Practical Anatomic Pathology
Series Editors: Fan Lin · Ximing J. Yang

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Practical Cytopathology

Frequently Asked Questions

Springer

Practical Anatomic Pathology

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The proposed Book Series will be designed to provide a comprehensive, practical and state-of-the art review and update of the major issues and challenges specific to each subspecialty field of surgical pathology in a question and answer (Q&A) format. Making an accurate diagnosis especially from a limited sample can be quite challenging, yet crucial to patient care. The proposed Book Series, using the most current and evidence-based resources, will

1) focus on frequently asked questions in surgical pathology in day-to-day practice;
2) provide quick, accurate, terse, and useful answers to many practical questions encountered in daily practice;
3) emphasize the importance of a triple test (clinical, radiologic, and histologic correlation);
4) delineate how to appropriately utilize immunohistochemistry, in situ hybridization and molecular tests; and
5) minimize any potential diagnostic pitfalls in surgical pathology.

These books will also include highly practical presentations of typical case scenarios seen in an anatomic pathology laboratory. These will be in the form of case presentations with step-by-step expert analysis. Sample cases would include common but challenging situations, such as evaluation of well-differentiated malignant tumors vs. benign/reactive lesions; distinction of two benign entities; sub-classification of a malignant tumor; identification of newly described tumor and non-tumor entities; workup of a tumor of unknown origin; and implementation of best practice in immunohistochemistry and molecular testing in a difficult case. The Q&A format will be well accepted, especially by junior pathologists, for several reasons:

1) this is the most practical and effective way to deliver information to a new generation of pathologists accustomed to using the Internet as a resource and, therefore, comfortable and familiar with a Q&A learning environment;
2) it's impossible to memorialize and digest massive amounts of new information about new entities, new and revised classifications, molecular pathology, diagnostic IHC, and the therapeutic implications of each entity by reading large textbooks;
3) sub-specialization is a very popular practice model highly demanded by many clinicians; and
4) time is very precious for a practicing pathologist because of increasing workloads in recent years following U.S. health care reforms. This Book Series will meet all of the above expectations. These books will be written by established and recognized experts in their specialty fields and will provide a unique and valuable resource in the field of surgical pathology, both for those currently in training and for those already in clinical practice at various skill levels. It does not seek to duplicate or completely replace other large standard textbooks; rather, it will be a new, comprehensive yet concise and practical resource on these timely and critical topics.

More information about this series at http://www.springer.com/series/13808

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Practical Cytopathology

Frequently Asked Questions

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Practical Anatomic Pathology
ISBN 978-3-030-24058-5 ISBN 978-3-030-24059-2 (eBook)
https://doi.org/10.1007/978-3-030-24059-2

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

To my wife, Yunling, for her love and support.

— He Wang

To my husband, Chenyi, for his love, sacrifices, and support.

— Xiaohua Qian

To my parents; my husband, Feng; and my sons, Andrew and Kevin, for their unconditional love, support, and belief in me.

— Huihong Xu

Preface

We are honored and excited to join a group of expert pathologists and the Springer Publishing family in contributing *Practical Cytopathology: Frequently Asked Questions* as one of the volumes in the Practical Anatomic Pathology book series.

The goal of our volume is to provide a comprehensive, practical, and state-of-the art review addressing the major issues and challenges in the cytopathology practice using a question and answer (Q&A) format. Making an accurate diagnosis, especially on a limited cytology sample obtained by minimally invasive procedures, is often challenging yet crucial to patient care. In the era of precision medicine and genomic diagnostics, cytology is continuously expanding its role as an indispensable subspecialty in the multidisciplinary care team.

Using the most current and evidence-based approaches, this book (1) focuses on frequently asked questions in cytopathology's day-to-day practice; (2) provides quick, accurate, and useful answers; (3) emphasizes the importance of clinical, radiological, and cytological correlation, as well as cytohistological correlation; and (4) delineates how to judiciously use immunohistochemistry, molecular tests, flow cytometry, cytogenetics, and other established ancillary studies including next-generation sequencing and computer-assisted diagnostics.

This book includes highly practical presentations of typical gynecological and non-gynecological case scenarios with step-by-step analysis. Sample cases include common and challenging situations, such as distinguishing adenocarcinoma in situ vs. benign mimics in cervical cancer screening Pap tests; working up the basaloid neoplasm of salivary gland origin; recognizing mesenchymal tumors in a routine visceral organ on fine-needle aspiration specimens; updating new Bethesda terminology in reporting gynecologic cytology and thyroid cytology; and implementing best practice in ancillary studies in the workup of a tumor of unknown origin.

This book is written by established and widely recognized expert cytopathologists as well as rising stars in the field. We hope it will provide the most up-to-date information in the field of cytopathology for the trainees (residents, fellows), cytotechnologists, and cytopathologists at various skill levels. It does not seek to duplicate or completely replace other large standard cytopathology textbooks; rather, it serves as both a practical resource and guide to relevant references. We understand that other cytopathologists may have different opinions, approaches, experiences, and limitations in their clinical practices. Thus, we hope that this book will also provide a new platform for further exploring the evidence-based satisfying practice in cytopathology. Besides a handy version of quick search for particular topics, this book will also have an online version available for a quick reference.

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Cytology Techniques

1

Jing He and Yun Gong

Contents

List of Frequently Asked Questions

1. What types of stains are the routinely and commonly used for diagnostic cytology?
Two types of stains are typically performed: Romanowsky stains using air-dried, methanol postfixed smears and Papanicolaou stains using smears fixed in 95% ethanol or Carnoy's solution. Both stains are complementary for cytologic diagnosis.

Romanowsky stains allow better estimation of relative cell and nuclear sizes, preferably highlight cytoplasmic details, smear background elements, and intercellular matrix components. Romanowsky stains are also useful for detecting microorganisms and diagnosing hematolymphoid neoplasms.

Papanicolaou stains allow better visualization of nuclear characteristics such as the nuclear membrane, chromatin, and nucleoli. Papanicolaou stains also yield well-stained cytoplasmic transparency and differential cytoplasmic counterstaining. Many modifications of the original Papanicolaou stains staining method have been made for the Papanicolaou stains [1].

2. What is regressive Papanicolaou staining method?
Papanicolaou Technique I uses Harris hematoxylin regressively. The cells are intentionally overstained and then excess hematoxylin is removed by differential extraction in HCl. Running water bath is required during the staining process.

3. What is progressive Papanicolaou staining method?
Papanicolaou Technique II uses hematoxylin progressively. Since the reduction in staining time prevents overstaining of the cytoplasm, differential extraction in HCl is not necessary. Mayer hematoxylin and Gill hematoxylin are always used progressively because they rarely overstain nuclei, regardless of staining time. Progressive staining is usually recommended for cell samples that do not adhere well to glass slides, since the running water step used for regressive staining can be avoided.

4. What are the dyes used in Papanicolaou stain?
Papanicolaou stain includes both acidic and basic dyes. Acidic dye stains the basic components of the cell and basic dye stains the acidic components of the cell. The multichromatic Papanicolaou stain involves the following dyes:

- Hematoxylin (e.g., Harris hematoxylin, Gill hematoxylin, hematoxylin S) is the nuclear stain which stains cell nuclei blue. It has affinity for chromatin, attaching to sulfate groups on the DNA molecule.
- Orange green (OG) is an acidic counterstain (cytoplasmic stain) which stains matured and keratinized cells. The target structures are stained orange in different intensities.

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_1

- Eosin azure (EA) is the second counterstain which is a polychrome mixture of eosin Y, light green SF, and Bismarck brown. Eosin Y gives a pink color to cytoplasm of superficial epithelial squamous cells, nucleoli, red blood cells, and cilia. Light green SF stains blue to cytoplasm of other cells such as nonkeratinized squamous cells. Staining solutions commonly used in cytology are EA-36, EA-50, and EA-65. The number denotes the proportion of the dyes. EA-50 and EA-36 contain twice the amount of light green used in EA-65. EA-65 is preferred for the thick nongynecologic smears since EA-50 and EA-36 stain the background intensely. Bismarck brown Y stains nothing and is often omitted.

5. What is the hydration step used in Papanicolaou stain?

The series of graded alcohols (50%, 70%, 80%, and 95%) are used for hydration and dehydration which helps to minimize cell distortion.

6. What is the fixative used for Papanicolaou method? How long should the fixation time be?

- Ethanol (95% ethyl alcohol) fixative demonstrates excellent results and is employed by most laboratories. This fixative can be applied for all smears, including nongynecologic specimens such as fine-needle aspiration, as well as gynecologic specimens, such as cervical or vagina smears. This fixative can also be used for the final fixation of all smears of either fresh fluids or those initially collected in 50% alcohol or other preservatives.
- Prior to staining, the smears should be kept in the 95% ethyl alcohol fixative for a few minutes. However, the appearance of the smear will not be altered if the specimen is left in the fixative for several days or even weeks.

7. What is the optimal time between collection and preparation of the cytology samples?

- Urine and CSF need to be processed as soon as possible. These specimens can endure only 1 or 2 hours delay. Refrigeration does not protect cells.
- Unfixed cells from samples containing thick mucus, such as bronchial aspiration and sputum, remain intact longer because mucus coating helps preserve cell structure. Refrigeration can slow growth of bacteria that can damage the cells and break down the mucus. Respiratory samples can be preserved in the refrigerator for 12–24 hours.
- Body cavity effusions, including pleural, peritoneal, and pericardial fluids, may be preserved for 24–48 hours without refrigeration. Body cavity effusions have a high protein content which serves as a tissue culture medium and helps preserve cell morphology.
- Fixation of smears made from FNA specimens varies with the type of stain being utilized. Slides for Romanowsky staining can be left at room temperature until the time for postfixation and staining. Those to be Papanicolaou stained should be placed in 95% ethanol (or equivalent fixative) within a few seconds of preparation. The cell block can be made from the remaining specimen in the fixative.

8. What are the advantages and disadvantages of prefixation?

- Prefixation is recommended when the specimen has to be sent to the distant laboratory.
- Prefixative or preservative is added to the fresh sample until the time of slide preparation. Prefixation can better preserve cellular morphology which is essential for making accurate diagnosis. Some specimens can be preserved for days without deterioration of cells. But the length of preservation time depends on the PH, protein content, and enzymatic activity of the specimens.
- There are disadvantages of using prefixatives. Prefixation can harden the cells in spherical shapes, condense chromatin, and precipitate protein. If the specimen can be processed immediately, no prefixatives are needed.

9. What solutions are used for prefixation?

- Ethyl alcohol (50% solution) is commonly used as prefixative for fluid and can be added in equal volume to the fluids. Higher concentration (>50%) of ethyl alcohol should not be used in fluids rich in protein since the sediment becomes hardened and difficult to be spread on slides. However, 95% ethyl alcohol can be used in collection of gastric washing.
- Methanol can improve preservation of cellular details in specimens with PH higher than 4.5.
- Saccomanno's fixative can be used as prefixative or fixative. Equal volume is added to the specimen.
- Mucoliquefying preservative is designed for use in the collection of mucoid and fluid specimens.
- Cytospin Collection Fluid (Thermo Electron Corporation) is available.
- CytoRich Red was developed for use with automatic cytology system. There is a marked reduction of erythrocytes and background material, when compared to slides prepared from the same specimens collected in Cytospin Collection Fluid.

10. What are the methods used to lyse erythrocytes prior to slide preparation?

Bloody smears can be difficult to interpret. Prior to slide preparation, lysing erythrocytes helps for better recovery of epithelial cells. Carnoy's fixative has been used for hemolyz-

ing erythrocytes. A newer method has been developed for erythrocyte hemolysis. Commercial agents are now available to lyse red blood cells and fix the cellular elements. Two commonly used fixatives are CytoRich Red and CytoLyt Solution. The fixative (1 ml) is added to the sample (per 25–50 ml). The supernatant is poured off after centrifugation. An alternative way is to add solution to the bloody sediment after centrifugation and the sample recentrifuged when the erythrocytes are lysed.

11. What are the components of Carnoy's fixative? How do we use this fixative? What are the advantage and limitation of using this fixative?

- Carnoy's fixative contains 95% ethanol (60%), chloroform (30%), and glacial acetic acid (10%). Carnoy's fixative can hemolyze red blood cells and is thus useful for processing bloody specimens.
- The bloody smears are placed in Carnoy's fixative for 3–5 minutes, until the sediment becomes colorless. Then the smears are transferred to 95% alcohol.
- Carnoy's fixative must be prepared fresh when needed and discarded after each use. This fixative loses its effectiveness on standing, and the chloroform can react with acetic acid to form hydrochloric acid.
- Carnoy's fixative is rapid acting and gives good nuclear preservation and retains glycogen. However, it lyzes red blood cells and dissolves lipids and can produce excessive hardening and shrinkage. Shrinkage of the epithelial cells is greater in Carnoy's fixative than those specimens fixed in 95% ethanol.

12. How are the solutions maintained and how often does it need to be replaced?

- The solutions used for Papanicolaou stain need to be replaced in order to ensure a crisp and well-delineated staining quality. The frequency of replacement depends on the volume and nature of the materials processed.
  - Alcohols for rehydrating and dehydrating need to be changed weekly or discarded each day to avoid filtering these solutions.
  - Hematoxylin solution may be used for a longer period of time if it is stored in a dark bottle. Only a small amount of fresh solution needs to be added every day to replace the solution lost due to evaporation.
  - OG-EA needs to be replaced weekly, or as soon as the cells appear dull and lose crisp contrasting.
  - Bluing solution needs to be changed at least once a day.
  - Xylene should be replaced as soon as it appears tinted with any of the cytoplasmic stains.
  - Water rinse should be replaced after each use.

13. What is the routinely used method for urine or CSF specimen?

For the low cellular and low protein content cytology specimens such as urine and cerebrospinal fluid, various methods have been developed. The goals are prevention of cell loss and satisfactory preservation of morphologic details. Nowadays, cytocentrifugation combined with direct smears of the sediments have been widely used. The cytocentrifuge preparation method results in cell-rich monolayer slides with excellent cytomorphology. Specimens are sent to the cytology laboratory either fresh or having an equal volume of 50% alcohol added to help preserve the specimen. Refrigeration is recommended if transport to the lab or processing could be delayed. However, sample fixed in alcohol can cause cell loss up to 74–98% [2, 3].

14. What is Diff-Quik stain?

Romanowsky stains are neutral stains composed of a mixture of oxidized methylene blue (azure) dyes and eosin Y. The azures are basic dyes that bind acid nuclei and result in a blue to purple color. The eosins are acidic dyes that are attracted to the alkaline cytoplasm, producing red coloration. There are many commercially available Romanowsky stains modified for rapid performance.

Diff-Quik is a commercial Romanowsky stain variant, commonly used in cytological staining. The results produced with Diff-Quik are similar to those obtained with Wright-Giemsa. The three-step staining procedure (methanol, Hema-Diff solution 1, Hema-Diff solution 2) produces excellent staining results in just over 1 minute. So the Diff-Quik-stained smear is processed much quicker than the routine Papanicolaou stain.

15. What are the differences between conventional smear, SurePath, and ThinPrep?

- The conventional manner of collecting sample is spreading the sample on the glass slides with brush instruments.
- SurePath (Becton, Dickinson and Company, Franklin Lakes, NJ) and ThinPrep 2000 System (Hologic, Marlborough, MA) are two such liquid-based test systems currently approved by the FDA for cervicovaginal testing. By using the SurePath and ThinPrep methods, the sample is directly transferred to a vial of fixative after sample collection.
- Both ThinPrep and SurePath methods show a clean background without air-drying artifact. The ThinPrep procedure takes about 70 seconds per slide and results in a thin deposit of cells in a circle 20 mm in diameter (contrast with cytospin: diameter = 6 mm). SurePath prepares an evenly distributed deposit of cells in a circle 13 mm in diameter. In most cases, only a fraction of the sample is

used to prepare the slide used for diagnosis. If needed, the residual sample is available for additional ThinPrep slide preparation, cell block preparation, or molecular diagnostic testing (e.g., high-risk HPV, chlamydia, gonorrhea).

16. What is a cell block and what are the advantages and disadvantages of cell block?

- Cell block technique is an old method for preparing cytological materials. The basic steps of cell block preparation include fixation, centrifugation, and transferring a cell pellet for paraffin embedding. This technique is recommended for processing all residual cytological materials following cytological preparations. The materials suitable for cell block processing include materials obtained from fine-needle aspiration/biopsy or other sample types such as effusions, urine, bronchial brushing, and sputum.
- The cell block method uses histologic techniques for processing and thus offers the following advantages:
  - In cell block preparations, fresh cellular materials are fixed in formalin and processed via the identical procedures for regular paraffin-embedded tissues. Thus, the results are equivalent to other formalin-fixed and paraffin-embedded surgical specimens.
  - The cell block materials often contain tissue fragments which show the histologic patterns besides cytologic features, whereas cytosmears usually lack this useful information and may preclude a definitive diagnosis in some cases.
  - Multiple sections can be obtained from the cell block and can be utilized to perform different studies such as immunohistochemical stains, special stains, ultrastructural studies, and molecular studies. The control slides can be readily found from other paraffin-embedded pathology tissue. Compared to direct smears, immunostaining on cell block sections is less likely to have background staining (Fig. 1.1).
  - Both cell block material and smears yield comparable result for molecular testing.
- The disadvantages of the cell block technique are as follows:
  - Because of suboptimal cellular recovery, multiple needle or brushing passes may be needed in order to obtain adequate material for a good-quality cell pellet.
  - The turnaround time is increased, since the processing time for a cell block is longer than direct smears.
  - Cell block technique is more labor-intensive and thus increases medical expense.

17. What are the methods used in cell block preparation?

A good cell block preparation depends on the presence of adequate cellular aggregates or tissue fragments. The challenging step is to harden the cell pellet so that it can be easily transferred without losing diagnostic material. A variety of technical modifications have been reported and are still being improved [4].

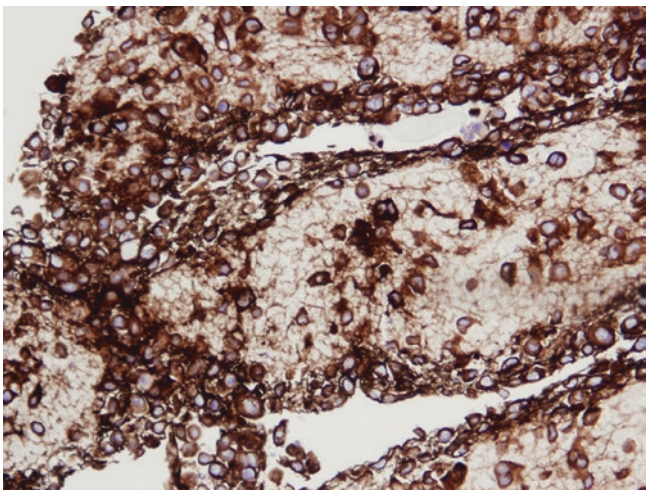

Fig. 1.1 Case 1. Tumor cells on cell block tissue sections are positive for CK-7 by immunohistochemical stain

- Normal saline needle rinse method:
  - This method is utilized to rinse the fine-needle aspiration needle in 20–30 ml of normal saline, followed by centrifugation and collection. Alternatively, formalin (10 ml) or paraformaldehyde or ethanol (50%) can be used to rinse the materials for cell block [5], or an equal amount of 95% ethanol and 10% formalin can be used as fixative.
- Tissue coagulum clot (TCC) method:
  - The TCC method allows the clot of tissue and blood mixture to form in the lumen of the needle. It is used to obtain better recovery of cellular materials in cell block sections. As the coagulum streams out from the needle tip, it is collected onto a piece of filter paper and slightly air-dried. The tissue coagulum is then transferred into a formalin container and subsequently processed in the histology laboratory [5–7].
- Plasma thrombin or thrombin clot method:
  - Plasma and thrombin are added to the cellular pellet to enmesh the cellular material. Although this method may cause an uneven concentration of cells, the problems can be avoided by continuous agitation to disperse the cell population evenly throughout the fibrin mesh [8, 9].
- Agar embedding, collodion bag methods:
  - Due to the difficulties in the recovery and processing of small tissue fragments, manual cell block methods have been alternatively utilized. Basically, the concentrated sediments are supported by a substance such as

agar or a collodion bag. Since agar solidifies below 50 °C, this property can be used to form the cell pellet. The collodion bag effectively coats the tube before the cell pellet is added. This collodion bag method is recommended for scant cellular materials. Collodion is a nitrocellulose substance which can be used for friable tissue such as brain. Other alternative methods have been used including HistoGelTM, gelatin albumin, pre-gelatinized starch, sodium alginate, gelatin foam, and polyvinyl alcohol foam [10–13].

- ShandonTM CytoblockTM method:
  - This cell block preparation system can concentrate cells by cytocentrifugation in a Thermo Shandon Cytospin. The Cytoblock cassettes and reagents are available in the kit [14].
- Rapid cell block method and automatic cell block system:
  - A tissue cassette and filter assembly are designed to deposit the needle rinse in one plane for microtomy. This technique allows to yield a better tissue fragment. The automated cell block preparation system has been developed to recover the small tissue fragments and rapidly deliver the tissue in paraffin for histological sectioning in less than an hour. Cellient automated cell block system is a newly introduced CB technique, and more studies are required to validate the results of this system [15–18].

18. What are the advantages and disadvantages of using direct smears for the immunostains?

- Direct smears are sometimes the only available materials for diagnosis. In the absence of cell block, immunoperoxidase studies can be performed on the direct smears and provide useful information for diagnosis (Fig. 1.2). If the smears are cellular, the cells can be peeled off and transferred to multiple slides for a panel of immunostains. The previously Papanicolaou-stained slides can be used for immunostains.
- However, there are some disadvantages of direct smears:
  - It is more difficult than cell block to interpret the results due to thick smears, overlapping cells, poor cytoplasmic preservation, and the higher background staining that are frequently associated with staining on direct smears.
  - Cells can get lost during staining.
  - Cells might be disrupted during the mechanical process of making direct smears, which may cause leakage of antigens.
  - In contrast to cell blocks, staining on smears lack validated control tissue. This might cause difficulty in interpreting the staining result. Some immunostains cannot be reliably interpreted on smears fixed with alcohol-based fixative, such as S-100.
  - Three-dimensional groups of cells may trap antibodies leading to nonspecific staining. Because of lacking histologic pattern, the benign entrapped cells could be erroneously interpreted as tumor cells, leading to false-positive interpretation. False negative can also potentially occur on a small specimen if the tumor cells express some markers only focally or heterogeneously.

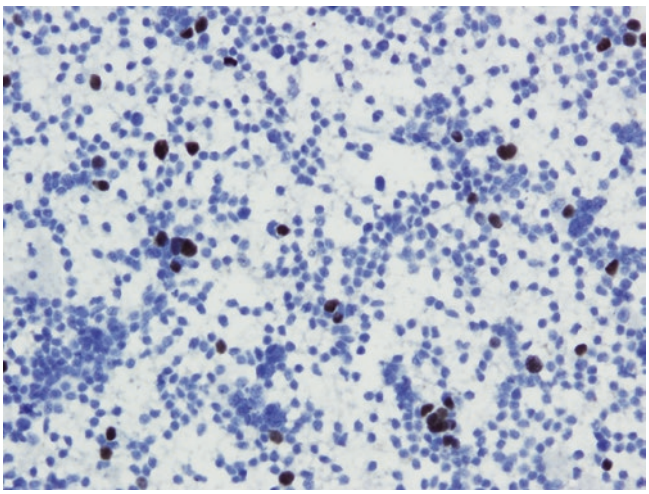

Fig. 1.2 Case 2. Tumor cells on a Papanicolaou-stained smear are positive for Sox-10 by immunohistochemical stain

19. What is a cytocentrifuge? What are the advantages and disadvantages of using cytocentrifuge for immunostains?

- A cytocentrifuge (cytospin) is a device that spins cells in a fluid suspension directly onto a glass slide. Multiple cytocentrifuge slides can be prepared at the same time.
- The advantages of using cytocentrifuges for immunostains are listed as follows:
  - Since cells are concentrated in a small area, only small amounts of antibodies need to be used.
  - There is less background staining in cytocentrifuge slides than in smears.
- The disadvantages of cytocentrifuges are as follows:
  - Loss of cells may occur during cytospin.
  - Lack of good control tissue for staining, similar to staining on smears.

20. What is PAS stain and PASD stain? What is GMS stain and how to interpret the stain? What methods are used to stain pigments, such as melanin and iron (hemosiderin)?

- Periodic acid-Schiff (PAS) is a commonly performed special staining method, which can detect polysaccharides and mucosubstances. Cells rich in glycogen and microor-

ganisms such as fungi are PAS-positive (magenta red) in standard PAS-stained slide. Extracellular mucin with glycoproteins and glycolipids are also PAS-positive.
- PASD stands for periodic acid-Schiff (PAS) with diastase digestion. Cells with glycogen can be determined if they are unstained in the diastase-treated slide. Therefore, PASD can be used to detect both intracellular and extracellular mucin as well as discriminate between mucinous and glycogen-rich tumors.
- Grocott-Gomori's (or Gömöri) methenamine is a silver stain, abbreviated GMS. Fungi and *Pneumocystis jiroveci* stain black sharply delineated. Glycogen and mucin stain rose to gray.
- Most common pigments are iron, hemosiderin, or melanin. Iron stain (Prussian blue) is used for staining iron or hemosiderin. It stains iron-containing pigments (hemosiderin) blue and stains nuclei red. Currently immunostains such as melan A and HMB45 are used for identifying melanoma cells with melanin pigment.

21. What is Oil Red O stain?

Oil Red O stain is a type of lipid stain. It stains fat orange to bright red and stains nuclei blue. Since histiocytes phagocytize fat in lipoid pneumonia, Oil Red O stain can be used to stain fat in sputum or bronchial washing specimens to determine if there is lipoid pneumonia. Sometimes it is also used to stain smears for fat.

22. What are universal precautions?

Universal precautions refer to disease prevention measures that should be utilized by all health care workers who may be exposed to blood or body fluids. Universal precautions are recommended by the *Centers for Disease Control* and Prevention (*CDC)* for all the patients because infection with HIV or other blood-borne pathogens cannot be reliably predicted by clinical findings alone.

23. What is the artifact called "cornflakes" and how does it solve the problem?

Cornflakes, also called "brown artifact," is a common artifact seen on coverslipped glass slides. It appears as a brown refractive pigment-like substance on the surface of the cells. It occurs when the mounting medium and coverslips are applied too slowly. This can result in evaporation of xylene before coverslipping since air is trapped and deposited on the surface of the cells, causing distinct appearance so called "cornflake." If this happens, the slide needs to be recoverslipped. The slides may be soaked in xylene and alcohol. Then the slides are rinsed in running water and restained.

24. Why do we need fixation before immunostaining?

Delayed in fixation may cause diffusion of antigens from the intracellular sites leading to reduced immunoreactivity. Autolysis can also cause nonspecific binding of antibodies. Thus, appropriate fixation is necessary for preservation of antigens and preserving morphological integrity. Buffered neutral formalin (10% formaldehyde) has been commonly used as fixative for cell block samples because of its preservation of morphological details, as well as its low cost and ease of preparation. The principle of formalin is the formation of hydroxylmethylene-type linkages between protein end groups and formation of coordinate bonds for calcium ions [19].

25. What are the antigen retrieval methods used for cytology specimen?

- Prolonged fixation in formalin may lead to reduced immunoreactivity of many antigens due to extensive crosslinking. Crosslinks may either directly affect the epitope itself or form between two or more different molecules masking the epitope. Antigen retrieval methods have been used to unmask the antibody-binding site, restore the epitope reactivity, and significantly enhance the immunostaining signal of the antigen.
- There are two main antigen retrieval techniques. One method is called proteolytic-induced epitope retrieval (PIER). Proteolytic enzyme digestion can break the crosslinks in the antigen and expose the masked epitopes. Optimization of proteolysis time, enzyme concentration, pH, and temperature are important to obtain optimal results. Over-digestion may result in false-positive staining; in contrast, under-digestion may cause a false-negative result.
- The other method, which is a major advance in antigen retrieval, is called heat-induced epitope retrieval (HIER). It is developed by using moist microwave heating of sections and has already been applied to cytological samples. The possible mechanism of this method is that heating can lead to disruption of formalin-induced bonds between calcium ions and proteins. However, heat retrieval methods could potentially expose unwanted epitopes of sequence in other antigens which could lead to unexpected cross-reactions and false-positive results. HIER and PIER can be combined in some cases [20–23].

26. Why is it important to use controls in immunohistochemistry stain?

It is important to use the positive and negative controls for immunohistochemistry stains for tissue sections as well as for cytology preparations. The positive controls can be obtained from the tissues with the antigens of interest. The negative controls can be acquired from tissue with absence of that antigen. If there is only a single slide available, internal control can be used as the negative control. The best positive and negative control reactions are those tissues present within the same patient. The controls should be processed in

the same manner as the test case. As part of the quality control process, positive and negative controls need to be validated before they could be used in the clinical assays. The validation of ambiguous results should be evaluated by using antibodies to different epitopes of the same molecule and by the use of antibodies to related markers [24–32].

Controls for non-formalin-fixed cytology smears ideally should be the cells that are prepared and stained in the same manner. However, in reality, it is difficult to obtain such controls and often histology samples are used for the controls in such situation. In addition, the antibody concentrations are not always customized for cytology specimens. Therefore, a disclaimer should appear in the bottom of the cytology report [33, 34].

27. What is immunofluorescence and its application in pathology?

Immunofluorescence techniques were first developed in the 1940s. The basis of immunofluorescence is labeling of antibodies with a fluorescent substance. The antigen-antibody product therefore can be visualized in fluorescence microscope in cells or tissues. This technique has been commonly used as a diagnostic tool in renal pathology as well as other immunologically related pathology. However, this technique has relatively low sensitivity, lacks morphological details, results in short storage time of the stained slides, and requires fresh frozen tissue, which limits its use in surgical pathology. In contrast, fluorescence in situ hybridization (FISH) depends on the formation of a hybrid between a fluorescently labeled DNA probe and its target chromosomal DNA, therefore permitting localization of a particular DNA sequence to a specific chromosome region [35, 36]. UroVysion, which is used in upper urinary tract surveillance, is a successful example of applying fluorescence in situ hybridization (FISH) technique in cytology.

28. What are TMA and CMA and what are their utilities?

Tissue microarrays (TMA) are assembled by taking preexisting formalin-fixed paraffin-embedded tissue blocks and re-embedded in a single recipient paraffin block. TMA blocks can hold up to several hundred tissue samples per slide which can contain a variety number of tissues including normal and tumor tissues [37–39]. Since a variety of samples are embedded on a single block, only a small amount of antibody is required for hundreds of samples examined. The results for these samples are more comparable because the conditions in the staining process are identical for all the samples, including incubation time, antibody concentration, and antigen retrieval conditions. A potential limitation of TMA is that only a relatively small tissue volume is represented in each core.

TMA may be used for quality control/quality assurance in the immunohistochemical lab. It can be used to analyze sensitivity and specificity of new antibodies, establish positive and negative control tissues, and discover new biomarkers. Molecular profiles can also be analyzed on the TMA blocks, and the results can correlate with diagnosis, prognosis, and management for tumors or disease. New genes and molecules may be potentially discovered on the samples. TMA also preserves archived tissues from rare tumors.

Cytology microarrays (CMA) have been constructed by some labs [37–39]. CMA is assembled by depositing cell suspensions on a glass slide in an array pattern. The process can apply to a wide range of cell suspension materials. CMA has a great potential in clinical research and practice.

29. What is flow cytometry?

Cytometry is the science of analyzing and classifying cells based on cell measurements. Flow cytometry technique plays an important role in defining cells and their functional status. Cells flow in single files through a measuring sensor, and cells in suspension are measured. Flow cytometry is able to make measurement and record a large number of cells in a short period of time and precisely control the stain conditions for all cells. It can not only measure the cell features but also quantify the cells' constitutions and functional characteristics.

30. What is the clinical application of flow cytometry technique? What is the minimum number of cells in fluid or FNA sample sufficient for flow cytometry test?

- Flow cytometry analysis has many applications in research and clinical laboratories. This technique has been carried out on any cell suspensions, including peripheral blood, bone marrow, effusions, and spinal fluid.
- The most common clinical application of flow cytometry is in the diagnosis of lymphoproliferative disorder. Flow cytometry plays a variety of roles in the field of lymphoma/leukemia, including diagnosis, classification, staging, minimal residual disease detection, evaluation of prognostic markers, detection of target molecules for therapies, molecular studies, and evaluation of multidrug-resistance markers. Flow cytometry has been proved to be an important diagnostic tool in cases of lymphoma from which the tissue provided was heavily involved with B cells and had monoclonal light chain restriction [40].
- The second application is DNA flow cytometry of solid tumors. Many solid malignant tumors are composed of cells with abnormal DNA contents, corresponding to abnormal chromosomes. Flow cytometry can analyze cellular DNA content and distinguish aneuploidy from diploidy in tumor cells. It can also measure proliferative activity of tumor cells (as expressed by S-phase fraction) by displaying tumor cell cycle distribution. The well-

differentiated neoplasms often show diploidy DNA modal pattern and demonstrate a more favorable clinical course. But it remains unclear if DNA contents are independent prognostic factor in poorly differentiated tumors.
- Only little sample preparation is required for flow cytometry analysis. A total of five million cells is usually sufficient for analysis.

31. What is in situ hybridization?

In situ hybridization (ISH) technique combines molecular and histochemical approaches to detect and localize DNA/RNA which may be present in the nucleus of a cell or on a chromosome. ISH can identify specific chromosomal abnormalities. It is a sensitive method since it has the ability to detect low copy gene expression on single cells and chromosome. It is also a specific tool because it can identify individual abnormal cells in a variety mixture of cell population [41]. ISH has been applied in a number of diagnostic situations, for example, in the determination of the presence of various types of HPV in precancerous cervical lesions and cancer.

32. What is the principle of FISH?

Fluorescence in situ hybridization (FISH) is a powerful molecular cytogenetic technique and is applicable to almost all types of cytological specimens. FISH technique depends on the formation of a hybrid between a fluorescently labeled DNA probe and its target chromosomal DNA sequences of interest, therefore permitting localization of a particular DNA sequence to a specific chromosome region [35, 42]. FISH has been widely used to detect chromosomal abnormalities. FISH can be applied in quantitation of chromosomes and genes, including chromosomal amplifications, deletions, translocations, and aneusomy in nuclei [35, 41–43].

The specific DNA probe is chosen to detect target DNA sequence (size ranging from less than 1 kb to several mega bases) on a specific chromosome site. The DNA probe is first labeled with an immunofluorescent compound such as biotin-11-dUTP or digoxigenin-11-dUTP. The targeted DNA and the probe are denatured. Then the labeled probe is hybridized to the target overnight either to metaphase chromosome or nondividing interphase preparations. The unbound probe is removed by washing. The resultant fluorescent signal is detected under fluorescence microscopy.

33. What is the application of FISH in cytology specimens? What are the requirements for the cytology preparation for FISH?

- FISH technique has many applications in cytology specimens. The initial application was the diagnosis of urothelial cancer in urinary sediment by UroVysion system [44, 45]. FISH techniques have currently been applied to a variety of cytology specimens (e.g., urine, effusion, sputum or bronchial washing, and lymph node aspirate) (Case 2, Fig. 1.3) [46, 47].
- FISH is applicable to almost all types of cytologic specimens including formalin-fixed paraffin-embedded cell block, air-dried or alcohol-fixed direct smear, cytospin preparation, cellular touch imprint, or liquid-based specimen (e.g., ThinPrep or SurePath).
- Direct smear and cytospin preparation appear to be superior to cell block sections because the gene copy number can be enumerated on monolayered tumor cells with entire nuclei without tissue section-associated truncating artifacts, thus yielding a more accurate score. Although formalin can be used for fixation, the best results are generally obtained with methanol/glacial acetic acid (Carnoy's solution) or acetone.
- The hybridized FISH specimens can be washed to remove the first set of FISH probes, followed by rehybridization with another set of FISH probes. If hybridization is performed within a few weeks after cytological preparation, greater than 95% the success rate can be achieved.

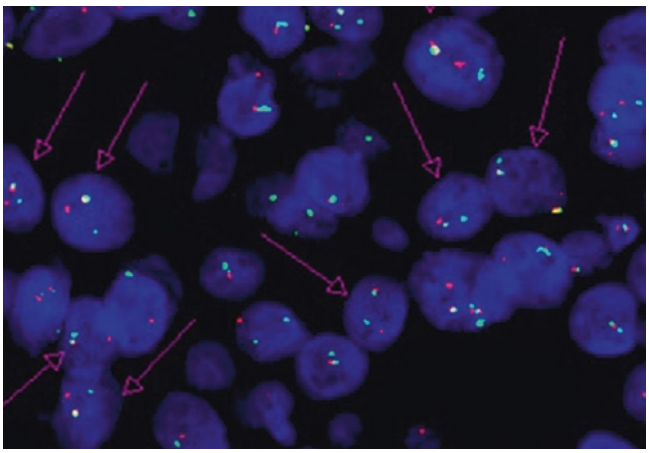

Fig. 1.3 Case 3. FISH study on the tumor cells within a cytospin shows t (11: 14) translocation

34. How many cells are required for FISH scoring?

There are many ways to score the FISH signals on a slide. In a research setting, the scoring of at least 100 or 200 consecutive cells is often considered as adequate. While in the standardized diagnostic analysis, the number of scored cells is usually lower. In the multitarget FISH assays, not more than 25 cells are required for scoring. It may be sufficient to score as few as 20 cells in amplification detection (e.g., HER2 FISH). The scoring should be performed on cells of interest such as malignant cells [48]. The current applications in cytology are scoring of HER2 gene amplification in breast cancer and aneusomy detection in urinary specimens in conjunction with UroVysion multitarget FISH testing.

35. What types of probes are used for FISH?

Two types of probes are used for FISH. The first type is locus-specific indicator (LSI), which hybridized to specific loci or genes of target. The LSI probe can detect translocation and rearrangement of genes or chromosomes. The abnormal signal patterns (e.g., fusion, split) in the nucleus can be visualized by using fluorescence microscope. The second type of probe is centromere enumeration probe (CEP). CEP can detect gain or loss of a specific gene region or chromosome. The numbers of copies of targeted chromosomes can be identified. FISH can detect the presence of microdeletions or duplications that are not apparent by conventional cytogenetic studies. Classical satellite, alpha-satellite centromeric, or beta-satellite centromeric probes are useful for the detection of copy number aberrations in interphase nuclei.

36. What is I-FISH?

Interphase FISH (I-FISH) allows assessment of chromosomal abnormalities in cells independent of their proliferative capacity. The I-FISH method is particularly useful for cytology specimens since it required only a few cells and it can facilitate a definitive diagnosis for the indeterminate cases due to scanty cellularity [49]. Cell block, direct smear (air-dried, alcohol-fixed, or archived), cytospin preparation, or cellular touch imprint are all suitable for I-FISH [50–53]. However, I-FISH is unable to identify "unexpected" chromosome abnormalities which are not designed.

37. What is the clinical significance of testing HER2 status?

Determining HER2 expression status is important for prognosis prediction and treatment decision in breast cancer. Overexpression of the HER2 protein (via immunostaining) or HER2 gene amplification (via FISH) is associated with a poor clinical outcome, and more importantly, is a prerequisite for anti-HER2 (e.g., trastuzumab/Herceptin) treatment. Dual-probe FISH is the preferred method over immunostaining [54]. HER2 status determined with FISH is more stable and reproducible and more strongly correlated with responsiveness to anti-HER2 therapy.

38. What are the differences between FISH, CISH, and SISH for HER2 test?

- Most of the HER2 gene tests are performed by immunohistochemistry (on cell block or tissue section) and FISH (on smears or cell block). However, there are some disadvantages of using FISH. Fluorescence microscope has to be used in the dark working area to visualize the cellular morphology, and the fluorescence signals fade with time.
- A new developed technique is called bright-field chromogenic in situ hybridization (CISH). CISH detects gene copy number by using a conventional peroxidase reaction and allows enumeration of the signals with simultaneous histologic examination under light-field microscopy. This technique is more straightforward than FISH for scoring. Signal counting is much easier than FISH because cellular morphology and histology are easier to be recognized by using counterstains in CISH. Although CISH has shown potential to replace the I-FISH technique in detection of HER2 gene amplification in tissue sections, its application in cytological specimens is still under investigation [48].
- Another technique is called silver in situ hybridization (SISH) which also uses bright-field technology. SISH can produce better contrasted signals than CISH.
- However, both CISH and SISH are required to have two separate slides, with one for HER2 counting and the other for chromosome 17 counting. In contrast, two-color FISH can count both on one slide. Two dual-color HER2 CISH/SISH assays have been developed recently [35, 42–48].

39. What is the principle of DNA microarray?

DNA microarray technology has emerged as a high-throughput approach for analysis of gene expression profiles and patterns. Thousands of oligonucleotides can be arrayed on a square centimeter chip, a solid supporting system. The mRNA is obtained from fresh tissue and serves as a template to create cDNA. Cancer cell cDNA is labeled with red fluorescence, while normal cell cDNA is labeled with green fluorescence. Labeled mRNA from the cells of interest are hybridized to their corresponding sequence on the array. Data is analyzed. The red signals indicate higher expression of the genes in cancer cells. While the green signals indicate higher expression of the genes in normal cells. The yellow signals indicate an equal expression of that gene in both normal and cancer cells. The matching genes are identified and the data analysis requires a computer analysis [55–57].

In contrast to the previous molecular methods, such as PCR, northern blot analysis, which can only study a specific gene expression level at a time, the microarray technology can study thousands of genes and a wide variety of tumor types which are contained in a single chip and detect which genes are overexpressed or active in cancer cell. This technique provides a new system for tumor classification and offers new information about which genes are involved in different forms of cancer and provides therapy-based molecular profiles.

40. What is the principle of polymerase chain reaction (PCR)? What are the applications of PCR in cytology?

Polymerase chain reaction, or PCR, is a molecular technique used to make multiple copies of DNA segment. The template DNA is heated to 94 °C, which separates the two strands of the double helix. When the temperature is lowered to 55 °C,

the primers anneal to the ends of the target sequence. Once the primers have annealed to the template, the temperature is increased to 72 °C. This is the optimum temperature for the Taq polymerase to elongate DNA using the primers as starting point. The newly synthesized DNA strands serve as template for the next PCR cycle. PCR is now often used in the cytology laboratory. The applications include identifying infectious organisms, detection of gene mutations in solid tumors, and diagnosis of lymphoma by B- and T-cell clonal gene rearrangement tests.

PCR-based methods have been successful in diagnosing viral infections in cytological samples, such as high-risk type of HPV infection and herpes virus infection in cervicovaginal cytological specimens. In *Chlamydia trachomatis* and *Neisseria gonorrhoeae* detection, PCR methods are also available for liquid-based gynecology cytological specimens. In addition, applications of PCR-based assays have been done on fine-needle aspiration specimen, such as identifying oncogene mutations or amplifications or activation, tumor suppressor gene deletions or mutations, and other genetic abnormalities, such as *BRAF* mutation in papillary thyroid carcinoma and unique translocations in sarcoma. Detecting gene mutations by PCR and other sequencing methods in a variety of cytopathologic samples has shown promise to increase the sensitivity in cytopathologic diagnoses. In addition, B- and T-cell clonality in malignant lymphomas can be identified by using DNA from fine-needle aspirates and other cytopathologic specimens.

41. How are the high-risk types of HPV tested by hybrid capture 2 (HC2) assay?

Hybrid capture 2 (HC2) assay is performed on cervical samples. Nucleic acids of samples are released and denatured, followed by HPV target DNA hybridization with a mixture of HPV-type-specific labeled RNA probes. The hybrids of DNA-RNA are captured on to the surface of a microtiter well coated by specific antibodies. Immobilized hybrids are then reacted with alkaline phosphatase-conjugated antibodies specific for RNA-DNA hybrids and detected with a chemiluminescent substrate. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted that is measured by a luminometer.

42. What is telecytology? What are the different types of digital cytology imaging?

Telepathology has been defined as "the practice of transmitting digital pathology images of microscopic or gross findings through telecommunication networks to remote viewing locations for diagnosis, storage or education." Telecytology is a branch of telepathology. Telecytology refers to "diagnostic cytopathology performed on digital images." Telecytology was first used with mainly cervical smears, and its application in cytology has been expanded [58, 59].

- There are three types of microscopic digital imaging:
  - The first is static (still images). The pathologist selects the areas, and the images are captured with a camera and are digitized. The image data is transmitted to a remote personnel through the Internet [60–62].
  - The second type is dynamic (real-time/live microscopy). The microscopic live images are directly transmitted to the recipient by live telecommunication. It finds application mainly in frozen section telepathology and rapid on-site evaluation for fine-needle aspiration [63–65].
  - The third type is virtual microscopy. The glass slides are scanned and converted to digital images. The specialized software allows simulation of panning around and zooming in or out using a conventional microscope. As whole slide imaging (WSI) technology has developed over the past years and it provides us with virtual microscopy that can be accessed anywhere in the world by using the Internet any time. WSI and virtual microscopy are increasingly being utilized in cytopathology.

43. What are the clinical applications of telecytology? What are the advantages and limitations of telecytology?

- Digital pathology has been applied to many fields, including remote frozen section diagnosis, rapid on-site evaluation (ROSE) of fine-needle aspiration, consultation, education, and slide archiving [66–72]. With the integration of LIS and the change of workflow, digital pathology may change the traditional practice of cytopathology in the future.
- The advantages of digital cytopathology are as follows:
  - In contrast to the tissue block in histology, every cytology slide is irreplaceable and unique. Implementation of digital cytology has a helpful aspect especially when the slides are damaged or defective. The permanent digital copy can be kept for archiving.
  - Digital data establishs a permanent reproducible record that can readily be communicated and displayed at variable magnifications. Digital imaging has been employed in evaluation of gynecologic material obtained by cytobrush technique (PAP tests) [73, 74] and nongynecologic material [75–77]. They are ideally suited for teleconsultation. Telecytology would diminish the need to physically send slides over long distances which could increase the chance of damaging the slides. Case consultation by transmitting images would reduce the time and cost.
  - Telepathology is an excellent teaching tool. Learning cytopathology with virtual microscopy is more efficient than from glass slides. A universal database with

digital cytology images of a variety of cases can be accessible to cytopathologists from everywhere. It can also be used in distance-based continuing education at teleconferences by using images accompanied by lectures, real-time microscopy sessions.
  - Telecytology has also been used as a tool for quality assessment and improvement in the evaluation of cytologic samples. It also finds application in cytology proficiency testing and various research purposes [78–80].
- The limitations of telecytology are as follows:
  - Cytology specimens offer unique challenges, particularly for direct smear preparations where three-dimensional groups are commonly encountered. Since cytological material tends to be distributed throughout the glass slides, intensive screening is required. Cytology relies on individual cell features evaluated at a higher magnification and good-quality images which telepathology may not fulfill. These factors become the barrier to the adoption of whole slide imaging for routine diagnostic cytopathology [81].

44. What are the circulating tumor cells and what is their clinical application?

The presence of circulating tumor cells in blood from metastatic carcinoma patients have been reported to be associated with short survival. Technical advances have facilitated the detection of rare circulating tumor cells. The CellSearch technique (Veridex) and CellSpotter assay systems were designed to detect rare tumor cells in whole blood. The principle of this system is based on the immunostaining method. The epithelial tumor cells are separated from the blood by antibody-coated magnetic beads and identified with the use of fluorescently labeled antibodies against cytokeratin. Then the epithelial tumor cells are enumerated. Circulating tumor cell detection for breast cancer patients have been reported to be an independent prognostic factor for early relapse [82–95].

45. What is next-generation sequencing (NGS)? What is the application of NGS in cytology?

Next-generation sequencing (NGS) is a DNA sequencing technology. Using NGS, an entire human genome (three billion base pair) can be sequenced within a short period of time. The NGS platforms perform sequencing of millions of small fragments of DNA in parallel. Bioinformatics analyses can piece together these fragments by mapping the individual reads to the human reference genome. Each of the three billion bases in the human genome is sequenced multiple times, providing high depth to deliver accurate data and an insight into unexpected DNA variation. NGS can be used to sequence entire genomes or constrained to specific areas of interest, including all 22,000 coding genes (a whole exome) or small numbers of individual genes.

Traditional Sanger sequencing is restricted to the discovery of substitutions, small insertions, and deletions. In contrast to Sanger sequencing, NGS captures a broader spectrum of mutations. The spectrum of variation comprises substitutions, insertions and deletions of DNA, large genomic deletions of exons or whole genes, and rearrangements such as inversions and translocations.

Rapid on-site evaluation (ROSE) for adequacy assessment is performed on the FNA materials to allow immediate triaging of materials for molecular testing such as NGS. The FNA sample tends to contain a purer population of tumor cells than tissue biopsy since cells are more easily aspirated than stromal components. In addition, there are better-quality nucleic acids from non-formalin-fixed direct smears. Therefore, aspiration cytology specimens provide an excellent source of cellular material for molecular studies. However, additional validation studies for a variety of cytology specimens, fixatives, and stains need to be performed on the cytology specimen for molecular study [96–105].

Case Presentations

Case 1

Case history

A 78-year-old female who has no prior history of malignancy and presented with abdominal discomfort. Abdominal CT showed a 12 × 8 cm necrotic mass in the right hepatic lobe in a background of cirrhosis and intrahepatic biliary dilatation and splenomegaly. The mass encases the right portal vein branches, and there is evidence of portal hypertension. The patient had elevated AST (49 IU/L; reference range: 15–46 IU/L) and Alkaline phosphatase (190 IU/L; reference range: 38–126 IU/L).

Specimen source

Ultrasound-guided fine-needle aspiration was performed. A Romanowsky-stained smear, a Papanicolaou-stained smear, and a cell block were made from the aspiration.

Cytologic findings

- Smears are cellular and contain well-preserved cells in loosely cohesive groups and singly dispersed forms. The tumor cells are polygonal with hepatoid appearance. The cells are uniform and have round to oval nuclei, coarsely granular chromatin, and small nucleoli. Cytoplasm is scant to abundant and appears granular. The neoplastic cells have a plasmacytoid appearance (Figs. 1.4 and 1.5).

- Cell block contains loosely cohesive neoplastic cells in a background of necrosis. The tumor cells have pleomorphic hyperchromatic nuclei, irregular nuclear membrane, prominent nucleoli, and pink cytoplasm. Apoptotic bodies are noted (Fig. 1.6).
- Needle core biopsy contains cohesive tumor cells forming glands. The tumor cells are round to polygonal shape with hepatoid appearance. The background shows desmoplastic stroma. Mitotic figures are seen (Fig. 1.7).

Differential diagnosis

- Cholangiocarcinoma
- Hepatocellular carcinoma
- Metastatic carcinoma

IHC and other ancillary studies

- Pan-CK positive
- CK-7 positive (Fig. 1.1)
- CK20 negative
- CDX-2 negative
- HepPar1, arginase, and glypican3 negative (Fig. 1.8)

Final diagnosis

- **Poorly differentiated adenocarcinoma**

Take-home messages

- Clinical and radiologic impression was hepatocellular carcinoma (HCC) versus cholangiocarcinoma; nonetheless, HCC is favored based on cytologic features.
- In well-differentiated hepatocellular carcinoma (HCC), the thickened cords of neoplastic hepatocytes are often surrounded by spindle-shaped endothelial cells. However, poorly differentiated HCC is difficult to distinguish from cholangiocarcinoma and metastatic carcinoma by cytomorphological features alone. In addition, there are rare cases of combined hepatocellular-cholangiocarcinoma (cHCC-CC) that present a special diagnostic challenge. Immunohistochemical studies are helpful in the challenging cases by using cytological material such as cell block or smears.
- Subsequent immunostains performed on cell block sections show tumor cells to be positive for PanCK and CK7 and negative for HepPar1, arginase, glypican3, CK20, and CDX2. Since the immunohistochemical profile was not specific for any primary origin, a differential diagnosis of poorly differentiated adenocarcinoma from the pancreaticobiliary tree, including primary intrahepatic cholangiocarcinoma, is rendered.
- This case demonstrates a well-established truth. Sometimes morphology and clinical findings are more useful than magic markers. This is the kind of case that is all too common and frustrates clinicians who want a definitive answer and pathologists who know that many times only a differential diagnosis can be rendered based on clinical judgement and morphology.

Reference: [105]

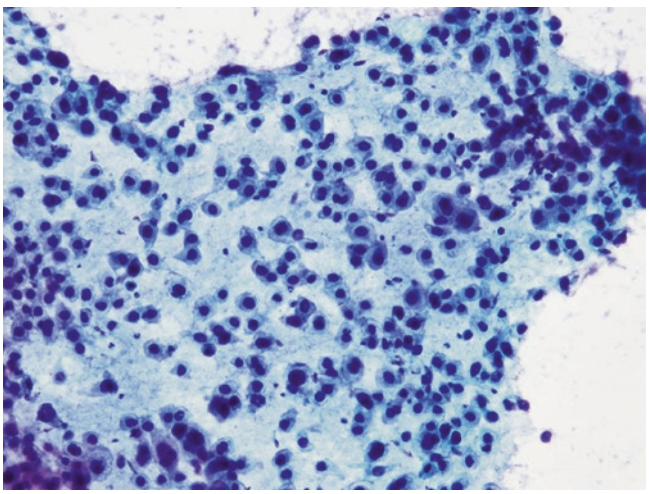

Fig. 1.4 Case 1. Cellular smears show well-preserved cells in loosely cohesive groups and singly dispersed forms. The tumor cells are polygonal with hepatoid appearance. The cells are uniform and have round to oval nuclei, coarsely granular chromatin, and small nucleoli. Cytoplasm is scant to abundant and appears granular. The isolated cells have plasmacytoid appearance (Papanicolaou stain)

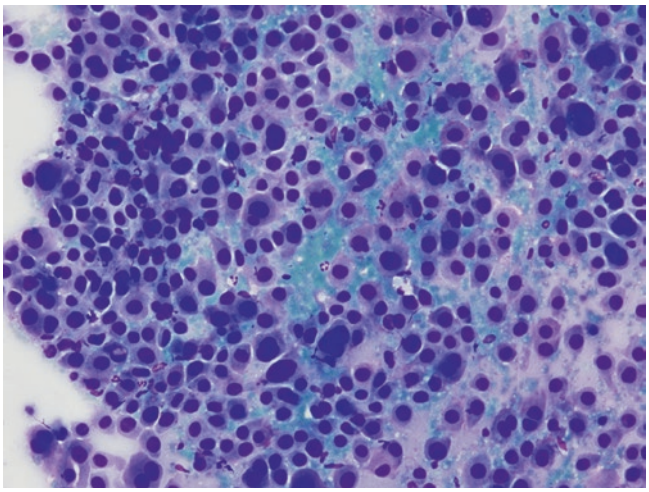

Fig. 1.5 Case 1. Cellular smears show well-preserved cells in loosely cohesive groups and singly dispersed forms. The tumor cells are relatively uniform round to polygonal nuclei and hepatoid appearance, coarsely granular chromatin, and small nucleoli. Cytoplasm is scant to abundant and appears granular. The isolated cells have a plasmacytoid appearance (DQ stain)

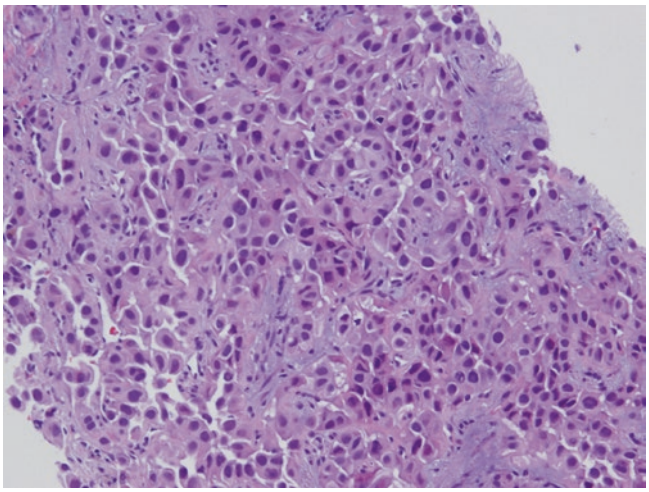

Fig. 1.7 Case 1. Needle core biopsy shows cohesive tumor cells forming glands. The tumor cells are round to polygonal shape with hepatoid appearance. The background shows desmoplastic stroma. Mitotic figures are seen

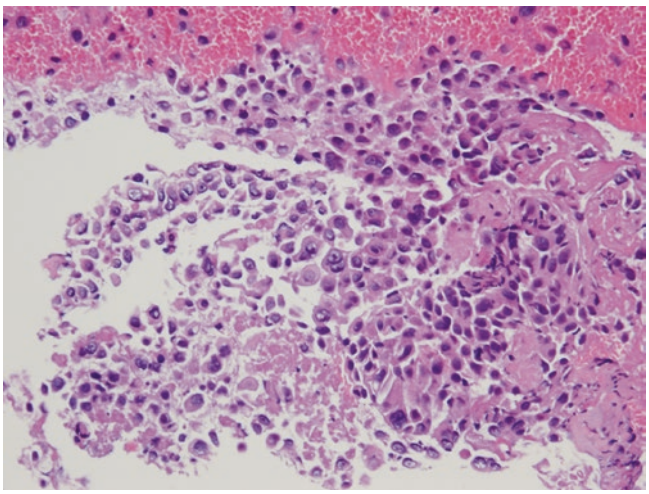

Fig. 1.6 Case 1. Cell block shows loosely cohesive neoplastic cells in a background of necrosis. The tumor cells show pleomorphic hyperchromatic nuclei, irregular nuclear membrane, prominent nucleoli, and pink cytoplasm. Apoptotic bodies are noted

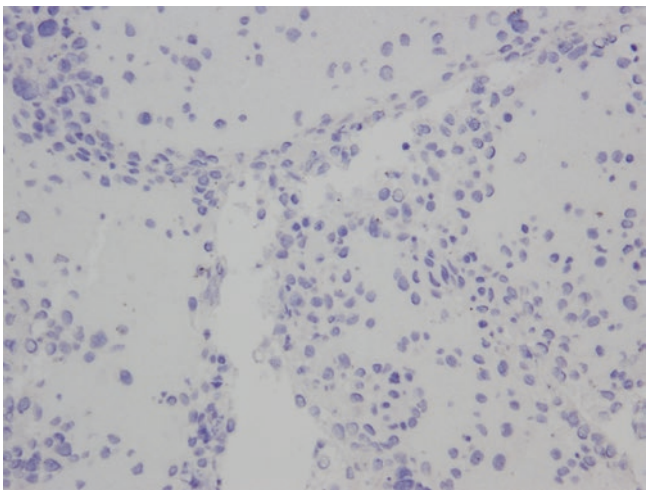

Fig. 1.8 Case 1. Tumor cells are negative for HepPar1 by immunohistochemical stain

Case 2

Case history

- A 71-year-old female had a history of melanoma of the vagina two years ago. Ultrasound showed an enlarged inguinal lymph node (1.8 cm).

Specimen source

Ultrasound-guided fine-needle aspiration was performed. A Romanowsky-stained smear and a Papanicolaou-stained smear were made from the aspiration.

Cytological findings

Pap-stained smears showed scattered slightly large cells present in a lymphoid tissue background. The atypical cells were scant and the degree of atypia was so subtle that they could be easily overlooked (Fig. 1.9).

Differential diagnosis

- Lymphoid proliferative disorder
- Metastatic carcinoma
- Metastatic melanoma

IHC and other ancillary studies

- Since cell block was not available, immunostaining for SOX10 was performed on a Pap-stained smear and the tumor cells were highlighted (Fig. 1.2).

Final diagnosis

- **Metastatic melanoma**

Take-home messages

- Clinical and radiologic impression was metastatic melanoma.
- Few atypical cells may be overlooked in a background of abundant lymphocytes.

Immunohistochemical study helps not only to highlight easily missed neoplastic cells but also to confirm the clinical suspicion of metastatic melanoma.

Reference: [106]

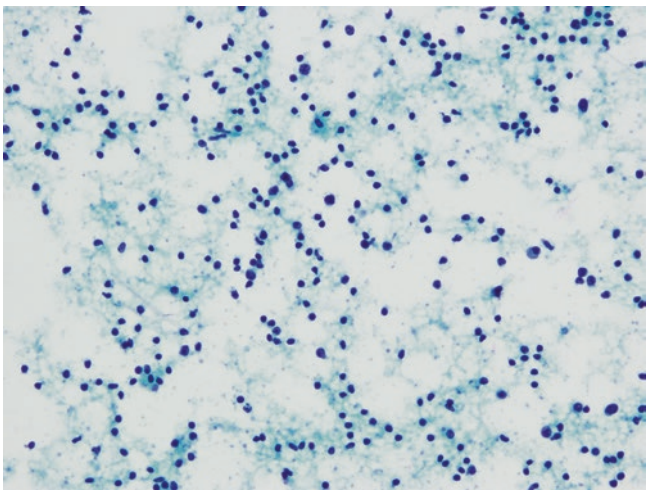

Fig. 1.9 Case 2. Smears showed lymphoid tissue with scattered slightly large cells. The atypical cells were so few and the degree of atypia was so subtle that they could be easily overlooked (Papanicolaou stain)

Case 3

Case history

A 77-year-old male with a history of mantle cell lymphoma two years ago was found to have a 1 cm new nodule in the soft tissue of the chest wall.

Specimen source

Fine-needle aspiration was performed. A Romanowsky-stained smear and a Papanicolaou-stained smear were made from the aspiration.

Cytologic findings

Smears contained a monotonous small- to medium-sized lymphoid population.

Differential diagnosis

- Lymphoma
- Soft tissue tumor

IHC and other ancillary studies

Flow cytometric immunophenotyping demonstrated a monotypic B-cell population with kappa light chain restriction (Fig. 1.10).

Immunohistochemical studies were performed. These cells were positive for CD19, CD20, CD22, CD79b, and CD5, while negative for CD10, CD11c, and CD30. SOX11 staining, which is a biomarker for

cyclin D1-negative mantle cell lymphoma, was positive on a Pap-stained cytospin smear (Fig. 1.11).

FISH study shows t (11: 14) translocation (Fig. 1.3).

Final diagnosis

- **Mantle cell lymphoma**

Take-home messages

Ancillary studies such as flow cytometry, immunohistochemical stains, and FISH help to establish the diagnosis.

Reference: [107]

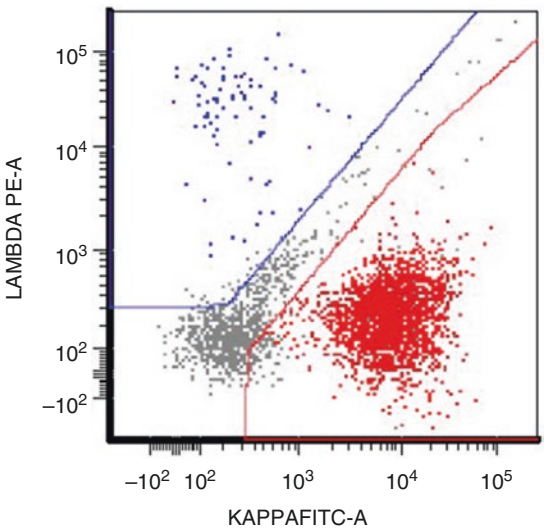

Fig. 1.10 Case 3. Flow cytometric immunophenotyping demonstrated a monotypic B-cell population with kappa light chain restriction

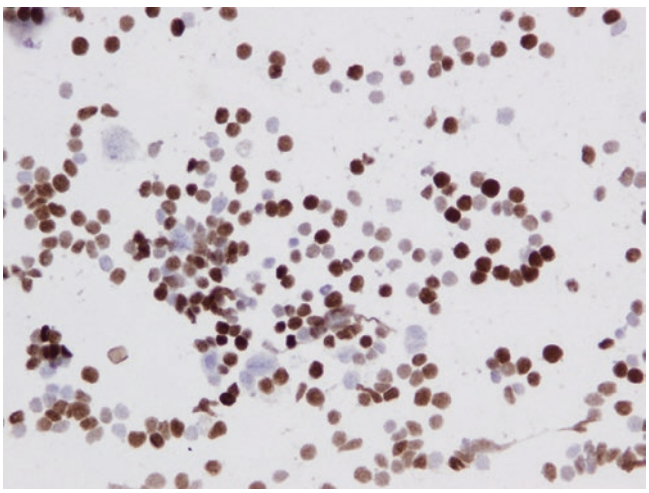

Fig. 1.11 Case 3. Immunohistochemical stain for Sox11 performed on the tumor cells within a Pap-stained cytospin was positive

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Cytopathology Laboratory Management

2

Theresa Castle

Contents

List of Frequently Asked Questions

1. What agencies are responsible for regulation and accreditation of cytopathology laboratories?
Cytopathology is one of the most highly regulated laboratories in the United States. It is regulated by the Clinical Laboratory Improvement Amendments of 1988 (CLIA 88) and the Health Insurance Portability and Accountability Act of 1996 (HIPPA). Cytopathology is rated as high complexity testing by the Food and Drug Administration (FDA). A CLIA certificate is required to receive payments from the Centers for Medicare and Medicaid Services (CMS). An on-site laboratory inspection from either The Joint Commission (TJC) or the College of American Pathologists is required to be issued a CLIA certificate, along with state licensure. TJC performs on-site surveys of hospitals every 3 years or laboratories every 2 years. The College of American Pathologists (CAP) Laboratory Accreditation Program surveys clinical laboratories on-site every 2 years, and a self-inspection is required between surveys. CAP checklists for Cytopathology, All Common, and Lab General are updated periodically and are available on the CAP website [1–7].

2. What are the staffing requirements for a cytopathology laboratory?
Adequate staffing of cytopathology lab is defined by CLIA 88. An adequately staffed lab includes a laboratory director who is responsible for the operation and administration of the laboratory. He or she must be licensed to practice medicine, be an anatomic pathologist or employ an anatomic pathologist as a technical supervisor, and have a license as a laboratory director by the state where the lab is located. Adequate staffing also requires a technical supervisor (TS), a general supervisor (GS), and cytotechnologists (CT). The TS is defined as a section director who is a qualified pathologist with a degree, MD or DO, board certification in cytopathology and/or surgical pathology, current license, and work history in related field (CYP.07700 21). The TS may sometimes also serve as the GS. The GS is a qualified CT with a BS or higher degree; CT certification; at least 3 years full-time experience in the past 10 years; state license, if required; and work history. CTs who are nonsupervisory personnel must be graduates of an accredited program of cytotechnology and certified in cytotechnology by a Health and Human Services approved agency such as the American College of Clinical Pathology (ASCP). To be eligible to register for the CT Board of Certification exam, ASCP requires a BS or BA degree or higher from an accredited college or university and successful completion of a Commission of Accreditation of Allied Health Education Programs accredited cytotechnology program within 5 years (ASCP Procedures Booklet, 10, ASCP.org).

3. What credentials are required of cytotechnologists?
Credentialing has become increasingly important in recent years. The cytology supervisor in conjunction with human resources is responsible for ensuring that potential new hires

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_2

provide documentation including diplomas, official transcripts, cytotechnologist certification, ThinPrep certification, BD SurePath certification, and Imaging System certifications. A state license is required in several states including California, Florida, and New York. At least two professional references are required for new hires. In addition, current PAP proficiency testing and documentation of continuing education at previous laboratory of employment are frequently required. Many employers require a slide test as part of the interview process.

4. What are the responsibilities of the section director/ technical supervisor?

The technical supervisor (TS) has a current license to practice medicine, is board certified in anatomic pathology or cytopathology, and has work experience in a related field. The TS is responsible for administrative and technical operations of the lab, as well as compliance with all regulatory agencies. Academic responsibilities of the TS include teaching and mentoring pathology residents, cytopathology fellows, medical students, and cytotechnology students. Operational responsibilities include:

- Selection of test methodology
- Establishment and verification of laboratory test performance specifications
- Enrollment and participation in proficiency testing
- Establishment of a quality control program to monitor ongoing test performance
- Resolution of technical problems and ensuring that remedial actions are taken
- Ensuring that patient test results are not reported until corrective actions are taken and test systems are functioning properly
- Reviewing statistical data
- Overseeing competency of personnel
- Performing semiannual workload limits for cytotechnologists

5. What are the responsibilities of the general supervisor?

The responsibilities of the general supervisor (GS) of the cytopathology lab include but are not limited to the day-to-day oversight of operations and personnel. Specially, the responsibilities include to ensure that the lab is adequately staffed and work with human resources to recruit, interview, and on-board new employees, to train employees and assess competencies twice in the first year of employment and annually thereafter, to conduct annual performance evaluations of personnel, and to coach and counsel employees and develop performance improvement plans as needed.

The GS should be accessible to provide on-site, telephone, or electronic consultation to resolve problems in accordance with procedures established by the technical supervisor (TS). He or she is involved in preview of Gyn and non-Gyn slides for quality assurance. The GS may need to assist in fine-needle aspiration (FNA) on-site adequacy evaluations and the preparation of smears and cytopreparation when required.

The GS is involved in quality control activities such as 10% prospective rescreen of gynecological pap tests, retrospective 5 years look back for high-grade squamous intraepithelial lesions, cytology-histologic correlation, and review of workload records. GS is also expected to assist the TS in proper participation, proctoring, and conduct of proficiency testing including distribution of slides, submitting results, and retaining documentation in compliance with the College of American Pathologists. GS also assists the TS in performance evaluation of the cytotechnologists using parameters detailed in the procedure manual and in cytotechnologist workload assessment. GS is responsible for maintaining the safety regulations and requirements of the lab as per the Occupational Safety and Health Administration and hospital or company policies. GS is involved in teaching and developing continuing education activities for cytotechnologists. Finally, GS is responsible for maintaining professional relationship among all colleagues in the lab and informing the TS in case of any leave of absence.

6. What workload limits and records are required?

CLIA 88 regulations set workload limits for cytotechnologists (CTs) working in the United States as a maximum number of slides at 100 in a 24-hour period, with a maximum of 12.5 slides/hour. States can establish lower workload limits, for example, California sets workload limits at 80 slides in a 24-hour period. Workload records are required of primary screeners of gynecologic and non-gynecologic slides. Primary screeners are CTs or the supervisor and in some instances may include pathologists who are primary screener in labs without CTs. Primary screening can be best described as having locator skills and diagnostic skills. Locator skills involve finding and marking diagnostic cells on the slide. Diagnostic accuracy is measured by the overlap between the CT interpretation and the pathologist's final diagnosis. The CT is responsible for recording the slide interpretation or diagnosis of each case examined, the total number of slides examined in all laboratories in each 24-hour period, and the number of hours spent screening slides in each 24-hour period. A CT who works at more than one lab in a 24-hour period is responsible for recording and reporting the number of hours and number of slides screened at all labs. Non-screening activities, such as participating in continuing education activities, assisting on fine-needle aspiration procedures, or preparing specimens, reduce the number of screening hours in an 8-hour day. To prorate maximum screening limits, use the formula:

$$\text{Number of hours screening slides} \times 100 / 8 = \text{maximum number of slides}$$

If a CT exceeds his or her screening limits, corrective action is necessary, including reeducation and retrospective rescreen if the cases on the day's workload limits were exceeded. If a variance is found on rescreening, the false-negative fraction is calculated to determine if a reduction of workload limits is required.

CTs are also responsible for recording the final diagnosis from the pathologist for gynecologic and non-gynecologic cases. Major and minor discrepancies are recorded. Major discrepancies are two or more variance between the CT diagnosis and the pathologist's final diagnosis. The CT's discrepant diagnosis is documented along with corrective action(s), for example, review of the case with the pathologist, calculation of false-negative fraction in the event of a two-step variance in Gyn cases, which may result in reduced workload limits.

7. What annual proficiency testing (PT) is required?

All cytopathology laboratories in the United States are subject to CLIA regulations. If the laboratory performs gynecological cytopathology, all personnel who examine gynecological preparations are required to participate in annual gynecologic peer interlaboratory comparison program. The CAP Gynecological Cytology Proficiency Test Program (PAP PT) and the ASCP Gyn PT are both approved by CMS. PT must be proctored by CAP- or ASCP-authorized proctors. A passing score is 90% on a ten-slide test. An individual who fails PT is required to retest on a second ten-slide test within 45 days of notification of failure. Failure of the second 10-slide test requires progressive documentation of remedial training and retesting on a 20-slide PT. If the 20-slide test is failed, 35 hours of remedial training and retesting on a second 20-slide PT will be administered until a passing score of 90% is achieved. The lab must maintain records of PT performance of all individuals, including retesting and remedial education for 2 years. CAP and CMS must be notified of leaving personnel who take PT before the next testing cycle and of new personnel who take PT, so that the testing roster is up to date.

8. What continuing education activities are required for cytotechnologists and technical staff?

Cytotechnologists (CT) who passed the CT Board of Certification exam in 2004 and in subsequent years are required by the American Society of Clinical Pathologists (ASCP) to participate in the Certification Maintenance program. They must submit evidence of 36 hours of continuing education (CE) every 3 years (ASCP website, CM). It is the best practice to ensure that all CTs participate in at least 12 hours of CE annually. Participation in CAP PAP PT includes two educational interlaboratory comparison program slide sets, PAPME, which provides up to 8 CE credits. Participation in the CAP interlaboratory comparison program in the four mailings of non-gynecological cytopathology slide sets, NGC, provides up to 20 CE credits annually.

A robust educational program can include journal reviews, participation in teleconferences, webinars, vendor educational sessions, microscopic review sessions, and annual safety training. Membership in regional cytology organizations and attendance at annual conferences by the ASCP and American Society of Cytopathology (ASC), as well as the Advanced Cytopathology Education (ACE) program offered jointly by ASCP and ASC, provide opportunities to actively learn during lectures and microscopic sessions, network with other cytotechnologists, exchange ideas about policies and procedures, and earn several hours of CE [5].

9. What should be included in the policies and procedures of a cytopathology lab?

There are two types of procedure manuals:

- *The Specimen Collection Manual* is a guide for preparation of patients, specimen collection, labeling, preservation, and transporting cytology specimens during and after cytopathology laboratory hours. It is made available to clinicians for collection of gynecologic and non-gynecologic specimens [6, 7].
- *The Policy and Procedure Manual* must include procedures for preanalytical, analytical, and postanalytical processes. There must be instructions for collection and receiving specimens in the lab, criteria for specimen rejection, how to handle irretrievable specimens, and documentation of notification of clinical team members in the event that a specimen is rejected or needs additional documentation. There must be a policy and procedures for preventing cross-contamination during specimen preparation and staining, especially highly cellular malignant specimens. Fine-needle aspiration smears may be stained for rapid evaluation and a preliminary diagnosis will allow positive cases to be separated from routine staining. Monolayer preparation of body fluids is an additional method of preventing cross-contamination. Detailed instructions for various specimen preparations, including different methodologies must be included in the manual, along with policies for daily quality control (QC) of stains and slide preparations [5] (Tables 2.1, 2.2, and 2.3).

Table 2.1 Preanalytical phase

Specimen collection, ordering, and receiving
Specimen rejection criteria and notification
Accessioning in laboratory information system (LIS)
Specimen preparation and slide staining
Cross-contamination prevention
QC stains and preparation
QC instruments and hoods
Environmental monitoring

Table 2.2 Analytical phase

Personnel requirements
Cytology workload
Individual maximum workload evaluation and competencies
PT and education

Table 2.3 Postanalytical phase

Records and reports of required elements
Records retention
Amended reports
Notification of significant and unexpected findings
Statistical records
Prospective 10% rescreen on negative gynecologic cases
Retrospective review of new HSIL or above
Cytologic-histologic correlation of Gyn and non-Gyn cases
Disparity resolution
Follow-up of new HSIL cases without histologic confirmation
Intra- and extra-departmental consultations
Safety-hazardous waste
Safety-formaldehyde and xylene exposure

10. Why must procedures reflect policies?

Procedures that are performed in the cytopathology lab should match what the written policies state. Procedures cannot be changed without validation studies. It may seem like a good idea to change, for example, the process in which cell blocks are prepared, but without validation studies and a change to the written policy, the lab is at jeopardy for being cited during an inspection.

11. What is document control and why is it important?

Document control is a method of safeguarding policies, procedures, and other documents that are related to laboratory testing. Document control ensures that only current policies, procedures, and forms are in place and has records of approval, review, and removal to archives. Electronic storage of current policies and procedures eliminates the need for hard copies of documents and safeguards the possibility that outdated documents are circulating in the lab. Permission to access policies is based on position. Technologists and lab staff are given permission to read and sign off on policies. Lab managers and supervisors are given permission to edit and upload new policies. Lab directors are given permission to edit, upload, and approve policies. Approvers and personnel are notified by e-mail of documents requiring review electronically [5, 7].

12. What is the workflow in a cytopathology lab?

Workflow involves preanalytical, analytical, and postanalytical phases. In the preanalytical phase, tests are ordered by a physician, a requisition is generated electronically or manually, the specimen is collected, and the specimen is transported to the lab with the requisition. In the lab, the specimen is received, accessioned, and processed into slides. The analytical phase is when the cytotechnologist and cytopathologist microscopically examine slides and enter a diagnosis in the laboratory information system, to generate a report. The postanalytical phase is when quality control is performed on staining and cytopreparation modalities and 10% prospective rescreening of negative gynecological tests. The final stage is record retention including filing and storage of reports, slides, and cell blocks. CAP requires retention of paper or electronic cytopathology reports for 10 years. Fine-needle aspiration glass slides must be retained for 10 years; all other non-gynecological and gynecological glass slides must be retained for a minimum of 5 years. Glass slides should be stored at room temperature for optimal preservation. Cell blocks must be retained for 10 years in a climate-controlled environment [5, 7].

13. What are the required elements in a cytopathology requisition?

The cytopathology requisition can be electronic or manual. It must include the name of the patient, the date of birth, and a unique identifying number, for example, a medical record number. The date of collection, the name of the ordering physician, and the anatomic source of the specimen are also required. Patient history is helpful, especially if there is a history or suspicion of malignancy, neoplasia, or dysplasia (Table 2.4).

14. What are the required elements in a final cytopathology diagnostic report?

- Name of patient and unique identifying number
- Date of birth of patient
- Date of collection
- Accession number
- Name of ordering physician or clinic
- Name of reviewing pathologist, when applicable
- Name and address of laboratory location where test was performed
- Date of report
- Test performed
- Anatomic source or type of specimen
- Cytopathological diagnosis and possible note
- Reporting of additional IHC stains, molecular and/or ancillary studies
- Basis for amendment, if applicable
- Pap disclaimer on Gyn reports [5]

Table 2.4 Requisition requirements

Name of patient and unique identifying number
Date of birth of patient
Date of collection
Ordering physician or clinic
Anatomic source and type of specimen
Requests for additional studies or stains

15. What is quality control in a cytopathology lab?

CLIA 88 Final Rule established quality control (QC) standards for gynecologic cytology. A minimum of 10% prospective rescreen of negative pap tests on randomly selected and targeted high-risk cases must be performed by a senior-level cytotechnologist, a supervisory cytotechnologist, or a cytopathologist. The method of randomly selecting QC slides must be in the policy as well as the criteria of defining high risk. In labs with high-volume gynecological cytology, best practice is to have the laboratory information system to automatically pull QC slides. Documentation must be maintained for 5 years for retrospective rescreen of negative PAP tests on newly diagnosed high-grade squamous intraepithelial lesion (HSIL) and in situ and invasive malignancy. Records of cytologic-histologic correlation of HSIL and above cases must also be kept for 5 years. Disparity between cytology and histology must be resolved by the technical supervisor. Records of notification to clinicians in the absence of follow-up biopsy on HSIL cases must be maintained and periodically reviewed.

Environmental monitoring of temperature and humidity is recorded daily in rooms where patient testing instruments and specimen storage equiptment are located. Records of room temperature, room humidity, and refrigerator and freezer temperatures are read on min/max National Institute of Standards and Technology (NIST)-certified thermometers. Min/max temperatures must be recorded during days when the department is closed. Corrective action for out of range values must be documented and reviewed by the general supervisor [7–9].

Records of daily function and maintenance on instruments, centrifuges, and hoods must also be maintained [7–9].

16. What data, reports, and statistics are required in a cytopathology lab?

Statistical records for non-gynecological cases should be reported separately from Gyn cases and reviewed at least annually by the laboratory director or designee. Statistical records for non-Gyn cases are listed as following:

1. The number of cases
2. Type/sources of specimens
3. Diagnostic categories:
   (a) Negative for malignancy
   (b) Atypical cytology
   (c) Suspicious for malignancy
   (d) Positive for malignancy
4. Records of cytology-histology correlation
5. Disparity resolution of significant discrepancies in cytology-histology correlation [6, 7]

For Gyn cytopathology cases, statistical records are maintained of the number of cases of the following cytopathology results:

1. Diagnostic category (including unsatisfactory cases), by preparation type
2. Significant cytologic/histologic discrepancies (as defined by laboratory policy)
3. Total number of negative cases rescreened before sign-out
4. Cases for which the rescreen resulted in reclassification as premalignant or malignant
5. Cases for which histopathology results are available to compare with malignant or high-grade squamous intraepithelial lesion (HSIL) cytopathology results
6. Records of the number of high-risk human papillomavirus (HR-HPV) reflex tests performed on ASC-US cases and the number of positive HR-HPV ASC-US cases
7. Records of ASC-US/SIL rates [5, 10]

Annual review of the cytology lab gynecological data in all modalities against College of American Pathologists (CAP) benchmark data is part of the statistical record. Outliers to the 5th and 95th percentile ranges, including ASC-US/SIL ratio, should be reported, along with an explanation. The patient population should be taken into consideration, as well as the number of cases annually. CAP suggests a minimum annual test volume of >300 cases for ThinPrep and SurePath preparations for comparison of lab data to the CAP 2013 benchmark data. Additionally, CAP suggests a minimum test volume of conventional pap smears >180 to compare cytology lab data to CAP 2013 benchmark data (Tables 2.5, 2.6, and 2.7).

17. What are quality management monitors in a cytopathology lab?

Quality management (QM) monitors are processes that are measured and reported in the pathology laboratory QM plan

Table 2.5 Conventional laboratory percentile-reporting rate

Category	5th	10th	25th	Median	75th	90th	95th
Unsatisfactory %	0.0	0.1	0.5	1.2	2.1	3.5	4.7
LSIL %	0.0	0.2	0.5	1.0	1.7	3.1	4.3
HSIL %	0.0	0.0	0.1	0.2	0.4	0.7	0.9
ASC-US %	0.2	0.5	1.4	2.6	4.8	6.9	8.8
ASC-H %	0.0	0.0	0.0	0.2	0.3	0.5	0.9
AGC %	0.0	0.0	0.0	0.1	0.3	0.5	0.8
ASC/SIL	0.4	0.6	1.2	2.0	3.2	5.3	6.5

Table 2.6 ThinPrep laboratory percentile-reporting rate

Category	5th	10th	25th	Median	75th	90th	95th
Unsatisfactory %	0.3	0.4	0.8	1.3	2.1	3.4	4.3
LSIL %	1.1	1.4	2.0	2.7	3.6	4.7	5.5
HSIL %	0.1	0.2	0.3	0.4	0.7	1.1	1.4
ASC-US %	2.1	2.7	3.9	5.4	7.5	10.3	12.5
ASC-H %	0.0	0.1	0.2	0.3	0.5	0.8	1.0
AGC %	0.0	0.0	0.1	0.2	0.3	0.5	0.9
ASC/SIL	0.8	0.9	1.4	1.8	2.5	3.2	3.8

Table 2.7 SurePath laboratory percentile-reporting rate

Category	5th	10th	25th	Median	75th	90th	95th
Unsatisfactory %	0.0	0.1	0.1	0.3	0.5	0.7	1.0
LSIL %	1.1	1.4	2.0	2.8	3.5	4.7	6.2
HSIL %	0.1	0.1	0.2	0.4	0.6	0.9	1.2
ASC-US %	1.9	2.4	3.7	5.0	6.9	9.2	11.5
ASC-H %	0.0	0.1	0.1	0.3	0.4	0.6	0.9
AGC %	0.0	0.1	0.1	0.2	0.3	0.6	0.7
ASC/SIL	0.7	0.9	1.3	1.7	2.2	2.7	3.3

From [5]

and reviewed at monthly performance improvement (PI) meetings. QM monitors are selected to improve processes and procedures and ensure best practices. Periodic and annual review of the dashboard will outline which departments are meeting their monitors and which need improvement. Examples of monitors include the number of amended cases in cytology and turnaround time (TAT) of non-gynecological cases. New procedures can be monitored to ensure quality requirements are being met. For example, a new procedure for the TAT of STAT bronchoalveolar lavage (BAL) samples states that TAT is 4 hours during operating hours. All non-STAT BAL will have a TAT of 24 hours. This can be accomplished by populating a spreadsheet with the data from the laboratory information system, including when the specimen was received in the lab and when the results were reported out. Additional information can be recorded, such as documentation of notification of the clinician in the event of a significant and unexpected finding such as positive for pneumocystis or malignancy. Outliers to TAT should be addressed at pathology performance improvement meetings and recorded in the minutes and QM dashboard.

18. What are the safety requirements in a cytopathology lab?

Cytopathology lab personnel are exposed to numerous potential hazards in the workday including biological, chemical, ergonomic, and physical hazards. There must be a safety policy and procedure manual approved by the laboratory director and documentation of employee review and training [7, 8, 11–13].

Federal regulatory standards are set by the Occupational Safety and Health Administration (OSHA) and the Department of Transportation (DOT). Other guidelines and standards for safety in the laboratory are set by the Centers for Disease Control/National Institutes of Health (CDC/NIH), The Joint Commission (TJC), and state and local agencies.

Requirements include a chemical hygiene plan (CHP) to protect laboratory workers from harm due to hazardous chemicals. The CHP is a written program stating the policies, procedures, and responsibilities for the proper storage of chemicals and an annual evaluation of chemical inventory. A supervisory-level technologist in the clinical laboratory is assigned to the role of chemical hygiene officer. All reagents used must be included in the inventory and evaluated for carcinogenic potential, reproductive toxicity, and acute toxicity [8, 9, 11–13]. Other required safety documents include:

- Spill management for biological and chemical spills
- Postexposure accident reporting and risk management investigation
- Waste management of biohazardous substances including body fluids and sharps
- OSHA's Bloodborne Pathogens Standard
- Availability and use of personal protective equipment (PPE)
- Annual vapor monitoring of personnel and areas for short- and long-term exposure to formaldehyde, xylene, and alcohols
- Fire safety and training
- Packing and shipping of dangerous goods
- Emergency preparedness including active shooter and bioterrorism training

19. What billing codes are used for fine-needle aspiration rapid on-site evaluation?

The billing codes used for non-gynecologic cytology are current procedural terminology (CPT) 5-digit codes for laboratory technical and physician professional services provided by the cytology lab. CPT codes for fine-needle aspiration (FNA) rapid on-site evaluation (ROSE) vary depending on the individual performing the FNA, whether the FNA is performed with or without image guidance, if additional stains are used for ROSE, and who is performing the on-site evaluation.

When the FNA is performed by a radiologist or clinician and the pathologist performs ROSE, every "evaluation episode" must be documented during the FNA procedure. The first evaluation episode by the pathologist is coded 88172. Each additional evaluation episode is coded 88177. If the pathologist is performing the FNA without imaging guidance, the CPT code is 10021. If the pathologist is performing the FNA with imaging guidance, for example, ultrasound, the CPT code is 10022. The code 76942 can be billed for use of ultrasound for needle placement. Additional billing includes 88312 for special stains of microorganisms and 88305 for cell block processing. Immunohistochemical stains are billed as necessary. The code for FNA interpretation and final cytopathology report is 88173, but should not be billed if the specimen is acellular.

If an FNA specimen is collected without ROSE, direct smears CPT 88104 can be received in the cytology lab. Liquid-based FNA collection concentration technique—cytospin, CPT 88108, or selective cell enhancement—and CPT 88112 for ThinPrep or SurePath slides can be prepared;

Table 2.8 CPT billing codes for fine-needle aspiration procedures

88172	FNA adequacy assessment by first episode by a pathologist
88177	FNA adequacy assessment for each additional episode by a pathologist
88173	FNA interpretation and report
10021	FNA performed by a pathologist without image guidance
10022	FNA performed by a pathologist with image guidance
76942	Use of ultrasound for needle placement
88305	Level IV surgical pathology gross and microscopic (cell block)
88312	Special stains for microorganisms
88313	Other histochemical stains
88342	Immunohistochemical stains, first stain (qualitative)
88341	Immunohistochemical stains, each additional stain (qualitative)
88630	Immunohistochemical stains (quantitative)
88104	Direct smear
88108	Concentration technique (cytospin)
88112	Selective cell enhancement for non-gynecologic specimens (ThinPrep, SurePath)

however, 88108 cannot be billed together with 88112. Also, 88112 selective cell enhancement cannot be billed with 88173 FNA interpretation and report [3, 10] (Table 2.8).

20. What are the new regulations in digital cytopathology?

Digital cytopathology is utilized in the real-time transmittal of digitalized or analog video to a cytopathologist at an off-site location for the rapid evaluation of FNA specimens for specimen adequacy. Digital cytopathology is also utilized to transmit images in consultation with off-site pathologists. There is no retention requirement for digital images if FNA glass slides are retained for 10 years.

Regulations by CAP include:

- Validation of telepathology systems before being used for clinical diagnostic purposes.
- Validation emulates real-world clinical environment and involves specimen preparation types and clinical settings relevant to intended use.
- Telepathology training required for all users of the system. Training is role specific.
- Policies and procedures for telepathology training and documentation of training.
- Procedures for patient confidentiality—HIPPA compliance, such as encryption, user authentication, and access restriction, especially when using mobile devices.
- Correct patient identification of slides/images communicated to reviewer verbally or by imaging of slide identifier.
- Access to pertinent clinical information at the time of slide/image review.
- Telepathology result records include statements of adequacy assessment, preliminary diagnosis, or recommendations for additional studies at the time of evaluation.
- Include telepathology services in the pathology laboratory's quality management program. The lab could monitor comparison of on-site evaluation to digital cytopathology [7, 14, 15].

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Overview of Cervical and Anal Cytopathology

3

Xinmin Zhang and Kathriel Brister

Contents

List of Frequently Asked Questions

1. What is Pap smear and what is its history?

Uterine cervical cytology, also known as Pap smear, Pap test, or cervical smear, is a screening test to detect precancerous lesions and cancers of uterine cervix, primarily for squamous precancerous lesions and carcinomas. The test was invented by and so named after Dr. Georgios Papanicolaou (May 13, 1883, to February 19, 1962), a Greek pioneer in cytopathology and early cancer detection. Dr. Papanicolaou came to the United States in 1913 to study cellular changes over the course of menstrual cycle at the Department of Anatomy of Cornell Medical College in New York. Part of his study included observing the cellular morphology of vaginal fluid in women where he discovered abnormal squamous cells under microscope. He also developed the Papanicolaou stain (Pap stain) to enhance the sensitivity of detecting abnormal squamous cells. Instead of two colors in routine hematoxylin and eosin stain (HE stain), the Pap stain is a multichromatic staining cytologic technique, additionally employing light green and orange G to differentiate the metabolic activities of a cell. The main advantages of this staining procedure include good definition of nuclear detail, cytoplasmic transparency, and indication of cellular differentiation of squamous epithelial cells.

He first reported his findings with the conclusion that uterine cancer could be diagnosed by means of vaginal smear at a medical conference in 1928, but the significance of his study was not well recognized until his publication in 1941 in collaboration with Dr. Herbert Frederick Traut, a gynecological pathologist at New York Hospital, followed by the book *Diagnosis of Uterine Cancer by the Vaginal Smear* 2 years later. Pap smear costs little, is easy to perform, and could be interpreted accurately with a rapid turn-around time, and therefore, it has been widespread nationally and worldwide, resulting in a significant decline in the incidence of cervical cancer.

References: [1, 2].

2. How is Pap smear collected and prepared?

The Pap test is a procedure used to collect cells from the cervix so that they can be screened under the microscope to identify cancer cells and dysplastic precancerous cells. Based on the way of collection and processing, Pap smears can be divided into the following types:

- *Conventional Pap smears*: A metal or plastic speculum is first placed inside the vagina. Next, using a small spatula, a sample of cells and mucus is lightly scraped from the ectocervix. A small brush or a cotton-tipped swab is then inserted into the opening of the cervix to take a sample from the endocervix. In patient whose cervix has been removed because of hysterectomy as a part of the treatment for a cervical cancer or precancerous lesion, cells will be sampled from the upper part of the vagina, known

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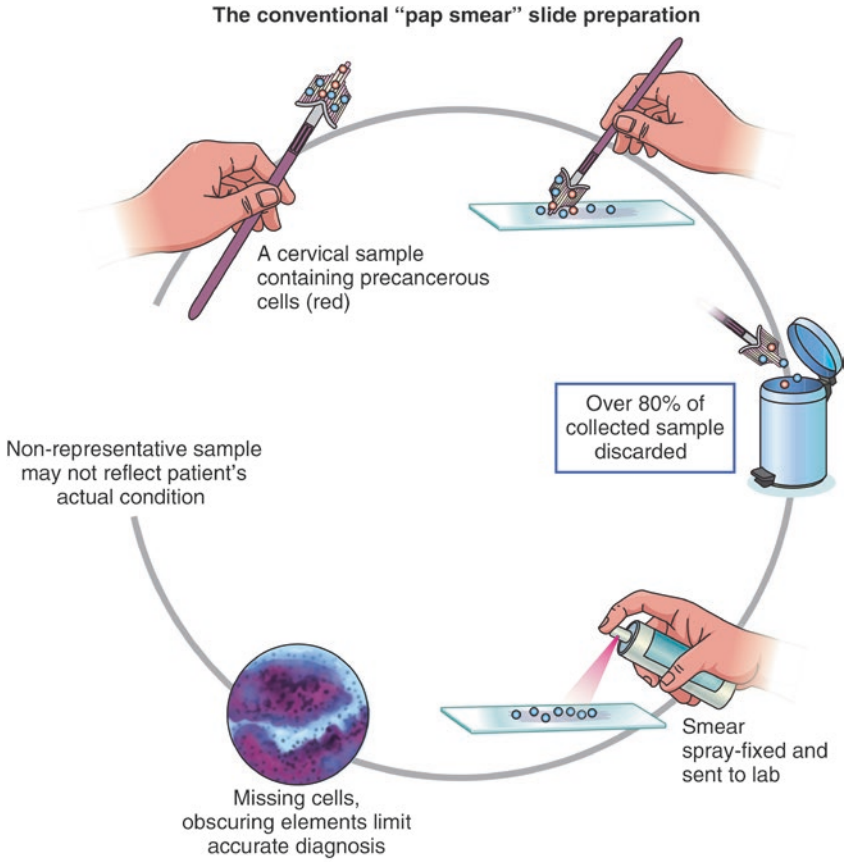
H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_3



In the traditional "smear" method of collecting cervical cells, only a small percentage of collected cells make it onto the slide itself, and abnormal cells may be discarded before they can be reviewed. During review of the slide, cells that have dried out and clumped together can obscure the view of abnormal cells.

Fig. 3.1 The conventional "Pap smear" slide preparation. In the traditional smear preparation, only a small percentage of collected cells make onto the slide, and abnormal cells may be discarded before they can be reviewed. During review of the slide, cells that have dried out and clumped together can obscure the view of abnormal cells. (Modified from: https://www.bing.com/images/search?q=Conventional+Pap+Smear+Technique&FORM=IRBPRS&=0)

as the *vaginal cuff*. The sample collected is directly rolled on a glass pathology slide to create a thin-layer smear followed by immediate fixation in 75% alcohol or an equivalent fixative (Fig. 3.1).

- *Liquid-based Pap smear*: Instead of "smearing" cervical cells onto a slide, the collected materials are immersed into a vial of liquid fixative provided by commercial companies and the specimen is then sent to cytology laboratory. At laboratory, the liquid is treated to remove other elements such as mucus and then placed onto slides by a special machine. Today, about 90% of Pap tests in the United States are liquid based.

Currently, the FDA-approved equipment for liquid-based Pap smear includes ThinPrep (Hologic, Marlborough, MA) and SurePath (BD Tripath, Burlington, NC). Although still controversial regarding its higher sensitivity in detecting high-grade squamous intraepithelial lesion (HSIL) than conventional Pap smear, liquid-based Pap smear does offer a few advantages. It improves the smear quality and screening easiness; enables preparation for duplicated smears; allows additional tests such as HPV, Chlamydia, and gonorrhea by using the excess materials collected; and facilitates the utilization of automated Pap smear screening devices (Fig. 3.2).

References: [3–5].

3. How is Pap smears evaluated?

A. *Manual screen by microscope*

- Manual microscopic examination of Pap smear is a systematic stepwise process to screen the entire smear and to detect the abnormal cells, microorganisms, or other findings primarily under 10× microscopic fields and with higher-power fields (20× and/or 40×) for confirmation. In order to maximize the visual accuracy, it is performed by trained cytotechnologist or cytopathologist and is required 20–30% overlap of each microscopic field in advancing to ensure evaluating cells at the central area of viewing field. The accuracy also depends on the quality of smears, particularly the conventional smears, and the experiences of screeners. Although conventional Pap smears contain more cells on each slide,

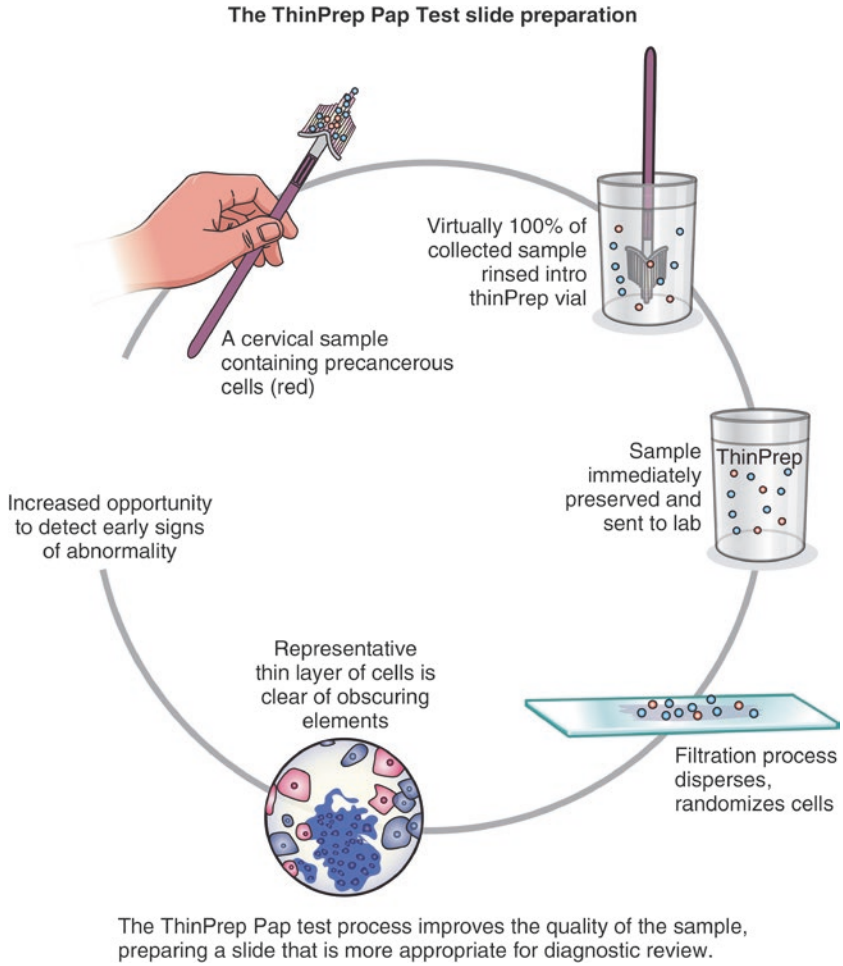


Fig. 3.2 The liquid-based smear slide preparation. In liquid-based smear preparation, majority of the cells are retrieved from brush/broom first and then suspended in liquid fixative. The smear forms a monolayer cells after homogenization, avoiding cell clumping, and is easier to screen. The excess materials can be preserved for additional smears or molecular studies, such as HPV test. (Modified from: https://www.bing.com/images/search?q=Conventional+Pap+Smear+Technique&FORM=IRBPRS&=0)

its unevenly smearing, air-dry artifact, and cover up of epithelial cells by mucoid materials and inflammatory cells add to the difficulty of screening. The job is tedious and time consuming, but so far is still the preferred screening method in majority of cytology laboratories.

B. *Automated screen by computer-based device*

- A more recent innovation has further improved Pap testing by automatically scanning for cells with large and dark nuclei that may be abnormal. It is basically a computer system that automatically scans the cells on the entire slide, and captures or electronically dots the potential abnormal cells. Finally, the cytotechnologist and/or cytopathologist have to review the dotted areas or the captured images, based on the device indication, to verify the abnormality and to decide any further screening actions before the final report is issued or released. The new method significantly increases the productivity and apparently improves disease detection. The currently FDA-approved automatic scanners are the ThinPrep Imaging System and the BD FocalPoint Guided Screening (GS) Imaging System.

References: [6, 7].

4. What is the efficiency of Pap smear evaluation?

Cervical cancer is the second most common cancer in women living in less-developed regions with estimated 445,000 new cases in 2012 (84% of the new cases worldwide). In 2012, approximately 270,000 women died from cervical cancer; more than 85% of these deaths occurred in countries as having low- and middle-income economies.

The Pap test is the most successful cervical cancer screening program. Besides cancer cells, more importantly it effectively detects the precancerous dysplastic cells, including low-grade squamous intraepithelial lesion (LGSIL), also known as cervical intraepithelial neoplasia (CIN 1) and high-grade squamous intraepithelial lesion (HGSIL, CIN 2, and CIN 3). With the introduction of Pap test in the mid-1950s, the incidence of invasive cervical cancer declined dramatically. Between 1955 and 1992, cervical cancer incidence and death rates in the United States declined by more than 60% and became the 14th common cancer, and the rate continues to decline (Fig. 3.3). A similar trend is shown worldwide, but the gratitude varies from region to region.

Although Pap test has been successful in preventing cervical cancer, it is not perfect. "False-positive" and "false-

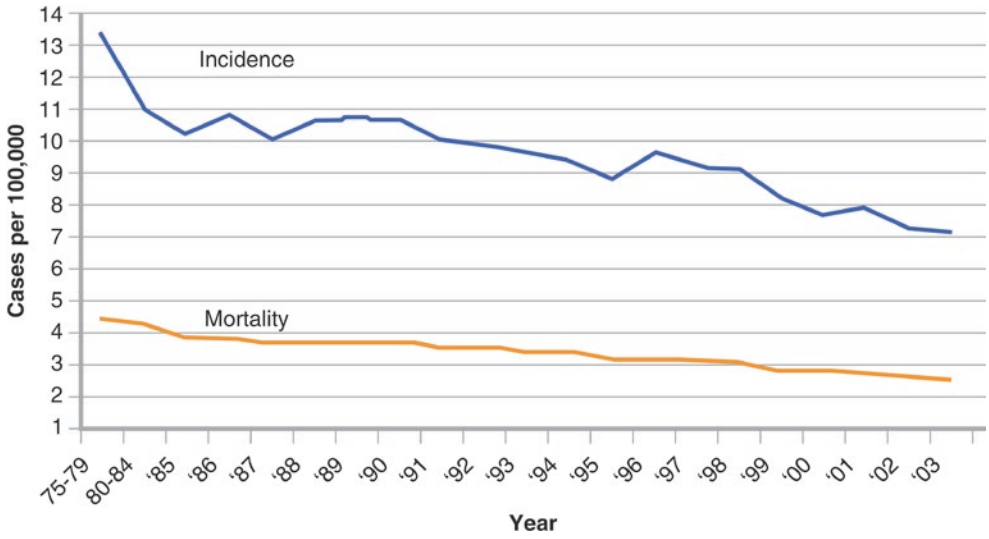


Fig. 3.3 SEER observed incidence, SEER delay adjusted incidence, and US death rates. Cancer of the cervix uteri, by race. With Pap smear intervention, cervical cancer incidence and mortality have straightly declined since the 1950s. (Modified from: https://seer.cancer.gov/csr/1975_2015/results_merged/sect_05_cervix_uteri.pdf)

negative" results do exist. The sensitivity of the test ranges from 30% to 80% and the specificity ranges from 80% to 100%. The false-negative rate varies from 6% to 20% (average 7.8%) and the false-positive rate is about 10–15% (average 10.3%). One of the limitations of the Pap test is that the results need to be examined by the human eye. The human eyes may get tired sometime and loss focus and may interpret the same subject variably by different persons with different trainings and experiences. So, an accurate analysis of the hundreds of thousands of cells in each sample is not always possible. The initial evaluation by automated scanner and followed by human confirmation seems to improve the accuracy.

References: [8–11].

5. What is the significance of HPV infection in the development of cervical dysplasia and carcinoma?

Human papillomavirus (HPV) infection is a common sexually transmitted disease. There are approximately 14 million new HPV infections in the United States each year, approximately 50% of which occur in 15- to 24-year-old young people. HPV typically causes no visible signs or symptoms, so males and females carrying HPV may spread it to others without knowing it. HPV could cause potentially serious diseases, including dysplasia and cancer of epithelial cells at cervix, vagina, vulva, and anus. Most HPV infection clears itself, but there is no way so far to predict who will or will not. Based on the frequency of their association with invasive cervical cancer, the genital HPVs are divided into high-risk (HR) and low-risk (LR) groups. By definition, the LR HPV types are those that have never been isolated from cervical carcinoma, compared to high-risk types which have been detected. The most common LR HPV types include 6, 11, 42, 43, 44, 53, 54, 61, 66, 72, 73, and 81. They are responsible for warts and low-grade squamous intraepithelial dysplasia, particularly the HPV types 6 and 11. The 13 types of

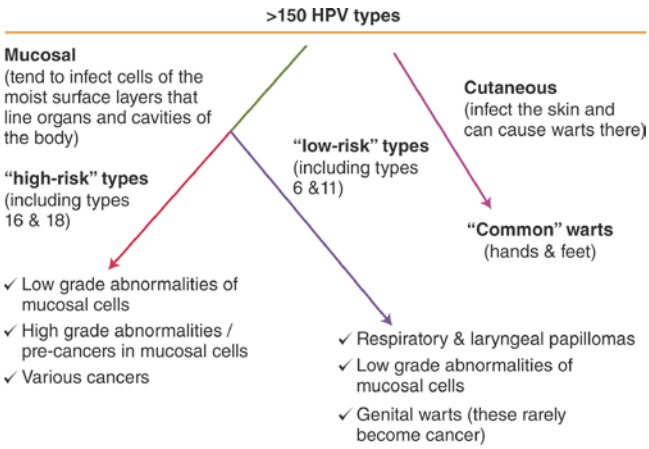


Fig. 3.4 There are more than 150 different types of HPV. This diagram shows the different groups of HPV types and the problems each group can cause. (Modified from: https://www.cancer.org/cancer/cancer-causes/infectious-agents/hpv/hpv-and-hpv-testing.html)

HR HPV that are of most concern are known by the numbers 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, among which just two HPV types, 16 and 18, are responsible for about 70% of all cancer cases. They are also detected in high-grade squamous intraepithelial dysplasia. There is sufficient evidence to indicate that HR HPV is a necessary cause of invasive cervical cancer worldwide (Fig. 3.4).

References: [12–14].

6. What is HPV test?

In contrast to Pap test that is used to find cellular changes or abnormal cells in the cervix, the HPV test checks for the gene transcriptions or DNA of the virus. Recent meta-analysis reported that HPV test improves the sensitivity of detecting HR HPV infection, as compared to the conventional cytology. It can be done at the same time as the Pap

test, with the same swab or a second swab. In 2014, HPV DNA test was approved by the Food and Drug Administration (FDA) to be used without a Pap test to screen for cervical cancer. However, it also can be used in combination with Pap test to screen for cervical cancer or precancerous changes. Pap test plus a HPV test (called co-testing) is the preferred way to find early cervical cancers or dysplasia in women 30 and older. It is not recommended to screen for cervical cancer in women under 30 years old, because women in their 20s who are sexually active are much more likely (than older women) to have an HPV infection that will go away on its own. For these younger women, results of this test are not as significant as those in the older age group and may be more confusing. The HPV test has also been used as the reflex test in women who have a Pap test result of atypical squamous cells of undetermined significance (ASCUS), to find out if more testing or treatment is needed. Multiple HPV tests with different molecular methods have been available in the market and even more are in the phase of development.

References: [15–17].

7. What are the currently FDA-approved high-risk (HR) HPV tests?

Up to now, FDA has approved the following molecular assays for detecting HR HPV in uterine cervical scraps: Hybrid Capture 2 High-Risk HPV DNA test (Digene), Cervista™ HPV HR and Gentind DNA Extraction (Hologic), Cervista™ HPV 16/18 (Hologic), CobasR HPV test (Roche Molecular Systems), RealTime HR HPV test (Abbot), APTIMAR HPV Assay (Gen-Probe), and Onclarity™ HPV Assay (BD Diagnostic Systems) (Sources: https://www.mlo-online.com/the-five-fda-approved-hpv-assays.php; https://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm598991.htm). HR HPV test is highly sensitive, but specificity depends on subsequent evaluation strategies and screening frequencies. The FDA-approved HPV tests include DNA-based assay and mRNA-based assay. The DNA assay either detects the full genome of HR HPV without individual virus genotyping (Digene Hybrid Capture 2, HC2) or targets the long control region 1 (L1) of the virus, which encodes a major structural capsid protein, either without genotyping (Cervista HPV HR test) or with partial genotyping (Cervista HPV test, Cobas 4800 HPV test, and Abbot RealTime HR HPV test). Compared to the early hybridization technique (HC2), invader assays (Cervista HPV HR test) show high sensitivity in the detection of HSIL, and Cervista HPV16/18 test demonstrates low false-positive rate with high sensitivity and specificity in genotyping HPV 16 and 18. The real-time PCR assays (Abbot RealTime HR HPV test) have the following features: high specificity with no cross-reaction with low-risk HPV types, high absolute clinical sensitivity for both CIN 2 and CIN 3 lesions, and comparatively clinical sensitivity and specificity relative to HC2. The Cobas 4800 HPV assay is better than HC2 assay in the detection of HR HPV with higher sensitivity in SurePath specimens in comparing with ThinPrep cytology specimens, and it is the one that is approved by FDA for HPV primary screening followed by the Onclarity HPV Assay. The BD Onclarity HPV assay was just approved (2/2018) for primary, secondary, and co-test with Pap smear.

The recently developed assays detect HPV E6/E7 mRNA from 14 HR HPV types, either without genotyping (APTIMA HPV test) or with partial genotyping (APTIMA HPV 16, 18/45 test). The APTIMA HPV test shows no cross-reaction with low-risk HPV types and has excellent performance and robustness. Besides the HR HPV 16 and 18 genotyping, APTIMA HPV 16, 18/45 test also includes genotyping for HPV type 45, a viral type more commonly identified in cervical adenocarcinoma.

References: [18–28].

8. Is cervical cancer preventable?

Because of the close association with HR HPV infection, invasive cervical cancer is theoretically preventable. World Health Organization (WHO) recommends a comprehensive approach to cervical cancer prevention and controls, and the recommended set of actions includes interventions across the life course. It should be multidisciplinary, including components from community education, social mobilization, vaccination, screening, treatment, and palliative care. Practically, the following strategies can be excised:

1. Control of HR HPV infection:
   - As a type of sexually transmitted infection, limiting HPV infection requires personal self-control. The comprehensive approach may include the following:
     (a) Education about safe sexual practices, including delayed start of sexual activity.
     (b) Promotion and provision of condoms for those already engaged in sexual activity.
     (c) Warnings about tobacco use, which often starts during adolescence, and which is an important risk factor for cervical and other cancers.
     (d) Male circumcision.
2. Early detection
   - Following guidelines to have routine Pap test and/or HPV test is an important preventive step one can take. As discussed above in details, the assays can detect the precancerous dysplasia and identify HR HPV infection, and therefore remarkably reduce the incidence of cervical cancer.
3. Vaccination
   - Vaccines have been a great success in preventing human infection by a variety of bacteria and virus.

Vaccines are available that can protect against certain types of HPV infections, including those most commonly linked to cancer, as well as some types that can cause anal and genital warts. Clinical trial results show that vaccines are safe and very effective in preventing infection with HPV 16 and 18. These vaccines only work to prevent HPV infection; they will not treat an infection that is already there. Therefore, to be most effective, the HPV vaccines should be given before a person becomes exposed to HPV. Some countries have started to vaccinate boys as the vaccination prevents genital cancers in males as well as females, and some available vaccines also prevent genital warts in males and females.

- Three vaccines are approved by the FDA to prevent HPV infection: Gardasil in 2006, Cervarix in 2007, and Gardasil 9 in 2014. All three vaccines prevent infections with HPV types 16 and 18, two high-risk HPVs that cause about 70% of cervical cancers. Gardasil also prevents infection with HPV types 6 and 11, which cause 90% of genital warts. Gardasil 9 prevents infection with the same four HPV types plus five additional high-risk HPV types (31, 33, 45, 52, and 58). Gardasil is quadrivalent and is approved for females and males 9–26 years of age. Cervarix is bivalent and is approved only for females 9–25 years of age. Gardasil 9 is 9-valent and is approved for females 9–26 years of age and males 9–15 years of age.
- In addition to providing protection against the HPV types included in these vaccines, the vaccines have been found to provide partial protection against a few additional HPV types that can cause cancer, a phenomenon called cross-protection. Because none of the currently available HPV vaccines protects against all HPV infections that cause cancer, or treats HPV infection or HPV-associated disease such as cancer, it is important for vaccinated women to continually undergo cervical cancer screening until further evidences warrant a change.
- The American Cancer Society (ACS) recommends the vaccine use for HPV vaccine as follows:
  (a) Routine HPV vaccination for girls and boys should be started at age 11 or 12. The vaccination series can be started as early as age 9.
  (b) HPV vaccination is also recommended for females 13–26 years old and for males 13–21 years old who have not started the vaccines, or who have started but not completed the series. Males 22–26 years old may also be vaccinated. It is important to know that vaccination at older ages is less effective in lowering cancer risk.
  (c) HPV vaccination is also recommended through age 26 for men who have sex with men and for people with weakened immune systems (including people with HIV infection), if they have not previously been vaccinated.
  (d) It is important to realize that no vaccine provides complete protection against all cancer-causing types of HPV, so routine cervical cancer screening is still necessary.
- Slightly different, WHO recommends vaccination for girls aged 9–13 years as this is the most cost-effective public health measure against cervical cancer. Some trials of HPV vaccination in women aged up to 55 years have shown almost 90% protection from cervical precancer caused by HPV16/18 among HPV16/18-DNA-negative women, and the results have led to a proposal to extend routine vaccination to women up to 30 years of age, providing that women will have at least one HPV-screening test at age 30 years or old.
- WHO has developed guidance on how to prevent and control cervical cancer, including through vaccination and screening. The Organization works with countries and partners to develop and implement comprehensive programs. By the mid of 2016, 65 countries had introduced HPV vaccines, most in developing countries, including increasing number of middle- and low-income countries. Given that the global burden still falls heavily on African and Asian countries where vaccination and screening programs are lacking, there is still a need for more countries to introduce the HPV vaccine.

References: [29–36].

9. What is the current guideline for Pap smear screening?

The American Society of Cytopathology recommends that cervical cancer can be diagnosed at early state if women follow these guidelines (Last Revised: December 9, 2016).

(a) All women should begin cervical cancer testing (screening) at age 21. Women aged 21–29 should have a Pap test every 3 years. HPV testing should not be used for screening in this age group (it may be used as a part of follow-up for an abnormal Pap test).
(b) Beginning at age 30, the preferred way to screen is with a Pap test combined with an HPV test every 5 years. This is called co-testing and should continue until age 65.
(c) Another reasonable option for women 30–65 is to get tested every 3 years with just the Pap test.
(d) Women who are at high risk of cervical cancer because of a suppressed immune system (e.g., from HIV infection, organ transplant, or long-term steroid use) or because they were exposed to DES in utero may need to be screened more often. They should follow the recommendations of their healthcare team.

(e) Women older than 65 years who have had regular screening in the previous 10 years should stop cervical cancer screening as long as they haven't had any serious precancers (like CIN2 or CIN3) found in the last 20 years (CIN stands for cervical intraepithelial neoplasia and is discussed later in the section "Work-up of an abnormal Pap test result" under the heading "How biopsy results are reported"). Women with a history of CIN2 or CIN3 should continue to have testing for at least 20 years after the abnormality was found.

(f) Women who have had a total hysterectomy (removal of the uterus and cervix) should stop screening (such as Pap tests and HPV tests), unless the hysterectomy was done as a treatment for cervical precancer or cancer. Women who have had a hysterectomy without removal of the cervix (called a supracervical hysterectomy) should continue cervical cancer screening according to the guidelines above.

(g) Women of any age should NOT be screened every year by any screening method.

Women who have been vaccinated against HPV should still follow these guidelines.

10. How is Pap smear result reported?

The most widely used system for describing Pap test results is the Bethesda System (TBS). TBS is a system for reporting cervical or vaginal Pap smear results. It was introduced in 1988 and revised in 1991 and 2001. The name of Bethesda system comes from the location (Bethesda, Maryland) of the conference that established the system. Additionally, the name of Bethesda System is also used for cytopathology of thyroid nodules.

During the past decade, substantial change in the realm of cervical cancer screening has occurred, including experiences gained with liquid-based technologies, automation, biomarkers, and other advances. In addition, the Pap test may be used as a "reflex test" after a more sensitive molecular (HPV) test. This has come close to reality as molecular testing options (primary HPV testing and co-testing) are now approved for cervical cancer screening. The positive predictive value may decrease in patients who are vaccinated against HPV infection. With the increase in vaccination, there is a need to advance education and performance. All these reasons led to the most recent update of the system in 2014. However, there are minimal changes relating to the terminology itself in the update. Of note is that Other Category reporting benign-appearing endometrial cells in a woman >40 years of age has been removed and it is now recommended to report benign-appearing endometrial cells for women aged ≥45 years.

The current Bethesda System for reporting cervical cytology is summarized in Table 3.1.

Table 3.1 Epithelial cell abnormalities

Squamous cell
Atypical squamous cells
Of undetermined significance (ASCUS)
Cannot exclude HSIL (ASC-H)
Low-grade squamous intraepithelial lesion (LSIL)
(*encompassing: HPV/mild dysplasia/CIN 1)*
High-grade squamous intraepithelial lesion (HSIL)
(*encompassing: moderate and severe dysplasia, CIS; CIN 2 and CIN 3)*
With features suspicious for invasion *(if invasion is suspected)*
Squamous cell carcinoma
Glandular cell
Atypical
Endocervical cells (NOS *or specify in comments)*
Endometrial cells (NOS *or specify in comments)*
Glandular cells (NOS *or specify in comments)*
Atypical
Endocervical cells, favor neoplastic
Glandular cells, favor neoplastic
Endocervical adenocarcinoma in situ
Adenocarcinoma
Endocervical
Endometrial
Extrauterine
Not otherwise specified (NOS)
Other malignant neoplasms: *(specify)*
Adjunctive testing
Provide a brief description of the test method(s) and report the result so that it is easily understood by the clinician
Computer-assisted interpretation of cervical cytology
If case examined by an automated device, specify device and result
Educational notes and comments appended to cytology reports (*optional*)
Suggestions should be concise and consistent with clinical follow-up guidelines published by professional organizations (references to relevant publications may be included)

Copied from Reference [43].

References: [37–43].

11. How does the government regulate cytology laboratories engaging in Pap smear examination?

The cytology laboratory is one of the most regulated laboratories providing patient service in this country, partially due to an extraordinary media attention in the 1980s on the issue of false-negative Pap tests. It promoted the legislation enacted by the US Congress named Clinical Laboratory Improvement Amendments of 1988 (CLIA 88). Congress charged the Center of Medicare and Medicaid Services (CMS) to implement the standard of cytology laboratory practice that sets daily workload limits for cytotechnologists (CT) and cytopathologists (CP) (if they are the primary Pap smear screener), stringent quality control procedures including 5-year retrospective rescreening of prior negative Pap

results if an HSIL or worse lesion is found, and assessment of cytologic-histologic correlations, pathologist review of all abnormal Pap results and those with reactive/reparative changes, 10% review of randomly selected cases that are interpreted by CTs as negative for epithelial cell abnormalities and other malignant neoplasm, proficiency test for Pap smear cytology, and unannounced specialized surveys. In addition, CLIA 88 also defined the qualifications and responsibilities of CPs and CTs.

CLIA 88 regulations at Section 493.855(a) state that "The laboratory must ensure that each individual engaged in the examination of gynecologic preparations is enrolled in a proficiency testing (PT) program approved by CMS." Starting form 2007, after a nationwide educational phase, cytology laboratory participation in annual proficiency test became mandatory. In 2006, CMS approved four cytology PT programes: State of Maryland Cytology PT program, American Society of Clinical Pathology (ASCP), Midwest Institute for Medical Education (MIME), and College of American Pathologists (CAP). State of Maryland Cytology PT program has recently drpped out of the game. The CMS-approved Cytology PT programs for calendar year 2016 are only the CAP and ASCP programs. Cytology laboratories can participate in the programs at their choice. Basically, each test consists of a set of 10 or 20 Pap smears (used to be direct smears, now ThinPrep or SurePath smears), with four categories of diagnosis, unsatisfactory for evaluation, negative, LSIL and HSIL and above. It is to be finished within 2 (10 slide set) or 4 (20 slide set) hours in an environment compatible to routine Pap smear screening, under the monitoring of specially certified proctors. The score deduction from the total of 100 points is designed differently based on the lesion one would miss and depend on CT or CP to manipulate the situation of real practice. The passing score is 90 points (90%) or higher. Retaking is allowed for the first-time failure and the second-time failure in PT will results in removal of individual from performing routine Pap smear examination until passing the test after a period of special training. This rule applies equally to CT and CP.

References: [44, 45].

12. What is the screening algorithm for anal neoplasia? Is it different from that of cervical neoplasia?

There are currently no consensus guidelines for anal neoplasia screening. No randomized controlled trials have assessed the efficacy of any screening algorithm for anal carcinoma. There are, however, multiple screening algorithms that have been recommended by organizations such as the New York State Department of Health AIDS Institute, American Society of Colon and Rectal Surgeons, and others. In the general population, anal cancer rates have been low traditionally, precluding a need for large population-based screening initiatives like those used for detection of cervical cancer. Since the 1970s and 1980s, however, the incidence of anal cancer has increased by 35% due to the prevalence of HIV and transplant patients. In the past 10 years, anal carcinoma rates have increased by an average of 2.2% per year.

Screening for anal dysplasia is particularly important in high-risk populations. Men who have sex with men (MSM), HIV-positive patients (both male and female), transplant patients (particularly renal), and women with multifocal squamous dysplasia or carcinoma of the lower genital tract are the highest risk groups. HIV-positive men have a 37-fold increased risk of anal cancer, and those with a history of receptive anal intercourse have a 60-fold increased risk. Renal allograft recipients without history of receptive anal intercourse also show an increased risk of anogenital malignancy. Thus, while receptive anal intercourse increases the risk of anal HPV infection and SIL, it is not a necessary prerequisite. The mechanism of HPV infection of the anus in the absence of receptive anal intercourse is hypothesized to be due to spread of body fluids during other sexual practices or spread from adjacent infected genitalia. However, despite the emphasis on MSM in the literature, women actually have a higher rate of anal carcinoma. The American Cancer Society estimates that in 2018, 5620 women and 2960 men will have new anal cancer diagnoses and there will be 1160 deaths (680 women and 480 men).

Due to its achievement in dramatically decreasing death rates from cervical malignancy and the similar HPV-mediated pathogenesis, cervical cancer screening has served as a model for anal cancer screening. Anal-rectal cytology (ARC), or the anal Pap test, was introduced in the 1990s and is considered the screening test of choice due to its relative ease and accessibility. In a 2010 study by Salit et al., in HIV-positive MSM, the sensitivity and specificity of an atypical squamous cells of undetermined significance (ASCUS) or above diagnosis on anal-rectal cytology for anal intraepithelial neoplasia (AIN) II or higher were 84% and 39%, respectively. In the same study, the negative predictive value (NPV) was 88% and positive predictive value (PPV) was 31%. A meta-analysis by Chiao et al. showed that the sensitivity and specificity of a diagnosis of ASCUS or above on ARC from HIV+ patients ranged from 69 to 93% and from 32 to 59%, respectively. The sensitivity of ARC for identification of anal squamous intraepithelial lesions has been found to be higher in HIV-positive men than in HIV-negative men, likely due to the higher incidence of extensive disease in this population. Compared to cervical Pap testing, a diagnosis of ASCUS on anal Pap is more frequently associated with the identification of any dysplasia on biopsy, and more frequently is associated with higher grade lesions.

Besides the ARC, other screening techniques exist for anal neoplasia including the digital anorectal exam (DARE). Invasive anal cancers are generally palpable on DARE, and it is a low-risk screening test. The New York State Department of Public Health AIDS Institute 2007 guidelines recommend screening high-risk groups annually with DARE and ARC.

References: [43, 46–56].

13. What is the role of HPV testing in anal cancer screening?

The pathogenesis of anal cancer, the vast majority (80%) of which consists of squamous cell carcinoma (SCC), is parallel to that of cervical SCC. High-risk HPV is associated with nearly all cervical cancers and over 90% of anal SCC. HPV types 16 and 18 predominate in both anal and cervical cancer; however, type 16 is more common in anal cancers than cervical cancers. Similarly to cervical SCC, anal SCC shows strong affinity for development within squamous metaplastic epithelium located at the transformation zone, and it exhibits an analogous progression from dysplasia to carcinoma upon infection with high-risk HPV genotypes. AIN II and III are generally considered the precursors to anal SCC and once identified, infrequently regress. Risk factors such as immunocompromise affect the risk of progression of these lesions. A recent series found that the risk of progression of premalignant lesions in immunocompromised patients managed expectantly was 13–50%. While there is ample evidence that HPV is strongly associated with endocervical adenocarcinoma, the link between HPV infection and colorectal adenocarcinoma thus far has not been definitively established.

In the HIV-positive population, CD4+ counts of less than 200 cells/mm3 are most closely linked to risk of progression of anal dysplasia. However, treatment with antiretrovirals to increase CD4+ counts does not appear to affect the risk of progression of dysplasia. Furthermore, extending the lifespans of these patients appears to increase the time available for progression of dysplasia. In the setting of HPV infection, immunosuppression brought on by low CD4+ counts is thought to inhibit the clearing of HPV-infected cells, leading to continued production of HPV proteins E6 and E7 which contribute to genomic instability. The ensuing persistent genetic alterations lead to the development of AIN II–III and carcinoma and are not affected by subsequent improved CD4 counts due to HAART therapy.

The use of HPV testing in cervical cytology testing is well studied and is a standard part of screening algorithms for cervical cancer. The role of HPV in the pathogenesis of anal cancer is just as important; however, formal guidelines for its use in anal testing are not in place. Furthermore, insurance coverage of HPV testing in anal cytology specimens is limited. While HPV testing can be performed using the same collection device as a liquid-based specimen, HPV testing of anal cytology specimens is not FDA approved and any laboratory performing this testing must internally validate the test. The highest risk population for anal cancer, HIV-positive MSM shows extremely high prevalence of HPV infection, and for this reason, HPV testing does not add significant useful information to screening of this group. HPV testing, however, has a good NPV in low-risk groups, and it may be useful for posttreatment follow-up.

The HPV vaccine is available as quadrivalent and bivalent formulations, and both have been shown to be over 90% effective in preventing persistent infection with HPV types 16 and 18 in vaccinated women who have received the complete series of vaccines. In 2009, the quadrivalent HPV vaccine was FDA approved for use in males ages 9–26 to prevent condylomata caused by HPV type 6 and 11. Research investigating the use of the vaccine to prevent AIN is ongoing, and the vaccine has been found to be immunogenic in HIV-positive patients.

References: [43, 46, 47, 52, 54, 57–61].

14. Are the cytologic features and nomenclature used for anal-rectal cytology specimens the same as those used for gynecologic cytology specimens?

The cytologic and histologic features of anal dysplasia and carcinoma are morphologically identical to those in the cervix. For this reason, the Bethesda nomenclature for classifying cervical cytology is also used for anal cytology specimens (negative for intraepithelial lesion or malignancy/NILM, atypical squamous cells of uncertain significance/ASCUS, atypical squamous cells of uncertain significance cannot rule out high-grade lesion/ASC-H, low-grade squamous intraepithelial lesion/LSIL, and high-grade squamous intraepithelial lesion/HSIL). The only significant difference when applied to anal cytology is that the adequacy criteria for anal cytology require fewer cells (2–3000 nucleated cells per liquid-based slide). When estimating cellularity using a ThinPrep slide, this is equivalent to approximately 1–2 nucleated squamous cells per high-power field (40x) and 3–6 with Surepath. Anal transformation zone sampling (colonic glandular cells or squamous metaplastic cells) should be reported, though as in cervical cytology, is not required for adequacy. The collection of ARC specimens is technically more challenging than that of the cervix due to the inability to visualize the transformation zone during collection. Liquid-based preparations are preferred due to the medium's ability to mitigate contamination by bacteria and fecal material. ARC specimens from high-risk populations commonly show a combination of LSIL and HSIL type cells.

Though the cytologic features of SIL in ARC specimens are identical to those of cervical cytology, there are differences in specimen composition due to location. Cytologic degeneration and keratinization of cells are more commonly seen in ARC. In general, keratinizing high-grade lesions are

more common in ARC than in cervical cytology. The detection of invasive SCCs is difficult on ARC due to the frequent lack of tumor diathesis in these specimens. This may be due to the presence of increased contamination by fecal material, bacteria, and exfoliated cellular debris in these specimens when compared to gynecologic specimens. In addition, reactive changes commonly seen in cervical cytology are not commonly identified unless herpetic lesions or ulcers are sampled. Glandular lesions can be detected on ARC, but are infrequent. Theoretically, as screened population age, detection of glandular lesions such as rectal adenocarcinoma may become more common. Viral infections such as herpesvirus and cytomegalovirus (CMV) can be detected in ARC specimens as well as other organisms such as Candida, amoebic trophozoites and cysts, Enterobius, and Strongyloides. Postablative therapy ARC specimens can contain increased macrophages which are morphologically similar to amebic cysts. Amebic cysts can be distinguished from HSIL or macrophages due to their refractile cyst wall.

It is worth noting that a recent review of results from the College of American Pathologists' Interlaboratory Comparison Program in Non-Gynecologic Cytology (CAP NGC) educational slide program from 2006 to 2011 showed poor interobserver agreement on anal cytology specimens, particularly when diagnosing HSIL and squamous cell carcinoma. The results highlight the need for improved pathologist education in interpreting ARC.

References: [15, 43, 46, 62–64].

15. How is anal-rectal cytology billed in the cytology laboratory?

In contrast to gynecologic cytology, ARC is not classified as a screening test by the insurance industry. ARC specimens incorrectly billed as gynecologic cytology specimens will be rejected by the insurance company. ARC specimens are classified as nongynecologic specimens for billing purposes. CPT (current procedural terminology) codes for these specimens are assigned based not on site sampled but rather on slide preparation technique. Each CPT code is used once per specimen, not per slide prepared. Hence, for an ARC specimen prepared as a ThinPrep slide, the billing code used would be 88112 ("Enriched/concentrated prep"). See Table 3.2 for common CPT codes used for ARC specimens.

References: [59, 63].

16. How are anal-rectal cytology specimens collected?

ARC specimens can be collected from female patients after gynecologic exam while in the dorsal lithotomy position. For male patients or when stirrups are not available, lateral recumbent position can be employed which consists of the patient laying on their side with knees flexed and pulled toward chest. Patients should be counseled to refrain from activities that may decrease cellular yield prior to sampling such as receptive anal intercourse, the use of douches, or enemas. Collection of the sample employs a moistened Dacron swab which is inserted into the rectum 5–6 cm to ensure sampling of the transformation zone. Lateral pressure is applied to the swab as it is rotated. A cytobrush can also be employed though may be more uncomfortable for the patient. A wooden handled cotton swab is not recommended due to the risk of breakage within the anal canal. If the specimen is being collected for liquid-based cytology, it is placed in the preservative solution and agitated to suspend cells. If direct smear is to be prepared, the swab can be directly smeared on the slide and spray fixative used akin to the preparation of gynecologic cytology specimens. A recent study showed that self-collection can be performed by the patient with 80% successful collection of an adequate sample on the first attempt.

References: [50, 54, 59].

Table 3.2 Common CPT codes used in ARC billing

Preparation	CPT code
Direct smear	88104
Concentrated prep (cytospin or Saccomanno)	88108
Enriched/concentrated prep (e.g., ThinPrep, SurePath)	88112
"Other source" (received as stained smear)	88160
"Other source" (received as unstained smear)	88161

Note: Table 3.2 *adapted from Cibas ES, Ducatman BS. Cytology: Diagnostic Principles and Clinical Correlates. 4th ed. Philadelphia, PA: Saunders/Elsevier; 2014 (page 530)*

17. How are squamous abnormalities of anal-rectal cytology (ASCUS and above) triaged and treated?

Patients who are found to have ASCUS or higher on ARC are recommended to proceed to high-resolution anoscopy (HRA) and biopsy. HRA is analogous to colposcopy. A high-magnification colposcope is used in conjunction with a transparent anoscope to visualize the anal canal and perianal skin. As in the cervix, the procedure employs the use of acetic acid solution; however, as the anus is more sensitive to this solution, a 3% dilution is used as opposed to the 5% used on the cervix. With the use of a disposable anoscope, the solution is applied with a cotton swab. The anoscope is removed and the solution is left in place for several minutes. The anoscope is replaced and the area is carefully examined for acetowhite epithelium and vascular changes, as in the cervix. Low-grade lesions are typically cauliflower-like and raised. High-grade lesions are more typically flat and are more commonly associated with vascular changes. The New York State Department of Health AIDS Institute recommends the use of HRA as the standard of care for patients with a history of anal Pap abnormalities. However, accessibility of the procedure limits its imple-

mentation, as the technique requires significant skill and there is a lack of trained practitioners.

Though AIN I does not progress directly to carcinoma, it is treated to reduce progression to AIN II or III, to eliminate disease when it is at a manageable size, to decrease symptoms, and to manage patient anxiety. AIN is generally asymptomatic, though it can be associated with pain and itching. There is limited research on the long-term efficacy of the treatment of AIN however. Treatment modalities include topical therapy, fulguration, cryotherapy, laser-based therapy, and surgery. Topical therapy is generally reserved for smaller lesions (<1 cm). Large diffuse and/or circumferential AIN lesions can be treated with surgery; however, this confers significant morbidity including pain, stenosis, and possible incontinence and is reserved for patients in whom invasive cancer needs to be excluded. Patients with AIN are recommended to be followed up every 4–6 months. Patients with HIV and AIN are particularly challenging to treat and may never achieve elimination of disease despite treatment.

References: [46, 47, 49, 50, 54].

Case Presentation

Case 1

Clinical history and morphological description: A 30-year-old woman had her first visit to gynecologist, and a conventional Pap smear was taken, which showed overlapping cells, inflammatory cells, cellular debris, and squamous cells with slightly nuclear enlargement (Fig. 3.5). It was interpreted as "Satisfactory for evaluation; endocervical/transformation zone components present (not shown in figure); partially obscuring inflammation present; and negative for intraepithelial lesion or malignancy. Reactive squamous cells were associated with inflammation." Concerning the quality of her specimen, the gynecologist repeated the Pap smear in a short-time period, and this time specimen was collected for liquid-based assessment. In the ThinPrep smear preparation, a few dysplastic squamous cells diagnostic of LSIL were detected in a background with much less inflammatory cells (Fig. 3.6). Follow-up biopsy confirmed the presence of cervical intraepithelial lesion, grade I (CIN I; Fig. 3.7). This is an example that the quality of smears might impact on the detection of abnormal cells.

Cytomorphologic findings: Figs. 3.5, 3.6, and 3.7

Take-home message:

This is an example that the quality of smears might impact on the detection of abnormal cells.

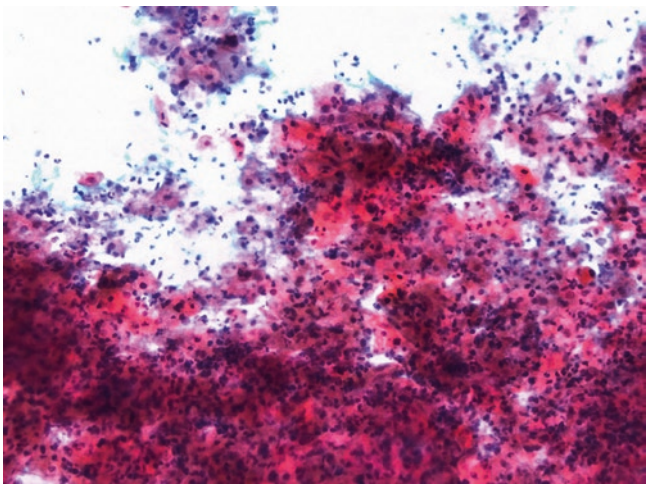


Fig. 3.5 Conventional Pap smear with overlapping cells and partially obscuring inflammatory cells. A few squamous cells with slightly nuclear enlargement and hyperchromasia, which can be interpreted as reactive squamous cells associated with inflammation. ×200

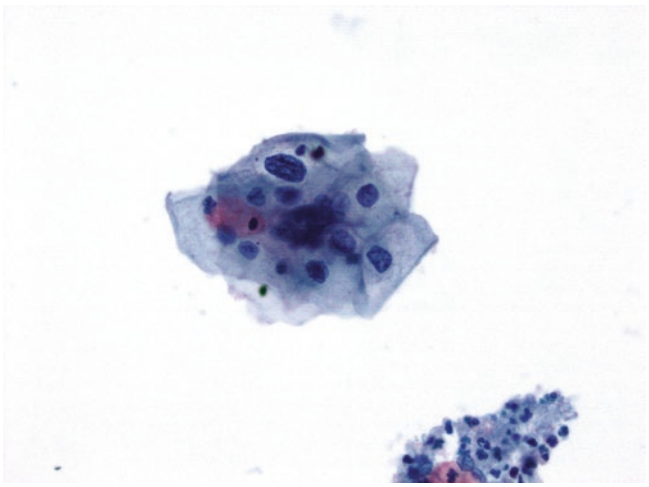


Fig. 3.6 Liquid-based (ThinPrep) Pap smear of the patient shows LSIL cells and much cleaner background. A cluster of inflammatory cells is also noted. ×400

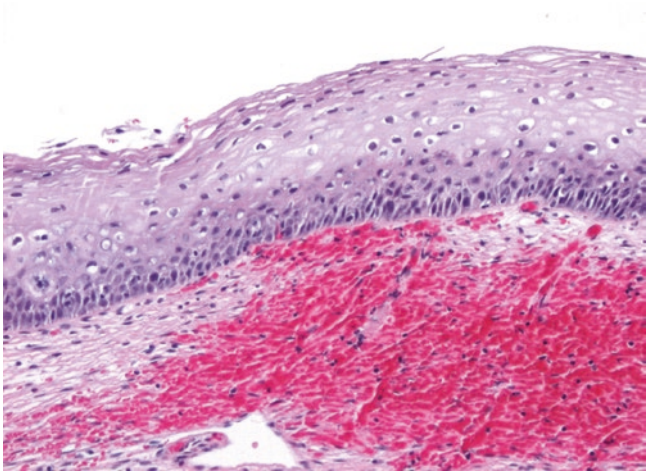


Fig. 3.7 Follow-up biopsy confirms the presence of CIN I. ×200

Case 2

Clinical history: The patient is a 28-year-old HIV+ female with a history of significant lower genital tract dysplasia. She has a history of cervical Pap smear showing LSIL. Prior biopsies of the cervix, labia, and vagina show changes ranging from VAIN/VIN/CIN II–III. An anal Pap smear is performed.

Cytomorphologic findings: Figs. 3.8, 3.9, 3.10, 3.11, 3.12, 3.13, and 3.14.

Description: The specimen is adequate and includes glandular cells consistent with transformation zone sampling. It contains multiple cells with mildly increased nuclear: cytoplasmic ratios and enlarged hyperchromatic nuclei with irregular nuclear contours and binucleation and multinucleation. Some cells show koilocytic halos. In addition, there is a minority of cells with high nuclear: cytoplasmic ratios, enlarged hyperchromatic nuclei with irregular contours. The atypical cells show variable keratinization.

The diagnosis is as follows:

- Specimen adequacy:
  - Specimen adequate for evaluation; transformation zone component(s) present
- Interpretation:
  - Atypical squamous cells cannot exclude a high-grade squamous intraepithelial lesion (ASC-H).
  - Background of low-grade squamous intraepithelial lesion (LGSIL). See note.
  - Note: Predominantly LSIL with rare abnormal cells suggesting a high-grade lesion (HGSIL). Suggest colposcopy/biopsy.
- Histology findings:
  - Description: Sections show anal transformation zone mucosa with full thickness atypia. Immunohistochemical stain for p16 shows strong block positivity within the atypical epithelium.
- Diagnosis:
  - Anal lesion, biopsy: Anal intraepithelial neoplasia (AIN) III
- Discussion: The patient is in two high-risk categories: HIV+ individuals and women with multifocal lower anogenital tract dysplasia. HIV infection is associated with persistence of HPV infection with increased risk of progression to AIN II or III and subsequent carcinoma. Women with preexisting HPV-mediated lesions in the lower genital tract are also at high risk of anal HPV infection and development of subsequent dysplasia and carcinoma. Anal Pap smears more commonly show atypical cells with keratinization and a mixture of low- and high-grade lesions. As in the cervix, strong diffuse block positivity for p16 immunohistochemistry highlights high-grade dysplasia.

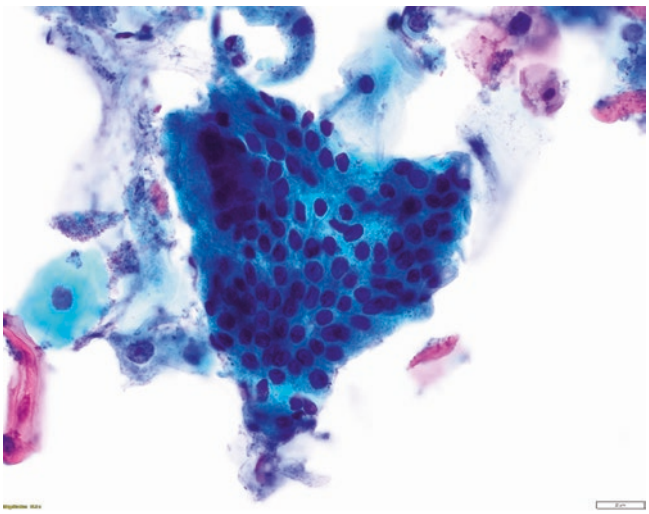


Fig. 3.8 Anal Pap, ThinPrep, 60×

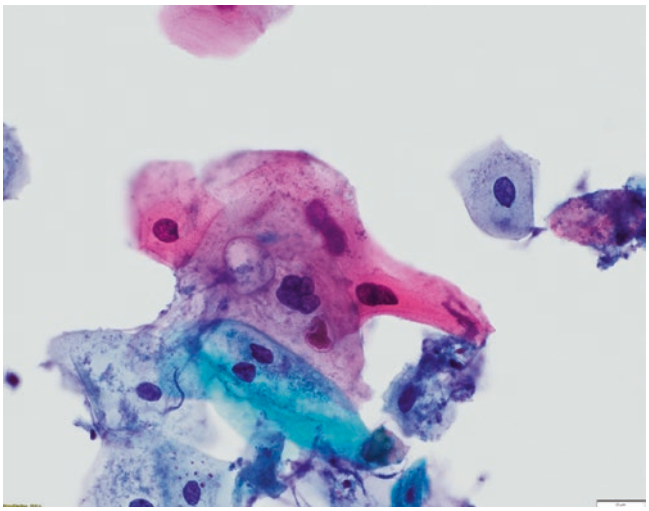


Fig. 3.9 Anal Pap, ThinPrep, 60×

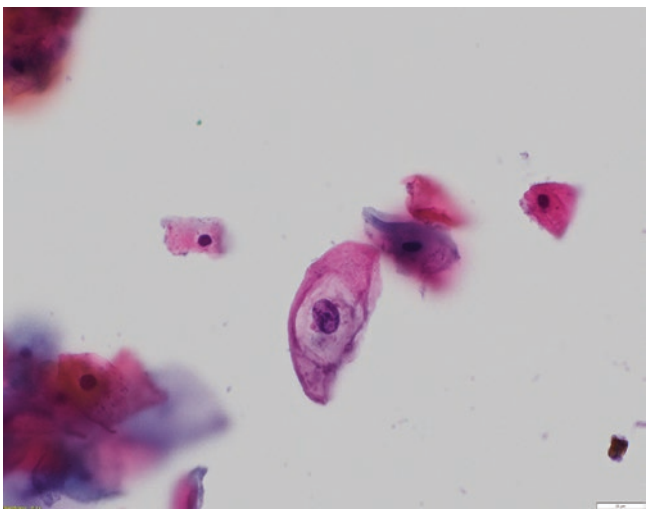


Fig. 3.10 Anal Pap, ThinPrep, 60×

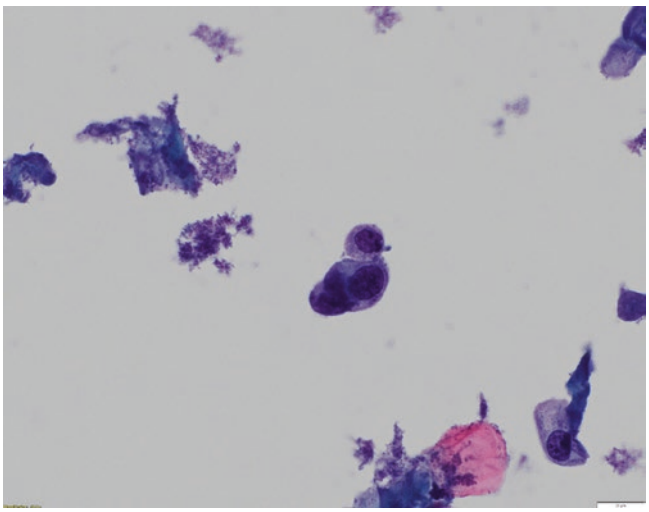


Fig. 3.11 Anal Pap, ThinPrep, 60×

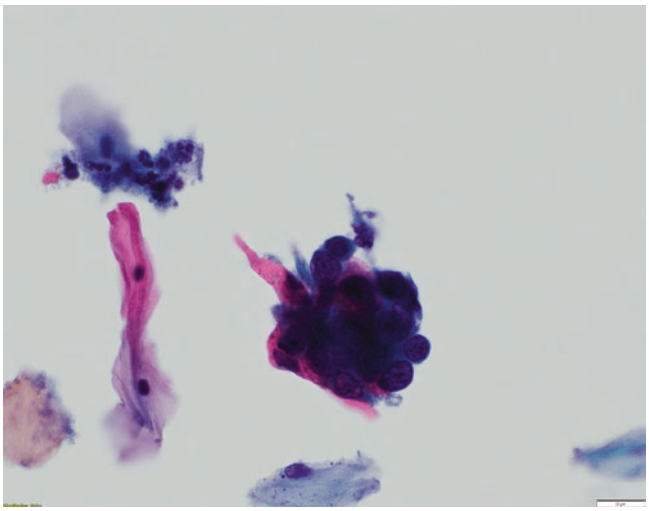


Fig. 3.12 Anal Pap, ThinPrep, 60×

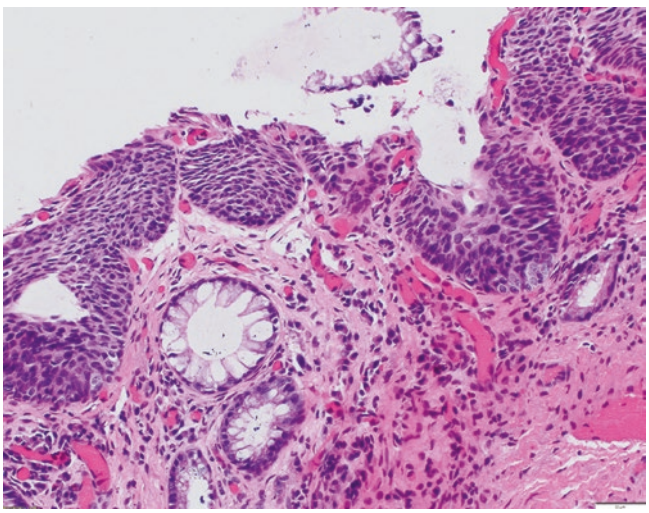


Fig. 3.13 H&E, 20×

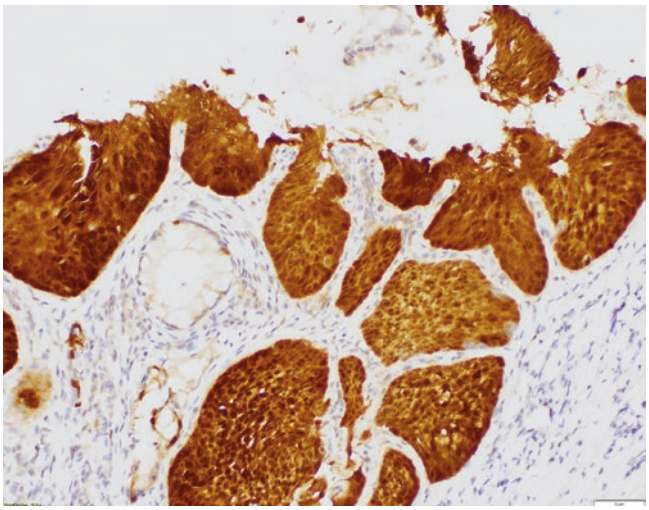


Fig. 3.14 p16 immunohistochemistry, 20×

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Normal and Benign Cervical Cytology

4

Rossitza Draganova-Tacheva and Kim HooKim

Contents

List of Frequently Asked Questions

1. What are the advantages of liquid-based preparations?

There are two methods for preparing a specimen for cervical cytology screening: the conventional Papanicolaou (Pap) smear (Fig. 4.1) and the liquid-based preparation (LBP) (Fig. 4.2). For conventional Pap smears, either a spatula or an endocervical brush or broom is used to make a smear on a single slide. For liquid-based cytology, the collecting device is placed into a liquid fixative solution. The vial with the liquid is sent to the cytology laboratory and centrifuged. After spin, the cells are trapped onto a filter and then plated in a monolayer onto a glass slide. The ThinPrep® Pap test (Hologic, Inc., Marlborough, MA) was the first of this methodology to be approved by the US Food and Drug Administration for use in cervical cancer screening. Subsequently, the SurePath® Pap test (Becton, Dickinson and Company, Franklin Lakes, NJ) was also approved by FDA for screening and detecting of cervical cancer and its associated precancerous lesions.

- Liquid-based technology provides a more representative cervical sampling than conventional smearing. Only a small portion of the sample taken from the cervix is transferred to the conventional Pap slide. Most of it is discarded with the sampling device, which can lead to inaccuracies in the diagnosis. In liquid-based technology, the sample is added to a vial with transport medium preservative. Samples are then processed using a filtration process for the slide preparation, where the sample is dispersed, randomized, and filtered, and a representative sample is transferred to the slide.
- This processing results in a monolayer preparation that may be analyzed by a computer-based imager and reviewed by a cytotechnologist and/or pathologist.
- The number of inadequate rates was reported to be lower after the introduction of liquid-based technology, and there have been many studies in the literature comparing liquid-based technology with conventional Pap tests. In LBP, the samples are fixed immediately after collection, resulting in fewer artifacts in cellular morphology. The mechanical distortions sometimes associated with smearing are missing. The process reduces obscuring inflammatory cells, mucus, blood, and debris. There is a FDA-approved reprocessing step for unsatisfactory specimens originally sampled with the ThinPrep test. This reprocessing procedure includes a wash step with 10% glacial acetic acid in CytoLyt solution for unsatisfactory ThinPrep specimens.
- Clinical studies have shown that sensitivity of LBP is improved compared with conventional Pap tests. A broad range of sensitivity (30–87%) has been reported with the conventional Pap test. A false-negative rate of about 14–33%, approximately two thirds of which is due to

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_4

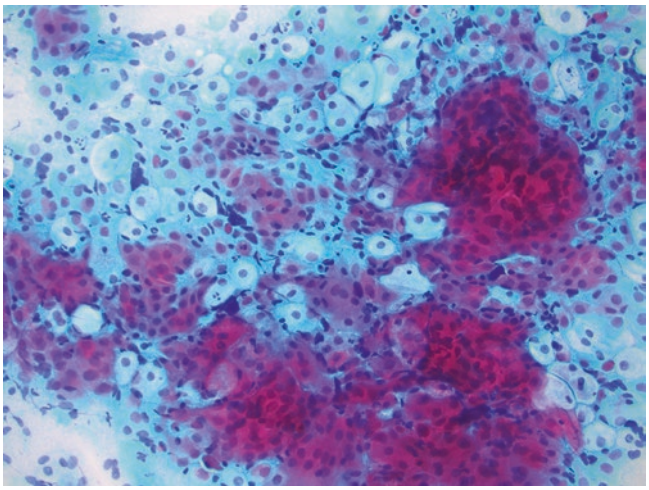

Fig. 4.1 Conventional Pap smear 40×

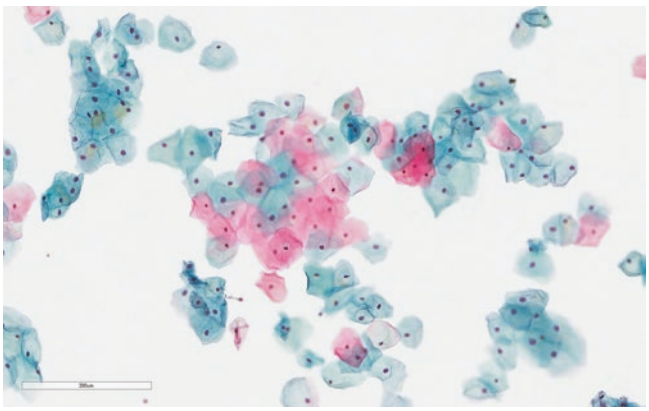

Fig. 4.2 Liquid-based Pap preparation 40×

limitations of sampling or slide preparation, is also reported. The pivotal trial for the ThinPrep Pap test demonstrated that the test provides a 65% increase ($P < 0.001$) in the diagnosis of low-grade squamous intraepithelial lesions (LSIL) or greater cytology and improvement in specimen quality compared with the conventional Pap test ($P < 0.001$). Subsequent studies supported this finding. The SurePath Pap test increases the detection rate of LSIL and high-grade squamous intraepithelial lesions (HSIL) by 47% ($P < 0.0011$) and 116% ($P < 0.0002$), respectively, compared with the conventional Pap test.

- The LBP method provides residual material in the collection media, which can be used for additional/adjunctive testing [e.g., high-risk human papilloma virus (HPV), Chlamydia trachomatis, and Neisseria gonorrhoeae]. The ThinPrep Pap test is approved by the FDA for use in testing for high-risk (HR) human papillomavirus (HPV), Chlamydia trachomatis, and Neisseria gonorrhoeae. The PreservCyt preservation medium is approved for use with molecular-based tests, including Cervista® HPV HR (Hologic, Inc), Cervista® HPV 16/18 genotyping (Hologic, Inc), Hybrid Capture® 2 (hc2; QIAGEN, Inc, Valencia, CA), Gen-Probe APTIMA COMBO 2® CT/NG (Gen-Probe, Inc, San Diego, CA), Roche COBAS AMPLICOR™ CT/NG (Roche Molecular Diagnostics, Pleasanton, CA), and BD ProbeTec™ CT/GC QX amplified DNA assay (Becton, Dickinson and Company). The SurePath Pap test is used in testing for Chlamydia trachomatis and Neisseria gonorrhoeae by using the BD ProbeTec™ CT/GC QX amplified DNA assay. In 2016, The US Food and Drug Administration approved the Roche Cobas HPV test as the first test for human papilloma virus (HPV) that can be used with cervical cells obtained for a Pap test and collected in SurePath preservative fluid.

References: [1–6].

2. What are the specimen adequacy criteria for liquid-based preparations and conventional smears?

The Bethesda System criteria for conventional Pap smears and liquid-based cytology (Nayar & Solomon 2015) should be used and, if a specimen is judged unsatisfactory, the reason should be provided on the cytology report. The presence of the transformation zone should be recorded, although this is not a requirement on its own for a satisfactory sample.

A specimen is satisfactory for evaluation if it has all of the following:

- Appropriate labeling and identifying information
- An "adequate number" of well-preserved, well-visualized squamous epithelial cells
- A comment should be made if there are the following:
  - Representation of the transformation zone
  - The presence of blood or inflammatory cells obscuring parts of the smear

A specimen is judged unsatisfactory for the following reasons:

- There is an inadequate number of well-preserved, well-visualized squamous epithelial cells (less than 8000–12,000 for conventional PAP smears, or 5000 for liquid-based cytology).
- A specimen is termed as inadequate if more than 75% of the squamous cells are obscured by blood, inflammatory cells, lubricant, thick clumps of cells, air-drying artifact, or poorly fixed cells. If less than 75% of the squamous cells are obscured, quality indicator comments should be made.

There are different reference methods to measure cellularity in conventional Pap smears and liquid-based cytology:

- The assessment of the adequacy of the squamous component of conventional Pap smears is known to be highly variable among observers. To increase the interobserver reproducibility, The Bethesda System for reporting cervical cytology provides reference images. The cytotechnologist/cytologist should compare these images to specimens in question to determine if there are sufficient numbers of fields with approximately equal or greater cellularity than the reference images.
- Cellularity is easier to assess on a liquid-based preparation, which is more evenly spread, either by comparison with reference images or by counting well-preserved squamous cells in a defined number of fields at high power (40×) or low power (10×). The minimal number of cells per field depends on the eyepiece used and the preparation diameter (SurePath or ThinPrep). A minimum of 10 microscopic fields should be assessed and the average number of cells per field estimated. If using a FN22 eyepiece and 13 mm preparation diameter, the minimum number of cells per field is 143.2 for 10× objective and 9.0 for 40×. For 20 mm preparation diameter, the minimum number of cells per field is 60.5 for 10× objective and 3.8 for 40×. If using a FN20 eyepiece and 13 mm preparation diameter, the minimum number of cells per field is 118.3 for 10× objective and 7.4 for 40×. For 20 mm preparation diameter, the minimum number of cells per field is 50.0 for 10× objective and 3.1 for 40×.

Transformation zone sampling is not regarded as a criterion for adequacy in The Bethesda System because longitudinal studies failed to indicate that women with negative samples without transformation zone have an increased risk of HSIL over time than women with negative tests that have an adequate transformation zone component. However, the presence or absence of the transformation zone component can be used as a quality assurance measure of the sample taking technique. An adequate number of endocervical cells for both conventional and liquid-based preparations (at least 10 well-preserved endocervical or metaplastic cells, singly or in clusters) confirms sampling of the transition zone.

References: [7–13].

3. How does the hormonal status affect the cells that are normally present?

A cervical smear or liquid-based cytology preparation that has been correctly taken will contain a variety of epithelial cells from the nonkeratinizing squamous epithelium, transformation zone, and endocervical canal. Benign squamous epithelial cells from the ectocervix include superficial squamous cells, intermediate squamous cells, and parabasal squamous cells. The transformation zone and the endocervical canal are represented by glandular endocervical cells and metaplastic squamous cells. The type of epithelial cells seen in a cervical sample is determined by the physiological effect of the sex hormones on the degree of maturation of the cervical epithelium and the location of the squamocolumnar junction. Throughout life, women undergo variations in type and level of sex hormones, which depends on age, pregnancy, menopause, exogenous hormonal intake, and function of pituitary–ovarian–adrenal axis. Estrogen is responsible for the proliferation and maturation of the squamous epithelial cells and the deposition of glycogen within the epithelium. Progesterone causes a rapid desquamation of the upper layer of the squamous epithelium, which leads to the exposition of the intermediate and parabasal cells to the surface.

- In the newborn (up to 8 weeks), there is increased number of intermediate cells with glycogen in the cytoplasm, similar to pregnancy due to the effect of maternal progesterone that crosses the placenta.
- During the infancy (8 weeks to puberty), cytology specimens show mainly parabasal cells similar to postmenopausal period, due to the low levels of estrogen.
- In the reproductive period (menstrual age), the type and the proportion of the different types of squamous cells depend on the day of the menstrual cycle.
  - During the menstrual phase (days 3–5), there is a slight predominance of the intermediate cells over superficial cells with RBCs, degenerated endometrial cells, and dirty background.
  - During the proliferative phase or preovulatory phase (days 5–14), the increased estrogen levels lead to gradual increase of superficial cells.
  - The increased levels of progesterone secreted by corpus luteum during the secretory or postovulatory phase (days 15–28) cause an increase in the intermediate cells, which are the predominant cell type.
- During pregnancy, marked increase of progesterone leads to increase number of intermediate cells.
- Menopause is associated with decrease in the levels of both estrogen and progesterone.
  - Early menopause: most cells are intermediate cells with progressive increase in the number of parabasal cells.
  - Late postmenopausal period: there is complete atrophy of vaginal and ectocervical epithelium with no intermediate and superficial cells. Predominantly parabasal cells either as discrete rounded cells or as sheets of cells are present. The presence of endocervical cells is diminished because of the inversion of the transformation zone. Inversion causes the endocervical area to move deeper into the endocervical canal where sampling can be out of reach of the spatula/brush.

As the cervical squamous epithelium, the vaginal squamous epithelium also responds to systemic levels of estrogen by progressively "maturing." This observation is the basis of the "Maturation Index" (MI). The MI, however, is not sensitive or specific and has largely been replaced by serum hor-

monal assays. A scraping of the mid-third lateral area of the vaginal wall mucosa, where the degree of maturation is hormone dependent, produces the best specimen. The MI is a ratio obtained by performing a random cell count of the three major types of squamous epithelium: parabasal, intermediate, and superficial cells. The MI is reported as relative percentages of these three cell types as a ratio: parabasal %: intermediate %: superficial %. The response of the squamous epithelium to various hormonal stimuli shows great variations from patient to patient and day to day in an individual patient. The only two absolute cell patterns are as follows: predominance of superficial cells that indicates the presence of estrogen (Fig. 4.3) and predominance of parabasal cells that indicates the absence of estrogenic stimulation (Fig. 4.4).

References: [14, 15].

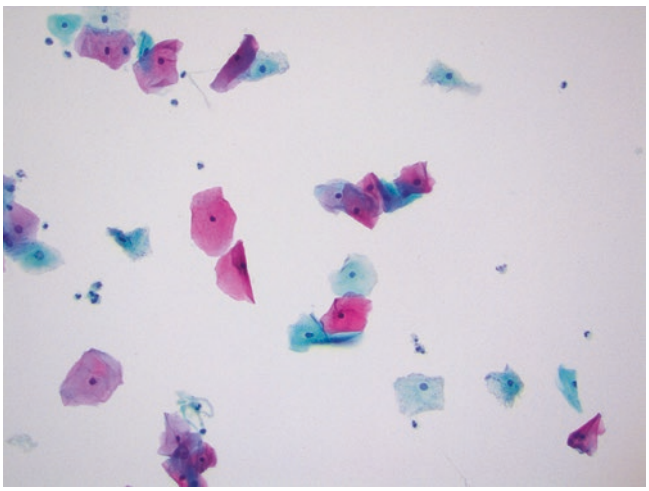

Fig. 4.3 Predominance of superficial squamous cells with small mature nuclei and abundant cytoplasm indicating the presence of estrogen stimulation 20×

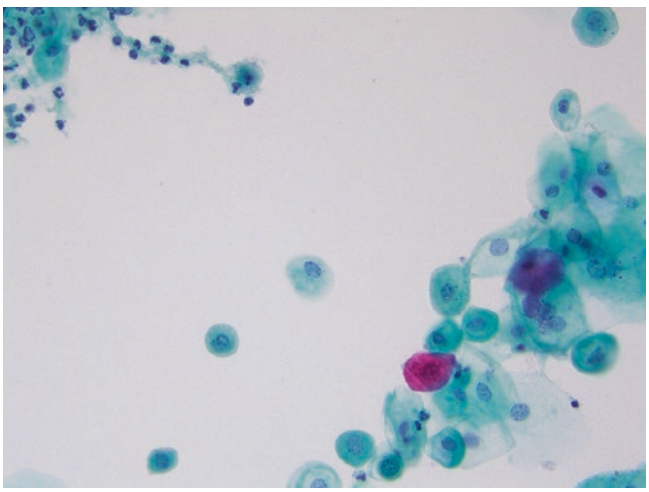

Fig. 4.4 Predominance of parabasal squamous cells indicating the absence of estrogen stimulation 40×

4. What are the clinically significant and commonly observed microorganisms? And what are the associated squamous changes?

The normal vaginal flora consists of predominately *Lactobacillus* sp. – a thin rod-shaped bacteria clinging to the squamous cells. Different factors can cause a shift in the vaginal flora to a predominance of other bacteria, the most common one being *Gardnerella vaginalis.* Cytology slides show squamous cells heavily covered by small coccobacilli, called "clue cells" (Fig. 4.5). There are predominantly intermediate cells, and the background is granular containing abundant coccobacilli and inflammatory cells. The squamous cells may show mild nuclear enlargement and a small perinuclear hallo.

There is a wide range of microorganisms, which can give inflammation and reactive changes in the epithelial cells. In some cases, the actual microorganism can be identified on the slide or will produce a distinctive cytopathic effect allowing identification of the specific organism. Specific infectious agents (besides the HPV) that are reported in cytology are *Trichomonas vaginalis*, *Candida albicans*, *Herpes simplex virus,* and sometimes *Actinomyces* sp.

