Overview of the Bethesda System for Reporting Thyroid Cytopathology

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Key Points

- Fine needle aspiration (FNA) is the most valuable screening and diagnostic test for thyroid nodules and plays a crucial role in managing patients with thyroid cancer.
- The Bethesda System for Reporting Thyroid Cytopathology classifies aspirates of thyroid nodules into six diagnostic categories.
- Each diagnostic category is associated with an evidence-based cancer risk and clinical management guidelines, which are endorsed by the American Thyroid Association.
- Incorporating the clinical and radiologic findings, thyroid FNA allows for a standardized, team-based approach to managing patients with thyroid nodules.

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Introduction

Thyroid nodules are very common, found in up to 70% of adults by imaging.

Although the majority are benign, it is important to identify the small percentage of nodules that are malignant and require surgical management. The recommended workup includes a dedicated ultrasound examination, which risk stratifies the nodule based on size and ultrasonographic features. If the criteria for biopsy are met, a fine needle aspiration (FNA) is performed. Since its introduction, FNA has become the most valuable screening and diagnostic test for thyroid nodules. Many are now performed under ultrasound guidance, with optional rapid on-site evaluation to ensure proper placement of the needle, which improves accuracy and decreases the number of nondiagnostic specimens. The results are then reported using The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC).

TBSRTC is the most widely used standardized system for reporting thyroid fine needle aspiration biopsy results. TBSRTC is a six-tiered scheme with evidence-based anticipated risk of malignancy (ROM) and standard treatment approaches associated with each diagnostic category.

Since its introduction, TBSRTC has been widely adopted worldwide and endorsed by the American Thyroid Association. It outlines six distinct diagnostic categories and provides a standardized, well-defined approach to reporting thyroid cytopathology. Each category is associated with an anticipated risk of malignancy (ROM) and linked to evidence-based clinical management guidelines (Table 4.1). As a result, TBSRTC not only allows for improved communication within a healthcare team, but also provides guidance for appropriate clinical management. A brief discussion of each diagnostic category within TBSRTC follows.

Table 4.1 The 2017 Bethesda system for reporting thyroid cytopathology

Diagnostic category	Risk of malignancy if NIFTP is not considered malignant (%)	Risk of malignancy if NIFTP is considered malignant (%)	Usual management
Nondiagnostic or unsatisfactory	5–10	5–10	Repeat FNA with ultrasound guidance
Benign	<3	<3	Clinical and sonographic follow-up
Atypia of undetermined significance or follicular lesion of undetermined significance	6–18	~10–30	Repeat FNA, molecular testing, or lobectomy
Follicular neoplasm or suspicious for a follicular neoplasm	10–40	25–40	Molecular testing or lobectomy
Suspicious for malignancy	45–60	50–75	Near-total thyroidectomy or lobectomy
Malignant	94–96	97–99	Near-total thyroidectomy or lobectomy

NIFTP noninvasive follicular thyroid neoplasm with papillary-like nuclear features

Table adapted from: Cibas ES, Ali SZ. The 2017 Bethesda System for Reporting Thyroid Cytopathology. Thyroid. 2017;27(11):1341–1346. doi:10.1089/thy.2017.0500

Discussion

Nondiagnostic

This category includes specimens that are unsatisfactory for interpretation due to scant cellularity or are compromised by obscuring blood, air-drying artifact, or overly thick smears (Fig. 4.1a).

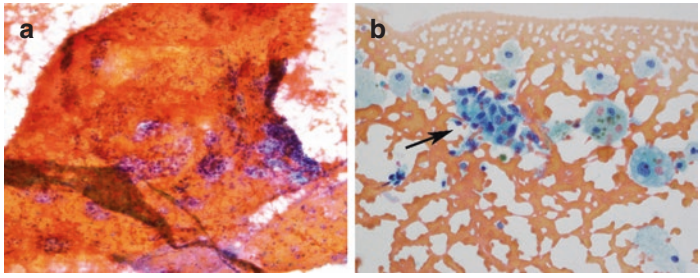

Fig. 4.1 Nondiagnostic. (**a**) Extensive blood and clotting artifact obscures the follicular cells and distorts architecture, precluding evaluation. (**b**) Nodules with cystic degeneration often contain abundant hemosiderin-laden macrophages and cyst lining cells (arrow). If insufficient follicular cells are present, cystic aspirates are classified as nondiagnostic

