Minimizing the Diagnosis of "Follicular Lesion of Undetermined Significance" and Identifying Predictive Features for Neoplasia

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We used proposed standard morphologic criteria as a guideline to conduct a 10-year retrospective review of thyroid fine-needle aspiration specimens that were originally interpreted as "follicular lesion of undetermined significance" and followed by surgical intervention. We sought to investigate whether the indeterminate diagnosis could be minimized by assessing various cytomorphologic features and identifying the features predictive of neoplasia. Using the standard morphologic criteria, we semiquantitatively assessed a total of 24 cytomorphologic features in 123 aspirates and recorded an overall interpretation on completion of the review. Cyto-histologic correlation was evaluated and logistic regression model was performed to identify cytomorphologic features predictive of neoplasia. Although 32 of 123 aspirates remained in the indeterminate category, the retrospective review reclassified 64 aspirates as non-neoplasia and 27 aspirates as neoplasia. Histologic confirmation was achieved in 47 (73.4%) non-neoplastic and 15 (55.6%) neoplastic aspirates with a diagnostic accuracy of 68.1%. Furthermore, our analysis demonstrated that neoplasia is positively associated with the presence of syncytial tissue fragments, isolated microfollicles, follicles with scalloped borders, scant cytoplasm, irregular nuclear membranes, nuclear overlapping, coarse chromatin, and increased cellularity. On the contrary, the presence of honeycombing tissue fragments, background colloid, and histiocytes inversely correlated with neoplasia.

Overall, using proposed standard morphological criteria can minimize the diagnosis of "follicular lesion of undetermined significance," and allow for more accurate cyto-histologic correla-

Fine-needle aspiration (FNA) of thyroid is an effective triage method in the management of thyroid nodules. It aims to distinguish neoplastic nodules that require surgical intervention from non-neoplastic nodules that may be conservatively managed by clinical and radiologic follow-up.[1–4] It is well known that a straightforward cytologic diagnosis may not be rendered for every aspirate partially due to inherent cytological similarities between lesions.[5–7] The recent National Cancer Institute (NCI) Thyroid FNA State of the Science Conference recommended use of the category "follicular lesion of undetermined significance" for thyroid aspirates in which cytomorphologic findings are neither convincingly benign nor sufficient for interpretation of neoplasia/malignancy.[8] Previous studies claimed that the use of this category reduced the false-negative rate of FNA[6] while eliminating this category substantially increased both false-positive and false-negative rates.[9] Judicious use of this diagnostic category is encouraged and follow-up with repeat aspiration and correlation with clinical and radiologic findings is suggested by the committee members of the NCI conference.[8]

FNA of thyroid nodules has been a growing practice within our institution with 600–800 thyroid FNAs being evaluated annually in recent years. Nearly 6% were classified as "follicular lesion of undetermined significance." Although this indeterminate interpretation represents a minority of the total FNA diagnoses, the predictive uncertainty for malignancy, inherent in this diagnosis, is problematic

and frequently results in unnecessary surgical treatment. Using proposed standard morphologic criteria, we conducted a 10-year retrospective review of thyroid FNAs previously categorized as "follicular lesion of undetermined significance" with surgical follow-up to assess whether the indeterminate interpretations could be minimized and cytomorphologic features predictive for neoplasia could be identified.

Materials and Methods

Between January 1998 and December 2007, our institution performed a total of 3,809 thyroid FNAs among which 220 (5.8%) aspirates were categorized as "follicular lesion of undetermined significance." A SNOMED search was performed in the electronic pathology database and retrieved a total of 123 aspirates in which the indeterminate interpretation was rendered and hemithyroidectomies or total thyroidectomies were subsequently performed. Routine cytomorphologic examination of thyroid FNAs consisted of analysis of air-dried Diff-Quik smears, alcohol-fixed Papanicolaou smears, and ThinPrep and/or cell block preparations. The indeterminate diagnoses were originally rendered as the cytopathologists initially determined that the cytomorphologic features were equivocal and insufficient for definitive classification into either non-neoplastic or neoplastic categories. Suboptimal specimens due to insufficient cellularity, poor preservation, and/or obscuring blood were not categorized into this particular group.

