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CYTOTOLOGY

Diagnostic Principles and Clinical Correlates

Fourth Edition

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Cytology

Diagnostic Principles and Clinical Correlates

FOURTH EDITION

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Cytology: Diagnostic Principles and Clinical Correlates

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Dedication

To Todd Bryant Stewart and Alan M. Ducatman

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Preface

We hope this book will serve as a useful guide for the pathologist in practice and for the trainee—resident or fellow—who is looking to obtain expertise in the subspecialty of cytopathology.

It has been four years since the publication of the third edition of *Cytology: Diagnostic Principles and Clinical Correlates*. Since then, cytology has continued to grow and evolve as a discipline devoted to the diagnosis of cellular tissue obtained by minimally invasive methods (e.g., scraping, brushing, aspiration), thus the need for this updated edition. However, we have retained many of the qualities of the prior editions. This edition again aims to be concise yet comprehensive. We have emphasized brevity and clarity. The text is grounded in an understanding of surgical pathology and current diagnostic terminology. Where relevant, we have illustrated the value of established ancillary studies. Although the book is multi-authored, the chapters follow a similar format: indications, sample collection and preparation methods, recommended terminology for reporting results, accuracy (including common pitfalls that lead to false-negative and false-positive diagnoses), a description of normal elements, and, finally, a how-to guide for the diagnosis of benign and malignant lesions with an emphasis on differential diagnosis. We have retained the bulleted “capsule summaries,” particularly for summarizing cytomorphologic features and differential diagnoses. We have continued to emphasize clinical correlation (hence the title). For example, [Chapter 1](#) includes the recently revised guidelines of the American Society for Colposcopy and Cervical Pathology for managing women with abnormal cervical cytologic diagnoses. Good cytologists are those who understand the clinical implications of their interpretations.

A major enhancement of this new edition is the inclusion of a dedicated chapter on fine-needle aspiration technique and specimen handling, accompanied by a video demonstration. We hope trainees and even practicing pathologists will find this especially useful.

Once again, we hope we have conveyed the beauty, strength, and challenge of cytology. With this book we have strived to take some of the mystery out of cytology, but mysteries remain, their solutions still obscure. If this text inspires the reader to explore and even solve some of them, we will consider ourselves doubly rewarded.

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2013

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We express our deep appreciation to Mr. Dennis Padget of DLPadget Enterprises, Inc., for his help with the complexities of billing in [Chapter 18](#). We relied extensively on his *Pathology Service Coding Handbook* for the information set forth in that chapter. Readers who want more information on pathology coding questions can contact Mr. Padget at DennisPadget@EmbarqMail.com (502-693-5462) for information about subscribing to that comprehensive electronic text.

We are indebted to many members of the staff of the Brigham and Women's Hospital and West Virginia University School of Medicine and Hospital—the cytotechnologists, cytopathologists, and trainees—who inspire us with their devotion to cytopathology and who continue to challenge us. In particular, we acknowledge Dorothy Nappi, CT (ASCP), and Grace Goffi, CT, MIAC, who have helped us train so many pathology residents and fellows over the years. Without their help we would not have our extraordinary collections of cytology teaching cases from which so many of the images in this book are derived.

Finally, to our friends, families, and loved ones, especially Todd Stewart and Alan Ducatman, who tolerated the long evening and weekend hours that

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Edmund S. Cibas

Barbara S. Ducatman

CHAPTER 1

Cervical and Vaginal Cytology

Edmund S. Cibas

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The 20th century witnessed a remarkable decline in the mortality from cervical cancer in many developed countries. This achievement is attributable to the implementation of the Papanicolaou (Pap) test. In the 1930s, before Pap test screening was introduced, cervical cancer was the most common cause of cancer deaths in women in the United States.¹ Today, it is not even in the top 10.²

There are approximately 12,000 new cases of cervical cancer in the United States each year, with 4000 deaths.² Worldwide, however, the cervical cancer incidence (over 500,000 cases annually) and mortality (275,000 deaths per year) are second only to those for breast cancer.³ Screening programs, unfortunately, are rudimentary or nonexistent in many parts of the world. Less than 5% of women in developing countries have ever had a Pap test.⁴ By contrast, 89% of women in the United States report having had a Pap test in the preceding 3 years.

Around the world, Pap test screening is implemented in two different ways, commonly referred to as *opportunistic* versus *organized*.⁵ An *organized screening program* is planned at the national or regional level. It specifies a target population and screening intervals and has a mechanism for inviting women to attend screening services, informing them of their result, and referring them for treatment. *Opportunistic screening*, the system in place in the United States, for example, is done independently of an organized or population-based program, on women who are often visiting health services for other reasons. Screening is recommended during a consultation or requested by the woman. Opportunistic screening tends to reach younger, lower-risk women who are

attending family planning and antenatal services. It is generally accepted that organized screening is more cost-effective than opportunistic screening, making better use of available resources and ensuring that the greatest number of women benefit.

History of the Papanicolaou Test and Its Current Practice

The Pap test is considered by many to be the most cost-effective cancer reduction program ever devised.¹ Credit for its conception and development goes to George N. Papanicolaou, an anatomist and Greek immigrant to the United States. In 1928 he reported that malignant cells from the cervix can be identified in vaginal smears.⁶ Later, in collaboration with the gynecologist Herbert Traut, who provided him with a large number of clinical samples, Papanicolaou published detailed descriptions of preinvasive cervical lesions.^{7,8} Pathologists and clinicians initially greeted this technique with skepticism, but by the late 1940s Papanicolaou's observations had been confirmed by others. The Canadian gynecologist J. Ernest Ayre suggested taking samples directly from the cervix with a wooden spatula, rather than from the vagina with a pipette as originally described by Papanicolaou.⁹ Eventually, cytologic smears were embraced as an ideal screening test for preinvasive lesions, which, if treated, would be prevented from developing into invasive cancer.

The first cervical cancer screening clinics were established in the 1940s.¹⁰ The Pap test was never evaluated in a controlled, prospective study, but several pieces of evidence link it to the prevention of cervical cancer. First, the mortality rate from cervical cancer fell dramatically after screening was introduced, by 72% in British Columbia¹¹ and 70% in Kentucky.¹² Second, there was a direct correlation between the intensity of screening and the decrease in mortality. Among Nordic countries, the death rate fell by 80% in Iceland, where screening was greatest; in Norway, where screening was lowest, the death rate fell by only 10%.¹³ A similar correlation was observed in high-and low-screening regions of Scotland¹⁴ and Canada.¹⁵ In the United States, the decrease in deaths from cervical cancer was proportional to the screening rates in various states.¹⁶ Finally, women in whom invasive cancer does not develop are more likely to have had a Pap test than women with cancer. In a Canadian study, the relative risk for women who had not had a Pap test for 5 years was 2.7,¹⁷ and screening history was a highly significant risk factor independent of other factors such as age, income, education, sexual history, and smoking. In Denmark, a woman's risk of developing cervical cancer decreased in proportion to the number of negative smears she had had—by 48% with just one negative smear, 69% with two to four negative smears, and 100% with five or more smears.¹⁸

Screening guidelines differ around the world. In the United States, revised

cervical cancer screening recommendations were issued in 2012 by the American College of Obstetricians and Gynecologists (ACOG),¹⁹ the U.S. Preventive Services Task Force (USPSTF),²⁰ and a consortium of the American Cancer Society, the American Society for Colposcopy and Cervical Pathology, and the American Society for Clinical Pathology (ACS/ASCCP/ASCP).²¹ Their guidelines differ in minor ways, but there is general agreement on the larger points, including longer screening intervals and a later age to start screening (age 21) than had been recommended in the past ([Table 1.1](#)). The U.S. Department of Health and Human Services (DHHS) offers a web-based National Guideline Clearinghouse that synthesizes the guidelines of the different organizations.²² The guidelines address women with an average risk for cervical cancer. Women at higher risk—those with a history of cervical cancer, in utero diethylstilbestrol (DES) exposure, and/or immunocompromise (due to organ transplantation, chemotherapy, chronic corticosteroid treatment, or infection with the human immunodeficiency virus [HIV])—may benefit from more frequent screening. Because women with HIV infection/acquired immune deficiency syndrome (AIDS) have higher rates of cervical cancer than the general population, it is recommended that HIV-seropositive women have a Pap test twice during the first year after diagnosis of HIV infection and, if the results are normal, annually thereafter.²³ Adherence to screening guidelines is critical for cervical cancer prevention. In Sweden, for example, women who had not had a Pap smear within the recommended screening interval were at higher risk for development of cervical cancer than those who had been screened (odds ratio 2.52).²⁴

TABLE 1.1
CERVICAL CANCER SCREENING GUIDELINES IN THE UNITED STATES (FOR WOMEN AT AVERAGE RISK)

Circumstance	Recommendation
Age to begin screening	Age 21. Women younger than age 21 should not be screened, regardless of the age of sexual initiation
Women aged 21 to 29 years	Every 3 years with cytology (liquid-based or conventional) alone
Women aged 30 to 65 years	Every 3 years with cytology alone, or Every 5 years if cotesting with cytology and human papillomavirus (HPV) assay (preferred by ACOG and ACS/ASCCP/ASCP)
Discontinuation of screening	Age 65 years if adequate prior screening and no history of cervical intraepithelial neoplasia (CIN) 2 or higher*
Screening after total hysterectomy	Not recommended if no history of CIN 2 or higher

ACOG, American College of Obstetrics and Gynecology; ACS/ASCCP/ASCP, American Cancer Society/American Society for Colposcopy and Cervical Pathology/American Society for Clinical Pathology; CIN 2, cervical intraepithelial lesion grade 2.

*ACOG and ACS/ASCCP/ASCP define “adequate prior screening” as *three consecutive negative cytology results or two consecutive negative co-test results within the previous 10 years, with the most recent test performed within the past 5 years*. “No history of CIN 2 or higher” is defined by ACS/ASCCP/ASCP as *within the last 20 years*.

In 2012, the ASCCP revised its guidelines for the management of women with abnormal cervical cytology, human papillomavirus (HPV), and histopathologic results.²⁵ These guidelines, mentioned throughout this chapter in the relevant sections, apply only to women whose abnormalities are detected during screening. Management is individualized for women with postcoital or unexplained abnormal vaginal bleeding, pelvic pain, abnormal discharge, or a visible cervical lesion.

Two prophylactic HPV vaccines provide a new opportunity for cervical cancer prevention. Both vaccines consist of empty protein shells called *viruslike particles* that are made up of the major HPV capsid protein L1. They contain no DNA and are not infectious. One of the vaccines, Gardasil (Merck & Co., Inc.), is a quadrivalent vaccine that protects against HPV types 6, 11, 16, and 18. The other is the bivalent vaccine Cervarix (GlaxoSmithKline), which protects against HPV 16 and 18. They have shown extraordinary efficacy in preventing type-specific histologic cervical intraepithelial neoplasia (CIN) grade 2/grade 3 lesions, with no difference in serious adverse effects from placebo.²⁶ The vaccines are administered in three doses to females prior to the initiation of sexual activity. Screening guidelines, however, are no different for the vaccinated population than for those not vaccinated. Continued Pap screening, even for the vaccinated population, remains important because these vaccines do not protect against 30% of cervical cancers (i.e., those not related to HPV 16 or 18); the duration of protection is unknown; they are not effective in treating prevalent HPV infections; and the cost of the vaccines might limit their use in some populations. The American Cancer Society recommends routine HPV vaccination principally for females aged 11 and 12 years, and also for females aged 13 to 18 to “catch up” those who missed the opportunity to be vaccinated.²⁷ According to the 2011 National Immunization Survey of Teens, 53% of female adolescents aged 13 to 17 years in the United States had initiated HPV vaccination, and 35% had completed the recommended three doses.²⁸

Sampling and Preparation Methods

To obtain an ideal Pap specimen, the American Cancer Society recommends the following patient instructions²⁹:



Patient instructions

- Try not to schedule an appointment for a time during your menstrual period. The best time is at least 5 days after your menstrual period stops.
- Do not use tampons, birth-control foams, jellies, other vaginal creams, or douches for 2 to 3 days before the test.
- Do not have sexual intercourse for 2 days before the test.

Once the patient is positioned, a bivalve speculum of appropriate size is gently inserted into the vagina.³⁰



Specimen collection

- The speculum can be lubricated with warm water or sparingly applied water-soluble lubricant.
- Excess mucus or other discharge should be removed gently with a cotton swab.
- The sample should be obtained before the application of acetic acid or Lugol's iodine.
- An optimal sample includes cells from the ectocervix and endocervix.

Water-soluble gel lubricant, if used, should be applied sparingly to the posterior blade of the speculum, avoiding the tip; excessive lubricant can result in an unsatisfactory specimen.³⁰⁻³⁴ When visible, different lubricants have different effects and different appearances on cytologic preparations.³⁴⁻³⁶ It can be helpful to check any guidelines issued by the manufacturers of liquid-based

cytology instruments with regard to recommended lubricants.

There are no clinically important differences between conventional smears and liquid-based cytology (LBC) methods, so either is considered acceptable for cytologic screening.^{20,21}

Conventional Smears

Conventional smears are often obtained using the combination of a spatula and brush. The spatula is used first. Although a wooden or plastic spatula is acceptable, the plastic spatula is recommended, because wooden fibers trap diagnostic material.³⁰ The spatula is rotated at least 360°. The sample can be smeared on one half of a slide and spray fixed (the other half should be covered to avoid coating it with fixative before the endocervical sample is applied). Alternatively, one may set aside the spatula sample momentarily while the endocervical brush sample is obtained.

After the brush is inserted in the endocervical canal, some bristles should still be visible. If it is inserted too far, there may be inadvertent sampling of the lower uterine segment (LUS), which causes diagnostic difficulties because its epithelium resembles a high-grade intraepithelial lesion (HSIL) and adenocarcinoma in situ (AIS). The brush should be rotated gently only one-quarter turn. A larger rotation is unnecessary because the circumferential bristles are in contact with the entire surface the moment the brush is inserted.

The spatula sample, if not already applied and fixed, should be applied to the slide, then the brush sample rolled over the slide, followed by immediate fixation. The two samples can be placed in quick succession on two separate halves of the slide, or the endocervical sample can be rolled directly over the spatula sample, both covering the entire slide. Immediate fixation (within seconds) is critical in order to prevent air-drying artifact, which distorts the cells and hinders interpretation.

The broomlike brush (“broom”) has a flat array of plastic strips contoured to conform to the cervix, with longer strips in the middle. This design allows simultaneous sampling of the endocervix and ectocervix. The long middle strips are inserted into the os until the shorter outer strips bend against the ectocervix. The broom is rotated three to five times. To transfer the material, each side of the broom is stroked once across the slide in a painting motion.

The cotton swab moistened with saline is no longer recommended because its fibers trap cells, reducing the efficiency of cell transfer onto slides.

There are two options for smear fixation. Coating fixatives contain alcohol

and polyethylene glycol and are applied by pump sprays, by droppers from dropper bottles, or by pouring from an individual envelope included as part of a slide-preparation kit. Alternatively, the smear can be immersed directly into a container filled with 95% ethanol.

Samples for LBC are obtained as just described, except that instead of smearing the cells on a slide, the collection device is rinsed in a vial containing a liquid fixative. In the United States, the liquid-based Pap test is more common than the smear.

Liquid-Based Cytology

In 1996, the U.S. Food and Drug Administration (FDA) approved the **ThinPrep** (Hologic, Marlborough, MA) as an alternative to the conventional cervicovaginal smear. This was followed 3 years later by approval of the AutoCyté Prep (now **SurePath**) (BD TriPath, Burlington, NC). LBC was an important step in the development of automated Pap screening devices—an improved preparation was needed to minimize cell overlap so that automated instruments would perform better in identifying abnormal cells. But LBC performed so well in clinical trials against conventional smears that it found a market independent of automated screening. Although a number of studies showed an increased detection of cytologic low-grade squamous cell intraepithelial lesion (LSIL) and/or HSIL with LBC,³⁷ subsequent metaanalyses and prospective randomized trials failed to demonstrate a significant difference between conventional smears and LBC in the detection of histologic CIN 2/3.^{38,39} Nevertheless, LBC offers several advantages over conventional smears: the opportunity to prepare duplicate slides and even cell block preparations from the residual sample^{40,41}; the option of “out-of-vial” aliquoting for HPV, *chlamydia*, and gonorrhea testing; an improved substrate for automated screening devices; and a thinner cell preparation that most pathologists and cytotechnologists find less tiring to review than smears.

ThinPrep Papanicolaou Test

The practitioner obtains the ThinPrep Pap sample with either a broom-type device or a plastic spatula/endocervical brush combination. The sampling device is swirled/rinsed in a methanol-based preservative solution (PreservCyt) for transport to the cytology laboratory and then discarded. Red blood cells are lysed by the solution. The vials are placed one at a time on the ThinPrep 2000

instrument. The entire procedure (Fig. 1.1A) takes about 70 seconds per slide and results in a thin deposit of cells in a circle 20 mm in diameter (contrast with cytocentrifuge: diameter = 6 mm). A batch-processing version (the ThinPrep 3000) is also available. It uses the same consumables (filters and solutions) but allows automated processing of 80 samples at one time. 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).

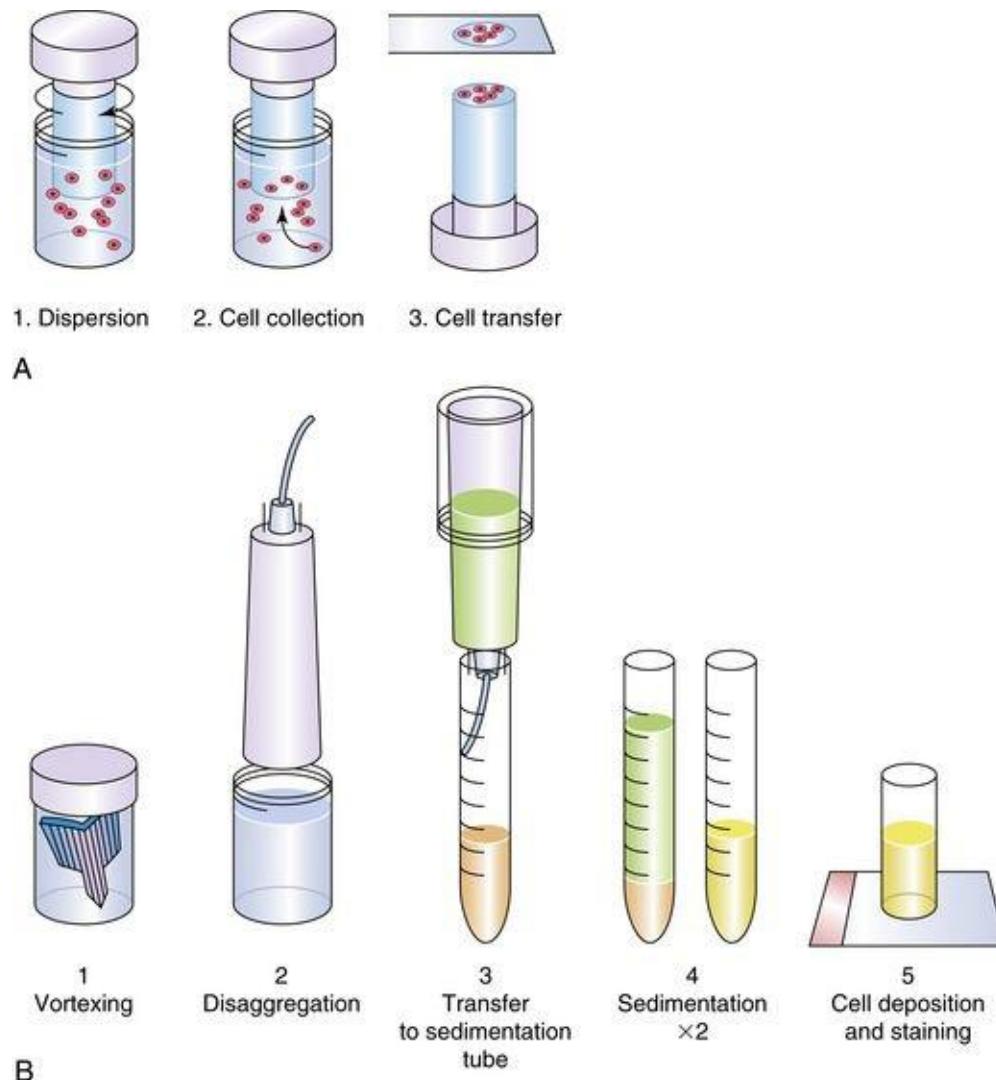


FIGURE 1.1 Liquid-based slide preparation methods.

A, ThinPrep method. 1. The sample vial sits on a stage, and a hollow plastic cylinder with a 20 mm diameter polycarbonate filter bonded to its lower surface is inserted into the vial. A rotor

spins the cylinder for a few seconds, dispersing the cells. 2. A vacuum is applied to the cylinder, trapping cells on the filter. The instrument monitors cell density on the filter. 3. With continued application of vacuum, the cylinder (with cells attached to the filter) is inverted 180°, and the filter pressed against a glass slide. The slide is immediately dropped into an alcohol bath. *B*, SurePath method. 1. The sample is vortexed. 2. Cell clusters are disaggregated by syringing the sample through a small orifice. 3. The sample is poured into a centrifuge tube filled with a density gradient reagent. 4. Sedimentation is performed in a centrifuge. A pellet is obtained and resuspended, and the sedimentation is repeated. 5. The tubes are transferred to the PrepStain instrument, where a robotic arm transfers the fluid into a cylinder. Cells settle by gravity onto a cationic polyelectrolyte-coated slide. The same robotic arm also dispenses sequential stains to individual cylinders.

A multicenter, split-sample study found that the ThinPrep detected 18% more cytologic cases of LSIL and more serious lesions as compared with conventional smears, with no significant difference in the detection of organisms.⁴² A number of studies have shown significant increases in the detection of cytologic HSIL after the implementation of the ThinPrep.^{37,43-47} Subsequent metaanalyses and a prospective randomized trial, however, failed to demonstrate a significant difference between conventional smears and ThinPrep in the detection of histologic CIN 2/3.^{38,39} Data suggest that the ThinPrep is equivalent to the conventional smear in the detection of endocervical AIS and endometrial pathology.^{48,49}

The ThinPrep collection vial has been approved by the FDA for testing for HPV, useful for primary screening alongside the Pap (so-called cotesting), and for managing women whose Pap specimen shows atypical squamous cells (ASCs).^{25,50}

SurePath Papanicolaou Test

TriPath Imaging (acquired by Becton Dickinson in 2006) developed the SurePath Pap test (formerly AutoCyté Prep) for samples collected in an ethanol-based transport medium. The process is shown in [Figure 1.1B](#). In contrast with the ThinPrep method, the practitioner snips off the tip of the collection device and includes it in the sample vial. The equipment to prepare slides includes a Hettich centrifuge and the PrepStain robotic sample processor with computer and monitor. The PrepMate is an optional accessory that automates mixing the sample and dispensing it onto the density reagent. Red blood cells and some leukocytes are eliminated by density centrifugation. In addition to preparing an evenly distributed deposit of cells in a circle 13 mm in diameter, the method incorporates a final staining step that discretely stains each individual slide.

A multicenter, split-sample clinical trial showed a 7.2% increase in the

detection of cytologic LSIL and more serious lesions, as well as a significant decrease in the percentage of unsatisfactory specimens.⁵¹ Subsequent metaanalyses, however, failed to demonstrate a significant difference between conventional smears and SurePath in the detection of histologic CIN 2/3.³⁹

Automated Screening

Historical Overview

Automated cytology screening devices have been under development since the 1950s. The first computerized screening system was developed in the United States by Airborne Instruments Inc. and was called the Cytoanalyzer.⁵² In preclinical trials it did not perform as well as expected, and the project was discontinued. The difficulty of the task was soon appreciated, especially the inherent problems with analyzing smears prepared in the conventional manner. Despite setbacks, research into cervical cytology screening continued throughout the following decades, with the development of the TI-CAS,⁵³ Quantimet,⁵⁴ BIOPEPR,⁵⁵ CERVIFIP,⁵⁶ CYBEST,⁵⁷ DIASCANNER,^{58,59} FAZYTAN,⁶⁰ and LEYTAS.⁶¹ Some of these instruments are now in museums, but others have served as prototypes for systems that are now commercially available.

In the 1990s, researchers in the United States and Canada established private enterprises supported by venture capital in order to develop a commercial automated screening instrument. Foremost in the field were AutoCyte (formerly Roche Image Analysis Systems), Cytac, Neopath, and Neuromedical Systems. A three-way merger took place in 1999, when AutoCyte, after purchasing the intellectual property of Neuromedical Systems, merged with Neopath to form a new company called TriPath Imaging, acquired in 2006 by Becton Dickinson. In 2007, Cytac Corporation, developer of the ThinPrep Pap Test and ThinPrep Imaging System, merged with Hologic Inc. and became a wholly owned subsidiary of Hologic.

In 1998, the FDA approved the AutoPap System (now called the FocalPoint Slide Profiler; BD TriPath Imaging, Burlington, NC) as a primary screener for conventional cervicovaginal smears, followed by approval in 2002 for use with SurePath slides. In 2003, the FDA approved the ThinPrep Imaging System (Hologic, Marlborough, MA) as a primary screener for ThinPrep Pap slides, and in 2008 it approved the FocalPoint Guided Screening (GS) Imaging System. Neither is approved in the United States for automated screening of nongynecologic cytology specimens.

ThinPrep Imaging System

The ThinPrep Imaging System (TIS) uses the principle of *location-guided*

screening to aid the cytotechnologist in reviewing a ThinPrep Pap slide. TIS consists of two components, the image processor (“imager”) and the Review Scope ([Fig. 1.2A and B](#)). Stained and coverslipped ThinPrep slides are placed in a cartridge (each cartridge holding 25 slides), and up to 10 cartridges are loaded onto the bench-top imager. The imager has the capacity to screen more than 300 slides per day. It scans the slides and identifies 22 fields of view (FOV) on each slide that, based on optical density measurements and other features, are the most likely to harbor abnormal cells. The x and y coordinates of the 22 FOV are stored in a database and retrieved at a later time. The server is electronically linked to one or more Review Scopes in the laboratory. A Review Scope resembles a standard microscope but is augmented with an automated stage, a pod that controls the stage and objectives, and a keypad. The scope also has a camera that reads the slide identifier when the slide is loaded onto the stage. When a valid slide identifier is recognized, the server sends its coordinate information to the scope, permitting the cytotechnologist to navigate to the 22 FOV using the pod. Navigation to each FOV is done geographically—that is, using the shortest distance from one FOV to the next. The cytotechnologist uses the pod to advance forward or return back through the FOV, changing objectives as needed. If no abnormal cells are found in any of the FOV, the case has been completed and can be reported as negative. If any abnormal cells are found in any of the FOV, a review of the entire slide must be performed. This can be done using the autoscan function on the Review Scope, with preset, customized user screening preferences. The Review Scope has both electronic and physical slide dotting capabilities.



FIGURE 1.2 Automated cytology screening devices.

A, ThinPrep Imaging System: the imager. The imager consists of (*left to right*): the imaging station, an image processor and server, and a user interface consisting of a monitor, keyboard, and mouse. B, ThinPrep Imaging System: the Review Scope. Imaging data are electronically linked to a customized microscope called the Review Scope. After the ThinPrep slides have been imaged, they are brought to the RS for location-guided review. In addition to a microscope, there is a console (with display and keypad) and a navigator pod. C, BD FocalPoint Slide Profiler. The FocalPoint Slide Profiler consists of two main components (*left to right*): the workstation (computer, monitor, keyboard, mouse, modem, and printer) and the floor-standing instrument (slide processor). D, BD FocalPoint Guided Screening Review Station. After SurePath slides have been imaged, they are brought to the Review Station for location-guided review. Imaging data are electronically linked to a customized microscope. In addition to the microscope, there is a barcode scanner and a monitor with keyboard and mouse. (A and B courtesy Hologic, Inc. and affiliates. C and D courtesy BD Diagnostics Inc.).

The accuracy of the TIS was evaluated in a clinical trial at four laboratories. ThinPrep slides were first screened manually, and the results recorded. They were then rescreened using the TIS. Truth adjudication was performed by expert review of all abnormal cases and a proportion of negative slides. The TIS detected significantly more abnormal slides (atypical squamous cells of

undetermined significance [ASC-US] or greater) than manual review (82% versus 76%).⁶² A later split-sample study comparing conventional smear cytology versus the TIS for ThinPrep slides showed a significantly higher detection rate of histologic HSIL (CIN 2/3) with the TIS.⁶³

Because 22 FOV represent approximately 25% of the ThinPrep cell spot,⁶⁴ implementation of the TIS enhances productivity.^{62,65,66}

Implementing the TIS requires adopting the proprietary ThinPrep Pap stain, to which some adjustment is necessary because it yields darker nuclear staining of metaplastic and endocervical cell clusters than most traditional Pap stains. The TIS does not eliminate false-negatives, which are still encountered, albeit less frequently than in the absence of imaging.⁶² A number of postapproval studies have shown significant increases in the detection of cytologic LSIL and HSIL after implementation of the TIS.⁶⁷⁻⁶⁹

BD FocalPoint Guided Screening Imaging System

The BD FocalPoint Guided Screening (GS) Imaging System ([Fig. 1.2C](#) and [D](#)) uses programmed algorithms to measure cellular features like nuclear size, integrated optical density, nuclear-to-cytoplasmic ratio, and nuclear contour—morphologic features established using planimetry and ocular micrometry for the diagnosis of squamous and glandular lesions.⁷⁰

AutoPap, the predecessor of the BD FocalPoint GS Imaging System, was originally intended as a primary screening device that would eliminate the need to manually screen as many as one half of all smears. It was temporarily redesigned as a quality control rescreening device called the AutoPap 300 QC System and obtained FDA approval for this function in 1995. The AutoPap 300 QC System did not find a wide audience, however, and became obsolete in the year 2000. A redesign resulted in a new instrument (the AutoPap System-Primary Screener, later renamed BD FocalPoint Slide Profiler) which obtained FDA approval as a primary screening device in 1998. In this mode, the device is used in the initial screening of smears. It identifies up to 25% of slides as requiring “no further review.” Of the remaining slides that require manual review, it also identifies at least 15% for a second manual review, which may be used as a substitute for the 10% review of negative Paps required of all U.S. laboratories (see [Chapter 18](#)). A barcode is applied to each slide, and slides are loaded into slide trays. Up to 288 slides can be loaded at a time (8 slides per tray, 36 trays). Each slide is analyzed using preset algorithms at $\times 4$ magnification for a visual map of the entire slide, then 1000 fields are captured at $\times 20$

magnification. After analysis, the device assigns a score (from 0 to 1.0) to each slide according to the likelihood of an abnormality. Slides with scores below a cut off are considered “no further review,” and those above the cut-off are triaged for full manual review. Any slide deemed unsuitable for analysis because of preparation or coverslipping problems requires manual review.

The accuracy of the BD FocalPoint Slide Profiler was evaluated in a clinical trial at five laboratories.²¹ Each slide was first evaluated in the conventional manner. The same slides were then processed by the AutoPap System, which detected significantly more abnormal slides (ASC-US or greater) than conventional practice (86% versus 79%). Of importance, the BD FocalPoint Slide Profiler is not approved for women at high risk for cervical cancer. Thus, a laboratory that uses the BD FocalPoint Slide Profiler for primary screening must set aside all Paps from high-risk women for manual screening. It is up to the laboratory to define what constitutes a Pap from a high-risk patient. False-negative results are occasionally encountered with the BD FocalPoint Slide Profiler. In the clinical trial, there were 10 false-negatives (5 ASC-US, 4 LSILs, and 1 HSIL) in the 1182 cases considered “no further review,” and another study found 9 false-negatives (5 ASC-US and 4 LSILs) in the 296 cases considered “no further review.”²² The productivity gain is modest, because in practice the FocalPoint Slide Profiler archives only about 16% to 17% of Paps without full manual review.^{21,23}

The most recent phase in BD FocalPoint development occurred in 2008 with FDA approval of the BD FocalPoint GS Imaging System. The BD FocalPoint GS Imaging System consists of the BD FocalPoint Slide Profiler plus a BD FocalPoint GS Review Station and, like the TIS, uses the principle of location-guided screening to aid the cytotechnologist in reviewing a slide. A SurePath slide is first examined by the BD FocalPoint Slide Profiler, which uses algorithms to identify the 10 FOV most likely to harbor abnormal cells. These FOV slides are presented to a cytotechnologist for review at the microscopic Review Station; if no abnormality is detected in the FOV, the slide is reported as negative without any further review. But if any abnormality is seen in any of the FOV samples, or if specimen adequacy cannot be confirmed, the slide is triaged for full manual review.

The accuracy of the BD FocalPoint GS Imaging System was evaluated in a clinical trial at four laboratories. The detection of cytologic HSIL+ increased by 19.6% and of cytologic LSIL+ by 9.8% in the computer-assisted arm, with small but statistically significant decreases in specificity. For cytologic ASC-US+ sensitivity and specificity, the study arms were not statistically different.²⁴ As with the TIS, implementation of the BD FocalPoint GS Imaging System

enhances productivity.⁷⁵

Accuracy and Reproducibility

The sensitivity of cytology for detecting preinvasive squamous and glandular lesions is difficult to establish, but it is clearly far from perfect. Most studies of preinvasive lesions suffer from verification bias (i.e., cases are referred for biopsy on the basis of an abnormal smear, and biopsy is not performed in women with negative Pap test results). The few relatively unbiased studies show that the mean sensitivity of the Pap test is 47% (range 30% to 80%), and the mean specificity is 95% (range 86% to 100%).⁷⁶

The sensitivity of cytology is less than ideal for invasive cancers as well, and estimates range widely (16% to 82%). Many women with cervical cancer have a history of one or more negative smears.⁷⁷⁻⁸⁸ The relative contributions of sampling and laboratory error vary from one study to another and likely depend on how carefully retrospective rescreening is performed.

False-positive diagnoses of cervical cancer occur in 10% to 15% of cases.^{89,90} The chief culprits are the atrophic smear with benign squamous atypia in a granular, pseudonecrotic background; reparative changes; and keratinizing HSILs.

The interobserver reproducibility of cytologic interpretations is also less than perfect. In a large study of women, most of whom had mild cytologic abnormalities, the unweighted κ statistic for four categories of diagnosis—negative, atypical, LSIL, and HSIL—was 0.46, indicating moderate reproducibility.⁹¹ (Roughly, a κ of 0 or less represents poor agreement; 0 to 0.2, slight agreement; 0.2 to 0.4, fair agreement; 0.4 to 0.6, moderate agreement; 0.6 to 0.8, very good agreement; and 0.8 to 1.0, almost perfect agreement.) In the same study, the reproducibility of histologic interpretations of cervical biopsies, also for four categories of diagnosis, was identical (0.46). The greatest disagreement with Paps involved those originally interpreted as showing ASC-US; the second reviewer agreed with only 43% of cases. The greatest disagreement with biopsies involved those originally interpreted as CIN 1; the second reviewer concurred in only 43% of cases.⁹¹

A graphic demonstration of the relative reproducibility of various cytologic findings is available on the Bethesda System Web Atlas, which contains the results of the Bethesda Interobserver Reproducibility Project. A large number of images were reviewed by hundreds of observers, who were asked to place the images into one of the Bethesda System categories. The results are displayed for each image as a histogram.⁹²

Diagnostic Terminology and Reporting Systems

Papanicolaou devised a numerical system for reporting cervical smears, which was originally intended to convey his degree of suspicion that the patient had cancer: **class I**, absence of atypical or abnormal cells; **class II**, atypical but no evidence of malignancy; **class III**, suggestive of but not conclusive for malignancy; **class IV**, strongly suggestive of malignancy; and **class V**, conclusive for malignancy. Over time, however, the Papanicolaou class system underwent many modifications and was not used in a uniform fashion.⁹³ It nevertheless persisted in many laboratories well into the 1980s. In other laboratories it was replaced (or supplemented) by descriptive terms borrowed from histologic classifications of squamous lesions. Squamous cancer precursors were originally divided into **carcinoma in situ**, a high-risk lesion of immature, undifferentiated atypical cells, and **dysplasia** (subdivided into mild, moderate, and severe), the latter a lower-risk lesion of more mature squamous cells. In the 1960s, Richart challenged the duality of dysplasia/carcinoma in situ and proposed a new term, **cervical intraepithelial neoplasia** (CIN). CIN was graded from 1 to 3, but Richart believed that CIN 1 (mild dysplasia) had a strong propensity to progress to CIN 3 and cancer. The high rate of progression found in his study most likely related to stringent entry criteria: for inclusion, CIN 1 had to be confirmed on three consecutive Paps.⁹⁴ The study data showed a higher progression rate for mild dysplasia than most other natural history studies.⁹⁵ The CIN concept was highly influential, however, and for many years squamous precursors were treated as much on the basis of their size and location as on their grade.

In 1989, the Bethesda System was introduced to standardize the reporting of cervical cytology results and incorporate new insights gained from the discovery of HPV.⁹⁶ The name for a squamous cancer precursor was changed to **squamous intraepithelial lesion (SIL)**, subdivided into only two grades (low and high), based on the evolving understanding of the biology of HPV. In this system, LSIL encompasses CIN 1, and HSIL encompasses CIN grades 2 and 3. This was a shift away from the CIN concept, one based on a reevaluation of the existing evidence, which demonstrated that most LSILs are, in fact, transient HPV infections that carry little risk for oncogenesis, whereas most HSILs are associated with viral persistence and a significant potential for progression to invasive cancer.

The first Bethesda System workshop, in 1988, was followed by two others, in

1991 and 2001, which made modifications to the original framework and terminology. The 2001 workshop broadened participation by using a dedicated website on the Internet, and an electronic bulletin board received more than 1000 comments regarding draft recommendations. The 2001 Bethesda System, like its predecessors, recommends a specific format for the cytology report, starting with an explicit statement on the adequacy of the specimen, followed by a general categorization and an interpretation/result.^{97,98}

The Bethesda System

Specimen Adequacy

One of the most important advances of the Bethesda System is its recommendation that each Pap report begin with a statement of adequacy. In 1988, the Bethesda System proposed three categories for specimen adequacy: “satisfactory,” “less than optimal” (renamed “satisfactory but limited by” in 1991), and “unsatisfactory.” The 2001 Bethesda System eliminated the middle category because it was confusing to clinicians and prompted unnecessary repeat Pap tests. Nevertheless, the 2001 Bethesda System advocates mentioning the presence or absence of a transformation zone component and permits comments on obscuring elements. The 2001 Bethesda System criteria for adequacy are listed in [Table 1.2](#). They are somewhat arbitrary, because scientific data on adequacy are limited, particularly regarding the minimum number of cells needed for an adequate sample.

TABLE 1.2

THE 2001 BETHESDA SYSTEM CATEGORIES FOR SPECIMEN ADEQUACY

SATISFACTORY FOR EVALUATION

A satisfactory squamous component must be present. Note the presence/absence of endocervical/transformation zone component.

Obscuring elements (inflammation, blood, drying artifact, other) may be mentioned if 50% to 75% of epithelial cells are obscured.

UNSATISFACTORY FOR EVALUATION

Specimen rejected/not processed because [specify reason].

Reasons may include

- Lack of patient identification
- Unacceptable specimen (e.g., slide broken beyond repair)

or:

Specimen processed and examined, but unsatisfactory for evaluation of an epithelial abnormality because [specify reason]. Reasons may include

- Insufficient squamous component
- Obscuring elements covering more than 75% of epithelial cells

It is easy to determine whether a specimen is adequate or unsatisfactory in most cases. Slides received without patient identification or broken beyond repair should be rejected as unsatisfactory. An appropriately labeled smear with an adequate complement of well-preserved squamous and endocervical cells is clearly satisfactory. About 1% or less of Pap specimens are interpreted as unsatisfactory.^{99,100} Unsatisfactory Paps can be finalized by a cytotechnologist and need not be reviewed by a cytopathologist (see [Chapter 18](#)).

One of the components of an adequate Pap specimen is an *adequate squamous component*. In the 1988 and 1991 Bethesda Systems, the requirement for an adequate squamous component was defined as “well-preserved and well-visualized squamous epithelial cells should cover more than 10% of the slide surface.”¹⁰¹ This guideline, however, was interpreted differently by different cytologists. Even in laboratories that interpreted it literally, observers consistently overestimated the percentage of slide coverage by squamous cells.¹⁰² With the 2001 Bethesda System modification, the requirement was redefined as a minimum “estimated number of squamous cells,” the minimum being different for conventional and liquid-based preparations:



The minimum number of squamous cells for adequacy depends on the preparation method:

- liquid-based: 5000
- conventional: 8000 to 12,000

The minimum number of 5000 squamous cells for an adequate LBC Pap was based on correlations made between the false-negative rate and squamous cell cellularity.¹⁰³ Because LBCs likely represent a more homogeneous representation of the material obtained by the collection device,¹⁰⁴ a more stringent squamous cellularity requirement was imposed on conventional smears.

The cellularity of the squamous cell component is *estimated*; laboratories are not expected to count individual cells. With experience, an adequate squamous cell component is apparent in most cases. In borderline cases, techniques are available for estimating adequacy: reference images for conventional smears and a spot-counting procedure for liquid-based preparations. Reference images of known cell counts are useful for estimating cellularity.¹⁰² Accordingly, the 2001 Bethesda System published images to assist in the estimation of squamous cellularity on conventional smears.⁹⁸

A spot-counting method is used to evaluate LBCs with borderline squamous cellularity. A minimum of 10 fields are counted along a diameter that includes the center of the slide ([Fig. 1.3A](#)). If the cell circle has blank spots, these should be represented in the fields counted ([Fig. 1.3B](#)). The average number of squamous cells is then compared against tables that take into account the objective, the eyepiece field number, and the diameter of the circle that contains cellular material.⁹⁸ For example, with an FN20 eyepiece, and a $\times 40$ objective, the sample is adequate if the average number of cells counted is greater than 3.1 for a ThinPrep slide.

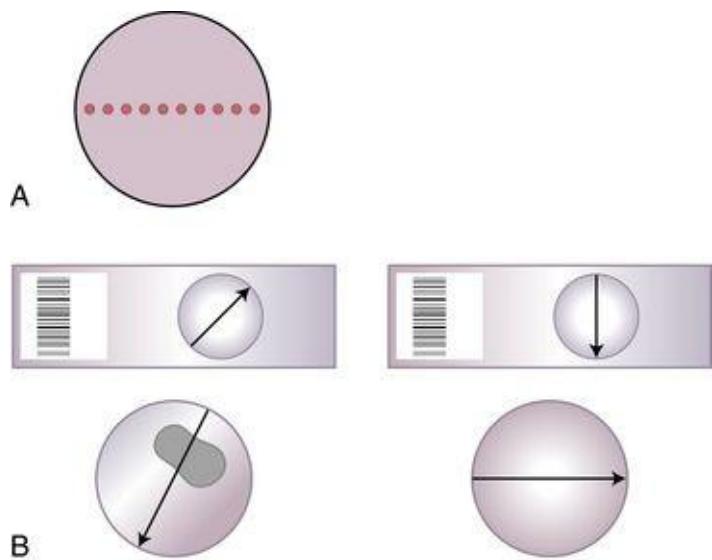


FIGURE 1.3 Method for estimating the adequacy of the squamous component of liquid-based preparations.

A, At $\times 40$, 10 fields are counted starting at the edge (horizontal or vertical) and including the center of the preparation. B, An attempt is made to include “holes” in proportion to their size, making sure that the fields counted cover both cellular and sparsely cellular areas in proportion to their size.

Additional slides can usually be generated from the residual vial of an LBC sample. In some laboratories, an additional slide is prepared when the initial slide has insufficient cellularity. The addition of a washing step with 10% glacial acetic acid increases the percentage of satisfactory ThinPrep Paps, uncovering occasional cases of SIL and invasive cancer.^{105,106}

Squamous cellularity is sometimes difficult to estimate, for example, when there is marked cell clustering or cytolysis. In certain clinical settings, particularly in women with atrophy, a lower number may be adequate. In these situations, cytologists are expected to use their judgment when evaluating adequacy.⁹⁸

For women with an unsatisfactory Pap result, repeat cytology in 2 to 4 months is recommended. In women with an unsatisfactory Pap and a positive HPV test, either a repeat Pap in 2 to 4 months or colposcopy is acceptable.²⁵

In the 2001 Bethesda System, the presence or absence of an *endocervical/transformation zone component* is noted on the report. An endocervical component is considered present if 10 or more endocervical or squamous metaplastic cells, either isolated or in groups, are present. The data on the endocervical component as a measure of adequacy are contradictory.¹⁰⁷ The importance of endocervical cells was first suggested by cross-sectional studies, which showed that smears are more likely to contain SIL when endocervical

cells are present.^{108–110} Data from retrospective case-control studies, however, do not support this; investigators have found no association between false-negative Pap and the absence of endocervical cells.^{111,112} Retrospective cohort studies have shown that women whose initial smears lack endocervical cells do not develop more lesions on followup than women whose smears do have an endocervical component,^{113–115} implying that an endocervical component is not essential. Currently, a smear without endocervical cells is not considered unsatisfactory, although the absence of an endocervical/transformation zone component is mentioned as a “quality indicator.” A repeat Pap is not necessary.²⁵

General Categorization

The general categorization is an optional component of the 2001 Bethesda System.



Three categories

- negative for intraepithelial lesion or malignancy
- epithelial cell abnormality
- other

The 1991 Bethesda categories “within normal limits” and “benign cellular changes” were combined into a single “negative” category in 2001. “Other” includes cases that do not fit neatly into one of the other two categories: non-epithelial malignancies like melanoma and lymphoma, and benign-appearing endometrial cells in women over 40 years of age.

Specimens are categorized according to the most significant abnormality identified.

Interpretation and Results

Recommended terminology for reporting findings is listed in [Table 1.3](#).

TABLE 1.3
THE 2001 BETHESDA SYSTEM FOR REPORTING CERVICAL CYTOLOGY

SPECIMEN ADEQUACY (see Table 1.2)

GENERAL CATEGORIZATION (Optional)

Negative for intraepithelial lesion or malignancy (NILM)

Epithelial cell abnormality

Other

INTERPRETATION/RESULTS

NILM

Organisms

Trichomonas vaginalis

Fungal organisms morphologically consistent with *Candida* species

Shift in flora suggestive of bacterial vaginosis

Bacteria morphologically consistent with *Actinomyces* species

Cellular changes consistent with herpes simplex virus

Other non-neoplastic findings

Reactive cellular changes associated with: inflammation (includes typical repair); radiation; intrauterine contraceptive device (IUD)

Glandular cells status post hysterectomy

Atrophy

Epithelial cell abnormalities

Squamous cell

Atypical squamous cells (ASC)

- of undetermined significance (ASC-US)
- cannot exclude HSIL (ASC-H)

Low-grade squamous intraepithelial lesion (LSIL)

High-grade squamous intraepithelial lesion (HSIL)

Squamous cell carcinoma (SQC)

Glandular cell

Atypical glandular cells (AGC) (specify if endocervical,
endometrial, or not otherwise specified)

AGC, favor neoplastic (specify if endocervical or not
otherwise specified)

Endocervical adenocarcinoma in situ (AIS)

Adenocarcinoma

Other

Endometrial cells in a woman older than 40 years of age

AUTOMATED REVIEW AND ANCILLARY TESTING

EDUCATIONAL NOTES AND SUGGESTIONS (Optional)

Including nonneoplastic findings, other than organisms, is optional, given that many clinicians desire the Pap test report to be as concise as possible. Findings of no clinical consequence, if mentioned, may result in confusion and even unnecessary repeat testing. Nevertheless, many cytologists believe it is important to document that certain findings were interpreted as benign, particularly those that can mimic a neoplasm.

The Normal Pap

A normal Pap test result begins with a statement of adequacy, followed by “negative for intraepithelial lesion or malignancy” (NILM). Additional findings (e.g., reactive changes, infectious organisms) are listed subsequently. Approximately 90% of Pap specimens are interpreted as NILM.¹¹⁶ NILM Paps, with the exception of those specimens that show reactive/reparative changes, can be finalized by a cytotechnologist and need not be reviewed by a pathologist (see [Chapter 18](#)). In the United States, a pathologist is required to review cases that feature reactive/reparative changes and any abnormality at the level of ASC-US or higher. This represents about 10% to 20% of the total Pap volume in most laboratories.

Squamous Cells

The ectocervix is lined by a stratified squamous epithelium that matures under the influence of estrogen. The most mature squamous cells are called *superficial cells*. They have a small, pyknotic nucleus that is 5 to 6 μm in diameter. *Intermediate cells* have a larger nucleus measuring 8 μm in diameter, which is not pyknotic but instead has a finely granular texture. Intermediate cells are occasionally binucleated and even multinucleated. Both superficial and intermediate cells are large polygonal cells with transparent pink or green cytoplasm ([Fig. 1.4](#)). Superficial and intermediate cells are the predominant cells in cytologic samples from women of reproductive age.

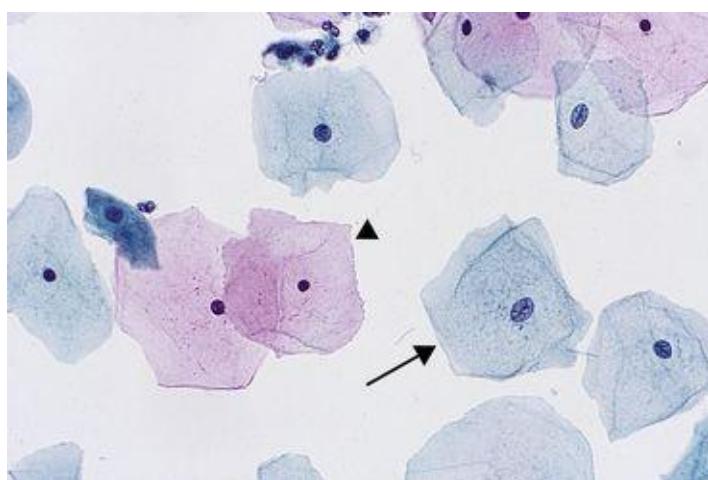


FIGURE 1.4 Superficial and intermediate squamous cells.

The mature squamous epithelium of the ectocervix in women of reproductive age is composed throughout most of its thickness by superficial (arrowhead) and intermediate (arrow) cells.

Immature squamous cells are called *parabasal cells* and *basal cells*. Because a Pap test does not usually scrape off the entire thickness of the epithelium but only the upper few layers, immature cells near the base of a mature epithelium are not usually sampled. An immature epithelium, however, is composed throughout its thickness by parabasal-type and/or basal-type cells. Immature epithelium is common at the transformation zone, where it is called *squamous metaplasia*, and whenever there is squamous epithelial atrophy due to a low estrogen state. Thus, parabasal and basal cells are typically obtained from squamous metaplasia or atrophic epithelium.

Squamous atrophy is encountered in a variety of clinical settings associated with a low estrogen state.



Low estrogen states include:

- premenarche
- postpartum
- postmenopause
- Turner syndrome
- status post bilateral oophorectomy

Immature, parabasal cells are round or oval rather than polygonal and have a variably sized nucleus that is usually larger than that of an intermediate cell. Basal cells are even smaller and have very scant cytoplasm ([Fig. 1.5](#)).

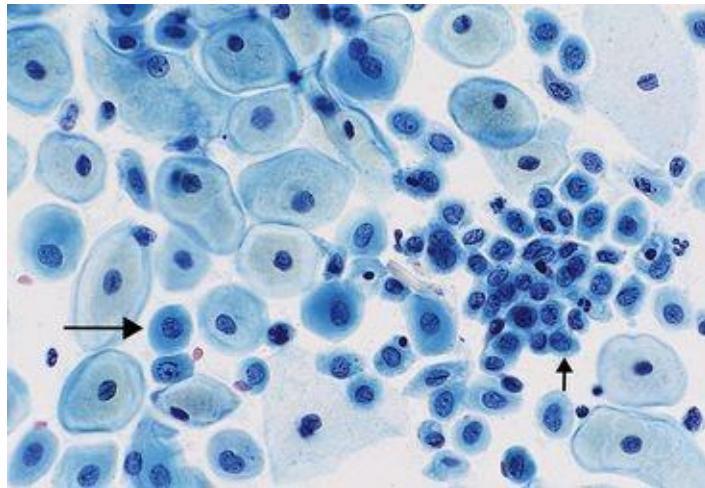


FIGURE 1.5 Parabasal and basal cells (postpartum smear).

Parabasal cells (*large arrow*) are oval and typically have dense cytoplasm. Basal cells (*small arrow*) are similar but have less cytoplasm. Many cells have abundant pale-yellow staining glycogen, a characteristic but nonspecific feature of squamous cells of pregnancy and the postpartum period.

Basal and parabasal cells are the hallmark of atrophy. With a deeply atrophic cervical epithelium, no superficial or intermediate cells are seen, only parabasal and basal cells. In addition, atrophic epithelium, particularly in postmenopausal women, is prone to injury and inflammation and often shows a spectrum of changes that should be recognized as normal and not confused with a significant lesion. Sheets of immature cells are crowded and syncytium-like, mimicking the crowded cells of an HSIL (Fig. 1.6A). Nevertheless, the chromatin texture in atrophy is finely granular and evenly distributed, nuclear contours remain mostly smooth and thin, and mitoses are generally absent. A curious variant, *transitional cell metaplasia*, is notable for prominent longitudinal nuclear grooves (“coffee-bean nuclei”), wrinkled nuclei, and small perinuclear halos (Fig. 1.6B).¹¹⁷ Cellular degeneration is seen in some cases of atrophy (Fig. 1.7A). Dark blue, rounded, amorphous masses known as “blue blobs,” thought to represent either condensed mucus or degenerated bare nuclei, are sometimes seen (Fig. 1.7B), as is a granular background (see Fig. 1.7A) that resembles the necrosis associated with invasive cancers.

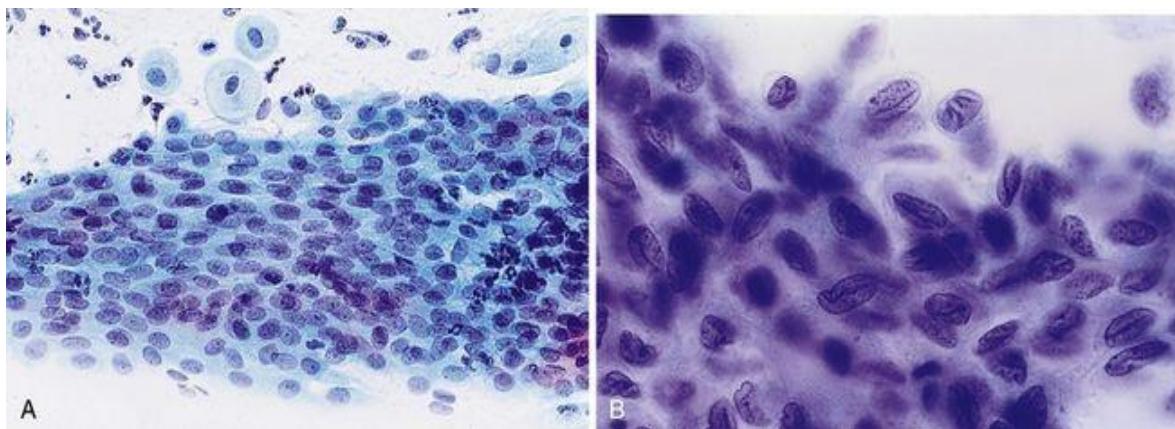


FIGURE 1.6 Parabasal cells (postmenopausal smear).

A, Atrophic epithelium is composed almost exclusively of parabasal cells, often arranged in broad, flowing sheets. B, Transitional cell metaplasia. In this uncommon condition, the atrophic epithelium resembles transitional cell epithelium by virtue of its longitudinal nuclear grooves. Nuclear membrane irregularities raise the possibility of a high-grade squamous intraepithelial lesion (HSIL), but the chromatin is pale and finely textured.

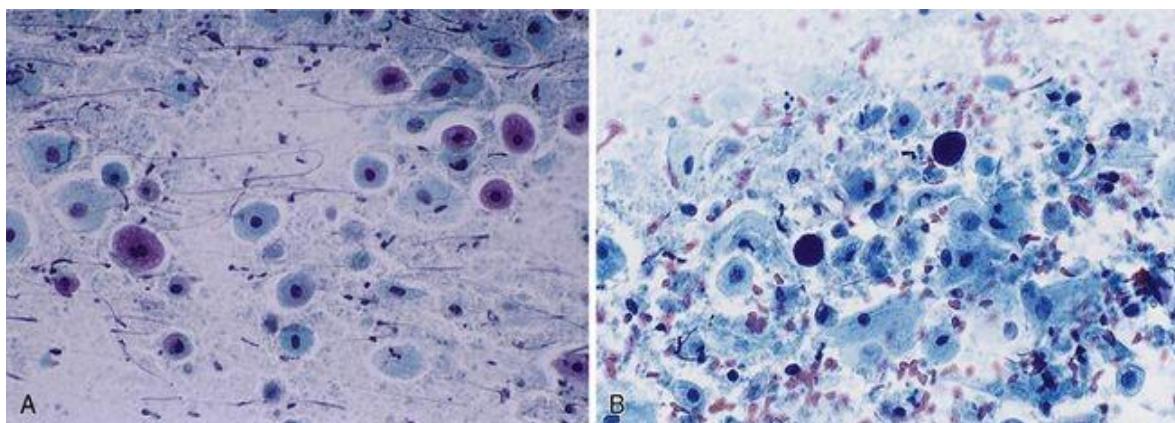


FIGURE 1.7 Parabasal cells (postmenopausal smear).

A, Degenerated parabasal cells in atrophic Paps have hypereosinophilic cytoplasm and a pyknotic nucleus. Note the granular background, which is commonly seen. B, Dark blue blobs are seen in some atrophic smears. These featureless structures should not be interpreted as a significant abnormality.

Parabasal cells are also the constituents of squamous metaplasia of the endocervix. Squamous metaplasia is a common morphologic alteration of the endocervical epithelium usually limited to the transformation zone in women who otherwise have good squamous maturation. It is identified on smears as flat sheets of immature squamous cells (parabasal cells), often arranged in an interlocking fashion like paving stones ([Fig. 1.8](#)). The parabasal cells may show

mild variation in nuclear size, with slightly irregular contours and slight hyperchromasia.

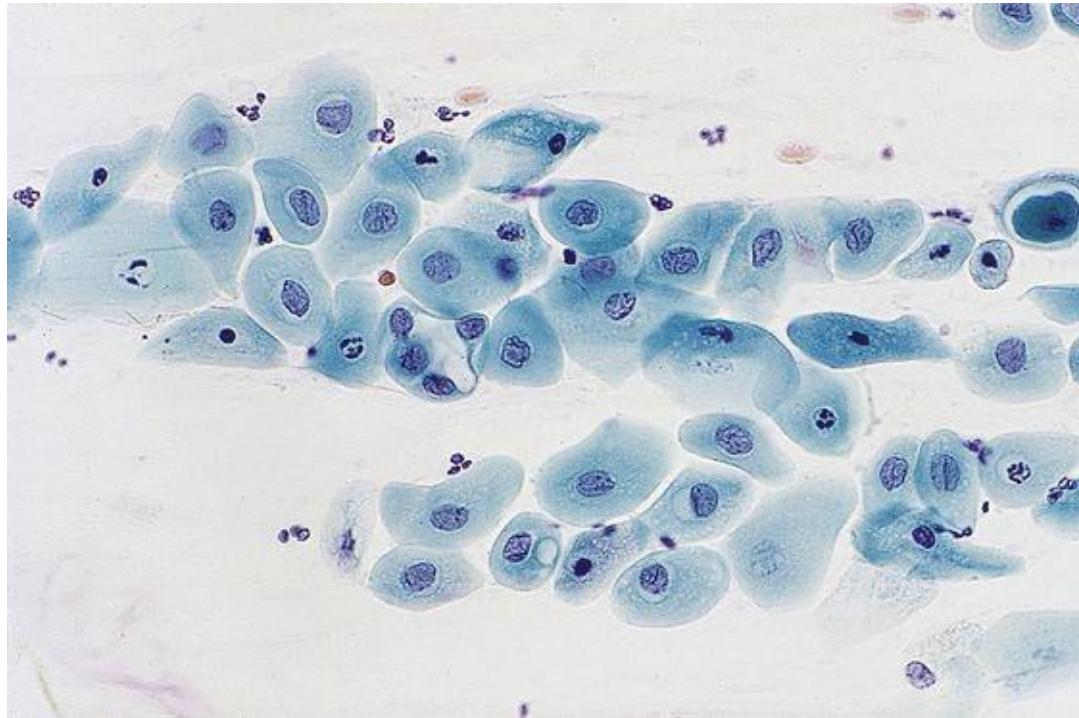


FIGURE 1.8 Squamous metaplasia.

Interlocking parabasal-type cells, as seen here, represent squamous metaplasia.

Squamous metaplasia, as defined cytologically, is always composed of parabasal cells (immature squamous cells). So-called mature squamous metaplasia, a histologic term describing mature squamous epithelium overlying endocervical glands, is not recognized as such on cytologic preparations.

Other normal changes of squamous cells are hyperkeratosis and parakeratosis. *Hyperkeratosis* is a benign response of stratified squamous epithelium due to chronic mucosal irritation, as in uterine prolapse. Anucleate, mature, polygonal squamous cells appear as isolated cells or plaques of tightly adherent cells ([Fig. 1.9A](#)). Such cells are benign and should not be considered abnormal. This cytologic picture is mimicked by contamination of the slide by squamous cells of the vulva or skin from the fingers of persons handling the slide.

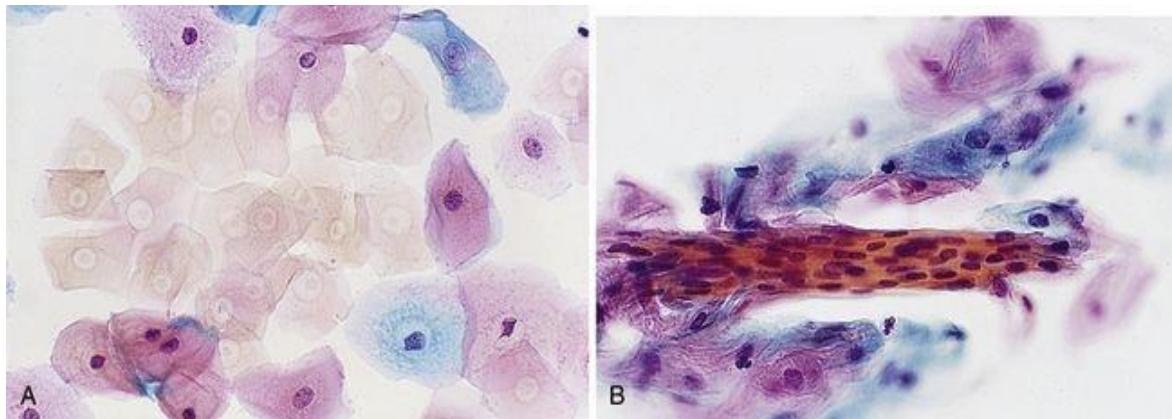


FIGURE 1.9 Keratosis.

A, Hyperkeratosis. Anucleate squames are a protective response of the squamous epithelium.
B, Parakeratosis. Parakeratosis appears as plaques, as seen here, or isolated cells.

Parakeratosis, a benign reactive change also caused by chronic irritation, is characterized by small, heavily keratinized squamous cells with dense orangeophilic cytoplasm and small, pyknotic nuclei ([Fig. 1.9B](#)). When such densely keratinized cells show nuclear atypia in the form of enlargement and membrane irregularity, they are called “atypical parakeratosis” and should be categorized as an epithelial cell abnormality (see further on).

Endocervical Cells

The endocervix is lined by mucin-producing columnar cells that have an eccentrically placed nucleus with a finely granular chromatin texture and abundant vacuolated cytoplasm. Nucleoli are inconspicuous but become very prominent in reactive conditions like cervicitis. Endocervical cells are often identified in strips or sheets rather than as isolated cells ([Fig. 1.10](#)). When arranged as strips, the cells have the appearance of a picket fence. When in sheets, they resemble a honeycomb because of the well-defined cell borders and uniform cell arrangement. Rarely, mitoses are identified. They should not raise suspicion of a neoplasm if the cells are otherwise normal in appearance. Tubal metaplasia is a benign alteration of the endocervical epithelium found in about 30% of cone biopsy and hysterectomy specimens ([Fig. 1.11](#)).¹¹⁸

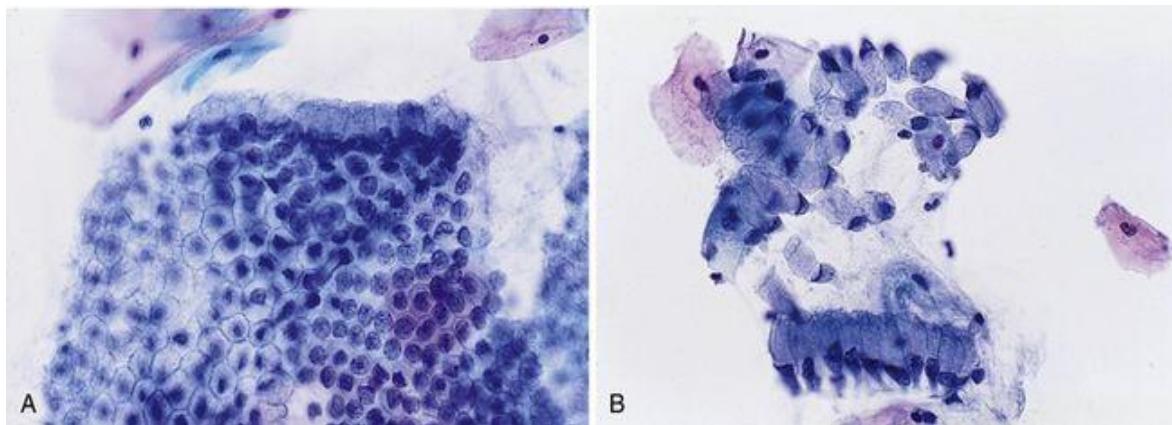


FIGURE 1.10 Endocervical cells.

A, Normal endocervical cells are often arranged in cohesive sheets. Note the even spacing of the nuclei, their pale, finely granular chromatin, and the honeycomb appearance imparted by the sharp cell membranes. B, Sometimes they appear as strips or isolated cells. Abundant intracytoplasmic mucin results in a cup-shaped nucleus.

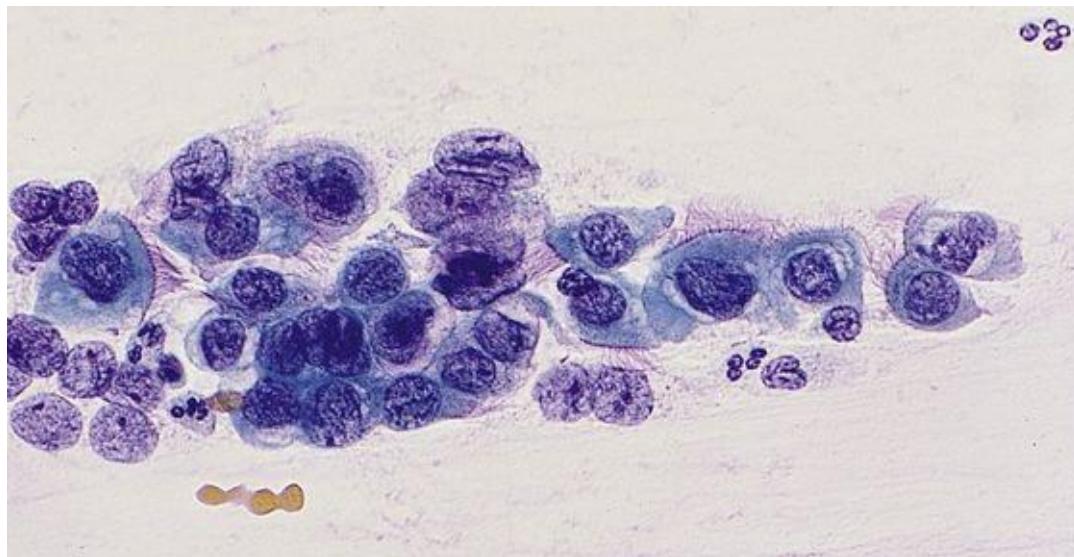


FIGURE 1.11 Tubal metaplasia.

Ciliated endocervical cells are occasionally seen.

Exfoliated Endometrial Cells

Spontaneously exfoliated, menstrual endometrial cells are seen if the Pap sample is taken during the first 12 days of the menstrual cycle.¹¹⁹



Cytomorphology of exfoliated endometrial cells

- balls of small cells
- isolated small cells
- scant cytoplasm
- dark nucleus
- nuclear molding
- nuclear fragmentation

Exfoliated endometrial cells are most easily recognized when they are arranged in spherical clusters ([Fig. 1.12](#)). They are small, with a dark nucleus and (usually) scant cytoplasm. Occasional cells may have more abundant clear cytoplasm. Clusters have a scalloped contour owing to the slight protrusion of individual cells. Apoptosis is common. Isolated endometrial cells are also seen, but they are less conspicuous because of their small size.

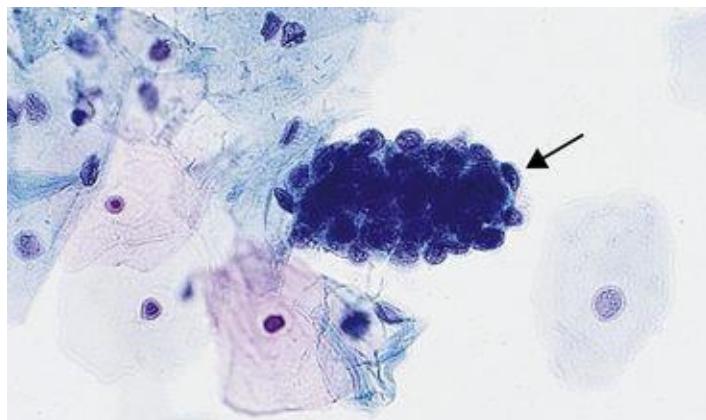


FIGURE 1.12 Endometrial cells.

Spontaneously exfoliated endometrial cells, as in menses, are small cells arranged in balls. Cytoplasm is scant. Nuclei around the perimeter appear to be wrapping around adjacent cells (*arrow*), a characteristic but nonspecific feature.

Occasionally, endometrial cell clusters consist of an obvious dual cell population, with small, dark stromal cells (in the center) and larger glandular cells (around the edges). Most endometrial cell clusters, however, do not have this dual population. Clusters like that in [Fig. 1.12](#) might be glandular endometrial cells, stromal endometrial cells, or a mix of both.¹²⁰

Shedding endometrial cells after day 12 (“out of phase”) is associated with endometritis, endometrial polyps, and intrauterine devices (IUDs). In a young woman, abnormal shedding is almost never due to endometrial adenocarcinoma.^{[121,122](#)} For this reason, endometrial cells need not be mentioned in the report for women younger than 40 years of age. Some laboratories do so anyway, to document that the cells were identified and interpreted as benign endometrial cells. Endometrial cells are notorious for their ability to cause diagnostic difficulty, because a variety of neoplastic cells resemble endometrial cells. In a woman 40 years of age or older, benign-appearing endometrial cells are reported because of the small risk of endometrial neoplasia.



Differential diagnosis of exfoliated endometrial cells

- HSIL
- squamous cell carcinoma
- AIS
- small cell carcinoma

The differential diagnosis includes a number of very significant lesions that mimic endometrial cells and thus are sometimes mistakenly interpreted as normal, particularly if the woman is in the first 12 days of her menstrual cycle. Attention to certain cytologic details can help avoid some if not all of these misattributions. A minority of HSILs are composed of relatively small cells. Like endometrial cells, their nuclei are dark, and they have scant cytoplasm ([Fig. 1.13A](#)). HSIL cells, even when small, are usually bigger than endometrial cells, vary more in size, and have denser cytoplasm. HSIL clusters are usually less well circumscribed and not as spherical as endometrial cell balls. Some poorly differentiated squamous cell carcinomas (SQCs) are comprised of small, dark cells that mimic endometrial cells to perfection ([Fig. 1.13B](#)). In such cases, suspicious clinical findings (e.g., postcoital bleeding) might be the only clue to the correct interpretation. Most adenocarcinomas *in situ* have a columnar cell morphology, but a minority are made up of smaller and rounder cells ([Fig. 1.13C](#)), particularly on liquid-based preparations. Careful examination for focal columnar differentiation and mitoses can be very helpful. The rare small cell carcinoma of the cervix may display crush artifact ([Fig. 1.13D](#)), which is rarely seen with endometrial cells.

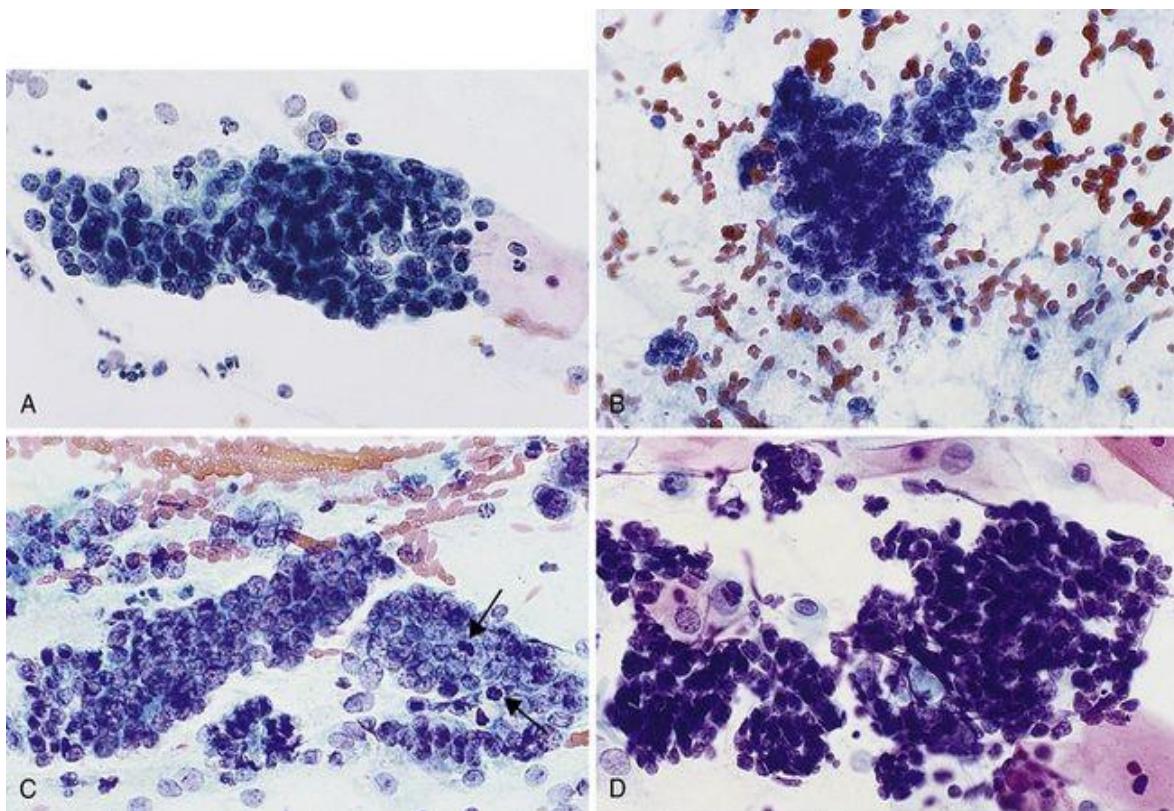


FIGURE 1.13 Mimics of exfoliated endometrial cells.

A, High-grade squamous intraepithelial lesion (HSIL). The cells of some HSILs are small, but still larger than endometrial cells and usually arranged in flatter aggregates rather than spheres. B, Squamous cell carcinoma (SQC). Some poorly differentiated SQCs are indistinguishable from endometrial cells. The granular debris (“tumor diathesis”) seen here can also be seen in normal menstrual Pap samples. C, Adenocarcinoma in situ (AIS). Some cases of AIS have an endometrioid appearance, but mitoses (arrows) are distinctly uncommon in exfoliated endometrial cells. D, Small cell carcinoma. The cells resemble endometrial cells but are even darker. There is nuclear smearing, which is not characteristic of benign endometrial cells.

Abraded Endometrial Cells and Lower Uterine Segment

The endocervical sampling device occasionally inadvertently samples the LUS or endometrium.¹²³ This is especially likely when the endocervical canal is abnormally shortened, such as after a cone biopsy or trachelectomy.^{124,125}



Cytomorphology of abraded endometrium and lower uterine segment

- large and small tissue fragments
- glands and stroma
- stromal cells

- uniform
- oval or spindle-shaped
- finely granular chromatin
- occasional mitoses
- capillaries traversing larger fragments
- glands
 - tubular
 - straight or branching
 - mitoses (some cases)
 - extreme nuclear crowding
 - scant cytoplasm

The characteristic feature is the combination of glands and stroma, often in large fragments ([Fig. 1.14A-C](#)), either together or separated. Glandular cells of the LUS resemble endocervical cells but have a higher nuclear-to-cytoplasmic ratio, are more hyperchromatic, and can be mitotically active. Because of their very high nuclear-to-cytoplasmic ratio, they can be confused with a significant squamous or glandular lesion.¹²³

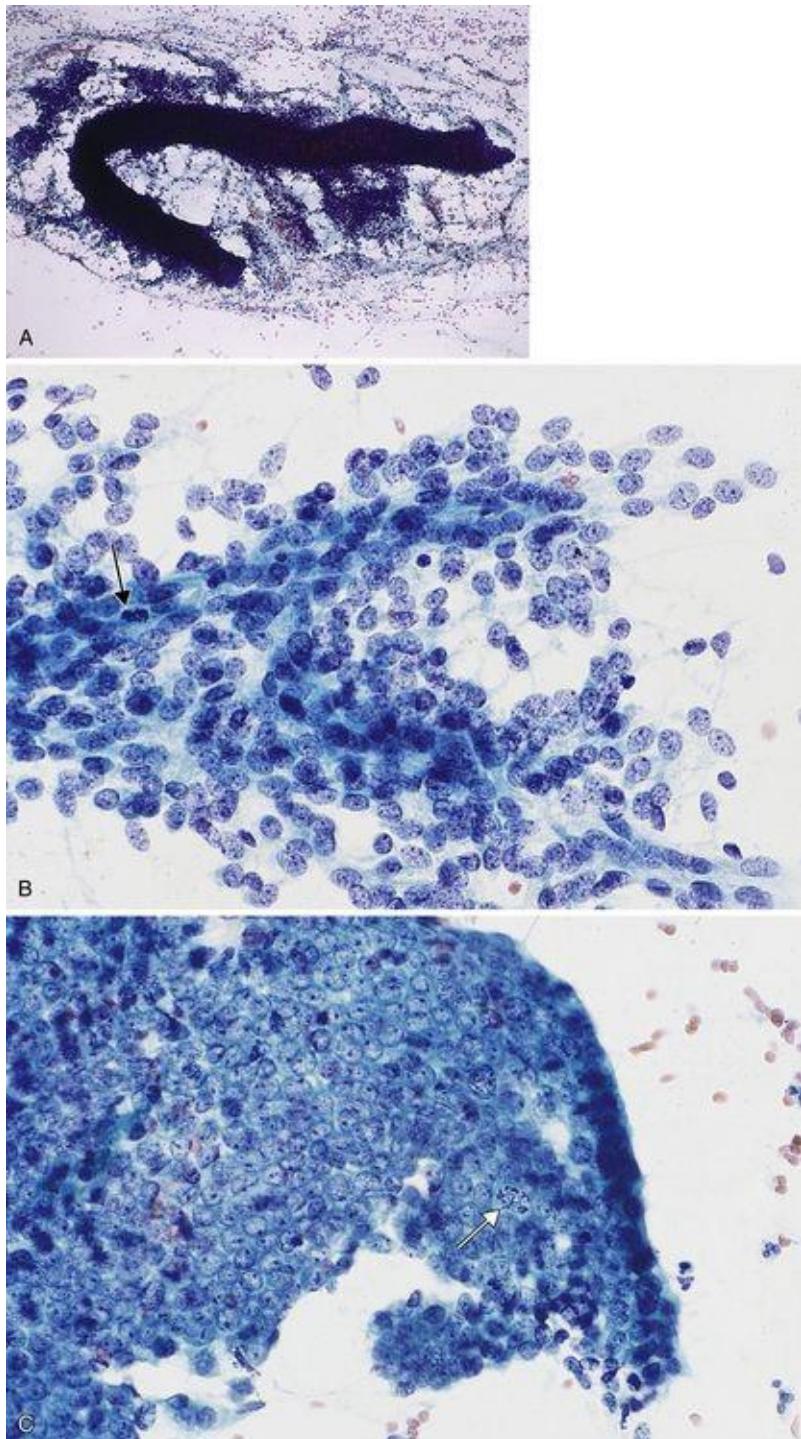


FIGURE 1.14 Endometrial cells, directly sampled.

A, An intact endometrial tubule is surrounded by well-preserved endometrial stromal cells. B, Benign stromal cells are elongated and mitotically active (arrow) and might suggest a high-grade squamous intraepithelial lesion (HSIL) or malignancy. The pale, finely granular chromatin and the association with intact endometrial glands are clues to a benign interpretation. C, The glandular cells are crowded and mitotically active (arrow) but evenly spaced.

Trophoblastic Cells and Decidual Cells

Syncytiotrophoblastic cells from placental tissue are seen very rarely, perhaps in about 0.1% of Paps from pregnant women.¹²⁶ The cells are large, with abundant blue or pink cytoplasm. They have multiple nuclei that have a granular chromatin texture and slightly irregular contours. Trophoblastic cells can be distinguished from multinucleated histiocytes because their nuclei are darker and more irregular in contour (Fig. 1.15). They do not show the prominent molding and ground-glass appearance of nuclei associated with herpes simplex infection. Immunostains for human chorionic gonadotropin and human placental lactogen can be used to confirm their identity as trophoblastic cells. The presence of syncytiotrophoblastic cells is not a reliable predictor of an impending abortion.¹²⁶

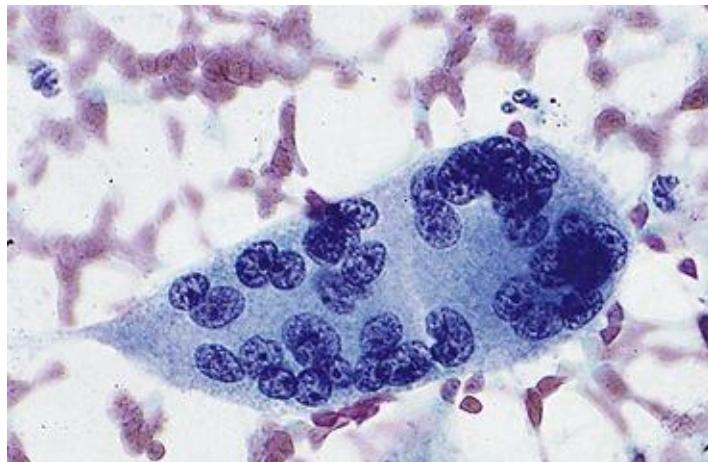


FIGURE 1.15 Syncytiotrophoblast.

The nuclei of these multinucleated cells are dark and coarsely granular, unlike those of histiocytes.

Decidual cells are isolated cells with abundant granular cytoplasm, a large vesicular nucleus, and a prominent nucleolus. They often show degenerative changes.

Inflammatory Cells

Neutrophils are seen in all Pap samples and do not necessarily indicate infection, but they are present in increased numbers after injury or infection. Lymphocytes

and plasma cells are rare, but occasionally—most often in older women—they are numerous (Fig. 1.16A and B). This pattern is called *follicular cervicitis* because biopsy specimens show lymphoid follicle formation. The lymphocytes of follicular cervicitis can be confused with HSIL cells, endometrial cells, and lymphoma. Histiocytosis are associated with myriad conditions (e.g., menses, pregnancy, foreign bodies, radiotherapy, and endometrial hyperplasia and carcinoma) (Fig. 1.17), but by themselves are a nonspecific finding of no clinical significance.

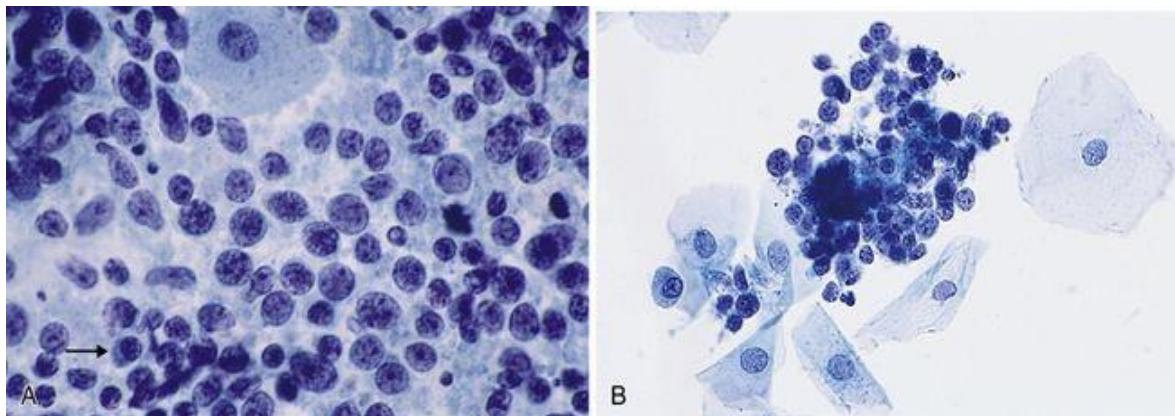


FIGURE 1.16 Follicular cervicitis.

A, This smear from a 61-year-old woman contains numerous lymphocytes in various stages of maturation, including an occasional plasma cell (arrow). Most normal lymphocytes have a round nuclear contour, unlike the cells of a high-grade squamous intraepithelial lesion (HSIL), to which they bear a superficial resemblance. B, Lymphocytes are also a mimic of exfoliated endometrial cells. They are roughly the same size or a bit smaller, more heterogeneous in size, and less tightly clustered than most endometrial cells.

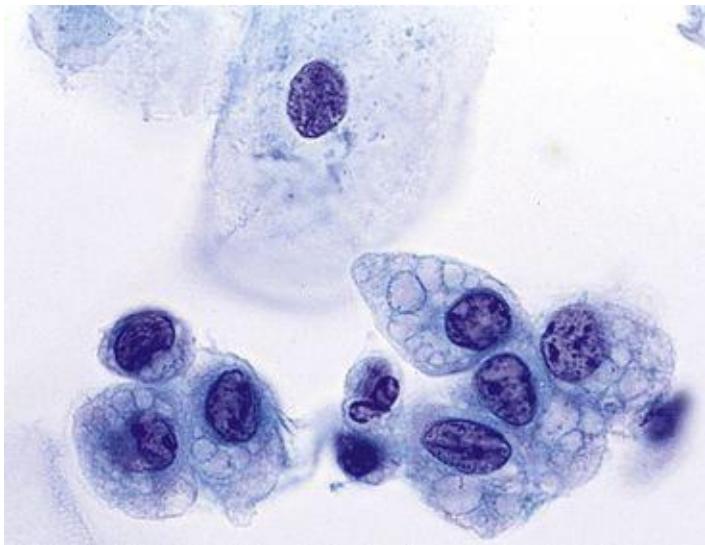


FIGURE 1.17 Histiocytes.

Histiocytes have abundant multivacuolated cytoplasm and an oval, occasionally folded nucleus.

Lactobacilli

The vagina is colonized by gram-positive rod-shaped bacteria of the genus *Lactobacillus*. They are beneficial because they produce lactic acid, which reduces the ambient pH and possibly protects from infection by *Candida* and other pathogens. Lactobacilli metabolize the glycogen contained within exfoliated squamous cells. The resulting cellular pattern, commonly seen during the second (luteal) phase of the menstrual cycle, is known as cytolysis—bare intermediate cell nuclei, fragments of squamous cytoplasm, and abundant bacterial rods ([Fig. 1.18](#)). Cytolysis can interfere with one's ability to evaluate the nuclear-to-cytoplasmic ratio, an important criterion in grading SILs.

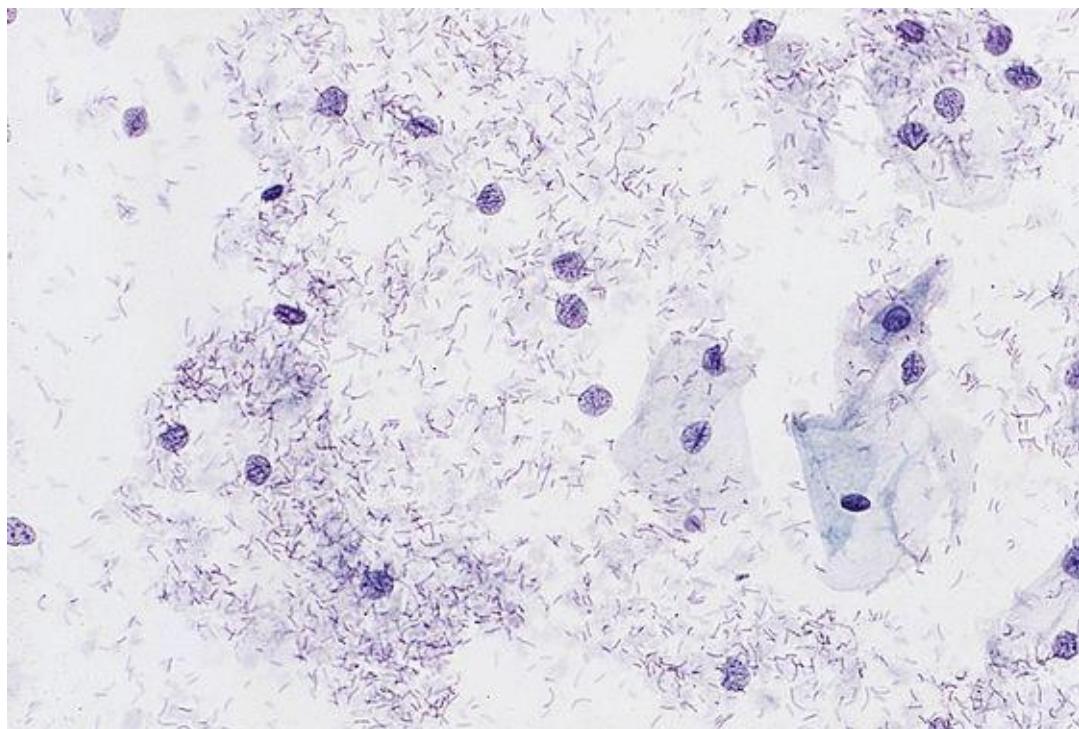


FIGURE 1.18 Lactobacilli.

These bacteria are part of the normal flora of the vagina. Note the bare nuclei of the intermediate cells, which are subject to cytolysis by these organisms.

Artifacts and Contaminants

The more commonly encountered artifacts and specimen contaminants are illustrated in [Figure 1.19](#).

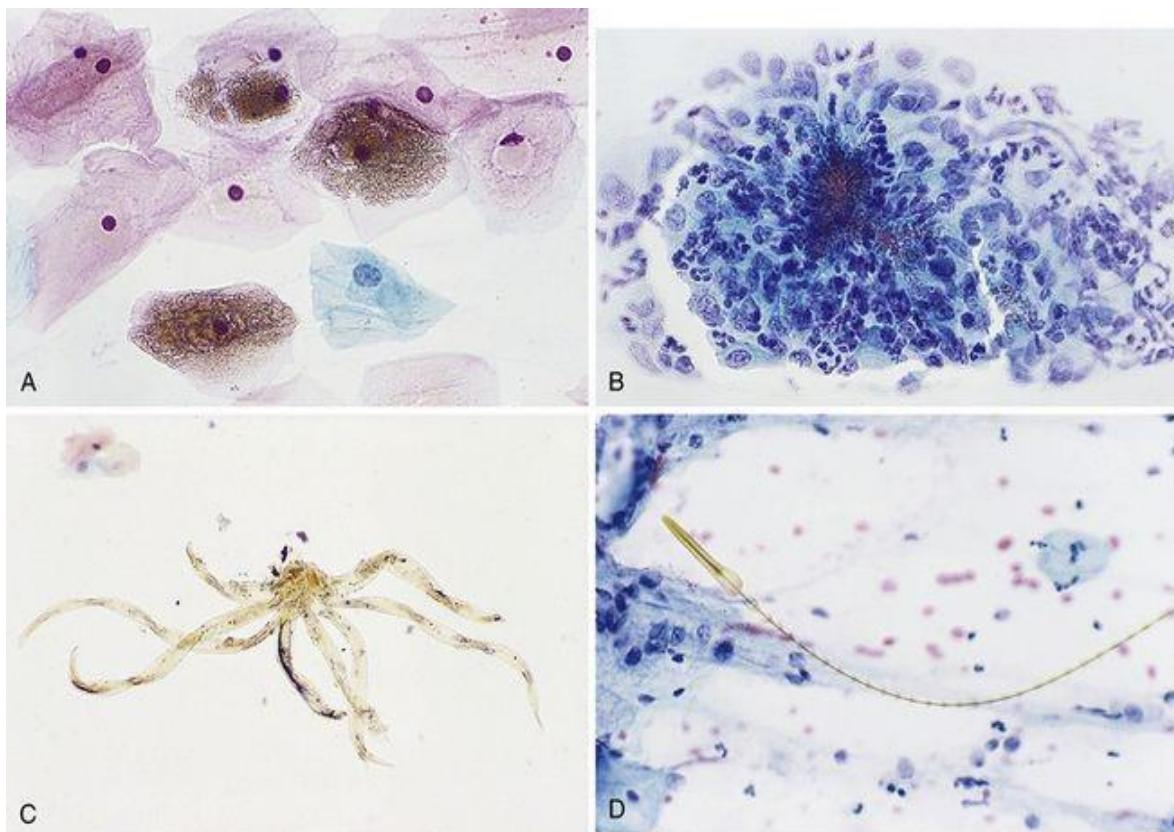


FIGURE 1.19 Artifacts and contaminants.

A, “Cornflaking.” This refractile brown artifact results from bubbles of air trapped on superficial squamous cells, resulting in obscuring of the nuclei. It can be reversed by returning the slide through xylene and alcohol to water, then restaining and recoverslipping. B, “Cockleburrs.” This is the name given to radiating arrays of club-shaped orange bodies composed of lipid, glycoprotein, and calcium, surrounded by histiocytes. They are most commonly associated with, but not limited to, pregnancy. They have no clinical significance. C, Trichome. These large star-shaped structures are derived from plants. They stain a pale yellow and have from three to eight legs. Trichomes are produced by many different plants and vary in color, size, and shape. D, Carpet beetle parts. These arrow-shaped structures are contaminants from sources such as gauze pads and tampons.

Organisms and Infections

Shift in Flora Suggestive of Bacterial Vaginosis

A steep reduction in the proportion of lactobacilli, with a concomitant predominance of coccobacilli, is associated with bacterial vaginosis, a disorder characterized by a thin, milky vaginal discharge and a foul, fishy odor. At one time attributed solely to *Gardnerella vaginalis*, it is now clear that bacterial vaginosis can be caused by other bacteria as well.¹²⁷ The diagnosis is made by correlating morphologic findings on a Pap or wet prep with other test results (vaginal pH and the amine-odor “whiff” test after addition of potassium hydroxide [KOH]).¹²⁸



Cytomorphology of a shift in flora

- short bacilli (coccobacilli), curved bacilli, or mixed bacteria
- no lactobacilli
- “filmy” appearance
- “clue cells”

The cytologic hallmark is the replacement of the normal lactobacilli by shorter bacilli (coccobacilli), curved bacilli, and mixed bacteria ([Fig. 1.20](#)). These small organisms are numerous and give a “filmy” appearance to the preparation. They frequently adhere to squamous cells, completely covering them like a shag carpet (“clue cells”). Clue cells are not specific for the diagnosis. Requiring at least 20% clue cells may increase the specificity of the diagnosis.¹²⁹ Neutrophils are often scarce.

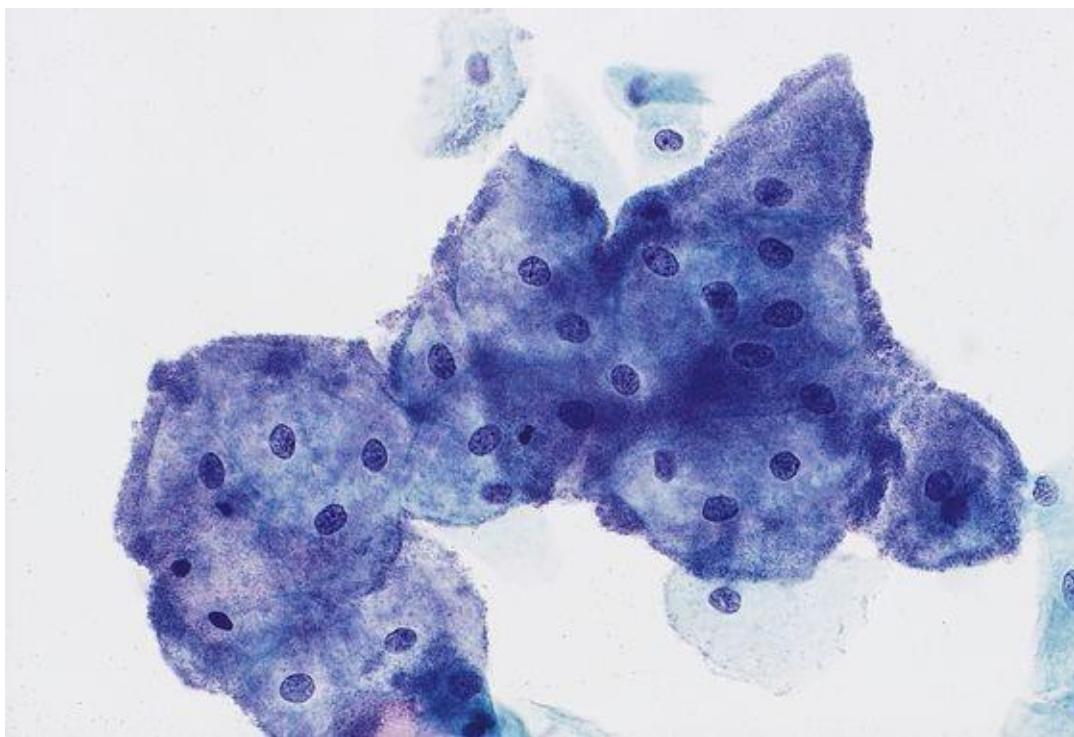


FIGURE 1.20 Shift in flora suggestive of bacterial vaginosis.

