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.

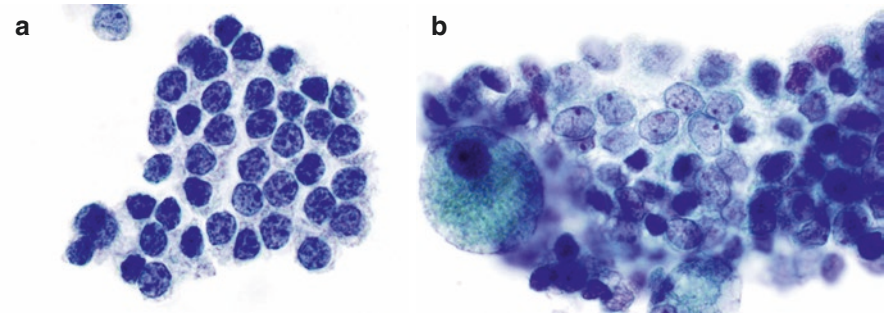

Fig. 4.1 Atypia of Undetermined Significance with nuclear atypia. (**a**) Most of the follicular cells are arranged in benign-appearing macrofollicle fragments. (**b**) Rare cells have pale nuclei and mildly irregular nuclear membranes. When such cells are very few in number, an atypical interpretation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

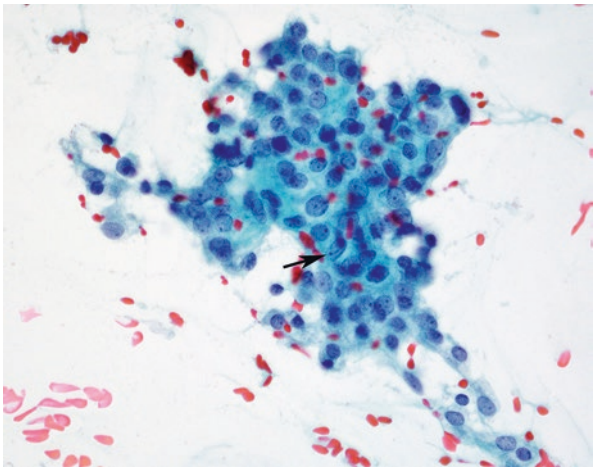

Fig. 4.2 Atypia of Undetermined Significance with nuclear atypia. Follicular cells show mild enlargement, small distinct nucleoli, and pale chromatin. Nuclear contours are uniform with only a rare nuclear groove (arrow). Molecular testing identified an *HRAS* mutation. Noninvasive follicular thyroid 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

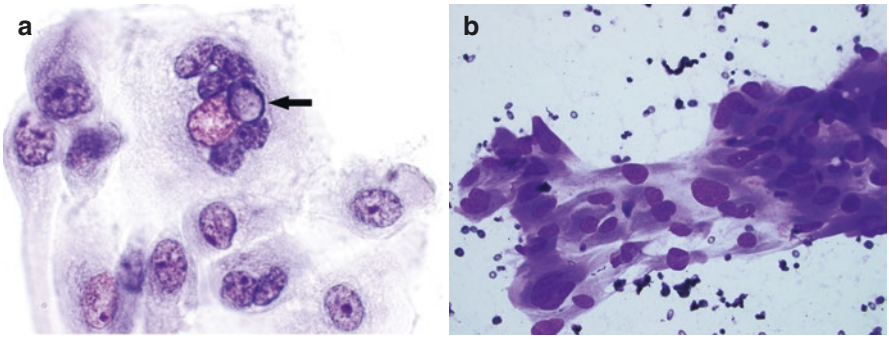

Fig. 4.3 Atypia of Undetermined Significance with nuclear atypia. (**a**) In this sparsely cellular specimen, some cells have abundant cytoplasm, enlarged nuclei, and prominent nucleoli. One cell has an apparent intranuclear pseudoinclusion (arrow). Such changes may represent atypical but benign cyst 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)

