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Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS): Interpretation and Algorithm for Follow-Up

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Abstract

The Bethesda System for Reporting Thyroid Cytology (TBSRTC) has proven to be an effective and robust thyroid fine needle aspiration (FNA) classification scheme to guide the clinical treatment of patients with thyroid nodules. However, a tendency of increasing diagnosis of atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS) is observed. This is commensurate with the incorporation of new molecular tests for classifying indeterminate thyroid nodules. Moreover, a sizable portion of AUS/FLUS is correlated with follicular variant papillary carcinoma (FVPTC). A suggestion of reclassifying noninvasive FVPTC (NI-FVPTC) or noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) as a neoplasm rather than a carcinoma would significantly change the risk of malignancy in AUS/FLUS category. We review the diagnostic criterion and subclassifying suggestions of AUS/FLUS, features indicating follicular variant neoplasm in AUS/FLUS category, and commercially available molecular tests for AUS/FLUS subgrouping. We propose a multidisciplinary approach to AUS/FLUS follow-up.

Keywords: atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), diagnosis, risk of malignancy, follow-up

1. Introduction

Fine needle aspiration (FNA) is continuously and widely considered to be the most accurate method for the evaluation of a thyroid nodule [1, 2]. To address a significant variability in the

reporting of cytological findings in thyroid FNA samples, a consensus recommendation known as the Bethesda System for Reporting Thyroid Cytology (TBSRTC) was provided in 2007. It includes six diagnostic categories, which are associated with varying risks of malignancy: I = nondiagnostic (ND), II = benign, III = atypia/follicular lesion of undetermined significance (AUS/FLUS), IV = follicular neoplasm/suspicious for a follicular neoplasm (FN/SFN), V = suspicious for malignancy, and VI = malignant [3]. This reporting system is compatible with that recommended by the British Thyroid Association [4] (**Table 1**). The BSRTC has proven to be an effective and robust thyroid FNA classification scheme to guide the clinical treatment of patients with thyroid nodules [5–9]; it is endorsed as a standard practice in reporting thyroid aspiration cytology by the 2015 American Thyroid Association (ATA) guidelines [2]. The Bethesda reporting system for thyroid FNA was used in 90% of practices in a recent survey [10].

Bethesda diagnostic category [3]		British Thyroid Association [4]		Cancer Risk [3]	Management [3]
I	Nondiagnostic or unsatisfactory	Thy1	Nondiagnostic	1–4%	Repeat FNA with U/S
II	Benign	Thy2	Nonneoplastic	1–3%	Follow-up clinically
III	Atypia of undetermined significance (AUS) or follicular lesion of undermined significance (FLUS)	Thy3a	Atypical features present	~5–15%*	Repeat FNA
IV	Follicular neoplasm (FN) or suspicious for a follicular neoplasm (SFN)	Thy3f	Follicular neoplasm suspected	20–30%*	Lobectomy
V	Suspicious for malignancy (SM)	Thy4	Suspicious of Malignancy	60–75%*	Lobectomy or total thyroidectomy
VI	Malignant	Thy5	Diagnostic of malignancy	97–99%	Total thyroidectomy

* Reclassifying non-invasive follicular variant papillary thyroid carcinoma (NI-FVPTC) as neoplasm, and renaming it as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) would have most pronounced effect on the indeterminate categories: a decrease of cancer risk 5.2-17.6% in AUS/FLUS, 8-15.1% in SF/FN and 17.6-41.5% in suspicious for malignancy [22, 23] (see 3.3).

Table 1. Thyroid fine needle aspiration diagnostic category.

However, with implementation of BSRTC there is a tendency of increasing diagnosis of AUS/FLUS [6–12], and the risk of malignancy (ROM) associated with AUS/FLUS seems to be higher than estimated before [6–17]. These trends are commensurate with the incorporation of new molecular tests for classifying indeterminate thyroid nodules [16–20], and increased diagnosis of follicular variant papillary thyroid carcinoma (FVPTC) by surgical pathology [21]. Of note, a significant portion of AUS/FLUS has a histologic diagnosis of FVPTC [11, 12, 22]. If non-invasive FVPTC (NI-FVPTC) is considered as a neoplasm rather than a carcinoma, e.g.

noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) as recently suggested, the risk of malignancy for AUS/FLUS would further change [16, 22, 23].

Although “indeterminate” thyroid nodules include AUS/FLUS (III), FN/SF (IV), and suspicious for malignancy (V) categories, AUS/FLUS remains the most “*undetermined*” in terms of management. The recommendations for FN/SF (IV) and suspicious for malignancy (V) are lobectomy or total thyroidectomy [3] with 61.2 and 86.0% of them potentially referring to surgery in practice [22], while the suggestion for AUS/FLUS cases is repeated FNA biopsy [3]. The risk of malignancy for AUS/FLUS diagnosed postoperatively ranges from 5 to 48% [24–27]. The wide range has posed anxiety to patients and difficulty in reaching clinical decision.

Hürthle cells are oncocytic cells, present in normal thyroid (increase with age), goiter, Hashimoto thyroiditis, Graves disease, radiation, chemotherapy, adenoma and carcinoma [29]. A Hürthle cell carcinoma is diagnosed if morphologic features indicative of malignancy are obvious. Otherwise, it is categorized as SN/FSN if the nodule is composed exclusively of Hürthle cells. It is downgraded to AUS/FLUS in a background of Hashimoto thyroiditis [3, 28, 29]. Hürthle cell lesions are considered “non-predictable” or “with higher malignant risk” [30], despite the fact that the risk of malignancy diagnosed by FNA in Hürthle cell lesions is similar to non-Hürthle cell lesions by other studies [28, 31].

We would discuss the scenarios triggering AUS/FLUS diagnosis, characters helpful for subclassifying AUS/FLUS, cytological features of FVPTC, and Hürthle cell lesion. An algorithm with integrated approaches of clinical, radiologic, pathologic, and molecular findings will be proposed for indeterminate thyroid nodules.

2. AUS/FLUS interpretation

2.1. Diagnosis of exclusion and incorporation of biomarkers

The AUS/FLUS thyroid nodules represent those not clearly benign or malignant [3]. The cytologic findings are not convincingly benign, yet the degree of cellular or architectural atypia is not sufficient for an interpretation of SF/FN, suspicious for malignancy, or malignancy [28]. It is a diagnosis of exclusion (**Figure 1**).

Case 1 (Figure 2): The aspiration of a 2.6 cm × 2.5 cm × 1.7-cm isthmus solid nodule is from a 46-year-old female. The smears are cellular with scant colloid, unlike a benign thyroid nodule. Nuclear enlargement and overlapping resembling SF/FN are present, but are focal. Rare microfollicles are identified in cell-block section. There are no papillary carcinoma features such as nuclear groove, pseudoinclusion, or papillae. This case was categorized cytologically as AUS (case courtesy of Dr. Jaklyn McClendon, Anaheim Health Medical Center, CA).