- *Trichomonas vaginalis*: Pear-shaped or rounded flagellated protozoan with a single karyosome, coating squamous cells or single in the background (Fig. 4.6a, b). Reactive changes in the squamous cells include nuclear enlargement and small perinuclear halo.
- *Candida albicans*: Pseudohyphae and yeast are often seen in clusters of squamous cells where they align around the hyphae, forming so-called "shish kebab" structures (Fig. 4.7). Changes in the squamous cells include nuclear enlargement and small perinuclear halo.
- *Herpes simplex virus*: The classic viral cytopathic changes include cytomegaly, multinucleation, molding of the

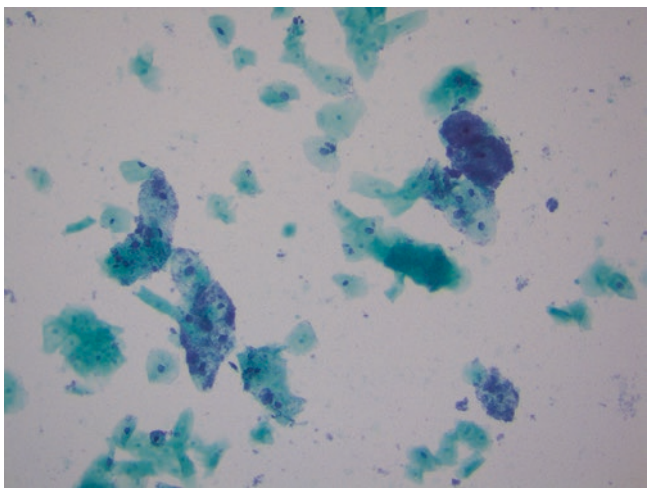

Fig. 4.5 "Clue cells" squamous cells covered by small coccobacilli 40×

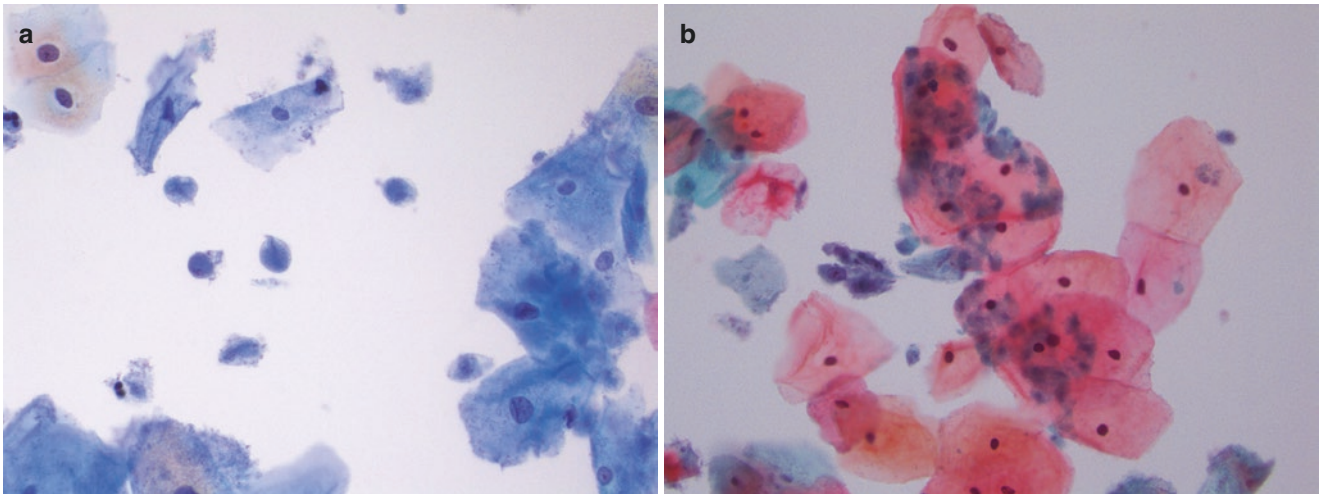

Fig. 4.6 (**a**) *Trichomonas vaginalis*. Pear-shaped protozoan with a single karyosome 60×. (**b**) *Trichomonas vaginalis* coating squamous cells and single in the background 40×

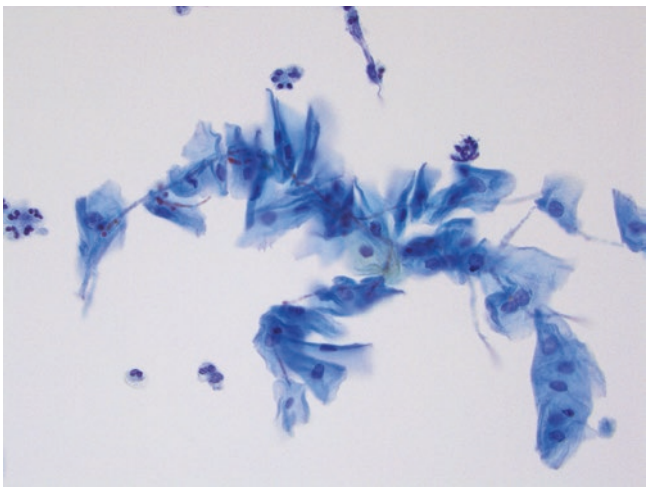

Fig. 4.7 *Candida albicans*. Pseudohyphae in clusters of squamous cells forming so-called shish kebab structures 40×

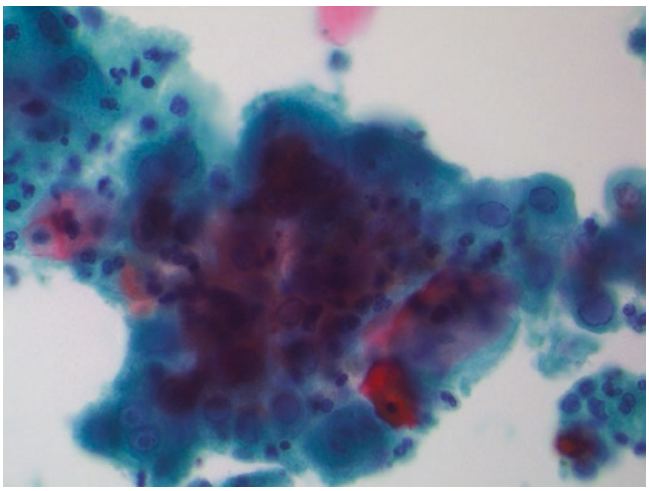

Fig. 4.8 *Herpes simplex virus*. Multiple cells with glassy nuclear clearing and margination of the chromatin. In the left lower corner, two cells with nuclear inclusions "Cowdry bodies" can be seen. 60×

nuclei, and glassy nuclear clearing with margination of the chromatin. Nuclear inclusions called "Cowdry bodies" can be seen as well (Fig. 4.8).

- *Actinomyces* sp.: Often associated with the presence of IUD. Characteristic wooly balls of filamentous bacteria are seen (Fig. 4.9). The squamous and glandular epithelium can show nonspecific reactive changes.

The importance to recognize these microorganisms is not only because they can give a clinically significant infection but also because they can cause reactive cytologic changes that can be mistaken for intraepithelial neoplasia. One of the pitfalls is the presence of a perinuclear halo in the squamous cells that can be mistaken with the koilocytic change associated with HPV infection (Fig. 4.10). The inflammatory halos are much smaller and not that sharp. The enlarged nucleus often seen in infectious reactive changes lacks the characteristics of HPV infection – significant enlargement (three times the size of the intermediate cell), hyperchromasia, and membrane irregularity. It is important to remember, though, that the infection and the dysplasia can coexist.

References: [16–19].

5. What morphologic findings can be considered as reactive/reparative changes related to the presence of inflammation?

Cellular changes seen in cervical specimens in the background of inflammation are a common source of diagnostic pitfalls. The differential diagnosis includes glandular atypia

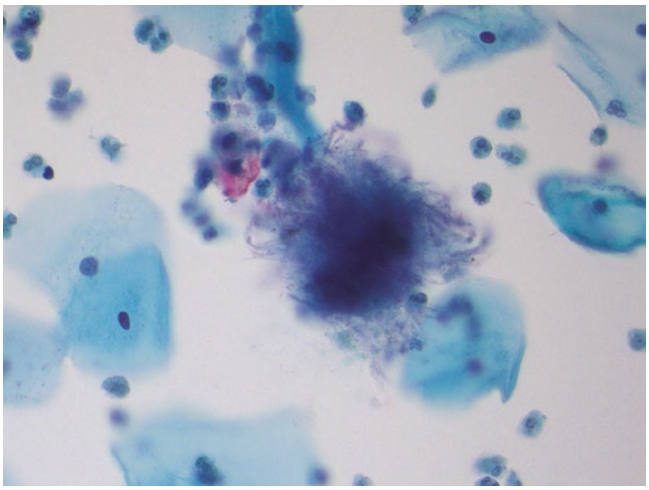

Fig. 4.9 *Actinomyces* sp. Characteristic wooly balls of filamentous bacteria 60×

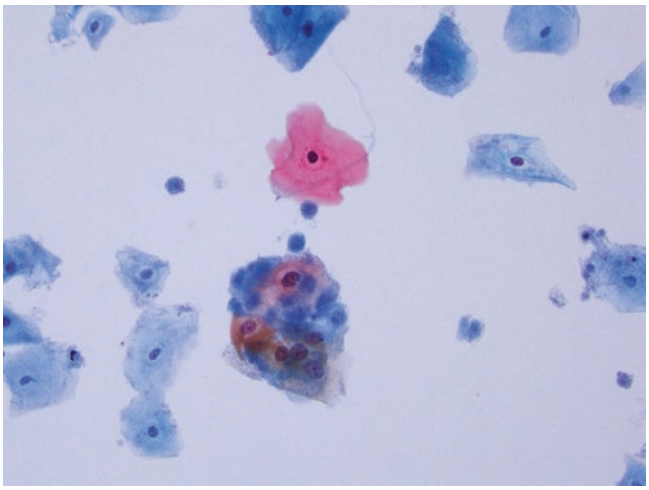

Fig. 4.10 Perinuclear inflammatory halo in the squamous cells. The inflammatory halos are small and not sharp, the nuclei are small, with regular membrane and fine chromatin 40×

and squamous intraepithelial lesions. Commonly seen reactive changes in association with inflammation are as follows:

- A variable degree of nuclear enlargement is present in the squamous and endocervical components. Sometimes, the size of the nucleus can reach the range seen in squamous intraepithelial neoplasia. Nuclear size variation can be seen in one cellular group, giving an impression of pleomorphism.
- Binucleated and multinucleated cells can be present.
- Mild hyperchromasia of the chromatin and single or multiple small prominent nuclei can be seen.
- Cytoplasmic changes may include small perinuclear halo, vacuolization, and polychromasia.

Among all benign cellular changes, repair frequently elicits a false-positive laboratory results. Vice versa underdiagnosing epithelial abnormalities as repair is a common source of false-negative Pap test results. Repair is characterized by the following:

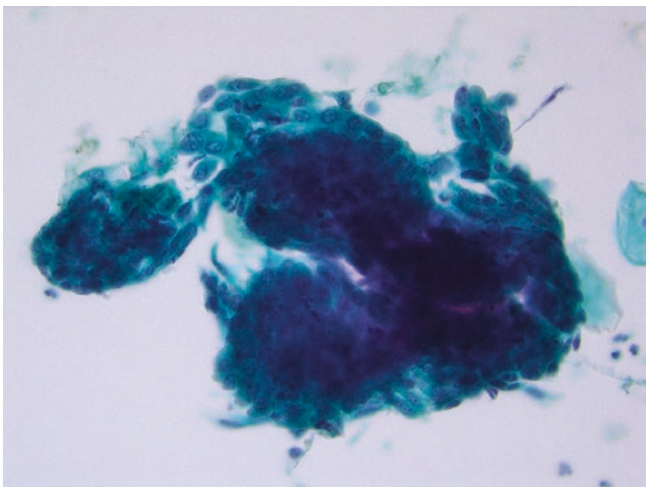

Fig. 4.11 Repair. Cohesive group of cells with well-defined borders, enlarged nuclei, finely granular chromatin, prominent nucleoli, normal N/C ratio, and characteristic streaming of the nuclei and interdigitation of the cytoplasm ("school of fish" appearance) 40×

- Cohesive monolayers of cells with well-defined or indistinct borders, abundant cytoplasm, uniform enlarged nuclei, finely granular and evenly distributed chromatin, prominent nucleoli, and normal N/C ratio.
- Streaming of the nuclei, and streaming and interdigitating cytoplasmic processes, gives the characteristic appearance of "school of fish" (Fig. 4.11).
- Very often intracytoplasmic polymorphonuclear leukocytes can be seen.

The main features that will help to differentiate reactive/reparative changes from dysplasia are as follows:

- Smooth nuclear membrane.
- Vesicular or hypochromatic nuclei.
- Finely granular and evenly distributed chromatin.
- Most of the time well-defined cytoplasmic borders.
- If a perinuclear halo is present, it is small without peripheral thickening, characteristic for koilocytes.
- Streaming of the cells in the groups.

Real diagnostic challenges are the PAP specimens in which both features of repair and dysplasia are seen. The presence of atypical reparative cells can be present in a wide variety of reactive and neoplastic conditions. The Bethesda System recognizes these changes as "atypical reparative changes" and recommends categorizing these cases as "atypical squamous cells" (ASCUS and ASC-H) or "atypical glandular cells" (AGC). Reflex molecular analysis for high-

risk HPV performed on liquid-based cytology samples would be helpful in predicting the possible association with an underlying intraepithelial lesion or carcinoma.

References: [20–22].

6. What are the characteristics of reactive cellular changes associated with radiation?

Radiation exposure, such as that occurring during radiotherapy for cervical cancer, produces significant changes in the appearance of the cervical and vaginal epithelium. Such changes can be mistaken for intraepithelial neoplasia or carcinoma. They can disappear with time or persist for many years. It is important to keep in mind that radiation changes and residual/recurrent dysplasia/carcinoma can coexist. The characteristic morphologic findings include the following:

- Cytomegaly – markedly increased cell size (both cytoplasm and nucleus) without change in the nuclear to cytoplasmic ratio
- Bizarre cell morphology
- Nuclear pleomorphism – some small and some large nuclei in the same cell group
- Binucleation and multinucleation
- Slight nuclear hyperchromasia (smudgy chromatin) with single or multiple prominent nucleoli
- Nuclear vacuolization (Fig. 4.12)
- Cytoplasmic vacuolization and polychromasia (two-tone cytoplasm), sometimes intracytoplasmic neutrophils

If the radiation treatment was given for squamous cell carcinoma or adenocarcinoma of the cervix, the differential diagnosis is recurrent/residual carcinoma with radiation changes. Morphologically, postradiation squamous intraepithelial lesions are diagnosed using the usual criteria. The high-grade dysplasia is often of keratinizing type, with cells showing high nuclear to cytoplasmic ratio and dark chromatin. However, in the setting of postradiation, it may be difficult to differentiate low-grade dysplasia from radiation effect. It is highly recommended to correlate with colposcopic and pelvic examination findings.

References: [20–23].

7. What differentiates atrophic changes from squamous intraepithelial lesion?

Atrophy is a physiological process, as a result of lack of hormonal stimulation, which is needed for the maturation of the squamous epithelium. It can be seen in postmenopausal, pregnant, postpartum, and contraceptive-use patients. Atrophy-related epithelial changes in perimenopausal and postmenopausal women often pose a diagnostic difficulty, related to the increased number of basal and parabasal cells, with an eventual diagnosis of ASCUS or ASC-H. Studies showed that a diagnosis of ASCUS and ASC-H in women over 50 years old is less likely to be associated with dysplasia than that diagnosis in younger patients. Giving topical estrogen treatment before obtaining the specimen will cause the atrophic cells to mature, but dysplastic cells will not respond, which can decrease the number of false-positive cytology. High-risk HPV co-testing is also recommended in diagnostically challenging cases. The diagnostic features for atrophy are as follows:

- Increased number of parabasal and basal cells, which form sheets and syncytial-like aggregates with preserved nuclear polarity and little overlap (Fig. 4.13).
- Single parabasal cells may predominate (Fig. 4.14).
- Naked nuclei may be seen due to autolysis.

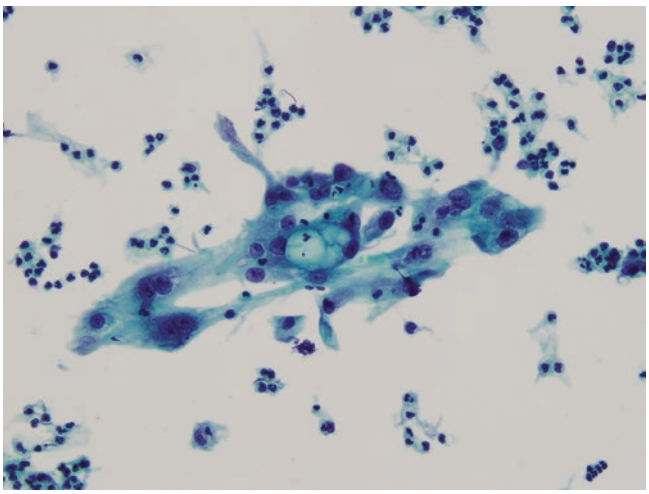

Fig. 4.12 Radiation changes. Cytomegaly, bizarre cell morphology, nuclear hyperchromasia (smudgy chromatin), and nuclear and cytoplasmic vacuolization 40×

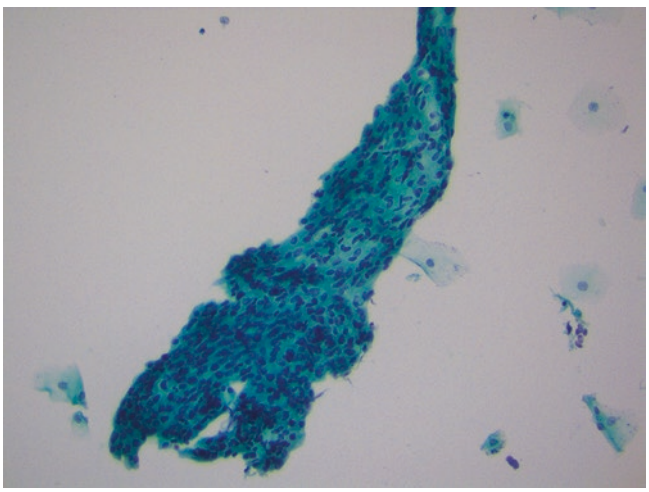

Fig. 4.13 Parabasal and basal cells forming crowded sheets with increased nuclear to cytoplasmic ratio, but preserved nuclear polarity and little overlap 20×

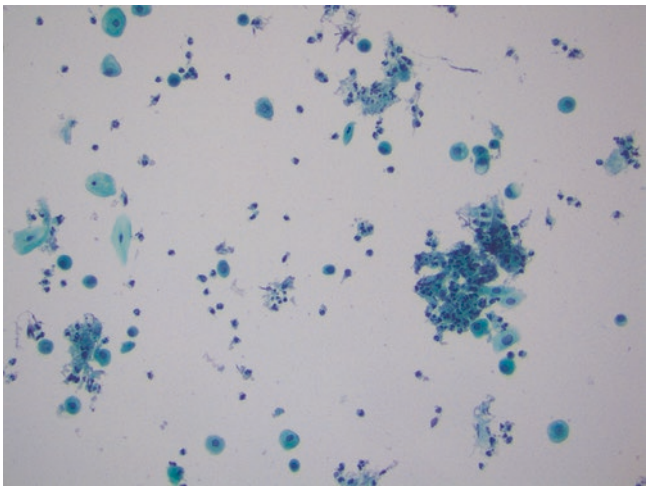

Fig. 4.14 Predominance of single parabasal cells 20×

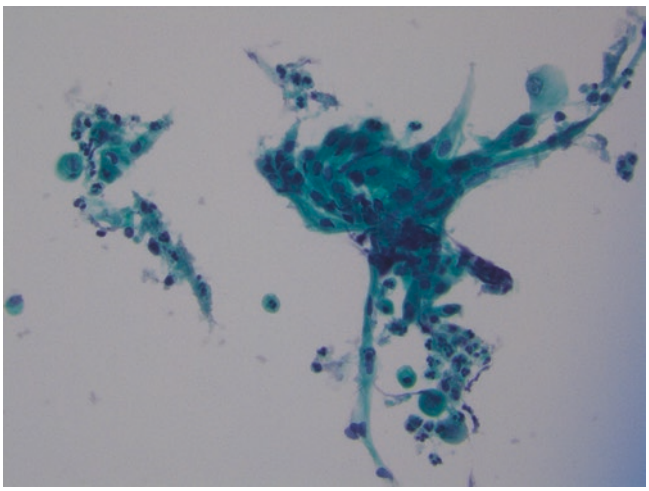

Fig. 4.15 Atrophic vaginitis with inflammation, debris, and old blood, resembling tumor diathesis. Reactive/reparative changes with streaming of the nuclei and cytoplasm "school of fish" appearance is also present 20×

- Nuclear enlargement with increased N/C ratio but uniform smudgy chromatin and smooth nuclear contour.
- Pseudokeratinized cells (orangeophilic cytoplasm) due to degeneration.
- Severe atrophy can show dirty background with inflammation, debris, old blood, and blue blobs (amorphous basophilic material), called atrophic vaginitis, which resemble tumor diathesis (Fig. 4.15).
- Histiocytes with round to epithelioid nuclei and giant cells may be seen.

Due to the immediate fixation of the liquid-based preparations, usually there is less nuclear enlargement and naked nuclei. Granular background material in atrophic vaginitis tends to clump and cling on the cells, giving a cleaner background. In conventional preparations, air-drying artifact can give more prominent nuclear enlargement. In general, in smears, there are more debris and blue blobs, giving the impression of "dirty" or "necrotic" background.

Granular background, enlarged nuclear size, hyperchromasia, and abnormal chromatin pattern can be associated with atrophic changes. The most reliable morphological features favoring HSIL in atrophic smears include increased number of abnormal single cells with high nuclear/cytoplasmic ratio and irregular nuclear membrane.

References: [20, 24–26].

8. What differentiates squamous metaplasia from squamous intraepithelial lesion?

Squamous metaplasia is a common response to hormonal changes and chronic irritation in glandular endocervical epithelium. It is a common finding in almost every cervix and is located in the transformation zone. Metaplasia is characterized by replacement of endocervical epithelium by subcolumnar reserve cells, which differentiate into immature and then mature squamous epithelium. Metaplastic cells may be immature (parabasal-like cells), intermediate, or mature (differentiated intermediate or superficial cells). They are characterized by the following:

- Larger mean nuclear size, about 50 μm (similar to the parabasal cell).
- Round to oval nucleus with smooth nuclear membrane (slight irregularity in the nuclear shape and membrane can be seen in immature squamous metaplasia).
- Fine, evenly distributed chromatin.
- Distinct cytoplasmic membrane.
- Dense, cyanophilic cytoplasm often vacuolated in immature squamous metaplasia.
- "Spider cells," cells with spindled cytoplasmic projections, most commonly seen in conventional smears.
- Metaplastic cells can be single or arranged in sheets of cells with polygonal distinct borders (Fig. 4.16a, b).

Distinguishing between squamous metaplasia and high-grade squamous intraepithelial lesion can be extremely difficult, given the presence of increased N/C ratio in both entities. Nuclear enlargement without any other nuclear abnormalities, like irregular contours or dark chromatin, favors benign squamous metaplasia. A high N/C ratio together with hyperchromasia and nuclear irregularities is consistent with high-grade intraepithelial lesion or ASC-H.

References: [20–23].

9. What are the characteristics and significance of the keratotic cellular changes?

The squamous mucosa covering the cervix is normally non-keratinized. Keratinization can occur as a result of chronic

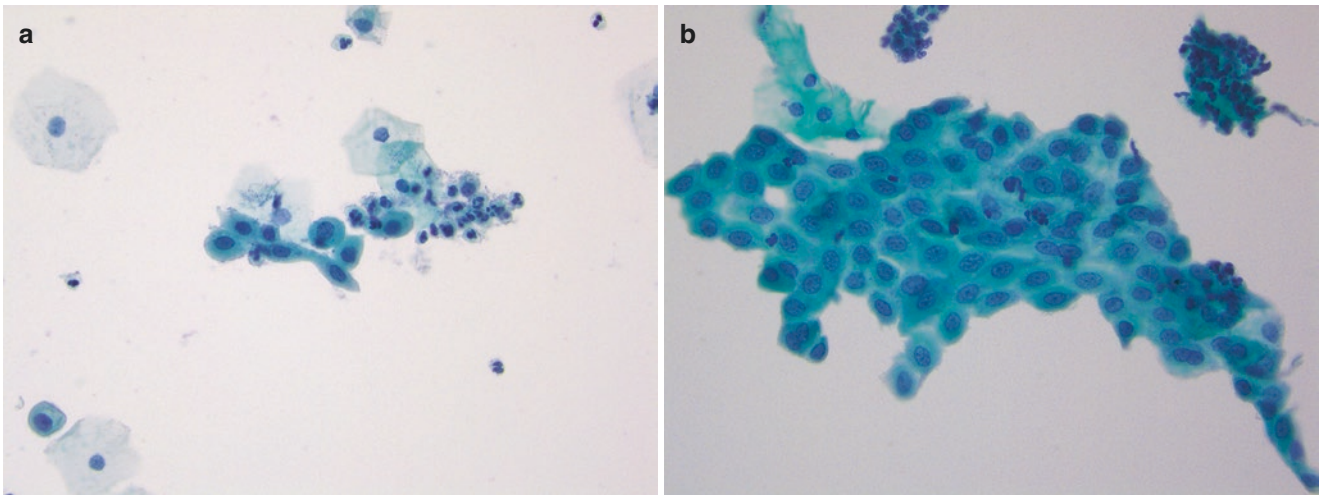

Fig. 4.16 (**a**) Metaplastic cells showing higher nucleus to cytoplasmic ratio, darker chromatin, but smooth nuclear membrane, fine chromatin, and round dense cytoplasm 40×. (**b**) Metaplastic cells arranged in sheet with polygonal distinct borders, smooth nuclear membranes, and no overlap 40×

irritation as a protective mechanism, and it is also seen in association with human papilloma virus (HPV) infection. It is a result of hypermaturation of the squamous epithelium and can be represented by hyperkeratosis and parakeratosis. Both keratotic changes are commonly detected in cervical specimens. While there is no morphologic or clinical confirmation that hyperkeratosis corresponds to precursor lesions, keratotic changes can take place in association with cervical neoplasia. The key morphologic features of hyperkeratosis are as follows:

- Anucleated mature polygonal squamous cells can have empty spaces or "ghost nuclei."
- Orangeophilic, yellow or pink cytoplasm.
- Can contain keratohyalin granules.
- Seen singly or in overlapping thick sheets (Fig. 4.17).

Parakeratosis cells are characterized by the following:

- Sheets of cells with dense orangeophilic or eosinophilic cytoplasm with small dense (pyknotic) nuclei
- Single round, oval, polygonal, or spindle cells with dense orangeophilic cytoplasm and dark small nuclei (Fig. 4.18a)
- Whorls of keratinized cells (Fig. 4.18b)

All the abovementioned changes are considered benign reactive, unless the cells demonstrate nuclear pleomorphism, irregularities, severe hyperchromasia, and enlargement. These findings, also called "atypical parakeratosis", are consistent with epithelial cell abnormality. A diagnosis of ASCUS or squamous intraepithelial neoplasia should be considered, depending on the degree of atypia. While isolated, anucleated squamous cells may have no clinical importance; thick plaques of hyperkeratotic cells with irregular contours may rarely be associated with underlying squamous cell carcinoma.

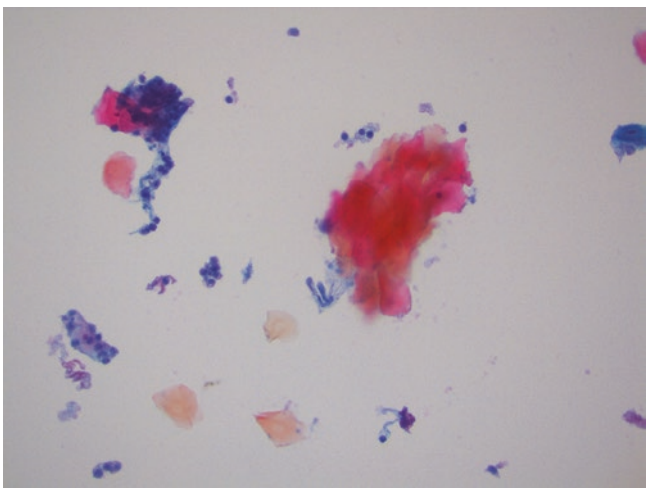

Fig. 4.17 Hyperkeratotic squamous cells. Thick sheets of orangeophilic, anucleated mature polygonal squamous cells. In the left lower corner, three single cells with empty nuclear spaces or "ghost nuclei" 40×

References: [20, 27, 28].

10. What differentiates tubal metaplasia from endocervical atypia or neoplasia?

Tubal metaplasia is a metaplastic process, which involves endocervical glands, and it is a frequent finding in the upper endocervical canal and lower uterine segment. The normal simple columnar endocervical epithelium is replaced by epithelium that recapitulates benign fallopian tube epithelium,

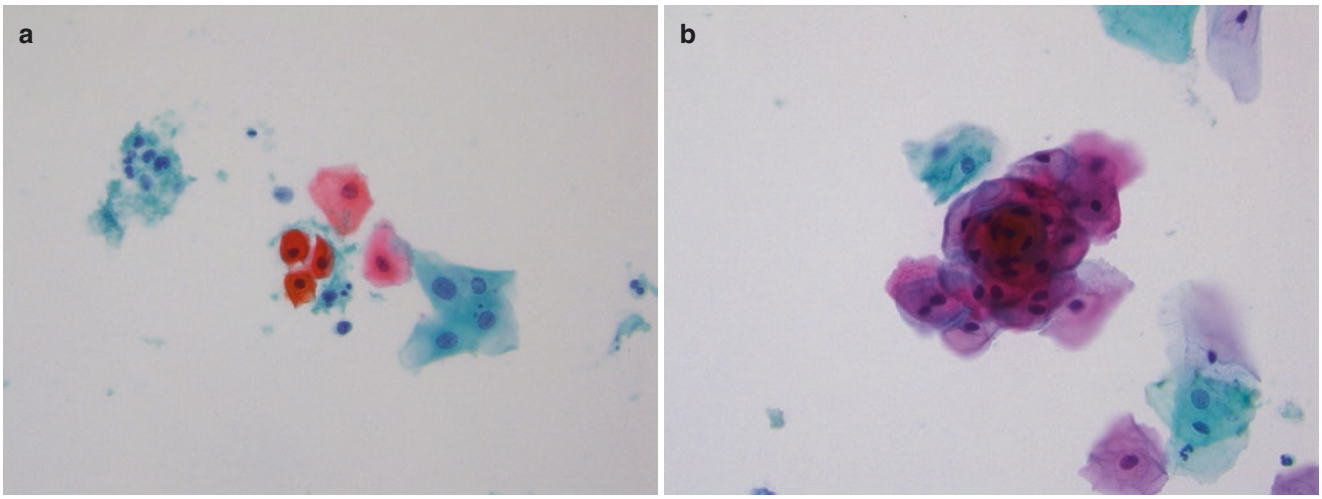

Fig. 4.18 (**a**) Single polygonal parakeratotic cells with dense orangeophilic cytoplasm and dark small nuclei 40×. (**b**) Whorls of keratinized cells 40×

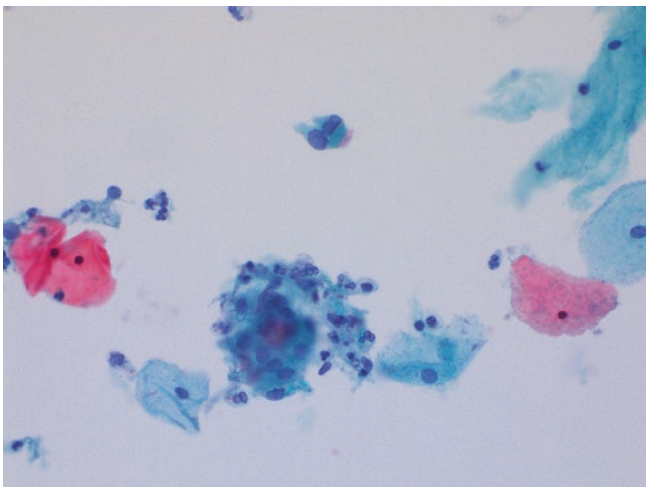

Fig. 4.19 Tubal metaplasia. Two ciliated columnar cells with large pleomorphic nuclei 40×

composed of three cell types: secretory cells, ciliated cells, and peg cells. This metaplastic process is a potential pitfall in the cytologic diagnosis of endocervical glandular atypia/dysplasia. The morphologic features that favor benign metaplastic process are as follows:

- Small groups or crowded groups of ciliated columnar cells.
- Vacuolated or granular cytoplasm may have goblet cell change.
- Round to oval nuclei, which may be pleomorphic and enlarged (higher N/C ratio).
- Dark chromatin, but evenly distributed without prominent nucleoli.
- The presence of cilia and/or terminal bar is characteristic (Fig. 4.19).

Because of the nuclear characteristics, like nuclear enlargement, dark chromatin, and stratification, tubal metaplasia is one of the most common reasons for the diagnosis of atypical glandular cells in cervical cytology. The atypical/dysplastic endocervical cells are arranged in sheets, dyscohesive clusters, and crowded groups with palisading and rosette formation. The nuclei are pleomorphic, with irregular contours and prominent nucleoli, a feature not seen in tubal metaplasia. However, the most reliable benign finding is the presence of the cilia and terminal bars.

References: [20, 29, 30].

11. What are the pregnancy-related changes that can be misinterpreted as neoplastic abnormalities?

Pregnancy-related epithelial and nonepithelial cell changes can make interpretation and distinguishing normal from abnormal findings challenging in cytology. Both squamous and glandular epitheliums undergo changes, due to the altered hormonal stimulation. The endocervical glands become hyperplastic and hypersecretory and can have changes consistent with Arias-Stella reaction. This reflects in increased number of endocervical cells. The transformation zone moves outward, and more cells with morphologic features of immature squamous metaplasia can be seen. The decreased estrogen stimulation leads to incomplete maturation of the squamous epithelium with predominance of the intermediate cells (Fig. 4.20). The changes in squamous epithelium include the following:

- Predominance of boat-shaped intermediate squamous cells with glycogen-rich cytoplasm (navicular cells) with folded edges (Fig. 4.21)
- Eccentric vesicular nuclei with delicate chromatin

The navicular cells may mimic koilocytes, because of the clearing of the cytoplasm, but they lack the sharp outline of

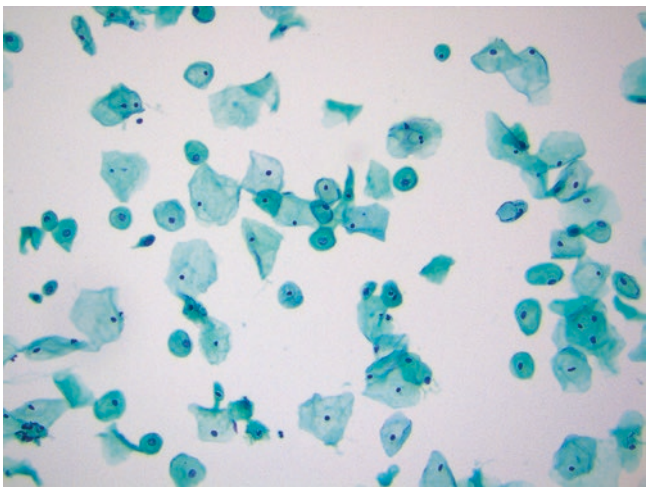

Fig. 4.20 Pregnancy-related changes. Predominance of intermediate squamous cells 40×

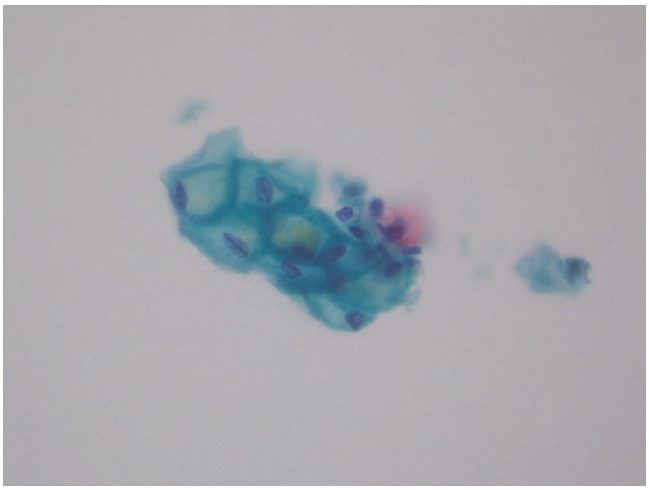

Fig. 4.21 Boat-shaped intermediate squamous cells with glycogen-rich cytoplasm (navicular cells) 60×

the perinuclear halo with the condensation of the cytoplasm at the periphery. More importantly, there are no dysplastic nuclear changes such as significant nuclear enlargement, irregularities, and hyperchromasia.

Endocervical cell changes in pregnancy usually resemble reactive/reparative changes related to other causes. In some cases, the reactive glandular atypia cannot be distinguished from dysplastic changes, rendering a diagnosis of atypical glandular cells. Commonly seen alterations are as follows:

- Increased number of endocervical cells
- Nuclear enlargement, multinucleation, and small prominent nucleoli

Arias-Stella reaction is an exaggerated physiologic response of the glandular epithelium to the increased hormonal levels. These cells are rarely seen in cervical cytology specimens. When present, they can be easily mistaken for in situ or invasive adenocarcinoma. Obtaining a history of pregnancy or postpartum status is very important to avoid a false-positive diagnosis. Arias-Stella cells are characterized by the following:

- Usually single cells or in small clusters of cells with nuclear crowding and overlap
- May have vacuolated cytoplasm
- Enlarged nuclei, dark smudgy chromatin, prominent nucleoli, nuclear irregularities (grooves and pseudoinclusions)

Another rare finding in cervical samples during pregnancy or postpartum that can mimic epithelial cell abnormalities are decidual cell, derived from hormonally stimulated endocervical stroma. The features of Decidual cells are as follows:

- Large, oval to polygonal, single or in small clusters
- Abundant granular or finely vacuolated cytoplasm with cytoplasmic extensions
- Large nuclei, at least 3–4 times the size of the intermediate cell, sometimes multinucleated
- Finely granular chromatin with prominent basophilic nucleoli and smooth contours

Decidual cells can be misinterpreted as ASCUS or LSIL, when the cytoplasm is abundant; or ASC-H or HSIL, when the nuclei are markedly enlarged giving a higher N/C ratio. In general, the decidual cells are larger than the dysplastic cells, with fine evenly distributed chromatin and smooth nuclear membrane.

Rarely during the pregnancy, postpartum or abortion, cytotrophoblasts or syncytiotrophoblasts can be present in the cervical specimens. Cytotrophoblasts are rarely recognized as such because of their resemblance of small metaplastic squamous cells or endometrial cells. Since having high N/C ratio and hyperchromatic nuclei, they can be mistaken for HSIL. Syncytiotrophoblasts are large cells with up to 50 or more uniform nuclei and granular cytoplasm tapering at one end of the cell. The differential diagnosis is herpes infection, but the nuclei of syncytiotrophoblasts are overlapping, not molding, and lack the ground-glass inclusions. Both types of cells, cytotrophoblasts and syncytiotrophoblasts, are usually seen in an inflammatory and bloody background.

References: [20, 31, 32].

12. Can benign-appearing glandular cells be present in posthysterectomy specimens?

Although glandular cells are not expected to be seen in posthysterectomy vaginal cuff specimens, studies show that benign-appearing glandular cells can be present in about 13% of such PAP tests. The exact origin of these cells often remains unknown, and different sources have been reported

in the literature, including vaginal endometriosis, vaginal adenosis, rectovaginal fistula, tubal prolapse, and mesonephric duct remnants. The presence of glandular cells shows a strong association with inflammation/repair as a background finding, and without the abovementioned sources, most probably represent a regenerative process in response to hysterectomy or therapy. The presence of glandular cells should not be misdiagnosed as a recurrent or a new primary adenocarcinoma in the absence of cytologic atypia.

The glandular cells can be found singly, in rows and honeycomb groups. The glandular cells can have the morphology of endocervical-type cells, squamous metaplastic-like cells, or goblet-type cells.

If a recurrent or new adenocarcinoma is present, the glandular cells will demonstrate malignant features such as irregular nuclear contours, increased nuclear to cytoplasmic ratio, hyperchromasia, and marked nuclear pseudostratification and overlap.

References: [33–35].

13. How and when to report endometrial cells?

Benign endometrial cells are present in a wide range of Pap tests depending on a patient's age and menstrual status. The finding usually represents a physiologic shedding, and it is most commonly seen in the first half of the menstrual cycle or during the menstrual period in premenopausal women. In postmenopausal women, the presence of endometrial cells is an abnormal finding and may be a sign of endometrial neoplasia. This is the reason why the 1991 Bethesda System for Reporting Cervical Cytology recommended reporting benign endometrial cells in cervical cytology in postmenopausal women. Very often the information regarding menstrual status is not available or is incorrect, and the Bethesda system 2001 recommends reporting normal endometrial cells in women 40 years or older. As many of these women are menstruating, routine biopsy at or after age of 45, may lead to unnecessary procedures for benign endometrial cells. To improve the predictive value of benign endometrial cells, the 2015 Bethesda System for Reporting Cervical Cytology recommends reporting normal endometrial cells in women 45 years or older.

Studies show that the incidence of clinically significant endometrial lesions associated with the presence of endometrial cells in Pap tests in women aged 40–50 years is very low, and women should undergo endometrial sampling only when additional clinical indicators are present. Although optional, the Bethesda System for Reporting Cervical Cytology recommends the use of educational note, explaining the significance of the finding. It should emphasize that the detection of exfoliated endometrial cells is usually a benign finding and only a small proportion of women have endometrial abnormalities. If the date of the last menstrual cycle is provided, the comment may state that the findings are consistent with the menstrual history.

It is important to emphasize that all symptomatic women and women with atypical endometrial cells on Pap should undergo endometrial sampling to exclude endometrial hyperplasia or carcinoma.

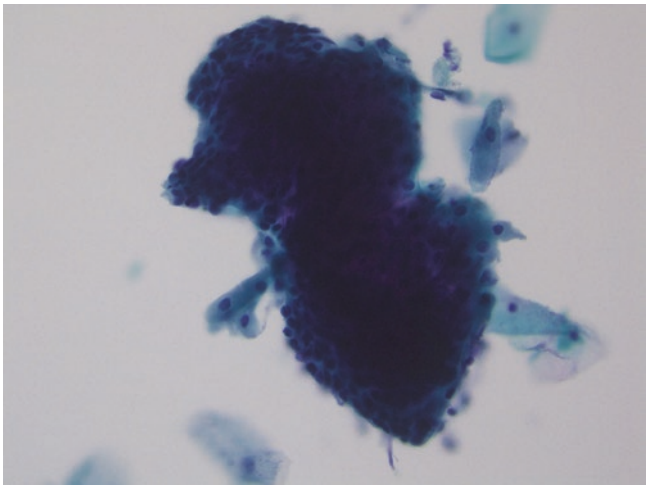

Fig. 4.22 Endometrial cells. Three-dimensional cluster of small round cells with dark nuclei and scant cytoplasm 60×

Characteristic features of exfoliated endometrial cells are (Fig. 4.22) as follows:

- Tight ball-like clusters, three-dimensional clusters, and rarely single cells.
- Cells are small, cuboidal, or round.
- Nuclei are small and dark, similar in size to the size of an intermediate squamous cell nucleus.
- Inconspicuous nucleoli.
- Cytoplasm scant and finely vacuolated.
- Ill-defined cell borders.
- Exodus – spherical cell cluster with a central core of tightly packed stromal cells and periphery of glandular cells.

References: [36–38].

Case Presentation

Case 1

Learning objectives:

1. Describe cytologic changes associated with intrauterine contraceptive device (IUD) on cervical Pap smears.
2. Distinguish IUD-related features from high-grade intraepithelial lesion (HSIL).
3. Distinguish IUD-related features from adenocarcinoma.

Case history: The patient is a 38-year-old woman who presented to her family doctor for removal of IUD

and an annual Pap smear. Her last Pap smear was negative for intraepithelial lesion (NILM) and positive for other high-risk HPV (non-HPV16/HPV18). She also has irregular menses and her last normal menstrual period was 10 years ago.

Cytological findings: Crowded glandular clusters containing cells with mild-to-moderate nuclear enlargement, focally vacuolated ample cytoplasm, and occasional prominent nucleoli. Rare neutrophils are also identified within some groups (Fig. 4.23).

Differential diagnosis: Adenocarcinoma, HSIL involving glands, HSIL, reactive cellular changes associated with IUD.

Final diagnosis: Reactive cellular changes associated with IUD.

Take-home messages:

- IUD cellular changes can mimic endometrial, endocervical, or squamous malignancies. One should exercise caution when examining specimens from patients with current or past history of IUD, as cytologic changes may persist for months after removal.
- Epithelial components may be exfoliated or shedded as single cells, papillary fragments, or clusters. These include endocervical cells, metaplastic squamous cells, and endometrial cells with or without stromal components.
- IUD-induced reactive changes that mimic adenocarcinoma include crowded glandular groups containing cells with large nuclei, increased N:C ratio, prominent nucleoli, hyperchromasia, and cytoplasmic vacuoles. Intracytoplasmic neutrophils can also be seen, which may suggest leukophagocytosis typically associated with adenocarcinoma.
- Single endometrial cells with enlarged nuclei and dark, smudgy degenerated chromatin may also resemble HSIL.
- Other common findings include *Actinomyces* organisms, calcifications, and mixed inflammatory background consisting of neutrophils, lymphocytes, histiocytes, and giant cells.
- Attention to pertinent clinical history may help resolve difficult cases. Also, the presence of an inflammatory reactive background, degenerative changes, rarity of atypical cells, and absence of other squamous dysplastic cells are other helpful clues which favor IUD-related changes. Otherwise, cases may be cautiously assigned an atypical category with recommendation of follow-up and repeat Pap smear.
- Despite concerning cytologic changes, IUD is not associated with development of HSIL or carcinoma.

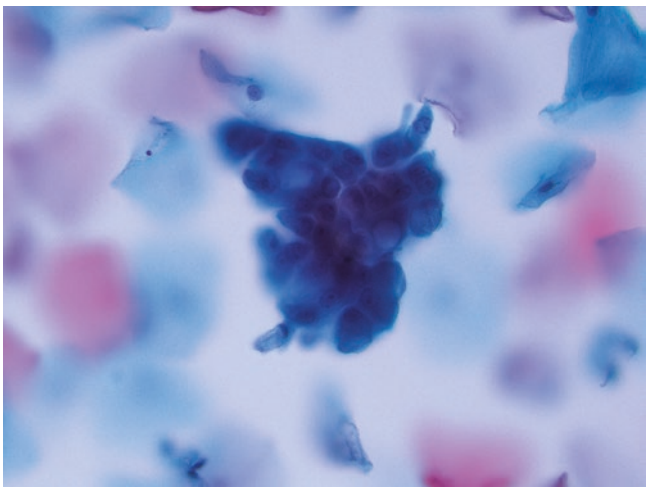

Fig. 4.23 Crowded cluster of cells with moderate nuclear enlargement, focally vacuolated abundant cytoplasm and prominent nucleoli 60×

Case 2

Learning objectives:

1. Recognize cytologic features of follicular cervicitis.
2. Describe potential causes of follicular cervicitis.
3. Distinguish follicular cervicitis from other clinically significant cellular entities.

Case history: The patient is a 50-year-old woman who presents for annual Pap smear. She has no history of abnormal Paps. Her last normal menstrual period was 1 year ago. Since then, she has had occasional irregular bleeding.

Cytologic findings: Loose aggregates of cells with scant cytoplasm, uniform, dark, round nuclei, and indistinct nuclei. Few larger cells with abundant cytoplasm containing basophilic debris "tingible body macrophages" are also seen (Fig. 4.24).

Differential diagnosis: HSIL, follicular cervicitis, endometrial cells present in woman ≥45 years, endometrial adenocarcinoma, and lymphoma.

Take-home messages:

- Follicular cervicitis is chronic inflammation of the cervix with formation of lymphoid follicles with germinal centers.
- Causes of follicular cervicitis may be of noninfectious or infectious etiology. Noninfectious causes include chemical irritation from douches, local mechanical trauma from tampons, IUD, dia-

Reference: [39].

phragms, and pessaries. Bacterial and chlamydial infections are the most common causes of infectious cervicitis. Follicular cervicitis due to *Chlamydia trachomatis* infection is also particularly common in younger women.

- The cytologic features of follicular cervicitis are loose aggregates of reactive lymphoid cells.
- In this case, variable-sized loose clusters were seen. The largest group is depicted in photomicrograph. The cells contain scant cytoplasm with round nuclei and dark chromatin with fine chromocenters, features characteristic of lymphoid cells. Unlike endometrial clusters, the aggregates are not tight and three-dimensional with smooth outlines. Furthermore, the population also lacks features of lymphoma and consists of polymorphous lymphocytes with no cytologic atypia and tingible body macrophages containing nuclear debris. Plasma cells may also be seen in some cases.

References: [40, 41].

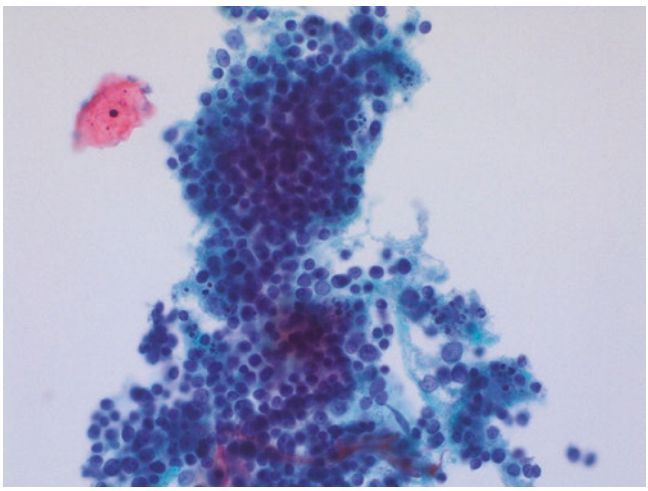

Fig. 4.24 Loose aggregate of cells with scant cytoplasm, dark round nuclei, and indistinct nuclei. Few larger cells with abundant cytoplasm containing basophilic debris "tingible body macrophages" 40×

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Cervical Cytology with Squamous and Glandular Abnormalities

5

Shuyue Ren

Contents

Frequently Asked Questions

1. What are the categories in the squamous cell abnormalities in cervical cytology?

According to the recent third edition of The Bethesda System for Reporting Cervical Cytology, squamous cell abnormalities are categorized into atypical squamous cell (ASC), squamous intraepithelial lesion (low-grade and high-grade), and squamous cell carcinoma. ASC is further separated into atypical squamous cells – of undetermined significance (ASC-US) and atypical squamous cells – cannot exclude high-grade squamous intraepithelial lesion (ASC-H). Low-grade squamous intraepithelial lesion (LSIL) includes both mild dysplasia/cervical intraepithelial neoplasia (CIN 1) and cytopathic effects associated with human papillomavirus (HPV). High-grade squamous intraepithelial lesion (HSIL) includes moderate dysplasia (CIN 2), severe dysplasia (CIN 3), and carcinoma in situ (CIS).

Reference: [1].

2. What are atypical squamous cells (ASC)? What are their cytomorphological features?

Squamous cells demonstrate uncertain diagnostic findings suggestive of squamous intraepithelial lesion (SIL), but which are qualitatively or quantitatively insufficient for a definitive interpretation as SIL (see diagnosis criteria below). The ASC is separated into two categories: atypical squamous cells – undetermined significance (ASC-US) and atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion (ASC-H).

There are three essential features: (1) squamous differentiation; (2) increased nuclear to cytoplasmic ratio; and (3) minimal nuclear changes, which may include hyperchromasia, chromatin clumping, irregularity, smudging, and/or multinucleation.

References: [1, 2].

3. What are atypical squamous cells – undetermined significance (ASC-US)?

ASC-US refers to changes that are suggestive of LSIL.

The cytomorphological features are as follows (Figs. 5.1, 5.2, and 5.3):

1. Atypical squamous cells with mature, intermediate cell-type cytoplasm.
2. Nuclei are approximately two and one half to three times the size of the nucleus of a normal intermediate squamous cell or a squamous metaplastic cell.
3. Slightly increased nuclear to cytoplasmic ratio.
4. Minimal nuclear hyperchromasia and irregularity in chromatin distribution or nuclear shape.
5. Nuclear abnormalities associated with dense orangeophilic cytoplasm (atypical parakeratosis), cytoplasmic changes that suggest HPV cytopathic effect (incomplete koilocytosis) but with absent or minimal nuclear changes.

References: [1–7].

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_5

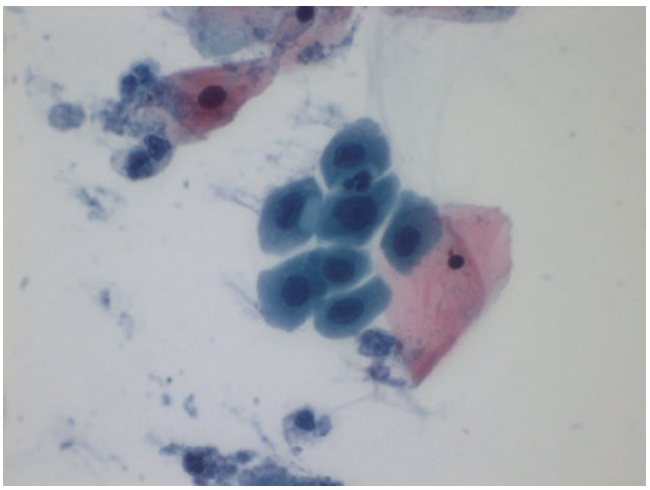

Fig. 5.1 Atypical squamous cells – undetermined significance (ASC-US). Squamous cells with enlarged nuclei, increased nuclear to cytoplasmic ratio, and mild nuclear irregularity

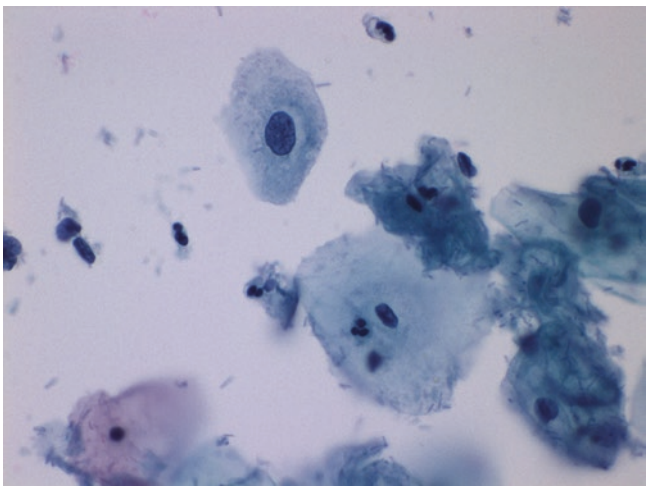

Fig. 5.3 Atypical squamous cells – undetermined significance (ASC-US). Intermediate squamous cells with nuclear enlargement, hyperchromasia, and nuclear membrane irregularities

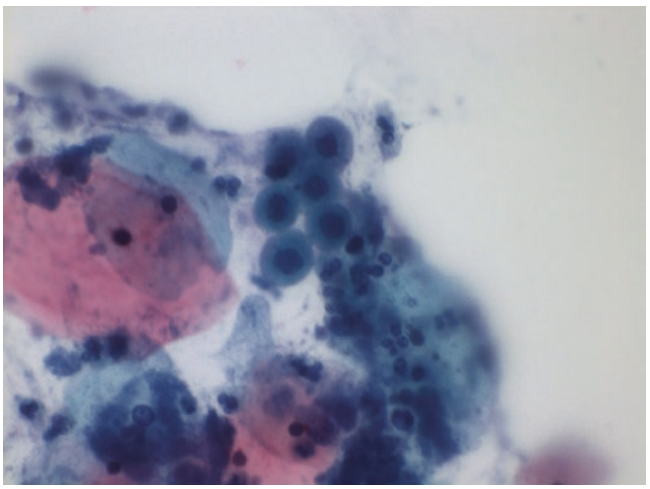

Fig. 5.2 Atypical squamous cells – undetermined significance (ASC-US). Squamous cell with enlarged nuclei, nuclear hyperchromasia, and mild nuclear irregularity

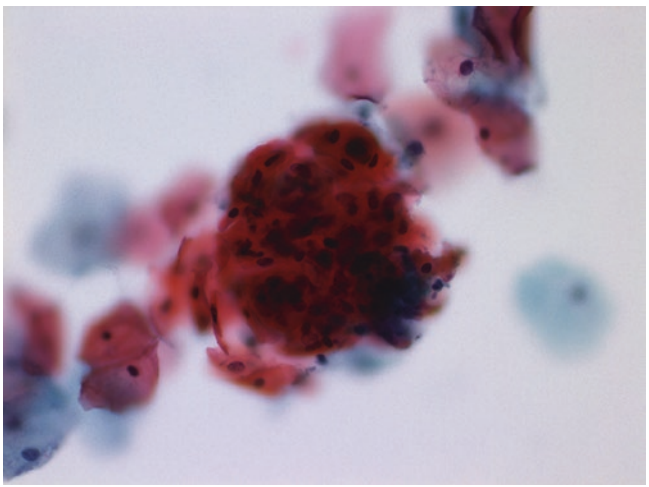

Fig. 5.4 Atypical squamous cells – undetermined significance (ASC-US) – atypical keratinized cells. Keratotic cells with mild nuclear enlargement, hyperchromasia, nuclear membrane irregularities, and in three-dimensional cell cluster

4. What are the atypical cytomorphological patterns of squamous cells – undetermined significance (ASC-US)?

1. Atypical parakeratosis: parakeratosis with mild nuclear enlargement, hyperchromasia, nuclear membrane irregularities, or in three-dimensional cell clusters (Fig. 5.4).
2. Atypical repair: marked repair reactions with some degree of cellular overlapping, dyscohesion, marked variation in nuclear size, prominent and irregular nucleoli, and irregular chromatin distribution.
3. Atypical squamous cells in atrophy: atrophic squamous cells showing nuclear enlargement, hyperchromasia, membrane irregularities, and marked nuclear pleomorphism.
4. "Atypia" in poorly preserved specimens, decidual cells, trophoblastic cells, and others.

References: [7–11].

5. What are the outcomes of ASC-US and ASC-H? How to manage these patients according to American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines?

Observational studies on women whose cervical smears showed ASC-US without treatment (minimum follow-up is 6 months) show the following results:

- 68.1% of women regress.
- 7.1% progress to HSIL.
- 0.25% progress to invasive carcinoma.

According to ASCCP, the women (>24 years old) with ASC-US may have HPV testing or have repeat Pap cytology at 12 months. If HPV testing is positive or repeat Pap cytology again shows ASC, colposcopy should be performed.

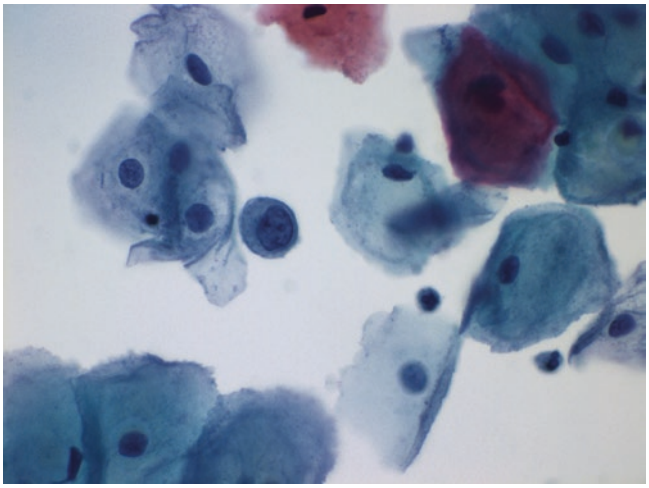

Fig. 5.5 Atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion (ASC-H). An isolated small cell with high nuclear to cytoplasmic ratio and enlarged and irregular nucleus

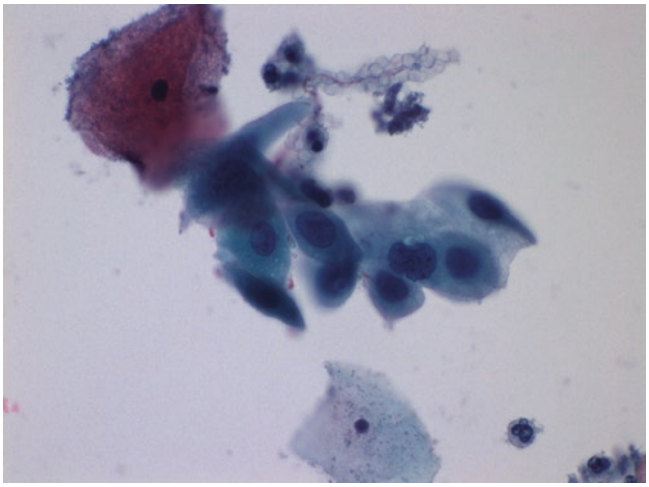

Fig. 5.6 Atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion. (ASC-H). A group of atypical metaplastic cells with dense cytoplasm, high nuclear to cytoplasmic ratio, enlarged nuclei, and irregular nuclear membranes

Colposcopy should be performed in the women diagnosed with ASC-H regardless of HPV status.

References: [12, 13].

6. What are atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion (ASC-H)?

ASC-H is designated for the minority of ASC cases in which the cytological changes are suggestive of HSIL.

The cytomorphological features are as follows:

1. Atypical squamous cells usually present singly (Fig. 5.5) or in small groups of less than ten cells.
2. The size of the cells is similar to that of the metaplastic cells. The nuclei are about 1.5–2.5 times larger than normal, and show significant nuclear membrane irregularity.
3. Marked increase in nuclear to cytoplasmic ratio (similar to HSIL).

References: [1, 10].

7. What are the cytomorphological patterns of atypical squamous cells – cannot exclude a high-grade squamous intraepithelial lesion (ASC-H)?

1. Atypical squamous metaplastic cells with small cell pattern: few immature (small) squamous cells with high nuclear to cytoplasmic ratio, and mild-to-moderate nuclear atypia.
2. Atypical crowded sheets pattern: crowded squamous cells showing atypical nuclear features (enlargement, mild hyperchromasia, irregularity, and abnormal chromatin), loss of polarity (Fig. 5.6), or difficult to visualize.
3. Markedly atypical reparative like changes: atypical repair raising concerns about the possibility of HSIL or invasive carcinoma, especially in high-risk patients.
4. Atypical cells with atrophic like pattern: atrophic like cells showing nuclear enlargement and hyperchromasia.
5. Atypical cells in a background of postradiation changes: concerning for recurrent or residual carcinoma.

8. What are the mimics of ASC-H?

1. Nonsquamous cells: histiocytes (eccentric oval and round nuclei, fine chromatin, and foamy cytoplasm), isolated endocervical cells (degenerated changes, regular chromatin, and evenly distributed chromatin), exfoliated endometrial cells either singly or in groups (smaller cells without pleomorphism), and decidualized stromal cells (usually pregnancy-related large cells, oval to polygonal, three to four times of nuclei of intermediate squamous cells and distinct nucleoli).
2. Basal cell hyperplasia, reserve cell hyperplasia and immature squamous metaplasia: these cells may have increased nuclear to cytoplasmic ratio, but with no prominent hyperchromasia and irregular nuclear membranes.

Reference: [11].

9. What are the 5-year risks of ASC for histologic diagnosis of HSIL/CIN 3?

- ASC-US with negative HPV: 0.44%
- ASC-US with positive HPV: 6.8%
- ASC-H with negative HPV: 3.0%
- ASC-H with positive HPV: 28%

Reference: [14].

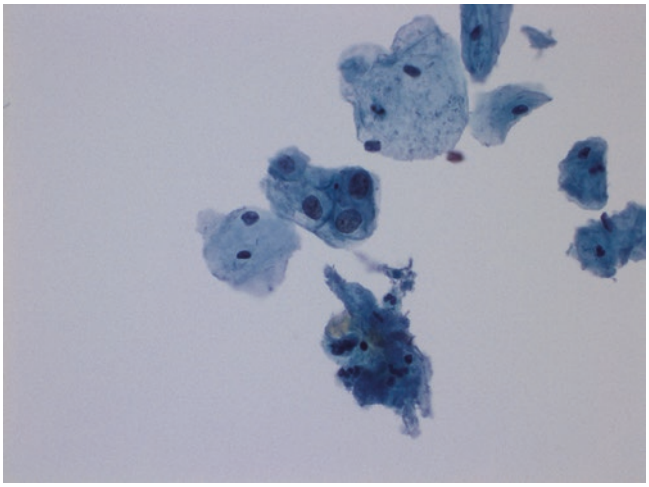

Fig. 5.7 Low-grade squamous intraepithelial lesion (LSIL). Intermediate-sized squamous cells with nuclear enlargement, hyperchromasia, irregular nuclear contour, and koilocytosis

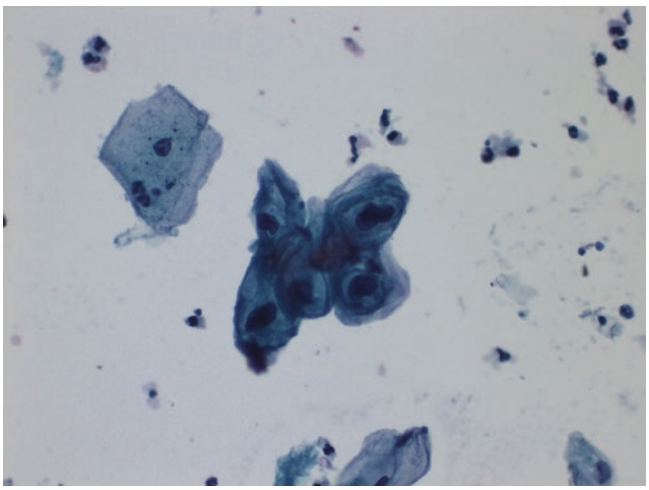

Fig. 5.8 Low-grade squamous intraepithelial lesion (LSIL). Intermediate-sized squamous cells with nuclear enlargement, hyperchromasia, irregular nuclear contour, and koilocytosis

10. What are the cytomorphological features of low-grade squamous intraepithelial lesion (LSIL)?

LSIL is a low-risk squamous intraepithelial lesion, and is associated with a large number of different HPV infections including low-risk and high-risk types, which cause mild dysplasia (CIN 1) in the squamous epithelium.

The cytomorphological features are as follows (Figs. 5.7, 5.8, and 5.9):

1. Intermediate-sized squamous cells with fairly abundant "mature" intermediate or superficial cell-type cytoplasm, occurring singly, in clusters, or in sheets.
2. Nuclear atypia.
   (a) Enlargement more than three times the area of normal intermediate nuclei.
   (b) Slightly increased nuclear to cytoplasmic ratio.

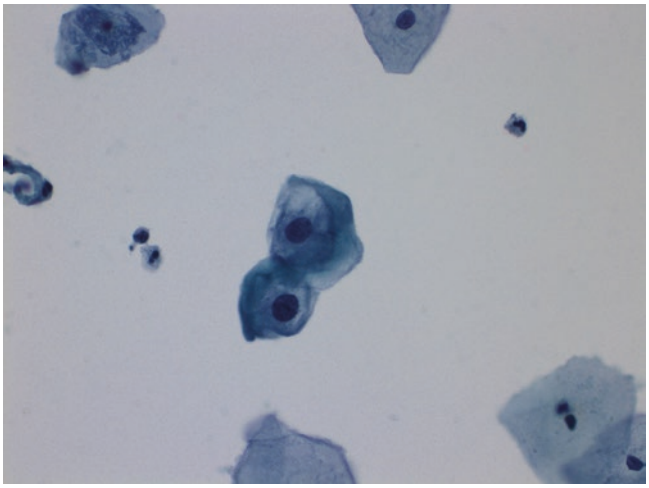

Fig. 5.9 Low-grade squamous intraepithelial lesion (LSIL). Intermediate-sized squamous cells with nuclear enlargement, hyperchromasia, irregular nuclear contour, and koilocytosis

   (c) Generally hyperchromatic but may be normochromatic.
   (d) Variable nuclear size.
   (e) Slight chromatin coarseness.
   (f) Irregular nuclear contour.
   (g) Absent or inconspicuous nucleoli.
   (h) Binucleation and multinucleation are common.
3. Koilocytosis or perinuclear cavitation is a characteristic viral cytopathic feature but is not required.
4. Keratinizing variant: abnormal cells may show increased keratinization with dense, eosinophilic cytoplasm, and with little or no evidence of koilocytosis.

References: [1, 15, 16].

11. What are the differential diagnoses of low-grade squamous intraepithelial lesion (LSIL)?

1. Reactive squamous cells.
   These cells are commonly seen in perimenopausal women, show nuclear enlargement, but with no hyperchromasia or irregular nuclear contour.
2. Pseudokoilocytosis.
   These cells normally show small nonspecific perinuclear halos but with no atypical nuclear features. They are usually associated with infections.
3. Reactive endocervical cells.
   These cells may show greater nuclear enlargement, and single or multiple nucleoli, but with smooth nuclear membranes. Enlarged cells often form cohesive sheets that interdigitate in classic "school of fish" architecture.
4. ASC-US.
   These cells show changes that are suggestive of LSIL but insufficient evidence (nuclear size is smaller than LSIL, mild irregular nuclear contour, and mild hyperchromasia).

5. Herpes cytopathic effect.
   - Classical herpes cytopathic effect shows multinucleated cells with nuclear molding, chromatin margination and clear, ground glass nuclei. However, early herpes cytopathic effect may show nuclear enlargement and degenerative hyperchromatic chromatin and may be mistaken for LSIL.
6. Radiation changes.
   - These changes manifest as low nuclear to cytoplasmic ratio, same nuclear size as LSIL, two-toned and vacuolated cytoplasm that lacks the perinuclear clearing and peripheral condensation present in a typical koilocyte, and history of radiation therapy.

12. What are the outcomes of LSIL? How to manage the patients with LSIL according to ASCCP guidelines?

Most LSIL patients represent a transient HPV infection with low risk of oncogenesis, 47.3% of LSIL will regress (especially in adolescents), 20.8% will progress to HSIL, and very few (0.15%) will progress to invasive squamous cell carcinoma if untreated.

Other studies reported that the 5-year risks of LSIL for histologic diagnosis of HSIL/CIN 3 are as follows:

– LSIL with negative HPV: 1.8%
– LSIL with positive HPV: 6.1%

According to ASCCP, women (>24 years old) with LSIL and negative HPV test will have repeat co-testing at 12 months. If HPV test is positive, colposcopy is recommended. For women aged 21–24 with LSIL, repeat cytology at 12 months is preferred. If the result is negative, ASC-US or LSIL, repeating cytology at 12 months. If Pap test demonstrates ASC-H, AGC or HSIL, colposcopy is recommended.

References: [12–14].

13. What are the cytomorphological features of high-grade squamous intraepithelial lesion (HSIL)?

HSIL is a squamous intraepithelial lesion, virtually all (97%) positive for high-risk HPV tests and encompassing moderate-to-severe dysplasia (CIN 2–3). It carries a significant risk of progression to invasive squamous cell carcinoma if untreated.

The cytomorphological features are as follows (Figs. 5.10, 5.11, 5.12, 5.13, 5.14, and 5.15):

1. The cells are smaller, like parabasal cells, show less cytoplasmic maturity than cells of LSIL, occur singly, in sheets, or in syncytial-like aggregates (hyperchromatic crowded groups).
2. Nuclear atypia.
   (a) Marked increase in nuclear to cytoplasmic ratio.
   (b) Nuclear enlargement, more variable than that seen in LSIL, some showing the same nuclear size as LSIL and others may be considerably smaller than that of LSIL.
   (c) Hyperchromatic nuclei.
   (d) Irregular nuclear contour and frequent indentations.
   (e) Coarsely granular or fine chromatin.
3. Cytoplasm can appear "immature," or densely metaplastic.
4. Keratinizing HSIL with nuclear atypia, irregular hyperchromasia, "mature" and densely keratinized cytoplasm and disorganized growth pattern.

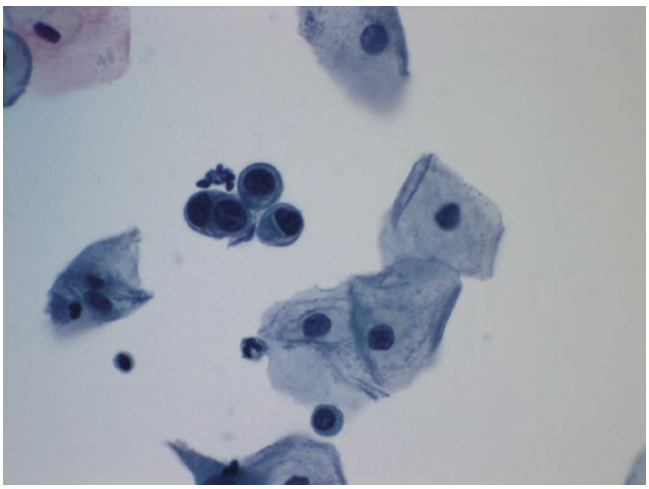

Fig. 5.10 High-grade squamous intraepithelial lesion (HSIL). A few small cells with high nuclear to cytoplasmic ratio, nuclear enlargement, hyperchromasia, and irregular nuclear contour

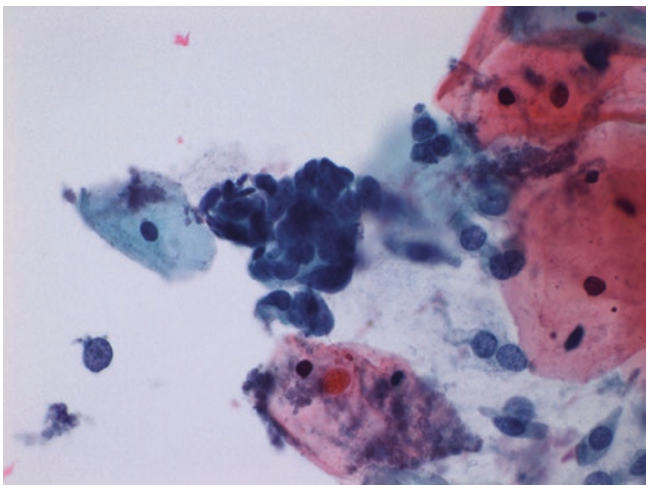

Fig. 5.11 High-grade squamous intraepithelial lesion (HSIL). Hyperchromatic crowded squamous cell three-dimensional group with high nuclear to cytoplasmic ratio and nondiscernible cytoplasmic borders

References: [1, 16, 17].

14. What are the patterns of HSIL?

1. Syncytial-like aggregates/hyperchromatic crowded groups (Fig. 5.11).
   (a) Cellular three-dimensional aggregates with no discernable cytoplasmic borders, scant cytoplasm, and loss of polarity.
   (b) Nuclei showing features of HSIL.

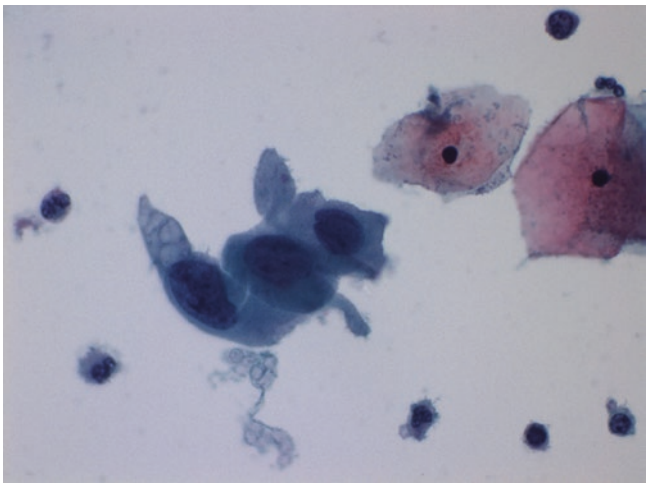

Fig. 5.12 High-grade squamous intraepithelial lesion (HSIL). HSIL extending into endocervical glands

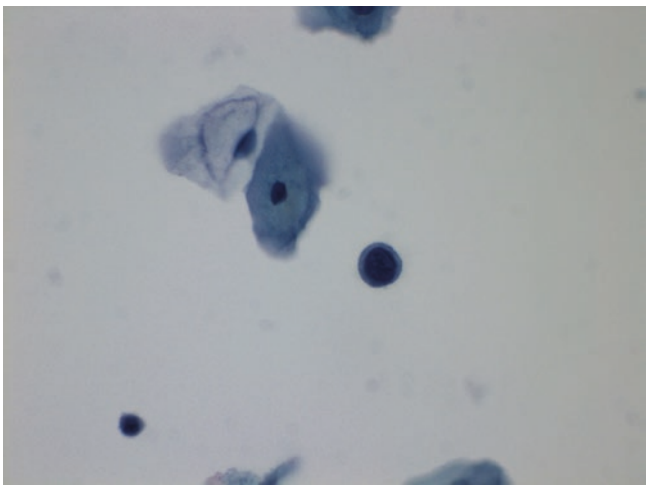

Fig. 5.14 High-grade squamous intraepithelial lesion (HSIL) . Isolated high nuclear to cytoplasmic ratio and hyperchromatic cells lie between benign cell clusters

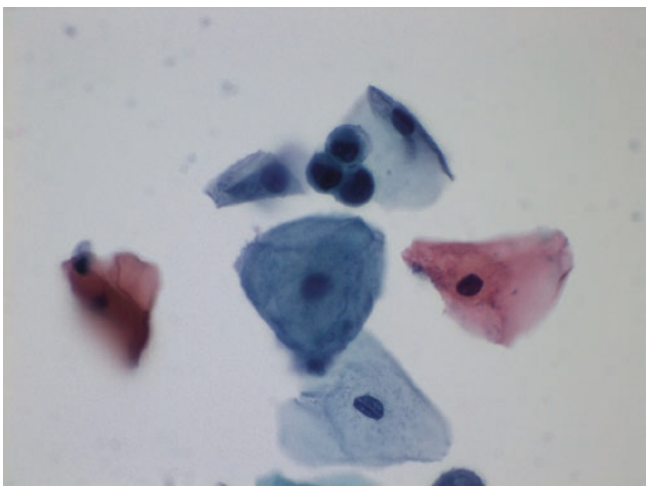

Fig. 5.13 High-grade squamous intraepithelial lesion (HSIL). A few small cells with high nuclear to cytoplasmic ratio, nuclear enlargement, hyperchromasia and irregular nuclear contour

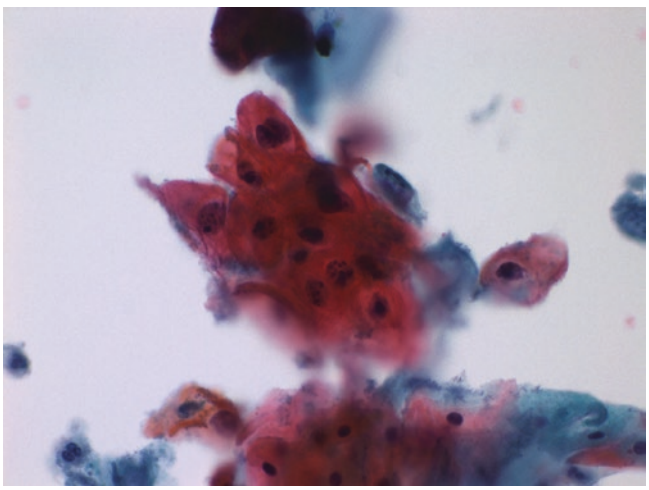

Fig. 5.15 High-grade squamous intraepithelial lesion (HSIL). Keratinizing squamous cells with nuclear atypia, irregular, hyperchromasia, "mature" and densely keratinized cytoplasm. Suspicious for invasive squamous cell carcinoma

2. HSIL extending into endocervical glands (Fig. 5.12).
   (a) Can be misinterpreted as glandular abnormalities.
   (b) Loss of central polarity and piling within cell groups, but lack of peripheral feathering and discohesiveness such as seen in AIS.
   (c) Nucleoli may be visualized, but not prominent.
3. Resembling endometrial cells and repair like pattern.
   (a) Clusters of endometrial-like cells showing small cells with degenerated nuclei, pyknotic nuclei, scant cytoplasm, and tapered ends.
   (b) Repair-like cells showing abundant cytoplasm, enlarged nuclei, and prominent nucleoli.
   (c) Classic HSIL cells usually present on the same slide.
4. Single and rare small HSIL cells (Figs. 5.13 and 5.14).
   (a) Rare small detached cells with high nuclear to cytoplasmic ratio present between normal cells (HSIL vs ASC-H).
   (b) Nuclei with HSIL cell features.
5. Abnormal stripped nuclei.
   (a) Stripped nuclei with atypical features, not intermediate nuclei seen in cytolysis.
   (b) Thorough review for HSIL.
6. Keratinizing high-grade lesions**.**
   (a) This pattern showing single or three-dimensional clusters of HSIL cells with abundant abnormally keratinized cytoplasm, pleomorphic shapes including elongated, spindle, caudate, and tadpole cells.

(b) Nuclei showing enlargement, pleomorphism, hyperchromasia, dense or opaque chromatin, and absence of nucleoli.
(c) No tumor diathesis.

7. HSIL in atrophy.
   (a) Small cells, the size of basal/parabasal cells or immature squamous metaplastic cells.
   (b) Nuclei overlapping with HSIL cell features.

References: [1, 5, 18–23].

15. What are the mimics/differential diagnoses of high-grade squamous intraepithelial lesion (HSIL)?

1. Isolated cells including endometrial cells, endocervical cells, reserve cells, parabasal cells, and immature squamous metaplastic cells. These cells may have high nuclear to cytoplasmic ratio but with no nuclear atypia seen in HSIL.
2. Inflammatory cells such as histiocytes or lymphocytes. Histiocytes have oval-kidney bean nuclei, normochromatic, with longitudinal grooves and foamy cytoplasm. Mature lymphocytes have small nuclei, and germinal center lymphocytes often have larger nuclei associated with tingible body macrophages.
3. Metaplastic cells such as squamous metaplastic cells and transitional metaplastic cells usually show lower nuclear to cytoplasmic ratio, and no nuclear atypia seen in HSIL.
4. Decidualized stromal cells with high nuclear to cytoplasmic ratio but without HPV cytopathic effect.
5. Endocervical polyp cells may have reactive changes showing high nuclear to cytoplasmic ratio but with no nuclear atypia seen in HSIL.
6. Adenocarcinoma in situ.
   (a) Columnar cells with parallel nuclei.
   (b) Nuclei showing hyperchromasia, fine-to-coarse chromatin, irregularity and high nuclear to cytoplasmic ratio.
7. Atrophic cells may have large nuclei with characterized smudgy or degenerative chromatin and a very high nuclear to cytoplasmic ratio, but with minimal variability in nuclear size and no mitosis.
8. Tubal metaplasia.
   (a) Crowded groups but with polarity, terminal bar, and cilia (diagnostic features).
   (b) Metaplastic cell resembling squamous metaplastic cell in size with basally placed nuclei, smooth membrane, slightly increased nuclear to cytoplasmic ratio.
9. IUD changes.
   (a) Small clusters of cells.
   (b) Variable nuclear to cytoplasmic ratio, usually low but can be high.
   (c) Degenerated nuclei with smudgy dark chromatin.
   (d) Vacuolated cytoplasm.

References: [1, 6, 11, 24].

16. What features are suspicious for invasive squamous cell carcinoma?

1. Highly pleomorphic HSIL cells with keratinized cytoplasm (Figs. 5.15).
2. Macronucleoli.
3. Necrosis, tumor diathesis, and granular proteinaceous debris.

17. What are the outcomes of HSIL? How to manage the women with HSIL according to ASCCP guidelines?

It is reported that 35% of women with HSIL regress and 1.4% progress to invasive carcinoma if untreated.

Other studies reported that the 5-year risks of HSIL for histologic diagnosis of HSIL/CIN 3 are as follows:

– HSIL with negative HPV: 29%
– HSIL with positive HPV: 50%

According to ASCCP, the women (> 24-year-old) with HSIL should have colposcopy with endocervical assessment or immediate loop electrosurgical excision (if they are not pregnant). Colposcopy is recommended for women aged 21–24 years with HSIL or ASC-H (immediate loop electrosurgical excision is unacceptable).

References: [12–14].

18. What are the cytomorphological features of keratinizing squamous cell carcinoma?

1. Predominantly as isolated, single cells and less commonly in cellular aggregates (Fig. 5.16).
2. Marked pleomorphic cells, with caudate and spindle cells ("tadpoles" and "fiber cells") containing dense orangeophilic cytoplasm (Fig. 5.17).
3. Variable nuclei, irregular nuclear membranes, dense opaque nuclei, and coarsely granular chromatin (Figs. 5.17 and 5.18).
4. Macronucleoli may be seen.
5. Tumor diathesis may be present.

References: [1, 4, 6].

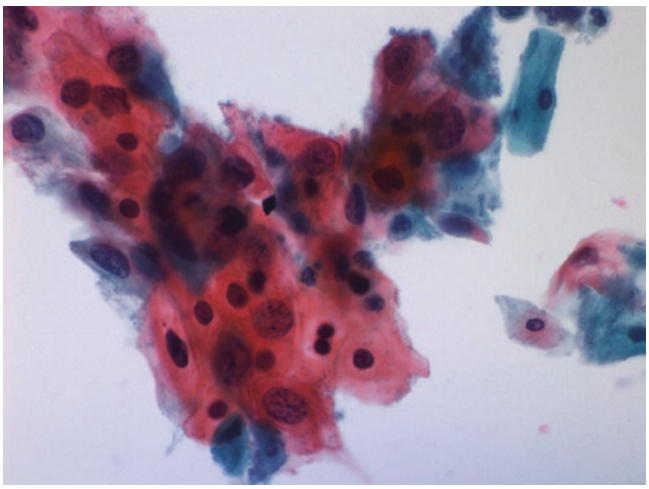

Fig. 5.16 Squamous cell carcinoma – keratinizing. Aggregates of keratinizing squamous cells with marked pleomorphic cells, atypical nuclei, and dense orangeophilic cytoplasm

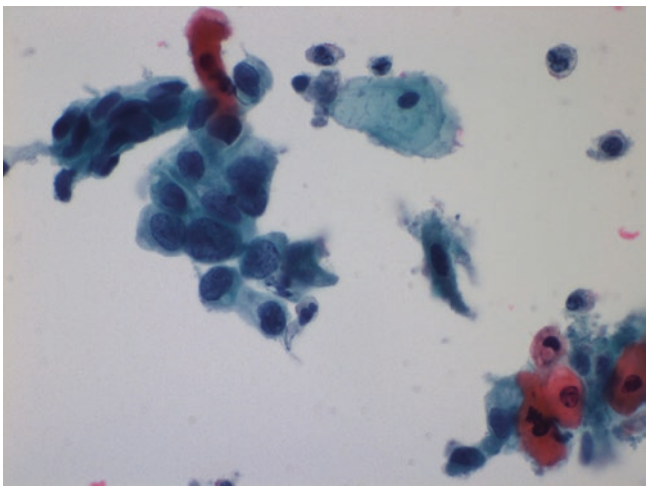

Fig. 5.18 Squamous cell carcinoma. Disorganized malignant cell clusters with marked pleomorphism

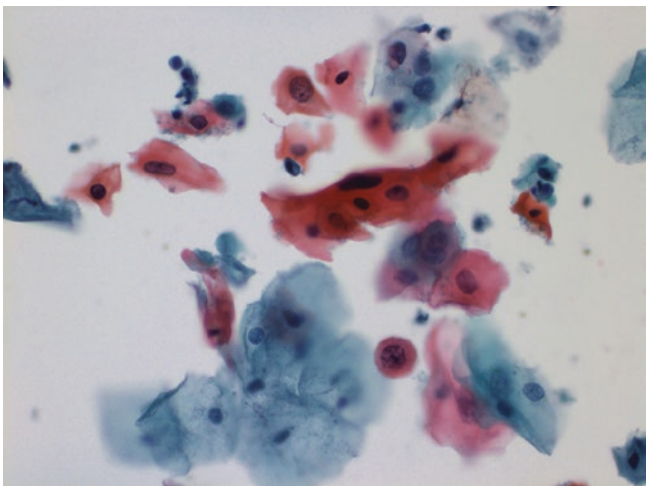

Fig. 5.17 Squamous cell carcinoma – keratinizing . Marked pleomorphism of cell size and shape and cytoplasmic keratinization

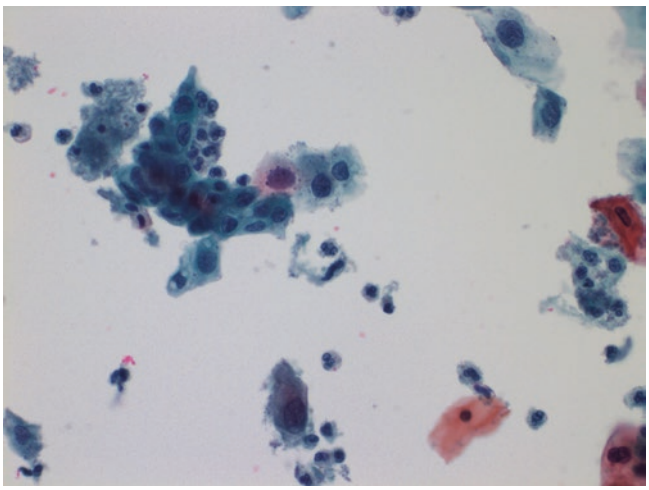

Fig. 5.19 Squamous cell carcinoma – nonkeratinizing. Predominantly relatively smaller nonkeratinizing squamous cells present singly and in aggregates with poorly defined cell borders, atypical nuclei with coarsely clumped chromatin, and tumor diathesis in the background

19. What are the cytomorphological features of nonkeratinizing squamous cell carcinoma?

1. Tumor cells are relatively smaller in size than those of many HSIL cells, occur singly or in syncytial aggregates with poorly defined cell borders (Fig. 5.19).
2. Variable nuclei size, irregular nuclear membranes, and coarsely clumped chromatin with chromatin clearing.
3. Nucleoli may be prominent.
4. Tumor diathesis is often present.

References: [1, 4, 6].

20. What are the differential diagnoses of squamous cell carcinoma?

1. Adenocarcinoma.
   - Nonkeratinizing squamous cell carcinoma and poorly differentiated squamous cell carcinoma show features which make their differentiation from adenocarcinoma difficult. Immunohistochemical studies performed on cell blocks can be helpful for differentiation, such as p40, CK5/6, p16, PAX8, etc.
2. Atrophy with atypia.
   - Scattered cells with large and dark nuclei, smudgy chromatin, eosinophilic or orangeophilic cytoplasm

present in a background of atrophy and inflammation (should be interpreted as ASC-US).

3. Atypical repair.
   - Repair usually shows flat cohesive sheets of cells commonly with nucleoli, finely textured chromatin, and no tumor diathesis. But atypical repair may be similar to invasive carcinoma and is better to be interpreted as ASC-US for close follow-up.
4. Benign endometrial cells.
   - Nonkeratinizing squamous cell carcinoma may show endometrial cell-like small cells. Endometrial cells have small nuclei, high nuclear to cytoplasmic ratio but minimal anisonucleosis and no mitosis.
5. *Behçet's disease.*
   (a) A chronic disease of uncertain etiology with oral and genital ulcers.
   (b) Numerous isolated, keratinized cells with dark pleomorphic nuclei and large nucleoli.
6. Pemphigus vulgaris.
   (a) May mimic poorly differentiated squamous cell carcinoma.
   (b) Clinical history and/or with blisters.

References: [23–27].

21. What are atypical glandular cells?

Atypical glandular cells (AGC) are used to diagnose cases where glandular abnormalities morphologically fall between benign reactive process and definite neoplasia on a smear specimen. The quantity of the atypical cells or the mild degree of atypia is insufficient but raises the suspicion of malignancy. AGCs should be categorized according to their sites of origins (endocervical or endometrial) whenever possible as the clinical management for patients may vary significantly depending upon the cell type; otherwise, AGC is used.

Reference: [1].

22. What are atypical endocervical cells and what are the diagnostic cytomorphological features?

Endocervical-type cells with nuclear atypia exceed obvious reactive changes but lack unequivocal features of endocervical adenocarcinoma in situ or invasive adenocarcinoma. Atypical endocervical cells should be further qualified when a particular entity is favored.

Cytomorphological features of atypical endocervical cells, NOS (Fig. 5.20):

1. Abnormal cells in sheets or strips with discernible cell borders and crowding.
2. Nuclei showing enlargement (three to five times of normal endocervical nuclei), nuclear overlapping, pseudostratification, pleomorphism, mild hyperchromasia, mild irregularity, occasional nucleoli, and rare mitosis.

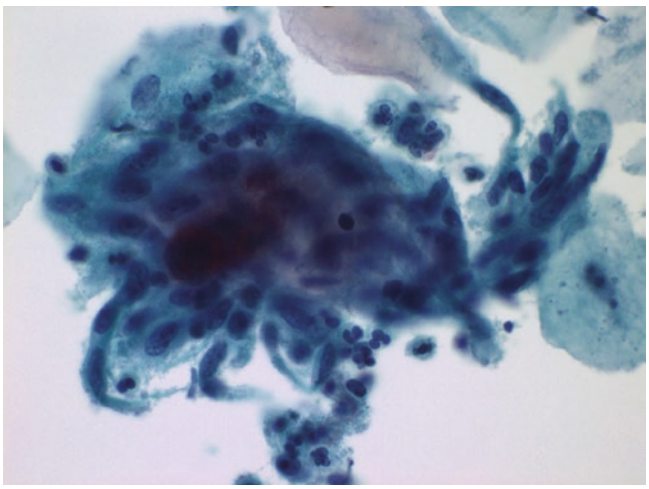

Fig. 5.20 Atypical endocervical cells. Sheets of crowded cells with increased nuclear to cytoplasmic ratio and feathering at the edges of the sheet

3. Increased nuclear to cytoplasm ratio.

Cytomorphological features of atypical endocervical cells, favor neoplastic:

1. Abnormal cells in sheets or strips with discernible cell borders, crowding, glandular formations, and feathering.
2. Nuclei showing enlargement, nuclear overlapping, pseudostratification, pleomorphism, hyperchromasia, coarse chromatin, and occasional mitosis.
3. Increased nuclear to cytoplasm ratio.

References: [1, 4].

23. What are the features of endocervical adenocarcinoma in situ?

Endocervical adenocarcinoma in situ is a noninvasive high-grade endocervical glandular lesion that is characterized by nuclear enlargement, hyperchromasia, chromatin abnormality, pseudostratification, and mitotic activity.

The cytomorphological features are as follows:

1. Abnormal cells in sheets, clusters, pseudostratified strips, columnar-shaped, and glandular formation with "feathering" or "bird-tail" appearance (nuclear and cytoplasmic tags protruding from the periphery).
2. Palisading nuclei are enlarged, hyperchromatic, coarsely granular chromatin, apoptotic bodies, mitoses, inconspicuous nucleoli, crowding and overlapping.
3. Nuclear to cytoplasmic ratios are increased; the quantity of cytoplasm and cytoplasmic mucin is decreased.
4. Background is clean with no tumor diathesis.

References: [1, 6].

24. What are the differential diagnoses of endocervical adenocarcinoma in situ/ atypical endocervical cells?

1. Endometrial cells/endometriosis. Three-dimensional groups of smaller cells with nuclear overlapping, mild hyperchromasia and spindled stroma, but no pleomorphism or feathering.
2. Endocervical polyps. These cells may have reactive changes showing high nuclear to cytoplasmic ratio but with no nuclear atypia or feathering.
3. Microglandular hyperplasia has glandular cells with no nuclear overlapping or atypia.
4. Reactive endocervical cells are flat sheets of glandular cells with no marked atypia or feathering.
5. Tubal metaplasia: usually strips of glandular cells with mild hyperpchromasia and diagnostic features of terminal bar and cilia.
6. Reparative changes: flat sheets of glandular cells with nucleoli, but no hyperchromasia or feathering.
7. Arias-Stella reaction cells are usually associated with pregnancy.
8. HSIL. These cells are in syncytial groups or singly, round irregular nuclear contour, with no glandular formation or feathering.
9. Invasive adenocarcinoma show features of adenocarcinoma in situ, together with findings of invasion (such as macronucleoli, tumor necrosis, and tumor diathesis).

25. What are atypical endometrial cells and the diagnostic cytomorphological features?

Atypical endometrial cells are endometrial cells with enlarged nuclei and are generally not further qualified as favor neoplastic.

The cytomorphological features are (Fig. 5.21):

1. Cells occur in small groups, usually 5–10 cells per group, with ill-defined cell borders.
2. Nuclei are slightly enlarged, mild hyperchromasia, chromatin heterogeneity, and occasional nucleoli.
3. Cytoplasm is scant or moderately abundant and vacuolated.

References: [1, 4].

26. What are the common conditions associated with atypical endometrial cells?

1. Endometrial polyps.
2. Chronic endometritis.
3. Intrauterine device (IUD).
4. Endometrial hyperplasia.
5. Endometrial carcinoma.

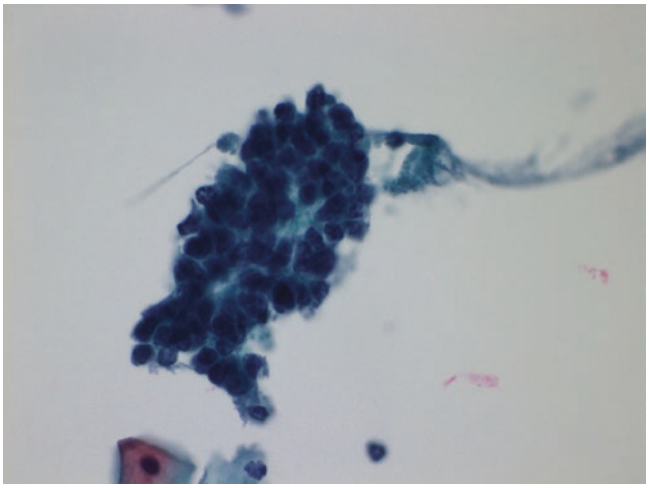

Fig. 5.21 Atypical endometrial cells. Three-dimensional group of small cells with crowded round or oval nuclei, scant cytoplasm and ill-defined borders

27. How to manage the women with atypical glandular cells, atypical endocervical cells, and adenocarcinoma in situ and atypical endometrial cells according to ASCCP guidelines?

The women with atypical glandular cells, atypical endocervical cells, or adenocarcinoma in situ are recommended to have colposcopy with endocervical sampling and endometrial sampling (if ≥35-year-old or at risk for endometrial neoplasia).

Endometrial and endocervical sampling are recommended for the women with atypical endometrial cells. If no endometrial pathology is identified, further colposcopy is recommended.

Reference: [13].

28. What are the cytomorphological features of endocervical adenocarcinoma?

1. Abnormal columnar cells in sheets, three-dimensional clusters, syncytial aggregates or singly.
2. Nuclei are enlarged, round, pleomorphic, uneven chromatin with clearing, membrane irregularities, and prominent nucleoli.
3. Abundant finely vacuolated cytoplasm.
4. Tumor diathesis.

29. What are the cytomorphological features of endometrial adenocarcinoma?

Pap test is mainly a screening test for cervical lesions and fortuitously pick up exfoliated cells from some endometrial carcinomas and the findings are largely dependent upon the grade and type of the carcinoma.

The cytomorphological features are as follows:

1. Single or small tight clusters of cells.
2. Nuclei are enlarged, hyperplastic, variable, loss of polarity, chromatin clearing, small-to-prominent nucleoli (Fig. 5.22).

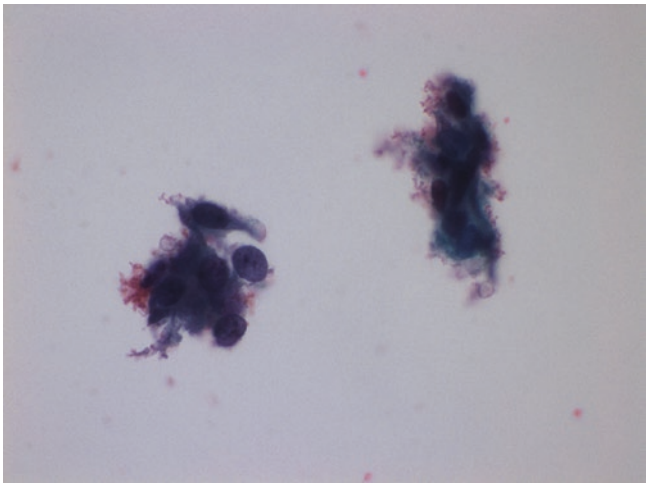

Fig. 5.22 Adenocarcinoma. Cohesive groups of glandular cells with nuclear crowding and vacuolated cytoplasm

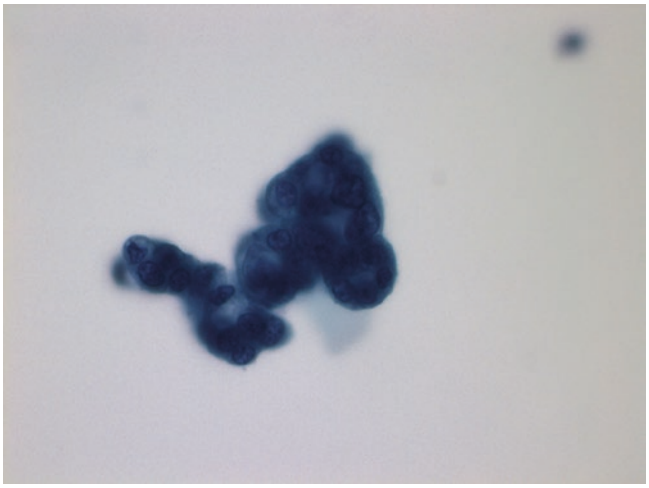

Fig. 5.23 Adenocarcinoma. Cohesive groups of glandular cells with nuclear crowding and cytoplasmic vacuoles present in a clean background

3. Scant, cyanophilic and vacuolated cytoplasm.
4. Intracytoplasmic neutrophils ("bags of polys").
5. Finely granular or "watery" tumor diathesis.

Reference: [1].

30. What are the differential diagnoses of endocervical/endometrial adenocarcinoma?

1. Endocervical adenocarcinoma: columnar appearing and glandular cells with enlarged, hyperchromatic, and palisading nuclei, coarsely granular chromatin and feathering.
2. Adenocarcinoma of other sites: vagina, ovary (Fig. 5.23), fallopian tube, and metastasis. Metastatic tumors have clean background and features of primary tumor.
3. Squamous cell carcinoma, especially nonkeratinizing squamous cell carcinoma.
4. IUD effect: small clusters of cells show low nuclear to cytoplasmic ratio, degenerative nuclei, and smudgy chromatin.
5. Endocervical polyp atypia may have reactive changes, such as high nuclear to cytoplasmic ratio but no marked nuclear atypia.
6. Reactive endocervical cells are flat sheets of glandular cells with no marked nuclear atypia.
7. Adenocarcinoma in situ: columnar cells with hyperchromatic parallel nuclei, fine-to-coarse chromatin, irregularity, high nuclear to cytoplasmic ratio, and feathering.
8. Pemphigus vulgaris may mimic carcinoma but with distinctive clinical history and/or with blisters.

31. How is endometrial adenocarcinoma distinguished from endocervical adenocarcinoma?

1. Endometrial adenocarcinoma usually shows scant small-sized tumor cells in small clusters with round and irregular nuclei, degenerated vacuolated cytoplasm, and no squamous intraepithelial lesion. Immunohistochemical stain for p16 is usually patchy but is positive in serous carcinoma.
2. Endocervical adenocarcinoma usually occurs in younger patients than that of endometrial adenocarcinoma, and shows hypercellular sheets/strips/rosettes of columnar/oval shaped tumor cells with feathering, elongated/oval/pleomorphic nuclei, cytoplasmic mucin, and is sometimes associated with concurrent squamous intraepithelial lesion. High-risk HPV test is usually positive and immunohistochemical stain for p16 is positive (>70% diffuse and strong).

32. What are the rare malignant tumors of uterus?

1. Malignant melanoma.
   (a) Cellular Pap smear with abnormal cells showing pleomorphic, discohesive, round/oval/spindled shaped, large nuclei, binucleation, intranuclear pseudoinclusions, and prominent nucleoli.
   (b) Well-defined cytoplasm with or without melanin pigments.
   (c) Melanophages and tumor diathesis may be present.
   (d) Immunohistochemical stains performed on cell block for melanoma markers (S100, HMB45, Melan A and SOX 10) are helpful for the diagnosis.
2. Neuroendocrine tumor.
   (a) Classified as low-grade neuroendocrine tumors (carcinoid and atypical carcinoid) and high-grade neuroendocrine carcinomas (small-cell carcinoma and large-cell carcinoma).
   (b) Tumor cells with cytomorphological features of neuroendocrine neoplasm, variable dependent upon the type.
   (c) Immunohistochemical stains performed on cell block for neuroendocrine markers (CD56, synaptophysin, and chromogranin) are positive.
3. Germ cell tumors: choriocarcinoma, yolk sac tumor and teratoma.
4. Malignant mixed mesodermal tumor (carcinosarcoma).
   (a) Biphasic tumor composed of malignant epithelial and mesenchymal components.
   (b) The morphological features depend upon the tumor type.

(c) The epithelial component can be endometrioid, serous or clear cell type.
(d) The mesenchymal sarcomatous component can be endometrial stromal sarcoma, fibroblastic sarcoma, leiomyosarcoma, rhabdomyosarcoma, chondrosarcoma, or osteosarcoma.

5. Sarcomas.
   (a) Leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, endometrial stromal sarcoma, Ewing/primitive neuroectodermal tumors (PNET), and myeloid sarcoma.
6. Lymphoma.
   (a) Lymphoma can be primary or disseminated disease.
   (b) Discohesive cells with cytomorphological features correlate with the type of lymphoma.
   (c) Immunohistochemical stains performed on cell block for lymphoma markers are helpful for a definitive diagnosis.
7. Metastatic tumor.

References: [3, 6, 28–38].

33. What are the features of extrauterine carcinomas on Pap specimen?

1. Tumor from extrauterine sites through direct invasion or lymphatic and/or hematogenous metastasis, such as bladder, ovary, fallopian tube, gastrointestinal tract, breast, and kidney.
2. Usually rare cells unless direct extension or extensive metastasis.
3. Usually clean background without tumor diathesis unless direct extension or extensive metastasis.
4. Pattern and morphologic features depending upon the primary tumor type.

References: [32, 39].

Case Studies

Case 1
Learning objectives:

1. Recognize cytomorphological features of high-grade squamous intraepithelial lesion (HSIL).
2. Describe the cytomorphological patterns of HSIL.
3. Distinguish HSIL from its mimics.

Case history: The patient is a 31-year-old woman with a history of abnormal Pap showing low-grade squamous intraepithelial lesion (LSIL) 11 years ago, who presented for follow-up examination. She also had vulvar condyloma 7 years ago and was resected.

Cytomorphological findings: Smaller cells occur singly and in small aggregates, like parabasal cells, and show less cytoplasmic maturity than cells of LSIL. The cells show marked increase in nuclear to cytoplasmic ratio, nuclear enlargement, hyperchromatic nuclei, irregular nuclear contour, and coarsely granular chromatin (Fig. 5.24).

Differential diagnosis: Isolated cells including endometrial cells, endocervical cells, reserve cells, parabasal cells, and immature squamous metaplastic cells; inflammatory cells such as histiocytes; decidualized stromal cells; endocervical polyp cells; adenocarcinoma in situ; IUD changes.

Final diagnosis: high-grade squamous intraepithelial lesion (HSIL).

Take-home messages:

- There is a significant risk of invasive carcinoma development in patients with HSIL if not treated.
- It is very important to recognize HSIL cells and the patterns of HSIL (can be in hyperchromatic crowded groups, single and rare small cells) and to distinguish them from LSIL cells and other mimics in Pap test.
- In general, HSIL occurs at an older age than LSIL. In this case, the patient has a history of LSIL 11 years ago but whether HSILs develop from LSILs or devolve independently is controversial.
- High-risk HPVs are found in over 90% of cervical HSILs.

References: [1, 17].

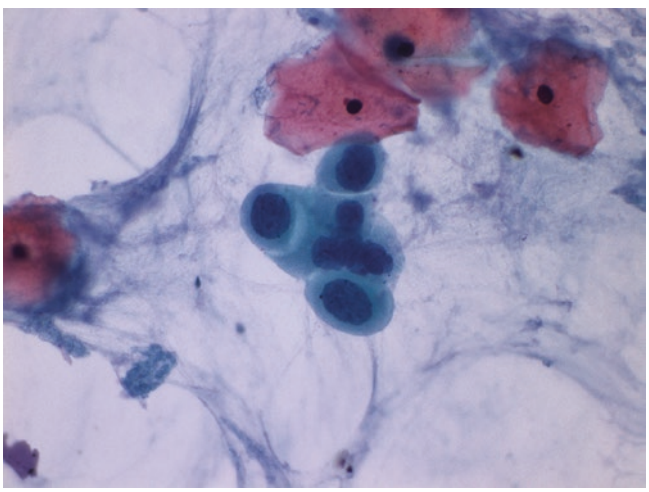

Fig. 5.24 The cells show marked increase in nuclear to cytoplasmic ratio, nuclear enlargement, hyperchromatic nuclei, irregular nuclear contour, and coarsely granular chromatin

Case 2

Learning objectives:

1. Recognize cytomorphological features of atypical endometrial cells.
2. Distinguish features of atypical endometrial cells from atypical endocervical cells.
3. Describe the common conditions associated with atypical endometrial cells.

Case history: The patient is a 60-year-old woman who presented for postmenopausal bleeding for a few weeks. She has no history of abnormal Pap smear. Ultrasound demonstrated 8-mm-thick endometrium. Endometrial biopsy was performed weeks later and endometrial adenocarcinoma, FIGO grade 2, was identified.

Cytological findings: Few small tight clusters of cells show enlarged, hyperplastic, and variable nuclei, loss of polarity, chromatin clearing, and small-to-prominent nucleoli. Cytoplasm is cyanophilic and vacuolated with intracytoplasmic neutrophils ("bags of polys") (Fig. 5.25).

Differential diagnosis: Endometrial polyps; chronic endometritis; endometrial hyperplasia; endometrial carcinoma; IUD effect; endocervical adenocarcinoma in situ and adenocarcinoma.

Final diagnosis: atypical endometrial cells.

Take-home messages: Atypical endocervical cells (NOS or favor neoplastic) are abnormal cells in sheets or strips with discernible cell borders, crowding, glandular formations, feathering, nuclear atypia and increased nuclear cytoplasm ratio.

- Atypical endometrial cells occur in small groups with ill-defined cell borders, slightly enlarged nuclei, mild hyperchromasia, chromatin heterogeneity, and occasional nucleoli. Cytoplasm is scant or moderately abundant and vacuolated.
- Atypical endometrial cells are generally not further characterized as favor neoplastic since this is difficult and poorly reproducible.
- The clinical history is helpful. In younger patients with history of squamous intraepithelial lesion and positive high-risk HPV testing, HPV-associated endocervical adenocarcinoma in situ and adenocarcinoma should be considered in the differential diagnosis. In postmenopausal patients, carcinoma of endometrial or other primary sites is more likely.
- In this case, the patient is a postmenopausal woman and endometrial adenocarcinoma is identified in the later endometrial biopsy.

References: [1, 4].

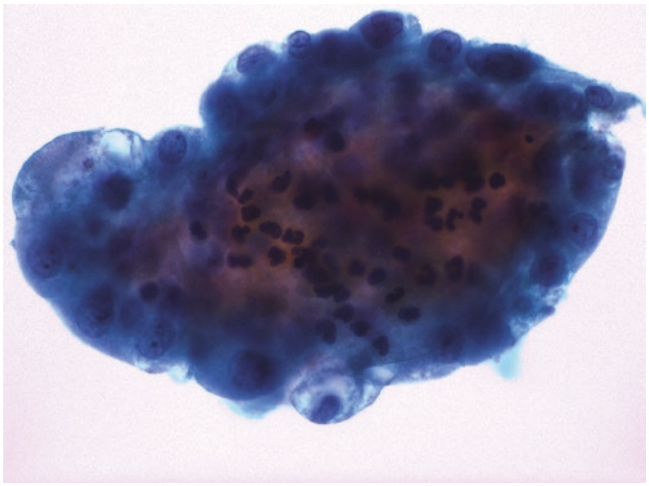

Fig. 5.25 A cluster of atypical cells show enlarged, hyperplastic, and variable nuclei, loss of polarity, chromatin clearing, and small-to-prominent nucleoli. Cytoplasm is cyanophilic and vacuolated with intracytoplasmic neutrophils ("bags of polys")

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Gastrointestinal, Pancreas, and Bile Ducts Cytology

6

Zhongren Zhou, Cory T. Bernadt, and Huihong Xu

Contents

List of Frequently Asked Questions

1. What are the changes over the years of gastrointestinal cytology practice?

Answer:

GI cytology is traditionally considered as a cost-effective ways for the rapid evaluation, interpretation, and triaging of a variety of neoplastic and non-neoplastic gastrointestinal tract lesions [1, 2]. Over the years, the advances of the new technology enable the gastroenterologist to better visualize the superficial mucosal lesion and submucosal deep-seated lesion. The cytology specimen is not limited to routine brushing and direct fine-needle aspiration; more and more endoscopic ultrasound- or CT-guided fine-needle aspiration methods are used to increase diagnostic accuracy. However, a successful cytologic examination is determined by the skill of endoscopist, specimen collection and preparation, and the expertise of the cytopathologist.

2. What are the most common upper GI cytology in immunocompromised patient population?

Answer:

The most common upper GI disorder in immunocompromised patient population is the infectious esophagitis. The most frequent cause is *Candida albicans*, which causes dysphagia and white plaque under endoscopy. The esophageal brushing smear [3] is often more sensitive than tissue biopsies, to reveal the presence of pseudohyphae with constriction along the long axis and small round budding yeast forms in a background of acute inflammatory exudate and reparative squamous cells. The characteristic diagnostic feature ("shish kebab" effect) is commonly seen as eosinophilic to gray-brown-colored pseudohyphae embedded or spearing within the stacked squamous cells in Papanicolaou-stained smears. The most frequent viral cause of esophagitis is herpes simplex virus, which causes mucosal sloughing and multiple shallow, sharp-edged ulcers under endoscopy. The characteristic viral cytopathic changes are the classic Cowdry type A intranuclear eosinophilic inclusion bodies with a clear halo, thickened nuclear membrane due to chromatin margination, ground-glass homogeneous chromatin, multinucleation, and nuclear molding in the infected cells. The background is often composed of neutrophilic debris and squamous cells with reactive atypia. In practice, due to the enlargement of the virocytes, the common differential diagnosis includes squamous cell carcinoma, radiation-induced damage, and other viral infections such as CMV [4]. Refer to Table 6.1 for the cytologic differential diagnosis. In most cases, endoscopic biopsies and brushings are complementary for the diagnosis of viral esophagitis.

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_6

Table 6.1 Cytomorphologic differential diagnosis in esophageal brushings

	Cell type	Multinucleation	Chromatin	Inclusions	N/C ratio	Nucleomegaly Cytomegaly
HSV esophagitis	Squamous	Present in a compressed pattern	Ground-glass	Intranuclear (eosinophilic) with halo	Normal to mild increase	Mild
CMV esophagitis	Stromal, glandular	Absent	Thick marginated	Intranuclear (basophilic) with halo, cytoplasmic (eosinophilic)	Marked increase	Marked
Squamous cell Carcinoma	Squamous	Absent	Granular and hyperchromatic	Absent	High	Pleomorphic, macronucleoli
Small cell carcinoma	Neuroendocrine	Present with molding	Salt and pepper	Absent	Very high	Absent
Radiation- or Chemotherapy-induced esophagitis	Squamous	Present in a disordered pattern	Pale, structureless	Absent	Normal	Proportional
Repair	Squamous	Absent	Pale, fine granularity, even	Absent	Normal to mild increase	Mild

Table 6.2 Immunohistochemistry differential of GI tract spindle cell lesions

	CD117	DOG1	S100	Pan CK	Synaptophysin	Chromogranin	SOX10
GIST	+	+	–	–	–	–	–
Leiomyoma	–	–	–	–	–	–	–
Schwannoma	–	–	+	–	–	–	–
Spindle cell carcinoma	–	–	–	+	–	–	–
Spindle cell carcinoid tumor	–	–	–	+[a]	+	+	–
Metastatic melanoma	–	–	+	–	–	–	+
Plexiform fibromyxoma	–	–	–	–	–	–	–

[a]Cytoplasmic dotted pattern

3. What are the cytological features of GIST tumor? How to differentiate its subtypes and other GI spindle cell tumors?

Answer:

Gastrointestinal stromal tumor (GIST) is the most common mesenchymal tumor of the GI tract. The neoplastic cells are the precursors to the cells of Cajal, arising in the wall of the GI tract, usually due to the activation mutation of c-kit. Stomach is the most common tumor site. Since the common mural location of the gastric tumors, routine gastric brushing is less sensitive unless the lesions involve submucosa and mucosa resulting ulceration. Recently EUS-guided FNA is able to reach the deep-residing lesion in the submucosa and muscle wall, which significantly increased the diagnostic sensitivity and specificity with this minimal invasive approach.

Based on cytomorphology, the lesional cells can be separated into spindle cell type and epithelioid cell type. The spindle cell type GIST often presents with loose irregular clusters of spindle-shaped cells, some forming interlacing bundles. Some other features include single elongated nucleus with squared-off ends, thin wispy pale to eosinophilic cytoplasm with long extension, scattered stripped nuclei, and numerous perinuclear vacuoles. The epithelioid cell type GIST is less common, and usually shows sheets of epithelioid cells with a condensed rim of eosinophilic cytoplasm, peripheral cytoplasmic clearing, and round nuclei with small nucleoli. Some cases illustrate mixed cell types with both spindle and epithelial cells. About 10–30% of GIST tumors are malignant with intra-abdominal dissemination and distant metastases. The cytology sample is often quite cellular, with necrotic background, frequent cellular atypia including increased N/C ratio, and mitotic figures [5].

The most common differential diagnosis for spindle cell lesion besides GIST are leiomyoma, schwannoma, spindle cell carcinoma, spindle cell carcinoid tumor, plexiform fibromyxoma, and metastatic melanoma. Immunohistochemistry studies on cell block or smear slides help to differentiate the diagnosis; refer to Table 6.2 [6–9].

4. What are the common pancreatic cytology specimens, adequacy criterion, and their clinical utilities?

Answer:

Pancreatic cytology specimens include pancreatic duct or common bile duct brushing and fine-needle aspiration.

Common bile duct (CBD) or pancreatic duct brushings are obtained through endoscopic retrograde cholangiopan-

creatography (ERCP), which is very useful to collect samples from pancreatic or bile duct stricture without mass lesion. However, the common CBD brushings are the most challenging cytologic specimens and have diagnostic limitations. Inflammation, stone, or stents usually cause the reactive or reparative changes in superficial biliary mucosa [10]. Frequently, the reactive/reparative cytological changes overlap with neoplastic process. Therefore, the rate of false-negative diagnosis is relatively high in routine practice. The diagnosis of atypical or malignant cytological changes needs cytology expert consensus opinion.

EUS-FNA is a well-established method to collect pancreatic cytological samples. It came to use in 1991 for pancreatic cancer and now is performed on a routine basis at many endoscopic centers. It is evident that this procedure has a major impact on the clinical management of patients, by obtaining diagnostic tissue from lesions outlined by EUS-FNA [11]. EUS can easily locate the pancreatic mass, and then use a needle through the stomach or duodenum to sample the lesions. However, the quantity or quality of the EUS-FNA sample varies greatly by the endoscopists' experience. Among all the pancreatic lesions, the sensitivity and specificity for pancreatic ductal adenocarcinoma are relatively high [12]. The rapid on-site evaluation of EUS-FNA sample is strongly recommended, which greatly improves the quality and quantity of diagnostic sample. For pancreatic cystic lesion, the fluid also should be collected for further mucin, cytology, biochemical, and molecular tests. In addition, fine-needle aspiration in conjunction with biopsy is developed to lower the insufficient rate, and increase the sensitivity and specificity [13].

5. What are the normal components of a pancreatic FNA specimen?

Answer:

The pancreatic FNA specimen usually contains scant cellularity, including acinar cells, ductal cells, and rare islet cells. The acinar cells are polygonal cells forming small grape-like groups with punctate granular cytoplasm in Romanowsky-stained smear. Nuclei are round with small nucleoli. Only a few ductal cells are present in the normal pancreatic FNA. The ductal cells are often arranged in cohesive sheets and strips with a honeycomb pattern. The cells are evenly spaced inside sheets or strips with relatively small nuclei and clear cytoplasmic borders.

6. What are the contaminated components of a pancreatic FNA specimen?

Answer:

The EUS-FNA needs to penetrate through the stomach or duodenum into pancreatic lesions. Therefore, the gastric or duodenal mucosal epithelium is commonly present in cytological samples. The gastric mucosa component is generally present as sheets of columnar epithelial cells forming honeycomb pattern without goblet cells. The duodenal mucosa component is also present as sheets of columnar cells with intermingled "fried eggs"-like goblet cells. In addition, smooth muscle cells or lymphocytes are occasionally present in the sample as well.

7. What is the adequacy criterion for a pancreatic FNA sample?

Answer:

In normal pancreatic specimen, the cellularity is usually low with mixed acinar cells and ductal cells. There is no clear cut-off number about pancreatic cells for adequacy. However, we consider that it is non-diagnostic if no glandular cells or endocrine cells are present with suspicious mass lesion on US or CT imaging. For radiographic cystic lesions, we usually do not call non-diagnosis since most of cystic lesions are difficult to collect cyst wall lining cells. The cystic fluid having either thick mucin or clear fluid and elevated carcinoembryonic antigen (CEA) level is sufficient to make a diagnosis [14, 15].

8. What are the cytological features for a chronic pancreatitis?

Answer:

The chronic pancreatitis, which is often caused by gallbladder stone or alcohol abuse, can mimic a mass-like lesion by radiological imaging study, and usually requires EUS-FNA to make a definitive diagnosis. However, the cytological sample of chronic pancreatitis is often sparsely cellular in a background of chronic inflammation and cellular debris. The cytological features are not distinctive, including scant flat sheets of ductal cells or single scattered neuroendocrine cells with relatively large nuclei and little variation. Occasionally atypical cells show prominent nucleoli, but with smooth nuclear membrane and low nuclear/cytoplasmic ratio [16]. The stricture of the common bile duct in chronic pancreatitis is a common complication, and often requires to place stents inside the biliary tract to prevent obstruction. However, the stents may cause acute inflammation, necrosis, and atypical reactive changes in the biliary mucosa.

9. What are the types of primary pancreatic tumors in WHO Classification?

Answer:

Benign [17]

- Acinar cell cystadenoma
- Serous cystadenoma

Premalignant lesions

- Pancreatic intraepithelial neoplasia, grade 3 (PanIN-3)
- Intraductal papillary mucinous neoplasm (IPMN) with low- or intermediate-grade dysplasia

- Intraductal papillary mucinous neoplasm (IPMN) with high-grade dysplasia
- Intraductal tubulopapillary neoplasm (ITPN)
- Mucinous cystic neoplasm (MCN) with low- or intermediate-grade dysplasia
- Mucinous cystic neoplasm (MCN) with high-grade dysplasia

Malignant lesions

- Ductal adenocarcinoma
  - Adenosquamous carcinoma
  - Mucinous adenocarcinoma
  - Hepatoid carcinoma
  - Medullary carcinoma
  - Signet ring cell carcinoma
  - Undifferentiated carcinoma
  - Undifferentiated carcinoma with osteoclast-like cells
- Acinar cell carcinoma
- Acinar cell cystadenocarcinoma
- Intraductal papillary mucinous neoplasm (IPMN) with an associated invasive carcinoma
- Mixed acinar ductal carcinoma
- Mixed acinar neuroendocrine carcinoma
- Mixed acinar neuroendocrine ductal carcinoma
- Mixed ductal neuroendocrine carcinoma
- Mucinous cystic neoplasm (MCN) with an associated invasive carcinoma
- Pancreatoblastoma
- Serous cystadenocarcinoma
- Solid pseudopapillary neoplasm

Pancreatic endocrine tumor

- Nonfunctioning (nonsyndromic) neuroendocrine tumors
  - Pancreatic neuroendocrine microadenoma
  - Nonfunctioning pancreatic neuroendocrine tumor
- Insulinoma
- Glucagonoma
- Somatostatinoma
- Gastrinoma
- VIPoma
- Serotonin-producing tumors with and without carcinoid syndrome
  - Serotonin-producing tumor
- ACTH-producing tumor with Cushing syndrome
- Pancreatic neuroendocrine carcinoma (poorly differentiated neuroendocrine neoplasm)
  - Neuroendocrine carcinoma (poorly differentiated neuroendocrine neoplasm)
  - Small cell neuroendocrine carcinoma
  - Large cell neuroendocrine carcinoma
- Mixed neuroendocrine nonneuroendocrine neoplasms
  - Mixed ductal neuroendocrine carcinoma
  - Mixed acinar neuroendocrine carcinoma

10. What is the cytological classification of pancreatic lesions?

Answer:

Recently, the Papanicolaou Society of Cytopathology has developed a set of guidelines for pancreatobiliary cytology, including indications for endoscopic ultrasound (EUS) guided fine-needle aspiration (FNA) biopsy, techniques of EUS-FNA, terminology and nomenclature of pancreatobiliary disease, ancillary testing, and post-biopsy treatment and management [14, 15]. The proposed terminology scheme recommends a six-tiered system: Non-diagnostic, negative, atypical, neoplastic (benign or other), suspicious and positive for malignancy (refer to Table 6.3). The positive or malignant category is reserved for high-grade, aggressive malignancies including ductal adenocarcinoma, acinar cell carcinoma, poorly differentiated neuroendocrine carcinomas, pancreatoblastoma, lymphoma, and metastases. This proposed guideline provides terminology that standardizes the category of the various diseases of the pancreas, some of which are difficult to give specific diagnosis by cytology alone [14].

Table 6.3 Pancreatic cytology terminology, definition, and diagnostic criteria

Terminology category	Definition	Diagnostic criteria
Category I: Non-diagnostic	No diagnostic or useful information about the solid or cystic lesion sampled	Gastrointestinal contamination only; non-specific cyst contents with insufficient cyst fluid volume for ancillary testing; evaluation limited by scant cellularity
Category II: Negative (for malignancy)	Adequate cellular and/or extracellular tissue to evaluate	Benign pancreatobiliary tissue including acute pancreatitis; chronic pancreatitis; autoimmune pancreatitis; pseudocysts; lymphoepithelial cyst; spleen/accessory spleen
Category III: Atypical	Cells present with cytoplasmic, nuclear, or architectural features that are not consistent with normal or reactive cellular changes of the pancreas or bile ducts and are insufficient to classify them as a neoplasm or suspicious for a high-grade malignancy	Atypical ductal cells: cells with crush artifact; scant population of small monomorphic polygonal cells of unclear origin; atypical bile duct epithelium with nuclear features suggestive of repair in a background of acute inflammation

Table 6.3 (continued)

Terminology category	Definition	Diagnostic criteria
Category IVA: Neoplastic: Benign	The presence of a cytological specimen sufficiently cellular and representative, with or without the context of clinical, imaging and ancillary studies, to be diagnostic of a benign neoplasm	Serous cystadenoma: scant non-mucinous cuboidal epithelium and scant hemosiderin-laden macrophages in a non-mucinous cyst fluid
Category IVB1: Neoplastic: Mucinous neoplasm	Premalignant such as intraductal papillary neoplasm of the bile ducts (IPN-B), IPMN or MCN with low, intermediate or high-grade dysplasia by cytological criteria	MCN: multiloculated cysts with mucin-producing epithelial cells and subepithelial ovarian-type stroma that does not communicate with the pancreatic ductal system; in almost all cases occurs in women; located in the body or tail; easily removed comparing life-long surveillance; by cytology alone, it is difficult to separate from IPMN IPMN: papillary mucinous cells with or without high N/C ratio or irregular nuclear contour; in radiology imaging, ductal dilatation or cyst formation and/or a mass lesion, including main-ductal IPMN and branched ductal IPMN; GNAS mutation in most of IPMN IPN-B: mucinous cells in ductal brushing or FNA with or without increasing N/C ratio, irregular nuclear contour or pleomorphism; similar to IPMN
Category IVB2: Neoplastic: Non-mucinous neoplasm	A low-grade malignant neoplasm such as well-differentiated PanNET, SPN, or rare GIST	PanNET (pancreatic endocrine tumor and pancreatic endocrine neoplasm): numerous single and small clusters of bland cells with high or moderate N/C ratio, salt and paper chromatin; cells with positive synaptophysin, chromogranin or CD56 immunostain SPN: bland cuboidal cells with loosely cohesive groups or singly dispersed cells adjacent to vascular structures; PAS-D positive hyalin globules; nuclei with nuclear grooves GIST: Spindle cells or mixed spindle cells and epithelioid cells from the interstitial cell of Cajal; c-kit protein (CD117), DOG1 and CD34 by immunohistochemistry; located in a peripancreatic location
Category V: Suspicious (for malignancy)	When some, but an insufficient number of the typical features of a specific malignant neoplasm are present, mainly pancreatic adenocarcinoma	Rare markedly atypical epithelial cells with all features for adenocarcinoma or acinar cell carcinoma; rare mucinous cystic cells with high-grade epithelial atypia and abundant coagulate necrosis
Category VIA: Malignancy: PDAC and variants	A group of neoplasms that unequivocally display the pancreas cytological characteristics and include PDAC and its variants	PDAC: abundant pleomorphic ductal cells forming three-dimensional clusters with variable size of nuclei (largest nucleus/smallest nucleus ≥ 4) in the same group (Case 2, Fig. 6.1); tumor cells with high N/C ratio, irregular nuclear contour, prominent nucleoli, hyperchromasia or clear/washout chromatin, necrosis, or occasional mitoses
Category VIB: Malignancy: Others	A group of neoplasms that unequivocally display malignant cytologic characteristics excluding PDAC and its variants, including acinar cell carcinoma, high-grade neuroendocrine carcinoma (small cell and large cell), cholangiocarcinoma, pancreatoblastoma, lymphomas, sarcomas, and metastases to the pancreas	Cholangiocarcinoma: same as ductal adenocarcinoma; usually diagnosis by bile duct brushings with high false-negative rate due to overlying benign epithelium, insufficient sampling, reactive change with stent; degeneration due to bile Acinar cell carcinoma: tight "grape-like" clusters highly composed of loose aggregates and singly dispersed cells, tumor cells with granular cytoplasm, round to oval nuclei, and often a single prominent nucleolus Poorly differentiated neuroendocrine carcinoma (small cell carcinoma or large cell neuroendocrine carcinoma): high N/C ratio, nuclear pleomorphic; salt and pepper chromatin, nuclear molding; tumor cells with positive synaptophysin, chromogranin, and CD56 Pancreatoblastoma: including epithelial and stromal components – the epithelial component with syncytial aggregates and singly dispersed epithelial cell with acinar, endocrine, or ductal differentiation; the stromal component, immature mesenchymal cells, squamoid corpuscles within the epithelial aggregates such as primitive spindle-shaped cells, and heterologous elements such as cartilage may be seen

MCN Mucinous cystic neoplasms, *IPN-B* Intraductal papillary neoplasm of the bile ducts, *IPMN* Intraductal papillary mucinous neoplasm, *PanNET* Pancreatic neuroendocrine tumor, *SPN* Solid pseudopapillary neoplasms, *PDAC* Pancreatic ductal adenocarcinomas

11. What are the diagnostic cytological features of pancreatic ductal adenocarcinoma?

Answer:

Pancreatic ductal adenocarcinoma (PDAC) is the most common pancreatic cancer (85–90%) and usually located in the pancreatic head. (If you do not know the detailed subclassification of a malignant tumor in pancreatic EUS-FNA samples, the pancreatic ductal adenocarcinoma is a good guess.) The key diagnostic feature of PDAC is groups of pleomorphic ductal cells forming three-dimensional clusters with variable size of nuclei (largest nucleus/smallest nucleus ≥4) in the same group. In addition, PDAC consists of tumor cells with high nuclear/cytoplasmic ratio, irregular nuclear contour, prominent nucleoli, hyperchromasia or clear/washout chromatin, necrosis, or occasional mitoses. In the well-differentiated PDAC, tumor cells shows a disorganized cellular sheet (drunken honeycomb) architectures, and with abundant mucinous cytoplasm and pleomorphic nuclei.

12. What are the sensitivity and specificity of a pancreatic EUS-FNA for diagnosing pancreatic malignancy?

Answer:

In one meta-analysis of a total 4984 cases with solid pancreatic mass by EUS-FNA cytological diagnosis, the sensitivity for malignant cytology was 85% and the specificity was 98%. If atypical and suspicious cytology results were included to determine true neoplasms, the sensitivity increased to 91%, but the specificity was reduced to 94% [18]. For pancreatic cystic lesions in another meta-analysis of 1024 cases, the sensitivity and specificity for malignant cytology were 51% and 94%, respectively. If the suspicious or potential malignancy were included, the sensitivity and specificity were 52% and 97%, respectively [19].

13. What is the typical immunohistochemical staining profile of pancreatic ductal adenocarcinoma?

Answer:

There are many immunohistochemical markers applied in diagnosis of pancreatic ductal adenocarcinoma, including von Hippel-Lindau tumor suppressor (pVHL), placental S100 (S100P), mammary serine protease inhibitor (maspin), insulin-like growth factor II messenger RNA-binding protein-3 (IMP3), mesothelin, prostate stem cell antigen (PSCA), annexin A8, fascin, claudin 4, claudin 18, p53, SMAD family member 4 (DPC4/SMAD4), carcinoembryonic antigen (CEA), cytokeratin (CK) 17, CK19, mucin (MUC) 1, MUC2, MUC5AC, cancer antigen 19-9 (CA19-9), and EpCAM. More recently, three additional biomarkers (annexin A10, plectin 1, and aldo-keto reductase family 1 member B10 [AKR1B10]) have been reported as useful markers to differentiate pancreatic PDACs from benign/reactive pancreatic ducts [20]. Based on that study and review of the literature, they concluded that pVHL, maspin, S100P, IMP3, CK17, MUC5AC, and DPC4/SMAD4 were the best diagnostic panel of immunomarkers for confirming the diagnosis of pancreatic PDACs in both surgical and FNAB specimens [20].

14. What are the biomarker tests for pancreatic ductal adenocarcinomas and their clinical significance?

Answer:

CA19-9, which is a carbohydrate found on multiple carrier proteins, is useful for monitoring response to therapy, but not very useful as an early detection biomarker [21]. Poruk and colleagues assessed the utility of secreted proteins *OPN* (osteopontin) and *TIMP1* (tissue inhibitor of metalloproteinase 1) plus *CA19-9* as diagnostic or prognostic biomarkers, by using commercially available ELISA kits and reached diagnostic sensitivity of 87% and specificity of 91%. In addition, MUC5AC was also confirmed to be able to differentiate early pancreatic cancer from benign controls and chronic pancreatitis [22]. A novel three-protein biomarker panel (LYVE-1, REG1A, and TFF1) was tested in a multicenter cohort urine samples, which showed promising data in detecting patients with early-stage pancreatic cancer [23]. Genomic changes are highly recurrent in PDAC. Assay consisting of *ctDNA*, *CA19-9*, *CEA*, *HGF*, and *OPN* showed the best combination to detect PDAC with a sensitivity of 64% and specificity of 99% [21]. They also found that the panel had prognostic value beyond clinical and histopathology in current practice. Patients with poorer survival were more likely to have a positive test. It is very challenging to get ideal biomarkers for early detection of PDAC. However, the progress made to-date in finding biomarkers for early detection of PDAC provides optimism and invigoration to the field.

15. What are the diagnostic cytological features and differential diagnosis of pancreatic neuroendocrine tumor?

Answer:

Pancreatic neuroendocrine tumors (PanNETs) are uncommon and represent only 1–2% of all pancreatic neoplasms. They can occur at any age but are most common in adults with a mean age of about 40 years. PanNETs are generally small circumscribed masses (1–3 cm in diameter) and can be partially cystic. A small minority (less than 5%) is completely cystic which can lead to misclassification and result in a false-negative FNA. Aspirate smears of PanNETs are usually highly cellular and composed of singly dispersed cells and loosely cohesive groups with occasional pseudorosettes. The cells have uniform, round to oval, eccentrically placed nuclei with a plasmacytoid appearance. The chromatin is finely stippled ("salt and pepper"). Nucleoli can range from inconspicuous to prominent. There is moderate to

abundant cytoplasm that is usually finely granular, but also can be vacuolated. The differential diagnosis is wide, including solid-pseudopapillary neoplasm, acinar cell carcinoma, non-Hodgkin lymphoma, melanoma, plasmacytoma, metastatic renal cell carcinoma, and sampling of benign pancreatic acini, particularly in paucicellular aspirates.

16. What are the cytological features and differential diagnoses for pancreatic solid pseudopapillary tumor?
Answer:

Solid-pseudopapillary neoplasm (SPN) is an uncommon tumor that occurs almost exclusively in young women. The FNA specimens are usually highly cellular with bland cuboidal cells arranged as loosely cohesive groups, singly dispersed cells, or characteristically myxoid or hyalinized vascular structures lined by neoplastic cells. The neoplastic cells have fine granular cytoplasm that may contain PAS-D-positive hyalin globules. The nuclei are round to oval with nuclear grooves and inconspicuous nucleoli. The differential diagnosis of SPN includes PanNET and acinar cell carcinoma, both of which tend to yield aspirates composed of loosely cohesive groups of bland epithelioid cells.

17. How to differentiate between pancreatic neuroendocrine tumor and solid pseudopapillary tumor by using immunohistochemical markers?
Answer:

PanNETs and SPNs often have similar and overlapping cytomorphologic features. A panel of immunohistochemical (IHC) stains can be used to distinguish these two entities. The vast majority of SPNs harbor mutations in the β-catenin gene. This results in an abnormal pattern of labeling with antibodies to the β-catenin protein. SPNs demonstrate cytoplasmic and nuclear staining with β-catenin, whereas PanNETs show cytoplasmic staining only. The endocrine markers synaptophysin, CD56, and neuron-specific enolase are consistently positive in both SPNs and PanNETs; however, chromogranin, being more specific, is usually negative in SPNs. Finally, antibodies to cytokeratins (CAM 5.2, AE1/AE3) are typically either negative or only focally positive in SPNs. In contrast, PanNETs show strong diffuse labeling with these markers.

18. What are the cytological features and differential diagnoses for acinar cell carcinoma?
Answer:

Acinar cell carcinoma is a rare pancreatic exocrine tumor that tends to occur in older adults. It has a poor prognosis with an overall 5-year survival of less than 10%. FNA demonstrates highly cellular smears composed of loose aggregates and singly dispersed cells. The tumor cells have a moderate amount of granular cytoplasm with round to oval nuclei and often a single prominent nucleolus. Stripped naked nuclei are commonly seen in the background. The differential diagnosis includes benign acinar cells which tend to form tight "grape-like" clusters and usually have small, inconspicuous nucleoli. PanNET and SPN also yield highly cellular aspirates composed of loose aggregates and singly dispersed cells. Loosely acinus-like aggregates of acinar cell carcinoma can resemble the rosettes of PanNETs. Special stains and immunohistochemistry can be used to aid in the diagnosis. In the malignant acinar cells, the periodic acid-Schiff stain highlights the cytoplasmic granules, and the immunohistochemical stains for the pancreatic enzymes trypsin, chymotrypsin, and lipase are typically positive as well. Neuroendocrine markers such as synaptophysin, chromogranin, and CD56 are negative or scatter positive for acinar cell carcinoma.

19. What are the cytological features and differential diagnoses for pancreatoblastoma?
Answer:

Pancreatoblastoma is a rare epithelial malignancy that occurs primarily in young children. FNA yields both epithelial and stromal components. The epithelial component is characterized by syncytial aggregates and singly dispersed epithelial cells. The epithelial cells may have acinar, endocrine, or ductal differentiation. Characteristically, squamoid corpuscles may be present within the epithelial aggregates. The stromal component is composed of primitive spindle-shaped cells, and heterologous elements such as cartilage may be seen. The differential diagnosis includes other primary pancreatic neoplasms such as acinar cell carcinoma, PanNET, and SPN, as well as morphologically similar childhood small round blue cell tumors that occur in the vicinity of the pancreas, including Wilms tumor and neuroblastoma.

20. What are the pancreatic cystic lesions?
Answer:

Pancreatic cystic lesions consist of a wide variety of categories including benign and malignant cystic lesions, and further dividing into true cysts, pseudocysts, and cystic neoplasms [15]. In adults, 85–90% of these lesions are pseudocysts, and they usually occur as a complication of chronic pancreatitis. Most of the true cysts are neoplastic. A true cyst is distinguished by the presence of an epithelial lining, indicating its benign natural history and developmental origin [24]. Because some pancreatic cysts have more of a malignant potential than others, it is absolutely essential that an accurate diagnosis is rendered so that effective care can be given to each patient. Recently, Pitman et al. published a "standardized terminology and nomenclature for pancreatobiliary cytology: The Papanicolaou Society of Cytopathology Guidelines" [14]. Based on their guideline, we summarized the cystic lesions in pancreas in Table 6.3 [14, 15].

21. What are the cytomorphologic features and genetic findings of intraductal papillary mucinous neoplasm?
Answer:

The intraductal papillary mucinous neoplasm (IPMN) is usually diagnosed by endoscopic retrograde pancreatography (ERCP), which demonstrates a segmental or diffusely dilated pancreatic duct with filling defects corresponding to mucin or mural nodules. The IPMN is divided into main-duct IPMN and branch IPMN. Most of the IPMN (about 70%) is located in pancreatic head. EUS-FNA samples usually show thick mucin with or without mucinous lining cells. It is difficult to differentiate low-grade IPMN mucinous lining from contaminated gastrointestinal mucosa. However, the cytology is helpful in identifying high-grade dysplasia/invasive carcinoma, which shows high N/C ratio, 3-D clusters or "drunken honeycomb" pattern, irregular nuclear contour, pleomorphism (1–4 ratio), and washout clear chromatin with prominent nucleoli. However, it is indistinguishable between high-grade dysplasia and invasive adenocarcinoma in cytology specimen due to lack of histological evaluation. The ancillary studies including CEA (cut-off level: 192 ng/ml) and *KRAS* mutation analysis each had high specificity (100% and 93.2%), but low sensitivity (48.3% and 56.3%) for the diagnosis of cystic mucinous neoplasm (Kadyifci Endosc Int Open 2016). *KRAS* mutation is significantly more frequent in malignant cystic mucinous neoplasm (73%) compared to non-malignant (37%). GNAS mutation is predominantly in IPMN but not in MCN. For the branching IPMN, it is difficult to differentiate from MCN.

22. How to differentiate between IPMN and MCN?
Answer:

It is impossible to differentiate the IPMN from MCN only by cytological morphology from EUS-FNA of pancreatic cysts. However, GNAS mutation test from pancreatic cyst content can be used to separate IPMN and MCN (refer to the answer of question 21). In addition, the radiology imagings also help to identify if pancreatic cysts connect to main pancreatic duct. The location of cysts are also helpful since IPMN is more often located in pancreatic head (70%) and MCN is usually in the pancreatic body and/or tails. Also, MCN is found exclusively in women, but IPMN is present equally in men and women.

23. What is the serous cystadenoma of the pancreas?
Answer:

Serous cystadenoma (SCA) of pancreas accounts for 1–2% of all cystic neoplasms of the pancreas. Most of them are benign cystic lesion including serous microcystic adenoma, serous macrocystic adenoma, and serous solid adenoma. Only rare cases of serous cystadenocarcinoma have been reported [25, 26]. Some SCAs are associated with von Hippel-Lindau syndrome (VHL) with *VHL* tumor suppressor gene mutation (3p35). SCAs are located in the pancreatic tail (61%), body (12%), and head (15%) [25]. The cytological samples usually consist of scant cuboid cells containing intracytoplasmic glycogen and contaminated GI tract cells. The fluid is clear and CEA level is <192 ng/ml. With EUS-FNA, it is challenging to make a definite diagnosis of serous microcystic adenoma due to the scant cellularity and bland cytomorphology [27, 28]. The differential diagnosis includes pseudocyst, mucinous cystic neoplasm, intraductal papillary mucinous neoplasm, lymphoepithelial cyst, or rare dermoid or epidermal inclusion cyst. Mucinous cystic lesions can be ruled out if the smear is lack of mucin in the background. Lymphoepithelial cysts, dermoid, or epidermal inclusion cysts show a predominant squamous component. Pseudocyst is almost impossible to differentiate from SCAs if it is hypocellular and lack of cellular debris.

24. What is the lymphoepithelial cyst?
Answer:

Lymphoepithelial cyst (LEC) of the pancreas is a rare benign pancreatic cystic lesion and is mostly found in male patients [29]. The LEC aspiration smear typically consists of numerous squamous cells in the background of variable number of lymphocytes, squamous, and keratinous debris [30–32]. The differential diagnosis includes contaminated squamous cells from esophagus, dermoid, or epidermal inclusion cyst, well-differentiated squamous carcinoma, ectopic splenic tissue, and serous cystic neoplasms. The contaminated squamous cells usually present as scattered squamous cells without lymphocytes or keratinous debris in the background. Dermoid or epidermal inclusion cysts are also very rare and show numerous squamous cells and keratinous debris, but no lymphocytes in the background. Well-differentiated squamous cell carcinoma usually shows dysplastic squamous cells with changes including relative high N/C ratio, irregular nuclear contours, and enlarged nuclei. Serous cystic neoplasm usually has scant cellularity without squamous cells and lymphocytes. It is important to differentiate this benign lesion from its malignant mimics.

25. What is the most common metastatic malignancy to the pancreas and its corresponding distinctive cytological features?
Answer:

Pancreatic metastases from other primary sites are uncommon and account for only 2% of pancreatic malignancies. Renal cell carcinoma (RCC) is the most common metastatic tumor in pancreas (74%) [33–36]. EUS-FNA samples of metastatic RCC consist of three-dimensional loose cell clusters with transpassing vessels, and background of blood and necrotic cellular debris. Some lesional cells line along the vessels and some form loose group or single cells. The lesional cells show abundant wispy clear cytoplasm with ill-

defined edges and vacuoles. The nuclei are small or moderate in size, and have fine chromatin with inconspicuous nucleoli. Some lesional cells have amphophilic cytoplasm. The differential diagnosis includes solid pseudopapillary tumor, pancreatic neuroendocrine tumor, acinar cell carcinoma, chronic pancreatitis with reactive macrophages, and pancreatic ductal carcinoma. The immunohistochemistry test is the best way to differentiate RCC from other pancreatic tumors. Renal cell carcinoma is positive for PAX8, RCC, vimentin, CD10, and EMA, and negative for synaptophysin and chromogranin. The cytology of metastatic RCC is very tricky since the morphology of RCC tumor cells is bland, and is easily to be missed by routine practice. It is important to check patient's history for other malignant tumors and collect good cell blocks to confirm these macrophage-like cells by immunohistochemistry study.

26. What is the common differential diagnosis for bile duct brushing specimen?

Answer:

Bile duct brushing specimens are common samples for the diagnosis of cholangiocarcinoma or pancreatic carcinoma. The common differential diagnosis of bile duct brushing includes cholangiocarcinoma, pancreatic carcinoma, mucinous cystadenoma, IgG4 cholangitis, and reactive atypia from stone, stent, or inflammation. The major concern is the reactive atypia versus malignancy. The cytomorphology of bile duct cells in both reactive atypia and adenocarcinoma is overlapping. Both reactive atypia and well-differentiated adenocarcinoma show the nuclear enlargement, prominent nucleoli, irregular nuclear contour, and open chromatin. However, well-differentiated adenocarcinoma usually shows pleomorphic changes (nuclei size ratio of the largest and the smallest cells is over 4:1 in the same group), high N/C ratio, irregular nuclear contour, and necrosis without extensive inflammation. Also, one of the best guides is to compare the cells of benign or reactive bile duct with the abnormal bile duct cells. With the clinical history of stent placement, the definitive diagnosis of adenocarcinoma should be very cautious, since the severe cellular atypia due to the stent can mimic the well-differentiated cancer cells.

Case Presentations

Case 1

Learning Objectives:

1. To become familiar with cytologic features of the spindle cell lesion of the GI tract
2. To generate the differential diagnosis

Case History:

A 66-year-old female presented to the clinic with diarrhea, nausea, vomiting, and profound weight loss over the past 4 weeks. Her past medical history was significant for hypothyroidism and breast cancer status post right breast lumpectomy. Abdominal CT scan showed hypoattenuating, partially exophytic gastric mass up to 5.3 cm. This was seen to extend from the body of the stomach.

Specimen Source:

Ultrasound-guided fine-needle aspiration was performed on this mass lesion of the stomach. A SurePath smear and cell block were made from the aspiration. Immunohistochemistry studies have been performed on the cell block tissue sections.

Cytomorphological Findings (Fig. 6.1a–d):

- Moderate cellularity
- Scattered individual tumor cells, some in loose clusters
- Epithelioid tumor cells with oval nuclei, bland chromatin, and inconspicuous nucleoli
- Peripheral cytoplasmic clearing
- Increased inflammatory cells and red blood cells

Differential Diagnosis:

- Leiomyoma
- Schwannoma
- Poorly differentiated carcinoma
- Gastrointestinal stromal tumor

IHC and Other Ancillary Studies:

- CD117 and DOG1 are positive in lesional cells.
- Smooth muscle actin, desmin, and S-100 are negative in lesional cells.
- PDGFRA mutation p.D842V is detected.

Final Diagnosis:

Gastrointestinal Stromal Tumor (GIST), Epithelioid Type

Take-Home Messages:

- GISTs are the most common gastrointestinal mesenchymal tumors. About 70% of the GISTs are spindle-shaped cells; the epithelioid cell types (30%) are usually less commonly seen.
- Cytology of FNA specimen with the assistance of ancillary immunohistochemistry studies on cell

block tissue is sufficient to make a definitive diagnosis.

- Over 90% of GIST are immunoreactive for CD117/c-KIT, which is not a totally specific marker. CD117 marker positive can also be found in some carcinomas or sarcomas, such as adenoid cystic carcinoma of salivary glands or angiosarcoma. CD117 also can be found negative in some GIST tumor. DOG1 is another sensitive and specific marker to confirm the diagnosis of GIST in both primary and metastatic sites, especially in the hypocellular cytology specimen.
- Approximately 90% of sporadic GISTs have somatic gain of function mutations of the proto-oncogene c-KIT. About half of the c-KIT wild-type GISTs harbor gain of function mutations of platelet-derived growth factor receptor alpha (PDGFR-α). PDGFR-α-mutated GISTs predominantly arise in the stomach and present with an epithelioid or mixed morphology. Recent studies have shown that about 15% of GISTs in adults and 90% in children lack c-KIT or PDGFR-α mutations, the so-called "wild-type" GISTs. Some of them is associated with succinate dehydrogenase (SDH) complex dysfunction, which can be found in familial GISTs.

References: [37–39].

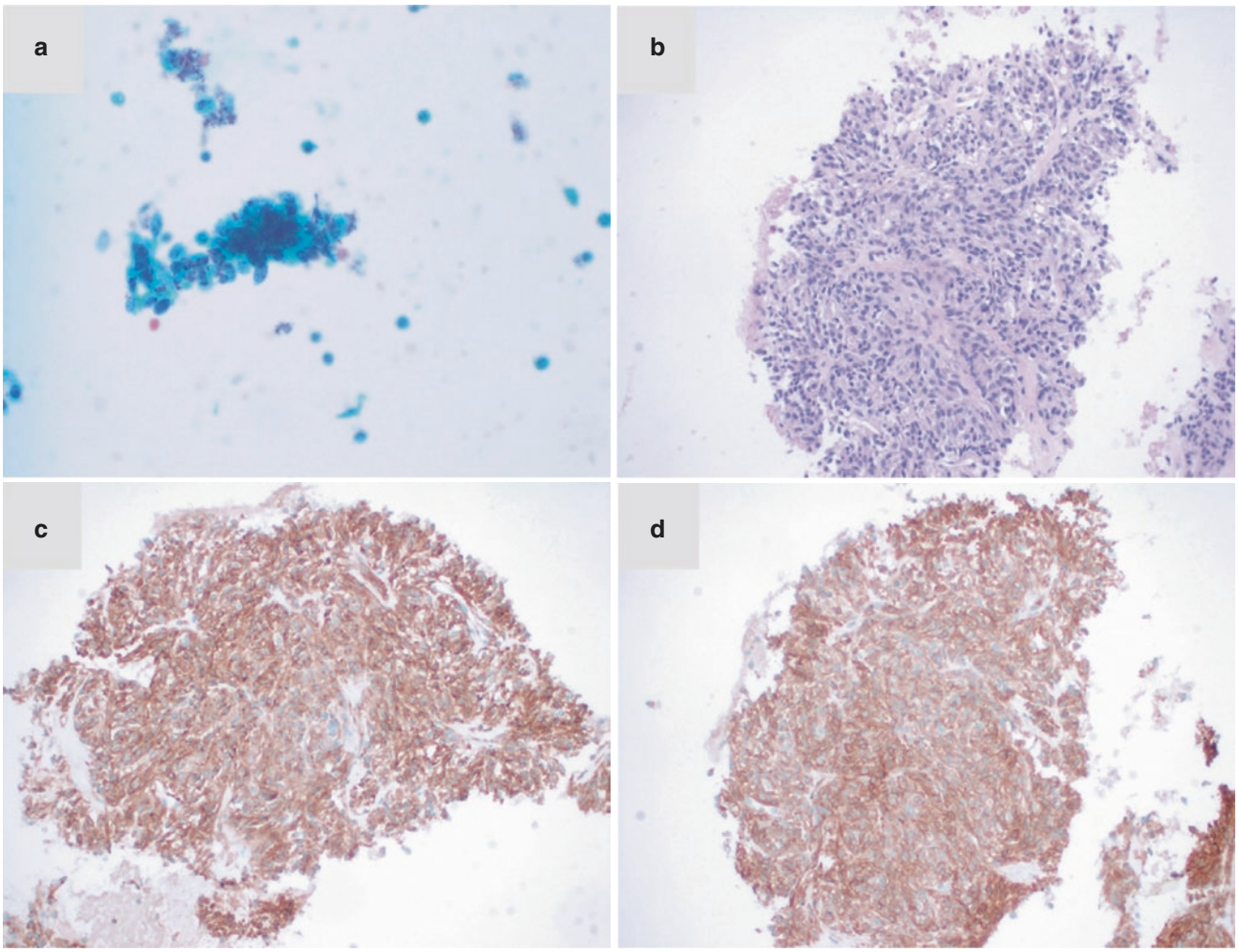

Fig. 6.1 (**a**) Case 1. Single and clusters of epithelioid-like cells with round nuclei and small nucleoli are present (Papanicolaou stain). (**b**) Case 1. Lesion composed of predominantly sheets of epithelioid cells with well-defined cell membranes, ample eosinophilic cytoplasm, round nuclei, and small nucleoli (Cell block, H&E stain). (**c**) Case 1. The lesional cells are positive for CD117 immunostain (Cell block, Immunohistochemical stain). (**d**) Case 1. The lesional cells are positive for DOG1 immunostain (Cell block, Immunohistochemical stain)

Case 2

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this pancreatic tumor
2. Discuss differential diagnosis of pancreatic mass

Case History:

A 54-year-old female had back pain and jaundice. CT reveals a 1.7 cm mass in the pancreatic head. The patient underwent endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) by a gastroenterologist.

Specimen Source:

EUS-FNA was performed. A Diff-Quick (Romanowsky stain) smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic Findings (Fig. 6.2a–d):

- The smears show abundant three-dimensional cohesive clusters with pleomorphic, crowding, and overlapping cells. Isolated cells and naked nuclei are scattered in the background.
- Nuclei of the cells show enlargement, irregular nuclear membrane and contour, anisonucleosis (nuclear size varies 4:1 in the same group), hyperchromasia, and prominent nucleoli.
- Some groups of lesional cells form a disordered honeycomb pattern (drunken honeycomb), focal overlapping, inconspicuous nucleoli, and open nuclear chromatin.

Differential Diagnosis:

- Ductal adenocarcinoma
- Acinar cell carcinoma
- Chronic pancreatis with reactive or reparative atypia
- Metastatic adenocarcinoma

IHC and Other Ancillary Studies:

- p53 positive
- SMAD4 loss
- Trypsin negative

Final Diagnosis:
Pancreatic Ductal Adenocarcinoma
Take-Home Messages:

- In pancreas, 90% malignant tumors are pancreatic ductal adenocarcinoma and the 5-year survival is only around 8%. There is very narrow disease progression window from the symptom to metastasis. Without catching the disease at the early stage, the prognosis is dismay. Therefore, the adequacy check is important during the EUS-FNA procedure and we need to make sure to collect enough samples for diagnosis.
- The reactive atypia from pancreatitis, stone artifact, radiation therapy, or stent sometimes show significantly enlarged nuclei with prominent nucleoli and occasionally irregular nuclear contour, which mimics the well-differentiated adenocarcinoma. However, nuclear sizes usually are not significantly variable in the reactive atypia. The criteria for the diagnosis of adenocarcinoma require 1–4 ratio or greater variance between the largest nucleolus and the smallest nucleolus in a same group.
- We usually do not use immunohistochemistry to confirm our diagnosis or subclassify the category of adenocarcinoma. In some occasions, the definite diagnosis has to be confirmed between reactive atypia and well-differentiated adenocarcinoma; p53 and SMAD4 immunohistochemistry can be used to support the diagnosis. SMAD4 is a tumor suppressor gene that is inactivated in more than 50% of pancreatic cancer [40]. p53 mutation or p53 protein overexpression is present in about 60–70% of PDAC [41, 42]. The p53 overexpression or SMAD4 loss support the diagnosis of PDAC.

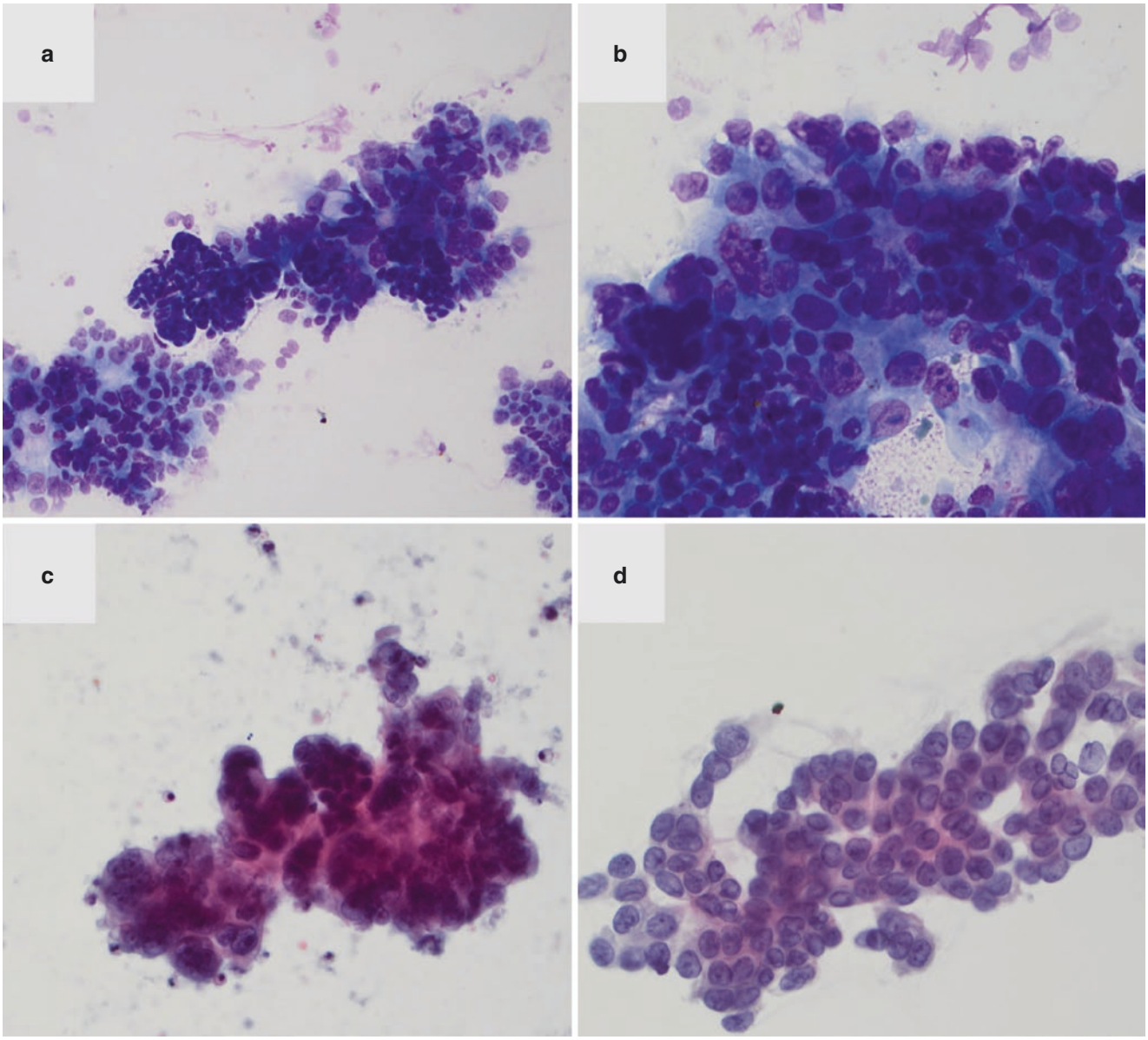

Fig. 6.2 (**a**) Case 2. Some ductal cells are arranged in three-dimensional cohesive clusters. These ductal cells appear pleomorphic, crowding. and overlapping. Scattered singled ductal cells and naked nuclei are present in the background (Diff-Quick stain). (**b**) Case 2. Under high power, nuclei of the ductal cells show marked pleomorphism and anisonucleosis (nuclear size varies 4:1 in the same group) (Diff-Quick stain). (**c**) Case 2. The ductal cells are enlarged, high N/C ratio, irregular nuclear membrane, hyperchromasia, and prominent nucleoli (Papanicolaou stain). (**d**) Case 2. Ductal cells in a group form a disordered honeycomb pattern (drunken honeycomb), with focal overlapping, inconspicuous nucleoli, and open nuclear chromatin (Papanicolaou stain)

Case 3

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this pancreatic mass
2. Discuss differential diagnosis of this pancreatic mass
3. Discuss immunostain to differentiate primary or metastatic carcinoma in pancreas

Case History:

A 74-year-old female had back pain and hematuria. CT revealed two adjacent pancreatic masses in pancreatic head (2.5 cm) and body (1.3 cm), respectively. An endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) was performed on the larger pancreatic lesion by a gastroenterologist.

Specimen Source:

EUS-FNA was performed. A Diff-Quick (Romanowsky stain) smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic Findings (Fig. 6.3a–d):

- The Diff-Quick stain smear consists of some three-dimensional loose clusters with trespassing blood vessels. Blood and debris is present in the background.
- The lesional cells show abundant wispy clear cytoplasm with ill-defined edges and vacuoles. The nuclei are small or moderate in size, eccentrically placed, with fine chromatin and inconspicuous nucleoli. Some cells are lining along the vessels, and some others form loose clusters or scattered around.

Differential Diagnosis:

- Neuroendocrine tumor
- Solid pseudopapillary tumor
- Chronic pancreatitis with reactive macrophages
- Metastatic renal cell carcinoma

IHC and Other Ancillary Studies:

- PAX8 positive
- RCC positive
- Vimentin positive
- Pancytokeratin positive
- Synaptophysin negative
- Chromogranin negative
- CD68 highlights macrophages

Final Diagnosis:

- **Metastatic Renal cell Carcinoma**

Take-Home Messages:

- The renal cell carcinoma (RCC) is the most common metastatic tumor in pancreas, which may present with single or multiple masses. The cytology diagnosis of metastatic RCC is very tricky since the cytomorphology of tumor cells is bland, which is easily to be missed in routine practice. It is important to check patient's personal history of neoplasms and to collect good cell blocks to perform additional immunohistochemistry studies.
- We usually use a panel of immunohistochemistry markers, which include PAX8, RCC, CD68, β-catenin, synaptophysin, and chromogranin, to differentiate this metastatic RCC from other mimics (such as reactive macrophages, neuroendocrine tumor, or solid pseudopapillary tumor).