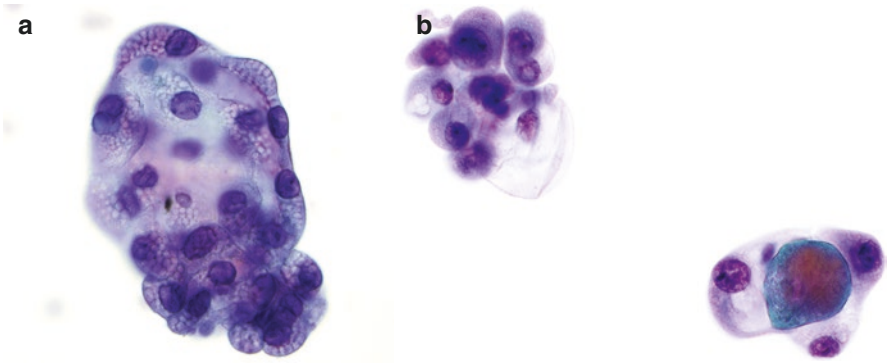

Fig. 4.4 Atypia of Undetermined Significance with nuclear atypia. (**a**) Cystic papillary carcinoma cells often show degenerative vacuoles; these cells have been termed "histiocytoid." A useful feature for recognizing them and distinguishing them from histiocytes is the sharply defined edges of the 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].

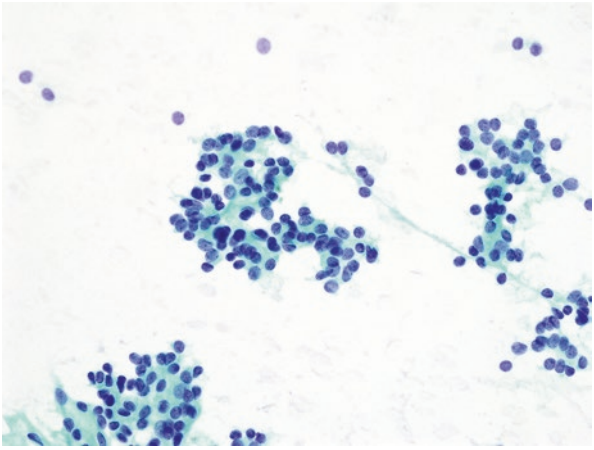

Fig. 4.5 Atypia of Undetermined Significance with nuclear and architectural atypia. Nuclear atypia is evident, with nuclear enlargement, crowding, and chromatin pallor, and infrequent nuclear grooves. Architectural atypia is manifested by a crowded three-dimensional configuration of follicular cells.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

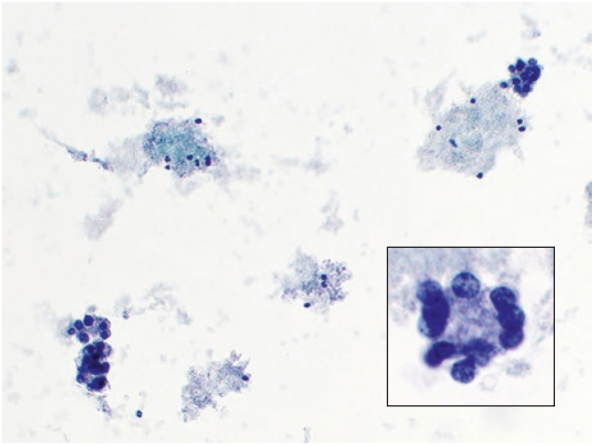

Fig. 4.6 Atypia of Undetermined Significance with architectural atypia. Scanning magnification reveals a sparsely cellular specimen with a predominance of microfollicles (Inset: high magnification of a microfollicle) (ThinPrep, Papanicolaou stain)

