Von G. Samedi
Thèrése Bocklage

Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features

Essentials in Cytopathology
Series Editor
Momin Siddiqui

Springer

Essentials in Cytopathology

Series Editor
Momin T. Siddiqui

More information about this series at http://www.springer.com/series/6996

Von G. Samedi • Thèrése Bocklage

Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features

Springer

Von G. Samedi
Department of Pathology
Health Sciences Center
University of New Mexico
Albuquerque, NM, USA

Thèrése Bocklage
Department of Pathology
Health Sciences Center
University of New Mexico
Albuquerque, NM, USA

ISSN 1574-9053 ISSN 1574-9061 (electronic)
Essentials in Cytopathology
ISBN 978-3-319-39807-5 ISBN 978-3-319-39809-9 (eBook)
DOI 10.1007/978-3-319-39809-9

Library of Congress Control Number: 2016944623

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer International Publishing AG Switzerland

Dedication (from Thèrése J. Bocklage)
To my dear sisters, Cam and Paula, alternately Louise and Diabola

Dedication (from Von G. Samedi)
To my wife Sachielle, my mother Marie-Thérèse, and my father Lucien

Foreword

Health care is rapidly moving toward precision medicine, an approach that takes into account individual variability in genes, environment, and lifestyle for each person. Over the past 50 years, cytopathology has made progress toward increased precision through refinements in diagnostic criteria, combined with sophisticated molecular testing. But despite this, we remain haunted by a spectrum of cellular changes that lurk between definitively benign and malignant cellular features. These include a variety of cellular responses to such perturbing factors as infections, inflammation, ischemia, therapeutic agents, and mechanical irritation. Unfortunately the morphologic changes that accompany these irritants often include increased mitotic activity, nuclear enlargement, chromatin alterations, and nucleolar modifications, all of which overlap with features we rely on to diagnose malignancy. Cytology pioneers recognized these challenges even when the field was in its infancy. Dr. John K. Frost, in his seminal book, *The Cell in Health and Disease*, used the term proplasia to describe "an abnormal response to repeatedly and chronically encountered injurious agents which can bear connotation of neoplastic development" [1]. False-positive malignant diagnoses (and the potential over-treatment of the patient) associated with reactive changes haunt all students and practitioners of cytopathology, leading many to the seductive security of an "atypical" diagnosis. If cytopathologists are to participate fully in precision medicine, we must constantly strive to minimize such imprecise diagnoses. This volume represents an important step in the systematic characterization of these benign mimics of malignant cells and will serve as a useful guide in navigating this treacherous terrain.

1. Frost JK. The cell in health and disease: an evaluation of cellular morphologic expression of biologic behavior. 2nd rev. ed. 1986.

Douglas P. Clark
Chair of Pathology, UNM School of Medicine
UNM Health Sciences Center
Albuquerque, New Mexico
March 16, 2016

Preface to Pitfalls in Diagnostic Cytopathology with Key Differentiating Cytologic Features

What is a "pitfall"? According to the Merriam-Webster Dictionary online resource, a pitfall is broadly defined as "a hidden or unrecognized danger or difficulty." More literally, the Oxford English Dictionary online resource defines pitfall as "a covered pit used as a trap." Our goal with this concise book was to write a "trail guide" identifying the snares and hazards on the route to the correct cytologic diagnosis. The book thus maps the more common to esoteric entities that can mimic or confound the unwary or uninformed. There are many, but they can be managed. The "mimics" comprise benign entities that can be mistaken for malignancies (the suspiciously, slightly too helpful false signage leading to a pit). The "confounders" comprise malignant tumors that can be mistaken for benign lesions (the innocent-appearing but unnaturally tousled leaves and grass that cover a pit). Familiarity with the diagnostic terrain (obtained by consulting this guide) combined with alertness to subtle features (acquired by careful review of the case) reduces the risk of plummeting into a pit. Space prevents illustrating each entity, but awareness of the various pitfalls and their key features (provided in tables in this book) is a key start to exercising appropriate diagnostic caution and avoiding them.

We acknowledge that sometimes the cytologic hazards are too great to enable reaching the diagnostic destination: in such cases, other routes (other sample types) can lead to the diagnosis. For example, some entities in breast cytopathology cannot be distinguished from malignant tumors: in such cases, needle core biopsy is recommended. However, for many pitfalls in cytology, it is possible to successfully bridge over them with ancillary techniques such as immunocytochemistry and molecular genetic testing to reach the correct diagnosis. This book also briefly lists these additional diagnostic aids. Such aids, of course, are not always needed: a strong, well-informed leap knowing only the pitfall features can be just as successful.

We hope you enjoy the diagnostic journey, deftly sidestepping these now uncovered pitfalls.

Albuquerque, NM, USA

Von G. Samedi
Thèrése J. Bocklage

Acknowledgments

Thanks go to Teresa Quintana, our ever enthusiastic and excellent administrative assistant, and Michael Grady, our accomplished visual media specialist.

Contents

Breast Cytology

1

Brief Introduction

Most breast carcinomas and benign lesions can be diagnosed relatively easily. The sensitivity and specificity of breast FNA for correctly identifying carcinoma is ~95 % according to several recent reviews. The cytologic diagnosis of breast lesions *must* occur with input from the clinical impression and imaging findings. This "triple test" for malignancy in breast lesions shows very high specificity and sensitivity when concordant in all three tests (~99 %). Marked discordance such as low BI-RADS (Breast Imaging-Reporting and Data System, American College of Radiology) score with "positive for malignancy" cytology requires surgical tissue biopsy confirmation before definitive therapy (Table 1.1).

The number of breast FNAs is declining especially in areas of the world with ready access to core needle biopsy (CNB) facilities. However, even in settings where CNB has replaced FNA for initial diagnosis of radiologically suspicious breast lesions, cytology still has a role: (1) in ROSE (rapid on site evaluation) in the form of touch imprints of CNB in lesions that may be otherwise challenging for the interventional radiologist to biopsy to ensure that lesional tissue is obtained, (2) in recurrent tumors, (3) in satellite tumors, and in (4) in small, atypical lesions with relatively low BI-RADS suspicion for carcinoma (repeat BI-RADS 3 on consecutive exams, for example). A convincing argument is still made that breast FNA is more cost effective than core needle biopsy even if performed in only a subset of a clinic's patients.

Unfortunately, a lower caseload of breast FNA specimens leads inversely to a higher rate of unsatisfactory and inconclusive ("atypical" and "suspicious") diagnoses. A typical large volume practice staffed by experienced cytopathologists reports an inconclusive diagnostic rate of 4–8 %. However, in small practices or with less experienced practitioners, the rate can creep above 20 % or more. Importantly, not even an expert breast cytopathologist can definitively diagnose every breast FNA specimen. This is because an actual irreducible "gray zone" exists in which features of a few benign lesions and a few malignant lesions overlap (see Fig. 1.1). For expert breast cytopathologists, this zone encompasses 2–5 % of all breast FNAs. Especially for these irrefutably ambiguous lesions that are inconclusive on cytology, the clinical and radiologic features must be carefully integrated to determine the best patient recommendation: (1) repeat imaging at a later time; typically 6 months later, (2) immediate use of different type of imaging (such as MRI), (3) repeat FNA or (4) surgical tissue biopsy/excison confirmation.

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_1

Table 1.1 BI-RADS (Breast imaging-reporting and data system classification; (American College of Radiology) applicable for mammography, ultrasound and MRI with correlative European cytology diagnostic categories)

BI-RADS diagnostic category	Results	Significance	Management of BI-RADS score	Likelihood of malignancy based on BI-RADS score	European recommended cytology diagnostic categories
B0	Incomplete	Inadequate for interpretation	Review other studies or recall patient for repeat imaging	N/A	C1
B1	Negative	Normal tissue	Routine screening	Essentially 0 %	C2
B2	Benign findings	Benign findings such as cysts present	Routine screening	Essentially 0 %	C2
B3	Probably benign	Some atypical features	Short interval F/U (repeat at 6 months)	2 %	C3
B4	Suspicious abnormality	Suggestive of malignancy but not definitively diagnostic: • 4a: low suspicion (>2 % to ≤10 %) • 4b: moderate suspicion (>10 % to ≤50 %) • 4c: high suspicion (>50 % to ≤95 %)	Pathology specimen diagnosis	20–35 % overall	C4
B5	Highly suggestive of malignancy	Patient is very highly likely to have a malignancy	Pathology specimen diagnosis	95 %	C5
B6	Known biopsy-proven malignancy	Imaging is performed in a patient with pathology proven malignancy	Surgical excision when clinically appropriate	N/A	–

BI-RADS breast composition modifiers include the following features:
[a]Breasts are almost entirely fatty
[b]Scattered areas of fibroglandular density
[c]Breasts are heterogeneously dense which may obscure small masses
[d]Breasts are extremely dense, which lowers the sensitivity of mammography

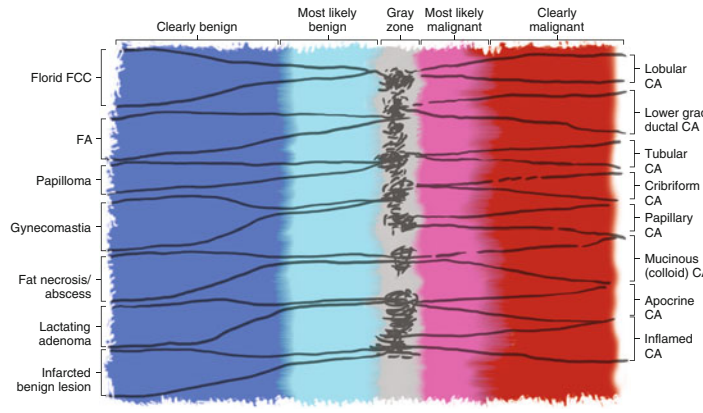


Fig. 1.1 The most common lesions that enter the gray zone of breast cytology

The "Gray Zone" of Breast Cytology

The most common breast lesions that mimic malignancy include fibroadenomas, florid fibrocystic change, papillary lesions and atypical ductal hyperplasia. The most common malignant lesions that could be misinterpreted as benign include examples of low grade ductal carcinoma not otherwise specified, low grade cribriform carcinoma, tubular carcinoma, lobular carcinoma, papillary carcinoma, low grade apocrine carcinoma, and low grade adenosquamous carcinoma. Tables 1.2 and 1.3 describe in more detail common to rare mimics of malignant tumors and common to rare mimics of benign lesions, respectively.

As is true for cytologyic findings from other body sites, some breast cytology features may strongly support a benign or malignant diagnosis but none have 100 % sensitivity or specificity. In breast cytology samples, features that support a benign interpretation include: (1) abundant bipolar stripped nuclei, (2) bland appearing apocrine cells, (3) well polarized sheets of epithelial cells that contain scattered, small hyperchromatic nuclei which come into focus above the plane of the epithelial cells (these are presumed myoepithelial cell nuclei), (3) cyst-like debris comprising a proteinaceous background, macrophages and foam cells, (4) moderately cellular stromal fragments in the case of fibroadenomas, (5) staghorn-like large folded branching sheets of small epithelial cells common to fibroadenomas and benign phyllodes tumors but also found in examples of FCC and a few other lesions, and (6) acute inflammation. Features that support a diagnosis of malignancy include: (1) complete absence of bipolar stripped nuclei, (2) abundant intact isolated or paired atypical epithelial cells, (3) marked nuclear atypia that can comprise hyperchromasia, macronucleoli, irregular chromatin distribution, irregular nuclear shapes, sharp angled nuclear notches, and marked variation in nuclear size, (5) atypical mitotic figures and (6) signet ring cells.

It is important to reiterate that none of the above listed features, if found in isolation, enable a definitive diagnosis of benign or malignant. Six general rules to follow before rendering an unequivocal "positive for malignancy" diagnosis comprise the following: (1) a definitive diagnosis can be made when the specimen is moderately to highly cellular with many malignant cells that are present on more than one slide, (2) a definitive diagnosis can be made when single malignant cells are present in addition to atypical clusters and groups, (3) a definitive diagnosis CANNOT be made when marked acute inflammation and inflammatory cellular debris are present, (4) a definitive diagnosis CANNOT be made if the only atypical cells are small with only mild nuclear atypia (MNA), (5) a definitive diagnosis CANNOT be made if atypia is mild to moderate, but abundant intermixed obvious benign elements are present. And, of course, all cytology findings must be interpreted in the light of the clinical and radiologic findings which leads to general rule (6) a definitive diagnosis of malignancy should be made WITH GREAT HESITANCY when radiologic and clinical features are unsupportive. Despite a cautious, expert approach, "False negative diagnoses are mainly due to sampling error [while] false positive diagnoses are rare but constitute a practical reality." [2]. This chapter provides the information to minimize the false positive rate and to constrain the inconclusive rate (ideally to no more than 10 % of a practice's breast FNAs). To begin, Fig. 1.2 highlights the spectrum of nuclear atypia found in breast lesions, while Fig. 1.3 highlights the spectrum of architectural atypia found in breast lesions.

Lesions that May Show Low Grade Epithelial Atypia

Benign Lesions that Mimic Carcinoma (Mimics of Malignancy)

Florid Fibrocystic Change Differentiating between a low grade ductal carcinoma (in situ or invasive) and florid ductal hyperplasia is not always possible in a cytology specimen. In a cellular sample consisting of crowded tubules with loss of polarity, mild nuclear atypia (MNA = mild nuclear enlargement, +/− small nucleoli, + mild

Table 1.2 Benign breast lesions that mimic malignant breast tumors = benign confounders

Mimicker/confounder		What it mimics	Architecture/cellularity	Nuclear features	Cell/cytoplasmic features	Background	Differentiating features	Additional findings/steps
Cellular epithelial lesions	Florid fibrocystic changes	LGDC	• Low to moderate cellularity • Clusters and LFBS containing M-EN • Clusters = cribriform, micropapillary, tubular, or flat fragments • Some may be syncytial (loss of polarity) with nuclear crowding and overlap	• Mild atypia (MNA = mild nuclear enlargement, +/− small nucleoli, + mild variation in nuclear shape +/− mildly increased hyperchromasia) • Macronucleoli usually absent	• No special features except apocrine cells have granular cytoplasm • Cells of columnar cell hyperplasia have columnar shape	• Stripped M-EN, +/− apocrine cells • May have microcalcifications (especially if columnar cell component present) • +/− hemorrhagic or proteinaceous background	• *Usually* M-EN prominent in epithelial fragments and as stripped nuclei	• Low BI-RADS score in most cases • May require CNB in rare cases with diffuse nuclear atypia, syncytial clusters
	Radial scar/ complex sclerosing lesion	• LGDC • TubCa	• Low to moderate cellularity (most cases) • May have small angulated tubules • May have syncytial fragments	• MNA • Micronucleoli may be present	• No special features except apocrine cells, when present, have granular cytoplasm • No columnar cells	• Stripped M-EN, +/− dense hypocellular stromal fragments	• Reported to be less cellular than most cases of TubCa with less angulation and complexity to ductal groups • Mixed cell types including apocrine cells and M-EN	• BI-RADS score may be deceptively high • MRI may be helpful • Usually does not present as a palpable mass
	Nipple adenoma	• LGDC • Sclerosing adenosis	• Low to moderate cellularity • Many features similar to sclerosing adenosis • +/− 3-D frags with rounded contours (but lacking FVC)	May have mild nuclear atypia (MNA)	Round to columnar cells	+/− hypocellular stromal fragments	M-EN present in cell clusters	• Confirm subareolar location • May clinically simulate Paget disease of nipple with nipple erosion and discharge • Often requires IHC on CNB to make correct diagnosis

	Sclerosing adenosis	• LGDC • TubCa	• Low to moderate cellularity • Cell clusters usually small with round, angular or tubular shapes	• Usually not atypical to mildly atypical • Rare cases show moderate nuclear atypia	No special features	• Stripped M-EN • M-EN also present in at least some of the epithelial cell clusters • Often with dense, hypocellular stromal fragments • SIEC +/−, usually few if present	• Clusters not as angulated or complex as in tubular carcinoma • M-EN present in at least some of the clusters	• Usually does not present as a mass lesion • May require CNB if atypical calcifications on imaging or diffuse atypia on cytology
	Microglandular adenosis	• LGDC • TubCa	• Small angulated tubules often present • Tubules may contain eosinophilic secretions	Mild nuclear atypia may be present	No special features	• Sparse stripped M-EN • SIEC may be present	• Tubules with eosinophilic secretions should prompt consideration of the diagnosis	• May require CNB and IHC • BI-RADS score usually low • Usually does not present as a mass lesion
	Atypical apocrine metaplasia	• Apocrine Carcinoma • Apocrine DCIS	• Cell sheets and clusters • +/− papillae with FVC • Often mixed with FCC, FA, sclerosing adenosis, radial scar or intraductal papilloma components	• Moderate to marked nuclear size and shape variation • Prominent nucleoli	• Granular cytoplasm • N/C ratio low to moderate	+/− inflammatory background	• Lacks the necrosis found in many cases of apocrine DCIS • Benign elements present	• May present as multiple breast masses • BI-RADS score may be high
	Atypical ductal hyperplasia (ADH)	• LGDC • Florid FCC	• Cell clusters with mild loss of polarity • +/− cribriform, micropapillary, solid sheets	Mild to moderate nuclear atypia	No special features	• Intermixed benign components (such as FCC) often present • Often some SIEC	• Intermixed benign components • Some M-EN in cell groups and as stripped naked nuclei	Specific dx of ADH cannot be made reliably on cytology
	Lactating adenoma	HGDC	• Moderate to high cellularity • Cellular clusters with knoblike extensions = hyperplastic lobules • On monolayer: lacy fragments with tissue paper like folds, smaller cell clusters and lobules compared to direct smears	• Nuclei often round with prominent nucleoli that may appear cherry red on Pap stain	• Fragile cells often with torn or stripped cytoplasm • Cytoplasm often scant, vacuolated and/or frothy	• Proteinaceous background (milky) often with many embedded stripped epithelial nuclei • On monolayer the proteinaceous material is clumped and globular • Scant to absent M-EN	• Milky background • Uniform round shape of nuclei	• Nearly all patients are lactating or pregnant • BI-RADS score usually low

(continued)

Table 1.2 (continued)

Mimicker/confounder		What it mimics	Architecture/cellularity	Nuclear features	Cell/cytoplasmic features	Background	Differentiating features	Additional findings/steps
	Gynecomastia	• LGDC • FA • FCC	• Moderate to high cellularity • Clusters and sheets of overlapping small cells	Absent to mild nuclear atypia	• No special features • Few cases will have apocrine cells (granular cytoplasm) and/or squamous cells (glassy keratinized cytoplasm)	• M-EN cells present in cell clusters and in background • +/− fibrotic or vascular stromal fragments • +/− apocrine cells, squamous metaplasia and foam cells	• Absent to rare SIEC • M-EN cells in cell clusters and as stripped nuclei	• Generally has fairly characteristic imaging findings • Clinical hx can reveal pre-disposing factors
	Gynecomastoid change	• LGDC • FA • FCC	Similar to gynecomastia	Similar to gynecomastia	No special features	May be associated with other benign lesions such as FCC	M-EN cells in clusters and as stripped nuclei as in gynecomastia	Only reported as a pure lesion in a patient with Turner syndrome
Papillary lesions	Large duct papilloma	• LGDC PapCa • FA	• 3-D frags with FVC often but not always present • May just have LFBS	No to mild nuclear atypia	• Round to oval to columnar cells • +/− apocrine cells • +/− squamoid cells	• Hemorrhagic, cystic or less commonly proteinaceous background • M-EN present in cell clusters and in background as naked nuclei • SIEC may be prominent	• Diagnosis is possible if 3-D frags with FVC present and also benign components such as apocrine metaplasia	If unilocular simple cyst on imaging, then no excision required (BI-RADS 1 score)
	Papillary hyperplasia in FCC	PapCa	• 2-D pseudopapillary cluster (no FVC, rounded bulbous shape) • FCC type clusters and sheets	No to mild nuclear atypia	Mixed round to oval to columnar cells	• Hemorrhagic, cystic or proteinaceous background • Stripped ME-N in background and in ductal cell groups	Other features of FCC present	BI-RADS score usually low

Mucinous lesions	Mucocele like lesion (MLL)	Colloid Ca	• Low to moderate cellularity • Small groups and clusters of ductal epithelial cells	Mild nuclear atypia (MNA)	• Lesional cells small round to cuboidal • Lack cytoplasmic mucin vacuoles • Benign epithelial elements may be present	• Mucinous background • +/− microcalcs • M-EN present in background in most cases	• Mucin usually lacks embedded blood vessels • SIEC absent to very sparse • May have apocrine and benign ductal cells intermixed	• Presents as microcalcs in post-menopausal pt • Mass in pre-menopausal pt • BI-RADS score may be equivocal or suspicious
	FA with mucinous background	Colloid Ca	• Moderate to high cellularity • LFBS and 2-D pseudo-papillae with bulbous or rounded contours	Mild nuclear atypia (MNA)	• Few to no SIEC • Abundant M-EN in cell clusters	• Abundant M-EN • Mucinous background described as more metachromatic on Giemsa stain than mucin in colloid carcinoma	• Often has typical features of FA with LFBS containing many M-EN and abundant M-EN in background	• Mucinous background is mucicarmine negative (positive in colloid carcinoma) • BI-RADS score usually low
	FCC with mucin or mucinous cells	LGDC	• Low to moderate cellularity • Other FCC components present	No to mild nuclear atypia (MNA)	• Few to no SIEC • +/− Few cells with intracellular mucin	M-EN present in background in most cases	• SIEC absent to very sparse • Other components of FCC present	BI-RADS score usually low
	Ductal adenoma with mucinous cells	LGDC	• Moderate to high cellularity • Cohesive clusters	Anisonucleosis in apocrine cells, when present	• Some cells with intracellular mucin • Granular cytoplasm in apocrine cells	• M-EN present in groups and in background • No extracellular mucin	• SIEC absent to sparse • M-EN present	May require CNB for diagnosis

(continued)

Table 1.2 (continued)

Mimicker/confounder		What it mimics	Architecture/cellularity	Nuclear features	Cell/cytoplasmic features	Background	Differentiating features	Additional findings/steps
	Rare infarcts or abscesses with pseudo-mucinous cells	• LGDC • HGDC	Infarcted benign epithelial clusters	Degenerative and reactive atypia present	• May have SIEC • M-EN may be inconspicuous or absent • Rare cells may have cytoplasmic vacuoles mimicking mucin vacuoles	• Inflammatory background with cell debris • No extracellular mucin	• Inflammatory background, especially neutrophils precludes unequivocal malignant diagnosis • Range of cell atypia • +/− reactive fibroblasts or myofibroblasts	Clinical history and BI-RADS score may be concerning
Biphasic epithelial and fibrous lesions	Fibroadenoma (FA)	• LGDC • Phyllodes tumor • FCC	• Moderate to high cellularity • LFBS with embedded M-EN • No true 3-D papillae with FVC	~5% of cases have mild nuclear atypia (MNA)	Edges of LFBS may have columnar cells	• Cellular stromal fragments that may appear slightly myxoid • Abundant stripped M-EN	• Numerous M-EN • Only slight nuclear atypia, if present • SIEC absent to very sparse	BI-RADS score usually low
	Benign phyllodes tumor	• FA • Malignant phyllodes tumor	• As above for FA • May have more prominent staghorn 2-D pseudopapillae than most FA	+/− mild nuclear atypia (MNA)	As for FA	• Cellular stromal frags usually present • SIEC sparse to common • Numerous stripped M-EN in background	• No marked nuclear atypia • May have more SIEC than FA but not diagnostically reliable finding	• Clinical history usually of low suspicion • BI-RADS score usually low

	Adenomyo-epithelioma	• LGDC • FA • Adenoid cystic carcinoma	• Moderate to high cellularity • Large clusters some appearing similar to FA • LFBS • Also clusters of pure M-E cells • +/− Branching tubules	• No to mild to moderate nuclear atypia • Some cases have nuclear pseudoinclusions or nuclear grooves	• M-E cells may be epithelioid • +/− cytoplasmic vacuoles (“soap bubble cells”)	• Some groups comprise cells surrounding metachromatic fibrillary globules similar to collagenous sperulosis • M-EN usually present in groups and background • Fibromyxoid stromal frags common	• Fibrillary globules and M-EN clues to the diagnosis	BI-RADS score may be suspicious but most have low score
	Pseudoangi-omatous stromal hyperplasia (PASH)	• LGDC • FA	Similar to FA	Similar to FA	• Similar to FA but may have increased number of intact, non-atypical elongate spindle cells	• Stromal fragments similar to FA • Fewer stripped M-EN than in most FA but still present	Similar to FA	• BI-RADS score usually low • Cannot distinguish from FA on cytology
Benign mesenchymal lesions	Mammary myofibroblas-toma	• Metaplastic Ca • Desmoid tumor • Schwannoma • Metastatic melanoma	• High cellularity • Clusters and single cells • Some clusters may show peripheral palisading	• Mild to moderate nuclear atypia with hyperchromasia and/or vesicular nuclei • +/− intranuclear pseudoinclusions and nuclear grooves	• Cells are spindled, plump or oval in shape • Single intact tumor cells common	• +/− stromal fragments that are fibrotic, myxoid or adipocytic • +/− mast cells	• Epithelial cells absent (but rarely, the tumor cells of myofibrob-lastoma may appear epithelioid) • No M-EN	• BI-RADS score usually low • ICC: CD34 positive and RB1 negative unlike other entities in the differential diagnosis

(continued)

Table 1.2 (continued)

Mimicker/confounder	What it mimics	Architecture/cellularity	Nuclear features	Cell/cytoplasmic features	Background	Differentiating features	Additional findings/steps
Nodular fasciitis	• Metaplastic CA • Granulation tissue	• Moderate to high cellularity • Single or clustered spindle cells	• No to mild nuclear atypia: vesicular nuclei with fine chromatin and small nucleoli • Mitotic figures may be present (none are atypical)	• Cells are spindled to plump • N/C ratio is moderate • Cytoplasm pale eosinophilic to amphophilic on cell block	• Lymphocytes and red blood cells often present • Heterogeneous stromal matrix from myxoid to fibrous • +/− Multinucleated histiocytic giant cells	• Epithelial cells absent • No M-EN	• Lesion is usually small but may be rapidly growing • Usually occurs in younger patients (<40 years)
Deep fibromatosis (desmoid tumor)	• LG metaplastic ca • Schwannoma • Exuberant scar	• Low to high cellularity • Single cells and loose clusters	• Nuclei similar to those in nodular fasciitis • No to very rare mitotic figures	• Cells are spindled to plump • N/C ratio is moderate • Cytoplasm pale eosinophilic to amphophilic on cell block	• A few lymphocytes may be present • Mast cells present in most cases • No M-EN • +/− Heterogeneous stromal fragments	• Epithelial cells absent • No M-EN • No significant nuclear atypia	• Usually occurs in younger patients (<40 years) • ICC: 70 % of cases show aberrant nuclear beta-catenin expression
Granular cell tumor	• Low grade apocrine ca • Apocrine metaplasia	• Moderate to high cellularity • Most cells disrupted but usually some intact single cells and loose clusters	• Nuclear atypia ranges from minimal to marked with prominent nucleoli but not coarse chromatin	• Cells are often disrupted • N/C ratio is low to moderate • Cell shape is plump (polygonal) to spindled • Cytoplasm is eosinophilic granular on Pap and H&E stains and dense and oncocytic on DQ	• Background often comprises disrupted granular cytoplasm (Pap stain) and abundant stripped tumor nuclei with nucleoli • Necrosis and blood absent • M-EN absent	• GCT cells are generally larger, may be spindled with much dissolution and less granularity on DQ stain than apocrine cells	• BI-RADS score may be high • Large age range; average 30 years • ICC: positive S100; negative keratins

Inflamed lesions	Spontaneous infarct with reactive atypia in a benign lesion	• LGDC • HGDC	• Most commonly occurs in FA, papillary lesions, phyllodes tumor • Dyscohesive intact lesional cells present • Often non-infarcted components present • +/− Fibroblast clusters in late stage	• Nuclear enlargement and irregular nuclear shapes often present • Shrunken pyknotic (ischemic) nuclei also often present • Non-infarcted epithelial cells show typical features of the underlying lesion	• Reactive and ischemic cells may show cellular enlargement • Non-infarcted components show typical features including embedded M-EN in cell clusters • Late stage of infarction may include spindled fibroblasts	• Necrosis common • Ghost cells common • Stromal fragments common • Inflammation +/−	Background and non-infarcted components are clue to diagnosis	BI-RADS score usually low
	Fat necrosis/ traumatic infarct	• LGDC • HGDC	• Cellularity varies with phase of lesion: most cells are inflammatory • Fragments of fat with degenerative changes	• No epithelial components • Histiocytes and multinucleated giant cells have vesicular nuclei without atypia • +/− myospherules (degenerating red blood cells contained in sac-like structure)	• Acute inflammation • Histiocytes and MNGC common • +/− foamy histiocytes • +/− siderophages • Later phases contain fibroblasts that may be spindled	• +/− Red blood cells, • +/− hemosiderin, +/− hematoidin • +/− fibrous stromal fragments • +/− dystrophic calcifications • +/− cellular debris	• No atypical epithelial cells • Inflammation and degenerated fat fragments characteristic	• Most are superficial, central and occur in large pendulous breasts • BI-RADS score may be high • MRI may show findings consistent with fatty lesion • Most regress over time

(continued)

Table 1.2 (continued)

Mimicker/confounder		What it mimics	Architecture/cellularity	Nuclear features	Cell/cytoplasmic features	Background	Differentiating features	Additional findings/steps
	Mammary duct ectasia (MDE)	• LGDC • Infection	• Low epithelial cellularity • Few clusters of ductal cells	• +/− reactive nuclear atypia	• Ductal cells are cuboidal or round • +/− ochrocytes (histiocytes with brown granular lipofuscin pigment)	• Granular debris • +/− cholesterol crystals or clefts • +/− dystrophic calcifications • Inflammation includes histiocytes, lymphocytes and plasma cells • +/− MNGC	• No significant nuclear atypia • Groups of ductal cells are sparse and should have M-EN • M-EN in background may be absent or difficult to identify	• Palpable subareolar mass often with thick nipple discharge • May rarely occur in infants/children with bloody nipple discharge
	Squamous metaplasia of lactiferous ducts (SMOLD)	• Metaplastic Ca • Necrotizing carcinoma • LGM	• Low epithelial cellularity with few groups of ductal cells • Squamous cells	+/− reactive nuclear atypia	• Squamous cells are not atypical but may be degenerated • +/− MNGC • Histiocytes, neutrophils, lymphocytes common	• Background of inflammation • Keratinous debris	• Clinical history • Benign features of ductal cells and squamous cells • Inflammation	Patient usually young to middle aged adult and smoker with painful peri-areolar mass and nipple discharge
	Lobular granulomatous mastitis (LGM)	• LGDC • Infection	• No or low epithelial cellularity • +/− non-caseating epithelioid granulomas	No nuclear atypia in epithelial cells	• Cell types include lymphocytes, plasma cells, histiocytes, MNGC • +/− neutrophils and eosinophils	• +/− debris • Inflammation always present	• No squamous cells • No keratinous debris • Few to absent epithelial cells • Inflammation	Patient usually young adult and pregnant/lactating within last several years

	Intramammary lymph node	Lymphoma	• Moderate to high cellularity • Dyscohesive cells and unorganized clusters	• Mixed nuclear features corresponding to the varied lymphocytes in a normal or reactive node	Usually scant cytoplasm	• +/− tingible body macrophages • +/− stromal and endothelial cells • No lymphoglandular bodies	• No epithelial cells • Polypmorphous population of lymphocytes • Tingible body macrophages	• BI-RADS score low • Flow cytometry: negative for monoclonality
	Reactive seroma in the setting of prior surgery or breast implant	• Lymphoma • Infection	• No epithelial cells • Dyscohesive inflammatory cells	• No significant nuclear atypia in inflammatory cells	• Mixed inflammation either neutrophil or lymphocyte rich	• Usually no lymphoglandular bodies	• No atypical lymphoid cells	• BI-RADS score low • ICC negative for monoclonality

Note: Descriptions are based on direct smears; monolayers and cell blocks may show other features

MNA mild nuclear enlargement, +/− small nucleoli, + mild variation in nuclear shape +/− mildly increased hyperchromasia, *FA* fibroadenoma, *M-EN* myoepithelial nuclei, *LFBS* large folded and/or branching epithelial sheets with good polarity, *3-D frag* three dimensional epithelial fragment, *FVC* fibrovascular core, *SIEC* single intact epithelial cells, *Infl* inflammation, *LGDC* low grade ductal carcinoma, *HGDC* high grade ductal carcinoma, *PapCa* papillary carcinoma, *TubCa* tubular carcinoma, *CribCa* cribriform carcinoma, *LobCa* lobular carcinoma, *FCC* fibrocystic changes (includes florid ductal hyperplasia), *Ca* carcinoma, *CNB* core needle biopsy, *IHC* immunohistochemistry, *ICC* immunocytochemistry, *MNGC* multinucleated giant cells, *BI-RADS* Breast Imaging-Reporting AND Data System

Table 1.3 Malignant breast tumors that mimic benign breast tumors = malignant confounders

Mimicker/confounder		What it mimics	Architecture cellularity	Nuclear features	Cell/cytoplasmic features	Background	Differentiating features	Additional findings/steps
Low grade carcinomas	LGDC	• FCC • FA	Small tight ductal epithelial clusters with overlapping (loss of polarity)	Mild nuclear atypia with mild enlargement, hyperchromasia and mild variation in shape (MNA)	• Cuboidal cells • N/C ratio usually high	• Sparse to absent M-EN • SIEC	• Many SIEC (but not always present) • Absence of M-EN in groups and in background • Nuclear hyperchromasia may be more prounounced than in FCC or FA	• Some cases require CNB for diagnosis • BI-RADS score usually at least suspicious
	TubCa	FCC	• Rigid tubes with abrupt diameter change and pointed tips • Abnormal, sharp-angled branching	• Mild nuclear atypia (hyperchromasia, mild size variety) • +/− nucleoli	No special features	• Very sparse to absent stripped M-EN • SIEC and intact epithelial cell doublets present	• More monotonous than benign mimickers • More angulation and rigidity to tubules • No M-EN	• May require CNB for accurate diagnosis • BI-RADS score usually at least suspicious
	Cribriform Ca	FCC	• Sheets of epithelial cells with punched out spaces • Clusters present also	Mild nuclear atypia (MNA)	No special features	• Very sparse to absent M-EN • SIEC and intact epithelial cell doublets present	• Lack of M-EN • SIEC present • Mild nuclear atypia	• May require CNB for diagnosis • BI-RADS score usually at least suspicious
	LobCa	FCC	• Often low cellularity • Dyscohesive cells • Small cell clusters • Single file strands	• Mild nuclear atypia (MNA) • Moderate to high N/C ratio • Pleomorphic variant shows more range in nuclear size and shape with grooved and cerebriform nuclei	• +/− signet ring cells or other cells with vacuolated cytoplasm • +/− targetoid mucin droplets in cytoplasmic vacuoles • +/− plasmacytoid cells with eccentrically placed nuclei	• Absent ME-N • SIEC • May have benign ductal cells in background due growth of lobular carcinoma interweaving with benign tissue	• Signet ring cells should prompt consideration of the diagnosis but are not specific • Dyscohesion and strands suggestive of diagnosis	• Sparse cellularity and mild atypia may require CNB for diagnosis • BI-RADS score usually high • Mass may be ill-defined or multifocal

	Adenoid cystic Ca	• Collagenous spherulosis • Adenomyoepi-thelioma	• Cribriform sheets with balls of matrix surrounded by tumor cells (solid variant not distinguishable from IDC). • May have small tubules embedded among disorganized basaloid cells	Nuclei fairly uniform, hyperchromatic with granular chromatin and usually inconspicuous nucleoli	• Cells are small with sparse cytoplasm • Cells forming small tubules are larger with more cytoplasm • +/− clear cytoplasm	• Matrix material forming balls surrounded by a peripheral rim of basaloid cells • +/− dispersed matrix appears like mucoid or myxoid material	• More cellular than collagenous spherulosis • Adenomyoepithelioma usually has fibromyxoid stromal fragments but also contains hyaline balls surrounded by tumor cells (may need CNB and or ICC for diagnosis)	• ICC: c-kit, CD43 positive in adenoid cystic carcinoma • Collagenous spherulosis: negative for above and positive for calponin
	Secretory Ca	Lactating adenoma	• Cellularity low to high Clusters and mats of medium sized cells • Honeycomb pattern may be present containing balls of secretions	• Nuclei fairly uniform and round with small nucleoli • Binucleation may be prominent	• Cells medium sized with prominent small to large clear cytoplasmic bubbly vacuoles and granular to foamy eosinophilic to amphohilic cytoplasm • Cells may appear plasmacytoid or signet ring-like	• Matrix material may be present and colloid-like (hyaline globules) • Absent ME-N	• More cellular than LA • No intact lobules as can be seen in LA • Absent bipolar stripped M-EN in background	• Clinical history is helpful (not pregnant or lactating, may be post-menopausal but also occurs in children and young adults) Secretions often PAS/D positive
	Cystic hypersecretory Ca	• Lactating adenoma • Secretory carcinoma	• Cellularity low to high • Clusters of cells	• MNA if the lesion is in-situ • Marked nuclear atypia if the lesion has become invasive	Cells range in size without specific features	• Colloid-like matrix material • Scattered SIEC often present	• Invasive form shows marked nuclear atypia • Colloid-like material • No stripped nuclei with giant red nucleoli as in LA • No stripped M-EN as in most LA	BI-RADS score low to intermediate if invasion is absent

(continued)

Table 1.3 (continued)

Mimicker/confounder		What it mimics	Architecture cellularity	Nuclear features	Cell/cytoplasmic features	Background	Differentiating features	Additional findings/steps
	Scirrhous Ca	• FCC • FA	May appear similar to lobular carcinoma	Mild to marked nuclear atypia ranging from MNA to large pleomorphic nuclei with irregularly distributed coarse granular chromatin	No signet ring cells or other mucinous cells	• Absent ME-N • SIEC present • +/− fibrous stromal fragments	• Nuclear atypia may be marked enough to allow definitive diagnosis • SIEC	BI-RADS score usually high Sparse cellularity may require CNB for diagnosis
	Low grade Adenosquamous Ca	• FCC • SMOLD • Mammary duct ectasia	Irregular clusters	MNA	Some cells are metaplastic with squamous features	Keratin debris	Difficult to definitively diagnose on cytology	Often requires CNB and IHC to make correct diagnosis
Mucinous lesion	Mucinous (colloid) Ca	MLL	• Cellularity usually moderate to high • Variable patterns including sheets, small balls, strands, and pseudopapillae	• MNA to moderate nuclear atypia • Marked nuclear atypia should suggest mixed colloid-ductal carcinoma	• Cells small to medium sized • Usually lack intracytoplasmic mucin	• Wispy or colloid like mucoid material often with embedded thin walled branching vessels and malignant cells • Calcifications may be present	• Usually more cellular than MLL • Nuclear atypia greater than in MLL • Mucoid material often contains thin-walled branching vessels • SIEC more common than in MLL • Absent to sparse stripped M-EN	Mammogram often shows a rounded mass Adding MRI may improve diagnostic accuracy
Papillary tumor	Papillary Ca	Duct papilloma	• May have 3-D papillae with FVC • Some cases have only LFBS	• MNA • +/− increased hyperchromasia • Large rounded stripped nuclei may be numerous	• Round to columnar cells • Columnar cells may be abundant	• Sparse to absent M-EN • SIEC may be abundant • +/− hypocellular dense stromal frag • Often cystic or hemorrhagic background +/− calcs including psammoma bodies	• Cannot distinguish some PapCA from ductal papillomas • Some PapCa have more SIEC, more nuclear hyperchromasia, no stripped M-EN, and abundant columnar cells compared to papilloma	Radiology findings may be helpful

Mixed epithelial fibrous tumors	Low grade phyllodes tumor	Benign phyllodes tumor or FA	Similar to FA and benign phyllodes tumor	• Nuclear atypia usually mild • Mitotic figures rarely found in cytology specimen	• Cells range from cuboidal to columnar • Intermixed M-EN often present	• Cellular fibrous or myxoid matrix • Matrix may be sparse or absent • May have abundant stripped spindled nuclei	Cannot distinguish most examples of low grade phyllodes tumor from benign phyllodes tumor or FA	Rapid growth on radiology studies a clue to diagnosis of phyllodes tumor
	Low grade metaplastic carcinoma	• Scar • Deep fibromatosis (desmoid tumor)	• Cellularity low • Most cells aggregated in loose clusters	MNA	Cells mostly round to polygonal with rare spindle cells	M-EN absent	ICC can reveal keratin expression in the cells	Difficult to diagnose on cytology Clinical hx and radiology findings may be helpful
Sarcoma	Low grade angiosarcoma	• LGDC • FCC	• Cellularity varies • Loose non-polarized clusters and mats • No tubules or ducts	• Mild nuclear atypia (MNA) • Nuclei often spindle shaped	• Cells often spindled but oval or round cells can be present • Cytoplasm may be abundant and vacuolated • Cytoplasm may contain hemosiderin deposits	• Single intact spindle cells may be numerous • No M-EN • Background may be bloody with neutrophils	May not be diagnosable on FNA	MRI may be helpful
Inflamed carcinomas	Breast carcinoma with chronic inflammation	• Infarcted FA • Infarcted papilloma	• Cellularity varies • Clusters, small 3-D groups	• Nuclear atypia ranges from MNA to marked with vesicular nuclei and nucleoli • +/− mitoses	• Cells range in size but most are medium to large • N/C ratio medium to low	• Abundant lymphoplasmacytic infiltrate • +/− necrosis • SIEC often present	• Unequivocal malignant nuclear features are required to enable definitive diagnosis of malignancy • Infarcted benign lesion may have typical components also present	Radiology and clinical findings may be helpful
	Breast Ca with acute inflammation	• Infarcted FA • Infarcted papilloma • Abscess	• Cellularity varies • Clusters, small 3-D groups	Nuclear atypia often prominent with vesiculation, macronucleoli or coarse chromatin	As above	• Abundant neutrophils +/− eosinophils, lymphocytes • Necrotic cells and debris common	• Exercise extreme caution interpreting atypia in the setting of marked acute inflammation • Infarcted benign lesion may have typical components also present	Radiology and clinical findings may be helpful

(continued)

Table 1.3 (continued)

Mimicker/confounder		What it mimics	Architecture cellularity	Nuclear features	Cell/cytoplasmic features	Background	Differentiating features	Additional findings/steps
Hematopoietic lesions	Primary mammary lymphoma/leukemia	Intramammary lymph node	• Cellular • Dyscohesive and loosely clustered cells	• Usually no to minimal variation in nuclear size; most often nuclei are small and hyperchromatic	High N/C ratio	• Lack of tingible body macrophages • Lymphoglandular often bodies present	• Lack of polymorphous population • ICC may support cytologic impression of monotonous cell population • Flow cytometry may reveal monoclonality	Radiology often suggests the diagnosis
	Anaplastic large cell lymphoma next to breast implant	Seroma	• Cellular • Dyscohesive and loosely clustered cells	• Hallmark cells present: large cells with horseshoe, eccentric or kidney-bean shaped nuclei and eosinophilic paranuclear region • +/− macronucleoli • Mitoses common	• Hallmark cells have abundant pale to eosinophilic cytoplasm	• Background often includes lymphocytes, eosinophils and histiocytes	• Hallmark cells • ICC supportive • Flow cytometry supportive	Most commonly occurs 2–10 years after implant placement ICC: CD30 positive; may express EMA, ALK1 negative

Note: Descriptions are based on direct smears; monolayers and cell blocks may show other features

MNA mild nuclear enlargement, +/− small nucleoli, + mild variation in nuclear shape +/− mildly increased hyperchromasia, *FA* fibroadenoma, *M-EN* myoepithelial nuclei, *3-D frag* three dimensional epithelial fragment, *LFBS* large folded and or branching epithelial sheets with good polarity, *FVC* fibrovascular core, *SIEC* single intact epithelial cells, *Infl* inflammation, *LGDC* low grade ductal carcinoma, *HGDC* high grade ductal carcinoma, *PapCa* papillary carcinoma, *TubCa* tubular carcinoma, *CribCa* cribriform carcinoma, *LobCa* lobular carcinoma, *FCC* fibrocystic changes (includes florid ductal hyperplasia), *Ca* carcinoma, *CNB* core needle biopsy, *IHC* immunohistochemistry, *ICC* immunocytochemistry, *MNGC* multinucleated giant cells, *BI-RADS* Breast Imaging-Reporting AND Data System

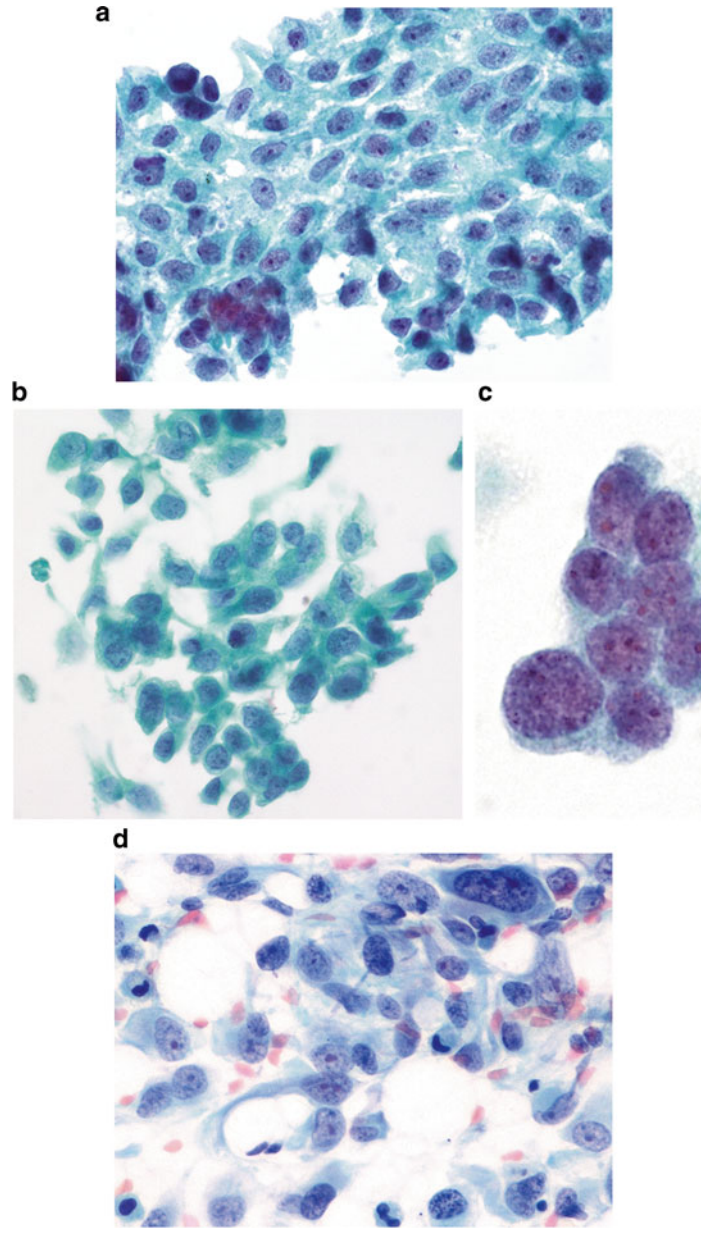


Fig. 1.2 Spectrum of nuclear atypia in breast lesions: (**a**) No nuclear atypia: lesional nuclei are small, uniform with fine evenly dispersed chromatin and inconspicuous nucleoli (Pap stain, direct smear); (**b**) Mild nuclear atypia: nuclei show mild enlargement and range in nuclear size and shape (Pap stain, monolayer), (**c**) Marked nuclear atypia: marked variation in nuclear size and shape with hyperchromasia and coarse blotchy chromatin (Pap stain, direct smear); (**d**) Marked nuclear atypia with vesicular nuclei and prominent nucleoli (Pap stain, direct smear)

variation in nuclear shape +/– mildly increased hyperchromasia) and few or no stripped bipolar nuclei but with intermixed benign clusters, a diagnosis of "atypical" should be rendered. Clinical follow-up with histologic evaluation should be recommended. Figure 1.4 provides an example of florid fibrocystic changes.

Fibroadenoma Approximately 5% of FAs contain epithelial groups with nuclear atypia which is usually only of focal mild degree and mixed with classic staghorn clusters (large folded branching sheets), bipolar stripped nuclei and cellular stromal fragments. The majority of fibroadenomas with mild nuclear atypia are

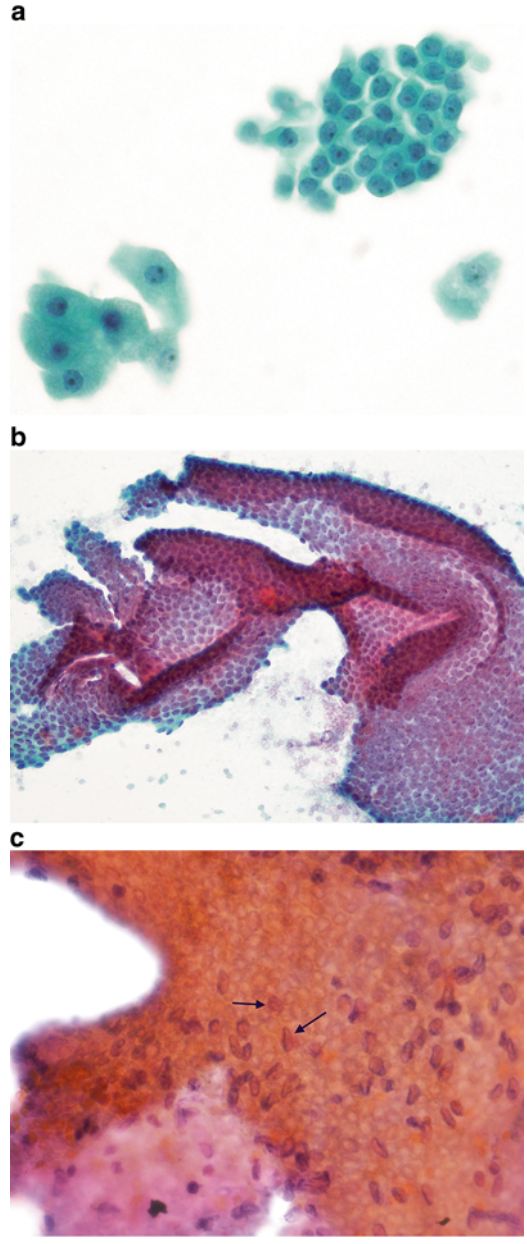


Fig. 1.3 Spectrum of architectural atypia in breast lesions: (**a**) Benign ductal cells forming a small polarized flat sheet and loosely clustered benign apocrine cells (Pap stain, monolayer); (**b**) benign large folded and branching complex sheet (Pap stain, direct smear); (**c**) *arrows* point to two of numerous myoepithelial nuclei present in a benign large folded sheet (Pap stain, direct smear); (**d**) atypical cluster with loss of polarity and nuclear crowding (Diff Quik stain, direct smear); (**e**) syncytial cluster with marked loss of polarity and ragged edges (Pap stain, direct smear); (**f**) degenerating loosely cohesive tumor cells with necrotic debris (Pap stain, direct smear); (**g**) syncytial group with numerous naked tumor nuclei in background that should not be mistaken for bipolar stripped myoepithelial nuclei (*arrows*) (Diff Quik stain, direct smear); (**h**) abundant single intact epithelial cells with no background bipolar stripped nuclei (Pap stain, monolayer)

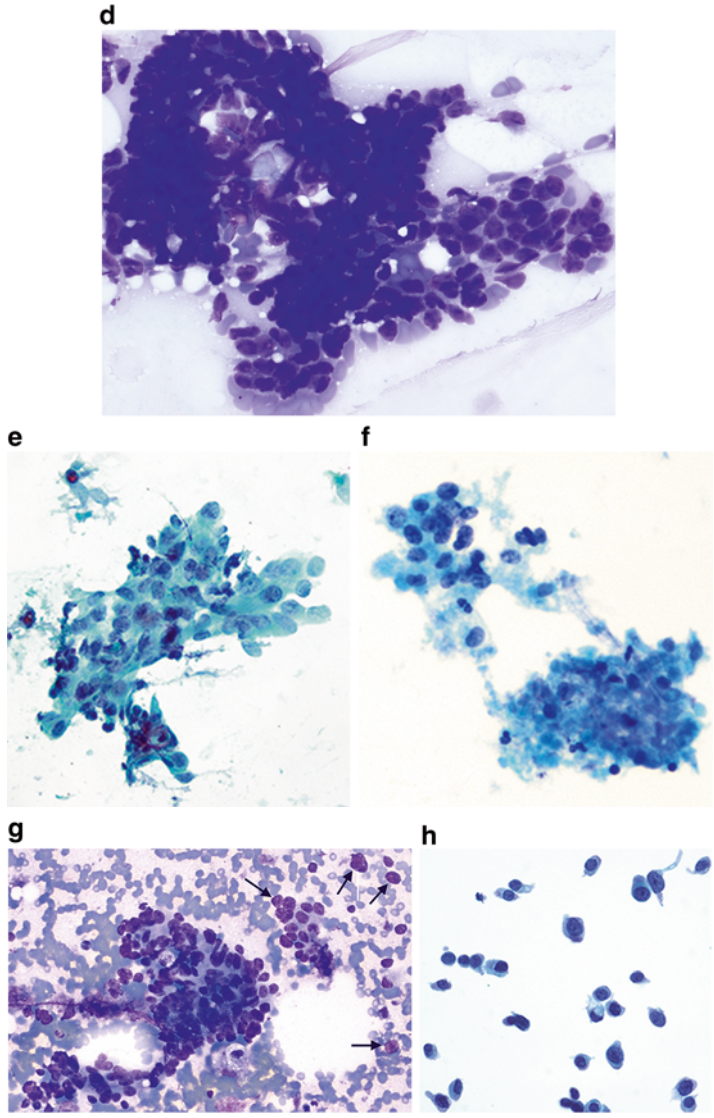


Fig. 1.3 (continued)

benign on resection. However, cases of FA with extensive mild or focal moderate to marked nuclear atypia should be diagnosed as "atypical" or "suspicious", because some of these will comprise DCIS or invasive carcinoma in or adjacent to the FA on tissue biopsy/resection. Importantly, some fibroadenomas lack stromal fragments on FNA but still can be called benign and suggestive of FA if large folded branching sheets are present and radiology is supportive. In practice, florid fibrocystic changes may be indistinguishable from FA on cytology, as both may contain large folded branching sheets and fragments of stroma. Figures 1.5 and 1.6 provide examples of fibroadenomas with and without mild nuclear atypia.

Sclerosing lobular hyperplasia (also termed fibroadenomatoid change) is reported as generally indistinguishable from FA on cytologic examination. A few examples may lack stromal fragments and the abundant number of stripped nuclei characteristic of FA. On imaging studies it can appear similar to FA.

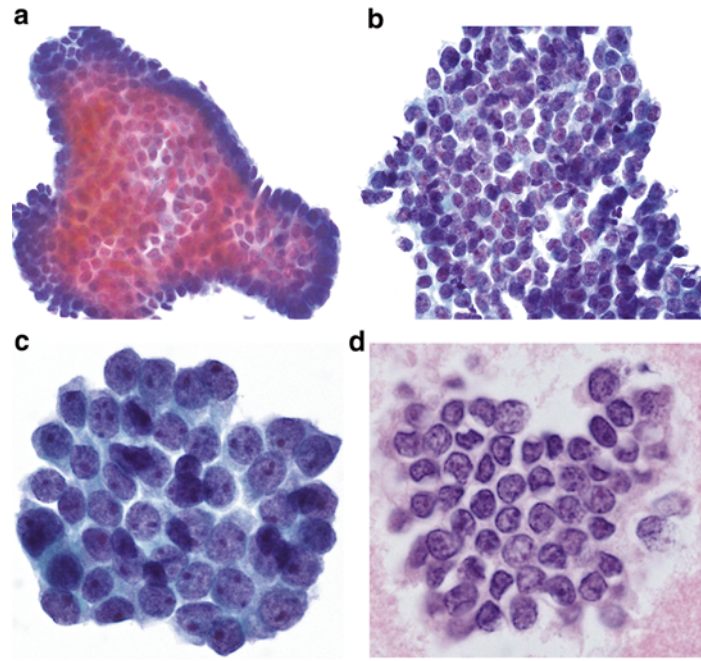


Fig. 1.4 Florid fibrocystic changes: 36 year old woman with BI-RADS 3 right breast mass. (**a**) 3-D group of tightly cohesive small cells with mild loss of polarity (Pap stain, monolayer); (**b**) mild nuclear atypia with nuclear crowding and overlap (Pap stain, monolayer); (**c**) reassuring benign appearing flat fragment with good polarity and intermixed myoepithelial nuclei (Pap stain, monolayer); (**d**) benign group with no significant nuclear atypia and good polarity (H&E stain, cell block)

Radial Scar/Complex Sclerosing Lesion These lesions look similar to florid fibrocystic changes but can also include fragments of dense, hypocellular stroma and glands with angulated contours resembling tubular carcinoma. Compounding diagnostic assessment is the common radiologic finding of a suspicious stellate lesion. Many examples will contain obviously benign components such as bland apocrine cells and benign clusters with intermixed myoepithelial (M-E) cells. However, a few cases with architectural atypia (crowded overlapping cells without intermixed M-E cells and with a few scattered isolated intact epithelial cells) and mild nuclear atypia (MNA as defined in the florid fibrocystic change paragraph above) may need to be referred for core needle biopsy for definitive diagnosis (and CNB diagnosis itself can be difficult often requiring immunohistochemistry to confirm the presence of myoepithelial cells in the pseudo-infiltrating glands and tubules).

Sclerosing Adenosis Most examples can be diagnosed as benign. However, those cases with angulated tubules, scant bipolar stripped nuclei (equated with myoepithelial nuclei), scattered intact epithelial cells and mild nuclear atypia (MNA) should be diagnosed as inconclusive (either atypical or suspicious) because they may be indistinguishable from low grade carcinoma, tubular carcinoma or DCIS colonizing sclerosing adenosis. Tubular carcinoma usually but not always is more cellular than sclerosing adenosis with a greater proportion of abnormal angulated and/or branched tubules with tapered ends and more abundant dyscohesive epithelial cells. Radiologic findings of sclerosing adenosis may include suspicious calcifications and BI-RADS scores of 4 and 5 in a few cases.

Microglandular Adenosis The cytology specimen may be cellular with small clusters of epithelium showing mild nuclear atypia (MNA) and few to no myoepithelial cell nuclei mimicking a low grade ductal carcinoma. However, dense rounded secretions within cell clusters are often present and are absent in most cases of ductal carcinoma.

Atypical Apocrine Metaplasia Apocrine cells often are found in cyst aspirates. Despite nuclear enlargement, variability in nuclear size and

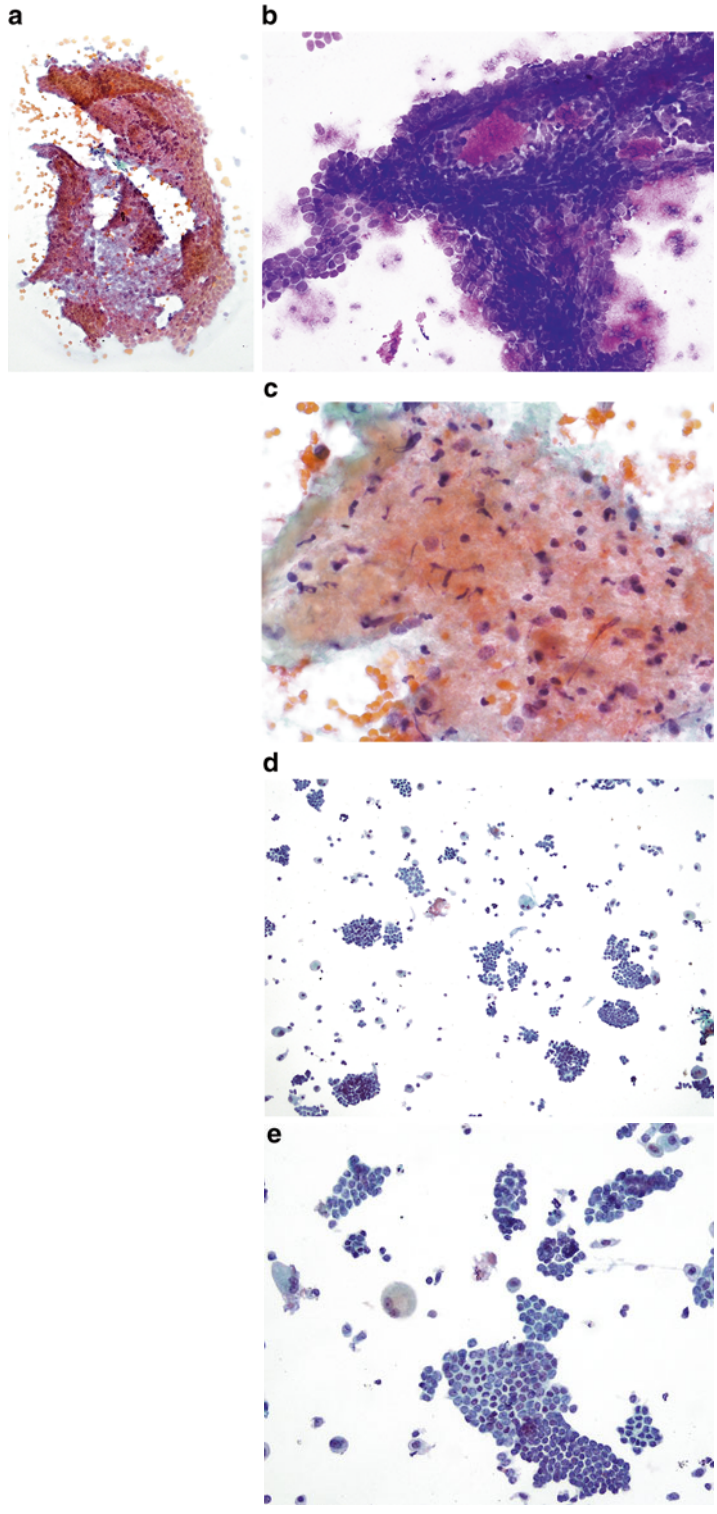


Fig. 1.5 Fibroadenoma showing varying appearances on direct smears and monolayer: (**a**) Large complex folded sheet typical of FA (Pap stain, direct smear); (**b**) metachromatic myxoid stroma that can be seen in some FA and can simulate mucin (Diff Quik stain, direct smear); (**c**) appearance of myxoid stroma on Pap stain (direct smear); (**d**) The large complex folded sheets on the direct smears are small and much more fragmented on the monolayer (Pap stain); (**e**) The monolayer at higher magnification shows fairly numerous individual intact epithelial cells and only rare stripped myoepithelial nuclei. These findings might be worrisome without knowledge of the disruptive effects of the monolayer preparation

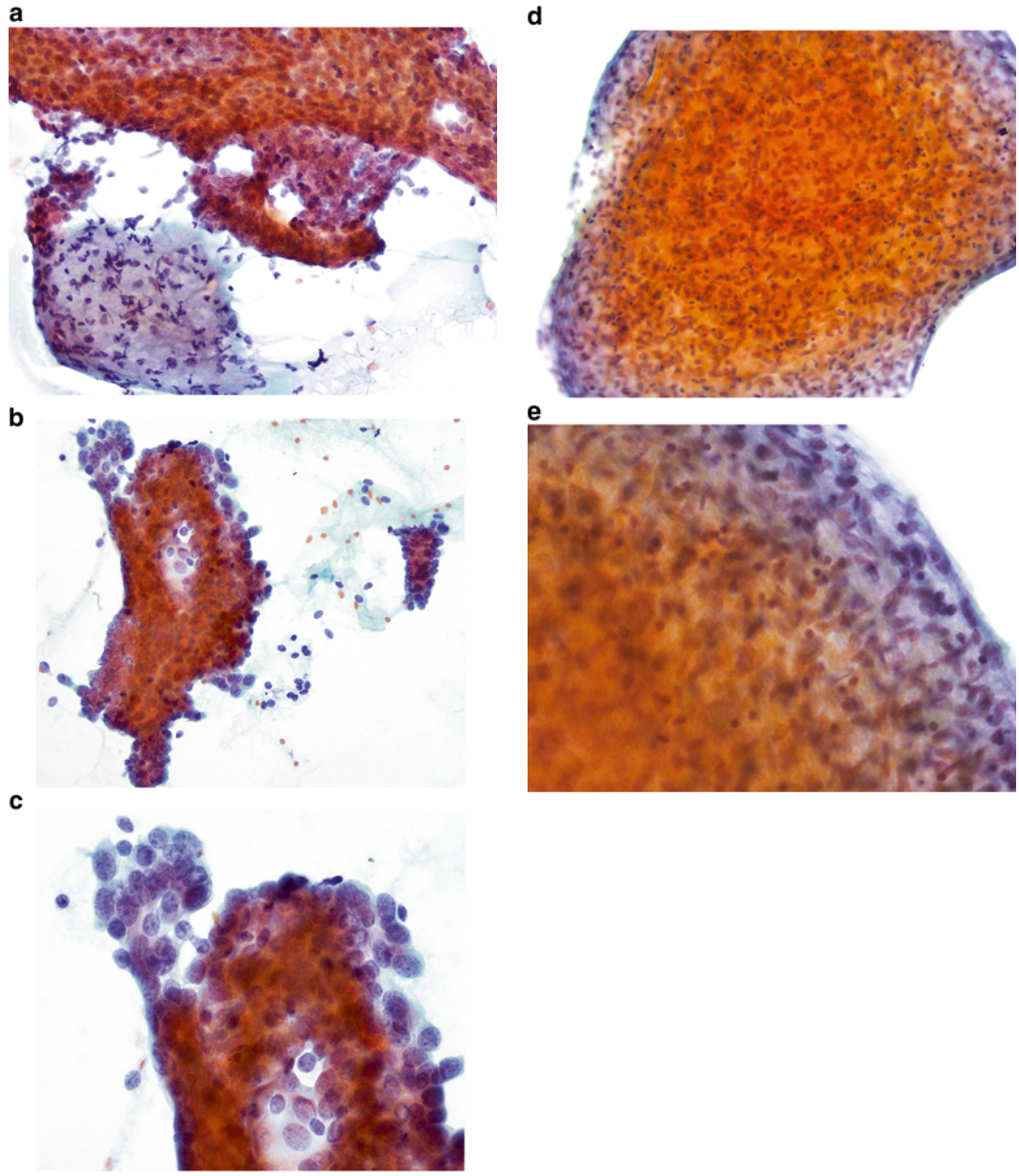


Fig. 1.6 Fibroadenoma with focal mild nuclear atypia: 41 year old woman with bilateral well circumscribed breast nodules consistent with fibroadenomas on mammograms (BI-RADS score 2). (**a**) Typical large folded sheet without MNA and dense stroma fragment (Pap stain, direct smear); (**b**) a minority of the specimen contained folded sheets with increased nuclear atypia; however, a reassuring finding is many stripped bipolar nuclei in the background; (**c**) higher magnification of atypical group shows mild nuclear atypia comprising mild range in nuclear size, mild hyperchromasia and slightly coarse chromatin; (**d**) some stromal fragments are hypercellular (Pap stain, monolayer), but (**e**) show no evidence of nuclear atypia in the stromal cells as would be present in a malignant phyllodes tumor (a benign phyllodes tumor is not able to be excluded based on these images). According to a recent study, approximately 80 % of FA with atypia are benign on resection (usually with florid fibrocystic changes in or adjacent to the FA). However, FA with diffuse mild or marked nuclear atypia should be diagnosed as atypical or suspicious and may contain DCIS or low grade invasive carcinoma on resection

prominent nucleoli, when these cells derive from a simple unilocular cyst, the lesion has a >99 % follow-up benign diagnosis. However, atypical apocrine metaplasia can occur mixed with other lesions such as FA, radial scar, sclerosing adenosis and FCC. It may also rarely form a small mass or multiple masses identified by screening mammography. Cytologic features include anisonucleosis, macronucleoli and granular cytoplasm. Inflammation may be present. Differentiation from apocrine DCIS can be difficult, although the former often also has comedo type necrotic debris and grade 3 nuclear atypia (including coarse granular chromatin, irregular chromatin distribution, marked variation in size and shape of nuclei and sharp angled notches). On the opposite end of the spectrum, differentiation from low grade apocrine carcinoma can also be challenging. Intermixed, obviously benign elements are a clue to the diagnosis of atypical apocrine metaplasia.

Atypical Ductal Hyperplasia (ADH) ADH cannot be accurately diagnosed by cytology. The features may overlap with fibrocystic changes (and often ADH is associated with florid fibrocystic changes in a tissue resection). An inconclusive diagnosis is appropriate in samples with some atypical features mixed with benign elements.

Gynecomastia Most cases of gynecomastia can be accurately diagnosed on cytology and clinical presentation. FNA may exhibit slightly increased nuclear atypia with increased epithelial cell dyscohesion. These may be interpreted as "atypical". As usual, clinical and radiologic features contribute to correct interpretation of the cytologic features. Figure 1.7 provides an example of gynecomastia with dyscohesion that could be misinterpreted as suspicious for malignancy.

Gynecomastoid Change This lesion mimics gynecomastia histologically but occurs in women. It does not usually form a mass. However, cytologic features simulating gynecomastia have been reported in a patient with Turner syndrome and bilateral breast nodules.

Lactating Adenoma Smears are often cellular and strewn with many round bare epithelial nuclei containing single prominent nucleoli. A lipid laden background (on direct smears) and fragments of multiple small balls of epithelium or even entire lobules complete with fibrovascular supporting tissue are also characteristic. Monolayer preparations are described as showing lacy fragments with tissue paper quality and globular clumps of milky background material containing bare epithelial nuclei with cherry red macronucleoli. Radiologic findings and clinical history are helpful in confirming the diagnosis. Figure 1.8 provides an example of lactating adenoma.

Large Duct Papilloma Most duct papillomas are correctly diagnosed as benign lesions. A few cases (~5–10 %) may have features that raise the possibility of low grade carcinoma, papillary or otherwise. These atypical lesions feature increased cellularity, mild nuclear atypia (MNA), and increased intact single epithelial cells. Benign features are also usually present. Cases with atypia should be signed out as "atypical" if radiology is not conclusively benign. A background of foam cells, bipolar stripped nuclei (myoepithelial nuclei), and bland apocrine cells suggests a benign diagnosis. An example of a benign cyst with pseudopapillary structures mimicking large duct papilloma is provided in Fig. 1.9.

Nipple Adenoma Cytologic features are not well reported. However, one study noted that the lesions are often cytologically diagnosed as atypical or suspicious. Correlation with imaging should be helpful.

Malignant Tumors that May Be Difficult to Diagnose Conclusively on Cytology (Confounders)

Low Grade Ductal Carcinoma, Not Otherwise Specified Well-differentiated ductal carcinoma can appear similar to ADH and florid epithelial hyperplasia on cytology. Intermixed benign elements should prompt a cautious diagnosis of

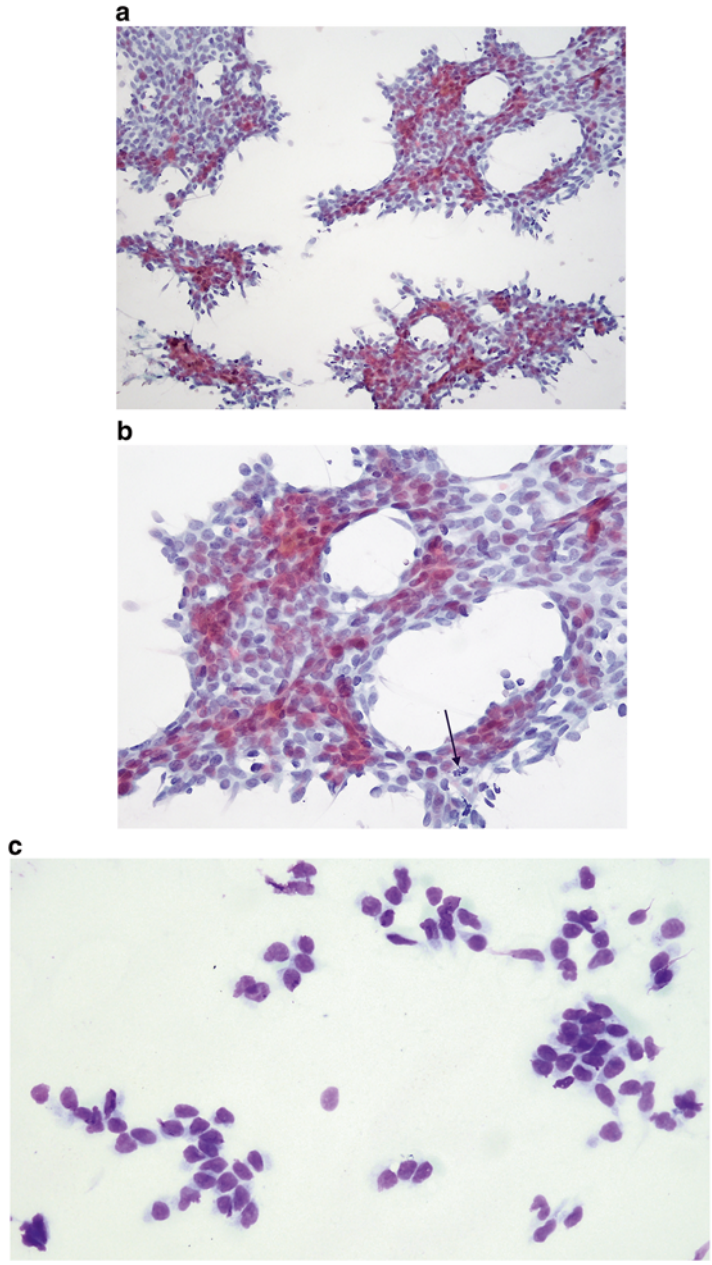


Fig. 1.7 Gynecomastia with dyscohesion called suspicious on FNA: 50 year old man with a 1 cm retroareolar mass. (**a**) The FNA is hypercellular with many fenestrated clusters showing mild loss of polarity and absent myoepithelial cells (Pap stain, direct smear); (**b**) the lesion is mitotically active (*arrow*), although most groups show little nuclear atypia (Pap stain, direct smear); (**c**) Dyscohesion is present which can be a worrisome finding (Diff Quik, direct smear); (**d**) some of the more dyscohesive cells exhibit mild nuclear atypia (Diff Quik stain, direct smear). Excision revealed the active phase of gynecomastia with marked hyperplasia and mitotic activity

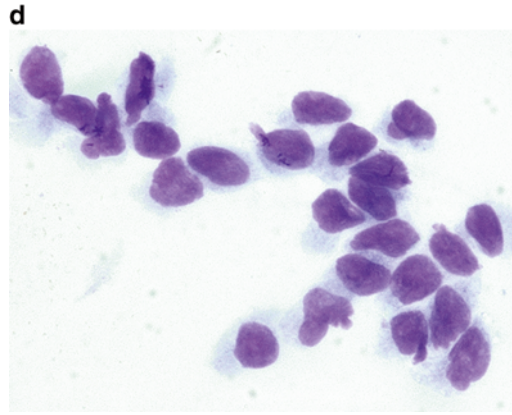


Fig. 1.7 (continued)

"atypical" or "suspicious". Small carcinomas (1 cm or less) may be contaminated by adjacent fibrocystic change, so the finding of benign components is not always an indicator of overall benignancy. An example of low grade ductal carcinoma is provided in Fig. 1.10.

Tubular Carcinoma According to several reviews, tubular carcinoma is usually hypercellular with more rigid, angulated, and tapered branching tubules than its histologic mimics of florid FCC, radial scar/complex sclerosing lesion and sclerosing adenosis. Most cases will also have abundant intact isolated or doublet epithelial cells. Nuclear atypia ranges from mild to moderate. Some cases may show sparse cellularity and may be confused with a benign lesion. A few cases may contain fragments of elastotic stroma, a non-specific finding that can also be seen in benign entities such as sclerosing adenosis. A classic sharply angulated tubule of tubular carcinoma is illustrated in Fig. 1.11.

Lobular Carcinoma Lobular carcinoma accounts for 7–20 % of all cytologic inconclusive diagnoses in some reviews. Cytology samples may be under-diagnosed because of sparse cellularity and only mild nuclear atypia. Close attention to small cells with an elevated N/C ratio and nuclei with increased complexity (folds, wrinkles, nipples) can suggest the diagnosis. Scattered small cells with mucin vacuoles, some suggesting signet ring cells, should prompt careful evaluation of nuclear features. However, not all lobular carcinomas have such mucinous cells and degenerated benign cells can have single large cytoplasmic vacuoles. Correlation with radiologic imaging and clinical history can be helpful. The pleomorphic variant of lobular carcinoma is more readily diagnosed as malignant because of marked nuclear atypia, but sparsely cellular specimens may be diagnostically challenging. Figure 1.12 illustrates features of invasive lobular carcinoma.

Others Adenoid cystic carcinoma can be mistaken for collagenous spherulosis or myospherulosis. Immunocytochemistry can be used to resolve the diagnosis (c-kit positive and calponin negative in adenoid cystic carcinoma versus the opposite in collagenous spherulosis). Secretory carcinoma and cystic hypersecretory carcinoma could be mistaken for lactational change in a benign epithelial proliferative lesion. Radiologic and clinical correlation is usually helpful.

Mixed Epithelial and Fibrous Lesions

Benign Lesions that Mimic Carcinoma (Mimics of Malignancy)

Fibroadenoma with Myxoid Features (Myxoid or Mucinous FA) This could be confused with colloid (mucinous carcinoma). However, most

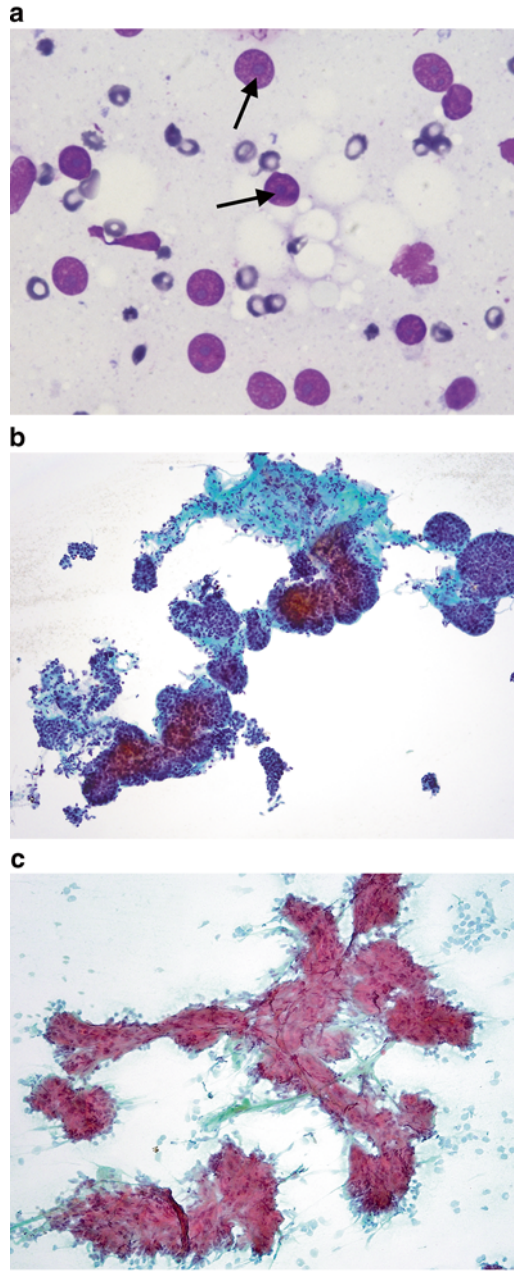


Fig. 1.8 Lactating adenoma: 23 year old pregnant woman with new breast mass. (**a**) The ductal cells are fragile and easily burst leading to stripped round nuclei with single prominent nucleoli (*arrows* point to nucleoli) floating in a proteinaceous, lipidic background (Diff Quik, direct smear); (**b**) whole lobules can be aspirated made of bulbous terminal ductules and supporting intralobular stroma (Pap stain, direct smear); (**c**) Sometimes the supporting fibrovascular stroma is denuded of the fragile hyperplastic epithelial cells which instead splay across the slide (Pap stain, direct smear); (**d**, **e**) groups can appear atypical with ragged edges, loss of polarity, inconspicuous myoepithelial nuclei, and high N/C ratio with nuclear hyperchromasia (Pap stain, direct smear); (**f**) proteinaceous background appears thicker and more clumped on the monolayer but still contains characteristic intermingled intact and stripped epithelial nuclei (Pap stain); (**g**) the cell block shows a single population of foamy epithelial cells with macronucleoli that might be mistaken for a neoplastic process (H&E stain)

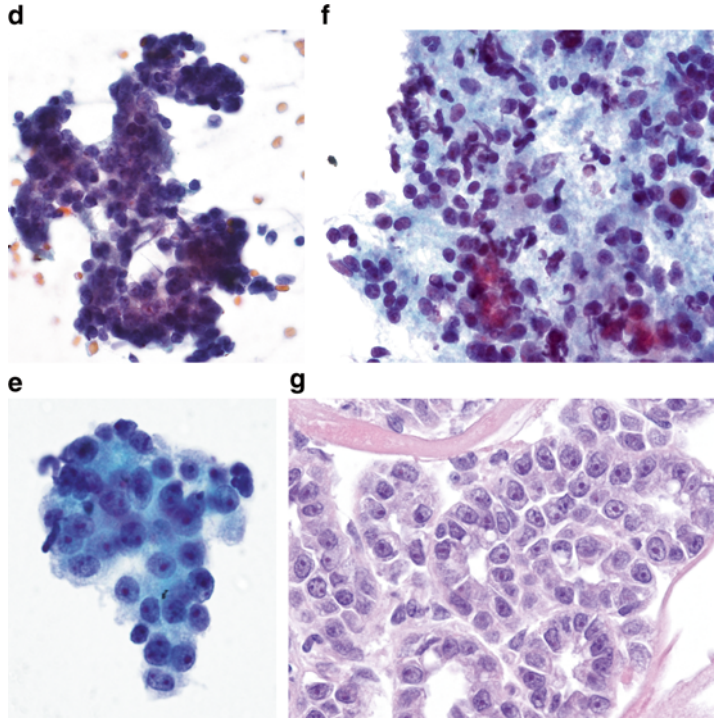


Fig. 1.8 (continued)

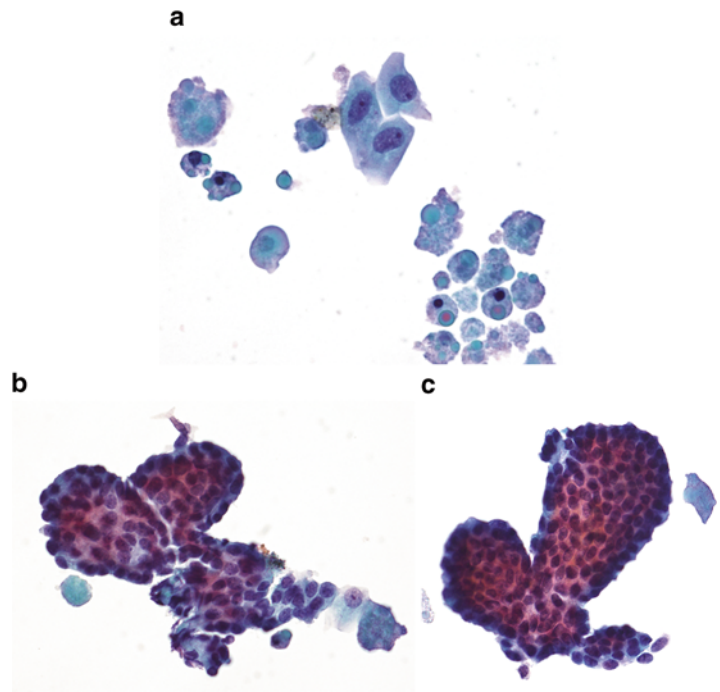


Fig. 1.9 Benign cyst with pseudopapillary structures: 49 year old woman with a simple cyst (BI-RADS 2) lesion. (**a**) Macrophages, proteinaceous debris and benign appearing apocrine cells account for most of the aspirate (Pap stain, monolayer). (**b**, **c**) Bulbous pseudopapillary ductal epithelium (Pap stain, monolayer). The presence of pseudopapillae in a radiologically simple cyst is acceptable for a benign lesion and does not require excision

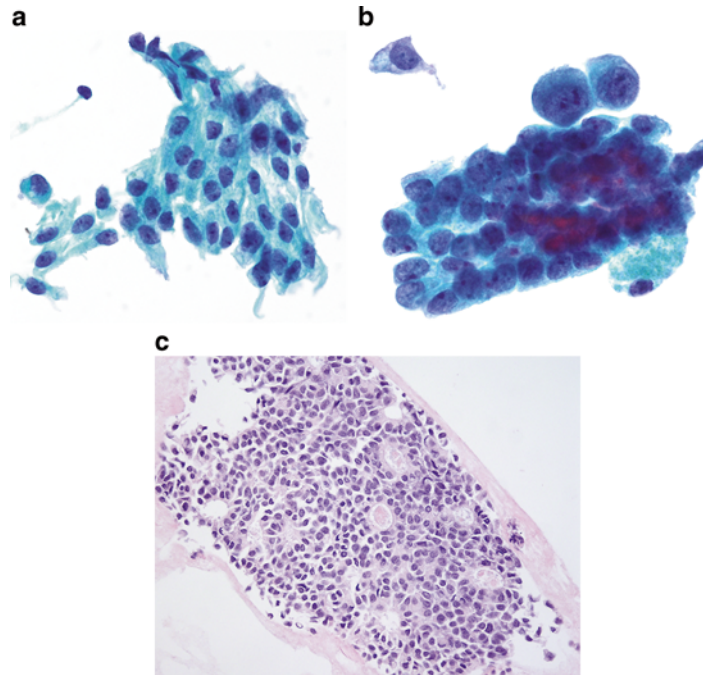


Fig. 1.10 Low grade invasive ductal carcinoma: 53 year old woman with breast mass and BI-RADS 4 score. (**a**) Small sheet of ductal cells without myoepithelial cells but with deceptively bland nuclei (Pap stain, monolayer); (**b**) tubule with mild nuclear crowding and adjacent large single intact epithelial cells with more pronounced atypia (Pap stain, monlayer); (**c**) cell block reveals low grade cribriforming monotonous sheets of cells (H&E; cell block section). Resection showed low grade invasive cribriform carcinoma and comedo DCIS

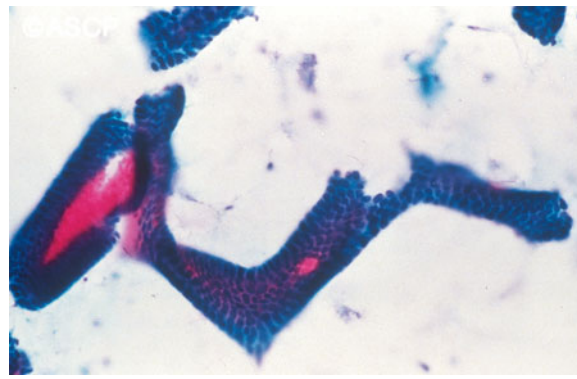


Fig. 1.11 Tubular carcinoma: classic appearance of neoplastic tubules showing rigid sides and sharp angled branching with only mild nuclear atypia (Pap stain, direct smear; courtesy of ASCP). Tubular carcinoma can be diagnosed if these angulated tubules are the sole cell population, single intact tumor cells are present, stripped bipolar myoepihthelial nuclei are absent and radiology is supportive

cases also feature classic large, complex, folded sheets of minimally atypical epithelial cells and abundant stripped bipolar nuclei +/− spindled nuclei. The myxoid component is strandy and mucicarmine negative in FA compared to the matrix in colloid carcinoma. On DQ staining, the matrix of myxoid FA is reported to be more prominently metachromatic than the matrix of colloid carcinoma.

Benign Phyllodes Tumor These may exhibit mild nuclear atypia (MNA), although most are diagnosed on FNA as a fibroadenoma. The presence of abundant stripped bipolar nuclei should support a benign diagnosis.

Adenomyoepithelioma This is a rare benign mixed myoepithelial/epithelial tumor that produces hypercellular aspirates with epithelial groups and

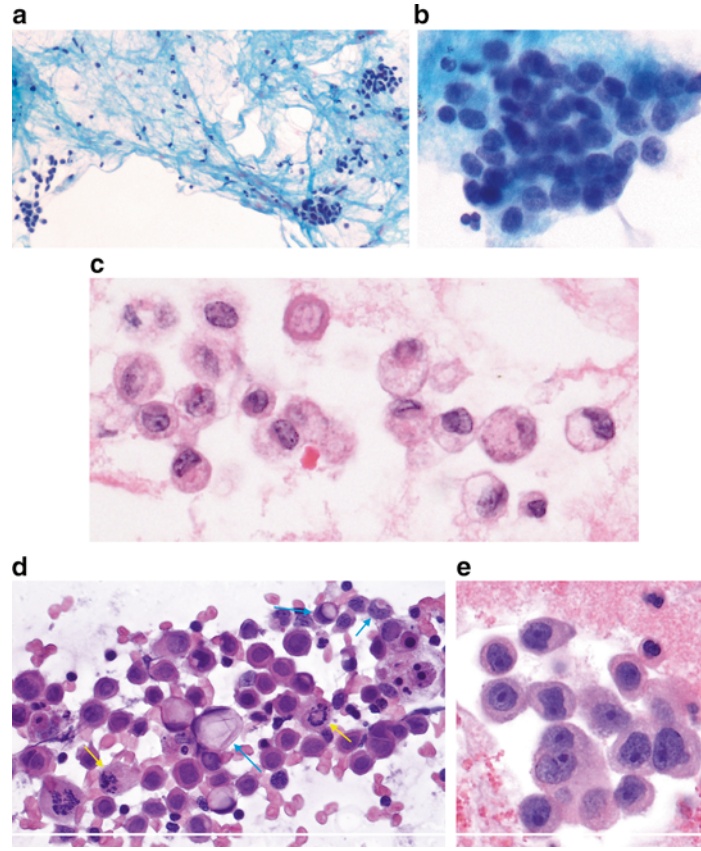


Fig. 1.12 Lobular carcinoma: suspicious breast mass in a 69 year old woman. (**a**) Classic invasive lobular carcinoma: Tumor cells are sparse and loosely cohesive (Pap stain, direct smear); (**b**) high magnification shows a loose syncytium of small cells with minimal nuclear atypia (Pap stain, direct smear); (**c**) small dyscohesive tumor cells with eccentric nuclei (H&E stain, cell block section); (**d**) pleomorphic lobular carcinoma with obvious malignant nuclear features, a few cells with intracytoplasmic mucin (*blue arrows*) and several mitotic figures (*yellow arrows*) (rehydrated H&E stain, direct smear); (**e**) same patient, cell block specimen showing similar dyscohesive highly atypical cells (H&E stain, cell block section)

spindle cells. The spindle cells may be intact but dispersed singly. Necrosis may be present. Intranuclear cytoplasmic inclusions should prompt consideration of the diagnosis. Immunocytochemistry can be performed to demonstrate the dual nature of the lesion (myosin heavy chain, smooth muscle actin, keratin 5/6 and p63 reactivity in the ME cells and ER receptor positivity in the epithelial cells) and suggest the diagnosis.

Pseudoangiomatous Stromal Hyperplasia (PASH) This is a relatively common lesion but with few reports of the cytologic findings. It is reported to resemble fibroadenoma.

Malignant Tumors that May Be Difficult to Diagnose Conclusively on Cytology (Confounders)

Low Grade Phyllodes Tumor Cytologic features overlap considerably with fibroadenoma and benign phyllodes tumor. The diagnosis could be suggested in the setting of abundant stripped *spindled (rather than bipolar)* nuclei and hypercellular stromal fragments. Clinical and radiologic features can be helpful.

Scirrhous Carcinoma (Ductal Carcinoma with Marked Desmoplasia) These tumors can be sparsely cellular. Caution is warranted with a hypocellular sample especially in the setting of a BI-RADS 4 or 5 radiologic interpretation. Dense, hypocellular sclerotic fragments may be present, but are not specific.

Low Grade Adenosquamous Carcinoma This clinically indolent tumor may be mistaken for a benign lesion not only in a cytology sample but also in a core needle biopsy specimen. The following features are noted: irregularly clustered cell groups, minimal nuclear atypia, no necrosis and no mitotic activity with metaplastic spindle cells and keratinous debris. Although not specific, the last two components should prompt consideration of the diagnosis.

Papillary Lesions

Benign Lesions that Mimic Carcinoma (Mimics of Malignancy)

Intraductal Papilloma Most cases can be diagnosed as benign. A few cases will feature numerous intact single epithelial cells. These cases should not be interpreted as malignant in the absence of other malignant features. Unfortunately, true papillary lesions of the breast may lack diagnostic 3-D fragments with fibrovascular cores. When they are present, the diagnosis of a papillary lesion can be made, and the lesion can be confirmed as benign if nuclear atypia is absent. A few papillomas may be associated with collagenous spherulosis, a possible clue to the diagnosis.

Papillary Hyperplasia in FCC A low power appreciation of mixed benign components including bland epithelial clusters with ME cells, stripped bipolar naked nuclei, and benign apocrine cells should prevent a misdiagnosis of papillary carcinoma.

Malignant Tumors that May Be Difficult to Diagnose Conclusively on Cytology (Confounders)

Papillary Carcinoma In many examples, the unequivocal diagnosis of malignancy is not achievable due to overlap of features with ductal papilloma. However, the diagnosis can be suggested when the following components are present: (1) 3-D groups of cells without ME cells and with fibrovascular cores, (2) hyperchromatic nuclei compared to duct papilloma, (3) abundant columnar to round, intact, dispersed epithelial cells, (4) absence of bipolar stripped nuclei, +/− (5) stripped nuclei that are round and *of various sizes*.

Lesions with Mucin

Benign Lesions that Mimic Carcinoma (Mimics of Malignancy)

Mucocele Like Lesion (MLL)) Compared to colloid carcinoma, these lesions are hypocellular and contain mucin *without* embedded, thin, branching blood vessels. However, FCC may accompany MLL and cause greater cellularity. Mammography screening-detected MLL differs from symptomatic MLL in that it can show microcalcifications and occurs in postmenopausal patients. The microcalcifications can be scored as atypical or suspicious on radiology. Calcifications can also be seen in colloid carcinoma, both on imaging and in the cytology specimen. Some MLL are associated with ductal carcinoma in situ, so significant nuclear atypia should lead to a diagnosis of "atypical" or "suspicious" depending on the quantity and degree of atypia.

FCC with Mucinous Cells or Mucin Other features of FCC are present.

Ductal Adenoma with Mucinous Cells These can be mistakenly diagnosed as "suspicious" due to hypercellularity and mucin containing cells combined with the radiologic impression of a mass. However, benign components are also present such as apocrine cells, ME cells in the epithelial groups and bipolar stripped naked nuclei.

Rare Infarcts or Abscesses with Pseudo-Mucinous Cells Fat necrosis can release lipids that may be engulfed by macrophages. These should not be interpreted as signet ring cells. Inflammation and reactive fibroblasts are clues to the diagnosis, and the radiologic impression is usually BI-RADS 1 or 2 (negative or benign).

Malignant Tumors that May Be Difficult to Diagnose Conclusively on Cytology (Confounders)

Some Colloid (Mucinous) Carcinomas Most colloid carcinomas are moderately to highly cellular with at least mild nuclear atypia and dispersed intact epithelial cells and few to no stripped bipolar naked nuclei. The mucin is described as often containing branching, thin-walled vessels. However, a sparsely sampled tumor may cause confusion with MLL. Cases with all the above features can be diagnosed as malignant. However, an unequivocal specific diagnosis of colloid carcinoma should not be made on cytology, as some carcinomas are mixed

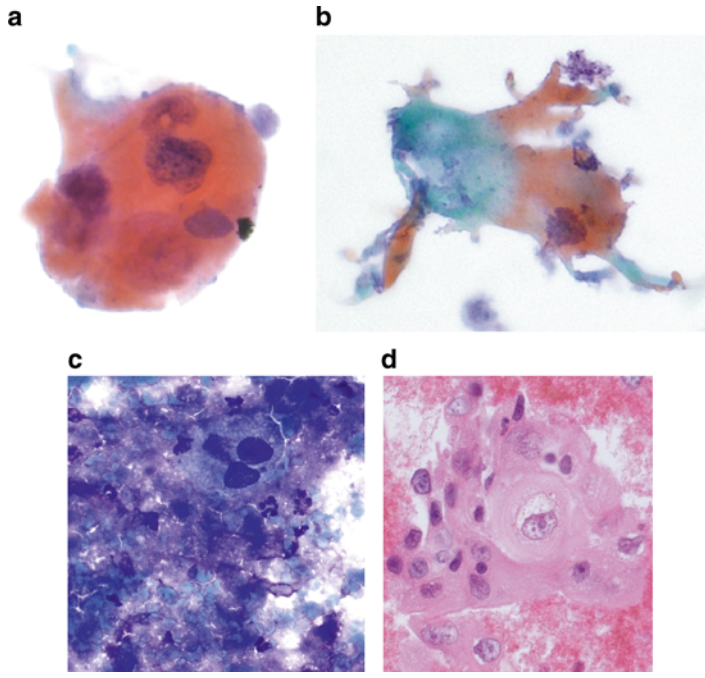


Fig. 1.13 Metaplastic carcinoma: 55 year old woman with metaplastic breast carcinoma with high grade nuclear features and areas of squamous differentiation. (**a**, **b**) Scattered, degenerated atypical squamous cells (Pap stain, monolayer); (**c**) malignant squamous cell floating among granular debris (Diff Quik stain, direct smear). (**d**) Flat plate-like group of malignant cells with vague cytoplasmic lamellations consistent with squamous differentiation (H&E stain, cell block section)

mucinous/ductal carcinoma. Undersampling of the conventional ductal carcinoma component could cause an erroneous diagnosis of pure colloid carcinoma. Figure 1.13 provides an example of mucinous carcinoma.

Mesenchymal Lesions and Non-mesenchymal Spindle Cell Only Lesions

Benign Lesions that Mimic Carcinoma (Mimics of Malignancy)

Mammary Myofibroblastoma This is an uncommon tumor that may preferentially occur in men. Cytology specimens are typically cellular with dispersed and aggregated spindle to plump to oval cells with up to moderate nuclear atypia. The groups also contain blood vessels and metachromatic stroma. Nuclear pseudoinclusions and mast cells may be present. Epithelial elements are absent. Cases may be mistakenly signed out as suspicious. Immunocytochemistry (CD34 and desmin positive) and radiologic findings can be diagnostically helpful.

Nodular Fasciitis The lesion is typically small, rapidly growing and more superficial. A mix of lymphocytes and round to spindle cells with varying amounts of heterogeneous stromal matrix is characteristic. Marked nuclear atypia and epithelial elements are absent. Mitoses may be identified.

Desmoid Tumor (Deep Fibromatosis) Desmoid tumors may occur in the breast. Most patients are young to middle aged adults. Smears may be cellular comprising plump to elongate spindle cells

without nuclear atypia. Mast cells may be present. Epithelial components are absent. Immunocytochemistry for beta-catenin may reveal diagnostic, abnormal nuclear expression in some of the tumor cells.

Granular Cell Tumor (GCT) This tumor is most often confused with apocrine lesions of the breast. It most commonly presents as a palpable breast mass in young to middle-aged women. It can occur in the deep tissue of the breast and radiologically can show worrisome features such as spiculation, indistinct margination and acoustical shadowing. FNA smears contain loose clusters and single intact granular cells with small round to oval nuclei. Stripped tumor nuclei and granular cell contents can be strewn in the background. The absence of bipolar stripped naked (ME) nuclei but presence of dispersed intact single tumor cells and mild nuclear atypia with range in size and shape can strongly suggest a diagnosis of low grade apocrine carcinoma. Awareness of this diagnostic possibility in the setting of what appears to be a pure apocrine epithelial population should prompt performing differentiating antibody staining. GCT cells express S-100 protein and do not express keratins.

Others Other benign mesenchymal tumors may rarely occur in the breast such as schwannoma and neurofibroma. These are usually superficial, comprise spindled to oval cells and lack significant nuclear atypia. Clinical history and immunocytochemistry can be diagnostically helpful.

Malignant Tumors that May Be Difficult to Diagnose Conclusively on Cytology (Confounders)

Low Grade Metaplastic Carcinoma Even on tissue sections, low grade metaplastic carcinoma may be difficult to distinguish from exuberant scar tissue or fibromatosis. Subtle nuclear atypia may be present. The specimen may be hypocellular. Older patient age and high BI-RADS score with immunocytochemical staining revealing keratin expression are diagnostically helpful. For contrast, a high grade metaplastic carcinoma is illustrated in Fig. 1.14.

Low Grade Angiosarcoma These can occur de novo, most commonly in young adult women or may occur after breast-conserving therapy. Smears may be scant to cellular and may primarily contain rounded or oval tumor cells with minimal nuclear atypia. Spindled tumor cells may be rare or absent. Unfortunately, helpful cytologic findings of neoplastic vessels or tumor cells with intracytoplasmic vacuoles containing red blood cells are usually lacking. In the setting of a history of breast carcinoma, such lesions could be mistaken for recurrent carcinoma. In young women, the lesion could be interpreted as benign. Immunocytochemistry revealing vascular marker (CD34, CD31) expression may be suggestive, but tissue biopsy is required to make the correct diagnosis.

Apocrine carcinoma could be interpreted potentially as apocrine metaplasia with atypia. However, nuclear atypia is marked, necrosis is often present, and by imaging, a high BI-RADS score has usually been rendered. Figures 1.15 and 1.16 provide examples of apocrine carcinoma and apocrine metaplasia with superimposed infarction, respectively.

Lesions with Inflammation

Benign Lesions that Mimic Carcinoma (Mimics of Malignancy)

Spontaneous Infarct with Reactive Atypia in a Benign Lesion Spontaneous infarction can occur in fibroadenomas, large duct papillomas and other benign lesions. Worrisome findings include dyscohesion, necrosis, ghost cells and reactive nuclear changes. Reassuring findings include typical features of the underlying lesion, smudgy or pyknotic nuclei, and inflammation. Clinical correlation and imaging features can be helpful.