Numerous small bacteria cover large portions of the slide. In some but not all cases, these bacteria adhere to squamous cells (“clue cells”), giving the appearance of a shag rug, as seen here. Lactobacilli are absent.

This pattern is common and seen in about 50% of patients referred to a dysplasia clinic.¹²⁷ Clinical correlation is required for a definite diagnosis of bacterial vaginosis, because the cytologic pattern is neither sufficient nor necessary for the diagnosis. Women who are symptomatic are treated with metronidazole or clindamycin.

Trichomonas Vaginalis

Trichomonas vaginalis is a primitive eukaryotic organism, a parasitic protozoan that causes trichomoniasis, a sexually transmitted disease. Patients may experience burning and itching, with a malodorous vaginal discharge, but up to 50% are asymptomatic.¹³⁰ Although regarded primarily as a disease of women, it also occurs in men, most of whom are asymptomatic.



Cytomorphology of *Trichomonas vaginalis*

- 15 to 30 μm long
- pear-shaped

- pale, eccentrically placed nucleus
- red cytoplasmic granules

The organism is a 15 to 30 μm pear-shaped protozoon that has a small, very pale, eccentrically placed nucleus ([Fig. 1.21](#)). The cytoplasm often contains tiny red granules. It is commonly accompanied by *Leptothrix*, a nonpathogenic, long, filamentous bacterium. Some squamous cells have a small, narrow, indistinct perinuclear halo that calls to mind the cytopathic changes of HPV, but *Trichomonas*-related halos are smaller and accompanied by only minimal nuclear atypia.

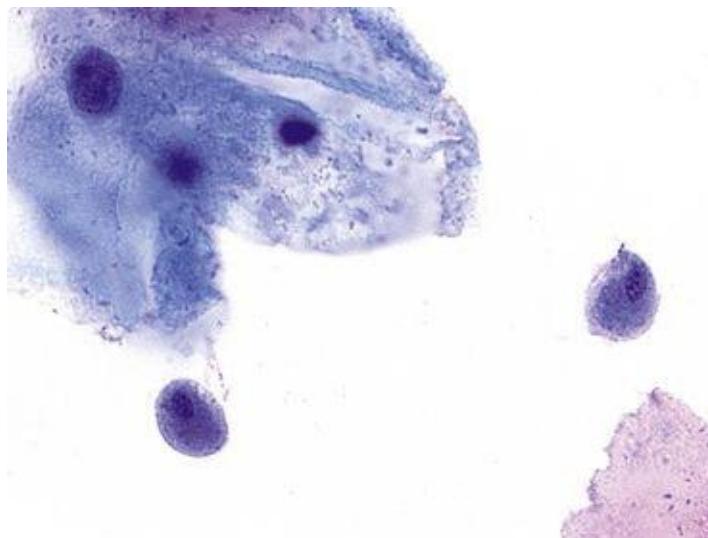


FIGURE 1.21 *Trichomonas vaginalis*.

This organism has an indistinct, ghostly appearance, with a pale oval nucleus and faint red granules.

Patients and their sexual partners are treated with metronidazole.^{[130](#)}

Candida

Candida albicans and *Candida glabrata* are fungal species that infect the vulva, vagina, and cervix. Patients may be asymptomatic, or they may complain of burning, itching, and a thick, cheesy discharge.

These fungi are eosinophilic and often interspersed among squamous cells ([Fig. 1.22](#)). In many cases, some

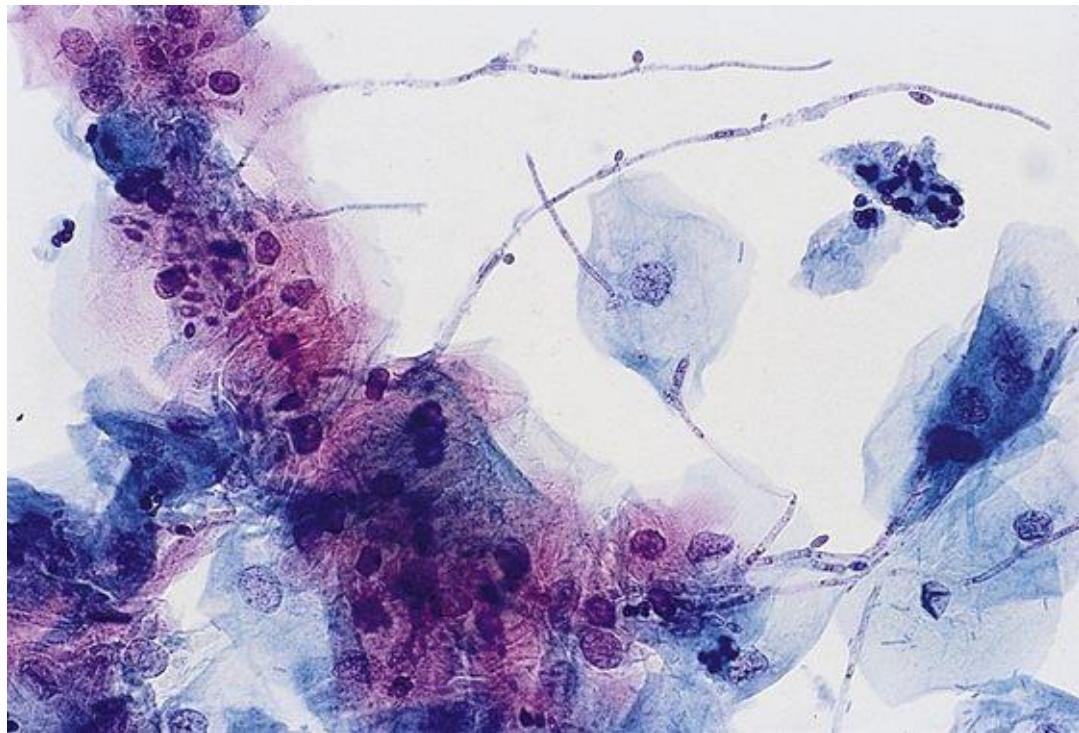


FIGURE 1.22 *Candida*.

Pseudohyphae and yeast forms, some of them budding from pseudohyphae, are seen. Note the skewered squamous cells.



Cytomorphology of *Candida*

- pink
- yeast forms (3 to 7 μm diameter)
- long pseudohyphae and true hyphae
- tangles of pseudohyphae and yeast forms (“spaghetti and meatballs”)
- skewers of squamous cells around pseudohyphae (“shish kebabs”)

squamous cells appear in linear arrays, as if skewered by the pseudohyphae. Tangles of pseudohyphae (“spaghetti”) admixed with

yeast forms (“meatballs”) are common. Thin mucus strands are a common mimic of *Candida* pseudohyphae, but they are pale blue rather than pink like *Candida*.

Not all women with this finding are symptomatic, and usually only symptomatic women are treated.

Actinomyces

Actinomyces organisms are gram-positive anaerobic bacteria that are normal inhabitants of the mouth and bowel. They are uncommon in the cervix and vagina, where they are almost always associated with a foreign body, most commonly an IUD. It is estimated that 7% of women with an IUD have *Actinomyces* bacteria on their Pap,¹³¹ and the frequency is related to the duration of continuous IUD use. When found incidentally on a Pap test, they are almost always harmless. In a small number of cases, however, women with an IUD develop pelvic actinomycosis, usually a tubo-ovarian abscess, presumably through ascending infection. Case reporting has not been systematic, so it is impossible to judge the risk of this significant complication, but pelvic actinomycosis due to an IUD is considered exceedingly rare.¹³²



Cytomorphology of *Actinomyces*

- tangled clumps of bacteria (“cotton balls,” “dust bunnies”)
- long, filamentous organisms

If *Actinomyces* organisms are seen on a Pap ([Fig. 1.23](#)), removal of the IUD is not necessary, and treatment of asymptomatic women is not recommended.¹³¹

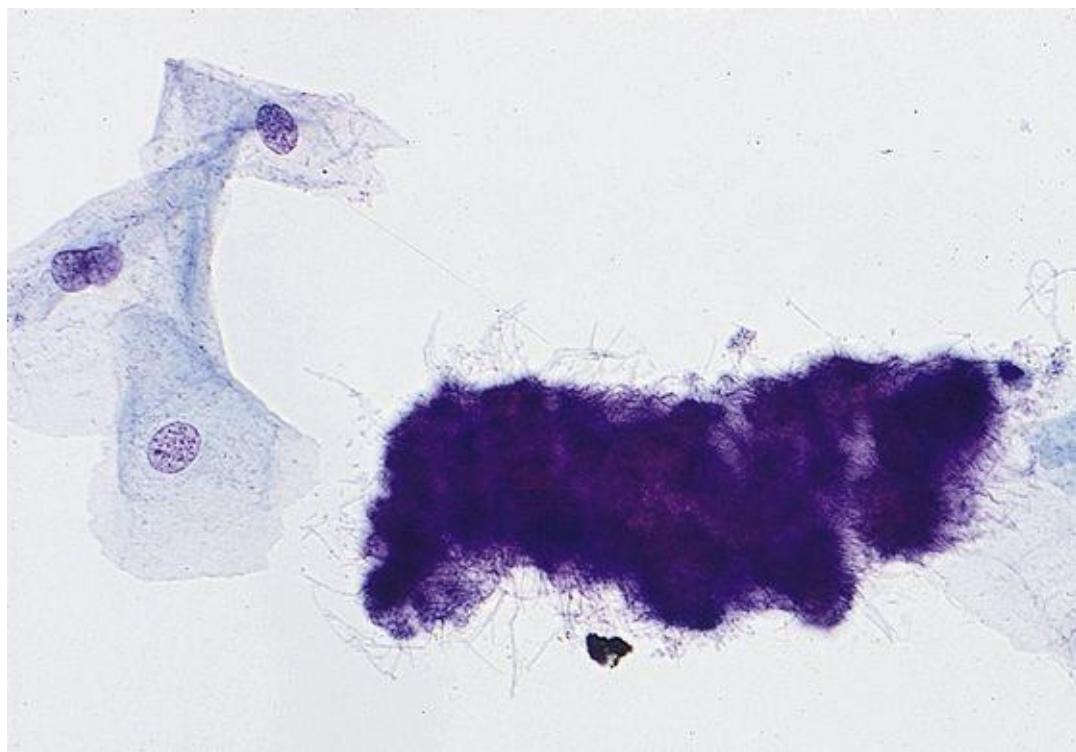


FIGURE 1.23 *Actinomyces* organisms.

These bacterial colonies resemble dark cotton balls. The organisms are filamentous, shown here protruding from the mass of bacteria.

Herpes simplex virus

Infection by the herpes simplex virus (HSV) is identified by the characteristic nuclear changes of infected epithelial cells.



Cytomorphology of herpes simplex cytopathic changes

- the 3 Ms:
 - multinucleation
 - molding of nuclei
 - margination of chromatin
- ground-glass nuclei
- eosinophilic intranuclear inclusions

The nucleus has a homogeneous, glassy appearance ("ground-glass"), and nuclear membranes are thick due to peripheral margination of chromatin ([Fig.](#)

[1.24A](#)). Multinucleation is common, with molding of nuclei. Eosinophilic intranuclear inclusions may be present.

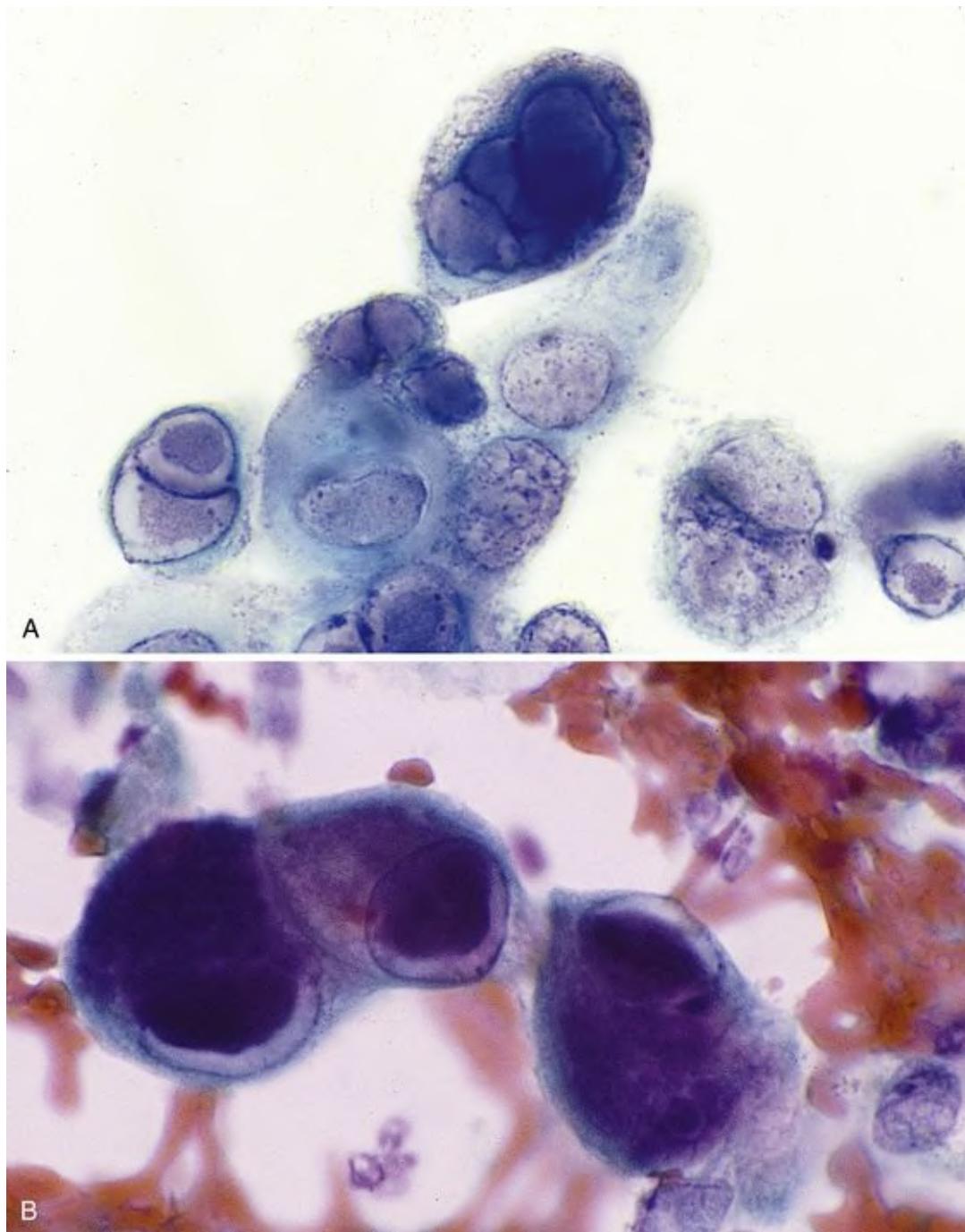


FIGURE 1.24 Viral cytopathic changes.

A, Herpes simplex virus. The nuclei of infected cells are filled with viral particles, which impart a pale, homogeneous appearance. Nuclear chromatin is visible only at the periphery of

some nuclei. Some have a well-defined eosinophilic intranuclear inclusion. *B*, Cytomegalovirus. Each cell has a large basophilic intranuclear inclusion that is surrounded by a halo; the cytoplasm contains multiple small basophilic inclusions as well. This patient was immunocompetent and asymptomatic, and the inclusions were identified in only a few cells.

Cytomegalovirus

Exposure to and infection by cytomegalovirus (CMV) are common in the general population, but clinical manifestations, such as mononucleosis, are relatively uncommon. The cytologic changes of CMV infection can be seen on cervical–vaginal preparations from immunocompetent as well as immunocompromised patients.¹³³ In immunocompetent patients, the infection is transient and usually asymptomatic.



Cytomorphology of cytomegalovirus cytopathic changes

- mononuclear cells
- markedly enlarged
- basophilic intranuclear inclusion
- small granular cytoplasmic inclusions

Infected cells are enlarged, and the nuclei have a solitary basophilic inclusion surrounded by a halo. Multiple small, granular cytoplasmic inclusions are also present (Fig. 1.24B). The infected cells are endocervical and/or ectocervical in origin.¹³⁴

Chlamydia Trachomatis

Chlamydia trachomatis is one of the most common sexually transmitted pathogens and a leading cause of cervicitis, endometritis, and pelvic inflammatory disease. Cytologic criteria for diagnosis, such as cytoplasmic vacuolization or an inflammatory infiltrate composed of transformed lymphocytes, have been shown to have low diagnostic accuracy.¹³⁵ Laboratories have therefore abandoned cytologic diagnosis in favor of microbiologic testing methods.

Rare Infections

Amebiasis of the female genital tract caused by *Entamoeba histolytica* is uncommon; 10% to 20% of cases have been associated with neoplasms.¹³⁶ The organisms, which range in size from 12 to 40 µm and have a small, eccentric nucleus and abundant vacuolated cytoplasm, may be misinterpreted as large histiocytes. Erythrophagocytosis is common. Unlike *E. histolytica*, *E. gingivalis* is not associated with a pathogenic role in genital infections, although it has been described as accompanying *Actinomyces* spp. in patients using IUDs.¹³⁷

Granuloma venereum (granuloma inguinale) is a sexually transmitted, ulcerative condition that usually involves the labia but can cause cervical lesions. The causative organism (*Calymmatobacterium granulomatis*, also known as the Donovan body) is an encapsulated gram-negative bacterium that is concentrated in macrophages and difficult to see with the Papanicolaou stain. A Giemsa stain demonstrates the intracellular organisms.¹³⁸ Another condition in which intracellular bacteria are seen is malakoplakia, which rarely involves the cervix.¹³⁹

Benign and Reactive Changes

Trauma, infections, hormonal stimulation, radiation, and other factors cause a variety of morphologic alterations of squamous and endocervical cells that range from the very mild to the alarmingly exuberant. At their most extreme, reactive epithelial changes mimic malignancy. For this reason, federal regulations require that a cytotechnologist refer all cases with “reactive or reparative” changes to a pathologist for review (see [Chapter 18](#)). Because the word *reactive* is rather nebulous, defining precisely which morphologic alterations require pathologist review is up to the laboratory director, and implementation rests on the judgment of the cytotechnologist. Thus, familiarity with the characteristic morphology of reactive changes is important and helps prevent misdiagnosis. Inflammatory changes affect both squamous and endocervical cells, but the changes are often more dramatic in endocervical cells.

Benign Squamous Changes

Mature squamous cells can show a variety of nuclear and cytoplasmic changes, most commonly *simple nuclear enlargement* of intermediate squamous cells without hyperchromasia or nuclear membrane irregularity. The nuclear enlargement is usually slight (one-and-a-half to two times the area of a normal intermediate cell nucleus), but sometimes is greater. Despite the nuclear size increase, the chromatin is finely and uniformly granular. Bland nuclear enlargement of intermediate cells is particularly common in Paps from perimenopausal women (aged 40 to 55 years). Because of this association they have been termed *PM* (for *perimenopausal*) cells ([Fig. 1.25](#)). Without accompanying hyperchromasia or nuclear membrane irregularity, these cells are unlikely to represent a significant squamous lesion.¹⁴⁰ The cause of nuclear enlargement in squamous cells from perimenopausal women is not known.

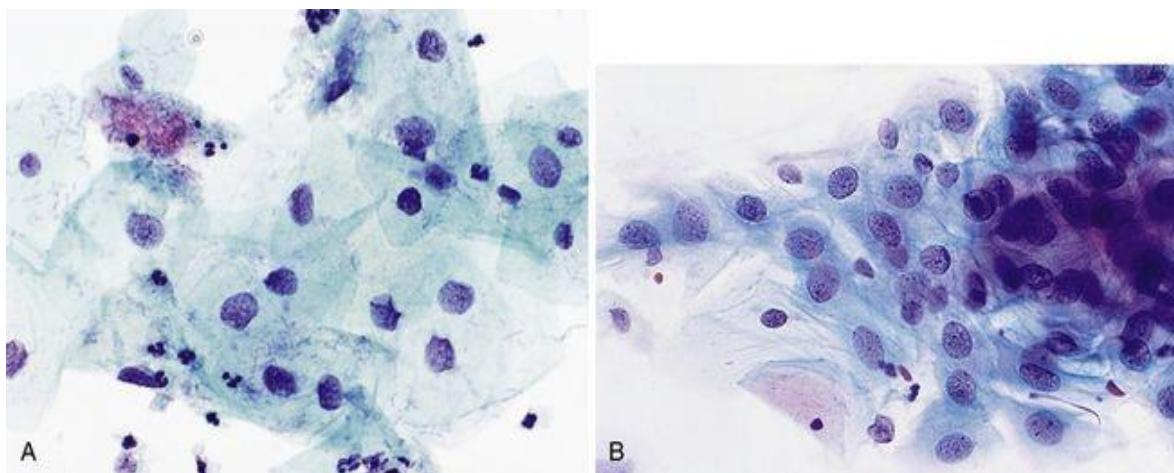


FIGURE 1.25 Benign squamous cell changes.

A, PM cells. Nuclear enlargement, with little in the way of nuclear membrane irregularity or hyperchromasia, is a common finding in intermediate squamous cells from perimenopausal women. Such bland nuclear enlargement should not be mistaken for a significant atypia. B, A similar bland nuclear enlargement can occur in squamous metaplastic cells.

Nonspecific perinuclear cytoplasmic clearing in superficial and intermediate squamous cells is associated with inflammatory conditions such as *Trichomonas* infection, but it can also be a slide preparation artifact. It is distinguished from koilocytosis by the small size of the halo and the absence of increased cytoplasmic density outlining the cavity ([Fig. 1.26A](#)). Large cytoplasmic clearings occur in squamous cells with abundant cytoplasmic glycogen. They are distinguished from LSIL cells in that they have a normal intermediate cell nucleus ([Fig. 1.26B](#)).

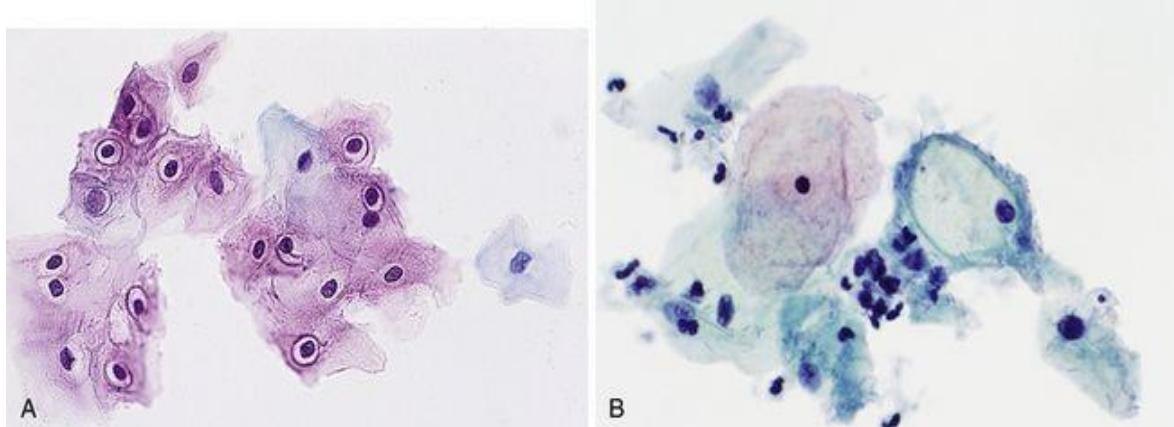


FIGURE 1.26 Nonspecific halos.

A, Small halos around the nuclei of squamous cells are nonspecific and do not represent human papillomavirus (HPV)-related changes. B, Some normal squamous cells have abundant

glycogen that mimics koilocytosis. Note the normal nucleus.

Squamous metaplastic cells are particularly prone to reactive changes. There can be nuclear enlargement and variation in nuclear size, and nucleoli are sometimes prominent. Smooth nuclear membranes and finely textured chromatin are reassuring. In some cases, however, the alterations in metaplastic squamous cells are more marked and overlap with the features of HSIL. Such borderline cases are referred to as *atypical squamous metaplasia*.

Benign Endocervical Changes

Reactive endocervical cells often show much greater increases in nuclear size than squamous cells. Some reactive endocervical cell nuclei are four or five times larger than normal, usually with an accompanying increase in cytoplasm. The enlarged nuclei remain round or oval, but they frequently have a large nucleolus ([Fig. 1.27](#)). Such changes are not uncommon in *pregnancy*, where in their extreme form they represent the Arias-Stella reaction.¹⁴¹ They are also seen in patients with endocervical polyps and inflammation of any cause.

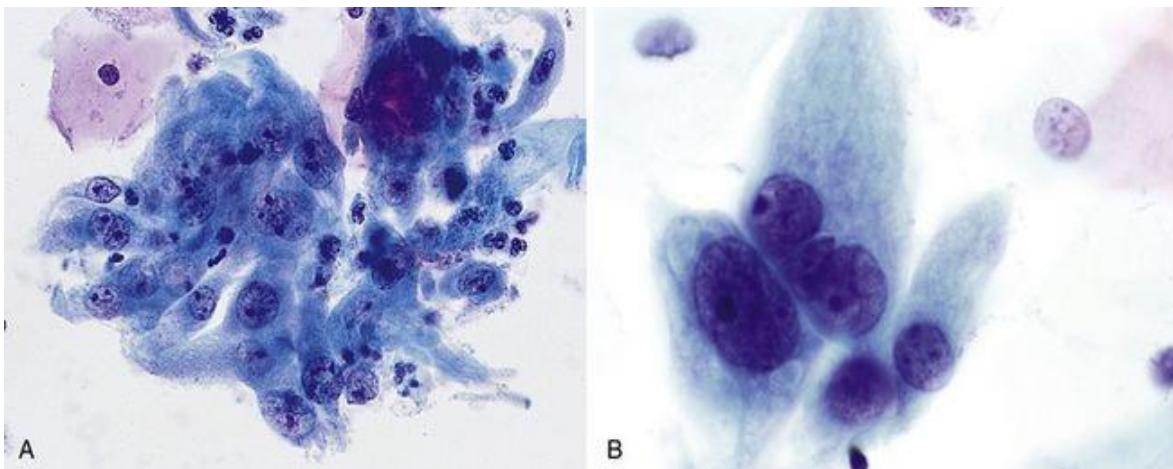


FIGURE 1.27 Reactive endocervical cells.

A, A common finding, reactive endocervical cells are enlarged and have a prominent nucleolus. B, Isolated cells can be as big as mature squamous cells and mimic a low-grade squamous intraepithelial lesion (LSIL), but a prominent nucleolus is uncharacteristic of an LSIL.

Reactive endocervical cells are also seen in *microglandular hyperplasia*, a benign alteration of endocervical epithelium associated with oral contraceptive

use. Microglandular hyperplasia was originally described in histologic material, where it was sometimes confused with adenocarcinoma. Cytologic changes range from entirely normal endocervical cells to marked nuclear enlargement, often with prominent nucleoli and cytoplasmic vacuolization (Fig. 1.28).¹⁴² Clinical correlation is useful. Knowledge that the patient is pregnant or has a visible endocervical polyp can alert the cytologist to the possibility of reactive changes and provide a rational explanation for the alterations. In their most extreme forms, however, reactive endocervical cells raise a differential diagnosis that includes LSIL, HSIL, AIS, and invasive cancer. The differential diagnosis of reactive endocervical cells is discussed in greater detail in the corresponding sections that follow. Ultimately, the benign nature of reactive endocervical cells is betrayed by the roundness of the nucleus, its fine chromatin granularity, and the normal nuclear-to-cytoplasmic ratio.

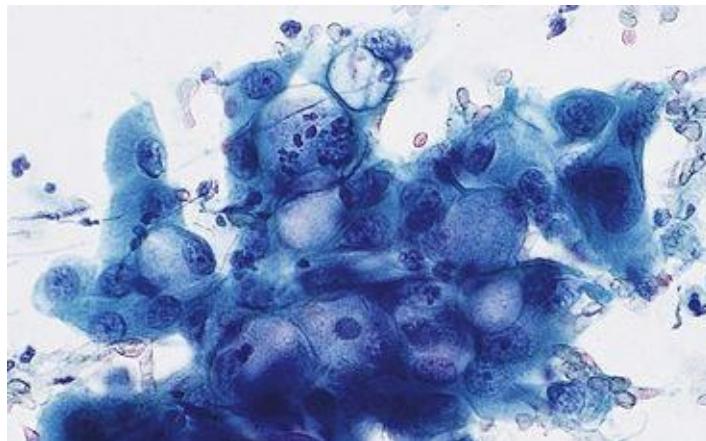


FIGURE 1.28 Reactive endocervical cells (microglandular hyperplasia). These cells are enlarged and have a prominent large cytoplasmic vacuole.

Repair

Reparative changes result from injury to the cervical epithelium and the proliferation of reserve cells, which grow to reepithelialize a focus of ulceration.



Cytomorphology of repair

- cohesive, flat sheets
- streaming appearance
- large nucleus with marked size variation

- large nucleolus, sometimes irregular in shape
- pale chromatin
- mitoses

Typical repair is composed of flat sheets of cells that have an enlarged nucleus, a prominent nucleolus, and occasional mitoses. Repair cells often maintain a uniform polarity that gives the sheets the appearance of streaming (like a school of fish) or being pulled out (like taffy) (Fig. 1.29). Because the sheets are very cohesive, individual abnormal cells—so characteristic of carcinomas—are generally absent in repair reactions. Nevertheless, some repair reactions are so extensive, with unusual features such as crowded nuclei and a coarsely granular chromatin texture, that doubt about their benign nature is raised. Such a specimen is best interpreted as “ASC-US, with features of atypical repair.”

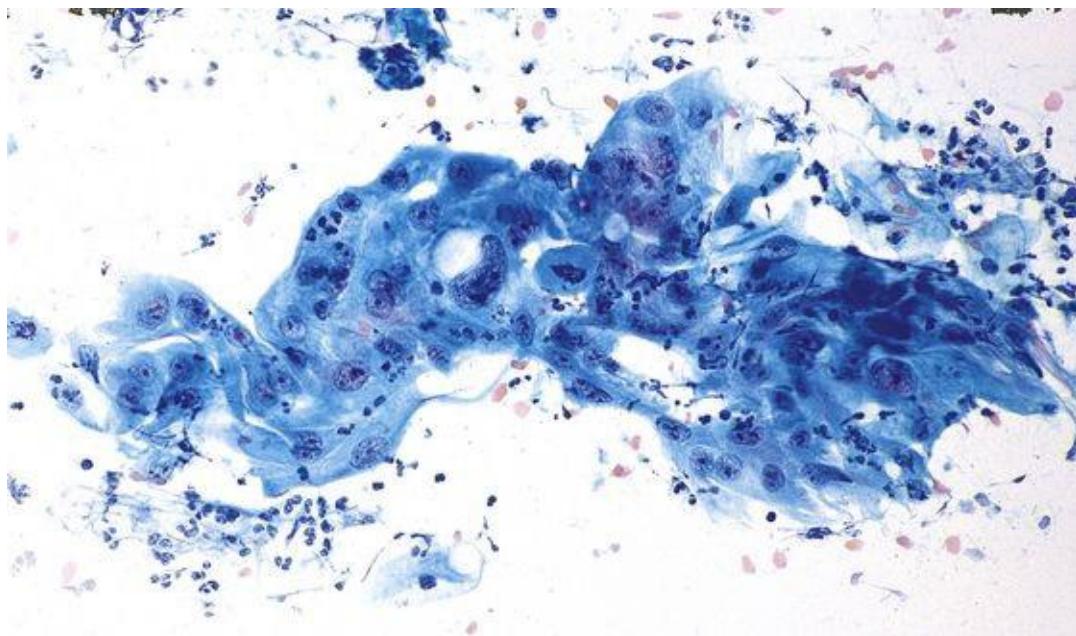


FIGURE 1.29 Typical repair.

Reparative epithelium is cohesive and arranged in a monolayered, streaming sheet.



Differential diagnosis of repair

- nonkeratinizing squamous cell carcinoma

- endocervical adenocarcinoma

Reparative epithelium does not resemble LSIL, HSIL, or AIS. Rather, it leapfrogs over precursor lesions and audaciously mimics invasive cervical cancers, both nonkeratinizing squamous cell carcinoma and adenocarcinoma. The resemblance stems from the combination of large, round nuclei; prominent nucleoli; and mitoses. The distinction from cancer is usually straightforward, however. Reparative epithelium may be associated with inflammation, but the necrotic debris typical of invasive cancers is absent. Invasive cancers often contain sheets and clusters of malignant cells, but there are usually numerous isolated malignant cells as well, whereas reparative epithelial cells are famously cohesive. Nonkeratinizing squamous cell carcinomas have coarsely textured chromatin rather than the fine granularity of repair cells.

Radiation Changes

Radiation induces changes in cells that either disappear with time or persist for many years.



Cytomorphology of radiation changes

- large, bizarre cells
- normal nuclear-to-cytoplasmic ratio
- cytoplasmic vacuolization and polychromasia
- multinucleation

The characteristic changes are marked cellular and nuclear enlargement with preservation of the nuclear-to-cytoplasmic ratio, cytoplasmic vacuolization, and cytoplasmic polychromasia (“two-tone” cytoplasm) ([Fig. 1.30](#)). Nuclei have finely granular chromatin or show smudgy hyperchromasia, and there can be nuclear as well as cytoplasmic vacuolization. Cells are isolated or arranged in groups, and multinucleation is common. Reparative epithelium commonly accompanies radiation changes. Some chemotherapeutic drugs induce similar changes.

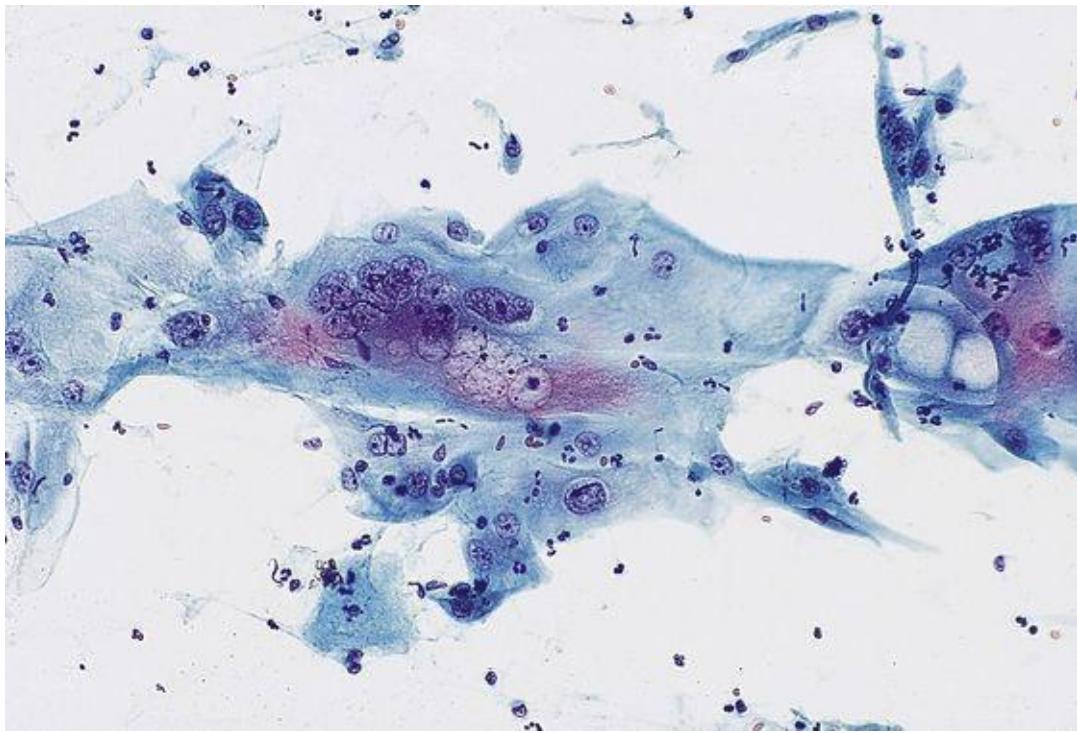


FIGURE 1.30 Radiation effect.

Radiation effect looks like a wild reparative reaction, with very large cells, multinucleation, cytoplasmic vacuolization, and a curious “two-tone” (pink and blue) cytoplasmic staining pattern.



Differential diagnosis of radiation changes

- herpes cytopathic changes
- recurrent cancer
- LSIL

Radiation changes superficially resemble herpes cytopathic changes. Multinucleation occurs in both conditions, but radiation effect lacks the ground-glass nuclear appearance or Cowdry A type inclusions typical of herpes. If the radiation was given for a cervical cancer, the differential diagnosis includes recurrent squamous cell carcinoma or adenocarcinoma of the cervix, with superimposed radiation changes. The cells of a recurrent squamous cell carcinoma and adenocarcinoma are typically more numerous than the scattered radiation cells. Recurrent cancers show more significant nuclear atypia than is seen in radiation. Coarsely textured chromatin (rather than smudgy hyperchromasia) is typical of nonkeratinizing squamous cell carcinoma.

Cellular Changes Associated with Intrauterine Devices

There are two distinct cell types that are associated with IUD use.¹⁴³



Cytomorphology of intrauterine device effect

- vacuolated cells
- small dark cells with scant cytoplasm

The first type of “IUD cell” is a glandular cell with abundant vacuolated cytoplasm, and in some cells a large vacuole displaces the nucleus. These cells may be arranged in small groups (5 to 15 cells) or occur as isolated cells (Fig. 1.31). Nuclei are enlarged and nucleoli are usually visible. The second type is the isolated small cell with a hyperchromatic nucleus and a high nuclear-to-cytoplasmic ratio. Sometimes reparative changes are also present, and the background is inflamed.

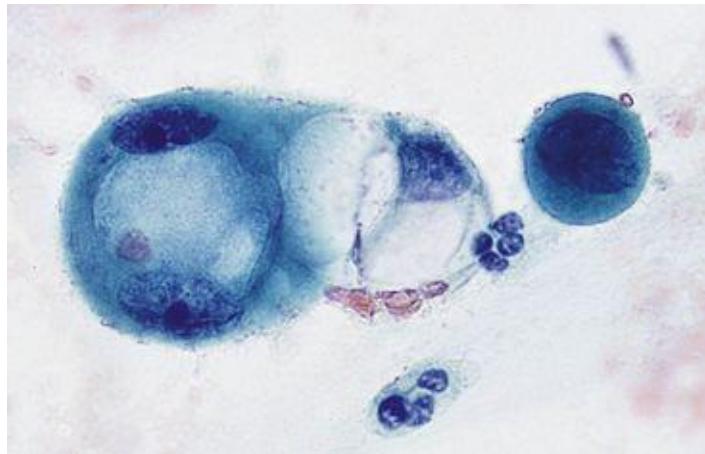


FIGURE 1.31 Intrauterine device (IUD) effect.

The two types of cells are seen here: a vacuolated cell and a small dark cell with scant cytoplasm. This combination is characteristic of IUD effect.



Differential diagnosis of intrauterine device effect

- adenocarcinoma

- HSIL

The histogenesis of IUD cells is uncertain. The vacuolated type is virtually indistinguishable from the cells of an adenocarcinoma, particularly those of endometrial origin. The small IUD cells resemble HSIL cells except that they have a more prominent nucleolus.¹⁴⁴ If the woman has an IUD, and the cells in question are few in number, it is common practice to interpret the specimen as benign. But an interpretation of “atypical glandular cells” (AGCs) or “atypical squamous cells” might be considered if the cells are more numerous or atypical than usual; a repeat Pap after removal of the IUD might be helpful in such circumstances.

Glandular Cells Status Post Hysterectomy

Glandular cells resembling normal endocervical cells are seen in approximately 2% of vaginal Paps from women who have had a total hysterectomy.¹⁴⁵ This finding is more common in women who have had postoperative radiotherapy and may therefore represent a therapy-induced metaplasia of squamous epithelium. If they resemble normal endocervical cells, they are entirely benign (Fig. 1.32) and need not raise the possibility of an adenocarcinoma, even if the hysterectomy was carried out for an adenocarcinoma of the cervix or endometrium. A line in the report noting “benign glandular cells status post hysterectomy” is appropriate. No further evaluation is recommended.²⁵

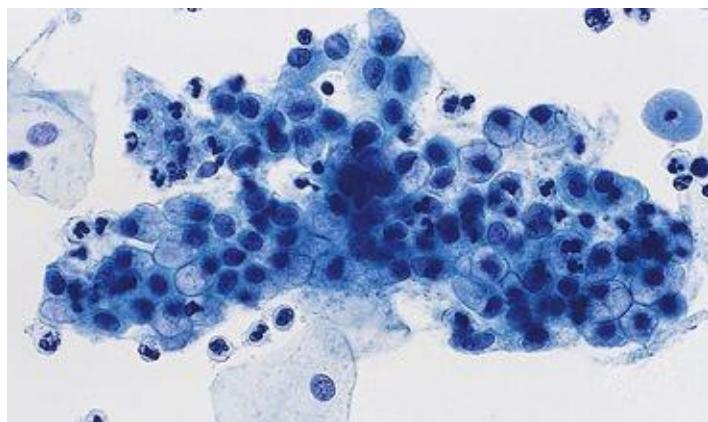


FIGURE 1.32 Glandular cells status post hysterectomy.
The squamous mucosa of the vagina has undergone focal mucinous metaplasia.

Given that some hysterectomies are supracervical, sometimes endocervical cells on a Pap sample labeled “vaginal” from a woman who has had a hysterectomy are truly cells from the cervical stump. Careful review of the operative notes can help clarify this possibility.

Other Benign Changes

The cells of tubal metaplasia of the endocervix often look like normal endocervical cells, except that they have cilia. Sometimes they have a higher nuclear-to-cytoplasmic ratio and slight hyperchromasia and may be mistaken for a significant squamous or glandular lesion if a careful search is not made for cilia.¹⁴⁶ Cilia are very reliable evidence that the cell they are attached to is benign because ciliated adenocarcinomas of the endocervix are very uncommon.^{147,148} Endometriosis of the cervix (Fig. 1.33) resembles directly sampled endometrium.

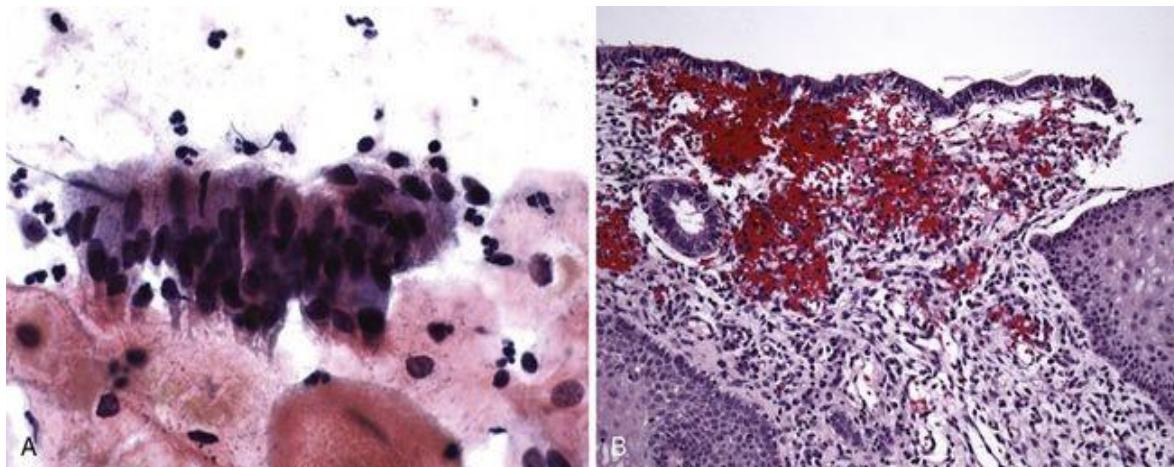


FIGURE 1.33 Endometriosis of the cervix.

A, The dark and pseudostratified nuclei of endometrial-type epithelium mimic endocervical neoplasia. B, A cervical biopsy reveals the tell-tale mix of benign endometrial glands and stroma.

Vaginal Specimens in “DES Daughters”

The daughters of women who were given diethylstilbestrol (DES) during pregnancy to prevent a threatened abortion are at risk for a variety of abnormalities, most of them benign, of the vagina, cervix, and uterus. About one third of these DES daughters develop vaginal adenosis, the presence of glands in the vagina. Mucinous epithelium is the most frequently encountered type of glandular epithelium, but tubal and endometrial-type epithelium is sometimes seen. A diagnosis of vaginal adenosis is supported by the presence of glandular or squamous metaplastic cells on a direct sample from the wall of the vagina.

Clear cell carcinoma of the vagina is the least common but most dreaded complication of inutero DES exposure.

Squamous Abnormalities

Squamous Intraepithelial Lesions

The term *squamous intraepithelial lesion* (SIL) encompasses the spectrum of precursors to invasive squamous cell carcinoma, previously called *dysplasia*, *carcinoma in situ*, *borderline lesion*, and *CIN*. Strong evidence links SIL with invasive squamous cancer. Epidemiologic risk factors (e.g., sexual history) are similar for patients with SIL and those with invasive cancer and both are associated with HPV. Both SIL and cancer have similar chromosomal abnormalities as measured by cytogenetic or image analysis methods. Women with SIL are at least 10 years younger on average than those with invasive cancer; this chronology suggests progression of SIL to invasion. Finally, SIL resembles cancer morphologically and is often present in histologic sections directly adjacent to invasive cancer.

The natural history of SIL is not easy to study. Ethical considerations prohibit using a control group, especially women with high-grade lesions.¹⁴⁹ Many studies have chosen a high-grade lesion as their end point for investigating the behavior of low-grade lesions, because allowing a lesion to progress to invasive cancer is out of the question. Yet it is precisely the risk of progression to invasion that is of paramount interest. A biopsy itself interferes with the natural history of a lesion by removing it entirely or by causing a surrounding inflammatory reaction that can destroy it.¹⁵⁰ Followup biopsy specimens or Pap samples may not be representative of the underlying lesion, and followup time may be inadequate. Finally, the criteria for diagnosing and grading SIL differ among observers. A metaanalysis of this large and heterogeneous body of data suggests that about 50% of LSILs regress and that only about 0.15% progress to invasive cancer in 2 years.⁹⁵ Fewer HSILs regress, and many more progress to invasive cancer ([Table 1.4](#)). The 5-year cervical cancer risk in women 30 years of age or older with HSIL is 8%.¹⁵¹

TABLE 1.4
THE NATURAL HISTORY OF CYTOLOGIC PREINVASIVE SQUAMOUS LESIONS (FOLLOWUP AT 24 MONTHS)

	Regress (%)	Progress to HSIL (%)	Progress to Invasive Cancer (%)
ASC-US	68	7	0.25
LSIL	47	21	0.15
HSIL	35	—	1.4

ASC-US, atypical squamous cells of undetermined significance; *HSIL*, high-grade squamous intraepithelial lesion; *LSIL*, low-grade squamous intraepithelial lesion; *HSIL*, high-grade squamous intraepithelial lesion.

From Melnikow J, Nuovo J, Willan AR, Chan BK, Howell LP. Natural history of cervical squamous intraepithelial lesions: a metaanalysis. *Obstet Gynecol* 1998;92(4 Pt 2): 727-735.

The sexual transmission of HPV explains the well-known epidemiologic association between sexual history and increased risk of cervical cancer. Although detected in virtually all cervical cancers by current molecular techniques,¹⁵² HPV was originally identified in association with a distinctive altered squamous cell known as a *koilocyte*. This unusual cell was first described in 1949 by Ayre, who called it “precancer cell complex,” speculating that it was a precursor to cancer.¹⁵³ In 1960 he correctly suggested a viral etiology. They were recognized by Papanicolaou, who illustrated them with “dyskaryotic” cells in his *Atlas of Exfoliative Cytology*.¹⁵⁴ The term *koilocytosis* was coined by Koss and Durfee in 1956 after the Greek *koilos* (“hollow”), because of the prominent, sharply defined cytoplasmic cavities of the cells.¹⁵⁵ Two decades later, two groups of investigators working independently made the connection between koilocytes and HPV.^{156,157} Subsequent ultrastructural,¹⁵⁸ immunocytochemical, and in-situ hybridization¹⁵⁹ studies confirmed the presence of virus within koilocytes (Fig. 1.34, inset). When it was first realized that these changes were due to a virus, an attempt was made to separate them from dysplasia and CIN.¹⁵⁶ Ultimately, it became apparent that a morphologic distinction was not possible,¹⁶⁰ and evidence began to accumulate linking HPV to the pathogenesis of squamous cancer.¹⁶¹⁻¹⁶³ Currently there is little doubt that HPV plays a central role in causing cervical cancer.

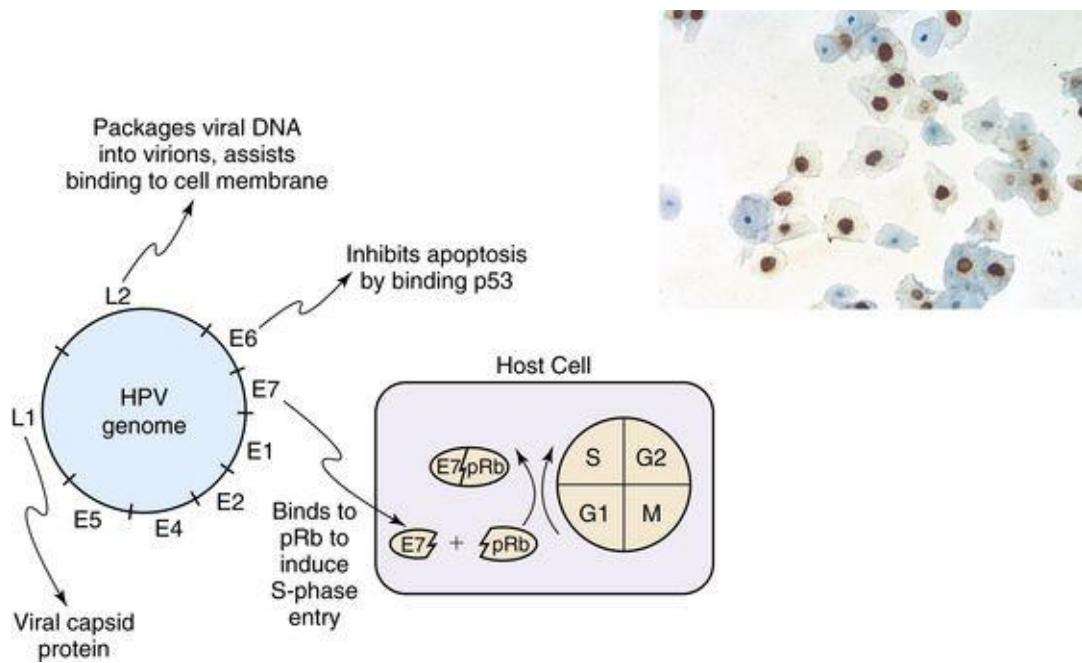


FIGURE 1.34 The human papillomavirus (HPV) genome and its effects on the host cell. The HPV genome has early (E) and late (L) genes. The E6 and E7 genes are most responsible for the transforming effects of integrated HPV DNA on the host cell. *Inset:* Detection of HPV by *in situ* hybridization. The dark brown signal is centered around the nucleus of infected cells. (Courtesy Miu-Fun Chau, DakoCytomation, Carpinteria, CA.)

The small HPV genome consists of about 8000 base pairs of circular double-stranded DNA. It codes for only eight genes (Fig. 1.34), which are classified as “early” (E) or “late” (L) depending on the timing of their expression in the epithelium. HPV infection is established in the basal layers of the epithelium, where the HPV genome is maintained, with expression of the E genes. As the epithelium matures toward the surface, gene amplification and viral assembly occur, with expression of L1 and L2, with eventual viral release. L1 is the major viral capsid protein and is the principal component of HPV vaccines. The E6 and E7 gene products play the most significant part in cervical oncogenesis. They have a number of cellular targets, with a multitude of effects that lead to malignant transformation.¹⁶⁴ The two most important appear to be (1) the binding of E6 to p53, which results in the blocking of apoptosis, and (2) the binding of E7 to the retinoblastoma tumor suppression protein pRB, which abolishes cell-cycle arrest and leads to unscheduled cellular proliferation.^{164,165}

More than 100 types of HPV have been isolated, of which more than 40 infect the female genital tract. Only a minority cause cervical cancer. The genital HPVs are divided into low-risk and high-risk types based on the frequency of their association with invasive cervical cancer. By definition, an HPV is low risk if it has never been isolated from a cervical carcinoma, and high risk if it ever has

been. Persistent infection with any one of about 15 high-risk (carcinogenic) types accounts for virtually all cervical cancers.¹⁶⁴



Examples of low-risk and high-risk human papillomaviruses

- low-risk: 6, 11, 42, 43, 44, 53, 54, 57, 66
- high-risk: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68

HPV 16 is the prototype of the high-risk viruses and the one most commonly detected in cervical cancers.

The risk of HPV infection per sexual contact is not known but is probably fairly high. Most women, if they are sexually active, are infected with one or more HPV types at some point in their lives. For unclear reasons, the virus has a strong predilection for the transformation zone. Serology is not an accurate measure of infection, because only 50% to 60% of infected women have circulating antibodies to HPV.¹⁶⁶ Clearly, only a minority of HPV infections persist and lead to cancer. Cellular immune responses play a role in clearing infection, but how they work is still poorly understood.

Clinical HPV testing is generally restricted to high-risk types, and in this context *HPV testing* refers to testing for high-risk types. Testing for low-risk types has no role in screening or management of women with abnormal cytology.²⁵ A variety of molecular techniques—hybrid capture, target amplification, invader chemistry—can be used to detect HPV. The Hybrid Capture 2 test (Qiagen), which was evaluated in the multicenter ASCUS/LSIL Triage Study (ALTS) trial, uses a cocktail of probes to the 13 high-risk HPV types listed in the example box, which account for nearly 90% of HPVs detected in HSIL and invasive cancers.⁵⁰ Other FDA-approved HPV tests include Cervista HPV HR (Hologic), the cobas HPV test (Roche Molecular Diagnostics), and the Aptima HPV assays (Hologic Gen-Probe).

Grading Squamous Intraepithelial Lesions

The Bethesda System recommends a low-grade/high-grade approach to grading SIL. This is based on the evidence that most LSILs are transient infections that carry little risk for oncogenesis, whereas most HSILs are associated with viral

persistence and a significant potential for progression to invasive cancer.

LSIL encompasses lesions previously described separately as koilocytosis (flat condyloma) and mild dysplasia (CIN 1). The distinction between condyloma and CIN 1 is not reproducible,^{167,168} and both lesions contain a heterogeneous distribution of low-risk and high-risk HPV types. HSIL encompasses lesions previously described as moderate dysplasia (CIN 2) and severe dysplasia/carcinoma in situ (CIN 3). HPV typing plays no role in the grading of SIL. Although low-risk viruses are more common in LSIL than in HSIL, high-risk viruses predominate in both.^{50,169} Morphologic assessment by conventional light microscopy is still the gold standard for grading SILs.

Low-Grade Squamous Intraepithelial Lesion

LSIL is a low-risk intraepithelial lesion that is encountered in approximately 2.5% of all Pap specimens.¹¹⁶ LSIL is caused by a large number of different HPVs, including low-risk and high-risk types. Many LSILs regress spontaneously (see [Table 1.4](#)), but some persist for long periods of time. Approximately 21% progress to HSIL, but it is possible that at least some of these may have been HSILs from the beginning but were initially misclassified as LSILs. In fact, 18% of women with an LSIL Pap result prove to have HSIL (CIN 2/3) on biopsy.¹⁷⁰ Less than 1% of untreated LSILs progress to invasive cancer.⁹⁵



Cytomorphology of low-grade squamous intraepithelial lesion

- intermediate-size cells
- nuclear atypia
 - enlargement
 - irregular contour
 - hyperchromasia
 - slight chromatin coarseness
- cytoplasmic cavities (koilocytes)
- keratinizing variant

LSIL is a lesion of intermediate or superficial cells that show nuclear

enlargement accompanied by moderate variation in nuclear size and slight irregularities in nuclear shape and contour. Hyperchromasia is present and can take the form of either a uniformly granular increase in chromatin or the smudgy hyperchromasia seen in some koilocytes. Nucleoli are inconspicuous. Classic koilocytes have large, sharply defined perinuclear cytoplasmic cavities surrounded by a dense rim of cytoplasm. Their nuclei are usually enlarged, but they are diagnostic of LSIL even in the absence of nuclear enlargement ([Fig. 1.35](#)). Some LSILs show prominent keratinization manifested by deeply orangeophilic cytoplasm and squamous pearls ([Fig. 1.36](#)).

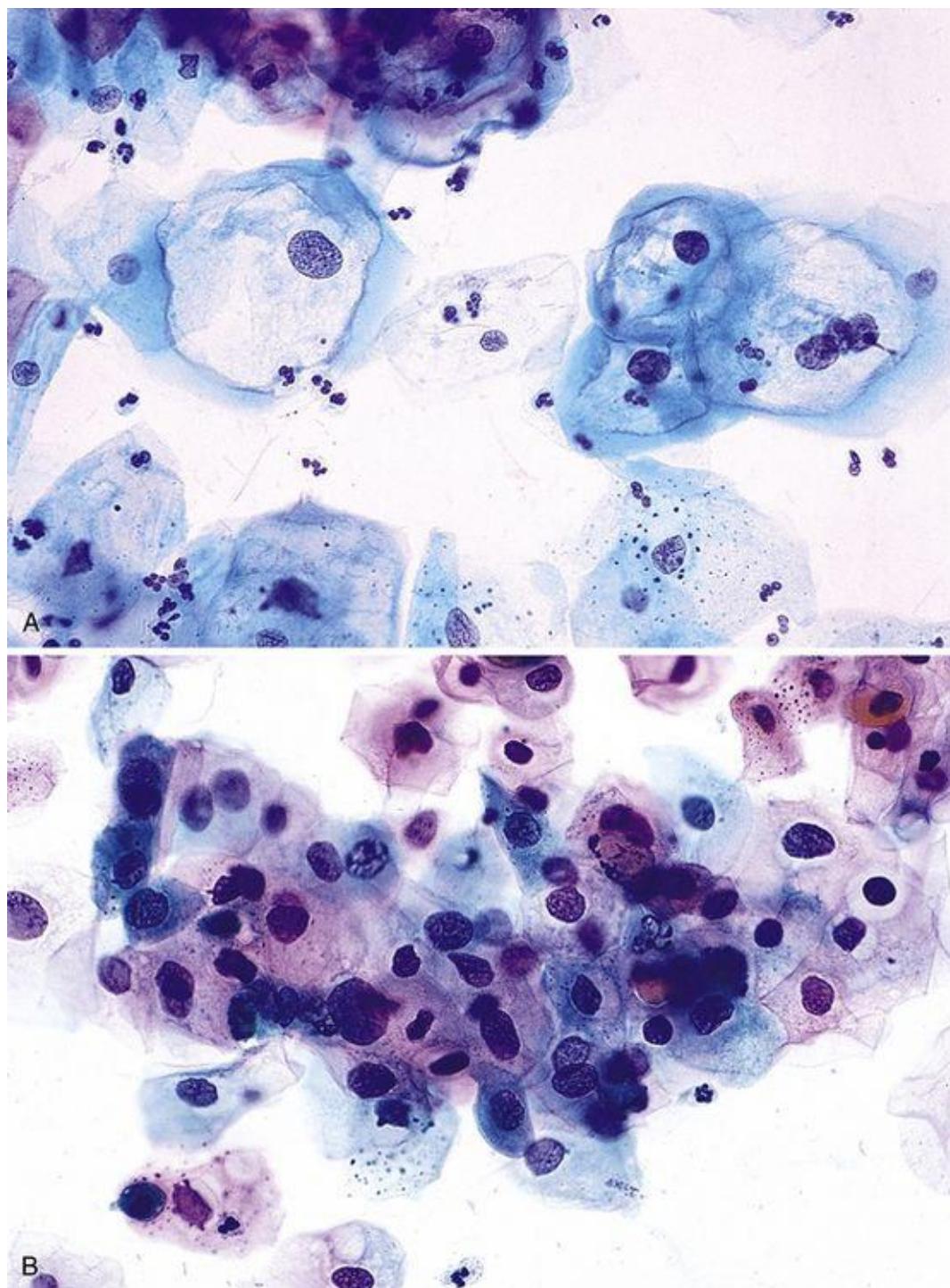


FIGURE 1.35 Low-grade squamous intraepithelial lesions (LSILs).
A, Classic koilocytes, as seen in this LSIL, have a large cytoplasmic cavity with a sharply defined inner edge and are frequently binucleated. Nuclear enlargement may not be as marked as in the nonkoilocytic LSILs. B, “Nonkoilocytic” LSIL. Nuclei are significantly enlarged and show mild hyperchromasia and nuclear contour irregularity. No definite koilocytes are seen. This pattern was once called *mild dysplasia* or CIN 1.

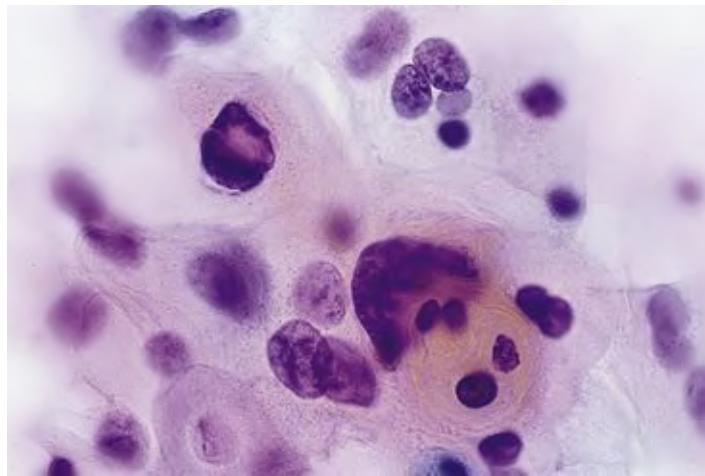


FIGURE 1.36 Low-grade squamous intraepithelial lesion (LSIL), keratinizing type. A squamous pearl is being formed.



Differential diagnosis of low-grade squamous intraepithelial lesion

- reactive squamous cells
- squamous cells with nonspecific halos
- reactive endocervical cells
- ASC-US

Nuclear enlargement by itself is not diagnostic of LSIL. It is common with benign squamous cells, particularly those seen in perimenopausal women (PM cells) (see [Fig. 1.25A](#)). Similarly, small, nonspecific halos mimic the cavities of koilocytes. They are seen in association with *Trichomonas* and other infections, and they can be an artifact of slide preparation. Nonspecific halos are often smaller than koilocyte cavities and unassociated with nuclear atypia (see [Fig. 1.26A and B](#)). Some markedly enlarged reactive endocervical cells have the size and polygonal shape of a squamous cell. With their enlarged nucleus they mimic an LSIL (see [Fig. 1.27B](#)). They are recognized by the company they keep (arranged alongside smaller, more recognizable endocervical cells) and by their granular rather than smooth cytoplasm.

Mild but noticeable nuclear changes and larger cytoplasmic cavities raise the possibility of LSIL but sometimes fall short qualitatively or quantitatively. Squamous cells that are suspicious but not conclusive for LSIL are reported as ASC-US.

Management. The management of a woman with an LSIL Pap depends on her particular circumstances. HPV testing to triage women with an LSIL Pap result is not generally recommended, because the high rate of positivity (77%) limits its usefulness.²⁵ Colposcopy is recommended in most cases.²⁵ If the patient is pregnant, it is acceptable but not necessary to defer colposcopy until 6 weeks post partum. Postmenopausal women with LSIL cytology can be managed by immediate colposcopy, but repeat Pap testing at 6 and 12 months or HPV testing are also acceptable.²⁵ With the latter two options, the postmenopausal woman is referred for colposcopy only if the HPV test result is positive or any one of the Pap specimens is ASC-US or greater. After two consecutive negative Pap test results, return to routine screening is recommended.

High-Grade Squamous Intraepithelial Lesion

HSIL is an intraepithelial lesion that is encountered in about 0.5% of all Pap specimens. Virtually all women (97%) with an HSIL Pap result test positive for high-risk HPV.⁵⁰ If left untreated, it carries a significant risk of progression to cervical cancer (see [Table 1.4](#)).



Cytomorphology of high-grade squamous intraepithelial lesion

- usually parabasal-sized cells
- discrete cells or syncytium-like groups (hyperchromatic crowded groups)
- nuclear atypia
 - enlargement
 - marked irregularity in contour
 - usually marked hyperchromasia
 - marked chromatin coarseness
- keratinizing variant

HSIL is usually a lesion of immature squamous cells. Patten divided HSILs into three categories based on cell size (frequencies in parentheses): large cell (20%), intermediate (70%), and small cell (10%).¹⁷¹ These subtypes have no biologic significance but are helpful to keep in mind in considering what cells

might mimic an HSIL. Nuclear enlargement is generally in the same range as in LSILs, but the nuclear-to-cytoplasmic ratio is higher because the cells are smaller ([Fig. 1.37](#)). In general, hyperchromasia, irregular chromatin distribution, and membrane contour irregularity are all more severe than in LSIL. In any given HSIL, one or more of the characteristic nuclear changes predominate. Thus, some HSILs have very irregular nuclear contours but only mild to moderate hyperchromasia. Architecturally, the cells of HSIL are arranged in two main patterns: as distinct individual cells ([Fig. 1.37](#)), or as cohesive groups of cells with indistinct cell borders (syncytium-like clusters) ([Fig. 1.38](#)). They may have dense, squamoid cytoplasm, but HSIL cells are often completely undifferentiated in appearance and lack any defining squamous features. In fact, cytoplasmic transparency and vacuoles ([Fig. 1.39](#)) and an elongated configuration ([Fig. 1.40](#)) can cause them to be mistaken for cells of glandular origin. Although usually a lesion of small, immature squamous cells, mature keratinizing cells with marked nuclear atypia are classified as HSIL ([Fig. 1.41](#)).

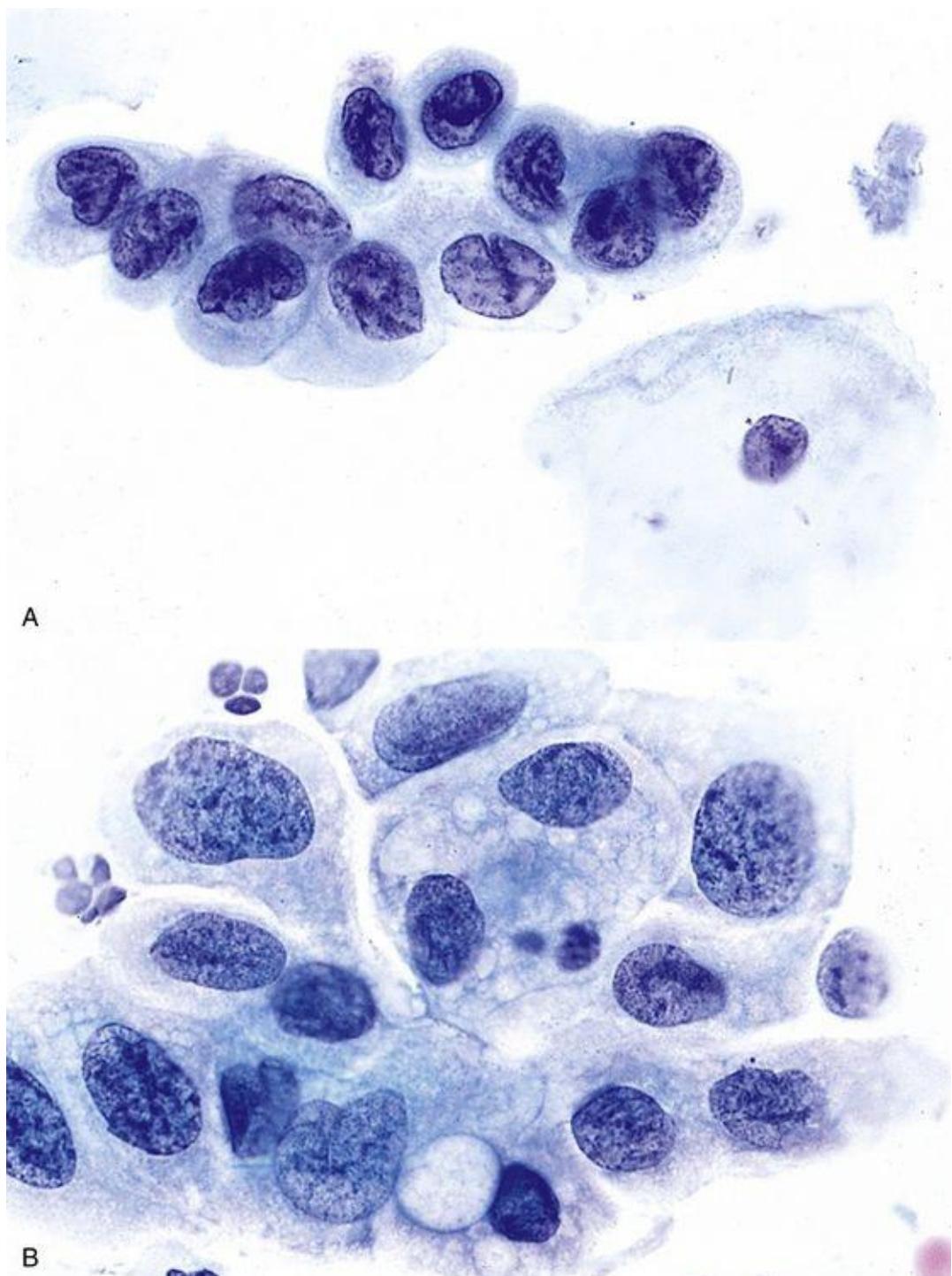


FIGURE 1.37 High-grade squamous intraepithelial lesion (HSIL).
A, These cells have scant cytoplasm and a markedly hyperchromatic nucleus with highly irregular nuclear contours. B, Cells with a moderate amount of cytoplasm, formerly called *moderate dysplasia* or *CIN 2*, are incorporated in the HSIL category.

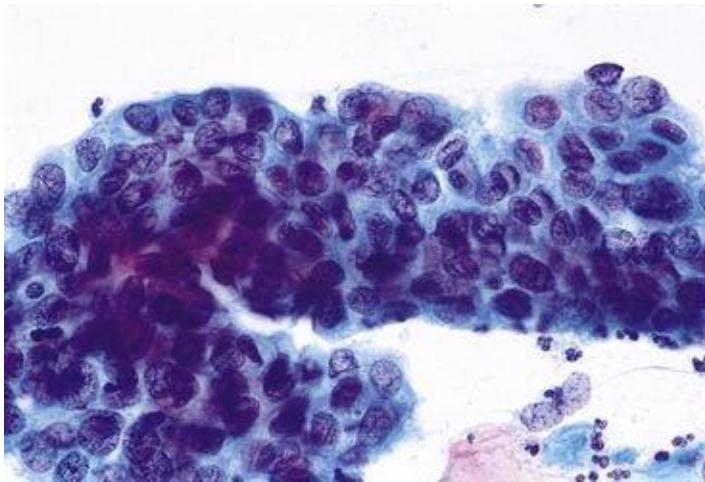


FIGURE 1.38 High-grade squamous intraepithelial lesion (HSIL).
The cells of an HSIL are often arranged in dark, three-dimensional groups in which individual cell borders are indistinct (syncytium-like).

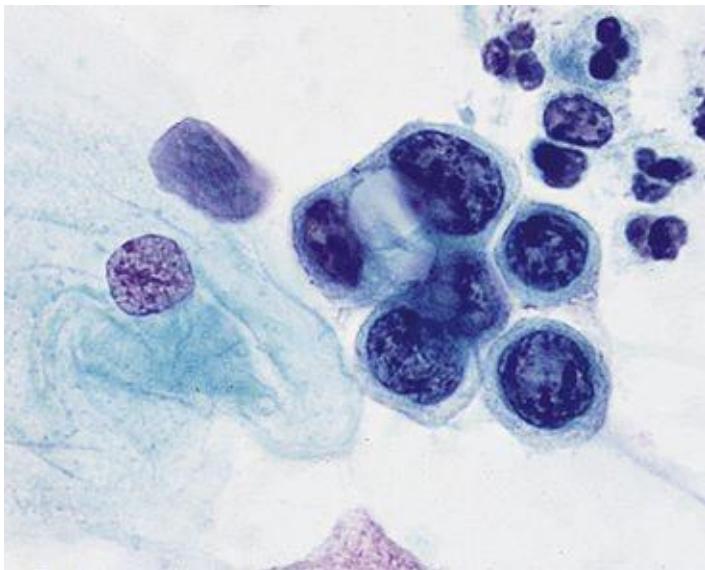


FIGURE 1.39 High-grade squamous intraepithelial lesion (HSIL).
Some HSILs are composed of very small, dispersed, highly atypical cells. The nucleus of these small cells is not much larger than that of normal intermediate cells. They are nevertheless identified as abnormal because of their hyperchromasia, markedly irregular nuclear outline, or both. Some HSIL cells have cytoplasmic vacuoles. These do not indicate a glandular lesion.

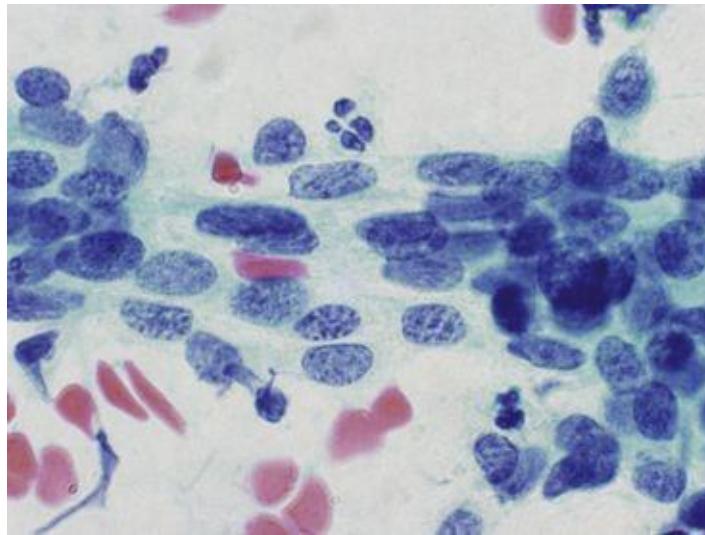


FIGURE 1.40 High-grade squamous intraepithelial lesion (HSIL).
The cells of some HSILs have an elongated configuration that makes them look columnar. In the absence of strips, rosettes, or feathering, this should not be taken for evidence of glandular differentiation (i.e., an adenocarcinoma in situ [AIS]).

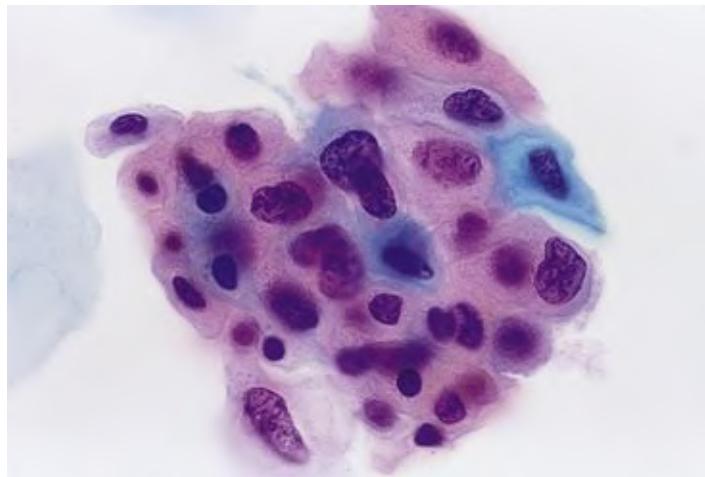


FIGURE 1.41 High-grade squamous intraepithelial lesion (HSIL), keratinizing type.
Although the cells show differentiation by keratinizing, they are classified as HSIL if the nuclei are sufficiently abnormal.



Differential diagnosis of high-grade squamous intraepithelial lesion

- squamous metaplasia
- atrophy
- transitional metaplasia

- exfoliated endometrial cells
- follicular cervicitis
- histiocytes
- IUD effect
- endocervical polyp atypia
- adenocarcinoma in situ
- squamous cell carcinoma
- ASC-H
- ASC-US associated with atrophy

Distinguishing HSIL from its many mimics is an important skill of the cytologist. As with histologic sections, one of the most frequent cytologic mimics is squamous metaplasia. Squamous metaplastic cells commonly show mild degrees of nuclear enlargement, nuclear membrane irregularity, and even chromatin coarsening. These changes rarely rise to the level of atypia seen in HSIL. In postmenopausal women, sheets of atrophic squamous epithelium mimic the syncytium-like clusters of HSIL (see [Fig. 1.6A](#)). Although atrophic squamous cells have a high nuclear-to-cytoplasmic ratio, their nuclei are usually very regular, with finely textured chromatin. Transitional cell metaplasia, associated with Paps from older women, is likely to raise the possibility of HSIL because of the irregularity of the nuclear outlines and prominence of nuclear grooves (see [Fig. 1.6B](#)). The minimal hyperchromasia and the abundance of coffee bean-shaped nuclei is a clue to the benign metaplastic nature of these cells. HSIL cells, even those of the small-cell type,¹⁷ are usually bigger than endometrial cells (compare [Figs. 1.12](#) and [1.13A](#)), vary more in size, and have denser cytoplasm. HSIL clusters are usually less well circumscribed and not as spherical as endometrial cell clusters. Lymphoid cells, commonly seen in postmenopausal women, are smaller than HSIL cells, their chromatin is even more coarsely textured, and there are often admixed plasma cells, dendritic cells (with a larger, pale nucleus) and tingible-body macrophages (see [Fig. 1.16](#)). Histiocytes are roughly the same size as HSIL cells, and many have irregular nuclear contours, but their chromatin is finely textured and cytoplasm is often abundant and fluffy (see [Fig. 1.17](#)). The small cells of IUD effect are usually few in number and have a more prominent nucleolus than is commonly seen with HSIL (see [Fig. 1.31](#)). Occasional inflamed endocervical polyps are lined by a single layer of highly atypical, hyperchromatic endocervical cells that are easily overinterpreted as HSIL. Their true nature is often clarified only after histologic correlation ([Fig. 1.42](#)).

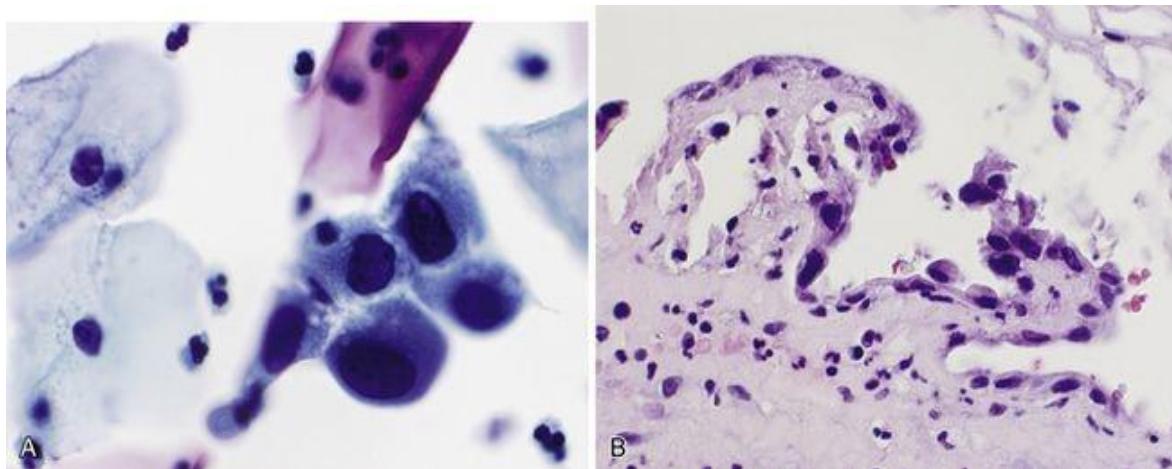


FIGURE 1.42 Endocervical polyp atypia mimicking high-grade squamous intraepithelial lesion (HSIL).

A, The slide contains scattered isolated cells with dark nuclei. B, The surface of the endocervical polyp reveals a single layer of reactive endocervical cells.

The neoplastic cells of adenocarcinoma in situ (AIS) share many of the nuclear features of HSIL. Clusters of neoplastic cells are more likely to represent HSIL rather than AIS unless there is clear columnar differentiation in the form of feathering or rosette formation. Squamous cell carcinoma should be considered whenever the cytologic criteria for HSIL are fulfilled, but in addition one finds prominent nucleoli and/or necrotic debris. Cell block preparations from a residual liquid-based sample can help by providing a “histologic” look at hyperchromatic cell clusters (Fig. 1.43A and B).⁴⁰ Immunohistochemistry for p16 and Ki-67 (MIB1) helps distinguish benign from neoplastic cells;¹⁷² p63, which highlights squamous but not glandular lesions, helps distinguish between squamous and glandular lesions.¹⁷³

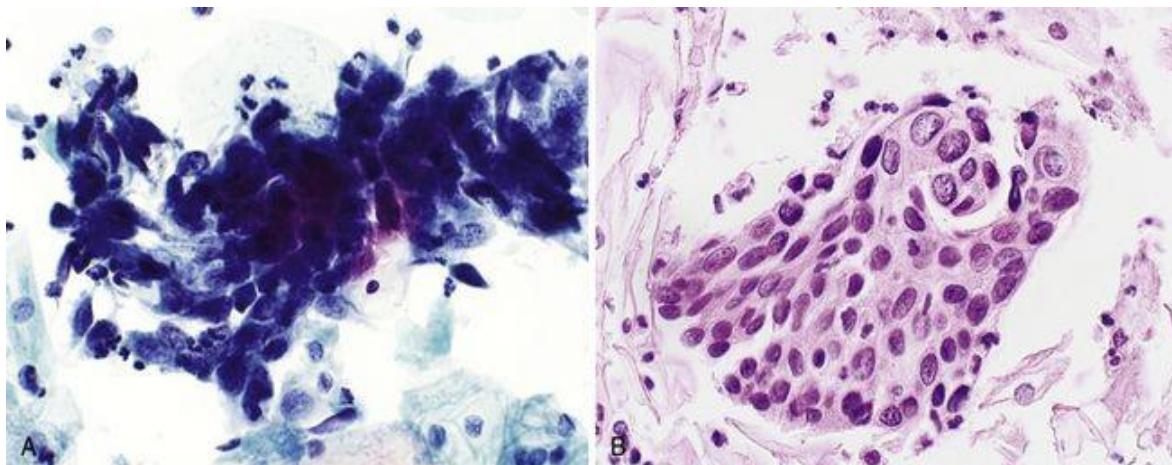


FIGURE 1.43 Utility of cell blocks in evaluating hyperchromatic cell clusters.

A, The distinction between a squamous and a glandular lesion can be problematic, especially when individual cells in dark cell clusters are poorly visualized. B, A cell block prepared from the residual liquid-based sample in this case clarified the findings as a high-grade squamous intraepithelial lesion (HSIL).

In some cases, uncertainty remains regarding the true nature of the cells examined. Cells with the features of squamous metaplasia sometimes show a degree of nuclear atypia that makes it impossible to exclude an HSIL. These cases of *atypical squamous metaplasia* are reported as “ASC, cannot exclude HSIL.” Another diagnostically difficult pattern is the marked squamous atypia associated with a deeply atrophic Pap. Atrophic cervical epithelium sometimes displays a marked squamous atypia that is impossible to distinguish from HSIL. The recommended approach is to call such cases ASC-US.

Management. Management is more aggressive for cytologic HSIL than for LSIL because HSIL carries a higher risk of progression to invasive cancer. With the exception of younger women (ages 21 to 24) and those who are pregnant, an immediate loop electrosurgical excision (LEEP) (the “see-and-treat” approach) is acceptable as the initial treatment if the woman has an HSIL Pap, but not LSIL. An alternative to LEEP is colposcopy with endocervical assessment (evaluating the canal using the colposcope or tissue sampling).²⁵ In younger and pregnant women, colposcopy is recommended. Histologic CIN 2+ is found in 60% of women with cytologic HSIL, and cervical cancer in about 2%.²⁵

Problems in the Diagnosis of Squamous Intraepithelial Lesions

Avoiding Overdiagnosis of LSIL. Care must be taken not to overinterpret nonspecific halos (see Fig. 1.26A and B) or the minimal nuclear changes of benign cells like PM cells (see Fig. 1.25A).¹⁴⁰ Without hyperchromasia or nuclear membrane irregularity, such cells are best called negative. Cellular changes that include some hyperchromasia and/or nuclear membrane irregularity are suggestive of LSIL and should be categorized as ASC-US.

Distinguishing LSIL from HSIL. The distinction between cytologic LSIL and HSIL is an important one, with significantly different implications for clinical management. Proficiency in this distinction is an important skill of the cytology practitioner. As mentioned previously, HSIL is usually a lesion of immature squamous cells, and nuclear atypia (hyperchromasia, irregular chromatin distribution, and membrane contour irregularity) is more severe than in LSIL. If a specimen is composed of both LSIL and HSIL, it should be reported as an HSIL even if the HSIL cells are less numerous than the LSIL

cells. In a small percentage of cases, the presence of morphologic features intermediate between typical LSIL and HSIL makes grading difficult.¹⁷⁴ Although there are generally fewer abnormal cells in an LSIL than in an HSIL, the quantity of cells is an unreliable discriminator.



Cytomorphologic patterns of “SIL, grade cannot be determined”

- few dysplastic cells
- extensive cytolysis
- LSIL, with a small number of equivocal HSIL cells
- extensively keratinized SILs, without definite HSIL

Grading is difficult when dysplastic cells are few in number, when the cytoplasm of the dysplastic cells is obviously affected by cytolysis, or when an LSIL is accompanied by a small number of cells suggestive of but not conclusive for HSIL. Extensively keratinized SILs without definite HSIL are especially difficult to grade¹⁷⁵ (Fig. 1.44). In all such cases, a diagnosis of “SIL, grade cannot be determined” (or “LSIL, cannot exclude HSIL”) is appropriate,¹⁷⁶ and about one half of surveyed laboratories report using such a category.¹¹⁶ This interpretation accounts for 3% to 12% of all cytologic SILs.^{170,177–179} Patients with this diagnosis have an intermediate risk (between that of cytologic LSIL and HSIL) of harboring histologic HSIL (CIN 2/3).^{176–178}

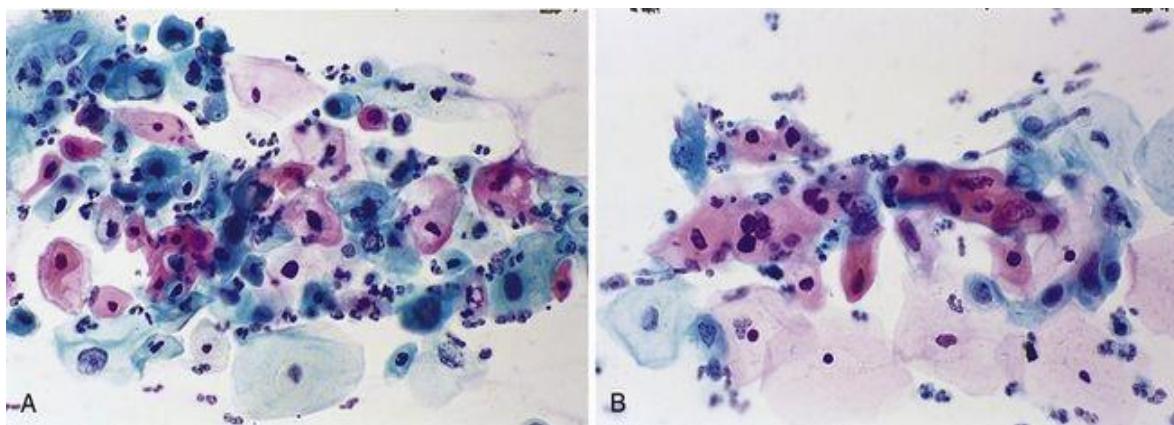


FIGURE 1.44 Squamous intraepithelial lesion (SIL), cannot determine grade.

When a lesion is extensively keratinized and there is no definite high-grade squamous intraepithelial lesion (HSIL), it is difficult to grade. Colposcopically directed biopsies showed, cervical intraepithelial neoplasia (CIN) 1 (A) and CIN 2/3 (B).

Distinguishing HSIL from Invasive Carcinoma. The criteria used to distinguish HSIL from invasive carcinoma are by no means perfect. Occasionally, a classic case of HSIL on cytology will turn out to be invasive squamous cancer on biopsy. Conversely, the possibility of invasive cancer is often raised in cases of HSIL in which the cells have marked nuclear abnormalities associated with abundant, heavily keratinized cytoplasm and unusual cell shapes, but the lesion turns out to be just a keratinizing HSIL on biopsy.⁹⁰ Clinicians understand that no diagnosis of HSIL on cytologic material excludes the possibility of invasive cancer, and that colposcopy and biopsy are necessary for confirmation. HSILs with features worrisome for invasive cancer can be reported as “HSIL, with features suggestive of invasive cancer.”

Squamous Cell Carcinoma

SQC is the most common malignant tumor of the cervix, accounting for about 75% of cervical cancers.¹⁸⁰ Although most patients are between the ages of 35 and 55 years, invasive tumors occur in younger patients, including those under 30 years of age.²⁹ HPV 16 accounts for about 50% to 60% of SQCs worldwide, and HPV 18 for an additional 10% to 15%.¹⁸¹ Early invasive tumors can be asymptomatic, but as the tumor grows, patients can develop abnormal vaginal bleeding, vaginal discharge, and pain during intercourse (dyspareunia). With more advanced tumors there can be back pain, sciatica, tenesmus, and hematuria.

Histologically and cytologically, SQCs range from well-differentiated, keratinizing tumors to poorly differentiated, nonkeratinizing tumors.¹⁸² Some SQCs cannot be distinguished cytologically from HSIL, particularly the smaller, less deeply invasive tumors.¹⁸³ Others can be confidently diagnosed as invasive cancers, however.



Cytomorphology of squamous cell carcinoma

- HSIL features, plus:
 - macronucleolus
 - irregular chromatin distribution
 - tumor diathesis
 - “tadpoles” and “fiber cells” (keratinizing type)

The classic pattern of SQC shows abundant necrotic debris: a granular, amorphous precipitate with nuclear debris and red blood cells called *tumor diathesis* ([Fig. 1.45](#)). It is not specific for invasive cancers; a similar pattern is seen in some atrophic smears and even during heavy menstrual bleeding. When associated with hyperchromatic crowded groups of atypical cells or abundant atypical keratinized cells with unusual shapes (“tadpoles,” “fiber cells”), the pattern is diagnostic.

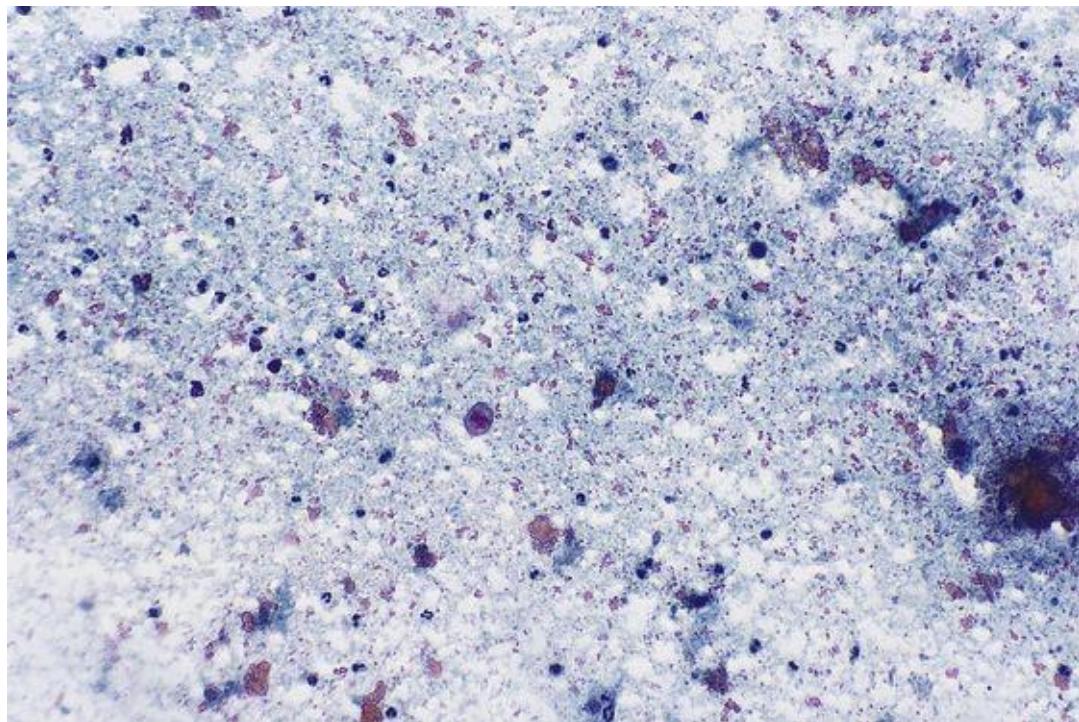


FIGURE 1.45 Squamous cell carcinoma (SQC).

Slides from deeply invasive tumors show abundant tumor diathesis, a granular precipitate of lysed blood and cell fragments. In some cases, the malignant cells can be hard to identify. In other cases, the tumor diathesis is focal, and if this feature is missed, the tumor may be misclassified as a high-grade squamous intraepithelial lesion (HSIL).

The cells of a nonkeratinizing SQC look like modified HSIL cells ([Figs. 1.46](#) and [1.47](#)). Like HSIL, they are hyperchromatic and have scant cytoplasm, but they have a prominent nucleolus and a highly irregular pattern of chromatin distribution. The cells of a keratinizing SQC are often bizarrely elongated ([Fig. 1.48](#)). Some are long and spindle-shaped, with small condensed nuclei (fiber cells). Others have a larger cytoplasmic body with a long tail (so-called tadpole cells). Such cells are uncommon in keratinizing HSILs.

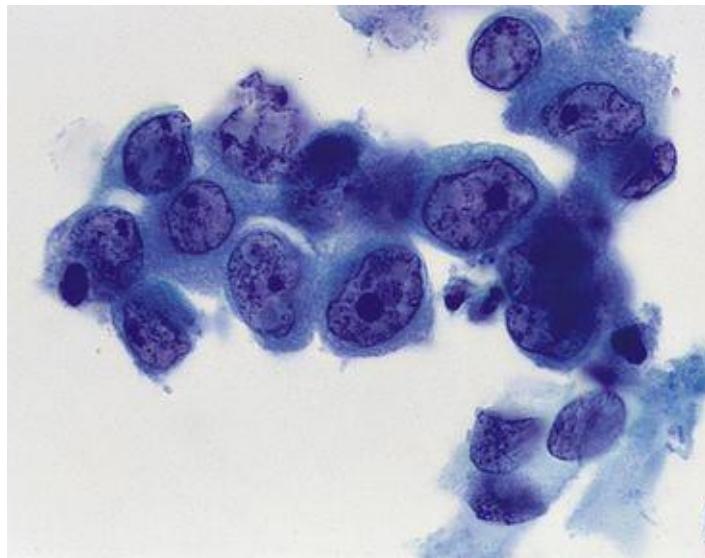


FIGURE 1.46 Squamous cell carcinoma (SQC), nonkeratinizing.
The malignant cells have irregularly distributed chromatin and a prominent nucleolus, characteristic features of invasive SQCs.

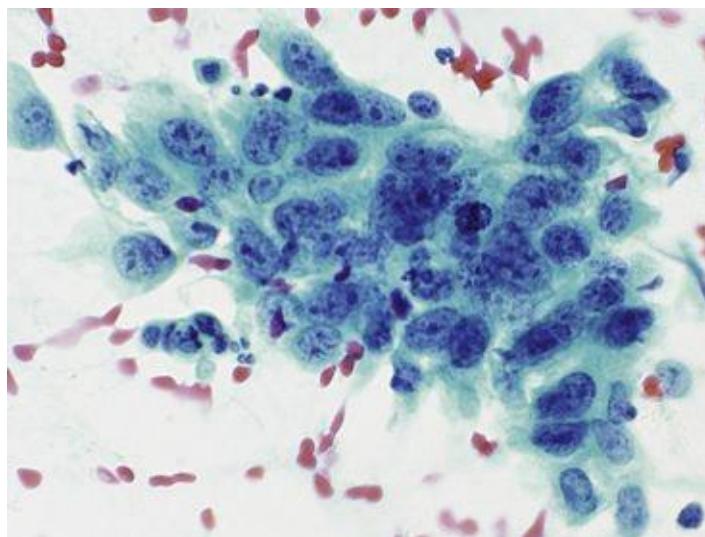


FIGURE 1.47 Squamous cell carcinoma (SQC), nonkeratinizing.
The sheetlike arrangement of poorly differentiated squamous carcinoma cells with nucleoli and mitoses mimics the appearance of reparative epithelium, but the crowding and haphazard arrangement of the cells are not typical of repair.

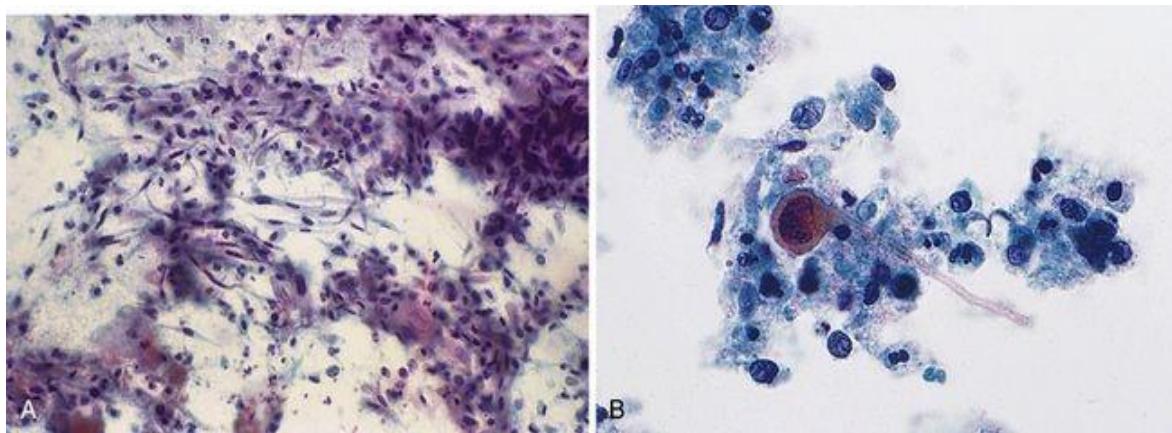


FIGURE 1.48 Squamous cell carcinoma (SQC), keratinizing.