The specimen was forwarded for ThyGenX Thyroid Oncogene Panel and ThyraMIR Thyroid miRNA Classifier. ThyGenX™ Oncogene Panel detected one NRAS point mutation (Q61R). The ThyraMIR™ microRNA Classifier was negative. Thyroid nodules with AUS diagnosis and NRAS mutation are at increased risk for malignancy (42–65% compared to 7–37% in NRAS-negative AUS, $p = 0.038$) [32]. However, RAS mutation has limited value-predicting malignancy in the absence of BRAF mutation among thyroid nodules with AUS/FLUS cytology [33];

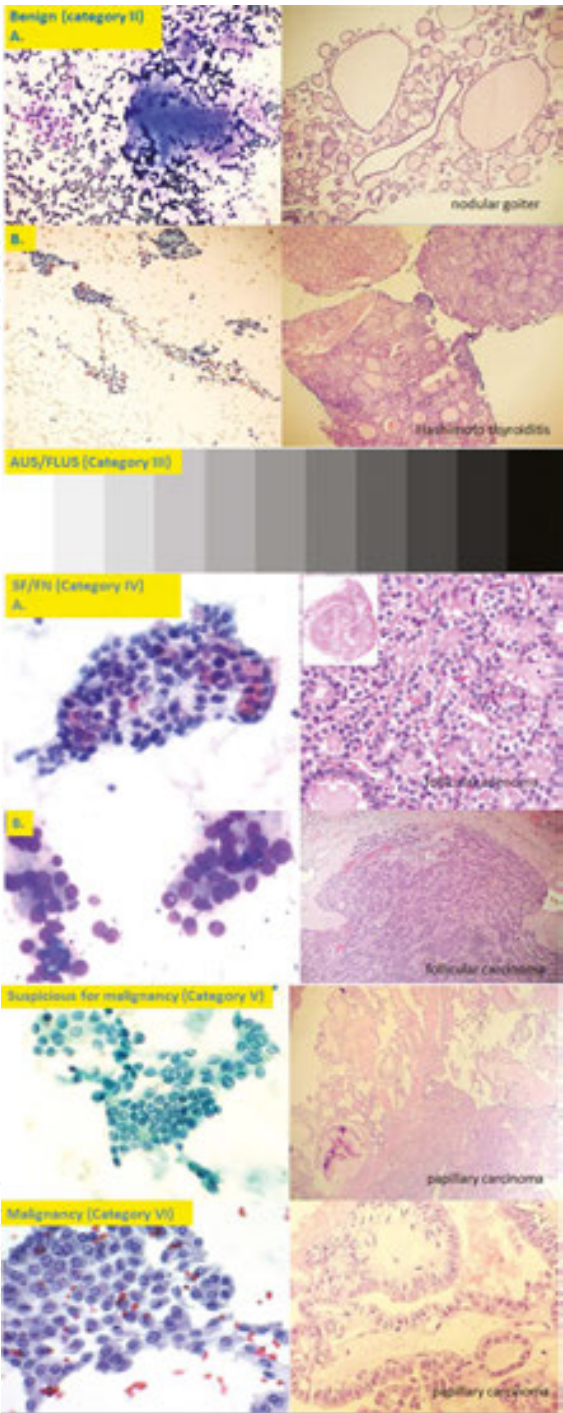


Figure 1. AUS/FLUS in the Bethesda Thyroid Diagnostic Category. The cytological interpretation is on the left, the corresponding histological diagnosis is on the right. Category I nondiagnostic or unsatisfactory is not illustrated. Category II is a group of benign lesions including nodular goiter with abundant colloid and honeycomb sheets of follicular cells (IIA), and Hashimoto thyroiditis characterized by follicular cells, Hürthle cells, lymphocytes and plasmacytes (IIB). Category IV indicates follicular lesions (SF/FN) featured by increased cellularity and microfollicles. Category V applies to aspirations with some but not all features of malignancy. Category VI includes lesions meeting diagnostic criteria of malignancy. AUS/FLUS: Atypia of undetermined significance/follicular lesion of undetermined significance SF/FN: suspicious for follicular neoplasm/follicular neoplasm.

RAS mutation has been detected in follicular adenoma (8–48%) and follicular carcinoma (0–52%) [34]. The probability of malignancy is reduced to 50:50 in combination with the mild atypia and negative ThyraMIR™ finding in this case.

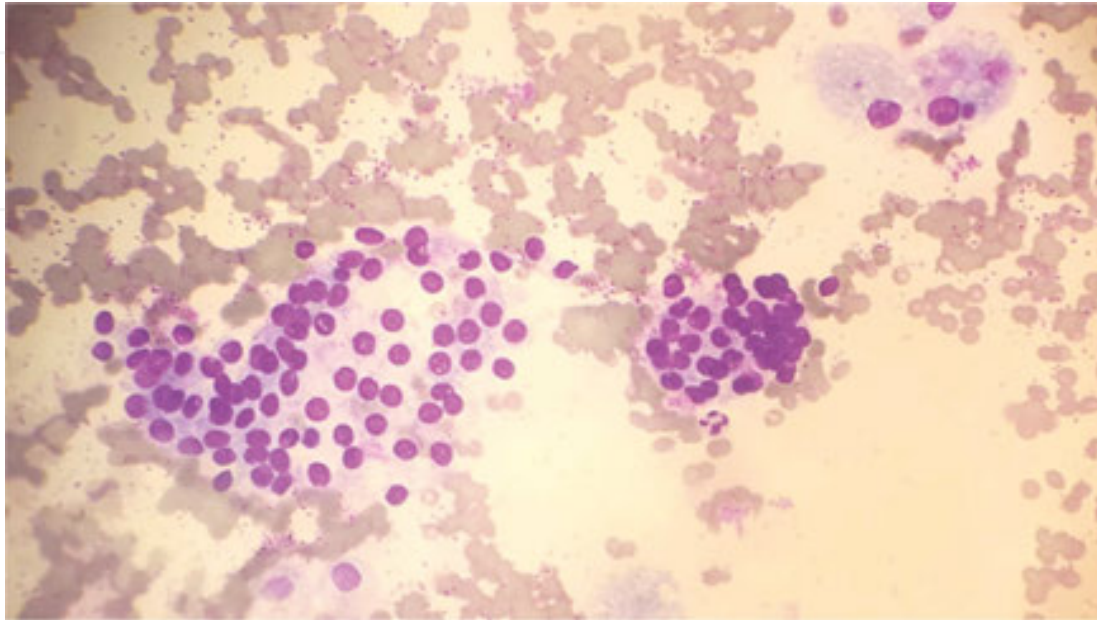


Figure 2. Atypia of undetermined significance. Focal crowded follicular cell clusters with scant colloid. (Case courtesy of Dr. Jaklyn McClendon, Anaheim Health Medical Center, CA.)

This case has also brought up a question of how ancillary studies would help subclassify AUS/FLUS. The various performances in molecular tests could be due to the nature of AUS/FLUS, which is a group of heterogeneous borderline lesions in transit from hyperplastic or adenomatoid nodule to adenoma, adenoma with atypia, and carcinoma. There is no single magic marker for subclassifying AUS/FLUS. Most tests use a panel of markers. New tests are emerging and extensive validation is required. Therefore, the 2015 American Thyroid Association (ATA) guidelines recommend ancillary studies but do not specify particular test(s) for AUS/FLUS follow-up [2].