Fat Necrosis/Traumatic Infarct Most examples are superficial, central and occur in large pendulous breasts. Clinical findings can be misleading, as patients may also have lymphadenopathy and may not recollect trauma. Radiologic findings can be worrisome including showing

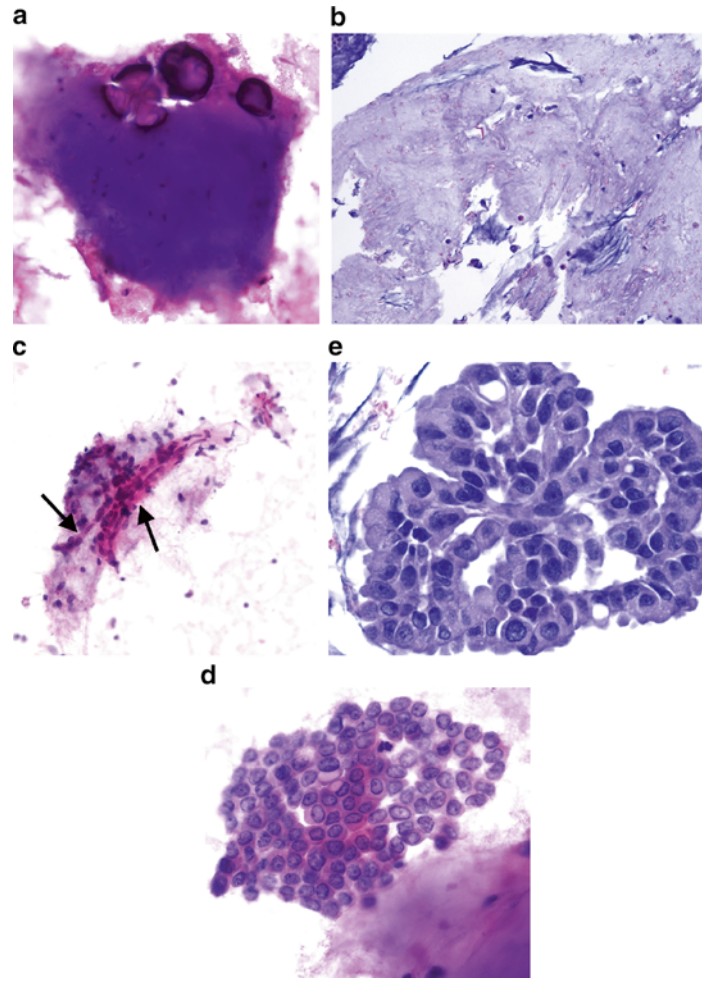


Fig. 1.14 Mucinous carcinoma: 67 year old woman with a BI-RADS 5 12 cm breast tumor. (**a**) Abundant mucin with scattered psammoma bodies is present (H&E stain, direct smear); (**b**) the mucin lacks branching thin-walled vessels reported to be common in mucinous carcinoma and a diagnostic clue but missing in this patient's tumor (H&E stain, direct smear); (**c**) for comparison, another patient with colloid carcinoma in which the mucin does contain blood vessels (*arrows*) (H&E stain, direct smear); (**d**) some groups show only mild nuclear atypia (H&E stain, direct smear); (**e**) floret like arrangement of tumor cells showing moderate nuclear atypia (H&E stain, cell block section). The tumor expressed ER and PR and was HER2 negative by immunohistochemistry

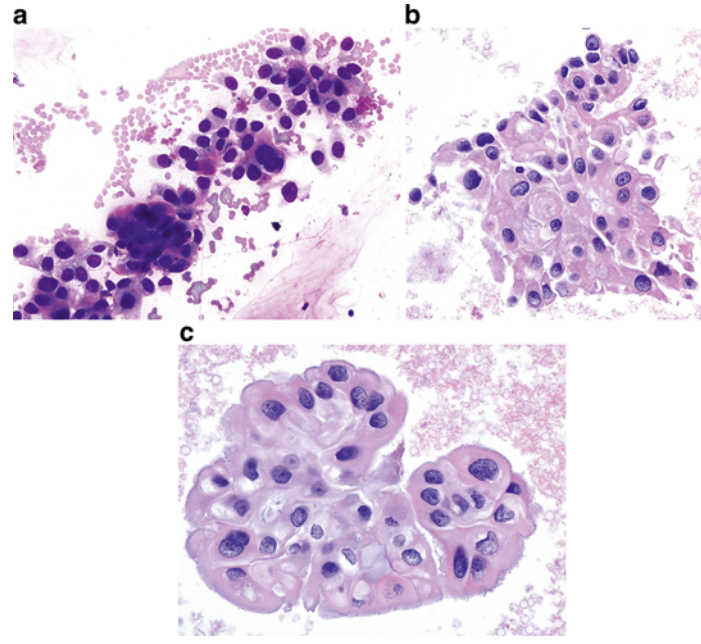


Fig. 1.15 Apocrine carcinoma: 59 year old woman with a 10 cm ulcerated breast mass, long neglected. (**a**) The tumor is cellular with loosely cohesive tumor cells with marked range in nuclear size and abundant cytoplasm (Diff Quik stain, direct smear); (**b**) tumor cell cytoplasm is eosinophilic and granular on the cell block (H&E stain); (**c**) The degree of nuclear atypia is moderate but is not greater than can be seen in some atypical apocrine metaplastic lesions (H&E stain; cell block section). The tumor was negative for ER, PR and HER2 expression by immunohistochemistry

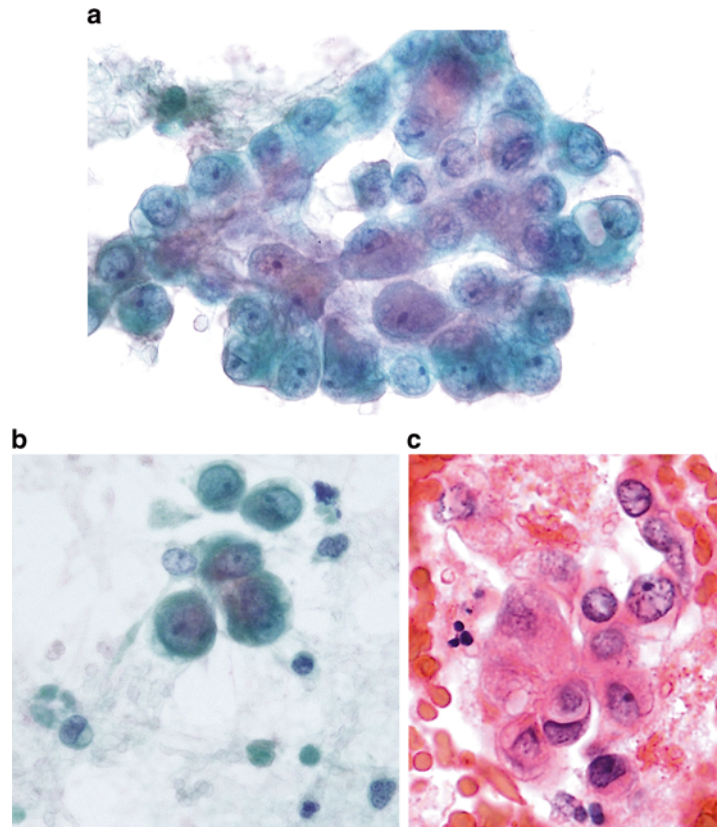


Fig. 1.16 Apocrine metaplasia with degenerative atypia and infarction: 35 year old woman with 2 cm, BI-RADS 3, complex breast mass. (**a**) Clusters of degenerating apocrine cells in a bloody, debris filled background (Pap stain, direct smear); (**b**) individual apocrine cells are present mixed with degenerated inflammatory cells and blood; (**c**) a few groups of variably preserved and degenerated apocrine cells are present on the cell block (H&E stain). The differential comprises atypical apocrine metaplasia and apocrine carcinoma. The excision showed an infarcted focus of florid apocrine metaplasia

suspicious calcifications. Usually, these lesions can be recognized on cytology as they typically show degenerating fat, cellular debris, foamy histiocytes and occasionally multi-nucleated giant cells.

Mammary Duct Ectasia The findings are similar to fat necrosis but inflammation can be more prominent and benign-appearing metaplastic squamous cells are common.

Squamous Metaplasia of Lactiferous Ducts (SMOLD) This lesion usually affects younger adult women who smoke. The clinical presentation can be alarming with a red, painful, periareolar mass and radiology findings may be inconclusive (asymmetric density, mass, distortion, or hypoechoic ill-defined mass with sinus and duct continuity). The cytologic findings comprise mixed inflammation with bland appearing metaplastic squamous cells, reactive duct epithelium, keratin debris and multi-nucleated giant cells. Figure 1.17 provides examples of various benign inflammatory conditions including SMOLD.

Lobular Granulomatous Mastitis (LGM)) Most patients are young adult females with a preceding pregnancy within the last 4–7 years who present with a unilateral mass. Cytology findings include non-caseating granulomas, multi-nucleated giant cells, plasma cells, lymphocytes, varying numbers of neutrophils and no metaplastic squamous cells. Eptihelium is scant and not atypical.

Intramammary Lymph Node Radiologic studies can suggest the diagnosis but may be inconclusive. A polymorphous population of lymphocytes is characteristic.

Reactive Seroma in the Setting of Prior Surgery or Breast Implant These may occur years after the implant was placed. Cytology

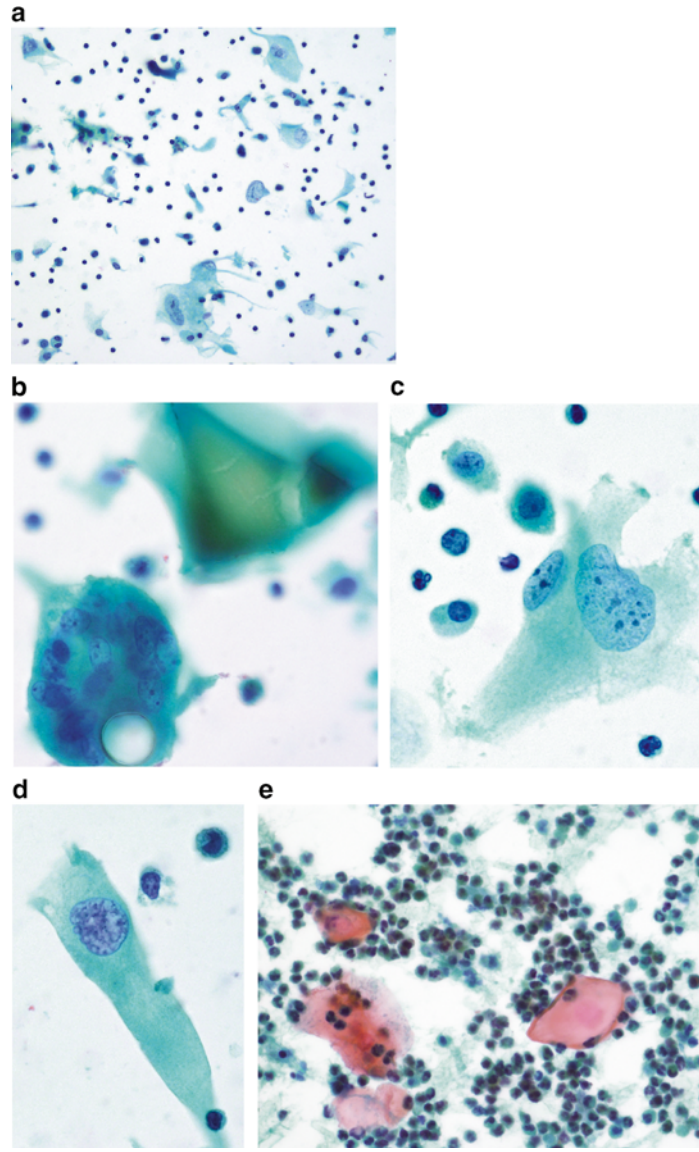


Fig. 1.17 Inflammatory conditions: (**a**) 71 year old woman with a mass near a breast implant shows a mix of chronic inflammatory cells and large polygonal wispy cells consistent with reactive fibroblasts (Pap stain, direct smear); (**b**) multinucleated giant cell engulfs foreign implant material with more foreign material in background (Pap stain, direct smear); (**c**) and (**d**) atypical fibroblasts are large with irregularly shaped nuclei and nucleoli but not hyperchromasia or coarse chromatin (Pap stain, direct smear). Resection showed mixed inflammation and foreign body giant cell reaction to ruptured implant material. (**e**) Thirty year old woman with heavy smoking history and painful central breast mass: squamous metaplasia of lactiferous ducts (SMOLD). Note the marked neutrophilic infiltrate and bland appearing, well-keratinized squamous cells

reveals mixed inflammatory cells without atypical cells. Flow cytometry can be performed and is negative for a monoclonal proliferation.

Malignant Lesions that May Be Difficult to Diagnose Conclusively on Cytology (Confounders)

Breast Carcinoma with Marked Chronic Inflammation and Giant Cells Some breast carcinomas are infiltrated by large numbers of lymphocytes and/or plasma cells or osteoclastic like giant cells. In the setting of such marked inflammation, unequivocal nuclear features of malignancy must be present to enable a confident diagnosis of malignancy. Otherwise, an inconclusive diagnosis is appropriate.

Breast Carcinoma with Acute Inflammation Only rarely, are breast carcinomas associated with marked neutrophilic inflammation. The typical scenario is a high grade carcinoma with spontaneous infarction or hemorrhage. Diagnostic caution is advised in the setting of atypical epithelial cells mixed with abundant neutrophils. Concordance in

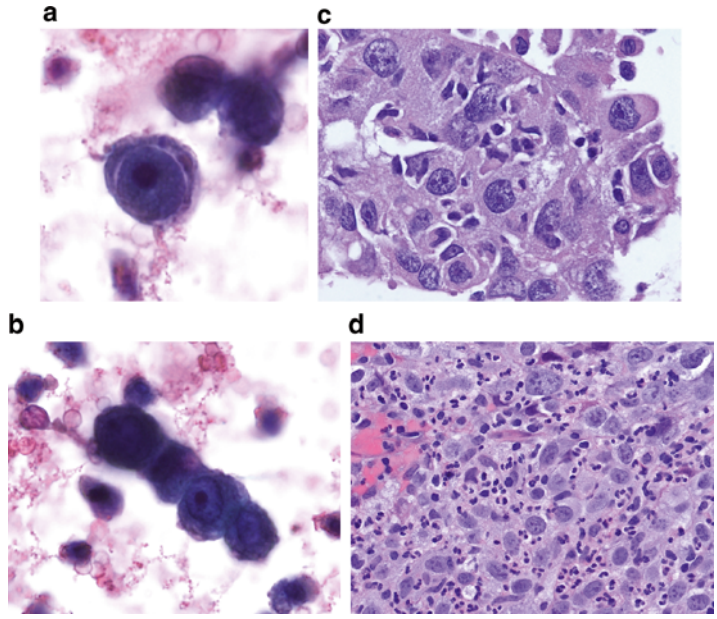


Fig. 1.18 High grade invasive ductal carcinoma with abundant acute inflammation: 32 year old woman with rapidly growing breast mass. Because of massive acute inflammation, a diagnosis of "suspicious" rather than "positive" for malignancy was made on the FNA. This was an appropriate degree of caution. (**a**, **b**) Tumor cells are dyscohesive on the Pap stained direct smears and obscured by abundant blood and acute inflammation; (**c**) cell block shows large cells with marked nuclear atypia (H&E stain); (**d**) resected tumor confirms high nuclear grade and abundant acute inflammation. The patient was triple negative on ER, PR and HER2 immunohistochemical staining

clinical and radiologic findings is necessary before rendering a positive diagnosis. Figure 1.18 provides an unusual example of high grade ductal carcinoma with acute inflammation occurring in a young woman.

Primary Mammary Lymphoma or Leukemic Infiltrate (Choristoma) Radiologic findings are inconclusive. Immunocytochemistry and often flow cytometry are required to make the correct diagnosis. Core needle biopsy is often performed to subtype the hematopoietic process.

Anaplastic Large Cell Lymphoma in the Setting of Prior Breast Implant This is a relatively newly described rare complication in patients with breast implants of either the saline or silicone type. It presents as a late-onset (median time interval of 8 years) peri-capsular seroma, mass attached to the capsule, an erosion through the skin or may be discovered on revision surgery. The cytologic features include highly atypical dyscohesive large lymphoid cells with abundant pale to eosinophilic cytoplasm and irregular nuclei with dispersed chromatin and scattered prominent nucleoli. "Hallmark cells" may be present: these have horseshoe-, eccentric or kidney bean-shaped nuclei and a paranuclear eosinophilic region. The background is inflammatory comprising lymphocytes, eosinophils and histiocytes. Most cases show strong expression of CD30 and BCL2 and no expression of ALK. Confusingly, up to 70 % of cases show expression of EMA, but keratin is not expressed. Molecular studies often reveal T-cell gene rearrangements.

Metastatic Tumors

Malignant Tumors that May Be Difficult to Diagnose Conclusively on Cytology (Confounders)

Low Grade Metastatic Carcinoma Clinical history and features unusual for typical ductal carcinoma are clues to the diagnosis.

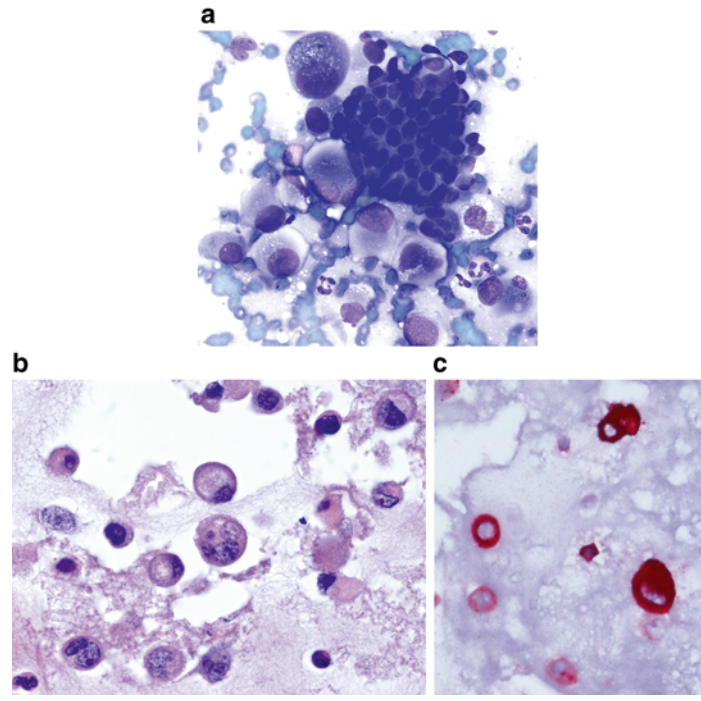


Fig. 1.19 Metastatic melanoma: 51 year old woman with known history of melanoma and new breast mass. (**a**) Malignant melanocytes surround a benign breast ductal group (Diff Quik stain, direct smear); (**b**) dyscohesive large tumor cells with marked nuclear atypia including macronucleoli and multinucleation (H&E stain, cell block); (**c**) because the tumor cells mimic a high grade breast carcinoma, HMB45 ICC staining was performed and is positive (HMB IHC stain, cell block section)

Metastatic Melanoma Melanoma is notorious for its varied cytologic and histologic appearances. It can appear epithelial, mixed epithelial and spindled, pure spindled or as a small round blue cell tumor. Pigment is often lacking in metastatic lesions. Clinical history and radiologic findings (widespread metastases) are diagnostically helpful. Immunocytochemistry (expression of S-100 protein, MelanA, HMB45, MIT-F and lack of expression for keratins) may be required to confirm the diagnosis. Molecular studies can be carried out on the cytology specimen (such as for BRAF-V600E mutation). Figure 1.19 provides an example of metastatic melanoma.

Conclusions

1. *Practice makes (nearly) perfect.* As can be appreciated from the long list above, many lesions in the breast can present diagnostic difficulty. Fortunately, the common breast lesions (90–95 % of all breast cytology specimens) can be diagnosed with relative ease *by experienced cytopathologists who continuously refresh their skills.*
2. *Stay on good terms with your clinicians and radiologists.* A cytologic diagnosis should not be made in a vacuum: the clinical and radiologic features must be factored into the interpretive process. Clinical notes and radiology reports must be reviewed, and in some cases, conversations with the clinician and radiologist are necessary to ensure the correct diagnosis is made.
3. *A bad diagnosis often accompanies a bad sample*. The most accurate diagnosis occurs with optimal sampling of the lesion and appropriate smear technique. Overzealous compression of a drop of aspirate may result in falsely elevated isolated intact epithelial cells artifactually raising concern for malignancy. Under-sampling may yield a sparse specimen that inadequately represents the lesion.
4. *Screening can pose diagnostic problems*. Small, non-palpable lesions picked up on screening and subsequently sampled by cytology may be contaminated by normal or

FCC groups. This can and often should prevent a conclusive diagnosis of malignancy.

5. *You can't skirt the "Gray Zone"*. The Gray Zone in breast cytology is real and unavoidable. A few cases actually should be signed out inconclusively. This is appropriate. This is because overlap exists in the cytologic features of some benign and malignant specimens of such entities as low grade ductal carcinoma, papillary carcinoma, tubular carcinoma, fibroadenoma, florid fibrocystic changes and duct papilloma, among others.
6. *Core needle biopsies are hard to beat*. Newer breast cytology techniques may be diagnostically useful but may be impractical if a CNB can be quickly performed instead. Image analysis, quantitative expression of certain proteins, and quantitation of chromosomal instability features such as micronuclei and nuclear buds compared against benign pairs of myoepithelial nuclei may be diagnostically useful but only in a research setting to date. Core needle biopsy can settle most inconclusive cytologic diagnoses.

Suggested Readings

1. Shabb NS, Boulos FI, Chakhachiro Z, Abbas J, Abdul-Karim FW. Inconclusive or erroneous fine-needle aspirates of breast with adequate and representative material: a cytologic/histologic study. Diagn Cytopathol. 2014;42(5):405–15.
2. Orell SR, Miliauskas J. Fine needle cytology of breast lesions: a review of interpretive difficulties. Adv Anat Pathol. 2005;12:233–45.
3. Weigner J, Zardawi I, Braye S. The true nature of atypical breast cytology. Acta Cytol. 2013;57(5): 464–72.
4. Willis SL, Ramzy I. Analysis of false results in a series of 835 fine needle aspirates of breast lesions. Acta Cytol. 1995;39(5):858–64.
5. Nayar R, De Frias DVS, Bourtsos EP, Sutton V, Bedrossian C. Cytologic differential diagnosis of papillary pattern in breast aspirates: correlation with histology. Ann Diagn Pathol. 2001;5:34–42.
6. Simsir A, Waisman J, Thorner K, Cangiarella J. Mammary lesions diagnosed as "papillary" by aspiration biopsy 70 cases with follow-up. Cancer. 2003; 99:156–65.
7. Chai SM, Kavangh S, Ooi SS, Sterrett GF, Cull G, Plunkett M, Spagnolo D, Amanuel B, Joske D, Leslie C, Barham T, Frost F. Anaplastic large-cell lymphoma associated with breast implants: a unique entity within the spectrum of peri-implant effusions. Diagn Cytopathol. 2014;42(11):929–38.
8. Lui PCW, Tse GMK, Tan PH, Jayaram G, Putti TC, Chaiwun B, Chan NHL, Lau PPL, Mak KL, Khin AT. Fine-needle aspiration cytology of metaplastic carcinoma of the breast. J Clin Pathol. 2007;60:529–33.
9. Bataillon G, Collet JF, Voillemot N, Menet E, Vincent-Salomon A, Klijanienko J. Fine-needle aspiration of low-grade adenosquamous carcinomas of the breast: a report of three new cases. Acta Cytol. 2014;58(5): 427–31.
10. Jarboe E, Layfield LJ, Collins B. Fibromatosis-like metaplastic carcinoma of the breast: a diagnostic pitfall for fine needle aspiration cytology: a case report. Acta Cytol. 2010;54:712–6.
11. Ventura K, Cangiarella J, Lee I, Moreira A, Waisman J, Simsir A. Aspiration biopsy of mammary lesions with abundant extracellular mucinous material review of 43 cases with surgical follow-up. Am J Clin Pathol. 2003;120:194–202.
12. Heymann JJ, Halligan AM, Hoda SA, Facey KE, Hoda RS. Fine needle aspiration of breast masses in pregnant and lactating women: experience with 28 cases emphasizing Thinprep findings. Diagn Cytopathol. 2014;43:188–94.
13. Jimenez-Heffernan JA. Fine needle aspiration cytology of atypical apocrine adenosis of the breast. Cytopathology. 2009;20:133–41.
14. Álvarez-Rodriguez F, Gimeno-Aranguez M, López-Varela C, González-Peramato P, Tortoledo-Bruni A, Rodríguez-Costa J, Perna C, Jiménez-Heffernan JA. Fine-needle aspiration cytology of mammary myofibroblastoma: a report of six cases. Acta Cytol. 2012;56(5):501–5.
15. Hata S, Kanomata N, Kozuka Y, Fukuya M, Kobayashi TK, Ohno E, Moriya T. Cytologic appearance of myospherulosis of the breast diagnosed by fine-needle aspirates: a clinical, cytological and immunocytochemical study of 23 cases. Diagn Cytopathol. 2011;39(3):177–80.
16. Papeix G, Zardawi IM, Douglas CD, Clark DA, Braye SG. The accuracy of the 'triple test' in the diagnosis of papillary lesions of the breast. Acta Cytol. 2012;56(1):41–6.
17. Farshid G, Pieterse S, King JM, Robinson J. Mucocele-like lesions of the breast: a benign cause for indeterminate or suspicious mammographic microcalcifications. Breast J. 2005;11(1):15–22.
18. Kundu UR, Guo M, Landon G, Wu J, Sneige N, Yun G. Fine-needle aspiration cytology of sclerosing adenosis of the breast a retrospective review of cytologic features in conjunction with corresponding histologic features and radiologic findings. Am J Clin Pathol. 2012;138:96–102.
19. Simsir A, Walsman J, Cangiarella J. Fibroadenomas with atypia: causes of under- and overdiagnosis by aspiration biopsy. Diagn Cytopathol. 2001;25:278–84.
20. Agnihotri M, Naik L, Kothari K, Fernandes G, Ojha S. Fine-needle aspiration cytology of breast lesions

with spontaneous infarction: a five-year study. Acta Cytol. 2013;57(4):413–7.
21. Gupta RK. Fine needle aspiration cytology of granulomatous mastitis: a study of 18 cases. Acta Cytol. 2010;54(2):138–41.
22. Aqel NM, Howard A, Collier DS. Fat necrosis of the breast: a cytological and clinical study. Breast. 2001; 10:342–5.
23. Goel S, Bhatia A, Dey P. Spontaneously occurring micronuclei in infiltrating ductal carcinoma of breast: a potential biomarker for aggressive phenotype detection? Diagn Cytopathol. 2013;41(4):296–302.
24. Verma S, Dey P. Correlation of morphological markers of chromosomal instability in fine needle aspiration cytology with grade of breast cancer. Cytopathology. 2014;25(4):259–63.
25. Subbaiah RM, Dey P, Nijhawan R. Artificial neural network in breast lesions from fine-needle aspiration cytology smear. Diagn Cytopathol. 2014;42(3): 218–24.
26. Nagar S, Iacco A, Riggs T, Kestenberg W, Keidan R. An analysis of fine needle aspiration versus core needle biopsy in clinically palpable breast lesions: a report on the predictive values and a cost comparison. Am J Surg. 2012;204:193–8.
27. Pieterse AS, Mahar A, Orell S. Granular cell tumour: a pitfall in FNA cytology of breast lesions. Pathology. 2004;36:58–62.

Female Reproductive System Cytology

2

Brief Introduction

This chapter describes mimics (benign lesions that can be mistaken for dysplasia or carcinoma) and confounders (malignant lesions that can be mistaken for benign entities) occurring in a Pap-stained monolayer preparation of the cervix/vagina.

Dr. Georgios Papanikolaou's deceptively simple test has saved the lives of millions of women worldwide through enabling early detection of pre-invasive and early invasive tumors. Advances in sample collection and preparation such as the monolayer prep (SurePath™, Becton, Dickinson and Company, Franklin Lakes, New Jersey and ThinPrep™, Hologic, Bedford, MA) have reduced the percentage of equivocal Pap test diagnoses. Automated pre-screening (ThinPrep Imaging System, Hologic and others) consistently detect small numbers of atypical cells aiding in diagnostic sensitivity. Ancillary testing for high risk human papilloma virus (hrHPV) has further refined the approach to diagnosis and treatment of women with potential cervical, vaginal and vulvar dysplasia/carcinoma and cervical adenocarcinoma. Monolayer preparations, in particular, appear to increase the sensitivity of detection of endometrial neoplasia. In addition, specific immunocytochemical (ICC) staining using antibodies to p16, Ki67 and others may improve diagnostic accuracy in challenging cases of cytologically atypical glandular or squamous cells. Large, well-conceived, evidence-based studies have contributed to improving cognitive assessment of Pap tests. However, even with technical and experiential advances, changes in hormonal state, repair after mechanical injury or inflammation, some non-neoplastic lesions, and some infections may provoke epithelial alterations that can be misinterpreted as worrisome for dysplasia or malignancy (atypical squamous cells of undetermined significance = ASCUS, atypical glandular cells of undetermined significance = AGUS), misinterpreted as dysplastic (low grade squamous intra-epithelial lesion = LGSIL or high grade squamous intra-epithelial lesion = HGSIL) or misinterpreted as malignant (squamous cell carcinoma = SCC, AIS = adenocarcinoma in situ, adenocarcinoma).

Cervical and endometrial glandular lesions pose more diagnostic challenges than squamous dyplasia and carcinoma. Factors that contribute to diagnostic difficulty include sometimes scant number of atypical cells, poor preservation, overlap with benign entities such as glandular or squamous cell repair, microglandular hyperplasia and tubal metaplasia and bland appearance of some low grade glandular tumors. Glandular cell abnormalities diagnosed on Pap tests occur infrequently compared to ASCUS and squamous intra-epithelial lesion (SIL) diagnoses. However, the incidence of endocervical glandular carcinoma is rising both in proportion to cervical squamous cell carcinoma and in actual numbers

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_2

(cervical adenocarcinoma accounts for ~25 % of all cervical neoplasias) [26].

For both medical and economic reasons, correctly identifying mimics of dysplasia and carcinoma is essential. However, in some instances, a definitive assessment is not possible, and a diagnosis of AGUS or ASCUS acknowledges that some Pap tests yield ambiguous but atypical findings. Because the Pap test is a screening procedure, the false positive diagnosis carries a less severe consequence than a false negative diagnosis, as a confirmatory biopsy is typically performed, so that sensitivity is prioritized over specificity. Although indeterminate themselves, AGUS and ASCUS diagnoses trigger defined clinical follow-up plans that usually resolve the patient's diagnosis and guide appropriate treatment.

Most women with a Pap test diagnosis of AGUS have benign changes on follow-up. Based on several recent studies, a diagnosis of atypical glandular cells of undetermined significance (AGUS and its subcategories) is associated with dysplasia or carcinoma in 15–35 % of patients on further cytologic or histologic follow-up [5, 13, 22, 24, 27]. A recent large review of 662 diagnoses of AGUS representing 0.41 % of Pap test cases at the University of Pittsburgh Magee-Womens hospital over a 26 month period (247,131 total Pap tests) reported a 15.3 % follow-up rate of a significant abnormality. The significant abnormalities comprised squamous dysplasia, endocervical adenocarcinoma, endometrial carcinoma, endometrial hyperplasia, and ovarian carcinoma. Benign causes of AGUS in this study encompassed endometrial polyp, endometritis, endocervical polyp, endocervical tubal metaplasia, microglandular hyperplasia and lesions identified as nonspecific chronic cervicitis or reactive squamous metaplasia (this last diagnosis was the most common follow-up diagnostic category accounting for ~85 % of subsequent diagnoses) [28]. In this and other studies, it was found that the type of significant abnormality, when present, tended to differ according to patient age: women over age 50 with a diagnosis of AGUS were more likely to have endometrial hyperplasia/carcinoma, while women under 40 with a diagnosis of AGUS were more likely to have SIL, AIS or endocervical adenocarcinoma [28].

Some patients diagnosed on Pap with AGUS also have a synchronous diagnosis of ASCUS, atypical squamous cells of undetermined significance cannot exclude a high grade lesion (ASCUS-H), or HGSIL. Coexisting squamous lesions (dysplasia, carcinoma) occur in ~25 % of glandular lesions [20]. When both AGUS and a squamous cell abnormality are diagosed on the Pap test, the rate of follow-up abnormality in the cervix is significantly higher than for a diagnosis of AGUS alone [28].

In challenging AGUS cases, additional hrHPV testing in liquid based Pap samples can be sensitive (83 %) and specific (78–82 %) for significant lesions (HGSIL, AIS, or cervical adenocarcinoma) [29]. Additionally, cell block preparations of monolayer specimens can reveal more architectural features which can clarify the diagnosis [22]. Immunocytochemical staining with antibodies to p16 and Ki67 can aid in differentiating atrophic atypia (negative or rare cells reactive) from squamous dysplasia (many cells reactive), for example. This panel showed a higher positive predictive value for the presence of high grade squamous dysplasia on follow-up biopsy than Hybrid Capture 2 HPV testing in a recent multi-site European study (PALMS study) [7]. Another recent small study of 34 cases of AGUS diagnoses reported that a dual panel of ProExC™ (Becton, Dickinson and Company) and IMP3 immunocytochemical staining performed on ThinPrep™ MLPs yielded a high positive predictive value for glandular lesions diagnosed on subsequent biopsies and therefore could reduce the number of indeterminate cytology-based diagnoses streamlining patient management [12].

Historically, identifying endometrial neoplasia on a Pap test was considered difficult with reported low diagnostic sensitivity. However, recent studies suggest that monolayer preparations exhibit improved sensitivity ranging from approximately 60–94 % [20, 21]. Especially for low grade endometrial adenocarcinoma, mimickers include normal endometrial cells and various benign neoplastic, reactive and inflammatory conditions including atypical repair, endometrial or endocervical polyp, IUD effect, and endometritis, among others. Knowledge of the features of these mimickers gained in part from in-house,

rigorous cytologic-histologic correlation could make the Pap test a significantly useful screening test for endometrial carcinoma in at-risk populations. Additionally, judicious use of ancillary tests such as the aforementioned antibody panels and cell blocks could further improve the sensitivity of the Pap MLP as a screening test for endometrial neoplasia.

Mimics of Dysplasias, Hyperplasias and Tumors Found in Cervical/Vaginal Pap Smears and Pap-Stained Monolayer Preparations

Infectious/Inflammatory (See Table 2.3)

Common Reactive and Reparative Changes

Reactive and reparative changes commonly occur in squamous cells and endocervical glandular cells in response to various infections and inflammatory processes. Because these are highly prevalent changes that are sometimes mistaken for dysplasia or carcinoma, the characteristic cellular changes are listed in Table 2.1 contrasting reactive changes to changes more commonly associated with dysplasia and carcinoma. Table 2.2 lists common challenging cell patterns in the Pap test and the differential diagnosis. This table includes the entities discussed in greater detail below. Figures 2.1, 2.2, 2.3, and 2.4 provide examples of reactive and reparative changes and confounders (see Table 2.3).

Follicular Cervicitis

Follicular cervicitis (FC) may mimic high grade squamous dysplasia because cells aggregate in groups and display a high N/C ratio and hyperchromatic nuclei. However, epithelial-type cohesion is absent, tingible body macrophages are often intermixed and the lymphocyte nuclei exhibit more variability in shape than in many high grade squamous dysplasias. Also, lymphocytes are usually smaller then most cells of HGSIL.

Trichomonas and Candida Infections

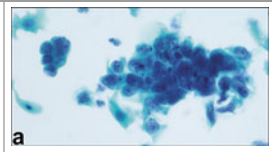
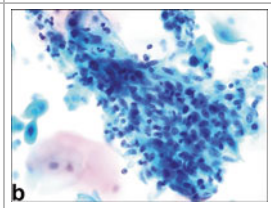
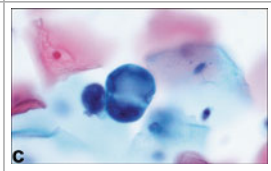
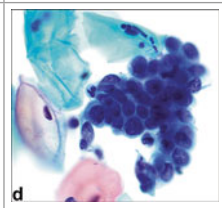
In most cases, the changes induced by these infections do not pose diagnostic difficulties. However, mild nuclear enlargement, parakeratosis, thin perinuclear halos lacking a rolled sharp cytoplasmic edge, and florid reparative changes may simulate ASCUS or AGUS. Two or more pathologic processes may also co-exist such as Trichomonas with squamous dysplasia. Careful, complete assessment may indicate the presence of defensible ASCUS, AGUS or dysplasia (see Figs. 2.5, 2.6, and 2.7).

Table 2.1 Reactive and reparative changes in cervical squamous cells and endocervical glandular cells

Cell	Nuclear features	Cytoplasmic features	Features that are usually absent
Reactive/ reparative squamous cell	• Mild nuclear enlargement (MNE) • Binucleation • Variation in nuclear size • Macronucleoli • Prominent chromocenters • Chromatin degeneration • Pyknosis • Karyorrhexis	• Single to multiple vacuoles • Emperipolesis • Paradoxical increased or decreased cytoplasmic maturation • Thin, blurry perinuclear halo • Abnormal eosinophilia • Dual coloration (polychromasia)	• Nuclei: markedly increased N/C ratio, coarse chromatin, irregularly distributed chromatin, atypical mitotic figures • Cytoplasm: thick perinuclear halo with rolled cytoplasmic rim
Reactive/ reparative endocervical glandular cell	• Nuclei enlarged but remain round or oval • Multinucleation • Macronucleoli (single or multiple) • Mitotic figures +/– but are NOT atypical	• May appear damaged: shredded or wispy • N/C ratio may be increased • Goblet cell metaplasia may be focally present • Tubal metaplasia may be present +/– ciliocytophthoria (tufts of cilia and terminal bars)	• Nuclei: as above but a few reactive/reparative ECC may have slight chromatin coarsening • Apoptotic bodies usually absent or rare • Architecture: feathering, rosettes, pseudostratification usually absent, nuclear overlapping only in multinucleated endocervical cells

ECC endocervical cells, *MNE* mild nuclear enlargement

Table 2.2 Differential diagnosis of common patterns in a Pap test

Pattern	Example	Benign entities that can have this pattern	Dysplastic and malignant entities that can have this pattern
CROWDED HYPERCHROMATIC GROUPS of cells with a high N/C ratio	 a	• Tight overlapping clusters of stripped nuclei (of reserve cell origin) • Benign endometrial cell group in or out of phase • Cervical endometriosis • Endometritis • Endometrial polyp • Follicular cervicitis • Transitional metaplasia	• High grade non-keratinizing squamous dysplasia • Dysplasia superimposed on atrophy • Adenocarcinoma in situ, some types • Endometrial hyperplasia • Endometrial carcinoma • Ovarian or tubal carcinoma
SHEETS OF IMMATURE-APPEARING CELLS with a moderate N/C ratio and no keratinization	 b	• Transitional cell metaplasia • Reserve cell hyperplasia • Immature metaplasia • Atrophic epithelium • Endometrial stroma • Decidual tissue[a]	• Non-keratinizing dysplasia
ISOLATED RARE ATYPICAL CELL, DOUBLETS OR TRIPLETS with hyperchromatic nucleus and high N/C ratio	 c	• IUD effect • Single markedly atypical cells in marked atrophy • Tubal metaplastic cells with inconspicuous terminal bar and stripped cilia • Cytotrophoblasts in pregnancy or post-partum[a] • Endometritis with or without IUD	• Non-keratinizing high grade dysplasia shedding as rare cells only • Endometrial carcinoma shedding as rare cells • Endocervical carcinoma shedding as rare cells • Ovarian or tubal carcinoma shedding as rare cells • Rare cervical or vaginal melanoma with small cell morphology
MITOTICALLY ACTIVE GLANDULAR GROUPS with slightly increased N/C ratio	 d	• Reactive endocervical glandular cells • Tubal metaplasia • Reparative glands after recent LEEP, curettage or punch biopsy • Reparative glands after vaginal delivery[a] • Normal proliferative endometrial glands inadvertently sampled	• Adenocarcinoma in situ, some types • Low grade cervical adenocarcinoma such as minimal deviation adenocarcinoma or villoglandular adenocarcinoma

[a]Associated with pregnancy or lactation

Iatrogenic (See Table 2.4)

Hormone Replacement Therapy (HRT)

HRT may cause atrophic changes that could mimic non-keratinizing squamous dysplasia or may cause breakthrough bleeding with endometrial cells shed out of phase. As in other causes of squamous atrophy, the nuclei are usually mildly enlarged and may be slightly hyperchromatic. However, coarse chromatin, irregular nuclear borders and mitotic activity are usually absent. The cells also are arranged uniformly without extensive nuclear overlapping when focusing in one plane. HPV testing can be diagnostically helpful.

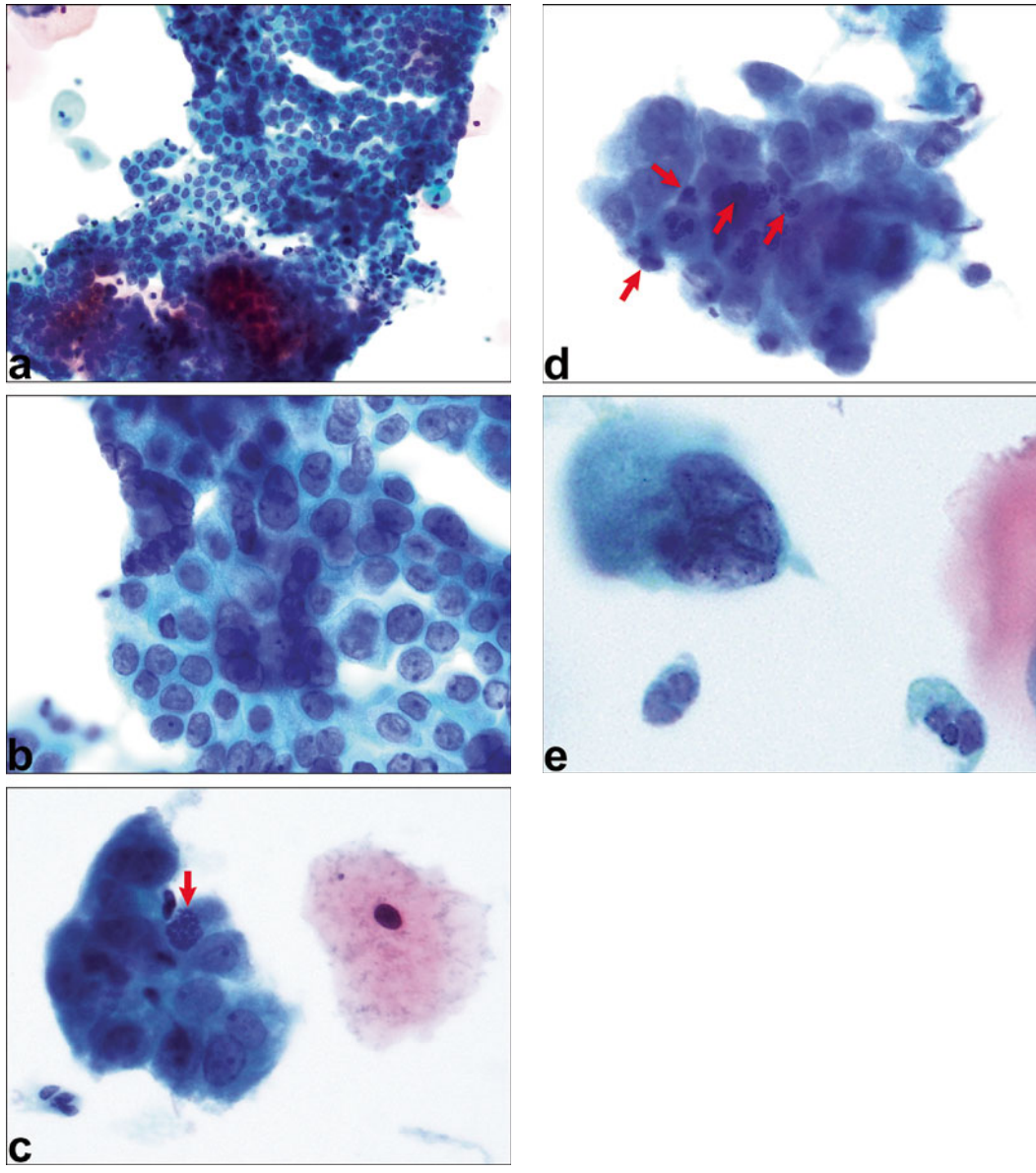


Fig. 2.1 Endocervical cells with reactive changes and confounder. (**a**) Sheet of reactive endocervical cells shows mild loss of polarity at medium magnification (Pap stain, MLP); (**b**) High magnification reveals multinucleation in some cells and small nucleoli but coarse chromatin and hyperchromasia are absent; (**c**) A small clump of reactive endocervical cells contains a mitotic figure (*arrow*). Mitoses by themselves do not indicate a significant lesion such as AIS or adenocarcinoma; (**d**) Another small poorly organized group of reactive endocervical cells with intermingled neutrophils (*arrows*). Neutrophils and neutrophil debris should not be mistaken for apoptotic bodies which are a worrisome finding in endocervical glandular groups; (**e**) Multinucleated endocervical adenocarcinoma cell. Most multinucleated endocervical glandular cells are benign and reactive. However, AIS and cervical adenocarcinoma cells can be multinucleated (MN). In contrast to the benign MN cells in (**b**), the nucleus of this cell exhibits coarse chromatin and parachromatin clearing, two features typical of a malignant nucleus

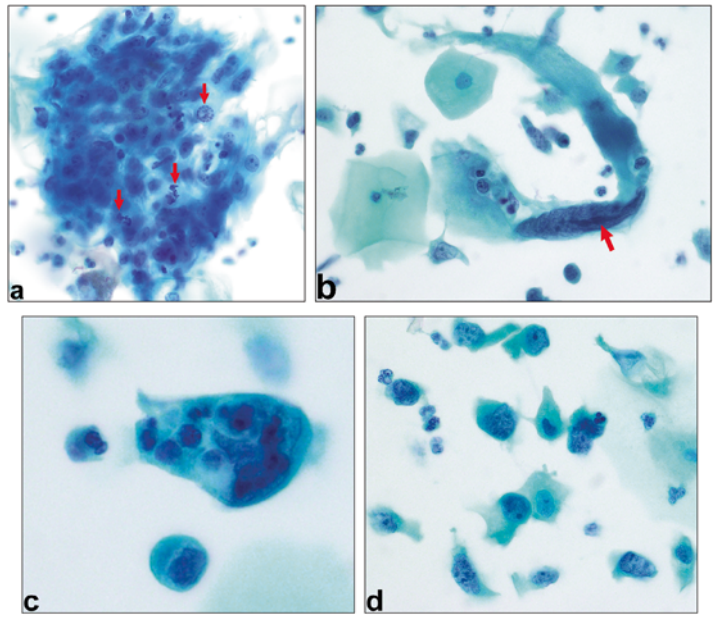


Fig. 2.2 Squamous cells with reactive changes and confounders. (**a**) This loose but vaguely flowing cluster of reactive metaplastic squamous cells shows an alarming number of mitotic figures (*arrows*). However, nuclear shapes are simple, chromatin is predominantly fine, and nucleoli and chromocenters support an activated but reactive state, in combination with the other features of benignancy. Figure (**b**) contains a gigantic epithelial cell altered by pelvic irradiation and chemotherapy. The nucleus is also enlarged, but the N/C ratio remains low. In irregularly shaped nucleolus (*arrow*) is common in these cells. (**c**) Radiation-induced atypia in this glandular cell includes leukophagocytosis, a non-specific finding that occurs in benign reactive and neoplastic conditions. (**d**) These dispersed, atypical glandular or metaplastic cells occurring after radiation therapy and surgery for endocervical adenocarcinoma feature slight nuclear contour irregularities, an increased N/C ratio, and slightly coarse chromatin. Diagnoses of AGUS and ASCUS-H were rendered and supported by the findings. Follow-up tissue biopsies showed reactive atypia both of small, metaplastic squamous cells and cuboidal glandular cells

Young women on oral contraceptives may show glycogenated "pseudo-koilocytes" on a ThinPrep MLP [15]. These cells feature perinuclear clearing with an incomplete thickened cytoplasmic rim, a clear or partly clear perinuclear zone and mild nuclear enlargement with mild to moderate nuclear hyperchromasia. The cells lack coarse chromatin or irregular nuclear borders. PAS without diastase can identify cytoplasmic glycogen, a clue to the diagnosis of a pseudo-koilocyte. High risk HPV testing can be diagnostically helpful.

Patients on HRT or OCP may also develop microglandular hyperplasia (MGH), a benign lesion of the cervix than can occasionally be mistaken for HGSIL. MGH is described in detail below.

Intra-Uterine Device (IUD)

Intra-uterine devices cause inflammatory and reactive changes in the endometrium. Endometrial cells may be shed at any stage of the cycle and notoriously may mimic HGSIL and endometrial or endocervical glandular neoplasia. Most often, the cells are medium-sized and round with enlarged "swollen" nuclei with variable vacuolation of cytoplasm sometimes resembling signet ring cells or histiocytes. A few cells exhibit bulging vacuoles of cytoplasm described as "bubble gum"-like. The background can include neutrophils, *Actinomyces* colonies and psammoma bodies formed around IUD particles. Uncommonly, fibroblasts, foreign body-type multinucleated

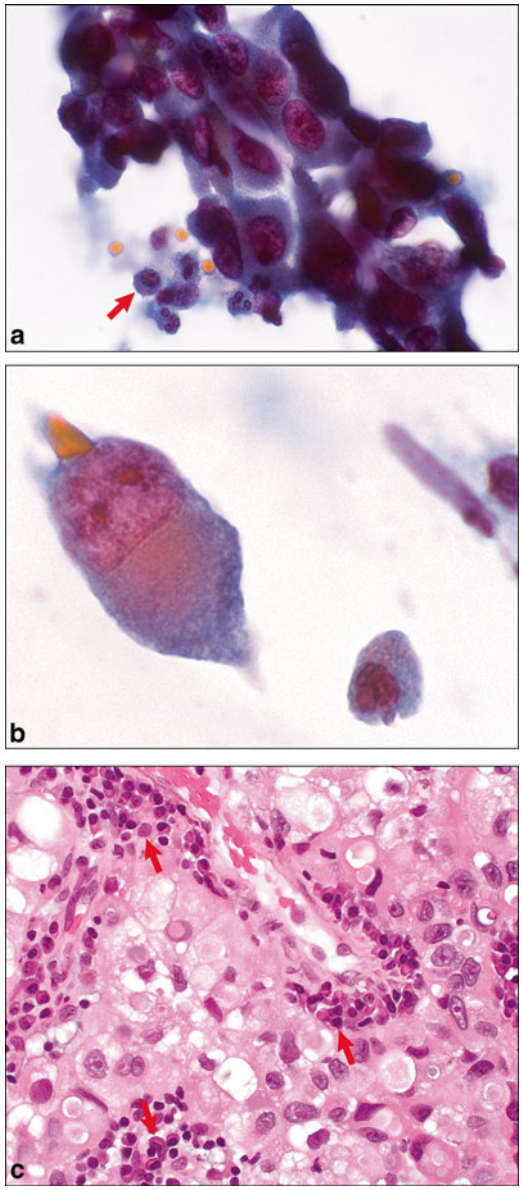


Fig. 2.3 Confounder of reactive squamous cells: glassy cell carcinoma. (**a**) This cluster of malignant cells in a case of glassy cell carcinoma mimics florid atypical repair. Note the extremely large size of the cells compared to the adjacent plasma cell (*arrow*). Plasma cells may be prominent in the background of GCC. (**b**) The same case of GCC also shed isolated, well-preserved malignant cells as in this field. Such single malignant cells supported the diagnosis of a significant pathologic abnormality rather than florid repair. (**c**) Histologic section of the GCC depicted in (**a**) and (**b**) confirm the large tumor cell size, nuclear features that may mimic repair and large numbers of infiltrating plasma cells (*arrows*)

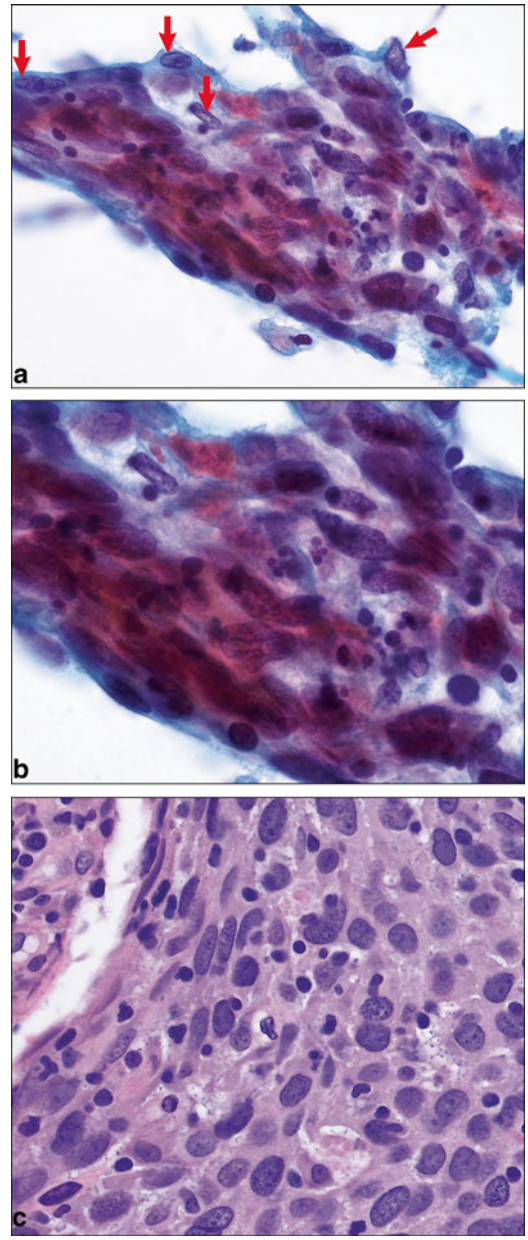


Fig. 2.4 Confounder of reactive squamous cells: high grade non-keratinizing dysplasia. (**a**) High grade, non-keratinizing dysplasia shows cohesive flowing cellular arrangement and polychromasia with neutrophils mimicking reparative changes. (**b**) Higher magnification shows sharply angled nuclear notching (see *arrows* in photo (**a**)) and parachromatin clearing worrisome for dysplasia. (**c**) Subsequent tissue biopsy confirmed the diagnosis of non-keratinizing HGSIL

Table 2.3 Infectious/inflammatory mimickers in cervical/vaginal cytology specimens

Mimicker	What it mimics	Architecture	Cell features	Background	Key differentiating features	Ancillary studies
Follicular cervicitis	HGSIL especially of small cell type	• Unorganized mats or groups • Dyscohesive	• Variation in cell size • Round nuclei • Intermixed histiocytes with reniform nuclei	• Intermixed tingible body macrophages	• Lack of irregularly shaped nuclei • Lack of coarse chromatin	• Cell block for CD45 staining (positive in follicular cervicitis)
Atrophic vaginitis with atrophic atypia	HGSIL	• Sheets • Single cells	• Scattered cells with cytoplasmic orangophilia (pseudomaturation) • N/C ratio often slightly increased • A few nuclei may have suspicious features including enlargement, hyperchromasia and coarser chromatin	• May have grainy, diathesis-like background • Chronic inflammation	• Most nuclei are pale with fine chromatin • Some with dark but smudgy chromatin consistent with degenerative atypia	• HPV test on monolayer residual sample • Cell block can be made and stained with p16 (negative in atrophic cells)
Trichomonas cervicitis	ASCUS LGSIL AGUS	• Aggregates and single cells like in normal smear	• "Pseudo-koilocytes": thin perinuclear halo mimics koilocytic halo but is much smaller without thickened cytoplasmic rim	• Trichomonads • Inflammation • Degenerated vacuolated squamous cells • *Leptothrix* in some cases	• No Nuclear features of LGSIL (enlargement, coarse chromatin, membrane irregularities)	None required
Candida cervicitis	ASCUS LGSIL	• "Shish-kebab": squamous cells skewered on strands of Candida pseudohyphae • "Spaghetti and meatballs" : Candida pseudohyphae and yeast forms • Disorganized mats of squamous cells	• Mild nuclear enlargement (MNE) • "Pseudo-koilocytes": thin perinuclear halo mimics koilocytic halo but is much smaller without thickened rim • Hyperkeratosis and/or parakeratosis in some cases	• Inflammation	• No Nuclear features of LGSIL (enlargement, coarse chromatin, membrane irregularities)	Negative HPV test

LGSIL low grade squamous intraepithelial lesion, *HGSIL* high grade squamous intra-epithelial lesion, *AIS* adenocarcinoma in situ, *EcA* endocervical adenocarcinoma, *MNE* mild nuclear enlargement

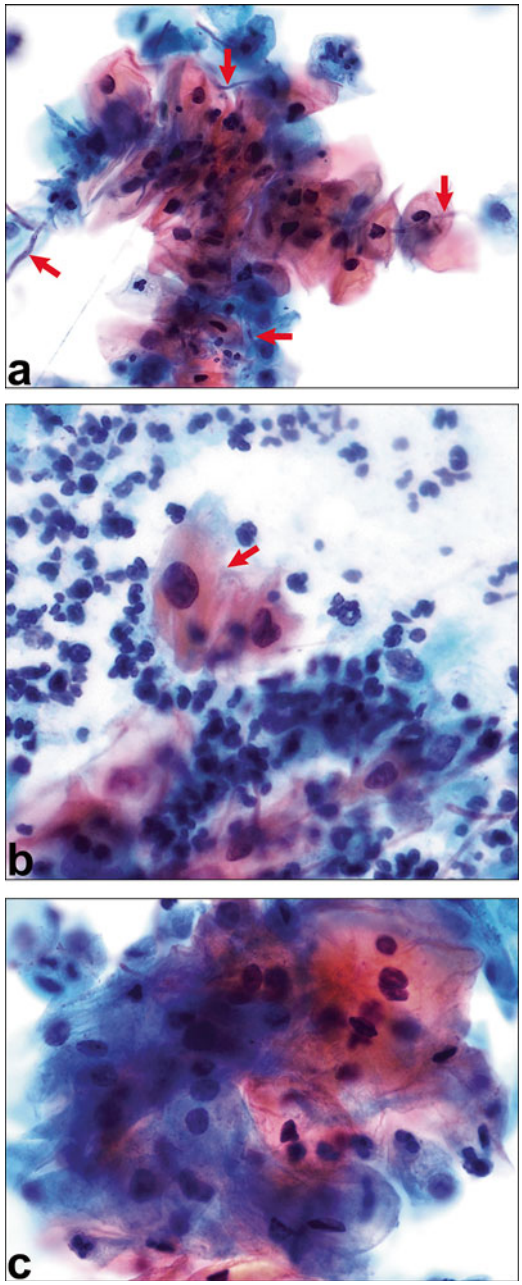


Fig. 2.5 Common infections: Candida. (**a**) A classic "shish-kebab" arrangement of squamous cells "skewered" on fungal hyphae (*arrows*) enables a rapid diagnosis of Candida infection. (**b**) Inflammation in infections may be obscuring, especially in a traditional Pap slide. Visible cells may show degenerative changes rendering interpretation difficult. Note the polychromasia, cracks and fraying of the degenerated squamous cell (*arrow*). (**c**) Cells in a typical case of cervical Candidiasis often exhibit reactive changes such as mild nuclear enlargement, hyperchromasia, and retention of relatively large nuclei in more mature cytoplasm. However, marked nuclear enlargement and increased N/C ratio are absent

giant cells and histiocytes with phagocytosed debris are also present. A clinical history of IUD can prevent a misdiagnosis of AGUS, ASCUS-H or HGSIL. In most cases, the number of atypical cells is small (in contrast to many cases of HGSIL), and hrHPV testing can be diagnostically helpful in especially problematic cases. These atypical cells may persist in a Pap specimen at least 6 months after removal of the device. Figure 2.8 provides examples of IUD effect and other benign processes that may shed atypical endometrial cells.

Laser Ablation; Tissue Biopsies

Loop electrosurgical excision procedure (LEEP) damages the cervical epithelium, and resulting reparative changes can appear very atypical with nuclear enlargement, pleomorphism, prominent nucleoli and mitotic activity. Pap MLPs usually return to normal by 8 weeks after the procedure; however, in some cases florid changes may be indistinguishable from true dysplasia. HPV testing may not be useful in this setting, as the patient has had dysplasia.

Approximately 70 % of cervical cone biopsies also contain foci of tubal metaplasia: groups of crowded, small hyperchromatic cells with an increased N/C ratio and slightly coarse chromatin. Degenerative nuclear features such as a pale "washed-out" appearance or smudgy chromatin may also be present. Most groups will lack nuclear feathering (taffy-like pulling outward of nuclei around the edge of a cell group), apoptotic bodies and nucleoli. However, worrisome strips with nuclear palisading can be seen. The key to the correct diagnosis of metaplasia rather than AIS or HGSIL is the detection of cilia and/or terminal bars in some of the cells. Tubal type adenocarcinoma does occur, however, and is in the differential of ciliated cell groups with: (1) severe nuclear atypia, (2) mitotic activity and (3) apoptotic bodies. Fortunately, tubal type endocervical adenocarcinoma is rare (as is ciliated endometrial adenocarcinoma), and these two carcinomas should only be seriously considered in cases with the above three findings the triad of which would otherwise occur only infrequently in typical tubal metaplasia.

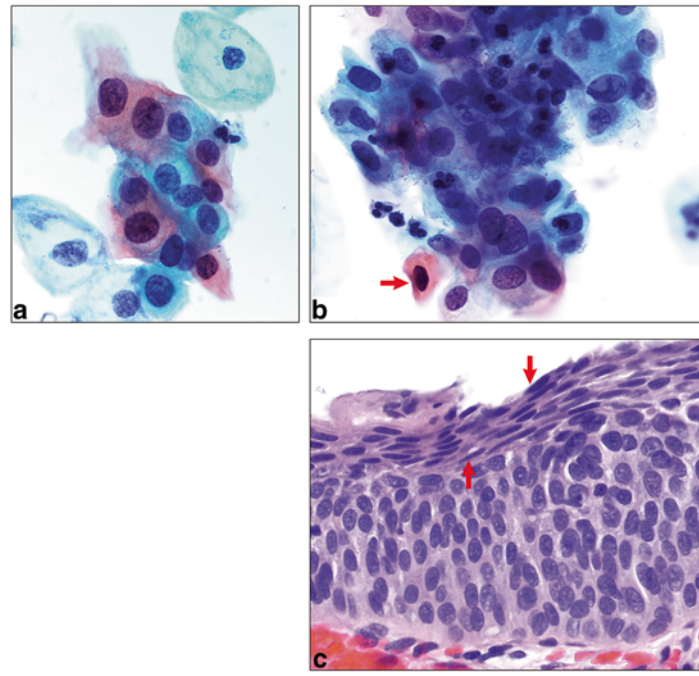


Fig. 2.6 Confounder of Candida infection: keratinizing high grade dysplasia in the setting of Candidiasis. (**a**) By contrast, the group of cells in a patient with abundant fungi shows a worrisome increase in N/C ratio even in well-keratinized cells. (**b**) Other groups of cells in this patient's MLP showed unequivocal features of HGSIL including nuclear contour irregularities combined with coarse chromatin, an N/C ratio >50 % and a suggestion of parachromatin clearing in some nuclei. Note the parakeratotic cell (*arrow*). (**c**) Follow-up biopsy revealed high grade dysplasia with atypical parakeratosis (*arrows*)

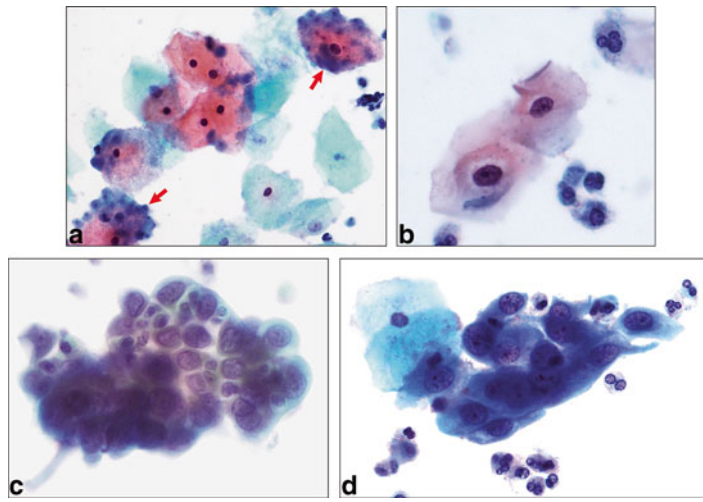


Fig. 2.7 Trichomonas infection and reactive changes. (**a**) A florid case of Trichomonas infection showed innumerable organisms that studded the surface of squamous cells (*arrows*). (**b**) Thin partially transparent perinuclear haloes lacking a crisp, rolled cytoplasmic edge are commonly seen in Candida and Trichomonas infections and should not be mistaken for true koilocytes. (**c**) Reactive changes in an endocervical gland in the setting of Trichomonas shows an increased N/C ratio and nuclear contour irregularities and mild nuclear overlap. An appropriate diagnosis of AGUS favor reactive was followed by a negative tissue biopsy confirming inflammatory induced atypia. (**d**) Metaplastic squamous cell with chromatin coarsening and small nucleoli in another example of Trichomonas infection. Note the relatively simple shape of the nuclei and generally low (<50 %) N/C ratio. Follow-up biopsy revealed inflammatory changes superimposed on immature squamous metaplasia

Table 2.4 Iatrogenic mimickers in cervical/vaginal cytology specimens

Mimicker	What it mimics	Architecture	Cell features	Background	Key differentiating features	Ancillary studies
Hormone replacement therapy (HRT) with nuclear atypia	• Non-keratinizing LGSIL • Non-keratinizing HGSIL	• Evenly spaced clusters and sheets	• Mild nuclear enlargement • +/− mild hyperchromasia	No specific features	• Absence of: coarse chromatin, irregular nuclear contours • Mitoses usually absent	High risk HPV test helpful in some cases (negative)
Tamoxifen therapy	• AGUS • Endometrial neoplasia • HGSIL	• Tight round balls • Clusters of stripped nuclei with overlapping	• Small cells with high N/C ratio (endometrial cells) • Stripped nuclei are small with fine chromatin +/− small nucleoli on MLP	• Post-menopausal: squamous maturation • Pre-menopausal: atrophy	Lack of nuclear atypia in small cells and stripped nuclei	Negative hrHPV test in cases resembling AGUS or HGSIL
Other drugs	Unknown effect with most agents	• Unknown effects for most drugs	• Digitalis: squamous maturation • Tetracyclines: parabasalar cell prominent	• Inflammatory in some cases	Lack of convincing nuclear features of dysplasia[a]	Negative hrHPV test in cases suggesting ASCUS-H
Intra-uterine device (IUD)	• Endometrial neoplasia • HGSIL	• Small 3-d groups • Isolated cells	• Cells round, medium in size with hyperchromatic chromatin and high N/C ratio • Cells with cytoplasmic vacuolation "bubble gum" like in some • Nucleoli +/−	• Inflammatory in some cases • Actinomyces rarely • Psamomma bodies • Normal endometrial cell clusters may be present	• Sparse atypical cells • History of IUD in place or recently (within 6 months) removed	• Negative hrHPV test • Negative IMP3 and ProExC ICC in cell blocks from MLP sample
Prior laser ablation or cervical biopsies	• HGSIL, subtype with vesicular nuclei +/− nucleoli • AGUS	• Florid reactive or reparative clusters or sheets • Large gland fragments	• Reactive reparative cells show enlarged vesicular nuclei +/− nucleoli, +/− mitotic figures • Large glands may be too thick to evaluate more than the peripheral rim of columnar cells	• Inflammation +/− • Tubal metaplasia often present	• Lack convincing nuclear features of dysplasia[a]	• May have a recent history of positive hrHPV test
Radiation or chemotherapy	• HGSIL (subtype with vesicular nuclei) • SCC (type with vesicular nuclei and nucleoli) • AGUS	• Clusters • Mats • Isolated cells	• Large cells with normal or low N/C ratio • Dense eosinophilic often vacuolated cytoplasm • Large clear to vesicular to smudgy nuclei • Macronucleoli	• Inflammation • More obvious reparative changes in squamous +/− endocervical cells	• Lacks mitoses • Lacks markedly high N/C ratio • Lacks apoptotic bodies	• Negative for hrHPV • Negative for p16 (in cell block prep)

LGSIL low grade squamous intra-epithelial lesion, *HGSIL* high grade squamous intra-epithelial lesion, *SCC* squamous cell carcinoma, *AIS* adenocarcinoma in situ, *EcA* endocervical adenocarcinoma

[a]Convincing nuclear features of dysplasia comprise a combination of two or more of: hyperchromasia, irregular nuclear contours, irregular chromatin distribution, parachromatin clearing, N/C ratio >50 %, but clinical context is also a factor

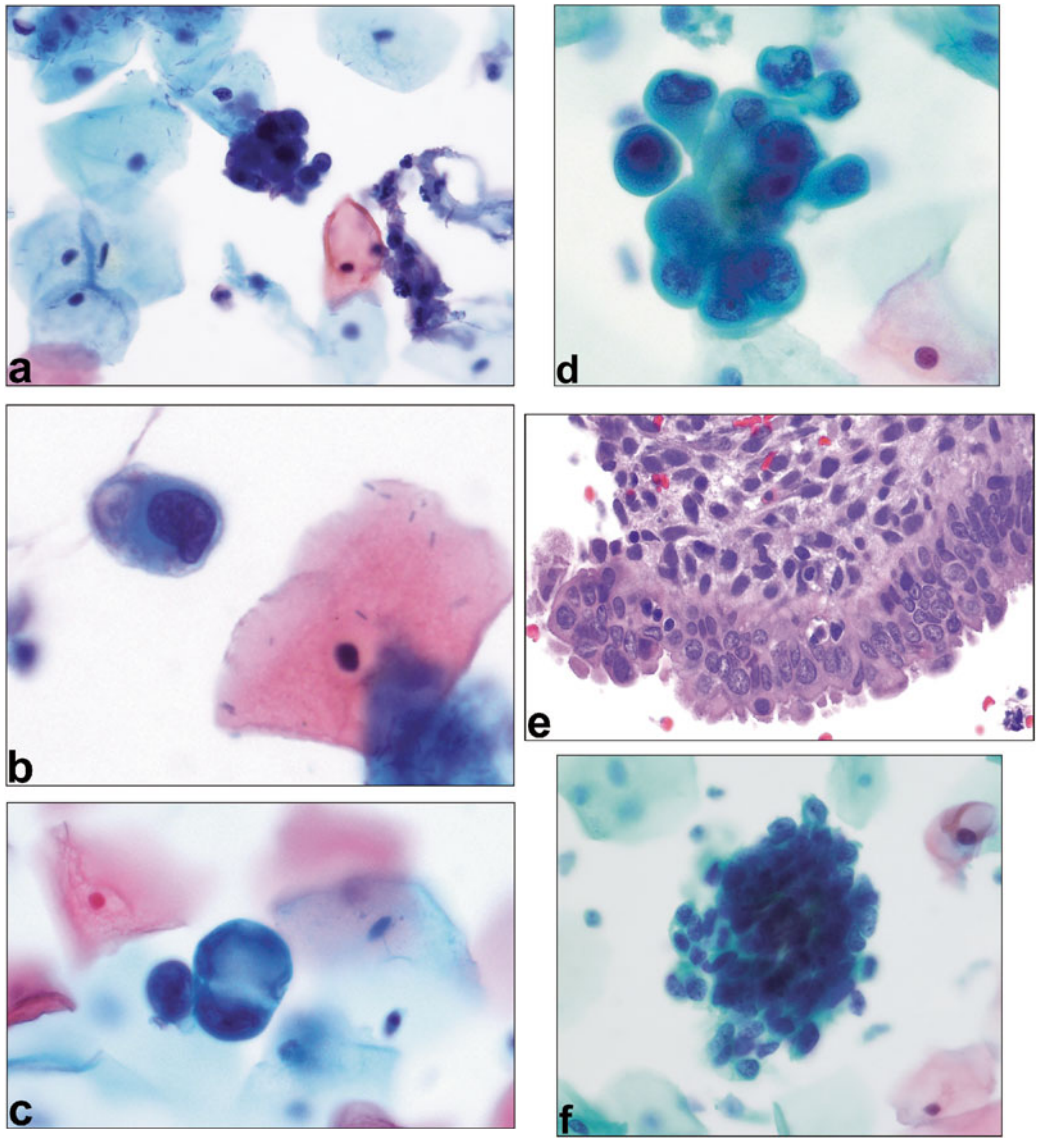


Fig. 2.8 Mimics of AIS and adenocarcinoma (AGUS cases with benign follow-up). (**a**) Degenerated glandular cells form a loose cluster in a Pap MLP from a patient with an IUD in place. Without the history of IUD, the cells could be interpreted as endometrial cells shed out of phase or AGUS. Clinical history is immensely helpful in this setting. (**b**) Single atypical "IUD cell" with hyperchromasia, irregular nuclear contour and cytoplasmic vacuole. HGSIL is in the differential, but hrHPV testing was negative and subsequent cervical tissue biopsies were negative for dysplasia and carcinoma. (**c**) Rare, markedly atypical doublet of endometrial glandular cells in a patient with chronic endometritis. A diagnosis of "AGUS-endometrial" is appropriate, as the cells are identical to examples of endometrioid adenocarcinoma. (**d**) Atypical glandular cells form a loose cluster. The size of the cells and markedly irregular nuclear contours suggest possible endometrial origin. Again, a diagnosis of AGUS-NOS or AGUS- endometrial is reasonable. (**e**) Follow-up endometrial biopsy revealed an endometrial polyp with surface abrasion and reparative changes to the surface mucosa. The features of these cells match those in the patient's Pap MLP. (**f**) Endometrial cell group from a patient with several foci of cervical endometriosis. The cells are identifiable as endometrial and do not exhibit marked atypia. However, the cells were shed out of phase, prompting cervical and endometrial biopsies. Note that on an MLP, endometrial nuclei may have better preserved nuclei that may feign atypia such as mild contour irregularities, granular chromatin and small nucleoli. Apoptotic bodies may also be present

Post-radiation or Chemotherapy

Changes seen post-irradiation and after some types of chemotherapy are fairly characteristic to the degree that the clinical history can be ascertained in many cases. Affected squamous cells are enlarged, sometimes markedly so, as are the nuclei which are pleomorphic and vesicular. Importantly, the majority of the altered cells retain a low N/C ratio unlike true malignant cells. Cell cytoplasm can show degenerative changes including two-tone color (polychromasia) and cytoplasmic vacuolation. Cell borders may be frayed or wispy. Nuclear features also may include multinucleation, degenerative smudgy chromatin, and karyorrhexis, and macronucleoli may be single to multiple and even pleomorphic. Florid changes can persist 20 years but often abate over time.

The differential diagnosis comprises residual or new malignany, AGUS or HGSIL. Persistent malignant cells may also undergo the above-described reactive/reparative/degenerative changes but critically will retain very coarse chromatin and uneven chromatin distribution combined with an increased N/C ratio. A few of the dysplastic or malignant cells may appear similar to original tumor without secondary superimposed treatment effect. In a few cases, definitive diagnosis may be impossible. In these instances, tissue biopsy is indicated.

A particularly difficult differential occurs in the setting of allogeneic bone marrow transplant (BMT) patients treated with busalfan and conditioning total body irradiation. Without a history of BMT, the cytologic findings on Pap can appear identical to keratinizing high grade dysplasia or squamous carcinoma, as the affected cells display nuclear enlargement, pleomorphism, hyperchromasia and stripped atypical nuclei, and the background can simulate a tumor diathesis with granular debris and inflammation [17]. Since immunosuppression predisposes to new HPV infections or rapid progression of residing HPV lesions, distinction between chemotherapy induced atypia and dysplasia/carcinoma is especially relevant. Concurrent hrHPV testing, clinical history and possibly p16/Ki-67 dual staining can be diagnostically helpful.

Tamoxifen Therapy

Postmenopausal women on Tamoxifen may show maturation of the squamous cells in a clean background on a Pap, while premenopausal patients exhibit an immature squamous epithelial pattern. Patients are at risk for endometrial hyperplasia and carcinoma, usually after several years of treatment. A history of Tamoxifen use should prompt a careful search for atypical appearing endometrial cells. Clusters of stripped nuclei have been reported in patients taking Tamoxifen; the most recent interpretation of these small round blue nuclei aggregates posits they represent degenerated reserve cells of the cervical epithelium. Such stripped nuclei aggregates may be found in older women not taking Tamoxifen. In both instances (women on Tamoxifen and women >50 years without a history of Tamoxifen use) these nuclei show no atypical features, are small with fine chromatin and inconspicuous nucleoli and should be differentiated from the stripped, cytologically atypical nuclei that can signal the possible presence of HGSIL, squamous carcinoma or a glandular lesion.

Other Drugs

Quite probably, a number of over-the-counter and prescribed drugs affect the cervical and endometrial epithelium to a degree that would cause observable changes in a Pap test. However, little is known regarding the effects of most of these many thousands of compounds. Some studies note that digitalis causes squamous maturation, while tetracyclines cause excessive exfoliation with only parabasal cells present on the MLP. Unusual maturation patterns may thus be accounted for in some patients by use of a specific medication.

Miscellaneous

Extensive Immature Squamous Metaplasia

Especially when inflammatory changes are superimposed, immature squamous metaplasia is probably the most common mimic of squamous dysplasia and AGUS. An N/C ratio <50 %, smooth nuclear contours and even distribution of

chromatin favor a benign interpretation. Inflammatory changes introduce more diagnostic difficulty and may lead to a diagnosis of ASCUS. Negative high risk HPV test results and no expression of p16 by immunocytochemistry support a "negative for intra-epithelial lesion or malignancy" (NILM) diagnosis. Figure 2.9 provides examples of metaplasia and a confounder. Table 2.5 lists miscellaneous conditions that can be mistaken for dysplasia or carcinoma.

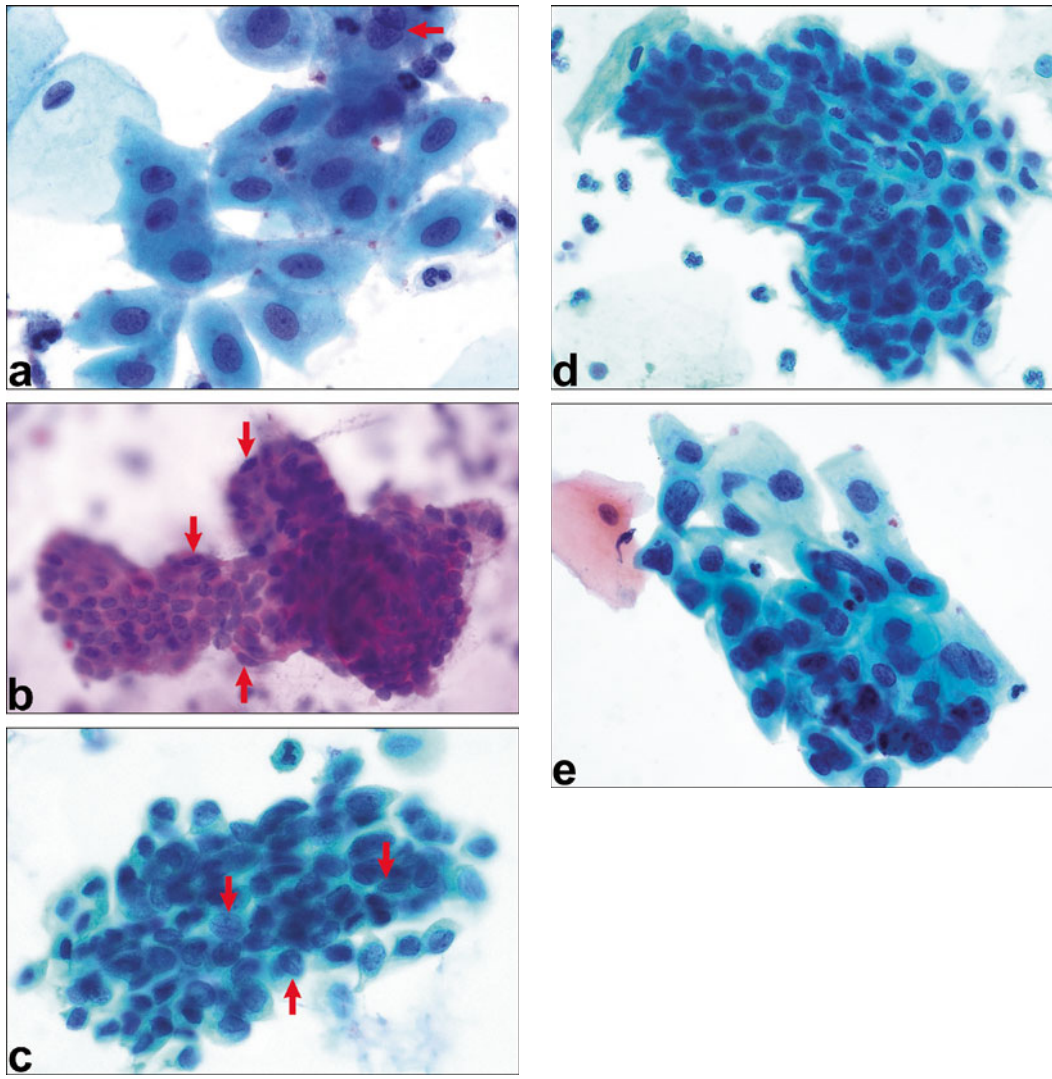


Fig. 2.9 Squamous and transitional metaplasias and confounder. (**a**) Normal squamous metaplastic cells show mild nuclear enlargement and binucleation (*arrow*) without marked hyperchromasia, nuclear contour irregularities, or irregular chromatin distribution. (**b**) Metaplastic squamous cells may assume the shape of endocervical glands as they replace the glandular cells. Notice the focally flattened cell border (*arrows*). (**c**) Numerous nuclear grooves (*arrows*) suggest the possibility of transitional metaplasia in this group of cells from a postmenopausal patient. (**d**) Sometimes atrophy is difficult to distinguish from metaplasia. This group derives from the Pap of a 58 year old woman status post chemotherapy and radiation for cervical carcinoma. Although the N/C ratio is elevated, the chromatin is fine, evenly distributed, and nuclear shapes are simple. (**e**) These metaplastic squamous cells show too much nuclear atypia (marked variation in nuclear contours, N/C ratio in some cells >50 %, slightly irregular chromatin clearing) to be considered reactive. Follow-up biopsy revealed moderate squamous dysplasia

Table 2.5 Miscellaneous conditions that may mimic significant lesions in cervical/vaginal cytology specimens

Mimicker	What it mimics	Architecture	Cell features	Key differentiating features	Ancillary studies
Arias-Stella change[a]	• AGUS • AIS • HGSIL, non-keratinizing type • Exuberant repair • Non-keratinizing SCC	• Isolated single cells • Irregular tissue fragments with crowding and nuclear overlap • Atypical cells or groups are usually sparse	• N/C ratio often but not always high • Cytoplasmic vacuoles common ("bubble gum" look in some) • Leukophagocytosis +/− • Nuclei range from vesicular to hyperchromatic • May have nucleoli • May have nuclear pseudoinclusions and grooves • Mitoses +/−	• Lack of coarse chromatin • Lack of parachromatin clearing • Lack of prominent nuclear pleomorphism • History of pregnancy • Sparse number of atypical cells	• Negative hrHPV test • Negative for p16 by ICC on cell block
Decidual change (decidualized stromal cells of either endometrium or cervix)	• LGSIL • Metaplastic squamous cells	• Flat clusters, sheets • Single cells	• Cells round to polygonal with dense eosinophilic to pale finely granular cytoplasm • N/C ratio low • Nuclei 3–5× size of intermediate nucleus with fine granular chromatin; may be hyperchromatic	• Low N/C ratio • Smooth nuclear contours • Fine chromatin evenly distributed • May have secondary degenerative changes	• Negative hrHPV test • Negative for p16 on ICC • Negative for keratin on ICC
Trophoblastic cells[a]	• LGSIL • HGSIL	• Isolated single cells	• Cytotrophoblasts: round with evenly spaced chromatin, +/− nucleoli, +/− hyperchromasia, +/− smudgy change; N/C ratio may be >50 % • Syncytotrophoblasts: very large, multinucleated, +/− cytoplasmic vacuoles, nuclei may have small nucleoli	• Lack parachromatin clearing • Lack marked nuclear pleomorphism • Usually only sparse but if numerous may indicate risk for pregnancy loss	• Negative hrHPV test
Post-partum or lactational squamous cells[a]	HGSIL	• Single cells • Small clusters • Sheets	• Can appear similar to atrophy with small size and lack of keratinization • May have higher N/C ratio	• Lack coarse chromatin • Lack parachromatin clearing	• Negative hrHPV test • Negative for p16 on cell block

(continued)

Table 2.5 (continued)

Mimicker	What it mimics	Architecture	Cell features	Key differentiating features	Ancillary studies
Degenerated seminal vesicle cells	HGSIL	Single cells	• Round to polygonal cells • Hyperchromatic with coarse chromatin • N/C ratio often high • May have degenerative changes (smudgy nuclei)	• Usually sparse • Associated with sperm • Golden yellow cytoplasmic pigment	• Negative hrHPV test • Negative for p16 by ICC on cell block
Endometriosis	• Endometrial neoplasia • AGUS NOS • AGUS—endocervical • Endometrial cells from endometrium shed out of phase	• Gland fragments • Small tight balls of small cells • Spindle cell stromal fragments • Mixed gland/stromal fragments • Fragments may have protruding blood vessels	• Tubal metaplasia often present and may have small cells with high N/C ratio, hyperchromasia and nuclear contour irregularities • Endometrial nuclei usually small, high N/C ratio, +/− mitoses and apoptotic bodies • May have hematoidin pigment and histiocytes in background	• Cilia and/or terminal bars in tubal metaplastic cells • Lack of marked nuclear atypia in the endometrial epithelial and stromal cells	Benign features may be appreciated on a cell block Positive for vimentin and ER on
Inadvertent sampling of lower uterine segment (LUS)	• Endometrial neoplasia • Endocervical neoplasia	Same findings as for endometriosis	Same findings as for endometriosis except lack hematoidin pigment and prominent histiocytes	Same as for endometriosis	Same as for endometriosis
Tubal and tuboendometrioid metaplasia (TEM)	• HGSIL • AGUS, AGUS favor neoplastic • Endometrial neoplasia	• Isolated single cells • Small clusters • Uncommonly strips with nuclear overlap and pseudostratification	• Round to oval nuclei with high N/C ratio • Hyperchromasia often present • Some nuclei may be washed out • Pleomorphism may be present	• Lacks apoptotic bodies • Lack coarse chromatin, uneven chromatin distribution/parachromatin clearing • Cilia or terminal bars in some cells • Goblet cells +/−	• Negative CEA and negative to patchy p16 (cell block prep) • Vimentin and usually ER diffusely positive (cell block prep) • Cell block may clearly show benign features including cilia, peg cells etc.
Transitional metaplasia	HGSIL	• Streaming fascicles • Organized sheets	• Oval to elongate nuclei • Fine evenly distributed chromatin	• Polarized groups with lack of nuclear overlap in one plane of section • Lack of coarse chromatin and parachromatin clearing • Lack of pleomorphism	• Negative hrHPV test • Cases with superimposed dysplasia will have nuclear features of dysplasia[b] and positive hrHPV test

Microglandular hyperplasia	• AGUS • AIS • ASCUS • Repair with inflammation	• Tight clusters • Acini	• Mild nuclear enlargement • Mild nuclear contour irregularities • Mild hyperchromasia • Small parakeratosis +/− • Inflammation	• Lacks convincing nuclear features of dysplasia[b]	• Negative hrHPV test • May express p16 on ICC • Positive for ER unlike most cases of endocervical AIS/adenocarcinoma
Lobular endocervical glandular hyperplasia (LEGH)	• AGUS • AIS • Minimal deviation adenocarcinoma	• Polarized sheets • Strips • Both can be numerous	• Cells round with abundant granular cytoplasm +/− golden brown granular pigment • +/− goblet cells • N/C ratio usually <50 % • Mild nuclear enlargement • Conspicuous nucleoli • Nuclear pseudoinclusions +/−	• Typical examples lack marked nuclear atypia • Atypical examples may be indistinguishable from AGUS	• Negative hrHPV test (does not help exclude adenoma malignum) • MRI findings may be helpful in differentiation from adenoma malignum
Endocervical polyp	• AGUS • ASCUS-H • HGSIL	Florid reactive/reparative changes including disorganized clusters often with intermixed neutrophils	• Nuclear enlargement • Prominent nucleoli • Mitotic figures • Inflammatory background • Immature metaplastic cells may have hyperchromasia	• Lack coarse chromatin • Lack marked nuclear contour irregularities • Lack parachromatin clearing	• Negative hfHPT test • Negative for p16 by ICC on cell block
Endometrial polyp	• AGUS • HGSIL • Endometrial cells shed out of phase	• Clusters • Small 3-d tight balls • Large gland and stromal fragments (mixed or pure)	• Nuclear atypica ranges from none (look like normal endometrial cells) to marked atypia in cases of polyps with surface abrasion • Nuclear enlargement • Increased N/C ratio • Hyperchromasia • Mitoses +/− • Apoptotic bodies +/−	May not be able to distinguish markedly atypical examples from AGUS	• Negative hrHPV test • Cell block may show benign architectural features • Positive for ER, vimentin • ProExC and IMP3 both negative in 100 % of glandular lesions and ~80 % of SIL

(continued)

Table 2.5 (continued)

Mimicker	What it mimics	Architecture	Cell features	Key differentiating features	Ancillary studies
Atrophy with rare isolated markedly atypical cells	• HGSIL • AGUS • AIS • ADENOCA	• Isolated single cells • Rarely, clusters of 2–5 cells	• Small to medium-sized cells with high N/C ratio • Hyperchromasia • Nuclear contour irregularities +/– • Degenerative nuclear changes +/– • Stripped nuclei +/–	• Extreme rarity of markedly atypical cells • Markedly atrophic background • Negative clinical history of bleeding or carcinoma	• Negative hrHPV test • Cells disappear after course of topical estrogen therapy
Extensive immature metaplasia	• HGSIL • LGSIL	• Sheets of non-keratinizing with N/C ratio <50 %	• Minimal nuclear overlapping when focusing in one plane	• Lack coarse chromatin • Lack apoptotic bodies • Lack markedly irregular nuclear contours	• Negative hrHPV test • Negative for p16 by ICC on cell block
Reserve cell hyperplasia	• HGSIL • AGUS, AIS	• Type of immature metaplasia composed of sheets of small cells with a relatively high N/C ratio • May be mixed with typical immature metaplasia fragments	• Minimal nuclear overlapping when focusing in one plane • Hyperchromatic	• Lack coarse chromatin • Lack apoptotic bodies • Lack markedly irregular nuclear contours	• Negative hrHPV test • Negative for p16 by ICC on cell block
Atypical oxyphilic metaplasia (of superficial endocervical glands)	• AIS	• Glandular fragments • Clusters	• Medium sized cells with eosinophilic, dense cytoplasm • Cytoplasmic vacuoles +/– • Nuclear enlargement • Hyperchromasia • Nuclei may be multilobated or multinucleated • Prominent nucleoli	• Usually sparse in sample • Usually lacks nuclear pseudostratification • Lacks apoptotic bodies • Lacks mitoses	• Negative hrHPV test • Negative for p16 by ICC on cell block

LGSIL low grade squamous intra-epithelial lesion, *HGSIL* high grade squamous intra-epithelial lesion, *SCC* squamous cell carcinoma, *AIS* adenocarcinoma in situ, *EcA* endocervical adenocarcinoma

[a]Associated with pregnancy or lactation

Reserve Cell Hyperplasia

Reserve cell hyperplasia is a type of immature squamous metaplasia composed of small crowded cells with a relatively high N/C ratio. Apoptotic bodies, coarse chromatin, nuclear overlapping when focusing in one plane of section, and markedly irregular nuclear contours are absent.

Transitional Cell Metaplasia

Transitional metaplasia is most often found as streaming fascicles or sheets of oval to elongate nuclei with characteristic nuclear grooves similar to transitional epithelium of the bladder. Most patients are post-menopausal. The clusters are well organized. Nuclei are uniform with fine, evenly distributed chromatin. Consistent with the atrophic nature of the process, mitotic activity and apoptotic bodies are absent. The differential diagnosis includes HGSIL or ASCUS-H. Dysplasia may be superimposed on transitional metaplasia, and such dysplastic cells would exhibit more hyperchromasia with coarse chromatin, greater variation in nuclear size, marked nuclear overlapping when focusing in one plane, scattered mitotic figures and would test positively for HPV.

Tubal and Tuboendometrioid Metaplasia (TEM)

Tubal and tuboendometrioid metaplasia often may be mistaken for HGSIL or AGUS. The process is common, and many architectural and cytologic features mimic those of a significant epithelial lesion. The cells may be arranged singly, in small groups or clusters and disorganized strips. Uncommonly, some cell groups exhibit pseudostratification and nuclear overlapping worrisome for AGUS. Moreover, many cells exhibit significant hyperchromasia and a high N/C ratio. Nuclear pleomorphism may be present. Reassuringly, apoptotic bodies are nearly always absent, and mitotic figures are usually rare. In contrast to typical AIS, the nuclei are mostly round to oval rather than elongate and they usually lack coarse chromatin and uneven chromatin distribution. A few nuclei may show a washed out pale appearance consistent with degeneration. Scattered goblet cells or cells with discrete cytoplasmic vacuoles can occur. Cilia and/or terminal bars in at least some of the cells of obvious similarity in other ways to the most atypical cells are key diagnostic cues. The differential diagnosis comprises AGUS, conventional endocervical AIS/adenocarcinoma, typical endometrial adenocarcinoma and the very rare ciliated endometrial adenocarcinoma or tubal type endocervical adenocarcinoma. Tubal type adenocarcinoma can only be suggested when the ciliated cells exhibit extreme nuclear atypia, mitotic activity and apoptotic bodies with loss of normal polarity in cell groups. Both tubal metaplastic cells and malignant tubal cells are negative for hrHPV, and by immunocytochemical staining express bcl2, vimentin, PAX2, ER and PR compared to endocervical AIS which does not express these antigens [14]. Figure 2.10 provides examples of tubal metaplasia.

Tuboendometrioid metaplasia includes a mixture of tubal type epithelial cells and cells with features of endometrium. Figures 2.11 and 2.12 provide examples of normal endometrium to compare with pathologic endometrial conditions.

Atrophic Vaginitis

Atrophic vaginitis yields large numbers of immature, intermediate or parabasal cells. The cells may exhibit "red atrophy" corresponding to cytoplasmic eosinophilia, "blue blobs" comprising fragments of cytoplasm, and form syncytia. A higher N/C ratio and smudgy hyperchromatic nuclei may require a diagnosis of ASCUS in some cases. Rare, isolated cells may appear particularly suspicous for HGSIL and most likely represent rounded up, degenerated endocervical cells or reserve cells. High risk HPV testing on the current specimen, possibly p16/Ki67 staining, or a course of estrogen followed by a repeat Pap test can resolve initial ambiguous findings.

Degenerated Seminal Vesicle Cells

Seminal vesicle cells may pose a diagnostic challenge as they also do in urine specimens. The cells are round to polygonal with a high N/C ratio and hyperchromasia. Nuclear degeneration such as smudgy chromatin and nuclear holes may be present. Cytoplasmic granular golden pigment and sperm provide diagnostic clues, when pres-

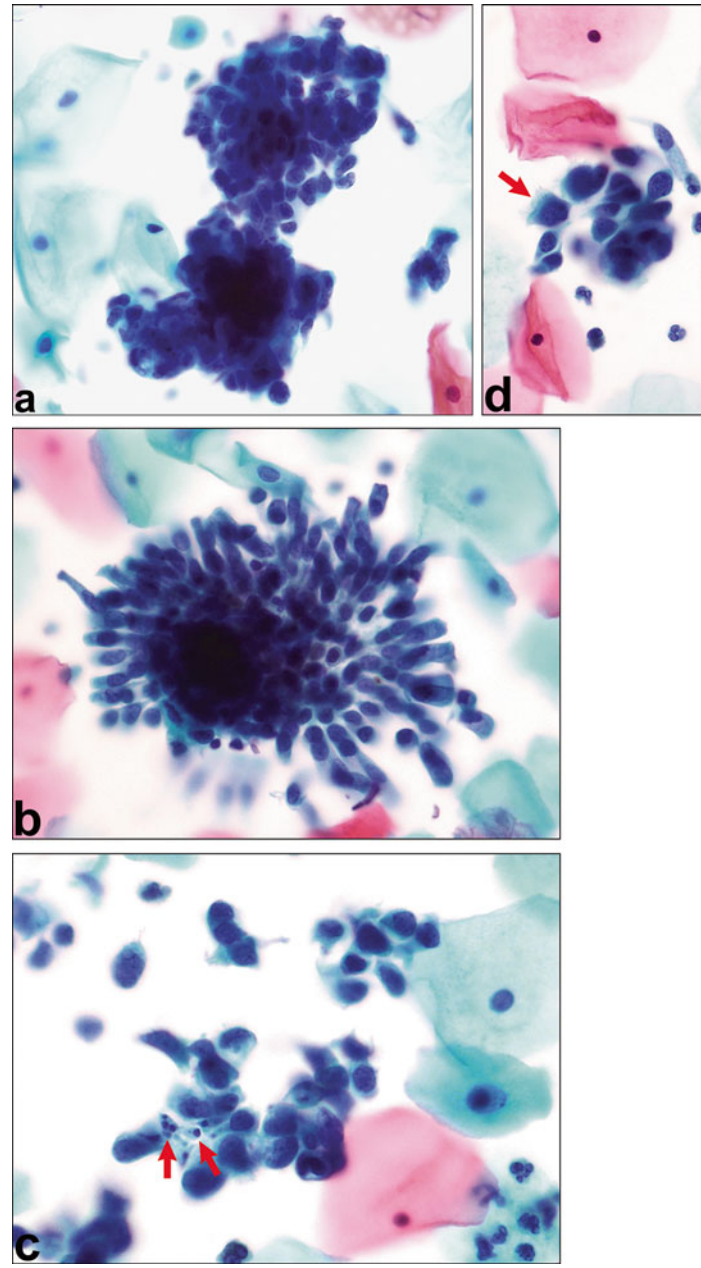


Fig. 2.10 Tubal metaplasia. (**a**) shows a tight cluster of metaplastic tubal cells arranged in a 3-d ball or gland-like structure. These cells in isolation could be interpreted as endometrial or HGSIL growing into an endocervical gland. (**b**) A floret-like cluster of metaplastic tubal cells shows cell feathering but importantly not nuclear feathering at the edges. (**c**) Neutrophilic debris could be misinterpreted as apoptotic bodies (*arrows*). However, mitotic activity and marked nuclear changes such as coarse, irregularly distributed chromatin are absent. The cells are also overall relatively small compared to most cases of cervical AIS/adenocarcinoma. (**d**) The key diagnostic finding supporting a diagnosis of tubal metaplasia is the presence of cilia (*arrow*) and/or terminal bars in at least some of the atypical cells

ent. Seminal vesicle cells are usually very few in number and do not form cohesive clusters. Sperm and seminal vesicle cells can persist several days after intercourse.

Pregnancy Related Findings

(a) *Post-partum and lactation changes*: Many squamous cells are immature and may be atrophic or small and "active" with a higher N/C ratio. Coarse chromatin and para-chromatin clearing are absent. However, these cells could be mistaken for HGSIL. Figure 2.13 provides examples of atrophy and a confounder. Squamous atrophy generally occurs in latter half of pregnancy and may persist into the post partum period if the patient is breast feeding

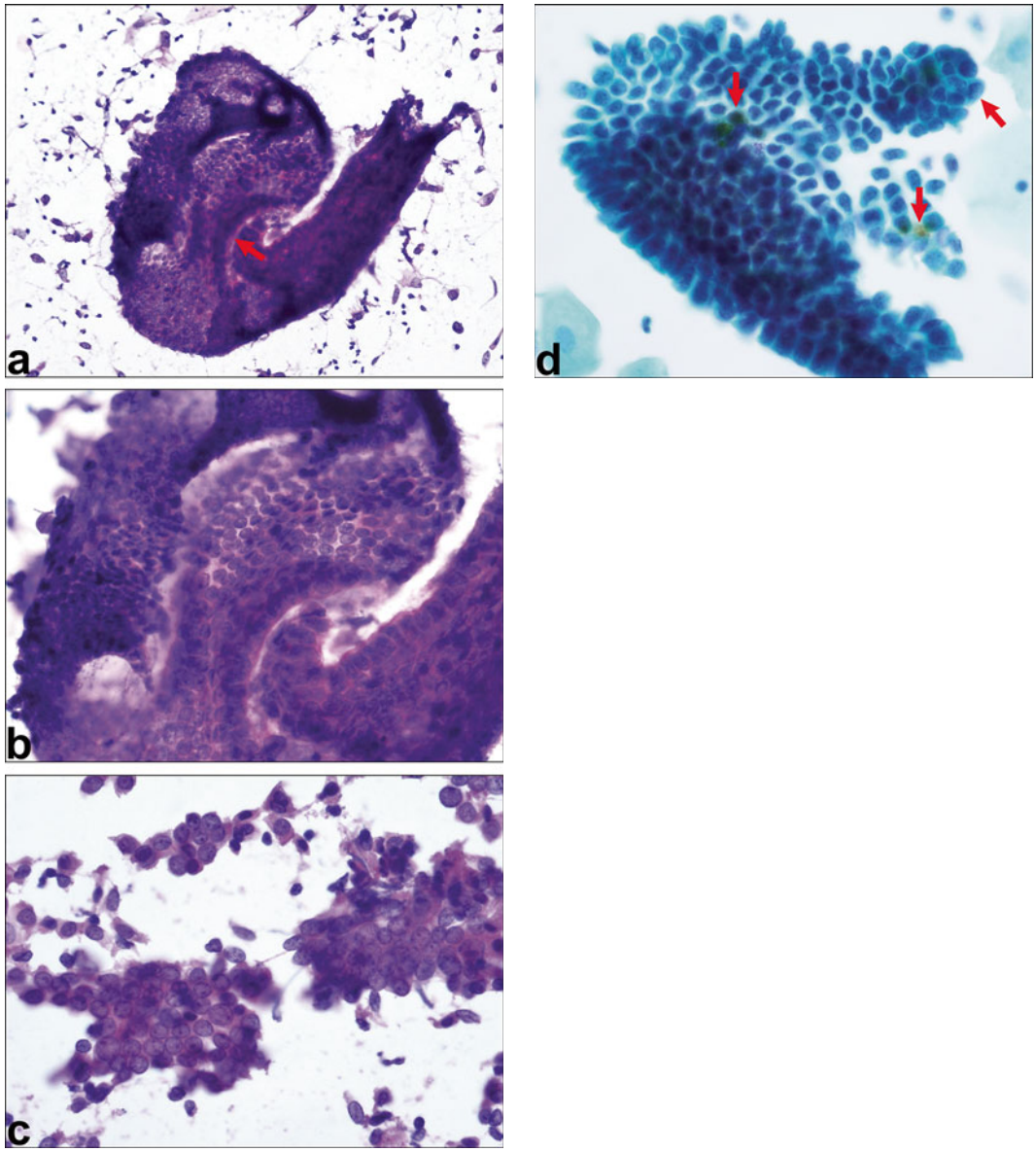


Fig. 2.11 Normal endometrium and endometriosis. Directly sampled endometrium can occur in the Pap with an aggressive brushing technique or may occur in the setting of prolapsed endometrial polyp, chronic endometritis or endometriosis. These figures derive from direct scrapes of the endometrium and are stained with hematoxylin and eosin except (**d**). (**a**) Shows a smoothly contoured folded gland with peripheral palisading of columnar epithelium (*arrow*). (**b**) Nuclear crowding is present, but the nuclei are uniform with fine chromatin and a simple ovoid shape. (**c**) Mild nuclear size variation is appreciable in this smaller frayed glands. (**d**) This well-formed gland shed into the Pap in a patient with cervical endometriosis contains foci of hematoidin pigment (*arrows*) and one edge is starting to ball-up

In some instances, ASCUS-H or ASCUS is an appropriate diagnosis. Clinical history and concurrent HPV testing may be helpful.

