The Bethesda System for Reporting Thyroid Cytopathology

Definitions, Criteria, and Explanatory Notes

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Third Edition

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Foreword

The excitement was palpable: when the 2-day meeting in October 2007 ended, the participants knew something good and durable had been accomplished in Bethesda, Maryland: they had come up with a framework for reporting thyroid cytopathology results that could be adopted nationwide and even internationally, leading to better communication between pathologists and clinicians, and ultimately better outcomes for patients with a thyroid nodule.

A decade earlier, in 1996, the Papanicolaou Society of Cytopathology had published guidelines for fine needle aspiration (FNA) of thyroid nodules. Other countries, most notably the United Kingdom and Italy, had adopted standardized, category-based reporting systems nationwide for thyroid cytopathology in their countries. And yet, in 2007, there was still an astonishing heterogeneity in the way thyroid results were reported in the United States. Some laboratories used a limited number of categories for reporting results, but their number and their names varied from one laboratory to another. Still other laboratories reported results using descriptive terminology, without explicit categories. This variability created confusion and hindered the sharing of data among institutions. Something had to be done to bring order out of chaos.

And so, the National Cancer Institute (NCI), under the able leadership of NCI chief cytopathologist Dr. Andrea Abati, sponsored an interdisciplinary, 2-day conference on thyroid cytopathology at the NCI campus in Bethesda. Preparations for the conference began 18 months earlier with the establishment of an open-access website dedicated to closely monitored comments and discussion. A steering group was appointed, and a Terminology and Morphologic Criteria Committee was assigned responsibility for preparing a summary document based on literature review. The committee members were Zubair W. Baloch (chair), Sylvia L. Asa, Richard M. DeMay, William J. Frable, Virginia A. LiVolsi, Maria J. Merino, Gregory Randolph, Juan Rosai, Mary K. Sidawy, and Philippe Vielh. The first draft of the Terminology Committee's proposal was posted on the website for online forum discussion from May 1 to June 30, 2007. Committee members were tasked with monitoring the forum discussion threads and evaluating any suggested modifications. Several revised drafts and online discussion periods followed. The culmination was a 2-day in-person conference on October 22 and 23, 2007, which was attended by 154 registrants, including pathologists, endocrinologists, surgeons, and radiologists. The Terminology Committee presented its refined proposal, and a

lively debate of controversial areas followed. Based on the discussions and majority consensus at the meeting, the committee's summary document was revised one final time and published the following year. The conclusions regarding terminology and morphologic criteria from that historic 2007 meeting provided the framework for the original edition of this atlas in 2010, edited by Syed Z. Ali (Chair, Atlas Committee) and Edmund S. Cibas (Figs. 1, 2, 3, and 4).

Fig. 1 Dr. Andrea Abati, chief cytopathologist at the National Cancer Institute in 2007, was the guiding force behind the Bethesda conference

Fig. 2 Bethesda conference moderators Drs. Edmund Cibas and Susan Mandel display the conference program book

Fig. 3 Dr. Zubair Baloch (at podium), chair of the Terminology Committee, with committee members (from left to right) Drs. Mary Sidawy, Sylvia Asa, Virginia LiVolsi, Richard DeMay, and Juan Rosai (Committee members William Frable, Maria Merino, Gregory Randolph, and Philippe Vielh not shown)

Fig. 4 Conference attendees participate in discussion of the Committee's presentation

The editors and chapter authors of the atlas were very much inspired and guided by the highly successful and influential NCI conferences that led to the Bethesda System for Reporting Cervical Cytology. We had a shared belief that it is critical for the cytopathologist to communicate an interpretation to the referring physician in terms that are succinct, unambiguous, and clinically useful. We also recognized that the terminology was a flexible framework that would evolve as our understanding of thyroid nodules grew.

The second edition of the atlas, published in 2017, was inspired by new developments in the field of thyroid cytopathology since the publication of the first edition in 2009. These included revised clinical guidelines for the management of patients with thyroid nodules, the introduction of molecular testing as an adjunct to cytopathologic examination, and the reclassification of the noninvasive follicular variant of papillary thyroid carcinoma as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Much of the groundwork for the second edition was laid by a symposium entitled "The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC): Past, Present, and Future" at the 2016 International Congress of Cytology in Yokohama, Japan. Preparations for the

symposium began 12 months earlier with the designation of a steering group and the appointment of an international panel composed of 16 cytopathologists and an endocrinologist, whose task was to review and summarize the published literature in English since the first edition. The symposium was moderated by Drs. Syed Ali and Philippe Vielh (Fig. 5), and the discussions and recommendations from the symposium were published later that year. Based on the panel's recommendation, the six original general categories remained unchanged in the second edition. The chapters devoted to these categories were expanded, with refined definitions, morphologic criteria, and explanatory notes.

Fig. 5 Dr. Syed Ali and Philippe Vielh, moderators of the Yokohama Symposium at the 19th International Congress of Cytology in 2016

This most recent edition simplifies the reporting structure by settling on just one name for each of the six categories and aligning terminology with the most recent classification of thyroid tumors by the World Health Organization. It's gratifying to see that TBSRTC has been widely adopted and endorsed by the American Thyroid Association. It has gone far towards improving communication between cytopathologists and our clinical colleagues and has provided a uniform template for the sharing of data among investigators. May this new edition continue to inspire advances in thyroid cytopathologic diagnosis and the betterment of patients with thyroid nodular disease.

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Contents

Overview of Diagnostic Terminology and Reporting

1

Zubair Baloch, David Cooper, Martin Schlumberger, and Erik Alexander

With its inception, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) established a uniform, tiered reporting system for thyroid fine needle aspiration (FNA) specimens. Using TBSRTC, the cytopathologist can communicate thyroid FNA interpretations to the referring physician in terms that are succinct, unambiguous, and clinically useful [1, 2].

Since the widespread acceptance of TBSRTC into clinical practice, further refinement of the diagnostic categories, recommended management strategies (e.g., molecular testing, repeat FNA vs. surgery), and their implied risks of malignancy continued to occur [3–5]. The goal of preoperative FNA and cytologic analysis is to inform conservative management of thyroid nodules unlikely to cause harm, while conversely leading to surgical management aimed at effectively treating thyroid cancer. Data increasingly support comparable efficacy in applying less invasive management strategies to certain thyroid cancers [6]. In fact, among nodules shown

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to be malignant, TBSRTC classification may predict the aggressiveness of the tumor. With this in mind, clinicians are increasingly favoring surgical lobectomy, limiting routine use of radioactive iodine for ablative purposes, and even considering nonoperative monitoring approaches for small thyroid malignancies [3, 7, 8]. The new TBSRTC third edition notes these options for each category, though acknowledges that cytology alone should not dictate the full management of thyroid nodule care. Integrated multivariable assessment of each impacted patient should occur, allowing the most informed and individualized treatment decisions [6, 8, 9].

New to this third edition are the following:

1. Unification of diagnostic categories under a single name. The diagnostic category of "Nondiagnostic/Unsatisfactory" is now termed as "Nondiagnostic" only, the category "Atypia of Undetermined Significance/Follicular Lesion of Undetermined Significance (AUS/FLUS)" termed as "Atypia of Undetermined Significance (AUS)" only, and the category "Follicular Neoplasm/Suspicious For a Follicular Neoplasm (FN/SFN)" termed as "Follicular Neoplasm (FN)" only.
2. Data informing use of TBSRTC in the pediatric population is now included. The risk of malignancy (ROM) is higher in children compared to adults, and while TBSRTC should still be used for interpreting pediatric thyroid nodule cytology, adjusted risk of malignancy estimates should be applied [10–18].
3. Refined risk of malignancy estimates, incorporating more extensive published data since the second edition of TBSRTC.
4. More formalized subcategorization of AUS based on ROM: AUS with nuclear atypia vs. AUS-other.
5. Whenever possible, the terminology used in TBSRTC has been harmonized with the latest 2022 WHO classification of Thyroid Neoplasms.
6. A broadening of Chap. 10 to incorporate all high-grade follicular-derived carcinomas, including poorly differentiated thyroid carcinoma (PDTC) as well as differentiated high-grade thyroid carcinoma (DHGTC).
7. Brand new chapters covering clinical perspectives and imaging studies (Chap. 13) and the use of molecular and other ancillary tests (Chap. 14).
8. New and updated images to better illustrate diagnostic criteria and cytologic features.

Format of the Report

For clarity of communication, each thyroid FNA report should begin with a general diagnostic category. TBSRTC diagnostic categories are shown in Table 1.1.

Each category has an implied cancer risk, which ranges from 1% to 2% overall for the "Benign" category to virtually 100% for the "Malignant" category. As a function of these risk associations, each category is linked to evidence-based clinical management guidelines, as shown in Table 1.2 and discussed in more detail in the chapters that follow.

Table 1.1 The Bethesda System for Reporting Thyroid Cytopathology; diagnostic categories

I. Nondiagnostic
Cyst fluid only
Virtually acellular specimen
Other (obscuring blood, clotting artifact, drying artifact, etc.)
II. Benign
Consistent with follicular nodular disease (includes adenomatoid nodule, colloid nodule, etc.)
Consistent with chronic lymphocytic (Hashimoto) thyroiditis in the proper clinical context
Consistent with granulomatous (subacute) thyroiditis
Other
III. Atypia of Undetermined Significance
Specify if AUS-nuclear atypia or AUS-other
IV. Follicular Neoplasm
Specify if oncocytic (Hürthle cell) type
V. Suspicious for Malignancy
Suspicious for papillary thyroid carcinoma
Suspicious for medullary thyroid carcinoma
Suspicious for metastatic carcinoma
Suspicious for lymphoma
Other
VI. Malignant
Papillary thyroid carcinoma
High-grade follicular cell-derived non-anaplastic thyroid carcinoma
Medullary thyroid carcinoma
Undifferentiated (anaplastic) carcinoma
Squamous cell carcinoma
Carcinoma with mixed features (specify)
Metastatic malignancy
Non-Hodgkin lymphoma
Other

It is important to note that the traditional method of estimating the ROM, which is based on histologic follow-up (i.e., dividing the number of patients with cancer by the total number of patients with surgical follow-up), overestimates the risk of malignancy, particularly for the Nondiagnostic, Benign, and AUS categories, where there is selection bias given the relatively small proportion of nodules that undergo excision. On the other hand, when calculated using the total number of FNA specimens (with and without surgical follow-up) as the denominator, assuming that unresected nodules are benign, the ROM is most certainly underestimated. The actual ROM is expected to be in the midrange of the values obtained using these calculations, which take into account only cytologic-defined risk, though optimal risk determination should be individualized and incorporate as many predictive variables as possible. The best current risk estimates based on surgically resected nodules are depicted in Table 1.2, with footnotes clarifying ROM estimates provided when appropriate.

Table 1.2 The Bethesda System for Reporting Thyroid Cytopathology: implied risk of malignancy (ROM) with expected ranges based on follow-up of surgically resected nodules with recommended clinical management [19–47]

Diagnostic category	ROM[a] Mean% (range)	Usual management[b]
Nondiagnostic	13 (5–20)[c]	Repeat FNA[d] with ultrasound guidance
Benign	4 (2–7)[e]	Clinical and sonographic follow-up
Atypia of Undetermined Significance[f]	22 (13–30)	Repeat FNA[d], molecular testing, diagnostic lobectomy, or surveillance
Follicular Neoplasm[g]	30 (23–34)	Molecular testing[h], diagnostic lobectomy
Suspicious for Malignancy	74 (67–83)	Molecular testing[h], lobectomy or near-total thyroidectomy[i]
Malignant	97 (97–100)	Lobectomy or near-total thyroidectomy[i]

[a] These ROM estimates are skewed by selection bias, as many thyroid nodules (especially those diagnosed as Benign or AUS) may not undergo surgical excision

[b] Actual management may depend on other factors (e.g., clinical, sonographic) besides the FNA interpretation

[c] The risk of malignancy varies with the type/structure of the nodule, i.e., solid vs. complex vs. ≥50% cystic. Nondiagnostic aspirates from solid nodules are associated with a higher risk of malignancy as compared to those showing ≥50% cystic change and low-risk ultrasonographic features. See Chap. 2 for discussion

[d] Studies have shown diagnostic resolution with repeat FNA [48–50]

[e] This ROM estimate is based on follow-up of surgically resected nodules, which is skewed by selection bias since the vast majority of thyroid nodules classified as benign do not undergo surgical excision. Based on long-term follow-up studies, the best overall ROM estimate for a benign FNA is approximately 1–2% [51–55]

[f] This category can be further subclassified into specimens with nuclear vs. non-nuclear atypia; the ROM appears to be higher for cases with nuclear atypia. See Chap. 4 for discussion [56, 57]

[g] Includes cases of follicular neoplasm with oncocytic features (Hürthle cell neoplasm) [58, 59]

[h] Molecular analysis can be performed to assess the type of surgical procedure (lobectomy vs. total thyroidectomy)

[i] In the case of "Suspicious for metastatic tumor" or a "Malignant" interpretation indicating metastatic tumor rather than a primary thyroid malignancy, surgery may not be indicated

As noted above, TBSRTC can be applied for reporting pediatric thyroid FNA specimens. The implied risk of malignancy for each diagnostic category based on published studies to date is depicted in Table 1.3.

The reclassification of some encapsulated follicular patterned thyroid neoplasms as noninvasive follicular thyroid neoplasm with papillary like nuclear features (NIFTP) has implications for the implied ROM, as NIFTP tends to behave in a more indolent fashion. Based on published literature to date, the overall reduction in ROM for each category is accounted for in Table 1.4 [19, 66, 76–78].

For some of the general diagnostic categories, subcategorization can be informative and is often appropriate; recommended terminology is shown in Table 1.1. Additional descriptive comments (beyond such subcategorization) are optional and left to the discretion of the cytopathologist. Notes and recommendations can be useful, especially with relation to the NIFTP terminology (see Chaps. 4, 5, 7, and 8). Some laboratories, for example, may wish to state the risk of malignancy associated

Table 1.3 The Bethesda System for Reporting Thyroid Cytopathology in Pediatric Patients with implied risk of malignancy (ROM) and possible management recommendations [10, 12–18, 60–65]

Diagnostic category	ROM Mean% (range)	Possible management recommendations
Nondiagnostic	14 (0–33)	Repeat FNA with ultrasound guidance
Benign[a]	6 (0–27)	Clinical and sonographic follow-up
Atypia of Undetermined Significance	28 (11–54)	Repeat FNA or surgical resection
Follicular Neoplasm[b]	50 (28–100)	Surgical resection
Suspicious for Malignancy	81 (40–100)	Surgical resection
Malignant	98 (86–100)	Surgical resection

[a] ROM is skewed by selection bias since a majority of thyroid nodules classified as benign do not undergo surgical excision

[b] Includes cases of follicular neoplasm with oncocytic features (Hürthle cell neoplasm)

Table 1.4 Reported decreases in the risk of malignancy (ROM) of TBSRTC diagnostic categories if excluding nodules diagnosed on surgical pathology to be "Noninvasive Follicular Thyroid Neoplasm with Papillary Like Nuclear Features (NIFTP)" [19, 66–75]

Diagnostic category	% Decrease in ROM if excluding NIFTP[a] Mean% (range)	Estimated final ROM if excluding NIFTP[b] Mean%
Nondiagnostic	1.3 (0–2)	12
Benign	2.4 (0–4)	2
Atypia of Undetermined Significance	6.4 (6–20)	16
Follicular Neoplasm	7.1 (0.2–30)	23
Suspicious for Malignancy	9.1 (0–40)	65
Malignant	2.6 (0–13)	94

[a] Based on weighted average (mean) reduction in malignancy with expected ranges calculated from refs. [19, 66–75]

[b] Based on estimated average (mean) ROM values from Table 1.2 minus values presented in this table

with the general category, based on their own cytologic–histologic correlation or that found in the literature (Table 1.2). Sample reports, which we hope will be a useful guide, are provided in the remaining chapters.

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Nondiagnostic

2

Barbara Crothers, Daniel Johnson, Laurence Leenhardt, Steven Long, and Sevgen Önder

Background

Fine needle aspiration (FNA) is the most accurate and cost-effective method for evaluating thyroid nodules. In order to provide useful diagnostic information for clinical management, an FNA sample of a thyroid nodule should be representative of the underlying lesion. Retrospective studies have reported lower rates of both nondiagnostic and false-negative cytology from FNA procedures performed

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using ultrasound guidance compared to palpation [1, 2]. Therefore, for nodules with a higher likelihood of either a nondiagnostic cytology (those with greater than 25–50% cystic component) [3] or sampling error (those that are difficult to palpate or posteriorly located nodules), US-guided FNA is preferred. If the diagnostic ultrasound confirms the presence of a predominantly solid nodule corresponding to what is palpated, the FNA may be performed using palpation or US guidance.

Cellularity/adequacy is dependent not only on the technique of the aspirator, but also on the inherent nature of the lesion (e.g., solid vs. cystic). High-quality specimens require proficient collection combined with excellent slide preparation, processing, and staining. In general, the adequacy of a thyroid FNA is defined by both the *quantity* and *quality* of the cellular and colloid components.

The terms "nondiagnostic" (ND) and "unsatisfactory" have been used interchangeably by many pathologists and clinicians. Some interpret these terms to mean different things [4, 5]. This third edition of The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) reiterates the distinction between the terms. An unsatisfactory specimen is always ND, but some technically satisfactory/adequate specimens may also be considered nondiagnostic; that is, showing nonspecific features not conclusively representative of a particular entity. At the 2007 NCI Thyroid State of the Science conference, the terms ND and "unsatisfactory" were equated and recommended for the category that conveys an inadequate/insufficient sample [6]. In the updated system, "nondiagnostic" is the sole descriptive diagnostic term and statements on overall adequacy are reported separately. For the sake of simplicity, ND is the preferred diagnostic category term used throughout this monograph to convey a sample that does not meet the adequacy criteria outlined below.

An assessment of specimen adequacy is an integral component of a thyroid FNA interpretation because it conveys the degree of certainty with which one can rely on the result. Stringent criteria of adequacy, when appropriately applied, ensure a low false-negative rate. Whereas the *quality* of a specimen is irrefutably critical to proper interpretation, rigid numerical criteria for cell *quantity* have not been clinically validated and remain controversial. TBSRTC recommends a minimum number of follicular cells (see Definition, below) based on criteria developed at the Mayo Clinic [7] and their long-term successful implementation.

Definition

Nondiagnostic is the diagnostic line term for specimens that fail to meet the following adequacy requirements.

Criteria for Adequacy

Criteria A thyroid FNA sample is considered adequate for evaluation if it contains a minimum of six groups of well-visualized (i.e., well stained, well preserved, undistorted, and unobstructed) follicular epithelial cells with at least ten cells per group. These six groups of ten follicular cells could be either on one slide or distributed among several for adequacy determination. These criteria apply to all cytologic preparations (including conventional smears and liquid-based samples). Liquid-based preparations often have higher numbers of dissociated single follicular cells or smaller groups as opposed to more intact cell fragments.

Exceptions to this requirement apply to a limited number of case types, including:

1. **Aspirates with cytologic atypia**: Any sample that contains significant nuclear or cellular atypia should never be considered ND and must be reported in the appropriate TBSRTC diagnostic category (i.e., TBSRTC categories III–VI). A minimum number of follicular cells is not required.
2. Aspirates from **solid nodules with inflammation**. Nodules in patients with lymphocytic (Hashimoto) thyroiditis, granulomatous thyroiditis, or thyroid abscess may contain only inflammatory cells, and a minimum number of follicular cells is not required. These samples are reported as Benign (TBSRTC category II).
3. **Colloid nodules**. Specimens that consist of abundant, easily identifiable colloid are considered Benign (TBSRTC category II) and satisfactory for evaluation. A minimum number of follicular cells is not required if colloid predominates.

Nondiagnostic

The following scenarios describe cases considered nondiagnostic:

1. Fewer than six groups of well-preserved, well-stained follicular cell clusters with ten or fewer cells each (see exceptions above).
2. Poorly prepared, poorly stained, or significantly obstructed or distorted follicular cells (Fig. 2.1).
3. Cyst fluid (with or without histiocytes) and fewer than six groups of ten benign follicular cells (Fig. 2.2).
4. No cellular material present.
5. Blood only (Fig. 2.3).
6. Ultrasound gel precipitate only (Fig. 2.4).
7. Lack of collection of cells from the targeted lesion (e.g., skeletal muscle, respiratory cells, or cartilage only) (Fig. 2.5).

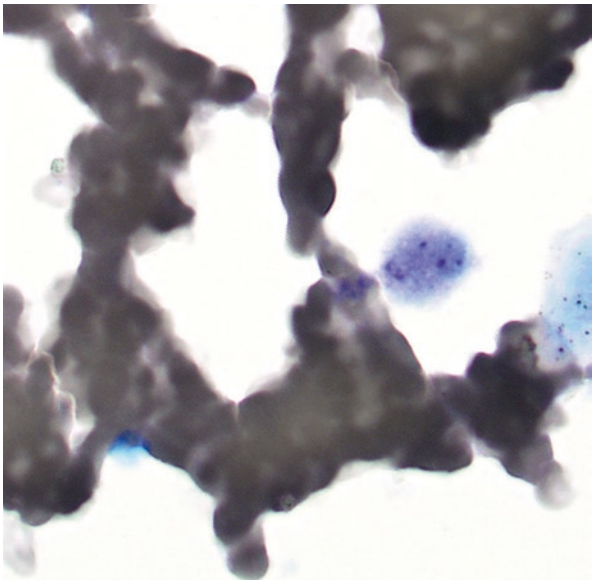

Fig. 2.1 Nondiagnostic. Excessive air-drying artifact and insufficient staining. Air-drying is due to the lack of sufficient alcohol fixation, most frequently in Papanicolaou stains, and may obscure cellular features or render cells uninterpretable (smear, Diff-Quik stain)

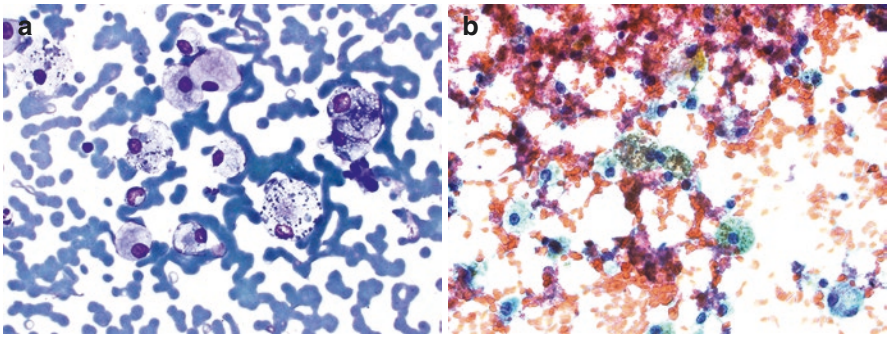

Fig. 2.2 Nondiagnostic. Cyst fluid only. Thyroid lesions with cystic degeneration, common in hyperplastic nodules, may yield only histiocytes, with or without hemosiderin. Histiocytes have abundant, finely vacuolated cytoplasm, uniform oval nuclei, and may contain cytoplasmic cell degeneration products (**a**: smear, Diff-Quik stain; **b**: smear, Papanicolaou stain)

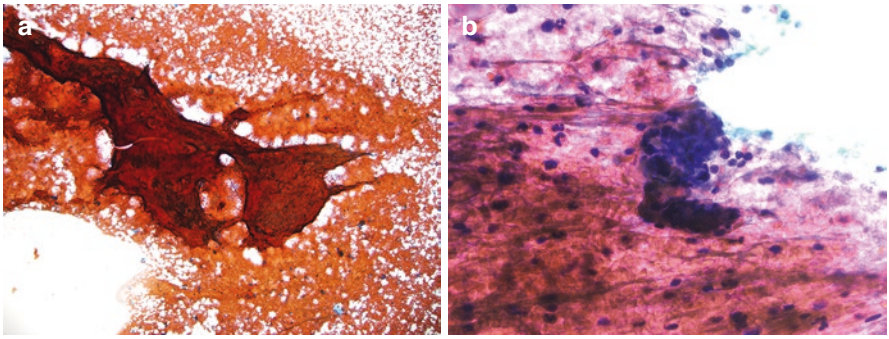

Fig. 2.3 Nondiagnostic. Obscuring blood. Thyroid is a vascular organ and excessive blood collection may result in clots (**a**) that obstruct cellular features (**b**). Using a smaller gauge collection needle (26 or 27 gauge), avoiding negative pressure, and shortening the lesion dwell time may improve cellularity (smears, Papanicolaou stains)

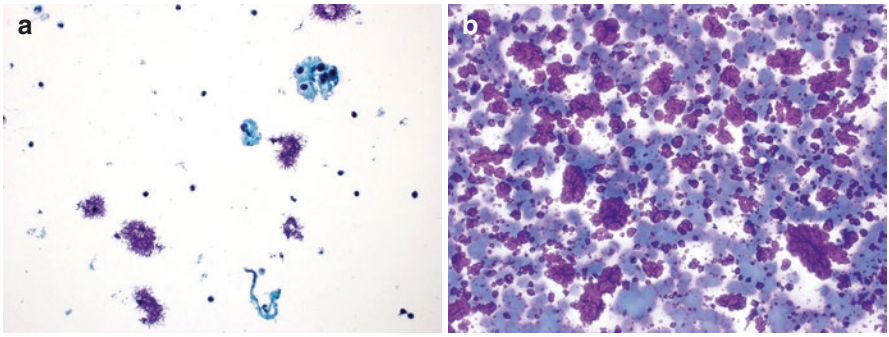

Fig. 2.4 Nondiagnostic. Ultrasound gel. Different brands of gel may appear differently, but all can mimic dense colloid. Most gels have a slightly filamentous (**a**) or angulated (**b**) appearance. It is best to wipe gel from the skin prior to tissue collection to prevent this finding (**a**: ThinPrep, Papanicolaou stain; **b**: smear, Diff-Quik stain)

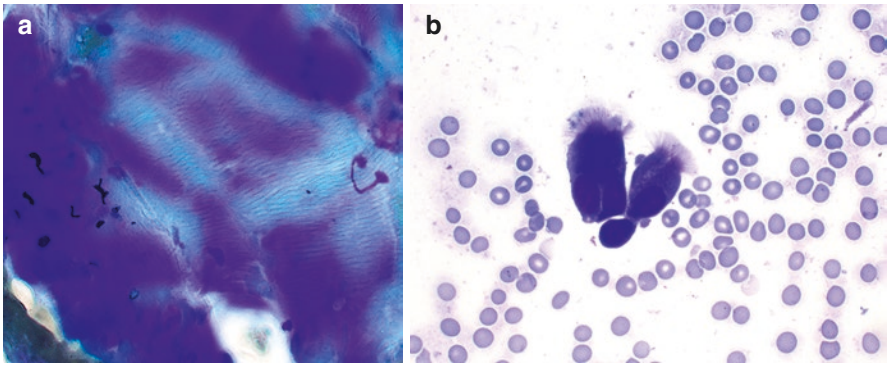

Fig. 2.5 Nondiagnostic. Skeletal muscle and ciliated cells. Collection of muscle tissue around the target is most recognizable by fine cross-striations (**a**), as evident in the pale areas of this sample, and should not be mistaken for dense colloid. Ciliated cells (**b**) or cartilage may be collected from the trachea. When these are the only findings, they indicate that the target (thyroid) has not been sampled (smears, Diff-Quik stains)

Explanatory Notes

Adequate samples minimize false-negative reports of thyroid lesions [8, 9]. Despite continued controversy regarding adequacy criteria, TBSRTC criteria have been successfully employed for over a decade with low reported false-negative rates [10, 11].

Given that most ND nodules are benign (see Management below), some investigators have proposed lowering the required number of follicular cells to reduce ND interpretations and questioned the need for repeat FNA. Only a few studies have assessed the number of follicular cells and their impact on specimen adequacy. One study reported that decreasing the required number of follicular cells from 60 to 10 improved the specificity of a benign diagnosis without significantly affecting sensitivity, but only if oncocytic (Hürthle) cells and atypical follicular cells were excluded from the count [12]. In a study of liquid-based thyroid samples, lowering the number of required follicular cells also did not affect the test performance if atypical features were excluded [13]. These data suggest that requiring a smaller number of follicular cells, or eliminating the requirement for groups of cells, would significantly reduce ND interpretations without significantly impacting the false-negative rate. However, the prior criteria have been retained with the understanding that this is an evolving area that would benefit from more robust evidence.

Adequacy assessments rely on the quantity of thyroid follicular cells and exclude consideration of macrophages, lymphocytes, and other nonmalignant cellular components [14, 15]. The ability to obtain follicular cells by FNA is dependent, in part, upon the nature of the lesion. The number of follicular cells necessary for a diagnosis is contingent upon the lesion aspirated, because some lesions such as benign cysts, thyroiditis, or colloid-abundant nodules do not yield many follicular cells. Others are comprised of additional cell types that may define the lesion but that overwhelm the follicular component.

Thyroid cancers are predominantly solid. Solid nodules and partially cystic nodules with nuclear atypia should always be considered adequate and reported as abnormal (Atypia of Undetermined Significance, Suspicious for Malignancy, or Malignant, depending on the findings), with a comment describing any limiting factor(s) such as scant cellularity [16]. Follicular cells are not always present in aspirates of inflammatory lesions such as lymphocytic thyroiditis or granulomatous thyroiditis. Therefore, there is no minimum requirement for a follicular component when inflammation predominates. The presence of abundant colloid (as opposed to serum) (Fig. 2.6) reliably identifies most benign processes despite scant follicular cells [17]. One group of follicular cells with features sufficient for the diagnosis of papillary thyroid carcinoma may constitute an adequate specimen in the proper clinical setting and should not be considered ND despite scant cellularity [18, 19].

Cyst fluid may yield only macrophages (Fig. 2.2), but the risk of malignancy is low for these lesions if they are simple and under 3 cm [14, 16, 20, 21]. The cytopathologist is not always privy to clinical or sonographic information, however, and in isolation the possibility of a cystic papillary thyroid carcinoma cannot be excluded

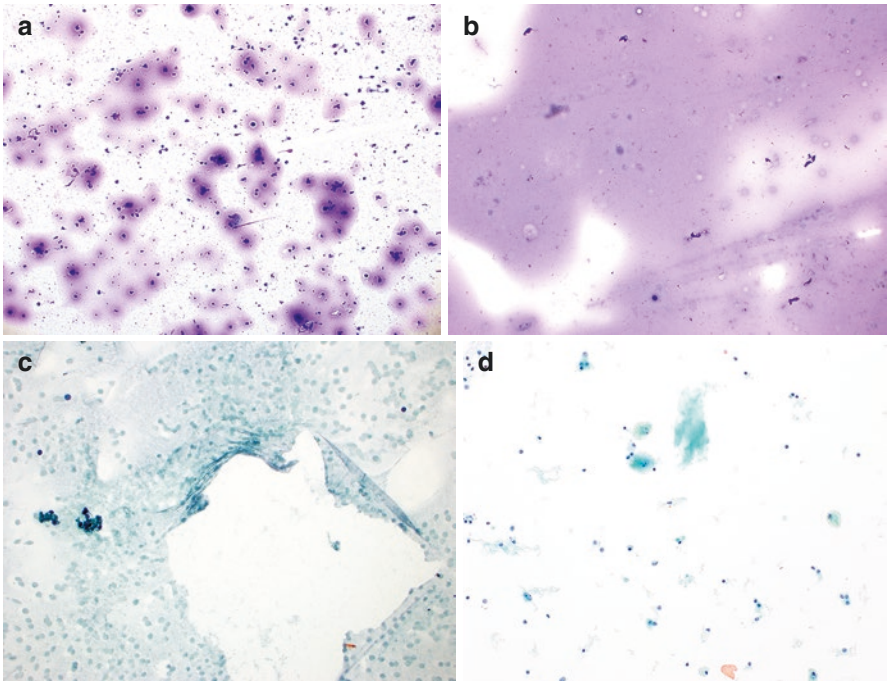

Fig. 2.6 Benign. Watery colloid. The presence of abundant colloid, whether thick or watery, indicates a colloid nodule and is not considered "nondiagnostic." Thin, watery colloid often shows sharp margination, condensation around cells (**a**, **b**), cracks and folds (**c**). Colloid in liquid-based preparations is usually clumped (**d**) (**a**, **b**: smears, Diff-Quik stains; **c**: smear, Papanicolaou stain; **d**: ThinPrep, Papanicolaou stain)

if a sample consists entirely of fluid and histiocytes. Younger patients with only cystic fluid have been shown to have a slightly higher risk of malignancy, primarily papillary carcinoma [21–23]. For this reason, these cases are reported as ND followed by the subcategory "Cyst fluid only" (see section on "Sample Reports", Example 2). In the proper clinical setting (e.g., ultrasound evidence of a simple, unilocular cyst), some specimens may be considered clinically adequate, even though they are reported as ND [14, 22, 24].

Occasionally, an adjacent anatomic site is aspirated, such as the trachea (ciliated respiratory cells) or sternocleidomastoid muscle (Fig. 2.5), yielding only non-thyroidal tissue and are reported as ND. Ultrasound gel should be wiped from the skin surface before needle insertion to prevent gel from obscuring the cellular component (Fig. 2.4), whether the sample is prepared by smearing or using liquid-based methods. Overall specimen adequacy is similar for smeared preparations and liquid-based preparations (LBP), but an additional LBP slide may decrease the number of inadequate results [25]. In TBSRTC, unless a sample is interpreted as ND, it is considered satisfactory for evaluation. The frequency of ND interpretations varies considerably from laboratory to laboratory (range 3–34%) [26, 27].

Management

The risk of malignancy for ND nodules is difficult to calculate precisely, because most ND nodules are not resected. Among surgically excised nodules initially reported as ND, the malignancy rate is broad, ranging from 7% to 32% [28–30]. Surgically resected nodules, however, represent a selected subset of nodules that were either repeatedly ND, had worrisome clinical/sonographic features, or both. Thus, surgically resected ND nodules over-represent malignancies compared to the entire cohort of ND nodules. A reasonable extrapolation of the overall risk of malignancy for lesions in the ND category is likely around 10–13% (also see Chap. 1) [31].

There are predictive factors impacting ND results, including patient factors (e.g., obesity, anticoagulation), nodule characteristics (e.g., small, cystic, or deep), and FNA biopsy skill. Ultrasound-based risk stratification systems (RSS), including the Thyroid Imaging, Reporting, and Data System (TIRADS), play an important role in triaging ND nodules, with the risk of malignancy increasing with the RSS score. In a Korean series comparing four RSSs, a high-risk score (score 5) in European (EU)-TIRADS, American Thyroid Association (ATA), American College of Radiology (ACR)-TIRADS, and Korean (K)-TIRADS RSSs conferred a 63%, 81%, 80%, and 81% risk for malignancy in operated patients, respectively. More interesting is the proportion of malignancy in operated patients with a low-risk score: 28% in EU-TIRADS-3 (low risk), 50% in ATA "very low suspicion" sonographic pattern, 15% in ACR TIRADS-2 (not suspicious), and 50% in K-TIRADS-2 (low suspicion) [30].

Small nodule size and purely cystic nodules are two main ultrasound criteria that impact the cytological results. The likelihood of a ND result increases with increasing cystic content in nodules, and high cystic content of nodules is an independent predictor of a nondiagnostic cytology result [3]. Regarding cyst fluid only cases, one study [32] reported that the risk of malignancy was low (2%), and all subsequent malignant cases had associated suspicious ultrasound findings. If ultrasound findings are not suspicious, "cyst fluid only" cases may be clinically separated from ND as a subtype of benign nodule [33]. The only predictor of subsequent malignancy in cyst fluid specimens is the presence of atypical or suspicious follicular epithelium [16].

For FNA procedures, it is well established that ultrasound guidance provides significant improvement in cytological results and is now systematically required. Most thyroid FNA in the USA are performed under ultrasound guidance by radiologists, endocrinologists, or pathologists [34]. Ultrasound guidance with rapid on-site evaluation (ROSE) for adequacy is preferred for repeat aspirations after an initial ND specimen, especially for solid nodules [31]. Most studies have demonstrated that ROSE (whether on-site or using telecytology) significantly reduces the unsatisfactory rate by over 50%, even in experienced groups [35–39]. Although one study did not find a decrease in ND rates with ROSE, there was a significant decrease in the number of needle passes and procedural time [40]. In the absence of ROSE, obtaining a minimum of three separate samples of the nodule may reduce the rate of unsatisfactory specimens [27]. However, larger nodules (≥3 cm) often require

additional passes to obtain diagnostic specimen [41]. Performing a cell block from the residual LBP sample can convert some initially ND FNAs into a satisfactory sample [42]. Despite the recommendation to repeat FNA in order to obtain a diagnostic sample, one retrospective study showed that the probability of obtaining a diagnostic result sequentially decreased after repeated aspirates following an initial ND result [29]. This may be in part due to poor observer diagnostic agreement for multiple samples [43]. ND rates have been found to decrease with increasing procurer experience [44] and procedural volume [39].

Nodules with an initial ND result should be re-aspirated unless the nodule is purely cystic [31, 45, 46]. Repeating the FNA results in a diagnostic interpretation in up to 60–80% of cases, primarily in lesions with a smaller cystic component [31, 45, 47, 48]. Most nodules with a ND interpretation prove to be benign [18, 49]. Nevertheless, repeat FNA yielded a different category 65% of the time in one study of Canadian patients. Of 194 patients with initial ND results, 35% of them remained ND, 37% shifted to benign, 26% to indeterminate results, and 2% to malignancy [50].

Although historically it had been customary to wait several months before repeating an FNA to allow for resolution of biopsy-induced inflammation and potentially confounding atypia, intervals shorter than 3 months do not seem to increase the frequency of atypical results [51–53]. Data are conflicting on whether repeat FNA within 3 months increases the likelihood of a second ND interpretation. One study reported that a repeat in under 3 months did not affect the diagnostic yield of cells [54] but another [55] determined that it may, even when the repeat was performed by experienced pathologists. Those patients whose FNA was repeated within 3 months of an initial ND result had a fivefold greater likelihood of another ND result compared with patients repeatedly aspirated after 3 months, hypothetically due to increased hemorrhage [55]. In total, there does not appear to be compelling evidence to delay a repeat thyroid FNA procedure following an initial ND.

ND specimens should not be submitted for molecular diagnostics, since the likelihood of insufficient cellularity for testing is high, although some specimens may contain sufficient nucleic acid for testing [56]. Additional dedicated thyroid passes should be obtained to ensure sufficient tissue and nucleic acid for molecular evaluation. Molecular testing is recommended only for indeterminate nodules classified as Atypia of Undetermined Significance (AUS), Follicular Neoplasm (FN), or Suspicious for Malignancy (SFM), TBSRTC categories III, IV, and V, respectively. Despite limitations, molecular testing may be of value in selected cases and may influence clinical management decisions [13].

After two successive ND specimens, close clinical follow-up with ultrasound or surgery should be considered, depending upon the clinical and imaging findings. Because the risk of malignancy in cystic lesions is low, re-aspiration of most cystic nodules with an initial ND result should be performed only if the ultrasound findings are suspicious. For cases in which repeated FNAs result in ND results or for those ND with suspicious ultrasound features, surgery is a valid option. Ultrasound characteristics and individual patient factors should be considered in a broader context to guide clinical decision-making.

Sample Reports

Example 1 (Solid Nodule)
NONDIAGNOSTIC.
Specimen processed and examined, but unsatisfactory due to insufficient cellularity.
Note: Clotting blood obscures the rare follicular groups and their cytologic features. A repeat aspiration should be considered if clinically indicated.

Example 2 (Cystic Lesion)
NONDIAGNOSTIC.
Cyst fluid only.
Note: The specimen is nondiagnostic because it consists almost exclusively of histiocytes. Recommend correlation with cyst size and complexity on ultrasound to assist with further management of the lesion.

Example 3 (Processing Issue)
NONDIAGNOSTIC.
Specimen processed and examined, but nondiagnostic due to poor fixation and cellular preservation.
Note: A repeat aspirate should be considered if clinically indicated.

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Benign

3

Tarik Elsheikh, SoonWon Hong, Christian Nasr, and Elena Vigliar

Introduction

Thyroid fine needle aspiration (FNA) derives much of its clinical value from its ability to reliably identify benign thyroid nodules, thus sparing many patients with nodular thyroid disease unnecessary surgery. Because most thyroid nodules are benign, a benign result is the most common FNA interpretation (approximately 60–70% of all cases) [1, 2].

To report benign thyroid cytopathology results, the diagnostic category terminology of "Benign" is preferred over other terms such as "Negative for malignancy" and "Non-neoplastic" [3, 4]. Benign cytopathology is associated with a very low risk of malignancy (less than 4%, and closer to 1.5% if papillary microcarcinomas are excluded) [5], and patients are usually followed conservatively with periodic clinical and radiologic examinations [2, 6, 7]. Benign results can be further subclassified as follicular nodular disease, thyroiditis, or other less common entities.

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Nodular goiter is the most commonly sampled lesion by FNA, and chronic lymphocytic or Hashimoto thyroiditis is the most often encountered form of thyroiditis.

Follicular Nodular Disease

Background

Nodular goiter (NG) is a clinical terminology that refers to an enlarged thyroid gland with single or multiple nodules. NG is not recommended as a terminology for pathologic diagnosis, since a variety of lesions including hyperplasia, thyroiditis, and neoplasm can produce clinically enlarged nodular thyroid gland. The term "follicular nodular disease" (FND) has been recommended by the 2022 WHO classification of thyroid neoplasms to refer to the spectrum of changes previously designated as colloid nodule, hyperplastic nodule, adenomatous nodule, or benign follicular nodule [8]. Several studies have shown that these nodules may or may not be clonal; therefore, they may represent an admixture of hyperplastic nodules and true adenomas. The terminology of FND avoids designating these lesions as hyperplastic or neoplastic in nature.

FND is the most encountered entity in thyroid cytopathology and encompasses a group of benign lesions with similar cytologic features that are classified histologically as nodular hyperplasia, hyperplastic (adenomatoid) nodules, colloid nodules, nodules in Graves' disease, and an uncommon subset of follicular adenomas composed predominantly of macrofollicular or normofollicular architecture. The distinction among these different histologic entities may not be possible by FNA, but this is of little importance because they are all benign, and can be managed in a similar, conservative manner. In surgical pathology, the noncommittal term "benign follicular nodule" has been previously suggested for benign cellular nodules where distinction between follicular adenoma and hyperplastic nodule (HN) is not possible on histologic examination [9]; however, the recently proposed alternative terminology of FND avoids defining such lesions as hyperplastic or adenomas [8]. Cytologically, FND is characterized by benign-appearing follicular cells, variable amounts of colloid, oncocytic cells, and macrophages.

Definition

The designation "follicular nodular disease" applies to a cytologic sample that is adequate for evaluation and consists of benign-appearing follicular cells and colloid in varying proportions. The term FND may be utilized in cytology reporting as a subclassification of a more generalized "benign" diagnosis. Further subclassification such as colloid nodule or Graves' disease may also be used, depending on the cytomorphologic findings and associated clinical presentation (see section on "Sample Reports").

Criteria

Follicular cells are present, often with sparse to moderate cellularity.

Colloid is present, often in moderate to abundant amounts.

Colloid is viscous, shiny, and light yellow or gold in color (resembling honey or varnish) on gross examination. It is dark blue-violet-magenta with Romanowsky-type stains and green or orange-pink with Papanicolaou stain (Figs. 3.1 and 3.2). It may be thin or thick in texture (Fig. 3.3).

Thin, watery colloid often forms a "thin membrane/cellophane" coating or film with frequent folds that impart a "crazy pavement," "chicken wire," or mosaic appearance (Fig. 3.1). At times, it forms lacunae (Fig. 3.4).

Thick (dense, "hard") colloid has a hyaline quality and often shows cracks (Fig. 3.2a).

Follicular cells are arranged predominantly in monolayered sheets and are evenly spaced ("honeycomb-like") within the sheets (Figs. 3.3 and 3.4a).

Occasionally, follicular cells are arranged in intact, 3-dimensional, variably sized balls/spheres, spherules, and/or microtissue fragments (Fig. 3.5).

Oncocytes may be present, in flat sheets and/or as isolated cells, and at times may predominate in a specimen (Fig. 3.6).

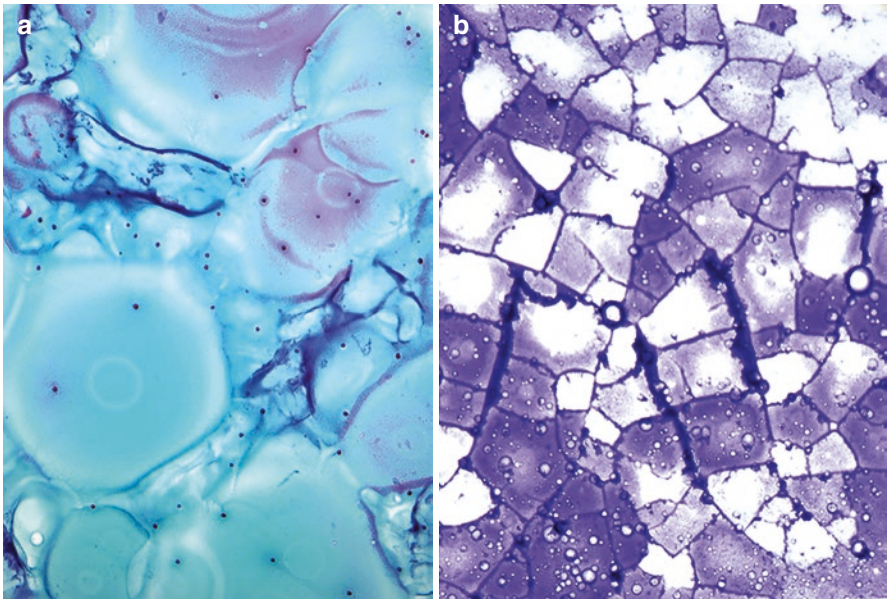

Fig. 3.1 Follicular nodular disease/colloid nodule: watery colloid. (**a**) Watery colloid is light green or pink with alcohol-fixed, Papanicolaou-stained preparations and has a "thin membrane" or "cellophane coating" appearance, often with coalescing "puddles" (smear, Papanicolaou stain). (**b**) Colloid stains blue-violet with air-dried, Romanowsky-stained preparations and often shows a chicken wire appearance (smear, Diff-Quik stain)

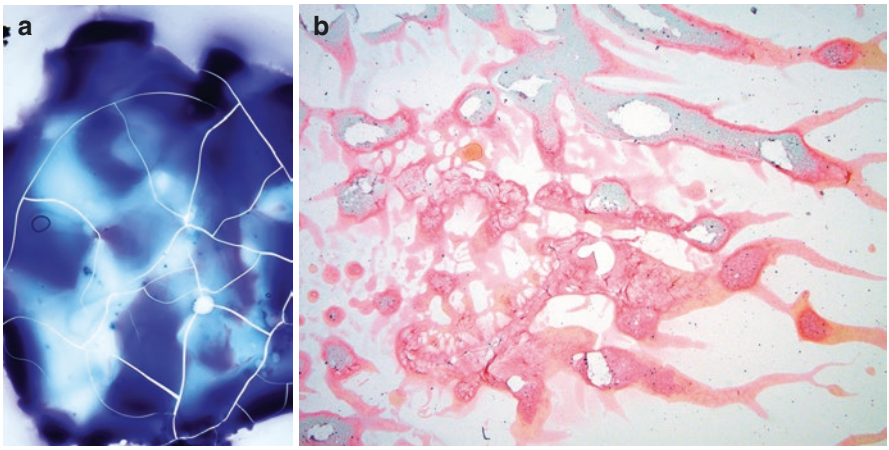

Fig. 3.2 Follicular nodular disease: thick colloid. (**a**) Colloid demonstrates a "stained glass cracking" appearance (smear, Diff-Quik stain). (**b**) Colloid is orange-pink and grayish-green with alcohol-fixed Papanicolaou-stained preparations and covering a major part of the glass slide surface in this case (smear, Papanicolaou stain)

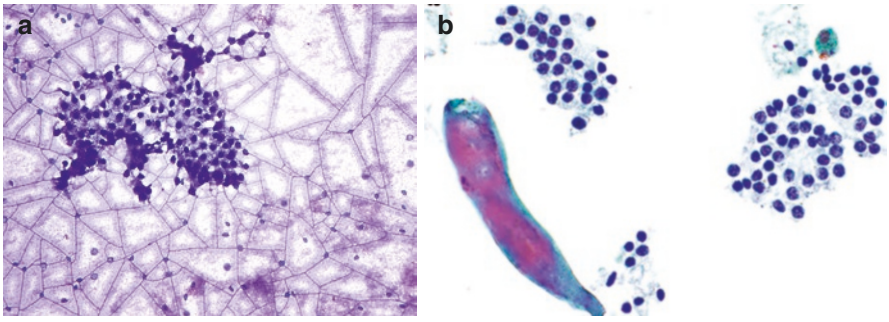

Fig. 3.3 Follicular nodular disease. Monolayered sheets of evenly spaced follicular cells have a honeycomb-like arrangement. (**a**) Watery colloid is present (smear, Diff-Quik stain). (**b**) Thick colloid is present (ThinPrep, Papanicolaou stain)

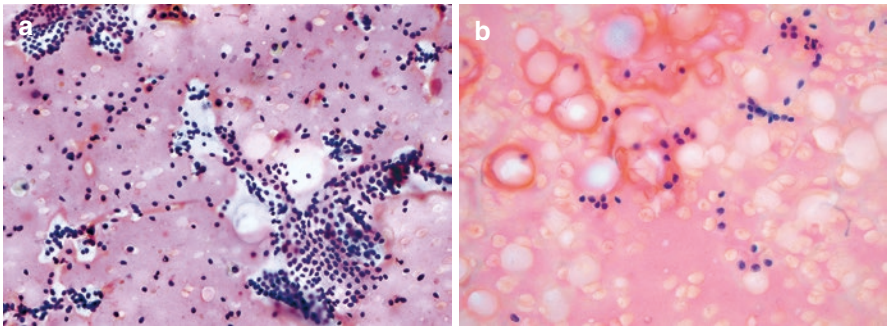

Fig. 3.4 Follicular nodular disease. (**a**) Monolayered sheets of follicular cells are the predominant finding. Stripped follicular cell nuclei are present in the background. When watery colloid is admixed with blood (note the pale-staining red blood cells), it can be difficult to recognize (smear, Papanicolaou stain). (**b**) Colloid is easier to recognize when it forms characteristic folds and lacunae (smear, Papanicolaou stain)

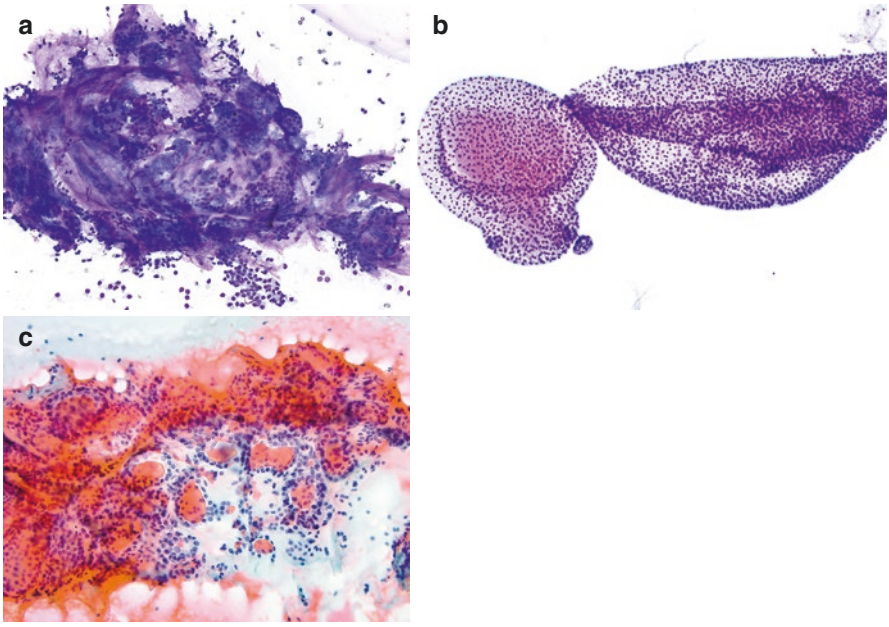

Fig. 3.5 Follicular nodular disease. (**a**, **b**) Three-dimensional, variably sized balls/spheres are admixed with flat sheets. Within the spheres there is maintenance of polarity, including a relatively evenly spaced nuclear arrangement (**a**: smear, Diff-Quik stain; **b**: ThinPrep, Papanicolaou stain). (**c**) Microtissue fragments are admixed with flat sheets, spherules, and colloid. The spherules show follicle formation, but these are not microfollicles, because there is maintenance of polarity, and the nuclei are evenly spaced without nuclear overlapping and crowding (smear, Papanicolaou stain)

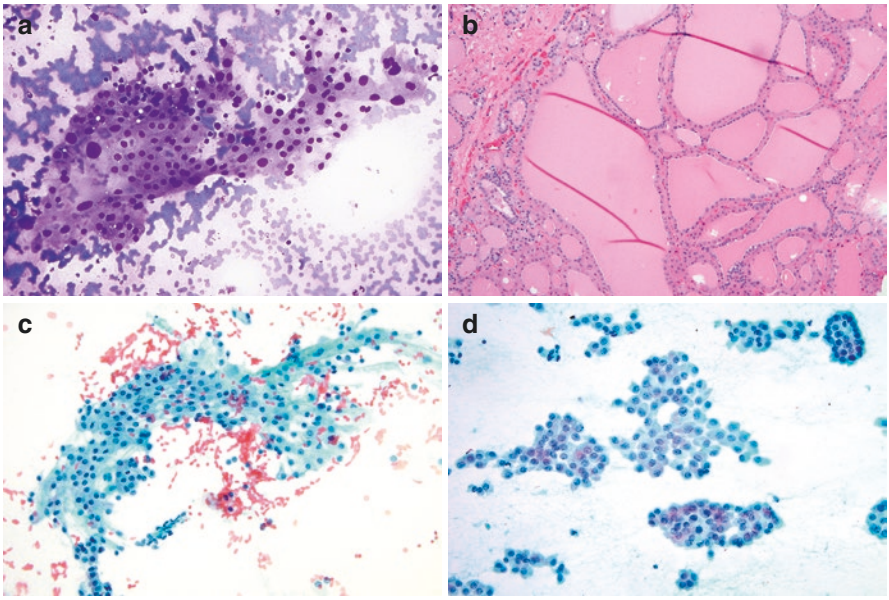

Fig. 3.6 (**a**, **b**) Follicular nodular disease (FND). (**a**) Oncocytes (Hürthle cells) can be a prominent component of FND and may show large-cell dysplasia. There was abundant colloid present elsewhere on the smears (smear, Diff-Quik stain). (**b**) The corresponding histologic specimen showed a predominantly macrofollicular architecture with oncocytic metaplasia (hematoxylin and eosin stain). (**c**) This aspirate of a 1.7 cm nodule showed low cellularity, uniform oncocytes (absence of anisonucleosis), and significant colloid (smear, Papanicolaou stain). Follow-up revealed FND (not shown). (**d**) This hypercellular specimen from a 2.5 cm nodule showed a uniform oncocytic cell population and some colloid (not shown) (smear, Papanicolaou stain). Follow-up revealed FND (not shown)

Microfollicles may be present but comprise a minority of the follicular cell population.

The follicular cells have scant or moderate amounts of delicate cytoplasm (Figs. 3.7 and 3.8).

Green-black cytoplasmic granules may be seen, representing lipofuscin or hemosiderin pigment (Fig. 3.7b).

Follicular cell nuclei are round to oval, approximately the same size or slightly larger than a red blood cell or lymphocyte (7–10 μm in diameter), and show a uniformly granular chromatin pattern (Fig. 3.8).

Minimal nuclear overlapping and crowding can occur (Fig. 3.9a). Anisonucleosis is appreciated in some cases, but there is no significant nuclear pallor or nuclear membrane irregularity.

Small-sized follicles comprised of flat sheets of follicular cells without nuclear overlapping or atypia may be present and do not represent neoplastic microfollicles (Fig. 3.9b).

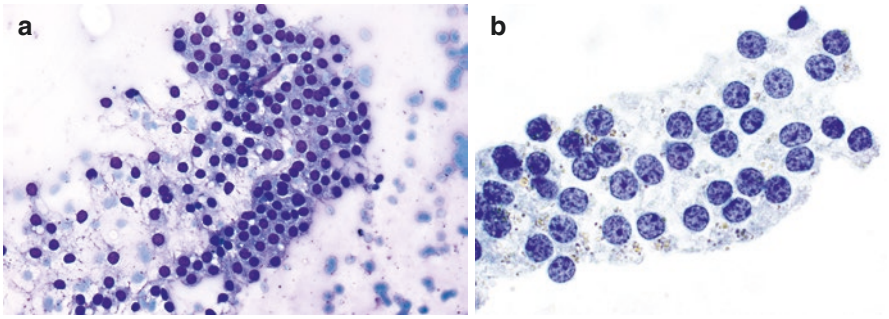

Fig. 3.7 Follicular nodular disease. (**a**) Benign follicular cells have delicate cytoplasm and ill-defined borders. The nuclei are uniformly spaced and approximately the size of red blood cells. Watery colloid is present in the background (smear, Diff-Quik stain). (**b**) Follicular cells may contain golden-brown cytoplasmic hemosiderin pigment (ThinPrep, Papanicolaou stain)

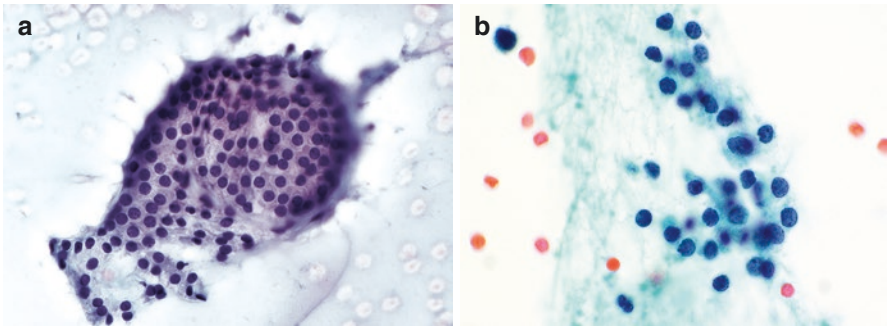

Fig. 3.8 Follicular nodular disease. Benign follicular cells have round to oval, monomorphic nuclei with finely granular chromatin and inconspicuous or absent nucleoli (**a**: smear, Papanicolaou stain; **b**: SurePath preparation, Papanicolaou stain)

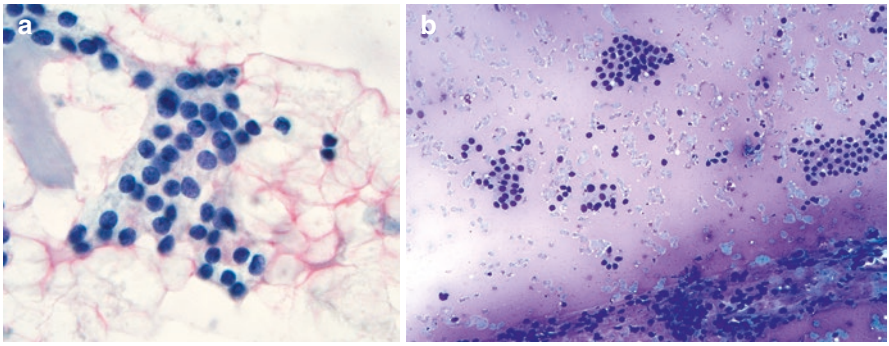

Fig. 3.9 Follicular nodular disease. (**a**) Nuclear overlapping, crowding, and slight anisonucleosis may be observed in some clusters, but there is no significant nuclear enlargement or atypia (smear, Papanicolaou stain). (**b**) Small-sized follicles without significant nuclear overlapping or atypia of follicular cells (middle and left side of photo) represent small fragments of macrofollicles, not neoplastic microfollicles; they are lined by cells of same size and shape as those of benign flat sheets present in upper and right side of photo. Watery colloid is seen in the background (smear, Diff-Quik stain)

Follicular cells trapped in fibrin may show artifactual nuclear overlapping and crowding (Fig. 3.10a), and nuclei may be stripped and mistaken for lymphocytes (Fig. 3.10b).

Papillary hyperplasia is occasionally seen (Fig. 3.11).

Follicular cells may appear shrunken, spindled, and degenerated when associated with abundant colloid (Fig. 3.12).

Macrophages are commonly present and may contain hemosiderin pigment (Fig. 3.13).

Focal reparative changes are sometimes observed, especially in cystic lesions, including cyst lining cells with enlarged nuclei, finely granular chromatin, and a squamoid or spindle-shaped ("tissue-culture cell") appearance (Fig. 3.14). Occasionally, there is focal nuclear atypia (Fig. 3.14c).

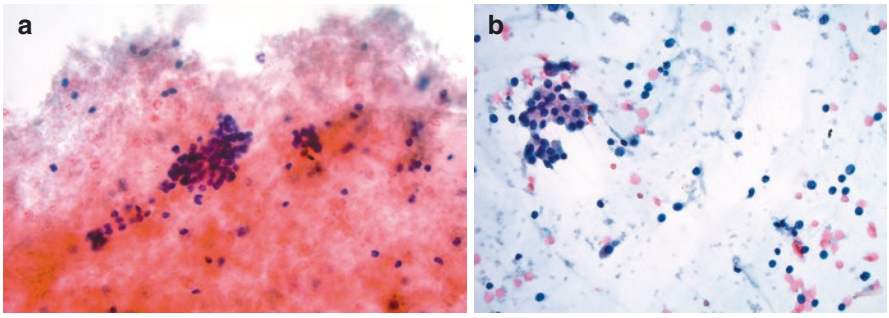

Fig. 3.10 (**a**) Thyroid follicular cells entrapped and distorted by fibrin clot in a hypocellular bloody aspirate. A specimen dominated by such artificial pseudo-complexity should be signed out as "nondiagnostic," rather than "benign" or "atypical" (smear, Papanicolaou stain). (**b**) Stripped ("naked") thyroid follicular cell nuclei may be seen in background; care must be taken not to mistake them for lymphocytes (smear, Papanicolaou stain)

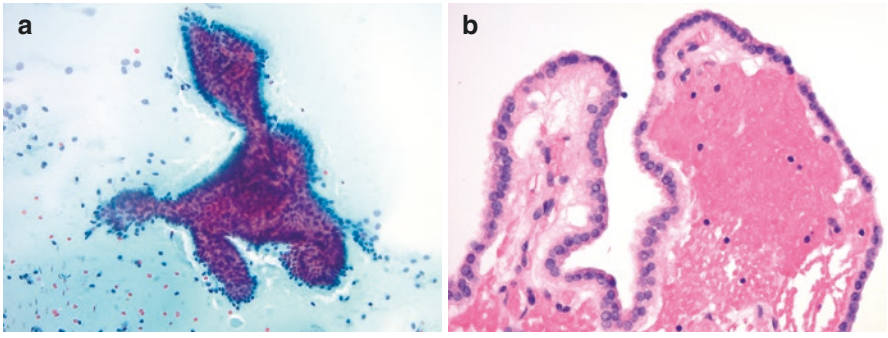

Fig. 3.11 Follicular nodular disease. Papillary hyperplasia may be seen in association with a hyperplastic nodule or follicular adenoma. (**a**) The follicular cells usually remain arranged in flat sheets; true papillae with fibrovascular cores are rarely apparent. Nuclear features of papillary thyroid carcinoma are absent (smear, Papanicolaou stain). (**b**) Follow-up histology revealed papillary hyperplasia associated with follicular nodular disease (H&E stain)

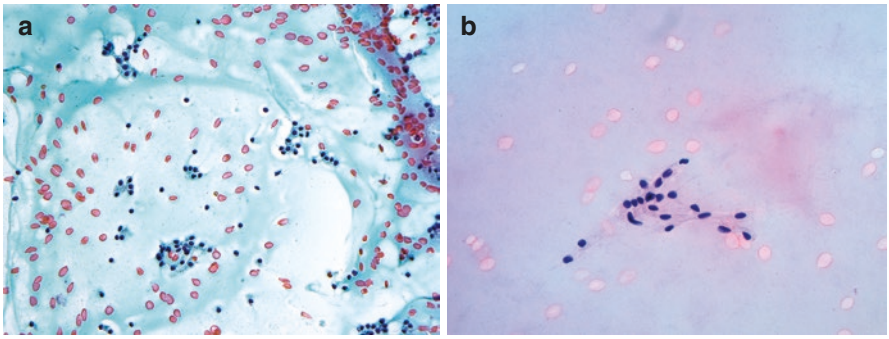

Fig. 3.12 Follicular nodular disease. Follicular cells suspended in abundant colloid tend to dissociate and may appear shrunken and spindled (**a**, **b**: smears, Papanicolaou stain)

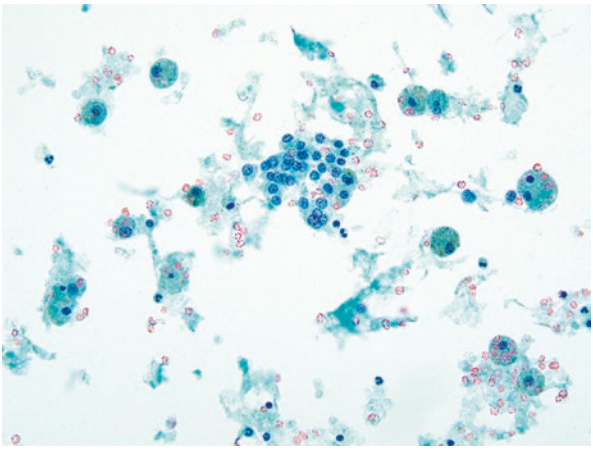

Fig. 3.13 Benign thyroid cyst. Prominent cystic degeneration often occurs in follicular nodular disease. Abundant macrophages and few benign thyroid follicular cells are present (smear, Papanicolaou stain)

Explanatory Notes

The major differential diagnosis of a circumscribed follicular-patterned nodule in surgical resection specimens is hyperplastic/adenomatoid nodule (HN), follicular adenoma (FA), follicular thyroid carcinoma (FTC), follicular variant of papillary thyroid carcinoma (FVPTC), and noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). FVPTC and NIFTP are recognized primarily by their characteristic nuclear features. The great majority of FAs and FTCs are solitary nodules and encapsulated, with a predominantly trabecular/solid or microfollicular architecture; therefore, when aspirated they are most likely to be reported cytologically as FN or AUS. Less frequently, a FA or FTC may display a prominent macrofollicular or normofollicular pattern. Thus, in surgical pathology specimens most nodules with a trabecular, solid, or microfollicular growth pattern are diagnosed as either FA or FTC, depending on the absence or presence of invasion, respectively, whereas most nodules with a normofollicular or macrofollicular

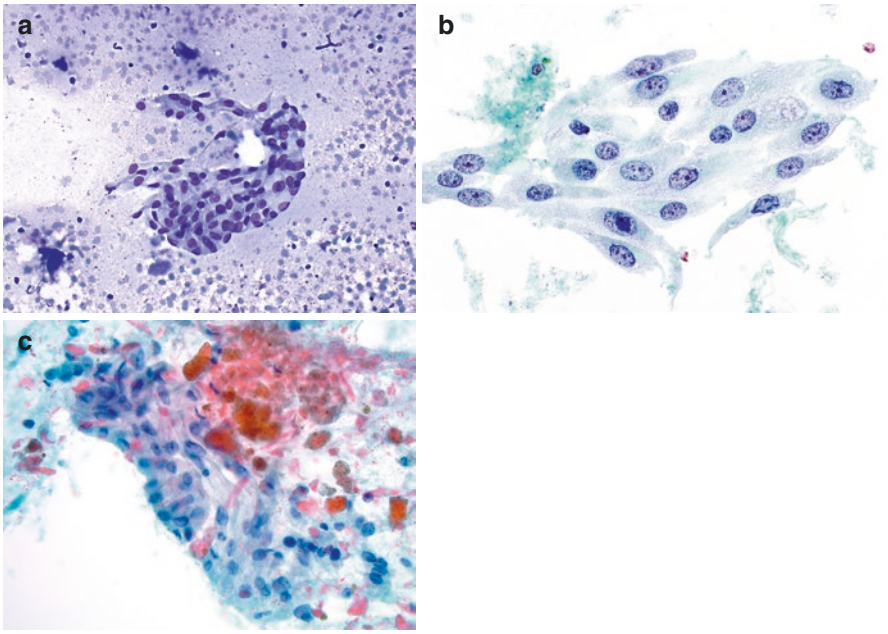

Fig. 3.14 Follicular nodular disease: cyst lining cells. (**a**, **b**) Reparative changes are commonly associated with cystic degeneration. Cyst lining cells are usually a small component of the benign aspirate and easily recognized because of their elongated shape and cohesive, flat and/or squamoid appearance, low nuclear/cytoplasmic ratio, and small prominent nucleoli. (**a**: smear, Diff-Quik stain; **b**: Papanicolaou stain). (**c**) Occasionally, these cells show elongated nuclei with nuclear grooves and powdery chromatin. When the changes are focal and mild, particularly if the background is overwhelmingly benign, they are easily recognized as reactive, but when more advanced and widespread they may raise a concern for papillary thyroid carcinoma (smear, Papanicolaou stain). This case was initially signed out as AUS, but follow-up revealed regressive/reparative changes in a benign cyst

pattern are called HN [9]. Frequently there is histologic overlap of FA and HN, and their distinction may not be possible. The latter observations have been confirmed by molecular studies showing admixture of clonal and non-clonal nodules with highly variable architecture. The term FND has been proposed by the World Health Organization (WHO) to refer to those lesions and avoid defining them as hyperplastic or neoplastic [8].

The term FND is especially apt for cytology reporting because many of the histologic distinctions described above (solitary vs. multiple nodules, encapsulated vs. unencapsulated) are not apparent on an aspiration sample. Thus FND conveniently describes a morphologically diverse group of benign histologic lesions, ranging from the colloid nodule or nodular goiter with minimal cellularity and abundant colloid to the hyperplastic (adenomatoid) nodule with moderate cellularity and scant colloid [10–13]. The predominance of honeycomb-like sheets of benign follicular cells, admixed in some cases with oncocytic cells (Figs. 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 3.10, 3.11, and 3.12), and variable amount of colloid is the hallmark of FND.

Cytoplasmic lipofuscin and hemosiderin pigment granules (paravacuolar granules) are more commonly associated with benign nodules (Fig. 3.7b), but they can be found in malignant neoplasms and so have no diagnostic significance [14].

Watery colloid is most apparent with one of the Romanowsky-type stains like the commonly used Diff-Quik stain; it is less conspicuous but still visible with Papanicolaou-stained preparations (Figs. 3.1, 3.2, 3.3, and 3.4) and can sometimes be confused with serum in bloody specimens. Helpful clues to recognizing watery colloid on smears are the presence of cracking and folding in colloid, as well as its tendency to surround follicular cells and occasionally form lacunae (Fig. 3.4), whereas serum accumulates at the edges of the slide and around platelets, fibrin, and blood clots. Specimens consisting of abundant colloid only (i.e., colloid covering the majority of the surface of a smear), with rare or no follicular cells, are considered FND, reported as "Benign," and may be further described as "suggestive of" or "consistent with" a colloid nodule (Fig. 3.2b); a diagnosis of FND is appropriate in this setting, even if one cannot find six groups of well-preserved, well-visualized follicular cells, each with at least ten follicular cells. Occasionally, the benign thyroid follicular cells are spindled and show evidence of dissociation when suspended in abundant colloid (Fig. 3.12).

The cytologic features and diagnostic accuracy of FND are generally similar between smears and liquid-based preparations (LBP), but there are a few differences [15, 16]. The amount of colloid is diminished in LBP when compared with smears, but nuclear detail may be superior [17, 18]. In LBP, benign-appearing follicular cells are arranged in relatively smaller monolayer sheets, usually with less than 20–25 cells per sheet, and the cells have pale cytoplasm and smaller and darker nuclei (Fig. 3.15). Thick colloid appears as dense, dark blue-orange/red droplets; and watery colloid as thin, delicate, blue to pink tissue paper-like sheets (Fig. 3.16) [16]. Macrophages may have more abundant pale cytoplasm, enlarged, pale nuclei, and prominent nucleoli. Oncocytes (Hürthle cells) may be arranged in a more dissociative pattern and appear shrunken compared to conventional smears, with irregularly shaped, variably sized nuclei and more prominence of the nucleoli (Fig. 3.26b).

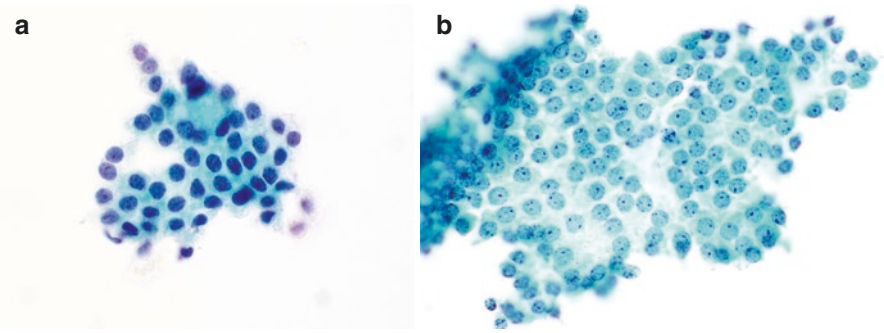

Fig. 3.15 Follicular nodular disease (liquid-based preparations). The follicular cells have pale cytoplasm and small, round, evenly spaced nuclei. (**a**: ThinPrep, Papanicolaou stain; **b**: SurePath, Papanicolaou stain). (The case illustrated in Fig. 3.15b is courtesy of Douglas R. Schneider, MD, Department of Pathology, Steward St. Elizabeth's Medical Center, Boston, MA, USA)

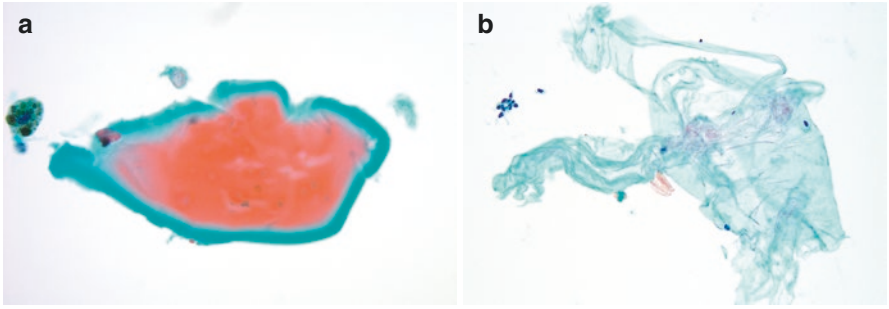

Fig. 3.16 Colloid in follicular nodular disease (liquid-based preparations). (**a**) Thick colloid on liquid-based preparations resembles its counterpart on smears (ThinPrep, Papanicolaou stain). (**b**) Watery colloid has a thin, "folded tissue paper" appearance (ThinPrep, Papanicolaou stain)

Thyroid cysts with an inadequate number of follicular cells should be interpreted as "Nondiagnostic," with a comment pertaining to the "cyst fluid only" nature of the aspirate (see Chap. 2) [19].

Thyroglossal duct cyst enters in the differential diagnosis of thyroid cystic lesions when an aspirate consists predominantly of proteinaceous material, inflammatory cells, and rare degenerated squamous or ciliated columnar cells (Fig. 3.17). The diagnosis can be suggested in the appropriate clinical presentation (anterior midline neck cyst, usually above the thyroid isthmus and below the hyoid bone, or rarely just lateral to midline). Occasional benign-appearing squamous cells may also result from squamous metaplasia associated with lesions such as lymphocytic thyroiditis and cystic papillary thyroid carcinoma.

Mature squamous cells and anucleated squames rarely predominate. If they do, the cyst may be indistinguishable from branchial cleft cyst [20]. A cellular sample comprised almost exclusively of mature, benign-appearing squamous cells has been associated with benign follow-up in the available but limited literature, and such cases may be reported as benign in the appropriate clinical setting (Fig. 3.17) [21, 22].

A not infrequently encountered cystic lesion is the parathyroid cyst, which may be clinically and cytologically mistaken for a thyroid cyst. Aspirated fluid from a parathyroid cyst, however, has a characteristic watery, clear gross appearance, is often acellular or hypocellular, and may show rare cohesive groups of small round cells with dark nuclei and scant cytoplasm, arranged in sheets or microfollicles (Fig. 3.18). The diagnosis of a parathyroid cyst can be confirmed by immunohistochemistry (positive staining for parathormone, GATA3, and chromogranin; negative for thyroglobulin and TTF-1) and/or demonstration of elevated parathormone levels in the cyst fluid [23, 24].

A cellular FND may prompt consideration of a follicular neoplasm, but high cellularity alone is not enough to merit the interpretation "Follicular neoplasm (FN)." Follicular cell crowding and overlapping, nuclear enlargement, and syncytial/microfollicle formation affecting a majority of the follicular cell population are the important diagnostic features of FN [25]. The mere presence of microfollicular

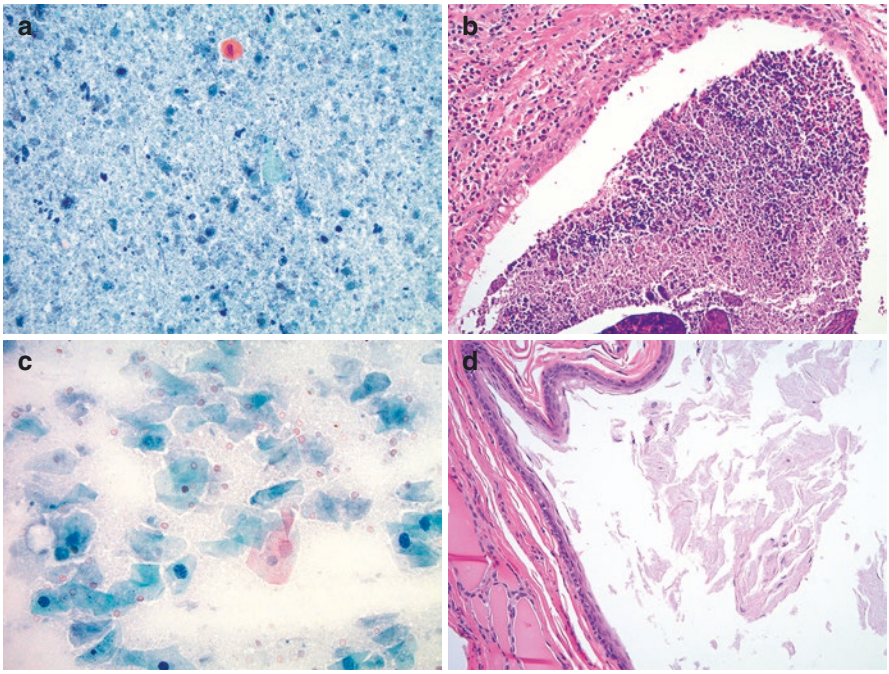

Fig. 3.17 Mature squamous cells in thyroid aspirates. (**a**, **b**) *Thyroglossal duct cyst*. (**a**) Proteinaceous material, inflammatory cells, and a rare degenerated squamous cell are present (smear, Papanicolaou stain). (**b**) The corresponding histopathologic specimen shows cyst contents (as observed in the fine needle aspirate) and a cyst wall lined by mixed squamous and cuboidal/columnar epithelium (hematoxylin and eosin stain). (**c**, **d**) *Benign squamous-lined cyst of thyroid*. (**c**) The cellular aspirate consists entirely of benign-appearing, mature nucleated and anucleated squamous cells (smear, Papanicolaou stain). (**d**) Histologic follow-up demonstrated a simple intrathyroidal squamous-lined cyst with contents similar to those observed in the aspirate (H&E stain)

structures is not equated with neoplasia or Atypia of Undetermined Significance (AUS). Some FND contain a minor component of microfollicles, but these tend to show no significant nuclear enlargement or nuclear overlapping and crowding. When microfollicles comprise a minority of the sample and are accompanied by a predominance of macrofollicle fragments, the sample is interpreted as "benign." Macrofollicle fragments range in size from small to large; a small fragment of benign-appearing follicular cells should not be misconstrued as a neoplastic microfollicle (Fig. 3.9b). Occasionally, pseudo-microfollicular architecture and/or pseudo-complexity of follicular cells are seen as a result of fibrin clot distortion in a bloody specimen (Fig. 3.10a); it's best to render a diagnosis of "nondiagnostic" rather than AUS or FN if this pattern predominates a specimen [26]. Based on limited literature, spherules, defined as small follicles with rounded smooth ball-like contours, have been reported to be exclusively associated with benign nodules [27]. The even spacing of nuclei within the spherule distinguishes it from neoplastic microfollicles (Fig. 3.5c).