Proposed standard morphologic criteria was established according to Kini's description[10] with minor modifications. Prior to the retrospective review, images illustrating the standard morphologic criteria with explanations were circulated among the reviewers and an agreement was achieved on the morphologic criteria for cytologic interpretation. Four cytopathologists (X.J., M.H.R., S.M.K., and C.W.M.) participated in the retrospective review. Reviewers' experience ranged from junior to senior level. Subsets of 123 aspirates were randomly distributed among the reviewers who were blinded to corresponding histologic findings at the time of retrospective assessment. Each reviewer performed an independent assessment and a total of 24 individual cytologic features (Table I and Fig. 1) were semi-quantitatively assessed for each aspirate with a score of 0 (absent), 1 (noticeable), 2 (easily seen), and 3 (prominent). Using the institutional eight thyroid FNA diagnostic categories[11] as a guideline, the reviewers recorded their interpretation on completion of assessment.

Cyto-histologic correlation was performed between the diagnoses rendered at the retrospective review and the corresponding histologic diagnoses. Furthermore, statistical analysis was performed using the logistic regression model to identify cytomorphologic features predictive of histologic diagnosis of neoplasia (both adenoma and carcinoma). A *P* value of less than 0.05 was considered statistically significant.

Table I. Cytomorphologic Features Assessed During the Retrospective Review

Overall cellularity
Architecture
  Honeycombing tissue fragments
  Syncytial tissue fragments
  Papillary tissue fragments
  Intact follicles
  Follicle with scalloped borders
  Microfollicles within tissue fragments
  Isolated microfollicles
Cytoplasm
  Scant cytoplasm
  Squamoid/dense cytoplasm
Nuclear features
  Enlargement
  Pleomorphism
  Overlapping
  Irregular membranes
  Clearing chromatin
  Coarse chromatin
  Nucleolus
  Pseudoinclusion
  Grooves
Background
  Colloid
  Hurthle cell metaplasia
  Lymphocyte
  Histiocytes
  Multinucleated giant cells

Results

The retrospective review of a total of 123 thyroid aspirates previously diagnosed as "follicular lesion of undetermined significance" using proposed standard morphologic criteria reclassified 64 (52%) aspirates as non-neoplastic lesions (nodular hyperplasia or chronic lymphocytic thyroiditis) and 27 (22%) aspirates as neoplasms. Of these 27 neoplasms, 22 were reclassified as "follicular neoplasm" and five as "positive for papillary thyroid carcinoma." However, the indeterminate diagnosis remained unchanged in the remaining 32 (26%) aspirates. Table II shows correlation between the cytologic diagnoses rendered by the retrospective review and corresponding histologic diagnoses. Among the 64 aspirates reclassified as non-neoplastic, 47 (73.4%) were confirmed as benign thyroid nodules on corresponding surgical specimens. Of 27 aspirates reclassified as neoplastic, 15 (55.6%) were confirmed as neoplastic by histologic examination (Fig. 2) including nine follicular adenomas, one follicular carcinoma, and five papillary thyroid carcinomas. The diagnostic accuracy reached 68.1% (62/91).