Normal thyroid aspirates consist of follicular cells and colloid. A thyroid FNA requires six or more groups of well-visualized follicular cells, with at least ten cells in each group, to be considered adequate for evaluation. Specimens that fail to meet the criteria for adequacy, including cystic lesions (Fig. 4.1b), are considered nondiagnostic.

The three exceptions to this rule include cases with any atypia, abundant colloid indicative of a benign colloid nodule, or specific diagnostic conditions such as chronic lymphocytic (Hashimoto) thyroiditis. The precise ROM for nondiagnostic nodules varies but is estimated as 5–10%. Nondiagnostic nodules are generally managed with a repeat FNA under ultrasound guidance with rapid on-site evaluation. In up to 60% of cases, a subsequent FNA results in a diagnostic interpretation, with a majority proving to be benign. However, FNAs that are repeatedly nondiagnostic may require surgery depending on other clinical and radiologic factors.

Benign

Since most thyroid nodules are benign, the most common FNA interpretation is a benign result. This category comprises approximately 60–70% of all cases and may be subclassified further into

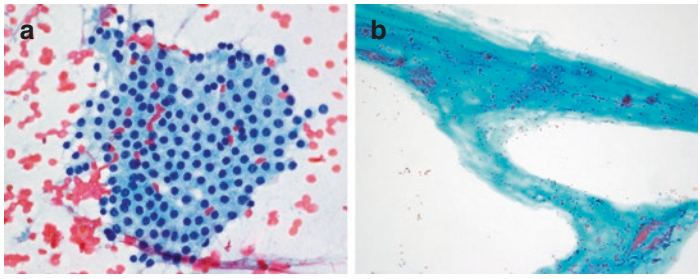

Fig. 4.2 Benign follicular nodule. (**a**) Macrofollicles rupture with FNA and appear as flat, monolayered sheets of evenly spaced follicular cells on cytologic preparations. Benign follicular cells have round to oval nuclei, finely granular chromatin, smooth nuclear contours, inconspicuous nucleoli, and scant amounts of delicate cytoplasm. (**b**) Colloid may have a watery or dense appearance staining pink or green-blue on alcohol-fixed Papanicolaou-stained slides. Abundant colloid favors a benign process and, if sufficiently plentiful, may be called benign even if follicular cells are absent

specific entities. A diagnosis of "benign follicular nodule" on cytology encompasses a group of histologic entities with identical cytologic features, including nodular hyperplasia in multinodular goiter, adenomatoid nodules, and colloid nodules. These are all predominantly composed of macrofollicles that once aspirated rupture to release luminal colloid and appear as flat sheets of unremarkable follicular cells (Fig. 4.2).

Benign follicular cells have small and round nuclei, smooth nuclear contours, uniformly granular chromatin, and scant to moderate amounts of delicate cytoplasm. Mild nuclear size variation and oncocytic (Hürthle cell) or cystic degenerative changes with hemosiderin-laden macrophages are acceptable. A colloid nodule may be sparsely cellular, but is considered benign as it also consists of macrofollicles with abundant colloid.

Hashimoto thyroiditis aspirates are characterized by numerous lymphoid cells admixed with normal follicular cells and occasional Hürthle cells (Fig. 4.3). Although generally straight forward on cytology, the diagnosis should be confirmed clinically by serologic tests. Examples of other less common benign conditions