A, In keratinizing carcinomas, the cells have markedly aberrant shapes, as seen here. So-called fiber cells are numerous. B, Tumor diathesis and a tadpole cell are seen in this tumor.

Most SQCs are associated with an adjacent or overlying HSIL, so cytologic preparations from SQCs often contain a population of HSIL cells as well.



Differential diagnosis of squamous cell carcinoma

- HSIL
- atypia of atrophy
- atypia of repair
- benign endometrial cells
- Behçet disease
- pemphigus vulgaris

The differential diagnosis of SQC includes HSIL. Prominent nucleoli and tumor diathesis are the principal cytologic features that help distinguish SQC from HSIL, but these features are not present in all smears from patients with SQC. A significant number of women with an SQC are diagnosed as having HSIL because prominent nucleoli and tumor diathesis are absent.¹⁸³ Conversely, a granular, tumor diathesis–like background is not specific for invasive cancers and is seen in women with atrophic vaginitis⁹⁹ (see Fig. 1.7), severe cervicitis, and rare cases of HSIL.⁹⁰

In postmenopausal women, marked atrophy atypia is one of the most common benign mimics of a keratinizing SQC. The benign atypia of atrophy is characterized by scattered cells with large, dark nuclei and eosinophilic or

orangeophilic cytoplasm. The large, dark nuclei are alarming, but chromatin is usually smudgy. Such cells, if seen in a deeply atrophic squamous background, should be interpreted as ASC-US and not HSIL or invasive cancer.

Marked repair atypia is another good mimic of nonkeratinizing SQC. Both repair and SQC feature large cells with prominent nucleoli, and mitoses are seen in both. Repair cells are recognized by their finely textured chromatin pattern and the flatness and cohesion of the sheets. If the nuclei have coarsely textured chromatin, show marked crowding, or demonstrate significant dyshesion, an SQC should be considered.

A minority of nonkeratinizing SQCs are composed of small cells that are indistinguishable from endometrial cells (see [Fig. 1.13B](#)). The blood that accompanies menstrual endometrial cells resembles the granular necrosis that is tumor diathesis, adding to the similarity. Mitoses, if identified, should raise the suspicion of SQC. In some cases, knowledge that the patient has a suspicious cervical mass or suggestive clinical symptoms (e.g., dyspareunia) may be the only clue to the correct interpretation.

Behçet disease, a chronic disease of uncertain cause that is characterized by oral and genital ulcers, can mimic SQC. Slides may show numerous isolated, keratinized cells with dark, pleomorphic nuclei and large nucleoli. A history of this disorder may be critical for correct diagnosis.^{[184](#)} Smears from patients with pemphigus vulgaris, a blistering disorder that involves mucosal surfaces, may mimic a poorly differentiated SQC. A complete history may be important to avoid making an overcall, although cases of coexisting SQC and pemphigus vulgaris have been reported.^{[185](#)}

Management. Treatment choices for women with cervical cancer depend on tumor stage.^{[186](#)} Hysterectomy (simple or radical, depending on histologic findings) is the treatment of choice for lower stage and smaller (IA, IB1, selected IIA1) tumors. Women with early-stage disease who wish to preserve fertility have the option of trachelectomy (surgical removal of the cervix) with lymph node dissection. Concurrent chemoradiation therapy using a cisplatin-based regimen is the primary treatment of choice for women with higher-stage SQC (IB2 to IVA).

Atypical Squamous Cells

Since the days of Papanicolaou, cytology laboratories have used a borderline category to report findings of uncertain significance. Terminology was inconsistent and often confusing, however, because benign changes were

sometimes reported as “benign atypia.” In the Bethesda System, recognizably benign cases, previously called “benign atypia,” “inflammatory atypia,” or “reactive atypia,” are excluded from this category. The 1988 and 1991 Bethesda Systems used the term *atypical squamous cells of undetermined significance* (ASC-US) to designate “cellular abnormalities that were more marked than those attributable to reactive changes but that quantitatively or qualitatively fell short of a definitive diagnosis of SIL.” In the 2001 Bethesda System, ASC-US was replaced by *atypical squamous cells* (ASC) and redefined in a subtle way. Instead of being a diagnosis of exclusion, ASC is a diagnosis conveying *a suspicion of SIL*.

Most cytologists agree that this category is essential. Eliminating ASC would result in increased reporting of LSIL (which probably contributes little to cancer prevention) and decreased recognition of CIN 2/3.¹⁸⁷ It is risky to eliminate an equivocal category because of the large number of women with underlying HSIL who are discovered through a work-up for an equivocal cytology reading. In fact, histologic HSIL is found in 10% to 20% of women with ASC Paps,^{50,170} and ironically, ASC Paps, because they are more common, detect more cases of histologic CIN 2+ than HSIL Paps.¹⁸⁸ Finally, the elimination of an equivocal category seems imprudent in view of the expectations in the United States and elsewhere for a very sensitive Pap test.⁹⁷

ASC diagnoses should be kept to a minimum. There is no “correct” rate of ASC, but expert consensus suggests that this diagnosis be kept to less than 5% of all Pap cases. For labs that serve high-risk populations, a better gauge is the ASC/SIL ratio, which should not exceed 3:1.¹⁰¹ The ASC rate can be kept low through education, optimal sample preparation, and the monitoring (with feedback) of individual ASC/SIL ratios.^{189,190} According to a 2006 College of American Pathologists survey, most U.S. labs are complying with the foregoing recommendations: ASC accounts for 4.6% of all Paps, and the median ASC/SIL ratio is 1.5.¹¹⁶

In the 2001 Bethesda System, ASC is qualified as either **“of undetermined significance” (ASC-US)** or **“cannot exclude HSIL” (ASC-H)**. The higher risk of the latter category is well recognized.^{191,192}

Atypical Squamous Cells of Undetermined Significance

The cases described in this section on ASC are daily dilemmas for cytologists. ASC-US, the most common non-negative interpretation, constitutes 4.3% of all Pap test interpretations.¹¹⁶ The decision to categorize a Pap specimen as negative

(NILM), ASC-US, ASC-H, LSIL, or HSIL rests on the quantity of the altered squamous cells, the severity of the abnormalities, the state of preservation of the specimen, and the clinical setting. If the changes are suspicious but not conclusive for SIL, the findings are reported as ASC-US.



Cytomorphologic patterns of ASC-US

- atypical cells with mature, intermediate-type cytoplasm, including cells suggestive of koilocytes
- atypical squamous cells in atrophy
- atypical parakeratosis
- atypical repair
- “atypia” due to a compromised specimen

Atypical mature squamous cells with features suspicious for an SIL are classified as ASC-US ([Fig. 1.49A](#)). Some cases have incomplete features of koilocytosis ([Fig. 1.49B](#)).

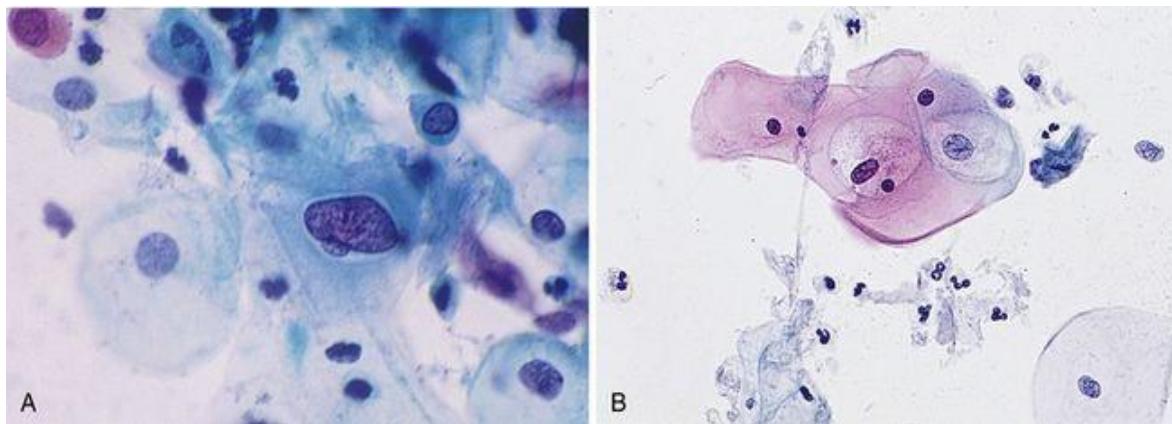


FIGURE 1.49 Atypical squamous cells of undetermined significance (ASC-US).
A, The nucleus of this mature squamous cell is significantly enlarged, and there is moderate hyperchromasia. Cells like this, particularly if few in number, are suggestive but not diagnostic of a squamous intraepithelial lesion (SIL). B, Some cells have large cytoplasmic cavities but minimal nuclear atypia. It is preferable to diagnose such cases as ASC-US when abnormal cells are few and the changes minimal.

Atypical squamous cells associated with atrophy are diagnosed as ASC-US when there is nuclear enlargement with hyperchromasia, when nuclei are

irregular in contour and chromatin distribution, and when there is marked cellular pleomorphism with unusual shapes. In extreme cases, the changes seen in atrophy with inflammation are difficult to distinguish from an SIL or invasive cancer ([Fig. 1.50](#)). Management options include a course of intravaginal estrogen cream (e.g., 1 g estrogen cream three times a week for several months), followed by a repeat Pap test a week after completion of the regimen.¹⁹³ A significant squamous lesion will be more easily detected among the mature cells, whereas a benign alteration due to atrophy will be transformed into normal epithelium.

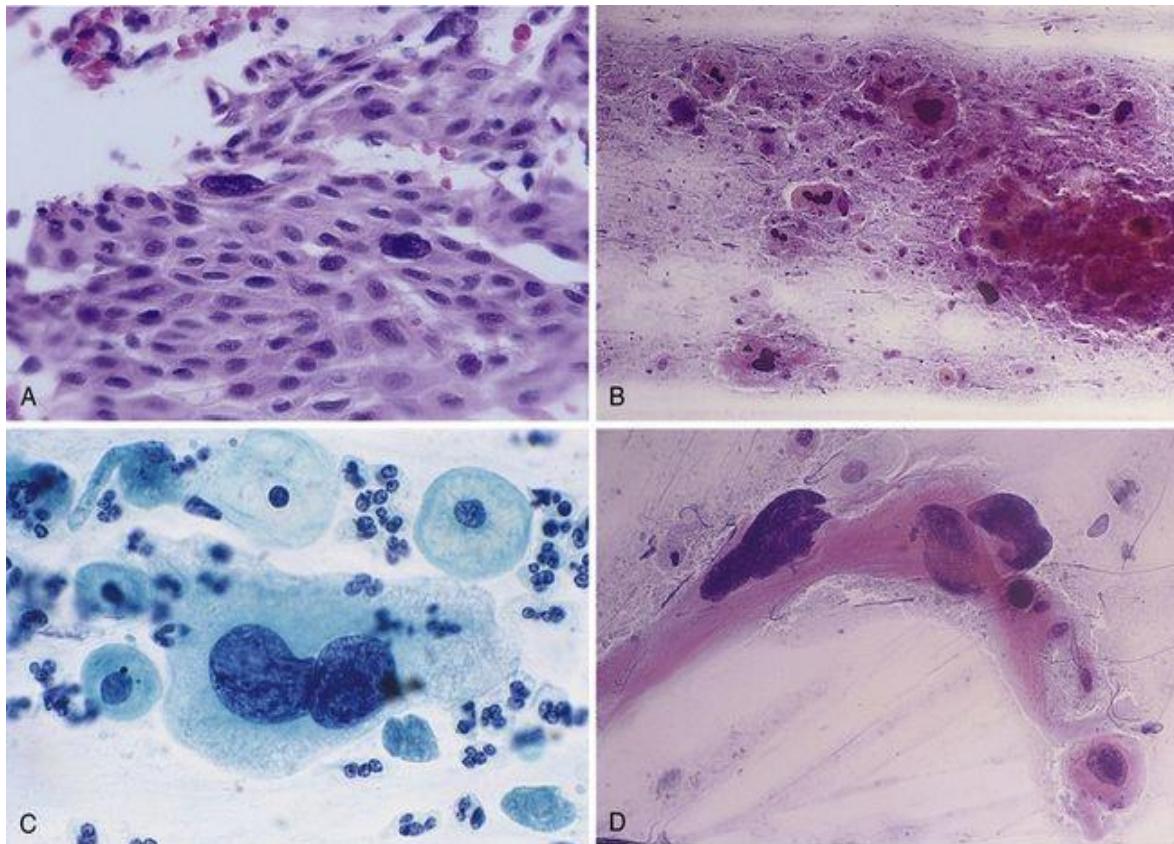


FIGURE 1.50 Atypical squamous cells of undetermined significance (ASC-US), associated with atrophy.

A, Histologic section of benign atrophy-associated atypia. B, Cytologic smear shows scattered large atypical cells in a granular background. C, Some cells have a markedly enlarged, hyperchromatic nucleus. D, Often cells are poorly preserved, with smudgy nuclei and hypereosinophilic cytoplasm. Followup in all cases was benign.

Squamous atypia in a postmenopausal woman is less often associated with a biopsy-proven SIL (17%) than in a premenopausal woman (46%).¹⁹⁴ Further, the

rate of HPV detection in women with atypia is lower (10% versus 50%). In another study, squamous atypia in women over the age of 50 was associated with histologic CIN in less than 5% of cases.¹⁹⁵

Parakeratosis with mild nuclear enlargement and mild to moderate nuclear membrane irregularity (*atypical parakeratosis*) suggests an SIL ([Fig. 1.51](#)). In some cases such cells are accompanied by other changes diagnostic of an SIL, but when the changes are mild, such cases are best classified as ASC-US.

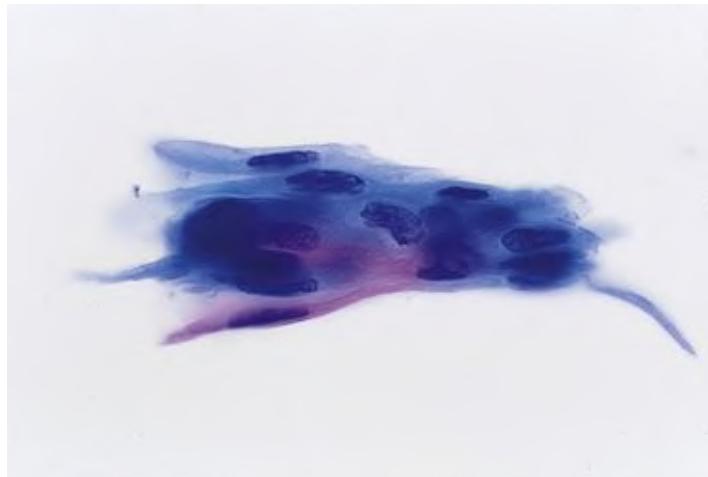


FIGURE 1.51 Atypical squamous cells of undetermined significance (ASC-US), with features of atypical parakeratosis.

Small, keratinized squamous cells with mild variation in nuclear size and contour may represent either a reactive process or a significant squamous lesion.

Highly exuberant atypical repair reactions can demonstrate cellular crowding and overlap (in contrast with typical repair, which is in flat sheets), marked variation in nuclear size, prominent and irregular nucleoli, and irregular chromatin distribution ([Fig. 1.52](#)). Such cases are difficult to distinguish from invasive carcinoma. Carcinomas often have a tumor diathesis and many isolated atypical cells, features that are usually absent in repair reactions.

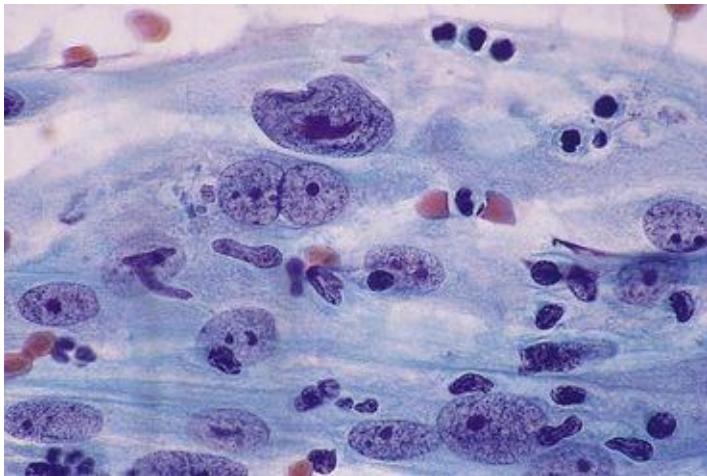


FIGURE 1.52 Atypical squamous cells of undetermined significance (ASC-US), atypical repair reaction.

In some cases of repair there is such striking nuclear atypia that an invasive cancer cannot be excluded. This lesion proved to be benign.

Management. Reflex HPV testing is the preferred approach for managing most women with ASC-US.²⁵ Women who test positive for HPV should be referred for colposcopy with directed biopsies. Women who test negative for HPV return for cotesting in 3 years. Repeat cytology in 1 year is an acceptable option, but reflex HPV testing is preferred for managing a woman with ASC-US, because it is more sensitive than a single repeat Pap test.⁵⁰ If repeat Pap testing is selected, colposcopy is recommended if the result is ASC-US or worse; if the result is negative, return to testing every 3 years is recommended.

There are minor variations in the recommendations for younger (ages 21 to 24) and pregnant women. In younger women with an ASC-US Pap, cytology at 12 months is preferred, but reflex HPV testing is acceptable. If the cytology option is selected, only women with an ASC-H or HSIL Pap (or worse) at the 12-month followup should be referred for colposcopy. The recommendations for pregnant women are the same as the general recommendations, except that it is acceptable to defer colposcopy until 6 weeks post partum. Endocervical curettage in pregnant women is unacceptable.

Atypical Squamous Cells, Cannot Exclude HSIL

ASC-H is the second, by far less common subtype of ASC, representing only 0.3% of all Pap test interpretations.¹¹⁶ This category is reserved for Pap specimens that are specifically *suspicious for HSIL*. The most common pattern is that of immature (small) squamous cells with mild to moderate nuclear atypia

(enlargement, hyperchromasia, membrane irregularity), commonly called *atypical squamous metaplasia* ([Figs. 1.53 and 1.54](#)).

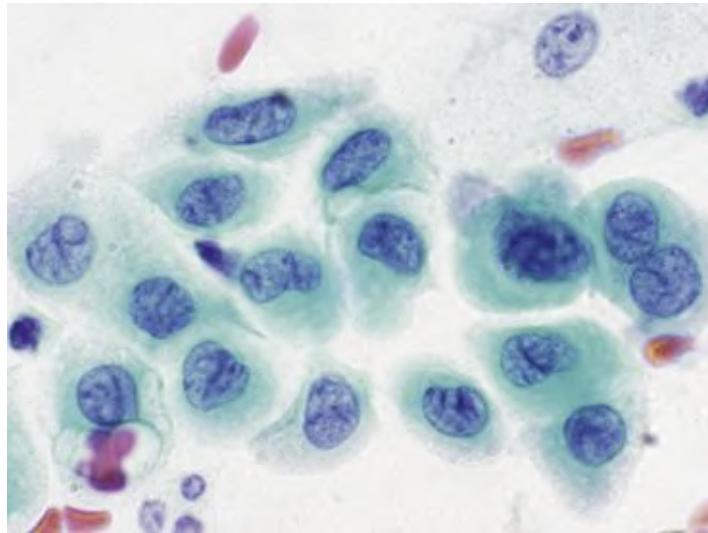


FIGURE 1.53 Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (HSIL).

These metaplastic-like cells show significant nuclear membrane irregularity. There is no hyperchromasia or significant nuclear size variation, however, which makes the diagnosis of HSIL uncertain.

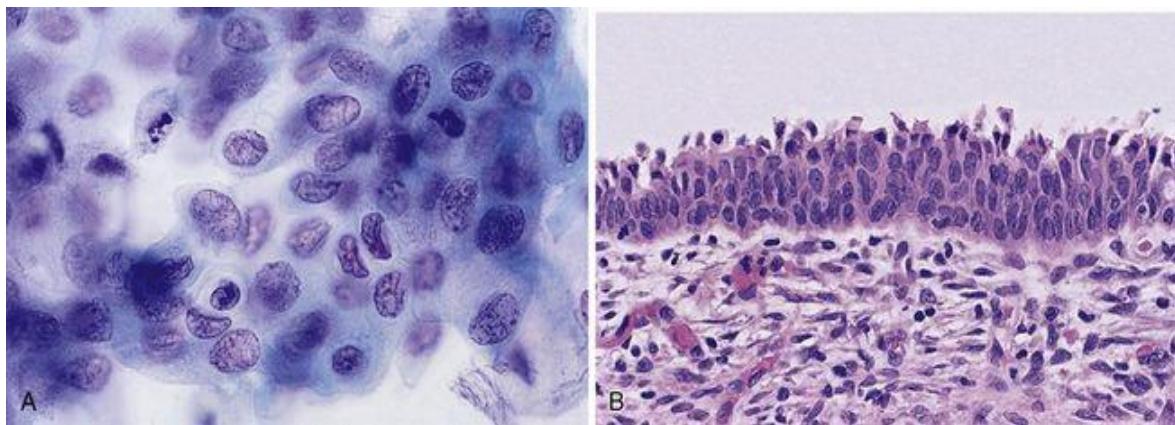


FIGURE 1.54 Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H).

A, Immature squamous metaplastic cells sometimes show some nuclear atypia that raises the possibility of HSIL, but the degree of nuclear enlargement, hyperchromasia, and membrane irregularity is insufficient for a definite diagnosis. B, Subsequent colposcopy revealed benign immature squamous metaplasia, and a human papillomavirus (HPV) test was negative for

high-risk HPV.

Management. ASC-H cytology carries a higher risk for histologic CIN 2/3 than ASC-US (50% versus 17%).¹⁹⁶ For this reason, women with an ASC-H Pap should be referred for colposcopy, regardless of HPV testing result.²⁵

Glandular Abnormalities

Endocervical Adenocarcinoma in Situ

Endocervical AIS is the recognized precursor to endocervical adenocarcinoma. The evidence linking them is similar to that linking SIL to squamous cell carcinoma; women with AIS are on average 13 years younger than those with adenocarcinoma (39 versus 52 years of age); AIS resembles adenocarcinoma morphologically and is often found in histologic sections adjacent to invasive carcinoma. AIS has been discovered retrospectively in biopsies originally called negative in women who later develop invasive adenocarcinomas.¹⁹⁷ and HPV 16 and 18 have been identified in AIS and adenocarcinomas in similar proportions.

The concept of endocervical AIS was first introduced in 1953, when its histologic features were convincingly illustrated by Friedell and McKay.¹⁹⁸ Widespread recognition of the cytologic characteristics of AIS came only in the late 1970s and 1980s, when a group of Australian investigators published their experience with a large number of histologically confirmed cases.^{199–201} It was not until 2001 that the cytologic criteria for AIS were considered sufficiently reliable to merit a separate, explicit diagnostic category in the Bethesda System. There has been a steady increase in the incidence of AIS, owing in part to better cytologic recognition of AIS. But cytologic diagnosis remains a challenge, mainly because it is still a very uncommon lesion. The incidence of AIS is a mere 1.25/100,000, as compared with 44.4/100,000 for SQC in situ.¹⁸⁰ Therefore, in practice, one is likely to see one case of AIS for every 36 cases of HSIL.



Cytomorphology of adenocarcinoma in situ

- hyperchromatic crowded groups
- glandular differentiation
 - columnar cells
 - strips and rosettes
 - “feathering”
- neoplastic nucleus
 - hyperchromasia
 - crowding, stratification
 - inconspicuous nucleolus
 - apoptosis
 - mitoses

- no tumor diathesis

In the 2001 Bethesda System, AIS is a separate diagnostic category because there is a consensus that the cytologic criteria are accurate and reproducible.²⁰¹⁻²⁰³ Examination of the slide under low magnification reveals hyperchromatic crowded groups similar to those of HSIL (Fig. 1.55). Closer inspection reveals evidence of glandular differentiation: columnar cells arranged in strips and/or rosettes (Fig. 1.56A). Columnar cells in sheets reveal their glandular nature by “feathering,” a splaying out around the edges (Fig. 1.56B). Nuclei are hyperchromatic and crowded and there is scant cytoplasm. Apoptotic bodies are seen in most cases and are a useful clue to the diagnosis.²⁰⁴ Mitoses are seen in some cases and are helpful, but only if accompanied by the typical nuclear changes as described.

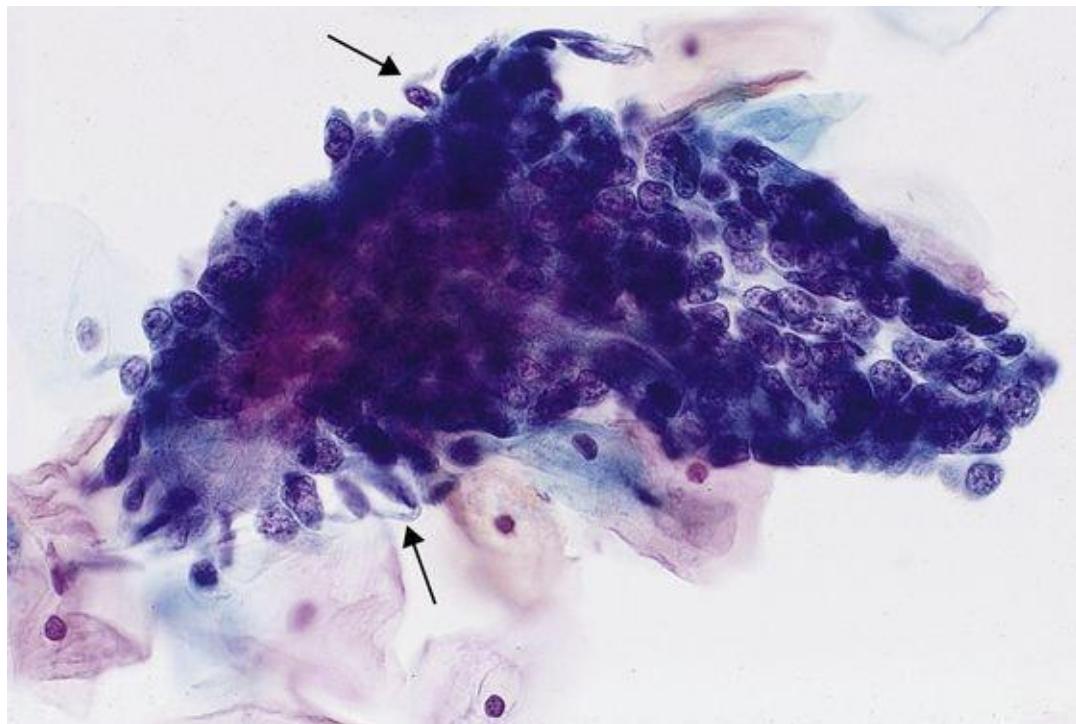


FIGURE 1.55 Adenocarcinoma in situ (AIS).

At first glance, some groups of neoplastic cells resemble the hyperchromatic crowded groups of a high-grade squamous intraepithelial lesion. Only slight feathering is seen (arrows).

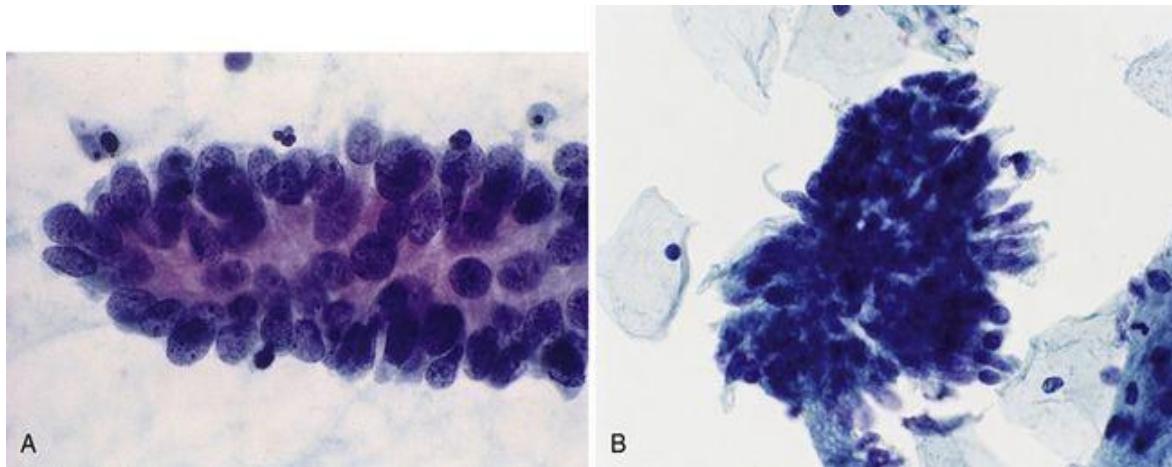


FIGURE 1.56 Adenocarcinoma in situ (AIS).

A, Rosettes are highly characteristic of AIS and virtually never seen with high-grade squamous intraepithelial lesion (HSIL), benign endocervical cells, lower uterine segment (LUS), or endometrial epithelium. B, The glandular nature of these neoplastic cells is betrayed by “feathering.”



Differential diagnosis of adenocarcinoma in situ

- exfoliated endometrial cells
- tubal metaplasia
- abraded endometrial cells and LUS
- reactive endocervical cells
- reparative changes
- HSIL
- invasive adenocarcinoma

A serious and not uncommon problem is mistaking AIS for benign cells.^{205,206} Some cases of AIS, in fact, strongly resemble menstrual endometrial cells (“endometrioid” AIS),²⁰⁷ and apoptosis is a feature of both (see Fig. 1.13C). The cells of AIS are generally better preserved and have a coarser chromatin texture. Feathering, rosettes, and mitoses are virtually never seen in menstrual endometrium. AIS resembles tubal metaplasia (Fig. 1.57A-D), but tubal metaplasia is recognized by the presence of terminal bars and cilia. In addition, tubal metaplasia lacks mitoses and apoptosis. AIS also strongly resembles directly sampled endometrium and LUS, particularly because mitoses can be seen in both (see Fig. 1.14B and C). The intact tubules alongside stroma that are typical of endometrium and LUS are rarely seen in AIS (see Fig. 1.14A),

however, and the nuclei of endometrium and LUS, although crowded, are arranged in an orderly rather than a haphazard pattern.

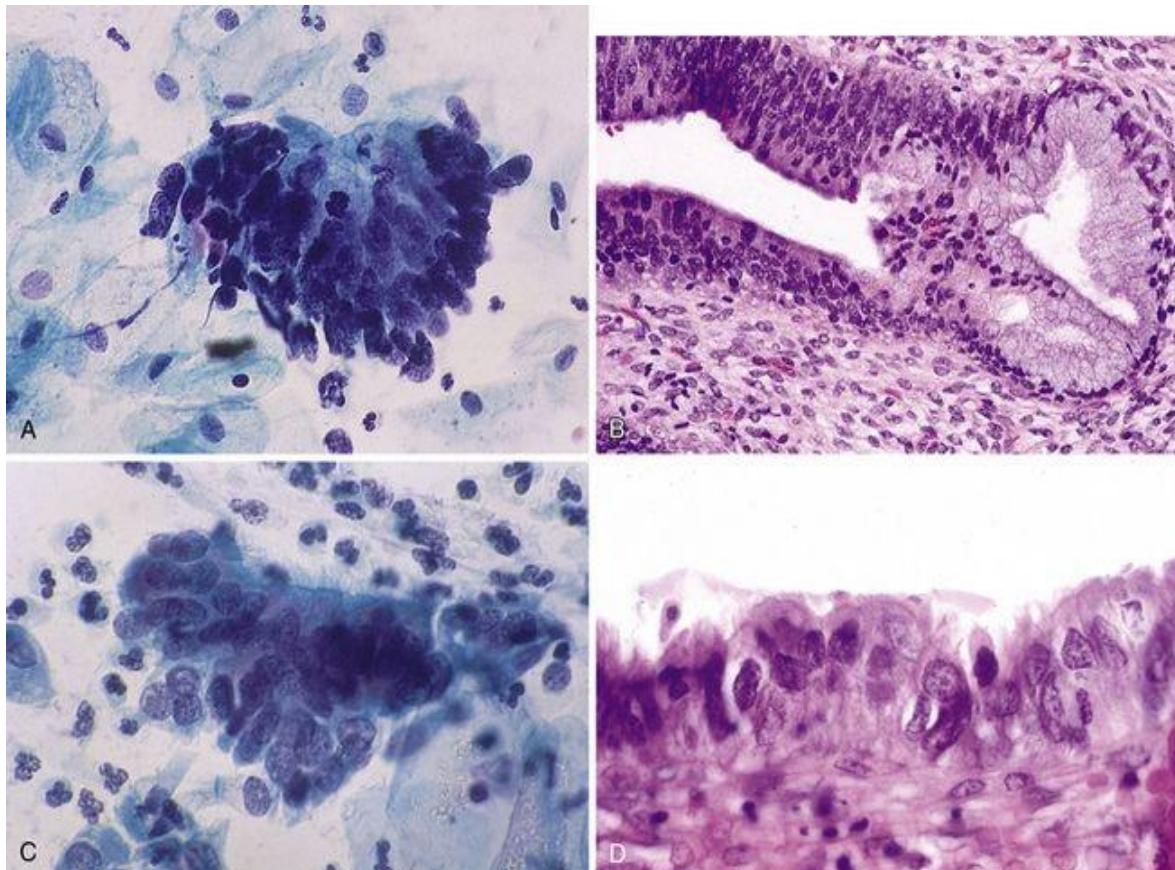


FIGURE 1.57 Adenocarcinoma in situ (AIS) compared to tubal metaplasia.
A, Endocervical AIS. Cells are columnar in shape, dark, crowded, and arranged in a curved strip. B, A cone biopsy revealed AIS. C, Tubal metaplasia. Atypical glandular cells bear a resemblance to those in A, except that cilia are identified. D, Subsequent biopsies showed tubal metaplasia of surface endocervical epithelium.

Reactive endocervical cells and reparative epithelia show a greater range of nuclear size and less hyperchromasia than AIS, in which cells generally have strikingly uniform dark nuclei. The nuclei of reactive endocervical cells typically have prominent nucleoli, a feature seen in only a very small proportion of AIS cases.

AIS can resemble HSIL almost to perfection (see Fig. 1.55). Both are characterized by hyperchromatic crowded groups, mitoses, apoptosis, and coarse chromatin. The cells of HSIL, like those of AIS, can have pale and/or foamy cytoplasm. The diagnosis of AIS should be reserved for cases with clear

columnar glandular differentiation: strips of columnar cells, rosettes, and feathering. If AIS is suspected, cell block preparations from a residual liquid-based sample can help by providing a “histologic” look at hyperchromatic cell clusters⁴⁰ (see Fig. 1.43A and B). Immunohistochemistry for p16 helps to identify HPV-related neoplastic cells;¹⁷² p63, which highlights squamous but not glandular lesions, helps distinguish between them.¹⁷³

The distinction between AIS and adenocarcinoma is problematic. Some cases of adenocarcinoma are clearly invasive because the cells are large, with abundant cytoplasm and prominent nucleoli, and a tumor diathesis is present. These features are absent in some cases of adenocarcinoma, however, resulting in significant morphologic overlap between AIS and cervical adenocarcinoma. Thus, the cytologic diagnosis of AIS does not exclude invasive adenocarcinoma; histologic evaluation is necessary for a definite distinction. Even the histologic distinction between AIS and adenocarcinoma is often a difficult judgment based in part on whether the lesion extends below the normal location of endocervical glands.

Management. A woman with a cytologic diagnosis of AIS should undergo colposcopic examination with endocervical sampling.²⁵ For women older than 35 years and younger women with certain clinical indications such as unexplained vaginal bleeding, endometrial sampling should be included. If there is no evidence of invasive disease, she should have a diagnostic excision procedure, one that yields an intact specimen with interpretable margins.

Adenocarcinoma

Adenocarcinomas of the endocervix, endometrium, vagina, and even the ovaries and fallopian tubes are sometimes detected with the Pap test. There is significant overlap in their morphologic features, so that a precise site of origin often cannot be established. Additional testing (imaging studies, histologic sampling) is usually required for definitive classification and treatment.

Endocervical Adenocarcinoma

Adenocarcinoma of the endocervix represents approximately 15% of cervical cancers in the United States.¹⁸⁰ Some patients complain of bleeding or vaginal discharge, but others are asymptomatic. As with cervical squamous cell carcinomas, HPV is commonly present in endocervical adenocarcinomas. HPV 16 accounts for about 40% and HPV 18 for an additional 30%.²⁰⁸ There are many

histologic subtypes, which include but are not limited to mucinous (the most common, and subdivided into endocervical, intestinal, signet ring cell, minimal deviation, and villoglandular variants), endometrioid, adenosquamous, clear cell, serous, and mesonephric types.¹⁰² The myriad subtypes make cytologic recognition particularly challenging. Some cases of invasive endocervical adenocarcinoma are cytologically indistinguishable from AIS, but in many cases the diagnosis of an invasive adenocarcinoma can be made or at least suggested: 93% of endocervical adenocarcinomas have either suspicious or positive cytology.²⁰⁹



Cytomorphology of endocervical adenocarcinoma

- tumor diathesis (half or fewer of cases)
- large, round nucleus
- prominent nucleolus
- abundant cytoplasm

The cells of *mucinous endocervical adenocarcinomas* are often arranged in sheets. Well-differentiated endocervical mucinous adenocarcinomas are composed of columnar cells with abundant, foamy cytoplasm and a basally located nucleus ([Fig. 1.58A and B](#)). They are sometimes difficult to distinguish from reactive endocervical cells ([Fig. 1.59A-D](#)). Nuclei are pale or hyperchromatic, and mitoses are sometimes present. In moderately and poorly differentiated tumors there is greater variation in nuclear size and shape, and nucleoli are prominent ([Fig. 1.60](#)). A tumor diathesis is present in only about one half of cases²¹⁰ (see [Fig. 1.58A and B](#)), which contributes to the difficulty in distinguishing AIS from invasive adenocarcinoma.

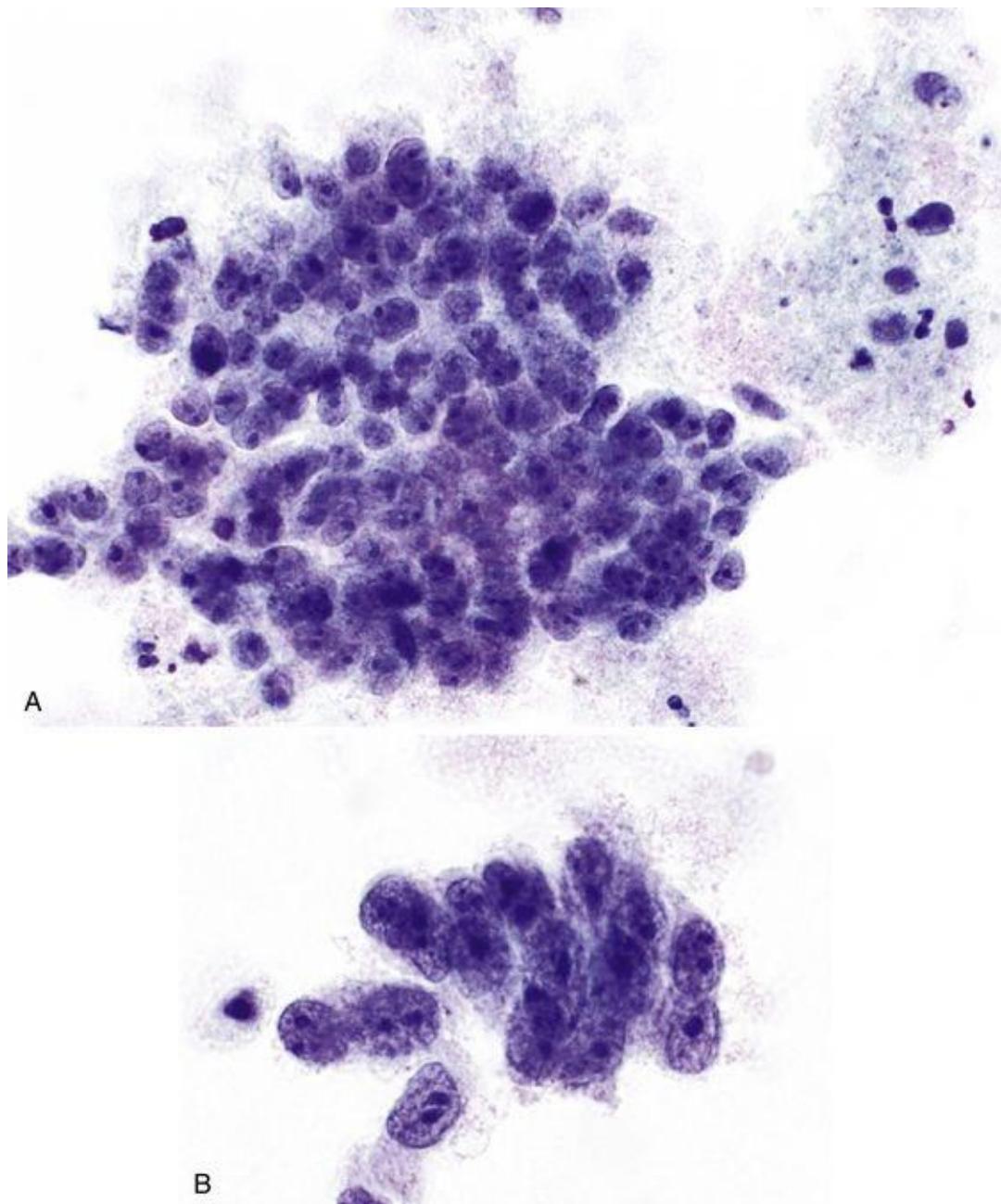


FIGURE 1.58 Endocervical adenocarcinoma.

A, The cells are round rather than elongated as in adenocarcinoma in situ (AIS). They are crowded and hyperchromatic, and a tumor diathesis is present. Tumor diathesis on liquid-based preparations appears as clumps and as a granular ring around groups of malignant cells (“clinging diathesis”). B, High magnification reveals nuclear crowding and very large nucleoli.

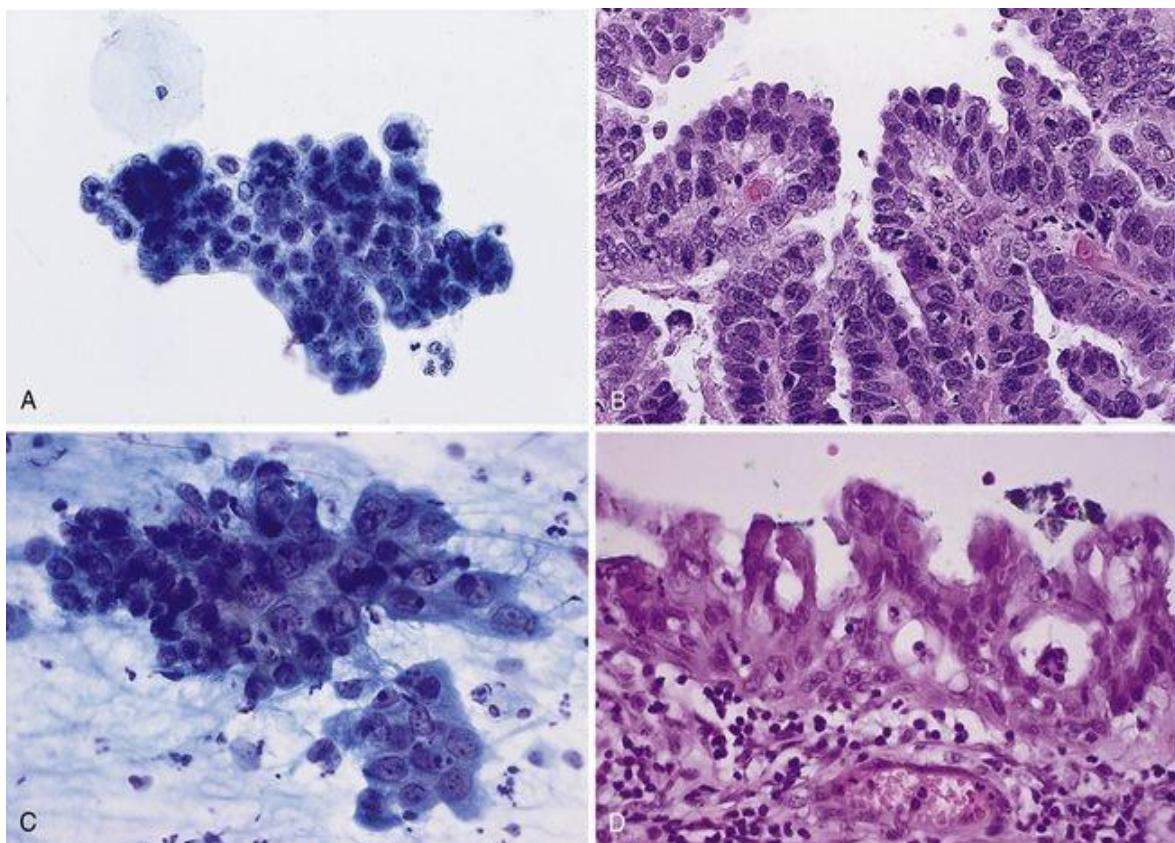


FIGURE 1.59 Endocervical adenocarcinoma compared with reactive endocervical cells. *A*, Endocervical adenocarcinoma, well-differentiated. The cells are enlarged and crowded, but the features are not conclusive for malignancy (note the absence of tumor diathesis). A diagnosis of atypical glandular cells (AGCs) was made. *B*, Histologic sections showed adenocarcinoma. *C*, Reactive endocervical cells. These cells appear similar to those in *A*. *D*, Biopsy in this patient confirmed reactive changes due to inflammation.

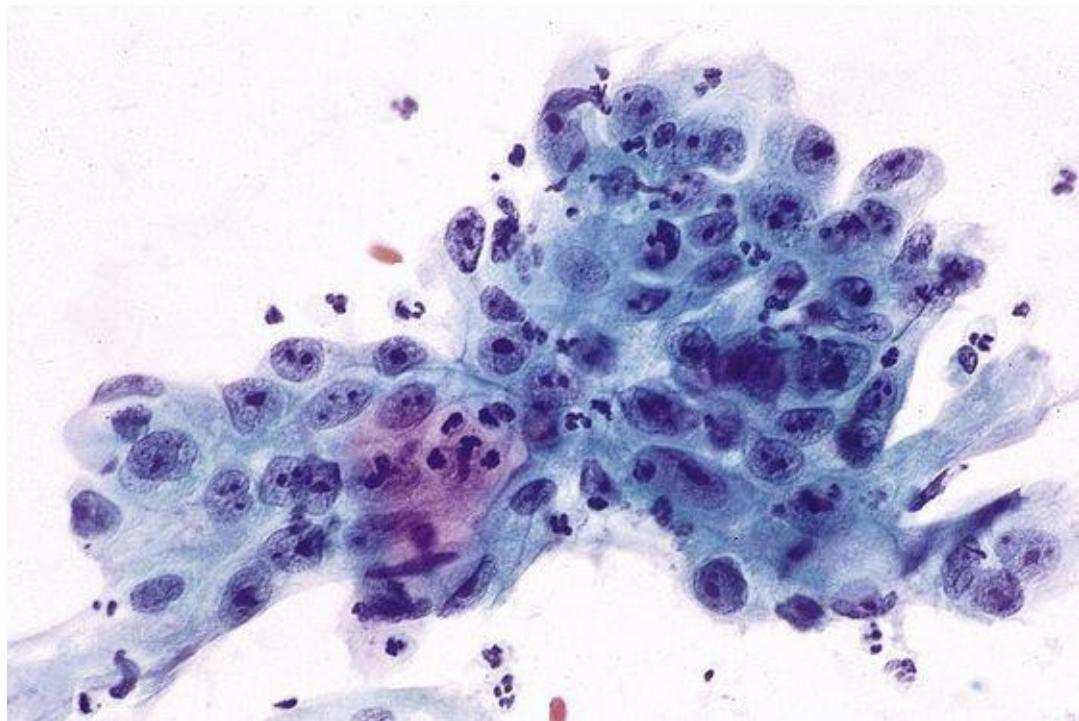


FIGURE 1.60 Endocervical adenocarcinoma.

These malignant cells show variation in nuclear size, with very prominent nucleoli and coarsely granular chromatin.

Adenosquamous carcinoma is composed of malignant squamous and glandular cells arranged in sheets of large pleomorphic cells with abundant dense cytoplasm and prominent macronucleoli. *Clear cell carcinomas* of the endocervix and the vagina are morphologically identical. Both are composed of round cells with pale nuclei, prominent nucleoli, and abundant foamy or finely granular cytoplasm.

The rare, extremely well-differentiated tumor known as *minimal deviation adenocarcinoma* (or adenoma malignum) is composed of mucinous glands that show little if any atypia, and yet, if untreated, invade deeply and metastasize. Patients sometimes present with vaginal discharge. In most cases, the neoplastic cells on the Pap test look like entirely normal endocervical cells²¹¹ ([Fig. 1.61A](#)). Frequently, even cervical biopsy specimens and endocervical curettings are misinterpreted as benign. A correct diagnosis often requires at least a cone biopsy to appreciate the invasive nature of the lesion ([Fig. 1.61B](#)).

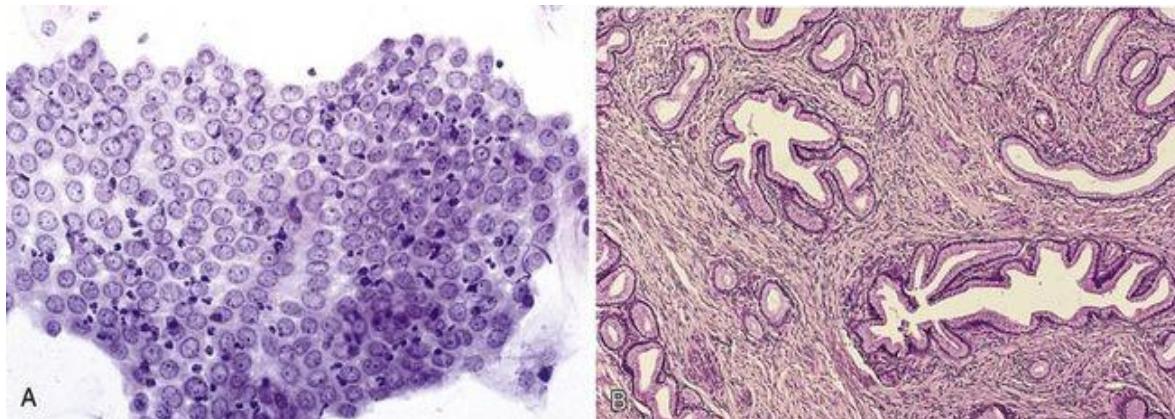


FIGURE 1.61 Minimal deviation adenocarcinoma.

A, The cells are sometimes impossible to distinguish from normal endocervical cells, as in this case. B, A cone biopsy revealed deeply invasive, misshapen neoplastic glands.

Villoglandular adenocarcinomas are rare low-grade neoplasms that rarely if ever metastasize. Cytologically, they resemble AIS in that the cells appear uniform and crowded, with mild to moderate atypia. As in AIS, strips and rosettes are seen,¹⁴⁹ and there is no tumor diathesis.²¹² Very few are diagnosed prospectively as an adenocarcinoma by cytology. Most are reported as benign or as “atypical glandular cells.”²¹²

The cytologic features of the rare *mucoepidermoid carcinoma* and *adenoid cystic carcinoma* of the cervix are similar to their counterparts in the salivary gland and elsewhere (see [Figs. 11.17, 11.18, 11.20-11.22](#)).

Adenocarcinomas of the cervix are treated in a similar manner to that for squamous cancers.¹⁸⁶

Endometrial Adenocarcinoma

Endometrial adenocarcinoma is predominantly a tumor of postmenopausal women, with a peak incidence in women in their late 50s and early 60s; it is rare in women younger than 40. About 90% manifest with postmenopausal bleeding, but some are asymptomatic. Most endometrial adenocarcinomas are of the endometrioid type. Less common types include serous and clear cell adenocarcinomas, which manifest at a more advanced stage and carry a worse prognosis. The mucinous type of endometrial carcinoma, by contrast, behaves like the endometrioid type.

The Pap test is mainly a screening test for cervical lesions and is not intended for the detection of endometrial lesions.⁹⁷ Nevertheless, the Pap test does fortuitously pick up cells from many endometrial cancers. The cells that

exfoliate from high-grade endometrial adenocarcinomas, particularly those of papillary serous or clear cell type, are obviously malignant, and such cases can be and are reported as adenocarcinomas (or, if there is doubt, interpreted as “atypical endometrial cells”). Cervical Pap cytology is atypical, suspicious, or positive for malignancy in 38% to 90% of endometrial adenocarcinomas.^{213,214}



Cytomorphology of endometrial adenocarcinoma

- round cells
- enlarged nucleus
- hyperchromatic
- prominent nucleolus
- scant or abundant vacuolated cytoplasm
- cytoplasmic neutrophils (“bags of polys”)

In many cases of the endometrioid type of endometrial cancer, the malignant cells are not at all numerous, and only about one third of cases contain a tumor diathesis.²¹⁵ The malignant cells are round, isolated or in groups, and larger and more vacuolated than benign endometrial cells (Fig. 1.62A). Histiocytes frequently accompany the atypical cells and in some cases outnumber them.

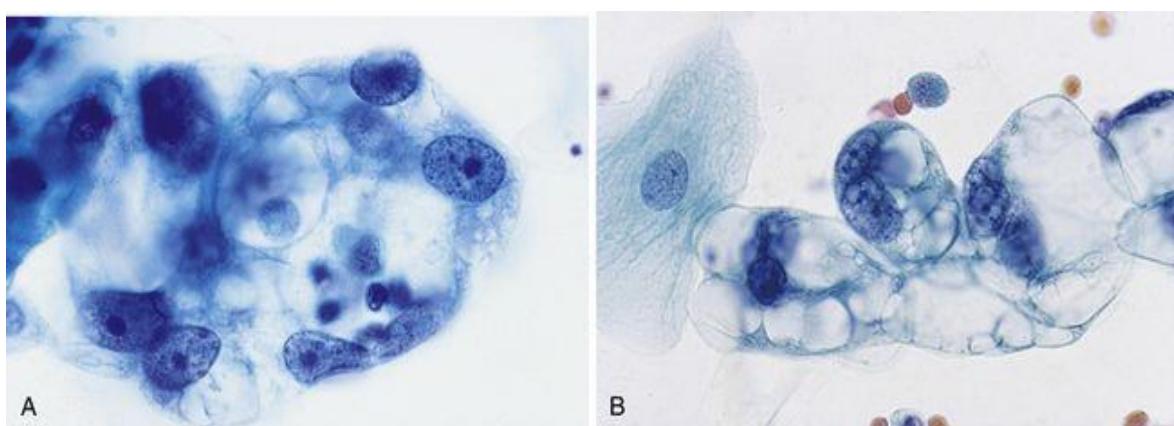


FIGURE 1.62 Endometrial adenocarcinoma compared with intrauterine device (IUD) effect. A, Endometrial adenocarcinoma, endometrioid type. These malignant cells are large, vacuolated, and associated with neutrophils. B, IUD effect. Benign cells in women with an IUD are indistinguishable morphologically from those of endometrial adenocarcinomas.

Cells from serous adenocarcinoma of the endometrium are typically very large, pleomorphic, and easily recognized as malignant. Numerous bare nuclei in a necrotic background are characteristic. Compared with smears from the endometrioid type, smears from papillary serous adenocarcinomas contain more malignant cells.²¹⁵ Psammoma bodies are present in only 25% of cases.²¹⁵

Pap slides are more likely to contain malignant cells in patients with a serous rather than an endometrioid type of endometrial adenocarcinoma.^{216,217}

Differential Diagnosis of Adenocarcinoma

Because there is significant morphologic overlap among adenocarcinomas of the cervix, endometrium, and other sites, they are considered together.



Differential diagnosis of adenocarcinoma

- endocervical adenocarcinoma
- endometrial adenocarcinoma
- adenocarcinoma of other sites
 - vaginal
 - ovarian
 - tubal
 - metastatic
- squamous cell carcinoma
- IUD effect
- endocervical polyp atypia
- reactive endocervical cells
- adenocarcinoma in situ
- pemphigus vulgaris

When adenocarcinoma cells are identified on a Pap slide, the two principal suspects are endocervical and endometrial adenocarcinoma. The age of the patient is helpful: The older the patient, the more likely it is that the tumor has arisen in the endometrium. Morphologic features are also helpful. Endometrial adenocarcinoma cells are rounder and tend to exfoliate as isolated cells and smaller clusters, often arranged as spheres, whereas the cells of endocervical adenocarcinomas are more columnar and more commonly are shed as sheets of cells. Histiocytes commonly accompany endometrial carcinomas and not endocervical carcinomas. Ultimately, the cytologist can usually only suggest the

possibilities, favoring one site over another. The final classification rests on histologic examination.

Adenocarcinoma of the vagina is very rare and often associated with a maternal history of DES use during pregnancy.

Adenocarcinomas from the ovaries and fallopian tubes are more commonly associated with psammoma bodies,²¹⁸ but this finding is not entirely reliable, because endocervical and endometrial cancers sometimes contain them as well.

Nonkeratinizing squamous cell carcinomas resemble endocervical adenocarcinomas. Unless focal keratinization is identified, a definite distinction is not possible. The cells of IUD effect are indistinguishable from those of endometrial adenocarcinoma ([Fig. 1.62B](#)). If the woman has an IUD, it is likely that they represent IUD effect rather than an adenocarcinoma.

Enlarged, vacuolated cells with engulfed neutrophils (“bags of polys”) are seen with inflamed endocervical polyps and represent reactive endocervical cells ([Fig. 1.63](#)), yet they mimic a similar cell that is characteristic of endometrial carcinoma. Morphologic distinction can be impossible, and knowledge that the patient has an endocervical polyp may be the only clue to correct interpretation.

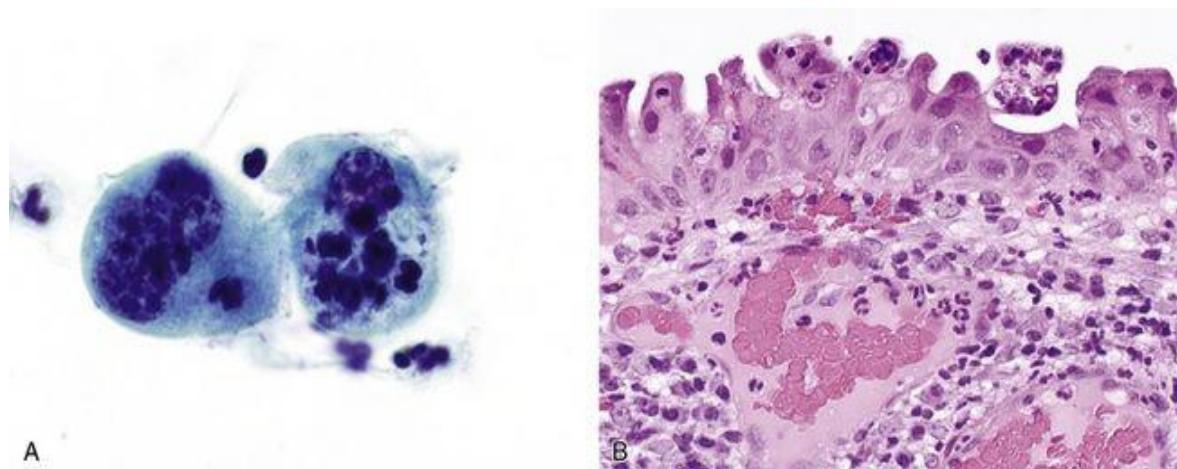


FIGURE 1.63 Inflamed endocervical polyp mimicking endometrial adenocarcinoma. *A*, The large vacuolated cells are associated with neutrophils, just like the cells of endometrial adenocarcinoma. *B*, Histologic sections reveal an acutely inflamed polyp lined by reactive endocervical cells infiltrated by polys.

Reactive endocervical cells and atypical repair (see [Figs. 1.52](#) and [1.59C](#)) mimic adenocarcinomas and vice versa.⁸³ Reactive cells, paradoxically, often show more marked variation in nuclear size and nucleolar size and shape than adenocarcinomas, which are often deceptively uniform.⁸³ Reactive cells have thin

nuclear membranes compared with those of adenocarcinomas, which are often thick and sometimes irregular in contour. Reactive cells form sheets but rarely balls of cells, as is seen with many adenocarcinomas. There are cases, however, where doubt remains; these are diagnosed as “atypical glandular cells.”

The distinction from AIS is problematic and not possible in many cases. If a tumor diathesis is present or the cells are round and have prominent nucleoli, the tumor is more than likely an invasive adenocarcinoma.

Pemphigus vulgaris is a rare blistering disorder that involves mucous membranes, including the cervix. The squamous cells of the cervix lose their squamous morphology and take on a pseudoglandular appearance, with a pale nucleus and prominent nucleolus. The features resemble those of repair except that isolated cells are prominent.²¹⁹

Atypical Glandular Cells

The category AGC is reserved for cases in which the cellular changes fall between those of a definite benign reactive process and those of an unequivocal AIS or adenocarcinoma. It represents 0.2% of all Pap interpretations¹¹⁶ and should be used only when the atypia raises the suspicion of AIS or adenocarcinoma; any case that is recognized as clearly benign should be reported as NILM. These atypical cells are subclassified as “atypical endocervical,” “atypical endometrial,” or “not otherwise specified.”

About 30% of patients with AGCs have a significant lesion. Although some are AIS or invasive adenocarcinoma, many if not most of the lesions turn out to be CINs.^{25,220} This underscores the resemblance of HSIL and AIS. Cell block preparations from the residual LBC sample can be helpful by providing a “histologic” look at the atypical cells.⁴⁰ Similarly, immunohistochemistry for p63, which highlights squamous but not glandular lesions, can be helpful in selected AGC cases.¹⁷³

Atypical Endocervical Cells

This category includes cases in which an endocervical cell atypia raises the possibility of endocervical AIS or adenocarcinoma, but a benign endocervical reaction such as atypical endocervical repair, pregnancy-related change (e.g., Arias-Stella), endocervical polyp atypia, and microglandular hyperplasia¹⁴² cannot be excluded (see Fig. 1.59C and D). Depending on the severity of the atypia, one can leave the interpretation of “atypical endocervical cells”

unqualified (“not otherwise specified”), or qualify it as “favor neoplastic” if a neoplasm is strongly favored.



Differential diagnosis of atypical endocervical cells

- reactive endocervical cells
- ASC-H
- HSIL

The differential diagnosis of atypical endocervical cells includes reactive endocervical cells and squamous lesions. If endocervical cells have enlarged nuclei, but the nuclei are round and regular in contour, with finely textured chromatin and prominent nucleoli, they are most likely reactive (see [Fig. 1.27B](#)). To qualify as atypical, endocervical cell nuclei should raise the suspicion of AIS, meaning they should be elongated, hyperchromatic, and crowded, often with an elevated nuclear-to-cytoplasmic ratio. A common error is mistaking squamous lesions, particularly HSILs, for atypical endocervical cells. Many HSILs have transparent and even vacuolated cytoplasm (see [Fig. 1.39](#)). Atypical cells with a rounded contour are more likely to be HSIL than AIS, and for such cases ASC-H is a more appropriate interpretation. The cells of AIS are usually recognizably columnar. For this reason, “atypical endocervical cells” should be reserved for cells with a recognizably columnar morphology. If AIS is suspected, cell block preparations from a residual liquid-based sample can help by providing a “histologic” look at hyperchromatic cell clusters⁴⁰ (see [Fig. 1.43A and B](#)). Immunohistochemistry for p16 helps to identify HPV-related neoplastic cells;¹⁷² p63, which highlights squamous but not glandular lesions, helps distinguish between them.¹⁷³

Management. Because of the high incidence of significant lesions in women with atypical endocervical cells, colposcopy with endocervical sampling is recommended regardless of HPV result.²⁵ For women older than 35 years and younger women with unexplained vaginal bleeding, endometrial sampling should be included. If, after colposcopy, CIN 2+ is not identified for atypical endocervical cells (unqualified), cotesting at 12 and 24 months is recommended, and return to repeat cotesting in 3 years is recommended if both cotests are negative. (If any test is abnormal, however, colposcopy is recommended.) If the Pap diagnosis was qualified as “favor neoplasia” and invasive disease is not identified during the initial colposcopic workup, a diagnostic excisional

procedure is recommended.

Atypical Endometrial Cells

Atypical endometrial cells are isolated cells or rounded clusters of cells with an enlarged nucleus and one or more additional features of nuclear atypia (e.g., membrane irregularity, prominence of nucleoli). Cytoplasm is scant or moderately abundant and vacuolated. Such cells are suspicious for endometrial adenocarcinoma, but the quantity of the altered cells or the mild degree of atypia prevents a conclusive for malignancy ([Fig. 1.64](#)). Similar changes are known to be caused by endometrial polyps, chronic endometritis, IUDs, and endometrial hyperplasia.

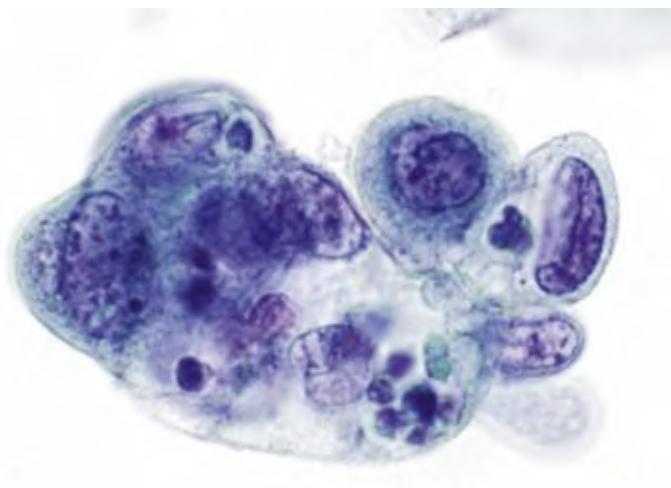


FIGURE 1.64 Atypical endometrial cells.

These cells have enlarged nuclei with slightly irregular contours and some infiltration by neutrophils.

Management. Because atypical endometrial cells carried a significant risk of cancer,²¹ endometrial and endocervical sampling is recommended.²⁵ If no endometrial pathology is identified, colposcopy is recommended.

Other Malignant Neoplasms

Small Cell Carcinoma

Tumors that resemble small cell carcinomas of the lung arise in the uterine cervix. Some have concomitant evidence of squamous cell differentiation and are considered variants of poorly differentiated squamous cell carcinoma. True small cell carcinomas, however, are a distinct entity, commonly associated with HPV type 18.^{[22](#)} They are highly aggressive, with a predilection for the early development of distant metastases.



Cytomorphology of small cell carcinoma

- clusters of small cells
- hyperchromatic nucleus
- nuclear molding
- scant cytoplasm
- mitoses
- nuclear smearing

Cytologic preparations show clusters of small cells with hyperchromatic nuclei and finely granular chromatin. Cytoplasm is scant. Nuclear molding is present (see [Fig. 1.13D](#)), as are mitotic figures. Like their counterparts in the lung, these cells are fragile and show nuclear smearing. Often poorly preserved, the cells are easily confused with menstrual endometrial cells. Nuclear smearing and mitoses, however, are very uncommon with endometrial cells and provide a good clue to the diagnosis of a small cell carcinoma.

Malignant Melanoma

Although more common in the vulva, melanomas can arise in the vagina and, even less frequently, the cervix. Vaginal melanomas occur predominantly in the elderly and are aggressive tumors.



Cytomorphology of melanoma

- isolated cells
- large cells, epithelioid or spindled
- round or oval nucleus
- melanin (not all cases)

The malignant cells are often isolated rather than clustered, and this pattern is helpful in distinguishing them from the more common carcinomas ([Fig. 1.65](#)). Tumor cells are large, with a round or oval nucleus and often have a very prominent single nucleolus. Cytoplasm is scant or abundant and in some cases demonstrates the tell-tale fine, brown granularity of melanin. Melanophages—histiocytes with abundant coarse ingested pigment—may be present.

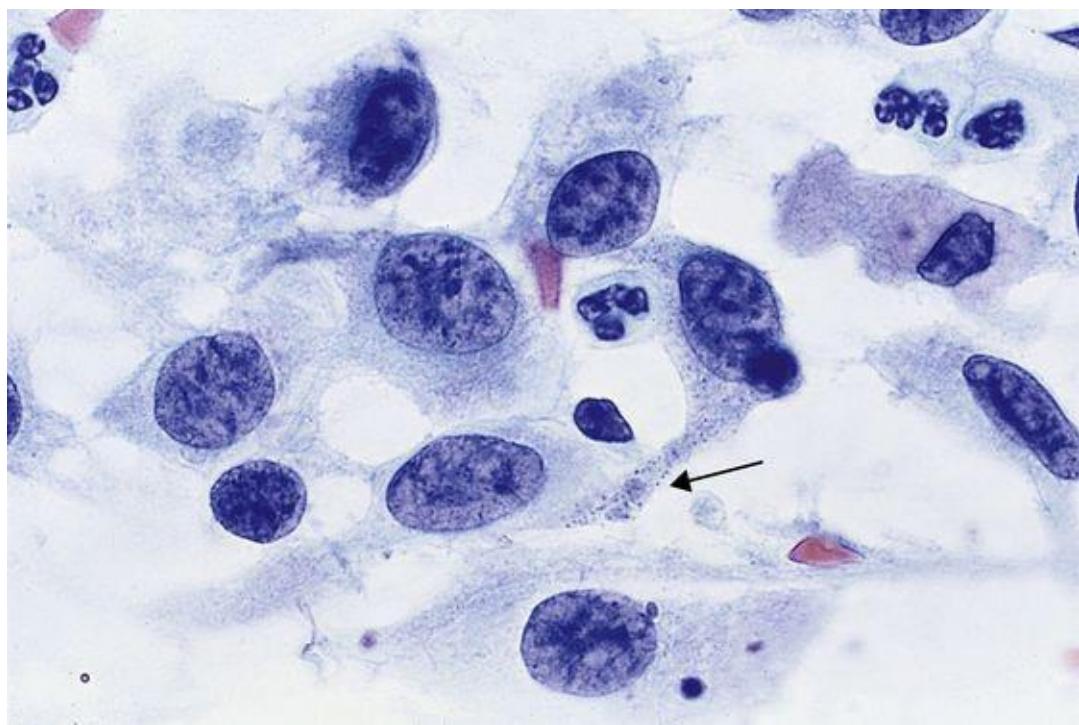


FIGURE 1.65 Malignant melanoma of the vagina.

The malignant spindled and epithelioid cells are noncohesive. There is focal finely granular melanin pigment (arrow).

Malignant Lymphoma

Non-Hodgkin lymphoma frequently involves the cervix and vagina when the disease is advanced. Rarely, it may arise as a primary tumor at these sites.²²³ Cytologic samples are negative if the mucosa is not ulcerated. The tumor cells are larger than small, mature lymphocytes, with a nucleus that is irregular in contour and coarsely granular. The differential diagnosis includes follicular cervicitis (see Fig. 1.16A and B), which is composed of a mixed population of lymphocytes in various stages of maturation, in contrast with many lymphomas, which are composed of a uniform population of atypical lymphoid cells.

Malignant Mixed Mesodermal Tumors

Malignant mixed mesodermal tumors arise much more commonly in the endometrium than in the cervix. Many cases in which the cervix is involved represent extension from an endometrial primary. As with endometrial carcinoma, the most common symptom is vaginal bleeding. The tumors are composed of malignant glands admixed with malignant spindle cells; the latter may show features of stromal sarcoma, leiomyosarcoma, rhabdomyosarcoma, chondrosarcoma, or liposarcoma. Much of the tumor is made up of undifferentiated cells. Smears are often highly cellular and contain malignant glandular or undifferentiated cells with scant cytoplasm. Malignant spindle cells may be present, but are usually a minor component of the specimen.

Metastatic Tumors

Tumors from many sites can metastasize to the cervix or vagina and be detected on smears. Perhaps the most common are stage III or IV adenocarcinomas of the ovary and fallopian tube, which make their way to the cervix and vagina via the endometrial cavity.²⁰⁹ These tumors are most commonly serous in type and resemble the serous carcinoma of the endometrium described previously. Tumors of the ovary and fallopian tube identified on cervical or vaginal smears may give a clean background if large, necrotic tumor implants have not been formed in the cervix.

Psammoma bodies are small, concentrically laminated calcifications that stain dark blue with the Papanicolaou stain. They are commonly seen in some tumors of the ovary, fallopian tube, endometrium, and peritoneum, but are extremely rare in routine cervical or vaginal smears.²¹⁸ Their presence should prompt a search for a neoplasm, especially if they are associated with atypical cells²¹⁸ (Fig.

[1.66\).](#)

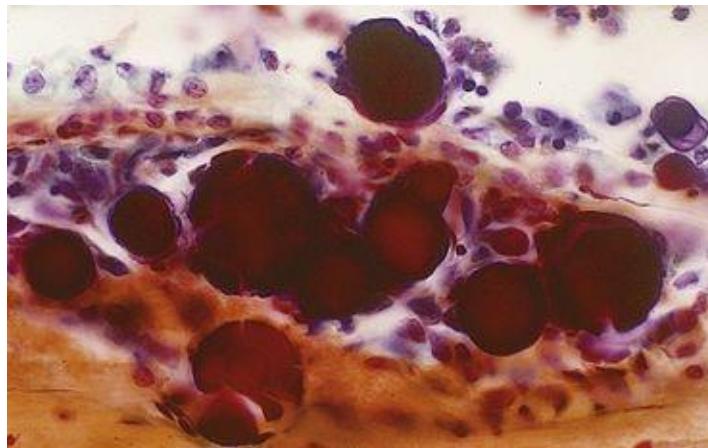


FIGURE 1.66 Psammoma bodies.

These calcific spheres are dark blue or purple and have concentric laminations. They are often fractured, as seen here. These psammoma bodies originated from a borderline serous tumor of the ovary. When cells from ovarian or tubal neoplasms travel through the endometrial cavity, they can be seen on cervical or vaginal Pap samples.

Carcinomas of the colon and rectum can spread directly to the vagina. Tumor cells frequently have a columnar shape with large, very hyperchromatic nuclei and a high nuclear-to-cytoplasmic ratio. Isolated cells can have a signet ring cell appearance. Tumor necrosis may be present.

Carcinomas of the bladder and urethra can also spread to the vagina. Tumor cells are large, often with hyperchromatic nuclei. Clinical correlation is needed for determining the site of origin.

Tumors from distant sites like the breast, kidney, pancreas, and lung can metastasize to the female genital tract. In general, precisely identifying the primary site is impossible without the clinical history and previous biopsy material for comparison.

Endometrial Cells in Women Older than 40 Years of Age

Although the Pap test is not employed as a screening test for endometrial cancer, it has been known for decades that benign-appearing endometrial cells in an older woman may be a sign of endometrial cancer. Studies, some of them dating back to the 1970s, have shown that 6% of women with benign-appearing endometrial cells have endometrial carcinoma, and 12% have hyperplasia ([Table 1.5](#)). Most of these women come to medical attention because of vaginal bleeding, but 10% to 25% are asymptomatic^{224,225} ([Table 1.6](#)). It is not known whether the exfoliated endometrial cells are even neoplastic, or whether they represent just glandular and stromal breakdown associated with the neoplasm.

TABLE 1.5
METAANALYSIS OF BENIGN-APPEARING ENDOMETRIAL CELLS IN POSTMENOPAUSAL WOMEN: PREDICTIVE VALUE FOR ENDOMETRIAL HYPERPLASIA AND CARCINOMA (DATA PRE-2001)

Study	Definition of Postmenopausal	No. of Patients	Biopsy Findings		
			Hyperplasia (%)	Cancer (%)	Hyperplasia or Cancer (%)
Cherkis et al., 1988 ²²⁴	≥40	179	23	20	43
Gomez-Fernandez et al., 1999 ²³⁷	Unknown	84	6	6	12
Gondos and King, 1977 ¹²¹	≥40	147	23	2	25
Ng et al., 1974 ²²⁷	≥40	501	52	23	75
Sarode et al., 2001 ²³⁸	>55	81	4	4	8
Yancey et al., 1990 ¹²²	Unknown	74	9	0	9
Zucker et al., 1985 ²²⁵	Unknown	23	10	6	16
TOTAL		1089	127 (12%)	61 (6%)	188 (17%)

TABLE 1.6
HISTORY OF BLEEDING IN POSTMENOPAUSAL WOMEN WITH ENDOMETRIAL CELLS AND BIOPSY-PROVEN ENDOMETRIAL CANCER

Study	No. of Patients	No. with History of Bleeding (%)	
		YES	NO
Cherkis et al., 1988 ²²⁴	20	15	5 (25%)
Zucker et al., 1985 ²²⁵	18	16	2 (11%)
Gomez-Fernandez et al, 1999 ²³⁷	6	6	0 (0%)
TOTAL	44	37	7 (16%)

Because of the associated risk, the 1991 Bethesda System recommended that benign-appearing endometrial cells in postmenopausal women be reported as an epithelial cell abnormality. The recommended terminology was “endometrial cells, cytologically benign, in a postmenopausal woman.” This presented an unanticipated difficulty, however, because menopausal history was not always provided. If the menopausal status was not given, could this diagnosis be made based on age? If so, how old should a woman be for this interpretation to apply? The median age of final menstrual period is 51 years, but the coefficient of variation is large.²²⁶

In the 2001 revision of the Bethesda System, the diagnosis is recommended for all women aged 40 and older, irrespective of menstrual status.⁵⁰ This threshold was selected to optimize sensitivity, because cases of endometrial carcinoma have been detected in women between the ages of 40 and 50 who have benign-appearing endometrial cells on cytology.²²⁷

Whether the risk applies to postmenopausal women on hormone replacement therapy is not clear. Data are sparse, but some investigators have found that Paps with benign-appearing endometrial cells do identify a small number of asymptomatic women on hormone replacement therapy with endometrial adenocarcinoma and hyperplasia.²²⁸

It was once believed that histiocytes alone convey an increased risk for endometrial cancer.²²⁹ This has been widely refuted.^{225,230,231} Thus, only spontaneously exfoliated endometrial cells (Fig. 1.67) are considered significant. Directly abraded endometrium or LUS, like histiocytes, should not be reported under this heading.

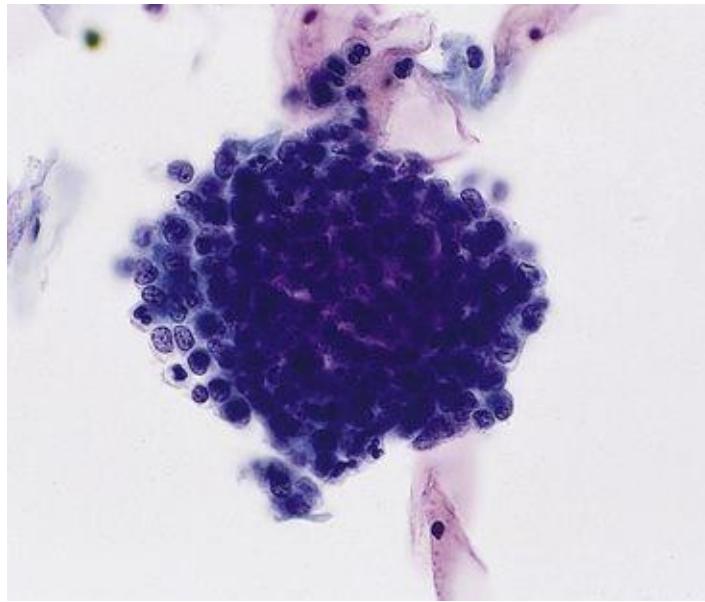


FIGURE 1.67 Endometrial cells in a woman older than 40 years of age. These cells are indistinguishable from menstrual endometrial cells (see [Fig. 1.12](#)).

An educational note can be particularly helpful, as in this example:



Sample report

Satisfactory for evaluation.

Endometrial cells, cytologically benign, in a woman greater than or equal to 40 years of age.

Negative for squamous intraepithelial lesion.

Note: Endometrial cells after age 40, particularly out of phase or after menopause, may be associated with benign endometrium, hormonal alterations, and less commonly, endometrial abnormalities. Suggest clinical correlation.

In the 2001 Bethesda System, this interpretation is no longer categorized as an epithelial cell abnormality, and because of the small but definite risk of a significant endometrial lesion, neither is it categorized as NILM. This orphan diagnosis, therefore, falls into the general categorization “Other,” a heading some laboratories simply omit from the report, as in the foregoing example. Because the primary goal of the Pap test is the identification of squamous precursors, the explicit statement “negative for squamous intraepithelial lesion”

is included.

This Pap interpretation represents 0.5% to 1% of all Pap reports.^{232–235} The associated risks of endometrial hyperplasia and cancer since the implementation of the 2001 Bethesda System are shown in [Table 1.7](#).

TABLE 1.7

METAANALYSIS OF BENIGN-APPEARING ENDOMETRIAL CELLS IN WOMEN OVER 40: PREDICTIVE VALUE FOR ENDOMETRIAL HYPERPLASIA AND CARCINOMA (DATA POST 2001)

Study	No. of Patients	Biopsy Findings		
		No. with Hyperplasia (%)	No. with Cancer (%)	No. with Hyperplasia or Cancer (%)
Browne et al., 2005 ²³⁵	211	1 (0.5)	6 (2.8)*	7 (3.3)
Thrall et al., 2005 ²³²	159	9 (5.7)	0	9 (5.7)
Bean et al., 2006 ²³⁴	140	2 (1.4)	0	2 (1.4)
Kapali et al., 2007 ²³³	499	4 (0.8)	4 (0.8)	8 (1.6)
TOTAL	1099	16 (1.4)	10 (0.9)	26 (2.4)

*Two women with cancer were premenopausal and asymptomatic.



Differential diagnosis of endometrial cells in women over 40

- crushed endocervical cells
- follicular cervicitis
- small blue nuclei

A common mimic of endometrial cells in older women is the cluster of crushed, atrophic endocervical cells. They are recognized on the basis of some residual columnar shape. The lymphoid cell clusters of follicular cervicitis (see [Fig. 1.16A and B](#)) are another mimic. Lymphoid cells are about the same size as or smaller than exfoliated endometrial cells and less tightly cohesive. Admixed larger, paler dendritic cell nuclei and tingible-body macrophages are typical of follicular cervicitis. Clusters of naked squamous cell nuclei ([Fig. 1.68](#)) are easily mistaken for endometrial cells but can be identified because they have no cytoplasm. Naked squamous cell nuclei (often called *small blue cells*) are common in postmenopausal women and thus are a frequent mimic of endometrial cells. They are seen in 21% of Paps from women over the age of 50, and their prevalence is proportional to patient age.²³⁶ At one time their presence was associated with tamoxifen, a nonsteroidal antiestrogen used in the treatment

and prevention of breast cancer, but the frequency of small blue nuclei is no higher in these patients than in women who are not taking tamoxifen.^{[236](#)}

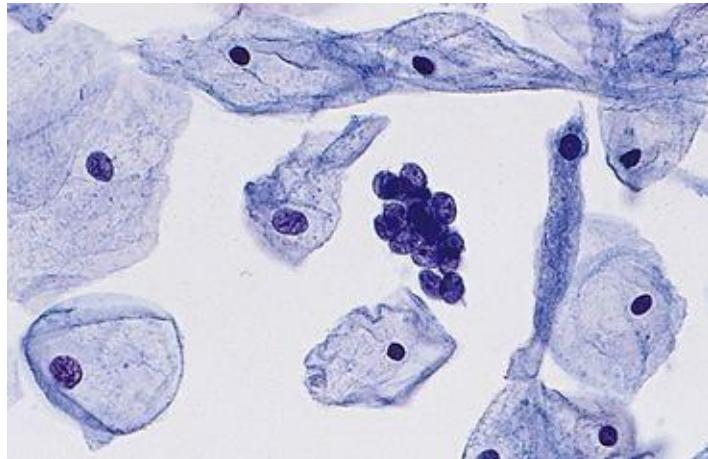


FIGURE 1.68 Bare squamous cell nuclei.

They are about the size of endometrial cells and sometimes aggregate. Cells that lack cytoplasm should not be interpreted as endometrial cells.

Management. Benign-appearing endometrial cells in women over 40 are usually not from a cancer or hyperplasia (see [Tables 1.5](#) and [1.7](#)). In most women, they are physiologic (the woman is still cycling, either naturally or because of hormone replacement therapy), or a result of benign endometrial pathology (e.g., an endometrial polyp). For this reason, an endometrial sample is not indicated for all women with this diagnosis. The woman's physician, who knows her menstrual and menopausal status, clinical risk factors for endometrial cancer, and whether or not she is on hormone replacement therapy, should use his or her clinical judgment in deciding whether or not to take a histologic endometrial sample. Consensus guidelines recommend endometrial assessment for women who are postmenopausal.^{[25](#)}

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CHAPTER 2

Respiratory Tract and Mediastinum

Christopher A. French

[Normal Anatomy, Histology, and Cytology of the Respiratory Tract Sampling Techniques, Preparation Methods, Reporting Terminology, and Accuracy](#)

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Exfoliative cytology was first used to study cells of the respiratory tract in 1845.¹ The ability to diagnose pulmonary diseases cytologically was appreciated as early as 1919,² but it was not until the 1950s and 60s that pulmonary cytology came into its own as a diagnostic discipline. Its emergence was bolstered by the introduction of direct sampling methods via bronchoscopy and fine-needle aspiration (FNA),³ resulting in an impressive armamentarium of sampling techniques. Since then, the improved sensitivity (and common application) of thoracic imaging has created an ever-increasing need for the cytologic evaluation

of pulmonary lesions.

Normal Anatomy, Histology, and Cytology of the Respiratory Tract

The respiratory tract can be categorized into upper and lower compartments. The upper airway extends from the sinonasal region to the larynx. The lower respiratory tract, which is the major focus of diagnostic respiratory cytopathology, extends from the trachea to the lungs. The tracheobronchial tree divides into progressively smaller units: bronchi, bronchioles, and respiratory acini.



Anatomy and cellular components of the respiratory tract

Upper respiratory tract

- ciliated columnar cells
- squamous cells

Lower respiratory tract

- trachea and bronchi
 - ciliated columnar cells
 - goblet cells
 - basal/reserve cells
 - neuroendocrine cells
- terminal bronchioles
 - nonciliated cuboidal/columnar cells (Clara cells)
- alveoli
 - type I and II pneumocytes
 - alveolar macrophages

The trachea and bronchi are lined by a pseudostratified epithelium. The predominant cell is the *ciliated columnar cell*, which has a basally placed nucleus with finely textured chromatin. The luminal surface has a thick terminal bar with cilia ([Fig. 2.1](#)). *Goblet cells*, present in a ratio of approximately one per six ciliated cells, also have a basally located nucleus, but they lack cilia, and

their cytoplasm is distended by mucus. Goblet cells secrete mucus, whereas ciliated cells move the mucus and entrapped contaminants up the airway. Adjacent to the basement membrane are *basal or reserve cells*: small, undifferentiated cells that are the presumed forerunners of the ciliated and goblet cells. *Neuroendocrine cells*, or Kulchitsky cells, are also present in the respiratory epithelium, but they are identified only with special stains or ultrastructural examination: They are argyrophil-positive and possess dense core granules.

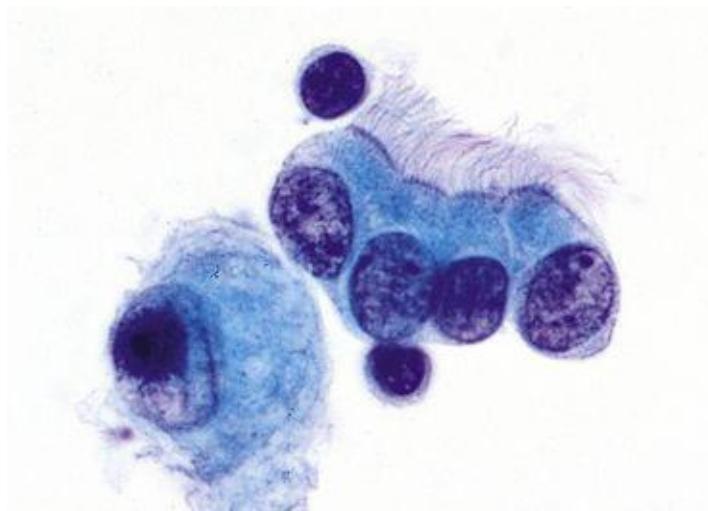


FIG. 2.1 Normal ciliated bronchial cells (bronchial brushing). These columnar cells have oval nuclei and finely stippled chromatin. Numerous cilia project from the apical surface (Papanicolaou stain).

The terminal bronchioles are lined by nonciliated cuboidal to columnar cells called *Clara cells*; they are not sufficiently distinctive with routine cytologic preparations and thus are not specifically identified. The alveolar lining consists of *type I* and *type II pneumocytes*. Type I pneumocytes, which are more numerous, are paper thin and cover the gas exchange portion of the alveolar surface. The type II pneumocyte is more conspicuous: plump and cuboidal rather than flat. It secretes pulmonary surfactant, seen ultrastructurally as osmiophilic lamellar bodies. After lung injury, these cells function as reserve cells for the delicate type I pneumocyte. On cytologic preparations, type II pneumocytes are round and have vacuolated cytoplasm; they can be difficult to distinguish from macrophages.

Alveolar (pulmonary) macrophages vary in appearance depending on the amount and type of phagocytosed cytoplasmic material. In general, they have

one or more round to oval nuclei and lacy or bubbly cytoplasm ([Fig. 2.2](#)). Numerous alveolar macrophages must be present for a sputum sample to be judged adequate. Under normal circumstances, a few white blood cells, such as neutrophils and lymphocytes, are also found within the alveolar compartment. An increased number of inflammatory cells is abnormal: Abundant neutrophils indicate an acute pneumonia, and numerous lymphocytes are usually associated with chronic inflammation.

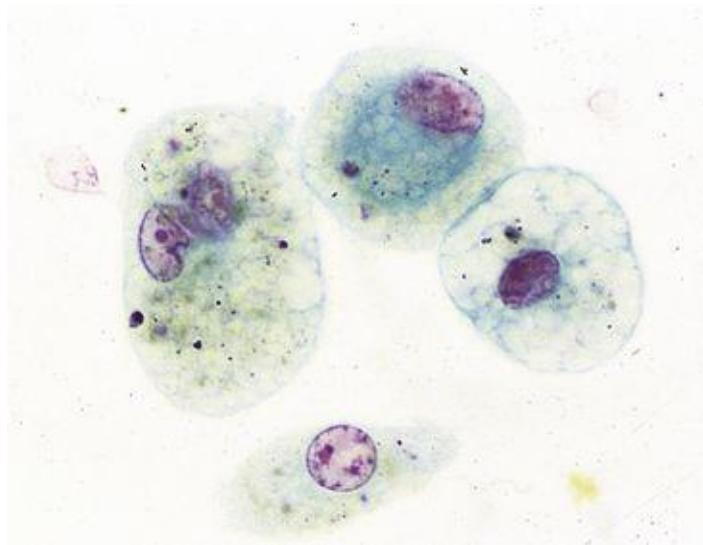


FIG. 2.2 Pulmonary alveolar macrophages (sputum).
Pulmonary macrophages have abundant foamy cytoplasm that often contains black carbon particles, as seen here. Macrophages contain hemosiderin pigment following pulmonary hemorrhage. Hemosiderin is golden brown rather than black (Papanicolaou stain).

Sampling Techniques, Preparation Methods, Reporting Terminology, and Accuracy

Familiarity with the variety of sampling and preparation methods is crucial for cytologic interpretation, because cytomorphology is different depending on the sampling and preparation method. The accuracy of respiratory cytology also varies depending on the specimen type.

As with other nongynecologic cytology specimens, respiratory tract diagnoses are typically reported as “negative for malignant cells,” “positive for malignant cells,” or “nondiagnostic (unsatisfactory),” followed by a descriptive diagnosis. Inconclusive findings are commonly reported as “atypical cells present” (connoting a low degree of suspicion) or “suspicious for malignancy” (connoting a high degree of suspicion). Cancer is confirmed in 40% of “atypical” respiratory specimens and in almost 70% of those reported as “suspicious.”⁴ Atypical/suspicious cases usually remain inconclusive even after careful retrospective reexamination, which fails to reveal any morphologic features to reliably distinguish benign from malignant specimens.⁵

Sputum

Sputum consists of a mixture of cellular and noncellular elements that are cleared by the mucociliary apparatus. It was once the most common respiratory tract specimen because it is relatively easy to obtain, with little discomfort to the patient. Sputum cytology is generally reserved for symptomatic individuals: As a screening test (e.g., in asymptomatic smokers), sputum cytology is not effective in decreasing mortality from lung cancer. With the advent of bronchoscopy and FNA, its use as the mainstay in respiratory cytology has declined significantly.

Collecting multiple sputum samples over several days optimizes sensitivity. Early morning, deep cough specimens are preferred.⁶ If the patient is not able to expectorate adequately, expectoration can be induced by having the patient inhale nebulized water or saline. Sputum induction increases the detection of lung cancer.⁷ When prompt preparation of sputum is not possible, the patient can expectorate into a 70% ethanol solution, which prefixes the specimen.

A simple method of sputum preparation is known as the “pick and smear” technique, whereby fresh sputum is examined for tissue fragments, blood, or both. Smears are prepared from areas that contain these elements and

immediately fixed in 95% ethanol. A modification of this is the Saccomanno method, which calls for sputum to be collected in 50% ethanol and 2% carbowax.⁸ The specimen is then homogenized in a blender and concentrated by centrifugation. Improved sensitivity has been demonstrated by the use of dithiothreitol (DTT) for homogenization.⁹ Smears are made from the concentrated cellular material. The Saccomanno method must be performed in a biologic safety hood due to the risks of infection from aerosolization. Sputum can also be processed using thinlayer methods or embedded in paraffin for cell block sections.¹⁰

The adequacy of a sputum sample is established by finding *numerous* pulmonary macrophages.⁹ Specimens consisting merely of squamous cells, bacteria, and *Candida* organisms are unsatisfactory because they represent only oral contents. Even ciliated cells, which also line the sinonasal passages, do not guarantee that a sample is from the lower respiratory tract. The presence of numerous macrophages indicates that a satisfactory, deep cough specimen of the lower respiratory tract has been obtained. In an adequate sample they should not be difficult to find: If they are absent or few in number, the sample should be reported as unsatisfactory.

The sensitivity of sputum cytology for the diagnosis of malignancy increases with the number of specimens examined, from 42% with a single specimen to 91% with five specimens.¹¹ The specificity of sputum examination is high, ranging from 96% to 99%, and the positive and negative predictive values are 100% and 15%, respectively.¹² Thus, negative sputum results do not guarantee the absence of a malignancy, especially in a patient suspected of having lung cancer. The sensitivity of sputum cytology depends also on the location of the malignant tumor: 46% to 77% for central lung cancers but only 31% to 47% for peripheral cancers.^{13,14} Surprisingly, sensitivity is independent of tumor stage and histologic type. Accuracy in tumor classification is 75% to 80%¹⁵ and is tumor type-dependent.¹⁶

Bronchial Specimens

A pivotal improvement in sampling the lower respiratory tract occurred with the development of the flexible bronchoscope in the late 1960s. Today, any part of the respiratory mucosa can be sampled with this device.

Complications of bronchoscopy are rare (0.5% and 0.8% for major and minor complications, respectively)¹⁷ and include laryngospasm, bronchospasm, disturbances of cardiac conduction, seizures, hypoxia, and sepsis. The incidence

of major complications is higher for transbronchial biopsy (6.8%).¹⁷

Bronchial Aspirations and Washings

Bronchial secretions can be aspirated directly from the lower respiratory tract through the bronchoscope, but an alternative (and more common) method is to “wash” the mucosa by instilling 3 to 10 mL of saline and suctioning the washings. The fluid is centrifuged and the concentrate used to make smears, thinlayer preparations, and/or cell blocks; the latter are particularly useful when special stains are needed.

Bronchial Brushings

Fiberoptic bronchoscopy allows direct visualization and sampling of the tracheobronchial tree. A brush is applied to the surface of an endobronchial lesion, and the entrapped cells are either smeared onto a glass slide or rinsed in a collection medium for thinlayer and/or cell block preparation. If smears are made, immediate fixation (by immersion into 95% ethanol or by spray fixation) of the smears is essential to preserve morphologic detail.⁶

The diagnostic accuracy of bronchial washing/brushing cytology is comparable to that of bronchial biopsy.¹⁸ Brushings with cell block preparation sometimes detect malignancy more reliably than bronchial biopsies.¹⁹ Accuracy improves when clinical history is provided with the specimen.²⁰ The diagnostic yield also improves when several different sampling methods are used in concert.^{21,22}

Bronchoalveolar Lavage

The choice between bronchoalveolar lavage (BAL) and bronchial washing depends on the location of the airway one desires to sample. With BAL, the bronchoscope is wedged into position as far as it will go in order to sample the distal airways, which are flushed with sterile saline.

BAL is particularly useful for the diagnosis of opportunistic infections in immunocompromised patients. The specimen can be examined cytologically and a portion also submitted for microbiologic studies. The distinction between oral contamination and a real bacterial infection can be difficult, but an abundance of normal squamous cells usually indicates contamination by oral flora, whereas neutrophils imply a real infection.²³ In immunocompromised patients, the

diagnostic yield for infectious pathogens is 39%, the sensitivity 82%, and the specificity 53%.²⁴ In patients with acquired immunodeficiency syndrome (AIDS), BAL has a sensitivity for documenting infection comparable to that for transbronchial biopsy (86%); when used in combination with biopsy, sensitivity increases to 98%.²⁵ Historically, the most common pulmonary pathogens detected by BAL in human immunodeficiency virus (HIV)-seropositive individuals were *Pneumocystis jirovecii* (78%) and bacteria (19%); the remainder were *Mycobacterium tuberculosis*, atypical mycobacteria, *Histoplasma*, and *Cryptococcus*.²⁶ The frequency and distribution of infections has changed since the widespread use of highly active antiretroviral therapy (HAART) to treat HIV.²⁷ Among HIV-seropositive individuals with nonspecific cytologic results, 27% prove to have pathogens, usually bacterial or fungal, by either culture or biopsy,²⁸ which emphasizes the importance of a multimodal approach to diagnosis in this setting.