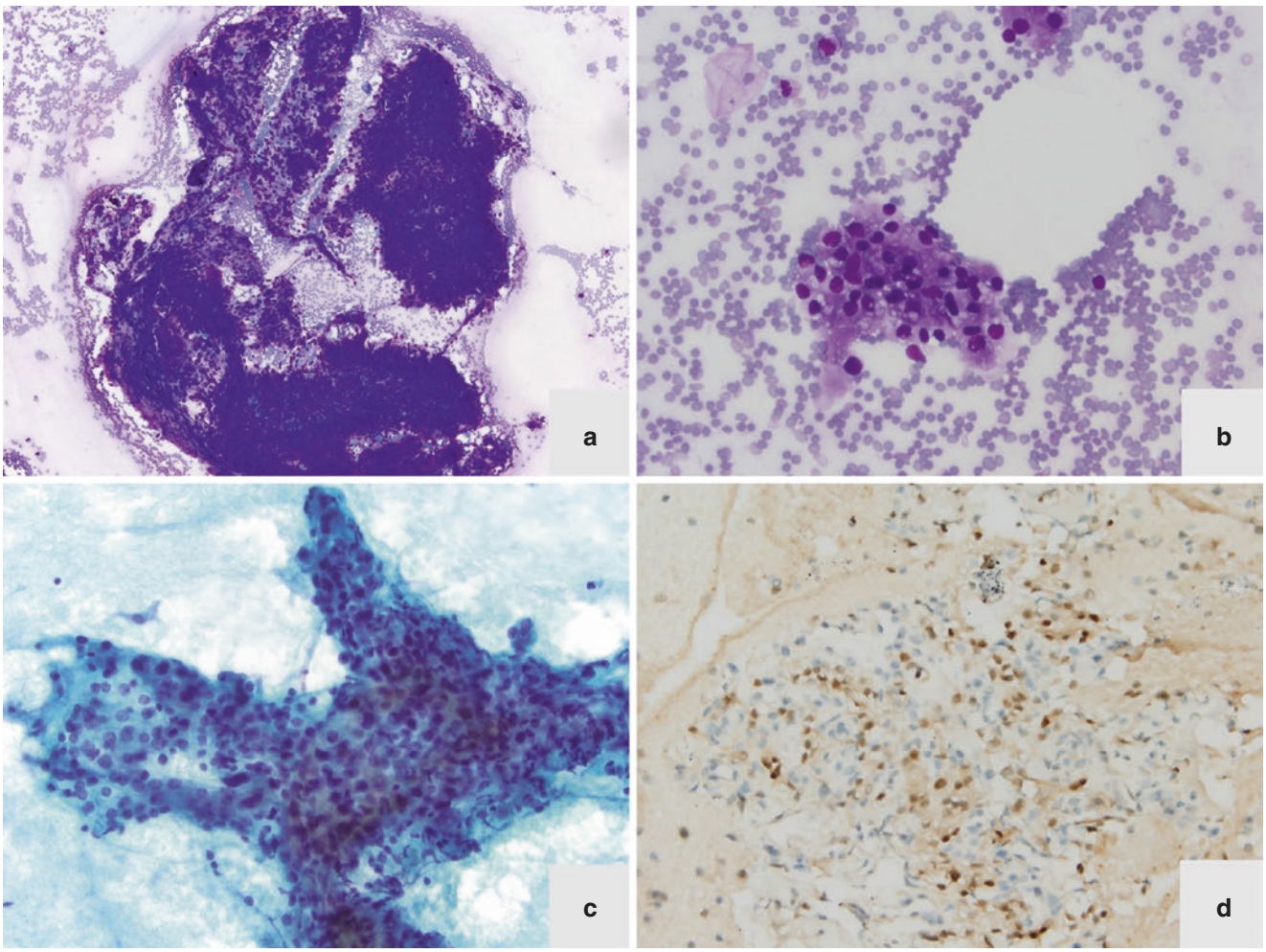

Fig. 6.3 (**a**) Case 3. A few three-dimensional loose clusters with trespassing vessels are present in the background of blood and debris (Diff-Quick stain). (**b**) Case 3. The cells form loose clusters and show abundant wispy clear cytoplasm with ill-defined edges and vacuoles (Diff-Quick stain). (**c**) Case 3. Some cells are lining along the vessels and some others show loose clusters or single cells. The cytoplasm of the cells is clear and wispy. The nuclei are small or moderate in sizes, eccentrically located, fine chromatin with inconspicuous nucleoli (Papanicolaou stain). (**d**) Case 3. The tumor cells are immunoreactive for PAX8 (Cell block, Immunohistochemistry stain)

Case 4

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this tumor
2. Discuss differential diagnosis of this tumor
3. Discuss immunostains to differentiate primary or metastatic adenocarcinoma

Case History:

A 54-year-old male had a single episode of pancreatitis and peripancreatic fluid collection. MRI/MRCP imaging study revealed fluid collection at the anterior aspect of the pancreas. Follow-up EUS test identified an 18-mm mass in the pancreas body.

Specimen Source:

EUS-FNA was performed. A Diff-Quick (Romanowsky stain) smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic Findings (Fig. 6.4a–c):

- Numerous loosely cohesive groups and sheets of cells are present on the smear slides.
- Some of these cells form distinct acinar-like configuration and others show nests, single cells, or naked nuclei. Ample cytoplasm with amphophilic to eosinophilic granules are evident. The nuclei are round or oval with smooth contour, prominent nucleoli, and occasional mitosis. The size of nuclei is variable.

Differential Diagnosis:

- Neuroendocrine tumor
- Solid pseudopapillary tumor
- Pancreatic ductal adenocarcinoma
- Acinar cell carcinoma

IHC and Other Ancillary Studies:

- Trypsin positive
- Synaptophysin negative
- Chromogranin negative
- Beta-catenin nuclei negative

Final Diagnosis:

- **Cytology: adenocarcinoma, favor acinar cell carcinoma**

Whipple Resection (Fig. 6.4d):

- Surgical resection from this patient shows the pancreatic lesion with disorganized acinar pattern. In some areas, the lumens are more dilated resulting in glandular or acinar-like pattern. The acinar cells contain moderate to abundant cytoplasm with amphophilic to eosinophilic granules. The nuclei are round or oval and relatively uniform.

The final surgical diagnosis is acinar cell carcinoma.

Take-Home Messages:

- Acinar cell carcinoma is a rare pancreatic carcinoma. It is challenging to differentiate this tumor from well-differentiated adenocarcinoma without immunostain. Since the clinical management for both tumors is surgery, the diagnosis of adenocarcinoma without classification will not change the patient's treatment plan. However, the trypsin immunostain or PAS-D special stain could be used to make a definitive diagnosis if cell block has sufficient diagnostic cellular tissue.

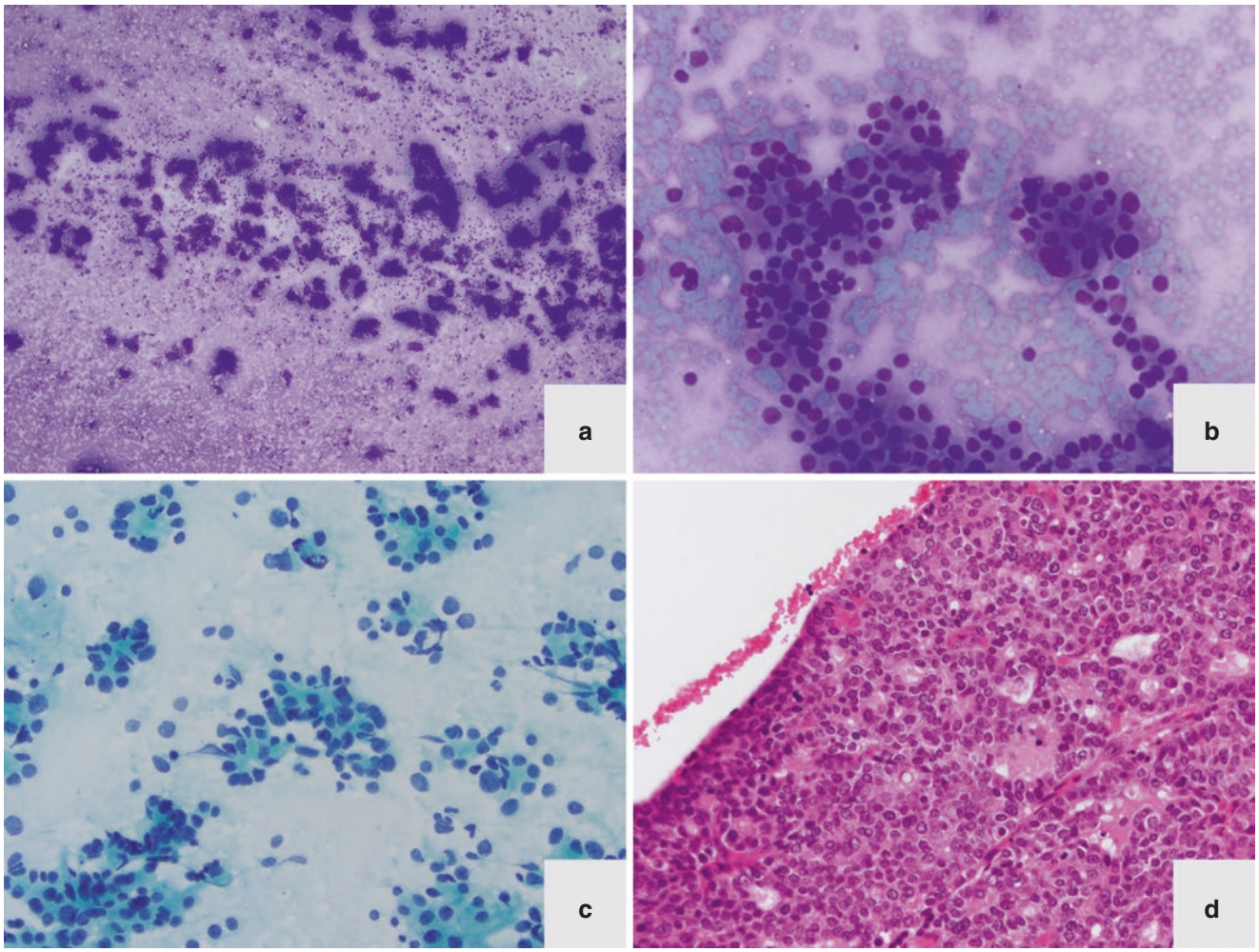

Fig. 6.4 (**a**) Case 4. Numerous loosely cohesive groups and sheets of cells are present (Diff-Quick stain). (**b**) Case 4. Some of these cells form distinct acinar-like configuration and others show nests or dispersed single cells. Moderate to abundant cytoplasm with amphophilic to eosinophilic granules are evident (Diff-Quick stain). (**c**) Case 4. Lesional cells arranged in glandular or acinar-like pattern are very obvious in this area. The nuclei are round or oval with smooth contour, prominent nucleoli, and occasional mitosis. The size of nuclei is variable. (Papanicolaou stain). (**d**) Case 4. Tissue sections from Whipple resection show tumor cells form the pancreatic lesion with disorganized acinar pattern. In some areas, the lumens are more dilated resulting in glandular or acinar pattern. The tumor cells contain moderate to abundant cytoplasm with amphophilic to eosinophilic granules. The tumor nuclei are round or oval and relatively uniform (Tissue H&E stain)

Case 5

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this tumor
2. Discuss differential diagnosis of this tumor
3. Discuss immunostains to differentiate primary or metastatic neoplasms

Case History:

A 39-year-old male with history of status post-pheochromocytoma resection. Recently, MRI/MRCP follow-up revealed a 1.3-cm partially cystic and partially solid pancreatic mass.

Specimen Source:

EUS-FNA was performed. A Diff-Quick (Romanowsky stain) smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic Findings (Fig. 6.5a, b):

- The specimen is cellular, consists of loosely cohesive groups, dispersed bland single cells, and bare nuclei.
- The nuclei of the lesional cells are small, eccentrically located with moderate granular or oncocytic cytoplasm (plasmacytoid appearance) and fine stippled chromatin ("salt and pepper").

Differential Diagnosis:

- Neuroendocrine tumor
- Solid pseudopapillary tumor
- Pancreatic ductal adenocarcinoma
- Acinar cell carcinoma
- Metastatic pheochromocytoma

IHC and Other Ancillary Studies (Fig. 6.5c):

- Trypsin negative
- Synaptophysin positive
- Chromogranin positive
- B-catenin nuclei negative

Final Diagnosis:

- **Cytology: pancreatic neuroendocrine tumor**

Whipple Resection (Fig. 6.5d):

The tumor of the Whipple resection specimen consists of two components. One is microcystic, and the other is mainly solid nests. The lining cells of the microcystic component are uniform, cuboidal with single or double layers. The cuboid cells contain clear or vacuolated cytoplasm and small oval or round nuclei with inconspicuous nucleoli (Fig. 6.5d insert α). The tumor cells in the solid component are arranged in nest, trabecular, and occasional glandular patterns. The cells are relatively uniform and show fine eosinophilic cytoplasm and eccentrically located nuclei with fine stippled chromatin (Fig. 6.5d insert β).

Final Diagnosis:

Pancreatic neuroendocrine tumor and serous microcystic adenoma

Take-Home Messages:

Questions: Which syndrome possibly has both serous microcystic adenoma and PEN?

Answer: Von Hippel-Lindau Syndrome

- Von Hippel (1911) and Lindau (1926) first reported this syndrome
- Melmon and Rosen (1964) named it as VHL disease
- 1/36,000 live births
- Mutations of the *VHL* tumor suppressor gene (3p25-26) [43]
- Related tumors and cysts in multiple organs
  - Retinal angiomas or CNS hemangioblastoma
  - Clear cell RCC
  - Pheochromocytoma
  - Pancreatic neuroendocrine neoplasm
  - Pancreatic cysts and serous adenoma
- 5–12% *VHL* patient with PEN; 25% of PEN with metastasis [25, 44–47]
- 35–75% with cysts and multiple microcystic adenoma
- 11% combined lesions of PEN and cysts

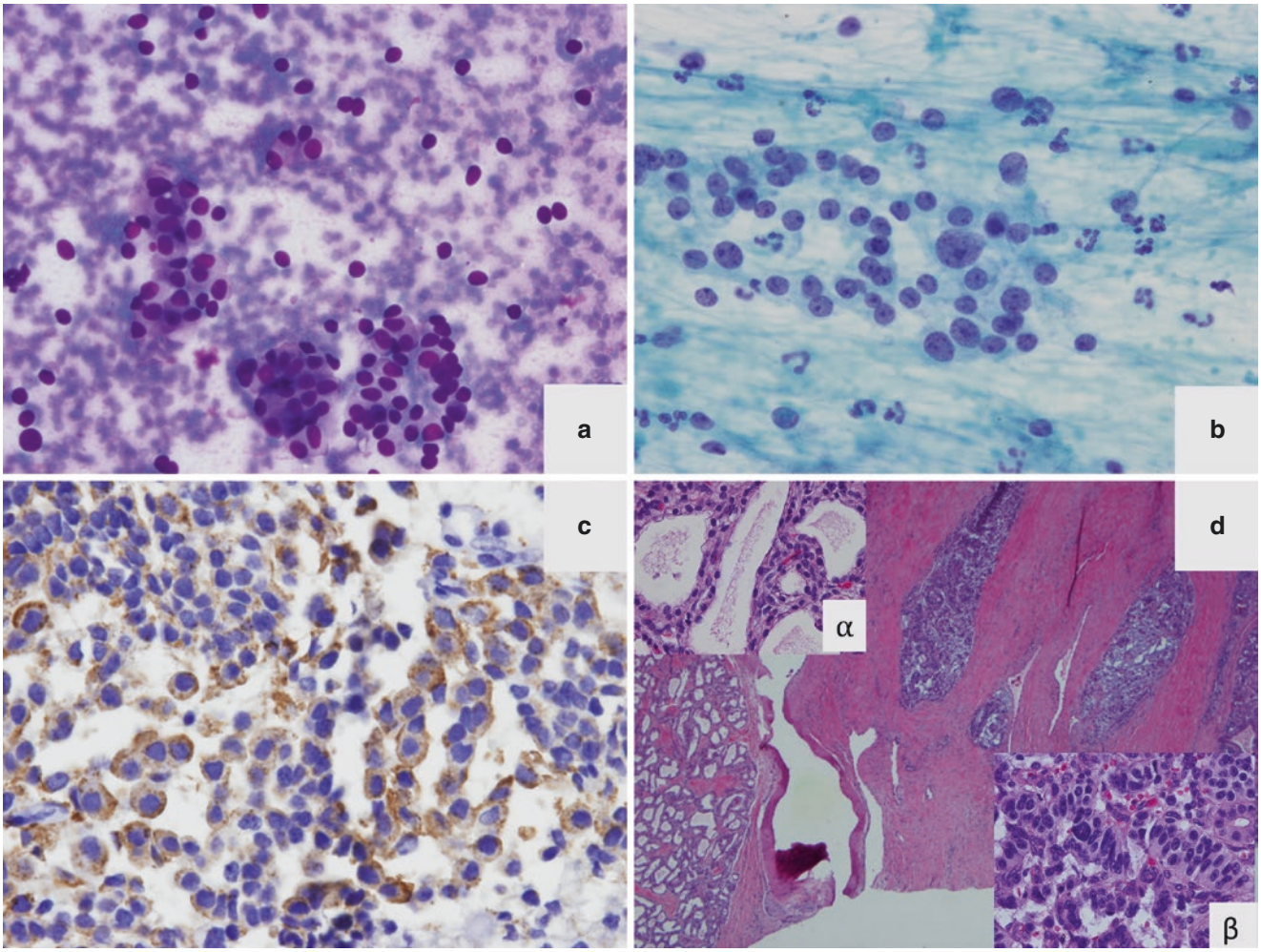

Fig. 6.5 (**a**) Case 5. Smear consists of loosely cohesive groups, numerous dispersed bland single cells, and small naked nuclei (Diff-Quick stain). (**b**) Case 5. The nuclei of the lesional cells are eccentrically located with moderately granular or oncocytic cytoplasm (plasmacytoid appearance) and fine stippled chromatin ("salt and pepper") (Papanicolaou stain). (**c**) Case 5. The tumor cells are positive for chromogranin immunostain (cytoplasmic dot staining pattern) (Cell Block, Immunohistochemistry stain). (**d**) Case 5. Tissue section from Whipple resection shows tumor consisting of two components. One component appears microcystic; the other forms solid nesting pattern. The lining cells of the microcystic component are uniform, and cuboidal in shape with single or double layers. The cuboid cells contain clear or vacuolated cytoplasm and small oval or round nuclei with inconspicuous nucleoli (Fig. 6.5d, insert α). The tumor cells in solid component arrange in nest, trabecular, and occasional glandular patterns. The cells are relatively uniform and show fine eosinophilic cytoplasm and eccentrically located nuclei with fine stippled chromatin (Fig. 6.5d, insert β) (Tissue, H&E stain)

Case 6

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this tumor
2. Discuss the key differential diagnosis of this tumor
3. Discuss the importance of immunostains to rule out differential diagnosis

Case History:

A 42-year-old male was incidentally identified a 4.1 cm mass in the body/tail of the pancreas by abdominal CT scan. The clinical diagnosis is suspicious for neuroendocrine neoplasm.

Specimen Source:

EUS-FNA and biopsy were performed. A Diff-Quick (Romanowsky stain) smear, a Pap-stained smear, a cell block, and a tissue biopsy were made from this procedure.

Cytologic Findings (Fig. 6.6a–d):

- Hypercellular specimen shows numerous loosely cohesive groups, single cells, and bare nuclei present in the bloody background. Some neoplastic cells are lining along the thickened myxoid or hyalinized vessels. The cells are monotonous, with lack of pleomorphism, and anisonucleosis. The cytoplasm is vacuolated, clear or foamy, and with not well-defined cell borders. The nuclei are uniform, round to oval, with stippled chromatin and occasional grooves.
- The tissue section of fine-needle biopsy shows poorly cohesive cells and single cells around fibroid or myxoid stromal material. The cytoplasm of the neoplastic cells is eosinophilic, and the nuclei are uniform with stippled chromatin.

Differential Diagnosis:

- Neuroendocrine tumor
- Solid pseudopapillary tumor
- Pancreatic ductal adenocarcinoma
- Acinar cell carcinoma

IHC and Other Ancillary Studies (Fig. 6.6d):

- Synaptophysin focal positive
- Chromogranin negative
- CD 56 positive

Final Diagnosis:

- **Cytology: pancreatic neuroendocrine tumor**

Whipple Resection (Fig. 6.6e, f):

Tissue sections from the pancreatic mass show solid nests of loosely cohesive lesional cells surrounding thickened and hyalinized blood vessel wall and forming a pseudopapillary architecture. The tumor cells are positive for β-catenin, CD56, and synaptophysin. The tumor cells are negative for chromogranin.

Final Diagnosis: Solid pseudopapillary tumor

Take-Home Messages:

- Solid pseudopapillary tumor is a rare pancreatic carcinoma. It is challenging to differentiate this tumor from neuroendocrine tumor since both tumors have clusters and single cells. In addition, both tumors are positive for CD56 and synaptophysin immunostains. However, the solid pseudopapillary tumors are negative for chromogranin and positive for β-catenin (stain both membrane and nuclei). In classic scenario, solid pseudopapillary carcinomas present predominantly in young female. In this case, the patient is a male and 45 years old. This is why the cytologist did not perform a full immunohistochemical panel. Remember that the ratio of male to female in solid pseudopapillary tumor is 1–10, and patients are usually in the third to fourth decade (mean age 35 years). A full immunohistochemical panel should be done to rule out differential diagnosis.

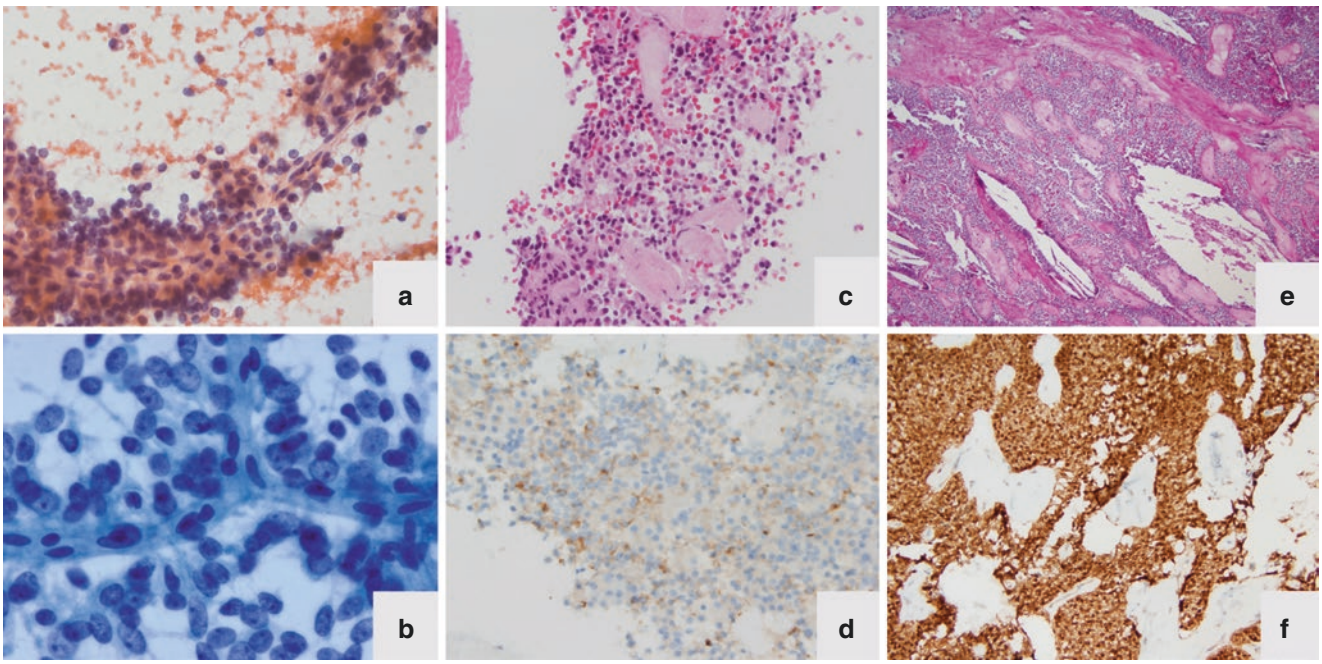

Fig. 6.6 (**a**) Case 6. Cellular smear shows loosely cohesive groups, single cells, and bare nuclei present in the bloody background (Diff-Quick stain). (**b**) Case 6. Neoplastic cells are cuffing along the thickened hyalinized blood vessel located at the center of the figure. The cells appear monotonous, with lack of pleomorphism, and anisonucleosis. The cytoplasm is vacuolated, clear, or foamy, and with indistinct cell borders. The nuclei are uniform, round to oval, with stippled chromatin, and occasional grooves (Papanicolaou stain). (**c**) Case 6. Poorly cohesive cells and single cells are found around fibroid or myxoid stromal material. The cytoplasm of the neoplastic cells is eosinophilic, and the nuclei are uniform with stippled chromatin (FNAB, H&E stain). (**d**) Case 6. The tumor cells are focally positive for synaptophysin immunostain (FNAB, Immunohistochemistry stain). (**e**) Case 6. The tumor mass shows solid nests of loosely cohesive lesional cells surrounding thickened and hyalinized blood vessel wall and forming a pseudopapillary architecture (Tissue, H&E stain). (**f**) Case 6. The tumor cells are positive for β-catenin immunostain (Tissue, Immunohistochemistry stain)

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Lung and Respiratory Tract Cytology

7

Katie Dennis and Fang Fan

Contents

List of Frequently Asked Questions

1. What are the common types of lung cytology specimens and their clinical utilities?
Lung cytology specimens include the following:

- Sputum
- Bronchial washings
- Bronchial brushings
- Bronchoalveolar lavage
- Fine-needle aspirations

Sputum samples are relatively easy to obtain and used in symptomatic patients, but not used as a screening method of lung cancer in asymptomatic smokers due to low sensitivity. Early morning deep cough specimens are preferred. Recently sputum cytology has become less popular due to the wide use of bronchoscopy and fine-needle aspiration. Bronchial washings are obtained by instilling 3–10 mL of saline during a bronchoscopy, and then suctioning the fluid to prepare smears, liquid-based cytology, and/or cell blocks for examination. Bronchial brushings are also obtained during bronchoscopy and can directly sample visualized endobronchial lesions for evaluation. Diagnostic accuracy of bronchial washing and brushing is superior to sputum and comparable to bronchial biopsy. Bronchoalveolar lavage (BAL) is utilized during bronchoscopy to sample distal airways by flushing with sterile saline and suctioning the fluid for evaluation. BAL is often used to diagnose infections, especially in immunocompromised patients, but can also be used to diagnose malignancy.

Guided by a CT scanner, X-ray, or ultrasound machine, fine-needle aspiration (FNA) of lung lesions may be obtained transbronchially, transesophageally, or percutaneous transthoracically. These modalities can eliminate the need for more invasive and expensive surgical diagnostic procedures and are used to diagnose malignancy and stage cancer patients by sampling regional lymph nodes. The relatively new diagnostic procedure endobronchial ultrasound-guided fine-needle aspiration (EBUS-FNA) is used to sample mediastinal and paratracheal lymph nodes as well as peribronchial lung lesions or mediastinal lesions. One advantage of EBUS is the increased access to lower station lymph nodes which cannot be reached by other FNA methods [1, 2].

2. What are the normal components of a BAL specimen?
Normal components of a BAL specimen include pulmonary alveolar macrophages, lymphocytes, and occasional neutrophils [3]. Respiratory epithelial cells may also be present. An adequate BAL specimen should contain abundant pulmonary macrophages (Fig. 7.1).

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_7

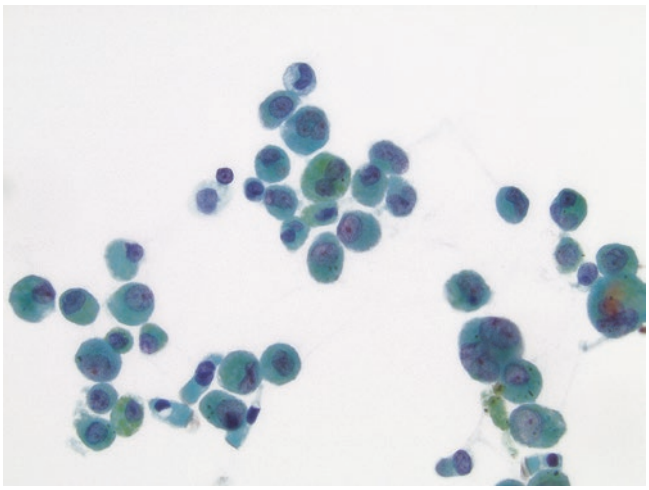

Fig. 7.1 Normal BAL. An adequate BAL specimen contains numerous pulmonary macrophages which have abundant foamy cytoplasm and occasionally golden-brown hemosiderin pigment. A few columnar ciliated respiratory epithelial cells are also present. (ThinPrep 600×)

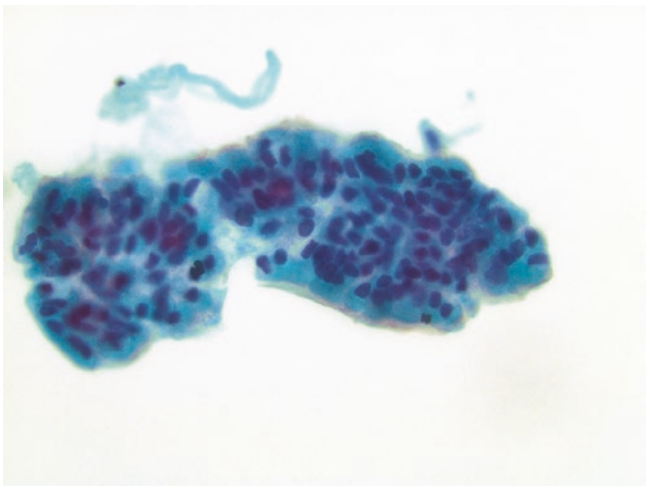

Fig. 7.2 Normal ciliated bronchial cells-bronchial wash. An aggregate of benign bronchial epithelial cells is present, showing columnar-shaped cells with small round nuclei and cilia along the apical surface. (ThinPrep 600×)

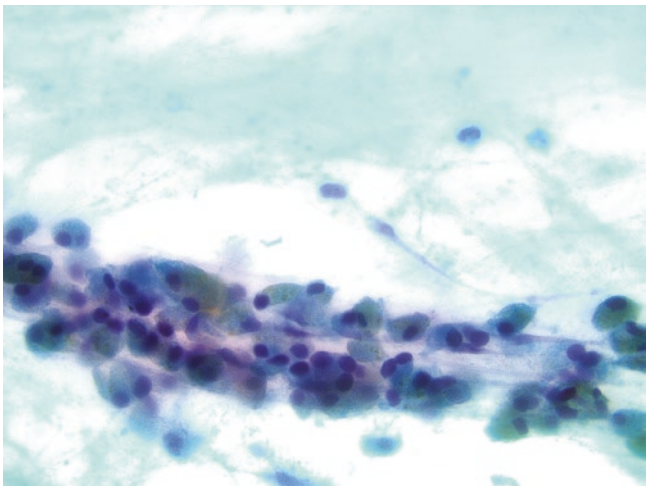

Fig. 7.3 Adequate sputum sample. Numerous alveolar macrophages are present, with abundant foamy cytoplasm, round nuclei, and occasional hemosiderin pigment. (Direct smear, Papanicolaou stain 600×)

Table 7.1 Cellular components, adequacy criteria, and sensitivity/specificity in diagnosing malignancy of various lung cytology specimens

	Cellular components	Adequacy criteria	Sensitivity	Specificity
Sputum	Alveolar macrophages, squamous cells	Numerous alveolar macrophages	42–91%	96–99%
Bronchial washing	Ciliated columnar cells, goblet cells, basal/reserve cells	Numerous bronchial cells	30–97%	95–99%
Bronchial brushing	Ciliated columnar cells, goblet cells, basal/reserve cells	Numerous bronchial cells	30–97%	95–99%
Bronchoalveolar lavage	Alveolar macrophages, pneumocytes, inflammatory cells	Abundant alveolar macrophages	35–70%	91–95%

3. What are the normal components of a bronchial brushing specimen?

Normal components of a bronchial brush specimen include ciliated columnar cells, as well as occasional goblet cells and basal/reserve cells (Fig. 7.2).

4. What is the adequacy criterion for a sputum sample?

Numerous alveolar macrophages must be present for a sputum sample to be considered adequate (Fig. 7.3). Specimens consisting primarily of squamous cells, bacteria, and Candida-like fungal organisms represent oral contamination and are considered unsatisfactory sampling.

5. What is the sensitivity of a bronchoalveolar lavage specimen in diagnosing malignancy?

The sensitivity of BAL in diagnosing malignancy ranges from 35% to 70%, and is higher for multifocal or diffuse tumors [4, 5] (Table 7.1).

6. What are the common fungal organisms that may be seen in lung cytology specimens?

Candida species are commonly found in respiratory cytology specimens, and are generally considered to be oral content contaminants, although they may cause opportunistic infections, especially in immunocompromised patients. Other pathogenic

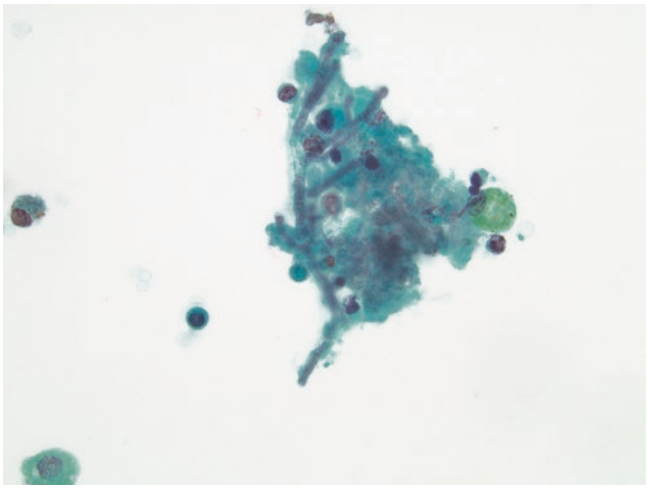

Fig. 7.4 Aspergillus, bronchial brush. Fungal organism with hyphae, acute angle branching, and septations. (Direct smear, Papanicolaou stain 600×)

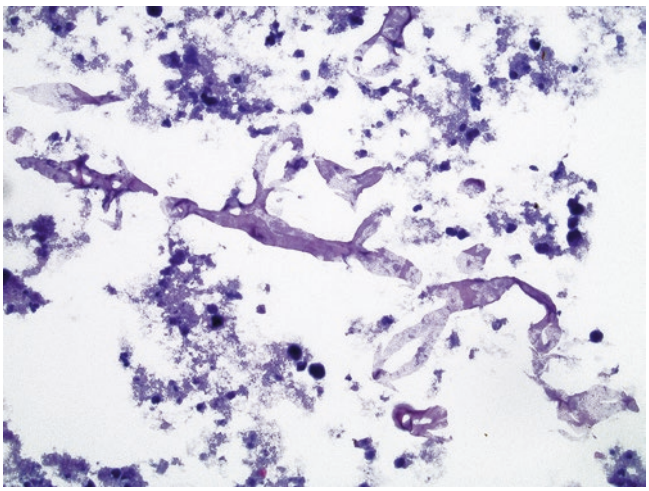

Fig. 7.5 Zygomycosis, bronchial brush. Large non-septated fungal organism with ribbon-like morphology and 90-degree angle branching. (Direct smear, Papanicolaou stain 600×)

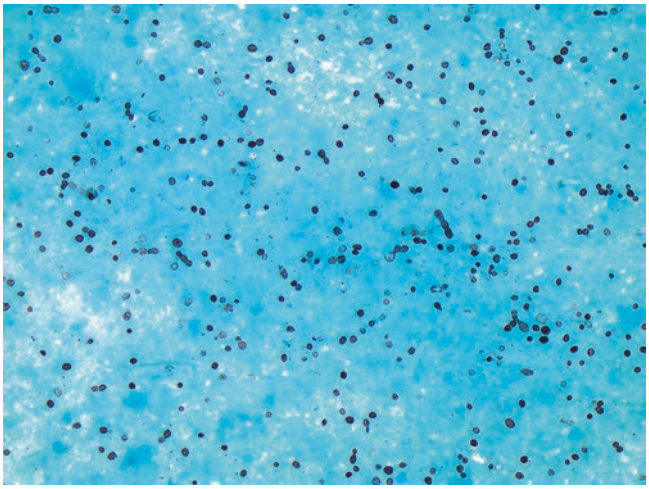

Fig. 7.6 Histoplasmosis, FNA. Round-to-oval-shaped yeast forms with narrow-based budding. (GMS stain 600×)

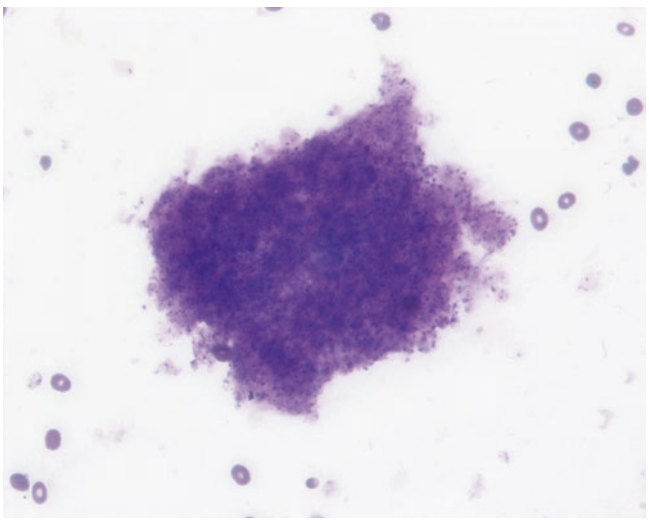

Fig. 7.7 *Pneumocystis jirovecii*. A large foamy proteinaceous cast is present with small central dark intracystic bodies visible at the edge of the cast. (Modified Giemsa 600×)

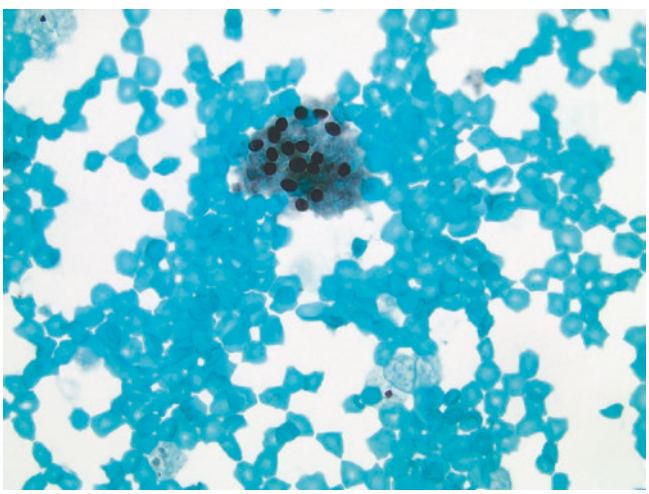

Fig. 7.8 *Pneumocystis jirovecii*. The organism appears as non-budding cup-shaped oval cyst forms, highlighted by silver stain. (GMS stain 600×)

fungal infections can be diagnosed by cytology including cryptococcosis, blastomycosis, coccidiomycosis, paracoccidioidomycosis, sporotrichosis, aspergillosis (Fig. 7.4), zygomycosis (Fig. 7.5), histoplasmosis (Fig. 7.6), and pneumocystis (Figs. 7.7 and 7.8). Pulmonary infections should be considered in cases of granulomatous inflammation or immunocompromised patients, and special stains on smears or cell block material can help highlight the characteristic microscopic appearance of each species. BAL, in particular, is noted for its favorable sensitivity in detecting *Pneumocystis jirovecii* [6] (Table 7.2).

7. What are the morphologic features of common viral infections in lung?

Morphologic features of viral infections in cytology specimens are similar to those seen in histologic specimens. Herpes simplex virus (HSV) typically infects the respiratory tract of immunocompromised patients. Cytopathic

Table 7.2 Differential diagnoses of pulmonary fungal organisms

Organisms	Geographic distribution	Morphology	Size
Cryptococcus neoformans	Worldwide	Yeast; narrow-based budding, mucin capsule, refractile center	4–15 μm diameter
Histoplasma capsulatum	Americas: Ohio and Mississippi river valleys	Small intracellular budding yeast	1–5 μm diameter
Blastomyces dermatitidis	North America	Broad-based budding yeast	8–20 μm diameter
Coccidioides immitis	North American deserts	Spherules; endospores	15–60 μm
Aspergillus fumigatus	Worldwide	Septate hyphae; 45-degree angle branching	10–30 μm width
Mucor; *Rhizopus*	Worldwide	Variably sized hyphae, ribbon-like nonseptate, 90-degree angle branching	10–30 μm width
Candida albicans	Worldwide	Budding yeast; pseudohyphae	2–10 μm width

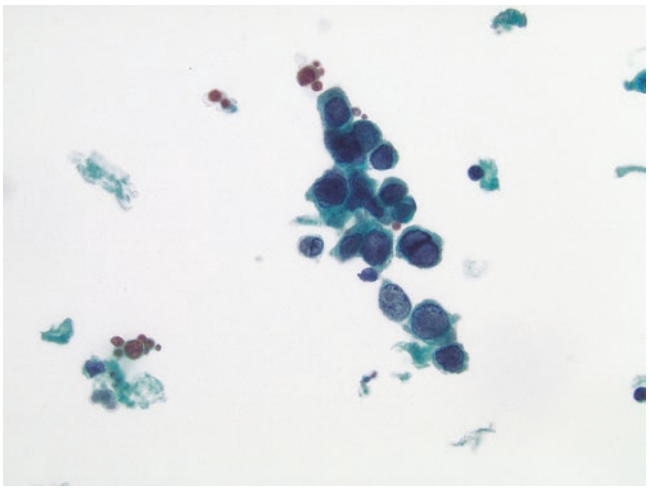

Fig. 7.9 Herpes simplex virus. Infected cells show chromatin margination, glassy smudged nuclei, nuclear molding, and focally binucleation. (ThinPrep, bronchial brush 600×)

changes include multinucleation, nuclear molding, chromatin margination, and nuclear inclusions (Fig. 7.9). Herpes zoster shows identical cytomorphology. Cytomegalovirus (CMV) may also be diagnosed in lung cytology specimens. Typical viral cytopathic changes in CMV include cytomegaly and large basophilic nuclear inclusions (Fig. 7.10). Less frequently encountered viral infections include measles and respiratory syncytial virus, both of which show large multinucleated giant cells. The giant cells in measles infection have eosinophilic intranuclear and intracytoplasmic inclusions. The giant cells in respiratory syncytial virus infection have basophilic cytoplasmic inclusions with halo. Adenovirus infection may have two types of nuclear inclusions. One is large intranuclear basophilic inclusions leading to a smudged appearance of the nuclei. The other is eosinophilic intranuclear inclusion resembling Cowdry A inclusion of HSV. Adenovirus infection may cause ciliocytophthoria which is the decapitation of the terminal bar and cilia.

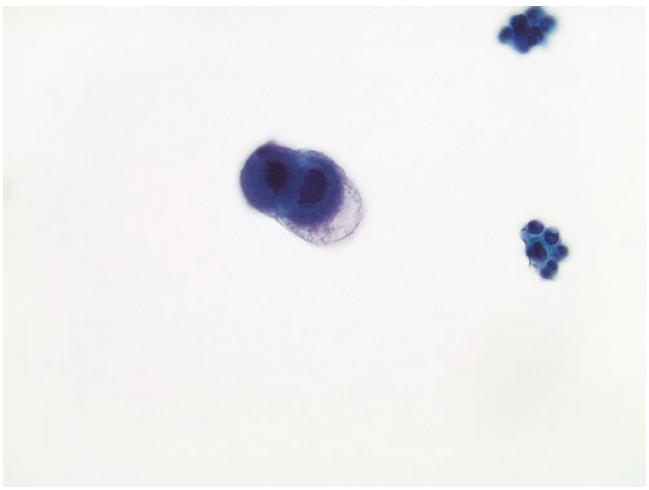

Fig. 7.10 Cytomegalovirus. Infected cells are enlarged and show basophilic nuclear inclusions. (ThinPrep, BAL 600×)

8. What does type II pneumocyte hyperplasia may mimic and how to make the distinction?

Acting as the alveolar reserve cells, type II pneumocytes proliferate in response to lung injury caused by various entities including pneumonia, pulmonary emboli, interstitial lung disease, diffuse alveolar damage, chemotherapy, radiation, and others. Hyperplastic type II pneumocytes may show atypical features including enlarged cell size, nucleomegaly, coarse chromatin, and prominent nucleoli (Fig. 7.11), and can mimic adenocarcinoma [7]. Clinical history of acute illness and diffuse pulmonary infiltrates is critical in avoiding overdiagnosis of these reactive cells, and repeat sampling may be useful after the inflammatory process has abated.

9. What are the Creola bodies?

Large clusters of reactive bronchial cells often seen in chronic inflammatory airway diseases are termed Creola bodies (Fig. 7.12). The clusters may be cohesive and spherical, and mimic the cells of adenocarcinoma. However, Creola body cells will have normal nuclear features as well as cilia which helps distinguish them from a malignant process.

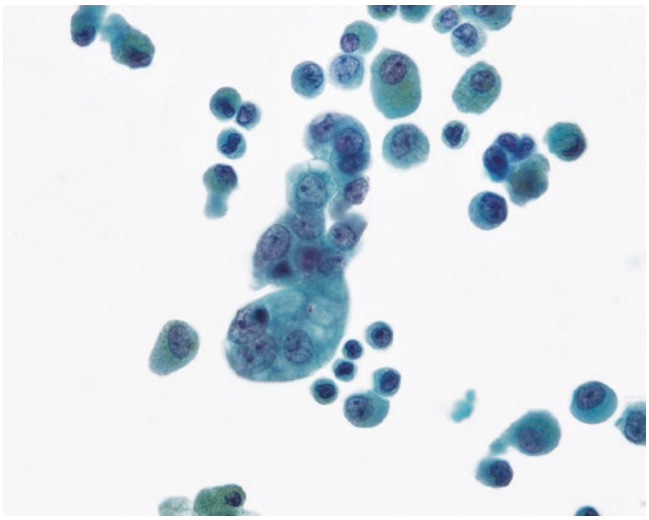

Fig. 7.11 Type II pneumocytes. Isolated cells and small three-dimensional clusters are present showing enlarged nuclei with prominent nucleoli and scattered background inflammatory cells. (ThinPrep, BAL 600×)

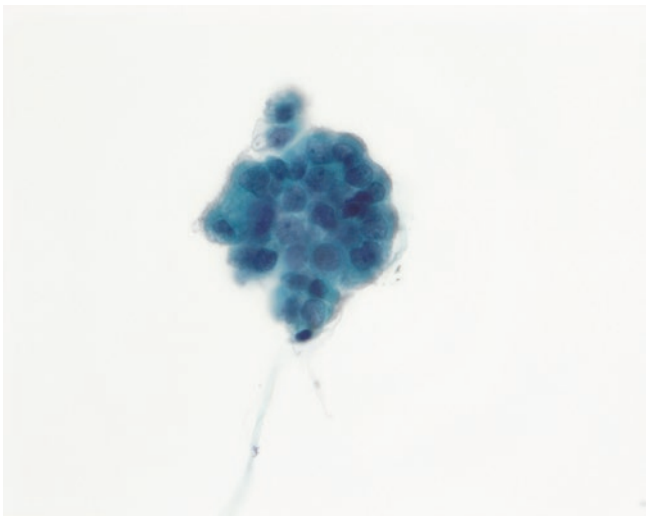

Fig. 7.12 Creola body. A tight spherical cluster of cells is present with scant cytoplasm. Normal nuclear features and the presence of cilia are present, indicating a benign process. (ThinPrep 600×)

10. What does ciliocytophthoria mean?

Decapitation of ciliated columnar cells is termed ciliocytophthoria. Decapitation leads to fragments of detached terminal bars and cilia without nuclei, and can be prominent in certain infections such as adenovirus [8].

11. What are the morphologic features of reserve cell hyperplasia?

Reserve cells are also termed basal cells and are found adjacent to the basement membrane. During lung injury from any cause, the surface epithelium is shed and in response, reserve cells proliferate. These cells may be seen in cytologic specimens, especially bronchial washings and brushings. Reserve cells are small and dark, and hyperplasia of these cells may mimic small cell carcinoma which is a common differential diagnosis. Features of reserve cell hyperplasia include tightly packed small cells with smudged dark chromatin, nuclear molding, and scant cytoplasm (Fig. 7.13). Mitoses and necrosis which are present in small cell carcinoma should not be seen in reserve cell hyperplasia.

12. What are Curschmann spirals, Ferruginous bodies, and Charcot-Leyden crystals?

Curschmann spirals are coiled strands of inspissated mucus that are commonly found in respiratory specimens and are considered a nonspecific finding (Fig. 7.14). Ferruginous

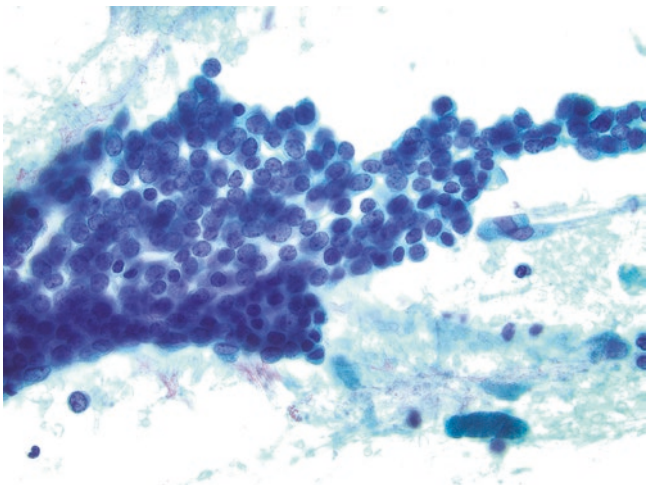

Fig. 7.13 Reserve cell hyperplasia. An aggregate of small benign cells with hyperchromatic nuclei and scant cytoplasm is present. The absence of necrosis or mitoses helps indicate a benign process. (Direct smear, bronchial wash, Papanicolaou stain 600×)

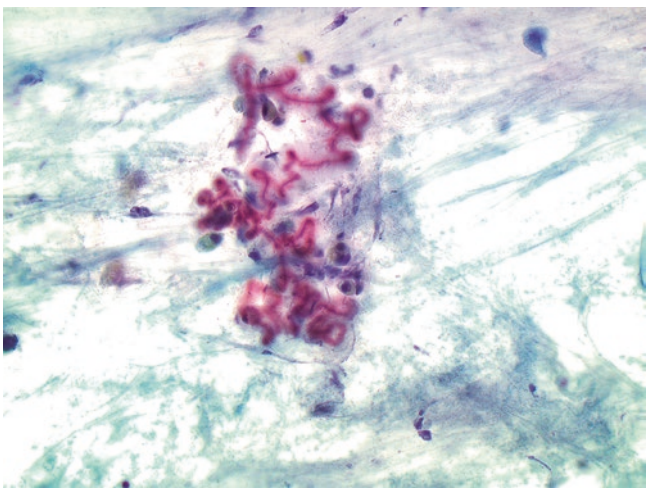

Fig. 7.14 Curschmann spiral, sputum. Coiled strand of inspissated mucus. (Papanicolaou stain 400×)

bodies are composed of mineral fibers and ferroproteins and are typically found in BAL specimens of patients with known asbestos exposure. They stain golden yellow to black on Papanicolaou stain, and are dumbbell shaped measuring 5–200 μm in length (Fig. 7.15). Charcot-Leyden crystals are a product of degenerating eosinophils that are rhomboid and needle shaped. They are pink or orange on Papanicolaou stain, and are seen in patients with allergic disorders and asthma.

13. What are the differential diagnoses for granulomas seen in a lung cytology specimen?

Granulomas are often seen in lung cytology specimens (Figs. 7.16 and 7.17). Granulomas are aggregates of epithelioid histiocytes and sometimes multinucleated giant cells. Epithelioid histiocytes have elongated "foot-print"-like nuclei and abundant pale cytoplasm. The nuclei are elongated with finely granular chromatin, sometimes longitudinal nuclear grooves and small nucleoli. In contrast, macrophages have round nuclei and abundant foamy cytoplasm. Dendritic cells in lymph nodes have round nuclei, coarsely granular chromatin, and distinct small nucleoli. They are intermingled with lymphocytes. Non-caseating granulomas can be seen in sarcoidosis, while caseating granulomas are more often reported in necrotizing infections such as tuberculosis or fungal infections, or in Wegener granulomatosis. Granulomas can also be seen associated with neoplasms such as Hodgkin lymphoma, germ cell tumor, and squamous cell carcinoma. Special stains, microbiologic cultures, and clinical history can help determine the underlying etiology.

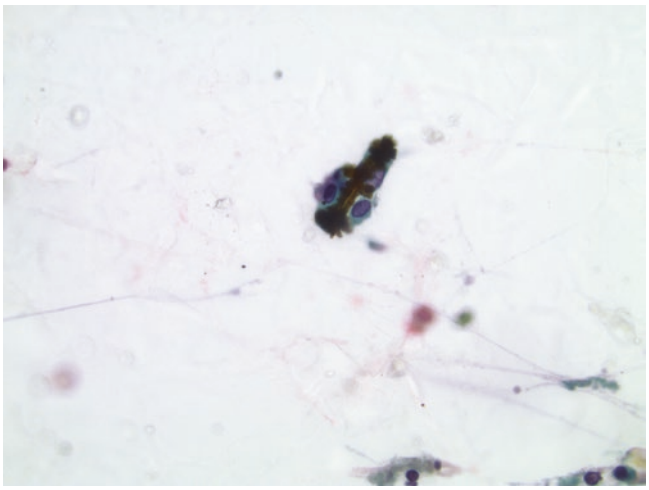

Fig. 7.15 Ferruginous body, bronchial wash. Dumbbell-shaped golden black mineral fiber. (ThinPrep 600×)

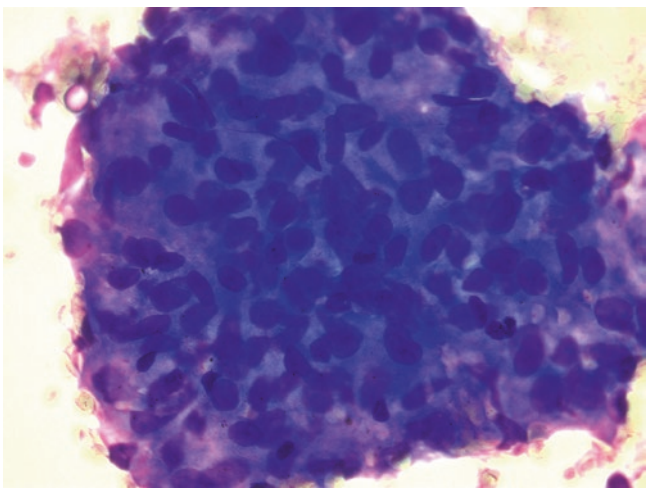

Fig. 7.16 Granuloma, FNA. Aggregate of epithelioid histiocytes with round, curved, and spindle-shaped nuclei. (Modified Giemsa 600×)

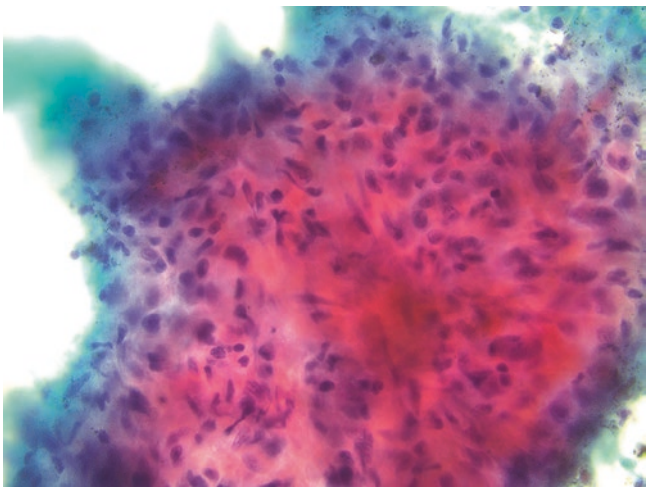

Fig. 7.17 Granuloma, FNA. Aggregate of epithelioid histocytes with round, curved, and spindle-shaped nuclei, abundant cytoplasm, and smooth nuclear contours. (Papanicolaou stain 600×)

14. What are the findings of alveolar proteinosis in a BAL specimen?

Pulmonary alveolar proteinosis (PAP) is a rare condition in which lipoproteinaceous material, derived from surfactant, accumulates within alveolar spaces. PAP may be associated with a variety of underlying conditions such as chronic infection, malignancy, or immune deficiency syndromes, or occur in otherwise healthy adults [9]. The diagnosis of PAP is made on BAL and/or transbronchial biopsy specimens. The BAL specimen has a characteristic opaque milky gross appearance with white particles. The smears show large acellular globular aggregates (Fig. 7.18), alveolar macrophages, and scattered inflammatory cells. This material stains cyanophilic on the Papanicolaou stain, and stains basophilic on the modified Giemsa stain. Both the granular debris and the globules are intensely PAS positive and resistant to diastase. Pulmonary macrophages may be filled with PAS-positive material (Fig. 7.19).

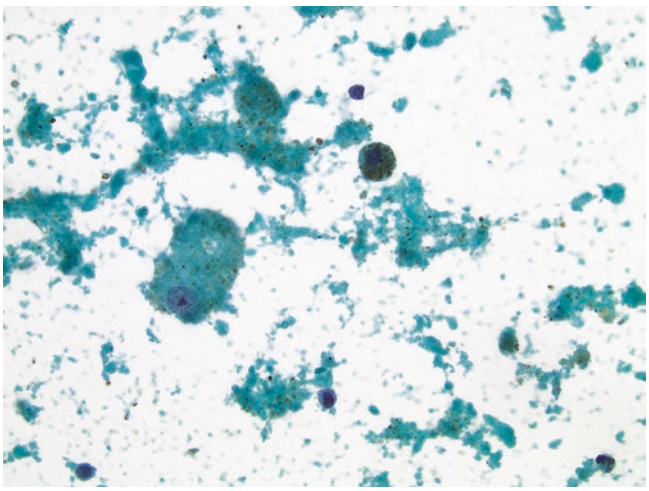

Fig. 7.18 Pulmonary alveolar proteinosis, BAL. Abundant acellular aggregates of light blue globular material and occasional macrophages. (Papanicolaou stain 600×)

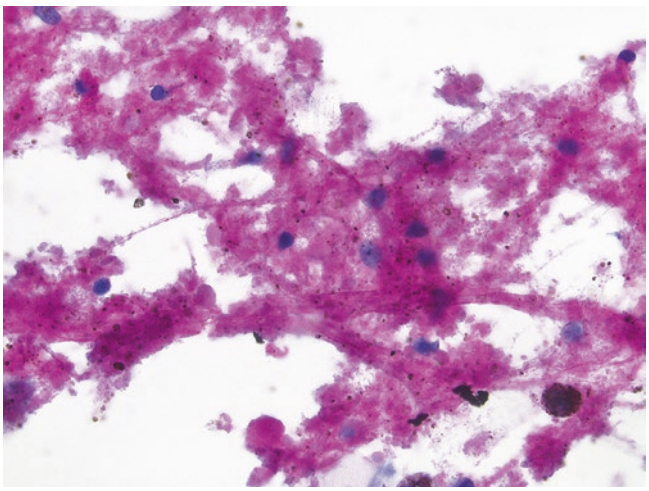

Fig. 7.19 Pulmonary alveolar proteinosis, BAL. Acellular material stains bright pink on PAS stain. (PAS stain 600×)

Differential diagnoses include *Pneumocystis jirovecii*, amyloid, and organizing pneumonia. Special stains with PAS/PAS-D, GMS, and Congo red are helpful.

15. What are the types of primary lung carcinomas and their prevalence?

Four histologic types of carcinoma account for the vast majority of primary lung carcinoma: Squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and small cell carcinoma. Although they may occur in pure form, nearly half of all lung cancers contain more than one histologic type. Adenocarcinoma is the most common subtype of lung carcinoma, accounting for 40% of all lung cancer cases overall. Squamous cell carcinoma has an overall incidence of 30%, while small cell carcinoma and large cell carcinoma have incidences of 20% and 9%, respectively [10, 11].

16. What are the risk factors for lung adenocarcinoma?

The majority of lung adenocarcinomas are attributed to tobacco smoking. However, the presence of lung adenocarcinoma in patients who have never smoked means other risk factors exist. Other reported risk factors include various environment exposures and genetic factors [11, 12].

17. What are the diagnostic cytological features of lung adenocarcinoma?

Architecturally, the tumor cells may form cohesive three-dimensional groups, sheets, and loose clusters with disordered arrangements (Fig. 7.20). Singly dispersed cells are also seen. Tumor cells have enlarged nuclei with vesicular or finely granular chromatin, irregular nuclear membrane, and prominent nucleoli (Fig. 7.21). Cytoplasm is scant to abundant and appears vacuolated, foamy, or translucent (Fig. 7.22). The background is clear or necrotic.

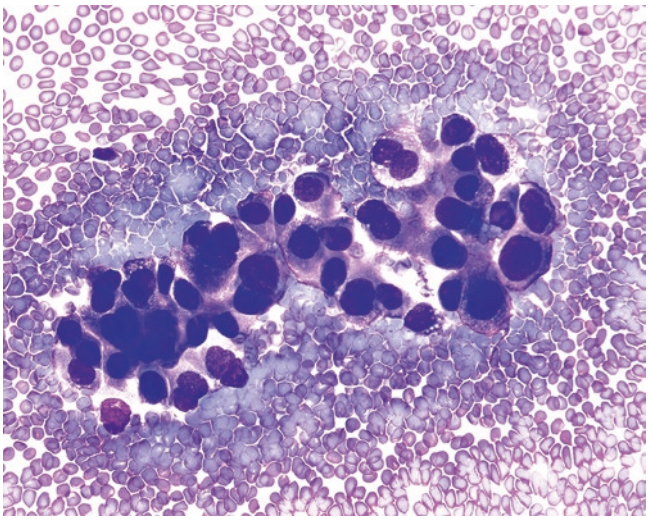

Fig. 7.20 Adenocarcinoma. A loosely cohesive epithelial group showing disordered arrangement and pleomorphism. (Modified Giemsa 400×)

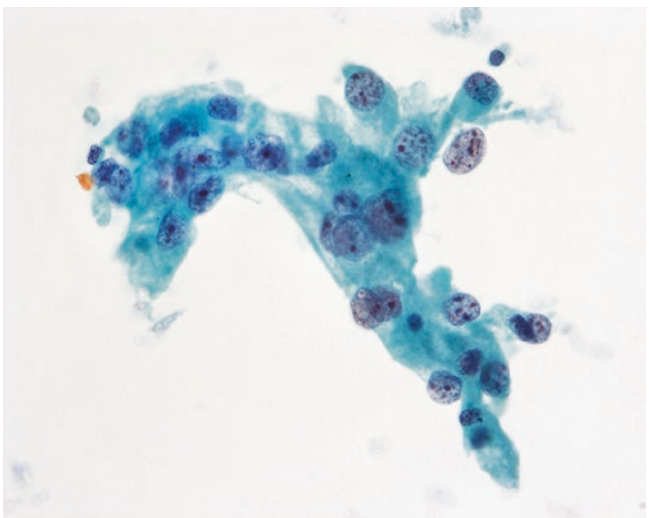

Fig. 7.21 Adenocarcinoma. Cells have enlarged nuclei, clumped chromatin, and prominent nucleoli. (Papanicolaou stain 600×)

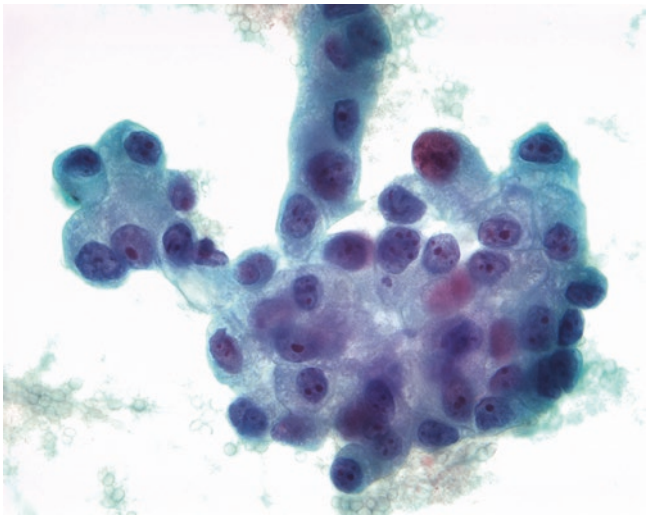

Fig. 7.22 Adenocarcinoma. Cytoplasm is abundant, foamy, and vacuolated. (Papanicolaou stain 600×)

18. What molecular tests should be performed in lung adenocarcinomas and their clinical significance?

In patients with advanced-stage lung adenocarcinoma, analysis of gene mutations for *EGFR* (epidermal growth factor receptor), *KRAS*, *BRAF*, and *MET*, and gene rearrangements for *ALK* (anaplastic lymphoma kinase) and *ROS1* may be performed.

Somatic mutations in the exons 18–21 of the tyrosine kinase domain of EGFR occur in approximately 10–20% of Caucasian patients and 35–45% of Asian patients with adenocarcinomas [13, 14]. These mutations are associated with sensitivity to treatment with EGFR tyrosine kinase inhibitors (TKIs). Lung tumors associated with EGFR-mutated lung cancer more typically occur in women who did not smoke. These tumors are more common in women of East Asian ethnicity than in those belonging to other ethnic groups. EGFR mutations are more common in adenocarcinomas with lepidic and papillary patterns.

ALK rearrangements (not mutation) resulting in EML4-ALK fusion gene have been identified in some patients with non-small cell carcinoma of the lung (approximately 4–5% of patients with lung non-small cell carcinoma, typically light or never smokers and relatively younger at presentation). The presence of EML4-ALK seems to be mutually exclusive to that of EGFR and KRAS mutations [15]. Various methods can be used for detection of EML4-ALK rearrangements, including FISH, RT-PCR, and immunohistochemistry, though currently FISH is the most widely used method. There are tyrosine kinase inhibitors in clinical use for these tumors including crizotinib and ceritinib.

ROS1 rearrangements are present in approximately 1–2% of lung adenocarcinomas and these tumors have shown response to crizotinib. These mutations are more frequent in patients who are light or never smokers and are relatively younger [16].

KRAS mutations are mutually exclusive with EGFR and ALK alterations and occur in 20–30% of lung adenocarcinomas, especially those with mucinous differentiation. KRAS mutation testing may be performed first because it is a quicker, easier, and less expensive test. Currently, no targeted therapy is available for KRAS mutations.

BRAF mutations in lung non-small cell carcinomas serve as targetable alterations for BRAF inhibitors. It occurs in approximately 5% of lung adenocarcinomas and about half of the mutations involve V600E. MET amplification is associated with response to crizotinib and cabozantinib.

19. What cytologic material is suitable for molecular testing?

All cytologic material including the air-dried smears, alcohol-fixed smears, and cellblocks are suitable for molecular testing including all the tests mentioned in Q20.

When an adequate pass is seen (tumor cells are seen in the smear) during the on-site adequacy assessment, it is essential to request additional passes (at least two) for cellblock preparation. Use limited stains (if necessary) to subtype the carcinoma. Preserve tissue for molecular testing. It is important to establish a team-type approach to achieve timely and clinically necessary molecular testing.

20. Which gene mutations are predictors of failure to EGFR tyrosine kinase inhibitor (TKI) therapy?

KRAS mutations occur more often in adenocarcinomas of smokers and are adverse prognostic factor. They are predictors of resistance to EGFR TKI therapy [17]. Mucinous differentiation of adenocarcinomas appears to strongly correlate with KRAS mutations.

21. What are the differential diagnoses of lung adenocarcinoma in a cytology specimen?

Differential diagnoses for lung mucinous adenocarcinoma include the following (see Table 7.2):

- Goblet cell hyperplasia
  - Cells are bland. Cilia is identified.
- Metastatic adenocarcinoma
  - Clinical history and immunohistochemical stains (such as negative for TTF-1, and positive for CDX2 in colorectal adenocarcinoma)

Differential diagnoses for lung non-mucinous adenocarcinoma include the following:

- Reactive pneumocytes (very important!) (Fig. 7.23)
  - Clinical history of a reactive process and lack of a mass lesion

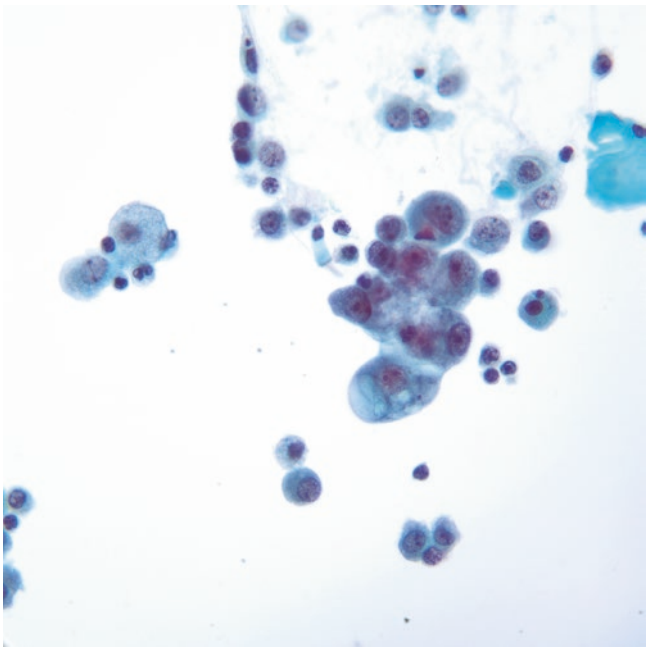

Fig. 7.23 Reactive type II pneumocytes. Reactive type II pneumocytes may show atypical features including enlarged nuclei, coarse chromatin, prominent nucleoli, and vacuolated cytoplasm, mimicking adenocarcinoma. Clinical history is critical in avoiding overdiagnoses in these cases. (Papanicolaou stain 400×)

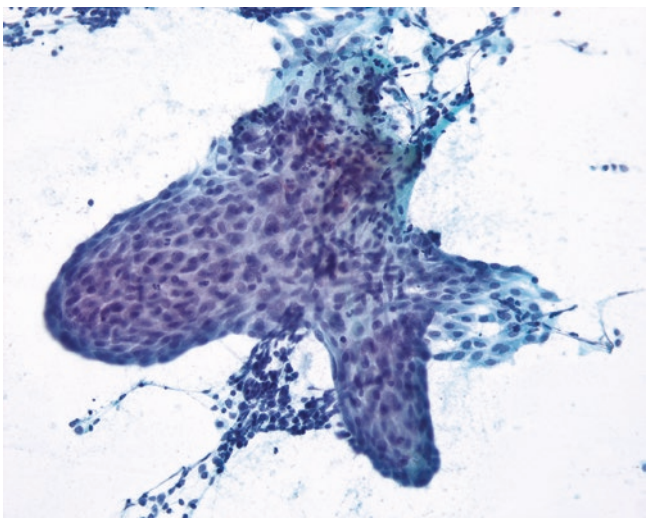

Fig. 7.24 Benign mesothelial cells. CT-guided lung FNAs may obtain mesothelial cells. Mesothelial cells have a flat-sheet arrangement with cells streaming. Nuclei have smooth nuclear membrane and inconspicuous or small nucleoli. (Papanicolaou stain 200×)

- Non-keratinizing squamous cell carcinoma
  - Immunohistochemical stains for p40 and TTF-1
- Benign mesothelial cells (Fig. 7.24)
  - Flat sheets of cells with streaming. Cells have round nuclei with smooth nuclear membrane.
- Pulmonary sclerosing pneumocytoma (PSP)

Table 7.3 Differential diagnoses of lung adenocarcinoma

	Architecture and background features	Cytologic features	IHC staining
Reactive pneumocytes	Small clusters and singly dispersed cells	Enlarged nuclei, prominently nucleoli, vacuolated cytoplasm	PanCK+ TTF-1+
Benign mesothelial cells	Flat sheets with streaming	Round nuclei with smooth nuclear membrane and small nucleoli.	PanCK+ TTF-1− Calretinin+ D2–40+
Lung adenocarcinoma	Cohesive three-dimensional groups, sheets, and loose clusters with disordered arrangements. Singly dispersed cells are also seen	Tumor cells have enlarged nuclei with vesicular or finely granular chromatin, irregular nuclear membrane and prominent nucleoli. Cytoplasm is scant to abundant and appears vacuolated, foamy, or translucent. The background is clear or necrotic	PanCK+ CK7+ CK20− TTF-1+ Napsin+ Calretinin− D2–40−
Non-keratinizing SCC	Cohesive cell groups with crowded arrangements. Background necrosis present	Round to oval nuclei, irregular nuclear membrane, dense cytoplasm. Prominent nucleoli may be present	PanCK+ TTF-1− Calretinin− D2–40− P40+
Metastatic adenocarcinoma	Crowded epithelial groups and single cells. Necrotic background may be present	Enlarged nuclei with irregular nuclear membrane and prominent nucleoli (see Table 7.6)	PanCK+ TTF-1− Napsin− (see Table 7.6)

22. What is the difference between invasive mucinous adenocarcinoma and colloid adenocarcinoma of the lung?

Invasive mucinous adenocarcinoma, formerly called mucinous bronchioloalveolar carcinoma, is composed of monolayer sheets of bland epithelium with cytoplasmic mucin. On immunohistochemical staining, tumor cells are positive for CK7 and CK20, and negative for TTF-1 and Napsin A. Approximately 75% of lung mucinous adenocarcinoma is associated with *KRAS* mutation.

Colloid adenocarcinoma of the lung is characterized by abundant extracellular mucin and scant bland tumor cells floating in the mucin pools. Colloid adenocarcinoma of the lung expresses similar markers as intestinal epithelium. It is positive for CK20 and CDX2, and negative for CK7 and TTF-1. In contrast to invasive mucinous adenocarcinoma, the mucin pools in colloid adenocarcinoma replace the underlying alveolar architecture [18].

Differential diagnosis between these two entities based on cytology alone is difficult. Invasive mucinous adenocarcinoma typically shows monolayer sheets of bland tumor cells forming "drunken honeycomb" in the background of mucin. Colloid carcinoma has scant single cells or cells in small clusters floating within abundant mucin pools. Immunohistochemical stains may be of help. Definitive diagnosis is best made on surgical resection specimens.

23. What are the risk factors for lung squamous cell carcinoma?

The major risk factor for lung squamous cell carcinoma is smoking. Squamous cell carcinoma is related to smoking amount, duration, and tar level [19]. Exposure to several occupational agents, especially arsenal, has been associated with lung squamous cell carcinoma [20]. Recent literature also suggests an association between lung squamous cell carcinoma and HPV infection, but this association remains controversial [21].

24. What are the diagnostic cytological features of lung squamous cell carcinoma?

Keratinizing squamous cell carcinoma (Fig. 7.25):

- Sheets, syncytial, and isolated cells
- Dense, glassy, orangeophilic cytoplasm with sharp borders
- Coarse chromatin
- Prominent nucleoli present or absent
- Necrotic or inflammatory background

Non-keratinizing squamous cell carcinoma (Fig. 7.26):

- More sheets and syncytial groups
- Dense cytoplasm with sharp borders
- Coarse and hyperchromatic chromatin
- Prominent nucleoli may be present
- Necrotic or inflammatory background

25. What immunohistochemical stains help in the differential diagnosis between poorly differentiated adenocarcinoma and non-keratinizing squamous cell carcinoma of the lung?

Immunohistochemical stains for TTF-1, Napsin, p63, p40, and CK5/6 help in the differential diagnosis between poorly differentiated adenocarcinoma and non-keratinizing squamous cell carcinoma of the lung. Adenocarcinoma is positive for TTF-1 and Napsin, and negative for p63, p40, and CK5/6. Squamous cell carcinoma is positive for p63, p40, and CK5/6 and negative for TTF-1 and Napsin.

TTF-1 and Napsin are comparable in sensitivity for diagnosing lung adenocarcinomas. However, TTF-1 is also positive in small cell lung carcinoma, large cell neuroendocrine carcinoma, and thyroid carcinomas. Napsin A may be expressed in other tumors such as renal cell carcinoma.

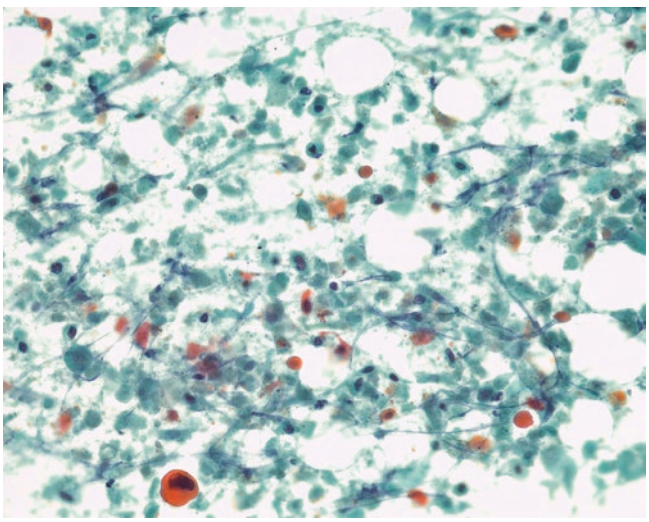

Fig. 7.25 Keratinizing squamous cell carcinoma. Keratin debris and atypical keratinizing squamous cells are seen. (Papanicolaou stain 400×)

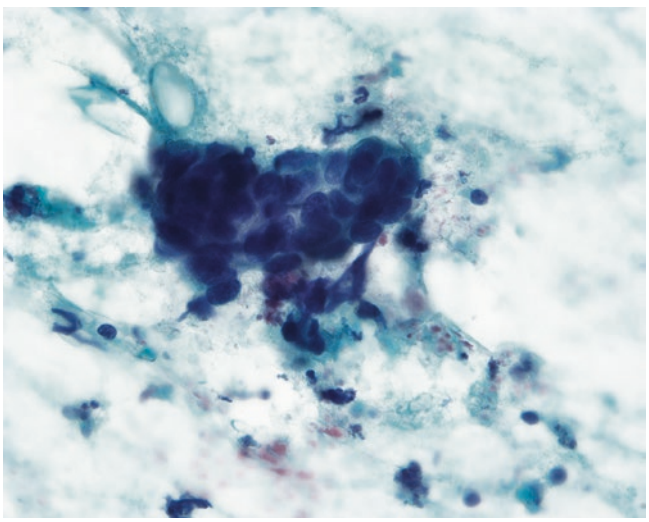

Fig. 7.26 Non-keratinizing squamous cell carcinoma. Cells have high nuclei-to-cytoplasmic ratio, hyperchromatic chromatin, and dense cytoplasm. Immunohistochemical stains are usually needed to confirm non-keratinizing squamous cell carcinoma (positive for CK5/6, p63, and p40). (Papanicolaou stain 600×)

P40 is a more specific marker for squamous cell carcinoma than p63; p63 can be positive in up to 30% of lung adenocarcinomas.

26. What are the differential diagnoses for lung squamous cell carcinoma?

Differential diagnoses for keratinizing squamous cell carcinoma include the following:

- Atypical squamous metaplasia (Fig. 7.27)
  - Atypia insufficient for a definitive diagnosis of malignancy; clinical history of inflammation and lack of a mass lesion
- Metastatic squamous cell carcinoma
  - Clinical history of squamous cell carcinoma

Differential diagnoses for non-keratinizing squamous cell carcinoma include the following:

- Poorly differentiated adenocarcinoma (Fig. 7.28)
  - Immunohistochemical stains for p40 and TTF-1 are helpful
- Metastatic carcinoma
  - Clinical history and immunohistochemical stains are helpful
- Small cell carcinoma
  - Immunohistochemical stains for neuroendocrine markers

27. How are neuroendocrine tumors of the lung classified?

The neuroendocrine tumors of the lung are classified as carcinoid tumor (typical carcinoid and atypical carcinoid), small cell carcinoma, and large cell neuroendocrine carcinoma [11]. The terminology of well-differentiated neuroendocrine carcinoma (grade 1 neuroendocrine carcinoma) or moderately differentiated neuroendocrine carcinoma (grade 2 neuroendocrine carcinoma) is not recommended.

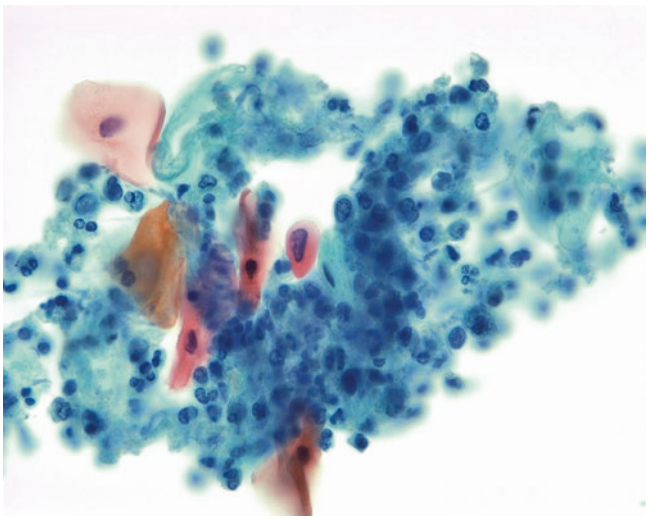

Fig. 7.27 Atypical squamous metaplasia. Atypical squamous metaplasia can occur in inflammatory and reactive conditions. Knowing the clinical history helps with the differential diagnosis. (Papanicolaou stain 600×)

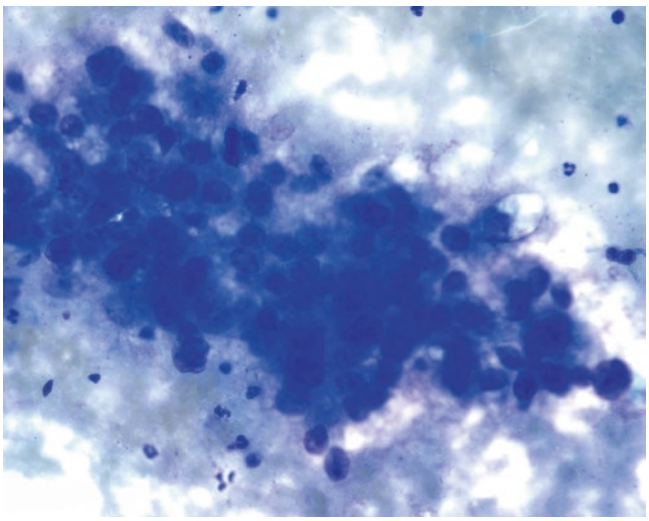

Fig. 7.28 Poorly differentiated adenocarcinoma. Crowded groups with disordered arrangement. Cells have high N/C ratio and irregular nuclear contour. (Modified Giemsa stain 400×)

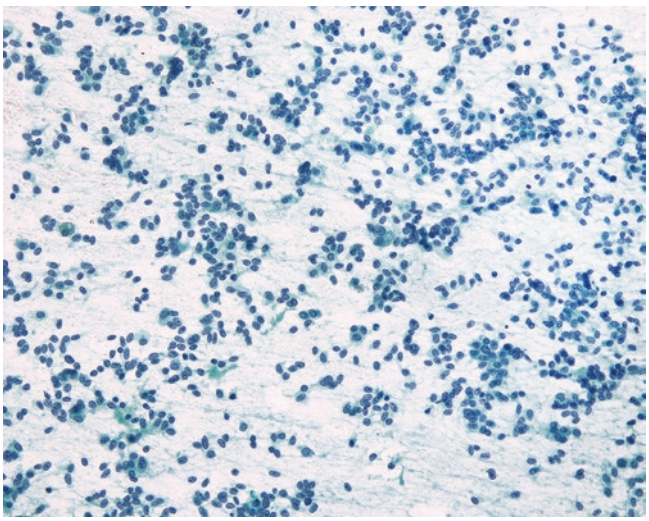

Fig. 7.29 Carcinoid tumor. Cellular smear showing loosely cohesive and singly dispersed cells. Cells have a monomorphic morphology. The background is clean without necrosis. (Papanicolaou stain 200×)

28. How to separate carcinoid tumor from atypical carcinoid tumor in lung cytology specimens?

The cytomorphological features of lung carcinoid tumor are usually bland and monomorphic. It can be misdiagnosed as benign bronchial cells which have cilia and more cohesive. Atypical carcinoid tumors are more aggressive clinically, and have increased mitosis, prominent nucleoli, and necrosis (see Table 7.4) [11, 22].

Table 7.4 Lung carcinoid tumor and atypical carcinoid tumor

Cytomorphological and IHC features	Carcinoid (Fig. 7.29)	Atypical carcinoid (Fig. 7.30)
Cell size	Small to medium	Medium
Nuclear shape	Round to oval	Round to oval, elongated
Chromatin	Coarsely granular	Coarsely granular
Nucleoli	Inconspicuous or small	Sometimes prominent
Cytoplasm	Abundant, granular	Scant to moderate
Mitoses (per 10 HPFs)	Rare (<2)	Present (2–10)
Necrosis	Absent	Present, focal
Proliferation marker Ki-67	Up to 5%	Up to 20%
Epithelial markers (pancytokeratin and EMA)	Positive (dot-like pattern)	Positive (dot-like pattern)
Neuroendocrine markers (synaptophysin, chromogranin, and CD56)	Positive	Positive
Thyroid transcription factor-1 (TTF-1)	Negative	Negative

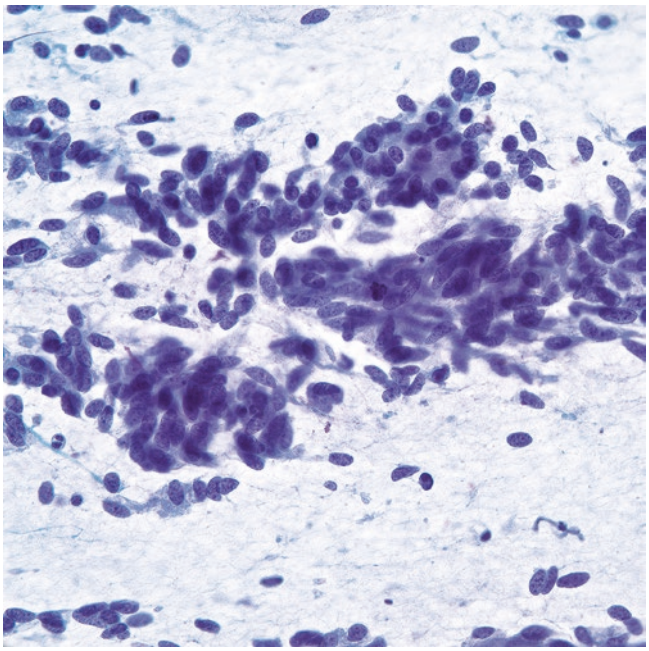

Fig. 7.30 Atypical carcinoid. Cells have elongated nuclei and scant cytoplasm. Chromatin is coarsely granular. Focal apoptotic cell and necrosis is present. (Papanicolaou stain 600×)

29. What are the cytological features and typical immunohistochemical staining patterns of lung small cell carcinomas?

Typical cytologic features of small cell carcinomas include (Figs. 7.31 and 7.32):

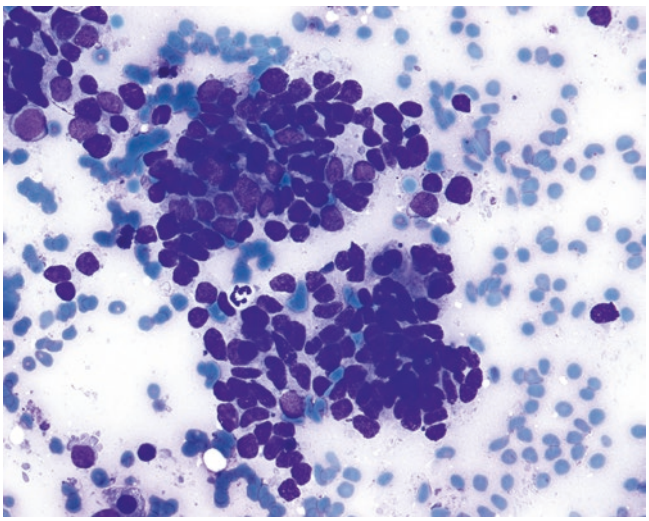

Fig. 7.31 Small cell carcinoma. Tumor cells are small with scant cytoplasm and nuclear molding. (Papanicolaou stain 400×)

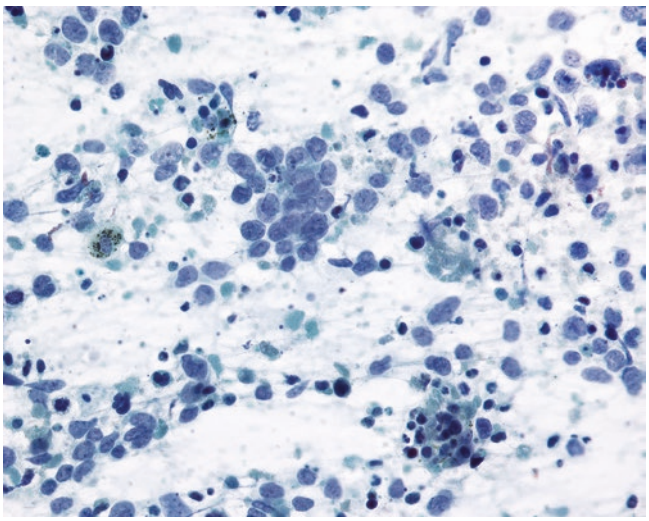

Fig. 7.32 Small cell carcinoma. There are abundant apoptotic cells and necrotic debris. Tumor cells have finely granular chromatin and inconspicuous nucleoli. (Papanicolaou stain 400×)

- Small cells with scant cytoplasm (nuclear diameter < 3 resting lymphocytes)
- Oval or carrot-shaped nuclei
- Salt and pepper chromatin, indistinct nucleoli
- Nuclear molding, paranuclear blue bodies
- Abundant mitoses, single-cell necrosis
- Background crush artifact and nuclear debris

Small cell carcinomas are typically positive for NSE (71%), chromogranin (44%), synaptophysin (55%), CD56 (89%), and TTF-1 (90–95%). Ki-67 is usually greater than 50%.

Table 7.5 Differential diagnoses of small cell carcinoma

	Architecture and background features	Cytologic features	IHC staining
Small cell carcinoma	Cohesive and dispersed cells with nuclear molding Apoptotic bodies and necrosis may be present. Crush artifact common	Round to oval nuclei, scant cytoplasm, finely granular chromatin, and indistinct nucleoli. Mitoses frequent	PanCK+ CD45− Chromogranin+ Synaptophysin+ CD56+ TTF-1+ Ki-67 > 50%
Lymphoma	Discohesive cells. Crush artifact common. Background lymphoglandular bodies	Scant to moderate cytoplasm, finely granular to clumped chromatin. Prominent nucleoli may be present	PanCK− CD45+ Chromogranin− Synaptophysin− TTF-1−
Non-keratinizing SCC	Cohesive cell groups with crowded arrangements. Background necrosis present	Round to oval nuclei, irregular nuclear membrane, dense cytoplasm. Prominent nucleoli may be present	PanCK+ CD45− Chromogranin− Synaptophysin− P40+ TTF-1−
Poorly differentiated adenocarcinoma	Cohesive cell groups with crowded arrangements	Round to oval nuclei, irregular nuclear membrane, translucent or vacuolated cytoplasm, and prominent nucleoli	PanCK+ CD45− Chromogranin− Synaptophysin− P40− TTF-1+ Napsin+

30. What are the differential diagnoses for small cell carcinomas in a lung cytology specimen?

Differential diagnoses of small cell carcinoma in a lung cytology specimen include lymphoma, non-keratinizing squamous cell carcinoma, and poorly differentiated adenocarcinoma. Immunohistochemical staining is helpful in the differential diagnosis (Table 7.5).

31. What are the key morphologic features for large cell neuroendocrine cell carcinoma on a cytology specimen?

In large cell neuroendocrine cell carcinomas, cells are large (>3 lymphocytes), have finely granular chromatin, and prominent nucleoli. There is frequent mitosis. Apoptosis or necrosis is seen in the background (Fig. 7.33). Diagnosis is confirmed by positive IHC staining for neuroendocrine markers.

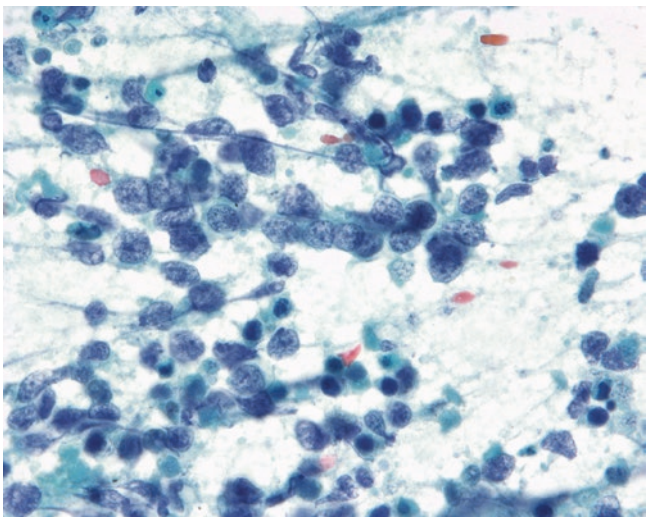

Fig. 7.33 Large cell neuroendocrine carcinoma. Tumor cells are large with finely granular chromatin and small nucleoli. (Papanicolaou stain 600×)

32. Can a large cell carcinoma of the lung be diagnosed on a cytology specimen?

Large cell carcinoma is defined as an undifferentiated carcinoma that, even on extensive sampling, does not show morphologic or immunohistochemical features of adenocarcinoma, squamous cell carcinoma, or neuroendocrine carcinoma. This must be assessed on a resection specimen and the diagnosis is not made on a cytology specimen [11, 22]. On the other hand, the diagnosis of non-small cell carcinoma, possible large cell neuroendocrine carcinoma can be made if the carcinoma shows appropriate cytologic features (including nuclear palisading, molding, rosettes, distinct nucleoli, greater amount of cytoplasm than small cell carcinoma, necrosis, apoptosis) and expresses neuroendocrine markers (synaptophysin, chromogranin, CD56).

33. What are the morphologic features and IHC findings of a lung clear cell tumor (Sugar tumor, PEComa)?

Clear cell tumor of the lung is believed to arise from perivascular epithelioid cells and therefore stains positive for HMB-

45, a feature it shares with angiomyolipoma and PEComa. Tumor cells are bland with polygonal or spindle shape, oval nuclei, and abundant clear cytoplasm. Differential diagnoses include lung squamous cell carcinoma or adenocarcinoma with clear cell features, and metastatic tumors with clear cell morphology such as renal cell carcinoma. Immunohistochemical stains are helpful in making the distinctions.

34. What are the morphologic features of lung hamartoma on a fine-needle aspiration specimen?

FNA sampling of a pulmonary hamartoma shows a characteristic mixture of benign epithelial cells and mesenchymal material. Immature fibromyxoid material is often present admixed with bland spindle cells, and benign epithelial cells. Occasionally, mature cartilaginous elements and adipocytes will also be seen (Fig. 7.34).

35. What are the morphologic features and genetic findings of inflammatory myofibroblastic tumor (IMT)?

Inflammatory myofibroblastic tumor is a tumor composed of bland spindle cells. It may be seen in the lungs, as well as other locations and is most common in young patients under age 40. Cytologic features include bland spindle cells arranged in a storiform pattern admixed with a prominent inflammatory cell component (Fig. 7.35). There is minimal, if any, mitoses and necrosis, and little pleomorphism. Reported translocations involve the ALK gene and the two tropomyosin genes TPM3 and TPM4 [23]. Differential diagnoses include other primary and metastatic spindle cell lesions such as smooth muscle tumors and nerve sheath tumors. IMT is negative for desmin, myogenin, myoD1, and S100, and may show focal positivity for SMA.

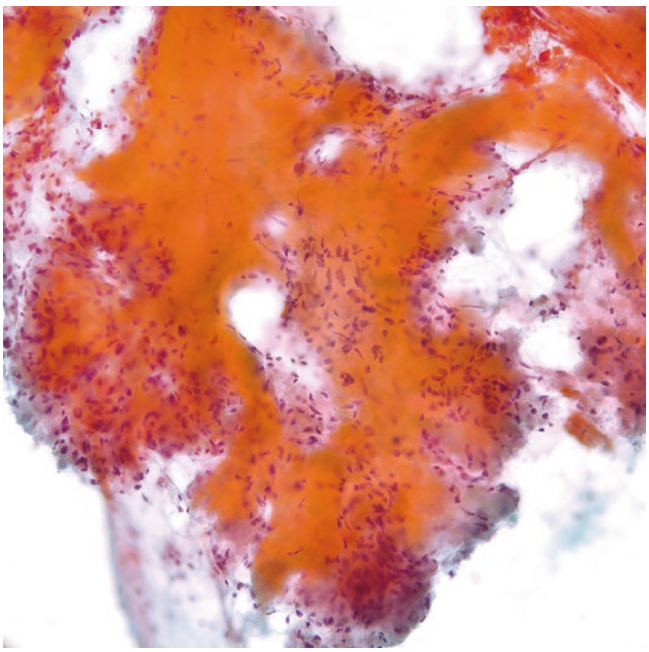

Fig. 7.34 Pulmonary hamartoma. Benign spindle cell aggregates are present in a background of immature fibromyxoid material. (Papanicolaou stain 400×)

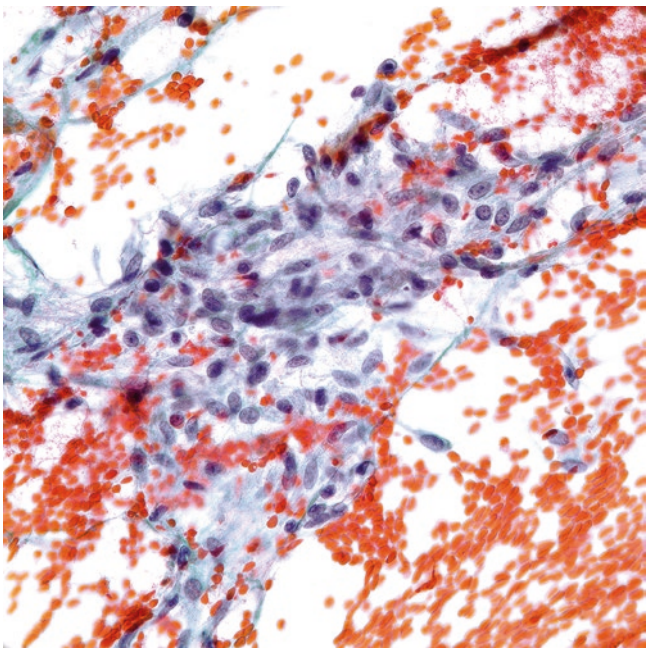

Fig. 7.35 Inflammatory myofibroblastic tumor. Aggregate of bland spindle cells in a storiform pattern. (Papanicolaou stain 600×)

36. What do cells in endobronchial granular cell tumor resemble in cytology smears?

The cells in endobronchial granular cell tumor have small round to oval nuclei and abundant foamy or granular cytoplasm. The cells resemble macrophages, and therefore may be overlooked. Cells appear uniform and form small clusters. Immunohistochemical stain shows positivity for S-100, SOX-10, and CD68. PAS stain is positive for cytoplasmic granules which are resistant to diastase.

37. What is the most common primary non-Hodgkin lymphoma of the lung?

The most common primary non-Hodgkin lymphoma of the lung is extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma), followed by diffuse large B-cell lymphoma [24].

38. What is pulmonary lymphomatoid granulomatosis?

Pulmonary lymphomatoid granulomatosis (PLG) is an EBV-associated T-cell-rich B-cell lymphoproliferative

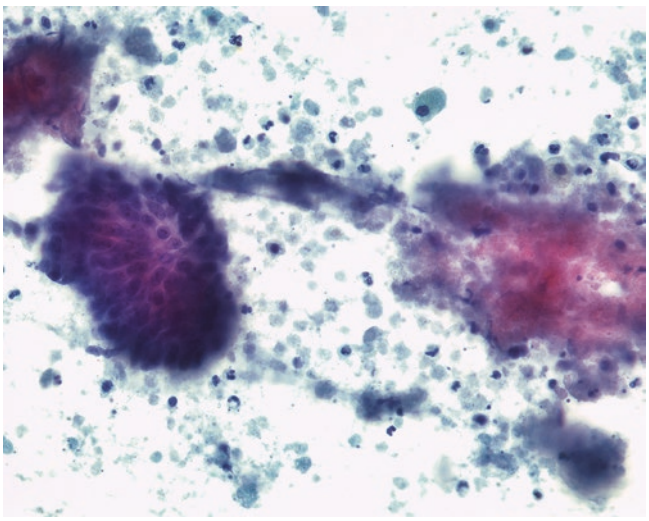

Fig. 7.36 Metastatic colonic adenocarcinoma. Tumor cells are columnar in shape and associated with a background of dirty necrosis. (Papanicolaou stain 400×)

disorder. Patients present with fever, cough, dyspnea, and chest pain. Imaging study reveals an opaque area in the lung. Fine-needle aspiration shows large atypical lymphocytes admixed with small mature lymphocytes. The large atypical lymphocytes are B lymphocytes and positive for Epstein-Barr virus in situ hybridization (EBER) [25]. The background small mature lymphocytes are T lymphocytes.

39. What are the common differential diagnoses of lymphoma in lung cytology specimens?

The common differential diagnoses of lymphoma in lung cytology specimens include small cell carcinoma, adenocarcinoma, melanoma, and inflammatory lesions.

40. What are some of the most common metastatic malignancies to the lung and their corresponding distinctive cytological features?

The common metastatic malignancies in the lung include metastatic squamous cell carcinomas from the head and neck region, metastatic melanoma, and metastatic carcinomas from breast, colon, kidney, and urinary bladder (Fig. 7.36) [26] (see Table 7.6).

Table 7.6 Metastatic tumors to the lung: morphologic features and immunohistochemical (IHC) staining results

	Architecture and background features	Cytologic features	IHC staining
Metastatic SCC	Cohesive sheets and crowded groups. Keratin debris may be seen in keratinizing SCC. Necrosis often present	High N/C ratio, irregular nuclear membrane, finely granular to clumped chromatin, and dense cytoplasm	CK5/6+ p63+ p40+
Metastatic breast cancer	Loosely cohesive and singly dispersed plasmacytoid cells. Bare atypical nuclei may be present in the background	Irregular nuclear membrane, vesicular chromatin, and prominent nucleoli	GATA3+ TTF-1- Napsin-
Metastatic colonic cancer	Crowded epithelial groups. The background has characteristic "dirty necrosis"	Columnar cells with hyperchromatic nuclei and prominent nucleoli	CK7- CK20+ CDX2+
Metastatic melanoma	Loose clusters and singly dispersed cells with a plasmacytoid morphology	Tumor cells have prominent nucleoli. Sometimes, cytoplasmic melanin pigments are present (dark blue on DQ stain and greenish-blue on Pap stain)	PanCK− HMB45+ SOX10+
Metastatic RCC	Loosely cohesive cell groups with extracellular magenta-colored matrix material	Tumor cells have abundant vacuolated cytoplasm, irregular nuclear membrane, and small to prominent nucleoli	PanCK+ PAX8+ CD10+

41. What are the morphologic features of thymoma and thymic carcinoma on FNA specimens?

Thymoma is the most common tumor of anterior mediastinum. On FNA smears, type A thymomas have bland spindle-shaped epithelial cells, while type B thymomas have round to polygonal epithelial cells admixed with variable amounts of lymphocytes. The epithelial cells in thymomas are positive for p63, CK5/6, and PAX-8. Lymphocytes are T-cells and stain positive for CD3 and TdT.

Thymic carcinomas are poorly differentiated carcinomas and subtypes include squamous cell carcinoma, neuroendocrine carcinoma, and others. Differential diagnoses are with metastatic carcinomas. Thymic carcinomas are positive for CD5 and CD117, which distinguishes it from other carcinomas.

42. What are the common types of mediastinal germ cell tumors and their morphologic features?

Mediastinal germ cell tumors include teratoma, seminoma, and non-seminomatous germ cell tumors. Their morphologic features and immunohistochemical staining patterns are listed in Table 7.7.

Table 7.7 Differential diagnosis of mediastinal germ cell tumors

	Morphologic features	IHC staining
Teratoma	Benign squamous epithelium, anucleated squamous cells, keratin debris, ciliated cells, mucous cells	PanCK+ OCT3/4−
Seminoma	Clusters and single large tumor cells with round to oval nuclei, clear cytoplasm, and prominent macronucleoli. Small lymphocytes are seen in the background. Characteristic foamy "tigeroid" background may be seen in air-dried smears due to fragile cytoplasm rich in glycogen vacuoles	EMA− PANCK− OCT3/4+ SALL4+ CD117+
Non-seminomatous germ cell tumor	Large malignant cells form glandular or papillary-like architectures. Tumor cells have vesicular chromatin and prominent nucleoli	EMA+ OCT3/4± SALL4± CD30+ CD117− AFP+ (yolk sac) Beta-HCG+ (choriocarcinoma)

Case Presentations

Case 1

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this tumor
2. Discuss differential diagnosis of this tumor
3. Become familiar with immunohistochemical staining features of this tumor

Case History:

A 52-year-old female was hospitalized for COPD exacerbation. CT-chest reveals a 2.7 cm right middle lobe nodular opacity. The subsequent PET scan demonstrates a hypermetabolic right middle lobe pulmonary nodule consistent with primary pulmonary neoplasm.

Specimen Source:

CT-guided fine-needle aspiration was performed. A modified Giemsa-stained smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic Findings:

- Cellular smears showing abundant well-preserved cells in loosely cohesive groups and singly dispersed forms (Fig. 7.37).
- The cells are uniform and have round to oval nuclei, coarsely granular chromatin, and small nucleoli (Fig. 7.38).
- Cytoplasm is scant to abundant and appears granular.
- The isolated cells have a plasmacytoid appearance.

Differential Diagnosis:

- Adenocarcinoma
- Carcinoid tumor
- Lymphoma

IHC and Other Ancillary Studies:

- TTF-1 negative
- Chromogranin positive (Fig. 7.39)
- CD45 negative
- Ki-67 2%

Final Diagnosis:

- **Carcinoid Tumor**

Take-Home Messages:

- In carcinoid tumor, the tumor cells are small and uniform with round to oval nuclei, coarsely granular chromatin, and small nucleoli.
- Carcinoid tumor cells may be singly dispersed and have a plasmacytoid appearance or form loosely cohesive groups or rosettes.
- By immunohistochemical stain, the cells are positive for pancytokeratin and neuroendocrine markers (synaptophysin, chromogranin, and CD56). The tumors are usually negative for TTF-1. However, TTF-1 expression is common in small cell carcinomas of lung.
- Carcinoid tumors present with two distinctive features: low proliferation rate (0–1 per 2 mm^2 mitotic rate and <5% ki-67 proliferative index) and lack of necrosis. These features make them to differ from atypical carcinoid tumors and small cell carcinomas. Some carcinoid tumor cells are small and plasmacytoid, resembling lymphoid cells. But they usually have more cytoplasm, form clusters or rosettes patterns, and are negative for lymphoid lineage markers.

References: [23, 27, 28].

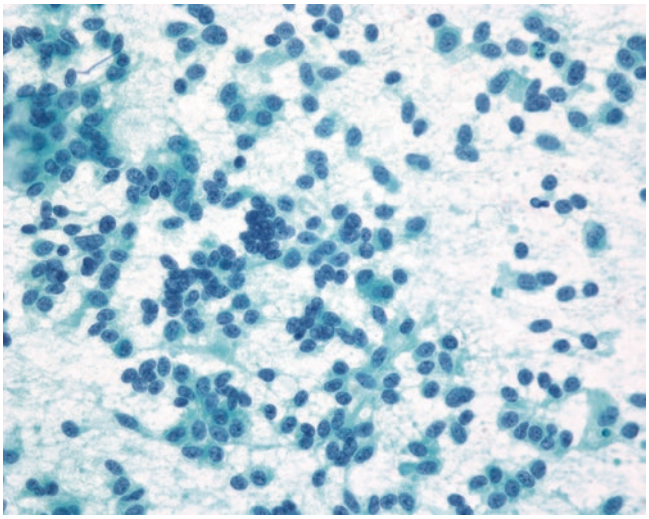

Fig. 7.37 Case 1. Abundant monomorphic cells in loose clusters and single forms. (Papanicolaou stain 400×)

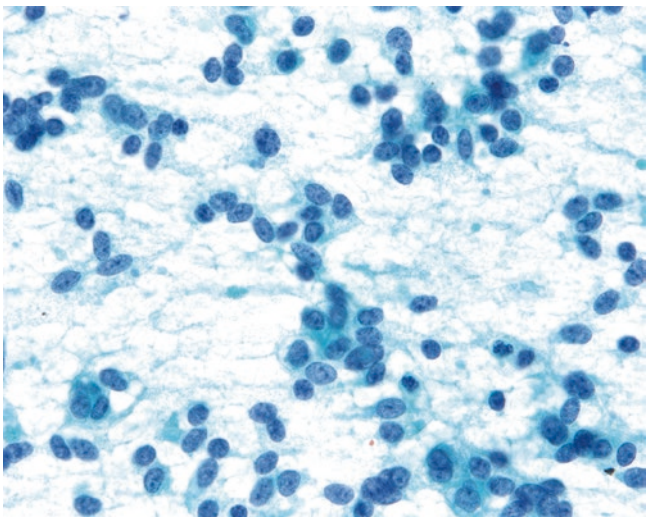

Fig. 7.38 Case 1. Cells have round to oval nuclei and coarsely granular chromatin. (Papanicolaou stain 600×)

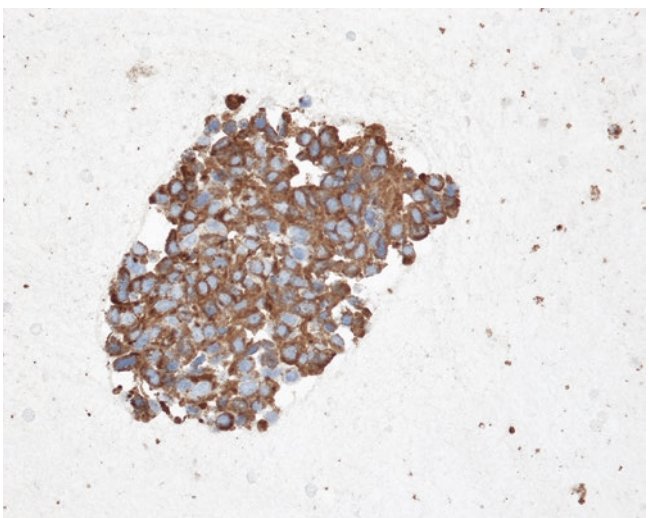

Fig. 7.39 Case 1. Immunohistochemical stain for chromogranin. (IHC stain 400×)

Case 2

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this tumor
2. Discuss differential diagnosis of this tumor
3. Become familiar with immunohistochemical staining features of this tumor

Case History:

A 73-year-old female developed symptoms of cough approximately 9 months ago. He recently developed increasing cough symptoms and chest wall pain. CT of the chest revealed a left lower lobe lung mass. A PET scan revealed a metabolically active left lower lobe lung mass, as well as extensive metabolically active mediastinal and bilateral hilar lymphadenopathy.

Specimen Source:

EBUS-FNA of an enlarged mediastinal lymph node was performed. A modified Giemsa-stained smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic Findings (Figs. 7.40 and 7.41):

- Abundant cell groups arranging in flat sheets and loose clusters.
- The cells are highly pleomorphic with enlarged nuclei, irregular nuclear membrane, vesicular chromatin, and prominent nucleoli.
- The cytoplasm is vacuolated and translucent.

Differential Diagnosis:

- Adenocarcinoma
- Mesothelioma
- Non-keratinizing squamous cell carcinoma

IHC and Other Ancillary Studies:

- TTF-1 positive
- P40 negative
- EGFR mutational analysis positive
- Immunohistochemical stain for PD-L1 shows low expression in tumor cells

Final Diagnosis:

Lung adenocarcinoma

Take-Home Messages:

- Tumor cells in lung adenocarcinomas may form sheets or clusters. Tumor cells have enlarged nuclei with irregular nuclear membrane, vesicular chromatin, and prominent nucleoli.

- Tumor cells are positive for TTF-1 and negative for p40. A limited panel of TTF-1/p40 can help make the final diagnosis and save tissue for molecular testing.
- EGFR/ALK/ROS molecular testing may be performed in advanced-stage lung adenocarcinomas to guide therapy.
- Standard PD-L1 immunohistochemical staining (PD-L1 IHC 22C3 pharmDx test) is recommended in all advanced lung non-small cell carcinomas for selection of immunotherapy with Pembrolizumab. PD-L1 expression in at least 50% of tumor cells is required in the first-line setting and PD-L1 expression in at least 1% of tumor cells is required in the second-line setting.

References: [29–33].

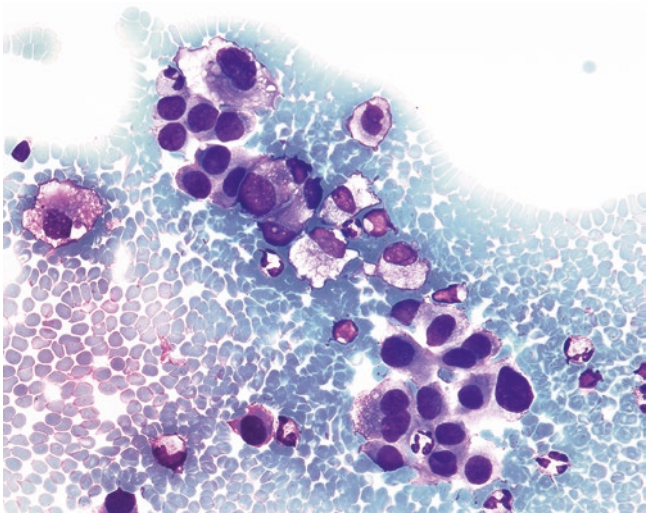

Fig. 7.40 Case 2. Loosely cohesive cell clusters with pleomorphism. (Modified Giemsa stain 400×)

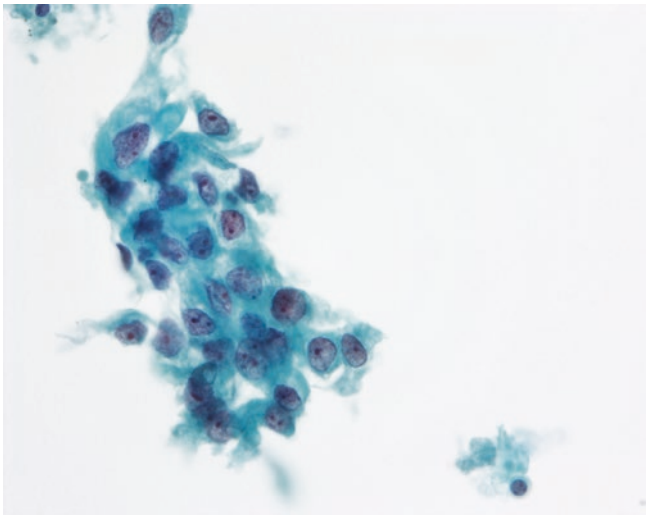

Fig. 7.41 Case 2. Cells have irregular nuclear membrane, vesicular chromatin, and prominent nucleoli. (Papanicolaou stain 600×)

Case 3

Learning Objectives:

1. Become familiar with the typical clinical presentation of this lesion
2. Recognize characteristic cytomorphologic features of this lesion
3. Discuss differential diagnosis of this lesion

Case History:

A 42-year-old man who is HIV-positive presents with dry cough, fever, and dyspnea. Chest image shows bilateral diffuse infiltrates.

Specimen Source:

A stat bronchoalveolar lavage was performed. A modified Giemsa-stained smear and a GMS-stained smear were made from the lavage.

Cytologic Findings:

The smear shows foamy proteinaceous spheres (Fig. 7.42). GMS stain demonstrates groups of cup-shaped cysts and a central dark zone (Fig. 7.43). There

is no budding. These features are usually described as "crushed Ping-Pong balls" or "soap and bubbles."

Differential Diagnosis:

- *Pneumocystis jiroveci*
- Histoplasmosis
- Alveolar proteinosis

IHC and Other Ancillary Studies:

- GMS positive
- PAS diastase negative

Final Diagnosis:
Pneumocystis jiroveci
Take-Home Messages:

- *Pneumocystis jiroveci* infection occurs in immunocompromised patients.
- Clinical presentation includes dry cough, dyspnea, and fever.
- The organisms are recognized as circumscribed foamy proteinaceous alveolar casts. On GMS stain, the cysts are cup-shaped with a central dark zone. There is no budding. Histoplasma are small intracellular budding yeasts.

References: [34, 35].

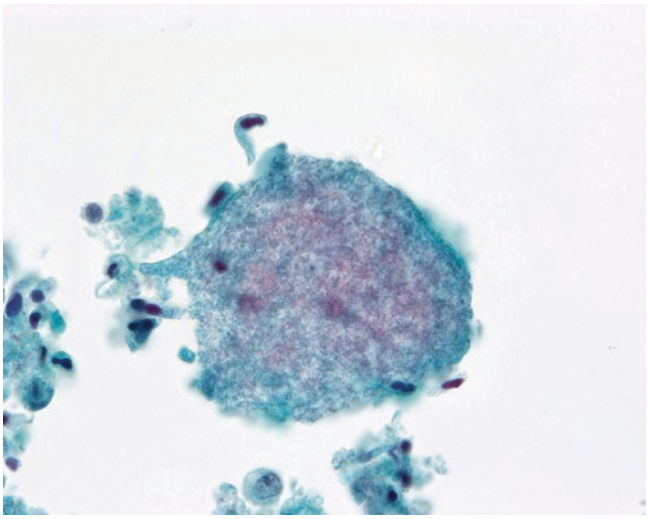

Fig. 7.42 Case 3. The smear shows foamy proteinaceous spheres (soap and bubble appearance). (Papanicolaou stain 600×)

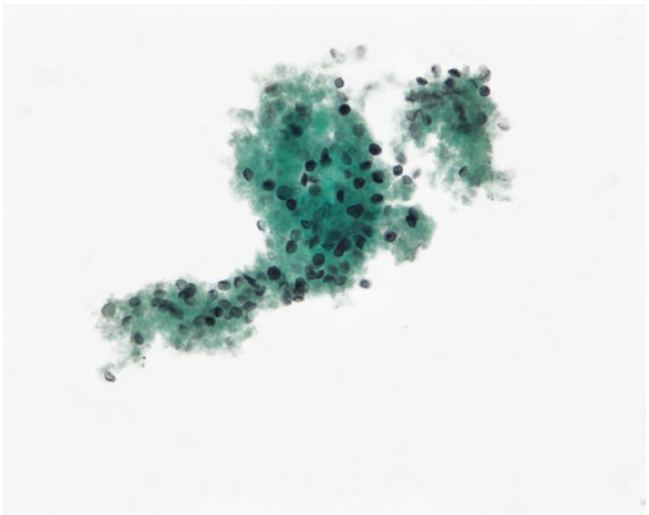

Fig. 7.43 Case 3. GMS stain demonstrates groups of cup-shaped cysts and central dark zone. (GMS stain 600×)

Case 4

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this tumor
2. Discuss differential diagnosis of this tumor
3. Become familiar with immunohistochemical staining features of this tumor

Case History: A 65-year-old male presented with shortness of air. CT scan showed a 3 cm lung mass.

Specimen Source: Transbronchial fine-needle aspiration was performed. A modified Giemsa-stained smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic Findings:

- Cellular specimen showing loose aggregates of cells with high nuclear-to-cytoplasmic ratio, nuclear molding, and prominent crush artifact (Fig. 7.44).
- Small round cells with scant cytoplasm, scattered bare nuclei, powdery chromatin, and indistinct nucleoli (Fig. 7.45).
- Background appears necrotic.

Differential Diagnosis:

- Lymphoma
- Small cell carcinoma

- Reserve cell hyperplasia
- Non-small cell carcinoma

IHC and Other Ancillary Studies:

- Cell block shows clusters of similar small round blue cells with high nuclear-cytoplasmic ratio and molding (Fig. 7.46).
- Chromogranin (Fig. 7.47) and CD56 positive.
- CD45 negative.

Final Diagnosis:
Small cell carcinoma
Take-Home Messages:

- Small cell carcinoma is a high-grade neuroendocrine tumor and will stain for neuroendocrine markers such as synaptophysin, chromogranin, and CD56.
- Cytologic features include small blue cells with high nuclear-to-cytoplasmic ratio, molding, powdery or "salt and pepper" chromatin, and indistinct nucleoli.
- The cells are fragile and often appear stripped or crushed.
- High mitotic activity and necrosis are typically present, and help distinguish small cell carcinoma from lower grade neuroendocrine tumors.
- In rare cases, small cell carcinoma is diagnosed after TKI therapy of an EGFR-mutated adenocarcinoma. It may be due to "transformation" or "dedifferentiation" of the adenocarcinoma; or that the original tumor is a mixed tumor with an adenocarcinoma component and a small cell carcinoma component and the "transdifferentiation" is not associated with the TKI treatment.

References: [23, 36, 37].

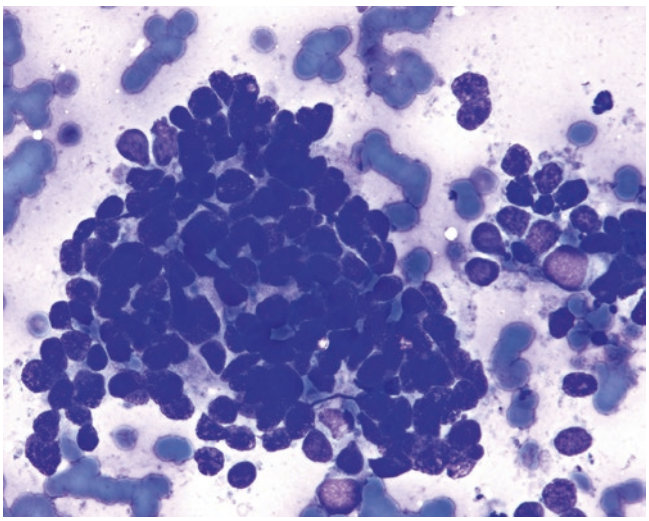

Fig. 7.44 Case 4. Cellular aggregate of cells with high nuclear-to-cytoplasmic ratios, nuclear molding, and crush artifact. (Modified Giemsa 600×)

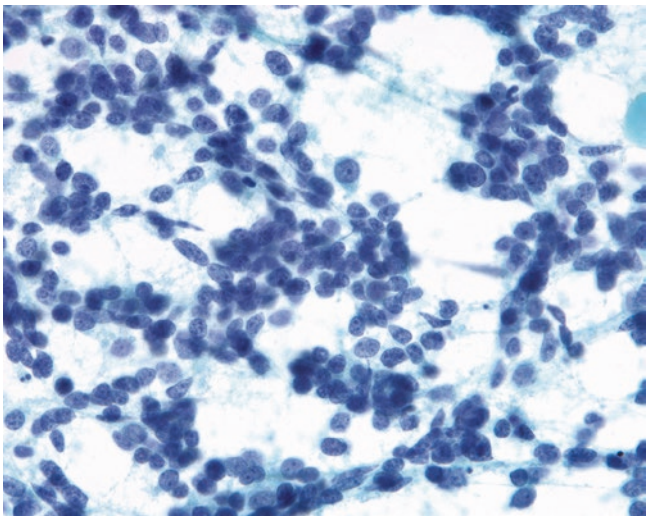

Fig. 7.45 Case 4. Cellular specimen showing small round to oval cells with scant cytoplasm, powdery chromatin, and indistinct nucleoli. (Papanicolaou stain 600×)

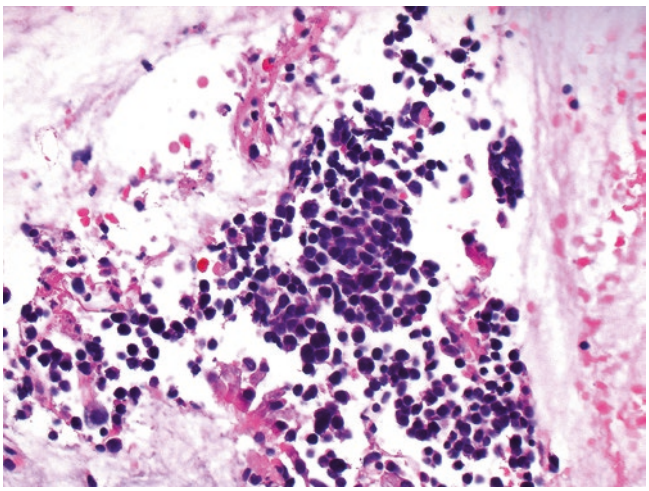

Fig. 7.46 Case 4. Cell block specimen shows clusters of small blue cells with nuclear molding. (H&E stain 200×)

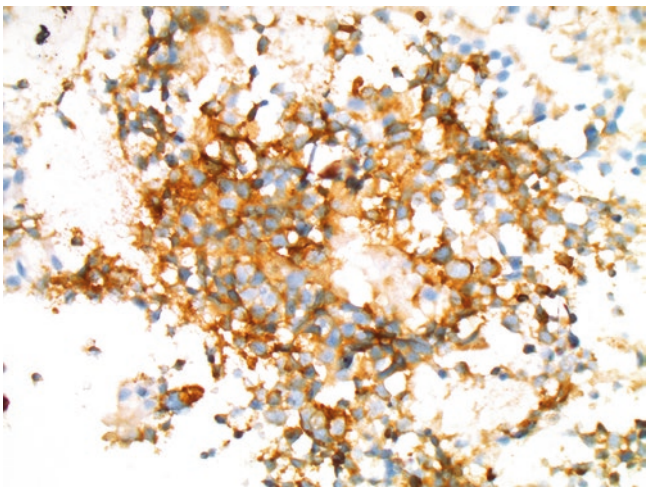

Fig. 7.47 Case 4. Chromogranin immunohistochemical stain is positive in the tumor cells. (400×)

Case 5

Learning Objectives:

1. Recognize characteristic cytomorphologic features of this tumor
2. Discuss differential diagnosis of this tumor
3. Become familiar with immunohistochemical staining features of this tumor

Case History: A 50-year-old nonsmoking male presented with hemoptysis and cough. CT scan showed a 1.8 cm hilar mass.

Specimen Source: EBUS – fine-needle aspiration was performed. A modified Giemsa-stained smear, Pap-stained smear, and a cell block were made from the aspiration.

Cytologic Findings:

- Cellular smears with large epithelioid and pleomorphic cells (Fig. 7.48).
- Dispersed isolated cells and loose aggregates of epithelioid cells with pigment (Fig. 7.49).
- Round or oval nuclei, often eccentrically placed, with macronucleoli (Fig. 7.50)
- Occasional binucleated cells and nuclear inclusions.

Differential Diagnosis:

- Carcinoma
- Lymphoma
- Melanoma

IHC and Other Ancillary Studies:

- S-100 and SOX10 positive
- Pancytokeratin and CD45 negative

Final Diagnosis:

- Metastatic melanoma

Take-Home Messages:

- The cytomorphology in melanoma can vary from epithelioid to spindle to pleomorphic and often mimics other tumors.
- Typically, cells are dyscohesive or loosely aggregated with a plasmacytoid appearance.
- Prominent cherry-red nucleoli are characteristic, and nuclear inclusions may be present.
- Melanin pigment, melanophages, and finely vacuolated cytoplasm are helpful when present.
- Immunohistochemistry is useful; the tumor will stain positive for melanoma markers such as S-100, SOX-10, and/or MART-1, and will be negative for keratin markers and CD45.

References: [38, 39].

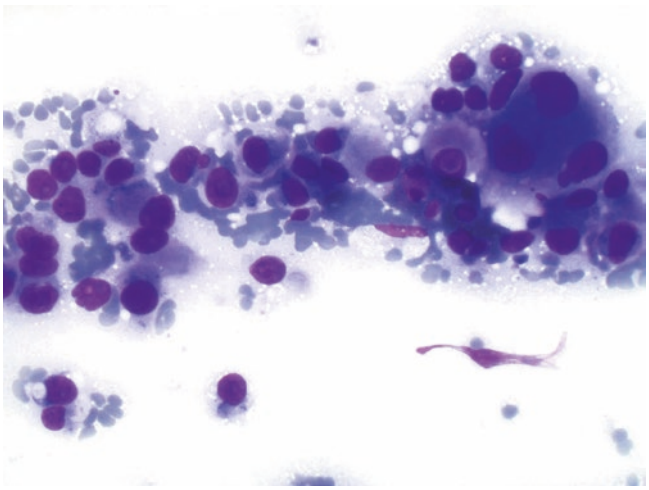

Fig. 7.48 Case 5. Large epithelioid and pleomorphic cells are present which are loosely cohesive. (Modified Giemsa 400×)

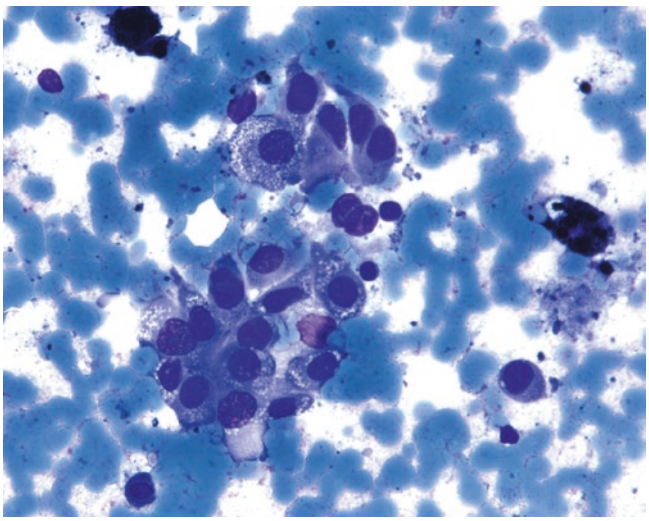

Fig. 7.49 Case 5. Loose aggregates of atypical epithelioid cells with plasmacytoid morphology. Melanin pigment, which appears blue to black on modified Giemsa stain, is present. (Modified Giemsa 600×)

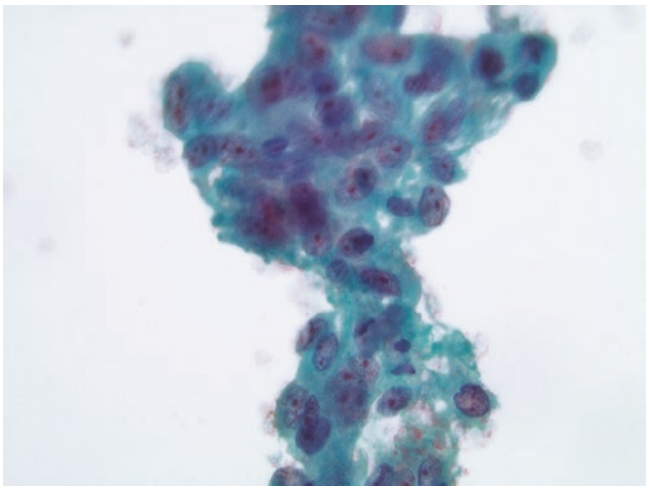

Fig. 7.50 Case 5. Aggregate of large epithelioid cells with prominent red macronucleoli. (Papanicolaou stain 600×)

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Urine Cytology

8

Nikolina Dioufa, Gina Prochilo, and Suad Taraif

Contents

List of Frequently Asked Questions

1. What are the types of urine specimens commonly received in the cytology laboratory?

Urine samples can be divided into two broad categories, voided and instrumented. Voided urine is ideally collected midstream and clean catch to avoid bacterial and cellular contaminants from the penile/distal urethra in males and vulva/vagina in females. Poor preservation and cellular degeneration are common features of voided specimens. First morning specimens should be avoided due to overnight cellular deterioration.

Alternatively, instrumented urine can be collected using catheters or via washes of the upper and/or lower urinary tracts. Bladder washes are usually obtained during cystoscopy, and upper tract (renal pelvis and ureteral) washes are obtained during cystoureteroscopy. Instrumented urine samples are more cellular and naturally containing tissue fragments due to forceful exfoliation of the barbotage/irrigation procedure. Instrumented specimens usually have fewer contaminants. However, lubricant gel material used to introduce the cystoscope, if excessive, can sometimes obscure the cellular elements limiting the usefulness and/or interpretation of the test.

Loop urine is also an instrumented sample and is most commonly obtained from patients who underwent cystectomy for urothelial carcinoma. The cardinal feature of these specimens is severe degeneration and the presence of other contaminants and cell types originating from the intestinal loops or "neobladder." Some of the advantages and disadvantages of the various types of urine specimens are summarized in Table 8.1.

References: [1–3].

2. What are the available cytopreparation methods for urine cytology specimens?

Direct smears were historically used to prepare slides from urine samples. However the availability of cytoconcentration techniques, cytocentrifugation, and thin-layer preparations

Table 8.1 Types of cytologic urine specimens

Urine specimen type	Advantages	Disadvantages
Voided urine	Noninvasive Simple Inexpensive Less bacterial-cellular contaminants	Poor preservation Cellular degeneration Low cellularity
Instrumented urine	Good cellularity Fewer contaminants Good cellular preservation Precise localization of the lesion	Invasive Obscuring elements
Loop urine	Follow up of patients with history of urothelial carcinoma	Severe degeneration Cellular contaminants

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_8

largely replaced direct smears. In both former techniques, a concentrated cell sample is deposited on a glass slide which can be stained using modified Giemsa, Papanicolaou, or even hematoxylin and eosin stains. The thin-layer method increases the sensitivity of the test by removing obscuring elements, such as blood and inflammatory cells. Cytocentrifugation, on the other hand, while concentrating the cells in a smaller area preserves much of the obscuring elements.

References: [1, 4].

3. What is an adequate urine sample?

Literature regarding what constitutes an adequate urine sample for cytological examination is sparse. In general, there are attempts to standardize adequacy criteria, both qualitative and quantitative. However, no one single proposal has successfully taken off as of yet. A sample with abnormal cells is always adequate. Adequacy issues usually arise in sparsely cellular specimens, since low cellularity or inadequate representation may pose potential for missing a significant pathological process including malignancy.

The Paris System for Reporting Urinary Cytology tried lately to address adequacy. Using complex mathematical information related to the surface area and capacity of the bladder as well as the thickness of the lining urothelium, the group came up with "the adequacy algorithm." The focus of this adequacy algorithm though, like the rest of the Paris System, is the ability of the sample to confidently confirm or exclude a diagnosis of high-grade urothelial carcinoma.

References: [5–10].

4. What are the types of cells that can be seen in a urine cytology specimen?

The degree of cellularity in any given sample is a product of both collection and preparation methods. Voided urine samples are generally hypocellular or moderately cellular at best. Cells are mostly small to intermediate urothelial cells with scattered large mononuclear, binucleate, or multinucleated large umbrella cells. Variable numbers of squamous cells are also usually present. Instrumented urine is usually more cellular and benign urothelial tissue fragments that are forcefully exfoliated with the fluid jet are commonly seen. Degenerated inflammatory cells are almost invariably present in the background. Red blood cells may also be present, especially in patients clinically presenting with hematuria – the commonest indication for urine cytology.

5. I see squamous cells in the specimen; what is their significance?

Benign squamous cells are considered normal constituents, especially in voided urine samples. Squamous cells in females mostly originate from the vulva or vagina. However, squamous cells can also be derived from the trigone region of the urinary bladder where squamous metaplasia is frequent. In males, besides the bladder trigone, the distal urethra is an additional source.

It is not uncommon to find dysplastic/HPV-infected squamous cells in urine samples, especially in females. These cells must be interpreted in the context of the entire clinical picture, as they most likely represent cervicovaginal contamination. However, other lesions (e.g., vulvar or penile condylomas) can also yield dysplastic/HPV-infected squamous cells in urine samples. Another cervicovaginally derived contaminant is *Trichomonas Vaginalis*, especially in cases of heavy infection.

Besides benign squamous cell contaminants, squamous cell carcinomas can arise in the urinary bladder and along the rest of the urothelial tract. This happens usually in the setting of chronic irritation due to stones or chronic infections. A classic example is squamous cell carcinoma arising in the background of long-standing *Schistosoma haematobium* infection of the urinary bladder in some endemic areas of the Middle East.

References: [11–13].

6. What is the difference in cellular constituents between a male and a female urine sample?

Other than the abundance of contaminant squamous cells from the vagina in females, samples from both genders are indistinguishable. Sperm cells can sometimes be seen in male urine samples, especially in adolescent men. Sperm cells may also be seen in normal women's urine following intercourse due to vaginal contamination.

7. How do we report benign cellular fragments in urine?

Classical cytology teaching preached that cellular fragments are abnormal in voided urine. With time, it was realized that some cellular fragments can be spontaneously exfoliated and subsequently seen in voided urine samples. Cellular fragments are commonly seen and expected in instrumented urine samples. According to the Paris System for Reporting Urinary Cytology, all benign cellular fragments, irrespective of the method used to obtain the sample, are to be classified as benign cellular fragments and accordingly diagnosed as "negative for high-grade urothelial carcinoma." Only fragments with unquestionable fibrovascular cores are to be considered in the rare cytological diagnosis of "low-grade papillary urothelial carcinoma."

References: [10, 14, 15].

8. What are some of the potential organisms that can be detected in a urine cytology preparation?

Contaminant bacteria from the vulvar, perineal, or penile skin can be frequently seen, usually cocci in clusters. They can be seen in the background or more commonly adherent to squamous epithelial cells. Other organisms that can be seen include *candida*, mostly as a vaginal contaminant, but

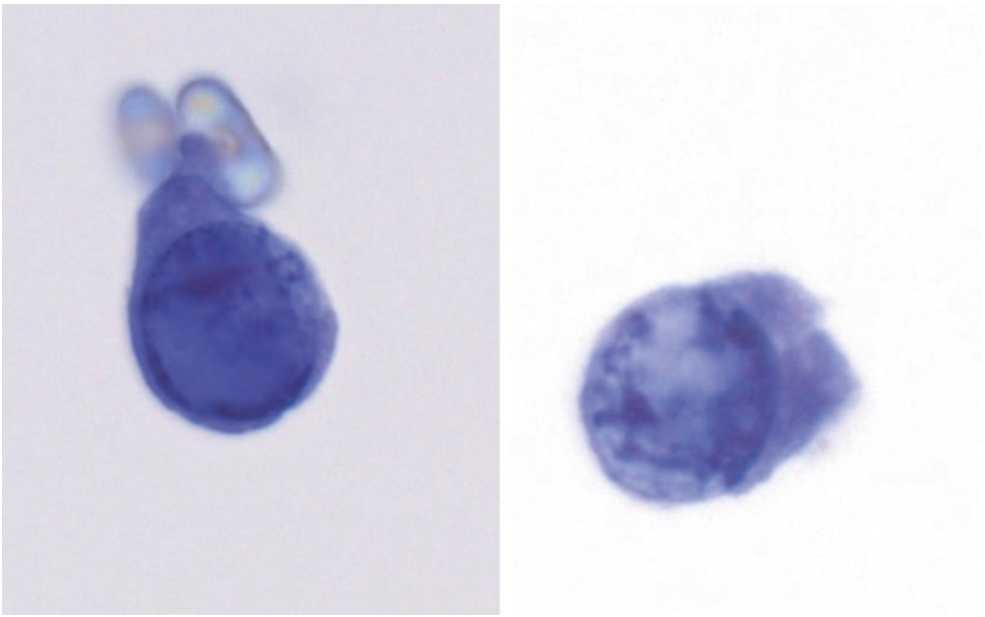

Fig. 8.1 Polyoma virus-infected "decoy" cells

may represent a pathological finding. Both yeast and pseudohyphal forms may be seen. *Trichomonas* in the urine is not rare either, and if vaginal infection is severe, then numerous organisms including trichomonas colonies can contaminate the urine. Other less frequently encountered organisms include parasites like *Schistosoma haematobium* and rarely *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma* spp., and *Ureaplasma* spp.

References: [16, 17].

9. What are the cytological changes associated with polyoma virus infection and what is their significance?

Polyoma virus-infected urothelial cells can masquerade as malignant urothelial cells with enlarged, hyperchromatic, and eccentrically placed nuclei. However, other accompanying cellular and background features are usually reassuring. The sample will usually show striking degenerative changes. The smudged nuclei will lack any additional nuclear detail other than hyperchromasia. Nucleoli are not usually visible. The nuclear contours are either smooth or disrupted due to disintegration. The cytoplasm can be tapered to one end like a comet tail (Fig. 8.1).

The significance of polyoma virus-infected cell identification in urine is twofold. One is that they can be mistaken for malignant urothelial cells, and subsequently false-positive results may be obtained. The other is their significance as an indicator of (re)activation of latent polyoma viruses in urothelial cell layers, especially in the setting of the immune-compromised patients, transplant, chemotherapy, diabetic, elderly, or rarely healthy adults. The presence of polyoma virus-infected cells in urine is sometimes associated with upper urinary tract disease, including tubulitis or glomerulonephritis. The absolute count of polyoma virus-infected cells (or their actual counts per microscopic field) may be used to adjust the dose of chemotherapy/immunosuppression.

References: [18–21].

10. Are urinary crystals significant? Do we have to report them?

The identification of urinary crystals is not a routine part of urine cytological examination, but it is helpful to get accustomed to some of the most common and frequently encountered crystals. These include:

- Calcium oxalate: Oval, dumbbell, or envelope shaped
- Uric acid: Variable, barrel, plate-like, or diamond shaped
- Struvite/triple phosphate: Rectangular prism or coffin-lid shaped
- Cysteine: Hexagonal shaped

Reference: [1].

11. Are urinary casts significant? Do we have to report them?

Casts in urine can be physiological or may indicate upper urinary tract disease. There are several types of casts (Table 8.2). Not all casts have clinical significance. Recognizing the different morphological variants of casts and reporting them are not usual parts of urine cytological examination, at least not under the current Paris System for Reporting Urinary Cytology, which places the emphasis on diagnosing high-grade urothelial carcinoma. However, some degree of familiarity with at least a few important casts may prove useful (Fig. 8.2).

Reference: [22].

12. What is the spectrum of benign reactive changes that can be seen in urine samples?

Mild cytological atypia in the form of enlarged nuclei and prominent nucleoli are the hallmark of reactive changes. The most common causes of reactive changes are lithiasis and urinary tract infections. The nuclear to cytoplasmic ratio of reactive urothelial cells is usually low. Nuclear membranes are smooth and nuclei are usually vesicular. Significant hyperchromasia, clumped chromatin, and high nuclear to cytoplasmic ratio are not usually seen and are more indicative of a neoplastic process.

References: [23, 24]

Table 8.2 Common types of urinary casts

Type	Composition	Clinical significance
Hyaline casts	Tamm-Horsfall mucoprotein	Dehydration Exercise
Granular casts	Degenerate cellular casts Aggregates of plasma proteins IgG light chains	Strenuous exercise Chronic renal disease Acute tubular necrosis (ATN)
Waxy casts	Degenerate cells (renal failure casts)	Severe chronic renal disease Amyloidosis
Fatty casts	Lipid droplets	Tubular degeneration Nephrotic syndrome Hypothyroidism
Red blood cell (RBC) casts	RBCs	Pyelonephritis Glomerulonephritis Acute interstitial nephritis Lupus nephritis
Epithelial casts	Tubular epithelial cells	Renal tubular necrosis Viruses Transplant rejection
Bacterial casts	Bacteria	Acute pyelonephritis
White blood cell (WBC) casts	WBC	Glomerulonephritis

13. What are the cytological changes seen secondary to lithiasis?

Florid reactive changes of the urothelial cells in the form of enlarged vesicular nuclei with prominent nucleoli are usually seen along with some exfoliated small urothelial tissue fragments. Urinary crystals can also be seen in the background along with red blood cells especially in cases with frank hematuria. If there is an associated urinary tract infection, then some neutrophils and bacteria can also be expected. This constellation of features was in the past referred to as "stone atypia." However, the term "atypia" always brought along some degree of confusion to the treating physicians including how to follow up these cases after the resolution of the acute nephrolithiasis episode. In the current Paris System for Reporting Urinary Cytology, stone atypia is classified as part of the benign/reactive cellular changes and accordingly diagnosed as "negative for high-grade urothelial carcinoma."

References: [25–27].

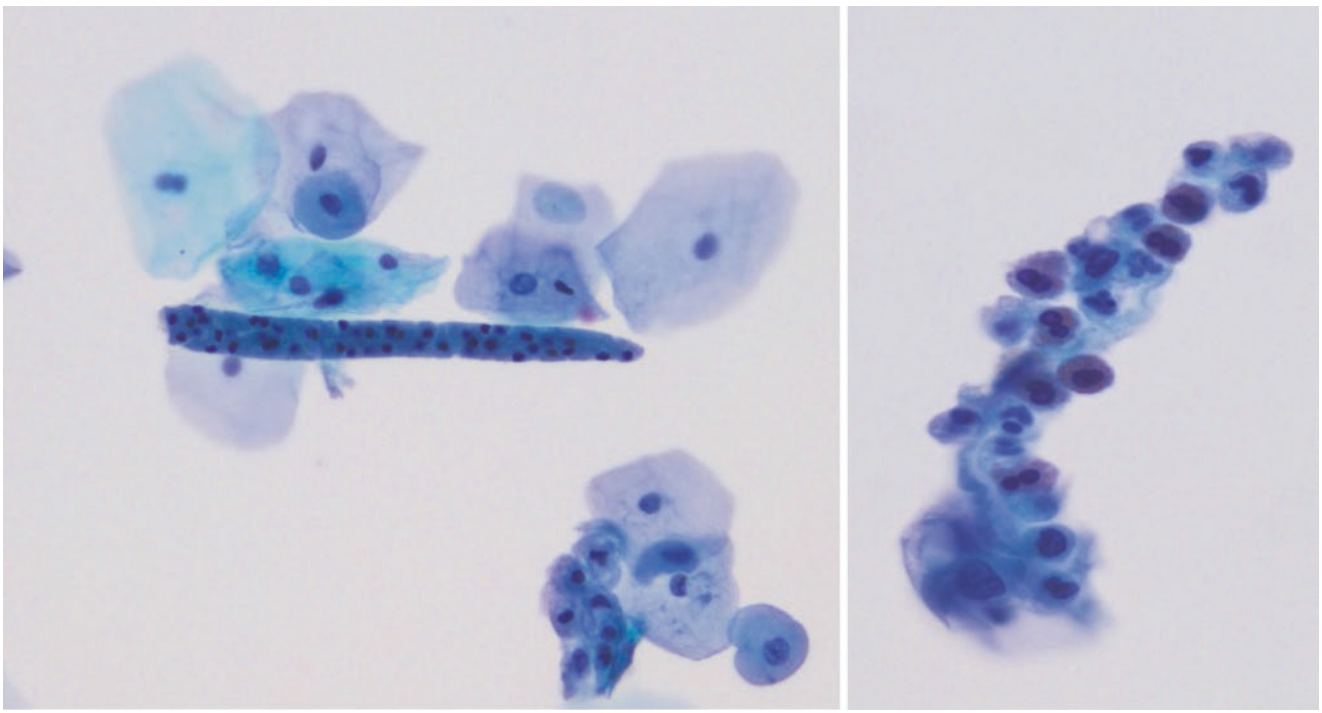

Fig. 8.2 Representative casts in urine

14. What are the cytological features of cystitis cystica et glandularis?

Usually seen in the setting of chronic inflammation and irritation, cystitis cystica et glandularis presents in the urine as scattered benign glandular epithelial cells.

15. Can we diagnose renal lesions using urine cytology?

The purpose of urinary cytology is to survey the urothelial tract including the detection of upper tract lesions. However, cells from other non-urothelial lesions and tumors can also shed in the urine, especially if those lesions encroach upon the pelvicalyceal system. This commonly includes cells from renal cell carcinomas and rarely lymphomas and angiomyolipomas.

16. Can we diagnose prostatic lesions using urine cytology?

Sperms, seminal vesicle cells, prostatic secretions, benign prostate gland epithelial cells, and prostatic carcinoma are very rare but can all be seen in urine cytology samples.

Sperms will be visible in the background, either individually or in small groups/clusters. These samples cannot be used for fertility evaluation, but may give an indication of retrograde ejaculation. Seminal vesicle cells can be recognized using the same cytomorphological criteria used to separate them from prostate cancer in prostate needle core biopsies. Most importantly is the recognition of the golden-brown cytoplasmic lipofuscin pigment. Seminal vesicle cells are usually larger than the average prostate glandular cell, with occasional atypical/bizarre cells (so-called monster cells). There may be prominent nucleoli.

Corpora amylacea may be seen in the background, especially in the setting of benign prostatic hyperplasia.

Benign prostate gland epithelial cells are extremely rare to identify. However, prostatic adenocarcinoma can be seen, especially in the setting of locally advanced prostatic carcinoma directly invading the urinary bladder. The cells will have the usual features of prostatic adenocarcinoma, namely, enlarged vesicular nuclei with prominent nucleoli. Absence of a basal cell layer can be confirmed using immunohistochemistry on cell block preparations.

References: [28–30].

17. How is the Paris System for Reporting Urinary Cytology different from prior reporting schemes?

The main focus of the Paris system was to increase the standardization and simplify the reporting of urine cytology. The authors of the Paris system achieve this by utilizing the strengths of high specificity of urine cytology for the detection of high-grade urothelial carcinoma and by eliminating the weakness of low specificity for the detection of low-grade lesions. The most important goal of the Paris system is to report the presence or absence of the clinically significant lesion which is the high-grade urothelial carcinoma. Prior reporting schemes failed to be widely accepted because of complexity and lack of reproducibility.

The system classifies urinary samples into benign (including reactive changes and stone-related atypia), atypical urothelial cells, suspicious for high-grade urothelial carcinoma and low-grade urothelial carcinoma. The low-grade urothelial carcinoma diagnosis is strictly limited to urine samples containing epithelial fragments with fibrovascular cores (Fig. 8.3).

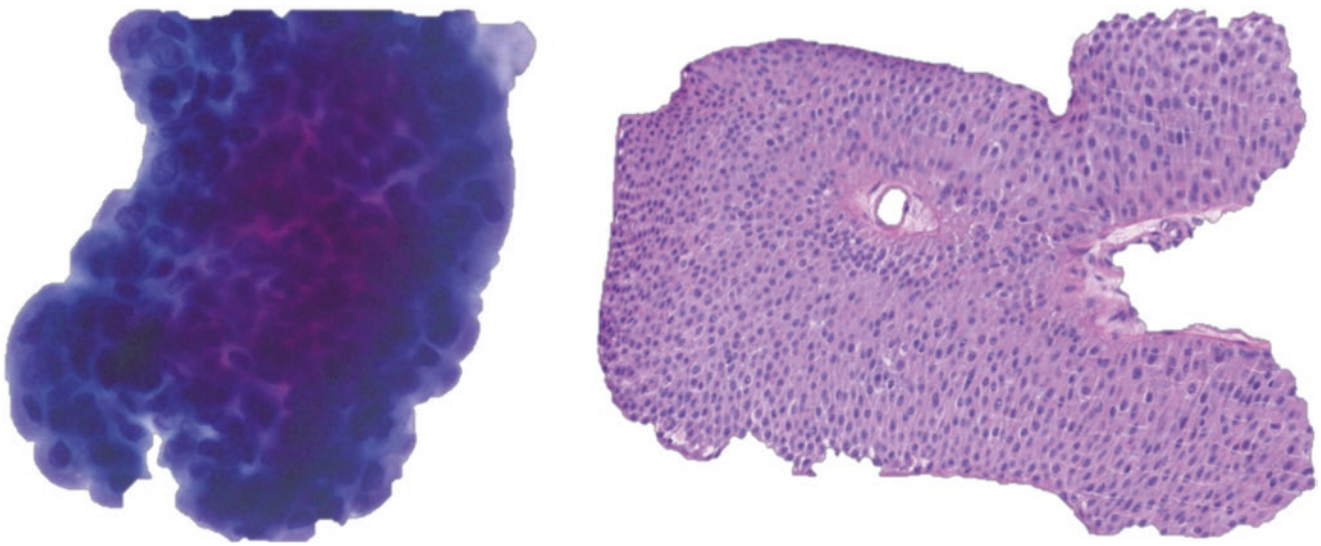

Fig. 8.3 Low-grade urothelial carcinoma (Pap smear and corresponding histological biopsy)

18. Can urothelial papillomas and papillary urothelial neoplasm of low malignant potential (PUNLUMP) be reliably diagnosed using urine cytology?

The diagnosis of both papillomas and PUNLUMPs relies on a combination of architectural and cytological features. The current Paris System for Reporting Urinary Cytology identifies a category for benign (including benign tissue fragments) and another for low-grade papillary urothelial carcinoma (for cellular clusters with fibrovascular cores). It is very likely that both papillomas and PUNLUMPs are being placed in the atypical category as they are more stratified in terms of cell thickness than the average urothelium. At the same time, they fall short in terms of cytological atypia, in comparison to both low- and high-grade urothelial carcinoma. Some PUNLUMPs may end up with the low-grade papillary lesions if fibrovascular cores are seen.

Reference: [31].

19. What are the cytological features of carcinoma in situ and high-grade urothelial carcinoma?

Urine has a very high sensitivity and specificity to diagnose high-grade urothelial carcinoma and carcinoma in situ (CIS). Features are those of highly cellular samples consisting of cells with high nuclear to cytoplasmic ratio, nuclear hyperchromasia, irregular nuclear contour, prominent nucleoli, and cytological and nuclear polymorphism. Malignant cells are usually scattered individually and in variably sized cell groups and tissue fragments. Fibrovascular cores may be present, but are not necessary for the diagnosis. The background may be dirty, with red blood cells, neutrophils, or tumor necrosis. However, this tumor diathesis only brings attention to the cells. The diagnosis relies on identifying high-grade nuclear features.

Compared to urinary tract biopsies, urine has the advantage of being able to "survey" the entire length of the urothelial tract. In CIS, the urothelial cells tend to shed in the urine due to loose adherence to the basement membrane, rendering urine cytology at times more sensitive than tissue biopsy. Bladder biopsies may show mostly a denuded epithelium, and the diagnosis could rely solely on the presence of high-grade urothelial cells in the urine sample.

20. What do we see in a urine sample from an ileal conduit (loop urine)?

Samples from ileal conduits and neobladders are usually poorly preserved with a dirty background. Degenerating glandular cells derived from the intestinal epithelium are common, sometimes simulating histiocytes. Bacteria are also commonly seen along with mucus and other debris (Fig. 8.4).

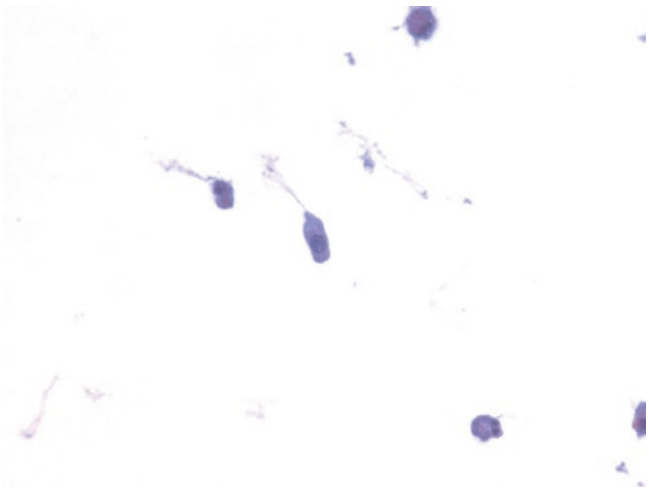

Fig. 8.4 Urine sample from ileal conduits

21. What are the cytological changes associated with chemoradiation therapy?

Classic chemoradiation therapy-related cytological features, like all other body sites, include proportional nucleocytomegaly. Cells are usually huge, with enlarged, often bizarre-shaped nuclei and multinucleation, as well as smudged/degenerative-looking nuclei. The cytoplasm is equally voluminous. Mitotic figures may be seen. The diagnostic challenge usually rests in differentiating therapy effect in tumor/malignant cells from therapy effect on benign urothelial lining cells. The distinction at times may not be straightforward or always feasible.

Other therapy changes include those related to intravesical bacillus Calmette-Guerin (BCG) treatment. BCG induces a granulomatous response in the bladder where loose collections of epithelioid histiocytes or even frank well-formed granulomas may be seen. These findings are best interpreted in clinical context. However, if additional cytological material (e.g., additional cytocentrifugation, thin-layer, or cell block material) is available, the performance of special stains to rule out potential fungal or mycobacterial infection is prudent. Additional features of urine cytology following BCG treatment include a clean background, lacking caseation-type necrosis, or significant inflammation.

22. Can we diagnose urinary tract metastatic carcinomas using urine cytology?

Although it is not the primary purpose of urinary cytology, tumor metastasis can involve the kidney, bladder, and the rest of the urinary tract and subsequently show up in urine. Any type of malignancy (epithelial, mesenchymal, lymphoid, or otherwise) can present in the urine, including melanomas. Examples in the literature of individual case reports are numerous and represent potential challenges to the cytologist in the absence of pertinent clinical history (Fig. 8.5).

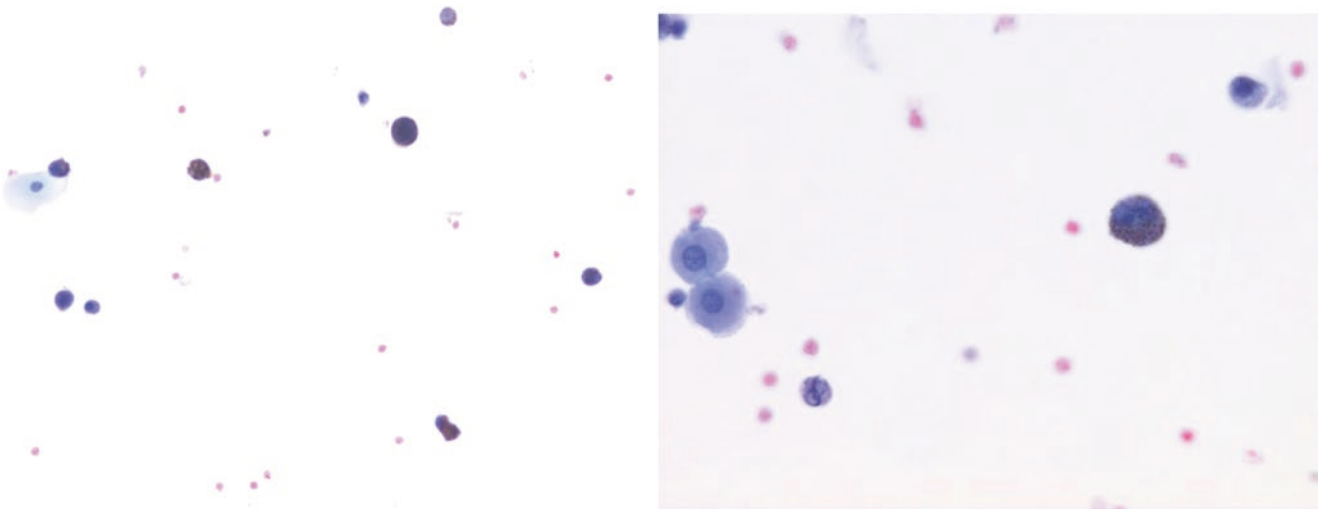

Fig. 8.5 Metastatic melanoma in urine

Case Presentation

Case 1

Case history: A 50-year-old male with intermittent microhematuria for 1 year. Cystoscopic examination showed no obvious papillary or mass lesions, but noted a few reddish spots in the urothelial mucosa. Voided urine was collected and submitted to cytology for review (Figs. 8.6 and 8.7).

Description: The ThinPrep of the urine sample is highly cellular. In addition to the benign urothelial cells, clusters and single cells with hyperchromatic nuclei and high nuclear/cytoplasmic ratio are noted. Careful examination of the nuclei revealed obvious pleomorphism, prominent nucleoli, uneven chromatin distribution, and irregular nuclear contour. The cells are overlapping in three dimensional arrangements.

Diagnosis:

- High-grade urothelial carcinoma

Differential diagnoses:

- Metastatic carcinoma
- Urothelial cells infected by virus, especially polyoma virus
- Urothelial cells with degenerative changes

Take-home message:

- Urine cytology is more sensitive than cystoscopy in identifying urothelial carcinoma in situ.

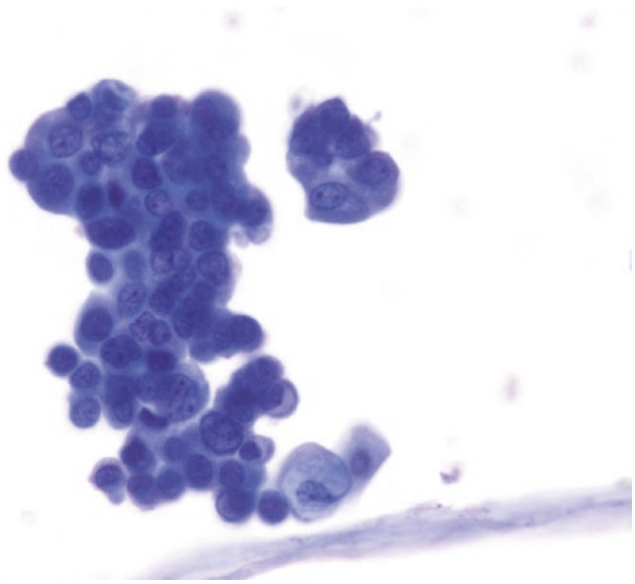

Fig. 8.6 ThinPrep of urine ×20

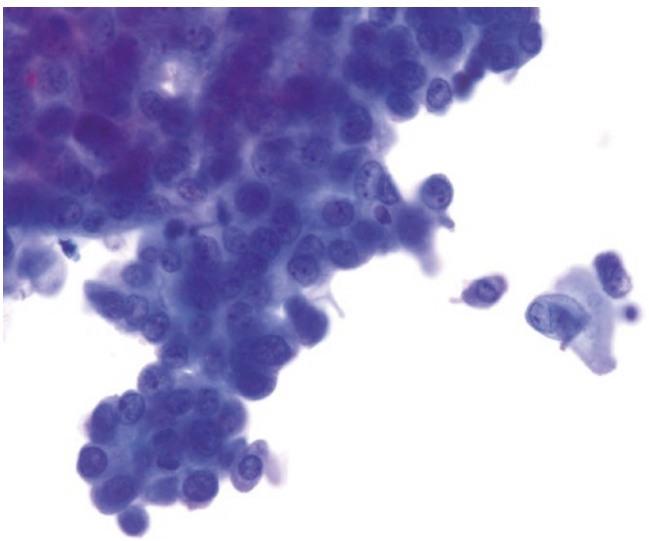

Fig. 8.7 ThinPrep of urine ×40

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Body Cavity Effusions and Washings

9

Xin Jing

Contents

Frequently Asked Questions

1. What is the main purpose of cytologic examination of body fluids? How to report cytologic findings?

The cytologic examination of effusions collected from pericardial, pleural, and peritoneal cavities or fluids obtained from peritoneal/pelvic washings is performed in order to determine the presence or absence of malignant cells. The examples of benign conditions causing effusions include congestive heart failure, hepatic cirrhosis, chronic renal failure, hypoalbuminemia, infection, trauma, etc. The malignant entities include carcinomas, mesothelioma, lymphomas, melanoma, soft tissue malignancy, etc. In general, "positive for malignant cells" and "no malignant cells identified" are reported when malignant cells are definitively present and absent, respectively. When metastatic malignant cells are identified, ancillary tests (i.e., immunostains, flow cytometry) may be applied for categorization of the malignancy (i.e., carcinoma, lymphoma, melanoma, etc.) and determination of primary site. When the cells show atypical features concerning for malignancy, however, the extent of atypia is quantitatively and/or qualitatively insufficient to be categorized as malignancy, a diagnosis of atypical cells or suspicious for malignant cells may be rendered. The definitive criteria for distinguishing the two less-definitive categories (atypical vs. suspicious) are lacking. As a result, both intraobserver variation and interobserver variation are commonly seen in real-life practice.

2. What is the minimum volume of effusion fluid required for an optimal cytologic evaluation?

There is no consensus regarding minimum fluid volume submitted for an optimal cytologic assessment. It is considered adequate regardless of the specimen volume if malignant cells are identified. A large-scale study of pleural fluids demonstrates that a minimum fluid volume of 75 mL is required to ensure a true benign diagnosis. Accordingly, fluid volumes of less than <75 mL increase the risk of a false-negative, indeterminate, or non-diagnostic result. The authors recommend a disclaimer to all benign specimens of <75 mL, suggesting that the low volume may have compromised specimen evaluation (Rooper et al. 2014) [1].

3. What cytologic preparations are commonly employed?

Effusion fluids are often processed using conventional cytopreparatory technique and/or liquid-based cytology. Various results have been reported in terms of cellularity, cell distribution, and cytomorphology in comparison of these two processing methods. With regard to the cytomorphology, some authors have observed that liquid-based cytology (i.e., ThinPrep preparation) demonstrated better nuclear chromatin morphology and significant shrinkage of cell size. It is noteworthy to mention that liquid-based cytology has cleaner background and less screening time. However, both methods offer compatible diagnostic sensitivity [2–4]. In addition, part of the fluids may be processed as cell block which may

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_9

show histology pattern and be utilized for immunocytochemical staining if needed [5].

4. What are collagen balls? Do collagen balls have any clinical significance?

Collagen balls are tissue fragments with a smooth contour that are composed of mesothelial cells intermingling with or surrounding collagenous stroma. The mesothelial cells surrounding the stroma often become flat. They are found in women only and present in pelvic washing or peritoneal washing specimens. The collagen balls are probably originated from the surface of the ovaries and any structure covered by mesothelium. Collagen balls are non-specific findings and should not be mistaken as neoplasms [6] (Fig. 9.1a, b).

5. Does the presence of psammoma bodies indicate malignancy?

A psammoma body appears as a sphere with a laminated calcification and may present in pleural, peritoneal, and pericardial fluids involved by metastatic carcinomas of the thyroid, lung, ovary, and uterus. However, the presence of psammoma bodies does not necessarily imply a malignant condition and its presence may also be associated with benign conditions, i.e., ovarian cystadenoma or cystadenofibroma, papillary mesothelial hyperplasia, endosalpingiosis, and endometriosis, etc. [7] On Diff-Quik-stained smears, a psammoma body does not pick up stain and appears as colorless, refractile material. On Papanicolaou-stained conventional smears and liquid-based preparations, a psammoma body appears as a sphere with a laminated calcification (Fig. 9.2a, b).

6. What are the cytomorphological features of endometriosis and endosalpingiosis?

Müllerian epithelial cells may appear in pelvic washing specimens from women with benign conditions, i.e., endometriosis and endosalpingosis. The presence of endometrial epithelial cells and hemosiderin-laden histiocytes is suggestive of endometriosis. The epithelial cells along with stromal cells are arranged as honeycomb or syncytial sheets or tight clusters [8]. However, stromal cells and hemosiderin-laden histiocytes may be absent in some cases [9].

Typical features of endosalpingiosis include epithelial cells arranged as small clusters or branching tubular structures. Commonly, the epithelial cells have a cuboidal to columnar appearance with uniform nuclei, smooth nuclear membrane, and fine chromatin. Occasionally, the epithelial cells exhibit moderate cytologic and/or nuclear atypia which pose diagnostic challenges [9]. The presence of ciliated epithelial cells favors benign endosalpingiosis (Fig. 9.3).