(b) *Arias-Stella change (A-S change) in glands*: Arias-Stella secretory/hypersecretory epithelial changes more commonly occur in endometrial glands but can also involve

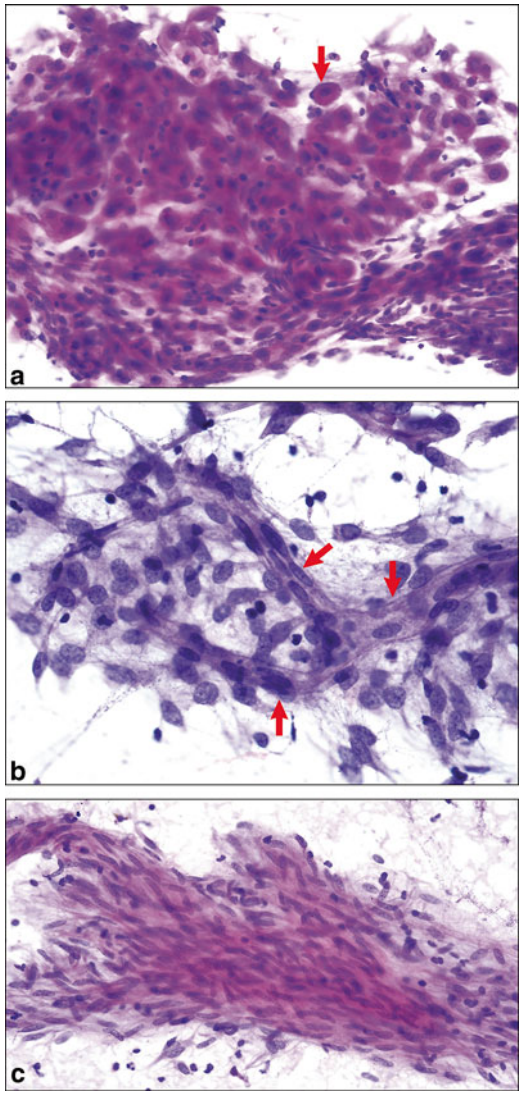


Fig. 2.12 Normal endometrial stroma. (**a**) Decidualized stromal cells may exhibit a convex almost glassy appearance (*arrow*) mimicking intermediate or metaplastic cells. (**b**) Some sheets of stroma or combined stroma/epithelium will contain branching blood vessels (*arrows*) and suggest the diagnosis of endometrial tissue. (**c**) Endometrial stromal cells may form flowing spindle cell aggregates that should not be misinterpreted as spindle cell squamous dysplasia

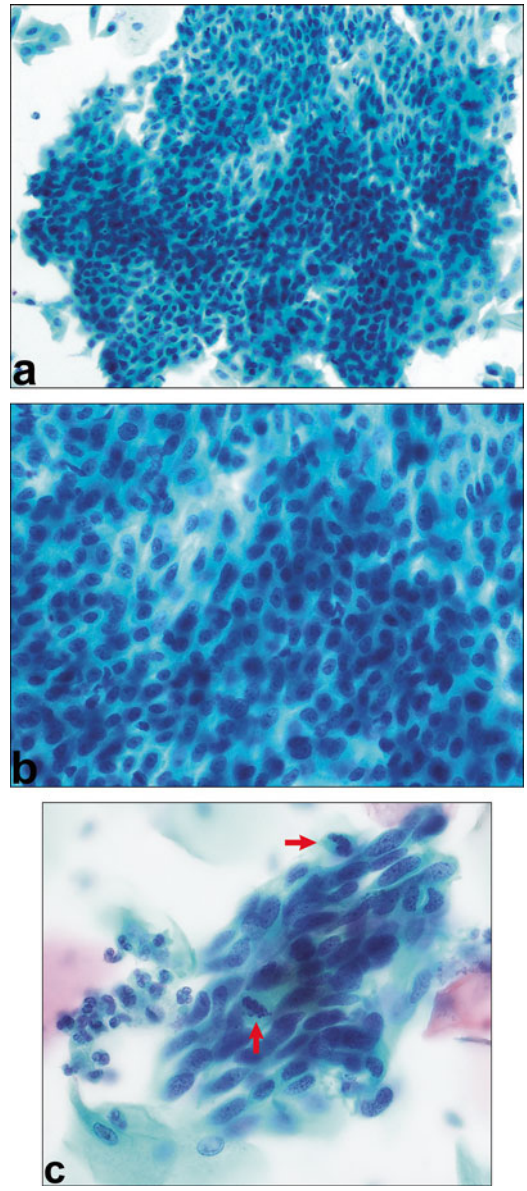


Fig. 2.13 Squamous atrophy and confounder. (**a**) At low magnification, sheets of atrophic squamous epithelium may appear alarmingly crowded and monotonous. (**b**) However, at higher magnification, the nuclei have a simple shape, the chromatin is relatively fine, and there is no significant nuclear overlap when focusing in the same plane. Small nucleoli may be present. Mitotic figures and apoptotic bodies are absent, even in such a hypercellular field. (**c**) Non-keratinizing high grade dysplasia mimicking atrophy can show streaming at low magnification. However, nuclear overlap in the same plane of focus, coarse chromatin and mitotic figures (*arrows*) enable the correct diagnosis

endocervical glands. Changes described include atypical cells arranged as isolated single cells or irregular, disorganized tissue fragments with crowded and overlapping nuclei (see Fig. 2.14). Cell size ranges as does the amount of cytoplasm imparting a low to high N/C ratio (often it is high). Cytoplasmic vacuolation is common, and leukophagocytosis may occur. Nuclei are commonly enlarged, round to oval, may be hyperchromatic or vesicular, and may contain prominent nucleoli (single or multiple), nuclear pseudo-inclusions and nuclear grooves [6]. The differential diagnosis includes exuberant repair or regeneration, AIS, HGSIL and non-keratinizing SCC. Key features that help distinguish Arias-Stella from significant lesions include lack of coarse chromatin, lack of parachromatin clearing, lack of prominent nuclear pleomorphism, a history of pregnancy and the general rarity of the atypical cells. Mitotic figures are not helpful, as they occur both in A-S change and significant lesions. Arias-Stella reaction is not just confined to pregnant patients but can occur in HRT, patients on oral progestational agents for the management of dysfunctional uterine bleeding, or treatments for infertility. Moreover, the change may develop early in pregnancy before the patient knows she is pregnant. In some cases, clear-cut distinction of Arias-Stella change from a significant glandular or squamous lesion is impossible, and a diagnosis of AGUS or ASCUS (depending on the specific set of features present) is warranted.

(c) *Trophoblastic cells*: Cytotrophoblasts are round cells with an elevated N/C ratio that are usually sparse and shed as single isolated cells. The nuclei may be hyperchromatic, but chromatin is evenly spaced although it can be coarse or smudgy due to degeneration. Macronucleoli may be present. Irregular nuclear contours and parachromatin clearing are absent. The differential diagnosis comprises HGSIL or endometrial cells. Rarely, the cells may persist for months after delivery. HPV testing, clinical history of pregnancy or postpartum state, and immunocytochemical staining for p16 may be diagnostically useful. A diagnosis of ASCUS or ASCUS-H may be indicated in some cases with increased cellularity or particularly atypcal, well-preserved nuclear features. Syncytiotrophoblasts may also be shed during pregnancy. These are large multinucleated cells that are usually indistinguishable from multinucleated histiocytes (see Fig. 2.14). It might be surmised that shed intermediate trophoblasts (ITs) would be difficult to distinguish from dysplastic squamous cells because of nuclear enlargement and pleomorphism characteristic of ITs. However, there are no reports of the specific appearance of intermediate trophoblastic cells in Pap smears or MLPs.

(d) *Decidualized stromal cells*: Decidual cells occur as isolated single cells or in small clusters or sheets. The cells are round to polygonal and may exhibit dense "convex" cytoplasm, or the cytoplasm may be granular or finely vacuolated. The N/C ratio is usually low, and cells contain abundant cytoplasm. While sometimes appearing "dense," the cytoplasm may also appear pale due to a high glycogen content and small, non-optically clear perinuclear halos may be seen (see Fig. 2.14). Decidual cell nuclei are generally larger than intermediate squamous cell nuclei (3–5×), contain fine chromatin that is evenly distributed and may be normo- to hyperchromatic. Nucleoli are often present, and nuclear rims are usually smooth. Degenerative nuclear features may affect some cells. The cells are most commonly mistaken for metaplastic squamous cells but could be confused with ASCUS. HPV testing and p16 immunocytochemical staining (both negative in decidual cells) could be used in difficult cases.

(e) *Pseudokoilocytes of high estrogen and/or progesterone states*: As previously noted (see Section "Hormone replacement therapy (HRT)" above) some intermediate squamous cells on ThinPrep™ MLPs with abundant glycogen may exhibit mild hyperchromaticity, condensed cytoplasmic rims and non-optically clear large halos that could mimic

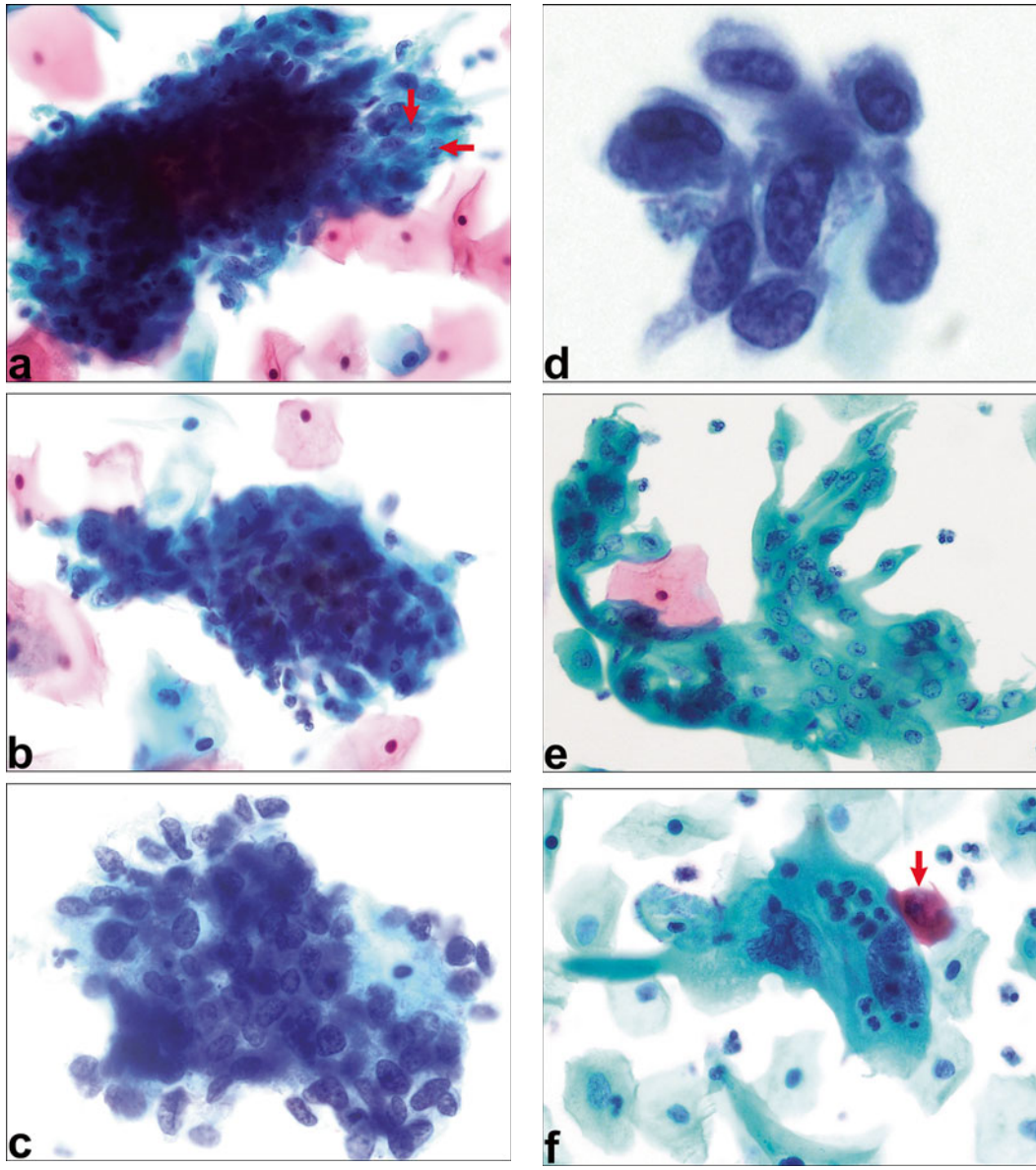


Fig. 2.14 Pregnancy and post-partum changes and confounder. (**a**) A fragment of decidual cells in a post-partum Pap shows a ragged edge and the constituent cells exhibit hypochromasia and small nucleoli (*arrows*). (**b**) Another decidual fragment which contains tumbled cells with slightly more granular chromatin and variable hyperchromasia. Careful examination of the cells confirms a relatively low N/C ratio in intact cells. (**c**) Atypical glandular cluster in a 37 year old woman's post-partum Pap shows crowding, irregular nuclear contours, and coarse chromatin. However, the groups are rare in the smear, the patient tests negative for hrHPV, and repeat Pap 3 months later is without abnormalities. This could represent degenerated Arias-Stella change in an endocervical gland. (**d**) Another post-partum Pap test with atypical glandular cells and a negative hrHPV test. A diagnosis of AGUS is prudent in cases with this much nuclear atypia. Interestingly, the cells look somewhat similar to the activated, hyperfunctional epithelium of lactating adenoma except lack macronucleoli. (**e**) Probable syncytiotrophoblast in a Pap smear obtained in mid-pregnancy. Note the large size, irregular shape and cytoplasmic vacuolations. A patient with abundant syncytiotrophoblasts in a Pap and bleeding may be at risk for pregnancy loss. (**f**) Thirty three year old woman with markedly atypical large cells in a post-partum Pap. The differential could include intermediate trophoblasts as well as squamous dysplasia. Note the leukophagocytosis and additional, possibly keratinized atypical cell (*arrow*). Follow-up tissue biopsy revealed high grade dysplasia composed primarily of large, non-keratinizing cells

koilocytes [15]. This is considered a processing artifact. Nuclear details less marked nuclear enlargement and finer chromatin than true koilocytes. Cytoplasmic glycogen in the perinuclear pale area also suggests the diagnosis of benign changes. High risk HPV testing is helpful in especially difficult cases.

Endometriosis of Cervix/Vagina

This uncommon condition can cause shedding of endometrial cells out of phase or reveal three dimensional glands and fragments of spindled stroma, sometimes with hemotoidin pigment and histiocytes in the background. Tubal type cells and cell groups are common and include cells with cilia and/or terminal bars, goblet cells, and slender columnar cells. The differential diagnosis includes an endometrial glandular lesion or less commonly, an endocervical glandular lesion. Immunocytochemical staining in a cell block can confirm the endometrial origin of the non-tubal epithelium (ER positive, vimentin positive), while p16 reactivity is absent or patchy. In some cases, a diagnosis of AGUS is unavoidable.

Endocervical Polyp

Patients with an endocervical polyp may have a normal Pap test or may shed cells with enough reactive/reparative changes that could prompt consideration of AGUS or ASCUS-H. Changes include disorganized clusters of cells with nuclear enlargement and nucleoli, mitotic activity and an inflammatory background. The cells importantly lack coarse chromatin, parachromatin clearing, and marked nuclear membrane irregularities. High risk HPV and p16 ICC testing can be helpful. A clinical history of polyp on colposcopy also aids in correct interpretation.

Endometrial Polyp

The findings on Pap of an endometrial polyp range from normal appearing endometrial cell balls shed out of cycle to highly worrisome clusters of cells due to surface abrasion of the polyp. Nuclear enlargement, hyperchromasia and elevated N/C ratios in glandular strips can be misinterpreted as AGUS or AGUS favor neoplasia. Mitotic activity and apoptotic bodies may be present. Some polyps shed three dimensional mixed stromal and glandular fragments identical to those seen in inadvertent sampling of the lower uterine segment (see below). Distinction from a significant glandular lesion is often not possible.

Inadvertent Sampling of Lower Uterine Segment (LUS)

Large three-dimensional but organized crowded sheets, strips and tubules may be present, especially in a SurePath™ specimen compared to a ThinPrep™ MLP. The aggregates often exhibit ragged trailing borders, and small vessels may protrude from the surface of the fragment. The small, uniform glandular cells and spindled stromal cells may form cohesive structures or glandular and spindled cells may exclusively form fragments of one cell type. Nuclear pleomorphism, hyperchromasia, coarse chromatin and loss of polarity with overlapping of nuclei when focusing in one plane are absent. Concurrent HPV testing or p16/Ki-67 dual ICC staining may be diagnostically helpful in challenging cases.

Microglandular Hyperplasia (MGH)

This benign cellular change in the cervix is composed of cuboidal endocervical cells and intermixed immature metaplastic squamous cells puddled by clusters of neutrophils. The Pap test usually contains only sparse numbers of the cells which are typically organized in tight clusters and acine. In most instances, the components resemble repair with inflammation. Mild nuclear enlargement, nuclear membrane irregularities, mild hyperchromasia and small parakeratotic cells may suggest a diagnosis of AGUS, ASCUS or AIS in the few florid examples. Lesional cells are negative on hrHPV testing but may express p16 on ICC. Unlike conventional endocervical cells and most cases of conventional endocervical AIS, MGH cells will diffusely express ER.

Lobular Endocervical Glandular Hyperplasia (LEGH)

LEGH represents a type of gastric foveolar metaplasia. When florid in the cervix, numerous polarized sheets and strips may be found in the Pap test. LEGH cells characteristically exhibit mild nuclear enlargement, conspicuous nucleoli and

fairly abundant cytoplasm which in some cells has a lacy or finely granular golden appearance due to accumulation of gastric type mucin. Some examples are atypical and can manifest as groups of cells with loss of polarity, apoptosis and mitotic activity with nuclear enlargement, hyperchromasia and nucleoli; these are worrisome findings and may require a diagnosis of AGUS. It has been reported that nuclear pseudoinclusions are common in LEGH in contrast to adenoma malignum or gastric type cervical adenocarcinoma, which could help differentiate LEGH from these two types of malignant gastric epithelial carcinomas of the cervix, but other types of AIS can have nuclear pseudo-inclusions, so this finding does not necessarily prove benignancy [10].

Tumors or Dysplasias that may be Difficult to Identify as Malignant

High Grade Dysplasia of Small Cell Non-keratizing Type

In pure form, this type of squamous dysplasia could easily be mistaken for metaplastic cells, endometrial cells or congregates of lymphocytes as in follicular cervicitis. The dysplastic cells derive from reserve or basal cells and invest the Pap as single cells or small, often loose clusters. Nuclear features typify dysplasia in that hyperchromasia and coarsely granular chromatin are present. Nucleoli may or may not be seen. Concurrent testing for hrHPV is positive. Many cases of small cell non-keratinizing HGSIL contain a component of more easily recognizable HGSIL or LGSIL. Figures 2.15, 2.16, and 2.17 include examples of lesions exhibiting a hyperchromatic crowded group pattern on the MLP. Table 2.6 lists and describes key features of some deceptively bland dysplasias and rare malignant tumors that may be mistaken for benign lesions or other malignancies.

Stratified Mucin-Producing Intraepithelial Lesion (SMILE)

SMILE is a recently described type of high grade dysplasia featuring a mixture of squamous and mucinous cells. Cytologic features include three dimensional cell clusters with nuclear stratification and crowding. Nuclei are only mildly atypical, but mitotic figures and apoptotic bodies are present. Some cells are vacuolated consistent with mucin production [4]. The constellation of features can be subtle. Fortunately, for diagnostic purposes, SMILE is almost always associated with a more easily recognizable lesion such as HGSIL, AIS or adenocarcinoma (present in ~95 % of 60 cases in a recent histologic review) [8].

Verrucous Carcinoma (Rare)

This rare, well-differentiated keratinizing squamous cells carcinoma features large numbers of anucleate squamous cells as fragments of thick keratin, while the nucleated cells may have pyknotic features only [19]. Correlation with clinical history and colposcopy findings can be critical in suggesting a significant lesion, as the features on the MLP imitate low grade dysplasia or just reactive parakeratosis and orthokeratosis. Abundant amounts of abnormal keratosis on a Pap test should be reported even in the absence of cytologic atypia.

Minimal Deviation Mucinous Endocervical Adenocarcinoma (Adenoma Malignum; Rare)

This non-HPV related carcinoma often features abundant, complex folded or fenestrated sheets on the Pap corresponding to numerous, abnormally shaped glands on histology. Cells are mucinous with a low N/C ratio and may contain pale to golden yellow cytoplasmic pigment (gastric type mucin). The background may be mucin-rich with or without a clinging diathesis on MLP. Slight atypia of groups at higher magnification includes crowding and mild nuclear overlap. Nuclear atypia is mild with minimal pleomorphism and nuclear contour irregularities. These subtle findings are usually accompanied by some aggregates showing more marked atypia including nuclear pseudostratification, a higher N/C ratio, hyperchromasia and coarser chromatin. Importantly, hrHPV is negative and should not lull the reviewer into a diagnosis of NILM. The differential diagnosis includes lobular endocervical glandular hyperplasia (LEGH): both entities can have lacy or granular golden pigment (gastric/pyloric type mucin), but most cases of LEGH lack nuclear atypia. Interestingly, some investigators assert

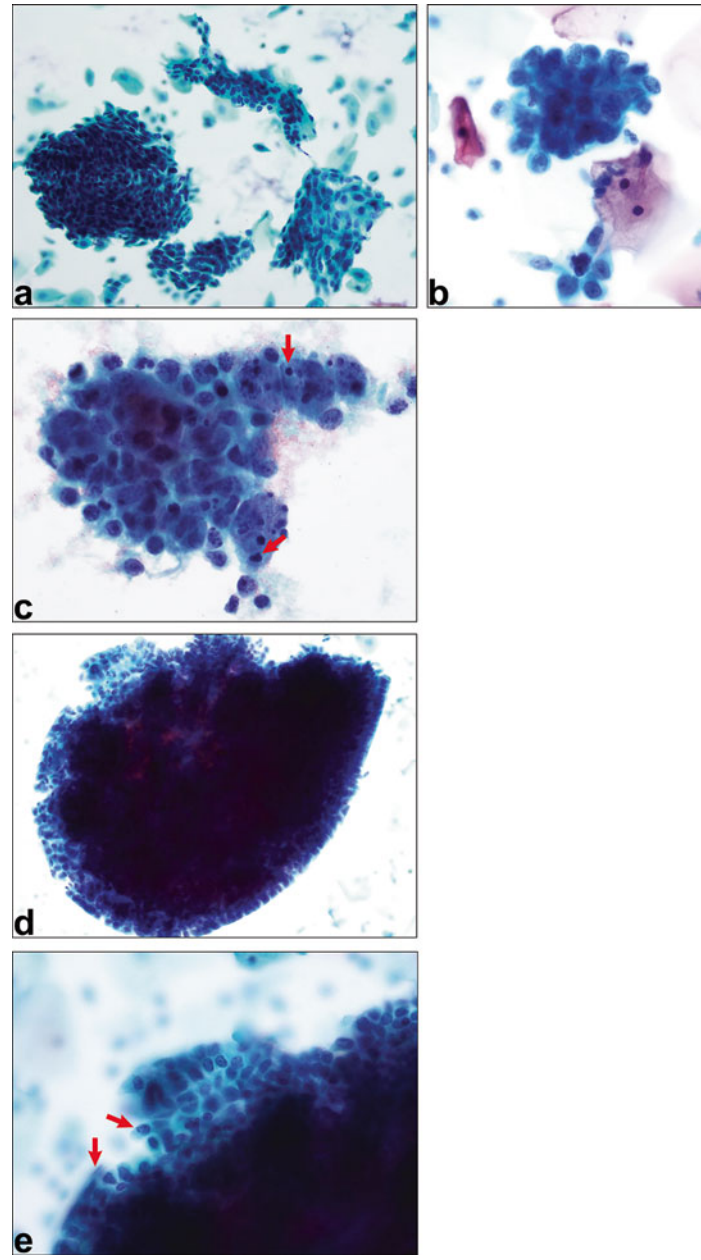


Fig. 2.15 Hyperchromatic crowded group pattern (part 1). This pattern of cell arrangement poses diagnostic challenges. Careful attention to polarity of cells, relationship of cells in one plane of focus, and nuclear features usually enables the correct interpretation. Equivocal cases require a diagnosis of ASCUS, ASCUS-H or AGUS. (**a**) Varying degrees of cell crowding and NC ratios from a Pap of a post-menopausal woman. (**b**) Small crowded hyperchromatic ball of endometrial cells with a peripheral hobnail appearance in a Pap from a patient with abnormal uterine bleeding and chronic endometritis on tissue biopsy. The cells do not appear particularly atypical, but may not be distinguishable from shed cells of low grade endometrial adenocarcinoma or normal endometrial cells shed up to 12 days post LMP. Clinical history and patient age must be factored into the assessment. (**c**) Another patient with chronic endometritis, copper type IUD and degenerated decidualized endometrial stromal fragment. Note the numerous apoptotic bodies (*arrows*). Note also the granular eosinophilic debris consistent with lysed blood. (**d**) A vigorous brushing technique can extract large intact whole glands that are especially well-preserved on SurePath™ slides. (**e**) Higher magnification of the edge of the group reveals a columnar shape and small, non-atypical nuclei (*arrow*). A terminal bar is present in one cell (*arrow*). Tubal metaplasia is common in the upper endocervical canal

that atypical LEGH may represent in-situ adenoma malignum. MRI findings may help in this differential, as adenoma malignum and LEGH reportedly differ in imaging appearance. Cases may require tissue biopsy for definitive diagnosis. Figures 2.18, 2.19, 2.20, 2.21, and 2.22 provide examples of various types of AIS and cervical and endometrial adenocarcinoma including minimal deviation adenocarcinoma.

Villoglandular Subtype of Cervical Adenocarcinoma

This variant of conventional cervical adenocarcinoma can display numerous, complex, smooth-edged branching structures that could suggest inadvertent sampling of LUS, endometrial polyp or endometriosis. However, at higher magnification, the small nuclei exhibit pseudostratification, hyperchromasia, apoptotic bodies, irregular

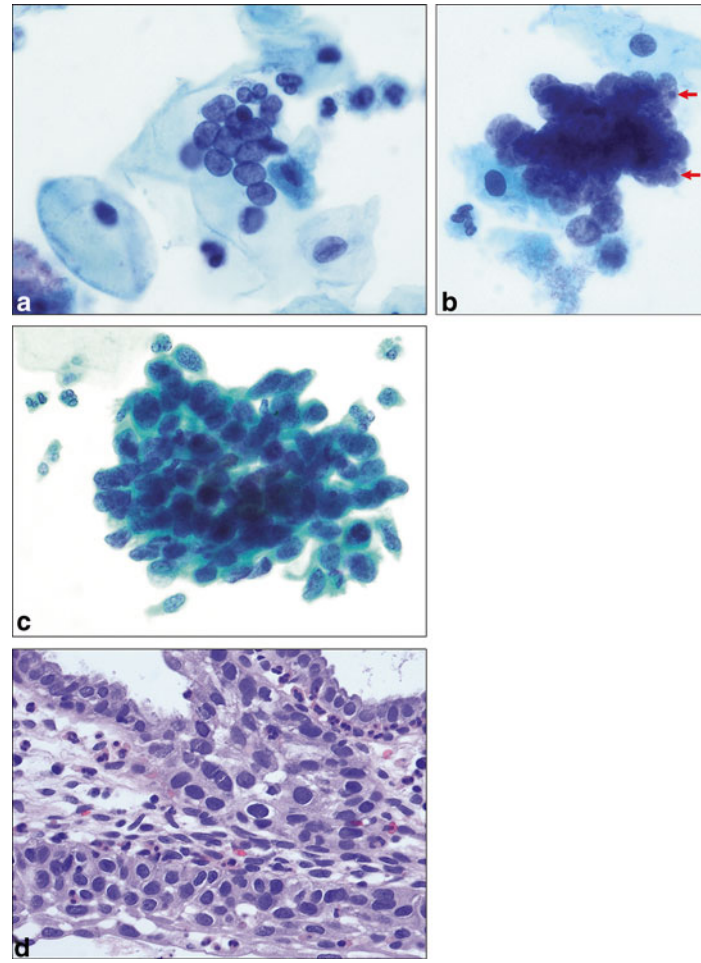


Fig. 2.16 Hyperchromatic crowded group pattern (part 2). (**a**) Stripped bare nuclei in a post-menopausal woman on Tamoxifen should not be mistaken for HGSIL or atypical endometrial cells. (**b**) A post-menopausal patient with no history of Tamoxifen therapy also has stripped small round blue nuclei on a cervical/vaginal MLP. Note the small nucleoli (*arrows*). (**c**) HGSIL composed of small cells with a high N/C ratio, irregular nuclear contours and hyperchromasia. Note the marked nuclear overlapping in the same plane of focus. (**d**) Follow-up biopsy reveals HGSIL extending into endocervical glands

nuclear membranes and small to large nucleoli suggesting the diagnosis [9]. Nuclear overlapping in one plane is also present. The sample is hrHPV positive. Colposcopy often reveals an abnormal exophytic growth.

Tubal Type Endocervical Adenocarcinoma (Very Rare)

This diagnosis shoule only be entertained in the setting of extreme atypia of glandular cells and groups which also contain cilia and/or terminal bars [18]. Marked nuclear pleomorphism, high mitotic activity and convincing apoptotic bodies could enable a diagnosis of AGUS. An unequivocal diagnosis of malignancy should not be rendered: tissue biopsy is required.

Clear Cell Adenocarcinoma of the Cervix (Rare)

Low grade clear cell adenocarcinoma may be difficult to identify on a Pap test, as it could resemble reactive endocervical glandular cells. However, most clear cell carcinomas are at least intermediate nuclear grade and will show enough atypia to enable a diagnosis of AGUS or AIS/adenocarcinoma. Atypical, stripped, bare nuclei often are widely dispersed, and sometimes a tigroid appearance is also present because of rupture of the fragile, glycogenated tumor cells. Intact cells retain fairly abundant, clear to granular cytoplasm, and the nuclei commonly contain macronucleoli. Leukophagocytosis may be present. Concurrent hrHPV testing is positive in 40 % of cases.

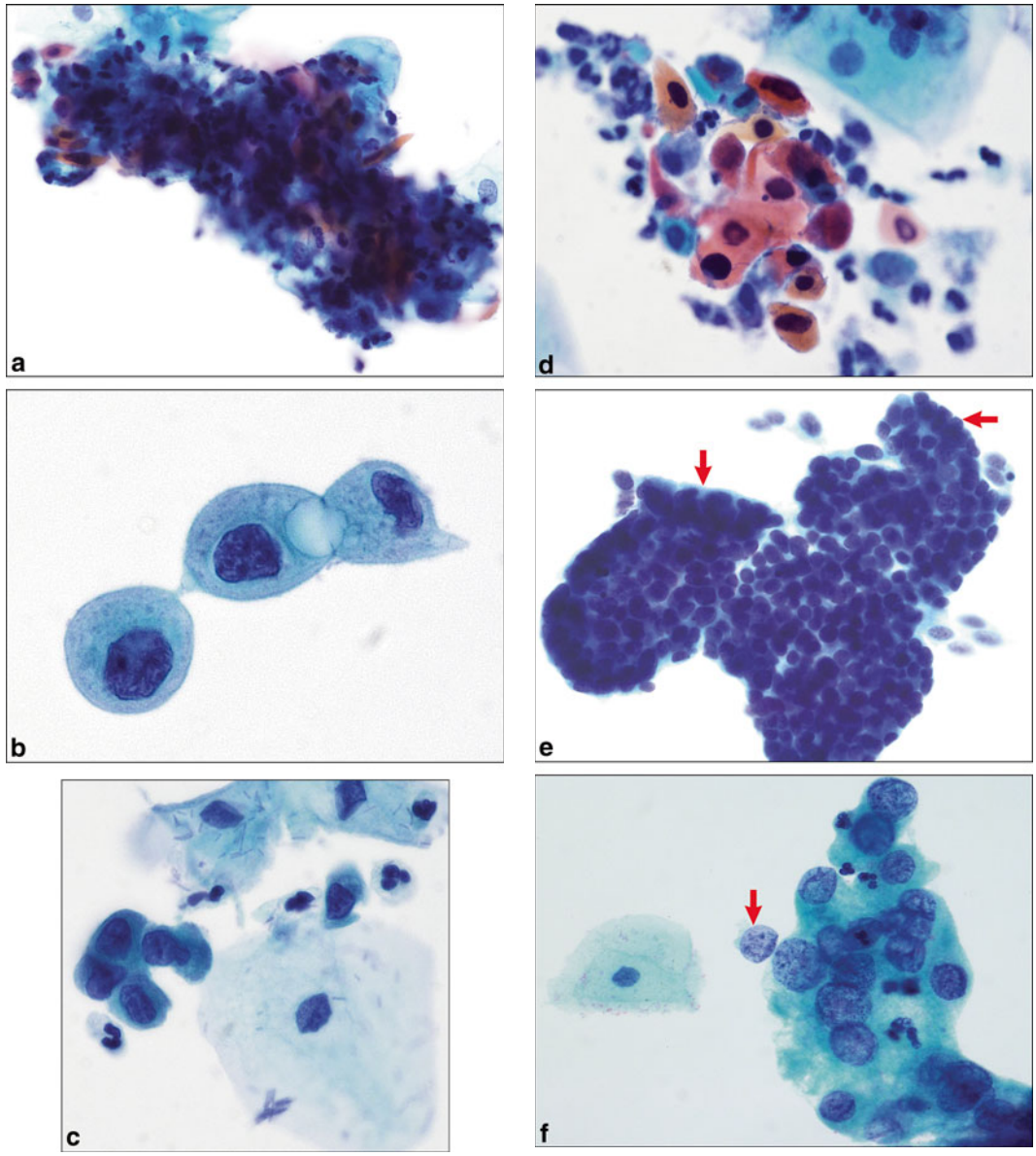


Fig. 2.17 Hyperchromatic crowded group pattern (part 3). (**a**) A thick crowded group may be impossible to assess because individual cell features are obscured. Attention should shift to other clusters and single atypical cells. (**b**) The same patient has hyperchromatic metaplastic cells with nuclear contour irregularities worrisome for dysplasia. (**c**) The same patient also has a few cells with unequivocal features of HGSIL. (**d**) The same patient also has loose clusters of atypical parakeratosis. A confident diagnosis of HGSIL could thus be made based on examination of all atypical cells and groups. (**e**) This relatively subtle example of conventional endocervical AIS shows a community border (*arrows*) and glandular configuration. However, excessive loss of polarity (marked nuclear overlap in one plane of focus), an extremely high N/C ratio and marked hyperchromasia imparted by increased, relatively coarse chromatin signal the group is at least "AGUS endocervical, favor neoplastic". (**f**) A crowded group of HGSIL is undergoing cellular dissolution. The *arrow* shows one cell almost devoid of cytoplasm. Large atypical stripped nuclei, while not diagnostic of dysplasia, should prompt careful search for intact diagnosable dysplastic cells

Table 2.6 Neoplastic and dysplastic confounders in cervical/vaginal cytology specimens

Mimicker	What it mimics	Architecture	Cell features	Background	Key differentiating features	Ancillary studies
HGSIL of small cell non-keratinizing type	• Follicular cervicitis • Reserve cells • Endometrial cells	• Clusters • Single cells	• Small cells with very high N/C ratio • Nuclei: dark, coarse chromatin, often irregular membranes • Nucleoli uncommon	• +/− large keratinized dysplastic squamous cells • +/− atypical glandular cells	• Dysplastic nuclear features	• Positive for p16 • HPV positive
Endometrioid adenocarcinoma, low grade	• Normal Endometrial cells • IUD effect • Endometrial polyp • Endocervical polyp • Endometritis	• Tight small 3-D balls	• Small cells with high N/C ratio • Some cells may mimic histiocytes with foamy cytoplasm • Some cells may have large vacuoles • N/C ratio ranges • Hyperchromasia • Mitoses +/− • Apoptotic bodies +/−	• Diathesis may be absent or of "clinging" type in monolayer preps • Lysed blood background in some cases	• Nuclei may be 2–3× larger than normal endometrial cell nuclei • Can be indistinguishable from conditions such as endometrial polyp, endometritis, and IUD effect. • Clinical history may be helpful	• May be positive for ProExC and IMP3 by ICC on cell blocks or additional MLP slides
Minimal deviation adenocarcinoma (adenoma malignum; AM) of cervix	• Normal ECC groups • LEGH (lobular endocervical glandular hyperplasia)	• Clusters with "drunken honeycomb" loss of polarity • Strips with pseudostratification • 3-D groups • Single cells	• Cells may have only mild nuclear atypia including enlargement • A few cells may have macronucleoli • Cytoplasm: abundant, "lacy" golden yellow with vacuolation	• Background may be bloody • Background may be floridly mucinous • Background may contain "clinging diathesis" in MLP	• Mild architectural and nuclear atypia • A few cells may show marked nuclear atypia (hyperchromasia, enlargement, increased N/C ratio, pseudostratification)	• Usually HPV and p16 negative • Cell block IHC: positive for p53, Ki67 (50%), and HIK-1083 (pyloric phenotype) • MRI shows cysts in LEGH which are absent in AM
Tubal type endocervical AIS	• Tubal metaplasia • Reactive endocervical cells	• Strips, rosettes, feathered groups • Single cells and small clusters	• Cells round to columnar with cilia and/or terminal bars • High N/C ratio • Hyperchromasia • Coarse chromatin • Parachromatin clearing • Mitoses • Apoptotic bodies	• Background may include benign tubal metaplasia • May be mixed with conventional type AIS	• Must have markedly atypical nuclear contours, coarse chromatin, mitoses and apoptotic bodies to enable a diagnosis of AGUS-favor neoplastic	• Extremely rare tumor: diagnosis of exclusion • Requires tissue biopsy for confirmation of diagnosis
Verrucous carcinoma of cervix	• Normal squamous cells • Parakeratosis • Hyperkeratosis	Abundant disorganized mats of orthokeratotic and parakeratotic cells	• Plate-like cells with low N/C ratio • Minimal nuclear atypia	• Lysed blood background +/− • Diathesis +/−	• Abundance of OK and PK should prompt at least a comment in the PAP report • Rare cells with more atypia may be present	• May be negative for hrHPV • Clinical history and colposcopy findings may be helpful

Glassy cell carcinoma	• Repair • Atypical repair (a type of ASCUS or AGUS)	• Sheets • Loose clusters • Single cells	• Large cells with granular cytoplasm • Low N/C ratio • Nuclei large and pleomorphic, vesicular with macronucleoli	• Heavy inflammatory infiltrate of lymphocytes, plasma cells, eosinophils common	• Irregular nuclear membranes • Single cells with marked nuclear atypia	Typically associated with barrel-shaped cervix on colposcopy
Microglandular hyperplasia-like endometrioid adenocarcinoma	• Microglandular hyperplasia • Histiocytes (muciphages)	• Tight clusters • Acini • Single cells	• Some cells have features of microglandular hyperplasia • Additional cells show hyperchromasia • Mitoses • Apoptotic bodies	• Lysed blood background +/− • Clinging diathesis (in MLP) +/− • Inflammation +/−	• Unlike most patients with microglandular hyperplasia, patient is post-menopausal • Nuclear atypia exceeds that of microglandular hyperplasia	• Requires tissue biopsy for diagnosis • Both MGH and MGH-like endometrial adenocarcinoma express ER
Small cell neuroendocrine carcinoma of cervix	• Follicular cervicitis • Reserve cell hyperplasia • HGSIL	• Sheets • Single cells • Gland-like structures	• Cells: small to large with high N/C ratio • Nuclei: dark with “salt and pepper chromatin” • Nuclear molding	• Nuclear strands/ streaks due to high fragility • Tumor diathesis • Apoptotic bodies	• Nuclear molding • Hyperchromatic with high N/C ratio	• Positive for HPV16 or 18 • P16 positive • Positive for CD56, synaptophysin, chromogranin
Serous ovarian or tubal adenocarcinoma	• Atrophic atypia • IUD effect • AGUS-EM • EM Ca including serous carcinoma	• Single cells • Small clusters • Atypical cells are sparse	• Markedly atypical cells with marked nuclear contour abnormalities • Vesicular to hyperchromatic • Macronucleoli +/−	• Psamomma bodies in 25% (not entirely specific but helpful)	• Nuclear features warrant at least a diagnosis of AGUS • Determining specific origin relies on clinical history, other pathology specimens and imaging findings	• Cannot distinguish endometrial serous and clear cell carcinoma from primary ovarian, peritoneal or tubal carcinomas
Malignant melanoma	• Poorly differentiated adenocarcinoma • Poorly differentiated squamous carcinoma	Single cells predominantly	• Cells range from epithelioid to spindled • Nuclei: +/− pseudoinclusions and grooves • Macronucleoli • Fine dusty or grainy cytoplasm • Tumor giant cells common	• +/− Melanophages • Granular debris (tumor diathesis)	• Melanin pigment helpful if present • Macronucleoli and binucleation	• Cell block positive for melanocytic markers

ECC endocervical cells, *LEGH* lobular endocervical glandular hyperplasia, *LGSIL* low grade squamous intraepithelial lesion, *HGSIL* high grade squamous intra-epithelial lesion, *SCC* squamous cell carcinoma, *AIS* adenocarcinoma in situ, *EcA* endocervical adenocarcinoma, *LEGH* lobular endocervical glandular hyperplasia, *3-D* three dimensional, *EM Ca* endometrial carcinoma

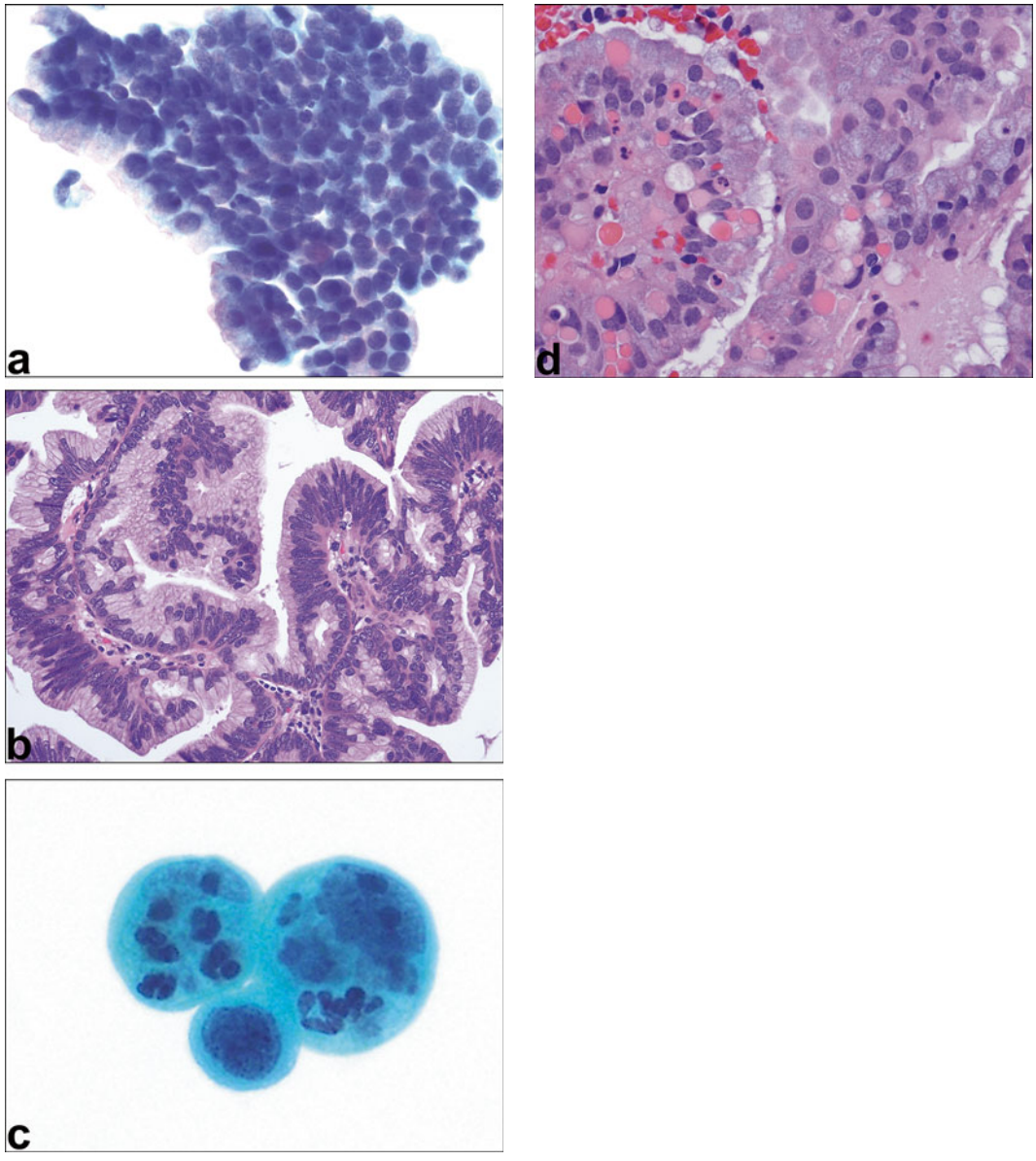


Fig. 2.18 Confounders—deceptively bland AIS and adenocarcinomas (AGUS cases with neoplastic follow-up, part 1). (**a**) Strip of AIS, conventional type shows marked loss of polarity (nuclear overlapping in one plane of focus), an increased N/C ratio and hyperchromasia but no significant nuclear contour abnormalities. (**b**) Follow-up biopsy confirms AIS, conventional type. Notice the nuclear uniformity and increased N/C ratio matching the features on the Pap MLP. (**c**) Atypical large round glandular cells with leukophagocytosis and nuclear atypia including irregular contours and macronucleoli. The patient was post-menopausal, without an IUD and presented with vaginal spotting. The atypical cells were sparse. (**d**) Endometrial biopsy revealed a low grade mixed endometrioid and mucinous adenocarcinoma. Leukophagocytosis and degenerative changes are present in the tissue biopsy corresponding to the Pap findings

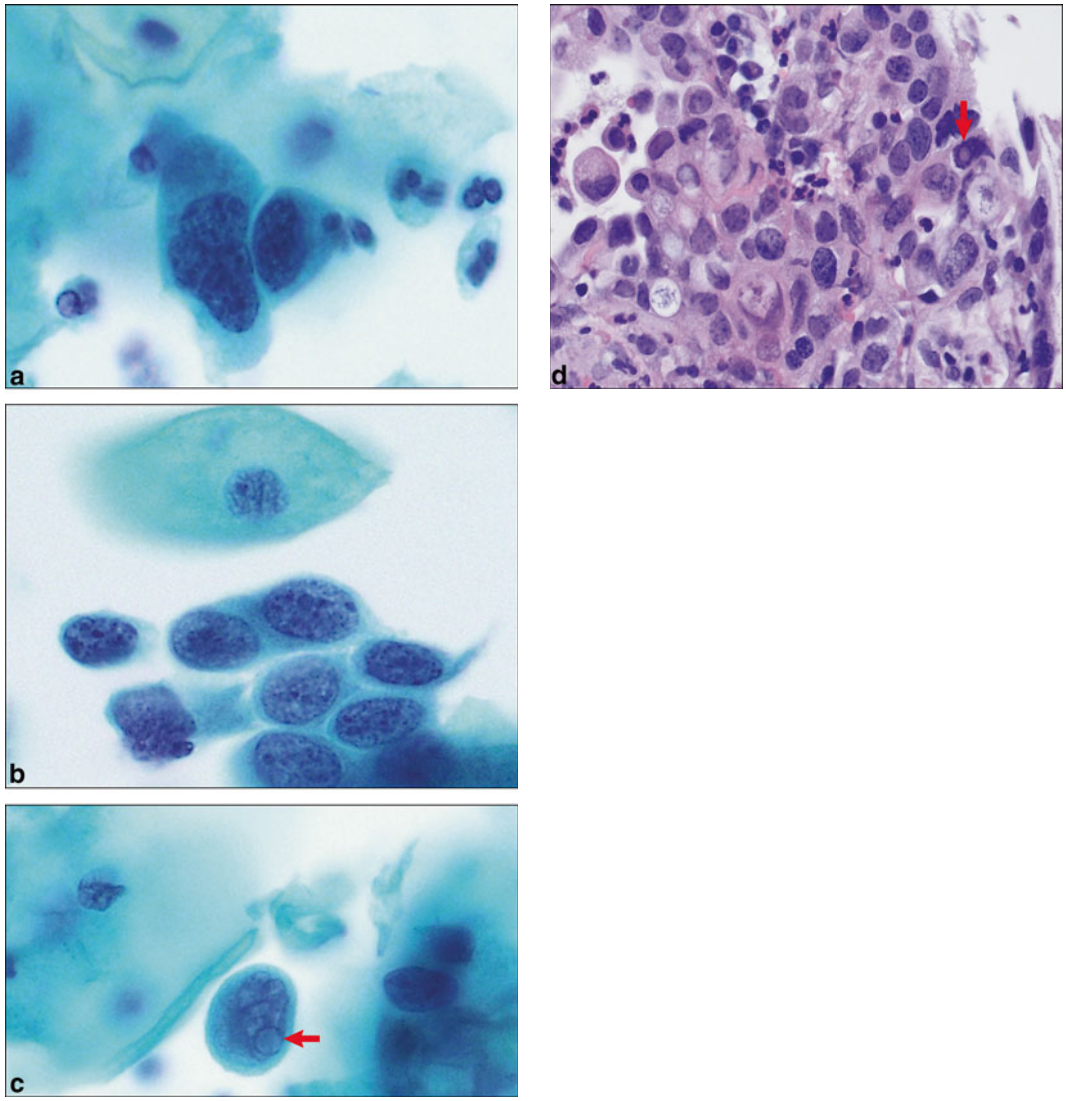


Fig. 2.19 Confounders—deceptively bland AIS and adenocarcinomas (AGUS cases with neoplastic follow-up, part 2). (**a**) Atypical uni-and multinucleated glandular cells in a patient with AIS. (**b**) The same MLP contains non-overlapping loose clusters of endocervical glandular cells with nuclear enlargement and coarse chromatin. (**c**) A few of the malignant cells contain nuclear pseudoinclusions (*arrow*). Pseudo-inclusions may be seen in benign and malignant glandular cells. (**d**) Follow-up biopsy revealed AIS with high grade nuclear features including coarse chromatin and scattered nuclear pseudo-inclusions (*arrow*)

Glassy Cell Carcinoma (GCC; Rare)

This rare variant of adenosquamous carcinoma can mimic florid atypical repair (see Fig. 2.23). Cells are arranged as sheets and clusters and as isolated individual cells. Some of the clusters exhibit a streaming, repair like orientation of tumor cells. The finding of markedly abnormal, non-degenerated, single dispersed cells are a clue to the diagnosis of GCC. The cells are comparatively very large with markedly pleomorphic nuclei, macronucleoli and coarse chromatin that is often irregularly distributed. Prominent acute and chronic inflammation is also typical and can consist of predominantly eosinophils and/or plasma cells. On colposcopy, the cervix is usually abnormally enlarged with a "barrel" shape. Concurrent hrHPV testing is positive.

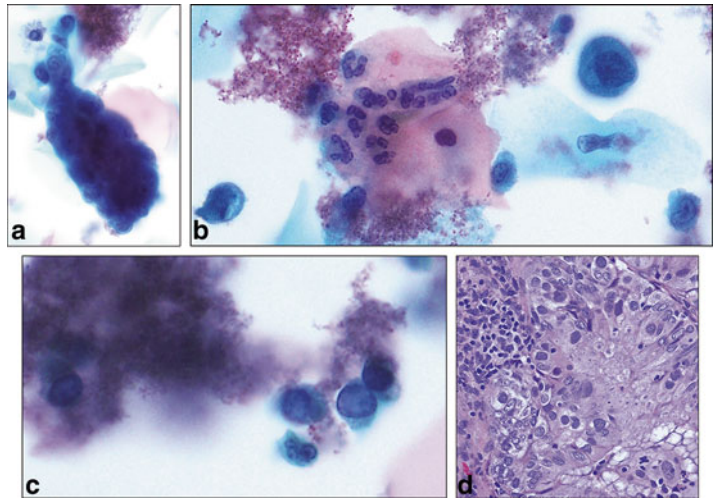


Fig. 2.20 Confounders—deceptively bland AIS and adenocarcinomas (AGUS cases with neoplastic follow-up, part 3). (**a**) Atypical epithelial cells forming a glandular structure in a patient with AIS. This group alone could also represent florid immature metaplasia replacing an endocervical gland or metaplastic squamous dysplasia. (**b**) In the same patient, these dispersed atypical, non-keratinizing cells could also represent HGSIL. (**c**) Same patient specimen with additional small markedly atypical cells with a high N/C ratio and nuclear pallor with beading of the chromatinic rim. Notice the background contains granular debris, even on an MLP. (**d**) Follow-up biopsy and LEEP contained multiple foci of AIS characterized by columnar to round cells, some cells with nuclear clearing and moderate nuclear pleomorphism similar to the cells on the Pap MLP

Small Cell Neuroendocrine Carcinoma (Rare)

Small cell carcinoma most often develops in pure form in the cervix but can be mixed with malignant squamous or glandular components. As at other body sites, the tumor comprises small cells with very high N/C ratio, nuclear molding and nuclear fragility causing streaking of nuclear material in a conventional smear. In most cases, mitoses are numerous as is nuclear debris. The differential diagnosis includes follicular cervicitis (lack atypical nuclear features and molding and typically only a few clusters are present in the slide), lymphoma (lacks typical "salt and pepper" granular chromatin and usually lacks nuclear molding). Immuocytochemistry for chromogranin, synaptophysin, and keratins can be diagnostically helpful. Concurrent hrHPV testing is usually positive.

Well-Differentiated Endometrioid Adenocarcinoma

The most common gynecologic cancer can be indistinguishable from benign lesions such as endometrial polyp, endometritis, tubal metaplasia and endometrial cells shed out of phase. Cellularity may be low. Cells most often shed in small tight balls with mild nuclear pleomorphism with or without nucleoli making them difficult, in some cases, to distinguish from normal endometrial cells. Individual cells may contain cytoplasmic vacuoles and resemble histiocytes. However, nuclei are usually more hyperchromatic, may contain nucleoli, and the N/C ratio is usually higher than in histiocytes. A diathesis is usually absent in MLPs, but the background may retain a lysedblood appearance. A clinical history of postmenopausal bleeding and/or thickened endometrial stripe on ultrasound examination shoud raise the diagnostic possibility and prompt careful search for rare atypical groups.

Adenoma Malignum-Type Well-Differentiated Mucinous Endometrial Adenocarcinoma (Very Rare)

The cytologic findings of this carcinoma have not been described. Since it can be combined with typical endometrial endometrioid adenocarcinoma,

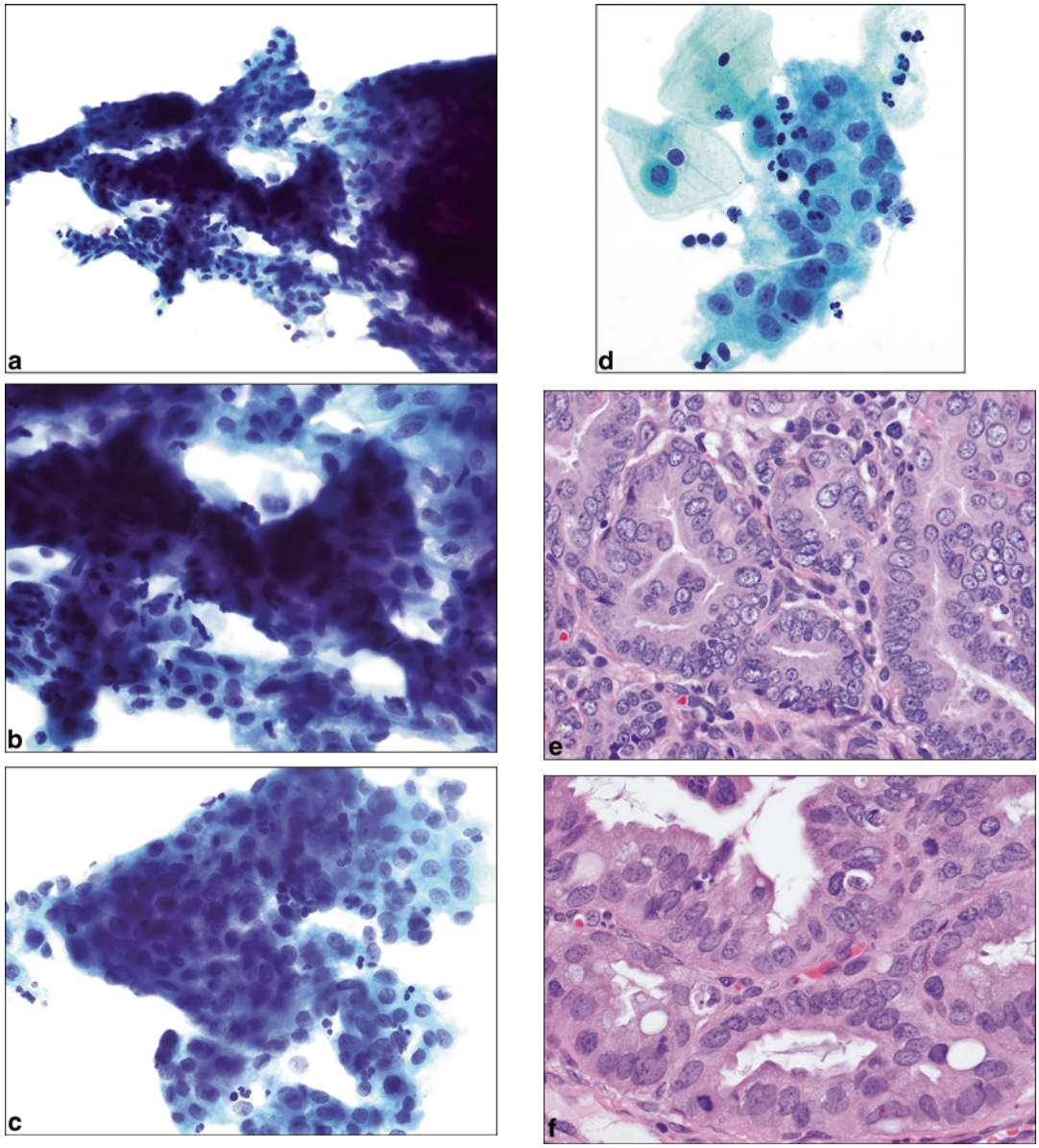


Fig. 2.21 Confounders—deceptively bland AIS and adenocarcinomas (AGUS cases with neoplastic follow-up, part 4). (**a**) This patient's lesion shed multiple complex folded and branching structures on the MLP. (**b**) The constituent cells suggest an appearance of relatively bland squamous metaplasia. (**c**) Other branching structures featured delicate cells with fine foamy cytoplasm suggestive of possible endocervical glandular differentiation. Atypia is mild but nuclear overlapping in one plane of focus is apparent. (**d**) High magnification of another loose group emphasizes the monotonous character of the cells and their equivocal cytoplasmic features and cell shape which could be consistent with glandular or metaplastic cells. (**e**) Subsequent biopsy revealed a mixed conventional and intestinal type adenocarcinoma. In this field, the tumor cells show the monotony captured in the MLP. (**f**) High magnification confirms some cell shapes which could be mistaken for squamous on the MLP. (**g**) Goblet cells, while focally prominent in the LEEP, were not identified on the preceeding MLP. (**h**) Other areas showed typical endocervical AIS, which also was not appreciated on the MLP. Some cervical tumors may have multiple architectural and cytologic features; not all of which may be demonstrable on the MLP

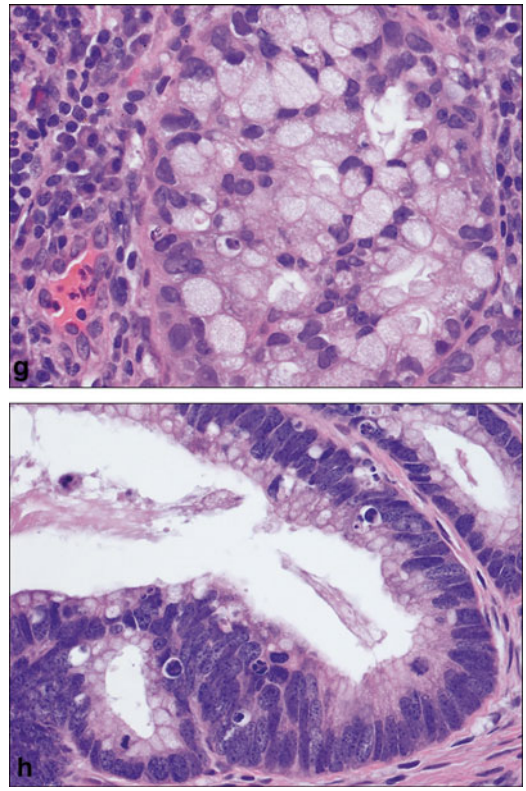


Fig. 2.21 (continued)

it is likely that some cases could be diagnosed as malignant based on correctly identifying the conventional component.

Microglandular Hyperplasia-Like Endometrioid Adenocarcinoma (Uncommon)

Only one case report of the cytologic findings of this tumor have been published. This low grade subtype of mucinous endometrial adenocarcinoma shares some of the appearance of microglandular hyperplasia in a Pap test but additionally produces scattered, single atypical cells with hyperchromasia, mitoses and apoptotic bodies [25]. Suspicion should be raised if findings of microglandular hyperplasia with or without atypia are seen in the Pap test of a postmenopausal patient.

Metastatic Carcinomas or Ovarian/Fallopian Tube Carcinomas Shed After Transit Through the Uterus (Rare)

Patients are usually middle-aged or older and may present with post-menopausal bleeding. Lesional cells are rare to sparse unless the tumor has metastasized to the vaginal wall or cervix and subsequently ulcerated. Marked nuclear atypia and degenerative changes are common. If recognized as malignant, the tumor cells can appear identical to primary gynecologic carcinomas. Carcinomas only rarely metastasize to the cervix, but most the common ones in descending order are: stomach, ovary, colon, lobular carcinoma of breast, pancreas and clear cell renal cell carcinoma [16]. The clinical history and directed immunocytochemical staining are nearly always required to render a specific diagnosis.

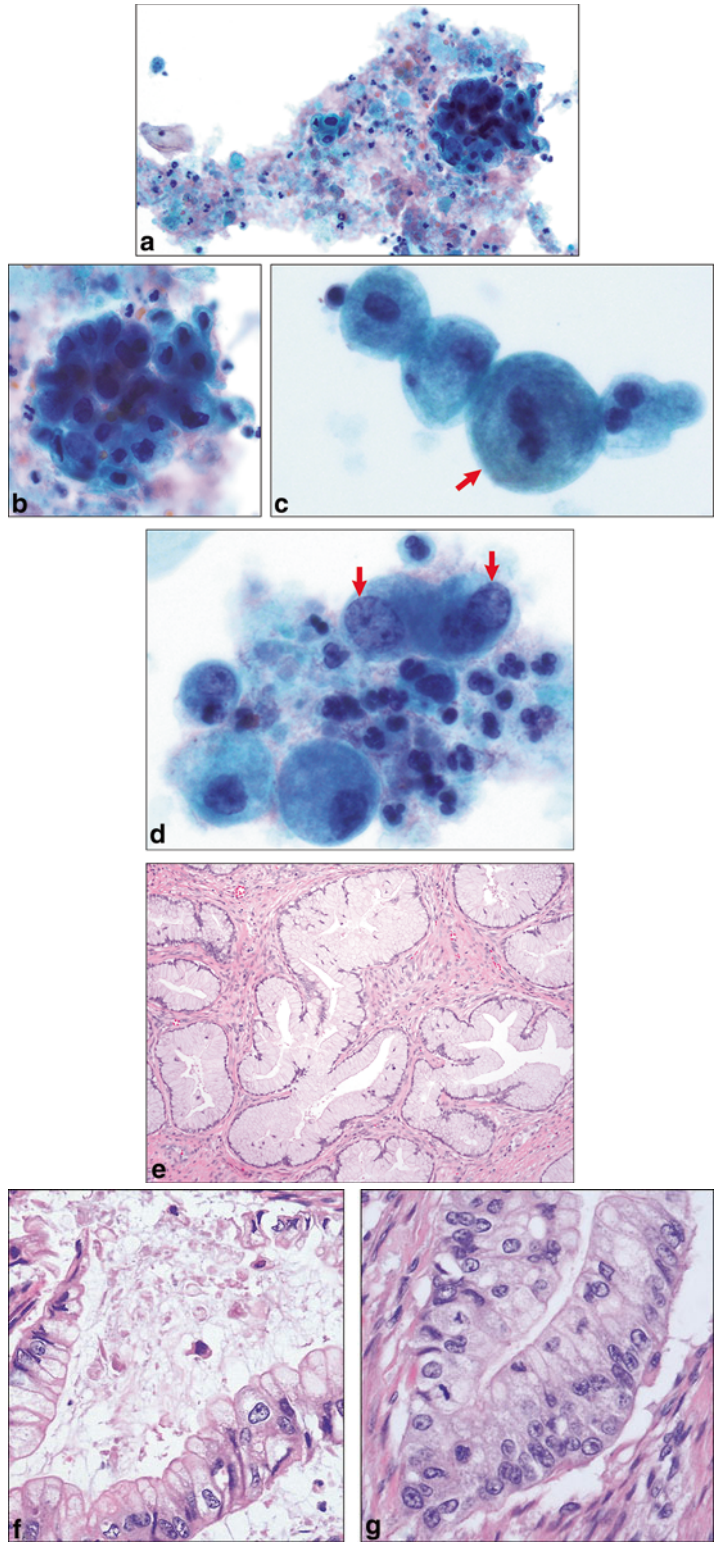


Fig. 2.22 Confounders—deceptively bland AIS and adenocarcinomas (AGUS cases with neoplastic follow-up, part 5). (**a**) Loose cluster of atypical cells in a background of prominent cellular debris detectable even on an MLP. (**b**) At high magnification, the atypical cells have abundant finely granular cytoplasm and irregularly shaped, degenerated but hyperchromatic nuclei. (**c**) One of the lesional cells exhibits a subtle golden brown color to the cytoplasm (*arrow*). (**d**) The tumor cells resemble histiocytes but are larger with coarse, irregularly distributed chromatin (*arrows*). (**e**) Biopsy revealed minimal deviation adenocarcinoma of the cervix (adenoma malignum). (**f**) The abundant cellular debris noted on the MLP corresponded to malignant glands containing cellular debris mixed with mucin. (**g**) As in most cases of adenoma malignum, a few glands were lined by more atypical cells, some of which were also seen on the patient's Pap

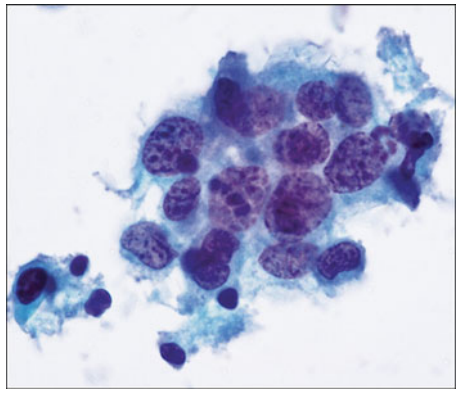


Fig. 2.23 HGSIL cannot rule out squamous carcinoma. This example of invasive squamous carcinoma highlights the very clumped and coarse chromatin that suggests the diagnosis. Some squamous carcinomas lack nucleoli, as in this case

Conclusions

1. *Diagnoses Abound in Paps as Doublets and Triplets and More, Galore*. The Pap test samples a complex, dynamic environment, and patients may present with several pathologic processes synchronously. Examples include atypical squamous cells induced by Trichomonas and/or Candida infections mingling with actual dysplastic squamous cells, radiation/chemotherapy- engendered atypical cells intermixing with residual cancer cells or new dysplastic cells, normal endometrial cells and/or tubal metaplastic cells co-shedding with cells of endometrial carcinoma, concurrent AIS and HGSIL and reparative changes and many other permutations. Vigilant cytotechnologists and pathologists, aware of the potential of multiple lesions in one test, carefully assess the features of all altered cells and render the appropriate interpretations. One diagnosis does not always fit all the findings.
2. *Clinical History Hones Assessment, Especially in Specific Circumstances.* A Pap with equivocal but worrisome findings should prompt review of the patient's history. Such information as a prior gynecologic malignancy/dysplasia or non-gynecologic malignancy, presence or recent removal of an IUD, history of radiation therapy or chemotherapy, menopausal status, pregnant or postpartum state, HRT/OCP use, ultrasound findings of a thickened endometrial stripe or MRI findings of abundant cervical cysts, and colposcopy findings of a polyp, exophytic mass, erosion, barrel-shaped cervix etc. provide diagnostic assistance, guide choice of any additional testing and permit recommendations for follow-up.
3. *MLPs Afford Myriad Opportunities for Clarifying the Diagnosis(es)*. In addition to enabling high risk HPV testing, residual monolayer Pap samples can be used to make additional slides and cell blocks and run specific molecular genetic tests. Key architectural features can be revealed in the cell block, while immunocytochemistry using antibodies to p16, Ki-67, IMP3 and others can confirm the presence or absence of a significant lesion such as AIS versus reactive glandular changes, among others. Such testing is not routinely performed currently but may become more prevalent if high sensitivity and specificity and favorable cost-benefit ratios are demonstrated. The samples are eminently suitable if ancillary tests are desired.
4. *Old-Fashioned, Yes, But Cytologic-Histologic Correlation Still Rocks*. It may seem antiquated in these days of genomic arrays, but simple visual comparison of follow-up histologic biopsies with the preceeding unusual or unexpected Pap findings can significantly improve diagnostic skills and consequently improve patient care. There's a reason why the hand axe, a modified rock, was in use about a million years; it could not be readily improved upon.
5. *The Pap Test Is Not Going Away*. Even with HPV vaccination programs, the Pap test will remain a common cytology test for years to come (but probably not a million). Potential new formalized indications such as screening for endometrial carcinoma may empower its use outside the realm of detection of HPV-related disease. All the more reason that familiarity with mimics and confounders of this profoundly facile, reliable test continues to be a critical cognitive asset for practicing cytotechnologists and cytopathologists.

Suggested Readings

1. Nayar R, Wilbur DC, editors. The Bethesda system for reporting cervical cytology: definitions, criteria, and explanatory notes. 3rd ed. Cham: Springer; 2015.
2. Cibas ES, Ducatman BS. Cytology: diagnostic principles and clinical correlates. 4th ed. Elsevier: Philadelphia; 2014.
3. Mody DR, editor. Diagnostic pathology: cytopathology. Salt Lake City: Amirsys; 2014.
4. Backhouse A, Stewart CJ, Koay MH, Hunter A, et al. Cytologic findings in stratified mucin-producing intraepithelial lesion of the cervix: a report of 34 cases. Diagn Cytopathol. 2016;44:20–5.
5. Behtash N, Nazari Z, Fakhrejahani F, Khafaf A, Azar EG. Clinical and histological significance of atypical glandular cell on Pap smear. Aust N Z J Obstet Gynaecol. 2007;47:46–9.
6. Benoit JL, Kini SR. "Arias-Stella Reaction"- like changes in endocervical glandular epithelium in cervical smears during pregnancy and postpartum states- a potential diagnostic pitfall. Diagn Cytopathol. 1996;14:349–55.
7. Bergeron C, Ikenberg H, Sideri M, et al. Prospective evaluation of p16/Ki-678 dual-stained cytology for managing women with abnormal Papanicolaou cytology: PALMS study results. Cancer Cytopathol. 2015;123(6):373–81.
8. Boyle DP, McCluggage WG. Stratified mucin-producing intraepithelial lesion (SMILE): report of a case series with associated pathological findings. Histopathology. 2015;66:658–63.
9. Choi Y, Kim H, Choi H, Hwang D, et al. Liquid-based cytology of villoglandular adenocarcinoma of the cervix: a report of three cases. Korean J Pathol. 2012;46:215–20.
10. Hashi A, Yuminamochi T, Xu JY, Kondo T, et al. Intranuclear cytoplasmic inclusion is a significant diagnostic feature for the differentiation of lobular endocervical glandular hyperplasia from minimal deviation adenocarcinoma of the cervix. Diagn Cytopathol. 2008;36:535–44.
11. Khalbuss WE, Pantanowitz L, Monaco SE. Cytomorphology of unusual primary tumors in the Pap test. Cytojournal. 2013;10:17–42.
12. Lastra RR, Ou JJ, Reilly ME, Lawrence WD, et al. Utility of ProExC and IMP3 immunocytochemical staining in atypical glandular cells of undetermined significance in liquid-based cervical cytology. Diagn Cytopathol. 2014;42:375–9.
13. Lee CY, Ng WK. Follow-up study of atypical glandular cells in gynecologic cytology using conventional Pap smears and liquid-based preparations: impact of the Bethesda System 2001. Acta Cytol. 2008;52:159–68.
14. Loureiro J, Oliva E. The spectrum of cervical glandular neoplasia and issues in the differential diagnosis. Arch Pathol Lab Med. 2014;138:453–83.
15. Morrison C, Prokorynm P, Piquero C, Wakely PE, Nuovo GJ. Oral contraceptive pills are associated with artifacts in ThinPrep Pap smears that mimic low-grade squamous intraepithelial lesions. Cancer Cytopathol. 2003;99:75–82.
16. Mulvany NJ, Mitchell G, Allen DG. Adenocarcinoma in Pap smears. Pathology. 2009;41:411–5.
17. Negri G, Herz M, Deola S, Piccin A, et al. Abnormal cervical cytology after allogeneic bone marrow transplantation. Am J Clin Pathol. 2014;142:222–6.
18. O'Connell F, Cibas ES. Cytologic features of ciliated adenocarcinoma of the cervix: a case report. Acta Cytol. 2005;49:187–90.
19. Pantanowitz L, Upton MP, Wang HH, Nasser I. Cytomorphology of verrucous carcinoma of the cervix. A case report. Acta Cytol. 2003;47:1050–4.
20. Raab SS. Can glandular lesions be diagnosed in the Pap smear cytology? Diagn Cytopathol. 2000;23:127–33.
21. Remondi C, Sesti F, Bonanno E, Pietropolli A, et al. Diagnostic accuracy of liquid-based endometrial cytology in the evaluation of endometrial pathology in postmenopausal women. Cytopathology. 2013;24:365–71.
22. Risse EKJ, Holeierhoek JP, Meijer-Marres EM, Ouwerkerk-Noordam E, et al. Increased diagnostic accuracy of atypical glandular cells in cervical liquid-based cytology using cell blocks. Cytopathology. 2011;22:253–60.
23. Sams SB, Currens HS, Raab SS. Liquid-based Papanicolaou tests in endometrial carcinoma diagnosis: performance, error root cause analysis, and quality improvement. Am J Clin Pathol. 2012;137:248–54.
24. Schnatz PF, Guile M, O'Sullivan DM, Sorosky JL. Clinical significance of atypical glandular cells on cytology. Obstet Gynecol. 2006;107:701–8.
25. Shidham VB, Dayer AM, Basir Z, Kajdacsy-Balla A. Cervical cytology and immunohistochemical features in endometrial adenocarcinoma simulating microglandular hyperplasia. A case report. Acta Cytol. 2000;44:661–6.
26. Smith HO, Tiffany MF, Qualls CR, Key CR. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States- a 24 year population-based study. Gynecol Oncol. 2000;78(2):97–105.
27. Tam KF, Cheung AN, Liu KL, Ng TY, et al. A retrospective review on atypical glandular cells of undetermined significance (AGUS) using the Bethesda 2001 classification. Gynecol Oncol. 2003;91:603–7.
28. Zhao C, Florea A, Onisko A, Austin RM. Histologic follow-up results in 662 patients with Pap test findings of atypical glandular cells: results from a large academic womens hospital laboratory employing sensitive screening methods. Gynecol Oncol. 2009;114:383–9.
29. Krane JF, Lee KR, Sun D, Yuan L, Crum CP. Atypical glandular cells of undetermined significance. Outcome predictions based on human papillomavirus testing. Am J Clinical Pathol. 2004;121:87–92.

Salivary Gland Cytology

3

Brief Introduction

The human body harbors both major and minor salivary glands. The major ones are paired and consist of the parotid, the submandibular and the sublingual glands. The minor salivary glands are numerous and are estimated to be from 500 to 1000. Approximately 40 subtypes of neoplasms (benign and malignant) have been described. Fine needle aspiration is considered to be one of the best modalities for initial evaluation of these neoplasms. However, assessing these specimens can be a challenge for the cytopathologist because of significant cytomorphologic diversity and overlap between benign and malignant salivary gland tumors. Judicious attempt is always made to categorize neoplastic entities to best guide appropriate therapeutic interventions. However, one has to also be mindful of the non-neoplastic conditions that share many cytomorphological features with neoplasms. Ancillary studies are often of limited use, but certain cytomorphological features when evaluated in the context of the clinical and radiographic impression may help prevent erroneous interpretation.

Classification of Salivary Gland Neoplasms Based on Predominant Cytomorphological Features

A bevy of cytomorphological features are recognized in any given neoplastic salivary gland tumor. However, any of these features can be seen in diverse salivary gland tumors. Furthermore, numerous benign non-neoplastic states may share some of these features. For the purpose of discussing the major confounders encountered in salivary gland cytology, it is helpful to classify these neoplasms based on these features (Table 3.1).

Confounders Based on Cytomorphological Findings

Matrix-Producing Lesions

A number of salivary gland tumors tend to produce matrix that is readily appreciated with Romanowsky staining. The morphologic appearance of these extracellular components and their

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_3

Table 3.1 Common cytomorphological features of salivary gland tumors

Matrix-producing
Lymphocyte-rich
Oncocytic
Basaloid
Mucinous
Inflammatory background
Granulomatous changes
Other non-oncocytic metaplastic changes

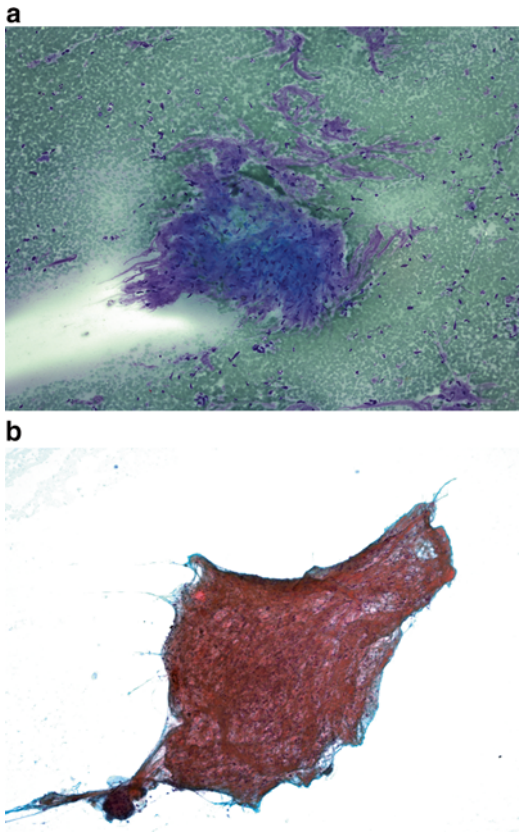


Fig. 3.1 (**a**) Fragments of inflamed stroma in acute sialadenitis (Smear; Diff-Quik, Medium Magnification); (**b**) Fragment of inflamed stroma in acute sialadenitis (Smear; Papanicolaou, Medium Magnification)

association with the cellular components can help in defining the tumors. Nonetheless, the presence of Romanowsky-stained extracellular materials does not always translate to the presence of neoplasms. Inflamed and irritated salivary gland tissue can yield fragments of inflamed stroma (Fig. 3.1) mimicking extracellular matrix produced by salivary gland tumors (Table 3.2).

Lymphocyte-Rich Lesions

Lymphocytes are frequently seen in association with many salivary gland tumors. They are also present with equal frequency in many benign non-neoplastic lesions. Inflamed non-neoplastic states (Fig. 3.2) or sampling of intra-salivary gland lymph node (see Fig. 3.3) will lead to inflammatory cells in the aspirate. Good clinical and radiological correlation will prove most helpful in these situations (Table 3.3).

Oncocytic Lesions

Separating true oncocytic salivary tumors from non-oncocytic salivary tumors with oncocytic metaplasia and from benign salivary glands with oncocytic metaplasia can be challenging. Fortunately, the true neoplasms in this category have additional characteristic cytomorphological and clinical features. Establishing the absence of these features is key to correctly interpreting non-neoplastic salivary glands with oncocytic metaplasia (Fig. 3.4). In addition, oncocytic cells from metaplasia will be immunoreactive to myoepithelial cell markers, such as calponin, p63, and smooth muscle actin. Most oncocytic tumors will be negative for these markers These include tumors such as oncocytoma, oncocytic carcinoma, Warthin's tumor, acinic cell carcinoma, and oncocytic mucoepidermoid carcinoma (Table 3.4).

Basaloid Lesions

Chronic sialadenitis often has small angulated groups of ductal-type cells (Fig. 3.5) that can mimic a basaloid tumor. Special attention to the cellularity and clinical history may avoid misinterpreting the aspirate as neoplastic (Table 3.5). Basaloid neoplasms of the salivary glands that are commonly diagnosed by cytology include cellular pleomorphic adenoma, solid variant of adenoid cystic carcinoma, basal cell adenoma and basal cell adenocarcinoma. Differentiating these entities in cytological specimens can be difficult, but it is worth elaborating on their respective specific features. In general, a basaloid neoplasm is suspected when a cellular aspirate

Table 3.2 Matrix-producing lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Pleomorphic adenoma • Adenoid cystic carcinoma • Carcinoma ex-pleomorphic adenoma • Basal cell adenoma • Basal cell adenocarcinoma • Polymorphous low grade adenocarcinoma • Myoepithelioma • Epithelial-myoepithelial carcinoma • Ectomesenchymal basaloid neoplasm	Acute sialadenitis	• Infection: virus, bacteria • Duct obstruction	• Parotid most common • Neutrophils • Histiocytes • Necrosis • Amylase crystalloids • Fibrin • Organisms • Inflamed stroma scant	• Clinical correlation • Microbiology
	Chronic sialadenitis	• Duct obstruction • Radiation therapy • Trauma • Autoimmune disease • Bulimia	• Submandibular most common • Low cellularity • Lymphocytes • Increase in ductal-type cells • Amylase crystalloids • Inflamed stroma scant	• Clinical correlation
	Granulomatous sialadenitis	• Infection: mycobacteria, fungi • Sarcoidosis • Calculi	• Epithelioid histiocytes • Giant cells • Inflamed stroma scant	• Clinical correlation • Microbiology • Special stains for organisms
	Lymphoepithelial sialadenitis	• Autoimmune disease • Sarcoidosis	• Lymphoepithelial lesions (reactive ductal cells admixed with lymphocytes) • Lymphoplasmacytic infiltrate • High cellularity	• Clinical correlation
	Necrotizing sialometaplasia	• Radiation therapy • Trauma	• Minor salivary glands most common • Low cellularity • Squamous metaplasia • Inflamed stroma scant	• Clinical correlation

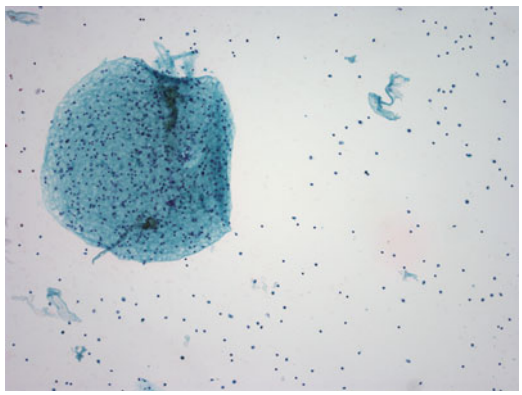


Fig. 3.2 Aspirate from chronic sialadenitis showing inflamed stroma and lymphocytes in the background (Smear; Papanicolaou, Medium Magnification)

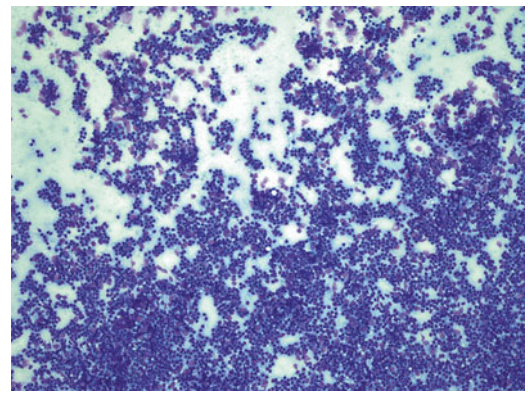


Fig. 3.3 Sampling of intraparotid lymph node (Smear; Diff-Quik, Medium Magnification)

Table 3.3 Lymphocyte-rich lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Warthin tumor • Acinic cell tumor • Lymphoepithelial carcinoma • Lymphadenoma • Mucoepidermoid carcinoma • Metastatic carcinoma to intra-salivary lymph node • Lymphoma	Acute sialadenitis	• Infection: virus, bacteria • Duct obstruction	• Parotid most common • Neutrophils • Histiocytes • Necrosis • Amylase crystalloids • Fibrin • Organisms	• Clinical correlation • Microbiology
	Chronic sialadenitis	• Duct obstruction • Radiation therapy • Trauma • Autoimmune disease • Bulimia	• Submandibular most common • Low cellularity • Lymphocytes • Increase in ductal-type cells • Amylase crystalloids	• Clinical correlation
	Granulomatous sialadenitis	• Infection: mycobacteria, fungi • Sarcoidosis • Calculi	• Epithelioid histiocytes • Giant cells	• Clinical correlation • Microbiology • Special stains for organisms
	Lymphoepithelial sialadenitis	• Autoimmune disease • Sarcoidosis	• Lymphoepithelial lesions (reactive ductal cells admixed with lymphocytes) • Lymphoplasmacytic infiltrate • High cellularity	• Clinical correlation
	Intra-salivary lymph node	• Normal	• Parotid gland • Polymorphous lymphoid population	• Clinical correlation • Flow cytometry • Ultrasound-guided FNA
	HIV-associated cysts	• HIV-positive patients	• Parotid glands (bilateral) multiple cysts • Polymorphous lymphoid population • Foamy histiocytes • Occasional squamous cells	• Clinical correlation

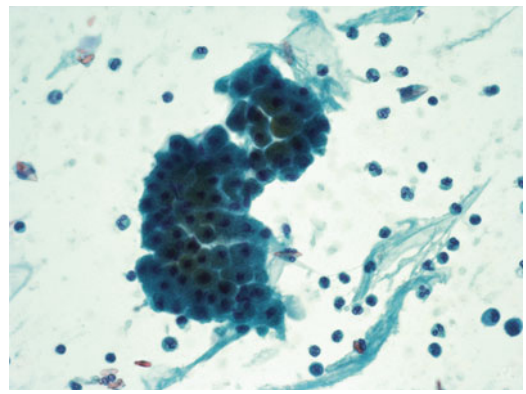


Fig. 3.4 Oncocytic metaplasia in benign oncocytosis (Smear; Papanicolaou, High Magnification)

Table 3.4 Oncocytic lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Warthin tumor • Pleomorphic adenoma • Oncocytoma • Oncocytic carcinoma • Acinic cell carcinoma • Mucoepidermoid carcinoma • Cystadenoma • Cystadeno-carcinoma • Metastasis	Oncocytosis	• Metaplasia (metaplastic and oncocytic) in older patients	• Lack of cytomorphological features associated with other salivary gland neoplasms	• Clinical correlation • Cell block • Immunohisto-chemistry: S100, calponin, SMA
	Sclerosing polycystic adenosis	• Benign, slowly growing parotid mass	• Parotid glands • Apocrine metaplasia • Flat sheets of evenly spaced squamoid cells	• Clinical correlation

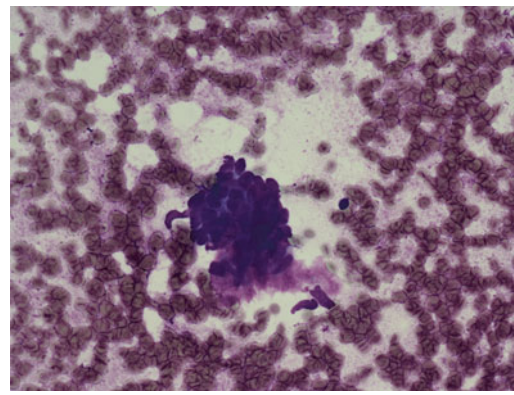


Fig. 3.5 Small angulated ductal cells in chronic sialadenitis (Smear; Diff-Quik, High Magnification)

consists of cells with minimal cytoplasm and hyperchromatic nuclei with minimal to no extracellular matrix.

Cellular pleomorphic adenoma is characterized by minimally identifiable fibrillary extracellular matrix. Aspirate of this tumor tends to show more single discohesive myoepithelial cells in addition to clusters. The presence of any fibrillary matrix, even sparse, would favor this type of basaloid neoplasm. In the complete absence of the matrix, a definitive diagnosis is not possible.

Solid variant of adenoid cystic carcinoma is equally challenging to diagnose on cytology. The presence of any three-dimensional cylindrical structures containing extracellular matrix would favor adenoid cystic carcinoma.

Basal cell neoplasms (adenoma and adenocarcinoma) regardless of subtype (solid, tubulotrabecular and membranous) are separated cytologically from the other basaloid tumors by the presence of two populations of neoplastic cells. These include oval smaller cells with scant cytoplasm and dark nuclei and larger cells with moderate amount of cytoplasm and less hyperchromatic nuclei. Depending on the subtype, the cytoarchitecture can be more supportive of basal cell neoplasms. Such architecture includes trabeculae, branching tubules, squamous morules, cellular clusters with palisading of basaloid cells that are surrounded by a band of matrix in the periphery. However, the distinction between basal cell adenoma and basal cell adenocarcinoma by cytology is not always possible without integrating the clinical and imaging impression, such as evidence of invasion in the latter. If obvious malignant features (e.g., nuclear pleomorphism, atypical mitoses, necrosis) are observed, basal cell adenocarcinoma can be favored.

Table 3.5 Basaloid lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Basal cell adenoma • Basal cell adenocarcinoma • Adenoid cystic (solid variant) • Pleomorphic adenoma • Sialoblastoma • Metastasis	Chronic sialadenitis	• Duct obstruction • Radiation therapy • Trauma • Autoimmune disease • Bulimia	• Submandibular most common • Low cellularity • Lymphocytes • Increase in ductal-type cells (small angulated groups) • Amylase crystalloids	• Clinical correlation

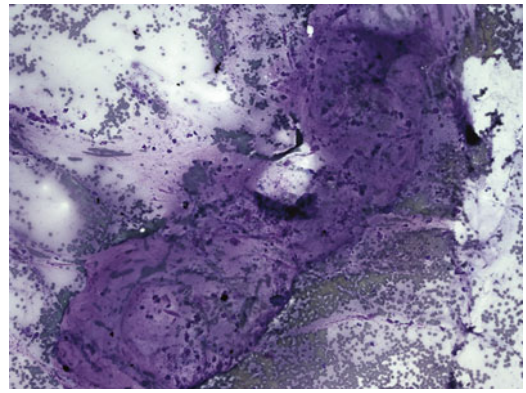


Fig. 3.6 Mucin from aspirate of a retention cyst (Smear; Diff-Quik, Medium Magnification)

Mucinous Lesions

Benign non-neoplastic entities, such as mucocele and retention cyst, can produce mucin (Fig. 3.6). This always raises the possibility of malignant neoplasms such as mucoepidermoid carcinoma or metastatic disease. However, location of the lesion, cellularity, types of cells, and inflammatory background will help prevent mis-diagnosis (Table 3.6).

Lesions with Inflammatory/Necrotic Background

High grade tumors often are accompanied by an inflammatory and necrotic background. As expected, acute sialadenitis (Fig. 3.7) may have a similar background. The important clues to avoid calling inflammatory non-neoplastic entities tumors are the lack of other features associated with these high grade tumors (Table 3.7).

Table 3.6 Mucinous lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Mucoepidermoid carcinoma • Warthin tumor • Cystadenoma • cystadenocarcinoma • Metastasis	Mucocele	• Excretory duct obstruction/damage	• Minor salivary glands • Lack of true epithelial cyst lining • Chronic inflammatory background • Histiocytes • Low cellularity	• Clinical correlation
	Mucus retention cyst	• Dilated excretory duct	• True epithelial cyst lining	• Clinical correlation
	Sclerosing polycystic adenosis	• Benign, slowly growing parotid mass	• Parotid glands • Apocrine metaplasia • Flat sheets of evenly spaced squamoid cells	• Clinical correlation

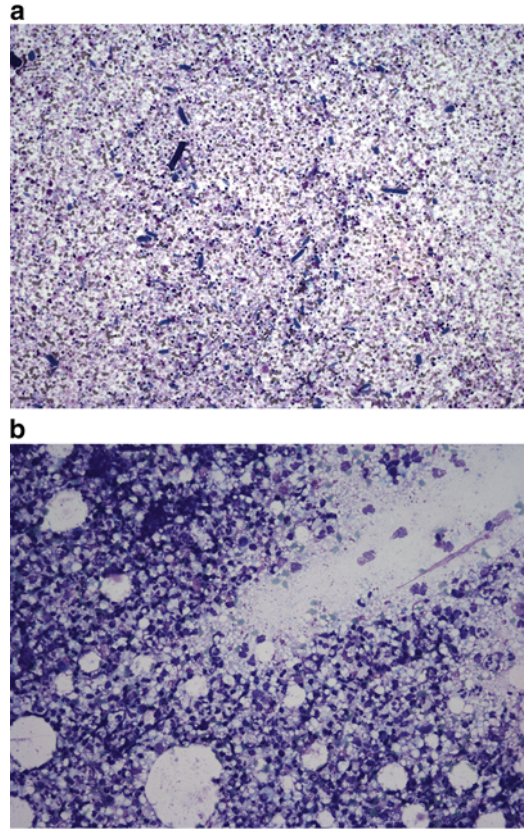


Fig. 3.7 (**a**) Chronic and acute sialadenitis with amylase crystalloids (Smear; Diff-Quik, Low Magnification); (**b**) Acute sialadenitis aspirate with an acute inflammatory background (Smear; Diff-Quik, Low Magnification)

Table 3.7 Lesions with necrotic/inflammatory background

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Salivary duct carcinoma • Adenoid cystic carcinoma • Carcinoma ex-pleomorphic adenoma • Basal cell adenocarcinoma • Epithelial-myoepithelial carcinoma • Lymphoepithelial carcinoma • Mucoepidermoid Carcinoma • Lymphoma • Warthin tumor • Metastasis	Acute sialadenitis	• Infection: virus, bacteria • Duct obstruction	• Parotid most common • Neutrophils • Histiocytes • Necrosis • Fibrin • Amylase crystalloids • Organisms • Inflamed stroma scant	• Clinical correlation • Microbiology
	Granulomatous sialadenitis	• Infection: mycobacteria, fungi • Sarcoidosis • Calculi	• Epithelioid histiocytes • Giant cells • Inflamed stroma scant	• Clinical correlation • Microbiology • Special stains for organisms
	Necrotizing sialometaplasia	• Radiation therapy • Trauma	• Minor salivary glands most common • Low cellularity • Squamous metaplasia • Inflamed stroma scant	Clinical correlation

Conclusion

Many of the pitfalls in salivary gland cytology may be avoided by assessing the different components, both cellular and extracellular, present in the aspirate, in the given clinical and radiological context. One or two cytomorphological features alone are not sufficient, especially if in low quantity, to classify a lesion as being neoplastic.

Suggested Readings

1. Arduino PG, Carrozzo M, Pentenero M, et al. Non-neoplastic salivary gland diseases. Minerva Stomatol. 2006;55(5):249–70.
2. Barnes L, Eveson JW, Reichart P, Sidransky D. World health organization classification of tumours of head and neck tumours. Lyon: IARC Press; 2005.
3. Cajulis RS, Gokaslan ST, Yu GH, et al. Fine needle aspiration biopsy of the salivary glands. A five-year experience with emphasis on diagnostic pitfalls. Acta Cytol. 1997;41(5):1412–20.
4. Campbell JB, Morgan DW, Oates J, et al. Tumors of the minor salivary glands. Ear Nose Throat J. 1989; 68(2):137–40.
5. Chang A, Harawi SJ. Oncocytes, oncocytosis, and oncocytic tumors. Pathol Annu. 1992;27(Pt 1): 263–304.
6. Krane JF, Faquin WC. Sailvary gland. In: Cibas ES, Ducatman BS, editors. Cytology: diagnostic principles and clinical correlates. 4th ed. Edinburgh: Saunders; 2014. p. 299–332.
7. Daneshbod Y, Daneshbod K, Khademi B. Diagnostic difficulties in the interpretation of fine needle aspirate samples in salivary lesions: diagnostic pitfalls revisited. Acta Cytol. 2009;53(1):53–70.
8. Geyer JT, Ferry JA, Harris NL, et al. Chronic sclerosing sialadenitis (Küttner tumor) is an IgG4-associated disease. Am J Surg Pathol. 2010;34(2):202–10.
9. Hughes JH, Volk EE, Wilbur DC. Pitfalls in salivary gland fine-needle aspiration cytology: lessons from the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology. Arch Pathol Lab Med. 2005;129(1):26–31.
10. Mair S, Leiman G, Levinsohn D. Fine needle aspiration cytology of parotid sarcoidosis. Acta Cytol. 1989;33(2):169–72.

11. Malhotra P, Arora VK, Singh N, et al. Algorithm for cytological diagnosis of nonneoplastic lesions of the salivary glands. Diagn Cytopathol. 2005;33(2):90–4.
12. Mohan H, Tahlan A, Mundi I, et al. Non-neoplastic salivary gland lesions: a 15-year study. Eur Arch Otorhinolaryngol. 2011;268(8):1187–90.
13. Rice DH. Noninflammatory, non-neoplastic disorders of the salivary glands. Otolaryngol Clin North Am. 1999;32(5):835–43.
14. Sáenz-Santamaría J, Catalina-Fernandéz I, Fernandez-Mera JJ. Sialadenitis with crystalloid formation. Fine needle aspiration cytodiagnosis of 15 cases. Acta Cytol. 2003;47(1):1–4.
15. Wax TD, Layfield LJ, Zaleski S, et al. Cytomegalovirus sialadenitis in patients with the acquired immunodeficiency syndrome: a potential diagnostic pitfall with fine-needle aspiration cytology. Diagn Cytopathol. 1994;10(2):169–72.
16. Young NA, Mody DR, Davey DD. Misinterpretation of normal cellular elements in fine-needle aspiration biopsy specimens: observations from the College of American Pathologists Interlaboratory Comparison Program in Non-Gynecologic Cytopathology. Arch Pathol Lab Med. 2002;126(6):670–5.

Gastrointestinal Tract

4

Brief Introduction

With advances in of endoscopy and tissue biopsy techniques, cytology has had an appreciably diminished role in the diagnosis of neoplasms of the gastrointestinal tubal tract (oral cavity to anus). However, because cytology of the GI tract still has a few advantages over histology, cytolopathologists will continue to encounter a number of these specimens in their practice. Some of these advantages include sampling a wider surface area, reaching less accessible areas (e.g., strictured tubal segments), yielding better preserved materials, and diminishing risks of procedural complications. In fact, cytology is the main diagnostic modality in certain clinical scenarios such as infectious and submucosal lesions. In this chapter, the focus will be on exfoliative cytology specimens dealing with mucosal-based lesions (Table 4.1). For those obtained from fine needle aspiration, essentially from submucosal lesions (Table 4.2), refer to the corresponding chapters on salivary gland, lymphoid, neural, neuroendocrine and mesenchymal neoplasms. In addition, anal cytology will be discussed along with uterine cervical cytology in the appropriate section.

Common Neoplasms of the Gastrointestinal Tract Diagnosable by Cytology

All gastrointestinal tract cytological specimens are acquired during endoscopy. Most are exfoliated with the possible exceptions of oral and deep submucosal lesions that are instead aspirated with fine needles. When exfoliative cytology is the chosen sampling method, brushing of mucosal-based lesions is performed. The mucosal-based neoplasms (Table 4.1) amenable to cytological evaluation are essentially squamous cell carcinoma and adenocarcinoma. The deeper seated submucosal neoplasms (Table 4.2) understandably yield more cytological material via fine needle aspiration. The clinical index of suspicion for malignancy and concurrent histology usually contributes to reaching an appropriate cytological interpretation. Unfortunately, many reactive

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_4

Table 4.1 Common mucosal based GI tract neoplasm diagnosable by cytology

Location	Neoplasms
Oropharyngeal	• Squamous cell carcinoma
Esophagus	• Squamous cell carcinoma • Adenocarcinoma
Stomach	• Adenocarcinoma
Small and large intestine	• Adenocarcinoma
Anus	• Squamous cell carcinoma

Table 4.2 Common submucosal based GI tract neoplasm diagnosable by cytology

Location	Neoplasms
Oropharyngeal	• Salivary gland neoplasms • Lymphoid neoplasms
Esophagus	• Salivary gland neoplasms • Mesenchymal neoplasms • Neural neoplasms
Stomach	• Neuroendocrine neoplasms • Mesenchymal neoplasms • Lymphoid neoplasms • Neural neoplasms
Small intestine	• Neuroendocrine neoplasms • Mesenchymal neoplasms • Lymphoid neoplasms • Neural neoplasms
Large intestine	• Neuroendocrine neoplasms • Mesenchymal neoplasms • Lymphoid neoplasms • Neural neoplasms

conditions can lead to worrisome cytomorphological atypia and thus can veer the cytopathologist to an inappropriate false-positive diagnosis.

Confounders Based on Cytomorphological Findings

Oropharyngeal Space

Exfoliative cytology of the oropharyngeal mucosa is rarely performed because tissue biopsy is the gold standard for diagnosing any underlying lesions. Nonetheless, because it is a non-aggressive technique, it remains an attractive option for the early diagnosis of oral cancer in selected instances for both patients and clinicians. Most cytologists therefore have limited experience with these specimens. The diagnostic challenge is further compounded by the existence of diverse non-neoplastic conditions (Table 4.3) with cytomorphological features usually seen in neoplastic states, primarily squamous cell carcinoma.

Reactive, atypical squamous cell changes can be seen in ulcers regardless of the etiology (Fig. 4.1). Nuclear enlargement and macrocytes are often seen in oral mucosa of patients with megaloblastic anemia, history of chemoradiation, and diabetes. Hyperkeratosis can be present with mechanical or chemical irritation, certain autoimmune diseases such as the hereditary mucosal syndromes, and candidiasis. Squamous mucosa of pemphigus vulgaris patients is cytologically remarkable for marked reactive, repair-like changes. Sometimes, an underlying true neoplasm such as a granular cell tumor can elicit worrisome alterations in surface squamous epithelial cells.

Esophagus

Besides the anorectal region, the esophagus is by far the most sampled segment of the tubal GI tract by exfoliative cytology, primarily when an infectious process is suspected.

Esophageal Adenocarcinoma when brushed sheds abundant dysplastic cells. The latter are characterized by nuclear enlargement, nuclear membrane irregularities, and a high nucleocytoplasmic ratio. A tumor diathesis may be present. Frequently, these malignant cells can be seen in a single cell pattern (Fig. 4.2).

