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REVIEW

The Bethesda system for reporting thyroid cytopathology: into the clinic

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Abstract: Fine-needle aspiration (FNA) remains the most effective and safe method of evaluating thyroid nodules for potential surgical management. Since 2007, the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has standardized nomenclature for thyroid FNA and provided an evidence-based malignancy risk for each of its diagnostic categories. Using TBSRTC criteria, most thyroid nodules can effectively be categorized as either "benign" or "malignant" and referred for definitive management without further testing. However, many thyroid nodules fall into an indeterminate TBSRTC category, most notably atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS). Efforts have been made to elucidate further clinical utility from indeterminate cases, including nomenclature modifications and molecular-based testing modalities. The use of "atypia qualifiers" in AUS/FLUS cases appears to refine the diagnosis to provide a more specific risk of malignancy. Notably, AUS qualifiers of "cannot exclude papillary thyroid carcinoma" and "cannot exclude follicular neoplasm" appear to carry a higher risk of malignancy than other AUS qualifications in multiple studies. Molecular panels appear to hold particular promise as adjuncts in helping to delineate worrisome from non-worrisome thyroid nodules with indeterminate cytology. In particular, the miRInform test (Asuragen), an oncogene mutation panel, appears to show utility in its ability to "rule in" a malignant or neoplastic process, although it is limited by a relatively high false-negative rate. Conversely, the Afirma test (Veracyte), a gene expression classifier (GEC), appears to show clinical promise due to its high negative predictive value; albeit with a significant false-positive rate. Herein, we provide an overview of TBSRTC diagnostic categories and a literature review of new attempts to further refine indeterminate categories; as well as a review of the most commonly used commercial molecular panels used in thyroid cytopathology.

Keywords: thyroid, FNA, the Bethesda system, cytopathology

Introduction

Thyroid carcinoma is by far the most common endocrine malignancy.[1] It is estimated that as many as 7% of the adults have palpable nodules and up to 50% of adults have thyroid nodules detectable by ultrasonography. However, only 5% of thyroid nodules are found to be malignant; therefore, surgical removal of every thyroid nodule is unnecessary, expensive, and potentially risky.[2]

Fine-needle aspiration (FNA) is the most cost-effective and safe method to diagnose thyroid carcinoma and to stratify thyroid nodules for surgical management. Before the routine use of thyroid FNA, the percentage of surgically resected thyroid nodules that were malignant was 14%.[3] With current thyroid FNA practice, the percentage of resected nodules that are malignant surpasses 50%.[4] It is important for

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pathologists to communicate thyroid FNA interpretations to refereeing physicians in terms that are unambiguous and clinically useful. However, the terminology for thyroid FNA has traditionally varied significantly among laboratories and created confusion in some cases.

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) is the offspring of the 2007 National Cancer Institute State of Science Conference on thyroid FNA that defines consensus diagnostic terminology and morphologic criteria.[5–8] TBSRTC provides uniform diagnostic terminology for pathologists to communicate with clinicians. Per TBSRTC, there are six diagnostic categories including nondiagnostic or unsatisfactory (ND/UNS); benign; atypia of undetermined significance (AUS) or follicular lesion of undetermined significance (FLUS); follicular neoplasm (FN) or suspicious for FN; suspicious for malignancy (SM); and malignant. Each diagnostic category is associated with a specific risk of cancer and a recommendation for the management (Table 1). A detailed online image bank with illustrations corresponding to each Bethesda category can be seen at http://www.papsociety.org./atlas.html (courtesy of the Papanicolaou Society of Cytopathology). With its employment of universal morphological criteria and corresponding malignant risk stratification, TBSRTC provides useful information to clinicians, allowing them to dictate management in an equitable fashion.

TBSRTC diagnostic categories

Nondiagnostic or unsatisfactory

This category applies to specimens that are UNS due to an inadequate number of follicular cells, obscuring blood, overly thick smears, and poorly fixed smears. For a thyroid FNA specimen to be satisfactory for evaluation, it requires at least six groups of well-preserved follicular cells, preferably all six groups of follicular cells are on the same slide and each group composed of at least ten cells. At least 200 cells are required for a liquid-based specimen.