7. How to distinguish reactive mesothelial cells from adenocarcinoma?

Adenocarcinomas account for the majority of malignant pleural, pericardial, and peritoneal effusions. Malignant pleural effusions are often caused by carcinoma of lung, followed by breast, ovarian, and gastrointestinal origin. Adenocarcinomas of lung and breast are also the first and second most common metastatic carcinoma in malignant

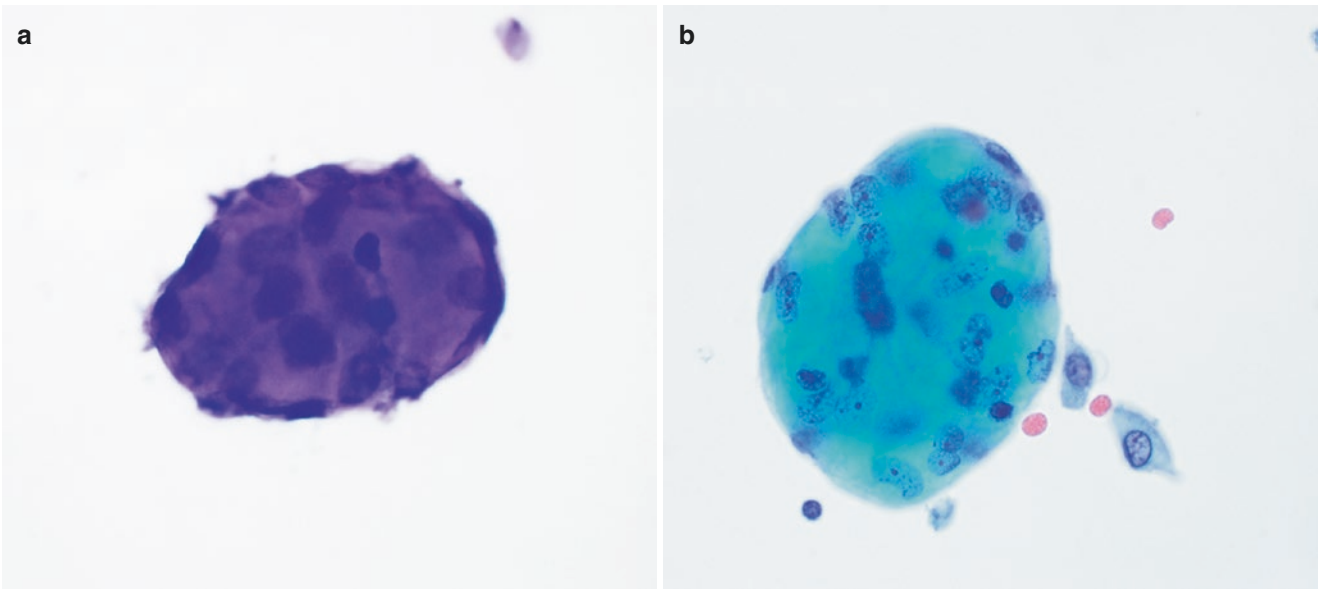

Fig. 9.1 Collagen balls. Mesothelial cells which are intermingling with or surrounding collagenous stroma. (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively)

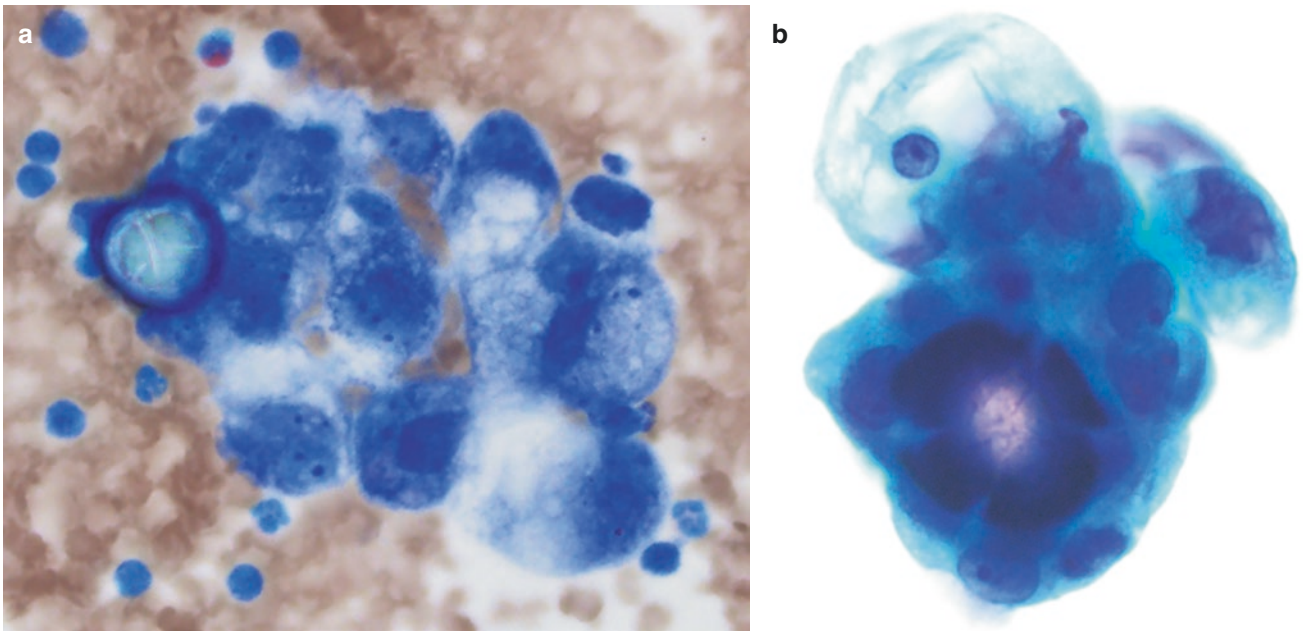

Fig. 9.2 Psammoma bodies. Acellular, calcified spheres within the clusters of epithelial cells, with colorless refractile (**a**, Diff-Quik stain) and laminated appearance (**b**, Papanicolaou stain)

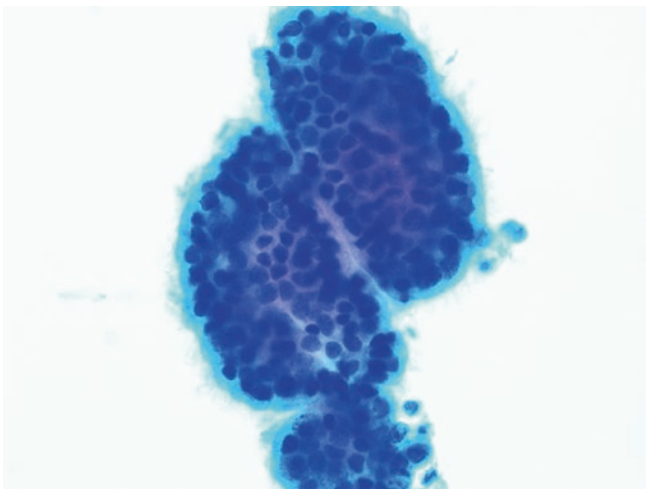

Fig. 9.3 Endosalpingiosis. Cohesive group of ciliated epithelial cells with moderate cytologic/nuclear atypia. Cilia and terminal bar are very prominent (Papanicolaou stain)

pericardial effusions [10]. Malignant peritoneal effusions are commonly associated with adenocarcinoma of ovary followed by gastrointestinal, pancreatic, or breast [11, 12].

Reactive mesothelial cells may show atypical features, including a notable amount of cell clusters, vacuolated cytoplasm, irregular nuclear membrane, and prominent nucleoli. Unlike adenocarcinoma, reactive mesothelial cells appear monotonous with fine chromatin and lack marked variation in cell/nuclear size and shape. When in doubt, workup with a panel of immunostains consisting of 2–3 mesothelial markers (i.e., WT-1, D2-40, calretinin, CK5/6, desmin) and 2–3 epithelial markers (i.e., MOC-31, EMA, B72.3, BerEp4) may help to distinguish reactive mesothelial cells from adenocarcinoma. Adenocarcinoma cells stain positive for epithelial cell markers while being negative for mesothelial markers. After adenocarcinoma is confirmed, immunostains with organ-associated markers may be added if the specimen is collected from the patient with adenocarcinoma of known origin. In case of unknown origin, more organ-associated markers should be attempted in hope of identifying the primary site of adenocarcinoma.

8. How to distinguish mesothelial cell hyperplasia from mesothelioma?

It is not uncommon that florid hyperplasia of mesothelial cells may present in effusions associated with benign conditions. Features favoring reactive hyperplasia are cellular specimens with one-cell population, monotonous cells arranged as single cells or loose clusters, with a subtle variation in size and shape of the cells/nuclei. Features favoring mesothelioma include hypercellularity, monotonous cells arranged as single cells, spheres with smooth borders, or tight/loose clusters with scalloped borders, numerous multinucleated giant mesothelial cells, a wide range of cell size and markedly enlarged cells/nuclei, as well as very prominent nucleoli. However, mesothelioma cells can also be deceptively bland and mimic benign mesothelial cells. Thus, distinguishing reactive mesothelial cells from mesothelioma based on morphologic features can be a challenge

and performing a panel of immunostains on the cell block preparation may be necessary in order to establish a definitive diagnosis. In this regard, the combination of positive expression for EMA with an enhanced membranous staining pattern and negative expression for desmin strongly favors mesothelioma; on the other hand, a combination of negative reaction with EMA (non-membranous staining) and positive reaction with desmin favors reactive mesothelial cells. Further, strong membranous positivity for GLUT-1 and/or strong nuclear staining for p53 favor mesothelioma. A Ki67 proliferative index showed no significant difference between reactive mesothelial hyperplasia and mesothelioma [13]. In addition, a greater expression for insulin-like growth factor-II mRNA-binding protein 3 (IMP3) has been seen in mesothelioma compared to reactive mesothelial cells [14].

9. What are the common immunostaining markers used for distinguishing epithelioid mesothelioma from carcinomas?

Very useful, positive mesothelioma markers include calretinin, CK5/6, WT-1, and D2-40. However, each of these markers has some limitations. In this regard, Calretinin and WT-1 has either limited or no value for distinguishing mesothelioma from serous or breast carcinomas; CK5/6 is not useful for distinguishing mesothelioma from serous, squamous, or breast carcinomas; D2-40 has little or no value for distinguishing mesothelioma from serous or squamous cell carcinomas. Very useful, positive carcinoma markers include MOC-31, BerEp4, BG-8, and CEA. Other markers are B72.3 and CD15. Among these markers, MOC-31, BerEp4, BG-8, CEA, and B72.3 have no values for discriminating mesothelioma from renal cell carcinoma. In addition, CEA and B72.3 are not useful while differentiating mesothelioma from serous and squamous cell carcinomas, respectively (Ordonez 2013) [15]. It is recommended to use a panel of immunostaining that consists of 2–3 mesothelial markers and 2–3 epithelial markers while making a distinction between mesothelioma and carcinomas.

10. What are the cytomorphological features favoring adenocarcinoma?

The presence of foreign (malignant) cell populations and background reactive mesothelial cells raises concerns for adenocarcinoma. The malignant cells may appear as single cells and/or present as various sizes of clusters which may show three-dimensional arrangement with smooth community outlines or papillary configuration with scalloped borders. Intercellular windows are lacking. The malignant epithelial cells contain granular to vacuolated cytoplasm, enlarged nuclei with high N/C ratio, moderate to severe nuclear atypia which is manifested by marked variation in nuclear size and shape, irregular nuclear membrane, coarse chromatin, and prominent nucleoli.

11. What are the cytomorphological features and immunocytochemical profile of malignant effusions associated with metastatic breast carcinoma?

Effusion with metastatic ductal carcinoma of the breast shows various amounts of malignant cells. Some of the cells appear as single cells while others may be arranged as three-dimensional, loose clusters with irregular contours, or tight spheres with smooth borders resembling cannonballs. Nuclear pleomorphism, nuclear enlargement, high N/C ratio, irregular nuclear membrane, coarse chromatin, and prominent nucleoli are evident. Metastatic lobular carcinoma usually presents as single, dispersed cells and a linear pattern may be seen. Vacuolated cytoplasm may be present and large vacuoles may push the nuclei to the side, resulting in an appearance of signet-ring cells. The cells have mild nuclear atypia, granular to coarse chromatin, and inconspicuous nucleoli. In a difficult case, immunostaining for gross cystic disease fluid protein-15 (GCDFP-15), mammaglobin, ER, PR, and GATA-3 may be useful to confirm the breast origin. Metastatic breast carcinoma may stain positive for these markers in various degree (Fig. 9.4a–e).

12. Does the presence of cannonballs in effusion fluids specifically indicate metastatic ductal adenocarcinoma of breast?

Metastatic breast carcinoma cells exfoliated into effusion fluids may show a cohesive, three-dimensional ball-like pattern with smooth community contours, resembling cannonballs. The malignant cells may appear relatively bland without marked nuclear atypia. Although the presence of cannonballs favors ductal adenocarcinoma of breast, it may be seen in metastatic carcinoma of primary sites other than breast, such as metastatic small cell carcinoma of lung [16] and well-differentiated neuroendocrine carcinoma of thymus [17].

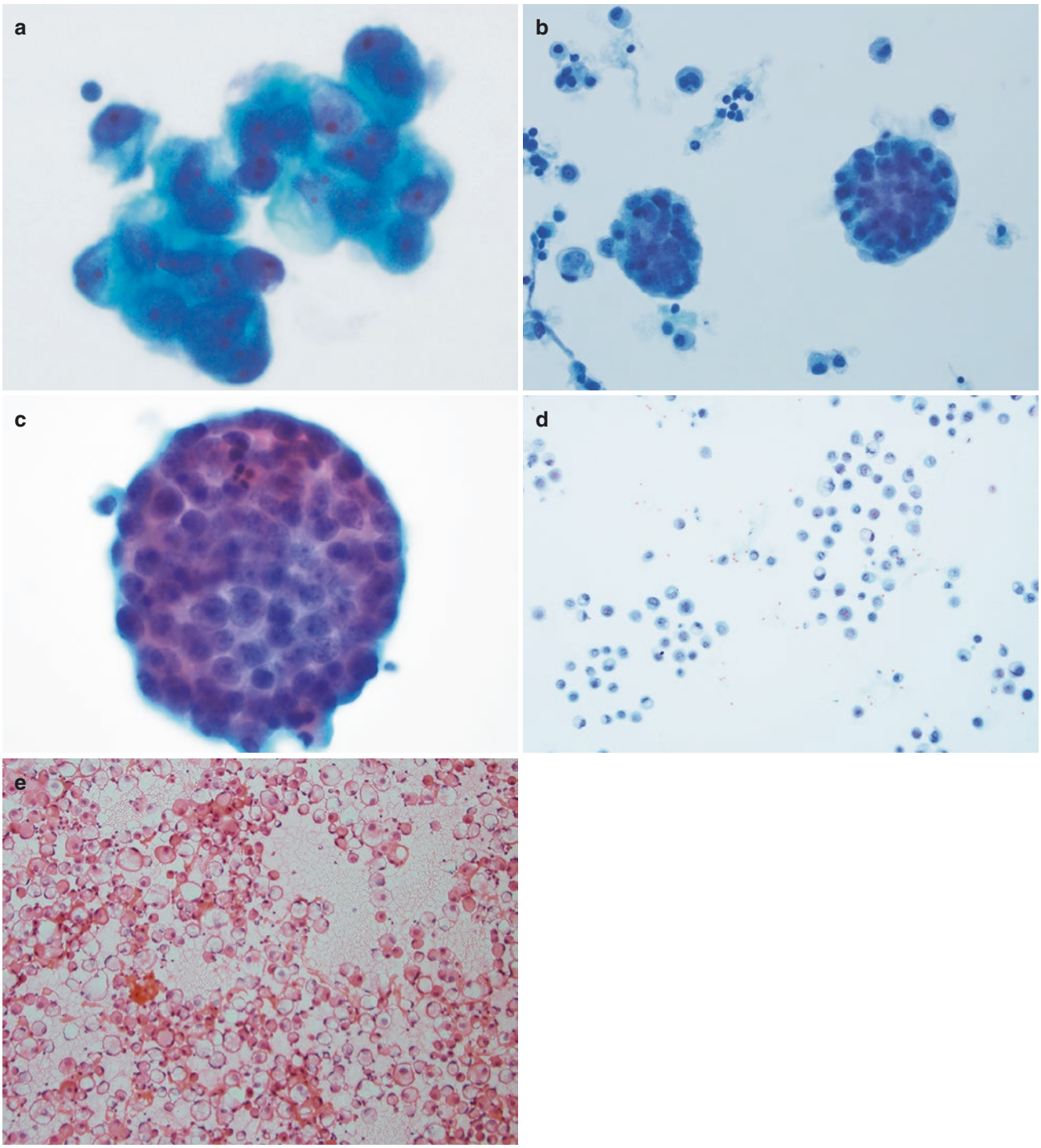

Fig. 9.4 Metastatic breast carcinoma. Ductal carcinoma cells are arranged as disorganized, three-dimensional clusters, cannonballs, and single cells (**a**, **b** and **c**, Papanicolaou stain). Lobular carcinoma shows single dispersed cells with eccentrically located nuclei. Some cells have an appearance of signet-ring cell (**d** and **e**, Papanicolaou and HE stain, respectively)

13. What are the cytomorphological features and immunocytochemical profile of malignant effusions associated with metastatic adenocarcinoma of lung?

Effusion with metastatic pulmonary adenocarcinoma contains malignant cells which appear as single cells or are arranged as three-dimensional clusters with nuclear overlapping/crowding. Delicate cytoplasm and cytoplasmic vacuoles, variation in nuclear size and shape, nuclear enlargement with high N/C ratio, fine to coarse chromatin, and prominent nucleoli are present. TTF-1 and napsin A are useful markers which show positive expression in metastatic adenocarcinoma of lung (Fig. 9.5a, b).

14. How to distinguish metastatic non-keratinizing squamous cell carcinoma from mesothelioma and adenocarcinoma?

Non-keratinizing squamous cell carcinoma shows nuclear pleomorphism, nuclear enlargement, high N/C ratio, coarse chromatin, and prominent nucleoli. Compared to adenocarcinoma, non-keratinizing squamous carcinoma cells have well-defined cell borders and dense cytoplasm with characteristic endo-ectoplasmic demarcation. The cells are arranged as single cells and/or flat groups. Three-dimensional clusters may be occasionally seen. When facing the challenge of distinguishing the cells of squamous cell carcinoma from benign or malignant mesothelial cells, immunostains for CK5/6, p40, and p63 may be performed on the cell block material. All three markers are expressed by squamous cell carcinoma, whereas mesothelial cells stain positive for CK5/6 while being negative for p40 and p63. To make a distinction from adenocarcinoma, applying a panel of immunostaining consisting of p63 and/or p40, TTF-1, and napsin A is helpful. Squamous cell carcinoma is diffusely and strongly positive for p40 and/or p63 while being negative for TTF-1 and napsin A (Fig. 9.6a–c).

15. What are the features of malignant effusions associated with metastatic small cell carcinoma of lung?

Effusion fluids involved by metastatic small cell carcinoma contain single cells and/or groups of cells with nuclear molding. The tumor cells show a scant amount of cytoplasm, high N/C ratio, salt and pepper chromatin, and inconspicuous nucleoli. However, features resembling non-small cell carcinoma may be present, including large cell clusters, coarse chromatin, and conspicuous nucleoli. To differentiate it from poorly differentiated non-small cell carcinoma and non-Hodgkin lymphoma, immunostains for pancytokeratin, CD45, and neuroendocrine markers including synaptophysin, chromogranin A, and CD56 should be performed. Small cell carcinoma shows positive dot-like cytoplasmic staining pattern for pancytokeratin and some if not all three endocrine markers while being negative for CD45 (Fig. 9.7a, b).

16. What are the cytomorphological features of malignant effusions associated with metastatic pancreatic adenocarcinoma?

Metastatic adenocarcinoma of pancreas often shows features that are seen in typical adenocarcinoma. The cells appear as

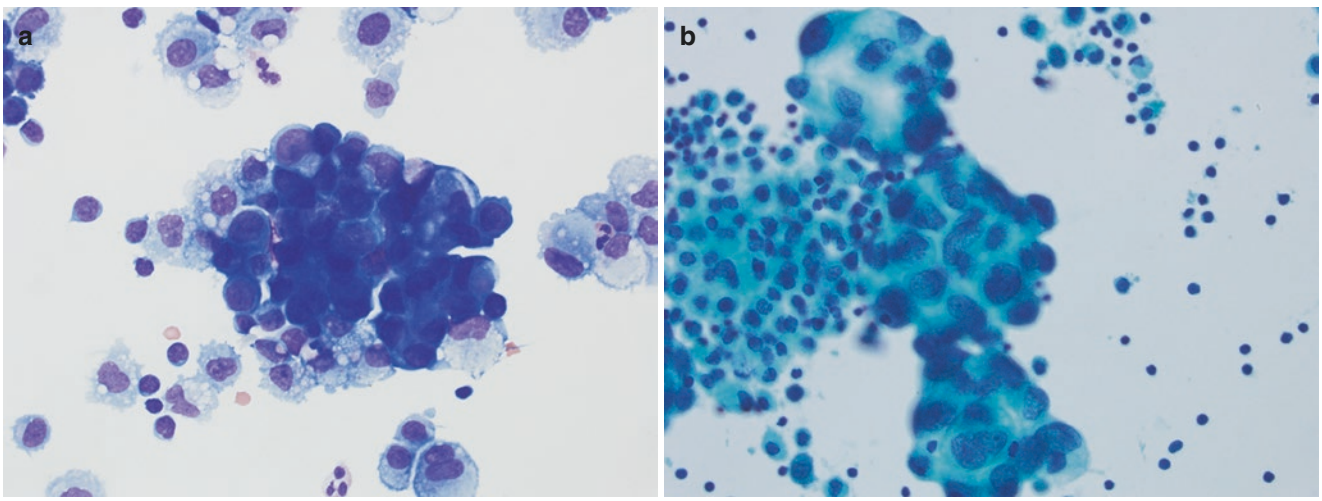

Fig. 9.5 Metastatic pulmonary adenocarcinoma. Carcinoma cells are arranged as single cells or three-dimensional clusters with nuclear overlapping. Delicate and vacuolated cytoplasm, nuclear pleomorphism, fine chromatin, and conspicuous nucleoli are present (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively)

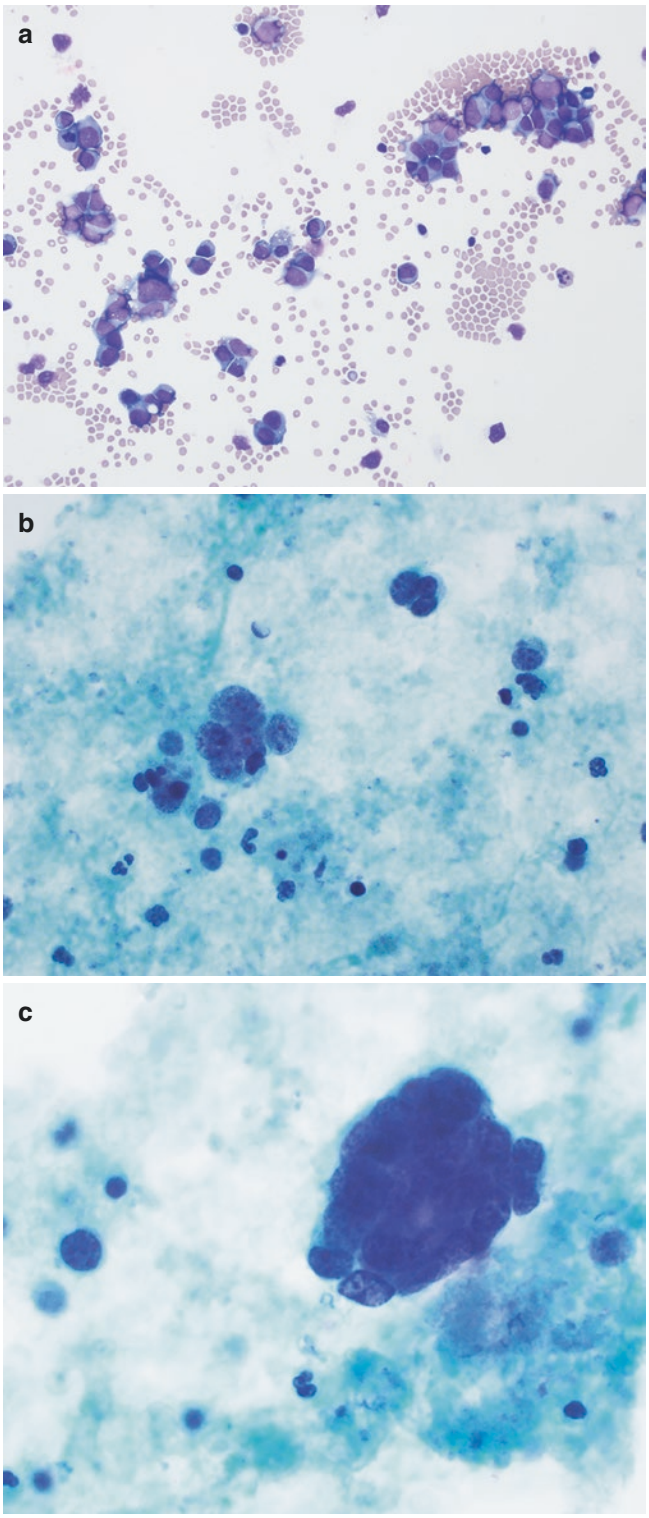

Fig. 9.6 Non-keratinizing squamous cell carcinoma. The cells are arranged as single cells and/or flat groups with well-defined cytoplasmic borders and dense cytoplasm (**a**, Diff-Quik stain). Occasionally, three-dimensional clusters may be seen (**b** and **c**, Papanicolaou stain)

single cells or three-dimensional disorganized clusters. Granular to vacuolated cytoplasm, high N/C ratio, marked variation in nuclear size and shape, irregular nuclear membrane, coarse chromatin and prominent nucleoli are present. Positive immunohistochemical reaction for CK7, CK19, Mesothelin, napsin, placental S100 (S100P), and insulin-like growth factor-II mRNA-binding protein 3 (IMP3) in pancreatic ductal carcinoma has been reported [18]. However, clinical and imaging correlation is important to confirm pancreatic origin (Fig. 9.8a).

17. What are the cytomorphological features of malignant effusions associated with high-grade papillary serous carcinoma of the female genital tract?

Regardless of its origin (i.e., ovary, uterus, or fallopian tube), the carcinoma shows similar cytomorphological features. The cells are often arranged as papillary clusters with crowded nuclei and slit-like spaces. Some papillae may contain psammoma bodies. Vacuolated cytoplasm, high N/C ratio, marked variation in nuclear size and shape, irregular nuclear membranes, coarse chromatin, and prominent nucleoli are evident. Immunostains may be helpful for determining primary site. In this regard, positive staining for WT-1 is seen in a significant proportion of ovarian serous carcinomas compared to the serous carcinomas originating from the uterus and fallopian tube. Further, HER2/neu overexpression is seen exclusively in serous carcinomas of endometrial origin [19] (Fig. 9.9a–c).

18. What are the cytomorphological features of pseudomyxoma peritonei?

Typically, the effusion will contain predominantly thick mucin. The epithelial cells show various degrees of atypia depending on the differentiation of the original mucinous neoplasm. Epithelial cells with bland appearance or mild atypia are noted in low-grade mucinous neoplasm whereas markedly atypical epithelial cells are present in mucinous adenocarcinoma (Fig. 9.10).

19. What are the organ-associated immunostaining markers that are commonly used during the workup of primary site(s) of metastatic carcinomas?

TTF-1 and napsin A are useful for identification of adenocarcinoma of lung. Some clear cell and papillary renal cell carcinomas also express napsin A. Both PAX-8 and PAX 2 are expressed in renal cell carcinoma and serous carcinomas. PAX-8 is also positive in carcinomas that develop from thyroid follicular cells. CDX-2 is positive in adenocarcinoma of gastrointestinal or pancreatobiliary origin [15]. Over 50% of breast carcinomas express GCDFP-15 and/or mammaglobin. Further, GATA3 has been shown to be a sensitive marker for

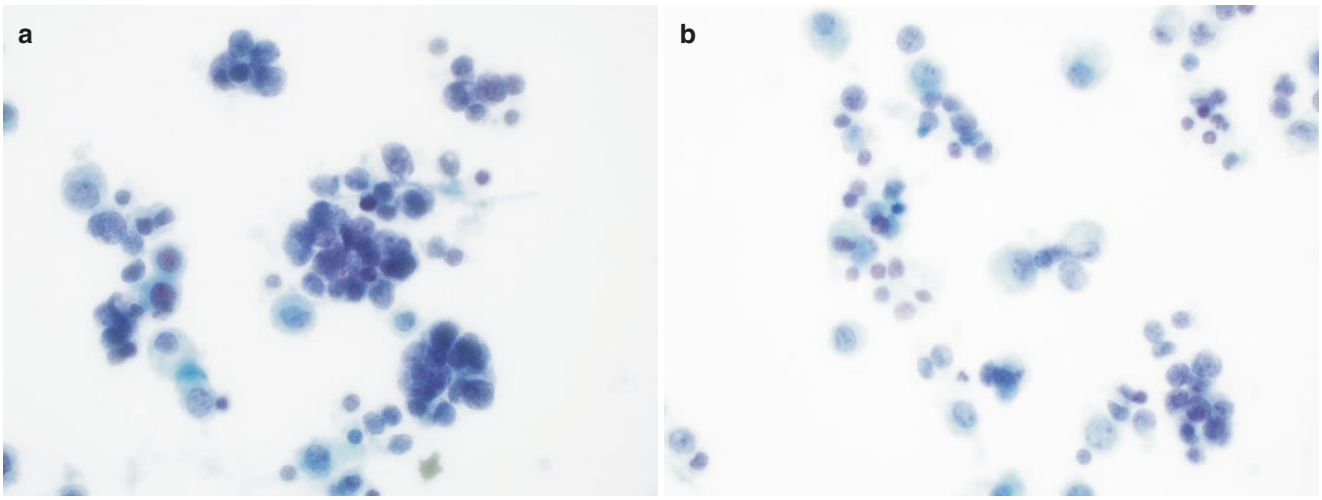

Fig. 9.7 Small cell carcinoma. Single cells and/or groups of cells with nuclear molding are present. The cells show scant amount of cytoplasm, high N/C ratio, salt and pepper chromatin, and inconspicuous nucleoli. The size of the tumor cell is usually one and a half to four times that of a lymphocyte (**a** and **b**, Papanicolaou stain)

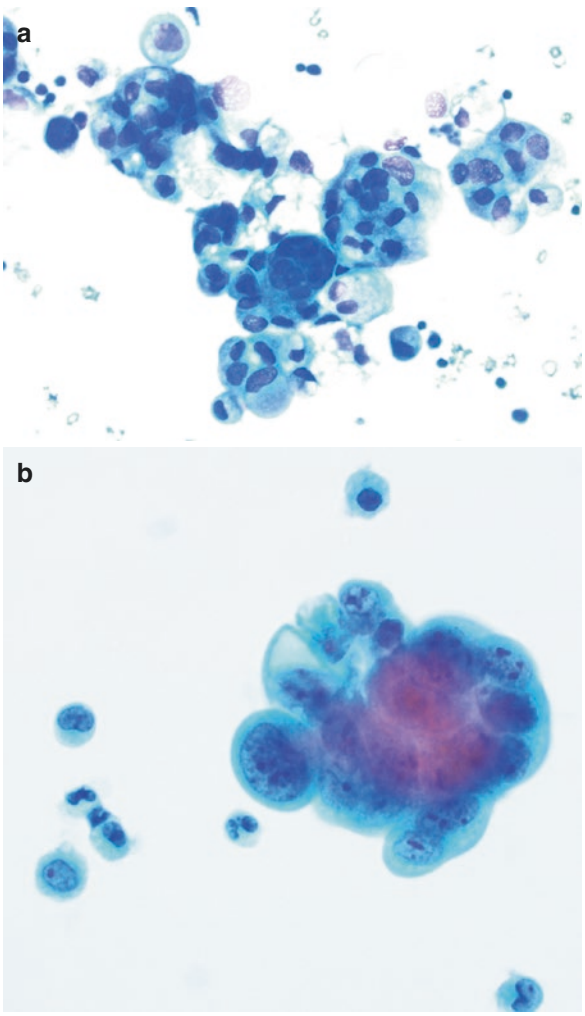

Fig. 9.8 Metastatic adenocarcinoma of pancreas. The cells are arranged as three-dimensional disorganized clusters. Granular to vacuolated cytoplasm, high N/C ratio, marked variation in nuclear size and shape, irregular nuclear membrane, coarse chromatin, and prominent nucleoli are present (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively)

metastatic breast carcinomas in effusions. However, it is not specific for breast origin and positive staining for GATA3 may be seen in carcinomas from other primary sites [20, 21]. It is noteworthy to mention that GATA3 may be positive in mesothelioma and over half (58%) of malignant mesotheliomas showed nuclear GATA3-positivity on histology specimens [22].

20. What are the features of metastatic papillary thyroid carcinoma?

There are a couple of case studies documenting cytomorphological features of effusion involved by metastatic thyroid carcinoma (Olson et al. 2013; Lew et al. 2015) [23, 24].

Accordingly, psammoma bodies were seen in both studies; one study observed classic features of conventional PTC, such as abundant papillae, nuclear enlargement and overlapping, intranuclear grooves, and pseudoinclusions. On the contrary, the malignant cells did not reveal the aforementioned classic features in the other study; instead, the metastatic papillary thyroid carcinoma cells showed a moderate amount of delicate and/or vacuolated cytoplasm, ovoid nuclei, and irregular nuclear contours. The lack of hallmark features of papillary thyroid carcinoma raises a great diagnostic challenge. In addition, these malignant cells may show positive staining for epithelial markers (i.e., MOC31, CEA, BerEp4), TTF-1, and napsin A. Without additional markers including thyroglobulin and/or PAX-8, misinterpretation of the findings as metastatic adenocarcinoma of lung origin may occur. Taken together, including PAX-8, thyroglobulin, and TTF-1 when performing immunostaining workup in an appropriate clinical context is crucial for establishing an accurate diagnosis (Fig. 9.11a, b).

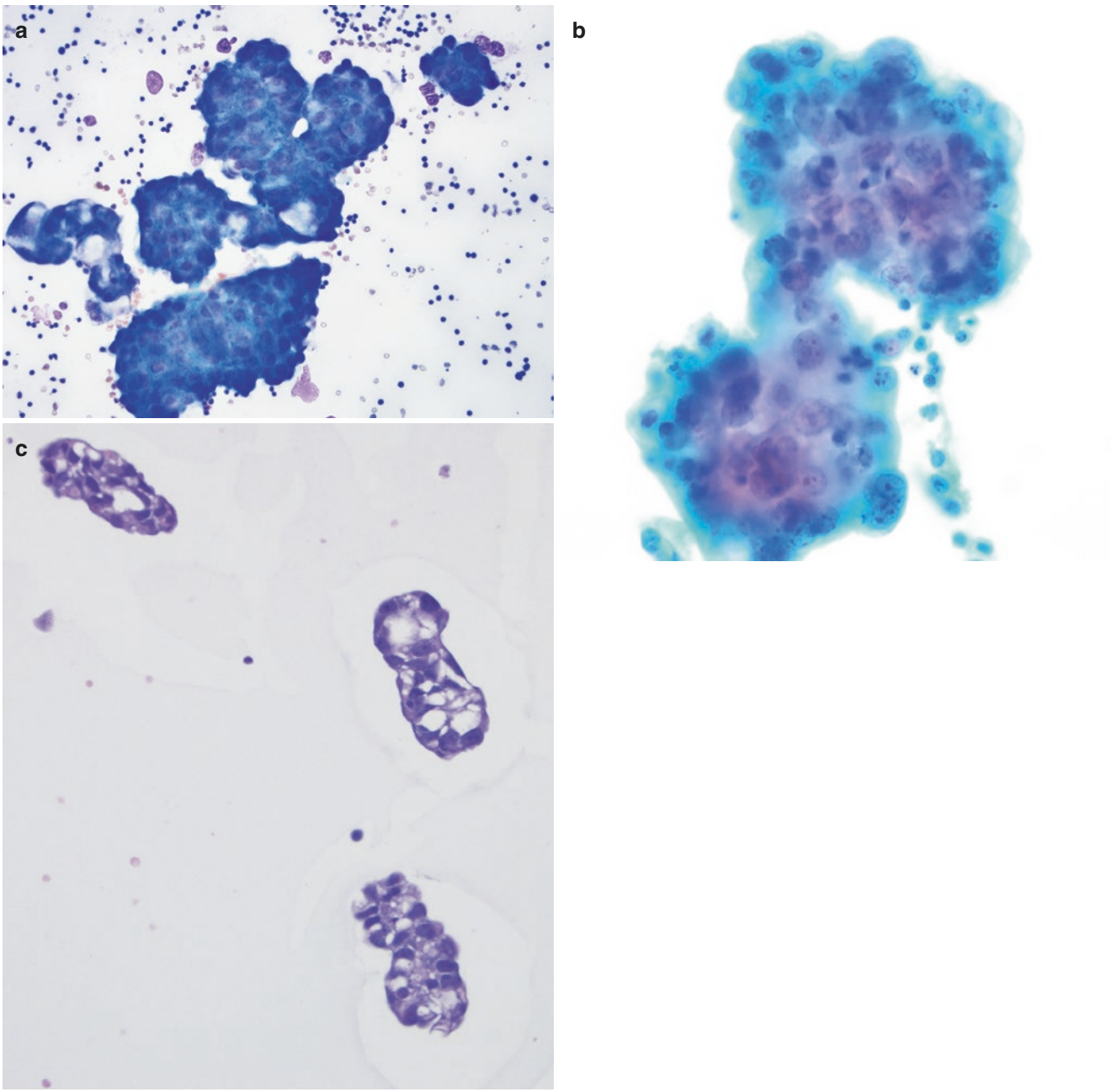

Fig. 9.9 Metastatic papillary serous carcinoma of endometrium. The carcinoma cells are arranged as complex, papillary clusters with crowded nuclei and slit-like spaces. Vacuolated cytoplasm, high N/C ratio, marked variation in nuclear size and shape, irregular nuclear membranes, coarse chromatin, and prominent nucleoli are present. (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively; **c**, H&E stain cell block)

21. What are the cytomorphological features of metastatic urothelial cell carcinoma?

Metastatic urothelial cell carcinoma in effusions is commonly poorly differentiated. The cells are arranged as single cells or clusters. Well-defined cell borders, dense cytoplasm, nuclear pleomorphism, nuclear enlargement with high N/C ratio, irregular nuclear membranes, coarse chromatin, and prominent nucleoli are easily appreciated. Occasionally, vacuolated cytoplasm and cell-in-cell pattern may present. Metastatic urothelial cells may show positive for staining for GATA-3 and p63 (Fig. 9.12a–c).

22. What are the cytomorphological features of metastatic melanoma? What ancillary study can be used to make a definitive diagnosis?

Melanoma is notorious for its great variety of morphological features. The specimens have various amounts of malignant cells which are arranged as single cells, acini, and loose or three-dimensional clusters. The malignant cells appear epithelioid and pleomorphic with well-defined cytoplasmic borders. Larger neoplastic cells show various shaped nuclei, bi- or multinucleations, and cell-in-cell engulfment. The smaller neoplastic cells have a plasmacytoid appearance with round,

eccentrically located nuclei, coarse chromatin, and prominent nucleoli. Dark, coarse pigments may or may not be seen. Occasionally, intracytoplasmic vacuoles may present and some cells may show a signet-ring appearance, mimicking adenocarcinoma. A panel of immunostains consisting of melanoma markers (S-100, Mart-1/Melan-A, HMB-45, or SOX10) should be performed on the cell block material in order to establish a definitive diagnosis [25] (Fig. 9.13a, b).

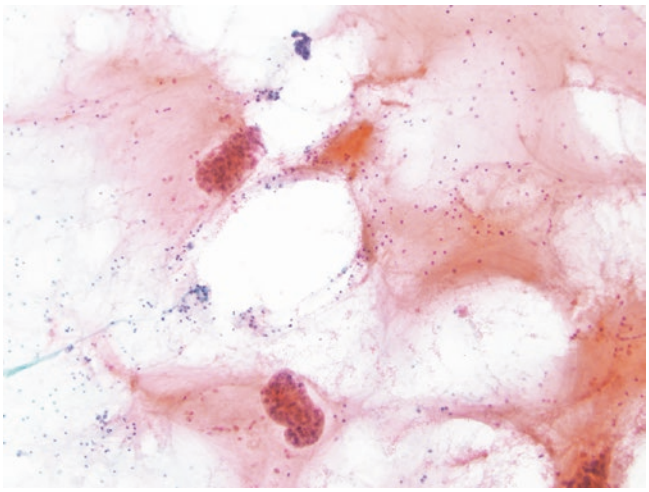

Fig. 9.10 Pseudomyxoma peritonei. Abundant thick mucin in the background. There are rare clusters of epithelial cells with mild atypia (Papanicolaou stain)

23. What role does flow cytometry or immunocytochemistry play in the diagnosis of non-Hodgkin's lymphomas involving effusion? How often is peritoneal involvement of lymphoma? pericardial?

Pleural effusion occurs in 20–30% of non-Hodgkin's lymphomas with a wide variation in rate of positive cytologic findings (22.2–94.1%) [26]. The involvement of peritoneal and pericardial cavities is less common. Flow cytometry analysis or immunocytochemistry as an adjunct to cytologic evaluation of effusions plays an important role in the diagnosis of non-Hodgkin's lymphomas, especially in situations of low to intermediate grade lymphoma involving the serous cavity and/or presence of a small proportion of malignant lymphoid cells. In these clinical scenarios, a definitive diagnosis of lymphomas is difficult to make based on cytomorphological finding alone as the atypical/suspicious cells may mimic reactive cells [27].

24. What are the cytomorphological features suggestive of multiple myeloma involving effusion?

The effusion contains single, dispersed cells, nuclear pleomorphism, bi- or multinucleation, eccentrically located nuclei, prominent nucleoli, and characteristic clock-face chromatin (Fig. 9.14).

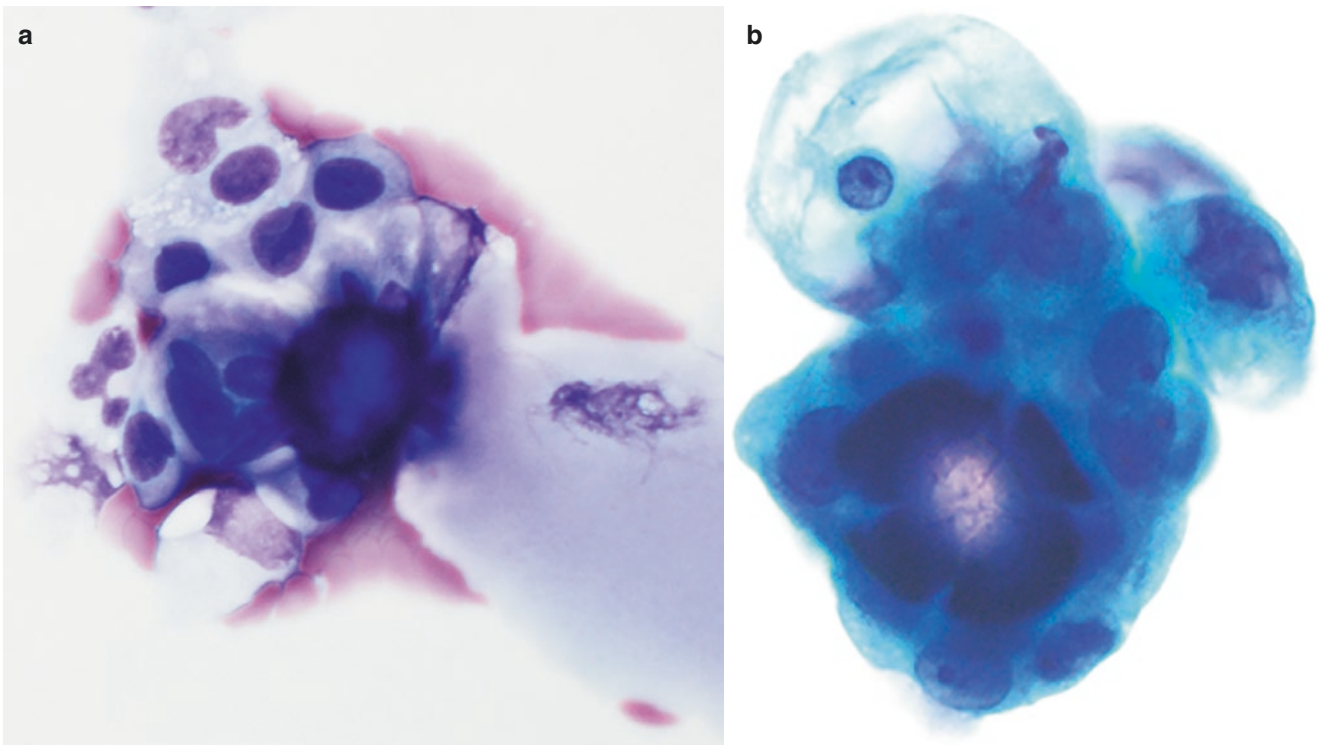

Fig. 9.11 Metastatic papillary thyroid carcinoma. Clusters of malignant cells with Psammoma bodies. Nuclear enlargement, irregular nuclear membrane, and intranuclear grooves are present. The presence of vacuolated cytoplasm mimics adenocarcinoma (**a** and **b**, Diff-Quik and Papanicolaou stain, respectively)

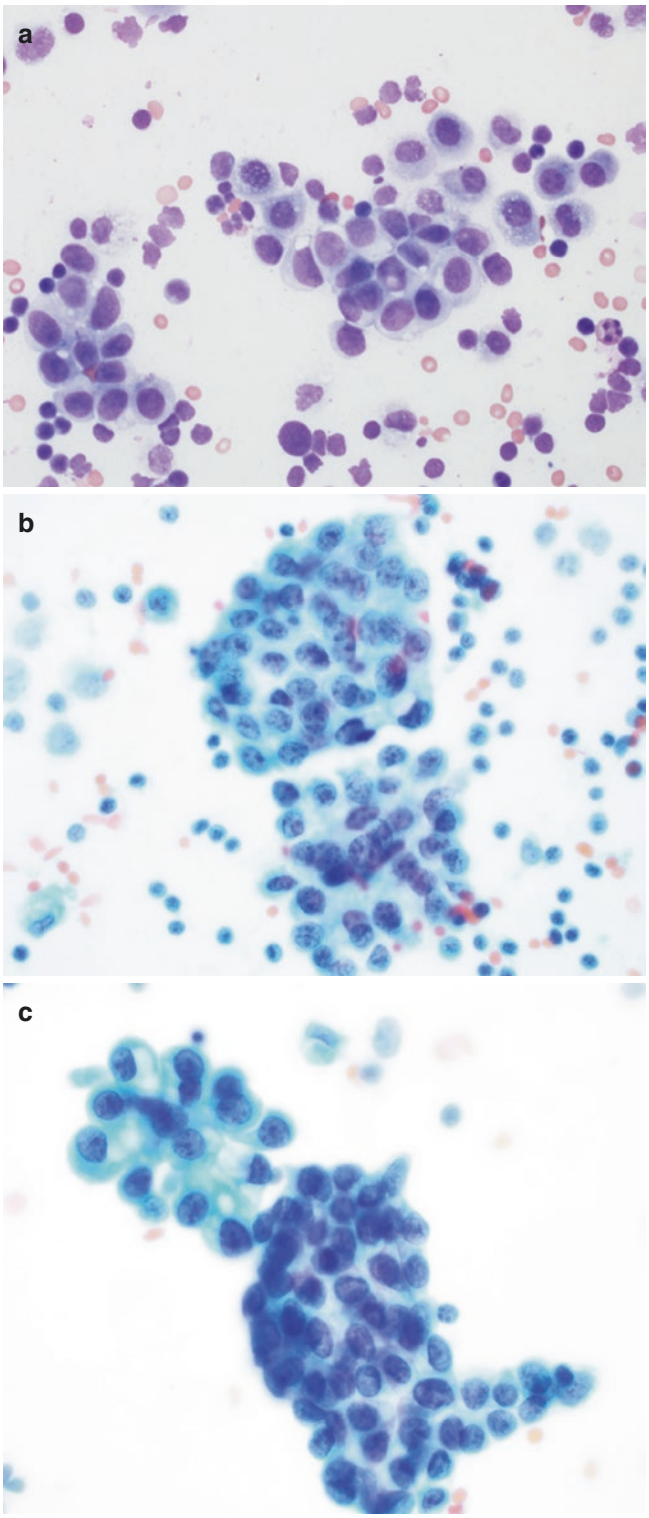

Fig. 9.12 Metastatic urothelial cell carcinoma. The cells are arranged as single cells or clusters. Well-defined cell borders, dense cytoplasm, nuclear pleomorphism, nuclear enlargement with high N/C ratio, irregular nuclear membrane, coarse chromatin, and prominent nucleoli are easily appreciated. Vacuolated cytoplasm may present (**a**, Diff-Quik stain; **b** and **c**, Papanicolaou stain)

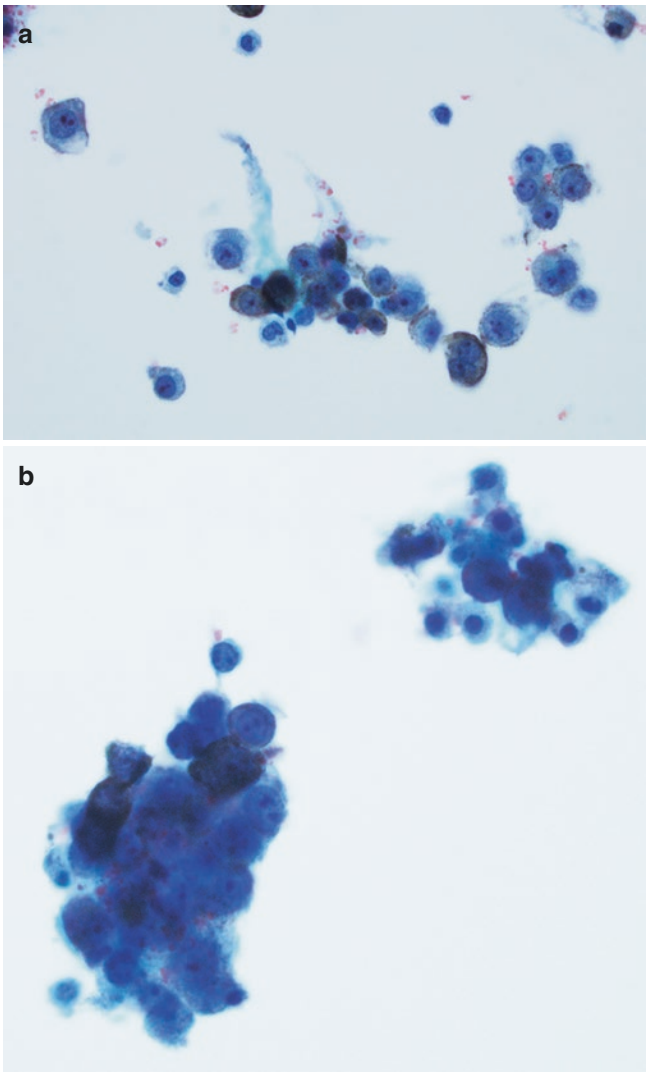

Fig. 9.13 Metastatic melanoma. Single and clusters of malignant cells have enlarged nuclei, coarse chromatin, and prominent nucleoli. Some cells contain dark, coarse pigments (**a** and **b**, Papanicolaou stain)

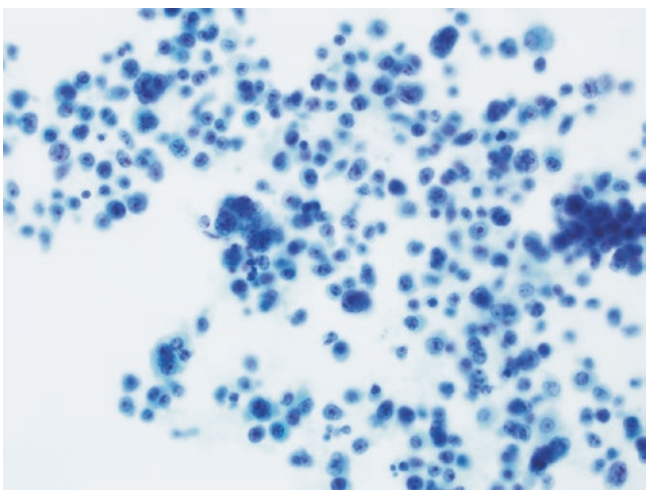

Fig. 9.14 Multiple myeloma. Single, dispersed cells, nuclear pleomorphism, bi- or multinucleation, eccentrically located nuclei, prominent nucleoli, and characteristic clock-face chromatin (Papanicolaou stain)

Case Presentation

Case 1

Clinical history:

- A 36-year-old female presents with left pleural effusion. She has a history of esophageal adenocarcinoma. Thoracentesis was performed and a total of 1000 mL of fluid is submitted for cytologic evaluation.

Cytomorphological findings:

- Both the Diff-Quik-stained conventional smear and Papanicolaou-stained ThinPrep® smear reveal scattered, single malignant-looking cells with enlarged nuclei, high N/C ratio, nuclear pleomorphism, irregular nuclear contour, coarse chromatin, and prominent nucleoli.

Differential diagnosis:

- Metastatic carcinoma
- Reactive mesothelial cells
- Mesothelioma

Immunostains performed on the cell block material:

- Positive for CK7, BerEp4, EMA, MOC-31, and CDX-2

Negative for TTF-1, GATA-3, and ER (Fig. 9.15a–e)

Final diagnosis:

- Metastatic adenocarcinoma, consistent with esophageal origin

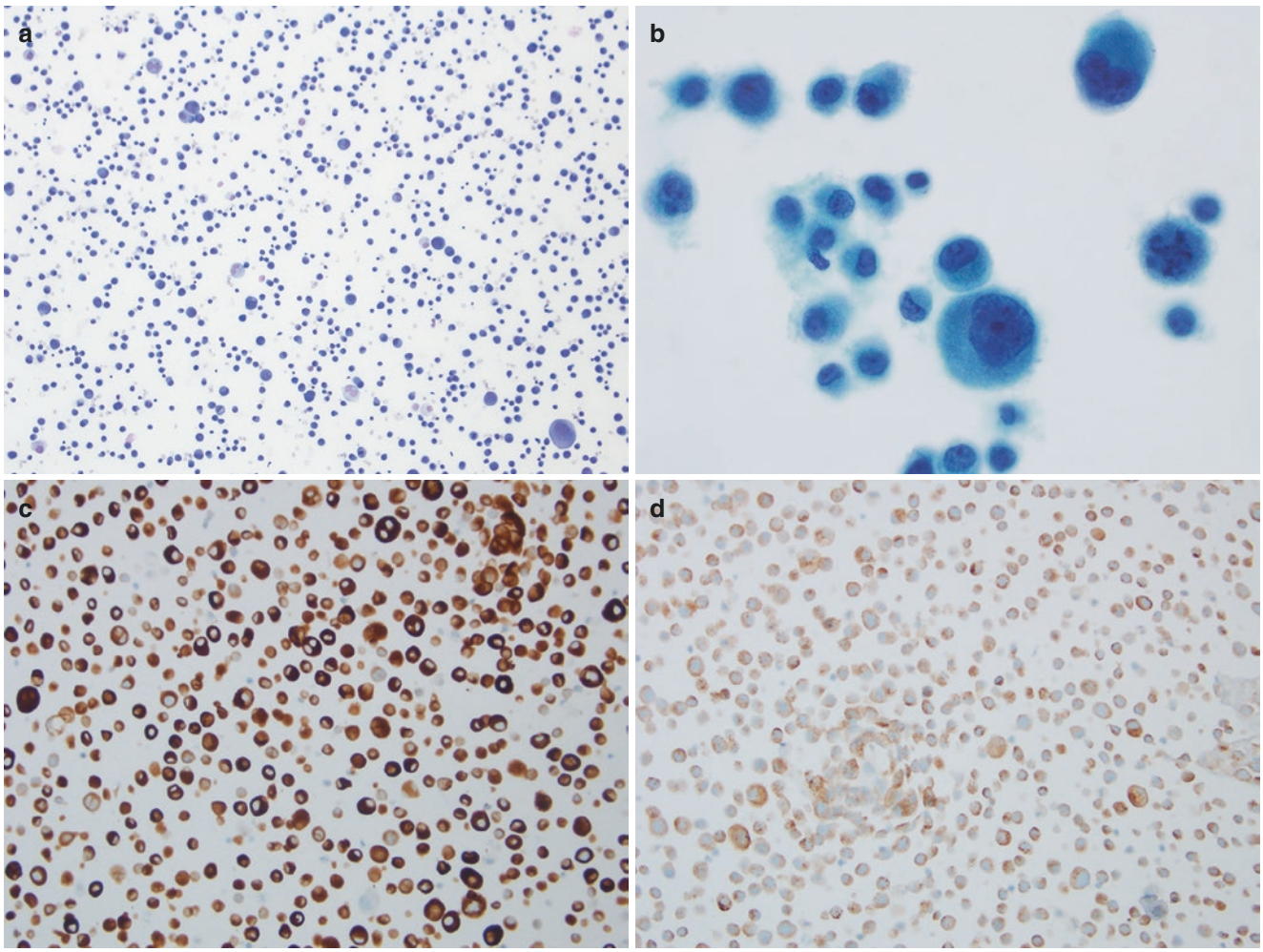

Fig. 9.15 Metastatic adenocarcinoma of esophagus. Single, dispersed malignant cells are present in both Diff-Quik- (**a**) and Papanicolaou-stained smears (**b**). The malignant cells contained in the cell block are positive for EMA (**c**), MOC-31 (**d**), and CDX-2 (**e**)

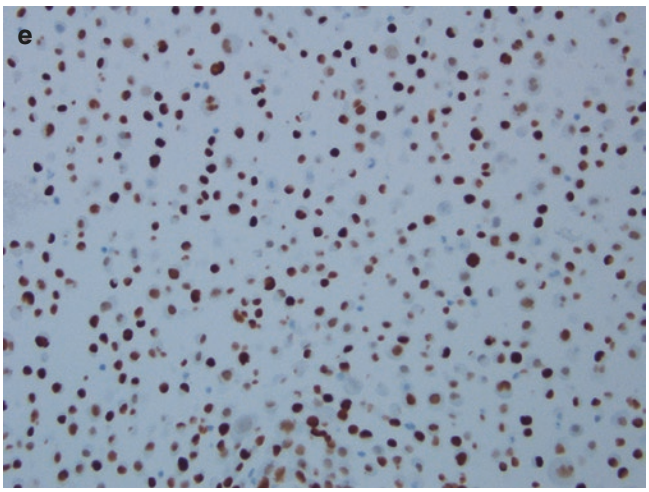

Fig. 9.15 (continued)

Case 2

Clinical history:

- A 76-year-old female presents with dyspnea. Imaging studies show right pleural effusion and thickening endometrium. Her past medical history is non-significant. Thoracentesis was performed and a total of 400 mL of fluid is submitted for cytologic evaluation.

Cytomorphological findings:

- The Papanicolaou-stained ThinPrep® smear reveals numerous single dispersed malignant-looking cells with enlarged nuclei, high N/C ratio, nuclear pleomorphism, irregular nuclear contour, coarse chromatin, and prominent nucleoli. Extremely large nuclei and multinucleation are seen.

Differential diagnosis:

- Metastatic carcinoma
- Mesothelioma
- Malignant neoplasm other than carcinoma

Immunostains performed on the cell block material:

- Positive for EMA, MOC-31, BerEp4, PAX-8, CK7, and CK20

Negative for WT-1, TTF-1, GATA-3, ER, and CDX-2 (Fig. 9.16a–d)

Final diagnosis:

- Metastatic adenocarcinoma, suggestive of Müllerian origin

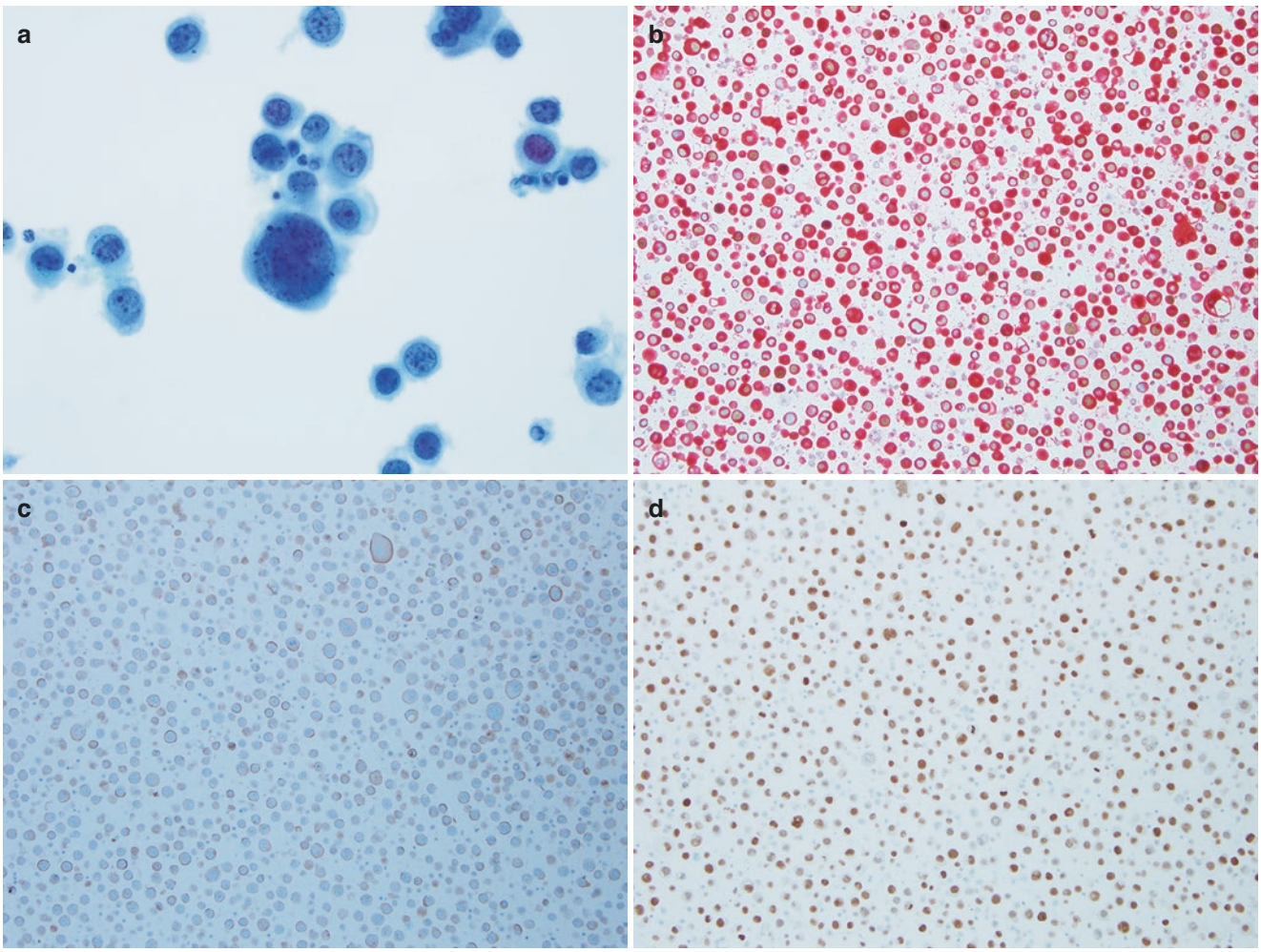

Fig. 9.16 Metastatic adenocarcinoma of Müllerian origin. The Papanicolaou-stained ThinPrep® smear reveals single dispersed malignant cells with enlarged nuclei, high N/C ratio, nuclear pleomorphism, irregular nuclear contours, coarse chromatin, and prominent nucleoli (**a**). The malignant cells are positive for EMA while being negative for WT-1 (**b**). The malignant cells are also positive for MOC-31 (**c**) and PAX-8 (**d**)

Case 3

Clinical history:

- An 87-year-old female presents with pleural effusion. Cytology smears and cell block prepared from the pleural fluid at the referring institution are received for consultation.

Cytomorphological findings:

- The provided smears show numerous malignant cells which are arranged as single cells or tight clusters with scalloped borders. The malignant cells show marked pleomorphism in nuclear size and shape, nuclear enlargement along with high N/C ratio, coarse chromatin, and prominent nucleoli. Cell-in-cell pattern and gigantic, multinucleated cells are easily seen.

Differential diagnosis:

- Metastatic carcinoma
- Mesothelioma
- Malignant neoplasm other than carcinoma

Immunostains performed on the cell block material:

- Positive for D2–40, WT-1, and EMA (enhanced membranous staining)
- Negative for desmin, B72.3, CEA, CD15, and PAX-8 (Fig. 9.17a–d).

Final diagnosis:

- Positive for malignant cells, favor mesothelioma

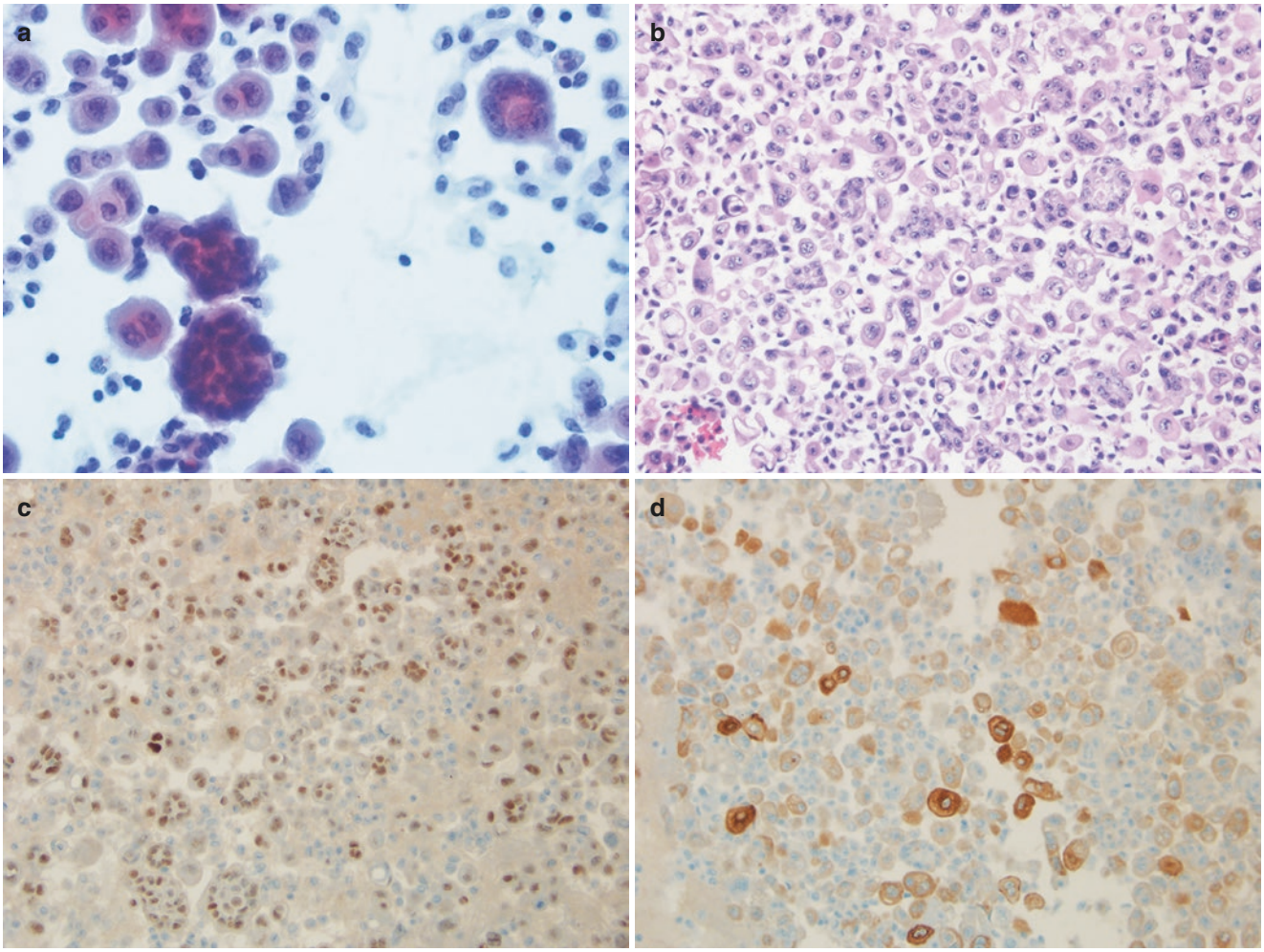

Fig. 9.17 Mesothelioma. Numerous malignant cells present in Papanicolaou-stained smear (**a**) and cell block (**b**). The cells are arranged as single cells or tight clusters with scalloped borders. Nuclear pleomorphism, gigantic multinucleated cells, cell-in-cell pattern, nuclear enlargement along with high N/C ratio, coarse chromatin, and prominent nucleoli are easily seen. The malignant cells stain positive for WT-1 (**c**, nuclear staining) and EMA (**d**, enhanced membranous staining)

Acknowledgment The author thanks Brian Smola, senior cytotechnologist at University of Michigan for proofreading and editing the chapter.

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Cerebrospinal Fluid

10

Matthew Torre

Contents

Frequently Asked Questions

1. What are the common ways to prepare cerebrospinal fluid specimens?

Most cerebrospinal fluid (CSF) specimens are collected by lumbar puncture (LP). In this procedure, a needle is inserted into the lumbar subarachnoid space through the L3–L4 or L4–L5 intervertebral space. In rare instances, clinicians may opt to perform a suboccipital puncture into the cisterna magna, though this procedure is associated with a higher risk of vascular injury [1]. CSF can also be collected from subcutaneously implanted Ommaya reservoirs, which are catheters that communicate with the lateral ventricles and are used to deliver intrathecal chemotherapeutic agents. Ventricular CSF cytology may be more sensitive than lumbar CSF at detecting intracranial tumors [2]. Regardless of the collection method, at least 1 mL of CSF should be collected for cytologic evaluation, but 3 mL or more (up to 10 mL) is desirable.

The three most common ways to prepare CSF specimens for cytologic evaluation are cytocentrifugation, membrane filtration, and liquid-based preparations (e.g., ThinPrep). During cytocentrifugation, cells within the CSF are centrifuged onto slides and then either air-dried or alcohol-fixed and stained. During membrane filtration, CSF is transferred to a funnel with a filter at the bottom. Suction is applied to the funnel, collecting cells at the filter. The cells are fixed and mounted, along with the filter, onto slides and stained. Further steps may be necessary to reduce background staining of the filter. To prepare ThinPrep slides, CSF is added to a vial containing a liquid preservation medium. The vial is placed in a processor with a disposable filter. After the sample is homogenized, a vacuum concentrates the cells on the filter. The filter is inverted onto a slide, and the cells transferred to the slide are then stained.

There are pros and cons of each of these preparation methods. Membrane filtration allows for high cell concentration and good preservation of cytologic detail; however, this method is time-consuming, requires greater technical expertise, is prone to shrinkage or cell contraction artifact, and shows background staining of the filter [3]. Cytocentrifugation is rapid, technically simple, and inexpensive, but produces less cellular slides and can cause significant cytologic distortion [3, 4]. Liquid-based preparations produce cellular slides, preserve cell morphology and cytologic detail, have cleaner backgrounds by reducing obscuring blood elements, and can be used for immunohistochemistry (IHC) and molecular tests; the drawbacks include loss of certain architectural features, compression artifact along the circumference of the cellular portion of the slide, inability to run flow cytometry on leftover fluid, and higher costs [5]. Compared to cytocentrifugation, liquid-based preparations may be more sensitive at detecting leptomeningeal metastases from solid tumors [6] and are less likely to be nondiagnostic [7].

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_10

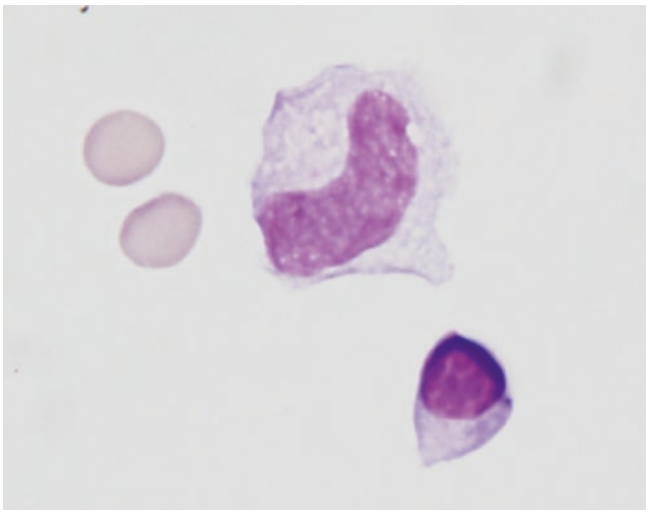

Fig. 10.1 Normal cellular elements of cerebrospinal fluid include lymphocytes (bottom right), with round nuclei, dense chromatin, and scant to small amounts of cytoplasm, and monocytes (top center), with kidney bean-shaped nuclei and moderate amounts of cytoplasm (Giemsa-Wright stain)

2. What are normal elements in CSF specimens?

Normal CSF is hypocellular and primarily composed of lymphocytes and monocytes (Fig. 10.1). In adults, the expected cellularity is fewer than 5 cells/mm^3, with lymphocytes generally comprising 60–70% of the total cellularity, though estimates among studies vary [8]. The CSF of neonates is twice as cellular and is monocyte predominant [9, 10].

Lymphocytes are recognized by their small size, round nuclei with dense "smudgy" chromatin, inconspicuous nucleoli, and scant basophilic cytoplasm. In contrast, monocytes are larger and have characteristic lobulated or kidney bean-shaped nuclei with pale chromatin and moderate amounts of cytoplasm.

Less common CSF elements include choroid plexus/ependymal cells, brain tissue, and germinal matrix cells.

The choroid plexus consists of modified ependymal cells overlying a basal lamina composed of capillaries and connective tissue with macrophages and dendritic cells. The choroid plexus and ependymal cells line the ventricles of the brain and the central canal of the spinal cord. They are involved in CSF production, maintenance of the CSF milieu and the CSF-blood barrier, and play a role in normal brain development. Choroid plexus/ependymal cells are observed in less than 0.4% of CSF cytology specimens and are most often seen in CSF collected from the cisterna or ventricles, pediatric patients, or patients with hydrocephalus [11, 12].

It is diagnostically challenging to distinguish choroid plexus from ependymal cells. Cytologically, they appear as round-to-cuboidal cells occurring singly or in clusters, with round nuclei, vesicular chromatin, and small-to-moderate amounts of cytoplasm (Fig. 10.2a).

Brain tissue fragments can contain neurons, glial cells, and blood vessels. When brain tissue is seen in CSF specimens, it is usually in the context of ventricular/reservoir taps, brain trauma, or recent brain surgery.

Brain tissue can be identified by its characteristic fibrillary neuropil (Fig. 10.2b). Glial cells have round nuclei, with astrocytes having larger nuclei with open chromatin and oligodendrocytes having smaller nuclei with denser chromatin. Neurons vary in size and have round nuclei, fine-to-slightly granular chromatin, prominent nucleoli, and variable amounts of basophilic, angulated cytoplasm (Fig. 10.2c).

The germinal matrix, present in the subependymal cell layer of the lateral ventricles in the developing brain, is composed of neural and glial precursors. It is most prominent at 20–26 weeks gestation, but largely involutes by around 35 weeks gestation [13]. Highly vascular, the germinal matrix is prone to rupture, resulting in intraventricular hemorrhage. Germinal matrix cells can be observed in the CSF of infants, most frequently in those with a history of prematurity or hydrocephalus [14, 15].

Germinal matrix cells are immature-appearing cells, commonly occurring in clusters with high nuclear:cytoplasmic (N:C) ratio, nuclear molding, fine chromatin, inconspicuous nucleoli, and scant basophilic cytoplasm with admixed hemosiderin-laden macrophages (Fig. 10.2d). These benign immature cells can be mistaken for malignancy like medulloblastoma.

Contaminants that can be seen in CSF specimens include bone marrow elements, chondrocytes, squamous cells, skeletal muscle, peripheral blood, and starch granules. These contaminants are artifacts of CSF procurement. The LP needle can pick up squamous cells and/or fragments of skeletal muscle as it passes through skin and soft tissue. Incorrect placement of the LP needle can result in inadvertent sampling of chondrocytes (Fig. 10.3a) from the intervertebral disc or of bone marrow elements (Fig. 10.3b) from the vertebral body. Peripheral blood can be introduced in the CSF from a traumatic tap or from a central nervous system (CNS) hemorrhage; the presence of neoplastic cells in these samples could represent involvement of the CSF, peripheral blood, or both. Powdered surgical gloves, now banned by the FDA [16], can contaminate CSF specimens with starch granules.

3. What are abnormal inflammatory cells in CSF specimens?

Abnormal inflammatory cells in CSF specimens include macrophages, plasma cells, eosinophils, and basophils. Elevated numbers of neutrophils are also abnormal.

Macrophages are mononuclear cells with abundant cytoplasm (Fig. 10.4a). Conditions in which they can be seen in the CSF include hemorrhage, cerebral infarction, demyelinating diseases, and meningitis. The contents of their cyto-

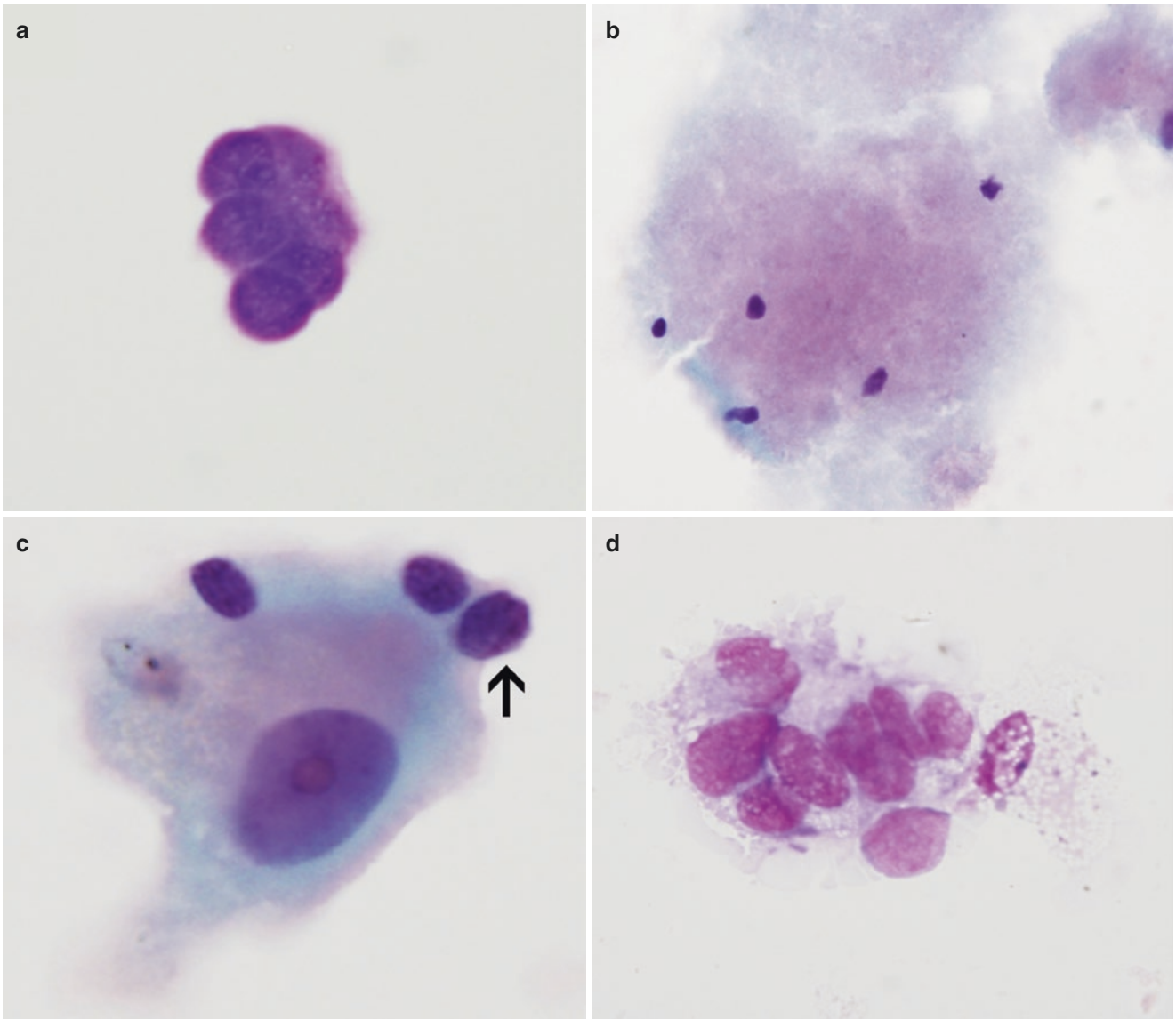

Fig. 10.2 (**a**) Choroid plexus/ependymal cells are round to cuboidal with small-to-moderate amounts of cytoplasm and can form small, dense clusters (Giemsa-Wright stain). (**b**) Brain tissue has characteristic fibrillary neuropil (Giemsa-Wright stain). (**c**) Neurons can have large nuclei, prominent nucleoli, and abundant amounts of angulated cytoplasm. Glial cells (arrow) have small, round nuclei (Giemsa-Wright stain). (**d**) Germinal matrix cells can appear as highly cellular aggregates of cells with prominent nuclear molding (Giemsa-Wright stain)

plasm correspond to the underlying disease process. For instance, macrophages with phagocytosed red blood cells ("erythrophages") can be present in the acute setting after traumatic taps [17] and intracranial hemorrhage [18]. Macrophages containing hemosiderin pigment ("siderophages") are suggestive of intracranial hemorrhage of at least 2 days duration and can persist in the CSF for weeks [19, 20]. Lipid-laden macrophages can be seen with cerebral infarction, meningitis, and demyelinating diseases such as multiple sclerosis [21, 22].

Plasma cells have eccentrically placed nuclei with clock-face chromatin and perinuclear hofs (i.e., Golgi apparatus) (Fig. 10.4b). Plasma cell pleocytosis can be identified in myriad infections [23–25] (e.g., Lyme disease, Herpes encephalitis, HIV, syphilis, tuberculosis, neurocysticercosis, and fungal infections such as *Cryptococcus*), inflammatory conditions [21, 26, 27] (e.g., multiple sclerosis, Guillain Barre syndrome, and neurosarcoidosis), and in rare cases of multiple myeloma with leptomeningeal involvement [28].

Eosinophils are typically bilobed with eponymous large eosinophilic granules within the cytoplasm (Fig. 10.4c). The presence of CSF eosinophilia, defined as more than 10 eosinophils per μL or a CSF differential of greater than 10% eosinophils [29], is most commonly due to parasitic infections, such as *Angiostrongylus cantonensis* [30]. Other causes include viral meningitis, fungal or *Rickettsial* infec-

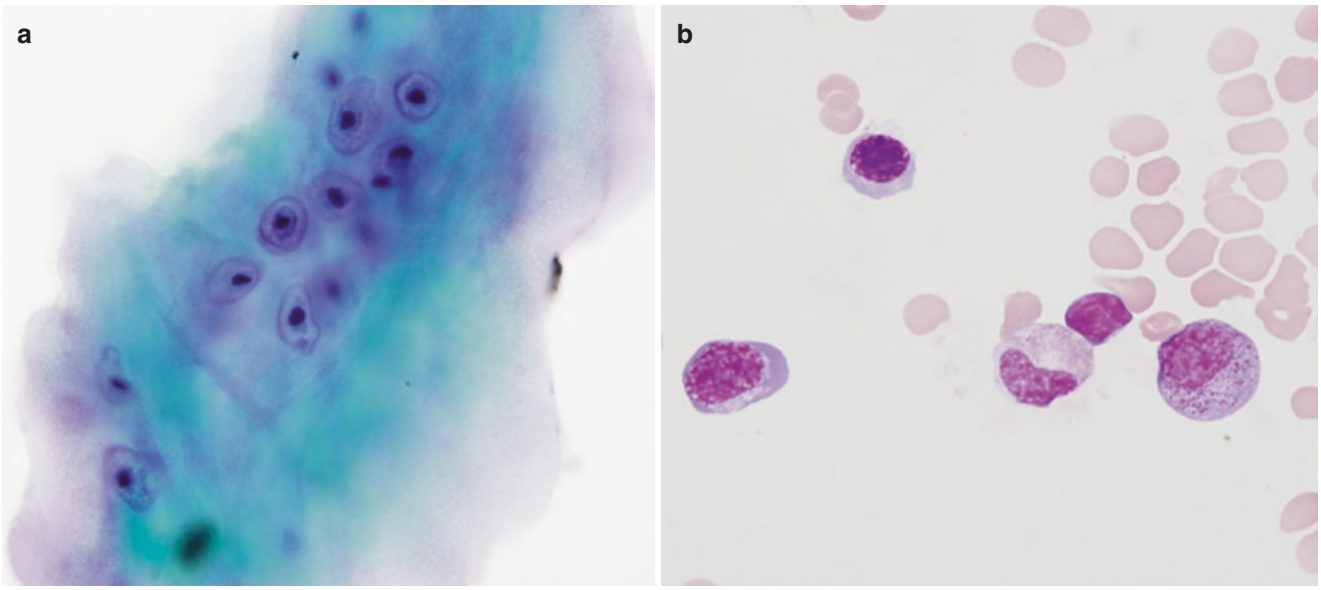

Fig. 10.3 CSF contaminants include (**a**) chondrocytes from the intervertebral disc (Papanicolaou stain) and (**b**) immature erythroid and myeloid elements from the vertebral body bone marrow (Giemsa-Wright stain)

tion, tuberculous meningoencephalitis, drug reaction (e.g., ibuprofen, ciprofloxacin, vancomycin, and gentamicin), hematologic malignancy, reaction to foreign material (e.g., VP shunts), acute polyneuritis, and neurosarcoidosis [30–32].

Basophils are identified by their eponymous cytoplasmic basophilic granules. Basophilic pleocytosis of the CSF is rare but has been described in cases of lymphocytic meningitis, tuberculous meningitis, neurocysticercosis, allergic reactions, and various hematologic disorders [33–35].

Neutrophils have single multilobed nuclei and fine cytoplasmic granules. Elevated neutrophils in the CSF can be due to a number of infectious etiologies [36–40] such as bacterial meningitis (Fig. 10.4d), acute viral, tuberculous or fungal meningitis, toxoplasma meningoencephalitis, cerebral abscess, and ventriculitis. Neutrophilic pleocytosis can also be observed in reactive conditions [38, 41–43], such as after seizure and cerebral infarction. Peripheral blood contamination from traumatic taps can result in falsely elevated numbers of neutrophils in the CSF.

4. What are the common causes of aseptic meningitis?

Aseptic meningitis is defined as inflammation of the meninges in the absence of pyogenic bacteria in the CSF. The most common cause of aseptic meningitis, contrary to its name, is infection. However, in many cases, the underlying etiology of aseptic meningitis remains unknown even after extensive workup [44]. Enteroviruses (including echoviruses, coxsackieviruses, and polioviruses) are the most frequently identified causative pathogen in patients with aseptic meningitis, responsible for over 80% of cases [45]. Other pathogens include flaviviruses (e.g., Saint Louis encephalitis virus, West Nile virus), herpesviruses (e.g., HSV1, HSV2, ZVZ, EBV, and HHV-6), mumps, HIV (in the setting of seroconversion), fastidious bacteria (e.g., mycobacteria, *Borrelia burgdorferi*, *Treponema pallidum*), fungi (e.g., *Cryptococcus neoformans*), and various parasites (e.g., *Toxoplasma gondii*) [46]. Herpesvirus infection can result in a rare form of recurrent aseptic meningitis called Mollaret meningitis [47]. Bacterial meningitis partially treated with antibiotics can also manifest as aseptic meningitis [48].

Less common causes of aseptic meningitis include systemic diseases (e.g., systemic lupus erythematosus, Kawasaki disease, sarcoidosis, Behçet's disease, and granulomatosis with polyangiitis), neoplastic meningitis, migraine, vaccines, and drugs (e.g., NSAIDS, trimethoprim-sulfamethoxazole, and intravenous immunoglobulin) [47, 48]. Patients with systemic or inflammatory diseases might be at higher risk of developing drug-induced aseptic meningitis [49].

The cytologic features of aseptic meningitis can vary. Although, by definition, some degree of pleocytosis is present in all cases, the cell differential can vary. In the acute setting, the CSF can have a neutrophilic predominance [50]. For most examined cases, however, the CSF contains mature, normal-appearing lymphocytes, plasma cells, monocytes, and/or activated lymphocytes. In contrast to mature lymphocytes, activated lymphocytes are larger and have irregular nuclei, denser chromatin, variably prominent nucleoli, and a greater amount of cytoplasm [21, 51] (Fig. 10.5).

In some instances, the pathogen responsible for aseptic meningitis can be identified in the CSF cytology preparation,

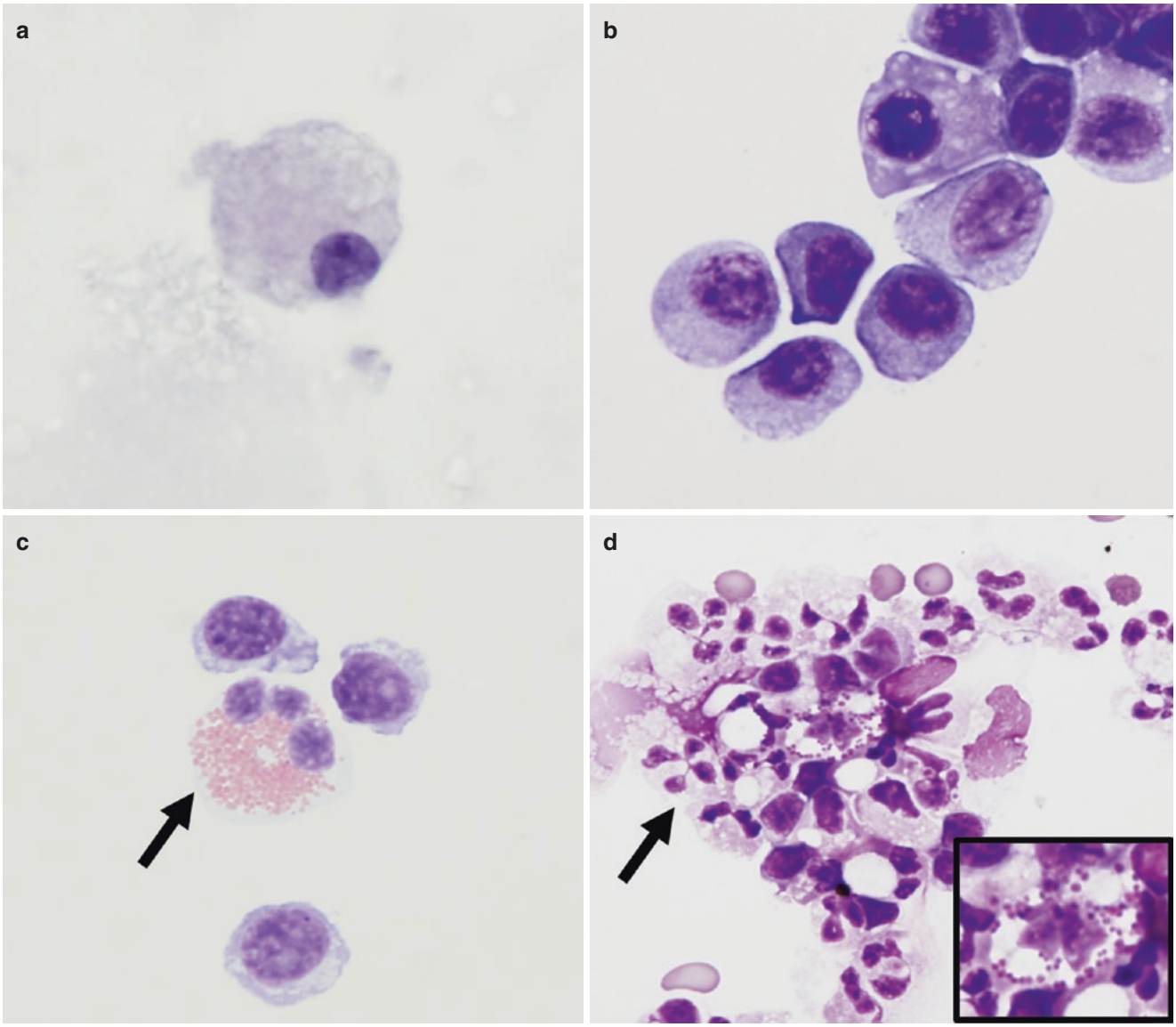

Fig. 10.4 Abnormal inflammatory cells in CSF. (**a**) Macrophages are characterized by their abundant cytoplasm and round-to-folded nuclei with pale chromatin (Giemsa-Wright stain). (**b**) Plasma cells have eccentrically placed nuclei, clock-face chromatin, and perinuclear hofs (Giemsa-Wright stain). (**c**) Eosinophils are multilobated and have large eosinophilic cytoplasmic granules (Giemsa-Wright stain). (**d**) Neutrophils have a multilobated nucleus. This patient's neutrophilic leukocytosis is due to bacterial meningitis. Clusters of cocci (*inset*) are present (Giemsa-Wright stain)

such as *Cryptococcus* (Fig. 10.6a) and *Toxoplasma* (Fig. 10.6b). Evaluating the CSF specifically for viral cytopathic changes is unlikely to yield positive results [52].

5. What findings or diagnoses are considered "critical values"?

In clinical pathology, there are well-established guidelines for lab results that, because of their significant implications for patient care and management, require immediate clinician notification (i.e., "critical values"). No such codified guidelines exist in surgical pathology or cytopathology. According to a consensus statement by the College of American Pathologists (CAP) and the Association of Directors of Anatomic and Surgical Pathology (ADASP), individual institutions should develop their own policies for defining urgent critical values and for notifying clinicians of these results [53]. However, based on multi-institutional surveys of cytopathologists and members of ADASP, several CSF findings are widely considered to require timely clinician notification, including unexpected malignancy, abundant neutrophils, and the presence of bacteria, fungi, and/or acid-fast bacilli [54, 55].

6. It is possible to do ancillary tests on CSF samples?

Several different types of ancillary tests can be performed on CSF samples for the workup of infection or malignancy.

These tests can provide important diagnostic information to guide clinical care and management.

Pathogens involving the CNS (i.e., viral, bacterial, and fungal) generally have characteristic CSF lab values and white blood cell differentials, but these findings are nonspecific. Ancillary tests can assist in the speciation of the disease-causing organism and include CSF cultures, microbial polymerase chain reaction (PCR), antigen testing, serology, and Gram, silver, and AFB stains. Although CSF cultures might be the gold standard for diagnosing CNS infections, the amount of time required for cultures to grow can be prohibitively long when urgent clinical decisions need to be made [56].

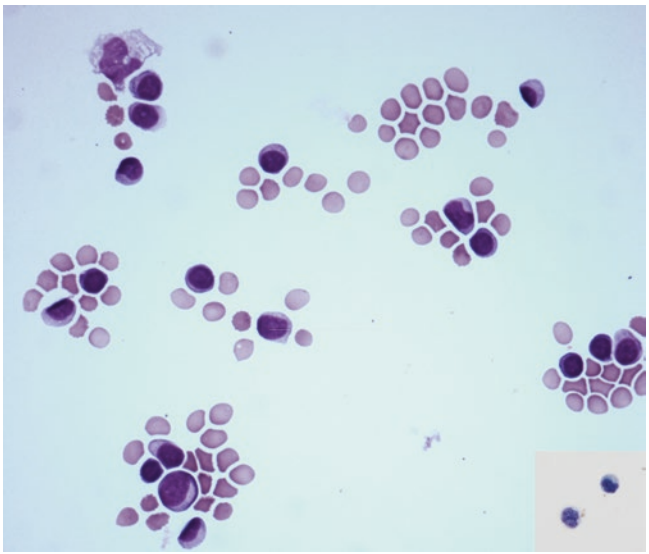

Fig. 10.5 Hypercellularity and polymorphous lymphocytosis are common features of aseptic meningitis (Wright-Giemsa stain). The activated larger lymphocytes often have irregularly shaped nuclei best seen on Papanicolaou stain (*inset*)

For patients with suspected bacterial meningitis, Gram stain of the CSF can be performed rapidly and has a reported sensitivity of 60–90%, though this varies with the species of bacteria and whether the patient has received antibiotics prior to CSF collection [57]. The utility of performing bacterial antigen tests in addition to Gram stain appears to be limited [58]. Broad-range PCR is being increasingly used for the workup of bacterial meningitis, with one study demonstrating a sensitivity of 100% and a specificity of 98% [59]. PCR is particularly useful for identifying fastidious bacteria and for evaluating CSF in patients who have been previously treated with antimicrobials [60].

Viral PCR is the preferred assay for speciation during the workup of viral meningitis. It has a sensitivity of greater than 95% for enteroviruses, HHV-6, HSV, and EBV [61]. The utility of performing viral cultures, which are less sensitive and have a longer turn-around time compared to PCR, has been questioned [62, 63]. In contrast, viral serology still has an important role in the evaluation of viral meningitis, particularly when arboviruses are suspected [64].

The use of dedicated cultures for fungal meningitis has limited value, as the two most common meningitis-causing fungi (*Cryptococcus* and *Candida*) grow on bacteria media [65]. Furthermore, cryptococcal antigen latex agglutination assays have sensitivities ranging from 90% to 100% [66, 67], with many enzyme immunoassays having a similar level of performance [68].

For the workup of malignancy, ancillary tests can be helpful when there are only rare neoplastic-appearing cells or

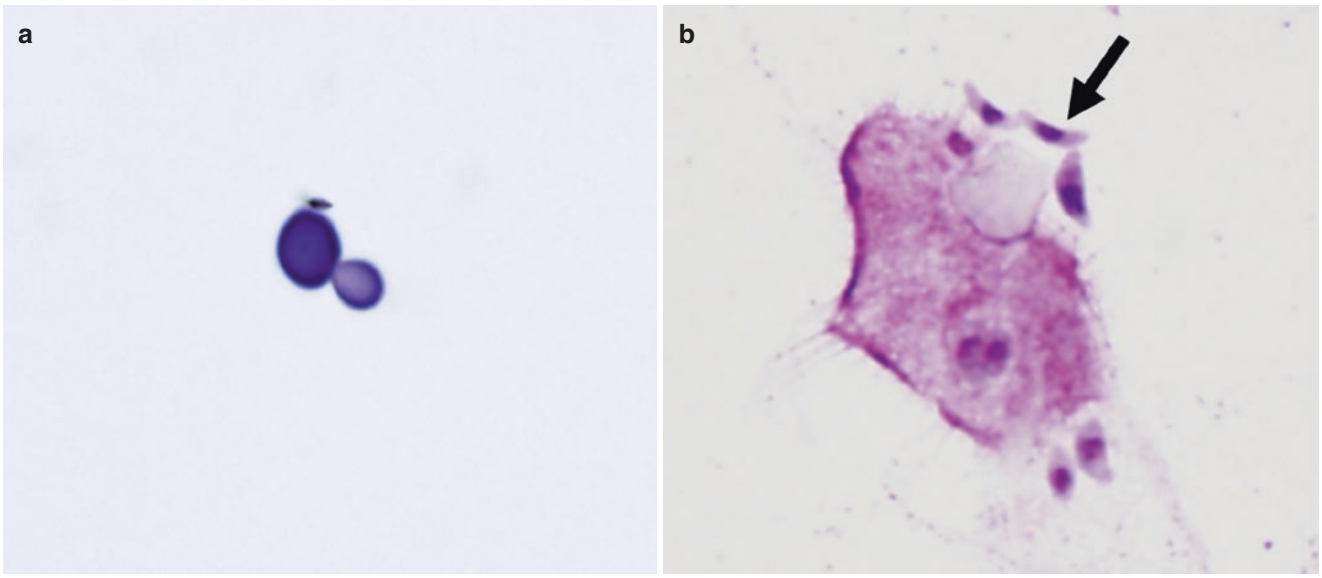

Fig. 10.6 Organisms can be occasionally identified in CSF and include (**a**) *Cryptococcus neoformans*, an encapsulated narrow-based budding yeast (Wright-Giemsa stain), and (**b**) *Toxoplasma gondii*, a crescent-shaped protozoan (arrows, Wright-Giemsa stain)

when neoplastic cells might mimic benign cells, making a definitive diagnosis difficult based on cytology alone.

Tumor cells can enter the CSF through a variety of mechanisms, including seeding of the leptomeninges via hematogenous, lymphatic, or perineurial spread and direct tumor extension to the subarachnoid space or ventricular system [69]. Approximately, 5% of all cancer patients will have leptomeningeal involvement at some point during the course of their disease [70], and this prevalence is expected to increase as the survival rates of cancer patients improve. Diagnosing neoplastic leptomeningeal involvement, of which CSF cytology is the gold standard, is clinically important because it has an exceptionally poor prognosis. Metastatic spread to the leptomeninges is associated with a median survival of 4–6 weeks for untreated patients and 2–6 months for patients receiving treatment [71]. Patients with known or suspected leptomeningeal involvement can receive intrathecal chemotherapy or monoclonal antibodies that could potentially improve survival time.

Conventional CSF cytology has limited sensitivity for detecting leptomeningeal involvement. The sensitivity is only approximately 50% for the first lumbar puncture but increases to 80% with subsequent evaluations [72]. The sensitivity can be maximized by sending at least 10.5 mL of CSF for cytologic evaluation, reducing delays in specimen processing, and collecting CSF from a site (e.g., lumbar or ventricular) that is closest to the tumor [73]. In contrast, CSF cytology has a very high specificity for leptomeningeal involvement [74].

Axillary tests for detecting or further characterizing neoplastic cells in the CSF include flow cytometry and IHC.

Flow cytometry is more sensitive than CSF cytology at detecting hematologic malignancies [75, 76], though positive CSF cytology might provide additional prognostic information, such as worse progression-free survival [77].

IHC can be performed on liquid-based CSF preparations. However, many labs have not optimized staining protocols for these preparations, so only a limited panel of immunostains may be able to be performed. Immunostains can be helpful for workup of hematologic malignancies, especially when neoplastic cells can be mistaken for reactive inflammatory cells (and vice versa). Demonstrating kappa or lambda light chain restriction, for instance, would be consistent with a clonal B-cell population by either IHC or flow cytometric analysis. Involvement by acute myeloid leukemia or acute promyelocytic leukemia can be confirmed by the myeloperoxidase (MPO) positivity by either IHC or cytochemistry in tumor cells (Fig. 10.7). Keratin immunostains can demonstrate the presence of epithelial cells in CSF specimens to support a diagnosis of metastatic carcinoma, which can be useful when only scattered suspicions cells are identified (Fig. 10.8). Characterizing the immunophenotype of tumor cells can assist in the diagnosis of occult primaries. In practice, the utility of routinely supplementing CSF cytology with IHC to diagnose leptomeningeal involvement is quite modest, with an added sensitivity of less than 10% [78].

New molecular and immunomagnetic assays might provide additional diagnostic and therapeutic information. Genetic sequencing of tumor cells can identify targetable genetic alterations [79]. Immunomagnetic assays, in which a patient's fluid specimen is mixed with magnetic beads coated with antibodies such as epithelial cell adhesion marker, can be used to identify rare circulating tumor cells. The detection and quantification of circulating tumor cells in peripheral blood has a well-documented role in diagnosing early metastasis and predicting treatment response [80, 81]. While not currently approved by the FDA for clinical use in CSF, these immunomagnetic assays have superior sensitivity at detecting leptomeningeal carcinomatosis compared to conventional CSF cytology [82, 83].

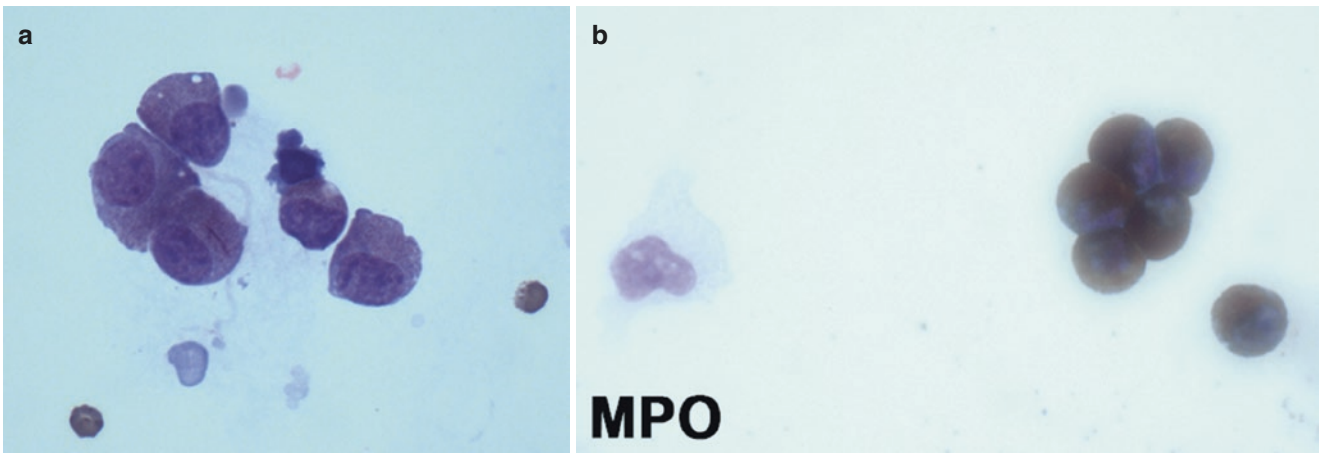

Fig. 10.7 Acute promyelocytic leukemia. (**a**) Promyelocytes show large nuclei (sometimes divided into two lobes) with variably prominent nucleoli and moderate amounts of cytoplasm with azurophilic granules and bundles of Auer rods. Note a small lymphocyte in the center (Giemsa-Wright stain). (**b**) MPO cytochemical stain is positive in the cytoplasm of promyelocytes but not in a monocyte (left)

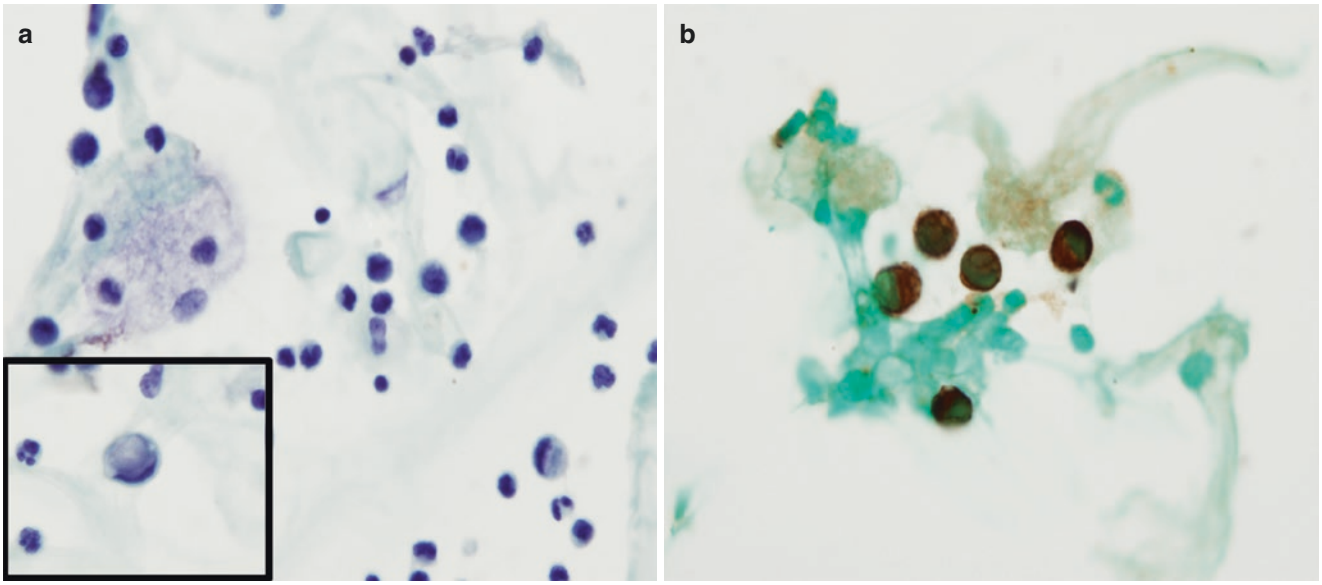

Fig. 10.8 Immunohistochemistry can confirm metastasis. (**a**) Scattered atypical cells suspicious for carcinoma, including cells with signet-ring cell morphology (*inset*) (Papanicolaou stain). (**b**) AE1/AE3 keratin immunostain highlights the suspicious cells, supporting the diagnosis of metastatic carcinoma. After additional workup, the patient was diagnosed with gastric adenocarcinoma

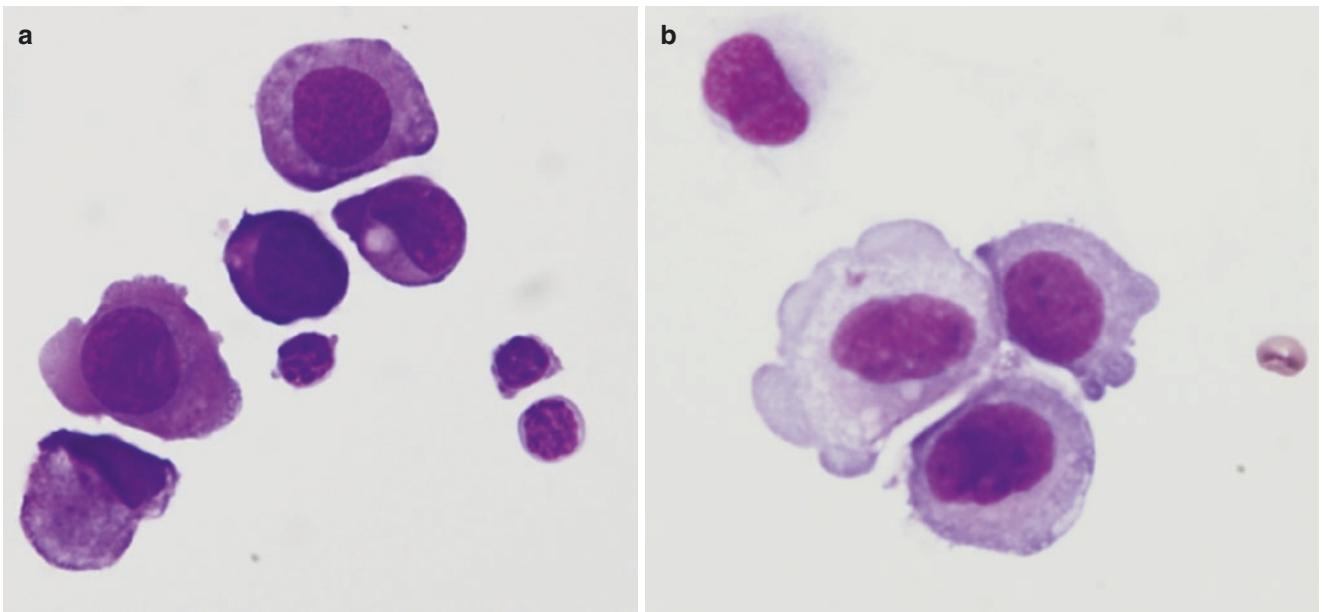

Fig. 10.9 Breast carcinoma. (**a**) Invasive lobular carcinoma has discohesive cells, some with eccentrically placed nuclei (Giemsa-Wright stain). (**b**) Invasive ductal carcinoma is composed of larger, more cohesive cells with cytoplasmic blebs (Giemsa-Wright stain)

7. What are the top three primary sites for a positive CSF in a patient with metastatic disease?

The solid tumors that most commonly metastasize to the CNS resulting in positive CSF cytology are breast cancer, lung cancer, and melanoma [84, 85].

Breast carcinoma is responsible for 12–34% of all positive CSF specimens and metastasizes to the leptomeninges in approximately 5% of patients [72]. Invasive lobular breast carcinoma and triple negative breast carcinoma have a higher risk of involving the leptomeninges [86–88]. Cytologically, invasive lobular breast carcinoma appears as discohesive small-to-intermediate-sized cells with eccentrically placed nuclei and usually occur as single cells, tiny clusters, or cords (Fig. 10.9a). Intracytoplasmic lumina and signet-ring-cell morphology can be present. Invasive ductal breast carcinoma appears as large cells, occurring singly and in small clusters, with cytoplasmic blebs and variable pleomorphism depending on the histologic grade (Fig. 10.9b).

Lung carcinoma is responsible for 10–26% of all positive CSF specimens and metastasizes to the leptomeninges in approximately 10–25% of patients with small cell lung carcinoma and 1% of patients with non-small cell lung carcinoma [72]. Leptomeningeal involvement by squamous cell carcinoma of the lung is uncommon [89]. Small cell lung carcinoma appears as small cells occurring singly or in clusters with high N:C ratios, prominent nuclear molding, coarse chromatin, inconspicuous nuclei, frequent mitoses, and necrosis (Fig. 10.10a). Lung adenocarcinoma appears as large cells occurring singly or in clusters, with eccentrically placed nuclei, variably prominent nucleoli, and moderate-to-abundant amounts of cytoplasm (Fig. 10.10b). Intracytoplasmic mucin vacuoles can also be observed.

Melanoma is responsible for 17–25% of all positive CSF specimens and metastasizes to the leptomeninges in approximately 22–46% of patients [72]. Cutaneous melanomas with deeper Clark's level of invasion and primary melanomas of the head and neck might have an increased risk of metastasizing to the CNS [90]. The characteristic cytologic appearance of melanoma is of large, discohesive, pleomorphic cells with prominent macronucleoli and brown intracytoplasmic melanin pigment (Fig. 10.11). Nuclear pseudoinclusions, scattered mitoses, and necrosis can also be seen.

Positive CSF cytology from metastatic involvement of the leptomeninges can be the first manifestation of an extra-CNS malignancy in 5–10% of patients [91]. The most common occult primaries include lung carcinoma, gastric adenocarcinoma, and melanoma [92–95]. Gastric adenocarcinomas that metastasize to the leptomeninges are almost always poorly differentiated [96]. Cytologically, gastric adenocarcinoma often appears as single cells or small clusters of cells with intracytoplasmic mucin vacuoles that displace hyperchromatic nuclei to the side (i.e., signet-ring-cell morphology) (Fig. 10.8a, inset).

8. What do you look for when you encounter a CSF with features of high-grade lymphoma?

CNS lymphomas can either be primary or secondary. Primary CNS lymphomas involve the brain, spinal cord, leptomeninges, and/or eyes, and are rare, constituting 2.2% of primary brain tumors [97]. Secondary CNS lymphomas spread to the CNS from a systemic site and can occur in up to 5–10% of

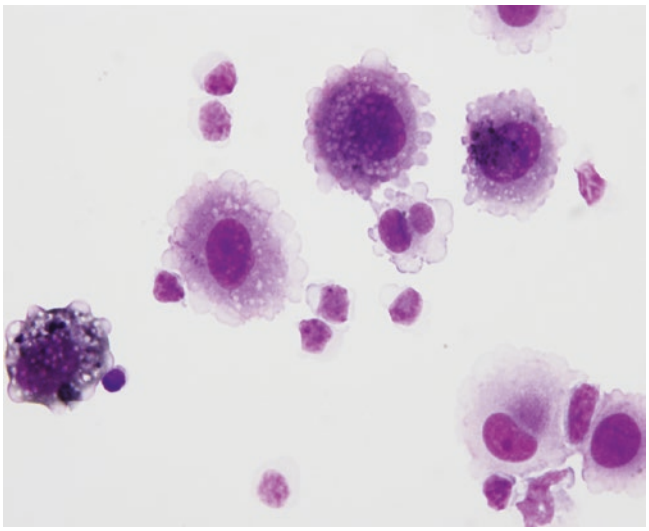

Fig. 10.11 Melanoma can appear as large, pleomorphic cells with macronucleoli and intracytoplasmic melanin pigment (Giemsa-Wright stain)

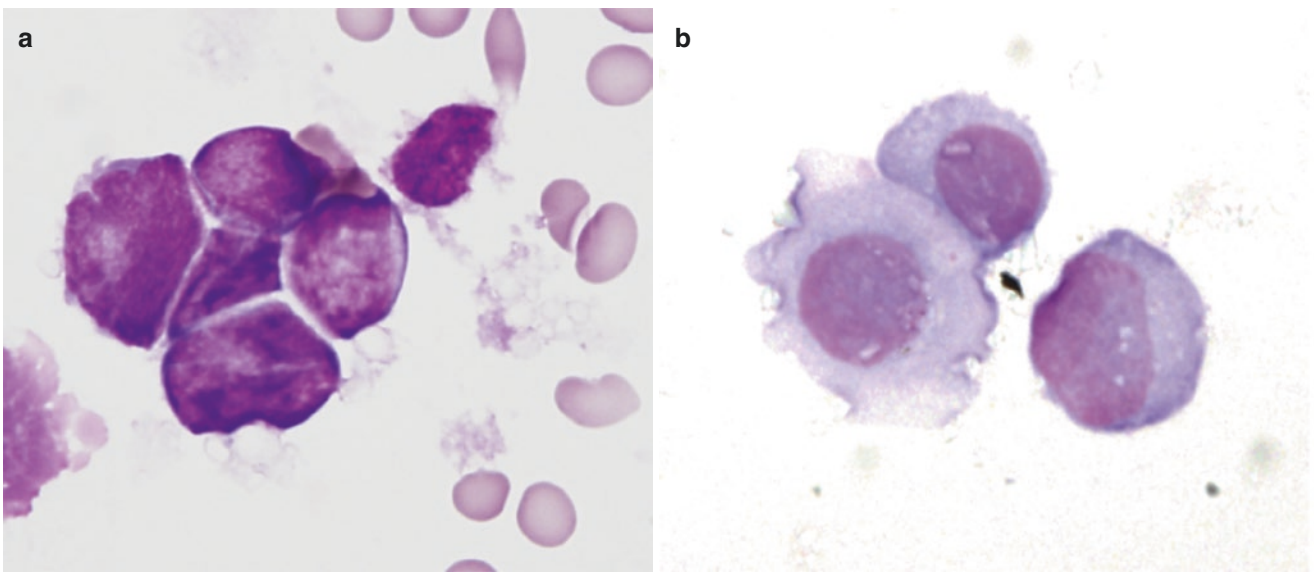

Fig. 10.10 Lung carcinoma. (**a**) Small cell lung carcinoma is composed of cells with high N:C ratio and nuclear molding (Giemsa-Wright stain). (**b**) Lung adenocarcinoma has large cells with moderate-to-abundant cytoplasm, some with eccentrically placed nuclei (Giemsa-Wright stain)

patients with lymphoma, depending on the histologic type and a variety of other risk factors [98].

The most common lymphoma of the CNS, both primary and secondary, is aggressive large B-cell lymphoma, namely diffuse large B-cell lymphoma (DLBCL). For patients with aggressive large B-cell lymphoma of the CNS, it is recommended that *MYC*, *BCL-2*, and *BCL-6* translocations be assessed by fluorescence in situ hybridization (FISH) [99]. Around 5% have a translocation involving *MYC* and a translocation involving *BCL-2* or *BCL-6*, and are referred to as double hit lymphoma (DHL). Differentiating between DHL and DLBCL is clinically important because DHL is associated with a worse prognosis and overall survival, higher incidence of CNS involvement at time of diagnosis, increased risk of CNS relapse, and attenuated response to R-CHOP therapy [100–102].

The cytology of aggressive large B-cell lymphomas is variable but tends to consist of intermediate-to-large cells with round-to-irregular nuclear contours, occasional nuclear clefting, variably prominent nucleoli, coarse chromatin, and cytoplasmic vacuoles, with mitoses and apoptotic bodies [103] (Fig. 10.12). While positive CSF cytology might be a useful tool for diagnosing CNS involvement, CSF flow cytometry is more sensitive [75].

9. What leukemia most commonly involves the CSF?

Acute lymphoblastic leukemia (ALL) has disseminated disease to the leptomeninges in approximately 5% of cases at time of diagnosis [104]. The prevalence of CNS relapse has decreased substantially since the routine use of CNS prophylactic therapies – from up to 75% [105] to around 5% [106]. ALL patients with disease involving the CNS have a reduced 5-year overall survival compared to patients without CNS involvement [107]. Several risk factors predict CNS involvement, including elevated lactate dehydrogenase, high tumor proliferative index, mature B-cell phenotype, CD56 expression on neoplastic cells, and various cytogenetic abnormalities including Philadelphia chromosome positivity with *BCR-ABL1* fusion [108].

ALL has a variable cytologic morphology depending on the subtype, and there are no unique cytomorphologic features for specific subtypes. B-lymphoblastic leukemia/lymphoma (B-ALL) is primarily a childhood disease, while T-ALL is more common in adolescents. The typical lymphoblasts are small to medium-sized cells with round nuclear contours, fine chromatin, small-to-inconspicuous nucleoli, and scant basophilic cytoplasm. Some lymphoblasts may have moderate amounts of basophilic, vacuolated cytoplasm (Fig. 10.13).

10. What is the clinical significance of CSF involvement in a patient with a primary CNS tumor?

Although leptomeningeal involvement is associated with poor prognosis in primary CNS tumors and necessitates more aggressive chemotherapeutic regimens, the precise role of CSF cytology is unclear, particularly when MRI studies are available. Many of the investigations examining the clinical significance of positive CSF cytology for primary CNS tumors have focused on pediatric brain tumors, three of which are described below. Around 20% of children with primary brain tumors have disseminated disease to the leptomeninges [109].

Medulloblastoma accounts for approximately 20% of all pediatric brain tumors and has disseminated disease to the

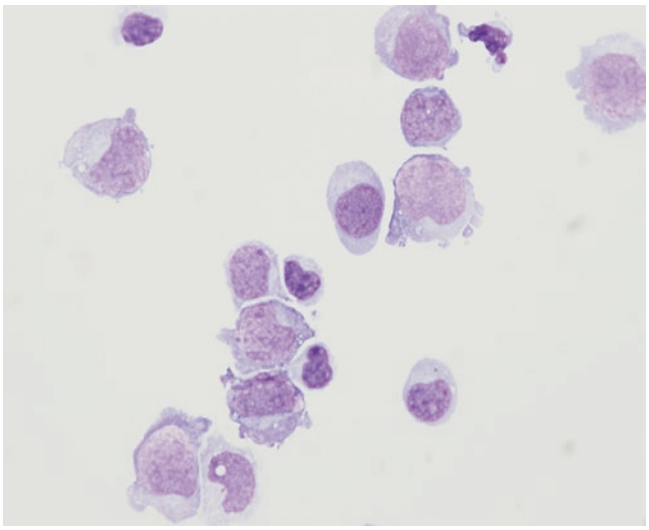

Fig. 10.12 Diffuse large B-cell lymphoma is the most common CNS lymphoma and is composed of intermediate-to-large discohesive cells with round-to-irregular nuclei and coarse chromatin. Nuclear clefts, prominent nucleoli, and cytoplasmic vacuoles can be present (Giemsa-Wright stain)

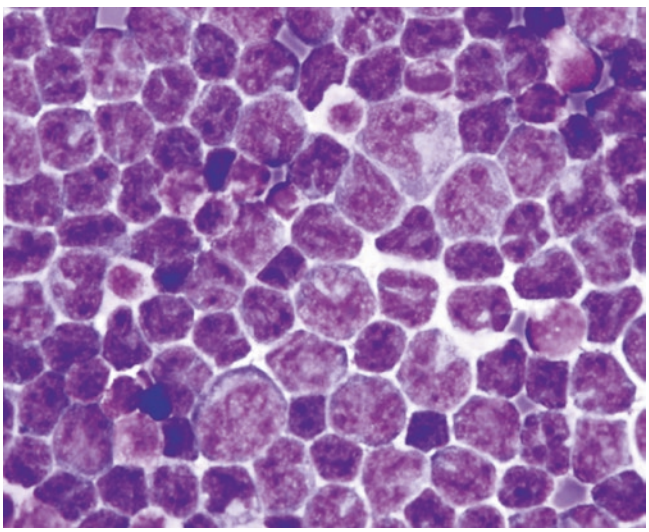

Fig. 10.13 Acute lymphoblastic leukemia can have a widely variable morphologic appearance (Giemsa-Wright stain)

leptomeninges in 30% of cases at time of diagnosis [110]. Leptomeningeal involvement is associated with worse overall survival [111, 112] and is an indication for high-dose chemoradiation. MRI has a higher sensitivity for detecting early disseminated disease compared to CSF cytology, even when serial CSF specimens are evaluated [111]. However, CSF cytology can detect a subset of positive cases not identified by MRI [113].

Cytologically, medulloblastoma appears as small-to-intermediate-sized cells with high N:C ratio, nuclear molding, coarse chromatin, variably prominent nucleolus, and scant cytoplasm occurring singly or in tightly packed clusters (Fig. 10.14).

Ependymoma comprises 6–10% of all pediatric brain tumors and has disseminated disease to the leptomeninges in 10–30% of cases at time of diagnosis [114]. The ability of CSF cytology to detect disseminated disease not identified by MRI is poor [114, 115]. Regardless, comprehensive staging of ependymoma and evaluation of disease recurrence requires cytologic evaluation of the CSF, the results of which have implications for patient outcome and treatment [116]. Patients with both radiologic and cytologic evidence of leptomeningeal disease have a particularly bad prognosis [117]. In one bi-institutional study of patients with ependymoma, the rate of positive CSF cytology was 16.7% overall (6.7% in adults, 21.2% in children), with a higher rate in high-grade ependymomas compared to low-grade ependymomas (66% vs. 8%) and in samples collected intraoperatively compared to samples collected postoperatively (21.7% vs. 1.8%) [118].

The cytologic appearance of ependymoma depends on subtype. For instance, classic ependymoma appears as cuboidal epithelial cells with round-to-oval, occasionally eccentrically placed nuclei, and inconspicuous nucleoli, occurring singly or in aggregates with variably prominent gland-like structures (Fig. 10.15); the tumor cells of tanycytic ependymoma are notable for long, hair-like glial processes; myxopapillary ependymoma appears as large epithelioid cells with oval nuclei, small nucleoli, and large amounts of cytoplasm, with fragments of acellular myxoid material in the background [118].

Atypical teratoid/rhabdoid tumor (AT/RT) comprises 1–2% of all pediatric brain tumors and has disseminated disease to the leptomeninges in up to 30% of cases at time of diagnosis [119]. The overall prognosis of patients with AT/RT is poor, with a median survival time ranging from 6 to

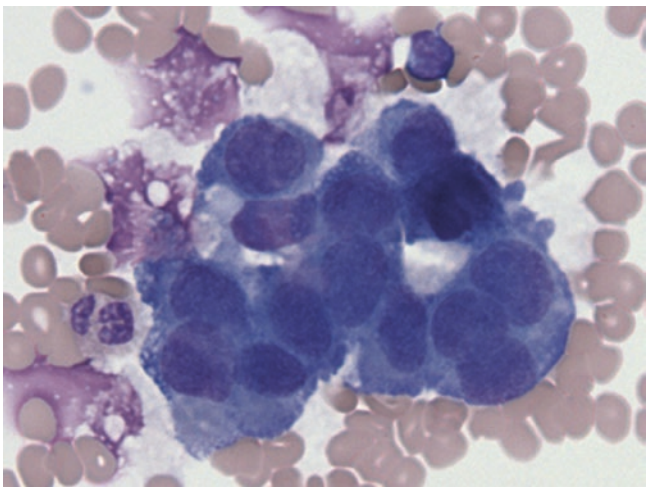

Fig. 10.15 Ependymoma has a variable morphology depending on the subtype. This is a classic ependymoma with cells with oval nuclei and inconspicuous nucleoli forming a three-dimensional gland-like structure (Giemsa-Wright stain)

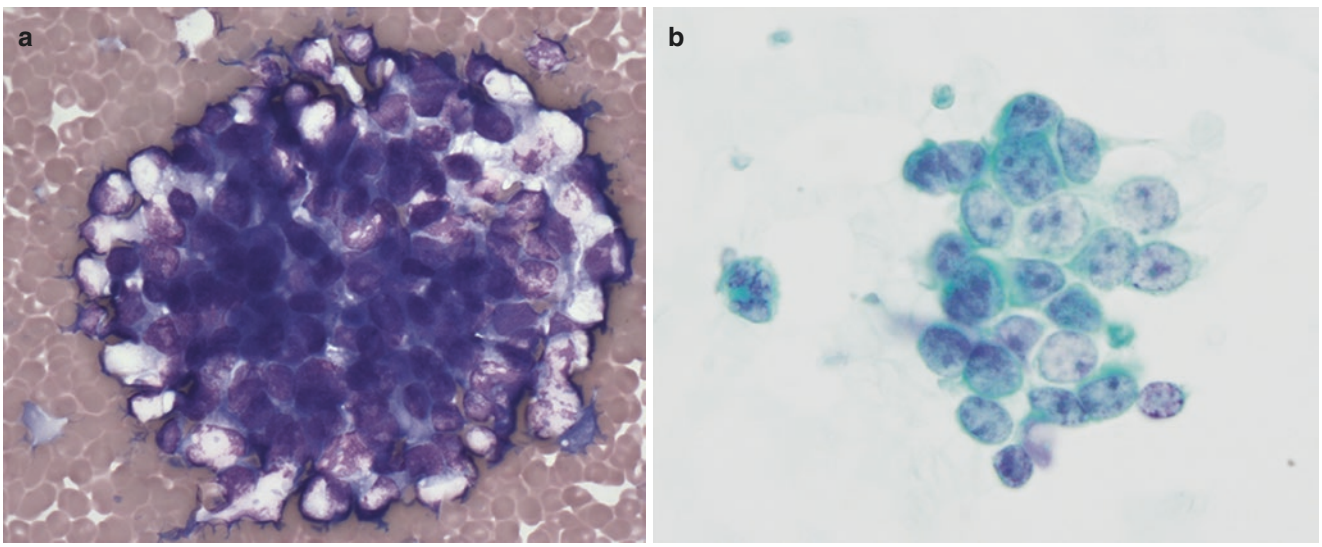

Fig. 10.14 Medulloblastoma is composed of cells with high N:C ratio, nuclear molding, and variably prominent nucleoli, and can form tightly packed clusters (**a** Giemsa-Wright stain; **b** Papanicolaou stain)

17 months [120, 121]. The presence of leptomeningeal disease (determined by positive CSF cytology or by radiology) at time of diagnosis is associated with reduced overall survival [122, 123]. Although comparisons of the sensitivities of CSF cytology and MRI to detect leptomeningeal disease are limited, one study found that out of eight patients with AT/RT who had positive CSF cytology, only four had evidence of disseminated disease by imaging [124].

The cells of AT/RT have a varied cytologic appearance. Two different neoplastic cell populations can be identified: large rhabdoid cells with eccentrically placed nuclei, prominent nucleoli, and eosinophilic cytoplasm, and small cells with high N:C ratio, prominent nucleoli, and variable amounts of cytoplasm; tumor cells generally occur singly, and specimens tend to be hypercellular with an inflammatory background [125].

Other primary CNS neoplasms that can involve the leptomeninges include gliomas, lymphomas, choroid plexus tumors, germ cell tumors, pineal tumors, and meningiomas.

11. Is regular CSF surveillance required in any type of CNS malignancy?

Although standardized routine CSF testing has been proposed for patients with CNS tumors [126, 127], there do not appear to be any widely adopted surveillance protocols. Instead, the extent and duration of posttherapeutic follow-up is usually determined by regional preferences, enrollment status in clinical trials, and clinical necessity [128].

There is a field of literature debating the utility of CSF surveillance in patients with ALL. CNS relapse in ALL is associated with an approximately threefold increase in risk of subsequent bone marrow relapse and death [129]. Routine CSF monitoring can detect early asymptomatic CNS relapse [130]. However, the clinical utility of detecting subclinical relapse in this population is unclear [131], with no benefit observed in measures such as median survival [132]. Other investigations suggest that surveillance testing does not have diagnostic or prognostic value [133] and may actually fail to detect a significant proportion of relapses [134]. Furthermore, surveillance CSF cytology can be financially costly [135].

Case Presentations

Case 1

Learning Objectives:

1. To become familiar with the cytologic features of the neoplasm
2. To generate a differential diagnosis

Case History:

- A 42-year-old female presents with headache, double-vision, and 40-pound unintentional weight loss over the past year. A brain MRI showed a large frontal lobe mass, and a spine MRI showed multiple foci of enhancement along the lumbar nerve roots. A lumbar puncture is performed.

Histologic Findings:

- Large atypical epithelioid cells occurring singly and in small clusters, with round-to-oval nuclei, fine chromatin, inconspicuous nucleoli, highly variable amounts of vacuolated basophilic cytoplasm, and cytoplasmic blebs (Fig. 10.16a)

Differential Diagnosis:

- Carcinoma, unknown primary
- Melanoma
- Macrophages

Ancillary Studies:

- S100 protein immunostain is strongly positive (Fig. 10.16b).

Final Diagnosis:

- Melanoma

Take-Home Messages:

- Melanoma has a variety of histologic appearances, so classic cytologic features such as macronucleoli and intracytoplasmic melanin pigment can be absent.
- The most frequent occult primaries to the CNS include lung carcinoma, melanoma, and gastric carcinoma.

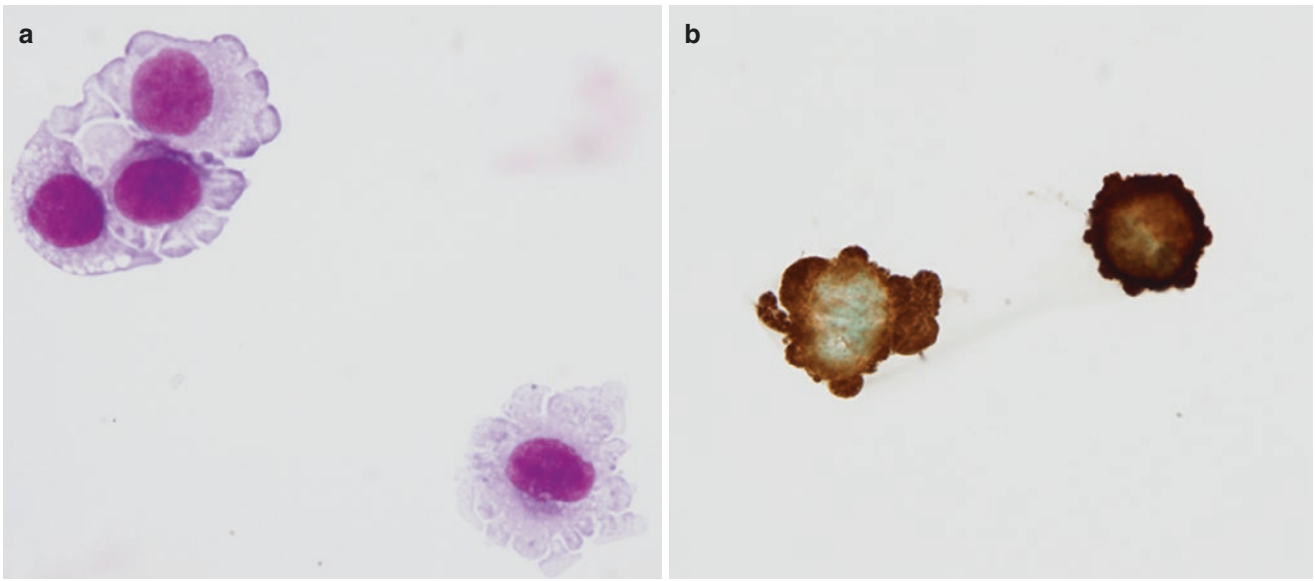

Fig. 10.16 Case 1. (**a**) Giemsa-Wright stain. (**b**) S100 immunostain

Case 2

Learning Objectives:

1. To become familiar with the cytologic features of this entity
2. To generate a differential diagnosis
3. To become familiar with the ancillary workup of this entity

Case History:

- A 63-year-old female with a 30 pack-year smoking history presents with ataxia, vertigo, and nausea. A brain MRI identified no lesion. A chest CT revealed a mass in the upper lobe of the right lung. The mass was biopsied, and pathology showed poorly differentiated adenocarcinoma. As part of the workup for her symptoms, a lumbar puncture had been performed.

Histologic Findings:

- Increased numbers of lymphocytes, including reactive forms characterized by increased size, irregularly shaped nuclei, occasionally prominent nucleoli, and moderate amounts of cytoplasm; there are scattered monocytes (Fig. 10.17).
- No malignant epithelioid cells are identified.

Differential Diagnosis:

- Aseptic meningitis of unclear etiology (infectious vs. inflammatory vs. drug reaction vs. other)

Ancillary Studies:

- Gram stain: Negative.
- HSV-1/HSV-2 PCR: None detected
- HSV-1/HSV-2 IgG: negative
- Lyme Elisa IgG and IgM: negative
- Coxsackie A and B antibody panels: negative
- EBV IgM and IgG: negative
- Anti-CV-2, anti-Yo, anti-HU, anti-Tr, anti-PCA-2, anti-Ma1, anti-Ma2, ANNA-3, anti-mGlu-R1: negative
- Anti-Ri: positive

Final Diagnosis:

- Aseptic meningitis, occurring in the setting of paraneoplastic syndrome (i.e., paraneoplastic cerebellar degeneration)

Take-Home Messages:

- The differential for aseptic meningitis is very broad, but the most frequent cause is infection, particularly by enteroviruses.
- The CSF in patients with paraneoplastic cerebellar degeneration often shows lymphocytic pleocytosis [136]. Correlation with clinical and other laboratory findings is key to diagnosis.
- Paraneoplastic cerebellar degeneration can present as ataxia, vertigo, diplopia, dysarthria, and dyspha-

gia and is due to antibody-mediated destruction of Purkinje cells [136].

- The detection of Anti-Ri antibody in serum or CSF indicates an otherwise unexplained neurological disorder such as autoimmune and paraneoplastic. A positive result also implies the presence of an underlying malignancy.

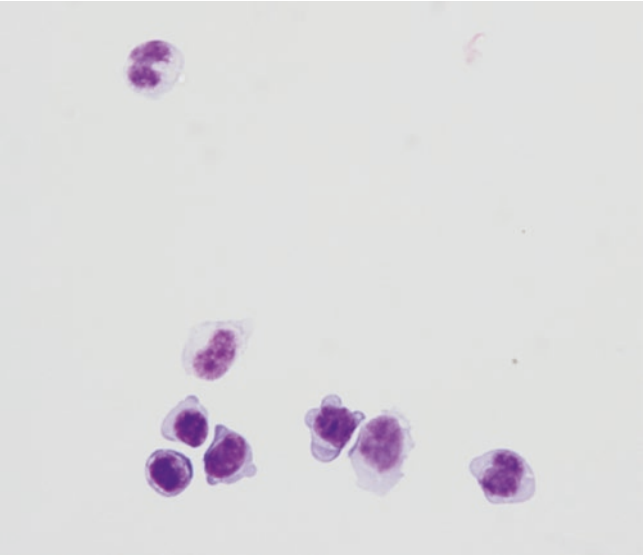

Fig. 10.17 Case 2. Giemsa-Wright stain

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Ovary and Peritoneal Washings

11

Kyle C. Strickland

Contents

Abbreviations

AFP	Alpha-fetoprotein
AGCT	Adult granulosa cell tumor
AJCC	American Joint Committee on Cancer
BSO	Bilateral salpingo-oophorectomy
CEA	Carcinoembryonic antigen
E2	Estradiol
ER	Estrogen receptor
FIGO	International Federation of Gynecology and Oncologists
FNA	Fine-needle aspiration
hCG	Human chorionic gonadotropin
HLM	Hemosiderin-laden macrophage
IHC	Immunohistochemistry
JGCT	Juvenile granulosa cell tumor
PR	Progesterone receptor
WHO	World Health Organization

List of Frequently Asked Questions:

Ovary

1. What are the indications for fine-needle aspiration of the ovary?

Fine-needle aspiration (FNA) of the ovary was first described in the early 1970s [1, 2]. The relative number of indications for ovarian FNA is limited but growing. Aspiration of the ovary is technically similar to other abdominal sites, and it is relatively safe and inexpensive. FNA of an ovarian mass is used in the following clinical scenarios:

- Diagnosis and therapy of a persistent ovarian mass in women of reproductive age [3].
- Drainage of extremely large benign-appearing cysts to allow for laparoscopic removal [4].
- Avoidance surgical intervention during pregnancy [5].
- Evaluation of malignancy in patients with a prior diagnosis or treated cancer, particularly in cases where laparoscopy is contraindicated.

For the cytologist, it is important to appreciate that a surgical approach is generally recommended for complex or solid lesions of the ovary. However, many patients present with a pelvic mass of unknown origin, and these occult ovarian lesions are often sampled by FNA. Ovarian FNA is contraindicated in the setting of acute abdominal/pelvic pain, as the procedure may delay treatment of serious conditions, such as torsion [6].

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_11

2. How are ovarian FNA samples obtained?
Aspiration can be performed transvaginally, transrectally, laparoscopically, at the time of laparotomy, or percutaneously through the abdomen, with or without imaging guidance. It is important to note the route of aspiration because contamination with normal tissue can occur and be somewhat problematic.

3. Why is aspiration of the ovary uncommon?
In the modern setting, ovarian aspiration is limited to a few circumstances, largely due to three main considerations:

1. There is concern that FNA of a malignant cyst can lead to tumor spillage and induce peritoneal seeding [7].
2. Aspiration as a therapeutic technique is not useful because a high percentage (up to 75%) of benign cysts will recur and ultimately require excision [8–10].
3. The diagnostic accuracy of ovarian FNA is controversial, and many malignant lesions are missed, especially in premenopausal patients [11].

As an additional concern, ovarian aspiration results in a high rate of unsatisfactory diagnoses (up to 20%), and ultrasound is as good or better at determining the malignant potential of ovarian lesions [12]. For cases in which radiologic imaging suggests malignancy, clinicians will recommend up-front surgery to evaluate an ovarian lesion, circumventing the need for FNA.

4. How accurate is ovarian FNA in the diagnosis of malignancy?
The answer to this question is somewhat unclear because various studies have defined the sensitivity and specificity in different ways. Although some report high values for sensitivity and specificity (up to 84% and 93%, respectively) [1, 13], these values are likely exaggerated because borderline tumors were excluded from analysis. Borderline tumors are a common cause of false-negative FNAs, because the cyst fluid is relatively acellular, perhaps due to greater cell-to-cell adhesions than their malignant counterparts. If borderline tumors are included in the analysis, the sensitivity ranges from 26% to 54% [14, 15]. Even though it is generally agreed that the test has a high specificity (>90%), the low sensitivity is a valid criticism of the technique because it limits the primary usefulness of the technique – as a rule out test for carcinoma.

5. What is the risk of iatrogenic peritoneal seeding following ovarian FNA?
Aspiration of ovarian cysts is considered taboo by some clinicians due to the risk of seeding an early stage ovarian cancer. However, the actual rate of seeding is not known [11]. The issue of seeding was first raised in a single but influential article that reported two cases in which surgical resection was delayed after fine-needle aspiration [7]. Iatrogenic peritoneal seeding was not confirmed in either case, but a strong argument was made that FNA has the potential to delay treatment, which may result in a worse prognosis for patients who already had peritoneal disease or cyst rupture prior to sampling. While seeding risk may in fact be minimal, the effect of malignant cyst rupture before and during surgery in patients with early stage ovarian cancer has been evaluated. One study of 60 patients with stage I epithelial ovarian carcinoma showed cyst rupture *during* surgery had no influence on survival rates (average follow-up 75 months) [16], with similar results shown in other studies as well [17, 18]. However, in patients who had cysts ruptured *prior to* surgery, there was a significant survival difference (10-year survival of 59% vs. 78% that had an intact capsule) [19]. The risk of seeding from an acute surgical spill seems to be small or nonexistent because surgeons irrigate spillage immediately. In contrast, slow and continuous spillage into the peritoneal cavity following disruption (biologic or iatrogenic) may create a favorable environment for peritoneal implantation. For this reason, ultrasound-guided FNA is considered to have more risk of a significant treatment delay than laparoscopic evaluation, which can often provide histologic information and therapy without much delay [7].

6. What are the other complications associated with ovarian FNA?
The risk of other major complications appears to be low for this procedure. In one of the largest studies, which included 893 patients, the most common complications of ovarian FNA were mild vagal symptoms (17, 2%) and transient mild-to-moderate pain (8, 0.9%) [20]. The most serious complications noted were acute abdominal pain (6, 0.7%) and infection (4, 0.4%). However, 6 (60%) of these patients required surgery, and no other life-threatening complications were reported. Severe pelvic infection following transvaginal and transrectal approaches has been seen in up to 1.3% of patients [13]. The findings of a low complication rate are similar to the complications of abdominal FNA in general [21].

7. Is there any therapeutic value to the aspiration of ovarian cysts?
Aspiration of small ovarian cysts has no apparent therapeutic benefit over other therapies. The vast majority (up to 71%) of ovarian cysts regress after a short-term course of oral contraceptives [22]. Of those masses that do not regress (endometriomas, benign neoplasia, benign para-ovarian cysts, hydrosalpinx, and malignant tumors), only para-ovarian cysts are appropriately treated by aspiration. As a rule of thumb, the larger the ovarian cyst, the greater the risk of recurrence [12]. Of note, endometriomas are generally not

acceptable to aspirate, as the underlying endometriosis ensures that the cysts will recur [3].

8. What are the ultrasonographic features of benign versus malignant ovarian lesions?

Benign and malignant ovarian masses demonstrate characteristic features on ultrasound. The following is a list from a comprehensive study of 211 adnexal masses (183 benign and 28 malignant) [23]. The authors found the following features associated with benign ovarian lesions:

- No solid component (54% benign vs. 0% malignant, $p < 0.001$)
- If present, a hyperechoic solid component (15% benign vs. 0% malignant, $p < 0.001$)
- An echogenic fluid component (58% benign vs. 21% malignant, $p < 0.001$)
- Thin (<3 mm) septations (26% benign vs. 4% malignant, $p = 0.02$)
- Thin (<3 mm) wall (50% benign vs. 29% malignant, $p < 0.001$)
- Normal free fluid in the abdomen (98% benign vs. 68% malignant, $p < 0.001$)
- Peripheral only or no flow detected by Doppler (83% benign vs. 14% malignant, $p < 0.001$)

In contrast, malignant ovarian lesions demonstrated the following ultrasonographic features:

- Nonhyperechoic solid component (32% benign vs. 100% malignant $p < 0.001$)
- An echogenic or no fluid component (43% benign vs. 61% malignant, $p < 0.001$)
- Thick (≥3 mm) septations (17% benign vs. 14% malignant, $p = 0.02$)
- Thick (≥3 mm) wall (43% benign vs. 32% malignant, $p < 0.001$)
- Abnormal amount of free fluid in the abdomen (2% benign vs. 32% malignant, $p < 0.001$)
- Central flow by Doppler (17% benign vs. 86% malignant, $p < 0.001$)

Using the above criteria, the authors developed a scoring formula with a sensitivity of 93% and specificity of 93% [23], which demonstrates why radiographic imaging is routinely used to identify lesions that require surgical evaluation.

Of note, some ultrasound findings are surprisingly *not* useful to predict a benign or malignant diagnosis ($p > 0.05$), including:

- Unilaterality (86% benign vs. 71% malignant)
- Bilaterality (7% benign vs. 14% malignant)
- Average size (5.1 cm benign vs. 5.9 cm malignant)
- Maximum size (6.0 cm benign vs. 6.9 cm malignant)

9. What is the role of ancillary testing to diagnose ovarian lesions?

Specimens obtained from ovarian aspiration are generally cyst fluids, which should be sent to the clinical laboratory for marker assessment. Measurements of estradiol (E2), CA-125, carcinoembryonic antigen (CEA), and alpha-fetoprotein (AFP) can be useful. E2 is a compound that is elevated in functional cysts but absent in epithelial lesions [24, 25]. CA-125 and CEA can be useful as tumor markers but are generally considered to be nonspecific. An elevated AFP is commonly associated with germ cell tumors of the ovary but can also elevated in teratomas, sex cord-stromal tumors, and other ovarian epithelial neoplasms.

10. What are the adequacy criteria for an ovarian FNA?

A nondiagnostic interpretation can be rendered in cases of low cellularity or in cases of poorly preserved cells. Although strict adequacy has not been established for ovarian FNA, studies have used the criteria of at least six groups of epithelial cells to make a diagnosis [11].

11. What cellular components are found in aspirates from normal ovaries?

- **Ovarian Stroma**: Fragments of normal ovarian stroma will appear as cohesive groups of small spindle cells containing elongated nuclei with blunt or tapered ends. Ovarian fibromas will have a similar cytologic appearance to normal ovarian stroma, although they are unlikely to yield much cellular material due to dense (and often abundant) intercellular collagen.
- **Germinal Epithelium:** If follicles are aspirated, germinal epithelium will appear as flat sheets of epithelioid cells with oval nuclei, indistinct nucleoli, and a small-to-moderate amount of watery cytoplasm.
- **Simple Nonfunctional Cysts:** Unilocular cysts are a normal finding and often diagnosed at resection as cortical inclusion cysts, paratubal serous cysts, hydrosalpinx, or benign simple cysts. These cysts are indistinguishable by cytology, yielding small groups of cuboidal epithelial cells accompanied by foamy histiocytes. These cysts are considering "nonfunctional," meaning that normal hormonal cycles do not influence their growth.
- **Cystic Follicles and Follicular Cysts:** Cystic follicles and follicular cysts differ only in the size of the lesion, with follicles larger than ~2 cm designated as follicular cysts. Follicular lesions may represent a potential pitfall for cytologists, especially since ovarian FNAs are an uncommon specimen. Follicular cysts are benign physiologic (or "functional") cysts composed of two cell types; an inner

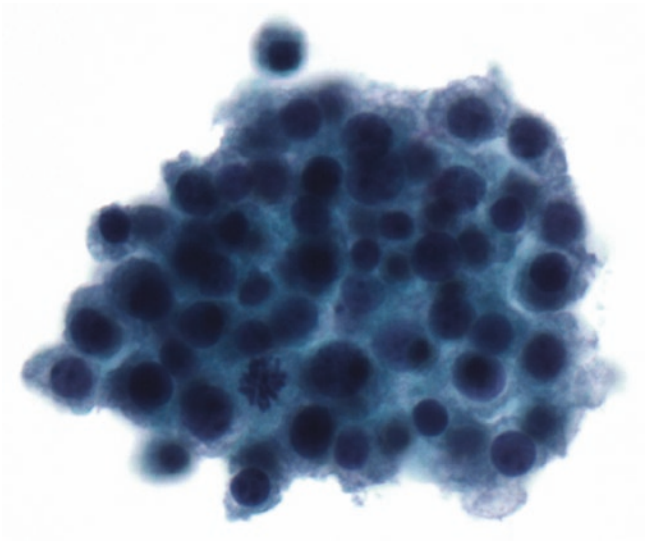

Fig. 11.1 Follicular cyst. Granulosa cells have granular and occasionally vacuolated cytoplasm and a large round-to-oval nucleus. These cells can appear immature, pyknotic, and mitotically active, mimicking malignancy (ThinPrep, Papanicolaou stain)

layer of granulosa cells overlying an outer layer of theca cells. Fluid from follicular cysts can be hypercellular, with the cells having granular and occasionally vacuolated cytoplasm and a large round-to-oval nucleus. The cells can appear immature, and mitotic figures are invariably present (Fig. 11.1); thus, it is not entirely surprising that follicular cysts are a common cause of false-positive results [26, 27]. The differential diagnosis includes granulosa cell tumor, carcinoid tumor, and serous neoplasia. Follicular cysts are composed of granulosa cells, so granulosa cell tumor should be excluded. Follicular cysts contain luteinized or nonluteinized granulosa cells, with the luteinized cells containing more abundant cytoplasm and larger nuclei.

- **Corpora Lutea and Corpus Luteum Cysts:** These functional cysts yield cellular aspirates with luteinized granulosa cells, appearing singly and in small clusters. These cells are epithelioid with round-to-oval eccentric nuclei and fine chromatin with prominent nucleoli. Luteal cells contain abundant granular cytoplasm with small vacuoles. The background may contain histiocytes, red blood cells, or hemosiderin-laden macrophages, especially in more advanced cysts. Corpus luteum cysts may arise during pregnancy and present as large lesions concerning for malignancy; aspirates may show marked cytoplasmic vacuoles or large cells with hyaline droplets [28].

12. What contaminants appear in ovarian aspirates?

Aspirates from ovarian lesions may contain contaminating epithelium from surround organs or those through which the needle passes. These include squamous epithelium indirectly sampled from transvaginal procedures and mesothelium from percutaneous abdominal aspirates. Columnar intestinal epithelium and mucus may be present if the needle pierces the intestines, and urothelial epithelium may be present if the bladder is punctured.

13. What are the diagnostic components of endometriomas (endometriotic cysts)?

Endometriomas are often referred to as "chocolate cysts" due to the characteristic thick dark brown fluid found within these lesions. The major component of endometriomas is hemosiderin-laden macrophages (HLMs) in a background of degenerated blood. Similar to endometriosis (discussed elsewhere), endometrioid cells can be found singly, in small clusters, or monolayered sheets. These cells represent ectopic endometrium, with benign round-to-oval nuclei with uniform chromatin and variable cytoplasm. Endometrial stromal cells, if present, appear as 3-dimensional cohesive clusters of spindles cells with oval nuclei and scant cytoplasm. Although most hemorrhagic cysts are endometriomas at histologic evaluation, benign and malignant neoplasia can also present in this way [3]. Thus, it is important to distinguish benign endometriomas from neoplastic hemorrhagic cysts, which also contain abundant HLMs.

14. How are benign follicular cysts distinguished from granulosa cell tumors by cytology?

Granulosa cell tumors exhibit nuclear atypia not seen in functional cysts, including pale and finely dispersed chromatin and nuclear membrane irregularities including grooves. However, nuclear grooves are not specific for granulosa cell tumor and are sometimes present in granulosa cells of functional cysts. Call-Exner bodies are frequently present in granulosa cell tumors. These are homogeneous aggregates of basement membrane surrounded by granulosa cells. Mitotic figures are not specific and can be found in either entity. Radiologic and clinical impressions of a benign cyst can be extremely helpful and reassuring.

15. What are the most common ovarian tumors?

Most ovarian tumors are benign (Table 11.1), including benign teratomas, cystadenomas, and stromal tumors, which comprise 71% of ovarian neoplasms [29].

Table 11.1 Most common ovarian tumors

Primary ovarian tumors	Frequency (%)
Benign cystic teratoma	32
Benign serous tumors	16
Benign mucinous tumors	14
Serous adenocarcinomas	9
Sex cord-stromal tumors	9
Borderline serous tumor	4
Endometrioid adenocarcinomas	3
Borderline mucinous tumor	1
Clear cell carcinoma	1
Mucinous adenocarcinoma	1

16. What are the most common histologic subtypes of epithelial ovarian tumors?

Tumor with serous histology is more common than other subtypes of epithelial ovarian tumors, followed by primary ovarian mucinous tumors (Table 11.2). Endometrioid and clear cell tumors of the ovary are relatively rare by comparison [30].

17. What are the key gross and cytologic features of benign epithelial-stromal tumors?

The most common benign epithelial-stromal tumors are serous cystadenomas. The adjective "serous" describes gynecologic epithelial tumors that appear similar to the ovarian surface and fallopian tube epithelium. Serous cystadenomas can be unilocular or multilocular, and the vast majority will contain clear fluid. The cyst wall will be smooth and may contain rounded nodules but will lack papillary excrescences. Many benign serous tumors are associated with mesenchymal stromal proliferation, and the term "-fibroma" is appended to the diagnosis to denote these lesions with a solid fibrous component (e.g., "serous cystadenofibroma").

Table 11.2 Histologic subtypes of ovarian epithelial tumors

Histologic subtype	Total (%)
Serous	46
Mucinous	36
Endometrioid	8
Clear cell	3
Other[a]	7

[a]Includes Brenner/transitional, undifferentiated, and mixed

Benign serous cysts contain cuboidal cyst-lining cells. In the absence of atypical features, ciliated cells with terminal bars, apical cytoplasm, and basal nuclei are diagnostic of benign serous cysts (Fig. 11.2). Psammomatous calcifications can be present. Benign-appearing spindle cells will be evident if the needle has sampled a solid fibrous component or normal ovarian stroma.

Brenner tumors are epithelial-stromal neoplasms that contain transitional (urothelial) type epithelium in a fibrous stroma. Benign Brenner tumors are typically solid and unilateral with a smooth cut surface. They can have microcysts, large cysts, or be associated with other benign (or malignant) ovarian neoplasms. Rarely, Brenner tumors can have borderline or malignant features (<1% of cases). The cytology of Brenner tumors can be difficult because the epithelial component can yield hypercellular aspirates whereas the fibromatous component may not sample well (Fig. 11.3). However, the epithelial cells are generally bland and polygonal with a generous amount of cytoplasm, and mitotic figures are rare. Brenner tumor epithelium is arranged in whorled nests inside fibrous stroma, a characteristic feature that make a cell block or concurrent core biopsy particularly helpful for the diagnosis.

18. How often are serous neoplasms benign?

Approximately, 50% of all serous tumors are benign at resection, and diagnostic entities include serous cystadenoma, serous adenofibroma, and serous cystadenofibroma (Table 11.3) [31]. Borderline tumors only comprise a small proportion of serous neoplasia.

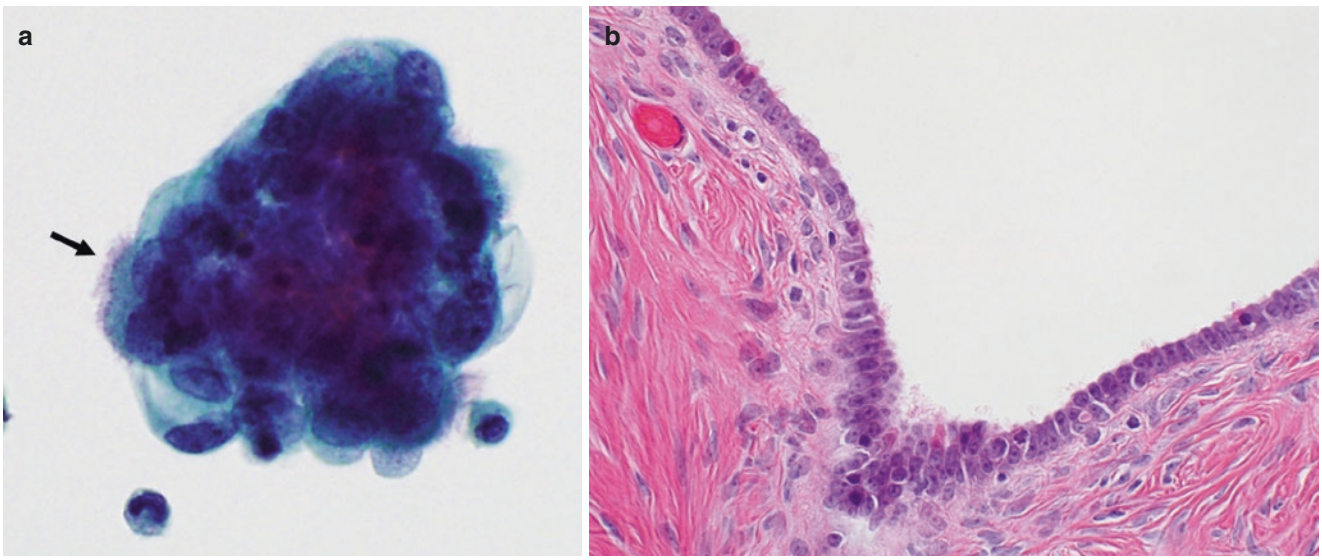

Fig. 11.2 Ovarian serous cystadenofibroma. (**a**) Groups of benign-appearing cells with cilia are typically seen in serous cystadenoma (ThinPrep, Papanicolaou stain). (**b**) Tumor demonstrates a simple lining of cuboidal epithelium overlying a dense fibrous component on resection. Note that many of the cyst-lining cells are ciliated (H&E stain)

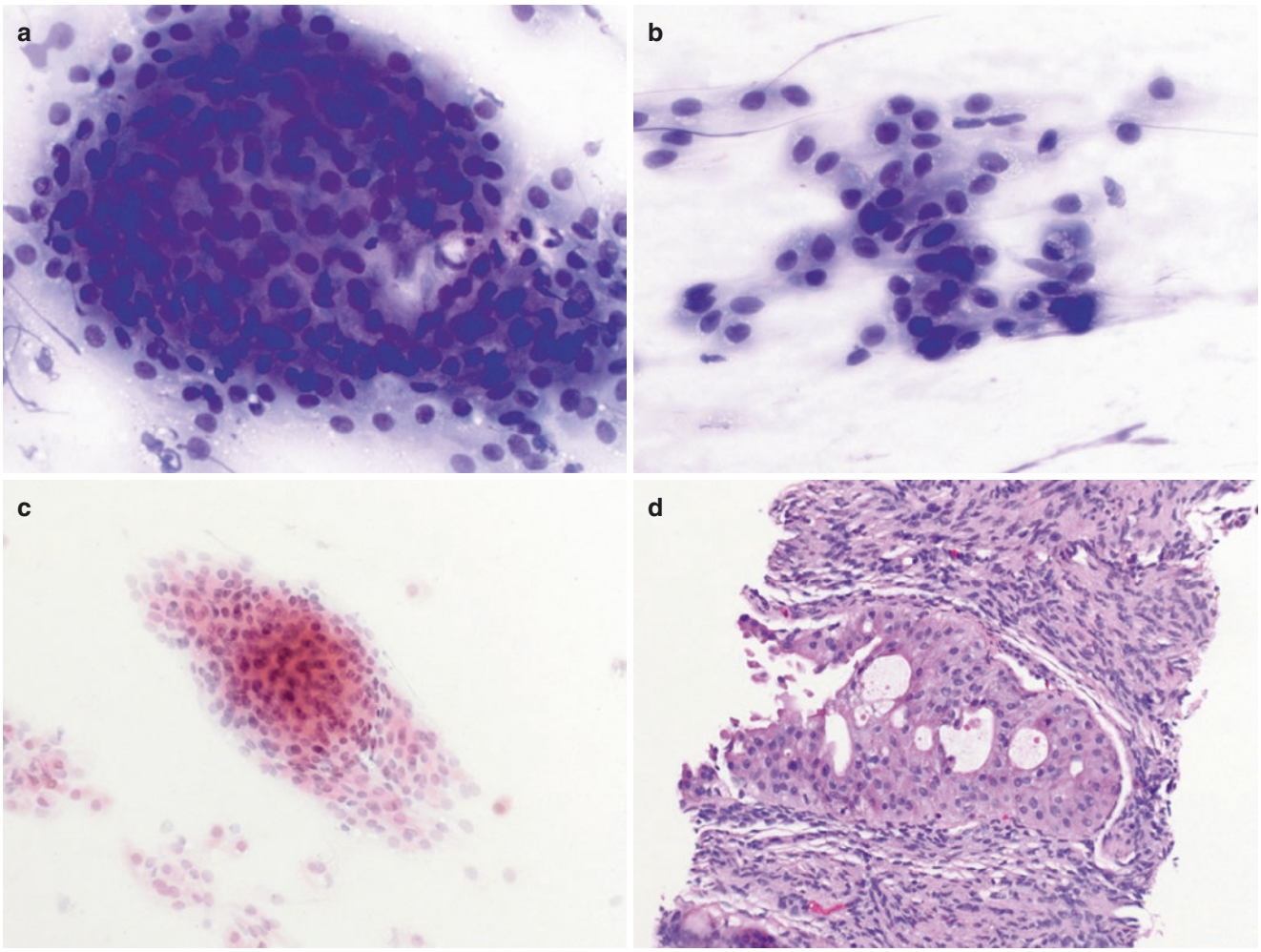

Fig. 11.3 Brenner tumor. (**a**) FNA of an ovarian Brenner tumor (air-dried smear) demonstrates whorled groups of benign-appearing cells with abundant cytoplasm (Diff-Quik stain). (**b**) Brenner tumors can be hypercellular, but the cells lack malignant features like mitotic figures and necrosis (Diff-Quik stain). (**c**) Alcohol-fixed preparation showing a whorled group of Brenner tumor cells (Papanicolaou stain). (**d**) Core biopsy demonstrates a rounded whorled group of epithelial cells in a background of cellular stroma (H&E stain)

Table 11.3 Histologic diagnoses of serous epithelial ovarian tumors

Diagnosis	Total (%)
Benign serous cysts	50
Serous borderline tumor	15
Serous adenocarcinoma	35

19. Is it possible to distinguish benign serous neoplasms, borderline serous tumors, and low-grade serous adenocarcinomas by cytology?

It is not possible to distinguish serous borderline tumors from low-grade serous adenocarcinomas by gross or cytologic examination. The histologic diagnosis of serous adenocarcinoma requires the presence of stromal invasion. For this reason, the term "low-grade serous neoplasia" is often used to describe lesions that have the cytologic appearance of borderline and low-grade serous adenocarcinoma. However, aspirates of serous borderline tumors (Fig. 11.4) will generally have less atypia and cellularity than those of low-grade serous adenocarcinomas (Fig. 11.5). The presence of nuclear atypia distinguishes low-grade serous tumors from benign serous cystadenomas, which exhibit bland and ciliated epithelium.