Of 32 aspirates in which the indeterminate interpretation remained unchanged, corresponding histologic examination identified 21 non-neoplastic lesions and 11 neoplasms, including nine follicular adenomas, one follicular carcinoma, and one papillary thyroid carcinoma. Malignancy was thus demonstrated in 6% of aspirates that remained as indeterminate on the retrospective review.
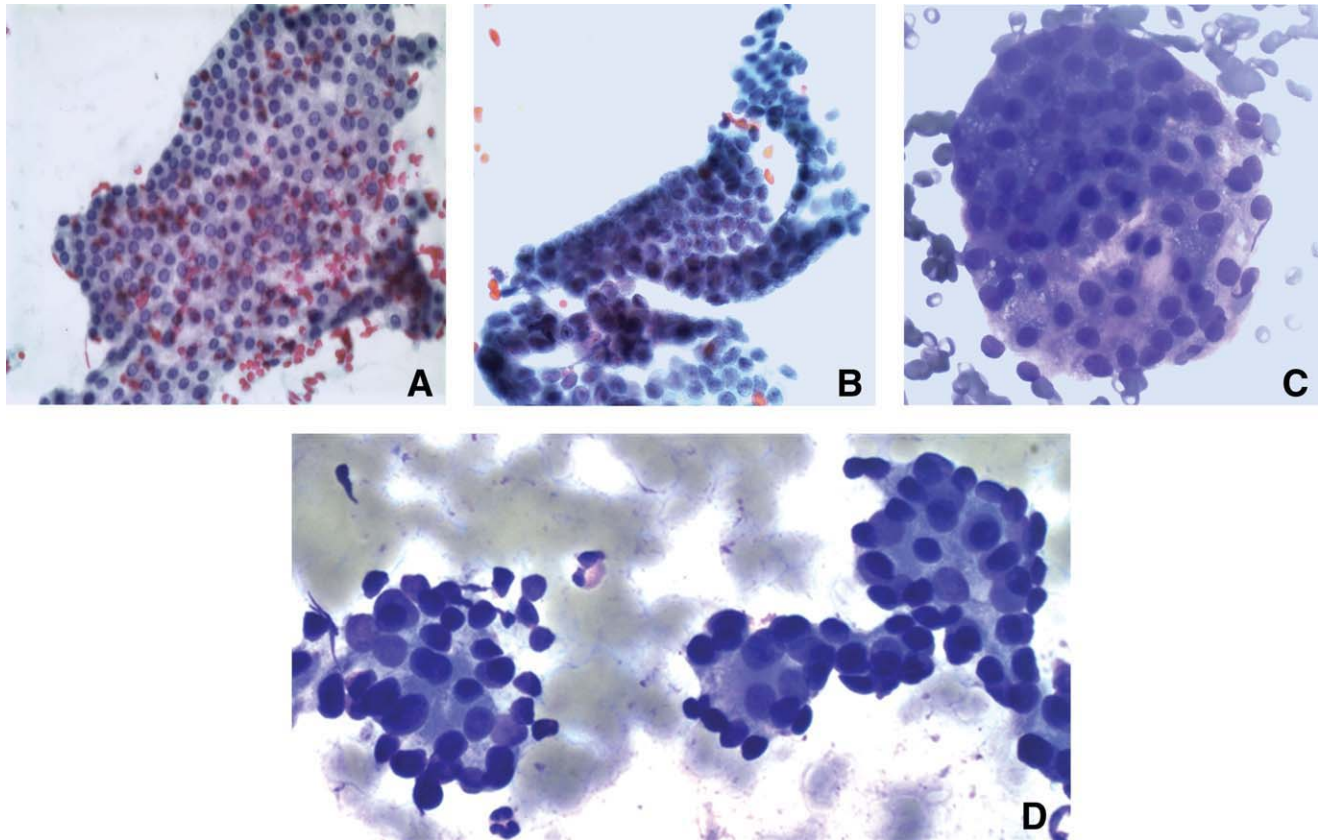
Fig. 1. Example of some cytomorphologic features assessed during the retrospective review. **A**: The honeycombing tissue fragment showing monolayer sheet of cells with well-defined cytoplasmic borders and evenly distributed nuclei. Nuclear polarity is maintained (Papanicolaou, ×600). **B**: Syncytial tissue fragments with cells that have poorly defined cytoplasmic borders. Nuclei are not evenly distributed. Nuclear crowding and overlapping is appreciated. Nuclear polarity is altered (Papanicolaou, ×600). **C**: This intact follicle shows a group of cells forming a sphere. The cells are arranged in a honeycombing pattern (Diff-Quik, ×600). **D**: Microfollicles. Cells comprising the small follicles show nuclear crowding and overlapping (Diff-Quik, ×600). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Correlation of Results of Retrospective Cytologic Review and Corresponding Histologic Diagnosis