BAL is also used for the diagnosis of malignancy, with sensitivity that ranges from 35% to 70%.^{29,30} The sensitivity of BAL for detecting malignancy is higher for multifocal or diffuse tumors.³¹ False-positive results are occasionally encountered due to atypical type II pneumocytes in the setting of pneumonia, diffuse alveolar damage,³² bone marrow transplantation,³³ and chemotherapy.³⁴

Transbronchial Fine-Needle Aspiration ('Wang Needle')

Transbronchial FNA is especially useful for the diagnosis of primary pulmonary lesions that lie beneath the bronchial surface and for staging lung cancer patients by sampling mediastinal lymph nodes.³⁵⁻³⁷ In these settings, the need for additional surgical procedures is eliminated in 20% of patients, and the cost is one-third that of mediastinoscopy.³⁸ The lesion is aspirated with a retractable (Wang) needle passed through a flexible catheter that is sent down the bronchoscope.

When transbronchial FNA is used to sample mediastinal lymph nodes, at least a moderate number of lymphocytes must be present to ensure the adequacy of the specimen and avoid a false-negative result.³⁹ Ciliated respiratory epithelial cells are common contaminants because the respiratory mucosa needs to be breached to reach the target. For this reason, ciliated cells should not be taken as evidence of adequate sampling. Complications from transbronchial aspiration are rare and include endobronchial bleeding, which is usually controlled by suctioning. Contraindications are coagulopathy, respiratory failure, and uncontrollable coughing.⁴⁰

Transbronchial FNA augments the diagnostic accuracy of bronchial washings, brushings, and endoscopic biopsies for the detection of primary pulmonary neoplasms.⁴¹⁻⁴² The sensitivity of transbronchial FNA by itself is 56% but increases to 72% when combined with bronchial brushing, washing, and biopsy. Specificity is 74%, and the positive and negative predictive values are 100 and 53% to 70%, respectively.

Transbronchial FNA is accurate in distinguishing small cell from non–small cell lung cancer.⁴³ For mediastinal staging of bronchogenic carcinoma, the negative predictive value of transbronchial FNA increases from 36% to 78% when negative specimens without sufficient lymphocytes are regarded as unsatisfactory for evaluation.³⁹ The most common cause of false negatives is sampling error.⁴²

Endobronchial Ultrasound-Guided (EBUS) Fine-Needle Aspiration

The accuracy of mediastinal staging by FNA improves with the use of ultrasound guidance.^{35,36} Endobronchial ultrasound-guided (EBUS) FNA is an enhanced procedure for sampling mediastinal and paratracheal lymph nodes and peribronchial lung or mediastinal lesions. Its primary indication is nodal staging of non–small cell lung cancer,⁴⁴⁻⁴⁷ but it is also indicated for sarcoidosis and metastases from extrapulmonary primaries.⁴⁸ This minimally invasive procedure is a safer alternative to cervical mediastinoscopy in selected patients. Using a bronchoscope equipped with an ultrasound probe tip, the operator performs an FNA with real-time ultrasound imaging of a lymph node or central lung mass. Sensitivity and specificity are 78% and 99%, respectively.⁴⁹ To optimize sensitivity, a cytologist can assess the sample on-site for adequacy. In the absence of lesional cells, adequacy is defined as the presence of lymphocytes (if a lymph node is being sampled) or pigmented macrophages (in the case of a lung mass).⁴⁸ As with transbronchial (Wang needle) FNA, ciliated respiratory epithelial cells are common contaminants and should not be taken as evidence of adequate sampling. The advantage of EBUS is that it increases accessibility to lower station lymph nodes: transbronchial (Wang needle) FNA can sample station 2 to 4 and 7 lymph nodes; transesophageal ultrasound-guided FNA can reach station 2 to 4 and 7 to 9 nodes; whereas EBUS can sample station 2 to 4, 7, and 10 to 12 nodes.⁴⁸ Thus, combining esophageal FNA with EBUS is often performed for full accessibility.

Although the EBUS FNA procedure itself is more expensive than

transbronchial (Wang) FNA, it saves downstream costs due to its greater sensitivity, thus reducing any need for more surgical staging.⁵⁰

Transesophageal Fine-Needle Aspiration

Mediastinal lymph node sampling can also be done endoscopically by passing the needle through the esophagus.⁵¹⁻⁵³ The addition of ultrasound guidance improves the accuracy of mediastinal lymph node sampling.⁵⁴ Like bronchoscopic FNA, endoscopic FNA realizes significant cost savings⁵³ and reduces the number of unnecessary thoracotomies.⁵²

The diagnostic yield for mediastinal staging is greatly improved when endoscopic transesophageal FNA is used in combination with transbronchial FNA, with an accuracy that approaches 100%.⁵⁵ As with transbronchial FNA, a moderate number of lymphocytes must be present to ensure the adequacy of the specimen and avoid a false-negative result.

Percutaneous Fine-Needle Aspiration

The ease, rapidity of diagnosis, and minimal morbidity of percutaneous FNA make it an attractive alternative to surgical biopsy in the evaluation of the patient with a peripheral pulmonary mass. FNA is of greatest benefit to patients for whom it spares a more invasive surgical procedure. Surgical intervention, in fact, can be avoided in up to 50% of patients with clinically suspected lung cancer.⁵⁶ There are some contraindications, however.



Relative contraindications to percutaneous fine-needle aspiration

- chronic obstructive pulmonary disease (COPD)
- emphysema
- uncontrollable coughing
- uncooperative patient
- bleeding diathesis (i.e., anticoagulant therapy)
- severe pulmonary hypertension
- arteriovenous malformation
- cardiac disease
- suspected echinococcal cyst⁵⁷

The most common complication of percutaneous FNA is pneumothorax. A radiographically detectable pneumothorax occurs in 21% to 34% of patients⁵⁸; only 10%, however, require intercostal drainage tubes.⁵⁸ The risk of a pneumothorax increases with the number of passes through aerated lung, and decreases if the path does not traverse aerated lung.⁵⁸ Transient hemoptysis occurs in 5% to 10% of patients. Other complications are rare and include hemopericardium, hemothorax, air embolism, tumor seeding, and death.^{58,59}

Percutaneous FNA is usually performed by a radiologist using computed tomography (CT) or ultrasound guidance, the latter best reserved for lesions that abut the pleura or chest wall. The needle gauge ranges from 18 to 25, and many different types of needle devices are available. Although many radiologists prefer 22-gauge Chiba needles, these require repuncture if more than one pass is needed. Another choice is the coaxial needle, with a large-bore outer needle serving as the guide for a small-bore inner needle. Once the outer needle is positioned, more than one aspiration can be performed using the inner needle.

It can be helpful if a cytotechnologist and/or cytopathologist attends the FNA procedure to assist with specimen handling and assess its adequacy on site. After smears are prepared, the needle is rinsed in a balanced electrolyte solution, Saccomanno's fixative, 50% ethanol, or commercial preservative solution. The cellular suspension can be processed as a cytospin, thinlayer preparation, or cell block, and it can be apportioned for flow cytometric analysis if needed. Formalin-fixed cell blocks are ideal for histochemical and immunohistochemical stains. A decision regarding the need, if any, for special studies can be made by the cytotechnologist or cytopathologist after examination of the smears.

Percutaneous FNA is a reliable and accurate way to diagnose many pulmonary neoplasms.



Accuracy of percutaneous fine-needle aspiration

- sensitivity: 89%
- specificity: 96%
- positive predictive value: 98%
- negative predictive value: 70%
- false-positive rate: 0.85%
- false-negative rate: 8%

In a study of more than 13,000 FNA specimens from 436 institutions, the

diagnostic sensitivity was 89% for the procedure itself and 99% for the pathologist's interpretation.⁶⁰ This difference indicates that most false-negative results are due to sampling error. About 15% of false-positive diagnoses and 5% of false-negative diagnoses have a significant, permanent, or grave influence on patient outcome.⁶⁰ The reliability of a negative FNA result is a matter of controversy, given that negative predictive values range from 34% to 88%.^{61–64} For this reason, most investigators recommend a repeat aspiration or tissue biopsy when a specific benign diagnosis that accounts for the lesion cannot be made with certainty. The small, cutting ("core") biopsy is no more accurate than FNA,^{61,65–67} but it is performed instead of FNA in some centers.

With regard to the management of patients with primary lung cancer, it is important to discriminate small cell from non-small cell carcinoma, and adenocarcinoma (ACA) from squamous cell carcinoma (SQC). The distinction between small cell and non-small cell carcinoma is possible in more than 95% of cases,⁶⁸ and between ACA and SQC in 88% of cases diagnosed by FNA.⁶⁹

A variety of benign cells occasionally contaminate a percutaneous FNA. Such cells need to be recognized as contaminants and not misconstrued as lesional. In particular, mesothelial cells from the pleura are common, and in some cases they can be numerous ([Fig. 2.3](#)). They resemble the cells of a well-differentiated adenocarcinoma (see "Adenocarcinoma") but are identified as benign mesothelial cells by their relative flatness, cohesion, and the characteristic slitlike "windows" that separate the mesothelial cells from each other.

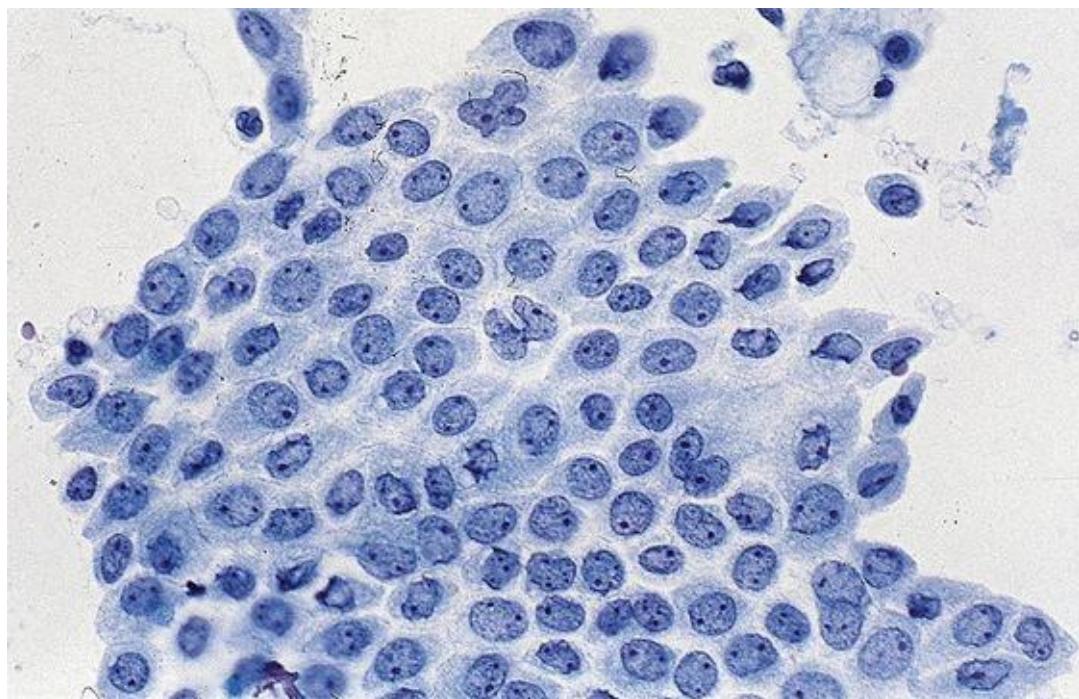


FIG. 2.3 Mesothelial cells (fine-needle aspiration [FNA]).

Benign mesothelial cells are occasionally seen in percutaneous fine needle aspirates. They are arranged in flat, cohesive sheets. The cells have round or oval nuclei, small nucleoli, and a moderate amount of cytoplasm. Slitlike spaces between the cells ("windows") can be appreciated (Papanicolaou stain).



Contaminants of percutaneous fine-needle aspiration

- mesothelial cells
- cutaneous squamous cells
- skeletal muscle
- fibroconnective tissue
- hepatocytes (transdiaphragmatic needle path)

If the specimen consists only of one (or more) of these contaminants, it should be interpreted as insufficient for evaluation (nondiagnostic) rather than negative, because there is no evidence that the lesion itself has been sampled.

Molecular Testing of Lung Cancers

As DNA sequencing technology has progressed to single base pair resolution, it is increasingly evident that lung cancer is not a single disease but rather a heterogeneous group of molecularly defined entities. Today some of these cancers are treated with more effective and less toxic “targeted therapies” (as compared to conventional chemotherapy); thus, accurate pathologic and molecular classification is needed. Because many molecular techniques use amplification methods, large amounts of tissue are not necessary, and molecular classification can be performed successfully on small biopsy and cytology specimens.²⁰ They require, however, that the sample have a minimum proportion of tumor to normal nuclei. Thus, assessment of the cytologic sample for its adequacy for molecular testing is a growing part of the cytologist’s job.

Many of the new therapies target genetic aberrations in the receptor tyrosine kinase pathways. Abnormally activated receptor tyrosine kinases are linked to a cascade of downstream effector pathways ([Fig. 2.4](#)) that result in transcriptional activation of genes involved in tumor growth, invasion, and angiogenesis.²¹ The pathway molecules implicated in lung cancer pathogenesis include epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor (HGFR, encoded by the proto-oncogene MET), vascular endothelial growth factor and receptor (VEGF, VEGFR), protein kinase ERBB2 (HER2), echinoderm microtubule–associated protein–like 4-anaplastic lymphoma kinase (EML4-ALK), and insulin-like growth factor 1 receptor (IGF-1R) (see [Fig. 2.4](#)). Bevacizumab, an anti-VEGF monoclonal antibody, and erlotinib, an EGFR inhibitor, are approved for clinical use; others are in clinical development.

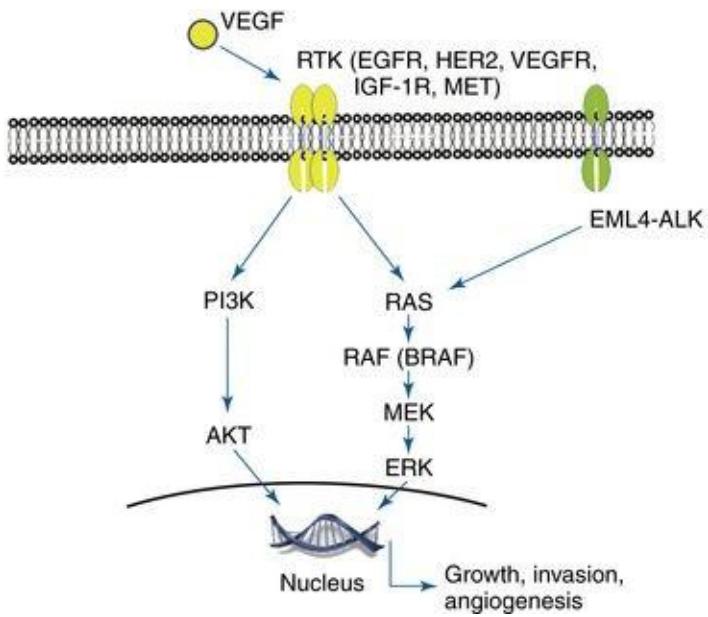


FIG. 2.4 Receptor tyrosine kinase signaling in cancer.
 This simplified diagram illustrates the pathways by which receptor tyrosine kinases (RTKs) stimulate growth, invasion, and angiogenesis (see text for details and abbreviations of individual RTKs). *PI3K*, Phosphatidylinositol 3-kinase; *AKT*, Protein Kinase B; *RAS*, Rous sarcoma oncogene; *RAF*, RAF proto-oncogene serine/threonine-protein kinase; *MEK*, MEK kinase; *ERK*, extracellular-signal-regulated kinase, also known as Mitogen-activated protein (MAP) kinase.

Epidermal Growth Factor Receptor

EGFR amplification drives growth, invasion, and angiogenesis through activation of PI3K/AKT and RAS/RAF/MEK signaling and is seen in 30% to 60% of non–small cell lung cancers.^{22–24} It is mutated in its tyrosine kinase domain in greater than 30% of Asians and 10% of Caucasians with non–small cell lung cancer.²⁵ Erlotinib, a U.S. Food and Drug Administration (FDA)–approved receptor tyrosine kinase inhibitor, significantly improves overall survival of previously untreated non–small cell lung cancer patients.²⁶ Moreover, erlotinib and gefitinib, the latter currently in clinical trials, are much less toxic than conventional chemotherapy. EGFR-mutated tumors respond much better than nonmutated tumors,²⁸ but unfortunately, a majority develop resistance within a year. Resistance is due to the acquisition of additional mutations within the tyrosine kinase domain of EGFR, most often a T790M amino acid substitution in exon 20.^{27,28} EGFR inhibitors are currently in development to overcome resistance.²⁹

MET

MET is a proto-oncogene that encodes another receptor tyrosine kinase, HGFR (see [Fig. 2.4](#)), and its amplification is associated with resistance to EGFR inhibitors. The mechanism of resistance is beyond the scope of this chapter, but MET is amplified in 5% to 20% of EGFR-mutated, EGFR-resistant non–small cell lung cancers.^{80–82} Therapeutic antiMET antibodies are in development.⁷⁹

Vascular Endothelial Growth Factor and Receptor

Bevacizumab is a highly effective monoclonal antibody targeting VEGF, a ligand implicated in tumor angiogenesis, significantly improving the overall survival of patients with non-squamous, non–small cell lung cancer when combined with carboplatin and paclitaxel.⁸³

Sunitinib and sorafenib, tyrosine kinase inhibitors of VEGFR, are in development.⁷⁹

ERBB-2 (HER2)

HER2, a member of the EGFR family, is amplified or overexpressed in up to 23% of non–small cell lung cancers, but oddly, these patients do not benefit from anti-HER2 single-agent therapy.^{84,85} In a subset of lung cancers (3% to 10%), however, HER2 is mutated in its kinase domain^{86,87}; for these patients, anti-HER2 antibodies and kinase inhibitors are in development.

Echinoderm Microtubule–Associated Protein–Like 4-Anaplastic Lymphoma Kinase

ALK is yet another receptor tyrosine kinase that plays an important role in a subset of lung cancers. When fused through chromosomal translocation to EML4, ALK becomes constitutively active, resulting in the activation of the pro-growth PI3K/AKT and RAS/RAF/MEK pathways (see [Fig. 2.4](#)). An EML4-ALK translocation is present in 5% to 7% of non–small cell lung cancers. These patients have a unique clinical and molecular profile: they are nonsmokers and relatively young (median age 52) males without EGFR or KRAS mutations.^{88,89} EML4-ALK translocated tumors are exquisitely sensitive to crizotinib.⁹⁰ The translocation is identified by fluorescence in situ hybridization ([Fig. 2.5](#)).

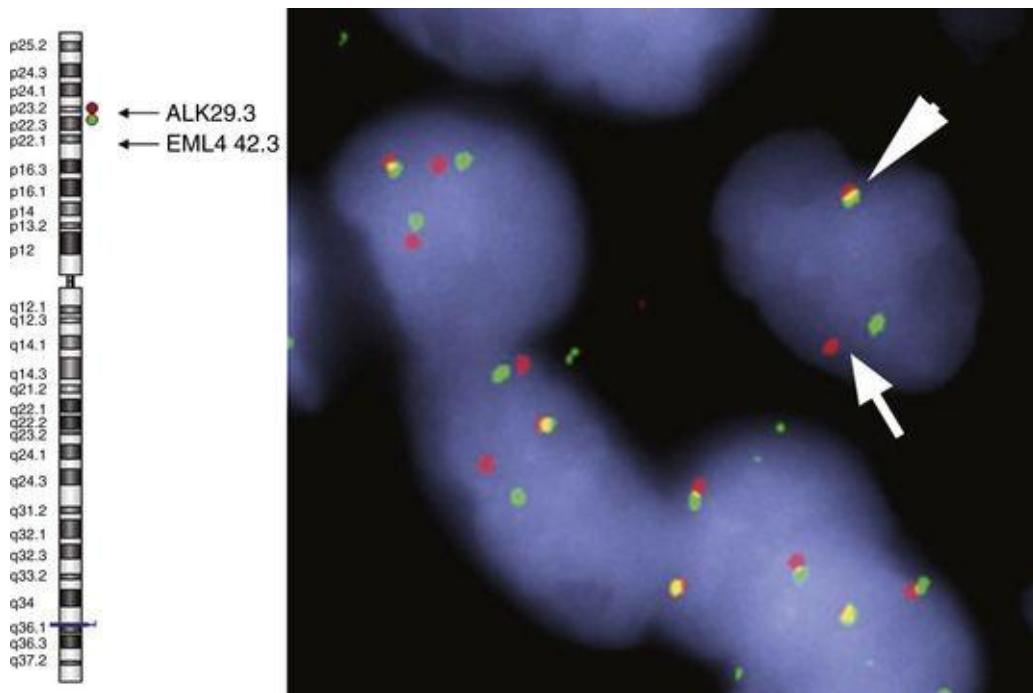


FIG. 2.5 Echinoderm microtubule–associated protein-like 4-anaplastic lymphoma kinase (EML4-ALK) intrachromosomal translocation identified by dual color split-apart fluorescence *in situ* hybridization (FISH).

The arrows (left portion) point to the breakpoints within chromosome 2 that result in the intrachromosomal translocation. On the right, red and green probes flanking the *ALK* locus on chromosome 2p23.2 reveal red–green doublets at the wild type *ALK* loci (arrow head) but are split apart when *ALK* is rearranged and fused to *EML4* on the same chromosome at 2p21 (arrow). The rearranged probes appear far apart because the chromatin of interphase nuclei is dispersed relative to that of chromosomal DNA. (Figure courtesy Dr. Lucian Chirieac, Department of Pathology, Brigham and Women's Hospital, Boston, Mass.)

BRAF

BRAF is a form of RAF, a serine-threonine kinase that activates MEK (see [Fig. 2.4](#)). Mutation of exons 11 or 15 in the kinase domain is implicated in the early development of lung cancer and is seen in 3% of non–small cell lung cancers.⁹⁰ These mutations are distinct from those seen in melanoma (V600E), raising concern that a BRAF-mutated lung cancer may not be responsive to the BRAF inhibitors currently used to treat melanoma.⁹¹ To circumvent this limitation, MEK inhibitors are in development.

Insulin-Like Growth Factor 1 Receptor

IGF-1R is a unique receptor tyrosine kinase in that deregulation of IGF-1R is associated with squamous lung cancers.⁹² Further, its amplification is associated with an improved prognosis.⁹³ Antibodies targeting IGF-1R are under clinical development.

PIK3CA

Amplification of PIK3CA occurs in 12% to 17% of non–small cell lung cancers and is believed to play a role in resistance to receptor tyrosine kinase inhibitor therapy.^{94,95} Moreover, activating mutations of the kinase or helix domain are seen in 2% to 13% of non–small cell lung cancers.^{76,95,96} PI3K inhibitors are currently in clinical development.

KRAS

KRAS-mutated cancers carry a worse prognosis and are typically absent in patients with receptor tyrosine kinase mutations. These patients are usually smokers, and evidence suggests that they do worse when erlotinib is added to their chemotherapy,⁹⁷ once more underscoring the importance of molecular testing in lung cancer. Preliminary evidence suggests that sorafenib, a weak inhibitor of downstream RAF, has efficacy in this group of otherwise treatment-resistant tumors.⁹⁸

Benign Cellular Changes

Reactive Squamous Cell Changes

Benign squamous cells from the oral cavity often contaminate sputum and bronchial cytology specimens. Inflammatory conditions of the mouth caused by trauma, candidiasis, or pemphigus can exfoliate mildly atypical squamous cells with hyperkeratinization and nuclear degeneration, usually in small numbers. Such minimal changes should not be misinterpreted as squamous cell carcinoma (SQC). More marked (but still benign) squamous cell atypias occur adjacent to cavitary fungal infections and stomas, and with almost any injury to the lung (e.g., infarction, radiation, chemotherapy, sepsis, diffuse alveolar damage) and might result in a false-positive interpretation of SQC.^{33,34} Another, uncommon source of false-positives is malignant cells from head and neck cancers that contaminate sputum and bronchial specimens.⁹⁹

Reactive Bronchial Cell Changes

Benign, reactive bronchial cell changes occur in response to noxious stimuli such as radiation, chemotherapy, and severe inflammation. Under such conditions, ciliated columnar cells can increase their nuclear area many times over, with multinucleation, coarsely textured chromatin, and large nucleoli ([Fig. 2.6](#)). Large clusters of bronchial cells known as *Creola bodies* (named after the first patient in whom they were recognized) are commonly seen in chronic airway diseases like asthma ([Fig. 2.7](#)).

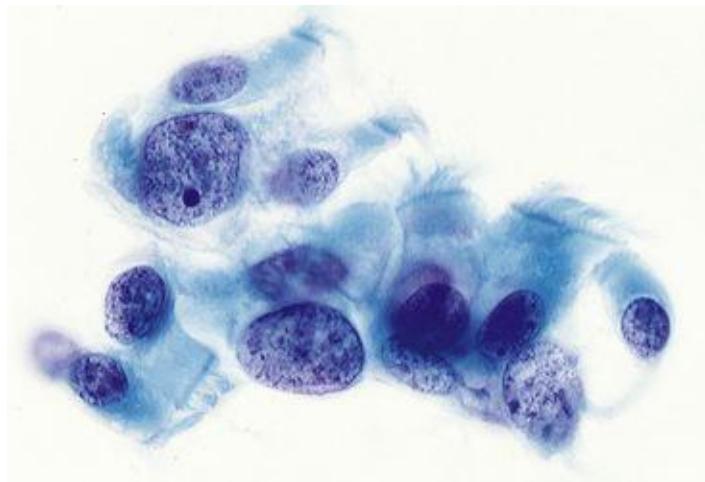


FIG. 2.6 Reactive bronchial cells (bronchial brushing).
Reactive bronchial cells can show marked nuclear size variation. Note that cilia—evidence of their benign nature—are retained (Papanicolaou stain).



FIG. 2.7 Reactive bronchial cells (Creola body; bronchial washing).
In chronic lung diseases, as in this case of asthma, clusters of reactive bronchial cells can assume a spherical shape and resemble the cells of an adenocarcinoma. Normal nuclear features and cilia indicate their benign nature (Papanicolaou stain).

Markedly reactive changes in benign bronchial cells mimic adenocarcinoma. Malignancy can be excluded if the atypical cells have cilia and/or demonstrate a spectrum of changes (from benign to markedly atypical) rather than the two distinct cell populations typical of a malignant sample obtained bronchoscopically. Note that in sputum and FNA specimens, a helpful dual cell population (malignant cells and bronchial cells) is usually not apparent.

Bronchial Reserve Cell Hyperplasia

As the surface epithelium of the respiratory tract is shed during lung injury, reserve cells proliferate and are seen in bronchial washings and brushings ([Fig. 2.8](#)).

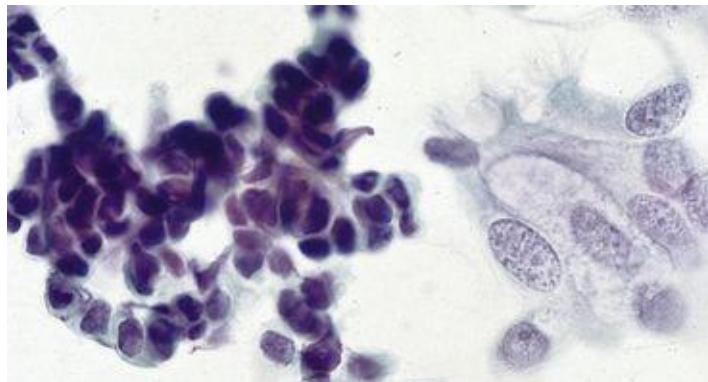


FIG. 2.8 Reserve cell hyperplasia (bronchial brushing).

These clusters of benign cells have hyperchromatic nuclei with nuclear molding. They are distinguished from small cell carcinoma by their extremely small size and the lack of necrosis. Compare these cells with the adjacent bronchial columnar cells (Papanicolaou stain).



Cytomorphology of reserve cell hyperplasia

- tightly packed cells
- very small cells
- smudged, dark chromatin
- nuclear molding
- scant cytoplasm
- no mitoses or necrosis

Reserve cell hyperplasia (RCH) is a mimic of small cell carcinoma but is usually easily distinguished from it. The cells of RCH show greater cohesiveness; they are smaller; and there are no mitoses or necrosis.

Repair

Repair represents reepithelialization of an ulcer created by trauma, radiation, burns, pulmonary infarction, and infections. Typical (and atypical) repair of the respiratory tract is very similar to that seen in the cervix and gastrointestinal (GI) tract.



Cytomorphology of repair

- flat, cohesive sheets
- abundant cytoplasm
- enlarged, often hypochromatic nuclei
- enlarged nucleoli
- mitoses

Reparative epithelium is most commonly seen in tracheobronchial brushings and washings. The differential diagnosis of repair includes malignancy: the non–small cell lung cancers, a metastasis, and other less common tumors. Malignant cells are usually less cohesive and orderly than reparative epithelium, and malignant cells are usually more numerous. Correlation with clinical history can be helpful; a conservative approach is recommended if the findings are not conclusive and there is a history of mucosal trauma or other lung injury.

Type II Pneumocyte Hyperplasia

Because type II pneumocytes function as alveolar reserve cells, they proliferate after lung injury.



Causes of type II pneumocyte hyperplasia

- pneumonia
- sepsis (diffuse alveolar damage)
- pulmonary embolus with infarction
- chemotherapeutic drugs
- radiation therapy
- inhalant damage (e.g., oxygen toxicity)
- interstitial lung disease

When floridly hyperplastic, as in diffuse alveolar damage, the cells of type II pneumocyte hyperplasia resemble those of adenocarcinoma ([Fig. 2.9](#)).

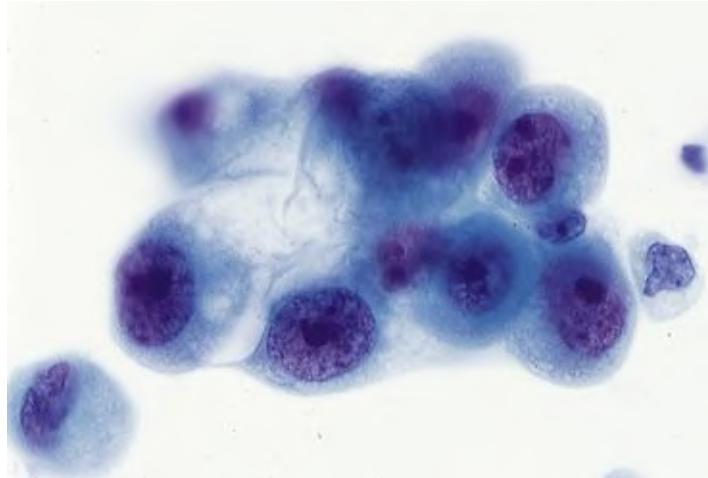


FIG. 2.9 Type II pneumocyte hyperplasia (bronchoalveolar lavage [BAL]). In patients with lung injury, type II pneumocytes are markedly enlarged and may mimic adenocarcinoma, as seen here. This patient had diffuse infiltrates and marked respiratory distress due to diffuse alveolar damage. In such clinical settings, an unequivocal diagnosis of malignancy should be avoided, inasmuch as most patients with lung cancer are not usually ill at presentation (Papanicolaou stain).



Cytomorphology of type II pneumocyte hyperplasia

- isolated cells and three-dimensional clusters
- large nuclei
- coarse chromatin
- prominent nucleoli
- scant to abundant cytoplasm

The only clue to avoiding an incorrect diagnosis of malignancy in a patient with type II pneumocyte hyperplasia may be the clinical history of respiratory distress and diffuse infiltrates. Thus, in an acutely ill patient with diffuse pulmonary infiltrates, markedly atypical cells should be interpreted cautiously.³³ Sequential respiratory specimens can be helpful, inasmuch as hyperplastic pneumocytes are not present in BAL specimens more than 1 month after acute lung injury.³²

Noncellular Elements and Specimen Contaminants

Noncellular and extraneous elements in respiratory material can be inhaled, produced by the host, formed as a host response to foreign material, or introduced as laboratory contaminants.

Curschmann spirals are coiled strands of mucus that stain purple with the Papanicolaou stain ([Fig. 2.10](#)). In the past they have been associated with chronic respiratory diseases, but they are, in fact, a nonspecific finding not worth mentioning in the report.

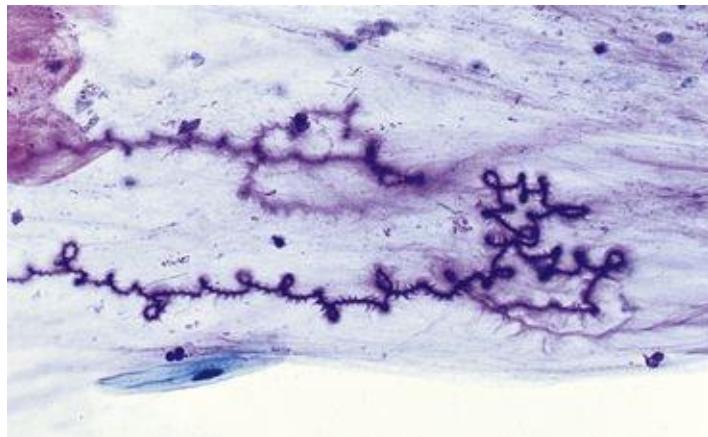


FIG. 2.10 Curschmann spiral (sputum).

These coils of inspissated mucus are commonly seen in respiratory specimens and are a nonspecific finding (Papanicolaou stain).

Ferruginous bodies are mineral fibers encrusted with ferroproteins. Dumbbell-shaped, ranging from 5 to 200 µm in length, they stain golden-yellow to black with Papanicolaou stain. Some but not all ferruginous bodies contain a core of asbestos. So-called *asbestos bodies* are distinguished from other ferruginous bodies by their clubbed ends and thin, straight, lucent core. *Asbestos fibers* are not visible by light microscopy but are usually much more numerous than asbestos bodies. Patients with known asbestos exposure usually have high numbers of ferruginous bodies in BAL fluid.^{[100](#)}

Charcot-Leyden crystals are rhomboid-shaped, orangeophilic structures derived from degenerating eosinophils in patients with severe allergic disorders like asthma ([Fig. 2.11](#)).

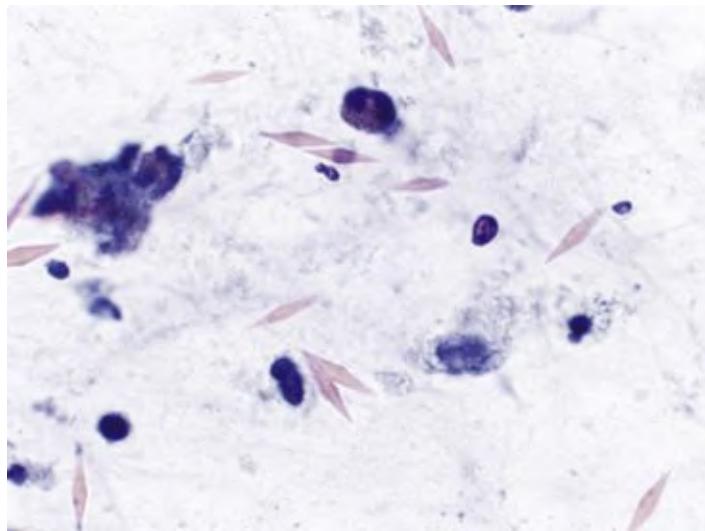


FIG. 2.11 Charcot-Leyden crystals (bronchial washing).

These needle-shaped crystals from a patient with asthma are a by-product of eosinophil degranulation (Papanicolaou stain).

Psammoma bodies are concentrically laminated calcifications seen in malignant tumors that have papillary architecture, like primary pulmonary adenocarcinoma, mesothelioma, and metastatic thyroid or ovarian cancer. They are also seen in benign conditions like pulmonary tuberculosis and alveolar microlithiasis.

Corpora amylacea are spherical structures with circumferential and radiating lines. They measure between 30 and 200 μm and are indistinguishable from those seen in the prostate. They have no known significance but are more commonly seen in older individuals ([Fig. 2.12](#)).

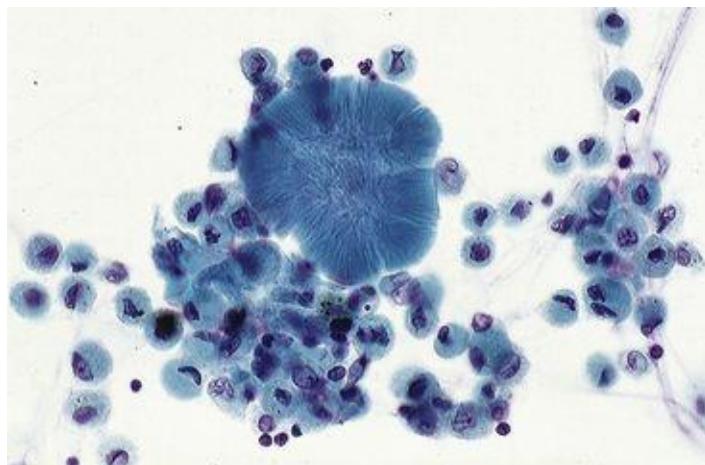


FIG. 2.12 Corpora amylacea (bronchoalveolar lavage [BAL]).

These large acellular bodies are somewhat variable in appearance. They may be spherical, as seen here, or angulated. They have fine radial striations and may have concentric laminations. Occasionally, there may be a central pigmented core. They are produced in the lung and other organs and have no known significance. Pulmonary corpora amylacea are not calcific, distinguishing them from psammoma bodies and the laminated spheres of pulmonary alveolar microlithiasis (Papanicolaou stain).

Amorphous protein in respiratory specimens may be a clue to the diagnosis of amyloidosis or alveolar proteinosis.

Specimen contaminants include vegetable matter ([Fig. 2.13](#)), pollen, and the pigmented fungus *Alternaria* ([Fig. 2.14A](#) and [B](#)).

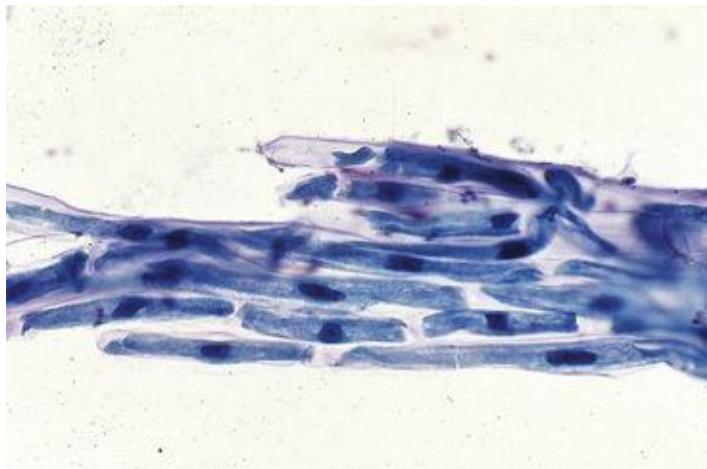


FIG. 2.13 Vegetable cells (sputum).

Some vegetable cells have elongated shapes and large nuclei, resembling the cells of keratinized squamous cell carcinoma. Their rectangular shape, uniform size, and refractile cellulose wall, however, help identify them as vegetable cells (Papanicolaou stain).

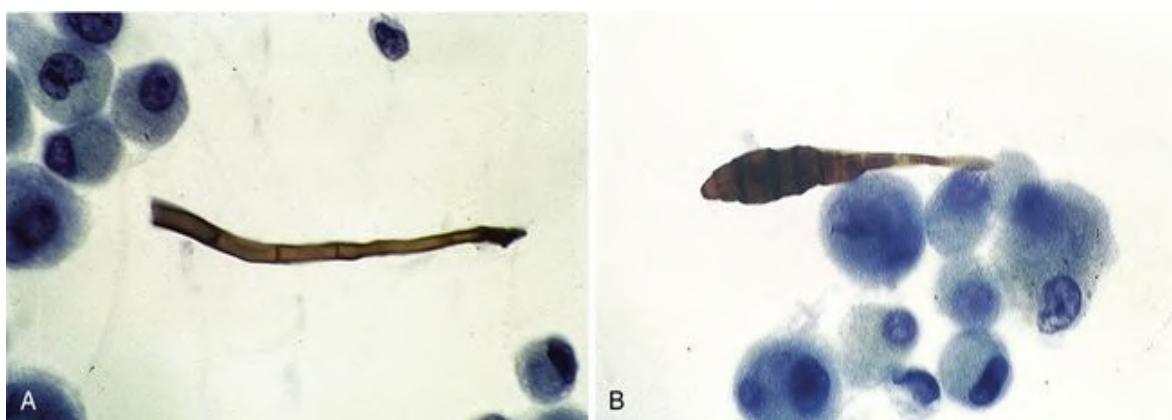


FIG. 2.14 Alternaria (bronchoalveolar lavage [BAL]).

This pigmented fungus is rarely pathogenic. It can contaminate virtually any cytologic specimen, including cervicovaginal smears, cerebrospinal fluid, and urine. *A*, The slender septate stalks (conidiophores) are occasionally branched (not shown). *B*, The conidia are snowshoe-shaped and have both transverse and longitudinal septations (Papanicolaou stain).

Infections

Cytology plays an important role in diagnosing infectious diseases, particularly those in immunocompromised patients, and is being utilized more frequently than ever because of improved sampling methods. It is important to know that conventional inflammatory responses can be much reduced, absent, or greatly altered in patients with immune deficiencies.

Viral Infections

Herpes Simplex

Herpes simplex virus (HSV) pharyngitis, laryngotracheitis, and pneumonia most commonly affect immunocompromised patients and neonates, and HSV-1 is the most common serotype to involve the respiratory tract. Ulcerative/necrotizing infections can involve the pharynx, larynx, tracheobronchial tree, or pulmonary parenchyma, and cytopathic changes are identical to those seen in other sites: multinucleation, nuclear molding, chromatin margination, and large nuclear (Cowdry A) inclusions ([Table 2.1](#)). The cytopathic changes of HSV are identical to those of herpes zoster. If the cytomorphologic changes are equivocal, the diagnosis can be confirmed by viral culture, immunohistochemistry, or in situ hybridization.^{[101](#)}

TABLE 2.1

PULMONARY VIRAL INFECTIONS

Virus	Nuclear Features	Inclusions	Other Changes
Herpes simplex and herpes zoster		Multinucleation; molding, peripheral margination of chromatin	Eosinophilic, intranuclear (Cowdry type A) —
Cytomegalovirus		Enlargement	Large intranuclear, basophilic, with halo; small cytoplasmic, basophilic
Measles virus		Multinucleation	Eosinophilic, intranuclear; multiple eosinophilic intracytoplasmic
Respiratory syncytial virus		Multinucleation	Cytoplasmic, basophilic, with halo Giant cells; necrosis
Adenovirus		Smudged appearance as a result of large inclusion that fills entire nucleus	Large intranuclear, basophilic Ciliocytophthora

Cytomegalovirus

Cytomegalovirus (CMV) is one of the most common opportunistic infections. Patients with CMV pneumonia often present with fever, dyspnea, cough, and diffuse nodular or reticular interstitial infiltrates. Viral cytopathic changes (cytomegaly, large basophilic nuclear and small basophilic cytoplasmic inclusions) are found in bronchial cells, pneumocytes, macrophages, endothelial cells, and fibroblasts (see [Table 2.1](#)). The diagnosis can be confirmed by viral culture, immunohistochemistry, in situ hybridization, or the polymerase chain reaction.^{[101-103](#)}

Measles Virus and Respiratory Syncytial Virus

Measles is a highly contagious, usually self-limited disease caused by the rubeola virus. The incidence has been curtailed due to the widespread use of a vaccine. Measles pneumonia occurs as an opportunistic complication, however, in children immunocompromised due to premature birth, cystic fibrosis, malignancy, or an immunologic disorder. Infection causes a giant cell pneumonia characterized by enormous multinucleated cells with cytoplasmic and nuclear inclusions (see [Table 2.1](#); [Fig. 2.15](#)).^{[104](#)} Similar findings are seen with infection by the respiratory syncytial virus (RSV). The diagnosis is usually confirmed by detecting RSV antigen in BAL specimens.

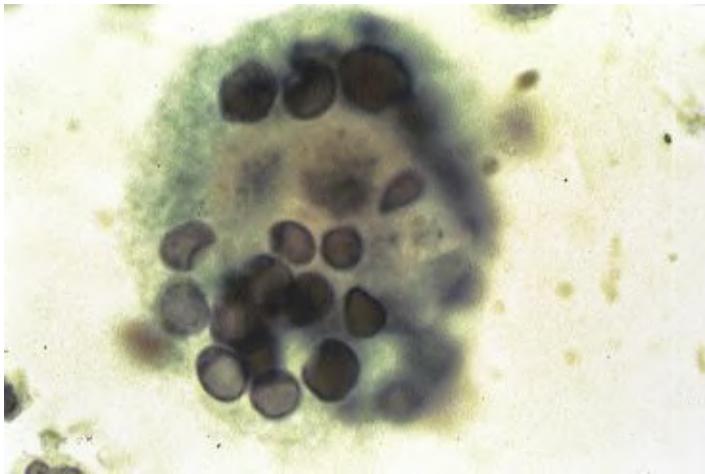


FIG. 2.15 Measles virus cytopathic effect (bronchoalveolar lavage [BAL], cell block). Measles virus infection causes a pneumonia with giant multinucleated epithelial cells that have eosinophilic intranuclear and intracytoplasmic inclusions. These cells are pathognomonic.

Adenovirus

Adenovirus infection usually produces only a minor febrile illness, but adenovirus pneumonia can be severe and fatal, particularly in the immunocompromised. The virus causes two types of nuclear inclusions. One is the smudge cell, in which a large basophilic inclusion usually fills the entire nucleus and obscures chromatin detail. The other is eosinophilic inclusions that resembles the Cowdry A inclusion of HSV infection. A curious morphologic decapitation of ciliated columnar cells, called *ciliocytophthoria*, can be prominent.¹⁰⁵ The detached cell apex, represented by only the terminal bar and cilia, without its nucleus, resembles a floating tuft of hair or eyelash (see [Table 2.1](#)).

Bacterial Pneumonias

Bacterial pneumonias are caused by a large number of bacteria, but most are characterized by a neutrophilic exudate. Common organisms include *Streptococcus pneumoniae* (pneumococcus), other streptococci, *Staphylococcus aureus*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Pseudomonas* sp., *Legionella* sp., *Nocardia* sp., *Actinomyces* sp., and some anaerobic bacteria. Many but not all bacteria can be seen with routine stains as well as with the Gram stain. Cytologic examination is not usually employed for the diagnosis of a bacterial pneumonia, which is typically established by correlating clinical findings with microbiologic studies.

Bacterial pneumonias often have a characteristic lobar or lobular distribution, but some pneumonias appear as round and circumscribed masses on imaging studies and thus mimic the appearance of a malignancy. In such cases, a cytologic specimen might be obtained, because the working clinical diagnosis is a suspected malignancy.

Several bacteria deserve special mention. *Actinomyces* species are a common inhabitant of the tonsillar area and thus a common contaminant of sputum and bronchial specimens (but not FNAs). Infection by *Actinomyces*, however, is uncommon. Pulmonary infection occurs by aspiration of oral contents or by direct extension from subdiaphragmatic abscesses. Actinomycosis is usually a chronic infection that may result in sinus tracts. The bacteria aggregate into grossly visible sulfur granules (so called because they look yellow on gross examination) and evoke a brisk neutrophilic response. When they appear in cytologic specimens as just an oral contaminant, *Actinomyces* bacteria are large blue “cotton balls” often associated with squamous cells, with no neutrophilic infiltrate, similar to what is occasionally encountered in Pap specimens (see [Fig. 1.23](#)). A true thoracopulmonary actinomycosis should be considered if the bacteria are associated with abundant neutrophils.

Nocardia are aerobic, filamentous bacteria that inhabit the soil and are acquired by inhalation. *N. asteroides* accounts for 80% of nocardial infections. Most patients are immunocompromised and have a subacute presentation. Cavitary nodules are seen in one third of patients. The organisms are found among abundant neutrophils. They are thin, filamentous, and beaded, with such extensive, predominantly right-angle branching that they resemble Chinese characters. They are gram-positive and stain with silver stains but are only weakly acid-fast and thus require modified acid-fast stains like the Fite-Faraco for visualization. The diagnosis is established by culture of a biopsy or BAL fluid.

Legionella pneumonia is caused by the aerobic gram-negative bacteria *Legionella* sp., of which the most common is *L. pneumophila*. The organisms are seen well with silver stains like the Steiner, Warthin-Starry, and Dieterle stains. A specific identification of *L. pneumophila* can be made in BAL samples using immunohistochemical or immunofluorescent methods.

Tuberculosis

Infection by *M. tuberculosis* commonly results in granulomatous inflammation ([Fig. 2.16](#)). Cytologic specimens contain aggregates of epithelioid histiocytes,

lymphocytes, and Langhans giant cells. Necrosis may or may not be evident. Granulomas by themselves, however, are a nonspecific finding and can be seen in other conditions like fungal infections and sarcoidosis. A definitive diagnosis of tuberculosis rests on identifying the organisms with the help of a special stain (Ziehl-Neelsen) or by microbiologic culture. Cell block preparations are particularly useful for special stains, but rarely are more than one or just a few organisms identified. (By contrast, infection by *M. avium-intracellulare*, as seen in immunocompromised patients, often yields innumerable acid-fast organisms.) A sensitive (93%) and specific (99%) assay called the Mycobacterium Tuberculosis Direct Test (MTD) is also available for the detection of *M. tuberculosis* and can be applied to respiratory specimens such as sputum.¹⁰⁶ The assay amplifies *M. tuberculosis* ribosomal RNA by the polymerase chain reaction.

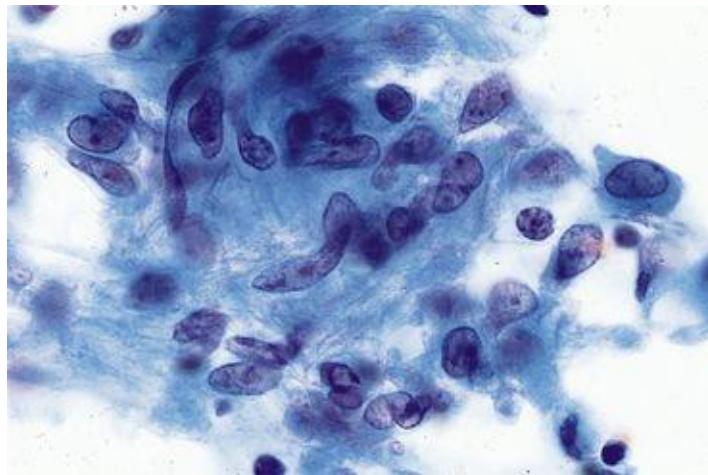


FIG. 2.16 Granuloma (fine-needle aspiration [FNA], *M. tuberculosis* infection). This aggregate of epithelioid histiocytes, the defining feature of the granuloma, has a syncytial appearance because individual cell borders are indistinct. Note the curved, elongated, boomerang-like shapes of some of the histiocytic nuclei. Interspersed lymphocytes are also seen (Papanicolaou stain).

In countries where *M. tuberculosis* is prevalent, the yield of acid-fast bacteria among all clinically suspicious lung masses can be very high.^{107,108} In immunocompromised patients with tuberculosis, there may be an abundance of acid-fast organisms but few well-formed granulomas. If Romanowsky-type stains are used in such cases, the abundant acid-fast organisms can be identified as negative images.

Pulmonary Fungal Infections

Pulmonary fungal infections are readily diagnosed by cytology, particularly in transthoracic FNAs, and should be suspected whenever there is granulomatous inflammation and/or necrosis. Cell block material can be used for silver or periodic acid–Schiff (PAS) stains. Many fungi have a characteristic microscopic appearance that enables a rapid, specific diagnosis ([Table 2.2](#)).

TABLE 2.2

PULMONARY FUNGAL INFECTIONS

Disease	Organism	Demographics	Morphology	Size	Immunocompetent Response	Appearance
Cryptococcosis	<i>Cryptococcus neoformans</i>	Worldwide	Yeast; variably sized; narrow-based budding; mucin capsule (immuno-competent); refractile center	4–15 µm (diameter)	Granulomatous	
Histoplasmosis	<i>Histoplasma capsulatum</i>	Americas, especially Ohio and Mississippi river valleys	Small budding yeast; intracellular	1–5 µm (diameter)	Granulomatous	
Blastomycosis	<i>Blastomyces dermatitidis</i>	North America	Broad-based budding yeast; thick cell wall	8–20 µm (diameter)	Neutrophilic/ granulomatous	
Coccidioidomycosis	<i>Coccidioides immitis</i>	North American deserts	Spherules; endospores	15–60 µm; 1–2 µm (diameter)	Granulomatous	
Paracoccidioidomycosis	<i>Paracoccidioides brasiliensis</i>	Central and South America	Yeast with multiple budding ("mariner's wheel")	4–40 µm (diameter)	Neutrophilic/ granulomatous	
Sporotrichosis	<i>Sporothrix schenckii</i>	Worldwide	Intracellular ovoid yeast with slight halo	2–4 µm (diameter)	Neutrophilic/ granulomatous	
Aspergillosis	<i>Aspergillus fumigatus; A. flavus</i>	Worldwide	Hyphae septate; 45-degree angle branching; occasional fruiting bodies in cavities	Hyphae 10–30 µm (width)	Tissue/vascular invasion; colonization; fungus balls	
Phycomycosis (zygomycosis)	<i>Mucor; Absidia; Rhizopus; Cunninghamella</i>	Worldwide	Hyphae variably sized, ribbon-like nonseptate; 90-degree angle branching	Hyphae 10–30 µm (width)	Tissue/vascular invasion	
Candidiasis	<i>Candida albicans; C. tropicalis; C. glabrata</i>	Worldwide	Budding yeast forming pseudohyphae ("sausage links")	2–10 µm (width)	Respiratory space colonization; tissue invasion	

Cryptococcosis

Cryptococcus neoformans is an inhabitant of the soil and is found in bird droppings. It can act as both a primary and an opportunistic pathogen and may involve numerous body sites. Pulmonary invasion by this fungus is heralded clinically by a productive cough, fever, and weight loss.

Histoplasmosis

Histoplasma capsulatum, another soil inhabitant, is contracted by the inhalation of spores and more commonly affects the immunocompromised. Histoplasmosis

can mimic tuberculosis clinically in that peripheral nodular lesions and mediastinal lymphadenopathy are relatively common. The organism, often present within the cytoplasm of macrophages, is so small that it may be overlooked if silver stains are not used.

Blastomycosis

Blastomyces dermatitidis inhabits wooded terrain. Although the lung is the primary target of infection, there may be distant spread to other organs, such as skin, bone, and the urinary tract.

Coccidioidomycosis

Coccidioides immitis infection is very common in endemic areas of the Southwest and Western United States, giving rise to positive skin tests in more than 80% of individuals in these areas. It produces a respiratory infection that usually resolves spontaneously but persists as a pulmonary mass in about 2% of patients. Multiorgan dissemination is more common in the immunocompromised patient. Because BAL or bronchial washings detect less than 50% of culture-positive cases,¹⁰⁹ cytologic diagnosis is best documented by transthoracic FNA. The organisms appear as mature (sporulating) or immature spherules, often with a fractured (broken ping-pong ball) appearance (Fig. 2.17), and as free endospores (see Table 2.2) in a background of granular eosinophilic debris with little inflammation. Hyphae are seen in 5% of cases. FNA cytology is diagnostically far superior to the culture of aspirated material.¹¹⁰

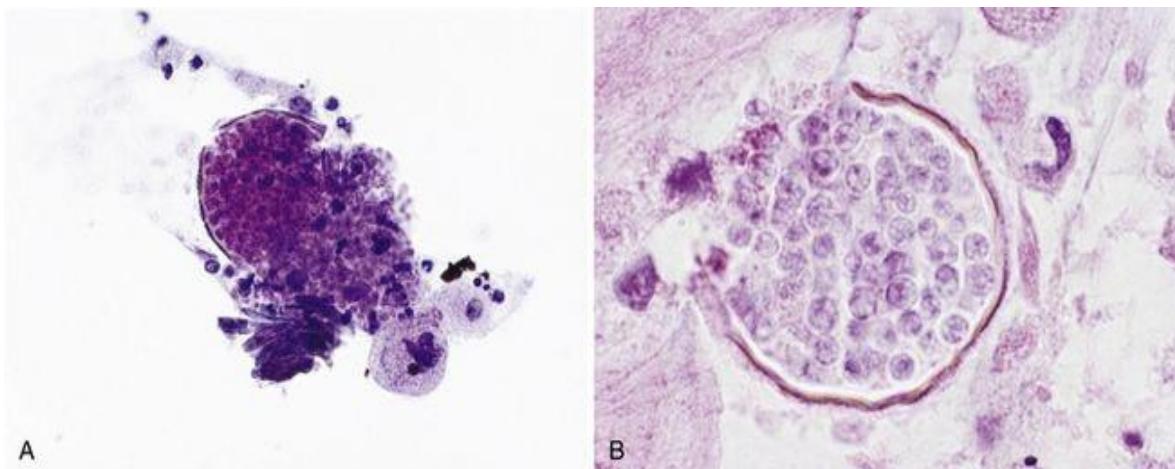


FIG. 2.17 *Coccidioides immitis*.

A, A fractured spherule releases endospores, resulting in the appearance of a broken ping-pong ball (Papanicolaou stain). B, A similar fractured spherule is seen in sections from the cell block (hematoxylin-eosin [H & E] stain).

Paracoccidioidomycosis

Paracoccidioidomycosis, also known as South American blastomycosis, is caused by the dimorphic fungus *Paracoccidioides brasiliensis* (Fig. 2.18) and is the most common systemic mycosis in Latin America.¹¹¹ It frequently involves the lungs and mucocutaneous areas of healthy individuals and clinically

simulates tuberculosis. There may be squamous metaplasia with atypia of the bronchial epithelium overlying granulomas, which may lead to an erroneous diagnosis of squamous cell carcinoma in cytologic material.¹¹² There is a high sensitivity (50% to 90%) for the detection of the organism in cell block preparations of sputum.¹¹³

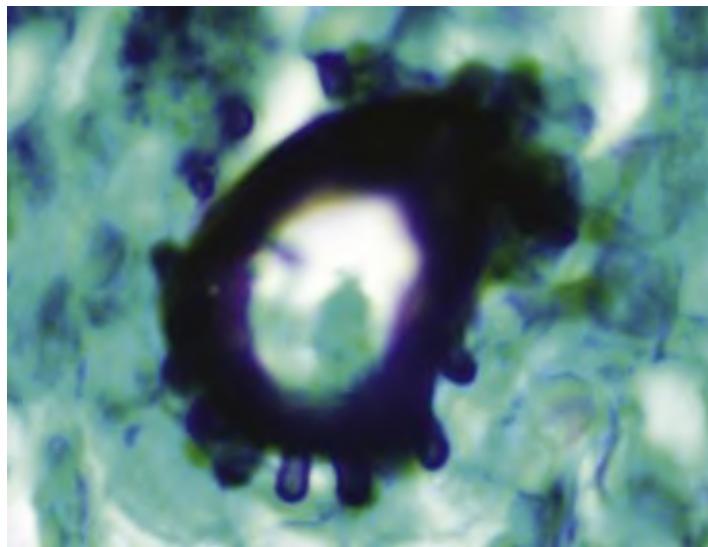


FIG. 2.18 Paracoccidioidomycosis.

A silver stain highlights the ship's wheel appearance of yeast forms budding off of the central parent yeast. The broad-based budding resembles that of *Blastomyces* species—hence its alternative designation, South American blastomycosis.

Sporotrichosis

Pulmonary infection caused by *Sporothrix schenckii* is uncommon and occurs mainly in immunocompromised patients, including diabetics and alcoholics. The clinical symptoms are nonspecific. Though often self-limited, these infections can become chronic, with mass lesions or cavitary nodule formation. The yeasts resemble *Cryptococcus*, *Histoplasma*, and *Candida*, and therefore culture or fluorescent antibody staining is necessary for definitive diagnosis.¹¹⁴

Invasive Fungi

This subgroup of fungi characteristically invades pulmonary tissue, especially blood vessels, of immunocompromised patients. Infections caused by these organisms are readily diagnosed by transthoracic FNA.

Aspergillosis. *Aspergillus* species can cause a variety of pulmonary disorders. Bronchopulmonary aspergillosis is characterized by the expectoration of mucous plugs containing fungal organisms, numerous eosinophils, and Charcot–Leyden crystals. When invasive, there may be hemorrhagic necrosis caused by mycelial invasion of vessels. In cavitary lesions, the organisms sporulate, producing fruiting heads (Fig. 2.19) and are associated with polarizable calcium oxalate crystals. There may be an associated squamous cell atypia that is indistinguishable from carcinoma, making clinical correlation imperative.³⁴

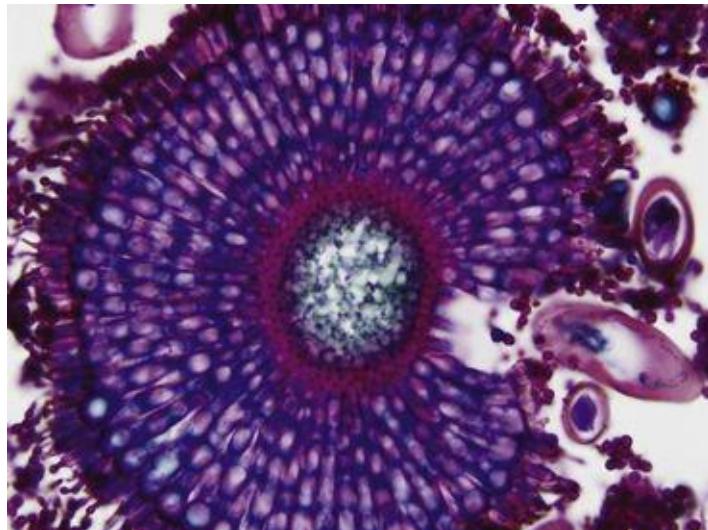


FIG. 2.19 *Aspergillus niger* fruiting body.

This sample was obtained by lung fine-needle aspiration (FNA) (cell block, periodic acid–Schiff [PAS] stain).

Zygomycosis. Zygomycosis is caused by several mycelia-forming fungi, including *Mucor*, *Absidia*, *Cunninghamella*, and *Rhizopus*. They are angioinvasive and often cause infarctions in the debilitated patient.

Candidiasis. *Candida* pneumonia is a common opportunistic infection. An elevated level of *Candida* antigen in BAL fluid suggests true infection rather than colonization.¹¹⁵

Pneumocystis jirovecii

The taxonomy of this organism, formerly called *Pneumocystis carinii*, has been debated, but microbiologists favor classifying it as a fungus, based in part on molecular evidence, hence the change in name to *Pneumocystis jirovecii*.^{116,117} The

pneumonia caused by *P. jirovecii* is particularly common in immunocompromised individuals but has decreased in frequency since the advent of novel therapies.²⁷ The clinical presentation includes dry cough, fever, and dyspnea. Pulmonary infiltrates are usually bilateral.

The organisms are well demonstrated in BAL material, which has a sensitivity that compares favorably with transbronchial biopsy.¹¹⁸ They can also be detected in bronchial washings and induced sputum.¹¹⁹ With Papanicolaou stains, the organisms themselves are not visible, but masses of organisms enmeshed in proteinaceous material result in green, foamy alveolar casts that are more circumscribed than debris or lysed red blood cells ([Fig. 2.20A](#)). The cysts are visualized with silver stains. They are cup-shaped, measure 5 to 7 μm in diameter, and often have a central dark zone ([Fig. 2.20B](#)). No budding occurs, which helps distinguish them from *Histoplasma*. The Giemsa stain highlights the cysts as negative images, but the eight 1.5 μm intracystic bodies or trophozoites are stained as discrete blue dots either within the cysts or as free organisms ([Fig. 2.20C](#)). In some cases, the foamy alveolar casts are absent, and the organisms may be present only in vacuolated macrophages.¹²⁰

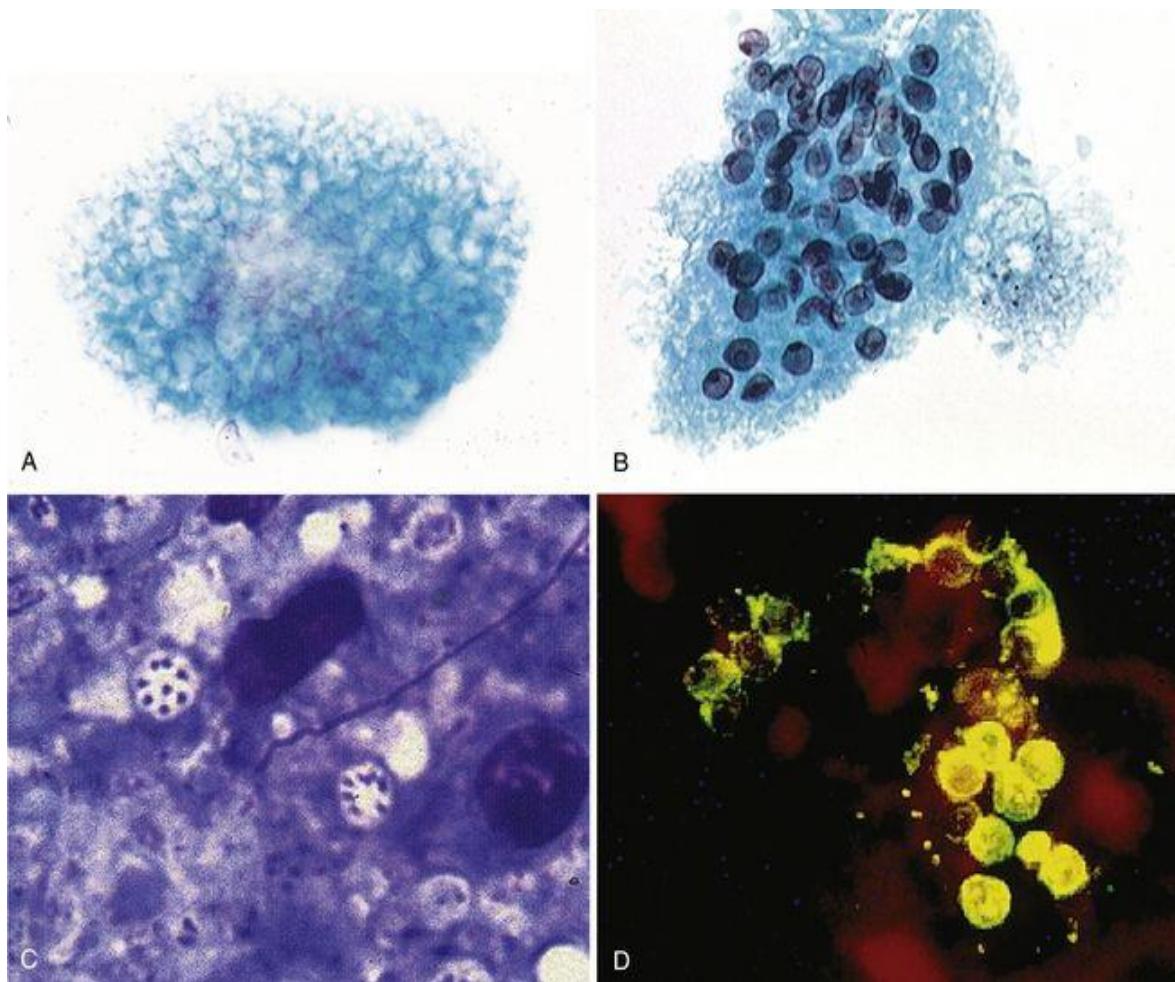


FIG. 2.20 *Pneumocystis jirovecii* (bronchoalveolar lavage [BAL]).

A, With the Papanicolaou stain, the *Pneumocystis* organisms are not seen, but foamy proteinaceous spheres characteristic of this infection are identified. B, The cysts, which have a cup-shaped configuration and a central dark zone, are seen with the methenamine silver stain. C, The Giemsa stain outlines the cysts as negative images and stains the intracystic bodies or trophozoites. Each cyst, as seen here, contains eight intracystic bodies. D, The direct immunofluorescence test is highly sensitive, revealing green fluorescent-stained organisms and their extracellular products.

Direct immunofluorescence has higher sensitivity (up to 92%) than use of Giemsa, toluidine blue, and silver stains.^{121,122} Application of this method to induced sputum (Fig. 2.20D) is the preferred initial diagnostic step because it is noninvasive and highly accurate.

Strongyloidiasis

Pulmonary strongyloidiasis is caused by the nematode *Strongyloides stercoralis*. It can affect immunocompetent people but is more common in the

immunosuppressed patient and presents as a pneumonitis with hemoptysis. Infection of the lungs is caused by the hematogenous migration of the infective larva (filariform larva) from the gut or skin. Histologically, there is a hemorrhagic pneumonia. This organism is identified in sputum or bronchial material by its large size and is differentiated from other hookworms by its notched tail and short buccal cavity ([Fig. 2.21](#)).

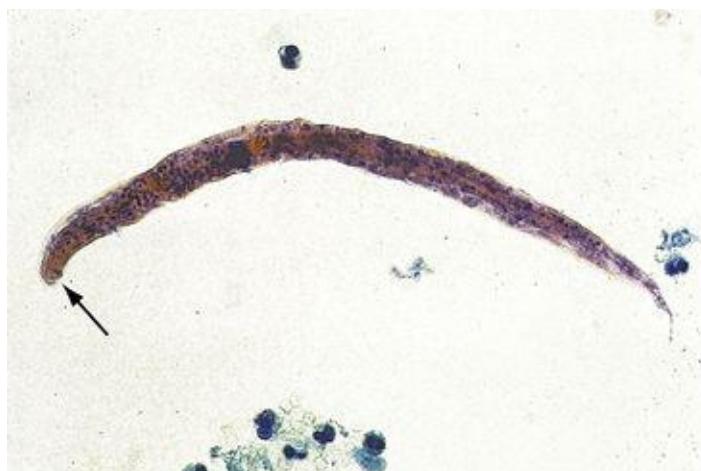


FIG. 2.21 Strongyloides (sputum).
These large roundworms are distinguished by their short buccal cavities (arrow) (Papanicolaou stain).

Dirofilariasis

Dog heartworm (*Dirofilaria immitis*) infection of the human lung has been documented by FNA.¹²³ This microfilarial disease is transmitted from dogs to humans via mosquitos, resulting in entrapment within small pulmonary vessels and subsequent pulmonary infarction. Diagnosis is made by aspiration of the discrete peripheral nodule, which shows necrotic material, fragments of infarcted pulmonary tissue, chronic inflammation, a granulomatous response, and rarely the worm itself.

Echinococcosis (Hydatid Disease)

The clinical and cytologic features of echinococcosis are described in [Chapter 12](#) (see [Fig. 13.4](#)). Sputum may contain scoleces if pulmonary hydatid cysts rupture. Because of the risk of anaphylactic shock, aspiration of a clinically suspected

hydatid cyst may be hazardous.^{57,124}

Nonneoplastic, Noninfectious Pulmonary Diseases

Sarcoidosis

A common disease of unknown etiology, sarcoidosis is characterized by noncaseating granulomas in many organs, most commonly the lung.



Cytomorphology of sarcoidosis

- aggregates of epithelioid histiocytes
- multinucleated giant cells
- lymphocytes

Cytologic specimens show noncaseating granulomas composed of epithelioid histiocytes, with scattered lymphocytes and multinucleated giant histiocytes (see [Fig. 2.16](#)).¹²⁵ The epithelioid histiocytes have round, oval, curved (boomerang-shaped), or spindle-shaped nuclei, with translucent, vacuolated cytoplasm, and are haphazardly arranged in a pseudosyncytial pattern.

Wegener Granulomatosis

This necrotizing vasculitis may present clinically as a lung mass with or without involvement of other organs like the nasal passages and kidneys. The histologic diagnosis of Wegener granulomatosis rests on the identification of necrosis, granulomatous inflammation, and vasculitis.



Cytomorphology of Wegener granulomatosis

- neutrophils
- giant cells
- necrotic collagen (“pathergic necrosis”)
- epithelioid histiocytes

The cytomorphologic findings are nonspecific but include chunks of necrotic tissue (Fig. 2.22), giant cells, granulomas, and neutrophils. The differential diagnosis includes a necrotizing infection (e.g., tuberculous, fungal), lymphomatoid granulomatosis, and other uncommon pulmonary diseases. If suspected on the basis of its characteristic cytomorphology, the diagnosis can be substantiated with serologic studies. The serum immunofluorescent antineutrophil cytoplasmic antibody (ANCA) test greatly aids in establishing the diagnosis of Wegener granulomatosis. Although the classic (cytoplasmic) pattern (c-ANCA) is considered more specific, neither the c-ANCA nor the perinuclear (p-ANCA) pattern is entirely sensitive or specific for the diagnosis of Wegener granulomatosis.¹²⁶

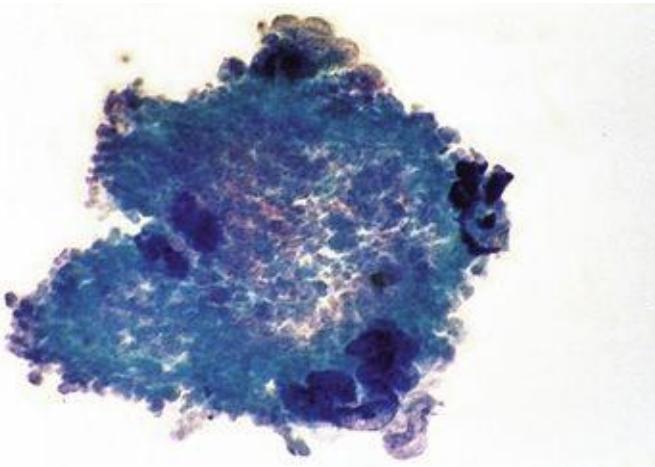


FIG. 2.22 Wegener granulomatosis (fine-needle aspiration [FNA]). A granular background consisting of necrotic collagen without acute inflammation is characteristic (Papanicolaou stain).

Pulmonary Amyloidosis

Pulmonary amyloidosis can be limited to the lung or represent a localized manifestation of systemic disease. Although it affects a wide age range, most patients are in their 50s and 60s. Pulmonary amyloidosis may manifest as nodular parenchymal amyloidosis (a mass lesion whose imaging characteristics mimic those of a neoplasm or granulomatous disease, hence “amyloid tumor”); tracheobronchial amyloidosis (in which the deposits are mostly submucosal and result in dyspnea, wheezing, and recurrent pneumonia); or diffuse parenchymal amyloidosis (with diffuse or multifocal deposits).

FNA yields irregular, waxy, amorphous, hypocellular material with a scalloped, occasionally cracked appearance (see [Fig. 10.11](#)). These deposits appear blue-green on Papanicolaou stains and show apple-green dichroism under polarized light after staining with the Congo red stain. In the nodular parenchymal type there may be multinucleated giant cells, and calcification and ossification are common.

Pulmonary Alveolar Proteinosis

Pulmonary alveolar proteinosis (PAP) is a rare disease characterized by an accumulation of a lipid-rich material within alveoli. PAP is most likely the result of impaired macrophage function due to the production of neutralizing autoantibodies to granulocyte-macrophage colony-stimulating factor (GM-CSF).^{[127,128](#)} Pulmonary alveolar proteinosis can also be secondary to a number of conditions like HIV infection or lung transplantation.^{[126](#)}

The onset is insidious, and one third of patients are asymptomatic despite impressive radiologic abnormalities. There can be a nonproductive cough, dyspnea, and expectoration of gelatinous material. Examination of BAL specimens can be helpful. The characteristic findings include an opaque, milky gross appearance; large, acellular, eosinophilic, PAS-positive blobs ([Fig. 2.23](#)); and pulmonary macrophages filled with PAS-positive material. The differential diagnosis includes pulmonary edema, *P. jirovecii* pneumonia, acute silicosis, and alveolar mucinosis. The diagnosis is made by correlating clinical and imaging findings with laboratory results and the characteristic cytologic findings. Symptomatic patients are treated with whole lung lavage, although recent data suggest that this condition can be treated effectively with GM-CSF replacement therapy.^{[129](#)}

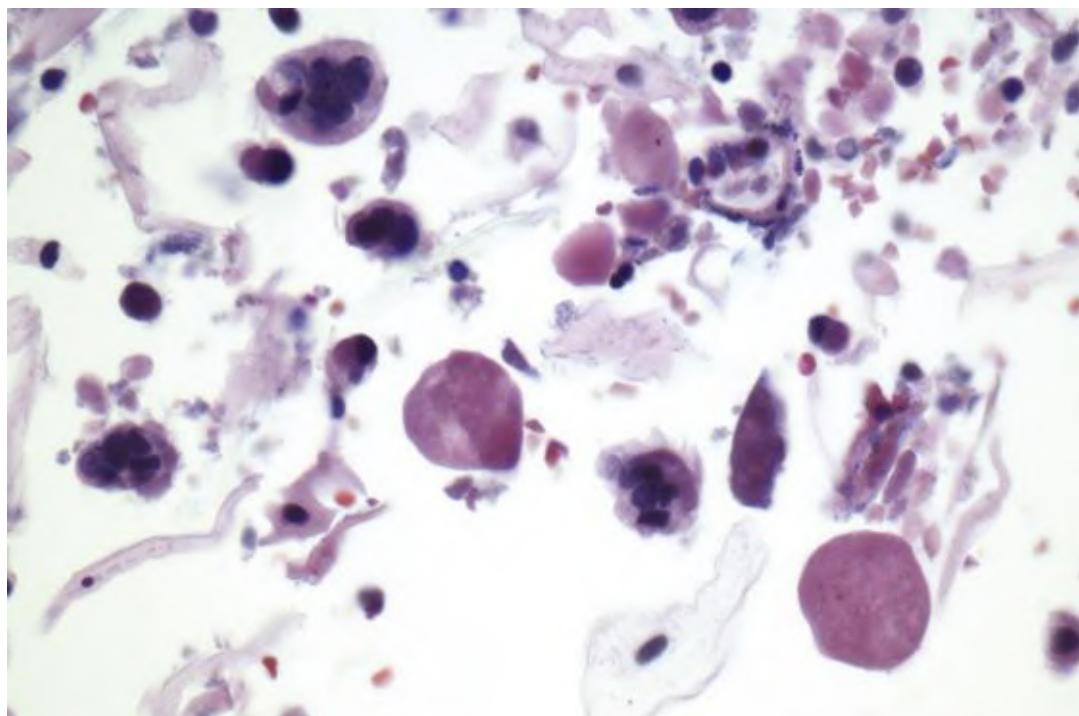


FIG. 2.23 Pulmonary alveolar proteinosis (bronchoalveolar lavage [BAL]). Hematoxylin-eosin (H & E)–stained cell block sections show numerous acellular eosinophilic aggregates.

Common Inflammatory Processes, Including Organizing Pneumonia

Some pneumonias respond slowly to antibiotic treatment, resulting in chronic mass lesions that mimic a neoplasm radiographically. *Organizing pneumonias*, therefore, are occasionally revealed by FNA.



Cytomorphology of organizing pneumonias

- tissue fragments composed of fibroblasts and collagen
- Masson bodies
- numerous pulmonary macrophages, some with hemosiderin
- lymphocytes
- pneumocytes

Because the clinical concern is often for malignancy, the cytologic features of

organizing pneumonias can be initially confusing, because they are unexpected and somewhat unusual. Cytologic preparations contain tissue fragments, often crushed, comprised of spindle-shaped fibroblasts embedded in collagen ([Fig. 2.24A-D](#)). Because the fibrous proliferation often fills alveoli, some fragments have a saccular appearance that corresponds histologically to Masson bodies (see [Fig. 2.24B](#)). Pulmonary macrophages, lymphocytes, and pneumocytes are often numerous; an abundance of such cells may provide the first indication that the lesion is inflammatory in nature rather than neoplastic. Because the findings are relatively nonspecific, however, at best one can only suggest the diagnosis of an organizing pneumonia. The possibility of sampling error cannot be entirely excluded, and it is prudent to mention this in the report.

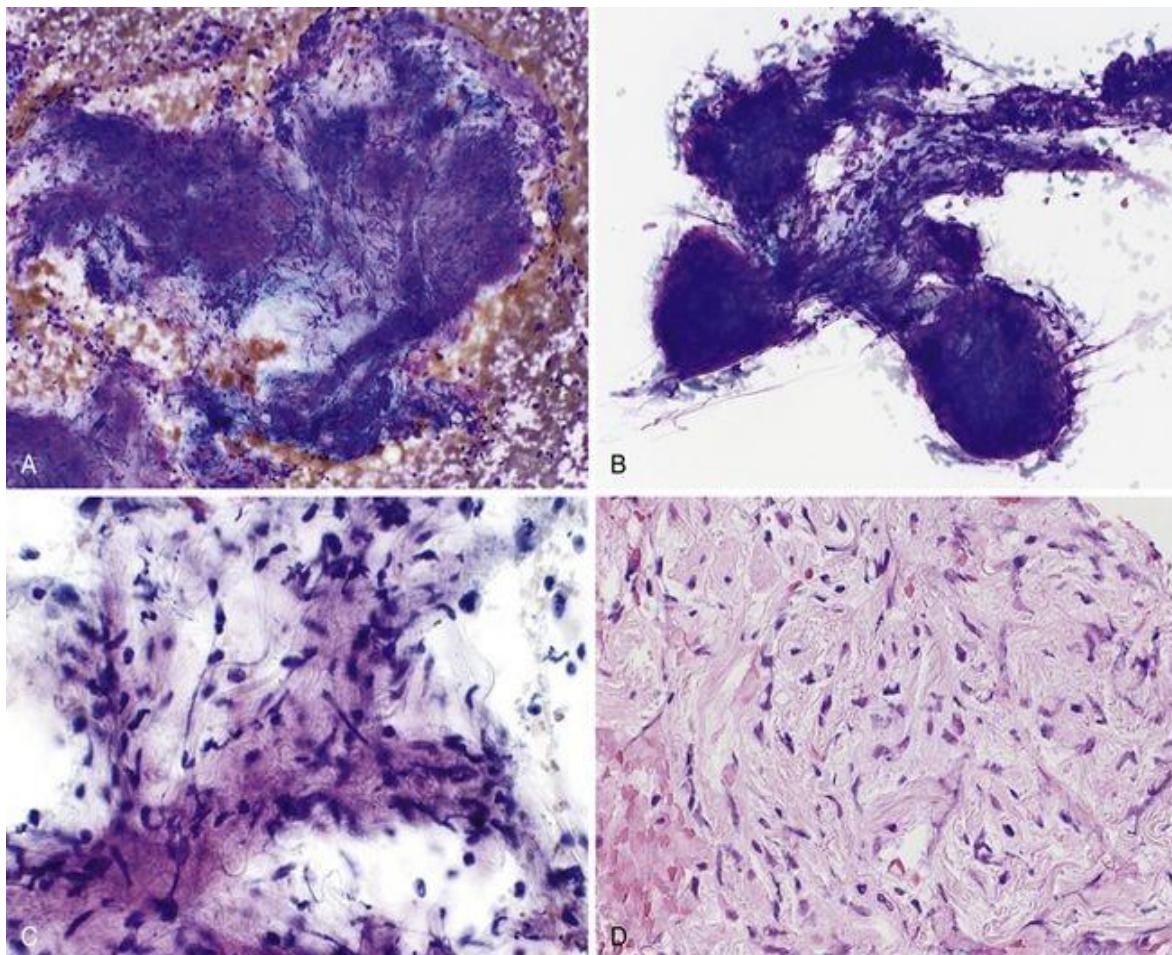


FIG. 2.24 Organizing pneumonia (fine-needle aspiration [FNA]).

A, Large, crushed tissue fragments composed of fibroblasts and collagen are characteristic (Romanowsky stain). B, Fibroblasts and collagen fill alveolar spaces, resulting in nodular masses (Masson bodies) (Romanowsky stain). C, High magnification reveals a mix of

fibroblasts, pneumocytes, macrophages, and fibrous tissue (Papanicolaou stain). *D*, Cell block preparations show fragments of fibrous tissue composed of loosely arranged fibroblasts admixed with chronic inflammatory cells (hematoxylin-eosin [H & E]).

There is considerable overlap in the cytologic features of common pulmonary diseases like organizing pneumonia, *bronchiolitis obliterans obstructing pneumonia* (BOOP, also otherwise known as *cryptogenic organizing pneumonia* (COP)), *diffuse alveolar damage*, and *transplant rejection*. All show fibrous tissue, pneumocytes, macrophages, and lymphocytes in various proportions.^{130–134} Reactive pneumocytes are especially prominent in organizing pneumonia and diffuse alveolar damage (see [Fig. 2.9](#)).

In the setting of lung transplantation, BAL is a routine monitoring procedure, especially for detecting infections, the most common of which are *Candida*, CMV, and HSV.¹³⁵ Neutrophils are normally seen within the first 3 months of transplantation,¹³⁴ and increased numbers of macrophages for years after the transplant.

Benign Neoplasms of the Lung

Pulmonary Hamartoma

Despite their time-honored name, pulmonary hamartomas are, in fact, neoplasms. Recurrent clonal rearrangements have been identified involving the *HMGA1* gene on chromosome 6p21.^{136,137} Most pulmonary hamartomas are discovered incidentally as a solitary discrete, round mass in the lung periphery on thoracic imaging studies. Less commonly, they are central and endobronchial; multiple hamartomas are rare.



Cytomorphology of pulmonary hamartoma

- benign glandular cells
- immature fibromyxoid matrix and bland spindle cells
- mature cartilage with chondrocytes in lacunae
- adipocytes

FNA specimens show a mixture of mesenchymal (fibromyxoid and cartilaginous material) and benign epithelial elements ([Fig. 2.25A-C](#)). In some hands, FNA has a sensitivity of 78% and a specificity of 100% in the diagnosis of a hamartoma.¹³⁸ A nationwide self-assessment testing program, however, has revealed a general lack of familiarity with this lesion, with a troubling false-positive rate (22%). The most common misdiagnoses are carcinoid tumor, adenocarcinoma, and small cell carcinoma.¹³⁹ Hamartoma should be considered for any well-circumscribed neoplasm that contains epithelial cells.

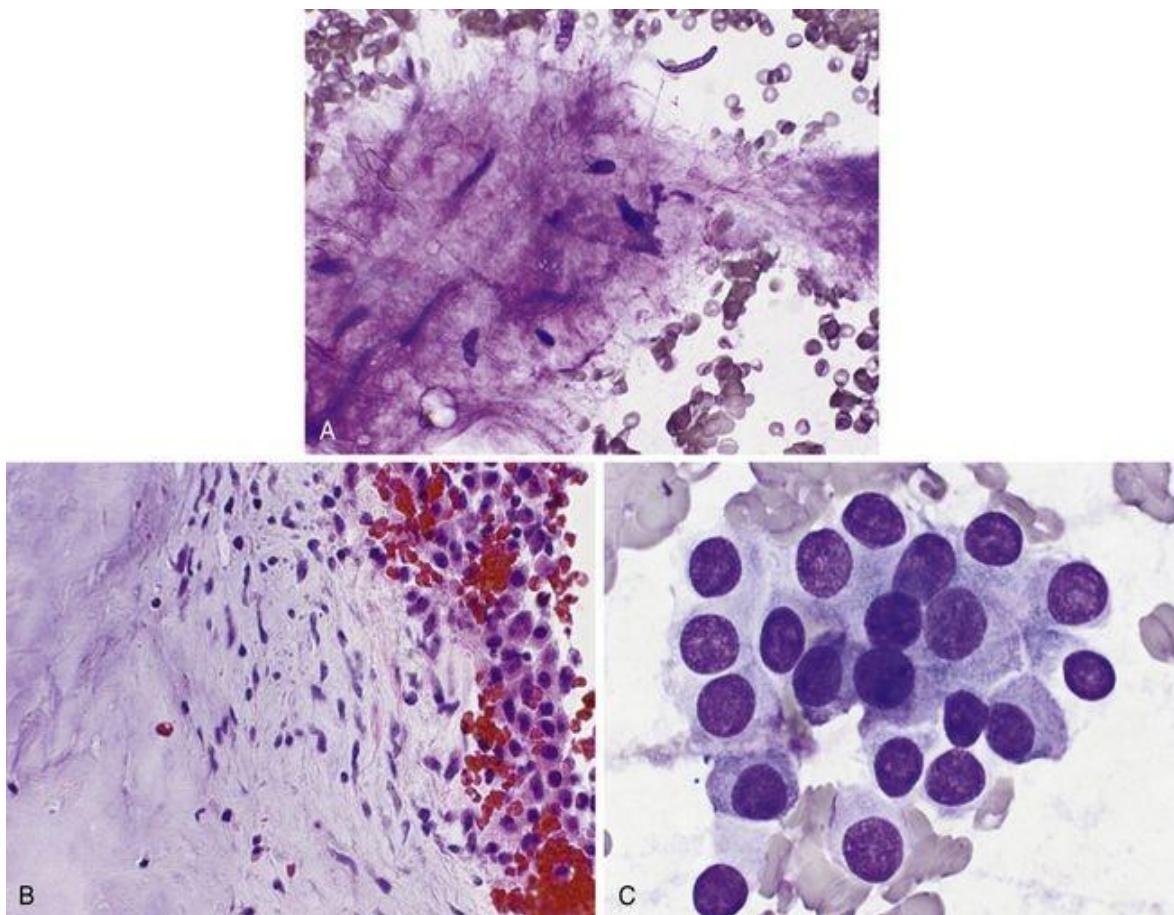


FIG. 2.25 Pulmonary hamartoma (fine-needle aspiration [FNA]).

A, Loose myxoid material is often present (smear, Romanowsky stain). B, The transition between fibromyxoid material and cartilage is seen. Benign epithelial cells are also present (cell block, hematoxylin-eosin [H & E] stain). C, The epithelial component can be monomorphic and cuboidal, as seen here, resembling benign mesothelial cells (smear, Romanowsky stain).

Inflammatory Myofibroblastic Tumor

Inflammatory myofibroblastic tumor (IMT), once known as inflammatory pseudotumor, is a neoplasm of cytologically bland spindle cells that occurs in the lungs and at other sites. It is most common under the age of 40. IMT is usually a peripheral, discrete, solitary nodule. These tumors behave unpredictably: Most patients have an excellent prognosis, but some tumors are locally aggressive.¹⁴⁰ The neoplastic nature of this lesion was confirmed by the cloning of translocations involving the anaplastic lymphoma kinase (ALK) gene (chromosome 2p23) and the two tropomyosin genes, *TPM3* and *TPM4*.¹⁴¹ ALK fusion partner variants include the clathrin heavy chain gene¹⁴² and *ATIC* gene.¹⁴³ Recent evidence suggests that IMT can be effectively treated with crizotinib, the

ALK inhibitor that is also effective in EML4-ALK–positive non–small cell lung cancer.¹⁴⁴



Cytomorphology of inflammatory myofibroblastic tumor

- spindle cells
- storiform pattern
- polymorphous inflammatory cells
- minimal if any necrosis

Cytologic preparations show bland spindle cells arranged as fascicles or in a storiform pattern.¹⁴⁵ There is little pleomorphism and few mitoses. Admixed with the spindle cells is an impressive infiltrate of inflammatory cells, including lymphocytes, plasma cells, histiocytes, and Touton type giant cells (defined as having a peripheral ring of nuclei). The differential diagnosis includes an organizing pneumonia and sarcomatoid carcinoma.

Endobronchial Granular Cell Tumor

Endobronchial granular cell tumors account for a small proportion of all granular cell tumors, which are thought to originate from Schwann cells. They are usually covered by bronchial epithelium and are composed of clusters of tumor cells with small nuclei and abundant, granular, eosinophilic cytoplasm and are surrounded by a thickened basement membrane.



Cytomorphology of endobronchial granular cell tumor

- small clusters of macrophage-like cells
- abundant granular cytoplasm
- small, uniform, round to oval nuclei

On cytologic preparations, the cells are easily overlooked because of their similarity to macrophages (see Fig. 17.29).^{146,147}

Precursor Lesions of the Respiratory Epithelium

Squamous cell carcinoma (SQC) of the lung is preceded by a precursor lesion akin to that of cervical cancer, with a progression from benign squamous metaplasia through dysplasia to invasive cancer. Unlike cervical cancer, however, lung cancer is only very rarely associated with human papillomavirus (HPV) infection,¹⁴⁸ and there is no successful method to screen for and treat precursor pulmonary lesions. Aberrant expression of specific cancer-associated proteins like p53 and the EGFR tyrosine kinase in dysplastic respiratory epithelium precedes the development of invasive carcinoma.^{149,150} Although the precursor lesions are not as well defined as they are for the uterine cervix, most authors acknowledge degrees (mild, moderate, severe) of dysplasia. Although the classification of dysplasia is subjective,¹⁵¹ the risk of developing bronchogenic carcinoma increases with the degree of atypia, which is paralleled by the development of aneuploidy.¹⁵² More than 40% of patients with dysplasia are later diagnosed with SQC.¹⁵³ Dysplastic changes have been demonstrated to regress in experimental systems.¹⁵⁴

The presumed precursor of pulmonary adenocarcinoma is *atypical adenomatous hyperplasia* (AAH).¹⁵⁵ AAH and nonmucinous adenocarcinoma of lepidic type have similar morphologic features and probably represent a continuum of pulmonary alveolar intraepithelial neoplasia. AAH, unlike adenocarcinoma, is usually an incidental finding, because AAHs are small (usually <5 mm in diameter) and lack interstitial inflammation and fibrosis. In AAH, the alveoli are lined by cuboidal cells with features of Clara cells and type II pneumocytes; ciliated cells and mucus cells are not seen. Intracellular inclusions and binucleation are common. Because AAH lesions are small and cytologic atypia is mild, cytologic samples may contain only a few mildly atypical cells ([Fig. 2.26A and B](#)). An outright interpretation of AAH is not used in cytologic samples and is restricted to larger tissue samples.

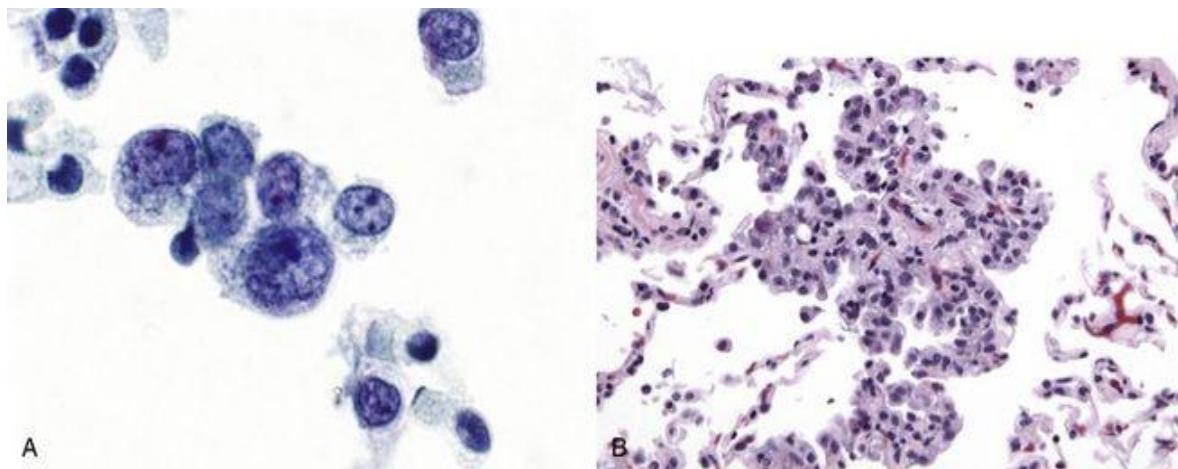


FIG. 2.26 Rare atypical cells (bronchoalveolar lavage [BAL]).

A, This case had a few small clusters of mildly atypical cuboidal cells like this one, showing anisonucleosis and prominent nucleoli. Benign bronchial cells with visible terminal bars are in the background. The case was appropriately interpreted as “atypical” (ThinPrep, Papanicolaou stain). B, A wedge resection from this lobe revealed atypical adenomatous hyperplasia. A minimally invasive adenocarcinoma was present in a different lobe (hematoxylin-eosin [H & E] stain).

Lung Cancer

Carcinoma of the lung is the most common cancer in the world today. Its geographic distribution parallels exposure to tobacco smoking, and, as a result, lung cancer is more common in developed countries. It is the most common fatal malignancy in both men and women in the United States, where it accounts for 29% of all cancer deaths in men and 26% in women.¹⁵⁶

The causes of lung cancer are many. In men, 85% of lung cancers are attributed to cigarette smoking.¹⁵⁷ Tobacco smoke contains numerous carcinogens, tumor initiators, and radioactive compounds, but the biologic mechanism by which tobacco substances initiate lung cancer is not known. Genetic alterations play an important role. Chromosome 3p deletion (seen commonly in neuroendocrine tumors)¹⁵² and p53, K-ras, and p16 mutations are common in lung carcinomas.^{158,159} p53 mutation, the most frequent mutation in human cancers, likely occurs in lung cancer via a G-C to T-A transversion induced by cigarette smoke.¹⁶⁰ Exposure to asbestos has a synergistic effect with smoking: Asbestos workers who smoke increase their risk of developing pulmonary cancer by at least 50-fold. The development of lung cancer has been linked to exposure to other substances, including radon, crystalline silica, nickel compounds, and organic compounds like benzene and vinyl chloride.

Universal screening for lung cancer with chest radiographs and sputum cytology is not cost-effective. The most comprehensive trial in the United States was undertaken by three medical centers under the auspices of the National Cancer Institute. Approximately 30,000 men were screened, and the overall mortality of the screened group was no lower than that of the control group.¹⁶¹ Nevertheless, high-risk individuals, such as workers in the asbestos or uranium industries, people over the age of 65 with a 20-year history of smoking, and those whose radiographs show a persistent abnormality¹⁶² do benefit from periodic screening. More recently, the National Lung Screening Trial evaluated low-dose CT as a modality for screening high-risk individuals and found that mortality could be reduced by 20%. Nodules greater than 4 mm were detected in 39% of enrolled subjects; 72% of the nodules were sampled, but only 1% were malignant.¹⁶³ The findings underscore the importance of safe and effective sampling techniques. Whether these data will change current screening practices remains to be seen; conclusions regarding the cost-effectiveness of this approach have not been finalized.¹⁶⁴

Patients with lung cancer often present with shortness of breath, cough, chest

pain, hoarseness, hemoptysis, or pneumonia. Some tumors, particularly adenocarcinomas, are detected incidentally in asymptomatic individuals.

Four histologic types account for 95% of all pulmonary tumors: squamous cell carcinoma (SQC), adenocarcinoma (ACA), large cell carcinoma (LCC), and small cell carcinoma. Because it is usually metastatic at the time of detection, small cell carcinoma is treated with systemic chemotherapy. The non–small cell carcinomas (SQC, ACA, and LCC) are more likely to be localized at the time of diagnosis, which allows for curative resection by lobectomy or pneumonectomy. The subclassification of nonsmall cell carcinomas—histologic and molecular—is becoming more important as novel therapies show success in the treatment of specific subtypes, like adenocarcinomas with EGFR mutations (see above and [Fig. 2.4](#)).