2.2. Reproducibility of AUS/FLUS

2.2.1. Adequacy check

Recognizing that some of the equivocal FNA cases are the result of inadequate number of cells or poorly visualized cells, the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has recommended adequacy evaluation with category I being nondiagnostic [3]. Blood diluted and obscured specimen may push the follicular cells into microfollicle-like structure, making the colloid imperceivable and masking the benign cytological details. A sparse preparation or interpretation hindered by sampling preparation artifact may not be

justified for a category of AUS/FLUS [3, 35]. It is important not to place those cases in AUS/FLUS group for the reasons: although re-biopsy is the same immediate outcome for ND or AUS/FLUS, the risk of malignancy for two AUS/FLUS diagnoses is higher than that for one AUS/FLUS diagnosis [10, 36], and most cases with two AUS/FLUS diagnoses will go to surgery [36].

2.2.2. *Correlation with clinical and ultrasound findings*

Clinical findings such as spiculated margin, microcalcification, hypoechogenicity of ultrasonography, larger size of mass, male gender, and increased thyroid-stimulating hormone (TSH) level may be associated with increased risk of malignancy in AUS/FLUS diagnosis. On the other hand, cystic or complex nodules have a lower risk of malignancy [37–42]. Considering those features, a more definitive diagnosis could be reached [35].

2.2.3. *Consensus diagnosis*

Expert consultation and group consensus reviews have been reported to minimize the diagnosis of FLUS [43]. A second opinion has an overall diagnostic resolution rate of 42.5% for “indeterminate” thyroid nodules and 71.5% reclassification accuracy in AUS/FLUS category [44].

2.2.4. *Quality assessment*

The use of the individual diagnostic categories within BSRTC varied up to 12.7-fold. The ratio of “AUS/FLUS” (A) to “malignant” (M) diagnoses varied at a range of 0.5–4.9 with a median ratio of 2.0. Based on this, an A:M ratio of 1.0–3.0 is proposed for the proper use of AUS/FLUS diagnosis. AUS:M ratios of >3.0 are likely because of overdiagnosis of AUS or underdiagnosis of M. AUS:M ratios of <1.0 are mostly due to low AUS rates, at the likely expense of sensitivity [45]. Some study supports this quality measure, but disputes exist [46].

3. Cytology hints for subgrouping AUS/FLUS

3.1. Reactive change and benign cellular components mimicking atypia

3.1.1. *Reactive atypia*

Reactive atypia in thyroid are associated with cystic degeneration, thyroiditis (inflammation), physical or chemical trauma, radiation, and other nonspecific causes, in a very similar way to cervical cytology [28, 29]. They present as metaplastic Hürthle cells, squamous cells, degenerative/regenerative follicular cells, or proliferative fibroblasts. Cells can be alarmingly bizarre with nuclear grooves or nucleoli but never have high N:C ratio, crowded nuclei, papillae, or microfollicle [47].

Case 2 (Figure 3): The smear is prepared from a 4-cm left-neck complex lesion on a 65-year-old female. The significant finding is a cluster of hemosiderin-associated cells with large elon-

gated or polygonal nuclei in contrast to a background of honeycomb-round follicular cells. Despite the presence of nuclear groove, no intranuclear pseudoinclusion or architectural disorder such as crowded nuclei or papillae is identified to further suggest malignancy. Concurrent Afirma gene expression classifier result is benign, and medullary thyroid cancer classifier is negative (Veracyte, Inc., San Francisco, CA).

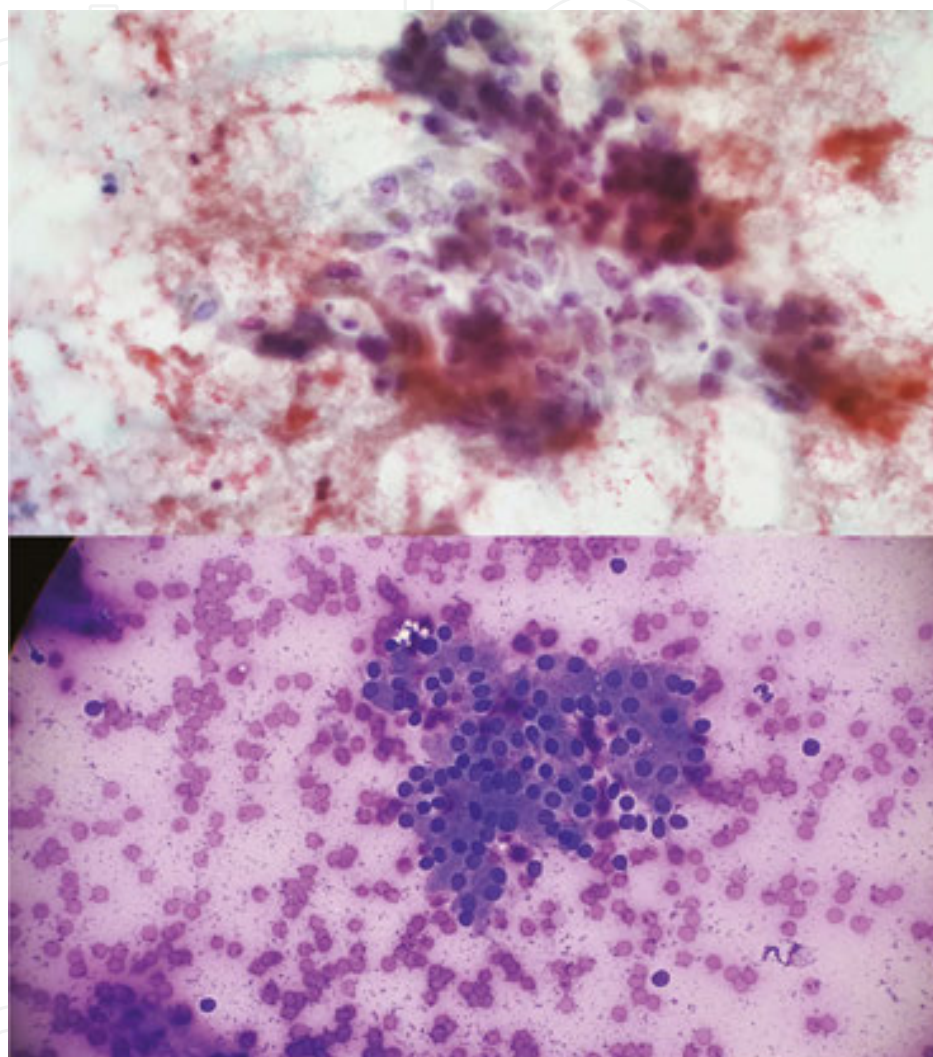


Figure 3. Atypical cells suggestive of benign cystic lining. The cells (upper) are larger compared to the rest of the follicular cells (lower), showing partially streaming appearance, irregular nuclear membrane and nuclear groove, pale chromatin, and small nucleoli. No nuclear overlapping or inclusion is present.

Atypical cystic lining cells need to be distinguished from cystic papillary carcinoma, which constitutes 10% of papillary carcinoma. The former does not have nuclear overlapping and inclusion [47].

Epithelioid cells in Hashimoto thyroiditis (**Figure 4**) or reparative fibroblasts can have atypical features such as large nuclei, taking an appearance of ugly ducklings among benign follicular cells. The low N:C ratio and non-clumping chromatin distinguish them from malignancy.