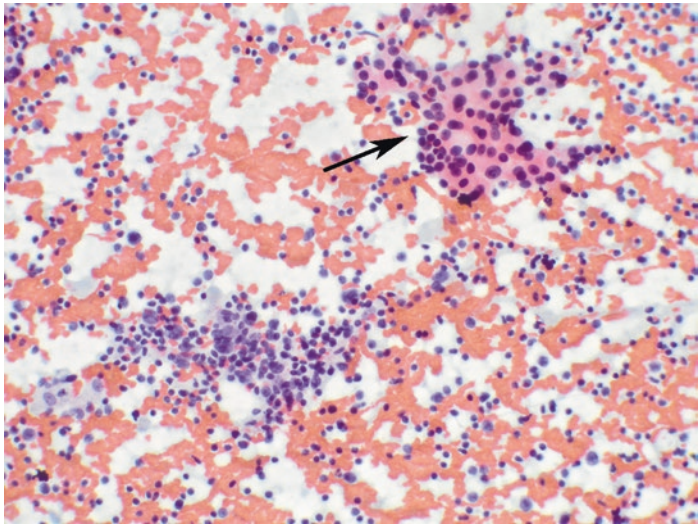

Fig. 4.3 Chronic lymphocytic (Hashimoto) thyroiditis. A group of Hürthle cells (arrow) with abundant granular cytoplasm is present in a background of abundant polymorphous lymphocytes

encountered in FNA specimens include subacute (de Quervain) thyroiditis, amyloid goiter, and black or pigmented thyroid.

A benign interpretation is associated with a very low ROM (<3%). These patients are managed conservatively, and follow-up intervals are determined by risk stratification algorithms based on ultrasound patterns.

Atypia of Undetermined Significance (AUS)/ Follicular Lesion of Undetermined Significance (FLUS)

This category encompasses a heterogeneous group of aspirates demonstrating a degree of atypia that is greater than normally attributable to benign, reactive changes, but insufficient for a malignant or suspicious diagnosis. Since "AUS" and "FLUS" are synonymous, one term should be selected for use by a laboratory

and further subclassified using descriptive language. Subclassification is encouraged to enhance communication between pathologists and clinicians and is particularly important for distinguishing the presence of cytologic/nuclear atypia, which has demonstrated a greater ROM than AUS with architectural atypia or a prominent Hürthle cell component.

TBSRTC defines six common patterns of AUS/FLUS and the preferred language used to describe the degree and nature of atypia. One pattern demonstrates focal or extensive, but mild **cytologic atypia**, raising the possibility of a papillary thyroid carcinoma (PTC) (Fig. 4.4a). In contrast, **AUS with architectural atypia** may be appropriate when the possibility of a follicular neoplasm cannot be ruled out, due to the diffuse presence of microfollicles (defined as 6 to 15 follicular cells arranged in a circular pattern around a central portion of colloid) in a scantly cellular specimen (Fig. 4.4b).

A combination of both **cytologic and architectural atypia** can be observed in the same specimen as these patterns are not mutually exclusive. Scantly cellular specimens may contain a pure population of Hürthle cells (**AUS, Hürthle cell type**), making it impossible to rule out the chance of a Hürthle cell neoplasm. Hürthle cells refer to oncocytic cells with enlarged, often eccentri-

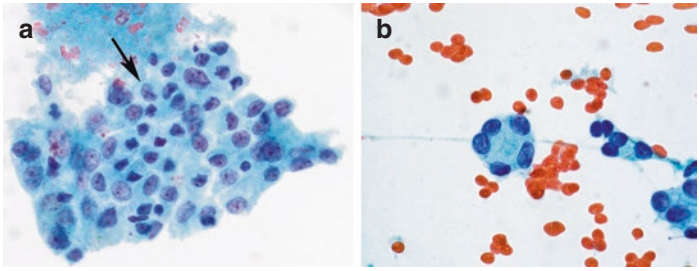

Fig. 4.4 AUS/FLUS. (**a**) Follicular cells show mild cytologic atypia associated with Hürthle cell change. Nuclear enlargement, pallor, pinpoint nucleoli, and occasional nuclear grooves (arrow) are seen. Intranuclear pseudoinclusions are absent. (**b**) High power image of a microfollicle. When predominant in a modestly cellular specimen, a diagnosis of AUS/FLUS with architectural atypia is warranted

cally located nuclei, prominent nucleoli, and abundant finely granular cytoplasm. **AUS, Hürthle cell type**, may also apply to cellular samples composed of bland Hürthle cells in a clinical setting that suggests benignity, such as Hashimoto thyroiditis or multinodular goiter.