There are several exceptions to the numeric requirement of benign follicular cells. Any specimen that contains abundant colloid is considered adequate even when less than six groups of benign follicular cells are identified, especially when the radiographic impression is that of a nodular goiter. Also, any cytologic atypia identified on a clinically solid nodule should not be classified as UNS; instead, either AUS or suspicious categories should be given. If there are abundant reactive lymphoid cells, even when only scant follicular cells are present, this lesion should be diagnosed as lymphocytic thyroiditis or Hashimoto's thyroiditis. At the 2007 National Cancer Institute Conference, it was also decided that cyst fluid only specimens should be considered as ND/UNS. The significance of a cyst fluid result depends in large part upon sonographic correlation. If the cystic nodule is small (<3 cm) and shows no worrisome sonographic features, an endocrinologist might proceed as if the cyst fluid diagnoses were a benign result. On the other hand, it might be clinically equivalent to an ND result if the sonographic features are worrisome, and the endocrinologist is not convinced that the sample is representative.[5–8]

A meta-analysis of eight studies showed 1.8%–23.6% of all thyroid FNAs were ND with an overall value of 12.9%.[9–17] Surgical resection was performed in 16.2% of cases, and the risk of malignancy was 16.8%. Usual management is clinical follow-up including repeat ultrasound-guided FNA in 6–18 months, preferably with on-site immediate specimen adequacy evaluation. American Thyroid Association (ATA) recommends correlation with sonography (increased vascularity or suspicious features) as a means of prioritizing nodules that yield ND FNAs for re-aspiration.[18,19] After two successive ND specimens, close clinical follow-up or surgery should be considered.

Benign

Benign thyroid aspirates typically contain abundant colloid and monolayered sheets of follicular epithelium with a

Table 1 The Bethesda system for reporting thyroid cytopathology: implied risk of malignancy and recommended clinical management

Diagnostic category	Risk of malignancy (%)	Usual management
I. Nondiagnostic or unsatisfactory		Repeat FNA with ultrasound guidance
II. Benign	0–3	Clinical follow-up
III. Atypia of undetermined significance or follicular lesion of undetermined significance	5–15	Repeat FNA
IV. Follicular neoplasms or suspicious for a follicular neoplasm	15–30	Surgical lobectomy
V. Suspicious for malignancy	60–75	Near-total thyroidectomy or surgical lobectomy
VI. Malignant	97–99	Near-total thyroidectomy

Abbreviation: FNA, fine-needle aspiration.

honeycomb appearance. The nuclei of benign follicular cells are small (equivalent to the size of red blood cells), rounded, and uniform with fine chromatin and smooth nuclear membrane. Abundant inflammatory cells, especially lymphocytes, also favor a benign thyroiditis. The diagnostic terminology for the benign thyroid lesions used in the literature includes nodular goiter, chronic lymphocytic thyroiditis, adenomatoid nodule, and colloid nodule.

A meta-analysis[9] showed the cases in this category ranged from 39% to 73.8% with an overall value of 59.3%, and a cumulative malignancy rate of 3.7%, which is slightly higher than that recommended by TBSRTC guidelines (0%–3%); however, it is within the 0%–5% range reported by the ATA guidelines.[18,19] Patients with a benign nodule are followed by clinical and radiological examination periodically and some patients may undergo repeat FNA due to increase in the size of nodule. It is recommended that serial ultrasound be used in follow-up of thyroid nodules to detect clinically significant changes in size (a 20% increase in nodule diameter with a minimum increase in two or more dimensions of at least 2 mm).[18,19]

Atypia of undetermined significance or follicular lesion of undetermined significance