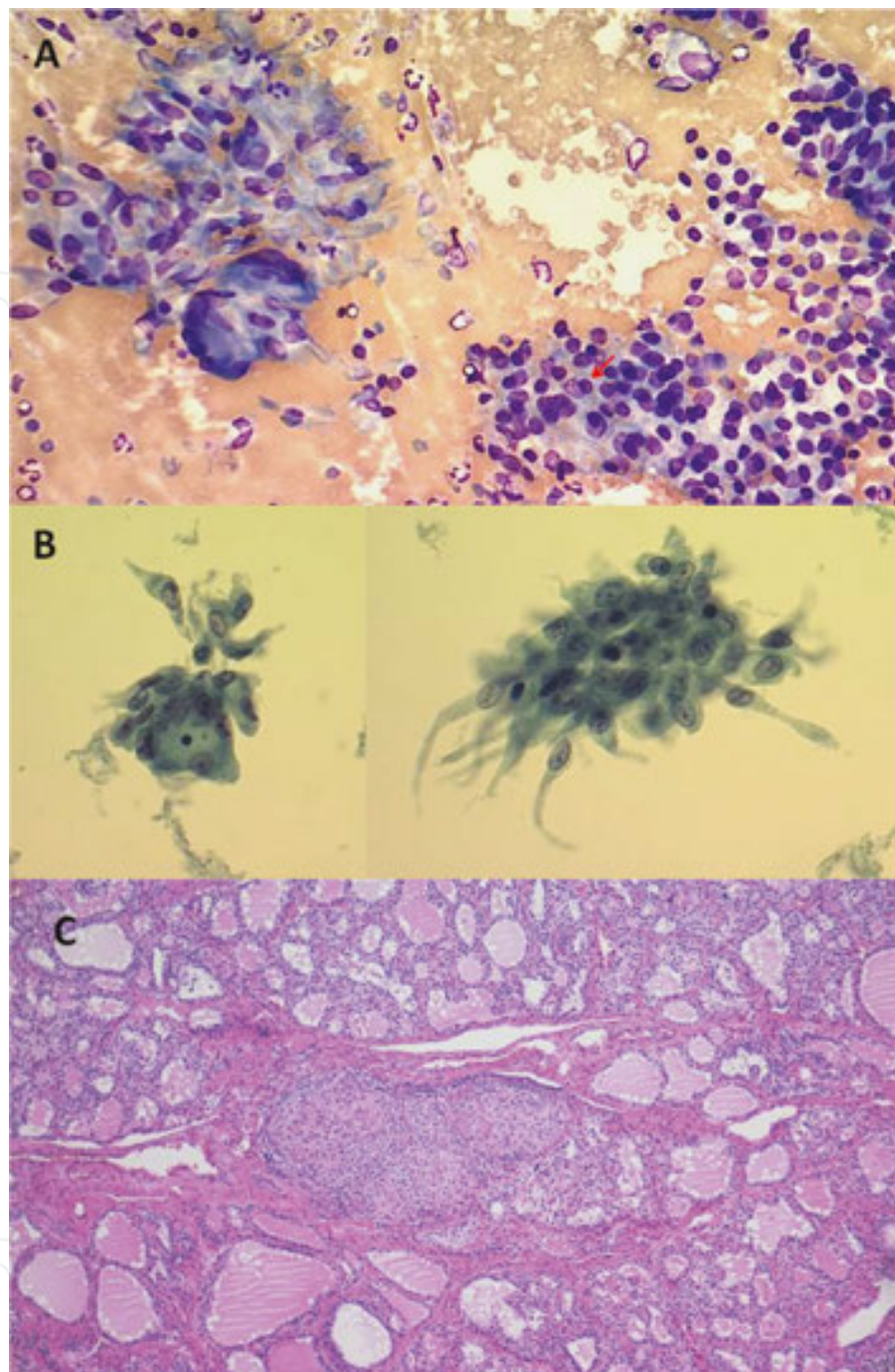


Figure 4. Granulomatous inflammation. (A) Epithelioid cells (left) in contrast to sheets of bland-appearing follicular cells mixed with several plasmacytes (arrow), Diff Quick stain. (B) Epithelioid cells in spindle-shaped and multinucleated form, Pap stain. (C) Resection shows Hashimoto thyroiditis with diffuse inflammation.

3.1.2. Perifollicular fibrosis

Perifollicular fibrosis refers to basement membrane-like material outlining follicles. Perifollicular fibrosis has been described in sporadic colloid nodule, adenomatoid hyperplasia, and in pediatric thyroid cancer following the Chernobyl disaster [24, 28, 48–50]. It is associated with

benign or low-grade thyroid lesions. The incidence of perifollicular fibrosis seems to be higher in elderly people (>60) than in other age population (5–10 vs 2%) ([51] and unpublished data). In the elderly, it is often linked to a paucicellular aspiration with fibrosis.

Cases 3 and 4 (**Figure 5**): Perifollicular fibrosis is illustrated in two cases with Bethesda category of atypia and benign, respectively. The first case (A) is a 38-year-old male with a left thyroid nodule status post chemoradiation for Hodgkin disease. Surgical resection shows an atypical adenomatoid hyperplasia. The second case (B) is a 61-year-old female with a benign 1.3-cm complex thyroid nodule.