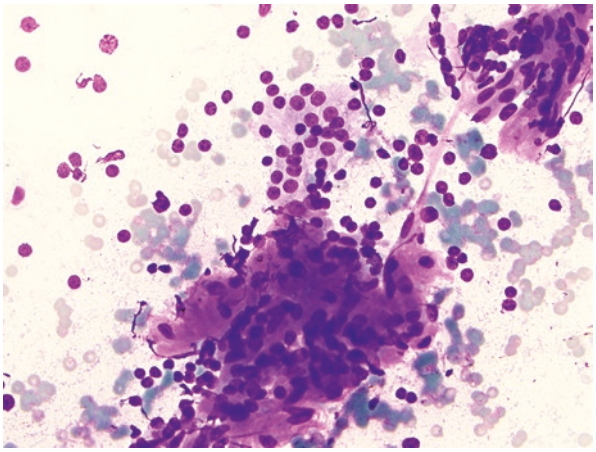

Fig. 4.7 Atypia of Undetermined Significance with architectural atypia. The smear shows cells arranged in a trabecular configuration with associated endothelial cells/blood vessels. Naked nuclei are prominent in the background and colloid is absent. This proved to be a parathyroid adenoma on resection (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.

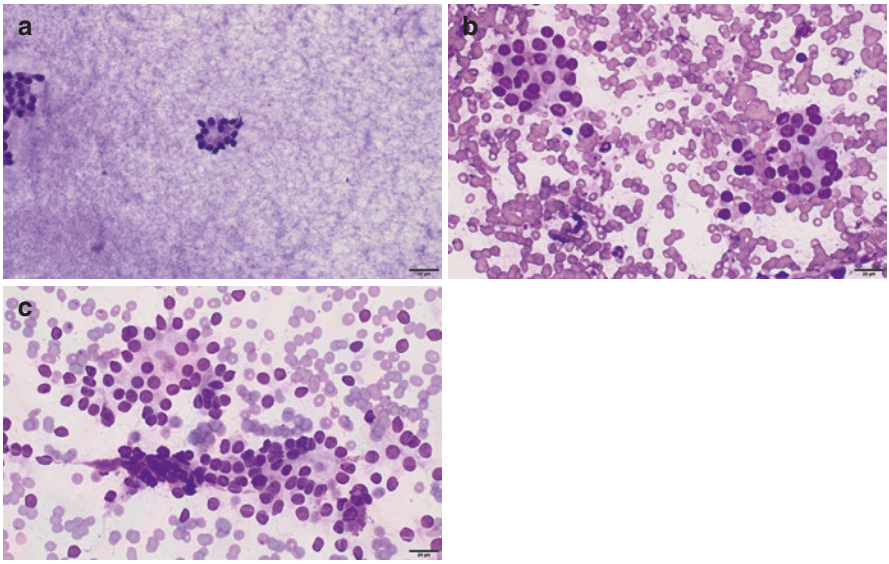

Fig. 4.8 Atypia of Undetermined Significance with architectural atypia. Three examples of *DICER1* mutated nodules in pediatric patients show variable cellularity and architectural atypia. Surgical resection identified a follicular adenoma (**a**), follicular carcinoma (**b**), and poorly differentiated 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

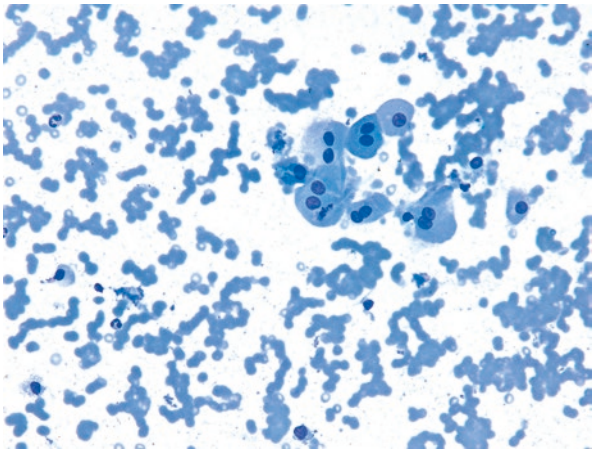

Fig. 4.9 Atypia of Undetermined Significance, oncocytic cell type. Sparsely cellular with abundant blood and predominantly oncocytic cells (smear, Diff-Quik stain)