This is a heterogeneous category that includes cases that cannot be classified as either benign or FN. The findings of cases categorized as AUS/FLUS are not convincingly benign, yet the degree of cellular or architectural atypia is insufficient for an interpretation of FN or SM. Some of these cases are placed in this category because of low cellularity or poor specimen quality due to poor fixation, obscuring blood, and thick smears.[8] The diagnostic category of AUS is based on cytomorphologic interpretation and is, therefore, highly subjective. In any given case diagnosed as AUS, a different pathologist may consider the observed atypia to be reactive in nature, while another may consider that degree of atypia to be diagnostic of papillary thyroid carcinoma (PTC). Many studies have found that the rate of AUS diagnoses varies significantly between different institutions as well as among pathologists depending on their experience.[15,21,22]

A meta-analysis showed FNA cases in this category ranged from 3% to 27.2% with an overall value of 9.6% and an overall rate of malignancy of 15.9%.[9] General clinical recommendations for an initial AUS/FLUS diagnosis are for "clinical correlation" and in most cases repeat aspiration after an "appropriate" interval. TBSRTC recognizes the equivocal diagnostic nature of this category, as well as the lack of clear clinical management direction an AUS/FLUS diagnosis portends and, therefore, sets a provisional institutional goal for most practice settings to limit the rate of AUS/FLUS diagnosis to a range of 7% of thyroid FNAs. More recent reviews have suggested that a range of 7%–12% of thyroid FNA cases may be a more typical representation of current practice.[20] Also interesting are some new data showing that the ultimate rate of malignancy in cases initially classified as AUS/FLUS may be higher than estimated by TBSRTC or the aforementioned meta-analysis, reaching 26.6%–37.8%.[23]

Owing to the inherent management uncertainty, an AUS/FLUS diagnosis causes, it has understandably been suggested that AUS could be further subclassified into more distinct subtypes, each conferring a different magnitude for the risk of malignancy.[24–29] Utilizing "atypia qualifiers" has been suggested as a means to subclassify the AUS diagnosis.[26,27] Renshaw subclassified the cases of "atypical follicular cells" in thyroid aspirates into four groups: AUS cannot exclude follicular neoplasm (AUS-FN), AUS cannot exclude Hürthle cell neoplasm (AUS-HCN), AUS cannot exclude papillary carcinoma (AUS-PTC), and AUS, not otherwise specified (AUS-NOS), and found that the risk of malignancy for cases of AUS-PTC was significantly higher (38%), whereas the risk of AUS-HCN was significantly lower at 7% than other subtypes of atypical follicular cells.[24] In a study by VanderLaan et al,[26] AUS with architectural atypia was used to describe an aspirate exhibiting focal or mild features that are similar to, but not sufficient for a diagnosis of "suspicious for a follicular neoplasm", a category that is equivalent to AUS-FN. The qualifier of AUS with cytologic atypia was broadly regarded as a specimen with features similar to but not sufficient for a diagnosis of "suspicious for papillary carcinoma", which is equivalent to AUS-PTC. It was also noted that cytologic atypia was associated with a malignant risk closer to 30% that is similar to Renshaw's study. Wu et al used the similar terminology and subclassified a total of 138 AUS cases into AUS-NOS (48), AUS-PTC (41), AUS-FN (32), and AUS-HCN (17) and further divided the AUS cases into high-risk and low-risk groups. The high-risk group includes AUS-PTC that carries a significantly higher risk of malignancy at 32% (not including papillary microcarcinoma [PMC], $P<0.001$) and 54% (including PMC, $P<0.001$) and AUS-FN with risks of malignancy at 25% (not including PMC) and 34% (including PMC) that is within the range of malignancy risk of FN suggested by the Bethesda system. The risks of neoplasm are significantly higher at 63% and 81% for AUS-PTC and AUS-FN, respectively. Both AUS-HCN and AUS-NOS had a relatively low-risk

profile with a follow-up malignant diagnosis in 0% and 8% of the cases (excluding PMC) and 18% and 19% of the cases (including PMC) and an intermediate risk of neoplasm of 53% and 44%, respectively. As expected, all the malignant cases associated with AUS-PTC were papillary carcinomas; including five cases of classical papillary carcinoma, eight cases of follicular variant of papillary carcinoma, and nine cases of PMC. On the other hand, follicular carcinoma is highly associated with AUS-FN, seen in six of eight malignant cases. Subclassification of the atypical thyroid aspirates based on cytomorphologic features into high-risk and low-risk group shows utility for management purposes. Patients with AUS-PTC and AUS-FN may warrant a thyroidectomy or lobectomy, while conservative follow-up with repeat FNA or molecular testing is an adequate management option for patients with low-risk lesions such as AUS-NOS and AUS-HCN.[29]