Rarely, patients with *Barrett's Esophagus* are screened by cytology. Caution is warranted in evaluating these specimens, since intestinal metaplastic epithelium can show some levels of atypical changes reminiscent of glandular neoplasms (Fig. 4.3). However, a tumor diathesis and single atypical cells should be minimal to absent. It is also prudent to be cognizant of the endoscopic findings as a cytological interpretation is being rendered. In the absence of a visualized mass, refrain from a cytodiagnosis of cancer.

Esophageal Squamous Cell Carcinoma, when present, yields sheets of cytologically malignant squamous, but often isolated atypical squamous cells are predominant (Fig. 4.4). The degree of keratinization and nuclear changes vary greatly on how well or poorly differentiated the tumor is. Endoscopic findings are often suggestive of a neoplastic process.

Table 4.3 Oropharyngeal mucosal-based lesions

Neoplasms	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Squamous cell carcinoma	Ulcers	• Infection • Trauma	• Numerous parabasal cells • Acute inflammatory background • Infectious agents • No predominant single atypical cells	• Clinical correlation • Microbiology
	Chemoradiation	• History of radiation and/or chemotherapy	• Nuclear enlargement • Macrocytes • Multinucleated squamous cells • Preserved nucleocytoplasmic ratio • Polychromasia • Cytoplasmic vacuolization	• Clinical correlation
	Underlying granular cell tumor	• Tongue mass consistent with granular cell tumor	• Atypical cohesive squamous cells • No predominant single atypical cells • No necrotic background	• Fine needle aspiration of tongue mass • Tissue biopsy for confirmation
	Pemphigus vulgaris	• Pemphigus vulgaris	• Tzanck cells with repair-like squamous cells • Round/polygonal uniform parabasal-sized cells • Round nuclei with smooth nuclear membranes • Dense cytoplasm with perinuclear acidophilic staining (red around nucleus)	• Clinical correlation • Tissue biopsy for confirmation
	Vitamin deficiencies (B12 and folate)	• Megaloblastic anemia (B12, folate deficiencies)	• Nuclear enlargement • Macrocytes • No predominant single atypical cells • No tumor diathesis	• Clinical correlation • Vitamin B12 and folate levels
	Hereditary mucosal syndromes	• Darier-white disease • White spongus nevus • Hereditary benign intraepithelial dyskeratosis • Pachyonychia congenita	• Cell-in-cell arrangements (corps ronds) • Orange squamous cells with elongated nuclei, dyskeratosis	• Clinical correlation
	Chronic irritation	• Dentures • Trauma • Chewing tobacco	• Nuclear enlargement • Hyperkeratosis • Nuclei relatively uniform with evenly distributed chromatin • No predominant single atypical cells	• Clinical correlation
	Diabetes mellitus	• Diabetes mellitus	• Nuclear enlargement • Preserved nucleocytoplasmic ratio • Binucleation • No predominant single atypical cells • No tumor diathesis	• Clinical correlation

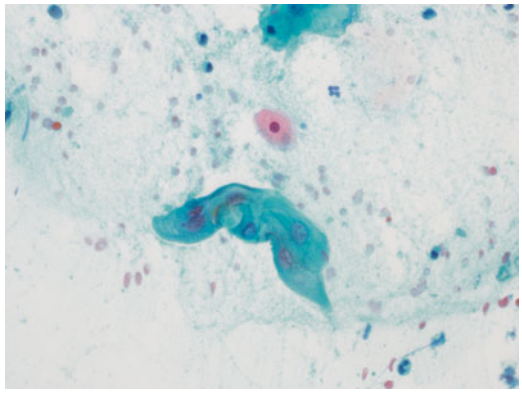


Fig. 4.1 Reactive squamous cells. Reactive and cohesive atypical squamous cells from an oral ulcer (Smear; Papanicolaou)

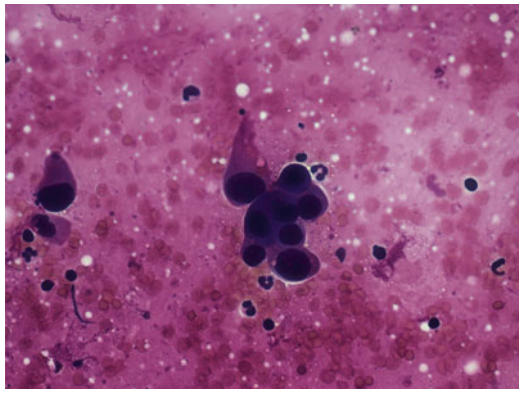


Fig. 4.2 Esophageal adenocarcinoma. Single malignant cells with high nucleocytoplasmic ratio (Smear; Papanicolaou)

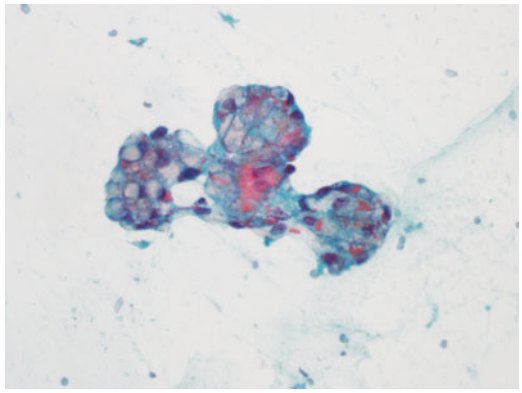


Fig. 4.3 Intestinal metaplasia in esophagus. Cohesive clusters of glandular epithelial cells with goblet cell metaplasia. The nuclei are hyper hyperchromatic and displaced to the side, mimicking a signet ring carcinoma to (Smear; Papanicolaou)

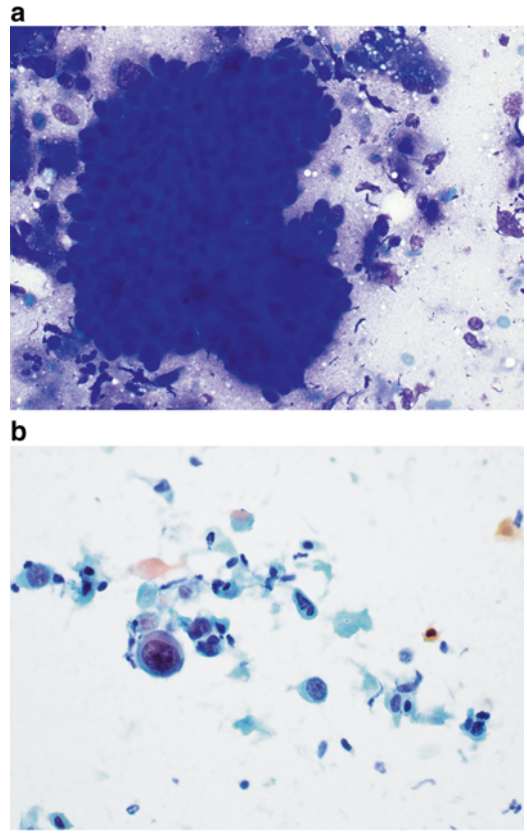


Fig. 4.4 (**a**) Squamous cell carcinoma. Sheet of cytologically malignant squamous cells (Smear; Diff-Quick). (**b**) Squamous cell carcinoma. Isolated malignant squamous cells (Smear; Papanicolaou)

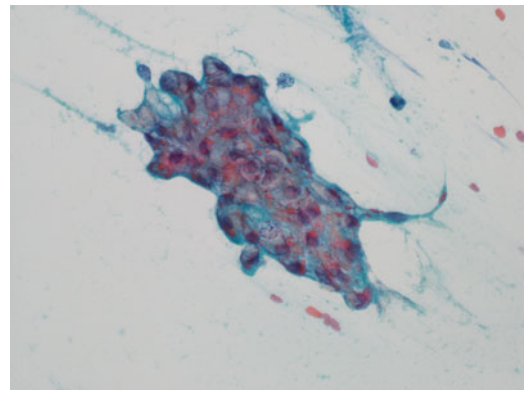


Fig. 4.5 Esophagitis. Reactive squamous cells in sheets from an infectious esophagitis (Smear; Papanicolaou)

Unfortunately, *Infectious Esophagitis* is often associated with atypical cytological changes (Fig. 4.5) associated with malignancies such as squamous cell carcinoma (Table 4.4). Identifying

Table 4.4 Esophageal mucosal-based lesions

Neoplasms	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Squamous cell carcinoma	Fungal esophagitis	• Immunosuppressed • Immunocompromised • Debility	• Fungal elements • Neutrophilic or eosinophilic infiltrates • Parabasal-sized cells • Hyperchromasia • Prominent nucleoli • Cohesive groups of squamous cells • Preserved nucleocytoplasmic ratio • Fine chromatin • Smooth nuclear membranes • No predominant single atypical cells	• Clinical correlation • Microbiology • PAS, GMS stains
	Viral esophagitis	• Immunosuppressed • Immunocompromised • Debility	• Viral cytopathic changes and inclusions • Parabasal-sized cells • Hyperchromasia • Prominent nucleoli	• Clinical correlation • Serology • IHCs (e.g., CMV, HSV, adenovirus)
	Ulcers	• Immunosuppressed • Immunocompromised • GERD • Chemical and mechanical trauma	• Neutrophilic infiltrates • Parabasal-sized cells • Hyperchromasia • Prominent nucleoli • Cohesive groups of squamous cells • Preserved nucleocytoplasmic ratio • Fine chromatin • Smooth nuclear membranes • Necrotic background • No predominant single atypical cells	• Clinical and endoscopic correlation
	GERD	• History of GERD	• Neutrophilic or eosinophilic infiltrates • Parabasal-sized cells • Hyperchromasia • Prominent nucleoli • Cohesive groups of squamous cells • Preserved nucleocytoplasmic ratio • Fine chromatin • Smooth nuclear membranes • No predominant single atypical cells	• Clinical and endoscopic correlation
	Chemoradiation	• History of radiation and/or chemotherapy	• Nuclear enlargement • Macrocytes • Multinucleated squamous cells • Preserved nucleocytoplasmic ratio • Polychromasia • Cytoplasmic vacuolization	• Clinical correlation
	Vitamins deficiencies	• Megaloblastic anemia (B12, folate deficiencies)	• Nuclear enlargement • Macrocytes • No predominant single atypical cells • No tumor diathesis	• Clinical correlation • Vitamin B12 and folate levels
	Pemphigus vulgaris	• Pemphigus vulgaris	• Tzanck cells with repair-like squamous cells • Round/polygonal uniform parabasal-sized cells • Round nuclei with smooth nuclear membranes • Dense cytoplasm with perinuclear acidophilic staining (red around nucleus)	• Clinical correlation • Tissue biopsy for confirmation
	Underlying granular cell tumor	• Esophageal mass consistent with granular cell tumor	• Atypical cohesive squamous cells • No predominant single atypical cells • No tumor diathesis	• Tissue biopsy for confirmation
• Adenocarcinoma	Barrett's esophagus	• History of GERD	• Goblet cells • Large, cohesive flat sheets of cells • No loss of polarity or cellular crowding • No predominant single cells • No nuclear irregularities	• Clinical correlation

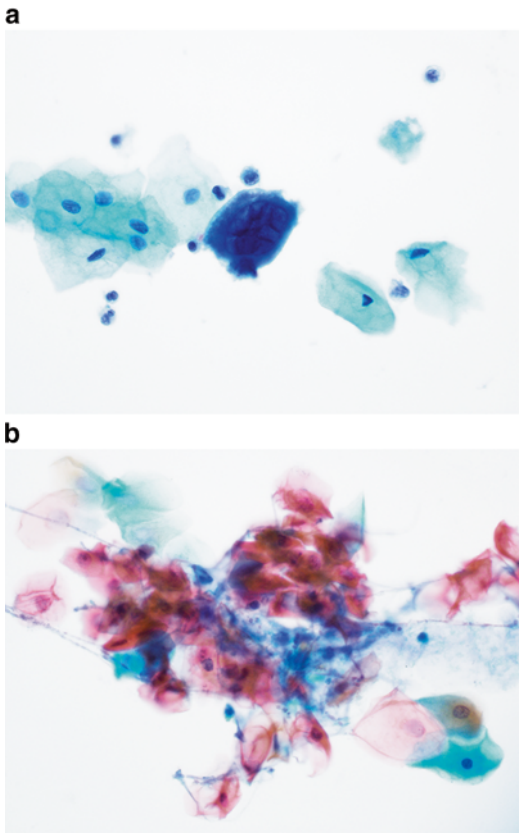


Fig. 4.6 (**a**) Herpes esophagitis. Viral cytopathic changes from herpes esophagitis (Smear; Papanicolaou). (**b**) Fungal esophagitis. Fungal elements associated with reactive squamous cells (Smear; Papanicolaou)

microorganisms such as yeast forms or viral inclusions can aid in the diagnosis (Fig. 4.6), but are not always present. As in the oral mucosa, reactive, atypical squamous cell changes can be identified by cytology in patients with esophageal ulcers, megaloblastic anemia, pemphigus vulgaris, underlying granular cell tumor or history of chemoradiation.

Stomach

Gastric Adenocarcinomas, which account for the majority of gastric malignancies, are exceedingly rare in cytology. However, if brushed, the tumor yields a high number of isolated cells in the setting of marked tumor diathesis (Fig. 4.7). Here again, a strong correlation with clinical and endoscopic impression is advised.

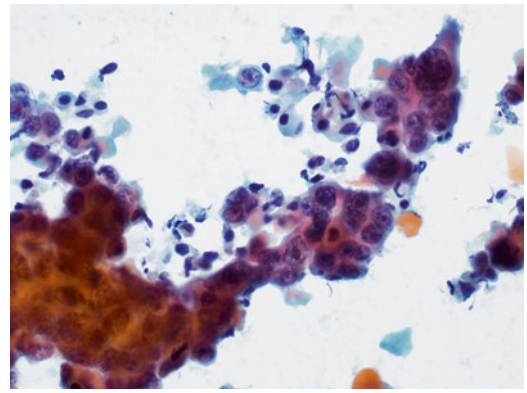


Fig. 4.7 Gastric adenocarcinoma. Malignant glandular cells with background of tumor diathesis (Smear; Papanicolaou)

An inflamed gastric mucosa, *Gastritis* (with or without an ulcer), can create reactive glandular atypia so severe that it can be difficult to exclude an adenocarcinoma. Chemoradiation, as expected, can also create marked cytological atypia. Besides correlating with the clinical and endoscopic impression, certain cytomorphological clues can be helpful in avoiding overdiagnosis (Table 4.5). In gastritis, the reactive epithelial cells tends to be cohesive, with more uniform cytological changes (Fig. 4.8).

Small and Large Intestine

Screening of the intestine for polyps or masses by cytology is virtually non-existent. Histology is the gold standard when assessing for intestinal mucosal-based malignancy such as an adenocarcinoma. There are nonetheless situations where exfoliative cytology is the preferred method. These include evaluating a stenotic lesion that is only accessible to a cytological brush and endoscopic screening of high risk bleeding patients.

Intestinal Adenocarcinomas (from both small and large intestine) resemble those of the esophagus and the stomach on cytology. The neoplastic cells have an increased nucleocytoplasmic ratio and marked pleomorphism with prominent nucleoli (Fig. 4.9). A highly dysplastic epithelium from a polyp can mimic

Table 4.5 Gastric mucosal-based lesions

Neoplasms	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Adenocarcinoma	Gastritis (chronic or active) with or without ulcers	• *H. pylori* infection • Autoimmune gastritis • Drugs (NSAIDS, aspirin, iron pills, bisphosphonates) • Other caustic agents • Mechanical trauma	• *H. pylori* organisms • Goblet cells • Neutrophils if active gastritis • Cohesive, orderly, flat, sheets • No loss of polarity or cellular crowding • Nuclear enlargement • No pleomorphism • Prominent nucleoli • No predominant atypical single cells	• Clinical correlation • Histochemical or IHCs for *H. pylori*
	Viral gastritis	• Immunosuppressed • Immunocompromised	• Viral cytopathic changes and inclusions • No predominant single atypical cells	• Clinical correlation • Serology • IHCs (e.g., CMV, HSV, adenovirus)
	Chemoradiation	• History of radiation and/or chemotherapy	• Nuclear enlargement • Macrocytes • Multinucleated cells • Preserved nucleocytoplasmic ratio • Cytoplasmic vacuolization	• Clinical correlation

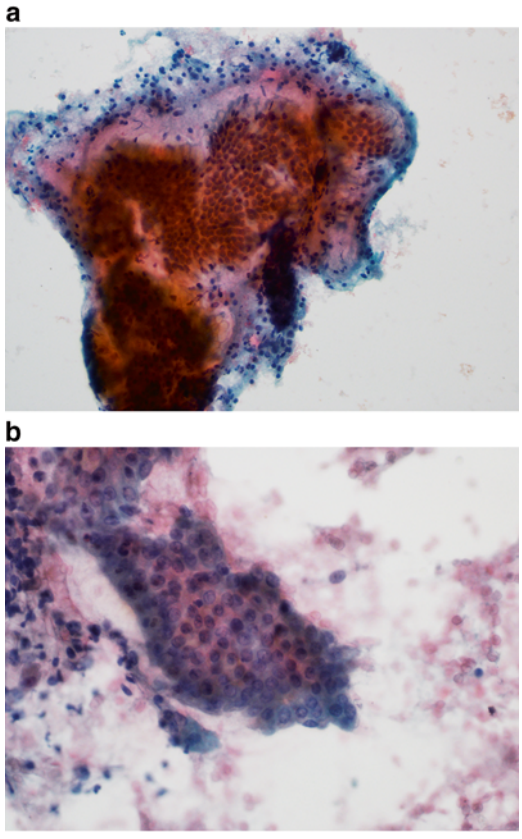


Fig. 4.8 (**a**) Gastritis. Reactive gastric epithelial cells with foveolar epithelium at the edge (Smear; Papanicolaou). (**b**) Gastritis. Reactive and cohesive gastric epithelial cells with mild nuclear enlargement (Smear; Papanicolaou)

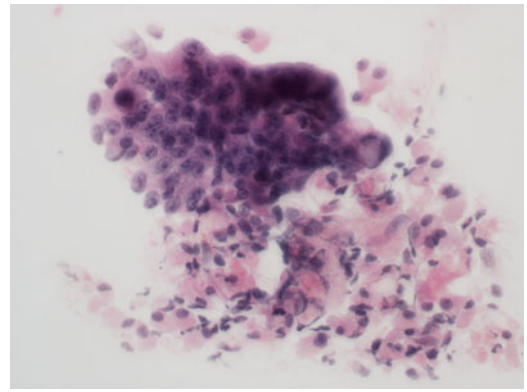


Fig. 4.9 Intestinal adenocarcinoma. Loose aggregate of malignant glandular cells with prominent nucleoli (Smear; Re-hydrated H&E)

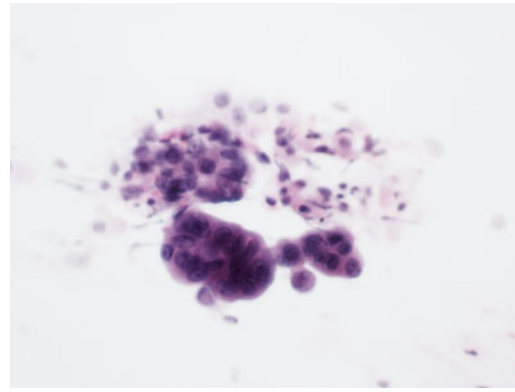


Fig. 4.10 Intestinal high grade dysplasia. Surface epithelium with cohesive cytologically atypical cells (Smear; Re-hydrated H&E)

carcinoma cytologically (Fig. 4.10). Distinguishing it from an invasive process is challenging without histological evaluation.

Differentiating the cytologic changes of *Epithelial Repair*, dysplasia, and carcinoma can virtually be impossible. As anywhere in the GI tract, an inflamed intestinal mucosa, regardless of etiology, is subject to marked reactive atypical changes mimicking adenocarcinoma (Table 4.6). Establishing the clinical and radiographic context can be beneficial.

Conclusions

Histology is essentially the primary manner to study any suspected gastrointestinal mucosal-based lesions. In the rare instance when exfoliative cytology is submitted for evaluation, one ought to be aware of the various infectious, inflammatory and iatrogenic states that tend to produce cytological changes similar to those expected in malignant states. In the majority of the cases, any pitfalls can be avoided when the

Table 4.6 Small and large intestinal mucosal-based lesions

Neoplasms	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Adenocarcinoma	Active inflammation and or ulcers	• Inflammatory bowel disease • Infection • Diverticulitis • NSAIDS • Ischemia	• Neutrophilic infiltrate • Cohesive, orderly, flat sheets of cells • No loss of polarity or cellular crowding • Nuclear enlargement • Preserved nucleocytoplasmic ratio • Coarse dark chromatin • Prominent nucleoli • No predominant atypical single cells	• Clinical and endoscopic correlation
	Chemoradiation	• History of radiation and/or chemotherapy	• Nuclear enlargement • Macrocytes • Multinucleated cells • Preserved nucleocytoplasmic ratio • Cytoplasmic vacuolization	• Clinical and endoscopic correlation

cytomorphological findings are assessed in the context of the clinical, radiographic and endoscopic impression.

Suggested Readings

1. Aste H, Pugliese V, Munizzi F, et al. Left-sided stenosing lesions in colonoscopy. Gastrointest Endosc. 1983;29(1):18–20.
2. Diniz-Freitas M, García-García A, Crespo-Abelleira A, et al. Applications of exfoliative cytology in the diagnosis of oral cancer. Med Oral. 2004;9(4): 355–61.
3. Galloro G, Mignogna M, de Werra C, et al. The role of upper endoscopy in identifying oesophageal involvement in patients with oral pemphigus vulgaris. Dig Liver Dis. 2005;37(3):195–9.
4. Geisinger KR, Teot LA, Richter JE. A comparative cytopathologic and histologic study of atypia, dysplasia, and adenocarcinoma in Barrett's esophagus. Cancer. 1992;69(1):8–16.
5. Isbister WH, Gupta RK. Colonoscopy, mucosal biopsy and brush cytology in the assessment of patients with colorectal inflammatory bowel disease. Surg Endosc. 1989;3(3):159–63.
6. Jhala N, Jhala D. Gastrointestinal tract cytology: advancing horizons. Adv Anat Pathol. 2003;10(5): 261–77.
7. Mokhtari M, Rasolmali R, Kumar PV. Pemphigus vulgaris of skin: cytological findings and pitfalls. Acta Cytol. 2012;56(3):310–4.
8. Mostaghni AA, Afarid M, Eghbali S, Kumar P. Evaluation of brushing cytology in the diagnosis of Helicobacter pylori gastritis. Acta Cytol. 2008;52(5): 597–601.
9. Navone R, Burlo P, Pich A, et al. The impact of liquid-based oral cytology on the diagnosis of oral squamous dysplasia and carcinoma. Cytopathology. 2007;18(6):356–60.
10. Padmavathy F, Siddaraju N, Sistla SC. Role of brush cytology in the diagnosis of Barrett's esophagus: an analysis of eight cases. Diagn Cytopathol. 2011;39(1): 60–4.
11. Prasad H, Ramesh V, Balamurali P. Morphologic and cytomorphometric analysis of exfoliated buccal mucosal cells in diabetes patients. J Cytol. 2010; 27(4):113–7.
12. Witkop CJ, Gorlin RJ. Four hereditary mucosal syndromes: comparative histology and exfoliative cytology of Darier-white's disease, hereditary benign intraepithelial dyskeratosis, white sponge nevus, and pachyonychia congenita. Arch Dermatol. 1961;84(5): 762–71.

Pancreaticobiliary Tract

5

Brief Introduction

Patients with suspected pancreaticobiliary lesions are managed according to the cytologic findings in concert with endoscopic, imaging and clinical impression. Nonetheless, a higher expectation is often placed on the cytology assessment. While "atypical" and "suspicious" categories have relatively well reported malignancy risks, some clinicians prefer "negative" or "positive" for definitive therapeutic management. Extreme caution is warranted to avoid false-positive diagnoses because numerous reactive conditions can elicit worrisome cytological atypia leading to over-interpretation. Therefore, cytologic diagnosis should always be rendered in conjunction with the endoscopic, imaging and clinical impressions. Fortunately, most benign inflammatory and reactive processes have distinguishing cytomorphological features.

Classification of Pancreaticobiliary Neoplasms Based on Imaging Features

A lesion in the pancreas or biliary tract is usually suspected due to symptoms, or when it is discovered incidentally during workup for another reason. Regardless of how it comes to medical attention, it will be evaluated by at least one of the many available imaging modalities. These include endoscopic retrograde cholangiopancreatography (ERCP), endoscopic ultrasound (EUS), multidetector computed tomography (MDCT), magnetic resonance imaging (MRI), magnetic resonance cholangiopancreatography (MRCP), and transabdominal ultrasound (TAU). This means that the radiological description of any suspected neoplasms will be available at the time of cytological evaluation. In fact, knowing a pancreaticobiliary lesion is solid, cystic, or heterogeneous (solid/cystic) helps in generating differential diagnoses. However, one ought to be mindful that mimickers of neoplasms can also be solid or cystic.

Confounders Based on Cytomorphological Findings

Solid Lesions (Table 5.1)

When a solid mass is suspected in the pancreas or in the biliary tree, it will be subjected to an array of diagnostic modalities, including cytological evaluation (aspiration and/or brushing). The list of possible neoplasms is unfortunately matched by a long list of possible non-neoplastic mimickers.

Aspirated *chronic pancreatitis* may yield predominantly ductal cells (Fig. 5.1) suggestive of a well-differentiated ductal carcinoma (Fig. 5.2). The key to avoid over-interpretation

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_5

Table 5.1 Solid lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Differentiating features	Additional steps
• Pancreatic ductal adenocarcinoma	Chronic pancreatitis	• Alcohol abuse • Gallstone • Medications • Infection • Trauma • Metabolic disorders • Autoimmune disorders • Eosinophilic • Tropical • Hereditary • Surgery	• Low cellularity • Rare single cells • No isolated atypical cells • Two-dimensional sheets • Acinar cells may be present • No nuclear crowding or overlapping • No nuclear enlargement • Fragments of inflamed fibrotic stroma	• Clinical correlation • Immunocytochemistry with DPC4 (SMAD4)
	Contaminants from chronic gastritis (active or inactive) Contaminant from chronic duodenitis (active or inactive)	• Pancreatic lesion in the body	• Enlarged glandular nuclei • Well-developed nucleoli • Intercellular cohesion • No isolated atypical cells	• Clinical correlation • Establish path of aspirating needle
		• Pancreatic lesion in the head	• Two-dimensional flat sheets with goblet cells • Enlarged and uniform glandular epithelium	• Clinical correlation • Establish path of aspirating needle
• Pancreatic neuroendocrine tumors • Solid pseudopapillary tumor • Lymphoid tumors • Acinar cell carcinoma • Pancreatoblastoma	Islet cell hyperplasia	• Diabetes mellitus • Chronic pancreatitis • Beckwith-Wiedemann syndrome	• Small size • Low cellularity • Pancreatic ductal and acinar cells may be present	• Clinical and imaging correlation • Immunocytochemistry • Flow cytometry
	Intrapancreatic Accessory Spleen	• Congenital	• Small Nodule (~1 cm) • Numerous dyscohesive lymphoid cells with minimal cytoplasm	• Clinical and imaging correlation • Immunocytochemistry • Flow cytometry
	Intrapancreatic lymphoid tissue	• Normal intra-parenchymal lymph node	• Numerous dyscohesive lymphoid cells with minimal cytoplasm	• Clinical and imaging correlation • Immunocytochemistry • Flow cytometry
• Mesenchymal tumors	Chronic pancreatitis	• Alcohol abuse • Gallstone • Medications • Infection • Trauma • Metabolic disorders • Autoimmune disorders • Surgery	• Low cellularity • Fragments of inflamed fibrotic stroma • Inflammatory background • No cytological atypia	• Clinical and imaging correlation • Immunocytochemistry
• Cholangiocarcinoma	Reactive Biliary Epithelium	• Primary sclerosing cholangitis • History of stents • History of stones	• Flat cohesive clusters and sheets • Uniform enlarged nuclei with fine chromatin • Inconspicuous nucleoli	• Clinical and imaging correlation

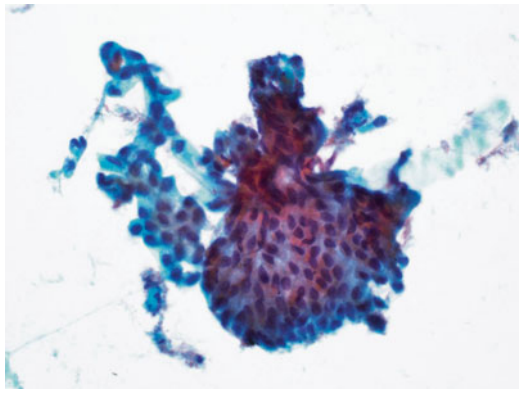


Fig. 5.1 Increased benign pancreatic ductal epithelium seen in chronic pancreatitis (Smear; Papanicolaou, High Magnification)

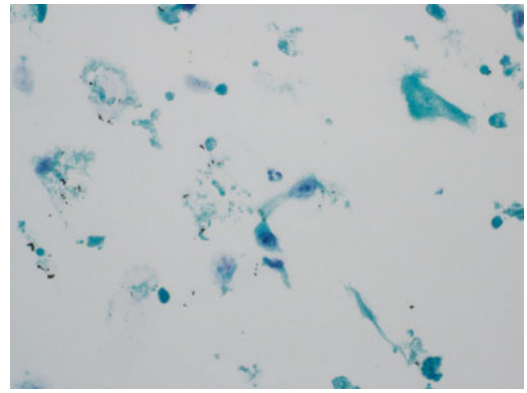


Fig. 5.3 Increased single atypical malignant ductal cells in pancreatic ductal adenocarcinoma (Smear; Papanicolaou, High Magnification)

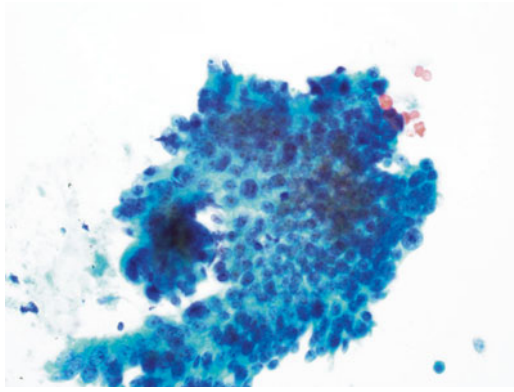


Fig. 5.2 Increased malignant ductal epithelium in pancreatic ductal adenocarcinoma, with nuclear pleomorphism, enlargement and overlapping (Smear; Papanicolaou, High Magnification)

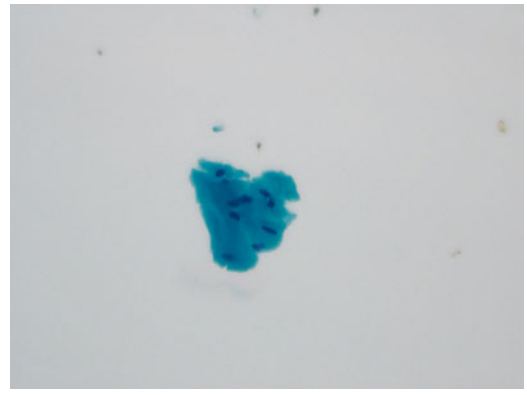


Fig. 5.4 Increased fragments of stroma in chronic pancreatitis (Smear; Papanicolaou, High Magnification)

is to recognize that those ductal cells will be in flat sheets without nuclear overlapping or atypia. Furthermore, the aspirate of chronic pancreatitis tends to have low cellularity. Single and isolated atypical cells (Fig. 5.3) are notably rare. Often, fragments of inflamed fibrotic stroma can be present (Fig. 5.4). Obtaining the history of chronic pancreatitis is beneficial. In difficult cases, immunohistochemical staining of a cell block with DPC4 (deleted in pancreatic carcinoma, also called smad4) potentially aids. Approximately 55 % of pancreatic adenocarcinoma and variants will lose expression of DPC4 while remnants of benign ducts will express the protein.

Anatomically, the pancreaticobiliary system is surrounded by many structures, primarily the stomach and duodenum providing an opportunity for *GI contamination*. The aspirating needle will undoubtedly collect epithelium, including reactive cells, from these structures on its way to a pancreaticobiliary lesion. It is therefore imperative to be aware of the exact path traversed by the sampling device to prevent interpreting reactive gastrointestinal epithelium as pancreaticobiliary neoplasm. While the enlarged glandular nuclei commonly found in reactive gastric (Fig. 5.5) and duodenal epithelium (Fig. 5.6) can be alarming, their benign nature is ascertained by preserved intercellular cohesion, architectural order, and the absence of isolated atypical cells (Fig. 5.3).

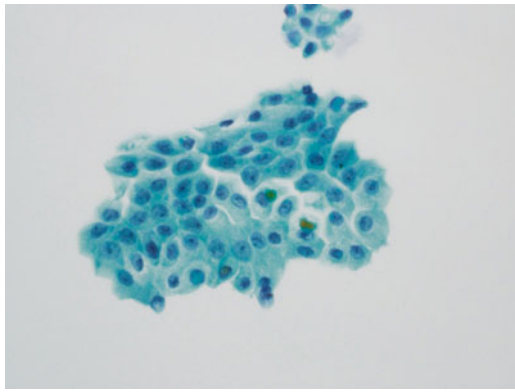


Fig. 5.5 Gastric epithelium collected while sampling a mass in the body of the pancreatic (Smear; Papanicolaou, High Magnification)

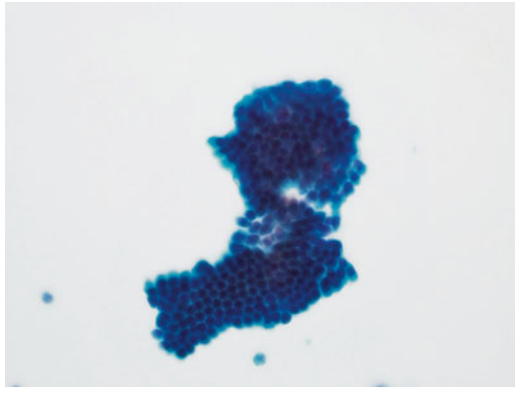


Fig. 5.6 Duodenal epithelium collected while sampling a mass in the head of the pancreatic (Smear; Papanicolaou, High Magnification)

Islet cell hyperplasia can mimic a neuroendocrine or solid pseudopapillary tumor. However, this is rarely an issue, as this entity often does not raise sufficient clinical concern for it to be aspirated. If sampled, the aspirate is hypocellular. By imaging, intrapancreatic *accessory spleen* and lymphoid tissue can cause some concerns. If sampled, the aspirate would resemble lymphoid tissue with numerous dissociated single cells with scant cytoplasm (Fig. 5.7). Neoplastic tumors of the pancreas with a similar dyscohesive pattern on cytological specimens include neuroendocrine tumors (Fig. 5.8), acinar cell tumors and solid pseudopapillary neoplasms.

Reactive biliary epithelium (regardless of etiology, Fig. 5.9) is notorious for mimicking

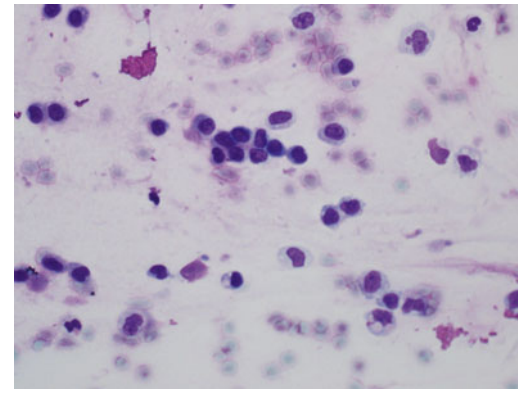


Fig. 5.7 Intrapancreatic accessory spleen with dyscohesive lymphoid cells (Smear; Papanicolaou, High Magnification)

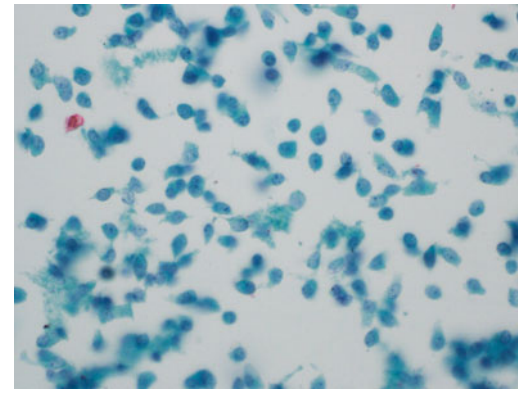


Fig. 5.8 Neuroendocrine tumor with numerous dyscohesive neoplastic cells (Smear; Diff-Quik, High Magnification)

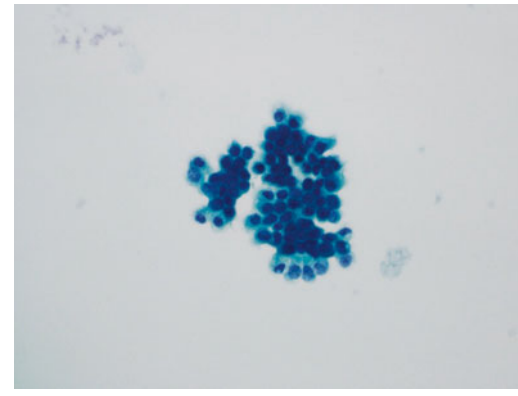


Fig. 5.9 Reactive biliary epithelium from a previously stented common bile duct (Smear; Papanicolaou, High Magnification)

carcinoma (Fig. 5.10). In the absence of any clinical and imaging impression, evaluation of a pancreaticobiliary brushing specimen should be avoided. Common biliary epithelial irritants include stents and stones. Inflammatory states, such as primary sclerosing cholangitis, will also cause a similar reactive atypical biliary epithelium. While clinical history is crucial, cytomorphological features favoring a benign process include flat cohesive clusters and sheets, retained polarity, cells with uniformly enlarged nuclei with fine chromatin and inconspicuous nucleoli.

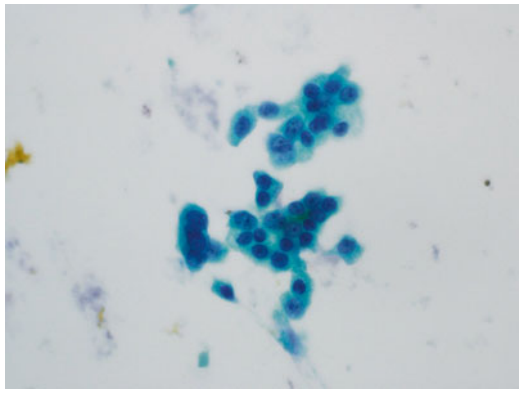


Fig. 5.10 Malignant biliary epithelium from a cholangiocarcinoma (Smear; Papanicolaou, High Magnification)

Cystic Lesions (Table 5.2)

Pancreatic neoplastic cystic lesions (including solid tumors with cystic degeneration) are commonly aspirated for cytological diagnosis. The latter is often complicated by the low cellularity of these aspirates. This hypocellularity can make it virtually impossible to distinguish true neoplastic cysts from benign cystic lesions.

Fortunately, one of the most common benign and non-neoplastic cysts, *pseudocyst of the pancreas*, occurs in a specific clinical setting, namely chronic pancreatitis. Often the clinical history is remarkable for alcohol abuse, gallstones or pancreatitis-induced medications. Cytomorphological findings favoring a pseudocyst include the absence of epithelium coupled with the presence of inflammatory cells (Fig. 5.11) and amorphous debris (Fig. 5.12).

Table 5.2 Cystic lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Differentiating features	Additional steps
• Intraductal papillary mucinous neoplasm • Mucinous cystic neoplasm	Gastrointestinal contaminants	• Sampling of suspicious mass via stomach or duodenum	• Thin mucus • Flat or sheets of cohesive epithelial cells with interspersed goblet cells • No isolated atypical cells	• Clinical and imaging correlation • CEA and amylase levels in aspirate
	Pseudocyst	• Chronic pancreatitis (alcohol and/or gallstones)	• No mucus • Inflammatory cells • Amorphous debris • No epithelium	• Clinical and imaging correlation • CEA and amylase levels in aspirate
• Serous cystic neoplasms • Lymphangioma	Pseudocyst	• Chronic pancreatitis (alcohol and/or gallstones)	• No mucus • Inflammatory cells • Amorphous debris • No epithelium	• Clinical and imaging correlation • CEA and amylase levels in aspirate
• Solid neoplasms with cystic degeneration	Pseudocyst	• Chronic pancreatitis (alcohol and/or gallstones)	• No mucus • Inflammatory cells • Amorphous debris • No epithelium	• Clinical and imaging correlation • CEA and amylase levels in aspirate
	Groove (paraduodenal) pancreatitis	• Developmental anomalies of minor ampulla • Alcohol abuse	• Giant cells • Fibrotic stroma	• Clinical and imaging correlation

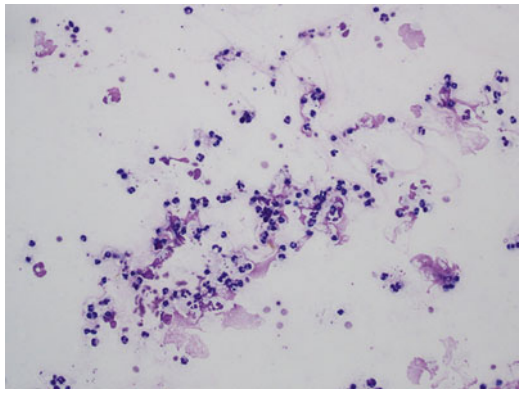


Fig. 5.11 Acute inflammatory cells in an aspirated pseudocyst (Smear; Diff-Quik, Intermediate Magnification)

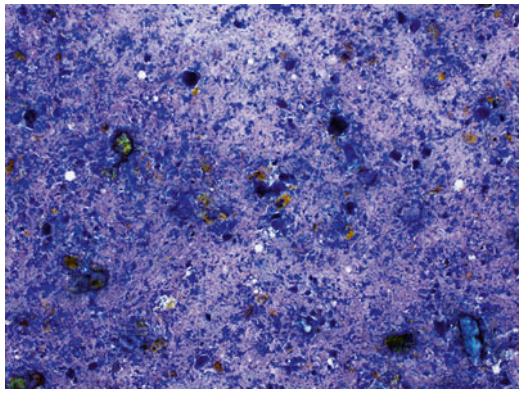


Fig. 5.12 Amorphous debris in an aspirated pseudocyst (Smear; Diff-Quik, Low Magnification)

Groove (paraduodenal) pancreatitis, while uncommon, has the potential of mimicking solid pancreatic neoplasms with cystic degeneration. Cytological features supporting groove pancreatitis include the presence of giant cells and inflamed fibrotic stroma. Gastrointestinal tract contaminants can make the aspirate of a pseudocyst resemble a true cystic neoplasm by the presence of epithelium. Special attention to the clinical history will be helpful.

Contaminant mucus from the gastrointestinal tract (Fig. 5.13) can also mimic mucin production by pancreatic mucinous neoplasms (Fig. 5.14). Here, integrating laboratory analysis of the aspirate will be helpful. Mucinous neoplasms of the pancreas tend to have an elevated CEA and a low to normal amylase level.

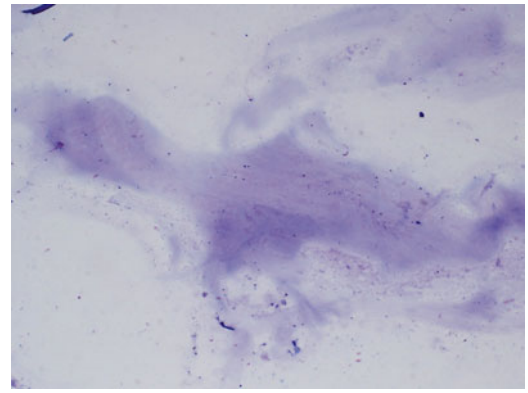


Fig. 5.13 Mucin collected from duodenum while sampling a mass in the head of the pancreas (Smear; Diff-Quik, Intermediate Magnification)

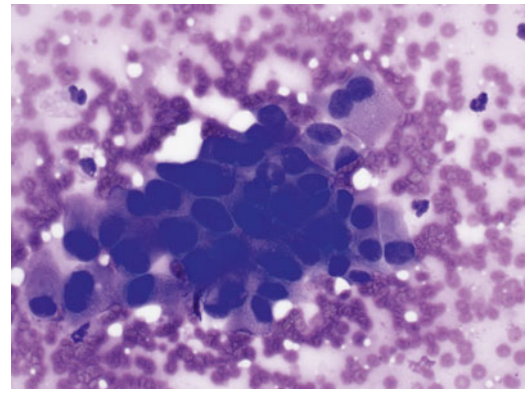


Fig. 5.14 Atypical columnar cells in a mucinous neoplasm of the pancreas (Smear; Diff-Quik, High Magnification)

Conclusion

Most pitfalls in cytological evaluation of pancreaticobiliary lesions can be successfully circumvented if the interpretation is rendered with consideration of the endoscopic, imaging, clinical and laboratory impressions. Because the pancreas and the biliary tree are surrounded by other structures, one has to be mindful of the path that the sampling device had to travel to reach its target. Thus, contaminants become an important group of neoplastic mimickers.

Suggested Readings

1. Adler D, Max Schmidt C, Al-Haddad M, et al. Clinical evaluation, imaging studies, indications for cytologic study, and preprocedural requirements for duct brushing studies and pancreatic FNA: the Papanicolaou Society of Cytopathology recommendations for pancreatic and biliary cytology. Diagn Cytopathol. 2014;42(4):325–32.
2. Brody JR, Costantino CL, Potoczek M, et al. Adenosquamous carcinoma of the pancreas harbors KRAS2, DPC4 and TP53 molecular alterations similar to pancreatic ductal adenocarcinoma. Mod Pathol. 2009;22(5):651–9.
3. Brosens LA, Leguit RJ, Vleggaar FP, et al. EUS-guided FNA cytology diagnosis of paraduodenal pancreatitis (groove pancreatitis) with numerous giant cells: conservative management allowed by cytological and radiological correlation. Cytopathology. 2014;26(2):122–5.
4. Chadwick BE, Layfield LJ, Witt BL, et al. Significance of atypia in pancreatic and bile duct brushings: follow-up analysis of the categories atypical and suspicious for malignancy. Diagn Cytopathol. 2014;42: 285–9.
5. Gonzalez Obeso E, Murphy E, Brugge W, et al. Pseudocyst of the pancreas: the role of cytology and special stains for mucin. Cancer. 2009;117(2):101–7.
6. Hollerbach S, Klamann A, Topalidis T, et al. Endoscopic ultrasonography (EUS) and fine-needle aspiration (FNA) cytology for diagnosis of chronic pancreatitis. Endoscopy. 2001;33(10):824–31.
7. Kroemer A, Sabet-Baktach M, Doenecke A, et al. Eosinophilic cholangitis and wirsungitis as cause of simultaneous bile duct obstruction and pancreatitis. Z Gastroenterol. 2012;50(8):766–70.
8. Midwinter MJ, Beveridge CJ, Wilsdon JB, et al. Correlation between spiral computed tomography, endoscopic ultrasonography and findings at operation in pancreatic and ampullary tumours. Br J Surg. 1999;86:189–93.
9. Megibow A, Zhou X, Rortterdam H. Pancreatic adenocarcinoma: CT versus MR imaging in the evaluation of resectability-report of the radiology diagnostic oncology group. Radiology. 1995;195(2):327–32.
10. Nagle JA, Wilbur DC, Pitman MB. Cytomorphology of gastric and duodenal epithelium and reactivity to B72.3: a baseline for comparison to pancreatic lesions aspirated by EUS-FNAB. Diagn Cytopathol. 2005; 33(6):381–6.
11. Pitman MB, Layfield LJ. Guidelines for pancreaticobiliary cytology from the papanicolaou society of cytopathology: a review. Cancer Cytopathol. 2014;122(6):399–411.
12. Tang ZH, Zou SQ, Hao YH, et al. The relationship between loss expression of DPC4/Smad4 gene and carcinogenesis of pancreatobiliary carcinoma. Hepatobiliary Pancreat Dis Int. 2002;1(4):624–9.
13. Ueda Y, Kurihara K, Kondoh T, et al. Islet-cell hyperplasia causing hyperinsulinemic hypoglycemia in an adult. J Gastroenterol. 1998;33(1):125–8.
14. Van Driel BE, Van Gulik TM, Strum PD, et al. Differential diagnosis of chronic pancreatitis and pancreatic cancer in brush cytology specimens. Cancer Res. 1999;59(22):5732–6.

Liver Cytology

6

Brief Introduction

Radiology, especially magnetic resonance imaging (MRI), in conjunction with the clinical context is an important modality for the assessment of newly discovered hepatic masses. Characteristic image findings are used to diagnose most of these lesions, in some instances negating the needs for cytological or histological evaluation. However, often these masses can be diagnostically challenging on imaging evaluation alone. In these cases, fine-needle aspiration (FNA) can be performed percutaneously with guidance by computed tomography (CT), ultrasound, or MRI to acquire cytological specimens. Core needle biopsy can similarly be used with imaging guidance. When the suspected tumor is in the hilum of the liver, brushings during endoscopic retrograde cholangiopancreatography (ERCP) are performed. Whether FNA, core biopsy or brushing, rapid on-site cytological assessment is frequently requested. Cytology, assisted by ancillary studies, is indeed appropriate to distinguish between primary and secondary liver neoplasia. However, a well-recognized difficulty in liver cytology is distinguishing non-neoplastic lesions from benign or malignant ones.

Classification of Hepatic Neoplasms Based on Cell of Origin

Any masses (Fig. 6.1) detected in the liver can potentially be evaluated by cytology to determine whether such masses represent primary or secondary tumors. The primary tumors of the liver are generally classified as epithelial and nonepithelial neoplasms. By far, epithelial neoplasms are the most common which include hepatocellular and bile duct types. The primary nonepithelial neoplasms of the liver consist primarily of mesenchymal tumors. Rarely, primary hepatic lymphomas are encountered in association with AIDS or as in post-transplantation lymphoproliferative disorders in the setting of Epstein-Barr virus infection. In this chapter, the discussions will be organized by cell types of primary lesions commonly found in the liver. Attention will focus on various non-neoplastic conditions that exhibit cytological features resembling these neoplastic hepatic lesions. For primary mesenchymal lesions of the liver, refer to the soft tissue chapter for more in depth coverage and discussion.

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_6

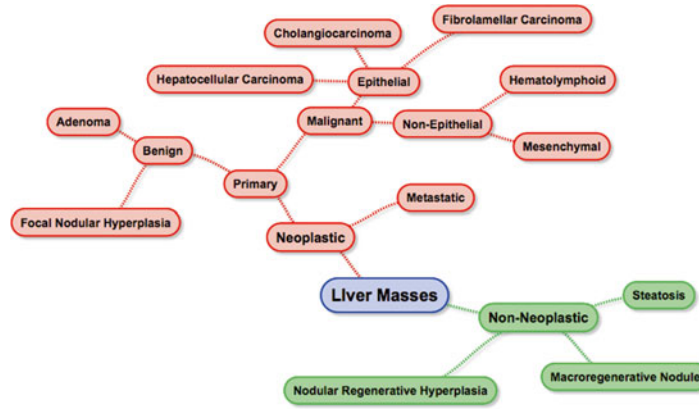


Fig. 6.1 Neoplastic masses of the liver

Confounders Based on Cytomorphological Findings

Hepatocellular Lesions

Epithelial neoplasms are predominantly hepatocellular tumors. In cirrhotic patients, *Hepatocellular Carcinoma* (HCC) is usually the suspected lesion (Table 6.1). Interpreting aspirates of this tumor can be problematic, especially when the tumor is very well-differentiated. However, regardless of the degree of differentiation, HCC tends to display characteristic cytomorphological features. These include high cellularity and abnormal cellular organization (Fig. 6.2). Histologically, the malignant cells can form trabeculae which is cytologically translated into balls of cells outlined by elongated non-neoplastic endothelial cells. Capillaries can also be seen traversing nests of malignant cells. The malignant cells can also be present singly (Fig. 6.3). With less differentiated carcinoma, stripped nuclei are often identified (Fig. 6.4). The HCC cells can be monotonous and often retain morphological similarity to benign hepatocytes. They are polygonal with well-defined borders, central nuclei and granular cytoplasm (Fig. 6.5). The nuclear to cytoplasmic ratio is frequently increased. Nucleoli can be prominent and numerous.

Malignant cells from well-differentiated HCC can resemble hepatocytes of *Normal Liver*. Similarly, aspirate of the latter can be mistaken for the former. Cytological findings favoring non-neoplastic aspirate of the liver include hypocellularity, presence of hepatocytes with low nuclear to cytoplasmic ratio, mild polymorphism, and micronucleoli (Fig. 6.6). Furthermore, the absence of endothelial cells wrapping balls of hepatocytes or capillaries traversing groups of hepatocytes argue against HCC.

Numerous other radiologically detectable non-neoplastic lesions have an indistinguishable cytological profile in comparison to normal liver. For example, cirrhotic liver can have a dominant *Macroregenerative Nodule* composed of benign hepatocytes. Heterogeneous blood flow within the liver can cause a *Nodular Regenerative Hyperplasia* composed of benign small and large hepatocytes (Fig. 6.7). Often, a fatty liver can display a nodule. In this case, the hepatocytes can have large cytoplasmic vacuoles (Fig. 6.8).

While HCC has some well-recognized differentiating cytologic features, it is highly recommended to correlate the cytological findings of any aspirates with clinical, laboratory and imaging impression. The majority of HCC arises in the setting of cirrhotic liver. Caution is advised when entertaining a diagnosis of HCC in a non-cirrhotic liver. HCC can have elevated alpha-fetoprotein (above 500 ng/mL). Because HCC is exclusively supplied by arterial blood, on dynamic imaging techniques it is typically hypervascular with washout in the venous phase. If a cell block can be obtained, immunocytochemistry has the potential to be helpful. Glypican-3, while negative in benign hepatocytes, is variably positive in HCC. Similarly,

Table 6.1 Hepatocellular lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Hepatocellular carcinoma and variants	Normal liver	• Normal liver or • Lesion with background of normal liver	• Thin ribbons of cells • Round to oval nuclei, variably sized • Abundant cytoplasm (low N/C ratio) • Mild pleomorphism • No significant stripped nuclei • Other cellular elements (bile duct epithelial cells, sinusoidal endothelial cells, and Kupffer cells)	• Clinical and radiographic correlation • Immunocytochemistry with glypican-3, glutamine synthetase and HSP70
	Macroregenerative nodule of cirrhosis	• Chronic liver disease, regardless of etiology	• Difficult to smear due to intact reticulum fiber network • Wide cytomorphologic spectrum from benign-appearing hepatocytes to markedly atypical ones • No significant stripped nuclei	• Clinical and radiographic correlation • Immunocytochemistry with glypican-3, glutamine synthetase and HSP70
	Nodular regenerative hyperplasia	• Heterogeneous blood flow within the liver, regardless of etiology	• Hepatocytes of varying size but with low N/C ratio • No significant stripped nuclei • Other cellular elements (bile duct epithelial cells, sinusoidal endothelial cells, and Kupffer cells)	• Clinical and radiographic correlation Immunocytochemistry with glypican-3, glutamine synthetase and HSP70
	Intrahepatic abscess	• Infectious (usually non-viral) • Inflammatory bowel disease • Immunocompromised or immunosuppresed	• Numerous neutrophils and necrotic debris if caused by bacteria or fungi • “anchovy paste” macroscopic appearance of cytological specimen for amebic abscess • No malignant cells seen	• Clinical correlation • Special stains to visualize micro-organisms • Microbiology
	Steatosis	• Fatty liver disease, regardless of etiology	• Hepatocytes with large cytoplasmic vacuoles filled with lipid • Lack of nuclear pleomorphism • No significant stripped nuclei • Other cellular elements (bile duct epithelial cells, sinusoidal endothelial cells, and Kupffer cells)	• Clinical and radiographic correlation • Immunocytochemistry with glypican-3, glutamine synthetase and HSP70
• Fibrolamellar hepatocellular carcinoma	Normal liver	• Normal liver	• Thin ribbons of cells • Round to oval nuclei, variably sized • Abundant cytoplasm (low N/C ratio) • Lack of nuclear pleomorphism • No bands of fibrosis between neoplastic cells	• Clinical and radiographic correlation • Immunocytochemistry with CD68, and cytokeratin 7
	Cirrhosis	• Chronic liver disease, regardless of etiology	• Difficult to smear due to intact reticulum fiber network • Wide cytomorphologic spectrum from benign-appearing hepatocytes to markedly atypical ones • No significant stripped nuclei	• Clinical and radiographic correlation • Immunocytochemistry with CD68, and cytokeratin 7
	Hepatitis B	• Chronic hepatitis B infection	• Ground-glass cells • Lack of nuclear pleomorphism • No bands of fibrosis between neoplastic cells	• Clinical and radiographic correlation • Immunocytochemistry with HBcAg, CD68, and cytokeratin 7
	Oncocytic hepatocytes	• Any chronic liver diseases	• Oncocytic hepatocytes • Lack of nuclear pleomorphism • No bands of fibrosis between neoplastic cells	• Clinical and radiographic correlation • Immunocytochemistry with CD68, and cytokeratin 7

continued

Table 6.1 (continued)

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Hepatoblastoma	Normal liver	• Normal liver or • Lesion with background of normal liver	• Thin ribbons of cells • Round to oval nuclei, variably sized • Abundant cytoplasm (low N/C ratio) • Lack of nuclear pleomorphism • No significant stripped nuclei	• Clinical and radiographic correlation • Immunocytochemistry with beta-catenin, AFP
• Hepatocellular adenoma	Normal liver	• Normal liver or • Lesion with background of normal liver	• Other cellular elements (bile duct epithelial cells, sinusoidal endothelial cells, and Kupffer cells)	• Clinical and radiographic correlation • Immunocytochemistry with beta-catenin, LFABP1, SAA and CRP
	Cirrhosis	• Chronic liver disease, regardless of etiology	• Difficult to smear due to intact reticulum fiber network • Wide cytomorphologic spectrum from benign-appearing hepatocytes to markedly atypical ones	• Clinical and radiographic correlation • Immunocytochemistry with beta-catenin, LFABP1, SAA and CRP
	Steatosis	• Fatty liver disease, regardless of etiology	• Other cellular elements (bile duct epithelial cells, sinusoidal endothelial cells, and Kupffer cells)	• Clinical and radiographic correlation • Immunocytochemistry with beta-catenin, LFABP1, SAA and CRP
	Nodular regenerative hyperplasia	• Heterogeneous blood flow within the liver, regardless of etiology	• Other cellular elements (bile duct epithelial cells, sinusoidal endothelial cells, and Kupffer cells)	• Clinical and radiographic correlation • Immunocytochemistry with beta-catenin, LFABP1, SAA and CRP
• Focal nodular hyperplasia	Normal Liver	• Normal liver or • Lesion with background of normal liver	• None	• Clinical and radiographic correlation
	Cirrhosis	• Chronic liver disease, regardless of etiology	• Difficult to smear due to intact reticulum fiber network • Wide cytomorphologic spectrum from benign-appearing hepatocytes to markedly atypical ones	• Clinical and radiographic correlation
	Nodular regenerative hyperplasia	• Heterogeneous blood flow within the liver, regardless of etiology	• None	• Clinical and radiographic correlation

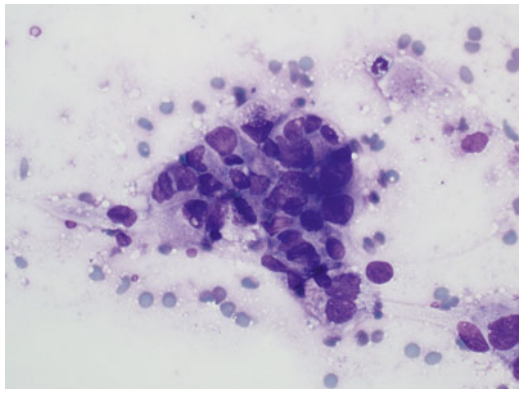


Fig. 6.2 Clusters of malignant hepatocytes with a high nucleocytoplasmic ratio and marked irregular nuclear membranes (Diff-Quik, high magnification)

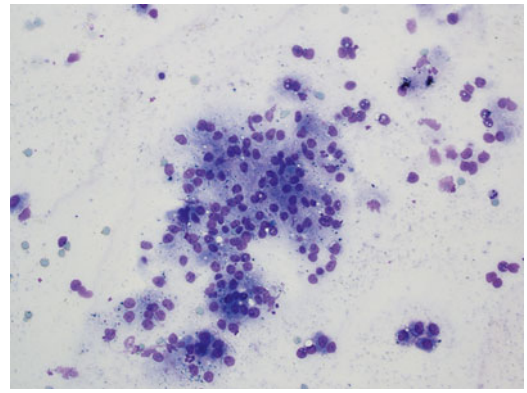


Fig. 6.5 Monotonous population of malignant cells from a hepatocellular carcinoma. The cells resemble benign hepatocytes (Diff-Quik stain, high magnification)

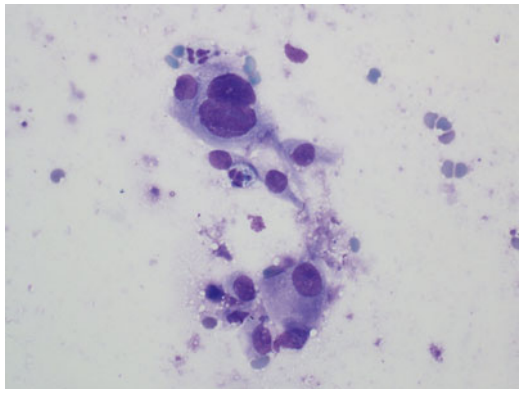


Fig. 6.3 Dispersed malignant hepatocytes with a high nucleocytoplasmic ratio and marked irregular nuclear membranes (Diff-Quik stain, high magnification)

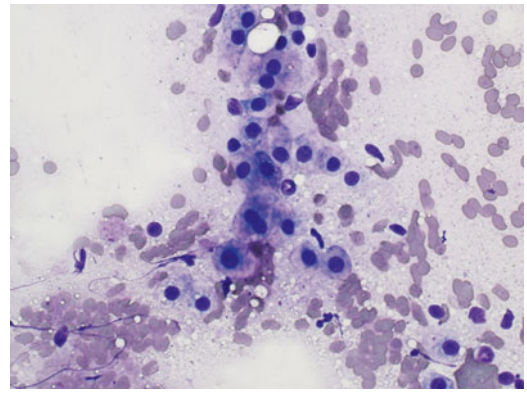


Fig. 6.6 Benign hepatocytes with a low nucleocytoplasmic ratio. Nuclear membranes are smooth (Diff-Quik stain, high magnification)

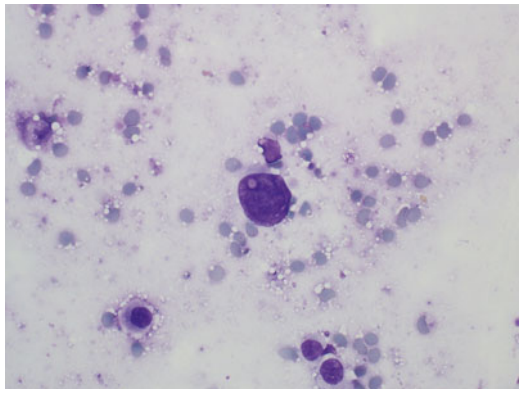


Fig. 6.4 Naked enlarged nuclei from a high grade hepatocellular carcinoma (Diff-Quik stain, high magnification)

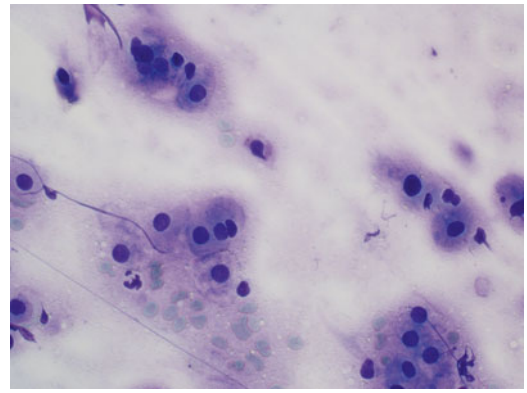


Fig. 6.7 Normal and atrophic hepatocytes from nodular regenerative hyperplasia. The atrophic hepatocytes have smaller nuclei and a decreased amount of cytoplasm (Diff-Quik stain, high magnification)

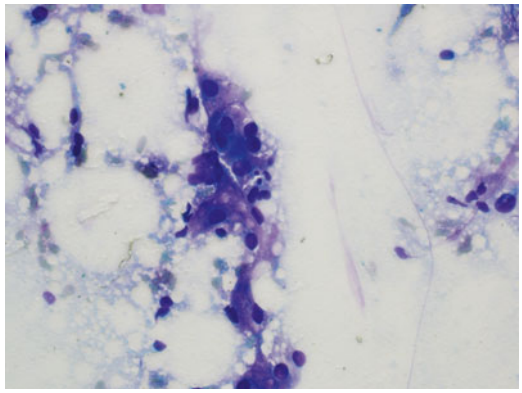


Fig. 6.8 Cytoplasmic vacuoles from a steatotic liver (Diff-Quik stain, high magnification)

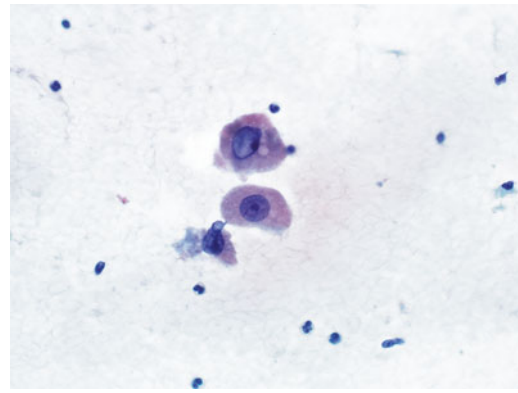


Fig. 6.9 Oncocytic malignant hepatocytes from a fibrolamellar hepatocellular carcinoma. The neoplastic cells have enlarged nuclei with prominent nucleoli and granular cytoplasm (Papanicolaou stain, high magnification)

HSP70 and glutamine synthetase staining are also seen in malignant hepatocytes.

One variant of HCC worth mentioning is *Fibrolamellar Carcinoma* (FLC). It is a rare variant with distinctive clinical, morphological, laboratory and radiological features. It occurs primarily in patients younger than 35 years of age, without association with cirrhosis. On imaging, occasionally a central scar is described. Serum alpha-fetoprotein elevation is normally not a laboratory finding like in ordinary HCC. The aspirate of FLC is remarkable for dyscohesive single neoplastic cells with a low nuclear to cytoplasmic ratio. The latter have abundant granular eosinophilic (oncocytic) cytoplasm and large nuclei with prominent nucleoli (Fig. 6.9). FLC has a characteristic immunophenotype with expression of cytokeratin 7 and CD68.

Benign hepatocytes of cirrhotic and non-cirrhotic livers have naturally granular cytoplasm and thus may mimic FLC (Fig. 6.6).

Chronic liver diseases, regardless of etiology, can have oncocytic cells again mimicking FLC. *Chronic hepatitis B* often contains ground glass hepatocytes reminiscent of neoplastic oncocytic cells of FLC (Fig. 6.10). Therefore, evaluation and interpretation of these aspirates needs to be done in conjunction with the clinical context.

Another malignant hepatocellular neoplasm also not related to background liver diseases or cirrhosis is *Hepatoblastoma*. It is seen in children younger than 5 years of age. Alpha-fetoprotein

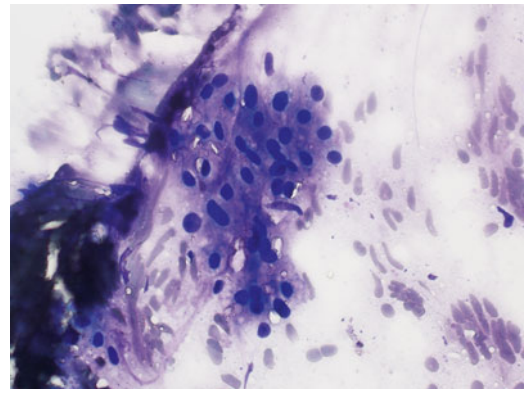


Fig. 6.10 Viral hepatitis B infected hepatocytes with ample granular cytoplasm (Diff-Quik stain, high magnification)

can be markedly elevated along with human chorionic gonadotropin. The cytological features depend on types of epithelial cells in the tumor which could be fetal, embryonic and/or anaplastic. Normal small hepatocytes are more likely to mimic the fetal type of epithelial cells. The distinguishing features are that neoplastic fetal cells of hepatoblastoma tend to be monomorphous and can form trabeculae, cords and nests of small blue cells (Fig. 6.11). Additionally, extramedullary hematopoiesis evidenced by erythropoietic cells and megakaryocytes is a characteristic feature of hepatoblastoma. Correlation with the clinical context will prevent erroneous assessment.

Two benign hepatocellular neoplasms that can be radiologically and histologically difficult to distinguish are *focal nodular hyperplasia* and *hepatocellular adenoma*. In a cytological specimen, distinguishing these tumors from normal non-neoplastic cells is virtually impossible. The presence of bile duct epithelium argues against these two entities; otherwise the cytomorphological profile is indistinguishable. Clinical and imaging findings can only at best support the diagnosis.

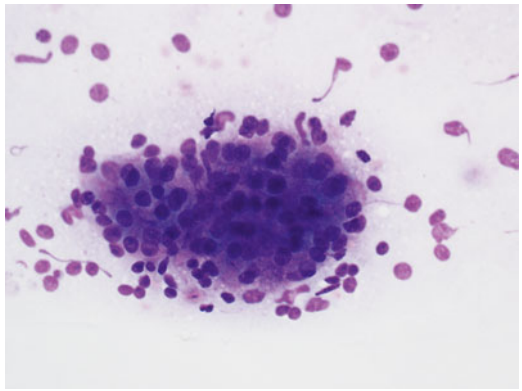


Fig. 6.11 Small blue cells with minimal cytoplasm present in a nested pattern in hepatoblastoma (Diff-Quik stain, high magnification)

Ductal-Type Lesions

The second major group of liver neoplasms is ductal-derived. *Cholangiocarcinoma* is the malignant ductal neoplasm (Table 6.2). It represents the second most common primary malignant hepatic tumor after HCC. Biliary brushing and/or fine needle aspiration are frequently used to provide cytological specimen for primary diagnosis. The latter is reached when the aspirate

Table 6.2 Ductal-type lesions

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discrimating features	Additional steps
• Cholangiocarcinoma	Normal liver	• Normal liver or • Lesion with background of normal liver	• Bile duct epithelial cells with low N/C ratio • Predominance of normal hepatocytes	• Clinical and radiographic correlation
	Cirrhosis			
	Bile duct harmartoma (Von Meyenberg Complex)	• Normal liver • Polycystic liver and kidney disease	• Hypocellular with rare bile duct epithelial cells with low N/C ratio	• Clinical and radiographic correlation
	Bile duct adenoma	• Subcapsular lesions • Incidental findings	• Hypocellular with rare bile duct epithelial cells with low N/C ratio • Inflamed stroma	• Clinical and radiographic correlation
• Mucinous cystic neoplasm	Normal liver	• Normal liver • Lesion with background of normal liver	• Bile duct epithelial cells with low N/C ratio • Predominance of normal hepatocytes • No mucin	• Clinical and radiographic correlation
	Solitary bile duct cyst	• Chronic liver disease, regardless of etiology	• Hypocellular • Cyst lining epithelial cells with low N/C ratio • No mucin	• Clinical and radiographic correlation
	Infectious parasitic cyst	• Infection withdog tapeworm *Echinococcus granulosus*	• Fragments of laminated membrane • Hooklets • Scolices • No epithelial cells • No mucin	• Clinical and radiographic correlation

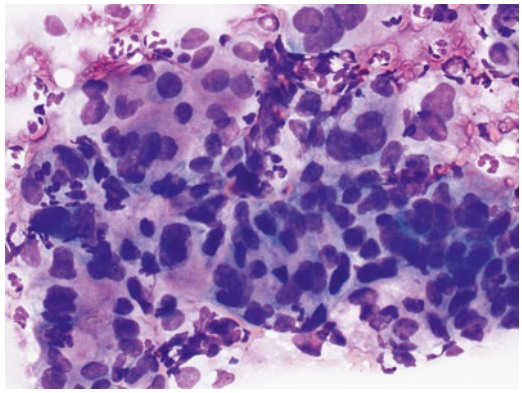


Fig. 6.12 Malignant ductal cells with enlarged irregular nuclei and cellular disorder in cholangiocarcinoma (Diff-Quik stain, high magnification)

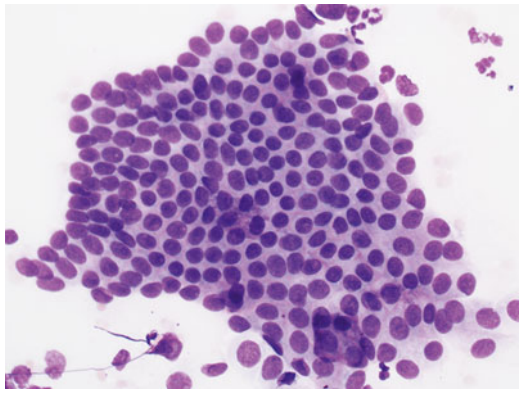


Fig. 6.13 Reactive bile duct cells arranged in an organized sheet

contains isolated cells and crowded groups or sheets of cells with glandular differentiation. Mucin production is not uncommon. The malignant cells can be mitotically active and have marked anisonucleosis and prominent nucleoli (Fig. 6.12). There is usually lack of hepatocytes.

Regardless of the etiology, *reactive bile duct epithelium* can be mistaken for cholangiocarcinoma. Besides the differentiating clinical and imaging features, reactive bile ducts can be correctly identified on cytology. They usually are present in more orderly sheets. The reactive bile duct cells have a low nuclear to cytoplasmic ratio without nuclear enlargement and irregularities (Fig. 6.13).

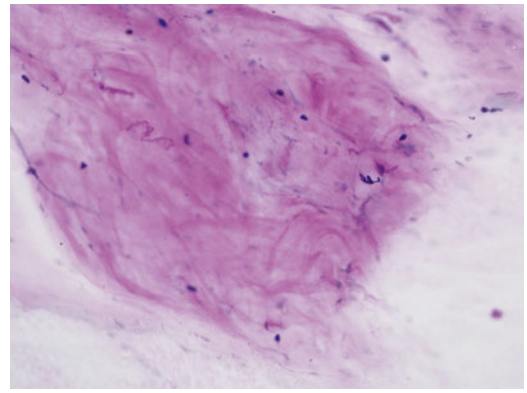


Fig. 6.14 Thick colloid-like mucin in a mucinous cystic neoplasm (Diff-Quik stain, moderate magnification)

Other nonneoplastic bile duct proliferations can cause similar diagnostic difficulties. *Bile Duct Adenoma* is often incidentally discovered and is a hepatic subcapsular lesion. When aspirated, the specimen is hypocellular with clusters of cuboidal to columnar cells. Mucinous metaplasia is not uncommon. Malignant features, such as nuclear membrane irregularities and mitotic activity, are absent. *Bile Duct Hamartomas* (von Meyenburg complex) located in or near portal tracts have similar cytomorphological findings and can be falsely interpreted as a malignant ductal neoplasm. As in the bile duct adenoma, malignant features are not present.

Hepatic *Mucinous Cystic Neoplasms* (MCN), found almost exclusively in women, are analogous to pancreatic MCN. Like in the pancreas, the aspirate often is acellular with abundant mucin and occasional columnar cells (Fig. 6.14). Rarely, spindle cells can be seen which represent sampling pericystic ovarian stroma commonly associated with MCN. Elevated CEA has been found to be helpful in MCN.

Solitary simple cyst is a nonneoplastic hepatic cystic lesion that can mimic MCN. Fortunately, the watery aspirate is quite distinct with lack of mucin. *Parasitic cysts*, such as those by a dog tapeworm *Echinococcus granulosus*, lack the mucinous content as well. In addition, scolices and hooklets can be seen.

Conclusion

Characteristic imaging findings are often sufficient to diagnosis and direct treatment in most hepatic lesions. When there is diagnostic uncertainty by imaging analysis alone, cytology evaluation is needed. Cytology, assisted by ancillary studies, is appropriate to distinguish between primary and secondary liver neoplasia. However, diagnostic challenges are not uncommon in cytology as well, due in part to the fact that non-neoplastic conditions of the liver can mimic benign or malignant neoplasms. The primary tumors of the liver are generally classified as epithelial and non-epithelial neoplasms. The epithelial type predominates. Knowledge of the characteristic clinical, imaging, laboratory and cytomorphological findings of hepatic neoplasms is critical to avoid over-diagnosis. Similarly, a firm appreciation of the common non-neoplastic conditions that exhibit cytological features resembling these neoplastic hepatic lesions is important.

Suggested Readings

1. Hill KA, Nayar R, DeFrias DV. Cytohistologic correlation of cirrhosis and hepatocellular carcinoma. Pitfall in diagnosis? Acta Cytol. 2004;48(2):127–32.
2. Clark T, Maximin S, Meier J, Pokharel S, Bhargava P. Hepatocellular carcinoma: review of epidemiology, screening, imaging diagnosis, response assessment, and treatment. Curr Probl Diagn Radiol. 2015;44(6):479–86.
3. Jain D. Tissue diagnosis of hepatocellular carcinoma. J Clin Exp Hepatol. 2014;4 Suppl 3:S67–73.
4. Crowe A, Knight CS, Jhala D, Bynon SJ, Jhala NC. Diagnosis of metastatic fibrolamellar hepatocellular carcinoma by endoscopic ultrasound-guided fine needle aspiration. Cytojournal. 2011;8:2.
5. Lagana SM, Salomao M, Bao F, Moreira RK, Lefkowitch JH, Remotti HE. Utility of an immunohistochemical panel consisting of glypican-3, heat-shock protein-70, and glutamine synthetase in the distinction of low-grade hepatocellular carcinoma from hepatocellular adenoma. Appl Immunohistochem Mol Morphol. 2013;21(2):170–6.
6. Thambi R, Devi L, Balachandran K, Poothiode U. Extramedullary hematopoiesis as a ‘clue’ to diagnosis of hepatoblastoma on fine needle aspiration cytology: a report of two cases. J Cytol. 2013;30(3):198–200.
7. Borzio M, Borzio F, Macchi R, Croce AM, Bruno S, Ferrari A, Servida E. The evaluation of fine-needle procedures for the diagnosis of focal liver lesions in cirrhosis. J Hepatol. 1994;20(1):117–21.
8. Longchampt E, Patriarche C, Fabre M. Accuracy of cytology vs. microbiopsy for the diagnosis of well-differentiated hepatocellular carcinoma and macroregenerative nodule. Definition of standardized criteria from a study of 100 cases. Acta Cytol. 2000;44(4):515–23.
9. Regev A, Reddy KR, Berho M, Sleeman D, Levi JU, Livingstone AS, Levi D, Ali U, Molina EG, Schiff ER. Large cystic lesions of the liver in adults: a 15-year experience in a tertiary center. J Am Coll Surg. 2001;193(1):36–45.
10. Yang GC, Yang GY, Tao LC. Distinguishing well-differentiated hepatocellular carcinoma from benign liver by the physical features of fine-needle aspirates. Mod Pathol. 2004;17(7):798–802.
11. Cohen MB, Haber MM, Holly EA, Ahn DK, Bottles K, Stoloff AC. Cytologic criteria to distinguish hepatocellular carcinoma from nonneoplastic liver. Am J Clin Pathol. 1991;95(2):125–30.
12. Bioulac-Sage P, Rebouissou S, Thomas C, Blanc JF, Saric J, Sa Cunha A, Rullier A, Cubel G, Couchy G, Imbeaud S, Balabaud C, Zucman-Rossi J. Hepatocellular adenoma subtype classification using molecular markers and immunohistochemistry. Hepatology. 2007;46(3):740–8.
13. Ruschenburg I, Droese M. Fine needle aspiration cytology of focal nodular hyperplasia of the liver. Acta Cytol. 1989;33(6):857–60.
14. Sola Pérez J, Pérez-Guillermo M, Bas Bernal AB, Mercader JM. Hepatoblastoma. An attempt to apply histologic classification to aspirates obtained by fine needle aspiration cytology. Acta Cytol. 1994;38(2):175–82.
15. Cibas ES, Ducatman BS. Cytology: diagnostic principles and clinical correlates, 4th ed., Ch 13; 2014.

Respiratory Tract Cytology

7

Brief Introduction

The respiratory tract is traditionally divided into upper airway (sinonasal space to larynx) and lower airway (trachea to lungs). In this chapter, the focus will be on the lower airway which accounts for the majority of specimens from the respiratory tract that cytopathologists are expected to review. These specimens have increased in number, partly due to the tremendous advances in thoracic imaging which have allowed radiologists to detect an ever-increasing number of thoracic lesions that need cytological evaluation. Often the differential diagnosis includes non-neoplastic conditions (e.g., infections, iatrogenic) vs truly neoplastic entities. The clinical and radiologic findings are however rarely conclusive for the treating physicians. Thus, histological and/or cytological diagnosis of these lesions are required for selecting the appropriate therapeutic management.

Fortunately, many of these respiratory tract lesions can be directly sampled via bronchoscopy which allows collection of bronchial secretions via aspiration, washing or bronchoalveolar lavage. Alternatively, sputum (spontaneous or induced) in symptomatic patients with adequate numbers of pulmonary macrophages can be used for cytological evaluation of any bronchial secretions. If an endobronchial lesion is visualized with bronchoscopy, it is brushed in order to exfoliate diagnostic cells. Fine needle aspiration is also an important sampling method for respiratory tract cytology. The aspiration can be transbronchial, transesophageal, endobronchial with ultrasound-guidance, or percutaneous.

Each of the aforementioned sampling techniques carries varying degrees of advantages and disadvantages in terms of the quality of the cytological specimens. However, the biggest challenge for the cytopathologist is the numerous reactive conditions that can elicit cytologic changes that remarkably mimic primary neoplastic states. In this chapter, these reactive conditions will be reviewed along with recommendations for avoiding common pitfalls in evaluation of respiratory tract cytological specimen.

Classification of Respiratory Tract Neoplasms Based on Cell of Origin

The lung is home to a number of primary neoplasms and to a vast list of non-neoplastic entities (Fig. 7.1). The commonly seen neoplasms include neuroendocrine tumors (typical carcinoid, atypical carcinoid, small cell neuroendocrine carcinoma, large cell neuroendocrine carcinoma); non-neuroendocrine epithelial tumors (adenocarcinoma, squamous cell carcinoma, adenosquamous cell carcinoma, large cell carcinoma, carcinosarcoma); and mesenchymal tumors (pulmonary hamartoma, inflammatory

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_7

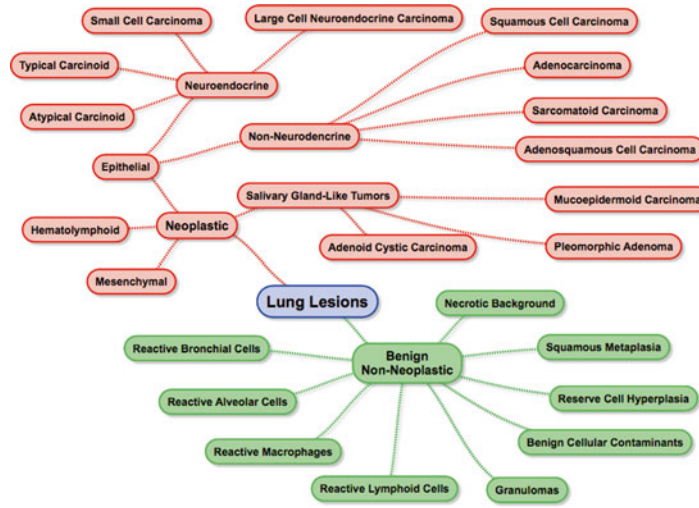


Fig. 7.1 Lower respiratory tract neoplasms and their primary mimickers

myofibroblastic tumors, solitary fibrous tumor). The less commonly primary respiratory tract neoplasms include salivary gland-like tumors (adenoid cystic carcinoma, mucoepidermoid carcinoma); and hematolymphoid tumors. Regardless of the type of tumor, cytology is often one of the main methods of diagnostic evaluation for these lesions. Clinical and imaging impression aid tremendously in the differential diagnosis. Nonetheless, reactive cellular changes are notorious for resembling neoplastic processes. In this chapter, these mimickers will be discussed along with clues for avoiding false-positive diagnosis.

Confounders Based on Cytologically Sampled Materials

Neuroendocrine Epithelial Tumors

This category of lung neoplasms includes typical carcinoid, atypical carcinoid, small cell carcinoma and large cell neuroendocrine carcinoma (Table 7.1). The *Typical Carcinoid* tumors (Grade I Neuroendocrine Carcinoma) tend to be centrally located and associated with cartilaginous airways in the overwhelming majority of the cases. When present, they can lead to localized obstruction and cause obstructive pneumonia or atelectasis. On imaging studies, a central mass adjacent to a large airway is often detected. On cytology, carcinoid tumors are architecturally remarkable for arrangements in trabeculae, cords, nests and rosette-like formations. Capillary structures can sometimes be observed around neoplastic nests (Fig. 7.2). The cells are monotonous and often single or in loose clusters (Fig. 7.3). They are round to plasmacytoid with scant to moderate cytoplasm; rarely they can be spindly (Fig. 7.4). The chromatin is finely speckled with inconspicuous nucleoli, nuclear molding; brisk mitotic activity and necrosis are not features of typical carcinoids. In addition to the features described, *Atypical Carcinoid* tumors (Grade II Neuroendocrine Carcinoma) show increased mitotic activity (Fig. 7.4). In cytologic preparations, it is difficult to accurately distinguish typical from atypical carcinoids.

High grade neuroendocrine tumors such as *Small Cell Carcinoma* (Grade III Neuroendocrine Carcinoma) differ cytologically from carcinoids by brisk mitotic activity and prominent background necrosis (Fig. 7.5). This tumor is usually centrally located as well. Furthermore, small cell carcinoma aspirates tend to be highly cellular. Neoplastic cells show marked nuclear molding (Fig. 7.6). They have minimal and delicate cytoplasm, but with variable size, but usually small. The nuclei are not unlike the carcinoid tumors, as they can be round, oval or spindly. The chromatin is dark. Often in small cell carcinoma, paranuclear

Table 7.1 Neuroendocrine epithelial tumors

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Carcinoid • Atypical carcinoid • Small cell carcinoma • Large cell neuroendocrine carcinoma • Granular cell tumor	Reserve cell hyperplasia	• Any irritations of to the respiratory mucosa	• Reserve cells are associated with ciliated columnar cells • Reserve cells are small • Lack of mitotic activity and necrosis	• Clinical correlation
	Lymphocytes	• Reactive lymph nodes	• Cells have minimal cytoplasm • Not clusters or rosettes formation • Brisk mitotic activity and necrotic background are absent	• Clinical and endoscopic correlation • Immunocytochemistry • Lymphocytes positive for CD45, but negative for neuroendocrine markers (synaptophysin, chromogranin, CD56, NSE) and epithelial markers (cytokeratin AE1/3, EMA, TTF-1)
	Granulomas	• Infection (e.g., mycobacteria, fungi) • Sarcoidosis	• Epithelioid histiocytes • Giant cells • Inflamed stroma scant	• Clinical correlation • Microbiology • Special stains for organisms • Immunocytochemistry: histiocytes positive for CD68
	Necrotic background	• Infection • Radiation	• Overt malignant cells are absent • Infectious organisms may be identified	• Clinical correlation • Microbiology • Special stains for micro-organisms (e.g., PAS, GMS, AFB)

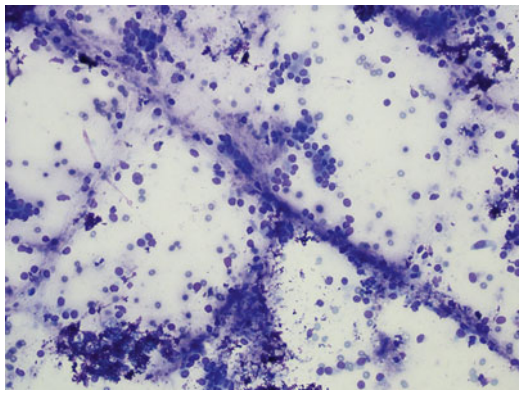


Fig. 7.2 Typical carcinoid. A capillary is seen traversing neoplastic monotonous cells in loose clusters. The nuclei are round with smooth membranes. No mitoses or necrosis is observed (Smear, Diff-quick)

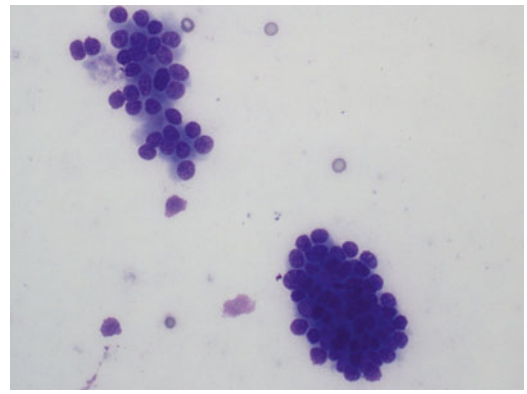


Fig. 7.3 Typical carcinoid. The neoplastic cells can form small three-dimensional nests (Smear, Diff-quick)

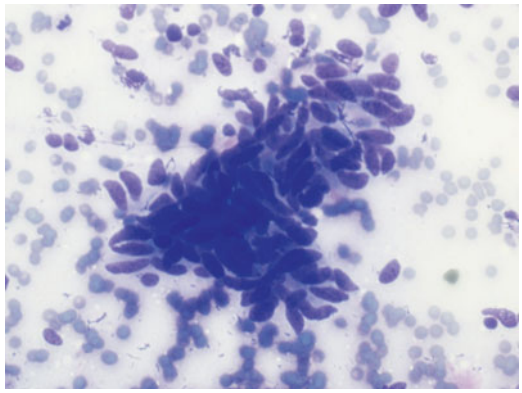


Fig. 7.4 Typical carcinoid. The neoplastic cells can be spindly with minimal cytoplasm (Smear, Diff-quick)

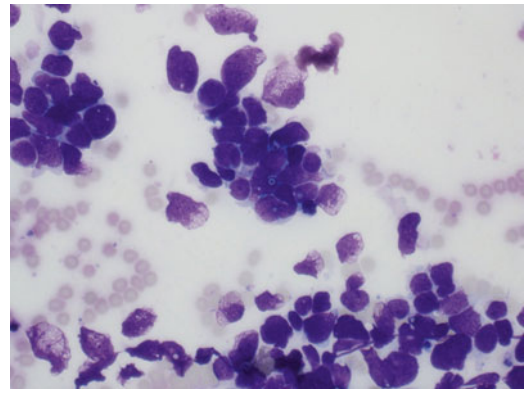


Fig. 7.7 Small cell carcinoma. Paranuclear cytoplasmic inclusions (blue bodies) often are detected (Smear, Diff-quick)

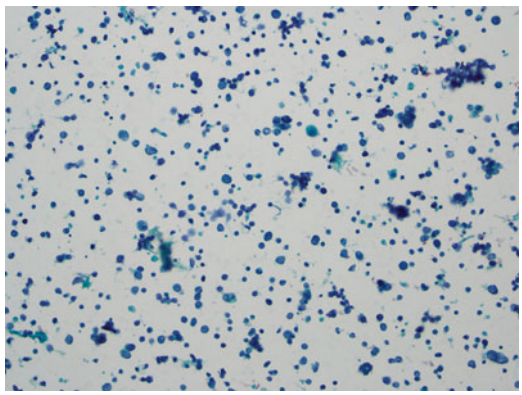


Fig. 7.5 Small cell carcinoma. A necrotic background is seen in this high grade neuroendocrine tumor. Small molded nests are present (liquid-based, Papanicolaou)

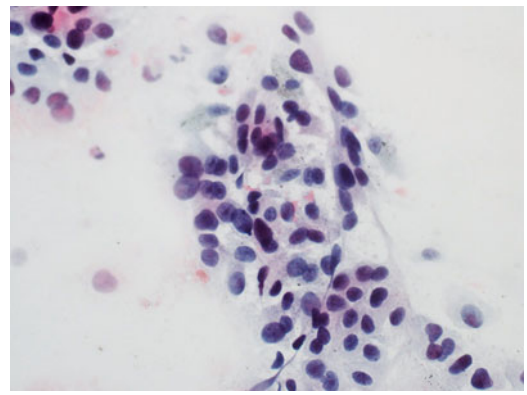


Fig. 7.8 Large cell neuroendocrine carcinoma. Conspicuous nucleoli in this high grade neuroendocrine tumor (Smear, Diff-quick)

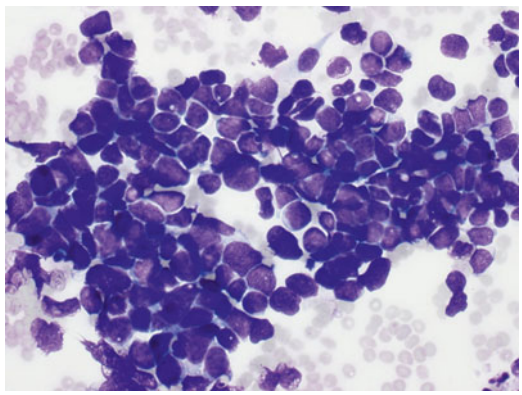


Fig. 7.6 Small cell carcinoma. Prominent nuclear molding characteristic of this tumor. The cells have scant cytoplasm with dark nuclei (Smear, Diff-quick)

cytoplasmic inclusions, also called blue bodies, are seen (Fig. 7.7). Necrotic background is an important diagnostic feature. *Large Cell Neuroendocrine Carcinoma* (also Grade III Neuroendocrine Carcinoma) shares many of cytologic characteristics of small cell carcinoma, but its cells tend to have more cytoplasm and prominent nucleoli (Fig. 7.8).

One of the main mimickers of neuroendocrine tumors in cytology is *Lymphoid Cells*. These has many cytological resemblances to neuroendocrine tumor cells. Lymphocytes have minimal cytoplasm and dark nuclei. When crushed, lymphocytes mimic the classic molding that is described in high grade neuroendocrine carcinoma, specifically small cell carcinoma (Fig. 7.9).

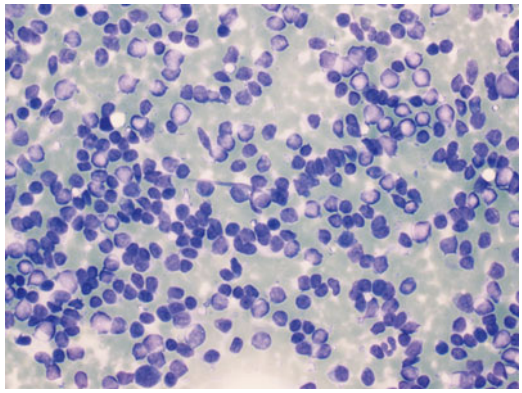


Fig. 7.9 Reactive lymphocytes. The cells are in a molding pattern similar to small cell carcinoma. However, mitoses, necrosis and blue bodies are absent (Smear, Diff-quick)

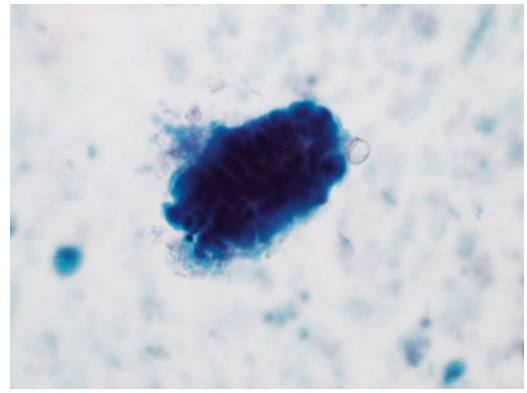


Fig. 7.10 Reserve cell hyperplasia. Cohesive cells with dense hyperchromatic nuclei, associated with benign ciliated columnar cells (Liquid-based, Papanicolaou)

Lymph node aspirates can be very cellular. However, lymphocyte cytoplasm volume is much less. While lymphocytes can be single and in loose aggregates, they do not form clusters or rosettes. Additionally, mitotic activity and necrotic background are absent in reactive lymph nodes. If there are any doubts, performing immunocytochemistry of the aspirate cell block can be very helpful. The lymphocytes will be positive for CD45, but negative for neuroendocrine markers (synaptophysin, chromogranin, CD56, NSE) and epithelial markers (cytokeratin AE1/3, EMA, TTF-1). Because neuroendocrine cells can have variable expression of these markers, it is important to use an IHC panel to exclude them.

Reserve Cell Hyperplasia is a condition seen when the respiratory mucosa is irritated by any chemicals. It consists of a proliferation of cohesive cells that are much smaller than the ciliated and goblet cells. Their minimal cytoplasm, dense hyperchromatic nuclei and cohesion make them look like small cell carcinoma (Fig. 7.10). One way to avoid overcalling these cells is to recognize the lack of mitotic activity and necrotic background. In addition, they can be associated with ciliated columnar cells or benign metaplastic squamous cells (Fig. 7.10).

Inflammatory Necrotic Background is a frequent cytological finding with high grade neuroendocrine carcinoma. This is characterized by abundant neutrophils and acellular debris. However, its mere presence is not pathognomonic

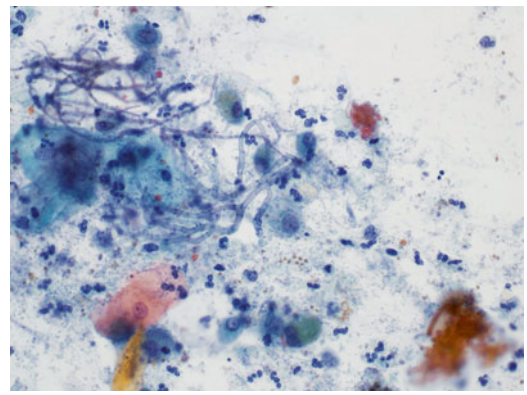


Fig. 7.11 Aspergillosis. Acute inflammatory infiltrate along with septated hyphae with 45° angle branching (Liquid-based, Papanicolaou)

for malignancy. Infectious processes should always be considered, especially if other overtly malignant features are absent (Fig. 7.11). Aspiration of foreign materials can lead to lung parenchymal abscess formation as well. Special stains (AFB, PAS, GMS) for microorganisms might elucidate the diagnosis. If additional specimen is available, it should be triaged for microbiologic studies, especially in rapid on-site evaluation (ROSE).

Granulomatous Inflammation often raises the possibility of an infectious or autoimmune condition. However, the spindle cells from the granulomas may also suggest spindle cells of neuroendocrine tumors (Fig. 7.12). Immunocytochemistry, in conjunction with clinical and

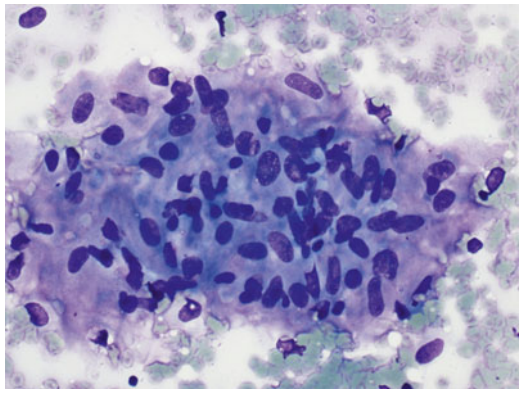


Fig. 7.12 Granuloma. Aggregate of epithelioid curvy histiocytes (Smear, Diff-Quick

imaging impression, is important in these situations. The spindle histiocytes of the granulomas will be positive for CD68 but negative for neuroendocrine and epithelial markers.

Non-neuroendocrine Epithelial Tumors

Adenocarcinoma is the most common form of lung cancer (Table 7.2). It tends to be more peripheral without association with tubular airways. Invasive adenocarcinomas of the lung have more than one histological presentation. They are classified according to the predominant pattern: lepidic, acinar, papillary, micropapillary, and solid. On cytological preparations, the malignant cells can be single or in sheets. Occasionally, acinar structures, or papillae can be detected (Fig. 7.13). The malignant cells have decreased cytoplasm, leading to an elevated nucleocytoplasmic ratio. The nuclei are often lateralized to one side, with cytoplasm in the more luminal aspect. Intracytoplasmic mucin can also be visualized (Fig. 7.14). Depending on the tumor differentiation, the nuclei can be enlarged with irregular nuclear membranes. Prominent nucleoli are not infrequent (Fig. 7.15). Nonmucinous lepidic predominant adenocarcinoma (previously bronchioloalveolar carcinoma) may show bland discohesive, hyperchromatic, and plasmacytoid cells. The nucleocytoplasmic ratio can be high to moderate (Fig. 7.16).

Unfortunately, many of the just described cytomorphological changes can appear in benign lung parenchyma with any injuries and inflammation occurring in the lung. For example following exposure to any injurious agents, *Ciliated Columnar Cells* can exhibit dramatic reactive changes mimicking adenocarcinoma. These columnar cells are frequently encountered in any lung cytological specimens. They have thick terminal bars with cilia on the luminal surface. Their nuclei are basally located with finely textured chromatin and smooth nuclear membranes. When irritated, the reactive changes include enlarged nuclei, multinucleation, nuclear membrane irregularities, prominent nucleoli and coarse chromatin (Fig. 7.17). The key to distinguishing them from malignant cells is their cilia if they are preserved. However, these reactive columnar cells can lose their cilia making the distinction impossible (Fig. 7.18). In those cases, it is best to compare the nuclear changes to those of columnar cells with cilia. If the changes are similar, a reactive process should be favored. Additionally, reactive bronchial cells, when in clusters, tend be in a two-dimensional configuration compared to malignant clusters that tend to form three-dimensional architectures (Fig. 7.19).

In chronic airway diseases such as asthma, *Creola Bodies,* which are spherical to oval clusters of reactive ciliated columnar cells, can mimic adenocarcinoma (Fig. 7.20). Their two-dimensional configuration and preserved cilia at their luminal edge betray their benign nature.

Alveolar Cells, especially type II pneumocytes, can also undergo florid hyperplasia in response to any noxious stimuli. These cells can eerily resemble an adenocarcinoma. They have enlarged nuclei, coarse chromatin with conspicuous nucleoli, and often are in a three-dimensional configuration (Fig. 7.21). Any potential history of acute lung injury should preclude a definitive malignant diagnosis.