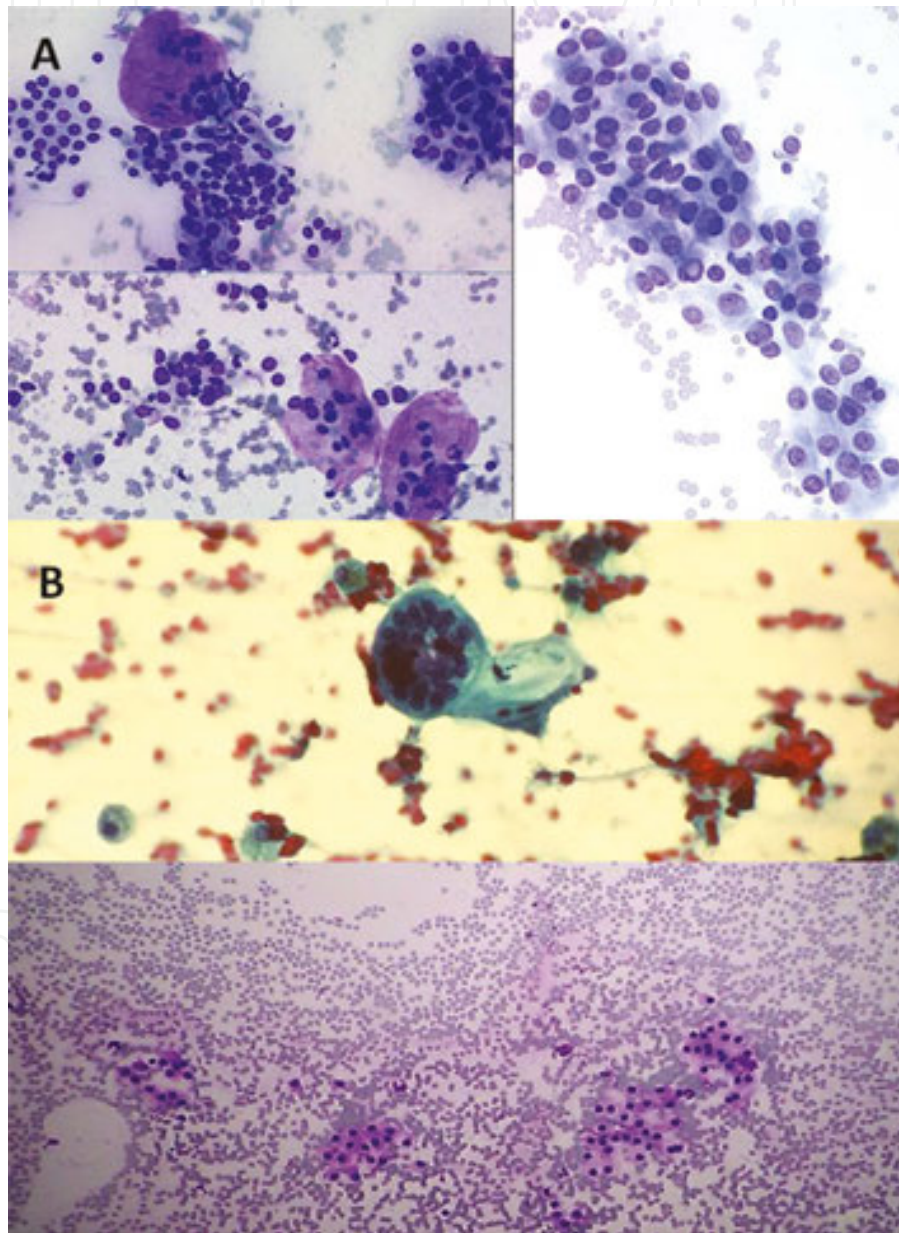


Figure 5. Perifollicular fibrosis (A) in a cellular specimen with atypical cells. Follicular cells are expelled off a balloon-like fibrous rind (perifollicular fibrosis, left, Diff Quick stain). The follicular cells are atypical with slightly larger, crowded nuclei and rare nuclear groove and inclusion (right, Pap stain). (B) In a benign thyroid nodule with sparser cells. A glassy hyaline rind is partially peeled off a follicle with hemosiderin macrophages (upper, Pap stain) and scattered benign follicular cells (lower, Diff-Quik stain) are seen in the background.

Perifollicular fibrosis was first described in post-Chernobyl nonneoplastic thyroid tissue [50]. The basement membrane-like structure surrounding follicles may inhibit tumor genesis and progression, although the mechanism underlying perifollicular fibrosis in radiation exposure might be different from that in natural aging.

3.1.3. Benign cellular components mimicking AUS/FLUS

Parathyroid gland is occasionally aspirated during thyroid nodule workup. In one molecular study for indeterminate thyroid nodules, three patients out of 441 who had a diagnosis of AUS/FLUS were found to have parathyroid rather than thyroidal origin after biochemical study and surgery [19].

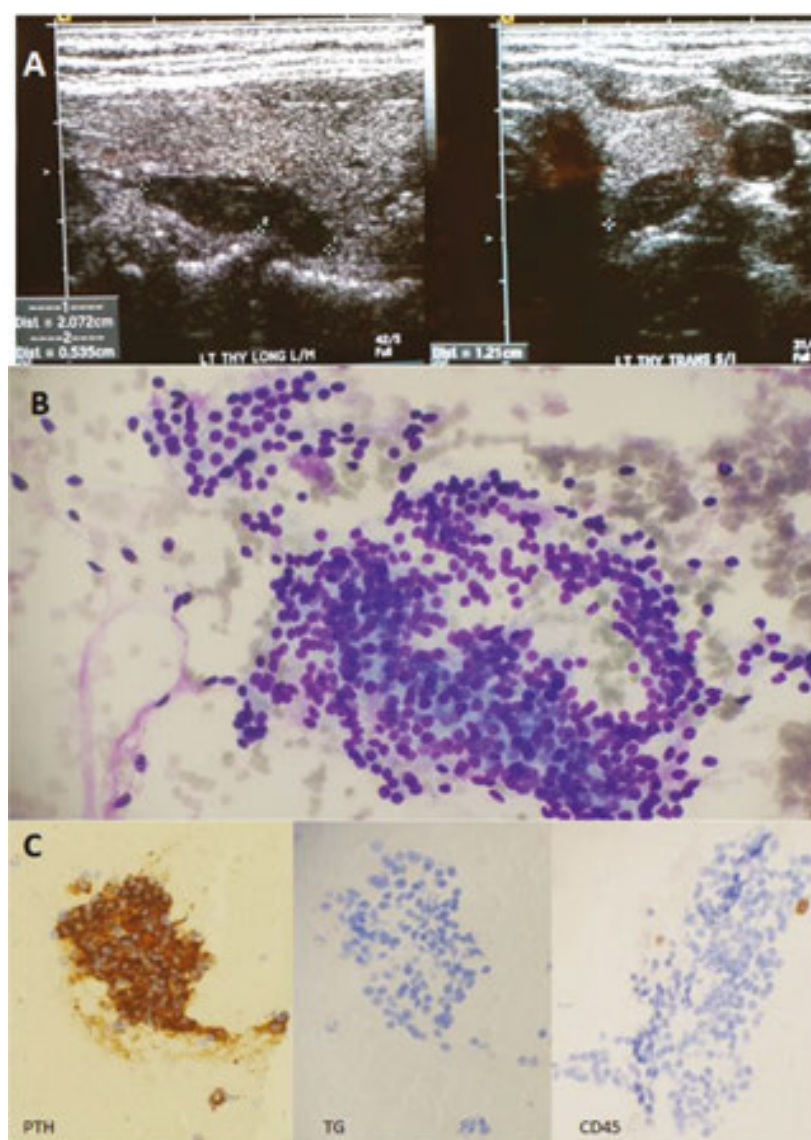


Figure 6. Parathyroid gland mimicking thyroid nodule. (A) An elongated nodule in the left posterior “thyroid” close to vessels. (B) Aggregates of structureless uniform cells with no colloid. (C) Immunohistochemistry performed on cell block confirmed parathyroid origin. PTH: parathyroid hormone; TG: thyroglobulin; CD45: white blood cell common antigen.

Case 5 (Figure 6): A 65-year-old female complained of “a lump in the throat.” Ultrasonography identified a right isthmus movable 1.9-cm nodule and a left posterior 2-cm nodule. Image and cytological features of the left posterior nodule are shown in A and B. The monomorphic small cells are bland appearing with scant cytoplasm and no identifiable colloid. The immunohistochemistry (IHC) confirms parathyroid origin.

If IHC information is not available, this case may be mistaken for FLUS in the absence of colloid or benign thyroid nodule if colloid from adjacent thyroid is mixed up.

Ultimobranchial body or solid cell nest is a developmental remnant derived from fourth to fifth pharyngeal pouch, considered as a normal component of thyroid. It is found in about 60% of serially sectioned thyroid with a male predominance [52–54]. Incidental aspiration of them may lead to AUS diagnosis [55].