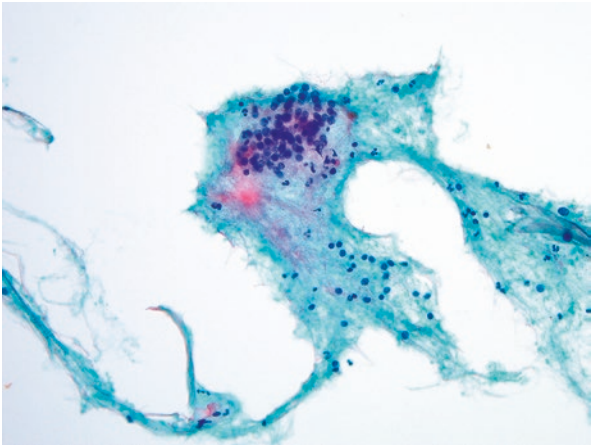

Fig. 3.18 Parathyroid cyst. This sparsely cellular specimen has rare groups of small round cells with dark overlapping nuclei and scant cytoplasm, suggestive of follicle formation (smear, Papanicolaou stain)

Papillary hyperplasia is defined histologically as a benign proliferation (either associated with HN or adenoma) notable for the arrangement of follicular cells, usually in a single layer, around fibrovascular cores. Fortunately, papillary hyperplasia in the form of true papillae (defined as having fibrovascular cores) is rarely encountered in aspirates, but when it is, it can be a diagnostic challenge [28]. More commonly, one sees large fragments of follicular cells associated with stromal tissue that only raises the question of a fibrovascular core. If there are no nuclear features of PTC, the case can be reported as benign (Fig. 3.11).

Oncocytic (Hürthle) cell predominance per se should not prompt the interpretation of an "oncocytic follicular neoplasm (OFN)." A minor population of oncocytic cells is a common finding in FND. Not uncommonly, oncocytic cells can be a prominent or even the predominant component of FND and in some cases there can be focal pronounced anisonucleosis and large-cell dysplasia (large cells with at least two times variability in nuclear size, and typically demonstrating hyperchromasia) of the oncocytic cells (Fig. 3.6). The interpretation of OFN should only be considered in hypercellular aspirates that consist exclusively (or almost exclusively) of oncocytic cells (see Chap. 6) [29]. An aspirate with a significant amount of colloid and a predominant uniform oncocytic cell population is consistent with FND in the appropriate clinical setting [29]. A recent study proposed a combination of four risk factors that was associated with neoplasm in 91% of cases (high cellularity, diffuse anisonucleosis, absent colloid, nodule size ≥2.9 cm) whereas the absence of four or three of those risk factors excluded neoplasia and malignancy in all sampled nodules (Fig. 3.6) [30]. Furthermore, large-cell dysplasia and transgressing blood vessels were found to be nonsignificant factors in discriminating non-neoplastic disease from neoplasm and malignancy [30].

It is important to evaluate colloid-rich aspirates for the presence of nuclear features of papillary carcinoma so as not to miss a macrofollicular variant of papillary carcinoma. This variant often presents with flat sheets without significant nuclear overlapping, resembling benign thyroid follicular cells on low magnification. Occasionally, an aspirate with the features of a FND will contain a subpopulation of cells with reparative changes, and it is important not to confuse those changes with those of papillary carcinoma (Fig. 3.14). When nuclear atypia (e.g., pallor, irregularity) goes

beyond what is accepted for reactive/reparative changes, such cases are interpreted as "Atypia of Undetermined Significance (AUS)" or "Suspicious for Malignancy," depending on the extent and degree of the atypia (see Chaps. 4 and 7).

"Black thyroid" is a benign pigmentation of thyroid follicular cells in patients on chronic treatment with antibiotics of the tetracycline family (e.g., minocycline) for conditions like acne. Follicular cells show abundant dark brown cytoplasmic pigment. It is darker than hemosiderin and likely represents a form of melanin as it stains with the Fontana-Masson stain (Fig. 3.19) [31, 32].

Amyloid goiter is a rare pathologic entity defined as a clinically apparent thyroid enlargement due to amyloid deposition. It is associated with both primary and secondary amyloidosis and results in a diffuse/bilateral involvement of the thyroid gland. Many patients present with symptoms of compression such as hoarseness, dysphagia, and dyspnea. FNA reveals abundant purple, pink/orange, or green amorphous material morphologically similar to colloid but recognizable due to the presence of embedded fibroblasts (Fig. 3.20) [33]. Focal amyloid deposits are also seen in medullary thyroid carcinoma.

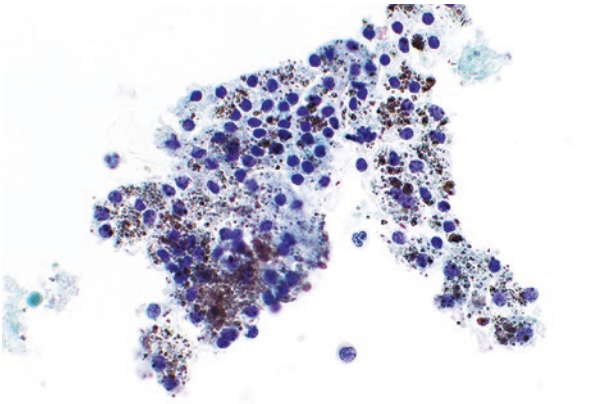

Fig. 3.19 Black thyroid. Follicular cells contain abundant dark brown pigment. Contrast with hemosiderin pigment in Fig. 3.7b (ThinPrep, Papanicolaou stain)

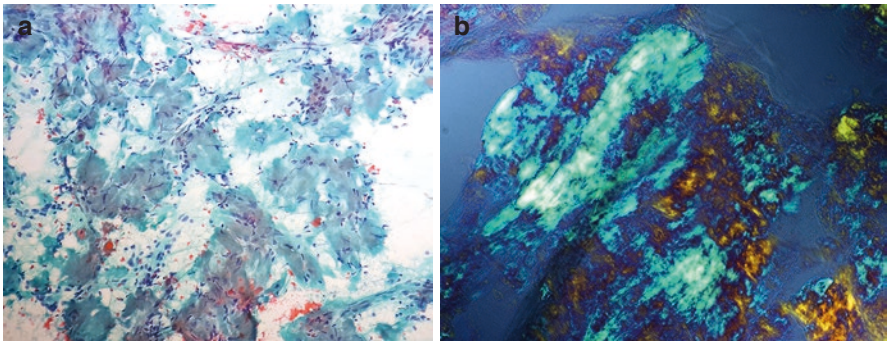

Fig. 3.20 Amyloid goiter. (**a**) Aspiration of abundant thick, glassy, amorphous material that stains green, pink/orange, or purplish (depending on the stain used) is observed. Amyloid deposits are mostly parenchymal and hence often display embedded fibroblasts, a characteristic feature (smear, Papanicolaou stain) (photograph is courtesy of Shipra Agarwal, MD, Department of Pathology, AIIMS, New Delhi, India). (**b**) A Congo red stain shows characteristic green birefringence upon polarization, confirming the diagnosis (cell block)

Graves' Disease

Graves' disease (GD) is an autoimmune diffuse hyperplastic thyroid disorder, commonly seen in middle-aged women and usually diagnosed clinically due to hyperthyroidism. Most patients have a diffuse rather than nodular enlargement of the thyroid gland and do not require FNA for diagnosis [34]. Occasionally, however, large and/or radiographically cold nodules develop that raise the suspicion of a coexisting malignancy and thus prompt FNA. The cytologic features of GD are nonspecific, and clinical correlation is needed for a definitive diagnosis. Aspirates are often cellular and show similar features to non-Graves' FND, including abundant colloid and a variable number of follicular cells. Occasionally, lymphocytes and oncocytes may be seen in the background.

Follicular cells are arranged in flat sheets and loosely cohesive groups, with abundant delicate, foamy cytoplasm (Figs. 3.21 and 3.22) [35]. Nuclei are often enlarged, vesicular, and show prominent nucleoli. Few microfollicles may be observed. Distinctive "flame cells" may be prominent and are represented by marginal

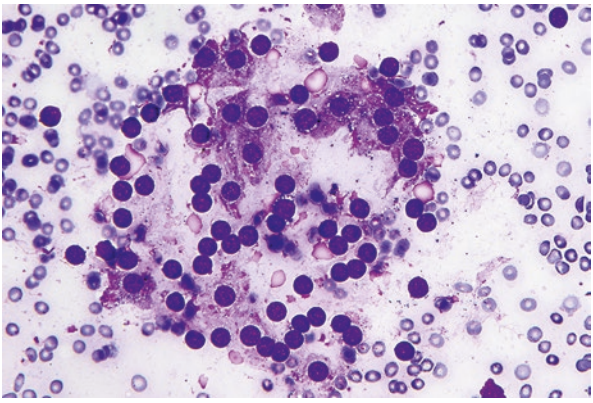

Fig. 3.21 Follicular nodular disease (patient with Graves' disease). Cells in monolayered sheets have abundant cytoplasm. Flame cells are distinctive for their marginal cytoplasmic vacuoles with red to pink frayed edges (smear, Diff-Quik stain)

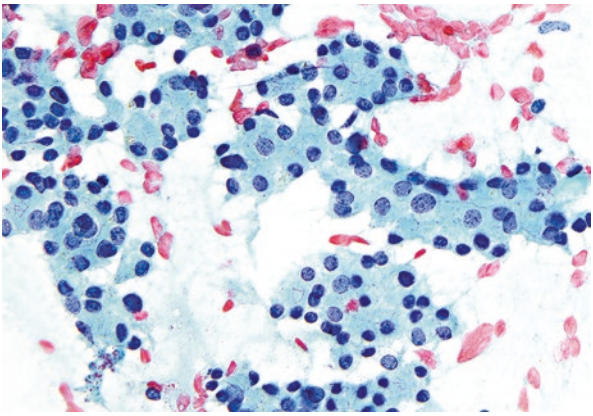

Fig. 3.22 Follicular nodular disease (patient with Graves' disease). The nuclei are often enlarged, vesicular, and show prominent nucleoli. Anisonucleosis is prominent. The cytoplasm has a granular, "oncocytoid" appearance (smear, Papanicolaou stain)

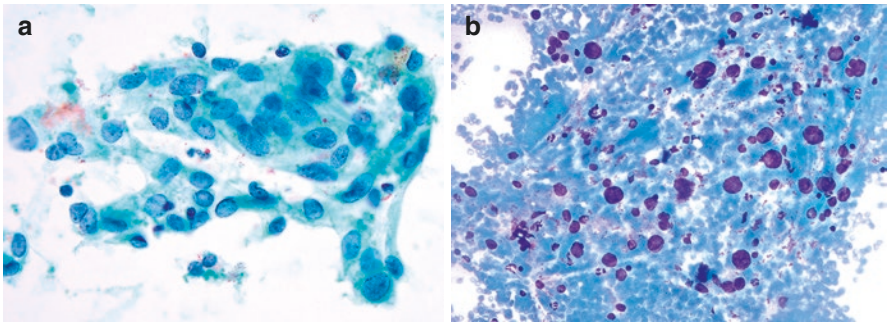

Fig. 3.23 Follicular nodular disease (patients with Graves' disease). (**a**) The follicular cells may display focal nuclear chromatin clearing and rare grooves. These changes are rarely diffuse, and other diagnostic nuclear features of papillary thyroid carcinoma are absent (smear, Papanicolaou stain). (**b**) There may be marked anisonucleosis associated with post-I^{131} therapy, so it's important to not overinterpret these changes as neoplastic or malignant in the appropriate clinical setting (smear, Diff-Quik stain)

cytoplasmic vacuoles with red to pink frayed edges (best appreciated with Romanowsky-type stains) (Fig. 3.21) [34, 36]. Flame cells, however, are not specific for GD and may be encountered in other non-neoplastic thyroid conditions, follicular neoplasms, and papillary carcinoma. Occasionally the follicular cells display focal chromatin clearing and rare intranuclear grooves (Fig. 3.23a). These changes are not diffuse, however, and other diagnostic nuclear features of papillary carcinoma are commonly absent [37]. Occasionally, treated GD shows prominent microfollicular architecture, significant nuclear overlapping and crowding, and considerable anisonucleosis. Care must be taken not to overinterpret these changes as malignant or neoplastic, and inquiry should be made regarding prior radioactive iodine therapy (Fig. 3.23b) [38, 39]. Lymphocytes are usually not prominent in GD, but in some cases, they may be present in significant numbers and mimic lymphocytic thyroiditis [36, 38].

Lymphocytic Thyroiditis

Background

Lymphocytic thyroiditis is a generalized terminology that encompasses a variety of conditions, including chronic lymphocytic (Hashimoto) thyroiditis, subacute lymphocytic thyroiditis (postpartum and silent thyroiditis), and focal lymphocytic (silent) thyroiditis [40]. Lymphocytic infiltrates may also be associated with Graves' disease, FND, and IgG4-related thyroiditis.

Chronic lymphocytic thyroiditis (CLT), synonymous with Hashimoto thyroiditis, is the most common of these conditions and typically affects middle-aged women but is also seen in both genders of any age. Patients often develop diffuse thyroid enlargement but only become candidates for FNA when they develop nodularity or an increasing thyroid volume which would raise the suspicion for

thyroid lymphoma. It is usually associated with circulating antithyroglobulin and antithyroid peroxidase (antimicrosomal) antibodies. Histologically, CLT shows diffuse infiltration of the thyroid gland by lymphoplasmacytic infiltrates, lymphoid follicles, oncocytic metaplasia, and variable fibrosis and atrophy.

Other types of autoimmune thyroiditis show an identical histologic appearance in a focal or diffuse pattern. Subtyping of lymphocytic thyroiditis by cytology, e.g., as CLT, requires clinical and serologic correlation.

Definition

The designation "lymphocytic thyroiditis" applies to a cytologic sample composed of many polymorphic lymphoid cells associated with benign thyroid follicular cells and/or oncocytic cells [41].

Criteria

Specimens are usually hypercellular, but advanced fibrosis or dilution with blood may decrease the apparent cellularity. An interpretation of lymphocytic thyroiditis does not require a minimum number of follicular or oncocytic cells for adequacy [19].

Oncocytic cells, when present, are arranged in flat sheets or as isolated cells. They have abundant granular cytoplasm, large nuclei, and prominent nucleoli (Figs. 3.24, 3.25, and 3.26).

Anisonucleosis of oncocytes may be prominent. Sometimes mild nuclear atypia is encountered, including scattered nuclear clearing and grooves (Fig. 3.25).

The lymphoid population is polymorphic, including small mature lymphocytes, larger reactive lymphoid cells, and occasional plasma cells. The lymphoid cells may be in the background or infiltrating epithelial cell groups (Fig. 3.26a).

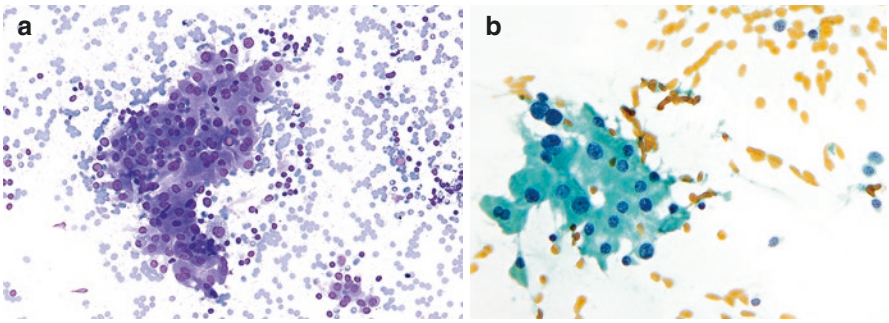

Fig. 3.24 Lymphocytic thyroiditis. (**a**) There is a mixed population of oncocytes and polymorphic lymphocytes (smear, Diff-Quik stain). (**b**) Oncocytic cells have abundant granular cytoplasm, large nuclei, and prominent nucleoli. There is mild anisonucleosis (smear, Papanicolaou stain)

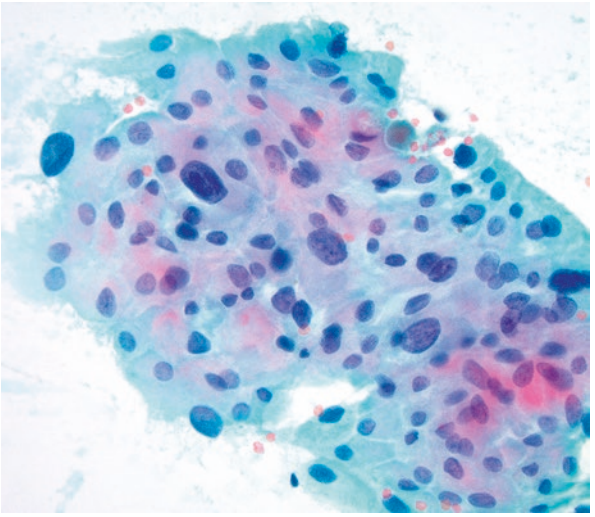

Fig. 3.25 Lymphocytic thyroiditis (LT). Focal large-cell dysplasia and prominent anisonucleosis of oncocytes are not uncommonly associated with LT (smear, Papanicolaou stain). If this appearance predominates in a specimen, however, a neoplasm may be suspected

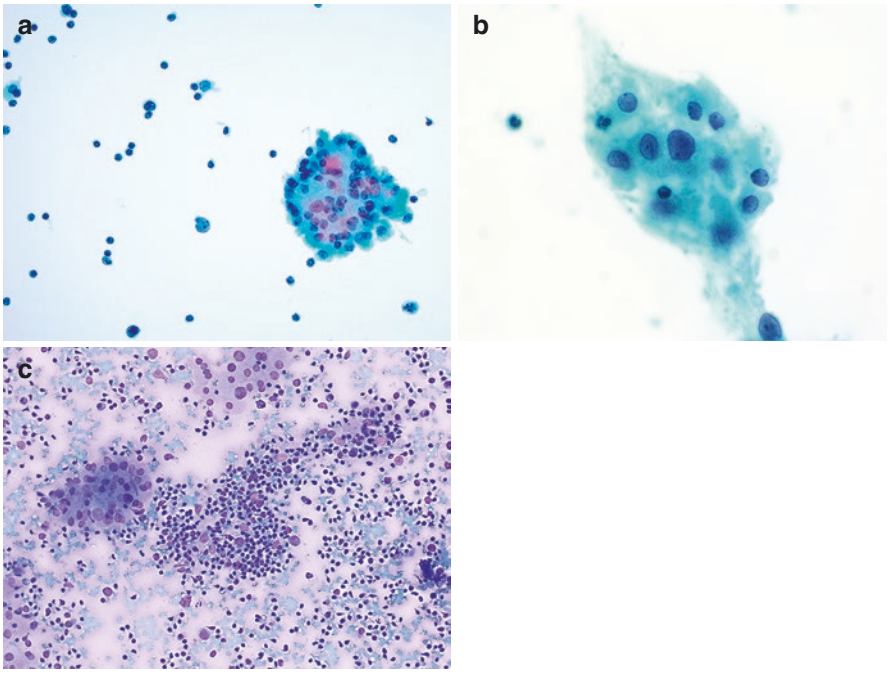

Fig. 3.26 Lymphocytic thyroiditis. (**a**) Lymphocytes are dispersed as isolated cells and infiltrate clusters of oncocytic cells (ThinPrep, Papanicolaou stain). (**b**) Oncocytes have abundant granular cytoplasm and prominent nucleoli (SurePath, Papanicolaou stain). (**c**) Germinal center fragments may be present, comprised of a heterogeneous mix of polymorphic lymphocytes and larger dendritic cells (smear, Diff-Quik stain)

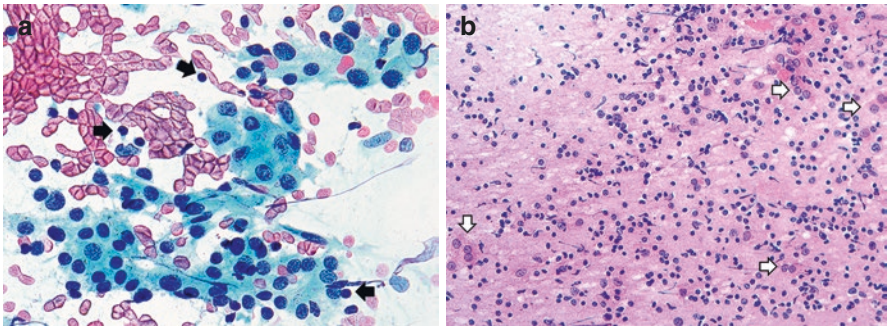

Fig. 3.27 Lymphocytic thyroiditis. (**a**) Oncocytic cells may predominate in any given sample, raising the possibility of an oncocytic neoplasm. Rare lymphocytes are present in the background (arrows) (smear, Papanicolaou stain). (**b**) Lymphoid cells may predominate in an aspirate, raising the possibility of lymphoma. Rare oncocytes are seen in the background (arrows) (smear, H&E stain)

Intact lymphoid follicles and lymphohistiocytic aggregates may be seen (Fig. 3.26c).

Oncocytic cells or lymphocytes may predominate in any given aspirate, raising the possibility of an oncocytic neoplasm or lymphoproliferative disorder, respectively (Fig. 3.27).

Granulomatous (de Quervain) Thyroiditis

Granulomatous (de Quervain) thyroiditis is a self-limited inflammatory condition of the thyroid that is usually diagnosed clinically and believed to be triggered by a viral infection, with influenza, adenovirus, and coxsackievirus being the most common triggers. FNA is generally performed only if there is nodularity that raises the possibility of a co-existing malignancy. In the absence of granulomas, the cytologic findings are nonspecific. The biopsy procedure, however, may be quite painful for the patient, preventing adequate sampling.

Criteria

The cellularity is variable and depends on the stage of disease.

Granulomas (clusters of epithelioid histiocytes) are present (Fig. 3.28), along with many multinucleated giant cells.

The early stage demonstrates many neutrophils and eosinophils, similar to acute thyroiditis.

In later stages the smears are hypocellular. They show giant cells surrounding and engulfing colloid, epithelioid cells, lymphocytes, macrophages, and scant degenerated follicular cells [42].

In the involutional stage, giant cells and inflammatory cells may be absent; some specimens may be insufficient for evaluation.

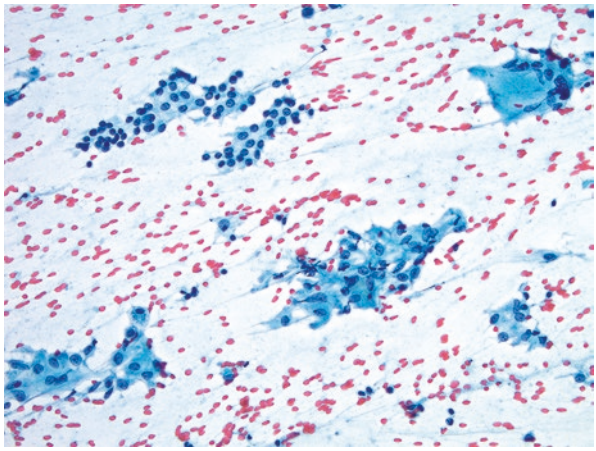

Fig. 3.28 Granulomatous thyroiditis. Loose granulomas, a multinucleated giant cell (right upper corner), mixed inflammatory cells, and benign thyroid follicular cells (left upper corner) are present (smear, Papanicolaou stain)

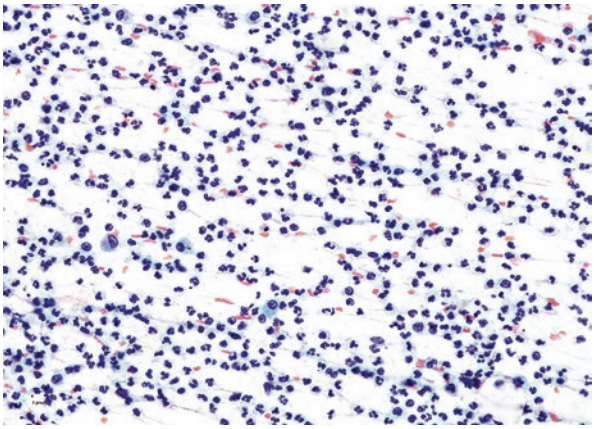

Fig. 3.29 Acute suppurative thyroiditis. There are numerous neutrophils and occasional macrophages (smear, Papanicolaou stain)

Acute Suppurative Thyroiditis

Acute suppurative thyroiditis (AST) is a rare but potentially fatal infectious condition of the thyroid, more commonly seen in immunocompromised patients or children with pyriform sinus fistula [43]. Other causes include generalized sepsis or rarely recent trauma or FNA. Bacterial infections are most common, but fungal infections are associated with the highest mortality. Patients most often present with neck pain and fever.

Criteria

Numerous neutrophils are associated with necrosis, fibrin, macrophages, and blood (Fig. 3.29).

There are scant reactive follicular cells and limited to absent colloid.

Bacterial or fungal organisms are occasionally seen in the background.

FNA and triage for cultures and special stains for organisms is the most definitive method to diagnose AST and manage accordingly.

Riedel Thyroiditis/Disease

This is the rarest form of thyroiditis and results in progressive fibrosis of the thyroid gland with extension into the soft tissues of the neck. Riedel thyroiditis (RT) is believed to be a manifestation of systemic IgG4 related disease in the thyroid, and one-third of patients develop fibrosing disorders in other organs [44]. A hard, fixed thyroid mass may clinically simulate anaplastic thyroid carcinoma and lymphoma.

Criteria

The thyroid gland feels very firm on palpation.
The preparations are often acellular.
Collagen strands and bland spindle cells may be present (Fig. 3.30).
There are rare chronic inflammatory cells.
Colloid and follicular cells are usually absent.

Explanatory Notes

Chronic lymphocytic (Hashimoto) thyroiditis (CLT), granulomatous (de Quervain) thyroiditis, and subacute lymphocytic thyroiditis are the most common clinically significant types of thyroiditis. "Lymphocytic thyroiditis" (LT) is a general term applied to chronic inflammation of the thyroid, but most cases represent autoimmune thyroiditis. Autoimmune thyroiditis includes CLT and subacute lymphocytic thyroiditis. Cytology cannot distinguish between the various subtypes of autoimmune thyroiditis.

CLT is the most frequently encountered autoimmune thyroiditis and globally the most common cause of hypothyroidism where iodine levels are sufficient [40].

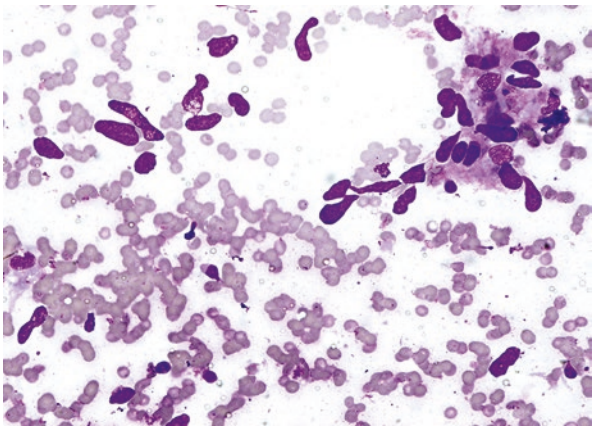

Fig. 3.30 Riedel thyroiditis/disease. This hypocellular smear contains scattered bland spindle cells and rare chronic inflammatory cells (smear, Diff-Quik stain)

Patients usually present with diffuse symmetric enlargement of the thyroid, but occasionally enlargement is localized and raises the suspicion of a neoplasm. CLT/Hashimoto thyroiditis had been characterized for many years as a well-defined clinicopathologic entity but now is considered a heterogeneous disease. IgG4-related thyroiditis is a new subtype of CLT characterized by inflammation rich in plasma cells with an increased ratio of IgG4 to IgG and marked fibrosis [45, 46]. A significant portion of cases of fibrosing Hashimoto thyroiditis and a minority of classic Hashimoto thyroiditis are now believed to belong to the spectrum of IgG4-related disease. Histologic features, however, remain the gold standard for establishing the diagnosis of IgG4-related disease, as cytology cannot render a specific diagnosis, and elevated IgG4 plasma cells have been described in other inflammatory and malignant conditions. Unlike most other IgG4-related diseases, IgG4-related thyroiditis appears to be mostly confined to the thyroid and lacks systemic manifestations.

Subacute lymphocytic thyroiditis is often referred to as painless thyroiditis, and patients can present with nodular enlargement. A similar process occurs in the postpartum period in up to 5% of women (postpartum thyroiditis) [40]. Most subacute lymphocytic thyroiditis patients have circulating antithyroid peroxidase antibodies or a family history of other autoimmune disorders.

Recent but limited literature, mostly case reports, has associated subacute and granulomatous thyroiditis with COVID-19 infection [47]. Complete remission of thyroiditis was recorded in most of these patients following steroid therapy.

In some patients with LT, the predominance of either the lymphoid or the oncocytic cell component may raise the possibility of lymphoma or oncocytic neoplasm, respectively (Fig. 3.27) [48, 49]. A monomorphic lymphoid population should raise the suspicion of lymphoma and prompt additional samples for flow cytometry to confirm the diagnosis (see Chap. 12). A polymorphic population of reactive lymphocytes raises the differential diagnosis of LT and intra- or peri-thyroid lymph node hyperplasia, but these can often be distinguished by their differing sonographic features. In pediatric patients, a population of small mature lymphocytes may represent intrathyroidal thymic tissue masquerading as a neoplasm, and immunohistochemistry and/or flow cytometry can be applied to confirm the clinical diagnosis and potentially avoid unnecessary surgery [50]. The diagnosis of AUS or "OFN" could be considered in cases with predominant oncocytic cell population and associated sparse or absent lymphocytic infiltrate (see Chap. 6). Nodule size may be a consideration, as the rate of malignancy appears to be lower in oncocytic nodules measuring less than 2.9 cm as compared to those equal or greater than 2.9 cm in size [30, 51]. The follicular or oncocytic cells occasionally demonstrate focal reactive changes and mild atypia, including nuclear enlargement, grooves, and chromatin clearing [48]. Therefore, the diagnostic threshold for papillary carcinoma should be raised slightly if there is cytomorphologic evidence of lymphocytic thyroiditis. In some cases, the features will be equivocal, in which case a diagnosis of AUS or "Suspicious for Malignancy" should be considered, depending on how well developed the nuclear changes of papillary carcinoma are. At times, stripped follicular cell nuclei of a FND may be misinterpreted as lymphocytes (Fig. 3.10b); care must be taken to identify the thin rim of cytoplasm surrounding true lymphocytes in order to avoid a misdiagnosis of LT.

The diagnosis of LT on liquid-based preparations can be challenging, as the chronic inflammatory background may be decreased or absent [16, 17, 52]. Because the lymphoid cells tend to be evenly dispersed in the background with liquid-based preparations, they are easy to overlook at low magnification. Liquid-based preparations, which are designed to eliminate red blood cells, are relatively enriched for white blood cells; therefore, care must be taken not to overinterpret the normal lymphocytes of blood as indicative of LT. If the lymphoid cells are present in the normal proportion to neutrophils of peripheral blood, then the lymphoid cells are merely blood elements. In LT, there will be a marked increase in the proportion of lymphoid cells to other inflammatory cells, sometimes accompanied by germinal center fragments. With liquid-based preparations, oncocytic cells occasionally have irregular nuclei.

The cytologic findings are often nonspecific in acute, subacute, and RT, and in some cases, there may be an overlap with LT [41, 53]. In the presence of granulomas, other causes of granulomatous inflammation besides granulomatous thyroiditis (de Quervain) should be considered, including sarcoidosis and infection. Careful examination should be undertaken to exclude the possibility of an associated malignancy such as a sclerosing lymphoma or fibrosing anaplastic carcinoma.

Management

The risk of cancer for cytologically benign thyroid nodules is difficult to assess because only a minority of nodules with benign cytology (approximately 10%) undergo surgery [54]. A reliable false-negative rate can only be calculated if all patients undergo surgery (the "gold standard") regardless of their FNA result; this is neither practical nor feasible, however. Most published studies have confirmed that a benign FNA diagnosis is associated with a very low false-negative rate, estimated to be in the range of less than 2–3% [55–61].

The 2015 American Thyroid Association (ATA) guidelines for the management of thyroid nodules strongly recommend that no further immediate diagnostic studies or treatment are required for benign cytology [7]. It is apparent from published literature and the ATA management guidelines that repeat FNA and/or surgery is considered only for a selected subset of thyroid nodules with benign cytology, including those that are large, symptomatic, have worrisome clinical and/or sonographic characteristics, including significant ultrasound (US) nodule growth (20% increase in at least two dimensions with a minimal increase of 2 mm or more than a 50% change in volume) or developing US abnormalities, such as irregular margins, microcalcifications, intra-nodular hypervascularity, and hypoechogenicity in solid areas [7]. Given the very low risk of malignancy associated with benign thyroid cytology, the ATA recommends that follow-up should be determined by risk stratification based on US pattern:

1. *Nodules with high suspicion US pattern:* repeat US and US-guided FNA within 12 months.
2. *Nodules with low to intermediate suspicion US pattern:* repeat US at 12–24 months. If there is evidence of growth or development of new suspicious sonographic features, the FNA could be repeated or observation continued with repeat US, with repeat FNA in case of continued growth.

3. *Nodules with very low suspicion US pattern*: the utility of surveillance US is limited. If US is repeated, it should be done at >24 months.

If a nodule has undergone repeat US-guided FNA with a second benign cytology result, US surveillance for this nodule is no longer indicated [7].

Sample Reports

If an aspirate is interpreted as Benign, it is implied that the sample is adequate for evaluation (an explicit statement of adequacy is optional). Descriptive comments that follow are used to subclassify the benign interpretation (see Examples 3.1–3.10 below). An educational note specifying the risk of malignancy for this interpretation, derived from the experience of the laboratory itself or from the literature, is optional.

Example 1
BENIGN.
Follicular nodular disease.

Example 2
BENIGN.
Benign-appearing follicular cells, colloid, and occasional oncocytic cells, consistent with follicular nodular disease.

Example 3
BENIGN.
Abundant colloid and rare follicular cells, consistent with follicular nodular disease (colloid nodule).

Example 4 (Clinical history of nodular goiter)
BENIGN.
Follicular nodular disease, consistent with nodular hyperplasia.

Example 5
BENIGN.
Numerous oncocytes and colloid, consistent with follicular nodular disease.

Example 6 (Clinical history not provided)
BENIGN.
Lymphocytic thyroiditis.

Example 7 (Clinical history not provided)
BENIGN.
Lymphocytes and benign follicular cells, consistent with lymphocytic thyroiditis.

Example 8 (Clinical history of Hashimoto thyroiditis provided)
BENIGN.
Consistent with chronic lymphocytic (Hashimoto) thyroiditis.

Example 9 (Not known if the patient has Hashimoto thyroiditis)
BENIGN.
Numerous polymorphic lymphoid cells and scattered oncocytic cells.
Note: The findings are suggestive of chronic lymphocytic (Hashimoto) thyroiditis in the proper clinical setting.

Example 10
BENIGN.
Proteinaceous material, macrophages, and rare benign-appearing but poorly preserved squamous cells.
Note: The findings are consistent with a benign developmental cyst such as a thyroglossal duct cyst. Clinical correlation advised.

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Atypia of Undetermined Significance

4

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Background

The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) defined and distinguished three different patterns of the so-called "indeterminate" aspirate, each with distinct cytologic features and follow-up risk of malignancy. Aspirates with

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cytologic features that are "Suspicious for Malignancy" (SFM) (see Chap. 7) have a higher risk of malignancy (ROM) than those classified as "Follicular Neoplasm" (FN) or "Oncocytic Follicular Neoplasm" (OFN) (see Chaps. 5 and 6). The Atypia of Undetermined Significance (AUS) category is reserved for cases with a lesser degree of atypia, nuclear and/or other in nature, which is insufficient to qualify for either the FN/OFN or SFM categories. AUS cases have an overall lower ROM, warranting separation from the other two indeterminate categories [1].

AUS has been extensively studied since the advent of TBSRTC, but calculating the ROM associated with this interpretation remains challenging. Since only a minority of AUS cases undergo surgical resection, estimating the ROM based on histologic follow-up alone overestimates ROM due to selection bias: AUS nodules are usually resected if they have worrisome clinical or sonographic features, an abnormal repeat aspiration result, and/or an abnormal molecular testing result. AUS nodules with a benign repeat aspiration and/or a benign molecular test result appropriately remain unresected. On the other hand, when ROM is calculated using the total number of AUS cases as the denominator, regardless of surgical follow-up, and assuming that unresected nodules are benign most certainly underestimates the ROM. The actual ROM is expected to be in-between the values obtained using these two different calculations and requires some extrapolation. There is evidence that the ROM of AUS has been further overestimated due to publication bias, since unexpected/discrepant results are more likely to be published than expected findings [2].

Despite these challenges, the overall low-risk nature of aspirates in this category has been borne out, and is clearly lower than that of the SFM category, but overlaps with the risks associated with the FN or OFN categories [3–5]. Follow-up studies since the introduction of TBSRTC and the AUS category demonstrate notable variability in the use of AUS [3–5]. The AUS interpretation is associated with a ROM that is higher (approximately 20–30%) than initially predicted (~5–15%) when TBSRTC was introduced in 2007. Furthermore, the risk differs according to the nature of the atypia prompting the AUS interpretation [6–14]. AUS aspirates with nuclear atypia (previously referred to as cytologic atypia in the second edition of this atlas) have an approximately twofold higher ROM compared with AUS cases with other types of atypia, including those with only architectural atypia [11, 12]. Oncocyte predominant AUS has a lower ROM than other AUS patterns [11, 12]. The introduction in 2016 of the terminology noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) altered these figures further [15]. Inclusion of NIFTP leads to a reduction in the ROM for AUS [16–21]. A recent meta-analysis indicates that AUS is the most frequent preoperative diagnosis for nodules that ultimately prove to be NIFTP (29.2% of all NIFTPs) and that NIFTP lowers the ROM for AUS by 8.2% [21]. Overall, NIFTP is estimated to reduce the ROM of an AUS diagnosis by 6–20% (see Chap. 1).

Definition

The diagnostic category "Atypia of Undetermined Significance" (AUS) is reserved for specimens that contain one or more of a heterogeneous group of findings that raise concern for neoplasm/malignancy but are insufficient to be classified as a

follicular neoplasm, suspicious for malignancy, or malignant. On the other hand, the findings are more marked than can be ascribed confidently to benign changes. Most frequently, AUS is due to atypia in follicular cells (typically nuclear and/or architectural in nature) or a predominance of oncocytic cells. Atypical lymphoid cells are a less common cause of AUS as is the finding of isolated psammoma bodies without accompanying atypical follicular cells.

Although follicular lesion of undetermined significance (FLUS) was previously considered an acceptable alternative for AUS, the inconsistent use of these two terms has been confusing, especially for subsequent management. To promote clarity and consistency, henceforth it is recommended that only the preferred AUS terminology should be used for this category.

The reproducibility of AUS remains at best only fair [22, 23]. In laboratories with very low AUS rates, the rates of FN and OFN are relatively elevated, suggesting that at least some cases that might have been placed in the AUS category are shifted into these categories [24–26]. Similarly, an inverse relationship often exists between use of AUS and the nondiagnostic category, indicating differing approaches to diagnostically limited material [25]. A multi-institutional review by board-certified practicing pathologists identified both cellular adequacy and Bethesda diagnosis as being significantly associated with the concordance rate [23]. High volume laboratories/pathologists with more experience in thyroid cytopathology are likely to be more comfortable calling an aspirate SFM or outright positive rather than AUS.

The criteria for using the AUS designation have been previously simplified to promote greater reproducibility [27]. At the same time, use of additional language to describe the nature of the atypia in the cytopathology report has been strongly encouraged [27]. The frequency and outcomes of the previously described subtypes of AUS have been reported as well as associated molecular findings [6–14, 28–36]. Overall, nuclear atypia accounts for 32% of AUS in these studies, architectural atypia for 41%, oncocytic atypia for 17%, and other types for 10%.

To further simplify subclassification while reflecting clinical risk and subsequent management, AUS diagnoses are now subclassified into one of two broad subcategories in this update: AUS with nuclear atypia that raises a low level of concern for papillary carcinoma or NIFTP (“AUS with nuclear atypia”) and that in which other (non-nuclear) features result in an AUS interpretation (“AUS—Other”).

Criteria

The heterogeneity of this category precludes describing all scenarios for which an AUS interpretation is appropriate. The most common situations, however, are outlined here. Subclassification of AUS aspirates is recommended to enable enhanced communication with other pathologists and clinical providers and to facilitate further refinement of the category as new information becomes available and new entities (like NIFTP) are defined. The use of descriptive qualifying language (e.g., “nuclear atypia” rather than “rule out papillary carcinoma”) is preferred since it

causes less concern for both physicians and patients and helps avoid overtreatment. Such descriptive terminology is therefore used exclusively throughout the following discussion and in the "Sample Reports" section. An aspirate with mild nuclear atypia that raises the possibility of papillary carcinoma, but is insufficient to warrant a SFM designation, poses a higher ROM than other patterns of AUS. Accordingly, it is recommended that AUS diagnoses be broadly subcategorized to indicate the presence or absence of such nuclear atypia and the scenarios outlined below are organized in this manner. Subclassification in this fashion is useful in guiding management.

It is also important to consider the adequacy of the specimen and specify if it is scant or otherwise compromised by limiting factors, and not use the AUS category if *bona fide* "atypia" is not identified. Such aspirates are often better classified as nondiagnostic or benign. However, if there is atypia in a scant or suboptimal aspirate, including this information in the report further guides management. For example, a repeat aspirate is more likely to be of benefit when the initial aspirate is scant or poorly preserved, whereas molecular testing may be preferred for follow-up of a cellular, well-preserved aspirate with diffuse mild nuclear atypia.

AUS with Nuclear Atypia

Focal Nuclear Atypia (Fig. 4.1)

Most of the aspirate appears benign but rare cells have nuclear enlargement, pale chromatin, and irregular nuclear contours, especially common in patients with lymphocytic (Hashimoto) thyroiditis. Intranuclear pseudoinclusions are typically absent. Rare pseudoinclusions by themselves should not prompt an AUS diagnosis; however, if they are accompanied by other compelling features of papillary carcinoma, the case should be considered suspicious for malignancy. Alternatively, a sample may be paucicellular and contain few cells as described above.

Extensive But Mild Nuclear Atypia (Fig. 4.2)

Many, if not most, cells have mildly enlarged nuclei with slightly pale chromatin and only limited nuclear contour irregularity. Intranuclear pseudoinclusions are typically absent.

Atypical Cyst Lining Cells (Fig. 4.3)

The cytomorphology of cyst lining cells has been well described, they are reparative follicular cells and/or mesenchymal cells, and the majority can be recognized as such and diagnosed as benign [36]. In rare cases, however, there is more atypia than usual, and it is appropriate to diagnose these as AUS. Cyst lining cells may appear atypical due to the presence of nuclear grooves, prominent nucleoli, elongated nuclei and pulled out cytoplasm, and/or rare intranuclear pseudoinclusions in an otherwise predominantly benign-appearing sample.

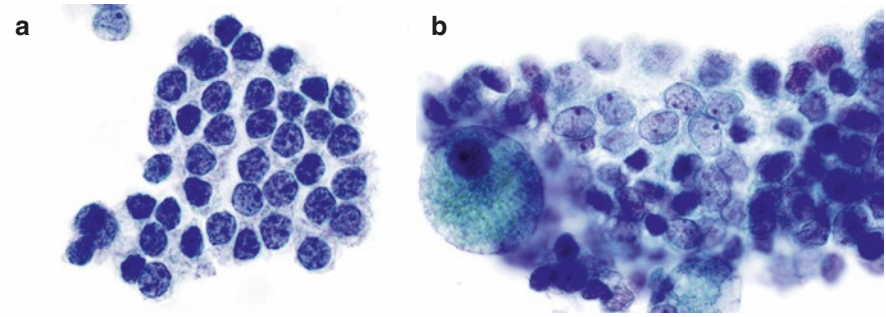

Fig. 4.1 Atypia of Undetermined Significance with nuclear atypia. (**a**) Most of the follicular cells are arranged in benign-appearing macrofollicle fragments. (**b**) Rare cells have pale nuclei and mildly irregular nuclear membranes. When such cells are very few in number, an atypical interpretation is more appropriate than "suspicious for malignancy" (ThinPrep, Papanicolaou stain)

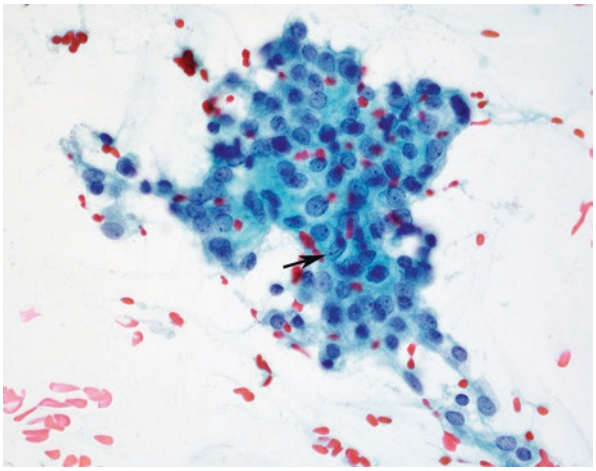

Fig. 4.2 Atypia of Undetermined Significance with nuclear atypia. Follicular cells show mild enlargement, small distinct nucleoli, and pale chromatin. Nuclear contours are uniform with only a rare nuclear groove (arrow). Molecular testing identified an *HRAS* mutation. Noninvasive follicular thyroid neoplasm with papillary-like nuclear features was diagnosed at lobectomy (smear, Papanicolaou stain). (Courtesy of Dr. Teresa Kim)

"Histiocytoid" Cells (Fig. 4.4)

These cells are often seen in cystic papillary carcinoma, which can be difficult to diagnose due to both sampling and interpretation issues [37–40]. Aspirates containing histiocytoid cells often have numerous histiocytes and few follicular cells. The atypical "histiocytoid" cells are larger than histiocytes, often isolated, but can be seen in a microfollicular arrangement or clusters. Compared with histiocytes, they usually have larger, rounder nuclei, a higher nuclear-to-cytoplasmic ratio, and "harder" (glassier) cytoplasm, without the hemosiderin or microvacuolization of histiocytes, although larger, more discrete, "septate" vacuoles can be seen. Epithelial

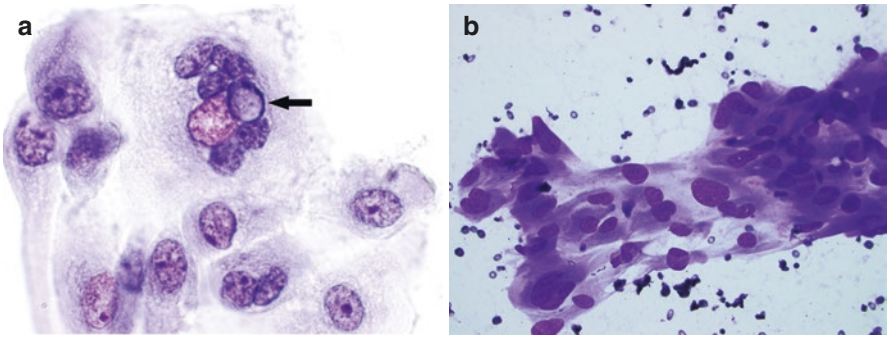

Fig. 4.3 Atypia of Undetermined Significance with nuclear atypia. (**a**) In this sparsely cellular specimen, some cells have abundant cytoplasm, enlarged nuclei, and prominent nucleoli. One cell has an apparent intranuclear pseudoinclusion (arrow). Such changes may represent atypical but benign cyst lining cells. However, a papillary carcinoma cannot be entirely excluded (ThinPrep, Papanicolaou stain). (**b**) Reparative-like changes of cyst lining cells can mimic some cytologic features of papillary carcinoma (smear, Romanowsky stain)

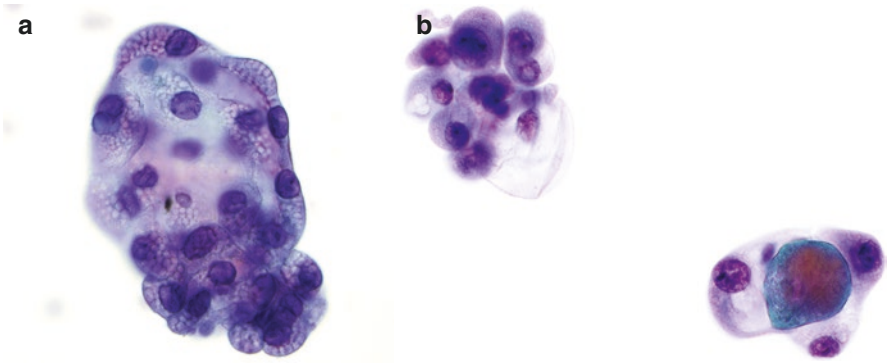

Fig. 4.4 Atypia of Undetermined Significance with nuclear atypia. (**a**) Cystic papillary carcinoma cells often show degenerative vacuoles; these cells have been termed "histiocytoid." A useful feature for recognizing them and distinguishing them from histiocytes is the sharply defined edges of the vacuoles, as opposed to the "fluffy" vacuoles of histiocytes (smear, Papanicolaou stain) (reproduced with permission from Ali SZ, Nayar R, Krane JF, and Westra WH. Atlas of Thyroid Cytopathology with Histopathologic Correlations, Demos Medical, New York, 2014). (**b**) In this example, a loose cluster and a microfollicular group exhibit both "hard" cytoplasm and large cytoplasmic vacuoles (ThinPrep, Papanicolaou stain)

(keratins) and histiocytic (CD68, CD163, PU.1) immunostains are potentially useful but often of limited value due to scant cellularity, unless a cell block has been made from the cyst fluid.

Nuclear and Architectural Atypia (Fig. 4.5)

Mild cytologic atypia as outlined above may coexist with architectural alterations, such as an increased presence of microfollicles or crowded three-dimensional

groups. Aspirates with both mild nuclear and architectural alterations are grouped with aspirates exhibiting only nuclear atypia since the ROM is similar regardless of the presence or absence of coexisting architectural atypia.

AUS-Other

Architectural Atypia (Figs. 4.6, 4.7, and 4.8)

1. A scantly cellular specimen with rare clusters of follicular cells, almost entirely in microfollicles or crowded three-dimensional groups and with scant colloid (Fig. 4.6). Although this pattern is low risk, AUS is warranted due to concern regarding limited sampling of a lesion that would merit an FN diagnosis if the specimen were more cellular. Sampling of an intrathyroidal parathyroid lesion may also present with this pattern and be difficult to separate from a thyroid follicular lesion based on morphology alone (Fig. 4.7).
2. A moderately to markedly cellular specimen exhibits architectural atypia as described above in most follicular cells (50–70% of follicular cells) but without a marked predominance (at least 70% of follicular cells) that would warrant a FN diagnosis. This pattern should not be confused with an overall mixed, but predominantly macrofollicular, aspirate, which should be called benign. *DICER1* mutated nodules may be associated with this pattern as they typically have architectural atypia with minimal-to-no nuclear atypia. This is especially true in pediatric samples where *DICER1* mutation is common in both multinodular goiter (MNG) and follicular neoplasms (Fig. 4.8) [41].

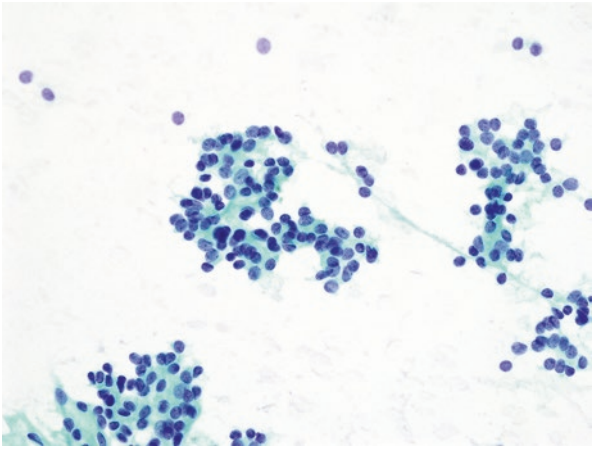

Fig. 4.5 Atypia of Undetermined Significance with nuclear and architectural atypia. Nuclear atypia is evident, with nuclear enlargement, crowding, and chromatin pallor, and infrequent nuclear grooves. Architectural atypia is manifested by a crowded three-dimensional configuration of follicular cells. The excised nodule was diagnosed as minimally invasive encapsulated follicular variant of papillary thyroid carcinoma (SurePath, Papanicolaou stain)

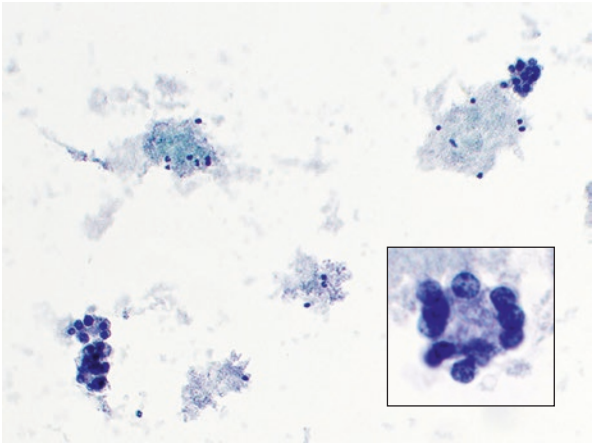

Fig. 4.6 Atypia of Undetermined Significance with architectural atypia. Scanning magnification reveals a sparsely cellular specimen with a predominance of microfollicles (Inset: high magnification of a microfollicle) (ThinPrep, Papanicolaou stain)

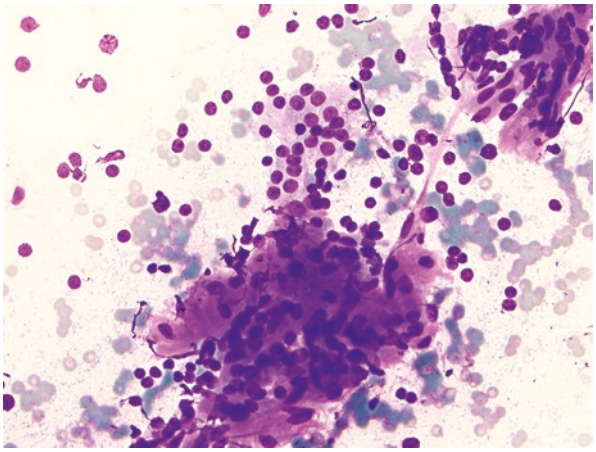

Fig. 4.7 Atypia of Undetermined Significance with architectural atypia. The smear shows cells arranged in a trabecular configuration with associated endothelial cells/blood vessels. Naked nuclei are prominent in the background and colloid is absent. This proved to be a parathyroid adenoma on resection (smear, Diff-Quik stain). (reproduced with permission from Ali SZ, Nayar R, Krane JF, and Westra WH. Atlas of Thyroid Cytopathology with Histopathologic Correlations, Demos Medical, New York, 2014)

3. Focally prominent microfollicles without nuclear atypia. A more prominent than usual population of microfollicles may be seen in a moderately or markedly cellular sample or in the clinical setting of MNG, but the overall proportion of microfollicles is not sufficient for a diagnosis of FN. This situation usually arises with direct smears and consists of a single FNA pass or a slide that looks different from the rest of the aspirate. This pattern also should not be confused with a mixed, but predominantly macrofollicular aspirate, more appropriately called benign.

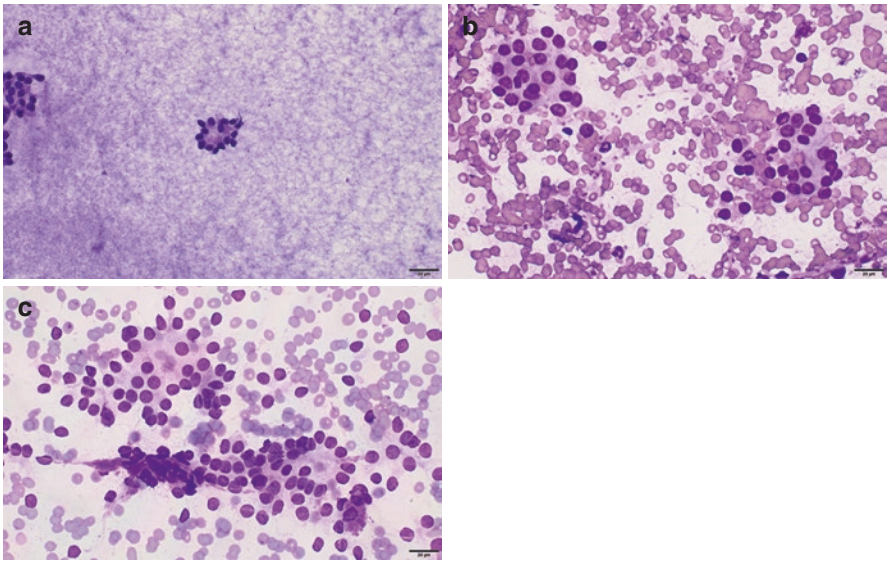

Fig. 4.8 Atypia of Undetermined Significance with architectural atypia. Three examples of *DICER1* mutated nodules in pediatric patients show variable cellularity and architectural atypia. Surgical resection identified a follicular adenoma (**a**), follicular carcinoma (**b**), and poorly differentiated carcinoma (**c**) (**a**: smears, hematoxylin and eosin stain; **b**, **c**: and Diff-Quik stain)

Oncocytic/Oncocyte Atypia (Figs. 4.9 and 4.10)

1. A sparsely cellular aspirate comprised exclusively or almost exclusively of oncocytic (previously termed Hürthle) cells with minimal colloid (Fig. 4.9). Although this pattern is very low risk, AUS is warranted due to concern for limited sampling of a lesion that would merit an OFN diagnosis if the specimen were highly cellular. Correlation with clinical/laboratory findings and radiologic risk stratification can be useful in determining the best diagnostic category.
2. A moderately or markedly cellular sample composed exclusively or almost exclusively of oncocytic cells (at least 70% of all follicular cells), in which the clinical setting suggests a benign oncocytic cell nodule, such as in lymphocytic (Hashimoto) thyroiditis or a multinodular goiter (MNG) (Fig. 4.10).
   (a) If the oncocytic cells are all in cohesive flat sheets without nuclear atypia and there is abundant colloid, a benign diagnosis is warranted in the absence of high-risk clinical or radiologic findings (see Chap. 6 for further discussion).
   (b) There may be clinical evidence of lymphocytic (Hashimoto) thyroiditis, but lymphocytes are absent (Fig. 4.10). Alternatively, a clinical diagnosis of Hashimoto thyroiditis has not been established, yet the presence of some lymphocytes (insufficient for a benign diagnosis) raises concern for

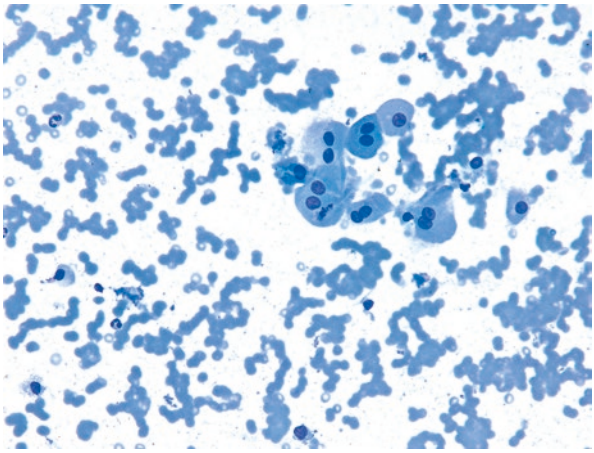

Fig. 4.9 Atypia of Undetermined Significance, oncocytic cell type. Sparsely cellular with abundant blood and predominantly oncocytic cells (smear, Diff-Quik stain)

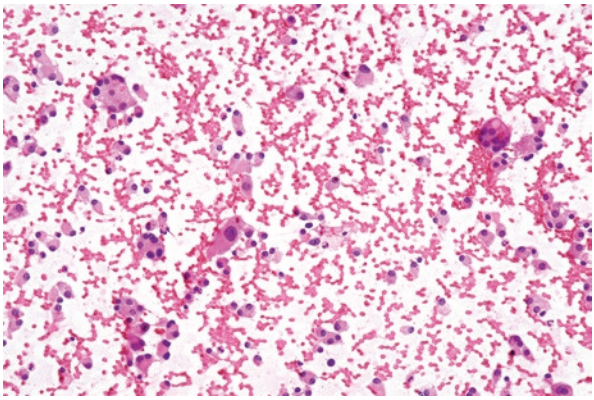

Fig. 4.10 Atypia of Undetermined Significance, oncocytic cell type (patient with history of Hashimoto thyroiditis). This patient had a subcentimeter nodule with modestly cellular smears showing exclusively oncocytic follicular cells without polymorphous lymphocytes in a bloody background (smear, hematoxylin and eosin stain)

Hashimoto thyroiditis. A repeat aspirate or additional clinical evaluation may resolve the diagnostic uncertainty.

(c) When multiple nodules in the same patient show features that would otherwise prompt a diagnosis of OFN, AUS may be preferred on the presumption that MNG with multiple hyperplastic oncocytic cell nodules and lymphocytic (Hashimoto) thyroiditis with oncocytic metaplasia are more probable than concurrent oncocytic type follicular neoplasms.

Atypia, Not Otherwise Specified (NOS) (Figs. 4.11, 4.12, and 4.13)

1. A minor population of follicular cells shows nuclear enlargement, often accompanied by prominent nucleoli (Figs. 4.11 and 4.12).

   This pattern of nuclear atypia does not raise concern for papillary carcinoma and is, therefore, best classified as NOS. Specimens from patients with a history of radioactive iodine, carbimazole, or other pharmaceutical agents can usually be diagnosed as benign, assuming that the appropriate clinical history is available, but AUS may be appropriate when the findings are particularly pronounced

or there is uncertainty regarding the clinical history. Similarly, metaplastic oncocytic cells may exhibit pronounced nuclear size variation, smudgy chromatin and/or nucleoli, especially in lymphocytic (Hashimoto) thyroiditis (Fig. 4.12). If not part of an aspirate that is comprised exclusively or almost exclusively of oncocytic cells, such findings should be considered benign and do not warrant an AUS classification.

2. Psammomatous calcifications in the absence of follicular cells with nuclear features of papillary carcinoma (Fig. 4.13).

   Psammoma bodies raise concern for papillary carcinoma and should prompt careful scrutiny of follicular cells to identify the nuclear features of papillary carcinoma. Free floating psammoma bodies may also be seen in cystic papillary carcinoma aspirates. However, when seen alone psammomatous calcifications should not be interpreted as SFM since there are a number of mimics, especially on radiology that are interpreted as worrisome "microcalcifications." "Lamellar bodies" of inspissated colloid may be indistinguishable from true psammomatous calcifications. In liquid-based preparations, small globules of thick colloid may display radial cracking, simulating psammoma bodies. The overall predictive value of psammoma bodies for papillary carcinoma is estimated to be about 50%, and in the absence of a concerning population of follicular cells, this finding is best classified as AUS [42].
3. Rare instances of atypia warranting an AUS designation not explicitly described elsewhere in this chapter.

Atypical Lymphoid Cells, Rule Out Lymphoma (Fig. 4.14)

There is an atypical lymphoid infiltrate for which a repeat aspirate for flow cytometry is desirable; however the degree of atypia is insufficient for the general category of "suspicious for malignancy." Besides lymphoma, other tumors such as thymic lesions may be in the differential diagnosis.

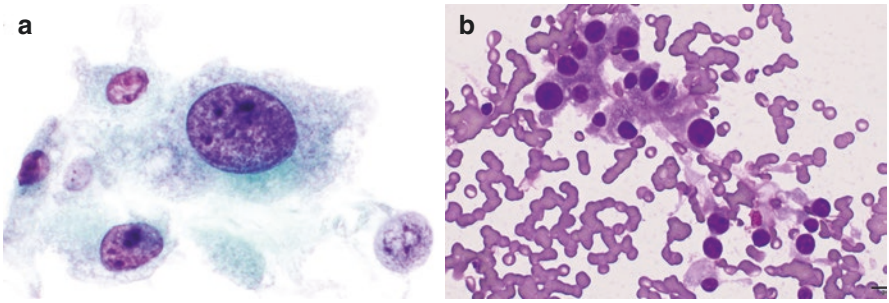

Fig. 4.11 Atypia of Undetermined Significance, not otherwise specified. The cytologic changes in these specimens do not raise concern for papillary carcinoma. (**a**) These follicular cells, in a patient with Graves' disease treated with methimazole (Tapazole®), show marked nuclear enlargement and anisonucleosis (ThinPrep, Papanicolaou stain). (**b**) These atypical follicular cells were obtained from a patient with a history of ionizing radiation to the neck (smear, Diff-Quik stain)

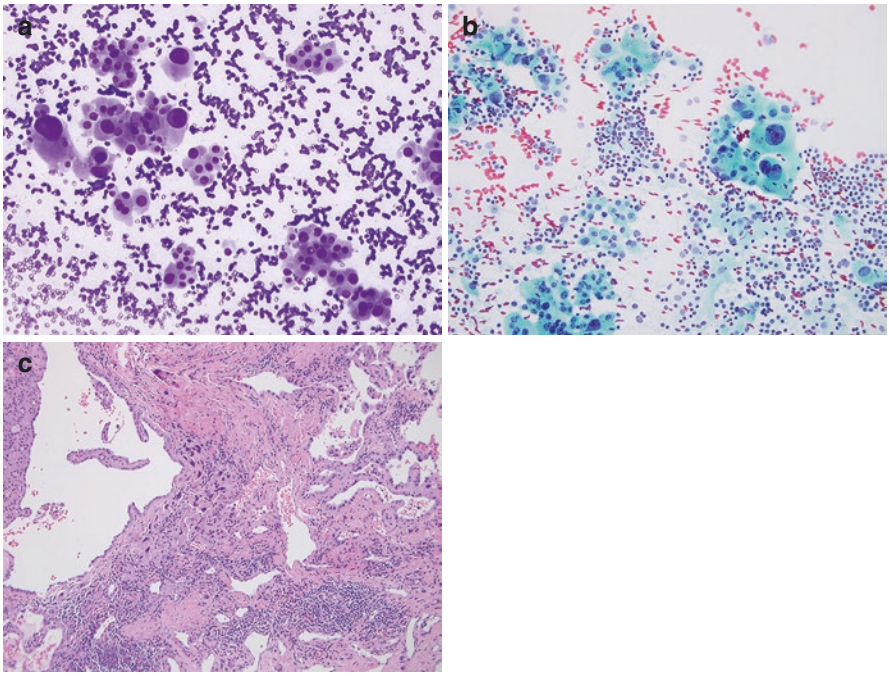

Fig. 4.12 Oncocytic cell atypia (patient with history of Hashimoto thyroiditis). These oncocytic cells show occasional marked nuclear enlargement. The findings in (**a**) show only oncocytic cells and could warrant an AUS diagnosis; however, the lymphocytic component of Hashimoto thyroiditis is readily seen in (**b**) so that a benign diagnosis would be preferable. The histology (**c**) confirms the presence of benign endocrine atypia in the metaplastic oncocytic cells of Hashimoto thyroiditis (**a**: smear, Diff-Quik stain; **b**: smear, Papanicolaou stain; **c**: histology, hematoxylin and eosin stain)

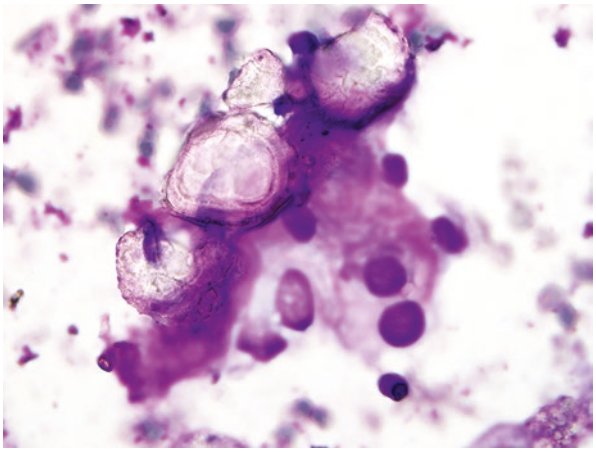

Fig. 4.13 Atypia of Undetermined Significance, not otherwise specified. Psammoma bodies are a characteristic feature of papillary carcinoma. They form at the tip of a papilla and consist of concentric dystrophic calcific lamellations. Psammoma bodies are non-birefringent and composed of calcium phosphate (smear, Diff-Quik stain). (reproduced with permission from Ali SZ, Nayar R, Krane JF, and Westra WH. Atlas of Thyroid Cytopathology with Histopathologic Correlations, Demos Medical, New York, 2014)

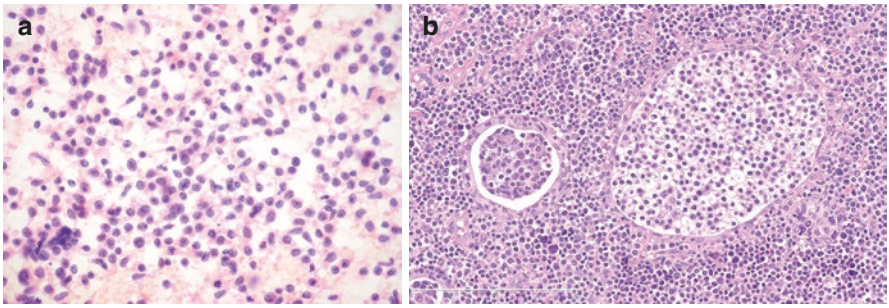

Fig. 4.14 Atypia of Undetermined Significance with atypical lymphoid cells. (**a**) Smear of a diffuse lesion suspected to be Hashimoto thyroiditis clinically shows extensive infiltration by monotonous small lymphocytes with slight variation in nuclear size and contour showing oval and occasionally kidney-shaped nuclei. Small distinct nucleoli can be observed in many cells; however, mitoses and necrosis are not seen. Clonality studies were not available in this case. (**b**) Follow-up thyroidectomy showed an extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) (smear (**a**) and histology (**b**), hematoxylin and eosin stain)

Explanatory Notes

AUS usage varies widely; this interpretation has been reported to account for as little as 1% to over 20% of thyroid FNAs [3]. Many initial studies of AUS were retrospective, with pre-TBSRTC terminology retrofitted to TBSRTC categories. Despite efforts to define this category and provide specific criteria, AUS has, at best, only fair reproducibility [22, 23]. A provisional goal of limiting AUS interpretations to approximately 7% of all thyroid FNAB interpretations was proposed in the first edition of TBSRTC atlas [1]. Since many laboratories struggled to achieve this figure, an upper limit of 10% was adopted as a more achievable target in the second edition and remains a reasonable figure [27]. Additionally, it has also been proposed that the AUS:Malignant ratio may be a useful laboratory quality measure that should not exceed 3.0 [43]. Other quality measures involving the AUS rate of the overall laboratory or individual practitioners have been proposed as well, including correlation of AUS rates with molecular testing outcomes [44].

TBSRTC recommends subclassification of AUS to improve risk stratification of malignancy and enable guidance for the next step in patient management: repeat FNA, molecular testing, or surgery/extent of surgery [27]. Several studies have confirmed the value of stratifying risk of malignancy by subclassification of AUS [6, 7, 11, 12, 34–36].

By themselves, compromising factors like sparse cellularity, air-drying artifact, obscuring blood, and excessive clotting artifact do not warrant an AUS diagnosis; such specimens should be classified as nondiagnostic if adequacy criteria are not satisfied and there is no atypia. Nevertheless, a diagnosis can be made on many compromised specimens: cases with prominent air-drying artifact, obscuring blood, and/or clotting artifact can still be diagnosed as benign if there are sufficient well-preserved, well-visualized follicular cells, and they can be diagnosed as abnormal

(e.g., AUS) if there is discernible atypia. There are several specimen preparation artifacts that may potentially raise concern for AUS. Inadvertent air-drying of alcohol-fixed smears may result in follicular cells with enlarged nuclei that have pale but slightly smudgy chromatin and irregular nuclear outlines (Fig. 4.15). Excessive blood clotting can impair the presentation of follicular cells, often giving the false impression of architectural crowding due to the entrapment of cells in the clot or the false impression of nuclear grooves due to fibrin strands (Fig. 4.16). These artifacts by themselves are not associated with an increased risk of malignancy. If the artifacts described above are focal, clearly recognizable, and associated with benign material elsewhere, such cases should be diagnosed as benign. Alternatively, when the artifacts are so pervasive as to preclude fulfilling standard adequacy criteria for well-preserved follicular cells, such aspirates should be deemed nondiagnostic for evaluation. Only rare cases where there is uncertainty as to whether the cytologic changes are artifactual in origin or truly atypical should result in an AUS diagnosis. Adequacy of cytologic specimens is an important component of cytopathology reports and may be valuable to include in specimens where an AUS diagnosis is considered, since such communication can provide further guidance for patient management. A compromised sample with artifactual changes should be acknowledged by including adequacy statements such as "Satisfactory but limited by …." within the report.

AUS is an interpretation of last resort and should be used judiciously. For example, the mere presence of some oncocytic cells (with or without nuclear size variation) or cyst lining cells, with their customary mild nuclear alterations (e.g., nuclear grooves, finely granular or pale chromatin), does not warrant an AUS designation if there is ample evidence of benign follicular cells and abundant colloid. Isolated

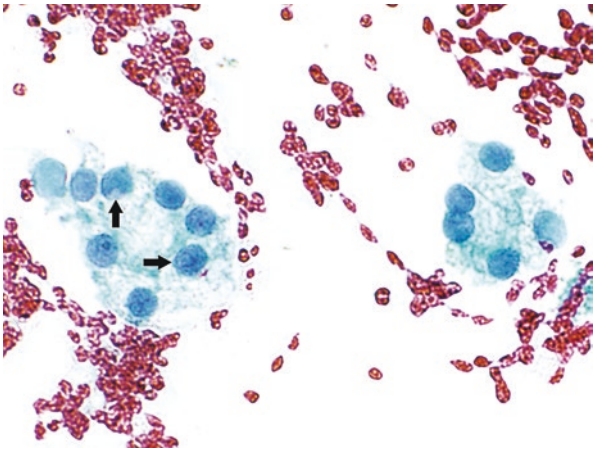

Fig. 4.15 Air-drying artifact. Inadvertent air-drying of alcohol-fixed smears leads to suboptimal nuclear detail (e.g., artifactual pallor, enlargement), including poorly defined, possible intranuclear pseudoinclusions (arrows). Except in rare instances, such changes can be recognized as artifactual and not diagnosed as AUS (smear, Papanicolaou stain)

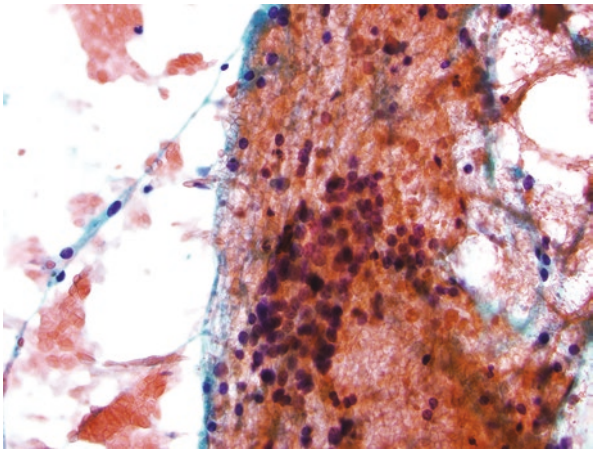

Fig. 4.16 Blood and clotting artifact. Extensive blood and clotting can distort the arrangement of follicular cells and make them look artifactually crowded. These findings should be discounted when assessing the architectural arrangement of the follicular cells. Without demonstrable atypia or sufficient benign follicular cells, such cases warrant a nondiagnostic interpretation (smear, Papanicolaou stain)

follicular cells with minimal alterations (isolated nuclear enlargement, pale chromatin, or nuclear grooves) or occasional microfollicles also do not merit the AUS category. Follicles may present as "spherules" which can be of variable size, with or without colloid, and have sharply outlined contours, usually highlighted by a basement membrane. The presence of these spherules, either dissociated or in tissue fragments (Fig. 4.17), is likely reflective of atrophic follicles in long-standing benign goiters, and should not be interpreted as AUS or FN, even when prominent, since they have consistently been associated with benign clinical outcomes [45]. Mixed, but predominantly macrofollicular, architectural patterns are best classified as benign, even when present in large tissue fragments. Papillae in the absence of any nuclear features of papillary carcinoma (Fig. 4.18) are indicative of papillary hyperplasia and should be interpreted as benign [46].

AUS specimens may be compromised by sparse cellularity that precludes a more definitive classification. A common example is the sparsely cellular aspirate with a predominance of crowded follicular cells in microfollicular or trabecular arrangements ("architectural atypia") (Fig. 4.6). In a moderately-to-markedly cellular specimen, most samples with a marked predominance of follicular cells in crowded microfollicular or trabecular groups and usually without the clinical setting of a MNG merit the interpretation of FN (see Chap. 5). In general, cytologists are appropriately reluctant to make that interpretation on a sparsely cellular sample because the lesion may not have been properly sampled. A similar example is the sparsely cellular aspirate that is comprised exclusively of oncocytic cells (Fig. 4.9). In a moderately or markedly cellular specimen, a sample that consists entirely of oncocytic cells and without the clinical setting of Hashimoto thyroiditis or MNG usually

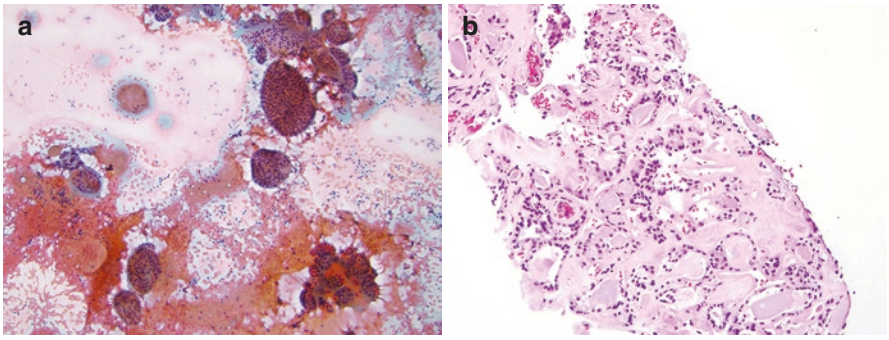

Fig. 4.17 Spherules. (**a**) Spherules of variable size are seen with sharply outlined contours. Even when small spherules predominate, these findings are associated with benign follicular nodules (**b**) and should not be classified as AUS with architectural atypia (**a**: smear, Papanicolaou stain; **b**: histology, hematoxylin and eosin stain)

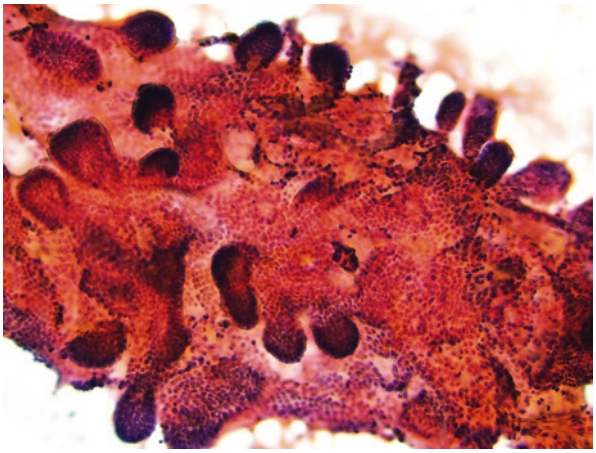

Fig. 4.18 Benign (papillary hyperplasia). Papillary projections are seen in papillary carcinoma, but Graves' disease and other hyperplastic thyroid nodules can show benign papillary proliferations. It is critical to carefully examine the cells, especially their nuclear features; a diagnosis of papillary carcinoma should not be rendered on architecture alone. In this case, the patient went to surgery and was found to have papillary hyperplasia in an involuting hyperplastic nodule (smear, Papanicolaou stain). (reproduced with permission from Ali SZ, Nayar R, Krane JF, and Westra WH. Atlas of Thyroid Cytopathology with Histopathologic Correlations, Demos Medical, New York, 2014)

merits the interpretation OFN (see Chap. 6). Most cytopathologists, again, are appropriately reluctant to make that interpretation in a sparsely cellular aspirate because of sampling concerns.