Follicular neoplasm/suspicious for follicular neoplasm

FNA cannot distinguish between benign and malignant non-papillary follicular and Hürthle-cell lesions. The diagnostic terminology of FN reflects the limitations of thyroid cytology since the diagnosis of follicular carcinoma is based only on the demonstration of capsular and/or vascular invasion. FNA smears of a FN are hypercellular as compared to most aspirates of benign colloid nodule and demonstrate a monotonous population of follicular cells with minimal or absent background colloid. The cells are usually arranged in three-dimensional, syncytial groups and microfollicles, defined as <15 follicular cells arranged in a circle that is at least two-thirds complete, with prominent nuclear overlapping and crowding. Nuclei are enlarged and often crowded with coarse chromatin and prominent nucleoli.[30]

The meta-analysis[9] showed that the percentage of cases classified into this category ranged from 1.2% to 25.3% with an overall value of 10.1%. More than two-thirds of these (~70%) underwent surgery, with a risk of malignancy of 26.1%. The discrepancy illustrated from the low malignancy rate among resected lesions is a reflection of the shortcomings of FNA to evaluate what is essentially a histological diagnosis. It appears that adjunctive testing of FNA specimens, including molecular studies, may hold promise in providing a more effective surgical triage (discussed later in further detail).

Hürthle cell neoplasm

Hürthle cells, also known as oxyphilic cells or oncocytes, are large polygonal cells with eosinophilic granular cytoplasm due to accumulation of mitochondria. The term HCN of the thyroid denotes a set of tumors, which are composed exclusively or predominantly of Hürthle cells with enlarged, round nuclei with prominent nucleoli. FNA specimens are cellular aspirate comprising monomorphic population of Hürthle cells (>90%) in a background of minimal or absence of colloid and lymphoid cells. Cells can be arranged in monolayer sheets, follicular groups, or as scattered single cells. Nuclei are round, eccentrically, or centrally located with finely granular chromatin and single-prominent nucleoli.

The following five cytologic criteria were shown to predict a Hürthle cell carcinoma on FNA smears that included a predominance of Hürthle cells with scant colloid and at least one of the following four cytologic features: small cell dysplasia (bland nuclei with cell diameter less than twice the nuclear diameter), large cell dysplasia (cells demonstrate at least twice the variation in the nuclear diameter, often with prominent nucleoli and irregular nuclear outlines), nuclear crowding, and dyshesion.[31,32] In addition, male sex and large tumor size also increase the risk for Hürthle cell carcinoma.[32] For Hürthle cell lesions, the presence of colloid and lymphocytes favors a benign non-neoplastic lesion, whereas nuclear enlargement and large tumor size are significantly more common in neoplasms than benign non-neoplastic Hürthle cell lesions.[32]

Surgical lobectomy is the recommended management option for either FNA diagnosis of FN or HCN. In cases found to be malignant on final histology, complete thyroidectomy is commonplace. It is accepted practice within many institutions for intraoperative consultation by frozen section to be performed on lobectomy specimens containing thyroid nodules with an FNA diagnosis of FN or HCN. This is due to the assumption that malignant findings on frozen section could prompt an immediate completion thyroidectomy. Multiple studies have found this practice not to be cost-effective and, in general, uninformative.[33,34]

Suspicious for malignancy

This category can be used as suspicious for papillary carcinoma, medullary carcinoma, other malignancies (eg, lymphoma, metastatic carcinomas), or neoplasm because of total necrosis of lesional cells (eg, anaplastic carcinoma). The meta-analysis[9] showed FNA cases in this category ranged from 1.4% to 6.3% with an overall value of 2.7%. The mean risk of malignancy in this category was 75.2%. Usual management option includes near-total thyroidectomy or surgical lobectomy.