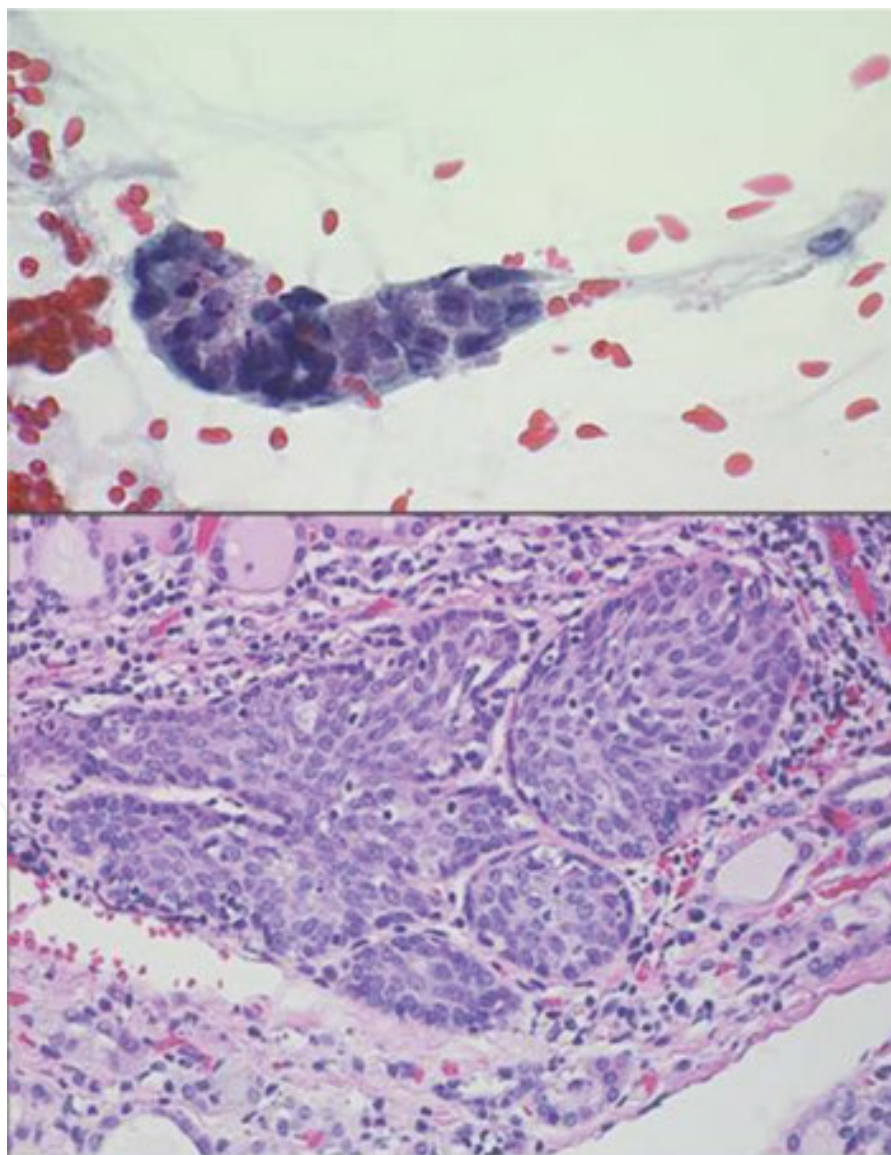


Figure 7. Ultimobranchial body. The aspiration shows cohesive polygonal cells. Histologic section shows a nest composed predominantly of similar polygonal or oval to spindle cells (main cells) mixed with a minor population of small cells with compact nuclei and clear cytoplasm.

Case 6 (**Figure 7**): The illustrated cell cluster is from a thyroid aspiration of a 46-year-old male. Cytological features distinguishing from malignancy include the absence of hyperchromatic nuclei or prominent nucleoli in tightly cohesive squamoid cells. Those cells are positive for Galectin-3, but negative for HMBE-1 [55].

3.2. Cytologic characters suggestive of malignancy

3.2.1. Nuclear atypia

It seems that nuclear atypia (a subgroup of AUS) is more predictive of malignancy than microfollicle (a subgroup of FLUS) [56]. Nuclear atypia suggestive of papillary carcinoma carry a higher risk of malignancy than AUS/FLUS interpretations made for other reasons, such as microfollicles, Hürthle cells, and suboptimal specimens [57]. This might be due to higher incidence of papillary carcinoma or follicular variant papillary carcinoma than follicular carcinoma, and incapability of cytology to distinguish adenoma from follicular carcinoma.

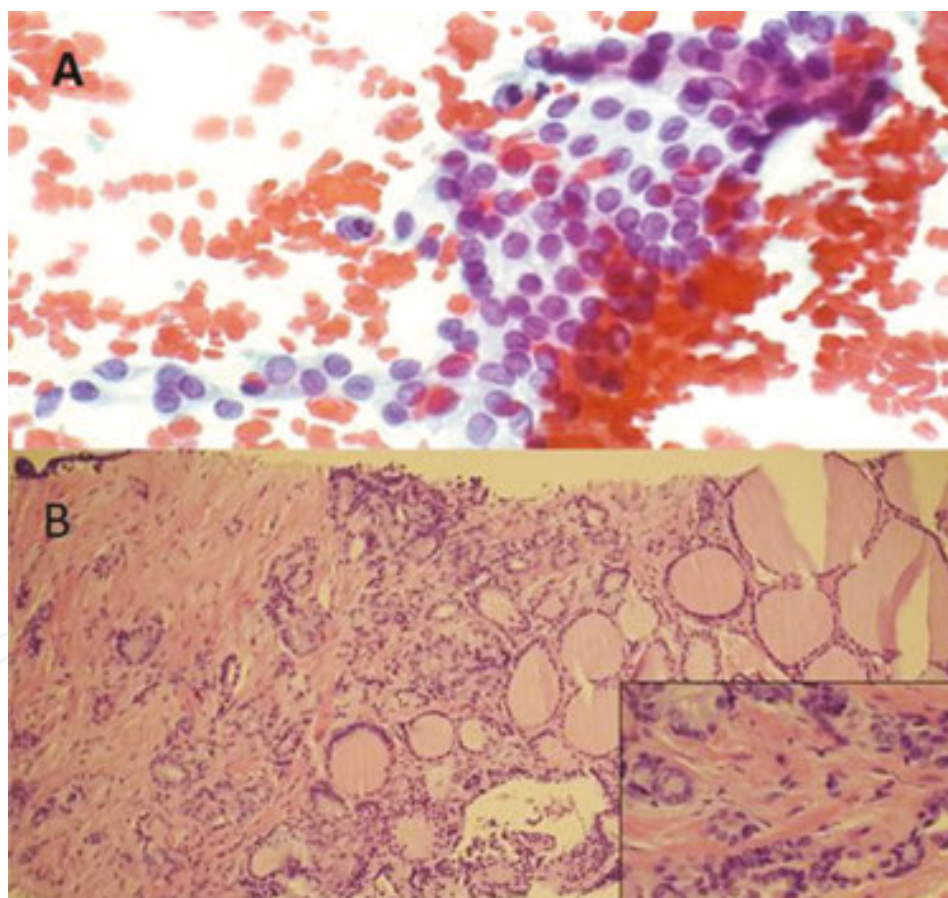


Figure 8. AUS upgraded to papillary carcinoma follicular pattern. (A) A combination of nuclear overlapping, groove, and pale chromatin indicates AUS, suspicious for malignancy. (B) A follow-up core biopsy confirmed papillary carcinoma follicular pattern with invasion.