The possibility of a parathyroid lesion should be considered when crowded three-dimensional clusters or trabecular arrangements are present [47–50]. About 25–30% of these lesions can be recognized based on the presence of "salt and

pepper" chromatin with or without abundant granular cytoplasm and accompanying crowded architecture. Ancillary studies such as parathyroid hormone assays, immunocytochemistry, and molecular studies can help in confirming the diagnosis when it is considered by the pathologist, radiologist, or clinician. However, without adequate clinicopathologic correlation many such nodules are not recognized as parathyroid in origin, especially when intrathyroidal. Certain molecular tests used for molecular testing of aspirates diagnosed as AUS (e.g., Afirma® gene sequencing classifier and Thyroseq®) recognize the expression profile of parathyroid cells [51, 52].

A moderately or markedly cellular aspirate from a solitary nodule that is composed almost exclusively of oncocytic cells is reported as OFN (see Chap. 6). However, a common subtype of AUS is also an aspirate predominated by oncocytic cells. In some clinical settings, such as lymphocytic (Hashimoto) thyroiditis and MNG, this pattern is believed to be more highly predictive of a hyperplastic oncocytic cell nodule and less predictive of an oncocytic cell neoplasm than usual [53, 54]. It is thus acceptable to diagnose a specimen composed exclusively of oncocytic cells in a patient with Hashimoto thyroiditis or MNG as AUS. If interpreted as AUS, an explanatory note that raises the possibility of oncocytic cell hyperplasia/metaplasia in these clinical settings can be very helpful (see section on "Sample Reports" Examples 4.4 and 4.5). In patients with known Hashimoto thyroiditis, the overwhelming percentage of carcinomas are papillary carcinomas whereas oncocytic metaplasia/hyperplasia is common and oncocytic cell adenoma/carcinoma are rare. As a result, cases with documented Hashimoto thyroiditis and a predominance of oncocytic cells with or without focal "atypia" should typically be diagnosed as benign. The note that accompanies an AUS interpretation in these settings is meant to reflect the underlying ROM more accurately, which, although not precisely characterized, is likely lower than that of OFN in general. The goal is to provide the clinician with the opportunity to avoid an unnecessary lobectomy in some of these patients. In this setting, the clinical decision to follow a patient rather than perform a lobectomy will often be based on clinical, sonographic, and molecular correlation; it is not clear whether a repeat aspiration is likely to add any helpful information.

The distinction between AUS and suspicious for malignancy is problematic in aspirates with nuclear atypia raising concern for papillary carcinoma. AUS with nuclear atypia is associated with malignancy, especially papillary carcinoma in 23–66% of cases [7, 11, 12, 29, 34–36]. A pooled cancer prevalence for AUS with "focal cytologic atypia" in a recent meta-analysis study [12] was 44%, while "extensive but mild cytologic atypia" had a similar ROM of 42%. As described, the focal nuclear pattern has rare cells, typically less than 20 in number, with enlarged, often overlapping nuclei, pale chromatin, irregular nuclear outlines, and nuclear grooves [55]. When accompanied by well-defined, intranuclear pseudoinclusions and/or psammomatous calcifications, these findings are even more highly associated with papillary carcinoma, and may warrant consideration of using the SUS diagnostic category [56].

The pattern of extensive but mild cytologic atypia is highly associated with the follicular variant of papillary carcinoma (FVPTC) and its indolent counterpart,

NIFTP. This pattern exhibits diffuse but subtle nuclear atypia including mild nuclear enlargement, focal nuclear irregularity, and only occasional intranuclear grooves. Although a recent meta-analysis study showed that NIFTP has a propensity for more frequent microfollicular architecture compared to FVPTC [21], distinction of NIFTP from FVPTC or other follicular patterned lesions cannot be made with certainty on cytology alone [18, 21, 57–59]. Such aspirates are usually better classified as SFM (see Chap. 7) when nuclear alterations are prominent, while classification as FN is more appropriate when microfollicular architecture is more pronounced (see Chap. 5). The presence of intranuclear pseudoinclusions is rare in NIFTP and, when present, may allow for a malignant diagnosis [57–59]. The AUS designation should be reserved for cases with few cells that have distinct but mild nuclear atypia (Fig. 4.1) and cases with more extensive but very mild nuclear atypia (Figs. 4.2 and 4.5). It must be acknowledged that precisely defining this distinction is difficult; pathologist experience influences the recognition and correct classification of these cases and expert consultation may be warranted, especially in challenging cases. With the advent of NIFTP, a subset of the above cases is no longer classified as carcinoma at resection [15, 16]. Since NIFTP remains a surgical rather than cytologic diagnosis, diagnostic lobectomy remains the appropriate clinical management for such cases.

Isolated nuclear enlargement, typically with prominent nucleoli, is not unusual in benign thyroid nodules and by itself does not indicate malignancy. In patients treated with radioactive iodine, carbimazole, or other pharmaceutical agents, nuclear enlargement can be especially prominent [60–62]. When the changes are mild and characteristic in a specimen accompanied by a clinical history of such treatment, a benign interpretation should be rendered. In some patients, however, the changes can be extreme and raise the possibility of malignancy (Fig. 4.11) [61, 62]. In such cases, an AUS interpretation is warranted. Significant nuclear size variation, often with smudgy chromatin and/or nucleoli, may also be seen in oncocytic cells, especially in the setting of Hashimoto thyroiditis and does not warrant an AUS diagnosis in the absence of other features to suggest an oncocytic neoplasm, particularly the presence of a pure or nearly pure population of oncocytic cells in the aspirate (Fig. 4.12).

Cyst lining cells are reactive follicular and/or mesenchymal cells associated with cystic degeneration of thyroid nodules. As such, they have very characteristic features and can be diagnosed as benign in most cases [37]. They are typically elongated, with pale chromatin, occasional intranuclear grooves, and relatively large nucleoli, and are virtually always associated with hemosiderin-laden macrophages. The spindle-shaped morphology of the cell and nucleus, reminiscent of reparative epithelium in cervical, bronchial, and gastrointestinal cytologic specimens, is helpful in distinguishing these cells from papillary carcinoma. In some cases, however, the cells are more closely packed, less elongated, and, as a result, more difficult to distinguish definitively from papillary carcinoma (Fig. 4.3) [37]. In these uncommon instances a diagnosis of AUS is appropriate.

Most AUS cases are based on the finding of follicular cell atypia, but in rare cases the AUS designation may be appropriate for non-follicular and even non-epithelial

atypia. An example of non-epithelial atypia that may warrant the AUS category is an atypical or monomorphous lymphoid infiltrate, especially in the setting of long-standing Hashimoto thyroiditis and/or a large or rapidly growing nodule. In some cases, the findings are not sufficiently concerning to warrant a suspicious or malignant diagnosis. Aspirates that have a prominent, somewhat polymorphous lymphoid component may raise concern for an extranodal marginal zone B-cell lymphoma (Fig. 4.14). If clonality studies are not available, an AUS diagnosis, with a recommendation for a repeat aspirate for flow cytometry, is appropriate.

Management

The 2015 American Thyroid Association guidelines recommend conservative management in most instances for an initial AUS interpretation in adults, with either repeat FNA or molecular testing [63]. A repeat FNA usually results in a more definitive cytologic interpretation; approximately 10–30% of AUS nodules are reported again as AUS on a repeat FNA [64–66].

Molecular testing of AUS nodules can reduce the need for diagnostic surgery. An increased number of patients may be managed with observation or surveillance because AUS aspirates frequently have negative molecular test results (referred to as a high benign call rate, BCR). Samples with negative molecular results typically have a low ROM of ~3–5% [52, 67–69]. Molecular test performance has improved dramatically over the past 10 years with the emergence of comprehensive diagnostic testing platforms offered by centralized reference laboratories. As discussed in Chap. 14, these tests include an evaluation of mutations, fusions, gene expression, copy number alterations, and microRNAs. The expanded testing platforms exhibit higher sensitivity and good specificity, despite the increased prevalence of *RAS* mutations within indeterminate samples. Molecular testing of AUS aspirates using the ThyroSeq® v3 genomic classifier leads to BCRs of 65–87% [67, 70, 71]. Studies using the Afirma® Genomic Sequencing Classifier (GSC) and Xpression Atlas (XA) demonstrate a BCR of 65–76% [68, 72–74]. Across studies, AUS with isolated architectural atypia is more likely to have a negative molecular result (higher BCR) than AUS with nuclear atypia. Oncocyte-predominate AUS aspirates have historically been difficult to assess with molecular assays due to our lack of understanding of the molecular drivers of oncocytic tumors. Recent studies show that oncocytic carcinomas and some adenomas have widespread copy number alterations with a near-haploid state and frequent mitochondrial DNA mutations [75–77]. The recent incorporation of copy number and mitochondrial DNA analyses in multiple commercial assays have improved the BCR and test performance for oncocytic lesions [52, 68, 69, 72, 73, 77–80].

The decision regarding surgery (typically lobectomy) versus continued observation is based on a synthesis of cytologic, molecular, clinical, and radiologic findings as well as clinical risk factors and patient preference. The ROM of an AUS nodule selected for surgical excision varies greatly and is dependent on the subtype of AUS, with a ROM of 36–44% for AUS with nuclear atypia and 15–23% for AUS with

other patterns [12]. The introduction of NIFTP terminology has further diminished the overall ROM for AUS, although it should be emphasized that surgical excision is indicated for potential NIFTP since this is a histologic diagnosis [15–21].

In contrast to the adult management guidelines, the 2015 American Thyroid Association pediatric guidelines recommended more aggressive management for an initial AUS in children to include diagnostic surgery [81]. In support of this more aggressive management are numerous studies over the last decade demonstrating that children with thyroid nodules are at increased risk of malignancy compared to their adult counterparts. The ROM within the AUS category, while variable across numerous small studies, ranges between approximately 15 and 50% [82–89], and is likely not altered significantly by NIFTP due to a low reported incidence in the pediatric population [90]. However, while the malignancy risk is higher in children across studies, more than half of the nodules in the AUS category likely represent benign disease. Proceeding directly to diagnostic surgery may lead to overtreatment of a large proportion of pediatric AUS nodules.

Recent evidence suggests that AUS subclassification in children, similar to that currently performed in adults, may provide further risk stratification. A systematic analysis of 68 AUS nodules with repeat FNA cytology demonstrated that nuclear atypia was associated with a malignancy rate of 59% (22/37 nodules) as compared to 6.5% for architectural atypia or oncocyte rich aspirates (2/31 nodules) [34]. This ROM for AUS subclassification is similar to that reported in adults. While additional larger studies are needed, it is reasonable to surmise that the presence of nuclear atypia in children, like that in adults, may help distinguish intermediate/higher risk from low-risk AUS lesions.

Similar to adults, molecular testing of pediatric thyroid AUS nodules may also provide further risk stratification prior to diagnostic surgery. The molecular landscape of pediatric thyroid cancer is distinct from that of adults and is composed largely of receptor tyrosine kinase fusions. Despite this difference, initial studies demonstrate that comprehensive molecular testing platforms may provide high sensitivity and adequate specificity for malignancy detection in pediatric aspirates [86, 91–93]. While diagnostic lobectomy may still be a reasonable approach, AUS subclassification, repeat FNA, and molecular testing may allow better risk stratification for more conservative management of some indeterminate nodules [34, 91, 94]. As of this writing, a revision of the ATA management guidelines for children with thyroid nodules and differentiated thyroid cancer is underway and expected to be published in 2023.

Sample Reports

If an aspirate is interpreted as AUS, it is implied that the sample is adequate for evaluation. An explicit statement of adequacy is optional but may be particularly beneficial when limiting factors contribute to the diagnostic interpretation. Additional narrative comments to qualify the nature of the AUS diagnosis are strongly recommended to provide risk stratification and guide next steps for management. Subclassification of AUS according to the presence or absence of nuclear

atypia is encouraged. A differential diagnosis and a recommendation may also be helpful for cases that fall into the AUS category. Generic descriptors (e.g., "focal nuclear atypia," "architectural atypia") are preferred over phrases associated with malignancy (e.g., "rule out papillary carcinoma," "pseudoinclusions"), which may prompt surgery rather than the intended more conservative management.

Example 1
Specimen adequacy is limited by scant epithelial cellularity.
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Other.
Sparsely cellular aspirate comprised of follicular cells with architectural atypia. Colloid is absent.
Note: A repeat aspirate may be helpful in order to further characterize the lesion.

Example 2
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Nuclear.
Both mild nuclear and architectural atypia are present.

Example 3
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Nuclear.
Follicular cells, predominantly benign-appearing, with focal nuclear atypia.
Note: Molecular testing or a repeat aspiration may be helpful in clarifying these findings.

Example 4
(FNAB of a patient with multiple, bilateral nodules; multinodular goiter)
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Other.
The specimen is moderately cellular and consists almost exclusively of oncocytic cells. Colloid is scant, and there is no apparent increase in lymphoid cells.
Note: In a patient with multiple nodules, the findings likely represent oncocytic cell hyperplasia in the setting of multinodular goiter; however an oncocytic follicular neoplasm cannot be entirely excluded. Molecular testing may be beneficial.

Example 5
(FNAB of a nodule in a patient with a history of Hashimoto thyroiditis)
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Other.
The sample consists exclusively of oncocytic cells with focal endocrine atypia.
Note: In a patient with Hashimoto thyroiditis, these findings more likely represent oncocytic cell metaplasia/hyperplasia; however an oncocytic follicular neoplasm cannot be entirely excluded. Molecular testing may be helpful in further clarifying the findings.

Example 6
(FNAB of a nodule in a patient with Graves' disease treated with ^{131}I)
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Other.
Follicular cells with likely treatment-related atypia.
Note: In the context of treatment of hyperthyroidism with radioiodine, these findings likely represent reactive, treatment-related changes. Suggest clinical/radiologic correlation and follow-up as warranted.

Example 7
(FNA of a nodule in a patient with a long-standing history of Hashimoto thyroiditis)
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Other.
Numerous relatively monomorphic lymphoid cells.
Note: The findings are atypical and raise the possibility of a lymphoproliferative process arising in the background of the patient's long-standing chronic lymphocytic thyroiditis. Immunophenotyping studies could not be performed since only smears were made from the aspirate. Repeat FNA with aspirate collected for flow cytometry would be helpful in reaching a more definite diagnosis.

Example 8
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Other.
Psammomatous calcifications are present in a background of benign-appearing follicular cells and colloid.
Note: Psammomatous calcifications in isolation are associated with both benign and malignant thyroid aspirates, including papillary thyroid carcinoma. Clinical and radiologic correlation and follow-up is recommended.

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Follicular Neoplasm

5

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Background

Since its first edition, The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has standardized the diagnostic terminology for the thyroid fine needle aspirates (FNAs) that suggest the possibility of a follicular neoplasm (FN), a diagnostic category that used to be reported with great variability, as demonstrated by a review of the literature published in 2008 [1]. In keeping with its overarching principle of a desired accrued simplification of the diagnostic categorization to ensure clear communication, the third edition of TBSRTC endorses only the terminology "FN" for the diagnostic category, which was also termed "Suspicious for FN" in the previous editions.

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Although it is acknowledged that there are overlapping cytologic features between various follicular-patterned lesions, including follicular nodular disease, follicular adenoma (FA), invasive follicular variants of PTC (FVPTC), follicular thyroid carcinoma (FTC), as well as the more recently described noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), there are nevertheless certain cytologic features that are very useful in raising the possibility of a neoplasm, and most importantly, those with the potential of being malignant. In this regard, FNA can be considered a screening test, selecting for surgery those nodules with a greater probability of malignancy. It is not the goal of FNA to identify all FN, because FA are clinically innocuous; in other words, the goal of the FN category is to identify all potential FVPTC, FTC, and NIFTP, and refer them for appropriate management [2]. The final diagnosis depends upon histologic examination of the surgical resection specimen because capsular and/or vascular invasion, the *sine qua non* of FTC and FVPTC, cannot be assessed by cytology. The majority of cases interpreted as FN will turn out to be FAs or follicular nodular disease on histologic follow-up (for more details, see section below "Cyto-Histologic Correlation").

The diagnostic terminology "FN" is recommended despite the fact that it is recognized that a certain proportion (up to approximately 30%) of cases that fulfill the criteria described herein prove not to be neoplasms but rather hyperplastic proliferations in the context of non-neoplastic conditions on cyto-histologic correlative studies; in view of these limitations, a facultative statement can be used to convey that uncertainty, if it is the preference of a laboratory (see Example 3) [3–5]. It is also important to point out that cytologic-histologic correlation is partly hindered by variability in the histopathologic classification of the follicular-patterned thyroid nodules [6–8].

The FN cytologic diagnosis is infrequent, accounting for only approximately 7% of the overall thyroid FNAs in most cytopathology laboratories [9].

Definition

The general diagnostic category "FN" refers to a moderately to markedly cellular aspirate that is comprised of follicular cells in which most are arranged in an altered architectural pattern characterized by microfollicle formation, and/or significant cell crowding, trabeculae, or single cells.

The majority of cases in the FN category consist of follicular cells without nuclear atypia: the nuclei are normal-sized and round, with clumpy or slightly hyperchromatic chromatin with absent or inconspicuous nucleoli. Nuclear grooves, intranuclear pseudoinclusions, and nuclear clearing are absent.

However, a minority of cases in the FN category consist of follicular-patterned aspirates with mild atypical nuclear changes, including increased nuclear size, nuclear contour irregularity (i.e., nuclear grooves), and/or chromatin clearing; such cases could still be classified as FN as long as true papillae are absent and intranuclear pseudoinclusions are either absent or very rare [10–13]. Such cases that exhibit the architecture pattern of typical FN but in which the subtle or focal nuclear

features are reminiscent of PTC raise the possibility of NIFTP or of FVPTC; as the latter two entities cannot be reliably distinguished prospectively by cytology, a facultative comment can be included in the report to alert the treating physicians of this diagnostic possibility (see Example 4) [14].

Exclusion from FN category-scenario #1

- The FNAs with the above-mentioned architectural pattern, but that are only sparsely cellular, are excluded from the FN category; they are best interpreted as "Atypia of Undetermined Significance (AUS)."

Exclusion from FN category-scenario #2:

- Cases with marked or unequivocal pronounced nuclear atypia (nuclear clearing and multiple nuclear pseudoinclusions) and/or presence of true papillae and/or psammoma bodies are excluded from the FN category and should be classified as "Malignant" or "Suspicious for Malignancy," depending on the quality or quantity of the changes (see Chaps. 7 and 8).

Criteria for FN Cases

Cellularity

Cytologic preparations are moderately or markedly cellular (Fig. 5.1a).

Architecture

There is a significant alteration in follicular cell architecture characterized by microfollicles and/or cell crowding, and less frequently, trabeculae or dispersed isolated cells (Fig. 5.1a–f).

Cytoplasm

The cytoplasm is scant or moderate.

Oncocytic changes should be absent, or present only focally (if present to any significant degree in a case exhibiting the above architectural features, the diagnostic category to consider is Follicular Neoplasm—Oncocytic Follicular Neoplasm (FN-OFN); see Chap. 6).

Nuclei

Most Common Scenario (i.e., The "Typical" FN Scenario)

The nuclei are normal-sized and round, with the chromatin clumpy/slightly hyperchromatic and an absent or inconspicuous nucleolus (Fig. 5.2a–c).

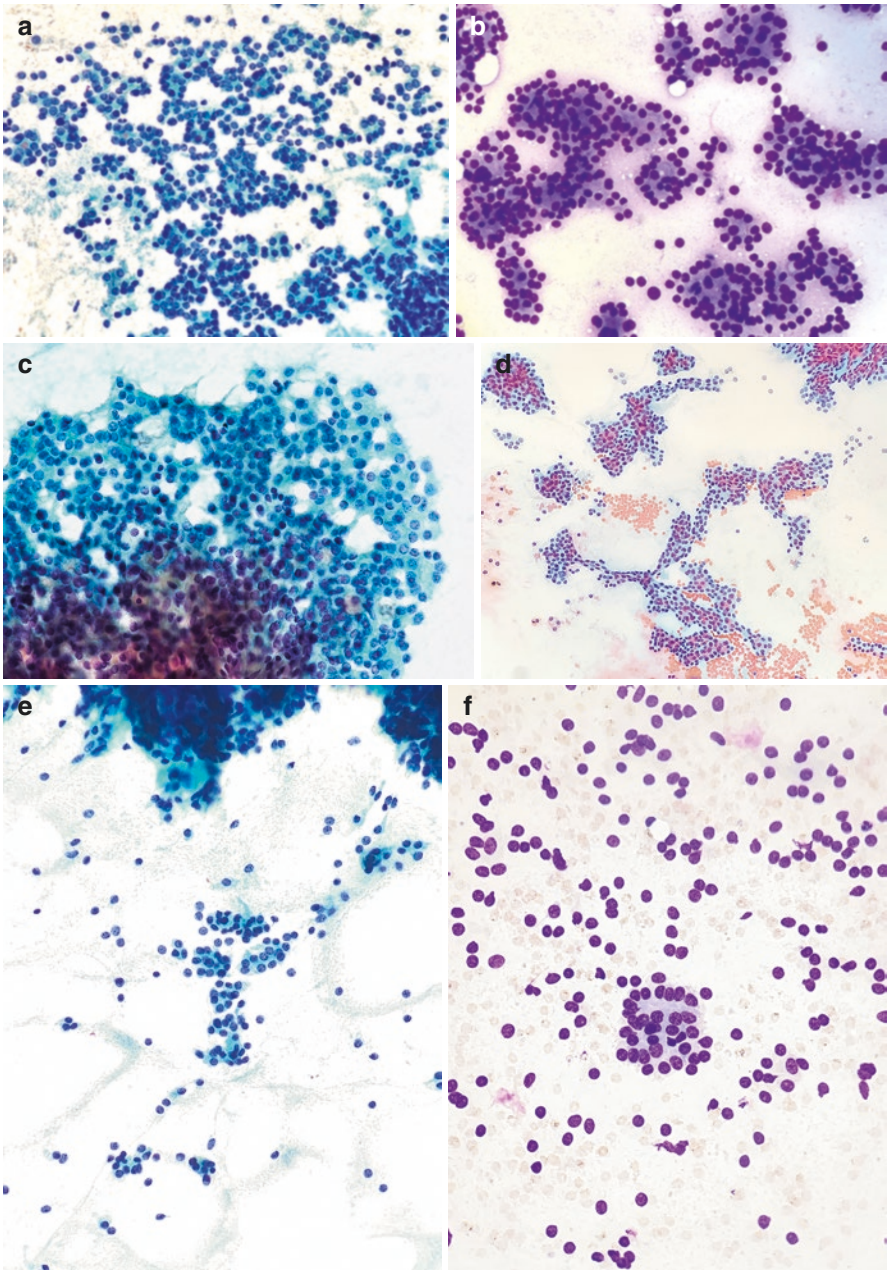

Fig. 5.1 Follicular neoplasm. Illustrating the various architectural alterations seen in FN. (**a**, **b**) Highly cellular aspirate composed of uniform follicular cells arranged in microfollicles (smears, **a**: Papanicolaou stain, **b**: Diff-Quik stain). (**c**) Follicular cells in crowded groups (smear, Papanicolaou stain). (**d**) Follicular cells in trabecular arrangement (smear, Papanicolaou stain). (**e**, **f**) Follicular cells in single cell pattern (smears, **e**: Papanicolaou stain, **f**: Diff-Quik stain)

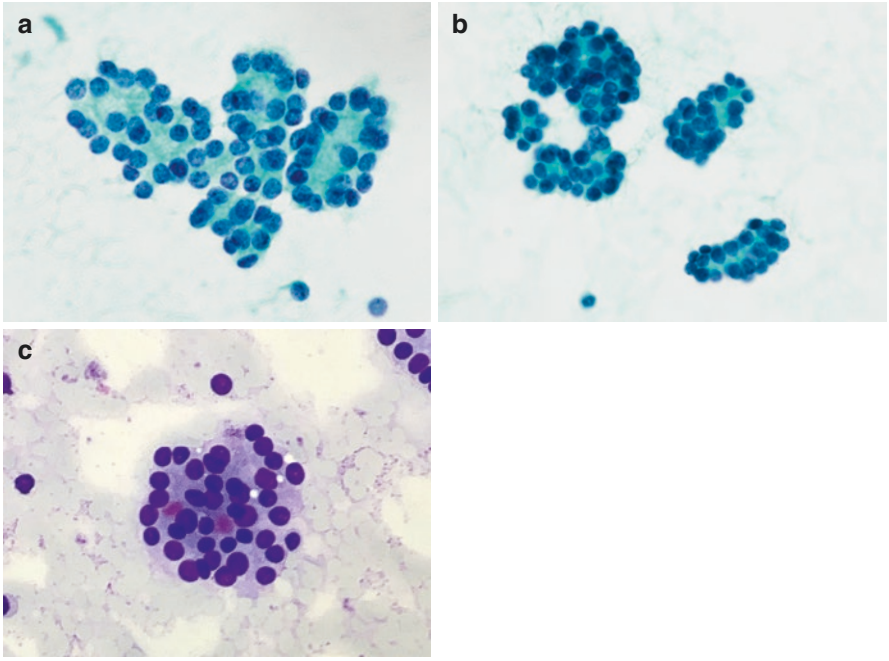

Fig. 5.2 Follicular neoplasm (*the "typical" FN scenario*). (**a**–**c**) The nuclei of the "typical" FN are normal-sized and round, with the chromatin clumpy/slightly hyperchromatic and an absent or inconspicuous nucleolus (the histologic follow-up for this case was follicular adenoma) (smears, **a**, **b**: Papanicolaou stain; **c**: Diff-Quik stain)

Infrequent Scenario (i.e., The "Potential NIFTP/FVPTC" Scenario)

Mild or focal nuclear atypia (papillary-like nuclear alterations) may be seen (Fig. 5.3a–d), manifested as either:

- Alterations of nuclear size and shape: enlargement, overlapping, and/or elongation.
- Nuclear membrane irregularities: irregular contours, grooves, and/or rare pseudo-inclusions.
- Alterations of chromatin characteristics: chromatin clearing, margination of chromatin to membrane, and/or glassy nuclei.
- A conspicuous nucleolus may be focally present.

Other Features

Colloid is scant or absent.

Cystic degeneration (i.e., foamy macrophages) may be present but is unusual; this is in contrast to FN-OFN, in which this feature is frequent.

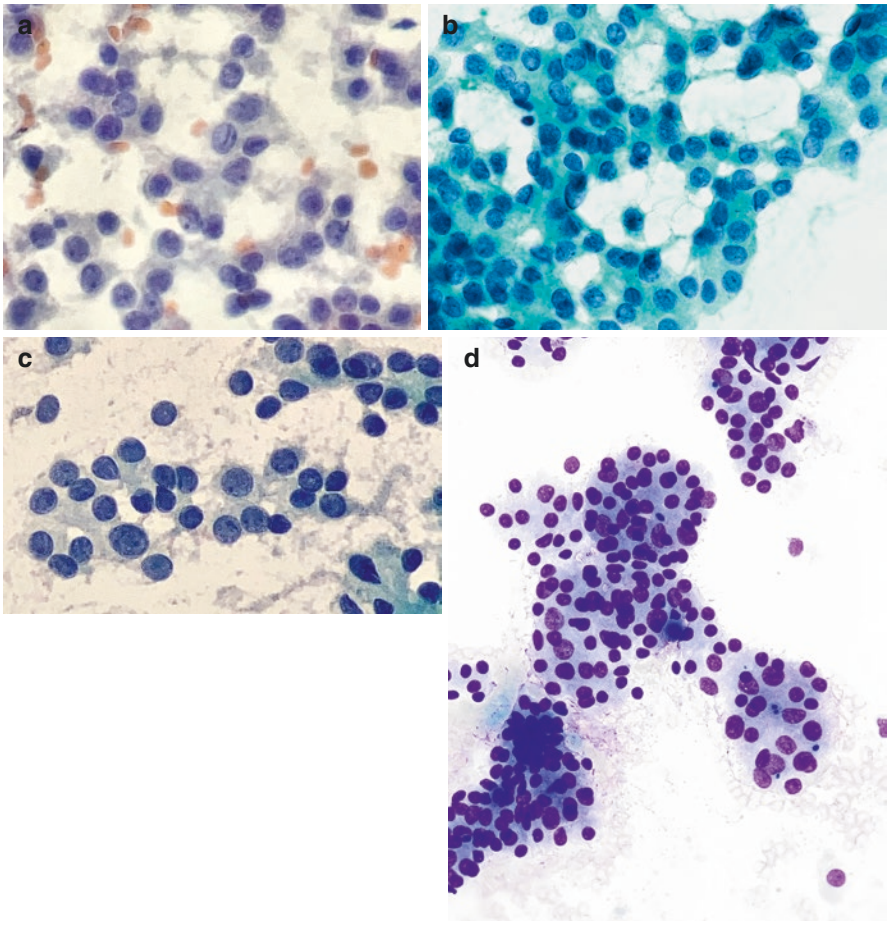

Fig. 5.3 Follicular neoplasm (*the "potential NIFTP/FVPTC" scenario*). (**a**–**d**) When the nuclei exhibit mild or focal nuclear atypia (i.e., papillary-like nuclear alterations), NIFTP/FVPTC comes into consideration (the histologic follow-up for this case was NIFTP) (smears, **a**–**c**: Papanicolaou stain; **d**: Diff-Quik stain)

Explanatory Notes

The hallmark of the FN case is the presence of a predominant microfollicular or crowded architectural pattern in the majority of the follicular cells in a moderately to markedly cellular specimen. Most commonly this is observed in the absence of nuclear atypia (no nuclear features reminiscent of PTC), or infrequently in the presence of mild/focal nuclear atypia (potential NIFTP/FVPTC scenario).

The microfollicles are composed of crowded and overlapping follicular cells (Figs. 5.1a and 5.2a–c). To improve reproducibility, it has been proposed that the designation of "microfollicle" be restricted to crowded, flat or 3-dimensional groups

with a circumference of less than 15 follicular cells arranged in a circle that is at least two-thirds complete [15]. A small amount of inspissated colloid may be present within the microfollicle (Fig. 5.2c). Microfollicles tend to be relatively uniform in size ("equisized"). Occasionally, crowded follicular cells form ribbons of overlapping cells ("trabeculae") that could be more prominent than the microfollicles (Fig. 5.1d). Although follicular cells can also be seen in a single cell pattern, it is infrequent (Fig. 5.1e, f); when present, the single cell pattern usually does not predominate, and the single cells are admixed with microfollicles, or less commonly trabeculae. In fact, when an aspirate exhibits a predominantly single cell pattern, other diagnostic entities (e.g., FN-OFN, medullary thyroid carcinoma, or even follicular nodular disease) should be considered instead, depending on the presence of other features (see Chaps. 6, 9, and 3, respectively).

It is important to recognize that rare macrofollicles may be present in FN specimens; however, if macrofollicles are easily identified in a specimen leading to a "mixed" macro- and micro-follicular architectural pattern, a diagnosis of FN should not be used; instead, either the "Benign" or "AUS" category should be considered depending on the other features seen (see Chaps. 3 and 4, respectively).

Although most FN are highly cellular, cellularity alone is insufficient to merit this designation. If the majority of follicular cells are arranged in macrofollicular fragments or honeycomb sheets (variably sized fragments without nuclear overlap or crowding), or if there is a significant mixture of both macrofollicles and/or honeycomb sheets along with microfollicles, the sample can be considered Benign. Of note, a small fragment of follicular cells is not necessarily a microfollicle: an important defining feature of the microfollicle is the crowding and overlapping of the follicular cells. Indeed, when a honeycomb sheet (which corresponds to a collapsed macrofollicle) is disrupted or fragmented, it may appear as a small fragment; however, in contrast to a "true" microfollicle, the nuclei of such a fragment should not be crowded or overlapping when looking at those that are located in the same focal plane (Fig. 5.4a–c).

A frequent dilemma is the sparsely cellular sample composed predominantly of microfollicles. Because of the low cellularity, it is more prudent to refrain from diagnosing such specimens as FN, and best to interpret them as "AUS" (see Chap. 4). In such cases, a repeat FNA to improve sampling and cellularity is a reasonable approach and is likely to resolve the discrepancy.

The majority of cases in the FN category consist of follicular cells exhibiting no nuclear atypia. The nuclei are normal-sized and round, the chromatin clumpy/slightly hyperchromatic with an absent or inconspicuous nucleolus. There are no nuclear grooves, intranuclear inclusions, nor nuclear clearing. In other words, there are no nuclear features reminiscent of PTC (Fig. 5.2a–c).

However, a minority of cases are those that exhibit an architectural pattern concerning for a FN, but in which the subtle/mild or focal nuclear features are concerning for PTC; such cases should also be diagnosed in the FN category as long as true papillae are absent and that intranuclear pseudoinclusions are either absent or very rare [10–13] (Fig. 5.3a–c). Such cases raise the possibility of NIFTP or FVPTC (see section below, for more details about NIFTP). Importantly, if the follicular cells

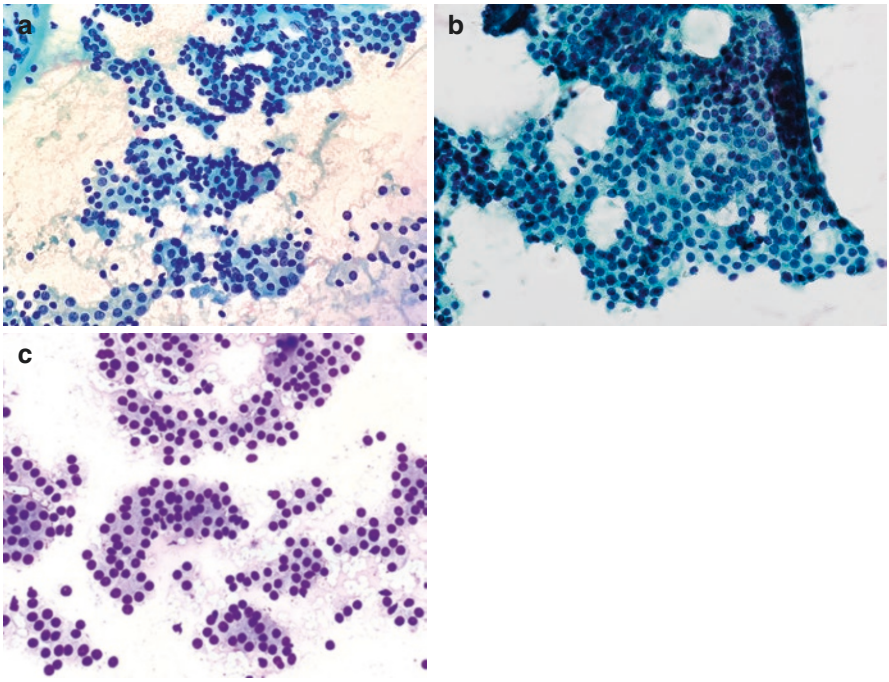

Fig. 5.4 Contrasting "true" microfollicle versus a "pseudofollicle." (**a**) A "true" follicle (with nuclei overlapping) from a follicular neoplasm (smear, Papanicolaou stain). (**b**, **c**) Pseudofollicles from a hyperplastic nodule (smears, **b**: Papanicolaou stain; **c**: Diff-Quik stain)

show definite or suspicious nuclear features of PTC, including frequent pseudoinclusions, and there are at least focal elements associated with classical PTC (psammoma bodies and/or true papillae), the specimen should not be interpreted as FN but rather as "Malignant—PTC," or "Suspicious for PTC," depending on the quality and quantity of the cytologic changes (see Chaps. 8 and 7, respectively). For lesions deemed borderline between FN and "Suspicious for malignancy," it may be more prudent to opt for the FN designation because the FN diagnosis is more likely to prompt a limited surgical approach (lobectomy).

More About NIFTP (and FVPTC)

NIFTP was originally described in 2016 in response to the increasing recognition that this well-demarcated or encapsulated follicular-pattern tumor has an indolent clinical behavior and should no longer be considered malignant nor labeled as "carcinoma" as it previously had been under the terminology of "encapsulated FVPTC" [7, 8]. Because a definitive diagnosis of NIFTP requires surgical excision for complete examination of the entire interface or capsule of the tumor with the

surrounding thyroid tissue to exclude capsular and/or vascular invasion, the distinction between NIFTP and FVPTC cannot be achieved by cytology.

Although now considered a non-cancer, NIFTP has an approximate prevalence of 9–10% of all retrospectively reviewed PTC cases worldwide; this percentage, however, shows significant geographic variation, ranging between 0.5% and 5% in Asia versus 13–20% in Western countries [16, 17].

The prospective cytologic diagnosis of NIFTP is problematic. In most series, the majority (approximately 75–80%) of FNAs of NIFTP cases are classified in the indeterminate diagnostic categories of TBSRTC; of those, approximately 50–75% are diagnosed as AUS, 25–30% as FN, while a minority are classified as suspicious or more rarely as malignant [7, 9, 13, 18]. A goal of this third edition is to increase awareness of diagnostic clues to a potential diagnosis of NIFTP. When such NIFTP are suspected, they should be put in the FN diagnostic category, with a facultative comment in the report raising this diagnostic possibility (see Example 4) [14].

Several studies have reported the cytological features of NIFTP [11–14, 18–22]. Although rare studies state that certain cytologic criteria can distinguish between NIFTP and FVPTC, the overall consensus is that there is too much overlap cytologically, but also ultrasonographically and molecularly, between the two entities to reliably distinguish them prospectively on cytology [11, 21]. However, there is general agreement that it is possible to distinguish most cases of NIFTP/FVPTC from benign follicular nodules and from conventional PTC on the basis of their cytological features. It is the nuclear, but not the architectural, features that allow the distinction of NIFTP/FVPTC from the benign nodules in a follicular pattern; although mild PTC-like nuclear changes will be seen in NIFTP/FVPTC, no nuclear atypia should be seen in benign nodules (with the exception of the context of lymphocytic thyroiditis) [13, 23]. At the other end of the spectrum, when trying to distinguish NIFTP/FVPTC from conventional PTC, the presence of marked/unequivocal pronounced nuclear atypia (nuclear clearing and multiple nuclear pseudoinclusions) and/or of any true papillae and/or psammoma bodies excludes NIFTP and strongly favors PTC [11, 12, 23, 24].

The ultrasonographic (US) features of most NIFTP are "benign," including wider than tall shape, well-circumscribed solid (hypo- or iso-echoic) nodules, and absence of microcalcifications; however, overall, these features are not specific and overlap with those of FA, FVPTC (more specifically, the invasive encapsulated subtype), and minimally invasive FTC. In contrast, US findings of thyroid malignancy including marked hypoechogenicity, taller-than-wide shape, micro-calcifications, and blurred or micro-lobulated margins argue against the diagnosis of NIFTP [14, 18, 25].

Short Notes About the Molecular Features of FN and NIFTP

Herein is a summary of the most pertinent molecular features of the FN category; more details are found in Chap. 14.

The follicular-patterned thyroid neoplasms, including FA, FTC, NIFTP, and FVPTC share many molecular alterations. The most common somatic mutations found are point mutations in the *RAS* gene family and *PPARG* rearrangements. In histologically proven cases, approximately 10% and 30–50% of FA and FTC respectively harbor mutations in the *RAS* genes, whereas *PAX8::PPARG* rearrangements occur in approximately 8% and 20–30% of FA and FTC, respectively [8, 21, 26, 27].

Molecular testing in thyroid FNAs diagnosed as FN reveals variation between studies in the percentage of cases exhibiting mutations, presumably partly due to inter-institutional differences in the type of thyroid FNA cases classified as FN. For example, using the same 7-gene panel test (*BRAF*, *RAS*, *RET::PTC*, and *PAX8::PPARG*), mutation rate in FN varied from 8.8% to 27.2.% between studies [26]. One constant in most series, however, is that *BRAF* V600E mutations and *RET::PTC* rearrangement (grouped together as *BRAF*-like mutations) are highly specific for malignant outcomes; similarly, *TERT* promoter mutations are usually associated with malignancy [27]. In contrast, *RAS* and *RAS*-like mutations (*PAX8::PPARG* without *BRAF* V600E alterations) are not malignant-specific since they are also detected in FA and low-risk neoplasms such as NIFTPs [21, 28].

NIFTP is characterized by alterations in either *RAS* (in up to 60% of cases), *PAX8::PPARG* (in up to 30% of cases), and *THADA* (in up to 30% of cases) gene fusions, or more rarely (in <10% of cases) by *BRAF* K601E, *EIF1AX*, *EZH1*, *DICER1*, *PTEN*, or *TSHR* mutations; however, *BRAF* V600E mutations and *RET* fusions (characteristic of conventional PTC) should be absent in NIFTPs [17, 21, 27–29]. The molecular profile of NIFTPs overlaps too much with that of other *RAS*-like tumors to allow a definitive preoperative identification.

Molecular testing of FN cases can be helpful to refining management decision-making. Molecular testing using a wider panel of genes (e.g., ThyroSeq® V3) can provide more refined risk stratification than pure cytomorphology, attributing different risk associated with different molecular signatures in the indeterminate nodules, including those classified as FN [30]. For example, in a study including FNAs categorized as AUS or FN, *PAX8::PPARG* fusion, or mutations in *BRAF*, *TERT*, and *PIK3CA* all carried a ROM of 100%; in contrast, *PTEN*, *DICER1*, *E1F1AX*, *TSHR*, and *TP53* genes were associated with benign pathologic follow-up, and the negative predictive value (NPV) for the FN cases was 95.5% [30]. In one study, the molecular-derived ROM for FN, when using Thyroseq v.3, was 32.6% [31]. The *Afirma*® Gene Sequencing Classifier (GSC) can also be used for risk stratification for FN, with a NPV of 95.8% for those classified as "benign" by *Afirma* GSC [32].

Risk of Malignancy

The re-classification of NIFTP as non-malignant has had an impact on the ROM of the indeterminate diagnostic categories, including the FN category, but the magnitude of the impact that the NIFTP has had on malignancy rates varies by institution. The range reported for the ROM of the FN category ranges from 20% to 50% with

an average of 30%, though this range can be 0.2–30% lower if excluding NIFTP in ROM calculations (see Chap. 1: Tables 1.2 and 1.4) [3–5, 9, 33]. Of note, the impact has been more significant in the West than in Asia where NIFTP is diagnosed much more sparingly; in one meta-analysis, the average relative decrease of the ROM for the FN category was 22% and 32% for Asian and Western studies, respectively [34].

Finally, although some studies have reported a higher ROM for the FN category in the pediatric population as compared to the adult one, meta-analytic data has shown no statistically significant difference between the two groups (see Chap. 1, Table 1.3) [35].

Differential Diagnosis of FN

A variety of relatively rare lesions (paraganglioma, hyalinizing trabecular tumor) and metastatic low-grade carcinomas from other primary sites can mimic FN; only parathyroid lesions are covered here.

Fine needle aspirations of parathyroid lesions (adenomas or hyperplasia) are composed of cells that resemble crowded and overlapping follicular cells, often in microfollicular arrangement, therefore mimicking FN (Fig. 5.5a). Even when the FNA is performed with ultrasound guidance, it may not be clear to the aspirator that the lesion arises from a parathyroid gland rather than the thyroid, particularly if the parathyroid gland is located within the thyroid parenchyma or thyroid capsule. When submitted as a "thyroid FNA" specimen, parathyroid samples are often misinterpreted as FN. If there is a clinical suspicion that the lesion may be parathyroid (such as in the context of hypercalcemia), or if there are cellular features suggesting that possibility (e.g., prominent "salt and pepper" chromatin, crowded trabeculae in an aspirate lacking colloid, or triangular clusters—the so-called "wedge pattern"), then the possibility of a parathyroid lesion may be suggested in the report (see Example 5) [36, 37]. Immunocytochemistry can be instrumental in reaching the correct diagnosis, in particular GATA3 and parathormone (PTH); parathyroid lesions are also usually positive for chromogranin, synaptophysin, and CD56, but are negative for

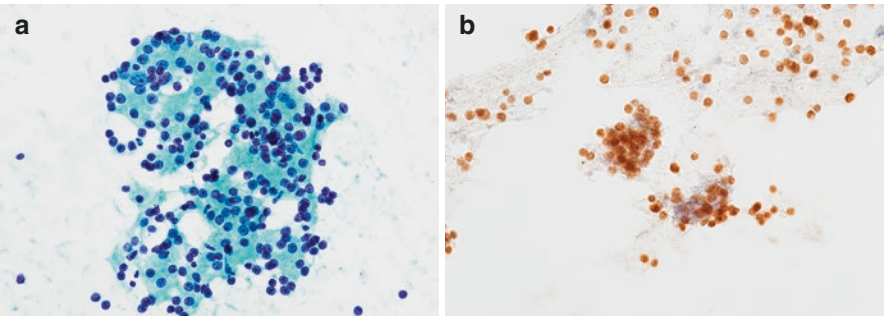

Fig. 5.5 Fine needle aspirate of a parathyroid adenoma, mimicking a follicular neoplasm (**a**: smear, Papanicolaou stain; **b**: Immunocytochemistry for GATA3)

thyroglobulin, TTF-1, and calcitonin [36, 37]. Assessment of PTH in the needle washout of FNA is also a powerful tool to rule in or rule out parathyroid lesions. The Afirma® GSC, which can be used as an adjunct to cytology for FN cases, includes a cassette that recognizes the gene expression profile of parathyroid neoplasms.

Cyto-Histologic Correlation

There are robust data on the predictive value of the FN interpretation because most patients with this FNA diagnosis undergo surgery. In most series, the most common histopathological diagnosis is FA, followed by adenomatous nodule/hyperplasia (follicular nodular disease), and much less frequently by FVPTC, NIFTP, and FTC. With the revised diagnostic criteria in the second and third editions of TBSRTC, however, it should be expected that the proportion of FVPTC and NIFTP would increase among cases diagnosed as FN. FA and follicular nodular disease account for approximately 40–45% and 30–35% respectively of the cases on follow-up [3–5]. The likelihood that the nodule is neoplastic (i.e., the risk of neoplasia, RON) is 65–75%, while the ROM is significantly lower (see section above "ROM," and Chap. 1: Tables 1.2 and 1.4) [3–5, 9, 33].

Management

According to the 2015 American Thyroid Association management guidelines, surgical excision (lobectomy) is the long-established standard of care for this diagnosis [2]. However, after consideration of clinical and sonographic features, molecular testing may be used to supplement malignancy risk assessment data in lieu of proceeding directly with surgery; informed patient preference and feasibility should be considered in clinical decision-making [2]. The extent of surgery may be expanded if the molecular testing results are considered high risk for malignancy. Conversely, a conservative approach could be chosen in patients who do not want surgery if the molecular data are of low suspicion for malignancy. Geographic variations on the use of molecular testing exist depending on availability and preferences; for example, in Japan, some FN cases are preferentially followed up without the use of molecular testing when a variety of triaging criteria/parameters (including ultrasonographic findings, tumor size, tumor volume doubling rate) suggest a low-risk FN (see Chaps. 13 and 14) [38].

Sample Reports

If an aspirate is interpreted as FN, it is implied that the sample is adequate for evaluation (i.e., an explicit statement of adequacy is optional). The general category FN is a self-sufficient interpretation and narrative comments that follow are optional; however, such comments are certainly suggested when there is concern for NIFTP/FVPTC. An educational note specifying the risk of malignancy for this interpretation, derived from the laboratory itself or from the literature, is optional.

Example 1
FOLLICULAR NEOPLASM.

Example 2
FOLLICULAR NEOPLASM.
Cellular aspirate of follicular cells with a predominantly microfollicular architecture, scattered isolated cells, and scant colloid. No nuclear features of papillary thyroid carcinoma are identified.

Example 3
FOLLICULAR NEOPLASM.
Cellular aspirate of follicular cells with a predominantly microfollicular architecture, scattered isolated cells, and scant colloid.
Note: Although the cytologic features are in keeping with a follicular neoplasm (FN), approximately 30% of cases diagnosed as Follicular Neoplasm (Bethesda IV) on FNA turn out to be benign follicular nodular disease on surgical resection. Potential histologic follow-up on resection include most commonly follicular adenoma, and less commonly (in decreasing order of frequency) hyperplastic nodules, follicular thyroid carcinoma, and much less likely NIFTP or FVPTC.

Example 4
FOLLICULAR NEOPLASM.
Note: Although the architectural features suggest a follicular neoplasm, some nuclear features raise the possibility of an invasive follicular variant of papillary carcinoma or its indolent counterpart, NIFTP; definitive distinction among these entities is not possible on cytologic material.

Example 5
FOLLICULAR NEOPLASM.
Cellular aspirate composed predominantly of crowded uniform cells without colloid.
Note: The features suggest a follicular neoplasm, but the possibility of a parathyroid lesion cannot be excluded. Correlation with clinical, serologic, radiologic, PTH level in the needle washout, and molecular test findings (if any) should be considered.

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Follicular Neoplasm (Oncocytic Follicular Neoplasm)

6

William Faquin, Claire Michael, Ilka Ruschenburg, and Kristine Wong

Background

Ewing coined the term "Hürthle cell" in 1928 based upon the description of a cell by Hürthle in 1894. The term has become entrenched in the thyroid lexicon, even though Hürthle's original description is now believed to represent a parafollicular or C-cell of the thyroid gland [1]. In 1898, Askanazy was the first to describe the follicular-derived Hürthle cell as we know it today [2]. The oncocyte or Hürthle cell (also called Askanazy cell and oxyphilic cell) is defined morphologically as a thyroid follicular cell with an abundance of finely granular cytoplasm that reflects an excessive number of mitochondria. In view of recent changes in the fifth Edition of the World Health Organization (WHO) Classification of Endocrine and Neuroendocrine Tumours, the term "oncocyte" will be used [3]. In oncocytic tumors, this striking cellular feature appears to be a consequence of alterations of

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mitochondrial and nuclear DNA that codes for proteins involved in oxidative phosphorylation, e.g., GRIM-19 [4–7]. Most oncocytes have an enlarged, round to oval nucleus and a prominent nucleolus.

Oncocytes are commonly seen in reactive/hyperplastic conditions like lymphocytic (Hashimoto) thyroiditis (LT) and multinodular goiter (MNG), where they are considered metaplastic, non-neoplastic follicular cells, but they can also be neoplastic (now designated as "oncocytic adenoma" and "oncocytic carcinoma" by the WHO) [3]. The WHO considers oncocytic adenoma and oncocytic carcinoma as encapsulated, follicular-patterned tumors composed of >75% oncocytic cells that lack characteristic nuclear features of papillary thyroid carcinoma (as in oncocytic PTC) and high-grade features (necrosis and ≥5 mitoses per 2 mm^2) [3]. In The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), FNA specimens that are suspicious for an oncocytic (Hürthle cell) neoplasm are distinguished from those suspicious for a non-oncocytic follicular neoplasm for two reasons: (1) there is a striking morphologic difference between these two cytologic patterns, which raises different diagnostic considerations, and (2) there are data to suggest that follicular and oncocytic carcinomas are genetically different neoplasms [4, 6–10]. For example, the *PAX8::PPARγ* rearrangement is seen in 26–53% of follicular carcinomas but rarely in oncocytic carcinomas [11, 12].

In TBSRTC, the term "Follicular neoplasm (oncocytic follicular neoplasm)" (FN-OFN) is used (see section on "Sample Reports" at the end of this chapter). It should be kept in mind, however, that a subset of cases (16–25%) in this category prove not to be neoplasms but rather hyperplastic proliferations of oncocytes in MNG or LT [13, 14].

Oncocytic carcinomas are uncommon, representing only 15–20% of all follicular carcinomas [15]. As with (non-oncocytic) follicular adenoma and carcinoma, the distinction between oncocytic adenoma and oncocytic carcinoma is based upon histologic evidence of transcapsular and/or vascular invasion. For this reason, thyroid FNA is used as a screening test for the detection of a probable oncocytic neoplasm that requires surgical excision (lobectomy) for precise histologic classification [16]. Although FNA is highly sensitive for detecting oncocytic carcinomas, its specificity is low: most nodules diagnosed by FNA as FN-OFN are benign, with a risk of malignancy of 25–50% [14, 17–19]. Updates to commercially available molecular testing platforms can be used to help guide management decisions for FNAs classified as FN-OFN [20, 21].

Definition

The interpretation "Follicular neoplasm (oncocytic follicular neoplasm)" refers to a cellular aspirate that consists exclusively (or almost exclusively) of oncocytes. Oncocytes with nuclear features of PTC are excluded from this category, although in some cases the distinction can be difficult (see Explanatory Notes).

Criteria

Specimens are moderately to markedly cellular.

The sample consists exclusively (or almost exclusively) of **oncocytes**:

- Abundant finely granular cytoplasm (blue or gray-pink with Romanowsky stains, green with Papanicolaou, pink with hematoxylin and eosin).
- Enlarged, central or eccentrically located, round nucleus.
- Prominent nucleolus.
- Small oncocytes with high nuclear/cytoplasmic (N/C) ratio.
- Large oncocytes with at least 2× variability in nuclear size.
- Oncocytes can be dispersed as isolated cells, arranged in sheets, or crowded groups.
- Binucleation is fairly common.
- There is usually little or no colloid.
- There are virtually no lymphocytes (excluding blood elements) or plasma cells.
- Transgressing vessels are present in some cases as well as intracytoplasmic "colloid" inclusions (lumens) [22].

Explanatory Notes

The FN-OFN aspirate is at least moderately cellular (Fig. 6.1), and excluding blood elements, is composed almost exclusively of oncocytes (Fig. 6.2) [8, 23–26]. Sparsely cellular samples do not qualify for this interpretation, and a diagnosis of AUS should be considered in this scenario instead. A small number of benign follicular cells may be present, but this is uncommon and usually represents sampling of the adjacent thyroid tissue. Similarly, lymphocytes are usually absent or rare. The oncocytes are often dispersed as isolated cells (Fig. 6.3) or as irregular three-dimensional groups (Fig. 6.2) [25, 26]. Oncocytic cells often show atypia, of which there are two dominant types. The atypia can be in the form of very large cells with

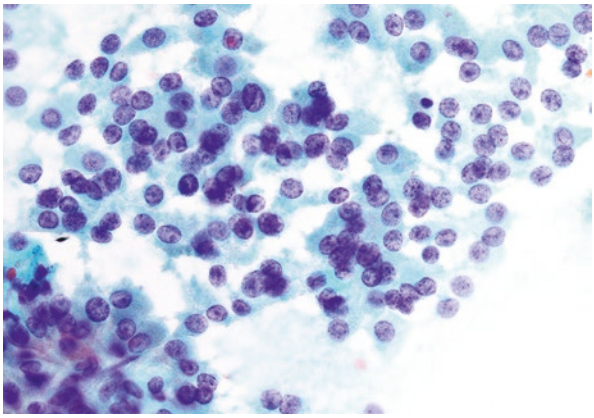

Fig. 6.1 Follicular neoplasm (oncocytic follicular neoplasm). The aspirate is hypercellular and consists of oncocytes of variable size arranged as isolated cells and in crowded groups; colloid is absent (smear, Papanicolaou stain)

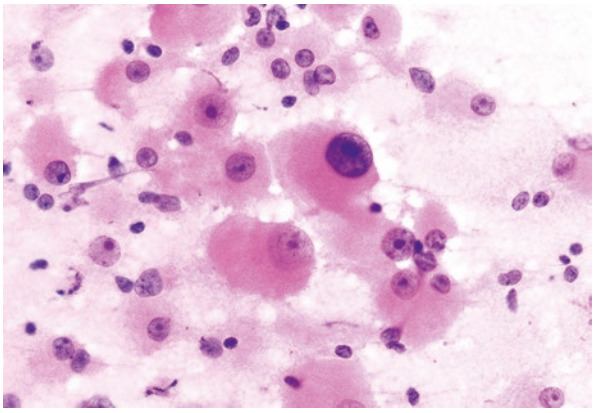

Fig. 6.2 Follicular neoplasm (oncocytic follicular neoplasm). The aspirate consists of a pure population of oncocytes with variation in cell size. The background lacks colloid and lymphocytes (smear, modified H&E stain)

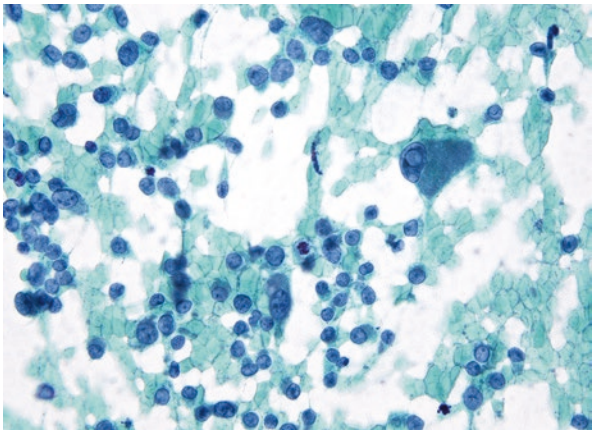

Fig. 6.3 Follicular neoplasm (oncocytic follicular neoplasm). The aspirate is cellular and consists exclusively of oncocytes in an isolated cell pattern simulating medullary thyroid carcinoma. An intranuclear pseudoinclusion is also observed (smear, Papanicolaou stain)

abundant granular cytoplasm that demonstrate at least twofold variability in nuclear size (Figs. 6.4 and 6.5) or relatively smaller oncocytes notable for less abundant granular cytoplasm and a higher nuclear to cytoplasmic ratio than the former (Figs. 6.6 and 6.7) [19, 27, 28]. Admixtures of small and large oncocytes are seen in some cases (Figs. 6.8 and 6.9). Importantly, oncocytic atypia by itself is an unreliable feature for the diagnosis, since very marked hyperchromasia, anisonucleosis, and nuclear membrane irregularity of oncocytes can be seen in MNG and LT [8]. Cellular cases lacking oncocytic atypia are suggestive of a benign nodule. Colloid is usually scant or absent, although a rare subset of oncocytic carcinomas with colloid has been described [28, 29]. Transgressing vessels are present in some cases and strongly support the diagnosis of a neoplasm over a non-neoplastic/metaplastic proliferation (Figs. 6.10 and 6.11) [22].

When an aspirate has all (or most) of the aforementioned features, the diagnosis of FN-OFN is straightforward. Problems arise with regard to: (1) the minimum necessary criteria for the diagnosis, (2) the best way to handle oncocytic proliferations in a patient with MNG or LT, and (3) the distinction from

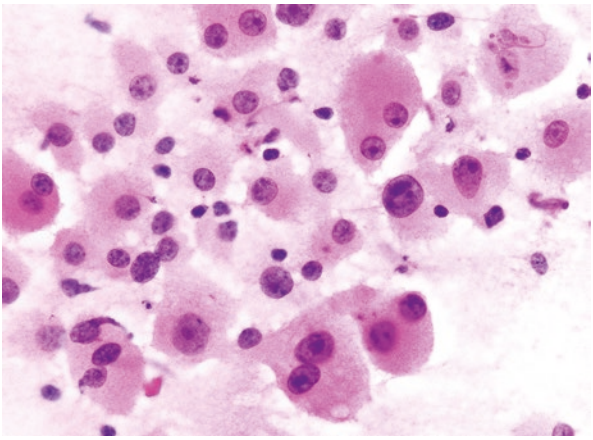

Fig. 6.4 Follicular neoplasm (oncocytic follicular neoplasm). The aspirate consists of numerous dispersed oncocytes. The nuclei are atypical and highly variable in size (smear, modified H&E stain)

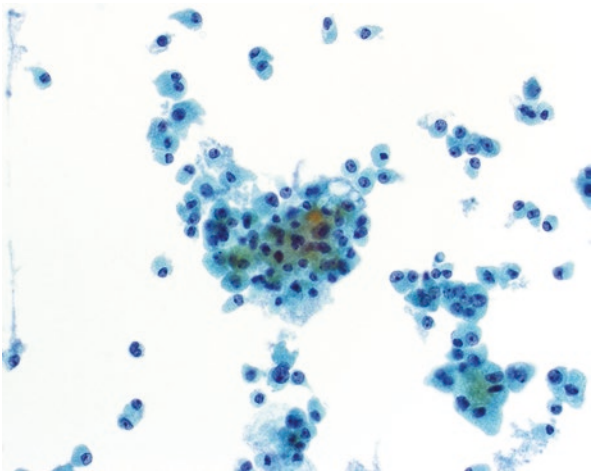

Fig. 6.5 Follicular neoplasm (oncocytic follicular neoplasm). The aspirate consists of oncocytes arranged in syncytial groups as well as dispersed neoplastic cells in the background. Colloid is absent (ThinPrep, Papanicolaou stain)

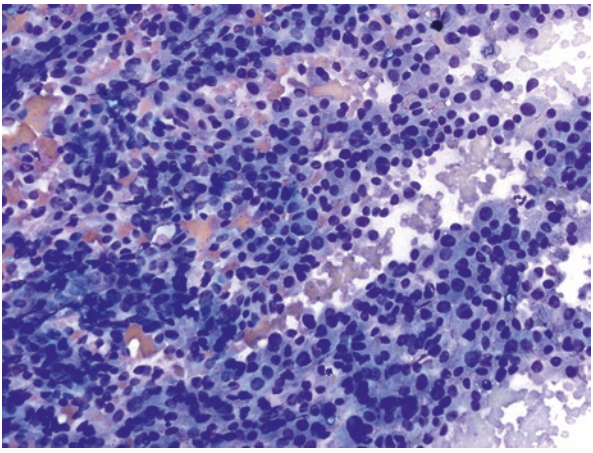

Fig. 6.6 Follicular neoplasm (oncocytic follicular neoplasm). Highly cellular aspirate comprised of oncocytes arranged in disorganized syncytial groups and an absence of background colloid (smear, Diff-Quik stain)

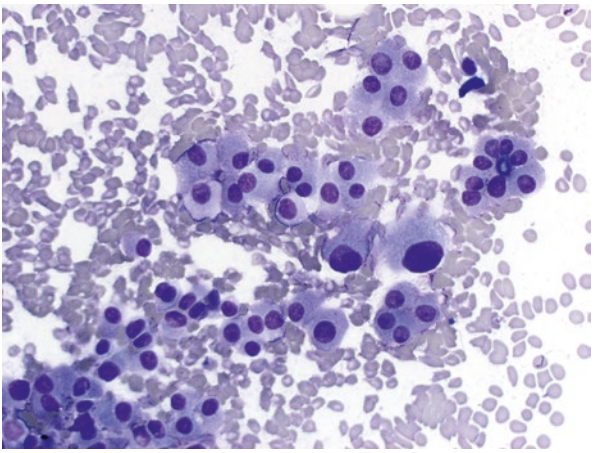

Fig. 6.7 Follicular neoplasm (oncocytic follicular neoplasm). The aspirate contains numerous loosely cohesive atypical oncocytes, which are highly variable in size (smear, Diff-Quik stain)

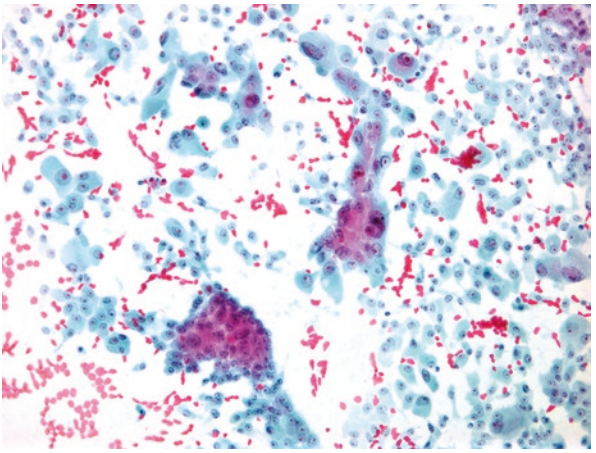

Fig. 6.8 Follicular neoplasm (oncocytic follicular neoplasm). The aspirate is highly cellular and consists of oncocytes with prominent nucleoli. The cells vary in size, and some are in small crowded groups with less than "usual" cytoplasm (smear, Papanicolaou stain)

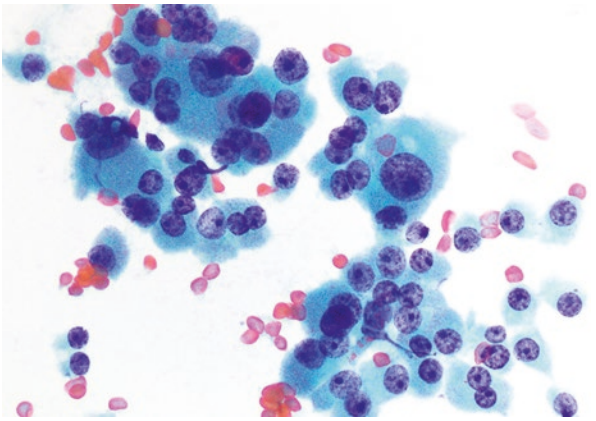

Fig. 6.9 Follicular neoplasm (oncocytic follicular neoplasm). The aspirate consists of loosely cohesive oncocytes. The cells are highly variable in size and amount of cytoplasm, transitioning from very large cells with abundant cytoplasm and macronucleoli to smaller, more uniform oncocytes with a high nuclear/cytoplasmic ratio (smear, Papanicolaou stain)

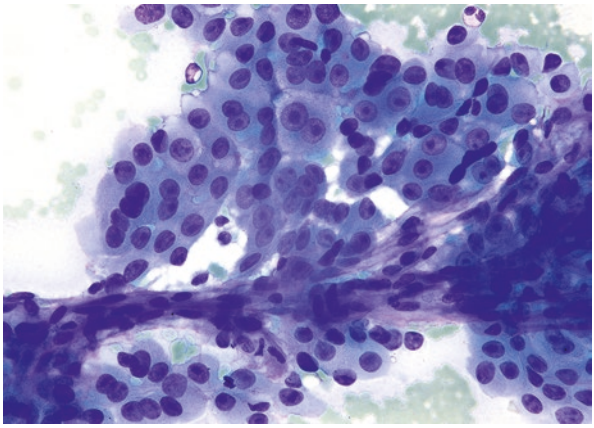

Fig. 6.10 Follicular neoplasm (oncocytic follicular neoplasm). This cellular aspirate consists of oncocytes with macronucleoli in crowded groups. Colloid is absent, and prominent transgressing vessels are present (smear, Diff-Quik stain)

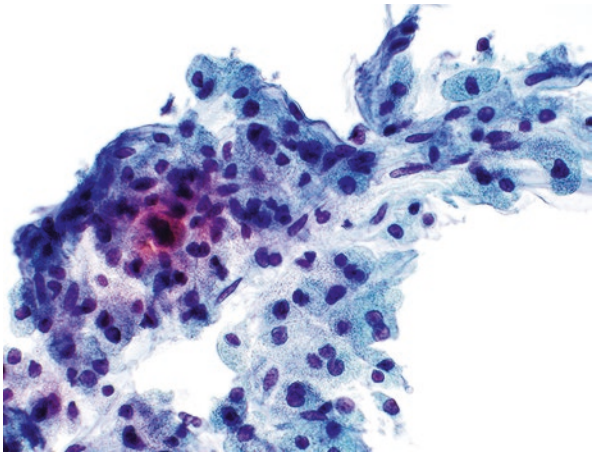

Fig. 6.11 Follicular neoplasm (oncocytic follicular neoplasm). Transgressing vessels associated with oncocytic follicular neoplasms can be seen with liquid-based preparations (ThinPrep, Papanicolaou stain)

follicular carcinoma, papillary carcinoma, medullary carcinoma, and parathyroid tumors.

Minimum Necessary Criteria

With regard to the *minimum criteria* for diagnosing FN-OFN, there are four general problematic scenarios.

The Sparsely Cellular Specimen Composed Entirely of Oncocytes and Frequently in a Background of Blood

A sparsely cellular aspirate certainly does not preclude an oncocytic carcinoma [27]. Most cytopathologists, however, are reluctant to make the diagnosis of FN-OFN on a scant aspirate; these aspirates are most appropriately diagnosed as

"Atypia of Undetermined Significance (AUS)" (see Chap. 4). A re-aspiration may resolve the diagnostic difficulty.

The Cellular Specimen Composed Entirely of Oncocytes Without Atypia

1. The cellular aspirate composed entirely of oncocytes but lacking atypia is more controversial. If the oncocytes are present as a uniform flat sheet of cells and accompanied by abundant colloid (particularly non-watery colloid), it is acceptable to interpret the sample as Benign [30]. It is important to note that some of these nodules may be PET-avid [31]. It is recognized, however, that some cytologists may prefer to classify these cases as AUS given that rare cases of oncocytic carcinoma with abundant colloid have been described [29].
2. If colloid is scant or absent, there are two different approaches to the cellular aspirate composed entirely of oncocytes without atypia. One approach is to diagnose such cases as FN-OFN. However, given that some investigators have shown that aspirates lacking atypia are almost never malignant [25, 27, 28], some cytopathologists diagnose pure oncocytic cases without atypia as benign or in some cases as AUS, accompanied by an optional note: "Although the predominance of oncocytes raises the possibility of an oncocytic neoplasm, the absence of atypia suggests that it is benign." Typically, such patients are followed clinically with periodic physical and sonographic examinations or, in AUS cases, reflex molecular testing can be considered.
3. It is not uncommon to encounter a relatively bland aspirate composed of cells with minimal atypia and intermediate amounts of cytoplasm (i.e., "oncocytoid" features). These cells have more granular cytoplasm than is seen in typical follicular cells but less than in usual oncocytes. Most of these cases can be diagnosed as benign with a similar (optional) note ("the findings raise the possibility of an oncocytic neoplasm, but the lack of atypia suggests it is benign").

The Clearly Abnormal Specimen with Partial or Minimal Oncocytic Differentiation

There are clearly abnormal cases (markedly cellular specimens with crowding and overlapping of cells, etc.) where oncocytic differentiation is focal rather than diffuse, or not as well developed as in most oncocytic neoplasms. In such cases, it is advisable to follow the guidelines of the WHO, which considers only those follicular neoplasms that are comprised of >75% oncocytes to be an oncocytic neoplasm [3]. Thus, a suspicious FNA in which <75% of the abnormal cells are well-developed oncocytes should be diagnosed as "Follicular Neoplasm" rather than FN-OFN. When oncocytic differentiation is not clear-cut (more granular cytoplasm than normal follicular cells, but less than usual oncocytes), it is often impossible to make a definitive distinction between follicular and oncocytic differentiation. Because the usual management is the same for both entities, a practical solution is to diagnose these aspirates as "Follicular Neoplasm," with the comment "Some oncocytic differentiation is present, and therefore an oncocytic neoplasm cannot be ruled out."

The Clearly Abnormal Specimen with Colloid

In some cases, an FNA sample may be at least moderately cellular, comprised exclusively of oncocytes with atypia in a background of colloid that is almost always "watery colloid" [18, 23, 29]. Despite the watery colloid, such cases should be diagnosed as FN-OFN. Finding hard colloid in this setting would be exceptional; such cases would be better diagnosed as AUS.

Oncocytic Proliferations in Patients with Multinodular Goiter or Lymphocytic Thyroiditis

The classic FN-OFN can be mimicked by a variety of other conditions, particularly MNG with oncocytic change and LT with oncocytic hyperplasia. Prominent oncocytic metaplasia often accompanies MNG, although typically one sees a mixture of elements: flat, cohesive honeycomb sheets of oncocytes admixed with normal follicular cells and a moderate to abundant amount of background colloid (Fig. 6.12). Aspirates with these features are easily recognized as benign and should not be interpreted as FN-OFN. Thus, whereas most aspirates of oncocytic neoplasms consist of a pure population of oncocytes, a mixture of oncocytes and non-oncocytic follicular cells is more indicative of a hyperplastic nodule. Exceptions to this rule occur and represent a limitation in the precise classification of these lesions by FNA. It is possible, for example, to aspirate normal follicular cells from adjacent thyroid tissue when aspirating an oncocytic neoplasm, particularly if the FNA is done without ultrasound guidance. Thus, a minor component of normal follicular cells does not exclude an oncocytic neoplasm. Conversely, some nodules in patients with MNG are composed exclusively of oncocytes (with or without significant nuclear atypia); such benign hyperplastic nodules masquerade as an oncocytic neoplasm. Clinical-cytologic correlation is a reasonable approach in such cases and can be performed by either the cytopathologist or the clinician. For example, in a patient

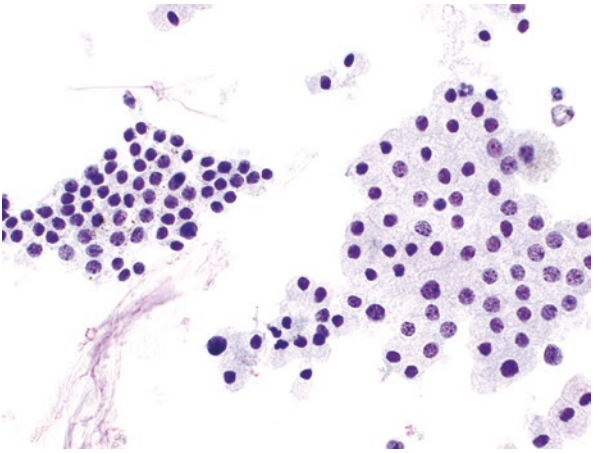

Fig. 6.12 Benign (multinodular hyperplasia with a prominent oncocytic component). There are benign follicular cells (*left*) and oncocytes (*right*) in cohesive flat sheets, with a moderate amount of watery ("tissue-paper") colloid. Such cases should not be called "Follicular neoplasm (oncocytic follicular neoplasm)" (ThinPrep, Papanicolaou stain)

known to have multiple nodules, it is acceptable to diagnose an exclusively oncocytic cell specimen as either FN-OFN or as AUS, although some have found similar rates of malignancy in the setting of solitary and multiple nodules [32]. If interpreted as AUS, an explanatory note that raises the possibility of oncocytic hyperplasia can be helpful (see Chap. 4, section on "Sample Reports", Example 4). The goal is to provide the clinician with the opportunity to avoid an unnecessary lobectomy in some of these patients. While a repeat aspiration in this setting of AUS is unlikely to add any helpful information, molecular testing could be considered.

In most nodules from patients with LT, lymphocytes predominate over oncocytes, and the benign aspirate is easily distinguished from an oncocytic neoplasm. In some patients with LT, however, oncocytic cell proliferations can produce nodules that exceed 1 cm in diameter and are composed of oncocytes with little or no lymphoid infiltrate (Fig. 6.13) [33]. When the lymphoid component is absent or inconspicuous, it can be difficult to exclude an oncocytic neoplasm. There may be a clue to the correct interpretation: in LT nodules, the oncocytes are "atypical" in a stereotypical way: they form small cohesive clusters of 3–10 cells containing large nuclei and smudgy, sometimes glassy chromatin. This nuclear atypia can mimic that found in papillary carcinoma, but it is not typical of oncocytic neoplasia.

Knowing that a patient has LT may impact the interpretation. In a patient known to have LT, it is acceptable to diagnose a specimen composed exclusively of oncocytes as either FN-OFN or AUS. Data suggest that the criteria for FN-OFN have a lower predictive value for malignancy when a patient has LT [34]. If interpreted as AUS, a note explaining that a benign oncocytic cell hyperplasia is favored can be very helpful (see Chap. 4, see section on "Sample Reports" Example 5). As in patients with MNG, the note that accompanies the AUS interpretation in a patient with LT is meant to more accurately reflect the underlying risk of malignancy, which although not well characterized or shown to be significantly different [32, 34] appears to be somewhat lower than for FN-OFN. The goal is to provide the clinician

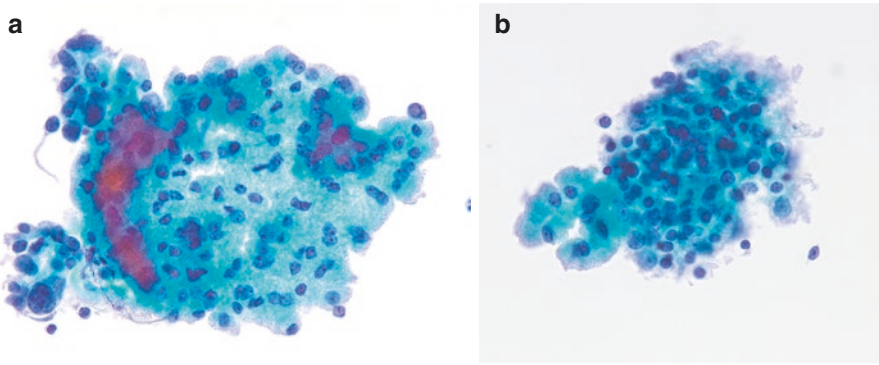

Fig. 6.13 Chronic lymphocytic thyroiditis. (**a**) Nodules in chronic lymphocytic (Hashimoto) thyroiditis can have a predominance of oncocytes. (**b**) Focally there were associated lymphoid aggregates. When the lymphoid component is scarce, cases may be interpreted as AUS (ThinPrep, Papanicolaou stain)

with the opportunity to avoid an unnecessary lobectomy in some of these patients. Note that in this AUS setting, a repeat aspiration is unlikely to provide any helpful information, but rather encourages clinical-cytologic correlation to more accurately predict the risk of malignancy. Molecular testing could also be considered in these cases.

Distinction from Other Tumors

The differential diagnosis of FN-OFN is broad and includes other follicular cell-derived neoplasms as well as non-follicular entities. Oncocytic follicular neoplasms can exhibit some of the architectural and nuclear features of papillary carcinoma, including papillary architecture (Fig. 6.14), mild nuclear atypia (pale chromatin, grooves, and rarely intranuclear pseudoinclusions), as well as occasional calcifications [35] (Fig. 6.15). Conversely, the cells of classic papillary carcinomas can show variable oncocytic change. This is particularly extensive in oncocytic papillary carcinoma (see Chap. 8). The abundance of granular cytoplasm in these neoplasms mimics that of oncocytic follicular neoplasms. Attention to nuclear details usually permits a distinction, but in some cases, it may not be possible to determine with certainty whether an aspirate is best classified as a papillary carcinoma or FN-OFN. Such aspirates can be diagnosed either as FN-OFN or "Suspicious for malignancy," accompanied by an explanatory note that includes the differential diagnosis of papillary carcinoma and an oncocytic follicular neoplasm. In such borderline cases, molecular testing could be considered (see Management, below). Even if the distinction cannot be made preoperatively, the threshold for total thyroidectomy for most low-risk differentiated (non-medullary) carcinomas has

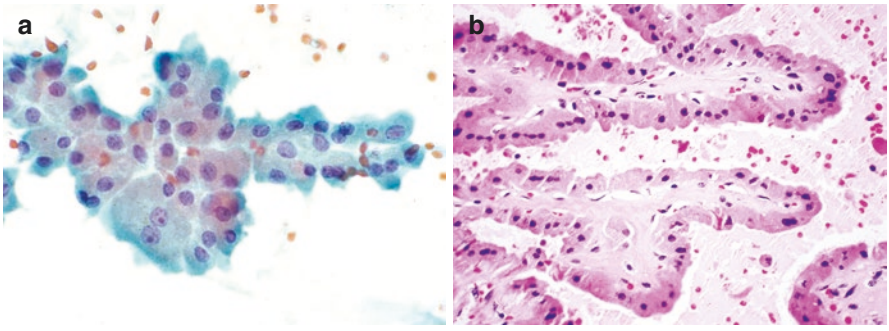

Fig. 6.14 Follicular neoplasm (oncocytic follicular neoplasm). (**a**) This cellular aspirate was comprised exclusively of oncocytes, without overt nuclear features of papillary thyroid carcinoma (smear, Papanicolaou stain). (**b**) Histologic examination revealed an oncocytic (Hürthle cell) carcinoma with papillary architecture. A subset of oncocytic neoplasms exhibits papillary architecture, with occasional cells that have an oval, pale, and grooved nucleus but lacking intranuclear pseudoinclusions. In such cases it can be difficult both cytologically and histologically to distinguish an oncocytic neoplasm from an oncocytic papillary thyroid carcinoma (H&E stain)

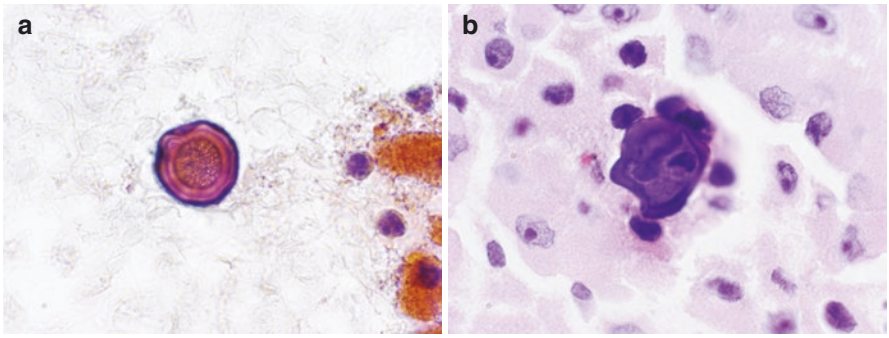

Fig. 6.15 Follicular neoplasm (oncocytic follicular neoplasm). (**a**) A minority of oncocytic neoplasms contain concentrically laminated concretions that are indistinguishable from psammoma bodies (smear, Papanicolaou stain). The correct diagnosis depends on the accompanying cellular features. (**b**) The histologic specimen revealed an oncocytic adenoma with similar concretions (H&E stain)

evolved such that lobectomy is often the initial procedure for tumors less than 4 cm regardless of subtype [36].