20. What are the key cytologic and immunohistochemical features of benign and malignant serous neoplasms?

It is not always possible to distinguish benign from malignant serous neoplasms by cytology, but there are some features common to either case (Table 11.4). Borderline neoplasms are tumors with uncertain malignant potential that still have a favorable prognosis even if they recur. Psammomatous calcifications are common in both serous borderline tumors and low-grade serous adenocarcinomas, found in approximately one-third of cases. Serous carcinomas are graded using a two-tier system that highly correlates

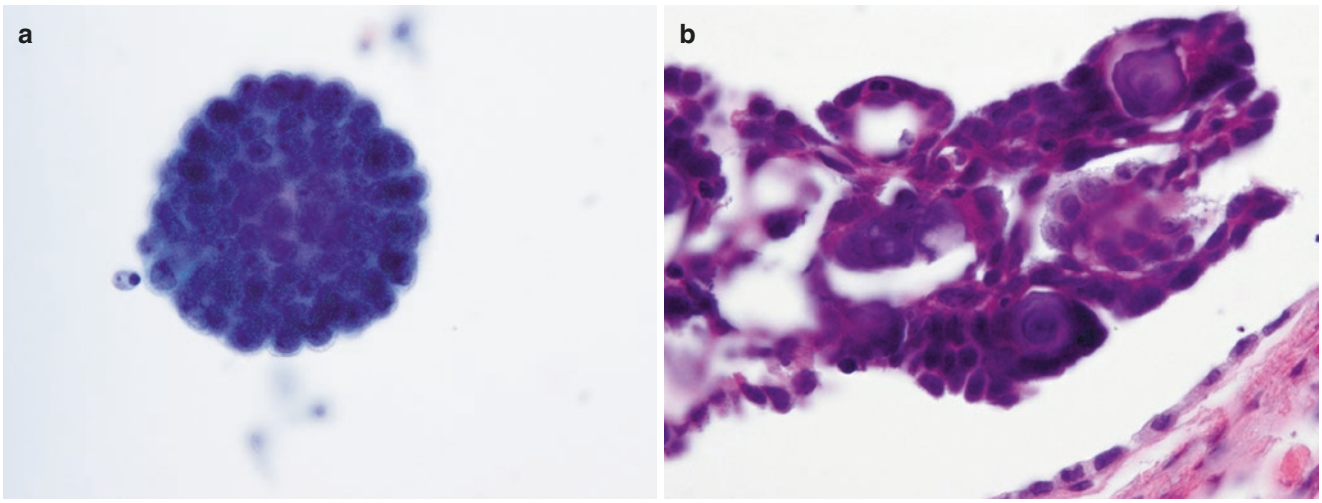

Fig. 11.4 Serous borderline tumor. (**a**) FNA of serous borderline tumors may be hypocellular with few cell groups, but lesional tissue should not demonstrate significant cytologic or nuclear atypia (Papanicolaou stain). In contrast to serous cystadenomas, cells do not have cilia. (**b**) Cell block preparation of the FNA demonstrated small strips of epithelial cells associated with psammoma bodies (H&E stain)

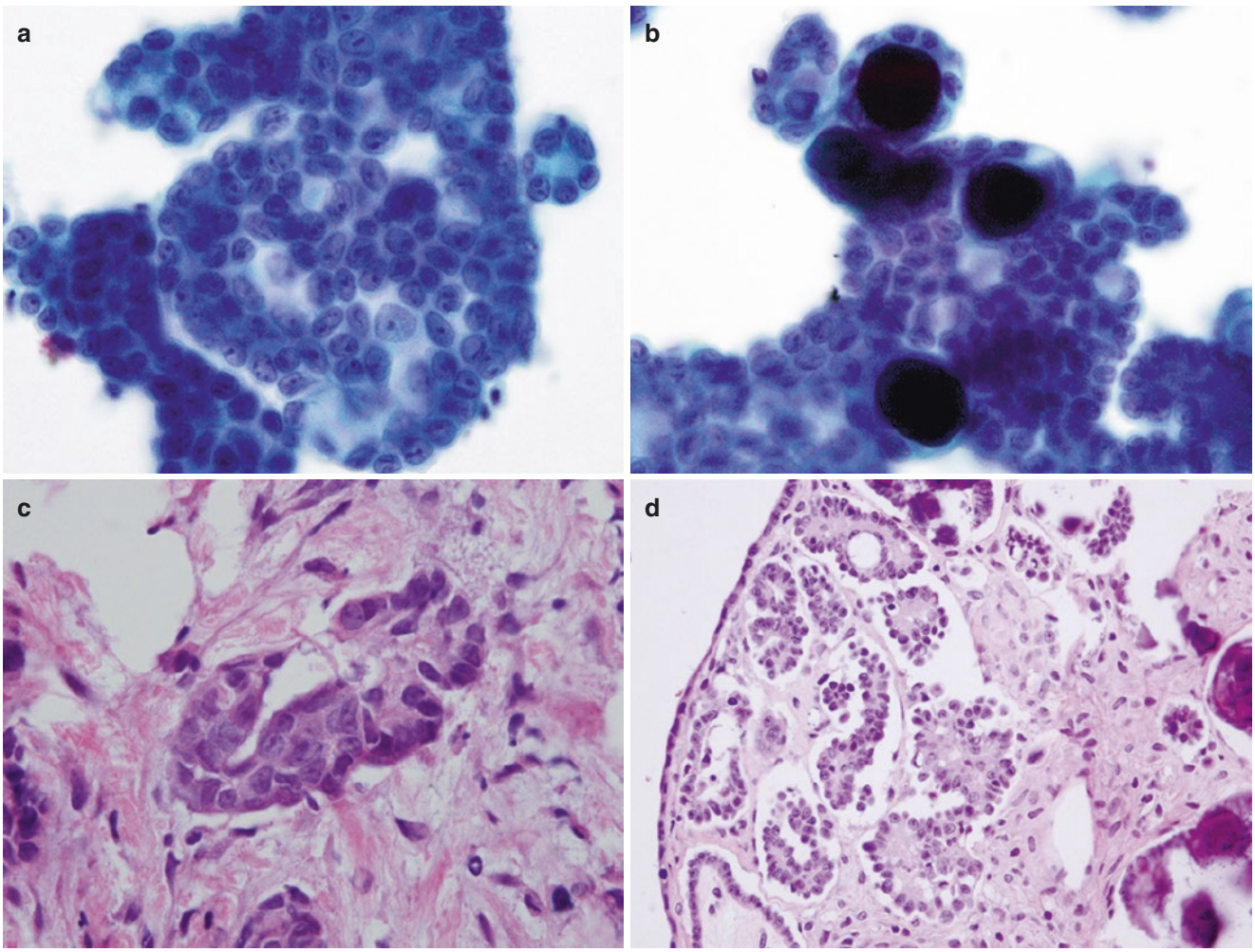

Fig. 11.5 Low-grade serous adenocarcinoma. (**a**) In contrast to serous cystadenomas and borderline tumors, this aspirate of a low-grade serous adenocarcinoma shows a crowded sheet of cells with enlarged irregular nuclei. (**b**) Psammoma bodies are frequent in low-grade serous adenocarcinomas. (**c**) Cell block preparations may reveal foci concerning for invasion. (**d**) Retraction artifact is commonly observed in low-grade serous adenocarcinomas and may be apparent in cell block fragments (**a**, **b**: Papanicolaou stain; **c**, **d**: H&E stain)

with long-term prognosis [32]. Low-grade serous carcinoma will appear in small clusters or in crowded sheets. Cytoplasmic vacuoles may be present, and cells will exhibit an increased nuclear: cytoplasmic ratio, with somewhat irregular nuclei and prominent nucleoli. High-grade tumors are more likely to yield positive washings than low-grade tumors and have frankly malignant cytology (Fig. 11.6) [33].

Table 11.4 Cytologic features of serous neoplasms

Benign serous tumors	Malignant serous tumors
Sparsely cellular	Higher cellularity associated with higher grade tumors; serous borderline tumors may be sparsely cellular and falsely negative
Bland, columnar, ciliated epithelium	Enlarged crowded cells with overlapping nuclei; irregular nuclear membranes; prominent nucleoli
Relatively clean background, some histiocytes; psammoma bodies rare	Psammoma bodies present in 30%
DDx includes cystadenoma, cystadenofibroma, cortical inclusion cyst, paratubal cysts, and hydrosalpinx	DDx includes serous borderline tumor and low-grade serous adenocarcinoma; overtly malignant cytologic features may indicate high-grade serous carcinoma

Serous neoplasms have characteristic immunohistochemical features that are sometimes essential for the diagnosis, especially when considering other tumor subtypes and metastatic lesions. Serous lesions are typically CK7 positive and CK20 negative, which can be helpful for distinguishing these from colorectal malignancies. Additionally, they are negative for CDX-2 and express PAX-8. WT-1 is a helpful positive marker because it distinguishes serous carcinoma from endometrioid adenocarcinomas and clear cell carcinomas of the ovary. Estrogen receptor (ER) and progesterone receptor (PR) exhibit variable positivity in serous carcinomas but are more likely expressed in low-grade neoplasms. In contrast to low-grade serous neoplasia, high-grade serous carcinomas almost always have a mutant (overexpression or loss) p53 phenotype and are diffusely and strongly reactive for p16.

21. What is the significance of p53 immunohistochemistry in high-grade serous carcinoma? How do I report p53 staining results?

The tumor suppressor gene, *TP53*, is mutated in ~96% of tubo-ovarian high-grade serous carcinomas [34], and immunohistochemistry for the protein product, p53, can act as a surrogate marker of *TP53* mutation status (Fig. 11.7).

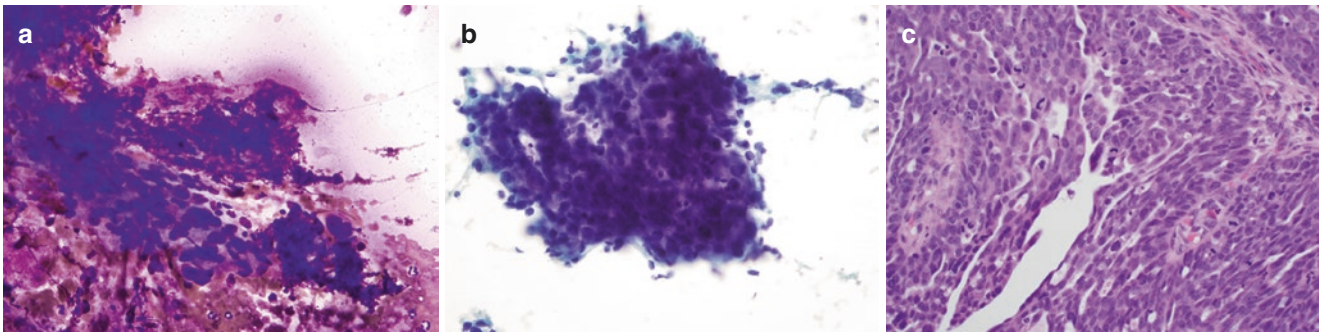

Fig. 11.6 High-grade serous carcinoma of the ovary. FNA smears demonstrate high-grade epithelial cells associated with necrosis (**a**: Diff-Quik stain; **b**: Papanicolaou stain). (**c**) Characteristic features of high-grade serous tumors include solid or papillary architecture, crowded overlapping cells, pleomorphic hyperchromatic nuclei, and abundant mitotic figures (cell block, H&E stain)

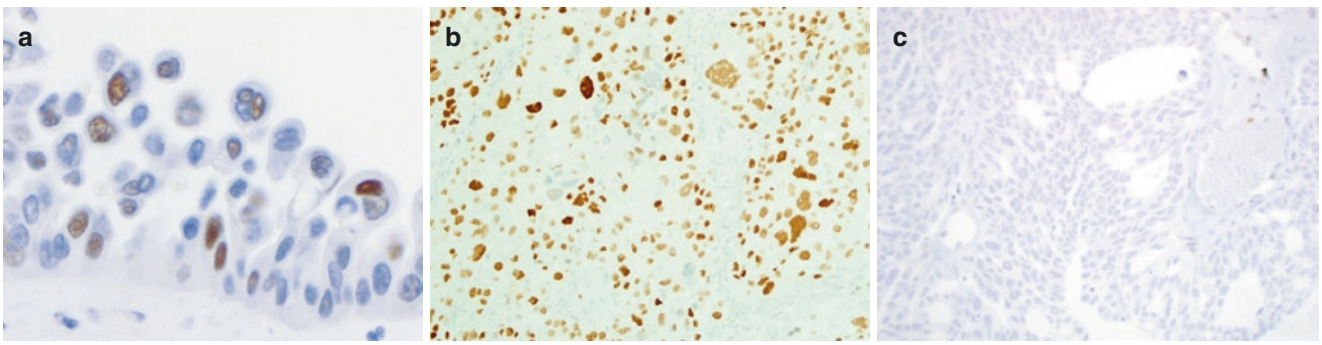

Fig. 11.7 Immunophenotypes of p53. (**a**) Wild-type staining pattern of p53 demonstrates heterogeneity, with variable strong and weak intensity seen in positive cells. (**b**) The most common mutant phenotype of p53 is a strong and diffuse staining pattern. (**c**) In a minority of cases, p53 demonstrates a null phenotype, indicating loss of the immunogenic portions of the p53 protein

Inactivating (missense) mutations of the gene result in increased nuclear expression of the p53 protein, yielding a diffusely and strongly positive staining pattern. Deleterious (nonsense and frameshift) mutations of *TP53* result in expression of a truncated protein, resulting in a null expression phenotype. The mechanism of p53 mutation is conceptually important because nonsense and frameshift mutations will still express N-terminal portions of the p53 protein, which may or may not be detectable by IHC. In one study correlating *TP53* mutation status with p53 expression, 62% had missense mutations (of which 100% had diffuse and strong p53 by IHC), 16% had nonsense mutations (of which 55% exhibited a null expression phenotype), and 13% had frameshift mutations (78% of which showed a null phenotype by IHC) [35].

If no mutations of *TP53* are present, p53 will display a wild-type staining pattern with weak expression in a heterogeneous distribution. Therefore, we recommend that p53 immunohistochemistry generally be reported in one of the following ways:

- POSITIVE p53 (diffuse and strong expression, mutant phenotype).
- POSITIVE p53 (heterogeneous expression, wild-type phenotype).
- NEGATIVE p53 (null expression, mutant phenotype).

22. How often is p53 mutated in gynecologic malignancies?

When confronted with adnexal masses and/or peritoneal implants, there is often a question of whether the tumor originated from the ovary, the uterus, or the peritoneum (the latter presumably arises from endometriosis or benign Müllerian inclusions). For the most part, the answer to this question is addressed after resection by surgical pathology. This is particularly true for primary peritoneal Müllerian disease because the diagnosis requires exclusion of ovarian and uterine primaries. However, p53 is a useful tool for separating low-grade from high-grade neoplasms, as well as high-grade serous from ovarian tumors with endometrioid and clear cell histology. This utility is best illustrated by examining the rate of p53 mutations in each of the tumor subtypes. Somewhat surprisingly, the p53 mutations occur at slightly different frequencies depending on the origin.

Primary Ovarian Tumors:

- 8% of borderline serous tumors have p53 mutations [36].
- 8% of low-grade serous carcinomas have p53 mutations [36].
- 96% of high-grade serous carcinomas have p53 mutations [34].
- 22% of endometrioid adenocarcinomas of the ovary have a mutant p53 immunophenotype [37].
- 0–3% of clear cell adenocarcinomas of the ovary have p53 mutations by sequencing [37, 38].

Primary Uterine Tumors:

- 90% of uterine serous carcinomas are found to have *TP53* mutations [39].
- 12% of endometrial endometrioid adenocarcinomas have a mutant p53 immunophenotype, including 40% of grade 3 tumors and only 3% of grade 1 and 2 tumors [40].
- 34% of clear cell adenocarcinomas of the uterus have a mutant p53 immunophenotype [41].
- 91% of uterine carcinosarcomas have p53 mutations (cBioPortal, TCGA provisional data).

Although useful for separating low-grade and high- grade serous tumors, p53 cannot distinguish high-grade tubo-ovarian from uterine serous carcinomas and carcinosarcomas. Only a small proportion of any endometrioid tumors will harbor p53 mutations, the vast majority of those being high grade. Clear cell carcinomas of the uterus exhibit p53 mutations more often than ovarian primaries.

23. What are the key cytologic and immunohistochemical features of endometrioid neoplasms of the ovary?

Endometrioid adenocarcinomas are usually cystic and solid tumors that are morphologically similar to endometrial endometrioid adenocarcinomas. In fact, these tumors are thought to arise from endometriosis and endometriomas, which are identified histologically in nearly half of ovarian endometrioid adenocarcinomas [31]. As seen in the endometrium, squamous differentiation is a common finding. Unlike serous and mucinous carcinomas, endometrioid borderline tumors are rarely encountered.

Cytologically, ovarian endometrioid adenocarcinomas are identical to those of the endometrium, with pseudostratified glandular cells in clusters with enlarged oval nuclei (Fig. 11.8). A cell block can be helpful to visualize well-differentiated endometrioid adenocarcinomas, but high-grade endometrioid lesions can be difficult to distinguish from other high-grade malignancies. Immunohistochemistry may be helpful, as these will typically have an immunoprofile strong positive for PAX-8, ER, and PR, patchy p16, wild-type p53 (unless high grade), and negative for WT-1.

24. What are the key cytologic and immunohistochemical features of clear cell neoplasms of the ovary?

Clear cell tumors of the ovary typically exhibit large, pleomorphic nuclei and abundant clear vacuolated cyto-

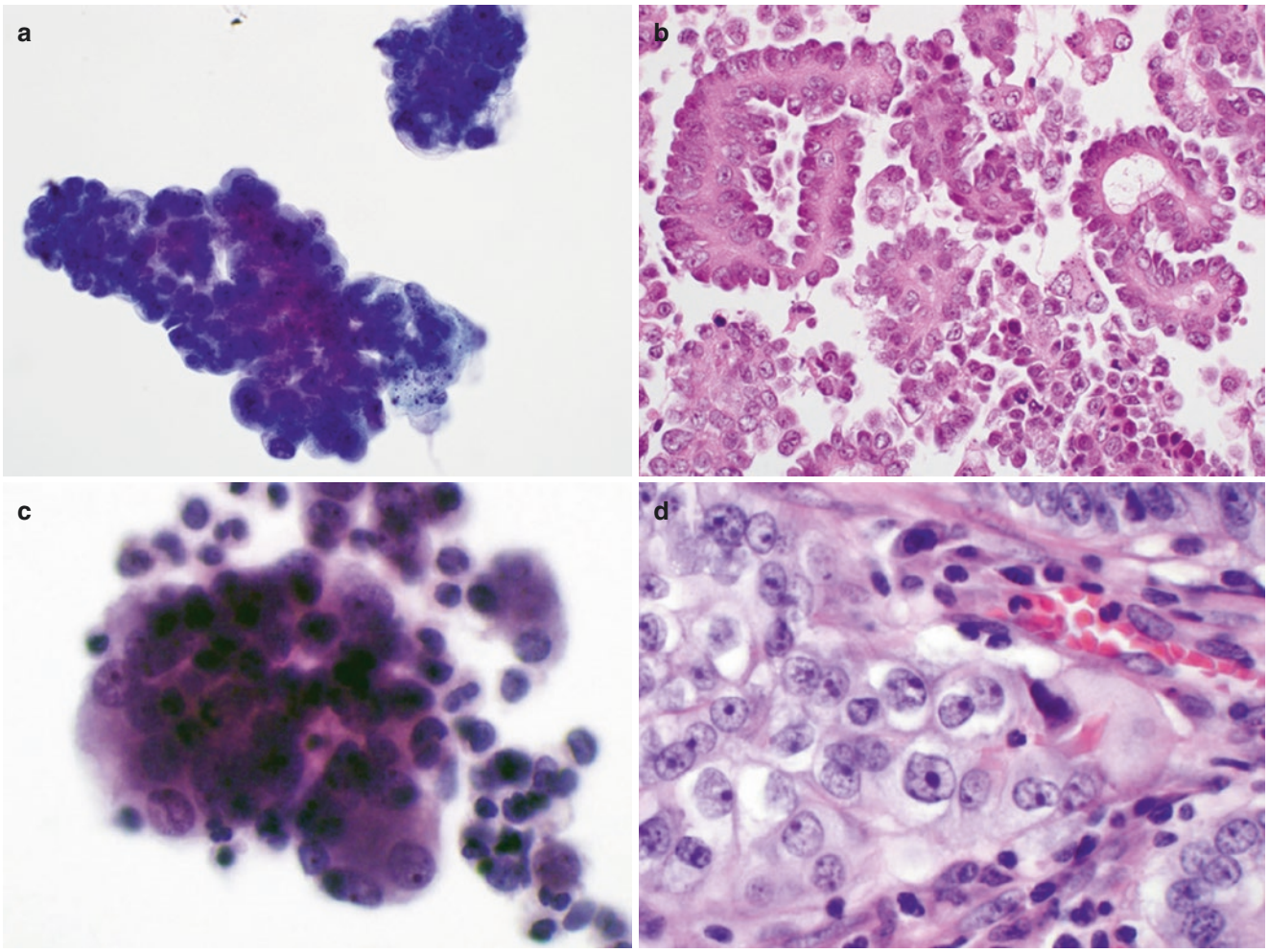

Fig. 11.8 Endometrioid adenocarcinoma of the ovary. (**a**) Peritoneal washing of a well-differentiated endometrioid adenocarcinoma of the ovary with clusters of atypical glandular cells (Papanicolaou stain). (**b**) The glandular architecture is best appreciated on the cell block sections (H&E stain). (**c**) FNA of a pelvic mass demonstrating high-grade endometrioid adenocarcinoma of the ovary (Papanicolaou stain). (**d**) Cell block preparation demonstrating high-grade pleomorphic nuclei with vesicular chromatin and prominent nucleoli (H&E stain)

plasm (Fig. 11.9). These tumors are considered high-grade malignancies, with <1% considered benign clear cell adenomas or borderline malignancies [31]. Immunohistochemistry is helpful if clear cell carcinomas are suspected, as they are typically positive for Napsin-A, AMACR, CK7, EMA, HNF1-B, wild-type for p53, and negative for ER, PR, and WT-1 [42]. Of note, clear cell carcinomas of both the ovary and kidney are positive for PAX-8, so other markers may be necessary if the primary site is uncertain.

25. What are the key cytologic and immunohistochemical features of ovarian mucinous tumors? Can they be distinguished from gastrointestinal metastasis?

Aspirates from mucinous tumors of the ovary often have a variable cytologic appearance (Fig. 11.10). Well-differentiated components may appear columnar with mucin vacuoles and exhibit only mild nuclear atypia. For this reason, it is difficult to distinguish mucinous borderline tumors from mucinous adenocarcinoma, and this is of minor concern to cytologists because as resection is required to exclude intramucosal carcinoma or invasion [43]. Mucinous ovarian tumors may cause pseudomyoxma peritonei, but the majority of adnexal mucinous tumors presenting with pseudomyxoma are in fact metastases from gastrointestinal sites. The finding of abundant mucin (in either peritoneal washings or an ovarian aspiration) is at least atypical, if not suspicious for a neoplastic process.

Mucinous adenocarcinomas of the ovary may arise from mucinous borderline tumors, and thus low-grade elements are suggestive of an ovarian primary rather than a metastasis [44]. Immunohistochemistry can be useful, as metastatic lesions from the colon and appendix will be positive for CK20, CDX-2, and SATB2 and largely negative for CK7, PAX-8, and WT-1. Often, however, a diagnosis of "mucinous cystic neoplasm" is sufficient to guide management [45, 46].

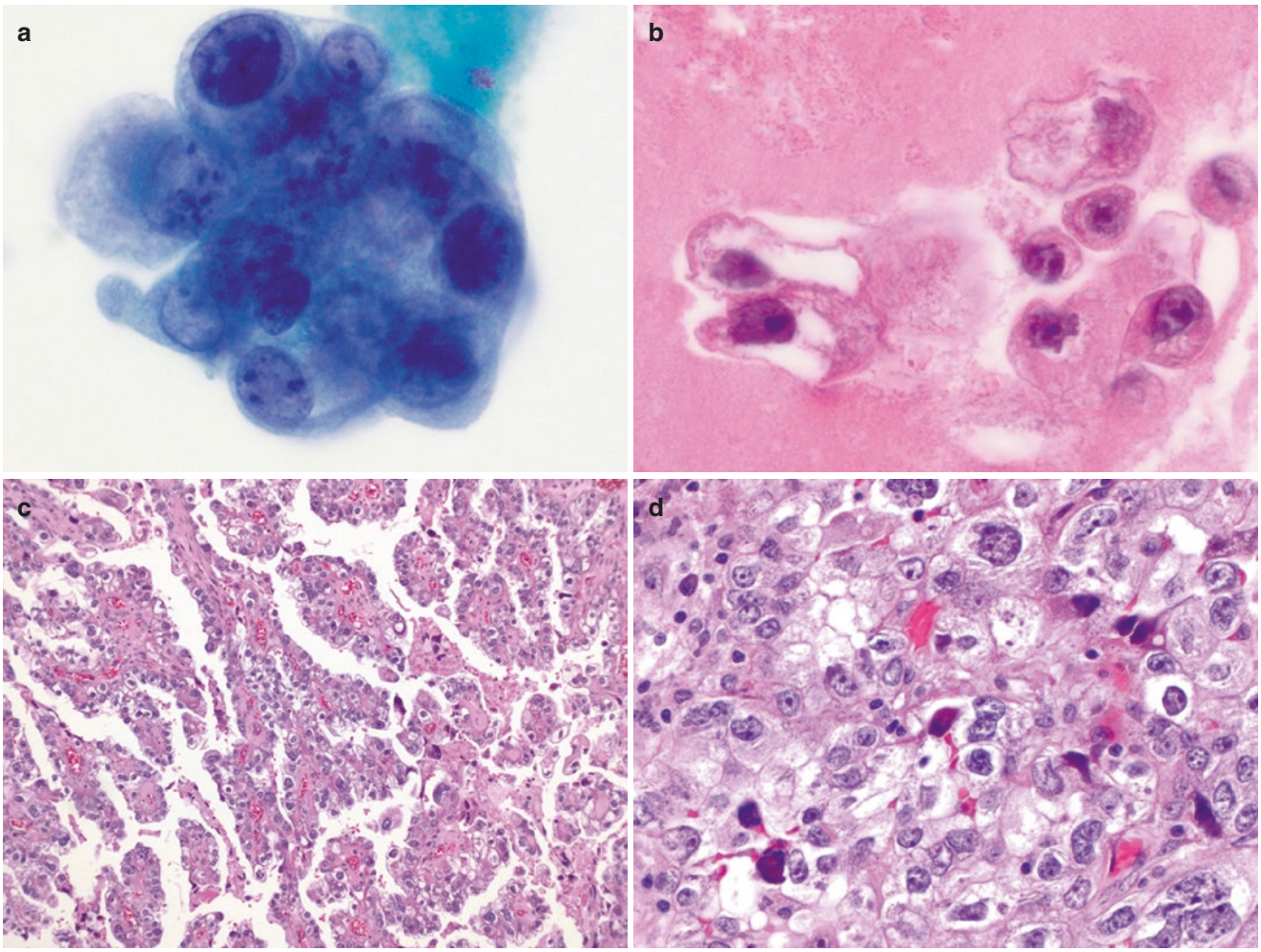

Fig. 11.9 Clear cell carcinoma of the ovary. Tumor cells have malignant nuclei and often exhibit abundant clear cytoplasm (**a**: ThinPrep, Papanicolaou stain; **b**: cell block, H&E stain). Resected tumor showed characteristic histologic features of clear cell carcinomas, namely malignant clear cells lining papillae in a "hobnail" appearance (**c**), and pleomorphic nuclei and abundant clear cytoplasm (**d**) (**c**, **d**: H&E stain)

26. What immunohistochemical markers are most helpful to subtype epithelial ovarian lesions? How should equivocal staining patterns be interpreted?

Subtyping of gynecologic malignancies has generally been reserved for surgical pathologists at the time of histologic resection, but advances in immunohistochemistry now provide cytopathologists with several tools for distinguishing gynecologic lesions (Table 11.5). Cytologic and histologic appearance and differential diagnosis should dictate the markers to be used. In the setting of equivocal staining patterns and a poorly differentiated gynecologic malignancy, a diagnosis of "high-grade Müllerian adenocarcinoma" can often be sufficient to guide further management.

27. What are the key cytologic and immunohistochemical features of germ cell tumors of the ovary?

Germ cell tumors of the ovary are analogous to those that arise in the testes. They most commonly present in women of reproductive age, and the vast majority of these are mature teratomas. However, care should be taken when examining specimens from pediatric patients, as malignant germ cell tumors are relatively much more common in this population.

Conceptually, there are three major categories of ovarian germ cell tumors, each of which have distinct cytomorphologic and immunohistochemical characteristics. If considering a germ cell tumor in the differential diagnosis, SALL-4 can be helpful first-line marker because it is positive in all three types of germ cell lesions. In cases of cytologically ambiguous germ cell tumors, the different types can often be distinguished by immunohistochemistry (Table 11.6).

- **Tumors with Embryonic Ectoderm, Mesoderm, and/or Endoderm Differentiation:** These tumors include mature and immature teratomas, the latter of which is malignant. Mature teratomas are not usually aspirated or biopsied because they frequently demonstrate characteristic ultrasonographic features. If aspirated, the most

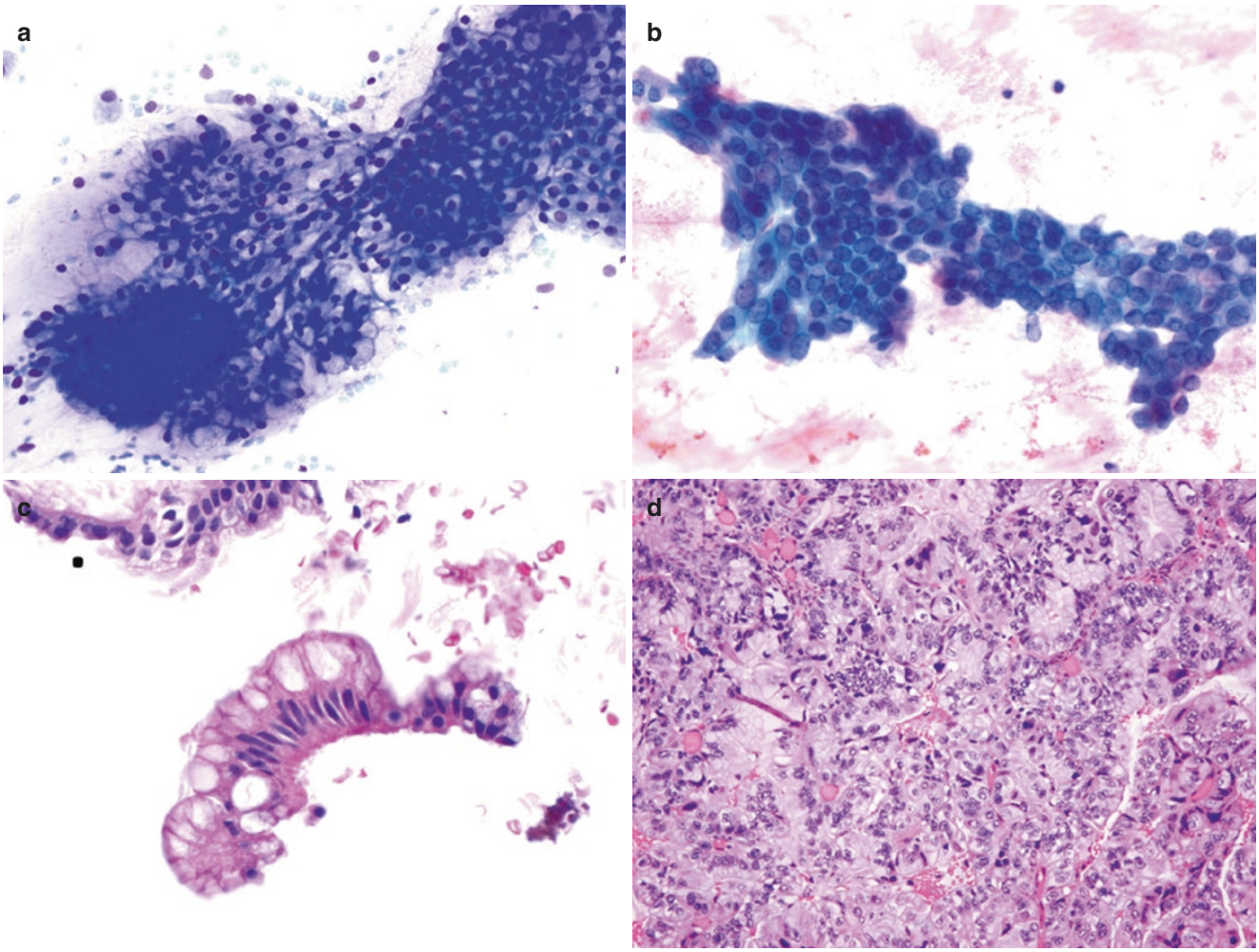

Fig. 11.10 Mucinous tumors of the ovary. FNA smear demonstrating sheets of benign-appearing mucinous epithelium (**a**: Diff-Quik stain; **b**: Papanicolaou stain) (**c**) Fragments of mucinous epithelium with goblet cells seen on a cell block, reminiscent of gastrointestinal epithelium. (**d**) At resection, mucinous tumors must be heavily sampled for focal areas of mucinous adenocarcinoma which are often missed on cytology (**c**, **d**: H&E stain)

Table 11.5 Summary of immunohistochemical staining of various epithelial ovarian tumors and mucinous metastases from gastrointestinal primaries

Ovarian tumor subtype	CK7	CK20	PAX-8	WT-1	CDX-2	SATB2	ER	PR	Napsin-A	AMACR	p53	p16
High-grade serous carcinoma	+	–	+	+	–	–	V	V	–	–	Mutant	Diffuse
Low-grade serous neoplasia	+	–	+	+	–	–	+	+	–	–	WT	-/F
Endometrioid type histology	+	–	+	–	–	–	+	+	–	–	V	V
Clear cell type histology	+	–	+	–	–	–	–	–	+	+	V	-/F
Mucinous (primary ovarian)	+	V	V	–	V	–	–	–	–	–	WT	-/F
Mucinous (metastatic)	–	+	–	–	+	+	–	–	–	–	V	-/F

Abbreviations and symbols: + positive, – negative, *V* variable, *WT* wild-type, *F* focal

Table 11.6 Immunohistochemical markers for malignant ovarian germ cell tumors

Malignant germ cell tumor	SALL-4	Pan-K	OCT-3/4	NANOG	c-Kit	AFP	hCG	GATA-3
Dysgerminoma (~50%)	+	Rare	+	+	+	–	–	–
Yolk sac tumor (~20%)	+	+	–	–	Rare	+	–	–
Embryonal carcinoma (3%)	+	+	+	+	–	–	–	–
Nongestational choriocarcinoma (1%)	+	+	–	–	–	–	+	+
Immature teratoma (20%)	+	–	+	–	–	–	–	–

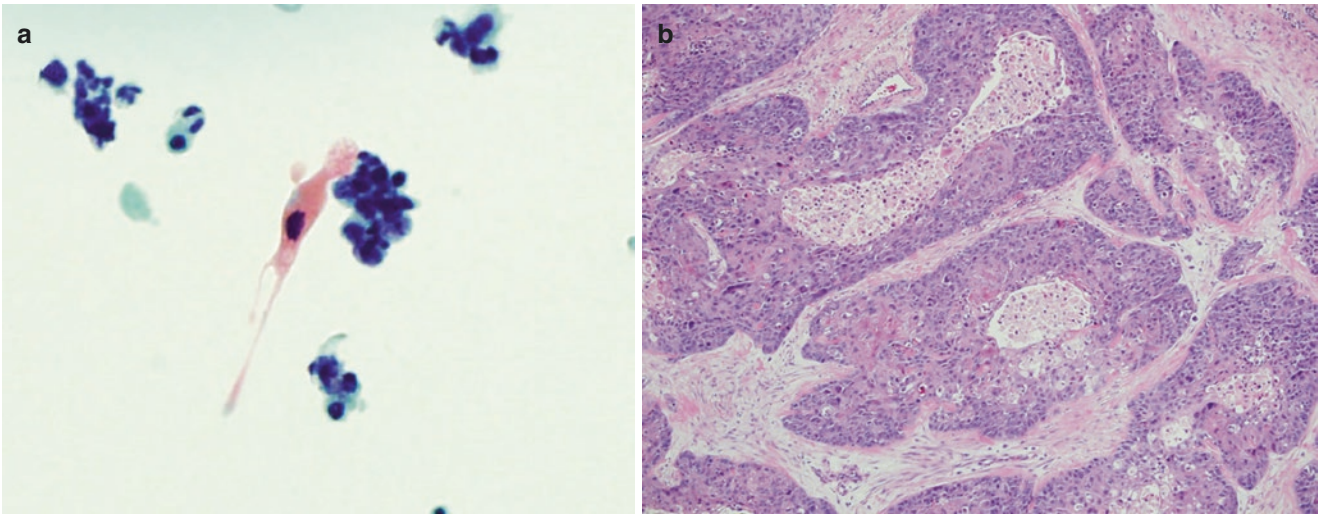

Fig. 11.11 Squamous cell carcinoma arising from mature teratoma. (**a**) Peritoneal washings from a patient showed dysplastic squamous cells in a background of reactive mesothelial cells (Papanicolaou stain). The differential diagnosis includes metastasis from a cervical lesion, but pap history and colposcopic findings may be negative. Immunohistochemistry with p16 may be helpful to exclude a cervical lesion. (**b**) Resection of an adnexal mass revealing squamous cell carcinoma arising from an ovarian mature teratoma (H&E stain)

common finding is anucleated squamous cells, which are indicative of ectodermal differentiation. Ectopic tissue from other organs can be present, including thyroid (struma ovarii), and, though uncommon, can undergo malignant transformation, leading to somatic-type malignancies such as primary ovarian thyroid carcinomas (positive for TTF-1 and PAX-8 by IHC) and squamous cell carcinomas (p63 positive by IHC, Fig. 11.11), which often portend a poor prognosis. All mature teratomas must be resected and evaluated for the presence of immature neuroectodermal elements, which are rare but diagnostic of malignancy.

- **Tumors that Express Transcription Factors of Pluripotency (i.e., OCT3/4 and NANOG):** This group of malignant germ cell tumors includes dysgerminomas (the ovarian equivalent of testicular seminomas) and embryonal carcinomas. Of the two, dysgerminomas are much more common, representing up to 5% of all ovarian malignancies. An accurate diagnosis of dysgerminoma is extremely important because these tumors respond well to therapy. Dysgerminomas often appear poorly differentiated cytologically, with large round nuclei, prominent nucleoli, and clear cytoplasm. Dysgerminomas will stain positive for the transcription factors associated with stem cell pluripotency, OCT-3/4 and NANOG. In fact, it is thought that dysgerminomas may be precursors of other germ cell tumors, a theory which may explain why 10% of germ cell tumors have mixed cell types. Embryonal carcinomas may stain positive for these markers as well. Cytologically, embryonal carcinoma will exhibit large round cells with irregular pleomorphic nuclei and multiple chromocenters. The two can often be distinguished by IHC, as dysgerminomas are typically keratin-negative and exhibit membranous staining for c-Kit (CD117).
- **Tumors with Extraembryonic Differentiation:** The germ cell tumors in this category include yolk sac tumor and nongestational choriocarcinoma. Both can appear poorly differentiated malignant epithelioid neoplasms, and the fact that they are keratin-positive can lead to a potential pitfall by rendering a diagnosis of carcinoma. Yolk sac tumors will be positive for alpha-fetoprotein and SALL-4. Choriocarcinomas will be positive for human chorionic gonadotropin (hCG), GATA-3, and SALL-4.

28. What are the key cytologic and immunohistochemical features of sex cord-stromal tumors of the ovary?

Sex cord-stromal tumors of the ovary are benign in 90% of cases. The class of benign sex cord-stromal tumors is comprised of fibromas (which derived from ovarian stromal fibroblasts), thecomas (derived from hormone-secreting ovarian stromal cells), or mixed tumors with features of both fibromas and thecomas. Aspirates of these tumors are generally hypocellular, because they are often solid tumors with abundant intracellular collagen. Fibromas are composed of spindle-shaped cells with an unsurprising fibroblast-like appearance. Pure thecomas are rare, but thecomatous cells generally have a monomorphic appearance with clear cytoplasm and varying degrees of vacuolization.

Sex cord-stromal tumors of the ovary with malignant potential include adult granulosa cell tumor, juvenile granulosa cell tumor, Sertoli-Leydig cell tumor, and steroid cell tumors. Not all sex cord tumors of the ovary are easily cate-

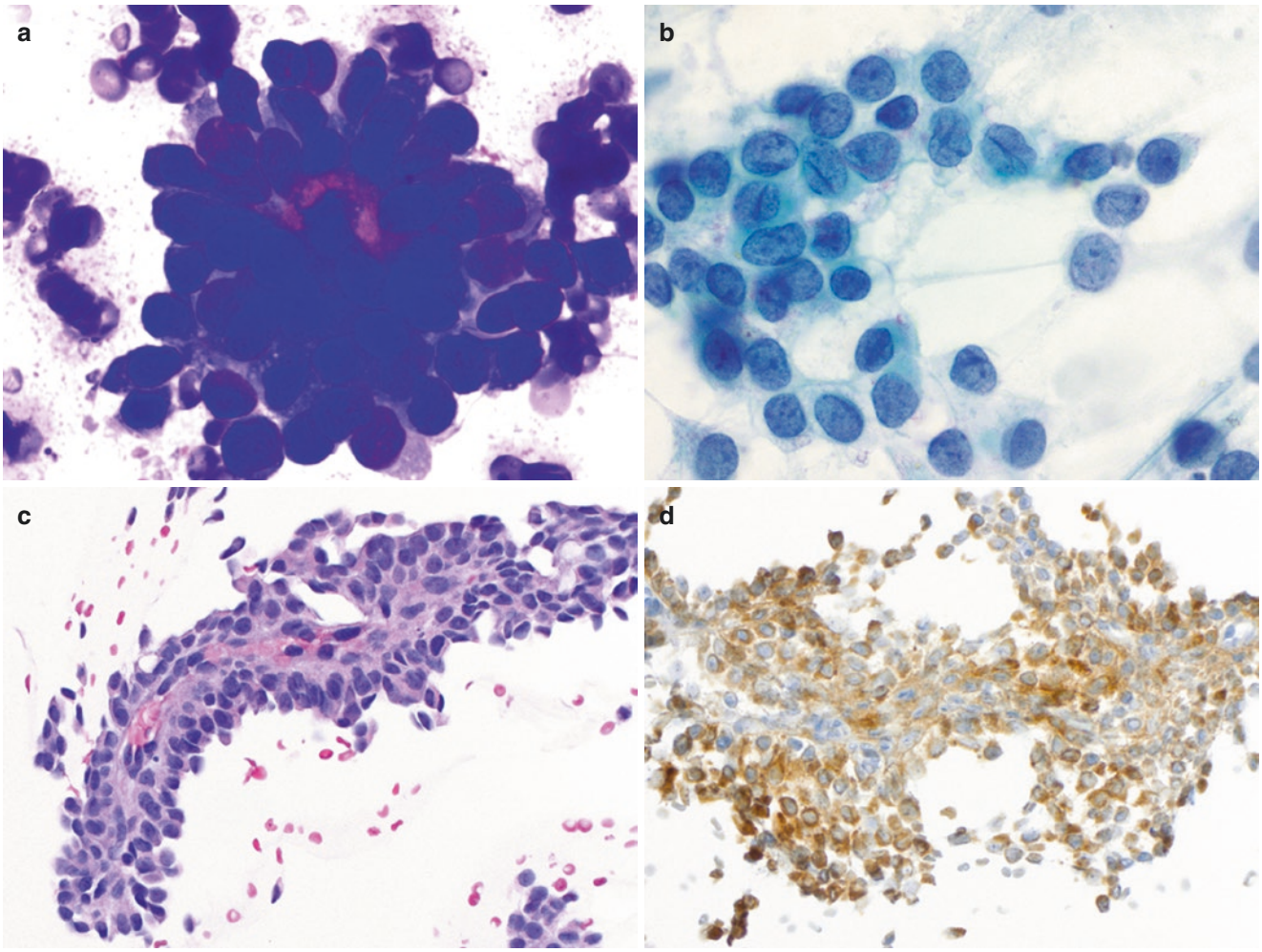

Fig. 11.12 Adult granulosa cell tumor (AGCT). (**a**) Diff-Quik stain of an AGCT illustrating a pseudofollicular arrangement (Call-Exner body). (**b**) The nuclear grooves and irregularities ("coffee bean nuclei") of AGCT are best visualized on alcohol-fixed preparations (Papanicolaou stain). (**c**) Cell block of AGCT, demonstrating a papillary-like architecture (H&E stain). (**d**) Inhibin IHC is commonly used to diagnose AGCT, which will demonstrate cytoplasmic positivity

gorized, sometimes prompting a diagnosis of "unclassified sex cord-stromal tumor."

Adult granulosa cell tumors (AGCT) are typically unilateral and confined to the ovary, but patients can present with ruptured tumors or peritoneal spread. These tumors are predominantly solid, composed of monomorphic neoplastic cells with scant cytoplasm that can appear singly, as naked nuclei, in loose clusters, in cords, or in a pseudofollicular pattern (Fig. 11.12). This latter architecture, termed Call-Exner bodies, describes a pattern of granulosa cells arranged around small globules of eosinophilic hyaline. Other helpful cytologic features of granulosa cells include prominent nuclear grooves and nuclear membrane irregularity. The distinction between AGCT and follicular cysts can be particularly challenging, as immunohistochemistry is unlikely to help, but the presence of predominantly normal granulosa cells can be helpful.

As the name suggests, juvenile granulosa cell tumors (JGCT) are more commonly seen in young patients. There are a few features which distinguish the juvenile from the adult variants. Foremost, there are genetic differences between the two because the vast majority of AGCTs harbor mutations of the transcription factor, *FOXL2*, which is wild-type in 90% of JGCTs [47]. From a cytologic perspective, the tumor cells of JGCT lack nuclear grooves and Call-Exner bodies, and the tumor nuclei appear round with fine chromatin and small chromocenters.

Immunohistochemistry can be very helpful in distinguishing sex cord-stromal tumors from other ovarian lesions. WT-1 and SF-1 are considered pan-markers of sex cord-stromal tumors [48]. Other markers, such as inhibin, calretinin, and CD99, are variably expressed in benign sex cord-stromal tumors, although they are more often positive in the malignant entities.

29. What are the key cytologic and immunohistochemical characteristics of small cell carcinomas of the ovary?

Small cell carcinomas of the ovary are malignant tumors composed of small round blue undifferentiated cells and are divided into two entities:

- **Small Cell Carcinoma of the Ovary, *Hypercalcemic Type*:** A poorly differentiated epithelial tumor that is associated with paraneoplastic hypercalcemia in 62% of cases. These tumors typically occur in younger patients (mean 24 years of age) and have a poor overall survival rate. Tumors will express CD56, synaptophysin, and, occasionally, parathyroid-related hormone. Inactivating mutations of the chromatin remodeling enzyme, SMARCA4, are often found in these tumors [49], and thus immunohistochemistry for SMARCA4 loss can particularly be helpful in the diagnosis, which distinguishes it from its pulmonary type counterpart.
- **Small Cell Carcinoma of the Ovary, *Pulmonary Type*:** A poorly differentiated tumor that expresses neuroendocrine markers analogous to small cell carcinoma of the lung. The primary differential diagnosis includes hypercalcemic type primary ovarian small cell carcinoma and metastasis from a lung primary. The presence of a lung mass and TTF-1 positivity by IHC favors a metastatic lesion.

30. What are the key features of metastatic tumors of the ovary?

Metastatic lesions to the ovary are common and account for almost 10% of malignant ovarian neoplasms found in women undergoing surgery for an adnexal mass. Common features of metastasis to the ovary include bilateral ovarian involvement, surface involvement, a nodular pattern of spread, small size (<10 cm), and history of a known nonovarian primary malignancy. In contrast, primary ovarian tumors are typically unilateral, large (>10 cm). Ovarian involvement commonly presents with metastases from colorectal (37%), breast (12%), gastric (9%), appendiceal (9%), pancreas (6%), and lung (2%) primaries.

Peritoneal Washings

1. What is the purpose of peritoneal washings?

The primary purpose of peritoneal washing cytology is to identify metastatic disease in the peritoneum that is not grossly visible, typically at the time of staging laparoscopy or resection. Peritoneal washings are obtained during benign gynecologic procedures to help exclude occult disease. In the setting of known metastatic disease, peritoneal washings can be used to monitor treatment response.

Washings are particularly important for gynecologic oncologists because cytologic evaluation is part of the staging system for fallopian tube and ovarian cancers [50]. Historically, peritoneal washings were evaluated in the staging of endometrial cancers (indicating stage IIIA disease), but the International Federation of Gynecology and Obstetrics (FIGO) revised staging criteria in 2009, and washing cytology was removed from the staging criteria [51].

2. What are the prognostic implications of positive peritoneal washings in gynecologic malignancies?

For endometrial cancers (stage I to IIIa), peritoneal washing cytology is an independent predictor of disease recurrence and mortality, and, in advanced stage patients, metastasis to the adnexa or uterine serosa does not seem to confer a worse prognosis than positive cytology alone [52]. It has been discovered that laparoscopically assisted vaginal hysterectomies have a higher incidence of positive peritoneal cytology compared to total abdominal hysterectomy, possibly due to retrograde dislocation of cancer cells during manipulation of the uterus [53], but the clinical significance of this appears to be minimal [54].

For cancers of the fallopian tube and ovary, tumors associated with positive peritoneal washings or ascites are classified as FIGO stage IC3, if they are otherwise confined to the adnexa and the washings are not associated with surgical spill intraoperatively (IC1), capsule rupture prior to surgery (IC2), or tumor on the ovarian surface (also IC2) [55]. Of note, the American Joint Committee on Cancer (AJCC) TNM staging does not make this distinction, and these three sub-stages are considered together simply as T1c. For invasive epithelial ovarian cancer, the 5-year survival for patients with stage IC is 81%, compared to 92% for IA and 14% for stage IV malignancies [56].

3. How often do peritoneal washings change the surgical staging in patients with gynecologic cancers?

In reality, surgical staging may not change very often, and this may be one of the reasons that washing status was eliminated from the FIGO staging criteria for endometrial cancer. A positive peritoneal washing upstages only 4.5% of patients and does not appear to affect outcomes [57].

For ovarian cancer, it has been estimated that peritoneal washings will upstage as many as 25% of patients with low stage disease [58], but it is important to note that the detection rate of peritoneal washings in otherwise stage IA or IB patients is heavily dependent on tumor subtype, with serous carcinomas more often positive than other variants [59]. FIGO stage IC (TNM T1c) accounts for 18.7% of all ovarian tumors [56].

4. Does ovarian cyst rupture during surgery lead to worse prognosis in the absence of surface involvement or positive ascites/washings?

This remains a controversial issue, with some studies finding a higher risk of recurrence and others not. Ovarian cyst rupture is always avoided if possible during primary resection of tumors confined to the adnexa because multivariate analysis has shown that capsule rupture and positive peritoneal washings are independent predictors of poor prognosis [55].

5. Are peritoneal washings obtained in any nongynecologic surgeries?

Although not part of the TNM staging of any nongynecologic malignancies, positive washings are often obtained during resection procedures because they are associated with poor prognosis in abdominal malignancies:

- In patients with gastric adenocarcinomas, positive washings are associated with advanced stage and poor overall survival [60].
- In patients undergoing surgery for colorectal cancer, positive peritoneal washings had a significantly higher rate of local recurrence and peritoneal carcinomatosis than those with negative washings [61], and long-term follow-up has revealed that the 10-year survival rate for patients with positive cytology is less than those with negative washings [62].
- In patients with pancreatic cancer, there is a significant correlation between positive peritoneal cytology and the presence of peritoneal metastases [63], and survival is typically worse than patients with negative cytology [64].

6. How accurate is peritoneal washing cytology versus ascites? If a peritoneal biopsy is positive for malignant cells, do peritoneal washings provide any additional information?

There are a number of important concerns about the accuracy of peritoneal washings. First, many patients with metastases to the peritoneum will have negative washings, with up to ~50% of patient's having false-negative cytology [65]. However, as stated above, the detection rate (and therefore the sensitivity and specificity) is highly dependent on tumor subtype [59]. For instance, in cases of low-grade serous neoplasia, the sensitivity is relatively high and strongly correlates with ovarian surface involvement and peritoneal implants [66].

Evaluation of ascites fluid has a false-negative rate that is about 6%, much less than that of peritoneal washings [65]. If histologic biopsy confirms peritoneal involvement, peritoneal washings provide no additional information, and the patient will be staged based on the results of the biopsy.

7. What common conditions lead to false-positive washings?

False-positive peritoneal washings occur in less than 5% of case [67], which can result in the following conditions:

- Mesothelial proliferation with psammomatous calcifications [68]
- Endometriosis, particularly with eosinophilic metaplasia [69]
- Endosalpingiosis [70]
- Ectopic pancreas [71]

The presence of Müllerian epithelium associated with psammomatous calcifications should prompt the cytologist to render an atypical diagnosis (Fig. 11.13).

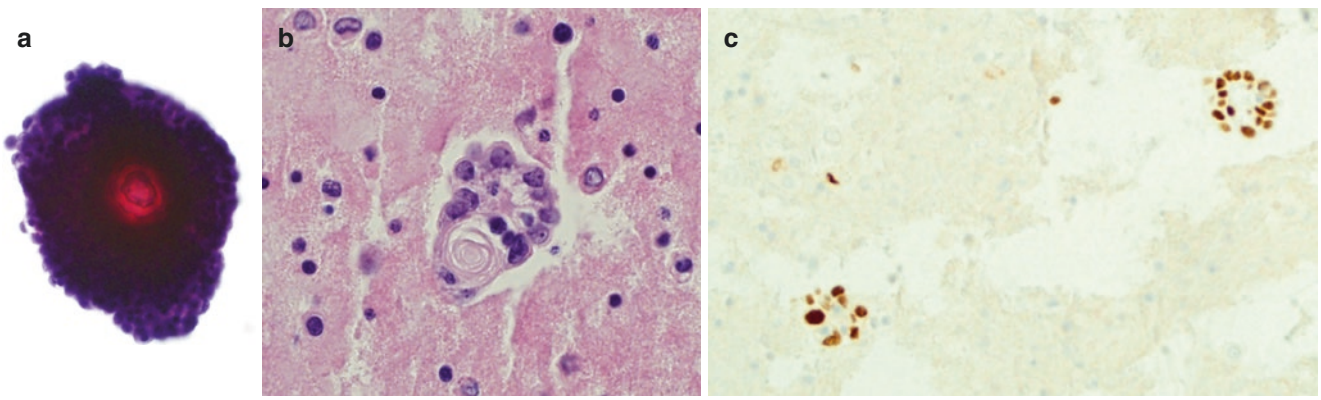

Fig. 11.13 Benign-appearing Müllerian proliferations associated with psammomatous calcifications. (**a**) Diff-Quik stain demonstrating a three-dimensional cluster of small, benign-appearing cells associated with a psammoma body. The differential diagnosis includes a mesothelial proliferation versus a benign Müllerian inclusion (such as endometriosis or endosalpingiosis) versus an implant of low-grade serous neoplasia. (**b**) Cell block with H&E stain demonstrating epithelium associated with a concentrically laminated calcification. Unless stroma is present, it is not possible to tell if the cells are derived from an invasive or a noninvasive implant. (**c**) Immunohistochemistry for PAX-8 will exhibit strong nuclear positivity in Müllerian epithelium, which is usually negative in mesothelial cells

8. Is there any reason to segregate washings from different peritoneal sites?
Peritoneal washings should be obtained from different peritoneal locations, which should be combined into a single specimen. There appears to be no benefit to segregating samples [72].

9. What are the adequacy criteria for a peritoneal washing specimen?
Strict adequacy criteria have not been established for peritoneal washings, but the presence of benign mesothelial cells should be identified before considering a specimen adequate. If malignant cells are present, the specimen should also be considered adequate [67].

10. How useful are atypical and suspicious interpretations of peritoneal cytology?
In general, peritoneal washings that are interpreted as "atypical" or "suspicious" are not useful to clinicians, and anything less than a malignant diagnosis is considered as a negative result [67].

Case Presentations

Case 1
Learning Objectives:

1. Review the cytology of mucinous tumors of the ovary.
2. Generate a differential diagnosis for mucinous tumors of the ovary.
3. Understand how IHC can differentiate primary ovarian from metastatic mucinous lesions in the ovary.

Case History:

- A 58-year-old female presents with abdominal distention and an elevated CA-125. CT reveals a 22-cm multiloculated cystic pelvic mass. The ovaries are not well visualized on imaging.

Specimen Source:

- U/S-guided FNA of a pelvic mass

Cytologic Findings:

- Abundant mucin admixed with inflammatory cells (Fig. 11.14a).
- Small group of mucinous cells without significant cytologic atypia (Fig. 11.14b).
- Cell block demonstrating strips of mucinous epithelium and stroma (Fig. 11.14c).
- Low-grade components are more likely to be found in primary ovarian mucinous tumors than metastases.

Differential Diagnosis:

- Mucinous cystadenoma of the ovary
- Mucinous borderline tumor of the ovary
- Mucinous adenocarcinoma of the ovary
- Metastatic mucinous adenocarcinoma

IHC and Other Ancillary Studies:

- Definitive subtyping requires resection and histologic evaluation for invasion.
- CK7 and PAX-8 positivity would favor an ovarian primary.
- CK20 and CDX-2 positivity would **not** exclude an ovarian primary.
- SATB2 would strongly favor metastasis from an appendiceal or a colorectal primary.

Final Cytologic Diagnosis:
Mucinous cystic neoplasm
Take-Home Messages:

1. Mucinous tumors of the ovary can be benign or malignant.
2. Mucinous tumors of the ovary require resection and histologic evaluation to correctly subtype.
3. Immunohistochemistry is often not definitive but can be helpful to favor an ovarian primary.

References: [43–46]

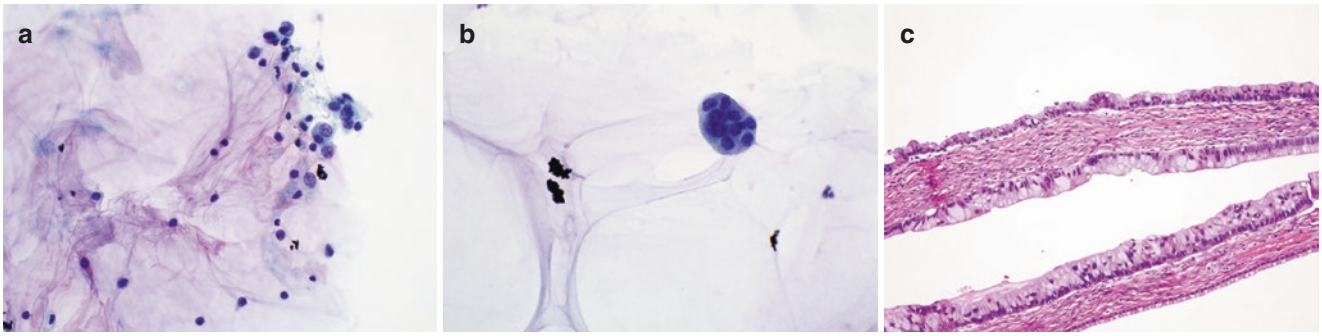

Fig. 11.14 Case 1: Pelvic mass, FNA. (**a**, **b**) Images of alcohol-fixed smear (Papanicolaou stain). (**c**) Image of cell block preparation (H&E stain)

Case 2

Learning Objectives:

1. Review the cytology of tubo-ovarian high-grade serous carcinoma.
2. Generate a differential diagnosis for tubo-ovarian high-grade serous carcinoma.
3. Review the immunohistochemical profile of tubo-ovarian high-grade serous carcinoma.

Case History:

- A 78-year-old female presents with a 5-cm solid and cystic ovarian mass.

Specimen Source:

- U/S-guided FNA of the ovarian mass

Cytologic Findings:

- Hypercellular aspirate with papillary structures (Fig. 11.15).
- Cells can also appear singly or in crowded clusters.
- Cells will exhibit a high N:C ratio, nuclear irregularities, and mitotic figures.

Differential Diagnosis:

- Tubo-ovarian high-grade serous carcinoma
- Ovarian endometrioid adenocarcinoma
- Clear cell carcinoma of the ovary
- Metastatic adenocarcinoma

IHC and Other Ancillary Studies:

- Gynecologic malignancies can often be distinguished from metastatic lesions and will often be positive for CK7 and PAX-8.
- Positivity for WT-1 and p16 may favor an ovarian primary, but uterine carcinomas can present with this immunophenotype as well.
- High-grade serous carcinomas of the ovary and uterus often demonstrates a p53-mutant phenotype, in contrast to low-grade endometrioid adenocarcinomas and clear cell carcinomas of the ovary.

Final Cytologic Diagnosis:
High-grade serous carcinoma
Take-Home Messages:

1. High-grade serous carcinoma will exhibit malignant cytologic features and papillary architecture. In contrast, benign and low-grade serous lesions will not exhibit this degree of cytologic atypia.
2. The primary site of high-grade serous carcinoma may be the ovary, the fallopian tubes, or the uterus, and it is not possible to make this distinction by cytology.
3. A diagnosis of "high-grade Müllerian adenocarcinoma" is often sufficient to guide management.

References: [32, 73, 74]

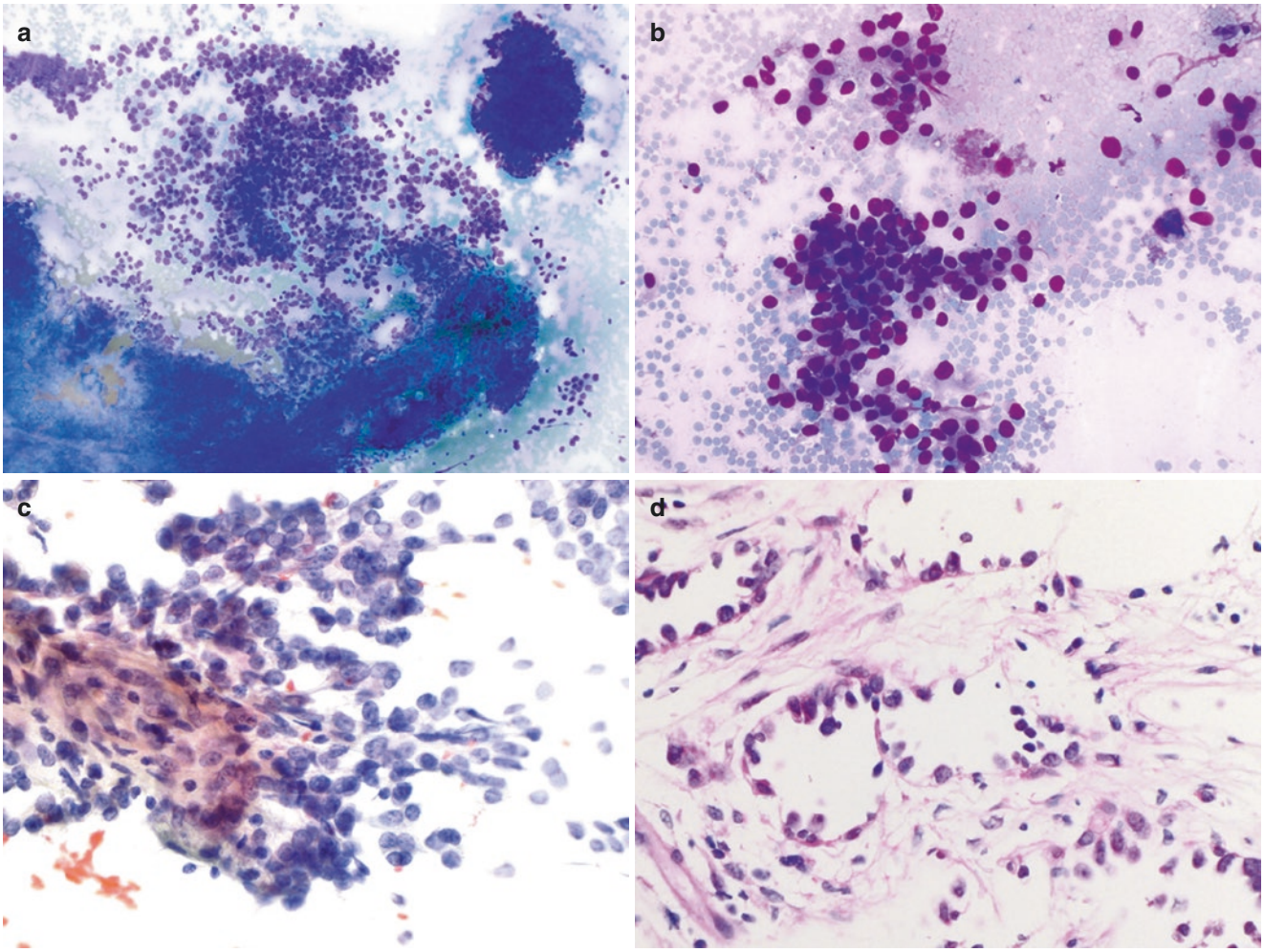

Fig. 11.15 Case 2: Ovarian mass, FNA. (**a**, **b**) Photomicrograph of air-dried smear (Diff-Quik stain). (**c**) Photomicrograph of alcohol-fixed smear (Papanicolaou stain). (**d**) Material from cell block (H&E stain)

Case 3

Learning Objectives:

1. Understand why peritoneal washings are obtained during gynecologic procedures.
2. Generate a differential diagnosis for endometrioid adenocarcinoma.
3. Understand the immunohistochemical profile of tubo-ovarian high-grade serous carcinoma.

Case History:

- A 40-year-old obese female with a history of cervical neoplasia presents with an adnexal mass and abnormal uterine bleeding. Endometrial biopsy demonstrates small fragments of adenocarcinoma, not otherwise specified. Hysterectomy and bilateral salpingo-oophorectomy are performed.

Specimen Source:

- Peritoneal washing

Cytologic Findings:

- Clusters of malignant glandular cells with increased N:C ratio, enlarged nuclei, and prominent nucleoli (Fig. 11.16a).
- Keratinized dysplastic cells are present in the washings (Fig. 11.16b).
- Malignant cells appear in the cell block, associated with numerous neutrophils (Fig. 11.16c). The source of keratinized cells is identified as squamous metaplasia on resection (Fig. 11.16d).

Differential Diagnosis:

- Endometrial versus ovarian endometrioid adenocarcinoma
- Serous carcinoma
- Endocervical adenocarcinoma

IHC and Other Ancillary Studies:

- Endometrioid adenocarcinomas are typically p53 wild-type, which distinguishes them from serous carcinomas.
- Immunohistochemistry for p16 will be negative or focal in endometrial adenocarcinomas, in contrast to HPV-related endocervical adenocarcinomas, which will be strong and diffusely p16 positive.

Final Cytologic Diagnosis:
Endometrioid adenocarcinoma

Take-Home Messages:

1. Peritoneal washings provide important prognostic information for ovarian and endometrial carcinomas, and washing status is a staging component of ovarian but not uterine cancers.
2. Endometrioid adenocarcinomas will have wild-type p53 and can exhibit squamous differentiation, which distinguishes them from gynecologic serous carcinomas.
3. Endocervical adenocarcinomas are often in the differential diagnosis for young patients who are HPV-positive or have a history of cervical dysplasia.

References: [75, 76]

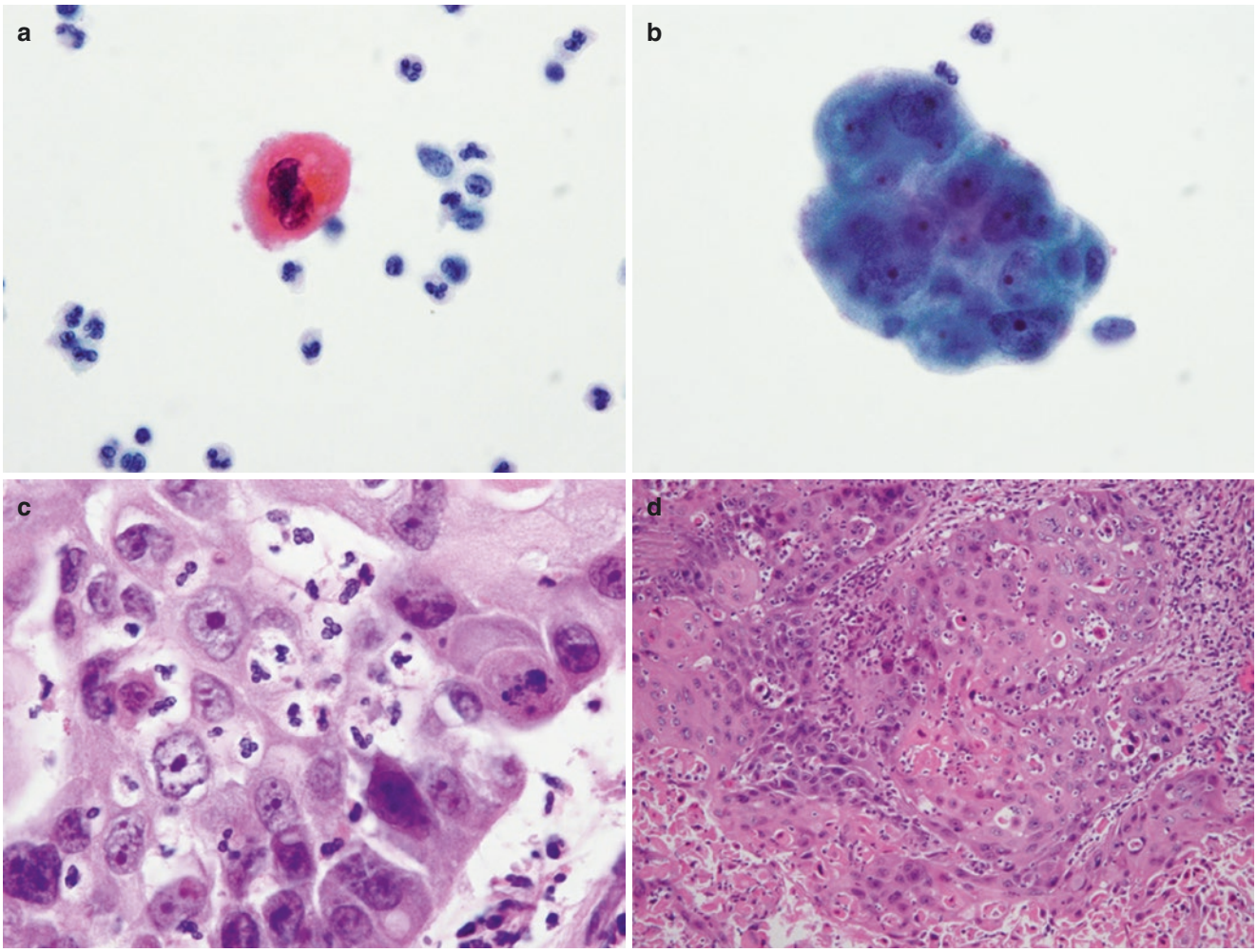

Fig. 11.16 Case 3: Cytology of pelvic washings obtained during hysterectomy. (**a**, **b**) Images taken from ThinPrep slide (Papanicolaou stain). (**c**) High-power image from cell block (H&E stain). (**d**) Resection of uterine tumor (H&E stain)

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Fine-Needle Aspiration Cytology of the Breast

12

Chen Zhou, Gang Wang, and Malcolm Hayes

Contents

List of Frequently Asked Questions

1. What are the advantages of FNA of the breast? What are the indications of FNA of the breast?

Breast FNA offers a safe, fast, inexpensive, and minimally invasive diagnostic solution to various breast lesions. It has few complications and is well accepted by patients. It does not require facility for tissue processing. When performed by aspirators trained with FNA technique and interpreted by cytopathologists experienced in reporting breast cytology, breast FNA cytology is highly accurate in diagnosing benign and malignant breast lesions, having a sensitivity and specificity almost similar to the core needle biopsy of the breast. A recent meta-analysis of 46 studies showed that breast FNA has a sensitivity of 92.7% and specificity of 94.8%.

In North America and in most developed countries, breast FNA has been replaced by core needle biopsy for preoperative diagnoses of breast palpable masses and impalpable radiologic abnormalities for the last 20 years. However, in developing countries, breast FNA is still being widely used for preoperative diagnoses of breast palpable mass and for some impalpable radiologic abnormalities. Breast FNA is also used in developed and developing countries for rapid on-site evaluation (ROSE), in "one-stop" diagnostic clinics, and for certain breast lesions (Table 12.1).

References: [1–4].

Table 12.1 Indications of breast FNA cytology

Indications	Purposes	Countries
Cyst of the breast	Diagnostic and therapeutic	Developing and developed
Palpable mass of the breast	Diagnostic	Developing and developed[a]
Impalpable radiologic abnormality	Diagnostic	Developing
Recurrent mass or metastasis after breast surgery	Diagnostic	Developing and developed
Preoperative axillary lymph node aspiration	Diagnostic and triage	Developing and developed
Nipple discharge	Diagnostic	Developing and developed

[a]Breast mass in pregnant and postpartum women or in women with contraindication to core needle biopsy; clinically inoperable or locally advanced breast mass

2. How are FNA techniques used to obtain cytology specimens of the breast?

Breast FNA can be performed with or without an image guidance. For palpable mass, manual aspiration without an image guidance is preferred. The mass can be fixed with one hand and aspirated using another hand. Needles of 23, 25, and 27 gauge are used for aspiration. We routinely use a 25-gauge needle and hold the needle hub in one hand to aspirate the lesion. To generate adequate aspirate material, more than three aspirations are performed with needle passing into the mass in different directions using a rapid back-and-forth oscillating motion. For sclerotic lesion or to make a good cell block, a syringe is attached in conjunction with a syringe holder or aspiration "gun" to provide suction for the aspira-

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_12

tion. The advantages of holding the needle hub by hand not only are having better control during the aspiration, less fearful to the patients, but also enabling the aspirators to feel the nature of the lesions through the aspiration needle. For example, the aspirator could feel the "gritty" sensation of carcinoma or fat necrosis, or the "sucked in" sensation of a benign fibrous scar. To avoid blood clot formed within the needle, the aspirator should stop aspiration once a small amount blood or aspirating material is accumulated within the needle hub.

In our experience as aspirators, we usually perform first 2–3 aspirations using the needle hub held by hand and last 1–2 aspirations using a syringe holder or aspiration "gun" to obtain adequate materials for cytology smears and cell block without causing excessive bleeding or trauma.

3. What are the preparation methods used to prepare the FNA cytology specimens of the breast?

Several preparation methods can be used to prepare breast cytology specimens, almost similar to FNA from other body parts.

After the aspirate, a syringe filled with air is used to connect the needle hub, and a drop of the aspirate is expressed onto a glass slide to make at least two cytological smears in a way similar to making a blood film. The cytology smear can be air-dried and stained with May-Grünwald Giemsa (MGG) stain or fixed immediate with alcohol spray or in alcohol solution and stained with Papanicolaou's (Pap) stain. For rapid on-site assessment, air-dried cytology smear can be stained with Diff-Quik solutions, and alcohol-fixed cytology smear can be stained with H&E staining.

Cytology aspirate and/or needle rinse can be collected in CytoLyt or other fixative solutions to prepare cytospin slides or monolayer liquid-based cytology slides such as ThinPrep or SurePath slides. The remaining material from the solution is used to prepare a cell block. This preparation method is helpful for facilities with no on-site support or a shortage of cytotechnologists or cytopathologists. As compared to conventional cytology smear, there are several other benefits of liquid-based cytology, including better cellular preservation, less interference from inflammatory cells, and more efficiency in screening cytology slides; however, there are also disadvantages of liquid-based cytology such as alterations in architecture and cell morphology and loss of myoepithelial cells and stromal fragments, which require modification in diagnostic criteria or additional training for interpretation of the liquid-based cytology slides, especially for those borderline lesions of the breast.

In our institution, we routinely prepare two cytology smears, one smear stained with MGG stain and another smear stained with Pap stain. We also use needle rinse or make dedicated passes of FNA to prepare a ThinPrep cytology slide and a cell block. For referral or sent in cytology specimens, we instruct the outside facilities to place FNA material directly into CytoLyt solution to send to our laboratory to prepare a ThinPrep cytology slide and a cell block.

Reference: [5].

4. How are the FNA cytological results of the breast reported? What is the minimal number of cells required for reporting FNA cytology of the breast?

In 1996, the National Cancer Institute Fine-Needle Aspiration of Breast Workshop Subcommittees proposed a uniform approach for reporting breast FNA cytology. A breast FNA cytology report should include (1) exact site of the FNA (side and position of the clock); (2) type of sample (FNA or nipple discharge); (3) a brief description of the cytological features; (4) conclusion of diagnosis using the following five categories (inadequate (C1), benign (C2); atypical, probably benign (C3); suspicious, favor malignancy (C4); and malignant (C5)); and (5) comments or recommendations. However, the NCI-recommended reporting has not been adopted widely and has not been updated after 10 years in its use. Recently, the International Academy of Cytology (IAC) brought together a group of cytopathologists, surgical pathologists, radiologists, surgeons, and oncologists to work on a standardized and comprehensive approach to breast FNA reporting. Because the reporting system was first proposed and discussed in 2016 at the19th IAC meeting in Yokohama, Japan, it is also called "Yokohama" reporting of breast FNA cytology.

The consensus for Yokohama reporting of breast FNA was to use five categories:

- Category 1: Insufficient material
- Category 2: Benign
- Category 3: Atypical, probably benign
- Category 4: Suspicious, probably in situ or invasive carcinoma
- Category 5: Malignant

The minimal cells required for a breast FNA cytology reporting varies according to different criteria proposed. Generally, 6 groups of ductal epithelial cells and at least 5–10 cells in each group are considered adequate. This rule does not apply to breast cystic lesions and inflammatory lesions, breast lipoma, or other stromal lesions. The Yokohama reporting of breast FNA cytology will also recommend the minimal number of cells required for the reporting of breast FNA cytology in its final version.

Reference: [2].

5. What are normal cytology components of FNA cytology of the breast?

Normal breast consists of large ducts (lactiferous, segmental, and subsegmental ducts), terminal duct-lobular units, and

fibroadipose stroma. The ducts and acini of lobules are lined by an inner layer of columnar to cuboidal epithelial cells and an outer layer of myoepithelial cells. In breast FNA specimens, components of normal breast cells and tissues can be seen in the background. The normal ductal or acinar epithelial cells are columnar to polygonal in shape and are arranged in cohesive groups or sheet with a honeycomb pattern. The epithelial cells have regular, oval nuclei, indistinctive or small nuclei, and a small amount of granular or clear cytoplasm. The myoepithelial cells appear in single dispersed pattern or within the groups of ductal epithelial cells. The cells have small, darkly stained, oval, or bipolar nuclei without cytoplasm (naked bipolar nuclei). The stromal components are mainly small fragments of adipose tissue (Figs. 12.1 and 12.2).

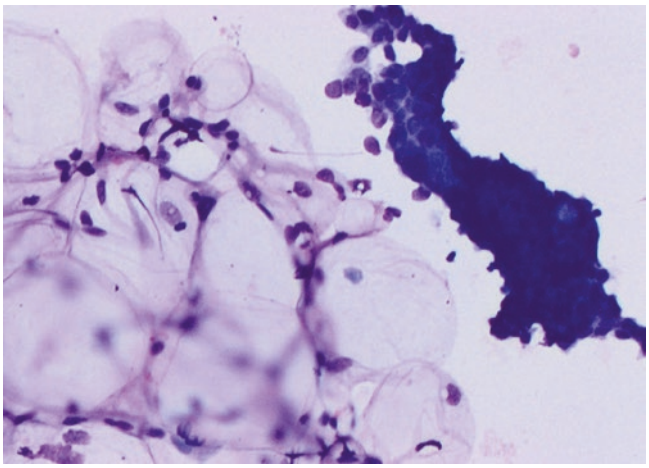

Fig. 12.1 Normal breast. The right upper shows a small duct, containing regular round nuclei of ductal epithelial cells and surrounded by a vague layer of darkly stained myoepithelial cells. The left lower shows a fragment of adipose tissue of normal breast (MGG stain)

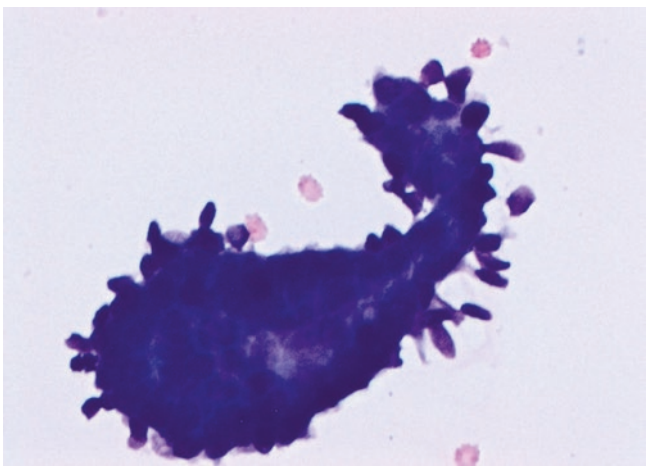

Fig. 12.2 Normal breast. An acinar and duct of terminal lobular unit is arranged in 3-D group, showing the pale nuclei of the inner layer of duct epithelial cells and the dark nuclei of the outer layer of myoepithelial cells (MGG stain)

6. What are the cytological features of a lactating adenoma?

Lactating adenoma is a nodular mass produced from secretory or lactational hyperplasia of lobules of breast during pregnancy or lactation. It is not a true neoplasm but rather nodular aggregates of hyperplastic lobules with lactation change. Clinically, FNA is performed to rule out malignancy that occurs during pregnancy or lactation. The cytological features of a lactating adenoma include (1) a moderately cellular specimen; (2) sheets of ductal epithelial cells with nuclear enlargement, prominent nucleoli, and foamy or vacuolated cytoplasm; and (3) many single epithelial cells and/or stripped round nuclei associated with a background of lipid droplets (Fig. 12.3).

Ductal epithelial cells of lactating adenoma are discohesive and have prominent nucleoli, which to some degree resemble malignant cells of breast carcinoma. However, the cells do not show variation in nuclear sizes and shapes and are present in a background of lipid droplets.

7. What are the cytological features of subareolar abscess? What are the cytological features of fat necrosis?

Subareolar abscess and fat necrosis are the two most common nonneoplastic mass lesions present for breast FNA.

Subareolar abscess is caused by plugging of lactiferous duct by ductal squamous material, resulting in acute inflammation, dilatation, and rupture of the duct with formation of a mass-like abscess. The cytological features of a subareolar abscess include (1) a cellular aspirate; (2) numerous acute inflammatory cells, histiocytes, and cell debris; (3) multinucleated histiocytes or loose formed granulomas; (4) anucleated squamous cells and/or benign squamous cells; and (5) occasional reactive ductal epithelial cells (Figs. 12.4 and 12.5).

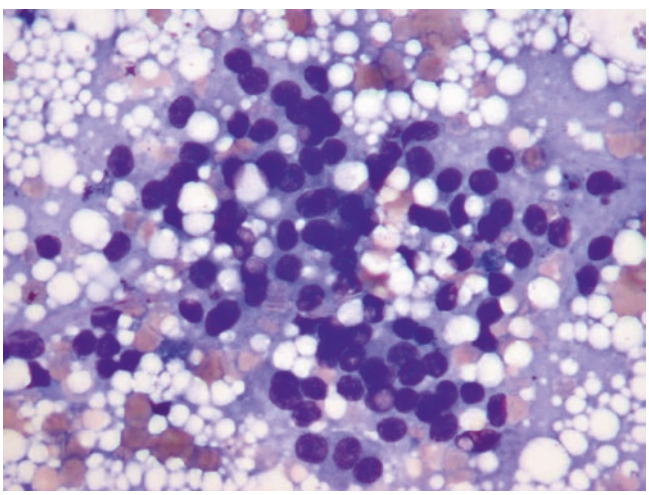

Fig. 12.3 Lactating adenoma. Many single dispersed, discohesive stripped nuclei without variation of nuclear sizes and shapes are present. Some of the nuclei contain prominent nucleoli. The background shows lipid droplets (MGG stain)

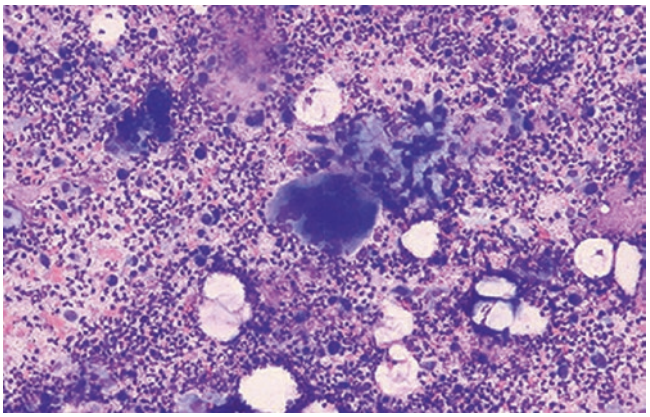

Fig. 12.4 Subareolar abscess. A hypercellular cytology smear shows multinucleated giant cells present in a background of numerous acute inflammatory cells (MGG stain)

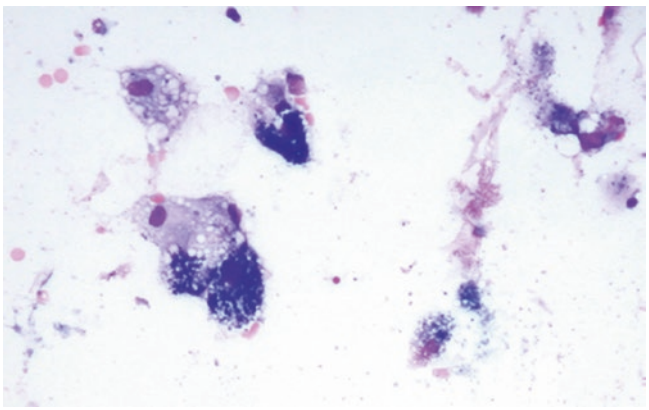

Fig. 12.6 Fat necrosis. Scattered foamy histiocytes; some have cytoplasmic blue-colored granular material present in a background of purple debris (MGG stain)

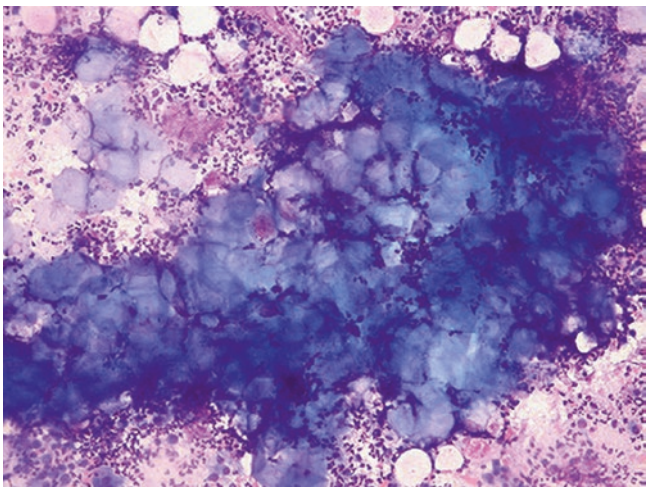

Fig. 12.5 Subareolar abscess. Sheets of anucleated squamous cells with sea blue cytoplasm are present in a background of numerous acute inflammatory cells (MGG stain)

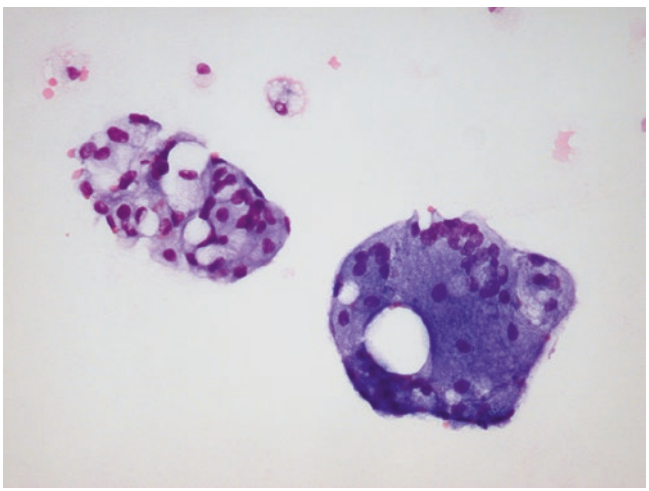

Fig. 12.7 Fat necrosis. One aggregate of foamy histiocyte containing several lipid vacuoles and a multinucleated giant cell containing a large round lipid vacuole are present. Several foamy histiocytes are present in the background (MGG stain)

The differential diagnoses of a subareolar abscess include a breast abscess associated with acute mastitis and an epidermal inclusion cyst. During breastfeeding, bacteria can enter the breast through traumatized nipple, causing an acute mastitis, breast abscess, and a tender mass. The FNA of breast abscess shows numerous acute inflammatory cells but no presence of anucleated squamous cells. An epidermal inclusion cyst of the breast can present as a breast mass and its FNA shows many anucleated squamous cells and a few multinucleated giant cells but does not have numerous acute inflammatory cells in the background.

Fat necrosis is caused by traumatic necrosis of breast or subcutaneous adipose tissue, resulting in a mass lesion. Clinically, fat necrosis is much more commonly caused by surgical trauma than by physical trauma. The cytological features of fat necrosis are as follows: (1) a hypocellular aspirate; (2) lipid debris and fat vacuoles; (3) foamy histiocytes and loose granuloma consisting of histiocytic aggregates; and (4) a few neutrophils, lymphocytes, and plasma cells (Figs. 12.6 and 12.7).

The differential diagnoses of fat necrosis include granulomatous mastitis and silicon granuloma; both of them contain foamy histiocytes and multinucleated giant cells. Besides the difference in clinical history, the FNA of granulomatous mastitis shows much more cellular specimen and contains many inflammatory cells; the FNA of silicon granuloma shows silicon globules within multinucleated giant cells and in the background.

8. What are the cytological features of a breast cyst? What are the cytological features of fibrocystic changes?

Breast cyst is a part of the fibrocystic change, which also typically displays changes of apocrine metaplasia, adenosis,

sclerosing adenosis, stromal fibrosis, and ductal hyperplasia. Large cysts can arise from the expansion of ducts into clinically palpable cystic masses. Clinically, fibrocystic disease presented by breast cysts is commonly seen in middle-aged and elderly women. FNA of breast cysts is not only a diagnostic test, but also a therapeutic procedure. After draining the content of the cyst, the aspirator should make sure that there is no palpable lesion left. Grossly, the cystic fluid is clear and yellow or dark and brown. FNA cytology of breast cysts typically shows (1) apocrine cells in cohesive sheets; (2) foamy histiocytes, some may have brown pigments; (3) cell debris (Figs. 12.8 and 12.9).

A breast cyst is reported as an apocrine cyst when apocrine cells are present, or a simple cyst when apocrine cells are absent and an inflamed cyst when inflammatory cells are present. Some of the inflamed cysts may contain cytological atypical apocrine cells or squamoid cells.

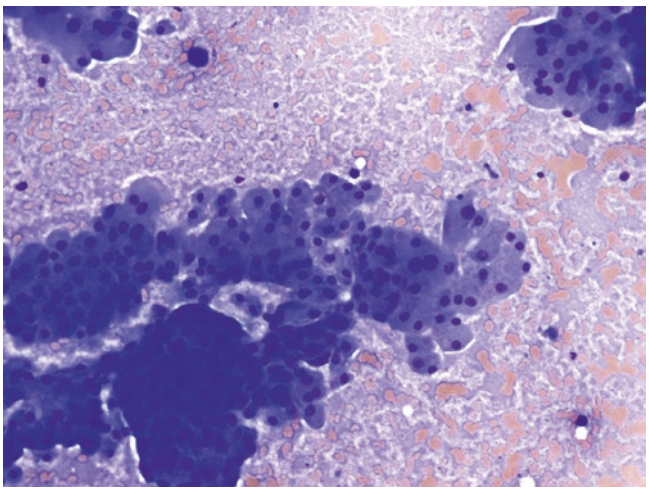

Fig. 12.8 Apocrine cyst. Large sheets and single apocrine cells are present. The cells have round nuclei, prominent nucleoli, abundant dense granular cytoplasm, and low N/C ratio (MGG stain)

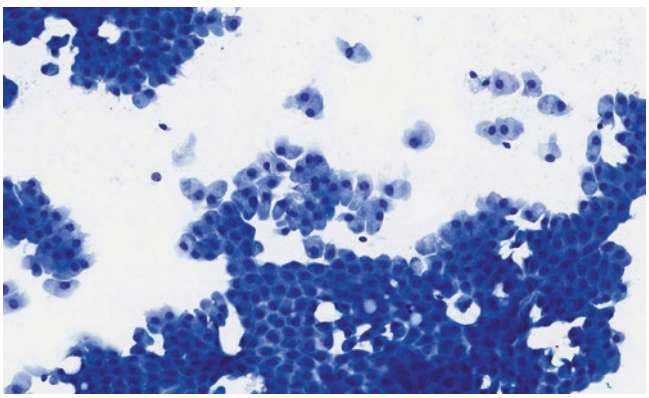

Fig. 12.9 Apocrine cyst. Sheets of benign apocrine cells are arranged in honeycomb pattern. The cells have round nuclei, prominent nucleoli, and abundant dense cytoplasm. The background is clean without necrosis (Pap stain)

9. What are the cytological features of proliferative lesion of the breast?

A proliferative breast lesion is fibrocystic change associated with epithelial hyperplasia, either usual ductal hyperplasia, atypical ductal hyperplasia, or atypical lobular hyperplasia. Fibrocystic change without epithelial hyperplasia is classified as nonproliferative breast lesions.

The cytological features of proliferative breast lesion without atypia are (1) cellular specimen; (2) cohesive sheets and large groups of benign ductal epithelial cells; (3) myoepithelial cells present within groups of epithelial cells or as single stripped nuclei in the background; and (4) no marked nuclear atypia and no dyshesive atypical ductal cells (Figs. 12.10 and 12.11).

The cytological features of proliferative breast lesions with atypia are (1) cellular specimen; (2) large and small

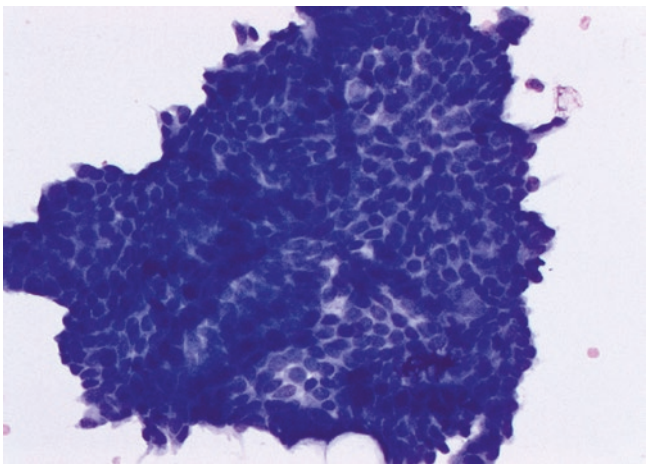

Fig. 12.10 Proliferative disease without cytologic atypia. Large cohesive sheet of ductal epithelial cells is present. The nuclei are regular, round to oval, without overlapping or crowding, and contain fine chromatin and indistinct nucleoli (MGG stain)

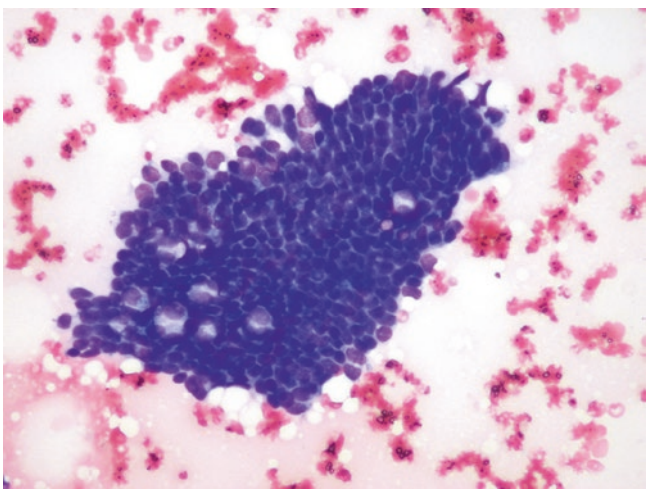

Fig. 12.11 Proliferative disease without cytologic atypia. Large flat sheet of ductal epithelial cells with regular, round to oval nuclei, without nuclear overlapping, and nucleoli are present (MGG stain)

groups of mild to moderately atypical ductal epithelial cells showing nuclear enlargement, nuclear crowding, loss of polarity, and prominent nucleoli; and (3) few myoepithelial cells in the background (Figs. 12.12 and 12.13).

Masood proposed a score index using six cytological features to classify breast lesions into nonproliferative breast disease, proliferative without atypia, proliferative with atypia, and carcinoma. Later, the Modified Masood Score Index was also proposed for such classification. Despite the efforts, it is still difficult to separate atypical proliferative breast lesion from low-grade in situ and invasive carcinoma; therefore, it is recommended that all atypical breast lesion should be excised or further investigated by core needle biopsy (Fig. 12.14).

References: [6, 7].

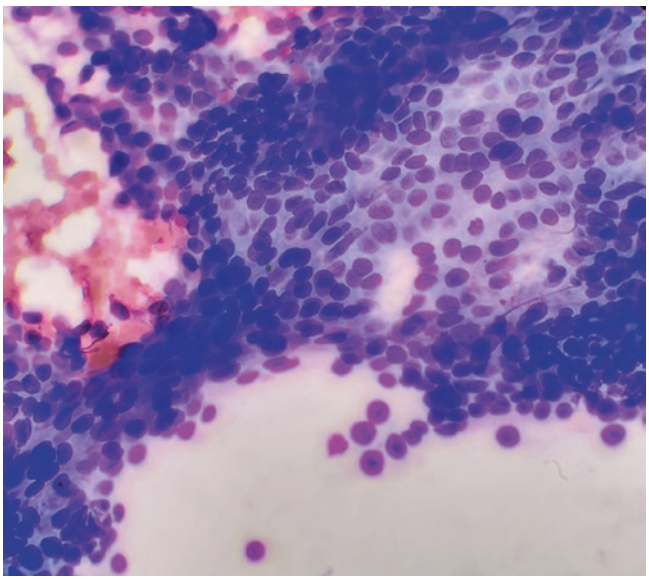

Fig. 12.12 Proliferative disease with cytologic atypia. Large flat sheet of ductal epithelial cells with regular, round to oval nuclei, and also with nuclear crowding and overlapping. Some scatter cells containing naked nuclei and prominent nucleoli are also present (MGG stain)

10. What are the cytological features of a fibroadenoma?

Fibroadenoma is the proliferation of both epithelial and stromal components of the breast. It is the most common type of benign breast nodule that underwent for FNA. Clinically, fibroadenoma typically occurs in young women but can also occur in middle-aged women. On palpation, it is a mobile rubbery nodule and, on imaging study, a round, well-circumscribed hypoechoic mass. The FNA of fibroadenoma typically has (1) a cellular aspirate; (2) cohesive branching sheets of ductal epithelial cells with "antler-horn" shapes; (3) numerous naked nuclei of myoepithelial cells in the background; and (4) fragments of fibromyxoid stroma with cloverleaf-like shape (Figs. 12.15, 12.16, and 12.17).

Because FNA from fibroadenoma is usually cellular, some of them display dispersed small groups of epithelial cells and single ductal epithelial cells with nuclear enlargement and prominent nucleoli, mimicking a low-grade ductal carcinoma. Such "atypical" fibroadenoma is difficult to

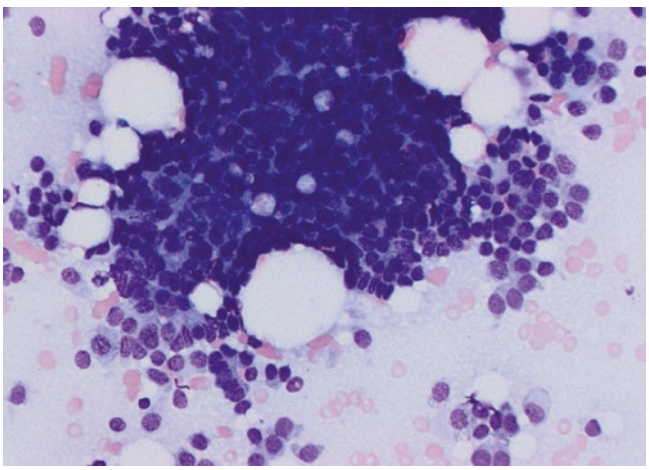

Fig. 12.13 Proliferative disease with cytological atypia. Cellular cytology smear displays large flat discohesive sheet of ductal epithelial cells with regular, round nuclei, and also with nuclear crowding and overlapping. Many scattered single cells containing pale cytoplasm are also present (MGG stain)

Cellular arrangement	Cellular pleomorphism	Myoepithelial cells	Anisonucleosis	Nucleoli	Chromatin clumping	Score
Monolayer	Absent	Many	Absent	Absent	Absent	1
Nuclear overlapping	Mild	Moderate	Mild	Micronucleoli	Rare	2
Clustering	Moderate	Few	Moderate	Micronucleoli and/or rare macro nucleoli	Occasional	3
Loss of cohesion	Conspicuous	Absent	Conspicuous	Predominantly macro nucleoli	Frequent	4
Total score						
Nonproliferative breast disease 6 – 10						
Proliferative breast disease without atypia 11 – 14						
Proliferative breast disease with atypia 15 – 18						
Carcinoma in situ / Carcinoma 19 – 24						

Fig. 12.14 Masood Score Index for assessment of breast FNA and for classification of breast lesions. (Modified from Masood and others)

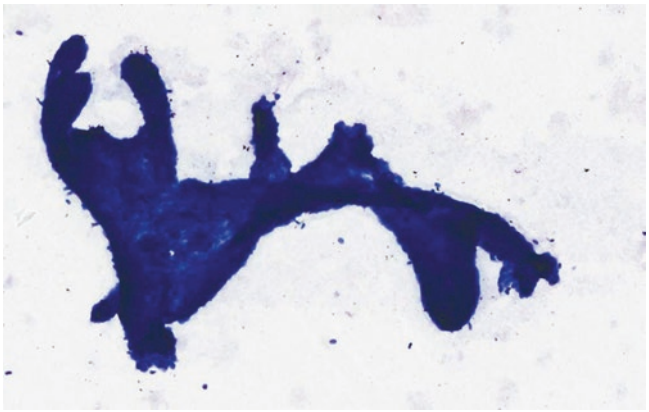

Fig. 12.15 Fibroadenoma. Low-power view of a fibroadenoma showing "antler-horn"-like 3-D structure (MGG stain)

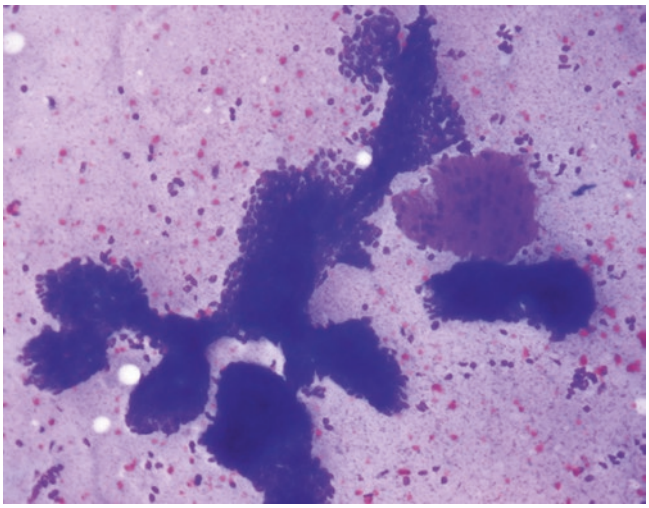

Fig. 12.16 Fibroadenoma. Under the low power, 3-D branching groups of ductal epithelial cells, a fragment of acellular stroma in round shape, and a background of stippled round to oval nuclei of myoepithelial cells are present (MGG stain)

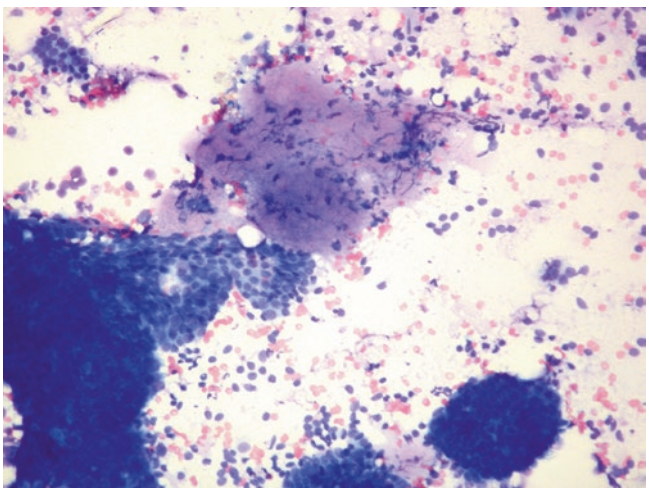

Fig. 12.17 Fibroadenoma. Large group of ductal epithelial cells with regular, round nuclei arranged in honeycomb pattern, round clover-shaped stroma, and a background of stippled round to oval nuclei of myoepithelial cells are present (MGG stain)

distinguish from a low-grade ductal carcinoma and is the most common cause of false-positive diagnosis in breast FNA cytology.

Reference: [8].

11. What are the cytomorphologic features of papillary neoplasm of the breast?

Papillary neoplasm of the breast encompasses a spectrum of benign and malignant papillary lesions: intraductal papilloma, atypical papilloma, papillary carcinoma in situ, encapsulated cystic papillary carcinoma, solid papillary carcinoma, and invasive papillary carcinoma. Clinically, papillary neoplasm presents with either symptom of nipple discharge or a subareolar solid mass. If both nipple discharge and breast mass are present, FNA of the breast mass should be performed because its sensitivity is much higher than those of nipple discharge. The cytomorphologic features of papillary neoplasm are characterized by (1) a cellular aspirate specimen; (2) three-dimensional papillary groups or tissue fragments with fibrovascular core; (3) flat sheets and cluster of epithelial cells surrounded by myoepithelial cells; (4) dispersed single or stripped nuclei of myoepithelial cells; (5) dispersed single or small cluster of uniform columnar cells; and (6) foamy histiocytes and hemosiderin-laden macrophages (Figs. 12.18, 12.19, and 12.20).

Although the presence of background myoepithelial cells and rare dispersed single columnar cells favor a diagnosis of benign papilloma and a lack of background myoepithelial cells and an increase in dispersed single columnar cells favor a diagnosis of a malignant papillary lesion, the cytological distinction of intraductal papilloma from atypical and malignant papillary lesion is unreliable. Tse et al. reported that the diagnostic accuracy was only 59% for papillary neoplasm, and there was no demonstrable quantitative difference between papilloma and papillary carcinoma using four cytological parameters: overall cellularity, epithelial cell ball

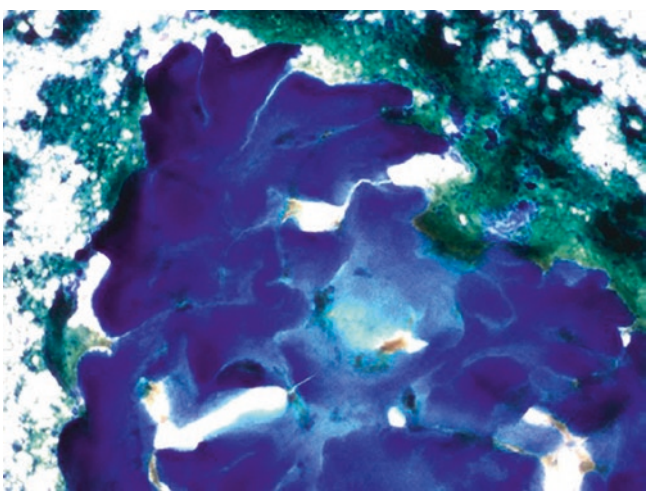

Fig. 12.18 Papillary neoplasm. Under low power, 3-D complex papillary structure is present (MGG stain)

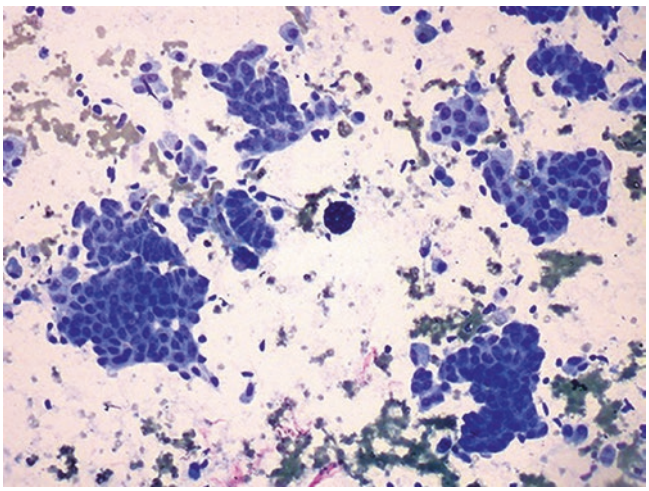

Fig. 12.19 Papillary neoplasm. Scattered small groups and single cells are present. The cells have regular round nuclei and moderate amount of cytoplasm. Occasional pigmented histiocytes are also present (MGG stain)

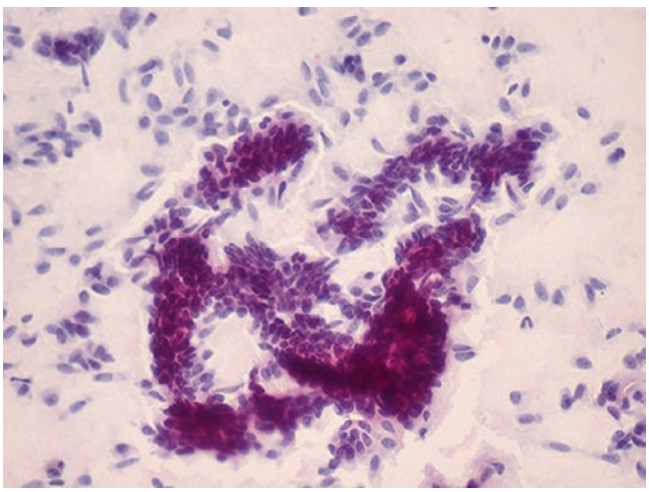

Fig. 12.20 Papillary neoplasm. Discohesive small groups of ductal epithelial cells and scattered single columnar cells are present. The cells have regular round nuclei and moderate amount of cytoplasm (MGG stain)

devoid of fibrovascular cores, background single cells, and papillary fragments and their morphology.

Fortunately, since all papillary neoplasm either papilloma or papillary carcinoma diagnosed on FNA or on core needle biopsy requires an excisional biopsy, a cytological reporting of papillary neoplasm is adequate for breast papillary lesions.

Reference: [9].

12. What are the cytomorphologic features of ductal carcinoma of the breast?

Invasive ductal carcinoma is the most common cause of malignant palpable mass of the breast, accounting for about 80% of invasive breast cancer. Ductal carcinoma in situ (DCIS) sometimes also presents as a mass lesion.

The cytomorphologic features of invasive ductal carcinoma and DCIS are basically the same, and their shared common features include (1) a hypercellular specimen; (2) loss of cohesion of ductal epithelial cells, forming loose small irregular clusters and many single isolated ductal epithelial cells; (3) absence of background myoepithelial cells; and (4) variable cytological atypia by displaying nuclear enlargement, overlapping, crowding, hyperchromatism, and pleomorphism as well as prominent nucleoli.

FNA of low-grade ductal carcinoma shows only mild nuclear atypia, small or distinct nucleoli, and discohesion of epithelial cells. As a result, false negative can occur. A recent study shows that the sensitivity of FNA is 80.9% for grade 1 ductal carcinoma and 57.1% for invasive tubular carcinoma. In contrast, FNA of high-grade ductal carcinoma usually shows marked cytological atypia and contains pleomorphic nuclei and visible mitosis (Figs. 12.21, 12.22, 12.23, and 12.24).

The presence of malignant cells embedded within adipose tissue and stroma on cytology smear was previously suggested to be a sign for invasion, but the claim is no longer accepted because malignant cells embedded in stroma could be produced by displacement from aspiration needle or by smearing artifact.

Reference: [10].

13. What are the cytomorphologic features of invasive lobular carcinoma?

Invasive lobular carcinoma accounts for less than 20% of invasive carcinoma of the breast. It can produce irregular thickening or lump of the breast. A majority of invasive lobular carcinoma is classic type, and its cytomorphologic fea-

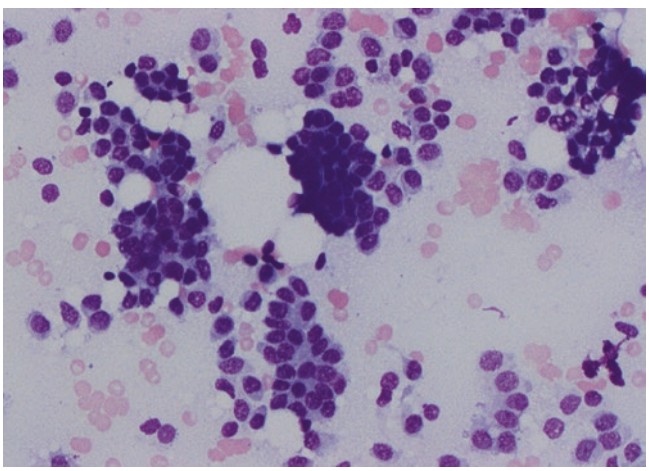

Fig. 12.21 Low-grade ductal carcinoma. A cellular cytology smear contains many discohesive ductal epithelial cells arranged in small groups and in single cell pattern. The cells display minimal cytologic atypia with regular round nuclei and indistinctive small nucleoli and small amount of pale cytoplasm. Occasional cells arranged in tubular glandular pattern are also seen (MGG stain)

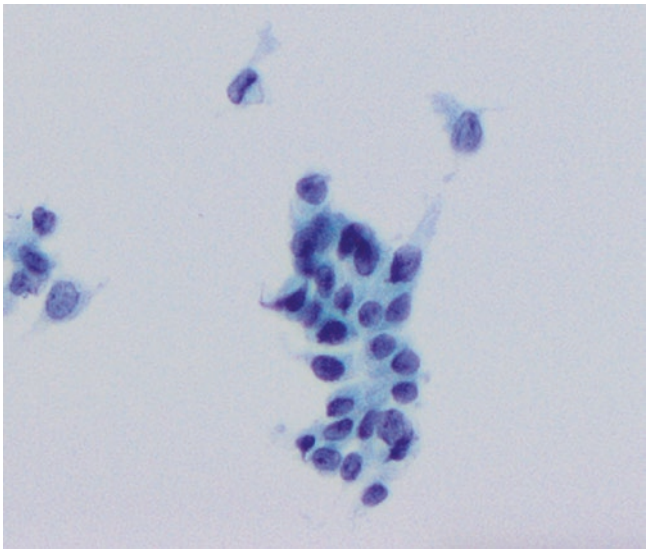

Fig. 12.22 Low-grade ductal carcinoma. ThinPrep slide shows mildly atypical cells with hyperchromatic, irregular nuclei arranged in small tubular pattern (Pap stain)

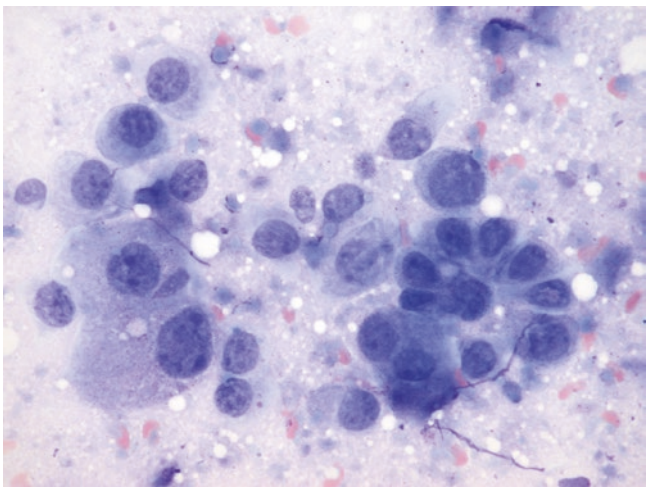

Fig. 12.23 High-grade ductal carcinoma. Discohesive small groups and single malignant cells with marked cytologic atypia are present. The cells show enlarged nuclei, variation of nuclear sizes, and crumped chromatin. Several cells display small glandular pattern (MGG stain)

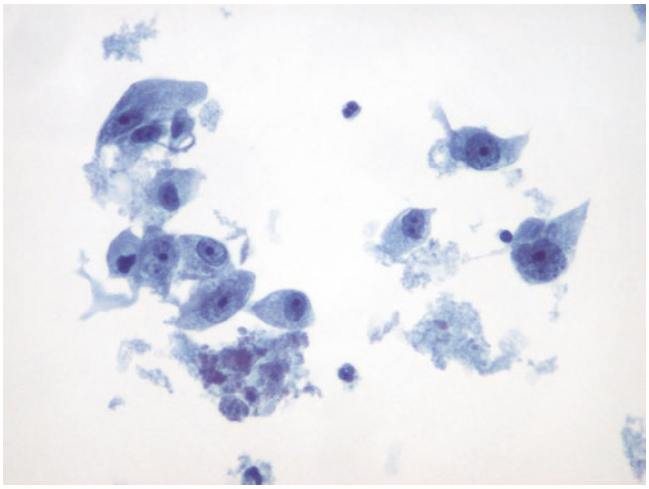

Fig. 12.24 High-grade ductal carcinoma. Markedly atypical large cells with enlarged nuclei, prominent nucleoli, and moderate amount of cytoplasm are present in the background of necrosis (Pap stain)

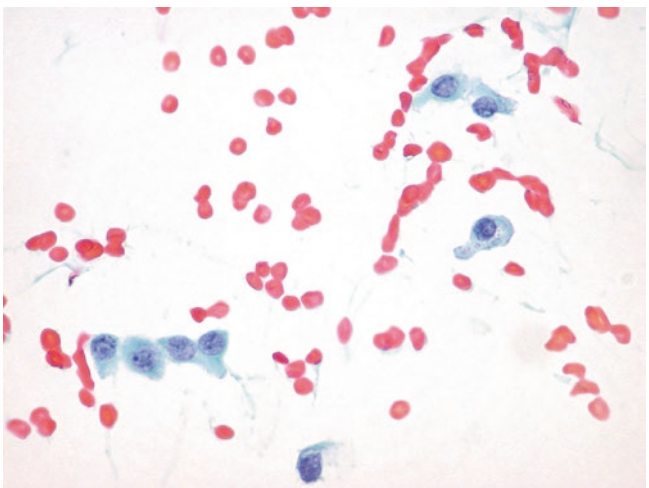

Fig. 12.25 Lobular carcinoma. Cytology smear shows discohesive epithelial cells with slightly enlarged round nuclei and moderate amount of cytoplasm present in a clean background; some of the nuclei are eccentric (Pap stain)

tures are (1) a hypocellular specimen; (2) dispersed non-cohesive single cells or small groups of cells arranged in linear shape; (3) cells with eccentric nuclei and cytoplasmic vacuoles (signet ring) or cytoplasmic vacuoles containing mucin with a central dot (targetoid) pattern; and (4) hyperchromatic nuclei with irregular outline (Figs. 12.25, 12.26, and 12.27).

FNA diagnosis of lobular carcinoma is a difficult task because it has low cellularity and minimal cytological atypia. Recent studies showed that the sensitivity of FNA of invasive lobular carcinoma is only 50% and it is much lower in classic type than in other variants of invasive lobular carcinoma. In contrast, the pleomorphic variant of invasive lobular carcinoma can be easily diagnosed because the tumor cells have significant cytological atypia, showing enlarged hyperchromatic nuclei, prominent nucleoli, nuclear pleomorphism, and moderate amount of cytoplasm with apocrine appearance.

References: [10, 11].

14. What are the cytomorphologic features of mucinous carcinoma of the breast?

Mucinous carcinoma accounts for about 2% of invasive breast carcinoma. It consists of scattered aggregates of malignant ductal epithelial cells floating within mucinous

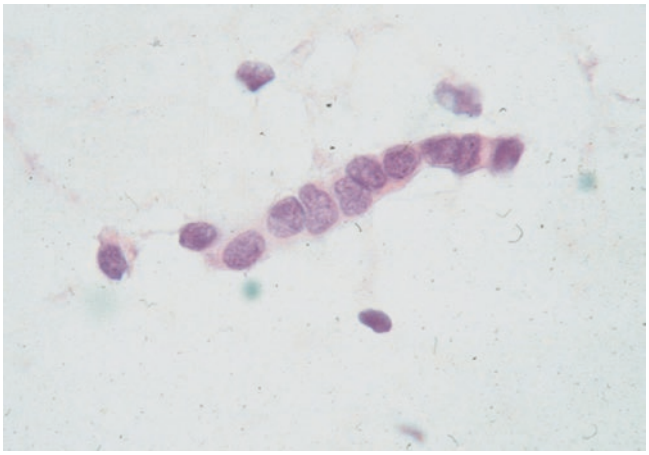

Fig. 12.26 Lobular carcinoma. Mildly atypical cells with slightly irregular nuclear outline are arranged in linear shape. A few discohesive epithelial cells are present in the background (MGG stain)

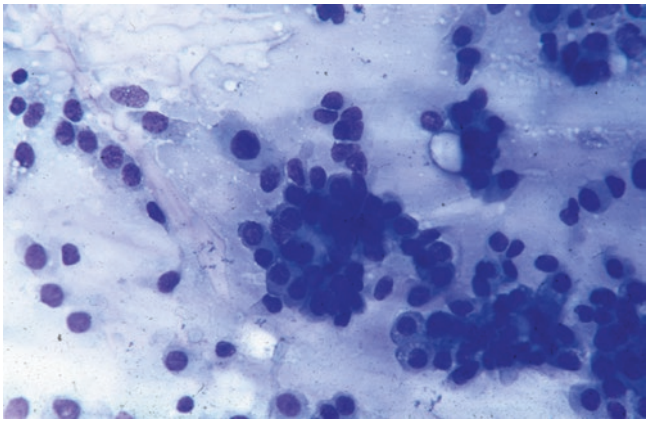

Fig. 12.28 Mucinous carcinoma. Small groups and single epithelial cells with regular round nuclei, small to moderate cytoplasm are present in a mucinous background. Some cells have stripped bare nuclei (MGG stain)

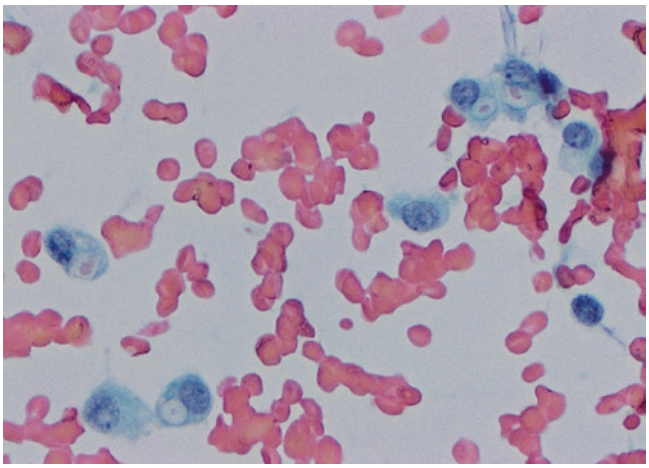

Fig. 12.27 Lobular carcinoma. Discohesive epithelial cells with slightly enlarged round eccentric nuclei and moderate amount of cytoplasm are present. Some cells contain cytoplasmic round vacuolation and have a red dot within it (targetoid pattern) (Pap stain)

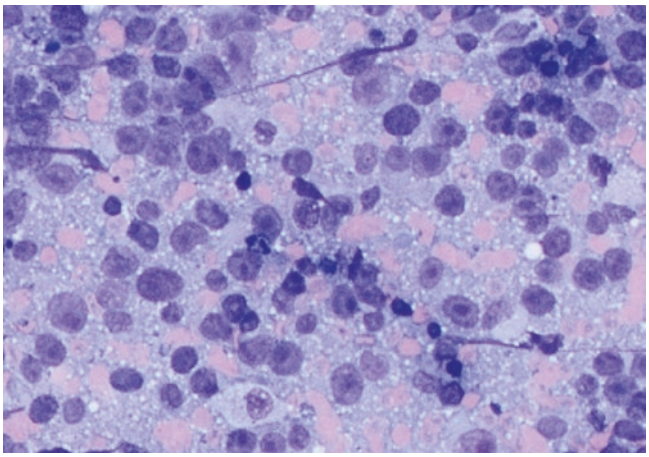

Fig. 12.29 Medullary carcinoma. A cellular cytology smear contains many dispersed stripped malignant nuclei displaying large nuclei and prominent nucleoli. A few lymphocytes are present within malignant cells (MGG stain)

pools. Clinically, the tumor usually presents as a soft, well-circumscribed palpable mass, simulating a fibroadenoma or a cyst. The cytomorphologic features of mucinous carcinoma are (1) three-dimensional clusters of ductal epithelial cells with mild cytological atypia; (2) abundant mucinous materials surrounding ductal epithelial cells; and (3) no high-grade nuclear atypia (Fig. 12.28).

Separation of pure mucinous carcinoma from mixed mucinous carcinoma on cytology specimens is difficult. It was reported that pure mucinous carcinomas have cytological features of abundant mucin, small nuclei, and/or regular nuclear outline, while mixed mucinous carcinomas have sparse mucin, large nuclei with irregular nuclear outline, or presence of nucleoli.

Reference: [12].

15. What are the cytomorphologic features of medullary carcinoma of the breast?

Medullary carcinoma accounts for about 1% of invasive breast carcinoma. It consists of aggregates of high-grade invasive ductal carcinoma surrounded by heavy lymphocytic infiltrate. Clinically, it usually presents as a soft, well-circumscribed mass simulating a fibroadenoma. The cytomorphologic features of medullary carcinoma include (1) a hypercellular aspirate; (2) single and small cluster of large malignant vesicular nuclei, prominent nucleoli, and scanty cytoplasm; (3) bizarre stripped nuclei with prominent nucleoli; and (4) numerous lymphocytes in the background (Fig. 12.29).

Because atypical medullary carcinoma and some poorly differentiated invasive ductal carcinoma of basal cell type

can mimic medullary carcinoma histologically, a cytological diagnosis of medullary carcinoma is often not possible; therefore, a cytological reporting of a "medullary-like carcinoma" is adequate, followed by an explanation note raising the possibility of a medullary carcinoma.

Reference: [13].

16. What are the cytomorphologic features of metaplastic carcinoma of the breast?

Metaplastic carcinoma of the breast accounts for less than 1% of invasive breast carcinoma. It is an invasive carcinoma with squamous cell or mesenchymal differentiation. Histologically, it has low-grade and high-grade types. The low-grade type consists of components of low-grade malignant squamous cells and spindle cells; in contrast the high-grade type consists a mixture of high-grade carcinoma and matrix-producing sarcoma. The cytomorphologic features reflect the spectrum of the metaplastic carcinoma of the breast: (1) hypocellular specimen in low-grade lesion or hypercellular specimen in high-grade lesion; (2) malignant spindle cells and squamous cells; (3) large pleomorphic malignant cells or sarcomatoid cells in high-grade lesion; and (4) malignant cartilage and bone in high-grade lesion (Figs. 12.30 and 12.31).

Reference: [14].

17. What are the cytomorphologic features of the breast implant-associated anaplastic large cell lymphoma?

Breast implant-associated anaplastic large cell lymphoma (BI-ALCL) is a newly described entity of primary breast lymphoma, occurring rarely but more commonly in women with breast implants. Patients usually present with a late-onset seroma or an effusion around implant and infrequently with a breast mass. Because of the risk associated with BI-ALCL, it is now recommended that late seroma of breast implant should be aspirated and investigated.

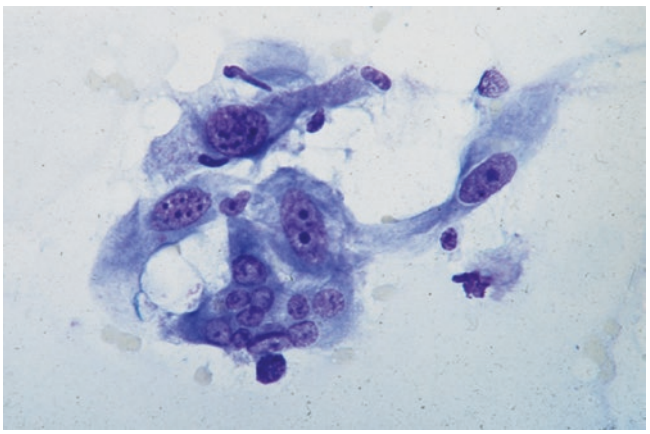

Fig. 12.30 Metaplastic carcinoma. Pleomorphic malignant spindle cells and multinucleated malignant cells are present. The cells have round to elongated nuclei and abundant blue cytoplasm, displaying squamous differentiation or squamoid appearance

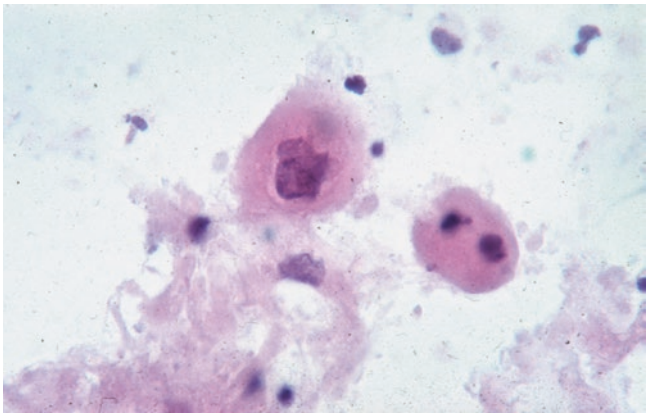

Fig. 12.31 Metaplastic carcinoma. Two cells of atypical chondrocytes are present. The cells have pink dense cytoplasm, sharp cell outline, and dark-stained irregular nuclei

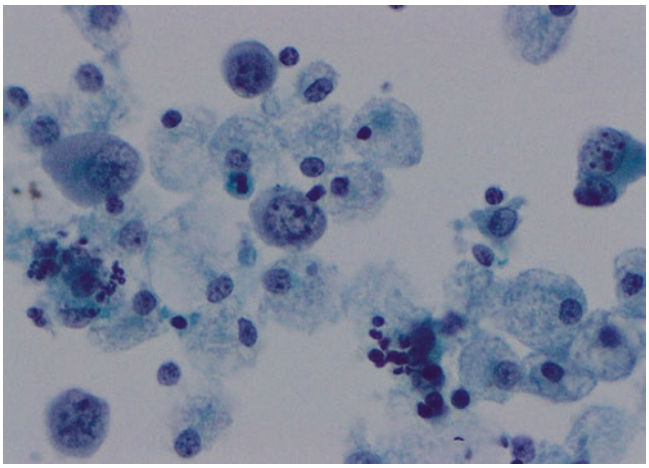

Fig. 12.32 Breast implant-associated anaplastic large cell lymphoma. The ThinPrep cytology slide shows some dispersed large malignant cells containing enlarged hyperchromatic round nuclei and small to moderate amount of basophilic cytoplasm, foamy histiocytes, and other chronic inflammatory cells (Pap stain)

The cytomorphologic features of BI-ALCL are (1) cellular specimens; (2) non-cohesive large pleomorphic cells with irregular, lobulated nuclei, prominent nucleoli, and basophilic cytoplasm; and (3) a background of variable inflammatory cells (Figs. 12.32 and 12.33).

Cell block is useful for the diagnosis and differential diagnoses of BI-ALCL which include chronic inflammation, poorly differentiated carcinoma, and other lymphomas. Tumor cells of BI-ALCL show strongly and diffusely positive staining for CD30 and EMA, variable positive staining for CD4 and CD45 but negative for ALK and cytokeratin. A majority of tumor demonstrate T-cell receptor gene rearrangement.

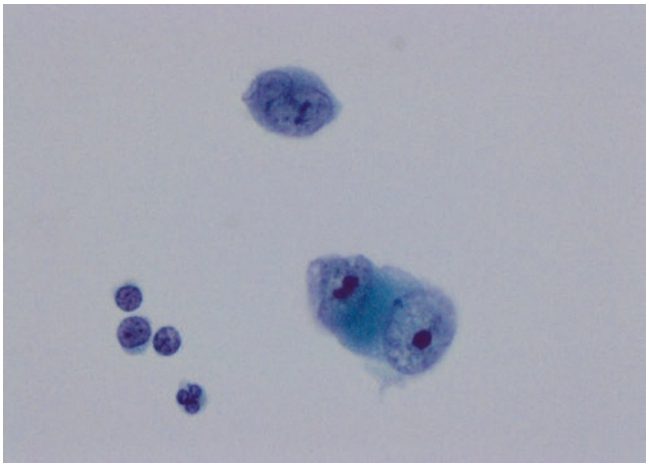

Fig. 12.33 Breast implant-associated anaplastic large cell lymphoma. Two binucleated large cells with abundant cytoplasm are present. One of the large cells has the "Reed-Sternberg" cell or Hodgkin cell-like appearance (Pap stain)

Once ALCL is diagnosed on cytology specimens, systemic ALCL and cutaneous ALCL also need to be ruled out using patient's clinical history and axillary tests.

Patients diagnosed with BI-ALCL in breast effusion/seroma cytology specimens need to have immediate removal of implant and excision of the fibrous capsule around the implant.

References: [15, 16].

18. What are the limitations of FNA cytology of the breast? What is the triple test?

There are three limitations that exist in breast FNA cytology. First, it is the inadequate cytological sample, frequently due to FNA performed by inexperienced or inadequately trained aspirators and infrequently due to sclerotic breast lesions such as sclerotic fibroadenoma, sclerosis adenosis, radial scar, and invasive lobular carcinoma. Second, it is the cytological borderline lesions of the breast, which poses a diagnostic hardship even for the most experienced cytopathologists. The challenges of borderline lesions include "atypical" fibroadenomas, various papillary lesions, atypical ductal hyperplasia, low-grade carcinoma, and others. Third, cytologically it is impossible to separate DCIS from invasive ductal carcinoma.

Because of the limitations of breast FNA cytology, triple test has been applied to improve the diagnostic accuracy. Triple test is the consideration of results from three parameters: clinical, radiologic, and cytological. Besides cytological results, clinical history, and physical examination, imaging results from ultrasound and/or mammography and/or MRI should also be considered before rendering a cytological diagnosis. If any of the three parameters is positive, triple test is positive. If all of the three parameters are negative, triple test is negative. Triple test has a sensitivity of 99.6% and specificity of 93%.

19. What are the common pitfalls of FNA cytology of the breast? When is the "atypical" category used for reporting breast cytology?

Recognizing common pitfalls of FNA cytology of the breast could prevent cytopathologists from making false-negative and false-positive diagnoses. False-negative diagnosis occurs due to inadequate sampling or sampling error, or due to interpretation error. Certain carcinomas (e.g., invasive lobular carcinoma, low-grade metaplastic carcinoma) and low-grade carcinoma (e.g., low-grade ductal carcinoma, invasive tubular carcinoma, and invasive mucinous carcinoma) are the common sources of interpretation error. False-positive diagnosis occurs in "atypical" fibroadenoma, atypical ductal hyperplasia, and lactating adenoma and rarely in fat necrosis.

Atypical category (C3) based on NCI reporting of breast cytology accounts for about 5% of FNA cytology specimens and reveals about 30–40% of malignancy in the follow-up histology. However, there are significant inter-observer and intra-observer variations of atypical cytological diagnosis. Masood and others reported using Masood Score Index (MSI) and Modified Masood Score Index (MMSI) to quantitatively assess six cytological parameters to define cytological atypia. As shown in Fig. 12.14, an MSI score of 15–18 was considered proliferative breast disease with atypia. Recently, IAC Breast Group attempted to define the use of atypia in the following scenarios: (1) epithelial hyperplasia with marked dispersed often columnar cells but minimal nuclear atypia (differential diagnosis is epithelial hyperplasia or low-grade DCIS); (2) intraductal papillomas with diagnostic stellate papillary fragments but again marked dispersal of cells (differential diagnosis is low-grade DCIS); (3) epithelial hyperplasia with more complex possibly cribriform or micropapillary tissue fragments (differential diagnosis is low-grade DCIS); (4) stromal hypercellularity without nuclear atypia or necrosis in the otherwise typical fibroadenoma raising a possibility of a low-grade phyllodes tumor; and (5) low cellularity smears with minute epithelial tissue fragments and single cells showing eccentric cytoplasm that raise a concern for lobular carcinoma.

References: [2, 6, 7, 11, 17–21].

20. How is FNA cytology of axillary lymph node interpreted?

Preoperative FNA of the axillary lymph node is performed for both diagnostic and triage purposes. For women with a suspicious breast mass and suspicious axillary lymph node, aspiration of axillary lymph node at the same time could provide not only a cytological diagnosis but also information for decision on axillary sentinel lymph node biopsy procedure. Because FNA of axillary lymph node has a very low false-positive rate (<1.5%), women with a positive cytology diagnosis of axillary lymph node will bypass the procedure of axillary sentinel lymph node biopsy and directly receive axillary lymph node dissection. In some institutions, rapid

on-site assessment of cytology smear of FNA of axillary lymph node is performed at the time of the breast surgery.

The cytomorphology of a positive axially lymph node is almost similar to adenocarcinoma metastatic to a lymph node: (1) a hypercellular specimen; (2) epithelial groups or single epithelial cells with cytological atypia; and (3) a background of small mature lymphocytes and small lymphohistiocytic aggregate. In cell block, the metastatic carcinoma can be further confirmed by immunostaining using ER, GATA-3, or keratin antibodies.

Reference: [22].

21. What are the cytological diagnoses of nipple discharge?

Cytology of nipple discharge does not involve FNA procedure. The specimen is prepared from touching the droplet of nipple secretion/discharge on to the surface of a glass slide and making cytology smears.

Nipple discharge occurs in physical conditions from hormonal imbalance and also in breast neoplasms, such as intraductal papillary lesions and ductal carcinoma. Bilateral nipple discharge of milky, serous fluid is more commonly associated with hormonal effect, while unilateral nipple discharge of bloody fluid is more likely associated with a neoplastic breast lesion, especially an intraductal papillary lesion.

The cytomorphology of nipple discharge due to hormonal effect includes (1) a hypocellular smear; (2) foamy histiocytes; and (3) background of inflammatory cells and/or red blood cells (Fig. 12.34).

The cytomorphology of nipple discharge due to intraductal papillary lesions includes (1) a hypocellular smear; (2) single and small three-dimensional clusters of ductal epithelial cells with mild cytological atypia; and (3) background of inflammatory cells and/or red blood cells (Fig. 12.35).

A cytological reporting of "suspicious for papillary neoplasm" is warranted for such lesion, which will lead to an excisional biopsy.

The cytomorphology of nipple discharge caused by ductal carcinoma is similar to those of ductal carcinoma: (1) a cellular smear; (2) dispersed single and small clusters of ductal epithelial cells with marked cytological atypia; and (3) necrotic debris and/or red blood cells (Fig. 12.36).

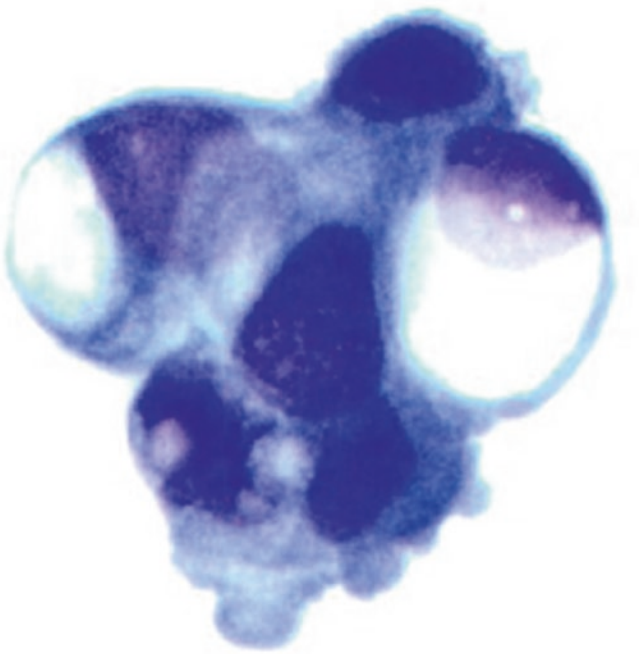

Fig. 12.35 Nipple discharge. A hypocellular specimen contains a small papillary cluster of ductal epithelial cells with mild nuclear atypia and cytoplasmic vacuolation (MGG)

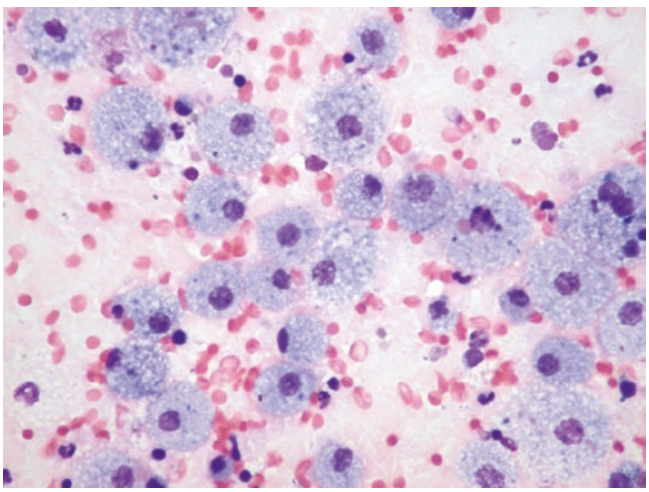

Fig. 12.34 Nipple discharge. Many foamy histiocytes containing small round nuclei and abundant foamy cytoplasm are present in the cytology smear (MGG stain)

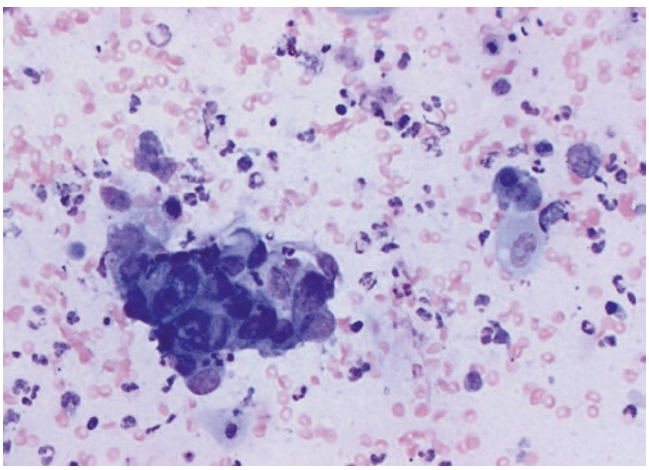

Fig. 12.36 Nipple discharge. A rather cellular cytology smear contains several dispersed single cells and a small 3-D cluster of cells displaying nuclear crowding, overlapping, and variation of nuclei. The background shows necrotic debris and inflammatory cells (MGG)

22. How is the cell block of breast FNA cytology specimens used to assist diagnosis?

A cell block could be applied to assist cytological diagnoses, as it reveals histologic/architectural pattern in borderline lesions and enables immunocytochemistry testing in a way similar to those used for histology specimens.

To differentiate atypical hyperplasia or low-grade DCIS from usual ductal hyperplasia, including differentiating a malignant papillary lesion from intraductal papilloma with usual ductal hyperplasia, cytopathologists could use the cell block to perform immunostain using ER and high molecular weight keratin such as CK5/6 and CK34beta. Cells of atypical hyperplasia or low-grade DCIS show diffusely and strongly positive staining for ER and negative staining for high molecular weight keratin; in contrast cells of usual hyperplasia show patchy positive staining for ER and diffusely and strongly positive staining for high molecular weight keratin.

To differentiate in situ carcinoma from invasive carcinoma including separating papillary carcinoma in situ from invasive papillary carcinoma, cytopathologists could also use the cell block to perform immunostain using p63, heavy-chain smooth muscle actin, and CK5/6 to demonstrate the presence or absence of the myoepithelial cell layer. For example, a papillary lesion with an intact basal layer of myoepithelial cell is considered a benign intraductal papilloma (Figs. 12.37 and 12.38).

Cell block could also be used to differentiate invasive ductal carcinoma from invasive lobular carcinoma by E-cadherin immunostain.

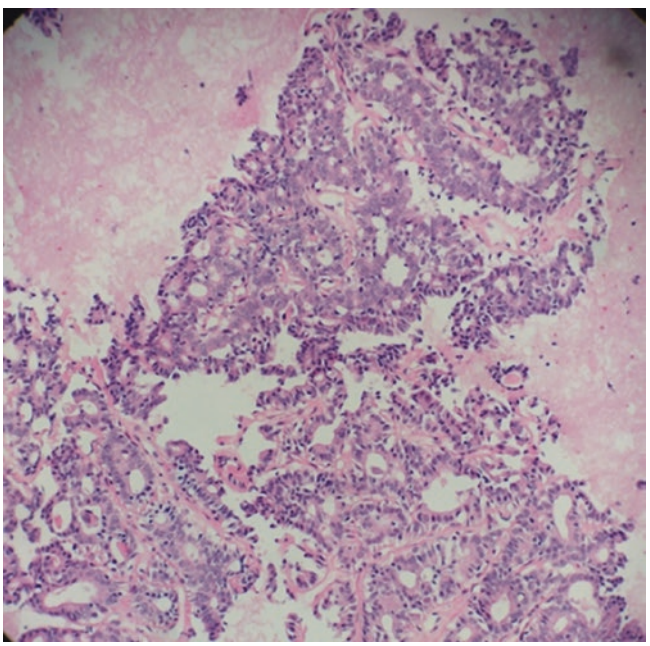

Fig. 12.37 Cell block of an atypical cytology smear was prepared, and the H& E slide shows a cellular lesion, raising a concern for papillary lesions or adenosis or others

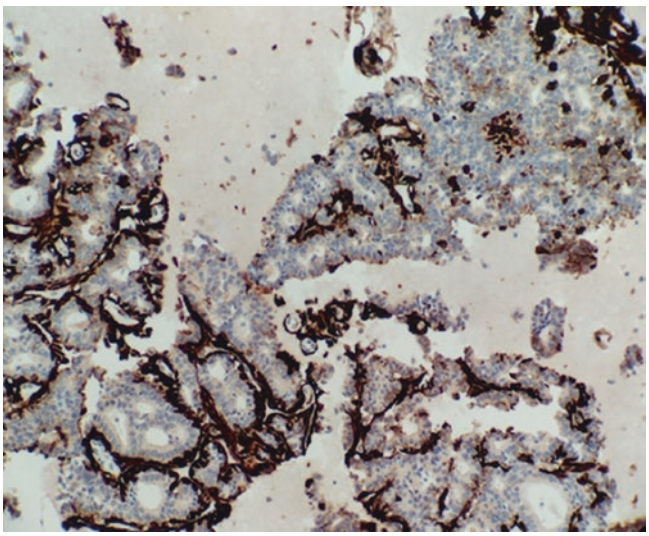

Fig. 12.38 p63 and heavy-chain myosin double staining highlights the basal cell layer of the lesion, indicating that this is a benign tumor

Cell block is frequently used to differentiate primary from metastatic carcinoma. To confirm the breast primary, ER, GATA-3, GCDFP-15, and mammaglobin antibodies have been used. Recent studies showed that GATA-3 is the most sensitive marker for breast carcinoma, being 100% positive in ER-positive breast carcinoma and positive in some triple negative breast carcinoma in cell block cytology specimens. Because GATA-3 is negative in ER-positive gynecologic cancer, a panel of GATA-3 and ER offers the most sensitive and specific conformation test for a primary breast carcinoma.

References: [23, 24].

23. Could we use FNA cytological specimens for ER, PR, and Her2 testing and for other predictive marker testing?

Breast FNA cytology specimen is a good source of material for breast biomarker testing when the specimen is adequately fixed and well prepared. Testing of ER, PR, and Her2 has been reported in a variety of cytology specimens including air-dried cytology smear without fixation, alcohol-fixed cytology smear, alcohol- or formalin-fixed cytospin specimen, alcohol-fixed liquid-based cytology slides, and cell block made from cells fixed in alcohol or formalin. The concordance of ER between immunocytochemistry and immunohistochemistry is highest in cell block specimens followed by cytospin and liquid-based cytology slides and lowest in air-dried cytology smear. Therefore, for biomarker testing especially ER testing, cell block made of cells fixed in formalin is the specimen of choice, offering highest concordance (98%) to the histologic specimens.

Although Her2 testing using immunocytochemistry is not recommended unless it is done using cell block made of

formalin-fixed cells, Her2 FISH testing could be performed using various cytology specimens because the procedure is not fixation dependent.

One of the drawbacks of using cytology specimens for breast biomarker testing is that malignant cells of invasive carcinoma and DCIS cannot be separated in the testing; therefore, breast biomarker testing using cytology specimens is only recommended for metastatic carcinoma or recurrent invasive carcinoma and is not for preoperative primary carcinoma of the breast.

Testing of Ki-67 of breast cancer on cytology specimens is not recommended because of its low concordance to immunohistochemistry, and its clinical value still awaits further confirmation.

Recently, rapid development in targeted therapy and immunotherapy for cancer treatment has called for new genetic testing and new molecular marker testing of breast cancer. FNA from breast is a good source of material for these new genetic and molecular testing.

References: [25, 26].

Case Presentation

Case 1

Clinical History

A 21-year-old college student noticed a lump in her right breast 2 weeks ago. On physical examination, the lump was mobile, firm, and well circumscribed. Mammogram showed a 2 cm well-defined mass and reported a benign BIRAD 2 lesion. Because of her anxiety, the patient was referred to FNA clinic for breast FNA.

Cytomorphologic Findings

Under the low power, the MGG-stained cytology smear displays branching fragments and groups of ductal epithelial cells, stromal fragments with a broad round smooth border surrounded by ductal epithelial cells, and scattered stripped naked nuclei in the background. Under the high power, ductal epithelial cells show mild nuclear crowding, nuclear variation in sizes and shapes, and focal distinct nucleoli. A few small darkly stained nuclei of myoepithelial cell are present with the cell group (Figs. 12.39 and 12.40).

Differential Diagnosis

Fibroadenoma
Phyllodes tumor
Well-differentiated ductal carcinoma
Papilloma

Final Diagnosis: Fibroadenoma

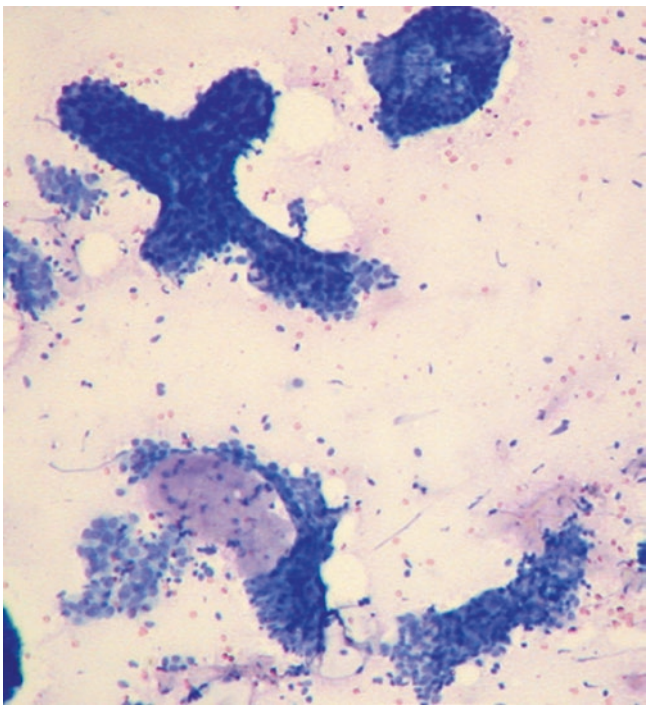

Fig. 12.39 Cytology smear of breast FNA of the 21-year-old college student displays, under the low power, branching fragments and groups of ductal epithelial cells, stromal fragments with a broad round smooth border, and scattered single naked nuclei in the background (Case 1, MGG stain)

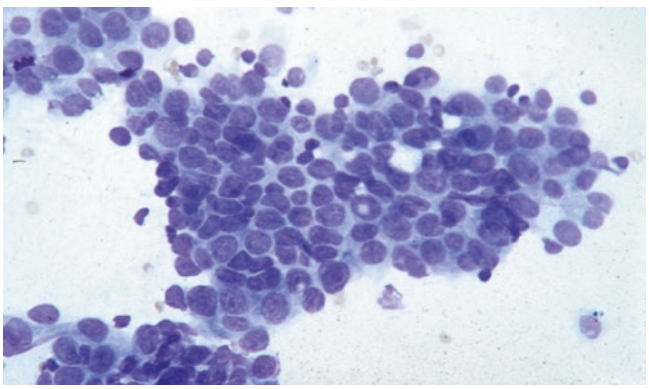

Fig. 12.40 Cytology smear of breast FNA of the 21-year-old college student shows, under the high power, ductal epithelial cells with mild nuclear crowding, mild variation in nuclear sizes and shapes, and focal distinct nucleoli (Case 1, MGG stain)

Case 2

Clinical History

A 45-year-old woman presents with a painless, slow-growing lump in her left breast for 6 months. Physical examination reveals a 3.5 cm well-circumscribed firm mass in left low quatrant of her

breast. There is no axillary lymphadenopathy. Mammogram showed a well-defined mass and suggested a benign tumor. FNA of the breast mass was performed.

Cytomorphologic Findings

Under the low power, the MGG-stained cytology smear reveals a multibranching 3-D structure or group of ductal epithelial cells and scattered single cells in the background. Under high power, both MGG-stained slide and ThinPrep cytology slide contain dispersed small groups or single columnar cells. A few columnar cells are arranged in strips. Occasional hemosiderin-laden macrophages are present in the background. A cell block was prepared which contains small fragments of tissue showing a hyalinized fibrovascular core surrounded by a sheet of monotonous ductal epithelial cells (Figs. 12.41, 12.42, 12.43, and 12.44).

Differential Diagnosis

Fibroadenoma
Invasive lobular carcinoma
Papillary neoplasm

Final Diagnosis: Papillary Neoplasm

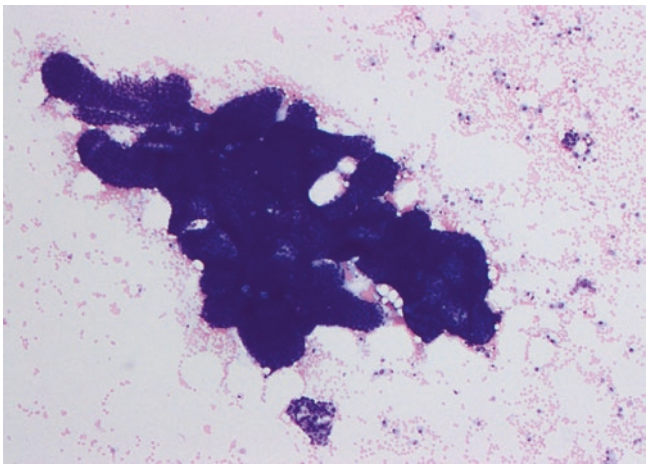

Fig. 12.41 Cytology smear of breast FNA of the 45-year-old woman reveals, under the low power, a multibranching 3-D structure of ductal epithelial cells and scattered single cells in the background (Case 2, MGG stain)

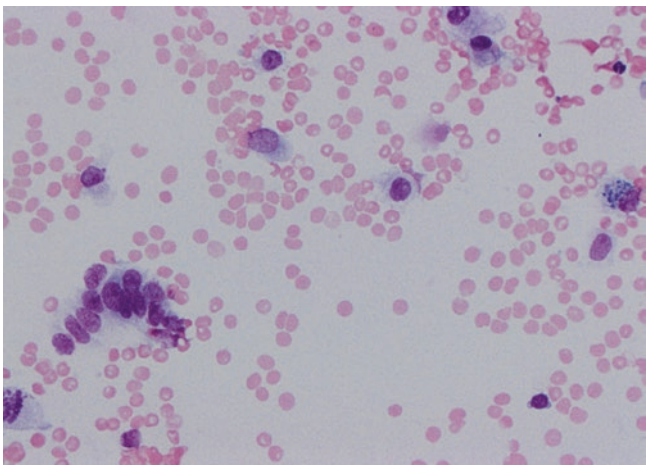

Fig. 12.42 Cytology smear of breast FNA of the 45-year-old woman displays, under the high power, scattered small strips and single columnar cells. Occasional hemosiderin-laden macrophages are present in the background (Case 2, MGG stain)

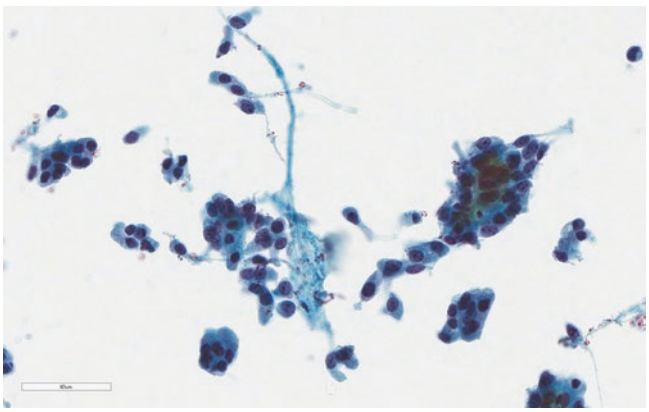

Fig. 12.43 ThinPrep cytology slide of breast FNA of the 45-year-old woman exhibits a cellular specimen containing small groups and single columnar cells. The ductal cells have moderate amount of cytoplasm, round to oval nuclei with mild nuclear crowding, and occasional distinct nucleoli (Case 2, Pap stain)

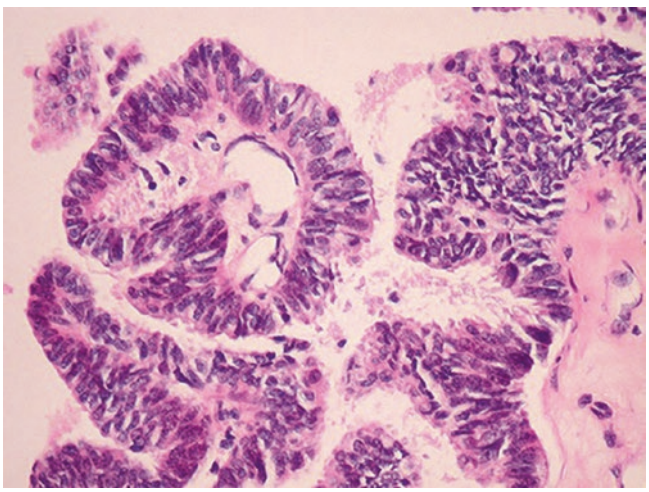

Fig. 12.44 Cell block made from the breast FNA of the 45-year-old woman contains small fragments of tissue showing a hyalinized fibrovascular core surrounded by monotonous stratified columnar ductal epithelial cells (Case 2, H/E stain)

Case 3

Clinical History

A 42-year-old nurse told her family doctor that she might have fibrocystic disease of the breast because she felt a small soft cystic nodule in her right breast for the past 5 months. The nodule was painless and slow growing. Physical examination showed a 1.2 cm soft well-circumscribed mass. Ultrasound showed a hypoechoic lesion. A FNA was performed trying to drain the "cyst."

Cytomorphologic Findings

Under the low power, MGG-stained cytology smear reveals many blue staining pools of mucin. Some groups of ductal epithelial cells are "buried" with the pools of mucin. Under high power, MGG-stained cytology slide reveals small groups of ductal epithelial cells that are closely associated with mucin. The cells have small nuclei, indistinct nucleoli, mild nuclear crowding, and focal irregular nuclear outline (Figs. 12.45 and 12.46).

Differential Diagnosis

Apocrine cyst
Fibroadenoma
Invasive mucinous carcinoma

Final Diagnosis: Invasive Mucinous Adenocarcinoma

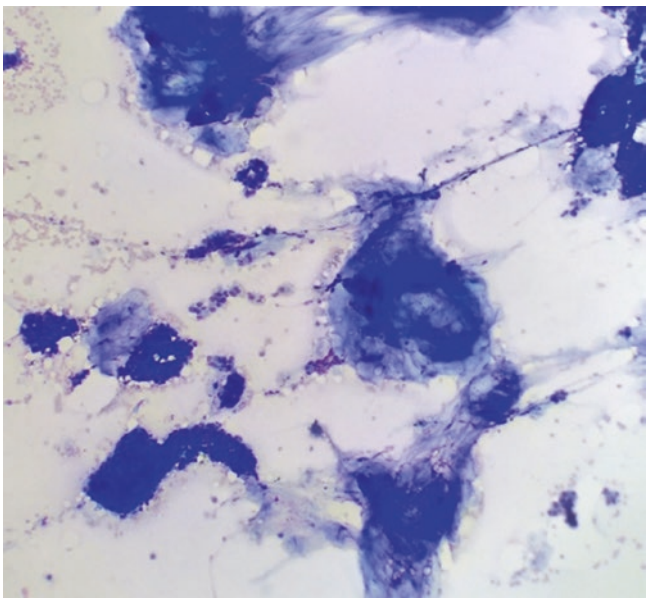

Fig. 12.45 Cytology smear of breast FNA of the 42-year-old nurse exhibits, under the low power, many blue staining pools of mucin; some have groups of ductal epithelial cells "buried" with them (Case 3, MGG stain)

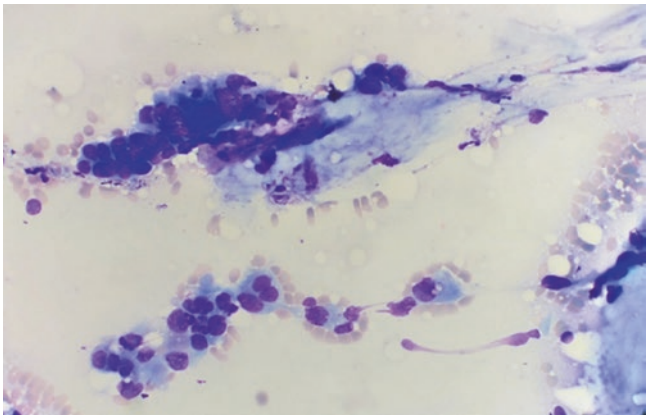

Fig. 12.46 Cytology smear of breast FNA of the 42-year-old nurse displays, under the high power, small groups of ductal epithelial cells closely associated with mucin. The cells have small nuclei, mild nuclear crowding, and focal irregular nuclear outline (Case 3, MGG stain)

Case 4

Clinical History

A 68-year-old woman with a past history of right breast carcinoma treated with surgical excision and chemotherapy 10 years ago now presents with a small subcutaneous nodule in her right upper chest wall. The nodule is 0.5 cm, painless, and firm. The patient also had a history of basal cell carcinoma on her face and melanoma in situ in her left arm diagnosed a year ago. The subcutaneous nodule is aspirated.

Cytomorphologic Findings

ThinPrep cytology slide shows many scattered isolated large atypical cells containing eccentric round nuclei, occasional binucleation, and abundant cytoplasm. The cells have somewhat "plasmacytoid" appearance but do not have prominent nucleoli. The H&E slide of cell block contains similar "plasmacytoid" cells, but a few cells also have signet ring cell appearance. Immunostaining was performed using the cell block, and the tumor cells show positive staining for ER (Figs. 12.47, 12.48, and 12.49).

Differential Diagnosis

Melanoma
Recurrent breast carcinoma
Plasma cell-rich skin lesions

Final Diagnosis: Recurrent Breast Carcinoma

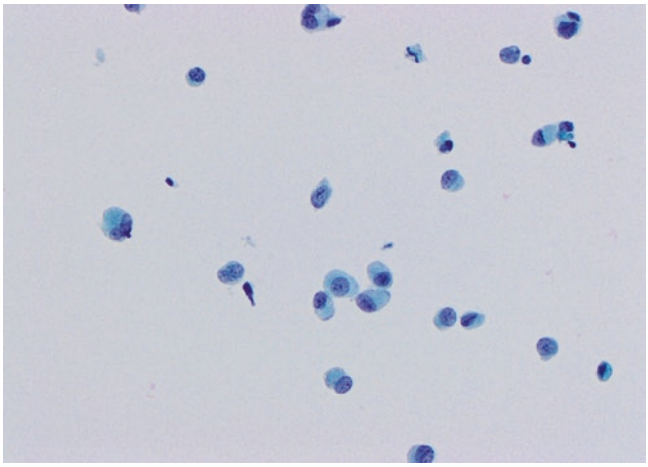

Fig. 12.47 ThinPrep cytology slide of breast FNA of the 68-year-old woman shows many scattered isolated large atypical cells containing eccentric round nuclei, occasional binucleation, and abundant cytoplasm, having somewhat "plasmacytoid" appearance (Case 4, Pap stain)

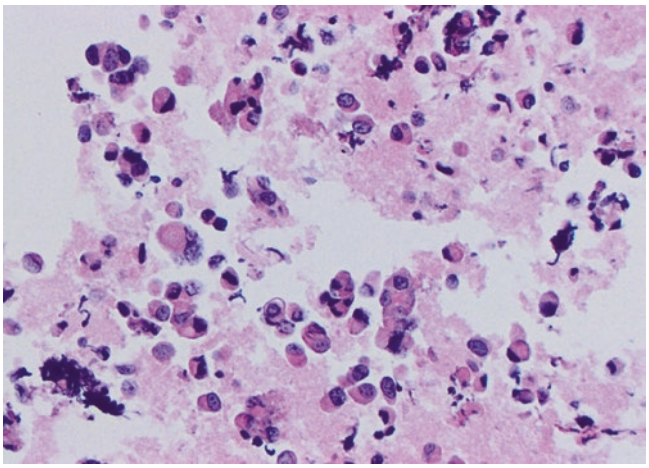

Fig. 12.48 Cell block made from the breast FNA of the 68-year-old woman contains many large atypical "plasmacytoid" cells and a few signet ring-like cells (Case 4, H/E stain)

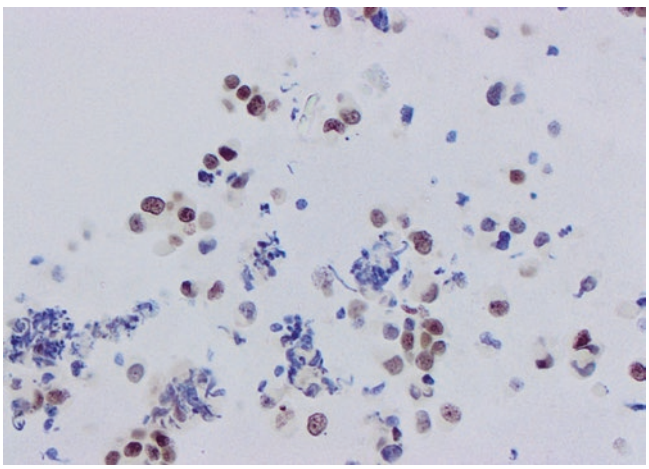

Fig. 12.49 Immunohistochemistry (IHC) performed on the cell block of the breast FNA of the 68-year-old woman shows that the nuclei of the cells are stained positively for ER (Case 4, IHC stain)

Case 5

Clinical History

In follow-up visit, a 60-year-old woman presents with a small solid mass at the site of her previous left breast lumpectomy performed 9 months ago for an invasive ductal carcinoma. Physical examination shows a 1.5 cm hard mass at the edge of previous surgical site. Clinically recurrent breast carcinoma is suspected and a FNA is performed

Cytomorphologic Findings

Both MGG-stained and Pap-stained cytology smears display multinucleated giant cells containing several round to irregular nuclei, distinct nucleoli, and abundant foamy cytoplasm. The Pap cytology smear also contains several small aggregate and isolated cells; some have round nuclei and prominent nucleoli (Figs. 12.50 and 12.51).