Malignant

The cytologic smears are diagnostic of malignancy including PTC, medullary thyroid carcinoma, poorly differentiated (formerly "insular") carcinoma, anaplastic carcinoma, lymphoma, and metastatic carcinoma. The reported rate of malignancy in all publications in a meta-analysis[9] ranged from 2% to 16.2% with an overall value of 5.4% and a risk of malignancy of 98.6%. These high positive predictive values are most likely attributable to the fact that approximately 80% of thyroid malignancies are PTC. Most aspirates from PTC exhibit features that have been well described in the literature and provide a high sensitivity and specificity for the diagnosis of conventional PTC.[35]

Adjunctive studies in thyroid cytopathology

Immunocytochemistry

While benign and malignant diagnoses rendered by FNA are quite accurate, there remains a group of diagnostically challenging indeterminate lesions that make up ~30% of thyroid FNAs (including AUS/FLUS, FN/suspicious for follicular neoplasm [SFN], and SM). A number of studies have attempted to evaluate the role of immunocytochemistry in further elucidating malignant risk in such indeterminate follicular (nonmedullary) lesions.

The most commonly utilized markers for delineation of increased malignant risk in thyroid cytopathology include antihuman mesothelial cell 1 (HBME-1), galectin-3, and cytokeratin 19 (CK19).[36] Each of these markers has touted reasonable sensitivity in identifying PTC and/or FC. Studies have reported sensitivities for HBME-1 ranging from 70% to 97% of PTCs and 63%–88% of FCs.[36–41] Galectin-3 has shown sensitivities of 73%–99% in PTC and 21%–88% of FCs.[36,39–42] CK19 is reported to stain 72%–97% of PTCs and 21%–44% of FCs.[37,40–43] Lack of diagnostic specificity in these markers, however, has presented itself to be a barrier to widespread clinical utility.[36] HBME-1 and CK19 are reported to stain as many as 55% and 33% of follicular adenomas, respectively, as well as 33% and 17% of nodular hyperplasia cases.[40,41] Galectin-3 does appear to retain a greater degree of specificity in some studies;[40,41] however, it's diagnostic utility have been challenged in others.[44] Some studies have advocated the use of a panel of these immunostains as a means to increase their utility by amplifying the sensitivity and specificity.[36–38,40,41] Our personal institutional experience with immunocytochemistry in indeterminate follicular thyroid lesions on FNA is that they fail to provide any definitive prognostic information to the clinician and thus are not often utilized.

Molecular studies

With increasing knowledge about molecular mechanisms involved in carcinogenesis and lack of definitive cytologic characterization of indeterminate lesions, utilization of molecular techniques has become an attractive option to clinicians. Recent studies have demonstrated the utility of a molecular panel, rather than a single marker, in improving the diagnostic accuracy of the thyroid cytological sample.[45–49] The best commercial tests available today are 1) miRInform thyroid panel (Asuragen, Austin, TX, USA) and 2) Afirma GEC (Veracyte, South San Francisco, CA, USA). Most clinicians use the former panel to "rule in" the diagnosis of malignancy, and the latter panel to "rule in" benign diagnoses.[48–51]

The miRInform test (Asuragen) is composed of a panel of oncogene mutations, including the BRAF V600E mutation, RAS mutations (HRAS, KRAS, NRAS), RET/PTC rearrangements (RET/PTC1 and RET/PTC2), and PAX8/PPARF fusion. This 4-gene panel test has excellent specificity but is only associated with up to 30% negative predictive value (NPV).[52,53] The largest prospective trial study performed by Nikiforov et al[47] showed positive predictive values of 88%, 87%, and 95% and specificity of 99%, 97%, and 96%, for the FNA diagnoses of AUS, FN/SFN, and SM, respectively. However, a significant false-negative rate was found to be associated with these diagnostic categories, which ranged from 6% to 28%. The authors suggested that the presence of any mutation, especially BRAF and RET/PTC, would be a strong indication of cancer, therefore justifying a total thyroidectomy.