Alveolar Macrophages, like other macrophages, are cytologically recognizable by their vacuolated cytoplasm. The latter may contain phagocytosed debris. Their nuclei are round to oval without nuclear membranes irregularities. In lipid pneumonia, the reactive histiocytes can

Table 7.2 Non-neuroendocrine epithelial tumors

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Adenocarcinoma • Squamous cell carcinoma • Adenosquamous cell carcinoma • Large cell carcinoma • Sarcomatoid carcinoma	Reactive ciliated columnar cells	• Inadvertently collected during sampling of lower respiratory tract • Any lung diseases (e.g., infection, trauma, radiation, inflammation)	• Smooth nuclear membranes • Cilia may be preserved • Two-dimensional configuration • No overt malignant cytological features (e.g., necrosis, pleomorphism, atypical mitoses)	• Clinical correlation
	Reactive alveolar cells	• Inadvertently collected during sampling of lower respiratory tract • Any lung diseases (e.g., infection, trauma, radiation, inflammation) • Acutely ill patients	• Atypical cells are rare	• Clinical correlation
	Reactive Alveolar Macrophages	• Lipid pneumonia	• Low nucleocytoplasmic ratio • Vacuolated cytoplasm • No overt malignant cytological features (e.g., necrosis, pleomorphism, atypical mitoses)	• Clinical correlation • Immunocytochemistry; histiocytes are positive for CD68
	Squamous metaplasia	• Chronic irritation of the bronchial mucosa (e.g., chemicals, smoke)	• No overt malignant cytological features (e.g., necrosis, pleomorphism, atypical mitoses) • Only benign-appearing keratinized cells	• Clinical correlation
	Benign cellular contaminants	• Surrounding benign cells • Inadvertently collected during sampling of the lung	• These cells should lack over malignant features • They should be sparse and not abundant	• Clinical correlation • Immunocytochemistry (e.g., mesothelial markers if mesothelial cells are suspected)
	Necrotic background	• Radiation therapy • Infection	• No malignant cells in the inflammatory background • Microorganisms may be identified	• Clinical correlation

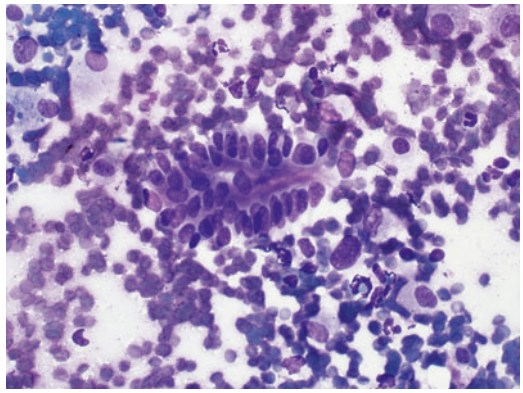


Fig. 7.13 Adenocarcinoma. Fragment of malignant cells in papillary configuration (Smear, Diff-Quick)

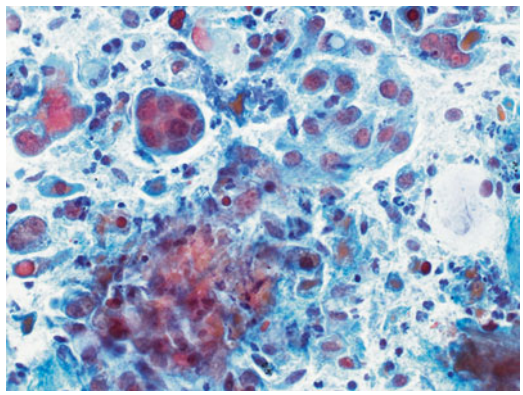


Fig. 7.14 Adenocarcinoma. Malignant glandular cells in three-dimensional configuration in a necrotic background. Vacuolated cytoplasm is present (Smear, Papanicolaou)

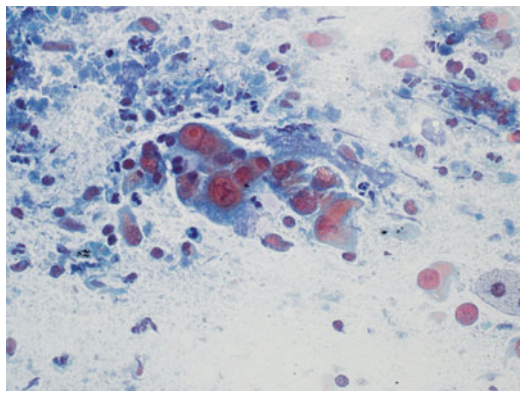


Fig. 7.15 Adenocarcinoma. Prominent nucleoli seen in the malignant glandular cells (Smear, Liquid-based, Papanicolaou)

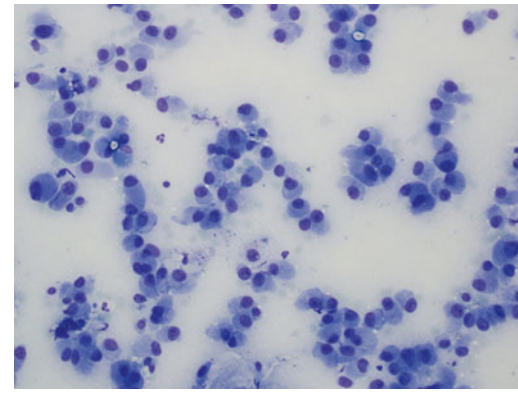


Fig. 7.16 Adenocarcinoma. Bland discohesive, hyperchromatic, and plasmacytoid cells with cellular monotony and a relatively high nucleocytoplasmic ratio (Smear, Papanicolaou)

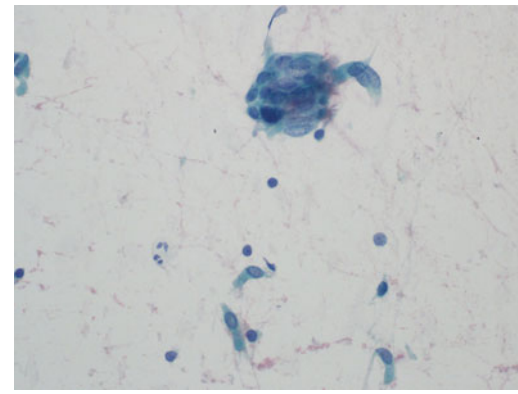


Fig. 7.17 Reactive columnar cells. Small cluster of columnar cells with retained cilia. The nuclei are moderately enlarged (liquid-based, Papanicolaou)

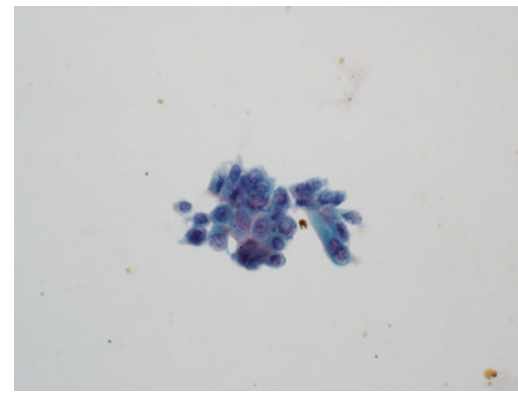


Fig. 7.18 Reactive columnar cells. Reactive columnar cells can be multinucleated (liquid-based, Papanicolaou)

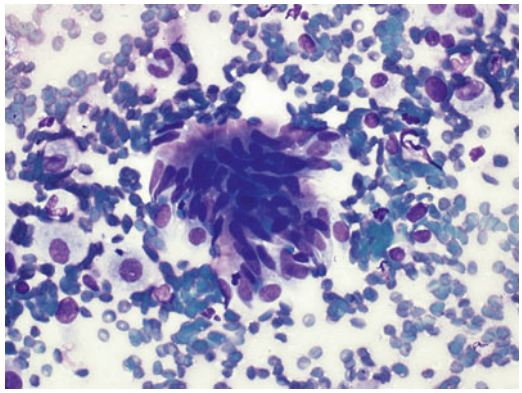


Fig. 7.19 Reactive columnar cells. Occasionally the reactive columnar cells can lose their cilia; however, their benign reactive nature is betrayed when they are compared to the nuclear changes in the columnar cells with cilia (Smear, Diff-Quick)

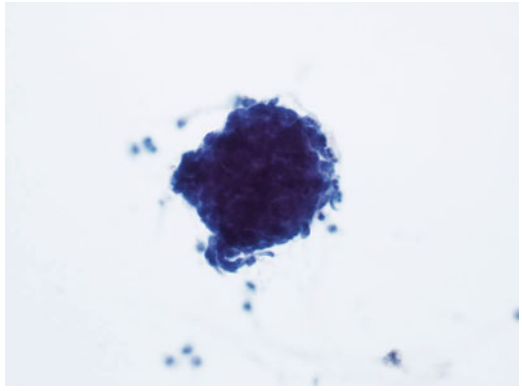


Fig. 7.20 Creola body. Spherical to oval cluster of reactive ciliated columnar cells in a two-dimensional pattern (Liquid-based, Papanicolaou)

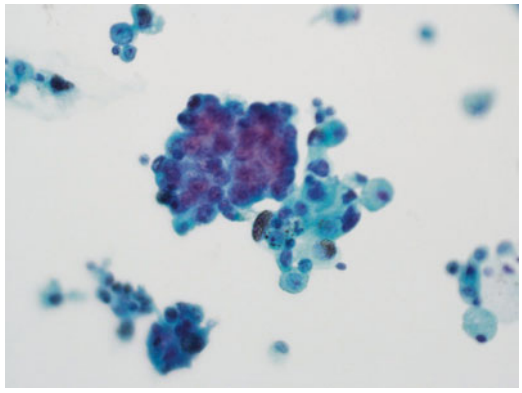


Fig. 7.21 Reactive alveolar cells. Florid alveolar cell hyperplasia in response to noxious stimuli. Note the enlarged nuclei with conspicuous nucleoli in a three-dimensional configuration (Liquid-based, Papanicolaou)

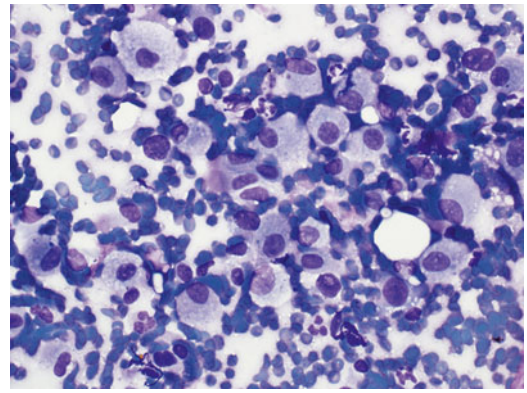


Fig. 7.22 Reactive macrophages. In lipid pneumonia, these histiocytes can be abundant. The nucleocytoplasmic ratio is low. The cytoplasm is finely vacuolated (Smear, Diff-Quick)

mimic malignant glandular cells (Fig. 7.22). Care should be taken to not interpret them as signet ring carcinoma. If there are doubts, immunocytochemical staining with CD68 will reveal their identity. Mucin staining should also be negative.

At the offset, in acutely or chronically ill patients, knowledge of any recent or concurrent injuries (e.g., pneumonia, respiratory distress, pulmonary infiltrates, radiation) to the lung should prompt caution during cytologic evaluation. If malignant cells are indeed present, two distinct cell populations, one malignant and one benign, should be identified.

Depending on the path of cytological specimen acquisition, *Mesothelial Cells* are not uncommon in percutaneous fine needle aspiration. Just like in fluid cytology, reactive mesothelial cells can mimic adenocarcinoma. The discriminating cytological features include knobby contours in cell clusters. The spaces between the cells "windows" would also be consistent with mesothelial cells (Fig. 7.23). Of course, as discussed in the fluid cytology chapter, the reactive changes include enlarged nuclei, multinucleation, cytoplasmic vacuolization, and mitotic figures (Fig. 7.24). If suspicion of heavy mesothelial cell contamination is present, obtaining a cell block followed by pertinent immunocytochemical staining will save the day. Mesothelial cells are immunoreactive for calretinin, WT1, D2-40, CK5/6, but negative for epithelial markers such as EMA, Claudin, and MOC-31.

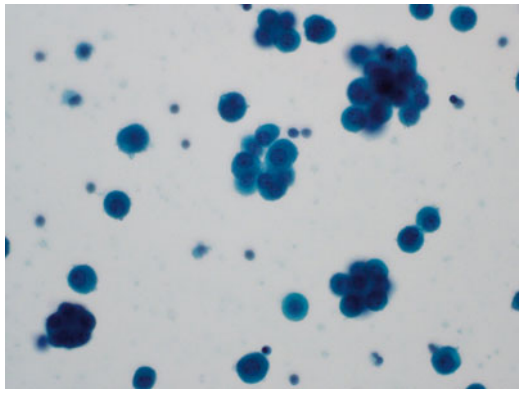


Fig. 7.23 Mesothelial cells. Inadvertently sampled mesothelial cells with "windows" between the cells. The nuclei have smooth membranes with prominent nucleoli (Liquid-based, Papanicolaou)

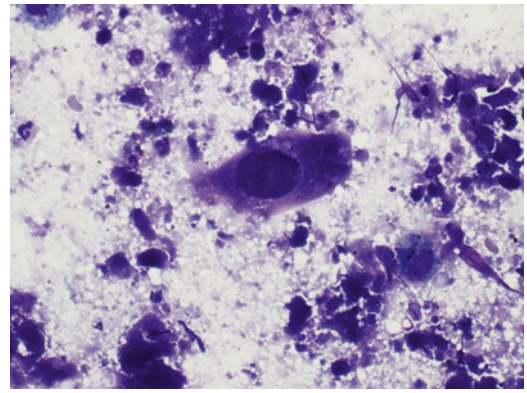


Fig. 7.25 Squamous cell carcinoma. A malignant and markedly enlarged squamous cell in a necrotic background (Smear, Diff-Quick)

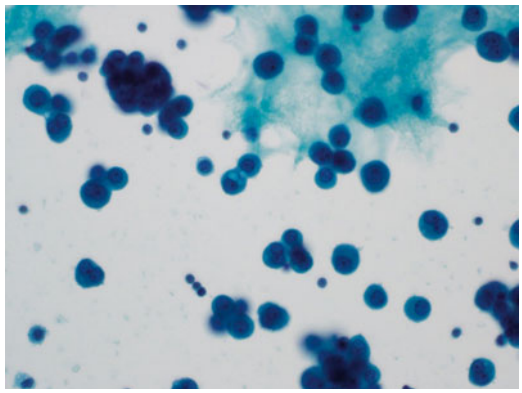


Fig. 7.24 Mesothelial cells. Reactive mesothelial cells may contain vacuolated cytoplasm (Liquid-based, Papanicolaou)

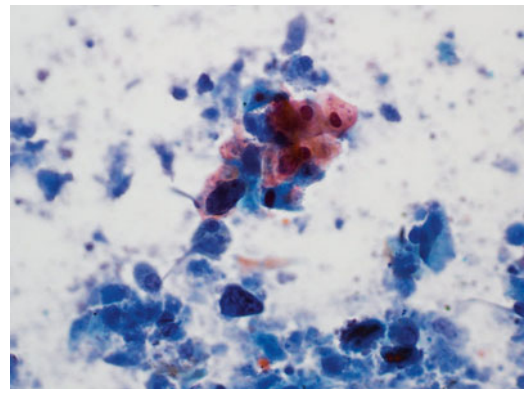


Fig. 7.26 Squamous cell carcinoma. Malignant squamous cells with orangophilic cytoplasm consistent with keratinization (Smear, Papanicolaou)

Squamous Cell Carcinoma is another important primary non-neuroendocrine epithelial pulmonary malignancy. It is strongly associated with cigarette smoking. It can occur anywhere in the lung parenchyma, but more frequently arises in a central location. When sufficiently large, cavitation of the tumor is common and can be appreciated by radiology studies. Several histologic variants are recognized, so the cytomorphologic features depend on finding squamous differentiation. Nonetheless, malignant squamous cells can occur singly or in dense sheets/clusters (Fig. 7.25). Their shape is reminiscent of normal benign squamous cells: polygonal, oval or spindly. Malignant squamous cells show varying degrees of pleomorphism and keratinization (Fig. 7.26). The latter is best seen with Papanicolaou stain, which shows as yellow-orange cytoplasmic staining. Nuclei are usually hyperchromatic with inconspicuous nucleoli or can be coarse with prominent nucleoli (Fig. 7.27). The nucleocytoplasmic ratio is variable but can be high. A necrotic and granular background can be seen. It is not uncommon for granulomas to be present, responding to spilled keratin. The neoplastic cells are immunoreactive to CK5, p63 and p40, consistent with squamous differentiation.

The upper respiratory tract is lined by non-keratinized squamous epithelium. However, with chronic irritation to the bronchial mucosa, the

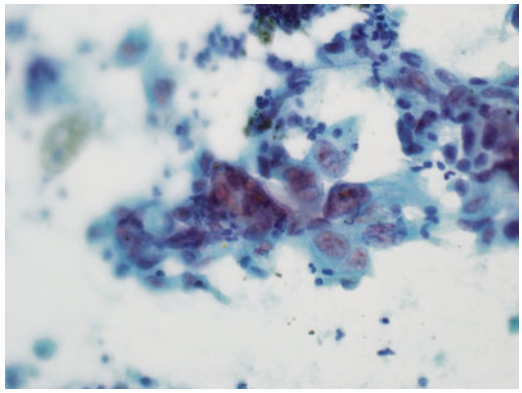


Fig. 7.27 Squamous cell carcinoma. Pleomorphic nuclei with prominent nucleoli (Smear, Papanicolaou)

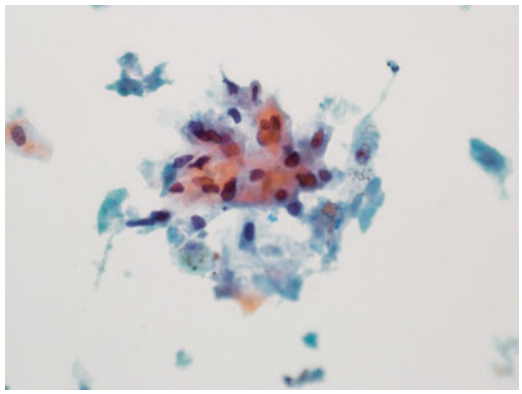


Fig. 7.28 Squamous metaplasia. Squamous cells with abundant orangophilic cytoplasm but without pleomorphism or nuclear enlargement (Smear, Papanicolaou)

lower respiratory tract undergoes *Squamous Metaplasia*. Therefore, in cytological specimens, the mere presence of squamous cells with orangophilic cytoplasm does not necessarily indicate squamous cell carcinoma. The absence of the above cytological changes associated with malignancy is a clue that the process may be reactive (Fig. 7.28). Besides chronic mucosal irritation, other conditions that can elicit squamous metaplasia include underlying infections, infarction, radiation, chemotherapy, sepsis, and diffuse alveolar damage. Therefore, obtaining the accurate clinical presentation is imperative to avoid overdiagnosis.

Granulomatous Inflammation, regardless of etiology, as previously discussed can lead to the presence of spindle cells from the granulomas. These cells can share the morphology of malignant squamous cells, especially if the nuclei are plump. Evidence of keratinization is absent. The spindle histiocytes of the granulomas will be positive for CD68 but negative for squamous epithelial markers.

Adenosquamous Cell Carcinoma is not as common as the other primary epithelial lung neoplasms. It is characterized by a combined malignant glandular and squamous component. It can occur anywhere in the lung parenchyma. The cytologic findings depend on which malignant component predominates and which aspect is sampled. As you might expect, the mimickers include those aforementioned that have the ability to resemble both adenocarcinomas and squamous cell carcinomas.

Sarcomatoid Carcinomas are uncommon primary epithelial neoplasms with sarcomatoid histomorphology. They are classified as carcinomas since they express epithelial markers. However, these tumors lack morphologic differentiation of either glandular or squamous origin. Five subtypes are recognized, pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma, and pulmonary blastoma (Fig. 7.29). The cytomorpholgical findings vary with the subtype. In pleomorphic carcinoma, the malignant cells can comprise giant and pleomorphic spindle cells. In spindle cell carcinoma, the malignant cells consist of a pure population of

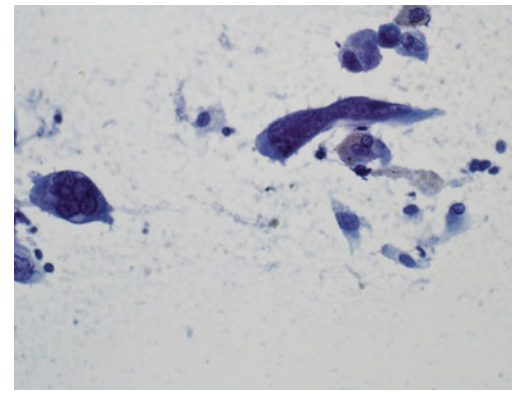


Fig. 7.29 Sarcomatoid carcinoma. Highly pleomorphic and multinucleated malignant cells with irregular nuclear membranes and spindled to polygonal shapes (Smear, Diff-Quick)

highly dysplastic single spindle cells. In giant cell carcinoma, the malignant cells consist of a pure population of predominantly single pleomorphic and bizarre-appearing giant cells. In carcinosarcoma, the tumor is biphasic with both malignant epithelial and mesenchymal cells expressing their respective markers. In pulmonary blastoma, the tumor is also biphasic with primitive epithelial and mesenchymal neoplastic cells. An extensive cytomorphologic description of these entities is beyond the scope of this book. However, the cytologist should avoid entertaining a diagnosis of sarcomatoid carcinoma when reactive-appearing spindle and/or giant cells are observed.

Any granulomatous inflammatory process could yield spindle and giant cells. Fortunately, these tumors are rare, but if they are being considered clinically and cytologically, proceed with immunocytochemistry to confirm the suspicious cells are not reactive histiocytes.

Large Cell Carcinoma is an undifferentiated carcinoma without morphological features of glandular, squamous or neuroendocrine differentiation. They are mostly found in the periphery of the lung. The malignant cells are large with a high nucleocytoplasmic ratio; the nuclei can have coarse chromatin, prominent nuclei, and irregular nuclear membranes. The cytoplasm can be vacuolated (Fig. 7.30).

The mimickers of this malignant neoplasm are the same that resemble adenocarcinoma, poorly differentiated squamous cell carcinoma and large cell neuroendocrine carcinoma. These include reactive bronchial, alveolar and squamous cells that arise in the setting of acute and chronic lung injury. Integrating the clinical picture with the cytologic impression can prevent false positive diagnoses.

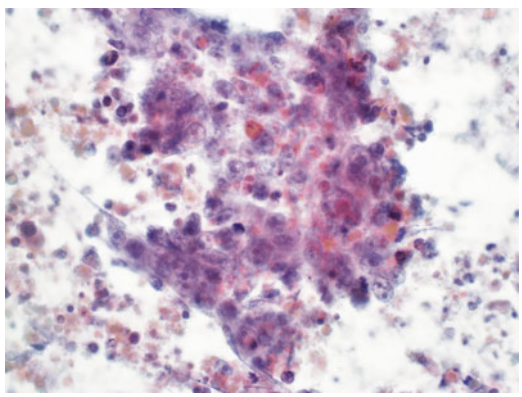


Fig. 7.30 Large cell carcinoma. Prominent nucleoli present in these cells with a relatively high nucleocytoplasmic ratio (Smear, Papanicolaou)

Mesenchymal Tumors

Primary *Mesenchymal Tumors* of the lung are exceeding rare. They are similarly classified as those that arise outside of the lungs. Refer to the chapter on soft tissue tumors for common and uncommon pitfalls.

Salivary Gland-Like Tumors

Similar to salivary glands, submucosal bronchial glands can give rise to a number of salivary gland tumors. They are histomorphologically similar to those from the head and neck, such as adenoid cystic carcinoma, mucoepidermoid carcinoma, acinic cell carcinoma, epithelial-mesenchymal tumors and pleomorphic adenoma. The cytologic characteristics of these tumors are discussed in Chap. 3.

These tumors are so uncommon that all other more common entities should be excluded first. These include not only other primary lung neoplasms already discussed, but other mimickers as well.

These include bronchial cartilage (Fig. 7.31) which may falsely resemble the myxoid extracellular matrix of pleomorphic adenoma (Fig. 7.32). Submucosal bronchial glands, (Fig. 7.33), if sampled, may give the appearance of adenoid cystic carcinoma (Fig. 7.34). However, the extracellular matrix will be absent, and the submcosal glands, will be diminutive in number. Degenerated metaplastic squamous cells can mimic the squamous component of mucoepidermoid carcinoma (Fig. 7.28). If a salivary gland-like tumor is being considered, it is still advisable to provide a descriptive diagnosis followed by a differential diagnosis.

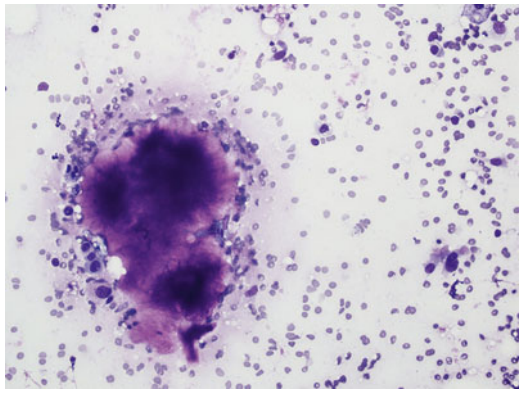


Fig. 7.31 Bronchial cartilage. Metachromatic cartilaginous material (Smear, Diff-Quick)

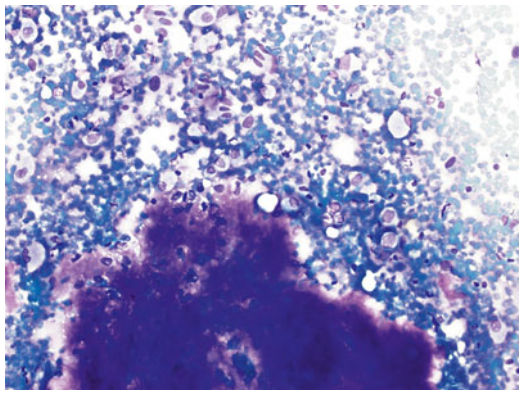


Fig. 7.32 Pleomorphic adenoma. Metachromatic extracellular matrix associated with myoepithelial cells from this salivary gland-like tumor of the lung (Smear, Diff-Quick)

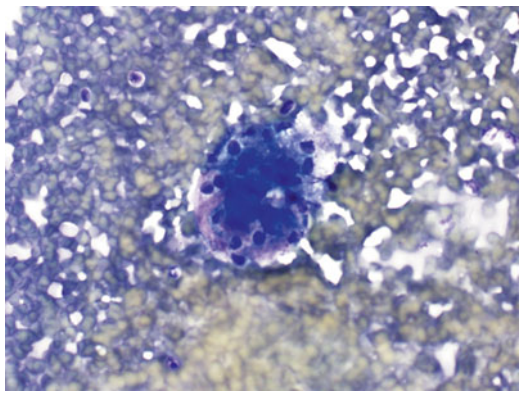


Fig. 7.33 Bronchial submucosal glands. Small nest of benign submucosal glands. The nuclei are not enlarged, and the cells have abundant cytoplasm (Smear, Diff-Quick)

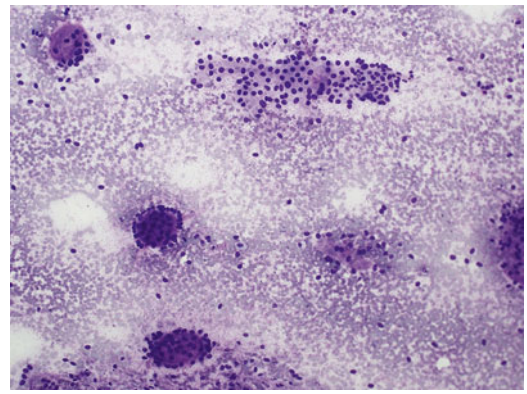


Fig. 7.34 Adenoid cystic carcinoma. Basaloid cells surround spheres of extracellular matrix with sharp edges from this salivary gland-like tumor of the lung (Smear, Diff-Quick)

Hematolymphoid Tumors

Lymphomas are not among the most common primary lung neoplasms but they do occur. When cytologic specimens are submitted with a clinical suspicion for hematolympoid malignancy, caution is warranted because there are certain conditions that are capable morphologically to present as lymphomas. Chief among them is *Lymphocytes* from inflammation regardless of the etiology. A pure population of lymphocytes on cytology should prompt a recommendation to the clinicians to submit additional materials for flow cytometry.

Histiocytes from granulomatous inflammatory processes can mimic Reed-Sternberg cells (Fig. 7.35). Histiocytes are however much smaller and can be identified with immunohistochemical studies for CD68.

Conclusion

The lower respiratory tract, from the trachea to the lungs, accounts for the majority of cytological specimens from the respiratory tract. Many of primary neoplasms arising from the lower airways have been described and can be grouped based on cell of origin as neuroendocrine, non-neuroendocrine, mesenchymal, salivary

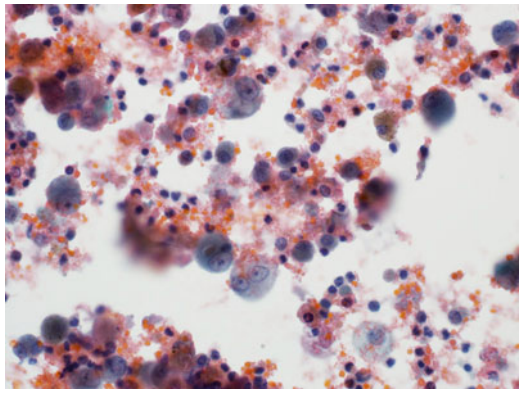


Fig. 7.35 Histiocytes. Binucleated histiocytes from granulomatous inflammatory processes (Smear, Papanicolaou)

gland-like and hematolymphoid. Cytologic evaluation of lung lesions can quickly become complicated whenever the bronchial, alveolar, histiocytes are exposed to noxious stimuli. Both acute and chronic lung injury can elicit reactive changes that are sometimes indistinguishable from malignancies. Ancillary studies can aid but not in all situations. Clinical correlation is the most important factor in making accurate cytodiagnosis in the respiratory tract.

Suggested Readings

1. Berman JJ, Murray RJ, Lopez-Plaza IM. Widespread post tracheostomy atypia simulating squamous cell carcinoma: a case report. Acta Cytol. 1991;35:713–6.
2. Beskow CO, Drachenberg CB, Bourquin PM, et al. Diffuse alveolar damage. Morphologic features in bronchoalveolar lavage fluid. Acta Cytol. 2000;44(4):640–6.
3. Cibas ES, Ducatman BS. Cytology: diagnostic principles and clinical correlates. 4th ed. Philadelphia, PA: Elsevier; 2014.
4. Crapanzano JP, Zakowski MF. Diagnostic dilemmas in pulmonary cytology. Cancer. 2001;93:364–75.
5. Dalton ML, Gatling RR. Peripheral adenoid cystic carcinoma of the lung. South Med J. 1990;83(5):577–9.
6. de Mattos MC, de Oliveira ML. Pseudoepitheliomatous proliferation, a pitfall in sputum cytology. Diagn Cytopathol. 1991;7(6):656–7.
7. Demay RM. The art & science of cytopathology. 2nd ed. ASCP Press: Chicago; 2011.
8. Frierson Jr HF, Covell JL, Mills SE. Fine needle aspiration cytology of atypical carcinoids of the lung. Acta Cytol. 1987;31:464–70.
9. Grotte D, Stanley MW, Swanson PE, et al. Reactive type II pneumocytes in bronchoalveolar lavage fluid from adult respiratory distress syndrome can be mistaken for cells of adenocarcinoma. Diagn Cytopathol. 1990;6:317–22.
10. Fiorella RM, Gurley SD, Dubey S. Cytologic distinction betweenbronchioloalveolar carcinoma and reactive/reparative respiratory ep-ithelium: a cytomorphometric analysis. Diagn Cytopathol. 1998;19:270–3.
11. Gulbahce HE, Baker KS, Kumar P, et al. Atypical cells in bronchoalveolar lavage specimens from bone marrow transplant recipients. A potential pitfall. Am J Clin Pathol. 2003;120(1):101–6.
12. Hughes JH, Young NA, Wilbur DC, Cytopathology Resource Committee, College of American Pathologists, et al. Fine-needle aspiration of pulmonary hamartoma: a common source of false-positive diagnoses in the College of American Pathologists Interlaboratory Comparison Program in Nongynecologic Cytology. Arch Pathol Lab Med. 2005;129(1):19–22.
13. Johnston WW. Percutaneous fine needle aspiration biopsy of the lung: a study of 1,015 patients. Acta Cytol. 1984;28:218–24.
14. Jordan AG, Predmore L, Sullivan MM, et al. The cytodiagnosis of well-differentiated neuroendocrine carcinoma. A distinct clinicopathologic entity. Acta Cytol. 1987;31(4):464–70.
15. Khajotia RR, Mohn A, Pokieser L, et al. Induced sputum and cytological diagnosis of lung cancer. Lancet. 1991;338(8773):976–7.
16. Lawther RE, Graham AN, McCluggage WG, et al. Pulmo-nary infarct cytologically mimicking adenocarcinoma of the lung. Ann Thorac Surg. 2002;73:1964–5.
17. Linder J. Lung cancer cytology. Something old, something new. Am J Clin Pathol. 2000;114(2):169–71.
18. Linssen KC, Jacobs JA, Poletti VE, et al. Reactive type II pneumocytes in bronchoalveolar lavage fluid. Acta Cytol. 2004;48:497–504.
19. Mullins RK, Thompson SK, Coogan PS. Paranuclear blue inclusions: an aid in the cytopathologic diagnosis of primary and meta-static pulmonary small-cell carcinoma. Diagn Cytopathol. 1994;10:332–5.
20. Naryshkin S, Young NA. Respiratory cytology: a review of non-neoplastic mimics of malignancy. Diagn Cytopathol. 1993;9:89–97.
21. Ogino S, Franks TJ, Yong M, et al. Extensive squamous metaplasia with cytologic atypia in diffuse alveolar damage mimicking squamous cell carcinoma: a report of 2 cases. Hum Pathol. 2002;33:1052–4.
22. Policarpio-Nicolas ML, Wick MR. False-positive interpretations in respiratory cytopathology: exemplary cases and literature review. Diagn Cytopathol. 2008;36(1):13–9.
23. Raab SS, Oweity T, Hughes JH, et al. Effect of clinical history on diagnostic accuracy in the cytologic interpretation of bronchial brush specimens. Am J Clin Pathol. 2000;114(1):78–83.

24. Saad RS, Silverman JF. Respiratory cytology: differential diagnosis and pitfalls. Diagn Cytopathol. 2010; 38(4):297–307.
25. Silverman JF. Inflammatory and neoplastic processes of the lung: differential diagnosis and pitfalls in FNA biopsies. Diagn Cytopathol. 1995;13:448–62.
26. Silverman JF, Weaver MD, Shaw R, et al. Fine needle aspiration cytology of pulmonary infarct. Acta Cytol. 1985;29:162–6.
27. Silverman JF, Marrow HG. Fine needle aspiration cytology of granulomatous diseases of the lung, including nontuberculous Mycobacterium infection. Acta Cytol. 1985;29:535–41.
28. Stanley MW, Henry-Stanley MJ, Gajl-Peczalska KJ, et al. Hyperplasia of type II pneumocytes in acute lung injury. Cytologic findings of sequential bronchoalveolar lavage. Am J Clin Pathol. 1992;97(5): 669–77.
29. Walker WP, Wittchow RJ, Bottles K, et al. Paranuclear blue inclusions in small cell undifferentiated carcinoma: a diagnostically useful finding demonstrated in fine-needle aspiration biopsy smears. Diagn Cytopathol. 1994;10(3):212–5.
30. Wax TD, Coogan AC, Johnston WW. Clinical significance of an inconclusive cytopathologic diagnosis: a five-year experience at Duke University Medical Center. Analysis and follow-up of specimens from the respiratory tract. Acta Cytol. 1997;41(4):1053–7.
31. Zaman S, van Hoeven K, Slott S, et al. Distinction between bronchioloalveolar carcinoma and pulmonary proliferations: a cytologic andmorphometric analysis. Diagn Cytopathol. 1997;16:396–401.

Urinary Tract Cytology

8

Introduction

The urinary tract is commonly subjected to stressors that can result in atypical and suspicious findings in a voided urine specimen (see Table 8.1). The most common stressors include indwelling catheters, various toxins, ischemic changes, calculi, bladder and kidney infections, immune mediated cystitis, and inflammatory reactions to radiation therapy, immunotherapy and chemotherapy.

As accurate assessment for malignancy is the major objective of voided urine cytology, it is highly desirable that the pre-analytical condition of the sample should be as pristine as possible, as urine caustically causes cells to rapidly degenerate and thereby acquire deceptive, atypical features. High quality precludes first morning voids and urine specimens sitting at room temperature without fixation for more than 2 h. Normal urothelial cells, when degenerated, may acquire suspicious features: nuclear hyperchromasia, increased N/C ratio, and wrinkled nuclear contours. In most cases, the atypia can be ascribed to degeneration, because the nuclear chromatin is smudgy, breaks are present in the chromatinic rim, or the cell margin is not intact. Unfortunately, cancerous samples may be impossible to diagnose as malignant if secondary degeneration is superimposed on the tumor cells. Care should be taken to immediately fix or refrigerate urine specimens prior to fixation to avoid degenerative atypia.

Good fixation but also accurate clinical history are essential for optimal employment of the new Paris system for reporting urinary cytology. This system de-emphasizes the diagnosis of lesions other than high grade urothelial carcinoma (in situ or invasive). Diagnoses in this system can be divided into four main groups: Group 1 consists of Positive for High Grade Urothelial Carcinoma (HGUC), Suspicious for High Grade Urothelial Carcinoma and Positive for Other Primary or Metastatic (high grade) Malignancy, Group 2 consists of Negative for High Grade Urothelial Carcinoma (NHGUC), Group 3 consists of Low Grade Urothelial Neoplasm (LGUN), and Group 4 consists of Atypical Urothelial Cells (AUC). Conceptually, the Paris system introduces more consistency in diagnosis of urine cytology findings and highlights its most valuable attribute: the ability to detect high grade urothelial lesions with high sensitivity. Moreover, the System aims to provide clear assessments to clinicians for optimal choice of the appropriate, standardized algorithm for patient treatment and follow-up. In terms of clinical significance, Group 1 contains the diagnoses that should prompt aggressive action, while Groups 2–4 consist of normal or insignificant findings. This includes the diagnostic category of LGUN, as it is biologically clear that nearly all low grade urothelial carcinomas do not transform to HGUC,

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_8

Table 8.1 Confounders for urothelial carcinoma and/or urothelial carcinoma in situ

Confounder/ mimicker	What it mimics	Architecture cellularity	Nuclear features	Cytoplasmic features	Associated conditions	Differentiating features	Additional steps
Calculus	LGUC	Cellular with single cells, flat fragments, clusters	• Usually mild range in nuclear size, fine chromatin, +/− small nucleoli • Degeneration often present causing smudgy or glassy nuclei, nuclear holes, breaks	• N/C ratio N to slightly high • Cytoplasm may be prominently vacuolated	• RBCs • Inflammation • Sometimes calcareous debris	Some LGUC have 3-d papillary groups with irregular ragged edges that would be unusual in reactive atypias	ALT, OCH, RevIM
Cystitis	LGUC	As above, but papillary cystitis may shed true papillary structures	As above	As above	• RBCs • Inflammation • Possibly calcareous debris in encrusted cystitis	• As above • Reactive papillae often feature smooth contours with a cytoplasmic collar or one smooth margin formed by umbrella cells	OCH RevIm (papillary cystitis lacks the complex coral appearance of LGUC on cystoscopy)
Polyoma virus	HGUC	Usually only a few atypical cells present	• Four types of nuclear change (1) Enlarged basophilic nuclei with ground glass look (most common) (2) Eosinophilic granular inclusion with halo (3) Finely granular enlarged nucleus without halo (4) Vesicular nucleus with clumped chromatin	N/C ratio very high with minimal to no cytoplasm apparent	• Little to no inflammation in most cases • No necrotic debris in most cases • No RBCs in most cases	• No well-preserved cells with coarse, irregularly distributed chromatin as in HGUC/CIS. • Necrosis and RBCs also may be present in HGUC/CIS	ICC for SV-40 antibody which is positive in the affected cells ALT: can have false positive with UroVysion
Instrumentation	LGUC	Similar to cystitis/ calculi	Similar to cystitis/ calculi	Similar to cystitis/ calculi	RBCs, inflammation	Similar to cystitis/ calculi	OCH

Indwelling catheter	LGUC and HGUC	Usually cellular Often many flat fragments	Marked nuclear atypia may be present but usually is only mild	N/C ratio variable	RBCs, inflammation	No necrosis	OCH
Radiation therapy	HGUC	• Usually only affects a few superficial cells • Variable cellularity	• Multinucleation, increased nuclear size, degenerative changes often present, often prominent nucleoli	• Cells often large but N/C ratio remains low • Polychromasia • Tattered edges	• RBCs • Inflammation	Cells with coarse irregularly distributed chromatin are absent	OCH ALT
Systemic chemotherapy	HGUC	• Usually only affects a few cells • Variable cellularity	• Changes as in radiation but more atypical cells some with coarse granular chromatin or ink black or with macronucleoli	Large cells with tattered edges	• RBCs • Inflammation	May be impossible to differentiate from HGUC/CIS	OCH ALT
BCG intravesical therapy	HGUC	• Variable cellularity • Flat fragments and clusters	Marked atypia may be present during treatment	N/C ratio may be elevated in reactive cells during therapy	Inflammation including giant cells and epithelioid histiocytes in some cases	During treatment may be impossible to differentiate from recurrent/residual HGUC/CIS	Repeat urine specimen several months after end of BCG ALT OCH
Lithotripsy	LGUC	Cellular specimen with flat fragments and clusters including papillary groups	Mild nuclear size increase, mild hyperchromasia and membrane irregularities	N/C ratio may be mildly elevated	RBCs, inflammation	Changes disappear by 2–3 months after treatment	Repeat urine specimen at 3 months OCH
Cystitis cystica/ glandularis	LGUC	• Normal cellularity • A few flat groups, gland strips or clusters	• Normal to mild variation in nuclear shape • Fine granular chromatin	Columnar or round shapes (goblet cells may be present with mucin vacuole)	Background usually clean	• Marked nuclear atypia is absent • Necrosis is absent	ALT Tissue biopsy

(continued)

Table 8.1 (continued)

Confounder/ mimicker	What it mimics	Architecture cellularity	Nuclear features	Cytoplasmic features	Associated conditions	Differentiating features	Additional steps
Mullerianosis	LGUC	• Normal cellularity • Few gland strips of columnar cells	• No significant nuclear atypia • Elongate to round nuclei with fine chromatin • May have degenerative changes	• Columnar or round shape • May have mucin or cilia	Background clean	May mimic normal spindled urothelial cells or LGUC which can have spindled urothelial tumor cells	ICC positive for ER, PR ALT negative
Prostatic epithelial polyp	LGUC	• Normal cellularity • Few flat fragments or papillary groups	No significant nuclear atypia	Small round cells with mildly high N/C ratio	Background may include inflammation or RBCs	• Rare • Cells resemble normal prostatic cells	• ICC positive for PSA or PSAP • ALT negative
Seminal vesicle cells	HGUC	• Cellularity variable • Usually only a few atypical cells • No fragments of seminal vesicle epithelium	• Nuclei enlarged and hyperchromatic • Degenerative changes often present • Macronucleoli may be present	• N/C ratio elevated • Large cells • Cytoplasmic pigment often present (lipofuscin)	Background may include sperm, prostatic epithelial cells, debris, corpora amylacea	• No necrosis • Cells are few and often degenerated • Ejaculate contaminants a clue to the diagnosis	ALT negative
Endometrial cells	LGUC	• Normal cellularity • Few strips of cells	• Nuclei elongate and hyperchromatic • May be degenerated	Elongate cell shape	Background often bloody	No significant nuclear atypia	OCH such as LMP

Papillary urothelial hyperplasia	LGUC	• Normal cellularity • Flat fragments and papillary clusters	• No significant nuclear atypia; may have mildly increased size	N/C ratio may be midlely elevated	Background clean	May not be able to distinguish from LGUC	• ALT • Tissue biopsy
Urothelial papilloma	LGUC	• May be hypercellular • Papillary clusters	As above	As above	As above	As above	• ALT • Tissue biopsy
Papillary urothelial neoplasm of low malignant potential	LGUC	As above	As above	As above	As above	As above	As above
Inverting papilloma	LGUC	As above	As above	As above	As above	As above	As above
Nephrogenic adenoma	LGUC	• Flat fragments • Variable cellularity	• Round with fine granular chromatin, often with nucleoli	Round to cuboidal shape	• May have inflammation and RBCs • Necrosis absent		• ALT • Tissue biopsy • RevIm • PAX2 and PAX8 ICC positive

ALT ancillary lab tests such as UroVysion, ImmunoCyt/uCyt, possibly others, *OCH* obtain clinical history, *RevIm* review cystoscopy +/− ultrasound, CT, MRI studies, *ICC* immunocytochemistry

and because it is not possible to distinguish benign urothelial neoplasms from low grade urothelial carcinomas by cytologic assessment [4].

Bladder washings compared to voided urine samples yield a cellular specimen with optimal preservation. Cells are often shed in clusters and mats. These should not be mistaken for a low grade papillary urothelial neoplasm (LGUN). Knowledge of the procedural nature of the sample will prevent an incorrect diagnosis of "atypical" or "suspicious". The presence of lubricant (pink to purple acellular material usually denser than mucin) indicates the sample is not a voided urine specimen. Catheterized urine samples and ureteral and renal pelvis brushings also characteristically yield tissue fragments and many more dispersed cells relative to a typical normal voided urine specimen.

Clinical history may be of critical importance in avoiding a serious diagnostic error. Medical history details that significantly affect the interpretation of atypical cells include renal transplant/immunosuppression, indwelling catheter, positive urine culture, prior instrumentation, type of sample (washings versus voided urine), pelvic irradiation, chemotherapy, immunotherapy, urinary tract calculi, lithotripsy within the last 2 months, intra-vesical chemotherapy, and history of bladder or other tumors, among many others. Any process or state that can irritate bladder mucosa can cause the shedding of atypical urothelial cells or cell clusters into the urine. This includes ischemic mucosal injury as in the setting of severe peripheral vascular disease, a common condition in the Western world that by itself may rarely be associated with an atypical urine specimen (in the setting of reactive pseudocarcinomatous hyperplasia). Obtaining the relevant clinical history can reduce misdiagnoses, sharpen diagnostic skills, and lower medical costs. Unfortunately, requisition forms seldom provide complete pertinent medical history. In this setting, the pathologist may need to make a phone call to the ordering caregiver or review the patient's medical chart in order to optimally assess the specimen. Although most mimickers of urothelial lesions cause confusion with LGUNs, a few mimickers can be mistakenly interpreted as HGUC. In these cases, only ancillary testing and/or scrupulous attention to medical history and imaging studies can forestall an erroneous diagnosis of "atypical", "suspicious for urothelial malignancy" or "positive for urothelial malignancy". Even with an ameliorating history, it is reasonable to render an atypical or suspicious diagnosis if the degree of architectural, cytoplasmic and nuclear changes seem too extreme for a mimicker.

Cytology enables rapid diagnosis of many types of primary urinary tract tumors (see Table 8.2). However, non-urinary tract tumors may uncommonly be found in a voided urine sample. These include vulvar, cervical, vaginal and uterine tumors, prostatic adenocarcinoma, and even tumors from faraway sites such as the lungs. Tumors that infiltrate the bladder directly may also shed cells into the urine and mimic a urothelial carcinoma. Colorectal adenocarcinoma, cervical squamous carcinoma, ovarian serous carcinoma and prostatic adenocarcinoma are the most common bladder invasive carcinomas to shed cells. Thus, in addition to infectious, inflammatory and iatrogenic conditions that can cause diagnostic confusion with primary urinary tract tumors, other malignancies can counterfeit urothelial carcinoma in urine specimens, albeit relatively rarely (see Table 8.3). Clinical history combined with directed immunocytochemical staining should enable the correct diagnosis in these relatively rare circumstances.

Ancillary tests such as UroVysion (Abbott Molecular Inc, Des Plaines, Illinois) for fluorescent in situ hybridization (FISH) may be worthwhile in confirming a diagnosis of urothelial neoplasm. Keep in mind that many LGUNs are not aneuploid, supporting the cytologic interpretation of non-neoplastic cells, and yielding a negative FISH result. Conversely, tetrasomy and occasionally aneuploidy may be found in benign samples requiring consilience in cytologic features, medical history and cystoscopy findings to achieve optimum diagnostic accuracy. For example, polyomavirus infection, radiation induced urothelial cell atypia, and contaminating seminal vesicle cells may cause a false positive UroVysion result. Other ancillary tests approved by the FDA

Table 8.2 Urinary tract neoplasms diagnosable by urine cytology

Location	Neoplasms	Diagnostic sensitivity	Diagnostic specificity
Urethra	Squamous cell carcinoma (SCC)	Low (only a few cells may be shed)	High (in terms of diagnosing squamous carcinoma but cannot exclude other primary sites or mixed type carcinoma)
	Urothelial cell carcinoma	Low for low grade	High (in terms of diagnosing high grade urothelial carcinoma but cannot exclude other primary sites)
		High for high grade	
Bladder	Low grade papillary urothelial neoplasm (LGUN)	Low	Low
	High grade urothelial carcinoma, (HGUC)	High	High
	Small cell undifferentiated bladder carcinoma	High	High (with supporting ICC)
	Primary bladder adenocarcinoma	Ranges from low to high with subtype	Low (require ICC and imaging studies)
	Primary bladder squamous cell carcinoma	High	High but cannot exclude other primary sites or mixed type carcinoma)
	LGUN	Low	Low
	HGUC	High	High
Kidney	Clear cell renal cell carcinoma	Very low	Low unless ICC used
	Other renal cell carcinomas	Very low	Low unless ICC used

for surveillance and evaluation of patients with hematuria with a clinical suspicion of bladder carcinoma include ImmunoCyt/uCyt (DiagnoCure Inc, Saint-Quebec City, Quebec, Canada), NMP-22 (BladderChek, Alere, Watham, Massachusetts) and BTA stat (Polymedco Inc, Cortlandt Manor, New York). None are 100 % sensitive, although sensitivity for HGUC is usually higher than that achieved by cytology alone but not typically enough to justify the additional cost of the test. Most of the ancillary tests are relatively specific (80–>90 %) for the diagnosis of high grade urothelial carcinoma or CIS, but then, so is urinary cytology. ImmuoCyt/uCyt may detect aneuploid low grade tumors but at the expense of reduced specificity compared to cytology or UroVysion. Two recent investigations of ProExC™ (a dual antibody combination of MCM2 and TOP2A, BD Diagnostics-TriPath, Burlington, North Carolina) have reported promising results in the stratification of atypical urothelial cells into two categories: urothelial carcinoma and reactive. An advantage of the ProExC™ test is that it is a chromogenic antibody test assessed by brightfield microscopy, although combination with uCyt (immunofluorescence assay) yields the best sensitivity for both LGUNs and HGUC (94 and 92 % respectively). Here again, cost-benefit analysis is recommended.

In addition to the improved sensitivity and specificity in urothelial carcinoma diagnosis achieved with *judicious* use of ancillary testing, cytologic classification of urothelial cell atypia into low suspicion and high suspicion categories for malignancy may further improve the sensitivity rate for malignancy and permit triaging those patients who would most benefit from cystoscopy and additional testing (see Table 8.4). Reproducible criteria that are evidence-based and carefully followed would optimally result in many confounders of malignancy in the urinary tract being consigned to the low suspicion/low risk category.

Notably, the Paris System for Reporting Urinary Cytology focuses on finding HGUC and de-emphasizing the LGUNs, since morphologic criteria are exquisitely sensitive for the high grade cells. Initial studies separating atypical urothelial cells into two categories of atypia (Atypical Urothelial Cells [AUC] or Suspicious for HGUC)

Table 8.3 Non-urinary tract tumors or dysplasias that may shed into or contaminate urine specimens

Original Location	Neoplasms	Cytologic features in urine (most cases have only a few atypical cells +/– red blood cells and inflammation)	Immunocytochemistry (* = may also be positive in urothelial neoplasms)	Incidence of positive urine specimen
Vulva, cervix, vagina	Squamous cell carcinoma and squamous dysplasia	• Primarily individual keratinized cells with nuclear hyperchromasia and variable nuclear shapes and sizes	• Positive: p40*, p63*, keratin 5 • Negative: keratin 7 • +/–: p16*	• Low (~4–6 % of squamous carcinomas) • Very low for dysplasias
Prostate	Prostatic adenocarcinoma	• Sheets and clusters of small round cells with elevated N/C ratio and prominent nucleoli • Glands and columnar cells may be present	• Positive: PSA, PSAP, AMACR	Low; does not require direct spread into bladder
Colorectal	Conventional colorectal carcinoma	• Degenerated clusters of elongate columnar cells with pseudostratification and nuclear hyperchromasia. • Individual tumor cells and debris may be present.	• Positive: keratin 20*, villin, CDX-2, SATB2	Can occur with direct spread of tumor into bladder
Ovarian	Ovarian serous carcinoma most common	• Marked pleomorphism of round large cells with coarse chromatin and macronucleoli	• Positive: ER (often), Pax8, keratin 7*	Can occur with direct spread of tumor into bladder or via vagina into urine
Endometrium	Endometrioid adenocarcinoma most common	• Mats and clusters of elongate cells with hints at gland formation. • Vesicular to hyperchromatic nuclei.	• Positive: keratin 7*, ER (often), vimentin* • Negative: keratin 20	Very low but can occur with direct spread of tumor into bladder more commonly than by contamination of random urine specimen
Breast	Invasive ductal carcinoma Invasive lobular carcinoma	• Variable cohesion. • Most cases consist of smaller cells with nuclear hyperchromasia. • +/– signet ring cells and cytoplasmic mucin	• Positive: keratin 7*, GATTA3 (90 %), mammaglobin (50 %), GCDFP (50 %) • Negative: keratin 20 unless mucinous	• Can occur with high stage disease. • Rare
Lung	Non-Small cell carcinoma	• Nonspecific cells of variable atypia and size with nuclear hyperchromasia; may form glands or mats; foamy or vacuolated cytoplasm • SCC of lung identical to other SCCs	• Positive in lung adenocarcinoma: TTF-1, napsin (~85 %), keratin 7* • Negative in lung adenocarcinoma: p40, keratin 20 (unless mucinous)	Can occur with high burden/high stage disease
	Small cell undifferentiated carcinoma (poorly differentiated neuroendocrine carcinoma)	• Dyscohesive cells with high N/C ratio, “salt and pepper” chromatin and nuclear molding	• Positive: TTF-1 (many cases), keratin 7*, chromogranin* (70 %), synaptophysin* (85 %)	Can occur with high burden/high stage disease
Skin	Melanoma	• Can appear only as abundant melanin pigment in histiocytes • Tumor cells may be of many morphologies including spindled cells but most often epithelioid with macronucleoli	• Positive: S100 protein (90 %), HMB45, MelanA • Negative: keratins	Can occur with high stage disease
Bone marrow or lymph nodes	Lymphoma of various types	• Dyscohesive small cells with elevated N/C ratio and usually mild nuclear size range	• Positive: CD45, B-cell or T-cell markers • Negative: keratins	Rare to occur in a voided urine specimen

Table 8.4 Proposed features of atypical urothelial cells cannot exclude high grade urothelial carcinoma (AUC-H)

Cellularity	Architecture	Nuclear features	Cytoplasmic features	Concurrent or F/U HGUC/CIS	Investigators (year) # type of specimens
–	–	• Hyperchromasia • N/C ratio >0.7 or both	_	• 38 % concurrent • 54 % developed lesion	• Piaton et al. [18] • 534 specimens, most NOT voided urines
–	–	• Hyperchromasia (often inky black) • High N/C ratio • Irregular borders • Anisonucleosis or all above features combined	–	• Hyperchromasia best predictor of HGUC • Criteria ONLY Worked in patients undergoing surveillance. • Criteria were not predictive of HGUC in patients presenting only with microhematuria.	• VandenBussche et al. [5] • 290 specimens
–	–	Nuclear features were easier to categorize with acid hematoxylin added to stain	–	• Negative: 13.3 % • Atypical favor reactive: 31.1 % • Atypical favor neoplastic: 37.6 % • Suspicious: 53.6 % • Malignant: 74.3 % • No statistical difference when atypia divided into two categories	• Bostwick and Hossain [7] • 10,473 with biopsy and followed for 1 year • 6427 with cystoscopy only
<10 atypical cells	Usually single cells	• Hyperchromasia and/or clumped chromatin • Intact and irregular nuclear membranes • N/C ratio >0.5	–	• Equated "suspicious" category with AUC-H • 92 % developed Carcinoma (HG or LG)	• Nhung Ton Nu et al. [19] • 773 specimens

"–" no specific features

have generally demonstrated that the Suspicious diagnosis shows increased specificity for predicting the presence or near-term development of HGUC compared with the diagnosis of AUC. However, specificity may be highest in patients with a known history of bladder tumors (eg patients under surveillance). This is because patients presenting with hematuria may have irritated mucosal changes that result in urothelial cells possessing the same features as described for Suspicious for HGUC. Once again, the clinical history can profoundly affect the interpretation of identical cytologic features as either strongly suspicious/indicative of HGUC (in a surveillance specimen) or indeterminate (in a microhematuria only, non-surveillance specimen).

From this point forward, terminology will be based upon The Paris System for Reporting Urinary Cytology. Table 8.5 lists the diagnostic categories.

Major Cytologic Confounders of Common Urinary Tract Neoplasms Diagnosable by Cytology

Urethra

Infectious or inflammatory conditions of the urethra may result in inflammation and atypical appearing squamous cells. Urethral carcinomas are seldom detected in a voided urine specimen.

Table 8.5 Diagnostic categories of the Paris system for reporting urinary cytology

Diagnostic category	Comment
Negative for high grade urothelial carcinoma	Urinary cytology cannot conclusively diagnose LGUC; therefore a "negative for LGUC" is not a component of this system
Atypical urothelial cells	Should have a low probability of HGUC on follow-up biopsy
Suspicious for high grade urothelial carcinoma	Should have a high probability of HGUC on follow-up biopsy
High grade urothelial carcinoma	
Low grade urothelial neoplasm	Cannot differentiate between LGUC, PUN-LMP, and urothelial papillomas on cytology
Other malignancy, primary or secondary	Most common are cervico-vaginal SCC, RCC, and PC

HGUC high grade urothelial carcinoma, *LGUC* low grade urothelial carcinoma, *PUN-LMP* papillary urothelial neoplasm of low malignant potential, *SCC* squamous cell carcinoma, *RCC* renal cell carcinoma, *PC* prostatic carcinoma

Bladder

Infectious/Inflammatory

Polypoid and Papillary Cystitis These conditions occur anywhere in the urinary tract but are most common in the bladder. Males are affected more often than females by a factor of ~3:1. Most patients are middle aged or older, but young adults may also be affected. The most common cause is an indwelling catheter, but any chronic mucosal irritation may result in the condition. These conditions do not usually result in atypical nuclear features, although mitotic activity may be increased. Inflammation mixed with clusters and possibly papillary groups are anticipated urine findings, although no reviews of the cytopathology of this type of cystitis have been published.

Bacterial Cystitis Inflammatory cells are numerous and usually consist mostly of neutrophils. Reactive/inflammatory changes in urothelial cells may lead to the inappropriate diagnosis of atypical urothelial cells. Bacteria, when present, may be difficult to identify and may be absent in the urine specimen despite a bacterial cause for the cystitis. Urine culture results are valuable. Figure 8.1 provides examples of reactive changes in the setting of cystitis and acute tubular necrosis.

Eosinophilic Cystitis Eosinophils may be less numerous than neutrophils. Other conditions causing urinary eosinophilia include acute interstitial nephritis, acute tubular necrosis, chronic renal failure, and Schistosoma infection. Reactive changes may be seen in the shed urothelial cells.

Viral Infections Including Polyoma Virus Approximately 90 % of people worldwide are infected by polyomavirus (BK in the bladder) usually by an asymptomatic infection occurring in childhood. Viral reactivation in the urine may occur in immunocompetent individuals as a clinically insignificant, asymptomatic event. However, in immunocompromised patients, BK virus may reactivate and cause significant disease such as polyomavirus-associated nephropathy (1–10 % of kidney transplant patients) and polyomavirus-associated hemorrhagic cystitis (5–15 % of allogenic hematopoietic stem cell transplant patients). Large numbers of polyomavirus altered urothelial cells may be associated with acute renal allograft dysfunction. Polyomavirus reactivation causes changes in the nuclei of urothelial cells that may be confused with high grade urothelial carcinoma/CIS, so-called "decoy cells". Figure 8.2 provides an example of a "decoy cell". Other viral infections that may be found in urine specimens include Herpes virus, Cytomegalovirus, Adenovirus (in the setting of immunosuppression or renal transplant) and Human papilloma virus (HPV). HPV and Herpes infection are usually due to cervicovaginal contamination and do not usually cause reactive urothelial cell changes. However, CMV may infect urothelial cells and in addition to shedding cells with typical nuclear and cytoplasmic inclusions, may cause reactive urothelial cells to be shed. Adenovirus infections may simulate BK viral induced nuclear changes and are associated with a hemorrhagic cystitis. HPV may infect urothelial cells but more commonly occurs in a urine specimen as cervicovagi-

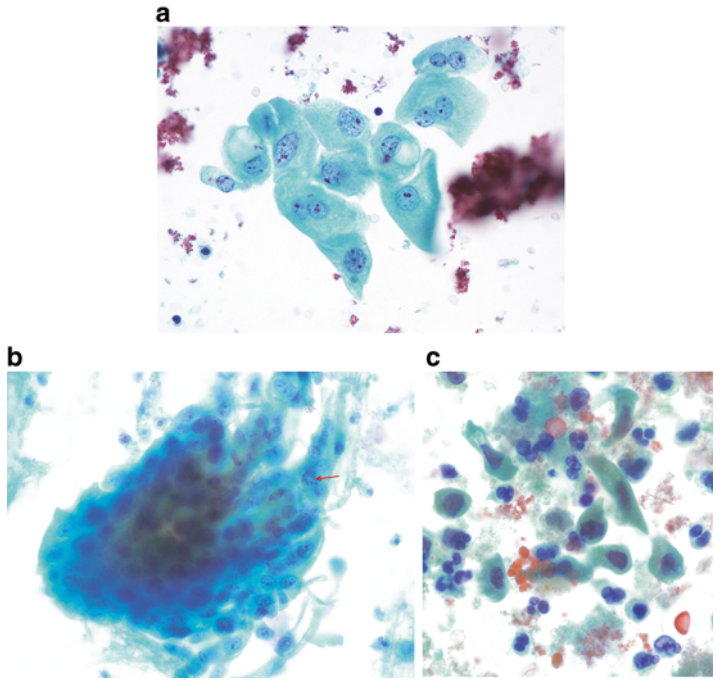


Fig. 8.1 Reactive urothelial cells. (**a**) Reactive umbrella cells in a patient with hemorrhagic cystitis. Note the nuclear enlargement but only minimal increase in N/C ratio, and evenly dispersed fine chromatin with reactive chromocenters/nucleoli. (**b**) Reactive cluster of urothelial cells in a patient with cystitis. Notice the vesicular nuclei with relatively fine chromatin, small nucleoli or chromocenters, and a mitotic figure. (**c**) Markedly atypical reactive and degenerated urothelial cells in a voided urine cytology specimen from a patient with acute tubular necrosis post-trauma. The urothelial cells exhibit hyperchromasia and irregular nuclear contours in a setting of marked acute inflammation, necrosis and hemorrhage. Granular casts and renal tubular cells were present in the background. Clinical correlation is helpful

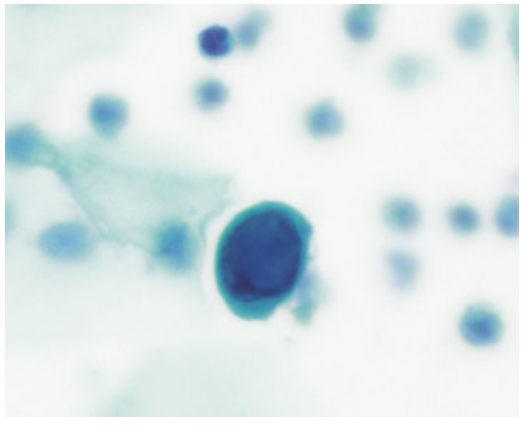


Fig. 8.2 Polyomavirus cell. These are usually found as isolated cells with only a few present in a well preserved voided urine specimen unless the patient is severely immunocompromised. Note the nuclear enlargement, marginated chromatin and slightly glassy nuclear center

nal squamous cell contaminants. The typical features of HPV infection can be identified in these cells such as nuclear enlargement, hyperchromasia, irregular nuclear borders and sharp, large perinuclear halos.

Cystitis Due To Other Organisms Candida is a common cervico-vaginal contaminant in urine specimens and thus usually should not be interpreted as pathogenic. However, in males and in debilitated/immunosuppressed individuals, it may cause a cystitis resulting in inflammation and reactive urothelial changes. Other rare causes of cystitis include Trichomonas (*T. vaginalis* in females and *T. hominis* in males), *Enterobius vermicularis*, and *Schistosoma hematobium*, as well as Mucor, Fusarium, and other fungal species.

Reactive urothelial cells may be shed with these infections leading to a diagnosis of atypical urothelial cells.

Follicular Cystitis This is a chronic cystitis defined by lymphoid follicle formation. It may cause reactive urothelial changes interpreted as atypical. The urine specimen contains many lymphocytes and also may contain fragments of germinal centers. Lymphoma may be a diagnostic consideration. This type of inflammatory reaction may also occur adjacent to urothelial tumors; therefore, a sample with features suggesting follicular cystitis should be carefully examined for evidence of neoplasia.

Interstitial Cystitis This immune mediated cystitis will feature neutrophils +/− eosinophils, degenerated urothelial cells, reactive urothelial cells and red blood cells in a voided urine sample. Cells resembling mast cells may be present.

Encrusted Cystitis This is a rare type of cystitis due to urea-splitting bacteria with most cases occurring after urological instrumentation. Calcifications that act as a biofilm form in the mucosa. Hematuria is the most common presentation. Laser ablation in addition to antibiotic therapy is required for cure. There are no reports of the cytology findings.

Inflammatory Pseudotumor This is a rare myofibroblastic lesion of young adults (most commonly) that can exhibit reactive urothelial changes, inflammation, spindled myofibroblasts, and red blood cells in a voided urine specimen. Only a few cases have been reported.

Iatrogenic

BCG Intravesical Therapy Urine cytology samples taken during treatment may yield highly atypical cells indistinguishable from residual high grade urothelial carcinoma/CIS (HGUC). Typical findings include lymphocytic and histiocytic predominant inflammation and red blood cells. Multinucleated histiocytic giant cells and spindled epithelioid cells can be shed into the urine but are less common (see Fig. 8.3). Since many patients treated by BCG may relapse or develop new tumors, careful scrutiny for atypical-malignant cells is prudent. Ancillary testing may be required to distinguish between true neoplastic cells and reactive atypia. A period of 3 months following the final BCG treatment is recommended to reduce the visual confusion between post-BCG reaction and residual/recurrent HGUC.

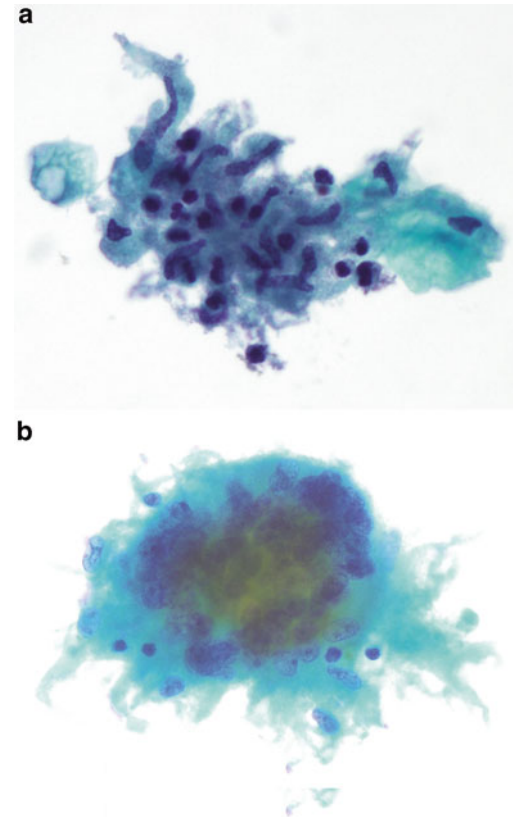


Fig. 8.3 BCG induced inflammatory cells. (**a**) This granuloma could be mistaken for a cluster of atypical spindled urothelial cells. Clinical history is helpful. (**b**) Multinucleated giant cells are nonspecific but indicate a chronic inflammatory process such as post BCG therapy or may rarely represent an inflammatory response to carcinoma

Radiation Induced Atypia Changes post radiation may persist for years. Urothelial cells appear atypical with cellular enlargement but usually with a preserved N/C ratio; nuclear hyperchromasia can be worrisome. Radiation cystitis may also be associated with pseudocarcinomatous urothelial hyperplasia.

Chemotherapy (Non-BCG) Induced Atypia Treatment with cyclophosphamide or busulfan notoriously often produces a hemorrhagic cystitis with markedly atypical cells showing suspicious features that in some cases exactly mimic HGUC.

Immunotherapy Induced Changes (Non-BCG) Cystitis with reactive urothelial cells can occur and lead to a diagnosis of atypical urothelial cells.

Laser Ablation Post treatment bladder wash specimens may contain spindled epithelial cells singly or in clusters that may be diagnosed as atypical urothelial cells.

Changes Post-lithotripsy Papillary clusters, single atypical cells, inflammation and red blood cells may first appear 24 h after the procedure but only persist for maximally 3 months.

Changes Post-flexible Cystoscopy Urine specimens taken within 24 h commonly contain papillary groups, columnar cells, increased cellularity and nuclear changes comprising an elevated N/C ratio and irregular nuclear contours. These may be interpreted as atypical or suspicious for neoplasia.

Ileal Conduit/Neobladder Neutrophilic and histiocytic inflammation, degenerated columnar cells, and debris are typical findings. Some degenerated lining cells may resemble histiocytes. Careful evaluation for recurrent or new urothelial carcinoma must be performed. A few patients with rectosigmoid neobladders may develop adenomas or adenocarcinomas, usually many years after the initial surgery, and squamous cell carcinoma has also been reported.

Normal Cell Contaminants from Other Sites

Vulvar, Vaginal and Cervical Squamous Cells Normal appearing squamous cells do not simulate a urothelial neoplasm. Dysplastic and carcinomatous squamous cells could contaminate a urine specimen necessitating further evaluation to determine site of origin of the abnormal cells. Squamous cells in urine specimens from women may also originate from metaplastic mucosa of the bladder trigone.

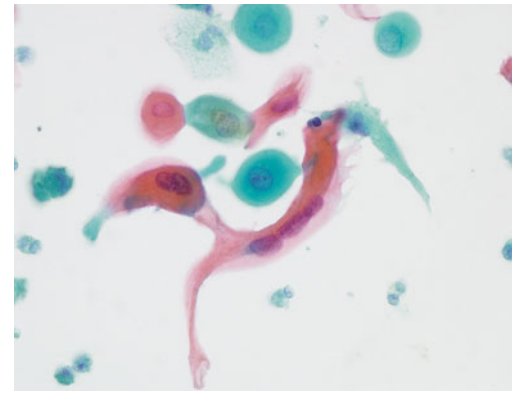


Fig. 8.4 Dysplastic appearing squamous cells representing the malignant squamous component of high grade urothelial carcinoma. In a female, these cells could also derive from a gynecologic squamous dysplasia or carcinoma. In this male patient, the atypical squamous cells greatly outnumbered the malignant urothelial cells in the urine specimen. This patient's resected urothelial carcinoma consisted of 20 % classic urothelial tumor cells, 60 % malignant squamous cells, and 10 % each glandular and sarcomatoid cells. The glandular and sarcomatoid elements were not identified in the urine cytology sample

Urethral Squamous Cells in Men Squamous cells occur far less commonly and in lower numbers per specimen in urine samples from men compared to urine samples from women. Some derive from the terminal portion of the urethra, but may also be due to metaplasia in the bladder secondary to prostatic hyperplasia. Dysplastic and malignant appearing squamous cells may be shed from any squamous lesion in the urinary tract or may be a component in an otherwise typical urothelial carcinoma (see Fig. 8.4). Primary pure bladder squamous carcinoma is usually associated with schistosomiasis but can occur outside that setting. Inflammation, red blood cells, and necrotic debris often accompany invasive carcinomas.

Endometrial Cells (Especially Menstrual Contamination) The columnar cells are usually

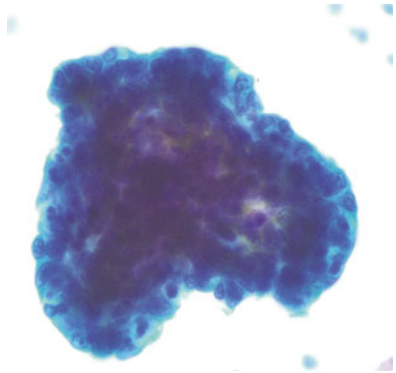


Fig. 8.5 Menstrual endometrium. The patient is a 34 year old woman with hematuria. A single papillary structure is present that could be mistaken for papillary urothelial carcinoma. However, the nuclei are pale and wrinkled (possibly due to degeneration) and inflammatory cells are intermixed. By history, the patient submitted the urine sample during her menses. No abnormalities were identified on cystoscopy. She's also at least a decade younger than most women diagnosed with bladder cancer

present as fragments or clusters, may show degenerative changes, and may be accompanied by red blood cells and debris. Clinical history is helpful. Figure 8.5 provides an example of menstrual contamination in a urine specimen.

Prostatic and Seminal Vesicle Cells Prostatic epithelial and seminal vesicle cells may be more commonly found in the urine of patients with prostatitis. Prostatic cells may be columnar or small and round and may shed in clusters or small sheets. Nuclear atypia is usually absent. Corpora amylacea and sperm may also be present in a granular background after a prostatic massage or simply a digital rectal examination (DRE). Seminal vesicle cells are large and may have hyperchromatic round nuclei or nucleoli and a higher N/C ratio than normal superficial urothelial cells. Pigment (lipofuscin) may be appreciated in the cytoplasm. The chromatin may be degenerated and smudgy, pasty, or glassy. Figure 8.6 provides examples of vesicle cells found in urine specimens. Prostatic and seminal vesicle cells are commonly present in the urine of patients post prostate manipulation.

Sperm These have typical features.

Renal Tubular Cells These are found in the urine and can be accompanied by casts of various types. The cells are small and round with a higher N/C ratio than superficial urothelial cells and may exhibit a clear, vacuolated or granular cytoplasm. Nuclear features may show degenerative atypia including hyperchromasia, slight enlargement or nucleoli. The cells may be mistaken for histiocytes. Acute tubular necrosis, other types of renal injury and active inflammatory conditions may increase the probability of shedding into the urine. Figures 8.7 and 8.8 comprise malignant tumors that might be mistaken for renal tubular cells (clear cell renal cell carcinoma and mucinous colonic adenocarcinoma, respectively).

Normal Variants

Papillary Urothelial Hyperplasia Cytopathology text authors report clusters and papillae of cells with minimal nuclear atypia in the absence of inflammation. There are no series of cases presented in the literature. Figure 8.9 illustrates the spectrum of atypia in papillary groups found in urine specimens.

Cystitis Glandularis and Cystitis Cystica Patients may shed clusters of benign glandular cells with or without intercalated goblet cells. Mucin may be present in the background. Some cells may be ciliated. Figure 8.10 illustrates the spectrum of atypia of cell clusters found in urine specimens.

Mullerianosis This is considered a metaplastic condition that comprises endometrial, tubal or endocervical epithelium replacing areas of transitional epithelium or present in the bladder muscularis. There is one report of the findings in a voided urine specimen. The lesion may form a polypoid mass up to 4.5 cm in size in the bladder, so it might be expected to shed Mullerian type cells and cell clusters.

Prostatic Epithelial Polyp Nonspecific clusters of benign columnar cells have been reported.

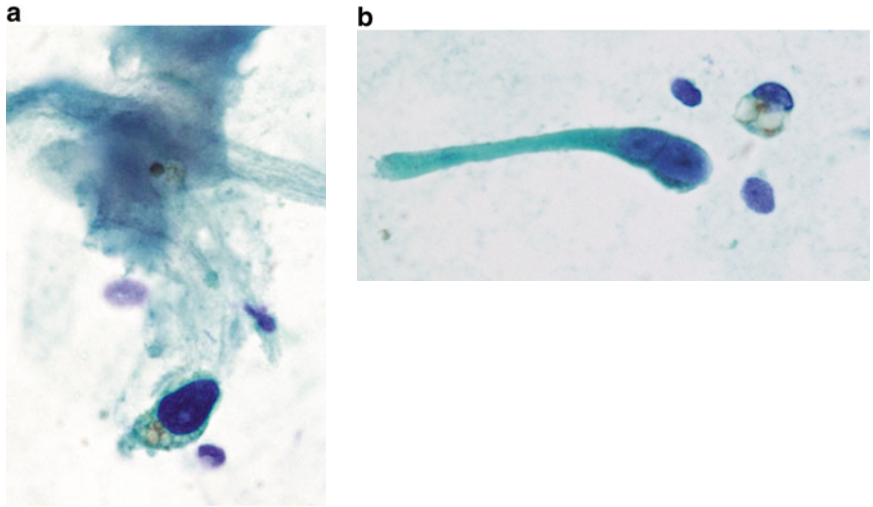


Fig. 8.6 Seminal vesicle cells. Seminal vesicle cells are typically cuboidal (**a**) to columnar (**b**) and may exhibit varying degrees of nuclear atypia including hyperchromasia and coarse granular chromatin. A diagnostic clue is intracytoplasmic golden translucent lipofuscin pigment (but this is not entirely sensitive or specific and may be absent in some cells: (**b**) contains a large columnar cell without pigment). Some specimens may also contain loose aggregates of fluffy sediment that also suggest seminal fluid contamination (**a**) Seminal vesicle cells will express PAX8 on ICC, in contrast to urothelial cells

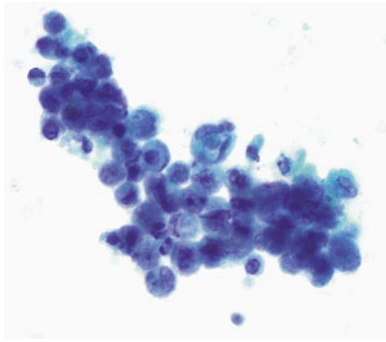


Fig. 8.7 Metastatic high grade mucinous colorectal carcinoma. These degenerated small malignant cells derive from a high grade mucinous colonic adenocarcinoma that invaded the bladder wall. Such groups could be mistaken for urothelial carcinoma, because malignant mucus cells can also be found in some urothelial carcinomas

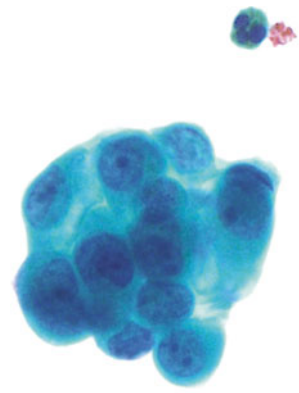


Fig. 8.8 Clear cell renal cell carcinoma. This three dimensional cluster of clear cell renal cell carcinoma can be mistaken for reactive atypia or a low grade urothelial neoplasm. The cytoplasm is frothy and macronucleoli are evident suggestive of clear cell RCC, but immunocytochemistry and correlation with clinical and imaging findings are necessary to confirm the correct diagnosis

Benign Tumors or Lesions

Bladder Calculus This can cause the release of highly atypical single cells and cell groups into the urine some of which may appear three dimensional or papillary. Some cases are associated with more clearly identifiable reactive groups (pale nuclei with fine chromatin, nucleoli and absent to only mildly increased N/C ratio). Calculus debris may be present, and acute

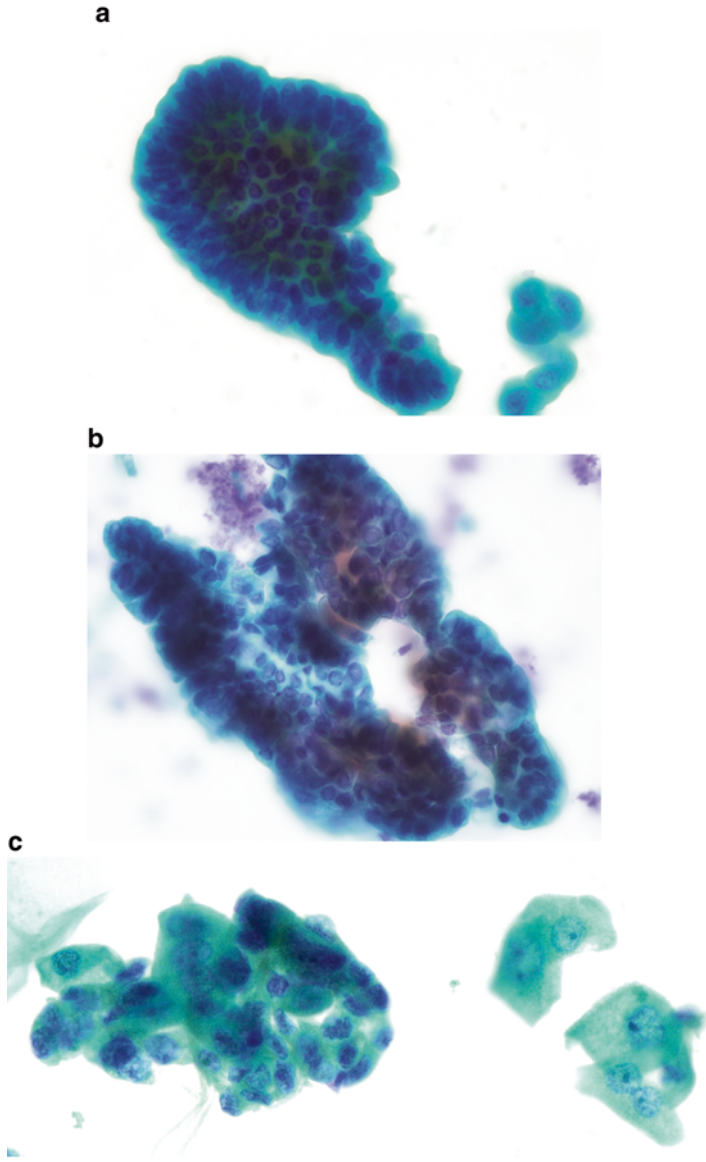


Fig. 8.9 Spectrum of benign to atypical to suspicious to positive papillary groups**.** (**a**) Benign urothelial papilla in voided urine sample from a 44 year old woman with hemorrhagic cystitis (on surgical biopsy) in the setting of radiation therapy for cervical carcinoma. (**b**) Atypical-appearing urothelial papilla in a bladder washing from a patient with bladder calculi. (**c**) Suspicious, ragged urothelial papilla in a voided urine sample from a 73 year old man with a history of recurrent urothelial carcinoma. Notice the atypical features of loss of cell polarity, variable N/C ratios, and complex folds and creases of the nuclei. However, hyperchromasia, coarse chromatin, and marked irregular chromatin distribution definitive for carcinoma are lacking. (**d**) Follow-up bladder biopsy reveals urothelial carcinoma in situ (CIS). The dysplastic cells exhibit similar nuclear features as the cells found in the patient's urine cytology specimen. (**e**) Normal bladder mucosa adjacent to the area of CIS shows less nuclear atypia and no mitotic activity, apoptotic bodies or hyperplasia. (**f**) For comparison, positive urothelial papilla in a patient with biopsy proven HGUC. Although the papilla is relatively well polarized, the tumor cell nuclei show marked hyperchromasia, coarse chromatin, prominent irregular chromatin distribution, and a uniform increased N/C ratio

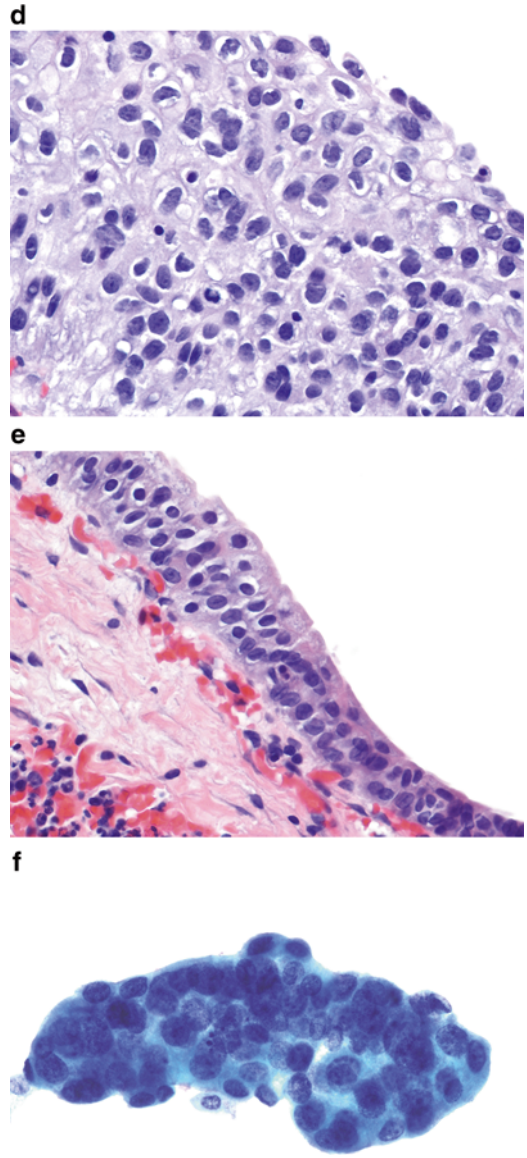


Fig. 8.9 (continued)

inflammation and red blood cells are common. Figure 8.11 illustrates the spectrum of atypia of single urothelial cells found in urine specimens.

Urothelial Papilloma and Papillary Urothelial Neoplasm of Low Malignant Potential Both urothelial papilloma and papillary urothelial neoplasm of low malignant potential may be indistinguishable from each other, from reactive causes of papillary groups, and from low grade papillary urothelial carcinoma.

Inverted Papilloma This benign tumor will rarely shed papillary clusters and sheets into a urine specimen. Mild degenerative atypia may be present. It may simulate a low grade urothelial papillary carcinoma or a reactive process.

Nephrogenic Adenoma Neoplastic cells usually have minimal nuclear atypia, but the urine sample may be hypercellular with many flat sheets or clusters (see Fig. 8.12).

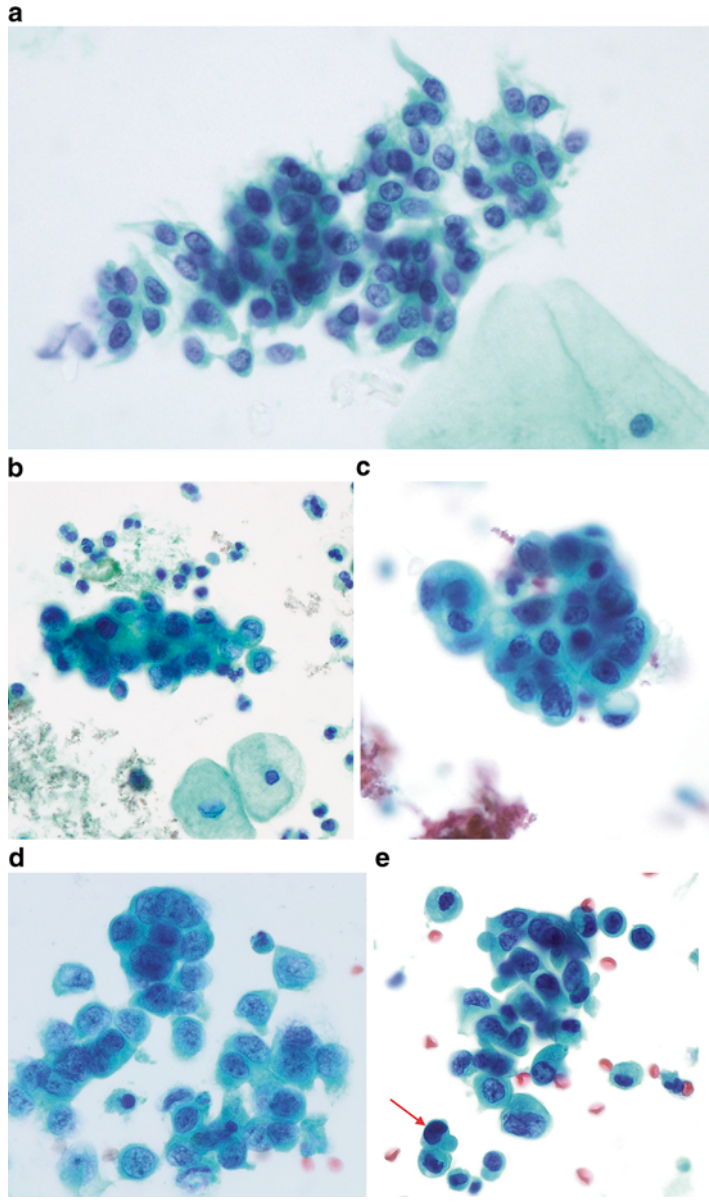


Fig. 8.10 Spectrum of benign to atypical to suspicious to positive cell clusters. (**a**) Benign cell clusters in a voided urine specimen. (**b**) Atypical-appearing cell cluster in a voided urine specimen from a patient with cystitis and no evidence of urothelial carcinoma or CIS. (**c**) Another example of atypical cell clusters in a patient with surgical biopsy-proven high grade urothelial carcinoma and carcinoma in situ. The N/C ratio is elevated and polarity is lost. However, the nuclear contours are regular, the chromatin is relatively fine, and marked hyperchromasia is absent. Thus, a definitive diagnosis of urothelial carcinoma/CIS was not rendered on the cytologic sample, even though 9p21 deletion was determined by FISH on some cells from the same sample. (**d**) Suspicious cell cluster in a elderly man presenting with hematuria. The urothelial cells exhibit an increased N/C ratio and mild anisonucleosis with only slightly granular chromatin. Because of the lack of coarse chromatin, marked variation in nuclear shape and lack of hyperchromasia, an unequivocal diagnosis of carcinoma was not made. (**e**) For comparison, positive cell cluster in a patient with resection proven, high grade urothelial carcinoma. The cells are well-preserved with a range of nuclear shapes, hyperchromasia (one with highly suspicious "inky black" chromatin, see *arrow*), and several show marked irregular chromatin distribution, all features consistent with high grade carcinoma/CIS

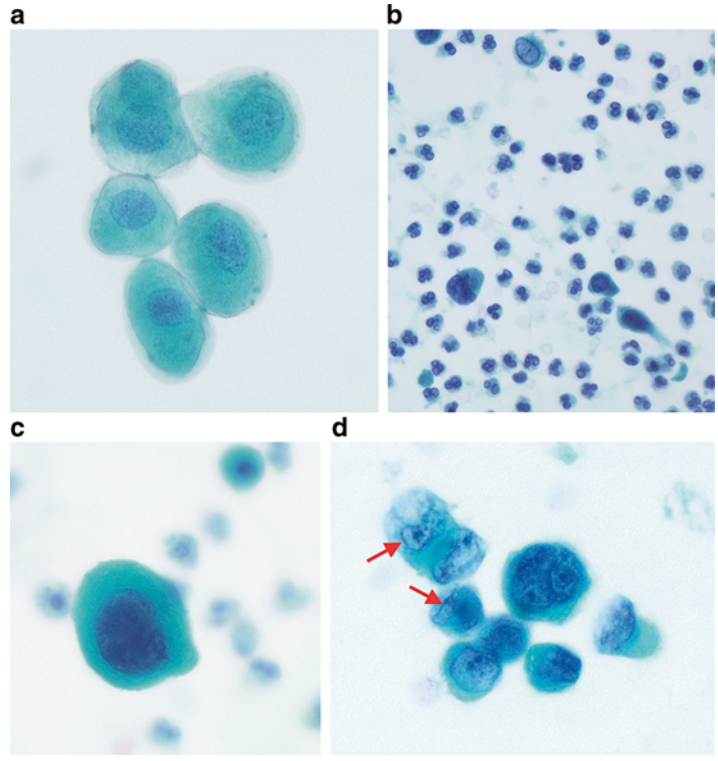


Fig. 8.11 Spectrum of benign to atypical to suspicious to positive single cells. (**a**) Benign single intermediate and superficial urothelial cells. (**b**) Atypical-appearing urothelial cells in a patient with cystitis. (**c**) Suspicious urothelial cells in a patient with a bladder calculus. (**d**) For comparison, positive urothelial cells in a patient with biopsy proven high grade urothelial carcinoma. Tumor cells are well-preserved. In addition to the usual nuclear features of malignancy, the cells also exhibit varying thickness of the chromatinic rim (*arrows* highlights the thickened rim) another feature suggestive of high grade carcinoma/carcinoma in situ

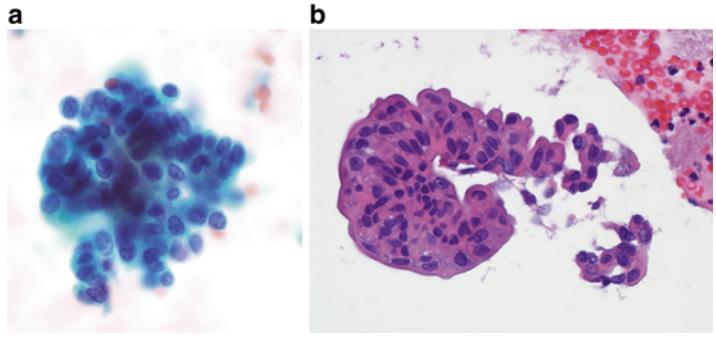


Fig. 8.12 Nephrogenic adenoma. (**a**) Nephrogenic adenoma can mimic a low grade papillary urothelial neoplasm. Three dimensional cell clusters with mild anisonucleosis and an increased N/C ratio are shed in a voided urine specimen. (**b**) A cell block preparation shows a frond of tumor. Immunocytochemistry can be readily performed and will reveal PAX2 and PAX8 expression in the tumor cells

Malignant Tumors That May Mimic Benign Processes

Plasmacytoid Urothelial Carcinoma This is an unusual form of urothelial carcinoma characterized by a small plasmacytoid, dyscohesive morphology. The tumor is often deeply invasive while showing no grossly identifiable tumor. It does not usually cause hematuria. There are no reports of the cytologic findings. Possibly, shed cells might be misinterpreted as plasma cells. Plasmacytoma has been described in one case report in which the diagnosis was rendered on the urine specimen. In this patient, a 71 year old man, the plasmacytoma formed a large mass in the wall that extended to the mucosal surface.

Leukemic/Lymphomatous Involvement of the Bladder Hematopoietic malignancies seldom involve the bladder. However, the mucosa is usually intact and therefore, little shedding into the urine would be expected.

Ureters and Renal Pelvis

Infectious/Inflammatory

Polypoid and Papillary Cystitis As with a bladder location, the urine specimen will contain red blood cells, inflammation (usually neutrophilic) and papillary clusters with absent to mild nuclear atypia.

Iatrogenic

Ureteral washings, Ureteral brushings, Renal pelvis brushings: All of these specimens are hypercellular and contain clusters of urothelial cells. Some may assume a papillary appearance. Typical papillary clusters have a smooth border and may exhibit a rim of cytoplasm ("cytoplasmic collar") Nuclear atypia will be minimal if the urothelium is benign.

Benign Tumors or Lesions

Ureteral or Renal Pelvis Calculus Markedly atypical single cells and cell clusters may be shed into the specimen (voided urine or washing) along with red blood cells, inflammatory cells, and sometimes calcareous debris.

Ureteral Papilloma, Papilloma of Uncertain Malignant Potential and Inverted Papilloma All three entities may shed papillary groups into the voided urine specimen or washing. Minimal nuclear atypia with or without degenerative features is present. These lesions cannot be morphologically distinguished from reactive atypia and low grade papillary urothelial carcinoma.

Kidney

Infectious/Inflammatory

Xanthogranulomatous Pyelonephritis This condition is relatively rare but may lead to a hypercellular urine specimen containing many histiocytes that could be mistaken for a clear cell renal carcinoma. Furthermore, positive immunocytochemical staining of these cells with RCC antigen has been reported. Clinical and imaging correlation is required.

Malakoplakia Cytology findings in a urine specimen have not been described. However, in FNA samples, the specimen features large foamy histiocytes, with intracellular and extracellular concentric laminated calcifications (Michaelis-Gutmann bodies) mixed with neutrophils, lymphocytes and plasma cells.

Acute Tubular Necrosis Small, degenerated renal tubular cells may mimic a low grade urothelial lesion. Granular, cellular and red blood cell casts are clues to the diagnosis but are not specific (see Fig. 8.1c).

Iatrogenic

There are no reports of the urine findings in patients after partial or complete nephrectomy.

Benign Conditions

Various types of renal cysts may shed slightly atypical clusters of small cells that could be misdiagnosed as renal urothelial cells. Cystic renal cell carcinoma may present as a benign cyst if neoplastic cells are of low Furhman grade, and the sample is hypocellular. Correlation with radiology findings is important.

Conclusions

High Grade Lesions Can Be Diagnosed Easily, But Not Low Grade Urothelial Lesions

The sensitivity and specificity for the detection of HGUC far surpass that of LGUN. High grade tumor cells exhibit the hallmarks of malignancy and are usually numerous in a well-preserved urine sample. However, many mimics of low grade tumors occur in the urine specimen as listed above and in the accompanying tables. Although some features hint at the diagnosis of a mimicker or confounder, in reality, for most urine

specimens, the pathologist cannot rely on cytologic characteristics alone to render the correct diagnosis of LGUN.

Fortunately, most LGUNs behave in an indolent fashion and usually can be diagnosed on cystoscopy. Therefore a false negative diagnosis on urine evaluation carries far less clinical impact than a false negative diagnosis of a high grade lesion. The primary objective of urinary cytology is to detect high grade lesions, lesions that are more likely to progress. The new Paris System of terminology clearly distinguishes the critical subsets of those patients who require immediate intervention and those who require close clinical monitoring from the subset of patients at low risk for aggressive disease.

The Microscope Is Only One of Three "Diagnostic" Instruments to Use When Assessing Atypical, Suspicious or Positive Cells in a Urine Specimen

The telephone and the computer can decisively influence the final diagnosis rendered on a urine specimen. The careful pathologist, dedicated to improving patient care and reducing medical costs, and inured or at least reconciled to some extra effort, evaluates atypical cells with full knowledge of clinical history, cystoscopy findings, and prior urine cytology diagnoses. In contrast, the pathologist conducting an uninformed review can readily be blindsided by highly impactful information, unfortunately after the case is signed out. Ureteral and pelvic washings with suspicious or positive features should be vetted in the cold light of the computer screen (accessing the electronic medical record or reading the secure e-mail from the ordering clinician) or after direct conversation with the urologist. A partial or complete nephrectomy could result from a false positive diagnosis, an event to strenuously avoid. Therefore, exercise informed caution in rendering a diagnosis of positive for HGUC, especially with upper urinary tract specimens.

Ancillary Testing Can Be Useful But Has Limitations

Tests such as UroVysion, and uCyt can improve sensitivity and specificity for the detection of HGUC in patients with suspicious or atypical urinary cytology. However, false positives and negatives still occur. Correlation with cytology features, medical history and cystoscopy findings optimizes the ability to accurately identify clinically significant lesions. These tests are inappropriate for cytology samples with cells of HGUC or a Negative urine.

Occam's Razor Should Bear a Blunt Edge When Dealing with Urine Samples

Parsimony dictates that atypical cells in a urine specimen derive from one condition. However, a few specimens may combine atypical cells that are truly reactive with atypical cells originating from a neoplasm. Examples include a renal pelvic calculus with adjacent urothelial carcinoma, an ileal conduit specimen with recurrent carcinoma, and a post-chemotherapy patient with atypical reactive cells mixed with malignant cells. Vigilant mental sorting of the types and degree of atypia in the sample should lead one to suspect the possibility of two extant processes which can then be analyzed further (via ancillary testing, correlation with clinical history, radiology results, etc).

Repeated Atypical Urine Specimens Should Prompt Careful Assessment of the Patient for Neoplasia: The "False False Positive"

"False false positives" (anticipatory positive results) are a known phenomenon in urine cytopathology. Unequivocal features of malignancy occasionally occur in the absence of positive cystoscopy, abnormal CT, MRI, and U/S imaging, positive tissue biopsies, or suspicious clinical

history. In some patients, the malignancy identified in the cytology specimen may take months or even years to declare itself by other means. Especially if a patient has repeated atypical or suspicious urine specimens without a predisposing condition, a concerted effort should be made to exclude a neoplastic process and to continue close follow-up. This could include use of ancillary testing. Some of these patients will eventually develop a clinically appreciable tumor. To best serve the patient, the pathologist needs to stand firm in the face of a negative clinical work-up but numerous repeat positive urine specimens. If marked, unequivocal atypia is present, it must be reported so the patient can be thoroughly evaluated to find the source of the recurrently shed, highly atypical cells.

Suggested Readings

1. Ubago JM, Mehta V, Wojcik EM, Barkan GA. Evaluation of atypical urine cytology progression to malignancy. Cancer Cytopathol. 2013;121:387–91.
2. Halling KC, Kipp BR. Bladder cancer detection using FISH (UroVysion assay). Adv Anat Pathol. 2008;15:279–86.
3. Tapia C, Glatz K, Obermann EC, et al. Evaluation of chromosomal aberrations in patients with benign conditions and reactive changes in urinary cytology. Cancer Cytopathol. 2011;119:404–10.
4. Rosenthal DL, Vandenbussche CJ, Burroughs FH, Sathiyamoorthy S, Guan H, Owens C. The Johns Hopkins Hospital template for urologic cytology samples: part I- creating the template. Cancer Cytopathol. 2013;121:15–20.
5. VandenBussche CJ, Sathiyamoorthy S, Owens CL, et al. The Johns Hopkins Hospital template for urologic cytology samples. Parts II and III improving the predictability of indeterminate results in urinary cytologic samples: an outcomes and cytomorphologic study. Cancer Cytopathol. 2013;121:21–8.
6. Voulgaris E, Pentheroudakis G, Pappa L, et al. Positive urinary cytology in patients with lung cancer in the absence of obvious urinary tract metastases. Lung Cancer. 2011;73:51–8.
7. Bostwick DG, Hossain D. Does subdivision of the "atypical" urine cytology increase predictive accuracy for urothelial carcinoma? Diagn Cytopathol. 2014;42:1034–44.
8. Kryvenko ON, Epstein JI. Pseudocarcinomatous urothelial hyperplasia of the bladder: clinical findings and follow-up of 70 patients. J Urol. 2013;189:2083–6.
9. Farinola MA, Lawler LP, Rosenthal DL. Plasmacytoma with involvement of the urinary bladder: report of a case diagnosed by urine cytology. Acta Cytol. 2003; 47(5):787–91.
10. Florescu MC, Miles CD, Florescu DF. What do we know about adenovirus in renal transplantation? Nephrol Dial Transplant. 2013;28:2003–10.
11. Goldstein ML, Whitman T, Renshaw AA. Significance of cell groups in voided urine. Acta Cytol. 1998;42: 290–4.
12. Guan H, Rosenthal DL, Erozan YS. Mullerianosis of the urinary bladder: report of a case with diagnosis suggested in urine cytology and review of the literature. Diagn Cytopathol. 2012;40:997–1001.
13. Vergara-Lluri ME, Hu E, Rao J-Y, et al. Comparative evaluation of ProEx C and ImmunoCyt/uCyt assays in atypical urine cytology. Arch Pathol Lab Med. 2014; 138:1215–22.
14. Mokhtar GA, Al-Dousari M, Al-Ghamedi D. Diagnostic significance of atypical category in the voided urine samples: a retrospective study in a tertiary care center. Urol Ann. 2010;2:100–6.
15. Yoshida K, Ishida M, Kagotani A, et al. Small cell carcinoma of the urinary bladder and prostate: cytological analyses of four cases with emphasis on the usefulness of cytological examination. Oncol Lett. 2014;7:369–72.
16. Nabi G, Greene D, Donnel MO. Suspicious urinary cytology with negative evaluation for malignancy in the diagnostic investigation of hematuria: how to follow up? J Clin Pathol. 2004;57:365–8.
17. Deshpande V, McKee GT. Analysis of atypical urine cytology in a tertiary care center. Cancer Cytopathol. 2005;105:468–75.
18. Piaton E, Decaussin-Petrucci M, Mege-Lechevallier F, et al. Diagnostic terminology for urinary cytology reports including the new subcategories 'atypical urothelial cells of undetermined significance (AUC-US) and 'cannot exclude high grade' (AUC-H). Cytopathology. 2014;25:27–38.
19. Ton Nu TN, Kassouf W, Ahmadi-Kaliji B, et al. The value of the 'suspicious for urothelial carcinoma' cytology category : a correlative study of 4 years including 337 patients. Cancer Cytopathol. 2014;122:796–803.