Differential Diagnosis

Fibrous scar
Recurrent carcinoma
Fat necrosis

Final Diagnosis: Fat Necrosis

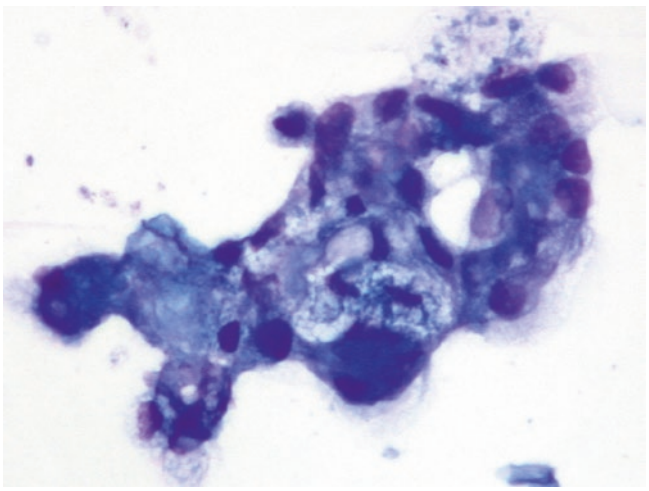

Fig. 12.50 Cytology smear of breast FNA of the 60-year-old woman displays multinucleated giant cells containing multiple round to irregular nuclei, distinct nucleoli, and foamy cytoplasm (Case 5, MGG stain)

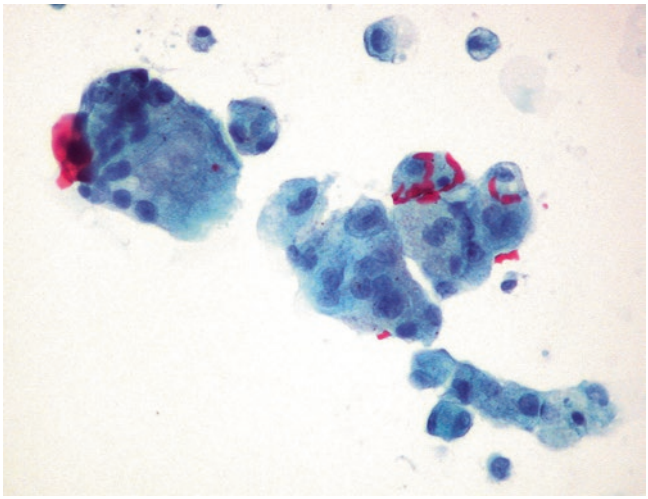

Fig. 12.51 ThinPrep cytology slide of breast FNA of the 60-year-old woman shows some multinucleated giant cells and scattered histiocytes. The giant cells contain multiple round pale stained nuclei with mild nuclear crowding, focal distinct nucleoli, and abundant foamy cytoplasm (Case 4, Pap stain)

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Thyroid

13

Huihong Xu and Sandra Cerda

Contents

Frequently Asked Questions

1. How is thyroid FNA used as a screening test for clinically or ultrasound-detected thyroid nodules?

As:

The prevalence of thyroid nodules is high in the general population, up to 50–60% [1]. However, the malignancy rate is only 5% [1]. The fine needle aspiration (FNA) serves as a minimal invasive test and as a gatekeeper for further management, as well as to select the appropriate surgical candidates. Based on ultrasonography (US), the thyroid nodules are categorized into three groups: low malignancy risk, intermediate malignancy risk, and high malignancy risk. The current American Thyroid Association (ATA) guidelines recommend FNA of thyroid nodules that are >10 mm diameter and lack of suspicious US and/or clinical findings but are not completely benign appearing (intermediate US risk thyroid nodules). FNA should be considered in thyroid nodules 5–10 mm diameter only when suspicious US signs are present (high US risk thyroid lesions). It is recommended that thyroid nodules <5 mm should be monitored with US (Table 13.1).

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2. What are the key procedure steps of thyroid aspiration and slide preparation?

As:

The FNA remains the gold standard for evaluation of thyroid nodules. The thyroid aspiration can be performed under ultrasound or palpation guidance. Ultrasound-guided thyroid fine needle aspiration can reduce the unsatisfactory rate, especially for those thyroid nodules with the following features, such as non-palpable, cystic, or unsatisfactory from previous aspiration. Aspiration nodules less than 1 cm are not recommended unless with suspicious features such as microcalcifications or lesion with heterogeneous cystic component (>25%) [3].

The key steps of the aspiration techniques are the same in both palpated and US-guided procedure. The skin should be cleaned by alcohol swab. Local anesthesia with lidocaine is optional. Regarding the aspiration needle, guidelines recommend the small size 25- or 27-gauge needle to avoid damaging the vessels. The needle should not go through gel if under US guidance; otherwise, it will interfere the cytomorphology. The aspiration can be performed with or without suction according to patient's condition and operator's preference and depending on the nodule structure and vascularization. When using suction, it should be released before the needle comes out. The number of aspiration needle passes varies, depending on the nature of the lesion, the expertise of the performer, and the availability of on-site evaluation, usually 2–5 passes [4].

Based on individual practice setting, a variable number of smear slides are prepared for alcohol fixed Papanicolaou stained smears, H&E smears, or air-dried and Romanowsky-

H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_13

Table 13.1 US rating system of the risk of malignancy and the indications for US-guided FNA [2]

	US classification systems	US features	Risk of malignancy	Indications for US-guided FNA
Class I	Low-risk thyroid lesion	Isoechoic spongiform appearance Simple cyst with thin margins Mostly cystic (>50%) nodules with comet-tail sign (colloid) Regular "eggshell" calcification	1%	>20 mm and increasing in size or associate with a risk history and before thyroid surgery or minimally invasive ablation therapy
Class II	Intermediate-risk thyroid lesion	Isoechoic or hyperechoic nodule with hypoechoic halo Mild hypoechoic nodule with smooth margin Peripheral vascularization Intranodular macrocalcification	5–15%	>20 mm
Class III	High-risk thyroid lesion	Marked hypoechogenicity Spiculated or microlobulated margins Microcalcifications Taller-than-wide shape Evidence of extrathyroidal growth or pathologic adenopathy Intranodular vascularization and well-defined halo	50–90%	≥10 mm Thyroid incidentalomas detected by positron emission tomography (PET)

type stained smears. As an adjunct to the smears, rinsing the needle in the cyto-collection fluid to make a cell suspension is a common practice. The cell suspension solution can be used for liquid-based preparation (either SurePath or ThinPrep), and cytospin and cellblock preparation. Additional passes may be required to rinse in a second tube for molecular studies (e.g., the Afirma Gene Expression Classifier, ThyroSeq, and ThyGenX) [5].

The Papanicolaou stain is used in liquid-based cytology (SurePath and ThinPrep) slides and other conventional alcohol fixed slides. It usually gives better nuclear features, such as inclusions, grooves, and chromatin texture. The Romanowsky-type stain is used in conventional air-dried slides and allows better evaluation of extracellular material (colloid and amyloid) and cytoplasmic granules. In some labs, an H&E stain is used for conventional alcohol fixed slides too. The advantage of the H&E stain is that the cytomorphology is comparable to the routine histology stain.

For the key procedures/steps, please also refer to the clinical management guidelines.

Table 13.2 Compare the cytomorphological features of colloid in thyroid FNA with different stains

	PAP (smear)	DQ (smear)	H&E (smear or cellblock)
Colloid (thin/watery)	A thin layer of blue, light green, or pink amorphous material with linear cracking artifact, lost on touch-prep slide	A thin layer of pink to purple amorphous material with linear cracking artifact, lost on touch-prep slide	Thin pink homogenous material
Colloid (thick/dense)	Round- or irregular-shaped chips displaying cracking artifact on the side with blue color. Two-tone color (pink/orange in the central area and blue/purple on the edge)	Round- or irregular-shaped chips displaying cracking artifact on the side with homogeneous pink/purple color	Dense pink homogenous material

3. What are the cytomorphological differences of colloid among different staining methods in thyroid FNA?

As:

Colloid, a sticky fluid, is present at the core of a thyroid follicle. It is basically a collection of large glycoprotein – thyroglobulin. It is synthesized by thyroid follicular cells under the stimulation of thyroid-stimulating hormone (TSH). The texture and quantity of the colloid reflect the metabolism status of the thyroid gland and the nature of the thyroid nodule. The cytomorphological features (Table 13.2) and quantity of colloid are among the key diagnostic criteria to determine the nature of the FNA targeted thyroid lesion. In general, benign thyroid nodule has abundant colloid, and the texture can be thin, watery, or thick. Some of the follicular lesions have balls of inspissated (bubble gum)-like colloid. And most of malignant lesions have scant thick colloid.

4. What are the complications of thyroid FNA?

As:

There is no general contraindication for thyroid FNA. Universal precautions are necessary to prevent the complications of thyroid FNA. Patients need to be prescreened and

given prophylaxis if there is history of bleeding disorder, uncontrollable coughing, infectious disease, mental disorder, etc. Complications are uncommon. Subcutaneous transient hematoma, local tissue or thyroid swelling, abscesses formation, and post-aspiration thyrotoxicosis can occur in some patients. Other uncommon ones are infarction of the nodule, tracheal injury, and damage to the local nerve and blood vessels. Seeding of neoplastic cells along the needle track after aspiration is very rare, mostly seen in papillary carcinoma, followed by follicular carcinoma and anaplastic carcinoma. [6].

5. What is the diagnostic accuracy of thyroid FNA? What are the main factors to cause false positive and false negative diagnosis?

As:

The sensitivity of thyroid FNA ranges from 65% to 98%. And the specificity ranges from 72% to 100%. The recent application of ancillary molecular tests has significantly increased both sensitivity and specificity.

False positive diagnosis is usually caused by overinterpretation of reparative changes, especially in the background of chronic inflammation or changes related to previous biopsy. In a hypercellular specimen, benign papillary hyperplasia could be overcalled as papillary thyroid carcinoma. These will result in the increase of unnecessary surgical rates or total thyroidectomy surgical rates. The false positive rate ranges from 0% to 7%.

False negative diagnosis rate is about 1–11%. Inadequate sampling, poor sample collection, lack of options of ancillary molecular test, diagnostic error, and sampling error due to occult small lesion, or heterogeneous cystic lesion, are the main reasons.

6. What is the relationship between Bethesda diagnostic categories and risk of malignancy (%)?

As:

The Bethesda reporting system for classifying thyroid cytology was proposed in 2007 by Dr. Edmund Cibas and Dr. Syed Ali at the meeting hosted by the National Cancer Institute and provides diagnostic categories with accompanying risk stratification and recommended clinical management. This standardized reporting system improves the communication between laboratories, surgeons, radiologists, cytopathologist, and clinicians toward management of patient [7, 8]. In 2017, a revised version was published by adding new knowledge in recent thyroid research advancement. Two major changes are added. One is applying ancillary molecular testing to assist cytomorphological diagnosis. The other is reclassifying the noninvasive follicular variant of papillary thyroid carcinoma as noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), to improve clinical management. In Table 13.3, the differences in risk of malignancy and recommended clinical management between 2010 and 2017 Bethesda System for Reporting Thyroid Cytopathology are listed.

7. What are the current ancillary studies available with thyroid FNA fluid specimen?

As:

The current ancillary studies include the following: thyroid hormone measurement on FNA washout (Table 13.4), immunocytochemistry (Table 13.5), and molecular testing [5].

The molecular testing in general is to complement, not replace, cytomorphological evaluation and to assist clinical

Table 13.3 The differences in risk of malignancy and recommended clinical management between 2010 and 2017 Bethesda System for Reporting Thyroid Cytopathology

Bethesda categories	Interpretation	Risk of malignancy (%) 2010	Risk of malignancy if NIFTP not CA (%) 2017	Risk of malignancy if NIFTP = CA (%) 2017	Decreased risk of malignancy after NIFTP reclassification (%)	Usual management 2010	Usual management 2017
I	Nondiagnostic or unsatisfactory	1–4	5–10	5–10	Not significant	Repeat FNA with US guidance	Repeat FNA with US guidance
II	Benign/negative	0–3	0–3	0–3	0.3–3.5	Clinical F/U	Clinical F/U
III	Atypical (AUS/FLUS)	5–15	6–18	10–30	5.2–13.6	Repeat FNA	Repeat FNA or molecular testing
IV	Follicular neoplasm or suspicious for a follicular neoplasm	15–30	10–40	25–40	9.9–15.1	Surgical lobectomy	Lobectomy or molecular testing
V	Suspicious for malignancy	60–75	45–60	50–75	17.6–23.4	Near-total thyroidectomy or surgical lobectomy	Near-total thyroidectomy or surgical lobectomy
VI	Malignant	97–99	94–96	97–99	2.5–3.2	Near-total thyroidectomy	Near-total thyroidectomy or surgical lobectomy

Adapted from Ali and Cibas [7, 9] with permission of Springer

Table 13.4 Utilization of hormone measurement in thyroid FNA washout

Hormone measurement	Indications	Comments
Thyroglobulin level	Recurrence of thyroid carcinoma s/p radical thyroidectomy Rule-out lymph node with metastatic thyroid carcinoma	*Fine needle aspiration thyroglobulin* (*FNA-Tg*) test increases FNA accuracy of lymph nodes which are suspicious for metastatic well-differentiated thyroid cancer Overall FNA-Tg sensitivity (95%) and specificity (94.5%) are good However the technique is not fully standardized and definite cutoff levels have not been validated
Calcitonin level	Clinical suspicion of medullary thyroid carcinoma (MTC) or multiple endocrine neoplasia type 2 (MEN2), screening lymph nodes with patients history of MTC	A diagnosis of MTC is highly indicated when the elevation of calcitonin is >100 pg/ml. The degree of calcitonin elevation correlates well with tumor volume.
Parathyroid hormone (PTH) level	Rule-out parathyroid adenoma	It has not been validated and definite cutoff values have not been established

Table 13.5 Utilization of immunocytochemistry (ICC) studies on thyroid FNA cellblock and smears

ICC studies	Indications	Comments
Markers include galectin-3, HBME-1, fibronectin-1, CITED-1, and cytokeratin-19	Differentiate thyroid lesions from nonfollicular origin (e.g., parathyroid gland, medullary thyroid carcinoma, lymphoma, metastasis from another organ origin)	These markers have not been adopted entirely to improve the DD of indeterminate nodules, due to absence of method standardization and overlap between follicular adenomas and differentiated thyroid carcinomas. The use of panels of IHC markers may reach a sensitivity and specificity of up to 90% if enough tissue is present in cellblock
PTH positive and TTF-1 negative	To confirm parathyroid gland origin	Need good quality of specimen
Positive: calcitonin, CEA, chromogranin, synaptophysin, TTF-1, and Congo red (amyloid component) Negative: thyroglobulin	To confirm medullary thyroid carcinoma	Need good quality of specimen
Lymphoma IHC panel	To confirm and classify lymphoma	Need good quality of specimen

management. It is not recommended in benign and malignant nodules with characteristic cytomorphological features. The uncertainty of incidental thyroid nodules (ITNs) can be resolved by molecular tests that are able to rule-in or rule-out malignancy. This ability to rule in and rule out depends on the test's PPV and NPV. BRAF, RET/PTC, PAX8/PPARG, and RAS mutations are often used to detect mutations in cytological indeterminate nodules. $BRAF^{V600E}$ is almost 100% PPV for papillary thyroid carcinoma. In Table 13.6, common mutations associated with thyroid neoplasia are listed.

One of the most exciting advancements in molecular studies is applying clinical utilization of next-generation sequencing platform (Table 13.7) [5].

8. How will changes in the new 8th edition AJCC cancer staging manual potentially affect our cytology reporting?

As:

In the 8th edition AJCC staging book [11, 12], the pN0 designation is clarified as one or more cytologically or histologically confirmed benign lymph node(s). This requisite will result in an increase in neck lymph node FNA or thyroid bed lymph node FNA for restaging. The challenge or the pitfall will be to differentiate between a metastatic tumor deposit and residual normal thyroid follicles, chronic thyroiditis versus metastatic lymph nodes, and normal parathyroid gland cells. Therefore, a definitive cytology diagnosis is important for further staging of the resected surgical specimen.

9. Can we diagnose NIFTP in thyroid cytology?

As:

Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP) is a recently proposed terminology for thyroid neoplasms which used to be called noninvasive encapsulated follicular variant of papillary thyroid carcinoma (PTC) [13].

The Endocrine Pathology Society performed a 10–25-year clinical follow-up study for patients with NIFTP diagnosis. This clinical study shows that this tumor has very low risk of recurrence and nodal metastasis. More conservative therapy like lobectomy only is recommended.

The diagnostic criteria for NIFTP are as follows:

- Encapsulation or clear demarcation.
- Follicular growth pattern with <1% true papillae formation; no psammoma bodies; <30% solid/trabecular /

Table 13.6 Common mutations in thyroid neoplasia [10]

	PTC, classical and tall cell	PTC, follicular variant	Follicular carcinoma	Poorly differentiated carcinoma	Anaplastic carcinoma	Follicular adenoma
BRAF V600E	+++			+	+	
BRAF K601E		+++	+			+
NRAS		+++	++	+	+	++
HRAS		++	+			+
KRAS	+	++	+			++
PTEN			+			++
TSHR			+			++
GNAS						++
RET/PTC	++ (PTC1/CCD6)					+ (PTC3/ NCOA4)
PAX8/PPARG		++	+++			+
ALK FUSIONS	+	+		++	++	
BRAF FUSIONS	+	+				
ETV6/NTRK	++					
NTRK FUSIONS	++					

Note: Number of "+" indicates reported frequency ranges in genetic mutations

Table 13.7 Utilization of next-generation sequencing platform on thyroid FNA specimen

Next-generation sequencing	Indications	Comments
Afirma (Veracyte): Gene expression classifier (GEC) test based on microarray technology used to analyze the mRNA expression of 167 different genes	Good as "rule-out test" with a NPV of 95% (Bethesda III) and 94% (Bethesda IV) categories (if the test is "benign" in ITN category, the patient could be followed up clinically with no need for surgery) Hurthle cell lesion specificity is high (58.8%)	Lower performance in lesions of Bethesda V (suspicious for malignancy) category; the NPV is only 85%, leaving a 15% risk of malignancy Lower performance as a "rule-in" test with a PPV of 38% for Bethesda II and 37% for Bethesda IV categories
ThyroSeq v.2. (new expanded version of the original ThyroSeq): next-generation sequencing-based gene mutation and fusion panel of DNA alterations (14 genes and >1000 mutations) and RNA alterations (42 fusions, 16 genes)	In the lesions with pretest probability of malignancy (14–34%), ThyroSeq has shown a reported PPV of 83% and NPV of 96% suggesting that it may potentially function as both "rule-out" and rule-in" test for nodules with indeterminate cytology A detection of a mutation highly predictive of malignancy (BRAFV600E, TERT, TP53, PIK3CA, gene rearrangement) could direct patients toward total thyroidectomy	In the lesions with a low pretest probability of malignancy (5–15%), although ThyroSeq v.2. would remain an effective "rule-out" test (good NPV of 98–99%), a relatively low PPV (40–69%) would make it an unsatisfactory "rule-in" test in indeterminate nodules There is an increased chance of detecting "false positive" molecular abnormalities with the expanded NGS-based mutational profile
ThyGenX (thyroid oncogene panel): using a next-generation sequencing (NGS) platform to identify more than 100 genetic alterations across 8 genes associated with thyroid malignancy	Only cases with Bethesda III and IV categories are accepted for ThyGenX analysis	ThyGenX requires only one dedicated FNA pass (50 ng of cellular material) More recently used in combination with the ThyraMIR test
ThyraMIR: based on the analysis of 10 different microRNAs	In conjunction with ThyGenX when the ThyGenX result is negative	A combination of ThyGenX and ThyraMIR demonstrated a NPV of 94% (good rule-out test) and PPV of 74% (good rule-in test) When both ThyGenX and ThyraMIR tests are negative, the residual risk of cancer is very low (6%)

insular growth pattern; no tall cell, columnar, or cribriform-morular morphology; and no necrosis.

- Nucleus with 2 or 3 following features (1-enlarged and elongated nucleus, nuclear overlapping; 2-nuclear membrane with irregular contours, grooves, and pseudoinclusions; 3-chromatin clearing with margination and glassy nuclei).
- No vascular or capsular invasion.
- No high mitotic activity (<3 mitotic figures per 10 HPF).

- NIFTP are BRAF V600E mutation negative; instead they often have RAS mutations like follicular adenoma/carcinoma [14].

However, it is not possible to make a definitive cytologic diagnosis of NIFTP on cytology FNA specimens. Studies show that NIFTP tumors have been diagnosed as all six categories of the Bethesda System for Reporting Thyroid Cytopathology. But most of them are clustered in the indeterminate categories: atypia of undetermined significance/follicular lesion of undetermined significance (BS III – AUS/FLUS), follicular neoplasm/suspicious for follicular neoplasm (BS IV – FN/SFN), and suspicious for malignancy (BS V – SFM) [8, 15].

The cytologic features of NIFTP are as follows[16]:

- Groups of follicular cells showing nuclear crowding/overlapping
- Some of the PTC nuclear features (1-enlarged and elongated nucleus, nuclear overlapping; 2-nuclear membrane with irregular contours, grooves, and rare pseudoinclusions; 3-chromatin clearing with margination and glassy nuclei)

If the following cytologic features are noticed, a NIFTP diagnosis should be excluded:

- Papillary architecture, such as true papillae, branching groups, capillaries, or psammoma bodies
- Frequent intranuclear pseudoinclusions

The thyroid FNA is still a screening test. A NIFTP lesion should be suspected on a cytology FNA specimen with a predominantly microfollicular pattern and some nuclear features of PTC. The possibility should be raised on a cytology report. So far there is no consensus as to what Bethesda category a NIFTP should be assigned to. The best approach is to make a comment [17].

In the current guidance of the management of indefinite thyroid FNAs, the molecular testing for rule-in or rule-out NIFTP is not very helpful. It usually harbors similar mutations such as RAS or PAX8/PPARγ to other follicular lesions (follicular adenoma and follicular carcinoma). PTC-associated BRAF V600E mutations and RET fusions are usually absent. Patients most likely will receive hemithyroidectomy alone [18].

10. What are the clinical practice guidelines of thyroid nodules?

As: Since the Bethesda System for Reporting Thyroid Cytopathology is linked to a malignancy risk, the clinical management of thyroid nodules is directed by the thyroid cytology reporting with six Bethesda diagnostic categories. The current clinical practice guidelines are listed in (Table 13.8).

Table 13.8 Clinical management of thyroid nodules [19]

FNA Diagnosis	Clinical management
Nondiagnostic – **Bethesda category I**	If the nodule is solid, repeating the FNA with US guidance is recommended If repeat FNA is inadequate, performing a US-guided CNB is recommended If FNA inadequacy is persistent, surgery may be considered in a minority of solid nodules with favorable clinical and US features If the nodule is predominantly cystic (>50%) with benign clinical and US features, follow up with US
Benign – **Bethesda category II**	Clinical follow-up Repeat FNA only if clinically symptomatic, having suspicious clinical or US features, or in nodules with an increase >50% in volume Medical treatment is not recommended in general Consider surgery if there is presence of local pressure symptoms, having suspicious US features Percutaneous ethanol injection (PEI) for thyroid cysts and complex nodules with a large fluid components, relapsing benign cystic lesions Consider laser or radiofrequency ablation, if the nodules are solid or complex, progressively enlarge, symptomatic, or having cosmetic concern Consider radioiodine therapy for hyperfunctioning and/or symptomatic goiter, high-risk surgical candidate
Indeterminate lesions (low risk) – **Bethesda III (AUS/FLUS)**	Consider conservative management if the clinical criteria are favorable Repeat FNA and review with experienced cytopathologist CNB may be considered Routine use molecular markers for ancillary testing is under investigating
Indeterminate lesions (high risk) – **Bethesda IV (FN/SFN)**	Consider surgery for most of the lesions Thyroid lobectomy plus isthmectomy is recommended; total thyroidectomy may be performed depending on clinical setting and patient preference Consider close clinical follow-up in a minority of cases with favorable clinical and US features
Suspicious nodules – **Bethesda V**	Surgical treatment is recommended Repeat FNA in cases (such as anaplastic thyroid carcinoma, metastatic lesions, and thyroid lymphoma) with inadequate cellularity or require more cellular material for further diagnostic work-up (such as anaplastic thyroid carcinoma, metastatic lesions, and thyroid lymphoma) Intraoperative frozen section may be considered
Malignant – **Bethesda VI**	Surgical treatment is recommended in the case of differentiated thyroid carcinoma Preoperative evaluation of concomitant suspicious nodule or lymph node with FNA/B

Case Presentation

Case 1

Learning objectives:

1. To become familiar with cytologic features of benign thyroid cytology
2. To generate a differential diagnosis

Case history:

- A 31-year-old female was found left neck mass incidentally on regular physical examination. Imaging studies show a 3 cm left thyroid nodule with solid and cystic US features. The patient, otherwise healthy, did not have any other clinical symptoms.

Specimen source:

- Ultrasound-guided fine needle aspiration was performed on the left thyroid nodule. A Pap-stained smear and SurePath smear were made from the aspiration. Corresponding surgical resection specimen was obtained 6 months later.

Cytomorphological findings:

- Adequate cellular specimen.
- The follicular cells arranged in clusters with microfollicular and macrofollicular patterns (Fig. 13.1a, c).
- Some follicular fragments show three-dimensional structure.
- Some colloid and scattered macrophages are seen in the background (Fig. 13.1b, c).

Differential diagnosis:

- Benign follicular nodule
- Follicular lesion
- Papillary thyroid carcinoma

Cytology final diagnosis:

- Benign (Bethesda category II)

- Multinodular goiter with cystic degeneration

Histological findings:

- Benign hyperplastic nodule with pseudopapillary hyperplastic changes (Fig. 13.1d)

Take-home messages:

1. Benign follicular lesion usually presents with variable proportion of colloid, microfollicles and macrofollicles, and some scattered macrophages.
2. Increased degenerative changes, stromal fragments, and foamy macrophage suggest of lesion with cystic degeneration.
3. In some benign hypercellular nodules, decreased colloid and abundant follicular cells can be present. Most of the follicular cells form monolayers, rosettes, microfollicles, or three-dimensional structure (pseudopapillary hyperplastic changes), but with bland nuclear features and without PTC-like nuclear features (such as nucleus enlargement, overlapping, and pseudoinclusions).
4. Very low false negative rate (<3%).

References: [20, 21].

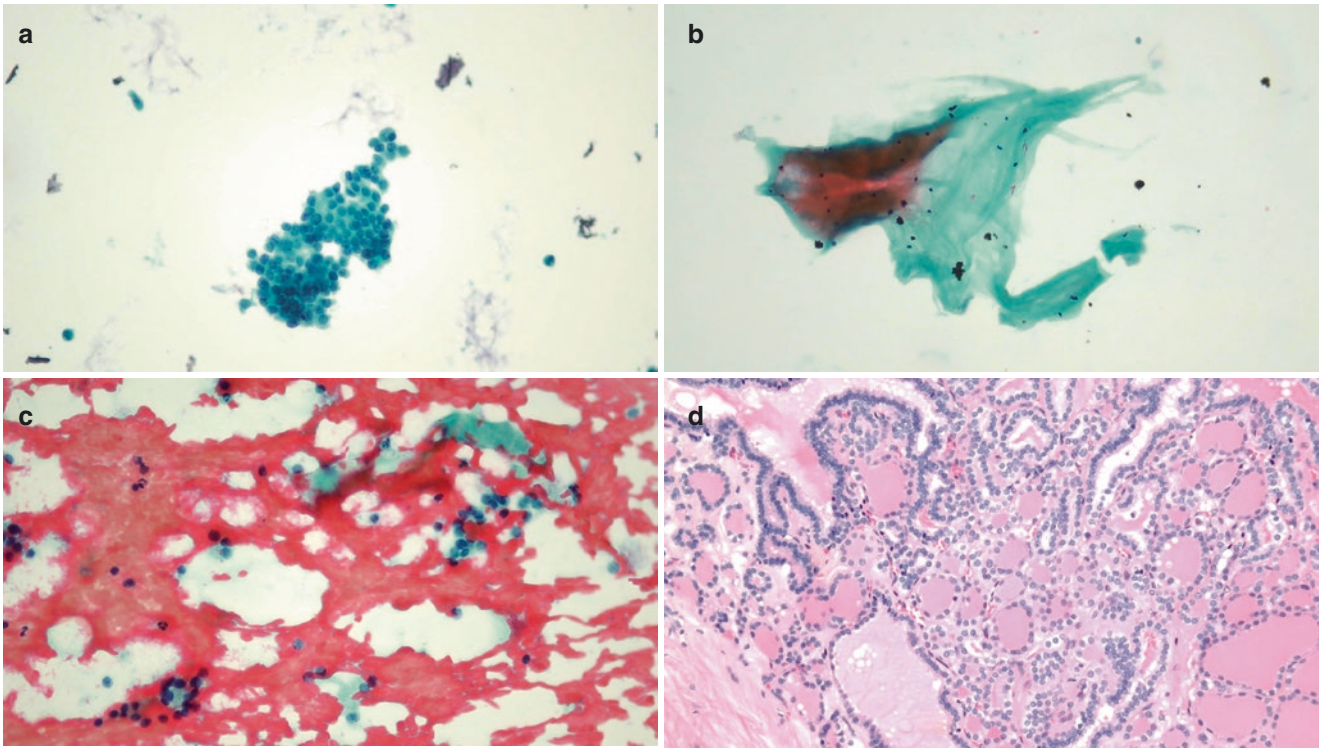

Fig. 13.1 Case 1. (**a**) Mixed microfollicles and macrofollicles (SurePath, Papanicolaou stain 400×). (**b**) Thin and thick colloid material (conventional smear, Papanicolaou stain 200×). (**c**) Mixed microfollicles and macrofollicles, some colloid and foamy macrophages (conventional smear, Papanicolaou stain 400×). (**d**) Benign hyperplastic thyroid nodule with focal papillary hyperplasia (histologic section, H&E stain 400×)

Case 2

Learning objectives:

1. To become familiar with the Bethesda System for Reporting Thyroid Cytopathology
2. To generate a differential diagnosis and recognize mimics of oncocytic follicular neoplasms
3. To become familiar with current clinical management guidelines

Case history:

- A 60-year-old male was found on imaging studies to have a 1.3 cm thyroid nodule in the right mid lobe. The patient, otherwise healthy, did not have any other clinical symptoms.

Specimen source:

- Ultrasound-guided fine needle aspiration was performed on the right thyroid nodule. A Pap-stained smear and SurePath smear were made from the aspiration. Corresponding surgical resection specimen was obtained 5 months later.

Cytomorphological findings (Fig. 13.2a–c):

- Hypercellular specimen with scant colloid.
- Follicular cells are arranged predominantly in microfollicular, trabecular, or syncytial sheet-like patterns.
- Some populations of microfollicles are arranged in crowded trabecular abnormal architectural groupings.
- Most of the follicular cells show oncocytic changes with finely granular cytoplasm, large and round central nuclei, and prominent nucleoli. Mild nuclear atypia and pleomorphism are noted.

Differential diagnosis:

- Hyperplastic proliferations of follicular cells in multinodular goiter
- Follicular lesions (follicular vs Hurthle cell)
- Chronic thyroiditis
- NIFTP
- Papillary thyroid carcinoma

Cytology final diagnosis:

- Follicular neoplasm or suspicious for a follicular neoplasm (Bethesda category IV)
- Hurthle cell (oncocytic) type

Histological Findings:

- Hurthle cell adenoma (Fig. 13.2d).
- There is no evidence of capsular or lymphovascular invasion.

Take-home messages:

1. The Bethesda category IV (follicular neoplasm or the synonymous term suspicious for a follicular neoplasm) is to identify a nodule that might be a follicular or Hurthle cell carcinoma and subject to surgical (lobectomy) follow-up. Distinction between a follicular adenoma and follicular carcinoma, which diagnostic criteria are based on capsular and/or vascular invasion, is not possible diagnosed on cytologic material.
2. It is important to differentiate the follicular lesions from the Hurthle cell lesions since they have different underlying genetics.
3. It is helpful to recognize the abnormal architectural patterns (predominantly single cells, syncytial-like sheets, and decreased colloid) which are suggestive of neoplasm instead of other benign mimics (e.g., Hurthle cell metaplasia in chronic lymphocytic thyroiditis and multinodular goiter).
4. If the lesion shows predominantly microfollicles and associated with mild focal nuclear changes, suspicious for FVPTC or NIFTP, it can be put in this Bethesda category.

References: [22–26].

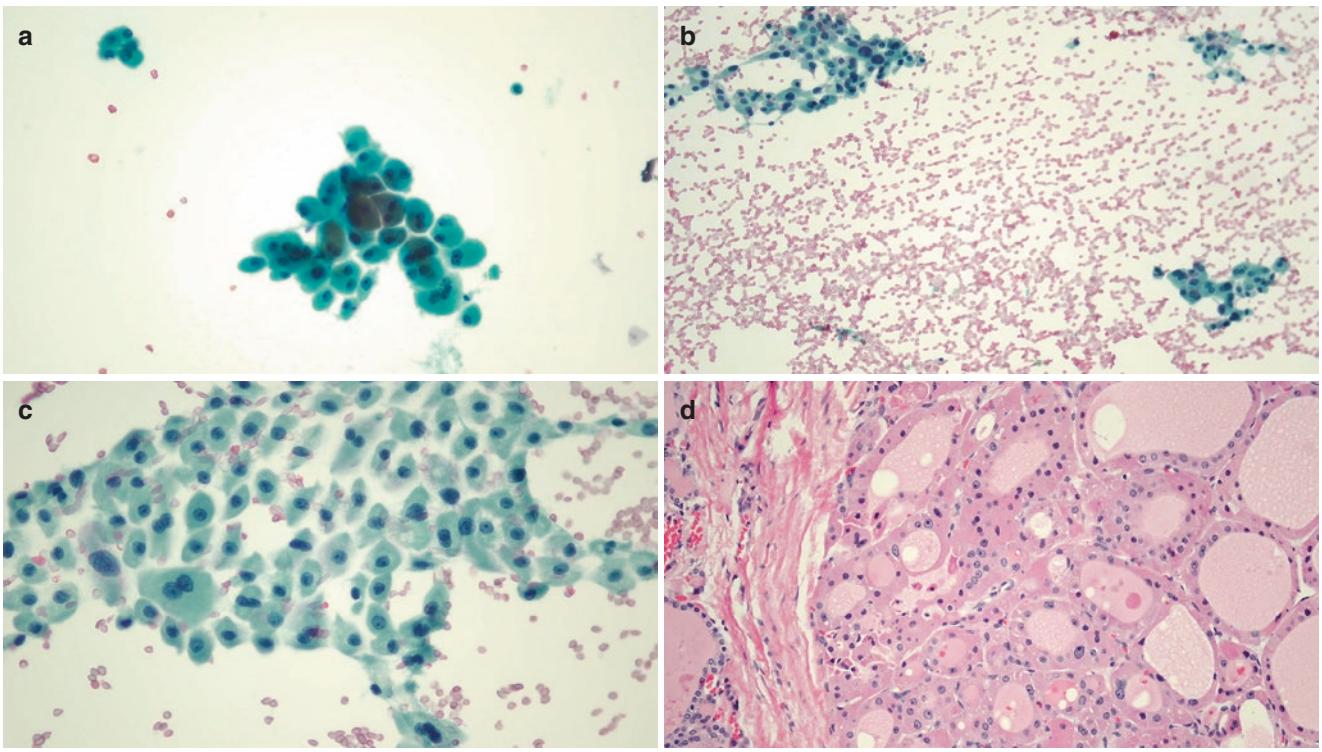

Fig. 13.2 Case 2. (**a**) Single and groups of Hurthle cells with predominantly microfollicular and trabecular patterns (SurePath, Papanicolaou stain 400×). (**b**) Some groups of the Hurthle cells show flat syncytial sheet-like pattern; other groups present slight overcrowded. Nuclei atypia and pleomorphism are present (conventional smear, Papanicolaou stain 200×). (**c**) Follicular cells form loose aggregate. The follicular cells show predominantly Hurthle cell changes with granular cytoplasm, well-defined cell border, centralized nuclei, and prominent nucleoli. Some cells show mild atypia with binucleation and nuclear pleomorphism (conventional smear, Papanicolaou stain 400×). (**d**) Hurthle cell adenoma, without capsular or vascular invasion (Histologic section, H&E stain 200×)

Case 3

Learning objectives:

1. To become familiar with the definition of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)
2. To learn how to generate an appropriate cytologic diagnosis

Case history:

- A 61-year-old woman in whom an incidental thyroid nodule was detected on MRI. Ultrasound study showed that the nodule was 2.5 cm, mostly solid and replacing the right thyroid lobe. The left thyroid lobe was normal. The patient, otherwise healthy, did not have any other clinical symptoms.

Specimen source:

- Ultrasound-guided fine needle aspiration was performed on the left thyroid nodule. A Pap-stained smear and SurePath smear were made from the aspiration. Corresponding surgical resection specimen was obtained 6 months later.

Cytomorphological findings (Fig. 13.3a, b):

- The follicular cells form microfollicular groups with slightly enlarged nuclei, crowding and overlapping.
- The nuclear chromatin appears pale and has occasional grooves and pseudoinclusions.
- Three-dimensional papillary structures or psammoma bodies are not seen.

Differential diagnosis:

- Benign follicular hyperplasia
- Follicular adenoma
- Follicular variant of papillary thyroid carcinoma
- Classic variant of papillary thyroid carcinoma

Other ancillary study:

- BRAF mutation analysis: BRAF V600E mutation absent

Cytology final diagnosis:

- Suspicious for a follicular neoplasm (Bethesda category IV)
- Note: Although the architectural features suggest a follicular neoplasm, some nuclear features raise the possibility of an invasive follicular variant of papillary carcinoma or its recently described indolent counterpart, NIFTP; definitive distinction among these entities is not possible on cytologic material.

Histological findings:

- The histologic sections of NIFTP at low power (Fig. 13.3c) and high power (Fig. 13.3d) show an encapsulated well-circumscribed lesion with no evidence of capsular invasion or invasion to the adjacent benign thyroid parenchyma. The lesion shows predominantly a microfollicular pattern, with no papillary architectures or psammoma bodies. The nuclear features are similar to those seen in a classic papillary thyroid carcinoma: nuclei are slightly enlarged or elongated, with nuclear crowding and overlapping, pallor chromatin, and irregular nuclear contour and/or grooves.

Final histological diagnosis:

- Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)

Take-home messages:

1. Most noninvasive follicular variant of papillary thyroid carcinomas is reclassified as NIFTP after surgical resection with histologic evaluation of the entire tumor capsule. The difficulty lies in avoiding overcalling this tumor in the thyroid FNA specimen as Bethesda category VI (malignant). Indeterminate categories (atypia of undetermined significance BC III, follicular neoplasm or suspicious for a follicular neoplasm BC IV, and suspicious for malignancy BC V) are recommended for reporting potential NIFTP like thyroid cytology.
2. Ancillary BRAF mutation study is recommended if diagnostic cytologic material is available.

References: [8, 15, 16].

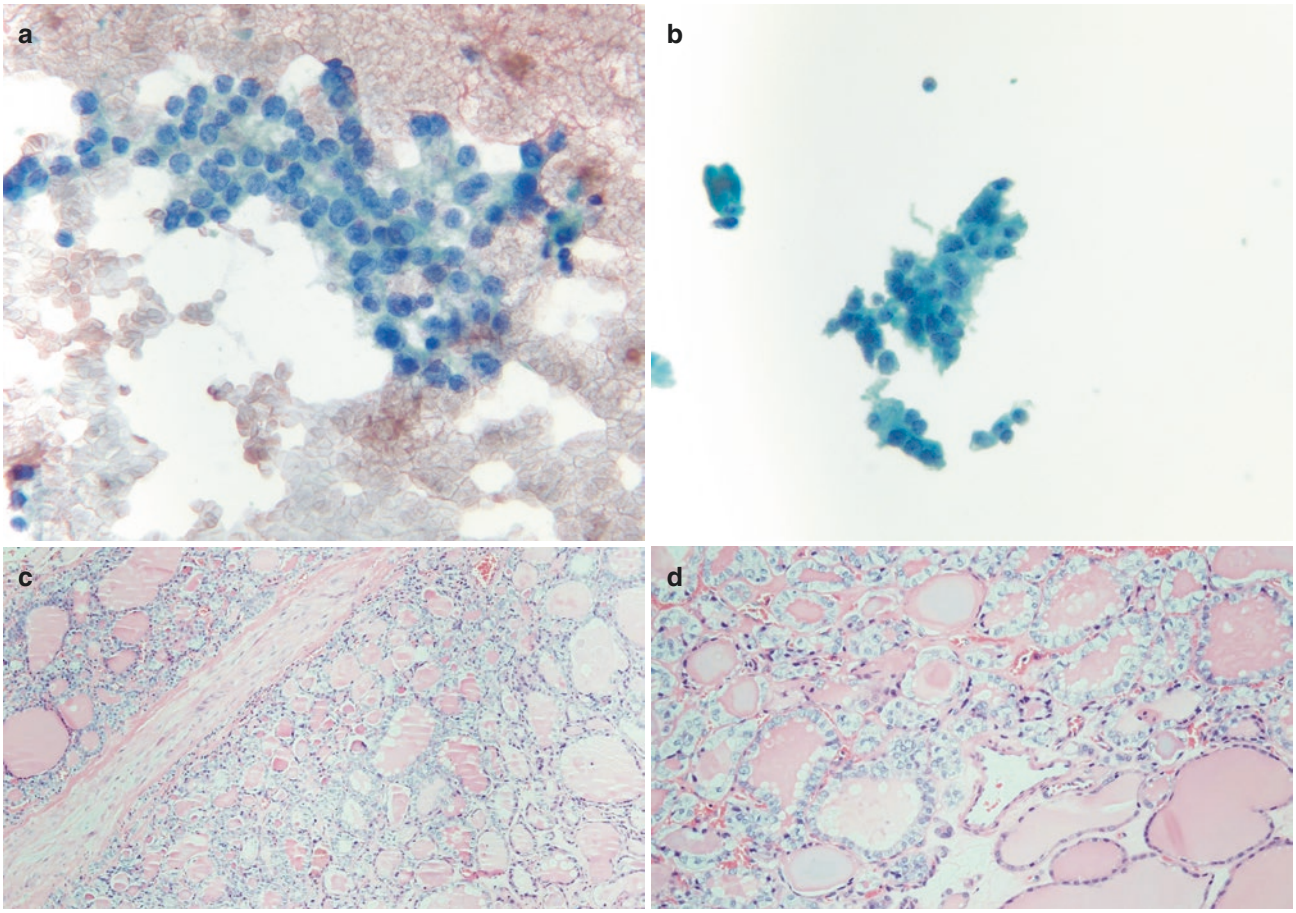

Fig. 13.3 Case 3. (**a**) Follicular cells form microfollicular groups with slightly enlarged, crowding, and overlapping nuclei. The nuclear chromatin appears pale with occasional grooves and pseudoinclusions. No three-dimensional papillary structures are seen (conventional smear, Papanicolaou stain 400×). (**b**) Follicular cells form microfollicular groups with slightly enlarged and overlapping nuclei. The nuclear chromatin appears pale. No true papillary structures are seen (SurePath smear, Papanicolaou stain 400×). (**c**) The lesion is encapsulated and well circumscribed. There is no evidence of capsular invasion or invasion to the adjacent benign thyroid parenchyma (histologic section, H&E stain 100×). (**d**) The lesion shows predominantly a microfollicular pattern, with no papillary architectures or psammoma bodies. The nuclei are slightly enlarged or elongated, with nuclear crowding and overlapping, pallor chromatin, and irregular nuclear contour and grooves (histologic section, H&E stain 200×)

Case 4

Learning objectives:

1. To become familiar with cytologic features of the thyroid carcinoma with neuroendocrine features
2. To generate a differential diagnosis

Case history:

- A 46-year-old male was found on imaging studies to have a 3.5 cm poorly defined right thyroid nodule. Further CT scan shows enlarged cervical lymph nodes.

Specimen source:

- Ultrasound-guided fine needle aspiration was performed on the right thyroid nodule. A Pap-stained smear and SurePath smear were made from the aspiration. Corresponding surgical resection specimen was obtained 1 month later.

Cytomorphological findings (Fig. 13.4a–e):

- Hypercellular specimen with scant colloid and some amorphous material which is positive for Congo red consistent with amyloid.
- Round or polygonal cells with mild pleomorphism form clusters, cords, or small follicles.
- Some of the tumor cells appear plasmacytoid and oncocytic with dense granular cytoplasm.
- The nuclei appear uniform round/oval with punctate chromatin.

Differential diagnosis:

- Benign adenomatous nodule
- Hurthle cell neoplasm
- Papillary thyroid carcinoma
- Medullary carcinoma of thyroid
- Metastatic neoplasm

Ancillary studies:

- Serum calcitonin is elevated.
- Positive IHC: calcitonin (Fig. 13.4e), TTF-1, chromogranin, and CEA.
- Negative IHC: thyroglobulin.
- Congo red staining is positive for amyloid component in the stroma.
- RET activation mutation is present.

Cytology final diagnosis:

- Malignant (Bethesda category VI)
- Medullary carcinoma of thyroid

Histological findings:

- Medullary carcinoma of thyroid.
- One lymph node is positive for metastatic medullary thyroid carcinoma (Fig. 13.4f).

Take-home messages:

1. The incidence of medullary thyroid carcinoma (MTC) is low, about 5–10% of all thyroid carcinoma. The tumor cell origin is the parafollicular cells or C-cells which produce calcitonin. About 90% of the MTCs are sporadic, and the rest have the background of familial genetic syndrome (such as MEN 2a, the Sipple syndrome). It is easy to miss the diagnosis if there is lack of clinical information of elevated serum calcitonin or no amyloid detected in the specimen. If cytologic features such as cord or nesting follicular cell groups with relative monomorphic nuclei and granular chromatin are present, a differential diagnosis of medullary thyroid carcinoma should be raised.
2. Sometimes normal follicular cells are entrapped in the lesion and can be sampled. The minor normal follicular component should not dissuade you including MTC as a differential diagnosis.
3. Ancillary studies such as immunohistochemistry studies and mutation analysis are helpful to characterize the lesion and confirm the diagnosis.

References: [27–31].

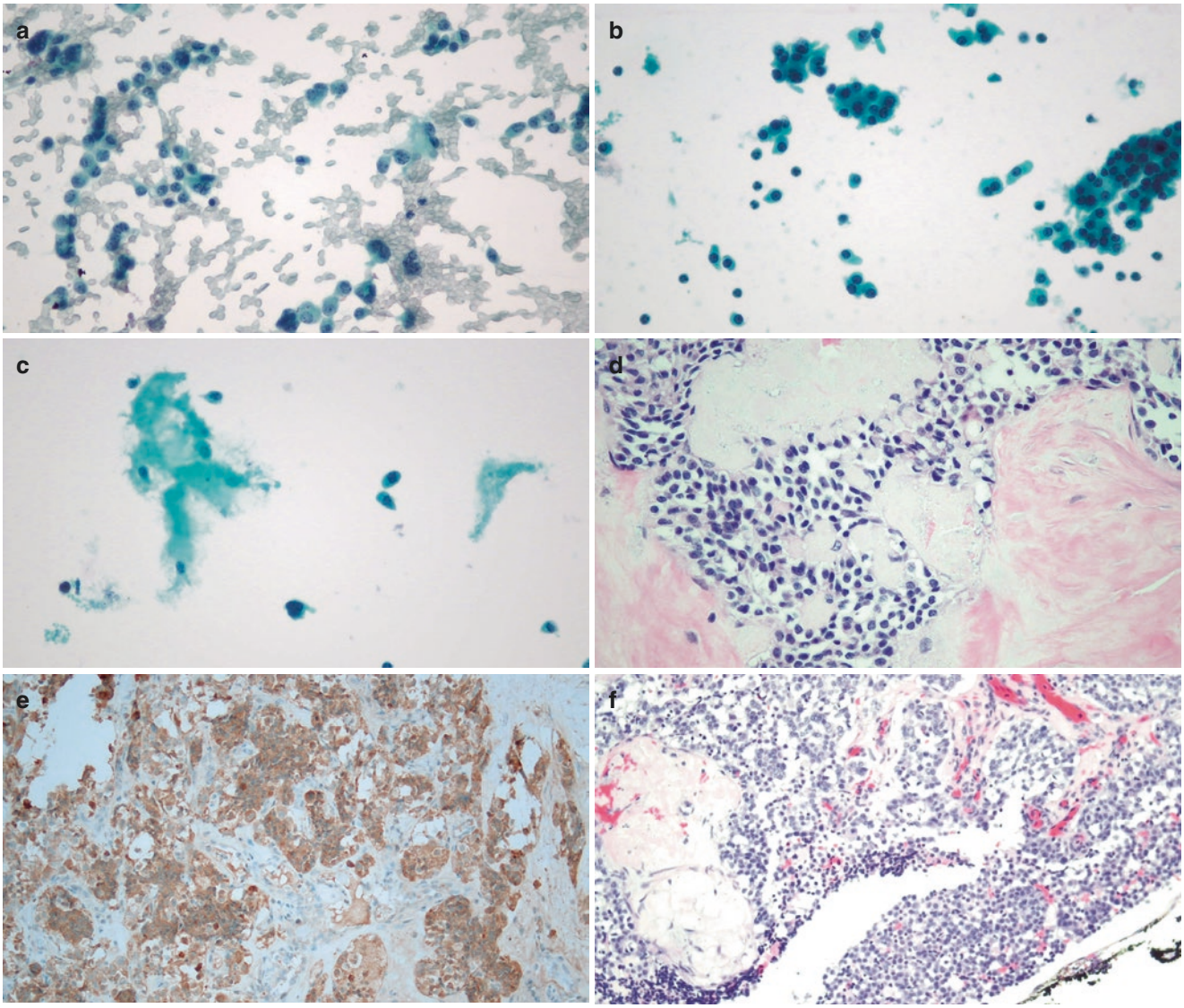

Fig. 13.4 Case 4. (**a**) Round or polygonal follicular cells with mild pleomorphism form clusters, cords, or small follicles (conventional smear, Papanicolaou stain 400×). (**b**) Some of the lesional cells appear plasmacytoid and oncocytic with dense granular cytoplasm (SurePath smear, Papanicolaou stain 400×). (**c**) Some of the lesional cells appear plasmacytoid and spindle. The nuclei are overall round/oval with punctate chromatin (SurePath smear, Papanicolaou stain 400×). (**d**) The cell-block shows pink amorphous material deposit in the stroma among the cords and ribbons of tumor cells. This material is positive for Congo red stain consistent with amyloid (histologic section, H&E stain 200×). (**e**) The lesional cells are positive for calcitonin immunostaining (histologic section, immunohistochemistry stain 200×). (**f**) Cervical lymph node is positive for metastatic medullary thyroid carcinoma. The tumor deposit shows similar cytomorphology as previous FNA specimen, fibrous band, and amyloid deposition are also noted (histologic section, H&E stain 100×)

Case 5

Learning objectives:

1. To become familiar with cytologic features of reactive atypia versus atypical malignancy
2. To generate a differential diagnosis of poorly differentiated thyroid tumor

Case history:

- A 59-year-old male was found on ultrasound to have a large heterogeneous but isoechoic right thyroid mass, more than 6 cm. The nodule showed minimal grade 2 peripheral vascular flow. No microcalcifications were noted. Otherwise, he was asymptomatic.

Specimen source:

- Ultrasound-guided fine needle aspiration was performed on the right thyroid mass. A Pap-stained smear and SurePath smear were made from the aspiration. Corresponding surgical resection specimen was obtained 1 month later.

Cytomorphological findings (Fig. 13.5a–c):

- Hypercellular specimen with scant colloid.
- Follicular cells are arranged in single and clusters; some groups have three-dimensional papillary structure.
- Most of the follicular cells are slightly enlarged; nuclear overlapping with pale, powdery chromatin; intranuclear pseudoinclusion; and nuclear groove. Follicular cells on some smear slides are predominantly singly and show slight pleomorphism, focal necrotic debris, and increased mixed inflammatory cells in the background.

Differential diagnosis:

- Chronic thyroiditis
- Papillary thyroid carcinoma
- Poorly differentiated carcinoma
- Lymphoproliferative disorder
- Metastatic carcinoma

Cytology final diagnosis:

- Malignant (Bethesda category VI)
- Poorly differentiated carcinoma

Histological findings (Fig. 13.5d):

- Poorly differentiated thyroid carcinoma with well-differentiated thyroid carcinoma component and areas of necrosis

Take-home messages:

1. When the cytological appearance is variable from field to field, or slide to slide, there is a possibility of different histologic patterns present in the same lesion.
2. When a greater degree of nuclear atypia or necrosis is present focally in a background of more differentiated carcinoma, poorly differentiated thyroid carcinoma component should be raised in the differential diagnosis.
3. Poorly differentiated thyroid lesion can be missed; our cytology report should alert the clinician to follow up with a surgical consult as the next step management as well as planning multimodality treatment.

References: [32–35].

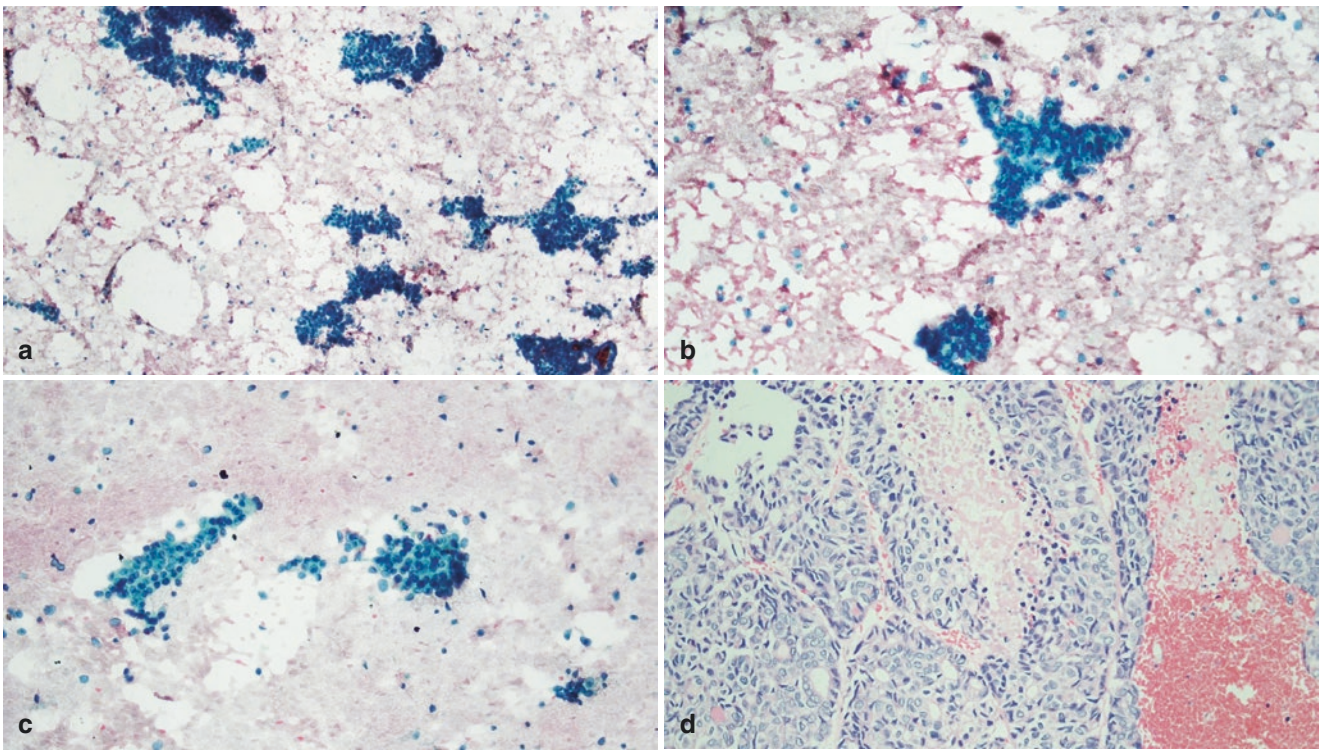

Fig. 13.5 Case 5. (**a**) Hypercellular specimen, follicular cells are arranged in single and clusters with trabecular and three-dimensional structures (conventional smear, Papanicolaou stain 100×). (**b**) Some follicular cells form three-dimensional papillary structure; the nuclei are slightly enlarged; overlapping with pale, powdery chromatin; pseudoinclusions; and nuclear groove (conventional smear, Papanicolaou stain 200×). (**c**) Follicular cells are either in loose aggregate or singly. Some cells show high N/C ratio and some degree of nuclear pleomorphism. Increased mixed inflammatory cells and cellular debris suggestive of necrosis are present (SurePath smear, Papanicolaou stain 200×). (**d**) Poorly differentiated thyroid carcinoma associated with necrosis and adjacent area with more differentiated papillary thyroid carcinoma coexist in this lesion (Histologic section, H&E stain 200×)

Case 6

Learning objectives:

1. To become familiar with cytologic features of the postsurgical and treatment-related changes and malignant tumor cell changes
2. To generate the differential diagnosis
3. To utilize ancillary studies in the cytologic diagnosis

Case history:

- A 48-year-old male with history of papillary thyroid carcinoma was status post total thyroidectomy, followed by radioactive iodine treatment. Posttreatment PET scan showed a thyroid bed mass with increased uptake around central left paratracheal area. The mass was about 1.1 × 1.0 cm. Serum test for thyroglobulin was elevated. A fine needle aspiration was performed on this mass lesion.

Specimen source:

- Ultrasound-guided fine needle aspiration was performed on the thyroid bed mass. A Pap-stained smear and SurePath smear were made from the aspiration.

Cytomorphological findings (Fig. 13.6a–d):

- Large sheets and three-dimensional groups of cells are present in a background of scattered histiocytes and small lymphocytes.
- Three-dimensional groups of lesional cells show crowed nuclei, nuclear membrane-bounded nucleoli, and pseudoinclusions. Psammoma bodies are present associated with the group of lesional cells.
- Cellblock tissue sections show fragments of lesional cells with typical cytologic features of papillary thyroid carcinoma.

Differential diagnosis:

- Parathyroid adenoma
- Reactive lymph node
- Reparative stromal tissue
- Lymph node with metastatic thyroid carcinoma
- Recurrent papillary thyroid carcinoma

Ancillary studies:

- BRAF V600E mutation is present.
- Thyroglobulin level is elevated in the FNA aspirate fluid.
- PTH is not detected in FNA aspirate fluid.

Cytology final diagnosis:

- Malignant (Bethesda category VI)
- Other: recurrent papillary thyroid carcinoma of the thyroid bed

Take-home messages:

1. FNA is a very important and practical diagnostic tool in monitoring changes in the thyroid bed for the recurrent thyroid carcinoma.
2. Clinical history is also very important to help us to reach the correct diagnosis. Changes induced by drugs, surgical or radiation treatments need to be included in the differential diagnosis.
3. Ancillary studies and aspiration fluid test for thyroglobulin and/or PTH are helpful.
4. The positive predictive value of thyroid bed FNA is very high in papillary and medullary thyroid carcinoma and less in follicular cell carcinoma.
5. False negative FNA results are usually due to low cellularity and lack of diagnostic tissue.

References: [36–38].

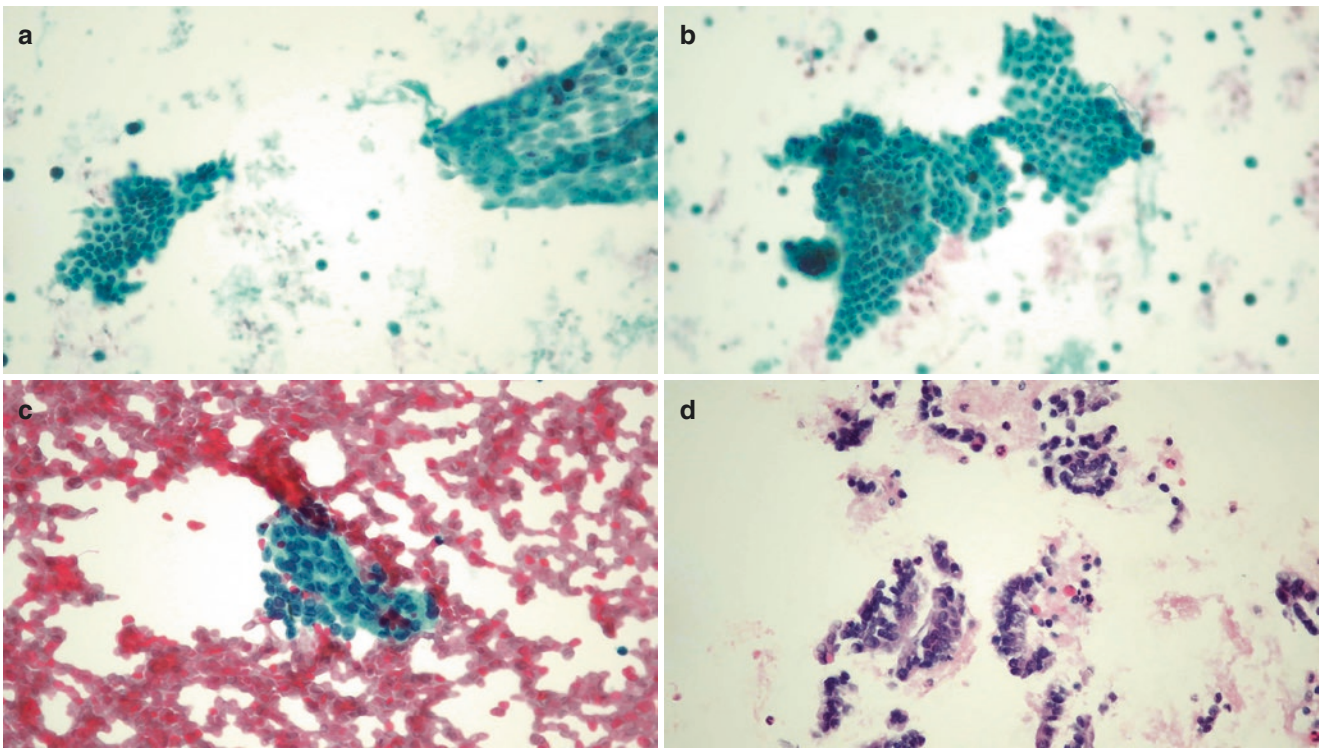

Fig. 13.6 Case 6. (**a**) Big sheets and three-dimensional groups of cells are present in a background of scattered histiocytes and small lymphocytes (SurePath smear, Papanicolaou stain 400×). (**b**) Follicular cells form three-dimensional papillary structure; the nuclei are slightly enlarged and overlapping. Psammoma bodies are present adjacent to the lesional cells (SurePath smear, Papanicolaou stain 400×). (**c**) Lesional cells form papillary structure, and the nuclei show overlapping, pseudoinclusions, and grooving (conventional smear, Papanicolaou stain 400×). (**d**) Aggregates of tissue fragments show partially fragmented papillae or epithelium lined by cuboidal cells with overlapping nuclei and clear chromatin consistent with papillary thyroid carcinoma (cellblock histologic section, H&E stain 400×)

Case 7

Learning objectives:

1. To become familiar with cytologic features of benign changes
2. To generate a differential diagnosis

Case history:

- A 53-year-old female was found on ultrasound to have a 4.9 cm mass/nodule in the posterior to left thyroid lobe. Serum test for PTH is elevated.

Specimen source:

- Ultrasound-guided fine needle aspiration was performed on the posterior left thyroid nodule. A Pap-stained smear and SurePath smear were made from the aspiration. Corresponding surgical resection specimen was obtained 2 months later.

Cytomorphological findings (Fig. 13.7a):

- Monomorphic lesional cells form microfollicular pattern.
- Scattered mixed inflammatory cells present in the background.

Differential diagnosis:

- Follicular lesion (follicular thyroid adenoma, parathyroid gland/adenoma)
- Reactive lymph node
- Lymph node with metastatic thyroid carcinoma

Ancillary studies

- PTH and calcium serum level is elevated.
- Thyroglobulin level is low.
- ThyroSeq v.2. study confirmed the presence of parathyroid follicular cells.

Cytology final diagnosis:

- Suspicious for a follicular neoplasm (Bethesda category IV)
- Favor parathyroid adenoma

Histological findings (Fig. 13.7b):

- Hypercellular parathyroid gland tissue consistent with parathyroid adenoma

Take-home messages:

1. It is challenging to distinguish parathyroid lesions from thyroid lesions. Based on characteristic cytologic features of parathyroid lesion, combined with clinical history, ancillary studies, or sestamibi scans, it is possible to reach the correct diagnosis.
2. Cytomorphology is important to generate the differential diagnosis. In this case, the FNA shows small uniform epithelial cells and some with oncocytic features and bare oval nuclei. The findings may represent parathyroid cells. However, the specimen is hypocellular. This interpretation cannot be confirmed without immunophenotyping or molecular studies. We feel that it is better to put this lesion in Bethesda category IV with a note. Surgical intervention is recommended.
3. Ancillary studies such as ThyroSeq v.2. and aspiration fluid test for thyroglobulin and/or PTH are helpful. Positive PTH and negative thyroglobulin confirm the diagnosis of parathyroid adenoma.

References: [39, 40].

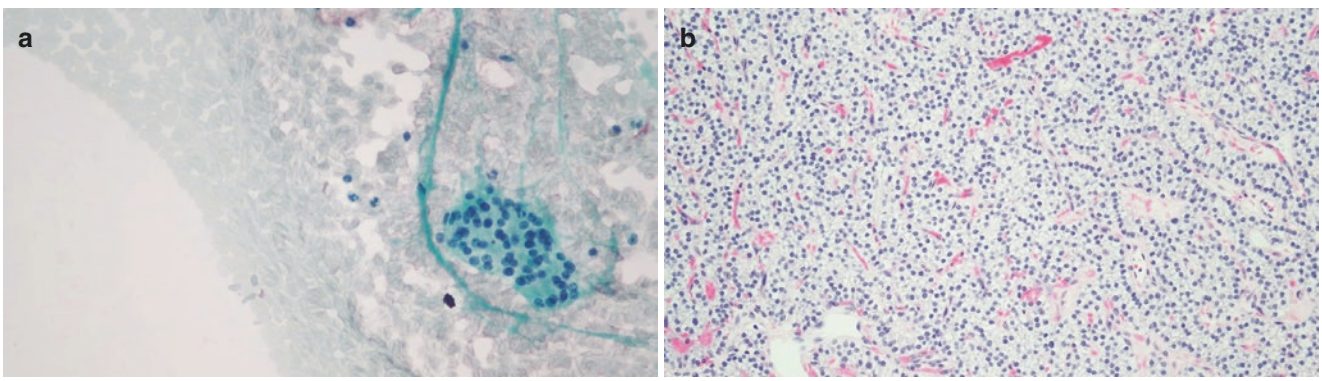

Fig. 13.7 Case 7. (**a**) Monomorphic follicular cells with microfollicular pattern (conventional smear, Papanicolaou stain 400×). (**b**) Hypercellular parathyroid gland tissue consistent with parathyroid adenoma (histologic section, H&E stain 200×)

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Salivary Gland

14

Huimiao Jiang, Elham Arbzadeh, Yun Gong, and He Wang

Contents

List of Frequently Asked Questions

1. Do we have adequacy criteria for salivary gland fine-needle aspiration (FNA)? What are factors influencing the ultrasound-guided FNA adequacy?

The exact number of cells that constitute "adequate" salivary gland FNA remains to be settled. Actually cellularity alone may not be sufficient to qualify a salivary gland FNA specimen as adequate if it does not correlate with clinical and radiological findings. In Milan system for reporting salivary gland cytopathology, it is proposed that rare or absent cells (<60 lesional cells) is generally not adequate for diagnosis. In a recent publication, Wang et al. proposed a salivary gland FNA specimen is considered as "adequate" if (1) 4 clusters or more of epithelial and/or mesenchymal cells are present, each cluster consisting of at least 10 cells; (2) 200 cells or more are present when only hematopoietic cells are observed.

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"Unsatisfactory" salivary gland FNA samples are most often caused by acellular or hypocellular specimens, but can also be due to a number of other factors, including obscuring blood, inflammation, necrosis, debris, fixation/staining artifacts, and preparatory artifacts such as severe crushing of cells. Diagnosing an aspirate as "satisfactory for evaluation" rather than "nondiagnostic" when there are inadequate cells present to explain a clinical mass should be avoided. Importantly, the presence of atypical cells, even if very few in number, should always be mentioned to prompt further evaluation. In effect, the presence of atypical cells outweighs other aspects of the specimen that might otherwise lead to an "unsatisfactory" designation; this approach is analogous to that taken in the evaluation of cervicovaginal specimens.

Among the more common salivary gland entities resulting in an unsatisfactory FNA sample are cystic lesions, both benign and malignant. In some instances, where only non-mucinous cyst contents are aspirated, the specimens are "nondiagnostic" or "unsatisfactory." For those cases where only normal salivary gland tissue is aspirated, the FNA specimen should be designated as either "nondiagnostic" or "unsatisfactory." In both situations, the FNA findings should be communicated with the treating clinical team. Usually, the nondiagnostic or unsatisfactory diagnosis is categorized from morphological changes in conjunction with clinical and/or radiological changes of the lesion.

References: [1–4]

2. What are clinical incidences of salivary gland tumors?

Salivary gland tumors (SGTs) are uncommon. The worldwide annual incidence ranges from 0.4 to 13.5 cases per 100,000 people. In general, women are more commonly

H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_14

affected than men. Epithelial tumors constitute 80–90% of all SGTs, with the majority being benign (75%) and pleomorphic adenoma (PA) being the most common (about 65% of all tumors).

- The sites of tumor occurrence with respect to the number of cases in descending order are parotid gland, submandibular gland, palate, cheek, and tongue.
- Tumors have the highest chance of being malignant if they arise from the retromolar area (89.7%), floor of mouth (88.2%), tongue (85.7%), sublingual gland (70.2%), and submandibular gland (50.0%), whereas only approximately 20% of all parotid tumors are malignant.
- Among the salivary gland carcinomas, the most common histological types in descending order are mucoepidermoid carcinoma (MEC), adenoid cystic carcinoma (AdCC), adenocarcinoma not otherwise specified (AdCa, NOS), and acinic cell carcinoma (AciCC).

Salivary gland tumors are generally rare in children. In patients under the age of 18 years old, half of the epithelial tumors are malignant, with low-grade MEC being the most common. In infants, mesenchymal tumors (hemangioma and lymphangioma) are the most common, and some unusual tumors such as sialoblastoma and salivary gland analog tumor occur almost exclusively in this age group.

References: [5–8]

3. How to distinguish normal salivary gland acini from AciCC?

Normal salivary gland parenchyma components commonly encountered in FNA samples are groups of acinar cells, although ductal cells and adipose tissue may occasionally be seen. They are most commonly from parotid gland and are of serous type. Acinar cells usually form polarized, cohesive grape-like groups with rare single cells. Individual cells are pyramidal shaped with foamy to granular cytoplasm and small round nuclei and inconspicuous nucleoli. Mucinous type acinar cells may be seen in submandibular gland or minor salivary gland aspiration.

Most AciCCs present as mobile, soft, well circumscribed 1–4 cm masses in parotid glands, and show serous type neoplastic cells with finely vacuolated to granular cytoplasm, somewhat resembling serous type acinar cells of benign parenchyma. However, in contrast to cohesive grape-like arrangement of benign acinar, the clusters of AciCC are more dyshesive and crowded in a haphazard arrangement. The neoplastic cells are monotonous, larger than benign counterpart with finely granular or vacuolated cytoplasm and round nuclei; the neoplastic cells show at least mild degree of cytological atypia. FNA smears of some AciCC demonstrate abundant naked nuclei and sometimes lymphocytes. In addition, adipose tissue and ductal cells are absent in AciCC (Fig. 14.1).

References: [5, 9–11]

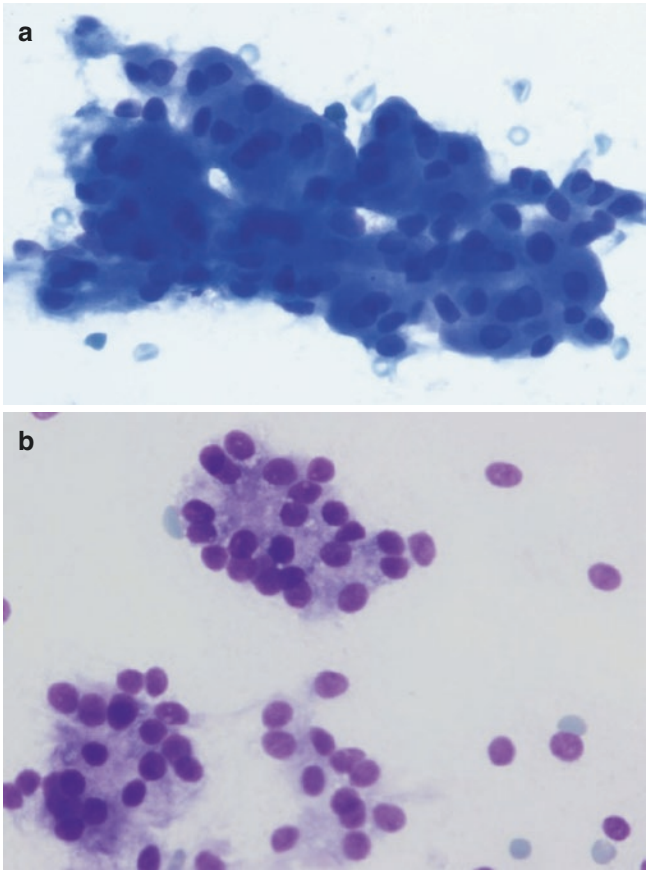

Fig. 14.1 Normal acini versus AciCC. (**a**) Acinar cells in normal salivary gland. The normal acinar cells in parotid glands are present in grape-like clusters with associated small inconspicuous tubules (Diff-Quik stain). (From Wang et al. [86], Fig. 1.2., with permission.) (**b**) Acinic cell carcinoma. Sheets of large polygonal epithelial cells with rich granular cytoplasm and indistinct cell borders. No well-formed "grape-like" structures are identified. The nuclei are round to oval, fairly bland, and uniform. Naked nuclei are also easily identified (Diff-Quik stain). (From Wang et al. [86], Fig. 1.33., with permission)

4. How to classify salivary gland FNAs and what are their corresponding histological entities?

Milan system for reporting salivary gland cytopathology is the product of an international group of cytopathologists, surgical pathologists and head and neck surgeons, aiming to produce a user-friendly and internationally accepted classification system for salivary gland FNA. Milan system classifies all salivary gland FNA into one of the six categories (listed in Table 14.1). Not all cytological diagnostic categories hold corresponding surgical pathology diagnoses.

References: [1–2, 5, 12]

5. What are currently established molecular markers for SGTs?

Characteristic molecular changes of SGT not only provide powerful diagnostic tools for pathologists, but are also potential therapeutic targets. Most recent molecular markers of SGTs are summarized in Table 14.2.

References: [2, 5, 13–25]

Table 14.1 The Milan system for reporting salivary gland cytopathology

FNA classification	Explanation	Histological entities
I. Nondiagnostic	This diagnostic category should only be used after all the material has been processed and examined. Exceptions include matrix material and mucinous cyst contents	
II. Non-neoplastic	Lacking cytomorphological evidence of a neoplastic process Inflammatory, metaplastic, and reactive changes Evidence of reactive lymphoid tissue	Inflammation (including granuloma) Benign cysts (including mucocele) Diffuse oncocytosis Necrotizing sialometaplasia Reactive lymph nodes
III. Atypia of undetermined significance	Samples are indefinite for a neoplasm A majority will represent reactive atypia or poorly sampled neoplasms	
IVA. Benign neoplasm	Include classic cases of pleomorphic adenoma (PA), Warthin tumor (WT), lipoma, etc.	(i) PA (ii) WT (iii) Oncocytoma (iv) Myoepithelioma (MyE) (v) Sebaceous tumors (vi) Ductal papilloma (vii) Canalicular adenoma (viii) Lymphoadenoma (ix) Benign mesenchymal tumor (vascular leiomyoma, infant hemangioma) (x) Basal cell adenoma (BCA)
IVB. Salivary gland neoplasm of uncertain malignant potential	Cases where a malignant neoplasm cannot be excluded A majority of the cases will be benign neoplasms, neoplasms with atypical features, and low-grade carcinomas	
V. Suspicious for malignancy	Report should state which type of malignant tumor is suspected or list differential diagnoses A majority of specimens will be high-grade carcinomas	
VI. Malignant neoplasm	An attempt should be made to subclassify the neoplasm into specific types and grades of carcinoma "Other" malignancies such as lymphomas, metastases, and sarcoma are also included in this category and should be specially designated	(a) MEC (b) AciCC (c) AdCC (d) Carcinoma ex-PA (e) Salivary duct carcinoma (SDC) (f) Clear cell carcinoma (CCC) (g) Secretory carcinoma (SC) (h) Polymorphous adenocarcinoma (PMAC) (i) Basal cell adenocarcinoma (BCAC) (j) Epithelial-myoepithelial carcinoma (EMC) (k) Myoepithelial carcinoma (MyEC) (l) Small cell carcinoma (m) Lymphoepithelial carcinoma (n) Malignant sebaceous tumors (o) AdCa, NOS (p) Oncocytic carcinoma (q) Squamous cell carcinoma (SCC) (r) Lymphoma involving the salivary gland (s) Secondary malignancy

6. What are the risks of malignancy and suggested clinical follow-up for various diagnostic categories according to the Milan system for reporting salivary gland cytopathology?

Major goals of Milan system for reporting salivary gland cytopathology include establishing the risk of malignancy (ROM) and recommending preferred clinical follow-up. Current recommendations are summarized in Table 14.3.

References: [1, 26–27]

7. What are examples of non-neoplastic category of salivary gland diseases?

Non-neoplasmic category refers to specimens lacking any evidence of a neoplastic process. This category includes various subcategories, including:

- Inflammatory
  - Acute sialadenitis: FNA is rarely performed for this lesion.
  - Chronic sialadenitis: including IgG4 sialadenitis.

Table 14.2 Common molecular biomarkers for salivary gland tumors

Tumor	Translocation	Genes involved	Prevalence
PA	t(3;8) t(3;12)	PLAG1-fusions HMG2-fusions	˃25% ~10%
CA-ex-PA		PLAG1-fusions HMG2-fusions HER2 amplification TP53 mutation	
MEC	t(11;19) (q21;p13) t(11;15) (q21;q26)	CRTC1-MAML2 CRTC3-MAML2	30–80% ~5%
AdCC	t(6;9) (q22-23;p23-24)	MYB-NF1B	˃80%
SC	t(12;15) (p13;q25)	ETV6-NTRK3	Translocation ˃80%
CCC	t(12;22) (q13;q12)	EWSR1-ATF1	~80%
BCA & BCAC	N/A	LOH/mutation 8q12 involving CTNNB1 LOH/mutation 16q12-13 involving CYLD1	~50% 75%
PMAC	N/A	PRKD1 mutation involving PRKD1	73%
SDC	N/A	17q21.1 amplification involving ERBB2	40%

Abbreviations: *PLAG1* pleomorphic adenoma gene 1, *HMG2* high mobility group AT-hook 2, *CA-ex-PA* carcinoma ex pleomorphic adenoma, *HER2* human epidermal growth factor receptor 2, *TP53* tumor protein p53, *CRTC1* cAMP response element-binding protein regulated transcription coactivator 1, *MAML2* mastermind-like 2, *CRTC3* cAMP response element-binding protein regulated transcription coactivator 3, *MYB* v-myb avian myeloblastosis viral oncogene homolog, *NF1B* nuclear factor 1/B, *ETV6* Ets variant gene 6, *NTRK3* neurotrophic tyrosine kinase receptor type 3, *HCCC* hyalinizing clear cell carcinoma, *EWSR1* Ewing sarcoma breakpoint region 1, *ATF1* activating transcription factor 1, *LOH* loss of heterozygosity, *PA* pleomorphic adenoma, *MEC* mucoepidermoid carcinoma, *AdCC* adenoid cystic carcinoma, *SC* secretory carcinoma, *CCC* clear cell carcinoma, *BCA* basal cell adenoma, *BCAC* basal cell adenocarcinoma, *PMAC* polymorphous adenocarcinoma, *SDC* salivary ductal carcinoma

Table 14.3 Risk of malignancy and recommended management

Diagnostic category	ROM	Management
Nondiagnostic	25%	Clinical and radiological correlation/repeat FNA
Non-neoplastic	10%	Clinical follow-up and radiological correlation
Atypia of undetermined Significance (AUS)	20%	Repeat FNA or surgery
Neoplasm		Surgery or clinical follow-up Surgery
(i) Benign	<5%	
(ii) Uncertain malignant potential (SUMP)	35%	
Suspicious for malignancy (low grade vs. high grade)	60%	Surgery
Malignant (low grade vs. high grade)	90%	Surgery

  - Granulomatous sialadenitis: aggregates of histiocytes and lymphocytes, sometimes with multinucleated giant cells.
- Benign cysts
  - Lymphoepithelial cyst: thick lymphoid tissue around epithelial lining
- Reactive lymph nodes: flow cytometry analysis is necessary to rule out low-grade lymphoma.
- Others: including ectopic thyroid, amyloidosis, and non-tyrosine crystalloids (Fig. 14.2)

References: [1–2, 28]

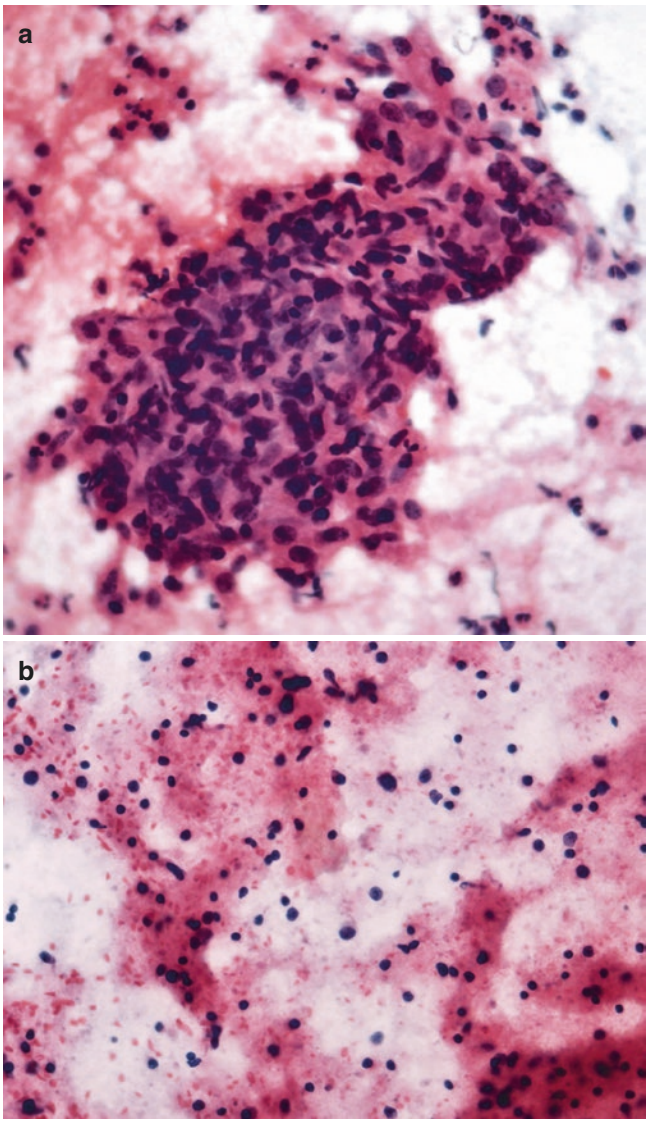

Fig. 14.2 Granulomatous sialendits and lymphoepithelial cyst. (**a**) Salivary gland in a HIV-positive female showing granulomatous inflammation on FNA (Papanicolaou). (From Michelow et al. [87], with permission.) (**b**) Benign lymphoepithelial cysts consisting of chronic inflammatory cells in a serous background (Papanicolaou). (From Michelow et al. [87], with permission)

8. How to report the malignancy category according to the Milan system for reporting salivary gland cytopathology?

Malignancy category of Milan system refers to aspirates which are diagnostic of malignancy and should be reported as "malignant" in the diagnostic line.

In the second line of diagnosis, effort should be made to subclassify into specific types and/or grades of carcinoma: for example, low grade versus high grade. The classification of high and low grade varies between different histological types of salivary gland malignancies. Generally, high mitoses, extensive tumor necrosis, and nuclear anaplasia are regarded as morphological features of high-grade tumors. Certain types of salivary gland tumor are classified as high grade by default, such as SDC, small cell carcinoma; other types contain both low- and high-grade tumors, such as MEC, carcinoma ex-PA; still others could undergo "high-grade transformation," including AciCC.

"Other" malignancies such as lymphomas, sarcomas, and metastases are also included in malignant category and should be specifically designated accordingly. Accurate diagnosis of the "other" malignancies usually needs application of ancillary tests and careful review of patient's clinical history. Flow cytometry is the most widely used ancillary test for diagnosis of lymphomas (except Hodgkin disease). Immunocytochemistry, together with molecular tests, are regularly employed for diagnosis of sarcoma, Hodgkin disease, and many metastatic malignancies.

References: [1, 29–34]

9. How many indeterminate categories are designated in the Milan system for reporting salivary gland cytopathology?

Milan System for Reporting Salivary Gland Cytopathology classifies the indeterminate salivary gland FNAs into three categories: Atypical, salivary gland neoplasm of uncertain malignant potential (SUMP), and suspicious for malignancy.

The "atypical" represents lesions where a clear distinction between non-neoplastic versus neoplastic cannot be made. Efforts should be made to reduce the use of this category to <10% of all salivary gland FNAs. Majorities of the atypical category FNAs will be reactive atypia or compromised specimens, and will turn out to be nonmalignant.

The SUMP includes all cases which are definitely categorized as neoplasm based on cytological features; however, clear distinction cannot be made between a benign and malignant neoplasm. This category is important for salivary gland FNA because cytomorphology alone is not sufficient to differentiate benign versus malignant rumors without histological evidence of invasion in certain tumors, including basal cell adenoma versus basal cell adenocarcinoma; oncocytoma versus oncocytic adenocarcinoma.

Suspicious for malignancy refers to specimens quantitatively and/or qualitatively fall short of criteria for malignancy: specimens with some but not all of the malignant criteria, or there is only scant and/or inadequate material with features suggestive of malignancy. It is expected that the risk of malignancy for this category should be 60–70%.

References: [1, 26]

10. What are matrix-containing/matrix-producing salivary gland tumors?

The presence and characteristics of matrix material in salivary gland FNAs provide important diagnostic information. The common matrix-producing SGTs include PA, AdCC, BCA, BCAC, MyE, PMAC, EMC, and CA-ex-PA.

A chondromyxoid matrix with sometimes embedded myoepithelial cells is characteristic of PA; acellular stromal spheres with sharp borders and surrounding tumor cells are typical of AdCC, but neither is entirely specific. For example, adenoid cystic like matrix can be encountered in 5% of PAs; solid and cylindrical AdCC may also show no matrix at all. Matrix in MyE and CA-ex-PA are identical to that in PA and show fibrillary chondromyxoid pattern, although myoepithelial cells are usually spindle-shaped.

It is interesting to note that while the matrix spheres in AdCC stains intensely with Romanowsky stain, the spheres can be subtle on Papanicolaou stain. This staining pattern is different from otherwise similar matrix material from basal cell neoplasms or MyEs, which is densely stained with both Romanowsky and Papanicolaou preparation.

Both EMC and AdCC contain acellular matrix globules. The matrix globules in EMC are reported as cohesive and intimately associated with surrounding neoplastic myoepithelial cells. In addition, a subtle, thin, pale-staining band of acellular material is apparently surrounding the matrix globules. In contrast, the matrix globules in cribriform AdCC are dyscohesive, loosely surrounded by basaloid cells, and lacked a peripheral rim of basement membrane material.

Extracellular matrix material in aspirates of PMAC is usually scant. But when it is present, it can be fibrillar or spherical, mimicking PA or AdCC, respectively. Evaluation of cytomorphological features and knowledge of the tumor sites are both essential to reach an accurate diagnosis (Fig. 14.3).

References: [5, 35–42]

11. What are immunocytochemical (ICC) markers useful for the diagnosis of AciCC?

AciCC is usually a low-grade, slow-growing tumor, but a subset of cases may occasionally develop recurrent and/or metastatic disease or undergo high-grade transformation. No specific molecular changes have been identified in AciCCs.

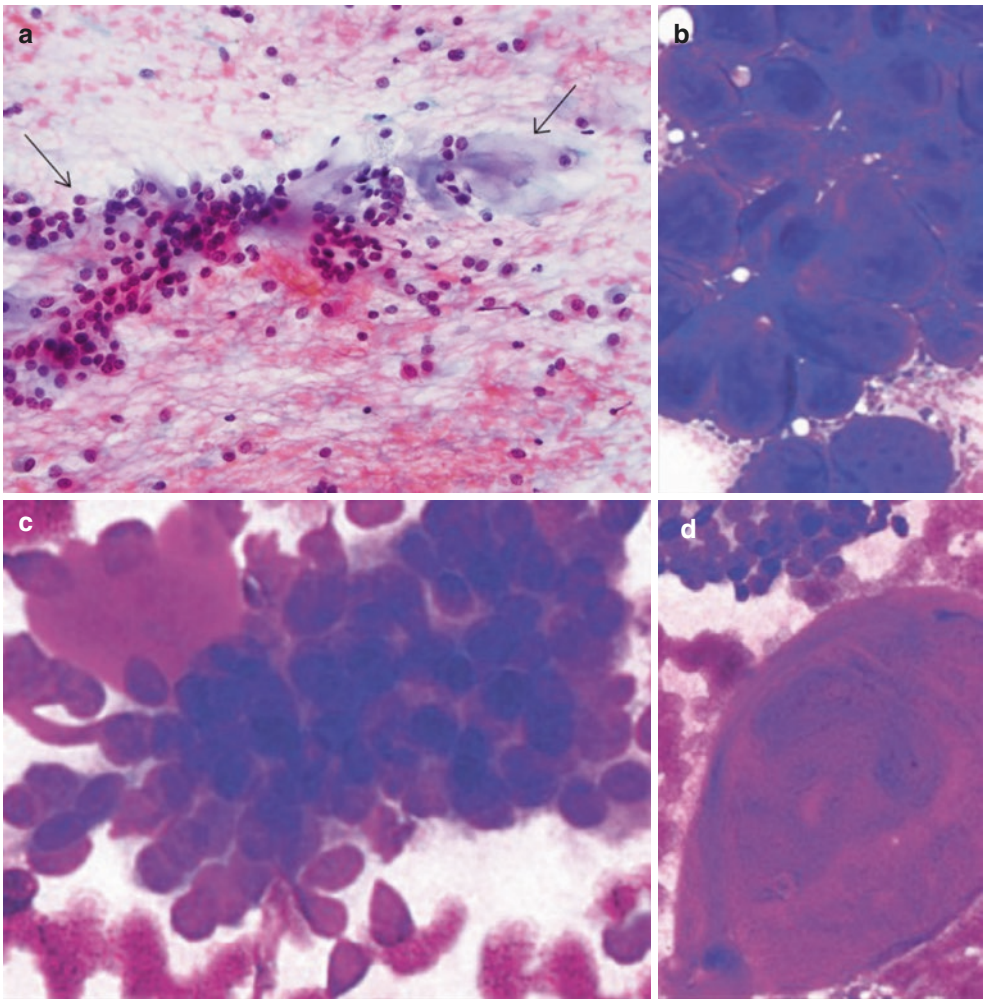

Fig. 14.3 (**a**) Hematoxylin and eosin stain of an FNA of a pleomorphic adenoma demonstrating a plasmacytoid myoepithelial cell (left arrow) and fibrillary stroma (right arrow). (**b–d**) Various extracellular matrix material from PMAC. (From Heaton et al. [88], with permission)

Most AciCC cells exhibit ICC evidence of differentiation toward acinar cells or ductal cells, such as positivity for low molecular weight cytokeratin. Recent studies show that AciCCs are positive for deletion of guanine-rich DNA 1 (DOG-1), SRY-related HMG-box 10 (Sox10), amylase, carbonic anhydrase VI, and salivary proline-rich proteins. The DOG-1 stain shows a complex mixture of intense apical membranous, cytoplasmic, and complete membranous staining. AciCC may have focal neuroendocrine staining, but no evidence of myoepithelial/basal cell differentiation. AciCC is negative for maspin and S100 stains.

DOG-1 is negative in SC; DOG-1 and SOX-10 are weakly and focally positive for MEC; DOG-1 and SOX-10 are predominantly negative in WT, oncocytoma, and oncocytic carcinoma.

Periodic acid-Schiff (PAS) and PAS with diastase (d-PAS) can be used to highlight the zymogen granules in the granular cytoplasm of AciCC.

References: [2, 43–44]

12. What are the diagnostic pitfalls that can lead to misinterpretation of WT?

Typical cytological features of WT include sheets of oncocytes, background lymphocytes, and granular debris. Occasionally, WT needs to be differentiated from SCC, low-grade MEC, low-grade lymphoma, and oncocytoma.

WT can show squamous metaplasia and sometimes associated with keratinization and even nuclear atypia. The atypical squamous cells, together with background of debris and lymphocytes, can lead to its misinterpretation as metastatic SCC in a lymph node, with or without cystic changes. Ancillary tests may also be helpful; in one report, carcinoembryonic antigen (CEA) positivity and c-kit positivity in WT were noted to be in 0% and 75.0% of cases, respectively, whereas metastatic SCC showed positive rates of 16.7% and 0%, respectively.

WT sometimes shows mucinous metaplasia ranging from abundant background mucin to mucin-containing epithelial cells, mimicking low-grade MEC. Careful evaluation of all smears and identification of typical WT cytological features

usually help to resolve this diagnostic dilemma. In difficult cases, a diagnosis of "atypical/SUMP" category should be considered with a note including the differential diagnoses of both WT and low-grade MEC.

When a WT is lymphocyte-predominant, an intraparotid lymph node or low-grade lymphoma may enter into the differential diagnoses. Careful identification of oncocytic epithelioid cells and granular background is helpful for an accurate diagnosis. In cases where sample consists of exclusively lymphoid cells, ancillary tests like flow cytometry, or immunocytochemistry might be considered to rule out lymphoma. When lymphocytes are rare or absent due to sampling error, WT may be indistinguishable from oncocytoma. Background granular debris is usually seen in WT, but not in oncocytoma.

References: [2, 45–46]

13. How to distinguish high-grade MEC from SCC?

The malignant nature of high-grade MEC is usually easy to be established based on mitosis and anisonucleosis. However, one challenging differential diagnosis of high-grade MEC, due to its cellular aspirates with predominant epidermoid and intermediate cells is SCC (either primary or metastatic). Most tumors with SCC appearing in salivary gland are metastasis and often have a known history of primary tumors. When SCC is keratinizing, differential diagnosis is simple since keratinization is not a feature of high-grade MEC. When SCC is nonkeratinizing, the distinction between both tumors can be difficult. Attention should be paid to identify the rare interspersed goblet cells or cells with intracytoplasmic mucin, features in favor of high-grade MEC.

References: [29, 47–50]

14. How to differentiate basaloid neoplasms encountered in salivary gland FNAs?

Basaloid salivary gland tumors have a very broad differential diagnosis, especially when aspirates are cellular and lack stromal component. The most common salivary gland basaloid neoplasms include BCA, BCAC, solid variant of AdCC, cellular PA, PMAC, cutaneous basal cell carcinoma (BCC), metastatic basaloid, and poorly differentiated SCC.

BCA is the prototype of salivary gland basaloid neoplasm, and is histologically divided into solid, tubular, membranous, and mixed patterns. Cytologically, BCA aspirates are composed of small and intermediate-sized basaloid cells, which sometimes show peripheral palisading; dense and nonfibrillary stroma is usually identifiable surrounding the cellular groups. In membranous type BCA, a thick ribbon of matrix around cellular groups is common. BCA and BCAC are morphologically identical except that the latter has an infiltrative growth pattern which cannot be assessed cytologically. It is interesting to note the stromal material surrounds BCA is hyalinized and dense, even in the Pap smears. While in contrast, matrix surrounded AdCC cells becomes transparent in Pap smears.

Table 14.4 Common ICC markers of basaloid salivary gland neoplasms

Entity	Positive ICC markers	Negative ICC markers
BCA/ BCAC	p63, p40, SMA, calponin, nuclear β-catenin, and LEF-1	MYB, PLAG1, and S-100
Solid variant AdCC	MYB, c-KIT, SOX-10, SMA, and calponin	PLAG1
Cellular PA	PLAG1, SOX-10, SMA, and calponin	MYB
PMAC	P63, S100	P40, SMA, calponin, LEF-1, and MYB
BCC of skin	Nuclear β-catenin and LEF-1	
SCC	p63, p40	SOX-10

Abbreviation: *LEF-1* Lymphoid enhancer-binding factor 1

An ICC panel can be useful in narrowing down the differential diagnoses. Myoepithelial cell markers are nonspecific and can highlight predominant component of a cellular biphasic tumor, as well as myoepithelial and basal cell neoplasms. More specific myoepithelial markers include SMA, calponin, and GFAP. More common ICC markers helpful in differential diagnoses of basaloid salivary gland neoplasms are listed in Table 14.4 below:

References: [1–2, 44]

15. How to differentiate salivary duct carcinoma from other high-grade salivary gland malignancies?

The high-grade salivary carcinomas include SDC, lymphoepithelial carcinoma, small cell neuroendocrine carcinoma, high-grade MEC, and high-grade Ca ex-PA. The most commonly encountered differential diagnoses of SDC in FNA practice include high-grade MEC, and CA-ex-PA. Differential diagnosis among the tumors may not always be easy due to inappropriate sampling. The other less commonly encountered high-grade salivary gland tumors include SCC, oncocytic carcinoma, and large B-cell lymphoma. High-grade AdCa, NOS also enters the differential diagnosis, but this is a diagnosis of exclusion, which should only be made after the other diagnostic considerations are excluded.

SDC, initially described by Kleinsasser, Klein, and Hubner as tumors analogous to ductal carcinomas of the breast, may arise de novo or represent malignant transformation of PA (CA-ex-PA) or be a component of a high-grade transformation of other low-grade salivary gland carcinomas. Infiltrative growth pattern and foci of necrosis are characteristic histological features of SDC. Cytological features of SDC can overlap with other high-grade salivary gland malignancies and include the following:

- Sheets, three-dimensional papillary like and cribriform groups of cells with overtly malignant cytological features; surrounded by single intact cells.

- Polygonal tumor cells are of medium to large sized with well-defined cell borders and abundant vacuolated or granular cytoplasm which commonly appear squamoid or oncocytic.
- Enlarged round-to-oval, pleomorphic nuclei with significant anisonucleosis, hyperchromasia, and prominent macronucleoli.
- Lack of matrix material in the smears.
- Mitoses are frequently seen.
- Necrotic background; naked enlarged nuclei may be present.

Immunostaining is very helpful to distinguish SDC from other high- or intermediate-grade salivary gland malignancies. SDC are positive for CK7, low-molecular-weight keratins, epithelial membrane antigen (EMA), GCDFP-15, GATA3, and androgen receptor (AR); and are usually negative for S100, CK5/6, p63, SMA, calponin, Her-2, ER, and PR.

Both high-grade MEC and SDC are composed of large pleomorphic epithelial cells; however, true squamous differentiation, intermediate cells, and mucinous (goblet) cells are not seen in SDC. ICC for AR may be additionally helpful, since it is negative in MEC. Molecular studies of the MECT/MAML translocation may also help to exclude MEC. Extensive keratinization is absent in both MEC and SDC; its presence indicates to the diagnosis of SCC. P63 and p40 staining are positive in MEC and SCC, but negative in SDC.

Chondromyxoid stroma, and possibly typical areas of PA, is essential to differentiate CA-ex-PA from SDC. Oncocytic carcinomas have diffuse oncocytic features; these features are usually only focally present in SDC. Singly dispersed large atypical cells and positive B-cell lineage markers by immunostains are characteristic of large B-cell lymphoma (Fig. 14.4).

References: [51–58]

16. What are the common secondary malignancies encountered in salivary gland FNA?

Secondary malignancies of salivary glands (SMSG) can either represent distant metastases or direct extensions from primaries in adjacent sites. SMSG can involve the parenchymal tissues of the major or minor glands, intraglandular or periglandular lymph nodes (usually in the parotid gland), or both. Various hematopoietic and lymphoid malignancies, including lymphomas, can constitute a significant portion of SMSG.

A recent study from six academic institutions identified 184 FNA cases of nonlymphomatous SMSG. Metastatic SCCs (47%) and melanomas (36%) constituted the majority of SMSG cases. Less frequent SMSGs were comprised of metastatic carcinomas from distant organs (9%), including breast, lung, kidney, thyroid, pancreatobiliary, prostate, and bladder. Other uncommon SMSGs including nasopharyngeal carcinoma, sarcoma, other metastatic skin-derived carcinomas, and metastatic chordoma were also observed. SMSG originate predominately from the head and neck origins.

References: [29, 31–33]

17. How to differentiate SGTs with oncocytic changes?

True oncocyte is morphologically characterized by having abundant granular eosinophilic cytoplasm and a round centrally located nuclei with a distinct nucleolus. The abundant granular cytoplasm is due to the presence of numerous mitochondria. The common differential diagnosis of salivary lesions with oncocytic features includes oncocytoma, AciCC, WT, the oncocytic variant of MEC, PA with oncocytic features, oncocytic carcinoma, and metastatic renal cell carcinoma.

Among the differential diagnoses, one of the most common challenges is to differentiate benign oncocytoma from low-grade AciCC. Key points of differentiation are listed in Table 14.5.

FNA aspirate of WT shows a characteristic granular opaque green brown “motor oil” appearance. Cytologically, aspirates of WT are characterized by three components: sheets of oncocytes and a mixed lymphoid population, and a “dirty” granular proteinaceous background. Occasionally, acute inflammation may be seen. In cases when oncocytes predominate, WT might be mistaken as an oncocytoma. An adequate sampling and a careful search for both cell components are helpful to make the correct diagnosis.

Aspirates of oncocytic variant of MEC are usually cellular and composed of bland oncocytic cells in a cystic and mucoid background. The key to the diagnosis is to carefully search for mucinous goblet cells, epidermoid, and intermediate cells. Mucicarmine stain can be performed on cell block to confirm the presence of intracellular mucin.

PA with oncocytic features is another mimicker in the differential diagnosis. In addition to oncocytic cells, aspirates of these cases also contain characteristic fragments of metachromatic fibrillar matrix material. Immunostain for myoepithelial markers (e.g., calponin, smooth muscle actin, S-100) performed on cell block is helpful. PA contains myoepithelial cells. In contrast, other most common oncocytic salivary gland lesions lack myoepithelial differentiation.

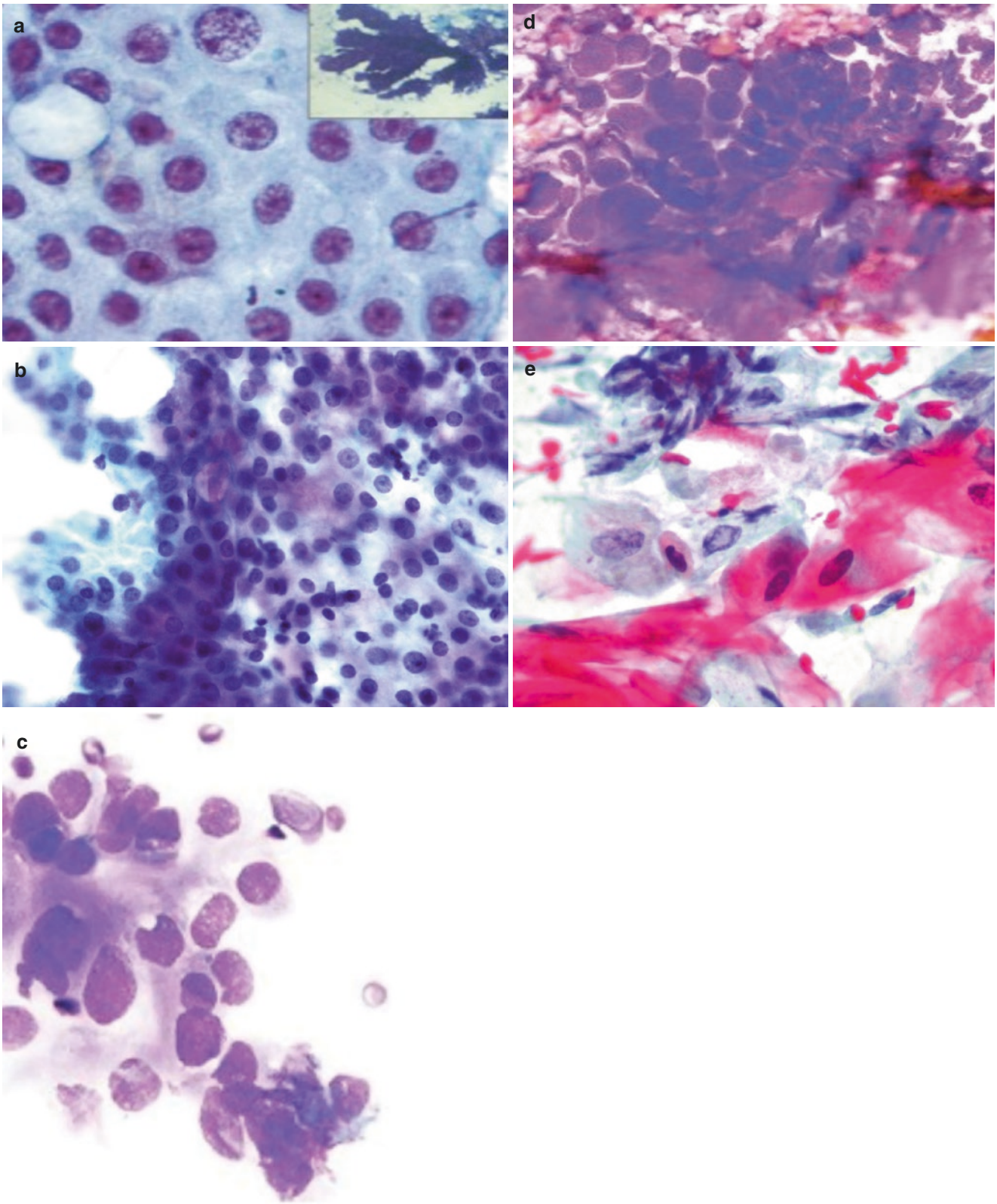

Fig. 14.4 High-grade MEC and keratinizing SCC. (**a**–**b**) High-grade MEC. Clusters of epidermoid cells with few mucus cells (Papanicolaou). (From Wang et al. [86], Figs. 1.27 and 1.28. with permission). (**c**–**d**) CA-ex-PA with focal classic chondromyxoid stroma (Diff-Quik stain). (**e**) Metastatic keratinizing squamous cell carcinoma of the parotid gland is observed on a Papanicolaou smear. The tumor cells show bizarre-shaped hyperchromatic nuclei, and irregular nuclear contours with sharp angles. Cytoplasm stains pink (Papanicolaou stain). The patient has a history of squamous cell carcinoma in the forehead. (From Wang et al. [86], Fig. 1.51 with permission)

Table 14.5 Key points of differentiation

	Oncocytoma	Low-grade AciCC
Distribution pattern	Most are cohesive clusters of uniform oncocytes; trabecular arrangement or single cells may also be seen	Clusters of crowded large polygonal cells arranged haphazardly
Nuclei	Centrally located with a distinct nucleolus No mitosis	Eccentrically located with small distinct nucleoli No or rare mitoses
Cytoplasm	Densely granular to waxy-appearing, no cytoplasmic vacuoles	Abundant and vacuolated; cytoplasmic zymogen granules
Background	Clean	Stripped naked nuclei may be seen
Special stain	Strong diffuse cytoplasmic PTAH Stain	Negative to weak PTAH stain; PAS+ diastase-resistant granules

Abbreviation: *PTAH* phosphotungstic acid-hematoxylin

Oncocytic carcinoma is a very rare carcinoma that can pose a diagnostic challenge in the cytological diagnosis of oncocytic SGTs. Aspirates may appear similar to those of benign oncocytomas. However, careful evaluation reveals atypical features including variation in cell size and shape, nuclear pleomorphism, mitosis, and necrotic background. Histological identification of tissue invasion is diagnostic.

Metastatic renal cell carcinoma in salivary glands can mimic a primary tumor. The presence of small basophilic cytoplasmic granules suggests AciCC. A good clinical history and immunostain performed on cell block help to make the definitive diagnosis (Fig. 14.5).

References: [2, 28, 44, 59–62]

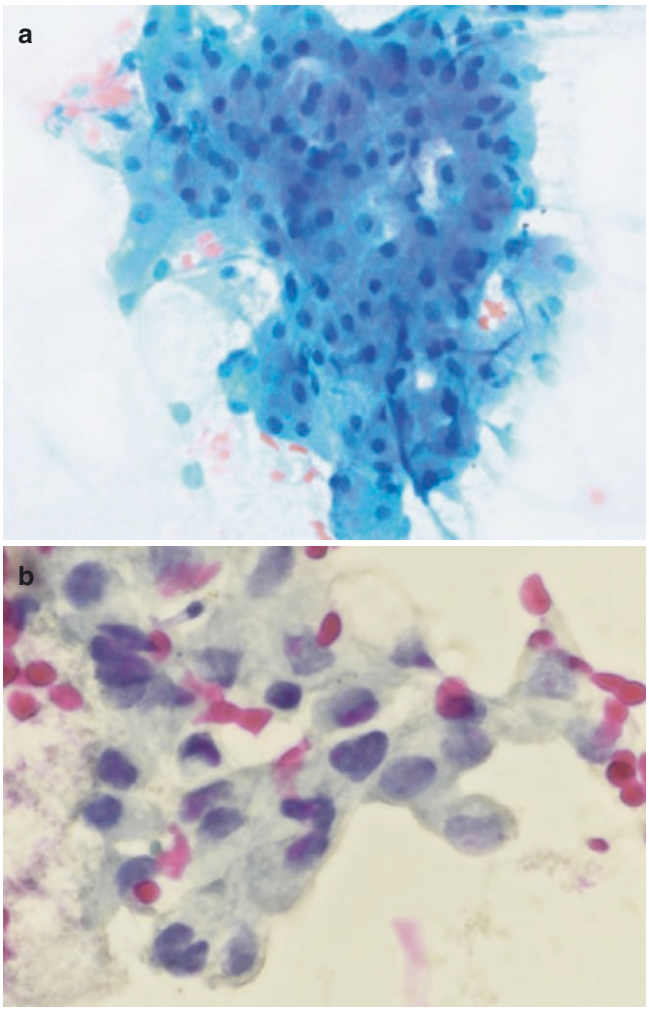

Fig. 14.5 Oncocytoma and RCC. (**a**) Oncocytoma. Tightly clustered large bland polygonal cells with centrally placed round nuclei without prominent nucleoli; abundant cytoplasm with pink fine granules. The background contains no matrix or lymphocytes (Papanicolaou stain). (From Wang et al. [86], Fig. 1.25. with permission.) (**b**) Metastatic renal cell clear cell carcinoma of the parotid gland is observed on Papanicolaou smears. (From Wang et al. [86], Fig. 1.53. with permission)

18. What are common differential diagnoses of mucinous lesion in the salivary gland?

The common differential diagnosis of mucinous lesion in the salivary gland includes mucocele, retention mucocele (retention cyst), WT with mucinous metaplasia, cystic PA with metaplasia, low-grade MEC, and SC. Among all these entities, cytological distinction between low-grade MEC and mucocele is the most commonly encountered diagnostic challenge.

Mucoceles are the most commonly acquired non-neoplastic salivary gland lesions. They are pseudocysts without true epithelial lining. Aspirates of mucoceles show variable amounts of mucoproteinaceous material, foamy histiocytes and muciphages, occasional giant cells, acute and chronic inflammatory cells, cholesterol crystals, and fibrous tissue fragments. In cases of mucoceles that occur secondary to sialolithiasis, fragments of salivary gland stones may be seen. In contrast, retention mucoceles are true cysts with an epithelial lining that can be cuboidal, ductal, oncocytic, or squamous. They represent accumulation of mucin within a dilated ductal space. The aspirates of retention cysts are similar to mucoceles but contain occasional groups of epithelial cells and have a cleaner background with fewer inflammatory cells (Table 14.6).

References: [1, 5, 63–65]

19. What are the salivary gland tumors (SGTs) with clear cells?

SGTs with clear cell features comprise a variety of entities including EMC, clear cell carcinoma, MyE, MyEC, sebaceous carcinoma, oncocytoma, MEC, AciCC, lipoma, and metastatic tumors such as renal cell carcinoma of clear cell type.

Table 14.6 Differential diagnoses of common mucinous tumors

	MEC	WT	PA	SC
Mucin	Mucoid globlet cells and extracellular mucinous matrix	Mucinous metaplasia in WT is not uncommon	Cystic PA with mucinous metaplasia	Eosinophilic filamentous matrix and mucin
Differential points	Goblet cells contain abundant mucoid cytoplasm and have indented eccentrically located nuclei	Sheets of evenly spaced oncocytes and lymphocytes in a background of granular debris	Metachromatic fibrillar matrix	Small cytoplasmic vacuoles: finely eosinophilic granular and/or mucin
Positive stains	Mucicarmine; keratin		Myoepithelial markers (smooth muscle actin, calponin, h-caldesmon)	Mammoglobin; GCDFP-15; STAT5

Aspirate of EMC is hypercellular and is characterized by a biphasic pattern of myoepithelial cells admixed with ductal cells. Myoepiothelial cells typically predominate and form loosely cohesive sheets and spheres. They are large and polygonal and contain abundant, clear, glycogen-rich delicate cytoplasm. The nuclei are uniform and oval with dispersed chromatin and small distinct nucleoli. The second population of intercalated ductal cells is usually minor. They are cuboidal and contain scant dense finely granular cytoplasm with high N:C ratios. The nuclei are round to oval with distinct nucleoli. Nuclear atypia is mild. Mitosis, apoptosis, and necrosis are rare. The background contains many naked stripped myoepithelial nuclei and dispersed single myoepithelial cells. In addition, spherical acellular concentrically laminated eosinophilic material, fragments of fibrous tissue, and dense hyalinized stroma may be seen. In challenging cases, ICC stains performed on cell block can highlight the biphasic populations of the tumor and facilitate the diagnosis. The ductal cells are positive for keratin and EMA and the myoepithelial cells are positive for smooth muscle actin, calponin, p63, and S-100.

CCC or hyalinizing/sclerosing CCC is a rare tumor that comprises of less than 1% of all salivary gland tumors. Histologically, it is characterized by monotonous clear epithelial cells within loose to densely hyalinized stroma. Aspirates show crowded groups of cells with oval bland nuclei and pale delicate glycogen-rich cytoplasm. These cells show no ductal or myoepithelial differentiation. This can be demonstrated by ICC stains performed on cell block and used to differentiate it from the EMC. Recently, a characteristic EWSR1-ATF1 gene transfusion is identified in ~80% of salivary CCC (Table 14.7).

References: [1, 2, 5, 66–71]

Table 14.7 Other salivary gland tumors with clear cell features

Sebaceous adenoma, sebaceous carcinoma	Oncocytomas	AciCC	MEC	RCC
Oil red-o positive lipid	PTAH-positive mitochondria	PAS-positive, diastase-resistant zymogens	PAS-positive, diastase-sensitive glycogen; mucicarmine-positive mucin	PAX-8, RCC, CD10

20. What are cytomorphological features of PMAC and differential diagnosis?

PMAC is a low-grade malignant neoplasm; majority of the cases occur in the minor salivary glands in the oral cavity, especially the palate. Aspirates of PMAC consist of monomorphic cuboidal to columnar cells with oval nuclei and moderate amounts of pale eosinophilic cytoplasm. Cells can be arranged in a variety of patterns including small cohesive ductal clusters, papillary groups, trabeculae, and single cells. Nuclei have open and finely stippled chromatin with small nucleoli. Extracellular matrix material is usually scant. But when it is present, it can be fibrillar or spherical, mimicking PA or AdCC, respectively. In contrast to PMAC, cells in adenoid cystic carcinoma are basaloid. In addition, the variety of cell arrangement pattern in PMAC provides a clue to distinguish it from both PA and AdCC.

ICC study has an increasing role in differentiating matrix-containing aspires of SGTs. Recently, it has been shown that AdCC is immunoreactive to CD117 and PA can be focally positive for CD117. In contrast, PMAC is negative for CD117. Therefore, CD117 may be helpful in distinguishing it from AdCC and PA (Fig. 14.6).

References: [72, 73]

21. What are common differential diagnoses of spindle cell salivary gland lesions?

The differential diagnosis of salivary gland aspirates showing a predominant spindle cell morphology are broad, including reactive lesions, benign and malignant neoplasms. The most commonly encountered benign neoplasms are spindled MyE, myoepithelial-predominant spindled PA, and schwannoma (Table 14.8 below).

Distinguishing benign from malignant lesions is important in the evaluation of spindle cell aspirates of salivary gland. Cytological features suggestive of malignancy include hypercellularity, nuclear pleomorphism, hyperchromasia, high mitotic rate, atypical mitoses, prominent nucleoli, and necrosis. The common malignant spindle cell tumors and their features are listed in Table 14.9 below.

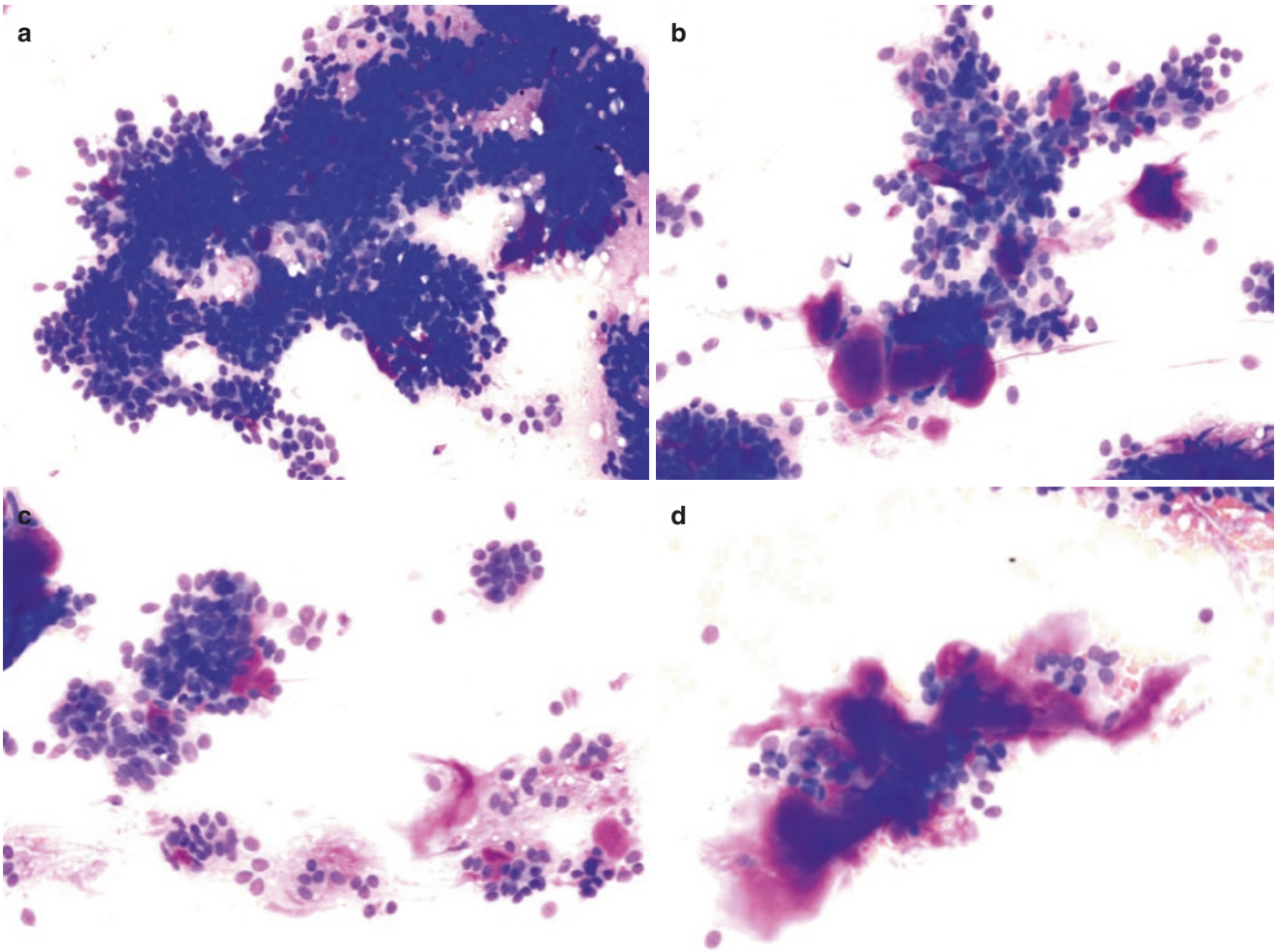

Fig. 14.6 Polymorphous adenocarcinoma. Cytomorphology of polymorphous adenocarcinoma. (**a**) Cohesive group of tumor cells with scattered magenta-stained hyaline globules. Note the finely granular material in the background. (**b**) High-power view demonstrating sharply demarcated hyaline globules closely mimicking adenoid cystic carcinoma. The dissociated tumor cells had ill-defined borders and often naked nuclei. Note the occasional arrangement of tumor cells at the periphery of the hyaline globules. (**c**) In this case, the background material ranged from globulated, granular, and fibrillary. (**d**) Among scattered tumor cells, this case demonstrated a background mimicking pleomorphic adenoma. (From Andreasen et al. [89], with permission)

Table 14.8 Differential diagnoses of benign spindle cell salivary gland neoplasms

	MyE	Myoepithelial-predominant spindled PA	Schwannoma
Characteristic points	Various cellular morphologies, including spindled, plasmacytoid, epithelioid, polygonal, stellate, and clear forms	Chondroid component and/or a ductal element	Palisading nuclei within the fibrillar stroma
Aspirate features	Cellular, groups of haphazardly arranged or singly dispersed spindled myoepithelial cells	Cellular, groups of myoepithelial cells and minor chondroid and ductal components	Hypocellular, clusters of spindled to rounded cells
Stroma	Small amounts of fibrillary, myxoid, or hyaline stroma	Small amounts of fibrillary, myxoid stroma	Myxoid to fibrillar stroma
Nuclei	Ovoid to fusiform nuclei, less elongate and have rounded ends	Ovoid to fusiform nuclei, less elongate and have rounded ends + minor round to oval nuclei	Long wavy or fishhook-shaped with tapering pointed ends
ICC	Positive: keratins, smooth muscle actin, p63, calponin; Weakly: S-100 and GFAP	Positive: PLAG1, keratins, smooth muscle actin, p63, calponin	Positive: S-100, vimentin Negative: keratin, smooth muscle actin, Leu-7, neurofilament

Table 14.9 Differential diagnoses of malignant spindle cell salivary gland neoplasms

MyEC	MPNST	Synovial sarcoma	Spindle cell ca	Spindle cell melanoma
More than half of cases arise from preexisting PA or MyE		Usually young patients and in their 20s to 40s	Often secondary to radiation therapy	
Variable degree of atypia, nuclear pleomorphism, coarse chromatin, prominent nucleoli, high mitotic activity; myxoid or hyaline stroma	Markedly hypercellular plump, elongate, and hyperchromatic nuclei, distinct nucleoli, and mitoses, delicate and fibrillary cytoplasm	Hypercellular, uniform spindle cells with mild nuclear atypia and scant pale cytoplasm. Mitoses are frequent. In biphasic forms, the epithelial component may exhibit glandular, sheet, or papillary structure	Loosely cohesive groups of spindle cells with high-grade nuclear features haphazardly arranged within dense fibrous stroma	Prominent nucleoli, intranuclear pseudoinclusions, and cellular dissociation; finely granular cytoplasmic melanin pigment
Background necrosis	Background naked stripped nuclei			
Positive for both myoepithelial and epithelial markers	Positive:Leu-7; Focal: S-100	Positive: cytokeratins, EMA, CD99, t(X;18) translocation;	Positive: vimentin, smooth muscle actin, and desmin Focal: keratin	Positive: S100, Mart-1, HMB45

Other less common benign spindle cell lesions in the differential diagnoses of salivary gland lesions include granulomatous inflammation, nodular fasciitis, fibromatosis, solitary fibrous tumor, and leiomyomas.

Epithelioid histiocytes in granuloma can form loose clusters and exhibit spindle cell morphology with kidney-shaped nuclei and moderate amounts of vacuolated cytoplasm. Birefringence for foreign body material and special stains for organisms should be attempted. If no specific etiology is identified after a thorough examination, noninfectious granulomatous diseases should be considered. The nature of the histiocytes is confirmed by positive ICC stain for CD68 and negative for other markers.

Nodular fasciitis is a self-limiting benign myofibroblastic lesion. Aspirates are characterized by collagenized groups of spindle-shaped and stellate myofibroblasts. Cells have plump oval to elongate bland nuclei with small distinct nucleoli and wispy bipolar cytoplasmic processes. Scattered single cells, acute and chronic inflammatory cells are seen in the background. Myofibroblasts in nodular fasciitis are positive for smooth muscle actin and negative for S-100 and keratin.

Fibromatosis usually presents in young patients. Aspirates show bland plump elongate fibroblasts admixed within myxoid and/or collagenous stroma. The fibroblasts and myofibroblasts comprising the lesion are immunoreactive for vimentin, smooth muscle actin, and sometimes desmin.

Solitary fibrous tumor is a rare neoplasm that occasionally involves the parotid gland. Aspirates are hypercellular, consist of groups of haphazardly arranged monotonous spindled cells and dense ropy collagen. The latter, when present, is a characteristic feature of this tumor. Immunocytochemically, solitary fibrous tumor is distinguished by its positivity with CD34 and negativity with S-100 and keratin.

Leiomyomas can rarely occur in the subcutaneous tissues of the head and neck and oral cavity. FNA is often painful. Aspirates comprise of groups of bland spindle cells with elongate blunt-end "cigar-shaped" nuclei and moderate amounts of eosinophilic cytoplasm. Stripped naked nuclei are seen in the background. Immunohistochemically, the tumor cells are positive for smooth muscle markers, including actin and desmin (Fig. 14.7).

References: [5, 28, 30, 74–78]

22. What are common differential diagnoses of cystic salivary gland lesions?

Cystic lesions account for up to 8% of all salivary gland masses and include a wide variety of entities from non-neoplastic lesions (mucocele, salivary duct cyst), to benign neoplasms (cystadenoma and WT), and to malignant neoplasms (low-grade MEC and cystadenocarcinoma).

The algorithm for diagnosing cystic salivary gland aspirate starts with the evaluation of the presence or absence of mucin. Entities with mucinous background have been discussed in Q 18.

For nonmucinous aspirates of salivary gland cysts, the presence or absence of background lymphocytes is a key feature. In the presence of lymphocytes, the differential diagnosis includes WT, lymphoepithelial sialadenitis (LESA), HIV-associated cysts, lymphoepithelial cysts (branchial cleft-like cysts) and metastasis. In the absence of lymphocytes, the differential diagnosis includes cystic PA, ductal cyst, AciCC, cystadenoma, cystadenocarcinoma, and ductal papilloma.

Lymphoepithelial cysts (branchial cleft-like cysts) are congenital cysts that are analogous to the branchial cleft cysts of the neck. Aspirates of lymphoepithelial cysts of the parotid gland usually contain bland mature and anucleate squamous cells, keratin debris, and macrophages in a back-

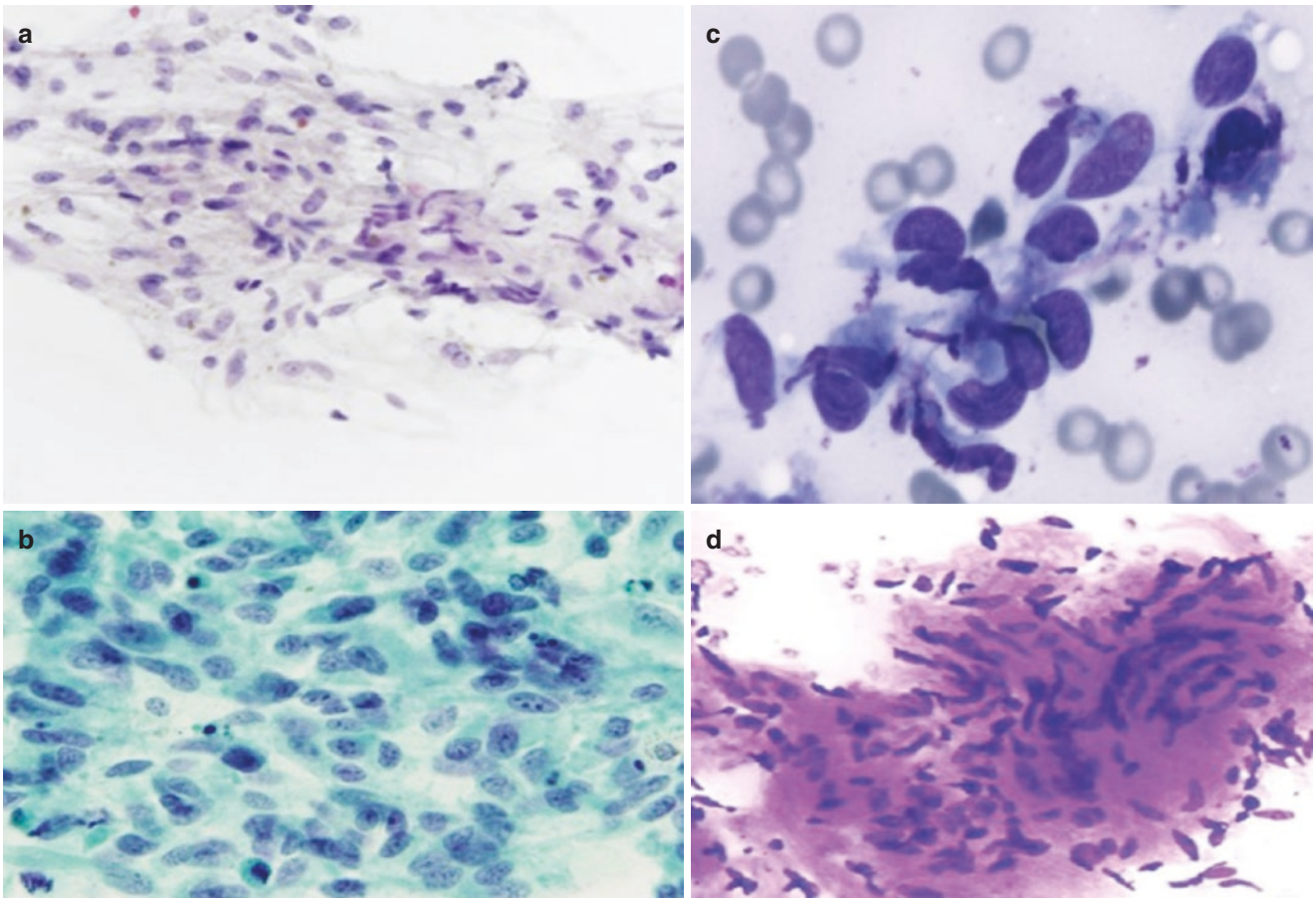

Fig. 14.7 Spindle cells neoplasms. (**a**) Myoepithelioma. Cohesive aggregates of spindle-shaped cells are embedded in a fibrillary matrix, tumor cells have barely perceptible cytoplasm and oval nuclei with inconspicuous nucleoli, Papanicolaou smear. (From Schneider et al. [44], with permission.) (**b**) Metastatic spindle cell melanoma of the parotid gland is observed on a Papanicolaou smear. (From Wang et al. [86], Fig. 1.52. with permission). (**c**) Leiomyoma with cigar-shaped nuclei. (**d**) Schwannoma. Hypocellular clusters of spindled cells with long wavy nuclei and fibrillar stroma

ground of turbid proteinaceous material and variable numbers of lymphocytes and lymphohistiocytic aggregates. Bland mucinous glandular cells and ciliated cells can occasionally be seen. Aspirates of LESA and HIV-associated lymphoepithelial cysts may be indistinguishable from lymphoepithelial cysts in salivary gland aspirates. Adequate clinical information helps to resolve the differential diagnosis. LESA is usually associated with Sjögren's syndrome and HIV-associated lymphoepithelial cysts occur in patients with HIV infections.

Cystic cystadenoma is a rare benign multicystic salivary gland tumor and is nearly indistinguishable from its malignant counterpart, cystadenocarcinoma, in aspirates. Cystadenoma and cystadenocarcinoma are distinguished by histological examination of invasion rather than by cytological atypia. Their aspirates are usually hypocellular and contain bland cuboidal to columnar cells in a background of cystic debris with eosinophilic material and sometimes psammoma bodies. The cytoplasms of the cells are often oncocytic, but may contain mucin. Epidermoid cells resembling low-grade MEC may even be seen. Therefore, when a cystadenoma is suggested in an aspirate, the differential diagnosis includes low-grade MEC, the papillocystic subtype of AciCC, cystic oncocytoma, ductal papilloma, and cystadenocarcinoma.

References: [79–85]

Case Presentation

Case 1

Clinical History:

A 49-year-old man with history of smoking noticed a slowly enlarging 3 cm complex cyst/mass in left parotid gland for 4 months. No significant pain is noticed. Ultrasound examination revealed focal calcification and extraparotid extension. Ultrasound-guided FNA was performed with rapid on-site evaluation (ROSE).

Cytomorphological Findings:

Smears show moderately cellular specimens, the cells have uniform round and vesicular nuclei with central nucleoli and eosinophilic pink vacuolated cytoplasm. Mild-to-moderate nuclear pleomorphism and mild nuclear contour irregularity are identified. Background proteinaceous material is common. These cells are arranged into microcystic, cribriform, tubular, papillary, follicular, or solid nests. Intraluminal secretions may be seen in the microcystic or tubular structures.

Differential Diagnosis:

- Acinic cell carcinoma
- Low-grade mucoepidermoid carcinoma
- Pleomorphic adenoma
- Secretory carcinoma
- Warthin tumor
- Metastatic renal cell carcinoma

Immunostains Performed on the Cell Block Material:

- Positive for AE1/AE3, mammoglobulin, S100, GCDFP-15, GATA3, DOG-1
- Negative for p63, ER, PR, Her-2

Molecular test (FISH): ETV6-NTRK3 translocation positive (Fig. 14.8)

Final Diagnosis:

- Secretory carcinoma

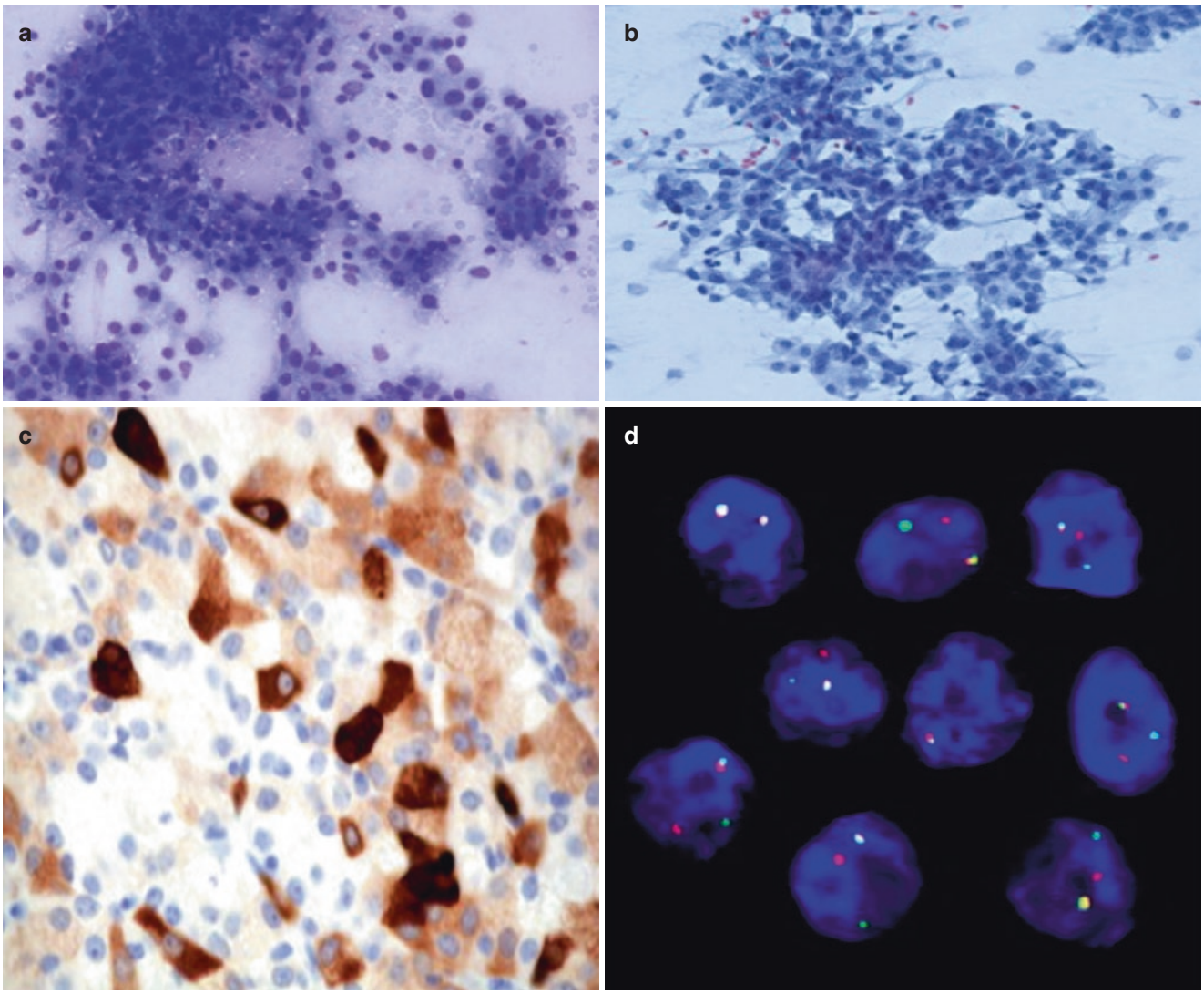

Fig. 14.8 Case 1. (**a**) Diff-Quick smear; (**b**) Pap smear; (**c**) mammoglobulin; (**d**) FISH: ETV6-NTRK3

Case 2

Clinical History:

A 51-year-old female presented with bilateral parotid swelling of 2-year duration, slowly progressing, 6 × 2.5 cm painless masses were noticed. Clinical impression was pleomorphic adenoma. All serum tumor markers were within normal limits and human immunodeficiency virus (HIV) status was negative. Magnetic resonance imaging: Bilateral enlarged parotid glands with multiple well-defined intraparotid focal lesions: differential diagnoses include pleomorphic adenoma, Warthin tumor, tuberculosis, and lymphoma.

Cytomorphological Findings:

FNA smears show highly cellular specimens. Heterogeneous population of atypical lymphoid cells and large dispersed monocytoid cells with scant cytoplasm, anisonucleosis with prominent nucleoli, irregular nuclear membrane, and numerous mitoses.

Differential Diagnosis:

- Warthin tumor
- Hodgkin lymphoma
- Melanoma
- Large B-cell lymphoma

Immunostains Performed on the Cell Block Material:

- Positive for CD20, B-cell lymphoma 6 protein (Bcl-6)
- Negative for AE1/AE3, CK8/CK18, S-100, Mart-1, CD3, CD5, CD10, and multiple myeloma oncogene-1 (MUM1)

Flow cytometry: positive for B-cell lymphoma (Fig. 14.9)

Final Diagnosis:

- Large B-cell lymphoma

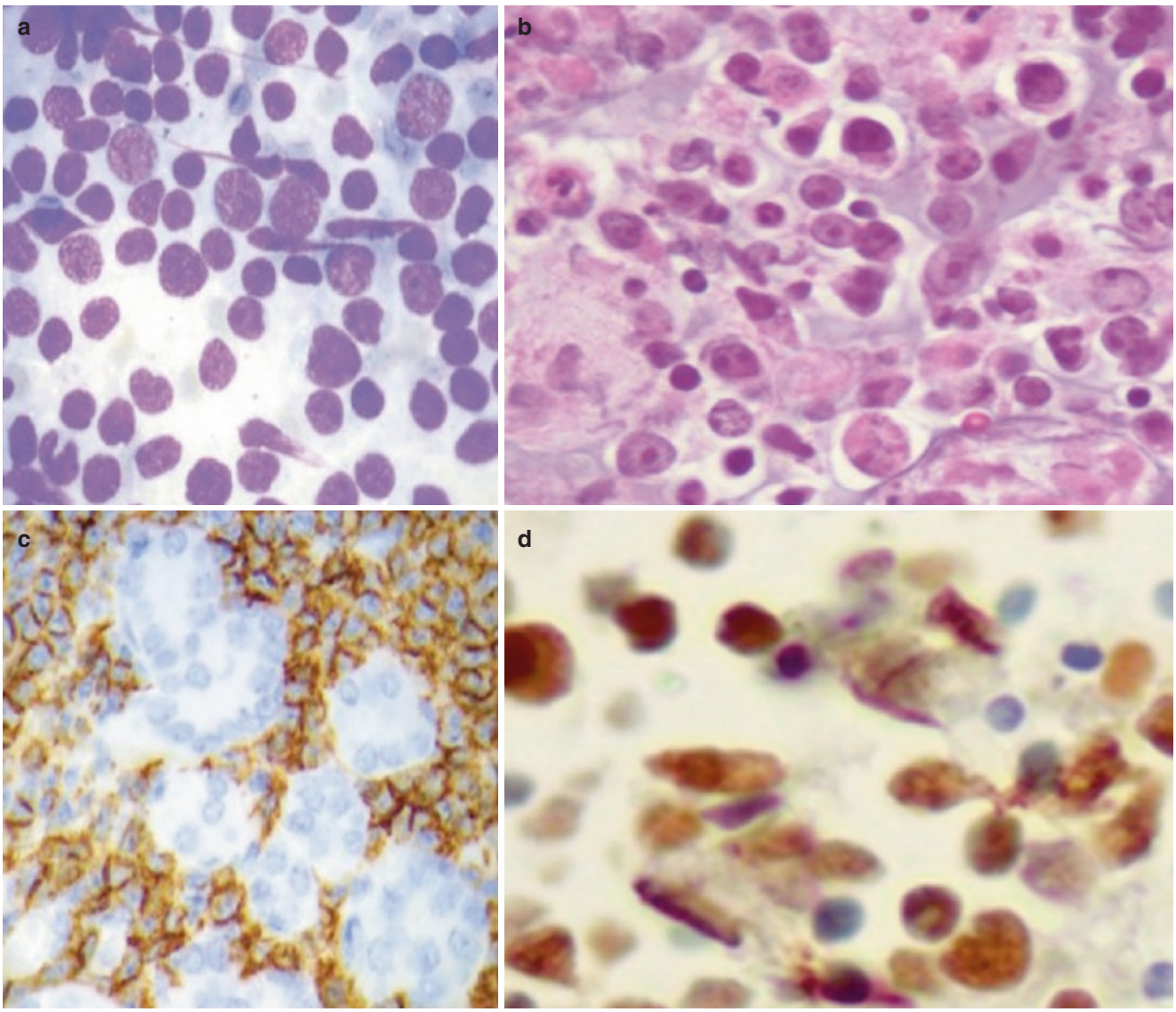

Fig. 14.9 Case 2. (**a**) Giemsa stain; (**b**) H&E cell block; (**c**) IHC-CD-20; (**d**) IHC-Bcl-6

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Lymph Nodes

15

John C. Lee and Xiaohua Qian

Contents

List of Frequently Asked Questions

1. Fine-needle aspiration (FNA) of lymph nodes for isolated lymphadenopathy or ruling out lymphoma should include what kind of tests?

Testing should include a minimum of cytomorphological evaluation and immunophenotypic studies. Cytomorphological evaluation can be achieved through on-site air-dried smears with Diff-Quik staining. If on-site analysis is not needed or performed, then the FNA material can be rinsed in either Roswell Park Memorial Institute (RPMI) medium or CytoLyt fixative. The soluble component of RPMI can then be used to make a Wright-Giemsa stained cytospin slide and/or for immunophenotyping by flow cytometry. Alternatively, the soluble component of the FNA material rinsed in CytoLyt can be used to make a Papanicolaou-stained SurePath or ThinPrep slide. Of note, CytoLyt-fixed FNA material cannot be used for flow cytometry, because antibody binding to cell surface antigens can only be achieved in the fresh unfixed state. If there is precipitate material in either the RPMI and/or CytoLyt sample, a cell block can be made to generate a hematoxylin-eosin (H&E) stained section for histological evaluation and for immunohistochemistry (IHC). A cell block H&E and IHC can provide some degree of architecture which can be very helpful in establishing a definitive diagnosis of either benign lymphoid tissue or lymphoma. If fluorescence in situ hybridization (FISH) is needed, smears, cytospins, monolayer slides, or cell block unstained slides can be used. At our institution, FNA passes in both CytoLyt and RPMI are submitted when lymphoma is in the differential diagnosis. In contrast, FNA passes are submitted only in CytoLyt when the metastasis is the primary concern. The above test triage process is illustrated in Fig. 15.1.

References: [1, 2].

2. What are the pros and cons of air-dried Diff-Quik staining versus air-dried Wright-Giemsa staining versus alcohol-fixed Papanicolaou staining?

Diff-Quik staining is the fastest to perform and is amenable for rapid on-site evaluations (ROSE). Staining with Wright-Giemsa or Papanicolaou stain takes longer to perform and is done in the cytology laboratories. Diff-Quik and Wright-Giemsa are Romanowsky stains and are performed on air-dried preparations. One of the benefits of air-dried preparations is that it maximizes the cell- and nuclear surface areas. For lymphoid and hematopoietic tissue, maximizing the cell size allows for the best appreciation of cytoplasmic features and some nuclear features. Alcohol-fixation shrinks cells to a smaller size due to dehydration (doing so for all cell types in a proportionate manner), so sometimes differentiating between small from intermediate size, or intermediate from large size can be more difficult. Diff-Quik stains chro-

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_15

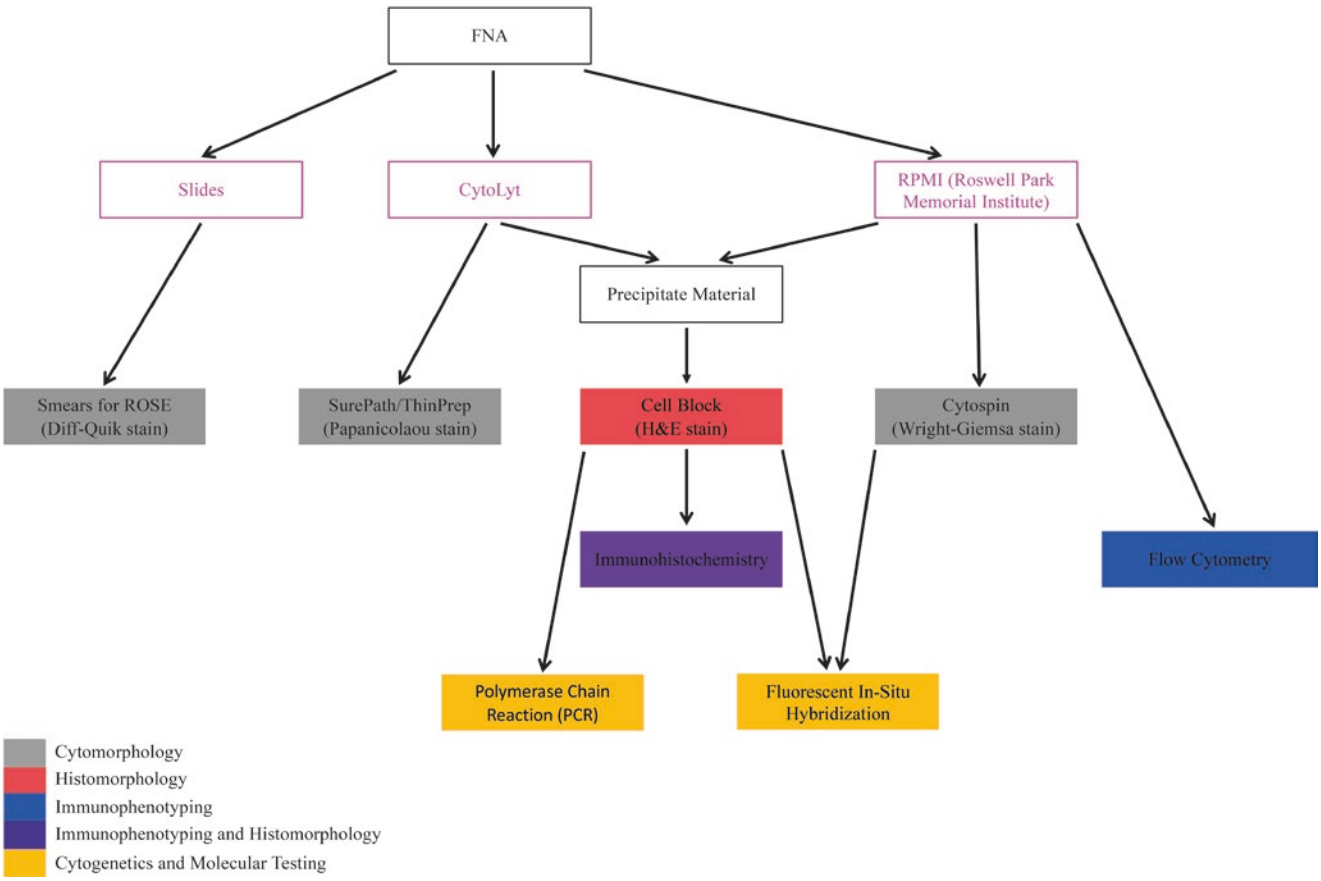

Fig. 15.1 Specimen triage and preparation of FNA material in the evaluation of lymphadenopathy

Table 15.1 Comparison of the pros and cons of different stains (air-dried Diff-Quik versus air-dried Wright-Giemsa versus alcohol-fixed Papanicolaou)

Air-dried Diff-Quik		Air-dried Wright-Giemsa		Alcohol-fixed Papanicolaou	
Pros	Cons	Pros	Cons	Pros	Cons
Rapid Larger cell size Cytoplasmic details	Overstained chromatin Lack nuclear details	Larger cell size Best for blastic chromatin and nucleolus Cytoplasmic details	Slow Mildly overstained Chromatin	Vesicular chromatin Prominent nucleolus Nuclear membrane characteristics	Slow Smaller cell size Lack cytoplasmic details

matin most intensely, followed by Wright-Giemsa and Papanicolaou stain. Therefore, the finely dispersed chromatin texture in blasts is best appreciated on air-dried, Wright-Giemsa-stained preparations, whereas the vesicular chromatin or chromatin clearing is best seen with Papanicolaou staining. Prominent nucleolus is most obvious with Papanicolaou, appreciable with Wright-Giemsa, and is difficult to appreciate with Diff-Quik stain. Nuclear membrane characteristics such as smooth, irregular, angulated, clefts, folded, or convoluted are best seen with Papanicolaou stain since chromatin staining is lighter and does not obscure the nuclear membrane. The cytoplasmic details are better appreciated with Romanowsky stains than with Papanicolaou stain (Table 15.1).

References: [3, 4].

3. What are the pros and cons of smear versus monolayer (cytospin, SurePath, ThinPrep) preparation? (Table 15.2)

References: [5].

4. What are the pros and cons of immunophenotyping using flow cytometry versus immunohistochemistry on cell block? (Table 15.3)

References: [1, 4].

5. In which types of lymphomas is flow cytometry either noncontributory or associated with false-negative finding?

Flow cytometry is often non-contributory in Hodgkin lymphomas because the neoplastic cells, namely the Reed-

Sternberg (RS) cells or lymphocyte predominant (LP) cells, are usually too few against a background of abundant mixed inflammatory cells to be evaluated by flow cytometry. Flow cytometry is known to yield false-negative results in a quarter to a third of large cell or high-grade lymphomas, both B-cell and T-cell types, because these lymphoma cells are fragile and may not survive the processing steps involved in flow cytometry.

References: [1, 4, 6, 7].

Table 15.2 Comparison of the pros and cons of smear versus monolayer preparations

Smear		Cytospin, SurePath, ThinPrep	
Pros	Cons	Pros	Cons
Rapid Larger cell size Suitable for ROSE	Need to be made on-site Crush artifact Artifactual variation in cell size Usage of diagnostic material which could be otherwise used for ancillary studies	Better preservation Better morphological uniformity Less material is needed	Slow Smaller cell size More epithelioid appearance

Table 15.3 Comparing the pros and cons of immunophenotyping (flow cytometry) versus immunohistochemistry on cell block

Flow cytometry		Immunohistochemistry on cell block	
Pros	Cons	Pros	Cons
Less material needed Multiple antibodies can be evaluated simultaneously Kappa and lambda evaluation More quantitative or objective	No correlation with morphology ("black box")	Correlation with morphology	More material needed Single antibody evaluation Kappa and lambda evaluation is often suboptimal More subjective

6. How would you triage a lymph node FNA based on the smear interpretation during a rapid on-site evaluation?

A triage flowchart is recommended based on the smear interpretation during ROSE (Fig. 15.2).

References: [1, 4].

7. How do you generally differentiate a benign lymph node from lymphoma based on cytomorphology?

The presence of an intact nodal architecture is supportive of a benign lymph node on histology. However, only a limited sense of architectural information can be gleaned from an FNA sample. Generally benign lymph nodes are either unremarkable ("resting") or reactive with secondary follicles. In benign lymph nodes, follicles are usually appreciable, even with cytomorphological preparations (Fig. 15.3). For unremarkable benign lymph nodes, lymphocytes outside of the follicles, are typically composed of small mature forms. The predominant presence of small mature lymphocytes is usually associated with a benign "resting" lymph node. If there is any degree of monomorphism associated with the small mature lymphocytes, that should give some concern for a "small cell" lymphoma. Reactive benign lymph nodes are associated with a polymorphous population of lymphocytes with a range of small to medium to large forms with a predominance of small mature forms.

Reference: [8].

8. Can lymph nodes produce a cohesive component on FNA?

The cohesive component in lymph node FNA are intact or fragmented follicles. We typically think of lymphocytes as being discohesive, singly scattered, and forming a monolayer on smear or touch prep. Follicles are three-dimensional structures that maintain cohesion due to the presence of follicular dendritic cell meshworks which provide a structural component and "housing" for lymphocytes. On touch prep, follicles exfoliate as entire intact units. On FNA, follicles can show a variable degree of disruption depending on aspiration technique, smearing and/or processing techniques.

References: [8, 9].

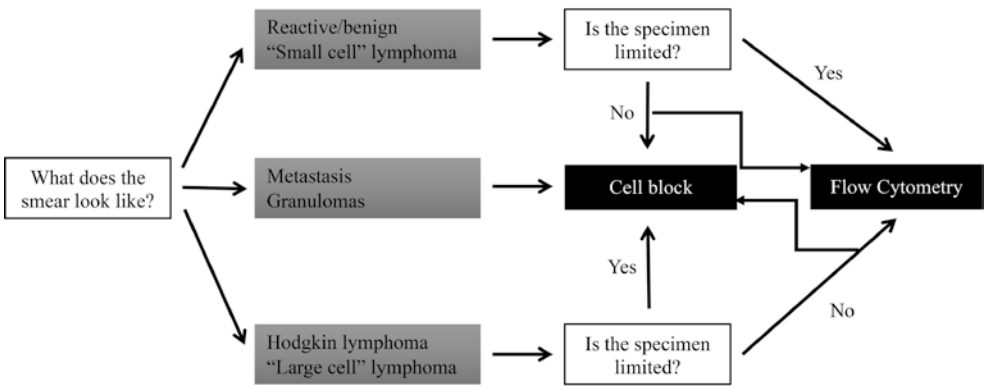

Fig. 15.2 Flowchart of FNA specimen triage based on the cytomorphology on rapid on-site evaluation (ROSE)

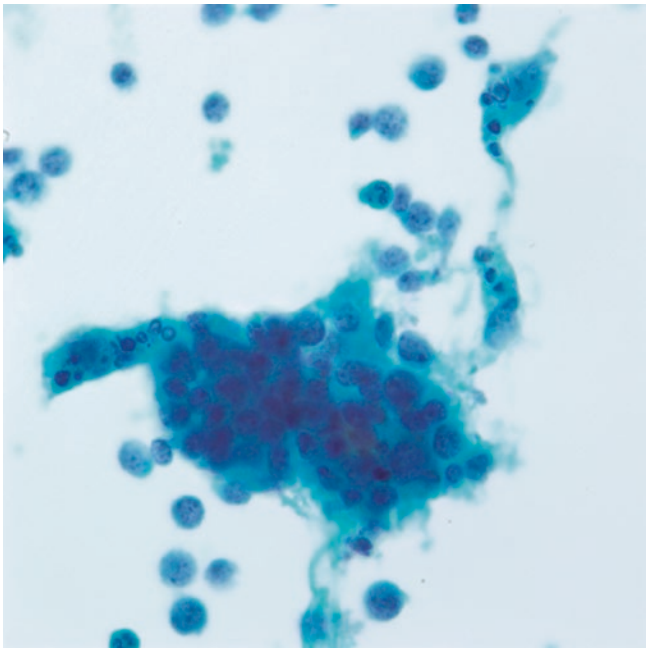

Fig. 15.3 Follicular hyperplasia. A 47-year-old female with recently diagnosed HIV presented with neck lymphadenopathy. FNA showed a cellular aspirate with large follicles in a background of polymorphous lymphocytes (SurePath preparation, Papanicolaou stain). Note the frequent tingible body macrophages (arrow)

9. How do you differentiate lymphoid follicles from epithelial structures?

Both follicles and benign/malignant epithelial structures are cohesive and may appear to be similar under low power magnification. Follicles are composed of follicular dendritic cells, small lymphocytes, centrocytes, and centroblasts, sometimes with tingible body macrophages and capillaries (Fig. 15.3). The lymphocytic nature of follicles can be difficult to appreciate in the center but is better visualized at the edges under high magnification.

10. Are tingible body macrophages (TBMs) always associated with benign lymph nodes?

The answer is no. TBMs are typically seen within reactive germinal centers, in association with follicular hyperplasia or hyperactivated follicles in benign lymph nodes. Of note, because germinal centers are cohesive and held together by follicular dendritic cell meshworks, TBMs may be sequestered within follicles on FNA smears. In the setting of high-grade lymphomas, TBMs can be recruited to clear out the dying or dead cells and are often associated with a high proliferation and apoptosis rate (i.e., Burkitt lymphoma).

References: [10, 11].

11. Why are granulomas difficult to visualize on FNA?

Granulomata are often not amenable to aspiration in the setting of granulomatous lymphadenopathy. The granulomas in sarcoidosis are well-formed, demarcated, and associated with fibrosis, which resists aspiration and yields paucicellular aspirates (Fig. 15.4). On smears, granulomata are cohesive structures due to the epithelioid nature of the histiocytes and the associated stromal fibrosis. They usually have a syncytial jumbled appearance with groups of oval to spindled cells seemingly sharing a common dense cytoplasm, yet the nuclei are distributed in a haphazard arrangement. Individual multinucleated giant cells can be seen. Similarly, necrotizing granulomata are often resistant to aspiration because the necrotic material is gummy and resists the negative pressure imparted by aspiration.

References: [12, 13].

12. What is in the differential diagnosis of granulomas in lymph nodes?

The differential diagnosis can be broadly divided as to whether the granulomata are necrotizing or non-necrotizing. Necrotizing granulomata are typically associated with an infectious etiology such as a fungal or mycobacterial lymphadenitis. Granulomata associated with neutrophilic abscesses is seen in association with cat-scratch (Bartonella henselae) lymphadenitis Non-necrotizing granulomata, especially those that are well formed and associated with fibrosis, are typically seen in sarcoidosis (Fig. 15.4). Granulomata are tricky because they are not always associated with a benign etiology. In rare circumstances, granulomata are associated with lymphomas and metastaic seminomas. Examples include classic Hodgkin lymphoma, nodular lymphocyte predominant Hodgkin lymphoma, and some T-cell lymphomas. In the lymphoepithelioid (or Lennert) lymphoma subtype of peripheral T-cell lymphoma NOS, non-necrotizing granulomata can be quite confluent (albeit poorly formed) and associated with cytologically bland neoplastic T-cells.

References: [3, 13–15].

13. What is in the differential diagnosis of Epstein–Barr virus (EBV) lymphadenitis?

The differential diagnosis of EBV lymphadenitis or infectious mononucleosis (IM) includes other viral lymphadenitis, classic Hodgkin lymphoma (cHL), diffuse large B-cell lymphoma (DLBCL), and other EBV-positive lymphomas. Viral lymphadenitis may cause distortion but not effacement of the nodal architecture. Subtle architectural changes noted on core biopsy, compounded by increased number of immu-

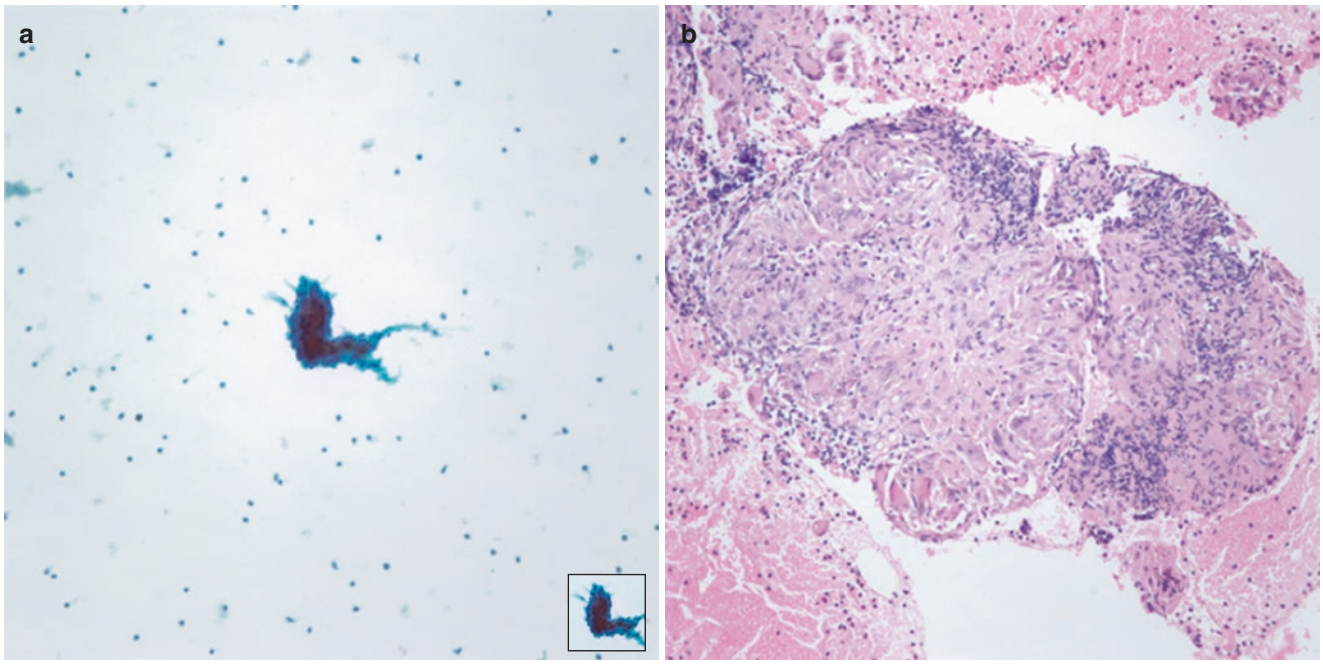

Fig. 15.4 Sarcoidosis**.** A 51-year-old female with mediastinal and abdominal lymphadenopathy. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) of a periduodenal lymph node was performed. (**a**) SurePath preparation showing hypocellular aspirate with few non-necrotizing granulomas in a syncytial arrangement (inset) (Papanicolaou stain). (**b**) Cell block preparation showing non-necrotizing granuloma surrounded by a rim of lymphocytes (Hematoxylin-Eosin [H&E] stain)

noblasts on FNA smears may be misinterpreted as "atypical" features mimicking lymphoma. Depending on the degree of immunoblastic proliferation, EBV-lymphadenitis can mimic cHL, especially when immunoblasts are Hodgkin-Reed-Sternberg (HRS)-like. EBV-lymphadenitis can also mimic DLBCL if the immunoblasts show clustering or sheeting. Even on an excision the distinction between an EBV-lymphadenitis and lymphoma can be challenging, which is why clinicians usually take a wait-and-watch approach when encountering a patient with symptoms of infectious mononucleosis. Any lymph node FNA from a teenager or young adult, especially from cervical lymph nodes, should be approached with caution. Overinterpretation of atypical features seen in EBV-lymphadenitis can be avoided by correlating with clinical history, complete blood count with differential, Monospot, and EBV serology.