	Histologic Diagnosis					
Cytologic diagnosis	Non-neoplasia	Follicular adenoma	Follicular carcinoma	Papillary carcinoma	Parathyroid adenoma	Total
Non-neoplasia	47	9	5	2	1	64 (52%)
Indeterminate	21	9	1	1	—	32 (26%)
Follicular neoplasm	9	9	1	3	—	22 (17.9%)
Papillary carcinoma	3	—	—	2	—	5 (4.1)
Total	80	27	7	8	1	123 (100%)

Table III shows cytomorphologic features that were significantly predictive for histologic diagnosis of neoplasia, along with odds ratios (95% confidential interval) and *P* values for each. As demonstrated by the logistic regression model, the histologic diagnosis of neoplasia positively correlated with the presence of the following cytomorphologic features: syncytial tissue fragments; isolated microfollicles; follicles with scalloped borders; scant cytoplasm; irregular nuclear membranes; nuclear overlapping; and coarse chromatin. Increased cellularity was a marginally significant feature that correlated with neoplasia. On the contrary, the presence of follicular cells displaying a hon-

eycombed pattern, abundant colloid, and histiocytes inversely correlated with neoplasia in a statistically significant manner.

Discussion

The committee of the recent NCI Thyroid FNA State of Science Conference defined "follicular lesion of undetermined significance" as a heterogeneous category consisting predominantly of cases in which the cytomorphologic findings are equivocal and neither convincingly demonstrate features justifying a definitive interpretation of a benign thyroid nodule nor demonstrate the degree of cellular/
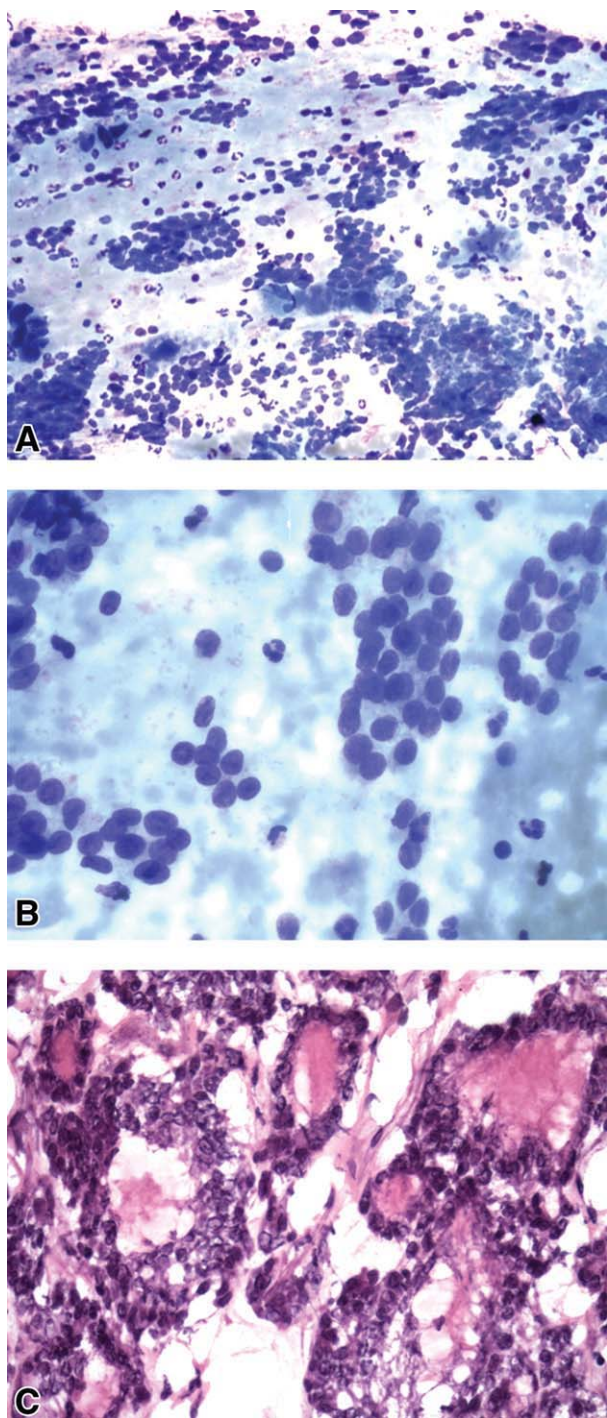
Fig. 2. Conventional smear showing **A**: hypercellularity with numerous microfollicles (Diff-Quik, ×200); **B**: the follicular cells exhibits nuclear crowding/overlapping, scant cytoplasm, nuclear enlargement with irregular nuclear membranes. No definitive intranuclear grooves and pseudo-inclusions are appreciated (Diff-Quik, ×600). It was interpreted as follicular neoplasm on the retrospective review. **C**: The corresponding histology (C) revealing follicular variant of papillary thyroid carcinoma (H&E, ×400). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table III. Predictive Cytomorphologic Features for Neoplasia