Some of the characteristic features of oncocytic follicular neoplasms also overlap with those of medullary thyroid carcinoma (Fig. 6.16), as many medullary carcinomas are comprised of isolated cells with abundant granular cytoplasm. The prominent nucleolus of many oncocytes, however, is absent from most medullary carcinomas. With Romanowsky stains, the cytoplasmic granules of oncocytes are typically blue, whereas those of medullary carcinoma are usually red. Intranuclear pseudoinclusions may be seen in medullary carcinoma and are typically absent or extremely rare in oncocytic follicular neoplasms. Immunohistochemistry is especially useful in the distinction between oncocytic follicular neoplasms and medullary carcinoma. Oncocytic follicular neoplasms are derived from follicular cells and are therefore positive for thyroglobulin but negative for calcitonin. In contrast, medullary carcinomas (derived from parafollicular C-cells) are negative for thyroglobulin but positive for calcitonin and chromogranin-A. When using immunostains, a panel including some that are expected to be positive (e.g., thyroglobulin) and others expected to be negative (e.g., calcitonin) is preferable to solitary antibody staining, as aberrant results can occur with any given antibody. Because the surgical approach to patients with medullary carcinoma often differs from that of oncocytic follicular neoplasms, cytopathologists should have a low threshold for confirmatory ancillary studies any time they consider a diagnosis of medullary carcinoma.

Aspirates of FN-OFN share cytologic features with oncocytic differentiated thyroid carcinomas with high-grade features (see Chap. 10). It can be difficult and in some cases impossible to distinguish FN-OFN from an oncocytic carcinoma with high-grade features by FNA; however, the presence of increased mitotic activity and/or background necrosis should raise the possibility of the latter [3, 37].

Parathyroid adenomas (and the rare carcinomas) usually mimic follicular neoplasms, but some have abundant granular to vacuolated cytoplasm that can mimic

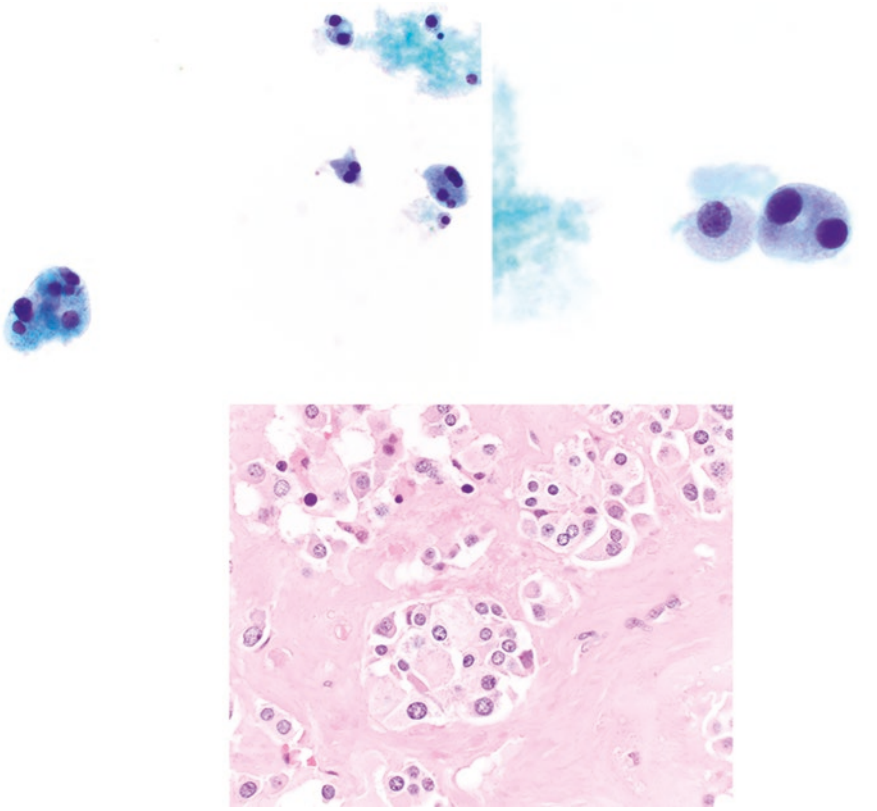

Fig. 6.16 Medullary thyroid carcinoma. Some medullary carcinomas can have abundant granular or oncocytic cytoplasm (ThinPrep, Papanicolaou stain). This case was diagnosed as follicular neoplasm (oncocytic follicular neoplasm), although subsequent molecular testing was consistent with medullary carcinoma. Resection demonstrated abundant granular, eosinophilic cytoplasm in the tumor (H&E stain)

instead oncocytic follicular neoplasms (Fig. 6.17). In some cases, imaging characteristics or serologic testing can suggest the diagnosis; however, when relying on morphologic evaluation, only 20–30% of parathyroid lesions are recognized [38–40]. In contrast to oncocytic follicular cells, the cells of a parathyroid adenoma, when composed of chief cells with abundant cytoplasm, are monomorphous with round, hyperchromatic nuclei and indistinct nucleoli. However, occasionally parathyroid adenomas can have extensive oncocytic change (oncocytic parathyroid adenoma) with cells that are morphologically indistinguishable from oncocytic follicular cells. If parathyroid tissue is suspected, immunohistochemistry can be used to confirm the diagnosis. Parathyroid tumors are immunoreactive for parathyroid hormone (PTH), GATA3, and chromogranin-A (and to a much lesser extent synaptophysin) [41] and are negative for thyroglobulin and TTF-1. If cyst fluid is obtained and submitted for chemical analysis, a high PTH

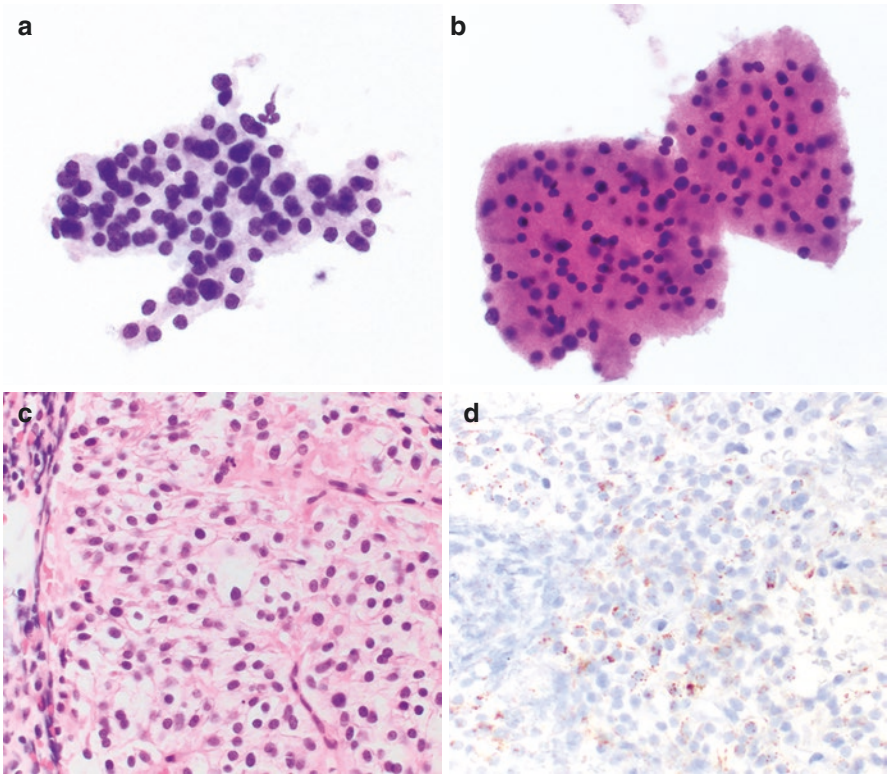

Fig. 6.17 Parathyroid tissue. (**a**, **b**) The amount of cytoplasm in parathyroid lesions can be variable, ranging from moderate to abundant (ThinPrep, Papanicolaou stain). (**c**) In cases where parathyroid tissue is suspected, a cell block can be made (H&E stain). (**d**) Immunohistochemistry for PTH was performed to confirm the diagnosis

level is diagnostically helpful [40]. In the event that a parathyroid lesion is misdiagnosed as FN-OFN, molecular testing would be able to distinguish it from a thyroid lesion. For example, the Afirma Genomic Sequencing Classifier (GSC) (Veracyte, Inc., South San Francisco, CA) (see Management, below) includes a "cassette" that recognizes the expression profile of parathyroid cells [20]. However, a distinction will not always be possible, especially if immunohistochemistry or molecular testing is not available or is inconclusive. In such cases, the possibility of a parathyroid tumor can be raised in the note that accompanies the interpretation.

Other less frequently encountered entities in the thyroid that can mimic oncocytic follicular lesions include metastases (e.g., clear cell renal cell carcinoma, see Chap. 12) and rare mesenchymal tumors, in particular granular cell tumor [42, 43] (Fig. 6.18). If suspected, the diagnosis of granular cell tumor can be confirmed by S-100 protein positivity and keratin negativity by immunohistochemistry.

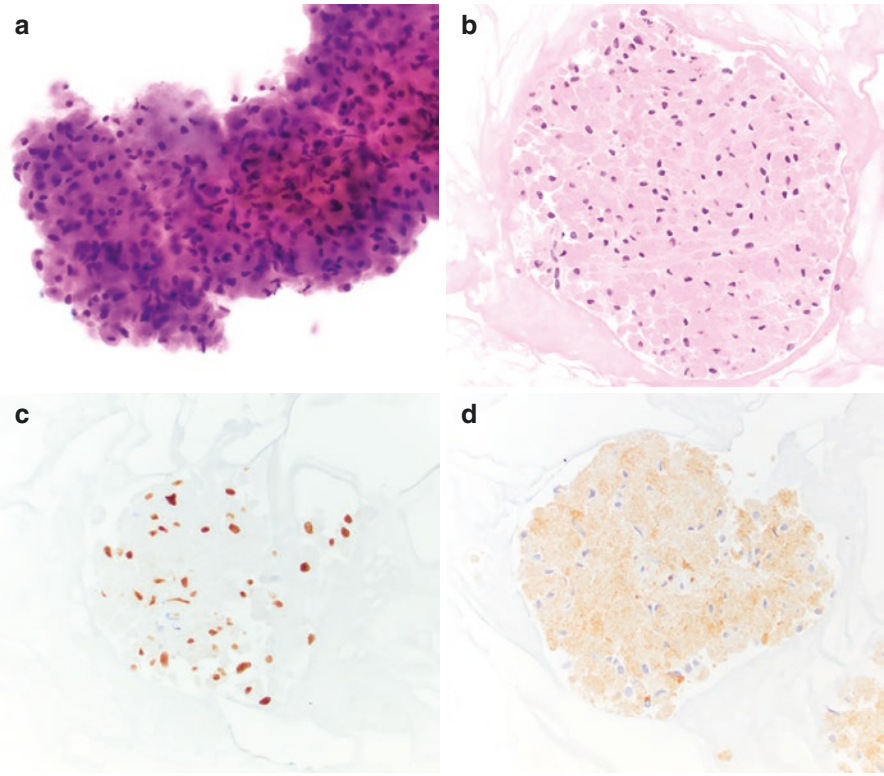

Fig. 6.18 Granular cell tumor. (**a**) Granular cell tumors have abundant granular cytoplasm, similar to oncocytic follicular cell-derived tumors (ThinPrep, Papanicolaou stain). (**b**) A cell block was made in this case and showed clusters of bland tumor cells with voluminous eosinophilic cytoplasm (H&E stain). (**c**) SOX10 and (**d**) CD68 stains were positive in the tumor cells, supporting the diagnosis of granular cell tumor

Management

As with all thyroid nodules, informed patient preference and clinical and sonographic features should be considered in the management decision of nodules interpreted as FN-OFN, although diagnostic surgical excision has historically been the standard of care. According to the 2015 American Thyroid Association guidelines, molecular testing may be used to supplement the malignancy risk assessment in lieu of proceeding directly to surgery [36], although limitations in molecular testing of oncocytic lesions initially prompted caution in its use [44]. However, improved performance of molecular diagnostics in oncocytic follicular cell aspirates has made this approach more viable in triaging these lesions [20, 45, 46].

Molecular test performance in oncocyte-predominant aspirates has greatly benefitted from the improved understanding of the pathogenesis of oncocytic follicular neoplasms. For example, unlike other follicular cell-derived tumors, oncocytic

follicular neoplasms frequently have mitochondrial DNA mutations and copy number alterations. They also demonstrate a lower frequency of *RAS* mutations, while *PAX8::PPARγ* rearrangements and *BRAF* V600E mutations (associated with follicular neoplasms and papillary carcinomas, respectively) are essentially absent [9, 10, 47]. The newest iteration of Afirma GSC (Veracyte, Inc., South San Francisco, CA) incorporates mRNA expression, sequencing, and copy number analysis into their updated algorithm that includes dedicated oncocytic cell (Hürthle cell) indices [20]. Thyroseq v3 (CBLPath/Sonic Healthcare, Rye Brook, NY), which includes sequencing and to a lesser extent gene expression and copy number analysis, has also demonstrated improved performance in oncocytic nodules [48].

Sample Reports

If an aspirate is interpreted as FN-OFN, it is implied that the sample is adequate for evaluation. (An explicit statement of adequacy is optional.) The interpretation FN-OFN is self-sufficient; narrative comments that follow are optional.

Example 1
FOLLICULAR NEOPLASM (oncocytic follicular neoplasm).

Example 2
FOLLICULAR NEOPLASM (oncocytic follicular neoplasm).
Cellular aspirate consisting predominantly of oncocytes in syncytial-like sheets and crowded clusters.

Example 3
FOLLICULAR NEOPLASM (oncocytic follicular neoplasm).
Cellular aspirate consisting of abundant isolated oncocytes in the absence of colloid.

Example 4
FOLLICULAR NEOPLASM (oncocytic follicular neoplasm).
Cellular aspirate of follicular cells with oncocytic features, including occasional nuclear grooves and focal papillary architecture.
Note: The findings raise the possibility of an oncocytic neoplasm with papillary features, but a papillary thyroid carcinoma cannot be excluded.

Example 5
FOLLICULAR NEOPLASM (oncocytic follicular neoplasm).
Cellular aspirate composed of cells with abundant granular cytoplasm.
Note: The findings raise the possibility of an oncocytic neoplasm, but a parathyroid tumor cannot be excluded. Correlation with clinical findings, imaging findings, and serologic testing results might be helpful.

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Suspicious for Malignancy

7

Fernando Schmitt, Ashish Chandra, Armando Filie, and Chiung-Ru Lai

Background

Most primary thyroid malignancies have distinctive cytologic features and are easily recognized on fine needle aspiration (FNA). The exceptions are follicular and oncocytic carcinomas (addressed in Chaps. 5 and 6). Although the cytologic features of papillary thyroid carcinoma (PTC), medullary thyroid carcinoma (MTC), and lymphoma are well established (see Chaps. 8, 9, and 12), in any given specimen they may be quantitatively and/or qualitatively insufficient for a definitive diagnosis. The reasons for diagnostic uncertainty in such cases include suboptimal sampling or cellular preservation, an unusual variant of PTC or MTC, overlapping cytomorphologic (particularly nuclear) features with other thyroid lesions, or inability to perform immunophenotyping for lymphoma. The reactive, involutional, and metaplastic changes of benign follicular cells in some cases of chronic lymphocytic

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(Hashimoto) thyroiditis can be difficult to distinguish from those of PTC, and the lymphoid cells of thyroiditis can be difficult to distinguish from those of a lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). A diagnostic category that conveys a strong suspicion for malignancy, therefore, is a necessity for thyroid FNA, and in the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) it is termed "Suspicious for Malignancy (SFM)." SFM is a heterogeneous category because it includes a variety of different primary and secondary malignancies. Most SFM cases are suspicious for PTC, although in many published series the type of suspected malignancy is not specified. SFM diagnoses account for approximately 3% (range 1.0–6.3%) of all thyroid FNAs [1–4]. As with any indeterminate diagnosis, this category should be used judiciously so that patients are managed as appropriately as possible.

The ultimate goal of separating a "suspicious" from a "malignant" category is to preserve the very high positive predictive value (PPV) of the malignant category without compromising the overall sensitivity of FNA. A SFM interpretation indicates to the clinical-surgical team the less than definitive nature of the diagnosis and allows for more conservative management options (e.g., surgical lobectomy) if indicated.

Rapid on-site evaluation (ROSE) of thyroid aspirates, ideally performed by a trained sonographer (radiologist or pathologist) assisted by a cytotechnologist, adds value to the FNA procedure by reducing the number of suspicious cases due to either low cellularity or those requiring confirmation on ancillary workup (medullary carcinoma, anaplastic carcinoma, lymphoma, or metastatic malignancies such as melanoma). Guided by ROSE, dedicated passes collected in appropriate transport media for immunochemistry on cell blocks or flow cytometry could allow a definitive diagnosis to be reached using the cytology sample, avoiding the need for another patient visit for a repeat FNA or an alternative method of sampling such as core biopsy.

A distinction between a malignant and suspicious diagnosis (and between suspicious and atypical) is admittedly subjective. A malignant diagnosis should be reserved for those cases that show sufficient cellularity and most, if not all, of the diagnostic features of the entity in question. An SFM interpretation is appropriately rendered when some of the diagnostic features are either absent or equivocal [5].

SFM is used for thyroid FNAs that are more likely than not malignant. The PPV of the SFM category is approximately 74% (range 50–80%) [1, 2, 6–15]. These numbers overestimate the malignancy risk because they don't account for the reclassification of the "non-invasive follicular variant of papillary thyroid carcinoma" as "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP), given the indolent behavior of this thyroid tumor [16]. The malignancy risk of the SFM category falls to approximately 65% when NIFTPs are not counted as malignant (see Chap. 1) [17, 18]. NIFTP is, nevertheless, a "surgical disease" (i.e., surgery is necessary for these nodules), and the higher risk estimate is arguably still appropriate for SFM if risk estimates are defined for surgical disease.

Definition

The diagnostic category "Suspicious For Malignancy" (SFM) is used when some cytomorphologic features (most often those of PTC) raise a strong suspicion of malignancy but the findings are not sufficient for a conclusive diagnosis. Specimens that are suspicious for a follicular or oncocytic neoplasm are excluded from this category (see Chaps. 5 and 6). For the category SFM, the morphologic changes are of such a degree that a malignancy is considered more likely than not.

Criteria

Suspicious for Papillary Thyroid Carcinoma

Patchy Nuclear Changes Pattern (Figs. 7.1 and 7.2)

The sample is moderately or highly cellular.

Unremarkable follicular cells (arranged predominantly in sheets and/or macrofollicular fragments) are admixed with cells that have nuclear enlargement, nuclear pallor, limited nuclear grooves, nuclear membrane irregularity, and/or nuclear molding.

Intranuclear pseudoinclusions (INPIs) are very few or absent, and psammoma bodies and papillary architecture are absent.

Incomplete Nuclear Changes Pattern (Fig. 7.3)

The sample is sparsely, moderately, or highly cellular.

There is generalized mild-to-moderate nuclear enlargement with mild nuclear pallor.

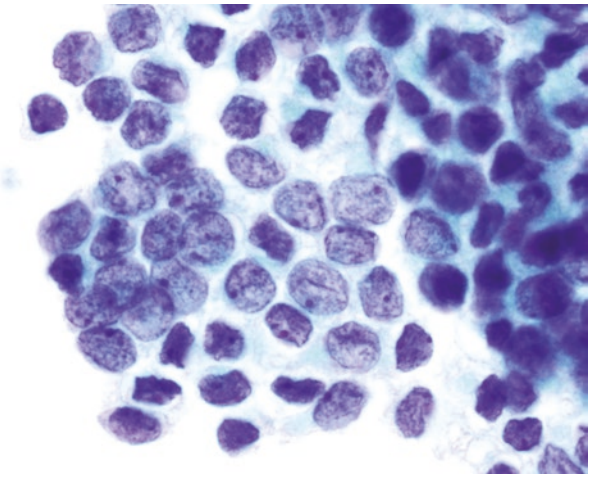

Fig. 7.1 Suspicious for papillary thyroid carcinoma. This sheet of follicular cells displays some features of papillary carcinoma, including nuclear enlargement, powdery chromatin, nuclear membrane irregularity, nuclear grooves and molding, and small nucleoli. These changes were patchy, however, and other follicular cells looked benign (ThinPrep, Papanicolaou stain)

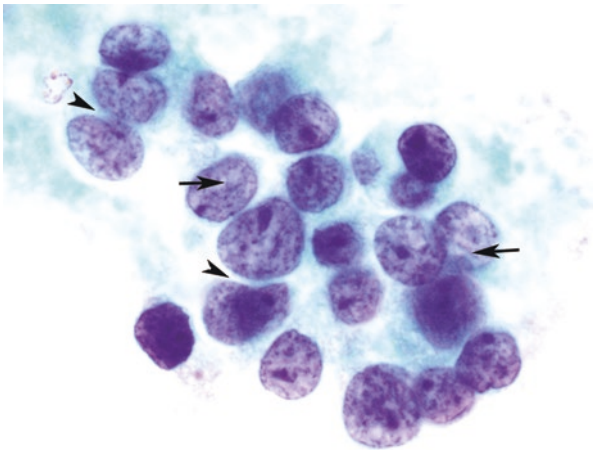

Fig. 7.2 Suspicious for papillary thyroid carcinoma. This loose sheet of follicular cells demonstrates enlarged nuclei, powdery chromatin, nucleoli, and nuclear grooves. There are some questionable (i.e., small, poorly defined) intranuclear pseudoinclusions (*arrows*) and slight nuclear molding (*arrow heads*). These changes were patchy, however, and other follicular cells looked entirely benign (ThinPrep, Papanicolaou stain)

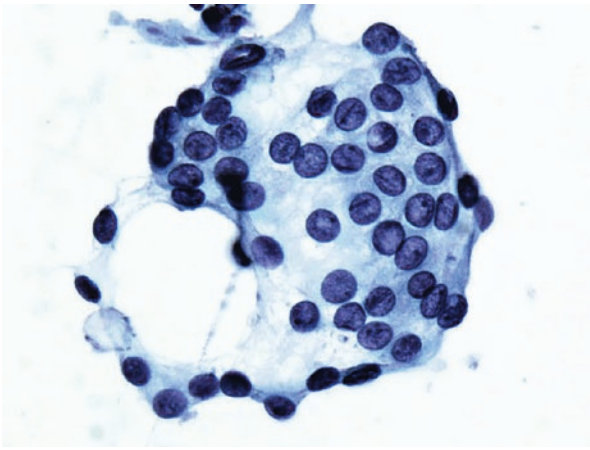

Fig. 7.3 Suspicious for papillary thyroid carcinoma. In this specimen, there were generalized but mild nuclear changes. A loose sheet of follicular cells shows slightly enlarged nuclei, variable chromatin pallor, small but prominent nucleoli, nuclear grooves, and minimal molding (ThinPrep, Papanicolaou stain)

Nuclear grooves may be evident, but nuclear membrane irregularity and nuclear molding are not apparent.

Intranuclear pseudoinclusions (INPIs) are very few or absent, and psammoma bodies or papillary architecture are absent.

Sparsely Cellular Specimen Pattern

Many of the features of PTC (see Chap. 8) are present, but the sample is very sparsely cellular.

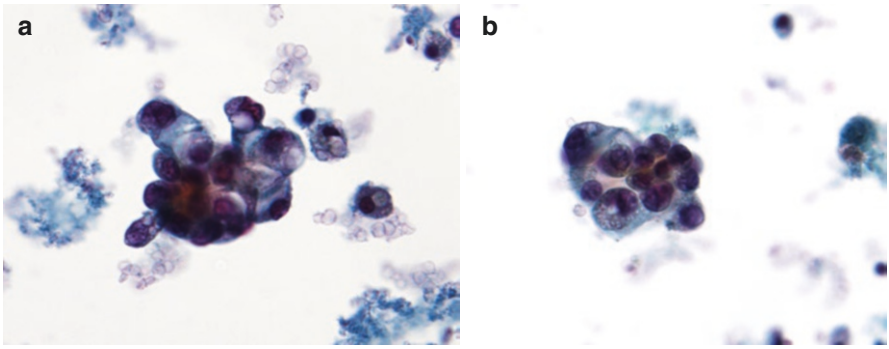

Fig. 7.4 (**a**, **b**) Suspicious for cystic papillary thyroid carcinoma. Groups of large, atypical, "histiocytoid" cells with enlarged nuclei and abundant vacuolated cytoplasm (ThinPrep, Papanicolaou stain)

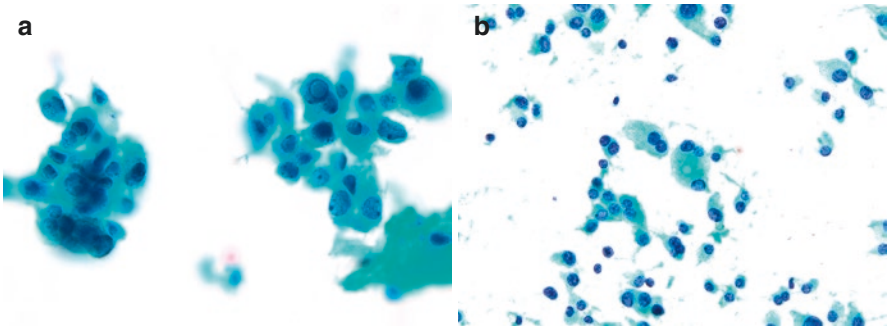

Fig. 7.5 (**a**, **b**) Suspicious for medullary thyroid carcinoma. Loose clusters and dyshesive monomorphic population of small or medium-sized cells with relatively increased nuclear/cytoplasmic (N/C) ratio, eccentrically located nuclei, rare INPIs, and smudgy chromatin (SurePath, Papanicolaou stain)

Cystic Degeneration Pattern (Fig. 7.4)

There is evidence of cystic degeneration based on the presence of hemosiderin-laden macrophages.

Scattered groups and sheets of follicular cells have enlarged, pale nuclei and some have nuclear grooves, but INPIs are very few or absent, and psammoma bodies or papillary architecture are absent.

There are occasional large, atypical, "histiocytoid" cells with enlarged nuclei and abundant vacuolated cytoplasm.

Suspicious for Medullary Thyroid Carcinoma (Figs. 7.5, 7.6, and 7.7)

The sample is sparsely or moderately cellular.

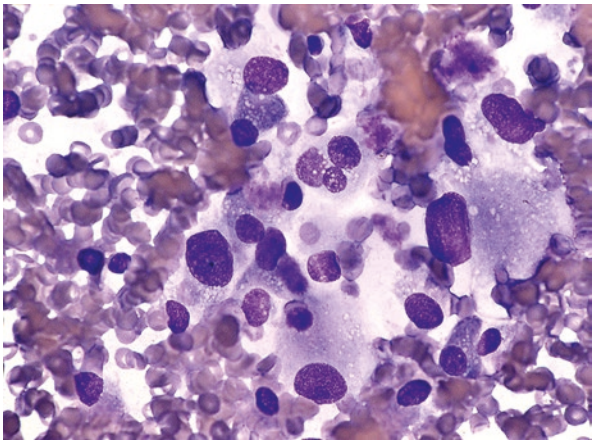

Fig. 7.6 Suspicious for medullary thyroid carcinoma. A loose cluster of cells with focal anisonucleosis, eccentrically placed (plasmacytoid) nuclei, and relatively abundant, finely vacuolated cytoplasm (smear, Diff-Quik stain)

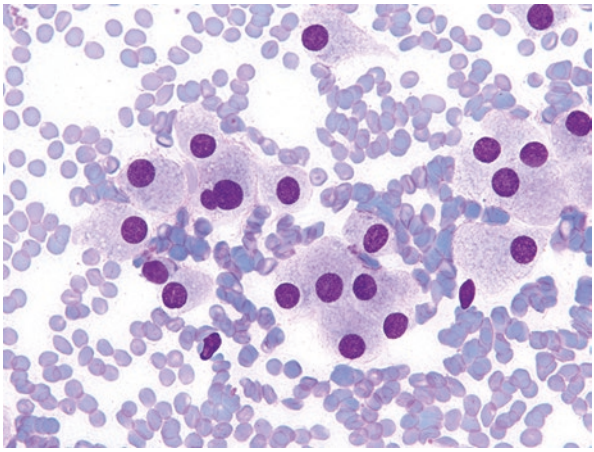

Fig. 7.7 Suspicious for medullary thyroid carcinoma. This hypocellular aspirate only showed few loosely cohesive, relatively uniform cells with granular and finely vacuolated cytoplasm and ill-defined cell borders (smear, Diff-Quik stain)

There is a monomorphic population of noncohesive small or medium-sized cells with relatively increased nuclear/cytoplasmic (N/C) ratio.

Nuclei are eccentrically located, with smudged chromatin due to suboptimal preservation; there are no discernible cytoplasmic granules.

There may be small fragments of amorphous material—colloid versus amyloid.

Neither adequate material for ancillary immunohistochemical studies nor FNA washout for calcitonin measurement to confirm a diagnosis of medullary carcinoma is available.

Suspicious for Lymphoma (Fig. 7.8)

The aspirate is composed of either numerous atypical small to intermediate-sized lymphoid cells or numerous atypical large-sized lymphoid cells.

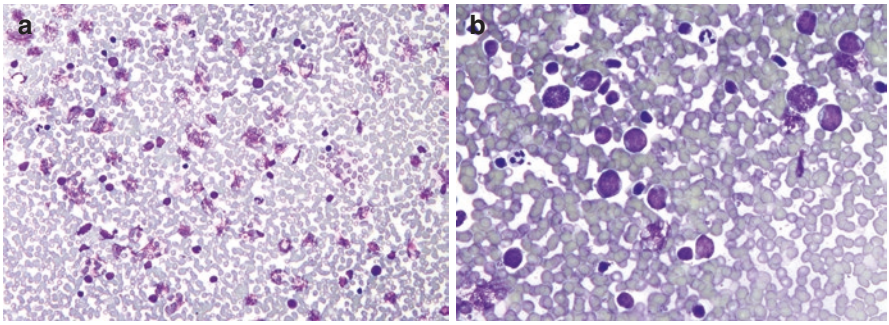

Fig. 7.8 Suspicious for lymphoma. (**a**) Scattered atypical large-sized lymphoid cells in a background of a few mature appearing lymphocytes, poorly preserved cells, and red blood cells. (**b**) At higher magnification in an area with better cellular preservation, the preserved atypical lymphoid cells show enlarged nuclei with round to irregular nuclear contours, open chromatin, and scant basophilic cytoplasm. In the absence of ancillary studies for immunophenotyping and clonality, the findings are suspicious but not conclusive for malignant lymphoma (smear, Diff-Quik stain)

Additional material for flow cytometry, molecular and/or immunohistochemical studies to confirm a diagnosis of lymphoma is inadequate or not available.

Or:

The sample is sparsely cellular and contains atypical lymphoid cells.

Suspicious for Malignancy, Not Otherwise Specified

See Explanatory Notes.

Explanatory Notes

Suspicious for Papillary Thyroid Carcinoma

The criteria for the most common general patterns of "SFM, suspicious for PTC" are outlined above. Because diagnostic histopathologic features of PTC can be patchy within a tumor nodule, FNAs of these nodules can also be heterogeneous. Unfortunately, this pattern is mimicked by a number of benign conditions like chronic lymphocytic thyroiditis, cystic degenerative changes, and radioiodine and carbimazole treatment [5]. The nuclear changes of follicular cells in chronic lymphocytic thyroiditis include focal enlargement, grooves, prominence of nucleoli, and chromatin clearing (Fig. 7.9); an abundance of lymphocytes and plasma cells does not exclude the possibility of a coexisting PTC [19].

Cyst lining cells associated with cystic degeneration have very characteristic features and can be diagnosed as benign in most cases [20]. These cells are typically elongated, with delicate chromatin, occasional intranuclear grooves, relatively large nucleoli, and are virtually always associated with hemosiderin-laden macrophages

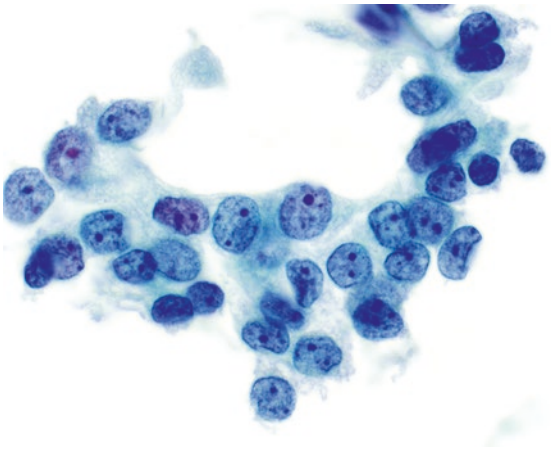

Fig. 7.9 Suspicious for papillary thyroid carcinoma (patient with Chronic Lymphocytic Thyroiditis). This sheet of unevenly distributed follicular cells shows nuclear enlargement, pale chromatin, nuclear irregularity, and prominent nucleoli (ThinPrep, Papanicolaou stain)

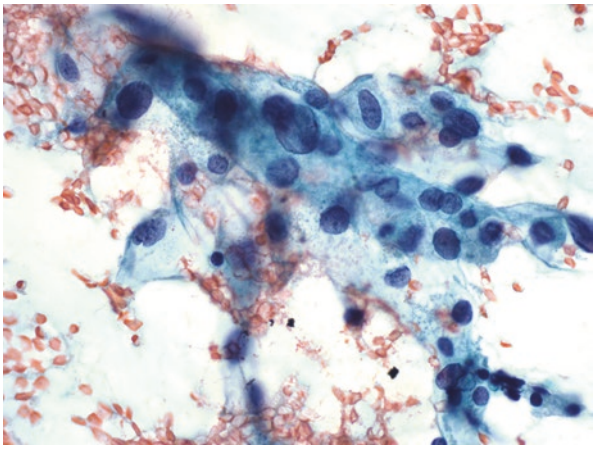

Fig. 7.10 Suspicious for papillary thyroid carcinoma. Follicular cells adjacent to areas of infarction, hemorrhage, and cyst formation ("cyst lining cells") can have nuclear changes similar to those of papillary thyroid carcinoma. When nuclear enlargement, pallor, and grooves are widespread throughout the specimen, the diagnosis "suspicious for malignancy" may be unavoidable (smear, Papanicolaou stain)

and benign-appearing macrofollicular fragments. The spindle-shaped morphology of the cell and nucleus, reminiscent of reparative epithelium in cervical Pap smears, is helpful in distinguishing these cells from PTC. In some cases, however, the distinction from PTC is more challenging. A diagnosis of AUS is appropriate for some cases (see Chap. 4), but in their most marked form they can be highly worrisome, and an SFM interpretation may be warranted (Fig. 7.10).

In patients treated with radioactive iodine, carbimazole, or other pharmaceutical agents, nuclear atypia can be especially prominent [21–23]. In some patients, the nuclear changes can be extreme and raise the possibility of PTC or other

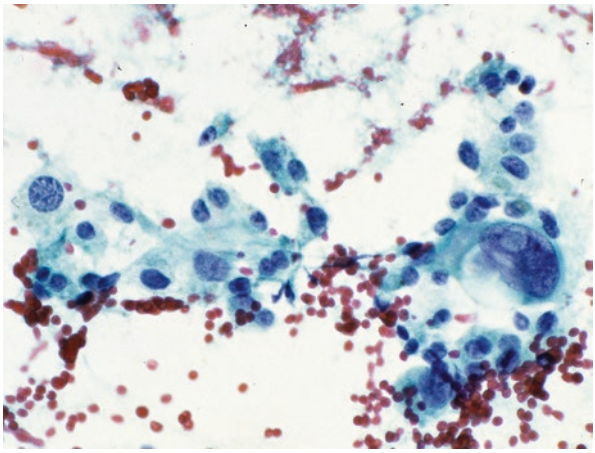

Fig. 7.11 Suspicious for papillary thyroid carcinoma (patient treated with radioiodine for nodular goiter). These follicular cells demonstrate marked anisonucleosis, pale chromatin, and a prominent INPI. Although the findings may represent treatment effect, the possibility of papillary thyroid carcinoma cannot be excluded when the atypia is as marked as in this case (smear, Papanicolaou stain)

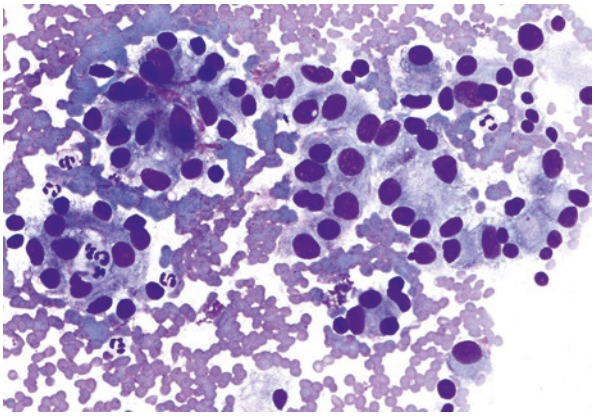

Fig. 7.12 Suspicious for papillary thyroid carcinoma. These representative microfollicular groups display nuclear enlargement, variable chromatin pallor, and rare nuclear grooves. Surgical resection of the nodule revealed a NIFTP (smear, Diff-Quik stain)

malignancy. As with cyst lining cells in their most extreme form, such cases warrant an SFM interpretation (Fig. 7.11).

Instead of being patchy, nuclear changes of follicular cells are sometimes generalized but mild and incomplete. Again, such relatively subtle generalized changes are seen in some PTCs, particularly the follicular variant, but can be mimicked by benign lesions like a follicular adenoma. For this reason, when generalized but mild, the findings are best interpreted as SFM, suspicious for PTC.

As discussed in detail in Chap. 8, a number of histologic subtypes of PTC are distinguished by some variation from the defining features of a classic PTC [24–31]. These include the common follicular variant (Fig. 7.12), as well as less frequently encountered subtypes like the oncocytic (Figs. 7.13) and columnar subtypes,

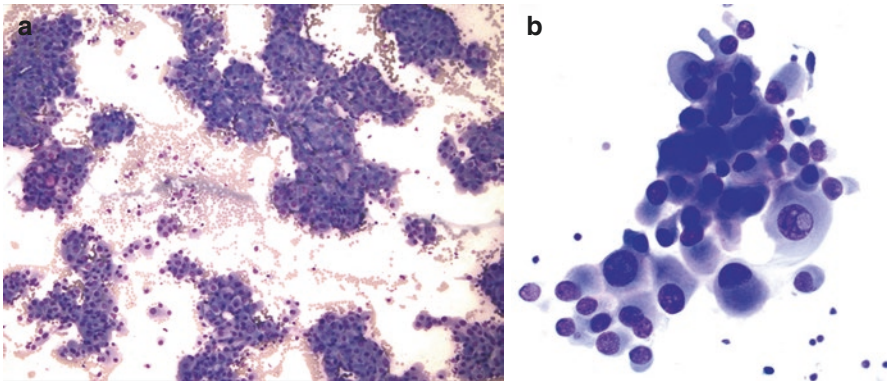

Fig. 7.13 Suspicious for papillary thyroid carcinoma, oncocytic subtype. (**a**) This low-magnification image reveals a hypercellular specimen with numerous groups of follicular cells with abundant cytoplasm (smear, Diff-Quik stain). (**b**) High magnification confirms the presence of abundant cytoplasm and reveals an INPI. Although the findings are suspicious for papillary carcinoma, an oncocytic neoplasm cannot be entirely excluded (smear, Diff-Quik stain)

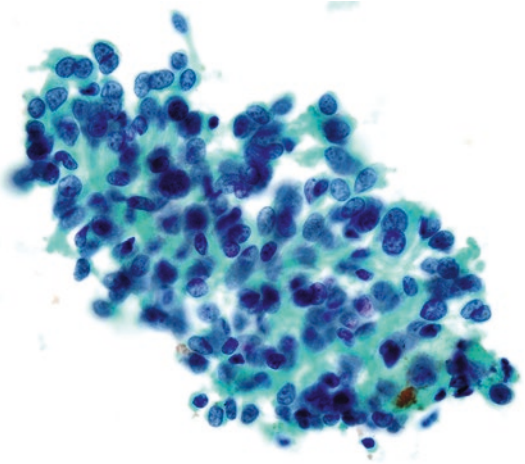

Fig. 7.14 NTRK-rearranged PTC with subtle nuclear grooving, infrequent nuclear elongation, and rare INPIs. (SurePath, Papanicolaou stain)

NTRK-rearranged [32], as well as PTCs with cystic degeneration, to name just a few. NTRK-rearranged PTC often demonstrated intermediate nuclear features, such as subtle nuclear grooving, infrequent nuclear elongation, and rare INPIs (Fig. 7.14). These morphologic differences are reflected in FNA specimens and can in some instances cause uncertainty in diagnosis. They may result in a sense of incompleteness or patchiness of expression of typical PTC features, leading to an interpretation of SFM rather than malignant.

The follicular variant of PTC (FVPTC) and NIFTP pose a challenge for FNA. These tumors, with follicular architecture but nuclear changes of PTC, have variable clinical behavior, ranging from the indolence of NIFTP to the aggressiveness of the invasive follicular variant of PTC. Just as for the distinction between

follicular adenoma and follicular carcinoma, histopathologic evaluation is necessary to identify the features of malignancy (i.e., invasion) that cannot be appreciated by FNA. Most FVPTCs and NIFTPs are diagnosed cytologically as either SFM, suspicious for a follicular neoplasm (SFN), or atypia of undetermined significance (AUS); relatively few are interpreted as Malignant [9, 18, 33]. The cytomorphologic features of NIFTP and FVPTC overlap across the diagnostic categories: predominantly microfollicular architecture and attenuated nuclear features of PTC (fine chromatin, pale nuclei, and nuclear grooves) are encountered in both the SFM and AUS categories [33–35]. It remains to be seen whether any collection of cytologic features is sufficiently reliable to allow prospective identification of NIFTP and its distinction from an invasive FVPTC by FNA alone. For the subset of FNAs diagnosed as SFM that have microfollicular architecture with some nuclear features of PTC but lack INPIs, papillae, or psammoma bodies, an educational note can be helpful (see Sample Reports, Example 2 below) [36].

Hyalinizing trabecular tumor (HTT) shares many morphologic features with PTC, including nuclear grooves and abundant INPIs (Figs. 7.15). Although it may be related to PTC, it is generally distinguished from PTC histologically based on its circumscription, trabecular growth pattern, and intratrabecular hyaline material [37]. These distinguishing features are difficult to appreciate by FNA, and many HTTs are interpreted as Malignant or SFM. Cytoplasmic staining for MIB-1 (as opposed to the nuclear staining pattern used in other contexts to establish a proliferative index) is a distinctive feature of HTT and can be helpful as an adjunct to cytomorphology [38].

Cystic PTCs, like other PTC subtypes, have unusual features that differ from those of the classic PTC, sometimes obscured by blood and macrophages. Some contain large cells with abundant dense or vacuolated cytoplasm and pleomorphic nuclei ("histiocytoid cells") (Fig. 7.4). Such PTCs can be difficult to diagnose with certainty as malignant [30, 31].

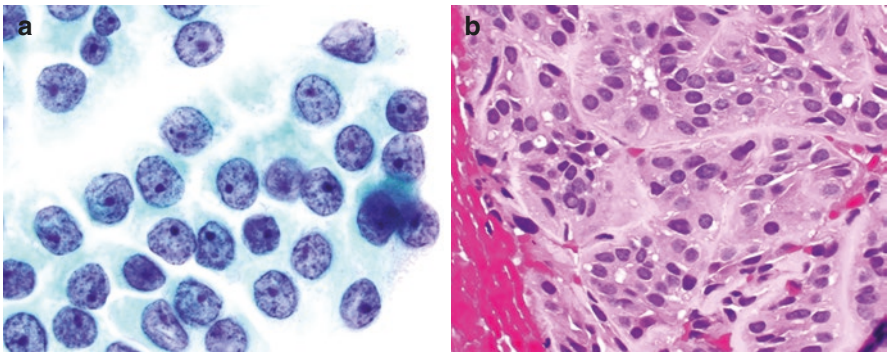

Fig. 7.15 Suspicious for papillary thyroid carcinoma. (**a**) A loose sheet of follicular cells shows nuclear enlargement, pale and powdery chromatin, nuclear grooves, and prominent nucleoli (ThinPrep, Papanicolaou stain). (**b**) A cell block preparation from the FNA reveals the nested pattern of the atypical cells, along with their pale chromatin and obvious INPIs. The subsequent thyroidectomy revealed a hyalinizing trabecular tumor (cell block, H&E stain)

Suspicious for Medullary Thyroid Carcinoma

A specimen may be less than definitive for the diagnosis of MTC due to technical issues like cellularity and preservation (Fig. 7.6) or unusual cytomorphologic presentations [39]. In such cases, a definitive diagnosis of MTC can be made if sufficient material is available for immunocytochemical stains (see Chap. 9), FNA washout for calcitonin measurement [40], or if the cytologic findings are interpreted in the proper clinical context (such as a markedly elevated serum calcitonin level).

Suspicious for Lymphoma

Most common primary thyroid (PT) lymphomas often show a monomorphic population of either mildly atypical small to intermediate-sized lymphoid cells (MALT lymphoma) or atypical large-sized lymphoid cells (diffuse large B cell lymphoma). PT MALT lymphoma may be difficult to distinguish from chronic lymphocytic thyroiditis [41]. The cytomorphological and immunophenotypical features of PT and secondary thyroid (ST) lymphomas are very similar and the aforementioned cytomorphological features might also be seen in other less common PT/ST B and T cell lymphomas [42]. Definite diagnosis of lymphoma would necessitate correlation of the cytomorphological features with clonality and immunophenotypical findings by flow cytometry, immunohistochemistry, and/or molecular analyses [41–44]. Lack of material available for ancillary studies, suboptimal cellularity, or suboptimal preservation can result in a less than definite diagnosis, leading to an "SFM, suspicious for lymphoma" interpretation (Fig. 7.8).

Suspicious for Malignancy, Not Otherwise Specified

Although uncommon, other malignancies like undifferentiated (anaplastic) thyroid carcinoma, poorly differentiated thyroid carcinoma, and metastasis, mostly adenocarcinomas of kidney, breast, lung and colon primaries and head and neck squamous cell carcinomas, are encountered in the thyroid [45] (Fig. 7.16). Definite diagnosis would require clinical correlation, review of prior tumor slides (for known primary malignancies), and/or ancillary studies findings [46]. Lack of one or more of these elements, suboptimal cellularity, or suboptimal preservation can lead to uncertainty and thus result in an "SFM, NOS" interpretation.

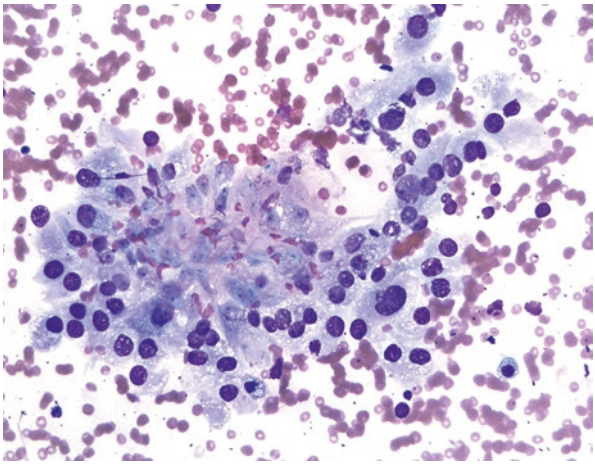

Fig. 7.16 Suspicious for malignancy, not otherwise specified. Cluster of atypical cells with enlarged nuclei and moderate to abundant pale to eosinophilic focally vacuolated cytoplasm. Although a history of renal cell carcinoma was provided, in the absence of ancillary studies and/or slides from primary renal cell carcinoma for review, the findings are suspicious but not conclusive for metastatic renal cell carcinoma (smear, Diff-Quik stain)

Management

In general, clinical-surgical recommendations relating to a "SFM, suspicious for PTC" FNA result have been framed by the relatively high rate of malignancy in this category, which would typically elicit consideration for offering a procedure intended for malignancy, most commonly a lobectomy or total thyroidectomy, which is well tolerated in expert hands. Important non-cytologic clinical data available through careful preoperative clinical and radiographic risk stratification should be introduced into the conversation as it relates to the recommendation for surgery and its extent (see Chap. 13). These factors improve the estimation of the prevalence of malignancy and include key historical features such as family history or history of irradiation, physical examination characteristics such as fixation of the mass to the adjacent cervical viscera, laryngeal exam including the finding of vocal cord paralysis ipsilateral to the mass [47], and the ultrasonographic appearance of the index nodule. Ultrasonographic preoperative stratification of nodules is increasingly important, as emphasized in the most recent American Thyroid Association

(ATA) guidelines for thyroid nodule workup, and is additive to cytologic evaluation in the estimation of malignancy in a given nodule [14]. ROSE of thyroid aspirates, ideally performed by a trained sonographer (radiologist or pathologist) assisted by a cytotechnologist, adds value to the FNA procedure by reducing the number of suspicious cases due to either low cellularity or those requiring confirmation on ancillary workup (medullary carcinoma, anaplastic carcinoma, lymphoma, metastatic malignancies, e.g., melanoma). Guided by ROSE, dedicated passes collected in appropriate transport media for immunochemistry on cell blocks or flow cytometry could allow a definitive diagnosis to be reached using the cytology sample, avoiding the need for another patient visit for a repeat FNA or an alternative method of sampling such as core biopsy.

ATA initiatives to reduce the extent of surgery for many low-risk thyroid cancers (4 cm or smaller, without extrathyroidal extension and lacking regional nodal metastasis) and decrease routine use of postoperative radioactive iodine treatment increasingly raise the possibility for lobectomy as initial surgical management [14]. Also, the recognition of the indolence of NIFTP further supports a less aggressive initial surgical procedure in some patients [16]. The reclassification of some nodules as NIFTP alters the rate of malignancy for SFM, with the emerging literature suggesting an overall decrease in the 15–20% range [17, 18]. The exact clinical and surgical impact of this is yet to be determined but clearly will help push the pendulum towards consideration of a more conservative initial surgical procedure in many circumstances. Many other factors relate to the decision of unilateral versus bilateral surgery, including the patient's acceptance of a more aggressive initial surgical procedure and thyroid hormone suppression, their willingness to have a potential second procedure, and the potential for surgery to affect the patient's use of voice in their profession. Intraoperative frozen section evaluation has limited utility for SFM nodules [48].

Role for Ancillary Studies

Ancillary studies can be very helpful for patients with the diagnosis of "SFM, suspicious for MTC" or "SFM, suspicious for lymphoma." An elevated serum calcitonin level and/or immunoreactivity of atypical cells for calcitonin or synaptophysin/chromogranin can lead to a conclusive interpretation of medullary thyroid carcinoma. A repeat FNA to obtain cells for flow cytometry, molecular and/or immunohistochemical studies can better characterize an atypical lymphoid cell population and can also help with providing a definite lymphoma diagnosis for patients with an initial "SFM, suspicious for lymphoma" interpretation.

Ancillary molecular studies have been more readily available and applied mainly to aspirates with TBSRTC indeterminate diagnoses, as their utility for a "SFM, suspicious for PTC" interpretation has been limited, given the relatively high risk of malignancy. Recent ATA statement on surgical application of molecular profiling to SFM cytology divulges that a seven-gene molecular panel (including BRAF, RAS, RET::PTC, PAX8::PPARγ) test (7-gene MP) would guide the surgical management

in which a positive 7-gene MP should be managed by initial oncologic thyroidectomy while a negative 7-gene MP should be managed by at least a diagnostic lobectomy. The ATA statement also discloses that an Afirma® genomic test is not routinely recommended but may be requested for SFM cytology if clinically indicated [15]. Of note, available commercial molecular tests performed on NIFTP aspirates tend to classify these lesions as "suspicious" and this may result in surgical overtreatment [49]. Most NIFTP harbor RAS mutations with a minority showing non-V600E BRAF mutations; the majority of NIFTP aspirates fall into the AUS category while only a small percentage of aspirates are interpreted as SFM [50]. The need for ancillary testing may be anticipated if ROSE is performed, allowing additional samples to be collected without the need to recall patients for repeat testing.

Sample Reports

If an aspirate is interpreted as SFM, it is implied that the sample is adequate for evaluation. An explicit statement of adequacy is optional. Narrative comments that follow are used to specify which malignancy/malignancies the findings are suspicious for. A microscopic description is optional.

Example 1
SUSPICIOUS FOR MALIGNANCY.
Suspicious for papillary thyroid carcinoma.

Example 2
SUSPICIOUS FOR MALIGNANCY.
Suspicious for papillary thyroid carcinoma.
Note: The overall cytomorphologic features are suggestive of a follicular variant of papillary carcinoma or its indolent counterpart, noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Definitive distinction between these entities is not possible on cytologic material.

Example 3
SUSPICIOUS FOR MALIGNANCY.
Suspicious for medullary thyroid carcinoma.
Note: Correlation with serum calcitonin level or re-aspiration for immunohistochemical studies or needle washout for calcitonin measurement might be helpful for definitive diagnosis if clinically indicated.

Example 4
SUSPICIOUS FOR MALIGNANCY.
Suspicious for lymphoma.
Note: Re-aspiration for flow cytometry, molecular and/or immunohistochemical studies might be helpful to better characterize the atypical lymphoid cell population, if clinically indicated.

Example 5
SUSPICIOUS FOR MALIGNANCY, NOT OTHERWISE SPECIFIED.
Note: Slides from the patient's known primary tumor are not available for review (for patients with known malignancies). The overall cytomorphologic features likely represent a metastatic carcinoma. Re-aspiration for immunohistochemical studies might be helpful for definitive diagnosis, if clinically indicated.

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Papillary Thyroid Carcinoma, Subtypes, and Related Tumors

8

Marc Pusztaszeri, Edward Stelow, William Westra, Maureen Zakowski, and Emmanuel Mastorakis

Background

Papillary thyroid carcinoma (PTC) is the most common malignant neoplasm of the thyroid gland in both adults and children, accounting for 80–85% of all thyroid cancers in adults and 90% in children [1]. It occurs in all age groups with a peak incidence in the fourth decade, and a M:F ratio of 1:3. Since the introduction of high-resolution imaging techniques (e.g., thyroid ultrasonography) into clinical practice, the incidence of thyroid cancer nearly tripled worldwide from 1975 to 2009, with PTC accounting for most of the surge [1–4]. The mortality rate remained stable during this time, however, suggesting that the more indolent forms of PTC were diagnosed and that overtreatment may occur. This has been referred to as an epidemic of overdiagnosis because of the prevalence of low-risk, non-lethal tumors that are often incidentally detected from a large subclinical reservoir of disease [4]. Recent epidemiological data suggests that the rising incidence and overdiagnosis of thyroid cancer is starting to slow down following several important developments in

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recommendations about the diagnosis, classification, and management of low-risk thyroid cancer in the last decade [4]. The reclassification of the noninvasive follicular variant of PTC (FVPTC) as "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) that was proposed in 2016 [5] and endorsed in the 2017 WHO classification may contribute to reduce this trend in overdiagnosis in the future, especially in areas where this tumor is commonly encountered.

Risk factors for PTC include external radiation to the neck during childhood, exposure to ionizing radiation, and genetic susceptibility [1, 2]. PTC usually presents as a thyroid nodule, often discovered incidentally on routine examination, but a minority of patients present with metastatic disease in neck lymph nodes. PTC spreads via lymphatics to the regional lymph nodes and less frequently to the lungs. It generally carries a good prognosis; death secondary to PTC is rare [1].

A malignant thyroid FNA diagnosis accounts for approximately 5% (range 2–16%) of all thyroid FNAs [2, 6], the majority of these are PTCs. When a diagnosis of PTC is made by FNA, 94–96% prove to be PTC on histologic follow-up, taking into consideration the reclassification of some FVPTCs as NIFTP (see also Chaps. 1 and 5) [2, 6–8]. Conventional (classic) PTCs are characterized histologically by numerous papillae lined by cuboidal to low columnar neoplastic follicular cells with distinctive nuclear features. A significant proportion of PTCs exhibit distinct architectural and/or cytologic features from those of conventional PTC, corresponding to different PTC subtypes. Furthermore, some PTC subtypes have different genomic alterations and biological behavior as compared to conventional PTC. An awareness of the cytomorphologic spectrum of PTC subtypes and related tumors helps prevent misdiagnosis, but it is not required to specify the subtype of PTC on an FNA specimen. In the following sections, conventional PTC and other PTC subtypes are described separately to highlight some of the morphologic heterogeneity in this family of tumors. Some uncommon thyroid neoplasms which have been related to PTC in the past, including hyalinizing trabecular tumor (HTT) and cribriform-morular thyroid carcinoma (CMTC), are also discussed in this chapter since they share some cytomorphological features with PTC. In the 5th edition of the WHO Classification of Endocrine and Neuroendocrine Tumors that relate to the thyroid gland, CMTC is no longer classified as a subtype of PTC but as a malignant thyroid tumor of uncertain histogenesis, while NIFTP and HTT are both classified as low-risk follicular cell-derived neoplasms [1].

Given the reclassification of some FVPTC as low-risk neoplasms rather than overt malignancies and the general consensus that FVPTC cannot be reliably distinguished from NIFTP on cytology [9–11], it is desirable to eliminate from the Malignant and Suspicious for Malignancy categories tumors likely to represent a NIFTP, in order to avoid possible overdiagnosis and overtreatment, since the recommended treatment for NIFTP is conservative surgery (e.g., lobectomy) in view of its indolent behavior (see Chap. 5). To accomplish this goal, follicular-patterned aspirates with nuclear changes that raise the possibility of FVPTC or NIFTP (e.g., mild enlargement, contour irregularity, and clearing) are best classified as Follicular

Neoplasm (FN) rather than Malignant or Suspicious for Malignancy, as long as true papillae are absent and intranuclear pseudoinclusions (INPIs) are either absent or very rare (see Chap. 5). In contrast, if the follicular cells show definitive nuclear features of PTC, including frequent INPIs, and there are at least focal elements associated with classical PTC (psammoma bodies and/or true papillae), the specimen should not be interpreted as FN but rather as "Malignant: PTC," or "Suspicious for PTC," depending on the quality and quantity of the cytologic changes. This approach leaves other subtypes of PTC in the Malignant category but minimizes the contribution of FVPTC and NIFTP.

Conventional (Classic) Papillary Thyroid Carcinoma

Definition

Conventional (classic) PTC is a malignant epithelial tumor derived from thyroid follicular epithelium that displays papillary architecture and characteristic nuclear alterations [1].

Criteria

Architecture:
Cells arranged in papillae and/or monolayer sheets and/or 3D groups.
Cellular swirls ("onion-skin" or "cartwheel" patterns) in some cases.

Nuclear features:
Enlarged and crowded nuclei, often molded.
Oval or irregularly shaped nuclei.
Longitudinal nuclear grooves.
Intranuclear pseudoinclusions.
Pale nuclei with powdery chromatin.
Thick nuclear membranes.
Macronucleoli or micronucleoli, central or marginally placed.

Other features:
Psammoma bodies.
Multinucleated giant cells.
Variable amount of colloid; may be stringy, ropy, or "bubble-gum"-like.
"Hobnail" cells.
Oncocytic (Hürthle cell) metaplasia.
Squamoid metaplasia.

"Histiocytoid" cells.

Liquid-Based Preparations

Liquid-based preparations (LBP) have been widely used for managing cytopathological specimens in the last three decades. Relative to conventional smears, LBP provides optimal cell preservation with fewer air-drying artifacts, fewer slides, shorter screening time, easier technical preparation, and a cleaner background because there is decreased obscuring material. In addition, cell block slides prepared from the well-preserved cellular remnants of LBP specimens can be used for further morphological examination, immunohistochemical (IHC) analysis, and molecular testing. There are some minor differences between smears and LBP with regard to the diagnosis of conventional PTC; they vary depending on the LBP methods and their fixatives [12–15]. Awareness of the cytomorphological features observed with the use of the LBP method is helpful because some of the classical features of PTC, such as background and large flat sheets, may not be seen in LBP and may make accurate interpretation more difficult for some cases.

Cytological features more frequent in LBP compared to smears:
Clean background.
High cellularity.
Convoluted nuclei.
Eosinophilic nucleoli.
Perinucleolar halo.
Trabecular and hobnail patterns.
Tall cells.
Collagenous stroma.
Naked capillaries.
Intercellular "window-like" spaces.

Cytological features less frequent or reduced in LBP compared to smears:
Pale/ground-glass nuclei and nuclear crowding/overlapping.
Intranuclear pseudoinclusions are smaller and less obvious in LBP.
Papillary pattern and tissue fragments.

Explanatory Notes

Although several nuclear alterations are characteristic, none are diagnostic of PTC in isolation or low frequency. Only when relatively widespread and in combination are they diagnostic of PTC, whether in direct smears or LBP. The minimum criteria and number of neoplastic cells necessary for an unequivocal diagnosis are uncertain and probably not definable, either cytologically or histopathologically. In other

words, the minimum quantitative threshold (e.g., the number of cells needed with nuclear grooves and/or INPIs) for a diagnosis of PTC in cytological or histologic specimens remains undefined. If, in the judgment of the cytologist, a case has some features of PTC but falls short of an unequivocal diagnosis, it is interpreted as "Suspicious for PTC," "Follicular Neoplasm," or "Atypia of Undetermined Significance (AUS)" (see Chaps. 7, 5, and 4, respectively), depending on the quality and quantity of the changes and the reviewer's degree of suspicion for PTC.

The cells of a conventional PTC are typically arranged in syncytial-like flat sheets or monolayers with crowded and overlapping nuclei (Figs. 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7 and 8.8). The latter feature often leads to conspicuous nuclear molding (Figs. 8.3, 8.4, and 8.5). Nuclear crowding, overlapping, and molding are important diagnostic features that help distinguish the cells of PTC from benign follicular cells. The monolayered sheet is characteristic of conventional PTC and mimics the flat sheet of a macrofollicular fragment typical of benign follicular nodules, such as those commonly seen in nodular hyperplasia (Fig. 8.8). The distinction requires particular attention to the arrangement of the cells in the sheets, evenly spaced vs.

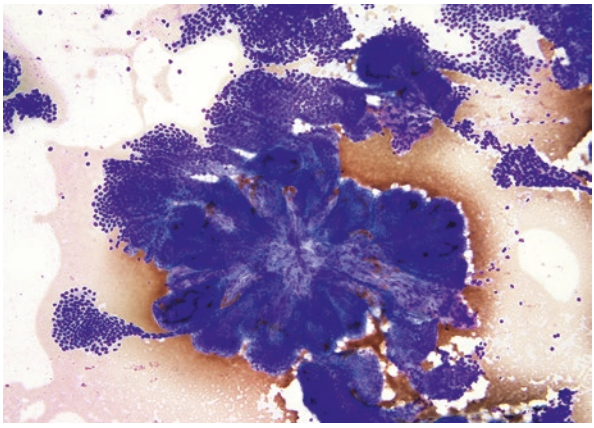

Fig. 8.1 Papillary thyroid carcinoma. Preparations are often highly cellular and composed of numerous monolayer sheets and occasional papillary-like fragments (smear, Diff-Quik stain)

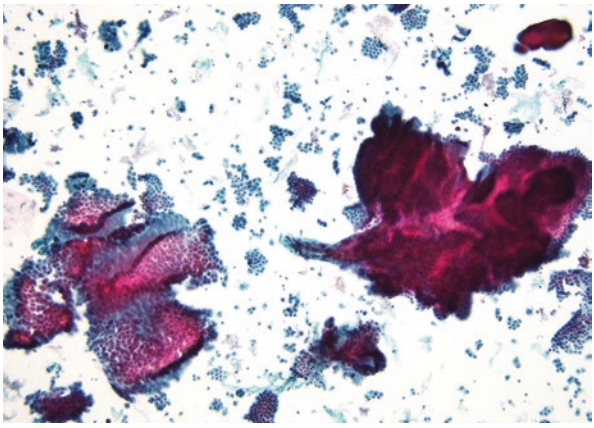

Fig. 8.2 Papillary thyroid carcinoma. Hypercellularity with mostly intact tissue fragments comprising of numerous papillary-like fragments (SurePath, Papanicolaou stain)

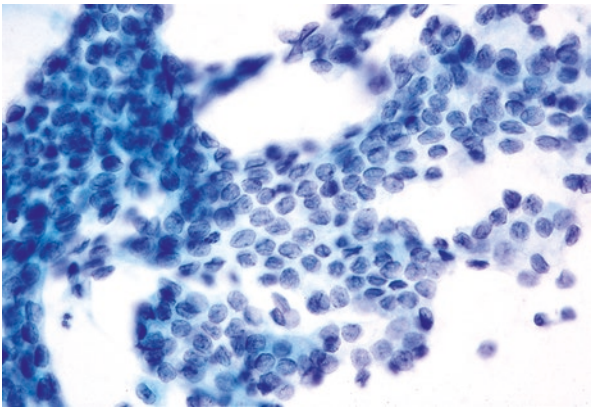

Fig. 8.3 Papillary thyroid carcinoma. Monolayer sheets with a syncytial-like appearance are characteristic of papillary thyroid carcinoma. These flat sheets resemble those of benign follicular nodules; attention to the nuclear features is essential for this distinction (smear, Papanicolaou stain)

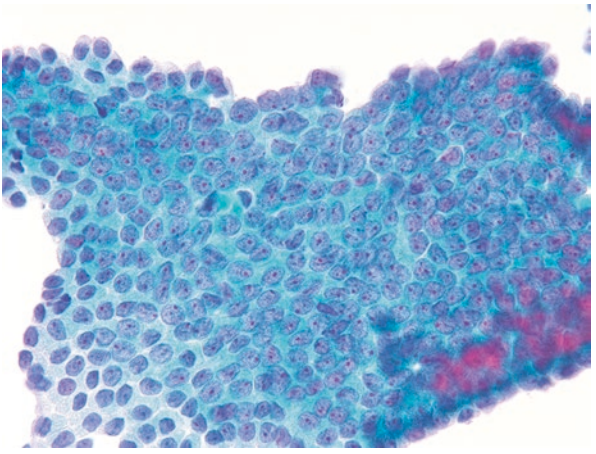

Fig. 8.4 Papillary thyroid carcinoma. This monolayer sheet is comprised of cells with irregular nuclei that show focal molding. Small nucleoli are also visible (ThinPrep, Papanicolaou stain)

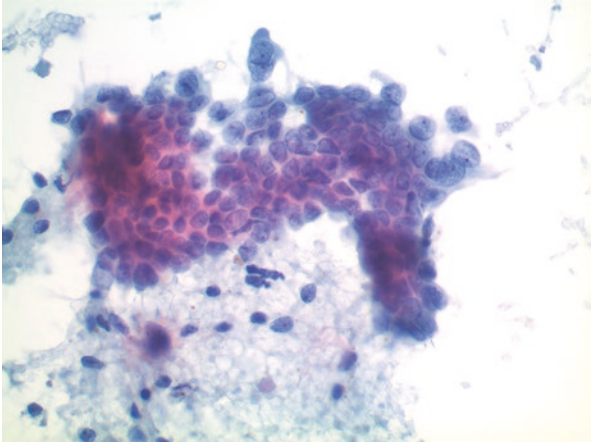

Fig. 8.5 Papillary thyroid carcinoma. This monolayer sheet is comprised of cells with irregular nuclei that show focal molding (Cytospin, Papanicolaou stain)

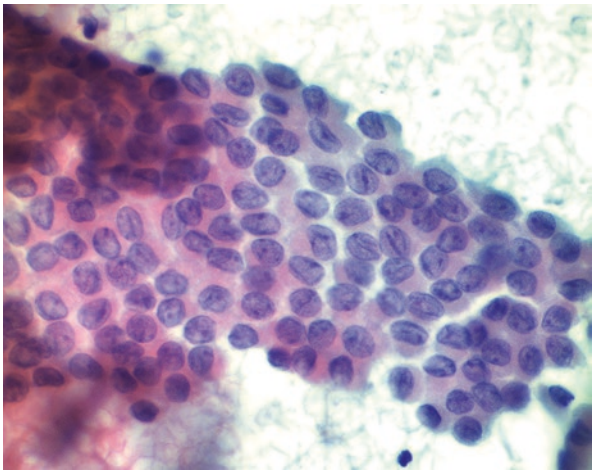

Fig. 8.6 Papillary thyroid carcinoma. This monolayer sheet is comprised of cells with irregular nuclei that show prominent grooving with coffee bean-like nuclei (Cytospin, Papanicolaou stain)

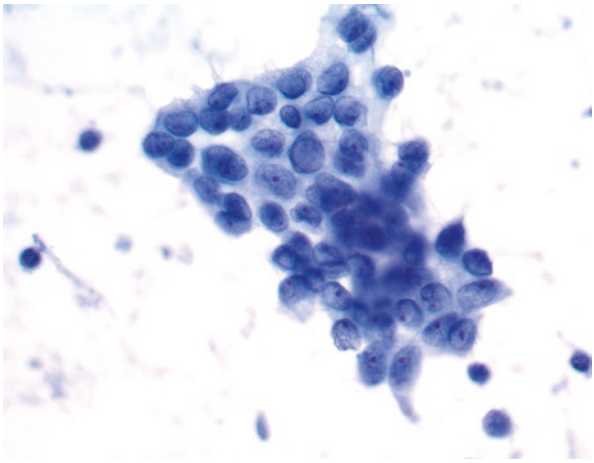

Fig. 8.7 Papillary thyroid carcinoma. This sheet is comprised of cells with irregular nuclei that show prominent nucleoli as well as intranuclear pseudoinclusions (Cytospin, Papanicolaou stain)

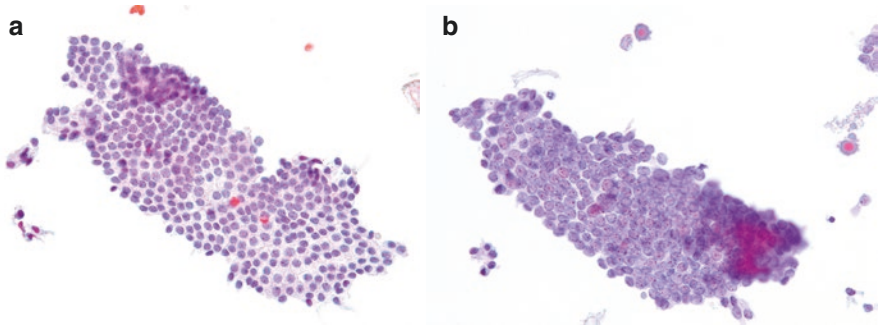

Fig. 8.8 Comparison of benign follicular cells with the cells of papillary thyroid carcinoma. (**a**) Benign follicular cells (nodular goiter). (**b**) Compared with those of the benign follicular cells, the nuclei of papillary carcinoma are larger, paler, more crowded, and more irregular in contour. (**a** and **b**, ThinPrep, Papanicolaou stain)

crowded, and to their nuclear features to avoid a false-negative diagnosis. The architectural pattern varies depending on the subtype of PTC (see below), but FNAs from a conventional PTC often display true papillary fragments with a fibrovascular core (Figs. 8.9 and 8.10), papillary-like fragments which have a rounded shape with smooth edges but lack a fibrovascular core (Fig. 8.11), and cellular swirls. Cellular swirls (Fig. 8.12) are flat, concentrically organized aggregates of about 50–200 tumor cells with a perpendicular arrangement of the most peripherally located ovoid cells relative to the radius of the swirl which is sometimes also called an "onion-skin" pattern [16]. Cellular swirls are a distinctive feature of the conventional PTC, seen in about 17% of cases, both in smears and LBP, and have not been reported in benign thyroid nodules [13, 16]. Although individually dispersed neoplastic cells are seen in PTC, a pattern of predominantly isolated cells is highly unusual, in contrast to the cells of medullary thyroid carcinoma (MTC).

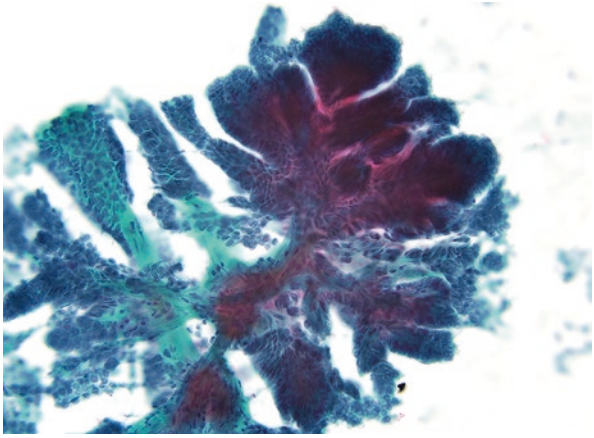

Fig. 8.9 Papillary thyroid carcinoma. True papillary tissue fragments, comprised of fibrovascular cores lined by neoplastic cells, are seen in the conventional type of papillary thyroid carcinoma (smear, Papanicolaou stain)

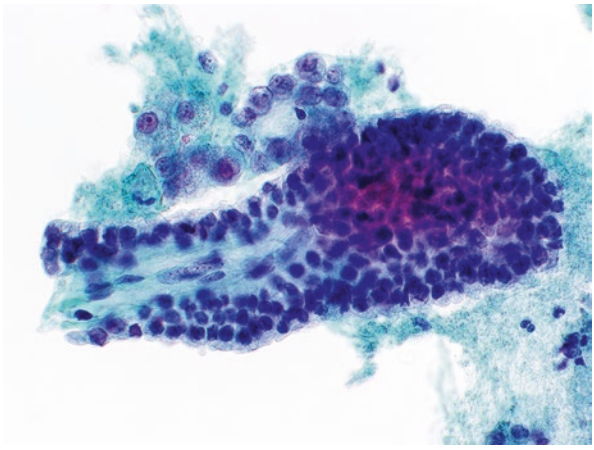

Fig. 8.10 Papillary thyroid carcinoma. The neoplastic cells surround a fibrovascular core (ThinPrep, Papanicolaou stain)

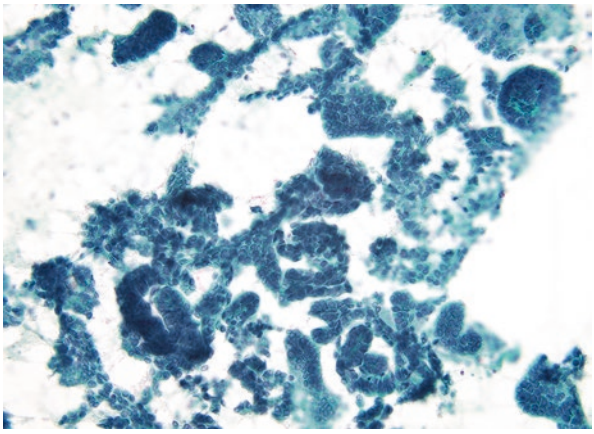

Fig. 8.11 Papillary thyroid carcinoma. There is a mixture of flat sheets and rounded, papillary-like fragments without fibrovascular cores (smear, Papanicolaou stain)

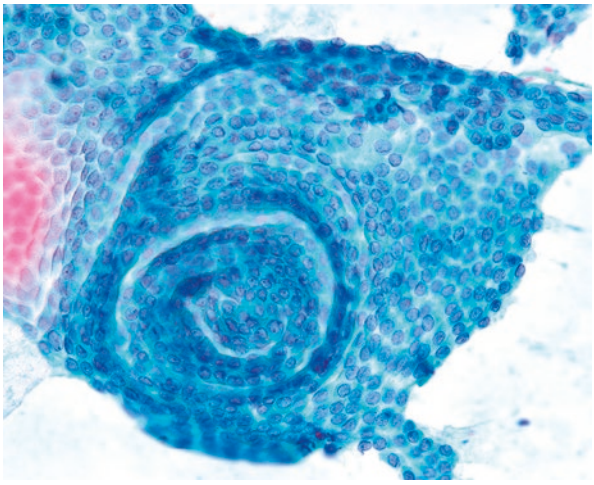

Fig. 8.12 Papillary thyroid carcinoma. Cellular swirls are highly characteristic of the conventional (classic) papillary thyroid carcinoma. They are a concentric aggregate of tumor cells in which many of the peripheral cells have ovoid (rather than round) nuclei and are oriented perpendicular to the radius of the swirl (smear, Papanicolaou stain)

The cells of PTC vary in size (from medium to large) and shape (cuboidal, columnar, polygonal, sometimes spindle-shaped and even histiocytoid). Cell borders are usually well demarcated. The amount and texture of cytoplasm can vary greatly. In some cases, the cells have scant cytoplasm, but abundant oncocytic or granular cytoplasm is common, although usually a focal finding. When extensive, it signals an oncocytic subtype of PTC. A hobnail pattern was suggested as a useful diagnostic criterion, especially on LBP [12, 13], and has been reported in several subtypes of PTC (hobnail, diffuse sclerosing, cystic). "Hobnail pattern" is the term employed to describe cells characterized by a high nuclear/cytoplasmic ratio and apical/eccentric placement of the nuclei that produces a surface bulge like hobnails [14, 17, 18]. Changes resembling squamous metaplasia, such as moderate to abundant dense cytoplasm and cells that fit together like pavement stones, are also seen, usually only as a focal finding in conventional PTC. Hyperkeratinized squamous

cells with orangeophilic cytoplasm on Papanicolaou stain and keratin pearls, however, are rare. Histiocytoid cells are characterized by extensive cytoplasmic vacuolation, like that seen in benign histiocytes, and typically arise in a PTC that has undergone cystic changes (Figs. 7.4 and 8.24).

The defining features of PTC are seen in the nuclei. They are generally slightly enlarged and can be round or oval but are often highly irregular in contour; the nuclear contour irregularity is often one of the first clues to the diagnosis (Figs. 8.6 and 8.8b). Convoluted nuclei, where more than half of the nuclear membrane is wrinkled, are very specific for PTC on LBP (97.3%) [12]. The chromatin of a conventional PTC nucleus is usually pale, finely textured, and evenly distributed (powdery), unlike the dark and coarsely textured benign follicular cell nucleus (Fig. 8.8). This chromatin characteristic is more easily appreciated with alcohol-fixed Papanicolaou-stained smears than air-dried Diff-Quik preparations or LBP, and it may be absent in some PTC subtypes such as columnar cell PTC. This pallor parallels the optically clear appearance of PTC nuclei in formalin-fixed tissue ("Orphan Annie eyes"), which is attributed to a fixation artifact that renders the nucleus practically empty in appearance.

Intranuclear pseudoinclusions are seen in 50–100% of aspirates of PTC, depending on the subtype of PTC (Figs. 8.7, 8.13, and 8.14). For example, INPIs are most frequent and florid in the tall cell PTC, whereas they are often rare or absent in FVPTC. INPIs are not specific for PTC, as they can be seen in aspirates of MTC, anaplastic thyroid carcinoma, HTT, and very rarely NIFTP or benign thyroid nodules (e.g., nodular goiter, follicular adenoma, lymphocytic thyroiditis). INPIs should therefore always be interpreted in light of the other architectural and nuclear features in a given FNA. Ultrastructurally, INPIs are membrane-bound spheroidal masses of cytoplasm that protrude into the nuclei. Thus, a true INPI displays the same color/texture of adjacent cytoplasm, is fully contained within the nucleus, and is sharply bordered by a rim of condensed chromatin like a "wire loop." These features help distinguish INPIs from common mimics: degenerative and artifactual vacuoles, fixation artifacts, and superimposed red blood cells.