Lung cancers often show histologic heterogeneity. Almost 50% of lung cancers contain more than one histologic type.¹⁵⁷ Thus, careful examination of all cytologic material for more than one histologic component is essential for correct classification.

Squamous Cell Carcinoma

SQC of the lung accounts for approximately one third of all primary pulmonary malignancies. Two thirds of these tumors occur in a central location, and the remainder arise in smaller bronchi. Postobstructive pneumonia and cavitation of the tumor are common. Of all the histologic subtypes, SQC is the one most commonly associated with hemoptysis. Apical SQCs in the superior sulcus are called Pancoast tumors, characterized by posterior rib destruction and neural invasion, resulting in severe pain and Horner syndrome (enophthalmos, ptosis, miosis, and ipsilateral decreased sweating). Several histologic variants (papillary, clear cell, small cell, basaloid) have been identified.¹⁵⁷



Cytomorphology of well-differentiated squamous cell carcinoma

- abundant noncohesive cells
- polymorphic cell shapes: polygonal, rounded, elongated (fiberlike), tadpole-shaped
- dense cytoplasmic orangeophilia (Papanicolaou stain)
- pyknotic nuclei
- frequent anucleate cells

The cytologic features vary depending on the degree of squamous differentiation. In a well-differentiated SQC, the malignant cells are noncohesive and come in a variety of shapes (polygonal, spindle, tadpole; [Fig. 2.27A and B](#)), with abundant smooth, dense cytoplasm filled with keratin. The cytoplasm stains green, yellow, or orange with the Papanicolaou stain and robin's egg blue with Romanowsky stains. Nuclei are usually small, hyperchromatic, and smudgy, and nucleoli are often inconspicuous.

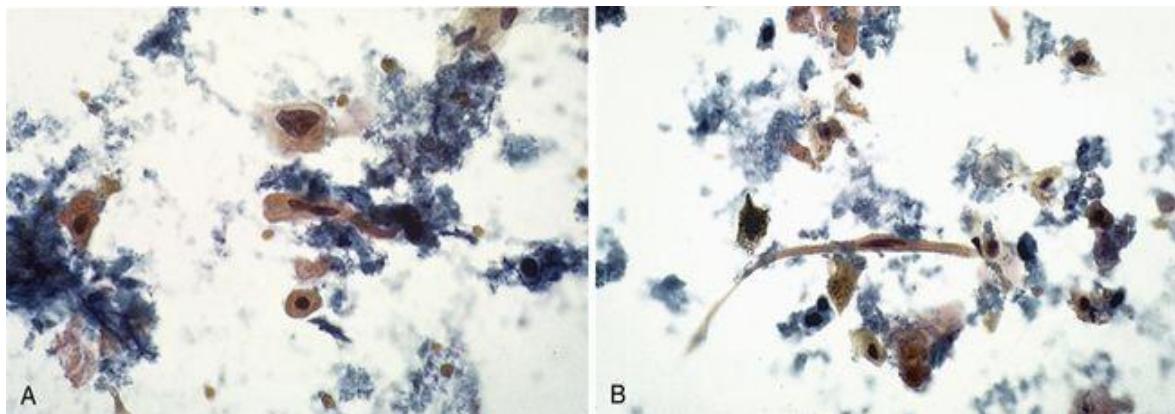


FIG. 2.27 Well differentiated squamous cell carcinoma (fine-needle aspiration [FNA]).
A, Well-differentiated squamous cell carcinomas are composed of cells with dense, orangeophilic cytoplasm and hyperchromatic, often pyknotic nuclei, some with angular contours. B, Bizarre, elongated, spindle-shaped cells are common. Often abundant granular debris, seen here, and inflammation are present.



Cytomorphology of moderately and poorly differentiated squamous cell carcinomas

- large, cohesive clusters of spindle cells
- rare to absent keratinization
- large nuclei
- coarse chromatin texture (“Idaho potato”)
- ± prominent nucleoli

In moderately and poorly differentiated SQCs, keratinization is less apparent or absent altogether. The best clue to squamous origin is the presence of spindled

cells within dense clusters. These clusters can have the streaming appearance of a school of fish but will show the typical nuclear features of malignancy. The cytoplasm may retain the characteristic dense, smooth appearance of most SQCs, but in some poorly differentiated SQCs it is granular and scant. Nuclei are larger, and nucleoli are prominent. Chromatin, rather than smudgy, is very coarsely textured and resembles the mottled and pitted surface of an Idaho potato ([Fig. 2.28](#)). The basaloid variant of SQC shows prominent palisading of nuclei around the perimeter of cell groups.

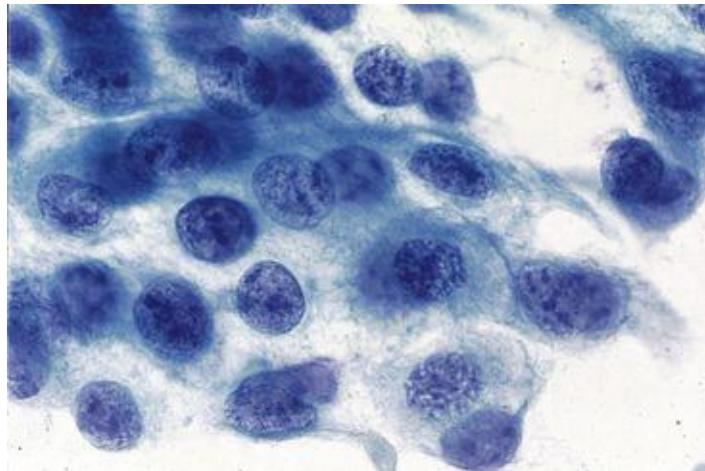


FIG. 2.28 Poorly differentiated squamous cell carcinoma (fine-needle aspiration [FNA]). Poorly differentiated squamous carcinoma cells are often arranged in thick groups of pulled-out, spindle-shaped cells, rather than as isolated cells. The nuclei are enlarged, and chromatin is coarsely granular, in contrast to the dense, frequently pyknotic nuclei of well-differentiated (keratinizing) squamous cell carcinomas (SQCs).



Differential diagnosis of squamous cell carcinoma

- squamous metaplasia
- degenerative changes
- reactive squamous atypia (e.g., aspergilloma)
- vegetable cells
- contamination from an upper airway cancer
- adenocarcinoma
- large cell carcinoma
- small cell carcinoma
- NUT (nuclear protein in testis) midline carcinoma
- metastatic squamous cell carcinoma

Squamous metaplastic cells (from chronic irritation to the bronchial epithelium) are recognizably benign because of their small, round, normochromatic nuclei. In sputum samples, degenerative changes impart a pyknotic, condensed appearance to the nuclei of benign squamous cells that mimics the pyknotic, smudgy nuclei of SQC. Benign degenerated nuclei, however, are small and do not vary in size and shape as much as those of SQC. Reactive squamous cell atypias occur adjacent to cavitary fungal infections ([Fig. 2.29](#)), stomas, and almost any injury to the lung (e.g., infarction, radiation, chemotherapy, sepsis, and diffuse alveolar damage).^{33,34} To avoid a false-positive, caution is advised when the atypical squamous cells are few in number, poorly visualized, or degenerated, or associated with a stoma, fungal infection, or any manner of severe lung injury. Vegetable food particles occasionally resemble keratinized squamous cells, but their cellulose wall and the regularity of their shape usually permit correct identification (see [Fig. 2.13](#)). Occasionally, malignant cells from an SQC of the upper airway may contaminate a specimen of the lower respiratory tract.²⁹

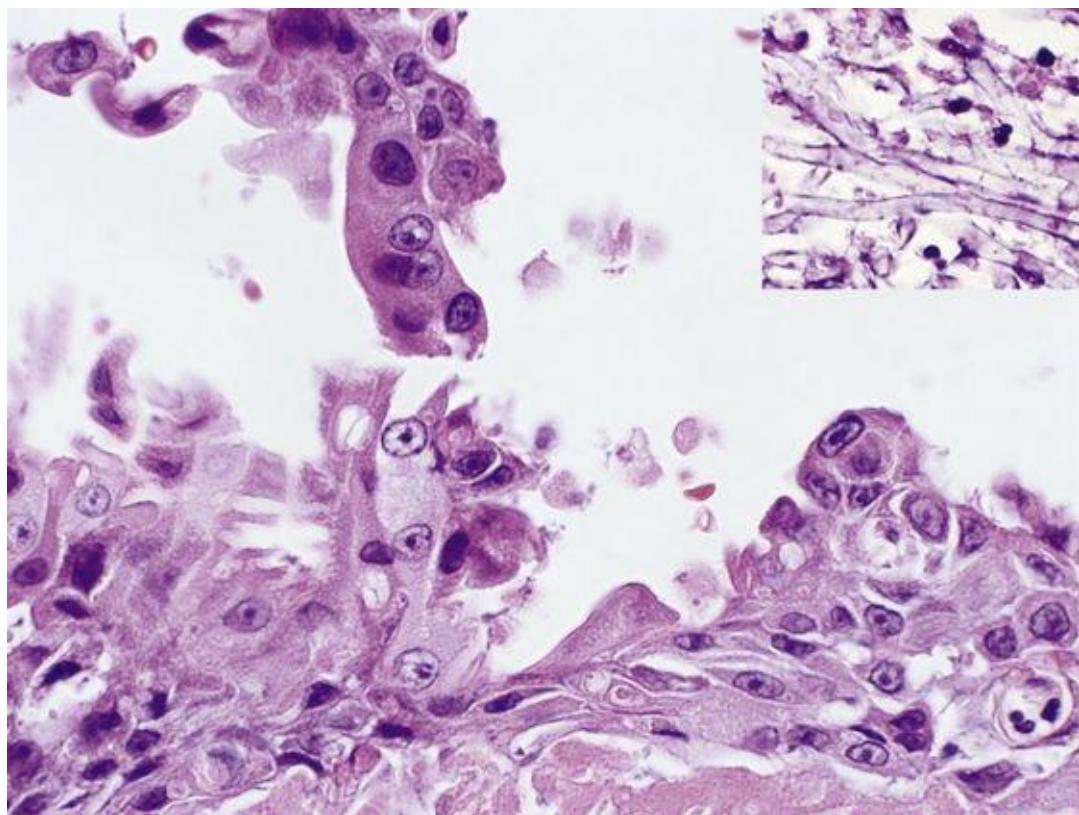


FIG. 2.29 Atypical squamous metaplasia (fine-needle aspiration [FNA]). Cavitary fungal infections, like this one by *Aspergillus* species (*inset*), are among the causes of reactive squamous atypia, a mimic of squamous cell carcinoma (SQC).

Because the distinction between SQC and adenocarcinoma (ACA) determines eligibility for molecular testing and the choice of targeted therapies, it is important to refine a malignant diagnosis as much as possible, even with limited samples.¹⁶⁵ Most SQCs and ACAs of the lung are easily distinguished from each other by cytomorphology, based on the presence of obvious keratinization (in squamous cancers) and abundant mucin and/or glandular structures (in adenocarcinomas). With poorly differentiated cancers, however, the distinction is more difficult. In general, nuclear chromatin is more finely textured in ACAs and coarser in SQCs. The cytoplasm is thinner, more foamy (vacuolated), and transparent in ACAs and denser in SQCs. Of note, focal intracellular mucin can be present in SQCs and cannot be relied upon for this distinction.

If the cytomorphology is not clear-cut, the International Association for the Study of Lung Cancer recommends immunohistochemistry for TTF-1 and p63 as the first line stains in the distinction between SQC and ACA (Table 2.3). (Other stains [CK5/6, CK7] are less sensitive and specific in this differential.)¹⁶⁵ If a cytologically indeterminate non–small cell carcinoma is immunoreactive for TTF-1, it is reported as “non–small cell carcinoma, favor adenocarcinoma.” If immunoreactive for p63 and negative for TTF-1, a cytologically indeterminate tumor is reported as “non–small cell carcinoma, favor squamous cell carcinoma.” Tumors positive for both TTF-1 and p63 are reported as “non–small cell carcinoma, favor adenocarcinoma.” If TTF-1 and p63 are positive *in different populations of tumor cells*, however, the tumor is reported as “non–small cell carcinoma, not otherwise specified,” with a comment that it might represent an adenosquamous carcinoma. If all markers are negative, the tumor is reported as “non–small cell carcinoma, not otherwise specified.” The report should clarify if the interpretation was made based on cytomorphology alone or whether special stains were required.

TABLE 2.3
TTF-1 AND P63 IN THE DIAGNOSIS OF SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA OF THE LUNG¹⁶⁵

Scenario	TTF1	p63	Diagnosis	Frequency	Next steps?
1	+ to +++	-	NSCLC, favor ACA	~90% of ACA	
2	+++	+ to +++ (in same cells)	NSCLC, favor ACA	0% of SQC	
3	-	+++	NSCLC, favor SQC*	>95% of SQC 0% of ACA	
4	+++	+++ (in different cells)	NSCLC, NOS, possible adenosquamous carcinoma	Rare (~1% of NSCLC)	Formal classification deferred to resection
5	-	-	NSCLC, NOS*	~10% of ACA 0% of SQC	Mucin stain, Napsin
6	-	+	NSCLC, NOS*	1% ACA 1% SQC	Mucin stain, CK5/6

ACA, adenocarcinoma; NOS, not otherwise specified; NSCLC, non–small cell lung cancer; SQC, squamous cell carcinoma ; *TTF1*, thyroid transcription factor 1.

*Exclude metastatic disease.

It is acceptable to substitute p40 for p63; p40 is much more specific for SQC than p63, which is at least focally positive in 30% of pulmonary adenocarcinomas.¹⁶⁶

The small cell variant of SQC is composed of smaller cells than the usual SQC. It is distinguished from small cell carcinoma because the cells of the small cell variant of SQC have coarse or pale chromatin, more prominent nucleoli, and distinct cell borders.

The possibility of a NUT (nuclear protein in testis) midline carcinoma, a subtype of squamous cell carcinoma, should be considered for any poorly differentiated malignancy that lacks glandular differentiation. This is true particularly when there is evidence of squamous differentiation (e.g., immunoreactivity for p63, keratinization). The diagnosis of NUT midline carcinoma is straightforward because, outside of the testis, nuclear immunohistochemical staining for the NUT protein is 100% specific and 87% sensitive for NUT midline carcinoma.¹⁶⁷

Metastatic SQCs are morphologically indistinguishable from primary SQCs of the lung. The distinction usually relies on clinical impression. In selected cases, molecular studies can be helpful: Testing for HPV can be helpful if there is a question of primary lung cancer versus metastatic SQC of the cervix.

Adenocarcinoma

ACA is the most common histologic subtype of lung cancer. Most ACAs occur in the periphery of the lung and are associated with a desmoplastic reaction and pleural puckering. Of all the histologic types, ACAs are most likely to be discovered incidentally in an asymptomatic individual. Some are detected based on clinically evident metastases. ACAs rarely show cavitation.

To improve upon the prior classification system for lung cancers and link therapeutically relevant molecular alterations with histologic and cytologic

findings, ACAs of the lung have been reclassified by an international consortium consisting of the International Association for the Study of Lung Cancer, the American Thoracic Society, and the European Respiratory Society.¹⁶⁵ A major goal of the new classification system is to eliminate the term *bronchioloalveolar carcinoma* due to a lack of agreed-upon criteria for its diagnosis and management. The new classification divides ACA into four groups based on surgical excision specimens: preinvasive lesions (including atypical adenomatous hyperplasia and adenocarcinoma in situ), minimally invasive ACA, invasive ACA, and variants of invasive ACA. ACA in situ (formerly bronchioloalveolar carcinoma) is defined by its growth along alveolar septa (lepidic growth) without destruction of the underlying alveolar architecture. There are two ACA in situ subtypes: *nonmucinous* (the great majority) and *mucinous* (a rarity). Metastatic pancreaticobiliary and colorectal ACAs can mimic a mucinous ACA in situ.

Invasive ACAs are classified histologically into five subtypes based on their predominant pattern: *lepidic predominant* (formerly nonmucinous bronchioloalveolar carcinoma), *acinar predominant*, *papillary predominant*, *micropapillary predominant*, and *solid predominant with mucin production*. The micropapillary predominant invasive ACA has a poorer prognosis than the others and is therefore important to recognize. Under “variants of ACA,” the new classification scheme lists the previously included *fetal* and *colloid* types and adds two new variants: *mucinous* (formerly mucinous bronchioloalveolar carcinoma) and *enteric*. Three prior variants—mucinous cystadenocarcinoma, clear cell, and signet ring—are now regarded as nondistinct entities. The rare fetal ACA resembles the epithelial component of the pulmonary blastoma (see “Sarcomatoid Carcinoma” below).¹⁶⁸ Mucinous ACA is now recognized as a clinically and genetically distinct variant of ACA, harboring KRAS mutations in 76% of cases^{165,169–172} and lacking EGFR mutations.^{165,169–172} Mucinous ACA is easily confused with metastatic colorectal ACA because it often (though not always) expresses CK20, not CK7.^{173–175}

The new classification applies to cytology and small biopsy specimens, with the understanding that specific ACA patterns and variants usually cannot be precisely identified and reported on limited samples. The principal recommendation of the new classification system is that the diagnosis “non–small cell carcinoma” be minimized and that a reasonable attempt be made to establish (or “favor”) either ACA or SQC. The rationale behind this expectation is that ACAs should be tested for therapeutically relevant targetable mutations. If the distinction is not possible by cytomorphology, limited, “first line” immunohistochemistry for just two markers (TTF-1 and p63) is recommended

(see above and [Table 2.3](#)). It is imperative that special stains be performed sparingly, so as to preserve tissue for molecular studies, if indicated. It is acceptable to substitute p40 for p63.



Cytomorphology of adenocarcinoma

- honeycomb like sheets, three-dimensional clusters, acini, papillae
- eccentrically placed, round or irregular nuclei
- finely textured chromatin
- large nucleoli
- mucin vacuoles
- translucent, foamy cytoplasm

There is great heterogeneity in the cytomorphology of lung ACAs, depending on the predominant histologic pattern. The malignant cells can be arranged in flat sheets with or without acinar formations, densely packed solid masses, true papillae with fibrovascular cores, or small, tight micropapillary balls ([Figs. 2.30 through 2.34](#)). These patterns are generally better appreciated on cell block preparations. Scattered isolated tumor cells are also present. Cytoplasm is usually abundant, with well-defined borders. It can be thin and translucent or foamy. Nuclei usually have round, smooth contours, but in some tumors the nuclear membranes are highly irregular. Chromatin is finely textured in most ACAs, but some poorly differentiated ACAs have coarsely textured chromatin. Nucleoli are prominent in many ACAs. Psammoma bodies can be seen in the micropapillary predominant pattern.

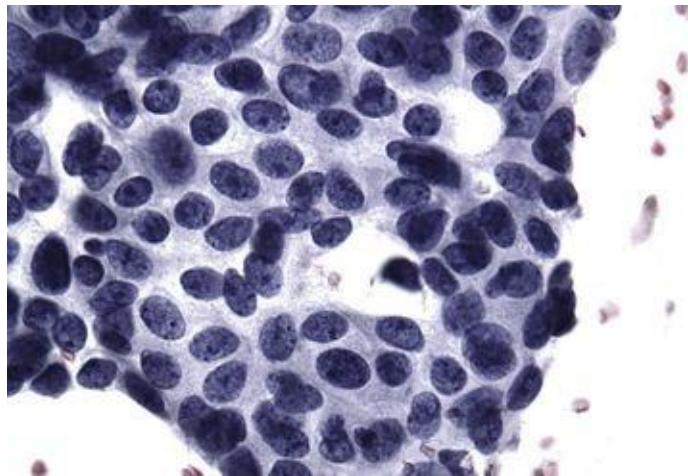


FIG. 2.30 Adenocarcinoma (acinar predominant).

The cells of this tumor are relatively uniform in size and shape, with small or medium-sized nucleoli. The finely textured chromatin is characteristic of adenocarcinomas as compared with squamous cancers. There are two prominent acinar spaces. Note the indistinct cell borders, imparting a syncytium-like appearance to this sheet of malignant cells. Contrast with the mesothelial cells in [Fig. 2.3](#), where slitlike spaces separate adjacent cells (smear, Papanicolaou stain).

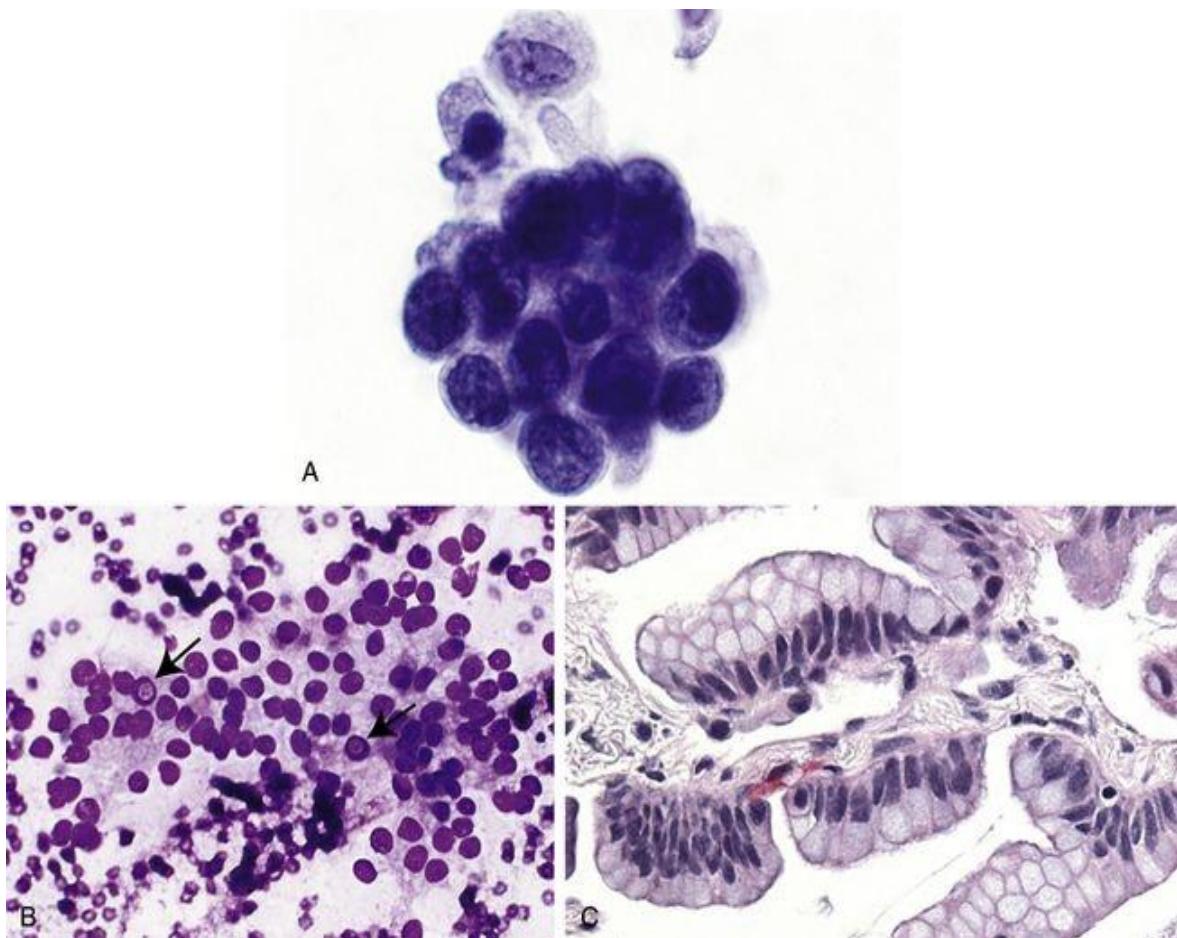


FIG. 2.31 Adenocarcinoma (ACA) (lepidic predominant).

A, Nonmucinous lepidic predominant ACA. These cells are hyperchromatic and have a high nuclear-to-cytoplasmic ratio, with scant microvacuolated cytoplasm. There are no clues to suggest a lepidic predominant pattern (bronchoalveolar lavage [BAL], Papanicolaou stain). B, Mucinous lepidic predominant ACA. The flat honeycomb like sheet of these uniform tumor cells is characteristic, as are the intranuclear pseudooinclusions (*arrows*). Nuclear grooves are better seen with the Papanicolaou stain (not shown). Overall, the nuclear changes are reminiscent of papillary carcinoma of the thyroid. Abundant extracellular mucin (not shown) is often present (smear, Romanowsky stain). C, Mucinous lepidic predominant ACA (histologic section). The tumor cells grow along alveolar septae, without tissue destruction (hematoxylin-eosin [H & E] stain).

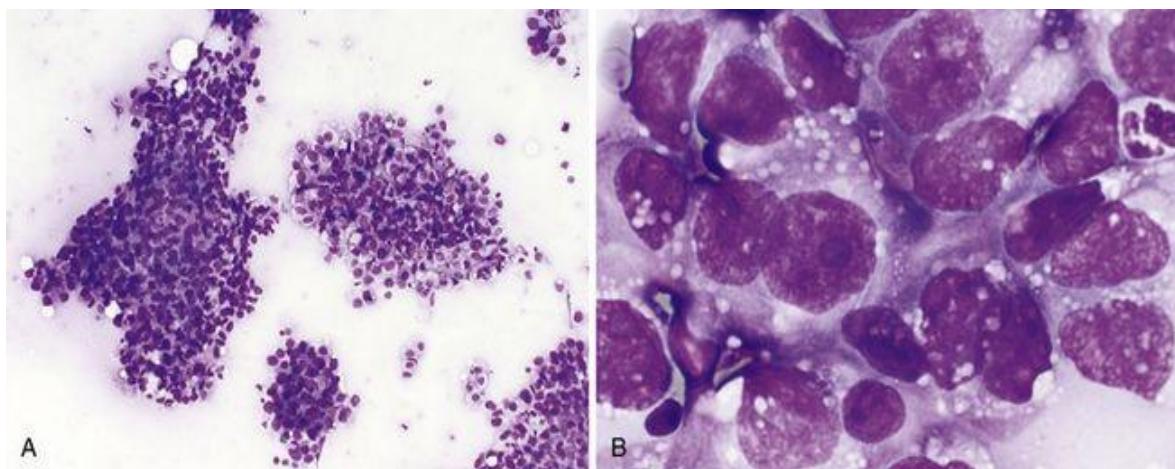


FIG. 2.32 Adenocarcinoma (solid predominant).

A, These malignant cells are arranged in large, densely packed, solid clusters. *B*, High magnification of this tumor reveals marked nuclear enlargement, with irregular nuclear contours and prominent nucleoli. Note the microvacuolated cytoplasm, a characteristic feature of adenocarcinomas as compared with squamous cancers.

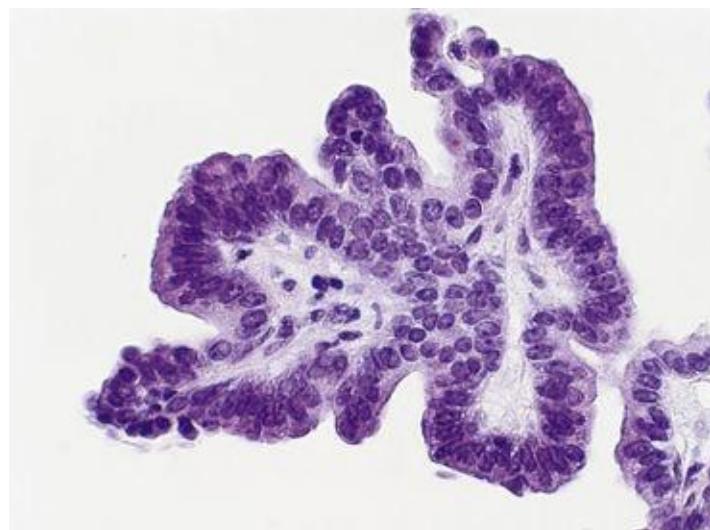


FIG. 2.33 Adenocarcinoma (papillary predominant).

These crowded malignant cells line a fibrovascular stalk (cell block, hematoxylin-eosin [H & E] stain).

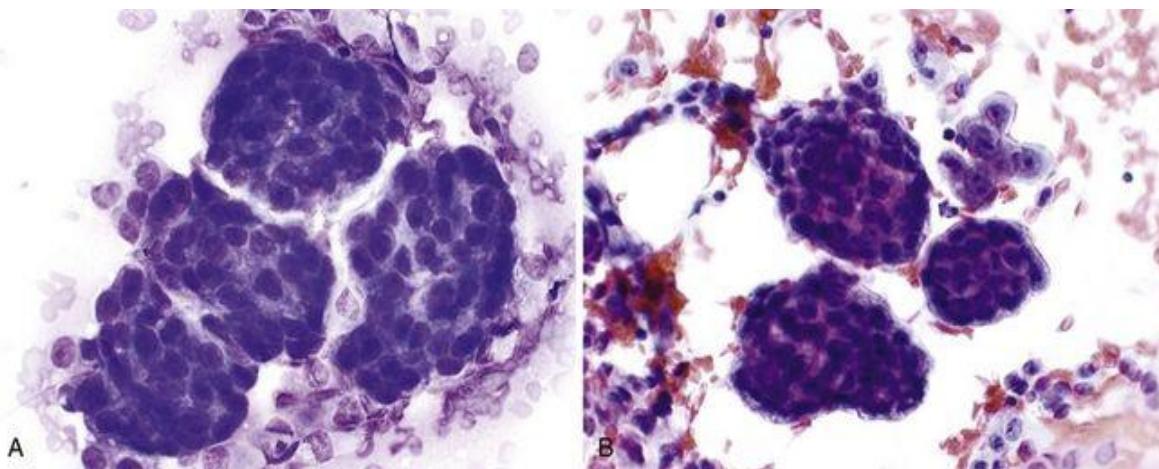


FIG. 2.34 Adenocarcinoma (micropapillary predominant).

A, The cells are arranged in very tight balls without a fibrovascular core (smear, Romanowsky stain). B, The nuclear features, including the prominent nucleoli, are better seen with alcohol-fixed preparations. This pattern can be mimicked by other adenocarcinomas, especially when samples are processed using liquid-based methods (see [Fig. 2.31A](#)) (smear, Papanicolaou stain).

Cytologic preparations from a mucinous ACA (invasive or *in situ*) show honeycomb like sheets of uniform cells with pale, optically clear nuclei and inconspicuous nucleoli. Grooves and nuclear pseudoinclusions are often present (see [Fig. 2.31B](#)). In some cases, the cells of a mucinous ACA are dispersed and resemble macrophages. The distinction among an ACA *in situ*, a minimally invasive ACA, and an invasive ACA cannot be made on a cytologic specimen. The cytologic interpretation “positive for malignant cells, adenocarcinoma” implies all three possibilities.^{[157,176](#)}



Differential diagnosis of adenocarcinoma

- reactive bronchial cells
- Creola bodies
- reactive pneumocytes
- atypical adenomatous hyperplasia
- mesothelial cells (FNA specimens)
- vegetable cells
- hamartoma
- squamous cell carcinoma
- large cell carcinoma
- small cell carcinoma
- epithelioid hemangioendothelioma and epithelioid angiosarcoma
- metastatic adenocarcinoma

Benign bronchial cell atypia and hyperplasia (anisonucleosis due to inflammation or injury, Creola bodies) can be recognized as a harmless mimic of adenocarcinoma if the atypical/hyperplastic cells have cilia (see [Figs. 2.6](#) and [2.7](#)) and/or demonstrate a continuous spectrum of changes (from benign to markedly atypical). In a bronchial specimen, where malignant cells are often intermixed with benign bronchial cells, it is usually straightforward to identify two distinct cell populations, one malignant, the other benign.

The cells of a florid type II pneumocyte hyperplasia resemble those of ACA (see [Fig. 2.9](#)). Attention to the clinical history (e.g., respiratory distress and diffuse infiltrates) can provide a clue that the cells are reactive. In an acutely ill patient with diffuse pulmonary infiltrates, markedly atypical cells should be interpreted with caution. Sequential respiratory specimens can help, because hyperplastic pneumocytes are not present in BAL specimens more than a month after the onset of acute lung injury.³² Atypical adenomatous hyperplasia (AAH) resembles reactive type II pneumocyte hyperplasia in cytologic samples. Unlike ACA, however, AAH is usually an incidental finding because AAHs are small (usually <5 mm) and lack interstitial inflammation and fibrosis. Cytologic samples may contain only a few mildly atypical cells (see [Fig. 2.26A](#)). An outright interpretation of AAH is restricted to larger tissue samples.

Mesothelial cells are common in percutaneous FNA specimens, and in some cases they can be numerous (see [Fig. 2.3](#)). They resemble the cells of a well-differentiated ACA, particularly a mucinous ACA, but are recognized as benign mesothelial cells by their cohesion and the characteristic slitlike “windows” that separate them from each other. Some percutaneous FNAs fail to sample the target lesion and obtain only mesothelial cells. In such cases it is important to identify the sample as insufficient (nondiagnostic).

Vegetable cells (see [Fig. 2.13](#)) and other contaminants can mimic the cells of an ACA but are recognized as contaminants because of their prominent capsule.

Some hamartomas have a conspicuous glandular component that resembles that of a well or even moderately differentiated ACA. Fragments of chondromyxoid matrix in the background are essential for establishing the diagnosis of a hamartoma and avoiding a false-positive interpretation.

Most ACAs are easily distinguished from SQCs because there is obvious keratinization, mucinous differentiation, and/or acinar formation. The cells of most ACAs are more cohesive than those of a keratinizing SQC. When a tumor is poorly differentiated, however, the distinction becomes more difficult. In general, nuclear chromatin is more finely textured in ACAs and coarser in SQCs. The cytoplasm is thinner and more vacuolated in ACAs and denser in SQCs. A stain for mucin can be helpful: Although focal intracellular mucin can be seen in

SQCs, abundant intracellular mucin is diagnostic of an ACA. Immunostaining for TTF-1 and p63 (or p40) helps classify cytologically indeterminate non–small cell carcinoma cases ([Table 2.3](#)).¹⁶⁵

The vascular tumors **epithelioid hemangioendothelioma** and **epithelioid angiosarcoma** occur as primary tumors in the lung and other sites. Epithelioid hemangioendothelioma is a low to intermediate grade tumor, and epithelioid angiosarcoma is high grade. They can present as a solitary mass or as multiple, bilateral pulmonary masses. Pleural involvement mimicking mesothelioma is not uncommon (see [Fig. 4.18](#)), and liver involvement is seen in 15% of cases. The cells of both tumors mimic carcinomas because they are epithelioid in shape. Epithelioid hemangioendothelioma is the more likely to be confused with ACA because it is lower grade.¹⁷⁷ The common occurrence of intracytoplasmic vacuoles and intranuclear inclusions in epithelioid hemangioendothelioma (and epithelioid angiosarcoma) only adds to the confusion. The diagnosis of epithelioid hemangioendothelioma and epithelioid angiosarcoma is established by immunohistochemistry for vascular markers like CD31, CD34, and ERG.¹⁷⁸ About 30% are also reactive for keratins, however, and might be misinterpreted as carcinomas if a wider antibody panel is not used.

The differential diagnosis also includes metastatic ACA. In a patient with a history of a previous neoplasm, it is helpful to compare the current specimen with the prior cytologic or histologic material to exclude a metastasis. Some cytologic features, such as the “dirty” necrosis and tall columnar cells of colorectal cancer, may suggest a specific primary site. Some metastatic tumors, however, are virtually indistinguishable from a primary lung ACA. Metastatic papillary thyroid carcinoma mimics some mucinous lung ACAs to perfection: Both tumors have intranuclear cytoplasmic pseudoinclusions, nuclear grooves, and optically clear nuclei. Immunohistochemistry can be essential. ACAs of the lung are typically positive for CK7 and negative for CK20, and 75% to 85% express TTF-1. Mucinous ACA is an exception, as it is often positive for CK20 and negative for TTF-1. Organ-specific antigens such as thyroglobulin, prostate-specific antigen, Hepar1, PAX-8, and renal cell carcinoma antigen are also helpful in selected circumstances.

Large Cell Carcinoma

LCC is an undifferentiated non–small cell malignancy that accounts for about 9% of all lung cancers.¹⁵⁷ It is a diagnosis of exclusion based on the absence of squamous, glandular, or small cell differentiation. The term “large cell

carcinoma” is not used for diagnosis in small biopsy or cytology specimens; rather, it is restricted to resection specimens after the tumor is thoroughly sampled.¹⁶⁵ Histologic variants include basaloid carcinoma, lymphoepithelioma-like carcinoma, clear cell carcinoma, LCC with rhabdoid phenotype, and large cell neuroendocrine carcinoma (LCNEC).¹⁵⁷ Most tumors are located in the periphery of the lung, with the exception of the basaloid subtype. The neuroendocrine nature of the LCNEC is confirmed by immunohistochemical studies.



Cytomorphology of large cell carcinoma

- syncytial clusters and dispersed cells
- irregular nuclei
- striking chromatin clearing
- prominent, often multiple nucleoli
- ill-defined, feathery cytoplasm

Cytologically, a diagnosis of malignancy is rarely difficult ([Fig. 2.35](#)). LCC cells are often arranged in large, syncytium-like sheets of crowded, overlapped cells. The nuclei are large and either round or markedly irregular, with irregularly distributed, coarse chromatin. Nucleoli are usually very prominent. LCNEC shows nuclear palisading, nuclear molding, and rosettes^{179,180} ([Fig. 2.36](#)). Mitotic counts are high, and there is usually necrosis. Confirmation of neuroendocrine differentiation is required, with clear-cut reactivity for at least one of the neuroendocrine markers (synaptophysin, chromogranin, and CD56). Like LCNEC, large cell **basaloid carcinoma** shows nuclear palisading around the margins of cell groups. It can be differentiated from LCNEC by the absence of neuroendocrine staining (<10% of cells) and the presence of cytokeratin 1, 5, 10, or 14, recognized by immunoreactivity for 34 β E12.^{157,181} It is not entirely clear, based on its current description, whether this entity is distinct from basaloid SQC. While basaloid carcinoma may not contain typical intercellular bridges or individual cell keratinization, abrupt squamous differentiation and staining for p63 occur in both.¹⁸² Moreover, both basaloid SQC and large cell basaloid carcinoma have similar very poor prognoses.¹⁸³ In contrast to small cell carcinoma, large cell basaloid carcinoma, like basaloid SQC, lacks nuclear molding, lacks staining for TTF-1, and shows greater cohesiveness.¹⁸² The very rare **lymphoepithelioma-like carcinoma** contains interspersed lymphoid cells.

Clear cell carcinoma is composed of large polygonal cells with abundant clear cytoplasm. The cells of **LCC with rhabdoid features** have large cytoplasmic globules.

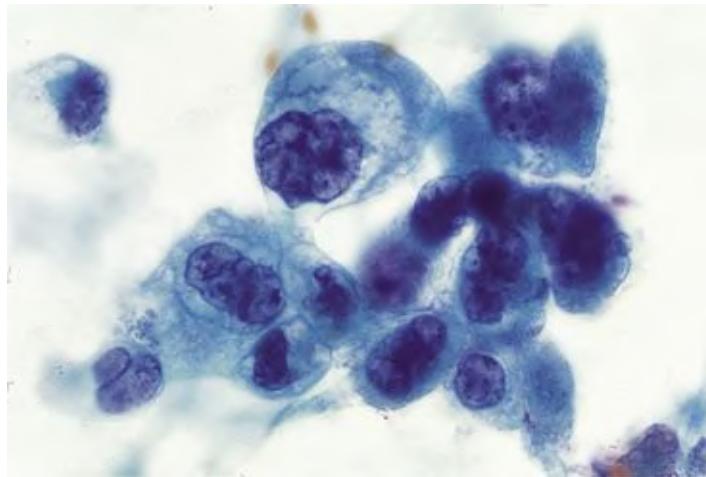


FIG. 2.35 Large cell carcinoma (bronchial washing).

The cells of this tumor are loosely arranged. Nuclei are markedly enlarged with focal chromatin clearing and multiple irregular nucleoli (Papanicolaou stain).

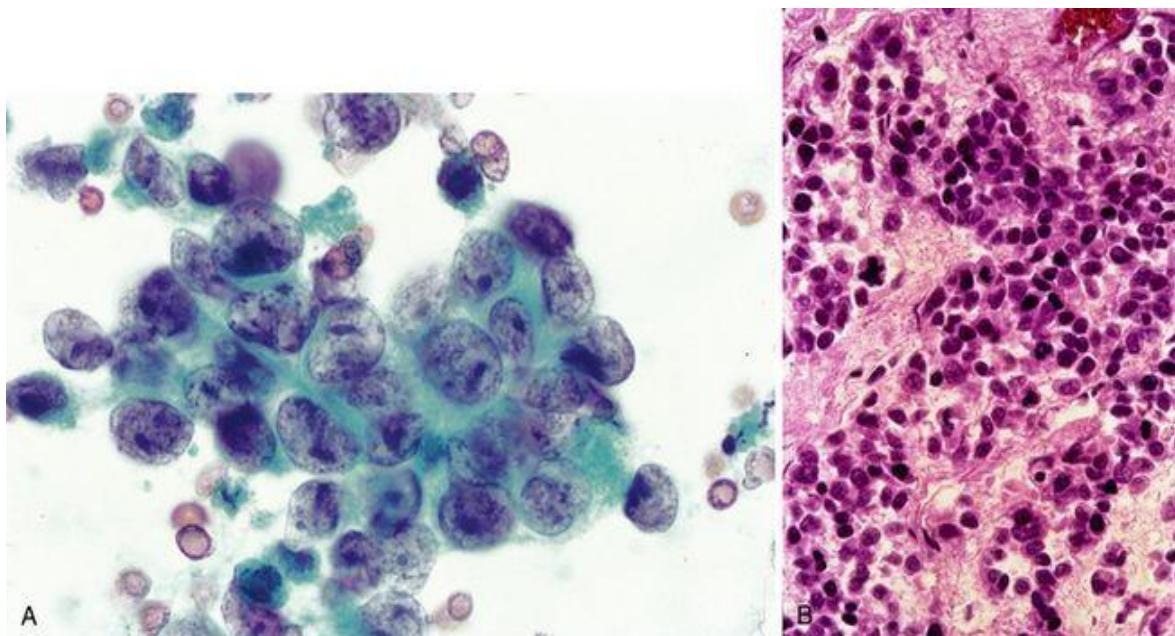


FIG. 2.36 Large cell neuroendocrine carcinoma (LCNEC).

A, The key cytologic features are rosettes, prominent nucleoli, carcinoid tumorlike nuclei with

marked enlargement and atypia, and a moderate or abundant amount of cytoplasm. Mitoses (not illustrated) are frequent. The diagnosis can be confirmed by immunohistochemistry for neuroendocrine markers (Papanicolaou stain). *B*, Histologic sections reveal the organoid growth pattern and brisk mitotic activity (hematoxylin-eosin [H & E] stain).



Differential diagnosis of large cell carcinoma

- reactive changes (e.g., radiation reaction)
- adenocarcinoma
- squamous cell carcinoma
- sarcomatoid carcinoma
- epithelioid angiosarcoma
- non-Hodgkin lymphoma
- metastatic carcinoma
- melanoma

Various types of lung injury (e.g., irradiation, infarction) can result in highly atypical bronchial cells or pneumocytes that mimic LCC and other tumors. The cells of a florid type II pneumocyte hyperplasia can be large and wildly pleomorphic, but most patients are acutely ill with diffuse alveolar damage. In acutely ill patients or those with other injury to the lung, a conservative approach to diagnosis is recommended. When there is no question that the lesion is malignant, and there is no evidence for ACA or SQC by cytromorphology and immunoprofile (see [Table 2.3](#)), the case is reported as “non–small cell carcinoma, not otherwise specified.” Sarcomatoid carcinoma should be considered if there is spindle or giant cell differentiation.

Epithelioid angiosarcoma occurs as a primary tumor in the lung and other sites. It is a particularly good mimic of LCC because it is a high-grade tumor, with large nuclei, prominent nucleoli, and brisk mitotic activity ([Fig. 2.37](#)). The diagnosis of epithelioid angiosarcoma is established by demonstrating vascular differentiation by immunohistochemistry for CD31, CD34, and/or ERG. About 30% are also reactive for keratins, however, so a wider antibody panel is essential.

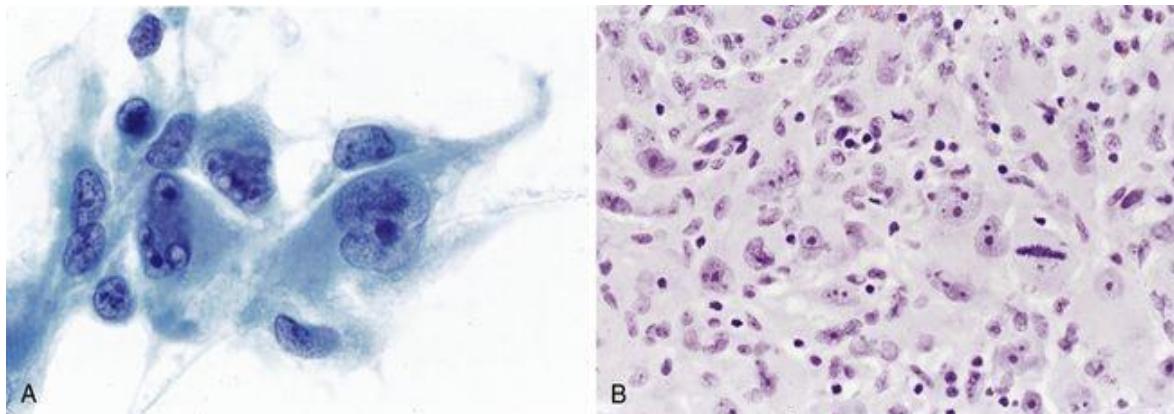


FIG. 2.37 Epithelioid angiosarcoma of the lung.

A, The large polygonal tumor cells resemble those of a large cell carcinoma (LCC; Papanicolaou stain). B, Cell block sections show sheetlike growth (hematoxylin-eosin [H & E] stain).

Diffuse large B-cell lymphoma, anaplastic large cell lymphoma, metastatic carcinoma, and melanoma can usually be distinguished from LCC with an immunohistochemical panel that includes CD20, CD3, ALK1, S-100, HMB45, keratins CK7 and CK20, and carcinoma-specific markers selected based on clinical history or findings.

Sarcomatoid Carcinoma

Sarcomatoid carcinoma is a family of poorly differentiated non–small cell carcinomas that show sarcomatoid or giant cell differentiation. The group includes pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma, and pulmonary blastoma. These tumors are important to recognize because they have a worse prognosis than the conventional non–small cell carcinomas.

Pleomorphic carcinoma is defined histologically as a poorly differentiated ACA, SQC, or LCC, at least 10% of which is a spindle or giant cell malignancy. The spindle cell component shows no differentiated sarcomatous elements. Cytologic preparations show, in addition to ACA, SQC, or LCC, a population of pleomorphic spindle or giant cells. Pleomorphic carcinoma is often a large peripheral tumor with a tendency to invade the chest wall.

Spindle cell carcinoma is a non–small cell carcinoma consisting only of spindle-shaped malignant cells with no differentiated spindle cell elements. Cytologic preparations show large malignant spindle cells with hyperchromatic nuclei.

Giant cell carcinoma is composed of enormous, often multinucleated cells

with no foci of ACA, SQC, or LCC. Cytologic preparations show dispersed, isolated, strikingly large, often multinucleated cells with round nuclei and prominent nucleoli (Fig. 2.38). Neutrophils are often prominent. It is important to recognize because it is associated with an aggressive clinical course.¹⁸⁴

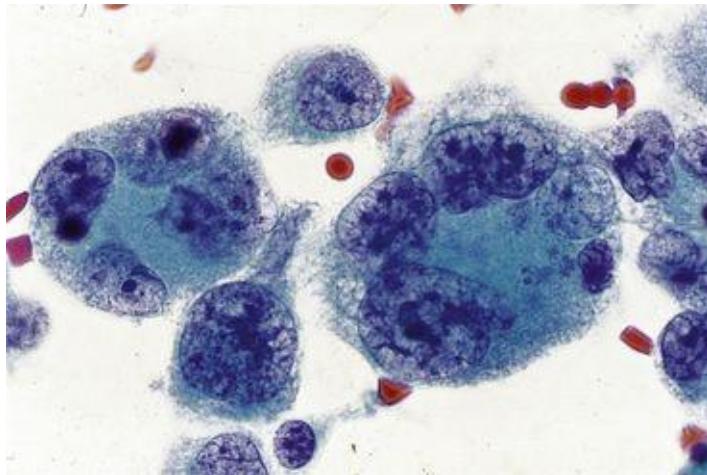


FIG. 2.38 Giant cell carcinoma (fine-needle aspiration [FNA]).
The huge multinucleated cells with striking nuclear atypia of this tumor are obviously malignant (Papanicolaou stain).

The diagnosis of a pleomorphic, spindle cell, or giant cell carcinoma can be suspected based on a cytologic specimen, but precise classification depends on extensive sampling and thus is best deferred to histologic examination.

The remaining two tumors are true biphasic neoplasms. **Pulmonary blastoma**, composed of primitive glandular and stromal elements, is a malignant neoplasm named for its resemblance to fetal lung. It is slightly more common in males, and the mean age is in the fourth decade.¹⁸⁵ Histologically there is a spindle cell component that can show myxoid, chondroid, osteoid, or rhabdomyoblastic differentiation, and an epithelial component consisting of tubules of cuboidal to columnar cells with frequent mitoses and subnuclear and supranuclear vacuoles. The vacuoles contain glycogen and impart a “piano key”–like, endometrioid appearance to the epithelioid component. Solid nests of squamoid cells (squamoid morules) are sometimes present. Tumors composed of just the epithelial component of the pulmonary blastoma are called fetal ACAs.¹⁶⁸ Pulmonary blastoma most likely arises from a totipotent precursor, because identical p53 mutations are present in both the epithelial and sarcomatous components within the same tumor.¹⁸⁶ Immunohistochemical demonstration of keratins in the epithelial component and muscle-specific actin in the spindle cells

helps to confirm the diagnosis.¹⁸⁷ Pitfalls in the cytologic diagnosis of blastoma occur when one of the components is poorly represented or when the stromal component is misinterpreted as a small cell carcinoma.

Carcinosarcoma differs clinically from blastoma in the mean age of patients at presentation (65 rather than 40 years). Morphologically, the spindle cell component includes differentiated elements like malignant cartilage, bone, or skeletal muscle.

Neuroendocrine Tumors

The neuroendocrine tumor (NET) group of neoplasms encompasses a morphologic and biologic spectrum and is divided by the World Health Organization (WHO) into four distinct diagnostic categories: carcinoid tumor, atypical carcinoid tumor, large cell neuroendocrine carcinoma (LCNEC), and small cell carcinoma.¹⁵⁷ By electron microscopy, all contain cytoplasmic dense-core, membrane-bound granules in variable quantities. NETs are immunoreactive for one or more of the neuroendocrine markers chromogranin A (most specific), synaptophysin, and CD56.

The critical feature that separates carcinoids and atypical carcinoids from LCNEC and small cell carcinoma is the mitotic rate ([Table 2.4](#)). Even if a tumor has morphologic features of small cell carcinoma, it should not be diagnosed unequivocally as a small cell carcinoma if it lacks frequent mitoses and necrosis. The proliferative rate of NETs is assessed as the number of mitoses per 10 high-power fields (hpfs) in a histologic section: Carcinoid tumors of the lung and thymus have less than 2 mitoses per 10 hpfs, atypical carcinoid tumors 2 to 10 per 10 hpfs, and high-grade tumors (small cell carcinoma and LCNEC) greater than 10 per 10 hpfs. (Note that these cut-off values are different for lung and thymus as opposed to gastroenteric and pancreatic NETs.) Staining for Ki67 (MIB-1) is a useful surrogate because it provides an accurate assessment of the proliferative rate and is readily applicable to smaller specimens. The proportion of MIB-1 immunoreactive cells in carcinoid and atypical carcinoid tumors is less than 25%, whereas small cell carcinomas show MIB-1 immunoreactivity in greater than 50% of neoplastic cells.¹⁸⁸ A grade assigned on the basis of an FNA or core biopsy, however, needs to take into account the possibility that a higher-grade focus may not have been sampled.

TABLE 2.4
CYTOLOGIC FEATURES OF PULMONARY NEUROENDOCRINE

TUMORS*

	Typical Carcinoid	Atypical Carcinoid	Large Cell Neuroendocrine Carcinoma	Small Cell Carcinoma
				
Cell size	Small to medium	Medium	Large	Small to medium
Predominant pattern	Tight clusters/rosettes	Loose clusters/rosettes	Loose clusters/rosettes	Dispersed cells
Cytoplasm	Moderately abundant	Scant to moderate, lacy	Scant or moderate, lacy	Scant
Plexiform vascularity	Common	Common	Not known	Rare
Nuclear molding	Rare	Slight to moderate	Slight to moderate	Prominent
Chromatin	Coarsely granular	Coarsely granular	Coarsely granular	Finely granular
Nucleoli	Small	Occasionally prominent	Prominent	Inconspicuous
Mitoses (per 10 high-power fields)	Rare (<2)	Uncommon (2–10)	Abundant (≥ 11 ; median 70)	Abundant (≥ 11 ; median 80)
Nuclear pleomorphism	Mild	Moderate	Marked	Moderate
Necrosis	Absent	Moderate	Marked	Marked
Nuclear crush	Absent	Mild	Moderate	Marked

*Cytologic features extrapolated from Travis WD, Linnoila RI, Tsokos MG, et al.: Neuroendocrine tumors of the lung with proposed criteria for large-cell neuroendocrine carcinoma: An ultrastructural, immunohistochemical, and flow cytometric study of 35 cases. Am J Surg Pathol 1991;15:529–553, and authors' unpublished observations.

LCNEC was previously discussed in greater detail (see “Large Cell Carcinoma”).

Carcinoids and atypical carcinoids are usually positive for keratins, but up to 20% can be negative.¹⁵⁷ Conflicting results have been obtained with TTF-1: Carcinoid tumors are positive in one third of cases.¹⁵⁷

Carcinoid Tumor

At one end of the spectrum of NETs is the carcinoid tumor (synonym, “typical carcinoid tumor”), a low-grade NET that accounts for 2% to 3% of all pulmonary tumors. Carcinoid tumors are uniformly distributed throughout the lungs. Centrally located tumors are often submucosal, with a prominent endobronchial component. When submucosal, the carcinoid tumor is usually covered by intact respiratory epithelium, and as a result sputum cytology is often negative. Carcinoid tumors have low metastatic potential: 10% to 15% of patients have regional lymph node involvement,^{157,189} and 5% to 10% eventually metastasize to distant sites,¹⁵⁷ but the prognosis is excellent with surgery, and 5-year survival is 90% to 98%.¹⁵⁷

Histologic examination reveals uniform polygonal cells with a variable growth pattern that may include nests, ribbons, papillae, and rosettelike arrangements. Occasionally the cells and nuclei are elongated (spindle cell carcinoid).¹⁹⁰



Cytomorphology of carcinoid tumor

- loosely cohesive groups and isolated cells
- rosettelike structures
- round, plasmacytoid, or elongated cells
- uniform nuclei with “salt and pepper” chromatin
- ample granular cytoplasm
- branching capillaries
- mitoses uncommon (<2 per 10 histologic high power fields)
- no necrosis

Cytologic preparations usually show a uniform population of isolated cells and loose clusters ([Fig. 2.39](#)). Large vascularized tissue fragments are sometimes present. Carcinoid tumors are very vascular, and sometimes a mesh of branching capillaries is encountered. In cell block sections, solid nests, trabeculae (ribbons), papillae, and rosettes can be appreciated. The tumor cells are round, oval, or elongated (as in the spindle cell carcinoid) and have moderate or abundant granular cytoplasm. Nuclei are round or oval, with smooth contours, finely speckled (“salt and pepper”) chromatin, and inconspicuous nucleoli. In some cases there may be focal nuclear atypia and pleomorphism, but this has no clinical significance.

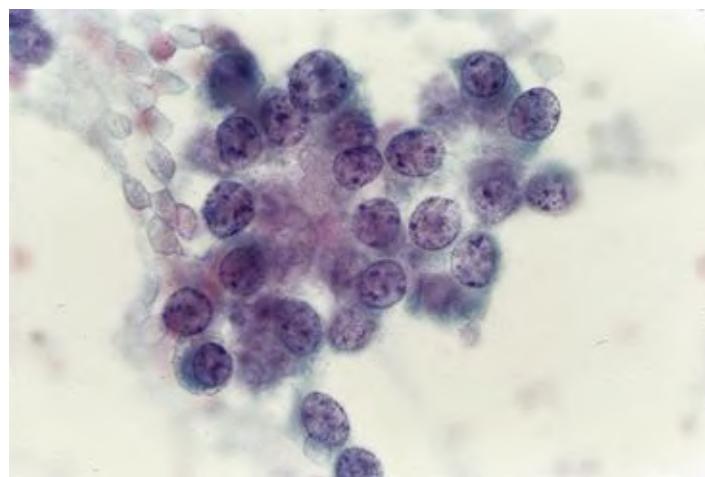


FIG. 2.39 Carcinoid tumor (fine-needle aspiration [FNA]).

The tumor cells have a moderate amount of coarsely granular cytoplasm and a “salt and pepper” chromatin pattern. Rosettes are seen in some carcinoid tumors (Papanicolaou stain).



Differential diagnosis of carcinoid tumor

- benign bronchial epithelial cells
- adenocarcinoma
- lymphoma
- small cell carcinoma and atypical carcinoid tumor

Because of their bland, monomorphous appearance, the cells of a carcinoid tumor may be mistaken for benign bronchial cells. In contrast to bronchial cells, carcinoid tumor cells are less cohesive and lack cilia. Because rosettes are seen in some carcinoids, they are sometimes mistaken for an adenocarcinoma. In most adenocarcinomas, however, there is greater variation in cell size and more grouping of cells into spheres and sheets than with carcinoid tumors. There may be a resemblance to lymphoid cells, but lymphoid cells have less cytoplasm and do not form clusters or rosettes. Carcinoid tumors resemble atypical carcinoids and small cell carcinomas but, in contrast to those tumors, carcinoids have a low mitotic rate and necrosis is absent (see [Table 2.4](#)). Although the WHO classification of lung tumors relies on the mitotic rate for the distinction between these three tumors, an accurate mitotic count is rarely possible in a cytologic sample. For this reason, an immunohistochemical proliferation index provides a more accurate assessment of the proliferation rate, and, in challenging cases, immunoreactivity for the proliferation marker MIB-1 (Ki-67) can be helpful, especially for confirming or ruling out small cell carcinoma. The proportion of MIB-1 immunoreactive cells in carcinoid and atypical carcinoid tumors is less than 25%, whereas small cell carcinomas show MIB-1 immunoreactivity in greater than 50% of neoplastic cells.^{[188](#)}

Atypical Carcinoid Tumor

The atypical carcinoid tumor is an intermediate-grade NET. It is biologically more aggressive than the carcinoid tumor, with a 5-year survival rate of 61% to 73%.^{[157](#)}



Cytomorphology of atypical carcinoid tumor

- cells and architecture similar to typical carcinoid
- focal necrosis
- mitoses (2 to 10 per 10 histologic hpf)

- prominent nucleoli

The cytomorphologic features of the NETs are listed in [Table 2.4](#). Atypical carcinoids resemble carcinoid tumors ([Fig. 2.40](#)). They differ morphologically, however, in several ways: The architectural arrangements tend to be looser; there is greater (but not brisk) mitotic activity; and there may be focal necrosis.¹⁹¹ In challenging cases, immunoreactivity for the proliferation marker MIB-1 (Ki-67) can be helpful, especially for ruling out small cell carcinoma. The proportion of MIB-1 immunoreactive cells in atypical carcinoid tumors, as with carcinoids, is less than 25%, whereas small cell carcinomas show MIB-1 immunoreactivity in greater than 50% of neoplastic cells.¹⁸⁸ Although helpful, these features do not always permit precise classification in cytologic and bronchial biopsy samples; final classification rests on thorough histologic sampling of a resected specimen. In view of this, surgical excision should be considered for all localized NETs.

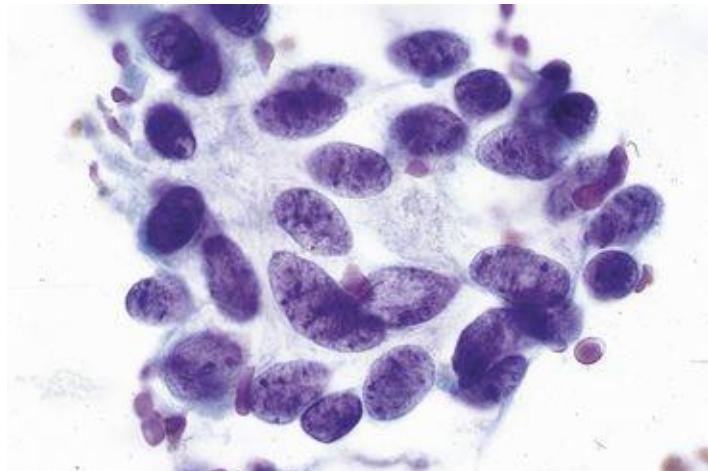


FIG. 2.40 Atypical carcinoid (fine-needle aspiration [FNA]). These tumors resemble carcinoid tumors, but they have more pleomorphism and nuclear enlargement, an increased number of mitoses, and focal necrosis, important distinguishing features (Papanicolaou stain).

Small Cell Carcinoma

Small cell carcinoma is a high grade NET (carcinoma). It accounts for 20% to 25% of all primary lung carcinomas, and 90% are centrally located. A majority

of patients are male smokers (80%), and their prognosis is poor (5-year survival less than 10%).¹⁹² A morphologic variant is the “combined small cell carcinoma” (e.g., small cell/squamous cell carcinoma, small cell/adenocarcinoma).

Histologically, small cell carcinoma consists of small, round to fusiform cells with scant cytoplasm. The tumor often undermines the bronchial mucosa, with extensive invasion of lung parenchyma and lymphatics. Necrosis, a very high mitotic rate, and nuclear encrustation of vascular walls are characteristic features. In some cases the cells are larger, with more cytoplasm and vesicular nuclei.

Cytologically, the cells are loosely aggregated and dispersed as isolated cells. The cells of small cell carcinoma are very fragile. Bare nuclei stripped of cytoplasm and crush artifact with smearing of nuclear DNA (chromatin streaks) are common. Crush artifact can be so marked that mitoses, which are abundant but delicate, may all be smeared and not identifiable. In such cases, apoptotic cells and single-cell necrosis are considered evidence of



Cytomorphology of small cell carcinoma

- small cells (twice the size of lymphocytes), occasionally larger
- carrot-shaped nuclei
- evenly dispersed, powdery chromatin
- nuclear molding
- small to indistinct nucleoli
- paranuclear blue bodies
- mitoses (>10 per 10 histologic high-power fields)
- scant cytoplasm
- nuclear debris and crush artifact

rapid cell turnover and abundant mitotic activity. The cells may be small or intermediate in size. Cytoplasm is scant and inconspicuous, and adjacent nuclei show frequent molding (Fig. 2.41A and B). Nuclei are round, oval, or stretched out (carrot-shaped). Chromatin is very finely textured, and nucleoli are absent or very uncommon. The cytologic appearance varies somewhat with the sampling method. In sputum, streams of small hyperchromatic cells form loose aggregates.

In bronchial brushings and FNA specimens, the cells are better preserved.

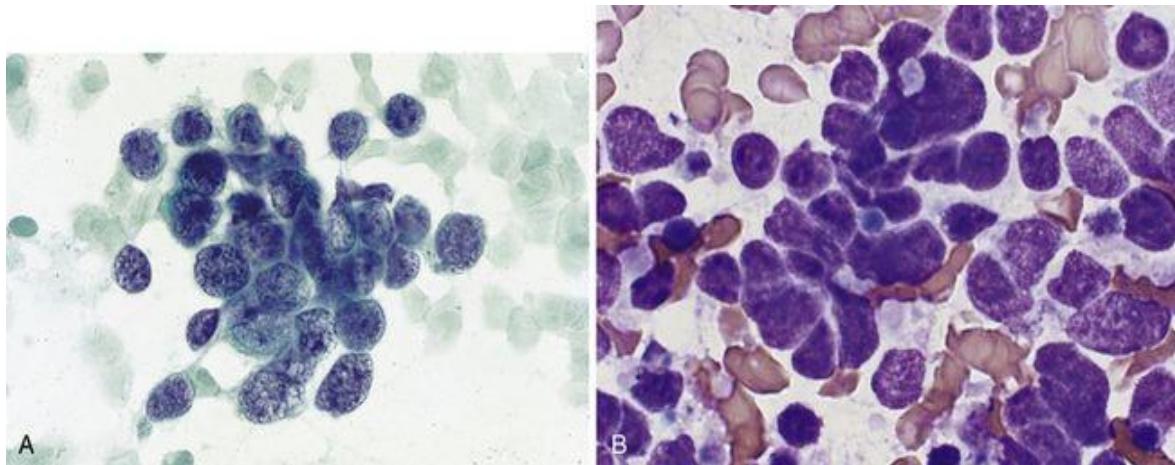


FIG. 2.41 Small cell carcinoma (bronchial brushing).

A, The cells of small cell carcinoma are tightly packed and show nuclear molding. They are fragile and occasionally degenerated. Well-preserved cells have a powdery chromatin texture (Papanicolaou stain). B, The small, so-called paranuclear blue body in the center of the field is a characteristic of small cell carcinomas (Romanowsky stain).

Some small cell carcinoma cells contain *paranuclear blue bodies*: solitary round, light to dark blue, homogeneous spheres that indent the nucleus (see Fig. 2.41B).¹⁹³ They are seen with air-dried preparations stained with Romanowsky-type stains, not with alcohol-fixed preparations stained with a Papanicolaou stain or formalin-fixed, hematoxylin-and-eosin–stained sections.¹⁹⁴ They are characteristic of small cell carcinomas but not pathognomonic, because they are occasionally seen in other cancers.¹⁹⁵



Differential diagnosis of small cell carcinoma

- reserve cell hyperplasia
- lymphocytes
- carcinoid tumor
- atypical carcinoid tumor
- non–small cell carcinomas
- NUT midline carcinoma
- Ewing sarcoma
- neuroblastoma
- Wilms tumor

- rhabdomyosarcoma
- pulmonary blastoma

The cells of reserve cell hyperplasia can show molding, but they are smaller and more cohesive than those of small cell carcinoma; there is no necrosis, mitoses are absent, and nuclei have smudged, featureless chromatin (see [Fig. 2.8](#)). Lymphoid cells, whether from an inflammatory process, an intrapulmonary lymph node, or lymphoma, can be mistaken for small cell carcinoma. Lymphoid cells generally do not form cell clusters, except artifactually on cytopsin or thinlayer preparations; they tend to be evenly spaced rather than molded together; and they are one-half the size of small cell carcinoma cells.

The distinction between small cell carcinoma and (typical) carcinoid tumor is usually straightforward, given that carcinoids lack nuclear molding, necrosis, and mitotic activity. In addition, the chromatin texture of carcinoid tumors is more coarsely granular than that of small cell carcinoma. Some carcinoid tumors, in particular the spindle cell variant, bear a superficial resemblance to small cell carcinoma. Therefore, care must be taken not to make a diagnosis of small cell carcinoma without abundant mitotic activity and necrosis. In challenging cases, immunoreactivity for the proliferation marker MIB-1 (Ki-67) is helpful. The proportion of MIB-1 immunoreactive cells in small cell carcinomas is greater than 50%, whereas carcinoids and atypical carcinoids show immunoreactivity in less than 25% of neoplastic cells.¹⁸⁸ The precise distinction between carcinoid and atypical carcinoid, and between atypical carcinoid and small cell carcinoma (see [Figs. 2.39](#) to [2.41](#)), is difficult, however, and distinguishing features are summarized in [Table 2.4](#). In general, atypical carcinoids have more numerous mitoses than carcinoid tumors, and small cell carcinomas have significantly more mitoses than typical and atypical carcinoids, but a final determination may not be possible without thorough histologic sampling.

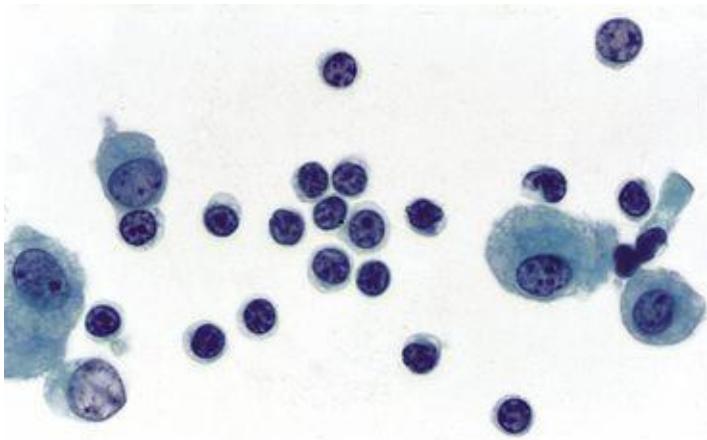


FIG. 2.42 Lymphocytic interstitial pneumonia (bronchoalveolar lavage [BAL]). This image demonstrates a polymorphous population composed of small and medium-sized lymphocytes. Although this cytologic pattern suggests a reactive process, a mucosa-associated lymphoid tissue (MALT) lymphoma cannot be excluded. Immunophenotyping is essential to confirm the reactive or neoplastic nature of the lesion (Papanicolaou stain).

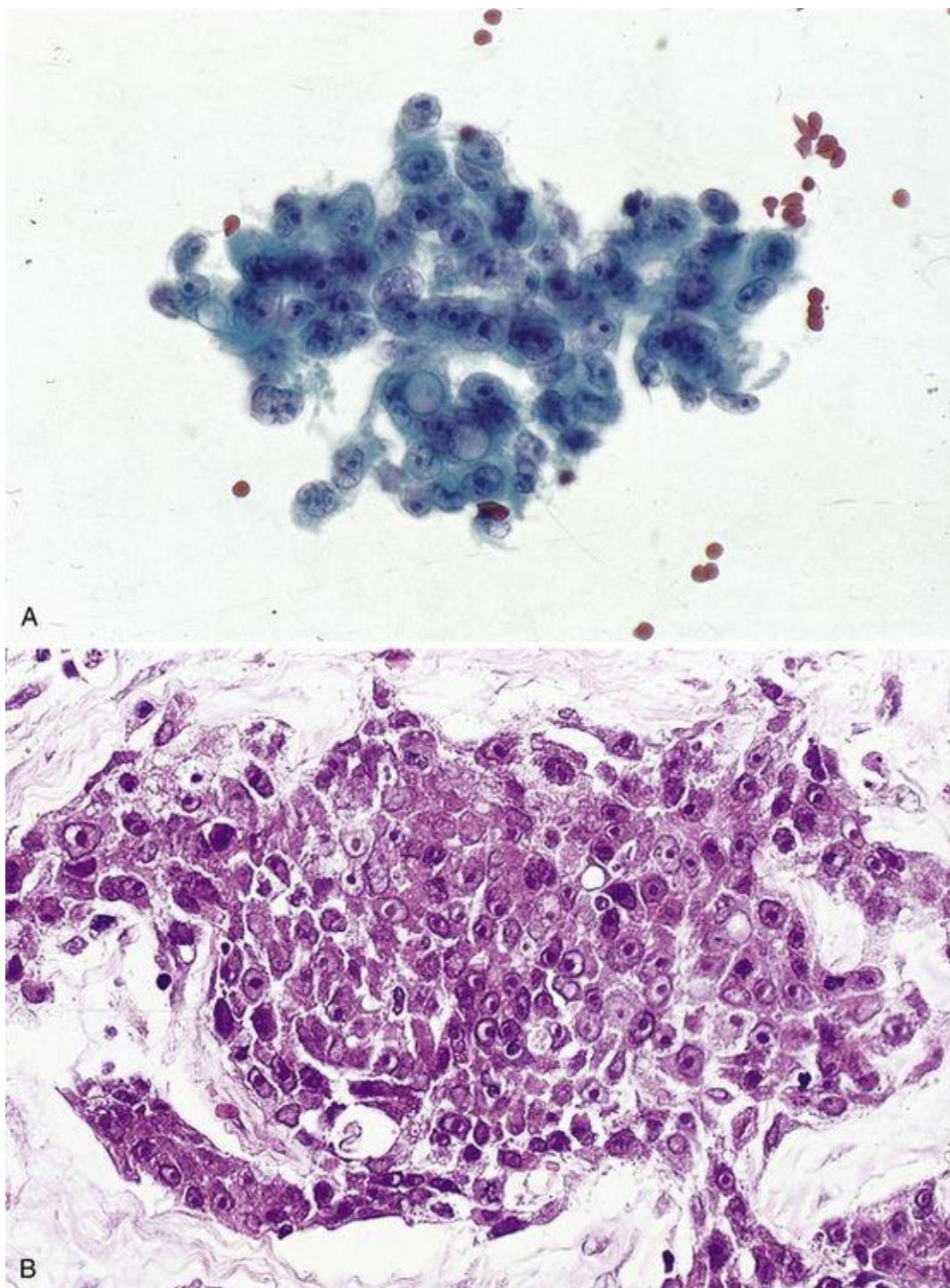


FIG. 2.43 Metastatic ductal carcinoma of the breast.

This patient had a history of ductal breast carcinoma. *A*, Fine needle aspiration of the lung. The smear shows clusters of tumor cells with prominent nucleoli and mucin-filled intracellular lumens (Papanicolaou stain). *B*, Breast lumpectomy. Comparison with the previously resected tumor shows close similarity, supporting the diagnosis of metastasis to the lung from the breast primary (hematoxylin-eosin [H & E]).

Small cell carcinoma is sometimes difficult to distinguish from the non–small cell carcinomas, particularly the small cell variant of squamous cell carcinoma and those adenocarcinomas composed of relatively small cells. The most helpful features in making this distinction are the powdery chromatin texture, inconspicuous nucleoli, prominent nuclear molding, and scant cytoplasm of small cell carcinoma. Care should be taken not to misinterpret the solid paranuclear blue bodies as mucin vacuoles (which are clear); otherwise, a small cell carcinoma might be misidentified as an adenocarcinoma.¹⁹³ Immunohistochemistry for p63 can be very helpful in distinguishing squamous cell carcinoma, which is immunoreactive, from small cell carcinoma, which is negative.^{196,197} Even after careful examination and immunohistochemical study, not all tumors are easily classified. In such cases, the possibility of a mixed tumor, a common occurrence in the lungs, should be considered.

A NUT midline carcinoma should be considered when faced with any poorly differentiated malignancy that lacks glandular differentiation, including small cell carcinoma. Immunohistochemical staining for the NUT protein is very helpful.¹⁶⁷

Clinical history, particularly patient age, provides clues to the diagnosis of the other small round cell tumors. The sarcomatous component of pulmonary blastomas resembles a small cell carcinoma; identifying the epithelial areas is crucial in making this distinction.

Uncommon Pulmonary Tumors

Adenoid Cystic Carcinoma and Other Bronchial Gland Tumors

The submucosal glands of the trachea and bronchi give rise to neoplasms that resemble those occurring in the salivary glands. The most common of these is the adenoid cystic carcinoma. This malignant tumor accounts for 20% to 35% of all cancers in the trachea but also occurs in the major bronchi. The tumors grow as polypoid masses, undermining intact overlying respiratory epithelium. They often infiltrate the cartilage and soft tissues of the major airways. Symptoms are related to obstruction and include cough, dyspnea, and hemoptysis. Treatment is surgical, but the long-term prognosis is poor, with 80% of patients dead of disease within 20 years.¹⁹⁸

These tumors are most often diagnosed by means of bronchial brushings or transbronchial needle aspiration. Cytologic preparations show cylinders or spheres of small, deceptively innocuous-looking epithelial cells that surround basal lamina material (see [Figs. 11.20](#) through [11.22](#)).

Mucoepidermoid carcinomas of the trachea and bronchi are uncommon, representing only 0.2% of lung tumors. They are defined by the presence of squamous, glandular, and intermediate cells, and by their origin within cartilage-containing airways. The diagnosis “adenosquamous carcinoma” should be restricted to tumors arising at the lung periphery beyond a recognizable bronchus. Mucoepidermoid carcinomas can develop in people of all ages, including children. They are endobronchial tumors like those of adenoid cystic carcinoma and other salivary gland tumors, and the presenting symptoms are similar. Cytologically, the tumors are diagnosed by finding squamous cells, intermediate cells, and mucinous cells (see [Figs. 11.17](#) and [11.18](#)). High-grade tumors show marked nuclear atypia. Mucoepidermoid carcinoma is one of a growing number of neoplasms for which a recurrent and tumor-specific genetic aberration is present: the t(11;19) translocation, forming the MECT1-MAML2 fusion transcript.¹⁹⁹

Other bronchial gland tumors include pleomorphic adenoma, acinic cell carcinoma, and oncocytoma.

Clear Cell Tumor ('Sugar Tumor,' PEComa)

The clear cell tumor is extremely rare and can occur in people of all ages, most of whom are asymptomatic. It is usually a peripheral mass and ranges from 1 to 7 cm in greatest diameter. Clear cell tumors most likely arise from perivascular epithelioid cells (PEC).^{[157,200](#)} Histologically and cytologically, the tumor consists of bland polygonal and spindle-shaped cells with a central, oval or elongated nucleus and clear, glycogen-rich cytoplasm.^{[201](#)} The differential diagnosis includes the clear cell variant of adenocarcinoma or squamous cell carcinoma of the lung, granular cell tumor, and metastatic tumors with clear-cell features (renal cell carcinoma, adrenal cortical carcinoma, some melanomas, and others).^{[201,202](#)} Immunohistochemistry is helpful for most of these distinctions except with melanoma: Most clear cell tumors are positive for HMB-45 and Melan-A, and are negative for cytokeratins and CEA.^{[200,203](#)}

Sarcomas

Primary malignant mesenchymal tumors of the lung are rare, occurring in adults of either sex. They can be large masses or small endobronchial lesions. One of the most common primary sarcomas is leiomyosarcoma.^{[204,205](#)} Others include fibrosarcoma, solitary fibrous tumor, chondrosarcoma,^{[206](#)} Kaposi sarcoma, angiosarcoma,^{[207](#)} epithelioid hemangioendothelioma,^{[177](#)} rhabdomyosarcoma, malignant peripheral nerve sheath tumor, and synovial sarcoma. Care must be taken not to mistake stromal and smooth muscle cells in ulcerative processes for sarcoma cells. Most sarcomas, both primary and metastatic, can confidently be distinguished from carcinomas by FNA.^{[208,209](#)}



Cytomorphology of sarcomas

- sheetlike, cellular aggregates
- single, highly atypical spindle cells



Differential diagnosis of sarcomas

- spindle cell carcinoma
- spindle cell carcinoid tumor
- spindle cell thymoma
- sarcomatoid mesothelioma
- melanoma

Whether the sarcoma is primary or metastatic is a distinction made mainly on clinical grounds. Immunohistochemistry for keratins, CEA, desmin, smooth muscle actin, CD31, CD34, ERG, calretinin, WT-1, S-100, and HMB45 can be very useful in the differential diagnosis.

Lymphomas and Leukemias

Hodgkin and non-Hodgkin lymphomas occur as primary tumors of the lung, but spread from an extrapulmonary primary is more common. Primary pulmonary non-Hodgkin lymphoma represents fewer than 10% of all extranodal lymphomas. By contrast, secondary lung involvement by non-Hodgkin lymphomas is noted in 20% to 50% of patients at some point in their clinical course.²¹⁰ The most common primary non-Hodgkin lymphoma of the lung (70% to 90%) is *extranodal marginal zone B-cell lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma)*, followed by *diffuse large B-cell lymphoma*. Much less common primary lymphoproliferative disorders include *lymphomatoid granulomatosis* and *peripheral T cell lymphoma*.²¹¹ MALT lymphomas are typically incidental solitary pulmonary masses and have an indolent clinical course: The 5-year survival rate is around 90%.^{157,211} By contrast, lymphomatoid granulomatosis, an Epstein-Barr virus (EBV)-associated, T-cell-rich B-cell lymphoproliferative disorder, is often associated with chest pain, cough, dyspnea, and neurologic symptoms,^{212,213} and the median survival is 2 years.



Differential diagnosis of pulmonary lymphoma and leukemia

- inflammatory lesions
- small cell carcinoma
- large cell carcinoma
- melanoma

MALT lymphoma can be particularly troublesome to diagnose by cytology alone because it is cytomorphologically indistinguishable from a reactive condition ([Fig. 2.42](#)). Identifying a diffuse large B-cell lymphoma is much more reliable because of the striking atypia of the large cells. Surface marker analysis

to demonstrate monoclonality, best determined by flow cytometric analysis,²¹⁴ is often essential to confirm the diagnosis of a MALT lymphoma. A diffuse large B-cell lymphoma, which is obviously cytologically malignant, need only stain for lymphoid markers (e.g., CD20 or leukocyte common antigen)²¹⁵ for diagnostic confirmation.

Primary pulmonary Hodgkin lymphoma is exceptionally rare, though secondary involvement occurs in 40% to 50% of patients.^{210,216} Primary pulmonary involvement is two-fold more common in women, who present with cough and B-type symptoms. Radiologic findings are equally divided among solitary lesions, multiple masses, and a pneumonia-like infiltrate. Patients who present with pulmonary involvement have a poorer prognosis than those with nodal disease only. The cytologic diagnosis of Hodgkin lymphoma rests on identifying Reed-Sternberg (RS) cells.²¹⁶ Mononuclear variants and a mixed background of lymphocytes, plasma cells, eosinophils, and histiocytes are typically present.

Granulomatous inflammatory processes need to be distinguished from Hodgkin lymphoma. Histiocytes can mimic RS cells, and granulomas can occur in Hodgkin lymphoma. RS cells are distinguished from histiocytes because RS cells have very large, inclusion-like nucleoli and highly irregular nuclei. Melanoma and large cell carcinoma can be excluded with immunohistochemical studies for keratins, S-100, and HMB45.

About 10% of diffuse pulmonary infiltrates in patients with leukemia result from leukemic involvement of the lung. At autopsy, infiltrates are seen in 25% to 65% of cases, most commonly acute leukemia. Leukemic involvement of the lung may also present as a mass lesion. The cells are dispersed as isolated cells, as with lymphomas, and cellular monomorphism is characteristic. BAL is useful for demonstrating leukemic involvement, especially when biopsy is contraindicated because of coagulopathy.²¹⁷ The diagnosis can be confirmed by immunohistochemistry or flow cytometry (e.g., blasts are commonly CD34+).

Other hematopoietic malignancies that can involve the lung include myeloma²¹⁸ and mycosis fungoides.²¹⁹

Metastatic Cancers to the Lung

The lungs receive the entire cardiac output, so it is not surprising that they are a common site for metastasis; 30% to 50% of extrapulmonary cancers involve the lungs at autopsy.²²⁰ Metastases are diagnosed in sputum in up to 70% of cases.²²¹ About 40% of metastatic tumors in exfoliative specimens are squamous carcinomas from an adjacent site in the aerodigestive tract. The remainder of the cases include adenocarcinomas (34%)—most commonly of the breast, kidney, and colon—and hematopoietic malignancies (8%).²²²

Because confirming metastatic disease is a major indication for transthoracic FNA, metastatic tumors account for between 14% and 26% of transthoracic FNA specimens.²²³ In a study of more than 1000 cases, malignant melanoma accounted for 27%, urinary and male genital tract neoplasms for 17%, breast carcinoma for 15%, female genital tract neoplasms for 13%, and GI tract neoplasms for 10%.²²² Another study reported that the most common metastases (in decreasing frequency) are those from breast cancer, colon cancer, renal cell carcinoma, bladder cancer, and melanoma.²²⁴

Primary sites for some metastatic carcinomas can be inferred based on their characteristic cytologic features. The cells of the usual type of colon carcinoma have a distinctive tall, columnar (“picket fence”) appearance, with hyperchromatic nuclei and “dirty” necrosis (from karyorrhexis). Malignant melanoma often demonstrates a single-cell arrangement, with cytoplasmic pigment, intranuclear cytoplasmic invaginations, and classic “mirror-image” binucleation. Breast carcinoma often show a single-file arrangement of cells, some with mucin-containing intracellular lumens ([Fig. 2.43A](#)); renal cell carcinoma cells are large cells with abundant clear cytoplasm and poorly defined cell membranes; and metastatic papillary thyroid carcinoma cells have nuclear grooves, nuclear pallor, and intranuclear pseudooinclusions, a morphologic appearance mimicked by the mucinous adenocarcinoma of the lung.

The distinction of primary lung carcinoma from a metastasis is often facilitated by immunohistochemistry. Most lung carcinomas are reactive for CK7 and nonreactive for CK20 (as contrasted with metastatic colon cancer, for example, which is CK7– and CK20+). Additionally, lung carcinomas can be distinguished from non-lung carcinomas, except those of thyroid origin, with antibodies to thyroid transcription factor 1 (TTF-1).²²⁵ Interestingly, whereas TTF-1 was once thought to be simply a marker of lung origin, there is now evidence that it is involved in the pathogenesis of lung cancer: 12% of lung

carcinomas have amplification of TTF-1.^{[226](#)}

Determining the primary site of the metastasis is greatly aided by comparing the patient's cytologic specimen with any previous pathologic material ([Fig. 2.43B](#)). It is particularly important to obtain sufficient cytologic material for cell block preparations so that tissue architecture and immunohistochemical markers can be assessed.

Tumors of the Mediastinum

The mediastinum is divided anatomically into three sections: anterior, middle, and posterior. Tumors of the anterior mediastinum are most likely to be malignant (59% of cases)^{[227,228](#)} and are the focus of this section. The anterior mediastinum is bordered by the pleura laterally, the thoracic inlet superiorly, and the diaphragm inferiorly. FNA of anterior mediastinal tumors of epithelial origin has an overall accuracy of 80%,^{[229,230](#)} compared with 100% for core biopsy, though subtyping is less accurate than biopsy.^{[231](#)} The differential diagnosis for tumors of this region is encapsulated in “the four Ts” mnemonic, which (awkwardly) stands for thymoma, teratoma (germ cell tumor), “terrible lymphoma,” and thyroid disease (goiter, accessory thyroid). An expanded differential diagnosis follows.



Differential diagnosis of anterior mediastinal masses

- thymoma
- thymic carcinoma
- germ cell tumor
- lymphoma
- parathyroid adenoma
- intrathoracic goiter
- lipoma
- lymphangioma
- aortic aneurysm
- NUT midline carcinoma

Thymoma

Thymoma is the most common neoplasm of the anterior mediastinum,^{[232](#)} comprising 20% of adult tumors.^{[233,234](#)} It is rare in children, and the average age is 50.^{[235](#)} Thymoma commonly occurs in association with several paraneoplastic syndromes, including myasthenia gravis, hypogammaglobulemia, and pure red blood cell aplasia.^{[236](#)}

Cytologic diagnosis of thymoma can be accomplished with high sensitivity on samples obtained using CT or ultrasound guidance. Ultrasound-guided biopsy has a sensitivity of 95% for the diagnosis of thymoma in experienced hands.^{[237](#)}

Thymomas are comprised of variable proportions of epithelial cells and lymphocytes (Fig. 2.44). The epithelial cells are distinguished from those of thymic carcinoma by the absence of unequivocally malignant cells.²³⁸ In 2004, the WHO proposed a thymoma classification that correlates with invasiveness and prognosis. Thymomas fall into two general categories based on cellular composition: type A and type B. Type A thymomas are comprised of bland spindle-shaped or ovoid cells, whereas Type B thymomas are comprised predominantly of round or polygonal cells.¹⁵⁷ Type B thymomas are further subdivided into types B1, B2, and B3 based on the proportion of lymphocytes and degree of epithelial atypia: B1 thymomas are richest in lymphocytes, and B3 are richest in epithelial cells. Type AB, as its name implies, is a mixture of types A and B.

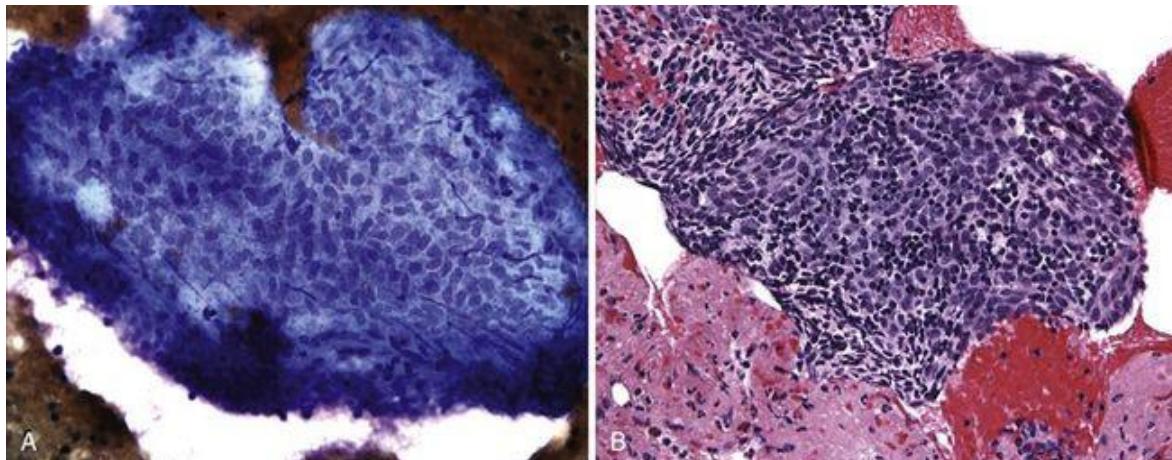


FIG. 2.44 Thymoma.

A, Smears reveal clusters of epithelioid cells (Romanowsky stain). B, Cell block sections show lymphocytes admixed with neoplastic thymocytes. A resection revealed mixed B2 and B3 type thymoma (hematoxylin-eosin [H & E]).

The type A epithelial cells are bland and often form whorling fragments with arborizing capillaries; rarely, Hassall corpuscles may be seen. The polygonal cells of type B thymoma are also bland but show mild atypia in the type B3, which have the poorest prognosis. The lymphocytes of type B thymoma are predominantly small and mature, but larger transformed forms may be present. For diagnosis by FNA, an adequate sample is essential to avoid mistaking normal thymic lymphocytes for thymoma. It is also important not to mistake mesothelial cells for thymic epithelial cells, particularly because thymoma, although negative for WT-1, is frequently positive for calretinin (43% of

cases).^{239,240} The lymphocytes of a thymoma, which are T-cells, stain for TdT, CD3, and CD99, but they lack the blastic chromatin of lymphoblasts. The epithelial cells are reactive for p63 and CK5. They do not react for CD5 or c-kit, as do thymic carcinomas.^{241,242}

Thymic Carcinoma

The term *thymic carcinoma* encompasses a heterogeneous group of rare, very aggressive tumors. Subtypes include squamous cell carcinoma, neuroendocrine carcinoma, mucoepidermoid carcinoma, basaloid squamous cell carcinoma, and undifferentiated lymphoepithelioma-like carcinoma. They have the cytomorphologic appearance of a poorly differentiated large cell carcinoma,^{243,244} thus metastatic carcinoma, which is far more common, must be ruled out. Thymic carcinoma distinguishes itself among carcinomas by its expression of CD5 and c-kit (CD117). It is negative for TTF-1.²⁴⁰

Medastinal Lymphomas

Primary mediastinal lymphomas are less common than secondary involvement by lymphoma and comprise only 10% of lymphomas involving the mediastinum. The most common primary mediastinal lymphomas are Hodgkin disease, primary mediastinal large B-cell lymphoma, and lymphoblastic lymphoma. Of these, Hodgkin lymphoma is the most common (50-70%).^{245,246} Hodgkin lymphoma of the mediastinum is more common in women, peaking in the third decade.²³⁶ The vast majority of mediastinal Hodgkin lymphomas are of nodular sclerosis type.

Primary mediastinal large B-cell lymphoma is twice as common in women as in men, and the mean age at presentation is 37.²³⁸ It is a unique entity derived from thymic B-cells. It lacks surface immunoglobulin and Bcl-6 mutations and shows no mantle or follicular center cell differentiation (negative for CD5 and CD10, respectively).²⁴⁷ Morphologically, it is indistinguishable from diffuse large B-cell lymphoma. Because sclerosis is common, FNA samples can be very hypocellular and often contain fragments of collagen. These lymphomas, like their extrathymic counterparts, stain for CD45, CD19, and CD20.²⁴⁷ The differential diagnosis includes anaplastic large cell lymphoma and acute lymphoblastic lymphoma. Anaplastic lymphoma is much more anaplastic, containing characteristic wreath cells, and stains for CD30, and lymphoblastic lymphoma is composed of blasts. Because of their T-cell lineage, both can be

distinguished from primary mediastinal large B-cell lymphoma by immunohistochemistry.

Acute lymphoblastic lymphoma/leukemia, uncommon in adults, is one of the top three lymphomas affecting children.²⁴⁸ The accuracy of FNA is nearly 100%.^{249,250} Eighty percent are T-cell derived, with an immunophenotype identical to that of mature thymic T cells. Cytomorphology, therefore, is critical in the distinction from normal thymic T cells. Lymphoblasts have a high nuclear-to-cytoplasmic ratio, with fine, reticulated chromatin, imperceptible to small nucleoli, smooth to irregular nuclear borders, and usually no cytoplasmic vacuoles. Lymphoblasts are usually numerous and 1.5 to 2 fold larger than mature lymphocytes.²³⁸

Germ Cell Tumors

Germ cell tumors occasionally arise in the mediastinum because of the failure of germ cells to migrate during embryonic development. They comprise 15% of adult mediastinal tumors, primarily in young adults, and are the second most common tumor of the mediastinum after thymoma.²²⁷ There are three general types: teratoma, seminoma, and nonseminomatous germ cell tumor, the last of which includes embryonal carcinoma, yolk sac tumor, and choriocarcinoma. Teratoma is the most common germ cell tumor of the mediastinum and is usually asymptomatic. The malignant germ cell tumors are far more common in men, making up 90% of cases.

Seminoma is the most common malignant germ cell tumor of the mediastinum²⁵¹ and occurs mostly in men between ages 20 and 40. It responds well to treatment, with an 85% 5-year survival rate, compared with 48% for nonseminomatous germ cell tumors.²⁵² Seminomas are usually hypercellular, consisting of loose clusters and isolated tumor cells with round to oval nuclei, coarse chromatin, and a single, large nucleolus, in a background of small, mature lymphocytes. The cytoplasm is usually moderately abundant and contains glycogen vacuoles that create blebs in the plasma membrane. Dispersal of the glycogen vacuoles due to cell disruption creates the classic “tigroid” background on smears ([Fig. 2.45](#)). Although very characteristic, the tigroid background is not pathognomonic of seminoma. It is occasionally seen with high-grade carcinomas of the lung and ovary and nonseminomatous germ cell tumors. Nonseminomatous components are frequently present, making precise classification challenging on a cytologic sample.

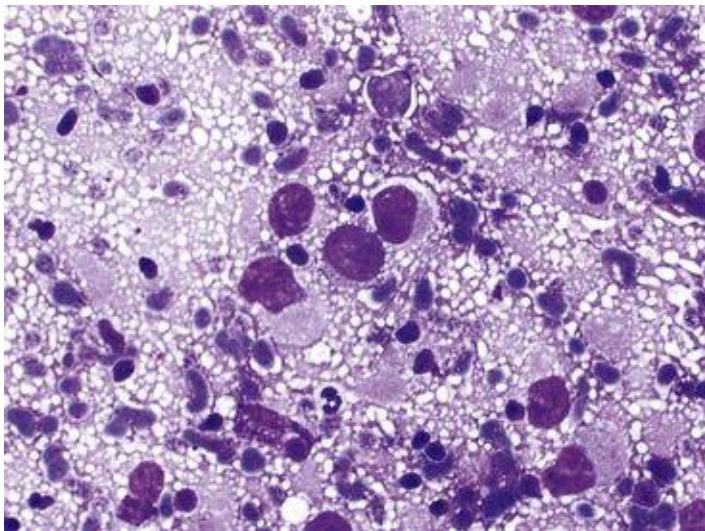


FIG. 2.45 Seminoma of the mediastinum.

Fragile large neoplastic cells and numerous small lymphocytes are surrounded by a “tigroid” background caused by spilled cytoplasmic glycogen (Romanowsky stain).

The distinction between a nonseminomatous germ cell tumor and a seminoma is an important one to make, however, because the treatment and prognosis are different. Seminomas are treated with radiation and/or chemotherapy,^{253,254} and nonseminomatous germ cell tumors are treated with intensive chemotherapy alone.²⁵⁵ FNA of nonseminomatous germ cell tumors of all three types reveals large malignant cells with large nucleoli, and moderate-to-abundant cytoplasm, forming loose or tight three-dimensional aggregates with papillary and glandlike features in a background of necrosis.²⁵⁶ The ability of FNA to subclassify these tumors is not well documented and may not be essential, perhaps because the treatment is similar for all types of nonseminomatous germ cell tumors.²⁵⁷

Immunohistochemistry is helpful in the differential diagnosis of germ cell tumors. Most germ cell tumors are positive for placental alkaline phosphatase, NANOG, and Oct 3/4.^{258,259} Nonseminomatous germ cell tumors can be distinguished from seminomas because the former express SOX2, CD30, alpha-fetoprotein, inhibin, and beta-HCG.^{258–260} Among nonseminomatous germ cell tumors, only choriocarcinoma stains for beta-HCG, whereas only yolk sac tumors and embryonal carcinomas stain for alpha-fetoprotein.^{259,261,262}

NUT Midline Carcinoma

NUT midline carcinoma is a rare, extremely aggressive subtype of squamous cell carcinoma that involves a variety of midline structures, most often the mediastinum.²⁶³ This disease is one of a growing number of cancers defined

purely by its distinctive molecular genetics, in this case a rearrangement of the *NUT* gene (aka NUTM1) on chromosome 15. In most cases, *NUT* (for nuclear protein in testis) is fused to *BRD4* on chromosome 19 as a result of chromosomal translocation, forming a *BRD4-NUT* fusion oncogene,^{[264](#)} but variant translocations occur in one third of cases.^{[263,265](#)} There is no cure, and the median survival is 6.7 months,^{[263](#)} but therapies targeting the fusion protein are in development.^{[266,267](#)} It affects all ages even though it was first described in children and young adults.^{[268](#)} The histopathologic and cytopathologic features are those of a poorly differentiated carcinoma: The malignant cells are predominantly noncohesive and small to moderate in size; nuclei are monomorphic, and cytoplasm is scant to moderate in quantity.^{[269](#)} Some cases are comprised of such monomorphic and poorly differentiated cells that they resemble Ewing sarcoma or leukemia.^{[270](#)} Because they frequently possess focal squamous differentiation, they can also be confused for metastatic squamous cell carcinoma or thymic carcinoma.^{[263](#)} Distinction from other entities, however, is straightforward because, outside of the testis, immunohistochemical staining for the *NUT* fusion protein is 100% specific and 87% sensitive for *NUT* midline carcinoma.^{[167](#)}

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CHAPTER 3

Urine and Bladder Washings

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Specimen Collection

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Summary

Urine cytology was popularized by George Papanicolaou in the 1940s as a way to detect and follow patients with bladder cancer.¹ By the 1960s, the cytologic, histologic, and clinical features of high-grade urothelial carcinoma (UC) were well established.² Cytology remains an inexpensive, quick, and reliable way to diagnose high-grade UC³ as well as a variety of less common tumors. Its reproducibility is variable among laboratories,⁴ however, and this is a significant weakness of the test. A number of ancillary tests have been introduced (e.g., UroVysion) in an attempt to improve accuracy, but their value and precise role are still debated.