This category is intended to be used as a last resort, and it is recommended to make up no more than 10% of all thyroid FNAs. The implied ROM ranges from 10% to 30%. Several options for managing AUS/FLUS nodules are available, including repeat FNA, molecular testing, or diagnostic lobectomy.

Molecular testing helps triage patients with AUS/FLUS or FN/SFN results and is recognized by TBSRTC and the ATA as a valid approach to further inform clinical management. Most classic and tall cell variants of PTC harbor a *BRAF* V600E mutation, while many follicular variants of PTC and follicular neoplasms have *RAS* or *RAS*-like mutations. Enhanced understanding of the genetic alterations underlying thyroid tumorigenesis has created a potential role for the routine use of commercially available molecular tests, such as the Afirma Genomic Sequencing Classifier and ThyroSeq v.3. The high negative predictive values of the most recent iterations of these tests make it appropriate to triage patients with negative results to conservative management.

Follicular Neoplasm (FN)/Suspicious for a Follicular Neoplasm (SFN) (Specify if Oncocytic/Hürthle Cell Type)

The FN/SFN category identifies nodules with significant architectural abnormalities that raise the possibility of a follicular carcinoma. On histologic evaluation, follicular carcinoma is distinguished from an adenoma by the presence of capsular and/or vascular invasion. However, these criteria cannot be reliably assessed on cytology. Therefore, FNA of follicular neoplasms is considered a screening, rather than a diagnostic test.

In contrast to benign follicular nodules that are comprised of predominantly macrofollicles with abundant colloid, FN/SFN lesions demonstrate follicular cells arranged in crowded, overlap-

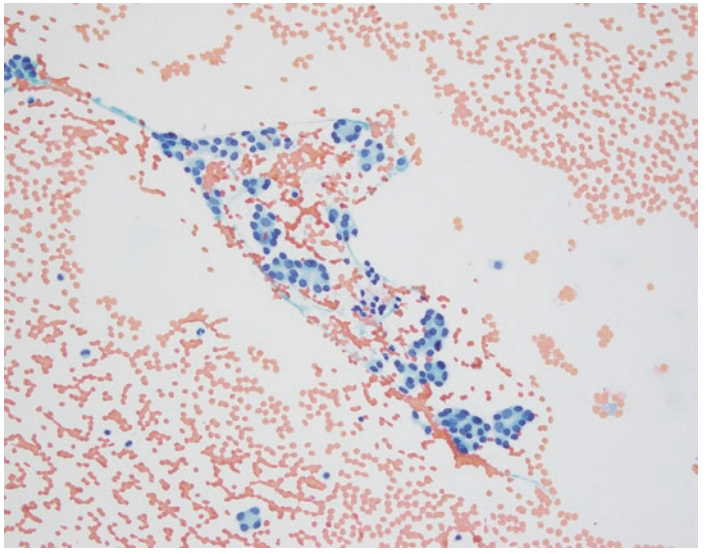

Fig. 4.5 FN/SFN. This cellular aspirate shows crowded, uniform follicular cells predominantly in microfollicular arrangements

ping groups, microfollicles, or trabeculae, with scant to absent colloid (Fig. 4.5).

Most cases diagnosed as FN/SFN are ultimately benign, but 25–40% of cases prove to be malignant. As a result, the differential diagnosis includes benign follicular nodules, a subset of which can have prominent microfollicles without clinical significance, as well as other follicular-patterned lesions, such as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Strict histologic criteria preclude a definitive diagnosis of NIFTP on FNA, but cytologic preparations often show a predominance of microfollicles in addition to mild nuclear changes that are abnormal enough to fall within the indeterminate categories (Fig. 4.6).