Nuclear pseudoinclusion and papillae are diagnostic of papillary carcinoma. Other nuclear features such as enlarged nuclei, pale chromatin, nuclear groove, and crowded nuclei are more

commonly seen in AUS/FLUS category. More prominence of these findings or a great of them should increase the suspicion of malignancy [57].

Case 7 (Figure 8): This is a case of AUS upgraded to malignancy in a 32-year-old female. The initial aspiration of a thyroid nodule demonstrates groups of follicular cells with various features of nuclear atypia including pale chromatin, nuclear groove, and nuclear overlapping. A core-needle biopsy (CNB) 1 month later confirmed papillary carcinoma of follicular pattern with invasion.

3.3. Follicular variant papillary thyroid carcinoma (FVPTC) and noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)

FVPTC is defined by histology as a tumor with PTC-type nuclei and follicular rather than papillary pattern (papillary architecture of <1%) [21]. Based on the presence or absence of invasion of capsule or parenchyma, it is subgrouped as invasive FVPTC (I-FVPTC) and noninvasive FVPTC. The NI-FVPTC is clinically indolent similar to adenoma [22, 58]. It is recently renamed as noninvasive follicular thyroid neoplasm with papillary-like nuclear features to avoid overtreatment [22, 23, 58]. The impact of reclassifying NI-FVPTC on the risk of malignancy would be most pronounced in the indeterminate categories: a decrease of ROM, 5.2–17.6%, in AUS/FLUS, 8–15.1% in SF/FN, and 17.6–41.5% in suspicious for malignancy [16, 22].

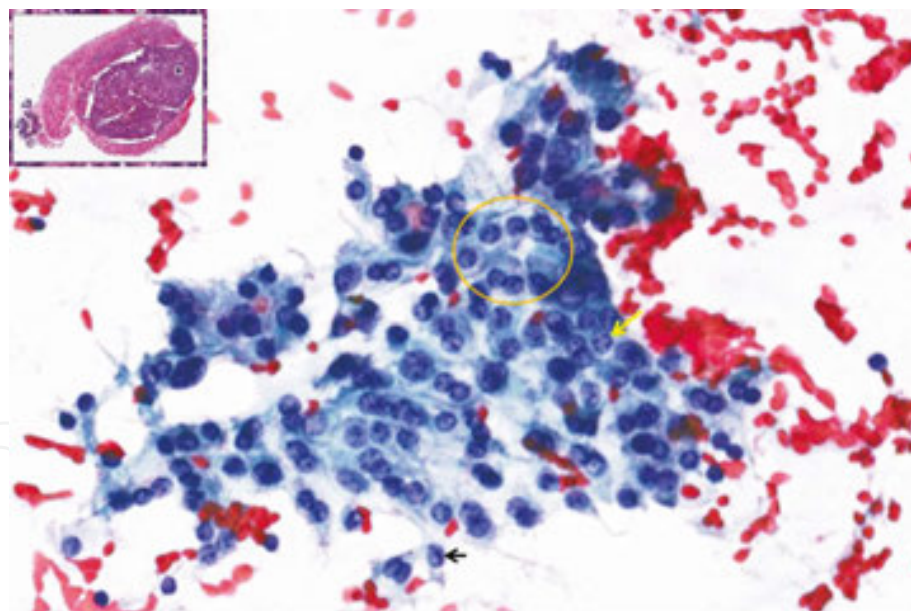


Figure 9. NIFNP/NI-FVPTC. NI-FVPTC characterized by pale nuclei with margined chromatin (yellow arrow), nuclear groove (black arrow), and microfollicle (orange circle) in an encapsulated nodule (left-upper corner) without capsule or parenchyma invasion.

NIFTP or NI-FVPTC has a molecular file of RAS mutation and PAX8/PPAR α translocation but lacking BRAF V600E mutation in keeping with follicular adenoma. I-FVPTC has an opposite molecular profile closer to classical papillary carcinoma than to follicular adenoma or NIFTP/NI-FVPTC (BRAFV600E > RAS mutations) [58].

Most NIFTP or NI-FVPTC are categorized as AUS/FLUS, SF/FN, or suspicious for malignancy in Bethesda system. The NIFTP/NI-FVPTC in AUS/FLUS category shows atypical PTC-type nuclear feature and microfollicles (**Figure 9**). However, I-FVPTC versus NIFTP/NI-FVPTC is a histologic diagnosis, not a cytology stratification. Whether the degree of nuclear atypia in FVPTC is predictive of invasion is unclear.

3.4. Hürthle cell lesion

Hürthle cell tumor of thyroid is a group of neoplasm with distinct biology, morphology, and natural history. However, it is not an independent entity in World Health Organization (WHO) category and therefore has been diagnosed as either benign adenoma, or papillary carcinoma or follicular carcinoma or poorly differentiated based on cytomorphology, architecture of papillae, follicle and solid/trabeculae, and evaluation of mitosis and necrosis [59]. Capsule invasion or vascular invasion is the criterion for malignancy, while pleomorphism by itself is not a feature of malignancy [28, 59]. Cytomorphological criteria have been shown to be helpful in distinguishing Hürthle cell neoplasm from Hürthle cell metaplasia (in nodular goiter or Hashimoto thyroiditis), but not Hürthle cell carcinoma from adenoma [60].

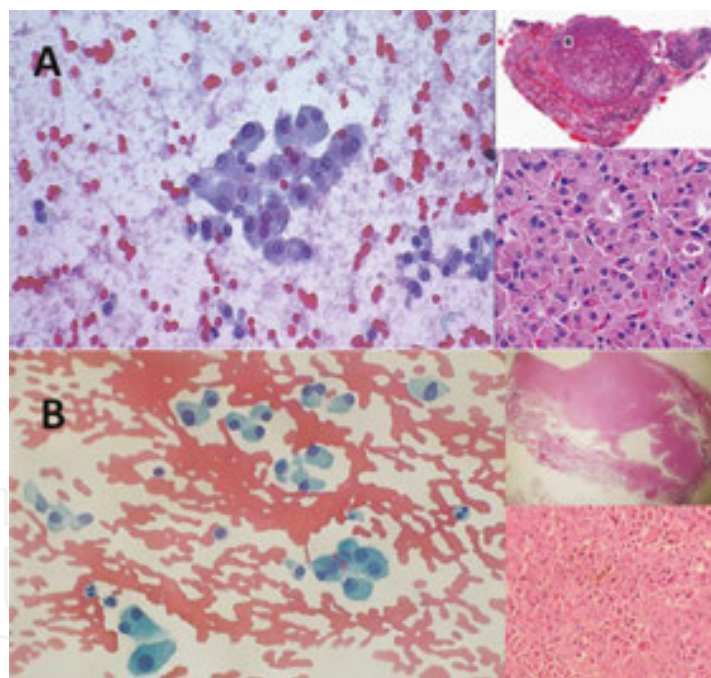


Figure 10. Hürthle cell lesion. (A) Predominant oncocytic cells on thyroid aspiration (left) and well-circumscribed nodule in the background of lymphocytic thyroiditis (Hashimoto disease) (right). (B) Loosely cohesive eosinophilic cells with binucleation on thyroid smears (left) and metastatic disease to the lung composed of the same cells (right) (case courtesy of Dr. Terry Welsh, Anaheim Health Medical Center, CA).