Features	Odds ratio (95% confidential interval)	P value
Syncytial tissue fragments	1.64 (1.11, 2.42)	0.01
Isolated microfollicles	1.82 (1.20, 2.75)	0.005
Follicles with scalloped borders	1.78 (1.02, 3.10)	0.04
Scant cytoplasm	2.08 (1.26, 3.44)	0.004
Irregular nuclear membranes	1.69 (1.00, 2.84)	0.05
Nuclear overlapping	1.47 (1.01, 2.16)	0.04
Coarse chromatin	2.56 (1.34, 4.97)	0.004
Honeycombing tissue fragments	0.59 (0.41, 0.85)	0.004
Colloid	0.49 (0.29, 0.81)	0.005
Histiocytes	0.57 (0.35, 0.94)	0.03

architectural atypia sufficient for a diagnosis of "follicular neoplasm" or "suspicious for malignancy." The committee proposed that, ideally, the classification of a thyroid FNA as indeterminate be used in less than 7% of cases.[8] However, a multi-institutional analysis has demonstrated marked variations in the use of this indeterminate diagnosis among both pathologists (2.5–28.6%) and institutions (3.3–14.9%).[12] Thyroid aspirates carrying the indeterminate interpretation comprised ~6% of all thyroid FNAs performed in our institution during the 10-year period of the retrospective review, thereby falling within the recommended range.

The marked variations in utilization of the indeterminate diagnosis are thought to be the consequence of the lack of precisely defined, objective morphological criteria.[9,12,13] Despite this fact, this interpretation should not be used too liberally. Recognition of various architectural patterns and cytologic features is essential in the cytomorphologic evaluation of thyroid FNAs. For example, a small fragment of follicular cells exhibiting evenly spaced, nonoverlapping nuclei should not be misinterpreted as a microfollicle simply because of its small size. There are also concerns related to threshold and level of stringency in applying the diagnostic criteria that may vary among institutions and observers. According to our institutional experience, the presence of a minor population of microfollicles in the setting of predominantly honeycombing tissue fragments, consistent with nodular hyperplasia, does not necessarily upgrade the interpretation to "follicular lesion of undetermined significance" or "suspicious for follicular neoplasm."