References: [3, 8, 16].

14. What is the differential diagnosis of plasmacytosis in lymph nodes?

The differential diagnosis of plasmacytosis is broad and includes both benign (majority) and malignant etiologies. Benign causes include infectious lymphadenitis such as syphilis, lymphadenopathy associated with autoimmune disorders such as rheumatoid arthritis, and Castleman disease including both the hyaline vascular and plasma cell variants. Plasmacytosis can also be one of the manifestations of IgG4-related diseases in lymph nodes. Though less common, but not rare, plasmacytosis can be associated with lymphomas. Any of the "small cell" B-cell lymphomas can show plasmacytic differentiation, with the most common being marginal zone lymphomas (MZL) and lymphoplasmacytic lymphoma (LPL). Nodal MZL is uncommon and a diagnosis of exclusion. Extranodal MZL of mucosa associated lymphoid tissue usually does not involve regional lymph nodes. Similarly, LPL typically does not involve lymph nodes. Other lymphomas in which there is a reactive plasmacytosis unrelated to the neoplastic clone, include classic Hodgkin lymphoma and angioimmunoblastic T-cell lymphoma. Lastly, plasma cell neoplasms (myeloma) can rarely involve extramedullary sites such as lymph nodes.

References: [3, 18, 19].

15. What is in the differential diagnosis of eosinophilia in lymph nodes?

The differential diagnosis of eosinophilia includes secondary reaction to a benign etiology such as hypersensitivities, medication, parasites, etc. Kimura disease, commonly seen in young men in Asia, is a specific pattern of lymphadenopathy with eosinophilia of unknown etiology. Eosinophilia can also be associated with neoplasms such as classic Hodgkin lymphoma, angioimmunoblastic T-cell lymphoma, adult T-cell leukemia/lymphoma, or Langerhans cell histiocytosis. Primary neoplastic eosinophilia, in which the eosinophils are part of the neoplastic clone, rarely causes lymphadenopathy.

References: [3, 20–23].

16. What panel of immunohistochemistry antibodies would you use on the cell block to confirm that you are dealing with a benign lymph node or a lymphoma?

The typical panel of immunohistochemistry antibodies includes PAX5, CD3, CD5, CD43, BCL2, BCL6 (or CD10), and CD21. These markers can help recreate the architecture of an otherwise disrupted and rearranged sample. A combination of a B-cell marker like PAX5, a germinal center marker like BCL6, and a follicular dendritic cell marker like CD21 can help recreate the follicles or the cortex. T-cell markers such as CD3, CD5, and CD43 can help recreate the paracortex. In addition, most lymph node FNAs are benign and composed predominantly of small mature lymphocytes. The most common malignant differential diagnoses for a benign lymph node, are the "small" B-cell lymphomas, and this panel is helpful for the distinction. CD5 and/or CD43 expression in B-cells is deemed to be aberrant and should not be seen in normal B-cells. In addition, markers such as CD5 and CD10 (or BCL6) can help in "small" B-cell lymphoma subtyping. CD23 can be added if the "small" B-cell lymphoma is CD5 positive. If the CD23 is positive, that would support chronic lymphocytic leukemia (CLL) / small lymphocytic lymphoma (SLL). However, if the CD23 is negative, that would support mantle cell lymphoma. Mantle cell lymphoma is confirmed with positivity for cyclin D1 and/or SOX11 (in general it is a good idea to do cyclin D1 for all CD5 positive B-cell lymphomas). If there is CD10 positivity in B-cells, Bcl-2 can be ordered to confirm if the follicles are reactive (Bcl-2 negative) or neoplastic (Bcl-2 positive). If there is an increase of plasma cells and clonality assessment by flow cytometry is not available, kappa and lambda in-situ hybridization (ISH) on cell block sections is recommended to determine the clonality of plasma cells.

References: [1, 4].

17. What types of lymphomas have a follicular or nodular pattern?

Follicles are B-cell rich and derive their structure from follicular dendritic cell (FDC) meshworks. In hematopathology, we refer to "nodules" as expanded follicles that maintain their structure from meshworks. In nodules, the connections between the FDC, are often attenuated. It is important to recognize follicles or nodules and their presence as either being the neoplastic component or related to the neoplastic component, which narrows our differential diagnosis. Lymphomas that have a follicular or nodular pattern include:

- Follicular lymphoma (FL): Most FLs have a predominantly follicular pattern with the FDC meshworks being tightly woven (Fig. 15.5).
- Marginal zone lymphomas (MZL) (extranodal, nodal, and splenic): Many MZL have a follicular or nodular pattern via a process called follicular colonization, in which the marginal zone cells colonize the follicle from inside out.
- Nodular lymphocyte predominant Hodgkin Lymphoma (NLPHL): Unlike FL or even MZL, the neoplastic cells of NLPHL are the lymphocyte-predominant (LP) or "popcorn" cells, which constitute a very small component of the nodules (Fig. 15.6). Identifying these cells requires a very cellular specimen with a decent cell block. It is not uncommon that NLPHL are missed on even cellular FNAs and/or core biopsies.
- Classic Hodgkin lymphoma (cHL), lymphocyte-rich subtype: It is specifically the lymphocyte-rich subtype of cHL that is characterized by follicles/nodules with few neoplastic Hodgkin-Reed-Sternberg (HRS) cells. cHL is known to be sometimes missed on FNA, particularly the lymphocyte-rich subtype.
- Angioimmunoblastic T-cell lymphoma (AITL): AITL has de-novo FDC meshworks that are recruited in the creation of a germinal center microenvironment outside the existing follicles. These meshworks are rich with arborizing endothelial venules containing neoplastic T-follicular helper cells, along with reactive B-cells, EBV transformed B-cells, reactive plasma cells, and eosinophils.
- Chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL) is often referred to as having a nodular pattern composed of proliferation centers that have increased numbers of prolymphocytes and paraimmunoblasts in addition to the typical CLL/SLL cells. However, these proliferation centers are not follicles and are not supplied by a FDC meshwork.

References: [17, 24–27].

18. How do you differentiate follicular hyperplasia from follicular lymphoma on cytology?

It is possible to distinguish follicular hyperplasia from follicular lymphoma (FL) on FNA when the specimen is cellular. It is strongly recommended to have immunophenotyping by either flow cytometry and/or immunohistochemistry to

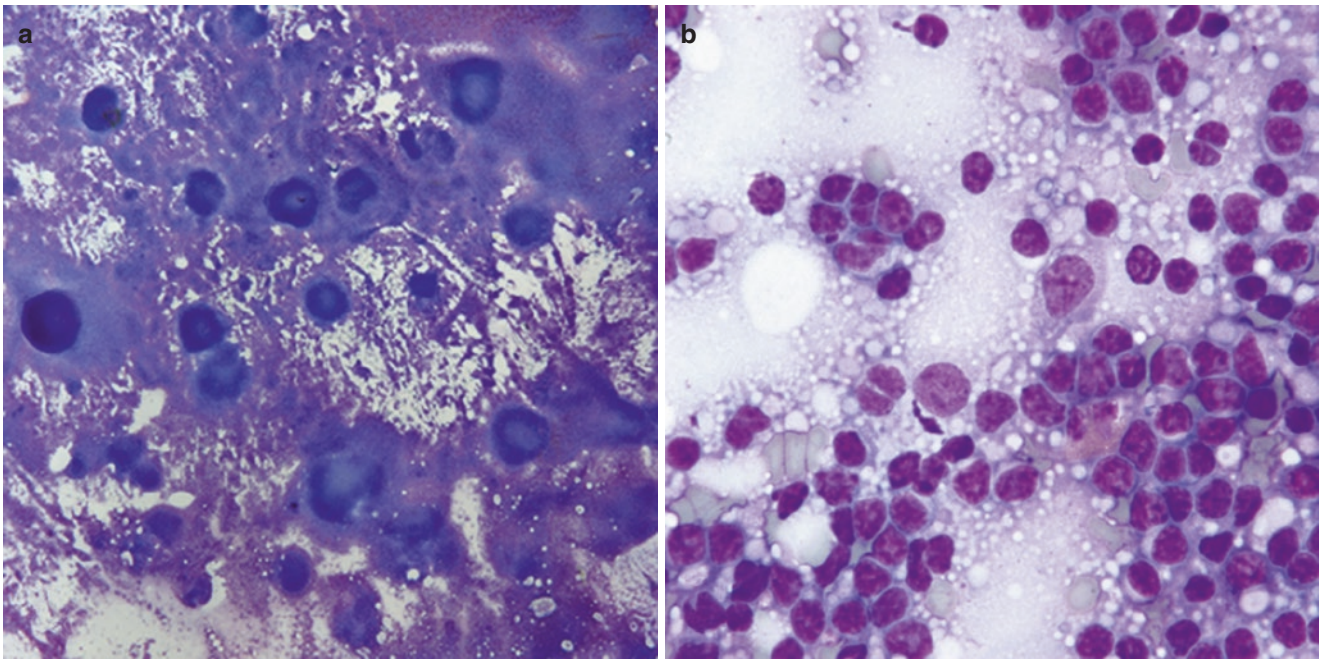

Fig. 15.5 Follicular pattern in follicular lymphoma. A 39-year-old female with a mesenteric lymph node excision. The touch prep with Diff-Quik staining shows an abundance of follicles at a low magnification (**a**, 20×) and a predominance of centrocytes and few centroblasts at a high magnification (**b**, 1000×)

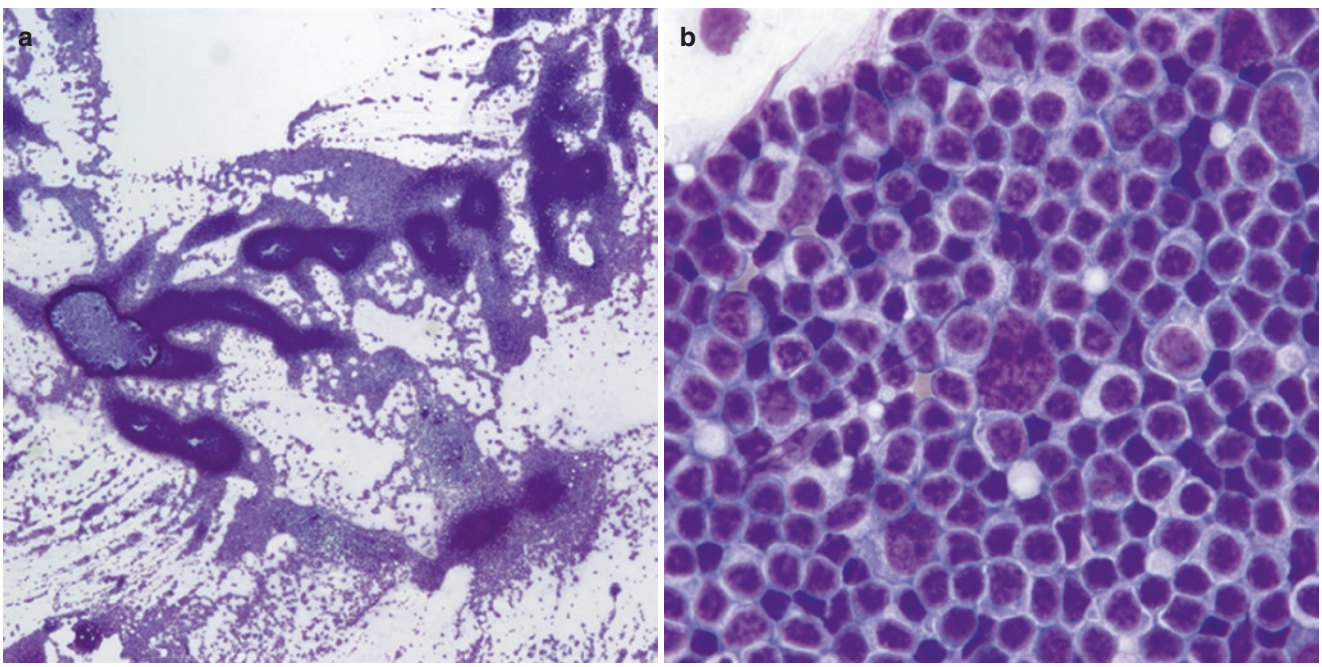

Fig. 15.6 Follicular pattern in nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) . The touch prep with Diff Quik stain from a neck lymph node excision shows an abundance of follicles with focal fusion at 40× (**a**) and presence of rare popcorn or lymphocytic histiocytic (L&H) or lymphocyte predominant (LP) cells in a background of small mature lymphocytes at 1000× (**b**)

Table 15.4 Cytomorphological comparison between follicular hyperplasia and follicular lymphoma

	Follicular hyperplasia	Follicular lymphoma
Number of follicles	Less	More
Cell types	Centrocytes and centroblasts	Predominantly centrocytes
Centrocytes with marked nuclear irregularity and clefts	Not present	May be present
Tingible body macrophages	More	Less

corroborate the cytomorphological impression (see Table 15.4).

References: [8, 28].

19. How do you grade follicular lymphoma (FL)?

FL grading is based on centroblast count on histological sections. An average of ≤15 centroblasts per high power field (hpf) in 10 hpf is considered low-grade (grade 1 and 2). This definition is based on tissue sections and it would be unusual for a cell block to consist of follicles having a sum area of 10 hpf or more. It is feasible to favor a low-grade FL based on cytomorphological evaluation and/or Ki67 immunohistochemistry. A statement of favoring low versus high grade may be sufficient for the oncologists to manage patients with the constellation of clinical parameters.

References: [28–30].

20. Are there problematic types of follicular lymphoma (FL)?

High-grade FL and pediatric-type FL are diagnostically problematic. The neoplastic follicles in these subtypes of FL can be Bcl-2 negative, and can be easily mistaken for follicular hyperplasia. In addition, high-grade FL can be CD10 negative. Finally, both subtypes of FL may lack a *IGH-BCL2* gene rearrangement or t(14;18). Because of these pitfalls, diagnosing these subtypes may ultimately require excisional biopsy.

References: [28, 31, 32].

21. How do you differentiate the lymphocytes in chronic lymphocytic leukemia (CLL) / small lymphocytic lymphoma (SLL) from benign lymphocytes?

Both CLL/SLL cells and benign small lymphocytes have granular or hyperchromatic chromatin. However, the chromatin in CLL/SLL is abnormally clumped or hyperclumped to the degree that there are zones of hyperchromatic chromatin separated by a thin intervening pale zone, imparting a "clotted," "soccer ball," "cracked plate," or "baked sugar cookie" appearance (Fig. 15.7a, b). A PAX5/CD5 double immunohistochemistry stain on the cell block can be particularly helpful by showing coexpression of CD5 in PAX-5-positive B-cells (Fig. 15.7c). The CD5+, CD23+, CD10− immunophenotype by flow cytometry is characteristic of CLL/SLL (Fig. 15.7d). CLL/SLL cells typically show dim or weak CD5, CD45, and CD20, which can be demonstrated on flow cytometry as well.

References: [1, 33, 34].

22. Which lymphomas are often associated with a polymorphous appearance?

The polymorphous appearance of a lymphoid population is often used as a supportive feature for a reactive lymph node. However, some lymphomas can also show a polymorphous appearance. They include marginal zone lymphoma (MZL), angioimmunoblastic T-cell lymphoma (AITL), Hodgkin lymphoma, and peripheral T-cell lymphoma, NOS. MZL, one of the "small" B-cell lymphomas, is composed of any combination of CLL/SLL-like, centrocyte-like, monocytoid, and plasmacytoid B-cells with some cases showing a scattering of large forms, often mimicking a reactive lymph node. The neoplastic cells in AITL are T-follicular helper cells, which, like HRS cells in cHL, are a minor component in AITL, with an inflammatory background composed of polyclonal B-cells, polyclonal plasma cells, eosinophils, EBV-transformed B-cells, HRS-like cells, and follicular dendritic cells. Sometimes oligoclonal or even monoclonal B-cells can be seen.

References: [17, 21, 22].

23. Can large cell transformation from a "small" B-cell lymphoma be reliably detected by FNA?

Diagnosis of large cell transformation usually requires tissue architecture. Any of the "small" B-cell lymphomas can have a variable amount of intermixed singly scattered large cells (including residual reactive centroblasts). Once large cells form clusters or sheets, then a large cell transformation can be considered. Any focus of large cell transformation can be disrupted, aspirated, and rearranged from FNA and is difficult to recognize by cytomorphology alone. For instance, an increased number of singly scattered large B-cells in a

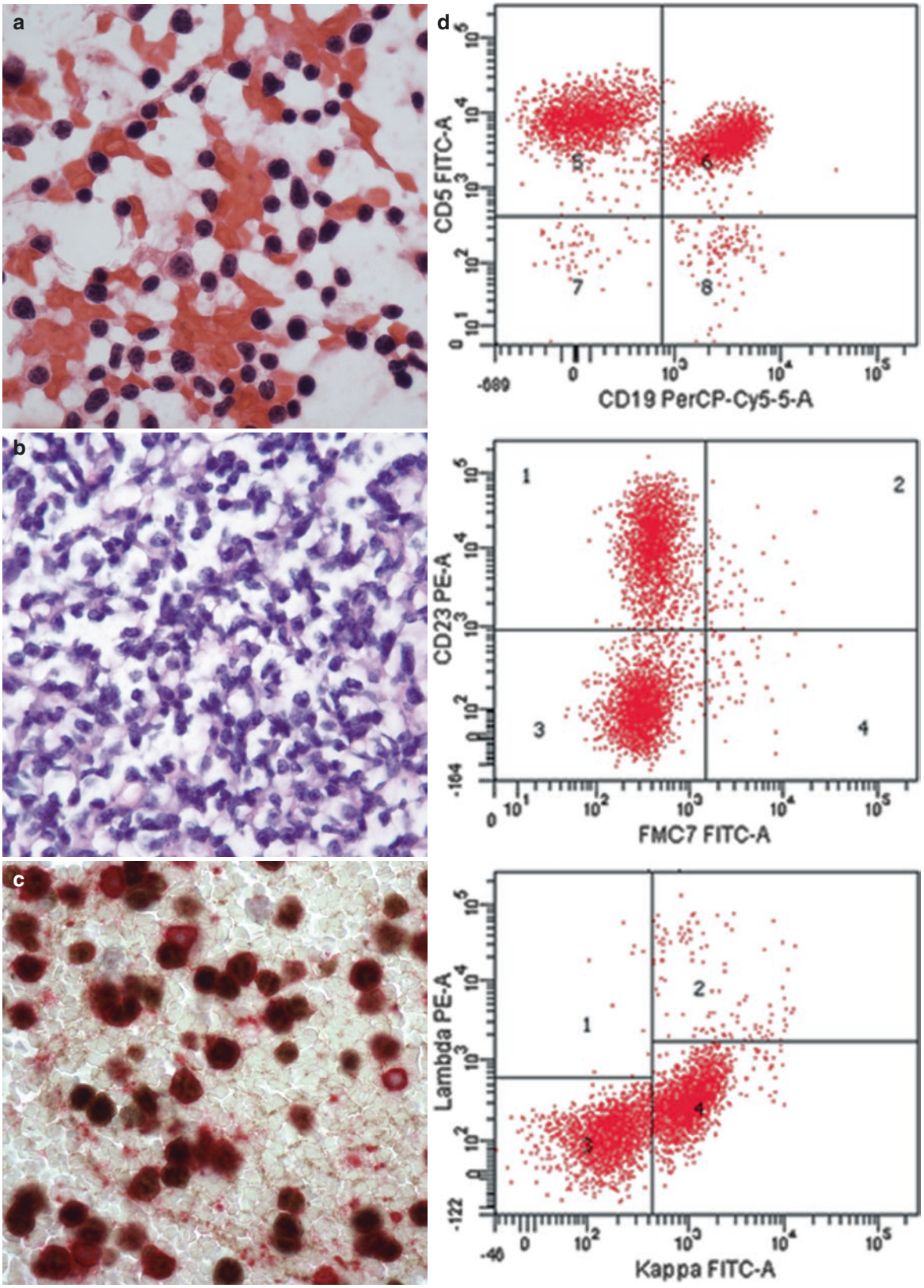

Fig. 15.7 Small lymphocytic lymphoma. (**a**) The monotonous population of small lymphocytes have scant cytoplasm and mostly round nuclei with dense chromatin, some with a "soccer ball" appearance (smear, H&E stain). A few large para immunoblasts and prolymphocytes are also present in both smears and cell block section (**b**, H&E stain). (**c**) A PAX5/CD5 double immunostain highlights numerous nuclear PAX5-positive B-cell (brown stain) with CD5 coexpression (weak pink stain), consistent with SLL/CLL cells. Reactive B-cells (brown PAX5 positive only) and T-cells (strong pink CD5 positive only) are also present (cell block). (**d**) Flow cytometric analysis after gating on lymphocytes shows the B-cell population aberrantly expressing CD5 (left panel), and are CD23 positive and FMC7 negative (middle panel), and with dim kappa restriction (right panel)

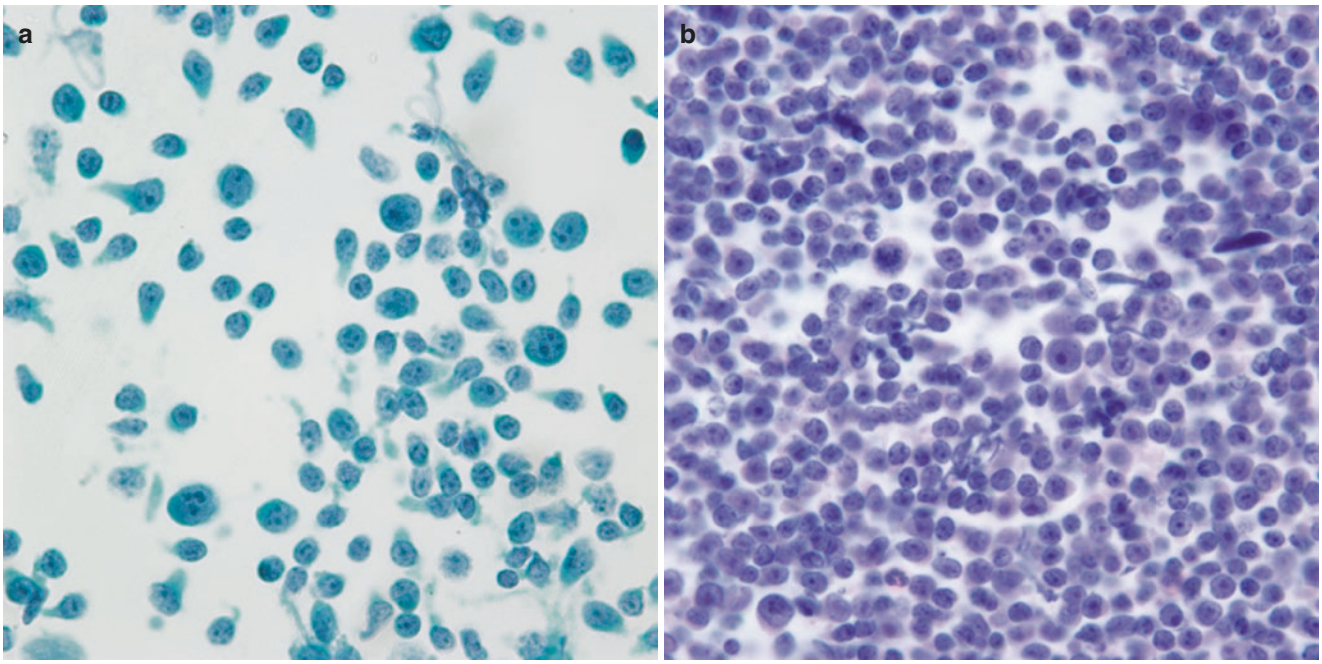

Fig. 15.8 Small lymphocytic lymphoma with an increased number of large cells could present either sampling of the proliferation center with many prolymphocytes or paraimmunoblasts (**a**, SurePath, Papanicolaou stain; **b**, cell block, H&E stain) or large cell transformation (Richter transformation). The distinction usually requires excisional biopsy to identify the sheets or clusters of transformed large cells

background of CLL/SLL could represent either aspiration of elements in the proliferation centers (Fig. 15.8) or large cell transformation. In such a setting, a comment on the possibility of large cell transformation could be mentioned. All "low-grade" lymphomas have the capacity to transform into a large cell lymphoma. A diffuse large B-cell lymphoma (DLBCL) transformation from CLL/SLL is known as a Richter transformation.

References: [27, 35].

24. What term may be used for high-grade B-cell lymphomas that are difficult to further subclassify in FNA?

"Aggressive B-cell lymphoma" is a broad category of clinically aggressive B-cell lymphomas including diffuse large B-cell lymphoma (DLBCL), pathology-specific subtypes of DLBCL such as T-cell/histiocyte-rich large B-cell lymphoma, location-specific subtypes (often with specific pathological features) like primary mediastinal (thymic) large B-cell lymphoma, virus-associated subtypes such as EBV-positive DLBCL NOS, and cytogenetic-specific subtypes such as high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements, as well as Burkitt lymphoma. It is not always possible to characterize a large cell lymphoma in a limited sample, even though cytomorphology can be defined (large) and lineage can be determined (B-cell); in such cases, an interim diagnosis of "aggressive B-cell lymphoma" is most appropriate.

References: [7, 24, 36].

25. What markers are used in the subclassification or prognostication for diffuse large B-cell lymphoma (DLBCL)?

DLBCL can be further subtyped based on cell of origin: GCB (germinal center B-cell like) and non-GCB, using markers such as CD10, BCL6, and MUM1. CD10 positivity is associated with a GCB origin. Isolated BCL6 positivity with MUM1 negativity is also associated with GCB origin. MUM1 positivity with or without BCL6 positivity is associated with a non-GCB origin. GCB DLBCL are associated with a better prognosis compared to non-GCB. DLBCL can also be further prognosticated based on MYC and BCL2 expression. An MYC and BCL2 double expressor DLBCL is associated with a worse prognosis compared to nondouble expressors. However, the double expressors have a better prognosis compared to the high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements ("double hit" lymphomas).

References: [37–41].

26. What is the differential diagnosis of Epstein–Barr Virus (EBV)-positive B-cell lymphomas and what additional testing could be useful in subclassification?

- Burkitt lymphoma: Intermediate cell size; cytoplasmic vacuolization seen on air-dried smears or touch prep; typical phenotype being positive for CD10, Bcl-6, CD43, and negative for Bcl-2; nearly 100% Ki-67 proliferation rate; and MYC rearrangement in an otherwise simple karyotype.
- EBV-positive diffuse large B-cell lymphoma NOS: Large cell size and exclusion of other more specific features seen in the other EBV-positive B-cell lymphomas.
- EBV-positive Hodgkin lymphoma: HRS cells are positive for CD30, CD15, MUM1, weakly positive for PAX5, and negative for CD45, CD20, CD3, EMA, and ALK.
- Post-transplant lymphoproliferative disorders (PTLD), polymorphic, monomorphic, or cHL: Clinical history.
- Plasmablastic lymphoma: CD138 positive.
- Primary effusion lymphoma (PEL) or extracavitary PEL: Human herpes virus 8 (HHV8) or latent nuclear antigen 1 (LANA1) positive.
- Lymphomatoid granulomatosis: Extranodal and T-cell rich.

References: [10, 24, 42–44].

27. Are Hodgkin-Reed-Sternberg (HRS) cells always specific to classic Hodgkin lymphoma (cHL)?

No, HRS or rather HRS-like cells are not entirely specific to cHL. The mononucleated Hodgkin cells show morphological overlap with immunoblasts, which can be abundant in reactive lymph nodes. The Reed-Sternberg cells are binucleated or multinucleated and can be seen in a variety of other lymphomas like anaplastic large cell lymphoma, angioimmunoblastic T-cell lymphoma, peripheral T-cell lymphoma NOS, "aggressive B-cell lymphomas", and even in reactive lymph nodes like those associated with infectious mononucleosis.

References: [45–48].

28. What serological test is always good to have clinicians order for any diagnosed T-cell lymphoma?

Human T-lymphotropic virus-1/2 (HTLV-1/2) serology is always good to order in any case of a T-cell lymphoma. Adult T-cell leukemia/lymphoma (ATLL) is typically composed of small to medium lymphoma cells that have the characteristic multiple invaginated or "flower cell" shape, which is best seen on peripheral blood smears. However, ATLL can sometimes show large cell morphology or even diffuse CD30 positivity. In these instances, HTLV-1/2 positive serology would be consistent with the diagnosis of ATLL.[107]

References: [24, 49].

29. What diagnosis should be considered when you encounter a poorly differentiated large cell malignancy in a lymph node FNA that is negative for CD45 (leukocyte common antigen or LCA), B-cell markers, T-cell markers, keratins, usual sarcoma markers, and melanoma markers?

Anaplastic large cell lymphoma (ALCL), both ALK-positive and ALK-negative, can fit this profile. ALCL is a T-cell lymphoma that can sometimes assume a "null cell" phenotype which means they can be negative for many of the pan T-cell markers such as CD3, CD5, CD2, or CD7 and are even negative for pan-lymphoid antigens such as CD45. In such a setting, additional markers such as CD4, CD30, ALK, epithelial membrane antigen (EMA), and cytotoxic markers (granzyme B, perforin, TIA1) should be employed.

References: [50, 51].

30. What class of hematolymphoid neoplasms is important to differentiate from mature B-cell and T-cell lymphomas, why is it uncommonly encountered, and why is it tricky?

Precursor lymphoid neoplasms or lymphoblastic lymphomas, both B-cell and T-cell types, must be differentiated from mature B-cell and T-cell lymphomas, respectively. It is also important to separate them from other high-grade lymphomas like "aggressive B-cell lymphoma" or T-cell lymphoma NOS because they are treated differently. Lymphoblastic lymphomas are uncommonly encountered in cytology because most of these neoplasms have a leukemic manifestation. Both B- and T-lymphoblastic leukemia/lymphoma can be differentiated from their mature lymphoma counterparts based on their immature marker expression profile, such as positivity for TdT, CD34, CD10 and dim expression for CD45. For B-lymphoblastic leukemia/lymphoma, they typically do not show either surface or cytoplasmic light chain expression. For T-lymphoblastic leukemia/lymphoma, they may show expression of immaturity marker like CD1a and CD99. Lymphoblastic lymphomas are additionally tricky because they range from small to intermediate in size and are often cytomorphologically more similar with the "small cell" lymphomas. Blastic morphology is best appreciated on air-dried, Wright-Giemsa-stained preparations. Finally, it is important to remember that CD10 positivity is not restricted to B-cell lymphomas of germinal center deri-

vation, such as follicular lymphoma, Burkitt lymphoma, and some diffuse large B-cell lymphoma. CD10 expression is also seen in B-lymphoblastic lymphoma, recapitulating the immunoprofile in normal precursor B-cells (hematogones).

References: [52–55].

31. How do you differentiate lymphoma from small cell carcinoma or other high-grade neuroendocrine carcinoma on cytomorphology?

It is common for small cell carcinoma of the lung to involve the hilar and mediastinal lymph nodes. Differentiating lymphoma from small cell carcinoma or high-grade neuroendocrine carcinoma on cytomorphology is possible but challenging (see Table 15.5). Small cell carcinoma tends to be poorly cohesive but still have a slightly greater degree of cohesion compared to lymphomas. Crush artifact can be seen in both small cell carcinoma and lymphomas; however, for the former, the crush tends to be large pulled-out tangles of chromatin material, whereas in the latter, the crush tends to be composed of individually blown up nuclei. Small cell carcinoma, though "small," tends to be larger than most lymphomas including large cell lymphomas. Small cell carcinoma tends to have rounded or smooth nuclear contours whereas lymphomas have some degree of slight notching or irregularity in nuclear contours. Finally, small cell carcinoma has the typical "salt and pepper" chromatin and frequent necrosis. Lymphomas/lymphoid tissue typically produce a background of lymphoglandular bodies.

References: [56–59].

32. What is the limitation of PAX5 as a marker in the evaluation of lymphadenopathy of unknown etiology?

PAX5 is a highly sensitive and specific marker for B-cells or lymphomas of B-cell lineage. T-cell lymphomas never express PAX5. In addition, PAX5 shows nuclear staining, which can be helpful in determining cell size. PAX5 is preferred over CD20 because the latter tends to be overstained (activated T-cells can be CD20 positive). However, some nonlymphoid neoplasms like small cell carcinoma, undifferentiated uterine carcinoma can be PAX5 positive. When encountering a small blue cell tumor that is only positive for PAX5 and negative for lymphoma cell of origin markers like CD10, BCL6, and MUM1, one should confirm the veracity of the PAX5 for B-cell lineage using another B-cell marker such as CD20 or CD79a.

Reference: [60].

Table 15.5 Cytomorphological differences between lymphoma and small cell carcinoma

	Lymphoma	Small cell carcinoma
Background	Lymphoglandular bodies	Necrosis
Cohesion	None	Mild
Crush artifact	Variable	Marked
Cell size	Smaller	Larger
Nuclear contours	Irregular even when smooth	Very smooth
Chromatin	Variable	"Salt and pepper"

33. What other malignancies can mimic lymphoma especially in head and neck lymph nodes?

Like lymphoma, metastatic nasopharyngeal carcinoma often presents as enlarged cervical lymph nodes. The smears show loose clusters of undifferentiated large cells, with large nuclei and pale chromatin, and moderate amount of cytoplasm. Large number of small lymphocytes and lymphoglandular bodies can be seen in the background. Positivity with keratins, coupled with negativity with pan-lymphoid marker CD45, is helpful in favoring metastatic carcinoma. Positivity for Epstein–Barr virus-encoded RNA (EBER) by in situ hybridization confirms nasopharyngeal carcinoma.

HPV-associated squamous cell carcinomas of the oropharynx or hypopharynx frequently present as cervical lymphadenopathy with unknown primary, clinically resembling lymphoma. FNA smears show clusters of basaloid cells with indistinct cell borders, a round to oval nucleus, and scant cytoplasm. Per the guidelines from the Collage of American Pathologists, all metastatic squamous carcinomas in cervical lymph nodes with or without known history of an oropharyngeal primary should be tested for HPV, which can be achieved by either RNA in situ hybridization or HPV PCR. If HPV testing is not available, P16 IHC can be used as a screen/surrogate marker for HPV-associated squamous cell carcinomas.

References: [61–64].

34. If a karyotype or chromosome analysis is desired, what kind of cytology specimen would be best for this ancillary testing?

A lymphomatous effusion could be a reliable source for a karyotype analysis because it is usually sufficient to generate 20 evaluable metaphases for a full karyotype, which is rarely achieved from an FNA sample. Lymphomatous effusions can be of large volume, often contain a lot of lymphoma cells, and are rich in nutrients to sustain the viability of the cells. A karyotype analysis is important for distinguishing Burkitt lymphoma (*MYC* rearrangement in a clean karyotype background), the "double hit" or "triple hit" lymphomas, and those lymphomas with variant translocations that are not detectable by FISH.

References: [2, 65].

35. When you encounter a spindle cell neoplasm on lymph node FNA and have excluded metastatic spindle cell carcinoma, melanoma, and sarcoma from elsewhere, what additional markers should you employ?

Dendritic cell sarcomas should be considered when metastatic diseases are excluded. Unlike most sarcomas, dendritic cell sarcomas commonly arise in lymph nodes. There are two main types: follicular dendritic cell sarcoma and interdigitating dendritic cell sarcoma. The former would stain for FDC markers such as CD21, CD23, and CD35. The latter would stain for S100 but would be negative for Langerhans cell markers such as CD1a and langerin.

Reference: [66].

Case Presentation

Case 1

Learning Objectives:

1. To be familiar with the distinct cytomorphology of this entity
2. To generate the differential diagnosis of this entity

Case History:

A 41-year-old female with fever and right neck lymphadenopathy with tenderness.

Specimen Source and Preparations:

FNA of the neck lymph node was performed. A SurePath slide and a cell block were made from the aspiration.

Cytological Findings:

- A polymorphous population of lymphocytes with an increased number of immunoblasts and plasmacytoid monocytes (Fig. 15.9a)
- Areas of necrosis and increased karyorrhexis (Fig. 15.9b)
- Many histiocytes with phagocytic debris and crescent-shaped nuclei (Fig. 15.9c)

Differential Diagnosis:

- Reactive follicular hyperplasia
- Necrotizing granulomatous lymphadenitis (infectious etiology)
- Lupus lymphadenitis

Immunohistochemistry (Fig. 15.9d–f)

A predominance of CD3-positive T-cells in the hyperproliferative and hyperapoptotic foci (not germinal centers), with few PAX5-positive B-cells and many CD8-positive cytotoxic T-cells.

Final Diagnosis:

Histiocytic necrotizing lymphadenitis, consistent with Kikuchi-Fujimoto lymphadenitis

Follow-Up:

One month after FNA, the patient had a lymph node excision which showed more confluent zones of necrosis. The diagnosis of histiocytic necrotizing lymphadenitis consistent with Kikuchi-Fujimoto lymphadenitis was confirmed.

Take-Home Messages:

1. Histiocytic necrotizing lymphadenitis is a morphological pattern due to two main etiologies: Kikuchi-Fujimoto lymphadenitis and lymphadenopathy associated with systemic lupus erythematosus (SLE).
2. The cytomorphology of Kikuchi-Fujimoto lymphadenitis is distinct and permits definitive diagnosis by FNA in the proper clinical setting.
3. Polymorphous lymphocytes with areas of necrosis, karyorrhexis, and characteristic histiocytic with phagocytic debris and crescent nuclei are typical FNA cytomorphological findings.

References: [67–69].

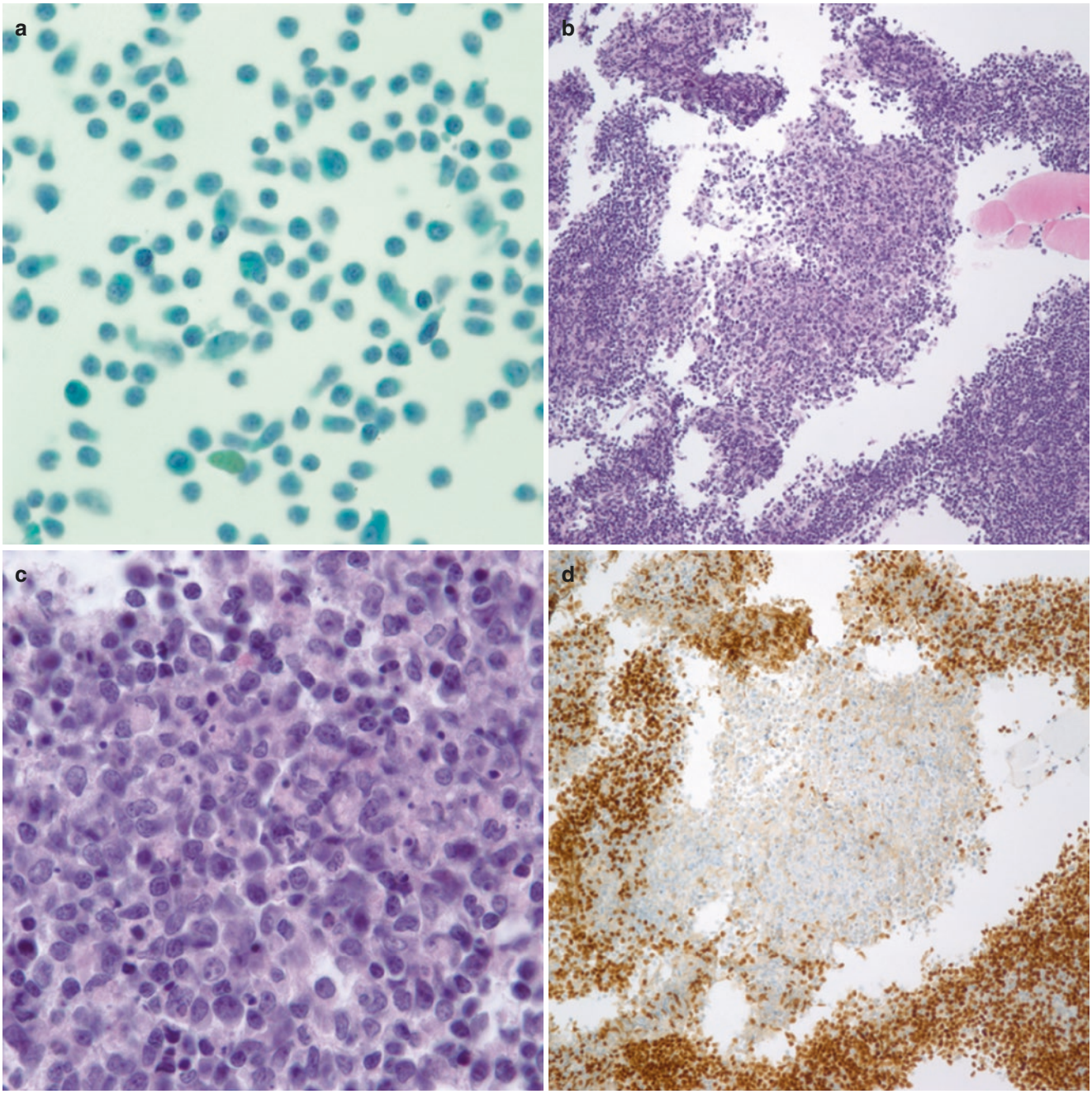

Fig. 15.9 Case 1. Neck lymph node, FNA. SurePath, Papanicolaou stain, 1000× (**a**); Cell block preparation, H&E stain, 200× (**b**) and 1000× (**c**); immunohistochemistry on cell block: PAX5 (**d**), CD3 (**e**), and CD8 (**f**)

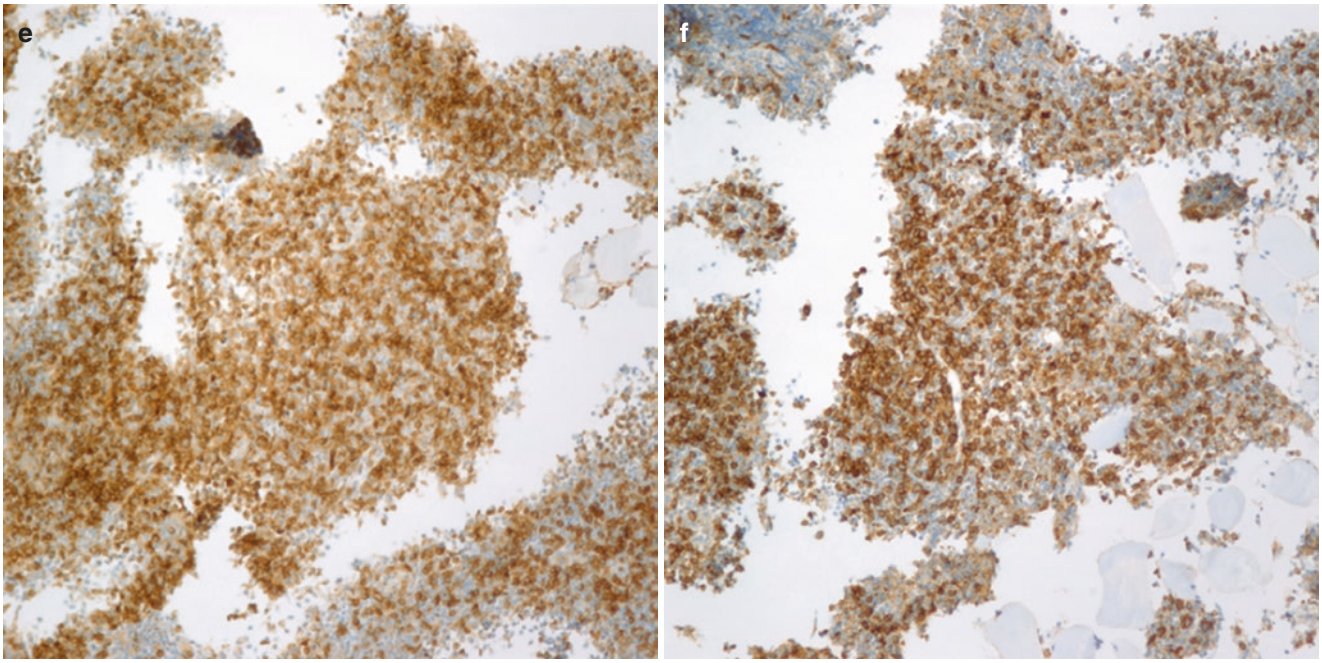

Fig. 15.9 (continued)

Case 2
Learning Objectives:

1. To be familiar with the cytomorphology of this entity
2. To learn how to work up of this entity with ancillary studies
3. To learn how to grade this entity with limited sample material

Case History:
A 29-year-old female with neck lymphadenopathy.
Specimen Source and Preparations:
Fine-needle aspiration of the neck lymph node was performed. A SurePath slide and a cell block were made from the aspiration.
Cytological Findings:

- Cellular sample with follicular structure (Fig. 15.10a)
- Lymphoid aggregates/follicles with no or a few tangible body macrophages Fig. 15.10a inset)
- Predominantly small centrocytes with clefted or cleaved nuclei and a few centroblasts (Fig. 15.10b)

Differential Diagnosis:

- Reactive follicular hyperplasia
- Marginal zone lymphoma with follicular colonization
- Diffuse large cell lymphoma
- Follicular lymphoma

Ancillary Studies:

- Immunohistochemistry (Figs. 15.10c–f): Vaguely nodular lymphoid fragments on cell block is composed predominantly PAX5-positive B-cells with coexpression of Bcl-2 and CD10. An immunostain for CD21 highlights the background follicular dendritic cell meshworks. A Ki67 proliferation index is low (25%).
- Flow cytometry (Fig. 15.10g): Flow cytometric analysis after gating on lymphocytes shows a predominant CD10 positive B-cell population and with lambda light chain restriction.
- Polymerase chain reaction (PCR) for *BCL2-IGH* rearrangement: Detected.

Final Diagnosis:
Follicular lymphoma, low grade (WHO grade 1–2).
Take-Home Messages:

1. FL, a common B-cell lymphoma in adults, is characterized genetically by a t(14:18)(q32;q21) translocation with *bcl2-IGH* rearrangement (up to 95% cases), and immunophenotypically by a germinal center derivation (CD10+, Bcl6+, CD5−), which can be detected on FNA samples by FISH/PCR and flow cytometry/immunohistochemistry (IHC), respectively.

2. Bcl2 immunostain is helpful in distinguishing FL from follicular hyperplasia only when the spatial relationship of a follicle can be visualized by either CD21 staining and or a Bcl6/Bcl2 two-color double stain.
3. Although the grading method for FL sampled by FNA has not been standardized, visual estimation of the percentage of small and large lymphocytes and/or Ki67 proliferation index can be potentially used for separating low-grade (WHO grades 1 and 2) from high-grade (WHO grade 3) tumors.

References: [25, 28–30].

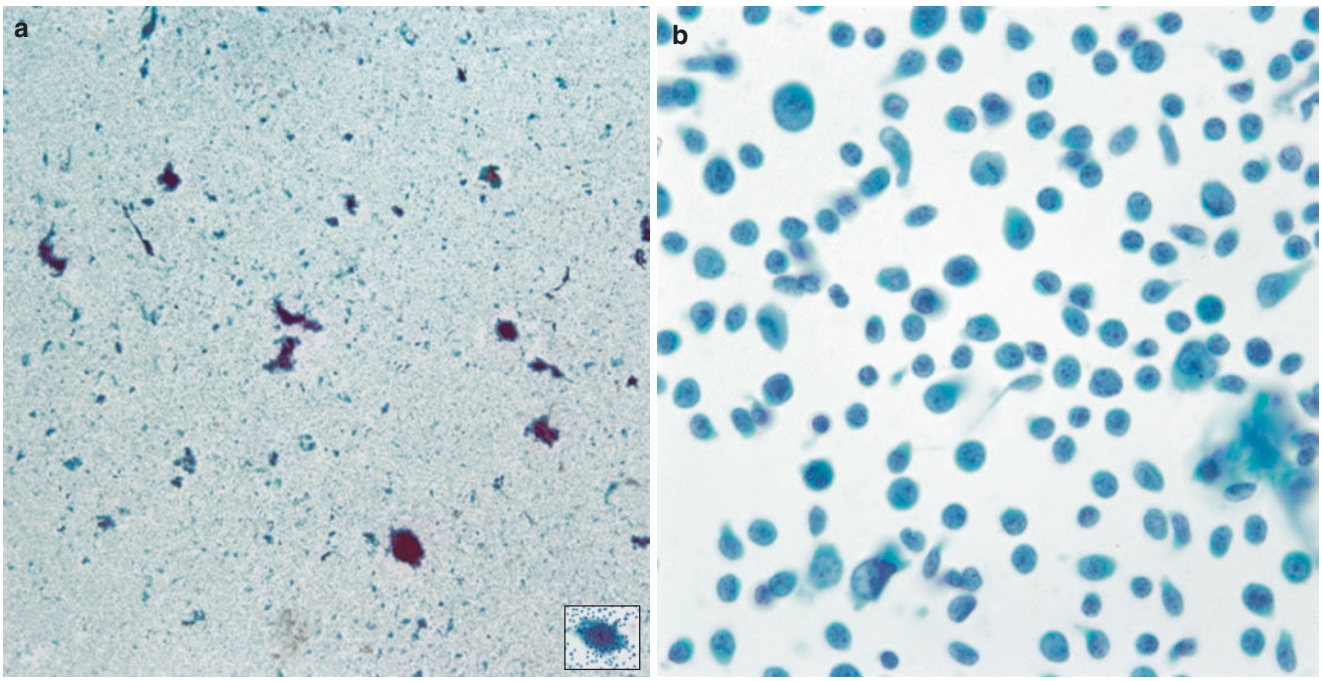

Fig. 15.10 Case 2. Neck lymph node, FNA. SurePath, Papanicolaou stain, 40× (**a**) and 1000× (**b**); immunohistochemistry on cell block: PAX5 (**c**), CD3 (**d**), CD21 (**e**), and Ki67 (**f**); Flow cytometric analysis (**g**)

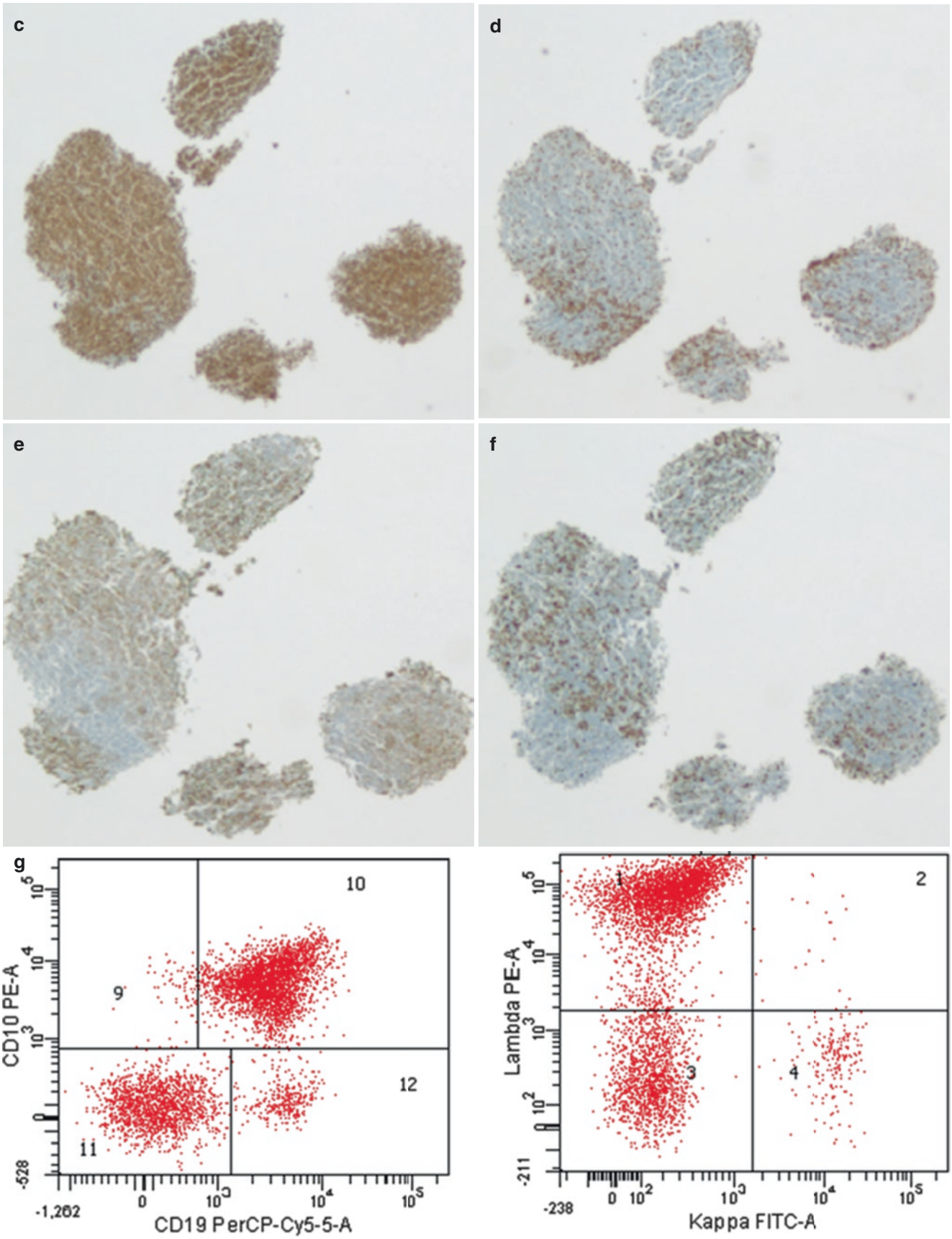

Fig. 15.10 (continued)

Case 3

Learning Objectives:

1. To be familiar with the cytomorphology of this entity
2. To learn how to work up of this entity with ancillary studies
3. To evaluate the prognostic factors of this entity

Case History:

A 63-year-old female with history of mesenteric and retroperitoneal lymphadenopathy.

Specimen Source and Preparations:

FNA of the mesenteric lymph node was performed. A SurePath slide and a cell block were made from the aspiration. Three days after the FNA, the pleural effusion was sent for cytology, and it was decided to send the remaining fluid for cytogenetic karyotyping.

Cytological Findings (Fig. 15.11a–b):

- Discohesive large atypical cells of varying sizes (2.5–5 times that of a small lymphocyte)
- Pleomorphic nuclei with irregular nuclear contours, vesicular chromatin, and prominent nucleoli

Differential Diagnosis:

- FL, WHO grade 3
- Double/triple hit lymphoma
- Other types of large B- or T-cell lymphomas
- Myeloid sarcoma
- Nonhematopoietic tumors like round cell sarcomas, poorly differentiated carcinoma

Ancillary Studies (Fig. 15.11c–f):

- Immunohistochemistry: Large cells in lymphoid tissue fragments are composed of predominantly PAX5-positive B-cells and few CD3-positive T-cells. An immunostain for CD21 shows the absence of follicular dendritic cell meshworks. A Ki67 proliferation index is high (85%). These large B-cells are also positive for Bcl 6, Bcl2, MUM1 and MYC and negative for CD10 (not shown).
- Flow cytometry: An abnormal B-cell population with aberrant CD5 expression and lambda light chain restriction (not shown).
- Karyotype (pleural fluid): Complex and including a t (3;22) (q27;q11.2) as well as add(3)(q27). No *MYC* rearrangement identified.

Final Diagnosis:

Diffuse large B-cell lymphoma, activated B-cell (ABC) type.

Take-Home Messages:

1. DLBCL, an aggressive but potentially curable B-cell lymphoma, can be subdivided into morphological variants (no clinical significance) and molecular subtypes (GCB type versus ABC type), which may predict survival after chemotherapy.
2. It is possible to diagnose and subtype DLBCL in cytology specimens with flow cytometry, IHC, and cytogenetic studies.
3. Lymphomatous body fluids are an excellent source for karyotyping.

References: [36, 38, 39, 70].

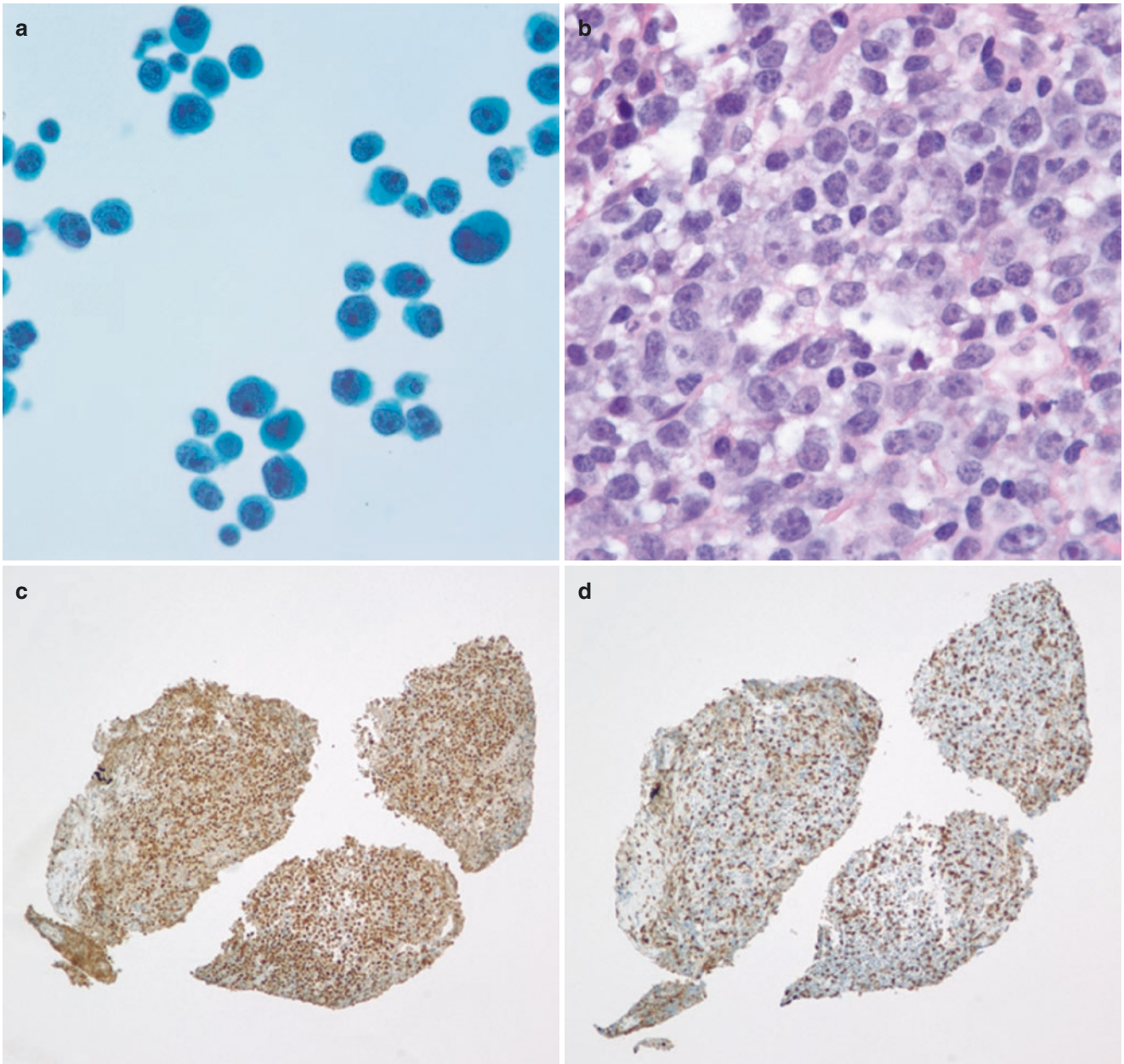

Fig. 15.11 Case 3. Mesenteric lymph node, FNA. SurePath, Papanicolaou stain, 1000× (**a**); Cell block preparation, H&E stain, 1000× (**b**); immunohistochemistry on cell block: PAX5 (**c**), CD3 (**d**), CD21(**e**), and Ki67(**f**)

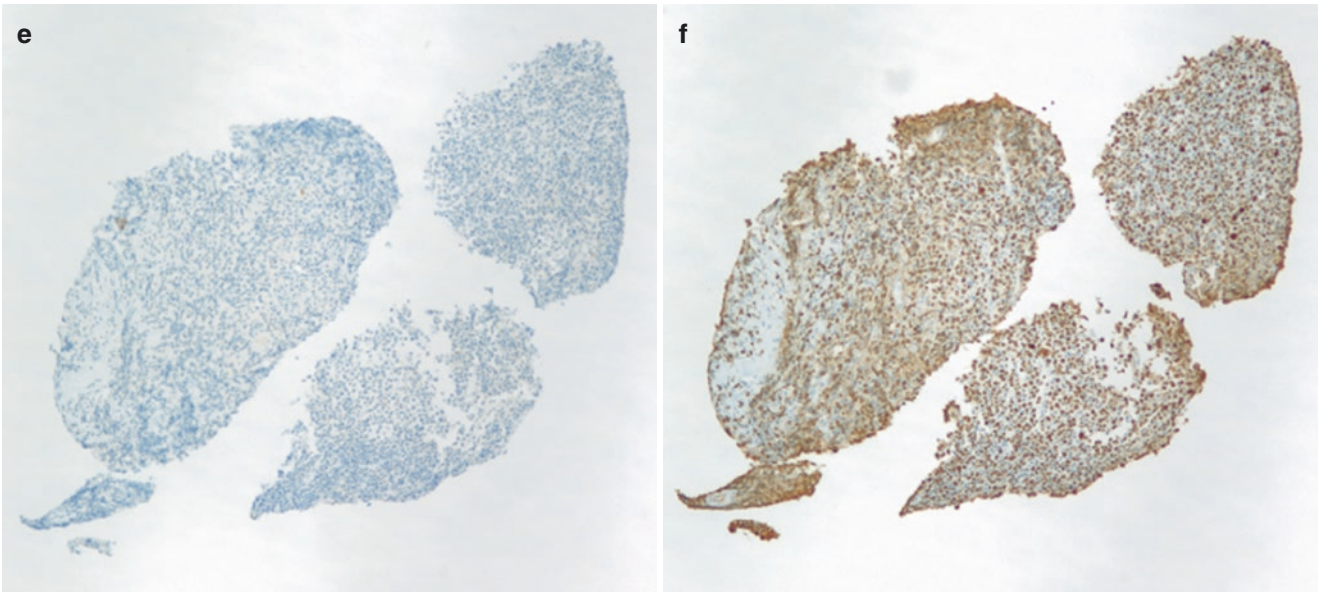

Fig. 15.11 (continued)

Case 4

Learning Objectives:

1. To be familiar with the cytomorphology of this entity
2. To be aware of the diagnostic pitfalls of this entity

Case History:

A 73-year-old male with an enlarged left neck lymph node.

Specimen Source and Preparations:

Fine-needle aspiration of the neck lymph node was performed. A SurePath slide and a cell block were made from the aspiration.

Cytological Findings (Fig. 15.12a–c):

- Rare binucleated large cells with prominent nucleoli
- Small lymphocytes and histiocytes in the background
- Granulomatous inflammation with necrosis

Differential Diagnosis:

- Reactive lymphoid hyperplasia
- Infectious mononucleosis
- Acute or granulomatous lymphadenitis
- T-cell-rich large B-cell lymphoma
- Hodgkin lymphoma

Ancillary Studies:

Immunohistochemisty: Large atypical cells are positive for CD30 (Fig. 15.12d), CD15, and PAX5 (dim), and negative for CD45, CD20, and CD3. In situ hybridization for Epstein–Barr virus-encoded RNA (EBER) is negative (not shown).

Final Diagnosis:

Classic Hodgkin lymphoma with necrosis and granulomatous inflammation.

Take-Home Messages:

1. The diagnosis of cHL is established by finding the few to occasional HRS cells in an inflammatory background.
2. Confirming the appropriate immunophenotype of Hodgkin-Reed-Sternberg (HRS) cells by IHC is essential to distinguish classic Hodgkin lymphoma (cHL) from morphological mimics.
3. Granulomatous inflammation can be uncommonly associated with cHL and is a diagnostic pitfall.-

References: [14, 45, 46, 48].

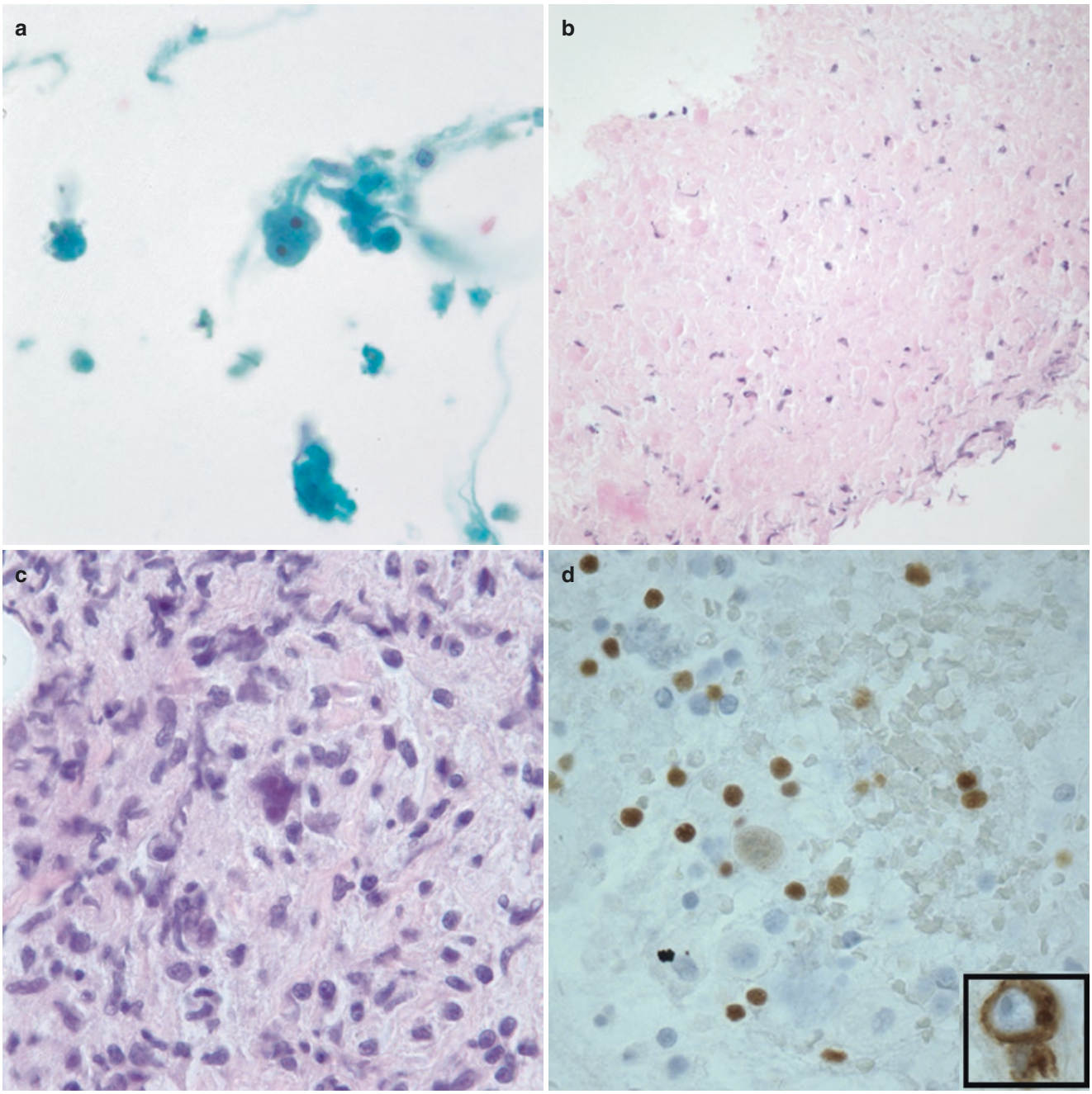

Fig. 15.12 Case 4. Neck lymph node, FNA. SurePath, Papanicolaou stain, 1000× (**a**); Cell block preparation, H&E stain, 400× (**b**) and 1000× (**c**); immunohistochemistry on cell block: PAX5 (**d**) and CD30 (*inset*)

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Liver Cytopathology

16

Jain Zhou, He Wang, and Nirag Jhala

Contents

List of Frequently Asked Questions:

1. How should we approach evaluation of liver FNA?

Fine needle aspiration (FNA) of the liver is used to obtain diagnostic material from focal liver lesions. It is a targeted approach to liver masses or mass-like lesions. It is not useful in the diagnosis of diffuse liver disorders, such as liver cirrhosis or hepatitis, which are preferably evaluated by a core biopsy.

In approaching liver FNA, the following scenarios are generally considered:

1. The aspirate contains entirely normal hepatic components, predominately normal hepatocytes, and the aspirate is representative of the targeted lesion. The differential diagnoses include hepatic adenoma, focal nodular hyperplasia (FNH), regenerative nodular lesions in cirrhosis, or in regenerative hyperplasia. Differentiation of these nodular lesions requires evaluation by core biopsy along with clinical and imaging study.
2. The aspirate contains hypocellular normal hepatic components, predominately benign ductal cells and some hepatocytes, and the aspirate is representative of the targeted lesion. The differential diagnoses include bile duct hamartoma and adenoma.
3. The aspirate contains predominantly cells other than those mentioned in the above scenarios, considering the following differential diagnoses: primary carcinoma (hepatocellular carcinoma, cholangiocarcinoma, hepatoblastoma in pediatric population, rarely angiosarcoma, and epithelioid hemangioendothelioma), metastasis (seen in most of the liver FNAs), and infection (hepatic abscess and hydatid cyst) (Figs. 16.1 and 16.2).

Reference: [1].

2. What are the morphologic features of normal cellular elements of FNA samples from the liver?

The normal cellular components of liver FNA include hepatocytes, bile duct cells, and rarely Kupffer cells and endothelial cells.

Normal hepatocytes are polygonal with granular eosinophilic cytoplasm. The hepatocytes often contain cytoplasmic pigments (lipofuscin, hemosiderin, bile pigment). The hepatocyte nucleus is round to oval with smooth nuclear contour, fine evenly distributed chromatin, and conspicuous nucleolus/nucleoli.

Normal bile duct epithelial cells appear as cohesive flat monolayer sheets. They are columnar to cuboidal cells and

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_16

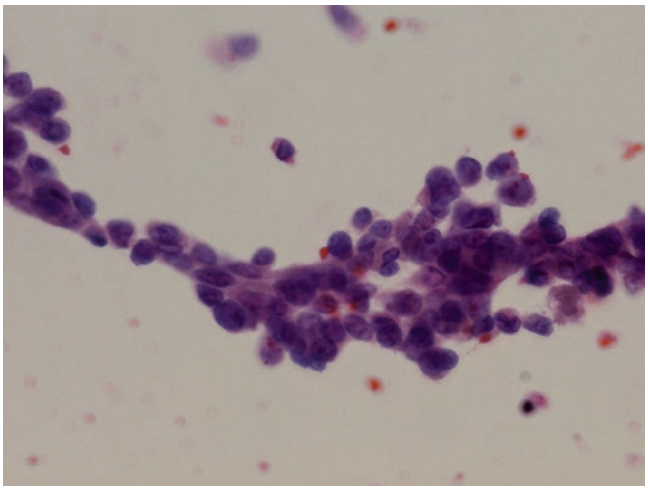

Fig. 16.1 Metastatic adenocarcinoma of the lung, forming small acini. Please note uneven chromatin distribution, prominent nucleoli, nuclear polymorphism

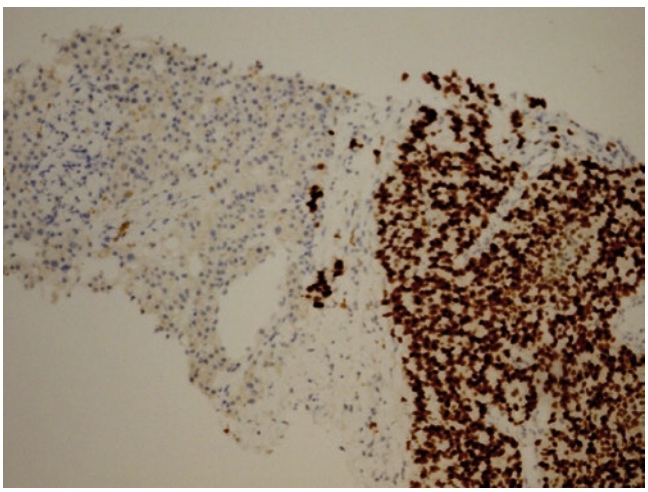

Fig. 16.2 Metastatic adenocarcinoma, positive nuclear stain for TTF-1. Please compare to the staining pattern of the neighboring hepatic tissue

smaller than hepatocytes (have less cytoplasm than hepatocytes) and contain round to oval nuclei with indistinct nucleoli.

Reference: [1].

3. What are possible contaminating cells noted on percutaneous liver FNA samples?

The possible contaminations from structures traversed on the way of the needle to the lesion include skin, skeletal muscle, diaphragm, stomach, bowel, or mesothelium. Such benign structural cells need to be recognized as contaminants and not misinterpreted as lesional cells. In particular, mesothelial cells may be seen in a relatively large number. They can resemble well-differentiated adenocarcinoma. Benign mesothelial cells are usually arranged in flat, cohesive sheets, and characteristic slit-like "windows" can be appreciated in Papanicolaou stain. Immunohistochemical study with mesothelial markers (calretinin, D2–40 and WT-1) can help in difficult cases.

If the sample consists only of one or more of these contaminants, it should be interpreted as insufficient for evaluation (nondiagnostic) rather than negative.

Reference: [2].

4. What are the pigments that could be seen in hepatocytes?

Lipofuscin, bile, and hemosiderin are the pigments that can be seen in liver cells.

Lipofuscin, a yellow-brown pigment, accumulates most prominently in centrilobular hepatocytes and is acid-fast and often diastase-PAS positive. Lipofuscin increases with advancing age, a normal "wear and tear" pigment. This pigment is of no pathologic significance.

Bile pigment is dark yellow-brown and is found in cholestatic liver disease. The presence of bile in a malignant process strongly supports hepatocellular origin.

Hemosiderin is present in hepatocytes, bile duct epithelial cells, and Kupffer cells. While hemosiderin is abundant in newborn livers, only small amount of hemosiderin is normally found in adults. Hemosiderin granules are coarse, golden-brown, and refractile and can be readily seen by the Prussian blue reaction for iron.

References: [3–5].

5. What are morphologic features of reactive hepatocytes?

There is nuclear size variation in the reactive hepatocytes. Macronucleoli may be present. However, the nuclear to cytoplasmic ratio is normal, the nuclear membranes are smooth, the chromatin is fine and granular, and mitotic figures are rare.

References: [1, 6].

6. What are the morphologic features of hepatocellular carcinoma (HCC)?

HCC is the most common primary carcinomas of the liver, and it can present as a solitary nodule, multiple nodules, or diffusely. Differentiation of HCCs ranges from well differentiated (resembling normal liver) to poorly differentiated with marked pleomorphism and tumor giant cells.

Thickened, cohesive broad trabeculae (more than two cell layers thick) of hepatocyte-like cells of increased nuclear to cytoplasmic ratio and granular cytoplasms, surrounded by endothelial cells, are characteristic of well-differentiated HCC. The tumor cells contain large, round nuclei with prominent nucleoli, sometimes with intranuclear pseudoinclusion. Hyaline inclusions may be seen in the tumor cell cytoplasm. Other typical features of well-differentiated HCC include cellular monomorphism, nuclear crowding, macronucleoli, loss of bile duct cells, and capillaries traversing tissue frag-

ments. The endothelium, either wrapping around cell groups or transgressing cell sheets, is highly specific for HCC.

Moderately and poorly differentiated HCCs show more single tumor cells and small cords, nests, or tubules. Tumor cells demonstrate moderate to marked pleomorphism, atypical mitosis, and tumor giant cells. The tumor cells contain hyperchromic large nuclei with irregular nuclear contour and prominent irregular nucleoli. Tumor cell necrosis may also be seen (Figs. 16.3 and 16.4).

References: [7, 8].

7. How can we differentiate reactive hepatocytes and well-differentiated hepatocellular carcinoma in FNA?

Well-differentiated hepatocellular carcinoma can closely resemble reactive conditions such as hepatocellular adenoma (HA), focal nodular hyperplasia (FNH), macroregenerative nodule, and dysplastic nodule. The presence of characteristic endothelial patterns (endothelial cells peripherally wrapping or transgressing groups of cells) is an important feature of well-differentiated HCC. Immunostaining with CD34 highlights these endothelial architectures. The cohesive broad trabeculae of the well-differentiated HCC usually have more than two-cell-layer thickness, which can be better appreciated on reticulin special stain. Immunohistochemistry for glypican-3, an oncofetal protein in cell growth and differentiation, can be helpful as well with 70–90% of HCC showing focal or diffuse cytoplasmic glypican-3 staining, whereas all HA and FNH were glypican-3 negative.

References: [9–13].

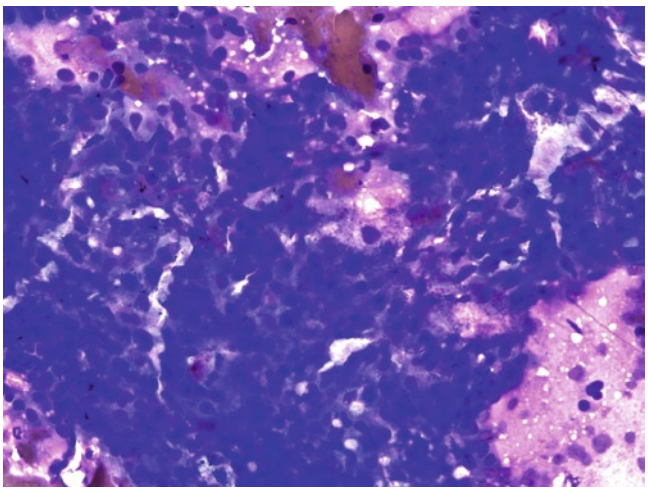

Fig. 16.3 Hepatocellular carcinoma, Diff-Quik stain. Please note the transgressing endothelial cells and nuclear polymorphism

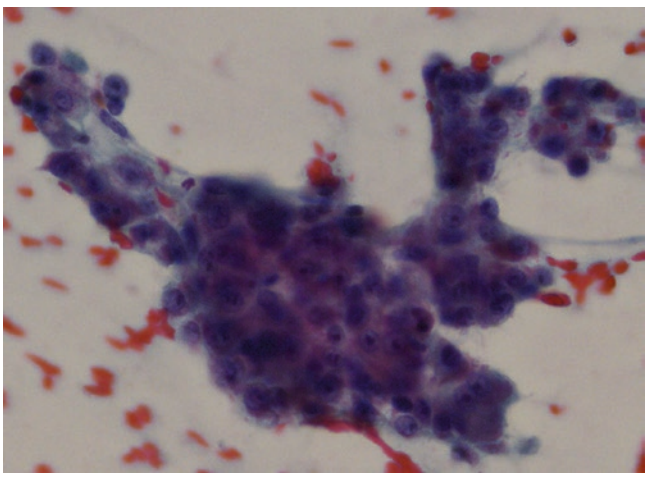

Fig. 16.4 Hepatocellular carcinoma, Pap stain. Please note the surrounding endothelial cells and hyperchromasia, prominent nucleoli, and nuclear polymorphism

8. What are morphologic features and ancillary studies that can help differentiate hepatic adenoma (HA) from hepatocellular carcinoma (HCC)?

Morphological differentiation between HA and moderately/poorly differentiated HCCs is usually straightforward because the HCCs show cytologic atypia. Differentiating HA from well-differentiated HCC can be very challenging by morphology alone. Ancillary studies including immunohistochemistry and chromosomal analysis have been used to separate these two entities.

Immunohistochemical panel to differentiate hepatic adenoma from HCC includes glypican-3, β-catenin, and glutamine synthetase. Glypican-3 is an oncofetal antigen that is normally expressed in fetal liver and placenta, but not in normal adult liver. Up to 90% of well-differentiated HCCs express glypican-3. Beta-catenin is an important player in Wnt signaling pathway. Abnormal nuclear expression has been reported in 20–40% of HCCs. Glutamine synthetase, in normal liver, is expressed in pericentral hepatocytes, but not in midzone or periportal hepatocytes. It is one of the genes that is upregulated as a result of β-catenin nuclear translocation. It is strong and diffusely expressed in carcinoma cells and is negative in most hepatic adenomas.

Gains of chromosomes 1q and 8q in well-differentiated HCC have been used to separate hepatic adenoma and HCC by fluorescence in situ hybridization (FISH) (Fig. 16.5).

References: [9, 12–16].

9. How do you differentiate hepatocellular carcinoma (HCC) with eosinophilic granular cytoplasm from its mimickers?

Tumors/lesions composed of cells with eosinophilic granular cytoplasm resembling HCC include focal nodular hyperplasia, epithelioid angiomyolipoma, metastatic renal cell carcinoma, pancreatic acinar cell carcinoma, salivary gland acinic cell carcinoma, neuroendocrine tumors, adrenal cortical carcinoma, and melanoma. Focal nodular hyperplasia can be separated from HCC by its

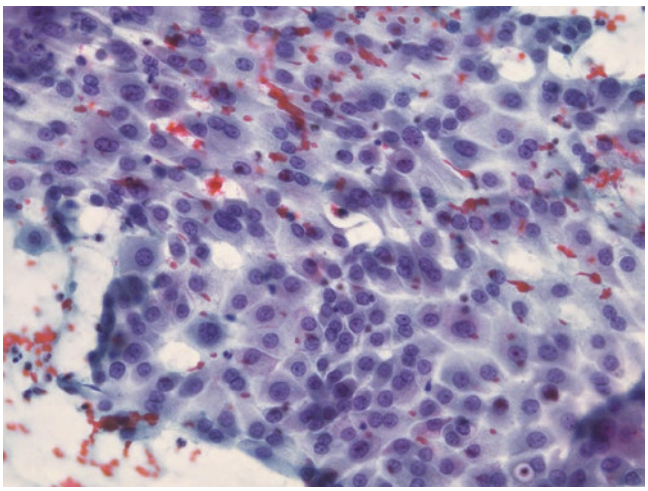

Fig. 16.5 Hepatic adenoma, Pap stain. Please note the nuclear size polymorphism and mild hyperchromasia

Table 16.1 Differentiation between HCC with eosinophilic granular cytoplasm from its mimickers

	Immunohistochemical and special stains
HCC	CK7-/Hep Par-1+/arginase+/ glypican-3+/AFP+/reticulin >2 cell plates
Fibrolamellar HCC	CK7+/Hep Par-1+/arginase+/ glypican-3+/AFP-
Renal cell carcinoma	Pax8+/RCC+/CAIX+/CK7-/vimentin +
Neuroendocrine tumor	Chromogranin+/synaptophysin+/CD56+
Melanoma	Malen A+/HMB45+/SOX10+
Acinic cell carcinoma of salivary gland	Dog 1 +/PAS+/PASD+
Acinar cell carcinoma of pancreas	Trypsin +
Adrenal cortical carcinoma	Inhibin +

Abbreviations: *HCC* hepatocellular carcinoma, *CK* cytokeratin

absence of cytologic atypia. An immunohistochemical/histochemical panel including arginase, HepPar-1, glypican-3, Pax8, chromogranin, synaptophysin, inhibin, S-100, Melan A, HMB45, dog 1, and trypsin is usually employed to differentiate the abovementioned tumors. See Table 16.1.

A primary hepatocellular carcinoma that predominantly affects children and young adults with no underlying liver disease is fibrolamellar HCCs. The tumor cells are large and polygonal with eosinophilic granular cytoplasm. Fibrolamellar HCCs are positive for CK7 and AFP (conventional HCCs are usually negative) and positive for markers of hepatocellular differentiation (arginase, HepPar-1, and glypican-3). Recently DNAJB1-PRKACA gene fusion transcript has been found to be a very sensitive and specific marker for fibrolamellar HCC.

References: [17–22].

10. How do you differentiate hepatocellular carcinoma (HCC) with clear cell change from its mimickers?

The HCCs with clear cell change are not an uncommon variant of HCC (prevalence of 0.4% to 37%) that can pose a diagnostic challenge, as many adenocarcinomas and epithelioid tumors have clear cell morphology. A panel of special stains and immunohistochemical studies are needed to differentiate tumors with clear cell histology, such as metastatic renal cell carcinoma, clear cell melanoma, clear cell sarcoma, adrenal cortical carcinoma, clear cell variant of pulmonary carcinoma, neuroendocrine tumor with clear cell feature, clear cell cholangiocarcinoma, and clear cell bile duct adenoma.

The HCCs with clear cell change express markers of hepatocellular differentiation including arginase, HepPar-1, glypican-3, and p-CEA (canalicular pattern). In the case of metastatic tumors with clear cell change, evaluation of a panel of antibodies including CK7, CK20, Pax8, CAIX, HMB45, Melan A, inhibin, chromogranin, synaptophysin, vimentin, thyroid transcription factor-1 (TTF-1), EMA, and S-100 should be helpful in establishing a possible primary site (Table 16.2).

References: [17, 23–25].

11. What are the features of benign and reactive bile duct epithelium in cytological specimens?

Bile ductal epithelium in cytological specimens usually presents in small flat monolayers with regular honeycomb arrangement and preservation of polarity. The medium-sized columnar epithelial cells possess a single, small, basally positioned nucleus. The chromatin is finely granular and pale stained, and nucleoli are inconspicuous in normal epithelium. The cytoplasm is pale, delicate, and scant to moderate in amount with well-defined cell borders. Goblet cells can also be seen occasionally. The benign ductal epithelial cells tend to round up when singly dispersed in the smears.

In response to inflammation, gallstones, instrumentation, and surgical manipulation, the biliary tract epithelium may undergo significant reactive/reparative changes. Benign reactive cells can demonstrate marked variation in nuclear size, and enlargement up to fivefold in the same cluster can rarely be seen. Nucleoli can become prominent and may be single or multiple. Normal mitotic figure and squamous metaplasia may also be identified. But in contrast to malignant changes, reactive bile duct epithelium usually present as cohesive monolayered sheets with fairly uniform cells that maintain polarity. Generally, a honeycomb arrangement with mild nuclear crowding, mildly enlarged round-to-oval nuclei, fine chromatin and smooth membranes is present. Nuclear hyperchromasia is usually mild. Most reactive epithelial cells still contain adequate cytoplasm. The background of reactive/reparative biliary smears may contain bile pigment, cholesterol, crystals, and a varying degree of inflammatory

Table 16.2 Differentiation between hepatocellular carcinoma with clear cells from its mimickers

	Immunohistochemical study
HCC	CK7-/Hep Par-1+/arginase+/glypican-3+/AFP+/reticulin >2 cell plates
Renal cell carcinoma	Pax8+/RCC+/CAIX+/CK7-/vimentin +
Neuroendocrine tumor	Chromogranin+/synaptophysin+/CD56+
Melanoma	Malen A+/HMB45+/SOX10+
Pulmonary adenocarcinoma	CK7+/CK20-/TTF-1 (nuclear)+/napsin A+
Cholangiocarcinoma	CK7+/CK19+/S100P-/pVHL+/MUC5AC/CK17−/glypican-3-
	Immunohistochemical study
Adrenal cortical carcinoma	inhibin

Abbreviations: *HCC* hepatocellular carcinoma, *CK* cytokeratin

cells. The presence of acute inflammation, especially intraepithelial neutrophils, favors a reactive process.

When differentiating reactive from malignant bile duct epithelial cells is difficult, ancillary tests including immunostain and molecular tests may be useful. In one study, mammary serine protease inhibitor (maspin) expression was much more frequently detected in malignant than in benign biliary epithelial cells, with malignant cells showing diffuse, strong/intermediate, and combined nuclear-cytoplasmic staining for maspin. A maspin+/ S100 calcium-binding protein P+/ von Hippel-Lindau gene product- staining profile was seen in 75% (35/45) of cholangiocarcinoma cases but in none (0/58) of the benign cases. In another study, maspin/p53 double immunostaining positive cells were observed in 88% (14/16) of morphologically malignant cases, 60% (6/10) of borderline cases, and 0% (0/18) of benign biliary brushing cases. In a study of 281 cholangioscopic biopsies by endoscopic ultrasound-guided fine needle aspiration biopsy for indeterminate biliary strictures, fluorescence in situ hybridization (FISH) polysomy/9p21 as a single modality was the most sensitive marker for malignancy. The sensitivity of FISH polysomy/9p21 and cytology was significantly higher than cytology alone at 63 versus 35% ($p < 0.05$).

References: [26–29].

12. What are the morphologic features associated with cholangiocarcinoma (CC) in cytological specimens?

The cytological (fine needle aspiration or brushing) diagnosis of CC is one of the challenging fields of cytopathology, especially for the well-differentiated carcinoma. This is partially because severe reactive/reparative changes are common in many biliary cytological specimens. Well-differentiated CC is morphologically reminiscent of normal bile duct epithelium but shows disorderly growth (piling up, crowding, loss of nuclear polarity, irregular or "drunken" honeycomb). Tumor cells are arranged in loose clusters, tubules, microacinar, as well as scattered singly. The cells are relatively small with ovoid hyperchromatic nuclei and a small amount of cytoplasm (high nuclear-cytoplasmic ratio). Large cells with irregular nuclear membrane and prominent nucleoli are sometimes identifiable. Less differentiated tumor cells show classic cytological features of malignant tumor cells (hyperchromasia, uneven chromatin distribution, prominent nucleoli, irregular nuclear contour) and typically display focal squamous features (density, distinct border) in their cytoplasm. Even within the same cluster, there is frequently a wide variety of tumor cell differentiation ranging from bland to obviously malignant in appearance. Spindle or giant tumor cells occasionally are seen. The tumor cells usually are mucin positive and may form signet rings, with vacuolated cytoplasm. Bile stasis is common. In some fine needle aspiration specimens, spindle-shaped fibroblastic cells are intimately admixed with tumor cells. Utilizing a multiple logistic regression analysis, a group of cytopathologists proposed that three key cytologic features are critical in separating benign from malignant bile duct smears, including nuclear molding, chromatin clumping, and increased nuclear-cytoplasmic ratio.

While a standardized reporting system for intrahepatic cholangiocarcinoma is not currently available, in 2014 Papanicolaou Society of Cytopathology proposed guidelines to report/classify pancreatobiliary cytopathology, including cholangiocarcinoma. The 6 diagnostic categories and their definitions in this guideline are listed below.

I. Nondiagnostic: specimen provides no diagnostic or useful information about the lesion sampled.
II. Negative (for malignancy): synonymous with the absence of malignancy and any cellular atypia in the cytology sample.
III. Atypical: The category of atypical should only be applied when there are cells present with cytoplasmic, nuclear, or architectural features that are not consistent with normal or reactive cellular changes of the pancreas or bile ducts and are insufficient to classify them as a neoplasm or suspicious for a high-grade malignancy. The findings are insufficient to establish an abnormality explaining the lesion seen on imaging. Follow-up evaluation is warranted.
IV. Neoplastic, benign: This interpretation category connotes the presence of a cytological specimen sufficiently cellular and representative, with or without the context of clinical, imaging, and ancillary studies, to be diagnostic of a benign neoplasm.

- Neoplastic, other: this interpretation category defines a neoplasm that is either premalignant or low-grade malignant neoplasm.

V. Suspicious (for malignancy): Some but an insufficient number of the typical features of a specific malignant neoplasm are present. The cytological features raise a strong suspicion for malignancy, but the findings are qualitatively and/or quantitatively insufficient for a conclusive diagnosis, or tissue is not present for ancillary studies to define a specific neoplasm. The morphologic features must be sufficiently atypical that malignancy is considered more probable than not.

VI. Positive/malignant: a group of neoplasms that unequivocally display malignant cytologic characteristics.

Immunocytochemically, cholangiocarcinomas are usually positive for mucicarmine, AE1/AE3 cytokeratin, cytokeratin 7, cytokeratin 19, CA19.9, and diffuse cytoplasmic staining with polyclonal CEA. FISH technique using UroVysion probe set is reported to increase diagnostic sensitivity of brush cytology of cholangiocarcinoma from 53.8% to 69.2%, while preserving specificity of 82.4%. The FISH assay uses a mixture of fluorescently labeled probes to the centromeres of chromosomes 3, 7, and 17 and chromosomal band 9p21 (Vysis UroVysion) to identify cells having chromosomal abnormalities (Figs. 16.6, 16.7, and 16.8).

References: [30–36].

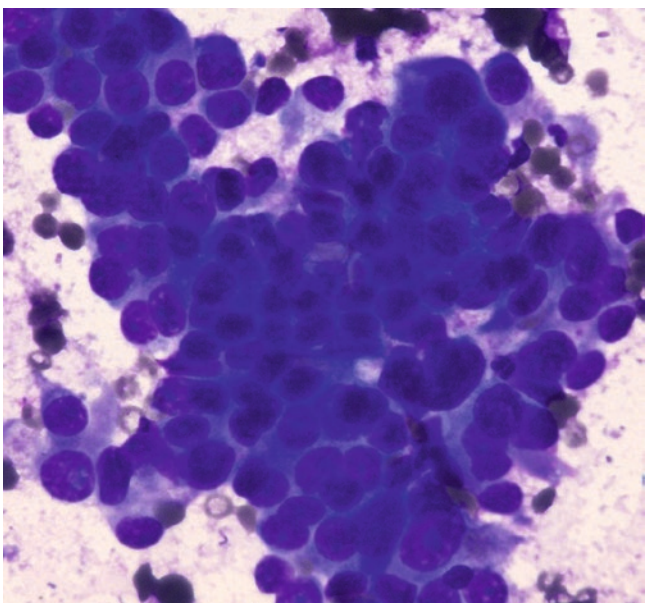

Fig. 16.6 Cholangiocarcinoma, Diff-Quik stain. Please note the nuclear polymorphism

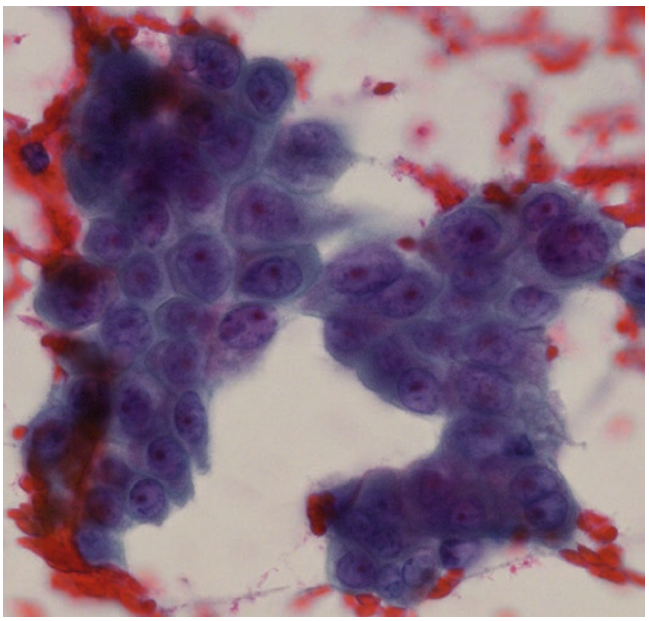

Fig. 16.7 Cholangiocarcinoma, Pap stain. Please note the uneven chromatin distribution, nuclear polymorphism, prominent nucleoli, and nuclear contour irregularities

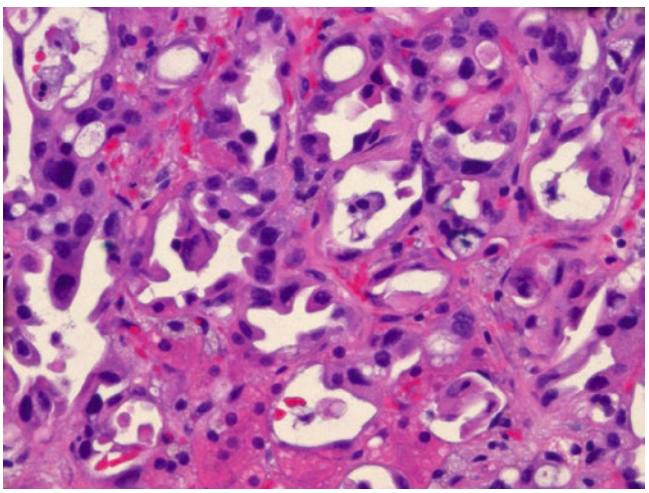

Fig. 16.8 Cholangiocarcinoma in core biopsy

13. How can you differentiate reactive bile ductules from bile duct adenoma and cholangiocarcinoma?

As mentioned earlier in Q 11, orderly cohesive sheets of cells with little or no nuclear crowding, regular ranks and files of cells, smooth nuclear membranes, and fine chromatin are characteristic of reactive cells. The FNA of a bile duct adenoma may be sparsely cellular due to fibrosis. The aspirate shows cohesive clusters of orderly bile duct cells, often columnar in shape. The cells are usually bland with occasional reactive changes but malignant features are absent. There is no cholestasis, and hepatocytes are not present. Fibroblasts or fragments of fibrous tissue may be seen. The above cytological changes sometimes

overlap with that of well-differentiated cholangiocarcinoma. In addition, adequate smears of intrahepatic CCs usually show ductular proliferation.

Sato et al. investigated heat shock protein (HSP)27 and HSP70 in tumor tissue, bile, and serum samples of patients with intrahepatic CC (ICC) and compared them to patient samples with inflammatory lesions and found an increase of HSP27 and HSP70 in cholangiocarcinoma and its precursor lesions compared with normal and reactive bile ducts. Gütgemann et al. demonstrated expression of cluster of differentiation 56 (CD56) in ductular proliferations and bile duct adenomas (BDAs), whereas most cholangiocarcinoma samples showed negativity for this marker. An immunohistochemical panel using antibodies for B-cell-lymphoma2 (BCL2), p53 and Ki67 index were proposed by Tan et al. for the discrimination of inflammatory, benign, and malignant bile duct lesions. In this study, an increase of p53 and BCL2 expression was demonstrated in the group of cholangiocarcinoma in combination with a higher ki67 proliferative index. In one new study, the expression of SerpinH1 was significantly higher in ICC than in bile duct adenomas and ductular reactions, whereas stress-induced phosphoprotein 1 expression in ICC was significantly higher than in ductular reactions, but not significantly higher than in bile duct adenomas. A proliferative index (ki67) of >5% might be used to distinguish malignant from benign or reactive bile duct lesions.

References: [37–41].

14. How do you distinguish cholangiocarcinoma from metastatic adenocarcinoma?

The liver is a common site for metastasis, and the majority of liver FNAs prove to be metastatic malignancies. Any lesions discovered on FNA that are suspected of being metastatic should be compared with histologic or cytopathological slides from the original tumor. The common sites of primary tumor include the colon, pancreas, stomach, breast, and lung. Clinically, serum alkaline phosphatase level is often elevated in metastatic tumors. Some authors suggest the presence of more than 10 ductular clusters to be associated with malignancy in FNA smears as a useful discriminator to separate CC from metastatic carcinoma. But differential diagnosis of cholangiocarcinoma from some metastatic carcinomas, particularly from pancreas, may be impossible on morphological ground alone. Immunocytochemical and molecular markers become more important in those situations (Table 16.3).

References: [42–45].

15. What are the cytological features associated with hepatoblastoma (HBL)?

HBL is the most common primary malignant neoplasm of the liver in children, particularly in those younger than 4 years old though it can also occur in older patients. Hepatoblastoma affects twice as many boys as girls and classically presents with marked elevation of α-fetoprotein (AFP) in the serum.

The epithelial components of HBL can show a wide range of differentiation from anaplastic to embryonal to fetal. Fine needle aspiration (FNA) allows accurate diagnoses of HBL with recognition of these histological subtypes. Histologically, anaplastic HBL tumor cells are "small blue cells" similar to those of other pediatric tumors, such as neuroblastoma and Ewing tumor. FNA smears of anaplastic type HBL show small cells with scant cytoplasm (thus highly elevated nuclear-cytoplasmic ratio), densely stained nuclei, irregular nuclear membrane, and coarse chromatin. Nucleoli are usually invisible.

Table 16.3 Differential cholangiocarcinoma from common hepatic metastatic malignancies

	Key points of cytomorphology	ICC tests
Cholangiocarcinoma	Nuclear molding, chromatin clumping, and increased nuclear-cytoplasmic ratio; "drunken" honeycomb	S100P-/pVHL+/MUC5AC-/CK17- / glypican-3-/CK7+/CK19+/CA125+
Colorectal	Columnar, hyperchromatic, and necrotic	CDX2+/ CK20+/CK7-
Gastric	Signet ring cells	
Well-differentiated neuroendocrine tumor	"salt-and-pepper" chromatin; abundant granular cytoplasm	Synaptophysin+/ chromogranin+/ CD56+/
Small cell carcinoma	Nuclear molding, hyperchromatic nuclei with finely granular chromatin and inconspicuous nucleoli	Synaptophysin+/ chromogranin+/CD56+/ Ki-67 high
Squamous cell carcinoma	Small, dark nuclei; abundant cytoplasm with a hard, glassy appearance	P40+/P63+/CK5/6+
Melanoma	Intranuclear pseudoinclusion and macronucleoli, melanin pigment	SOX10+/HMB45+/ Mart-1+
Pancreatic ductal adenocarcinoma	Morphologically identical to cholangiocarcinoma	S100P+/pVHL-/MUC5AC±/CK17+
Breast	Signet ring cells	GATA3+/ER+/PR+
Prostate	Microacini, prominent nucleoli	NKX3.1+/PSA+/PSAP+

Abbreviations: *CK* cytokeratin

Tumor cells form sheets and rosettes. Embryonal HBL tumor cells are small, oval to spindle-shaped cells and have large nuclei with coarse dark chromatin, prominent nucleoli, and a small amount of cytoplasm. They can be arranged in cords, rosettes, or papillae. The tumor cells appear crowded and disorganized with overlapping borders and mitotic figures are frequently seen. Fetal HBL tumor cells are larger than embryonal cells but smaller than mature hepatocytes. Usually there is little or no pleomorphism. The nuclei are round to oval, moderately hyperchromatic with fine chromatin, low nuclear-cytoplasmic ratio, and occasional nucleoli. Fetal HBL tumor cells can have granular or vacuolated cytoplasm, depending on the amount of glycogen and/or lipid present. The fetal cells are typically arranged in 2 or 3 cell layer thick tumor cords; loosely cohesive small sheets and acini or large disorderly clusters or trabeculae can also been seen. Fetal cells, but not embryonal cells, may contain bile, fat, or glycogen and may be associated with foci of extramedullary hemopoiesis, characterized by megakaryocytes, nucleated RBCs, and other immature blood cells. Many HBLs contain more than one subtype component.

The mesenchymal component of HBL, when present (i.e., mixed HBL), characteristically has a primitive, undifferentiated, and cellular appearance. Osteoid may be present. Less frequently, other differentiation, including squamous, skeletal muscle, and cartilage, may also occur. These metaplastic elements strongly favor a diagnosis of HBL over HCC. In contrast to HCC, marked pleomorphism and giant tumor cells are never seen in HBL. Both HBL and HCC can have hyaline inclusions in the tumor cell cytoplasm.

Most HBLs are positive for AFP. Activation of β-catenin in this tumor results in cytoplasmic and nuclear staining of both total and phosphorylated β-catenin. Cyclin D1 stains the nuclei of the mixed epithelial-mesenchymal type of HBL more than the pure fetal type. SERPINB3 is overexpressed in HBL, and this expression correlates with Myc expression and higher tumor stage. Recent gene profiling studies identified two apoptosis-associated genes, MYCN and BIRC5, that are highly upregulated in HBL.

References: [31, 46, 47].

16. How do you differentiate angiosarcoma from hemangioendothelioma (HE) in hepatic fine needle aspiration (FNA)?

Angiosarcoma is an uncommon but highly malignant tumor. It represents less than 1% of primary hepatic malignancies. A third of these tumors arise in a setting of cirrhosis. It is seen with increased frequency in patients who have been exposed to arsenic compounds, polyvinylchloride or thorotrast radiographic contrast agent. Tumors may be well or poorly differentiated. Aspirates of hepatic angiosarcoma are often very bloody. Suspect angiosarcoma when unexpected cellularity is obtained despite significant blood. Well-differentiated tumors show spindle cells. Less well-differentiated tumors show larger, pleomorphic, and bizarre cells, frequently with ingested cytoplasmic material. Tumor cells are arranged in individual cells, cords, loose clusters, or microacini with central lumens in a blood-rich background. Some tumor cells are plasmacytoid with intracytoplasmic amphophilic vacuoles, perinuclear clearing, or eosinophilic perinuclear condensation. However, obvious intracytoplasmic hemosiderin pigment or erythrophagocytosis is not frequently identified. Factor 8, CD31, and CD34 staining is helpful in confirming the diagnosis and differentiating the tumor cells from metastatic sarcomas (most commonly leiomyosarcoma). Epithelioid angiosarcoma (EAS) shows high-grade cytology with round, irregularly indented or lobulated nuclei with prominent nucleoli and scant cytoplasm. Features more commonly associated with EAS include capillary vessels, vascular lakes, and papillary growth.

HE is an extremely rare tumor that may arise in the liver. It is also a tumor of endothelial cells but behaves in a less malignant fashion than angiosarcoma. The HE smears usually show low cellularity comprised of singly dispersed cells and small tissue fragments. The tumor cells have variable cytomorphology, from epithelioid to spindle to scattered larger bizarre cells with hyperchromatic nuclei. Occasionally few tumor cells can show intranuclear inclusions. Intracytoplasmic vacuoles or lumina (ICLs) can been seen in cytology. Sometimes the ICLs are so large that the nucleus can become compressed, presenting with a signet-ring appearance. The HE tumor cells may form lumens of various sizes and can occasionally contain red blood cells. Some smears have been reported to contain single cells, pseudopapillary and pseudoglandular structures of varying sizes, complex branching cell groups with central stromal cores, as well as cell aggregates lacking sharp anatomic borders or scalloped outlines. Other vasoformative features like erythrophagocytosis, hemorrhagic background, and positive reaction to vascular markers can also been identified. Epithelioid HE (EHE) shows typical small bland-appearing polygonal (epithelioid) endothelial cells, often with intracytoplasmic lumina ("blister cells"), round or oval, hyperchromatic nuclei with smooth nuclear contours, delicate cytoplasm, and indistinct cytoplasmic borders. Osteoclastic giant cells and metaplastic bone have been reported in several cases. Intranuclear cytoplasmic inclusions are typically observed in EHE.

Although the cytologic features of HE may overlap with those of angiosarcoma, the predominance of individually dispersed cells, lower cellularity, intranuclear cytoplasmic inclusions, insignificant nuclear atypia, and lower mitotic

activity are considered cytomorphological features suggestive of HE.

Recurrent genetic aberrations in EHE include translocations of the *CAMTA1* gene on chromosome 1p and fusion with the *WWTR1* gene on chromosome 3q. The *YAP1-TFE3* fusion gene is generated from *YAP1* sequences on chromosome 11 and *TFE1* sequences from the X-chromosome. The genetic changes identified in the EHE have only been identified in extremely rare cases of EAS and thus may serve as markers to distinguish the two entities.

References: [1, 48–50].

17. How do you recognize hepato-cholangiocarcinoma (H-ChC) in liver fine needle aspiration (FNA)?

H-ChC is a special type of liver cancer with pathological features of hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), accounting for 1–14% of primary liver cancers. H-ChC has been known by various nomenclatures in the literature including mixed hepatocellular carcinoma-cholangiocarcinoma, hybrid HCC-CC, or combined liver and bile duct carcinoma. In 2010, the WHO updated the classification criteria for H-ChC: H-ChC was divided into the classical type and three subtypes with stem cell features (typical subtype, intermediate cell subtype, and cholangiolocellular subtype). The classical type contains areas of HCC, CC, and transitional zones.

Identification of a dual cell population in a hepatic FNA should raise the possibility of a H-ChC. Unequivocal HCC cells corresponding to Edmondson and Steiner's grade 3 lesions are a required component of the diagnosis. Adenocarcinoma, represented by cohesive columnar cells with ovoid, basal nuclei displaying nuclear palisading, acini, and/or papillary structures with variable intracytoplasmic, intra-acinar, or brush border mucin production, is another required diagnostic component. Intermediate cells with hybrid/polymorphic cytologic features straddling malignant hepatocytes and glandular cells are usually also identified in H-ChC. In practice, intermediate cells pose a great challenge to recognize and define: they tend to lose the classic cytologic features of malignant hepatocytes and acquire glandular characteristics.

Immunohistochemically, the hepatocellular component is positive for HepPar1, p-CEA, CD10, and glypican-3; the CC component shows expression of CK7, CK19, mucin-core protein 1 (Muc-1), and mucin/mucicarmine. CAM 5.2 and AE1 can also be useful to differentiate between HCC and CC component; both HCC and CC are positive for CAM5.2, while AE1 is positive in CC component only. Both components are negative for alpha-fetoprotein.

H-ChC with stem cell features, typical subtype, stains positively with CK7, CK19, CD56, cKIT, and/or EpCAM. H-ChC with stem cell features, intermediate subtype, is characterized by intermediate cells (between hepatocytes and cholangiocytes) and shows simultaneous expression of hepatocyte and biliary markers. Akiba et al. demonstrate that intermediate cells stain positively with arginase-1 and CK8, CK7, and CK19. H-ChC with stem cell features, cholangiolocellular subtype, is positive for stem cell markers—CK19, cKIT, CD56, and EpCAM.

The reliable diagnosis of H-ChC on cytologic preparations alone might be difficult. However, the addition of a cellblock or core biopsy, which may serve as a substrate for histochemical and immunohistochemical studies, may allow for a more accurate diagnosis. Serum markers for HCC, when elevated, may raise the suspicion of hepatocellular differentiation even if it is unsuspected on cytologic and/or histologic examination of a biopsy specimen.

References: [51–56].

Case Presentation

Case 1

Case history: A 46-year-old male with history of a large kidney mass presented with a liver mass.

Figures 16.9, 16.10, 16.11, 16.12, and 16.13, Case 1.

Description: Diff-Quik-stained conventional smear and Papanicolaou-stained smear show the sheets of tumor cells with eosinophilic and vacuolated cytoplasm. The nuclei are large, round, and eccentrically place.

Differential diagnoses:

- Metastatic renal cell carcinoma
- Metastatic melanoma
- Primary hepatocellular carcinoma
- Malignant mesothelioma
- Immunostains performed on the cellblock:
- Positive for EMA and vimentin
- Negative for S100

Final diagnosis:
Metastatic renal cell carcinoma
Take-home message:

- Majority of liver masses are metastatic malignancies.
- Adequate history of the patient is the key.
- Familiar with the immunohistochemistry panel for clear cell renal carcinoma (Pax8+/CK7−/vimentin +/ CAIX+/CD10+).

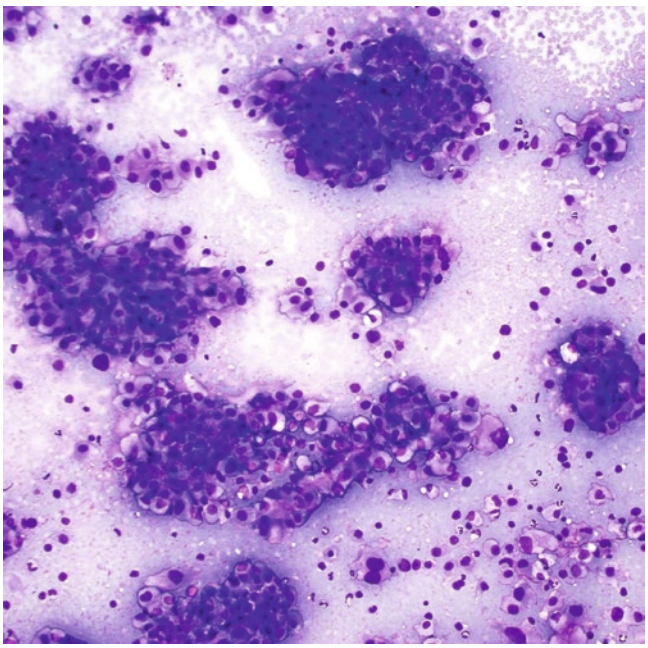

Fig. 16.9 Diff-Quik stain

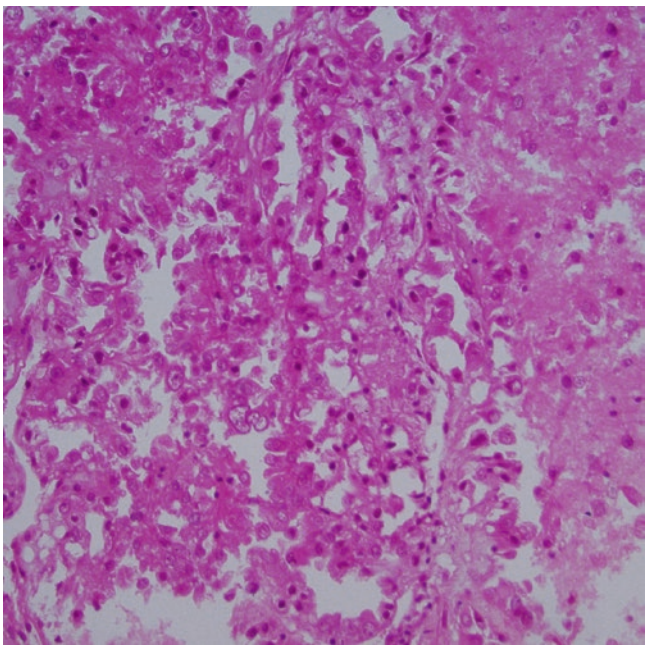

Fig. 16.11 Cellblock

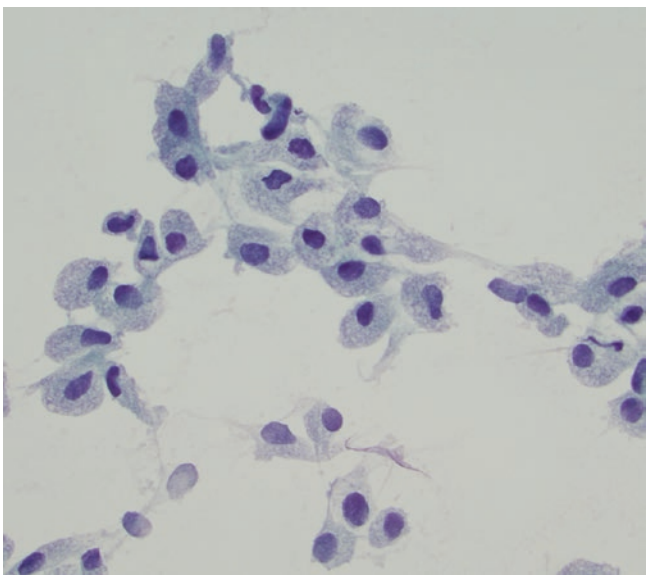

Fig. 16.10 Pap stain

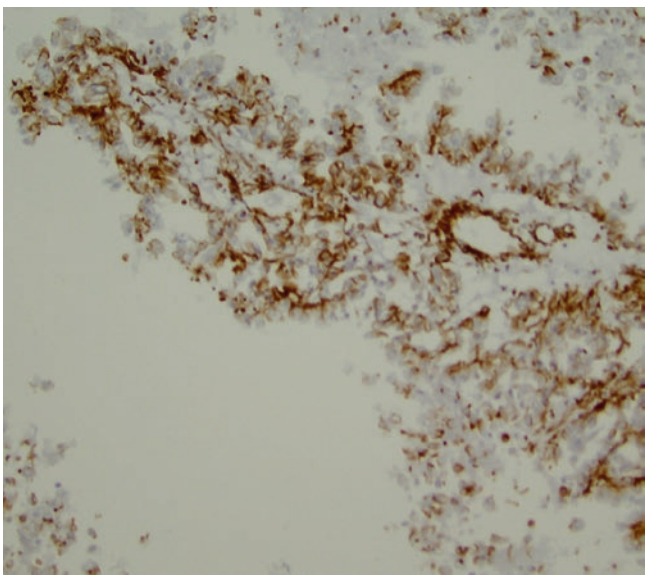

Fig. 16.12 ICC stain of vimentin

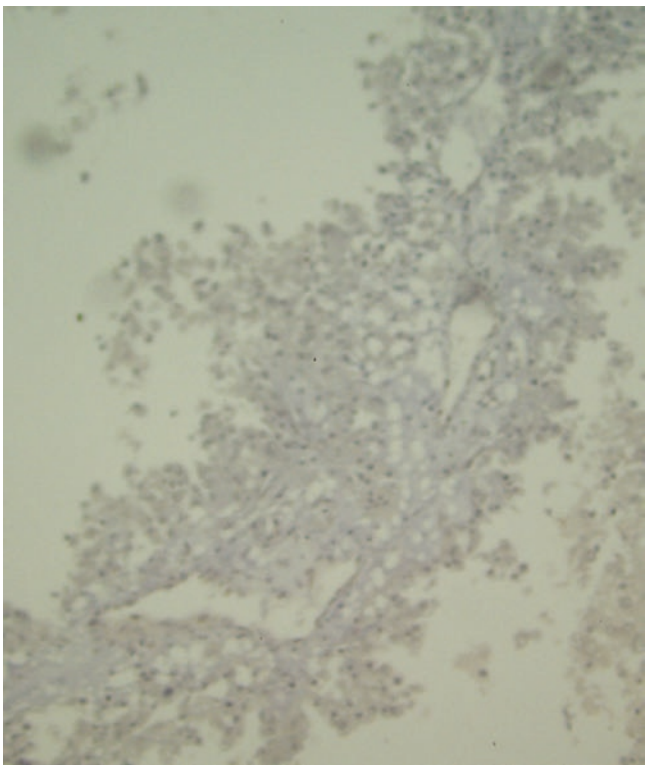

Fig. 16.13 ICC stain of S-100

Case 2

Case history: An 85-year-old man came to an outside hospital with complaint of nausea, vomiting, and weight loss. CT examination revealed multiple 2–3 cm nodules in his right liver. A CT-guided fine needle aspiration was conducted and material submitted to cytopathology department for review.

Figures 16.14, 16.15, 16.16, and 16.17, Case 2.

Description: H&E-stained conventional smears show the sheets of tumor cells with vacuolated cytoplasm. The tumor cells form small acini, or small 3-D structures; nuclei are large, round, mild nuclear contour irregularities. However, nuclear to cytoplasmic ratio is only slightly increased.

ICC stains show the tumor cells are Hep par1+/p-CEA+/GPC3+/SOX10-/PAX8−/inhibin-/TTF-1-/CK7-.

Differential diagnoses:

- Hepatocellular carcinoma (HCC), clear cell variant
- Metastatic renal cell carcinoma
- Clear cell melanoma
- Clear cell sarcoma
- Metastatic adrenal cortical carcinoma

Clear cell variant of pulmonary carcinoma
Neuroendocrine tumor with clear cell feature
Clear cell cholangiocarcinoma

Final diagnosis:
Hepatocellular carcinoma, clear cell variant
Take-home message:
Clear cell variant of tumor is diagnostic consideration for HCC and several other common malignant neoplastic types (see above).
Immunostain panel is critical for the differential diagnosis.

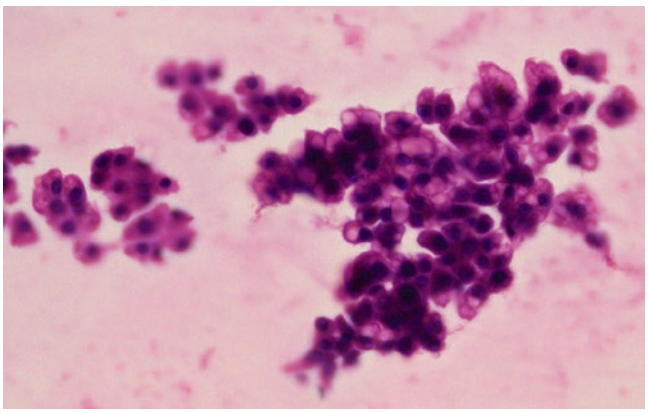

Fig. 16.14 H&E stain, X100

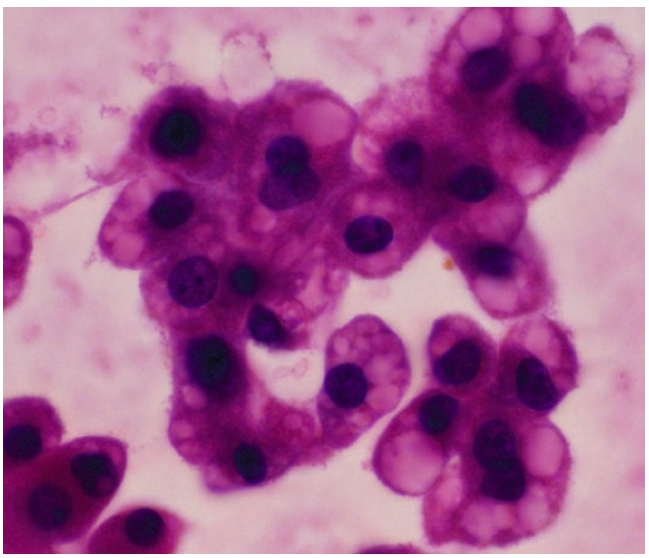

Fig. 16.15 H&E stain, X400

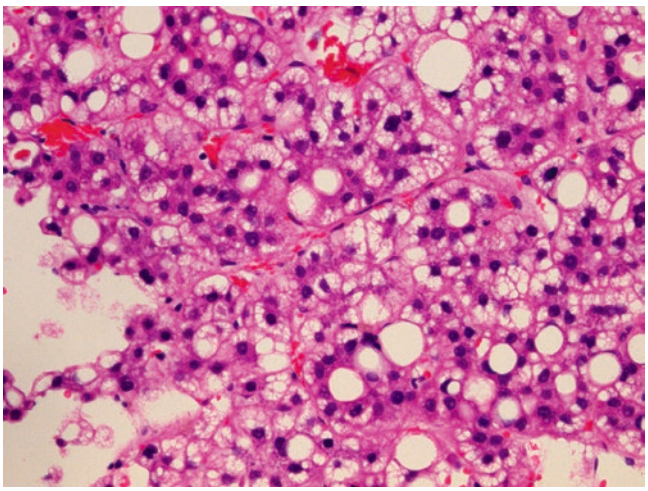

Fig. 16.16 Cellblock

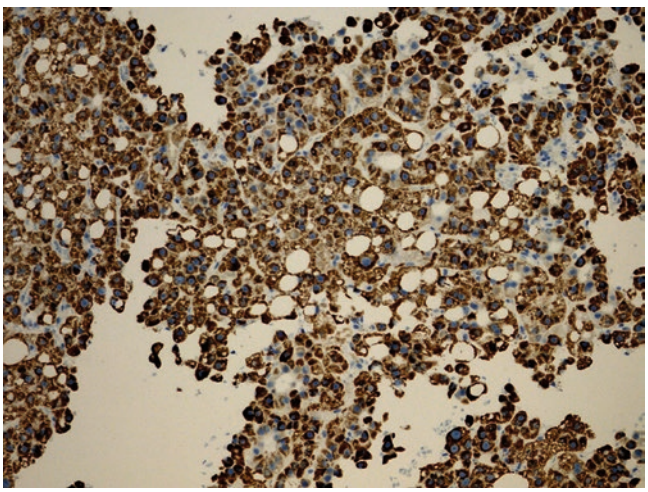

Fig. 16.17 ICC stain of Hep par-1

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Soft Tissue and Bone

17

Xiaohua Qian and Yaxia Zhang

Contents

List of Frequently Asked Questions

1. What are the advantages of using fine needle aspiration for soft tissue and bone lesions?

Fine needle aspiration (FNA) for the evaluation of soft tissue and bone tumors was first introduced in the early 1930s [1, 2]. Although FNA has been widely accepted as a means to document recurrent and/or metastatic soft tissue and bone tumors, its value as the initial diagnostic modality in patients with primary soft tissue and bone tumors remains controversial [3–5]. In recent years, minimally invasive biopsy techniques, namely, core needle biopsy (CNB) with or without accompanied FNA, have largely replaced incisional biopsy to establish histological diagnosis and grade for patients who are suspected to have a primary soft tissue and bone tumor. This advance is achieved in concert with the development in molecular genetic diagnostics, expansion of immunohistochemical biomarkers, and the maturation of various image-guided needle biopsy techniques [6]. Enhanced by rapid on-site evaluation (ROSE), and immediate tissue triage for flow cytometry and/or cytogenetic/molecular studies, FNA offers a valuable and fast diagnostic modality for lesions suspicious for high grade malignancy, especially for tumors with a broad differential diagnosis including carcinoma, lymphoma, and sarcoma [7, 8].

The advantages of using FNA for soft tissue and bone lesions include the following:

- Technically easy, cost-effective, office-based procedure.
- Exceedingly low risk of complications.
- Safe for difficult bone sites such as vertebrae and pelvic bones.
- Allows for ROSE, immediate tissue triage for ancillary studies, and initiation of treatment in emergent scenarios.
- Provides superior cellular material for molecular testing.
- No tumor contamination of tissue planes.
- On-site cytologic-radiologic correlation.
- Romanowsky-stained preparations are superior to other staining methods for demonstrating myxoid, cartilaginous, and osseous matrix.

2. What are the common challenges for evaluating primary soft tissue and bone tumors by FNA?

There are greater than 130 different soft tissue lesions, including more than 30 different entities of sarcomas in 2013 WHO Classification of Tumors of Soft Tissue and Bone [9]. The annual incidence of soft tissue sarcoma is <1% of all malignant tumors. It is, therefore, extremely challenging for a general practitioner to properly work up on infrequently encountered soft tissue or bone lesions with limited biopsy material. In addition, many mesenchymal tumors, like epithe-

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_17

lioid hemangioendothelioma and PEComa, can occur in visceral organs, mimicking carcinoma clinically and radiologically, and therefore pose significant diagnostic challenges when sampled unexpectedly by FNA [10, 11]. Awareness of diagnostic challenges and pitfalls is essential for successful needle biopsy diagnosis of soft tissue and bone tumors. These diagnostic challenges include the following:

- Lack of experience of cytopathologists with rare yet diverse soft tissue and bone lesions.
- Suboptimal material obtained from necrotic, cystic, or fibrotic lesions.
- Difficulty in sampling intramedullary, sclerotic lesions or extensively ossified bone lesions.
- Loss of characteristic architectural information and vascular patterns.
- Sampling error due to heterogeneity, bimorphic histology, and/or contamination of adjacent reactive tissue.
- Interpretation error due to significant morphologic, immunophenotypic, and genetic overlap among entities.
- Atypical clinical presentation and/or unusual morphologic features.
- Requirement of a close collaboration among members of a multidisciplinary team.
- Requirement of judicious ancillary work-up including immunohistochemistry and molecular cytogenetic studies.

3. What are the potential complications of needle biopsy of soft tissue and bone lesions?

Compared to excisional biopsy, needle biopsy, particularly FNA, is associated with a much lower rate of complications, ranging from 0% to 2% [12–14]. Hematoma is the most common complication. Persistent drainage from a needle tract and needle tract seeding by sarcoma are rare events, but may lead to increase the risk of local recurrence [14, 15]. To minimize needle tract seeding of tumor cells, a single needle insertion point at biopsy and complete resection of the biopsy tracts are recommended in cases with a high suspicion for sarcoma [16].

4. How to prepare FNA material for cytomorphologic analysis and ancillary testing?

Both alcohol-fixed, Papanicolaou-stained smears and air-dried, Romanowsky-type stained smears should be prepared for cytomorphologic analysis. Air-dried, Romanowsky-type preparations offer a simple fast staining process (<1 minute), good cytoplasmic details, and excellent visualization of extracellular matrix material, which is best suitable for ROSE. Both alcohol-fixed, Papanicolaou-stained smears and liquid-based preparations offer good nuclear details. But cells in liquid-based preparations can appear smaller, falsely epithelioid, even in spindle cell lesions; and useful cytomorphologic features like vascular patterns and myxoid matrix are usually lost. Cell block preparations usually contain mini tissue fragments, providing architectural information and reliable cellular material for immunohistochemical studies and molecular testing. FNA smears, liquid-based preparations, and cytospin slides are all suitable for ancillary testing including immunohistochemistry, fluorescence in situ hybridization (FISH), and next-generation sequencing (NGS) [17, 18].

5. What are cytologic elements that need to be analyzed in the evaluation of soft tissue and bone FNA samples?

- Background: tigroid (Fig. 17.1), lymphoglandular bodies, myxoid (Figs. 17.2, 17.3, and 17.4), cartilaginous, osteoid, inflammatory, or necrotic
- Cellularity: sparsely cellular or hypercellular; monotonous (Fig. 17.2) or pleomorphic (Fig. 17.3)
- Cell arrangement: dispersed single cell or in loose or tight clusters
- Predominant cell type: adipocytes (Fig. 17.4), spindle cells (Fig. 17.5), small round cells (Fig. 17.1), polygonal cells (Fig. 17.2), pleomorphic cells (Fig. 17.3), inflammatory cell rich (Fig. 17.6), or multinucleated giant cell rich (Fig. 17.7)
- Vascular pattern, prominent in myxoid tumors (Fig. 17.4a) or hidden in cellular clusters (Fig. 17.5)

6. What are the common morphologic patterns of soft tissue tumors on FNA samples?

Most soft tissue tumors, based on their predominant cell types and the presence or absence of myxoid stroma, can be

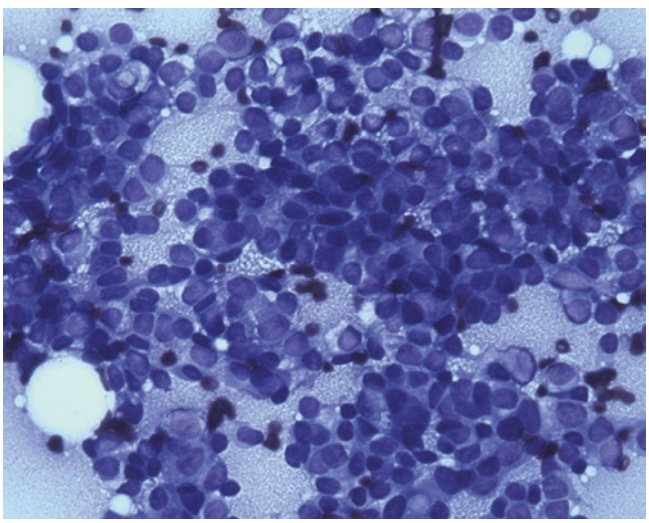

Fig. 17.1 The tigroid background is characteristic of Ewing sarcoma on FNA smears; the feature is better appreciated with air-dried Romanowsky-stained preparations. The hypercellularity, dispersed round "light and dark" cells, fine chromatin, nuclear molding, and occasional cytoplasmic vacuoles are also typical for Ewing sarcoma (Romanowsky stain)

cytomorphologically subcategorized into adipocytic, myxoid, spindle cell, round cell, epithelioid, and pleomorphic patterns. A pattern-based approach best serves as a guide to formulate the differential diagnosis and judicious application of immunohistochemical and molecular testing (Table 17.1).

7. What are the entities in the differential diagnosis of lesions with an adipocytic pattern?

Soft tissue tumors with an adipocytic differentiation include benign lipomatous tumors, liposarcomas, and nonlipomatous tumors with an adipocytic component. The diagnosis of these tumors is based on clinical presentation (age, site, superficial/deep, size), cytomorphology, and ancillary tests if needed [19].

Benign lipomatous tumors:

- Lipoma
- Hibernoma [20, 21]
- Spindle cell/pleomorphic lipoma [22]
- Lipoblastoma [23]
- Chondroid lipoma [24]

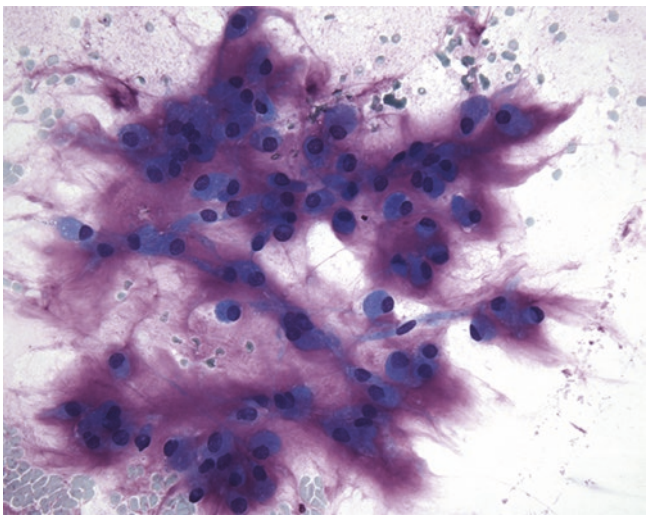

Fig. 17.2 The myxoid background is common for many soft tissue tumors. The fibrillary chondromyxoid matrix is characteristic of extraskeletal myxoid chondrosarcoma (EMC). Uniform epithelioid tumor cells with rhabdoid morphology can be seen in high-grade EMC. (Romanowsky stain)

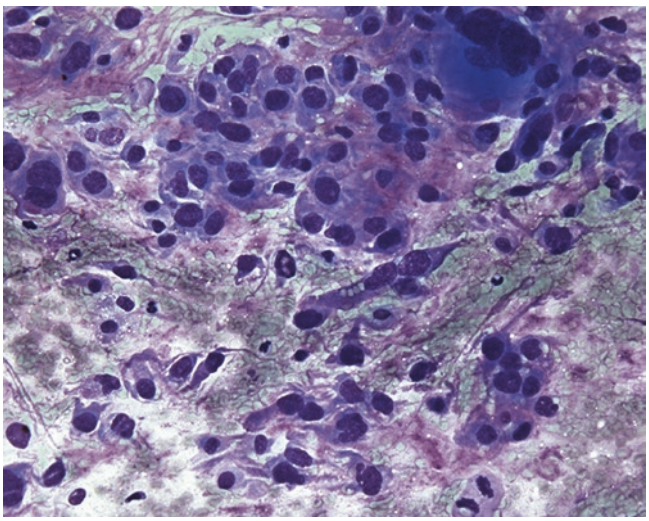

Fig. 17.3 The pleomorphic tumor cells show marked variation in size and shape, including multinucleated large bizarre forms. Frequent mitoses and myxoid stroma are also present. The findings are nonspecific and can be seen in any pleomorphic sarcomas, including myxofibrosarcoma, high grade (Romanowsky stain)

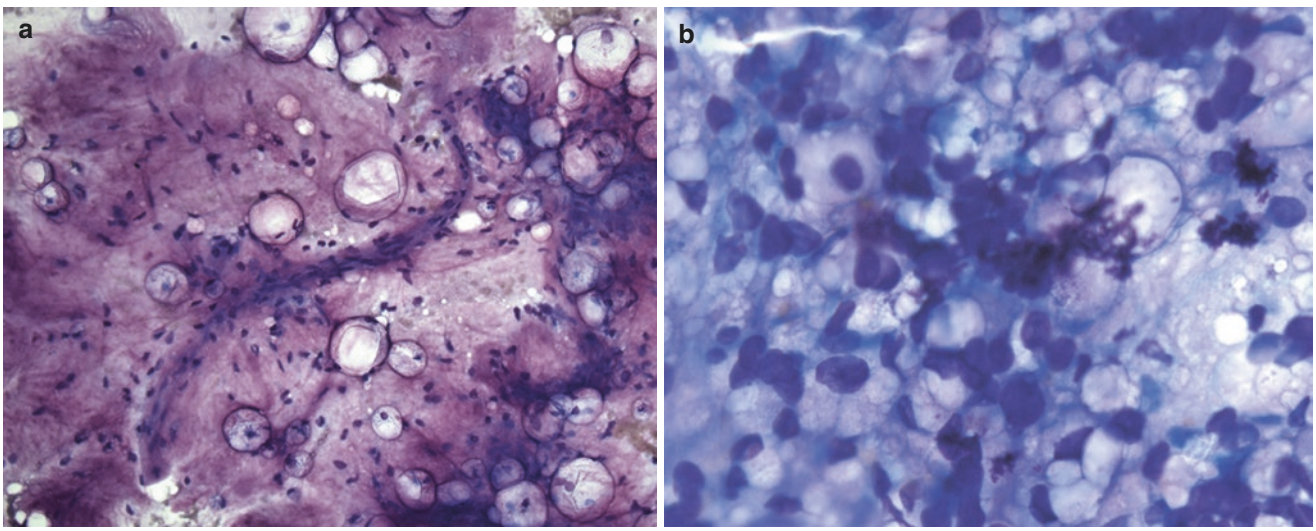

Fig. 17.4 (**a**) Myxoid liposarcoma. Tissue fragments contain loose myxoid matrix, vessels, histiocyte-like lipoblasts and uniform, nonlipogenic, oval tumor cells (Romanowsky stain). (**b**) Pleomorphic liposarcoma is characterized by clusters of pleomorphic cells, including numerous atypical lipoblasts (Romanowsky stain)

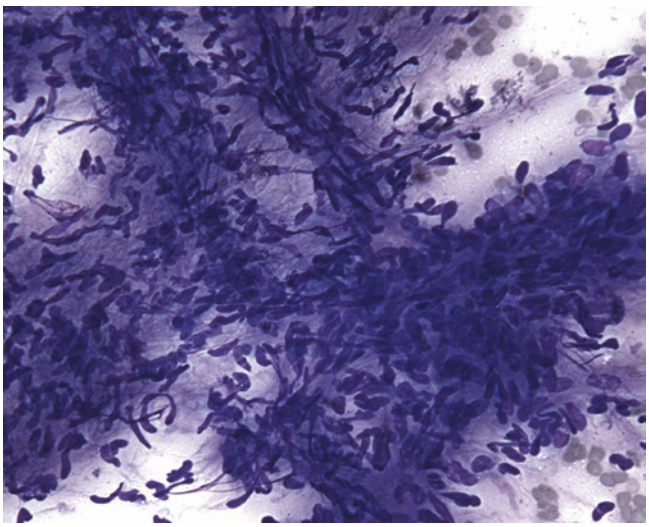

Fig. 17.5 Synovial sarcoma, monophasic. The distinctive pattern of dispersed cells alternating with cohesive cell clusters containing (hidden) branching vessels is characteristic for synovial sarcoma. The spindled tumor cells have bent nuclei with pointed ends resembling that of a schwannoma (Romanowsky stain)

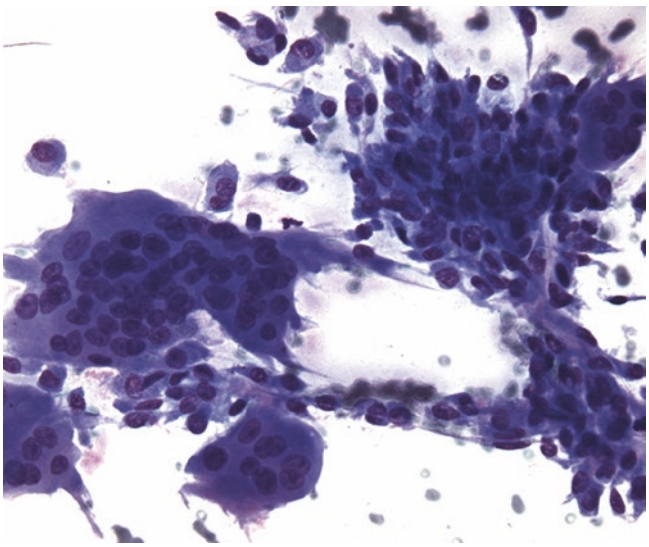

Fig. 17.7 Giant cell tumor of bone. Clusters of oval to spindled mononucleated cells bordered by multiple multinucleated giant cells, some of which contain more than 50 nuclei (Romanowsky stain)

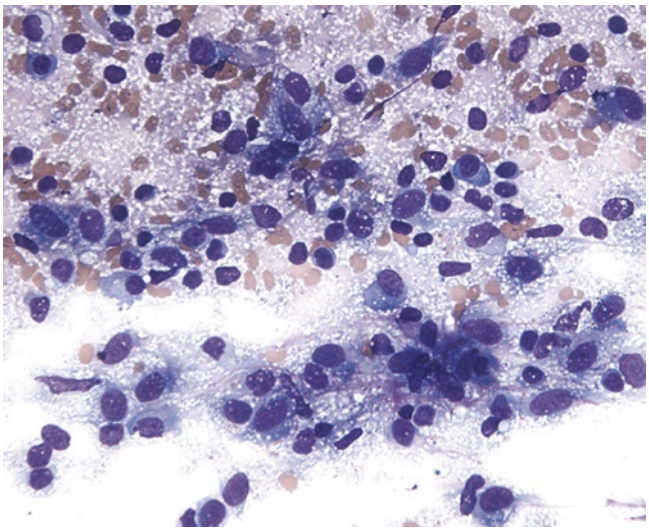

Fig. 17.6 Inflammatory myofibroblastic tumor. Dispersed and loose clusters of spindled fibroblastic cells with plump nuclei, conspicuous nucleoli, and frequent cytoplasmic tails are admixed with lymphocytes and plasma cells (Romanowsky stain)

Liposarcomas:

- Atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDLPS, Fig. 17.8) [25]
- Myxoid liposarcoma (MLPS, also in myxoid pattern, Fig. 17.4a) [26]
- Pleomorphic liposarcoma (PLPS, also in pleomorphic pattern, Fig. 17.4b) [27, 28]
- Dedifferentiated liposarcoma (DDLPS, also in pleomorphic/spindle cell pattern) [28]

Nonlipomatous tumors with an adipocytic component:

- Extrarenal angiomyolipoma/PEComa [29, 30]
- Extra-adrenal myelolipoma [31]
- Mammary-type myofibroblastoma
- Fat-forming solitary fibrous tumor [32–34]
- Intramuscular angioma
- Idiopathic retroperitoneal fibrosis [35]
- Fibrous hamartoma of infancy [36]
- Hemosiderotic fibrolipomatous tumor [37]

8. Are lipoblasts necessary for the diagnosis of liposarcomas?

Lipoblasts are multivacuolated adipocytes with a hyperchromatic, scalloped/indented nucleus (Fig. 17.4b). The presence of lipoblasts is a helpful finding in ALT/WDLPS and MLPS but is not required for the diagnosis. In addition, lipoblasts are commonly present in lipoblastoma/lipoblastomatosis [23] and can also be seen in DDLPS with homologous lipoblastic differentiation [28, 38]. In contrast, the presence of lipoblasts, is required for the diagnosis of pleomorphic liposarcoma [28]. It is important to recognize lipoblast-like cells or pseudolipoblasts in benign lipomatous tumors including hibernoma, spindle cell/pleomorphic lipoma, chondroid lipoma, and nonlipomatous tumors including myxofibrosarcoma. The lack of a hyperchromatic, sharply indented nucleus, and instead a small nucleus with smudged chromatin of a pseudolipoblast, distinguishes it from a true lipoblast.

Table 17.1 Cytomorphologic patterns of soft tissue tumors and commonly applied ancillary studies

Pattern	Immunohistochemistry	Molecular/genetic testing
Adipocytic	MDM2, CDK4, CD34, S100, RB1 loss, PLAG1, desmin, STAT6	FISH for *MDM2* amplification, *DDIT3* or *PLAG1* rearrangement
Myxoid	MUC4, EMA, CD34, S100, desmin	FISH for *EWSR1, FUS, NR4A3,* or *DDIT3* rearrangement
Spindle cell	SMA, desmin, β-catenin, S100, KIT, DOG1, CD34, TLE1, STAT6, ALK, H3K27*me*3	KIT mutational analysis, FISH for *ALK, USP6, SS18* or *PDGFB* rearrangement
Round cell	CD99, desmin, myogenin (myf4), SOX10, TdT, LCA, keratins, EMA, TLE1, WT-1 (C terminus), NKX 2.2, ETV4, CCNB3, BCOR, SATB2	FISH for *EWSR1, FOXO1*, *DDIT3*, or *SS18* rearrangement Detection of *HEY-1-NCO2*, *CIC-DU4*, or *BCOR-CCNB3* fusion
Epithelioid	S100, HMB45, SOX10, keratins, TFE3, CD34, SMARCB1/INI1 (loss), SMARCA4 (loss), CD31, ERG, CAMTA1, ALK, desmin, KIT, DOG1, SDH (loss), FOSB	FISH for *EWSR1, ALK, or TFE3* rearrangement
Pleomorphic	MDM2, CDK4, SMA, desmin, S100, SOX10, SATB2, keratins, Myogenin(myf4), myoD1, CD34	FISH for *MDM2* amplification

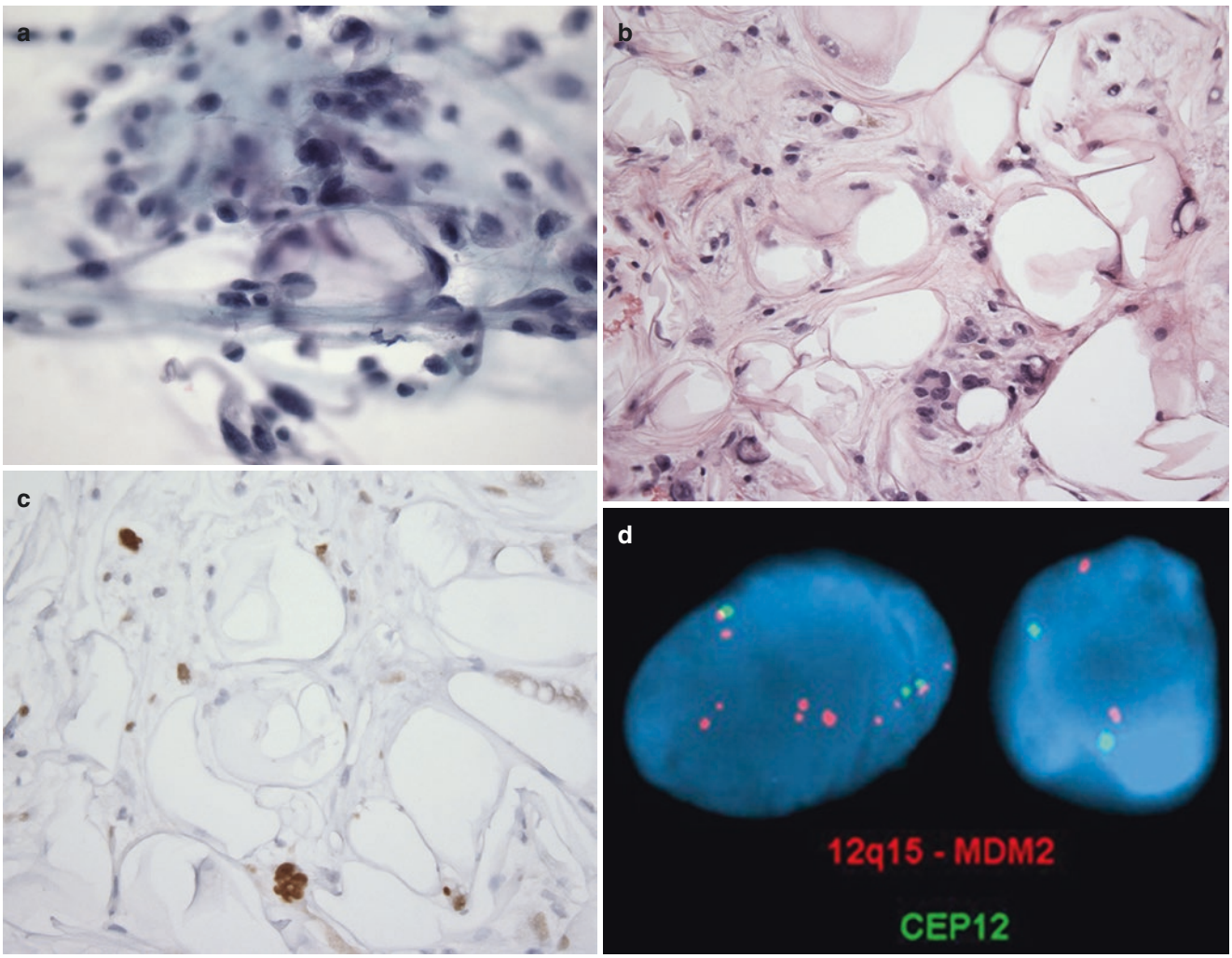

Fig. 17.8 Atypical lipomatous tumor/well-differentiated liposarcoma. Atypical spindled stromal cells and adipocytes with hyperchromatic nuclei (lipoblasts) are characteristic. Admixed histiocytes and inflammatory cells are present, resembling that of fat necrosis (**a**, smear with Papanicolaou stain; **b**, core needle biopsy with H&E stain). Nuclear overexpression of MDM2 (**c**, immunohistochemistry) in atypical tumor cells results from *MDM2* gene amplification, which can be confirmed by FISH (**d**, red MDM2 probe, green centromeric probe CEP12; courtesy of Paola dal Cin, PhD, Brigham and Women's Hospital, Boston, MA). NOTE: Histiocytes/macrophages in fat necrosis are weakly positive for MDM2 by IHC (nonspecific)

9. How is ancillary testing helpful in the differential diagnosis of lipomatous tumors?

The primary purpose of ancillary testing in lipomatous tumors is to:

- Confirm the diagnosis of certain benign lipomatous tumors, like CD34 positivity and RB loss of expression in spindle cell lipomas [39].
- Confirm the diagnosis of atypical lipomatous tumor/well-differentiated liposarcoma and dedifferentiated liposarcoma by demonstration of MDM2/CDK4 overexpression by immunohistochemistry (Fig. 17.8c) and/or *MDM2* amplification by FISH (Fig. 17.8d) [28, 40].
- Establish the diagnosis of myxoid liposarcoma by detecting *DDIT3* rearrangement [17, 18].
- Rule out/in a nonlipomatous tumor with a prominent fatty component, like STAT6 positivity in fat-forming solitary fibrous tumor [33, 41].

10. Is MDM2 positivity by immunohistochemistry specific for liposarcoma?

Absolutely not! ALT/WDLPS and DDLPS harbor amplification of chromosome 12q13–15 via supernumerary ring and giant marker chromosomes. The oncogene *MDM2* is encoded within the amplified locus, and the detection of *MDM2* amplification by FISH is the widely used approach to diagnosis of ALT/WDLPS and DDLPS. As a sensitive marker, MDM2 protein overexpression can also be demonstrated in intimal sarcoma and low-grade osteosarcomas like parosteal osteosarcoma with associated *MDM2* amplification and in a subset of other sarcomas like myxofibrosarcoma (MFS), PLPS, and malignant peripheral nerve sheath tumor (MPNST) without *MDM2* amplification [40, 42–44]. Of note, nonspecific MDM2 staining can even be seen in background histiocytes [45]. Therefore, care must be taken during interpretation to avoid misdiagnosis. Although *MDM2* amplification detected by FISH is the gold standard, MDM2 immunohistochemistry in combination with CDK4 and HMGA2, which are also encoded by genes within 12q13-15, increases the specificity [42]. Detection of *MDM2* amplification is especially helpful in separating ALT/WDLPS with a prominent myxoid stroma from MLPS; and DDLPS with a pleomorphic morphology from PLPS [43, 46, 47].

11. What are the entities in the differential diagnosis of lesions with a myxoid pattern?

Myxoid soft tissue tumors are characterized by the presence of abundant extracellular myxoid matrix material. This extremely diverse group of soft tissue tumors compasses benign tumors, those with a tendency for local recurrence/low malignant potential, myxoid sarcomas with a risk of distant metastasis, and myxoid variant of tumors with a specific line of differentiation.

Benign myxoid tumors:

- Ganglion cyst [48]
- Myxomas (intramuscular/cellular/juxta-articular) [48, 49]
- Other benign tumors often with myxoid stroma (spindle cell lipoma, nodular fasciitis) [22]

Myxoid tumors that are occasionally malignant or with a potential for local recurrence:

- Ossifying fibromyxoid tumor (OFMT) [50]
- Myoepithelioma of soft tissue (also seen in round cell pattern) [51]
- Atypical spindle cell lipomatous tumor [52]
- Myxoinflammatory fibroblastic sarcoma (MIFS, also in adipocytic and inflammatory patterns) [37, 59]

Myxoid sarcomas:

- Myxofibrosarcoma (MFS, low grade, high grade, also in pleomorphic pattern, Fig. 17.3) [53–55]
- Myxoid liposarcoma (MLS, also in adipocytic pattern, Fig. 17.4a) [26]
- Low-grade fibromyxoid sarcoma (LGFMS, also in spindle cell pattern, Fig. 17.9) [49, 56]
- Extraskeletal myxoid chondrosarcoma (EMC, also in epithelioid pattern, Fig. 17.2) [57, 58]

Myxoid variant of tumors with a specific line of differentiation:

- Nerve sheath tumors (neurofibroma, soft tissue perineurioma, microcystic schwannoma, epithelioid MPNST) [49, 60, 61]
- Myxoid solitary fibrous tumor
- Myxoid synovial sarcoma
- Dermatofibrosarcoma protuberans (DFSP), myxoid variant [17]
- Epithelioid inflammatory myofibroblastic sarcoma (E-IMS) [62]

12. What are the key features of myxoid tumors that are helpful in the differential diagnosis?

Because of significant clinical and morphologic overlap and limited value of immunohistochemistry, myxoid soft tissue tumors can pose considerable diagnostic challenges, especially in FNA samples. The key features that are helpful in the differential diagnosis and in the selection of proper ancillary studies in myxoid soft tissue tumors include the following:

- The location and depth of the lesion (distal or proximal extremity vs. retroperitoneum; dermal, subcutaneous, subfascial, or intramuscular)
- The extent and texture of myxoid stroma (abundant, granular in myxoma vs. myxochondroid in EMC) (Fig. 17.2)
- The presence (or absence) of nuclear pleomorphism (nuclear monotony indicating simple genetic events, such as translocations in MLPS and EMC (Fig. 17.2) vs. nuclear pleomorphism correlating complex karyotypes in MFS, Fig. 17.3)

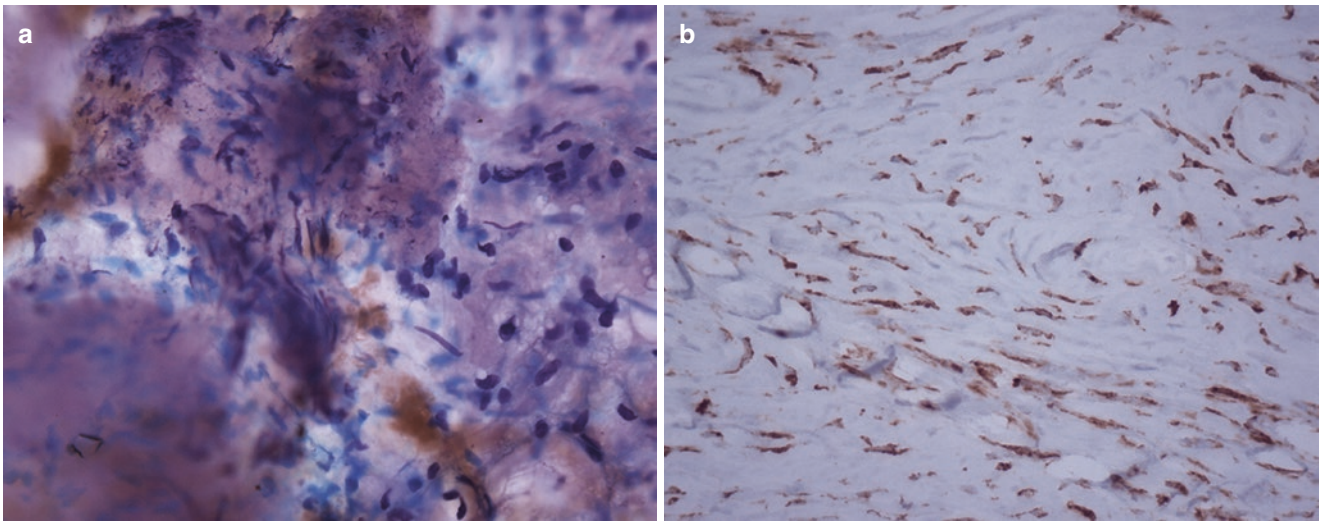

Fig. 17.9 Low-grade fibromyxoid sarcoma. Uniform, bland spindled cells are embedded in myxoid and hyaline matrix (left lower corner) (**a**, smear with Romanowsky stain). Cytoplasmic MUC4 expression distinguishes low-grade fibromyxoid sarcoma from its benign and malignant mimics, like perineurioma and myxofibrosarcoma, low grade, respectively (**b**, core biopsy with MUC4 immunohistochemistry)

- The pattern of vasculature (arborizing vascular network in MLPS (Fig. 17.4a) vs. curvilinear thin-walled vessels in MFS)
- The presence (or absence) of inflammatory cells (mixed inflammatory infiltrate in MIFS vs. lymphoplasmacytic infiltrate in IMT (Fig. 17.6) vs. neutrophil-rich infiltrate in E-IMS) [62]

13. How is ancillary testing helpful in the differential diagnosis of myxoid soft tissue tumors?

The primary purpose of ancillary testing in myxoid tumors is to:

- Confirm/exclude the diagnosis of LGFMS by showing MUC4 immunoreactivity/negativity when encountering a cytomorphologically uniform, low-grade, spindle cell tumor with myxoid stroma [49, 63].
- Confirm the diagnosis of MLPS (Fig. 17.4a), LGFMS (Fig. 17.9), or EMC (Fig. 17.2) by demonstrating *DDIT3*, *FUS*, or *NR4A3* rearrangement either by FISH and/or reverse-transcription polymerase chain reaction (RT-PCR), respectively, when encountering a cytomorphologically monotonous tumor with myxoid stroma [17, 64, 65].
- Establish the diagnosis of dedifferentiated liposarcoma by demonstrating MDM2/CDK4 overexpression by immunohistochemistry (Fig. 17.8c) and/or *MDM2* amplification by FISH (Fig. 17.8d) when encountering a myxofibrosarcoma-like tumor arising in the retroperitoneum [28].
- Establish a line of differentiation when encountering a soft tissue tumor that only occasionally displays prominent myxoid stroma, such as demonstrating EMA and claudin1 expression in soft tissue perineurioma [49] and CD34 expression and/or *PDGFB* rearrangement in myxoid variant of DFSP [17, 66].
- Rule out mimics of cellular myxoma, MFS, and MIFS, which are negative for most immunohistochemical markers.

14. How to distinguish low-grade fibromyxoid sarcoma from myxofibrosarcoma, low grade?

Low-grade fibromyxoid sarcoma (LGFMS), first described by Evans in 1987 [67], is characterized by a deceptively bland spindle cell morphology with a whorled growth pattern and alternating areas of myxoid and collagenous stroma (Fig. 17.9). It affects young to middle-aged adults and is defined genetically by *FUS-CREBL2* or *CREBL1* fusions [68]. In contrast, myxofibrosarcoma (MFS), the most common sarcoma of the elderly, is characterized by pleomorphic spindle cells associated with myxoid stroma and curvilinear vessels. Based on the degree of cellularity and the presence (or absence) of nonmyxoid areas, MFS can be further categorized into low grade, intermediate, and high grade [69]. These two distinct entities can be confused due to their similar sounding names and morphorlogic overlap in low-grade appearing tumors [70]. The distinctive clinical, morphologic, immunophenotypic, and genetic/molecular features of LGFMS and MFS are summarized in Table 17.2.

15. What are the entities in the differential diagnosis of lesions with a spindle cell pattern?

Soft tissue tumors with a spindle cell pattern form a large group of benign, intermediate biologic potential/locally aggressive or malignant mesenchymal tumors characterized by a fascicular growth pattern. The diagnosis of this group of tumors is usually straightforward with the integration of clinical data, cytomorphology, and a usually informative immunoprofile. It is important to recognize benign entities and to make a definitive diagnosis of these tumors to avoid evoking patient's anxiety, repeated biopsy, and unnecessary surgery (see Table 17.3). Depending on the clinical settings, diagnostic consideration should also include pseudosarcomatous

Table 17.2 Comparison between low-grade fibromyxoid sarcoma and myxofibrosarcoma

	Low-grade fibromyxoid sarcoma LGFMS	Myxofibrosarcoma MFS
Historical name	Hyalinizing spindle cell tumor with giant rosettes (HSCT)	Myxoid malignant fibrous histiocytoma (MFH)
Patient	Young adults (second to fourth decades) Equal gender distribution	Older adults (sixth to eighth decades) Slight male predominance
Site	Deep soft tissue of extremities and trunk	Superficial, multinodular mass in extremities and limb girdles
Histology	Mosaic architectural pattern Fibrous and myxoid areas with bland spindle cells Hyaline "giant" rosette	Pleomorphic spindle cells with myxoid stroma Curvilinear vessels Pseudolipoblasts
Cytomorphology	Variable, bland uniform ovoid to spindle cells with myxoid/hyaline stroma (Fig. 17.9a)	Pleomorphic spindle cells in a myxoid background (Fig. 17.3)
Immunohistochemistry	MUC4 + (sensitive and specific) (Fig. 17.9b) EMA+ (>60%)	Limited staining for CD34 or SMA
Cytogenetics	*FUS-CREB3L2* fusion (majority) *FUS-CREB3L1* fusion *EWSR1-CREB3L1* fusion	Complex karyotype
Treatment	Complete surgical excision	Complete surgical excision and/or radiation
Prognosis	Local recurrence rate: 10% (5 years)–64% (long term) Metastatic rate: 5% (5 years)–45% (long term)	Local recurrence rate: 50–60% Metastatic rate: 5–35%

Table 17.3 Summary of key clinical and cytomorphologic features, immunohistochemical markers, and common pitfalls of selected benign spindle cell tumors

Tumor	Key features	Immunohistochemistry	Common pitfalls Malignant mimics
Schwannoma	Cohesive tissue fragments with irregular edges; indistinctive cell borders; slender, wavy nuclei with pointed ends; long fibrillary cytoplasmic extensions; association with a peripheral nerve	*POS*: S100 and SOX10 (strong and diffuse), H3K27me3 (no loss) GFAP and AE1/AE3 (focal in deep-seated lesion) *NEG*: HMB 45, CD34, NFP, desmin	Degenerative nuclear atypia "ancient" changes mimicking MPNST; melanocytic schwannoma mimicking melanoma
Soft tissue perineurioma	Subcutis of limbs, slender cells with bipolar cytoplasmic processes, often myxoid stroma	*POS*: EMA, claudin1 CD34 (65%) *NEG*: S100, MUC4	LGFMS with EMA and/or claudin1 expression; DFSP with CD34 expression
Spindle cell/pleomorphic lipoma	Subcutis; middle-aged men; posterior neck/upper back; dispersed short spindle cells, long hyaline collagen fibers, fat cells, and myxoid background	*POS*: CD34 *NEG*: Desmin, Rb (loss)	Atypical spindle cell lipomatous tumor with CD34 expression and loss of Rb, MLPS with myxoid stroma and adipocytes
Nodular fasciitis	Rapid growth (<2 months), small (2–3 cm); subcutis; common sites: forearms and head and neck area; dispersed myofibroblasts in various shapes (bipolar, polygonal); myxoid background	*POS*: SMA (strong and diffuse), HHF35, calponin *NEG*: S100, CD34, β-catenin, desmin	Mitoses, ganglion-like cells and high ki67 index mimicking a variety of sarcomas; myofibroblastic proliferation mimicking desmoid fibromatosis
Leiomyoma	Large tissue fragments with smooth edges, clean background, bland cigar-shaped nuclei with blunt ends	*POS*: SMA, desmin, h-caldesmon *NEG*: S100, DOG1, KIT, β-catenin	Hyperplasia of interstitial cell of Cajal (KIT and DOG1-positive) in esophageal leiomyoma mimicking GIST

myofibroblastic proliferation (urinary tract), spindle cell/sarcomatoid carcinoma, spindle cell/desmoplastic melanoma, and sarcomatoid mesothelioma.

Benign spindle cell tumors:

- Schwannoma [60, 61]
- Neurofibroma [71]
- Soft tissue perineurioma [49]
- Nodular fasciitis (also in myxoid pattern) [72]
- Spindle cell lipoma [22]
- Leiomyoma [73, 74]
- Elastofibroma [75]

Spindle cell tumor with an intermediate biologic potential or locally aggressive:

- Desmoid fibromatosis [76, 77]
- Inflammatory myofibroblastic tumor (IMT, Fig. 17.6) [62, 78]
- Gastrointestinal stromal tumor (GIST)

- Dermatofibrosarcoma protuberans (DFSP) [66, 79]
- Solitary fibrous tumor (SFT) [33, 80]

Spindle cell sarcomas:

- Leiomyosarcoma [81]
- Low-grade fibromyxoid sarcoma (Fig. 17.9a, b) [49, 56]
- Malignant peripheral nerve sheath tumor (MPNST) [82, 83]
- Synovial sarcoma, biphasic or monophasic type (Fig. 17.5) [84, 85]
- Spindle cell/sclerosing rhabdomyosarcoma [86]
- Dedifferentiated liposarcoma (DDLPS) [28]
- Undifferentiated spindle cell sarcoma

16. How is ancillary testing helpful in the differential diagnosis of spindle cell tumors?

For the work-up of spindle cell tumors, immunohistochemistry is essential to determine the line of histologic differentiation (e.g., lipogenic, myogenic, neurogenic, or fibroblastic/myofibroblastic). FISH is usually applied to confirm the diagnosis in certain spindle cell tumors like synovial sarcoma, low-grade fibromyxoid sarcoma, inflammatory myofibroblastic tumor, and dedifferentiated liposarcoma when the results of immunohistochemistry are inconclusive. Although cytomorphologic features are not entirely specific, certain clinical presentations and discerning morphologic clues are useful in narrowing down the differential diagnosis and in selecting the right panel for immunohistochemistry [3, 40]. The key clinical and cytomorphologic features, helpful immunohistochemical markers, and common pitfalls of selected benign spindle cell tumors are summarized in Table 17.3.