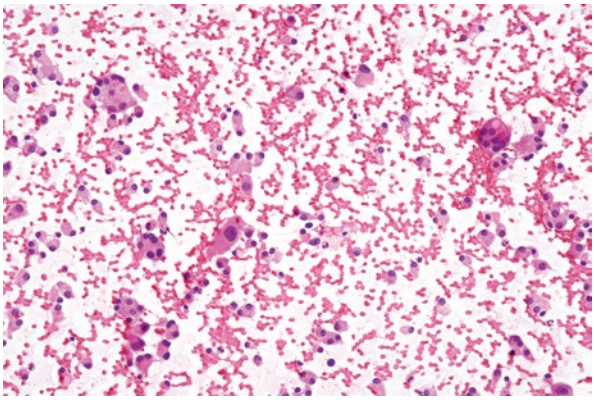

Fig. 4.10 Atypia of Undetermined Significance, oncocytic cell type (patient with history of Hashimoto thyroiditis). This patient had a subcentimeter nodule with modestly cellular smears showing exclusively oncocytic follicular cells without polymorphous lymphocytes in a bloody background (smear, hematoxylin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

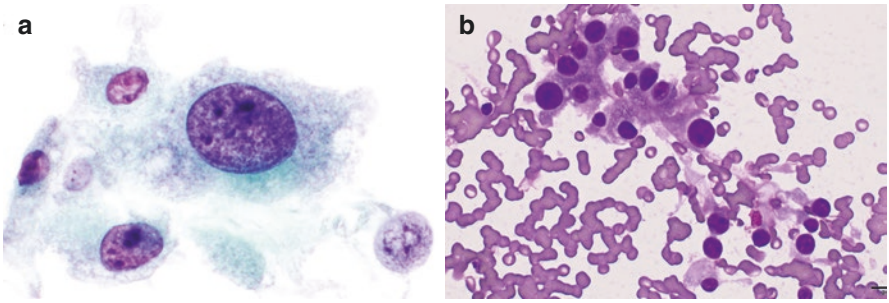

Fig. 4.11 Atypia of Undetermined Significance, not otherwise specified. The cytologic changes in these specimens do not raise concern for papillary carcinoma. (**a**) These follicular cells, in a patient with Graves' disease treated with methimazole (Tapazole®), show marked nuclear enlargement and anisonucleosis (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)

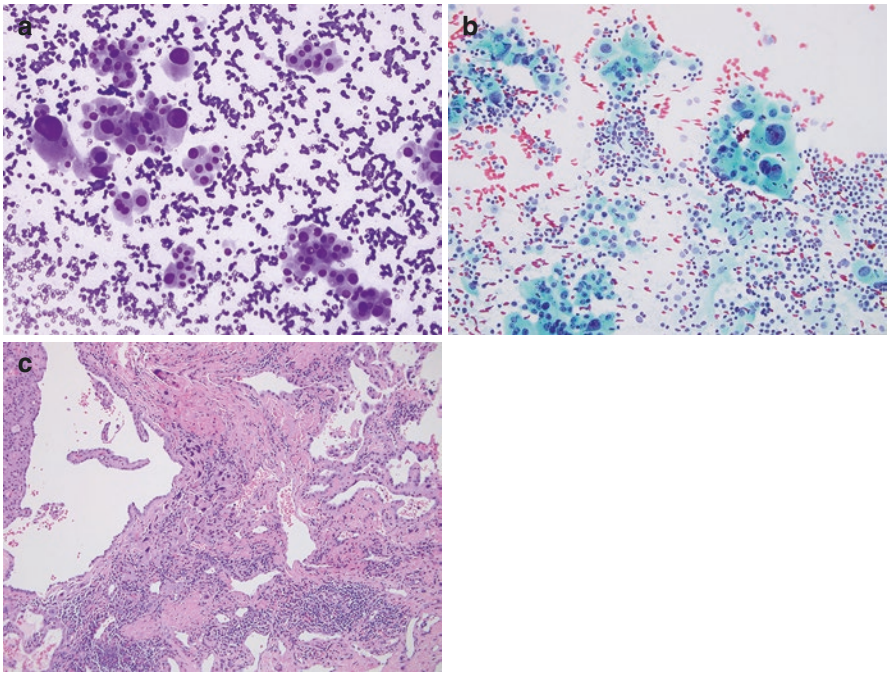

Fig. 4.12 Oncocytic cell atypia (patient with history of Hashimoto thyroiditis). These oncocytic cells show occasional marked nuclear enlargement. The findings in (**a**) show only oncocytic cells and could warrant an AUS diagnosis; however, the lymphocytic component of Hashimoto thyroiditis is readily 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)