These lesions are classified as FN/SFN when nuclear changes are mild and intranuclear pseudoinclusions, papillae, or psammoma bodies are absent. When nuclear changes are more pronounced and these suspicious features are present, a diagnosis of at least suspi-

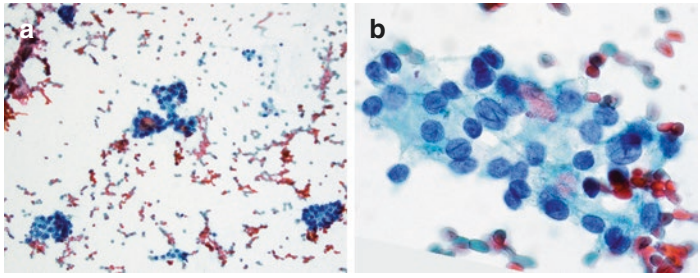

Fig. 4.6 NIFTP. (**a**) Aspirates show microfollicles with nuclear crowding and enlargement. (**b**) At high power, powdery chromatin and nuclear grooves are seen. The diagnosis of NIFTP requires histologic examination, but FNA specimens are usually classified as AUS/FLUS, FN/SFN, or suspicious for PTC. An explanatory note suggesting the possibility of NIFTP is recommended when the diagnosis is suspected

cious for malignancy is warranted. If NIFTP is suspected on FNA, TBSRTC suggests adding an explanatory note to encourage limited surgical management (typically diagnostic lobectomy).

The interpretation "suspicious for a follicular neoplasm/ follicular neoplasm, Hürthle cell type (FNHCT/SFNHCT)" refers to a subset of aspirates within the FN/SFN category that consists (almost) exclusively of Hürthle cells and raises the possibility of a Hürthle cell carcinoma (Fig. 4.7).

As with its conventional follicular counterpart, it is impossible to identify the presence of invasion to confirm malignancy on cytology. However, the mere presence of Hürthle cells is not diagnostic of FNHCT/SFNHCT. For example, Hashimoto thyroiditis often shows Hürthle cells admixed with numerous lymphoid cells. Similarly, Hürthle cell change is a common focal finding in benign follicular nodules. In addition to extensive Hürthle cell change, the most concerning lesions in the FNHCT/SFNHCT category also show the following worrisome cytologic or architectural features: small cell or large cell dysplasia, dyscohesion (loosened inter-cellular connections), or crowding. The background contains little to no colloid and transgressing blood vessels may also be seen.

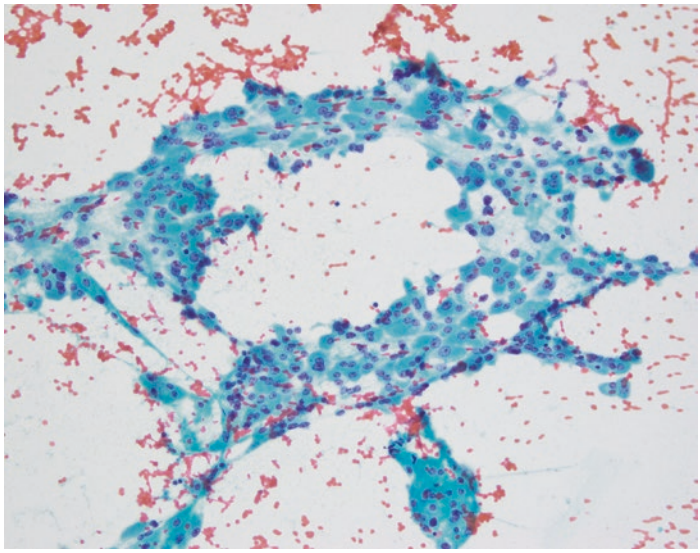

Fig. 4.7 FNHCT/SFNHCT. Cellular smears show a pure population of Hürthle cells arranged in loosely cohesive groups. Colloid and lymphocytes are absent from the background

As a minority of FN/SFN cases are malignant, they are typically managed conservatively with diagnostic lobectomy or molecular testing with the potential to avoid surgery.

Suspicious for Malignancy (SFM)

"Suspicious for malignancy" (SFM) is the last indeterminate category in TBSRTC and is indicated for cases with cytomorphologic features that are strongly suspicious for malignancy but are quantitatively and/or qualitatively insufficient for a conclusive diagnosis. SFM includes a variety of potential malignancies, although most cases are suspicious for PTC (Fig. 4.8).