Endocrine Cytology

9

Brief Introduction

The human endocrine system is extensive, encompasses many organs, and overlaps with many other physiological systems, such as the gastrointestinal and the reproductive ones. Strictly speaking, any organ capable of affecting other organs' activities by directly secreting hormones into the circulatory system belongs to the endocrine system. However, the major endocrine glands include the pineal gland, hypothalamus, pituitary gland, thyroid gland, parathyroid gland, adrenal glands, ovaries, and testes. Of these major organs, cytological specimens are commonly collected from the thyroid gland. Occasionally, lesions from the parathyroid, adrenal glands and ovaries are cytologically sampled. Thus, in this chapter, the focus will be mainly on the thyroid, and briefly on the parathyroid gland, the adrenal glands and the ovaries. Each of these organs is susceptible to non-neoplastic conditions with cytomorphological findings that have been described in neoplastic states in these organs. There are, however, key clinical, radiologic and cytologic aspects that can make such distinction possible.

Neoplastic Thyroid Nodules and Their Mimickers (Table 9.1)

Thyroid Nodules (Fig. 9.1)

Clinically or radiographically detected thyroid nodules are extremely common. With the advances of imaging modalities, numerous nodules of varying size that would have previously gone unnoticed are being identified. The majority of these nodules are either benign neoplastic or simply non-neoplastic in nature. The benign neoplastic nodules include follicular adenoma and Hürthle cell adenoma (considered variant of follicular adenoma).

Acquiring cytological specimens via fine needle aspiration, often guided by ultrasound imaging, has become the first line of screening of these nodules. Because the majority of the thyroid nodules do not require additional surgical or medical intervention, the role of the cytopathologist is to make the distinction between benign and malignant nodules in order to guide the appropriate therapeutic intervention. The diagnostic challenges are not insignificant because many of these non-neoplastic nodules may share some cytological features with the

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_9

Table 9.1 Thyroid nodules

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Papillary thyroid carcinoma • Hyalinizing trabecular adenoma	Follicular hyperplasia	• Goiter • Dietary iodine deficiency	• Predominantly macrofollicles • Lack of nuclear features of papillary thyroid carcinoma • Colloid is present	• Clinical correlation
	Granulomatous thyroiditis	• Subacute (De Quervain) thyroiditis • Infections (bacterial, fungal, viral or parasitic) • Palpation-induced thyroiditis • Sarcoidosis	• Epithelioid histiocytes and giant cells • Lack of nuclear features of papillary thyroid carcinoma	• Clinical and endoscopic correlation • Immunocytochemistry • Lymphocytes positive for CD45, but negative for neuroendocrine markers (synaptophysin, chromogranin, CD56, NSE) and epithelial markers (cytokeratin AE1/3, EMA, TTF-1)
	Graves' disease	• Autoimmune	• Lack of nuclear features of papillary thyroid carcinoma • Colloid is present	• Clinical and laboratory correlation
	Cyst lining cells	• Predominantly cystic lesion	• Abundant histiocytes • Lack of nuclear features of papillary thyroid carcinoma	• Correlate with imaging impression
	Pregnancy-related changes	• Pregnancy	• Lack of nuclear features of papillary thyroid carcinoma	
	Therapy-related changes	• History of head and neck radiation; antithyroid therapy; minocycline	• Lack of nuclear features of papillary thyroid carcinoma	• Clinical correlation
	Air-drying artifact	• Poor slide preparation	• Poorly preserved cytoplasm and nuclei • Nuclear and cytoplasmic staining pale	• Better smearing and fixation better

• Follicular adenoma • Follicular carcinoma	Follicular hyperplasia	• Goiter • Dietary iodine deficiency	• Predominantly macrofollicles	• Clinical correlation
	Dyshormonogenetic goiter	• Autosomal inborn error of metabolism	• minimal	• Clinical correlation
	Hyperplastic parathyroid	• Elevated PTH • High serum calcium	• Microfollicles • No colloid • Capillaries	• Clinical and laboratory correlation • Immunocytochemistry
• Hurthle cell adenoma • Hurthle cell carcinoma	Hurthle cell metaplasia	• Prior aspiration • Inflammatory condition	• 2-dimensional sheets and macrofollicles • Oncocytic cells not numerous	• Clinical correlation
	Hashimoto thyroiditis	• Autoimmune	• Background lymphocytes • Pleomorphic oncocytic cells	• Clinical and laboratory correlation
• Poorly differentiated carcinoma • Undifferentiated thyroid carcinoma	Acute thyroiditis	• Painful aspiration • Infections	• Necrotic background • Reactive stromal cells • Truly dysplastic follicle cells absent	• Clinical correlation
	Therapy-related changes	• History of head and neck radiation; antithyroid therapy	• Degree of atypia not as pronounced	• Clinical correlation
• Medullary thyroid carcinoma	Hashimoto thyroiditis	• Autoimmune	• Background lymphocytes • Pleomorphic oncocytic cells • No amyloid	• Clinical and laboratory correlation • Immunocytochemistry
	Riedel thyroiditis	• Systemic fibrosing diseases • Infiltrative borders on imaging	• Hypocellular aspirate • Spindle cells	• Clinical and laboratory correlation • Immunocytochemistry
• Lymphomas	Chronic thyroiditis	• Graves • Hashimoto	• Polymorphic lymphoid population • Follicle and oncocytic cells present	• Clinical and laboratory correlation • Immunocytochemistry • Flow cytometry

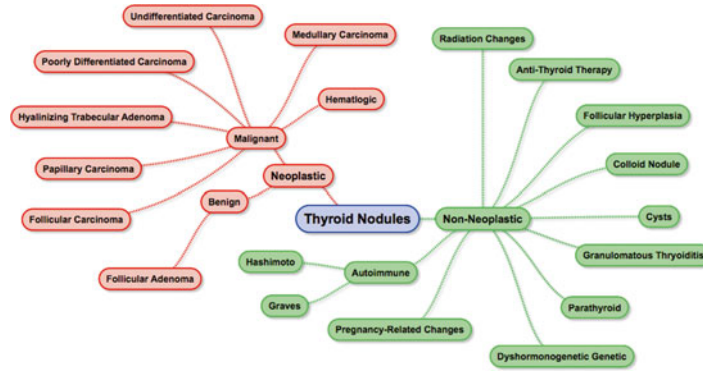


Fig. 9.1 Common thyroid nodules

malignant ones. The reasons are varied, from suboptimal specimens to preparation artifacts and to frank cytomorphological changes mimicking cancer. Concerns for false-positives and subsequently for unnecessary surgeries are shared among all those involved in the evaluation of these thyroid nodules. During such an evaluation, ascertain probability of malignancy by reviewing the clinical history, family history, concurrent laboratory findings, size and imaging characteristics of the nodule and known risk factors in the patient.

The malignant nodules are of numerous types. They include papillary thyroid carcinoma and its variants, follicular/Hürthle cell carcinoma, medullary carcinoma, poorly and undifferentiated thyroid carcinoma, and hematologic malignancies. Non-neoplastic nodules also comprise multiple types and occur more frequently. These include goiter, colloid nodules, autoimmune thyroiditis, granulomatous thyroiditis, cystic degeneration, radiation/chemotherapy-related changes, pregnancy-related changes, and intra-thyroid parathyroid. It is important to be mindful of these possible non-neoplastic nodules as the cytological specimen from a thyroid nodule is being interpreted.

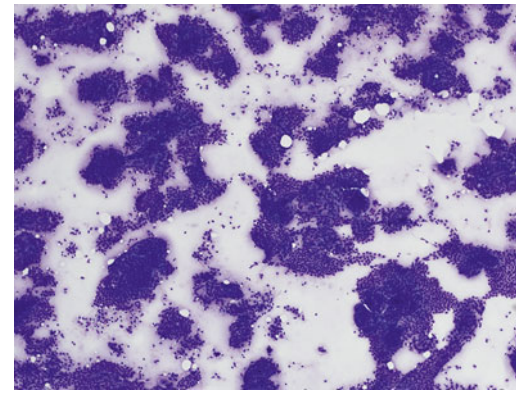


Fig. 9.2 Papillary thyroid carcinoma. Hypercellular aspirate with numerous sheets of follicle cells (Smear, Diff-Quick)

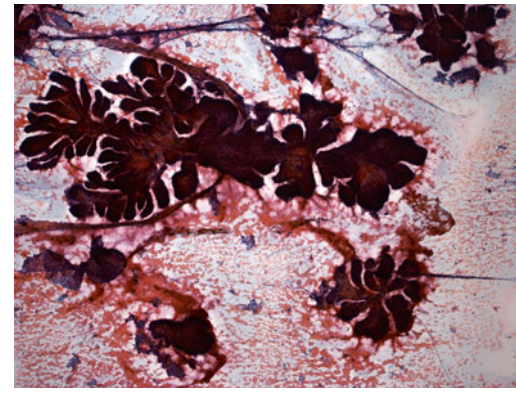


Fig. 9.3 Papillary thyroid carcinoma. Prominent papillary structures (Smear, Diff-Quick)

Papillary Thyroid Carcinoma

By far, the most frequently encountered malignant thyroid nodule seen by cytopathologists is *Papillary Thyroid Carcinoma*. On cytology, it is often epithelium-predominant and frequently hypercellular at low magnification (Fig. 9.2). Depending on the quality of the aspirate, papillae may be visualized (Fig. 9.3). The neoplastic follicular cells can have variable architectural

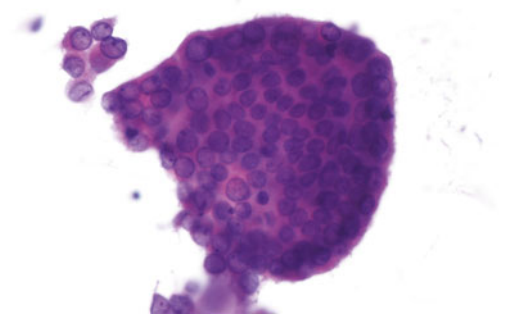


Fig. 9.4 Papillary thyroid carcinoma. Nuclear crowding present in the tip of a papillary structure (Smear, Diff-Quick)

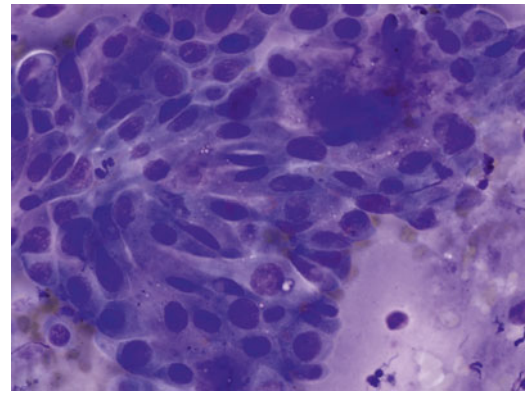


Fig. 9.6 Papillary thyroid carcinoma. Cytoplasm is dense and granular, reminiscent of squamous cells (Smear, Diff-Quick)

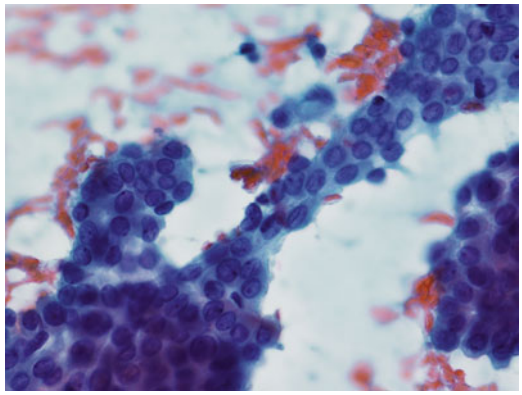


Fig. 9.5 Papillary thyroid carcinoma. Nuclei are oval with prominent grooves (Smear, Papanicolaou)

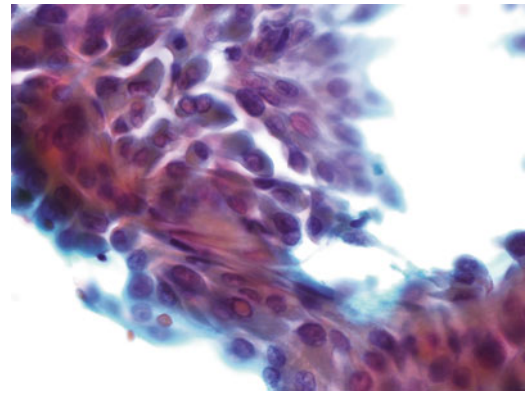


Fig. 9.7 Papillary thyroid carcinoma. Pseudoinclusions present in the nuclei (Smear, Papanicolaou)

configurations such as papillae, sheets, branching, clusters and microfollicles. When compared to benign cells of normal follicles, the neoplastic cells are more crowded (Fig. 9.4). At higher magnification, the nuclei are oval, enlarged, overlapping with irregular nuclear membranes and occasional grooves (Fig. 9.5). The cytoplasm can be dense (reminiscent of squamous cells) or can be oncocytic (Fig. 9.6). Nuclear pseudoinclusions are variably discerned (Fig. 9.7). Additionally, when the specimen is prepared with nuclear-enhancing stains (e.g., Papanicolaou), the chromatin appears pale and evenly distributed with occasional eccentric nucleoli (Fig. 9.5). A similar tumor, but still controversially classified, is the *Hyalinizing Trabecular Tumor*. This tumor shares similar cytological characteristics with papillary thyroid carcinoma.

A nodule-forming *Follicular Hyperplasia* from goiter can also yield a hypercellular aspirate. The increased cellularity should warrant further evaluation. However, careful assessment of the nuclear details will reveal lack of the just described nuclear cytologic features of papillary thyroid carcinoma. The cells are found in an orderly honeycomb pattern. Nuclei are not enlarged and contain inconspicuous nucleoli. Furthermore, in follicular hyperplasia, there should be a predominance of macrofollicles containing round nuclei with smooth nuclear membranes (Fig. 9.8). Abundant Colloid may also be observed (Fig. 9.9).

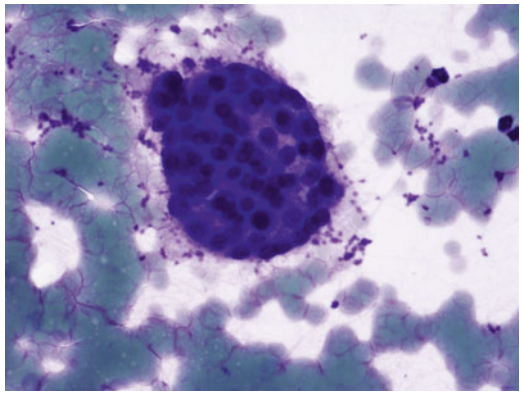


Fig. 9.8 Follicular hyperplasia. Macrofollicle with numerous follicle numerous without crowding or significant nuclear abnormalities (Smear, Diff-quick)

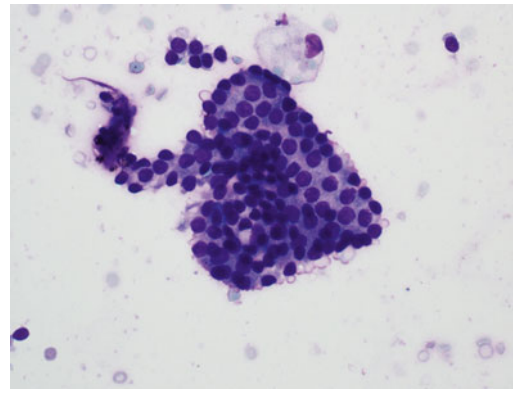


Fig. 9.10 Graves' disease. When untreated, hyperplastic papillae can be seen (Smear, Diff-Quick)

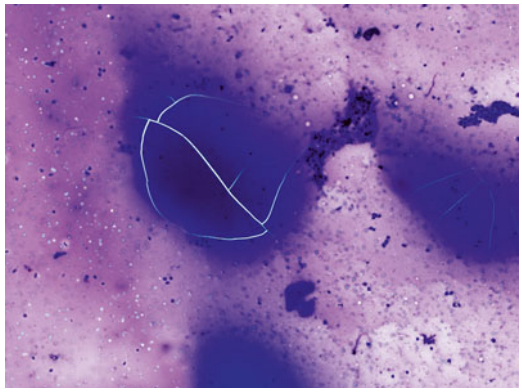


Fig. 9.9 Follicular hyperplasia. Abundant colloid is often seen (Smear, Diff-quick)

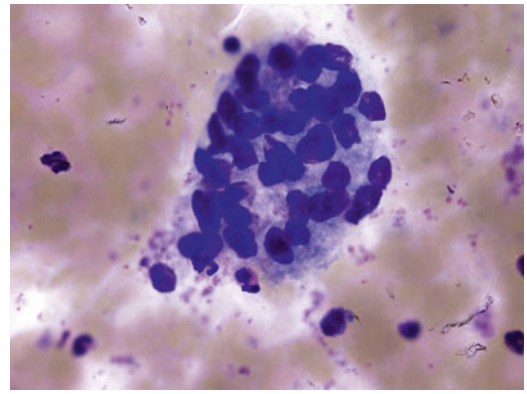


Fig. 9.11 Therapy-induced changes. Cellular crowding, nuclear enlargement and nuclear membrane irregularities secondary to radioactive iodine (Smear, Diff-Quick)

Patients with *Graves Disease* and thyroid nodules are rarely untreated. It is an autoimmune disorder characterized by IgG autoantibodies against thyroid-stimulating hormone. It is diagnosed clinically, biochemically and serologically. If an untreated Graves thyroid is aspirated, certain cytological findings may mimic PTC. These include papillary structures (Fig. 9.10). However, the nuclear characteristics of papillary thyroid carcinoma are absent.

Iatrogenic Changes in the thyroid following *Therapeutic Intervention* can easily lead to false-positives. Radioactive iodine is notorious for inducing cellular crowding, nuclear enlargement, nuclear membrane irregularities, nuclear grooves and even pseudoinclusions (Fig. 9.11). The history of radioactive iodine should automatically raise the threshold for calling lesions in these patients papillary thyroid carcinoma.

Benign non-neoplastic enlarging thyroid nodules often undergo cystic degeneration which is easily noted by ultrasound. On cytology, these cysts tend to yield histiocytes (Fig. 9.12). However, the cells lining these cysts, also called *Cyst Lining Cells* are notorious for mimicking the nuclei of papillary thyroid carcinoma. These include oval to elongated nuclei with nuclear grooves (Fig. 9.13). Caution is warranted when interpreting a cystic lesion. The cyst lining cells should not have pseudoinclusions, and the nuclei are not enlarged.

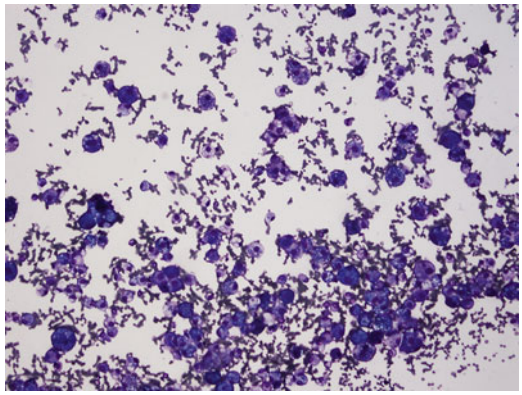


Fig. 9.12 Cyst. Numerous histiocytes present in a benign nodule with cystic degeneration (Smear, Diff-Quick)

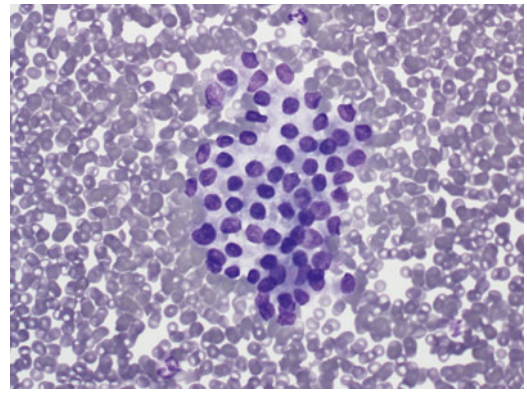


Fig. 9.14 Air-drying artifact. Follicle cells with artificially enlarged nuclei with poorly preserved cytoplasm (Smear, Diff-Quick)

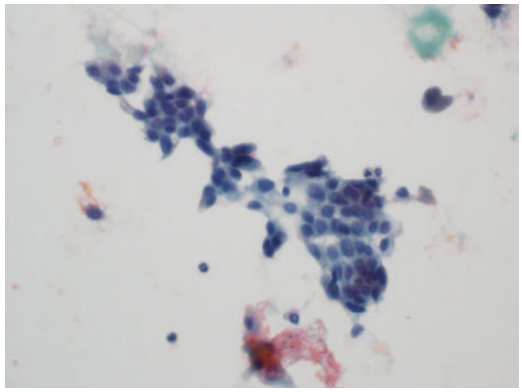


Fig. 9.13 Cyst lining cells. Reactive cyst lining cells with squamous features (Smear, Papanicolaou)

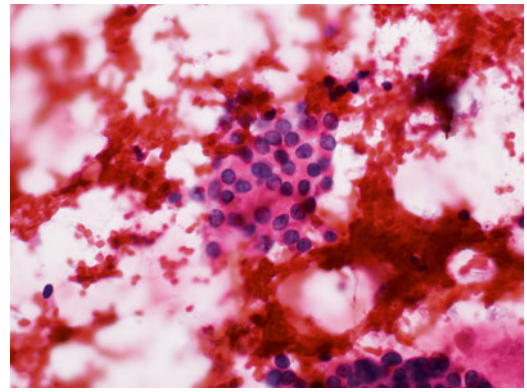


Fig. 9.15 Red blood cells. Bloody aspirate with a red blood cell on top of a follicle cell mimicking pseudoinclusion (Smear, Papanicolaou)

Smearing the aspirate is a common technique for dispersing the aspirate on a glass slide. One issue encountered in most cytology laboratories is the *Air Drying Artifact*. This is particularly true for slides that are stained with Romanowsky. The nuclei can be artificially enlarged, thus mimicking papillary thyroid carcinoma. Generally, the cytoplasm is not well preserved, hinting at this artifact (Fig. 9.14). It is therefore best to prevent such artifacts and obtain alcohol-fixed slides which complement the air-dried slides.

Thyroid aspiration is often a very bloody procedure, and the amount of blood increases with the increasing number of passes. Unfortunately, *Red Blood Cells* when sitting on top of follicle cells can give the morphological impression of pseudoinclusions (Fig. 9.15). If the latter is the sole feature of papillary thyroid carcinoma, it should be ignored. In fact, in a bloody aspirate, evaluation is best done assessing areas devoid of red blood cells.

Granulomatous Reactions are very nonspecific findings in a thyroid aspirate. They can be observed in papillary thyroid carcinoma, as well as in subacute (De Quervain) thyroiditis, infections (bacterial, fungal, viral or parasitic), palpation-induced thyroiditis, or sarcoidosis. In the case of subacute thyroiditis, the aspiration tends not be well tolerated by the patient. Clinically, the diagnosis is often evident and seems to arise following a viral illness. Other infections causing granulomatous reactions are

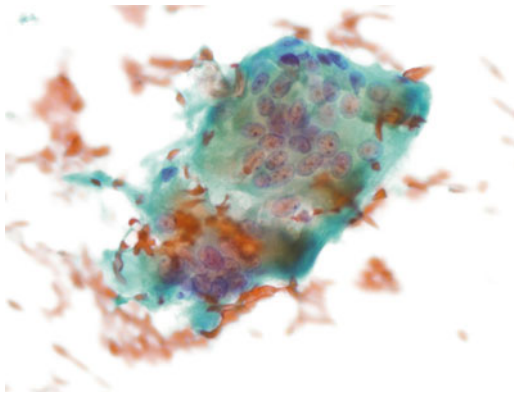


Fig. 9.16 Granulomatous thyroiditis. Multinucleated giant cell with epithelioid histiocytes (Smear, Papanicolaou)

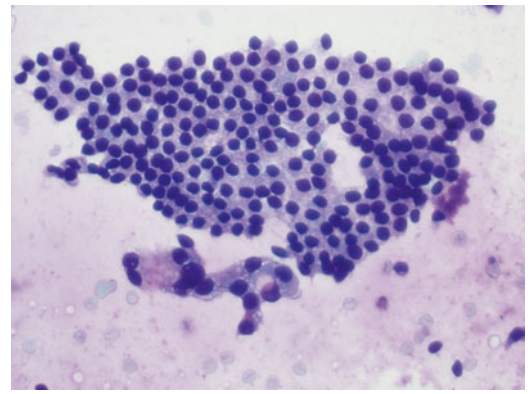


Fig. 9.17 Focal atypia. Small number of follicle cells with nuclear enlargement with a nearby sheet of normal follicle cells (Smear, Diff-Quick)

similarly diagnosed clinically, leaving little role for cytological evaluation. Palpation thyroiditis results from any trauma, including repeated palpations. Direct injury can force colloid outside the follicles eliciting a foreign-body giant cell reaction. The presence of non-caseating granulomas anywhere in the body, including in the thyroid, should always trigger consideration of sarcoidosis which should be clinically excluded.

Regardless of the reason for a granulomatous reaction, it is characterized by granulomas containing spindle to epithelioid histiocytes and occasional giant cells (Fig. 9.16). While these giant cells may be seen in papillary thyroid carcinoma, such interpretation should be reserved only when the other entities are not clinically evident and the above described cytological features of papillary thyroid carcinoma are clearly present.

Pregnancy-Related Thyroid Nodule is frequently aspirated because of concern for papillary thyroid carcinoma. Like Graves Disease, thyroid nodules in pregnant women can yield papillae on cytology from hyperplastic changes (Fig. 9.10). However, the nuclear characteristics of papillary thyroid carcinoma are absent.

Many aspirations are either unsatisfactory for evaluation or diagnostically equivocal (i.e., Atypical). If clinical suspicion for a neoplastic process exists, the nodule will be aspirated many more times until an unequivocal diagnosis is reached. *Prior Fine Needle Aspiration* has been reported to cause cytological changes in the thyroid that can mimic malignancy, including infarct related changes. These tend to be focal, such as nuclear enlargement and mild nuclear membrane irregularities (Fig. 9.17). It has been suggested that repeat thyroid FNA should be at least 3 months apart. In any event, if there is a history of prior FNA, focal to minimal nuclear abnormalities could be expected and do not warrant diagnosis of papillary thyroid carcinoma.

Follicular Neoplasms

The second most common type of neoplastic thyroid nodule is the *Follicular Neoplasm*. Distinction between adenoma and carcinoma is reserved for histological evaluation where presence of transcapsular or vascular invasion can be established. At low magnification, follicular neoplasm is remarkable for hypercellularity, architectural patterns of microfollicles, trabeculae, three-dimensional clusters, and minimal viscous colloid (Fig. 9.18). At higher magnification, the nuclei are overlapping (Fig. 9.19).

As described earlier, nodule-forming *Follicular Hyperplasia* can also yield a hypercellular aspirate. A follicular neoplasm is always in the differential. One cytomorphological finding favoring follicular hyperplasia is the predominance of macrofollicles or with a minor component of microfollicles. The nuclei are round with

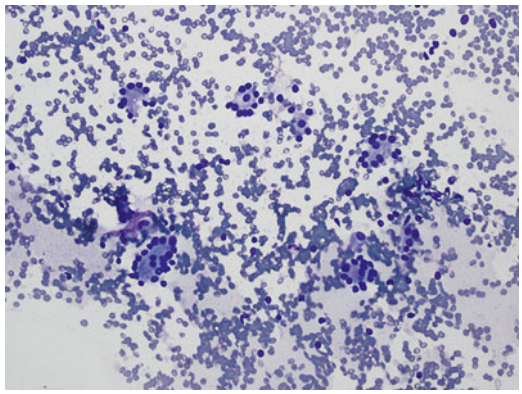


Fig. 9.18 Follicular neoplasm. Microfollicle-predominant aspirate (Smear, Diff-Quick)

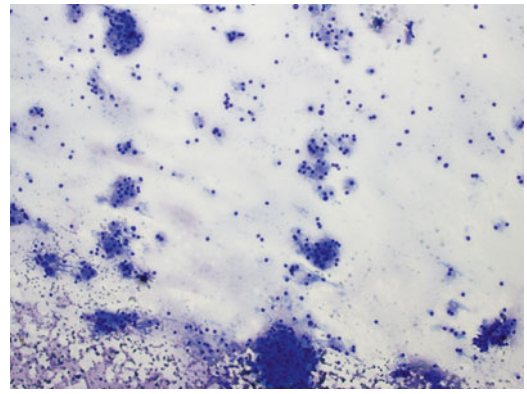


Fig. 9.20 Cellular parathyroid. Hypercellular with numerous microfollicles. The cells are small, round, uniform and resemble lymphocytes (Smear, Diff-Quick)

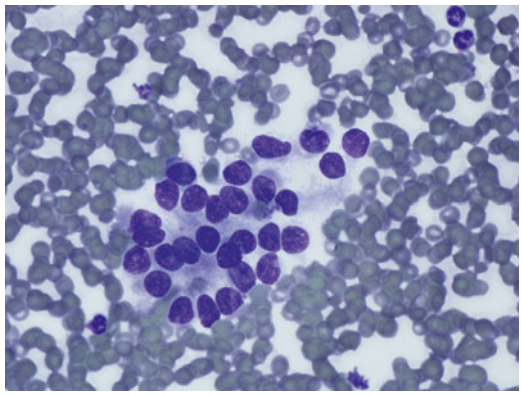


Fig. 9.19 Follicular neoplasm. Microfollicle with nuclear overlapping (Smear, Diff-Quick)

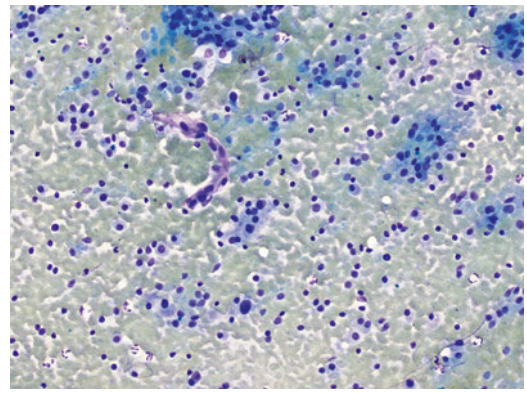


Fig. 9.21 Cellular parathyroid. Capillary with background parathyroid cells (Smear, Diff-Quick)

smooth nuclear membranes and do not overlap. Colloid may also be observed (Fig. 9.9).

Dyshormonogenetic Goiter, an autosomal recessive inborn error of metabolism, is a great mimicker of follicular neoplasm on cytology. When aspirated, it shows numerous microfollicles and minimal colloid which are features of a follicular neoplasm. The best clue to this diagnosis is correlating the findings with the clinical history. Cytologically it may be virtually impossible to exclude a neoplasm.

Occasionally, a *Hyperplastic Parathyroid* is mistakenly interpreted as a thyroid nodule and it is aspirated. The subsequent aspirate can be hypercellular with numerous microfollicles (Fig. 9.20). The cells are small, round, uniform with central nuclei and minimal cytoplasm, reminiscent of lymphocytes. If capillaries are observed, a parathyroid lesion can be favored (Fig. 9.21). Varying degrees of cytological atypia may be identified. A cytodiagnosis of a follicular neoplasm is often entertained. When confronted with such a possibility, it is best to exclude clinically a parathyroid adenoma or hyperplasia. The parathyroid hormone level will be elevated. A Cell block will enable immunocytochemistry with parathyroid hormone and thyroglobulin antibodies proving it to be a parathyroid lesion.

When the cytoplasm of the neoplastic follicular cells is predominantly or exclusively oncocytic (abundant granular cytoplasm with conspicuous nucleoli), the neoplasm is considered a *Hurthle Cell Neoplasm*. At low magnification, the aspirate is hypercellular with dyscohesive

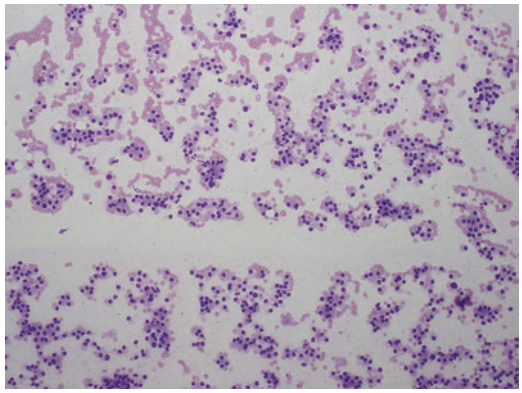


Fig. 9.22 Hurthle cell neoplasm. Hypercellular aspirate consistent with a monotonous population of oncocytic cells (Smear, Diff-Quick)

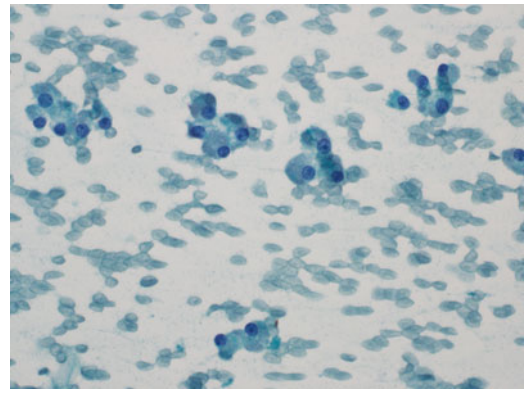


Fig. 9.24 Hurthle cell neoplasm. Nuclei are round and eccentrically placed, with conspicuous nucleoli (Smear, Papanicolaou)

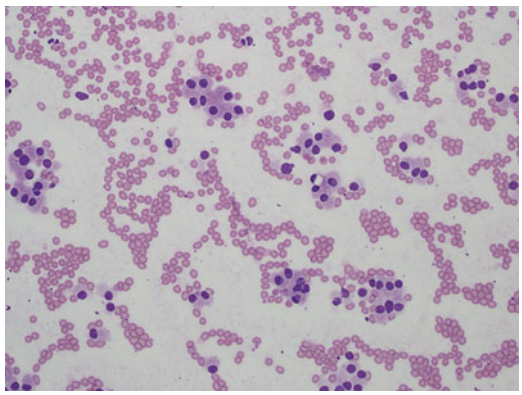


Fig. 9.23 Hurthle cell neoplasm. Oncocytic cells with distinct cell borders and abundant granular and dense cytoplasm (Smear, Diff-Quick)

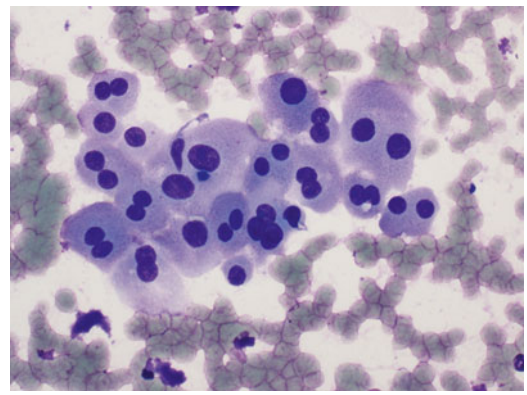


Fig. 9.25 Hurthle cell metaplasia. Oncocytic cells in two-dimensional sheets and macrofollicles (Smear, Diff-Quick)

single cells (Fig. 9.22). When in clusters, they tend to be 3-dimensional or form complex syncytial configurations. At higher magnification, the aspirate consists of a pure population of large cells displaying distinct cell borders with abundant granular and dense cytoplasm (Fig. 9.23). The pleomorphism is counterintuitively restricted in these neoplasms. The nuclei are round and eccentrically placed, with conspicuous nucleoli that are often prominent (Fig. 9.24).

Focal *Hurthle Cell Metaplasia* can occur in the thyroid and within benign nodules. When benign Hurthle cells are present, they are not numerous and their nuclei are not enlarged. They are more often present in 2-dimensional sheets and macrofollicles (Fig. 9.25). Oncocytic cell metaplasia occurs in a number of scenarios including prior aspiration and inflammatory condition such as Hashimoto thyroiditis.

Hashimoto thyroiditis is a common autoimmune disease of the thyroid. It clinically presents as hypothyroidism in the setting of diffuse goiter. Characteristic laboratory findings include serum antiperoxidase or antithyroglobulin antibodies. Even when this diagnosis is clinically established, the presence of a dominant nodule might trigger an aspiration for cytological evaluation. In these cases, the aspirate is remarkable for a variable amount of oncocytic (Hurthle) cells accompanied by a lymphoplasmacytic infiltrate (Fig. 9.26). In

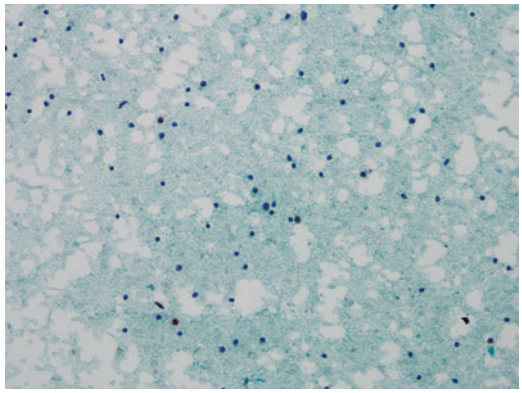


Fig. 9.26 Hashimoto thyroiditis. Lymphocytic background (Smear, Papanicolaou)

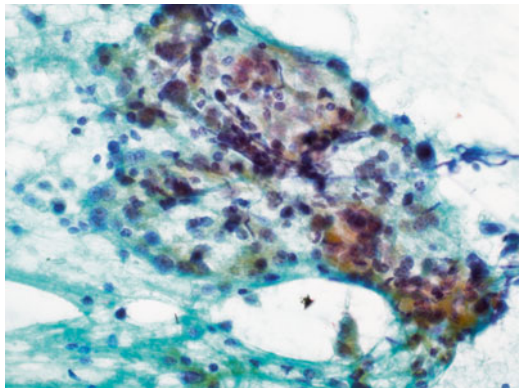


Fig. 9.27 Hashimoto thyroiditis. Lymphohistiocytic aggregate (Smear, Papanicolaou)

addition, lymphohistiocytic aggregates, tingible body macrophages, and lymphoid tangles may be observed (Fig. 9.27). Occasionally, the nuclei of the oncocytic cells may be enlarged, hyperchromatic, and contain variable large nucleoli and abnormal nuclear membranes (Fig. 9.28). Colloid is usually minimal. These features may thus suggest malignancy such as Hurthle cell neoplasm. Distinguishing Hashimoto thyroiditis from a true Hurthle cell neoplasm can be virtually impossible. Characteristics favoring neoplasm include a more uniform dyscohesive cytological population of oncocytic cells as opposed to pleomorphism that can be seen in non-neoplastic oncocytic cells. So when confronted with laboratory and clinical data that are consistent with autoimmune

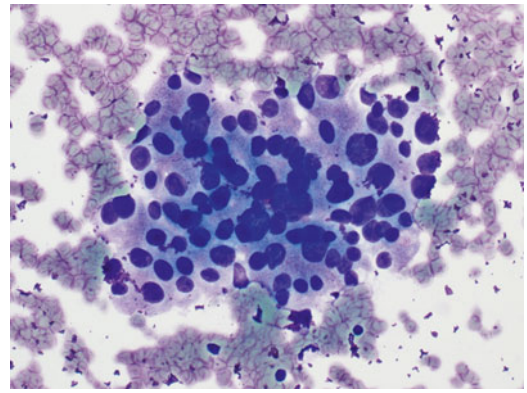


Fig. 9.28 Hashimoto thyroiditis. Oncocytic cells with enlarged, hyperchromatic nuclei with nuclear membrane irregularities (Smear, Diff-Quick)

thyroiditis, abnormal cytological findings may not necessarily represent a neoplasm.

Black Thyroid represents discoloration of thyroid parenchyma following long-standing use of minocycline. Morphologic findings include degenerative changes in follicular epithelial cells characterized by nuclear hyperchromasia and chromatin clumping. These features may easily be mistaken for neoplasia. In addition, pigmentation in follicular epithelial cells and macrophages is often appreciated. The clue to this diagnosis is a clinical history of chronic use of a tetracycline derivative.

Poorly Differentiated and Undifferentiated Carcinoma

A poorly differentiated follicular neoplasm of the thyroid is *Insular Carcinoma*. At low magnification, the aspirate is hypercellular with minimal to no colloid (Fig. 9.29). At higher magnification, the follicular cells are crowded, with a high nucleocytoplasmic ratio, and hyperchromatic nuclei. Frequent mitoses and a necrotic background are common (Fig. 9.30). An extremely more aggressive malignant thyroid neoplasm is the *Undifferentiated (Anaplastic) Thyroid Carcinoma,* seen in the middle-aged to elderly. The degree of abnormal morphological changes is pronounced. At low magnification, the aspirate is hypercellular with a marked necrotic

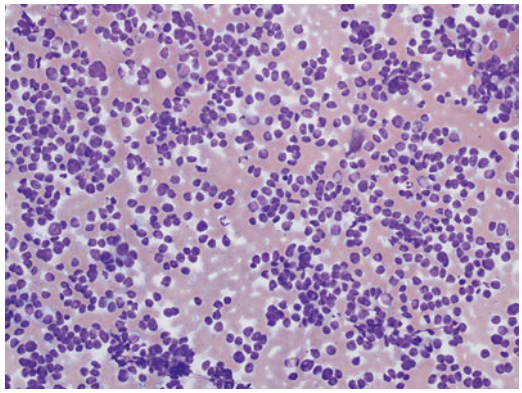


Fig. 9.29 Poorly differentiated carcinoma. Marked hypercellularity with dyscohesive cells (Smear, Diff-Quick)

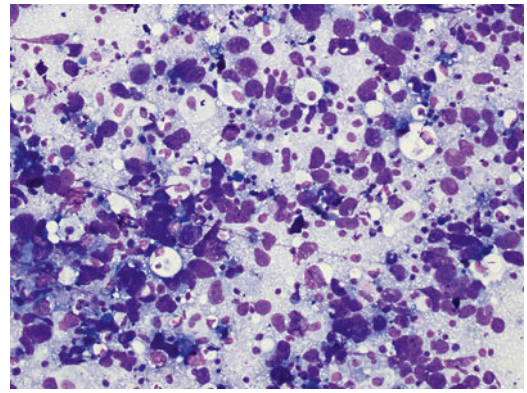


Fig. 9.31 Undifferentiated carcinoma. Highly pleomorphic enlarged nuclei with coarse clumped chromatin, and cell disruption (Smear, Diff-Quick)

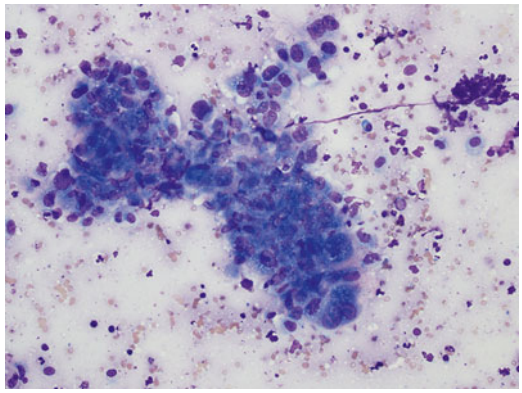


Fig. 9.30 Poorly differentiated carcinoma. Dysplastic cells with a necrotic background (Smear, Diff-Quick)

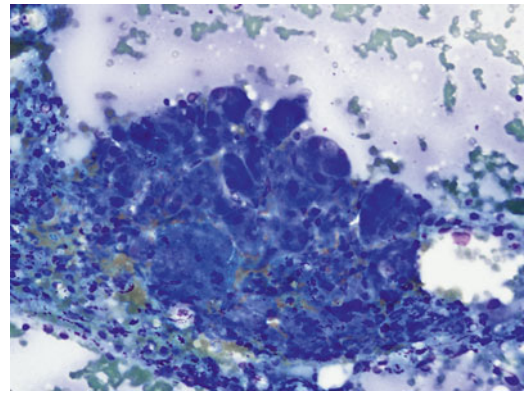


Fig. 9.32 Acute thyroiditis. Reactive follicle cells associated with neutrophils (Smear, Diff-Quick)

background. The neoplastic cells can be in a dyscohesive or clustering pattern. At higher magnification, the malignant features are often obvious and include highly pleomorphic enlarged nuclei with coarse clumped chromatin and occasional naked nuclei (Fig. 9.31). Mitotic figures are often easily identified. The cytodiagnosis of malignancy in both poorly differentiated and undifferentiated carcinoma is rarely a problem.

However, *Acute Thyroiditis* (if aspirated and they virtually never are) can yield a necrotic background reminiscent of these aggressive thyroid malignancies. The clinical diagnosis of acute thyroiditis is usually evident, and this should be a clue to a benign non-neoplastic process. If fine needle aspiration is attempted, it will be painful to the patient and often yield a neutrophilic infiltrate and fibrin. Such an aspirate should be submitted to the microbiology laboratory for analysis. Cytomorphologically, the aspirate contains neutrophils, histiocytes, reactive stromal cells such as fibroblasts, and necrotic debris (Fig. 9.32). The highly dysplastic epithelial cells of poorly differentiated and undifferentiated carcinoma should however be absent.

Similar to radioactive iodine therapy, *Antithyroid Therapy* can induce marked cytological changes mimicking malignancies, especially poorly differentiated and undifferentiated carcinoma. These changes include hyperchromasia, prominent nucleoli and mitotic figures (Fig. 9.33). Here, the clinical history is key to avoid overcalling such aspirates as positive for malignancy.

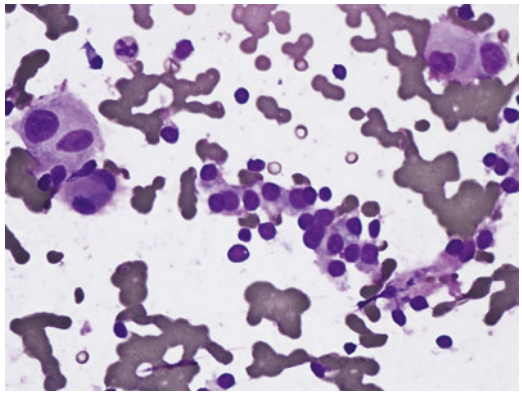


Fig. 9.33 Therapy-induced changes. Follicle cell with enlarged hyperchromatic nuclei following antithyroid therapy (Smear, Diff-Quick)

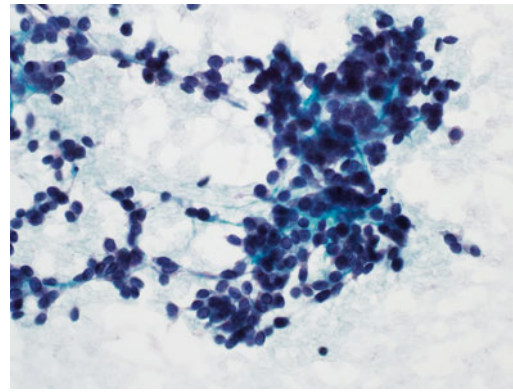


Fig. 9.35 Medullary carcinoma. Neoplastic cells are oval and plasmacytoid and multinucleated (Smear, Papanicolaou)

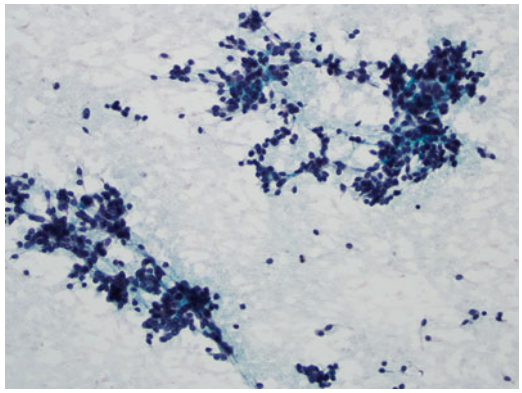


Fig. 9.34 Medullary carcinoma. Hypercellular aspirate with neoplastic cells arranged as loose aggregates and single cells (Smear, Papanicolaou)

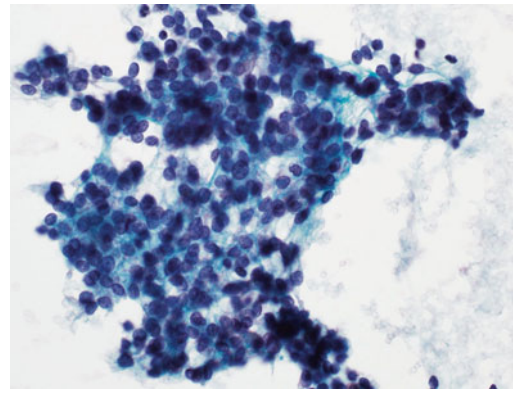


Fig. 9.36 Medullary carcinoma. Chromatin is coarse and granular (Smear, Papanicolaou)

Medullary Thyroid Carcinoma

Medullary Thyroid Carcinoma is a parafollicular C-cell derived malignant tumor. It is a neuroendocrine tumor that is for the most part sporadic, but in up to 30 % it can be associated with familial syndromes (i.e., multiple endocrine neoplasia, and familial medullary thyroid carcinoma syndromes). High clinical suspicion for this tumor can be achieved when serum calcitonin is elevated and there is a germline mutation of the RET oncogene in the setting of a thyroid nodule. At low magnification, the aspirate is hypercellular with neoplastic cells in a predominant single cell pattern or loose aggregates, with occasional amorphous globules of amyloid (Fig. 9.34). At higher magnification, the neoplastic cells can adopt various forms and shapes such as oval, plasmacytoid and spindle. Occasional, they can be multinucleated. The cells also display moderate nuclear enlargement and oval to spindled nuclei (Fig. 9.35). The nuclei commonly are situated eccentrically imparting a plasmacytoid appearance. The chromatin is coarse and granular, consistent with a neuroendocrine neoplasm (Fig. 9.36). The cytoplasm is granular, and small red granules in the cytoplasm have been described with Diff-Quik stains. The immunophenotype is consistent with neuroendocrine differentiation: positive for chromogranin, synaptophysin, neuron-specific enolase, CD56 and calcitonin.

Hashimoto Thyroiditis as described, demonstrates cells with abundant cytoplasm, and

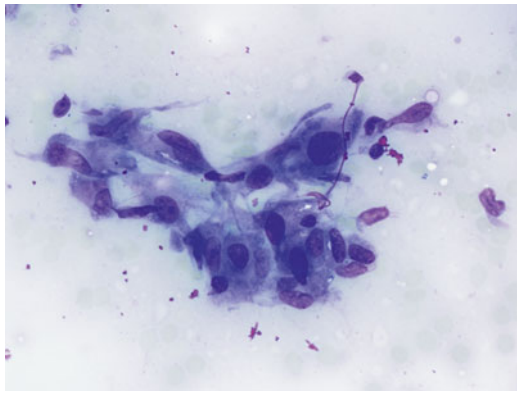


Fig. 9.37 Riedel thyroiditis. Reactive stromal cells (Smear, Diff-Quick)

eccentrically placed nuclei. These plasmacytoid cells may mimic a medullary thyroid carcinoma (Fig. 9.27). However, this should be the extent of similarity. Clinically, the patient with Hashimoto thyroiditis will usually have hypothyroidism. The aspirate will also include a lymphoplasmacytic infiltrate. The oncocytic cells will be positive for thyroglobulin but not for neuroendocrine markers.

Riedel thyroiditis is an invasive fibrosing lesion of the thyroid, often associated with other fibrosing lesions elsewhere in the body. It has been classified as part of the IgG4 sclerosing diseases. The thyroid in these patients is enlarged and hard on palpation. Imaging studies can suggest infiltrative borders. Because of the extensive fibrosis, fine needle aspiration tends not be very cellular. The aspirate can however contain spindle mesenchymal cells with enlarged nuclei, fragments of fibrous tissue (depending on the gauge of the needle used), lymphocytes, plasma cells, neutrophils and eosinophils (Fig. 9.37). These reactive stromal cells can be mistaken for neoplastic epithelial cells of medullary thyroid carcinoma. However, these patients will not possess the abnormal laboratory values seen in the malignant neoplasm. The atypical cells will also be scant.

Hematolymphoid Neoplasms

Primary Thyroid Lymphomas are uncommon, but usually arise in the background of Hashimoto thyroiditis. A rapidly enlarging or dominant nodule in a patient with Hashimoto thyroiditis tends to be suspicious and aspirated. Such a history should alert the cytologist to triage part of the aspirate for additional studies such as flow cytometric and gene rearrangement analysis. The cytomorphological features greatly depend on the type of lymphoma. In general, the aspirate is highly cellular with dyscohesive neoplastic lymphoid cells that can be monomorphic or polymorphic. Normal thyroid parenchyma such as follicles and colloid are minimally present. A definitive diagnosis of hematolymphoid neoplasm is best made in conjunction with ancillary studies and the clinical setting.

Chronic Thyroiditis such as Hashimoto thyroiditis, Graves disease, and Riedel thyroiditis yields a fair amount of lymphoid cells when aspirated (Fig. 9.28). Concern for a hematolymphoid neoplasm is not uncommon. The clinical scenario is the best clue for avoiding overinterpreting a benign lymphoid population as a malignant one. In all cases of chronic thyroiditis, the lymphoid cells tend to be polymorphic and are reactive by immunophenotyping. A special note about Hashimoto thyroiditis: up to 20 % of the cells have been reported to be monoclonal (either kappa or lamba-restricted). Therefore, caution is recommended when the clinical and ancillary tests are equivocal.

Neoplastic Parathyroid Nodules and Its Mimickers (Table 9.2)

Parathyroid Nodules (Fig. 9.38)

Intimately associated with the thyroid gland are the parathyroid glands. Abnormal pathology in the latter is often suspected when there are clinical signs of hyperparathyroidism, or when they are enlarged. This enlargement is detected radiologically as either an intrathyroidal or an extrathyroid juxtaposed nodule to the thyroid. They are infrequently aspirated for cytological evaluation; when they are it is usually because they are suspected of being thyroid nodules.

Table 9.2 Parathyroid nodules

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Parathyroid adenoma • Parathyroid carcinoma	Parathyroid hyperplasia	• Involves all four parathyroid glands	• Minimal	• Clinical correlation
	Parathyroiditis	• Inflammatory condition	• Lymphocytic background	• Clinical and correlation

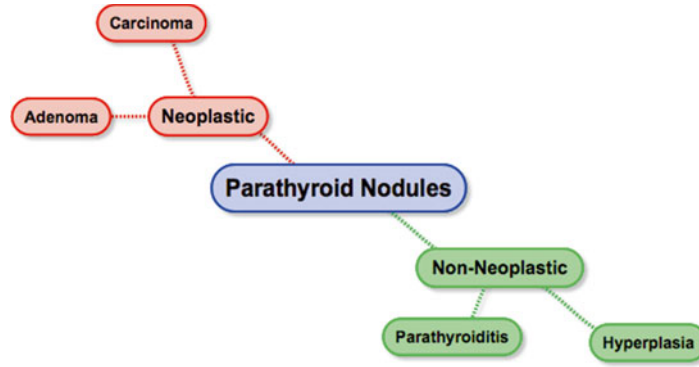


Fig. 9.38 Common parathyroid nodules

Parathyroid Adenoma and Parathyroid Carcinoma

Diagnosis of parathyroid neoplasms is not made on a cytological specimen. The clinical presentation in conjunction with laboratory data (e.g., parathyroid hormone and calcium) and imaging impression are better for uncovering pathological parathyroid lesions. *Parathyroid Adenoma* is suspected when a single parathyroid gland is enlarged and hyperfunctioning. The cytology is similar to normal parathyroid glands. The cells are small, round and uniform with frequent stripped nuclei (Fig. 9.20). Occasionally, a focal increase in pleomorphism may be noted. The characteristics of the cytoplasm may vary depending on the cell types. It can be granular (chief cells), clear (clear cells) or oncocytic (oxyphil cells). These cells may be seen aggregating around capillaries (Fig. 9.21). These cytomorphological features are those of *Parathyroid Carcinoma* as well. The distinction is clinical and often appreciated intraoperatively when local tissue invasion is visible.

The mimickers of these tumors include non-neoplastic parathyroid glands and any causes of parathyroid hyperplasia. The key to avoid false positives is to follow specific steps. First, identify the lesion as parathyroid and not a thyroid lesion. This is not always straightforward, but cytological features favoring parathyroid include naked nuclei, capillaries, mast cells, and minimal to no colloid. Second, be aware of the clinical context. Hyperparathyroidism is associated with specific signs and symptoms and laboratory data. Lastly, defer final characterization of the lesion to clinical and imaging impressions.

Neoplastic Adrenal Gland Nodules and Its Mimickers (Table 9.3)

Adrenal Gland Nodules (Fig. 9.39)

Masses in the adrenal glands can arise either in the cortex or in the medulla. Regardless of the location, diagnostic imaging can easily detect abnormal, even clinically insignificant, nodules in these glands and often as an incidental finding. Frequently, however, these nodules can secrete chemicals or hormones that give rise to clinical signs and symptoms suggesting their identities.

Table 9.3 Adrenal gland nodules

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Adrenal cortical adenoma	Adrenal hyperplasia	• Congenital	• Minimal	• Clinical correlation
	Normal adrenal cortical tissue	• Sampling error	• Minimal	• Clinical correlation
	Hepatocytes	• Contamination during sampling	• Ample cytoplasm • Round nucleus with conspicuous nucleolus • Pigment may be visible	• Clinical and imaging correlation • Immunocytochemistry
• Adrenal cortical carcinoma	Adrenal hyperplasia	• Congenital	• Naked nuclei	• Clinical correlation
	Normal adrenal cortical tissue	• Sampling error	• Naked nuclei	• Clinical correlation
	Hepatocytes	• Contamination during sampling	• Ample cytoplasm • Round nucleus with conspicuous nucleolus • Pigment may be visible	• Clinical and imaging correlation • Immunocytochemistry
• Pheochromocytoma	Normal adrenal medulla	• Sampling error	• Hypocellular	• Clinical, imaging and laboratory correlation
• Adrenal myelolipoma	Adipose tissue	• Sampling error	• Absence of hematopoietic elements	• Clinical and imaging correlation

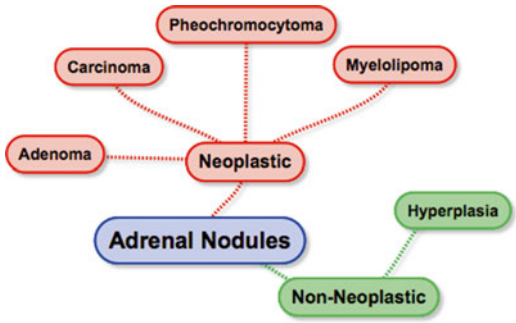


Fig. 9.39 Common adrenal gland nodules

While the majority of these nodules consists of benign neoplasms, there are a few non-neoplastic conditions that potentially can mimic radiologically and cytomorphologically both benign and malignant tumors. Definitive distinction between non-neoplastic and neoplastic entities by cytology is not always possible. Incorporating the clinical, imaging and laboratory impression is crucial during cytodiagnosis.

Adrenal Cortical Adenoma

Adrenal Cortical Adenoma tends to be solitary and rarely exceeds 10 cm. Aspiration yields a population of benign-appearing cortical cells. These have a low nucleocytoplasmic ratio with round small nuclei and moderate to ample clear to vacuolated cytoplasm. A predominance of naked nuclei is often identified. Occasionally, focal pleomorphism, hyperchromasia, and prominent nucleoli may observed (Fig. 9.40). Necrosis and mitoses are not cytomorphological features of adrenal cortical adenoma.

The aspirates of *Adrenal Hyperplasia* and *Normal Adrenal Cortical Cells* are virtually indistinguishable from those of an adrenal cortical adenoma. Clinical context, in conjunction with the imaging impression, are very important to suggest a cytodiagnosis of an adenoma.

Inadvertent sampling of the liver can lead to the presence of benign cells of hepatic origin that could be misinterpreted as adrenal cortical

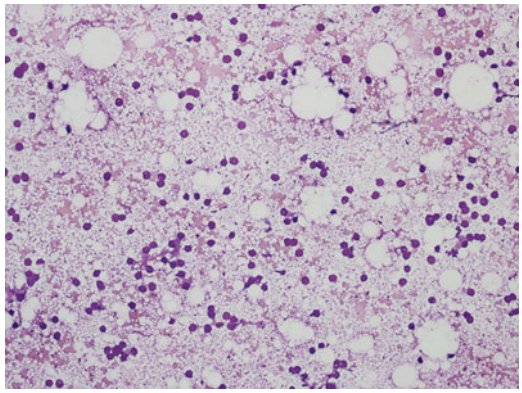


Fig. 9.40 Adrenal cortical adenoma. Numerous naked round nuclei with minimal pleomorphism and prominent cell fragility (dissolution) (Smear, Diff-Quick)

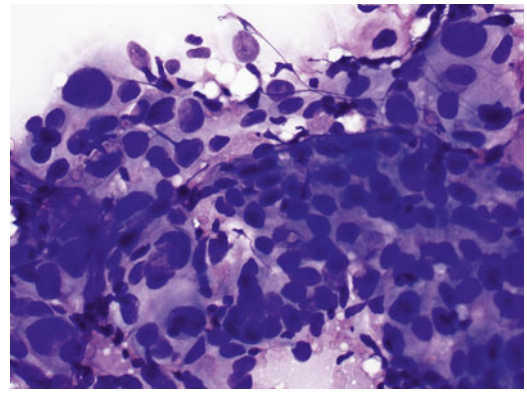


Fig. 9.42 Adrenal cortical carcinoma. Loose cluster of pleomorphism cells with retained cytoplasm (Smear, Diff-Quick)

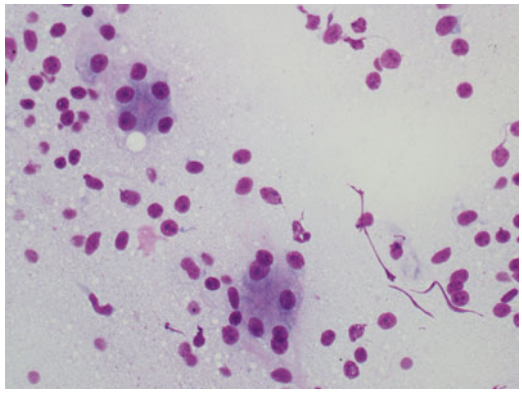


Fig. 9.41 Hepatocytes. The cells have a low nucleocytoplasmic ratio with ample granular cytoplasm. The nuclei may contain conspicuous nucleoli (Smear, Diff-Quick)

adenoma cells. The *Hepatocytes* will have ample cytoplasm. Their nuclei may contain inclusions. The nucleocytoplasmic ratio will be low (Fig. 9.41). If any pigments are detected in such cells, proceed to immunocytochemistry to rule out liver contamination. Hepatocytes are positive for Hep-Par1 and arginase.

Adrenal Cortical Carcinoma

This malignant counterpart of the adrenal adenoma is rare. *Adrenal Cortical Carcinoma* often exceeds 10 cm. The malignant cells tend to be dyscohesive or loosely cohesive. They retain their cytoplasm. Nuclear pleomorphism is not uncommon. Multinucleation and prominent nucleoli are often obvious (Fig. 9.42).

Adrenal Hyperplasia and *Normal Adrenal Cortical Cells* can be distinguished with much more ease. These two entities tend to present with diffuse nodularity or with a smaller unifocal nodule, respectively. The benign cortical cells have fragile cytoplasm yielding naked nuclei in the aspirate, similar to adrenal adenoma (Fig. 9.40).

Adrenal Pheochromocytoma

If clinical and laboratory assessment of a patient is consistent with *Pheochromocytoma*, then fine needle aspiration is contraindicated. However, if aspirated, it is hypercellular with variably spindled, epithelioid and plasmacytoid cells (Fig. 9.43). Immunocytochemistry on a cell block will reveal immunoreactivity to neuroendocrine markers (e.g., chromogranin, synaptophysin, CD56). While these cytological findings highly suggest this tumor, correlate with clinical and laboratory data.

In the rare event, the adrenal gland medulla is aspirated, the cells will have the above features. But a diagnosis of pheochromocytoma should not be made in the absence of the appropriate clinical context. Additionally, the aspirate will not be very cellular.

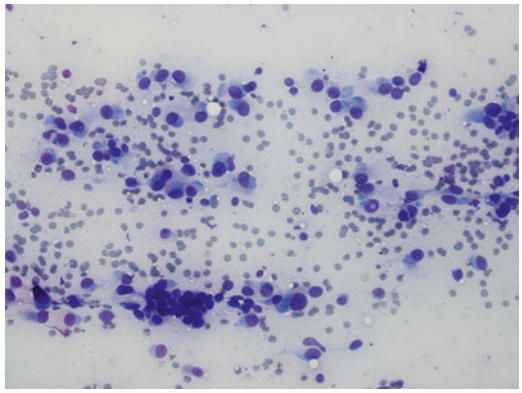


Fig. 9.43 Pheochromocytoma. Epithelioid and plasmacytoid cells (Smear, Diff-Quick)

Adrenal Myelolipoma

Adrenal Myelolipoma is a benign tumor of the adrenal gland. As the name implies, this neoplasm contains normal appearing bone marrow elements and mature adipose tissue. The former include megakaryocytes, nucleated red blood cells, and immature granulocytes.

The main mimicker of this tumor is from sampling error. Perirenal or periadrenal adipose tissue is aspirated. However, the absence of hematopoietic elements should preclude a definitive diagnosis.

Neoplastic Ovarian Masses and Its Mimickers (Table 9.4)

Ovarian Masses (Fig. 9.44)

Traditionally, frankly malignant-appearing or markedly enlarged ovaries by imaging analysis are not aspirated. Surgical resection is the next step, as it is often both therapeutic and diagnostic. However, a small incidental ovarian cyst or mildly enlarged ovary detected by ultrasound during an unrelated clinical work-up can be subject to fine needle aspiration. Therefore, the majority of cytological specimens encountered will have a high negative predictive value. Despite these odds, false-positives can occur, especially with hypercellular aspirates from follicle cysts showing mitotic activity. Correlation with clinical, sonographic and any adjunct laboratory analysis impression is advised to avoid false-positives.

Surface-Epithelial-Stromal Tumors

These tumors often have a characteristic sonographic appearance that negates the need for fine needle aspiration. They are generally classified as serous, mucinous, transitional, endometrioid and clear cells. Each of these neoplasms has benign, borderline and malignant counterparts. Rarely, the borderline and malignant ones are seen in cytology. More commonly, the benign types are aspirated. A detailed and comprehensive discussion of all surface-epithelial stromal tumors is beyond the scope of this book. Here, the focus will be the most commonly encountered types, namely serous and mucinous neoplasm with their corresponding mimickers.

Serous Tumors tend to produce clear fluid. In the benign variant, the aspirate is often hypocellular but may yield ciliated or non-ciliated columnar cells, or cuboidal cells. They are more likely to be in small crowded 3-dimensional clusters (Fig. 9.45). The cells have high nucleocytoplasmic ratios. The nuclear membranes are smooth. In the borderline and malignant variants, increased pleomorphism is appreciated. The cells are in sheets, branching structures, and in a single cell pattern. Cytoplasmic vacuoles can be seen covering more than one nuclei. The latter can be markedly enlarged with prominent nucleoli (Fig. 9.46).

Mucinous Tumors tend to produce mucinous, gelatinous fluid. In the benign variant, the neoplastic cells are columnar with apical mucin. No significant pleomorphism is present (Fig. 9.47). In the borderline and malignant variants, as with serous types, pleomorphism is conspicuous. The cells are arranged in sheets or in single cell pattern. Glandular formations can be observed. The enlarged nuclei contain conspicuous nucleoli (Fig. 9.48).

Follicle Cysts represent the most common non-neoplastic and physiological cystic lesions

Table 9.4 Ovarian masses

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Surface epithelial stromal tumors	Follicle cyst	• Physiologic	• No ciliated or mucinous epithelium • No dysplastic cells • Present in small 2- to 3-dimensional clusters and singly	• Clinical and imaging correlation • Immunocytochemistry
	Endometriotic cyst	• Endometriosis	• Abundant hemosiderin-laden macrophages	• Clinical correlation
	Contaminants	• Sampling device traversing different structures	• Cellular contaminants correspond to structures/organs traversed	• Clinical and imaging correlation • Immunocytochemistry
• Sex-cord stromal tumors	Follicle cyst	• Physiologic	• No ciliated or mucinous epithelium • No dysplastic cells • Present in small 2- to 3-dimensional clusters and singly	• Clinical and imaging correlation • Immunocytochemistry
	Contaminants	• Sampling device traversing different structures	• Cellular contaminants correspond to structures/organs traversed	• Clinical and imaging correlation • Immunocytochemistry
• Germ cell tumors	Contaminants	• Sampling device traversing different structures	• Cellular contaminants correspond to structures/organs traversed	• Clinical and imaging correlation • Immunocytochemistry

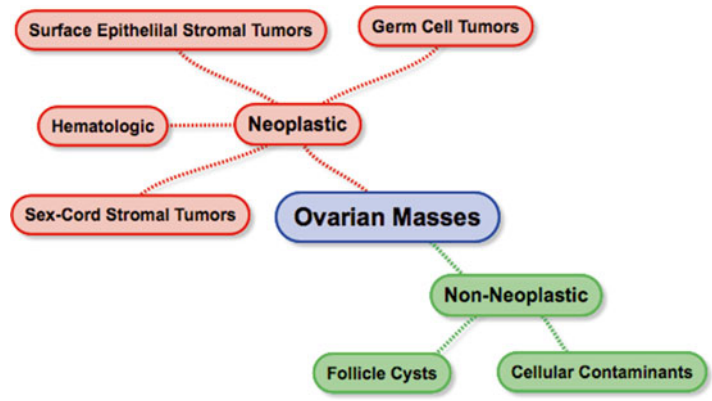


Fig. 9.44 Common ovarian masses

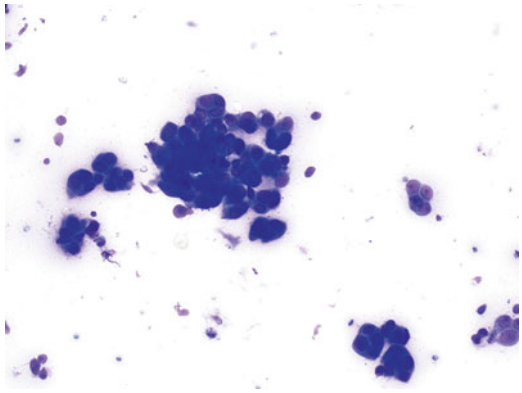


Fig. 9.45 Ovarian benign serous neoplasm. Cuboibal cells in a small crowded three-dimensional cluster (Smear, Diff-Quick)

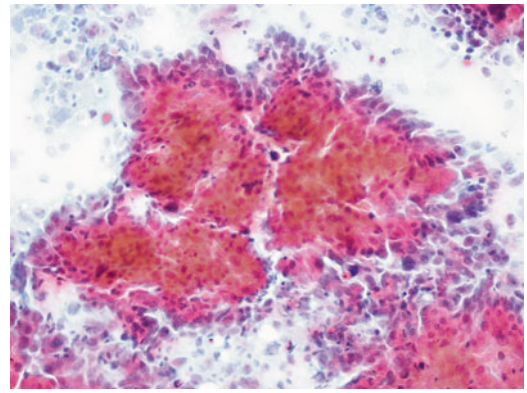


Fig. 9.48 Ovarian mucinous adenocarcinoma. Hypercellular aspirate of atypical columnar cells in sheets (Smear, Diff-Quick)

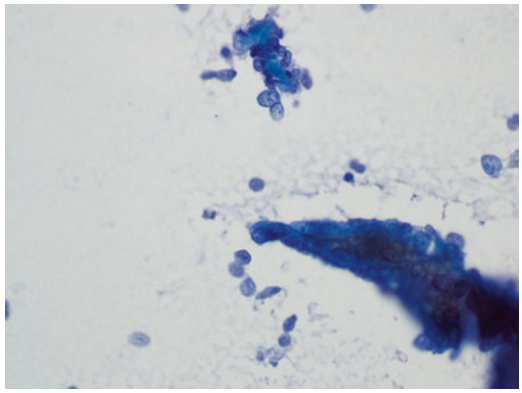


Fig. 9.46 Ovarian serous carcinoma. Dysplastic cells with increased nucleocytoplasmic ratio and in papillary configuration (Smear, Papanicolaou)

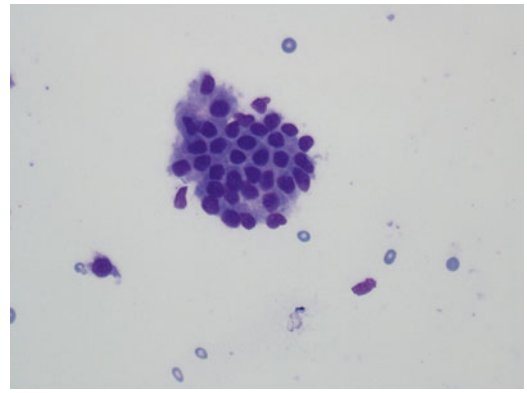


Fig. 9.49 Follicle cyst. Follicle cells dispersed singly and in small two-dimensional clusters (Smear, Diff-Quick)

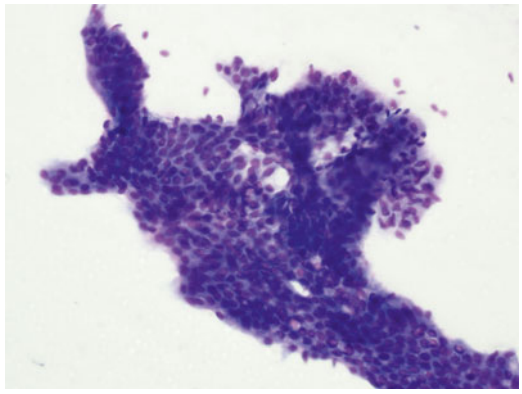


Fig. 9.47 Ovarian benign mucinous neoplasm. Sheet of neoplastic columnar cells without significant pleomorphism (Smear, Diff-Quick)

of the ovary. They are often detected incidentally by imaging studies or intraoperatively. These cysts are unilocular and non-complex. On cytology, they contain cells in small 2- to 3-dimensional clusters and singly. The cells have round nuclei with coarsely granular chromatin. Nucleoli may be appreciated (Fig. 9.49). The presence of mitotic figures is not uncommon. When granulosa cells are luteinized, they present with foamy cytoplasm. If the aspirate is cellular and contains numerous mitotic figures, it may raise the possibility of an epithelial tumor. However, follicle cysts do not contain ciliated or mucinous epithelium. Additionally, refer to the clinical and sonographic findings which may favor a benign

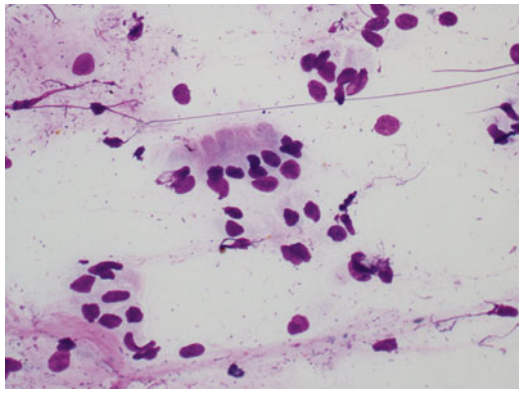


Fig. 9.50 Colorectal epithelium. Bland-appearing columnar cells (Smear, Diff-Quick)

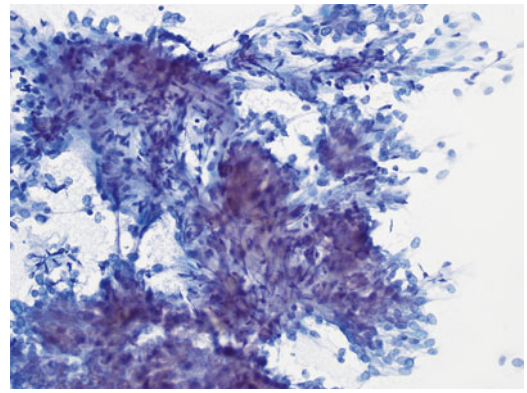


Fig. 9.51 Juvenile granulosa cell tumor. Sheet of oval neoplastic cells traversed by capillaries (Smear, Papanicolaou)

non-neoplastic lesion. If a cell block can be obtained, the granulosa cells of follicle cysts are positive for inhibin but negative for EMA and CK7. The opposite is seen in epithelial cysts. Check if fluid from the cyst was sent for laboratory analysis, as estradiol (E2) levels are elevated in follicle cysts.

Sampling contaminants can be a huge source of mimickers. The ovaries can be accessed via multiple pathways depending on the location and size of the targeted nodule. The cytological specimen can be collected transrectally, transvaginally, percutaneously or intra-operatively. *Colorectal Epithelium* may suggest a mucinous neoplasm; however these epithelial cells will not be abundant (Fig. 9.50). If present on the cell block, their immunophenotype will be consistent with intestinal differentiation (positive for CK20, CDX2 and SATB2).

Sex-Cord Stromal Tumors

Sex-cord stromal tumors are often not a clinical mystery, as they tend to secrete hormones or chemicals leading to characteristic signs and symptoms. The major tumors in this category include granulosa cell tumor, thecoma, fibroma, and Sertoli-Leydig cell tumor.

Granulosa Cell Tumors are derived, as the name implied, from granulosa cells. When the adult form is aspirated, the specimen is richly cellular with neoplastic cells present singly, in sheets and in clusters. The cells, with minimal cytoplasm, have round to oval nuclei with pale chromatin and occasional nucleoli. Nuclear grooves are frequently identified, in contrast to the juvenile type which shows no nuclear grooves (Fig. 9.51). They are immunoreactive to inhibin and calretinin.

As with surface-epithelial tumors, *Follicle Cysts* can mimic granulosa cell tumor. Follicle cysts contain granulosa cells. However, the nuclei are not oval and do not contain grooves. Reassuring features include benign clinical, laboratory and imaging interpretation.

Thecomas, Fibromas, Thecofibromas are very difficult to be diagnosed by cytology, as they commonly yield hypocellular aspirates. When cellular materials are present, they include benign-appearing spindle cells (Fig. 9.52). Therefore, a descriptive diagnosis is more appropriate followed by a differential diagnosis.

Germ Cell Tumors

Germ cell tumors are often clinically suspected and rarely aspirated. The list includes teratoma (mature and immature), dysgerminoma, embryonal cell carcinoma, yolk sac tumor, and choriocarcinoma. Here again only the most common types will be addressed.

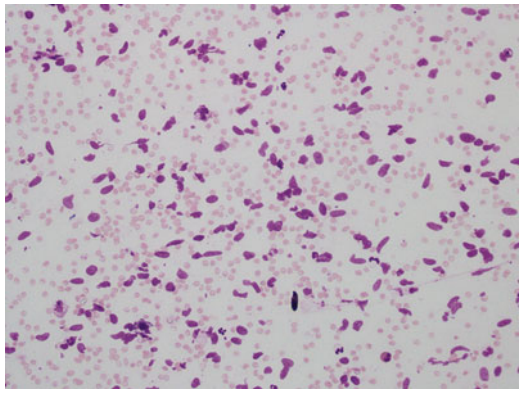


Fig. 9.52 Fibroma. Hypercellular aspirate with bland-appearing spindle cells (Smear, Diff-Quick)

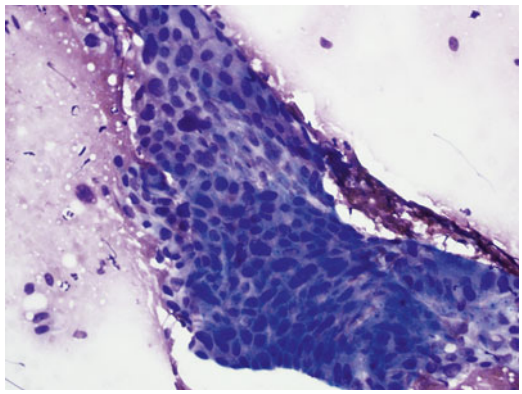


Fig. 9.53 Teratoma. Sheet of squamous epithelium (Smear, Diff-Quick)

Teratomas are neoplasms composed of tissue derived from endoderm, ectoderm and mesoderm. They can be mature or immature. These tumors are rarely sampled for cytology. In the mature variant, the aspirate is remarkable for numerous anucleated squamous cells, ciliated, mucinous cells or sheets of squamous cells (Fig. 9.53). The immature variant is exceedingly rare, and the diagnostic immature component may not be well represented on cytology.

As noted, sampling contaminants can be a huge source of mimickers. The cytological specimen can contain intestinal and squamous epithelium if the needle went through the colon and skin, respectively. These tissues can be seen in teratomas. However, these contaminants are unlikely to be present in abundance. Correlation with clinical and imaging is crucial.

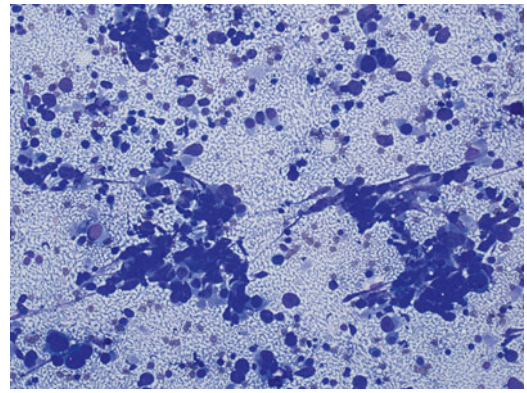


Fig. 9.54 Dysgerminoma. Epithelioid single cells with large nuclei, admixed with small mature lymphocytes in a tigroid background (Smear, Diff-Quick)

Dysgerminomas are solid ovarian masses akin to seminomas of the testes. Their aspirates are richly cellular composed of epithelioid single cells admixed with small mature lymphocytes. The epithelioid neoplastic cells have large nuclei with prominent nucleoli (Fig. 9.54). The cytoplasm tends to be granular. Immunophenotypically, tumor cells are positive for PLAP, CD117, SALL4, and OCT3/4.

Neoplastic Testicular Masses and Its Mimickers

Testicular masses are essentially not aspirated or biopsied. Concerns for seeding are high. Therefore, any masses suspicious by sonographic evaluation, clinical assessment and laboratory analysis are surgically resected.

Conclusion

When clinically or radiographically suspicious nodules are present in some of the major endocrine organs, cytology plays a key role in the initial evaluation. By far, the thyroid gland is the most commonly aspirated endocrine organ for cytological evaluation when nodules are identified. Occasionally, lesions from the parathyroid, adrenal glands and ovaries are cytologically sampled. Each of these organs is susceptible to

non-neoplastic conditions with cytomorphological findings that have been described in neoplastic states in these organs. However, intimate knowledge of the clinical, radiologic and laboratory assessment is critical when interpreting cytologic specimen from these or organs. Furthermore, subtle but definitive cytological features can be used in some case to distinguish a reactive from a neoplastic process.

Suggested Readings

1. Absher KJ, Truong LD, Khurana KK, et al. Parathyroid cytology: avoiding diagnostic pitfalls. Head Neck. 2002;24(2):157–64.
2. Allias F, Chanoz J, Blache G, et al. Value of ultrasound-guided fine-needle aspiration in the management of ovarian and paraovarian cysts. Diagn Cytopathol. 2000;22(2):70–80.
3. Baloch ZW, LiVolsi VA. Post fine-needle aspiration histologic alterations of thyroid revisited. Am J Clin Pathol. 1999;112(3):311–6.
4. Calderaro J, Andreiuolo F, Baudin E, et al. Fine needle aspiration cytology of oxyphilic parathyroid hyperplasia. Cytopathology. 2010;21(2):135–6.
5. Carcangiu ML, Steeper T, Zampi G, et al. Anaplastic thyroid carcinoma. A study of 70 cases. Am J Clin Pathol. 1985;83(2):135–58.
6. Centeno BA, Szyfelbein WM, Daniels GH, et al. Fine needle aspiration biopsy of the thyroid gland in patients with prior Graves' disease treated with radioactive iodine. Morphologic findings and potential pitfalls. Acta Cytol. 1996;40(6):1189–97.
7. Chen HI, Akpolat I, Mody DR, et al. Restricted kappa/lambda light chain ratio by flow cytometry in germinal center B cells in Hashimoto thyroiditis. Am J Clin Pathol. 2006;125(1):42–8.
8. Chen KT. Extraneous cells of hepatic origin in adrenal fine needle aspiration as a diagnostic pitfall: a case report. Acta Cytol. 2005;49(4):449–51.
9. Cibas ES, Alexander EK, Benson CB, et al. Indications for thyroid FNA and pre-FNA requirements: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of the Science Conference. Diagn Cytopathol. 2008;36(6):390–9.
10. Cibas ES, Ducatman BS. Cytology: diagnostic principles and clinical correlates. 4th ed. Philadelphia, PA: Elsevier/Saunders; 2014.
11. Demay RM. The art and science of cytopathology. 2nd ed. Chicago, IL: ASCP Press; 2011.
12. de Agustín P, López-Ríos F, Alberti N, et al. Fine-needle aspiration biopsy of the adrenal glands: a ten-year experience. Diagn Cytopathol. 1999;21(2):92–7.
13. Derringer GA, Thompson LD, Frommelt RA, et al. Malignant lymphoma of the thyroid gland: a clinicopathologic study of 108 cases. Am J Surg Pathol. 2000;24(5):623–39.
14. Ezzat S, Sarti DA, Cain DR, et al. Thyroid incidentalomas. Prevalence by palpation and ultrasonography. Arch Intern Med. 1994;154(16):1838–40.
15. Faquin WC, Cibas ES, Renshaw AA. "Atypical" cells in fine-needle aspiration biopsy specimens of benign thyroid cysts. Cancer. 2005;105(2):71–9.
16. Ganjei P, Dickinson B, Harrison T, et al. Aspiration cytology of neoplastic and non-neoplastic ovarian cysts: is it accurate? Int J Gynecol Pathol. 1996;15(2):94–101.
17. Gonzalez JL, Wang HH, Ducatman BS. Fine-needle aspiration of Hürthle cell lesions. A cytomorphologic approach to diagnosis. Am J Clin Pathol. 1993;100(3):231–5.
18. Gordon DL, Gattuso P, Castelli M, et al. Effect of fine needle aspiration biopsy on the histology of thyroid neoplasms. Acta Cytol. 1993;37(5):651–4.
19. Hamberger B, Gharib H, Melton LJ, et al. Fine-needle aspiration biopsy of thyroid nodules. Impact on thyroid practice and cost of care. Am J Med. 1982;73(3):381–4.
20. Harach HR, Zusman SB, Saravia Day E. Nodular goiter: a histo-cytological study with some emphasis on pitfalls of fine-needle aspiration cytology. Diagn Cytopathol. 1992;8(4):409–19.
21. Harigopal M, Sahoo S, Recant WM, et al. Fine-needle aspiration of Riedel's disease: report of a case and review of the literature. Diagn Cytopathol. 2004;30(3):193–7.
22. Hoda RS. Non-gynecologic cytology on liquid-based preparations: a morphologic review of facts and artifacts. Diagn Cytopathol. 2007;35(10):621–34.
23. Keyhani-Rofagha S, Kooner DS, Landas SK, et al. Black thyroid: a pitfall for aspiration cytology. Diagn Cytopathol. 1991;7(6):640–3.
24. Kuma S, Hirokawa M, Miyauchi A, et al. Cytologic features of hyalinizing trabecular adenoma of the thyroid. Acta Cytol. 2003;47(3):399–404.
25. McCorkell SJ, Niles NL. Fine-needle aspiration of catecholamine-producing adrenal masses: a possibly fatal mistake. AJR Am J Roentgenol. 1985;145(1):113–4.
26. Moran O, Menczer J, Ben-Baruch G, et al. Cytologic examination of ovarian cyst fluid for the distinction between benign and malignant tumors. Obstet Gynecol. 1993;82(3):444–6.
27. Lal A, Bourtsos EP, Nayar R, et al. Cytologic features of granulosa cell tumors in fluids and fine needle aspiration specimens. Acta Cytol. 2004;48(3):315–20.
28. Mulvany NJ. Aspiration cytology of ovarian cysts and cystic neoplasms. A study of 235 aspirates. Acta Cytol. 1996;40(5):911–20.
29. Nassar A, Gupta P, LiVolsi VA, et al. Histiocytic aggregates in benign nodular goiters mimicking cytologic features of papillary thyroid carcinoma (PTC). Diagn Cytopathol. 2003;29(5):243–5.

30. Odashiro AN, Nguyen GK. Fine-needle aspiration cytology of an intrathyroid parathyroid adenoma. Diagn Cytopathol. 2006;34(11):790–2.
31. Odronic SI, Reynolds JP, Chute DJ. Cytologic features of parathyroid fine-needle aspiration on ThinPrep preparations. Cancer Cytopathol. 2014;122(9):678–84.
32. Nguyen GK, Akin MR. Cytopathology of insular carcinoma of the thyroid. Diagn Cytopathol. 2001;25(5):325–30.
33. Nuñez C, Diaz JI. Ovarian follicular cysts: a potential source of false positive diagnoses in ovarian cytology. Diagn Cytopathol. 1992;8(5):532–6. Discussion 536–537.
34. Rossi ED, Revelli L, Giustozzi E, et al. Large nonfunctioning parathyroid cysts: our institutional experience of a rare entity and a possible pitfall in thyroid cytology. Cytopathology. 2015;26(2):114–21.
35. Saxena A, Alport EC, Moshynska O, et al. Clonal B cell populations in a minority of patients with Hashimoto's thyroiditis. J Clin Pathol. 2004;57(12):1258–63.
36. Selvaggi SM. Cytology of nonneoplastic cysts of the ovary. Diagn Cytopathol. 1990;6(2):77–85.
37. Shabb NS, Salti I. Subacute thyroiditis: fine-needle aspiration cytology of 14 cases presenting with thyroid nodules. Diagn Cytopathol. 2006;34(1):18–23.
38. Smejkal V, Smejkalová E, Rosa M, et al. Cytologic changes simulating malignancy in thyrotoxic goiters treated with carbimazole. Acta Cytol. 1985;29(2):173–8.
39. Stanley MW, Horwitz CA, Frable WJ. Cellular follicular cyst of the ovary: fluid cytology mimicking malignancy. Diagn Cytopathol. 1991;7(1):48–52.
40. Suen KC. How does one separate cellular follicular lesions of the thyroid by fine-needle aspiration biopsy? Diagn Cytopathol. 1988;4(1):78–81.
41. Tabbara SO, Acoury N, Sidawy MK. Multinucleated giant cells in thyroid neoplasms. A cytologic, histologic and immunohistochemical study. Acta Cytol. 1996;40(6):1184–8.
42. Trimbos JB, Hacker NF. The case against aspirating ovarian cysts. Cancer. 1993;72(3):828–31.
43. Tulecke MA, Wang HH. ThinPrep for cytologic evaluation of follicular thyroid lesions: correlation with histologic findings. Diagn Cytopathol. 2004;30(1):7–13.
44. Us-Krasovec M, Auersperg M, Bergant D, et al. Medullary carcinoma of the thyroid gland: diagnostic cytopathological characteristics. Pathologica. 1998;90(1):5–13.
45. Yang J, Schnadig V, Logrono R, et al. Fine-needle aspiration of thyroid nodules: a study of 4703 patients with histologic and clinical correlations. Cancer. 2007;111(5):306–15.
46. Wojcik EM, Selvaggi SM. Fine-needle aspiration cytology of cystic ovarian lesions. Diagn Cytopathol. 1994;11(1):9–14.
47. Wu HH, Cramer HM, Kho J, et al. Fine needle aspiration cytology of benign adrenal cortical nodules. A comparison of cytologic findings with those of primary and metastatic adrenal malignancies. Acta Cytol. 1998;42(6):1352–8.
48. Yee H, Greenebaum E, Lerner J, et al. Transvaginal sonographic characterization combined with cytologic evaluation in the diagnosis of ovarian and adnexal cysts. Diagn Cytopathol. 1994;10(2):107–12.
49. Young RH. New and unusual aspects of ovarian germ cell tumors. Am J Surg Pathol. 1993;17(12):1210–24.

Body Cavity Fluids

10

Brief Introduction

Various diseases, neoplastic and non-neoplastic, tend to cause fluid build-up in the cavities between surfaces of body walls (parietal) and of organs (visceral). These body cavities include the thorax, the pericardium, the abdomen, and the tunica vaginalis testis in men. Regardless of the anatomical cavities, the surfaces are lined by serous membranes composed of a single layer of cytologically identical, thin and flat mesothelial cells which, when irritated, become plump, cuboidal and hyperplastic.

Clinically significant fluid accumulation, or effusion, is defined as fluid exceeding 300–500 ml in volume. During clinical work-up of an effusion, a portion of the excess fluid is commonly aspirated by thoracentesis, pericardiocentesis, or abdominal paracentesis and submitted for cytological evaluation. Another major source of fluid cytology is a peritoneal washing. The latter is performed intraoperatively in staging of mullerian malignancies to evaluate involvement of the peritoneum by the tumor.

Therefore, an important question for the cytopathologist is whether the fluid represents a cancerous or a non-neoplastic process such as infection, intravascular pressure disequilibrium, trauma or connective tissue disorders (Fig. 10.1). Such distinctions can be especially challenging because fluids from any these non-neoplastic conditions can contain a reactive and worrisome population of mesothelial cells mimicking mesothelioma and metastatic carcinoma.

Fortunately, correlating the cytomorphological findings with the clinical and imaging impression of the fluid can significantly decrease false-positives. Furthermore, the physical characteristics of the effusion can also help in this endeavor, because transudate fluid is generally benign, whereas exudate fluid is generally more likely to be malignant. In this chapter, the discussion will focus primarily on cytomorphological features and ancillary tests that can make the distinction between malignant and reactive effusions more attainable.

Classification of Confounders Based on Common Malignancies Detected in the Body Cavities

The body cavities are frequently affected by malignancies, primary or metastatic. When involved, the body cavities' fluid volume increases. Alternatively, peritoneal washings can capture grossly and radiologically undetectable tumor in the peritoneum. Unfortunately, every subtype of tumor can be mimicked by non-neoplastic processes. For the purpose of discussion, the confounders will be grouped according to most common types of malignancies detected by cytology. By far, metastasis tops the list of

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_10

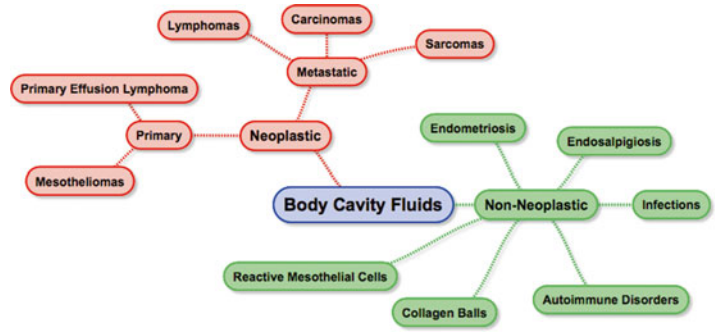


Fig. 10.1 Body cavity fluids lesions

malignant fluid and includes tumors of epithelial type (e.g., from lung, breast, gastrointestinal tract, hepatobiliary, mullerian, neuroendocrine origin), tumors of lymphoreticular type, and tumors of mesenchymal type. Mesothelioma is the major primary malignancy and deserves special attention. In the case of mesothelioma, cytomorphology is generally more important than ancillary testing. For mimickers of lymphoid and mesenchymal malignancies, please refer to the respective chapters for more in depth discussions.

Mimickers of Epithelial-Type Malignancy in the Body Cavities

Malignant epithelial neoplasms from anywhere in the body are capable of migrating into the body cavities (Table 10.1). When this occurs, the clinical and radiographic findings are associated with excess fluid accumulation in these cavities. An important but often overlooked piece of information is the general physical characteristic of the fluid being evaluated. Malignant effusions tend to be exudates (high protein content >3.0 g/dL, high specific gravity >1.015). The cytological findings will depend on the type of *Metastatic Carcinoma*. In general, the neoplastic metastatic cells are cytomorphologically distinct from background benign mesothelial cells. The former can be either larger or smaller than the latter, but with a significant increase nucleocytoplasmic ratio (Fig. 10.2). The malignant epithelial cells can be single or in clusters (Fig. 10.3). When in clusters, they tend to have a smooth "community" border (Fig. 10.4). If the fluid is involved by an adenocarcinoma, the neoplastic cells may show large vacuoles, often covering more than one nucleus (Fig. 10.5). Increased mitotic activity may be observed. Some carcinomas, like those arising from the breast, tend to form multiple tight clusters that can be appreciated at low power, often called "cannon balls" (Fig. 10.6). On cell block, clusters of malignant epithelial cells tend to retract from surrounding cell block material and other benign cells, forming lacunae (Fig. 10.7).

Correctly identifying a second population of metastatic malignant epithelial cells is however not always easy. By far, the biggest source of confusion in fluid cytology is the presence of *Reactive Mesothelial Cells* when assessing for involvement by adenocarcinoma. This is because mesothelial cells, in the setting of infection, inflammation or cellular degeneration, are notorious for resembling adenocarcinoma in a cytology specimen. Mesothelial cells from pericardial fluid are also notorious for being exuberantly reactive. Fortunately, certain cytomorphological features can help in the distinction. Benign effusions tend to be transudates (low protein content <3.0 g/dL and low specific gravity <1.015).The size of a mesothelial cell varies with the degree of reactive changes. Their individual cell border has been described as a "lacy skirt" secondary to surface microvilli and to the differential hue of the peripheral cytoplasm. The microvilli are best appreciated by electron microscopy, but occasionally discerned as metachromatic fuzzy edges on Romanowsky stain or Papanicolaou stain (Fig. 10.8). "Blebs," which are blunt and round cytoplasmic projections, can be seen when the mesothelial cells are degenerated (Fig. 10.9).