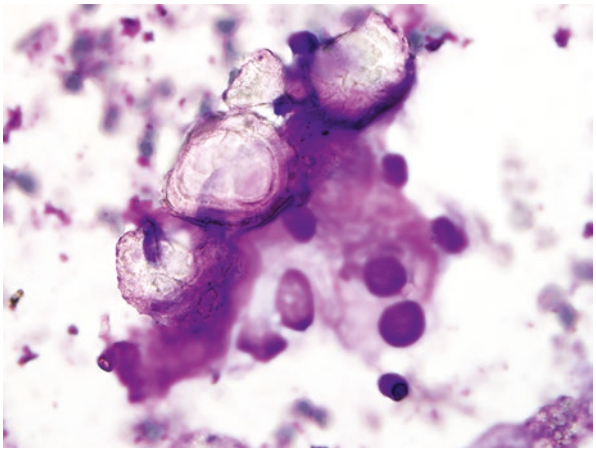

Fig. 4.13 Atypia of Undetermined Significance, not otherwise specified. Psammoma bodies are a characteristic feature of papillary carcinoma. They form at the tip of a papilla and consist of concentric dystrophic calcific lamellations. Psammoma bodies are non-birefringent and composed of calcium phosphate (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)

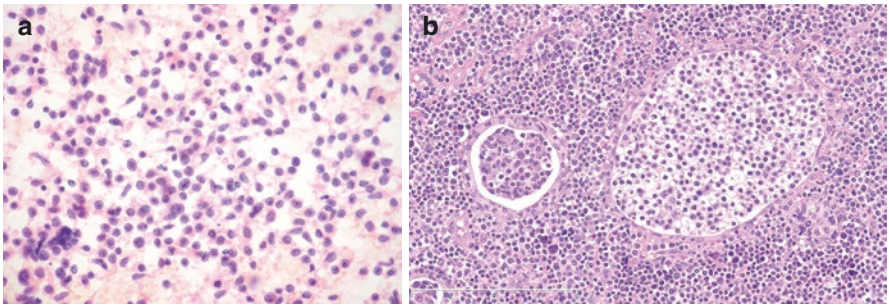

Fig. 4.14 Atypia of Undetermined Significance with atypical lymphoid cells. (**a**) Smear of a diffuse lesion suspected to be Hashimoto thyroiditis clinically shows extensive infiltration by monotonous small lymphocytes with slight variation in nuclear size and contour showing oval and occasionally kidney-shaped 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

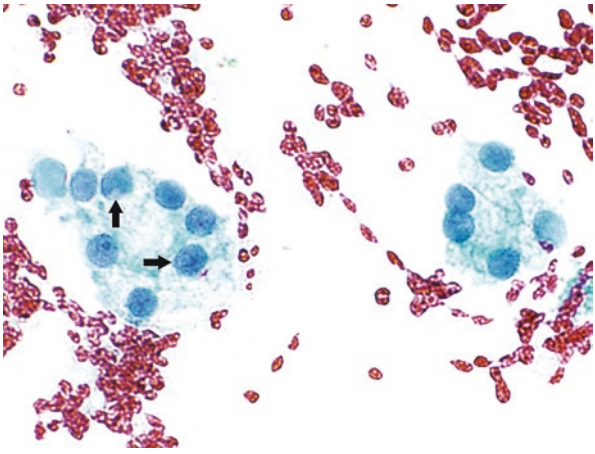

Fig. 4.15 Air-drying artifact. Inadvertent air-drying of alcohol-fixed smears leads to suboptimal nuclear detail (e.g., artifactual pallor, enlargement), including poorly defined, possible intranuclear pseudoinclusions (arrows). Except in rare instances, such changes can be recognized as artifactual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