As with AUS/FLUS, this interpretation should be used judiciously to ensure that patients are managed appropriately. Often the sample may lack cellularity or the characteristic features are

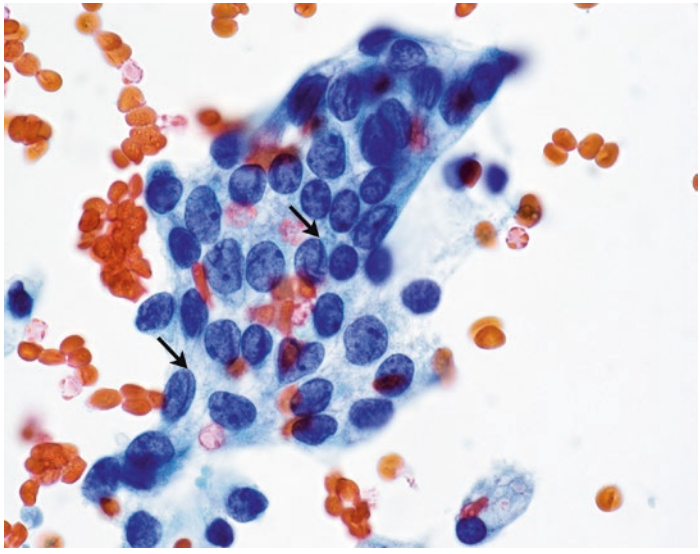

Fig. 4.8 Suspicious for papillary thyroid carcinoma. Sheets of follicular cells demonstrate nuclear enlargement, crowding, powdery chromatin, and nuclear grooves (arrows). Intranuclear pseudoinclusions, psammoma bodies, and papillary architecture are absent

patchy and incomplete. A SFM interpretation still conveys a degree of uncertainty but suggests that malignancy is considered more likely than not. Given the relatively high ROM (50–75%), ancillary molecular studies are not utilized for risk stratification. Instead, these cases are managed surgically, and the clinical and radiologic findings are crucial for determining the extent of surgery.

Malignant

PTC is the most common cancer of the thyroid, accounting for approximately 85% of malignancies. Its characteristic nuclear features are readily identified on cytology, making FNA biopsy an ideal diagnostic test for PTC (Fig. 4.9). Common features are

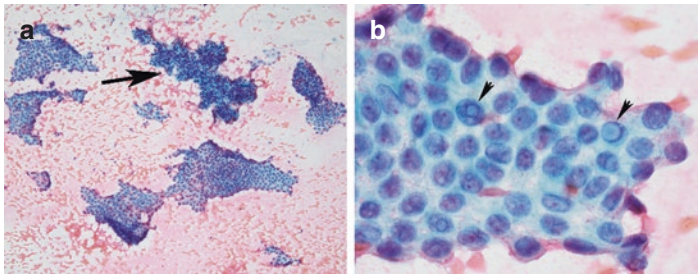

Fig. 4.9 Papillary thyroid carcinoma. (**a**) Hypercellular smears show sheets of malignant cells with a syncytial-like appearance. At low power, nuclear enlargement, pallor, and crowding are appreciated. A papillary structure with a fibrovascular core is present (arrow). (**b**) At high power, the nuclear features of papillary thyroid carcinoma, including nuclear membrane irregularities, grooves, and numerous pseudoinclusions (highlighted with arrows), are readily identified

nuclear grooves and pseudoinclusions, membrane irregularities, chromatin pallor, crowding, or overlapping. Classical PTC is the most common type and has papillary architecture, which is appreciable on FNA as fibrovascular cores lined by malignant cells. Other helpful features include psammomatous calcifications and multinucleated giant cells. In most cases, it is difficult and unnecessary to identify the specific subtype of PTC. However, some variants can demonstrate more focal or subtle nuclear changes. Patients with low-risk PTC up to 4 cm in size may be treated with lobectomy, according to current ATA guidelines, otherwise with near-total thyroidectomy, and prognosis is excellent overall.