Nuclear grooves are another hallmark of PTC [19]. Akin to INPIs, they are best seen with alcohol-fixed, Papanicolaou-stained preparations (Figs. 8.6 and 8.15) and are less conspicuous with air-dried Romanowsky-stained smears (e.g., Diff-Quik). Nuclear grooves and INPIs are manifestations of nuclear membrane increased plasticity which makes them less stiff and much more deformable than normal; a nuclear groove, for example, results from a nucleus folded onto itself [20]. Although a sensitive feature for the cytologic diagnosis of PTC, nuclear grooves are not specific and can be seen in a variety of other thyroid neoplasms such as oncocytic neoplasms and non-neoplastic conditions like lymphocytic thyroiditis. Quantification studies have shown that PTC tends to have more nuclear grooves than other lesions, but they have not shown that a specific number of grooves establish a definite diagnosis. For this reason, they should not be relied upon in isolation to make a diagnosis of PTC. In addition, nuclear grooves are useful only when identified within follicular epithelial cells; care must be taken not to misinterpret histiocytes or Langerhans

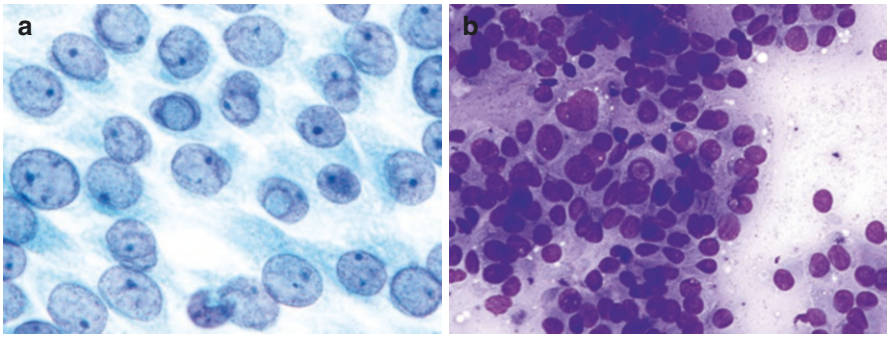

Fig. 8.13 Papillary thyroid carcinoma. (**a**) INPIs and micronucleoli are shown. Note that the two INPIs share the same aqua color and granular texture as the surrounding cytoplasm (smear, Papanicolaou stain). (**b**) A large INPI occupying most of the nucleus is seen in the center. The remaining nuclei show variation in size and shape (smear, Diff-Quik stain)

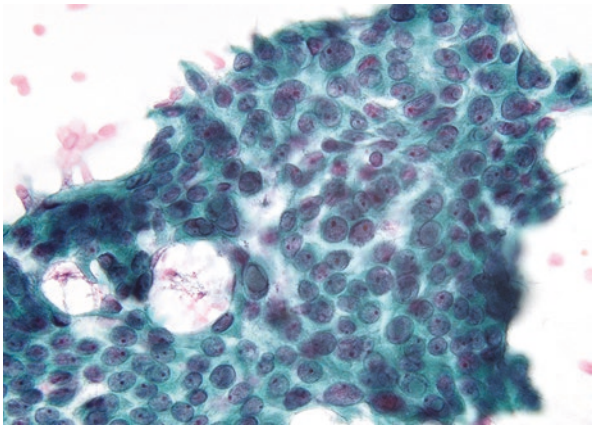

Fig. 8.14 Papillary thyroid carcinoma. Two intranuclear pseudoinclusions are seen. (smear, Papanicolaou stain)

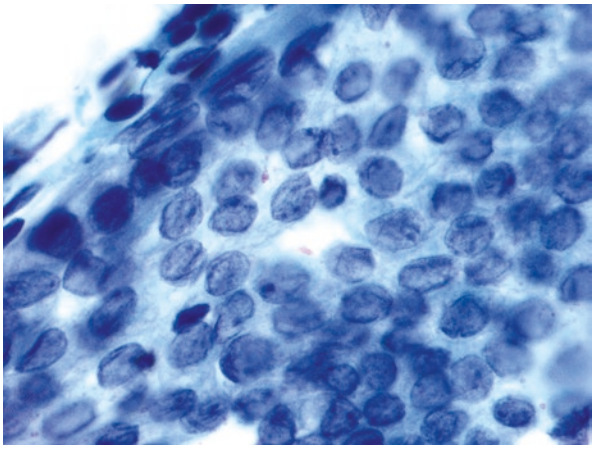

Fig. 8.15 Papillary thyroid carcinoma. Close inspection at high magnification shows frequent nuclear grooves, finely textured (powdery) chromatin, and micronucleoli (smear, Papanicolaou stain)

cells, which are characterized by elongated, oval nuclei with nuclear grooves, for the cells of PTC.

The nuclei of PTC typically display one to three micronucleoli, often positioned marginally underneath the nuclear membrane. On LBP, they are commonly eosinophilic (89%) and associated with a perinucleolar halo ("bare nucleoli") (63%) [12]. The latter has been reported to be very specific for PTC (96%) [12]. The eosinophilic nucleoli, however, are not only observed in PTCs but also in follicular nodular disease or follicular neoplasms, in particular those of the oncocytic type.

Multinucleated giant cells of histiocytic lineage are commonly seen in aspirates of PTC, even when cystic degeneration is not present (Fig. 8.16). Although common, they are not specific for PTC, and similar cells are seen in other conditions, both benign and malignant. The cells can be very large, and their nuclei can vary in number from few to numerous. They are part of the host response to the malignancy, along with other type of immune cells such as Langerhans cells, lymphocytes, and mast cells.

Psammoma bodies (PBs) are seen less frequently in FNA samples of PTC (4–20% of cases) than in histologic specimens (40–60%). They can be solitary or multiple, isolated, or attached to cells (Fig. 8.17). PBs alone (i.e., not associated with altered cells) are nonspecific and can be seen in MTC, lymphocytic thyroiditis, Graves' disease, and even nodular goiter. Calcifications resembling PBs occur in oncocytic neoplasms and represent calcification of colloid. The positive predictive value (PPV) for PTC of PBs in isolation is 50%; when seen in association with the cytologic features of PTC, the PPV is 100% [21].

The background usually contains relatively scant colloid, but some PTC subtypes (see below) can have abundant colloid. Colloid may be watery or dense and stringy with ropy pink strands, the so-called "bubble-gum" colloid (Fig. 8.18). The background is usually clean; the presence of necrotic debris is extremely uncommon in PTC and should raise the possibility of another malignancy. Hemosiderin-laden macrophages, representing hemorrhage and cystic changes, are common in PTC and can be prominent. Variable numbers of lymphocytes can be seen due to an underlying lymphocytic thyroiditis. When lymphocytes predominate, a

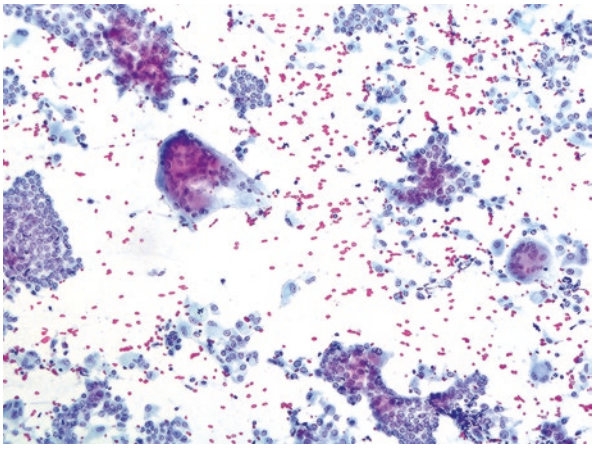

Fig. 8.16 Papillary thyroid carcinoma. Multinucleated giant cells accompany monolayered sheets of tumor cells. Although multinucleated giant cells are often seen in PTCs, they are a nonspecific finding (smear, Papanicolaou stain)

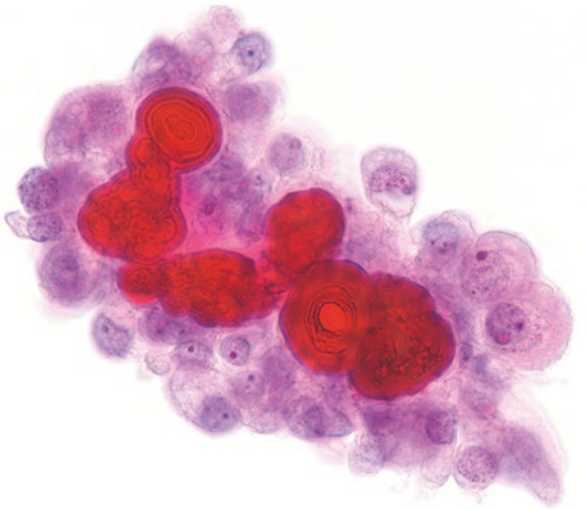

Fig. 8.17 Papillary thyroid carcinoma. Psammoma bodies are concentric rings and are lined here by atypical cells with oval, pale nuclei. Note that the tumor cells surrounding psammoma bodies show hobnail features (ThinPrep, Papanicolaou stain)

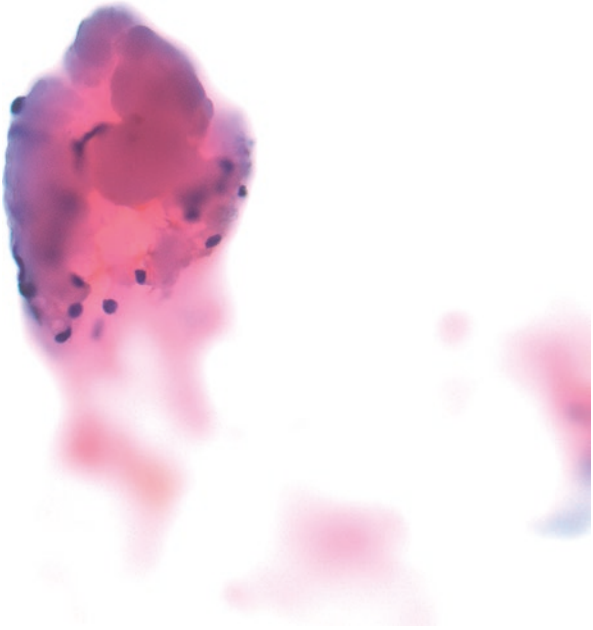

Fig. 8.18 Papillary thyroid carcinoma. The colloid in aspirates of PTC is often dense and stringy, the so-called "bubble-gum" colloid (Cytospin, Papanicolaou stain)

Warthin-like PTC or a diffuse sclerosing PTC should be considered (see below). Caution should be exercised when nuclear abnormalities are seen in follicular cell clusters with intimately admixed lymphocytes, as these nuclear changes may be reactive and not malignant.

Given an adequate sample, the majority of PTCs are straightforward to diagnose by FNA because most or all of the nuclear and architectural changes described

above are clearly identifiable and widespread. Such cases are reliably interpreted as malignant. In some PTCs, however, the nuclear changes are subtle and focal. Other PTCs may be incompletely sampled and yield only a small number of abnormal cells. If only one or two characteristic features of PTC are present, if they are only focal and not widespread throughout the follicular cell population, or if the sample is sparsely cellular, a malignant diagnosis cannot be made with certainty, and such cases are best classified as "Suspicious for Malignancy" (see Chap. 7).

Subtypes (Variants) of Papillary Thyroid Carcinoma (PTC)

Note: *In the latest WHO classification of thyroid tumors* [1], *the term "variant" has been replaced by "subtype" to allow for consistency with other WHO tumor classification schemes and avoid confusion with the molecular diagnostic term "genetic variant(s)."*

However, the term "variant" is still largely used, especially for FVPTC.

A substantial proportion of PTCs exhibit a variety of architectural and/or cytologic features that differ from those of conventional PTC. More than ten subtypes of PTC have been recognized considering: tumor size and delineation (encapsulated, invasive, and diffuse), architecture (follicular, macrofollicular, solid/trabecular, and micropapillary), cell type and shape (tall, spindle, columnar, oncocytic, clear, and hobnail), and associated stromal components (Warthin-like and fibromatosis/fasciitis-like stroma) [1, 2, 22]. Some PTC subtypes are associated with more aggressive and others with more indolent behavior than conventional PTC [1, 2, 22–24]. Columnar cell, hobnail, and tall cell subtypes are recognized as aggressive PTC subtypes by the American Thyroid Association (ATA) and WHO, with diagnosis of these subtypes impacting both risk stratification and clinical management [2]. The solid/trabecular subtype and the diffuse sclerosing subtype may be associated with a less favorable outcome, but the data remain conflicting [1, 2]. In contrast, the noninvasive FVPTC is indolent, with virtually no metastatic or recurrence potential following complete excision, and for this reason has been reclassified as NIFTP, a very low-risk neoplasm (see "Follicular Variant and NIFTP" below and Chap. 5) [1, 5].

Distinction between indolent and aggressive subtypes of PTC at the time of FNA contributes to risk stratification and may influence the management, depending also on the clinico-radiologic features in a given patient [2]. Precise subtyping, however, is rarely possible or reliable, because: (1) The predominant pattern may not have been sampled (many PTCs are heterogeneous with more than one growth pattern and/or cell type). (2) The architectural features of capsular and/or vascular invasion defining some of these subtypes cannot be assessed cytologically, akin to follicular thyroid adenoma/carcinoma. (3) The rarity of some of these subtypes makes it very difficult for the practicing cytopathologist to become familiar with their morphologic features which have significant overlap. (4) The PPV of any "specific" cytomorphologic features (described mostly in retrospective studies) is hard to predict since it is influenced by the incidence of the corresponding subtype in a given population [22, 23]. Nonetheless, the architectural and cytologic features that distinguish these subtypes from conventional PTC histologically are often observed cytologically, and

awareness of the phenotypic characteristics of the various subtypes of PTC can diminish the risk of misdiagnosis. Recognition of PTC subtypes at the time of FNA is generally not required; however, some of the more common subtypes such as tall cell PTC may be at least favored or suggested (see sample reports) [22–24].

Follicular Variant of PTC and NIFTP (See Also Chap. 5)

Definitions

The follicular variant of PTC (FVPTC) is completely or almost completely composed of small to medium-sized follicles lined by cells with variable nuclear features of PTC.

NIFTP is a noninvasive neoplasm with a follicular growth pattern and variable nuclear features of PTC. This terminology was introduced in 2016 to recognize the indolent behavior of thyroid neoplasms previously classified as noninvasive FVPTC and was included as a new entity in the 2017 WHO classification. Rigorous histologic criteria are applied for this diagnosis: the tumor must be well demarcated from surrounding normal thyroid (with or without a capsule) and it must have the nuclear features of PTC, although they are usually more subtle than with the classic PTC [1, 5]. The nuclear features may be focal, patchy, diffuse, or multifocal. Complete examination of the tumor capsule/interface is required to exclude capsular or vascular invasion. There are also several exclusion criteria including: >1% true papillae, PBs, tall cell or columnar cell features, >30% solid/trabecular/insular architecture, necrosis, or increased mitoses (≥3 per 10 high power fields) [1, 5].

Background

There are two distinct groups within FVPTC that differ morphologically, genetically, and clinically.

1. *FVPTC with an infiltrative growth pattern* is associated with frequent lymph node metastases, a risk of recurrence, and *BRAF* V600E mutations, similar to conventional PTC ("BRAF-like PTCs") [1, 2, 25]. *Diffuse FVPTC* is a rare and aggressive variant of infiltrative FVPTC that typically occurs in young females, extensively involving one lobe or both lobes in a multinodular fashion, with frequent distant metastases in the lungs and/or bones with or without concurrent regional lymph node metastases.
2. The *encapsulated FVPTC* is characterized by a follicular growth pattern with no papillae formation and partial or total tumor encapsulation, and the diagnosis rests on finding characteristic nuclear features of PTC. Historically, encapsulated FVPTC has been a controversial entity with poor diagnostic (cytologic and histologic) reproducibility. Most encapsulated FVPTCs show no invasive growth, whereas in about one-third of cases tumor capsular and/or vascular invasion are found [2]. These tumors, which frequently harbor *RAS* mutations, are biologically, genetically, and clinically closer to the follicular adenoma/carcinoma group than the PTC group ("RAS-like PTCs") [1, 25]. Encapsulated FVPTC with invasion tends to spread in a fashion similar to follicular thyroid carcinoma, with

distant lung and bone metastases and infrequent lymph node metastases [1]. In the absence of capsular or vascular invasion, encapsulated FVPTCs have a very low risk of recurrence or extrathyroidal spread, even in patients treated by lobectomy alone, provided that the tumor is completely excised [1, 2, 5]. Therefore, a carefully defined subset of encapsulated FVPTC has been reclassified as NIFTP, using strict histologic inclusion and exclusion criteria (see above) [1, 5].

NIFTP is a very low-risk tumor that likely represents a preinvasive stage of invasive encapsulated FVPTC [1, 5]. The paradigm shift in terminology has important clinical consequences and affects the cytologic diagnosis of thyroid nodules [7–11]. In several European countries and North America, NIFTP comprises approximately 10–20% of all tumors previously classified as thyroid malignancies, with significantly lower prevalence in Asia [1, 7–11]. Accordingly, adoption of this terminology lowers the frequency of a histopathologic diagnosis of thyroid cancer. It also causes an overall decrease in the risk of malignancy associated with thyroid FNA diagnoses, especially in the indeterminate diagnostic categories but also in the Malignant category, since NIFTP comprised a small subset (approximately 3%) of thyroid FNAs that were classified as Malignant in retrospective studies published shortly thereafter (see Chaps. 1 and 5) [7–11].

A NIFTP cannot convincingly be recognized as such by FNA because some of its defining features (like circumscribed margins) cannot be assessed cytologically. Nevertheless, the predominance of follicular architecture and associated nuclear changes (albeit mild) allow most NIFTPs to be recognized as abnormal, with about half of all NIFTPs diagnosed as FN and most of the remaining cases diagnoses as either Suspicious for Malignancy or AUS [7–11]. Because the nuclear changes are subtle (as with many invasive FVPTC), few NIFTPs are interpreted as malignant by FNA. To avoid overtreatment, it is highly desirable to exclude potential NIFTP cases from the Malignant category and limit this category to conventional and other subtypes of PTC. Nevertheless, given the histologic criteria for NIFTP [1, 5], it may not be possible to completely eliminate NIFTP from the Malignant category, even when using more stringent criteria. Thus, some pathologists may prefer to include an educational note to reinforce this limitation (see Chap. 1, Table 1.4, as well as "Sample Reports" below).

How to Distinguish NIFTP from PTC on Cytology?

The degree to which the characteristic nuclear features of PTC are displayed in FVPTC and NIFTP varies from case to case, with a wide quantitative and qualitative spectrum (Figs. 8.19 and 8.20) [9–11, 26–29]. Some FVPTCs, usually those that are infiltrative, have prominent classic nuclear features of PTC, but with others, especially the encapsulated FVPTC (including NIFTP), the features are only partially and focally displayed. Because of the significant overlap in the cytologic features between FVPTC and NIFTP, a definite distinction between these entities is not possible by FNA. FNA specimens from FVPTCs can be separated into two different

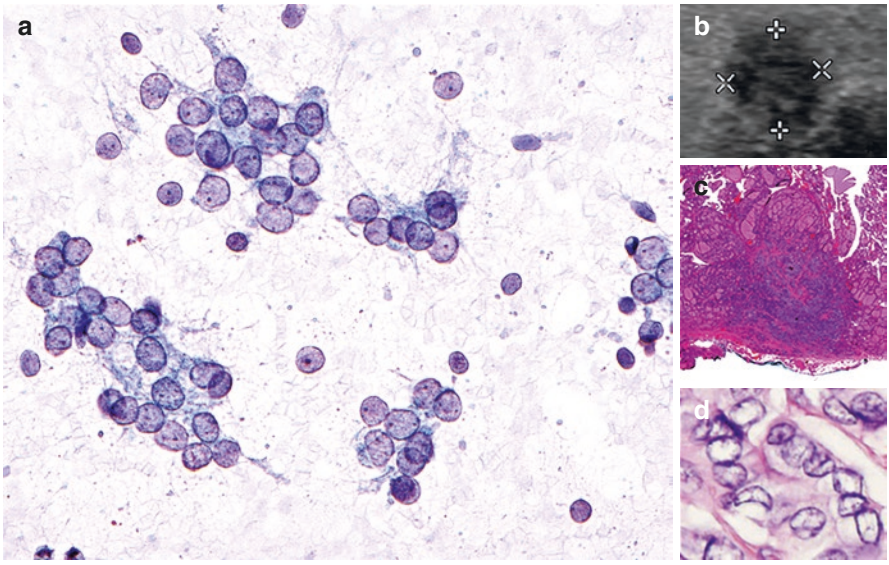

Fig. 8.19 Papillary thyroid carcinoma, follicular variant. (**a**) The aspirate shows microfollicles with crowded, enlarged clear oval nuclei (smear, Papanicolaou stain). (**b**) Ultrasound shows solid nodule with blurred margins. (**c**) This correlates with infiltrative margin on histology. (**d**) Histologically the tumor is composed of microfollicles with "Orphan Annie eye" clear nuclei (hematoxylin and eosin stain)

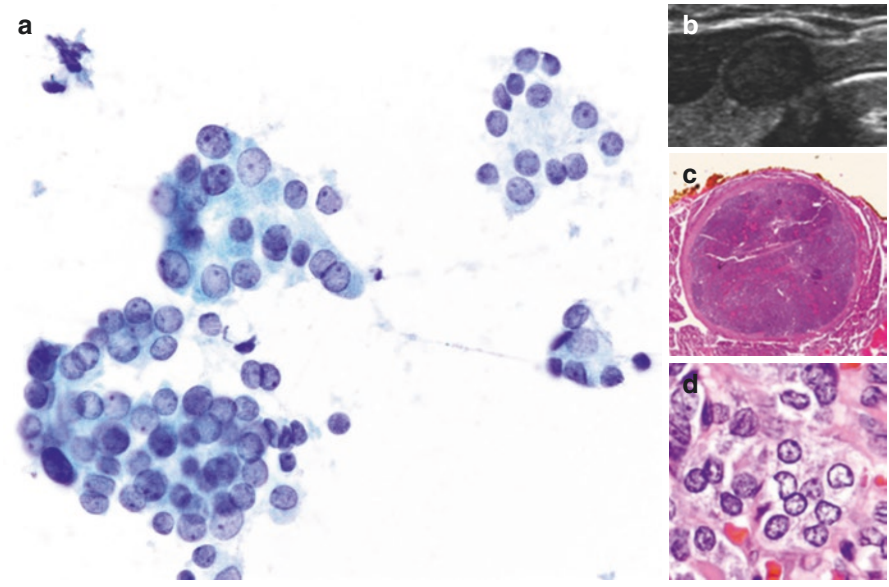

Fig. 8.20 Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (formerly called encapsulated follicular variant of papillary thyroid carcinoma). (**a**) The aspirate shows microfollicles with crowded, enlarged, clear, oval nuclei along with microfollicles with small dark nuclei (smear, Papanicolaou stain). (**b**) Ultrasound shows well-circumscribed solid nodule with a rim, correlating with encapsulation on histology (**c**). (**d**) Histologically, the tumor is composed of microfollicles with "Orphan Annie eye" clear nuclei (hematoxylin and eosin stain)

groups. In the first (30–40% of cases), the FVPTC shows widespread nuclear features of PTC and may be difficult to distinguish from a conventional PTC, especially from those that show a predominant follicular growth pattern. In the second group, which represents the majority of FVPTC and NIFTP cases, the tumor cells show only mild nuclear enlargement and elongation, chromatin clearing, and thick nuclear membranes, with INPIs and nuclear grooves rare or absent. These cytologic samples typically fall into one of the indeterminate categories: Suspicious for PTC (25–35%), FN (25–30%), or AUS (10–20%) [1, 7–11]. NIFTPs are often associated with more subtle nuclear features than classical PTC. Cytomorphologic features which are in favor of classic PTC and against the diagnosis of NIFTP are sheet-predominant architectural pattern, presence of true papillae and PBs, and easily identifiable INPIs. In addition, the nuclei of NIFTP are smaller, less elongated, and more rounded than those of conventional PTC. NIFTP nuclei are also not as crowded and have grooves that are more delicate and focal in comparison to its PTC counterpart [9–11].

In summary, a definitive cytologic diagnosis of PTC can be achieved by applying strict morphologic criteria. These criteria include high cellularity, predominant tumor sheets with papillae or swirls, marked nuclear enlargement with elongation of the nuclei, easily visualized grooves involving the long axis of the nuclei, fine chromatin, nuclear crowding, and the presence of more than rare INPIs (≥3). In contrast, a cytologic specimen raises the differential diagnosis of NIFTP if it exhibits low to moderate cellularity, a follicular architecture, mild nuclear enlargement, and delicate nuclear grooves, but lacks papillae, PBs, or definite INPIs (see also Chap. 5).

Macrofollicular Variant of PTC

Definition

The macrofollicular variant of PTC (MFVPTC) is a FVPTC in which over 50% of the follicles are arranged as macrofollicles (follicles measuring more than 200 μm in diameter).

Criteria

The sample consists of monolayered (two-dimensional) sheets of neoplastic epithelium and/or variably sized follicles.

Convincing nuclear changes of PTC must be present for a definite interpretation of malignancy.

In contrast to conventional PTC, the diagnostic nuclear features are often more subtle as seen in FVPTC.

Papillary structures and psammoma bodies are not seen.

Abundant thin colloid or fragments of thick colloid may be present.

Explanatory Notes

MFVPTC is one of the rarest histologic subtypes of PTC with less than 100 reported cases; it is commonly underdiagnosed as benign, both on histology and cytology,

due to the patchy nuclear features of PTC [30–32]. It is characterized by a low incidence of lymph node metastasis, but when metastases occur the macrofollicular architecture is usually maintained. Most MFVPTCs have an indolent behavior, but multiple bone and lung metastases may occur. The differential diagnosis of MFVPTC includes the benign follicular nodule seen with follicular nodular disease and the follicular adenoma of macrofollicular type. MFVPTC is easily

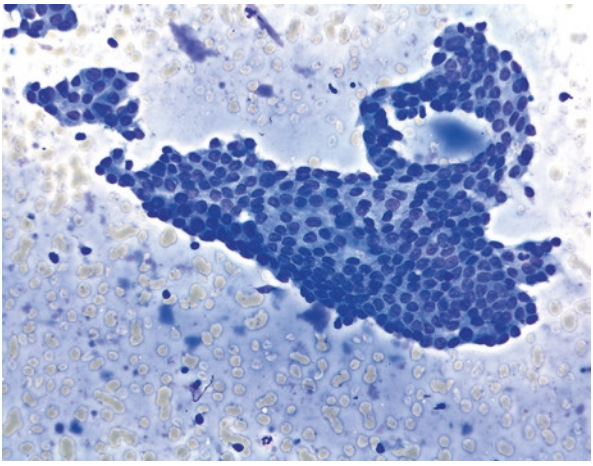

Fig. 8.21 Papillary thyroid carcinoma, macrofollicular. The neoplastic cells resemble those of a benign thyroid nodule at scanning magnification. In such cases there can be abundant thin colloid and relatively few sheets of cells. The difference lies in the nuclear features, which are better appreciated at high magnification (smear, Diff-Quik stain)

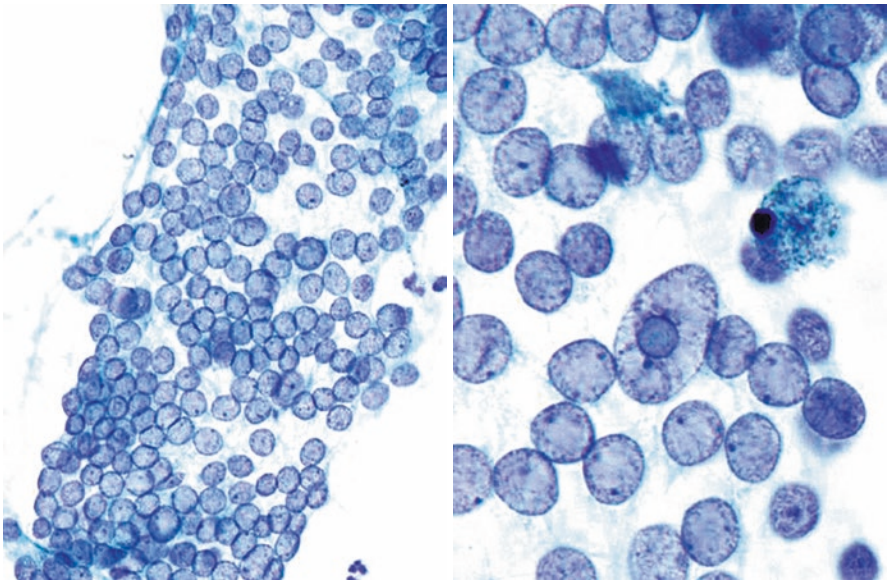

Fig. 8.22 Papillary thyroid carcinoma, macrofollicular. *Left*, There is a large sheet of tumor cells with crowded, "Orphan Annie eye" nuclei; *Right*, An intranuclear pseudoinclusion is present in the large oval nucleus. Note also the marginal micronucleoli (smear, Papanicolaou stain)

underappreciated at low magnification due to the abundance of thin colloid, the low cellularity, and the subtle and focal nuclear atypia. Thus, careful attention to nuclear features is necessary for all benign-appearing thyroid aspirates. Cytologically, the neoplastic cells usually have round/ovoid nuclei, either small or conspicuous eccentrically located nucleoli, chromatin clearing, nuclear overlapping, and nuclear grooves (Figs. 8.21 and 8.22) [30–32]. Only 45% of cases show INPIs, which range from rare to few [31]. Moderate-to-abundant thin and focally thick colloid and macrophages are often present. In contrast, PBs and papillary structures have not been reported. If follicular cells with round/ovoid nuclei, small-to-prominent, eccentrically located nucleoli, nuclear overlapping, and chromatin clearing are present in a background of abundant colloid, it is prudent to consider the possibility of MFVPTC and render a diagnosis of at least AUS, instead of a benign colloid nodule [31].

Cystic PTC

Definition

As the name implies, cystic PTC is a PTC that is predominantly cystic. It is a cytologic variant rather than a true histologic subtype, comprised of thin/watery fluid, abundant histiocytes, and hypervacuolated tumor cells. Cystic PTC on FNA correlates to a subset of the encapsulated (classic) PTC, a recognized PTC subtype in the WHO classification [1], with various stages of cystic degeneration.

Criteria

The neoplastic cells are typically arranged in small groups with irregular borders; sheets, papillae, or follicles may also be present.

Tumor cells look "histiocytoid" (i.e., hypervacuolated).

Macrophages, often containing hemosiderin, are present.

A variable amount of thin or watery colloid.

Convincing nuclear changes of PTC must be present for a definite diagnosis of malignancy.

In contrast to conventional PTC, fine/powdery chromatin is usually less prominent.

Explanatory Notes

The most common cystic lesion of the thyroid is cystic follicular nodular disease. On the other hand, PTC is the most common malignant neoplasm of the thyroid to undergo cystic changes. The amount of cystic change varies from case to case; approximately 10% of PTCs are almost entirely cystic [33, 34]. FNAs of cystic PTC show varying proportions of macrophages, colloid, and vacuolated "histiocytoid" neoplastic cells (Fig. 7.4) [33, 34]. A few small papillae comprised of viable tumor cells are sometimes present. The neoplastic cells of cystic PTC have more abundant, granular, or vacuolated cytoplasm than those of conventional PTC. The tumor cells

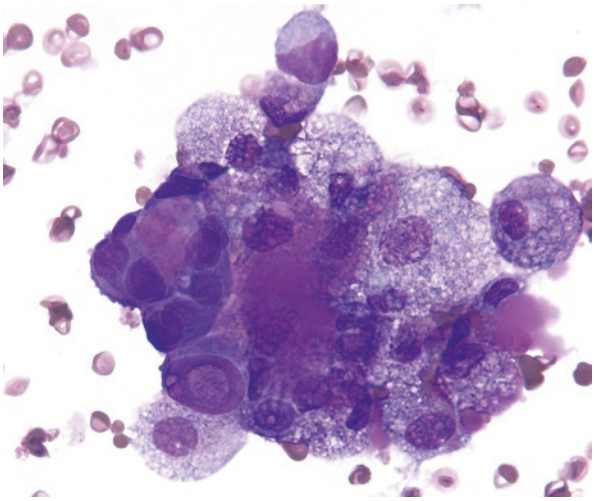

Fig. 8.23 Papillary thyroid carcinoma, cystic. There is prominent cystic change with numerous hemosiderin-laden macrophages. A small cluster of neoplastic cells has smooth, dense cytoplasm, and one cell has a large intranuclear pseudoinclusion (smear, Diff-Quik stain)

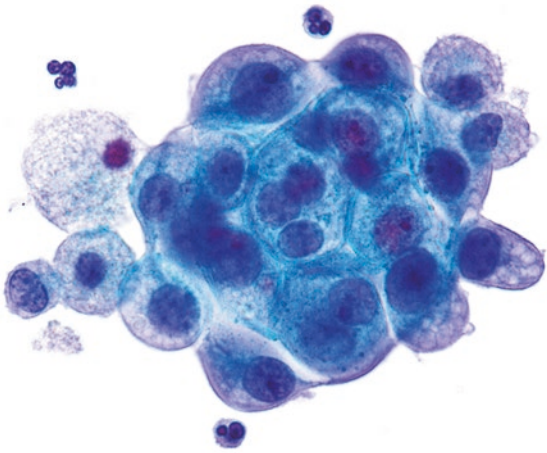

Fig. 8.24 Papillary thyroid carcinoma, cystic. Most of the cells in this image are neoplastic. They have abundant granular cytoplasm, hence the descriptor "histiocytoid." Classic nuclear features of papillary thyroid carcinoma are absent, but there is conspicuous nuclear enlargement (ThinPrep, Papanicolaou stain)

frequently appear more rigid and polygonal than normal follicular cells and display enlarged, oval to irregularly shaped nuclei with prominent nuclear grooves and occasional INPIs (Fig. 8.23). Some of the characteristic nuclear features of PTC, however, like pale, "powdery" chromatin, are often less apparent or even conspicuously absent (Fig. 8.24).

It should be noted that similar atypical cells are sometimes seen in benign follicular nodules with cystic change. These reactive cells may appear "histiocytoid" or they may be arranged in streaming sheets or cyst-lining cells which have enlarged nuclei, nucleoli, nuclear pallor, and occasional nuclear grooves. Their benign nature is betrayed by their elongated shape and the lack of nuclear crowding. In some

cases, however, the nuclear changes of cyst-lining cells can be extreme, and they occasionally show INPIs. Such cases are therefore properly diagnosed as "Suspicious for PTC" or AUS (see Chaps. 7 and 4, respectively).

Whereas some aspirates of cystic PTC are composed of abundant neoplastic cells and are readily interpreted as PTC, others have no neoplastic cells at all and are best interpreted as "Nondiagnostic; cyst fluid only" (see Chap. 2). Indeed, cystic PTC has long been recognized as a possible cause of false-negative thyroid FNAs. This concern may be less common in some centers with the precise sampling of a subcentimeter solid mural nodule within the cyst under high-resolution ultrasound guidance.

Oncocytic PTC

Definition

The oncocytic PTC is a thyroid tumor with nuclear changes characteristic of PTC but composed predominantly of oncocytic cells with variable architecture (follicular, papillary, or solid).

Criteria

The sample is composed predominantly of oncocytic cells (polygonal cells with abundant granular cytoplasm), arranged in papillae, sheets, microfollicles, or as isolated cells.

Convincing diagnostic nuclear changes of PTC must be present for a definite diagnosis of PTC.

Lymphocytes are absent or few in number.

Explanatory Notes

Focal oncocytic change is seen in many PTCs, including the conventional PTC. Only when the oncocytic changes are widespread (>75% of tumor cells) does the tumor

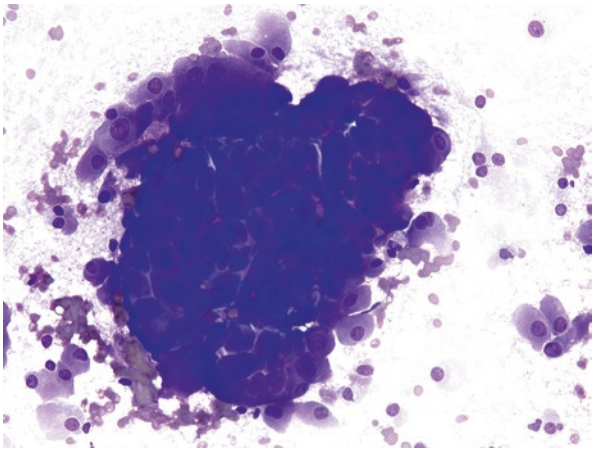

Fig. 8.25 Papillary thyroid carcinoma, oncocytic. The entire neoplasm is composed of oncocytic cells that have abundant granular cytoplasm. The nuclear features of papillary carcinoma are not readily apparent in this image; such cases are good mimics of oncocytic follicular neoplasms (smear, Diff-Quik stain)

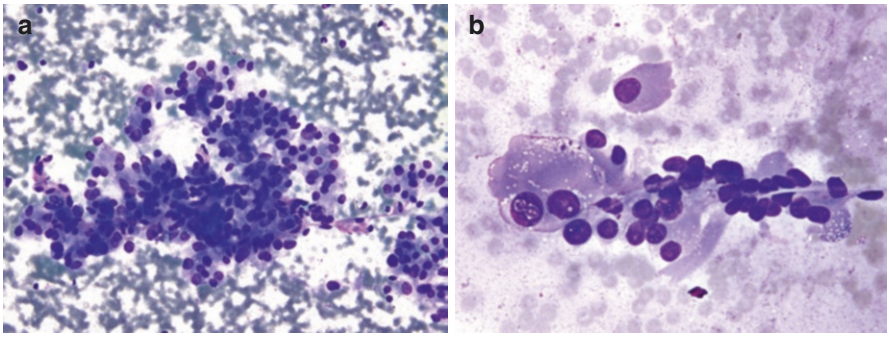

Fig. 8.26 Papillary thyroid carcinoma, oncocytic. (**a**) Loosely cohesive oncocytic cells have atypical, oval-shaped nuclei and rare intranuclear pseudoinclusions without nuclear grooves; such cases are good mimics of oncocytic follicular neoplasm or medullary thyroid carcinoma. (**b**) Multiple small and large intranuclear pseudoinclusions are seen in a large oncocytic cell with abundant granular cytoplasm (smears, Diff-Quik stain)

merit distinction as an oncocytic PTC (Figs. 8.25 and 8.26) [35, 36]. Aspirates of oncocytic PTC resemble those from other follicular cell-derived oncocytic proliferations, oncocytic MTC, and other oncocytic neoplasms (e.g., metastatic renal cell carcinoma). The characteristic nuclear features of PTC, therefore, must be searched for whenever an aspirate is composed predominantly of oncocytes. Non-PTC oncocytic lesions generally have rounder nuclei and more prominent nucleoli than the oncocytic variant of PTC. In addition, non-PTC follicular cell-derived oncocytic proliferations may have nuclear grooves and slight nuclear pallor, but INPIs are very rarely seen. When the full nuclear features of PTC are evident, oncocytic PTC can be readily diagnosed on FNA. When the nuclear features of PTC are not widespread, the case is best classified as "Follicular Neoplasm, Oncocytic Follicular Neoplasm" or as "Suspicious for PTC, oncocytic subtype." Lymphocytes are typically absent in FNAs of the oncocytic subtype of PTC; if present in large numbers, a Warthin-like PTC should be considered.

Warthin-Like PTC

Definition

The Warthin-like PTC (WL-PTC) is a circumscribed thyroid tumor with papillary architecture and lymphoid follicles that resembles a Warthin tumor of the parotid gland. It is often associated with Hashimoto's thyroiditis [1, 37–39]. The neoplastic cells have abundant granular eosinophilic (oncocytic) cytoplasm and the nuclear features of PTC.

Criteria

The neoplastic cells are oncocytic and arranged in papillae, monolayered sheets, and as dispersed cells.

A lymphoplasmacytic background is present; the lymphocytes and plasma cells permeate the fibrovascular stalk and are intimately associated with the tumor cells.

Convincing nuclear changes of PTC must be present for a definite diagnosis of malignancy.

Explanatory Notes

WL-PTC is a rare subtype with a prevalence of 0.2–1.9% of all PTCs, having a unique histomorphology simulating Warthin tumor of the parotid gland. Review of the available literature on cytological features of 28 cases of WL-PTC showed that while most (64.4%) were correctly diagnosed as Malignant-PTC, a smaller but significant percentage (10.7%) were erroneously labeled benign thyroid aspirates (thyroiditis) [39]. The rest were variably described as AUS or Suspicious for PTC. Even though most cases were appropriately diagnosed as PTC, they were not specifically subtyped as WL-PTC on cytology. Because of the mixture of oncocytes and lymphocytes, FNAs from WL-PTC resemble those from Hashimoto thyroiditis (Fig. 8.27) [37]. Also, the tumor itself is associated with Hashimoto thyroiditis more commonly than classical PTC (93% vs. 36%, respectively). The oncocytic cells of Hashimoto thyroiditis, however, typically have a round nucleus with a prominent single nucleolus; the nuclei of PTC (including the WL-PTC), by contrast, are more irregular in contour and nucleoli are less prominent. The oncocytic cells in Hashimoto thyroiditis may show nuclear clearing and grooves, but papillary fragments and INPIs are usually not seen. Processing of samples by LBP technique

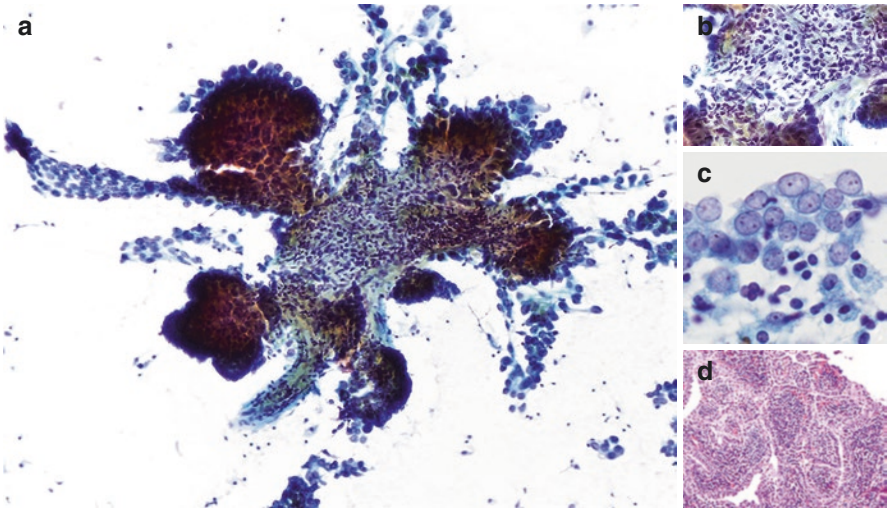

Fig. 8.27 Papillary thyroid carcinoma, Warthin-like. (**a**) The aspirate shows papillary fragments in a lymphocytic background (smear, Papanicolaou stain). (**b**) The fibrovascular cores are engorged with lymphocytes (smear, Papanicolaou stain). (**c**) The epithelial cells are also intimately associated with lymphocytes. The nuclei are enlarged, oval, and clear (smear, Papanicolaou stain). (**d**) Histologically, the tumor resembles a Warthin tumor of the salivary gland, with tumor epithelium surrounding lymphoid aggregates. Typical nuclear features of papillary carcinoma can be seen at high power (not shown) (hematoxylin and eosin stain)

increases the yield of the tumor cells; however, there is loss of the reactive lymphocyte-rich background. It may virtually be impossible to separate a WL-PTC from an oncocytic PTC associated with Hashimoto thyroiditis. Nevertheless, the distinction between WL-PTC and oncocytic PTC does not have any clinical implication in terms of management as well as prognosis [38, 39].

Tall Cell PTC

Definition

The tall cell PTC (TC-PTC) is an aggressive form of PTC composed of elongated "tall" tumor cells (on histologic samples their height is at least three times their width [1]) with abundant dense granular eosinophilic cytoplasm, prominent cell membranes, and the typical nuclear changes of PTC.

Criteria

The neoplastic cells are most commonly polygonal with centrally located nuclei but can be elongated and cylindrical with an eccentrically placed nucleus ("tail-like" cells or "tadpole" cells). They have granular cytoplasm with prominent cytoplasmic borders.

Some lymphocytes may be present.

Convincing nuclear changes of PTC must be present for a definite diagnosis of malignancy.

In contrast to conventional PTC:

- the nuclei tend to be larger and more elongated.
- the nuclear chromatin is sometimes less powdery and more granular.
- the nucleoli can be prominent and centrally placed.
- mitotic figures may be present.
- PBs are fewer in number.
- INPIs tend to be more frequent and more often multiple within a single nucleus, imparting a "soap bubble" appearance to the nucleus.

Explanatory Notes

The TC-PTC is the most common aggressive variant and accounts for 4–16% of all PTC cases. It tends to occur in elderly patients and is more common in men than other PTCs [2, 22, 24]. It frequently presents as a large and bulky tumor, often with extrathyroidal extension and vascular invasion [1, 2]. It is more aggressive than the conventional PTC and has a higher incidence of local recurrence, central neck involvement, and distant metastasis [1, 2, 22, 24]. TC-PTC accounts for a substantial portion of radioactive iodine refractory thyroid carcinomas [1]. According to the WHO classification, tall cells must account for ≥30% of all tumor cells for the diagnosis of TC-PTC [1]. However, if 10% or more of a PTC has tall cell features, the tumor is also associated with an adverse clinical outcome [1, 40]. Therefore, the identification of a minor tall cell component can be clinically significant. Up to 90%

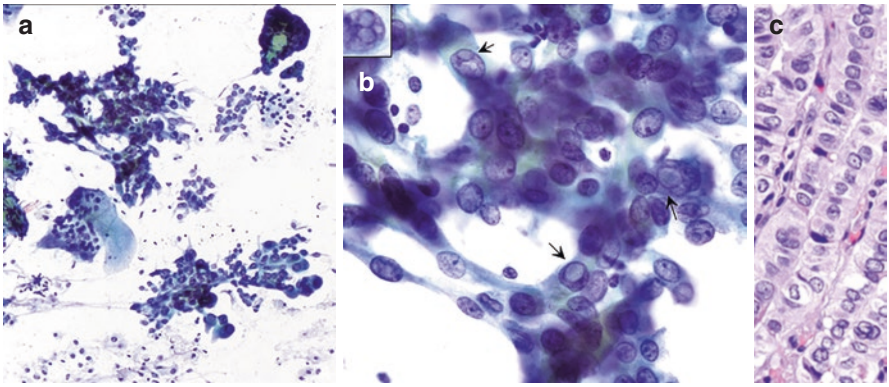

Fig. 8.28 Papillary thyroid carcinoma, tall cell. (**a**) The smear shows elongated cells in loosely cohesive arrangements (smear, Papanicolaou stain). (**b**) The cytoplasm is elongated, with frequent nuclear pseudoinclusions and rare soap bubble nuclei (inset) (smear, Papanicolaou stain). (**c**) Histologically, this variant is comprised of tall rectangular tumor cells with eosinophilic cytoplasm arranged in parallel rows (hematoxylin and eosin stain)

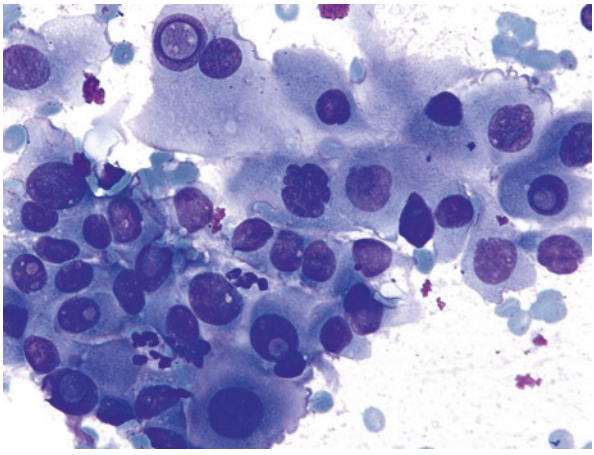

Fig. 8.29 Papillary thyroid carcinoma, tall cell. "Soap bubble-like" intranuclear pseudoinclusions (9 o'clock) are often seen in the tall cell variant of papillary thyroid carcinoma (smear, Diff-Quik stain)

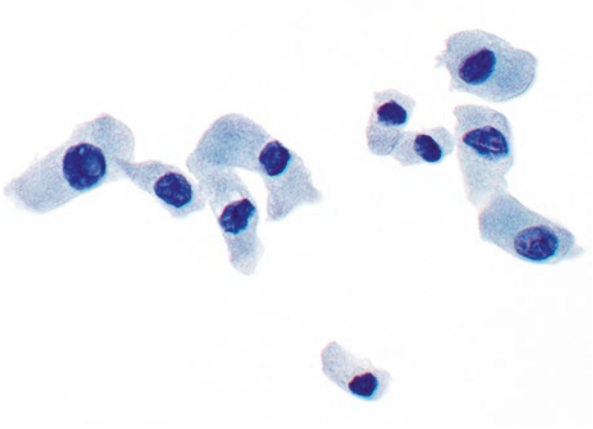

Fig. 8.30 Papillary thyroid carcinoma, tall cell. The "tallness" of these cells is readily appreciated. When this morphology is seen throughout the sample, one can raise the possibility of a tall cell variant in the FNA report (ThinPrep, Papanicolaou stain)

of TC-PTCs harbor the *BRAF* V600E mutation. *TERT* promoter mutations, which are associated with a worse outcome in PTCs, are also significantly more prevalent in TC-PTC (31%) compared to conventional PTC (<10%) [25]. TC-PTC is easily recognized as a PTC due to the prominence of the nuclear features of PTC, especially nuclear grooves and INPIs, which are frequent and easily identified (Figs. 8.28, 8.29, and 8.30) [14, 41–43]. Tall cell features may be easier to assess on LBPs than on conventional smears (Fig. 8.30) [12, 14, 44]. Tall cells are present in the majority of TC-PTC cases and are typically located at the periphery of cell clusters and as single cells [44]. Cytoplasmic cuffing along the periphery of cell clusters and soap bubble INPIs, when present, are highly suggestive of TC-PTC [44]. TC-PTC cases are more likely to show abundant oncocytic cytoplasm and distinct cell borders [44]. Finally, cytoplasmic tails are more likely to be present and more numerous in TC-PTC [44]. Although it is not essential to specify the variant of PTC by FNA in general, a TC-PTC (or tall cell features) may be at least suggested by FNA and it may influence the extent of surgery in a subset of cases along with other clinico-radiological factors.

Columnar Cell PTC

Definition

The columnar cell PTC (CC-PTC) is characterized by columnar cells with hyperchromatic, oval, and pseudostratified nuclei with supranuclear or subnuclear cytoplasmic vacuoles, reminiscent of a colonic adenoma or secretory-type endometrium [1]. The cells are typically arranged in papillae, but trabeculae and follicles can also be seen.

Criteria

Smears are cellular and generally lack colloid.

The neoplastic cells are arranged as papillae, clusters, and flat sheets, sometimes with small tubular structures.

The nuclei are elongated and pseudostratified.

Focal cytoplasmic vacuolization may be present.

Convincing nuclear changes of PTC must be present for a definitive diagnosis of malignancy.

In contrast to conventional PTC:

- the nuclear features of PTC (grooves, INPIs) are much less prominent.
- the nuclear chromatin tends to be hyperchromatic rather than pale and powdery.
- colloid and cystic changes (macrophages) are typically not seen.

Explanatory Notes

The CC-PTC is one of the least common subtypes of PTC (<0.4% of all PTCs). The majority of CC-PTCs occur in older male patients and are large, invasive tumors with extrathyroidal extension that pursue an aggressive clinical course [1, 45]. Rare well-circumscribed/encapsulated and intrathyroidal CC-PTC occur in younger

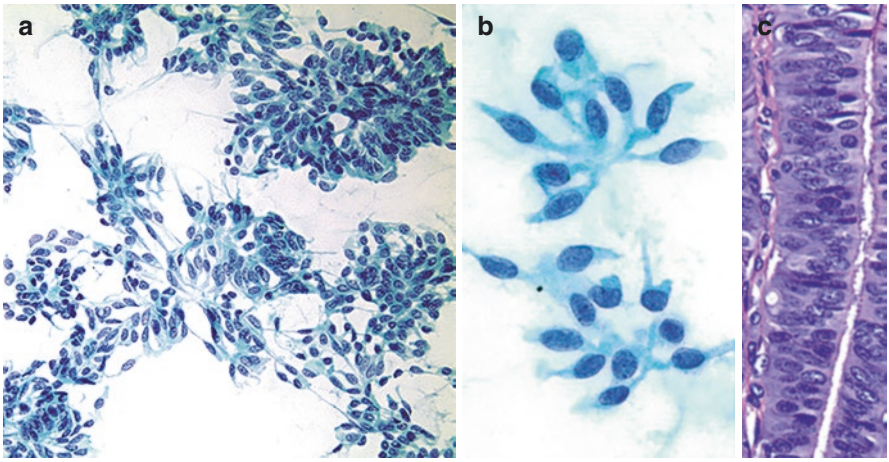

Fig. 8.31 Papillary thyroid carcinoma, columnar cell. (**a**) The aspirate shows loosely cohesive spindle-shaped cells (smear, Papanicolaou stain). (**b**) The cytoplasm is bipolar and wispy, and cigar-shaped nuclei have few characteristic features of papillary thyroid carcinoma (smear, Papanicolaou stain). (**c**) Histologic examination shows rows of pseudostratified columnar cells with elongated hyperchromatic nuclei and scanty cytoplasm (hematoxylin and eosin stain). (Courtesy of Dr. Tamar A. Giordgadze of Medical College of Wisconsin)

female patients and are comparatively indolent [1, 45]. Therefore, the mere presence of columnar cell features on cytology alone may not predict a worse outcome. CC-PTC does not show the typical nuclear features of PTC [1, 45–47]. The cells are usually large with pseudostratified oval or elongated nuclei and powdery chromatin (Fig. 8.31). Hypercellular smears composed almost exclusively of papillary structures with pseudostratified dark nuclei with a paucity of INPIs and nuclear grooves are highly suggestive of CC-PTC [47]. The unique morphology of CC-PTC generally allows it to be recognized as a neoplasm on FNA; however, due to lack of PTC nuclei, it may be underdiagnosed as FN or diagnosed as Malignant, but not typed as PTC. The dark and stratified nuclei of CC-PTC can mimic a metastasis from a colorectal or endometrial primary [46], but the necrotic background commonly present in metastatic disease from these primaries is unusual in CC-PTC. Clinicoradiological correlation, in addition to a limited IHC panel that includes thyroglobulin and TTF-1, can be very helpful. Importantly, PAX8 is expressed in both CC-PTC and gynecological carcinomas, and CDX-2 is expressed in up to 55% of CC-PTC [1], somewhat limiting the diagnostic value of these two markers. Occasionally, the neoplastic cells of CC-PTC may also be mistaken for MTC or even benign respiratory epithelial cells. Most CC-PTC demonstrate activating oncogenic driver alterations in *BRAF* including *BRAF* V600E mutation in 30–44% of cases, with most cases also harboring secondary oncogenic mutations, including *TERT* or *TP53*, and multiple chromosomal gains and losses [45].

Solid/Trabecular PTC

Definition

The solid/trabecular PTC (ST-PTC) is defined histologically by the presence of solid and/or trabecular and/or nested (insular) areas that lack papillae, follicles, and colloid storage and occupy >50% of the tumor [1]. The neoplastic cells have typical nuclear features of PTC.

Criteria

Smears are variably cellular and generally lack colloid.

The neoplastic cells may appear as cohesive, syncytial-type 3-dimensional tissue fragments, microfollicles/trabeculae, or non-cohesive, single cells.

The nuclei usually show the typical nuclear features of PTC, but they may be less elongated (rounder) and darker than those of conventional PTC.

True papillary formations with fibrovascular cores are scant or absent.

Explanatory Notes

ST-PTC is a rare PTC subtype (1–3% of adult PTCs) that is still poorly characterized. It is common in children and has been reported in >30% of children following the Chernobyl accident [1]. This variant is also more common in children without radiation exposure. The prognosis of ST-PTC appears to be less favorable in adults when compared to conventional PTC [1]. In a meta-analysis of 205 ST-PTCs, these tumors manifested a significantly higher risk for vascular invasion, tumor recurrence, and cancer mortality as compared to conventional PTC [48]. The genetic profile of ST-PTC is also distinct from that of conventional PTC. In general, the prevalence of *BRAF* mutations in ST-PTCs is rather low in comparison with those in conventional

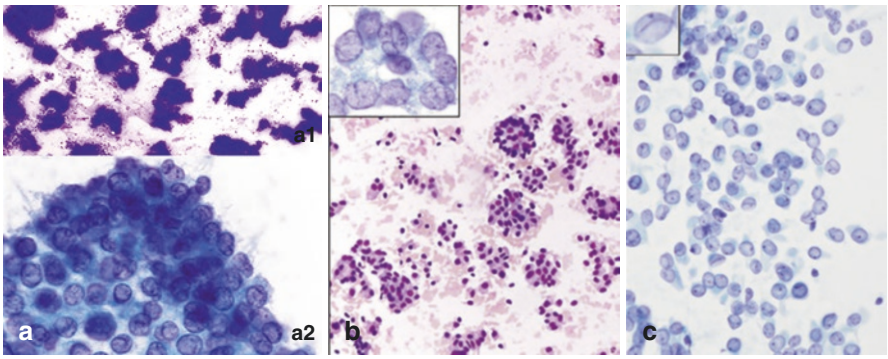

Fig. 8.32 Papillary thyroid carcinoma, solid/trabecular. This subtype may demonstrate three different cytologic patterns: (**a**) a cohesive, syncytial tissue fragment pattern, (**b**) a microfollicular/trabecular pattern, and (**c**) a non-cohesive, single-cell pattern. All three patterns have characteristic nuclear features of papillary carcinoma: convoluted clear nuclei in ***a2***, nuclear clearing and convolution in the inset of **b**, and nuclear clearing and grooves in **c**. (***a1*** and **b**: smears, Diff-Quik stain; ***a2***, **c** and **insets**: smears, Papanicolaou stain)

PTCs. In contrast, gene fusions such as *RET* or *NTRK1/3* are more prevalent in ST-PTCs [48]. The prevalence of *TERT* promoter mutation in ST-PTC is slightly higher than in conventional PTCs [48]. Because of the lack of criteria with high specificity and sensitivity, the preoperative diagnosis of SV-PTC is hardly ever made or suggested on cytology (Fig. 8.32) [49, 50]. Most cases of ST-PTC are diagnosed as Malignant or Suspicious for Malignancy (PTC or FVPTC) [49, 50]. The microfollicular pattern of ST-PTC is difficult to distinguish from other follicular-patterned lesions, including FVPTC and follicular neoplasms, and the typical nuclear features of PTC may be patchy in a subset of cases. In contrast, cohesive, syncytial, three-dimensional tissue fragments appear to be unique to ST-PTC and likely correlate with the nested pattern of the tumor cells observed histologically [49]. This pattern differs from the monolayered sheets typical of conventional PTC. A nonspecific single-cell pattern can also be seen in ST-PTC and may correlate with infiltrative tumor growth and more aggressive behavior [49]. This pattern can mimic MTC, but the two tumors can be distinguished by their nuclear features. There is also significant morphological overlap between ST-PTC and poorly differentiated thyroid carcinoma (PDTC). PDTC may have occasional nuclear grooves and INPIs, but the cells usually have more granular chromatin and scant cytoplasm, with a high nuclear:cytoplasmic ratio. The presence of mitoses and necrosis is helpful to suggest PDTC or differentiated high-grade thyroid carcinomas, but these features are not always present on cytology (see Chap. 10). Clinico-radiological correlation can also be very helpful. Although ST-PTC in children can have significant necrosis, they behave like a PTC and do not have the aggressiveness of a PDTC.

Diffuse Sclerosing PTC

Definition

The diffuse sclerosing PTC (DS-PTC) is characterized by diffuse involvement of one or both thyroid lobes, extensive lymphovascular invasion, numerous PBs, squamous metaplasia, marked lymphocytic infiltration, and prominent fibrosis (Fig. 8.33) [1].

Criteria

The smears are moderately to highly cellular with scant or absent colloid.

The neoplastic cells are arranged in three-dimensional ball-like clusters and cohesive clusters intermingled with inflammatory cells, but conventional monolayered syncytial and papillary clusters may also be present.

The neoplastic cells are round, polygonal, or columnar, with dense cytoplasm and distinct cytoplasmic borders; hobnail cells protruding from cell clusters are often present.

In contrast to conventional PTC:

- there is less chromatin pallor.

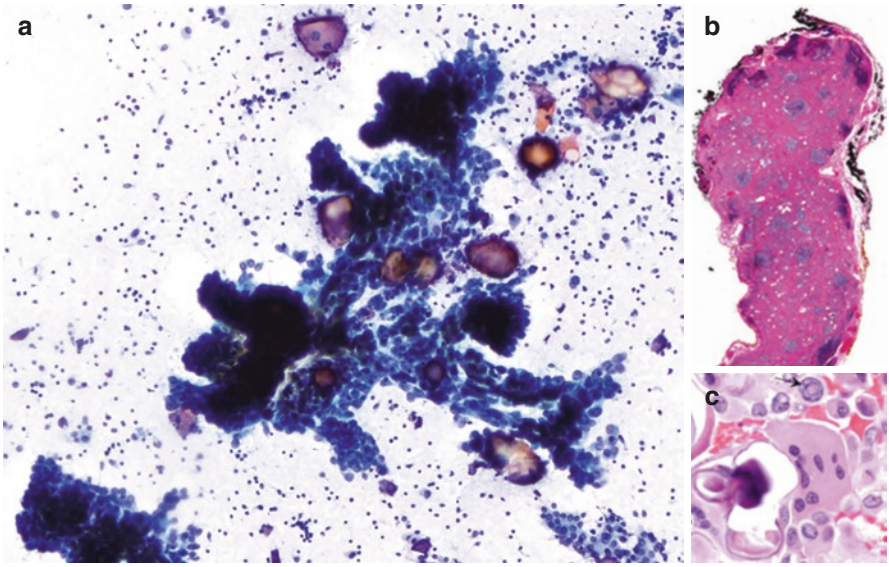

Fig. 8.33 Papillary thyroid carcinoma, diffuse sclerosing. (**a**) The aspirate shows papillary fragments associated with psammoma bodies in a lymphocytic background. The nuclear chromatin is darker than in the conventional papillary thyroid carcinoma (smear, Papanicolaou stain). (**b**) On histologic examination, the thyroid gland shows numerous lymphoid follicles and many small "holes." (**c**) The holes are from popped out psammoma bodies (**b**, **c**: hematoxylin and eosin stain)

- there are fewer INPIs and nuclear grooves (<50% of cases).
- large septate or unilocular cytoplasmic vacuoles are common.
- squamous metaplastic changes are common.
- numerous lymphocytes and PBs are present in the background.

Explanatory Notes

DS-PTC is a relatively uncommon PTC subtype (approximately 3% of all PTCs) [1]. This tumor is common in children and young adults and represents 10% of PTC seen in children exposed to the radioactive iodine following the Chernobyl accident [1]. It typically presents as a goiter without a dominant mass, reflecting a diffuse involvement of the gland that mimics Hashimoto's thyroiditis and/or lymphoma. Sonograms may reveal a characteristic "snowstorm appearance" due to numerous and widespread microcalcifications (PBs). DS-PTC is often associated with extrathyroidal extension, extensive cervical lymph node involvement, and distant metastasis [1]. Although DS-PTC has a lower disease-free survival than conventional PTC, its mortality rate is similar to conventional PTC [1]. Molecular analyses of DS-PTC have revealed *RET* translocations and especially *NCOA4*::*RET* in cases occurring after radiation fallout. *BRAFV600E* mutations are reported in 20% of cases and *ALK* rearrangements in 13% [1]. On FNA, the highly cellular aspirate is

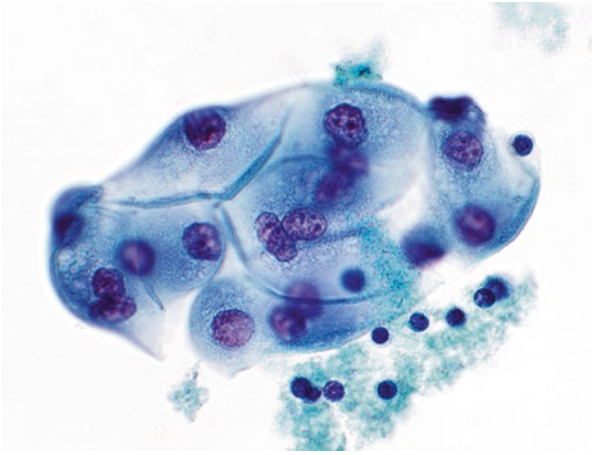

Fig. 8.34 Papillary thyroid carcinoma, diffuse sclerosing. The neoplastic cells in this image are "squamoid": they have a flat, polygonal shape with sharply demarcated cell membranes, and they fit together like jigsaw pieces, but there is no overt keratinization. This squamoid appearance is sometimes encountered as a focal finding in conventional (classic) papillary carcinomas, but in the diffuse sclerosing variant this feature is often widespread. Note that these cells lack the usual nuclear features of papillary carcinoma (ThinPrep, Papanicolaou stain)

notable for numerous monomorphic small lymphocytes (Fig. 8.34) and can be misleading for lymphocytic thyroiditis or malignant lymphoma [51]. It's worth remembering that in lymphocytic thyroiditis, atypical follicular cells are commonly encountered and nuclear grooves and INPIs are sometimes present. Furthermore, there is a lower incidence of characteristic nuclear features of PTC in DS-PTC. Three-dimensional clusters of tumor cells with hobnail features and cytoplasmic vacuoles, abundant PBs, and squamoid differentiation (Fig. 8.34) all suggest the possibility of a DS-PTC [51].

Hobnail PTC

Definition

The hobnail PTC (H-PTC) is an aggressive PTC subtype characterized histologically by complex papillary and micropapillary structures, which are covered with cells showing apically placed nuclei, bulging of the apical cell surface, and loss of cellular polarity/cohesiveness (hobnail features) [1]. Hobnail features must account for ≥30% of the tumor for the diagnosis of the hobnail variant of PTC [1].

Criteria

The neoplastic cells show loss of polarity and cohesiveness.

Single cells with eccentric nuclei and tapering cytoplasm (comet-like or tear drop-like cells) are present.

Neoplastic cells with an apically or eccentrically placed nucleus (hobnail features) can be seen in papillary or micropapillary clusters.

Multiple soap bubble-like INPIs and typical nuclear features of PTC are present.

Cell blocks may reveal papillary or micropapillary fragments lined by hobnail cells.

Explanatory Notes

H-PTC is a rare PTC subtype (<1% of all PTCs), first described in 2010 by Asioli et al. [18, 52]. Most patients with H-PTC demonstrate rapid disease progression and many die of disease within 5 years [1, 52]. As such, H-PTCs are extremely rare in a pure form and are usually associated with other aggressive subtypes of PTC (e.g., TC-PTC, CC-PTC, ST-PTC) and/or intermixed with areas showing progression to poorly differentiated or anaplastic thyroid carcinoma [1, 52]. Conversely, hobnail cells/features can be seen in conventional PTC with an indolent course, sometimes in >30% of the tumor where they are often associated with cystic and/or degenerative changes [53]. In contrast to H-PTC, these "hobnail-like" PTC occur in younger patients, have a low mitotic rate, and lack gross extra-thyroid extension and secondary pathogenic mutations [1, 53]. The *BRAF* V600E mutation is found in most (70–80%) cases, while *TP53* mutations, *TERT* promoter mutations, and *PIK3CA* mutations are also common [1]. On FNA, most H-PTC cases can be diagnosed as Malignant-PTC. However, the cytologic findings of H-PTC, described in a few retrospective studies, are essentially nonspecific and there is significant cytomorphological overlap with other aggressive subtypes of PTC such as the TC-PTC, CC-PTC, and DS-PTC [17, 18]. Hobnail morphology may occur also in the context of oncocytic, cystic/degenerative, and clear cell changes. As a result, a preoperative diagnosis of the H-PTC based on cytomorphology alone is not realistic. Even on histology, a diagnosis of H-PTC without the presence of aggressive clinico-pathologic features (such as extrathyroidal extension, vascular invasion, necrosis, and high mitotic count) should be rendered with caution [1]. The H-PTC also needs to be differentiated from metastases to the thyroid gland (or lymph nodes) that have hobnail and/or micropapillary growth patterns (e.g., breast, lung, ovary).

Related Tumors

In the fifth edition of the WHO Classification of Endocrine and Neuroendocrine Tumors that relate to the thyroid gland, the cribriform-morular thyroid carcinoma (CMTC) is no longer classified as a subtype/variant of PTC but as a malignant thyroid tumor of uncertain histogenesis, while hyalinizing trabecular tumor (HTT) is classified as a low-risk follicular cell-derived neoplasm, along with NIFTP and thyroid tumors of uncertain malignant potential [1].

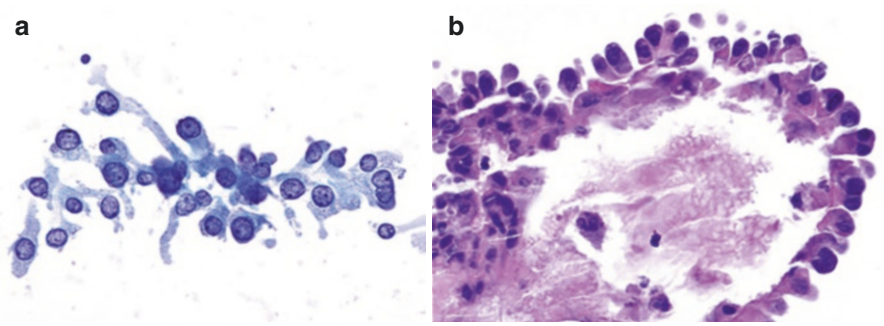

Fig. 8.35 Papillary thyroid carcinoma, hobnail subtype. (**a**) The tumor cells in this subtype are characterized by an eccentric location of the nucleus in elongated cytoplasm (hobnail-like) (smear, Papanicolaou stain). (**b**) The histologic counterpart shows similar features (hematoxylin and eosin)

Cribriform-Morular Thyroid Carcinoma

Definition

The CMTC is a rare, distinct thyroid malignancy characterized histologically by cribriform and solid architecture lacking colloid. The cells are tall and columnar or spindle-shaped, and squamoid morules are present. The tumor cell nuclei are often hyperchromatic and pseudostratified, although several nuclear features of PTC are also found. Some nuclei within the morules contain a peculiar nuclear clearing caused by biotin accumulation (Fig. 8.35).

Criteria

The smears are hypercellular.

Colloid is absent.

The tall, columnar neoplastic cells have a papillary-like arrangement.

Round to oval slit-like empty spaces formed by spindle to ovoid cells within cell clusters are present (cribriform pattern).

Cell clusters with eddy formation (morules) are present.

Spindle-shaped tumor cells are present in the background.

Pale staining nuclei with thickened nuclear membranes are present focally.

Nuclear grooves are present, but INPIs are less common than in the conventional PTC (58% of cases).

Foamy or hemosiderin-laden histiocytes are often present in the background.

Hyaline material can be seen within cell clusters or in the background.

PBs and multinucleated giant cells are absent.

The neoplastic cells usually express TTF-1; however, PAX8 immunoreactivity is weak, focal or negative, and tumors usually lack thyroglobulin reactivity.

The neoplastic cells show diffuse nuclear and cytoplasmic positivity for β-catenin in both inherited and sporadic forms of this tumor (due to germline or somatic alterations in the Wnt/beta-catenin pathway).

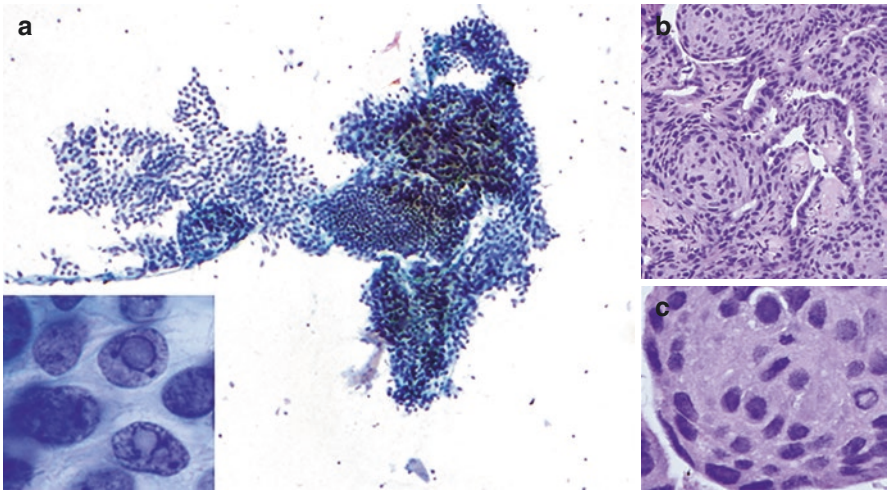

Fig. 8.36 Cribriform-morular thyroid carcinoma. (**a**) The aspirate shows large fragments of cohesive epithelium with a complicated arrangement (smear, Papanicolaou stain). The nuclear chromatin is dark, but intranuclear pseudoinclusions are present (inset). (**b**) Histologically, the tumor is characterized by cribriform morula formation (hematoxylin and eosin stain). (**c**) Higher magnification shows the characteristic morules (hematoxylin and eosin stain)

Explanatory Notes

CMTC was traditionally considered as a variant of PTC. Recent studies, however, showed that CMTC constitutes a clinicopathologically distinct category of thyroid carcinoma of uncertain histogenesis driven by Wnt/beta-catenin pathway activation [54, 55]. CMTC occurs almost exclusively in young women (97% of cases) and is frequently associated (up to 53%) with familial adenomatous polyposis (FAP) or Gardner syndrome and often precedes by several years the development of polyposis coli [1, 54–57]. A sporadic form occurs in patients who do not carry a germline mutation of *APC* gene. Generally, FAP-associated CMTC occurs in younger patients and is multifocal, whereas sporadic CMTC presents as a solitary thyroid nodule. CMTC is generally an indolent tumor, especially in its sporadic form. CMTC is associated with lymph node metastases at presentation in 12% and distant metastases in 3%, with overall mortality of 2% [1]. Most (95%) of CMTC aspirates showed features either diagnostic or suspicious of thyroid carcinoma [54, 57]. There is significant overlap between the architectural and nuclear features of CMTC and some PTC subtypes (conventional, TC-PTC, CC-PTC) (Fig. 8.36). Diffuse nuclear and cytoplasmic positivity for β-catenin is the hallmark of CMTC in both FAP-associated or non-FAP-associated cases, in contrast with other tumors and normal thyroid cells that show diffuse membranous β-catenin expression [1, 54, 55]. In addition, the neoplastic cells usually express TTF-1; however, PAX8 immunoreactivity is weak, focal or negative, and tumors usually lack thyroglobulin reactivity [1, 54, 55]. These findings raise questions about tumor cell origin and may indicate that these are not of thyroid follicular epithelial differentiation [54, 55]. Thus, in the appropriate

settings such as the presence of characteristic cytological features, relative young age of patients, clinical suspicious or confirmation of FAP as well as the presence of material for β-catenin IHC (nuclear and cytoplasmic positivity), the diagnosis of CMTC can be made or at least suggested preoperatively [56, 57]. The diagnosis of CMTC should alert the clinician for a possible diagnosis of FAP and initiation of genetic screening.

Hyalinizing Trabecular Tumor

Definition

The hyalinizing trabecular tumor (HTT) is a follicular cell-derived neoplasm composed of large trabeculae of elongated/polygonal cells with hyaline cytoplasm admixed with intra-trabecular hyaline material, and with nuclear features of PTC including prominent grooves, INPIs, and membrane irregularities [1].

Criteria

Cohesive neoplastic cells are radially oriented around amyloid-like hyaline stromal material.

Cells can be round or spindle-shaped.
INPIs and nuclear grooves are numerous.
Occasional PBs may be present.
Cytoplasmic paranuclear yellow bodies may be present.
Papillary and sheet-like fragments are absent.

Explanatory Notes

HTT represents <1% of thyroid neoplasms, has a strong female predominance (>80% of cases), and a mean age of 50 years (range: 21–79 years) [1]. Despite significant morphologic similarities with PTC, HTT is characterized by its unique

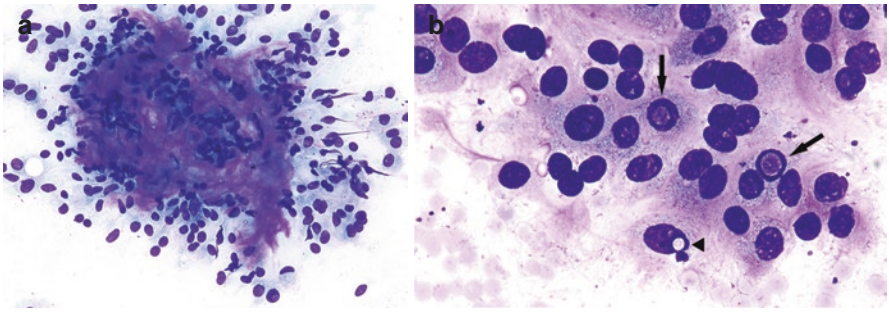

Fig. 8.37 Hyalinizing trabecular tumor. (**a**) A core of metachromatic hyaline material insinuates among cells with oval nuclei, anisonucleosis, and abundant cytoplasm (smear, Diff-Quik stain). (**b**) Oval neoplastic nuclei have occasional intranuclear pseudoinclusions (*arrows*). Note the clear hole in one of the adjacent nuclei (*arrowhead*), a mimic of INPIs, but recognizable as an artifact because the hole is white rather than the color and texture of cytoplasm (smear, Papanicolaou stain)

genetic profile with the presence of *GLIS* rearrangement (*PAX8::GLIS3* in most cases, less commonly *PAX8::GLIS1*) in virtually all cases analyzed and not in other thyroid tumors, when stringent diagnostic criteria are applied [1, 58, 59]. In contrast to PTC, *RAS* and *BRAF* V600E mutations have not been found in HTT. HTT is considered as a low-risk neoplasm, akin to NIFTP, and patients with HTT follow a benign clinical course in the vast majority of cases (>99%) even after long follow-up; complete excision is usually curative [1, 58–63]. Total thyroidectomy and/or radioiodine treatment are usually not warranted for HTT [1]. Because the morphologic features of HTT overlap significantly with those of PTC and MTC (see Chap. 9), HTT is very difficult to recognize as such in an FNA specimen (Fig. 8.37) [61–63]. Most HTTs are interpreted as PTC or Suspicious for PTC. Despite the presence of nuclear grooves, INPIs and irregular nuclear borders, all of which mimic PTC, the following diagnostic clues favor HTT: the presence of hyaline or amyloid-like material, loosely cohesive groups of tumor cells with a trabecular or syncytial pattern, radiating arrangement of neoplastic cells around a hyaline core, abundant eosinophilic or amphophilic cytoplasm, lack of papillae, and calcifications [61–63]. The presence of hyaline material may be misinterpreted as amyloid, therefore leading to an incorrect diagnosis of MTC, or may even thought to be colloid causing a false-negative diagnosis. It is important to remember that in MTC the tumor cells have eccentrically located nuclei with granular "salt and pepper" chromatin and inconspicuous nucleoli (see Chap. 9). IHC may be of help in discriminating HTT and MTC. HTT cells are positive for thyroglobulin and TTF-1 while they are negative for calcitonin. A peculiar cell membrane reactivity of the monoclonal MIB-1 to Ki-67, rather than nuclear staining, can further support the diagnosis of HTT. The specific *GLIS* fusion product can be identified by molecular techniques and also by IHC. While this biomarker is largely unavailable in most laboratories, the detection of a *GLIS* rearrangement or *GLIS* protein expression enables a preoperative diagnosis of HTT in cytology specimens [1, 62, 63]. The ultrasound findings of HTT usually show a well-defined iso- or hypoechoic solid nodule without microcalcifications that more closely resembles a follicular neoplasm or FVPTC than a classic PTC [1, 62].