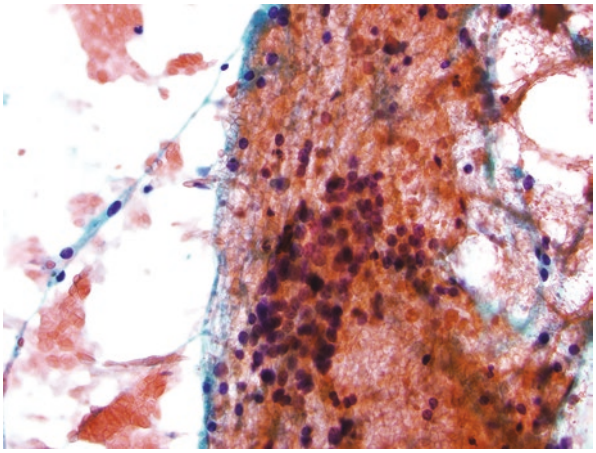

Fig. 4.16 Blood and clotting artifact. Extensive blood and clotting can distort the arrangement of follicular cells and make them look artifactually crowded. These findings should be discounted when assessing the architectural arrangement of the follicular cells. Without demonstrable atypia or sufficient 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

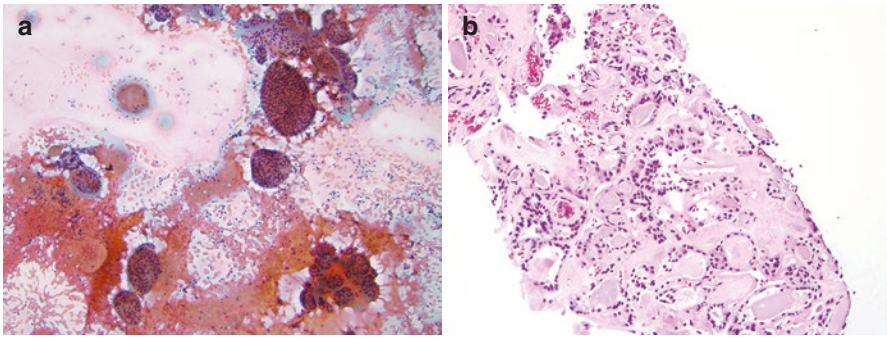

Fig. 4.17 Spherules. (**a**) Spherules of variable size are seen with sharply outlined contours. Even when small spherules predominate, these findings are associated with benign follicular nodules (**b**) and should not be classified as AUS with architectural atypia (**a**: smear, Papanicolaou stain;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

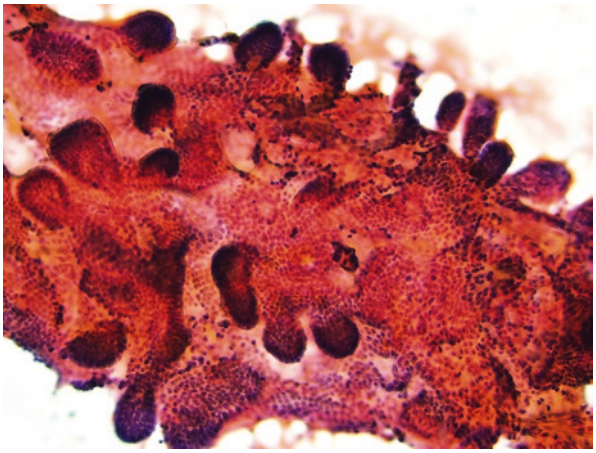

Fig. 4.18 Benign (papillary hyperplasia). Papillary projections are seen in papillary carcinoma, but Graves' disease and other hyperplastic thyroid nodules can show benign papillary proliferations. It is critical to carefully examine the cells, especially their nuclear features; a diagnosis of papillary 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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