17. Is it possible to grade sarcomas on FNA and/or core biopsy?

For many sarcoma types, grade is definitional: Ewing sarcoma, synovial sarcoma, and angiosarcoma are high-grade malignancies, whereas well-differentiated liposarcoma and dermatofibrosarcoma protuberans are low grade sarcomas. Some sarcomas are not readily gradable, such as epithelioid sarcoma and clear cell sarcoma. For sarcomas, histological typing does not provide sufficient prognostic information, the FNCLCC grading system, based on tumor differentiation, mitotic count, tumor necrosis, and histological grade, is widely used. Efforts should be made to grade sarcomas even with small biopsy samples, because the histological grade is the most important prognostic factor for sarcomas and provides guidance for therapy [87–90].

18. What are the entities in the differential diagnosis of lesions with a small round cell pattern?

Soft tissue tumors with a small round cell pattern are a group of highly aggressive sarcomas of children and young adults. Timely diagnosis and precise classification are required for clinical management. Although almost all the small round cell tumors yield cellular smears that are composed of dispersed round tumor cells with scant cytoplasm and many bare nuclei, some subtle but discerning morphologic clues are worthy extra attention: smear background (e.g., tigroid background in ES, lymphoglandular bodies in lymphomas) (Fig. 17.1), tumor cell size (e.g., small in DSRCT, large in alveolar RMS), the presence (or absence) of stromal material (e.g., osteoid in small cell osteosarcoma), and presence (or absence) of vessel-containing large tissue fragments (e.g., seen in synovial sarcoma). Certain small cell variants of sarcomas or one of the components of a sarcoma that exhibits small cell morphology are also included in this category. Depending on the clinical settings, differential diagnosis also extends to non-sarcomatous malignancies including lymphohematopoietic neoplasms, carcinomas, melanoma, and neuroblastoma.

Round cell sarcomas:

- Ewing sarcoma (ES, Fig. 17.1) [91–93]
- Desmoplastic small round cell tumor (DSRCT) [94–96]
- Embryonal rhabdomyosarcoma (embryonal RMS) [97]
- Alveolar rhabdomyosarcoma (alveolar RMS) [98–100]
- *CIC*-rearranged sarcoma [101]
- *BCOR*-rearranged sarcoma [102]

Other sarcomas with a small round cell variant/component:

- Synovial sarcoma (SS), poorly differentiated [85, 103]
- Osteosarcoma, small cell type [104, 105]
- Mesenchymal chondrosarcoma [106]
- Myxoid liposarcoma, high grade [26, 34, 107]

Non-sarcomas with a small round cell pattern:

- Small cell carcinoma (see Chap. 7)
- Merkel cell carcinoma [108, 109]
- Lymphomas and plasmacytoma (see Chap. 15)
- Myeloid sarcoma [110]
- Germ cell tumor
- NUT carcinoma [111]
- Neuroblastoma [112, 113]
- Small cell melanoma [114]
- Wilms tumor [115]

19. How is ancillary testing helpful in the differential diagnosis of small round cell sarcomas?

Given significant clinical, cytomorphologic, and immunophenotypic overlap among small round cell sarcomas, ancillary testing is often indispensable in establishing a specific diagnosis. An initial panel including CD99, keratins/EMA, S100 protein/SOX10, desmin, and TdT/LCA can exclude non-sarcomatous tumors and narrow down the differential diagnosis. Additional more focused markers to refine the diagnosis include myogenin/myoD1 (RMS) [116], TLE1 (SS) [117], WT-1 polyclonal against C-terminus (DSRCT)

[118], NKX2.2 (ES) [93], ETV4/DUX4 (*CIC*-rearranged sarcoma) [119], *BCOR*/CCNB3 (BCOR-rearranged sarcoma) [102], and SATB2 (small cell osteosarcoma) [120, 121]. Confirmatory molecular/genetic testing is essential when a small round cell sarcoma occurs at an unusual site (e.g., renal alveolar RMS), and/or at an unusual age (e.g., older than 40 years), and/or exhibits an unusual morphology (e.g., adamantinoma-like Ewing sarcoma) [122, 123]. Clinical, cytomorphologic, immunohistochemical, and molecular features of major differential diagnosis of small round cell sarcomas with relevant pitfalls are summarized in Table 17.4.

20. Is the presence of *EWSR1* rearrangement detected by FISH specific for Ewing sarcoma?

Absolutely not! *EWSR1* rearrangement is one of the "promiscuous" genetic alterations which can be seen in numerous and diverse tumor types. In addition to Ewing sarcoma, an *EWSR1* rearrangement can be identified in DSRCT, angiomatoid fibrous histiocytoma, clear cell sarcoma (CCS) of soft tissue, clear cell sarcoma-like tumors of the gastrointestinal tract, extraskeletal myxoid chondrosarcoma, myoepithelial tumors, and a subset of myxoid liposarcoma. *EWSR1* rearrangement can even be seen in clear cell carcinoma of salivary gland and rarely in mesothelioma and a subset of low-grade fibromyxoid sarcoma [124]. Therefore, a positive *EWSR1* FISH result is not entirely specific for Ewing sarcoma. The interpretation of FISH results must be made in conjunction with clinical, cytomorphologic, and immunophenotypic characteristics of each tumor.

21. What are the entities in the differential diagnosis of soft tissue tumors with an epithelioid/polygonal pattern?

Soft tissue neoplasms with an epithelioid or polygonal pattern are tumors composed of medium-sized or large, polygonal cells with moderate to abundant cytoplasm. They often yield cellular smears with both numerous dispersed neoplastic cells and cohesive cellular aggregates. This group of tumors includes well-defined entities (e.g., granular cell tumor, epithelioid sarcoma), the epithelioid variants of soft tissue tumors with a specific line of differentiation (e.g., epithelioid MPNST), and many vascular tumors (e.g., epithelioid hemangioendothelioma, epithelioid angiosarcoma).

Table 17.4 Differential diagnosis of small round cell sarcomas

Tumor	Cytomorphology	Immunohistochemistry and pitfall	Gene fusion/FISH or RT-PCR
Ewing sarcoma	Tigroid background, dispersed uniform, round cells with smooth nuclear membrane and fine chromatin	*POS*: CD99 (membranous) Fli1/ERG, NKX2.2 *Pitfall*: mimicking neuroendocrine tumors due to aberrant expression of keratin, synaptophysin, and chromogranin	*ESWR1-FLI1* *EWSR1-ERG* FISH with *ESWR1* break-apart probes
DSRCT	Abdominal masses in young male, clusters of small cells with occasional nuclear molding and vague acinar structure, fragments of stroma	*POS*: AE1/AE3, desmin, NSE, WT-1 (carboxyl-terminus) CD99 (variable) *Pitfall*: can occur in young women (25%) and/or at extra-abdominal sites, mimicking metastatic carcinoma due to strong keratin expression	*EWSR1-WT1* FISH with *ESWR1* break-apart probes
Alveolar RMS	Dispersed larger round cells with frequent single cell necrosis, binucleation, and wreath-like tumor giant cells	*POS*: desmin, myogenin/ myoD1 (strong and diffuse) *Pitfall*: mimicking neuroendocrine tumors and lymphoma due to aberrant expression of keratin, synaptophysin, and CD20, respectively	*PAX3-FOXO1A* *PAX7-FOXO1A* (rare) FISH with *FOXO1A* break-apart probes
Synovial sarcoma, poorly differentiated	Alternating large tissue fragments containing branching vessels and dispersed round to ovoid cells	*POS*: keratins/EMA (90%), TLE1 (strong and diffuse) *Pitfalls*: mimicking ES due to CD99 expression; mimicking carcinoma and adamantinoma due to keratin expression; overdiagnosis due to nonspecific TLE1 positivity in other tumors	*SS18-SSX1* *SS18-SSX2* FISH with *SS18* break-apart probes
CIC-rearranged sarcoma	Heterogeneity in nuclear shape and size, prominent nucleoli, and more cytoplasm and myxoid stroma	*POS*: CD99 (variable), WT-1, ETV4 *Pitfall*: mimicking ES due to considerable CD99 expression	*CIC-DUX4* *CIC-FOXO4* (rare) Detected by RT-PCR
BCOR-rearranged sarcoma	More common in bone, male predominance, wide morphologic spectrum with round to spindled cells in myxoid stroma	*POS*: CD99 (patchy), BCOR, CCNB3, TLE1, SATB2, CyclinD1 *Pitfall*: mimicking ES, SS, and small cell OS due to CD99, TLE1, and SATB2 expression, respectively.	*BCOR-CCNB3* *BCOR-MAML3* *BCOR-ZC3H7B* Detected by RT-PCR

Immunohistochemistry is central in establishing the diagnosis of most epithelioid soft tissue tumors and in excluding metastatic carcinoma, melanoma, mesothelioma, and even large cell lymphoma.

Well-defined soft tissue epithelioid tumors:

- Granular cell tumor [125]
- Glomus tumor [126]
- Clear cell sarcoma [127]
- Epithelioid sarcoma [128]
- Alveolar soft part sarcoma [129]
- PEComa [11, 30]
- Myoepithelioma/myoepithelial carcinoma of soft tissue [51]
- Extraskeletal myxoid chondrosarcoma (Fig. 17.2) [58]
- Sclerosing epithelioid fibrosarcoma [63]
- Extrarenal malignant rhabdoid tumor [130]
- SMACA4-deficient thoracic sarcoma [131, 132]

Epithelioid vascular tumors:

- Epithelioid hemangioma [133, 134]
- Epithelioid hemangioendothelioma (EHE) [10, 135]
- Epithelioid angiosarcoma (also in pleomorphic pattern) [136, 137]
- Pseudomyogenic hemangioendothelioma, AKA epithelial sarcoma-like hemangioendothelioma [138, 139]

Epithelioid variant of tumors with a specific line of differentiation:

- SDH-deficient gastrointestinal stromal tumor (GIST) [140]
- Epithelioid schwannoma [61]
- Epithelioid MPNST [141, 142]
- Epithelioid leiomyosarcoma [143]
- Epithelioid myxofibrosarcoma [144]
- Epithelioid inflammatory myofibroblastic sarcoma (E-IMS) [62]

22. What are the recent advances in ancillary testing for epithelioid soft tissue tumors?

In recent years, many advances have been made in defining the molecular alterations in numerous soft tissue tumors including many in this category of epithelioid soft tissue tumors [6]. These discoveries have guided the development of new surrogate markers which correlate well with the underlying genetic/molecular alterations, and many of which have been translated to routine diagnostic practice (Table 17.5) [45].

23. What are the mesenchymal tumors that express keratins and mimic carcinoma?

Keratins are intermediate filaments widely expressed in the cytoplasm of epithelial cells and hence the markers for carcinomas. However, certain distinctive soft tissue and bone tumors can express keratins and should be distinguished from carcinomas, especially at the head and neck region and/or in visceral organs [123, 153–155].

Table 17.5 Useful immunohistochemical markers that correlate with molecular alterations in selected epithelioid tumors

Marker	Epithelioid tumors	Molecular alterations
CAMTA1 [145]	Epithelioid hemangioendothelioma	*WWTR1-CAMTA1* fusion
TFE3 [146–148]	Epithelioid hemangioendothelioma Alveolar soft part sarcoma PEComa	*YAP1-TFE3* fusion *ASPSCR1-TFE3* fusion *SFPQ/PSF-TFE3* fusion
FOSB [149, 150]	Pseudomyogenic hemangioendothelioma Epithelioid hemangioma	*SERPINE1-FOSB* fusion *ZFP36-FOSB* fusion
ALK [62, 151]	Epithelioid inflammatory myofibroblastic sarcoma	*RANBP2-ALK* fusion
MUC4 [63]	Sclerosing epithelioid fibrosarcoma Hybrid SEF and LGFMS tumor	*EWSR1-CREB3L1* fusion *FUS-CREB3L2* fusion
SMARCB1 (INI1) [152]	Epithelioid sarcoma Malignant rhabdoid tumor Epithelioid malignant peripheral nerve sheath tumor	*SMARCB1* inactivation *SMARCB1* inactivation *SMARCB1* inactivation
SMARCA4 [132]	SMARCA4-deficient thoracic sarcoma	*SMARCA4* inactivation
SDHB/SDHA [140]	Epithelioid gastrointestinal stromal tumor Paraganglioma	Mutations in *SDHA, SDHB, SDHC, SDHD*

Modified from: Anderson and Hornick [48]

Soft tissue tumors with significant keratin expression:

- Epithelioid sarcoma (>99%, diffuse)
- Pseudomyogenic hemangioendothelioma (100%, diffuse)
- Extrarenal malignant rhabdoid tumor (>99%, diffuse)
- Synovial sarcoma (90%, diffuse in glands of biphasic, limited in other subtypes)
- Myoepithelioma (90%, diffuse)
- Desmoplastic small round cell tumor (90%, diffuse)
- Epithelioid hemangioendothelioma (up to 50%, patchy)
- Epithelioid angiosarcoma (up to 50%, diffuse)
- Alveolar rhabdomyosarcoma (up to 50%, patchy)
- Leiomyosarcoma (40%, patchy)
- Ewing sarcoma (30%, patchy)
- Inflammatory myofibroblastic tumor (30%, patchy)

Bone tumors with signifcant keratin expression:

- Chordoma
- Adamantinoma
- Intraosseous synovial sarcoma
- Dedifferentiated component of dedifferentiated chondrosarcoma
- Epithelioid hemangioma
- Epithelioid hemangioendothelioma
- Pseudomyogenic hemangioendothelioma
- Epithelioid angiosarcoma

24. What are the soft tissue tumors that can involve lymph nodes?

Most sarcomas metastasize via hematogenous spread to the lungs and bone and spare the lymph nodes. A subset of sarcomas, most commonly rhabdomyosarcoma, angiosarcoma, clear cell sarcoma, epithelioid sarcoma, and synovial sarcoma (in *acronym* RACES), can metastasize to the regional lymph nodes. SDH-deficient GIST can show early lymph node involvement but still have a relatively indolent clinical course [140]. Some mesenchymal tumors can arise de novo in lymph nodes (e.g., Kaposi sarcoma and follicular dendritic sarcoma). Interestingly, intranodal palisaded myofibroblastoma arises preferentially in inguinal lymph nodes [156].

25. What are the entities in the differential diagnosis of soft tissue tumors with a pleomorphic pattern?

Soft tissue tumors with a pleomorphic pattern are most commonly encountered in practice. They are characterized by morphologic pleomorphism (marked variation in tumor cell/nucleus size, shape, and type, but not necessarily the sign of malignancy) and often associated with underlying complex genetic alterations in sarcomas. Despite their obscured, nonspecific cytomorphology, most pleomorphic tumors can be classified according to the line of differentiation (e.g., lipogenic, myogenic, neurogenic) demonstrated by immunohistochemical studies (6–8 markers covering all lineages) [157]. Undifferentiated pleomorphic sarcoma is a diagnosis of exclusion and usually reserved to be diagnosed on surgical resection samples in which ample sampling and more comprehensive ancillary testing are achievable. Non-mesenchymal tumors like undifferentiated/anaplastic carcinoma, sarcomatoid mesothelioma, anaplastic large cell lymphoma, and melanoma also need to be excluded [158].

Benign and locally aggressive soft tissue tumors with morphologic pleomorphism:

- Nodular fasciitis (also in myxoid, spindle patterns) [72]
- Pleomorphic lipoma [159]
- Pleomorphic dermal sarcoma
- Myxoinflammatory fibroblastic sarcoma (also in myxoid pattern) [59]

Pleomorphic sarcomas with a line of differentiation:

- Pleomorphic liposarcoma (Fig. 17.4b) [27, 28]
- Pleomorphic leiomyosarcoma [81, 160]
- Pleomorphic rhabdomyosarcoma [161, 162]
- Malignant peripheral nerve sheath tumor
- Extraskeletal osteosarcoma [163]
- Angiosarcoma [164]

Dedifferentiated sarcomas:

- Dedifferentiated liposarcoma [28]
- Dedifferentiated solitary fibrous tumor [158]
- Dedifferentiated gastrointestinal stromal tumor [158]
- Dedifferentiated chondrosarcoma [165]
- Dedifferentiated chordoma [4]

Pleomorphic sarcomas with unknown line of differentiation:

- Myxofibrosarcoma, high grade (Fig. 17.3) [54]
- Radiation associated sarcomas [166]
- Undifferentiated pleomorphic sarcoma (UPS) [3]

26. Why do we need to subclassify pleomorphic sarcomas?

Pleomorphic sarcomas are a heterogeneous group of malignant mesenchymal tumors with diverse clinical presentation and a wide spectrum of prognosis (Table 17.6) [157]. Therefore, it is important to subclassify them with all the efforts even though it is not always possible on FNA/CNB.

27. What are the cytologic and molecular features of chondroblastoma?

- FNA smears are often hypercellular.

Table 17.6 Prognosis of common pleomorphic sarcomas

Tumor	Metastatic rate	5-year survival rate
Dedifferentiated liposarcoma	15–20%	70%
Myxofibrosarcoma	25–30%	70%
Undifferentiated pleomorphic sarcoma	30–35%	65–70%
Pleomorphic liposarcoma	50%	60%
Pleomorphic leiomyosarcoma	48–89%	35–50%
Extraskeletal osteosarcoma	60%	25%
Pleomorphic rhabdomyosarcoma	90%	27.1 months (median survival)

- Composed of round to slightly ovoid mononuclear chondroblasts, multinucleated osteoclast-like giant cells embedded in a myxoid matrix.
- The nuclei of the mononuclear cells are round, ovoid, or reniform with finely granular chromatin, irregular nuclear membrane, and longitudinal grooves or deep convolutions.
- Chicken wire pattern of calcification is infrequent in cytology sample.
- Mutations in *H3F3B* (located 17q25.1) have been identified in 95% of chondroblastomas.

References: [167–170].

28. What are the cytologic and molecular features of giant cell tumor of bone?

- FNA smears are often hypercellular.
- Composed of ovoid to spindle mononuclear cells and multinucleated osteoclast-like giant cells.
- The mononuclear cells appear to grow in a syncytium with ill-defined cell borders and little eosinophilic cytoplasm.
- The nuclei are round or ovoid with vesicular chromatin.
- The mononuclear cells may be mitotically active.
- The number of nuclei in innumerable multinucleated osteoclast-type giant cells is variable but may be as many as 50 or more (Fig. 17.7).
- Mutations *in H3F3A* (located at 1q42.12) have been identified in 92% cases of giant cell tumor of bone.

References: [4, 170, 171].

29. What are the common differential diagnoses of giant cell tumor of bone?

- Tenosynovial giant cell (aka PVNS)
- Brown tumor
- Giant cell-rich osteosarcoma
- Chondroblastoma
- Aneurysmal bone cyst

References: [4, 172].

30. What are the cytologic and immunophenotypic features of Langerhans cell histiocytosis of bone?

- The smears are moderate to high cellular.
- The histiocytes are round to ovoid containing large pale nuclei.
- The nuclei of the histiocytes are frequently kidney shaped and display a distinct irregular or folded nuclear outline.
- The chromatin pattern is bland, and the nucleoli are usually small and multiple.
- The cytoplasm of the histiocytic cells is often vacuolated or foamy and may contain phagocytosed debris.
- A modest number of eosinophils are commonly present in smears.
- These cells are positive for S100, CD1a, langerin, and cyclinD1.

References: [173, 174].

31. What are the three variants of chordoma histomorphologically? What are the cytologic and immunophenotypic features of conventional chordoma?

There are three histomorphologic variants of chordoma: conventional, chondroid, and dedifferentiated. Conventional chordoma is most commonly seen.

- The smears are characterized by myxoid matrix containing many dispersed large cells with abundant vacuolated cytoplasm.
- The large epithelioid cells arranged in cohesive nests or cords.
- The nuclei of the neoplastic cells are of moderate size with dark stain.
- The eosinophilic cytoplasm is abundant and contains multiple round clear vacuoles with "bubbly" appearance (physaliphorous cells named by Virchow in 1857).
- With Romanowsky-stained preparation, the vacuoles contain a densely metachromatic inclusion and magenta-colored background material.
- The neoplastic cells are positive for the epithelial markers (keratins and EMA, S100, and brachyury).

References: [175–178].

32. What are the three types of crystal deposit diseases of bone and joints, which can be evaluated by cytology?

- Monosodium urate crystals (gout) (Fig. 17.10)
- Calcium pyrophosphate crystals (pseudogout)
- Calcium hydroxyapatite (calcinosis)

References: [179, 180].

33. What are the most common clinicopathologic findings in periprosthetic pseudotumors?

- Patients often present with an enlarging painful mass status post total joint replacement.
- Imaging studies demonstrate the large heterogeneous mass with cortical bone destruction and soft tissue extension.

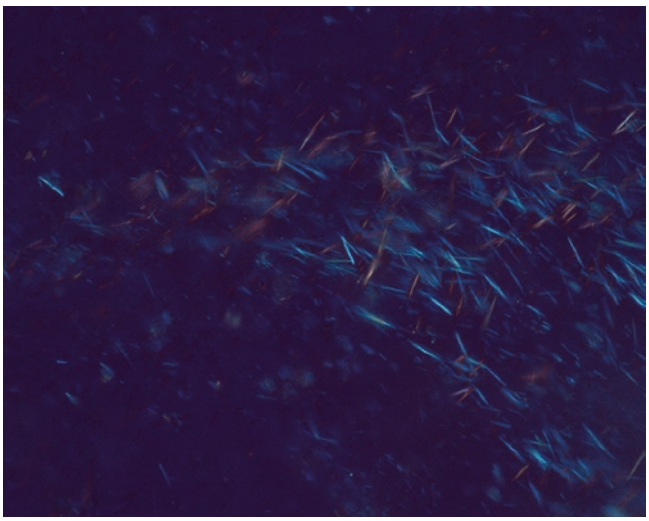

Fig. 17.10 Synovial fluid aspiration from left wrist shows numerous needle-shaped crystals (compensated polarized imaging 400×)

- Pathologic features include necrotic tissue, fibrin, fibrous tissue with foamy histiocytic and foreign-body giant cell reaction to metallic wear debris, methyl methacrylate orthopedic cement with residual radiographic contrast particles, and polyethylene.

References: [181, 182].

Case Presentation

Case 1

Learning objectives:

1. Recognize the key clinical and cytomorphologic features of this tumor.
2. Discuss the differential diagnosis of this tumor.

Case history:

A 60-year-old man with a right neck swelling for 7 years. Recently, it has slightly enlarged. A CT study shows a 1.5 cm subcutaneous nodule in the right posterior neck.

Specimen source:

Fine needle aspiration of the neck mass was performed by a cytopathologist. An air-dried, Romanowsky-stained smear and a Pap-stained smear were made from the aspiration.

Cytologic findings (Fig. 17.11a, b):

- A mixture of mature adipocytes, bland spindle cells, and hyaline, ropy collagen fibers in a background of myxoid matrix.
- Scattered short stubby vessels are noted.
- No pleomorphism, necrosis, or mitosis is identified.

Differential diagnosis:

- Benign lipoma with myxoid changes
- Spindle cell lipoma
- Atypical lipomatous tumor
- Myxoid liposarcoma
- Dermatofibrosarcoma protuberans (DFSP) with myxoid changes

Immunohistochemistry:

The tumor cells are positive for CD34 and show loss of nuclear expression of retinoblastoma protein (Rb).

Final diagnosis:

Spindle cell lipoma

Take-home messages:

1. Spindle cell lipoma typically occurs in the upper back, shoulder, or neck region in middle-aged to older men.
2. The tumor is subcutaneous and rarely exceeds 5 cm in greatest dimension.
3. In the appropriate clinical setting, FNA smears are diagnostic with four elements: mature adipocytes, bland spindle cells, ropy collagen fibers, and myxoid matrix.
4. The presence of floret-like multinucleated giant cells would suggest a pleomorphic lipoma.
5. The positivity of CD34 and loss of Rb expression support the diagnosis.

References: [22, 52, 183].

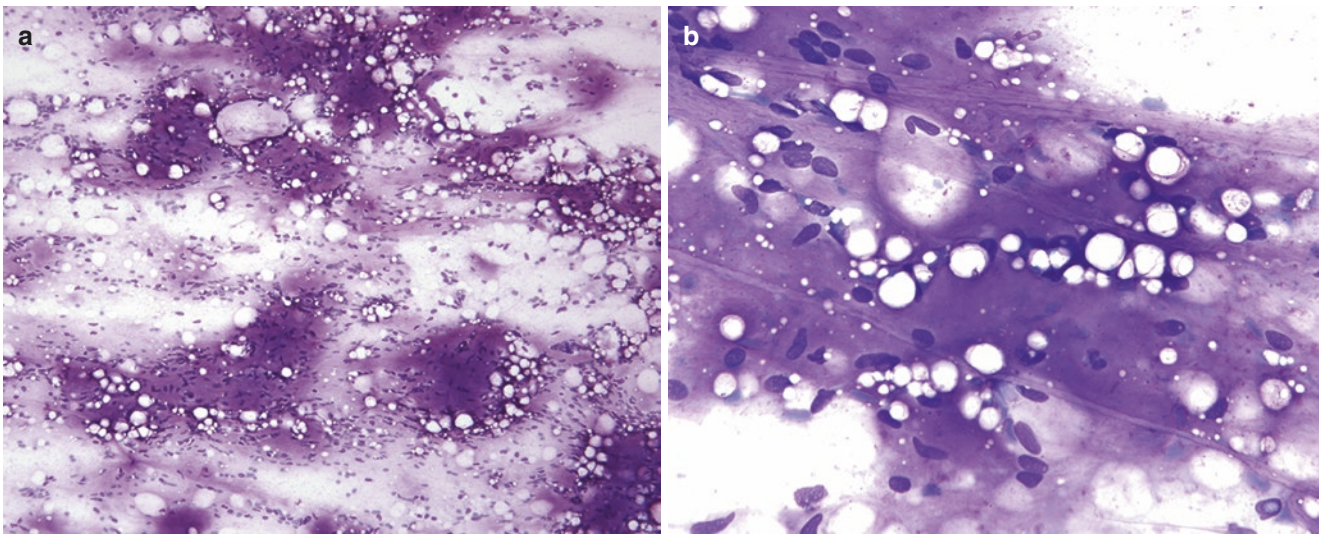

Fig. 17.11 Case 1. This tumor is characterized by a mixture of dispersed bland spindle cells and mature adipocytes in a myxoid background. The presence of long, ropy collagen fibers is a diagnostic clue, best seen at high magnification (**a**, **b**, Romanowsky stain)

Case 2

Learning objectives:

1. Recognize the characteristic clinical and cytopathology features of this entity.
2. Discuss the differential diagnosis of this entity.
3. Be aware of the diagnostic pitfalls of this entity.

Case history:

A 43-year-old woman presented with a newly discovered left arm mass. CT showed a subcutaneous lesion (2.3 × 1.5 × 1.2 cm) in the left elbow antecubital fossa.

Specimen source:

Ultrasound-guided fine needle aspiration of the left arm mass was performed by an interventional radiologist. An air-dried, Romanowsky-stained smear and an alcohol-fixed, Papanicolaou-stained smear were made from the aspiration. H&E stained slides of cellblock sections and core needle biopsy were also examined.

Cytologic findings:

- Cellular smears with clusters and dispersed plump spindled to stellate cells (Fig. 17.12a).
- Myxoid matrix, scattered osteoclast-like giant cells and mitoses are noted (Fig. 17.12b, c).
- Lack of hyperchromasia or necrosis.

Differential diagnosis:

- Low-grade myxofibrosarcoma
- Low-grade fibromyxoid sarcoma
- Myxoinflammatory fibroblastic sarcoma
- Dermatofibrosarcoma protuberans (DFSP) with myxoid changes
- Desmoid fibromatosis
- Nodular fasciitis

Immunohistochemistry:

Lesional cells are positive for SMA (strong and diffuse, Fig. 17.12d) and negative for desmin, S100, CD34, and β-catenin.

Final diagnosis:

Nodular fasciitis

Take-home messages:

1. Nodular fasciitis at early stage can be very proliferative, yielding very cellular smears. Mitoses, abundant myxoid stroma, osteoclast-like giant cells, and ganglion-like cells should not be mistaken for signs of malignancy.
2. Myofibroblasts in nodular fasciitis are strongly and diffusely positive for smooth muscle actin (SMA), muscle-specific actin (clone HHF35), and calponin; and negative for desmin, S100, and CD34, compatible with their myofibroblastic nature.
3. Nodular fasciitis is a self-limited or transient neoplasm, with recurrent gene rearrangements involving the *USP6* locus, which can be detected by FISH in difficult cases.
4. In patients with a typical clinical presentation (rapid growing mass, < 3 cm, subcutaneous, head and neck area or upper body/extremities), the characteristic cytomorphology by FNA allows for a definitive diagnosis to avoid surgical excision.

References: [72, 184, 185].

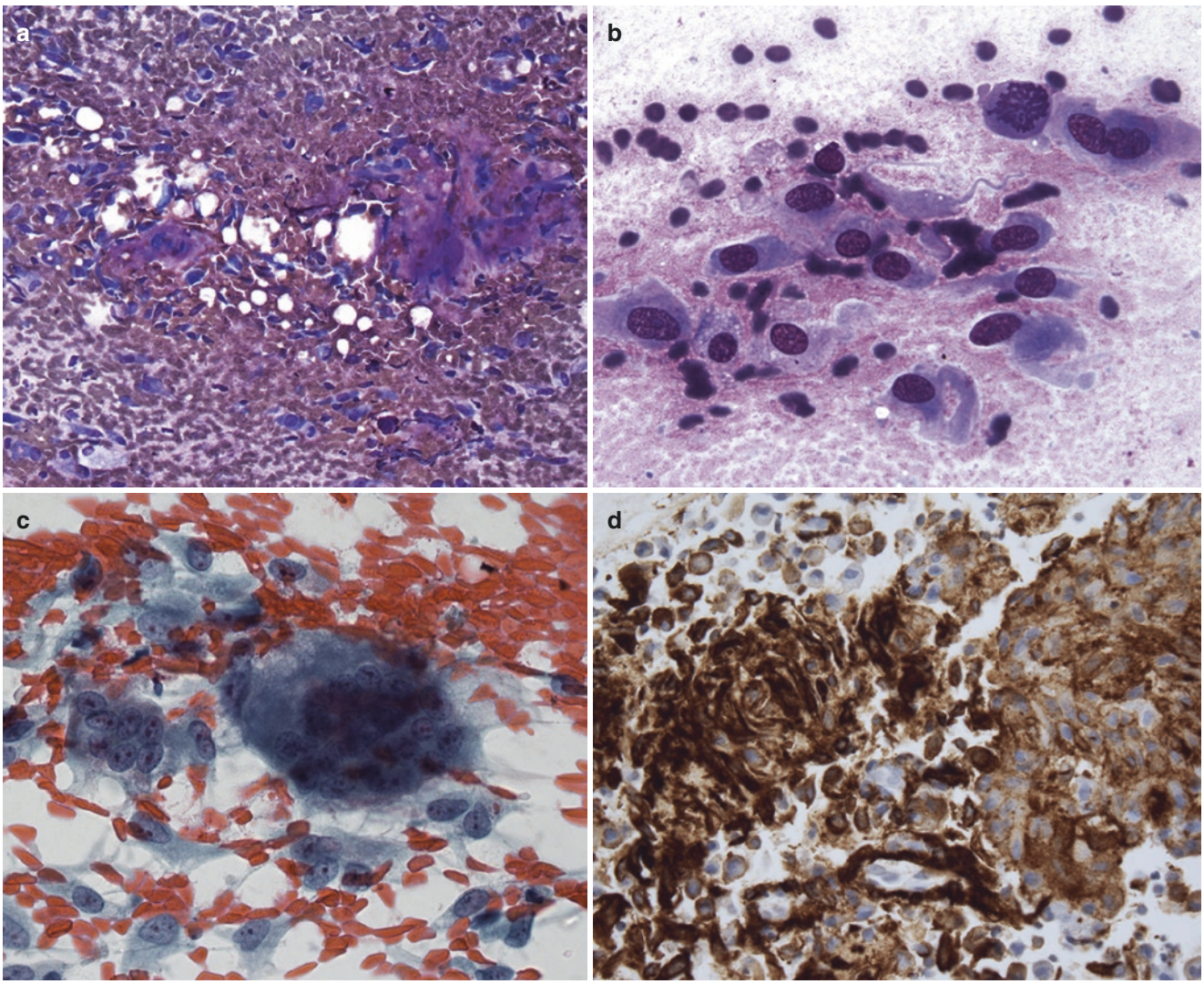

Fig. 17.12 Case 2. Cellular smears with clusters and dispersed plump spindled to stellate cells embedded in myxoid stroma (**a**, Romanowsky stain). Mitoses are not uncommon (**b**, Romanowsky stain). Scattered osteoclast-like giant cells are noted (**c**, Papanicolaou stain). Lesional cells are strongly and diffusely positive for SMA, compatible with their myofibroblastic nature (**d**, immunohistochemistry on cellblock)

Case 3

Learning objectives:

1. Recognize the clinical, radiologic, and cytomorphologic features of this tumor.
2. Discuss the broad differential diagnosis of this tumor.
3. Be aware of the diagnostic pitfalls of this tumor.

Case history:

A 60-year-old woman with a history of pT2 N1 M0 HPV associated tonsillar squamous cell carcinoma, status post radiation therapy 5 years ago. Now the patient presented with back and leg pain. PET/CT showed multiple lytic, FDG-avid osseous lesions highly suspicious for osseous metastasis. The largest lytic lesion is in the right ilium bone measuring 2.5 x 1.2 cm with cortical destruction.

Specimen source:

CT-guided fine needle aspiration and core needle biopsy of the right ilium mass were performed by an interventional radiologist. An air-dried smear with Romanowsky-stained smear and an alcohol-fixed, Papanicolaou-stained smear were made from the aspiration. H&E stained slides of cellblock sections and core needle biopsy were also examined.

Cytologic and histologic findings (Fig. 17.13a–c):

- Hypocellular smears.
- Small clusters and dispersed large, atypical epithelioid cells with an eccentrically located nucleus and abundant, basophilic, granular to microvacuolated cytoplasm. Binucleation is noted.
- Markedly pleomorphic cells with evidence of focal vasoformation on core needle biopsy.

Differential diagnosis:

- Metastatic carcinoma
- Metastatic melanoma
- Undifferentiated pleomorphic/spindle cell sarcoma
- Conventional osteosarcoma
- Dedifferentiated chondrosarcoma
- Pseudomyogenic hemangioendothelioma
- Epithelioid angiosarcoma

Immunohistochemistry:

The tumor cells are positive for AE1/AE3 (Fig. 17.13c), CD34, CD31, and ERG and negative for SATB2, S100, and FOSB.

Final diagnosis:

Epithelioid angiosarcoma

Take-home messages:

1. Epithelioid angiosarcoma, a rare, high-grade malignant neoplasm with endothelial differentiation, often occurs in older adults.
2. Primary bone epithelioid angiosarcoma often presents with multifocal lytic lesions and diffusely expresses keratins (up to 50%), mimicking metastatic carcinoma, especially in patients with a prior history of epithelial malignancy.
3. Epithelioid angiosarcoma should be considered in the differential diagnosis of any poorly differentiated epithelioid neoplasm, especially in visceral sites and bone.

References: [136, 164, 186, 187].

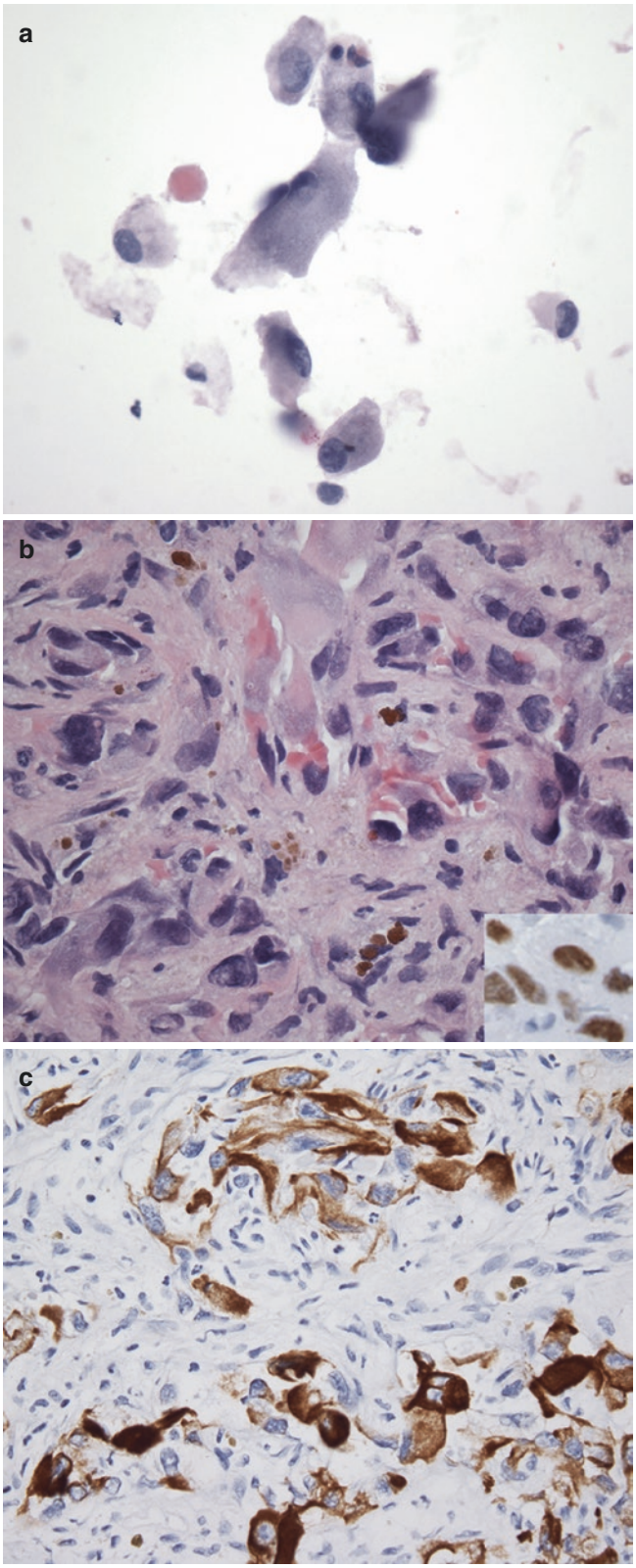

Fig. 17.13 Case 3. Hypocellular smears with scattered large, bizarre epithelioid cells (**a**, Papanicolaou stain). Markedly pleomorphic cells with evidence of focal vasoformation on core needle biopsy (**b**, H&E stain; inset: ERG immunostain). Tumor cells are strongly positive for keratins AE1/AE3, mimicking metastatic carcinoma (**c**, immunohistochemistry on core needle biopsy)

Case 4

Learning objectives:

1. Recognize the characteristic clinical, radiographic, cytopathology, and histopathologic features of this tumor.
2. Discuss the genetic alteration identified in this syndrome.

Case history:

A 24-year-old male presented with a left thigh lesion. X-ray showed a large calcified mass originating from the medial aspect of the proximal femur. MRI demonstrated a large heterogeneous mass with high intensity peripherally and low signal intensity for calcified areas on T2-weighted images. The marrow of the mass was connected to the bone marrow of the femur. Two additional cortical masses were also identified with one arising from the anterior aspect of the left femoral neck and the other from the posterior subtrochanteric region of the left femur.

Specimen source:

CT-guided fine needle aspiration and core biopsy were performed from the cartilaginous cap component.

Cytologic findings:

- The aspirate smears are paucicellular with opaque, glassy matrix.
- The cells are arranged in cohesive groups and singly dispersed forms (Fig. 17.14a, b).
- The aggregates of cohesive group contain lacunar spaces and the cells are uniform with centrally located, round, bland nucleus.

Differential diagnosis:

- Osteochondroma
- Periosteal chondroma
- Periosteal osteosarcoma
- Multiple hereditary osteochondroma
- Chondrosarcoma
- Chondroma of soft tissue
- Surface Osteosarcoma

IHC and other ancillary studies:

- S100 positive

Final diagnosis:

Low-grade chondrosarcoma arising in multiple hereditary osteochondroma

Take-home messages:

1. Multiple hereditary osteochondromas have inactivating mutations involving the genes *EXT1* and *EXT2* on chromosomes 8 and 11, respectively.
2. Multiple osteochondromas are manifestation of multiple hereditary osteochondromas, an autosomal dominant genetic disorder.
3. Osteochondromas are bulbous lesions attached to the underlying bone.
4. It is composed of a cartilage cap which merges with areas of cancellous bone.
5. Histologically, the cartilage cap varies in thickness with individual chondrocytes surrounded by abundant hyaline cartilage matrix.
6. Cytologically, the chondrocytes exhibit minimal cytologic atypia and no mitotic activity.

References: [188].

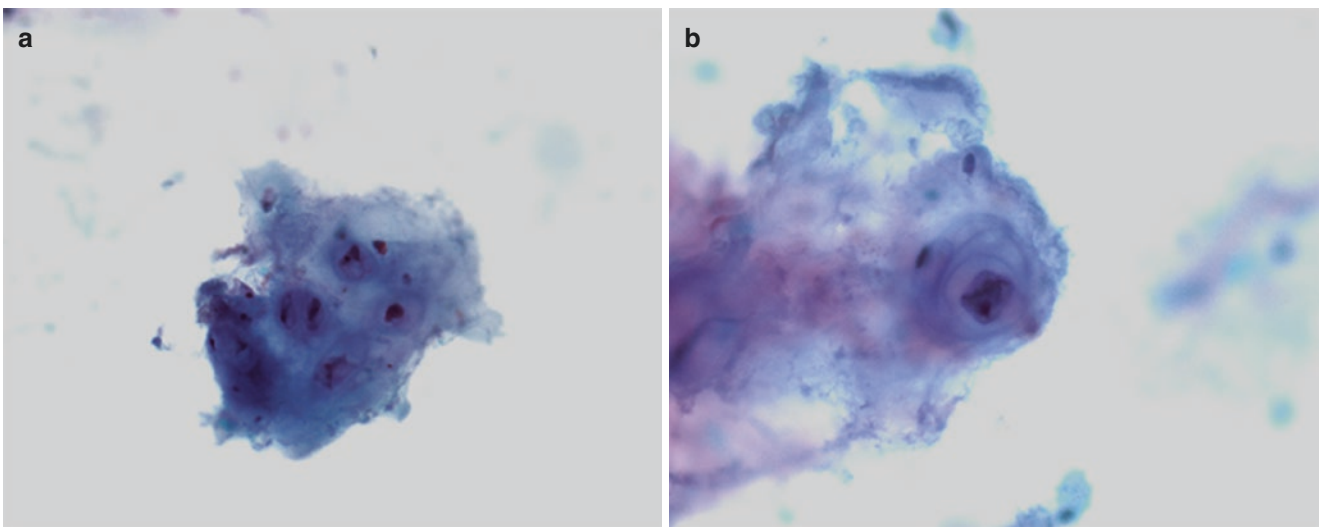

Fig. 17.14 Case 4. The aspirate smears are paucicellular with opaque, glassy matrix. The cells are arranged in cohesive groups and singly dispersed forms with lacunar spaces and centrally located, round, bland nucleus (**a** and **b**, Papanicolaou stain)

Case 5

Learning objectives:

1. Recognize the characteristic clinical, radiographic, cytopathology, and histopathologic features of this tumor.
2. Discuss the differential diagnosis of myxoid tumors.

Case history:

A 72-year-old male presented with a four-year history of sacral pain along with left numbness and tingling in lateral thigh. Images demonstrated a large destructive mass involving the sacral area.

Specimen source:

CT-guided fine needle aspiration and core biopsy were performed.

Cytologic findings (Fig. 17.15):

- The smears are characterized by myxoid matrix containing many dispersed large cells with abundant vacuolated cytoplasm.
- The large epithelioid cells arranged in cohesive nests or cords.
- The nuclei of the neoplastic cells are of moderate size with dark stain.
- The eosinophilic cytoplasm is abundant and contains multiple round clear vacuoles with "bubbly" appearance (physaliphorous cells named by Virchow in 1857).
- With Romanowsky-stained preparation, the vacuoles contain a densely metachromatic inclusion and magenta-colored background material.

Differential diagnosis:

- Chondrosarcoma
- Chordoma
- Myxopapillary ependymoma
- Myxoid liposarcoma
- Extraskeletal myxoid chondrosarcoma
- Myxofibrosarcoma
- Metastatic renal cell carcinoma

IHC and other ancillary studies:

The neoplastic cells are positive for the keratins, EMA, S100, and brachyury.

Final diagnosis:

Conventional chordoma

Take-home messages:

1. Chordoma is a slow-growing malignant neoplasm with a notochordal phenotype.
2. Chordoma develops in the axial skeleton, with the sacrum being the most common location.
3. Three histologic variants are present: conventional, chondroid, and dedifferentiated.
4. Physaliphorous cells and myxoid matrix are the characteristic features in cytology samples.

References: [22, 39, 175, 178, 189].

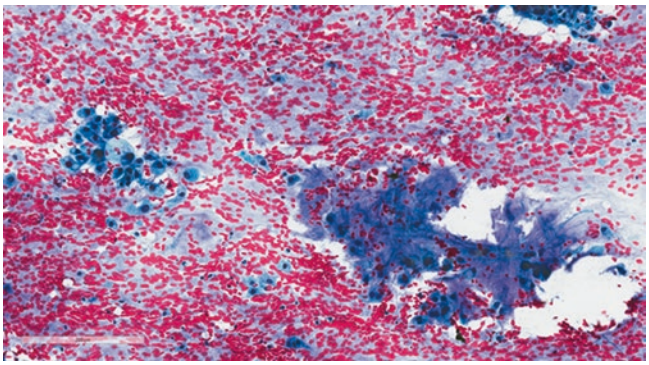

Fig. 17.15 Case 5. The smears are characterized by chondromyxoid matrix containing many dispersed large epithelioid cells with abundant vacuolated cytoplasm (Papanicolaou stain)

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Kidney, Adrenal Gland, and Retroperitoneum

18

Alarice C. Lowe

Contents

Abbreviations

CT	Computed tomography
EUS	Endoscopic ultrasound
FISH	Fluorescence in situ hybridization
FFPE	Formalin-fixed paraffin-embedded
FNA	Fine needle aspiration
GIST	Gastrointestinal stromal tumor
HLRCC	Hereditary leiomyomatosis and renal cell carcinoma syndrome
IHC	Immunohistochemistry
MR	Multiparametric magnetic resonance
N/C	Nuclear to cytoplasmic ratio
ROSE	Rapid on-site evaluation
WHO	World Health Organization

List of Frequently Asked Questions

Kidney

1. What entities are most likely to be evaluated by renal fine needle aspiration (FNA)?

Solid or partially solid/partially cystic renal masses, and those with radiologic findings which are equivocal for malignancy, will most frequently be evaluated by FNA [1]. Many renal lesions can be definitively characterized radiologically by computed tomography (CT) and multiparametric magnetic resonance (MR) imaging, and will not be biopsied. Most lesions found in the kidney are benign cysts which are readily identifiable by imaging. The remaining lesions include the partially cystic or solid renal masses. Solid renal masses with an identifiable fat component on imaging can also be diagnosed radiologically as angiomyolipoma. Fat is best seen on precontrast CT imaging [1].

Other lesions can be characterized with a high degree of certainty radiologically, but are likely to be biopsied for confirmation of diagnosis [1, 2]. Small lesions are more easily classifiable because the imaging characteristics are more easily attributed to the inherent nature of the tumor type. Clear cell renal cell carcinoma (RCC) is highly vascular and therefore enhancing during certain phases of CT and MR imaging. Papillary and chromophobe RCC show varying degrees of enhancement relative to clear cell RCC.

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_18

Table 18.1 Summary of selected renal malignant epithelial neoplasms and ancillary tools for classification

RCC subtype	Immunophenotype		Cytogenetic/molecular findings
	Positive stains[a]	Negative stains	
Clear cell (conventional) RCC	RCC+/CD10+/AMACR+/CA9+ (membranous)	CK7–/CD117–	Deletions in 3p
Papillary RCC	CK7+/AMACR+/CD10+	CD117–	Trisomies 7, 17; loss of Y
Chromophobe RCC	CD117+/CK7+	AMACR–	Multiple losses: −1, −2, −6, −10, −13, −17, −21
MiT family translocation RCC	RCC+/CD10+/AMACR+/variable HMB45/variable Melan A	EMA–/CK7–/CA9-	TFE3 or TFEB gene rearrangement
TFE3/Xp11 translocation RCC	TFE3+ (in addition)		Xp11 translocation with *TFE3* rearranegement
t(6;11)(p21;q12)	TFEB+ (in addition)		*t*(6;11)(p21;q12) with *TFEB* rearrangement
Mucinous tubular and spindle cell carcinoma	CK7+/AMACR+	CD10–/CD117–	[b]Multiple losses/gains
Oncocytoma	S–100A1+/CD117+/HNF1β	AMACR–/CK7– or weak	Loss of Y, X, or 1; 11q13 involvement
Clear cell papillary RCC	CK7+/CA9+ (cuplike)	AMACR-/CD117-/CD10-	N/A
Fumarate hydratase-deficient RCC		FH-/CK7-/RCC-/CD10- (except clear cell areas)	Germline 1q32 or 1q42–44 mutations
SDH-deficient RCC		SDHB- (all subtypes), SDHA- (SDHA-deficient tumors only)	Germline mutations in SDH subunits (A, B, C, or D)
Tubulocystic RCC	CK7+/AMACR+/CD10+		N/A
Collecting duct carcinoma	CK7+/P63+	RCC−/CD10−/AMACR−	Deletions in 1q, 6p, 8p, 13q, 21q
Urothelial Carcinoma	CK7+/CK20+/p63+	Variable PAX8	N/A

Abbreviations: *CA9* carbonic anhydrase 9; *FH* fumarate hydratase; *RCC* renal cell carcinoma; *SDH* succinate dehydrogenase
[a]All renal epithelial tumors are positive for PAX8 and PAX2
[b]Inconsistent results in the literature so far

Lesions larger than 4 cm may have associated necrosis, hemorrhage, and/or cystic change that results in a heterogeneous appearance; therefore the imaging findings are not as specific.

2. What information do clinicians need/want to know at the time of rapid on-site evaluation (ROSE)?

At the time of biopsy, the radiologist will want to know:

(i). If there is sufficient material to make a diagnosis
(ii). Whether the lesion is neoplastic or nonneoplastic (e.g., infection, malakoplakia)
(iii). How material should be appropriately allocated:
- If neoplastic, allocation of fresh tissue for ancillary testing (e.g., cytogenetics, flow cytometry) may be helpful for definitive diagnosis.
- If nonneoplastic, allocation of fresh tissue for microbiology may be helpful in the setting of acute or granulomatous inflammation.

3. What ancillary tests are important in the diagnosis of renal tumors?

Immunohistochemistry (IHC) can be helpful in separating metastatic tumors from primary renal tumors, and in classification of renal epithelial neoplasms in some settings. Some renal tumors cannot be classified by morphology and IHC alone. These tumors require cytogenetic results for definitive classification. Cytogenetic classification of renal tumors can be obtained by karyotype (requires fresh, unfixed tissue) or fluorescence in situ hybridization (FISH). FISH can be performed on smears, cytospin slides, liquid-based preparation slides, or formalin-fixed paraffin-embedded (FFPE) cell block material [3, 4]. Some institutions are beginning to utilize molecular analysis to obtain molecular-genetic information from FFPE material, but these are not yet commonly available (see Table 18.1).

4. How should renal FNA material be triaged and prepared for ancillary tests?

Material should be triaged according to whether the lesion is neoplastic or nonneoplastic.

For neoplastic entities, material should be allocated for IHC and cytogenetics, especially if the differential diagnosis includes a renal epithelial neoplasm. Most frequently a cell block is made for IHC. Fresh liquid material from the needle rinse can be submitted for karyotype and/or FISH pellet formation. Alternatively, material placed on direct smears, liquid-based preparations, or cytospin slides can be utilized directly for FISH analysis. Coverslipped and stained slides may be used [5, 6] as prior staining may not interfere with FISH testing [7]. Alternatively, if FISH testing is warranted

at ROSE, additional cytospin or smear slides may be kept unstained and uncoverslipped to facilitate FISH processing.

If the differential diagnosis includes lymphoma, fresh material from the needle rinse should be allocated for flow cytometry and cytogenetic analysis (karyotype/FISH).

For nonneoplastic entities, such as acute or xanthogranulomatous inflammation, fresh material should be allocated for microbiologic studies and cell block can be made to perform special studies for microorganisms.

5. Are there adequacy criteria for renal FNAs?

There are no universally accepted adequacy criteria for renal FNAs. As is practiced for other body sites that do not have a designated reporting scheme with defined adequacy criteria, samples are inherently adequate if a definitive diagnosis can be rendered [8].

Samples comprised entirely of benign renal parenchyma or cyst contents are most judiciously designated as inadequate/nondiagnostic. Clinical and radiologic correlation is needed to determine whether those findings alone may possibly represent the lesion of interest.

6. What information do clinicians need/want in the final report?

The final report should document a specific diagnosis, if possible, for benign and malignant neoplasms. For malignant diagnoses, information on classification and grading should also be included (please see Questions 7–9 for additional information).

7. Do malignant neoplasms need to be classified?

Classification and grading of malignant renal epithelial neoplasms on small biopsy is preferred to dictate treatment. Active surveillance, ablation, or neoadjuvant medical therapy are all emerging as viable options for patients with renal malignancies. In many instances, the original core or FNA biopsy may be the only material received for pathologic examination. Neoadjuvant chemotherapy is starting to be applied to locoregionally advanced and localized diseases [9]. Ablation (via cryotherapy or radiofrequency) is a viable option for localized renal neoplasms in poor surgical candidates and is feasible in the setting of metastatic disease as a form of locoregional control, and therefore FNA biopsy may represent the only tumor tissue available for grading. Tumor biopsy is recommended prior to ablation for definitive diagnosis. In fact, patients who will undergo medical or ablative therapy are more likely than surgical patients to have a renal biopsy performed [10–12]. In addition, lymphomas, sarcomas, and metastatic carcinomas/melanomas can occur in the kidneys and patients with these malignancies are treated differently from those with primary renal epithelial neoplasms. See Table 18.1 for the list of renal epithelial tumors and ancillary tools for classification.

8. Is grading necessary for malignant neoplasms?

Grading should be attempted for malignant neoplasms (please see question 7 above). The grade of the disease is one of the major predictors of clinical outcome and possible treatment options. Fuhrman nuclear grading is the most frequently used method for grading RCCs but has not been validated for most new WHO RCC subtypes. Fuhrman grading is possible on cytologic material [13]. Fuhrman grading should not be used in chromophobe RCC [14].

The 2016 WHO recommends using the four-tiered WHO/ISUP grading system which has been validated in CCRCC and papillary RCC [14, 15] and is similar to the Fuhrman system.

(i). Grades 1–3 are based on nucleolar presence and size:
   1. Grade 1 – inconspicuous or absent nucleoli at 400×
   2. Grade 2 – distinctly visible at 400×, but inconspicuous or invisible at 100×
   3. Grade 3 – distinctly visible at 100×

(ii). Grade 4 is based on the presence of marked nuclear pleomorphism, tumor giant cells, or rhabdoid or sarcomatoid differentiation.

In core biopsy samples, grading may be underestimated, but for the most part is accurate [16, 17]. Practically, because there is the concern of sampling error, grading on small samplings (FNA or core biopsy) should be qualified with a statement such as "in this limited sample."

9. Which benign neoplasms should be specifically reported?

The main benefit for specifically reporting a benign lesion is to understand the possible differential diagnosis. For example, a tumor misclassified as a metanephric adenoma (Fig. 18.1) is more likely to represent a nephroblastoma (Fig. 18.2) or the solid variant of papillary RCC than other malignant entities; and a tumor misclassified as an oncocytoma on small biopsy is more likely to represent a RCC with oncocytic features (Table 18.2) [18].

10. Which entities in the 2016 World Health Organization (WHO) Classification of Renal Neoplasms are new and how they be recognized?

Please note all of the following entities are infrequent with clear cell papillary RCC being relatively most common with a frequency of 4.1% in one series [19].

Hereditary leiomyomatosis and renal cell carcinoma syndrome (HLRCC)-associated RCC [20]

Also described as fumarate hydratase (FH)-deficient RCC. These tumors exhibit germline mutations in fumarate hydratase gene on 1q32 or 1q42–44. Unlike other germline mutant RCCs, these tend to be solitary.

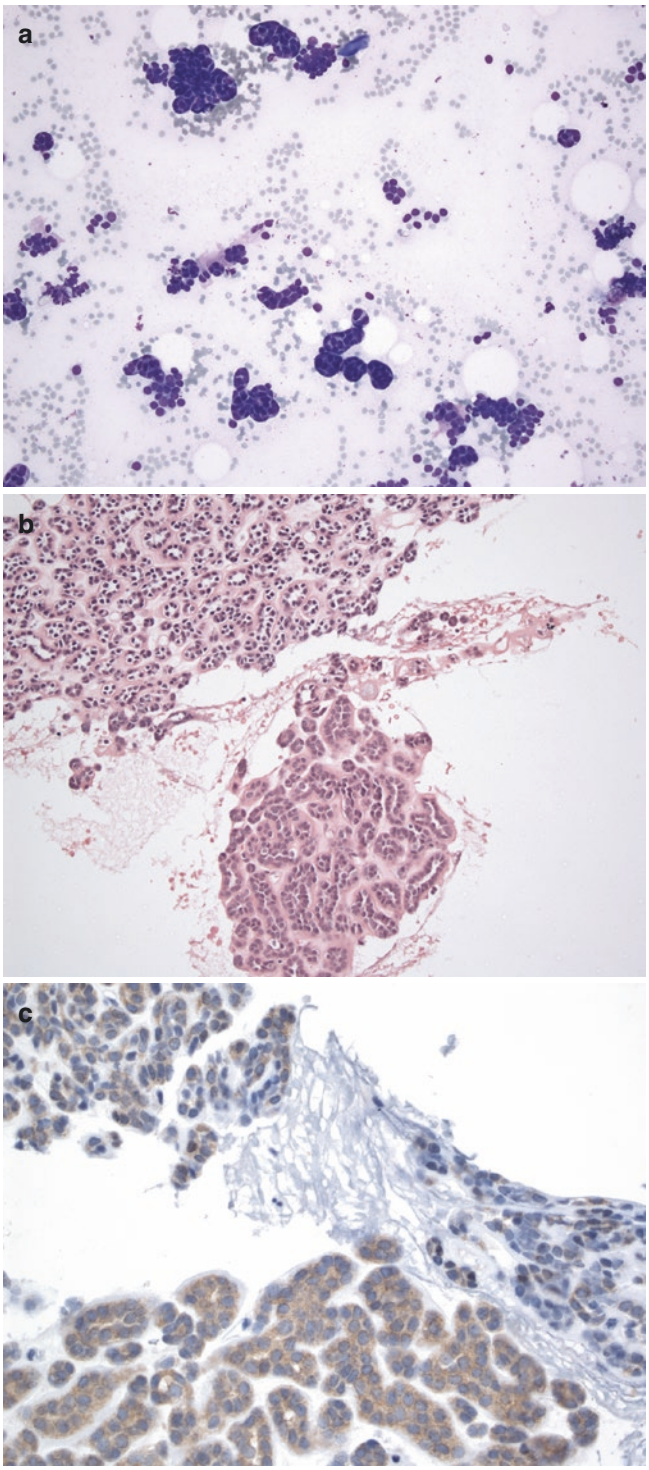

Fig. 18.1 Metanephric adenoma. (**a**) Numerous small tight rosette-like and tubular structures and other loosely cohesive groups. The cells are small with scant cytoplasm, dark homogeneous nuclear chromatin, and absence of nucleoli (Romanowsky stain, 200×). (**b**) Cell block shows acini and tubules with intervening stroma (H&E, 200×). (**c**) BRAF V600E immunostain performed on the cell block highlights the cytoplasm of the neoplastic cells (400×)

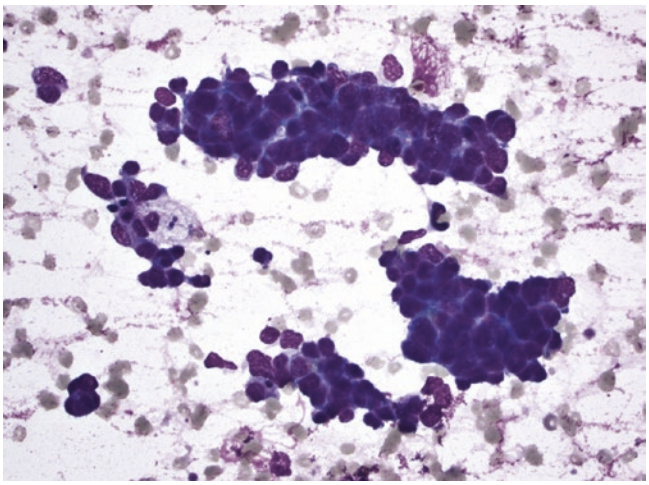

Fig. 18.2 Nephroblastoma (adult Wilm's tumor). Multiple small to large crowded aggregates of cells, which are variably sized and show hyperchromatic coarse chromatin. Associated apoptotic debris is present (Romanowsky stain, 200×)

Table 18.2 Benign renal neoplasms and their significant malignant differential diagnosis

Benign lesion	Differential diagnosis
Oncocytoma	Hybrid oncocytoma/chromophobe RCC Chromophobe RCC Tubulocystic RCC SDH-deficient RCC
Papillary adenoma	Papillary RCC
Angiomyolipoma	Other mesenchymal neoplasms
Epithelioid angiomyolipoma	Other epithelioid and/or mesenchymal neoplasms
Mixed epithelial stromal tumor	Other mesenchymal neoplasms
Metanephric adenoma	Nephroblastoma (Wilm's tumor) Solid variant of papillary RCC

The morphological hallmark of this tumor is a markedly enlarged eosinophilic or orangeophilic CMV-like nucleolus with perinucleolar clearing [20]. Most show papillary type 2 morphology with papillary architecture, abundant eosinophilic cytoplasm, and prominent nucleoli; a minority of cases may show clear cell component or a mixed pattern; rare cases show abundant desmoplasia. The cytomorphologic features of FH-deficient RCC described in a recent case series include strikingly enlarged malignant epithelial cells with abundant, voluminous cytoplasm with peripheral clearing, intranuclear cytoplasmic pseudoinclusions, and viral inclusion-like macronucleoli [21].

The differential diagnosis includes papillary RCC, collecting duct carcinoma, clear cell RCC, chromophobe RCC and Birt-Hogg-Dubé syndrome hybrid tumors (do not exhibit similar nuclear features, often multifocal, positive for C-kit),

and oncocytoma (benign nuclear features). FH-deficient tumors are negative for CK7, RCC, CD10 (except in clear cell areas), TFE3, UEA-1, and mucin (see Table 18.1).

Demonstration of complete loss of FH expression by IHC is helpful for the detection of FH-deficient RCC and HLRCC syndrome patients.

The clinical significance of recognition of these tumors is twofold. First, HLRCC patients exhibit germline mutations in fumarate hydratase on 1q, which is an autosomal dominant inherited condition with incomplete penetrance. Genetic counseling for the patient and family should be considered. Second, these tumors portend a poor prognosis. They are aggressive tumors that often metastasize to local and distant lymph nodes.

Succinate dehydrogenase (SDH)-deficient RCC [14, 22]

Morphologically, these tumors are unique for their eosinophilic flocculent cytoplasm with intracytoplasmic vacuoles/inclusions. Most SDH-deficient tumors show uniform cytology, solid or focally cystic growth, and round to oval, low-grade nuclei. The minority of cases with high-grade nuclei, sarcomatoid differentiation, or coagulative necrosis may behave more aggressively.

Immunophenotypically, SDH-deficient tumors show loss of cytoplasmic SDHB expression (regardless of the subunit that is mutated); SDHA-deficient tumors show loss of SDHA expression (in addition to loss of SDHB expression).

Germline mutations in SDH subunits (A, B, C, and D) disrupt mitochondrial complex II of the SDH enzyme. Mutations in the SDHB subunit are most frequent.

Diagnosis of this tumor is of clinical significance regarding screening and surveillance for at-risk patients or their family members for SDH-deficient RCC or other SDH-deficient tumors (pheochromocytomas/paragangliomas, GISTs, and pituitary adenomas).

Tubulocystic RCC

Tubulocystic RCC shows a predominantly cystic renal epithelial neoplasm with a macroscopically spongy cut surface, predominantly comprised of small to intermediate-sized cysts that are evenly distributed within a fibrotic background. Cysts are lined by a single layer of tumor cells with abundant eosinophilic and oncocytoma-like cytoplasm and WHO/ISUP grade 3 nucleoli. Solid components are absent [14, 23].

Cytologically, FNA material shows high-grade nuclei with oncocytic features, distinct cell borders, and intracellular windows [24]. The IHC profile is not specific. Clinically this tumor has a better prognosis than conventional RCC.

Acquired cystic disease-associated RCC [14]

By definition, these tumors occur in the context of end-stage renal disease and acquired cystic kidney disease. Tumors show eosinophilic or clear cell cytoplasm and prominent nucleoli and morphologically overlap other RCC types. They are not morphologically distinct and their cytologic features have not yet been reported. Clinically, these tumors tend to have indolent behavior. The key to identifying these tumors is the clinical context.

Clear cell papillary RCC (*CCPRCC*)

The morphologic hallmark of this clear cell tumor with low-grade nuclei is the presence of apical/luminal nuclear alignment (away from the basement membrane) [14, 25]. Cytologically, FNA samples of these tumors show sheets of monotonous bland-appearing cells in honeycomb arrangement or evenly distributed arrays. They may show eccentric nuclei placement. They show moderate clear cytoplasm that lacks abundant vacuolization, but small vacuoles may be seen. The nuclei are low grade [25, 26] (Fig. 18.3). Immunophenotypically, these tumors show a characteristic accentuated basolateral "cuplike" carbonic anhydrase IX staining (with relative sparing of the apical surface of the cell). They show diffuse CK7 staining and are negative for AMACR [25].

Distinguishing of CCPRCC from conventional clear cell RCC and papillary RCC on FNA is clinically significant because they appear to have a more indolent clinical course and are likely treated more conservatively.

11. Are there other changes in the 2016 WHO Classification of Renal Neoplasms that may affect cytology diagnosis/practice?

There are two additional changes that should be noted. First, multilocular cystic renal neoplasm of low malignant potential is the current WHO-recommended term for the lesion that was previously designated multilocular cystic RCC. These tumors should be comprised entirely of cysts lined by a single layer of low-grade tumor cells (WHO/ISUP grade 1 or 2) with abundant clear cytoplasm [14]. Second, the size for papillary adenoma has been changed from ≤0.5 cm to ≤1.5 cm. These tumors are unencapsulated with papillary or tubular architecture and low-grade tumor cells (WHO/ISUP grade 1 or 2). Because the presence of a capsule or higher-grade nuclei cannot be excluded on small biopsy, the diagnosis of papillary adenoma on a biopsy should be made with extreme caution [14]. The suggestion of papillary adenoma in the differential diagnosis may be most prudent.

Adrenal

12. What are the most frequent indications for biopsy of an adrenal lesion?

The most frequent indication for adrenal biopsy is to evaluate for metastatic disease in a patient with a known history of

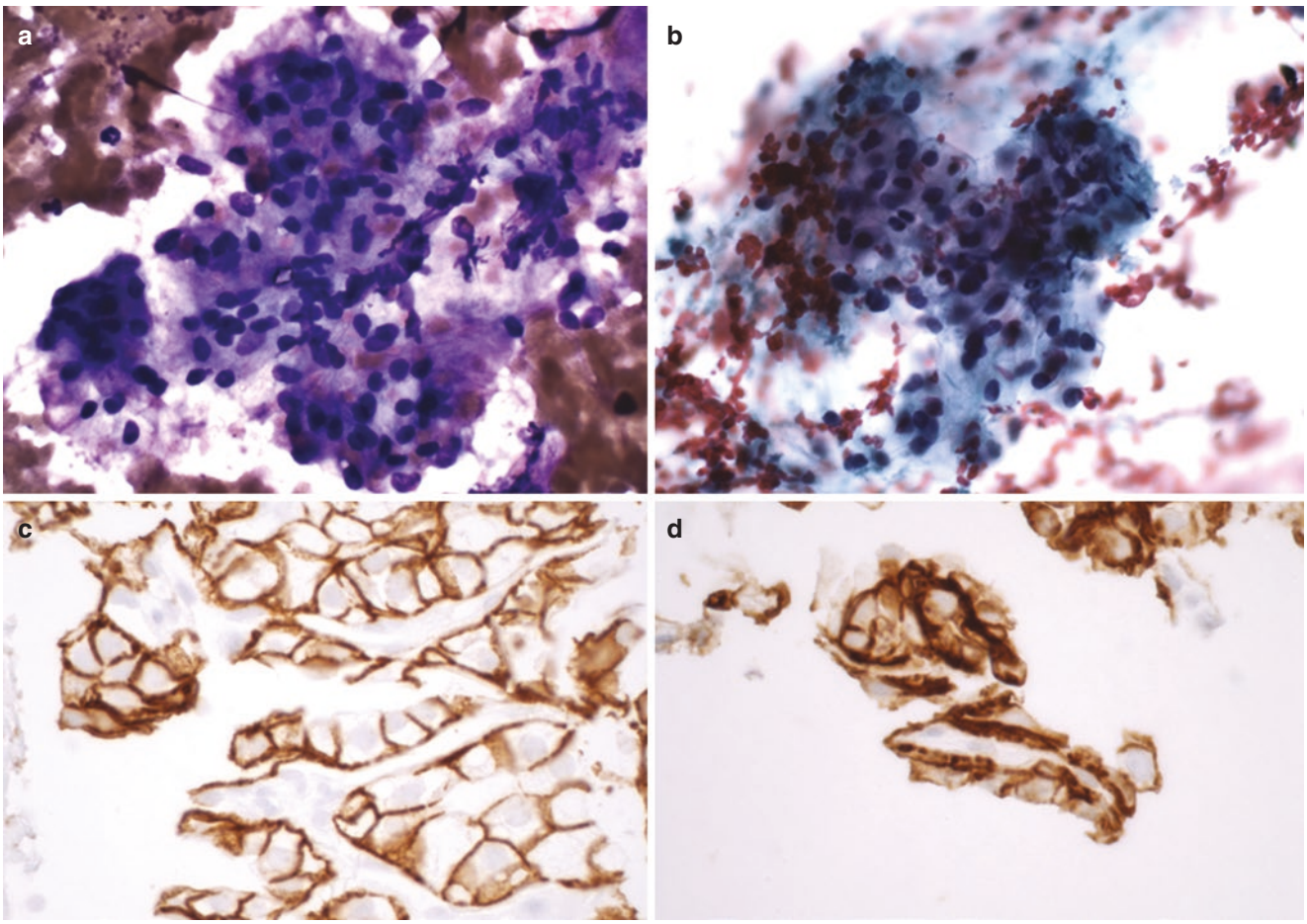

Fig. 18.3 Clear cell papillary renal cell carcinoma. (**a**) Well-organized cohesive aggregates of tumor cells with small round to oval nuclei showing fine dark chromatin and abundant cytoplasm. Cytoplasmic vacuolization is not as prominent as seen in conventional clear cell renal cell carcinoma (Romanowsky stain, 600×). (**b**) Tumor cells show low-grade features with small nuclei and even chromatin. Clear, vacuolated cytoplasm with distinct edges is better appreciated on alcohol-fixed preparations (Pap stain, 600×). (**c**) Tumors show a characteristic "cup-like" crisp membranous pattern of CA9 staining at the basolateral surface of the cells when cells are seen in a lateral position (**c**, core biopsy, 600×); however, the cells may only show a membranous staining pattern if the orientation of the cells is not "on edge" (**d**, cell block, 600×)

malignancy [27]. Adrenal biopsy may also be performed to evaluate an adrenal lesion with indeterminate or worrisome radiologic findings such as heterogeneity, necrosis, irregular margins, growth, or large size (generally larger than 4 cm) [27, 28]. In addition, adrenal biopsy can be used to establish the diagnosis of systemic infectious diseases like disseminated histoplasmosis.

13. Which entities are we most likely to encounter on adrenal biopsy/FNA?

A vast majority of adrenal lesions can be definitively characterized radiologically and therefore will not be biopsied. Most will be small adrenal cortical adenomas (homogeneously lipid-rich on unenhanced CT or lipid-poor on washout). Other less frequent entities that may be identified reliably by imaging include myelolipomas (exhibiting macroscopically identifiable fat) [29], hematoma/hemorrhage, and benign (uncomplicated) cysts [27].

In patients with a known history of malignancy, metastasis is the most frequent finding (50–70%) and most metastases are of lung origin (63–75%) [30–33]. The next most frequently encountered lesion is adrenal cortical adenoma (12–33%), followed by adrenal cortical carcinoma and pheochromocytoma (2 to ~10%) [30, 31, 33, 34].

In patients without known malignancy, adrenal cortical adenomas are the most frequent biopsied lesion; other less frequently identified lesions include pheochromocytoma, adrenal cortical neoplasm/carcinoma, occult metastasis, and hematoma [28, 35].

Rare lesions include sex cord stromal tumors (granulosa cell and Leydig cell tumors), adenomatoid tumor, schwannoma, and neuroblastic tumors (neuroblastoma, ganglioneuroblastoma, or ganglioneuroma) [36].

14. Is it safe to perform FNA/core biopsy of adrenal nodules?

Image-guided biopsy of adrenal lesions is safe with a complication rate of 0–9%, but consideration should be given to the expected lesion, approach taken, and operator experience [27, 30, 32, 33, 35, 37–42]. Alpha adrenergic blockade may be considered in suspected pheochromocytoma to prevent hypertensive crisis [28]. Known adrenal cortical carcinomas should not be biopsied due to the possibility of tumor seeding and lack of benefit [27, 43, 44].

15. What clinical information should be noted pre-procedurally?

Whether the patient has a prior known malignancy is important. Because most adrenal nodules from patients with known malignancies will be metastasis, knowledge of the patient's prior tumor can facilitate identification. Specific note should be made in patients with a history of lung carcinoma, which is by far the most frequent source of adrenal metastasis [30–33]. Note should also be made for patients with a history of clear cell renal cell carcinoma or hepatocellular carcinoma. Both may show similar radiologic findings to a lipid-rich adrenal cortical adenoma [27] and cytologic similarities to adrenal cortical tissue.

Biochemical studies are often underutilized and may be helpful in suggesting pheochromocytoma or adrenal cortical neoplasm. Excess catecholamines may be seen in functioning pheochromocytomas. Increased glucocorticoids (which may cause Cushing syndrome), aldosterone (Conn syndrome), or androgens/estrogens (virilization/feminization) may be seen in adrenal cortical hyperfunction due to adenoma or carcinoma and, in some instances, adrenal hyperplasia.

Biopsy approach and lesion laterality should be noted. Percutaneous image-guided biopsy of right-sided adrenal lesions may take a transhepatic approach; therefore, hepatocytes may be present in the sample as contamination [27]. EUS-guided FNA biopsy of adrenal nodules may have a duodenal or transgastric approach; therefore, gastrointestinal contamination may be encountered [37, 39, 41, 42].

16. What information do clinicians need/want to know at the time of ROSE?

At the time of ROSE, clinicians want to know whether sampling is sufficient to make a specific diagnosis. If metastasis is suspected and only adrenal parenchyma is sampled, additional passes should be performed. Since adrenal FNA is relatively infrequent and encountered entities are very diverse, there are no specific adequacy criteria for adrenal FNA.

17. How should adrenal FNA material be allocated?

Material should be allocated for cell block for IHC to confirm the lesional cells of origin. For suspected metastatic carcinomas, especially of pulmonary origin, additional material should be allocated for molecular testing (ie, next-generation sequencing) and PD-L1 study to direct targeted therapy. Material for flow cytometry and cytogenetic studies is appropriate if lymphoma is suspected at time of ROSE (see Chap. 15 Lymph Node for details). If an infectious process is suggested, fresh material should be allocated for microbiology.

Molecular differences have been noted that distinguish adrenal adenoma from carcinoma, but they have not been incorporated into the diagnostic algorithm of adrenal cortical lesions at the time of publication [36]. Although a significant fraction of pheochromocytomas are associated with known hereditary susceptibility genes, molecular or cytogenetic ancillary studies are not needed for diagnosis.

18. How can we best distinguish between primary adrenal neoplasms?

Primary adrenal neoplasms include adrenal cortical adenoma/carcinoma, pheochromocytoma, and myelolipoma. Adrenal cortical adenomas tend to have low cellularity specimens showing small aggregates of cells with low N/C ratio and abundant bubbly cytoplasm; nuclei are small and round and show no or single small nucleoli; the background is lipid-rich/vacuolated and contains many naked nuclei [40]. In our experience, rare large cohesive clusters of cells may be seen, which show similar cells with low N/C ratio, abundant vacuolated cytoplasm and bland nuclei. Adrenal cortical adenoma is morphologically similar to adrenal hyperplasia. Cytologically, adrenal cortical carcinoma may show well-differentiated or poorly differentiated morphology. Well-differentiated adrenal cortical carcinomas show solitary cells with vacuolated cytoplasm, occasional nucleomegaly, and rare mitoses [35]. These may be difficult to distinguish from adrenal cortical adenoma, even if sampled well. Poorly differentiated adrenal cortical carcinomas are high cellularity specimens, showing single cells with high N/C ratio, large nuclei with prominent nucleoli, necrosis, and mitoses [35, 40]. These may not be distinguishable morphologically from other poorly differentiated malignant neoplasms. IHC are often required.

It should be noted that the diagnosis of adrenal cortical carcinoma is based upon the Weiss criteria which incorporates some features that require histologic diagnosis [36]. Therefore, although high-grade nuclear features and the presence of necrosis and numerous mitoses of an adrenal cortical neoplasm may suggest an adrenal cortical carcinoma, definitive categorization should be deferred until excision.

Morphologically, pheochromocytomas often show cellular specimens with large, polygonal cells with anisocytosis, hyperchromatic nuclei with prominent nucleoli, and

granular/ill-defined cytoplasm; scattered cells with giant nuclei are often present [40].

Immunophenotypically, adrenal cortical lesions (whether hyperplasia, adenoma, or carcinoma) are positive for SF1, α-inhibin, Melan A, and calretinin. Synaptophysin is also positive in adrenal cortical lesions but is not specific. Pheochromocytoma tumor cells are positive for neuroendocrine markers (including synaptophysin) and GATA3 (80%). Sustentacular cells in pheochromocytoma are positive for S100 protein [36].

Retroperitoneum

19. Which entities may be encountered in retroperitoneal FNAs?

The English language literature describing FNAs of the retroperitoneum is sparse and predominantly describes Asian/South Asian patient cohorts and often encompasses renal, adrenal, and pancreatic FNAs [45–48].

In our own experience at Brigham and Women's Hospital, most retroperitoneal FNAs (excluding kidney, adrenal gland, and pancreatic FNAs) are performed on specimens designated as retroperitoneal lymph nodes, often in patients with a known primary malignancy. Metastases to retroperitoneal lymph nodes may arise from hematopoietic malignancies or carcinomas. Carcinomas are most frequently from the gynecologic or genitourinary primary sites. A minority of specimens are designated retroperitoneal masses. These most often represent enlarged lymph nodes but may also represent true retroperitoneal lesions.

The following organs are present in the retroperitoneum and primary tumors of these sites may be the source of masses in this space:

1. Kidney and ureter
2. Adrenal gland
3. Pancreas
4. Ascending and descending colon
5. Duodenum
6. Aorta and vena cava
7. Paravertebral nervous system

Primary retroperitoneal tumors not arising from the organs mentioned above include retroperitoneal sarcomas and other uncommon tumors. A vast majority of retroperitoneal sarcomas are dedifferentiated liposarcoma (37%), well-differentiated liposarcoma (26%), or leiomyosarcoma (19%). These may be suggested on imaging, respectively, due to the presence of both fat dense and solid components, a homogeneous fat dense mass, and a mass arising from a vessel with extensive necrosis or cystic change [49]. Solitary fibrous tumor (6%), malignant peripheral nerve sheath tumor (3%), undifferentiated pleomorphic sarcoma (2%), and other sarcomas (7%) are uncommonly encountered [49].

Other uncommon tumors encountered in the retroperitoneum include:

1. Paraganglioma – often cystic, peripancreatic [50, 51]
2. Gastrointestinal stromal tumor (GIST)
3. Masses containing hematopoietic elements (Fig. 18.4) – fat-containing by imaging and therefore are in the differential for liposarcoma [52]:
   (a) Extra-adrenal myelolipoma – encapsulated, well circumscribed
   (b) Mass-forming extramedullary hematopoiesis/sclerosing extramedullary hematopoietic tumor – most frequently seen in a patient with hematopoietic disorders, usually chronic myeloproliferative disorders, and located in the paravertebral region [53, 54]
4. Retroperitoneal fibrosis [55] – may be mass forming, idiopathic, or IgG4-related; requires clinical and serologic correlation [56]
5. Schwannoma [57], neurofibroma
6. Neuroblastoma, ganglioneuroblastoma, ganglioneuroma
7. Erdheim-Chester disease [58]

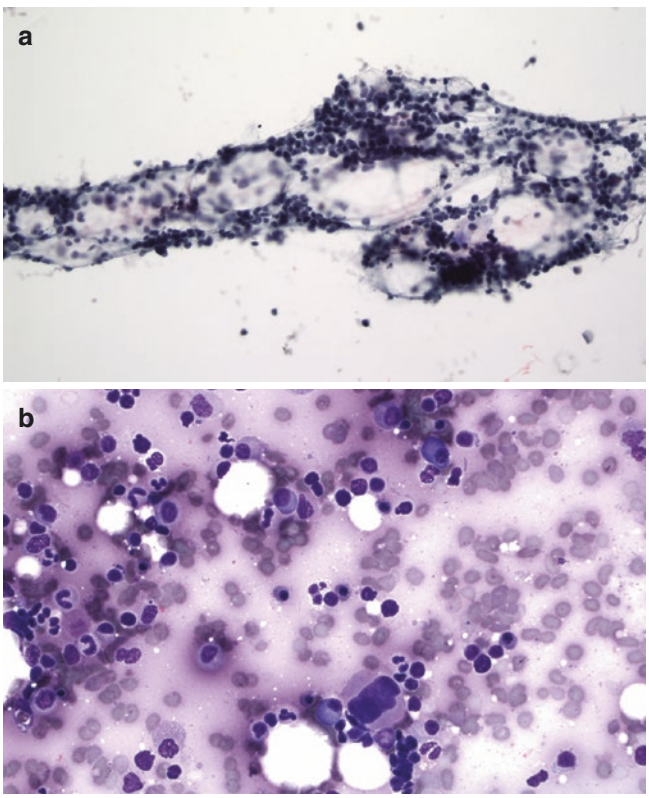

Fig. 18.4 Myelolipoma. (**a**) At scanning magnification, a polymorphous cellular infiltrate is intermixed with adipose tissue (Pap stain, 200×). (**b**) Higher magnification shows hematopoietic precursors at various stages of maturation (Romanowsky stain, 600×)

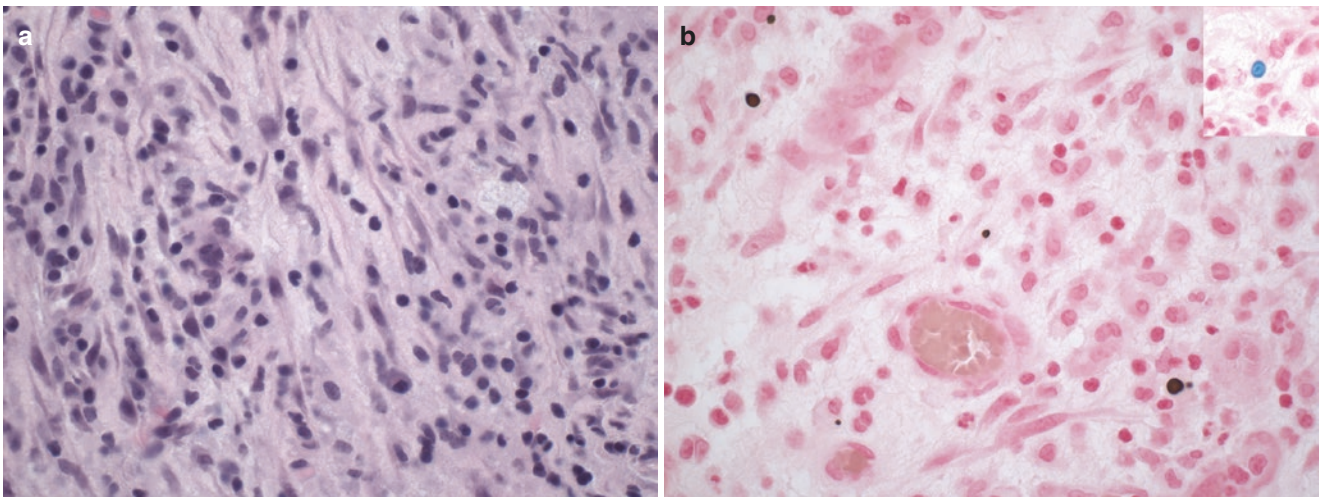

Fig. 18.5 Malakoplakia. (**a**) Cell block shows a population of spindled to epithelioid histiocytes with indistinct cell borders, which is partially obscured by a mixture of predominantly chronic inflammatory cells (H&E, 600×). (**b**) Characteristic Michaelis–Gutmann bodies are highlighted by both Von Kossa stain (dark brown) and iron stain (inset, blue) (600×)

Infectious/inflammatory conditions of lymph nodes or soft tissue may also be encountered. Malakoplakia (Fig. 18.5) is an uncommon entity that may be mass forming and mimic neoplasms in the retroperitoneum/perirenal/periadrenal space. This entity is being increasingly noted in the post-transplant setting [59, 60].

20. How frequently can a definitive diagnosis be obtained from a retroperitoneal FNA?

When sufficiently sampled, the diagnosis of metastasis is straightforward, especially with the aid of clinical history and immunohischemistry. Some primary retroperitoneal masses may be difficult to definitively diagnose on cytology. These often require an interdisciplinary approach including excellent communication with the radiologists and the clinical team. If a sarcoma is suggested clinically or at the time of ROSE, a core biopsy may increase diagnostic accuracy [61].

21. How should material be allocated for retroperitoneal FNAs.

In addition to smears/conventional cytologic preparations, a vast majority of biopsies should attempt to have material allocated for cell block.

- Depending on the lesion seen at the time of ROSE, allocation of fresh material for other ancillary testing may be warranted. For lymphoid lesions, flow cytometry should be performed. For mesenchymal neoplasms, cytogenetics should be attempted, but subsequent FISH testing may be sufficient (please see Question 4). If an infectious process is suggested, fresh material should be allocated for microbiology.

22. What are pitfalls in evaluating retroperitoneal FNAs?

One major pitfall is the ambiguity of the biopsy site/tissue, compounded by a lack of clinical history, especially for a prior malignancy. Lymphoid infiltrates can either be misinterpreted as chronic inflammation in the setting of a lymphoproliferative disorder with fibrosis or mask an underlying nonlymphoid neoplasm like seminoma. Another pitfall is contamination encountered upon sampling the lesion. For EUS-guided FNAs, GI contamination is common and should not be confused with a well-differentiated adenocarcinoma. Radiologist-performed percutaneous biopsies tend to take a posterior approach and traverse skin, subcutaneous fat, and possibly skeletal muscle before encountering the target. Contaminating fat, sometimes with fat necrosis, can be mistaken as a lipomatous neoplasm (or vice versa).

Case Presentations

Case 1

Learning objectives:

1. Recognize characteristic cytomorphologic features of this tumor.
2. Discuss differential diagnosis of this tumor.
3. Become familiar with immunohistochemical staining features of this tumor.

Case history:

An 86-year-old female who presented to the emergency room with lower back pain was found to have a 2 cm right renal mass.

Specimen source:

CT-guided fine-needle aspiration of the renal mass was performed. A modified Giemsa-stained smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic findings:

Cellular smears showing abundant large clusters, small clusters, and single cells (Fig. 18.6a).

The cells are uniform and have central or eccentric round to oval nuclei and fine chromatin and show abundant granular cytoplasm.

Rare cells show binucleation.

No significant atypia is present.

Differential diagnosis:

Chromophobe RCC

Hybrid oncocytoma/chromophobe RCC

Oncocytoma

Tubulocystic RCC

IHC and other ancillary studies:

S100A1 positive, showing a granular cytoplasmic pattern of staining

HNF1β positive showing diffuse nuclear staining (Fig. 18.6b)

Final diagnosis:

Oncocytoma

Take-home messages:

The tumor cells of oncocytoma show uniform with round to oval nuclei and abundant granular cytoplasm. Atypia is inconspicuous.

Architecturally, oncocytomas may show singly dispersed cells or form small or large cohesive groups. The characteristic solid nested pattern with surrounding loose stroma is best seen with cell block sections or core biopsies.

S100A1 and HNF1β immunostains could be helpful in difficult cases.

If greater atypia is present (prominent multinucleation, nuclear pleomorphism, and/or irregular nuclear membranes), a diagnosis of oncocytic neoplasm (including a differential diagnosis) may be most prudent.

Tubulocystic RCCs have high-grade nuclei and abundant eosinophilic cytoplasm, which cytologically show overlap with oncocytic renal epithelial neoplasms; however, they should show cystic architecture only, so large cohesive groups of cells are less likely to be present.

References: [14, 23, 62]

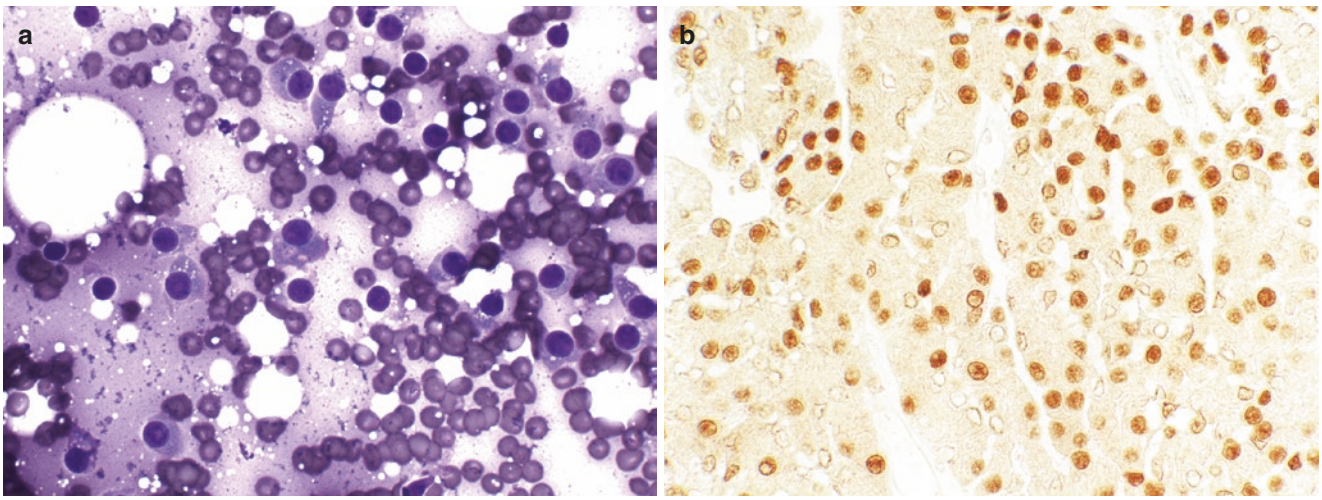

Fig. 18.6 Oncocytoma. (**a**) Smears show a monotonous population of epithelioid cells with abundant granular cytoplasm, distinct cytoplasmic edges, and round nuclei with fine chromatin (Romanowsky stain, 600×); (**b**) HNF1β immunostain shows diffuse nuclear staining (400×)

Case 2

Learning objectives:

1. Recognize cytomorphologic features of this tumor.
2. Discuss differential diagnosis of this tumor.
3. Become familiar with immunohistochemical and cytogenetic features of this tumor that may help to distinguish it from its mimics.

Case history:

A 51-year-old female presented with gross hematuria and abdominal pain and a negative cystoscopy. CT demonstrated a 4 cm endophytic infiltrative mass in the right kidney, abutting the renal sinus.

Specimen source:

CT-guided fine-needle aspiration of the kidney mass was performed. A modified Giemsa-stained smear, a Pap-stained smear, and a cell block were made from the aspiration. Half of the needle rinse was allocated for cytogenetic studies.

Cytologic findings:

Cellular smears showing abundant large aggregates, some with prominent papillary architecture, and small loosely cohesive epithelioid clusters (Fig. 18.7)

The cells show mild to moderate pleomorphism and have central or eccentric round nuclei with fine chromatin and abundant finely granular to vacuolated cytoplasm with indistinct cytoplasmic borders.

Differential diagnosis:

Clear cell RCC

MiT family translocation RCC

Papillary RCC

IHC and other ancillary studies:

TFE3 immunostain shows strong diffuse nuclear staining (Fig. 18.8a). CA9 stain is negative.

Karyotype revealed X;1 translocation.

By FISH, a *TFE3* (Xp11.23) rearrangement was observed in 80% of the tumor cell nuclei (Fig. 18.8b).

Final diagnosis:

MiT family translocation RCC (Xp11 translocation RCC)

Take-home messages:

The tumor cells of MiT family translocation RCC show eosinophilic granular to clear cell cytology, and the epithelial cells of some cytologic samplings may be cytologically indistinguishable from clear cell RCC.

The presence of papillary architecture or psammomatous calcifications in addition to clear cell RCC cytology should raise the possibility of a MiT family translocation RCC.

Immunophenotypically, strong and diffuse nuclear staining for TFE3 or TFEB supports the diagnosis. These tumors are typically negative for CA9 and CK7, which can help to distinguish them from clear cell RCC and papillary RCC, respectively.

Cytogenetic analysis via karyotype or FISH can identify TFE3 or TFEB abnormalities specific for this entity and can distinguish it from typical clear cell RCC (3p deletions) or papillary RCC (trisomies of chromosomes 7, 17).

References: [7, 63–65]

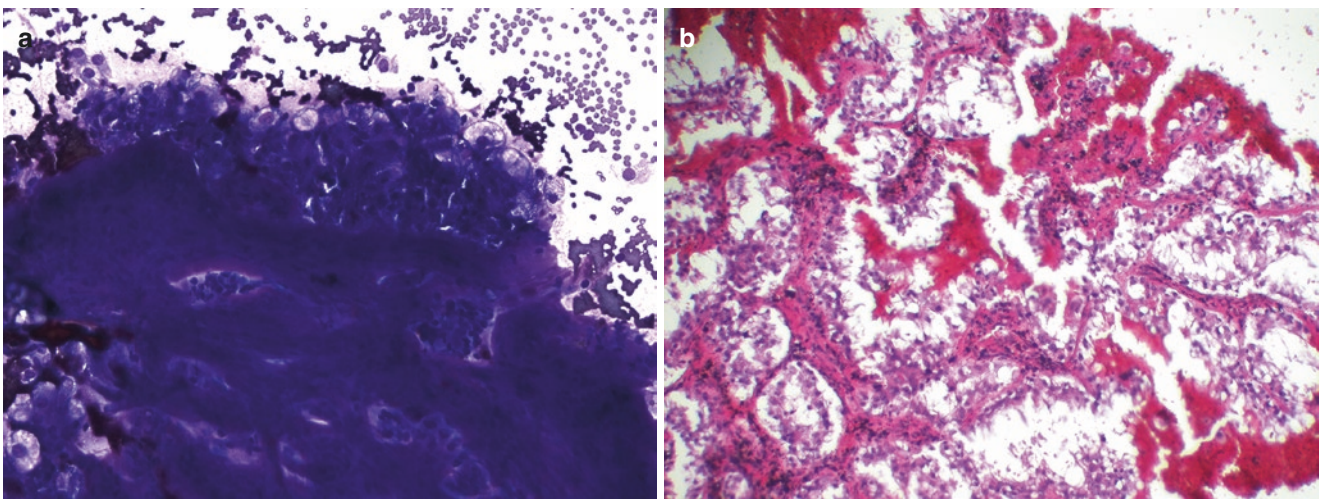

Fig. 18.7 MiT family translocation RCC. (**a**) Papillary architecture is prominent and the tumor cells at the periphery of papillary aggregates show low N/C ratio and abundant vacuolated cytoplasm (Romanowsky stain, 200×). (**b**) Cell block shows a mixture of clear cells and papillary architecture (H&E, 100×)

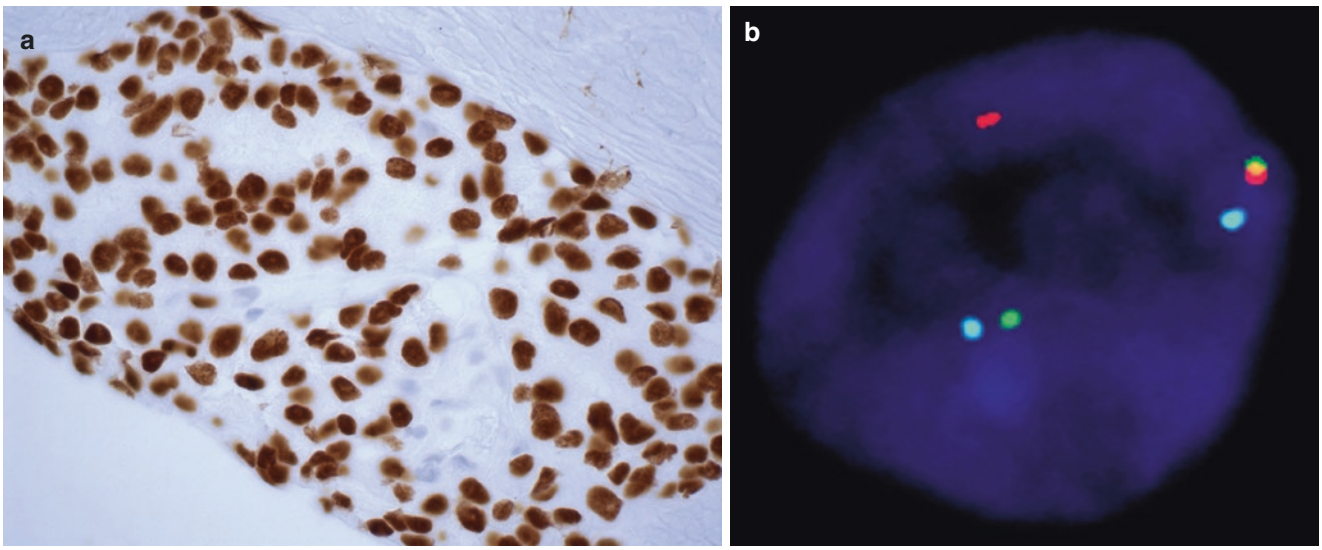

Fig. 18.8 Xp11/TFE3 translocation RCC. (**a**) TFE3 immunostain shows strong, diffuse nuclear staining (600×). (**b**) TFE3 break-apart FISH shows one normal fusion signal (red and green signals in close proximity creating a yellow signal) and one tumor-specific break apart signal (separate red and green signals). The CEP X centromeric probe (blue signal) confirms the specimen is from a female patient with two copies of the X chromosome (Courtesy of BWH Clinical Cytogenetics Laboratory)

Case 3

Learning objectives:

1. Recognize the cytomorphologic features seen in tumor.
2. Discuss differential diagnosis of this tumor.
3. Identify the optimal approach to evaluating the tumor morphologically and immunophenotypically.

Case history:

An 82-year-old male with a remote history of colorectal adenocarcinoma who presented with weakness and shortness of breath found on imaging to have pneumonia, numerous lung and liver lesions (up to 1 cm), and a 15 cm heterogeneous enhancing left suprarenal mass displacing and focally involving the superior pole of the left kidney and pancreas. The left adrenal gland was not identified. Biochemical workup was unremarkable.

Specimen source:

CT-guided fine-needle aspiration of the suprarenal mass was performed. A modified Giemsa-stained smear, a Pap-stained smear, and a cell block were made from the aspiration.

Cytologic findings:

Cellular smears showing abundant large clusters, small clusters, and single cells (Fig. 18.9).

The cells are markedly pleomorphic with hyperchromatic, irregular nuclei and focally coarse chromatin. Cytoplasm is abundant and focally vacuolated.

Differential diagnosis:

Adrenal cortical neoplasm (adenoma or carcinoma)
Metastatic carcinoma
Pheochromocytoma

IHC and other ancillary studies:

Tumor cells are positive for SF-1 (nuclear stain), α-inhibin, and synaptophysin (Fig. 18.10).

Tumor is negative for chromogranin, CA9, PAX8, CK7, and CK20.

Final diagnosis:

Adrenal cortical neoplasm, highly suggestive of adrenal cortical carcinoma

Take-home messages:

The differential diagnosis for tumors with high-grade features in the adrenal gland is broad and requires immunophenotyping for further characterization. (Please see Question 18 and Table 18.3 for IHC profile.)

Although the presence of mitoses and necrosis in an adrenal cortical neoplasm is highly suggestive of malignancy, definitive classification requires complete surgical pathology evaluation.

References: [66, 67]

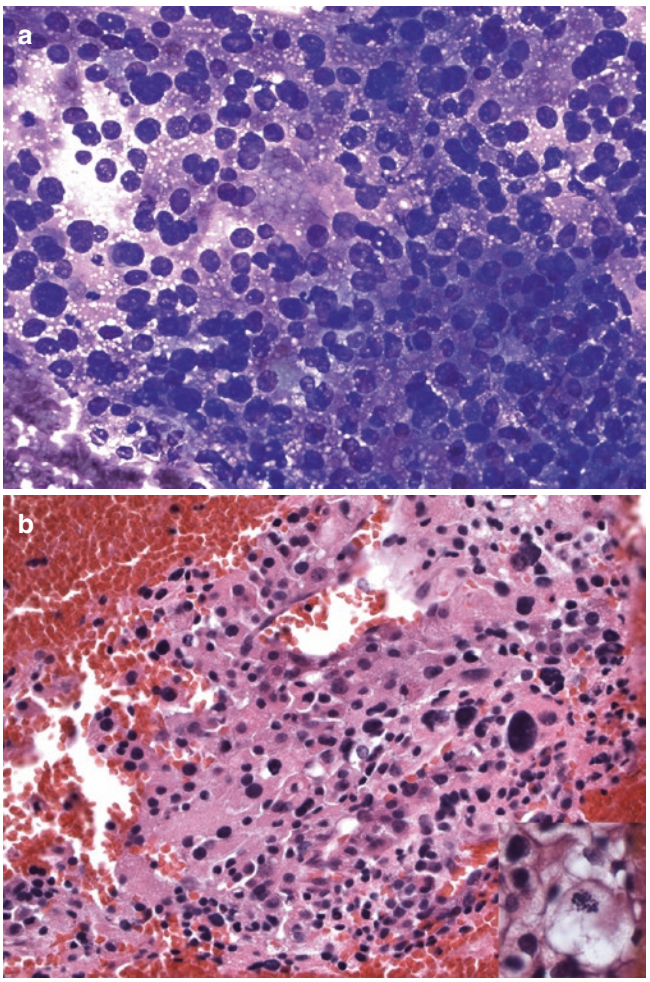

Fig. 18.9 Adrenal cortical carcinoma. (**a**) Cellular smears show highly atypical cells with architectural crowding, anisonucleosis, pleomorphism, and focally vacuolated cytoplasm (Romanowsky stain, 400×). (**b**) Cell block sections show tumor cells with eosinophilic cytoplasm and marked nuclear atypia and, sometimes, with abundant clear cytoplasm and abnormal mitosis (inset) (H&E, 400×)

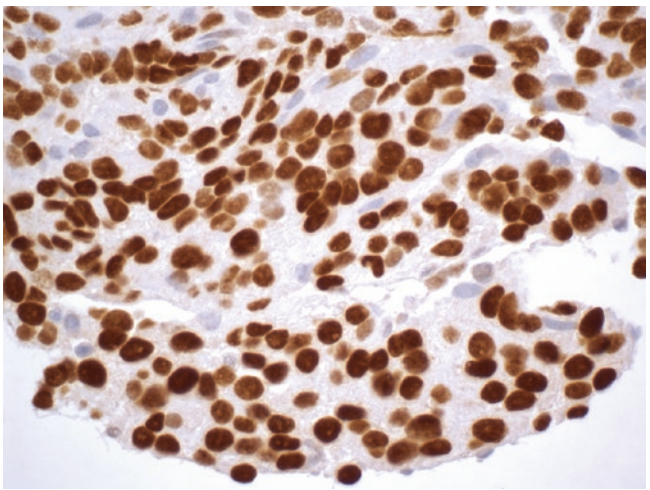

Fig. 18.10 SF1 immunostain shows diffuse nuclear positivity, confirming the diagnosis of adrenal cortical carcinoma (600×)

Table 18.3 Key features of primary adrenal neoplasms

Adrenal neoplasm	Morphology	Immunophenotype[a]
Adrenal cortical adenoma	Low cellularity specimens, low N/C cells, abundant vacuolated cytoplasm, small nuclei, background with naked nuclei, and extruded lipid	SF1+/α-inhibin+/ Melan A+/ calretinin+; Chromogranin-; Ki67 index <5%
Well-differentiated adrenal cortical carcinoma	Low N/C cells, single cells may be present, possibly more nuclear atypia than seen in adenoma, possible mitoses	SF1+/α-inhibin+/ Melan A+/ calretinin+; Chromogranin-; Ki67 index >5%
Poorly differentiated adrenal cortical carcinoma	Cells with high nuclear grade, necrosis, mitoses including atypical forms	SF1+/α-inhibin+/ Melan A+/ calretinin+; Chromogranin-; Ki67 index >5%
Pheochromocytoma	Variably pleomorphic cells	Chromogranin+, S100 highlights sustentacular cells
Myelolipoma	Adipocytes and benign hematopoietic elements	CD61 highlights megakaryocytes

[a]All primary adrenal neoplasms except for myelolipoma are positive for synaptophysin

Case 4

Learning objectives:

1. Recognize the cytomorphologic features of this lesion.
2. Discuss differential diagnosis.
3. Identify the optimal approach to evaluating these types of samples.

Case history:

A 49-year-old male with an incidentally identified retroperitoneal mass.

Specimen source:

CT-guided fine-needle aspiration of the suprarenal mass was performed. A modified Giemsa-stained smear, a Pap-stained smear, and a cell block were made from the aspiration. Fresh material was not allocated for microbiologic studies.

Cytologic findings:

Cellular smears showing abundant small and large granulomatous aggregates, with a background of numerous single histiocytes, few lymphocytes, and a minor population of atypical cells and atypical naked nuclei. The epithelioid cells are large with high N/C ratio, scant cytoplasm, large nuclei, some with prominent nucleoli (Fig. 18.11)

Cell block reveals abundant non-necrotizing granulomata and rare scattered and small aggregates of large atypical round cells.

Differential diagnosis:

Tumor (carcinoma, high-grade lymphoma, round cell neoplasm)

Infection

Sarcoid

IHC and special stains:

Tumor cells are positive for CD117, D2–40, and OCT3/4.

Special stains for microorganisms are negative.

Final diagnosis:

Seminoma with prominent associated granulomatous reaction

Take-home messages:

Retroperitoneal lymph nodes or masses may be involved by inflammatory/infectious etiologies or primary or metastatic tumors. The approach to the specimen will depend on the clinical findings and the morphology of the cells sampled.

Metastasis to retroperitoneal lymph nodes may be the first presentation of malignancy, as primary tumors are not always clinical identifiable.

Because rare atypical cells were seen at the time of ROSE, no material was allocated for microbiology. Instead, all the needle rinses were used to generate a cell block. If atypical cells favoring a neoplasm were not identified at ROSE and only granulomatous inflammation was seen, additional fresh material would have been allocated for microbiology.

Cell blocks are helpful in further characterizing rare atypical cells seen on smears and can provide material for special stains to workup possible infectious etiologies.

Sarcoid is a clinicopathological diagnosis of exclusion.

Reference: [68]

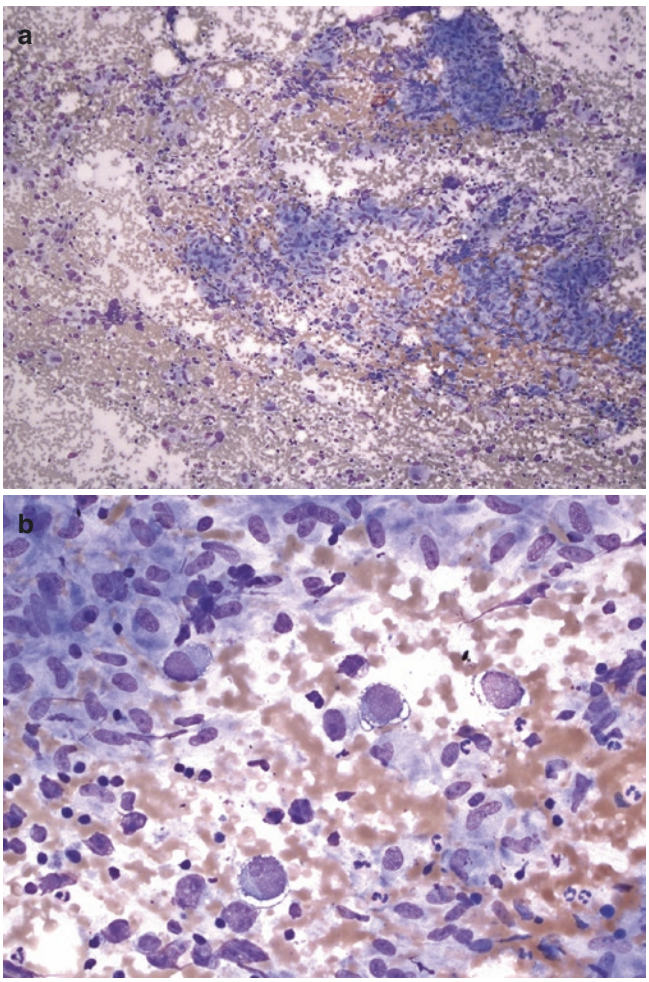

Fig. 18.11 Seminoma with marked granulomatous reaction. (**a**) Cellular smears show a polymorphous population dominated by large aggregates of cells with abundant cytoplasm (Romanowsky stain, 100×). (**b**) At higher power, the cellular aggregates (top left) are identifiable as granulomas. Rare highly atypical single cells and small aggregates are noticeable. These cells are large with small amounts of vacuolated cytoplasm (Romanowsky stain, 400×)

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Ocular Cytology

19

Manisha Mehta and Nora Laver

Contents

List of Frequently Asked Questions

1. What are the most common types of ocular specimens received in cytology?

Ocular cytology specimens are most commonly submitted for evaluation as (1) corneal or conjunctival scrapings, (2) aqueous fluid samples, (3) vitrectomy specimens, and (4) fine needle aspiration biopsies from the anterior and posterior segments of the eye (Table 19.1).

Conjunctival and corneal scrapings are usually submitted as direct smears or utilizing liquid-based cytology. The epithelial surface of the cornea and conjunctiva may be scrapped under local anesthesia with a small spatula or brush and the cellular yield smeared onto tissue slides. The smears may be alcohol-fixed or air-dried for the diagnosis of inflammatory, infectious, and neoplastic disorders. The cells may be prepared utilizing liquid-based cytology, placing the scraped cells in appropriate alcohol-based fixative.

The anterior chamber of the eye, located between the posterior surface of the cornea and the anterior surface of the iris, contains approximately 0.3 ml of aqueous fluid. Anterior chamber aqueous fluid aspirations are usually small samples (0.2 ml) and optimal handling is imperative. The undiluted aqueous fluid may be diluted with saline, and the majority of the specimen is then sent for molecular diagnosis of infections. Meanwhile, an alcohol-fixed or air-dried smear may be prepared from a single drop.

Table 19.1 Common ocular cytology specimens

Specimen site	Specimen type
1. Corneal and conjunctival cytology specimens/scrapings	Direct smears or liquid-based cytology (ThinPrep/SurePath)
2. Aqueous fluid samples	Undiluted fluid sample (0.3–0.4 ml)
3. Vitrectomy samples	
Therapeutic vitrectomy Diagnostic vitrectomy	Diluted vitreous Diluted and undiluted vitreous
4. Fine needle aspiration biopsies of tumors involving the uvea, retina, orbit, and ocular adnexa	Direct smears (Diff-Quik® staining or air-dried for cytogenetic testing) Liquid-based cytology (ThinPrep/SurePath) Cytospin and/or cell block Undiluted fresh tumor sample (molecular testing)

Vitrectomy is a type of surgery performed to remove some or all of the vitreous humor from the posterior segment. Vitrectomy specimens are submitted as diluted or undiluted specimens depending on the clinical suspicion and presumptive clinical diagnosis. Therapeutic and diagnostic vitrectomies differ in that when a diagnostic vitrectomy is performed, the initial 1 cc of undiluted vitreous sample is collected prior to the beginning of an infusion. A diluted vitreous sample is

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2_19

submitted in therapeutic and diagnostic vitrectomies; this sample has an irrigation solution added that is used during the removal of the vitreous. While processing therapeutic vitrectomy samples, the diluted vitreous sample can be used to prepare slides with liquid-based cytology methodology and a cell block when sufficient tissue is present.

Fine needle aspiration biopsies may be performed for the diagnosis of anterior chamber pathologies and for tumors involving the uvea, retina, orbit, and ocular adnexa. Multiple direct smears are prepared, by both air-dried and alcohol-fixed methods. The remainder of the specimen can be submitted for a cytospin, liquid-based cytology and/or a cell block preparation, if acquired volume permits. Both SurePath and ThinPrep liquid-based methods can be used. If molecular testing is needed, an undiluted sample is placed in a special fixative medium provided by a collection kit specific to the processing laboratory (such as DecisionDx-UM, Castle Biosciences Incorporated).

2. How does corneal epithelium differ from skin epithelium?

The corneal epithelium comprises of 5–6 layers of modified stratified non-keratinizing squamous epithelium, attached to a basement membrane. The basal cells, which are the closest to the basement layer, are smaller with a higher nuclear-cytoplasmic ratio compared to the overlying 2–3 layers of epithelial cells with interdigitating cytoplasmic processes. The superficial two layers are flattened epithelial cells with small round nuclei and inconspicuous nucleoli. Below the epithelial basement membrane lies Bowman's layer, a specialized layer of the corneal stroma that does not regenerate after injury (Figs. 19.1 and 19.2a). The corneal stroma is composed of collagen fibers arranged in compact organized layers which are secreted by keratocytes present in between collagen fibers. The stroma lies on Descemet's membrane, a thick, acellular basement membrane which is secreted by the endothelium. The endothelium is not true endothelium, but a single layer of non-regenerating cuboidal specialized cells on the undersurface of the Descemet's membrane derived from the neural crest. Surface epithelial smears from the normal cornea demonstrate cohesive sheets of non-keratinizing squamous epithelial cells (Fig. 19.2b). The presence of keratinized cells in smears from the cornea is abnormal (Fig. 19.2c) and should prompt cytologists to rule out regenerative changes in response to infectious/inflammatory processes, intraepithelial dysplasia, or squamous cell carcinoma.

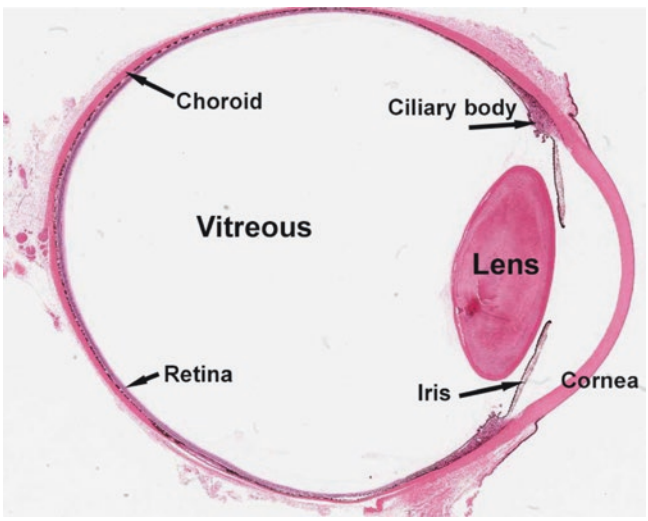

Fig. 19.1 Cross section of an adult eye showing the normal histology of ocular structures (H&E)

3. What are the common infections/parasites seen in the cornea and conjunctiva?

Common infections involving the conjunctiva and cornea include bacterial, fungal, viral, amoebic, rickettsial, and parasitic agents.

The most common etiologies of *bacterial keratitis* are *Streptococcus*, *Pseudomonas*, *Enterobacteriaceae* (includ-

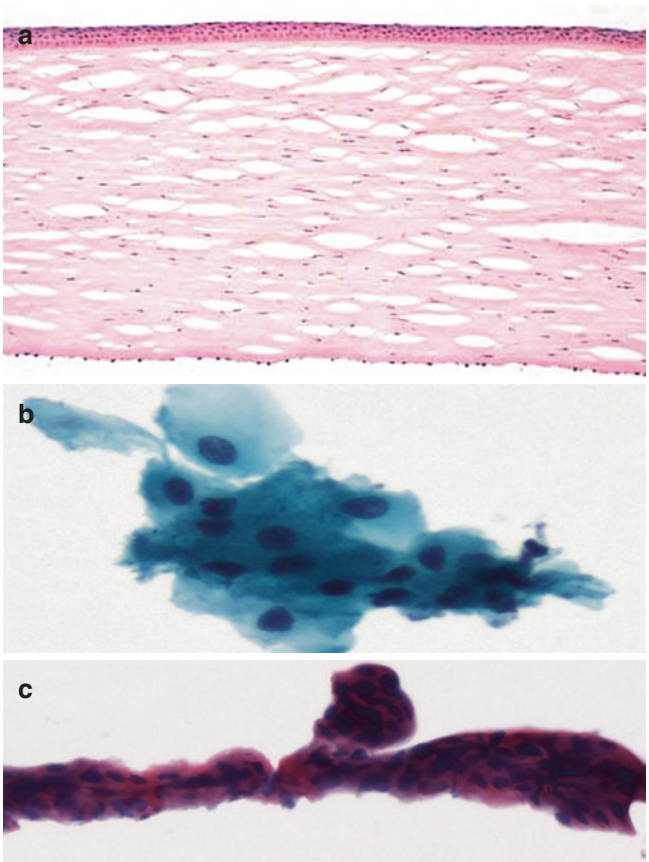

Fig. 19.2 (**a**) Normal cornea histology: Superficial non-keratinizing squamous epithelial cells, Bowman's layer, stromal fibers with keratocytes, Descemet's membrane, and endothelial cell layer (H&E). (**b**) Corneal epithelial cell smear in acute keratitis showing reactive changes (PAP). (**c**) Surface epithelial cells with abnormal keratinization in a cell block preparation (H&E)

ing *Klebsiella*, *Enterobacter*, *Serratia*, and *Proteus*), and *Staphylococcus* species [1]. Acute conjunctivitis due to bacterial infections predominantly shows neutrophils and bacteria in smears. Conjunctival scrapings and cultures are frequently obtained in severe bacterial conjunctivitis or in antibiotic-resistant cases. *Chlamydia trachomatis* infection of the cornea and conjunctiva results in trachoma, a contagious bacterial infection. Chronic untreated trachoma can cause severe scarring leading to blindness [2]. The characteristic finding in Giemsa-stained cytologic smears from trachoma are epithelial cells with multiple, small (0.5 μm) cytoplasmic basophilic inclusions with halos (Fig. 19.3a). Confirmatory ancillary tests supporting the diagnosis can be included such as chlamydial cultures from conjunctival cells, direct fluorescent antibody (DFA) staining of conjunctival scrapings, enzyme-linked immunosorbent assay, and serum immunoglobulin IgG titers against *Chlamydia* species [3].

Fungal keratitis may occur secondary to trauma (contact lens wear, foreign material), surgery-induced defects in the corneal epithelium, and endophthalmitis. Certain fungal infections have geographic prevalence with a preference for warmer climates [4–7]. In cases due to trauma or after surgery, the organisms penetrate the intact Descemet's membrane and can gain access into the anterior chamber or the posterior segment. Fungal keratitis can also occur secondary to fungal endophthalmitis, when fungal organisms extend from the posterior segment through Descemet's membrane and into the corneal stroma. The fungal infection can also spread to the cornea through the corneoscleral trabeculae present in the angle of the anterior chamber. Fungal keratitis is more commonly seen in developing countries than in the USA. Causative organisms include *Moniliaceae* (nonpigmented filamentary fungi, including *Fusarium* and *Aspergillus* species), *Dematiaceae* (pigmented filamentary fungi, including *Curvularia* and *Lasiodiplodia* species), and yeasts (including *Candida* species) [8]. In the USA 30,000 new cases are reported annually [9]. *Candida* and *Aspergillus* are the most common; *Fusarium* infection is most common in South Florida [10]. Up to 20% of cases of fungal keratitis (particularly candidiasis) can have bacterial coinfections.

Viral infections are a common cause of painful keratitis and conjunctivitis [11]. Specimens are obtained in chronic, recurrent infections or cases with atypical conjunctival reactions and failure to respond to treatment. Giemsa-stained conjunctival scrapings may show a chronic inflammatory response; mononuclear cells and lymphocytes are characteristically seen in viral infections. Adenoviruses usually involve the conjunctiva and a few small conjunctival cells may show multiple, small eosinophilic intranuclear inclusions. Herpes simplex and herpes zoster virus-induced inflammation involves both the cornea and conjunctiva and contains cells with typical eosinophilic, ground-glass intranuclear inclusions, multinucleation, margination of chromatin, and nuclear molding. Measles may involve the conjunctiva and show multinucleated cells with multiple eosinophilic inclusions surrounded by sharp halos.

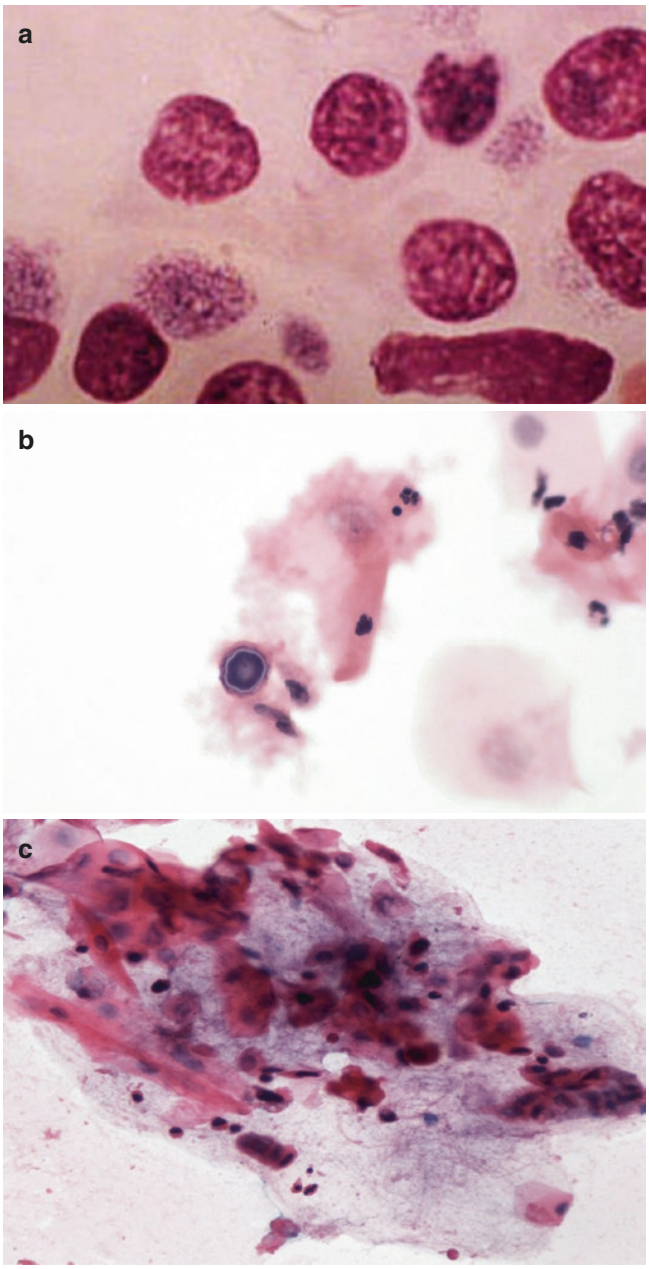

Fig. 19.3 (**a**) Conjunctival epithelial cells with multiple small cytoplasmic basophilic inclusions (Giemsa). (Original picture from the files of Moshe Lahav M.D., New England Eye Center Ocular Pathology Collection.) (**b**) Corneal keratitis smear showing *Acanthamoeba* double-walled cysts (H&E). (Courtesy of Nasreen A. Syed, M.D.) (**c**) Conjunctival smear in a case of allergic conjunctivitis showing numerous eosinophils (H&E)

Corneal amoebic keratitis is a rare but serious infection of the eye that can result in permanent visual impairment or blindness. The infection is caused by a microscopic, free-living amoeba called *Acanthamoeba*. *Acanthamoeba* amoebas

are very common in nature and can be found in bodies of water like lakes and oceans, soil, and air. They are usually present in contact lens wearers. Corneal scrapings along with confocal microscopy can aid in the diagnosis of the infection. Trophozoites, ameboflagellate measuring 15–30 μm, and the double-walled cysts of the parasite may be identified (Fig. 19.3b). Early diagnosis and aggressive therapy are essential to prevent visual loss [12].

Rare conjunctival and corneal infections in the USA can also be caused by tuberculosis, syphilis, and parasites.

4. What are the cytologic features of allergic conjunctivitis?

Allergic conjunctivitis is a common inflammation of the conjunctiva and cornea caused by a type I hypersensitivity reaction. There are two main types: acute (common condition occurring during allergic seasons) and chronic (less common condition occurring all year around). Allergic conjunctivitis is secondary to irritation due to allergens like pollen and mold spores. Cytological smears show inflammatory cells with predominant eosinophils [13] (Fig. 19.3c).

5. What are the main diagnostic challenges related to anterior chamber aqueous fluids?

Aqueous fluid samples may be submitted in the management of uveitis, to rule out infectious diseases, for the diagnosis of ghost cell and lens-induced glaucoma, epithelial downgrowth, iris cyst formation, juvenile xanthogranuloma, and tumor cells, among others. *Anterior uveitis* involves inflammation of the iris and ciliary body seen in association with autoimmune diseases or in otherwise healthy individuals. In anterior uveitis, there is an increase in the protein and cellular content of the aqueous causing an effect upon ophthalmologic examination known as flare. The smears show chronic inflammatory cells including lymphocytes, plasma cells, macrophages, and blood. Layering of white blood cells in the anterior chamber is known as hypopyon. Interleukin 6 measurements in these samples may be requested; increased levels of IL-6, IL-8, IFN-γ, and TNF-α have been found in idiopathic anterior uveitis, Behcet's disease, and ankylosing spondylitis. *Lens-induced uveitis* is caused by an immune complex reaction to lens protein exposure in cases of a disrupted lens capsule. Lens fragments may be retained in the anterior chamber during an apparent uncomplicated cataract surgery. Cytology specimens from the anterior chamber may show fragments of lens, neutrophils, macrophages, and granulomatous inflammation. *Ghost cell glaucoma* develops secondary to a vitreous hemorrhage with disruption of the anterior hyaloid of the vitreous. Senescent red blood cells or "ghost" erythrocytes may be found in the anterior chamber. Ghost erythrocytes cannot drain through the angle trabecular meshwork and their presence may lead to increased intraocular pressure, i.e., ghost cell glaucoma. Lens-induced glaucoma or phacolytic glaucoma occurs in advanced cataracts with leakage of lens cortex proteins into the anterior chamber through an intact lens capsule. These proteins may cause aqueous fluid drainage alterations through the trabecular meshwork and lead to open-angle glaucoma. In these samples, macrophages filled with liquefied lens cortical material are present. *Epithelial downgrowth* develops secondary to a perforating wound to the cornea or limbus. Squamous epithelial cells may grow into the wound and along the ocular surfaces lining the anterior chamber. This growth may result in blinding complications with angle-closure glaucoma and retinal detachment. *Juvenile xanthogranuloma* may present in children as iris nodules with spontaneous hemorrhages (hyphema) in the anterior chamber. Cytology samples of aqueous humor show histiocytes, occasional eosinophils, and rare Touton giant cells. *Iris epithelial cysts*, although uncommon, are usually paucicellular with pigmented iris cells and macrophages. Melanin granules in iris pigment epithelial cells are typically larger than those seen in melanoma cells and are uniform in size and shape. In contrast, melanin granules within macrophages tend to vary in size and shape. Iris epithelial cells may also be found in response to ocular injury, inflammation, and certain types of glaucoma (pigmentary glaucoma). *Tumor cells* in the anterior chamber may be seen in retinoblastoma with extensive vitreous and anterior chamber seeding. Most primary tumors of the iris and ciliary body are slow-growing nevi or low-grade melanomas. Tumor cells seldom shed from these tumors into the anterior chamber. Rarely however, metastasis may involve the iris and shed into the aqueous humor.

6. What is vitrectomy and what are the different types of vitrectomy samples?

Vitrectomy is an invasive procedure performed to remove some or all vitreous humor from the eye. The vitreous humor is a viscous substance that fills the cavity of the eye between the lens anteriorly and the retina posteriorly (Fig. 19.1). The average volume in adults is 4 ml and it is composed primarily of water (99%) and collagen types II and IX, glycosaminoglycans, soluble proteins, and glycoproteins.

A three-port pars plana vitrectomy is the standard procedure in which three openings/ports are made through the sclera to access the vitreous through the most avascular part of the ciliary body – the pars plana. A vitrector or vitreous cutting instrument, an illuminator (light source), and an infusion port with irrigating fluid are inserted into these ports. The vitrector simultaneously cuts and aspirates the vitreous, which is diluted with the irrigating fluid.

There are two types of vitrectomy samples: undiluted and diluted vitreous.

An undiluted vitreous sample is obtained before the start of a standard vitrectomy. This is done by inserting a needle through a self-sealing sclerotomy (opening in the sclera) and

aspirating approximately 1 ml of unadulterated/undiluted vitreous [14]. This undiluted vitreous is most useful for performing diagnostic tests.

The diluted vitreous sample is obtained during the vitrectomy procedure. It is the end result of the procedure wherein the fluid that is infused into the eyeball during vitrectomy to maintain its shape gets simultaneously aspirated with the vitreous. It is composed of the aspirated, cut vitreous which is diluted with the irrigating fluid. Depending on the complexity of the procedure and the time required, it can be of considerable volume (at least 200 ml).

7. What is the difference between a therapeutic and a diagnostic vitrectomy?

The type of vitrectomy performed and the type of vitreous sample obtained depends on the indication for a vitrectomy.

Therapeutic vitrectomies are performed to treat a cause of visual deterioration, to excise clinically significant vitreous opacities that cause visual loss or preclude adequate view of the posterior segment. Indications include vitreous hemorrhage, cataract, inflammatory vitreous opacification, lens-induced uveitis, endophthalmitis, epiretinal membrane excision, and sustained intravitreal drug delivery, among others. Commonly, only a diluted vitreous sample is collected during a therapeutic vitrectomy.

Diagnostic vitrectomies are performed to aid in diagnoses. In addition to vitrectomy, small retinal or choroidal fine needle aspiration biopsies may be performed during a diagnostic vitrectomy. The choroid is a highly vascular and pigmented layer containing melanocytes that lies between the sclera and the retina. It supplies the blood supply to the outer parts of the retina (Figs. 19.1 and 19.4a, b). The retinal pigment epithelium (RPE) is a cuboidal pigmented layer found between the retinal photoreceptors and the choroid. Indications for a diagnostic vitrectomy include infectious and noninfectious etiologies. Infectious etiologies include bacterial, fungal, viral, and parasitic uveitis [14, 15]. Noninfectious etiologies include autoimmune uveitis (or posterior uveitis), primary intraocular lymphoma, uveal melanoma, and metastatic tumors including infiltration by leukemia.

Both undiluted and diluted vitreous samples with or without retinal or choroidal fine needle biopsies can be collected during a diagnostic vitrectomy.

8. What are the components of a therapeutic vitreous sample?

While processing therapeutic vitrectomy samples, the diluted vitreous sample can be used to prepare Papanicolaou (PAP)- and periodic acid-Schiff (PAS)-stained slides with liquid-based cytology methodology and a cell block when sufficient tissue is available.

Normal cytologic components of a vitrectomy sample are the vitreous cells or hyalocytes; small, slender columnar cells with eosinophilic cytoplasm; vitreous fibers; and macrophages (Fig. 19.5a). Other components include fragments of peripheral retina (Fig. 19.5b), anterior uveal tissues, retinal pigment epithelial cells (RPE) (Fig. 19.5c), subretinal membrane, lens fragments (Fig. 19.5d), fibrovascular tissue fragments often seen in vitrectomies for tractional retinal detachment, and red blood cells. Conjunctival cells from the aspiration needle entry port can also be seen. Abnormal deposits can be seen in asteroid hyalosis (Fig. 19.5a inset), a degenerative condition in which calcium-lipid round complexes are found throughout the vitreous collagen fibers. Amyloid deposits can be found in familial amyloid polyneuropathy.

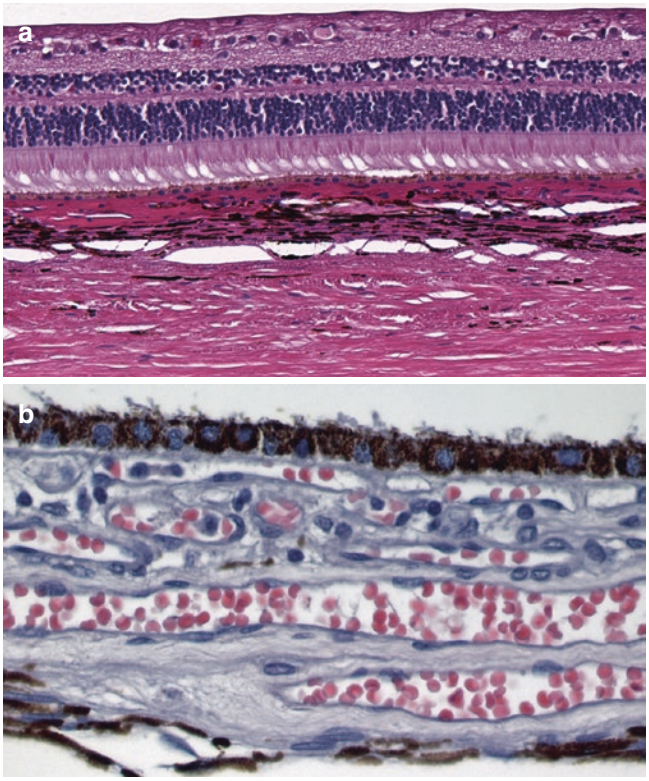

Fig. 19.4 (**a**) Histology of the normal retina, choroid, and sclera (H&E). (**b**) Histology of the normal choroid showing larger blood vessels closer to the sclera (bottom of the picture) and smaller vessels and capillaries under the retina; pigmented or nonpigmented melanocytes are normally present in a loose connective tissue matrix. The choroid is located under the retinal pigment epithelium, a layer of cuboidal pigmented cells (H&E)

9. How are diagnostic vitrectomy samples processed differently?

Vitrectomy is an invasive procedure with potential surgical complications that can cause blindness. The benefits of cytologic evaluation of a diagnostic vitrectomy must outweigh the risk of these complications. Furthermore, a diagnostic vitrectomy is usually the final test after multiple, equivocal, or negative ancillary systemic tests [15] and may yield little

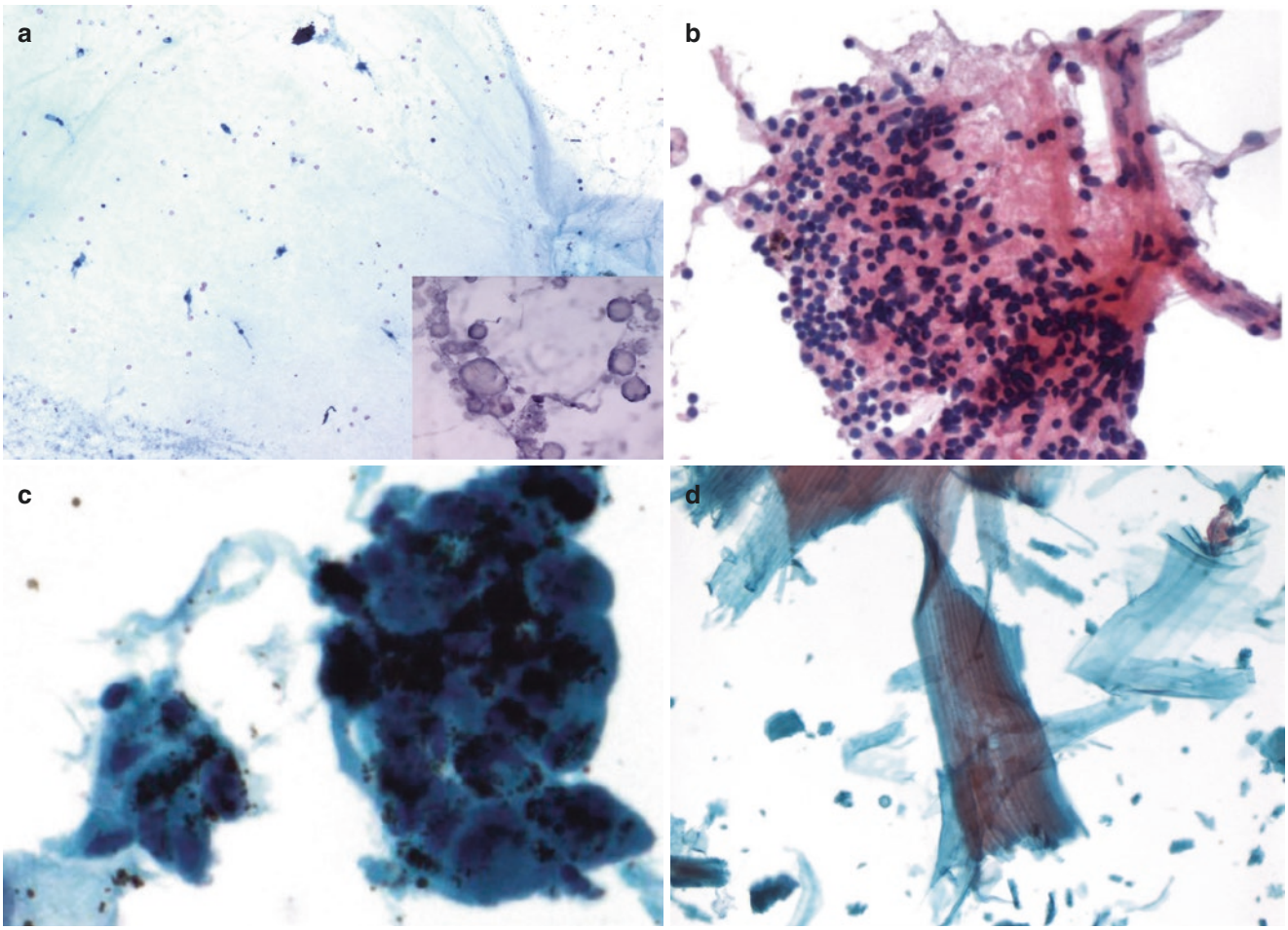

Fig. 19.5 Histologic components of a therapeutic vitrectomy. (**a**) Hyalocytes with vitreous fibers (PAP). Inset: Asteroid hyalosis (PAP). (**b**) Fragment of peripheral retina with vessels (H&E). (**c**) Retinal pigment epithelial cells (PAP). (**d**) Fragment of lens fibers (PAP)

fluid with low possibility of a repeat procedure, underscoring the importance of the optimal handling of this low-volume sample.

Appropriate handling of the low-volume (usually <1.5 ml) 'undiluted vitreous' sample is crucial and involves prioritizing diagnostic ancillary tests based on clinical suspicion and presumptive diagnosis [14]. Preoperative communication with the surgeon and supportive laboratories aids in optimal processing of the sample, such as sending a separate sample to microbiology for PCR studies and cultures in suspected cases of infectious uveitis. Special stains can be done on cell block preparations depending on the suspected etiology (e.g., a PAS stain for fungus infection). If lymphoma is suspected, adequate allocation of the sample to various tests (cytokine analysis, flow cytometry, molecular studies, and immunohistochemistry) should be done depending on the availability and accessibility of ancillary tests (discussed in question 10). Cytology from Papanicolaou- or Diff-Quik-stained smear slides, along with cytokine analysis, can aid diagnosis in autoimmune cases. Metastatic tumors are best diagnosed by cytology and immunohistochemistry performed on cell block preparations to determine the origin of the tumor. Table 19.2 includes recommendations for triaging diagnostic vitrectomy samples [14].

10. What is the workup for a vitrectomy specimen in a potential setting of lymphoma?

Both undiluted and diluted vitreous samples are usually obtained in suspected cases of lymphoma.

Prompt, appropriate transportation and refrigeration of the undiluted sample are essential to prevent cellular degeneration. If the available sample is sufficient, the undiluted vitreous sample can be allocated for 1–3 air-dried Diff-Quik-stained smears, polymerase chain reaction (PCR) analysis, and cytokine assays. PCR analysis, for heavy chain gene rearrangement (IgH) in B-cell lymphomas and TCR (T-cell receptor) rearrangement in the rare T-cell lymphomas, helps to identify monoclonality while cytokine assay for interleukins 6 and 10 (IL-6/IL-10) provides supportive diagnostic evidence. An IL6/IL10 ratio of less than 1 favors a diagnosis

Table 19.2 Recommendations for ancillary tests on diagnostic vitrectomy samples

	Clinical suspicion and presumptive diagnosis			
	Lymphoma	Infection	Autoimmune	Tumor metastasis
Undiluted vitreous	Cytology (smears) (PAP, Diff-Quik stains)	PCR: *Toxoplasma gondii*, HSV, VZV, TB complex, CMV	Cytology (smears) (PAP, Diff-Quik stains)	Cytology (smears) (PAP, Diff-Quik stains)
	PCR: IgH gene rearrangements, TCR rearrangements		Cytokine analysis (IL6/IL10 ratio)	
	Cytokine analysis (IL6/IL10 ratio)			
	Flow cytometry			
Diluted vitreous	Cytology (ThinPrep/SurePath) Cell block (H&E stain, immunohistochemistry for CD20 and CD3, in situ hybridization for kappa/lambda light chains, EBV)	Cytology (ThinPrep/SurePath) Cell block (PAS for fungus)		Cytology (ThinPrep/SurePath) Cell block (immunohistochemistry)
		Microbiology (cultures)		

of lymphoma, while an IL6/IL10 ratio of greater than 1 is most indicative of an inflammatory diagnosis. An undiluted sample of at least 0.5 ml is needed for PCR analysis and a volume of 2 ml is required for interleukin assay. If the undiluted sample is insufficient in volume, it may be diluted with balanced salt solution to reach a volume of 2.5–3 ml, of which 2 ml can be used for IL-10 and IL-6 analysis (1 ml each in two separate syringes) and 0.5–1 ml for PCR analysis. Cytokine measurements on vitreous samples are usually performed in a few laboratories with specific requirements for sample volume. Communication between surgeon, pathologist, and laboratory is key to determine whether all the requested tests will be performed by the same laboratory or if separate samples need to be sent out to different laboratories.

Flow cytometry is a powerful diagnostic tool for lymphoma in fluids with adequate cellularity and most laboratories require a minimum cell count (10,000–40,000/ml, depending on laboratory criteria) in the sample to obtain valid results [16, 17]. Flow cytometry can have very limited application in vitreous samples which are often very paucicellular [18] and may require customized criteria to improve cell collection, such as dilution of vitreous, collection in RPMI-1640 medium enriched with antibiotics, and centrifugation [19–21]. When such facilities exist, preoperative communication with the laboratory will assist in allocating the appropriate sample and volume for flow cytometry.

The diluted vitreous sample is used to prepare liquid-based cytology slides (ThinPrep/SurePath) stained with PAP stain. A cell block (for immunohistochemistry and in situ hybridization for kappa and lambda light chain monoclonality) is prepared if sufficient sample is available.

Primary lymphoproliferative disorder of the vitreous is usually the B-cell lymphoma type [22]. T-cell lymphoma rarely involves the vitreous. Lymphoma cells usually spill over from neoplastic infiltrates in the retina or sub-RPE and commonly present as posterior uveitis with overlapping

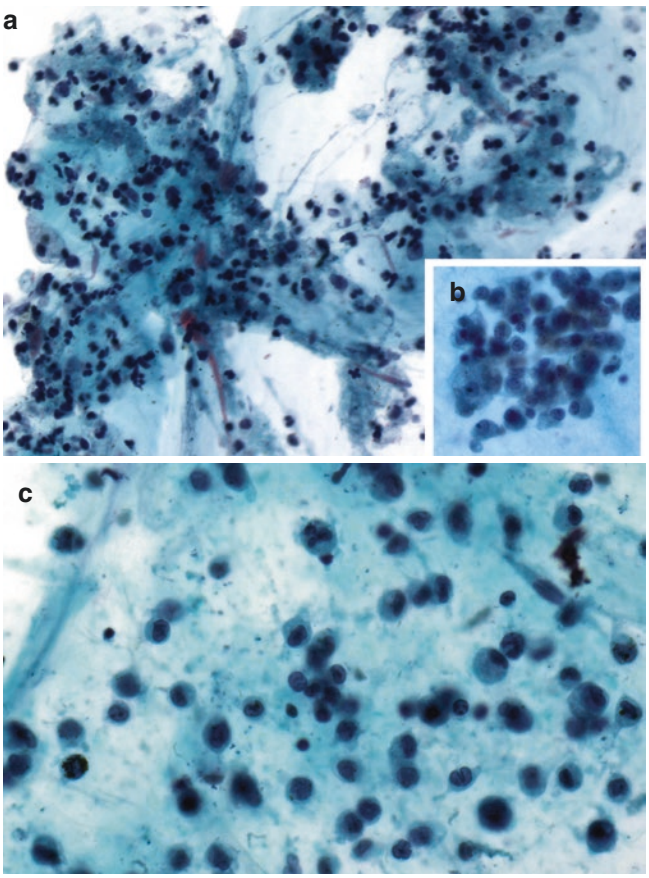

Fig. 19.6 Diagnostic vitrectomy. (**a**) Numerous neutrophils in a case of acute endophthalmitis (PAP). (**b**) Inset. Macrophages and lymphocytes present in a chronic HSV vitritis (PAP). (**c**) Large atypical lymphocytes in a B-cell lymphoma involving the vitreous (PAP)

symptoms between infectious, autoimmune, and neoplastic etiologies. Cytology (Diff-Quik- and PAP-stained slides) can be diagnostic and helpful in differentiating the inflammatory (acute and chronic) cellular infiltrate in infectious etiologies (Fig. 19.6a, b) and the neoplastic cells of lymphoma. The

diagnostic cytology features of intraocular lymphoma include a mixed population of cells with atypical large lymphoid cells which show convoluted nuclear membranes, multiple conspicuous nucleoli, and plasmacytoid-like scant cytoplasm (Fig. 19.6c). An accompanying infiltrate of small lymphocytes that are reactive T-cells is usually present.

11. What are the most common metastatic tumors to the eye?

Common tumors metastatic to the eye are breast and lung carcinomas in women; lung (Fig. 19.7a) and gastrointestinal carcinomas in men. These tumors show the same cytologic features found in samples from the organ of origin. Metastatic breast ductal carcinoma shows pleomorphic cells with enlarged eccentric hyperchromatic nuclei, fine or coarse granular chromatin, and small or large irregularly shaped nucleoli, while metastatic breast lobular carcinoma cells may be small- to medium-sized cells with hyperchromatic nuclei and large cytoplasmic vacuoles.

Among the metastatic carcinomas of the lung, squamous cell carcinoma cells may show keratinized or non-keratinized pleomorphic cells with dense cytoplasm and hyperchromatic nuclei; small cell carcinoma has small cells with characteristic nuclear molding, evenly dispersed powdery chromatin, and scant cytoplasm; adenocarcinoma cells show eccentrically placed nuclei with finely textured chromatin, large nucleoli, and foamy cytoplasm with mucin vacuoles. Metastatic gastrointestinal carcinomas show the similar cytomorphology of the primary tumor, the site of origin of which can extend from the stomach to the anal canal, as well as the pancreas and biliary tract.

Metastatic tumors can also rarely present as vitreous hemorrhage in which case pertinent clinical history and careful cytologic evaluation are important.

12. What are the cytologic features of a uveal melanoma versus a nevus?

Malignant uveal melanoma is the most common intraocular tumor in adults. The mortality of uveal melanoma averages nearly 50% as there is no effective form of chemotherapy or immunotherapy for metastatic uveal melanoma [23]. The clinical distinction between a melanoma and a benign choroidal nevus can be difficult at times and a fine needle biopsy can aid in the diagnosis. Choroidal nevi are benign common melanocytic lesions of the posterior uvea, with a prevalence in the USA ranging from 6% to 10% of the population [23]. The cytology of choroidal nevi shows spindle pigmented cells or amelanotic cells with bland oval or cigar-shaped nuclei, finely dispersed chromatin, without nucleoli, nuclear folds, or increased mitotic activity. Intranuclear cytoplasmic inclusions, balloon cell degeneration, or dendritic plump cells may be seen. If clinical evidence of growth of a choroidal nevus is present, then a malignant transformation must be ruled out.

The cytology of melanoma (Fig. 19.7b) shows atypical melanocytes seen in clusters and singly with spindle and/or epithelioid cell features. Epithelioid melanoma cells have abundant cytoplasm, large vesicular nuclei, and prominent centrally located nucleoli. Cytogenetic testing on air-dried smears can be performed to rule out the presence of monosomy 3 and or trisomy 8, allowing for stratification of the tumor into type 1 or type 2 depending on the absence or presence of monosomy 3. See question 13 for additional details.

13. How does uveal melanoma differ from melanoma seen in other parts of the body with regard to cytogenetic studies?

Uveal melanoma is usually characterized by point mutations in GNAQ and GNA11 genes that encode the G-protein α-subunit, whereas cutaneous melanomas show MAPK activation through mutations in BRAF (≅50 cases), NRAS (≅ 10–25% cases), or loss of function in NF1 (≅14% cases) [24].

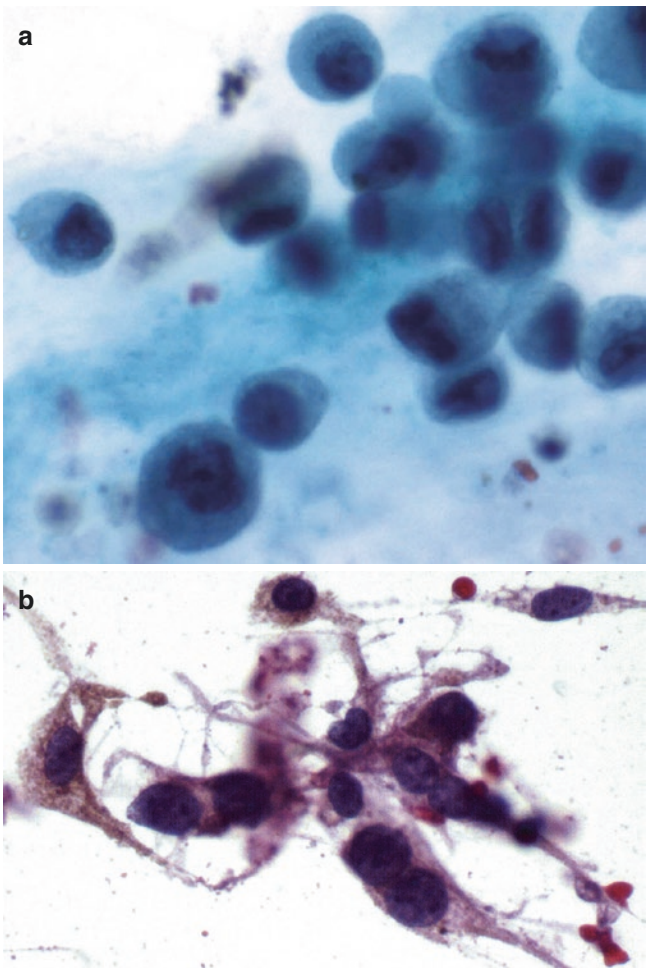

Fig. 19.7 Diagnostic vitrectomy. (**a**) Lung adenocarcinoma cells metastatic to the choroid and retina (PAP). (**b**) Fine needle aspiration biopsy cells showing pigmented atypical melanocytic cells consistent with uveal melanoma (smear, H&E)

BAP1 is a tumor suppressor gene located on chromosome 3 that is mutated in 47% of uveal melanomas [25]. BAP1-inactivating germline mutations are analogous to the frequent loss of chromosome 3 observed in high-risk sporadic disease [26].

If there is a clinical suspicion of uveal malignant melanoma, multiple slides (2–3) should be prepared with a single drop of fluid placed on the center of the slide and air-dried. These slides can be sent for cytogenetic studies to assess for loss of chromosome 3 (or monosomy 3), gain on 6p, loss on 6q, and gain on 8q. Additional molecular tests comprising of a gene expression profile can be done to classify malignant melanomas as class 1 (low metastatic potential) or class 2 (high metastatic potential), by utilizing undiluted fresh tumor fine needle aspiration samples (DecisionDx- UM, Castle Biosciences Incorporated) [27].

14. What are the pitfalls in the diagnosis of a nonpigmented uveal melanoma arising in the iris and ciliary body?

A uveal melanoma arising in the ciliary body can enlarge and cause significant symptomatology such as reduced visual acuity, lens subluxation, focal cataract, secondary glaucoma, pupillary abnormalities, and a blind and painful eye [28]. However similar symptomatology can be seen with rarely enlarging, benign entities such as melanocytoma, foreign body granuloma, metastatic disease, epithelial cyst, hemangioma, schwannoma, leiomyoma, and epithelial tumor of ciliary body origin [28].

Ciliary body pigmented adenomas are characterized by clusters of cytologically bland pigmented cells, which may show focal discohesion, but with uniform cells lacking significant nuclear atypia or nucleoli and with characteristic cytoplasmic, uniformly spherical, variably sized pigment granules [29]. Ciliary body melanocytomas are benign proliferations of plump polyhedral pigmented cells. Nonpigmented adenomas comprise of cohesive clusters of benign-appearing, nonpigmented cells embedded in a prominent extracellular matrix, representing basement membrane – like material, a characteristic feature of adenomas [30].

Pleomorphic adenoma of the ciliary body poses an additional degree of diagnostic difficulty due to its pleomorphic cytology that can closely resemble that of malignant melanoma. The FNA of a pleomorphic adenoma can show increased cellularity, cohesive cords and sheets of cells with significant nuclear atypia, discohesiveness, pigmentation, and rare intranuclear pseudoinclusions, but low nuclear-cytoplasmic ratios (Fig. 19.8a, b). The absence of mitotic figures and necrosis, and the presence of PAS-positive basement membrane, differentiates this entity from malignant melanoma. In addition, the immunohistochemical profile of pleomorphic adenoma includes diffuse positivity for vimentin, focal positivity for S-100 protein, and negative reactivity with cytokeratin, GFAP, HMB-45, and MSA [31].

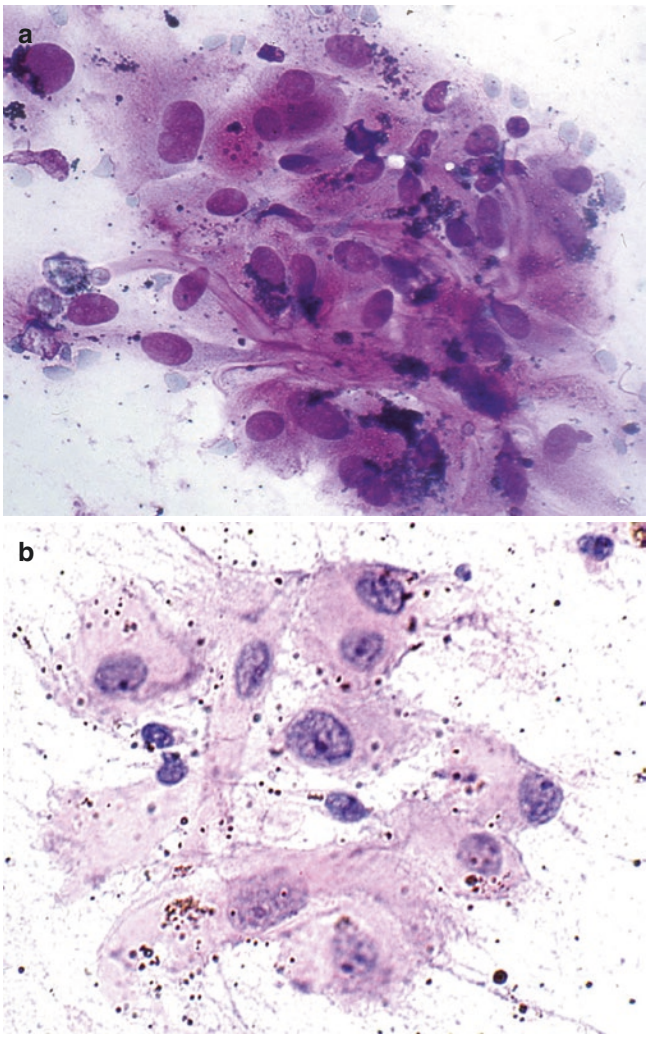

Fig. 19.8 Pleomorphic adenoma of the ciliary body, FNA. (**a**) Cohesive clusters of pleomorphic cells with nuclear atypia and basement membrane-like material with the absence of mitotic figures and necrosis (H&E). (**b**) Cells with intracytoplasmic pigment (H&E)

15. What other tumors are in the differential diagnosis of uveal and retinal lesions?

Uncommon diagnoses, such as congenital simple retinal pigment epithelium (RPE) hamartomas, choroidal nevi, and RPE adenomas and adenocarcinomas, may be sampled by fine needle aspiration biopsy. Congenital simple hamartoma of the RPE appears on ophthalmoscopy as a small localized, elevated black lesion that is usually located in the foveal region and on the surface of the retina [32]. The lesions are small, <1 mm in diameter. They are comprised of a proliferation of the RPE with benign cytological features (Fig. 19.9a).

Adenoma and adenocarcinoma of the RPE appear as an oval-shaped, abruptly elevated, usually pigmented mass arising from the peripheral RPE. The affected eye often has vitreous cells due to inflammation. As it enlarges, adenoma of the RPE can assume a retinal blood supply with large, dilated tortuous feeding vessels; intraretinal and subretinal

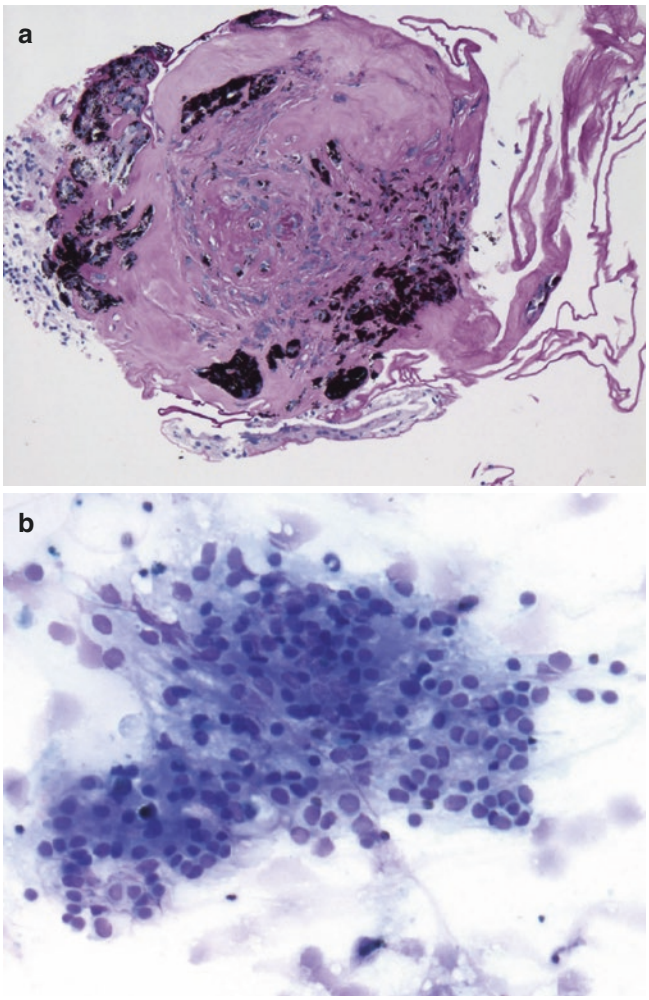

Fig. 19.9 Fine needle aspiration biopsy. (**a**) In congenital simple hamartoma, the retinal pigment epithelium shows proliferating cells with benign cytology and fibrous tissue deposits. (Cell block, PAS.) (**b**) FNA from a case of retinal pigment adenocarcinoma showing atypical cells, mitotic figures, and basement membrane material (smear, Diff-Quik)

exudation are commonly found adjacent to the tumor. Histopathologically, adenoma of the RPE usually shows cords of proliferating RPE cells, separated by fibrous stroma. The tumor cells are large and polyhedral with eccentric round-to-oval nuclei and abundant granular cytoplasm. Adenocarcinoma of the RPE (Fig. 19.9b) exhibits similar patterns, but the cells show increased pleomorphic features [32].

Case Presentation

Inflammatory/infectious vitritis versus lymphoma in a vitrectomy specimen.

- History
  - A 63-year-old white female patient presented with decreased vision and floaters for 1 month in the left eye. Her past medical history was significant for recently treated non-Hodgkin's lymphoma. Ophthalmic examination showed significantly reduced vision in the left eye (20/100) and inflammation throughout the eye: in the anterior chamber (anterior uveitis), vitreous (vitritis), and retina (retinitis). Signs of acute retinitis with necrosis raised the suspicion of viral retinitis. The right eye was normal.
  - Blood tests were inconclusive with negative serologies for infectious etiologies. The patient underwent a diagnostic vitrectomy.
- Specimen source
  - Diagnostic vitrectomy
- Cytology
  - Few atypical lymphocytes and normal lymphocytes
  - Histiocytes
- Differential diagnosis (based on past medical history and clinical impression)
  - Lymphoma
  - Viral vitritis
  - Toxoplasmosis (given the patient's recent treatment history for non-Hodgkin's lymphoma with immunosuppressant therapy)
- Ancillary tests and results
  - Tests for lymphoma: Cytokine analysis – IL-6/IL-10 ratio, greater than 1

    PCR analysis for IgH rearrangements: Negative

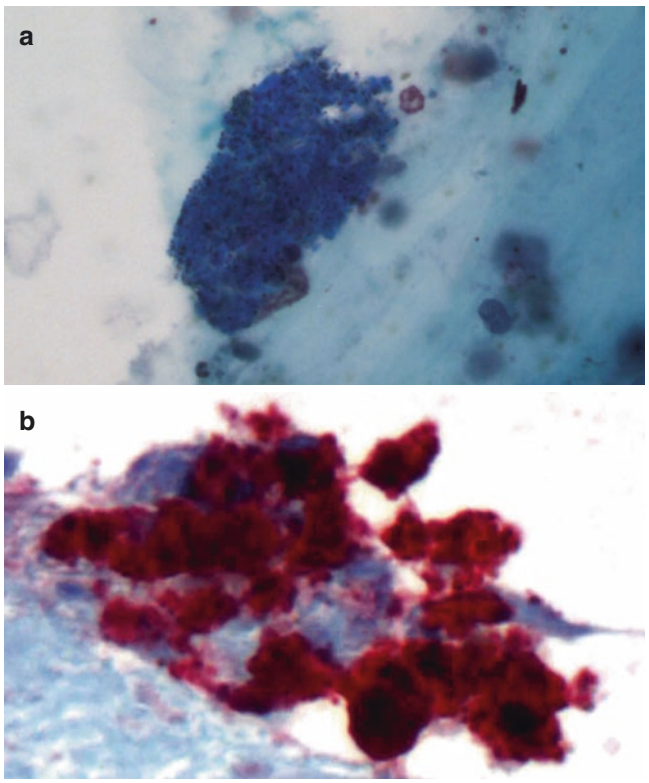

Fig. 19.10 Toxoplasmosis. (**a**) Toxoplasmosis tachyzoite-laden macrophages simulating cellular debris (PAP). (**b**) Positive immunoreaction with anti-toxoplasmosis antibody, red chromogen

  - PCR for viral etiologies (EBV, CMV, HSV, and VZV): Negative
  - PCR analysis for *Toxoplasma gondii*: Positive
- Treatment and outcome
  - The patient was treated with an anti-toxoplasmosis treatment regimen, after which the vision in the left eye significantly improved to near normal (20/25).
- Teaching points:
  1. Clinical history is of utmost importance in generating a differential diagnosis.
  2. Optimal processing of vitreous specimen can avoid repeated vitrectomy procedures and enhance diagnostic efficiency.

This case is an example of atypical ocular toxoplasmosis presenting with equivocal systemic tests. The differential diagnoses included inflammation, infection, and lymphoma etiologies, all with overlapping signs and symptoms. This case demonstrates how clinical correlation and appropriate allocation and processing of a vitreous sample can lead to a definitive diagnosis (toxoplasmosis) and vision-saving treatment.

Ocular cytology can be challenging due to the unique anatomy and clinical conditions affecting the eye. Clinicopathologic correlation, comprehensive knowledge of the various types of ocular specimens, and the appropriate processing and allocation of these specimens to select ancillary tests can greatly reduce the need for repeated sampling and enhance diagnostic efficiency.

Figure 19.10a, b is representative figures of toxoplasmosis vitritis with macrophages seen in vitreous fluid and positive immune reactivity for anti-toxoplasmosis antibody.

Acknowledgment We thank Dr. Stephen Foster for contributing the presented case of ocular toxoplasmosis.

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H. Xu et al. (eds.), *Practical Cytopathology*, Practical Anatomic Pathology, https://doi.org/10.1007/978-3-030-24059-2

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