The Afirma (Veracyte) test is a GEC that measures the expression of 167 RNA transcripts from "indeterminate" thyroid FNAs. Its main purpose is to identify lesions that are benign. A large, prospective, multicenter Veracyte validation study evaluated 265 indeterminate FNAs.[51] Seventy-eight of 85 malignancies were identified by the Afirma GEC as "suspicious" and showed a sensitivity and specificity of 92% and 52%, respectively. NPV was 93% among all indeterminate lesions, including 95% for AUS/FLUS, 94% for FN/SFN, and 85% for SM.

The cost of these newer commercial molecular panels may certainly be concerning, if not prohibitive, depending on the patient's insurance and financial situation. Single molecular tests for the individual oncogene mutations that are seen within the miRInform test (BRAF, RET/PTC, RAS, and PAX8/PPARF) are typically more widely available. In particular, BRAF mutation analysis by PCR can be employed in indeterminate cases displaying some cytomorphological features of PTC in an effort to evaluate for definitive

malignancy, as it is a highly specific test.[47] BRAF V600E mutation detection may even portend clinical importance in clear-cut malignant FNA cases, as its presence also correlates with poor prognostic features including "tall-cell" variant, as well as identifying a therapeutic target.[47]

Molecular tests should be considered only if the results could significantly change patient management. For FNA diagnoses of AUS/FLUS, molecular testing should be considered only if surgery is entertained. In this setting, Afirma GEC performs best due to its high NPV, as a benign GEC result could help rule in a benign diagnosis and avoid surgery. Repeat FNA evaluation is also a good option in this situation, as it was shown to have reclassified <50% of AUS/FLUS lesions as benign.[4] If surgery is inevitable, for instance, due to a very large nodule or because of the desire of the patient, then there is no need for molecular testing. For cytologic diagnosis of FN/SFN, generally associated with a 20%–30% risk of malignancy, the general management recommendation is lobectomy.[5,6] If the patient is clinically low risk for malignancy and/or not an ideal candidate for surgery, the Afirma GEC test could be utilized to "rule in" benign disease. However, if the patient is clinically high risk and could perhaps benefit from total thyroidectomy rather than lobectomy, then miRInform 4-gene panel could be utilized in "confirming" a malignant diagnosis and justifying total thyroidectomy. Perhaps a combination of both molecular panels is most ideal in this setting. Again, the cost of these tests should certainly be considered.

Both of these molecular panels are associated with significant false-positive and false-negative results, and management decisions should not entirely rely on molecular results. Cytologic diagnoses of SM are associated with a 60%–75% cancer risk on surgical follow-up.[4,5] Molecular testing in this setting plays a very limited role in patient management and should be discouraged.[49]

Conclusion

FNA of the thyroid remains the safest and most cost-efficient manner in which to stratify thyroid nodules for surgical excision. TBSRTC has standardized reporting nomenclature for thyroid FNA that corresponds with specific cytomorphologic criteria and risk of malignancy.

While most thyroid FNAs result in "benign" or "malignant" diagnoses, a significant percentage of thyroid FNAs still receive a diagnosis that is indeterminate for malignancy (AUS/FLUS, FN/SFN, HCN/SHCN, SM).[5] In the case of AUS/FLUS, specific nomenclature alterations including atypia qualifiers may be of use to communicate a more specific risk of malignancy to clinicians.[24–26] In particular, "AUS/FLUS, cannot rule out PTC" and "AUS/FLUS, cannot rule out FN" appear to be associated with a higher risk of malignancy than other AUS subclasses. Molecular panels appear to have particular promise in helping to provide additional information regarding the malignant risk of indeterminate thyroid lesions, specifically AUS/FLUS and FN/SFN. In particular, the miRInform test (Asuragen) appears to have promise in "ruling in" a malignant diagnosis for nodules with an indeterminate FNA diagnosis. Alternatively, the Afirma (Veracyte) panel may have utility in "ruling in" a benign diagnosis for similar lesions. Despite their potential clinical upside, utilization of such molecular tests should be limited to situations where results will directly affect patient management, and always in conjunction with cytologic thyroid FNA findings.

Disclosure

The authors report no conflicts of interest in this work.

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