Poorly differentiated (insular) thyroid carcinoma has an intermediate degree of cytomorphologic atypia and clinical behavior between a well-differentiated and an undifferentiated thyroid carcinoma. The diagnosis is difficult to make on FNA and rests on histologic evaluation. However, cytologic preparations are usually hypercellular and demonstrate nuclear overlapping, with nested, trabecular, or solid architectural patterns (Fig. 4.10). Tumor cells are typically uniform, but more pronounced atypia may also be appreciated. The presence of increased mitotic activ-

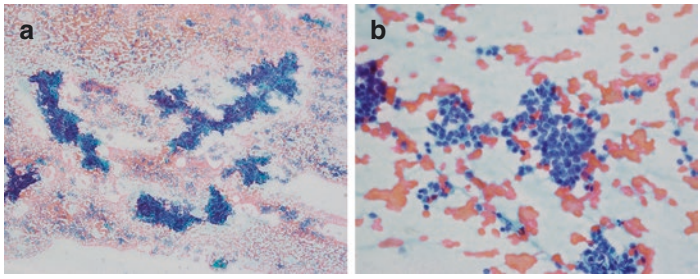

Fig. 4.10 Poorly differentiated thyroid carcinoma. (**a**) Low magnification image shows abundant groups of monotonous follicular cells arranged in crowded insulae. (**b**) Tumor cells show mild nuclear atypia with granular chromatin and overlapping nuclei. Scattered isolated tumor cells are present in the background

ity, apoptosis, and necrosis further suggests the diagnosis. Poorly differentiated thyroid carcinoma can resemble other entities, such as follicular neoplasms, medullary thyroid carcinoma, or anaplastic carcinoma. Immunohistochemical stains may help with the distinction on FNA, but surgical pathology evaluation is often necessary for definitive classification.

Undifferentiated (anaplastic) thyroid carcinoma is even more unfavorable than poorly differentiated thyroid carcinoma. This disease behaves aggressively and clinically presents as a rapidly enlarging mass, which has often already spread to adjacent structures or distant sites. The cytologic appearance is variable, comprised of large, markedly pleomorphic tumor cells that can demonstrate epithelioid, spindled, rhabdoid, or giant-cell morphology (Fig. 4.11). The nuclear features are undoubtedly malignant, and necrosis and abundant mitoses are also present. A proportion of cases focally demonstrate features of a well-differentiated precursor lesion, either PTC or follicular carcinoma. Prognosis is extremely poor, and the diagnosis relies on clinical and radiologic correlation.

In contrast to the previously described thyroid neoplasms which are derived from follicular cells, medullary thyroid carcinoma (MTC) arises from the parafollicular (C) cells of the thyroid. MTC is rare, accounting for 1–2% of all thyroid carcinomas.

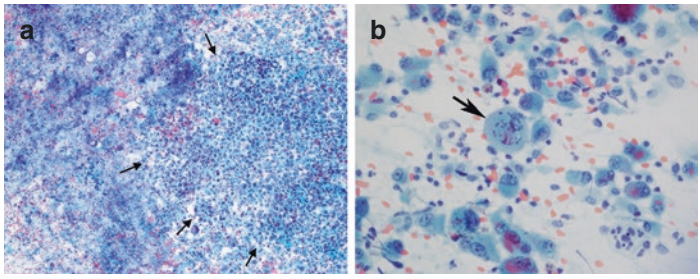

Fig. 4.11 Anaplastic thyroid carcinoma. (**a**) Hypercellular smear shows abundant tumor necrosis (left portion of image) with well-preserved, malignant cells (highlighted with arrows in right portion of image). (**b**) Significant pleomorphism is seen with enlarged, eccentrically located nuclei, irregular nuclear contours, coarse chromatin, prominent nucleoli, and abundant dense cytoplasm. Increased mitotic activity is present (arrow)