Some have argued that there is no significant cost benefit to urine cytology in specific settings⁵ like hematuria.⁶ Furthermore, the U.S. Preventive Services Task Force has found scant high-quality evidence in support of urine cytology and related ancillary studies.⁷ Perhaps it is not surprising that a test adopted more than 50 years ago does not meet contemporary standards for high-quality evidence. It is unlikely that such standards will be met any time in the near future, but alternatives to a randomized prospective trial have been proposed.⁸



Clinical indications for urine cytology

- hematuria
- followup of patients treated for urothelial carcinoma
- high risk of bladder cancer

The most common indication for urinary cytology is hematuria.^{9,10} The yield, however, is low: Hematuria is caused by a malignancy in only 5% to 10% of patients.¹¹⁻¹³ Another indication is surveillance for recurrent UC, because patients with a previously diagnosed and treated urothelial cancer are at risk for recurrence or a de novo primary elsewhere in the urinary tract.² Urine cytology is not used for screening asymptomatic individuals, because the benefits are outweighed by the considerable cost.¹⁴⁻¹⁶ It is used, however, when an individual has risk factors for bladder cancer, such as an occupational exposure to aniline dyes,¹⁷ the aromatic amines used in the petrochemical industry,¹⁸ or cyclophosphamide treatment for diseases like multiple sclerosis.¹⁹

Specimen Collection

There are six types of urinary specimens, each with its relative advantages ([Table 3.1](#)).

TABLE 3.1
RELATIVE ADVANTAGES AND DISADVANTAGES OF URINE SPECIMEN TYPES

Specimen Type	Advantages	Disadvantages
Voided urine	Noninvasive No instrumentation artifact	Low cellularity Vaginal contamination Poor preservation
Catheterized	High cellularity	Invasive Instrumentation artifact Poor preservation
Bladder washing	High cellularity Good cell preservation	Invasive Instrumentation artifact
Upper tract washing	High cellularity Good preservation Selective sampling	Invasive Instrumentation artifact
Brush cytology	Selective sampling	Invasive Air drying possible (if direct smear)
Ileal loop	Permits screening for recurrent bladder cancer	Low cellularity Poor preservation

Voided Urine

Voided urine should be obtained 3 to 4 hours after the patient has last urinated.²⁰⁻²² First morning voided urine specimens should be avoided, because cells in a stagnant, low pH, and hypertonic environment undergo degenerative changes, making cytologic assessment difficult. The minimum amount of urine necessary to ensure adequate cellularity is unknown, but it may be as high as 25 to 100 mL.²³

In women, voided urine can be contaminated by vaginal cells, but in most instances this does not compromise a diagnosis. Still, to help ensure the adequacy of the sample, a midstream (“clean catch”) specimen is recommended.

Catheterized Urine

Specimens obtained by catheterization have disadvantages for both the patient and the cytologist. First, catheterization carries a risk of urinary tract infection. Second, urine collected from an indwelling catheter is often a pooled specimen that has been at room temperature for many hours, and cellular degeneration can be pronounced. Third, the tip of the catheter often scrapes off benign urothelial cell clusters, which mimic a low-grade papillary neoplasm in appearance.

Bladder Washings

Bladder washings are obtained through a catheter by irrigating the bladder with 5 to 10 pulses of 50 mL of sterile normal saline, producing a cellular suspension of freshly exfoliated epithelial cells. This specimen is collected before any biopsy sampling. The chief advantages of bladder washings over voided urine are better cellular preservation, greater cellularity, and less chance of contamination by background debris.

Upper Tract Washings and Brushings

When an upper urinary tract malignancy is suspected, directed washings, brushings, and/or biopsies of a ureter or renal pelvis lesion can be performed. Although brushings obtained by direct visualization using an endoscope were introduced in 1973, they are rarely obtained. Nevertheless, the sensitivity and specificity of brushings compare favorably with those of other cytologic methods (voided, catheterized, irrigation).²⁴

The most common upper tract specimen is the directed washing with or without an upper tract biopsy.²⁵ Directed washing specimens are particularly challenging for urologists and cytologists.^{25–32} Urologists often cannot visualize lesions in the upper tract as well as those in the bladder; hence they rely on cytology here even more than for lesions in the bladder. (Although they may try to obtain a biopsy, often these specimens are small and crushed.) The stakes are high, because the operation of choice for a tumor in the upper tract is removal of the kidney and/or ureter. In most cases, the significant imaging finding is a filling defect, and the differential diagnosis is a tumor or a stone. Unfortunately, benign cytologic atypia produced by some stones can mimic the cytologic features of urothelial neoplasms. Finally, normal specimens from the upper tract often show diffuse nuclear enlargement, an elevated nuclear-to-cytoplasmic ratio, and very high cellularity. These entirely benign changes can suggest a

tumor and result in a false-positive diagnosis.²⁹ For these reasons, it is impossible to accurately diagnose low-grade lesions in upper tract specimens. For high-grade tumors, the sensitivity rates of ureteral washing cytology and ureteral biopsy for the detection of malignancy are similar and approach 70% to 80%.²⁵ With bilateral specimens, one can compare subtle changes between a lesion (on one side) and a presumably normal specimen (on the other side). The preparation of a “cell block” (a formalin-fixed, paraffin-embedded sediment of the urine sample) can be particularly useful, because small pieces of tumor are often easier to evaluate with this preparation method.^{25,33}

Ileal Conduit Samples

At the time of cystectomy for bladder cancer, a segment of ileum is isolated and reanastomosed to the ureters (or to one ureter if a nephrectomy is also performed) to provide a conduit for urine. Urine samples from these conduits contain a large number of degenerated intestinal epithelial cells. It is important that these specimens be screened for malignant cells, because patients with a history of bladder cancer have an increased risk for developing tumors of the ureters and kidneys.^{34,35}

Processing

Fresh specimens, between 1 and 12 hours old, do not need fixation. If it will take a specimen 12 to 24 hours to reach the laboratory, refrigeration is recommended, and if more than 24 hours, preservation with an equal volume of 50% to 70% ethanol \pm 2% carbowax is advised to avoid degeneration.²²

Slides can be prepared using a variety of concentration techniques, depending on the resources and preferences of the laboratory. These include sedimentation and smearing, membrane filtration, cytocentrifugation, and thinlayer methods. Slides prepared using one of these methods are fixed in ethyl alcohol and stained with the Papanicolaou stain. Urine samples and bladder washings can also be prepared using the cell block technique;²³ the centrifuged sediment is fixed in formalin, and the slides are stained using hematoxylin and eosin.

Reporting Terminology and Adequacy Criteria

Clinically meaningful adequacy criteria have not been defined. Nevertheless, there are four situations where the question of adequacy arises.



Patterns of nondiagnostic (unsatisfactory) specimens

- vaginal cells only
- obscuring inflammation or lubricant
- blood only
- marked degenerative changes

With the exception of ileal pouch specimens, a urine sample must contain at least some urothelial cells to ensure that it is indeed urine. For example, voided urine samples from women commonly consist almost entirely of squamous cells from the vagina. In some cases, the urothelial cells are obscured by abundant acute inflammation, presumably due to an infection, or by lubricant jelly. Some specimens consist only of blood. In all three cases, it is reasonable to require the identification of at least one urothelial cell in order to be sure the specimen is adequate and represents urine. Alternatively, one can report the case as benign, but append a note stating that urothelial cells are poorly represented, and clinical correlation concerning the adequacy of the specimen is advised.

Finally, degenerated cells are very common. If the specimen is so degenerated that an interpretation cannot be made (an uncommon circumstance), the specimen is inadequate. If only some cells are degenerated, and particularly if the degenerated cells appear atypical, it is advisable to interpret the specimen as abnormal rather than nondiagnostic (see “Diagnosing Difficult or Borderline Specimens: Common Patterns”).

In most laboratories, the results of urine cytology and bladder washings are reported using simple diagnostic categories such as “negative” (“no malignant cells identified,” or “benign”), “atypical” (“atypia of undetermined significance,” “mildly atypical urothelial cells, favor reactive;” “rare atypical cells, too few to further characterize”), “suspicious” (i.e., abnormal-appearing urothelial cells that are suspicious for carcinoma), and “positive” (i.e., conclusive for malignancy). Because urine cytology is an insensitive test for low-grade urothelial neoplasms,

some laboratories prefer the phrase “negative for a high-grade malignancy/carcinoma” rather than “negative for malignant cells.” For reasons given later (see “Diagnosing Difficult or Borderline Specimens: Common Patterns”), it is recommended that the “atypical” category be used as sparingly as possible.

Accuracy

Urine

Urine cytology is, at best, only moderately sensitive in detecting bladder cancer. A representative summary of published studies on the sensitivity of cytology is provided in [Table 3.2](#). These studies likely overestimate the sensitivity of cytology, because virtually all are affected by selection bias: Patients with a malignant biopsy are more likely to have had a suspicious or positive cytology. “Positive” results are obtained in 25% to 72% of patients with bladder cancer when all grades and stages of tumors are included in the analysis. Several variables affect the sensitivity of urine cytology. First, sensitivity is higher (37% to 89%) when suspicious diagnoses are included with positive diagnoses.^{36,37} Second, the sensitivity increases when more than one specimen is examined: Tumor cells may be absent from one urine specimen but present in subsequent specimens.^{36,38,39} For this reason, it has been recommended that at least three specimens per patient be examined.⁴⁰ Third, the sensitivity of urine cytology is highly dependent on the grade of the bladder tumor. Low-grade UCs are detected less reliably or not at all by cytology, as compared with high-grade carcinomas.^{36,37,41-43} Finally, the sensitivity of urine cytology may be reduced in patients who have been treated with radiation or chemotherapy.³⁷ Molecular cytogenetic analysis using the fluorescence in situ hybridization (FISH) method may be more sensitive than cytology but less specific (see “Ancillary Techniques”).

TABLE 3.2
SENSITIVITY OF URINE AND BLADDER WASHING CYTOLOGY FOR THE DIAGNOSIS OF BLADDER TUMORS

	Number of Specimens Examined per Patient	Number of Biopsy-Proven Bladder Cancers	Positive Cytology (%)	Suspicious and Positive Cytology (%)
URINE				
Umiiker, 1964 ^{46*}	NS	754	72	80
Schoonees et al., 1971 ¹¹⁹	1	114	70	80
Rife et al., 1979 ²¹	NS	634	64	76
Kern, 1984 ¹²⁰	NS	125	71	89
Koss et al., 1985 ³⁶	3	181	NS	83
Badalament et al., 1987†	1	228	49	64
Badalament et al., 1987†	3	149	62	74
Zein, Milad, 1991 ¹²¹	1	362	65	81
Wiener et al., 1993 ³⁷	NS	84‡	NS	71
Bastacky et al., 1999 ⁴¹	variable	180	NS	64
Halling et al., 2000 ⁴²	1	69	NS	58
Raab et al., 2007 ¹²²	variable	291	25	37
Caraway et al., 2010 ¹²³	variable	263	NS	39
Song et al., 2010 ¹²⁴	1	95	NS	28
Hajdinjak, 2011 ^{98*}	NS	NS	42	NS
BLADDER WASHINGS				
Esposti, Zajicek, 1972 ¹²⁵	1	274	71	82
Lewis et al., 1976 ¹²⁶	1	60	70	80
Loening et al., 1978 ^{45§}	1	262	77	NS
Zein et al., 1984 ⁵⁴	1	73	74	89
Badalament et al., 1987 ³⁸	1	59	66	NS
Curry, Wojcik, 2002 ⁴³	1	87	NS	53

NS, not specified.

*Review of multiple studies.

†Superficial bladder tumors (TIS, TA, T1) only.

‡Untreated patients only.

§Includes catheterized urine samples.

The moderate sensitivity of cytology is complemented by its very high specificity (range of most studies: 95% to 100%).⁴² False-positive results, in other words, are uncommon. Some investigators report finding no false-positive results in their studies^{36,44}; others describe rates ranging from 1.3% to 15%.^{40,45,46} False-positive results occur in patients with bladder stones,⁴⁷ human polyomavirus infection,⁴⁸ and chemotherapy.⁴¹ A positive cytologic result in the face of a negative biopsy result does not necessarily mean that the cytologic diagnosis is false. In many cases, a carcinoma that escaped histologic detection is discovered on a subsequent cystoscopic examination. Such a scenario (termed “anticipatory positives”) occurs for ancillary tests as well. Other sites besides the bladder can be the source of the malignant cells; a primary tumor of the ureters, kidneys, prostate, and other contiguous organs must be considered.^{21,49}

Urine cytology is complemented by cystoscopy. Low-grade tumors missed by cytology are papillary lesions readily visualized with the help of a cystoscope and thus earmarked for biopsy.^{50,51} Whether low-grade tumors can be reliably diagnosed on a voided urine sample, by cytology and/or an ancillary study, thus sparing the patient confirmatory cystoscopy, is not clear. Nevertheless, the chance of clinical progression of such a lesion is low. For high-grade carcinomas, however, particularly carcinoma in situ (CIS), which is more

difficult to detect by cystoscopy, cytology provides a high degree of diagnostic accuracy, even in voided urine specimens.

Bladder Washings

Bladder washings have the advantage over urine samples in improved cellularity and cell preservation. The sensitivity of a positive bladder washing cytology is slightly higher than that of urine cytology, ranging from 66% to 77% when all grades and stages of bladder tumor are included ([Table 3.2](#)). The superiority of bladder washings over voided urine cytology has been well documented.⁵²⁻⁵⁴ This is not to say that voided urine from patients undergoing cystoscopy can be neglected. From 7% to 13% of bladder tumors not detected by bladder washings are discovered in urine samples obtained before cystoscopic examination.^{53,55}

Bladder washing cytology is not without its drawbacks. Most significantly, catheterization is required to obtain the specimen. Bladder washings sample the bladder epithelium only, whereas urine contains cells exfoliated from the ureters and kidneys.

Washings of the ureter and pelvis have similar sensitivity (70% to 80% for high-grade lesions) but are particularly prone to false-positive results²⁹ because of the marked cellularity of these specimens.

Normal Elements



Normal elements

- urothelial cells
 - intermediate and superficial (“umbrella”) cells (voided urine)
 - intermediate, superficial, and basal cells (catheterized urine, washings)
- squamous cells
- seminal vesicle epithelial cells (very rare)
- degenerated intestinal epithelial cells (ileal conduit specimens)

A normal voided urine specimen is sparsely cellular, but urothelial (synonym: transitional) cells are usually present ([Fig. 3.1](#)). They are dispersed as isolated individual cells; tight clusters of urothelial cells are distinctly uncommon in a normal voided urine sample. In voided specimens, most urothelial cells are intermediate in size, with a moderate amount of homogeneously granular or finely vacuolated cytoplasm, round nuclei, and small nucleoli. In some cases they are columnar or spindled; this is a normal finding, although the reason for these shapes is not known ([Figs 3.2](#) and [3.3](#)). When degenerated, urothelial cells resemble histiocytes, especially because they sometimes contain round, red or green hyaline cytoplasmic inclusions called Melamed-Wolinska bodies⁵⁶ (see [Fig. 3.2](#)). They are seen in almost 50% of urine specimens and are more common in voided than in catheterized samples. The pathogenesis of these bodies is obscure, and they have no diagnostic value in urine, but they are useful in suggesting a urothelial origin for malignant cells in pleural effusions.⁵⁷ Umbrella cells are large and have abundant cytoplasm and large nuclei; binucleation and multinucleation are common (see [Fig. 3.3](#)). Although large and often multinucleate, umbrella cells have a very low nuclear-to-cytoplasmic ratio, relatively fine chromatin, and thin, smooth nuclear membranes, which helps to distinguish them from malignant cells. Some squamous cells are common in voided urine samples; they exfoliate from foci of squamous metaplasia in the trigone of the bladder (a normal finding, especially in women). Squamous cells can also be picked up as urine passes through the urethral orifice and is contaminated by cells from the vagina. Significant vaginal contamination of a voided urine sample (composed almost exclusively of squamous cells and bacteria, the latter either normal flora or coccobacilli) may necessitate

catheterization to obtain a more pure specimen.⁹

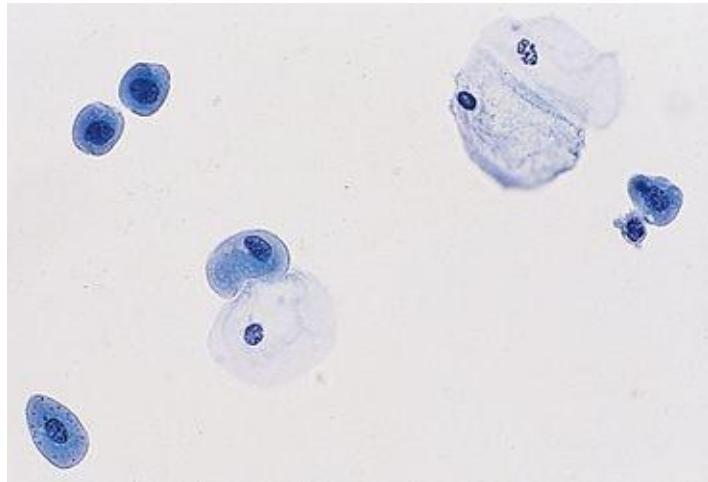


FIGURE 3.1 Normal voided urine.

Most benign voided urine samples show a mixture of urothelial cells and squamous cells. In voided urine, most of the urothelial cells are of “intermediate” type, with an oval or pyramidal shape.

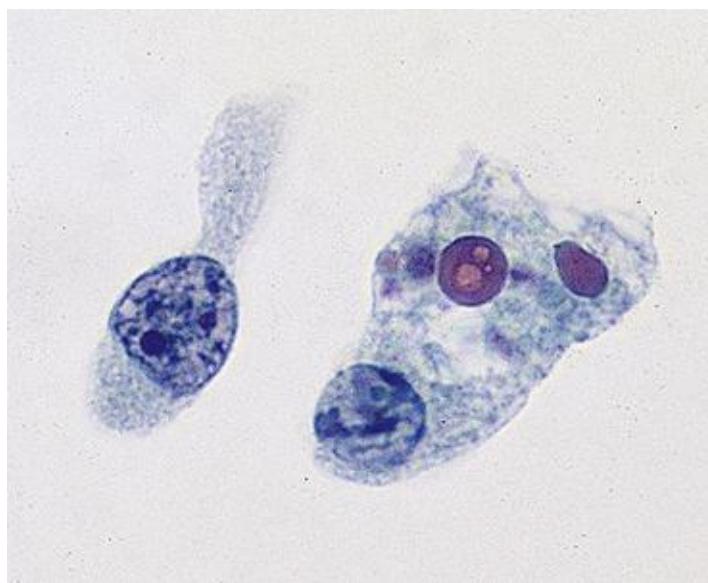


FIGURE 3.2 Cytoplasmic inclusions (Melamed-Wolinska bodies).

Degenerating urothelial cells frequently have round, eosinophilic (or sometimes green) cytoplasmic inclusions of varying sizes. A normal columnar-shaped urothelial cell is also present.

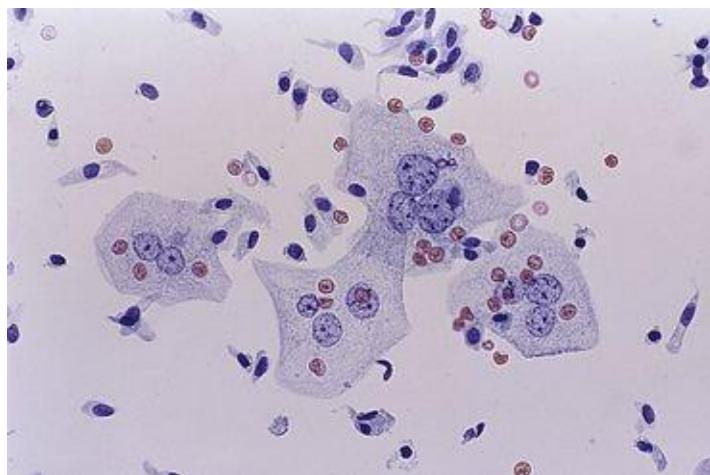


FIGURE 3.3 Umbrella cells.

These are the largest urothelial cells and cover the surface of the urothelium. Normal columnar urothelial cells are also present.

In catheterized samples, including washings and brushings, clusters of urothelial cells, some quite large, are an entirely normal finding: The instrument mechanically abrades the mucosal surface, resulting in large numbers of cells in fragments. Thus, the entire spectrum of basal, intermediate, and superficial (umbrella) cells is seen. Normal catheterized specimens, particularly washings and brushings, may appear worrisome for malignancy, particularly to the novice cytologist, because of the presence of intact mucosal fragments and the marked polymorphism of the cell population. Umbrella cells alone may be worrisome because of their size: They are among the largest of human epithelial cells. Even the small basal urothelial cells ([Fig. 3.4](#)), because of their scant cytoplasm and dark nuclei, are occasionally mistaken for carcinoma cells.

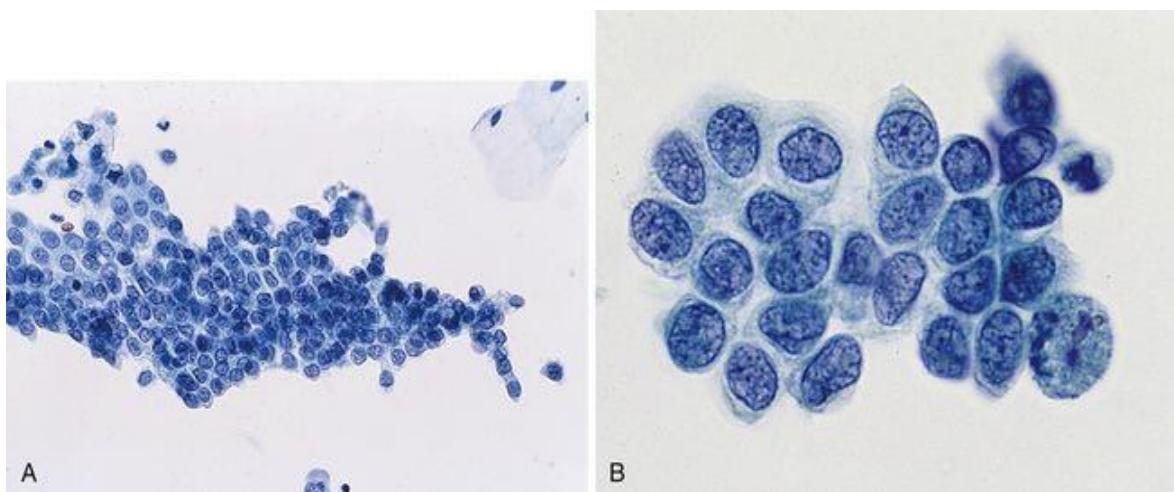


FIGURE 3.4 Basal urothelial cells (catheterized specimen).

A, These cells are rare in voided urine but common in catheterized specimens and usually tightly clustered. B, Higher magnification reveals predominantly round, regular nuclear contours.

On rare occasions, seminal vesicle epithelial cells are seen in urine samples from male patients. They sometimes have hyperchromatic nuclei and can be mistaken for malignant cells. The clue to their benign nature is the presence of lipofuscin, a golden-brown cytoplasmic pigment ([Fig. 3.5](#)).

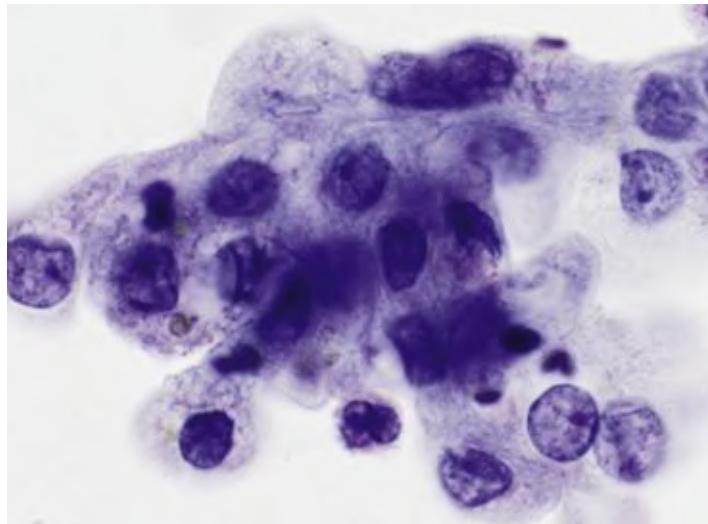


FIGURE 3.5 Seminal vesicle epithelial cells (voided urine).

These cells are recognizable as seminal vesicle cells because of their golden-brown pigment. Sometimes they are less well preserved than seen here.

The intestinal epithelial cells in ileal loop specimens are dispersed as isolated cells and show marked degenerative changes ([Fig. 3.6](#)), with eosinophilic intracytoplasmic inclusions such as those seen in degenerated urothelial cells. They are commonly mistaken for macrophages by the novice.

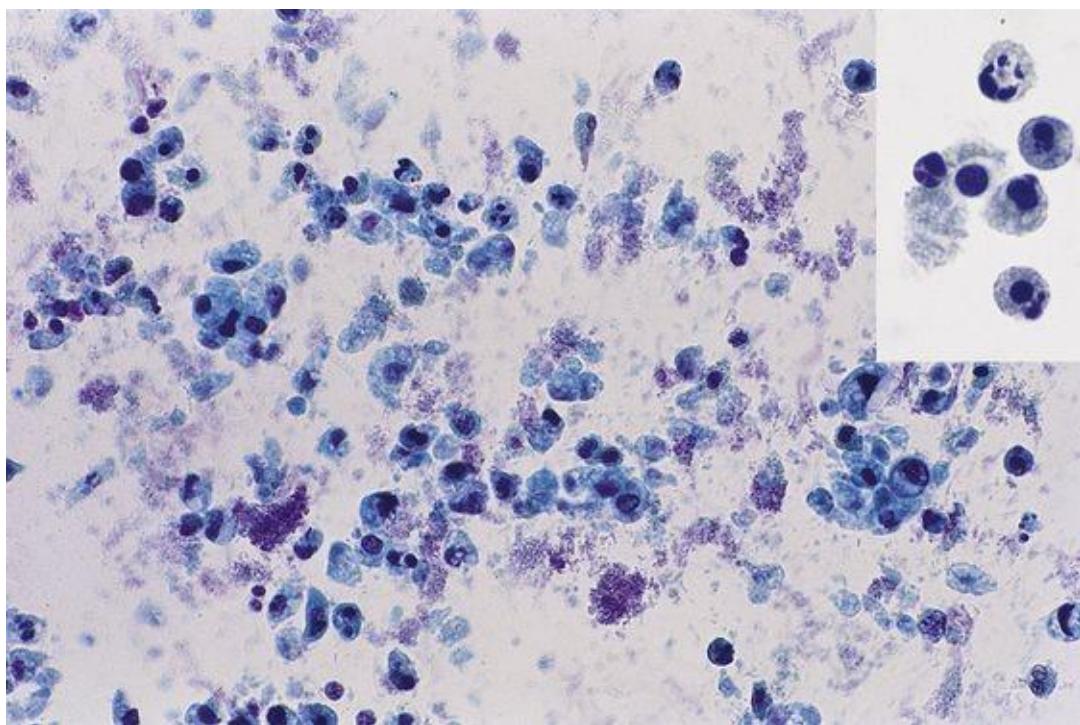


FIGURE 3.6 Ileal loop specimen.

Most cells in ileal loop specimens are degenerated intestinal cells which resemble macrophages (*inset*).

Crystals are commonly present. Occasional red blood cells are common, but the presence of many red blood cells is abnormal. Bacteria and *Candida* are commonly seen, usually without concomitant inflammation or clinical significance.

Benign Lesions

Infections



Infections

- bacteria, including malakoplakia
- fungi (especially *Candida*)
- herpes simplex virus
- cytomegalovirus
- *Trichomonas vaginalis*
- polyomavirus
- human papillomavirus

Infections of the bladder are caused by bacteria, viruses, parasites, or fungi. In the United States, bacterial infections are the most common. Cytologic preparations in cases of bacterial cystitis show a dense concentration of white blood cells (predominantly neutrophils), with plasma cells, lymphocytes, histiocytes, and numerous red blood cells. Bacteria are present, sometimes in overwhelming numbers. The inflammation can obscure the urothelial cells. The presence of bacteria in the absence of abundant neutrophils is nonspecific. This may be the result of vaginal or urethral contamination, but many patients, particularly females, have bacteria in the bladder without clinical symptoms of cystitis. Likewise, neutrophils are not necessarily indicative of a cystitis of infectious etiology.

Malakoplakia is an uncommon histiocytic inflammatory lesion of the bladder or upper respiratory tract that results from bacterial infection. Diagnosis by urine cytology is very uncommon. The cytologic hallmark is the presence of histiocytes, whose abundant granular cytoplasm is filled with bacteria and bacterial fragments. Often one finds basophilic, round, lamellated bodies, known as Michaelis-Gutmann bodies, which measure approximately 8 μm in diameter and may be intracellular within histiocytes or extracellular.

The most common fungus that infects the bladder is *Candida*. The organism is present as yeast forms and pseudohyphae, accompanied by a cellular, mixed inflammatory background. Urothelial cells usually show reactive changes. When *Candida* is present in the urine of female patients, the possibility of vaginal

contamination should be considered. Vaginal contamination, rather than true infection, is likely when the background contains numerous squamous cells and bacteria and few neutrophils. As with bacteria, some patients have *Candida* in their bladder without symptoms of cystitis.

Viral infections of the bladder include herpes simplex virus, cytomegalovirus (CMV), polyomavirus, and human papillomavirus (HPV). Herpetic infection of the bladder is uncommon, usually seen in the immunocompromised patient. The cytopathic changes include multinucleation, a ground-glass chromatin texture, and peripheral condensation of chromatin. In some cases the cells have a large eosinophilic nuclear inclusion which can be sharply angulated. Nuclear molding is often observed. Infected cells can be greatly enlarged and bizarrely shaped, with dense, opaque cytoplasm.

CMV affects urinary epithelium, most commonly renal tubular cells, in immunocompromised patients. Affected cells are markedly enlarged and have both nuclear and cytoplasmic inclusions. The nuclear inclusion is solitary, darkly basophilic, and surrounded by a zone of chromatin clearing. The multiple cytoplasmic inclusions are more variable in appearance: smaller, basophilic, and either finely or coarsely granular.

The parasitic protozoan *Trichomonas vaginalis* is responsible for one of the most common sexually transmitted diseases. It is usually associated with vaginitis, but it can cause urethritis and even prostatitis. In urine from a woman, the organisms are most likely contaminants from a vaginal infection if they are accompanied by abundant squamous cells and vaginal flora.⁵⁸ Rarely, they cause urethritis in men and are identified in urine cytology from such patients.^{59,60} The organism varies in size, with an average length and width of 10 and 7 µm, respectively. The nucleus is small and oval, and the cytoplasm contains fine red granules.

The human polyomaviruses (JC and BK viruses), members of the papovavirus family, commonly infect urothelial cells in both healthy and immunocompromised individuals, and characteristic viral cytopathic changes are seen in 4% of urine samples.⁶¹ The infection usually has no clinical significance, except in immunocompromised patients, particularly renal transplant recipients, but infected cells appear atypical and can be confused with malignant cells.⁴⁸ Infected urothelial cells have large, eccentrically placed nuclei with basophilic nuclear inclusions that completely replace the nucleus and appear glassy, opaque, or cloudy ([Fig. 3.7](#)). Nuclear membranes are markedly thickened. In children and immunosuppressed adults, the altered cells are numerous, whereas in immunocompetent adults, they are usually few in number.⁶² In contrast with CMV-infected cells, in which a halo surrounds the inclusion, the inclusion of

polyomavirus fills the entire nucleus. Because of their increased nuclear size and hyperchromasia, polyomavirus-infected cells can be confused with malignant cells—hence their pseudonym “decoy cells.” Unlike most malignant cells, however, decoy cells have perfectly smooth and round nuclei. In contrast with tumor cells, which often cluster to form groups, polyomavirus-infected urothelial cells are found only as isolated cells.⁴⁸ Because degenerated UC cells can resemble decoy cells, however, one should not interpret specimens as negative unless the morphology of the decoy cells is indeed classic.⁶¹ Testing for polyomavirus by immunohistochemistry or polymerase chain reaction is available and can be helpful in difficult cases. The differential diagnosis of decoy cells also includes degenerated benign urothelial cells. Polyomavirus-infected nuclei appear smudged and densely basophilic, and the chromatin is more uniform in texture than that of degenerated urothelial cells.

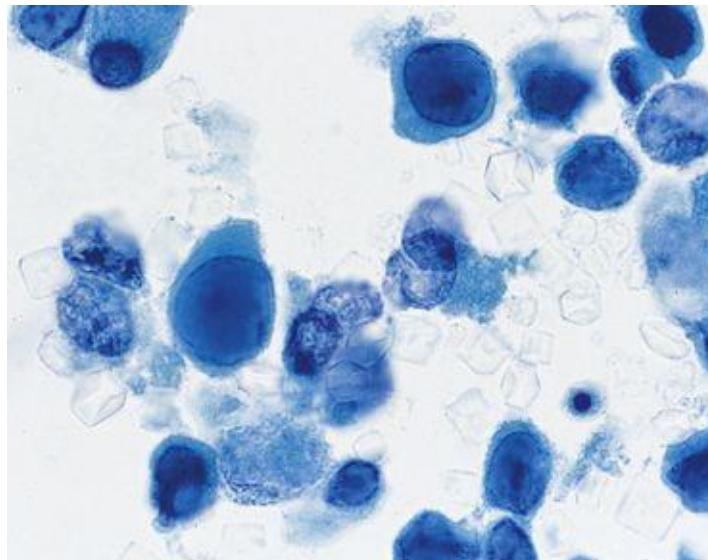


FIGURE 3.7 Polyomavirus infection.

Some enlarged, round nuclei are virtually replaced by a glassy, homogeneous inclusion.

HPV can infect the urinary tract,⁶³ but when cytopathic changes characteristic of this virus are seen in a voided urine specimen from a woman, the cells most likely have originated from the vulva or vagina. Koilocytes in a catheterized specimen, however, indicate a condyloma in the urinary tract.

Noninfectious Findings and Conditions

Crystals

Crystals are a common finding in urine specimens. Most have no clinical significance, and their existence depends on the concentration of their constituents and on the pH and temperature of the specimen. Crystals are reported and classified as a part of routine urinalysis, which is carried out on wet preparations rather than on cytologic ones. Most urologists do not expect interpretation of crystals on a cytology report. Still, many crystals retain their characteristic shapes on alcohol-fixed, Papanicolaou-stained preparations. Triple phosphate crystals are shaped like prisms and resemble coffin lids. Ammonium biurate crystals are spheres with protruding spicules (“thorn apples”). Uric acid crystals are the most common. They vary markedly in size and shape, and may look like many other crystals. Calcium oxalate crystals can be oval, dumbbell-shaped, or small and octahedral.

Other less common crystals include those composed of bilirubin (brown granules and needles), cholesterol, cystine (hexagonal plates), leucine (spheres with radiating striations), and tyrosine (slender needles).

Casts

Casts found in urine samples may be of no clinical significance, or they may be a manifestation of serious renal disease. As with crystals, most urologists do not expect an interpretation of casts on a cytologic specimen, but it is appropriate to comment on them if numerous. Hyaline casts and granular casts are physiologic and can be present in normal urine in large numbers, especially after physical stress. Hyaline casts have a homogeneous, glassy texture; granular casts are composed of finely or coarsely granular debris.

Red blood cell casts are characteristic of glomerular disease. White blood cell casts are seen in tubulointerstitial diseases and in association with transplant rejection. Epithelial casts, composed of degenerated renal tubular cells, can be seen in any disease, including acute tubular necrosis. Waxy casts are homogeneous and dense, often with sharp edges and fractures. Fatty casts contain lipid vacuoles and are seen in patients with the nephrotic syndrome.

Nonspecific Reactive Urothelial Cell Changes

Inflammation and injury to the urothelium result in reactive urothelial cell changes.



Cytomorphology of nonspecific reactive changes

- enlarged nuclei
- prominent nucleoli
- coarsely vacuolated cytoplasm

Cytologic features that support the diagnosis of reactive urothelial cells include coarsely vacuolated (as opposed to granular or finely vacuolated) cytoplasm, enlarged nuclei, and prominent nucleoli (Fig. 3.8). Although adenocarcinomas can have vacuolated cytoplasm, their nuclei are so atypical that they are rarely confused with reactive urothelial cells.

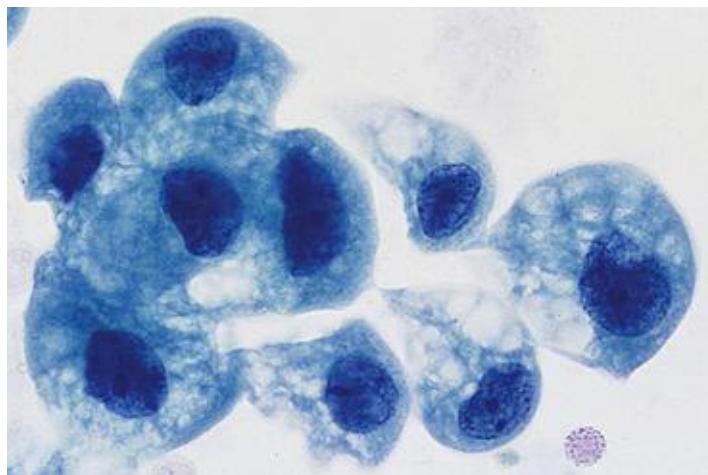


FIGURE 3.8 Reactive urothelial cells (catheterized urine).

Coarsely vacuolated cytoplasm is characteristic of benign, reactive changes and uncommon in malignancy.

Effects of Radiation and Chemotherapy

Radiation typically produces cellular and nuclear enlargement, although the nuclear-to-cytoplasmic ratio is not increased overall. Affected cells usually exfoliate as isolated cells rather than clusters. The cytomorphology varies greatly from one cell to the next. Cytoplasmic and nuclear vacuolization are common. Both cytoplasm and nucleus often show degenerative changes. The nuclear chromatin can be smudged and featureless, but the nuclear membrane remains

smooth, without the irregularity typical of malignant cells. Radiation effect can persist for weeks, months, or years after completion of treatment.

Thiotepa and mitomycin C, topical agents injected intravesically for the management of bladder cancer, produce striking cellular abnormalities similar to those seen in epithelia after radiation. Affected cells have enlarged cytoplasm and nuclei, but the normal nuclear-to-cytoplasmic ratio is preserved.⁶⁴ Nuclei are smooth in contour, round to oval in shape, and show chromatin smudging, but most affected nuclei are not hyperchromatic. Cells are frequently multinucleated, with vacuolization of nucleus or cytoplasm, which can have frayed borders. These changes have been observed in patients as early as 1 month after treatment with thiotepa.⁶⁵ Similar cytologic abnormalities are seen in patients receiving systemic chemotherapeutic agents such as cyclophosphamide and busulfan.

The changes induced by radiation and chemotherapy can mimic malignancy.⁴¹ Analysis of the nuclear-to-cytoplasmic ratio and nuclear chromasia is very important in this regard: Although the nucleus of a normal urothelial cell is enlarged after radiation or chemotherapy, a normal nuclear-to-cytoplasmic ratio is preserved, and the nucleus is normochromatic. In recurrent cancer, the nucleus is dark and the nuclear-to-cytoplasmic ratio is increased. Nevertheless, in some cases the distinction can not be made reliably either cytologically or histologically, only with followup.

Urothelial Atypia Associated with Urinary Calculi

Urinary tract calculi are the most frequent cause of ureteral obstruction. Patients present with hematuria, sometimes accompanied by severe pain. Large stones can be visualized on imaging studies. Some cytologic specimens have a background of blood. Inflammatory cells can be present, including neutrophils and lymphocytes. Tight groups of urothelial cells are common. In most instances these groups have smooth contours, and the cells appear benign: They have a centrally placed nucleus with a smooth nuclear border and finely granular chromatin. Such innocuous-looking cell clusters are impossible to distinguish from those of a papilloma, a papillary urothelial neoplasm of low malignant potential (PUNLMP), or even a low-grade UC. In a small proportion of patients with stones, the urothelial cells are atypical, some forming irregular and dyshesive clusters ([Fig. 3.9A and B](#)). They can show pleomorphism, coarsely granular chromatin, hyperchromasia, irregular nuclear staining, and occasional mitotic figures, and such findings can lead to a false-positive cytologic diagnosis.^{47,66,67}

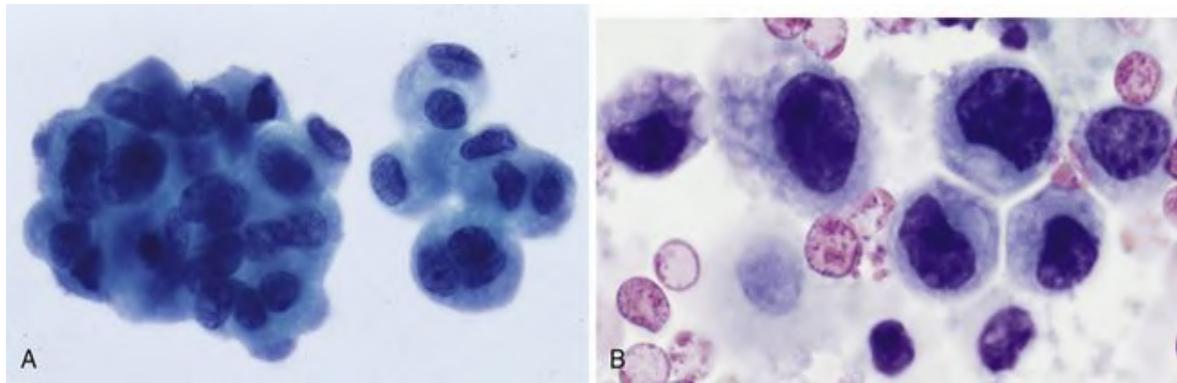


FIGURE 3.9 Benign stone atypia.

A, The mild atypia of these urothelial cells was induced by multiple bladder stones, which were removed 4 days later. Subsequent urine findings have been benign. B, In some cases, the urothelial cells are markedly atypical, with hyperchromatic and angulated nuclei. A distinction from urothelial carcinoma (UC) in such cases can be impossible.

Urothelial Neoplasms

For unclear reasons, the incidence of UC is rising. It accounts for 3% of all cancer-related deaths.⁶⁸⁻⁷⁰ There are over 73,000 new cases of bladder cancer in the United States each year and 14,880 deaths per year.⁷⁰ UC occurs three times as often in males, usually in patients over 50 years of age, and is associated with smoking and other risk factors, including a number of occupational exposures.^{2,68} Patients receiving cyclophosphamide, used for the treatment of nonurologic diseases like multiple sclerosis and Wegener granulomatosis, are at increased risk for developing bladder cancer.¹⁹ UCs typically have a large number of genetic alterations involving multiple different chromosomes (see “Ancillary Techniques”).



Occupational/environmental exposures and other risk factors for urothelial neoplasms

- aromatic amines
- phenacetin
- cyclophosphamide treatment
- alkylating agents
- smoking
- *Schistosoma hematobium*
- paralysis
- exstrophy
- diverticula

Several different histologic classification systems for urothelial neoplasms have been used in the past. The current standard is that of the World Health Organization (WHO) and the International Society of Urologic Pathologists (ISUP).²¹ Because their urologists prefer it, many pathologists, however, continue to use the prior WHO classification system, which divided bladder cancers into three grades: transitional cell carcinomas grade 1, 2, and 3. The current WHO/ISUP system recognizes PUNLMP, a lesion with a low risk of progression (<10%). Many of the lesions formerly called *transitional cell carcinoma, grade 1* are now considered PUNLMPs and are therefore not, strictly speaking, carcinomas. Although some authorities suggest that PUNLMPs should constitute approximately one third of all urothelial neoplasm, in many laboratories this

diagnosis is used much less frequently, and many such lesions are interpreted instead as low-grade UCs. Most of the formerly termed *transitional cell carcinomas grade 2* are now considered either low-grade or high-grade UC.



Current WHO/ISUP classification system for urothelial neoplasms

- flat lesions
 - dysplasia
 - carcinoma in situ
- papillary lesions
 - papilloma
 - papillary urothelial neoplasm of low malignant potential
 - low-grade urothelial carcinoma
 - high-grade urothelial carcinoma

Several points deserve emphasis regarding the current histologic classification of bladder tumors. First, papillomas, defined histologically as papillary lesions without cytologic atypia, are very rare and occur almost exclusively in young patients. Similar lesions in older patients likely represent PUNLMPs, which are defined histologically as papillary lesions with minimal to absent cytologic atypia but an increased cellular proliferation exceeding the thickness of normal urothelium. Second, urothelial dysplasia is a controversial, poorly defined entity, and the histologic and cytologic interpretation of dysplasia is irreproducible. For these reasons, an interpretation of “dysplasia” in a cytologic preparation of urine should be avoided. Because histologic dysplasia, such as it is, is almost always accompanied by CIS, its clinical significance is unclear. Finally, the threshold for the diagnosis of a high-grade tumor is set lower than in previous classifications.

The current WHO histologic classification system has important implications for cytologic diagnosis. In theory, the sensitivity of cytology for detecting malignancy should have improved with the adoption of the new system, because a lesion previously interpreted histologically as a grade 1 transitional cell carcinoma is now a PUNLMP. Recent studies, however, have not supported this (see [Table 3.2](#)). More importantly, in the new classification system, the term *low-grade carcinoma* is used for some lesions that in the older system were classified as *transitional cell carcinoma, grade 2*. Yet how much atypia is required for a histologic diagnosis of high-grade disease is not well defined. For practical

purposes, if a lesion can be recognized as malignant by cytologic atypia alone, we diagnose it as a high-grade lesion. The reproducibility of such a threshold is not known.

The stage of disease plays an important role in determining treatment. Superficial bladder cancers (i.e., noninvasive tumors or tumors that invade no deeper than the lamina propria) are treated conservatively, and patients followed at regular intervals (e.g., every 3 to 12 months) with cystoscopy and cytology for recurrence and progression. Patients with a UC that has invaded into the muscularis propria (“muscle-invasive bladder cancer”) are candidates for cystectomy or irradiation.

Papilloma

Although there remains considerable debate, most investigators believe that a papilloma is best defined as a rare papillary lesion without any cytologic atypia that occurs almost exclusively in young patients and never recurs. Because the lesion has no atypia, it cannot be recognized cytologically unless an intact papillary frond is identified.

Low-Grade Urothelial Lesions

Papillary Urothelial Neoplasm of Low Malignant Potential and Low-Grade Urothelial Carcinoma

In the latest WHO classification system, a PUNLMP is defined histologically as a papillary lesion with minimal to absent cytologic atypia but an increased cellular proliferation exceeding the thickness of normal urothelium. A low grade UC, on the other hand, is a neoplasm with mild nuclear atypia. The cytologic features of these lesions are similar and are summarized below.

The criteria for diagnosing low-grade lesions on cytologic material have been extensively studied over the past two decades. There are two general approaches: cytologic ([Fig. 3.10](#)) and architectural ([Fig. 3.11A](#) and [B](#)). The cytologic criteria^{49,72,73} are listed in the following box.



FIGURE 3.10 Cytologic criteria for the diagnosis of a low-grade urothelial lesion (catheterized specimen).
Homogeneous cytoplasm, an increased nuclear-to-cytoplasmic ratio, and irregular nuclear outlines are associated with low-grade lesions but are not specific.

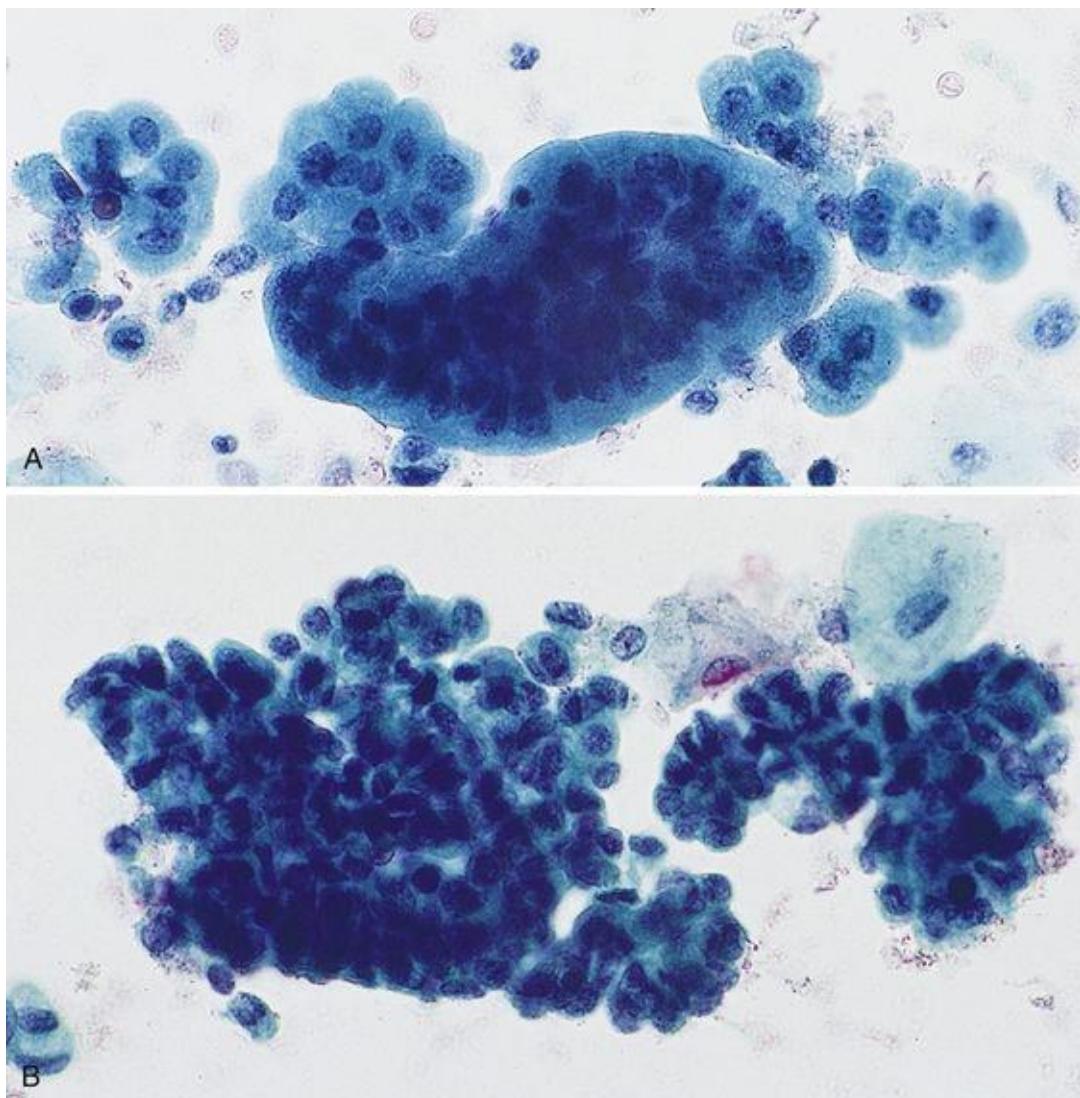


FIGURE 3.11 Architectural criteria for the diagnosis of a low-grade urothelial lesion (catheterized specimen).
A, Benign cell clusters have a smooth outline (“collared” groups). *B*, Low-grade lesions tend to exfoliate as clusters with irregular edges. Unfortunately, this feature lacks good specificity.



Cytologic criteria for diagnosing low-grade lesions

- cytoplasmic homogeneity
- high nuclear-to-cytoplasmic ratio
- irregular nuclear membranes

Although there is little argument over the criteria, there is disagreement on

how accurately low-grade lesions can be diagnosed. The sensitivity of cytology for detecting low-grade tumors using these criteria is about 30%, and the specificity about 80%.²³ In practice, specimens from patients without tumors far outnumber those from patients with tumors. As a result, most patients with a cytologic diagnosis of a low-grade lesion prove not to have a tumor.

The architectural criteria²³⁻⁷⁷ include the features listed in the following box.



Architectural criteria for diagnosing low-grade lesions

- papillary fragments with fibrovascular cores (diagnostic, but rare)
- cell clusters without cores (not specific: also seen with urolithiasis, catheterization)
- irregular cell clusters (more commonly associated with carcinoma than smooth cell clusters)

A papillary fragment with an intact fibrovascular core is diagnostic, but finding one is rare, occurring most often when urine is collected after a urologist has biopsied an obvious lesion. Numerous irregular fragments are associated with a sensitivity of 10% in voided urine and 44% in catheterized urine. The specificity is 83% in voided urine and 69% in catheterized urine. False-positive results are frequent if one uses these criteria to diagnose a low-grade lesion, because benign changes such as stone atypia mimic low-grade lesions (see [Fig. 3.9A](#)).

The cytologic and architectural criteria are unreliable in practice; these lesions have very little chance of progression, and, usually, the urologist can see these lesions cystoscopically and does not need or expect a cytologic diagnosis. For these reasons, there is little justification for attempting the cytologic diagnosis of low-grade lesions. On the other hand, urologists would welcome attempts at the diagnosis of low-grade tumors in the upper urinary tract (ureter and renal pelvis), where cystoscopic visibility is markedly reduced. Here the criteria, unfortunately, have proved even less useful. Thus, many laboratories do not even attempt to diagnose low-grade UC unless an intact papillary fragment is identified. In subtle cases, where sufficient cytologic atypia allows recognition of cells as neoplastic, the lesion is likely the low end of a high-grade UC. The presence of recognizable cytologic atypia is therefore a practical definition of the cutoff between the two grades. Ancillary studies like UroVysion are likely more sensitive for low-grade tumors than cytology is.

While these criteria are not very good for identifying low-grade tumors, they are helpful in designating a reactive pattern (see “Nonspecific Reactive Urothelial Cell Changes”).

Dysplasia

Although listed in the new WHO histologic classification system, dysplasia is a controversial lesion. There is no consensus on its morphologic features (opinions range from normal to high-grade nuclei). In addition, the diagnosis of dysplasia is of limited value in cytology, because almost all affected patients have a coexistent high-grade lesion. Finally, the term lacks precision, because some cytologists use it to refer to a specific entity and others to describe any atypia of undetermined significance. Therefore, it is advisable to avoid the term *dysplasia* in urinary cytology.

High-Grade Urothelial Carcinoma and Carcinoma In Situ

In the new WHO classification system, high-grade UC is defined histologically as a tumor with moderate to marked cytologic and architectural atypia; it can be an invasive tumor or a papillary, noninvasive tumor. Urothelial CIS is a flat, noninvasive lesion confined to the epithelium and composed of cytologically malignant cells. CIS and high-grade UC are indistinguishable cytologically and are considered together in the following discussion.



Cytomorphology of carcinoma in situ and high-grade urothelial carcinoma

- high nuclear-to-cytoplasmic ratio
- marked nuclear hyperchromasia
- coarsely granular chromatin
- irregular nuclear outline
- large nucleoli (some cases)

Urine and bladder washings contain predominantly isolated cells ([Fig. 3.12](#)), although occasional cell groups are seen ([Fig. 3.13](#)). The cells are large and highly atypical, often with a high nuclear-to-cytoplasmic ratio. Nuclei are

hyperchromatic, with coarsely granular chromatin and irregular nuclear membranes; enlarged and angular nucleoli are seen in some cases. Some malignant cells contain Melamed-Wolinska bodies.⁵⁶ The background can contain necrotic debris, blood, and inflammatory cells; their presence or absence does not, however, distinguish between an invasive versus in-situ tumor. High-grade UCs can show squamous or glandular differentiation ([Fig. 3.14A and B](#)).

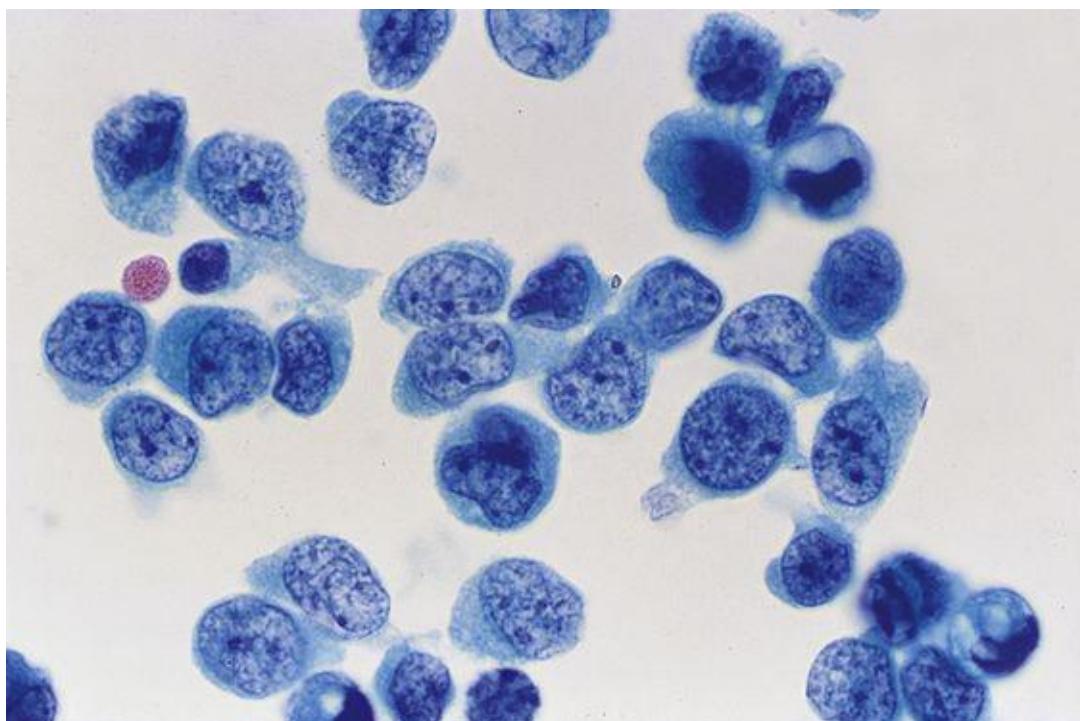


FIGURE 3.12 High-grade urothelial carcinoma (UC).
Numerous isolated malignant cells have enlarged nuclei with coarsely textured chromatin and an increased nuclear-to-cytoplasmic ratio.

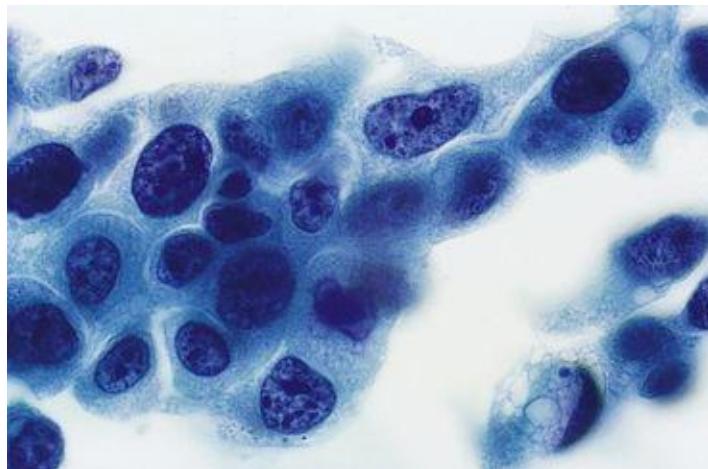


FIGURE 3.13 High-grade urothelial carcinoma (UC).
A cluster of malignant urothelial cells, some with prominent nucleoli.

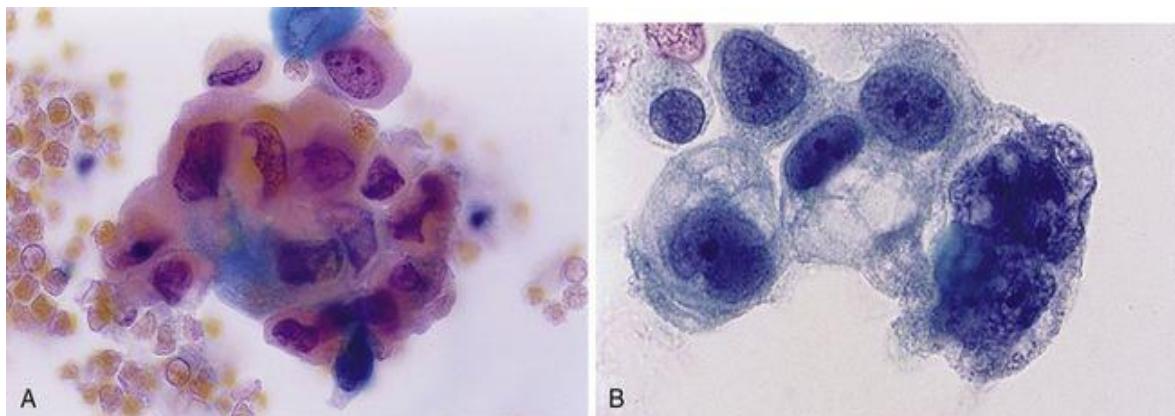


FIGURE 3.14 Urothelial carcinoma (UC) variants.
A, Some of the malignant cells show squamous differentiation, manifested by cytoplasmic orangeophilia. B, Some UCs have foci of adenocarcinoma.

In many cases the diagnosis is straightforward. The sensitivity of urine cytology for high-grade UC is 79%, and specificity is greater than 95%.⁴¹ Some studies, however, have reported a surprisingly low sensitivity for high-grade carcinoma, often no higher than 50%.⁴² These results suggest that, in some laboratories, high-grade carcinoma is underdiagnosed, with malignant cells reported, at best, as “atypical” because they were considered either too rare or poorly preserved to be diagnostic (or even suspicious).



Differential diagnosis of carcinoma in situ and high-

grade urothelial carcinoma

- polyomavirus
- stone atypia
- normal upper tract washings/brushings
- treatment effect
- nonspecific reactive changes

Because of their increased nuclear size and hyperchromasia, polyomavirus-infected cells (decoy cells) can be confused with malignant cells, particularly high-grade carcinoma (see [Fig. 3.7](#)). Malignant nuclei, however, are rarely as perfectly round as decoy cell nuclei. In contrast with tumor cells, some of which form groups, polyomavirus-infected urothelial cells are found only as isolated cells.⁴⁸ Kidney and bladder stones cause irritation of the urothelium, which in some cases results in a marked urothelial cell atypia that mimics carcinoma⁴⁷ (see [Fig. 3.9B](#)). If the patient is known to have a stone, a conservative approach to diagnosis is warranted.

Normal upper tract (ureteral and renal pelvic) washings are prone to false-positive diagnosis.²⁹ The cells are numerous, with large nuclei and a high nuclear-to-cytoplasmic ratio. Bilateral specimens are helpful because they allow comparison between a lesional and presumably normal specimen ([Fig. 3.15](#)). Preparing a cell block from the residual sediment is also particularly useful in this setting.²⁵

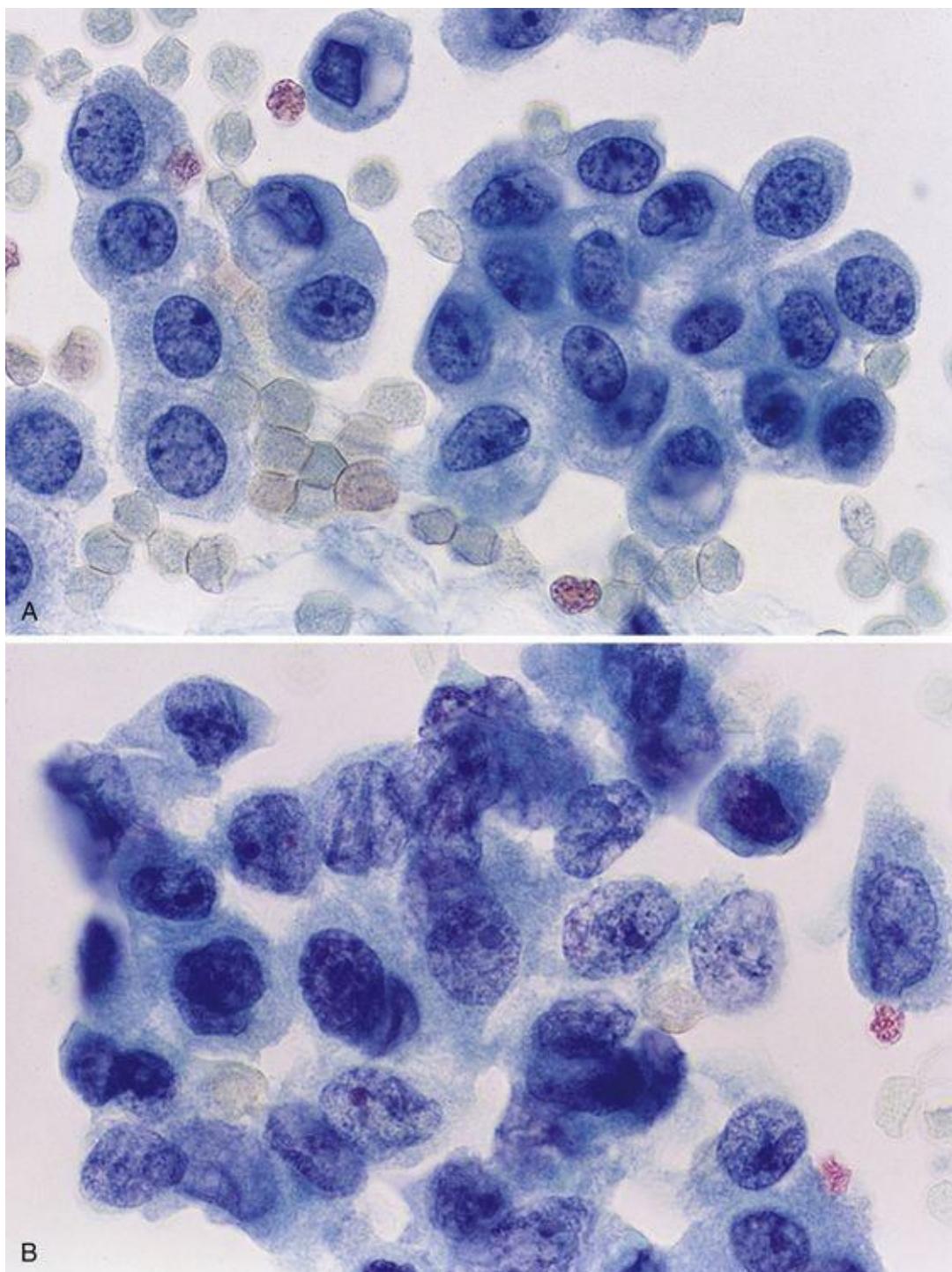


FIGURE 3.15 Bilateral ureteral washings.

A, A sample from the left ureter showing benign urothelial cells. B, A sample from the right ureter showing urothelial carcinoma (UC).

Radiation and chemotherapy produce cellular and nuclear enlargements that suggest UC, but the nuclear-to-cytoplasmic ratio is not increased, and there is

generally no significant hyperchromasia. There can be multinucleation, and cytoplasmic and nuclear vacuolization are common. The nuclei of treatment effect are sometimes smudged and featureless, but some carcinomas can have similar features.

Coarse cytoplasmic vacuolization is very uncommon in UC. When present, it is a clue that the atypia is due to benign reactive changes (see [Fig. 3.8](#)) rather than malignancy.

Urinary cytology is sometimes positive in the absence of a visible lesion.⁴⁹ There are two possible explanations. The lesion may be in the upper tract, for which radiologic studies and selective sampling are indicated or, less commonly, in the urethra, where it may not be well visualized at cystoscopy. Alternatively, a bladder lesion might be cystoscopically subtle or undetectable, in which case blind biopsies are of value. Even if blind biopsies are negative, a lesion is usually detected within the next few years.

Following treatment for UC, patients require continued surveillance for lesions that might develop elsewhere in the bladder, upper urinary tract, or urethra, given the multifocal nature of urothelial neoplasia. A patient whose urethra has not been removed at the time of radical cystectomy is screened by periodic urethral irrigation or swabbing.

Other Malignant Lesions

Other Primary Cancers of the Urinary Tract

Squamous Cell Carcinoma

Pure squamous cell carcinoma (SQC) is rare and strongly associated with *Schistosoma hematobium*. It is common in the Nile River valley but rare in the United States (<3% of all bladder cancers). Importantly, focal squamous differentiation is common in UCs (see [Fig. 3.14A](#)). A definite diagnosis of SQC should be deferred to biopsy or resection.



Cytomorphology of squamous cell carcinoma

- cytoplasmic keratinization
- pearls
- bridges
- angulated hyperchromatic nuclei

The differential diagnosis includes condyloma accuminatum of the bladder,⁶³ metastatic SQC, and an SQC of the gynecologic tract.

Adenocarcinoma

Adenocarcinomas of the bladder are rare and strongly associated with bladder extrophy and urachal remnants. They represent less than 2% of all bladder carcinomas. They resemble gastrointestinal adenocarcinomas, either well-differentiated tumors with isolated columnar cells, hyperchromatic nuclei, and amphophilic cytoplasm; or poorly differentiated tumors with signet ring cells or high-grade nuclei with prominent nucleoli. Abundant mucin can be present. Glandular differentiation is common in otherwise typical UCs (see [Fig. 3.14B](#)), and a definitive diagnosis of pure adenocarcinoma is best left to biopsy or resection. The differential diagnosis includes metastatic adenocarcinoma, particularly from the rectum.

Clear Cell Carcinoma

Clear cell carcinoma of the bladder is extremely rare⁷⁸ and resembles clear cell carcinoma of the female genital tract. When it occurs in the urologic tract it can be related to mullerian remnants, and it arises more often in or near the urethra than in the bladder.



Cytomorphology of clear cell carcinoma

- small rounded clusters of obviously malignant cells
- abundant clear cytoplasm
- large irregular nuclei
- vesicular chromatin
- large nucleoli

Small Cell Carcinoma

Small cell carcinoma is a very rare aggressive tumor, although the prognosis for affected patients is better than for those with small cell carcinoma of other sites.⁷⁹ The cytomorphology^{80,81} is identical to that of small cell carcinoma of other sites. The differential diagnosis includes metastatic lesions, especially from the lung.

Metastatic Cancers

Renal Cell Carcinoma

Some investigators have found malignant cells in the urine of approximately 50% of patients with renal cell carcinoma (RCC), including those with small tumors, but these studies predate the widespread use of computed tomography and magnetic resonance imaging.⁸² This high detection rate is at odds with other reports⁸³ and the personal experience of the author, who has reviewed the records from one large medical center over 10 years and found no such cases. Even those who more commonly detect RCC cells in urine acknowledge that urine cytology has no value in screening for RCC.^{22,82}

Prostatic Carcinoma

Prostatic carcinoma cells in urine specimens almost always occur in patients with poorly differentiated (Gleason score ≥ 8), unresectable tumors. There are two characteristic appearances. In some cases, the cells have prominent nucleoli and relatively abundant cytoplasm and are easy to identify as prostatic in origin⁸⁴ ([Fig. 3.16A](#)). In other cases, the cells have dark nuclei and resemble UC ([Fig. 3.16B](#))⁸³; without clinical information, the correct diagnosis is very difficult to achieve. Fortunately, the diagnosis of prostate cancer is already known in most cases. Nevertheless, in some cases the diagnosis is first suggested by urine cytology.⁸⁴

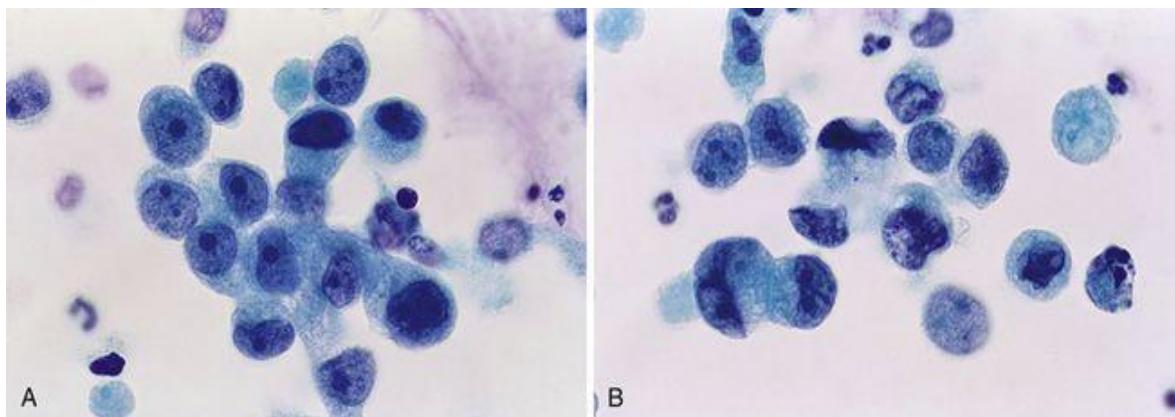


FIGURE 3.16 Prostatic carcinoma.

A, Some tumors have abundant cytoplasm and large nucleoli. B, Other high-grade prostate cancers are hard to distinguish from urothelial carcinoma (UC).

Colonic Carcinoma

Colonic carcinoma cells in urine specimens are often indistinguishable from those of a urothelial cancer. The cells are pleomorphic, with degenerated, hyperchromatic, irregular nuclei, and some cells can be vacuolated.⁸³ A clinical history of colon cancer may alert the cytologist to the possibility of metastatic colon cancer; cell block sections can be used to identify the characteristic immunophenotype of the cells ([Fig. 3.17A-C](#)).

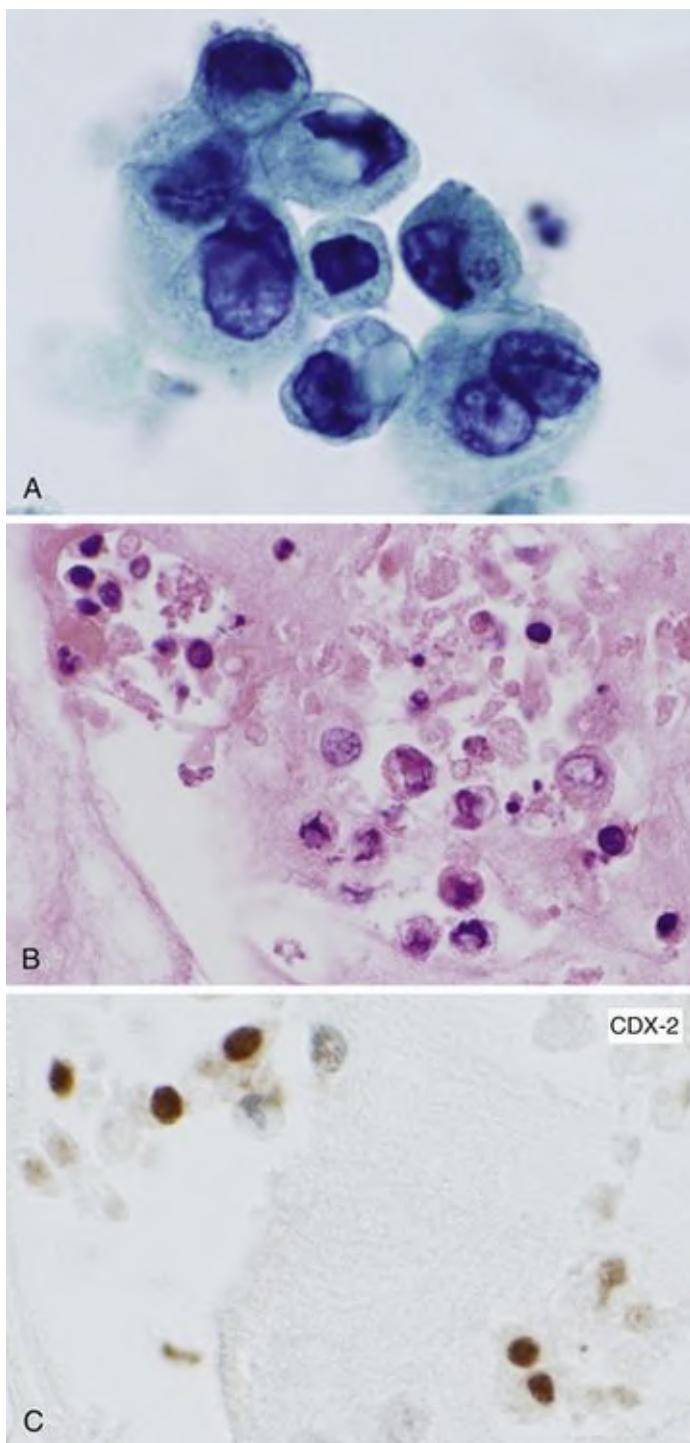


FIGURE 3.17 Metastatic colon cancer to the bladder neck.

A, A majority of the malignant cells are round, with dark, angulated nuclei. Cytoplasmic fragments from necrotic cells are present. Distinction from urothelial carcinoma (UC) is not possible by cytomorphology. B, A cell block preparation contains occasional degenerated malignant cells. C, The malignant cells show nuclear reactivity for CDX-2, commonly seen in colon cancers and usually absent in urothelial cancers. The malignant cells were also positive for cytokeratin 20 and negative for cytokeratin 7, the typical keratin profile of colon cancer.

Diagnosing Difficult or Borderline Specimens: Common Patterns

Not surprisingly, the diagnosis of “atypia” is used with great freedom in urine cytology: Many specimens show degenerative changes, and worrisome reactive changes are common. Although an atypical diagnosis appears wise from a clinical and risk management point of view, this may not be the case: atypical urines without further specification (such as “carcinoma cannot be ruled out”) are almost always regarded by urologists as negative (benign). Recent studies support this practice, because the risk of high-grade carcinoma is not significantly elevated by an atypical diagnosis.⁸⁵ For this reason, it is advisable to use *atypical* as sparingly as possible by classifying such cases instead as either negative or suspicious.

Most atypical urine specimens can be classified into one of five patterns.



Tips for common atypical urine patterns

- cell clusters in voided urine: diagnose as negative
- cytologic or architectural criteria for a low-grade lesion: diagnose as negative
- rare, small, highly atypical cells: diagnose as suspicious
- degenerated atypical cells with intact nuclear outlines: diagnose as suspicious
- rare, mildly atypical cells: try to diagnose as negative

Although it is traditional teaching that clusters of urothelial cells in a voided urine sample are associated with an increased risk of a low-grade urothelial neoplasm, data to support this contention are sparse.^{86,87} Anecdotal cases have been described, but in most studies, clusters of cells in voided urine specimens are no more common in patients with tumors than in those without. For this reason, it is advisable to diagnose such specimens as negative. If clusters of urothelial cells are particularly numerous, an explanatory comment such as the following can be helpful: “Clusters of minimally atypical urothelial cells are present. The underlying risk for UC is not well established. The finding is not specific and can be seen in a variety of conditions, including urolithiasis.”

The second pattern consists of the constellation of features suggestive of a PUNLMP and low-grade UC. As discussed above, the criteria are not accurate. Most urologists do not expect cytologists to diagnose these lesions, and their risk of progression to a high-grade carcinoma is so low that the value of this cytologic diagnosis is debatable. It is more important for a cytologist to focus on identifying subtle patterns of high-grade tumors. For a negative urine sample interpretation, some laboratories, albeit a minority, use the general heading “negative for a high-grade malignancy” rather than “negative for malignant cells” to reinforce the main purpose of urine cytology.

The next two patterns are associated with the greatest risk of a high-grade tumor. Although most high-grade tumors are easy to diagnose, some are more difficult. In one subtle pattern, the malignant cells are rare, small, sometimes hypochromatic, and occasionally obscured by blood. They have been termed *coy cells*.⁸⁸ A helpful clue to their malignant nature is significant nuclear membrane irregularity ([Fig. 3.18](#)). They are analogous to atypical squamous metaplastic cells of the cervix, which, although difficult to identify, are suspicious for a high-grade squamous intraepithelial lesion. Specimens with coy cells should be diagnosed as suspicious rather than atypical.

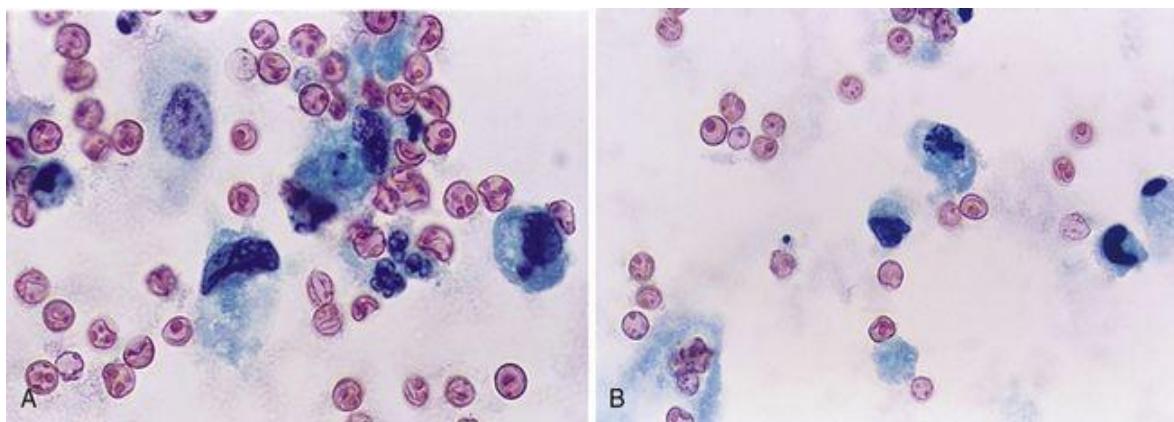


FIGURE 3.18 High-grade urothelial carcinoma (UC), difficult to detect.
A and B, In some cases, the malignant cells are hyperchromatic but rare and hidden by blood (“coy cells”). Nuclear outline irregularity is marked.

Another worrisome pattern is that of degenerated cells. If the entire specimen is poorly preserved (a very uncommon occurrence), it is uninterpretable and should be reported as inadequate. On the other hand, degenerative changes are common in high-grade tumors, resulting in dark, smudgy chromatin^{86,87} ([Fig. 3.19](#)). If some cells have an intact, irregular nuclear membrane, and benign cells

in the background are well preserved, the possibility of a high-grade lesion cannot be excluded, and the specimen should be diagnosed as suspicious rather than atypical.

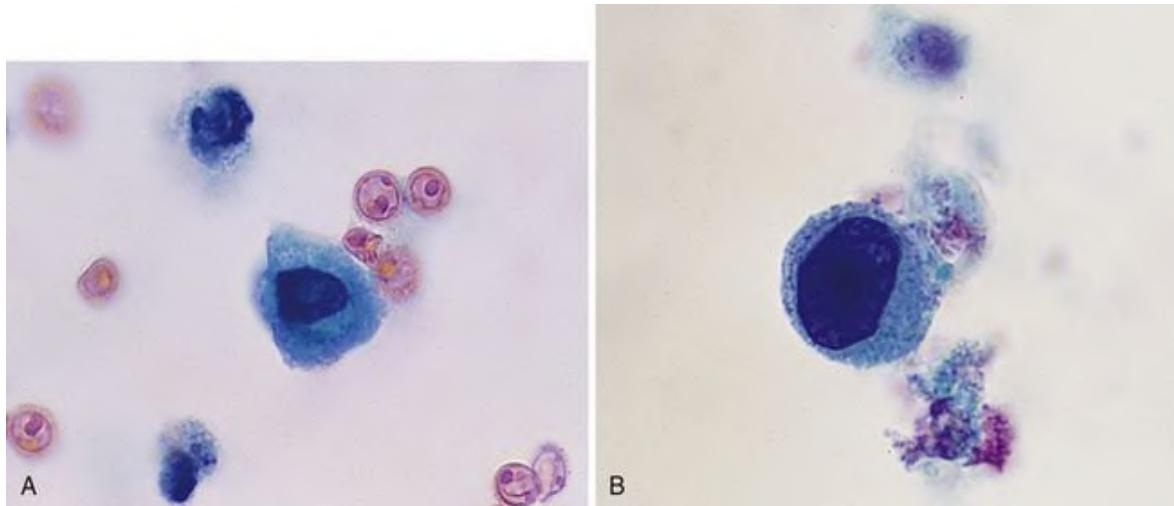


FIGURE 3.19 Degenerating cells of a high-grade urothelial carcinoma (UC).

A and B, Although the chromatin is smudgy, the nuclear membrane is intact. Such cells should not be ignored.

Finally, there is the urine specimen that contains only a few cells with enlarged, slightly irregular nuclei. This is the most common (and frustrating!) of the patterns. If these cells are few and the changes mild, the specimen should be diagnosed as negative. Although one would like to avoid an atypical diagnosis as much as possible, this is not always possible, and even experienced cytologists find the atypical category at times unavoidable.

Ancillary Techniques



Ancillary techniques

- DNA aneuploidy (flow cytometry, image analysis)
- Baud bladder tumor antigen (BTA) test
- nuclear matrix protein NMP22 test
- telomerase assays
- microsatellite instability assays
- hyaluronidase and hyaluronic acid
- growth factors
 - acidic fibroblast growth factor (FGF)
 - basic FGF
 - autocrine motility factor
 - epidermal growth factor
 - transforming growth factor- β
- cell adhesion molecules
- fibrinogen degradation products
- tumor-associated and blood group antigens
- fluorescence in situ hybridization (FISH)

Great efforts have been made to develop a test that either improves upon cytology in detecting UC or better predicts carcinoma progression.^{2,68,89,90} A partial list of commercially available or investigational tests is given above. The hope is that a more accurate test will eliminate the need for cystoscopy, which is costly and uncomfortable, in the followup of patients with conservatively treated, superficial cancers.

Many of these tests have greater sensitivity than cytology, but their less-than-ideal specificity remains a problem.⁹¹ Like cytologic examination, these tests have difficulty distinguishing reactive conditions, such as stone-induced urothelial atypia, from UC.

A test based on the genetic changes associated with bladder cancer, however, can be useful.⁹²⁻⁹⁶ Deletion of the p16 gene at chromosome 9p21 is one of the most common early alterations in low-grade carcinomas, and high-grade carcinomas are associated with aberrations of chromosomes 1, 3, 7, 9, 11, and 17. FISH technology can be applied to cytologic preparations to detect such cytogenetic abnormalities. Because no single abnormality is present in all

cancers, the success of the technique depends on using several probes. One multitarget FISH assay, called UroVysion (Abbott Molecular, Des Plaines, IL), combines centromeric probes to chromosomes 3, 7, and 17 with a locus-specific probe to band 9p21 ([Fig. 3.20](#)). UroVysion has been approved for the surveillance of patients treated for bladder cancer and as a screening tool in patients with hematuria.^{42,97} In preclinical trials for Food and Drug Administration (FDA) approval, the sensitivity of the test for low-grade lesions (transitional cell carcinoma grade 1) ranged from 48% to 61% (specificity 88% to 95%) and for high-grade lesions (transitional cell carcinoma grade 3) from 88% to 93% (specificity 80% to 95%). The method is reportedly more accurate than concurrent cytology, although the performance of cytology in these trials⁴² was significantly worse than has been reported in almost all previous large series (see [Table 3.2](#)). Recent studies suggest that the benefit of UroVysion is greatest for superficial tumors.⁹⁸

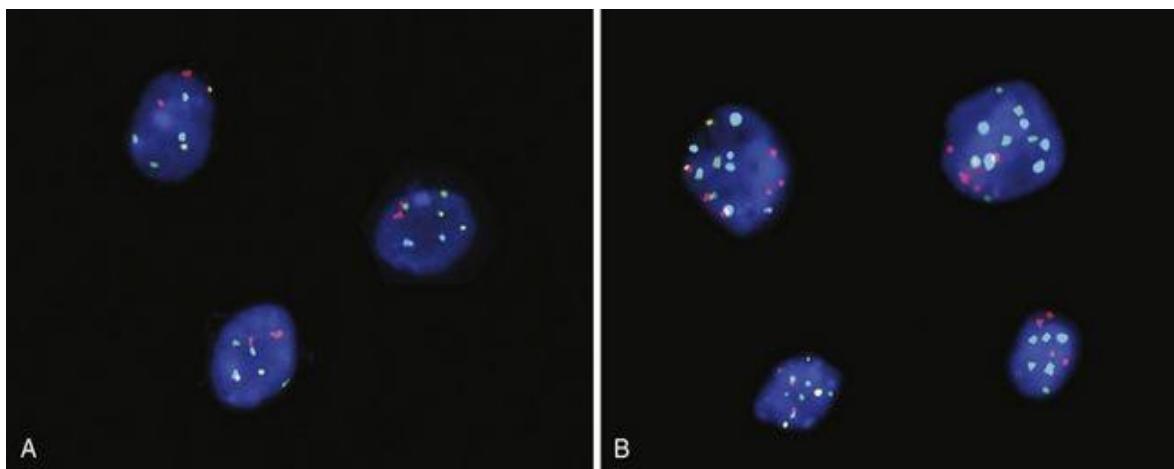


FIGURE 3.20 The UroVysion test for bladder cancer.
A, Benign urothelial cell nuclei show two signals with fluorescent centromeric probes for chromosomes 3 (red), 7 (green), and 17 (aqua), as well as two intact segments of 9p21 with a fluorescent locus-specific probe (yellow). B, Malignant urothelial cells show increased copy number for chromosomes 3 (red), 7 (green), and 17 (aqua), and some nuclei show loss of 9p21 (yellow).

UroVysion™ has its limitations. First, its performance (like that of cytology) varies considerably among laboratories.^{99–106} Sensitivity ranges from 50% to 89% and specificity from 29% to 89%. Although its performance has improved over the last 10 years, recent studies continue to show significant variation.¹⁰⁷ More importantly, the proportion of patients with truly false-positive results (i.e., no tumor on followup) versus those with “anticipatory positives” (i.e., tumor

eventually found on extended followup¹⁰⁸) is still debated. Obviously, the clinical utility of the test depends strongly on this ratio. Second, there is disagreement on the criteria for a positive result. Studies have shown that revised criteria, especially for tetraploid cells, can result in greater specificity than the original criteria used in the FDA trial,¹⁰⁷⁻¹¹⁰ yet application of the revised criteria is not uniform.¹⁰⁷ Third, many laboratories now use computer-assisted screening (e.g., BioView and Ikonisys) to interpret test results, a technology that was not available during the initial FDA trials.¹¹¹ The degree to which this method has altered the performance of the test is not well defined. Fourth, the performance of the test for upper tract lesions, where help is perhaps most needed, has been poor.¹¹² Finally, the value of UroVysion in clinical practice depends not only on the characteristics of the test itself and the patient population studied, but also on the performance characteristics of the urine cytology to which it is compared. In other words, it might be most useful where cytologic evaluation is least reliable.

Despite these caveats, there are settings where UroVysion can be of great benefit. In a manner similar to testing for HPV in women with equivocal cervical cytology, FISH helps clarify the significance of atypical cytologic findings,¹⁰⁹ inasmuch as patients with a positive FISH result can be screened sooner and more frequently than those with negative results. In patients under surveillance for recurrent bladder cancer, a positive FISH result occurs in 26% of those with an otherwise negative workup.¹⁰⁸ From 50% to 80% of these patients develop recurrent carcinoma within 29 months—their FISH result is thus considered an “anticipatory positive”—compared with 13% with negative FISH results. Studies have also shown a benefit to UroVysion in the followup of superficial recurrences in patients with a history of bladder carcinoma¹¹³; patients with positive FISH results can be triaged to surgery sooner than those with negative results.

Although UroVysion is FDA-approved to screen patients with hematuria for urothelial cancer, this application of the test is still debated. In some hands, UroVysion detects anticipatory positives (i.e., positive FISH result but negative urine cytology) in up to 30% of patients with hematuria, and 60% of these are later discovered to have UC. Other laboratories, however, find no difference in cancer detection between UroVysion and cytology. It has been suggested that the pretest probability of disease is so low in this setting that test performance is suboptimal.¹¹⁴ Other studies suggest that UroVysion adds little to the sensitivity of cystoscopy.^{115,116}

Taken together, the data suggest that where the quality of urine cytology is high, ancillary tests in general (and UroVysion in particular) provide little added benefit except in highly selected groups like high-risk patients. Where high-

quality cytology is not available, UroVysion, despite its own reproducibility issues, *may be* more reliable than cytology. A sensitivity of cytology for high-grade UC that is less than 50% is difficult to explain other than laboratory quality.^{[113,117](#)} This scenario is similar to that of HPV testing and cytology for cervical cancer screening, where the utility of HPV testing is a function of the quality of the cytology. Methods to define quality are increasingly recognized,^{[118](#)} and the future of cytology depends on accurate (and practical) criteria for quality. For laboratories with ready access to histologic followup, the sensitivity of cytology is easy to measure. If the sensitivity (using the sum of suspicious and positive cases to define a “positive” result) for the detection of histologically confirmed high-grade UC is less than 70%, the laboratory is likely not providing high-quality service. There are many possible reasons for this, including inefficient sample procurement, suboptimal specimen preparation, and overuse of the atypical category for cases that are better interpreted as suspicious or positive. The future of screening for UC may rest with newer, more innovative tests, but the near future is likely to focus on improving the quality of the tests we have.

Summary



Urine and bladder washings—summary

- Most urine specimens (for hematuria) are negative.
- The value of urine cytology for detecting high-grade carcinomas is high, but the quality of urine cytology varies among laboratories.
- The cytologic criteria for low-grade lesions lack specificity and should not be used.
- Clusters of urothelial cells per se are of limited value for the diagnosis of urothelial carcinoma in voided urine.
- The term *dysplasia* should be avoided in cytologic specimens.
- Upper tract specimens should be diagnosed conservatively.
- Atypical cells in patients known to have stones should be interpreted conservatively.
- Separating high-risk from low-risk patterns may be of value in reducing the number of atypical diagnoses.
- In defined clinical settings, a commercial FISH test (UroVysion) is a useful adjunct to cytology for the detection of urothelial carcinoma.

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CHAPTER 4

Pleural, Pericardial, and Peritoneal Fluids

Edmund S. Cibas

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The pleural, pericardial, and peritoneal cavities are lined by a single layer of flat mesothelial cells called the serosa. Normally, these cavities are collapsed and contain only a small amount of fluid, enough to lubricate the adjacent surfaces as they move over each other with respiration, heartbeats, and intestinal peristalsis. In disease states, a greater amount of fluid—an effusion—accumulates. Effusions are classified clinically as transudative or exudative. *Transudates* result from an imbalance of hydrostatic and oncotic pressures. Common causes are congestive heart failure (CHF), cirrhosis, and the nephrotic syndrome. Transudates have a low lactate dehydrogenase (LDH) and low total protein concentration. *Exudates* result from injury to the mesothelium, as occurs with malignancy, pneumonia, lupus, rheumatoid pleuritis, pulmonary infarction, or trauma. Exudates have relatively high LDH and total protein concentrations. The distinction is made by protein concentration measurements performed in the clinical laboratory. The distinction is important, because pleural involvement by a malignancy causes formation of an exudate, and therefore cytologic examination is not needed for a transudate.¹ Malignant tumors are a common cause of exudate formation because the serosal surfaces are a frequent site of metastasis for many tumors, as well as the site of origin for the asbestos-related tumor malignant mesothelioma.

Specimen Collection, Preparation, and Reporting Terminology

Specimens are obtained by inserting a needle into the pleural space (thoracentesis), pericardial space (pericardiocentesis), and peritoneal cavity (paracentesis). Care should be taken when withdrawing large amounts of pleural fluid because of the rare but life-threatening complication of reexpansion pulmonary edema.¹⁻³ By contrast, large volume paracentesis (e.g., 4 to 6 L) is relatively safe, and there are even reports of draining 20 L of ascitic fluid at one time.⁴ Although peritoneal fluid is usually obtained through the abdominal wall, in women it can also be aspirated from the cul-de-sac through the vagina (culdocentesis). Less commonly, fluid is obtained by suction during thoracic, abdominal, or cardiac surgery.

Fluid is collected in clean containers and sent unfixed to the laboratory. To prevent clotting, which widely disperses cells, thus hindering their evaluation, fluid can be collected in heparinized bottles containing 3 units of heparin per milliliter of capacity.⁵ If heparinized bottles are not available, the heparin should be poured into the bottle before the fluid is collected; specimen contact with glass results in rapid clotting.

Fluid is refrigerated at 4° C until the time of slide preparation. An effusion specimen is remarkably hardy—it can be refrigerated for 2 weeks or longer without compromising cellular morphology or antigenicity for immunostains.⁶ A variety of slide preparation methods are available. Slide preparation begins by shaking the container to evenly disperse the cells and then centrifuging a 50 mL aliquot (or the entire specimen if less than 50 mL). The supernatant is discarded and the sediment used to prepare smears, cytocentrifuge preparations,⁷ or thinlayer preparations.^{2,8} The slides are usually alcohol-fixed and Papanicolaou-stained. If a hematologic malignancy is suspected, air-dried cytocentrifuge preparations are helpful. One sample is stained with a Romanowsky-type stain, and the rest can be reserved, if needed, for immunocytochemical studies for lymphocyte surface markers.⁹ So-called cell blocks are especially useful as adjuncts to the “cytologic” preparations listed previously. To prepare a cell block, the sediment is wrapped in filter paper, placed in a cassette, embedded in paraffin, and cut and stained in the manner of histologic sections. Before placing it in a cassette, however, it is helpful to coagulate the sediment; one common way is to add a few drops of plasma and several drops of a thrombin solution,¹⁰⁻¹² but other methods are available,¹³ including an automated system called Cellient

(Hologic, Inc., Bedford, Mass.).¹⁴ Clotting the specimen with drops of plasma and thrombin does not disperse the diagnostic cells as does a spontaneously formed clot, but rather congeals the sediment into a compact mass. If the fluid was not heparinized and clots are present, they should be removed and placed in cassettes for processing as cell block material.