Lymphocytic thyroiditis is diagnosed based on a mixed cell population of lymphocytes, plasma cells, and follicular cells with Hürthle cell metaplasia. However, the differentiation of benign hyperplastic Hürthle cell nodule (or Hürthle cell adenoma) from Hürthle cell carcinoma can be very difficult based on cytology alone.

Case 8 (A and B) (**Figure 10**): A is a 56-year-old female presented with bilateral thyroid nodules. Surgical resection revealed hyperplastic oncocytic nodules in the background of lymphocytic thyroiditis. B is an 85-year-old female with one left thyroid nodule. Left lung wedge resection demonstrates metastatic lesion from thyroid (case courtesy of Dr. Terry Welsh, Anaheim Health Medical Center, CA). The smear preparations from A and B show similar oncocytic cells with the same cytological diagnosis of AUS/FLUS, Hürthle cell lesion.

After the lung lesion is diagnosed as thyroid metastasis (case B), a retrospective comparison of these two cytology cases was done. Hürthle cells in case B seem to be more loosely cohesive and have more binucleation. These architectural differences and cytological atypia are very subtle, requiring high grade of alert. Moreover, Hürthle cell metaplasia shows more pleomorphism than Hürthle cell carcinoma [27].

Retrospectively, the Hürthle cells in case A is positive for p27, but negative for HBME-1 and Galectin-3, in support of benign [54]. The triple immunostaining is not available for case B. The application of p27/HBME-1/Galectin-3 immunostaining in distinguishing benign Hürthle cell from Hürthle cell carcinoma needs to be studied.

A suspicious result from Afirma gene expression classifier does not increase the probability of malignancy in the Hürthle cell nodules. Patient should be counseled for the high possibility of unnecessary surgery based on suspicious interpretation of Afirma test for a Hürthle cell nodule [61, 62]. Molecular tests with both high sensitivity and specificity are needed for Hürthle cell nodules.

4. Algorithm for AUS/FLUS follow-up

4.1. Repeat FNA

Repeat FNA is the recommendation for AUS/FLUS in TBSRTC [3]. For an initial aspirate diagnosed as AUS/FLUS, repeat FNA is specifically endorsed by the American Thyroid Association as a suitable follow-up option, perhaps proving especially useful when limited cellularity contributes to the initial AUS/FLUS interpretation [2, 63].

For initial AUS/FLUS diagnosis, reclassification rate with repeat FNA is 56 and 69% [11, 64]. Most of the repeat FNA diagnoses are benign (69%, 47/74) [64]. The malignancy rate after surgery with or without repeat FNA for initial AUS/FLUS is 38.6 versus 15.6% [42, 65]. Repeat FNA helps the selection of patients with AUS/FLUS to triage to surgery, significantly reducing unnecessary surgery. Therefore, repeat FNA for nodules with AUS/FLUS on initial FNA is suggested.

Two consecutive AUS/FLUS diagnoses have higher malignancy risk of at least 31.0% than one AUS/FLUS diagnosis and a higher proportion of FVPTC [11, 36, 65]. Solid structure, increased nodular size (>2 cm), and irregular/microlobulated margins are found to be risk factors in two studies [42, 66] but not in the other [36]. Most cases with repeated AUS/FLUS will require surgery [11, 36, 42, 65].

For benign aspirates following initial AUS/FLUS, the malignancy risk is low (2 and 2.8%) so that clinical follow-up instead of surgical excision or continuous repeat FNA may be enough [64, 67], although one study has suggested a still higher risk of malignancy with one AUS/FLUS diagnosis compared to none [11]. The ultrasound features might be insignificant in predicting malignancy in this scenario [67].

A meta-analysis study has suggested that a core-needle biopsy has a higher conclusive rate than repeat FNA when the initial FNA produced inconclusive results [68]. This might be due to reduction in nondiagnostic category. Further prospective studies are necessary before follow-up CNB can be applied in daily practice.

4.2. Emerging molecular tests

Currently available commercial molecular tests for indeterminate (AUS/FLUS) thyroid nodules on fine needle aspiration have different strengths (Table 2). The Afirma gene expression classifier developed by Veracyte, Inc., is a “rule-out malignancy” test for the preoperative identification of benign thyroid nodules with indeterminate cytology. The Afirma test assesses gene expression from mRNA isolated from thyroid FNA samples by comparing the mRNA expression detected in a thyroid FNA against a panel of 167 molecular genes [17]. The other three later-developed assays test for 17 known thyroid cancer-related mutations and translocations [18], combined with miRNA, mRNA, and DNA mutation [19] or 14 thyroid cancer-related genes and 42 types of gene fusion related to thyroid cancer [20], aiming to increase diagnostic yields on positive-predictive value.

	Sensitivity (%)	Specificity (%)	Positive-predictive value (%)	Negative-predictive value (%)
Afirma gene expression classifier (Veracyte, Inc.) [17]	90	53	37.7	95
miRInform™ (Asuragen Inc.) [18]	63	99	94	88
Multi-Gene ThyroSeq Next-Generation Sequencing Assay (ThyroSeq®) [19]	90.9	92.1	76.9	97.2
ThyGenX and ThyraMIR™ [20]	94	80	68	97

Table 2. Commercial molecular tests for indeterminate (AUS/FLUS) thyroid nodules on fine needle aspiration.

The test performance may change when applied to individual clinics. One independent study has reported lower than previously reported negative- and positive-predictive value (75 compared to 95%, and 16 compared to 38%) for cytology diagnosis of AUS/FLUS and SN/FN combined [62]. One reason might be due to the lower malignancy rate for indeterminate thyroid nodules in the specialized academic center compared to the validation settings from Afirma (17 vs 24%). Adjusting the malignancy rate on Bethesda categories III and IV, another study from the same institution demonstrated still lower actual performance of Afirma™,

miRInform™, and ThyroSeq™ v2 tests compared to published sensitivity and specificity [69]. Assessing the institutional performance of each test is necessary along with the prevalence of malignancy. This has called for attention that customization is needed for the application of the molecular tests.

4.3. Clinical cytological rescore prior to surgery

Diagnostic excision has been performed for high-risk thyroid nodules, and surgery is an indication for large goiter with symptoms. The synthesis of cytological interpretation, clinical factors including age, gender, nodular size, results of molecular testing, ultrasound findings, personal and family history, and the presence of additional nodules will impact the determination of the appropriate extent of initial surgical management [2, 42, 63, 70].

Quantitative methods are available to assemble clinical, ultrasonographic, and cytological findings into a scoring system to evaluate the malignant risk of thyroid nodules, especially in cases with indeterminate or repeated nondiagnostic FNA [70]. A similar scoring system integrating molecular test results would be desirable.

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