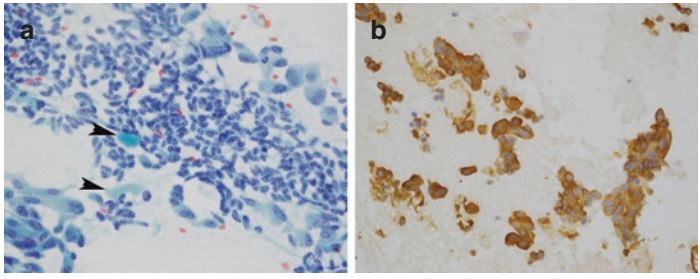

Fig. 4.12 Medullary thyroid carcinoma. (**a**) Hypercellular preparations are composed of abundant, loosely cohesive, and variably shaped (epithelioid, plasmacytoid, and spindled) cells. The background contains scattered amorphous material, consistent with amyloid (arrows). The malignant cells show stippled "salt and pepper" chromatin, anisonucleosis, small nucleoli, and scant to abundant amounts of delicate to granular cytoplasm. (**b**) Cell block preparations show tumor cells that are immunoreactive for calcitonin (brown cytoplasmic staining)

They are often sporadic in adults but may be associated with germline *RET* mutations and multiple endocrine neoplasia (MEN) syndromes. Aspirates show loose aggregates or singly dispersed cells with coarsely granular, "salt and pepper" chromatin, typical of neuroendocrine tumors (Fig. 4.12a). The cells are plasmacytoid

to spindled, with eccentrically located nuclei and occasional binucleation. They have moderate to abundant amounts of finely granular cytoplasm, which may appear metachromatic to reddish-brown, depending on the stain used, and require distinction from Hürthle cells. As some MTCs have large, pleomorphic cells or intranuclear pseudoinclusions, the differential diagnosis often includes poorly differentiated, anaplastic, or papillary thyroid carcinomas. Positive calcitonin, CEA, and chromogranin stains are confirmatory in virtually all cases (Fig. 4.12b). Amyloid can be highlighted by a Congo red stain. Elevated serum calcitonin level is diagnostic of MTC and measurement of this marker generally plays a first line role in confirmation of the diagnosis, particularly when immunohistochemical stains, which are not universally available, cannot be performed. MTC is usually treated with total thyroidectomy and regional lymphadenectomy.

Primary thyroid lymphomas are rare, comprising 1–5% of all thyroid malignancies, with up to 5% reported at some referral centers. These tumors usually occur in older-aged females with a longstanding history of Hashimoto thyroiditis. Patients present with an enlarging thyroid mass, often accompanied by compressive symptoms and involvement of cervical lymph nodes. Aspirates are highly cellular and consist of isolated lymphoid cells (Fig. 4.13). A majority are B-cell lymphomas, most commonly extranodal marginal zone B-cell lymphoma of mucosa-

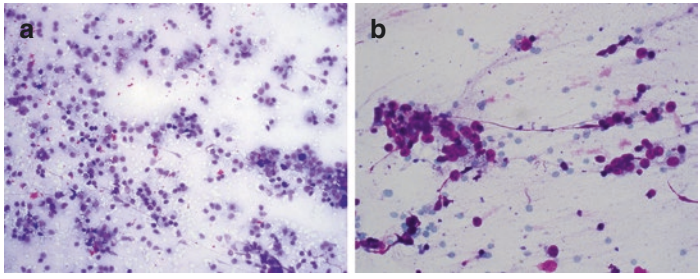

Fig. 4.13 Lymphoma. (**a**) MALT lymphoma of the thyroid showing a monotonous population of intermediate-sized lymphoid cells. (**b**) Diffuse large B-cell lymphoma (DLBCL) of the thyroid with large, atypical lymphoid cells with nuclear enlargement and prominent nucleoli

associated lymphoid tissue (MALT lymphoma) and diffuse large B-cell lymphoma (DLBCL). If the clinical presentation and/or cytomorphology suggests a lymphoma, ancillary studies such as immunohistochemistry, flow cytometry, and molecular genetics are useful for accurate diagnosis and further classification.

Lastly, metastatic disease to the thyroid can rarely occur and is usually recognized by the patient's clinical history or cytomorphologic dissimilarity to primary thyroid neoplasms. Immunohistochemistry is helpful in determining the origin.

Further Reading

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