Using more than one preparation method for effusions improves sensitivity for the detection of malignancy.¹⁵ A common preparation combination is one thinlayer slide and a cell block. Cell block sections are especially useful for special stains and immunohistochemistry because of the ease with which multiple duplicate slides can be prepared, the relative absence of obscuring background staining, and the standardization of the preparation for control slides.¹⁶ Cell blocks also make for excellent morphologic comparison with histopathologic sections (when, for example, the patient has had a prior breast biopsy) because they are fixed and stained in an identical manner.

In some laboratories, an unfixed wet smear stained with toluidine blue is prepared first to identify any fluid that contains large numbers of malignant cells. It is helpful to separate such fluids from the routine staining cycle to prevent cross-contamination.¹²

Leftover fluid is stored in the refrigerator in case additional slides are needed. Fresh fluid is sometimes useful for other studies such as flow cytometry,¹⁷⁻¹⁹ electron microscopy,²⁰ and cytogenetic or molecular genetic analysis.²¹⁻²³

General categories like “no malignant cells identified” and “positive for malignant cells” are commonly used to report results because they succinctly and unambiguously communicate an interpretation.²⁴ Inconclusive findings—when abnormal cells are too poorly preserved or too few to support a definite diagnosis of malignancy—are commonly reported as “atypical cells present” (connoteing a low degree of suspicion) or “suspicious for malignancy” (connoteing a high degree of suspicion). Suspicious diagnoses occur in about 5% of specimens.^{25,26} In this situation, if the patient does have a malignancy involving the serosal cavity, fluid is likely to reaccumulate, and the subsequent specimens may be diagnostic of malignancy.

Criteria for the adequacy of an effusion specimen have not been established.²⁴

Accuracy

Cytology is more sensitive than blind biopsy for detecting serosal malignancy (71% versus 45%),²⁷ presumably because fluid provides a more representative sample. Estimates of the sensitivity of cytology for diagnosing serosal malignancy range from 58% to 71%.^{26,27} The cancer detection rate by cytology is increased by 2% to 38% when multiple sequential specimens are examined.^{25,28,29} This still leaves a substantial false-negative rate. Thoracoscopy is the procedure of choice for patients with a strong clinical suspicion of pleural disease but a negative cytology result.³⁰

The specificity of a cytologic diagnosis is very high: False-positive diagnoses occur in less than 1% of cases.^{25,31} When they occur, false-positive and false-suspicious diagnoses are caused by mesothelial cell atypia in the setting of pulmonary infarction,²⁷ tuberculosis,²⁵ chemotherapy,³² acute pancreatitis,^{31,32} ovarian fibroma²⁵ and cirrhosis.³¹ In children, false-positives result from misinterpreting benign lymphoid cells as lymphoma or neuroblastoma.³³

Immunocytochemistry is an essential adjunct to cytomorphology in selected cases and substantially improves diagnostic accuracy.



When to use immunocytochemistry for effusions

- confirming malignancy when morphology alone is equivocal
- distinguishing adenocarcinoma from mesothelioma
- screening an effusion for lobular breast cancer
- establishing the primary site of a malignant effusion; a patient with:
 - an occult primary
 - multiple primaries
- assessing receptor status (e.g., HER2) for patients with breast and gastric cancers

The circumstances outlined above represent common applications for immunohistochemistry. Antibodies against carcinoembryonic antigen (CEA), B72.3, and a number of other markers have high sensitivity and specificity for malignancy and are extremely useful in a variety of settings, particularly for resolving cases that are cytologically equivocal.³⁴ Detailed discussion of specific

applications is found in the sections that follow.

Benign Elements

Benign effusions contain mesothelial cells, histiocytes, and lymphocytes in varying proportions. Because some bleeding is common during specimen collection, red and white blood cells are common contaminants.



Cytomorphology of mesothelial cells

- often numerous
- dispersed as isolated cells
- occasional small clusters with “windows”
- round cells
- round nucleus
- single nucleolus
- dense cytoplasm with clear outer rim (“lacy skirt”)

Mesothelial cells can be sparse or numerous in benign effusions ([Fig. 4.1](#)). They are mainly dispersed as isolated cells or occasional small clusters. Large clusters composed of more than 12 cells are highly unusual in benign effusions. Binucleation and multinucleation are common, and mesothelial cells in mitosis can be seen in benign effusions. The dense cytoplasm reflects the abundance of tonofilaments, and the clear outer rim (“lacy skirt” or “halo”) corresponds to long, slender microvilli, better visualized with electron microscopy. Two or more mesothelial cells in groups are often separated by a narrow space or “window.” Less commonly, mesothelial cells have one or more cytoplasmic vacuoles.

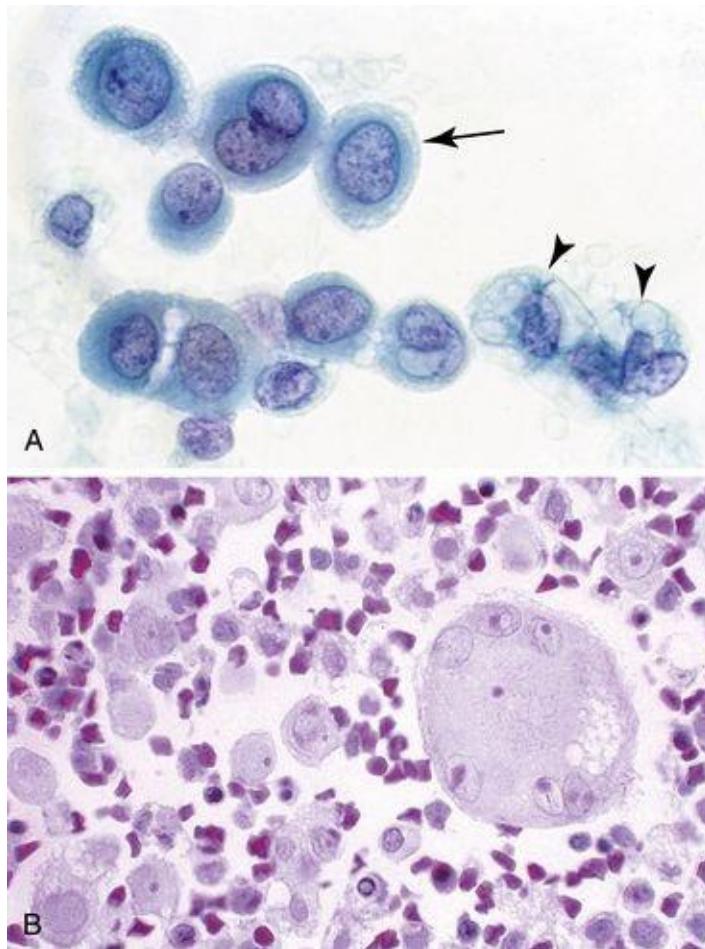


FIGURE 4.1 Mesothelial cells.

A, Many characteristic features of mesothelial cells are seen here: the peripheral lucent zone, or “lacy skirt” (*arrow*); the dense perinuclear zone; the occasional binucleation; and the slitlike separation (“window”) between adjacent cells. A few histiocytes (*arrowheads*) with folded nuclei and vacuolated cytoplasm are also present (Papanicolaou stain). B, Peripheral halos are well seen in this cell block section. Note also the large multinucleated mesothelial cell, a nonspecific finding (hematoxylin-eosin [H & E] stain).

With acute or chronic injury, mesothelial cells undergo hyperplasia and hypertrophy and can have significant nuclear atypia, but they remain predominantly dispersed as isolated cells. Such “reactive” mesothelial cells generally comprise a spectrum of cells that range from normal to atypical, with variation in nuclear size, a coarse chromatin texture, irregular nuclear contours, or prominent nucleoli ([Fig. 4.2](#)).

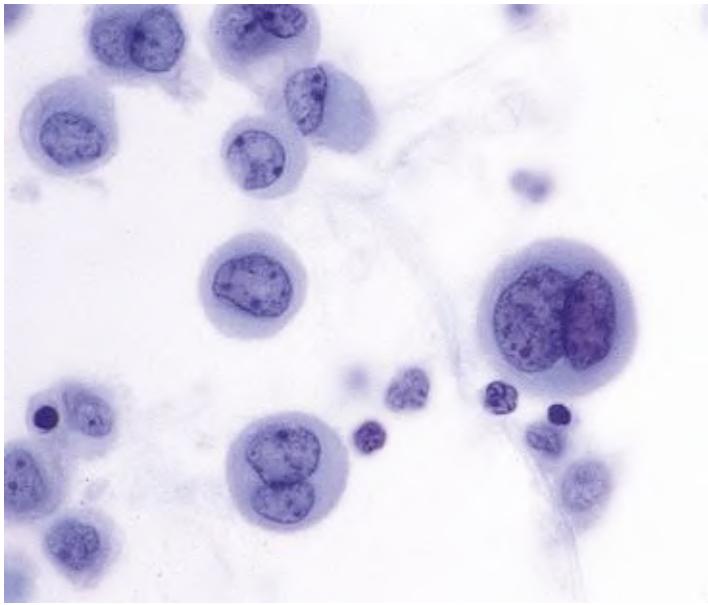


FIGURE 4.2 Reactive mesothelial changes (peritoneal fluid, cirrhosis). Some benign fluids contain a population of moderately enlarged mesothelial cells with large, hyperchromatic, irregular nuclei (Papanicolaou stain).



Differential diagnosis of reactive mesothelial cells

- mesothelioma
- metastatic malignancy

Malignant mesothelioma should be considered if there is marked atypia, particularly if the cells are much larger than normal, or if the fluid contains numerous clusters of 12 or more mesothelial cells, even if the cells themselves are not particularly atypical. Such large groups are uncommon in benign effusions. Clinical correlation is important because it may account for the atypia; some medical conditions, including anemia, cirrhosis, lupus, pulmonary infarction, renal failure, and acquired immunodeficiency syndrome (AIDS),³⁵ are notorious causes of mesothelial atypia. On the other hand, if the patient has a large, unexplained, unilateral effusion, particularly with radiographic evidence of pleural thickening, additional evaluation (pleural biopsy, cytogenetics), should be considered to exclude mesothelioma.^{21–23}

Metastatic malignancy should be considered when a population of cells is identified that is morphologically distinct from the mesothelial cells, histiocytes, and lymphocytes. In a minority of malignant effusions, a second population of

cells is not evident. This is particularly true with lobular carcinoma of the breast and melanoma, the cells of which mimic normal histiocytes or mesothelial cells. Special stains are then needed to resolve the case.



Cytomorphology of histiocytes

- smaller nucleus than that of mesothelial cells
- nucleus often folded
- cytoplasm granular and/or vacuolated
- no “windows” between adjacent cells
- dense aggregates (cell block sections)

Some effusions contain abundant histiocytes (Fig. 4.3A). A particularly marked histiocytic reaction to irritation of the serosal surfaces has been termed *nodular histiocytic/mesothelial hyperplasia*.^{36,37} This is a nonspecific chronic inflammatory reaction that should not be misconstrued as a malignancy in cytologic or histologic specimens.³⁷ When abundant, histiocytes can form aggregates on smears and liquid-based preparations,³⁷ and they tend to sediment together in cell block preparations, forming masslike aggregates that mimic malignancy. Immunohistochemistry can be useful to distinguish histiocytes from mesothelial cells and metastatic carcinoma: Histiocytes are immunoreactive for CD68 and CD163, and negative for keratin proteins (Fig. 4.3B); the reverse is true for mesothelial cells and metastatic carcinoma.

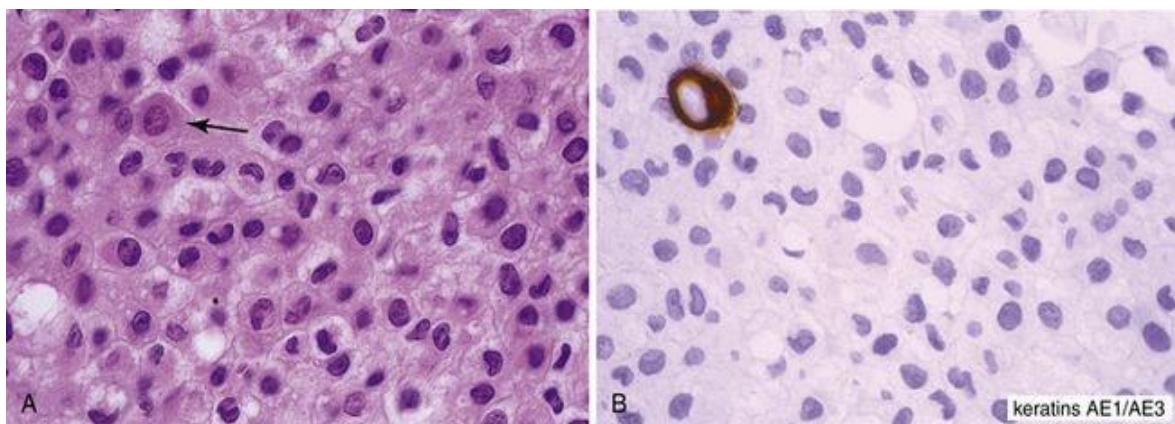


FIGURE 4.3 Histiocytes (pleural fluid, congestive heart failure [CHF]).

A, Like mesothelial cells, histiocytes are usually isolated cells, but centrifugation for cell block

sections compresses them into large groups. Compare the histiocytes, which have an oval or folded nucleus, with the mesothelial cell (*arrow*) (hematoxylin-eosin[H & E] stain). *B*, Only the mesothelial cell is immunoreactive for cytokeratins.

NonNeoplastic Conditions

In many benign disorders, effusions give a nonspecific cytologic picture. Thus, pleural fluid in CHF or pulmonary infarction is morphologically indistinguishable from pericardial fluid caused by renal failure and peritoneal fluid due to cirrhosis. Fortunately, the features of some benign conditions are sufficiently characteristic to narrow the differential diagnosis or even indicate the specific etiology. To give a very unusual example, finding undigested meat and vegetable matter in pleural fluid strongly suggests esophageal rupture (Boerhaave's syndrome).³⁸

Acute Serositis

Acute pleuritis, pericarditis, and peritonitis are usually the result of a bacterial infection. Bacterial infection of the pleura occurs in the setting of pneumonia, which secondarily involves the overlying pleura and results in a pleural empyema. Acute infection of the peritoneal cavity is often secondary to inflammation of or injury to the bowel, as in spontaneous bacterial peritonitis.

The fluid is a creamy pale yellow (purulent) and often foul-smelling. Cytologic preparations are highly cellular and composed almost exclusively of polymorphonuclear leukocytes. Bacteria are demonstrated with special stains in some cases.

It is important to screen such cases carefully for malignant cells because acute infection can be a complication of metastatic malignancy.

Eosinophilic Effusions

A pleural effusion is considered “eosinophilic” when eosinophils account for 10% or more of the nucleated cells present. Between 5% and 16% of exudative effusions are eosinophilic effusions.³⁹ The most common causes are pneumothorax and hemothorax.³⁰ The introduction of air or blood into the pleural space, so often the reason behind an eosinophilic effusion, can occur simply with repeated thoracenteses. Less common causes include drug reactions, parasitic infections, pulmonary infarction, and the Churg-Strauss syndrome.^{30,40} In about one third of cases the origin remains obscure.⁴¹ Most cases resolve spontaneously. Eosinophilic pericardial and peritoneal effusions are less common than eosinophilic pleural effusions.

Cytologic preparations are usually cellular and remarkable for a high concentration of eosinophils. On alcohol-fixed Papanicolaou-stained slides, the defining “eosinophilic” cytoplasmic granules are either orangeophilic or pale green and inconspicuous, and the cells are identified more on the basis of their bilobed nuclei ([Fig. 4.4](#)). The granules are brightly eosinophilic on cell block preparations stained with hematoxylin-eosin (H & E), however, and on air-dried Romanowsky-stained slides. Charcot-Leyden crystals (see [Fig. 2.11](#)) are present in some cases and, curiously, are more common in fluids that have been refrigerated for more than 24 hours.⁴²

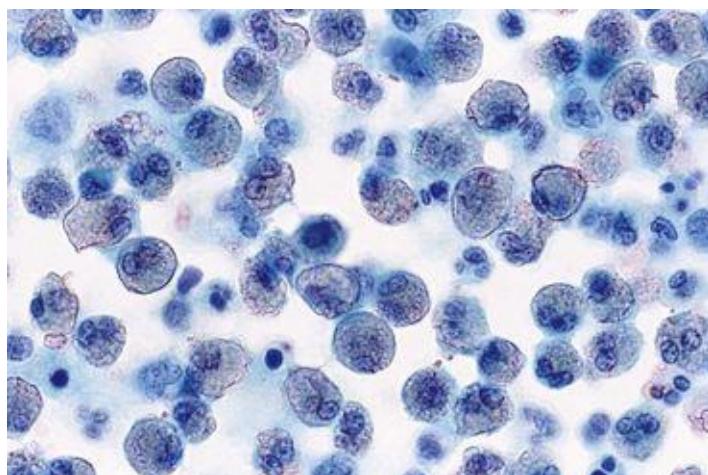


FIGURE 4.4 Eosinophilic pleural effusion.

Numerous eosinophils in pleural fluid are more commonly associated with benign conditions like a pneumothorax (as in this case) or hemothorax (Papanicolaou stain).

Lymphocytic Effusions

A pleural effusion consisting mostly of small lymphocytes is a relatively common but nonspecific finding (Fig. 4.5). Cytologic preparations are often highly cellular and composed almost exclusively of dispersed small lymphocytes.⁴³ Mesothelial cells and histiocytes are either conspicuously absent or present in only small numbers.

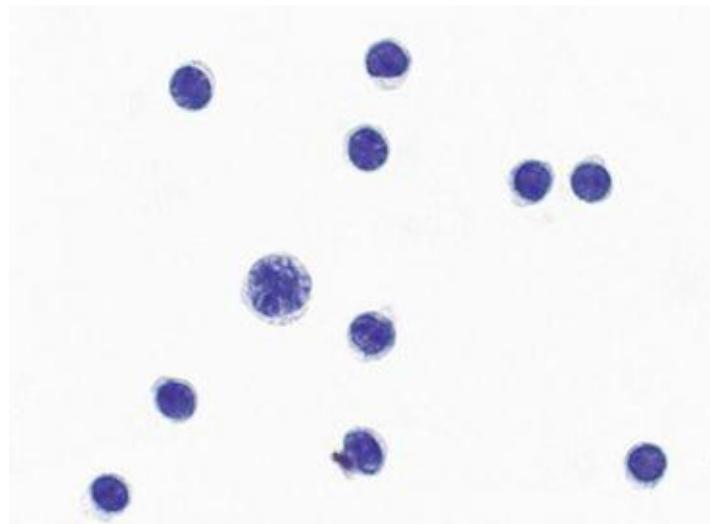


FIGURE 4.5 Lymphocytic pleural effusion.

The specimen consists almost exclusively of small lymphocytes with round nuclei and condensed chromatin. This finding is nonspecific (Papanicolaou stain).



Differential diagnosis of a lymphocytic effusion

- malignancy
- tuberculosis
- status post coronary artery bypass

Despite the absence of malignant cells, a malignancy is a common cause of a lymphocytic effusion. The malignancy may be nearby (e.g., in the lung) and may be obstructing lymphatic outflow but may not have spread to the pleural surfaces. Alternatively, a pleural malignancy may be evoking a peritumoral

lymphocytic response, but the tumor itself is not shedding cells into the effusion.³⁶ It is not uncommon for the initial pleural fluids in patients with a pleural mesothelioma to consist only of lymphocytes.⁴⁴

An effusion is very rarely the initial manifestation of a lymphoid malignancy. Thus, it is not cost-effective to evaluate every lymphocytic effusion consisting of mostly small round lymphocytes by flow cytometry or immunocytochemistry. On the other hand, if the patient is known to have a lymphoma or thymoma, an immunophenotypic workup is justified to exclude pleural involvement ([Fig. 4.6 A-D](#)).

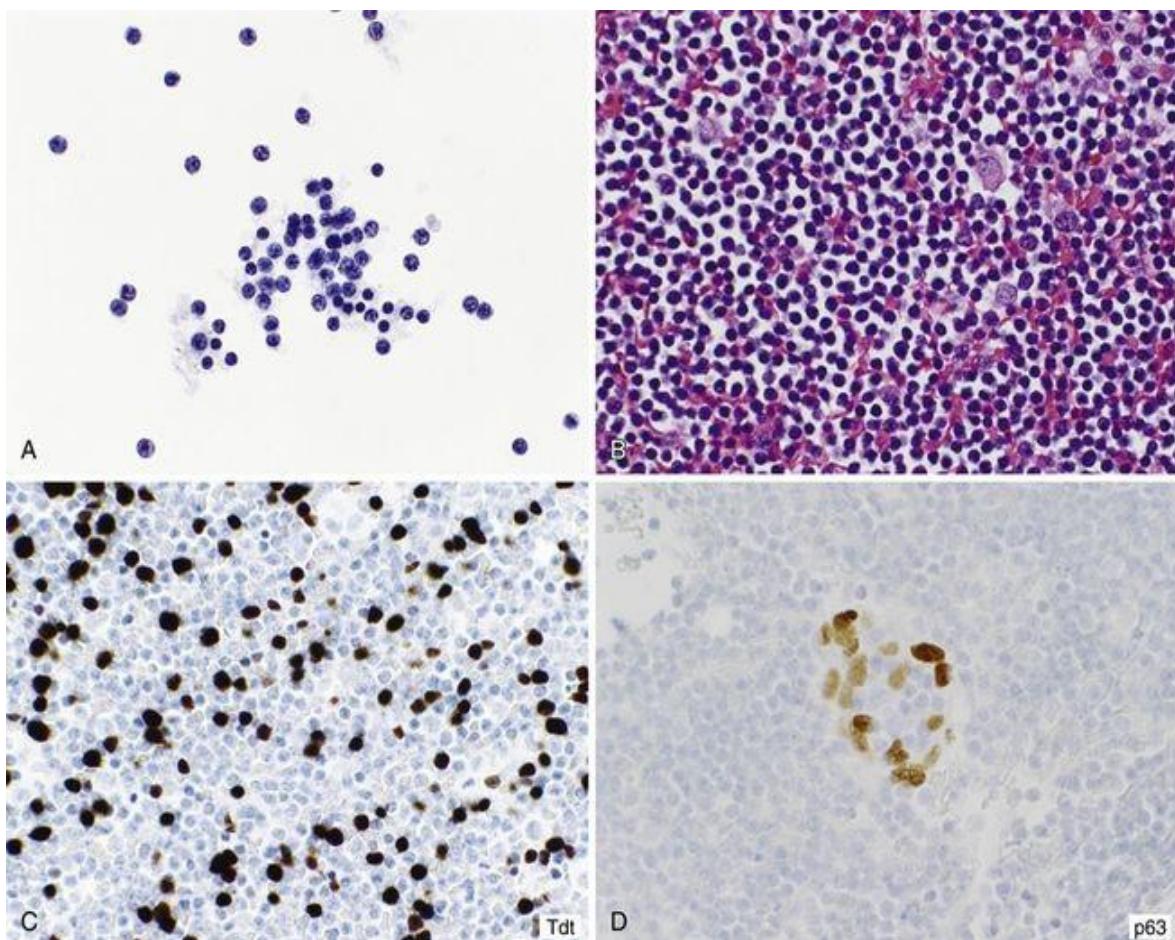


FIGURE 4.6 Metastatic thymoma, type B2 (pleural effusion).

A, The specimen looks like a nonspecific lymphocytic effusion, but the patient had a history of a thymoma (Papanicolaou stain). B, The cell block confirms the presence of numerous small lymphocytes (hematoxylin-eosin [H & E] stain). C, Numerous lymphocytes are positive for Tdt (terminal deoxytransferase), a marker of thymic lymphoid cells. D, A cluster of cells shows strong immunoreactivity for p63, a marker of squamous differentiation that is usually negative in mesothelial cells.

Effusions caused by small lymphocytic lymphoma and chronic lymphocytic leukemia are very uncommon. Because these are B-cell neoplasms, immunocytochemical or flow cytometric evaluation of lymphocyte surface markers is helpful in confirming the diagnosis. In a patient with chronic lymphocytic leukemia and a peripheral lymphocytosis, however, contamination of the effusion by peripheral blood during a traumatic tap should be excluded before diagnosing pleural involvement. Even a small amount of blood containing leukemic cells can result in a false-positive diagnosis.

The diagnosis of tuberculosis can be confirmed by microbiologic studies or pleural biopsy, which reveals caseating granulomas and acid-fast organisms. The differential diagnosis includes other benign effusions of nontuberculous origin, as in patients after coronary artery bypass surgery.³⁰

Rheumatoid Pleuritis

Less than 5% of patients with rheumatoid arthritis develop pleural involvement by their disease. In almost all cases, joint disease precedes the development of pleuritis, but occasionally pleuritis precedes or is synchronous with the onset of joint disease.^{45,46} The pleural effusion can be unilateral or bilateral, and some patients have a synchronous pericardial effusion. Radiographic studies reveal pulmonary nodules in a minority of patients; presumably, these are rheumatoid nodules. The effusion can last for days, months, or sometimes years.

The cytologic picture is so characteristic that it has been termed *pathognomonic*.⁴⁵ Examination of pleural fluid, therefore, can be extremely useful to confirm the diagnosis of rheumatoid pleuritis and exclude the possibility of coincident disease, especially a malignancy.



Cytomorphology of rheumatoid pleuritis

- abundant clumps of granular debris
- macrophages

Cytologic preparations are sparsely or moderately cellular. An abundant granular material dominates the picture ([Fig. 4.7A](#)). It can stain green, pink, red, or orange with the Papanicolaou stain, and it aggregates into small and large clumps with irregular edges. Large, islandlike masses can be appreciated in cell

block material. The predominant cell is the macrophage, which is round or spindle-shaped; multinucleated macrophages are seen in most but not all cases (see [Fig. 4.7A](#) and [B](#)). Lymphocytes and polymorphonuclear leukocytes may be seen. Mesothelial cells are noticeably absent.

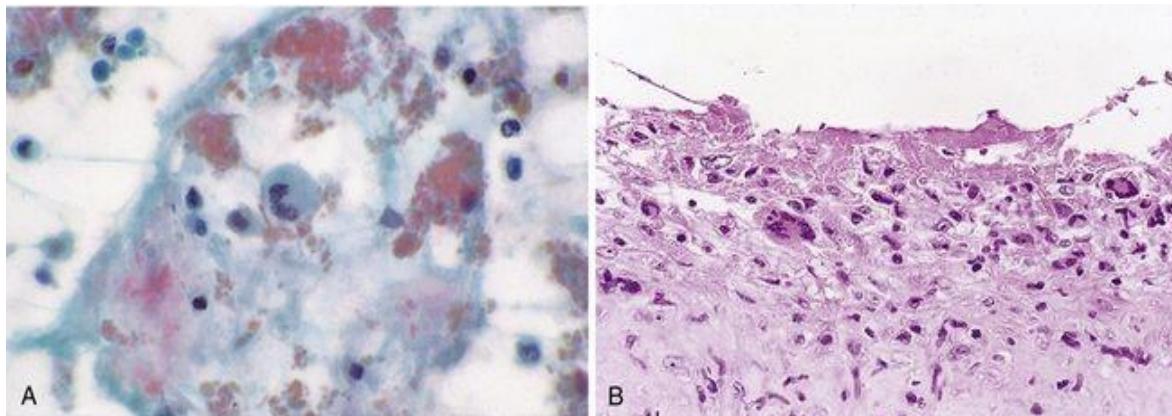


FIGURE 4.7 Rheumatoid pleuritis.

A, Scattered multinucleated histiocytes and clumped granular debris in the background are characteristic of pleural fluids in patients with rheumatoid pleuritis (Papanicolaou stain). B, A pleural biopsy has the appearance of an “opened-out” rheumatoid nodule, with epithelioid histiocytes, giant cells, and fibrinoid debris lining the pleural space (hematoxylin-eosin [H & E] stain).

The characteristic granular debris is different from fibrin, which is usually strandlike rather than coarsely granular. Although the elongated macrophages resemble the spindle cells seen in squamous and other cancers, their nuclei are normochromatic.

Lupus Pleuritis

About one third of patients with systemic lupus erythematosus (SLE) develop a pleural or pericardial effusion. Peritoneal effusions are less common but do occur. Rarely, an effusion is the initial manifestation.

The characteristic cell is the lupus erythematosus (LE) cell, a neutrophil or macrophage that contains an ingested cytoplasmic particle called a *hematoxylin body*. The hematoxylin body may be green, blue, or purple with the Papanicolaou stain and magenta with Romanowsky-type stains, and has a glassy, homogeneous appearance ([Fig. 4.8](#)). Filling the cytoplasm of the neutrophil or macrophage, it often pushes the nucleus to one side, indenting it into a crescentlike shape. Hematoxylin bodies are thought to represent degenerated

nuclei. Similar cells that contain ingested nuclei with a visible chromatin structure (rather than the glassy, structureless hematoxylin body) are called *tart cells* after the patient in whom they were first described.

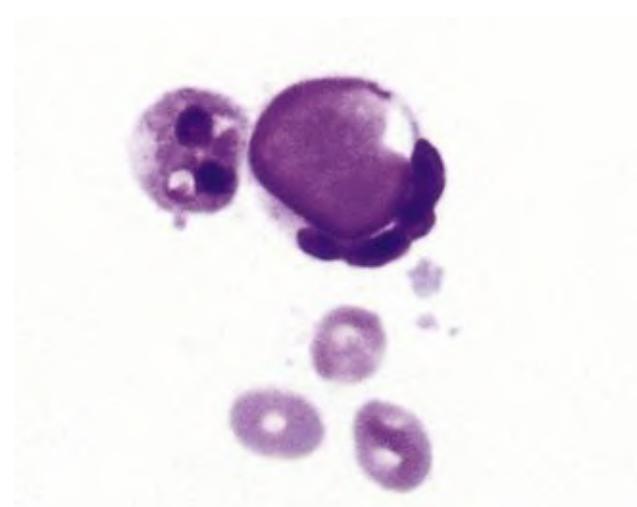


FIGURE 4.8 Hematoxylin body (lupus pleuritis).

The lobes of the nucleus are pushed against the side of the neutrophil by a large, homogeneous, intracytoplasmic body (Romanowsky stain).

LE cells are present in just 27% of effusions in patients with SLE, and only in those with a known diagnosis of SLE.⁴⁷

Other NonNeoplastic Conditions

Most viral pneumonias associated with a pleural effusion result in a nonspecific cytologic picture. The cytopathic changes characteristic of the herpesviruses and cytomegalovirus (CMV) are rarely seen in serous effusions. Although fungal infections are common in immunocompromised patients, organisms are rarely seen in pleural, pericardial, and peritoneal fluids. *Candida* species, *Cryptococcus neoformans*, *Coccidioides immitis*, *Blastomyces dermatitidis*, and *Aspergillus niger* have been described in fluids in rare instances.¹²

Pneumocystis jirovecii has been identified in pleural and peritoneal effusions from immunocompromised patients.⁴⁸⁻⁵⁰ Papanicolaou stains show foamy exudates similar to those seen in respiratory specimens. The trophozoites measure 2.5 to 5.0 µm and have pale cytoplasm and a dotlike nucleus. They may be intracellular (within macrophages) or extracellular, and are well seen on air-dried preparations stained with a Romanowsky-type stain. Cyst forms measure 4

to 7 μm and can be seen with special stains like the methenamine silver stain.

Malignant Effusions

Some tumors have a greater tendency than others to spread to the pleura, pericardium, or peritoneum. The most common are listed in [Table 4.1](#). In children, the most common cause of a malignant pleural or peritoneal effusion is non-Hodgkin lymphoma.³³

TABLE 4.1
**MOST COMMON TUMORS THAT CAUSE MALIGNANT EFFUSIONS,
BY SITE AND GENDER***

Site	Men	Women
Pleural	lung lymphoma/leukemia gastrointestinal tract sarcoma mesothelioma genitourinary (kidney, prostate, bladder) melanoma	breast lung lymphoma/leukemia ovary gastrointestinal tract endometrium sarcoma mesothelioma
Peritoneal	lymphoma/leukemia gastrointestinal tract pancreas lung sarcoma prostate melanoma germ cell tumors mesothelioma	ovary breast endometrium stomach lymphoma/leukemia colon and rectum pancreas mesothelioma

*Data from: Sears D, Hajdu SI. The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. *Acta Cytol* 1987;31: 85-97; and Johnston WW. The malignant pleural effusion: a review of cytopathologic diagnoses of 584 specimens from 472 consecutive patients. *Cancer* 1985;56: 905-909.

Most patients with a malignant effusion have a previously documented primary neoplasm. In some cases, however, a malignant effusion is the first manifestation of an occult malignancy. Lung cancer is the most common occult primary in women and men who present with a malignant pleural effusion. It is extremely uncommon for breast cancer to manifest itself initially as a malignant effusion.^{29,51,52} The most common occult sources of a malignant peritoneal effusion are gastrointestinal and pancreatic cancer in men and ovarian cancer in women.^{52,53} Other tumors that can manifest as a malignant effusion include lymphoma, melanoma, and mesothelioma.⁵² In some patients, the primary site is

never discovered.^{29,52}

Malignant cells in pleural, pericardial, or peritoneal fluid betoken a grim prognosis. The median survival for patients with a positive pleural or peritoneal effusion is less than 6 months.⁵⁴ Certain tumors, like estrogen-positive breast cancers and well-differentiated mucinous adenocarcinomas of the appendix, have a slightly better prognosis. Survival is improved in some patients with therapy targeted against the molecular profile of the cancer.⁵⁵

Systemic chemotherapy fails to alleviate most recurrent malignant effusions, with a few notable exceptions (e.g., those caused by lymphoma and small cell lung cancer). Because most malignant pleural effusions recur and impede respiration, chest tube placement or pleurodesis (sclerosis of the pleural cavity by injecting talc, doxycycline, or bleomycin) is often performed as a palliative measure.⁴ For patients with recurrent malignant ascites, palliative treatment may consist of either repeated paracenteses, intraperitoneal chemotherapy, placing a drainage catheter, or implanting a peritoneovenous shunt (usually into the superior vena cava).^{4,54} Surprisingly, there is no evidence that disseminating tumor cells via a peritoneovenous shunt decreases survival.⁴ The various treatment options have their advantages and disadvantages; selecting the best treatment option focuses on the patient's desires and improving the quality of life.



Tips for detecting malignant cells in effusions

- “second population”
- numerous large clusters
- lacunae (cell block sections)

A good way to identify malignant cells in effusions is to first locate some benign mesothelial cells. With these as a benchmark, one searches for a second population (not counting, of course, lymphocytes or histiocytes) that is clearly different. Malignant cells are not necessarily larger than mesothelial cells. Some are about the same size but are recognized because of their high nuclear-to-cytoplasmic ratio, nuclear hyperchromasia, or macronucleoli. Exceptions to this rule occur, notably mesothelioma, for which a sharp distinction between benign and neoplastic mesothelial cells is not appreciated.

Normal mesothelial cells virtually never form large cell clusters. Effusions with numerous large aggregates are easily spotted as malignant. Care must be

taken not to confuse loosely clustered cells, which are a common artifact of cytocentrifugation and liquid-based preparations; reliably malignant clusters are tightly cohesive.

Malignant cells in cell block sections are frequently situated in lacunae ([Fig. 4.9](#)). These clear spaces surrounding individual cells or groups of cells are seen in 75% of cell blocks of malignant effusions, mostly adenocarcinomas, but the finding is not specific; lacunae are also seen in one third of benign effusions.⁵⁶ Lacunae are helpful in locating potentially abnormal cells at low magnification, but inspection at high magnification is needed for definitive diagnosis.

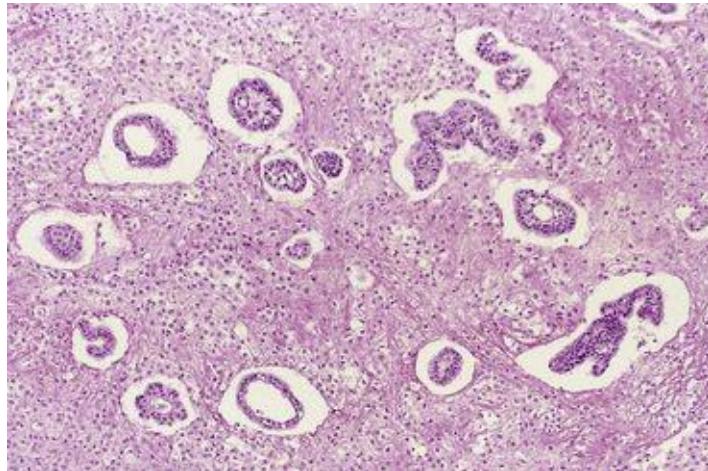


FIGURE 4.9 Cell block lacunae (pleural fluid).

In cell block sections, malignant cells are often situated in an empty space (lacuna); the reason for this artifact is unknown. It is commonly seen with adenocarcinomas, rarely with lymphomas and melanoma (hematoxylin-eosin [H & E] stain).

Primary Tumors

Primary tumors of the serosal surfaces are uncommon, being far outnumbered by secondary involvement by tumors from other locations. The two primary serosal malignancies considered here are mesothelioma and primary effusion lymphoma.

Diffuse Malignant Mesothelioma

Diffuse malignant mesothelioma (for simplicity, *mesothelioma*) accounts for less than 2% of malignant effusions.^{36,57} Strongly linked in most cases to asbestos

exposure, it arises most commonly in the pleura and less commonly in the peritoneum; primary tumors of the pericardium or tunica vaginalis of the testis are rare. The latency (time from first asbestos exposure to clinical disease presentation) is extremely long, with an average of 30 to 40 years. The peak incidence in the United States appears to have happened in the 1990s, but cases are still increasing in other countries such as Great Britain and Australia.³⁶

Malignant mesothelial cells grow as multiple plaques that coalesce into larger nodules visible radiographically as a thickening of the pleura. Histologically, these tumors are classified as epithelioid, sarcomatoid, desmoplastic, or biphasic types.⁵⁸ The epithelioid type comes in a variety of variant histologic patterns—tubulopapillary, adenomatoid (microglandular), sheetlike, deciduoid, small cell, and clear cell—but mixed patterns are common, and some of these are rare.^{36,58} Most mesotheliomas are, in fact, well-differentiated tumors and cytologically remarkably, deceptively bland. Mesotheliomas, like benign mesothelial cells, are immunoreactive for cytokeratins, desmin, calretinin, Wilms tumor protein 1 (WT1), and D2-40.

Common symptoms are chest pain and shortness of breath. Establishing the diagnosis is not always straightforward, with a median time from the onset of symptoms to diagnosis of 8 weeks.⁴⁴ Most patients have an effusion, usually unilateral, at the time of presentation. A positive mesothelioma effusion is often grossly described as having the color and consistency of honey. When suspicious and positive results are combined, the sensitivity of effusion cytology for the diagnosis of mesothelioma is only 32%.⁴⁴



Cytomorphology of mesothelioma

- two principal patterns:
 - **morular pattern:** large clusters with scalloped edges
 - **noncohesive cell pattern**
- cytomegaly
- round, centrally placed nucleus
- prominent nucleolus
- binucleation and multinucleation
- dense cytoplasm with peripheral “halo”
- normal nuclear-to-cytoplasmic ratio
- windows

Only the epithelioid and mixed (biphasic) types of mesothelioma are likely to exfoliate malignant cells; the pure sarcomatoid and desmoplastic types rarely exfoliate. When the malignant cells exfoliate, the most recognizable cytologic pattern is characterized by numerous large clusters (morulae) ([Fig. 4.10A](#)). The clusters are composed of up to hundreds of cells that are recognizably mesothelial in origin, with round nuclei, prominent nucleoli, and dense cytoplasm with a pale rim. The morulae have a knobby, scalloped contour (“mulberry clusters”), and some show branching ([Fig. 4.10B](#)). In most cases, the malignant mesothelial cells are larger than normal mesothelial cells, sometimes markedly so. Cytoplasm is abundant in most cases, and therefore the nuclear-to-cytoplasmic ratio is often deceptively normal ([Fig. 4.10C](#)), but cytoplasm can be scant in some cases ([Fig. 4.10D](#)). Occasionally, microvilli can be appreciated ([Fig. 4.11](#)). Nuclear atypia is mild in most cases. On cell block sections, the clusters are a solid mass of cells (see [Fig. 4.11](#)), or they may contain a collagenous or acid mucopolysaccharide core ([Fig. 4.12](#)).

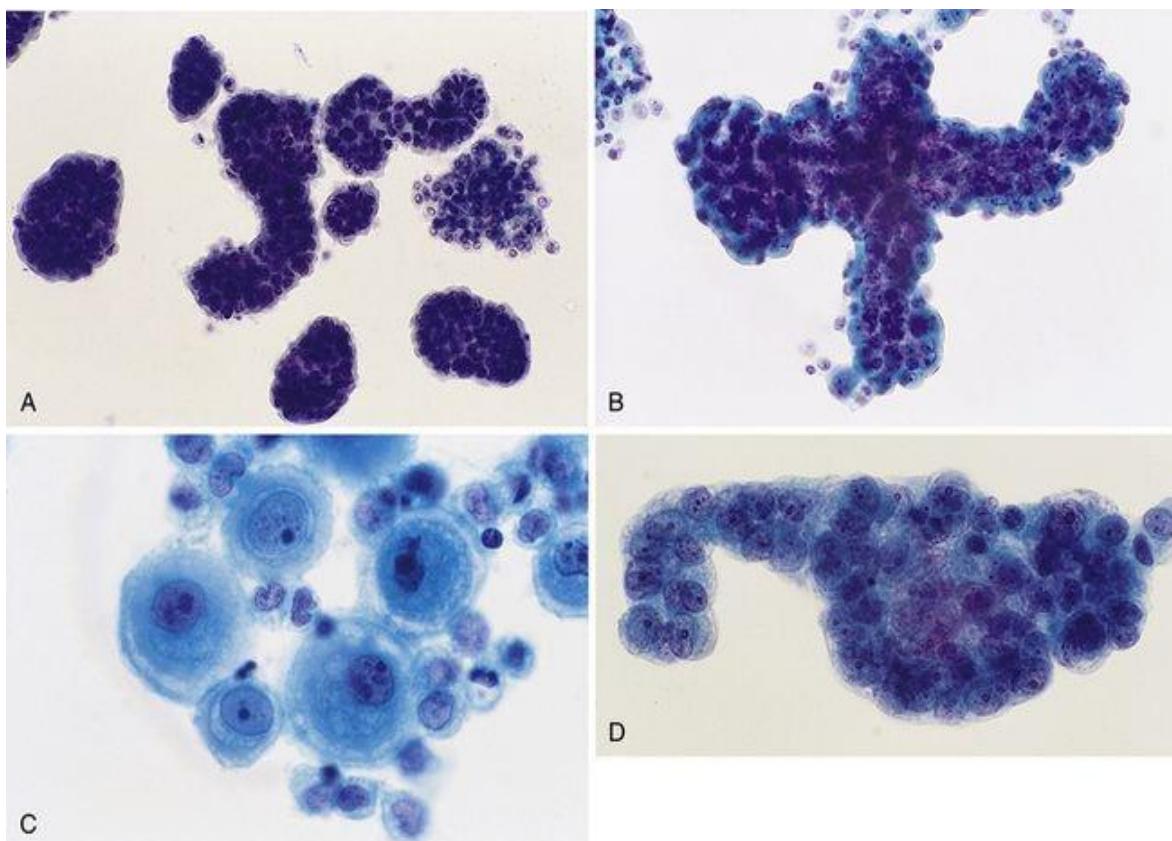


FIGURE 4.10 Malignant mesothelioma (pleural fluid).

A, Solid, morulelike spheres, some of them elongated, are composed of cells that resemble

normal mesothelial cells. A fluid composed of many large clusters is virtually always malignant (Papanicolaou stain). *B*, A branching pattern is seen in some cases. Note the knobby contours (Papanicolaou stain). *C*, In most mesotheliomas, the nuclear-to-cytoplasmic ratio of normal mesothelial cells is recapitulated (Papanicolaou stain). *D*, In other cases, the nuclear-to-cytoplasmic ratio is significantly increased (Papanicolaou stain).

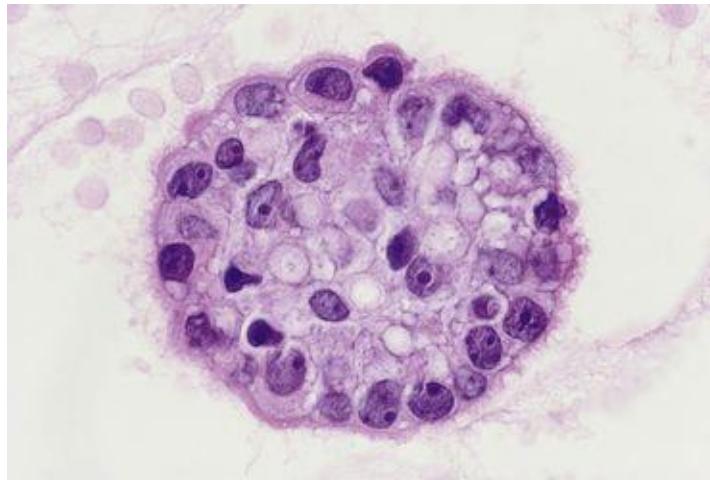


FIGURE 4.11 Malignant mesothelioma (pleural fluid).
In some cases, the characteristic microvilli can be appreciated (hematoxylin-eosin [H & E] stain).

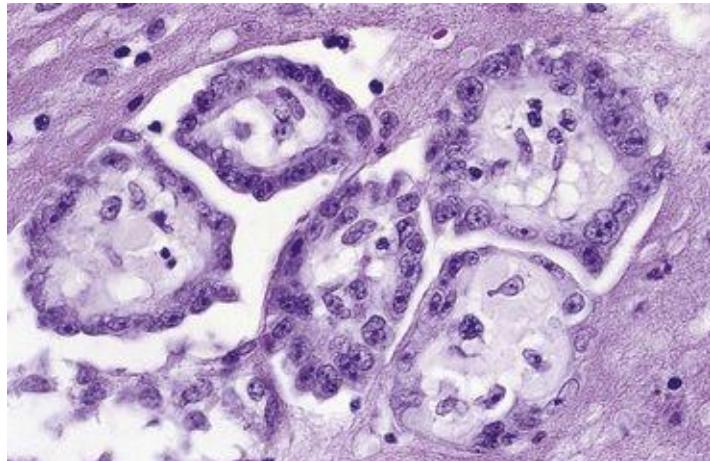


FIGURE 4.12 Malignant mesothelioma (pleural fluids).
In some cases, cell block sections show that clusters of malignant cells surround a collagenous core (hematoxylin-eosin [H & E] stain).

Not all mesothelioma fluids have the mulberry cluster pattern. In another common pattern, the malignant cells are not cohesive but instead are dispersed as isolated cells (see [Fig. 4.10C](#)).⁵⁹ More unusual variants include tumors composed predominantly of vacuolated cells ([Fig. 4.13A](#) and [B](#)), tumors that show small cell differentiation, those with an abundant lymphohistiocytic infiltrate, and those accompanied by psammoma bodies.⁶⁰

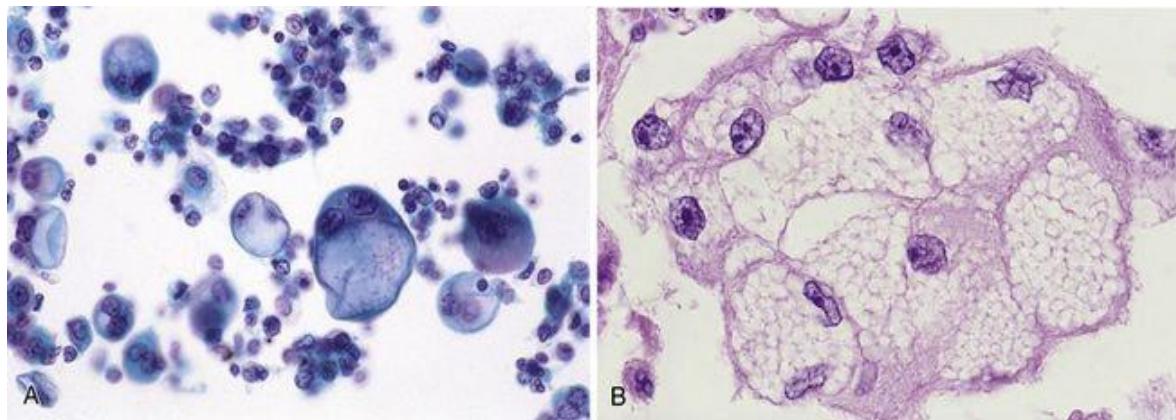


FIGURE 4.13 Malignant mesothelioma.

A, B, Rarely, the tumor cells show striking cytoplasmic vacuolization (A, Papanicolaou stain; B, hematoxylin-eosin [H & E] stain).



Differential diagnosis of mesothelioma

- reactive mesothelial cells
- metastatic tumor
 - adenocarcinoma
 - squamous cell carcinoma (SQC)
 - epithelioid hemangioendothelioma
 - epithelioid angiosarcoma

Mesothelioma versus Reactive Mesothelial Cells. Because reactive mesothelial cells of the pleura and peritoneum do not form numerous large morulae, the diagnosis of mesothelioma is straightforward when the specimen is highly cellular and contains many large clusters of enlarged mesothelial cells (see [Fig. 4.10A](#)). Such specimens can be called, at the very least, “suspicious for mesothelioma,” which will prompt a confirmatory biopsy. In the appropriate

clinical context (unilateral effusion, asbestos exposure, and pleural thickening) it can be argued such specimens can be accurately called “positive for malignancy.”

As mentioned previously, a strikingly morular pattern is not seen in all mesotheliomas. When the morular pattern is not present, the diagnosis is more problematic ([Fig. 4.14A](#) and [B](#)). There are precious few (if any) immunohistochemical markers on which one can rely for this distinction. The best marker for distinguishing benign from malignant mesothelial cells appears to be epithelial membrane antigen (EMA), but not all investigators report good specificity. Although some find excellent specificity,⁶¹⁻⁶⁶ especially for EMA clone E29,⁶¹ others do not.^{67,68}

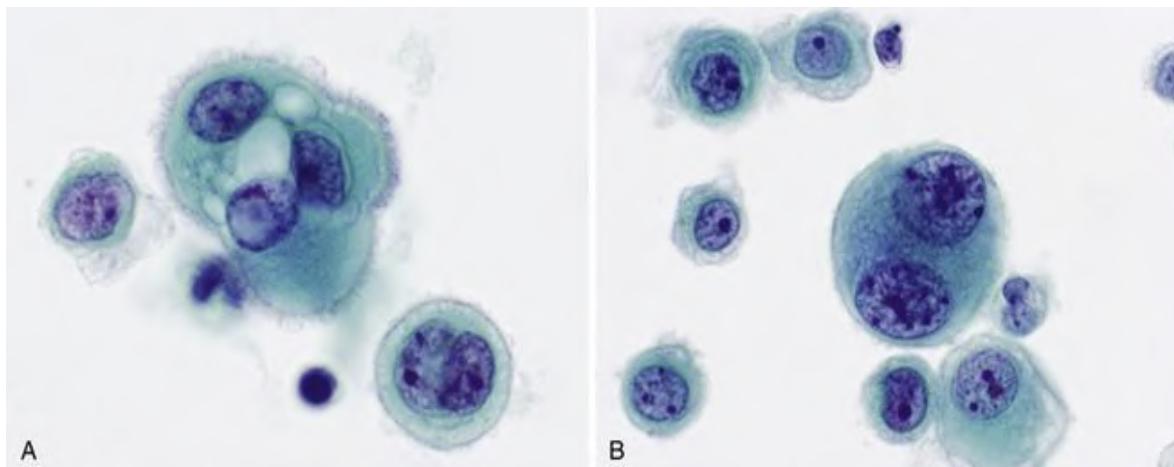


FIGURE 4.14 Reactive mesothelial cells versus mesothelioma.

A, Reactive mesothelial cells can show some variation in nuclear size and nuclear membrane irregularity (Papanicolaou stain). B, Mesotheliomas usually show greater cytomegaly, but this can be difficult to assess on a case-by-case basis (Papanicolaou stain).

Although time-consuming and not available in most laboratories, cytogenetic analysis has high sensitivity and specificity for the distinction between reactive mesothelial cells and mesothelioma. In almost all cases, mesotheliomas show clonal cytogenetic aberrations indicative of malignancy, the most common being deletions of 1p, 3p, 6q, 9p, and 22q.²¹ With a combination of appropriate probes, some deletions can be detected by fluorescence in situ hybridization (FISH) ([Fig. 4.15](#)).^{22,23,69-71}

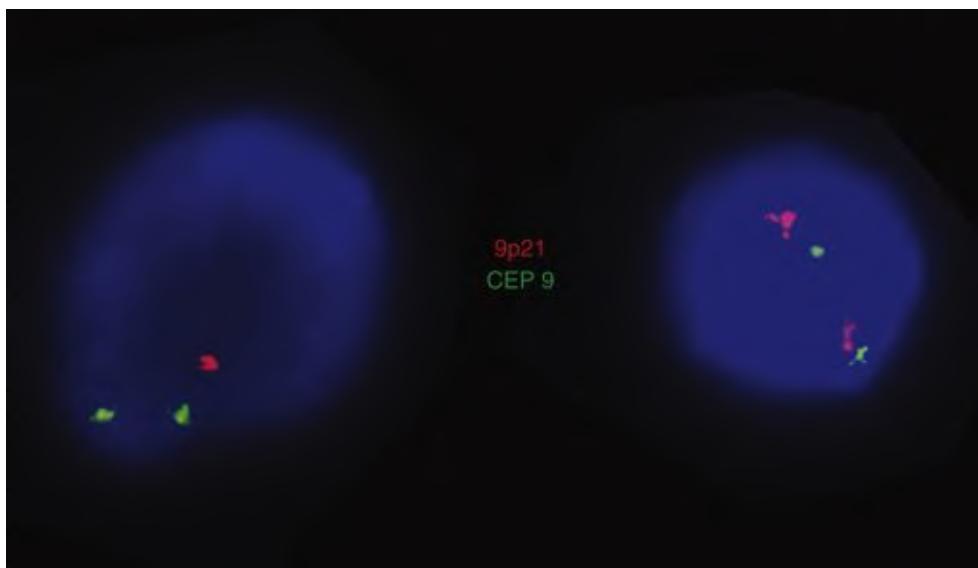


FIGURE 4.15 Malignant mesothelioma.

Mesotheliomas have clonal chromosomal deletions. Fluorescence in situ hybridization (FISH) of pleural fluid shows a normal cell on the right and a mesothelioma cell on the left. The preparation has been incubated with probes for the centromeric region (*green*) and the deleted region (*red*) of chromosome 9. Both cells show two centromeric regions, but the mesothelioma cell is missing a segment of the short arm of chromosome 9. (Courtesy of Dr. Paola Dal Cin, Brigham and Women's Hospital, Boston, MA.)

Mesothelioma versus Adenocarcinoma. Some adenocarcinomas spread to involve the serosal surfaces in a diffusely infiltrative, pseudomesotheliomatous pattern. The similarity extends to cytopathology: Exfoliated cells of both mesotheliomas and adenocarcinomas can be arranged in cohesive clusters or dispersed in a noncohesive pattern, and both can have vacuolated cytoplasm. A few morphologic clues exist, however. Mesothelioma cells form a morphologic continuum with benign-appearing mesothelial cells at one end, whereas fluids that harbor metastatic adenocarcinoma generally contain two distinct cell populations. Tumor cells that are separated by slitlike “windows” and have abundant, dense cytoplasm are more likely to be mesothelial in origin. On cell block sections, a core of edematous collagen and stromal cells, surrounded by neoplastic cells, is more commonly seen in mesothelioma than in adenocarcinoma (see Fig. 4.12). Conversely, ringlike structures with hollow cores, seen in some adenocarcinomas, are very uncommon in mesothelioma. Clusters with a knobby (mulberry-like) contour, rather than the smooth, cannonball-like edge of many adenocarcinomas, is characteristic of mesotheliomas (see Fig. 4.10B).

These morphologic clues, unfortunately, are not always reliable. Exceptions occur frequently enough that, in a given case, morphology alone cannot be depended upon for an unequivocal classification. Histochemical and

immunocytochemical stains can make the distinction in almost all cases and are indispensable in this regard (Table 4.2). Three mesothelial markers are particularly useful in this distinction. *Calretinin*, a calcium-binding protein, is strongly positive in most mesotheliomas, demonstrating a nuclear and cytoplasmic staining pattern (Fig. 4.16A). By contrast, only a small number of adenocarcinomas are positive, virtually always in a predominantly cytoplasmic pattern,^{22,23} although 15% of breast cancers show both nuclear and cytoplasmic staining for calretinin.²⁴ The *WT1* protein, the product of the *WT1* gene on chromosome 11p and implicated in the pathogenesis of Wilms' tumors and mesotheliomas, is strongly expressed in most mesotheliomas.^{25,26} *WT1* staining is nuclear, not cytoplasmic (Fig. 4.16B). With the exception of serous carcinomas, most adenocarcinomas are negative for *WT1*. *D2-40*, directed against a surface glycoprotein originally detected in germ cell neoplasms, is another mesothelial marker. A strong membranous staining pattern is characteristic of mesothelioma and serous carcinoma.²⁷

TABLE 4.2
COMMON HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL STAINING PATTERNS FOR MESOTHELIOMA AND METASTATIC ADENOCARCINOMA

Stain	Expected Result*	
	Adenocarcinoma	Mesothelioma
PAS-D	+	–
mucicarmine	+	–
CEA	+	–
MOC-31	+	–
Ber-EP4	+	–
Leu M-1	+	–
B72.3	+	–
TTF-1	+ (nuclear)**	–
calretinin	–	+ (nuclear and cytoplasmic)
WT1	–	+ (nuclear) [†]
D2-40	–	+ (membranous) [†]

CEA, carcinoembryonic antigen; *PAS-D*, Periodic acid-Schiff and diastase; *TTF-1*, thyroid transcription factor-1; *WT1*, Wilms' tumor protein 1.

*Result observed in most but not all cases

**Positive in adenocarcinomas of the lung and thyroid only. Also positive in small cell carcinomas

[†]Also positive in serous carcinomas of the ovary

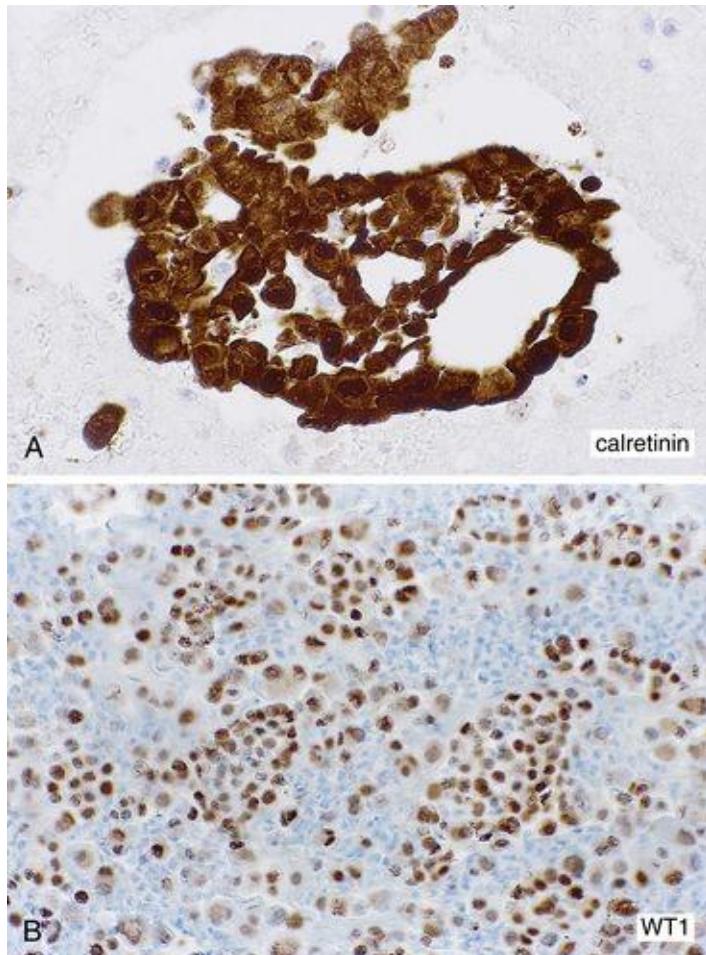


FIGURE 4.16 Immunoprofile of malignant mesothelioma.

A, Mesotheliomas show nuclear and cytoplasmic staining for calretinin. B, There is usually nuclear immunoreactivity for Wilms tumor protein 1 (WT1).

Mesotheliomas are typically negative for intracytoplasmic mucin with the mucicarmine and periodic acid–Schiff diastase (PAS-D) stains and negative for the carcinoma markers CEA, MOC-31, Ber-EP4, Leu-M1 (CD15), and B72.3.^{28–30} By contrast, at least half of adenocarcinomas in effusions are positive for cytoplasmic mucin, and most are immunoreactive for one or more of the carcinoma markers CEA, MOC-31, Ber-EP4, Leu-M1, and B72.3 (Fig. 4.17A). Thyroid transcription factor-1 (TTF-1) is particularly useful in the frequent distinction between lung adenocarcinoma and mesothelioma. Strong nuclear

staining is common in lung adenocarcinomas and absent in mesotheliomas ([Fig. 4.17B](#)).^{26,81}

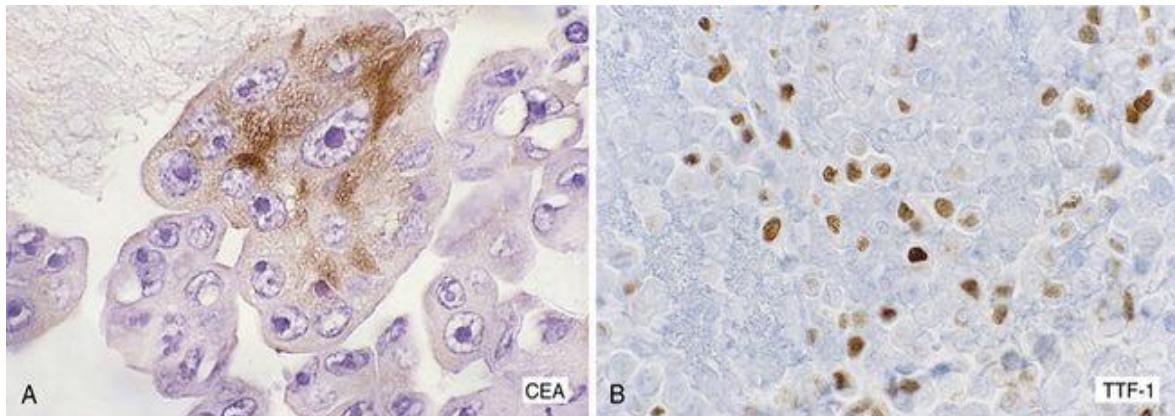


FIGURE 4.17 Immunoprofile of metastatic adenocarcinomas.

A, Most adenocarcinomas are immunoreactive for one or more of the carcinoma markers like carcinoembryonic antigen (CEA) (shown here), but often only some of the cells are positive. B, Most adenocarcinomas of the lung are immunoreactive for thyroid transcription factor-1 (TTF-1), as are most thyroid cancers.

A few caveats are in order. First, a critical analysis of the stains is important. Fine cytoplasmic granules seen with the PAS-D stain, the interposition of positively stained basement membrane material, and an extracellular localization of staining should not be misinterpreted as a positive reaction for mucin. A positive staining reaction for mucin is nevertheless encountered in rare instances of mesothelioma.^{36,82} For this reason, it is wise to use a panel of histochemical and immunocytochemical stains from those listed in [Table 4.2](#). A panel of four or five markers is sufficient in most cases and can be tailored to the particular differential diagnosis ([Table 4.3](#)).⁸⁰

TABLE 4.3
RECOMMENDED IMMUNOHISTOCHEMICAL PANELS BASED ON DIFFERENTIAL DIAGNOSIS*

Differential Diagnosis	Panel
Pleural mesothelioma versus lung adenocarcinoma	Mesothelial markers calretinin, WT1, and D2-40 and carcinoma markers MOC-31, Ber-EP4, B72.3, CEA, TTF-1
Peritoneal mesothelioma versus serous carcinoma	Mesothelial marker calretinin and carcinoma markers PAX-8, MOC-31 Ber-EP4, estrogen receptors
Mesothelioma versus squamous	Mesothelioma markers calretinin, WT1, and D2-40 and carcinoma

carcinoma	markers p63, MOC-31
Mesothelioma versus renal cell carcinoma	Mesothelioma markers calretinin, WT1, and D2-40 and carcinoma markers LeuM1 (CD15), RCC, PAX-8

*Modified from Ordonez NG. What are the current best immunohistochemical markers for the diagnosis of epithelioid mesothelioma? A review and update. Hum Pathol 2007;38(1): 1-16.

Mesothelioma can also be distinguished from metastatic adenocarcinoma by electron microscopy. The microvilli of mesothelioma cells are long and sinuous, with a length-to-diameter ratio of 15:1 or greater, whereas those of adenocarcinoma are short and stubby, with a smaller ratio.²⁰ This method requires special fixation of the specimen for optimal results, however, and is rarely used.

Mesothelioma versus Squamous Cell Carcinoma. In effusions as in histologic sections, certain mesotheliomas can resemble a squamous cell carcinoma (SQC) because both typically have dense cytoplasm. Immunohistochemistry can be helpful in this distinction (see [Table 4.3](#)).

Mesothelioma versus Vascular Tumors. Primary angiosarcomas and hemangioendotheliomas of the lung are rare, but they can have a pseudomesotheliomatous growth pattern that mimics mesothelioma not just clinically and radiologically but also cytologically.²¹ They arise in the lung but spread to involve the pleural surface in a diffuse pattern. Epithelioid hemangioendotheliomas and angiosarcomas bear an uncanny resemblance to carcinomas and epithelial mesotheliomas ([Fig. 4.18](#)). A panel of immunostains that includes vascular markers (CD31, CD34, ERG) and mesothelial markers (calretinin, WT1, D2-40) are helpful in this distinction.

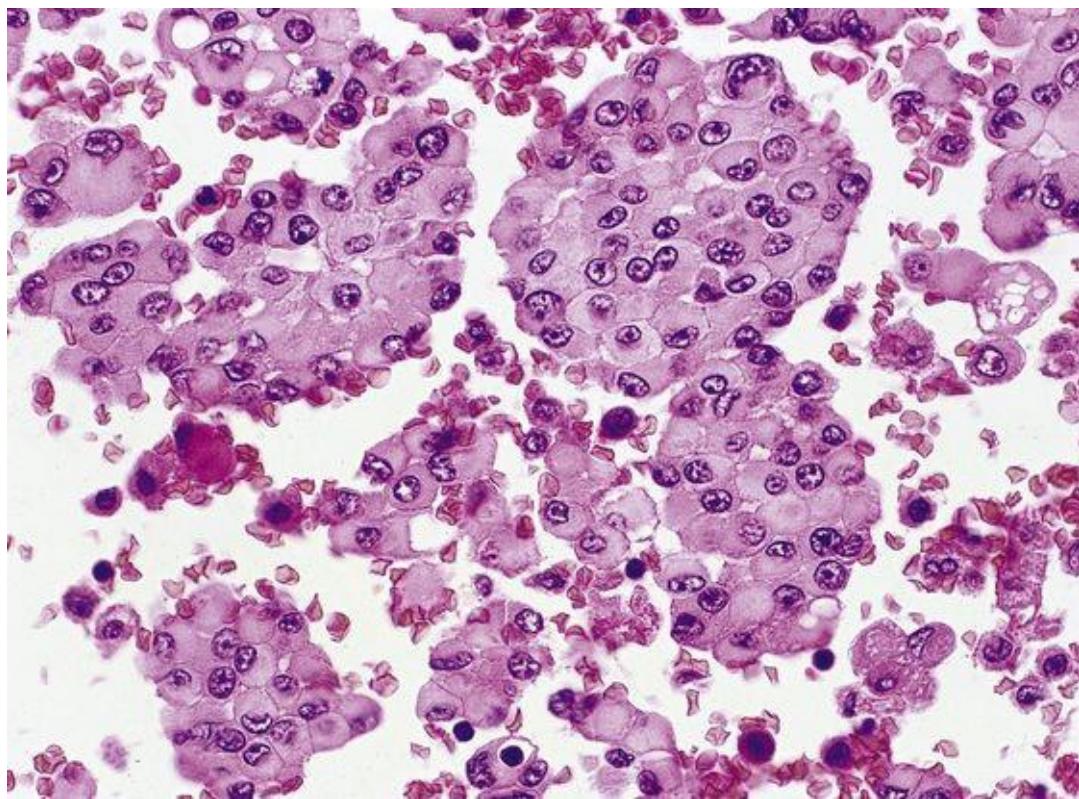


FIGURE 4.18 Epithelioid hemangioendothelioma of the lung (pleural fluid). This tumor is a good mimic of mesothelioma because the cells form large aggregates and have round, centrally placed nuclei and abundant cytoplasm (hematoxylin-eosin [H & E] stain).

Primary Effusion Lymphoma

Primary effusion (body cavity-based) lymphoma (PEL) is a rare subtype of diffuse large B-cell lymphoma (DLBL) that is associated with human herpesvirus 8 (HHV-8) and manifests with a pleural, pericardial, or peritoneal effusion.⁸⁴ Most cases have a null-cell immunophenotype; B-cell clonality is usually demonstrated by molecular studies. Rare PELs have a T-cell immunophenotype.^{85,86} All cases are positive for HHV-8; indeed, detection of HHV-8 is an essential step in confirming the diagnosis of PEL. By definition, this is a fluid-based malignancy without an associated mass lesion, lymphadenopathy, or organomegaly.⁸⁷ Most cases arise in the setting of human immunodeficiency virus (HIV) infection, but rare cases in transplant recipients have also been reported. The prognosis is poor: median survival is less than 6 months.^{87,88}



Cytomorphology of primary effusion lymphoma

- dispersed large cells
- round or irregular nucleus
- prominent nucleolus
- abundant basophilic cytoplasm (Romanowsky stain)

The cells are always large, ranging from plasmablastic/immunoblastic (round nucleus with prominent nucleolus) to anaplastic (large, irregular, multilobate nucleus) in morphology ([Fig. 4.19A](#)). Cytoplasm is abundant and may have a perinuclear hof or vacuoles. Mitoses and apoptotic bodies are present and can be numerous.⁸⁵ The diagnosis of malignancy is straightforward. In the usual clinical setting of an immunodeficiency, a lymphoma should be suspected; this can be confirmed by immunocytochemistry. PEL cells usually express CD45 (leukocyte common antigen) but are negative for the B-cell markers CD19 and CD20. Other lymphoid markers, like the activation marker CD30 and the plasma cell markers CD38 and CD138 are often present.⁸⁷ Demonstrating the presence of HHV-8 is essential for diagnosis ([Fig. 4.19B](#)).⁸⁶ In most cases the neoplastic cells are co-infected with Epstein-Barr virus (EBV), best demonstrated by EBV-encoded RNA (EBER) *in situ* hybridization.⁸⁶

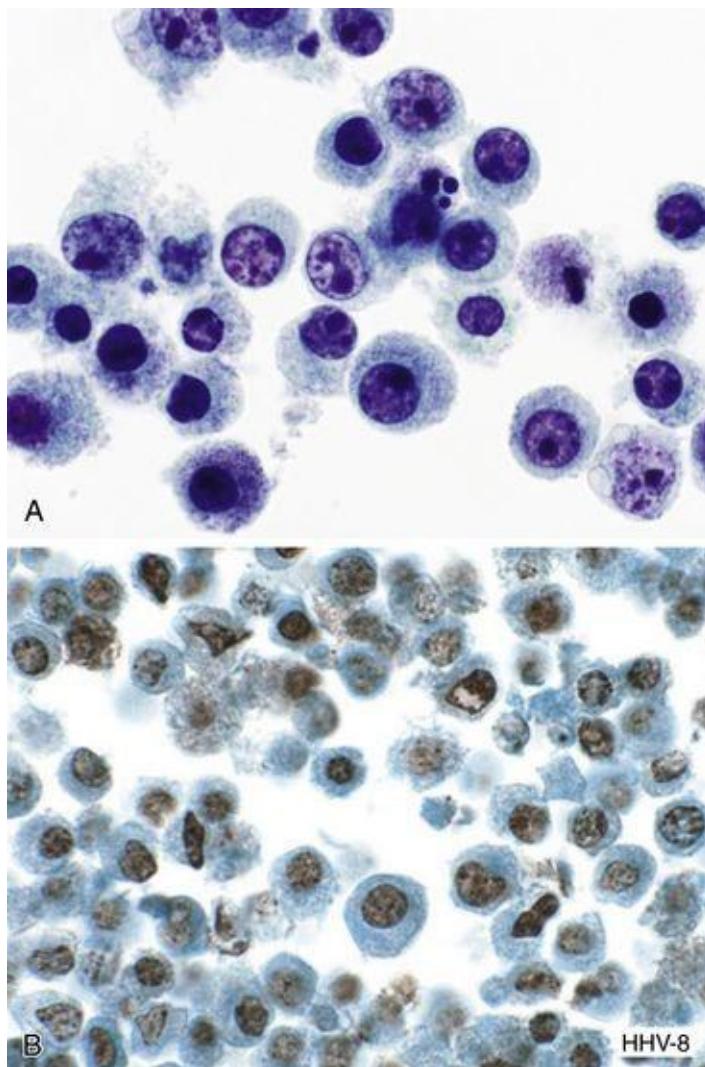


FIGURE 4.19 Primary effusion lymphoma (PEL).

A, The malignant cells are large, with thick nuclear membranes, irregularly distributed chromatin, and prominent nucleoli. Apoptotic bodies are present (Papanicolaou stain). *B*, The presence of human herpesvirus 8 (HHV-8), demonstrated here by immunohistochemistry, is a *sine qua non* of PELs.



Differential diagnosis of primary effusion lymphoma (PEL)

- diffuse large B-cell lymphoma (other than PEL)
- pyothorax-associated lymphoma
- anaplastic large cell lymphoma
- posttransplant lymphoproliferative disorder
- carcinoma
- melanoma

PEL cells are markedly atypical and obviously malignant. It is usually simply a question of classifying the malignancy. PEL is morphologically similar to the immunoblastic type of DLBL. If the patient has a mass lesion, lymphadenopathy, or organomegaly along with the effusion, the lymphoma is not classified as a PEL but is instead a conventional DLBL. Immunohistochemistry can also distinguish these entities, because DLBL expresses B-cell markers and is negative for HHV-8. *Pyothorax-associated lymphoma* (PAL) is a rare, EBV-associated subtype of DLBL that develops after longstanding chronic pleural inflammation, and there is usually an associated pleural mass. Like other DLBLs, it expresses B-cell markers and is negative for HHV-8. Anaplastic large cell lymphoma (ALCL) sometimes manifests with a pleural effusion. The “hallmark cells” (with horseshoe-shaped nuclei) typical of ALCL (see [Fig. 12.25](#)) can be seen in some cases of PEL, but ALCL is negative for HHV-8. In transplant recipients, the possibility of a posttransplant lymphoproliferative disorder (PTLD) should be considered (see [Chapter 12](#)). PTLDs are associated with EBV but are negative for HHV-8.⁸⁹ Carcinomas and melanoma can be excluded by the absence of immunoreactivity for keratins, S-100 protein, and HMB-45.

Metastatic Tumors

Adenocarcinoma

Metastatic carcinomas are by far the most common tumors found in effusions. Of these, adenocarcinomas are more common than squamous and small cell cancers. In most cases, carcinoma cells are easily recognized because, in one way or another, they are morphologically distinct from mesothelial cells ([Fig. 4.20](#)). This is the key to identifying them on cytologic preparations.

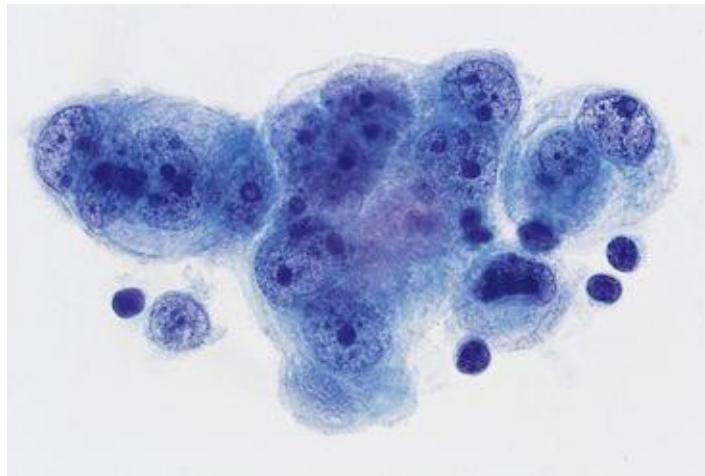


FIGURE 4.20 Adenocarcinoma of the lung (pleural fluid).

Clusters of very large, highly atypical cells like these are easily spotted and identified as malignant, but in the absence of a known primary, special stains might be needed for precise classification (Papanicolaou stain).

The cells of metastatic carcinoma are often but not necessarily larger, more pleomorphic, and more hyperchromatic than mesothelial cells. They can, in fact, be smaller and more uniform in size than the mesothelial cells around them. Most commonly, they are distinguished from benign mesothelial cells by their tendency to form cell clusters (see [Fig. 4.9](#)). Some carcinomas, however, exfoliate in a noncohesive way, as isolated cells instead ([Fig. 4.21](#)). In such cases, cell size, increased nuclear-to-cytoplasmic ratio, irregular nuclear contours, prominent nucleoli, or coarsely textured chromatin will distinguish them from mesothelial cells.

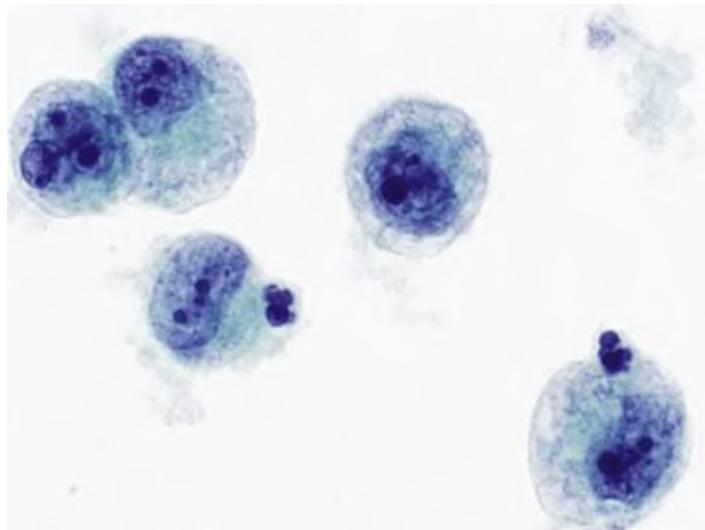


FIGURE 4.21 Adenocarcinoma of the lung (pleural fluid).

In cases like this, the malignant cells can be more difficult to recognize because they are dispersed as isolated cells. Careful examination reveals markedly enlarged nucleoli, irregular nuclear contours, and a cytoplasmic texture that is frothier and less dense than that of most mesothelial cells (Papanicolaou stain).



Cytomorphology of adenocarcinomas

- large spheres or isolated cells
- cytoplasmic vacuolization
- signet ring cells (gastric, breast)

Adenocarcinomas exfoliate as large spheres or as isolated cells. Cytoplasmic vacuoles are often present, but they are not specific for adenocarcinomas; they are seen in some mesotheliomas as well as other tumors. Some morphologic clues can point to the site of origin of an adenocarcinoma. For example, abundant hollow spheres (“cannonballs”) are a common pattern in metastatic breast cancer ([Fig. 4.22A and B](#)). Malignant signet ring cells in an effusion point to an origin from stomach cancer, but other primary sites, such as the breast, are also possible ([Fig. 4.23](#)). Most colorectal cancers are composed of elongated cells with hyperchromatic nuclei arranged in acinar formations; individual cell necrosis is a common finding. Large cells with prominent nucleoli and abundant lacy, vacuolated cytoplasm are typical of clear cell carcinomas of the kidney and the female genital tract ([Fig. 4.24](#)). Most metastatic prostate cancers exfoliate as isolated cells or loosely cohesive groups of cells with prominent nucleoli. Some high-grade metastatic prostate cancers resemble small cell carcinoma.²⁰

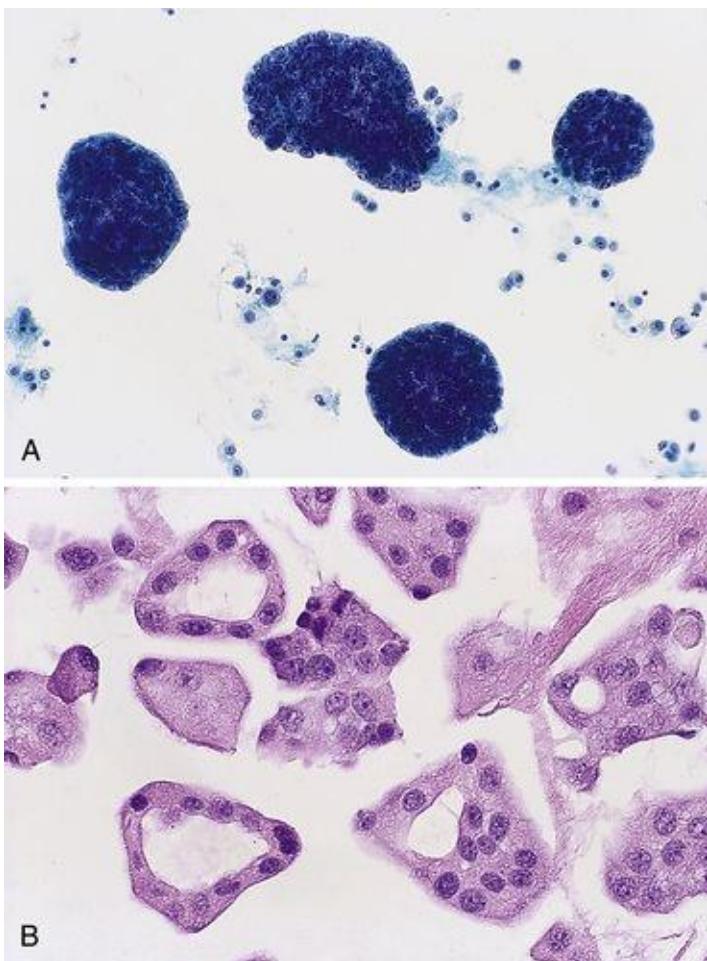


FIGURE 4.22 Ductal carcinoma of the breast (pleural fluid).
A, Ductal breast cancers often exfoliate as large spheres of malignant cells (Papanicolaou stain). B, The hollow nature of the spheres is apparent on cell block sections (hematoxylin-eosin [H & E] stain).

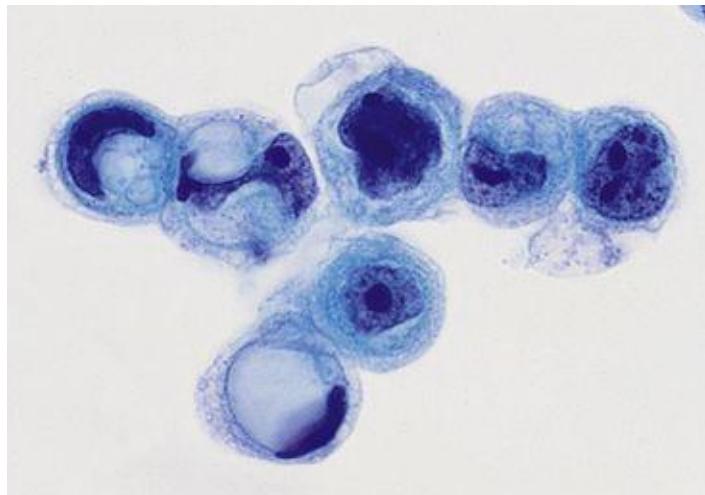


FIGURE 4.23 Adenocarcinoma of the stomach (pleural fluid).
Large numbers of isolated signet ring cells are characteristic of many gastric cancers
(Papanicolaou stain).

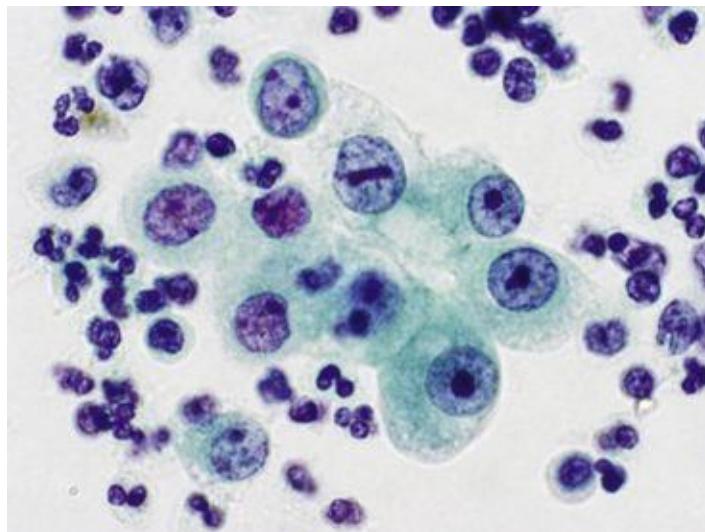


FIGURE 4.24 Clear cell carcinoma of the kidney (pleural fluid).
Large cells with round nuclei, prominent nucleoli, and abundant granular and vacuolated cytoplasm are typical of renal cell carcinoma (Papanicolaou stain).

Psammoma bodies are encountered in effusions caused by müllerian neoplasms, papillary carcinomas of the thyroid, adenocarcinomas of the lung, and mesotheliomas ([Fig. 4.25](#)). They are also seen in some benign proliferative reactions of the mesothelium, especially in the peritoneum,⁹¹ but also the pericardium,³⁵ and by themselves should not be taken as evidence of malignancy.

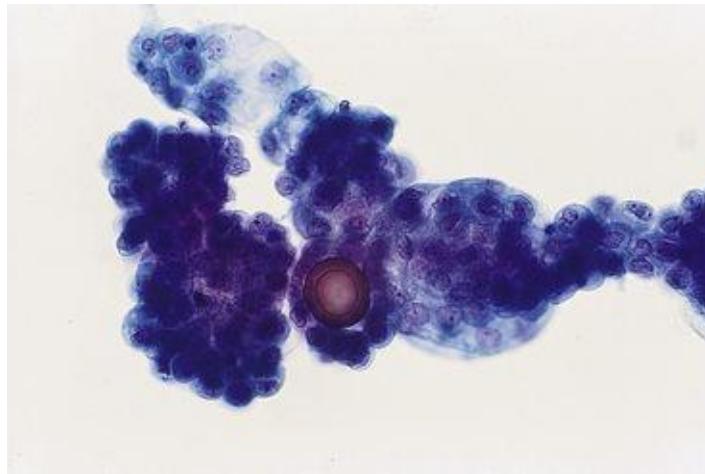


FIGURE 4.25 Metastatic papillary carcinoma of the thyroid (pleural fluid). This large irregular cluster of malignant cells is associated with a psammoma body. The diagnosis can be confirmed by positive immunoreactivity for thyroglobulin, TTF-1, and PAX8 (Papanicolaou stain).

When some mucinous adenocarcinomas, particularly those of the appendix, spread to involve the peritoneal surfaces, they produce a slow but relentless accumulation of extracellular mucin that eventually distends the peritoneal cavity, a condition known as *pseudomyxoma peritonei*. Peritoneal fluid from such patients is gelatinous and composed predominantly of mucin, which stains blue-green or purple with the Papanicolaou stain. Such specimens are often sparsely cellular and may contain only vacuolated histiocytes called muciphages ([Fig. 4.26A](#)). The tumor cells in the peritoneum that produce the mucin ([Fig. 4.26B](#)) often constitute just a tiny fraction of the volume of the specimen and may not be present in the slides examined. When there are no definite malignant cells (just mucin and muciphages), only a presumptive diagnosis of malignancy (i.e., “suspicious”) can be offered.

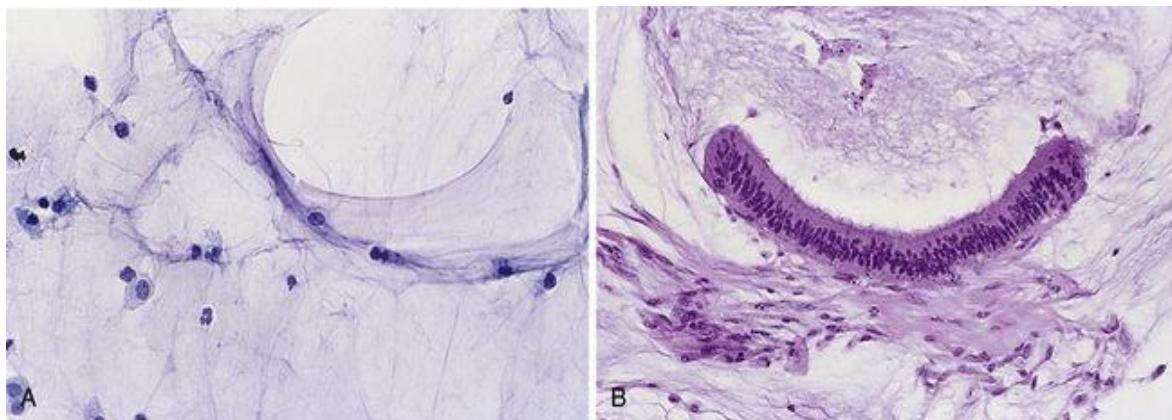


FIGURE 4.26 Pseudomyxoma peritonei (peritoneal fluid).

A, Extracellular mucin is present in abundance and stains blue or purple with the Papanicolaou stain. Malignant cells are often not seen; the few cells in this field are histiocytes (Papanicolaou stain). B, In this case, rare strips of neoplastic epithelium were identified in cell block sections (hematoxylin-eosin [H & E] stain).



Differential diagnosis of metastatic adenocarcinoma

- reactive mesothelial cells
- mesothelioma

It is usually easy to distinguish metastatic adenocarcinoma from reactive mesothelial cells. Malignant cells in cell block sections are frequently arranged in large clusters and/or are situated in lacunae (see [Fig. 4.9](#)). In some cases, however, metastatic adenocarcinoma cells are not easily recognized. A clear “second population” (morphologically distinct from mesothelial cells) may not be apparent; the tumor cells might mimic mesothelial cells; or the suspicious cells might be few in number or poorly preserved. In any doubtful case, special stains for mucin and immunohistochemistry for carcinoma markers ([Table 4.2](#)) are helpful for distinguishing reactive mesothelial cells (negative) from adenocarcinomas (positive).^{34,79} A panel of four or five immunomarkers is sufficient in most cases. The panel can be selected based on clinical history ([Table 4.3](#) can be applied for this purpose) and might include, in addition to carcinoma markers, two mesothelial markers like calretinin and WT1.⁸⁰

Lobular carcinoma of the breast is perhaps the most subtle of all the adenocarcinomas; with only routine stains, the cells of this tumor can be impossible to distinguish from histiocytes and mesothelial cells ([Fig. 4.27A](#)). For this reason, it is advisable to do special stains for mucin (mucicarmine and PAS-D) and a limited panel of immunostains (e.g., CEA, gross cystic disease fluid protein, gammaglobulin, estrogen receptor) on any effusion from a patient with lobular breast cancer ([Fig. 4.27B](#) and [C](#)).

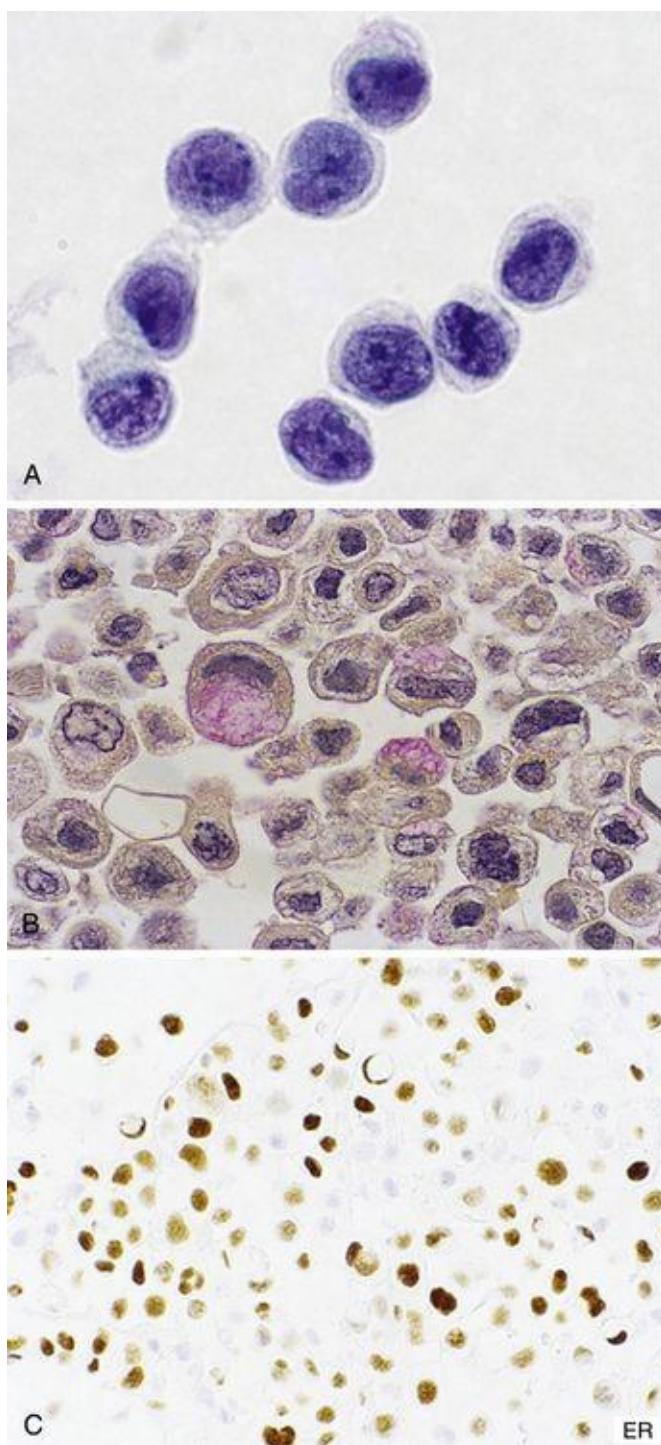


FIGURE 4.27 Lobular carcinoma of the breast (pleural fluid).

A, These malignant cells are extremely difficult to recognize because they resemble histiocytes or mesothelial cells (Papanicolaou stain). B, A mucicarmine stain reveals focal intracytoplasmic mucin. C, A positive immunostain for estrogen receptor (ER) not only identifies the presence of metastatic breast cancer, it may also guide treatment.

When the adenocarcinoma diagnosis is straightforward and the patient has a

single known primary, it is customary to assign the malignancy to the known primary (“positive for malignant cells, consistent with the patient’s known breast cancer”). Caution is advised, however, as patients with one cancer can develop a second or third malignancy. A limited immunohistochemical panel is advised in selected circumstances, especially if the morphologic features do not match those of the original tumor.⁹² Immunohistochemical markers are especially helpful in patients with a tumor of unknown primary or a history of two or more neoplasms. Useful tissue-specific antigens include TTF-1 (for adenocarcinoma and small cell carcinoma of the lung and thyroid cancers) (see [Fig. 4.17B](#)),⁸¹ estrogen and progesterone receptors (for breast and müllerian malignancies), thyroglobulin, prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), and PAX-8 (for müllerian, thyroid, renal, and thymic tumors).^{93–95} Negative staining is less helpful than positive staining, because none of these stains has 100% sensitivity for its associated malignancy. For example, negative PSA and PAP results do not exclude metastatic prostatic cancer, because less than 50% of metastatic prostate cancers in pleural effusions are immunoreactive for these markers.⁹⁰

The differential diagnosis of adenocarcinoma and mesothelioma has been discussed previously. Immunostains are indispensable for a definitive distinction (see [Table 4.3](#)).

Immunohistochemistry for Estrogen Receptor, Progesterone Receptor, and Human Epidermal Growth Factor Receptor 2. Determining the status of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) is recommended for breast cancer recurrences, not just primary invasive cancers.^{96,97} ER and PR status helps the oncologist evaluate the appropriateness of treatment of the recurrence with tamoxifen and aromatase inhibitors, and HER2 status determines appropriateness of treatment with trastuzumab (Herceptin). Because breast cancer recurrences sometimes involve the pleura and other serosal surfaces, ER, PR, and HER2 testing is often done on effusions.⁹⁸ ER and PR are considered positive if 1% or more of the tumor nuclei are positive.⁹⁶ The report should record the percentage of positively staining nuclei as well as the intensity of staining (weak, moderate, or strong). A positive HER2 result (3+) is defined as uniform, intense membrane staining of more than 30% of tumors cells ([Fig. 4.28](#)).

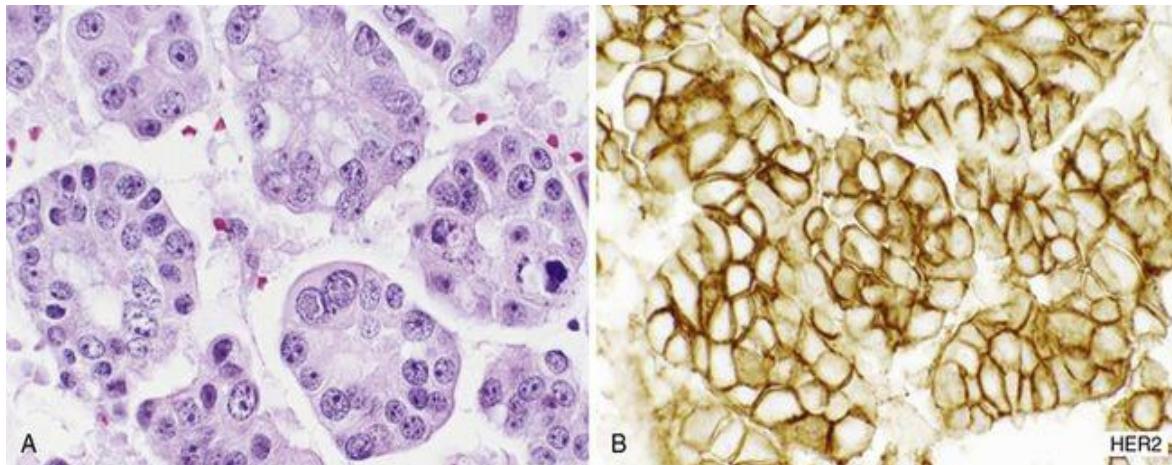


FIGURE 4.28 Ductal carcinoma of the breast (pleural fluid).

