeterminate category includes AUS/FLUS and FN/SFN, with a combined associated risk of malignancy >25%. Although most indeterminate lesions are referred for diagnostic thyroid lobectomy, more than two-thirds have no evidence of malignancy after final pathologic analysis. The rate of indeterminate results depends primarily on the expertise of the cytopathologist.

In recent years, a significant portion of the thyroid cytopathology-related literature has attempted to further characterize indeterminate nodules using adjunct molecular analysis. There are 2 main types of molecular tests for assessing thyroid nodules. So-called rule-in tests rely on the detection of single-gene point mutations involving \textit{BRAF} and \textit{RAS} and gene rearrangements involving \textit{RET/PTC} and \textit{PAX8-PPAR\gamma}, and these are seen in two-thirds of thyroid malignancies. Molecular testing for the detection of these mutations is highly specific, with a high positive predictive value but a very limited sensitivity, with a detection failure rate of approximately 33%.

So-called rule-out tests have a high negative predictive value and aim to reduce the rate of surgery when nodules have indeterminate cytology. The Afirma GEC test is a proprietary test developed by Veracyte, Inc (San Francisco, California). This test is based on the gene expression profiles of surgically proven benign and malignant thyroid nodules and identifies a benign gene expression profile.

In a large multicenter prospective blinded trial, the GEC test was found to have negative predictive values of 94% and 95% for FN/SFN and AUS/FLUS lesions, respectively. Additional studies from large institutions have corroborated and confirmed the validity of this test. Because the GEC test has negative predictive values similar to those of cytologically benign nodules, observation with follow-up ultrasonography can be confidently recommended. The avoidance of unnecessary operations means reduced morbidity and considerable cost savings.

Our case series describes the validity and usefulness of the Afirma protocol in a community setting. By centralizing the FNA procedure and cytopathology interpretation, we reduced the rate of indeterminate thyroid nodule cytologic interpretations from 26% to 10%. By further combining this protocol with molecular GEC testing, we were able to avoid surgery in 50% of the nodules with indeterminate cytology results and conservatively manage these patients by ultrasonography follow-up.

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**References**