Table 10.1 Mimickers of metastatic carcinomas in body cavity fluids

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Metastatic carcinomas	Mesothelial cells in aggregates	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• Aggregates have knobby contours • Aggregates not numerous • Windows (spaces) can be discerned within groups • No second distinct population of cells present	• Clinical and radiographic correlation • Transudate vs Exudate • Panel of immunocytochemistry specific for mesothelial cells and epithelial cells
	Mesothelial cells with vacuolated cytoplasm	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• No significant pleomorphism • Cytoplasm finely vacuolated	• Clinical and radiographic correlation • Transudate vs exudate • Panel of immunocytochemistry specific for mesothelial cells and epithelial cells • Mucicarmine (may be positive in adenocarcinoma)
	Mesothelial cells with nuclear enlargement	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• No significant pleomorphism • No second distinct population of cells present	• Clinical and radiographic correlation • Transudate vs exudate • Panel of immunocytochemistry specific for mesothelial cells and epithelial cells
	Mesothelial cells with mitotic figures	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• Mitotic figures are rare • No atypical mitosis • No second distinct population of cells present	• Clinical and radiographic correlation • Transudate vs exudate • Panel of immunocytochemistry specific for mesothelial cells and epithelial cells
	Mesothelial cells with degeneration	• Poorly processed and/or stored fluid	• No second distinct population of cells present • Cytoplasmic and nuclear details poorly visualized	• Clinical and radiographic correlation • Transudate vs exudate • Panel of immunocytochemistry specific for mesothelial cells and epithelial cells • Request fresh new fluid

(continued)

Table 10.1 (continued)

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
	Histiocytes with vacuolated cytoplasm	• Benign fluids	• Debris materials may be seen within the cytoplasm • No significant nuclear enlargement	• Clinical and radiographic correlation • Transudate vs exudate • Panel of immunocytochemistry specific for histiocytes and epithelial cells • Mucicarmine (may be positive in adenocarcinoma
	Histiocytes with multinucleation	• Granulomatous diseases • History of talc • History of trauma • History of radiation or chemotherapy	• Multinucleated cells with no significant pleomorphism • Cytoplasm is foamy	• Clinical and radiographic correlation • Transudate vs exudate • Panel of immunocytochemistry specific for histiocytes and epithelial cells • Mucicarmine (may be positive in adenocarcinoma
	Nodular histiocytic hyperplasia	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• No three-dimensional aggregates • Indistinct cell borders • Debris materials may be seen within the cytoplasm • No significant nuclear enlargement	• Clinical and radiographic correlation • Transudate vs exudate • Panel of immunocytochemistry specific for histiocytes and epithelial cells • Mucicarmine (may be positive in adenocarcinoma
	Endosalpingiosis	• No specific conditions	• Ciliated cells • No significant pleomorphism	• Clinical and radiographic correlation • Transudate vs Exudate • If non-mullerian malignancies suspected, use differential immu-staining
	Endometriosis	• History of endometriosis	• Hemosiderin-laden macrophages • Endometrial glands and stromal cells (sometimes best seen with cell block)	• Clinical and radiographic correlation • Transudate vs exudate • If non-mullerian malignancies suspected, use differential immu-staining
	Psamomma bodies	• Reactive mesothelial hyperplasia • History of endosalpingiosis and endometriosis	• Aggregates have knobby contours • Aggregates not numerous • Windows (spaces) can be discerned within groups • No second distinct population of cells present	• Clinical and radiographic correlation • Transudate vs exudate
	Collagen balls	• Benign occurrences	• Glassy hyaline core • Flat benign cell at the periphery	• Clinical and radiographic correlation • Transudate vs exudate

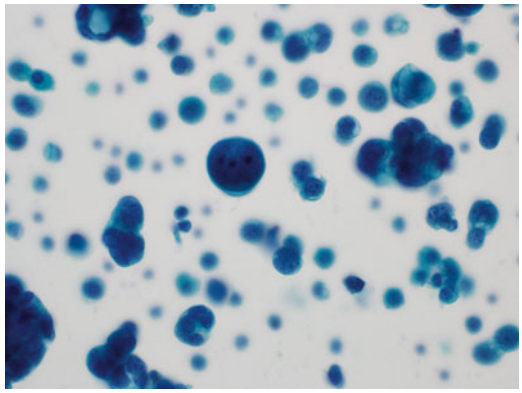


Fig. 10.2 Metastatic carcinoma. A second population of cells with high nucleocytoplasmic ratio that are larger than background cells (liquid-based; Papanicolaou)

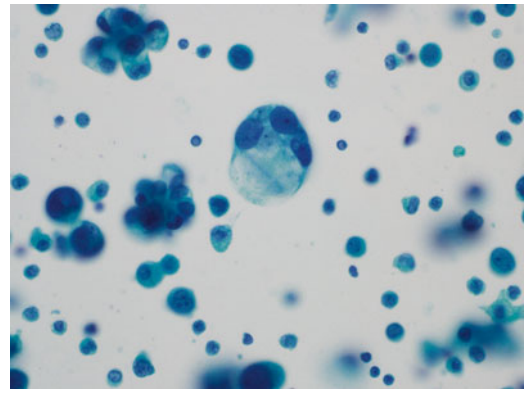


Fig. 10.5 Metastatic adenocarcinoma. Malignant epithelial cells in clusters with large vacuoles (liquid-based; Papanicolaou)

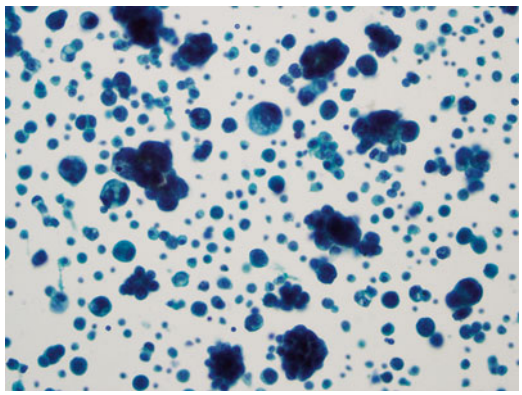


Fig. 10.3 Metastatic carcinoma. Malignant epithelial cells present singly and in clusters (liquid-based; Papanicolaou)

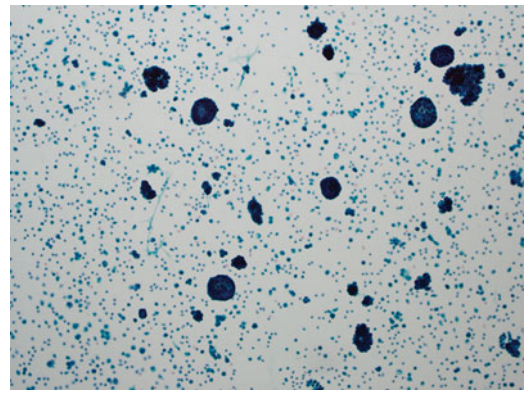


Fig. 10.6 Metastatic mammary carcinoma. Malignant epithelial cells in numerous tight round clusters in pleural fluid, also called "cannon balls" (liquid-based; Papanicolaou)

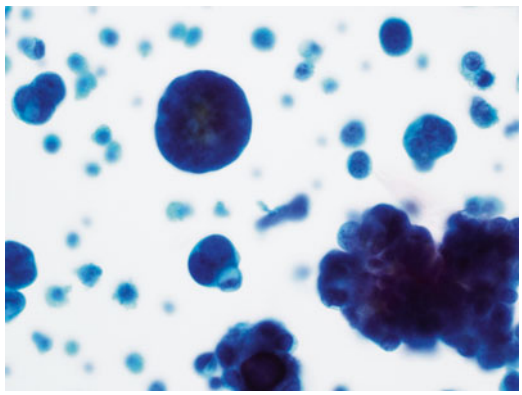


Fig. 10.4 Metastatic carcinoma. Malignant epithelial cells in clusters with smooth border (liquid-based; Papanicolaou)

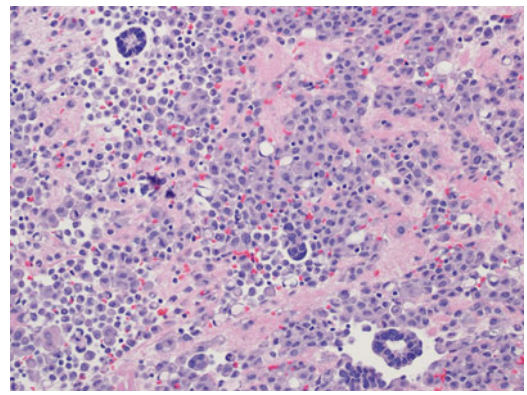


Fig. 10.7 Metastatic carcinoma. Malignant epithelial cells in clusters with retraction artifacts forming lacunae (cell block; hematoxylin and eosin)

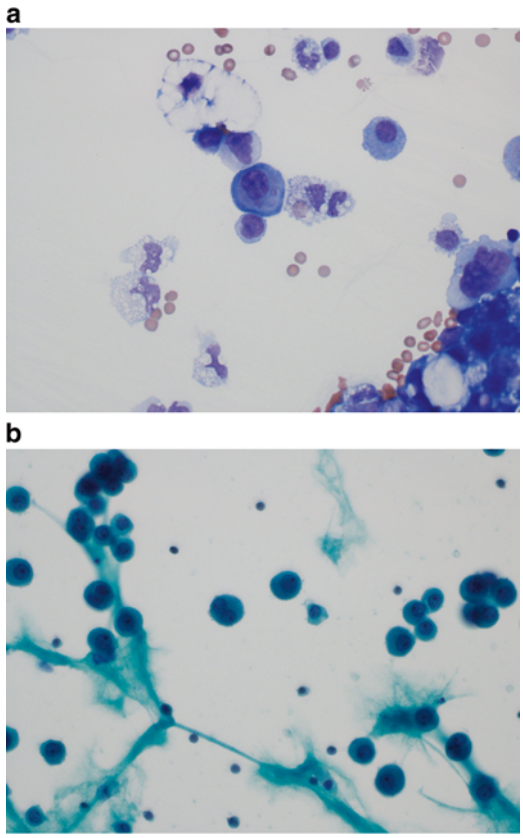


Fig. 10.8 (**a**) Mesothelial cells.. Cell border with fuzzy edges (cytopsin; Diff-Quik); (**b**) mesothelial cells. Cell border with fuzzy edges (liquid-based; Papanicolaou)

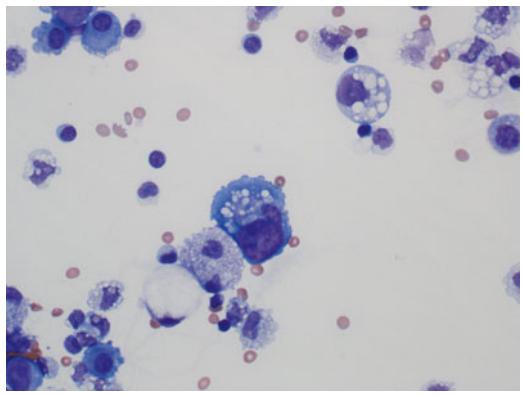


Fig. 10.9 Mesothelial cells. Peripheral blunt and round cytoplasmic projections from reactive and degenerated mesothelial cells (cytospin; Diff-Quick)

The cytoplasm of the mesothelial cell is granular and appears dense in the center and pale at the periphery (Fig. 10.8a). When the cell is degenerated, cytoplasmic vacuoles can form and be appreciated cytologically. Often a prominent vacuole can displace the nucleus from its natural central location to the side, in a signet ring fashion (Fig. 10.10). While benign mesothelial cells maintain a constant nuclear to cytoplasmic ratio, regardless of their size, they can be binucleated (Fig. 10.8a). The nuclei are round to oval. The nuclear membrane is conspicuous, smooth or mildly tortuous. In benign mesothelial cells, the chromatin is fine to coarse but always evenly distributed with occasional chromocenters. Nucleoli are usually inconspicuous. When mesothelial cells become reactive, regardless of the etiology, they exfoliate as single cells or small groups. The latter have lobulated, or commonly called "knobby" borders (Fig. 10.11). In peritoneal washings, the mesothelial cells are in orderly flat

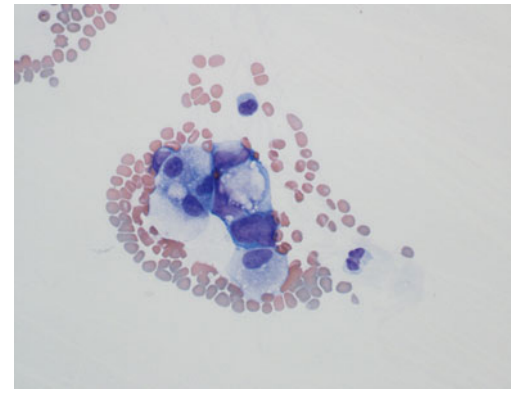


Fig. 10.10 Mesothelial cells. Vacuolated benign mesothelial cells (cytospin; Diff-Quick)

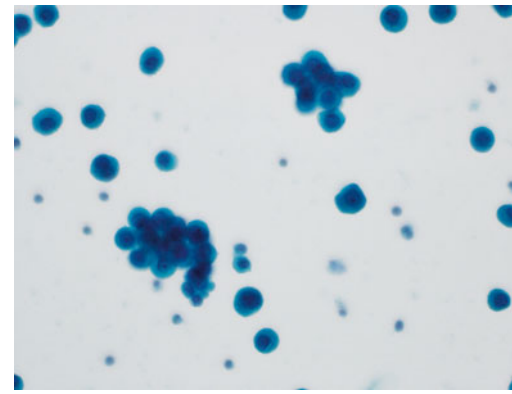


Fig. 10.11 Mesothelial cells. Clusters with knobby borders (liquid-based; Papanicolaou)

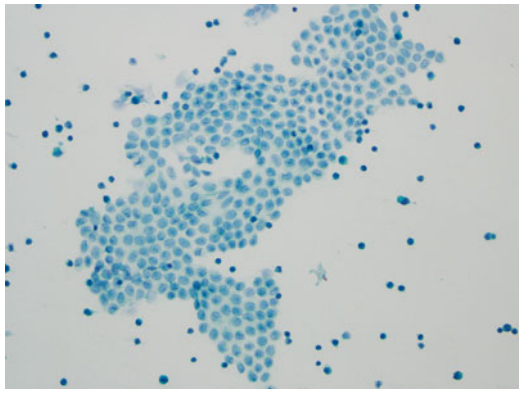


Fig. 10.12 Mesothelial cells.. Flat sheets from pelvic washing with "windows" between cells (liquid-based; Papanicolaou)

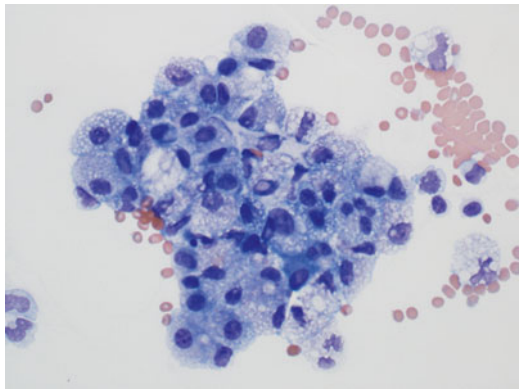


Fig. 10.13 Histiocytes. Collection of histiocytes with vacuolated cytoplasm (cytospin; Diff-Quick)

sheets with distinct spaces or "windows" between individual cells (Fig. 10.12). Mesothelial cells are immunoreactive to WT-1, calretinin, cytokeratins (particularly CK 5), D2-40, and mesothelin.

Histiocytes comprise an important group of cells normally found in body cavity fluids. They can also cause major diagnostic difficulties. The presence of vacuolated cytoplasm can also mimic vacuolated epithelial tumors. They are also of medium size, similar to mesothelial cells. However, their size and shape vary depending on their phagocytic activity (Fig. 10.13). Unlike mesothelial cells, histiocytes have indistinct cell borders and no microvilli. The cytoplasm is usually foamy or markedly vacuolated secondary to their phagocytic activity. Often phagocytized materials, such as hemosiderin, can be appreciated cytologically within the cytoplasm, especially in cases of endometriosis (Fig. 10.14). The nuclei of histiocytes are eccentrically located and are classically described as bean-shaped, but can be round or oval like mesothelial cells. Multinucleation is not uncommon. Giant cell histiocytes can be seen in association of granulomatous diseases, reaction to trauma, and radiation. Nuclear membranes are indistinct. The chromatin can be fine to coarse but evenly distributed. Nucleoli are usually inconspicuous, but can become conspicuous when reactive. Normally in effusions, histiocytes occur singly or in loose sheets, without grouping. However, they sometimes form tight aggregates also called nodules surrounded by fibrin bands. The cells are biphasic and composed of cohesive monotonous epithelioid clusters of polygonal or oval cells with round or grooved nuclei intimately associated with darker cuboidal cells. This finding is called "*nodular histiocytic hyperplasia*" (Fig. 10.15). Immunocytochemistry can help to correctly identify them as histiocytes. The latter express CD14, CD34, CD163 and CD68, but not cytokeratins which are expected in malignant epithelial cells.

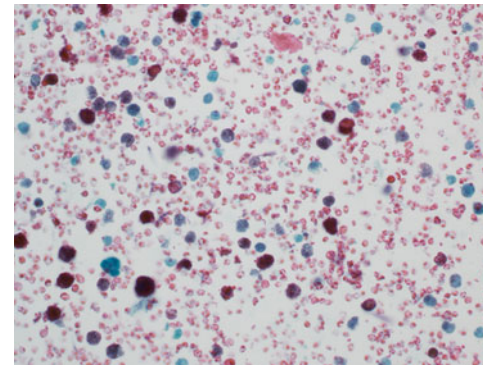


Fig. 10.14 Histiocytes. Hemosiderin-laden macrophages in endometriosis (liquid-based; Papanicolaou)

In women, some benign conditions, such as endometriosis or endosalpingiosis, can exfoliate a second population of cells distinct from background mesothelial cells, mimicking an adenocarcinoma. Immunocytochemistry will further complicate or suggest malignancy because these foreign cells will have a non-mesothelial immunophenotype. In *Endosalpingiosis*, the presence of ciliated epithelial cells is the distinguishing

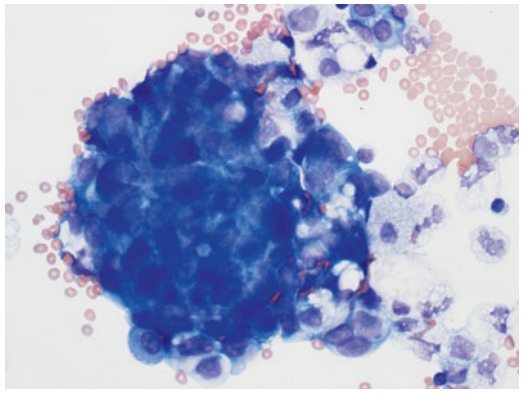


Fig. 10.15 Nodular histiocytic hyperplasia. Aggregate epithelioid histiocytes and darker round cells (cytopsin; Diff-Quick)

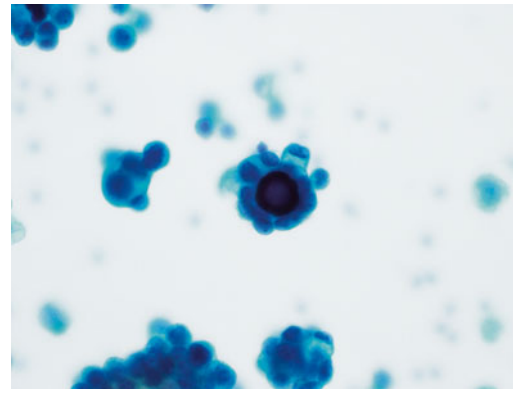


Fig. 10.17 Psammoma bodies. Calcific spheres with concentric lamination, surrounded by malignant epithelial cells (liquid-based; Papanicolaou)

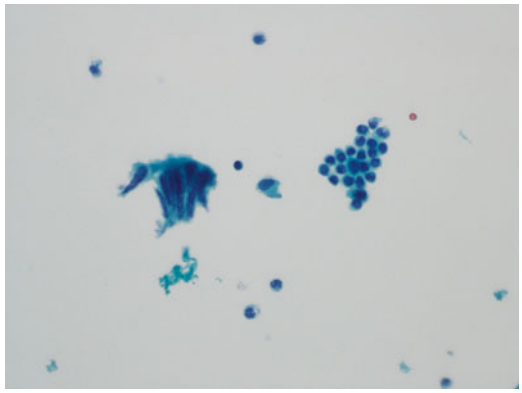


Fig. 10.16 Endosalpingiosis. Cluster of ciliated cells in fluid (liquid-based; Papanicolaou)

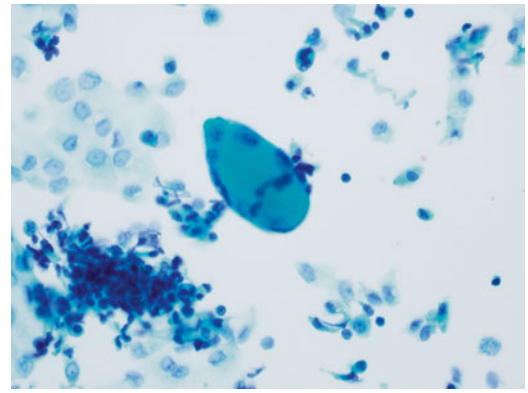


Fig. 10.18 Collagen ball. Sphere of collagen surrounded by mesothelial cells (liquid-based; Papanicolaou)

cytologic finding (Fig. 10.16). In *endometriosis*, hemosiderin-laden macrophages, endometrial glandular and stromal cells are not always found together in fluid cytology. Here, when the clinical history is suggestive, a cell block might provide the answer (Fig. 10.14).

Many adenocarcinomas that form papillary structures tend to form *Psammoma Bodies* which are concentrically laminated calcified structures (Fig. 10.17). Unfortunately, they can frequently be seen in benign effusions as well, especially those associated with but not limited to papillary mesothelial hyperplasia and endosalpingiosis. Thus a diagnosis of malignancy should not be made solely on the presence of psammoma bodies.

Occasionally, body cavity fluid will contain rare spherical to oval hyaline bodies/structures lined peripherally by flattened/cuboidal mesothelial cells. The smooth border is reminiscent of clusters of malignant epithelial cells in metastatic adenocarcinoma. These structures are called collagen balls (Fig. 10.18). The clue to their benign nature is their paucity in the fluid, the lack of abnormal cytology and the glassy hyaline appearance.

Patients with systemic lupus erythematosus can often present with increased fluid in their body cavities, raising concerns for a malignant process. Cytologically, their fluid might contain the *Lupus Erythematosus* cell which is a neutrophil or macrophage with an ingested cytoplasmic particle called a hematoxylin body.

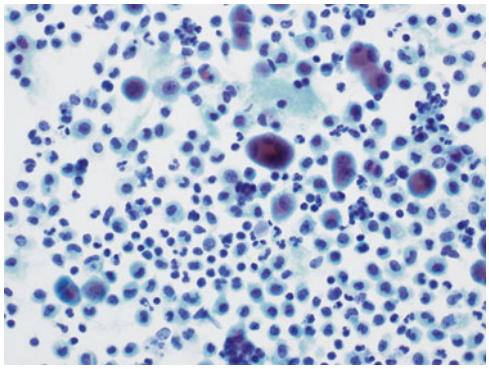


Fig. 10.19 Lupus erythematous cells. Intracytoplasmic body surrounded by the lobes of the nucleus (liquid-based; Papanicolaou)

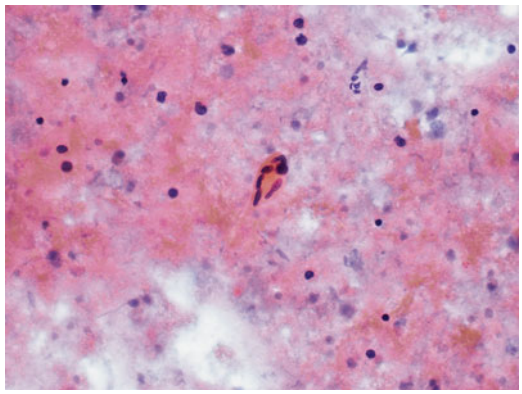


Fig. 10.20 Rheumatoid pleuritis. Multinucleated histiocyte in a granular background (liquid-based; Papanicolaou)

The nuclei are pushed to the side by such bodies (Fig. 10.19). These findings are rarely interpreted as malignant, but knowledge of the clinical story may prevent any confusion.

Effusions in patients with concurrent pulmonary nodules are concerning clinically and radiographically. Cytology plays a critical role in those patients to rule out malignant effusions. A minority of patients with rheumatoid arthritis can have such clinical presentation (i.e., effusions in the setting of pulmonary nodules). These lung lesions are called rheumatoid nodules, and the fluid accumulation is secondary to *Rheumatoid Pleuritis*. The characteristic findings are a background of granular material with scattered multinucleated histiocytes (Fig. 10.20). Here again, appropriate correlation with the clinical context will lead to the correct interpretation.

The diversity of epithelial tumors capable of metastasizing to body cavities is too broad for comprehensive coverage here. The constellation of cytomorphological findings thus needs to be interpreted in conjunction with the clinical and imaging impression. If there are any doubts about the nature of an effusion, especially in oncologic patients, obtaining a cell block is highly recommended. Adenocarcinoma is known to cause a characteristic retraction halo or lacunae around aggregates of neoplastic cells, not seen with mesothelial cells (Fig. 10.7). Additionally, immunocytochemistry is of extreme importance here. A panel of immunostains, including those mutually exclusively seen in mesothelial and epithelial cells are key diagnostic aids. Markers for the former were discussed earlier, and those for the former include cytokeratin CK 20 (rarely observed in mesothelial cells), MOC-31, claudin, Leu-M1, EMA, B72.3, and Ber-Ep-4. If the patient's malignancy is known and has been previously classified, it is therefore most useful to use markers specific for the tumor. Histochemistry can also be helpful, for example when vacuolated cells are present, mucicarmine staining can be beneficial in distinguishing malignant signet ring cells from macrophages.

Mimickers of Mesothelioma in the Body Cavities

Distinguishing reactive mesothelial cells from neoplastic ones on cytomorphological ground alone can virtually be impossible, especially at the well-differentiated end of the spectrum (Table 10.2). A foreign population of cells is not readily discerned as in the case of metastatic adenocarcinoma. Ancillary tests have limited benefits because reactive and malignant mesothelial cells are immunophenotypically similar. Nonetheless, there are cytological, though nonspecific, criteria that can aid in the distinction, depending on the variant of mesothelioma, i.e. epithelioid, sarcomatoid vs biphasic.

One important feature of effusion with *Mesothelioma* is high cellularity. The fluid will likely contains numerous mesothelial cells present singly but mostly in large clusters,

Table 10.2 Mimickers of mesotheliomas in body cavity fluids

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Mesothelioma	Mesothelial cells in aggregates	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• Groups are small in number and in size • No significant pleomorphism • Macromucleoli not a significant finding	• Clinical and radiographic correlation • Cytogenetics looking for deletions of 1p, 3p, 6q, 9p, and 22q (common in meso-theliomas)
	Mesothelial cells with multinucleation	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• Groups are small in number and in size • No significant pleomorphism • Macromucleoli not a significant finding • Mo more than two nuclei	• Clinical and radiographic correlation • Cytogenetics looking for deletions of 1p, 3p, 6q, 9p, and 22q (common in meso-theliomas)
	Mesothelial cells with nuclear enlargement	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• No significant pleomorphism • No second distinct population of cells present	• Clinical and radiographic correlation • Cytogenetics looking for deletions of 1p, 3p, 6q, 9p, and 22q (common in meso-theliomas)
	Mesothelial cells with mitotic figures	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• Mitotic figures are rare • No atypical mitosis • No second distinct population of cells present	• Clinical and radiographic correlation • Cytogenetics looking for deletions of 1p, 3p, 6q, 9p, and 22q (common in meso-theliomas)
	Mesothelial cells with degeneration	• Poorly processed and/or stored fluid	• No second distinct population of cells present • Cytoplasmic and nuclear details poorly visualized	• Clinical and radiographic correlation • Cytogenetics looking for deletions of 1p, 3p, 6q, 9p, and 22q (common in meso-theliomas)
	Nodular histiocytic hyperplasia	• Chronic and active liver disease • Pulmonary embolism • Pneumonia • Uremia • Pancreatitis • Pericarditis • Long-term dialysis • History of radiation, chemotherapy	• No three-dimensional aggregates • Indistinct cell borders • Debris materials may be seen within the cytoplasm • No significant nuclear enlargement	• Clinical and radiographic correlation • Transudate vs exudate • Panel of immunocytochemistry specific for histiocytes and mesohelial cells

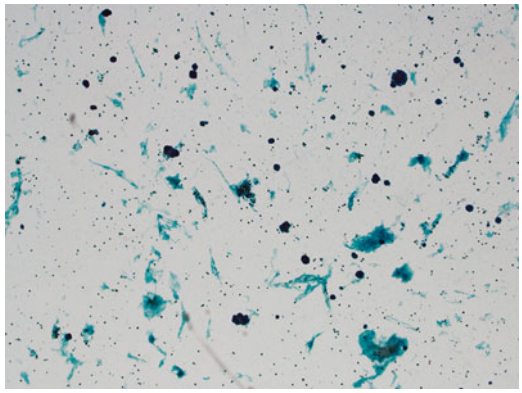


Fig. 10.21 Mesothelioma. Increased clusters of mesothelial cells at low power (liquid-based; Papanicolaou)

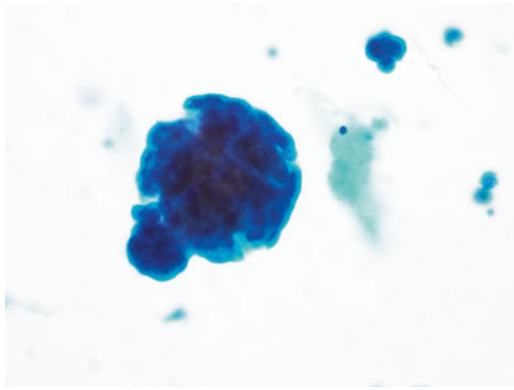


Fig. 10.22 Mesothelioma. Larger cluster of malignant mesothelial cells (liquid-based; Papanicolaou)

readily appreciable at low power (Fig. 10.21). Some of these groups can contain hundreds of cells and are more complex than those in benign effusions. These clusters can have varying configurations such as three-dimensional balls and papillae, but are exceedingly rare as flat two-dimensonal sheets. Central cores of collagen can occasionally be seen in the malignant clusters (Fig. 10.22). Malignant mesothelial cells typically have a variable size with a relatively constant nuclear to cytoplasmic ratio. Clusters of histiocytes in reactive effusions can mimic mesothelioma as well. Here, immunocytochemistry will prove beneficial in identifying these cells as histiocytes. Binucleation can sometimes be seen with reactive mesothelial cells, but more than three nuclei/cell are more suggestive of malignancy. Likewise, macronucleoli are more suggestive of mesothelioma than a benign reactive process (Fig. 10.23). Ultimately, strict correlation with clinical and imaging impression is needed. The majority of mesotheliomas occur in men. History of remote exposure to asbestos may be present. Imaging studies may suggest diffuse, nodular pleural or peritoneal thickening with growth by encasement of viscera in the body cavities, with or without a mass lesion.

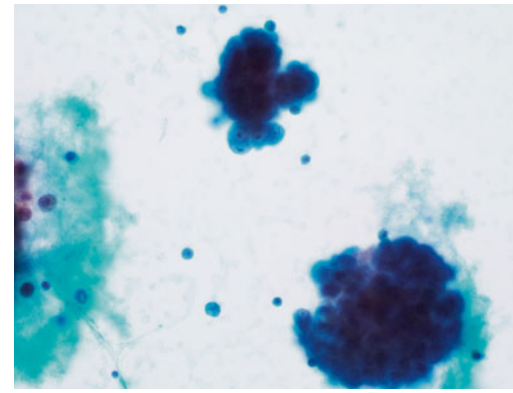


Fig. 10.23 Mesothelioma. Prominent nucleoli in the malignant mesothelial cells (liquid-based; Papanicolaou)

Conclusion

Distinguishing non-neoplastic fluids from neoplastic ones is challenging. However, this distinction becomes more attainable when interpretation is based on a constellation of findings as opposed to a single criterion. A clear clinical history is crucial (e.g., history of any malignancies in the patient). While often ignored, knowledge of the fluid's physical nature is important, because transudates are usually benign and exudates are more likely to be malignant. By far, metastasis tops the list of malignant fluids and includes predominantly tumors of epithelial type and mesothelioma. In the case of metastatic carcinoma, the main distinguishing feature is recognition of a separate non-mesothelial population of cells, coupled with appropriate immunocytochemistry. In the case of mesothelioma, cytomorphology is generally more important than ancillary testing, with special attention to the cellularity, the characteristic of the groups and the degree of atypia.

Suggested Readings

1. Chikkamuniyappa S, Herrick J, Jagirdar JS. Nodular histiocytic/mesothelial hyperplasia: a potential pitfall. Ann Diagn Pathol. 2004;8(3):115–20.
2. Choi YL, Song SY. Cytologic clue of so-called nodular histiocytic hyperplasia of the pleura. Diagn Cytopathol. 2001;24(4):256–9.
3. Cibas ES, Ducatman BS. Cytology: diagnostic principles and clinical correlates. 4th ed. Edinburgh: Saunders; 2014. Ch 4.
4. Cury PM, Butcher DN, Corrin B, et al. The use of histological and immunohistochemical markers to distinguish pleural malignant mesothelioma and in situ mesothelioma from reactive mesothelial hyperplasia and reactive pleural fibrosis. J Pathol. 1999;189(2):251–7.
5. Esteban JM, Yokota S, Husain S, et al. Immunocytochemical profile of benign and carcinomatous effusions. A practical approach to difficult diagnosis. Am J Clin Pathol. 1990;94(6):698–705.
6. Granados R, Cibas ES, Fletcher JA. Cytogenetic analysis of effusions from malignant mesothelioma. A diagnostic adjunct to cytology. Acta Cytol. 1994; 38(5):711–7.
7. Hsu C. Cytologic detection of malignancy in pleural effusion: a review of 5,255 samples from 3,811 patients. Diagn Cytopathol. 1987;3(1):8–12.
8. Johnston WW. The malignant pleural effusion. A review of cytopathologic diagnoses of 584 specimens from 472 consecutive patients. Cancer. 1985;56(4): 905–9.
9. Kho-Duffin J, Tao LC, Cramer H, et al. Cytologic diagnosis of malignant mesothelioma, with particular emphasis on the epithelial noncohesive cell type. Diagn Cytopathol. 1999;20(2):57–62.
10. Kobzik L, Antman KH, Warhol MJ. The distinction of mesothelioma from adenocarcinoma in malignant effusions by electron microscopy. Acta Cytol. 1985; 29(3):219–25.
11. Kutty CP, Remeniuk E, Varkey B. Malignant-appearing cells in pleural effusion due to pancreatitis: case report and literature review. Acta Cytol. 1981;25(4):412–6.
12. Naylor B. Cytological aspects of pleural, peritoneal and pericardial fluids from patients with systemic lupus erythematosus. Cytopathology. 1992;3(1):1–8.
13. Naylor B. The pathognomonic cytologic picture of rheumatoid pleuritis. The 1989 Maurice Goldblatt Cytology award lecture. Acta Cytol. 1990;34(4): 465–73.
14. Paintal A. The evolving role of effusion cytology in the diagnosis of malignant mesothelioma. Cytopathology. 2015;26(3):137–8.
15. Price BA, Ehya H, Lee JH. Significance of pericellular lacunae in cell blocks of effusions. Acta Cytol. 1992;36(3):333–7.
16. Renshaw AA, Dean BR, Antman KH, et al. The role of cytologic evaluation of pleural fluid in the diagnosis of malignant mesothelioma. Chest. 1997;111(1): 106–9.
17. Samedi V, White S, Zimarowski MJ, et al. Metastatic peritoneal mesothelioma in the setting of recurrent ascites: a case report. Diagn Cytopathol. 2010;38(9): 675–81.
18. Szeto MC, Disney B, Perkins P, et al. Ascites and other incidental findings revealing undiagnosed systemic rheumatoid arthritis. BMJ Case Rep. 2015:201. doi:10.1136/bcr-2014-207142.

Lymph Nodes Cytology

11

Brief Introduction

Lymphadenopathy, with or without history of malignancy, is often clinically concerning. However, complete surgical excision of an enlarged lymph node is not always practical for histological assessment. Fine needle aspiration is often performed first to determine whether the increase in the size of a lymph node is secondary to a benign or malignant process. While surgical excision may be subsequently performed, the advantage of an initial fine needle aspiration is that it is a rapid assessment and can be done as a much less invasive procedure. Certain shortcomings are nonetheless associated with lymph node cytology. Sampling error is always a possibility whenever a negative cytology is obtained. Inaccurate "false-positive" diagnosis unfortunately is also a real possibility. Reactive or inflamed lymph nodes can yield cellular material with features that can strikingly resemble a neoplastic process. In this chapter, these features will be discussed along with recommendations on how to avoid these pitfalls.

Classification of Lymph Node Neoplasms Based on Primary vs Secondary Origin

The reasons for lymph node enlargement can be classified into three main groups. First, a reactive process is usually secondary to a local or systemic inflammatory or infectious condition. A second important group consists of primary neoplasms of the lymph nodes: lymphomas. Lastly, metastatic diseases make up a major etiology in lymphadenopathy and one of the top indications for FNA. The mimickers of either primary or secondary neoplasms are reactive hyperplasia or lymphadenitis from the first group.

Confounders Based on Cytomorphological Findings

Mimickers of Malignant Lymphomas

There are many causes of lymphadenopathy besides lymphoma. However, many of the "non-lymphoma" conditions may have cytologic features reminiscent of lymphoma (Table 11.1) First of all, malignant lymphomas, depending on the subtype, have specific cytological features. It is most helpful to be aware of these features when evaluating a lymph node cytology specimen.

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_11

Table 11.1 Mimickers of neoplasms in the lymph node

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Malignant lymphomas	Acute lymphadenitis	• Local anatomical inflammation • Infectious conditions (e.g., bacterial and mycobacterial)	• Numerous neutrophils • Polymorphous lymphocytes • No epithelial cells present	• Clinical and radiographic correlation • Microbiology studies • Flow cytometry
	Chronic lymphadenitis	• Autoimmune diseases • History of infections • Post-dental procedures • Post-vaccination	• Polymorphous population of lymphocytes • Small, medium and large lymphocytes • Tingible-body macrophages • Dendritic cells • Dendritic-lymphocytic aggregates (“intact follicles”)	• Clinical and radiographic correlation • Microbiology studies • Flow cytometry
• Metastatic diseases	Acute lymphadenitis	• Local anatomical inflammation • Infectious conditions (e.g., bacterial and mycobacterial)	• Numerous neurtrophils • Polymorphous lymphocytes • No epithelial cells present	• Clinical and radiographic correlation • Microbiology studies • Cell block • Flow cytometry
	Chronic lymphadenitis	• Autoimmune diseases • History of infections • Post-dental procedures • Post-vaccination	• Polymorphous population of lymphocytes • Small, medium and large lymphocytes • Tingible-body macrophages • Dendritic cells • Dendritic-lymphocytic aggregates (“intact follicles”)	• Clinical and radiographic correlation • Cell block • Immunocytochemistry

In *Hodgkin lymphoma* (HL), most examples consist of small polymorphous lymphocytes with occasional Reed-Sternberg cells. The latter are large with two nuclei or nuclear lobes with a variable amount of cytoplasm (Fig. 11.1). The chromatin is coarse with peripheral margination. Hodgkin cells, also found in HL, are similar but smaller than Reed-Sternberg cells. The former are considered the mononuclear variant of the latter (Fig. 11.2). A background inflammatory infiltrate is often present (Fig. 11.3). Follicular aggregates or tingible-body macrophages are absent except in the lymphocyte-dominant subtype.

Acute and chronic lymphadenitis can potentially be mistaken for HL because of their inflammatory background. Furthermore, an immunoblast, in these conditions, can be up to four times the size of a small lymphocyte and resembles Reed-Sternberg cells. The immunoblasts have large round nuclei with large nucleoli and prominent nuclear membranes. In general, normal reactive lymph nodes contain polymorphous lymphocytes with minimal cytoplasm (Fig. 11.4). Most of these lymphocytes are small with round nuclei. The largest lymphocytes are the centroblasts with round, vesicular nuclei and multiple nucleoli. The centrocytes are intermediate-sized lymphocytes with cleaved nuclei. Two other large cells in reactive lymph nodes are tingible-body macrophages and dendritic cells. The tingible-body macrophages have abundant

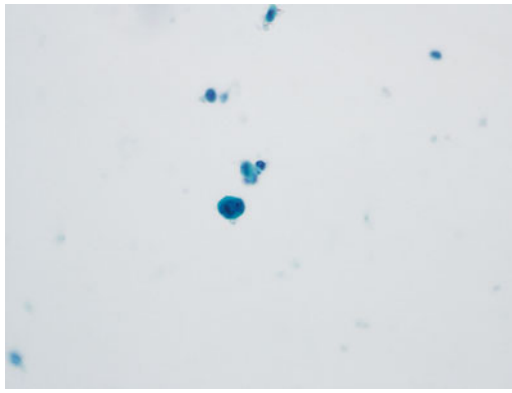


Fig. 11.1 Multinucleated Reed-Sternberg cell in Hodgkin lymphoma (Papanicolaou stain, high magnification)

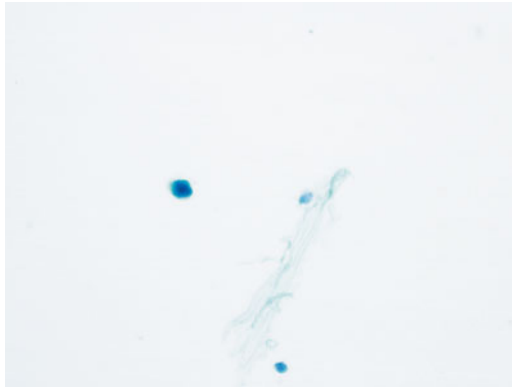


Fig. 11.2 Mononuclear variant of Reed-Sternberg cell in Hodgkin lymphoma, with prominent nucleoli (Papanicolaou stain, high magnification)

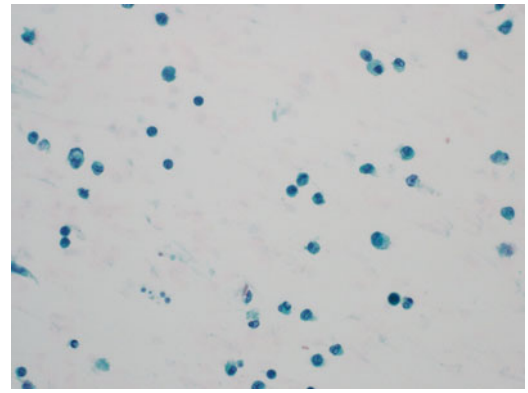


Fig. 11.3 Mixed inflammatory background in Hodgkin lymphoma (Papanicolaou stain, high magnification)

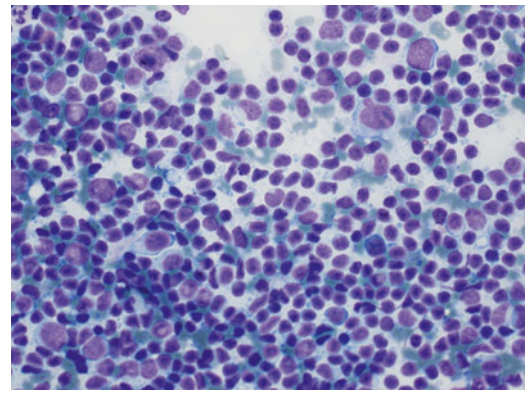


Fig. 11.4 Reactive lymphoid hyperplasia with a mixture of lymphocytes with varying size. Small lymphocytes, centrocytes and centroblasts are present. Immunoblasts and macrophages are also intermixed. (Diff Quik stain, high magnification)

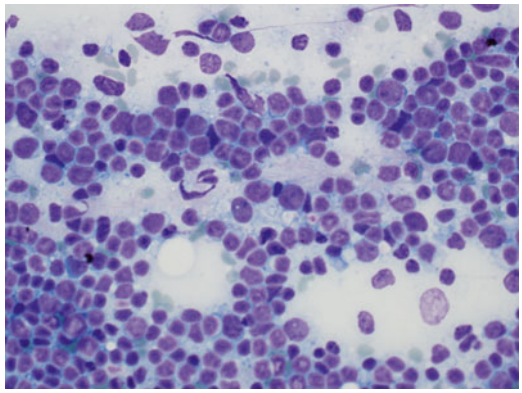


Fig. 11.5 A monomorphous population of lymphocytes consistent with a monoclonal process (Diff Quik stain, high magnification)

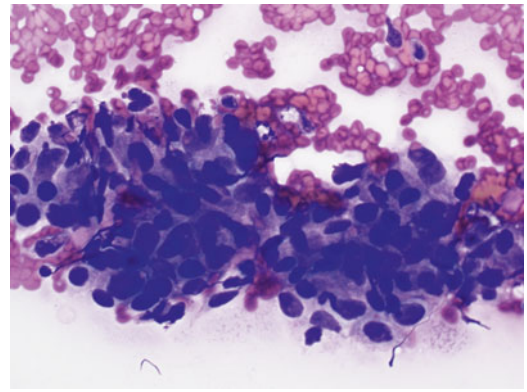


Fig. 11.6 Clusters of malignant epithelial cells in a lymph node consistent with metastatic squamous cell carcinoma (Diff Quik stain, high magnification)

cytoplasm (Fig. 11.4). Dendritic cells have large oval nuclei, small nucleoli, and cytoplasmic processes.

Non-Hodgkin lymphomas (NHL), require a very extensive discussion and are beyond the scope of this book chapter. However, some distinguishing cytomorphological features of NHL are worth discussing to avoid overdiagnosis. The aspirate tends to be highly cellular with a monomorphous population of lymphocytes (Fig. 11.5). The size of these lymphocytes vary depending on the subtypes (small vs medium vs large). A discohesive cell pattern is usually present. A polymorphous population, while favoring a reactive process, does not exclude a lymphoma. Similarly, acute and chronic lymphadenitis may be mistaken for NHL. In general, correlating cytomorphological findings with immunocytochemistry, flow cytometry, and molecular genetic studies will help avoid overcalling the mimickers as malignant lymphomas.

Mimickers of Metastatic Diseases

Fine needle aspiration for evaluation of *metastatic diseases* in patients with documented malignancy is very common. When malignant or benign-appearing non-lymphoid cells are aspirated from a suspected lymph node, the cytodiagnosis is simple even without further ancillary testing, except for subclassification. Virtually any

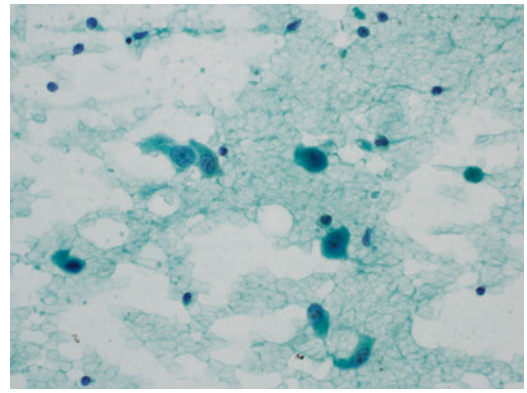


Fig. 11.7 Noncohesive single nonpigmented melanoma cells with plasmacytoid features and enlarged nuclei with prominent nucleoli in metastatic melanoma to the lymph node (Diff Quik stain, high magnification)

metastatic malignancy can be cytologically detected in lymph nodes. Caution is still needed, as the clinical and radiographic impression may bias the cytopathologist to overcall a reactive lymph node as positive for metastatic disease.

The cytomorphology of the metastatic tumor will depend on the type. For example, metastatic carcinoma will form clusters of cells (Fig. 11.6), while melanomas are more likely to be discohesive (Fig. 11.7). Lymphoglandular bodies (membrane fragments of lymphoid cytoplasm) are usually absent (Fig. 11.4).

Benign large cells from a reactive lymph node as described above have moderate to abundant cytoplasm that may simulate metastatic epithelial

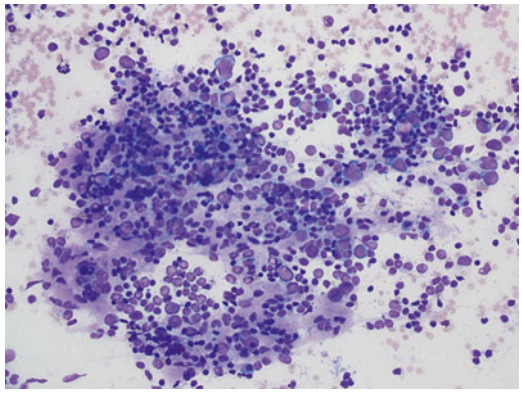


Fig. 11.8 A dendritic-lymphocytic aggregate consisting of a mixture of dendritic cells and a heterogeneous lymphocyte population (Diff Quik stain, high magnification)

tumor cells. Dendritic cells and immunoblasts are notorious confusing the novice. In addition, dendritic-lymphocytic aggregates, loose association of small lymphocytes and dendritic cells often mingled with a capillary, may mimic clusters of metastatic epithelial cells (Fig. 11.8). Once more, correlating cytomorphological findings with immunocytochemistry and molecular genetic studies will help avoid overcalling the mimickers as metastatic cancers.

Conclusion

Lymphadenopathy, with or without history of malignancy, is often clinically concerning. Fine needle aspiration is often initially performed to determine the etiology of the increase in the size of a lymph node. The reasons for lymph node enlargement can be classified into three main groups. First, a reactive process usually secondary to a local or systemic inflammatory or infectious conditions. A second important group consists of primary neoplasms of the lymph nodes: lymphomas. Lastly, metastatic diseases make up a major etiology in lymphadenopathy and one of the top indications for FNA. Inaccurate "false-positive" diagnosis on cytology unfortunately is also a real possibility. Reactive or inflamed lymph nodes can yield cellular material with features that can strikingly resemble a neoplastic process. In general, correlating cytomorphological findings with immunocytochemistry, flow cytometry, and molecular genetic studies will help avoid overcalling the mimickers as neoplastic entities.

Suggested Readings

1. Chen Y, Savargaonkar P, Fuchs A, Wasserman P. Role of flow cytometry in the diagnosis of lymphadenopathy in children. Diagn Cytopathol. 2002;26(1):5–9.
2. Wakely Jr PE. The diagnosis of non-Hodgkin lymphoma using fine-needle aspiration cytopathology: a work in progress. Cancer Cytopathol. 2010;118(5):238–43.
3. Glant MD. Cytopathology of lymph nodes in nonspecific reactive hyperplasia. Prognostication and differential diagnoses. Am J Clin Pathol. 1997;108(4 Suppl 1):S31–55.
4. Prasad RR, Narasimhan R, Sankaran V, Veliath AJ. Fine-needle aspiration cytology in the diagnosis of superficial lymphadenopathy: an analysis of 2,418 cases. Diagn Cytopathol. 1996;15(5):382–6.
5. O'Dowd GJ, Frable WJ, Behm FG. Fine needle aspiration cytology of benign lymph node hyperplasias. Diagnostic significance of lymphohistiocytic aggregates. Acta Cytol. 1985;29(4):554–8.
6. Yu GH, McGrath CM. Follow-up of morphologically reactive lymphoid proliferations in fine-needle aspirates of elderly patients. Diagn Cytopathol. 2000;23(4):249–52.
7. Flanders E, Kornstein MJ, Wakely Jr PE, Kardos TF, Frable WJ. Lymphoglandular bodies in fine-needle aspiration cytology smears. Am J Clin Pathol. 1993;99(5):566–9.
8. Gilani SM, Fathallah L, Al-Khafaji BM. Preoperative fine needle aspiration of axillary lymph nodes in breast cancer: clinical utility, diagnostic accuracy and potential pitfalls. Acta Cytol. 2014;58(3):248–54.
9. Mitra S, Ray S, Mitra PK. Fine needle aspiration cytology of supraclavicular lymph nodes: our experience over a three-year period. J Cytol. 2011;28(3):108–10.

Bone and Soft Tissue Cytology 12

Brief Introduction

Utility of Fine Needle Aspiration Biopsy of Bone and Soft Tissue Tumors

Cytopathologic diagnosis of bone and soft tissue tumors is unquestionably challenging. Some of the tumors are rare, tumors with markedly disparate behavior may resemble each other to a striking degree (such as a schwannoma with degenerative atypia and malignant peripheral nerve sheath tumor), and the complex categorization of these tumors continues to evolve. Fortunately, cytopathologic examination does not proceed in a vacuum: the patient's clinical history and imaging findings can narrow the differential significantly. Moreover, immunocytochemistry and molecular genetic testing can be performed on cytologic specimens and enable a more specific diagnosis. Molecular genetic based tests such as fluorescent in situ hybridization, PCR and high throughput sequencing are increasingly deployed in bone and soft tissue tumor diagnosis, and cytology specimens are often ideal for such tests.

Indications for sampling bone and soft tissue tumors comprise: (1) to confirm metastasis of a previously diagnosed sarcoma; (2) conversely, to confirm metastasis of carcinoma, melanoma or identify lymphoma in a bone or soft tissue site; (3) to triage a non-malignant process such as infection that presents as a bone or soft tissue mass; (4) to confirm that a needle core biopsy has adequately sampled the lesion (also termed ROSE: Rapid Onsite Evaluation); (5) to monitor for local recurrence in a previously excised primary bone or soft tissue tumor, and (6) to collect material for potential molecular genetic prognostic and treatment decision needs. Beyond these indications, the cytotechnologist and cytopathologist will encounter soft tissue or bone tumors as an unexpected tumor at an unusual site (such as the salivary gland region or in a lymph node) or as an unusual metastasis to a body cavity (such as in the pleural fluid or an ascites specimen). While these are relatively rare occurrences, familiarity with the pitfalls of bone and soft tissue tumor cytologic diagnosis can avert a serious medical error. As an example of the prevalence of this category of tumors at uncommon sites, a recent single-institutional review of intra-abdominal and intrathoracic EBUS procedures reported that 44 of 2400 specimens (~2 %) had a significant spindle cell or mesenchymal component [16].

Advantages of cytologic sampling over incisional biopsy and needle core biopsy include lower morbidity, much lower cost per procedure, low risk of seeding surrounding normal tissue, and the potential ability to sample multiple diverse areas within a large tumor. Disadvantages include difficulty obtaining adequate sample in predominantly cystic, necrotic, hemorrhagic or dense matrix-rich lesions, lack of experience

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_12

with the cytologic features of many of the tumors, and lack of experience with antibody reactivity differences in alcohol versus formalin fixed specimens. Additionally, standard karyotyping may be impossible to perform due to an insufficient number of tumor cells. However, for molecular genetic analysis, a cytology sample may be superior to formalin-fixed tissues [13]. DNA is well preserved in alcohol fixatives, FISH can be performed on smears (air-dried, fixed, and stained) without having to account for capitation effects, and cell blocks themselves are suitable for many of the newer genetic technologies.

Accuracy of Cytologic Diagnosis of Bone and Soft Tissue Tumors

The sensitivity and specificity for the diagnosis of bone and soft tissue tumors varies with sensitivity ranging from 25 to 100 % and specificity ranging from 83 to 100 % [1]. More recent studies report high sensitivity and specificity for diagnosing the type of tumor (e.g. carcinoma, melanoma, sarcoma, and lymphoma) and assessing whether it is benign or intermediate/malignant. Singh and colleagues reported ~95 % sensitivity and ~95 % specificity for the diagnosis of malignancy in the setting of adequate specimens, appropriate ancillary testing and experienced cytopathologists with 1 % false positive and 4 % false negative rates [6]. Khalbuss and colleagues reported 96 % sensitivity and 98 % specificity for accurate diagnosis of bone and soft tissue lesions (1100 total cases of which 105 were sarcomas and the remainder metastatic carcinomas, lymphomas, melanomas, infection and a few benign lesions) noting that the presence of a cytopathologist, concurrent needle core biopsies in some cases, and correlation with clinical and imaging findings greatly facilitated accurate diagnoses [3].

Performing ancillary studies is essential in many cases, as some melanomas, carcinomas and lymphomas assume the shape of the most common mesenchymal cell form: a spindle cell. Conversely, some sarcomas can appear epithelioid, melanocytic, or lymphocytic. Furthermore, some sarcomas appear bland on cytologic exam, while some benign mesenchymal tumors can show worrisome cytologic features. Morphologic exam alone may not suffice: accurate diagnosis commonly requires collecting extra sample to conduct immunocytochemical staining, molecular genetic testing and/or flow cytometric sorting.

A recent meta-analysis of studies comparing aspiration biopsy to needle core biopsy and incisional biopsy for the diagnosis of lesions of soft tissue and bone reported that the easiest diagnoses are metastatic carcinomas, the most challenging site for obtaining adequate sample is the vertebral column, and that it is easier to make a specific malignant diagnosis than a specific benign diagnosis [4].

Evidence-based review of five recent studies by members of the Musculoskeletal Tumor Society reported that incisional biopsy outperformed core needle biopsy which outperformed fine needle aspiration biopsy for the diagnosis of soft tissue masses but that the differences were NOT statistically significant. In this meta-analysis, infections, myxoid lesions and small round cell tumors were the most diagnostically challenging [2].

An additional challenge in cytologic diagnosis of bone and soft tissue tumors is that some features found histologically are not present on cytologic exam and vice versa. An example is the lack of alveolar architecture, lack of cytoplasmic crystalloids, but presence of cytoplasmic vacuoles and fine intercellular matrix in alveolar soft part sarcoma on cytologic as opposed to histologic exam [9]. Thus, it is important to recognize that some tumors lose key features that are well described in surgical pathology texts but gain new key features that may not be well described (as yet) in cytology texts.

Despite these cautionary notes, it is possible to successfully interpret most bone and soft tissue lesions *to the extent required for clinical purposes*. Achieving a specific diagnosis (52 % of adult and 92 % of pediatric sarcomas in one study) is less important than driving the appropriate clinical decision (83 % of all cases; n = 140 in the same study) [7].

As the genetic features of soft tissue and bone tumors become more well-characterized, corresponding molecular testing will ensue to enable a specific diagnosis. Progress to date permits specific diagnosis of ~50 % of the more common low grade and high grade spindle cell sarcomas and 100 % of small round blue cell sarcomas using a combination of cytologic and molecular genetic analyses [14]. This convergence of cytologic evaluation with molecular genetic testing will continue to expand.

General Categories of Soft Tissue Tumors That Can Be Sampled by FNA

Expert soft tissue/bone cytopathologists recommend dividing the FNA findings of bone and soft tissue lesions into five categories according to cell type or predominant matrix: (1) spindle cell; (2) polygonal/epithelioid cell; (3) small round blue cell; (4) pleomorphic; and (5) myxoid matrix rich. Four additional categories are included in this chapter because of the natural placement of mimicking lesions in these categories: (6) biphasic comprising mixed spindle cell and epithelioid/polygonal cells, (7) inflammatory cell rich, (8) giant cell rich, and (9) fatty. Figure 12.1 provides examples of these cell types in bone and soft tissue tumors, and Fig. 12.2 provides examples of the types of background found in bone and soft tissue tumors.

With these cytomorphologic categories, one can easily sort the specimen broadly then factor in the clinical history and imaging findings to select ancillary tests as needed: a set of steps culminating in a clinically actionable diagnosis and sometimes even a specific diagnosis. Tables 12.1 and 12.2 provide representative lists of tumors found in bone and soft tissue that can be sampled by fine needle aspiration biopsy. The tumors are arranged according to cell morphology or specific prominent background component.

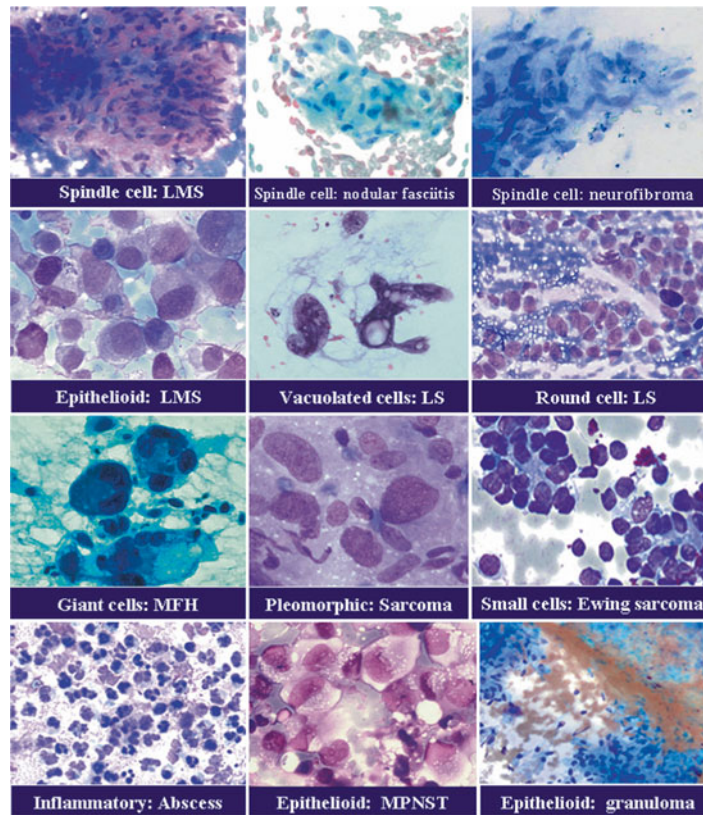


Fig. 12.1 Categorization of predominant cell type in soft tissue and bone FNAC. *LMS* leiomyosarcoma, *LS* liposarcoma, *UPS* undifferentiated pleomorphic sarcoma, and *MPNST* malignant peripheral nerve sheath tumor. (Images and text courtesy of Khalbuss and Parwani, *Introduction to Soft Tissue and Bone Cytopathology: A Practical Approach,* copyright 2011, with permission of Springer.)

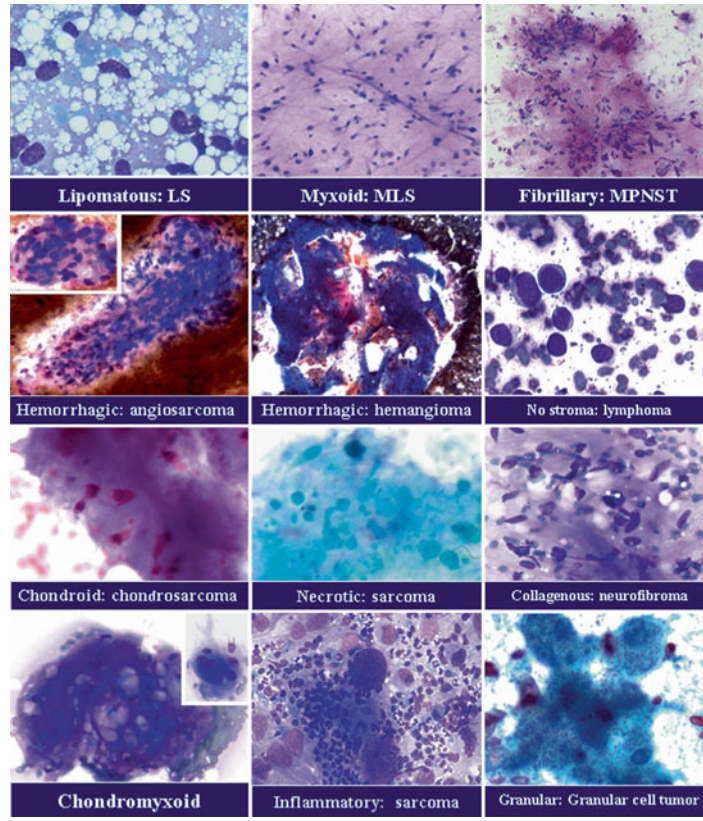


Fig. 12.2 Examples of categorization of background findings in soft tissue and bone FNAC. *LS* liposarcoma, *MLS* myxoid liposarcoma, *MPNST* malignant peripheral nerve sheath tumor. (Images and text courtesy of Khalbuss and Parwani, *Introduction to Soft Tissue and Bone Cytopathology: A Practical Approach,* copyright 2011, with permission of Springer.)

Table 12.1 Bone lesions that can be sampled by FNA

Cell morphology	Lesion	Matrix	Most common bone site	Most common radiologic imaging
Epithelioid lesions	Osteoid osteoma/ osteoblastoma	Osteoid, bone, rarely cartilage	M-D, D, spine, sacrum	Non-A, A
	Osteosarcoma	Osteoid, bone, +/− cartilage	M-D, gnathic bones in older adults	A
	Enchondroma	Cartilage	D, M-D	Non-A
	Chondrosarcoma	Cartilage +/− myxoid	D, pelvis, ribs	A or Non-A
	Chondroblastoma	Pink or blue osteochondroid	E	A or Non-A
	Clear cell chondrosarcoma	Pink or blue osteochondroid, bone, osteoid	E	A or Non-A
	Metastatic carcinoma	None, collagen	D, M-D	A usually
	Metastatic melanoma	None, collagen	D, M-D, pelvis, ribs	A usually
Spindle cell lesions	Osteosarcoma, fibroblastic variant	Osteoid, bone, none, collagen	M-D	A
	Chondrosarcoma, high grade	Hyaline cartilage, myxoid	D, pelvis, ribs	A
	Non-ossifying fibroma	None, collagen, osteoid	M-D	Non-A
	Aneurysmal bone cyst	None, collagen, osteoid	M-D	Non-A
	Metastatic melanoma	None, collagen	D, M-D, pelvis, ribs	A usually

(continued)

Table 12.1 (continued)

Cell morphology	Lesion	Matrix	Most common bone site	Most common radiologic imaging
Mixed epithelioid and spindle cell lesions	Fracture callus	Osteoid, bone, +/− cartilage	M-D, D	Non-A usually
	Osteosarcoma	Osteoid, bone, +/− cartilage	M-D	A
	Metastatic melanoma	None, collagen	D, M-D, pelvis, ribs	A usually
	Rare other primary bone sarcomas	None, collagen	M-D, D	A usually
Pleomorphic cell lesions	Osteosarcoma	Osteoid, bone, +/− cartilage	M-D	A
	Chondrosarcoma, high grade	Cartilage, myxoid	D, pelvis, ribs	A
	Rare other primary or metastatic high grade sarcomas	None, collagen	M-D, D	A
	Metastatic melanoma	None, collagen	D, M-D, pelvis, ribs	A usually
Giant cell rich lesions	Giant cell tumor of bone	None, collagen, osteoid	E	A or Non-A
	Osteosarcoma, giant cell rich	Osteoid, bone	M-D	A
	Chondroblastoma	Pink or blue osteochondroid	E	A or Non-A
	Osteomyelitis	Osteoid, bone, collagen, none	M-D, D, E	Non-A or A
Small round blue cell lesions	Ewing sarcoma family of tumors	None, collagen	D, pelvis	A
	Other translocation specific sarcomas	None, collagen	M-D, D, pelvis	A
	Lymphoma	None usually	D, M-D	A or Non-A
	Metastatic small cell carcinoma	None, collagen	D, M-D	A usually
	Metastatic melanoma	None, collagen	D, M-D, pelvis, ribs	A usually
	Metastatic pediatric small round cell tumors	None, collagen	D, M-D, pelvis, ribs	A usually
Inflammatory cell rich	Fracture callus	Osteoid, bone, +/− cartilage	M-D, D	Non-A usually
	Osteomyelitis	Osteoid, bone, collagen, none	M-D, D, E	A or Non-A
	Eosinophilic granuloma	None usually	D, skull	Non-A usually
	Rare carcinomas	None, collagen	D, M-D, pelvis, ribs	A usually
	Rare sarcomas	None, collagen	D, M-D	A usually
	Some lymphomas	None usually	D, M-D, pelvis, ribs, skull	A or Non-A

E epiphysis, *M* metaphysis, *D* diaphysis, *M-D* meta-diaphysis, *FB* flat bones, *A* aggressive on imaging, *Non-A* non-aggressive on imaging

Table 12.2 Soft tissue lesions that can be sampled by FNA

Cell morphology or dominant matrix	Lesion	Behavior	Site
Spindle cells	Some reactive pseudo-tumors such as mycobacterial pseudo-tumor	B	Sup, Dp, Retr, Visc
	Schwannoma	B	Sup > Retr >Dp
	Nodular fasciitis	B	Sup or Dp
	Neurofibroma	B	Sup or Dp
	Fibromatosis	I	Sup or Dp
	Spindle cell lipoma	B	Sup
	Dermatofibrosarcoma protuberans (DFSP)	I to M	Sup
	Some Malignant peripheral nerve sheath tumors (MPNST)	M	Dp
	Synovial sarcoma (SS), monophasic	M	Dp >> Visc = H&N
	Leiomyosarcoma (LMS)	M	Dp > Visc > Retr
	Some Gastrointestinal stromal tumors (GIST)	M	Visc
	Sarcomatoid carcinoma	M	H&N > Visc > Dp
	Some melanomas	M	H&N, Sup, Dp, Visc, Acr
Epithelioid and/or polygonal cells	Granular cell tumor	B	Sup > Dp > Visc
	MPNST, epithelioid variant	M	Dp
	LMS, epithelioid variant	M	Dp
	Epithelioid sarcoma	M	Sup, Dp
	Extra-renal rhabdoid tumor	M	Dp > Visc
	Alveolar soft part sarcoma	M	Dp, Visc
	Epithelioid hemangioendothelioma (EHE)	M	Sup, Dp, Visc
	Clear cell sarcoma (CCS)	M	Sup, Dp
	Sclerosing epithelioid fibrosarcoma (SEFS)	M	Dp
	Metastatic carcinoma	M	Sup, Dp
	Some metastatic melanomas	M	Sup, Dp, Visc
	Some lymphomas	M	Dp, Visc, Retr
Mixed spindle cells and epithelioid/polygonal cells	Synovial sarcoma, biphasic	M	Dp, Visc, H&N
	Some undifferentiated pleomorphic sarcoma	M	Dp, Retr
	Some myoepithelial tumors	B-M	Dp, Sup, H&N
	Some malignant peripheral nerve sheath tumors	M	Dp, Retr
	Some epithelioid sarcomas	M	Sup, Dp
	Some angiosarcomas	M	Sup, H&N, Dp, Visc
	Some rhabdomyosarcomas	M	Dp, H&N, Dp, Visc
	Some sarcomatoid carcinomas	M	Sup, H&N, Visc
	Some melanomas	M	Sup, H&N, Visc, Acr
Small round blue cells	Paraganglioma	B-M	H&N, Visc
	Glomus tumor	B	Acr, Sup
	Solitary fibrous tumor, cellular variant	I-M	Dp > H&B > Visc
	Extra-skeletal Ewing sarcoma family of tumors	M	Dp >> Visc
	Synovial sarcoma, poorly differerentiated	M	Dp, H&N, Visc
	Desmoplastic small round cell tumor (DSRCT)	M	Visc
	Other small round blue cell translocation specific sarcomas	M	Dp, H&N, Visc
	Pediatric undifferentiated sarcoma	M	Dp
	Some rhabdomyosarcomas	M	Dp, H&N, Dp, Visc
	Merkel cell carcinoma	M	Sup
	Metastatic small cell carcinoma	M	Sup, Dp, Visc
	Metastatic pediatric small round cell tumors such as Wilms tumor, neuroblastoma	M	Visc, Retr
	Endometrial stromal sarcoma (ESS)	M	Visc, Retr
	Some melanomas	M	Sup, H&N, Visc, Acr
	Lymphomas	M	Dp, Visc, Retr

(continued)

Table 12.2 (continued)

Cell morphology or dominant matrix	Lesion	Behavior	Site
Pleomorphic cells	Undiffererentiated pleomorphic sarcoma (UPS)	M	Dp, Retr
	Liposarcoma, pleomorphic	M	Dp
	Myxofibrosarcoma, high grade	M	Sup, Dp
	Rhabdomyosarcoma, pleomorphic	M	Dp, H&N
	Some melanomas	M	Sup, H&N, Visc, Acr
	Rare lymphomas, carcinomas	M	Sup, Dp, H&N, Visc, Acr
Myxoid matrix	Ganglion cyst	B	Near Acr, Dp
	Intramuscular myxoma	B	Dp
	Nodular fasciitis	B	Sup or Dp
	Schwannoma with myxoid change	B	Dp
	Myxoid liposarcoma (MLS)	M	Dp
	Chordoma	M	Base of skull, spine, sacrum almost exclusively
	Extraskeletal myxoid chondrosarcoma (EMCS)	M	Dp
	Metastatic mucinous carcinoma	M	Sup, Dp, Retr
Fat cells	Lipoma	B	Sup
	Fibrolipoma	B	Sup, often H&N
	Chondroid lipoma	B	Dp > Sup > H&N
	Intramuscular lipoma	B	Dp
	Myelolipoma	B	Visc
	Hibernoma	B	Dp
	Intramuscular hemangioma	B	Dp
	Some mesenchymal tumors that can contain fat such as fat-forming SFT and myofibroblastoma	B-M	Dp, Visc
	Liposarcoma	M	Dp, Retr
	Lipoblastoma	B-I	Dp > H&N > Visc
Inflammatory cell rich	Nodular fasciitis and other variants of fasciitis	B	Sup or Dp
	Some schwannomas	B	Dp, Retr
	Inflammatory myofibroblastic tumor	B-M	Dp, Visc, Retr
	Abscess	B	Any site
	Fat necrosis	B	Large fatty sites such as breast
	Granulation tissue	B	Any site
	Liposarcoma, inflammatory variant	M	Retr > Dp
	Leiomyosarcoma, inflammatory variant	M	Visc > Retr > Dp
	Sarcomas with necrosis	M	Any site
	Some carcinomas	M	Any site
	Some lymphomas	M	Any site
Giant cell rich	Granulomatous inflammation	B	Any site
	Myositis ossificans	B	Dp
	Tenosynovial giant cell tumor (localized and diffuse types)	B-M	Dp, Acr, H&N
	Some undiffererentiated pleomorphic sarcomas (UPS)	M	Dp, Retr
	Giant cell tumor of low malignant potential	I	Sup >> H&N
	Some metastatic carcinomas	M	Any site
	Some metastatic melanomas	M	Any site

B benign, *I* intermediate, *M* malignant, *Sup* superficial (supra-fascial) soft tissue, *Dp* deep soft tissue (sub-fascial), *Retr* retroperitoneum, *Visc* visceral, *H&N* head and neck, *Acr* acral

According to the cytomorphology categories above, the most common challenges in soft tissue FNAB diagnosis include (1) distinguishing benign spindle cell tumors from low grade spindle cell sarcomas, (2) distinguishing benign myxoid tumors from malignant myxoid tumors, (3) distinguishing benign fatty tumors from low grade malignant fatty tumors. Tables 12.3, 12.4, 12.5, 12.6, 12.7, 12.8, 12.9, 12.10, and 12.11 list the most common mimickers of bone and soft tissue tumors arranged by cell morphology or specific prominent background component. Some lesions appear in more than one table. Imaging is more helpful for bone than for soft tissue tumors and is essential for the diagnostic workup. The spindle cell and polygonal/epithelioid cell categories may be the most challenging to accurately subclassify and grade on cytology. Some tumors such as smooth muscle tumors that rely partly on mitotic counts to grade or render the lesion malignant cannot be definitively diagnosed on cytology.

When assessing a bone or soft tissue tumor, it is important to note that in terms of numbers, benign lesions >>> malignant (1/4 of adults develop a soft tissue mass, while only 1/200,000 develop a sarcoma), and the number of patients with metastatic carcinoma and melanoma >> the number of patients with primary bone tumors.

Special circumstances may require a particularly cautious diagnostic approach. For example, findings of post-radiation sarcoma can be impossible to separate from radiation-induced atypia: tissue biopsy is recommended in these cases.

Major Cytologic Mimics and Confounders of Bone Tumors

Benign Cells

Osteoblasts

These small cells can assume a plasmacytoid or epithelioid appearance and may cluster simulating a metastatic carcinoma. However, nuclear atypia is absent, the cells are relatively uniform and small compared to most malignant epithelial cells, and a characteristic badminton shuttlecock-like shape is usually found in a few of the cells (see Fig. 12.3). Osteosarcoma, by contrast, is usually high grade and features osteoblasts with prominent pleomorphism (Fig. 12.4).

Osteoclastic Giant Cells

If numerous, these cells could suggest the diagnosis of giant cell tumor of bone. Particularly in touch preps prepared from needle core biopsy specimens (when assessing for adequacy), osteoclasts seem to preferentially touch off on the slide. Even so, the number of these cells is still relatively few, and other components of giant cell tumor of bone such as the ovoid and spindled mononuclear tumor cells are absent. As with any bone lesion, careful correlation with imaging findings can avert a misdiagnosis. Giant cell tumor of bone, for instance, is one of the few tumors centered in the epiphysis, and patients are predominantly young to middle aged adults with a closed physis in the affected bone. Giant cells infiltrate neoplasms, may form as a reaction to foreign material or represent an inflammatory response to infection (see Fig. 12.5).

Infectious/Inflammatory and Reactive Processes

Epithelioid Granulomas

Epithelioid histiocytes are elongate spindle cells with wispy cytoplasm and ovoid to multiple or multi-lobulated nuclei that are hypochromatic with variably prominent nucleoli. Coarse nuclear chromatin, irregularly distributed chromatin, sharply angulated and creased nuclei and hyperchromasia (all features of nuclei usually found in malignant tumors) are absent. The cells may vaguely stream together and form mats or vague arrays corresponding to fragmented granulomas. Necrotic debris may or may not be present. The background may exhibit chronic inflammation and may contain dispersed histiocytes. Examples of atypia encountered in inflammatory processes are presented in Fig. 12.6. By contrast, a tumor mimicking a granulomatous process is presented in Fig. 12.7. Clinical history, imaging findings, and subtle nuclear features can prompt ancillary testing that may be required for correct diagnosis.

Table 12.3 Spindle cell mimickers/confounders of spindle cell mesenchymal tumors

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Nodular fasciitis (also known as a "pseudosarcoma")	Spindle cell sarcomas	Loose mats and single cells	• Spindled and polygonal cells • Cytoplasm often wispy or torn • Normal mitotic figures • Nuclei with fine chromatin +/− nucleoli	• Can be fibrotic, myxoid or hemorrhagic • May have intermixed chronic inflammatory cells	• Lacks coarse chromatin • Lacks atypical mitoses • Almost always lacks necrosis	• ICC: +/− actin; usually desmin negative • FISH:*USP6* translocation	• Rapid growth • Size almost always <4 cm • +/− hx of trauma • Usually in adolescents/young adults
Schwannoma with degenerative atypia	Spindle cell sarcomas especially LMS, GIST and FS arising in DFSP	• Tight mats +/− streaming and palisading • Relatively few dispersed single cells. Cells often disposed in fibrillary stroma	• Spindled cells with elongate buckled or sharply pointed nuclei AND cells with nuclear variability in size and shape; nuclear pseudo-inclusions	• May have a few inflammatory cells • Fibrillary stroma • Myxoid matrix +/−	• Degenerative nuclei have smudgy chromatin and nuclear holes • Usually lack coarse chromatin of malignant cells	ICC: S-100 protein diffusely positive (if performed on cell-block)	• Often superficial location • Usually <5 cm except in some deep sites
Inflammatory pseudotumor	Spindle cell sarcomas	Mats of plump to thin spindled cells and some dispersed cells	• Nuclei plump to elongate spindled • Chromatin usually fine • +/− nucleoli	Acute/chronic inflammation +/−	Nuclei lack coarse chromatin	Special stains may be positive for organisms such as mycobacteria	• Usually <5 cm • Patient may clinical features of infection
Sarcomatoid carcinoma	Spindle cell sarcomas	• Mats of spindled cells • May have epithelioid cells also	• Nuclear atypia usually high with pleomorphism • Coarse chromatin • Irregularly distributed chromatin	Necrosis often present	May not be able to distinguish sarcomatoid ca from spindle cell sarcoma	ICC: often positive for keratins but also vimentin or other ST markers	• Often located in head and neck in older patients • Often <5 cm
Melanoma with spindle cells	Spindle cell sarcomas	Usually dispersed spindled cells with few or no clumps	• Moderate to marked nuclear atypia • +/− macronucleoli • +/− tumor giant cells	Necrosis may be present	Melanin pigment may be present	ICC: variably positive for S-100, HMB45, MelanA, Mit-F	• Often superficial • May have clinical history of melanoma • May have lymphadenopathy
Fibromatosis	Spindle cell sarcomas	• Mats of plump spindle cells • Isolated cells • Stripped nuclei	Ovoid to plump spindled nuclei	• Some have metachromatic collagenous matrix fragments • +/− mast cells • +/− few lymphocytes	• Uniform cells • Lacks coarse chromatin	ICC: 70% have nuclear beta-catenin	• Often appears infiltrative on imaging • May be large

Spindle cell sarcomas include DFSP, LMS, MPNST, synovial sarcoma, some angiosarcomas, Kaposi sarcoma, pseudomyogenic hemangioendothelioma, low grade myofibroblastic sarcoma and others. Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

Table 12.4 Small round blue cell mimickers/confounders of round cell sarcomas

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Lymphoma	High grade small round cell sarcoma	• Dispersed cells • Some clumps if smears highly cellular	• Small to medium sized round cells usually with high N/C ratio • Round to cerebriform nuclei • +/− nucleoli	• Lymphoglandular bodies often present • Smeared nuclei	• Usually lacks nuclear molding • Often has lymphoglandular bodies	• ICC: lymphoid markers positive • Flow cytometry:can confirm diagnosis and aid in subtyping	• Patients may have lymphadenopathy • +/− peripheral blood abnormalities
Melanoma with small round cell morphology	High grade small round cell sarcoma	Mostly dispersed cells	Small round blue cells	• Melanin may be present • Necrosis +/−	May have some epithelioid cells with macronucleoli	ICC: melanocytic markers positive	• Patients may have lymphadenopathy • May have clinical history of melanoma
SFT, cellular variant	High grade small round cell tumor	• Tight clusters with prominent vessels • Stripped nuclei and few dispersed intact cells	• Small short plump spindled or round cells • Nuclei may be hyperchromatic with high N/C ratio	No specific background	• Usually lacks necrosis, apoptosis and coarse chromatin	ICC: positive for STAT6, +/− CD34	No specific clinical or imaging features
Endometrial stromal sarcoma (ESS)	High grade small round cell tumor	Mats and dispersed single small round cells Stripped nuclei	• Low grade tumors: uniformovoidnuclei+/− • High grade tumors: small angulated nuclei often with nucleoli	Matrix usually absent	• Lack of atypia • +/− spindled cells • Usually lacks the cytoplasmic vacuoles commonly found in Ewing sarcoma	• ICC: CD10, ER positive; SMA +/− • FISH: multiple specific translocations found in ESS	• Patient may have clinical history of ESS (in cases of metastatic ESS) • Imaging may reveal uterine mass
Merkel cell carcinoma	High grade small round cell tumor	Dispersed cells and clusters or clumps of variably cohesive tumor cells	• Round cells with high N/C ratio • High mitotic rare and apoptosis • Nuclear molding +/−	• Matrix usually absent • Rarely may have squamous cell component	May be impossible to diagnose without ancillary studies	ICC: Keratins, especially CK20 often paranuclear, chromogranin, synaptophysin, ALK, PAX8, PAX5, CD99 (can be membranous), MC polyoma virus positive; usually negative for TTF-1	• Dermal location • Older patients
Metastatic small cell carcinoma	High grade small round cell tumor	Mats and dispersed cells	• Round cells with high N/C ratio, nuclear molding, salt and pepper chromatin • Apoptotic bodies, mitoses	• Necrotic debris common • Smeared nuclear strands	• Neuroendocrine nuclear features • Nuclear molding suggestive but not specific as also occurs in round cell sarcomas	ICC: Keratin positive, chromogranin, synaptophysin usually positive, TTF-1 often positive	• Older patients • Lung lesion • Mediastinal adenopathy

High grade small round cell sarcomas include Ewing/PNET family of tumors, poorly differentiated synovial sarcoma, some rhabdomyosarcomas, desmoplastic small round cell sarcoma, CIC-DUX4 sarcoma, CCNB3-BCOR Ewing like sarcoma and others. Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

Table 12.5 Epithelioid/polygonal cell mimickers/confounders of epithelioid/polygonal cell sarcomas

Mimicker/confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/imaging findings
Proliferative myositis (a "pseudosarcoma")	Sarcoma with epithelioid cell morphology	Mats and dispersed cells	• Ganglion-like cells with amphophilic cytoplasm and prominent nucleoli • Polygonal cells with wispy or frayed cytoplasm	• Multinucleated degenerated muscle fibers • Chronic inflammation	• Nuclei have fine chromatin • Normochromatic without malignant features	ICC: often positive for SMA; usually negative for desmin	• Middle aged patient • Usually small size (most <6 cm) with rapid growth and history of injury in 50%
Osteoid osteoma/ osteoblastoma	Osteosarcoma	Small clusters and dispersed cells	Round osteoblasts with badminton shuttlecock shape	Osteoid chunks	May be impossible to distinguish from osteosarcoma	ICC: stains similarly to osteosarcoma	Imaging: often diagnostic
Chondroblastoma	Osteosarcoma	Small clusters and dispersed cells	Epithelioid tumor cells some with grooved nuclei and small nucleoli	• Pink chondroid matrix • Multinucleated osteoclasts	A few cases have more marked cytologic atypia	• ICC: S-100 protein, DOG-1, SMA, SATB2 may be positive • Genetic testing: PCR	• Imaging: epiphyseal lesion • Adolescent to young adult most commonly
Granular cell tumor	Alveolar soft part sarcoma Hibernoma Certain carcinomas	Dispersed cells and clumps	• Predominantly round to polygonal cells with low N/C ratio and granular cytoplasm • Few spindle cells • Nuclei round to oval +/– nucleoli +/– range in nuclear size and shape	Background may include many stripped tumor nuclei and aggregated pools of basophilic cytoplasm (on DQ)	• May be impossible to distinguish from alveolar soft part sarcoma or some carcinomas (RCC, HCC) • May be impossible to distinguish benign from malignant granular cell tumor	• ICC: S-100 positive, TFE3 positive (but so is ASPS), negative for keratins • Genetics: lacks *TFE3* rearrangement by FISH as found in ASPS (and also in a subset of RCC)	• Mostly in middle-aged adults in head and neck region but also extremities and viscera • Can be multiple in 10% simulating a more aggressive tumor
Clear cell chondrosarcoma	Osteosarcoma	Small clusters and dispersed cells	Epithelioid and polygonal tumor cells with prominent nucleoli	Osteoid	May be impossible to distinguish from osteosarcoma	ICC: S-100 protein, SATB2 positive	• Imaging: epiphyseal lesion • Young to middle ages adults most commonly
Melanomas with epithelioid/polygonal cell morphology	UPS	Clusters, mats and dispersed cells	• Epithelioid and polygonal tumor cells with macronucleoli • Tumor giant cells	• Melanin pigment sometimes present • Chronic inflammation+/–	May be impossible to distinguish without ancillary studies	ICC: S-100 protein, HMB45, MelanA usually positive but some cases are negative for all melanocytic markers	• Most patients middle aged or older • May have clinical history of melanoma even if remote
Metastatic carcinoma	UPS, Epithelioid sarcoma	• Tight clusters • Loose mats • Dispersed cells	• Medium to large cells with moderate NC ratio • Pleomorphic nuclei with coarse chromatin • +/– nucleoli • +/–mitoses	• Necrosis common • +/– collagenous fragments	May have lower grade component forming tubules or glands	ICC: positive for various epithelial markers including keratins, EMA, CEA, MOC31 etc	• May have clinical history of carcinoma • Middle aged or older patients

Sarcomas with epithelioid cell morphology (aside from epithelioid sarcoma) include some clear cell sarcomas, some malignant extra-renal rhabdoid tumors, some angiosarcomas, some malignant peripheral nerve sheath tumors, some leiomyosarcomas, sclerosing epithelioid fibrosarcoma, PEComas, some myxofibrosarcomas, some liposarcomas, some undifferentiated pleomorphic sarcomas (UPS) and others. Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

Table 12.6 Biphasic or mixed spindle cell and epithelioid/polygonal cell mimickers/confounders of mixed cell type sarcomas

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Granulation tissue	Mixed cell sarcoma	Mats and dispersed cells	• Spindled and polygonal fibroblasts and myofibroblasts • May sample epithelioid endothelial cells	Inflammation (acute or chronic) with histiocytes	Lacks malignant nuclear features	ICC: variably positive for SMA; usually negative for desmin	Clinical history of wound or injury
Fasciitis (a "pseudosarcoma")	Mixed cell sarcoma	Mats and dispersed cells	Spindled and polygonal fibroblasts/myofibroblasts	Chronic inflammation +/−	Lacks malignant nuclear features	ICC: variably positive for SMA; usually negative for desmin	Nodular fasciitis: rapidly growing, small (<4 cm) nodule in adolescents-young adults
Carcinoma with mixed morphology	Mixed cell sarcoma	Clusters, mats, dispersed cells	Round, pleomorphic and spindled to polygonal cells	• Necrosis common • Collagen fragments +/−	• Malignant nuclear features present • May have tubules or glands	ICC: usually positive for keratins	May have clinical history of carcinoma
Melanoma with mixed morphology	Mixed cell sarcoma	Mats and dispersed cells	• Round, pleomorphic and spindled to polygonal cells • Nuclei may have cherry red macronucleoli • May have nuclear pseudoinclusions	• Necrosis common • Inflammation+/− • Melanin +/−	May require ancillary testing to distinguish melanoma from other malignant tumors	ICC: melanocytic markers usually positive	May have clinical history of melanoma, even if remote (>20 years)

Some sarcomas with mixed spindle cell and epithelioid appearing cells (mixed cell sarcoma) include synovial sarcoma, some malignant peripheral nerve sheath tumors, some epithelioid sarcomas, some high grade examples of myxofibrosarcoma, leiomyosarcoma, liposarcoma. Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

Table 12.7 Fat cell mimickers/confounders of mesenchymal tumors with prominent fatty component

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Normal fat	Lipoma	• Fragments of fat cells with low N/C ratio • Intermingled small blood vessels	• Adipocytes have very low N/C ratio • Small, hyperchromatic nuclei Cells of Lockhern have larger, pale nuclei with multinucleation +/− pseudo-inclusions	Fat droplets	• May be impossible to distinguish from conventional lipoma • Some lipomas have scattered lipoblast-like cells	Karyotype can be abnormal in lipoma but in practical terms seldom performed	Ensure the needle is in the mass by imaging
Fat necrosis	Liposarcoma	Variably sized fat fragments with coursing vessels	• Some fat cells may be multivacuolated • Others typical	• Histiocytes including multivacuolated lipophages, +/− foreign body type giant cells, lymphocytes • Granular debris • Hemosiderin +/− • Fat droplets	• May be impossible to distinguish from atypical lipomatous tumor • Multivacuolated fat cells lack nuclear hyperchromasia	Not routinely performed	• Patient may have history of trauma • Lesion is usually small (<5 cm)
Lipoma with myxoid degeneration or fibrosis	• Myxoid LPS • Lipoblastoma • Spindle cell LPS	• Fat fragments with coursing vessels • Cells include normal fat cells and cells with multivacuolation	Multivacuolated cells may be regenerating lipoblasts or lipophages	• May have prominent myxoid matrix or fibrous fragments • Fat droplets	• Lacks malignant nuclear features • Lacks round cell component	• ICC: lacks significant MDM2/ CDK4 expression • Genetics: lacks translocations of myxoid LPS	• Tumor is often superficial and <10 cm • Almost all patients are adults
Spindle cell/ pleomorphic lipoma	ALT Spindle cell LPS	Fat fragments with coursing capillaries	• Adipocytes • Plump spindled cells usually with minimal atypia • Rare lipoblasts • Floret giant cells	• Myxoid stroma often prominent • Collagen fragments or fibers usually sparse • Mast cells	• Less cellular than spindle cell LPS • Lacks hyperchromatic lipoblasts	ICC: lack CDK4/ MDM2 expression and amplification Loss of normal RB1 expression in many cases	• Tumor is usually superficial in head and neck of middle aged patients (M>F) • Usually <10 cm
Intramuscular lipoma	Liposarcoma	Similar to lipoma except may have entrapped skeletal muscle fibers	Similar to lipoma	Entrapped skeletal muscle fibers may be atrophic and multinucleated	Difficult to distinguish from ALT but lacks hyperchromatic lipoblasts	• ICC: lacks significant reactivity for CDK4 and MDM2 • FISH: lacks amplification of CDK4, MDM2	Confirm that needle is in the lesion

(continued)

Table 12.7 (continued)

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Hibernoma	Liposarcoma	Large adipocytic fragments with coursing blood vessels	Tumor cells include microvacuolated brown fat cells and lipoblast like cells most commonly but also granular and spindle cells occur	Microvacuolated cells are clue to diagnosis but rare ALT can contain brown fat cells, too	Abundant brown fat cells suggest diagnosis	ICC: lacks MDM2/ CDK4 expression and amplification	Usually in young adults
Chondroid lipoma	• Liposarcoma including ALT and myxoid LPS • Extraskeletal myxoid CS • Hibernoma	• Clusters of cells and fragments intermixed with matrix • Vessels usually sparse	• Variably sized adipocytes • Chondroid cells appear microvacuolated	• Chondroid stroma (bluish gray on DQ stain) with intermixed cells • Myxoid stroma (magenta on DQ stain)	Nuclei lack malignant features	ICC: lacks CDK4/ MDM2 expression and amplification	• Rare • Superficial to deep tumor
Tumor infiltrating fat	Liposarcoma	Malignant cells intercalated with fat fragments	• Tumor cells often pleomorphic • May have multivacuolated regenerating fat cells simulating lipoblasts	• Necrosis +/− • Inflammation +/−	• Tumor nuclei have malignant features • Regenerating fat cells are not hyperchromatic	ICC: lacks MDM2/ CDK4 overexpression or amplification	Patients may have clinical history of carcinoma or melanoma

Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

Table 12.8 Myxoid mimickers/confounders of myxoid mesenchymal tumors

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Fasciitis (a "pseudosarcoma")	Sarcoma with myxoid matrix	• Mats and dispersed cells • Heterogeneous cell population	• Spindled to polygonal cells mostly • Proliferative myositis has ganglion-like cells	• Prominent myxoid matrix (magenta on DQ) • Collagenfragments+/– • Lymphocytes • Red blood cells	Nuclei lack malignant features	• ICC: variably positive for SMA and usually negative for desmin • Genetics: *USP6* mutation	• Rapidly growing in young adult (nodular fasciitis) and middle aged adult (proliferative myositis) • Almost all <5 cm and superficial
Schwannoma with myxoid degeneration	Sarcoma with myxoid matrix	• Mats with relatively few dispersed cells • Palisading +/–	• Buckled, wavy or pointy ended spindled cells • May have a few tumor cells with multilobulated nuclei • Scattered nuclear pseudoinclusions	• Myxoid matrix may be abundant • Inflammation +/–	• Scattered atypical cells lack malignant nuclear features and may show degenerative changes (nuclear holes, smudgy chromatin)	ICC: diffusely positive for S-100 protein	• Usually <5 cm when superficial • Often painful on aspiration • May show association with a peripheral nerve on imaging
Intramuscular myxoma	Low grade myxofibrosarcoma	• Relatively hypocellular in most cases • Elongate spindle cells	Spindle cells have long +/– branching processes	• Abundant myxoid matrix • +/– vacuolated histiocytes • +/– degenerated multinucleated muscle cells	• Low cellularity in most cases • Lack of prominent vessels in most cases • Lack of malignant nuclear features	ICC: not routinely performed	• Deep lesion usually with uniform appearance on imaging
Low grade fibromyxoid sarcoma	Fibroma, fibromatosis	Often hypocellular Plump spindle cells	• Nuclei ovoid to slightly tapered • Recurrent cases may show more nuclear atypia and variation	Myxoid matrix usually more common than fibrous fragments	• Often not recognizable as malignant • Nuclear features bland	• ICC: MUC4 positive and diagnostically helpful • FISH: *FUS* rearranged	• Occurs in young to middle aged adults • Related to sclerosing epithelioid FS
Metastatic mucinous carcinoma	Sarcoma with epithelioid cells and myxoid matrix such as myxofibrosarcoma, epithelioid variant	Usually small clusters and dispersed tumor cells	• Nuclei range from round to pleomorphic • Signetringcells+/–	• Abundant mucin • +/– necrotic debris • +/– inflammation	Malignant nuclear features usually present	ICC: positive for keratins and more specific markers depending on site of origin	• Patients are usually middle aged and older • May have clinical history of carcinoma

Sarcomas with myxoid matrix include myxofibrosarcoma, myxoid liposarcoma, low grade fibromyxoid sarcoma, some examples of leiomyosarcoma, malignant peripheral nerve sheath tumor, synovial sarcoma and others. Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

Table 12.9 Giant cell rich mimickers/confounders of mesenchymal tumors with prominent osteoclast-like giant cells

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Granulomatous inflammation	Mesenchymal tumor with giant cells	• Fragments of granulomas • Dispersed inflammatory cells	Heterogeneous cell population including spindled epithelioid histiocytes, round histiocytes, MNGC, lymphocytes	• Necrotic if granulomas are caseating • Foreign material in cases of foreign body granulatmous reaction	Mixed cell population a clue to the diagnosis	ICC: histiocytes express CD68 and not keratins (do both because some carcinomas express CD68)	History of inflammatory or infectious process
Sarcoidosis	Infection Low grade spindle cell sarcoma	Epithelioid granulomas	• Epithelioid histiocytes • MNGC	• Necrosis usually absent • Absent to sparse lymphocytic inflammation	Histiocytes lack malignant nuclear features	ICC: lesional cells express CD68	
Giant cell tumor of bone	Osteosarcoma, giant cell rich	Mixed MNGC and spindled to ovoid mononuclear cells	Tumor cells are ovoid to spindled	• Osteoid +/− • Inflammation +/−	• MNGC often have >20 nuclei • Usually only mild nuclear atypia	• ICC:SATB2 positive in 50%, MNGC positive for CD68 • Genetics: not routinely done but can be useful	Imaging very helpful=epiphyseal lesion in skeletally mature bone (closed physis)
Non-ossifying fibroma	• GCT of bone • Osteosarcoma	Mats and clumps of fibroblasts, inflammatory cells	• Tumor cells are spindled • MNGC +/−	• Osteoid +/− • Inflammation common • Collagenfragments+/−	Tumor cells lack malignant nuclear features	• ICC: not routinely performed • Genetics: not performed	Imaging very helpful and often suggests the diagnosis
Aneurysmal bone cyst	• GCT of bone • Telangiectatic osteosarcoma	• Usually hypocellular • Mats and clumps of fibroblasts, osteoblasts	• Tumor cells are spindled • MNGC common	• Osteoid +/− • Inflammation common • Collagenfragments+/−	Tumor cells lack malignant nuclear features	• ICC: not routinely performed • Genetics: *USP6* mutations but not routinely performed	Imaging very helpful and often suggests the diagnosis

Some mesenchymal tumors with prominent multinucleated osteoclast-like giant cells include giant cell tumor of bone, chondroblastoma, some osteosarcomas, clear cell chondrosarcoma, giant cell reparative granuloma (solid variant of aneurysmal bone cyst), giant cell tumor of low malignant potential, and some examples of undifferentiated pleomorphic sarcoma and leiomyosarcoma, among others. Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

Table 12.10 Pleomorphic cell mimickers/confounders of sarcomas composed of pleomorphic tumor cells

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Ischemic fasciitis	UPS and other pleomorphic sarcomas	Loose clumps and dispersed cells	• Polygonal and spindled cells • Multinucleated myofibroblastic giant cells	• Granular debris • Necrotic debris +/− • Inflammation common including lipophages	• May be difficult to distinguish from high grade sarcoma	ICC: variably positive for SMA; usually negative for desmin	Site is usually but not always similar to decubitus ulcer sites and may be associated with ulcer
Melanoma with pleomorphic cells	• UPS and other pleomorphic sarcomas • Some carcinomas • Some anaplastic lymphomas	Dispersed cells and loose clumps	• Pleomorphic cells some with multiple nuclei or multilobated nuclei • Macronucleoli common • Nuclear pseudoinclusions +/−	• Melanin +/− • Necrosis +/− • Inflammation common	May be impossible to distinguish from high grade sarcoma or carcinoma or anaplastic lymphoma	ICC: most cases positive for one or more melanocytic markers	May have history of melanoma even if remote (>20 years)
Metastatic carcinoma with pleomorphic cells	UPS and other pleomorphic sarcomas	• Dispersed cells • Mats, clumps, clusters	Pleomorphic cells with pleomorphic nuclei	• Necrosis common • Inflammation +/−	May be impossible to distinguish from high grade sarcoma or anaplastic lymphoma	ICC: most cases positive for keratins and other epithelial markers	May have clinical history of carcinoma

Some sarcomas with pleomorphic cells include undifferentiated pleomorphic sarcoma, dedifferentiated liposarcoma, high grade myxofibrosarcoma, and some examples of leiomyosarcoma, malignant peripheral nerve sheath tumor, dedifferentiated liposarcoma, and dedifferentiated solitary fibrous tumor, among others. Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

Table 12.11 Inflammatory cell rich mimickers/confounders of mesenchymal tumors with prominent inflammation

Mimicker/ confounder	What it mimics	Architecture	Cell features	Background	Differentiating cytologic features	Ancillary tests	Helpful clinical/ imaging findings
Abscess	Inflamed sarcoma	Predominantly dispersed cells	• Neutrophils, eosinophils lymphocytes histiocytes • Few spindled fibroblasts or myofibroblasts	• Nuclear and cytoplasmic neutrophilic debris • Hemorrhage • Hemosiderin +/−	• Lacks malignant nuclear features • Marked acute inflammation unusual in sarcomas	May do special stains for organisms	Clinical history may support infectious or inflammatory process
Granulation tissue	Inflamed sarcoma	Mats and dispersed cells	• Prominent acute, acute and chronic or chronic inflammation • Spindled fibroblasts and endothelial cells	• Hemorrhagic • Hemosiderin +/−	Lacks malignant nuclear features	Usually not helpful	Clinical history of recent injury or surgery
Fracture callus	Osteosarcoma	• May be hypocellular • Mixed inflammation • Round osteoblasts • Osteoclasts • Spindled cells	Osteoblasts and spindled cells may have nuclear size variability and nucleoli	• Osteoid • Mineralized bone fragments • Collagen fragments +/− • Myxoidmatrix+/−	• Osteoid may be lacelike like OS • Nuclear features may simulate osteosarcoma (may not be distinguishable)	• ICC: not helpful in differential of OS • Genetics: karyotype usually aneuploidy in high grade OS	• Clinical history of trauma • Some imaging features may be helpful
Fat necrosis	• Inflamed high grade sarcoma • Liposarcoma • Carcinoma if histiocytes mistaken for epithelial cells	• Fat fragments • Dispersed and loosely clustered cells	• Histiocytes including lipophages, MNGC, • Fat cells +/− scattered multivacuolated fat cells • Spindled cells	• Free fat droplets • Crumpled tissue paper- like structures (serum with fibrin) • Inflammation +/−	• Lesional cells lack malignant nuclear features • No large hyperchromatic stromal cells as found in ALT/LPS	ICC: histiocytes positive for CD68, negative for keratins	• Clinical history of trauma in ~50 % of cases • Most common in breast

Inflammatory myofibroblastic tumor	Inflamed high grade sarcoma	Dispersed and loosely clustered cells	• Spindled cells with oval hypochromatic nuclei +/− small nucleoli • +/− focal nuclear atypia • +/− ganglion like cells	• Lymphocytes • Plasma cells • (Neutrophils in the rare epithelioid inflammatory myofibroblastic sarcoma)	Lesional cells lack malignant nuclear features	• ICC: variably positive for SMA, usually negative for desmin, ALK positive in ~40% of patients, most commonly pediatric patients	• Wide age range but most commonly in children to young adults • Visceral sites such as lungs, bladder relatively common. • No specific imaging findings
Liposarcoma, inflammatory variant	• Granulation tissue • Abscess	Scattered atypical cells and clumps of tumor cells nearly obscured by inflammation	Tumor cells include lipoblasts with single and multiple vacuoles indenting a hyperchromatic nucleus and atypical stromal cells	Abundant neutrophils with fewer numbers of lymphocytes, plasma cells and histiocytes	• May not be possible to make the diagnosis if tumor cells are sparse or obscured • Some examples of pleomorphic and dediff LPS also have prominent inflammation, usually chronic	ICC: MDM2 and CDK4 usually positive but lesional cells may be too few to assess	Usually occurs in retroperitoneum in middle aged to older adults
Some lymphomas	Small round blue cell sarcoma	• Dispersed cells • Clumps usually due to thick smears	High grade lymphomas and Hodgkin lymphomas have large lesional cells often with marked nuclear pleomorphism	• Background can include eosinophils, mast cells, reactive T-lymphocytes and histiocytes • +/− blue bodies	Reed-Sternberg nuclear features can be seen in mimics	• ICC: malignant cells positive for lymphoid markers • Flow cytometry/ tissue biopsy: often necessary for subtyping	• Patient may have multifocal lymphadenopathy and B symptoms • Imaging may reveal lymphadenopathy

some sarcomas that can be markedly inflamed include some liposarcomas, some leiomyosarcomas, inflammatory myofibroblastic tumor, and any sarcoma with necrosis and secondary reactive inflammation; *OS* osteosarcoma, *LPS* liposarcoma. Malignant nuclear features include coarse chromatin, irregularly distributed chromatin, hyperchromasia, sharp angulations; marked variation in nuclear size and multinucleation are atypical only if chromatin is abnormal

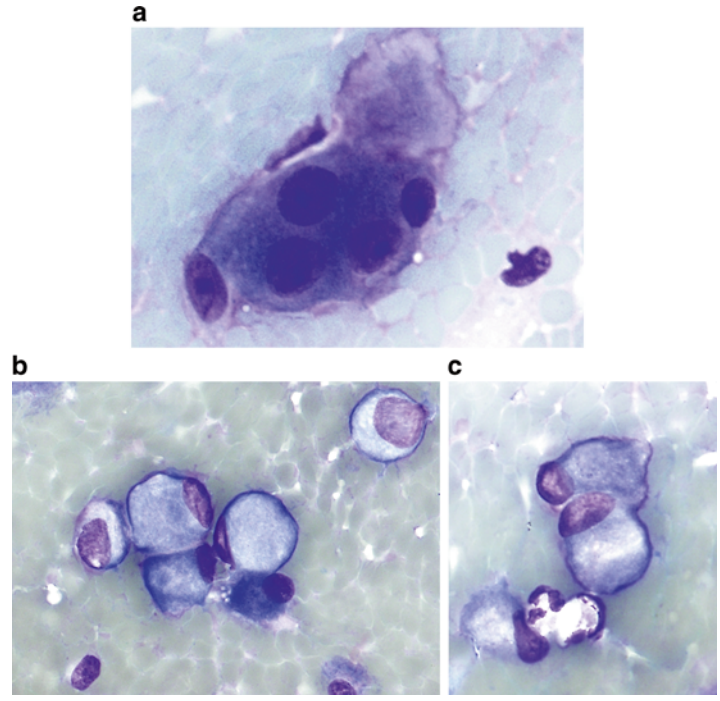


Fig. 12.3 Osteoblasts can mimic metastatic cells on smears. (**a**) Osteoblasts can cluster and mimic epithelial cells. (**b**) Osteoblasts can also appear plasmacytoid with a peripherally placed nucleus and a suggestion of a perinuclear hoff. (**c**) Reassuring features are the small size of the cells, the uniform appearance and the common presence of typical badminton shuttlecock-shaped osteoblasts

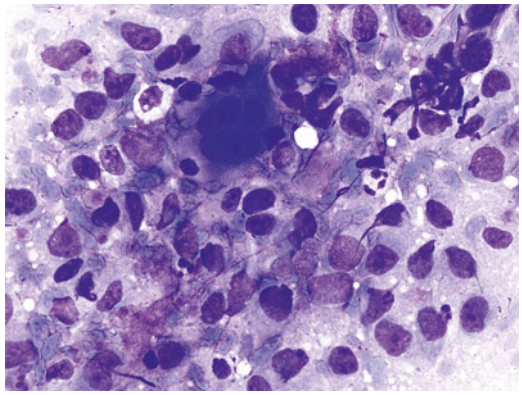


Fig. 12.4 Malignant osteoblasts in high grade osteosarcoma. In comparison with normal osteoblasts, malignant osteoblasts of high grade osteosarcoma exhibit variation in cell and nuclear size, a higher N/C ratio and often prominent nucleoli

Radiation Induced Atypia

The reactive fibroblasts in this condition can exhibit nuclear features suggestive of recurrent tumor or radiation-induced sarcoma. A needle core biopsy may be required to distinguish markedly atypical reactive changes from tumor. However, some examples are clearly reactive with wispy myofibroblasts and fibroblasts mixed with varying numbers of lymphocytes and myxoid to collagenous matrix fragments.