There are limited studies on the subclassification of the indeterminate category.[13,14] Our retrospective review of thyroid FNAs previously categorized as "follicular lesion of undetermined significance" using proposed standard morphologic criteria significantly reduced the number of indeterminate diagnoses from 123 to 32. The corresponding histologic diagnoses were concordant with the cytologic interpretation in nearly 75% of the non-neoplastic and more than one half of the neoplastic lesions that were reclassified by the retrospective review. A diagnostic accuracy of nearly 70% was achieved. Although surgery could have been avoided for nearly three-fourth of the non-neoplastic

lesions, the review failed to identify 16 neoplastic lesions of thyroid (false-negative). It was noted that the majority of the false-negative cases presented as a solitary nodule. The review also yielded false-positive rate of 20%, which is slightly higher than the previously reported false-positive rate (16%). The latter was generated from a study that examined thyroid nodules with definitive cytologic diagnosis of non-neoplasia and neoplasia/malignancy.[15] Although the diagnostic accuracy of the review appears to be less accurate than previously published data,[15,16] the differences were anticipated as the current study focuses on difficult thyroid lesions that exhibited equivocal features and thus cause diagnostic challenges. Whether the combination of clinical and cytologic examination is the best approach for selecting surgical candidates has yet to be investigated.[13]

Does the level of a pathologist's experience play a role in the frequency in which thyroid FNAs are interpreted as indeterminate? The results of a retrospective study of 21 indeterminate follicular lesions by Clary et al.[13] found that increased experience is related to use of more definitive diagnostic categories when a standardized answer form was provided. On the other hand, a multi-institutional study failed to show a relationship between the level of experience and usage of the indeterminate diagnostic category.[12] The results of the latter study were generated from review of original cytology reports. On the contrary, the results of the former study reported by Clary et al. were generated from a second review of previously diagnosed indeterminate lesions using a standardized answer form with four diagnostic categories (benign/favor benign, follicular lesion, favor neoplastic, and follicular neoplasm) and thus, selection bias existed.

In the current study, the morphologic features that are commonly encountered in cytologic samples and routinely assessed in an appropriate context were evaluated. Prior to the retrospective review, the reviewers discussed images illustrating the standard morphologic criteria and reached an agreement on the morphologic criteria for cytologic interpretation. We hoped that the approach for establishing uniformity among the reviewers would minimize both intraobserver and interobserver variability. Comprehensive assessment of interobserver variability is beyond the scope of the current study. A separate study is being conducted to evaluate interobserver agreement. However, the results of the current study provide a basis for the implementation of routine consensual evaluation of thyroid nodules categorized as "follicular lesion of undetermined significance" in cytology practice. We anticipate that the refined consensual approach will bring enhanced accuracy in the categorization of these difficult thyroid lesions.

Despite the efforts, there are certain lesions that fall into the indeterminate category due to intrinsic features of thyroid nodules and sampling variation, causing diagnostic difficulties during routine cytomorphologic examination. In our study, the interpretation of "follicular lesion of undetermined significance" remained unchanged in 32 aspirates after the retrospective rereview. The corresponding histologic examination revealed one follicular carcinoma and one papillary thyroid carcinoma. This low incidence of malignancy (6%) in our study is consistent with the risk of malignancy (5–10%) presented at NCI Thyroid FNA State of the Science Conference.[8] Because of the relatively low-predictive value of malignancy, patients in which this indeterminate cytologic interpretation is rendered on the initial thyroid FNA can benefit from repeat FNA and correlation with clinical and radiologic findings.[8] Several studies have shown that repeat aspiration yielded a definitive diagnosis for nodules initially diagnosed as indeterminate.[17–19] Lately, the potential of using molecular analysis as an adjunct of cytology to further refine an indeterminate interpretation of thyroid FNAs has been a topic of discussion.[20,21]

In summary, using proposed standard morphological criteria can help minimize the diagnosis of "follicular lesion of undetermined significance," allow for more accurate cyto-histologic correlation and thereby play a substantial role in reducing unnecessary surgical intervention.

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