Management

As a group, PTCs tend to be biologically indolent and have an excellent prognosis; survival rates of 96% at 5 years, 93% at 10 years, and >90% at 20 years have been reported [1]. Surgical consultation is recommended for patients with an FNA interpretation that is conclusive for PTC; subtyping the PTC cytologically is not essential and generally doesn't affect management [2]. The decision to perform surgery and the extent of surgery (lobectomy vs. total thyroidectomy) depend on the patient's age and overall health status and the size and sonographic characteristics of the tumor [2]. A cytologic diagnosis of PTC almost always leads to thyroid surgery. Active surveillance is an alternative to immediate surgery in a subset of patients, including those with very low-risk tumors (e.g., papillary microcarcinomas without

clinically evident metastases or local invasion, and no convincing cytologic or molecular evidence of aggressive disease) and patients at high surgical risk because of comorbidities, patients with an expected short remaining life span, or patients with concurrent medical or surgical issues that are more pressing than thyroid surgery [2]. For patients with thyroid cancer between 1 and 4 cm in diameter without extrathyroidal extension and without clinical evidence of lymph node metastases (cN0), the initial surgical procedure can be either a near-total/total thyroidectomy or a lobectomy [2]. Thyroid lobectomy alone may be sufficient initial treatment for low-risk PTCs, but the treatment team may choose total thyroidectomy to enable radioiodine therapy or to enhance follow-up based upon disease features and/or patient preferences [2]. If surgery is chosen for patients with microcarcinomas (<1 cm) without extrathyroidal extension and cN0, the initial surgical procedure should be a thyroid lobectomy unless there are clear indications to remove the contralateral lobe [2]. Thyroid lobectomy alone is sufficient treatment for small, unifocal, intrathyroidal carcinomas in the absence of prior head and neck irradiation, familial thyroid carcinoma, or clinically detectable cervical nodal metastases [2].

Sample Reports

The general category "Malignant" is used whenever the cytomorphologic features are conclusive for malignancy. If an aspirate is interpreted as Malignant, it is implied that the sample is adequate for evaluation. An explicit statement of adequacy is optional. Descriptive comments that follow are used to subclassify the malignancy and summarize the results of special studies, if any. If the findings are suspicious but not conclusive for malignancy, the general category "Suspicious for malignancy" should be used (see Chap. 7).

The positive predictive value of a conclusive malignant diagnosis of PTC is approximately 97–99%. This drops slightly, to 94–96%, if NIFTP is excluded from malignancies. Much of this drop in positive predictive value can be eliminated if the malignant category is limited to those cases with classical or tall cell PTC features.

Example 1
MALIGNANT.
Papillary thyroid carcinoma.

Example 2
MALIGNANT.
Papillary thyroid carcinoma.
Note: With the reclassification of a subset of indolent thyroid malignancies as "noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)," the positive predictive value of the malignant category for thyroid FNA

is expected to drop from 97–99% to about 94–96%. Thus, a small proportion of cases interpreted as malignant by FNA may prove to be NIFTP upon histologic examination.

Example 3
MALIGNANT.
Papillary thyroid carcinoma, favor tall cell variant.

Example 4
MALIGNANT.
Papillary thyroid carcinoma.
Note: Some cytologic features raise the possibility of a tall cell variant.

Example 5
MALIGNANT.
Thyroid carcinoma, most consistent with cribriform-morular thyroid carcinoma.
Note: The cytologic features and the immunoprofile (diffuse nuclear and cytoplasmic positivity for β-catenin, negativity for thyroglobulin) are compatible with the cribriform-morular thyroid carcinoma. Correlation with clinico-radiological findings is recommended. Genetic consultation should be considered to rule out the possibility of a familial syndrome.

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Medullary Thyroid Carcinoma

9

David Poller, Darcy Kerr, Maria Lozano, and Philippe Vielh

Background

Medullary thyroid carcinoma (MTC) is rare, accounting for approximately 1–2% of all newly diagnosed thyroid carcinomas [1, 2]. MTC occurs in sporadic and inherited forms [1, 2]. Sporadic MTC (70–80% of cases) typically presents as a solitary thyroid nodule in adults. In contrast, patients with hereditary MTC usually develop multifocal bilateral thyroid tumors, and the age of presentation varies with the syndrome. Hereditary syndromes include Multiple Endocrine Neoplasia (MEN) types 2A and 2B. Hereditary isolated MTC, formerly Familial Medullary Thyroid Carcinoma (FMTC), is considered a variant of MEN2A [3]. Table 9.1 summarizes the clinical and pathologic features of the sporadic and hereditary forms of MTC.

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Table 9.1 Clinical and pathologic features of hereditary and sporadic medullary thyroid carcinoma

	MEN2A	Hereditary isolated MTC	MEN2B	Sporadic MTC
Proportion	~20% of MTC (~80% of hereditary cases)	~4% of MTC (~15% of hereditary cases)	~1% of MTC (~5% of hereditary cases)	70–80% of MTC
		Considered a variant/ spectrum of MEN2A		
Age at presentation	Early adulthood (third–fourth decades)	Adulthood (fifth–sixth decades)	Infancy/Childhood	Adulthood (fifth–sixth decades)
Genetics	Germline *RET* mutation (most commonly exon 10 and exon 11)	Germline *RET* mutation (most commonly exon 10 and exon 11)	Germline *RET* mutation (95% with exon 16 codon M918T mutations; <5% with exon 15 codon A883F mutation)	Somatic *RET* mutations (most commonly codon M918T) in 25–45%
		Germline *MET* mutation possible		1–7% of presumed sporadic MTC found to have germline *RET* mutations
				Somatic *RAS* mutations have been identified in *RET* wild-type cases
Inheritance	Autosomal dominant	Autosomal dominant	Autosomal dominant	N/A
Associated diseases	Pheochromocytoma (50%); hyperparathyroidism (20–35%); variants with cutaneous lichen amyloidosis, Hirschsprung disease	Absence of pheochromocytoma, hyperparathyroidism, or other endocrinopathies	Aggressive MTC with early spread to lymph nodes; pheochromocytoma (50%); mucosal ganglioneuromas; Marfanoid habitus; everted eyelids; thick lips	N/A

Table 9.1 (continued)

	MEN2A	Hereditary isolated MTC	MEN2B	Sporadic MTC
Number of thyroid nodules	Usually multicentric/bilateral	Usually multicentric/ bilateral	Usually multicentric/ bilateral	Usually, a solitary nodule
C-cell hyperplasia	Present	Present	Present	Usually absent

MEN2A multiple endocrine neoplasia type 2A, *MTC* medullary thyroid carcinoma, *MEN2B* multiple endocrine neoplasia type 2B

MEN2 syndromes and hereditary isolated MTC show an autosomal dominant mode of inheritance and are associated with pathogenic germline mutations of the *RET* gene, encoded on chromosome 10, that result in constitutive activation of the RET receptor tyrosine kinase. Mutations in the extracellular domain of *RET* (e.g., codons 609, 611, 618, 620, and 634) result in kinase activation via ligand-independent receptor dimerization, and mutations in the catalytic domain of *RET* (e.g., codon 918) result in kinase activation independent of ligand or receptor dimerization. There is a strong correlation between specific pathogenic mutations and phenotype, vis-à-vis MEN2 subtype, aggressiveness of MTC, and association with other manifestations such as Hirschsprung disease and cutaneous lichen amyloidosis [2, 4]. Somatic *RET* mutations have been identified in up to 56% of sporadic MTC [5–7]. Among larger series, between 1 and 7% of patients with presumed sporadic MTCs are found to have hereditary disease, underscoring the importance of germline *RET* mutation testing in all patients diagnosed with MTC [4, 8, 9]. Germline *MET* mutation is also implicated in some hereditary isolated MTC cases [10].

Most cases of MTC demonstrate characteristic cytomorphology, a distinctive immunophenotype, and variable stromal amyloid deposition (Table 9.2). Nevertheless, MTC can show a wide variety of growth patterns, cell shapes, and cytoplasmic features, leading to the description of a large number of MTC variants, including papillary (or pseudopapillary), follicular, giant cell, spindle cell, small cell and neuroblastoma-like, paraganglioma-like, oncocytic, clear cell, angiosarcoma-like, squamous cell, melanin-producing, and amphicrine (mucin-producing) [11]. Recognizing and reporting any specific variant of MTC is not important for clinical management. This morphologic heterogeneity, however, leads to significant diagnostic challenges in the cytomorphologic evaluation of this neoplasm (Table 9.3) [12]. The 5th edition (2022) of the World Health Organization classification of endocrine and neuroendocrine tumors introduced grading of MTC (low- versus high-grade) as a recommended element, based on: mitotic count, Ki-67 proliferation index, and tumor necrosis [3]. Grading is not currently recommended on FNA samples.

Table 9.2 Cytologic differential diagnosis of medullary thyroid carcinoma

Tumor	Cyto-architecture and background	Cytoplasm	Nucleus	ICC/stains	
				Positive	Negative
Medullary thyroid carcinoma	Isolated, dispersed cells and/or syncytium-like clusters; amyloid	Plasmacytoid, epithelioid, giant, spindle-shaped. Red-purple granules with Romanowsky-type stains	Round, ovoid, or elongated, with granular chromatin. INPI and binucleation (some cases)	CT, CGRP, CEA, NE markers, TTF-1, PAX8 (variable but negative with monoclonal PAX8), Congo red (amyloid)	TG
Follicular neoplasm	Microfollicles/crowded groups	Scant to moderate	Round, hyperchromatic, variable nuclear enlargement	TTF-1, TG, PAX8	CT, CEA, NE markers
Oncocytic (formerly Hürthle cell) neoplasm	Isolated, dispersed cells and syncytium-like clusters	Abundant, finely granular (blue-gray with Romanowsky-type stains)	Round and enlarged, with moderate irregularity and prominent nucleolus	TTF-1, TG, PAX8	CT, CEA, NE markers
Papillary thyroid carcinoma	Papillae, sheets, microfollicles	Variable, depending on subtype	Enlarged and ovoid with irregular contours, grooves, INCI, and chromatin pallor	TTF-1, TG, PAX8	CT, CEA, NE markers
Hyalinizing trabecular tumor	Cohesive clusters associated with hyaline matrix	Epithelioid to spindled	Enlarged, with irregular contours, grooves, INPI, and chromatin pallor	TTF-1, TG, Ki-67 (membranous staining with MIB1)	CT, CEA, NE markers
Poorly differentiated thyroid carcinoma	Crowded groups, isolated, dispersed cells	Scant, occasionally plasmacytoid	Round, with variable enlargement and binucleation; apoptosis; mitosis	TTF-1, PAX8, TG (variable)	CT, CEA, NE markers
Anaplastic thyroid carcinoma	Isolated, dispersed cells and crowded groups. Variable necroinflammatory debris	Epithelioid, spindled, variable sizes	Enlarged, with variable pleomorphism, nucleolar prominence, multinucleation; necrosis/apoptosis; mitotic activity	PAX8 (variable)	CT, CEA, NE markers

Melanoma	Isolated, dispersed cells	Epithelioid, spindled, variable sizes. Variable melanin pigmentation	Enlarged, with prominent nucleoli	S-100 protein, HMB-45, Melan-A, SOX-10	CK, CT, CEA, NE markers
Parathyroid	Sheets, cords, acini	Moderate amount, often granular	Round, with granular chromatin	NE markers, PTH, GATA3	CT, CEA, TTF-1
Plasmacytoma	Isolated, dispersed cells; amyloid	Moderate amount with eccentric nuclei ("plasmacytoid")	Round, with granular chromatin	CD138, kappa or lambda Ig light chain restriction, Congo red (amyloid)	CT, TTF-1, NE markers
Paraganglioma	Isolated, dispersed cells and loose clusters	Moderate to abundant amount of delicate cytoplasm	Round, with granular chromatin, anisonucleosis	NE markers, S-100 protein (sustentacular cells)	CK, CT

CEA carcinoembryonic antigen, *CGRP* calcitonin gene-related peptide, *CK* cytokeratin, *CT* calcitonin, *ICC* immunocytochemistry, *Ig* immunoglobulin, *INPI* intranuclear pseudoinclusions, *NE* neuroendocrine, *PTH* parathyroid hormone, *TG* thyroglobulin

Table 9.3 Growth patterns of medullary thyroid carcinoma and associated differential diagnostic considerations

MTC growth pattern	Differential diagnosis
Amphicrine (mucin and calcitonin-producing cells)	Metastatic adenocarcinoma
Angiosarcoma-like	Angiosarcoma
Clear cell	Renal cell carcinoma, follicular neoplasm with clear cells
Encapsulated	Cystic PTC, metastasis
Follicular/tubular	Follicular neoplasm
Giant cell	Anaplastic (undifferentiated) thyroid carcinoma (ATC)
Melanin-producing/pigmented	Melanoma
Mixed follicular and medullary	Follicular neoplasm
Oncocytic (oxyphilic)	Oncocytic subtypes of follicular neoplasm and PTC
Papillary/pseudopapillary	Papillary thyroid carcinoma (PTC)
Paraganglioma-like	Paraganglioma, hyalinizing trabecular tumor
Small cell/neuroblastoma-like	Small cell carcinoma of the lung, lymphoma
Spindle cell	Sarcoma, ATC
Squamous	Squamous cell carcinoma, ATC, PTC with squamous differentiation/metaplasia

Table adapted from Pusztaszeri et al. Adv Anat Pathol. 2014;21:26–35
ATC anaplastic thyroid carcinoma, *MTC* medullary thyroid carcinoma, *PTC* papillary thyroid carcinoma

Definition

MTC is a malignant neuroendocrine neoplasm derived from the calcitonin-producing parafollicular cells (C-cells) of the thyroid gland.

Criteria

Aspirates show moderate to marked cellularity.

Numerous isolated cells alternate with syncytium-like clusters in variable proportions.

Cells are plasmacytoid, polygonal, round, and/or spindle-shaped. Long cell processes are seen in some cases.

The neoplastic cells usually show only mild to moderate pleomorphism.

Rare, bizarre giant cells may be seen; they can be numerous in the giant cell variant.

Nuclei are round, oval, or elongated and often eccentrically placed, with finely or coarsely granular ("salt and pepper") chromatin.

Binucleation is common. Multinucleation is less often observed.

Nucleoli are usually inconspicuous but can be prominent in some cells.

Intranuclear pseudoinclusions may be noted. Nuclear grooves are rare or absent.

Cytoplasm is granular and variable in quantity. Small red-purple granules are seen with Romanowsky stains in some cases. Rare cases show cytoplasmic melanin pigment.

Amyloid is often present and appears as dense, amorphous material that resembles thick colloid, mauve colored on Romanowsky stains, and pale green to eosinophilic on Papanicolaou stains.

With liquid-based preparations, fine cytoplasmic vacuolization can be prominent.

Cells are typically strongly immunoreactive for calcitonin, CEA, neuroendocrine markers (chromogranin, synaptophysin), and TTF-1. Immunoreactivity for PAX8 (polyclonal antibody) is variable; monoclonal PAX8 antibody is negative [13]. Cells are negative for thyroglobulin (aberrant results occasionally occur).

Explanatory Notes

Cytologic preparations from MTC are usually moderately or highly cellular and composed of a mixture of non-cohesive cells (Fig. 9.1) and crowded, syncytium-like aggregates (Fig. 9.2) [11, 12, 14–16]. Occasionally, rosette-forming, follicular, and pseudopapillary architecture can be seen. Most aspirates show a mixture of cell morphologies, including polygonal, round, and plasmacytoid shapes (all with round to ovoid nuclei) (Fig. 9.3), as well as spindled cells with elongated nuclei (Fig. 9.4) and some others may resemble follicular neoplasms (Fig. 9.5). Tumor cells have a variable amount of cytoplasm, sometimes replete with red-purple granules after Romanowsky-type staining of air-dried smears (Fig. 9.6). A minority of cases demonstrate cytoplasmic vacuoles, melanin-like pigment (Fig. 9.7), and intracytoplasmic lumina (Fig. 9.8) [11]. With liquid-based preparations, fine cytoplasmic vacuolization can be prominent (Fig. 9.7). Typically, most of the tumor cells demonstrate only mild to moderate nuclear pleomorphism, with occasional cells showing markedly enlarged or bizarre-appearing nuclei [11, 14]. Binucleation is common

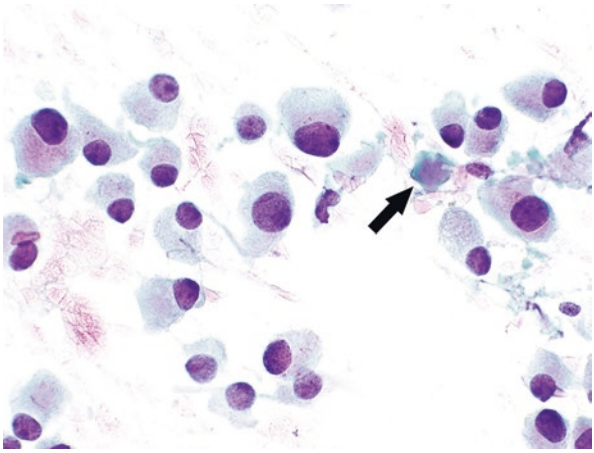

Fig. 9.1 Medullary thyroid carcinoma. Predominantly dispersed plasmacytoid or polygonal cells have granular ("salt and pepper") chromatin and small or indistinct nucleoli. A small fragment of amyloid is present (arrow) (smear, Papanicolaou stain)

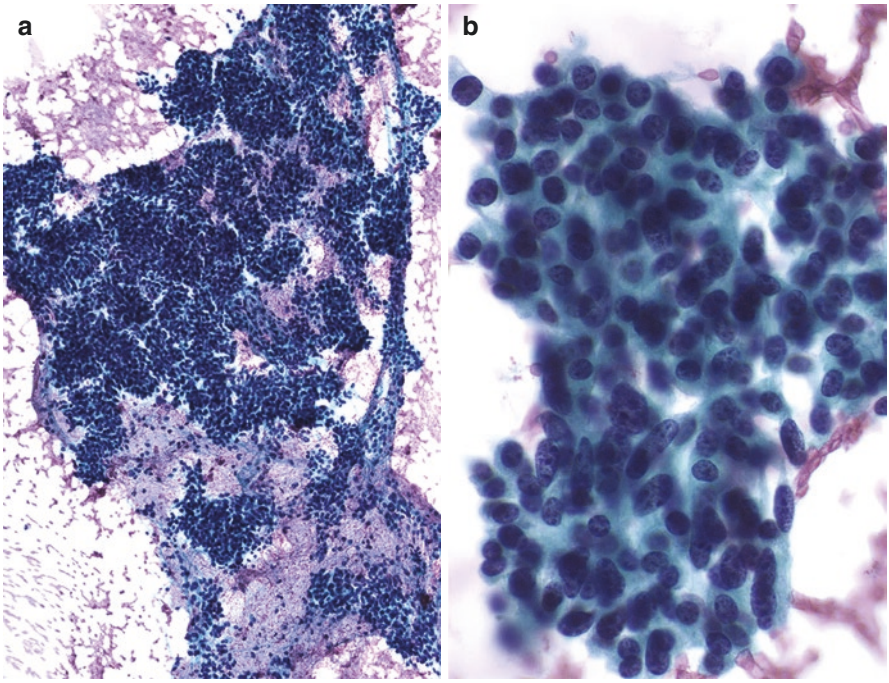

Fig. 9.2 Medullary thyroid carcinoma. (**a**) In some cases, a cohesive, syncytium-like pattern of crowded cells predominates, with few isolated cells. (**b**) In this example, tumor cells exhibit less abundant cytoplasm, round to ovoid nuclei, and coarse chromatin. Medullary thyroid carcinomas with this pattern mimic a follicular neoplasm, poorly differentiated thyroid carcinoma, and parathyroid neoplasms (smear, Papanicolaou stain)

[11, 14]. Intranuclear pseudoinclusions (indistinguishable from those seen in papillary thyroid carcinoma) are seen in a limited number of cells in ~20–50% of cases (Fig. 9.9) [11, 14, 17]. Nuclear grooves are rare [13]. Rare cases show scant cytoplasm and nuclear molding, resembling small cell carcinoma (Fig. 9.10). Amyloid is identified in approximately one-third to one-half of MTC aspirates (Figs. 9.1, 9.6, 9.11, and 9.12) [11, 14, 18]. It is virtually indistinguishable from colloid without the cellular context and is not diagnostic of MTC by itself, since amyloid may be present in papillary thyroid carcinoma (rarely) and amyloid goiter [19, 20]. Colloid is present in about 30% of MTC aspirates and is more frequent in medullary thyroid microcarcinoma, likely due to incidental colloid sampling from surrounding thyroid follicles [14, 21]. Calcifications (including psammoma bodies) have been reported in approximately 3% of MTC [11, 14].

Diagnosing MTC by FNA can be challenging given its diverse appearances and cytologic overlap with other tumors. Although the sensitivity of FNA for a definitive and specific diagnosis of MTC has been reported to be as high as 89% in a single-institution study, a meta-analysis revealed a substantially lower sensitivity of 56% (range 12–88%), thus highlighting the challenge that MTC poses to the cytologist

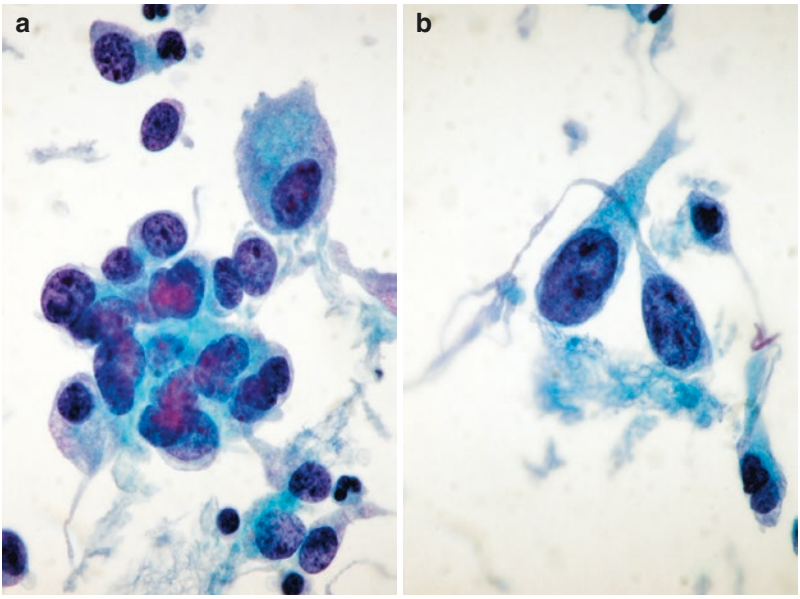

Fig. 9.3 Medullary thyroid carcinoma. A variety of shapes (round, polygonal, plasmacytoid (**a**), or spindled (**b**)) are seen in this population of tumor cells (smear, Papanicolaou stain)

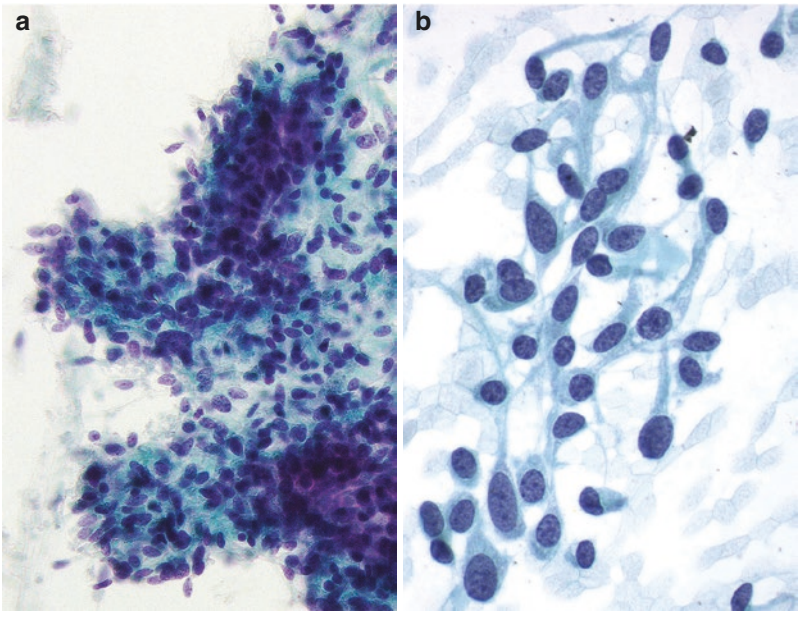

Fig. 9.4 Medullary thyroid carcinoma. (**a**) The spindle cell variant can have a syncytium-like arrangement (smear, Diff-Quik stain). (**b**) The spindle cell variant has prominent interdigitating cytoplasmic processes with oval nuclei. Smooth nuclear membranes, granular chromatin, and inconspicuous nucleoli are maintained (smear, Papanicolaou stain)

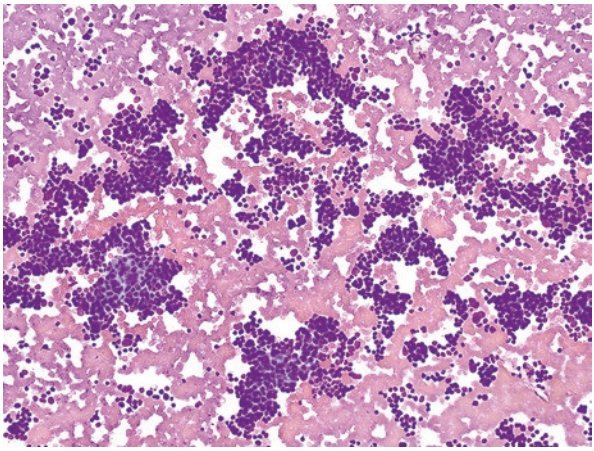

Fig. 9.5 Medullary thyroid carcinoma. Some cases such as this can resemble follicular neoplasms of the thyroid (smear, Diff Quick)

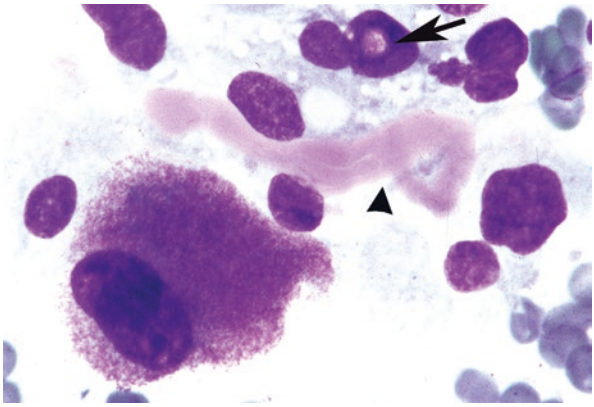

Fig. 9.6 Medullary thyroid carcinoma. A large tumor cell with abundant cytoplasm demonstrates red cytoplasmic granules with a Romanowsky-type stain. Note also the presence of amyloid (arrowhead) and a tumor cell with an intranuclear pseudoinclusion (arrow) (smear, Diff-Quik stain)

[14, 22]. Problems in diagnosis include cellular patterns that mimic follicular neoplasms, e.g., unremarkable chromatin, cohesive groups, lack of plasmacytoid morphology, and lack of multinucleation [23]. An Asian working group has proposed a set of seven parameters for the cytologic diagnosis of MTC: high cellularity, cellular pleomorphism, plasmacytoid cells, round cells, dyscohesive cells, salt-and-pepper chromatin, and binucleation or multinucleation, although this has not as yet been prospectively validated [24]. Immunocytochemistry (ICC) is extremely helpful for distinguishing MTC from its cytologic mimics (Table 9.2). Most MTCs are immunoreactive for "C-cell markers" (calcitonin, CEA), neuroendocrine markers (synaptophysin, chromogranin), and negative for thyroglobulin (Fig. 9.13) [25]. Insulinoma-associated protein (INSM1) may also be useful in confirming a neuroendocrine phenotype, although staining is not specific for MTC [26]. Staining for PAX8 varies considerably among studies (0–75%), with positive cases generally showing only focal immunoreactivity and typically seen with polyclonal antibodies [13, 27–30]. Therefore, TTF-1 and PAX8 are not helpful for distinguishing MTC

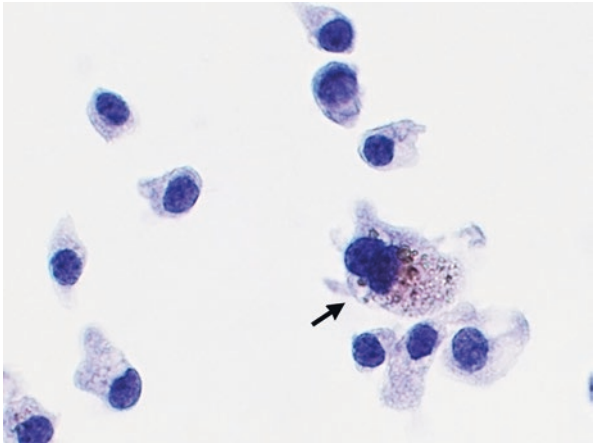

Fig. 9.7 Medullary thyroid carcinoma. Pigmentation and/or melanocytic differentiation can be seen in medullary thyroid carcinoma (arrow), which raises the possibility of a metastatic melanoma. Even without pigmentation, melanoma is a mimic of medullary thyroid carcinoma because both often demonstrate an isolated cell pattern, epithelioid or spindled morphology, and binucleation. Immunocytochemistry on the cell block in this case confirmed the diagnosis of medullary thyroid carcinoma (ThinPrep, Papanicolaou stain)

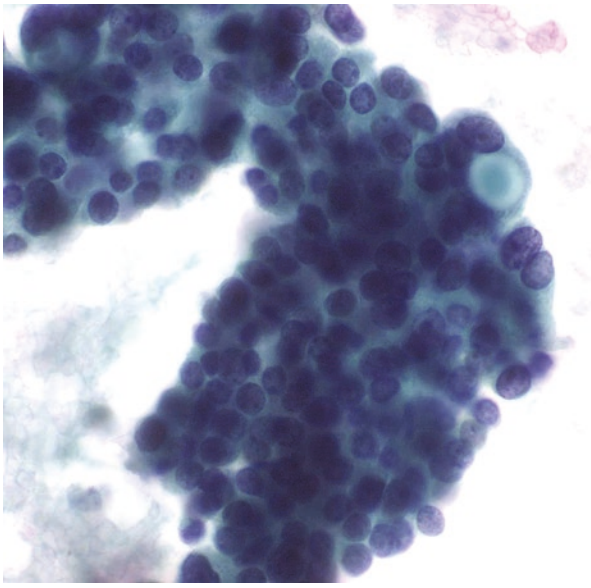

Fig. 9.8 Medullary thyroid carcinoma. Cytoplasmic vacuoles or lumina are occasionally seen in medullary thyroid carcinoma (smear, Papanicolaou stain)

from follicular cell-derived thyroid neoplasms. A Congo red stain can confirm the presence of amyloid, which supports the diagnosis of MTC in the context of characteristic malignant cells. The measurement of calcitonin levels in needle rinse (washout fluid) from FNAs of thyroid nodules, thyroidectomy beds, and/or lymph nodes can be helpful. This is particularly true in patients with elevated serum

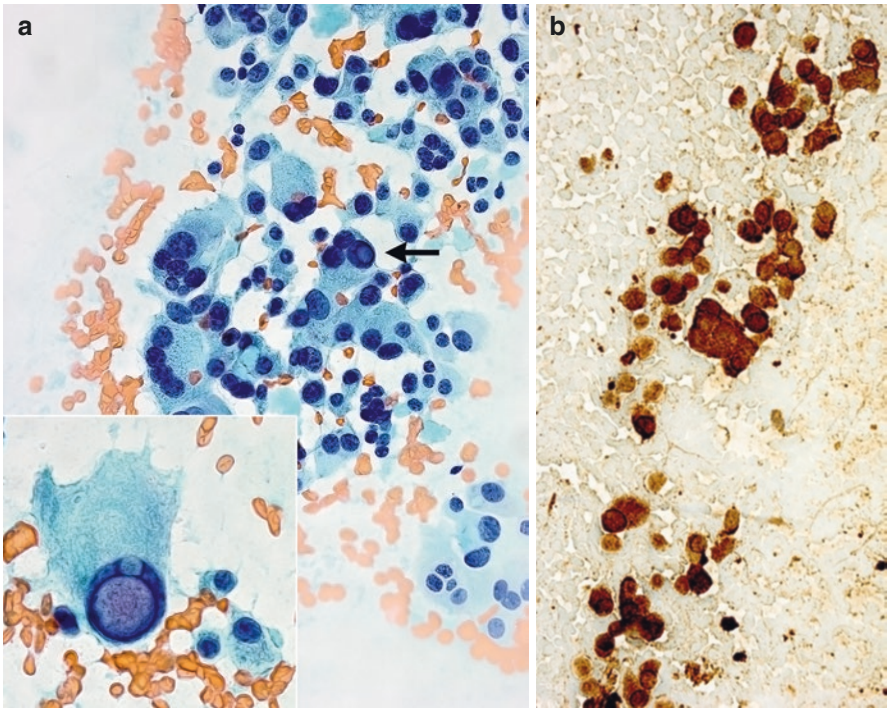

Fig. 9.9 Medullary thyroid carcinoma. (**a**) Intranuclear pseudoinclusions can be seen, mimicking papillary thyroid carcinoma (ThinPrep, Papanicolaou stain). (**b**) The diagnosis can be confirmed with immunocytochemical staining for calcitonin

calcitonin levels and/or when FNA findings are inconclusive for MTC, e.g., when confirmation by ICC is not possible given limited material or equivocal staining results [4, 31]. Overall, this test is reliable, inexpensive, and associated with fast turnaround time. A recent literature review suggests a diagnostic sensitivity of over 95% in most cases [32]. In order to avoid a repeat FNA for this purpose, calcitonin measurement in FNA washout fluid should be anticipated in all clinically suspected cases of MTC and for all patients with MEN2. The Afirma® Gene Expression Classifier (Veracyte, Inc., South San Francisco, CA), in common use for nodules interpreted as Atypia of Undetermined Significance (AUS) or Follicular Neoplasm (FN), includes an MTC classifier that is effective in identifying the MTCs hidden in the substantial population of nodules with indeterminate cytology [33, 34]. A multigene next-generation sequencing genomic classifier, Thyroseq 3, is also clinically effective for MTC [35, 36].

The differential diagnosis of MTC includes the full spectrum of follicular cell-derived thyroid tumors (Table 9.2). Aspirates of oncocytic (Hürthle cell) neoplasms often yield non-cohesive cells with abundant granular cytoplasm, resembling some MTCs (Fig. 9.14). Papillary thyroid carcinoma (PTC) and hyalinizing trabecular tumor (HTT) can also mimic MTC by virtue of their intranuclear pseudoinclusions

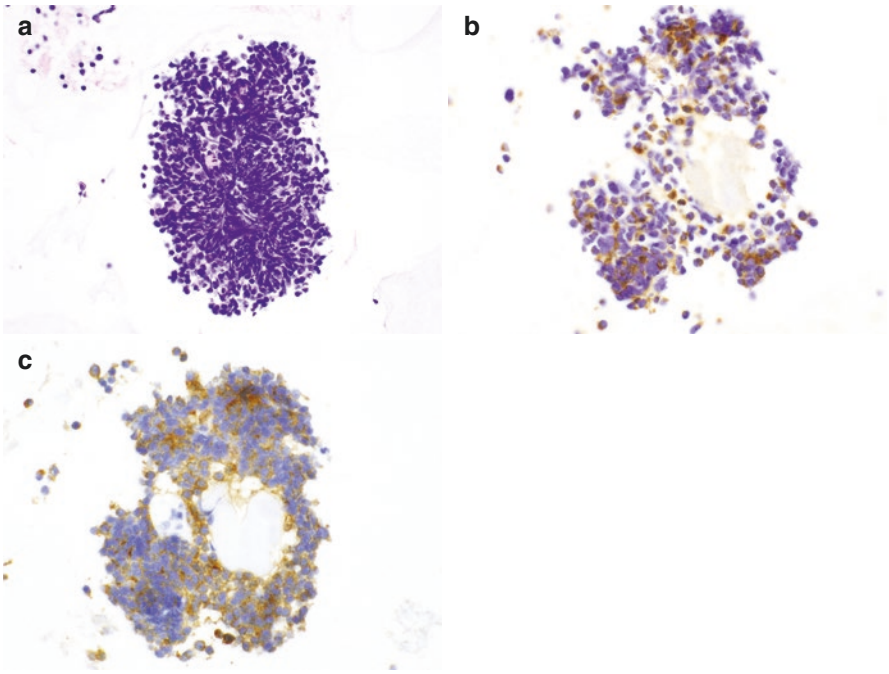

Fig. 9.10 Medullary thyroid carcinoma, small cell appearance. (**a**) Rare cases of medullary thyroid carcinoma exhibit scant cytoplasm and nuclear molding, resembling small cell carcinomas of the lung and of other sites (cell block, hematoxylin & eosin). (**b**) Immunoreactivity for calcitonin and (**c**) synaptophysin on cell block preparations supports the diagnosis of medullary thyroid carcinoma. Nevertheless, because their immunoprofiles can overlap and both tumor types can contain amyloid, the distinction requires correlation with clinical findings

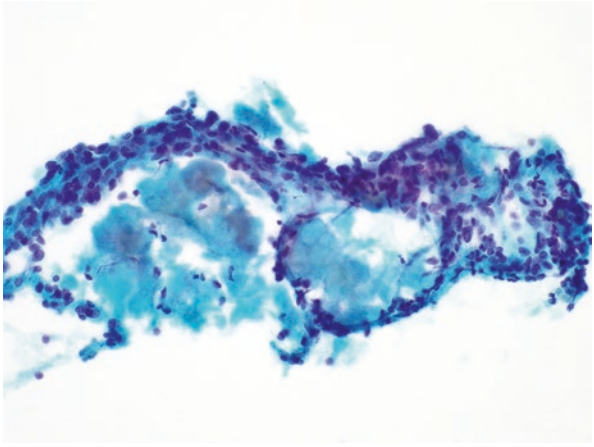

Fig. 9.11 Medullary thyroid carcinoma. Amyloid is abundant and readily appreciated as a light-green/blue amorphous deposit (smear, Papanicolaou stain)

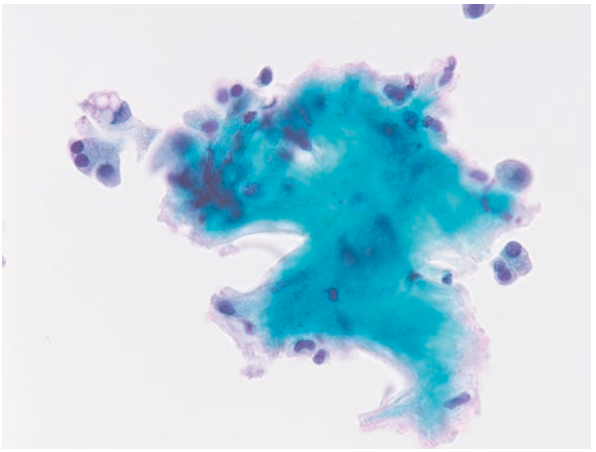

Fig. 9.12 Medullary thyroid carcinoma. Amyloid has the same dense, amorphous, and waxy appearance on liquid-based preparations as it does on smears (ThinPrep, Papanicolaou stain)

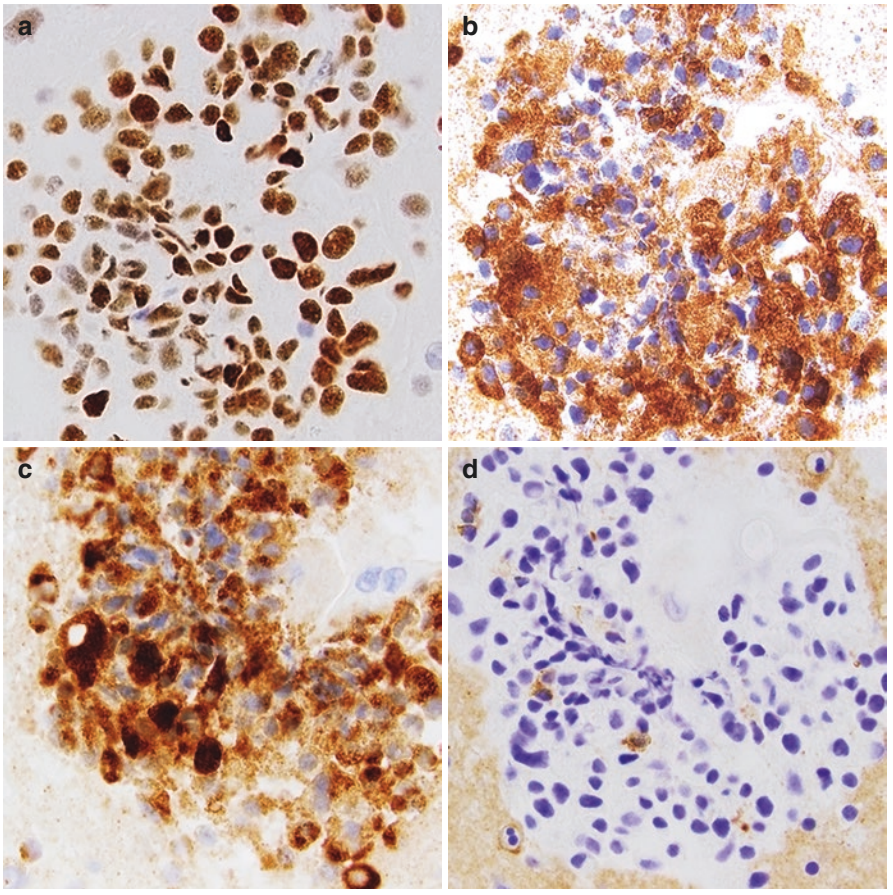

Fig. 9.13 Medullary thyroid carcinoma. The tumor cells (on cell block preparations) are immunoreactive for (**a**) TTF-1 (nuclear), (**b**) calcitonin (cytoplasmic), and (**c**) chromogranin (cytoplasmic). (**d**) Tumor cells are negative for thyroglobulin

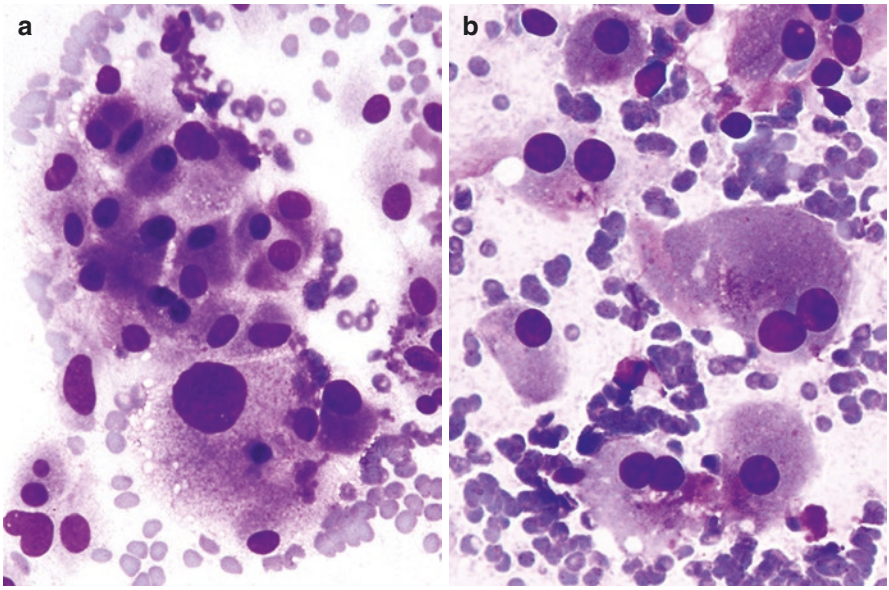

Fig. 9.14 Medullary thyroid carcinoma (left) versus oncocytic (Hürthle cell) neoplasm (right). (**a**) With Romanowsky-type stains, the cells of some (but not all) medullary thyroid carcinomas are noteworthy for abundant red cytoplasmic granules (smear, Diff-Quik). (**b**) In contrast, oncocytic follicular cells have blue-gray cytoplasmic granules with Romanowsky-type stains (smear, Diff-Quik)

[37]. HTT may produce hyaline material but this is periodic acid-Schiff (PAS) positive and negative with amyloid stains. HTT also has unique immunohistochemical and molecular features showing cytoplasmic Ki-67/MIB-1 (clone) staining and *PAX8*::*GLIS* translocation [38]. In particular, certain subtypes of PTC (tall cell, oncocytic) can have elongated or abundant cytoplasm, similar to that of some MTC cells [39]. Poorly differentiated thyroid carcinoma (PDTC) can resemble MTC; both have similar cytoarchitecture (crowded insular/nested groups and/or non-cohesive cells), variable binucleation and chromatin granularity, and occasional plasmacytoid shape (Fig. 9.15) [40]. Like MTC, cells of anaplastic (undifferentiated) thyroid carcinomas (ATC) can have a multitude of appearances, including epithelioid, plasmacytoid, spindled, and giant cell forms (Fig. 9.16) [41]. Increased mitotic activity, apoptosis, and necrosis should raise concern for PDTC; either differentiated high-grade thyroid carcinoma (DHGTC) or ATC rather than MTC. For each of the above possibilities, a panel of immunostains (calcitonin, CEA, synaptophysin, chromogranin) helps distinguish MTC from follicular cell-derived thyroid neoplasms.

Additional mimics of MTC include other neuroendocrine lesions in the head and neck, such as paraganglioma and parathyroid adenoma, both of which resemble MTC morphologically, and all are immunoreactive for synaptophysin and chromogranin. Additional immunostains help discriminate MTC (CEA+, calcitonin+, cytokeratin [CK]+) from paraganglioma (CEA-, calcitonin-, CK-, S-100 protein+ sustentacular cells) and parathyroid neoplasms/hyperplasia (CEA-, calcitonin-,

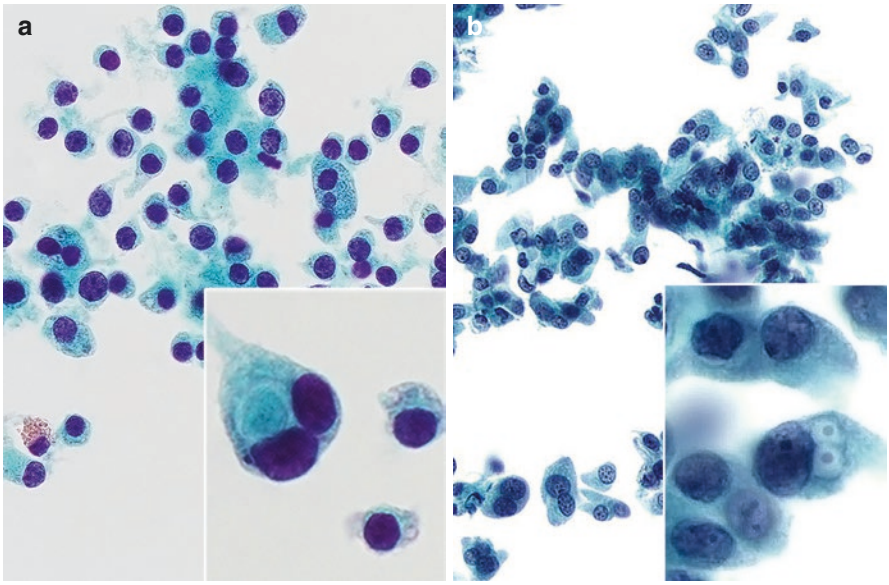

Fig. 9.15 Medullary thyroid carcinoma (left) versus poorly differentiated carcinoma (right). (**a**) Medullary thyroid carcinomas often demonstrate an isolated cell pattern, plasmacytoid cytomorphology, and they occasionally have cytoplasmic lumina (ThinPrep, Papanicolaou stain). (**b**) Poorly differentiated thyroid carcinoma can have similar features, and intracytoplasmic lumina are sometimes seen (ThinPrep, Papanicolaou stain)

PTH+, GATA3+) [42, 43]. Metastatic neuroendocrine tumors to the thyroid gland or cervical lymph nodes mimic MTC and can be associated with elevated serum calcitonin levels [44]. In particular, moderately differentiated neuroendocrine tumors (atypical carcinoid) of the larynx are frequently positive for calcitonin and/or CEA. In contrast to MTC, however, most laryngeal atypical carcinoids are negative for TTF-1 [45]. Correlation with clinical and radiographic findings plays a critical role in distinguishing MTCs from extra-thyroidal neuroendocrine tumors. Of other tumors metastatic to the thyroid, melanoma is a noteworthy mimic of MTC: the variable cell shape, frequent binucleation, intranuclear pseudoinclusions, and dispersed cell pattern of many melanomas are features shared by many MTCs. In a patient with a history of melanoma, MTCs can be recognized by their immunoreactivity for cytokeratins and C-cell markers, the lack of staining for melanocytic markers (HMB-45, S-100 protein, Melan-A, SOX-10), and the absence of macronucleoli. MTCs with a prominent spindle cell pattern resemble mesenchymal lesions; immunoreactivity for CK, calcitonin, and neuroendocrine markers can be used to confirm the diagnosis of MTC in this setting [46]. Finally, plasma cell neoplasms resemble MTC because a dispersed cell pattern, "plasmacytoid" morphology, and amyloid deposition are shared by both tumors. Plasmacytomas of the thyroid are exceptionally rare but have been described [47]. Expression of CD138 and immunoglobulin light chain restriction favor a plasma cell neoplasm, whereas expression of C-cell and neuroendocrine markers supports a diagnosis of MTC.

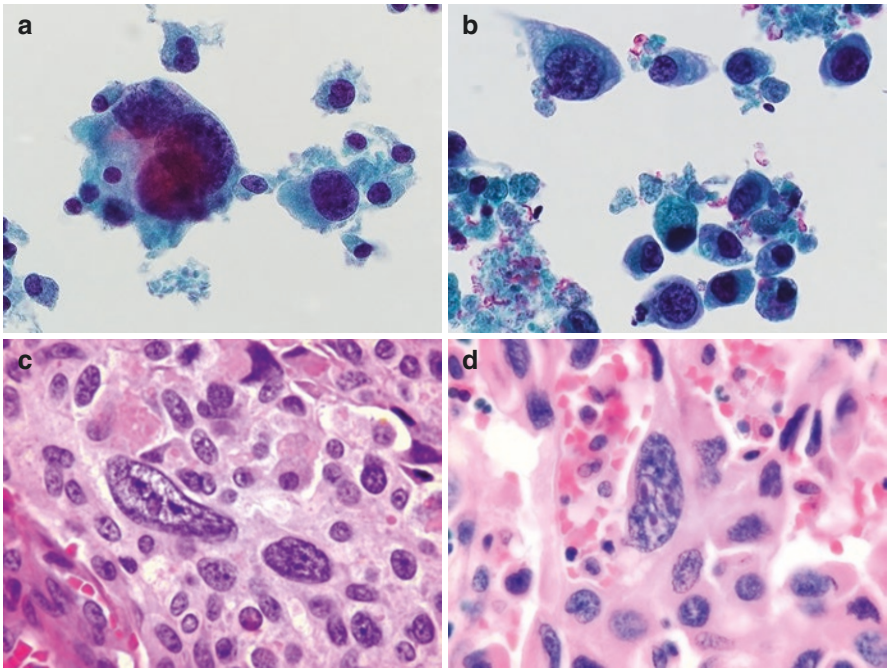

Fig. 9.16 Medullary thyroid carcinoma (left) versus undifferentiated/anaplastic thyroid carcinoma (right). (**a**) The giant cell variant of medullary thyroid carcinoma exhibits markedly enlarged, epithelioid tumor cells with pleomorphic nuclei, often admixed with more conventional-appearing tumor cells (ThinPrep, Papanicolaou stain). Multinucleation may be seen, as in this example. (**b**) Note the resemblance to undifferentiated (anaplastic) thyroid carcinoma, which can also exhibit an epithelioid cytomorphology and nuclear pleomorphism (ThinPrep, Papanicolaou stain). This rapidly growing primary thyroid tumor was positive for PAX8 and negative for TTF-1, calcitonin, synaptophysin, and chromogranin. Histologic images of medullary thyroid carcinoma (**c**) and undifferentiated thyroid carcinoma (**d**) demonstrate similar nuclear and cytoplasmic features (hematoxylin and eosin stain)

Management

Following a cytologic diagnosis of MTC, preoperative studies should include a neck ultrasound and measurement of serum calcitonin and CEA. Systemic imaging studies may be indicated for patients with clinical or laboratory evidence of metastatic disease. Genetic testing for germline *RET* mutations should also be performed, and patients with hereditary MTC should be evaluated for pheochromocytoma and hyperparathyroidism prior to thyroid surgery [4]. For patients with pheochromocytoma, alpha/beta-adrenergic blockade and resection of the adrenal tumor should precede thyroidectomy for MTC. Surgical treatment of MTC is usually total thyroidectomy and central lymph node dissection, with consideration of lateral cervical lymph node dissection depending on imaging studies and serum calcitonin levels. For patients with advanced, progressive MTC, tyrosine kinase inhibitors such as

vandetanib (targeting RET, EGFR, VEGFR) and cabozantinib (targeting RET, c-MET, VEGFR) can be used as single-agent first-line systemic chemotherapy [4].

Sample Reports

The general category "Malignant" is used whenever the cytomorphologic features are conclusive for malignancy. If an aspirate is interpreted as Malignant, it is implied that the sample is adequate for evaluation (an explicit statement of adequacy is optional). Descriptive comments that follow are used to subclassify the malignancy as MTC and summarize the results of special studies, if any. If the findings are suspicious but not conclusive for malignancy, the general category "Suspicious for malignancy" should be used (see Chap. 7).

Example 1
MALIGNANT.
Medullary thyroid carcinoma.
Note: A Congo red stain is positive for amyloid. Immunocytochemistry performed on cell block/cytocentrifuge/liquid-based preparations (choose one) shows that the malignant cells are immunoreactive for calcitonin, CEA, chromogranin, and TTF-1, and negative for thyroglobulin.

Example 2
MALIGNANT.
Consistent with medullary thyroid carcinoma.
Note: Cytomorphologic features are characteristic of medullary thyroid carcinoma, but tissue is insufficient for confirmatory immunocytochemical studies. Serum chemistry for calcitonin and CEA and/or repeat FNA for calcitonin measurement in the washout fluid warrant clinical consideration.

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High-Grade Follicular Cell-Derived Non-Anaplastic Thyroid Carcinoma

10

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Background

This chapter was called "poorly-differentiated thyroid carcinomas" in the previous editions of TBSRTC. The latest 5th edition of the WHO classification of thyroid neoplasms has now included poorly differentiated thyroid carcinoma (PDTC) in the same group as differentiated high-grade thyroid carcinoma (DHGTC) under the umbrella of "Follicular cell-derived non-anaplastic carcinomas, high-grade" in the chapter on malignant neoplasms [1]. This was the result of studies highlighting how non-anaplastic thyroid carcinomas that show necrosis and high mitotic activity have an aggressive clinical behavior intermediate between that of well differentiated thyroid carcinomas (WDTC) (papillary carcinoma, follicular carcinoma, and oncocytic carcinoma) and undifferentiated (anaplastic) thyroid carcinomas, despite the morphological architectural arrangement (follicular, papillary, or oncocytic) [2].

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Poorly differentiated thyroid carcinoma (PDTC) was first proposed as a distinct subtype of thyroid malignancy by Carcangiu et al. [3] These authors reinterpreted the original observation made in 1907 by Langhans, who described a locally aggressive tumor with a peculiar architecture: tumor cells arranged in large, round to oval formations, the so-called "insulae" [4].

In the past, high mitotic activity and tumor necrosis were necessary for the histologic diagnosis of PDTC [5]. To qualify histologically as PDTC, tumors must have a solid, trabecular, and/or insular pattern of growth; conventional nuclear features of papillary thyroid carcinoma should not be present throughout the tumor; and at least one of the following features must be present: mitotic activity ≥3/10 high-power fields (HPFs), tumor necrosis, and convoluted nuclei (known as the Turin criteria). Others considered PDTC as having ≥5 mitoses/10 HPF and/or tumor necrosis, independently from growth pattern (known as the Memorial Sloan Kettering Cancer Center criteria) [6]. PDTCs can also have prominent oncocytic features [7, 8].

DHGTC are carcinomas with high mitotic activity and necrosis and in which papillary or follicular architecture is still present and well identified (in contrast to PDTC, where usually these architectural features are lacking and solid, trabecular, and insular features are most common) [9].

PDTC and DHGTC are rare malignancies, accounting for 1–6.7% of all thyroid cancers [1, 10]. Both entities often present at an advanced stage, have a propensity for local recurrence, tend to metastasize to regional lymph nodes, and do not respond to radioactive iodine therapy. The disease-specific survival of patients with PDTC and DHGTC varies from 50% to 56% [1, 10]. A focal (10% or greater) PDTC component in an otherwise well-differentiated thyroid carcinoma designates a more aggressive clinical course than standard well-differentiated carcinomas of the thyroid [11].

Definition

PDTC is a thyroid carcinoma of follicular cell origin characterized by an insular, solid, or trabecular growth pattern with minimal colloid. The most classic architectural form of PDTC is the insular type, defined by its "cellular nests" or insular cell groups outlined by a thin fibrovascular border. In its pure form, PDTC lacks conventional nuclear features of papillary thyroid carcinoma and is distinguished from other well-differentiated thyroid neoplasms by the presence of poorly differentiated features: necrosis, mitoses, or small, round hyperchromatic nuclei with convoluted and irregular nuclear membranes [6]. The quantity and quality of cytoplasm of the tumor cells can vary, as a subset can have oncocytic features. While most PDTCs

contain a relatively monotonous population of malignant cells with limited pleomorphism, some are characterized by larger, more pleomorphic cells. However, there is no frank anaplasia as that seen in cases of undifferentiated (anaplastic) thyroid carcinoma. In cases where there is prominent pleomorphism, progression to undifferentiated (anaplastic) thyroid carcinoma should be a diagnostic consideration.

DHGTC still retain conventional nuclear features and structures of papillary thyroid carcinoma (or follicular architecture in a lesser percentage of cases), but are associated with more atypical cellular features, such as nuclear enlargement and/or pleomorphism and necrosis. Mitoses, which are almost never observed in well differentiated thyroid carcinomas, are more frequently present in DHGTC.

Criteria

Cellular preparations display an insular, solid, or trabecular cytoarchitecture (Figs. 10.1, 10.2, 10.3, and 10.4) suggestive of a PDTC morphology.

There is a uniform population of malignant follicular cells with scant cytoplasm, sometimes plasmacytoid (Fig. 10.5), or with oncocytic features (Fig. 10.6) and frequently arranged in microfollicles (Fig. 10.7).

The cells have a high nuclear/cytoplasmic (N/C) ratio with variable nuclear atypia (Figs. 10.8 and 10.9).

Colloid is scant/absent.

Apoptosis and mitotic activity are present (Fig. 10.10).

Necrosis is often present (Fig. 10.11).

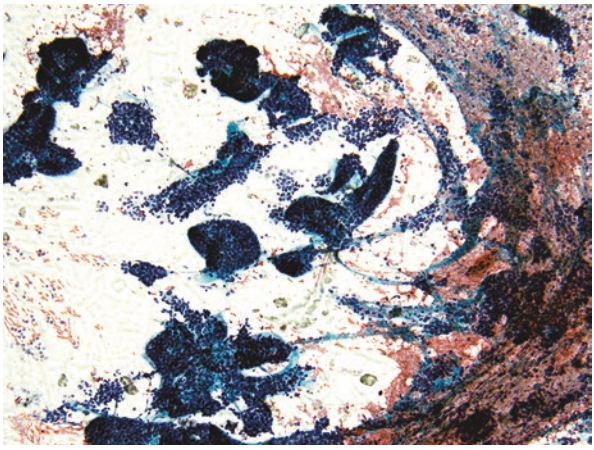

Fig. 10.1 Poorly differentiated thyroid carcinoma. A low magnification view reveals small follicular cells arranged in crowded insulae (smear, Papanicolaou stain)

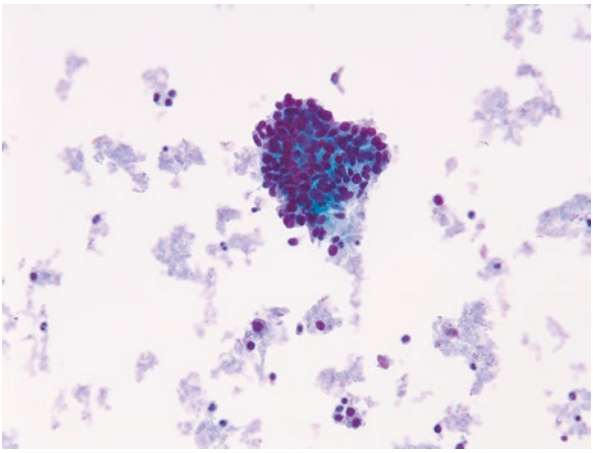

Fig. 10.2 Poorly differentiated thyroid carcinoma. The monomorphic cells are arranged in crowded 3-dimensional groups and scattered as isolated cells (ThinPrep, Papanicolaou stain)

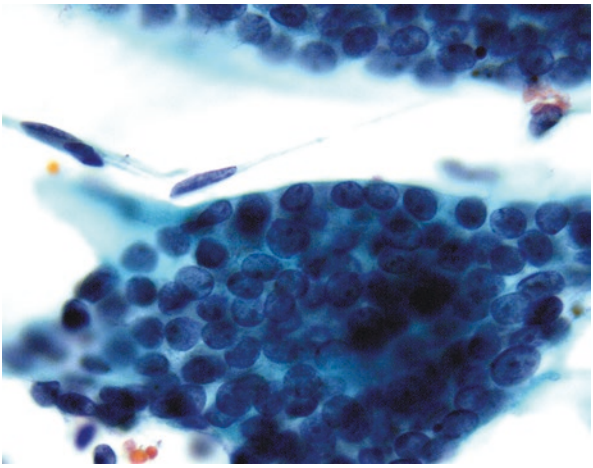

Fig. 10.3 Poorly differentiated thyroid carcinoma. Endothelium wrapping around cell groups can often be found highlighting the insular arrangements (smear, Papanicolaou stain)

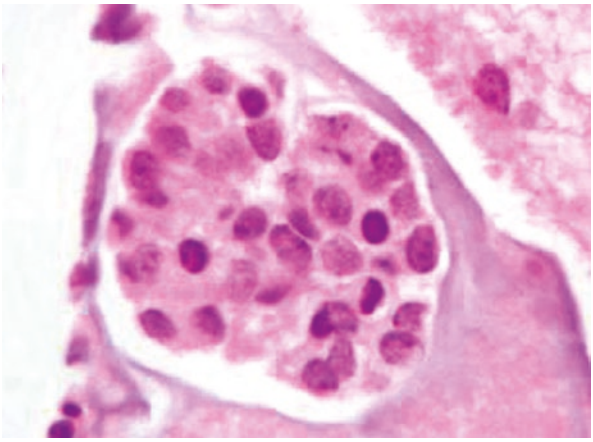

Fig. 10.4 Poorly differentiated thyroid carcinoma. The cell block demonstrates the arrangement of cells in insular groups (cell block, H&E stain)

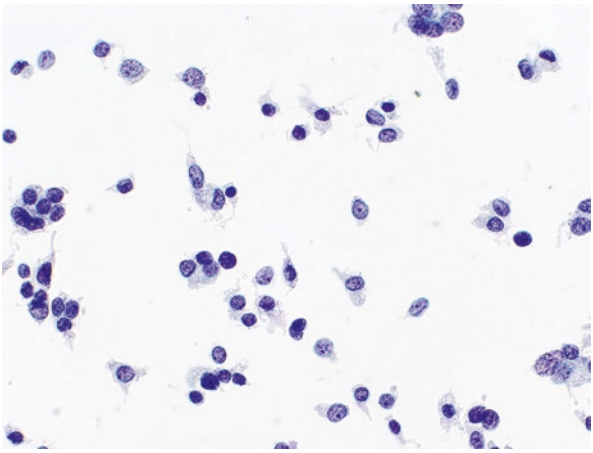

Fig. 10.5 Poorly differentiated thyroid carcinoma. In some cases, the malignant cells are arranged predominantly as isolated cells. They can have a plasmacytoid cytomorphology, as seen here (ThinPrep, Papanicolaou stain)

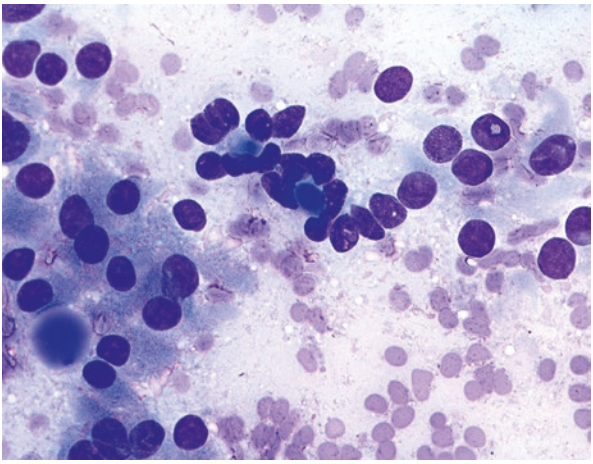

Fig. 10.6 Poorly differentiated thyroid carcinoma. In some cases, the cells have oncocytic cytoplasm. Some bare nuclei are also present (smear, Diff-Quik stain)

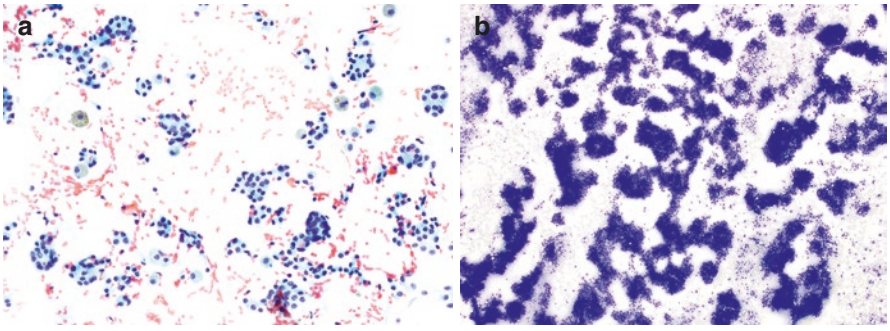

Fig. 10.7 High-grade follicular carcinoma. (**a**) The presence of microfollicles does not preclude the possibility of a poorly differentiated thyroid carcinoma or a high-grade follicular thyroid carcinoma (smear, Papanicolaou stain). (**b**) A hypercellular smear without colloid and large groups of follicular cells with an insular/solid component can be the only cytomorphological feature of a poorly differentiated thyroid carcinoma (smear, Diff-Quik stain)

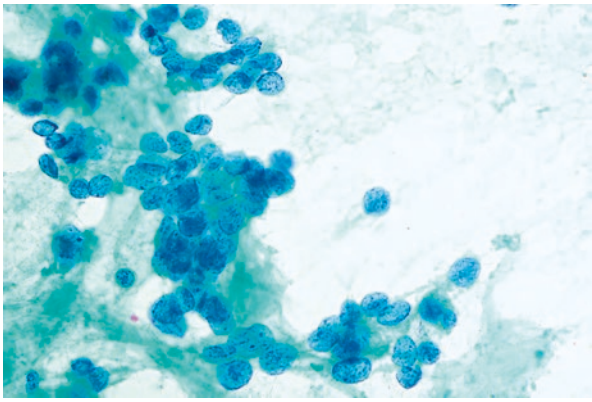

Fig. 10.8 Poorly differentiated thyroid carcinoma. Occasional tumors demonstrate only mild nuclear atypia, with small nucleoli and delicate chromatin (smear, Papanicolaou stain)

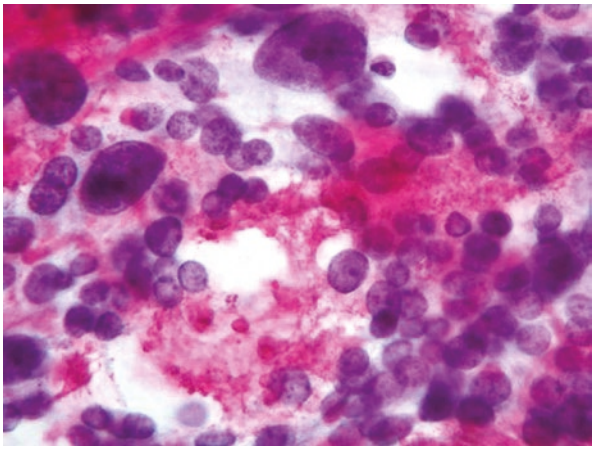

Fig. 10.9 Poorly differentiated thyroid carcinoma. Some aspirates exhibit marked nuclear atypia. In this example, there is impressive anisonucleosis (smear, Papanicolaou stain)

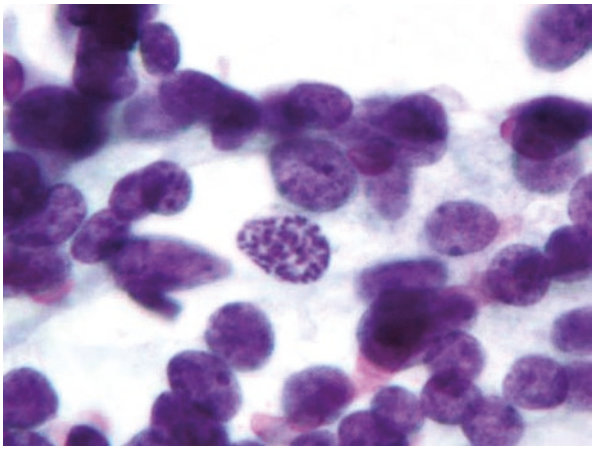

Fig. 10.10 Poorly differentiated thyroid carcinoma. Aspirates often contain mitotically active cells (smear, Papanicolaou stain)

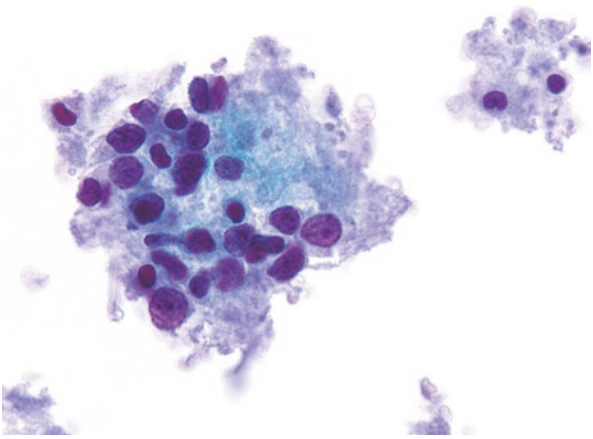

Fig. 10.11 Poorly differentiated thyroid carcinoma. Necrotic debris (cytoplasmic and nuclear fragments) is seen in occasional cases (smear, Papanicolaou stain)

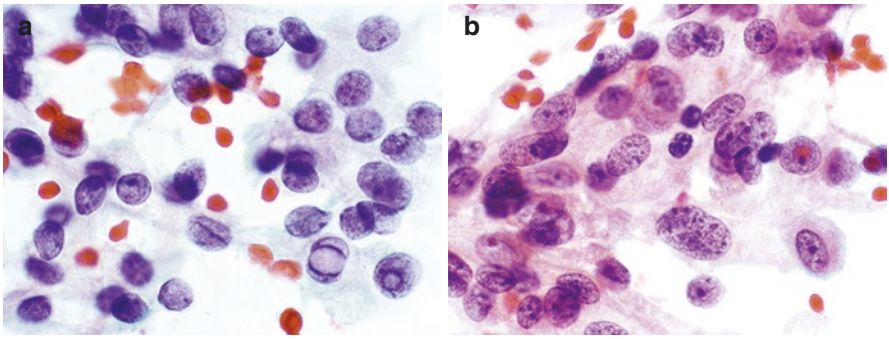

Fig. 10.12 High-grade follicular carcinoma. (**a**) In some cases, high-grade thyroid carcinomas show features of papillary carcinoma, including nuclear grooves and intranuclear pseudoinclusions. (**b**) There can be significant nuclear pleomorphism (smears, Papanicolaou stain)

Clearly malignant papillary carcinoma cells can display significant nuclear pleomorphism often accompanied by necrosis or necrotic debris (Figs 10.12 and 10.13).

In case of a predominant population of monotonous cell with plasmacytoid appearance, immunohistochemistry can be useful to exclude a medullary thyroid carcinoma or a metastasis (Figs. 10.14, 10.15, and 10.16).

In liquid-based cytology, PDTC exhibits the same cytomorphology, typically characterized by a population of cells with a high N/C ratio and focal nuclear atypia (Figs. 10.2 and 10.5).

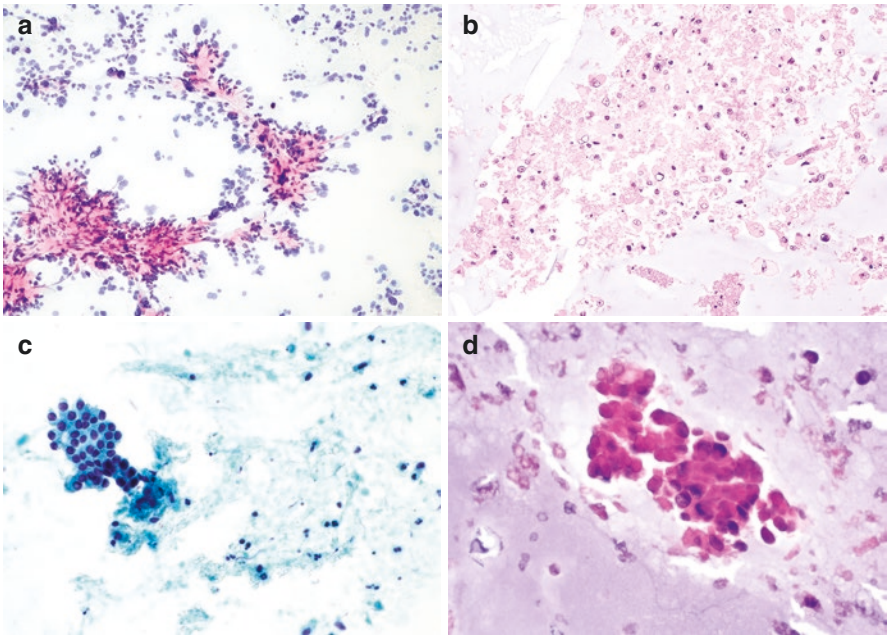

Fig. 10.13 High-grade follicular carcinoma. (**a**) High-grade tumors can show classic features of papillary carcinoma, including papillary architecture and intranuclear pseudoinclusions, but are associated with significant nuclear pleomorphism and dissociated cells (smear, Papanicolaou stain). (**b**) The cell block of the same case shows necrotic debris, cellular pleomorphism, and an oncocytic appearance of cells. Necrosis and pleomorphic tumor cells can also be the hallmark of anaplastic thyroid carcinoma, which should also enter in the differential diagnosis (cell block, H&E stain). (**c**) In a different case, papillary thyroid carcinoma cells lie in a necrotic background (smear, Papanicolaou stain) and (**d**) the corresponding cell block also shows necrotic cells (cell block, H&E stain)

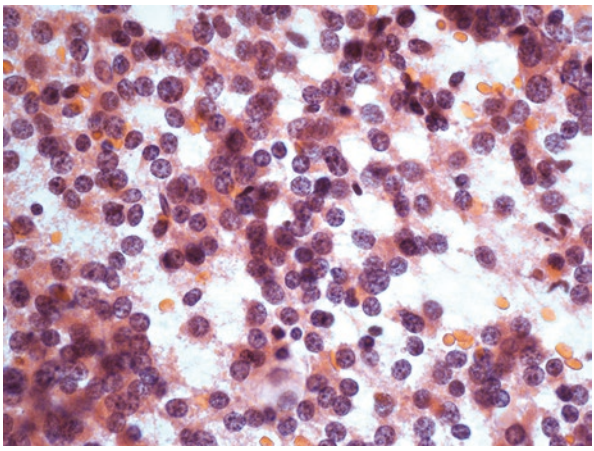

Fig. 10.14 Poorly differentiated thyroid carcinoma. Because some aspirates are comprised predominantly of isolated cells with granular chromatin, they mimic both medullary thyroid carcinoma and metastatic neoplasms (smear, Papanicolaou stain)

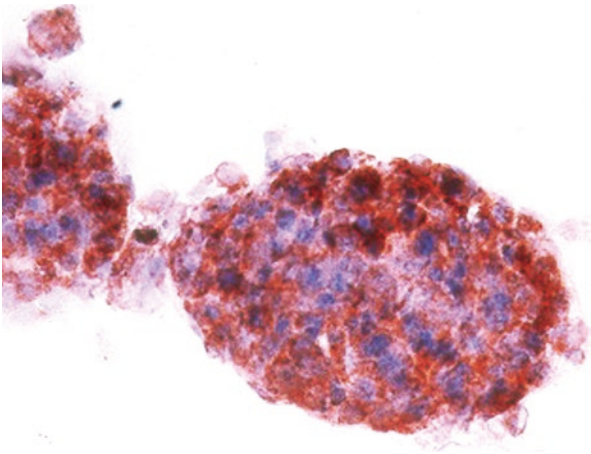

Fig. 10.15 Poorly differentiated thyroid carcinoma. Poorly differentiated thyroid carcinomas are positive for thyroglobulin, which helps to distinguish them from medullary thyroid carcinoma and metastatic tumors (ThinPrep, thyroglobulin IHC)

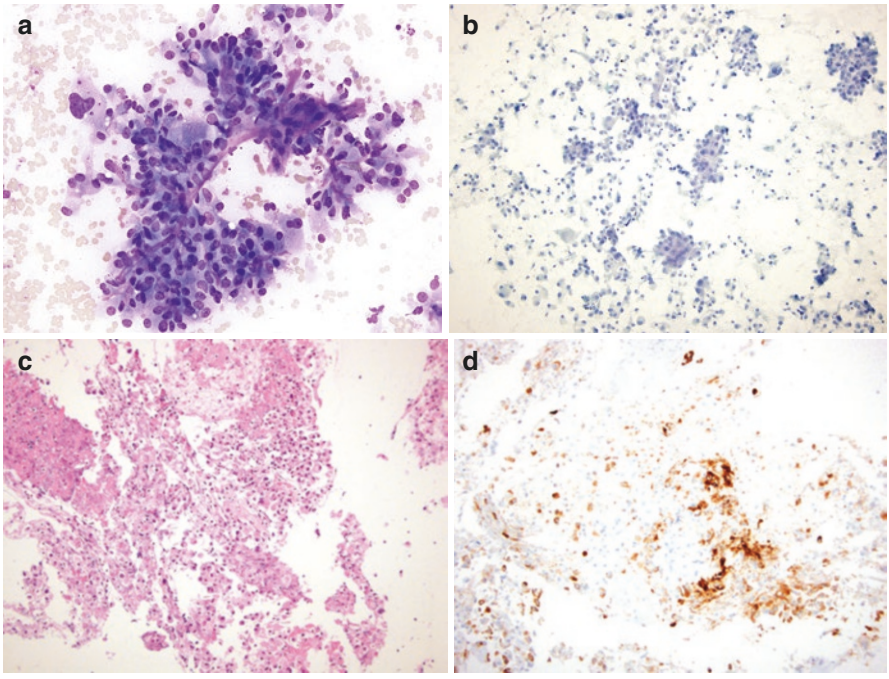

Fig. 10.16 High-grade follicular carcinoma. (**a**) Papillary architecture, plasmacytoid cells with nuclear pleomorphism (smear, Diff-Quik stain), (**b**) a more dissociated population of cells (smear, Papanicolaou stain) with (**c**) foci of necrosis (left corner) of the cell block, mimic medullary thyroid carcinoma (cell block, H&E). (**d**) Positive immunostaining for thyroglobulin confirms a high-grade papillary carcinoma (cell block, thyroglobulin immunostain)

Explanatory Notes

The below discussion will concentrate on the cytomorphological features of PDTC. On fine needle aspiration, PDTCs are difficult to recognize as such because they are rare and have cytomorphologic features that overlap with those of follicular neoplasms. DHGTC are more easily recognized from a cytological point of view, given the presence of characteristic papillary thyroid carcinoma nuclei and structures and/or follicular architecture; distinguishing from WDTC can be more challenging if tumor necrosis and mitotic activity are not readily apparent. Characteristic PDTC features on aspirate specimens do not have great specificity. A limited number of published case reports, small series, and institutional reviews yield just over 100 cases of PDTC, the aspirates of which are often hypercellular with scant amounts of colloid [12–23]. The tumor cells often have a monomorphic appearance at low magnification and consist of small to medium-sized cells with high N/C ratios. However, at higher magnification, variable degrees of atypia can be found along with abrupt nucleomegaly. While many PDTC cases show a single cell dispersion pattern, larger cell nests, trabeculae, sheets, and occasional follicular arrangements of neoplastic cells can be appreciated. The proportion of isolated cells versus larger fragments varies from case to case. As mentioned previously, the insular form of PDTC is identified histologically by its characteristic arrangement of cells in insulae with peripheral endothelial wrapping and peripheral alignment of nuclei and a similar pattern can be recognized in a subset of PDTC aspirates. Less frequently, some PDTC cases exhibit microfollicles (Fig. 10.7), nuclear grooves, and intranuclear pseudoinclusions (Fig. 10.12). Variable mitotic activity and apoptotic or necrotic debris can be appreciated but may be more frequently visualized on cell block or histologic material. Although necrosis is a concerning feature, it is important to differentiate tumor necrosis from that of infarct-type necrosis which can be seen after prior FNA. Morphologic findings such as a fibrovascular proliferation and/or the presence of hemosiderin-laden macrophages may be helpful in differentiating between these two etiologies of necrosis.