Fracture Callus

Fracture callus can mimic osteosarcoma on a needle core biopsy and similarly can mimic osteosarcoma on FNA. Osteoblasts may be pleomorphic in both conditions with nuclear hyperchromasia, coarse chromatin and macronucleoli. Debris, inflammation and osteoid likewise occur in both lesions. The osteoid in OS is often more filigree or lacelike compared to the chunkier osteoid of a fracture callus. However, clinical history, often imaging findings, and if necessary, tissue biopsy, can resolve the diagnosis.

Fractures may occur in the setting of metastatic carcinoma, benign bone lesions such as unicameral or aneurysmal bone cyst, and fibrous dysplasia, among others. In such instances, imaging findings may suggest a fracture has occurred, but microfractures may be undetectable. Depending on the size and stage of the

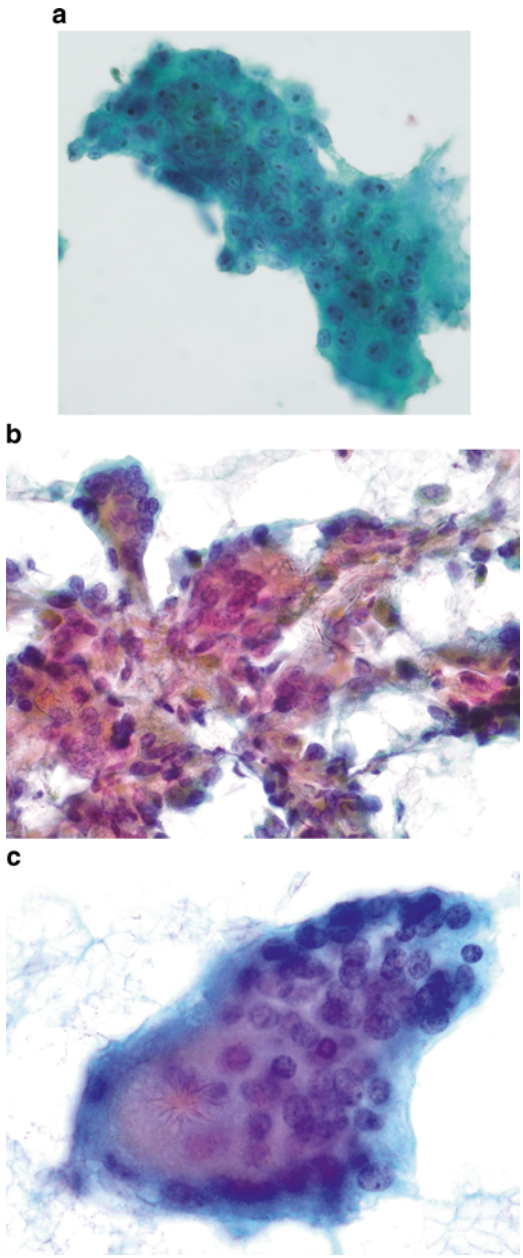


Fig. 12.5 Reactive multinucleated giant cells in normal and neoplastic conditions. (**a**) The multinucleated osteoclasts of giant cell tumor of bone (GCTB) are often described as gigantic, often with 40 or more nuclei. Although typical of GCTB, such huge osteoclasts can also be found in other tumors as is the case with this extremely large osteoclastic giant cell found in a chondroblastoma. Thus, giant multinucleated osteoclasts cannot be considered diagnostic of GCTB. (**b**, **c**) Multinucleated giant cells also occur in non-neoplastic conditions and can be a dominant feature. This patient with degenerating TMJ joint prostheses presented with growing firm bilateral masses. FNAB revealed abundant multinucleated giant cells, some aggregating around transparent filamentous foreign material (Pap stain) with others containing asteroidal bodies (which are nonspecific; Pap stain). The clinical history, imaging findings and presence of foreign medical material enabled the correct diagnosis of a reactive iatrogenic process

fracture, the underlying lesion will be contaminated by reactive osteoblasts, increased numbers of osteoclasts, fibroblasts/myofibroblasts, and inflammation.

Benign Mesenchymal Tumors Mimicking Sarcomas

Aneurysmal Bone Cyst (Solid ABC)

Both conventional and solid ABC can yield cellular specimens comprising a mix of fibroblasts, osteoclasts, inflammatory cells, and a few osteoblasts with fragments of osteoid or myxofibrous matrix. Malignant nuclear atypia is absent helping to differentiate the lesion from telangiectatic osteosarcoma (see Fig. 12.8). Imaging findings are often diagnostically supportive.

Non-ossifying Fibroma

The cell and matrix components are similar to ABC. Similarly, malignant nuclear features are absent, and imaging findings are fairly characteristic.

Fibrous Dysplasia

Aspirates may be hypocellular but contain spindled lesional cells mixed with various amounts of osteoid and mineralized bone fragments. Malignant nuclear features are absent. However, the findings can be indistinguishable from low grade central osteosarcoma. Imaging findings can be diagnostically helpful. Immunocytochemistry can be very helpful in that fibrous dysplasia shows lack of expression of MDM2 and CDK4 while diffuse expression is characteristic of low grade osteosarcoma.

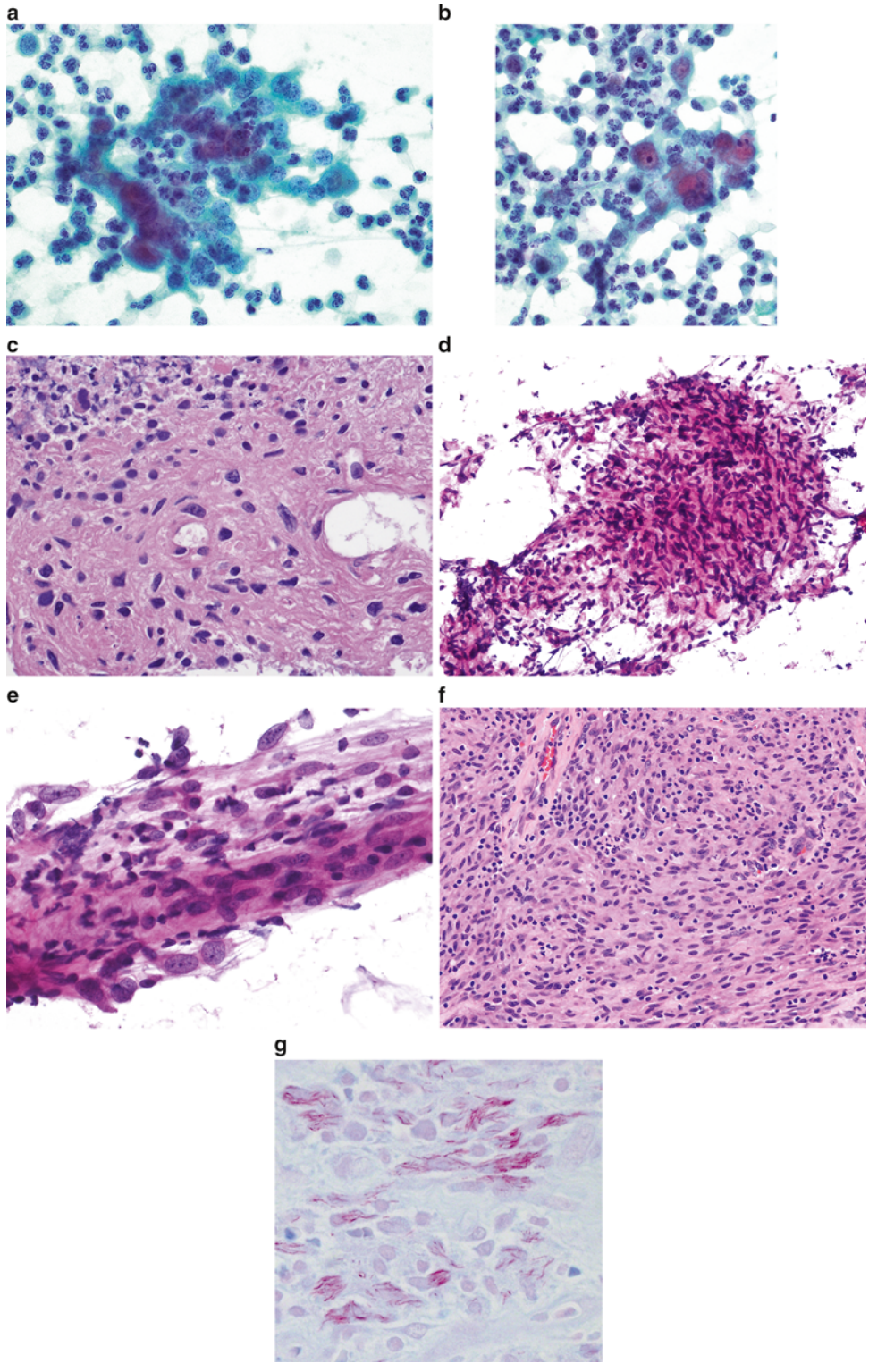


Fig. 12.6 Inflammatory or infectious processes in bone and soft tissue. (**a**–**c**) Abscesses may be associated with reactive histiocytes and fibroblasts that may show mild nuclear atypia in terms of mild variation in nuclear size, relatively high N/C ratio, and prominent nucleoli. The abundant intact and degenerated neutrophils and only rare atypical cells would be unusual in a neoplasm and warrant the diagnosis of abscess in the correct clinical setting.

Sarcomas Mimicking Benign Mesenchymal Tumors

Low Grade Osteosarcoma

As noted, low grade central osteosarcoma and paraosteal OS can mimic a benign spindle cell lesion such as fibrous dysplasia. In some cases, the patients may report symptoms extending several years further suggesting a benign process. Imaging features sometimes do not suggest an aggressive lesion. Immunocytochemical staining for MDM2 and CDK4 will reveal expression in osteosarcoma spindled tumor cells.

Post-radiation Sarcoma

While most post-radiation sarcomas consist of markedly pleomorphic tumor cells similar to those found in undifferentiated pleomorphic sarcoma, a few post-radiation sarcomas can appear deceptively bland on cytology and even tissue biopsy. The tumor cells may be few and resemble slightly hyperchromatic fibroblasts. The background may be inflamed with fragments of collagenized matrix. In these cases, molecular testing for aneuploidy (typical of post-radiation sarcoma) may be useful. Imaging findings of a new aggressive or growing mass in the radiated tumor bed should prompt a tissue biopsy.

Non-mesenchymal Tumors Mimicking Bone Sarcomas

Lymphomas

The differential here is primarily with small round blue cell sarcomas (SRBCS). Nuclear features of lymphomas may overlap with those of some SRBCS including conventional and atypical Ewing sarcoma, small cell osteosarcoma and *BCOR-CCNB3* Ewing-like sarcoma. Immunocytochemistry (ICC) results, the presence of spindle tumor cells in some of these tumors, and flow cytometry surface marker testing establishes the correct diagnosis.

Metastatic Melanoma

Melanoma notoriously can mimic carcinoma, lymphoma and sarcoma. This pertains equally to cytologic as well as tissue biopsy specimens because the malignant cells can form pure populations of small round blue cells, spindled cells, epithelioid/polygonal cells or highly pleomorphic cells. However, most commonly the metastatic deposit comprises a mix of spindled and epithelioid/polygonal cells. Nuclear pseudoinclusions and tumor giant cells with bulging nuclei and macronucleoli are also findings that should prompt diagnostic consideration of metastatic melanoma. Unfortunately, melanin pigment is only identified in a minority of metastatic lesions. Fortunately, immunocytochemical staining typically reveals expression of antigens found in melanoma such as S-100 protein, HMB45, and Melanocyte Antigen. Difficulty arises in the few metastatic melanomas lacking expression of these antigens. In such cases, clinical history of melanoma, even if remote (e.g. >10 years previously), should make metastatic melanoma a likely possibility especially in the setting of multiple lesions, lymphadenopathy and/or the lack of ICC markers pointing to another diagnosis.

Metastatic Carcinoma

The most common carcinomas to metastasize to the skeleton are lung, breast, prostate, kidney

Fig. 12.6 (continued) If numerous atypical cells are present, CD68 or SMA reactivity can alleviate concern for carcinoma. (**d**–**g**) Mycobacterial pseudotumor presenting as an ulcerated leg mass in a 45 year old man. The clinical suspicion for sarcoma was high, and the cytology specimen was signed out as low grade spindle cell neoplasm because of hypercellularity and a dominant population of spindled cells a few with mild nuclear variation. Resection revealed areas more suggestive of vague granulomas and confirmed the bland appearance of the spindled cells which prompted additional staining. An AFB stain shows numerous mycobacteria in the spindled histiocytes. (**a**, **b**, Pap-stained direct smears; **c**, H&E stained cell block; **d**, **e**, rehydrated H&E stained direct smears; **f**, resection histology; **g**, AFB stain on resected tumor.)

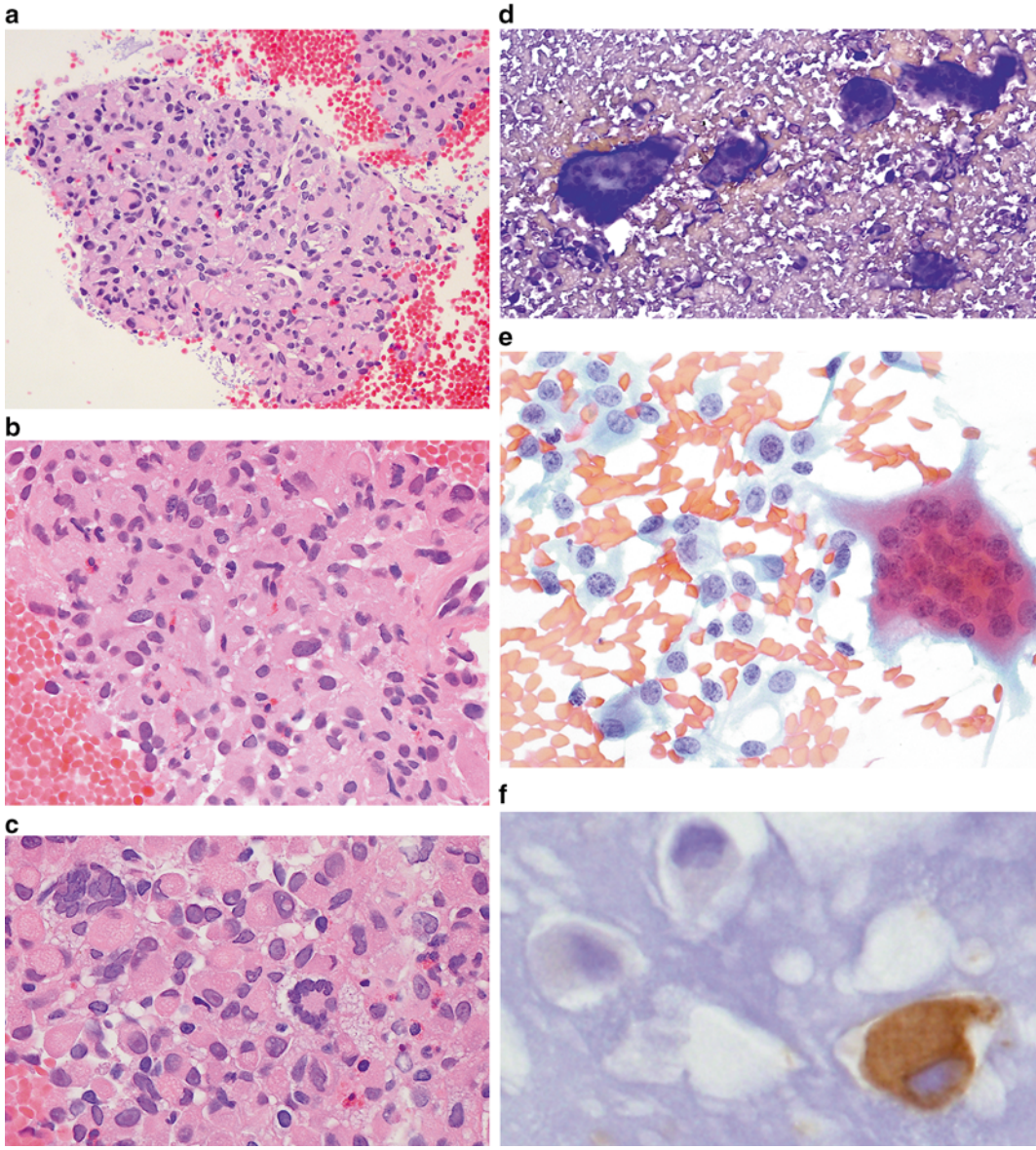


Fig. 12.7 Neoplasms mimicking a granulomatous process. (**a**–**c**) This recurrent pleomorphic hyalinizing angiectatic tumor (PHAT) of the ankle looks deceptively like a histiocytic inflammatory process with mats of eosinophilic cells at low magnification, cells that mimic round and epithelioid histiocytes at high magnification, and interspersed eosinophils. However, nuclear atypia is present including macronucleoli, multinucleation and nuclear pseudo-inclusions. The clinical history of prior PHAT was diagnostically helpful. (**d**, **e**) This giant cell tumor of tendon sheath (GCTTS) contains numerous multinucleated giant cells and mononuclear cells that resemble histiocytes mimicking a granulomatous process. Complicated assessment is the intermingling of true histiocytes among the mononuclear tumor cells. Some tumor cells may also contain hemosiderin crystals, also mimicking histiocytes. A few of the tumor cells may express desmin (**f**), a relatively common finding in GCTTS and which is also found in the diffuse type of this tumor (pigmented villonodular synovitis or tenosynovial giant cell tumor, diffuse type). The clinical history (23 year old woman with a toe mass) and imaging findings supported the diagnosis. (**a**–**c**, **e**, H&E stained cell blocks; **d**, DQ stained immediate smear.)

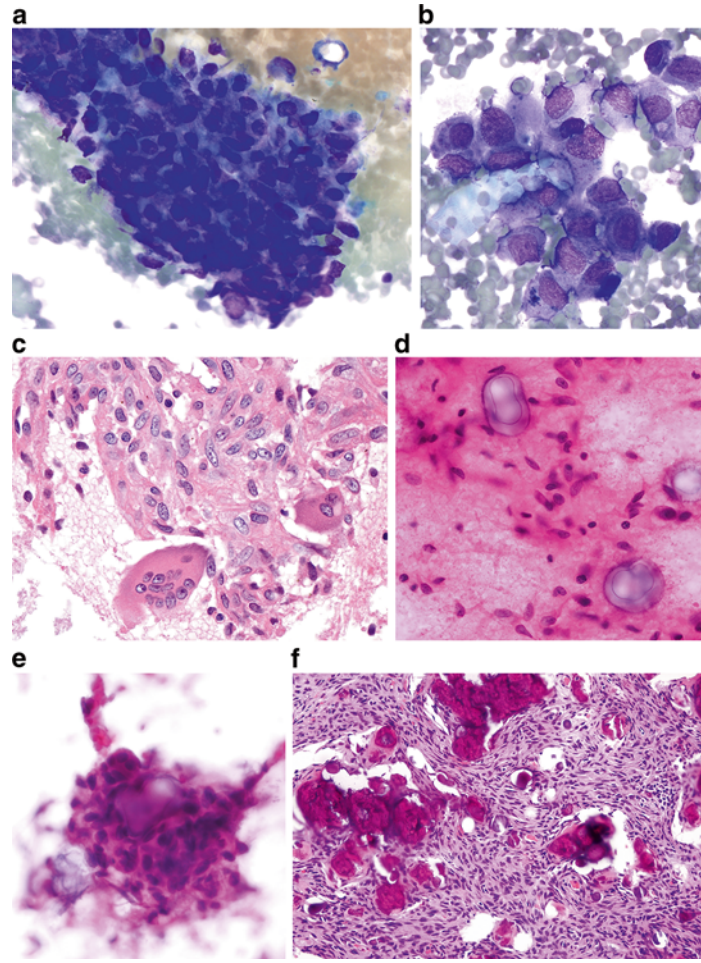


Fig. 12.8 Examples of benign bone tumors mimicking bone sarcomas. (**a**–**c**) This aneurysmal bone cyst (ABC) in the scapula of an 11 year old girl is hypercellular with clumps of round to spindled tumor cells (**a**) and osteoblast clusters forming new bone (**b**). Low grade osteosarcoma (LGOS) is in the cytologic differential. The cell block reveals multinucleated giant cells and spindle tumor cells without marked nuclear atypia (**c**). The imaging findings and lack of nuclear atypia help exclude LGOS. In difficult cases, directed genetic testing could be performed and would show a *USP6* gene mutation in ABC. (**d**–**f**) This example of fibrous dysplasis in the maxillary with cementum-like ossicles is hypercellular and could suggest the diagnosis of osteosarcoma (OS) (**d**, **e**). However, OS does not produce cementum like ossicles. The imaging findings supported a diagnosis of a benign tumor. Resection confirmed the cytology impression (**e**). (**a**, **b**, DQ stained immediate smears; **c**, H&E stained cell block; **d**, **e**, rehydrated H&E stained immediate smears; **f**, H&E stained section of resected tumor.)

and thyroid, although any carcinoma can spread to the bones. Metastatic carcinomas may mimic those sarcomas with epithelioid tumor cells such as some osteosarcomas, clear cell chondrosarcoma, and others. Sarcomatoid carcinomas (carcinomas with a spindle cell or mixed spindle cell-epithelioid/polygonal cell morphology) can mimic sarcomas with similar appearing cell populations. Small cell undifferentiated carcinoma can simulate lymphoma or a small round cell sarcoma. Making the correct diagnosis relies on integrating clinical history, imaging findings of multiple lesions and/or adenopathy, and immunocytochemistry results with the cytologic features.

Major Cytologic Confounders and Mimics of Soft Tissue Tumors

Benign Cells

Reactive Fibroblasts and Myofibroblasts

These cells are arguably the most common cells mistaken for a sarcoma cell. Characteristics they share with malignant cells include pleomorphism in cell size and shape, variation in nuclear size, number, and lobulation, often prominent nucleoli, and increased mitotic figures (see Fig. 12.9a–e). Such cells occurring in the base of intestinal

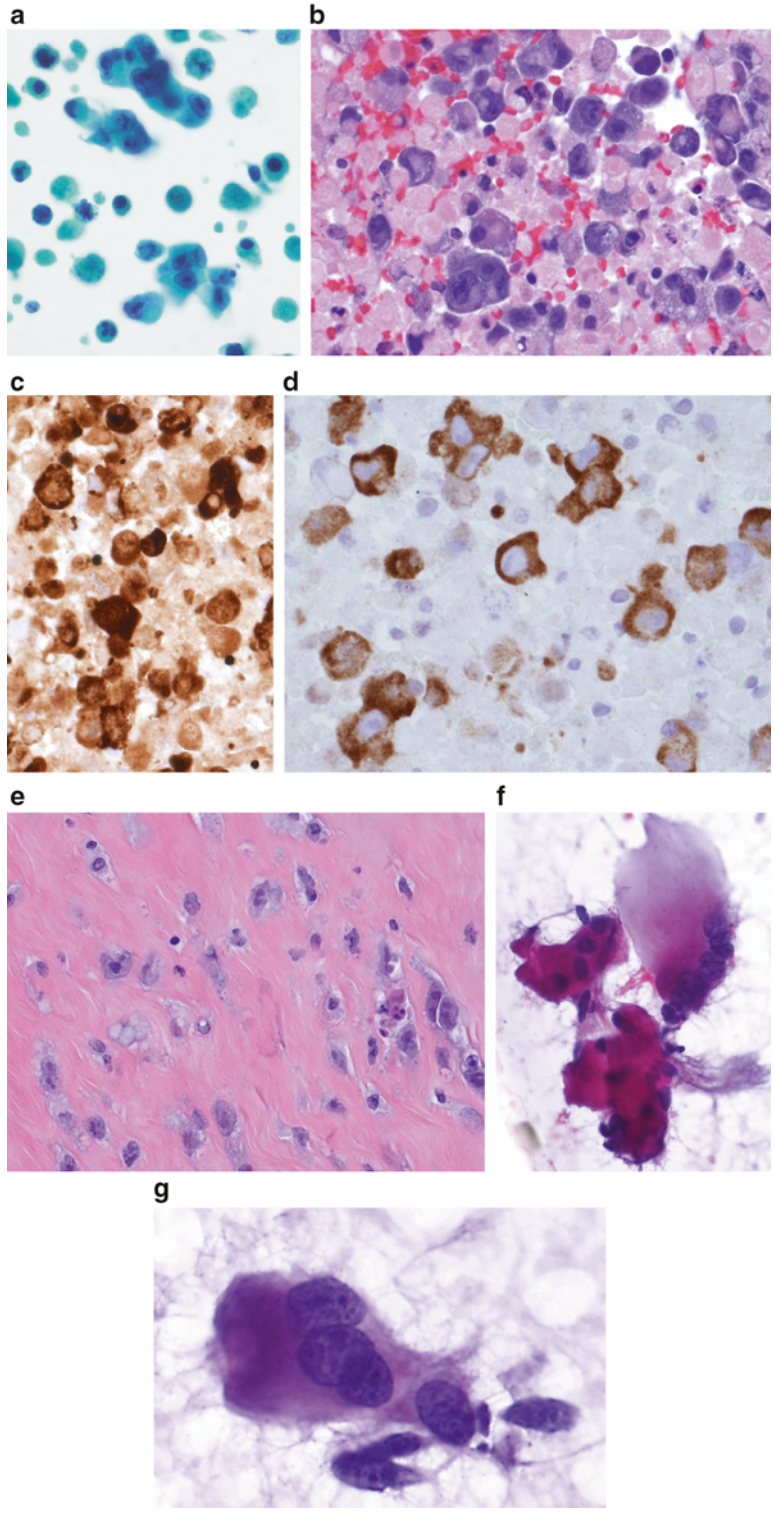


Fig. 12.9 Benign reactive or degenerating soft tissue cells that can mimic sarcoma. (**a**–**e**) Reactive myofibroblasts. This 80 year old woman with an unstable hip prosthesis had cystic and solid areas on imaging indeterminate for a mass. The monolayer and cell block contained many epithelioid cells with marked nuclear atypia including macronucleoli, irregularly shaped nucleoli and many necrotic cells (**a**, **b**). Immuhistochemical staining revealed both keratin (**c**) and smooth muscle actin (**d**) expression in the lesional cells. Although some sarcomas can express

ulcers have been dubbed "ulcerocytes" to highlight an association with tissue trauma. Clues that these are benign cells include: (1) frayed wispy amphophilic (on DQ stain) cytoplasm; (2) nuclear hypochromasia with fine chromatin; (3) reactive, irregularly shaped nucleoli in some cells; (4) inflammatory background.

Degenerating and Regenerating Skeletal Muscle Cells

Benign tumors such as deep myxomas, intramuscular lipomas and fibromatosis may infiltrate and damage surrounding skeletal muscle resulting in degenerated, multinucleated skeletal muscle cells that may exhibit nuclear pleomorphism, hyperchromasia and clumped chromatin (see Fig. 12.9f–g). Clues to that these are benign cells include: (1) range in N/C ratios with many cells having a very low N/C ratio; (2) at least some cells have bright eosinophilic cytoplasm; (3) at least some of the cells have small non-atypical nuclei arranged in a uniform lineup within the cytoplasm; (4) mitotic figures are absent.

Infectious/Inflammatory and Reactive Processes

Epithelioid Histiocytes

These have previously been described above. These cells can also appear identical to fibroblasts or myofibroblasts. The presence of large numbers of the cells or mats of cells could indicate a significant infectious process. Special stains for fungi or mycobacteria may be useful.

Plump Endothelial Cells

These cells may appear epithelioid or plump spindled. Nucleoli may be present. They are usually few in number and lack malignant nuclear features. They may form vessels discernable on smears and cell blocks.

Fat Necrosis

The most common site of fat necrosis presenting as a mass is the adult female breast. Imaging may be suggestive of carcinoma. The patient does not recall a history of trauma in ~50 % of cases. Cytology smears may be hypercellular but primarily consist of fragments of fat, lipophages, free fat droplets, and granular debris (see Fig. 12.10a for benign fatty lesions that may mimic liposarcoma). Multinucleated histiocytes, spindled fibroblasts, and flattened or collapsed, translucent disc like structures resembling crumpled tissue paper may be present. The fibroblasts lack malignant nuclear features.

Exuberant Scar and Repair

Smears can be cellular and contain large regenerating skeletal muscle cells with multinucleation and epithelioid to spindled myofibroblasts and fibroblasts. Some of the skeletal muscle cells may contain striations or eosinophilic material. The myofibroblasts may appear plasmacytoid, ganglion cell-like or rhabdoid but lack nuclear hyperchromasia, coarse chromatin and irregular chromatin distribution (e.g. malignant nuclear features). Patients may recall a traumatic event.

Fig. 12.9 (continued) both proteins, the most common cells to do so are myofibroblasts; these stain results supported the diagnosis of "atypical cells present; most likely reactive myofibroblasts". The resected specimen featured the same cells embedded in reactive fibrous tissue (**e**). (**f**, **g**) Degenerating and regenerating skeletal muscle cells. A multinucleated regenerating skeletal muscle cell is contrasted with normal skeletal muscle cells (**f**). The regenerating cell shows nuclear enlargement but the nuclei are aligned evenly and the cytoplasm retains an eosinophilic hue. Degenerated skeletal muscle cells can have atypical appearing nuclear features including hyperchromasia and coarse chromatin with an increased N/C ratio (**g**). Clues to the diagnosis include eosinophilia of the cytoplasm, a range of atypia in the cells, and ICC reactivity for desmin and myogenin (which can be performed in difficult cases). (**a**, Pap-stained monolayer slide; **b**, H&E stained cell block, **c**, pan-keratin IHC stain on cell block; **d**, smooth muscle actin IHC stain on cell block, **e**, H&E stained section of tissue biopsy; **f**, **g**, rehydrated H&E stained immediate smears.)

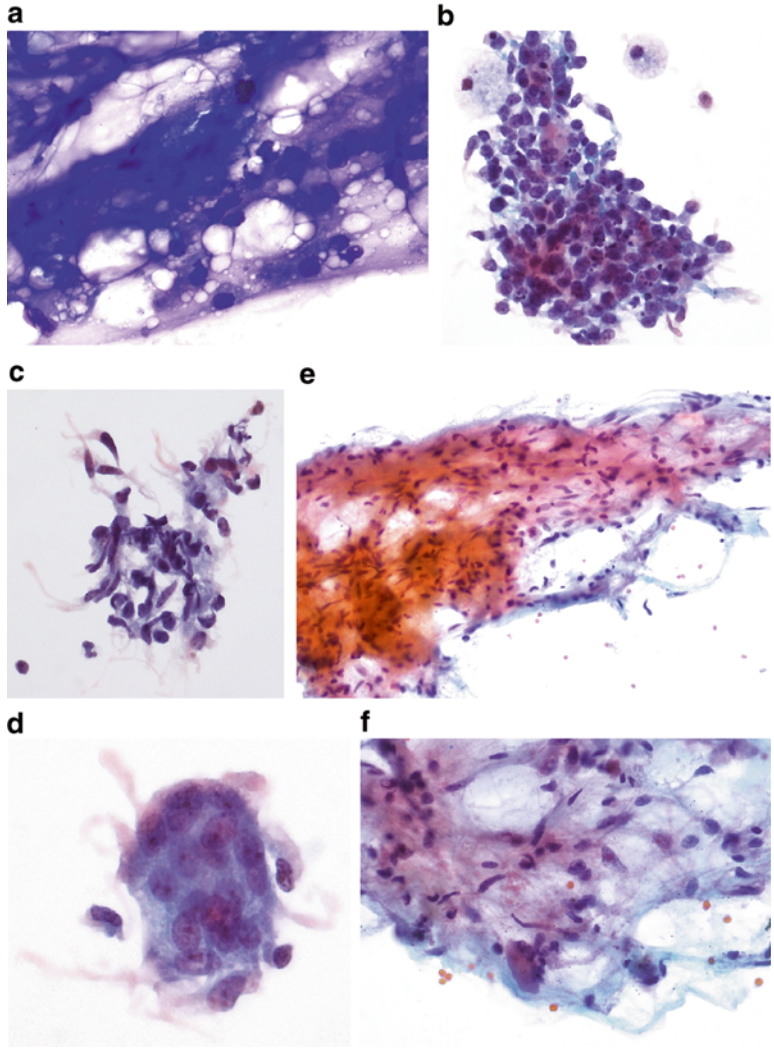


Fig. 12.10 Fat necrosis and benign adipocytic tumors mimicking liposarcoma. (**a**) DQ-stained immediate smear), (**b**–**d**) Pap-stained monolayer: fat necrosis in the breast of a 27 year old woman contains fragments of fat, inflammation, fibroblasts, and multinucleated giant cells. The smear (**a**) shows injured adipose tissue containing multivacuolated lipocytes and free fat globules mimicking the large range in size of malignant adipocytes. However, the presence of inflammation (even if sparse) and the non-malignant nuclear features support the diagnosis of a benign reactive process. (**e**, **f**) Spindle cell/pleomorphic lipoma (Pap-stained immediate smears). The neck mass of a 57 year old man contains fragments of fat with an increased number of spindle cells and with scattered atypical cells with nuclear enlargement, multinucleation and nucleoli. The clinical history of a mass in the neck of a middle aged man (common site for this type of lipoma) superficial location of the tumor and small size support a benign diagnosis. (**g**) Hibernoma (Pap-stained immediate smear): a large deep thigh mass in a 39 year old woman contains mature adipocytes and brown fat cells consistent with hibernoma, lipoma-like variant. The microvacuolated brown fat cells lack malignant nuclear features and do not intent the nuclei as would lipoblasts. (**h**, **i**) lipoma with myxoid degeneration (DQ-stained immediate smears): metachromatic myxoid matrix mimics that of myxoid liposarcoma (**h**), while free fat globules artifactually simulate lipoblasts. In difficult cases, FISH for the *DDIT3* translocation (positive in myxoid liposarcoma) can be performed

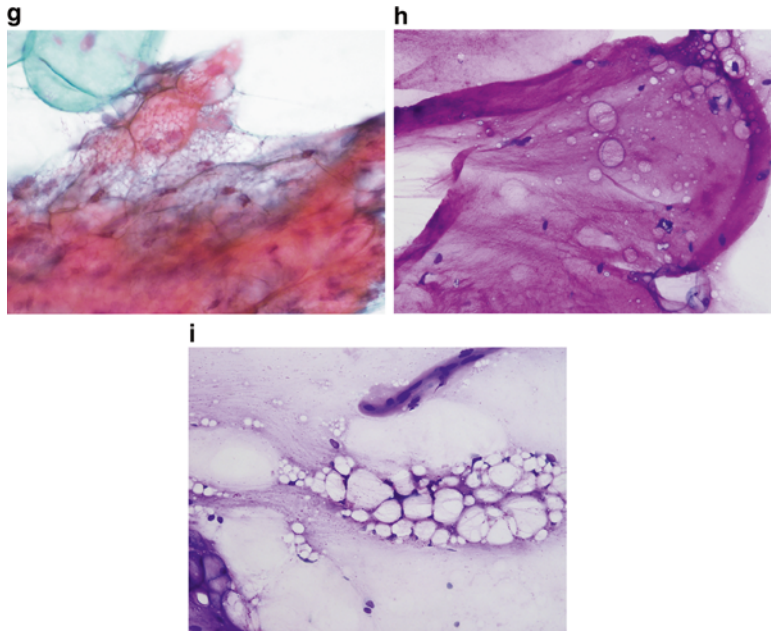


Fig. 12.10 (continued)

Benign Mesenchymal Tumors Mimicking Sarcomas

Pseudosarcomas (Nodular Fasciitis, Proliferative Fasciitis, Proliferative Myositis, Ischemic Fasciitis, Myositis Ossificans)

These lesions feature numerous fibroblasts and myofibroblasts exhibiting the range of shape and size found in exuberant scar tissue (see Fig. 12.11). In addition, chronic inflammatory cells and matrix such as osteoid may be present (especially in myositis ossificans). Stripped nuclei commonly invest the background. Some examples of nodular fasciitis contain prominent myxoid matrix. Malignant nuclear features are usually lacking, but nuclear features can be worrisome for malignancy in examples of ischemic fasciitis. A clinical history of trauma, small size (<4 cm), rapid growth, and, in some cases, imaging findings can be diagnostically helpful. These lesions differ from fibromatosis in being more heterogeneous. They lack nuclear beta-catenin as found in ~70 % of cases of fibromatosis. Genetic analysis reveals *USP6* gene alterations in cases of fasciitis.

Peripheral Nerve Sheath Tumors (Schwannoma, Neurofibroma, Mixed Type Peripheral Nerve Sheath Tumor)

These tumors vary in cellularity. Most of the cells clump or mat together with only a few dispersed single cells. Schwannomas and mixed type tumors may reveal nuclear palisading in the cell clumps (detectable in ~30 % of cytology specimens of schwannoma in one study; n = 116) [10, 12]. Although often described as containing elongate spindled Schwann cells with pointy ended, wavy or buckled nuclei, all types of peripheral nerve sheath tumor may also contain abundant round or oval cells. Nerve sheath tumors with "ancient change" contain scattered atypical cells with nuclear enlargement, multi-lobulation or multiple nuclei. These cells usually exhibit degenerative nuclear features such as smudgy chromatin, nuclear holes or breaks in the chromatinic rim. Myxoid change may also suggest a low grade myxoid sarcoma (see Fig. 12.12). However, in some cases distinction from sarcoma cannot be made on cytology exam alone.

Immunocytochemistry showing diffuse reactivity for S-100 protein diagnostically

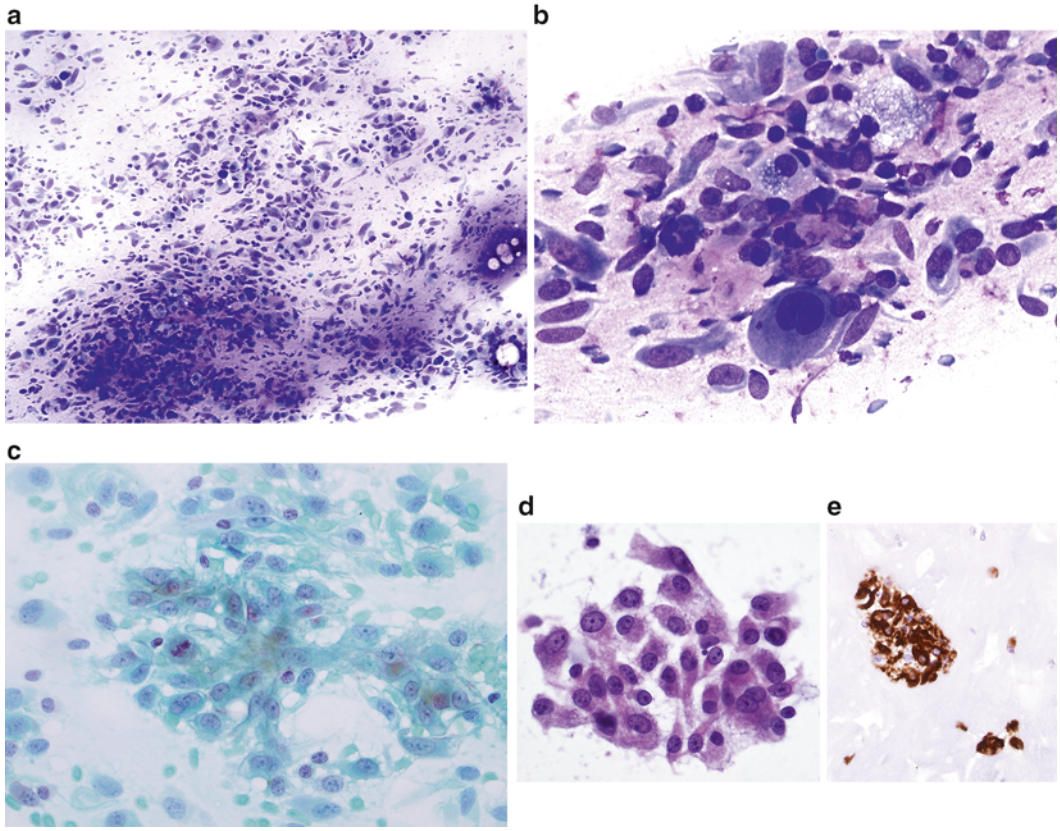


Fig. 12.11 Nodular fasciitis (a pseudosarcoma) mimicking sarcoma. This example of nodular fasciitis from a rapidly growing wrist mass in a 21 year old man is cellular and contains loose clusters of polygonal to epithelioid cells (**a**); even on the DiffQuik stain, the prominent nucleoli are visible (**b**). The Pap-stained immediate smear confirms the variation in cell size and shape. Lesional cells reveal wispy, frayed cytoplasm with indistinct cell borders. A mitotic figure is present but not atypical (**c**). The lesional cells can include binucleated forms and may resemble ganglion cells (**d**). The clinical history, the combination of variable cell shape with frayed delicate cytoplasm and lack of nuclear hyperchromasia and coarse chromatin are clues to the diagnosis. Smooth muscle actin is often positive (**e**) and can support the diagnosis. (**a**, **b**, DQ- stained immediate smear; **c**, Pap-stained immediate smear; **d**, rehydrated H&E-stained immediate smear; **e**, smooth muscle actin antibody in cell block section

distinguishes schwannoma from MPNST (except the epithelioid variant of MPNST).

An unintentional, iatrogenic diagnostic clue is that patients may also experience an elevated degree of pain or shooting pain when superficial lesions are aspirated.

Paraganglioma: Striking Atypia Can Occur in Paraganglioma Mimicking Carcinoma

The mimicry extends to architectural features, because tumor cells can shed in epithelial-like formations such as acini and pseupapillae. Nuclear atypia encompasses many of the features found in malignant cells including anisonucleosis, hyperchromasia, coarse chromatin and macronucleoli in some cases (see Fig. 12.13). Clues to the diagnosis include cytoplasmic pink granules, nuclear pseudoinclusions in some cases, plasmacytoid cells, spindled cells and a neuroendocrine "salt and pepper" distribution of chromatin ([17], n = 12 cases). Tumor location, clinical history and functional imaging findings are diagnostically helpful. Immunocytochemistry will reveal reactivity for synaptophysin and chromogranin with no reactivity for keratins.

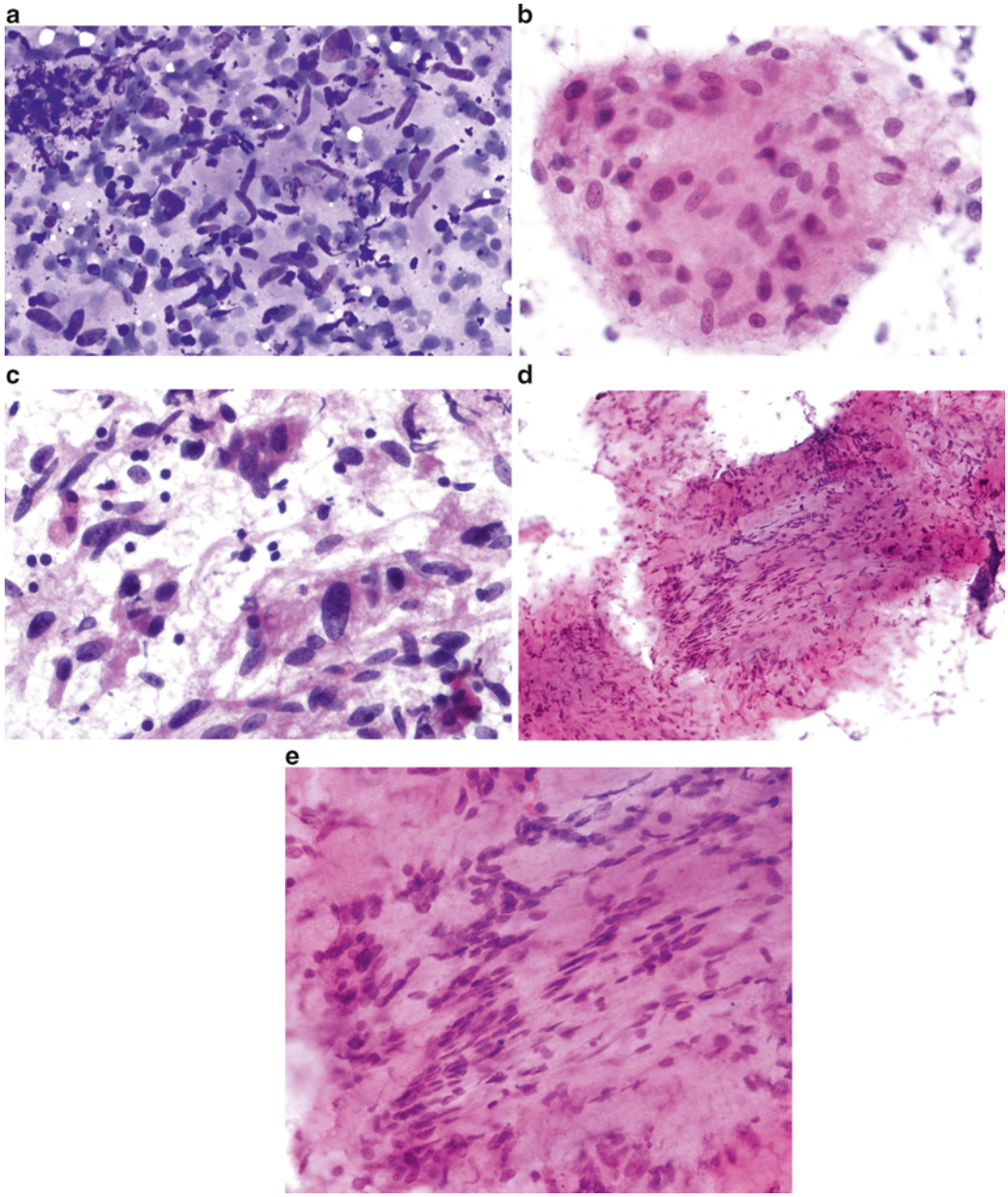


Fig. 12.12 Benign peripheral nerve sheath tumors mimicking sarcoma. Schwannomas can often yield cellular aspirates. Typically, there are few intact dispersed cells, but stripped nuclei may be common as in this smear. (**a**) Myxoid matrix is commonly present and can raise the possibility of a sarcoma such as low grade myxofibrosarcoma. (**b**) Degenerative nuclear atypia (increased N/C ratio, smudgy dark chromatin, irregular nuclear size and shape) can be worrisome for sarcoma, but unequivocal malignant nuclear features (coarse chromatin and granular hyperchromasia) are almost always absent. (**c**) Most of the intact cells are embedded in a collagenous or fibrillary matrix and about 33 % show alignment of cells consistent with Verocay bodies. (**d**, **e**) This is a clue to the diagnosis but can also be seen in some cases of malignant peripheral nerve sheath tumor, smooth muscle tumors and synovial sarcoma. (**a**, DQ-stained immediate smear, **b**–**d**, rehydrated H&E stained-immediate smears.)

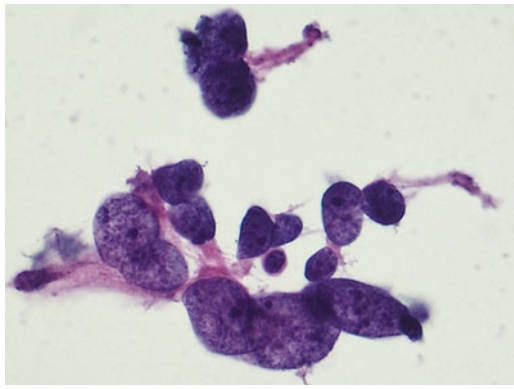


Fig. 12.13 Paraganglioma mimicking sarcoma. Paragangliomas may exhibit malignant nuclear features of marked hyperchromasia and coarse chromatin. Immunocytochemical staining, clinical history and imaging studies may be necessary to avoid an incorrect diagnosis of malignancy

Malignant paragangliomas paradoxically may show less cellular and nuclear pleomorphism than benign paragangliomas ([17], n = 12 cases). The typical Zellballen morphology of paraganglioma on histology is seldom found in cytology specimens.

Deep and Superficial Fibromatosis

Specimens may be hypercellular with many stripped nuclei, dispersed intact cells and thick mats of cells variably embedded in collagenized or myxoid matrix. Malignant nuclear features are absent. However, because of the cellularity and monotony of the cells, a low grade spindle cell sarcoma such as synovial sarcoma may not be excludable on cytology exam alone (see Fig. 12.14). Immunocytochemistry can be helpful and reveals nuclear beta-catenin expression in about 70 % of cases.

Lipomas with Degenerative Changes Including Fibrosis and Myxoid Stroma

Free, extracellular fat globules aggregating together as empty round spaces may appear to indent the nuclei of adjacent intact cells mimicking lipoblasts. Lipomas with or without fat necrosis may also harbor rare multivacuolated cells resembling lipoblasts. The nuclei of these multivacuolated cells are usually not hyerchromatic and lack coarse chromatin as would be present in true neoplastic lipoblasts.

Stromal changes may also raise other diagnostic considerations. Myxoid change may suggest a diagnosis of myxoma or myxoid liposarcoma. Fibrolipomas may mimic a fibroblastic tumor, as the fat component may not be appreciated in the cytology sample.

Longstanding clinical history, confirmation that the needle is in the lesion, and superficial location are all features that support a diagnosis of lipoma rather than a more significant lesion.

Sarcomas Mimicking Benign Mesenchymal Tumors

Low Grade Fibromyxoid Sarcoma

This tumor appears deceptively benign in both histologic and cytologic specimens. While tissue biopsies reveal a mixture of fibrous and myxoid stroma, most cytology specimens comprise only the myxoid component. The tumor cells are uniform without nuclear atypia, and cellularity may be low to moderate. Immunocytochemical diffuse expression of MUC4 can suggest the diagnosis. This tumor also has two specific fusion translocations (*FUS-CREB3L* or *FUS-CREB3L1*) that can be detected by FISH testing on smears [15].

Spindle Cell Sarcomas with Deceptive Low Grade Features Such As Some Examples of Leiomyosarcoma, Synovial Sarcoma, Malignant Peripheral Nerve Sheath Tumor and Low Grade Myofibroblastic Sarcoma, Among Others

For many of these tumors, a definitive diagnosis of malignant sarcoma can only be made with the support of ancillary testing, clinical history and imaging findings (see Fig. 12.15). Even so, for a few cytologically low grade sarcomas, the most refined cytology diagnosis may be rather rough: "low grade spindle cell mesenchymal neoplasm" being the most common diagnosis. Certain leiomyosarcomas, for example, require mitotic counts for the diagnosis, and such counting

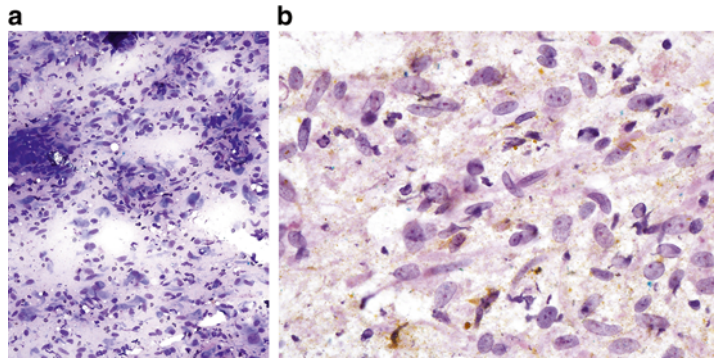


Fig. 12.14 Desmoid-type fibromatosis mimicking a low grade spindle cell sarcoma. (**a**) Desmoid tumors are a type of locally aggressive fibroblastic/myofibroblastic tumor that may attain a large size and show permeative growth on imaging. The smears may be cellular and can contain dispersed intact cells suggestive of a spindle cell sarcoma. However, the pale nuclei show fine, evenly dispersed chromatin consistent with a benign tumor. (**b**) Immunocytochemistry may be required to help exclude entities such as low grade leiomyosarcoma (positive for desmin and SMA), dermatofibrosarcomas protuberans (positive for CD34), and malignant peripheral nerve sheath tumor (negative for SMA; negative for nuclear beta-catenin). (**a**, DQ-stained immediate smear; **b**, rehydrated H&E-stained immediate smear.)

cannot be reliably performed on a cytology specimen.

This non-committal diagnosis of "low grade spindle cell mesenchymal neoplasm" may still be of value, as it can be made quickly, excludes entities such as infection, carcinoma, and melanoma and can ensure appropriate processing of the subsequent definitive biopsy.

Sarcomas Mimicking Carcinoma or Melanoma with Epithelioid Cell Morphology

From a pure cytologic vantage point, the quintessential sarcoma mimicking epithelioid melanoma or carcinoma is epithelioid sarcoma. However, numerous other sarcomas may contain epithelioid cells with round nuclei and which shed in clusters (see Fig. 12.16). Attention to clinical history (such as young adult age of patient with epithelioid sarcoma), physical exam, imaging findings and use of ancillary testing will result in the correct diagnosis. Importantly, many sarcomas with epithelioid appearing cells such as epithelioid sarcoma, myoepithelial sarcoma, epithelioid angiosarcoma and others may express simple or broad spectrum keratins. In such cases, the clinical presentation may be unusual for metastatic melanoma or carcinoma and will prompt additional ICC stains or molecular genetic anlaysis to enable the correct diagnosis. For example, epithelioid angiosarcoma expresses not just keratins but also vascular markers such as CD31, CD34, and ERG, and FLI1.

Of note, biphasic tumors such as synovial sarcoma and some examples of malignant peripheral nerve sheath tumor seldom shed the epithelial component in cytology samples, consisting instead of only spindled tumor cells [1]. For these biphasic sarcomas, a diagnosis of spindle cell sarcoma might be rendered on the cytology sample, and although technically a misdiagnosis, it would not be a clinically significant error, because the lesion would still be appropriately categorized as a malignant mesenchymal tumor.

Small Round Cell Sarcomas Mimicking Lymphoma, Small Cell Undifferentiated Carcinoma or Melanoma with Small Round Blue Cell Morphology

Especially on a rapid DiffQuik™ or MGG stain, a small round blue cell sarcoma could be mis-

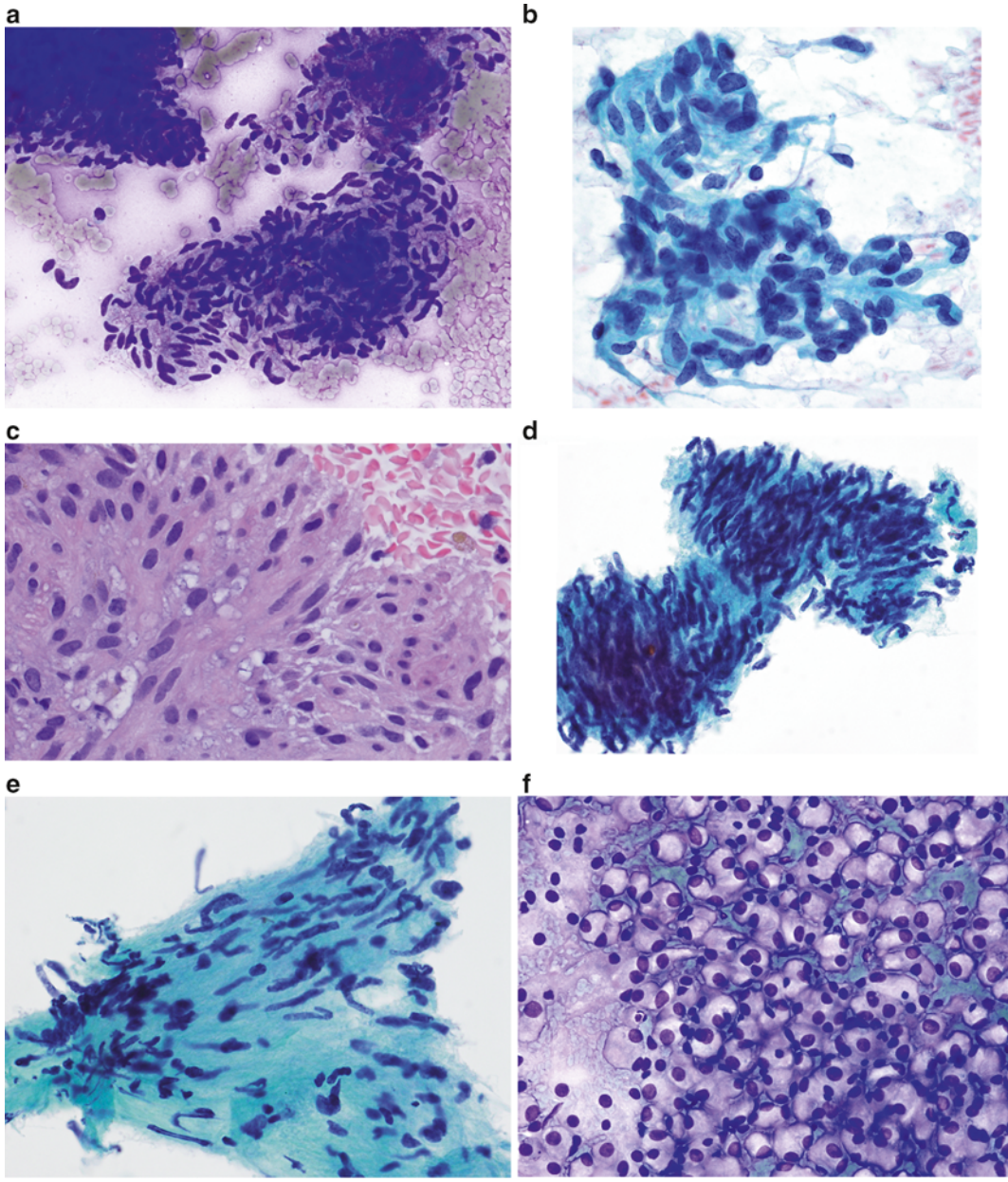


Fig. 12.15 Sarcomas mimicking benign mesenchymal tumors. (**a**) This metastatic leiomyosarcoma to the abdominal wall in a 49 year old woman 3 years post hysterectomy for her grade 2 leiomyosarcoma, is cellular with most cells clumping together as is often found in benign mesenchymal tumor aspirates. (**b**) Only mild nuclear atypia is noticeable on the Pap stained slide. The cigar-like shape of many of the nuclei is suggestive of smooth muscle differentiation. (**c**) Irregular cytoplasmic vacuolation is another feature suggesting smooth muscle differentiation. Note the minimal nuclear atypia despite the aggressive behavior of this tumor. (**d**, **e**) This gastrointestinal stromal tumor (GIST) of the duodenum could easily be mistaken for a benign peripheral nerve sheath tumor because of elongate buckled nuclei, fibrillary background matrix and minimal nuclear atypia. Immunocytochemistry can confirm the diagnosis of GIST. (**f**) Metastatic alveolar soft part sarcoma (ASPS) to the lungs in a 29 year old woman mimics histiocytic inflammation but shows monotonous round nuclei that would be unusual in histiocytes. (**g**) The corresponding cell block contains nests of granular eosinophilic cells with prominent nucleoli consistent with the diagnosis of ASPS. Architectural and nuclear features in a cell block can be diagnostically helpful.

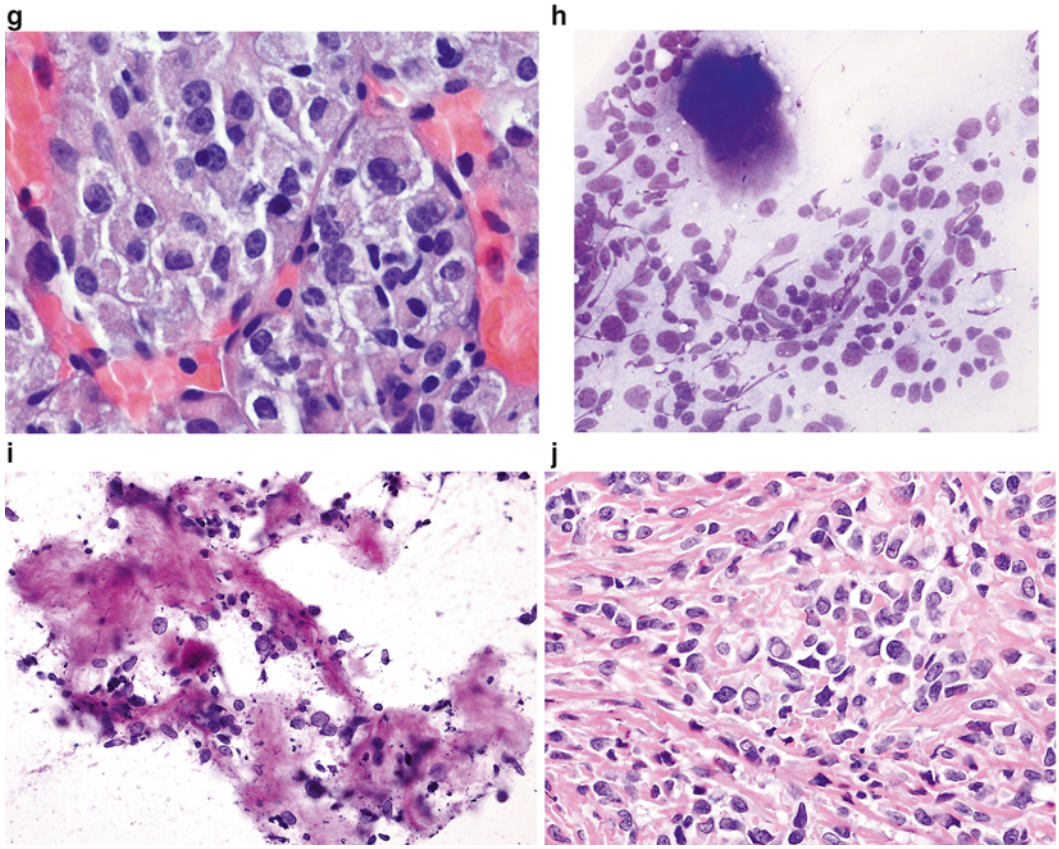


Fig. 12.15 (continued) (**h**) This sclerosing epithelioid fibrosarcoma (SEFS) resembles a benign process such as granulomatous inflammation due to the epithelioid cells and multinucleated giant cell. (**i**) The H&E stained smear of SEFS contains stripped nuclei that are ovoid with rare nuclear pseudo-inclusions. The cells are strewn among a collagenous matrix. These findings could be seen in a schwannoma. (**j**) Although this is a high grade sarcoma, the degree of nuclear atypia is usually mild. Note the nuclear pseudo-inclusions in two of the tumor cells. Unlike schwannoma, SEFS is usually a deep seated tumor, may be large, and does not elicit sharp or shooting pain on FNAB as would schwannoma. By ICC, SEFS expresses MUC4 but not diffuse S-100 protein. (**a**, DQ-stained immediate smear; **b**, Pap-stained immediate smear; **c**, (H&E-stained cell block; **d**, **e**, Pap-stained monolayer slide; **f**, DQ-stained immediate smear; **g**, H&E stained cell block; **h**, DQ-stained immediate smear; **i**, rehydrated H&E stained immediate smear; **j**, H&E stained section of resected SEFS.)

taken for a lymphoma, small cell undifferentiated carcinoma or melanoma. Some features of small round cell sarcomas may point to the correct diagnosis. The nuclei may show a subtle bipolar shape, the chromatin may be unusually diffusely coarse, and nuclear molding may be present; all features lacking in most lymphomas (see Fig. 12.17). Spindled tumor cells and the lack of blue bodies (bits of degenerated malignant lymphocytes) also suggest a non-lymphoma diagnosis. Clinical history including young patient age (in some cases), lack of melanin pigment and lack of reactivity for melanocytic markers but reactivity for other markers (such as myogenin or CD99) support a diagnosis of sarcoma over melanoma.

Non-mesenchymal Tumors Mimicking Sarcomas

Lymphoma, metastatic melanoma and metastatic carcinoma all may occur in soft tissue although far less commonly than in bone. Of these three

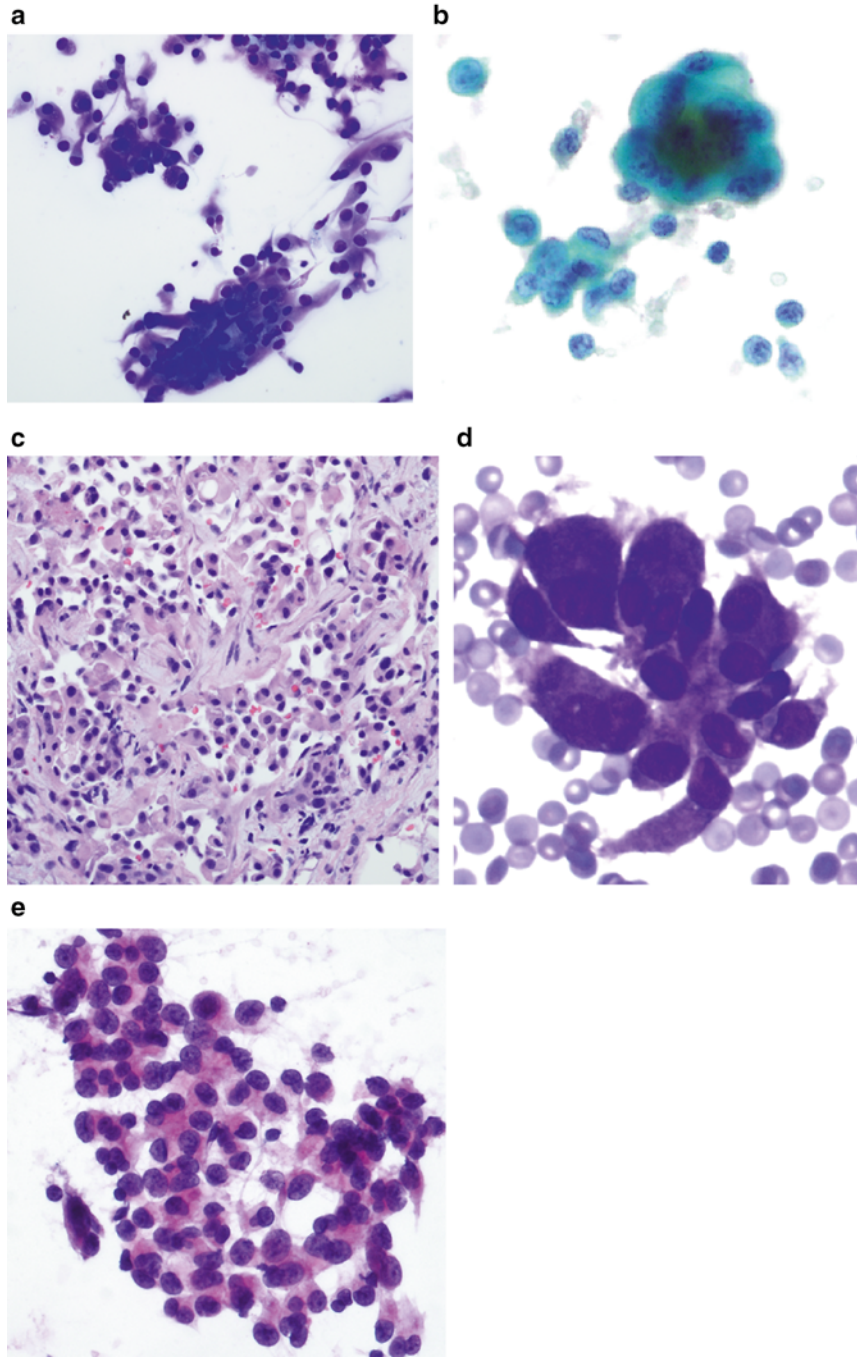


Fig. 12.16 Examples of sarcomas with epithelioid cells that mimic carcinoma or melanoma. These lesions can be especially difficult to identify as sarcoma when they occur at unusual or unexpected sites. (**a**) Epithelioid hemangioendothelioma (EHE) arising in liver. This liver mass in a 46 year old woman showed dyscohesive epithelioid cells on the touch prep and dispersed and clustered mildly atypical cells in the monolayer. (**b**) The needle core biopsy obtained simultaneously revealed a characteristic growth pattern of EHE. (**c**) Tumor cells expressed keratins and CD31 confirming the diagnosis. (**d**) Nasal angiosarcoma metastatic to cervical lymph node. The immediate smear contained loose groups and dispersed large epithelioid cells that could easily be mistaken for metastatic carcinoma. The epithelioid morphology and loose cohesion also mimics metastatic melanoma. (**e**) ICC would be warranted to make

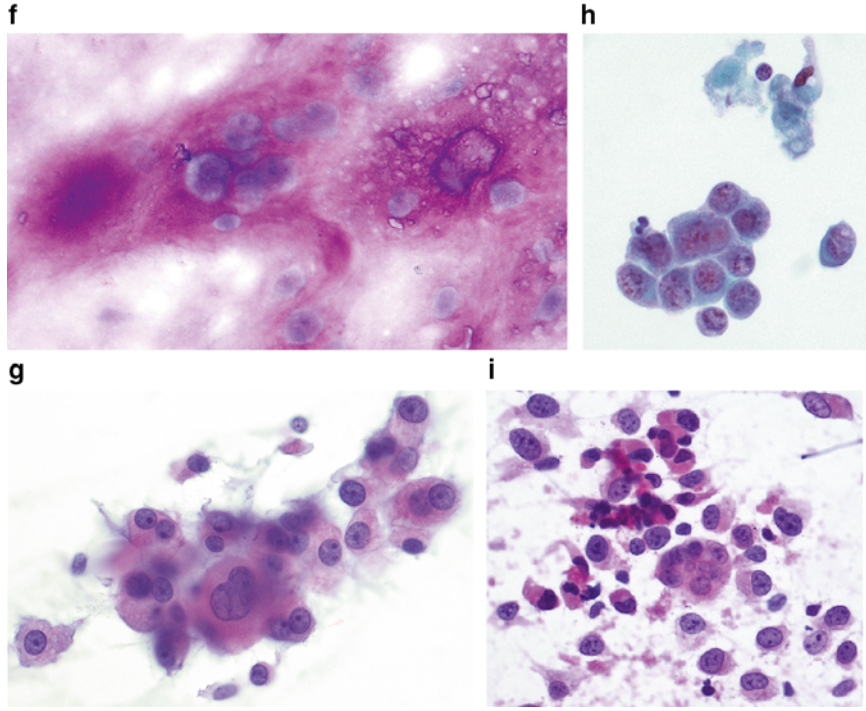


Fig. 12.16 (continued) the correct diagnosis: although epithelioid angiosarcoma is often reactive for keratins, it also expresses vascular markers. (**f**, **g**) Clear cell chondrosarcoma. This 4 cm aggressive appearing epiphyseal tumor in the femur of a 23 year old woman features epithelioid cells dispersed in metachromatic matrix simulating a metastatic mucinous carcinoma. The cells show obvious malignant nuclear features, but still resemble a carcinoma. However, the knowledge of the patient's age, tumor location and ICC ensured a correct diagnosis of clear cell chondrosarcoma. (**h**) Epithelioid sarcoma. This 34 year old man with epithelioid sarcoma presented with a longstanding lower leg ulcer and inguinal adenopathy mimicking an infectious process. The lymph node FNAB however, contained abundant malignant epithelioid appearing cells. The differential diagnosis includes metastatic melanoma and an unusual carcinoma. The tumor cells showed a range of size and multinucleation with a necrotic background, again simulating metastatic melanoma. (**i**) ICC with dual reactivity for keratins and vimentin suggested the diagnosis. (**a**, **d**, **f**, DQ-stained immediate smear; (**e**, **g**, **i**) rehydrated H&E-stained immediate smear); **h**, Pap-stained monolayer.)

major groups, metastatic melanoma is the most common to occur in soft tissue. It can comprise spindled cells, mixed spindled and epithelioid cells or epithelioid cells only. Melanin pigment is often absent. Macronucleoli, giant multinucleated tumor cells with peripherally placed bulging nuclei and nuclear pseudoinclusions can spark suspicion for the diagnosis. Clinical history can be helpful. Immunocytochemical expression of melanocytic markers is usually present. Even if absent, the diagnosis should be strongly considered in a patient with a history of melanoma.

Clinical history is likewise useful in the setting of lymphoma and carcinoma deposits in soft tissue. Immunocytochemical staining (using appropriate antibodies including expected negatively reacting antibodies) almost always can resolve the differential.

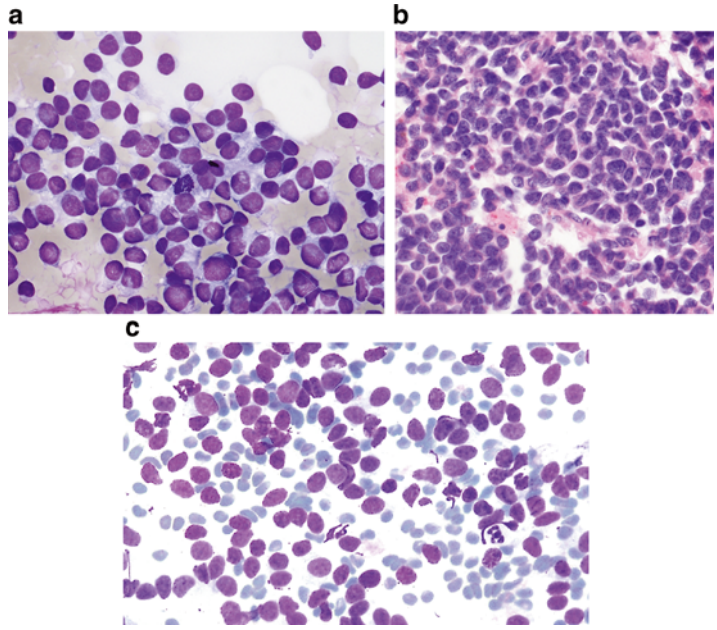


Fig. 12.17 Examples of small round cell sarcomas that mimic lymphoma. (a) Ewing sarcoma arising in the abdominal cavity of a 53 year old man. The resemblance to lymphoma is striking in a DiffQuik or MGG smear. However, the cells show nuclear molding and coarse granular chromatin on the cell block section; features that would be unusual in lymphoma. (**b**) Strong membranous reactivity for CD99 and *EWSR1* rearrangement by FISH confirmed the diagnosis. Metastatic endometrial stromal sarcoma to the lungs (**c**): the small size, high N/C ratio and dispersed pattern mimic lymphoma. However, nuclear molding is present and lymphoglandular bodies are absent, discordant findings for lymphoma. Immunohistochemical staining on the corresponding needle core biopsy combined with the clinical history of endometrial stromal sarcoma enabled the correct diagnosis. (**a**, DQ-stained immediate smear; **b**, H&E stained cell block; **c**, DQ-stained immediate smear.)

Conclusions

You can Quickly and Economically Drive the Clinical Decision in 80–90 % of cases

Most soft tissue and bone lesions can be diagnosed accurately if the specimen is representative. Rapid on site evaluation (ROSE) or performance of the FNAB by a pathologist produces the best specimen for diagnostic evaluation. Ultrasound guidance confirms correct placement of the needle and permits systematic sampling of the mass.

Always Invoke the Triple Test

The triple test applies to more than breast cytology samples. The patient's clinical history and imaging findings play key supporting roles in the diagnosis of a specimen obtained from bone or soft tissue. A major discordancy in diagnostic impression when comparing clinical history, imaging or cytologic findings (such as imaging shows malignant features but cytology appears benign) should prompt a tissue biopsy.

Collect Extra Sample for Potential Ancillary Testing

Several times yearly a new translocation or other specific genetic alteration is reported for a bone or soft tissue tumor. Some of these new findings show diagnostic utility. Fortuitously, cytology specimens yield high quality DNA and RNA for such testing [14]. Quantity of the nucleic acid varies directly with the cellularity of the specimen; therefore, always strive for a cellular sample.

Of course, the mainstay ancillary cytology technique is immunocytochemistry (ICC). ICC

can be performed on alcohol fixed samples, but reactivity may not be well characterized, especially for less common antibodies. Internal positive controls serve to verify reactivity, if pertinent. When possible, extra passes to prepare a cell block are desirable, as additional sections can be cut, and the sample can be fixed in formalin similar to tissue specimens substantiating the use of FFPE tissue positive controls.

References/Suggested Readings

1. Khalbuss WE, Parwani AV. Cytopathology of soft tissue and bone lesions, essentials in cytopathology 9. New York: Springer; 2011.
2. Traina F, Errani C, Toscano A, Pungetti C, Fabbri D, et al. Current concepts in the biopsy of musculoskeletal tumors. J Bone Joint Surg Am. 2015;7:97.
3. Khalbuss WE, Teot LA, Monaco SE. Diagnostic accuracy and limitations of fine needle aspiration cytology of bone and soft tissue lesions: a review of 114 cases with cytological-histological correlations. Cancer Cytopathol. 2010;118:24–32.
4. Rougraff BT, Aboulafia A, Biermann JS, Healey J. Biopsy of soft tissue masses: evidence-based medicine for the Musculoskeletal Tumor Society. Clin Orthop Relat Res. 2009;467:2783–91.
5. Domanski HA. Fine-needle aspiration cytology of soft tissue lesions: diagnostic challenges. Diagn Cytopathol. 2007;35:768–73.
6. Singh HK, Kilpatrick SE, Silverman JF. Fine needle aspiration biopsy of soft tissue sarcomas: utility and diagnostic challenges. Adv Anat Pathol. 2004;11:24–37.
7. Kilpatrick SE, Cappellari JO, Bos GD, et al. Is fine needle aspiration biopsy a practical alternative to open biopsy for the primary diagnosis of sarcoma? Experience with 140 patients. Am J Clin Pathol. 2001;115:59–68.
8. Dodd LG, Hertel J. Needle biopsy of mesenchymal lesions of the head and neck: evolving concepts and new strategies for diagnosis. Semin Diagn Pathol. 2015;32(4):275–83.
9. Wakely PE, McDermott JE, Ali SZ. Cytopathology of alveolar soft part sarcoma: a report of 10 cases. Cancer Cytopathol. 2009;117:500–7.
10. Domanski HA, Akerman M, Engellau J, et al. Fine-needle aspiration of neurilemoma (schwannoma). A clinicopathologic study of 116 patients. Diagn Cytopathol. 2006;34(6):403–12.
11. Pohar-Marinsek Z. Difficulties in diagnosing small round cell tumors of childhood from fine needle aspiration cytology samples. Cytopathol. 2008;19:67–79.
12. Domanski HA, Akerman M, Rissler P, Gustafson P. Fine-needle aspiration of soft tissue leiomyosarcoma: an analysis of the most common cytologic findings and the value of ancillary techniques. Diagn Cytopathol. 2006;34(9):597–604.
13. Klijanienko J, Couturier J, Brisse H, et al. Diagnostic and prognostic information obtained on fine-needle aspirates of primary neuroblastic tumors: proposal for a cytology prognostic score. Cancer Cytopathol. 2011;119:411–23.
14. Klijanienko J, Pierron G, Sastre-Garau X, et al. Value of combined cytology and molecular information in the diagnosis of soft tissue tumors. Cancer Cytopathol. 2015;123:141–51.
15. Domanski HA, Mertens F, Panagopoulos I, Akerman M. Low grade fibromyxoid sarcoma is difficult to diagnose by fine-needle aspiration cytolgy: a cytomorphological study of eight cases. Diagn Cytopathol. 2009;20(5):304–14.
16. Bean SM, Baker A, Eloubeidi M, et al. Endoscopic ultra-sound guided fine-needle aspiration of intrathoracic and intra-abdominal spindle cell and mesenchymal lesions. Cancer Cytopathol. 2011;119:37–48.
17. Varma K, Jain S, Mandal S. Cytomorphologic spectrum in paraganglioma. Acta Cytol. 2008;52(5):549–56.

Cerebrospinal Fluid Cytology 13

Brief Introduction

Imaging studies are critical and often are initially performed in evaluating patients presenting with signs and symptoms suggestive of central nervous system pathology. Tissue for diagnosis is not always practical to acquire except via surgery. Cytology, however, can offer the least invasive method to establish cellular diagnosis by examining cerebrospinal fluid (CSF). The latter may be obtained via lumbar puncture or directly aspirated from the cisterna magna at the base of the brain. Alternatively, CSF may be obtained from the lateral ventricles during surgical intervention. One of the main indications for requesting cytological evaluation of CSF is suspicion for malignancy, primary or metastatic. Neurologic symptoms, in the setting of a history of malignancy, makes leptomeningeal metastasis a strong possibility. The challenge for the cytopathologist is that numerous inflammatory, infectious, traumatic or iatrogenic conditions can lead to worrisome cells in the CSF that can be erroneously interpreted as tumor cells. In this chapter, these conditions are discussed.

Classification of Central Nervous System Neoplasms Based on Potential Cytological Finding Mimickers

CSF cytological specimens are normally hypocellular to virtually acellular. When cells are present, they are usually lymphocytes and monocytes. The list of conditions that can raise the cellularity, often associated with worrisome reactive changes, is long (Fig. 13.1). Tight correlation with clinical, radiological and noncellular characteristic of the CSF is the key to prevent inaccurate interpretation. Generally, the potential neoplastic mimickers can be classified into three groups. First, the normal non-pathological cellular constituents of CSF don't always cause difficulties when they are in low quantitation, but when slightly increased they can be problematic. Nonetheless, awareness of these normal components is needed before arriving at an interpretation of CSF cytology. Second, non-neoplastic but pathological cells deriving from inflammatory or infectious process can also spike the cellularity with both normal and unfamiliar cells causing

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9_13

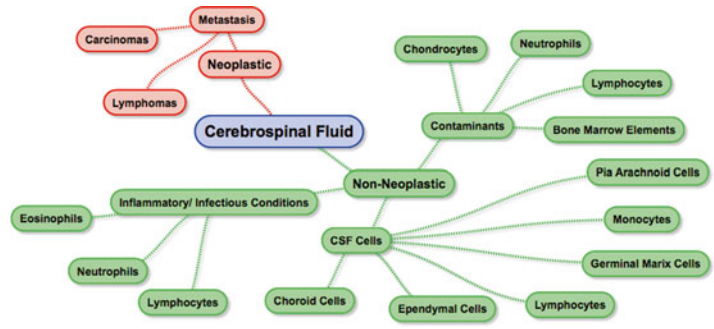


Fig. 13.1 Cerebrospinal fluid on cytology

diagnostic challenges. Lastly, aspiration of CSF can include contaminants. Knowledge of these is very important.

Normal Non-pathological CSF Cells as Potential Mimickers of Malignancy

Lymphocytes are the expected cells in a benign non-pathological CSF (Table 13.1). They are ordinarily small mature lymphocytes. These cells are recognized as lymphocytes by their minimal rim of cytoplasm leading to a high nucleocytoplasmic ratio. The nuclei are round with minimal nuclear membrane irregularities. The chromatin is coarse and dense (Fig. 13.2). They can be mistaken for any *Small Round Blue Cell Tumors*. These malignancies are recognizable by the presence of neoplastic cells that are larger than red blood cells in air-dried smears. This group of tumors includes Ewing sarcoma (EWS), peripheral neuroectodermal tumor, rhabdomyosarcoma, synovial sarcoma, non-Hodgkin's lymphoma, retinoblastoma, neuroblastoma, hepatoblastoma, nephroblastoma, medulloblastoma, small cell carcinoma and lobular breast carcinoma (Fig. 13.3). However, the low number of the small lymphocytes coupled with the lack of nuclear enlargement and significant mitosis should be a clue to their non-neoplastic nature. Immunocytochemistry is always desirable to confirm any suspected malignancy in the absence of definitive cytological features. This can be performed on cytospin preparations.

Monocytes, like lymphocytes, though in even smaller number, are normally found in CSF. They rarely cause diagnostic challenges. When their number increases and they are readily seen on CSF cytology, they can be problematic to interpret. Their larger size compared to lymphocytes may prompt a false positive diagnosis of small round blue cells or discohesive carcinomas like lobular breast carcinoma and signet ring carcinomas. Monocytes are recognized by their oval to bean-shaped nuclei. They have more cytoplasm than lymphocytes, usually not equally distributed around the nuclei (Fig. 13.4). Some reactive "atypical" changes can be seen with inflammation, infection, therapy or trauma. They can sometimes be mistaken for malignant epithelial cells. Here again, immunocytochemistry will be helpful.

Ependymal and choroid plexus cells are the lining cells of the ventricular system and can be collected and seen in the cytology specimen when CSF is aspirated directly from the ventricles. They can cause some diagnostic confusion, as they resemble malignant epithelial cells mimicking involvement by adenocarcinoma (Fig. 13.5). Ependymal and choroid plexus cells can be difficult to distinguish from each other. The cytopathologist is not expected to make such distinction. They both can occur in clusters or singly with cuboidal to columnar cells. These ventricular system lining cells have abundant cytoplasm (Fig. 13.6). The method of collection of CSF needs to be known at the time of cytological interpretation.

Germinal matrix cells, in premature babies with hydrocephalus from intraventricular brain hemorrhage, can be seen in CSF. They are usually arranged in molded clusters. The cells have round to oval nuclei with fine chromatin and scant basophilic cytoplasm. Thus, they can be

Table 13.1 Normal non-pathological CSF cells

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Small round blue cell tumors • Carcinomas in single cell pattern (e.g., lobular breast carcinoma, signet ring carcinomas)	Lymphocytes	• Normal CSF • Blood contamination • Prior aspiration or trauma	• Low cellularity • Small mature lymphocytes • Red blood cells may be present	• Clinical and radiographic correlation • Immunocytochemistry
	Monocytes	• Normal CSF • Blood contamination	• Low cellularity • Oval to bean-shaped nuclei • No mitosis	• Clinical and radiographic correlation • Immunocytochemistry
	Germinal matrix cells	• Premature babies with hydrocephalus • Intraventricular brain hemorrhage	• Hemosiderin-laden macrophages • Rare small blue cells with molding	• Clinical and radiographic correlation • Immunocytochemistry
• Adenocarcinoma	Ependymal cells	• CSF collected directly from the ventricles	• Single cell pattern or in clusters • Round to oval nucleus with moderate amount of cytoplasm	• Clinical and radiographic correlation
	Choroid cells	• CSF collected directly from the ventricles	• Single cell pattern or in clusters • Round to oval nucleus with moderate amount of cytoplasm	• Clinical and radiographic correlation

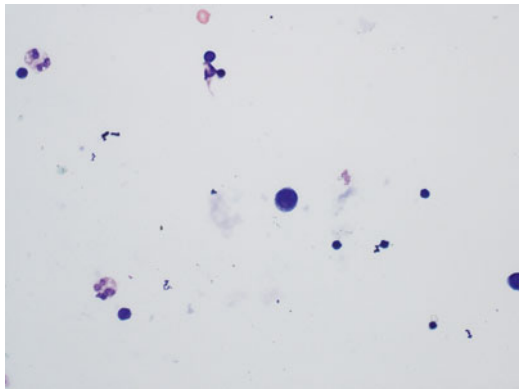


Fig. 13.2 Lymphocytes with round nuclei and coarse and dense chromatin. Scant rim of cytoplasm is seen (Diff-Quik, high magnification)

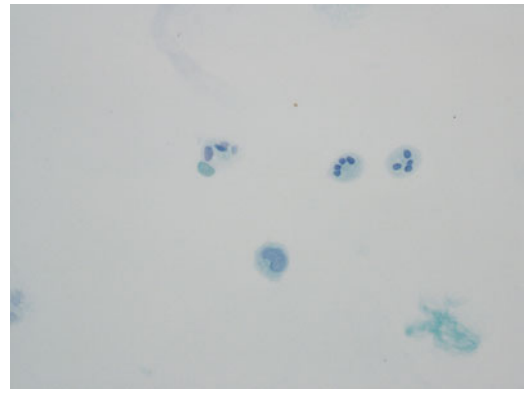


Fig. 13.4 Monocyte with bean-shaped nucleus, and moderate N/C ratio (Papanicolaou stain, high magnification)

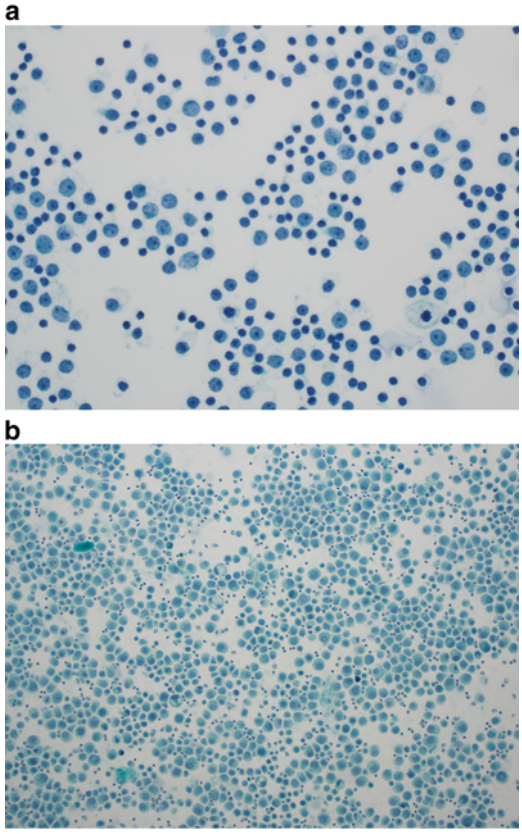


Fig. 13.3 (**a**) Second population of monomorphic malignant lymphocytes in lymphoma involving CSF (Papanicolaou stain, high magnification). (**b**) Dyscohesive malignant epithelial cells in a CSF with lobular breast carcinoma. The cells are vacuolated with signet ring features (Papanicolaou stain, high magnification)

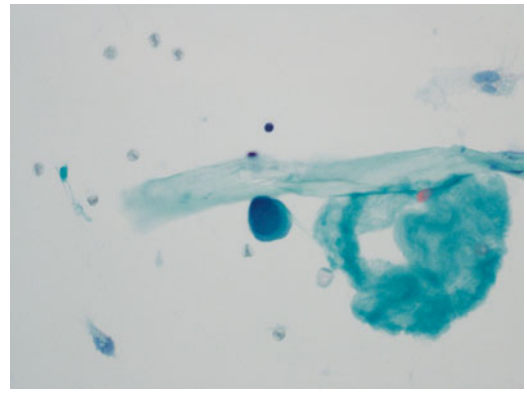


Fig. 13.5 Atypical reactive lymphocyte with cytomegaly, irregular nuclear membranes and increased cytoplasm (Diff-Quik stain, high magnification)

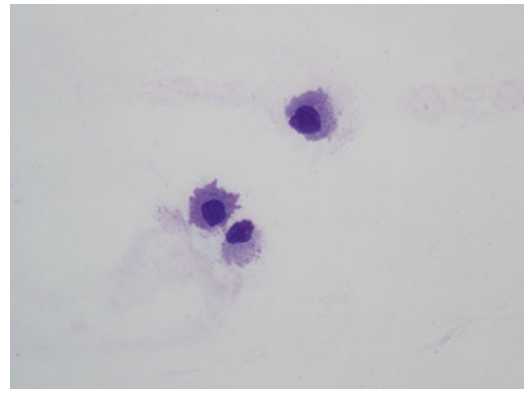


Fig. 13.6 Plasma cells with eccentrically placed nuclei and ample cytoplasm (Diff-Quik stain, high magnification)

mistaken for malignancy such as a small round blue cell tumor with its characteristic nuclear molding. The key to avoid overdiagnosis is the clinical history.

Non-neoplastic Pathological Cells in the CSF as Potential Mimickers of Malignancy

Macrophages can be identified in the CSF in almost any pathological condition affecting the central nervous system (Table 13.2). The fact they are not usually seen in normal non-pathological CSF can potentially lead to suspicion of malignancy. The larger size of macrophages in comparison to lymphocytes may make them appear worrisome. Furthermore, they can be in loose aggregates suggesting malignant epithelial cells, such as signet ring malignant cells. The clue to a benign non-neoplastic process is the low nucleocytoplasmic ratio and vacuolated cytoplasm (Fig. 13.7).

Neutrophils in the CSF often correlate with blood contamination from a traumatic procedure, but their presence in CSF fluid specimens may also indicate an underlying pathological condition. This is especially true when a relative pure population of neutrophils is seen. The causes of neutrophilia include an acute infectious process (bacterial, viral, fungal or tuberculous) and post-procedural changes (surgery, lumbar puncture, intratechal therapy). Any malignant tumor can cause neutrophilia as well. However, when interpreting CSF cytology with neutrophilia, a "Positive" diagnosis should be avoided in the absence of well-visualized malignant cells. Immunocytochemical studies can be helpful to rule out cytologically obscure malignant cells.

*Plasma cells, m*uch like macrophages, when present in the CSF are indicative of a pathological condition, usually of inflammatory type such as multiple sclerosis. Consideration of CNS involvement by multiple myeloma or plasmacytoma is always required. Sometimes, the plasma cells may also mimic a neuroendocrine tumor, or any dyscohesive carcinoma such as breast lobular carcinoma. Plasma cells have eccentrically placed nuclei with a perinuclear hoff. They have open chromatin and occasionally conspicuous nucleoli (Fig. 13.8). Correlation with the clinical context is crucial to avoid an erroneous diagnosis.

Table 13.2 Non-neoplastic pathological cells in the CSF

Neoplasms (benign and malignant)	Neoplastic mimickers	Associated conditions	Discriminating features	Additional steps
• Carcinomas • Myelomas	Macrophages	• Hemorrhage • Meningitis • Cerebral infarct • Any inflammatory conditions	• Low nucleo-cytoplasmic ratio • Vacuolated cytoplasm • Discohesive • Malignant cells not present	• Clinical and radiographic correlation • Immunocytochemistry
	Neutrophils	• Blood contamination • Meningitis	• Malignant cells not present • Microorganisms may be identified	• Clinical and radiographic correlation • Immunocytochemistry • Microbiology
	Plasma cells	• Meningitis • Infections • Any inflammatory conditions	• Discohesive cells	• Clinical and radiographic correlation • Immunocytochemistry • Microbiology
• Lymphomas	Atypical reactive lymphocytes	• Inflammation • Infection • Radiation	• Polymorphous lymphoid population	• Clinical and radiographic correlation • Immunocytochemistry • Microbiology

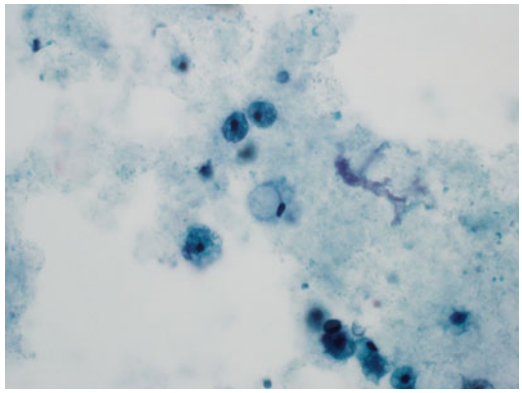


Fig. 13.7 Macrophages with vacuolated cytoplasm associated with small nuclei (Papanicolaou stain, high magnification)

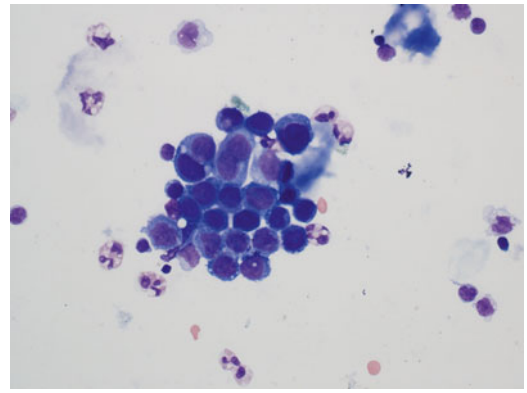


Fig. 13.9 Cohesive malignant epithelial cells with irregular enlarged nuclei, and increased nucleocytoplasmic ratios in an adenocarcinoma involving CSF (Diff-Quik stain, high magnification)

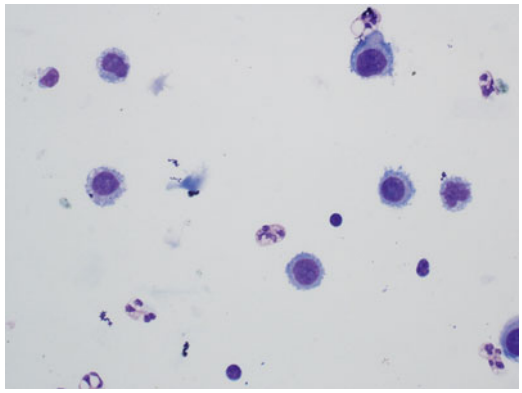


Fig. 13.8 Ependymal cells with epithelioid features (Papanicolaou stain, high magnification)

Atypical Reactive Lymphocytes can be appreciated in certain inflammatory and infectious processes which can also lead to lymphocytosis in the CSF. Additionally, reactive changes can be introduced in the CSF which can be concerning for a more ominous etiology. These reactive changes include cytomegaly and irregular nuclear membranes (Fig. 13.9). The clinical context will reveal an explanation for these changes. Conditions associated with reactive "atypical" lymphocytes include, but are not limited to, infections (viral, bacterial, fungal or tuberculous), inflammation (autoimmune disease such as multiple sclerosis) and history of radiation therapy. The cytological clue to the benign nature of a CSF fluid with reactive lymphocytes is the mixed lymphoid population.

Pia-arachnoid cells, when present in the CSF, occur singly or in flat sheets. The cytoplasm is abundant and dense. The nuclei are eccentric and bean-shaped, reminiscent of monocytes. Their presence is often indication of irritated meninges such as from inflammation and infection. Recognizing the cytomorphology and the clinical history will prevent overinterpreting these reactive changes as malignancy.

Non-neoplastic Contaminant Cellular Material as Potential Mimickers of Malignancy

Regardless of the method used to aspirate CSF for cytologic evaluation, contaminants surrounding normal cellular material can be seen and potentially confuse the diagnostic picture. For example, during lumbar puncture, the needle may inadvertently collect *Chondrocytes* from cartilage in the vertebral column (Fig. 13.10). They can be seen in association with cartilaginous fibrillar matrix mimicking mucinous carcinoma. Similarly, *bone marrow elements* can appear in a CSF cytological specimen. They can cause concern for a lymphoproliferative neoplastic process. The recognition of trilineage hematopoiesis should support a benign diagnosis.

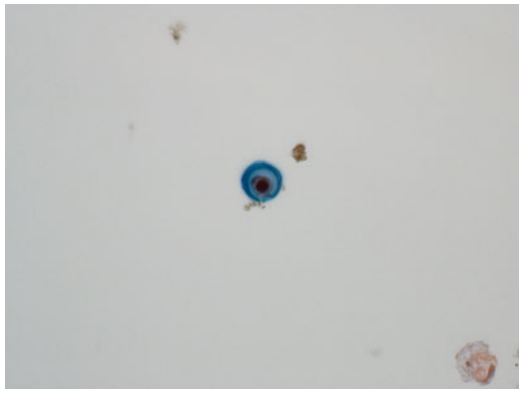


Fig. 13.10 Chondrocyte with pyknotic nucleus surrounded by a thick shell of extracellular mucopolysaccharide matrix (Papanicolaou stain, high magnification)

Conclusion

CSF cytology evaluation is frequently requested when there are neurological symptoms in a patient in order to determine the etiology especially when imaging studies are suggestive of central nervous system pathology. Tissue for malignancy confirmation is not always practical to acquire. In this circumstance, cytology has been able to vastly contribute to the work-up because it offers the least invasive method to establish cellular diagnosis by examining cerebrospinal fluid (CSF). There are however pitfalls in cytological evaluation of CSF cytology which can unfortunately lead to false-positives. Pitfalls can be avoided by being aware of the three main sources of confounders, namely: normal non-pathological cells of CSF; non-neoplastic but pathological cells; and aspiration of contaminants. Correlation with clinical, radiographic and non-cellular characteristics of CSF is also critical.

Suggested Readings

1. Rogers LR, Duchesneau PM, Nunez C, Fishleder AJ, Weick JK, Bauer LJ, Boyett JM. Comparison of cisternal and lumbar CSF examination in leptomeningeal metastasis. Neurology. 1992;42(6):1239–41.
2. Glantz MJ, Cole BF, Glantz LK, Cobb J, Mills P, Lekos A, Walters BC, Recht LD. Cerebrospinal fluid cytology in patients with cancer: minimizing false-negative results. Cancer. 1998;82(4):733–9.
3. Prayson RA, Fischler DF. Cerebrospinal fluid cytology: an 11-year experience with 5951 specimens. Arch Pathol Lab Med. 1998;122(1):47–51.
4. Gondos B, King EB. Cerebrospinal fluid cytology: diagnostic accuracy and comparison of different techniques. Acta Cytol. 1976;20(6):542–7.
5. Borowitz M, Bigner SH, Johnston WW. Diagnostic problems in the cytologic evaluation of cerebrospinal fluid for lymphoma and leukemia. Acta Cytol. 1981;25(6):665–74.
6. de Reuck J, Vanderdonckt P. Choroid plexus and ependymal cells in CSF cytology. Clin Neurol Neurosurg. 1986;88(3):177–9.
7. Fischer JR, Davey DD, Gulley ML, Goeken JA. Blast-like cells in cerebrospinal fluid of neonates. Possible germinal matrix origin. Am J Clin Pathol. 1989;91(3):255–8.
8. Chen KT, Moseley D. Cartilage cells in cerebrospinal fluid. Arch Pathol Lab Med. 1990;114(2):212.
9. Zeman D, Adam P, Kalistová H, Sobek O, Anděl J, Anděl M. Cerebrospinal fluid cytologic findings in multiple sclerosis. A comparison between patient subgroups. Acta Cytol. 2001;45(1):51–9.
10. Granter SR, Doolittle MH, Renshaw AA. Predominance of neutrophils in the cerebrospinal fluid of AIDS patients with cytomegalovirus radiculopathy. Am J Clin Pathol. 1996;105(3):364–6.
11. Choi J, Kim H, Kim SH. Atypical teratoid/rhabdoid tumor: analysis of cytomorphologic features in CSF, focused on the differential diagnosis from mimickers. Diagn Cytopathol. 2012;40(7):592–6.
12. Bigner SH, Jonston WW. The cytopathology of cerebrospinal fluid. I. Nonneoplastic conditions, lymphoma and leukemia. Acta Cytol. 1981;25(4):345–531.
13. Rajwanshi A, Radhika S, Gautam U. Malignant small round cell tumors. J Cytol. 2009;26(1):1–10.
14. Akhtar M, Iqbal MA, Mourad W, et al. Fine-needle aspiration biopsy diagnosis of small round cell tumors of childhood: a comprehensive approach. Diagn Cytopathol. 1999;21:81–91.

Index

V.G. Samedi, T. Bocklage, *Pitfalls in Diagnostic Cytopathology With Key Differentiating Cytologic Features*, Essentials in Cytopathology 27, DOI 10.1007/978-3-319-39809-9