In the majority of preoperative FNAs, PDTCs are diagnosed cytologically as “Follicular Neoplasm” or “Malignant” as a well-differentiated thyroid carcinoma. In two large FNA series of PDTCs, approximately 35% of cases were prospectively recognized as PDTC or “poorly differentiated carcinoma, NOS” [23, 24]. The other cases were diagnosed mostly as “suspicious for a follicular neoplasm” or as “carcinoma,” either papillary carcinoma, follicular variant of papillary carcinoma, or not otherwise specified. Using logistic regression analysis, Bongiovanni et al. noted that a combination of cytoarchitecture, single cell dispersion pattern, significant crowding, and high N/C ratio was most predictive of PDTC [23].

The combination of cytomorphologic features described above, however, is suggestive of PDTC in FNA specimens. Clinical and ultrasonographic correlation is also helpful in further clarification of the FNA diagnosis, as PDTCs are usually large tumors with extrathyroidal extension.

Other primary thyroid tumors and metastatic malignancies should be considered in the differential diagnosis. A subset of PDTCs exhibits a predominantly isolated cell pattern in FNA samples (Fig. 10.13). When this occurs, together with

a "salt and pepper-like" chromatin pattern, the possibility of medullary thyroid carcinoma should be excluded with immunohistochemical stains. In contrast to medullary thyroid carcinoma, most PDTCs are immunoreactive for thyroglobulin (Fig. 10.14) and PAX8, are negative for calcitonin and CEA [25], and are only rarely immunoreactive for the neuroendocrine markers like synaptophysin and chromogranin. Medullary thyroid carcinomas are most often positive for calcitonin, CEA, and neuroendocrine markers. They only rarely show positivity for PAX8 and are negative for thyroglobulin. TTF-1 is not useful for this distinction as it will be positive in both PDTC and medullary thyroid carcinoma. PDTC and DHGTC can display oncocytic features, raising the possibility of oncocytic neoplasms. Unfortunately, immunohistochemistry is not helpful in differentiating these two entities and clear delineation is deferred to subsequent resection specimens. Undifferentiated (anaplastic) thyroid carcinomas are also characterized by a variety of cytomorphologic patterns (see Chap. 11) together with necrosis and increased mitotic activity. In contrast, PDTCs lack the marked nuclear pleomorphism, high-grade features, and sarcomatoid features of undifferentiated (anaplastic) thyroid carcinomas. Additionally, undifferentiated (anaplastic) thyroid carcinomas only rarely stain positively for thyroglobulin and usually show focal TTF-1 positivity in contrast to the typical diffuse staining of both of these markers in PDTCs and DHGTCs [26].

Based purely on cytomorphology, a PDTC may resemble a metastasis from an extrathyroidal primary tumor: both typically yield cellular specimens with nuclear atypia and necrosis, and colloid is scant. The positive immunoreactivity of PDTCs for thyroglobulin and TTF-1 helps to exclude a metastasis. The subset of PDTCs with a predominantly isolated cell pattern and plasmacytoid cytomorphology can suggest a lymphoproliferative disorder, but PDTCs are negative for CD45 and markers of B cells (e.g., CD19, CD20) and plasma cells (e.g., CD138).

Molecular Genetics

High-grade follicular cell-derived non-anaplastic thyroid carcinomas most often develop from three distinct pathways: partial dedifferentiation or high-grade transformation of a papillary thyroid carcinoma, partial dedifferentiation or high-grade transformation from a follicular or oncocytic carcinoma, or de novo [27]. As expected, these tumors show a mutational burden that is higher than well-differentiated tumors but is less than observed in anaplastic carcinomas. Furthermore, these tumors show characteristic early somatic mutations that correspond to a well-differentiated counterpart. For example, tumors arising from PTC often show *BRAF* V600E mutations, tumors arising from a follicular carcinoma often show *RAS* mutations, and tumors arising from oncocytic carcinomas often show somatic copy number alterations and mutations in mitochondrial DNA [28, 29]. However, HGFDTCs are enriched for additional late event mutations that drive the process of high-grade transformation. These most commonly include *TP53* and telomerase reverse transcriptase (*TERT*) mutations, which are also detected in higher proportions in

Table 10.1 Common driver alterations and functions in poorly differentiated thyroid carcinomas

Gene	Hotspots/fusion Partner	Function	Prevalence
RAS	Point mutations in codon 61 of NRAS or HRAS	Activation of MAPK and PI3K/AKT signaling pathways	20–40% [30–32]
BRAF	V600E	Activation of MAPK signaling pathway	5–30% [28, 33, 34]
EIF1AX	Point mutations in codons 9–15 in exon 2 or at a splice site between exons 5 and 6	Typically, co-existing with RAS and causes altered initiation of protein translation; only 1–2% of conventional PTCs (26878173)	10% [28, 35]
PIK3CA	Point mutations in codons 542, 545, or 1047	PI3K/AKT signaling pathway	5–20% [28, 31]
PTEN	Inactivating mutations, insertions, deletions	PI3K/AKT signaling pathway	5–20% [27, 28]
AKT1	*AKT1*49G>A	PI3K/AKT signaling pathway	<5% [31]
TERT	C228T and C250T	Encodes catalytic subunit of telomerase which maintains the lengths of telomeres to preserve chromosome during replication	30–50% [28, 36–38]
TP53	Exons 5–8	Tumor suppressor that regulates the cell cycle, DNA repair, and apoptosis	10–30% [28, 39, 40]
ALK fusions	*ALK::STRN*	Receptor tyrosine kinase that activates multiple downstream signaling pathways, such as MAPK, PI3K/AKT, and JAK-STAT	5–10% [28, 41–43]
PPARG fusions	*PPARG::PAX8*	Fusion product is an oncoprotein which accelerates growth and decreases apoptosis	5–7% [28, 44, 45]
RET fusions	*RET::PTC1* and *RET::PTC3*	Receptor tyrosine kinase that activates multiple downstream signaling pathways, such as MAPK and PI3K/AKT	0–5% [28, 46, 47]

anaplastic carcinomas. The most common driver molecular alterations are shown in Table 10.1, along with a note about gene hotspots, gene function, and prevalence in PDTC. As molecular diagnostic testing continues to play an increasing role for patient management, particularly in thyroid FNA samples, it is important to have a basic understanding of the molecular alterations present in conventional differentiated thyroid carcinomas, as well as HGFDTCs.

Management

Because of their poor clinical prognosis and radioactive iodine resistance, HGFDTCs are usually managed more aggressively than well differentiated thyroid carcinomas, with total thyroidectomy and lymph node dissection. Currently, additional therapies based on molecular signature are available [48, 49].

Sample Reports

The general category "Malignant" is used whenever the cytomorphologic features are conclusive for malignancy. If an aspirate is interpreted as Malignant, it is implied that the sample is adequate for evaluation. (An explicit statement of adequacy is optional.) Descriptive comments that follow are used to subclassify the malignancy and summarize the results of special studies, if any. If the findings are suspicious but not conclusive for malignancy, the general category "Suspicious For Malignancy" should be used (see Chap. 7). Many PDTCs overlap morphologically with follicular neoplasms and are therefore inevitably interpreted as "Follicular Neoplasm."

Example 1
MALIGNANT.
Most consistent with differentiated high-grade thyroid carcinoma.
Note: Highly cellular aspirate with atypical follicular cells, necrosis, and scant colloid, most consistent with differentiated high-grade thyroid carcinoma. However, a poorly differentiated thyroid carcinoma cannot be excluded.

Example 2
MALIGNANT.
Papillary thyroid carcinoma with focal poorly differentiated features, suggestive of differentiated high-grade thyroid carcinoma.

Example 3
FOLLICULAR NEOPLASM.
Note: Atypical follicular cells are present with a prominently isolated cell component, focal necrosis, and mitotic activity. Immunostains on cell block sections are positive for thyroglobulin and TTF-1 and negative for calcitonin. The findings raise the possibility of a poorly differentiated thyroid carcinoma.

Example 4
MALIGNANT.
Papillary thyroid carcinoma.
Note: Papillary carcinoma cells are present in groups and isolated patterns with background necrosis. A differentiated (papillary) high-grade thyroid carcinoma could not be excluded.

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Undifferentiated (Anaplastic) Thyroid Carcinoma

11

Esther Diana Rossi, Justin Bishop, Vinod Shidham, Dinka Sundov, and Joseph Scharpf

Background

Undifferentiated (anaplastic) thyroid carcinoma (UTC), previously also called "giant and spindle cell carcinoma," is an extremely aggressive thyroid malignancy. Accounting for less than 5% of thyroid cancers [1–3], it carries the poorest prognosis of all, significantly worse than well differentiated and poorly differentiated thyroid carcinomas [4]. Most patients succumb to their disease within 6 months to 1 year of the initial diagnosis, typically as a result of tumor involvement of vital structures within the neck [2, 5]. Characteristic clinical features are associated with UTCs. These tumors are rarely seen in individuals below the age of 50 (<10% of cases), with a female predominance (2–4:1) [3–7]. Patients present with a hard,

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nodular thyroid gland, and most have a rapidly growing mass. Neck enlargement is due to marked tumor growth, with or without reactive fibrosis, which infiltrates into surrounding extrathyroidal soft tissues (e.g., muscle, trachea, esophagus, and adjacent skin, cartilage, and bone) [5]. Half of the patients with UTC report significant neck compression that can result in dyspnea, dysphagia, hoarseness, and/or pain [2, 5]. One-quarter to one-half of patients present with lymphadenopathy and/or distant metastases, most commonly to the lungs [2, 4, 6]. A history of long-standing goiter [2, 4, 6] and thyroid function tests indicating euthyroidism (despite extensive thyroid gland destruction) [2, 6] are common.

Definition

UTC is a high grade, pleomorphic, epithelial-derived malignancy with epithelioid and/or spindle cell features.

Criteria

Aspirates show variable cellularity but are usually moderately to markedly cellular.

Neoplastic cells are arranged as isolated cells and/or in variably sized groups (Figs. 11.1, 11.2, 11.3, 11.4, 11.5, 11.6, 11.7, 11.8, 11.9, 11.10, 11.12, 11.13, 11.14, 11.15, 11.16 and 11.17).

Cells are epithelioid (round to polygonal) and/or spindle-shaped and range in size from small to giant cells. "Plasmacytoid" and "rhabdoid" cell shapes may be seen (Figs. 11.3, 11.4, 11.5, 11.6, and 11.12).

Squamous cell carcinoma is considered a morphological subtype [7].

Nuclei show enlargement, irregularity, extreme pleomorphism, clumping of chromatin with parachromatin clearing, prominent irregular nucleoli, intranuclear

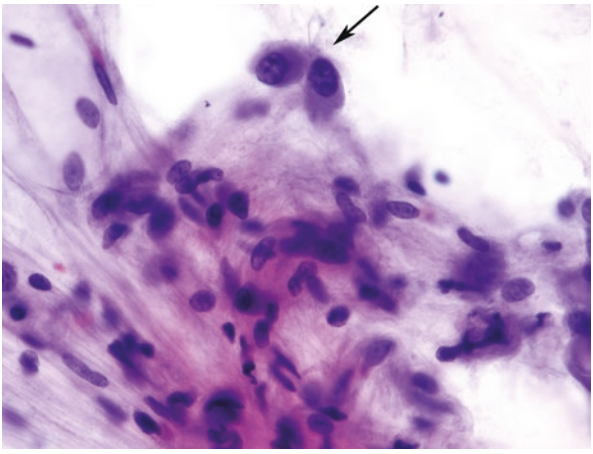

Fig. 11.1 Undifferentiated (anaplastic) thyroid carcinoma. Aspiration of tumors with abundant fibrosis can yield low cellularity. If cells lack marked nuclear atypia (arrow), rendering a definitive diagnosis can be difficult. Clinical correlation is important (smear, Papanicolaou stain)

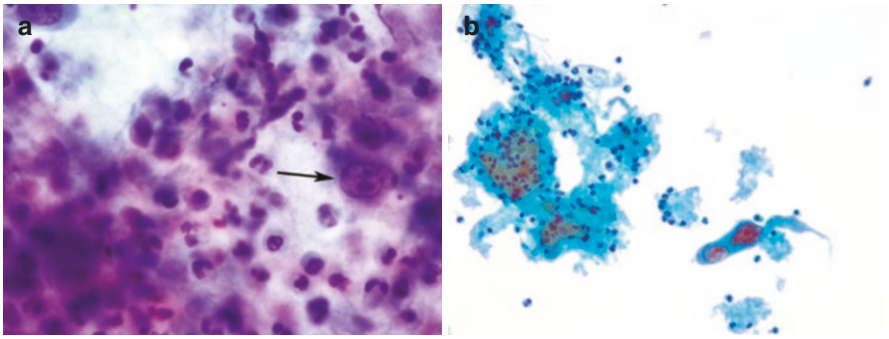

Fig. 11.2 (**a**, **b**) Undifferentiated (anaplastic) thyroid carcinoma. Widespread tumor necrosis and associated inflammation can hinder diagnosis because well-preserved malignant cells are few and far between (arrow) (**a**—smear, Papanicolaou stain) (**b**—ThinPrep, Papanicolaou stain)

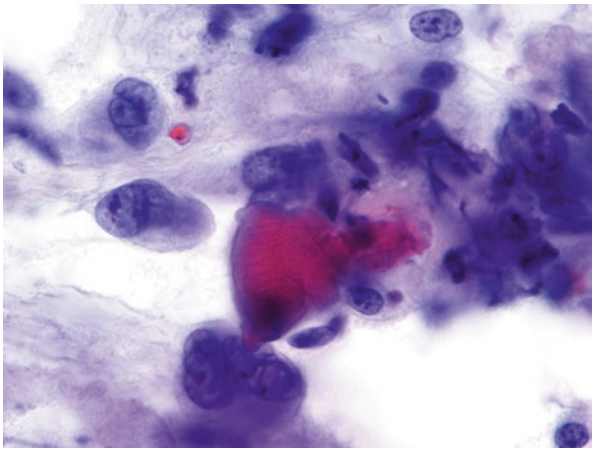

Fig. 11.3 Undifferentiated (anaplastic) thyroid carcinoma. Rapid tumor growth and invasion of extrathyroidal tissues is common. Aspiration samples can contain skeletal muscle fragments (center) as well as anaplastic tumor cells (smear, Papanicolaou stain)

pseudoinclusions, eccentric nuclear placement, and multinucleation (Figs. 11.7, 11.8, 11.9, 11.10, 11.11, 11.12, 11.13, 11.14, and 11.15).

Necrosis, extensive inflammation (predominantly neutrophils, "abscess-like"), and/or fibrous connective tissue may be present (Figs. 11.2 and 11.15).

Neutrophilic infiltration of tumor cell cytoplasm can be seen (Fig. 11.14).

Mitotic figures are often numerous and abnormal (Fig. 11.5).

Osteoclast-like giant cells (non-neoplastic) are conspicuous in some cases (Fig. 11.16).

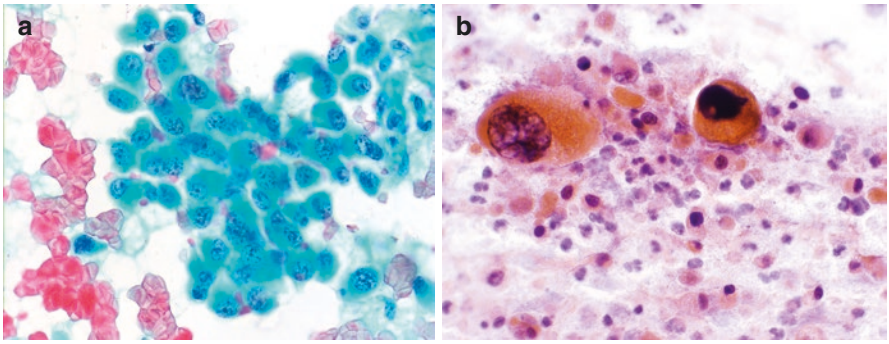

Fig. 11.4 (**a**, **b**) Undifferentiated (anaplastic) thyroid carcinoma. (**a**) Cells are epithelioid (polygonal) in appearance. Variation in cell and nuclear size is evident. Parachromatin clearing and nuclear contour irregularity are prominent (smear, Papanicolaou stain). (**b**) Undifferentiated (anaplastic) thyroid carcinoma, squamous cell carcinoma subtype. Large pleomorphic cells with conspicuous dense orangeophilia of the cytoplasm. There is abundant necrosis, and nuclei show degenerative changes (i.e., dark, smudged and/or marginated chromatin) (smear, Papanicolaou stain)

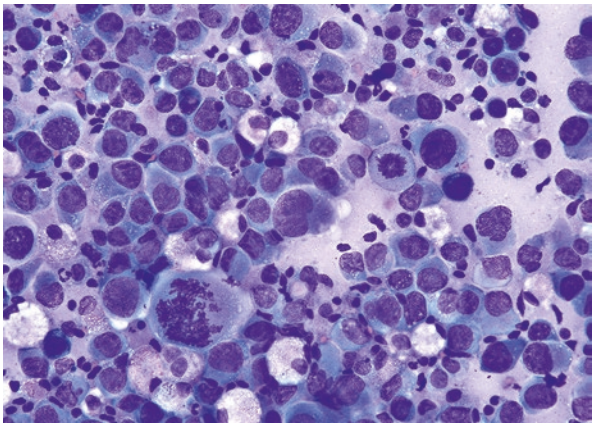

Fig. 11.5 Undifferentiated (anaplastic) thyroid carcinoma. The neoplastic cells are mostly round, with scant to moderate cytoplasm. There is less pleomorphism of nuclear size and shape than in most cases of UTC, but mitotic figures are easily found (smear, Diff-Quik stain)

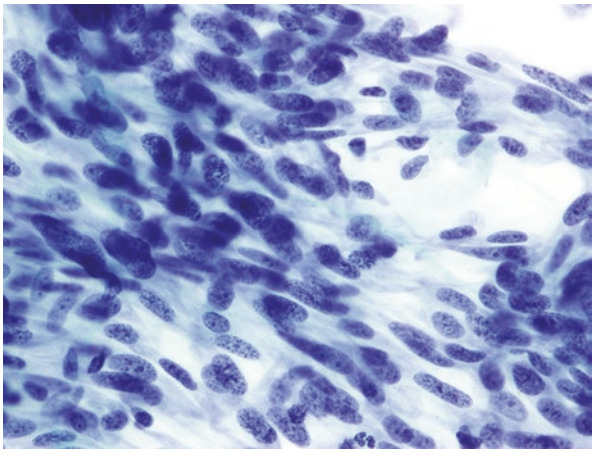

Fig. 11.6 Undifferentiated (anaplastic) thyroid carcinoma. All neoplastic cells are strikingly spindle-shaped, resembling the cells of a sarcoma. Although chromatin is coarse, parachromatin clearing, prominent nucleoli, and nuclear irregularity are not apparent (smear, Papanicolaou stain)

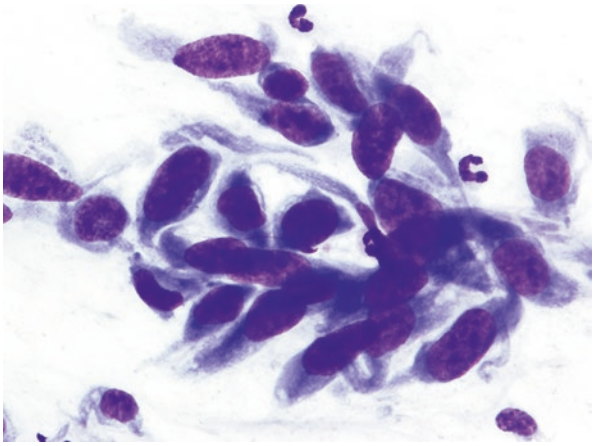

Fig. 11.7 Undifferentiated (anaplastic) thyroid carcinoma. Tumor cells are notably spindle-shaped, with long, tapering cytoplasmic processes (smear, Diff-Quik stain)

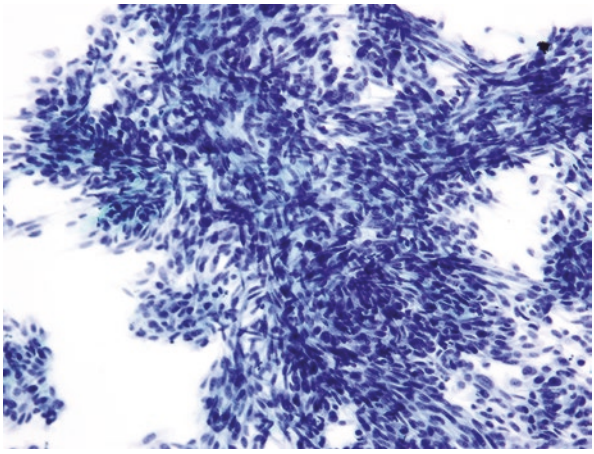

Fig. 11.8 Undifferentiated (anaplastic) thyroid carcinoma. Tumors with a predominantly spindle cell morphology can appear as microbiopsy fragments. A storiform pattern can be appreciated (smear, Papanicolaou stain)

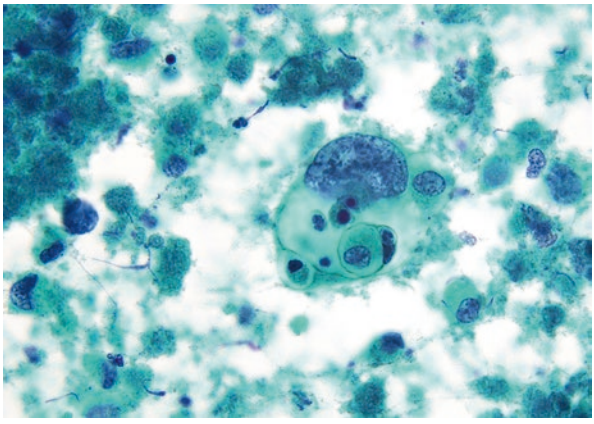

Fig. 11.9 Undifferentiated (anaplastic) thyroid carcinoma. These tumors can be associated with abundant necrosis often with infiltration by numerous neutrophils. A pleomorphic tumor giant cell with bizarre nuclear features and smaller, isolated, less anaplastic malignant cells are readily identifiable (smear, Papanicolaou stain)

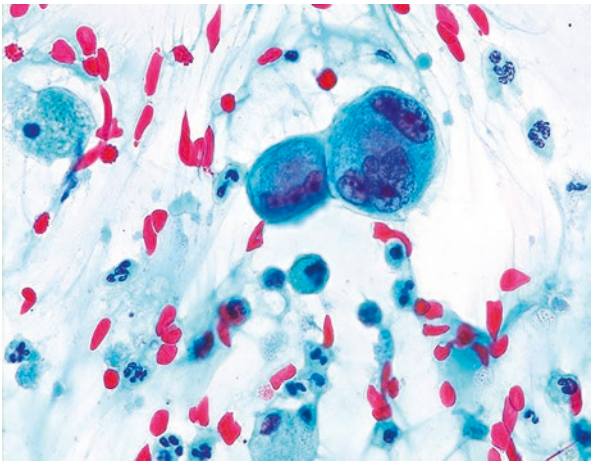

Fig. 11.10
Undifferentiated (anaplastic) thyroid carcinoma. Bizarre multinucleated tumor giant cells are found in some aspirations. The size of this tumor giant cell can be fully appreciated when compared to the adjacent neutrophils (smear, Diff-Quik stain)

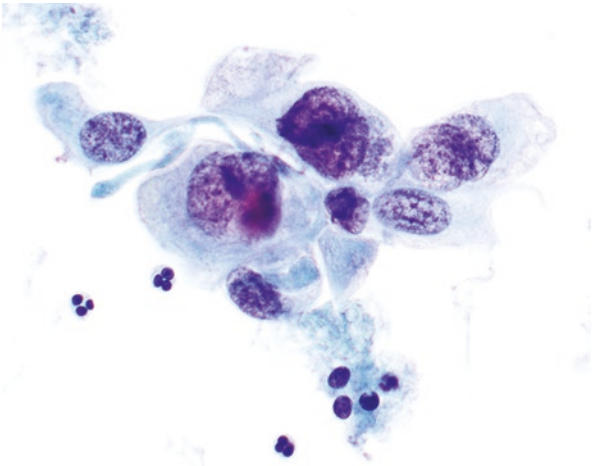

Fig. 11.11
Undifferentiated (anaplastic) thyroid carcinoma. Variably pleomorphic tumor giant cells with coarse chromatin are seen in a loosely cohesive cell group (ThinPrep, Papanicolaou stain)

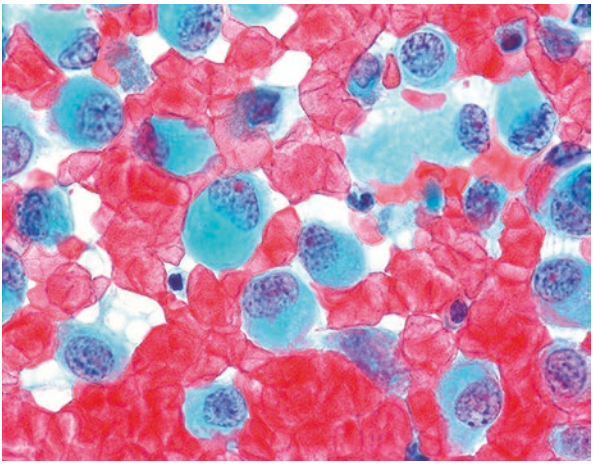

Fig. 11.12
Undifferentiated (anaplastic) thyroid carcinoma. In some cases, the epithelioid tumor cells have a conspicuously plasmacytoid or rhabdoid appearance (smear, Papanicolaou stain)

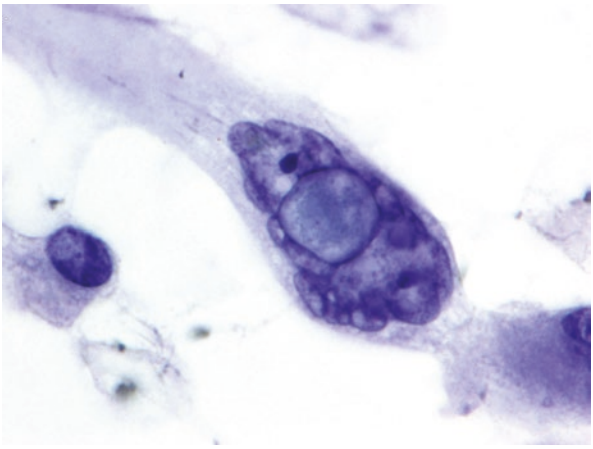

Fig. 11.13
Undifferentiated (anaplastic) thyroid carcinoma. A giant spindle-shaped tumor cell has a massive intranuclear pseudoinclusion. Other nuclear features include enlargement, contour irregularity, and a prominent nucleolus (smear, Papanicolaou stain)

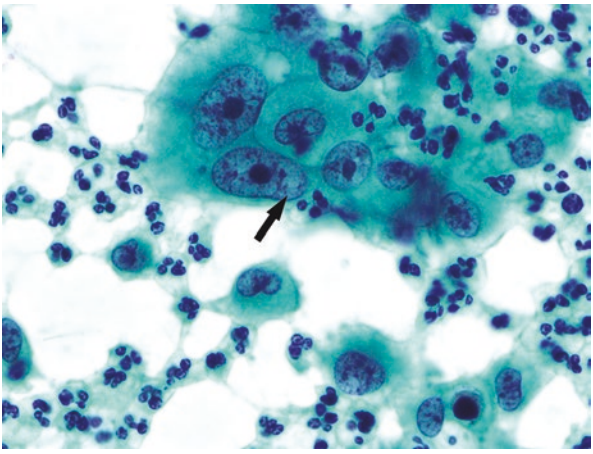

Fig. 11.14
Undifferentiated (anaplastic) thyroid carcinoma. Epithelioid tumor cells display size variation, mononucleated and binucleated forms, macronucleoli, and clumped chromatin with parachromatin clearing (arrow). Acute inflammatory cells are present in the background (smear, Papanicolaou stain)

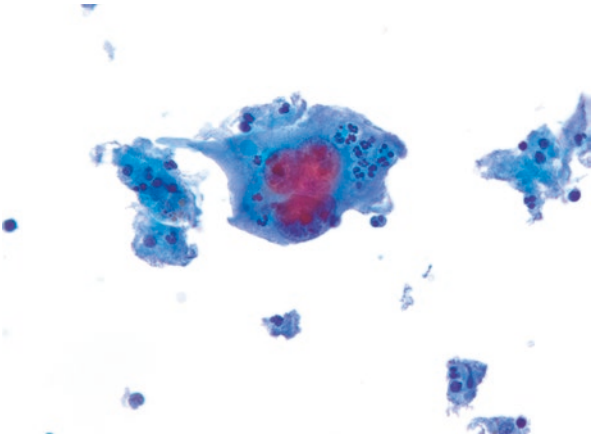

Fig. 11.15
Undifferentiated (anaplastic) thyroid carcinoma. There is conspicuous infiltration of a multinucleated tumor giant cell by neutrophils (ThinPrep, Papanicolaou stain)

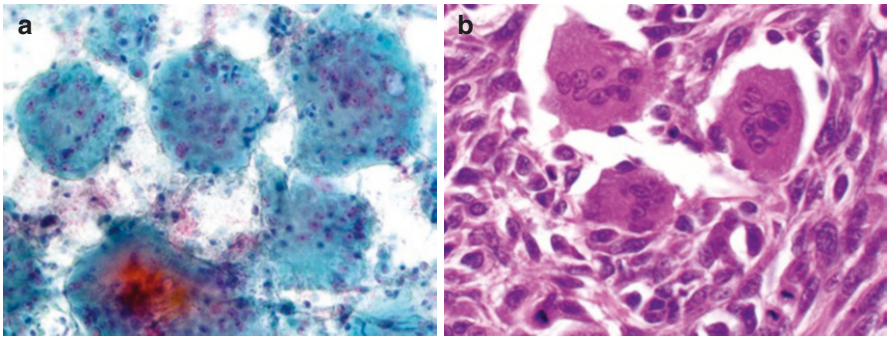

Fig. 11.16 Undifferentiated (anaplastic) thyroid carcinoma. (**a**) Some UTCs contain numerous non-neoplastic, osteoclast-like giant cells (smear, Papanicolaou stain). (**b**) The osteoclast-like giant cells are scattered among the malignant cells (thyroidectomy, hematoxylin and eosin stain)

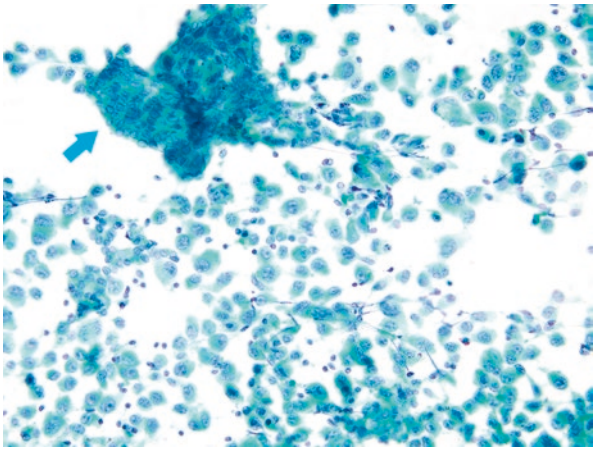

Fig. 11.17 Undifferentiated (anaplastic) thyroid carcinoma. The cohesive fragment (arrow) represents a residual focus of the well differentiated component of the tumor and is consistent with papillary thyroid carcinoma. The large population of the singly dispersed pleomorphic malignant cells are from the undifferentiated (anaplastic) carcinoma (smear, Papanicolaou stain)

Tumors have the following immunocytochemical and molecular profile:

- Pan-keratins, PAX8, and vimentin are often positive but can be focal (Fig. 11.18).
- Usually negative for TTF-1 and thyroglobulin.
- *TP53*, *CTNNB1*(β-catenin), *RAS* (i.e., *HRAS*, *KRAS*, or NRAS), and *BRAF* V600E mutations are seen in up to 80%, 70%, 50%, and 30% of cases, respectively.
- 65–75% of UTCs carry *TERT* promoter mutation.

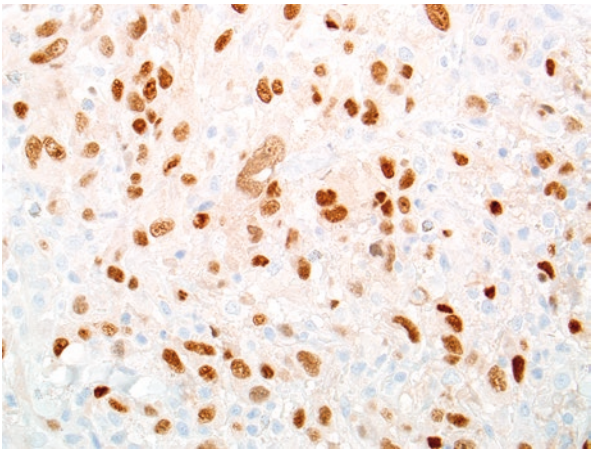

Fig. 11.18 Undifferentiated (anaplastic) thyroid carcinoma. PAX8, one of the most useful immunomarkers in this setting, displays crisp nuclear positivity (cell block, PAX8 immunostain)

Explanatory Notes

Cellularity is variable and it is not affected by the different cytologic preparations. Some aspirates are sparsely cellular, due in part to the marked fibrosis and hyalinization seen in some tumors [8–10]. When fibrosis predominates, the resulting low cellularity can hamper interpretation (Fig. 11.1). In other cases, widespread tumor necrosis yields a sparsely cellular sample with few viable malignant cells [8] (Fig. 11.2a, b). Due to rapid infiltrative tumor growth, aspirations can result in the acquisition of tumor cells admixed with extrathyroidal tissue such as skeletal muscle (Fig. 11.3).

Isolated cells and small to medium-sized cell groups can be found in most cases (Figs. 11.4, 11.5, 11.6, and 11.7). In spindle cell predominant UTCs, larger tumor tissue fragments can reveal a storiform-like pattern [6] (Fig. 11.8). Follicles, papillae, and trabecular/nested cell groups are not features of UTC. According to the 5th Edition of the WHO Classification of Endocrine Tumours, squamous cell carcinoma of the thyroid is now classified as a morphologic subtype of UTC [7]. UTC, squamous cell subtype is morphologically and immunohistochemically indistinguishable from squamous cell carcinomas of other organs (Fig. 11.4b). However, p16 immunoreactivity favors non-thyroidal origin with related prognosis. For this reason, correlation with clinical and imaging findings is essential for excluding a metastasis. The behavior of the squamous subtype is similar to that of UTC, as is the clinical management.

Small to gigantic malignant cells may be epithelioid (round to polygonal) or spindle-shaped [8, 11, 12] (Figs. 11.4, 11.5, 11.6, and 11.7). A given tumor often displays a mixture of cell shapes and sizes (Figs. 11.4 and 11.9). Nuclear pleomorphism can be striking, with giant, bizarre, hyperchromatic forms [8, 11, 12]

(Figs. 11.10 and 11.11). Nuclei may be variably positioned within the cells, but can be uniformly eccentric, resulting in a plasmacytoid morphology [7] (Fig. 11.12). Intranuclear pseudoinclusions (Fig. 11.13), prominent nucleoli (Fig. 11.14), coarse chromatin (Fig. 11.6), and parachromatin clearing (Fig. 11.14) may be identified [8, 11, 12]. Neutrophilic infiltration of tumor cells (Fig. 11.15), osteoclast-like giant cells (Fig. 11.16), necrosis, fibrotic tissue fragments, and mitotic figures (Fig. 11.5) may be present in variable proportions [8–12].

Some UTCs have a focus of co-existing well differentiated and/or poorly differentiated thyroid carcinoma, most often papillary thyroid carcinoma [5, 6, 10–12], but sometimes follicular carcinoma [5, 6, 13], oncocytic carcinoma [5, 6], poorly differentiated (insular) carcinoma [4, 10], and other types of poorly differentiated carcinomas (Fig. 11.17). Consequently, on occasion several components are observed in an aspirate. Hence, thorough sampling and attention to the possibility of multiple components are imperative so that the identification of the most significant (i.e., least differentiated) cellular pattern is made. The frequent co-existence of a nidus of well differentiated thyroid cancers within a UTC suggests that UTC represents dedifferentiation of a well differentiated thyroid cancer through a multistep process of carcinogenesis [2, 13]. This is supported by the occasional observation of UTC in metastatic foci from patients whose primary thyroid carcinomas were well differentiated [5, 13].

The most reliable immunostains yielding a positive result in UTCs are as follows: pan-keratins, with rates of expression ranging from 50 to 100% of cases [6, 14]; PAX8 (the most specific immunostain indicative of UTC's thyroid gland origin), seen in 76–79% of cases [15–17] (Fig. 11.18); and vimentin, present in 50–100% of cases, especially in the spindle cell tumor component [6, 15]. Because of frequent non-immunoreactivity for thyroglobulin and TTF-1 [6, 13] with non-immunoreactivity for PAX8 in a quarter of cases, challenges occur especially in cases with sparsely cellular samples or aspirates of spindle cell component that are non-immunoreactive for keratins. In these cases, an erroneous diagnosis of sarcoma might be entertained, but primary sarcomas of the thyroid are rare. Concerning the squamous cell carcinoma subtype, about 50% show PAX8 immunoreactivity. They are also immunoreactive for p63 and p40 with immunoreactivity for various cytokeratins including 7 and 20 and frequent overexpression of p53. Limited data on *BRAF* mutation analysis demonstrated mutation in some of them.

Other entities in the differential diagnosis of UTC include insular thyroid carcinoma, medullary thyroid carcinoma, lymphoma, and metastasis. Compared to UTC, poorly differentiated thyroid (insular) carcinoma has a lesser degree of nuclear atypia (lacking prominent nucleoli), a strikingly monotonous appearance with a trabecular/nested architecture, and lacks spindle-shaped cells and osteoclast-like giant cells. Medullary thyroid carcinoma, overall, is usually less pleomorphic than UTC, has finely stippled chromatin, and usually contains amyloid. Osteoclast-like giant cells and necrosis are absent in medullary carcinoma. If doubt remains after morphologic assessment, immunohistochemistry can be

helpful, as medullary carcinomas are reactive for calcitonin and chromogranin whereas UTCs are negative. The most difficult mimic to exclude is often a metastasis (e.g., melanoma, sarcomatoid renal cell carcinoma, squamous cell, or large cell carcinoma of the lung). Ruling out a thyroid metastasis requires knowledge of the patient's prior cancer history, clinical correlation (e.g., the size and the anatomic distribution of other extrathyroidal tumor masses), and selective immunostaining.

In paucicellular aspirates due to necrosis and/or fibrosis, an under-appreciation of rare malignant cells can lead to a misdiagnosis of a reactive process (e.g., Riedel thyroiditis) [9].

Molecular alterations that are seen with some frequency include the early thyroid carcinogenesis mutations of *RAS* and *BRAF* (up to 50% and 30% of cases, respectively), and the late carcinogenesis mutations of *TP53* and *CTNNB1* (β-catenin) which lead to progressive loss in thyroid differentiation (up to 80% and 70% of cases, respectively) [15, 18]. Mutations in the promoter region of the *TERT* gene are found with increasing frequency from well differentiated to poorly differentiated and to UTC. Approximately 65–75% of UTCs carry *TERT* promoter mutation. Some UTCs carry mutations and copy number alterations in the cell cycle genes *CDKN2A* and *CDKN2B*. Mutations in the *ATM*, *NF1*, and *RB1* genes can be found in 10% of UTCs. Five to 10% of UTCs carry mutations in the DNA mismatch repair genes such as *MSH*, *MLH1*, among others.

Management

The overall survival of patients with UTC has not changed significantly in over 20 years. One-fifth of patients require tracheostomy due to airway obstruction during the course of their disease [19].

Complete surgical resection, with or without preoperative hyperfractionated radiotherapy and/or chemotherapy to enhance resectability through tumor shrinkage, is the optimal treatment strategy [1, 19]. Suppression with radioactive iodine is largely ineffective for the treatment of UTC [1, 5, 19]. In cases where potential cure cannot be achieved, reducing the tumor burden through surgery facilitates the efficacy of postoperative radiation and/or chemotherapy [19]. In patients fit enough to tolerate these regimens, length of survival is improved [6, 19]. Not surprisingly, younger patients (<45 years old) and individuals with smaller tumors without extensive extrathyroidal extension or metastases have the best outcome [3, 5, 6]. The development of novel therapeutics such as molecular targeted therapies and immune checkpoint inhibitors holds promise for treating UTC [1, 19]. The presence of *BRAF V600E* mutation, detected by molecular or immunocytochemical staining under certain circumstances can guide treatment with dual BRAF/MEK inhibition (e.g., dabrafenib and trametinib). High tumor mutation burden (>10 mutations/Mb), high PD-L1 expression, or assessment of microsatellite instability or mismatch repair deficiency may aid in selection for immune checkpoint inhibitor therapy. Gene

fusion events including *ALK*, *NTRK*, or *RET* have implications for use of targeted therapies [1] (see Chap. 14).

Sample Reports

The general category "Malignant" is used whenever the cytomorphologic features are conclusive for malignancy. If an aspirate is interpreted as malignant, it is implied that the sample is adequate for evaluation. An explicit statement of adequacy is optional. Descriptive comments that follow are used to subclassify the malignancy and summarize the results of special studies, if any. If the findings are suspicious but not conclusive for malignancy, the general category "Suspicious for malignancy" should be used (see Chap. 7).

Example 1
MALIGNANT.
Undifferentiated (anaplastic) thyroid carcinoma.
Note: Immunocytochemistry shows that the malignant cells are focally immunoreactive for pan-cytokeratins AE1/3 and PAX8 and negative for thyroglobulin and TTF-1.

Example 2
MALIGNANT.
High-grade carcinoma, consistent with undifferentiated (anaplastic) thyroid carcinoma.
Note: Immunocytochemistry shows that the malignant cells are focally immunoreactive for cytokeratins AE1/3, PAX8, and vimentin and negative for thyroglobulin, TTF-1, HMB-45, and S-100 protein. The prior clinical history of malignant melanoma is noted.

Example 3
MALIGNANT.
Squamous cell carcinoma.
Note: The distinction between a primary thyroid squamous subtype of undifferentiated (anaplastic) thyroid carcinoma and a metastasis to the thyroid from a primary tumor elsewhere may not be possible; correlation with clinical and imaging findings is advised.

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Metastatic Tumors, Lymphomas, and Rare Tumors of the Thyroid

12

Lester Layfield, Kennichi Kakudo, and Ivana Kholovac

Background

Metastases from distant organs and direct extension from tumors in adjacent organs are uncommon but important to recognize in fine needle aspiration (FNA) samples of thyroid nodules. In rare cases, a metastasis to the thyroid can even be the initial presentation of a distant malignancy. Tumors of nearby structures that can involve the thyroid include those of the pharynx, larynx, esophagus, mediastinum, and regional lymph nodes [1]. The most common tumors that present clinically as metastases to the thyroid are cancers of the lung, breast, skin (especially melanoma), colon, and kidney [2–6]. The frequency varies in surgical vs. autopsy series (2.7–4.0%). Metastases, including micrometastases, are found in up to 10% of cancer autopsies [2]. Metastatic carcinomas characteristically present in one of three patterns: (1) multiple small discrete nodules less than 2 mm in diameter; (2) solitary large nodules; and (3) diffuse involvement. When small nodules are present, FNA

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samples reveal neoplastic cells admixed with native follicular epithelial cells. With routine and special stains, distinction from a primary neoplasm of the thyroid is often achievable, though clinical history and radiologic correlation are very helpful in this regard, especially when the cytopathologist is alerted to such findings on the FNA requisition form [7].

Malignant lymphomas occur as primary malignancies of the thyroid, but they can also involve the thyroid gland secondarily as a manifestation of systemic disease [8]. Most primary thyroid lymphomas are of B-cell lineage [8]. Lymphomas represent approximately 5% of thyroid neoplasms, usually associated with Hashimoto thyroiditis [8]. As primary tumors of the thyroid, plasma cell neoplasms, Hodgkin lymphoma, and Langerhans cell histiocytosis are rare but do occur.

Metastatic Renal Cell Carcinoma

Criteria

Samples show moderate to high cellularity.

Cells are dispersed individually and in small clusters, fragmented papillae, or sheets.

Cells have abundant pale, finely granular, clear or vacuolated cytoplasm.

Nuclei are round to oval, often with large nucleoli.

Smears are frequently bloody.

Explanatory Notes

A majority of the metastatic renal cell carcinomas (RCC) to the thyroid are of the clear cell type and present as either solitary or multiple nodules [9, 10], occurring as many as 20 years after the resection of the primary neoplasm [10].

Metastatic RCC displays moderately to highly cellular, often bloody, preparations [11]. Cells appear singly and in cohesive clusters, fragmented papillae, and sheets (Fig. 12.1). The individual cells have abundant pale, finely vacuolated cytoplasm with Romanowsky-stained preparations and abundant clear, finely granular cytoplasm with the Papanicolaou stain (Fig. 12.2). The nuclear/cytoplasmic ratio is low. The nuclei are round to oval and vary in size and shape. Nuclear chromatin is finely granular, and the prominence of nucleoli is directly proportional to the grade of the RCC. Intranuclear pseudoinclusions are found in a minority of metastatic RCCs. With air-dried, Romanowsky-stained smears, strands of pink, hyaline, or fibrillary stroma with attached fusiform cells are characteristic of high-grade RCCs.

The distinction between clear cell RCC and follicular or oncocytic neoplasms can be difficult, particularly if the RCC is occult or if the history of RCC is not provided [12]. Immunostaining for thyroid markers (e.g., thyroglobulin, TTF-1, and calcitonin) and RCC markers (e.g., RCC antigen, CD10) can aid in the differential diagnosis; however, for this particular differential diagnosis PAX8 is not helpful.

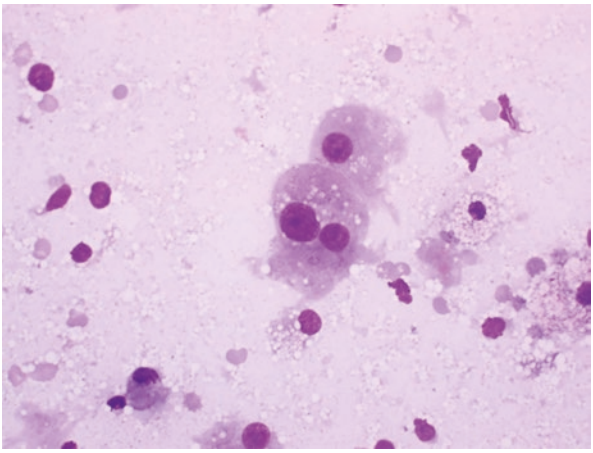

Fig. 12.1 Metastatic renal cell carcinoma, clear cell type. The malignant cells have finely vacuolated cytoplasm (smear, Diff-Quik stain)

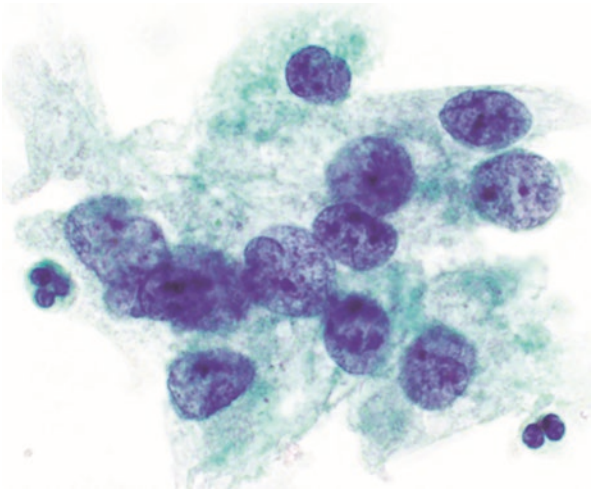

Fig. 12.2 Metastatic renal carcinoma, clear cell type. Cells in a small cluster have abundant finely granular cytoplasm. Note the adjacent neutrophils for size comparison (ThinPrep, Papanicolaou stain)

Metastatic Malignant Melanoma

Criteria

Aspirates are moderately or markedly cellular.

Most cells are non-cohesive.

Cells are variable in size and shape and include plasmacytoid, spindle-shaped, and anaplastic forms.

Nuclei are large and often eccentrically placed.

Intranuclear pseudoinclusions can be present.

Intracytoplasmic pigment is not common but can be seen as fine granules in neoplastic cells or coarse granules in histiocytes.

Cells are usually immunoreactive for S-100 protein, Melan-A, SOX-10, and HMB-45.

Explanatory Notes

Aspirates from metastatic melanoma are characterized by many dispersed cells, with marked variability in size; oval, plasmacytoid, fusiform, and anaplastic forms are typical (Figs. 12.3 and 12.4) [13]. Eccentrically positioned nuclei are usually round or ovoid and vary in size and number. The cells typically have well-defined cytoplasm. Intranuclear pseudoinclusions are seen. Intracytoplasmic melanin pigment is not common but may be seen as fine granules in the cytoplasm or dark staining in perinuclear areas. More often, melanin is found as coarse granules in histiocytes (Fig. 12.3b) [13]. Primary thyroid carcinomas with melanin pigment have been reported and are a histologic variant of medullary carcinoma of the thyroid, which can share many cytomorphologic features with melanoma (see Fig. 9.7 and Chap. 9). Immunocytochemistry can be helpful in this distinction: positivity for calcitonin favors a medullary thyroid carcinoma.

The distinction between melanoma and an undifferentiated (anaplastic) thyroid carcinoma can be difficult. Aspirates from melanoma are generally more cellular than those of undifferentiated carcinoma, with more intact isolated cells. Immunostains can be helpful: melanoma cells are positive for S-100 protein, HMB-45, SOX-10, and Melan-A; these markers are generally negative in undifferentiated carcinoma, which may retain immunoreactivity with PAX8.

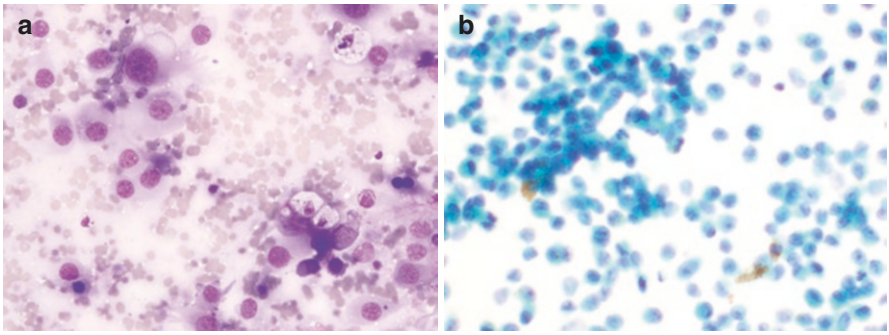

Fig. 12.3 (**a**) Metastatic melanoma. The malignant cells are isolated and loosely aggregated. They are large, oval and plasmacytoid cells with abundant granular cytoplasm, hyperchromatic nuclei, and prominent nucleoli. Foamy histiocytes are present (smear, Diff-Quik stain). (**b**) Malignant melanoma characterized by numerous non-cohesive plasmacytoid cells with some cytoplasmic melanin pigment present (smear, Papanicolaou stain)

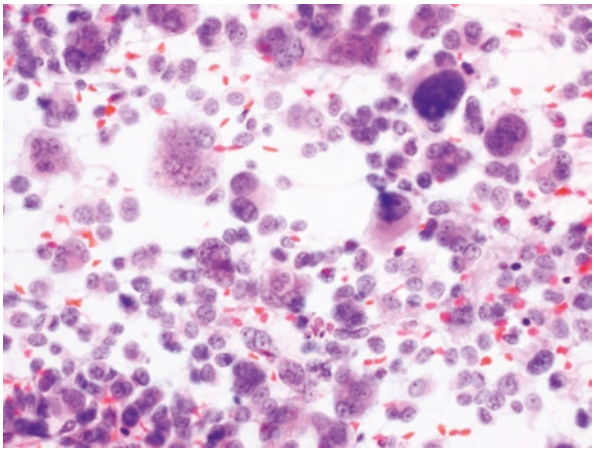

Fig. 12.4 Metastatic melanoma. Cellular aspirate composed of clearly malignant cells displaying marked anisonucleosis, multinucleation, and prominent nucleoli (smear, Diff-Quik stain)

Metastatic Breast Carcinomas

Criteria

Samples are of moderate to high cellularity with a uniform population of oval or polygonal cells.

Cells lie singly and in small clusters; the isolated cells retain their cytoplasm.

Cells are often immunoreactive for estrogen and progesterone receptors, GATA3, and mammaglobin and are negative for TTF-1, PAX8, and thyroglobulin.

Explanatory Notes

Adenocarcinoma of the breast is one of the most common tumors to metastasize to the thyroid [14], and the most common type is infiltrating ductal carcinoma. Smears are of moderate to high cellularity, with a uniform population of large polygonal or oval cells. The cells appear singly and in clusters. The single cells retain their cytoplasm, and the clusters are often angular.

With air-dried smears, purple cytoplasmic inclusions (magenta bodies) may be seen in metastatic breast cancer of both ductal and lobular types (Fig. 12.5). Many metastatic adenocarcinomas of the breast have cells similar in appearance to neoplastic follicular cells. The cells of infiltrating ductal adenocarcinoma are generally larger than those of follicular neoplasms but smaller than those of oncocytic neoplasms. The presence of microfollicles favors a thyroid neoplasm over a metastatic breast carcinoma.

Immunohistochemical stains for thyroid (e.g., thyroglobulin, TTF-1, calcitonin, and PAX8), breast (e.g., estrogen and progesterone receptors, mammaglobin, and GATA3), and parathyroid (parathormone and GATA3) markers can be helpful for

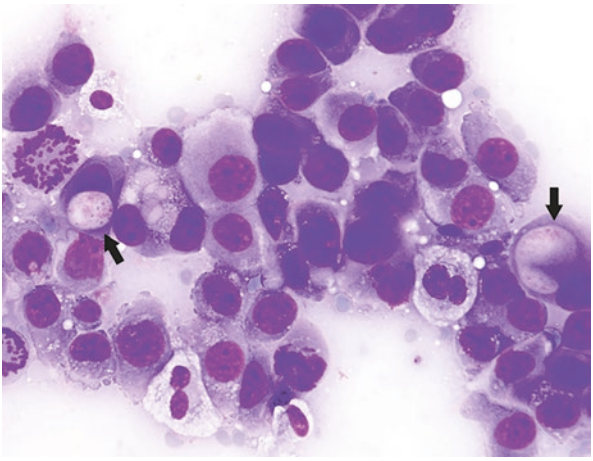

Fig. 12.5 Metastatic ductal carcinoma of the breast. Medium-sized cells have large eccentric nuclei and purple intracytoplasmic vacuolar granules (magenta bodies, *arrows*) that represent mucin vacuoles (smear, Diff-Quik stain)

the separation of metastatic mammary carcinoma from benign and malignant thyroid or parathyroid lesions.

Metastatic Pulmonary Carcinomas

Criteria

Non-small Cell Carcinomas

Isolated, dispersed cells and cell clusters.
- Large cells with variable amounts of cytoplasm, sometimes abundant.
- Nucleoli can be prominent.

Small Cell Carcinomas

Isolated, dispersed cells and cell clusters.
- Small cells with scant cytoplasm.
- Oval-to-elongated nuclei, nuclear molding.
- Finely granular chromatin.
- Inconspicuous nucleoli.
- Mitoses, necrosis.

Explanatory Notes

Metastatic small cell carcinoma (SmCC) of the lung may resemble a high-grade follicular derived carcinoma of the thyroid (poorly differentiated thyroid carcinoma) but has more fragile nuclei and cytoplasm and thus greater smearing artifact than primary thyroid neoplasms. Both metastatic pulmonary SmCC and poorly differentiated thyroid carcinoma are usually immunoreactive for TTF-1, and although the latter may express neuroendocrine markers like Synaptophysin, Chromogranin, or

INSM-1, poorly differentiated thyroid carcinomas are generally also positive for PAX8 and thyroglobulin.

Adenocarcinomas of pulmonary origin are composed of medium to large-sized cells in sheets or clusters/balls (Figs 12.6 and 12.7). The cells may be columnar, with round to oval, eccentrically positioned nuclei and prominent nucleoli. Both primary thyroid tumors and non-small cell lung adenocarcinomas may be TTF-1 positive, precluding this antigen as a distinguishing marker, though PAX8 positivity for thyroid tumors and Napsin-A positivity for lung adenocarcinomas can be helpful to distinguish these two entities. Metastatic lung adenocarcinomas are characteristically of higher nuclear grade than follicular neoplasms of the thyroid gland and are also more likely to contain intracytoplasmic mucin.

Squamous cell carcinomas typically demonstrate marked irregularities of nuclear shape and size. Keratinization, best seen with the Papanicolaou stain, is found in both well- and moderately differentiated metastatic squamous cell carcinomas.

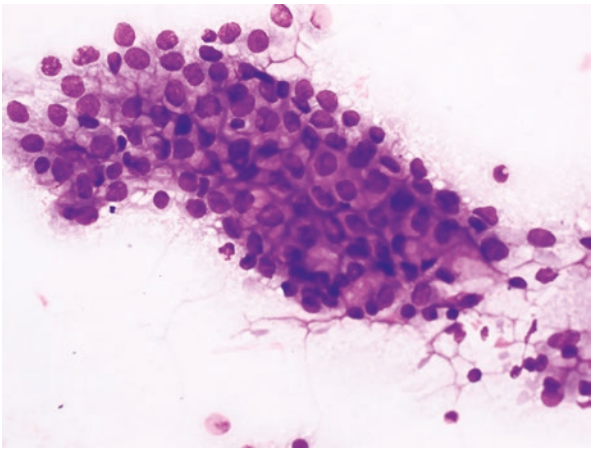

Fig. 12.6 Metastatic lung adenocarcinoma. Cohesive cluster of polygonal to columnar cells with mucinous differentiation (smear, Diff-Quik stain)

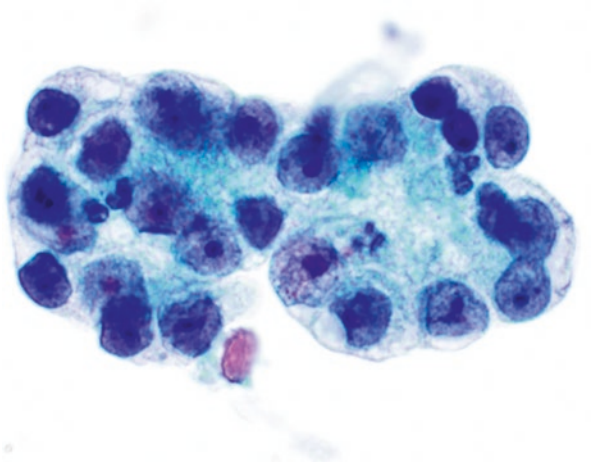

Fig. 12.7 Metastatic lung adenocarcinoma. Medium-sized cells have large nuclei, prominent nucleoli, and ample finely granular cytoplasm. The cells are arranged in spherical clusters (ThinPrep, Papanicolaou stain)

Immunohistochemistry for p40 can be helpful in identifying poorly differentiated squamous cell carcinomas. The distinction between a squamous cell carcinoma subtype of undifferentiated (anaplastic) thyroid carcinoma (see Chap. 11) and a metastasis from a squamous cell carcinoma of the lung, however, is not possible based on cytomorphology or immunoprofile. Clinical history and imaging findings are indispensable for separating these two neoplasms.

Other Metastatic Malignancies [14]

Examples of less common metastatic neoplasms to the thyroid gland are shown in Figs. 12.8, 12.9, and 12.10. Diagnosis by FNA is contingent upon the clinical history and radiographic findings, often with the support of immunohistochemistry.

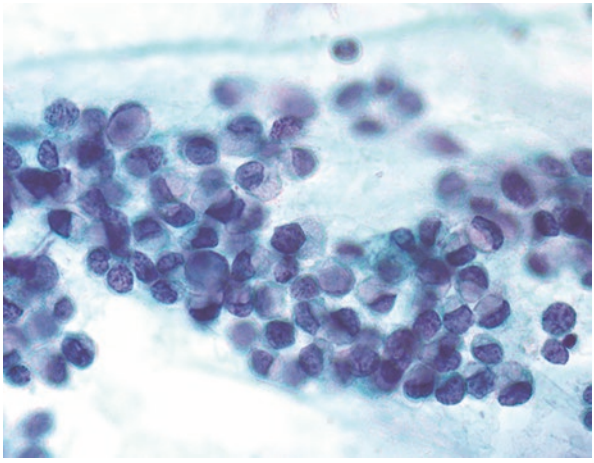

Fig. 12.8 Metastatic gastric signet-ring cell carcinoma. Uniform dispersed cells have a high N/C ratio and intracytoplasmic mucinous vacuoles (smear, Diff-Quik stain). (Photograph courtesy of Dr. QK Li, The Johns Hopkins Hospital, Baltimore, MD)

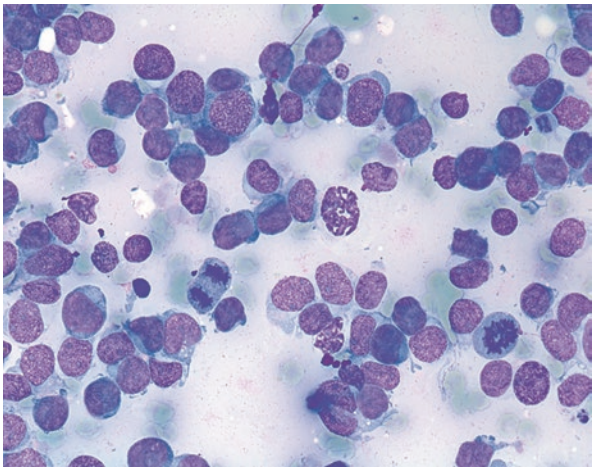

Fig. 12.9 Metastatic Merkel cell carcinoma. Dispersed small, round, blue cells have a high nuclear:cytoplasmic ratio and frequent mitotic figures, which can mimic metastatic small cell carcinoma of pulmonary origin (smear, Diff-Quik stain)

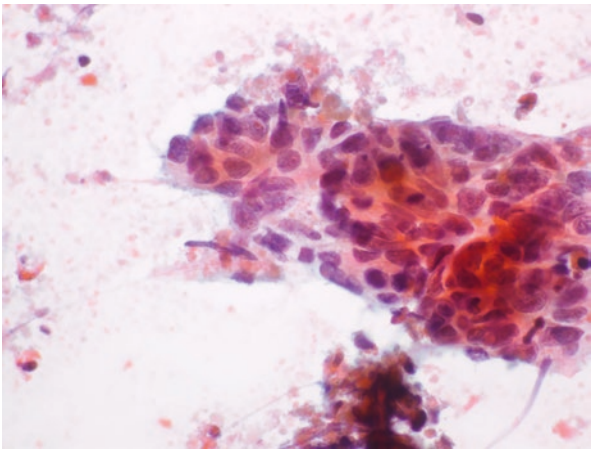

Fig. 12.10 Metastatic colorectal adenocarcinoma. Columnar cells with nuclear stratification are associated with granular necrotic debris in the background (smear, Papanicolaou stain)

Lymphoma Involving the Thyroid Gland

Criteria

Samples derived from lymphomas are often markedly cellular and composed of non-cohesive round to slightly oval cells.

The background contains numerous lymphoglandular bodies, best seen with a Romanowsky-type stain on air-dried preparations.

Cells of marginal zone lymphoma are about twice the size of a small mature lymphocyte.

Nuclei have vesicular ("open") chromatin (with Papanicolaou-stained preparations) and small nucleoli.

Diffuse large B-cell lymphomas contain cells with moderate to abundant basophilic cytoplasm on air-dried preparations stained with a Romanowsky-type stain.

Nuclei have coarse chromatin with one or more prominent nucleoli.

Explanatory Notes

The majority of primary lymphomas of the thyroid gland are non-Hodgkin lymphomas (NHL) of B-cell phenotype (98%) [15], and two-thirds are preceded by Hashimoto thyroiditis. Most NHLs of the thyroid gland are either diffuse large B-cell lymphomas or extranodal marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT). Distinction of thyroid lymphoma from Hashimoto thyroiditis may be difficult [16]. There are at least three different patterns of lymphoma on FNA [17]. One is characterized by a mixture of small

and large lymphocytes. This pattern can be seen in Hashimoto thyroiditis as well, but the absence of oncocytes, follicular epithelial cells, and plasma cells favors lymphoma. The second pattern is a monotonous population of large lymphocytes and is morphologically diagnostic of lymphoma. The third pattern is characterized by a monomorphous population of small lymphocytes, which may represent a low-grade lymphoma or chronic lymphocytic thyroiditis. Immunophenotyping studies are essential for the diagnosis of lymphoma in morphologically equivocal cases. A clonal relationship between Hashimoto thyroiditis and thyroid lymphoma may exist [18], and clonal B-cell populations by flow cytometry have been reported in patients with Hashimoto thyroiditis [19, 20]. Caution is advised, therefore, in interpreting flow cytometric results in isolation.

Secondary involvement of the thyroid gland by lymphoma is more frequent than primary disease. Approximately 20% of patients with disseminated lymphoma demonstrate thyroid involvement.

Extranodal Marginal Zone B-Cell Lymphoma (MALT Lymphoma)

FNA preparations from MALT lymphomas are markedly cellular and composed of lymphocytes in isolation and in clusters [21, 22]. Numerous lymphoglandular bodies are present on smear preparations. The cells are small to intermediate-sized, about twice as large as a small mature lymphocyte [21]. Most cells have a moderate amount of cytoplasm (Fig. 12.11). Small nucleoli are generally seen. A small number of larger cells with eccentrically placed nuclei, coarse chromatin, and prominent nucleoli may be present. These cells are admixed with lesser numbers of centrocytic cells, monocytoid B-cells, and plasma cells. In some cases, plasmacytoid cells dominate [23]. Often, a small number of thyroid follicular and oncocytic cells are admixed with the lymphocytes [21].

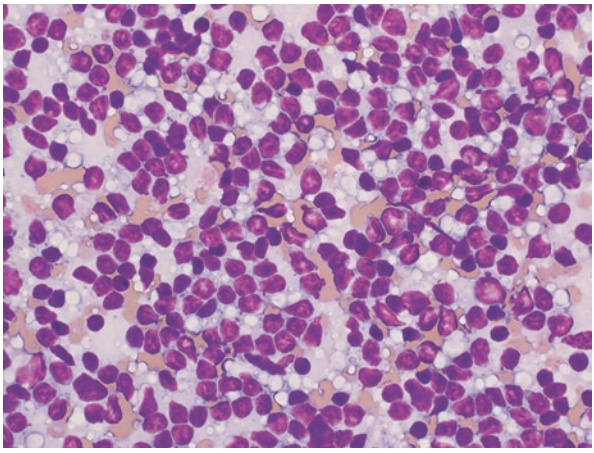

Fig. 12.11 Primary MALT-type lymphoma of the thyroid. There is an abundance of uniform intermediate-sized cells with small nucleoli and granular chromatin (smear, Diff-Quik stain)

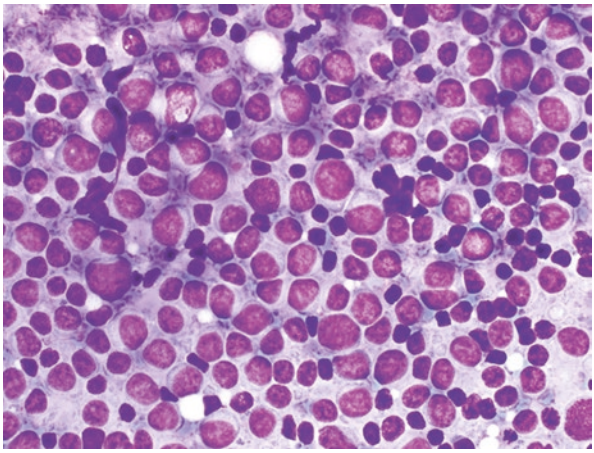

Fig. 12.12 Diffuse large B-cell lymphoma of the thyroid. The smear is cellular and composed mostly of large lymphoid cells whose nuclei are three to five times larger than those of the smaller lymphocytes (smear, Diff-Quik stain)

Diffuse Large B-Cell Lymphoma

Aspirates of diffuse large B-cell lymphoma are highly cellular, with many lymphoglandular bodies in the background of smear preparations. The cells are monomorphic non-cohesive large lymphocytes (Fig. 12.12) [21]. With air-dried smears and a Romanowsky-type stain, the cells have moderate to abundant basophilic cytoplasm. The nuclei possess coarse chromatin with one or more prominent nucleoli [21]. Nuclei striped of cytoplasm are numerous, and necrotic debris may be present. Flow cytometry may reveal light chain restriction of the CD45- and CD20-positive neoplastic cells [24]. Follicular epithelial cells are usually absent. Separation of these lymphomas from Hashimoto thyroiditis is generally straightforward [24].

Hodgkin Lymphoma

Hodgkin lymphoma of the thyroid is rare and can mimic a primary thyroid epithelial tumor or thyroiditis clinically and cytologically [25] (Fig. 12.13). The cellularity of FNAs varies from case to case. In some cases, Reed-Sternberg (RS) cells are present in a mixed background of small lymphocytes, plasma cells, eosinophils, histiocytes, fibroblasts, and capillaries, but the RS cells may not be recognized as such because primary Hodgkin lymphoma of the thyroid is such a rare disease. Instead, the RS cells may be considered atypical but of uncertain significance. In some cases, their large size raises the possibility of an undifferentiated (anaplastic) carcinoma, but clinical and imaging features can help distinguish these two very different neoplasms. In other cases, RS cells may be inconspicuous or absent, and such cases are often misinterpreted as thyroiditis; repeated FNAs are sometimes required for definitive diagnosis. Most cases prove to be of nodular sclerosis type; the marked fibrosis associated with this subtype may result in a paucicellular aspirate. Thus, FNA has relatively low accuracy for the diagnosis of thyroid Hodgkin lymphoma. Examination of incisional biopsy material is usually necessary to confirm the diagnosis, but when adequate material is obtained, FNA may be valuable in raising the possibility of Hodgkin lymphoma and thereby helping to guide subsequent clinical intervention.

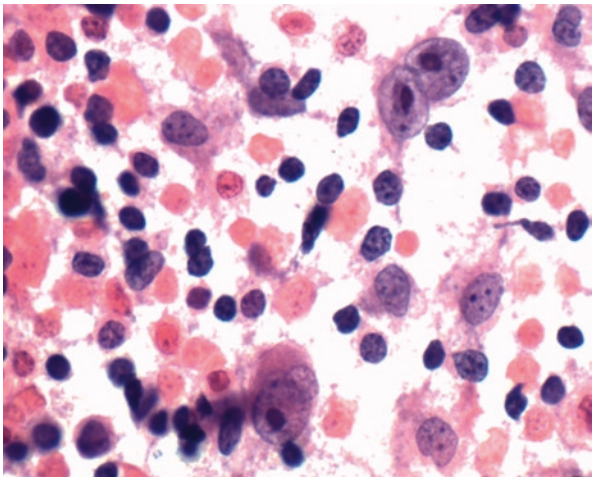

Fig. 12.13 Hodgkin lymphoma of the thyroid. The cells range widely in size and include scattered very large binucleated and multinucleated cells with features of Reed-Sternberg cells (smear, hematoxylin and eosin stain)

Rare Neoplasms of the Thyroid Gland

Paraganglioma

Definition

Paraganglioma of the thyroid is an intrathyroidal neuroendocrine tumor of paraganglionic origin.

Criteria

Moderately cellular smears.

Background rich in red blood cells.
Cells arranged in clusters, occasionally as microfollicles.
Lesser numbers of single cells and "naked" nuclei.
Intact cells have granular cytoplasm, often with wispy, poorly defined edges.
Cytoplasm may appear vacuolated.
Nuclei have stippled chromatin and may contain intranuclear pseudoinclusions.
Some cells have metachromatic cytoplasmic granules.

Explanatory Notes

Primary paragangliomas of the thyroid are very rare [26]. True examples likely arise from small paraganglia located beneath the thyroid capsule [27]. These neoplasms must be distinguished from paragangliomas extending into the thyroid gland from other sites as well as mimics like hyalinizing trabecular tumor of the thyroid and medullary thyroid carcinoma (MTC). Figures 12.14 and 12.15 demonstrate the characteristic cytomorphology of a primary paraganglioma. Negative immunocytochemistry for calcitonin to rule out MTC as well as CD5 to rule out neuroendocrine type intrathyroid thymic carcinoma solidifies the diagnosis of paraganglioma.

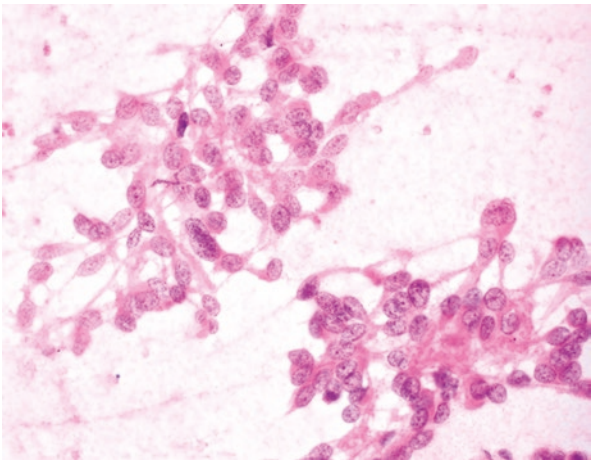

Fig. 12.14 Paraganglioma of the thyroid. FNA of an intrathyroidal paraganglioma is characterized by groups of bland spindle cells with scant wispy cytoplasm (smear, hematoxylin and eosin stain)

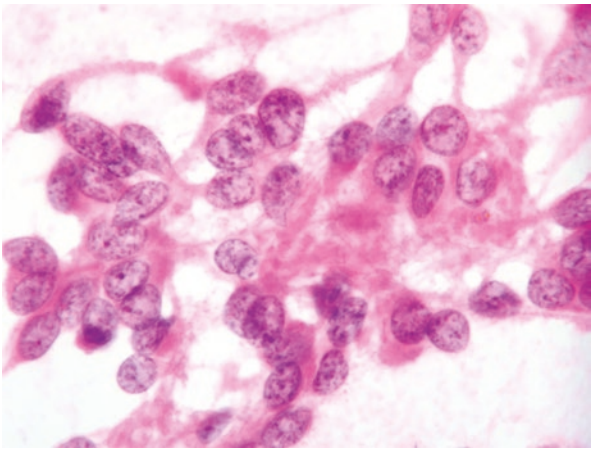

Fig. 12.15 Paraganglioma of the thyroid. Higher magnification of Fig. 12.14. Some aspirates from paraganglioma of the thyroid contain microfollicle-like structures composed of cells with pale wispy cytoplasm (smear, hematoxylin and eosin stain)

Langerhans Cell Histiocytosis

Definition

Langerhans cell histiocytosis is a proliferation of dendritic Langerhans cells with varying numbers of eosinophils.

Criteria

Moderate to marked cellularity.

Discrete, mostly isolated large mononucleated or multinucleated Langerhans cells with prominent nuclear membrane irregularity (deep nuclear clefting/grooves) and abundant pale vacuolated cytoplasm.

Eosinophils.

Scant or absent follicular cells and colloid.

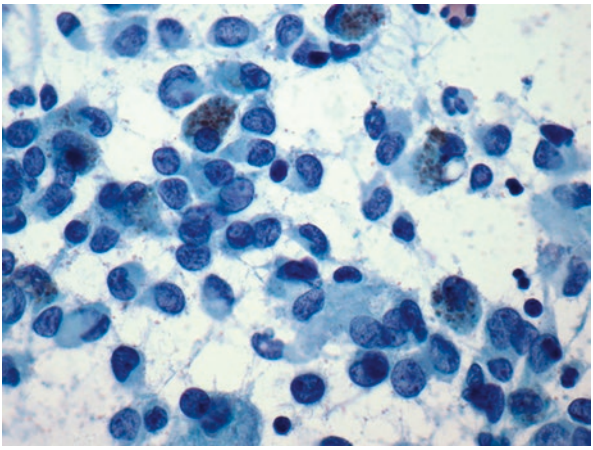

Fig. 12.16 Langerhans cell histiocytosis of the thyroid. The neoplastic cells have variably shaped nuclei, including some that are deeply folded, which mimic the nuclear grooves characteristic of papillary carcinoma. Hemosiderin-laden macrophages are also present. Eosinophils were prominent elsewhere on the smear (smear, Papanicolaou stain)

Explanatory Notes

Primary Langerhans cell histiocytosis (LCH) of the thyroid is rare; when encountered, its unusual features can cause diagnostic difficulties. The key to the diagnosis is recognizing the distinctive features of the neoplastic Langerhans cells, in particular, the strangely misshapen nuclei associated with abundant foamy cytoplasm [28]. Although there is a superficial resemblance to ordinary macrophages, the marked irregularity of LCH nuclei is not seen in benign macrophages (Fig. 12.16). If eosinophils are conspicuous, they are an additional clue. These tumors have been mistaken for papillary thyroid carcinoma, medullary thyroid carcinoma, poorly differentiated thyroid carcinoma, and a follicular neoplasm [28]. The diagnosis can be confirmed with immunohistochemistry: Langerhans cells are immunoreactive for CD1a and Langerin.

Mucoepidermoid Carcinoma

Definition

Mucoepidermoid carcinoma is malignant epithelial neoplasm with epidermoid and mucinous differentiation.

Criteria [29, 30]

Variable proportions of squamous cells (non-keratinized and keratinized) and mucous cells.

Keratin pearls.
Vacuolated mucus containing cells.
Extracellular mucin.
Eosinophils (some cases).

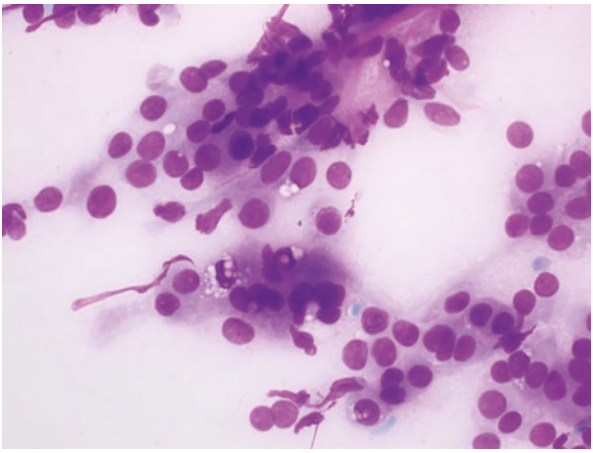

Fig. 12.17 Mucoepidermoid carcinoma of thyroid is characterized by sheets and clusters of cells, some of which are vacuolated while others have an epidermoid appearance (smear, Diff-Quik stain)

Explanatory Notes

Mucoepidermoid carcinoma (MEC) is most commonly a tumor of the salivary glands, but it also occurs at other sites. Primary MEC of the thyroid is rare, comprising about 0.5% of all thyroid malignancies. An association with a papillary thyroid carcinoma is seen in about half of cases [29]. Cytologic diagnosis is challenging and depends on identifying a mixture of so-called "intermediate-type" squamous cells (non-keratinized, cuboidal cells), keratinized cells, and mucous cells (Fig. 12.17). The differential diagnosis includes papillary thyroid carcinoma and squamous cell carcinoma subtype of undifferentiated (anaplastic) thyroid carcinoma. MECs of the thyroid are usually immunoreactive for thyroglobulin and TTF-1.

Sclerosing Mucoepidermoid Carcinoma with Eosinophilia (SMECE)

Definition

Sclerosing mucoepidermoid carcinoma with eosinophilia is a rare neoplasm of the thyroid resembling mucoepidermoid carcinoma of the salivary glands. It is molecularly distinct from follicular neoplasms of the thyroid and from salivary gland neoplasms. It appears to be more aggressive than other well-differentiated carcinomas of the thyroid.

Criteria [31, 32]

Hypercellular specimens.

Combination of isolated cells and solid squamoid nests.

Round to oval nuclei with prominent nucleoli.

Moderate amounts of dense cytoplasm.

Some cells show vacuolated cytoplasm and are associated with a glandular appearance.

Mixed lymphoid population.

Abundant eosinophils.

Background may have necrotic debris and mucin.

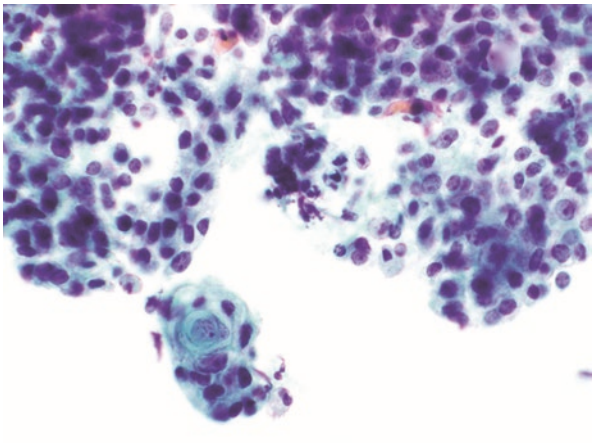

Fig. 12.18 Sclerosing mucoepidermoid carcinoma with eosinophilia. This neoplasm is comprised mainly of "intermediate cells": non-keratinizing immature, cuboidal squamous cells. A small squamous pearl is also present (ThinPrep, Papanicolaou stain)

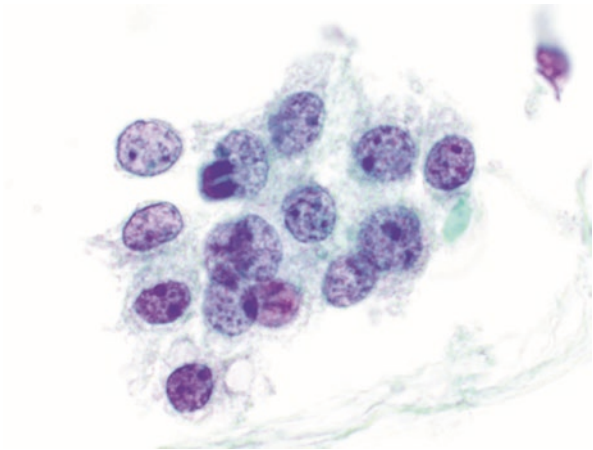

Fig. 12.19 Sclerosing mucoepidermoid carcinoma with eosinophilia. The intermediate cells have round nuclei, granular chromatin, and prominent nucleoli. Cytoplasm is thin and granular (ThinPrep, Papanicolaou stain)

Explanatory Notes

Sclerosing mucoepidermoid carcinoma with eosinophilia (Figs. 12.18 and 12.19) historically and cytologically resembles mucoepidermoid carcinoma. It is usually seen in patients with Hashimoto thyroiditis. SMECE is positive for TTF-1 but unlike MEC it is negative for thyroglobulin. It also lacks molecular features characteristic of follicular thyroid neoplasms [33].

Secretory Carcinoma of the Thyroid

Definition

Secretory carcinoma (previously termed mammary analog secretory carcinoma) is a malignant epithelial tumor that histologically and cytologically markedly resembles secretory carcinoma of the breast.

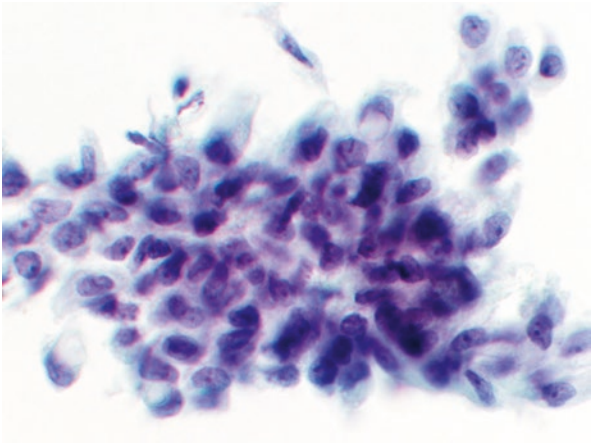

Fig. 12.20 Secretory carcinoma of the thyroid. The cells are arranged in groups, as seen here, and as isolated cells. Note that some cells have prominent large, solitary cytoplasmic vacuoles (ThinPrep, Papanicolaou stain)

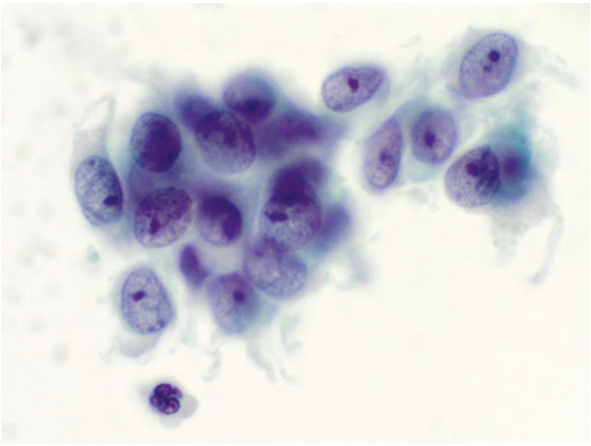

Fig. 12.21 Secretory carcinoma of the thyroid. The cells have prominent nucleoli (ThinPrep, Papanicolaou stain)

Criteria

Highly cellular specimen.

Cells arranged in sheets or branching pseudopapillae.
Round nuclei, prominent nucleoli, and grooves.
Granular and/or vacuolated cytoplasm.
Occasional solitary large cytoplasmic vacuole.

Explanatory Notes

Secretory carcinoma was first described as a tumor of the salivary glands in 2010. In 2016, Dogan et al. described three cases arising in the thyroid gland [30]. Cytologic preparations are highly cellular, and the cells are mostly cohesive, arranged in sheets and branching clusters lacking fibrovascular cores (Figs. 12.20 and 12.21). The

nuclei are round-to-oval, and nucleoli prominent; cytoplasm is vacuolated and/or granular. Occasional cells have a large solitary cytoplasmic vacuole. The cells are positive for mammaglobin, GCDFP-15, S-100 protein, p63, weakly positive for PAX8, and negative for TTF-1 and thyroglobulin. Like its salivary gland and breast counterparts, secretory carcinoma of the thyroid frequently harbors the *ETV6::NTRK3* fusion [34].

Ectopic Thymoma

Definition

A primary thymoma of the thyroid (ectopic thymoma) is a thymic epithelial tumor occurring in the thyroid.

Criteria [35–38]

The cytologic features depend on the type of thymoma present.

Type A Thymoma (Fig. 12.22)

Individual and tightly cohesive groups of spindle cells.
- Bland oval to spindle-shaped nuclei.
- Fine granular chromatin.
- Indistinct to absent nucleoli.
- Spindle cells often have scant or absent cytoplasm ("naked" nuclei).
- Small mature lymphocytes in background.

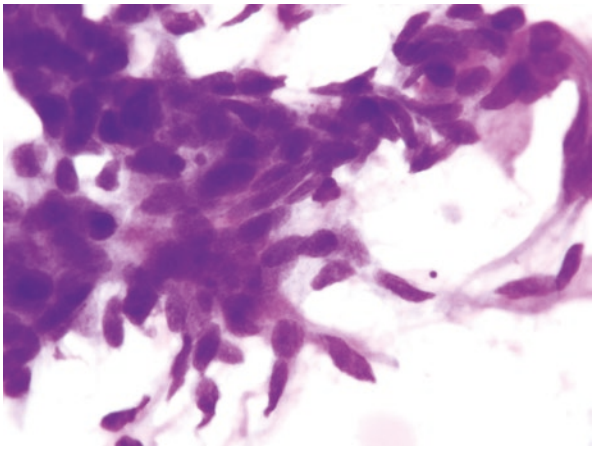

Fig. 12.22 Thymoma of the thyroid. Type A thymomas characteristically display variable proportions of lymphocytes and groups of spindle cells, often with scant cytoplasm and spindle-shaped nuclei with finely granular chromatin (smear, Diff-Quik stain)

Type B Thymoma (Fig. 12.23)

Variably cellular smears with a mixture of mature lymphocytes and clusters of polygonal epithelial cells.

Epithelial component characterized by bland round nuclei with fine granular chromatin.

Small or absent nucleoli.

Moderate to abundant amounts of cytoplasm in epithelial cells.

Explanatory Notes

Thymomas most commonly occur in the anterior mediastinum but may rarely involve the lower pole of the thyroid gland. Residual normal thymic tissue may accompany the thymoma within the thyroid.

Type B thymomas may resemble lymphoma or papillary thyroid carcinomas, whereas Type A thymomas can resemble a mesenchymal neoplasm or a carcinoid tumor [36]. Intrathyroid epithelial thymoma/carcinoma showing thymus-like differentiation (ITET/CASTLE) occurs in the thyroid (Figs. 12.24 and 12.25) [38]. This neoplasm is the malignant counterpart of thyroid thymoma and is a low-grade malignancy. The cytologic features of ITET/CASTLE have been described [36]. It should be separated whenever possible from squamous cell carcinoma (metastatic or primary), as these latter two neoplasms are high-grade malignancies.

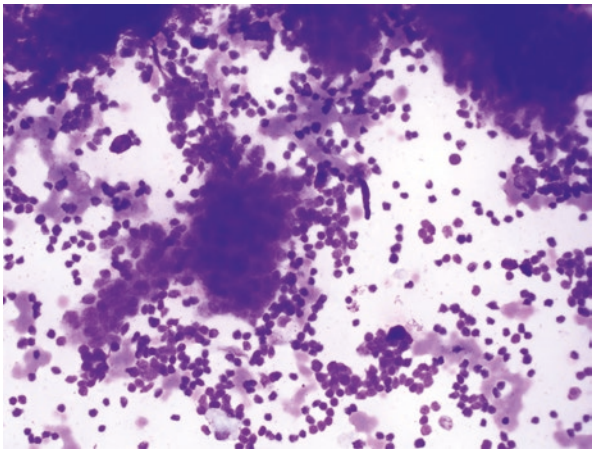

Fig. 12.23 Thymoma of the thyroid. Type B thymomas are characterized by prominent numbers of uniform small lymphocytes and groups of polygonal epithelial cells (smear, Diff-Quik stain)

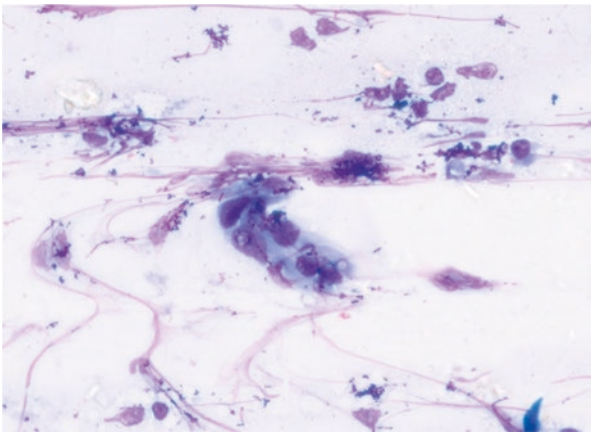

Fig. 12.24 Aspirate of CASTLE with small cluster of poorly differentiated neoplastic cells with scattered small lymphocytes in the background (smear, Diff-Quik stain). (Courtesy of Dr. Boris Rychly, Department of Pathology, Alfa Medical, Bratislava, Slovakia)

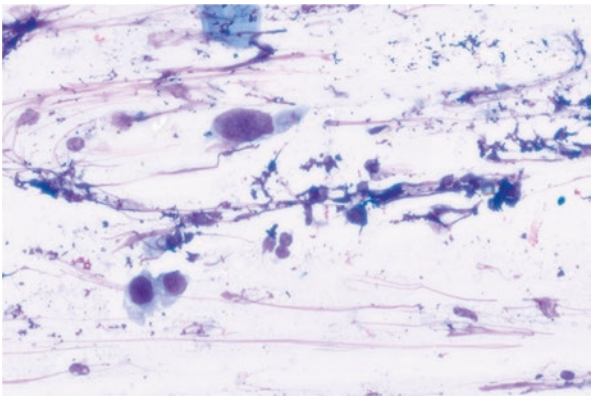

Fig. 12.25 Aspirate of CASTLE producing scant smears with individual cells having hyperchromatic nuclei and variable amounts of cytoplasm. Some tumor cells form tight clusters with marked nuclear crowding. Lymphoid cells are present in the background (smear, Diff-Quik stain). (Courtesy of Dr. Boris Rychly, Department of Pathology, Alfa Medical, Bratislava, Slovakia)

Spindle Epithelial Tumor with Thymus-Like Differentiation (SETTLE)

Definition

Spindle epithelial tumor with thymus-like differentiation (SETTLE), also called spindle cell tumor with thymus-like differentiation, is a rare malignant tumor of the thyroid characterized histologically by lobulated architecture and a biphasic cellular composition, with spindle-shaped epithelial cells that merge into glandular structures (Figs. 12.26 and 12.27) [39]. It probably develops from the branchial-pouch or a thymic remnant. Most reported cases have occurred in young male patients [39].

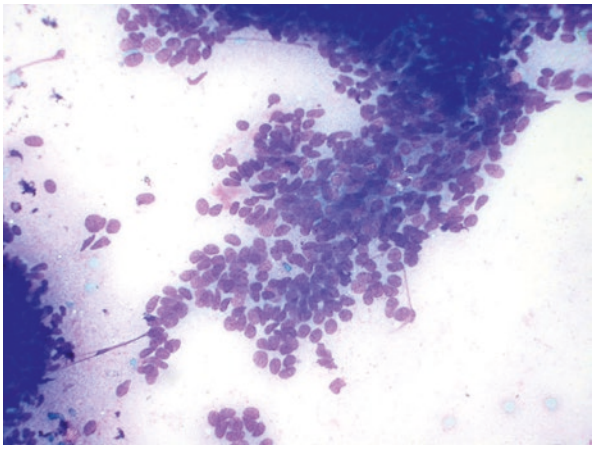

Fig. 12.26 Aspirate of a SETTLE showing oval to spindle cells forming groups and lying singly. Note magenta pink matrix (smear, Diff-Quik stain). (Courtesy of Professor Radhika Srinivasan, Department of Cytology and Gynecological Pathology Post Graduate Institute of Medical Education and Research, Chandigarh, India)

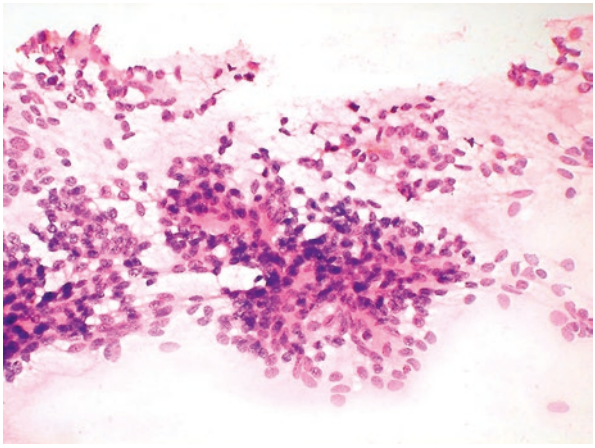

Fig. 12.27 Aspirate of a SETTLE showing numerous short spindle cells lying in a perivascular arrangement (smear, hematoxylin and eosin stain). (Courtesy of Professor Radhika Srinivasan, Department of Cytology and Gynecological Pathology Post Graduate Institute of Medical Education and Research, Chandigarh, India)

Criteria [39–41]

Highly or moderately cellular smears.

Dissociated uniform spindle cells with oval, bland nuclei.

Some groups and aggregates of spindle cells.

Occasional groups of epithelial cells.

Rare or absent mitotic figures.

Spindle cells are immunoreactive for cytokeratin and vimentin and non-reactive for thyroglobulin and calcitonin.

Explanatory Notes

SETTLE is difficult to specifically diagnose cytologically [39, 41]. It must be separated from medullary carcinoma of the thyroid and a variety of spindle cell neoplasms including primary synovial sarcoma of the thyroid and type A thymomas.

Other Rare Primary Neoplasms of the Thyroid Gland

A number of benign mesenchymal neoplasms occur in the thyroid gland, including lipomas, hemangiomas, schwannomas, and leiomyomas. Rarely, sarcomas arise in the thyroid, most frequently angiosarcomas, synovial sarcomas, osteosarcomas, and chondrosarcomas [42–46]. The cytomorphology of these primary thyroid sarcomas is identical to that of their more common counterparts in soft tissue.

Rarely, epithelial malignancies including mucoepidermoid carcinomas may arise primarily within the thyroid and have an appearance identical to those arising within the salivary glands [29].

Management

Metastases to the thyroid. Surgery is generally not indicated if the FNA is conclusive for a metastasis to the thyroid, and it may not be indicated if the results are suspicious for metastatic disease. Referral to an oncologist is appropriate.

Malignant lymphomas and Hodgkin lymphoma. Hodgkin lymphoma of the thyroid often requires surgical excision and chemotherapy, with or without radiation therapy [25]. For non-Hodgkin lymphoma of the thyroid, combined modality therapy (two or more of: surgery, radiation therapy, and chemotherapy) is the usual approach.

Rare primary tumors of the thyroid. Surgical excision (lobectomy or near total thyroidectomy) is generally indicated.

Sample Reports

The general category "Malignant" is used whenever the clinical and microscopic features are conclusive. The type of metastatic, lymphoid, or rare thyroid malignancy should be stated whenever possible. If features are suspicious but not conclusive for malignancy, the category "Suspicious for malignancy" is used. Some aspirates, particularly those that raise the possibility of a lymphoma of MALT-type but lacking corroborative immunophenotyping data, are more appropriately categorized as "Atypia of undetermined significance (AUS)" (see Chap. 4, Sample Report Example 7). If an aspirate is interpreted as Malignant, Suspicious for Malignancy, or AUS, it is implied that the sample is adequate for evaluation (an explicit statement of adequacy is optional).

Example 1
MALIGNANT.
Diffuse large B-cell lymphoma.
Note: Flow cytometry shows a CD45- and CD20-positive monoclonal B-cell population.

Example 2
SUSPICIOUS FOR MALIGNANCY.
Suspicious for metastatic adenocarcinoma of the breast.

Acknowledgment The authors would like to acknowledge the work in earlier editions of this chapter of Drs. Jerry Waisman and Kristen Atkins.

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Clinical Perspectives and Imaging Studies

13

Christopher VandenBussche, Syed Ali, Hossein Gharib, Hervé Monpeyssen, and Gilles Russ

Background: Thyroidology

Decades ago, endocrinologists primarily served as intermediaries between the attending physician and the surgeon or isotopist. Several developments have completely changed the situation:

- The segmentation of endocrinology into subspecialized areas, followed by further sub-segmentation by organ.
- The rise of ultrasound technology with increasingly powerful ultrasound scanners, the development of new tools such as Doppler and elastography, and the standardization of practice, all leading to the advent of the Thyroid Imaging Reporting & Data System (TI-RADS) score. Furthermore, the different versions of TI-RADS are in the process of being standardized.

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- The decline of systematic isotopic practices, but the persistent interest of scintigraphy to detect autonomous nodules and the development of ^{99}Tc-Mibi scintigraphy.
- Surgical de-escalation (common to all disciplines) with the abandonment of preventive total thyroidectomy, as well as the development of minimally invasive surgeries and the robotic procedures.
- The expansion of fine needle aspiration (FNA) to include ultrasound-guided techniques.
- The standardization of cytopathologic diagnosis and nomenclature with the release of TBSRTC.
- The emergence of and advancement of molecular diagnostics applied to thyroid FNA specimens.

This multi-modal approach, which has combined clinical evaluation, radiology, cytopathology, and molecular testing has caused endocrinologists to become thyroidologists; the approach to thyroid nodule has therefore changed considerably. The first step is *clinical*. Is the nodule palpable or bothersome? If so, it requires effective treatment. The second step is *biological*. The association of a nodule with a low TSH level should raise the suspicion of autonomous process and scintigraphy (ideally ^{123}iodine) can direct towards a functional nodule for which management is very specific. The third step is *ultrasound*. Is the nodule small or large? Is it a single nodule or part of a multinodular gland? Are there signs of tracheal compression? The TI-RADS score is assessed. The fourth step is *cytological*. TBSRTC category diagnosis is considered along with any other findings in the report.

All these findings are synthesized and discussed with the patient. Before a treatment plan is decided, additional diagnostics may be offered to the patient:

- Potentially a core-needle biopsy (if doable) in the case of a repeatedly nondiagnostic FNA specimen.
- A cervical cross-sectional CT scan in the case of a compressive nodule (e.g., intrathoracic goiter).
- A ^{123}Iodine scintigraphy if an autonomous nodule is suspected.
- In the event of an indeterminate nodule:
  - A re-assessment.
  - A ^{99}Tc-Mibi scan.
  - An additional FNA to allow sampling for molecular assays.

In the end, several possible solutions can be offered to the patient, along with an explanation of the advantages and constraints of each option:

- Active surveillance in the case of a benign nodule or microcarcinoma.
- Conventional management of thyroid cancer.

- Radioiodine therapy for autonomous or toxic nodules.
- For cystic nodules, one or two evacuations, possibly followed by one or more percutaneous ethanol injections.
- Surgery which may be a lobectomy or a total thyroidectomy. The choice can be given between conventional surgery or robotic surgery, and approach (e.g., transoral (TOETVA) or retroauricular). Discussion with a specialist surgery colleague is mandatory.
- Thermoablation using laser, HIFU, radiofrequency, or microwaves if the nodule is clearly benign.
- Multidisciplinary meetings for challenging or unusual cases.

Risk Stratification with Ultrasound

Ultrasound (US) risk stratification systems have two main aims. The first is to standardize the results of thyroid ultrasound reports, by using a quantitative cancer risk estimation approach. The second aim is to provide guidance regarding the indications for FNA. The value of US to evaluate a thyroid nodule has significantly improved, both in resolution but also in identifying specific features of malignancy. Major endocrine societies have introduced new ways to risk-stratify nodules based on US characteristics. The American Association of Clinical Endocrinologists (AACE) divides nodules into a simple, 3-risk category system: very low risk, 1%; intermediate risk, 5–15%; high risk, 50–75% [1]. On the other hand, the American Thyroid Association Sonographic Pattern System (ATASPS) recommends a 5-tier category: Benign, 1%; very low suspicion <3%; low suspicion 5–10%; intermediate suspicion 10–20%; high suspicion 70–90% [2]. These are useful when applied to individual patient with the sonographic finding of one or more nodules [3].

TI-RADS: Definition and Aims

The TI-RADSs (Thyroid Imaging Reporting and Data System) belong to the American College of Radiology (ACR): TI-RADS is a point-based system, which means that each US feature is attributed a certain number of points (Tables 13.1 and 13.2) [1]. For each nodule, the number of points is summed and the total gives the score which ranges from 1 to 5, with an increasing risk of malignancy. In contrast, the American Thyroid Association Sonographic Pattern System (ATASPS) is a pattern system, which consists of recognizing a grouping of US features in a single figure and then classifying the nodule in a category with a specific risk range of malignancy, from benign to high suspicion (Figs. 13.1, 13.2, 13.3, 13.4, 13.5, and 13.6) [2]. The European (EU)-TI-RADS and Korean (K)-TI-RADS also are pattern-based (Table 13.1) [3, 4].

Table 13.1 Comparison of ACR-TI-RADS and EU-TI-RADS systems

ACR-TI-RADS				EU-TI-RADS				
Category	Descriptor	Point value	FNA	Category	Descriptor	Risk	Radiologic findings	FNA
TR1	Benign	0 points*	N.I.	EU-TI-RADS 1	No nodule	N.A.	N.A.	N.A.
TR2	Not suspicious	2 points	N.I.	EU-TI-RADS 2	Benign	0%	Anechoic or entirely spongiform	N.I. unless compressive
TR3	Mildly suspicious	3 points	If >2.5 cm	EU-TI-RADS 3	Low risk	2–4%	Entirely isoechoic or hyperechoic	If >2.0 cm
TR4	Moderately suspicious	4–6 points	If >1.5 cm	EU-TI-RADS 4	Intermediate risk	6–17%	Mildly/partly hypoechoic	If >1.5 cm
TR5	Highly suspicious	7+ points	If >1.0 cm	EU-TI-RADS 5	High risk	26–87%	Non-oval shape, irregular margins, microcalcifications, or marked hypoechogenicity	If >1.0 cm FNA or active surveillance for <1.0 cm

Abbreviations: *N.I.* not indicated, *N.A.* not applicable, *cm* centimeter
*A total score of 1 point is not possible

Table 13.2 ACR-TI-RADS point assignments

Composition (choose 1)		Echogenicity (choose 1)		Shape (choose 1)		Margin (choose 1)		Echogenic foci (choose all that apply)	
Cystic/mostly cystic	0	Anechoic	0	Wider-than-tall	0	Smooth	0	None	0
Spongiform	0	Hyperechoic/isoechoic	1	Taller-than-wide	3	Ill-defined	0	Comet-tail artifacts	0
Mixed cystic/solid	1	Hypoechoic	2			Lobulated/irregular	2	Macrocalcifications	1
Solid/mostly solid	2	Very hypoechoic	3			Extra-thyroid extension	3	Peripheral ("rim") calcifications	2
								Punctate echogenic foci	3

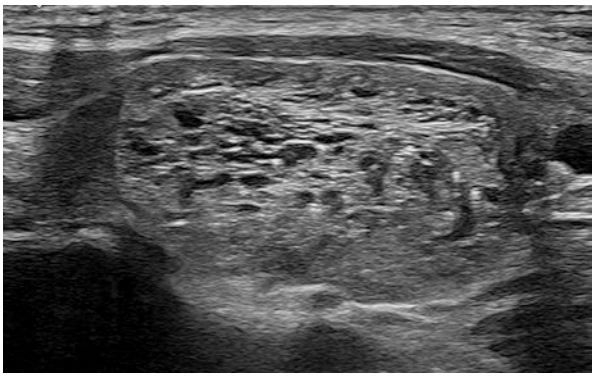

Fig. 13.1 Longitudinal plane. ACR-TI-RADS 2 nodule (2 points), ATASPS very low suspicion. Mixed cystic and solid, partially spongiform (tiny microcystic cavities representing less than 50% of the surface of the nodule), isoechoic, wider-than-tall, smooth margins, no hyperechoic foci

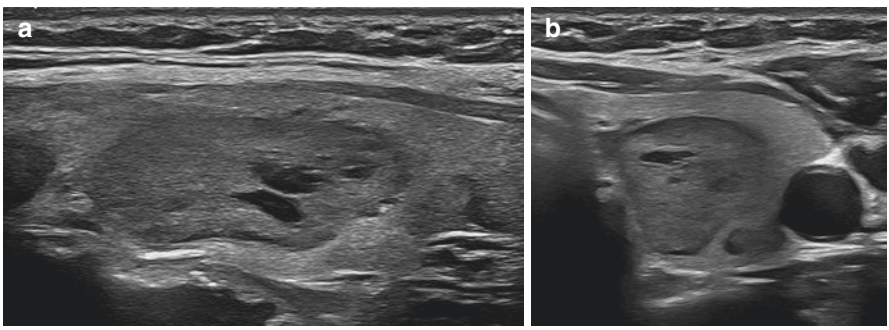

Fig. 13.2 (**a**) Longitudinal plane and (**b**) transverse plane. ACR-TI-RADS 3 nodule (3 points), ATASPS very low suspicion. Mixed cystic and solid, hypoechoic, wider-than tall, smooth margins, no hyperechoic foci

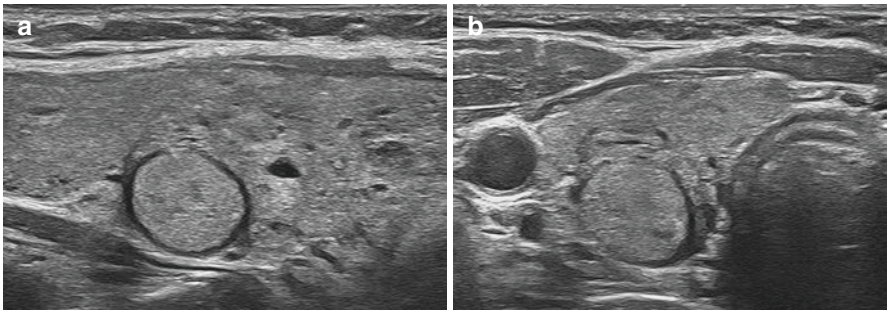

Fig. 13.3 (**a**) Longitudinal plane and (**b**) transverse plane. ACR-TI-RADS 3 nodule (3 points), ATASPS low suspicion. Solid, isoechoic to mildly hyperechoic, wider-than-tall, smooth margins, no hyperechoic foci

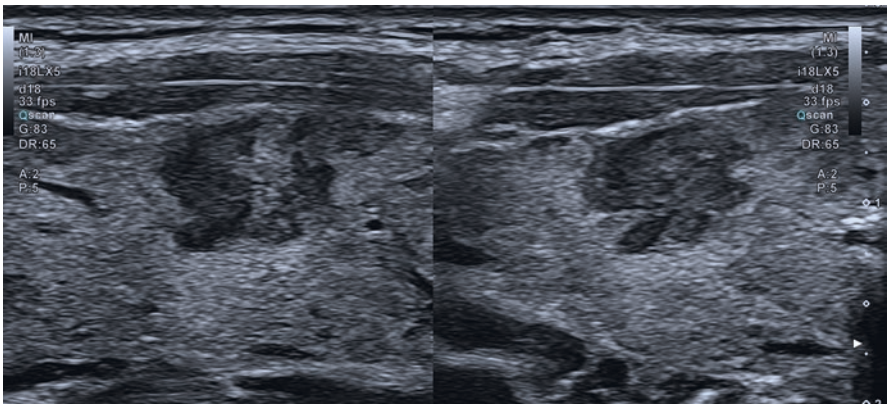

Fig. 13.4 Longitudinal and transverse planes, left and right, resp. ACR-TI-RADS 4 nodule (6 points), ATASPS high suspicion. Solid, hypoechoic, wider-than-tall, lobulated margins, no hyperechoic foci

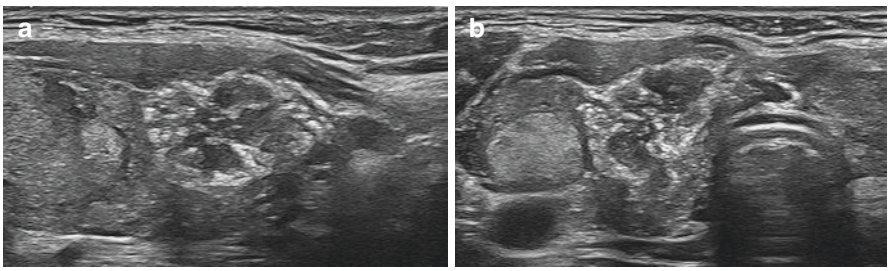

Fig. 13.5 (**a**) Longitudinal plane and (**b**) transverse plane. ACR-TI-RADS 5 nodule (7 points), ATASPS high suspicion. Solid, hypoechoic, wider-than-tall, smooth margins, peripheral and central punctate echogenic foci

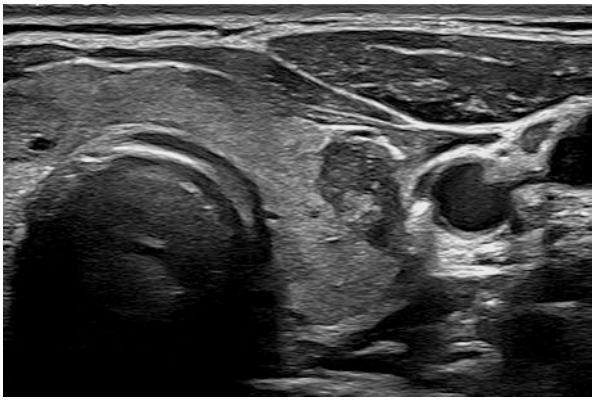

Fig. 13.6 Transverse plane. ACR-TI-RADS 5 nodule (12 points), ATASPS high suspicion. Solid, hypoechoic, taller-than-wide, lobulated and irregular margins, punctate echogenic foci

Indications for FNA

Thyroid nodule management should be based on a combination of clinical, US, and FNA information. Risk stratification is applied when selecting patients for follow-up or surgery. For example, a nondiagnostic sample with AACE or ATA class 1 US report should be followed without repeat FNA, due to negligible risk of malignancy.

The ACR-TI-RADS advises to use 10 mm, 15 mm, and 25 mm cut-offs for scores 5, 4, and 3, respectively. The ATASPS uses cut-offs of 10 mm for high- and intermediate-risk nodules, and 15 mm or 20 mm for low or very low-risk patterns, respectively.

Special circumstances of which the cytopathologist should be aware of for their interpretation, if sufficient data is provided by the sampler:

- Simple cysts. These are defined by US as having no significant solid part. Therefore the cytopathologist should not consider the relative lack of follicular cells as a nondiagnostic sample if a significant amount of colloid is present.
- Healing cyst. These are often markedly hypoechoic, with taller-than-wide shape, calcifications, and irregular margins. They can be classified as ACR-TI-RADS 5 or at high suspicion with the ATASPS. There again, if the cytopathologist is informed of this possibility, the relative lack of follicular cells is not necessarily nondiagnostic.
- Subacute thyroiditis. This is another cause of false positive scores with all RSSs, because of the hypoechogenicity and blurred margins, often mimicking a carcinoma.
- Hyperechoic foci. These can correspond to psammoma bodies, but much more often to microcystic cavities and therefore cause false positive high-risk scores.
- Large masses. These were previously inaccurately described by TI-RADS because they do not typically correspond to thyroid nodules. The latter are defined as lesions within the thyroid gland distinct from surrounding thyroid parenchyma whereas large masses are no longer distinct, because they occupy at

least an entire lobe. However, their US appearance is most often very suspect and they are usually categorized as high suspicion. Thus, a radiologic gamut comprising anaplastic carcinoma, lymphoma, metastasis, or even thyroidal invasion by another cervical cancer should systematically be evoked.

Challenges of the Cytopathologist in the Era of TI-RADS. What Is Expected from the Cytopathologist for each TI-RADS Score?

In the TI-RADS era, the cytopathologist's aims in interpreting thyroid samples are becoming more complex from the perspective of the treating physician. TI-RADS 3 nodules have a low probability of being cancer and most of the false negative cases correspond to follicular carcinomas or follicular variant of papillary carcinomas. Thus, the challenge for the cytopathologist is to detect these entities, while avoiding creating too many false positive cases. For TI-RADS 5 nodules, the aim is to avoid unnecessary surgical procedures. The high rate of indeterminate cytological results (25–50%) in TI-RADS 4 nodules also is a matter of concern.

Management of Nodules Post-FNA

For an ultrasound high-risk nodule with indeterminate cytology (AUS, FN, Suspicious for Malignancy), surgical treatment is preferred. For benign nodules, guidelines recommend an ultrasound follow-up 1–2 years after initial evaluation and thereafter at 3–5 year intervals [2, 5–7]. One recent study suggests that biopsy-proven benign nodules do not need US beyond 3 years since the risk of becoming malignant is exceedingly rare [8]. Repeat FNA should be considered when nodule volume increases 50% or more on follow-up exam. There is general consensus that thyroxine suppressive therapy is neither effective nor safe; its use in benign nodule has been largely abandoned.

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Molecular and Other Ancillary Tests

14

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Background

Insights into the genomic landscape of thyroid neoplasia have improved our ability to characterize thyroid tumors on FNA cytology samples [1]. These advances have led to several clinically relevant applications over the past decade, with gradual incorporation of FNA-based molecular testing into thyroid tumor management guidelines [2–5]. Chief among these applications has been the use of molecular diagnostics for the subset of thyroid nodules with indeterminate (AUS or FN) cytology. For these nodules, molecular testing complements cytomorphologic, clinical, and sonographic

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evaluation to fine-tune their risk of malignancy and to inform clinical decisions regarding active surveillance and diagnostic or therapeutic surgery. More recently, molecular testing of thyroid FNAs has also been leveraged to identify prognostic and predictive biomarkers for patients with an established diagnosis of thyroid cancer. This chapter will briefly summarize the key molecular changes in thyroid neoplasia, highlight the established and emerging uses of ancillary molecular testing for thyroid FNA specimens, and review the various lab-developed and commercially available molecular testing platforms that are currently used in clinical practice.

Overview of Molecular Changes in Thyroid Neoplasia

To date, ancillary molecular diagnostic tests for thyroid FNAs have largely focused on nucleic acid-based testing strategies. Genomic alterations in thyroid neoplasia include variants that occur at the DNA level as well as those that result in measurable changes in mRNA or microRNA expression profiles.

DNA-Level Alterations

Large-scale tumor genotyping studies have revealed recurrent single nucleotide variants, insertions and deletions, gene fusions, and copy number alterations in thyroid neoplasms [6–11]. Many of these variants result in overactivation of the MAPK (RAS-RAF-MEK-ERK) and/or PI3K/AKT/mTOR signaling pathways. Characteristic associations between driver alterations and thyroid tumor types are illustrated in Fig. 14.1 and summarized below.

- The detection of a *BRAF* V600E mutation and other driver alterations that confer *BRAF*-like gene expression profiles (e.g., *RET*, *BRAF* fusions) are highly specific for thyroid cancer and are typically associated with classical papillary thyroid carcinoma (cPTC) and tall-cell subtype of PTC [6].
- *ALK* and *NTRK* fusions are also highly specific for PTC, usually classical type with prominent follicular pattern or infiltrative follicular variant of PTC [12]. Rare cases of primary secretory carcinoma of the thyroid harboring *ETV6::NTRK3* fusion have also been described [12, 13].
- *RAS*-like alterations (e.g., mutations in *HRAS*, *KRAS*, *NRAS*, *BRAF* K601E, *EIF1AX*, *PTEN*, *DICER1*, and gene fusions involving *PPARG* or *THADA*) can be considered molecularly indeterminate for cancer, as such alterations are found in a broad spectrum of both benign and malignant follicular-patterned neoplasms, including follicular adenoma (FA), follicular thyroid carcinoma (FTC), noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), and invasive encapsulated follicular variant of papillary carcinoma (invasive EFV-PTC) [10, 14, 15].
- Mutations in *TP53*, *TERT* promoter, *AKT1*, and *PIK3CA* are generally considered late events in thyroid tumorigenesis that are associated with clinically

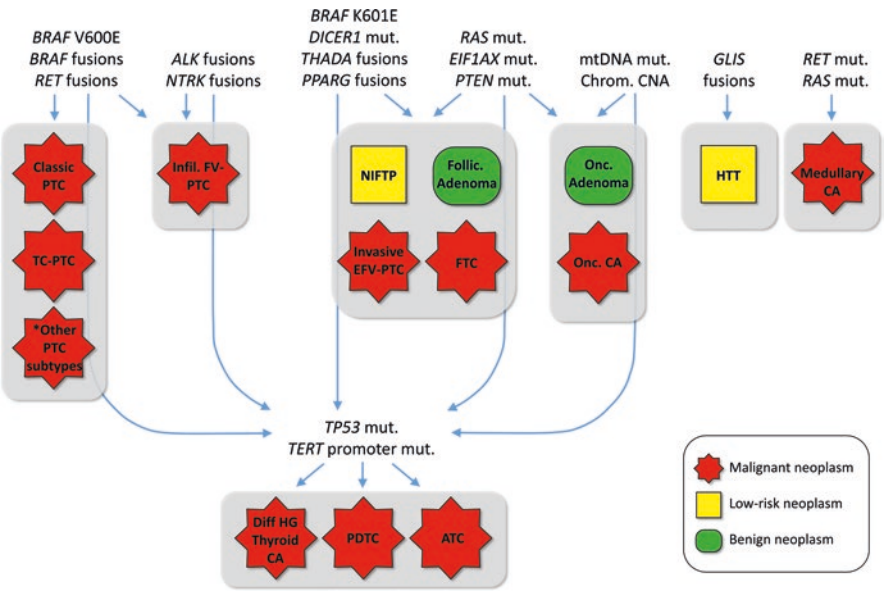

Fig. 14.1 Characteristic relationships between genomic alterations and thyroid tumor type. While *BRAF* V600E and other *BRAF*-like alterations are strongly associated with cancer, *RAS* mutations and other *RAS*-like alterations are associated with a range of benign, low-risk, and malignant neoplasms. Clinically aggressive thyroid cancers often harbor multiple mutations, including co-occurrence of driver mutations with *TP53* and/or *TERT* promoter mutations. *Other PTC subtypes with *BRAF*-like alterations include solid, diffuse sclerosing, columnar cell, hobnail, and Warthin-like subtypes. Abbreviations: *PTC* papillary thyroid carcinoma, *TC* tall-cell subtype, *Infil. FV-PTC* infiltrative follicular variant of PTC, *NIFTP* noninvasive follicular thyroid neoplasm with papillary-like nuclear features, *EFV-PTC* encapsulated follicular variant of PTC, *FTC* follicular thyroid carcinoma, *mtDNA* mitochondrial DNA, *Chrom.CNA* chromosomal copy number alterations, *Onc* oncocytic, *CA* carcinoma, *HTT* hyalinizing trabecular tumor, *Diff HG Thyroid CA* differentiated high-grade thyroid carcinoma, *PDTC* poorly differentiated thyroid carcinoma, *ATC* anaplastic thyroid carcinoma

aggressive cancers. Co-mutations of these genes with one of the driver alterations listed above are seen with increased frequency in differentiated thyroid cancers with distant metastasis as well as poorly differentiated thyroid carcinoma and undifferentiated (anaplastic) thyroid carcinoma [9, 16].

- Mitochondrial DNA mutations and recurrent chromosome-level copy number alterations are characteristic of oncocytic (formerly Hürthle cell) neoplasms [17, 18]. Additional oncogenic mutations (e.g., in *TERT* promoter, *TP53*, and *RAS* family of genes) superimposed on this background have been reported in oncocytic carcinomas [7, 8].
- Recent NGS studies of hyalinizing trabecular tumor (HTT) have identified *PAX8::GLIS3* and *PAX8::GLIS1* gene fusions, the detection of which may help distinguish HTT from papillary carcinoma on FNA cytology samples [19–21].
- Molecular alterations in medullary thyroid carcinoma include oncogenic *RET* mutations (germline or somatic) as well as somatic *RAS* mutations [22–24].

- Given these distinctive associations, genotyping-based tests for thyroid FNAs do not provide a binary "negative" or "positive" result. Instead, such tests offer a gradient of cancer probability (and information suggesting tumor type and prognosis as well) based on the type, number, and allelic frequency of the alterations that are identified.

Gene (mRNA) and microRNA Expression Alterations

A tumor's mRNA expression profile reflects which genes—and ultimately which proteins—are turned "on" or "off" to modulate cellular activity in response to various genetic, epigenetic, and environmental changes. High-throughput gene expression profiling studies have identified expression profiles that broadly distinguish benign and non-neoplastic lesions from cancer [25], as well as gene expression-based subgroups (e.g., *BRAF*-like and *RAS*-like) corresponding to particular genotypes and histologic subtypes of thyroid neoplasms [6, 10].

MicroRNAs are short (~22 nucleotide) noncoding RNAs that regulate gene expression at the post-transcriptional level. Certain microRNAs are divergently up- or down-regulated among different types of thyroid tumors [26–30]. Such differences in gene and/or microRNA expression profiles have been harnessed as a diagnostic tool for risk-stratifying cytologically indeterminate follicular cell-derived thyroid tumors on FNA samples [31]. To the extent that medullary thyroid carcinoma, parathyroid tissue, and metastases to the thyroid express genes and/or microRNAs that are distinct from follicular cell-derived tumors, expression profiling may also help identify these lesions on FNA material. The breadth of genes and microRNAs used in commercial expression profiling panels varies widely, from those that assay a small list of markers to those that interrogate thousands of genes using machine learning algorithms.

Current and Emerging Roles of Molecular Testing for Thyroid FNA Specimens

Refining Cancer Probability in Nodules Classified as AUS and FN

As discussed in Chap. 1, the usual management of thyroid nodules following FNA is informed by the implied risk of malignancy (ROM) associated with each Bethesda category. For nodules classified in the lower-risk indeterminate (AUS and FN) categories of TBSRTC, molecular testing can help refine ROM estimates and guide the decision between sonographic surveillance and diagnostic/therapeutic thyroidectomy (Fig. 14.2).

Various molecular testing formats have been applied to cytologically indeterminate thyroid FNAs, ranging from single-marker tests to extensive mutational and/or gene expression panels. While the currently available molecular tests offer different degrees of diagnostic stratification, test results can be generalized into one of three broad bins:

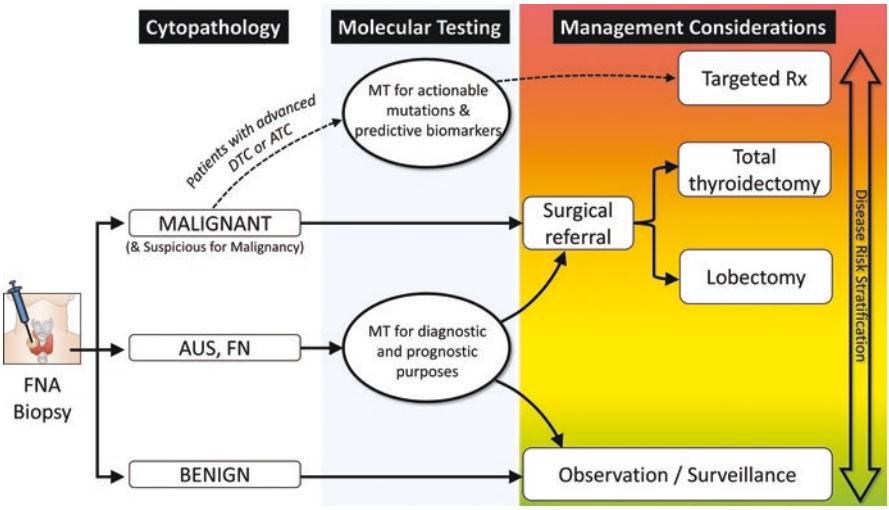

Fig. 14.2 Model summarizing the roles of FNA-based molecular testing (MT) for patients with thyroid tumors. The primary purpose of thyroid FNA-based MT over the past decade has been diagnostic/prognostic: to refine the cancer risk and guide management of lower-risk cytologically indeterminate (AUS and FN) nodules. For selected patients with advanced differentiated thyroid cancer (DTC) or anaplastic thyroid carcinoma (ATC), MT for actionable driver mutations and other predictive biomarkers can guide patients towards targeted therapies (Rx). (Model adapted from Nishino M and Krane JF. Updates in Thyroid Cytology. Surgical Pathology 2018; 11: 467–487 [32])

- *Low molecular probability of cancer* similar to the ~3% ROM associated with a cytologically benign nodule, for which clinical/sonographic surveillance would be appropriate. Tests capable of "ruling out" malignancy require high sensitivity and high (typically >95%) negative predictive value (NPV) for cancer.
- *Intermediate molecular probability of cancer*. These test results are often associated with neoplasia but lack the specificity to distinguish malignant neoplasms from benign ones. If surgery is pursued, lobectomy can be diagnostically helpful and therapeutically sufficient in most cases.
- *High molecular probability of cancer* similar to the 97–99% ROM associated with a cytologically malignant diagnosis, for which thyroidectomy is generally offered for therapeutic purposes. Tests capable of "ruling in" malignancy generally include markers strongly associated with classic papillary carcinoma (e.g., *BRAF* V600E mutation and *RET* fusions).

The use and interpretation of molecular tests for cytologically indeterminate thyroid nodules raise several important caveats.

- *The **population-based** ROM estimates provided by molecular test reports do not necessarily equal an **individual patient's** risk of thyroid cancer*. With respect to molecular testing, ROM estimates are generally derived from the positive and negative predictive values (PPV and NPV) observed in the clinical validation of

the test. PPV and NPV vary with the pre-test probability of disease, as described by Bayes' theorem. The approximate prevalence of cancer among the cytologically indeterminate categories of TBSRTC (i.e., 20–32% for AUS and 25–50% for FN) is often used as a practical stand-in for pre-test probability. However, a number of patient- and nodule-specific factors (e.g., age, sex, risk factors, family history, nodule size, sonographic features, and cytologic features) also influence pre-test probability. Accordingly, clinical and radiologic context should be considered together with cytopathology when (a) selecting nodules for molecular testing and (b) interpreting the results thereof.

- *The choice of molecular test for individual practices will depend in part on regional and global differences in management paradigms, as have been historically reported between Western and Asian countries* [33, 34]. For practices with a relatively low threshold to pursue diagnostic surgery for nodules with indeterminate cytology, large multigene test panels with high NPVs would be valuable to identify those nodules that can be spared unnecessary diagnostic surgery. In contrast, for practices where clinical guidelines favor active surveillance [35], a smaller panel of markers with high PPV for cancer (e.g., *BRAF* V600E single-gene test or 7-gene panel, discussed below) may suffice for selecting nodules that warrant resection.
- *In clinical validation studies of molecular tests, tumors ranging from indolent neoplasms (NIFTP) to high-grade carcinomas have been collectively classified as "Malignant" for the purposes of calculating binary test performance metrics such as sensitivity, specificity, NPV, PPV, and ultimately, "ROM."* While such groupings are convenient for statistical analysis, they obscure the prognostic spectrum that thyroid neoplasia spans (discussed below) [2, 36].

Prognostication of Tumors Based on Molecular Profiles

The use of molecular tests on thyroid FNA samples for preoperative risk stratification can be expanded beyond primary diagnosis to include tumor prognostication as well, with respect to structural disease recurrence, distant metastasis, and cancer-related mortality. The well-characterized associations between a tumor's molecular profile and its prognosis permit stratification of tumors into low, intermediate, and high molecular risk groups (MRG) [16]. Typically, the low MRG is represented by a single *RAS* mutation or *RAS*-like variant. The intermediate MRG includes the *BRAF* V600E mutation, other *BRAF*-like variants, or copy number alterations. The high MRG profile is characterized by the co-occurrence of one of the aforementioned driver alterations together with mutations in genes such as *TERT*, *TP53*, *AKT1*, and/or *PIK3CA*; this profile helps identify a subgroup of thyroid cancers with unfavorable outcomes. While routine molecular testing is not firmly established for thyroid FNA specimens that are suspicious or positive for malignancy, knowledge of a thyroid nodule's MRG profile in such cases, together with its clinical and radiologic features, may help select surgical options (e.g., lobectomy versus upfront total thyroidectomy) that are commensurate to tumor prognosis.

Identification of Systemic Therapy and/or Clinical Trials Tailored to a Tumor's Molecular Profile

For patients with advanced stage, locally recurrent, rapidly progressive, and/or metastatic disease who are not candidates for standard surgical and/or radioactive iodine (RAI) treatment, testing for actionable driver alterations may guide the selection of systemic therapy and/or clinical trials tailored to a tumor's particular molecular profile. For anaplastic carcinoma in particular, testing for targetable alterations (e.g., *BRAF*, *NTRK*, *ALK*, *RET*, tumor mutational burden, microsatellite instability, mismatch repair deficiency) may be useful in the neoadjuvant setting to convert an unresectable or borderline-resectable tumor into one that is amenable to surgery [3, 37]. The ability to detect these alterations on cytology and small biopsy samples obviates the need for more invasive surgical procedures in this context.

Targeted therapy strategies include (a) kinase inhibitors that selectively block constitutively activated receptor or cytoplasmic kinase signaling pathways [38–40], (b) redifferentiation therapy to enhance radioactive iodine uptake in RAI-refractory tumors [41, 42], and (c) immune checkpoint inhibition [43]. Table 14.1 lists examples of drugs targeting specific molecular alterations relevant to thyroid cancer.

Table 14.1 Summary of drugs targeting specific molecular alterations and possible application in clinical practice

Molecular alteration	Drugs	Clinical application
BRAF V600E mutation	Dabrafenib	Dabrafenib + trametinib (MEK inhibitor) *BRAF* V600E-mutated ATC, DTC, PDTC Redifferentiation of *BRAF* V600E-mutated PTC or PDTC
RAS mutation	Selumetinib, trametinib	Redifferentiation of RAS-mutated PTC or FTC or PDTC
RET mutation	Selpercatinib, pralsetinib	MTC
mTOR mutation	Everolimus	DTC, MTC, ATC
RET fusion	Selpercatinib, pralsetinib	RET fusion thyroid carcinoma Redifferentiation of RET-fused thyroid carcinoma
NTRK fusion	Larotrectinib	*NTRK* fusion thyroid carcinoma Redifferentiation of *NTRK*-fused thyroid carcinoma
	Repotrectinib	*NTRK* or *ALK* or *ROS* fusion thyroid carcinoma
	Entrectinib	*NTRK* or *ALK* or *ROS* fusion thyroid carcinoma
ALK fusion	Crizotinib	*ALK*-fusion thyroid carcinoma
	Repotrectinib	*NTRK* or *ALK* or *ROS* fusion thyroid carcinoma
	Entrectinib	*NTRK* or *ALK* or *ROS* fusion thyroid carcinoma
ROS1 fusion	Repotrectinib	*NTRK* or *ALK* or *ROS* fusion thyroid carcinoma
	Entrectinib	*NTRK* or *ALK* or *ROS* fusion thyroid carcinoma

Screening for Germline Alterations Associated with Hereditary Syndromes

Although thyroid FNA molecular testing is intended primarily for the detection of somatic alterations in tumor cells, these tests may identify germline mutations suggestive of hereditary cancer syndromes as well. Germline mutations associated with hereditary forms of thyroid cancer are summarized in Table 14.2 along with their

Table 14.2 Hereditary cancer syndromes associated with increased risk for thyroid cancer (*adapted from References* [44–48])

Syndrome	Germline mutation	Type of thyroid neoplasm	Incidence of thyroid lesions	Key extrathyroidal clinical features
MEN 2A and FMTC	*RET* (exons 10 and 11 most common)	MTC	90–100% (usually presents in adulthood)	MEN2A: pheochromocytoma, hyperparathyroidism, variants with cutaneous lichen amyloidosis and Hirschsprung disease FMTC: no association with pheochromocytoma or hyperparathyroidism
MEN 2B	*RET* (95% with exon 16 M918T mutation; <5% with exon 15 A883F mutation)	MTC	100% (usually presents in infancy/ childhood with early lymph node metastasis)	Pheochromocytoma, mucosal neuromas, GI ganglioneuromas, Marfanoid habitus, everted eyelids
Cowden syndrome	*PTEN*, *SDHB-D*, *KLLN* promoter methylation, *PIK3CA, AKT1, SEC23B*	PTC (classical and follicular subtypes), FTC	10%	Hamartomas and epithelial tumors of the breast, kidney, colon, endometrium and brain; mucocutaneous lesions; macrocephaly
FAP and Gardner syndrome	*APC*	Cribriform morular thyroid carcinoma	1–12% (usually women)	FAP: Multiple adenomatous polyps with malignant potential Gardner syndrome: FAP variant with extracolonic manifestations including supernumerary teeth, fibrous dysplasia of the skull, osteomas of the mandible, fibromas, desmoid tumors, epithelial cysts, hypertrophic retinal pigment epithelium, upper GI hamartomas, hepatoblastomas

Table 14.2 (continued)

Syndrome	Germline mutation	Type of thyroid neoplasm	Incidence of thyroid lesions	Key extrathyroidal clinical features
Carney complex	*PRKAR1A*	PTC, FTC, follicular adenoma	3%	Myxomas of soft tissues; skin and mucosal pigmentation (blue nevi); schwannomas, tumors of the adrenal and pituitary glands and testicle
Werner syndrome	*WRN*	FTC, PTC, ATC	18%	Premature aging; scleroderma-like skin changes; cataracts; premature graying and/or thinning of scalp hair; short stature
DICER1 syndrome	*DICER1*	Follicular nodular disease, FA, PTC, FTC, PDTC, particularly in pediatric patients	–	Pleuropulmonary blastoma; cystic nephroma; ovarian Sertoli-Leydig cell tumors

Abbreviations: *FAP* familial adenomatous polyposis, *FMTC* familial medullary thyroid carcinoma, *FTC* follicular thyroid carcinoma, *MEN* multiple endocrine neoplasia, *MTC* medullary thyroid carcinoma, *PTC* papillary thyroid carcinoma, *PDTC* poorly differentiated thyroid carcinoma, *ATC* anaplastic thyroid carcinoma

respective extrathyroidal manifestations. Awareness of the genotypes and clinical phenotypes of these syndromes can prompt genetic counseling, evaluation for germline testing, screening for associated malignancies, and consideration of screening or testing of relatives, as indicated by current guidelines.

Molecular Testing Platforms Available for Thyroid FNA Specimens

Molecular tests for thyroid FNA samples range from laboratory-developed ("in-house" or "home brew") tests to those performed in commercial reference laboratories. Laboratories performing clinical molecular tests should be certified and accredited by the appropriate national or international regulatory agencies [49]. The International Organization for Standardization (ISO) 15189 standard provides one benchmark for accreditation that is used in many countries [50]. Examples of regulatory compliance in the United States include Clinical Laboratory Improvement Amendments (CLIA) certification, College of American Pathologists (CAP) accreditation, as well as permits/licenses required by state departments of health.

All clinical laboratory tests should undergo analytical validation to establish accuracy and precision for detecting the analyte, reportable range, reference interval, analytic sensitivity, and analytic specificity. Analytical validation should be

performed for each specimen type (e.g., formalin-fixed paraffin-embedded cell-blocks, cells scraped from direct smears, fresh cells rinsed into nucleic acid preservative, etc.). In contrast, clinical validation defines the diagnostic performance characteristics of a test in a defined population (e.g., ability to distinguish benign thyroid tumors from malignant ones among nodules classified as AUS or FN). Ideally, clinical validation should be performed in prospective, blinded, multi-institutional studies to establish the diagnostic sensitivity, specificity, predictive values, and clinical utility of a test.

Tests for Oncogenic Mutations and Gene Fusions

Genotyping tests for thyroid FNA specimens have taken a variety of forms over the past decade, ranging from testing for a single variant (e.g., *BRAF* V600E mutation) to broader panels of oncogenic alterations. Traditional methods for evaluating a limited number of genomic alterations in thyroid FNA specimens include Sanger sequencing, real time PCR, allele-specific PCR, pyrosequencing, fluorescence melting curve analysis, fluorescence in situ hybridization for chromosomal rearrangements, and immunocytochemistry using mutation-specific antibodies (e.g., for the *BRAF* V600E mutation) [33, 51]. Traditional genotyping tests have been performed on various FNA sample types, including cells collected directly into nucleic acid preservative, cells lifted from direct smears, cellblocks, and residual material in liquid-based cytology samples post-slide preparation [52–56].

- *BRAF V600E mutation as single-gene test* can be incorporated into routine thyroid FNA practice [57, 58]. For patients with advanced or RAI-refractory thyroid cancer, testing for the *BRAF* V600E mutation can guide the selection of targeted therapy. The diagnostic utility of *BRAF* V600E testing alone for the risk stratification of cytologically indeterminate nodules is disputed and appears to vary geographically. In settings with an inclination towards active surveillance for cytologically indeterminate nodules and a relatively high prevalence of *BRAF* V600E among PTCs (as reported in some Asian practices), testing for this mutation alone may be cost-effective for ruling in cancer and directing patients towards thyroidectomy [33]. In Western practices, however, the relatively low sensitivity and NPV of *BRAF* V600E for thyroid cancer have limited its usefulness as a stand-alone marker for risk-stratifying nodules classified in the AUS, FN, and Suspicious for Malignancy categories [51, 59–61].
- A *7-gene test panel* comprising the most common driver mutations (involving *BRAF*, *HRAS*, *KRAS*, and *NRAS*) and gene fusions (*CCDC6::RET*, *NCOA4::RET*, and *PAX8::PPARG*) in thyroid neoplasia offers incremental improvements in refining the probability of cancer for a cytologically indeterminate thyroid nodule [53, 62]. Similar to single-gene testing for the *BRAF* V600E mutation, the use and limitations of the 7-gene panel for risk-stratifying cytologically indeterminate nodules may vary depending on the practice setting. For practices that prefer active surveillance for indeterminate nodules, detection of a *BRAF* V600E

mutation or *RET* fusion may steer management towards surgery, although these *BRAF*-like alterations are relatively infrequent compared to *RAS*-like alterations among AUS and FN aspirates [55]. In contrast, for practices that favor surgery for indeterminate nodules, the clinical impact of the 7-gene panel is less clear. Detection of one of the markers in the 7-gene panel would rule in neoplasia and only reinforce the recommendation for surgery (although the particular test result may influence extent of surgery). Moreover, a negative test result would be considered inadequate for steering nodules towards sonographic surveillance: among AUS and FN nodules in clinical validation studies, the 7-gene panel has exhibited a relatively low NPV (82–94%), corresponding to a residual cancer risk of 6–18% when the test is negative [53, 55, 63, 64].

- With the adoption of *next-generation sequencing (NGS) platforms*, massive parallel sequencing for a very large number of genomic alterations has become possible. Laboratory-developed NGS assays for cancer-related biomarkers are available for implementation in local molecular pathology laboratories, as are options to develop customized thyroid-specific NGS panels [65–67]. Different studies have demonstrated the analytical feasibility of NGS on various thyroid FNA specimen types, including the centrifuged supernatants usually discarded after the preparation of either cytospins or cell blocks [66, 68–70]. In the limited clinical validation studies reported to date for lab-developed NGS testing strategies for cytologically indeterminate thyroid FNAs, these tests showed variable NPV (81–100%) and PPV (29–81%) [67, 71, 72].

Combined Testing Platforms Offered by Reference Laboratories

In contrast to the traditional and NGS-based genotyping tests that can be performed locally in an institution's molecular pathology laboratory, several molecular diagnostic tests offered by centralized reference laboratories in the United States have emerged over the past decade: ThyroSeq® Genomic Classifier (University of Pittsburgh Medical Center and Sonic Healthcare USA, Inc.), Afirma® Genomic Sequencing Classifier & Xpression Atlas (Veracyte, Inc.), and ThyGeNEXT & ThyraMIR® (Interpace Diagnostics, Inc.). All three testing platforms combine NGS-based tumor genotyping panels with mRNA or microRNA expression profiling to varying degrees, although the core methodology and risk-stratification strategy differ among the tests (Fig. 14.3). Tables 14.3, 14.4, and 14.5 compare the methodology, pre-analytic considerations, biomarkers, and clinical validation studies for these tests.

- *ThyroSeq® Genomic Classifier (GC).* ThyroSeq GC uses high-throughput targeted DNA and RNA sequencing to test for an extensive panel of mutations and gene fusions associated with thyroid neoplasia. ThyroSeq also identifies chromosomal copy number alterations associated with oncocytic neoplasms. A limited gene expression panel via RNA sequencing is also used for confirming adequate sampling of thyroid follicular cells, identifying expression profiles

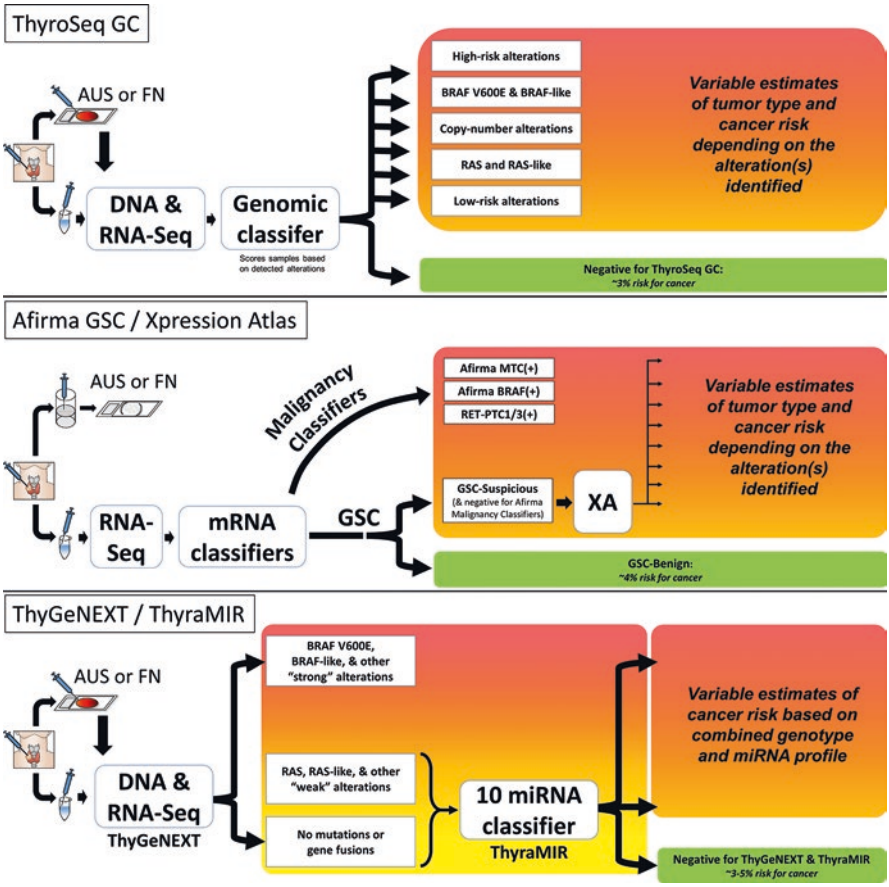

Fig. 14.3 Commercially available multigene panels and their use in risk-stratifying cytologically indeterminate (AUS or FN) thyroid nodules. Simplified schematic of ThyroSeq Genomic Classifier (GC), Afirma Gene Sequencing Classifier (GSC) and Xpression Atlas (XA), and ThyGeNEXT & ThyraMIR is shown. (Figure adapted from Nishino M and Nikiforova MN. Update on Molecular Testing for Cytologically Indeterminate Thyroid Nodules. Arch Pathol Lab Med. 2018;142(4):446–457 [73])

associated with *BRAF*-like or *RAS*-like alterations, and detecting lesions that are not derived from thyroid follicular cells, such as parathyroid, medullary thyroid carcinoma, and metastatic tumors. ThyroSeq GC is designated primarily for assigning thyroid nodules classified as AUS or FN into one of six molecular risk- and disease-stratified tiers based on the number, type, and allelic frequency of genomic and gene expression alterations that are detected. For tumors with molecular alterations, the test provides information about potential targeted therapies as well. In its clinical validation study, ThyroSeq demonstrated a NPV of 97% among AUS and FN nodules with a combined 28% prevalence of NIFTP and cancer [15]. In other words, such nodules that are negative for the ThyroSeq

Table 14.3 Comparison of methods, technology, and accepted starting materials for ThyroSeq, Afirma, and ThyGeNEXT/ThyraMIR

	ThyroSeq v3 Genomic Classifier (GC)	Afirma Genomic Sequencing Classifier (GSC) and Xpression Atlas (XA)	ThyGeNEXT and ThyraMIR
Core methodology	Tumor genotyping; detection of alterations in gene expression and chromosomal copy number	Gene expression profiling; tumor genotyping	Tumor genotyping; microRNA expression profiling
Primary technology used for the test	High-throughput DNA & RNA sequencing	High-throughput RNA sequencing	High-throughput DNA and RNA sequencing (ThyGeNEXT) RT-qPCR for microRNA profiling (ThyraMIR)
Accepted FNA sample types for nucleic acid extraction	Cellular material from FNA pass(es) collected directly into vendor's nucleic acid preservative -or- Direct smear slides (>200–300 follicular cells) -or- FFPE cellblock	Cellular material from FNA pass(es) collected directly into vendor's nucleic acid preservative	Cellular material from FNA pass(es) collected directly into vendor's nucleic acid preservative -or- Direct smear slides (>80 follicular cells) -or- FFPE cellblock

Abbreviations: *FFPE* formalin-fixed paraffin-embedded, *RT-qPCR* reverse transcription quantitative polymerase chain reaction

panel have an estimated NIFTP/cancer risk of approximately 3%, which is comparable to the NIFTP/cancer risk associated with cytologically benign nodules.

- *Afirma® Genomic Sequencing Classifier (GSC) and Xpression Atlas (XA)*. Afirma GSC uses high-throughput RNA sequencing to measure the expression levels of a broad panel of mRNA transcripts. The GSC includes biomarkers with high specificity for malignancy (e.g., gene expression profiles associated with medullary carcinoma and *BRAF* V600E-mutated papillary carcinoma, and RNA sequencing for *CCDC6::RET and NCOA4::RET* gene fusions), the detection of which is essentially diagnostic for malignancy. Expression profiles that confirm thyroid follicular cell sampling and flag sampling of non-thyroidal tissues (e.g., parathyroid tissue or metastatic tumors) are evaluated as quality control (QC) steps. RNA sequencing results that pass QC and are negative for the cancer-specific markers noted above undergo evaluation by the GSC's proprietary machine learning algorithms, which ultimately classify each sample as having either a "Benign" (low probability of malignancy) or "Suspicious" (intermediate probability of malignancy) transcriptional profile. Among AUS and FN nodules with a NIFTP/cancer prevalence of 24%, the Afirma GSC had a NPV of 96%,

Table 14.4 Comparison of biomarkers that are analyzed by ThyroSeq, Afirma, and ThyGeNEXT/ThyraMIR tests

	ThyroSeq v3 Genomic Classifier (GC)	Afirma Genomic Sequencing Classifier (GSC) and Xpression Atlas (XA)	ThyGeNEXT and ThyraMIR
Oncogenic mutations and gene fusions	112 genes (>12,000 variants and >150 gene fusions)	GSC: 1 mutation (*BRAF* V600E) and 2 fusions (RET-PTC1/3) XA: 593 genes (905 variants and 235 fusions)	13 genes (42 variants and 37 fusions)
Gene expression analysis	19 genes	10,196 genes (1115 genes for the GSC algorithm)	4 genes (housekeeping genes for QC)
microRNA expression analysis	N/A	N/A	10 microRNAs
Chromosomal copy number alterations	10 chromosomal regions	Loss-of-heterozygosity analysis	N/A
Prognostic markers	*TERT* promoter, *TP53*	*TP53*	*TERT* promoter
Markers of thyroid follicular cell sampling	mRNA of follicular cell-related genes	mRNA of follicular cell-related genes	mRNA of follicular cell-related genes
Markers of parathyroid sampling	mRNA of parathyroid-related genes	mRNA of parathyroid-related genes	N/A
Markers of medullary carcinoma	*CALCA*	*CALCA*, *CEACAM5*, *SCG3*, *SCN9A*, *SYT4*	miR-375, *RET* mutations

Table 14.5 Comparison of clinical validation studies for ThyroSeq, Afirma, and ThyGeNEXT/ThyraMIR

	ThyroSeq v3 Genomic Classifier (GC) [15]	Afirma Genomic Sequencing Classifier (GSC) [74]	ThyGeNEXT and ThyraMIR [75]
Sample source for clinical validation	Prospective, multi-institutional cohort of FNA material collected into nucleic acid preservative	Prospective, multi-institutional cohort (archival RNA samples remaining from 2012 validation study of the Afirma GEC)	Retrospective, multi-institutional cohort of archival cytology slides
# of AUS/FN cases	247	190	178
Prevalence of cancer	28%	24%	30%
Benign call rate	61%	54%	46%
Sensitivity	94%	91%	93%
Specificity	82%[a]	68%	62%[a]
NPV	97%	96%	95%
PPV	66%[a]	47%	52%[a]

[a]All test results with intermediate to high molecular probability of cancer were considered "positive" for purposes of comparing test performance

corresponding to a NIFTP/cancer probability of approximately 4% for nodules classified as "Benign" by the GSC [74].
  - While gene expression profiling remains the core methodology of the Afirma GSC, the RNA sequencing platform also permits evaluation for point mutations, insertions/deletions, and fusions involving the transcribed portion of the genome. The Afirma XA reports the detection of sequence variants with known associations with thyroid neoplasia [76–78]. Because RNA sequencing is confined the transcribed portion of the genome, *TERT* promoter mutations and other alterations in noncoding DNA are not identified by the Xpression Atlas. This test is intended for AUS and FN nodules with "Suspicious" Afirma GSC results, as well as for cytologically malignant (or Suspicious for Malignancy) aspirates for which tumor genotyping is desired for prognostic purposes and/or targeted therapy options.
- *ThyGeNEXT and ThyraMIR®*. ThyGeNEXT is a relatively focused genotyping panel that uses high-throughput DNA and RNA sequencing to identify thyroid neoplasia-related hotspot mutations in 10 genes (*ALK*, *BRAF*, *GNAS*, *HRAS*, *KRAS*, *NRAS*, *PIK3CA*, *PTEN*, *RET*, *TERT*) and 37 types of gene fusions involving 6 genes (*ALK*, *BRAF*, *NTRK*, *PPARG*, *RET*, *THADA*). mRNA expression levels of *PAX8* and *NKX2-1* (TTF-1) genes are included among a small gene expression panel to help confirm thyroid follicular cell sampling. The detection of variants with high specificity for malignancy (e.g., *BRAF* V600E mutation, *TERT* promoter mutations, *BRAF* fusions, *RET* fusions, *ALK* mutations and fusions) is reported as positive for a "strong" driver mutation and requires no further testing. Samples that are either (1) positive for a "weak" driver alteration (typically *RAS* mutations and other *RAS*-like variants) or (2) negative for any of the alterations in the ThyGeNEXT panel are considered molecularly indeterminate for malignancy and undergo additional testing with ThyraMIR, a quantitative RT-PCR-based microRNA expression classifier. ThyraMIR determines the expression profile of 10 microRNAs that are known to be up- or down-regulated in thyroid neoplasia and classifies samples into three tiers (negative, moderate, or positive) based on their projected probability of cancer. For AUS and FN nodules with a pooled 30% prevalence of NIFTP or cancer, the combined ThyGeNEXT and ThyraMIR tests had a 95% NPV (i.e., 5% risk of NIFTP/cancer for samples that are negative for both ThyGeNEXT and ThyraMIR) [75]. For the remaining permutations of ThyGeNEXT and ThyraMIR results, the test estimates NIFTP/cancer risk based on the particular driver alteration and microRNA profile that are identified.

While each of these tests use different methods to refine the preoperative cancer risk stratification of thyroid nodules, several common themes are emerging as these commercially available multigene tests have evolved over the past decade:

- *Combined testing approaches* that use aspects of multiplexed genotyping panels and gene or microRNA expression profiling.

- *High negative predictive value* for identifying nodules with molecular profiles associated with a very low probability of cancer, for which clinical/sonographic surveillance would be appropriate.
- Positive test results that cover *a range of cancer probabilities and tumor phenotypes*, including identification of biomarkers associated with increased risk of aggressive clinical behavior (e.g., metastasis and extrathyroidal extension).
- Inclusion of *actionable oncogenic driver alterations* in the genotyping panel.

A single-institution randomized clinical trial showed no significant differences in the diagnostic performance of ThyroSeq GC and Afirma GSC among nodules classified as AUS or FN [79]. On balance, each of these commercially available tests appears to provide similar information to the patient and treating physician for guiding clinical management decisions.

Notably, these commercially available tests are currently centralized in the United States and generally have high prices that may limit their accessibility to patients in countries with national health systems that do not cover the cost of the test [80]. Furthermore, most of the literature that has been published to date on these three commercially available tests have come from North American adult patient populations. Given the relatively high ROM for AUS reported in Asian compared to Western series [34], the NPV and PPV for these molecular tests may need to be adjusted accordingly when used in populations that differ from those represented in clinical validation studies.

Conclusions and Future Directions

Molecular testing offers an opportunity to refine the probability of malignancy for cytologically indeterminate nodules and may offer additional insights into tumor type, prognosis, and expression of predictive biomarkers on FNA cytology samples. Implementation of molecular testing in routine thyroid FNA practice and the selection of a particular testing platform will vary across practice settings. Test cost and accessibility are key considerations, as are regional and global differences in clinical practice, tolerance of risk and uncertainty, and thresholds for shifting from active surveillance to surgery [34, 35, 81]. If thyroid FNA molecular testing is to be used for clinical purposes, results must be integrated with each nodule's sonographic characteristics, cytologic features, patient characteristics, and patient's treatment preferences.

Looking ahead, the growing international adoption of TBSRTC will provide further opportunities to compare the safety profiles and the cost-effectiveness of different approaches to using molecular testing in the preoperative evaluation of thyroid nodules. Additional future directions include the inclusion of molecular data for estimating ROM for the indeterminate TBSRTC categories [82, 83], as well as the integration of molecular testing results as quality assurance metrics in cytopathology laboratory management [84, 85].

Sample Reports

The integration of molecular testing results into cytopathology reports is not standardized, given the differences in testing practices and assay platforms between cytopathology laboratories [86]. In general, cytopathologic diagnosis using TBSRTC categories should be made independently of molecular results. Molecular test results—whether issued as a part of the original cytopathology report, reported as an addendum (as shown in the examples below), or provided in a separate molecular pathology report—should be accompanied by an explanation of their clinical significance vis-à-vis probability of malignancy, tumor phenotype, prognosis, and/or therapeutic implications, as applicable.

Example 1 Positive for Low-Risk Mutation
FOLLICULAR NEOPLASM.
Cellular aspirate with follicular cells in microfollicular groups. Colloid is scant.
ADDENDUM: Molecular Test Result: *NRAS p.Q61R.*
Note: This mutation is associated with a 70–80% probability of cancer with low recurrence risk (usually follicular carcinoma or encapsulated follicular variant of papillary carcinoma) or pre-malignant neoplasm (NIFTP). Follicular adenomas typically comprise the remainder of tumors with this molecular profile. Surgical referral should be considered.

Example 2 Positive for Intermediate-Risk Mutation
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-Nuclear.
Scattered histiocytoid cells with nuclear atypia, present in a background of proteinaceous material and macrophages.
ADDENDUM: Molecular Test Result: *BRAF p.V600E.*
Note: *BRAF* p.V600E mutation is associated with a > 95% probability of papillary carcinoma. This mutation is associated with an intermediate risk of cancer recurrence. Surgical referral is advised, with consideration of oncologic thyroidectomy in the appropriate clinical and radiologic context.

Example 3 Positive for High-Risk Mutations
MALIGNANT.
Papillary thyroid carcinoma.
ADDENDUM: Molecular Test Result: *BRAF p.V600E* and *TERT C228T.*
Note: The presence of both *BRAF* and *TERT* mutations is associated with a > 95% probability of malignancy. This molecular profile is seen in more aggressive tumors with a high risk for disease recurrence. Surgical referral is advised, with consideration of oncologic thyroidectomy in the appropriate clinical and radiologic context.

Example 4 Negative for Oncogenic Alterations
ATYPIA OF UNDETERMINED SIGNIFICANCE.
AUS-other.
Hypocellular aspirate with follicular cells in microfollicular groups.
ADDENDUM: Molecular Test Result: *Negative for oncogenic alterations*.
Note: Based on clinical validation studies, the risk of malignancy is associated with an approximately [*]% risk of cancer. Nodules with a <5% risk of cancer are generally suitable for observation or surveillance in the appropriate clinical and radiologic context.
**Risk of cancer can be estimated by calculating 1 minus the NPV, as determined by the clinical validation of the test. Laboratories should confirm whether the prevalence of cancer among AUS nodules in a particular practice is within range of those analyzed in the clinical validation study.*

Example 5 Advanced Thyroid Cancer with Targetable Alteration
MALIGNANT.
Undifferentiated (anaplastic) thyroid carcinoma.
Note: By immunocytochemistry, tumor cells are positive for PAX8 and negative for thyroglobulin and TTF-1.
ADDENDUM: Molecular Result: *BRAF p.V600E.*
Patients with *BRAF p.V600E* mutated anaplastic carcinoma are eligible for the combination therapy with *BRAF* and MEK inhibitors.

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Correction to: Atypia of Undetermined Significance

Jeffrey Krane, Lan Chen, Ronald Ghossein,
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In the original version of Chapter 4, the first name for Dr. Song had been missed out.

Therefore, the author's name has been updated to Dong Eun Song in the revised version of the chapter.

The updated version of this chapter can be found at https://doi.org/10.1007/978-3-031-28046-7_4

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