A, Cell block section shows numerous malignant cells. This pleural recurrence occurred 4 years after treatment of the primary invasive tumor (hematoxylin-eosin [H & E] stain). B, Immunostaining for human epidermal growth factor receptor 2 (HER2) reveals uniform strong membrane staining in greater than 30% of the malignant cells. The patient's original breast excision had two breast cancers, one that was HER2-negative and another that was HER2-positive.

Amplification and/or overexpression of HER2 is also seen in up to one third of gastric and gastroesophageal junction (GEJ) cancers, and patients with HER2-positive gastric and GEJ cancers treated with trastuzumab have a significant increase in overall survival.⁵⁵⁻⁹⁹ Thus, HER2 testing of effusions positive for metastatic gastric or GEJ cancer might also help guide therapy.

Molecular Testing of Effusions with Metastatic Lung Cancer. Advanced stage lung cancers are increasingly being treated with “targeted therapies”—treatment targeted toward a specific genetic abnormality of the tumor—because such therapies are more effective and less toxic than conventional chemotherapy. Mutations in the epidermal growth factor receptor (EGFR), for example, are seen in 10% to 26% of non–small cell lung cancers, and patients with an activating mutation respond to tyrosine kinase inhibitors like erlotinib and gefitinib.¹⁰⁰⁻¹⁰² Randomized trials have shown significantly improved progression-free survival in patients with activating EGFR mutations who are treated with erlotinib as compared with standard chemotherapy, and patients treated with erlotinib have fewer severe adverse effects.¹⁰³ Accurate molecular classification of the tumor is essential to determine treatment. Lung cancer patients with positive pleural fluid cytology at the time of presentation are ideal candidates for such treatment, because curative surgical resection is not an option. Molecular classification can be performed successfully on small biopsy and cytology specimens,¹⁰⁴ but many of the molecular tests require that the sample have a minimum proportion of tumor to normal nuclei. Thus, assessment of the

cytologic sample for its adequacy and triaging the sample for molecular testing constitute a growing part of the cytologist's job.

Squamous Cell Carcinoma

SQCs rarely metastasize to the pleura, pericardium, or peritoneum. Those that do are most commonly carcinomas of the lung, larynx, and female genital tract.¹⁰⁵ The primary tumor is known in virtually all cases before an effusion develops.



Cytomorphology of squamous cell carcinoma

- large clusters or isolated cells
- keratinized or nonkeratinized
- dense cytoplasm

The cells of SQC either are arranged in large clusters ([Figs. 4.29A and B](#), and [4.30](#)) or occur as isolated cells. The cytologic appearance differs depending on the degree of keratinization of the tumor. The cytoplasm is usually dense and occasionally orangeophilic. Cells with a tadpole or spindle shape are uncommon. Nuclei are enlarged, hyperchromatic, and coarsely granular; nucleoli are usually not prominent. Nuclear pyknosis and karyorrhexis are seen in some cases. Anucleate

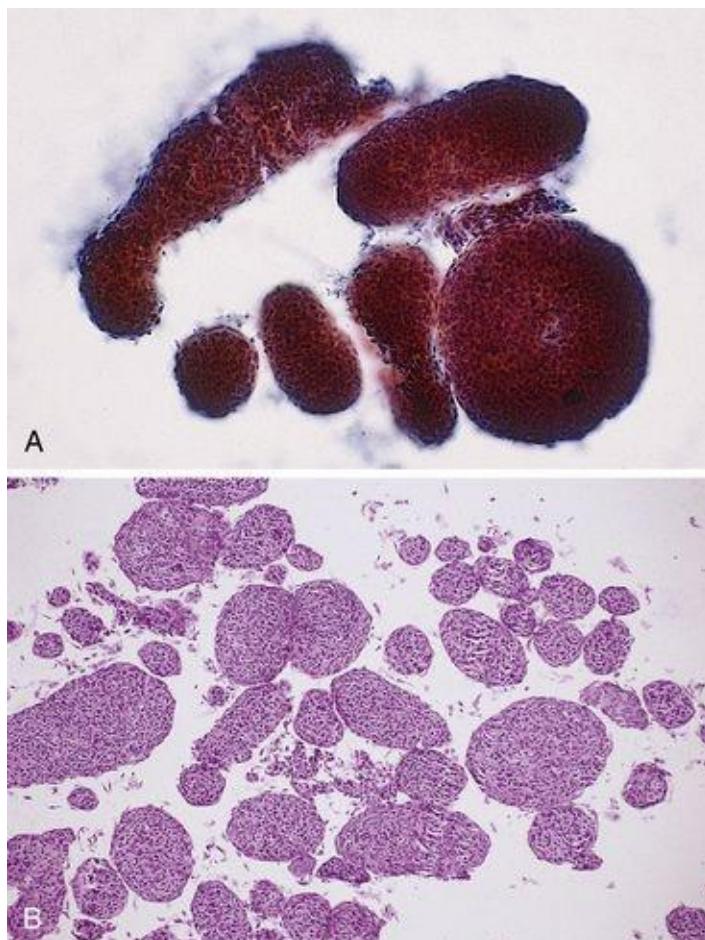


FIGURE 4.29 Squamous cell carcinoma (SQC) of the cervix (pericardial fluid). Nonkeratinizing squamous cell cancers shed large spheres of malignant cells (*A*, Papanicolaou-stained cytocentrifuge preparation. *B*, hematoxylin-eosin [H & E]-stained cell block preparation).

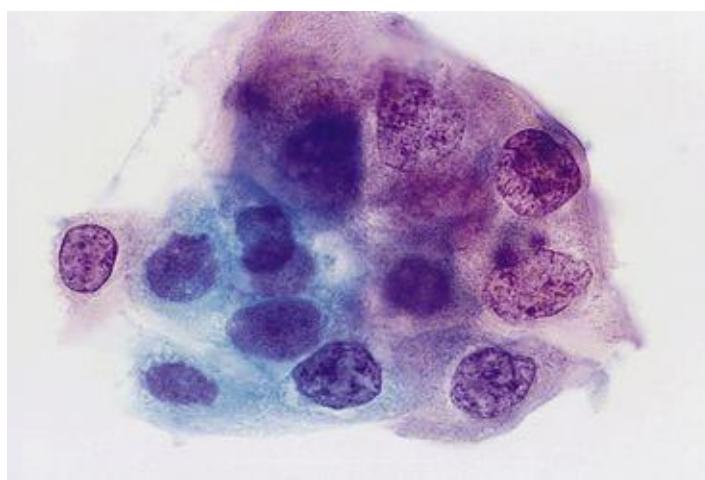


FIGURE 4.30 Squamous cell carcinoma (SQC) of the lung (pleural fluid). The malignant cells have coarsely textured chromatin and platelike cytoplasm (Papanicolaou stain).

squames may be present. Cytoplasmic vacuolization is sometimes seen, and it should not be interpreted as indicative of glandular differentiation.¹⁰⁵ A panel of immunostains that includes p63, MOC-31, and the mesothelial markers calretinin and WT1 can be useful to distinguish SQC from reactive mesothelial cells and mesothelioma (see [Table 4.3](#)).

Small Cell Carcinoma

Small cell carcinoma of the lung, despite its predilection for widespread metastases, causes a pleural effusion in less than 3% of patients.¹⁰⁶

The cells are dispersed as isolated cells and arranged in clusters and chains. They are small, with a diameter approximately two to three times that of small lymphocytes. Cytoplasm is scant. The nuclei are dark and have a finely granular chromatin texture; nucleoli are inconspicuous. Karyorrhexis is common. When



Cytomorphology of small cell carcinoma

- small cells (isolated, in chains and clusters)
- nuclear molding
- scant cytoplasm

arranged in groups, the cells are crescent-shaped and angulated as they wrap themselves around one another ([Fig. 4.31](#)).

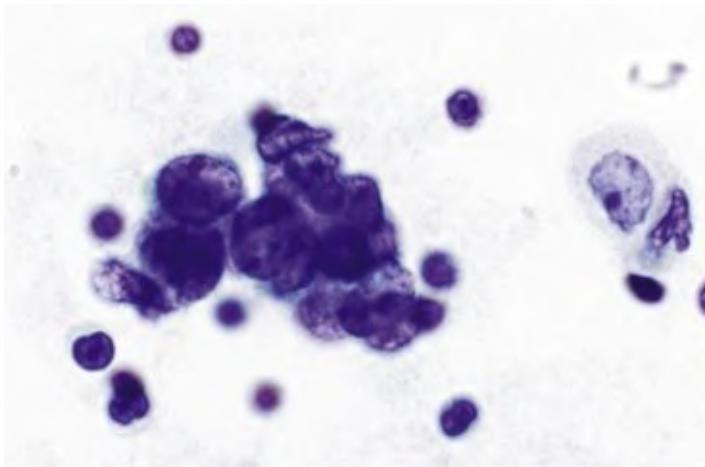


FIGURE 4.31 Small cell carcinoma of the lung (pleural fluid).

Some of the small malignant cells are isolated and others are molded together in clusters. The mesothelial cell on the right provides a size comparison (Papanicolaou stain).

The differential diagnosis includes lymphoma. The cells of small cell carcinoma are distinguished by their tendency to form clusters. Although the nuclei of lymphoma cells can be irregular in shape, they are rarely sharply angular like the cells of small cell carcinoma. Most small cell lung cancers, unlike lymphomas, are immunoreactive for TTF-1, but a negative result does not exclude the diagnosis. The differential diagnosis also includes other small cell malignancies like small cell carcinoma of the prostate⁹⁰ and Merkel cell carcinoma. In most cases, the clinical history of a specific malignancy points in the correct direction. Immunohistochemistry can be helpful if needed. For example, small cell carcinomas are immunoreactive for CK7 and negative for CK20; the reverse is true for Merkel cell carcinoma.

Melanoma

Most malignant melanomas arise in the skin, but extracutaneous tumors like ocular melanomas do occur. In 5% of cases, patients present with metastatic disease without a known primary or with only a remote history of a pigmented cutaneous lesion. To compound the problem, the diagnosis of melanoma can be subtle in effusions. The malignant cells resemble mesothelial cells: they are often isolated round cells with prominent nucleoli ([Fig. 4.32A](#)). In some cases, cells show a fine brown cytoplasmic pigmentation and/or intranuclear pseudoinclusions. Cell clusters are uncommon ([Fig. 4.32B](#)), and cell blocks rarely show pericellular lacunae. The distinction from reactive mesothelial cells is difficult in cases that show little nuclear pleomorphism and hyperchromasia

and no cytoplasmic pigmentation.¹⁰⁷ Immunocytochemistry is helpful; melanomas are positive for S-100 and HMB-45 and usually negative for keratin proteins.

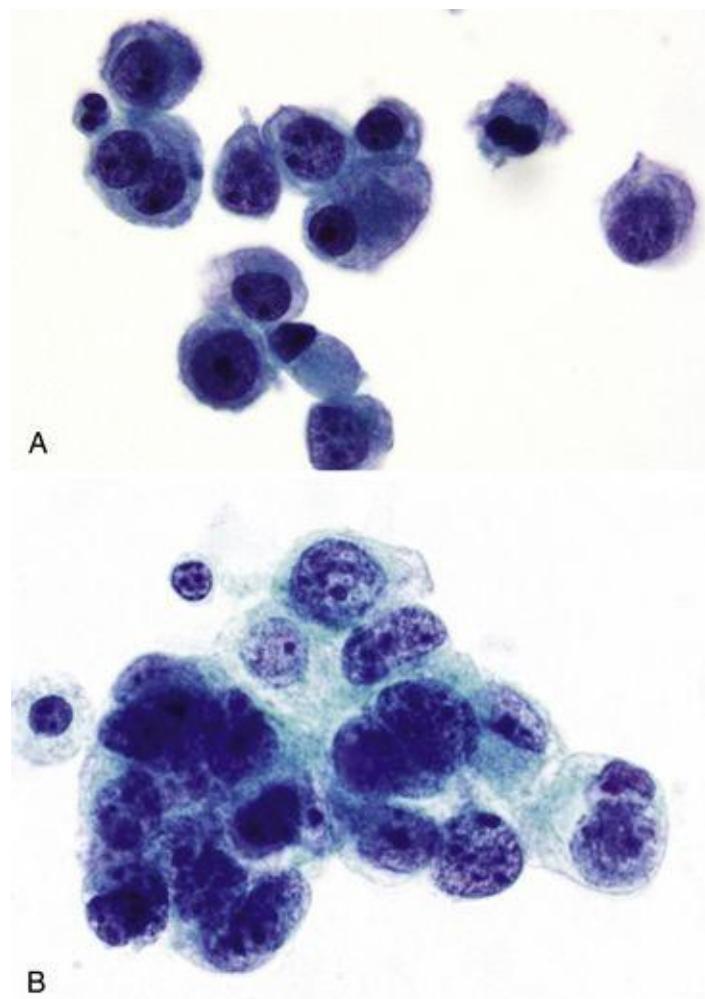


FIGURE 4.32 Melanoma (pleural fluid).

A, Nonpigmented melanoma cells are usually isolated, not clustered, and resemble mesothelial cells (Papanicolaou stain). B, Less often, they aggregate into large clusters (Papanicolaou stain).

Non-Hodgkin Lymphoma

A malignant effusion complicates the course of disease in many patients with non-Hodgkin lymphoma. In fact, 10% to 15% of malignant effusions are caused by lymphoma; the proportion is significantly higher in the pediatric population.^{29,108} Most represent secondary involvement of serosal surfaces; very

few are PELs. Certain subtypes have a greater propensity for involving serosal surfaces (e.g., lymphoblastic lymphoma).

Cytologic preparations are highly cellular and composed of dispersed lymphoid cells. Lacunar spaces are not seen in cell block sections with lymphomas. In many cases, mesothelial cells are conspicuously absent. The cells of DLBL are easiest to recognize, because they are larger than histiocytes and their nucleoli are usually prominent ([Fig. 4.33](#)). Nuclei are round or highly irregular, and the chromatin is coarsely textured. Cytoplasm is often abundant, pale, and vacuolated. The cells of small cell lymphomas are only slightly larger than normal lymphocytes. Those of follicular lymphomas have irregular and cleaved nuclei and scant cytoplasm. Lymphoblastic lymphomas are also composed of relatively small cells with round or irregularly shaped nuclei, finely dispersed chromatin, and scant or moderate amounts of cytoplasm ([Fig. 4.34](#)). Small lymphocytic lymphoma rarely involves serosal cavities. Burkitt and Burkitt-like lymphomas are high-grade neoplasms composed of lymphoid cells of intermediate size with round nuclei, prominent nucleoli, and coarsely textured chromatin. Mitoses are numerous.

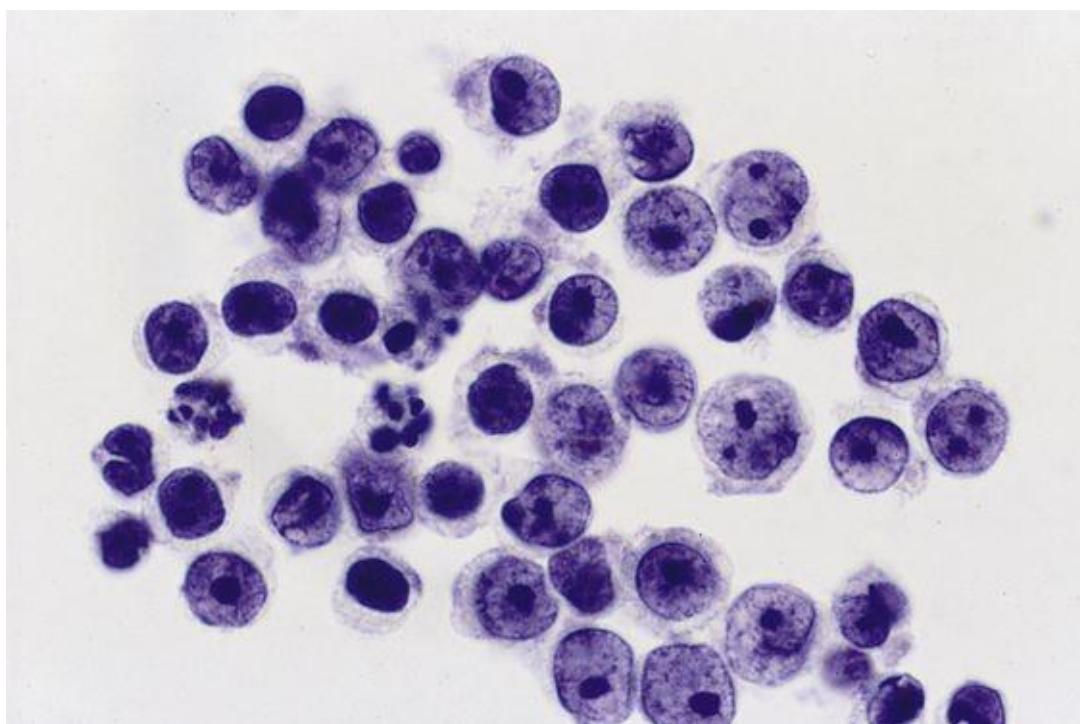


FIGURE 4.33 Diffuse large B-cell lymphoma (DLBL) (peritoneal fluid).

The lymphoma cells have predominantly round nuclei with prominent nucleoli. Note the karyopyknosis and karyorrhexis, characteristic of most lymphomas (Papanicolaou stain).

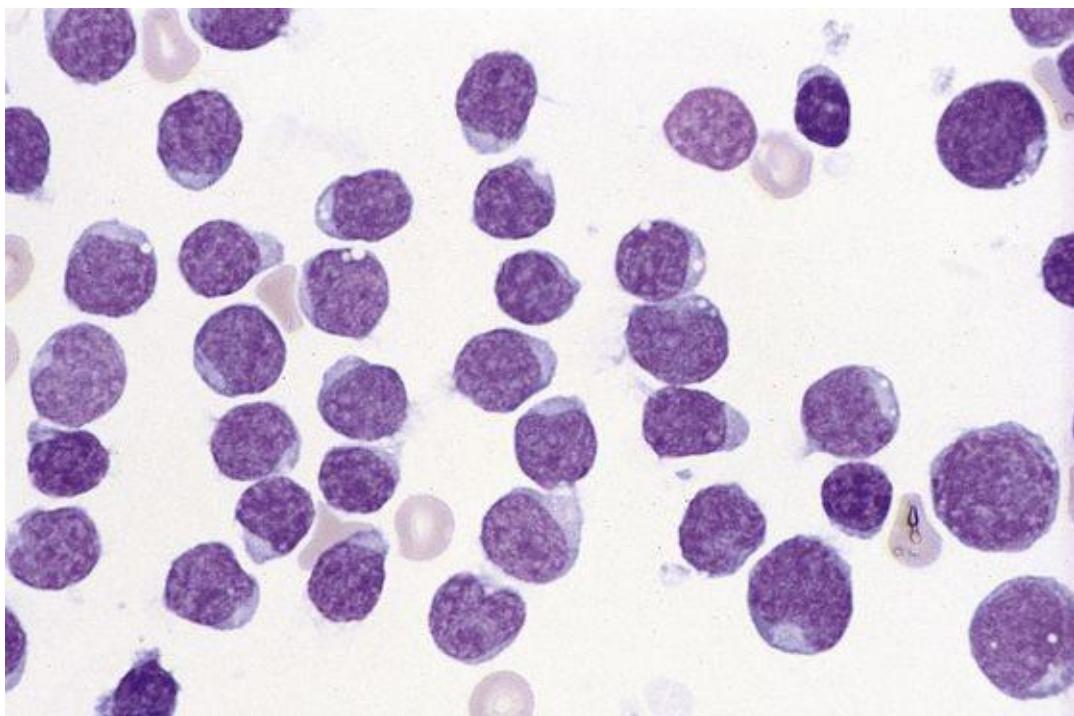


FIGURE 4.34 Lymphoblastic lymphoma.

Because lymphoblastic lymphoma cells are only slightly larger than normal lymphocytes, they are easily misdiagnosed. They are recognized by their more finely dispersed chromatin texture, better appreciated on air-dried preparations (Romanowsky stain).

Karyorrhexis is a conspicuous feature of many lymphomas (see [Fig. 4.33](#)). It is seen in both treated and untreated patients and is an uncommon finding in benign effusions or malignant effusions due to other tumors.

Precise classification of the lymphoma based on its morphology is rarely necessary with effusions because most patients have biopsy-proven disease before the effusion develops. Nevertheless, some degree of subclassification is usually possible based on the size of the cells, the degree of nuclear membrane irregularity (cleaved or noncleaved), and whether the cells resemble lymphoblasts or Burkitt cells. Such features can be appreciated especially well on air-dried Romanowsky-stained preparations (see [Fig. 4.34](#)).

● **Differential diagnosis of secondary involvement by lymphoma**

- primary effusion lymphoma
- benign lymphocytic effusion
- posttransplant lymphoproliferative disorder
- carcinoma
- mesothelioma

- melanoma
- small round blue-cell tumors

Secondary involvement by a large cell non-Hodgkin lymphoma like DLBCL or ALCL can be morphologically indistinguishable from a PEL. The absence of a documented primary or of any mass lesion, lymphadenopathy, or organomegaly in an immunocompromised patient should raise the possibility of PEL, which can be confirmed by demonstrating the presence of HHV-8. The differential diagnosis of secondary involvement by a lymphoma composed predominantly of small cells (e.g., lymphoblastic lymphoma) includes a benign lymphocytic effusion. The diagnosis of tuberculosis should be considered if there are suspicious clinical findings and the fluid is composed predominantly of small mature lymphocytes. The cells of small lymphocytic lymphoma are virtually impossible to distinguish from small, mature lymphocytes with Papanicolaou-stained preparations, even with the help of computer-assisted morphometry.¹⁰⁹ Given that serosal involvement by small lymphocytic lymphoma (or its leukemic counterpart, chronic lymphocytic leukemia) is a highly rare event (some authors who have reviewed large series of malignant lymphomas could find no autopsy-documented cases),¹¹⁰ it is wise to document immunoglobulin light chain restriction before rendering a diagnosis of malignancy. κ and λ light chain expression can be examined on cytocentrifuge preparations using immunocytochemistry⁹ or by flow cytometry.¹⁷ With immunocytochemistry, clonal excess is determined by visual inspection of the proportion of B cells that express κ or λ light chains ([Fig. 4.35A and B](#)). This method is a useful adjunct for the cytologic evaluation of lymphocyte-rich effusions that are cytologically equivocal for malignancy.¹⁸ In the case of the less common T-cell lymphoma, a T-cell phenotype with aberrant markers can be demonstrated by immunocytochemistry or flow cytometry, confirming a malignant diagnosis.⁹

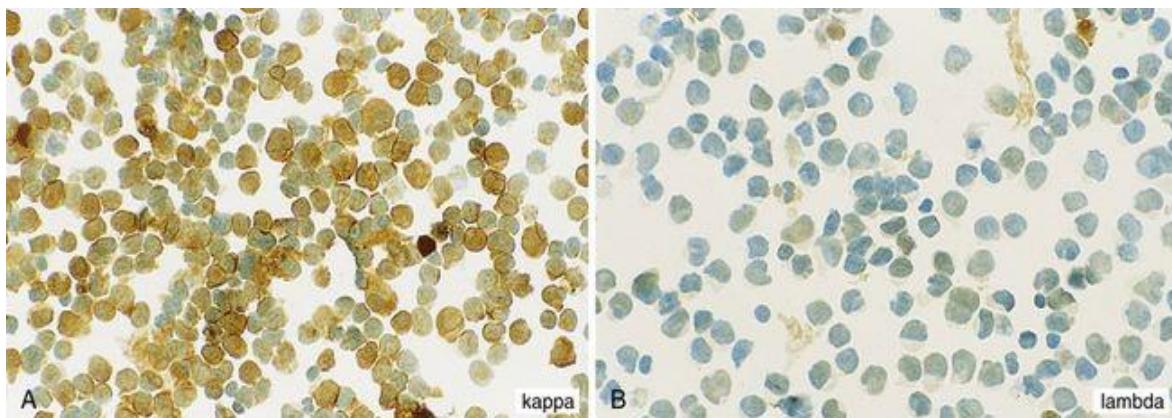


FIGURE 4.35 Non-Hodgkin lymphoma.

In equivocal cases, immunostains for immunoglobulin light chains, performed on cyt centrifuge preparations, can be helpful. Here the malignant lymphoid cells are immunoreactive for κ light chains (A) and negative for λ (B).

In a transplant recipient, the possibility of a PTLD must be considered whenever an effusion contains a prominent lymphoid population. PTLDs are associated with EBV, best demonstrated by EBER *in situ* hybridization, and are negative for HHV-8.

Lymphomas are rarely confused with other malignancies, because other tumors tend to form cell clusters in effusions. In some preparations, a crowding of the lymphoma cells mimics cluster formation, but such artifactual clustering is usually recognizable for what it is. Conversely, some carcinomas, mesotheliomas, and melanomas shed in a noncohesive pattern and can resemble the cells of a large-cell lymphoma (see [Figs. 4.10C](#) and [4.21](#)). If there is any doubt concerning the lymphoid nature of a malignant effusion, a panel of immunomarkers (e.g., leukocyte common antigen, keratin proteins, calretinin, WT1, TTF-1, S-100, HMB-45) can be helpful. Similarly, small round blue-cell tumors (e.g., neuroblastoma, Ewing sarcoma/PNET) can be ruled out with the appropriate immunohistochemical panel.

Hodgkin Lymphoma

Patients with Hodgkin lymphoma can develop benign and malignant effusions. The more common benign effusions are likely due to thoracic duct obstruction or impaired lymphatic drainage due to tumor that is nearby but not directly involving the serosal surface. Malignant effusions are relatively uncommon and are almost never the initial manifestation of the disease.

The cytologic hallmark is the Reed-Sternberg cell, a large, multinucleated cell with huge inclusion-like nucleoli ([Fig. 4.36](#)). Mononuclear variants are often

present, together with a mixed population of inflammatory cells that includes lymphocytes, plasma cells, eosinophils, neutrophils, and histiocytes.

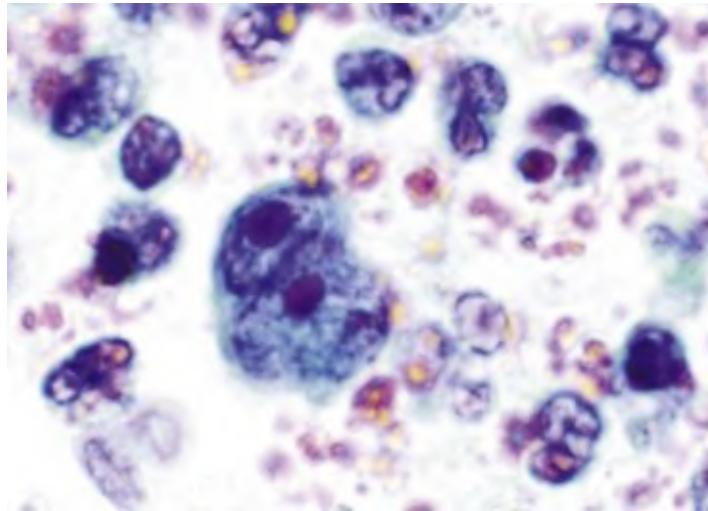


FIGURE 4.36 Hodgkin lymphoma (pleural fluid).

The classic Reed-Sternberg cell is a large, multinucleated cell with very prominent ("owl's eye") nucleoli (Papanicolaou stain).

In a patient with a history of Hodgkin lymphoma, a fluid composed of a mixed population of inflammatory cells but without Reed-Sternberg cells is considered suggestive of malignancy.

Multiple Myeloma

Multiple myeloma rarely involves the serosal cavities. The pleura is involved more commonly than the peritoneum or pericardium. Pleural involvement is sometimes a direct extension of the tumor from an erosive rib lesion.

The degree of plasmacytoid differentiation varies from one tumor to another. The cells are large and isolated. Nuclei are round and have coarsely textured chromatin with prominent nucleoli. Cytoplasm is usually abundant, nuclei are eccentrically positioned within the cell, and there is a perinuclear clear zone ([Fig. 4.37A](#)). The cells of poorly differentiated myelomas have less cytoplasm and vary in size and shape.¹¹¹

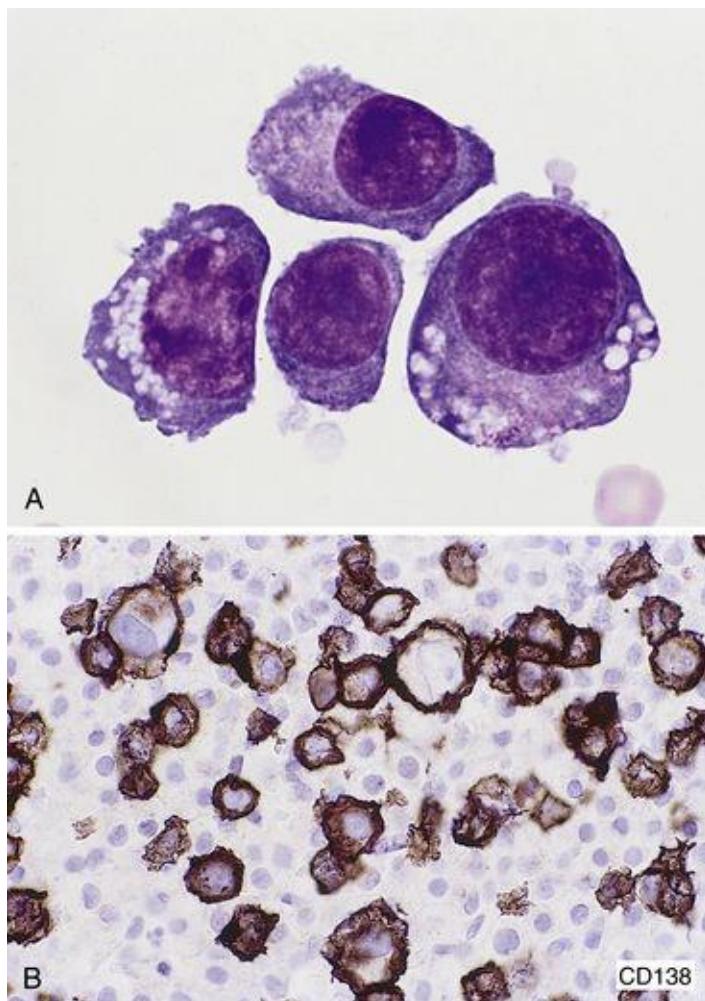


FIGURE 4.37 Multiple myeloma (pleural fluid).

A, The fluid is composed almost exclusively of malignant plasma cells with abundant cytoplasm and eccentrically placed nuclei (Romanowsky stain). B, The malignant cells are immunoreactive for CD138.

Immunocytochemistry reveals monotypic expression of either κ or λ light chains. Cell block sections are particularly well suited because the immunoglobulin is cytoplasmic in these tumors. Curiously, although the cells are negative for keratin proteins, they often express EMA.¹¹ They are also positive for the plasma cell marker CD138 ([Fig. 4.37B](#)).

Acute and Chronic Leukemias

Acute lymphoblastic and myeloblastic leukemias occasionally involve the serosal surfaces. Blasts are round, two or three times the diameter of lymphocytes, and dispersed as isolated cells. The nuclei are round or irregularly

shaped. The chromatin is pale and finely dispersed, and nucleoli are usually prominent.

Lymphoblasts cannot be distinguished from myeloblasts on Papanicolaou-stained preparations, but this is rarely if ever important, because the type of leukemia has usually been determined long before the patient develops an effusion. Auer rods, pink, rodlike structures in the cytoplasm, are diagnostic of myeloid differentiation and can be appreciated on air-dried Romanowsky-stained preparations.

The cells of chronic lymphocytic leukemia are indistinguishable from small, mature lymphocytes. Thus, immunophenotyping is necessary to establish this diagnosis.

In patients with circulating blasts, the possibility that the fluid was contaminated by peripheral blood during a traumatic tap is likely if the specimen contains red blood cells.

Myeloproliferative Neoplasms

Myeloproliferative lesions like primary myelofibrosis can lead to extramedullary hematopoiesis (EMH). The most common sites of EMH in patients with primary myelofibrosis (also called *chronic idiopathic myelofibrosis*, or *agnogenic myeloid metaplasia*) are the spleen and liver, but the pleura and other organs can also be involved.^{112,113} Megakaryocytes are the most conspicuous component of EMH and readily seen on alcohol-fixed, Papanicolaou-stained preparations and cell block sections.

In general, megakaryocytes are rare in pleural and peritoneal effusions and thus usually represent an unexpected and often puzzling finding¹¹⁴ (Fig. 4.38). Because of their large size and unusual morphology, they mimic malignant cells. Their identification can be aided with immunocytochemistry for the megakaryocyte marker CD61.

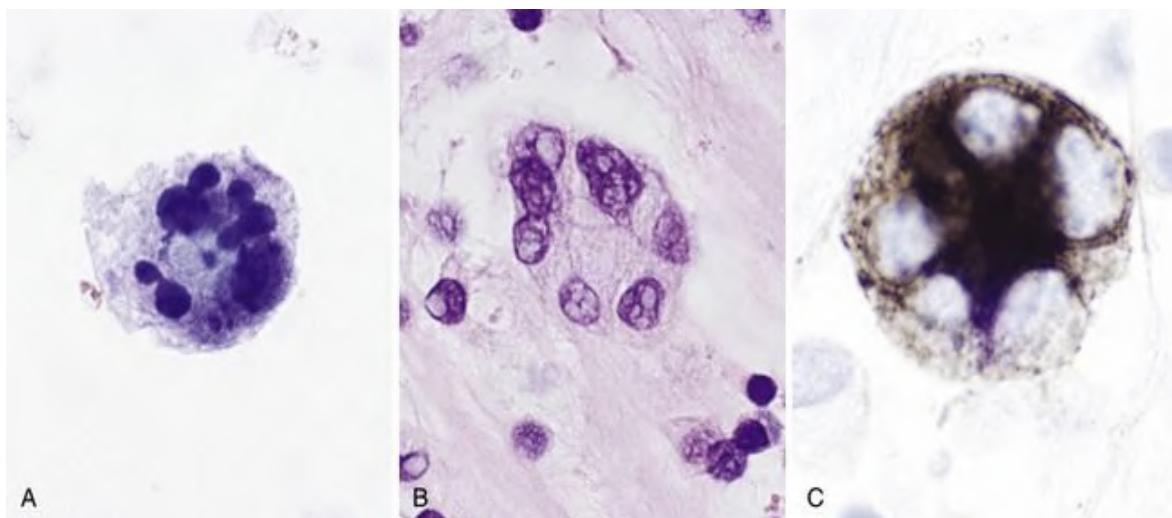


FIGURE 4.38 Extramedullary hematopoiesis (EMH) of pleura (pleural fluid).

A, This patient had a recent diagnosis of a squamous cell carcinoma (SQC) of the lung and developed a pleural effusion. The sample contained scattered megakaryocytes. A history of primary myelofibrosis was uncovered in the electronic medical record (Papanicolaou stain). B, The characteristic lobulated nucleus with threadlike connections between lobes is apparent (hematoxylin-eosin [H & E] stain). C, Megakaryocytes are immunoreactive for CD61. (The fluid was negative for metastatic SQC.)

Sarcomas

Virtually any sarcoma can metastasize to the serosal surfaces, although they do so much less frequently than other tumors. When they do, it is usually late in the course of the disease. Those seen in effusions range widely in morphology and include spindle cell sarcomas like leiomyosarcoma, synovial sarcoma, and malignant nerve sheath tumors; epithelioid sarcomas like epithelioid angiosarcoma; and small round-cell sarcomas like Ewing sarcoma/PNET, neuroblastoma, and embryonal rhabdomyosarcoma.⁵²

Not surprisingly, the cytomorphology of sarcomas in effusions is highly variable, depending on the sarcoma involved. The cells can be cohesive ([Fig. 4.39A](#); see also [Fig. 4.18](#)) or noncohesive ([Fig. 4.39B](#)), small or large. Small round-cell sarcomas like embryonal rhabdomyosarcoma exfoliate predominantly as dispersed cells, mimicking the pattern of a non-Hodgkin lymphoma. Immunocytochemistry is helpful in resolving equivocal cases.

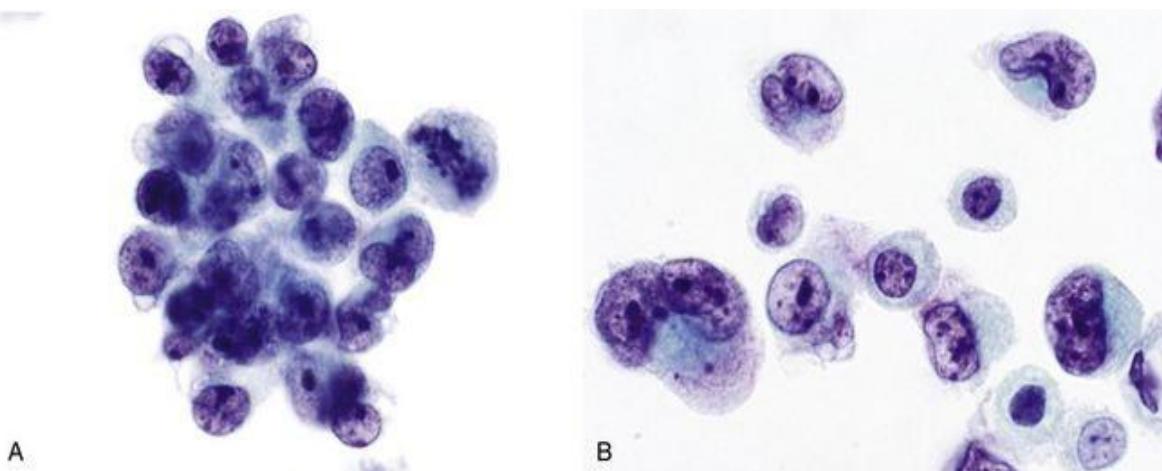


FIGURE 4.39 *A*, Epithelioid angiosarcoma (peritoneal fluid).

The patient had a history of squamous cell carcinoma (SQC) of the cervix. The new diagnosis of an intraabdominal angiosarcoma was made on this fluid and confirmed by positive staining for the vascular markers ERG and CD31. **B, Pleomorphic rhabdomyosarcoma (peritoneal fluid).** The malignant cells are noncohesive. Binucleation, eccentrically placed nuclei, and dense cytoplasm are typical of rhabdomyosarcoma. The cells were negative for keratin but positive for desmin (Papanicolaou stain).

Germ Cell Tumors

Like sarcomas, germ cell tumors rarely cause malignant effusions. When they do, it is usually late in the course of widely disseminated disease.

The *seminoma* of the testis, and its counterpart, the *dysgerminoma* of the ovary, are composed of noncohesive, uniform cells that resemble enlarged mesothelial cells. They have less cytoplasm than mesothelial cells, however, and more prominent nucleoli (see [Fig. 5.15](#)). Dysgerminoma/seminoma can be distinguished from embryonal carcinoma because the former has a characteristic membranous staining pattern for CD117.

Nonseminomatous germ cell tumors, which include embryonal carcinoma, endodermal sinus (yolk sac) tumor, choriocarcinoma, and malignant teratoma, are composed of large pleomorphic cells with pale, finely granular chromatin and prominent, often multiple, nucleoli. A cytologic diagnosis of malignancy in this group of tumors is straightforward, but correct subtyping and distinction from a poorly differentiated epithelial malignancy require correlation with serologic markers and immunocytochemical studies. Embryonal carcinoma, yolk sac tumor, and choriocarcinoma are keratin-positive but can be distinguished from epithelial malignancies because of their staining for PLAP and SALL4. Additionally, embryonal carcinomas and dysgerminomas are immunoreactive for Oct-3/4 and NANOG.

Immunohistochemistry is rarely necessary, however, because these tumors rarely manifest themselves initially as a malignant effusion.

Rarely, *sex cord–stromal tumors* can also involve serosal surfaces and cause an effusion, sometimes many years after treatment of the primary tumor ([Fig. 4.40](#)). Immunohistochemistry for inhibin, a marker of many sex cord–stromal tumors, along with comparison of the effusion specimen with sections from the original tumor, can be indispensable for correct interpretation.

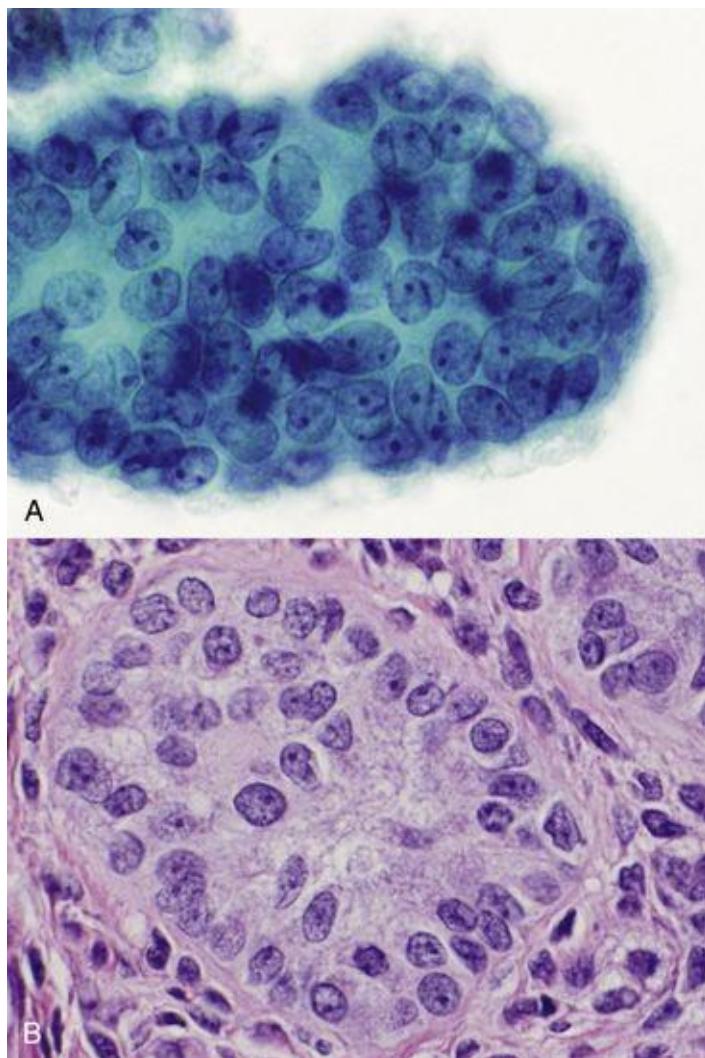


FIGURE 4.40 Adult granulosa cell tumor (ascites).

A, The malignant cells are uniform and bland and resemble benign mesothelial cells. The arrangement of cells in large clusters is suspicious, as is the prominence of nuclear grooves and focal follicle formation (Papanicolaou stain). B, Comparison with the original histologic sections was helpful in establishing the diagnosis (hematoxylin-eosin [H & E] stain).

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CHAPTER 5

Peritoneal Washings

Edmund S. Cibas

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[Monitoring Response to Treatment \('Second-Look Procedures'\)](#)

Peritoneal washing cytology (PWC) was introduced in the 1950s as a way to identify microscopic spread of cancer not visible by gross inspection of the peritoneal surface.¹ In some cancer patients, positive PWC may be the only evidence of metastatic disease to the peritoneum. Because positive results correlate with a poorer prognosis,^{2,3} cytologic findings are included in the staging systems for ovarian and fallopian tube cancers.^{4,5} The yield, however, is low: Positive washings by themselves change the surgical stage of only 3% to 5% of women with gynecologic cancers.^{6,7}

PWC is also used to exclude an occult cancer in patients who undergo laparoscopy or laparotomy for presumed benign gynecologic conditions, such as endometriosis or leiomyomata, and in women with BRCA1 and BRCA2 mutations undergoing risk-reducing salpingo-oophorectomy.⁸ PWC can be employed to monitor a patient's response to treatment for advanced ovarian

cancer and other malignancies (the “second-look” procedure), but this is usually limited to patients in research protocols.⁹⁻¹³ In some instances, PWC is used to detect peritoneal spread of nongynecologic malignancies like pancreatic and gastric cancer.



Indications for peritoneal washings (summary)

- staging gynecologic malignancies
 - ovarian
 - fallopian tube
- ruling out occult cancer
- assessing response to treatment (the “second-look” procedure)
- staging nongynecologic malignancies
 - pancreatic
 - gastric

Specimen Collection, Preparation, and Reporting Terminology

On entering the peritoneal cavity, the surgeon evacuates any preexisting peritoneal fluid and submits it separately for cytologic examination. Washings are obtained by instilling 50 to 200 mL of sterile saline or other physiologic solution into several different areas, usually the pelvis, the right and left paracolic gutters, and the undersurface of the diaphragm. The fluid is aspirated, and heparin is added to prevent clotting. Segregating washings from different sites does not have any advantage over combining them into a single specimen.¹⁴

The specimen should be delivered to the laboratory unfixed and refrigerated at 4° C until slides can be prepared. If a significant delay before cytopreparation is anticipated, an equal volume of 50% ethanol can be added. To prepare slides, the specimen is thoroughly mixed, and an aliquot (often 50 mL) is centrifuged to a cell sediment/pellet. From this sediment one can prepare smears, cytocentrifuge preparations, or thinlayer preparations, depending on the resources and preferences of the laboratory.¹⁵ The remaining (or separately centrifuged) cell sediment can also be fixed in 10% formalin, embedded in paraffin, and processed as a histologic specimen (“cell block”). Cell block sections are very useful, especially for morphologic comparison to the patient’s resected neoplasm.¹⁶

Results of PWC are commonly reported as “negative,” “atypical,” “suspicious,” or “positive.”¹⁷ “Atypical” (connoting a low degree of suspicion for malignancy) and “suspicious” (connoting a high degree of suspicion) interpretations should be avoided whenever possible, because they are not helpful to a physician faced with making a treatment decision.^{15,18} In most cases, only an unequivocally positive diagnosis is used for staging purposes—anything less is treated as a negative result. Side-by-side comparison with the corresponding resection specimen often helps resolve an equivocal case.

Criteria for specimen adequacy have not been established, but it seems reasonable to require some benign mesothelial cells before considering a peritoneal washing specimen adequate and negative for malignant cells.¹⁹ A specimen with malignant cells is always adequate.

Because peritoneal washings are obtained as part of a cancer staging procedure, there is usually a concurrent histologic specimen. For example, when washings are obtained as part of ovarian cancer staging, an oophorectomy is obtained by the laboratory at the same time. Representative slides from the

oophorectomy specimen can be helpful in a side-by-side comparison of a diagnostically difficult washing specimen.

Accuracy

Not all patients with metastases to the peritoneum have positive PWC results. In fact, 23% to 52% of patients with biopsy-proven peritoneal involvement have negative results.^{17,20-23} When peritoneal washings are examined as part of a second-look procedure, the false-negative rate is even higher, ranging from 31% to 86% of patients with biopsy-proven metastases.^{9,11,21,22,24-26} The higher false-negative rate of second-look procedures may be due to the poor distribution of fluid when the abdominal cavity is altered by adhesions.

False-positive diagnoses are uncommon but well documented,^{2,3,21,27-29} occurring in less than 5% of cases.^{2,23,30} Causes include reactive mesothelial proliferation with psammoma bodies^{23,27,28} and endometriosis.^{22,29} In particular, eosinophilic metaplastic atypia in endometriosis can be a source of atypical (but benign) cells that might be misconstrued as malignant.³¹ In one instance, ectopic pancreatic tissue was misinterpreted as adenocarcinoma in a patient with a mucinous ovarian cancer.³²

The Normal Peritoneal Washing

Peritoneal washings differ morphologically from peritoneal (ascitic) fluids in several easily recognizable ways. First, the washing procedure mechanically strips the peritoneal surface of entire sheets of mesothelial cells, whereas sheets of mesothelial cells are not seen in a benign ascitic fluid. Second, skeletal muscle and adipose tissue fragments are common in peritoneal washings, especially in cell block preparations.



Cytomorphology of benign peritoneal washings

- mesothelial cells in sheets
- collagen balls
- histiocytes
- skeletal muscle
- adipose tissue

Mesothelial cells in peritoneal washings are arranged predominantly in flat sheets, often large and occasionally folded ([Fig. 5.1](#)). In cell block sections the sheets are transected and appear as long, thin ribbons ([Fig. 5.2](#)). The cells are evenly spaced, with a moderate amount of cytoplasm. Nuclear membranes are thin, and the chromatin is pale and evenly dispersed; small nucleoli are often present. Although usually round or oval ([Fig. 5.3](#)), the nuclei are sometimes scalloped, wrinkled, or grooved,³³ possibly due to fixation artifact. Isolated mesothelial cells, similar to those seen in ascites, are also encountered.

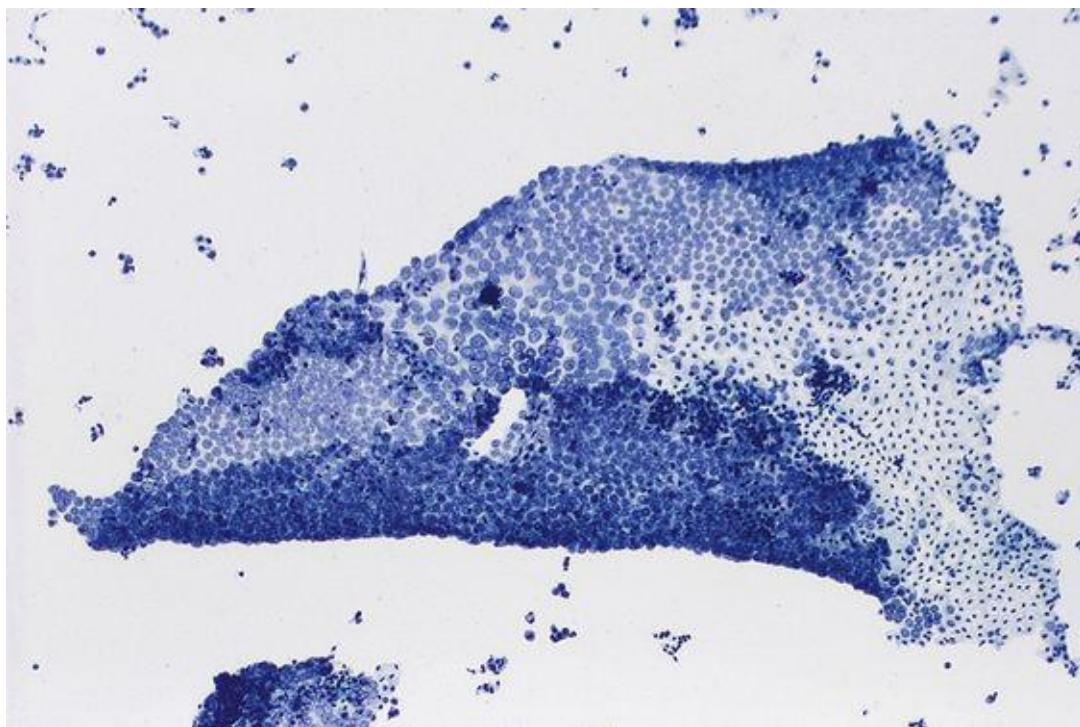


FIGURE 5.1 Mesothelial cells.

Normal mesothelial cells in peritoneal washings are often arranged in cohesive sheets. Although flat, the sheets can be folded on cyt centrifuge or thinlayer preparations. Isolated mesothelial cells are present in the background (Papanicolaou stain).

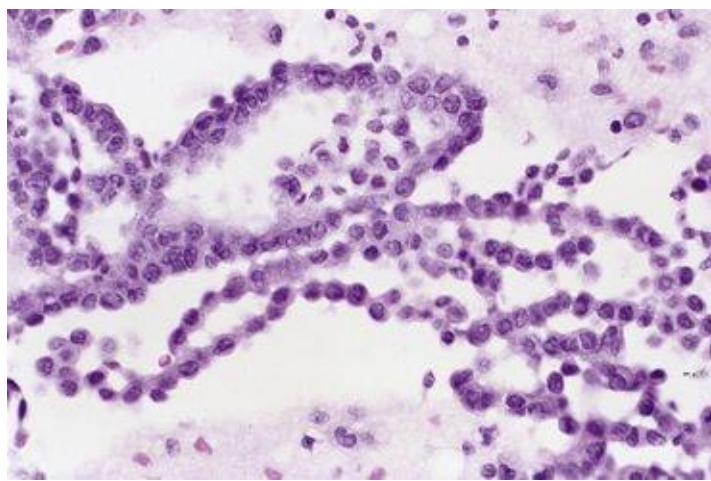


FIGURE 5.2 Mesothelial cells (cell block).

In cell block sections, a sheet of mesothelial cells is transected and looks like a string of pearls. When it curls around on itself it has a pseudoglandular appearance (hematoxylin-eosin [H & E] stain).

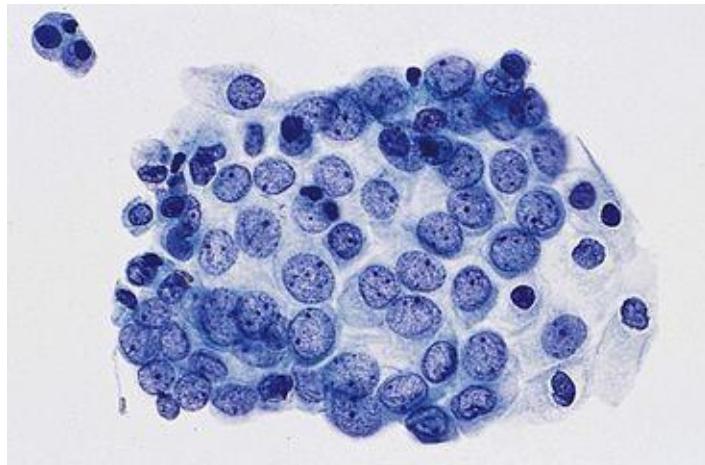


FIGURE 5.3 Mesothelial cells.

Usually, benign mesothelial cells have round or oval nuclei, but in some cases there are irregularities in nuclear contour. Note the spaces (“windows”) that separate adjacent cells, a common finding (Papanicolaou stain).

Spherical masses of collagen surrounded by benign, flattened mesothelial cells, known as *collagen balls*, are seen in up to 50% of peritoneal washings.^{33,34} Aqua in color with the Papanicolaou stain, they have round or bosselated contours (Fig. 5.4). They are usually few in number, but occasionally they can be abundant. They have no known significance (and therefore do not deserve mention on a cytology report). It has been suggested that they result from a pinching off of mesothelial-lined stromal projections known as *micropapillomatosis* on the surface of the ovaries.^{34,35}

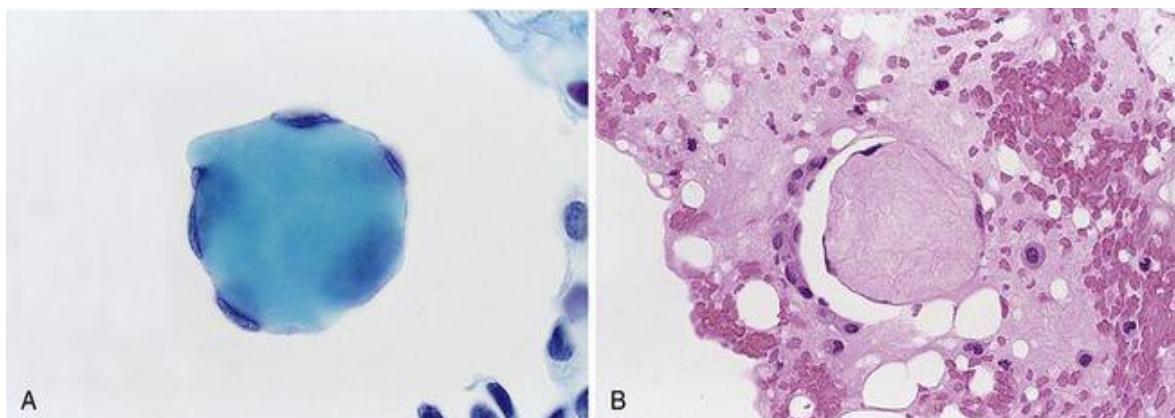


FIGURE 5.4 Collagen ball.

These spheres of collagen are surrounded by flattened mesothelial cells. A, Papanicolaou stain; B, hematoxylin-eosin (H & E)-stained cell block section.

Numerous *histiocytes*, scattered either as isolated cells or in variably sized aggregates, are often present ([Fig. 5.5](#)). Because they look different from mesothelial cells and are often haphazardly aggregated, they might be misinterpreted as metastatic cancer cells. Attention to the typical nuclear and cytoplasmic features of histiocytes (oval, folded, and kidney-shaped nuclei; pale chromatin; granular and microvacuolated cytoplasm) is helpful in correctly identifying them. *Skeletal muscle* and *adipose tissue* fragments are sometimes seen. (They fall into the peritoneal cavity when the abdominal incision is made and are suctioned along with the fluid.) *Detached ciliary tufts*, presumably of fallopian tube origin, are a relatively common incidental finding, especially when the washings are obtained during the secretory (luteal) phase of the menstrual cycle.^{[36](#)}

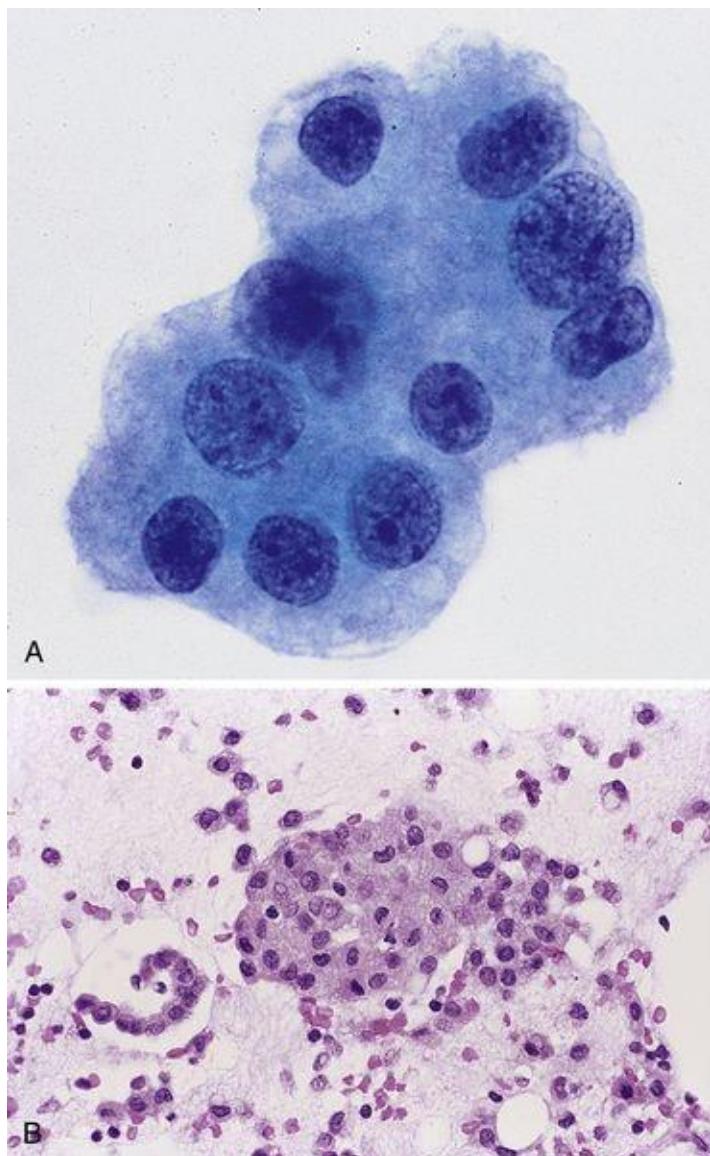


FIGURE 5.5 Histiocytes.

Histiocytes are often in groups, as seen here. They have indistinct cell borders (no “windows”), granular or vacuolated cytoplasm, and occasionally folded nuclei. A short strip of mesothelial cells is also present on the cell block section. *A*, Papanicolaou stain; *B*, hematoxylin-eosin (H & E)-stained cell block.

A typical interpretation of a benign peritoneal washing might read as follows: “negative for malignant cells; mesothelial cells and histiocytes.”

Benign Conditions

Endosalpingiosis and Similar Benign Proliferations

A group of benign conditions involving the peritoneal and ovarian surfaces can result in a proliferation of fallopian tubal-type epithelial cells or mesothelial cells, often with psammoma bodies. These conditions mimic peritoneal involvement by a serous borderline tumor and serous adenocarcinoma; familiarity with them is thus important.

Endosalpingiosis is a proliferation of benign glands and cysts lined by ciliated, fallopian tube-like epithelium. Common locations include the ovarian cortex, uterine serosa, peritoneal surface, and omentum. Psammoma bodies are often seen. Some authors reserve the term endosalpingiosis for proliferations that include tubal-type stroma as well as glands, and use the term *müllerian inclusion cysts* for cases that lack tubal-type stroma.³⁵ Both endosalpingiosis and müllerian inclusion cysts, however, are usually incidental, microscopic findings in a patient undergoing surgery for something else. Histologically, they raise the possibility of metastatic disease but are identified as benign proliferations because the cells are uniform and bland and lack mitotic activity.

Serous adenofibromas of the ovarian surface are benign ovarian tumors that, like endosalpingiosis, are composed of benign tubal-type glands and/or cysts, except that they are often a visible mass rather than a microscopic finding, and they have a broad fibrous stromal component. The epithelial portion of a serous adenofibroma, like endosalpingiosis, often contains psammoma bodies and can be confused with peritoneal involvement by a serous borderline tumor or even adenocarcinoma.

Prior surgery, pelvic inflammatory disease, a ruptured cyst, and other conditions cause *florid mesothelial hyperplasia*, sometimes accompanied by psammoma body formation.¹⁵



Cytomorphology of endosalpingiosis and similar benign proliferations

- cuboidal cells (\pm cilia) with minimal to mild atypia
- psammoma bodies

The conditions described above all have a similar appearance in peritoneal washings. Clusters of cuboidal mesothelial-like cells ([Fig. 5.6](#)), some arranged around a psammoma body ([Fig. 5.7](#) and [Fig. 5.8](#)) or nondescript calcification, are present and can be abundant.²⁸ Cilia may be present, but often they are absent or impossible to identify. Nuclei are round or oval, with a pale chromatin pattern and small nucleoli. Nuclear atypia is mild, and mitoses are very uncommon.

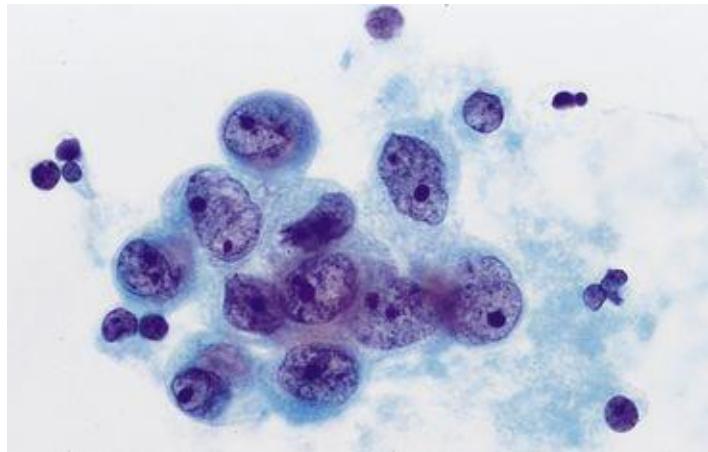


FIGURE 5.6 Reactive mesothelial cells (ovarian torsion).

The mesothelial cells are enlarged, with irregular nuclei and prominent nucleoli. Exploratory laparotomy revealed torsion of the right ovary with intraperitoneal adhesions.

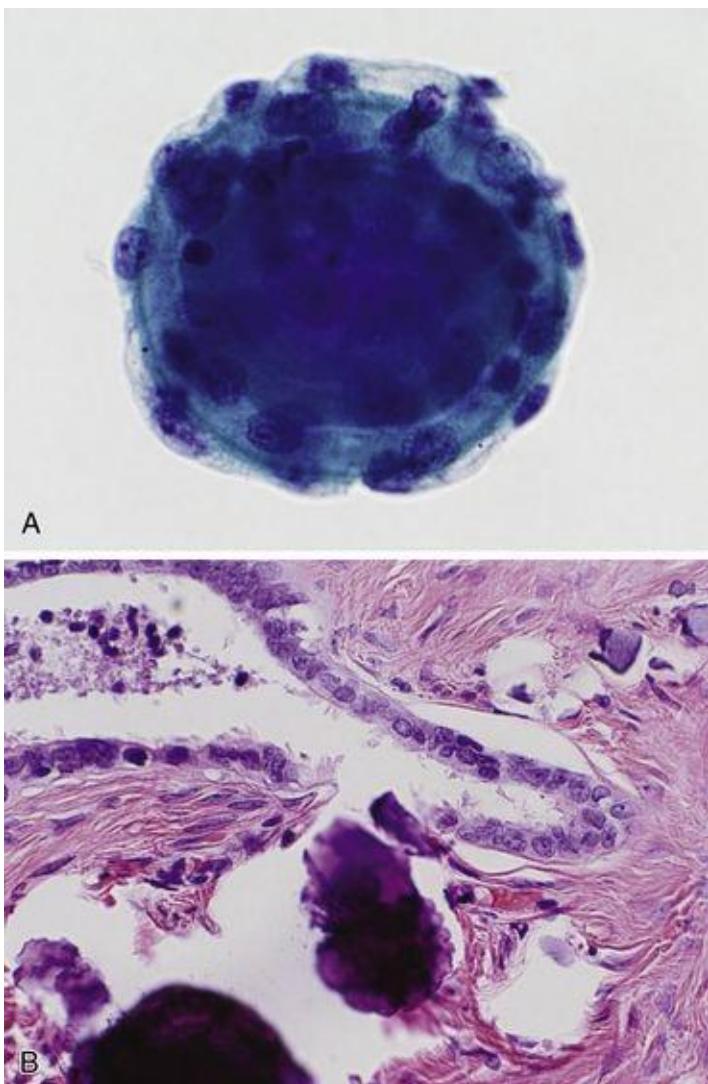


FIGURE 5.7 Endosalpingiosis.

A, The psammoma body has concentric lamination and is surrounded by cuboidal cells with small nucleoli and vacuolated cytoplasm. Cilia are not identified (Papanicolaou stain). B, The surface of the resected ovary shows benign ciliated glandular cells associated with psammoma bodies. There was no evidence of tumor (hematoxylin-eosin [H & E] stain).

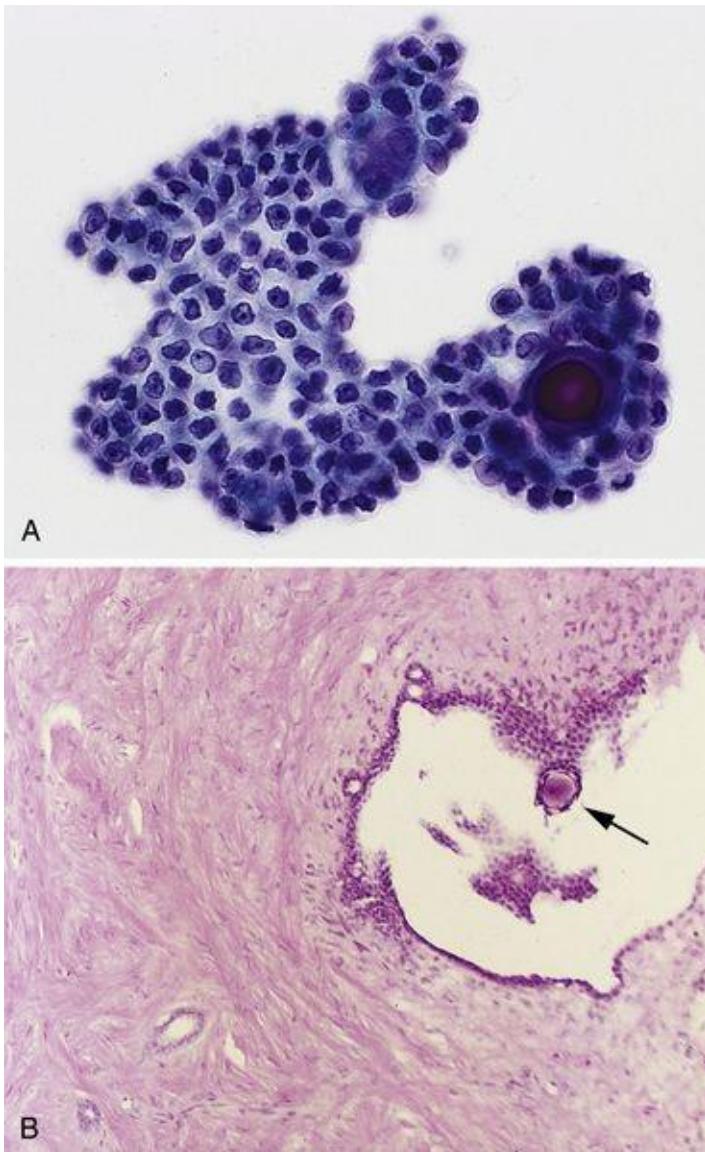


FIGURE 5.8 Serous adenofibroma of the ovary.

A, A psammoma body is surrounded by evenly spaced mesothelial cells with minimal nuclear atypia (Papanicolaou stain). B, The resected ovary shows a serous adenofibroma involving the ovarian surface, with an occasional psammoma body (arrow) (hematoxylin-eosin [H & E] stain).

These benign conditions are potential causes of a false-positive interpretation of involvement by a serous adenocarcinoma or borderline tumor.²⁷ To avoid a false-positive interpretation, a diagnosis of malignancy should not be based on the presence of psammoma bodies alone.^{22,27} Correlation with the concurrent histologic material is helpful in most cases. Certainly, if the patient does not have an adenocarcinoma or borderline tumor, one should avoid rendering such a diagnosis on the basis of the peritoneal washings alone. Some patients with endosalpingiosis or benign mesothelial proliferations, however, can also have a

serous borderline tumor confined to the ovary.¹⁸ Comparison of the peritoneal washings, particularly cell block preparations, with the histologic sections from the tumor often resolves equivocal cases, but the final interpretation is not always straightforward, and the pathologist is sometimes required to make the best judgment possible. There are few helpful morphologic clues, particularly because many serous borderline tumors have only mild cytologic atypia, equivalent to what can be seen with florid mesothelial hyperplasia and endosalpingiosis. One possible clue: In cell block sections, some papillary fragments of serous borderline tumors have broad stromal cores, a feature not seen in cell blocks containing only mesothelial hyperplasia or endosalpingiosis.

Endometriosis

Endometriosis, the presence of ectopic benign endometrial glands and stroma in the omentum, peritoneum, ovary, and elsewhere, is another potential pitfall in the evaluation of peritoneal washings.^{22,29}



Cytomorphology of endometriosis

- hemosiderin-laden macrophages
- endometrial glandular cells
- endometrial stromal cells
- tissue fragments containing endometrial glands and stroma (cell blocks)

The purpose of peritoneal washings in this setting is to exclude an occult malignancy, not to diagnose endometriosis. A diagnosis of endometriosis, in fact, can rarely be made by examining peritoneal washings alone. Hemosiderin-laden macrophages are seen in one third of women with endometriosis.³⁷ In a young woman, hemosiderin-laden macrophages are most likely due to endometriosis,³⁷ but by themselves they are a nonspecific finding seen in any condition associated with intraperitoneal bleeding. Some cases of endometriosis show a population of cells morphologically similar to but distinct (in subtle ways) from mesothelial cells and macrophages; these likely represent endometrial glandular and/or stromal cells. The endometrial-like glandular and/or stromal cells may, in fact, resemble the clusters of exfoliated endometrial cells seen in menstrual-phase

cervical-vaginal preparations ([Fig. 5.9A](#)), but a definitive distinction between mesothelial cells and endometrial cells is difficult.³⁷ On rare occasions, a diagnosis can be made when tissue fragments with endometrial-type glands and stroma are identified in cell block sections ([Fig. 5.9B and C](#)).

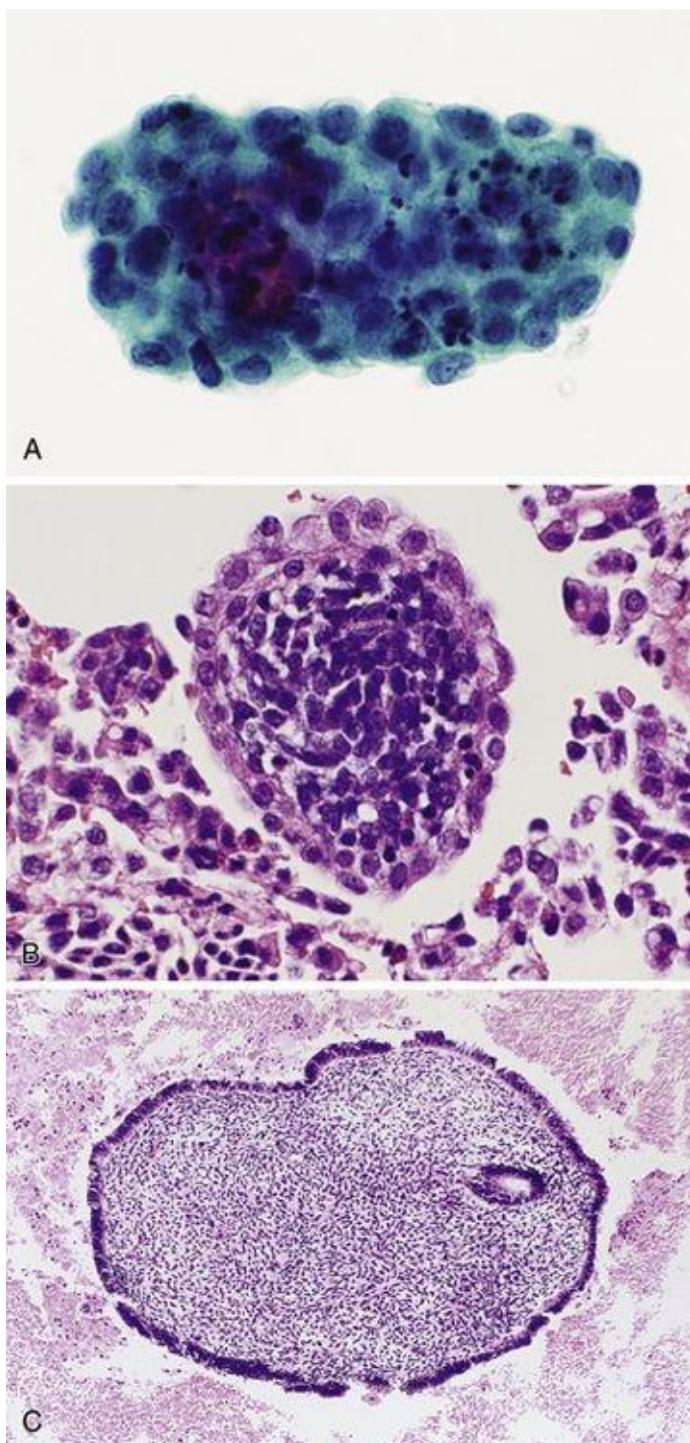


FIGURE 5.9 Endometriosis.

A, A cluster of cells has scalloped borders and contains nuclear fragments. Individually, the cells resemble mesothelial cells, but the nuclear debris suggests that they are degenerating endometrial cells. Such clusters are often recognized as endometrial cells only in retrospect, after reviewing cell block sections or correlating with laparoscopic or histologic evidence (Papanicolaou stain). *B*, The cell block from the same case as *A* shows endometrial glands surrounding a core of degenerated endometrial stromal cells (hematoxylin-eosin [H & E] stain). *C*, Occasionally, the cell block of peritoneal washings from a patient with endometriosis

shows diagnostic tissue fragments composed of intact endometrial-type glands and stroma (hematoxylin-eosin [H & E] stain).

Occasionally, the endometrial glandular cells are enlarged and atypical and might suggest a malignancy. Correlation with concurrent histologic material is very helpful in preventing a misdiagnosis.^{29,31}

Malignant Tumors

Ovarian Cancer

Ovarian cancer is the ninth most common cancer in women in the United States and accounts for 6% of cancer-related mortality.³⁸ The median age at diagnosis is 52 years. Most women (70% to 75%) with ovarian cancer present with stage III or IV disease (tumor spread beyond the pelvis). PWC is an important staging component; a positive finding modifies stage I and II tumors to stage Ic and IIc, respectively (Table 5.1). Primary treatment for presumed ovarian cancer is surgical staging and cytoreduction. Most women (except those with stage IA and IB, grade 1 tumors) receive postoperative chemotherapy, either systemic or intraperitoneal, depending on the stage of their disease. Platinum drugs (cisplatin, carboplatin) and taxanes (paclitaxel, docetaxel) are the most efficacious and are often used in combination. Novel strategies that target key molecular alterations are showing promise in the treatment of ovarian cancer.³⁹

TABLE 5.1
DEFINITIONS OF THE STAGES IN PRIMARY CARCINOMA OF THE OVARY

Stage I Growth limited to the ovaries

Ia Growth limited to one ovary; no ascites with malignant cells; no tumor on the external surface; capsule intact

Ib Growth limited to both ovaries; no ascites with malignant cells; no tumor on the external surfaces; capsules intact

Ic Tumor either stage Ia or Ib, but with tumor on surface of one or both ovaries; or with capsule ruptured; or with ascites present containing malignant cells or with **positive peritoneal washings**

Stage II Growth involving one or both ovaries with pelvic extension

IIa Extension and/or metastases to the uterus and/or tubes

IIb Extension to other pelvic tissues

IIc Tumor either stage IIa or IIb, but with tumor on surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with **positive peritoneal washings**

Stage III Tumor involving one or both ovaries with histologically confirmed peritoneal implants outside the pelvis and/or positive regional lymph nodes; superficial liver metastasis equals stage III;

tumor is limited to the true pelvis but with histologically proven malignant extension to small bowel or omentum

IIIa Tumor grossly limited to the true pelvis, with negative nodes, but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces or histologically proven extension to small bowel or mesentery

IIIb Tumor of one or both ovaries with histologically confirmed implants, peritoneal metastasis of abdominal peritoneal surfaces, none exceeding 2 cm in diameter; nodes are negative

IIIc Peritoneal metastasis beyond pelvis, greater than 2 cm in diameter, and/or positive regional lymph nodes

Stage IV Growth involving one or both ovaries with distant metastases; if pleural effusion is present, there must be positive cytology to allot a case to stage IV; parenchymal liver metastases equals stage IV

From: Current FIGO staging for cancer of the vagina, fallopian tube, ovary, and gestational trophoblastic neoplasia. *Int J Gynaecol Obstet* 2009;105(1):3-4.

The most common type of ovarian cancer—and the type that most often produces positive cytologic findings—is *serous adenocarcinoma*.



Cytomorphology of serous adenocarcinoma

- large or small clusters and isolated cells
- large cells
- marked variation in nuclear size
- nuclear hyperchromasia
- prominent nucleoli
- mitoses
- scant or abundant vacuolated cytoplasm

Serous adenocarcinoma is usually easy to recognize in peritoneal washings. The malignant cells are isolated and arranged in clusters ([Fig. 5.10](#)). Nuclei are enlarged and vary markedly in size. There is nuclear hyperchromasia, with coarsely textured chromatin and very prominent nucleoli ([Fig. 5.11](#)). Mitoses are common. Cytoplasm may be scant but is often abundantly vacuolated.

Psammoma bodies may be present. Morphologically identical tumors occur as primary neoplasms of the peritoneum (see [Fig. 5.11](#)).

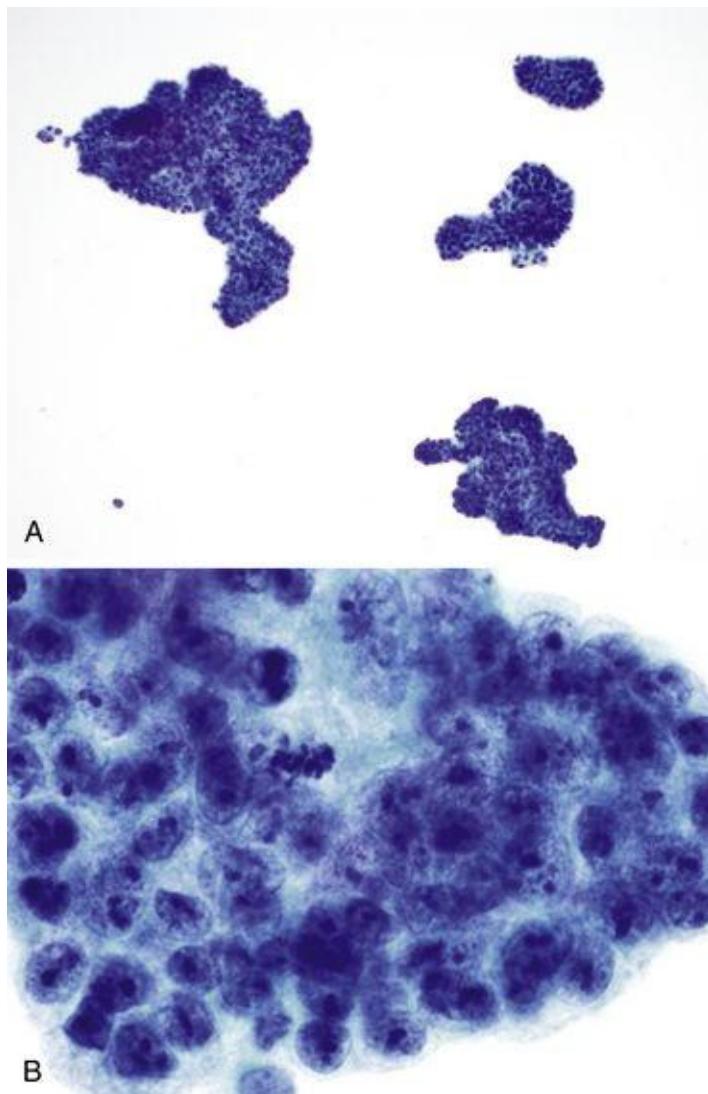


FIGURE 5.10 Serous adenocarcinoma of the ovary.

A, The malignant cells of serous carcinomas are often arranged in large, irregularly shaped three-dimensional clusters of crowded cells (Papanicolaou stain). B, Higher magnification reveals crowded large cells with round, dark nuclei; large nucleoli; and pale vacuolated cytoplasm. Mitoses are often present (Papanicolaou stain).

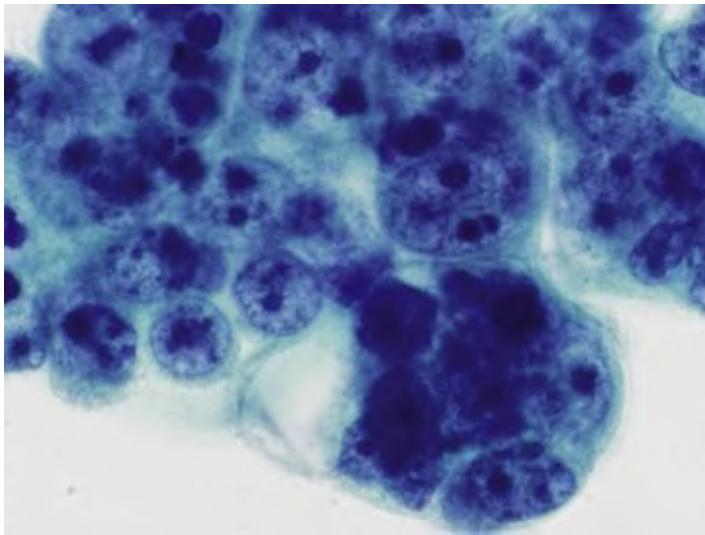


FIGURE 5.11 Serous adenocarcinoma of the peritoneum.

These tumors are morphologically indistinguishable from serous carcinomas of the ovary (Papanicolaou stain).

Serous borderline tumor is a low-grade neoplasm with a significantly better prognosis than serous adenocarcinoma (87% versus 50% 5-year survival, respectively).¹² The distinction is based on the presence or absence of stromal invasion within the primary tumor. Although the distinction cannot be made on the basis of peritoneal washings, some differences between the two diseases are apparent cytologically.^{33,40}



Cytomorphology of serous borderline tumor

- large or small clusters and isolated cells
- minimal-mild nuclear atypia
- cytoplasmic vacuoles
- psammoma bodies
- fibrovascular cores (cell block)

Positive peritoneal washings from patients with serous borderline tumors usually show large (Fig. 5.12A) or small (Fig. 5.12B), cohesive, three-dimensional, often branching clusters. Isolated cells are less conspicuous than in serous adenocarcinoma. Pleomorphism is slight or moderate. Cytoplasmic vacuoles can be seen, and the nuclear-to-cytoplasmic ratio is high. Nucleoli are inconspicuous, and mitoses are rare. As with serous adenocarcinomas,

psammoma bodies may be present. Cell blocks sometimes show thin or broad fibrovascular cores lined by neoplastic epithelium.

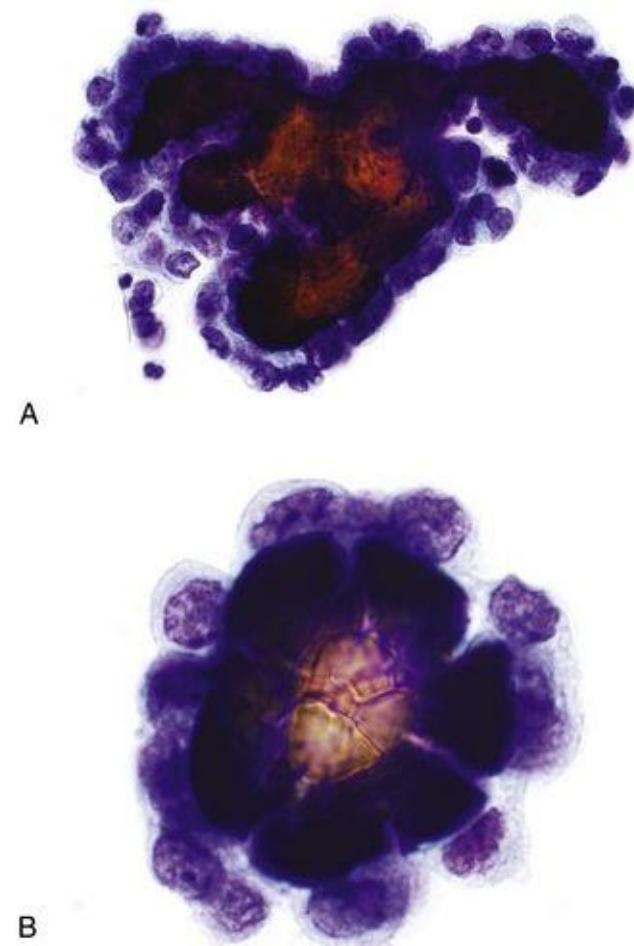


FIGURE 5.12 Serous borderline tumor.

A, Some neoplastic cells exfoliate as large branching micropapillary clusters with smoothly contoured or slightly scalloped edges. The centers are occupied by microcalcifications (Papanicolaou stain). B, A smaller cluster of cuboidal cells surrounding a cracked psammoma body from the same case demonstrates the minimal atypia characteristic of the serous borderline tumor. Biopsies showed implants of identical cells on the omentum and peritoneum. Compare with [Fig. 5.7](#): The distinction between endosalpingiosis and a borderline tumor is often very difficult (Papanicolaou stain).

The differential diagnosis, particularly for serous borderline tumors, includes endosalpingiosis and similar benign proliferations. A malignant diagnosis should never be made solely on the basis of psammoma bodies alone. The evaluation of peritoneal washings in patients with serous borderline tumors depends on a

careful comparison with the resected primary tumor. Cell block sections are especially useful in this regard. Broad fibrovascular cores lined by atypical epithelium are characteristic of serous borderline tumors and are very rarely seen in patients with endosalpingiosis or mesothelial hyperplasia ([Fig. 5.13](#)).

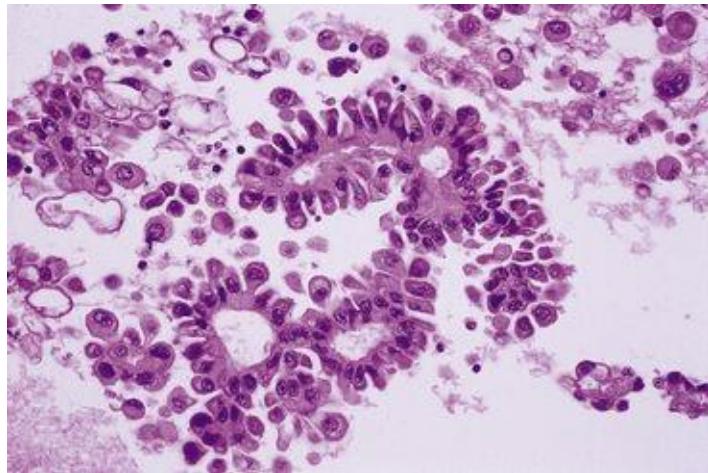


FIGURE 5.13 Serous borderline tumor.

Cell block sections are helpful because they may show papillary structures lined by atypical cells with stratification and tufting, features that are characteristic of these tumors (hematoxylin-eosin [H & E] stain).

Positive washings in a woman with a serous borderline tumor do not have the same clinical significance as in a woman with an adenocarcinoma. Postoperative chemotherapy offers no survival advantage in women with stage Ic or II serous borderline tumors, and the overall survival rate is very good even without treatment. In fact, “positive” washings in women with borderline ovarian tumors raise a question regarding terminology. Because these tumors are not, strictly speaking, carcinomas, it is preferable to report positive washings in a woman with a borderline tumor as “neoplastic cells present; consistent with involvement by the patient’s known serous borderline tumor.” The traditional phrase “positive for malignant cells” should be avoided, as it might imply that the cytologist is converting the borderline diagnosis (made after examination of the ovary) to a carcinoma diagnosis.

Less common than the serous tumors are the *mucinous*, *endometrioid*, *clear cell*, and *transitional cell tumors of the ovary*. There is significant overlap in the morphologic features of these tumors when they appear in peritoneal washings, such that distinction among them is not reliable (nor is it necessary) based on PWC. It is sufficient to report results as “positive for malignant cells; consistent

with adenocarcinoma.” Alternatively, if one holds reporting of the washings until the concurrent ovarian tumor resection has been examined and subtyped (e.g., as a clear adenocarcinoma), one can report the washings as “positive for malignant cells; consistent with clear cell adenocarcinoma.”

A mucinous tumor in the ovary, particularly one associated with pseudomyxoma peritonei, is often a metastasis (the appendix is a likely primary site) rather than a primary ovarian neoplasm ([Fig. 5.14](#)). Primary versus metastatic mucinous tumors of the ovary are difficult to distinguish by conventional histologic examination.

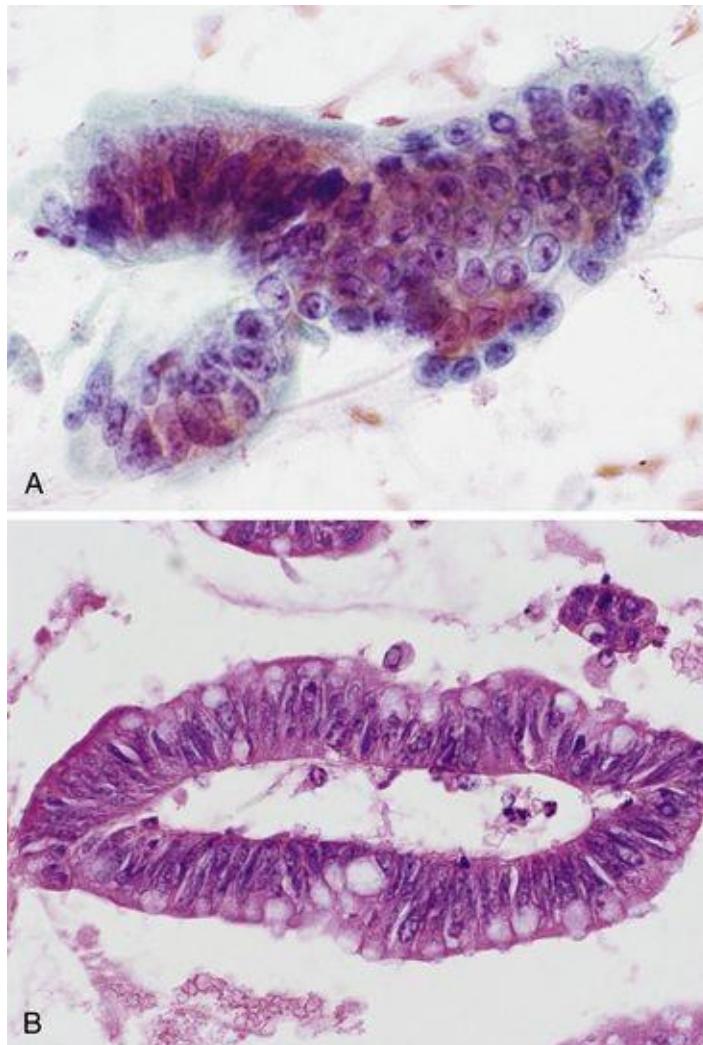


FIGURE 5.14 Mucinous adenocarcinoma of the appendix mimicking an ovarian mucinous adenocarcinoma.

The tumor cells are columnar, with elongated nuclei and a high nuclear to cytoplasmic ratio. The patient presented with pseudomyxoma peritonei and was found to have bilateral ovarian

metastases. A, Papanicolaou stain; B, hematoxylin-eosin (H & E)-stained cell block.

Germ cell (Figs. 5.15 and 5.16) and sex cord-stromal tumors of the ovaries can also involve peritoneal surfaces.⁴¹

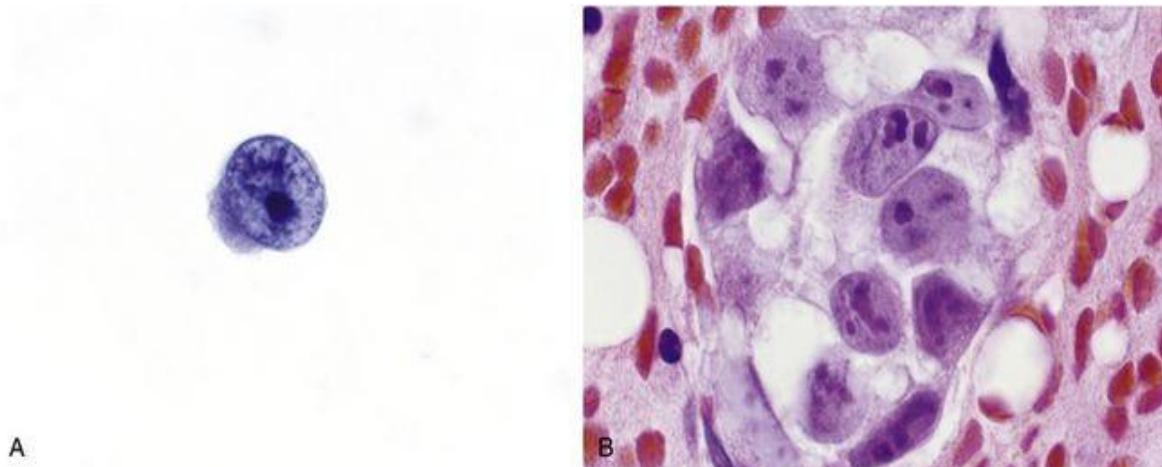


FIGURE 5.15 Dysgerminoma of the ovary.

A, The cells are large and relatively noncohesive, with a round, vesicular nucleus. There may be a single prominent nucleolus or multiple, irregular nucleoli (Papanicolaou stain). B, In cell block sections, the cells can be aggregated. Cytoplasm is moderately abundant and clear (hematoxylin-eosin [H & e] stain).

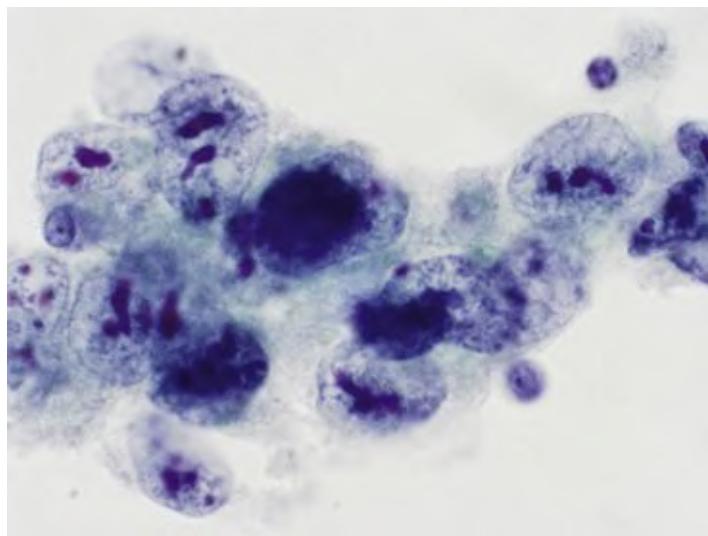


FIGURE 5.16 Embryonal carcinoma of the ovary.

The cells are large and pleiomorphic, with vesicular nuclei; prominent, irregular, and multiple

nucleoli; and pale cytoplasm (Papanicolaou stain).

Endometrial Cancer

Unlike ovarian cancers, most endometrial cancers are detected at an early stage and are therefore potentially curable. In 1988 the International Federation of Gynecology and Obstetrics (FIGO) adopted a surgical staging system in which peritoneal washings played an important role; positive results signified stage IIIA disease. In 2009, FIGO revised the surgical staging of endometrial cancer and eliminated PWC from the staging system, presumably because of controversy over the prognostic significance of PWC in women with endometrial cancer.⁴²⁻⁴³ Nevertheless, collection of peritoneal washings during surgery for endometrial cancer has its advocates and may provide useful information for treatment decisions.⁴⁴

Malignant cells from an endometrial cancer can spread to the peritoneal cavity by direct extension through the myometrium and serosa; by vascular or lymphatic invasion; or by retrograde migration through the fallopian tubes. After complete surgical staging, most women with endometrial cancer and positive PWC also have adnexal or lymph node involvement, and adjuvant therapy will be considered in such cases. (They are also more likely to have a positive cervical smear.⁴⁵) A small proportion (2% to 15%) of women with endometrial cancer limited to the uterus (and even to the endometrium) have positive PWC results.^{30,45-49} For this reason, it is thought that, at least in some cases, endometrial cancer spreads to the peritoneum by retrograde migration through the fallopian tubes. Malignant cells are often found in the lumina of the fallopian tubes in women with endometrial cancer.⁵⁰ Transtubal dissemination might be facilitated by the prior endometrial biopsy or curettage, or by preoperative intracavitary placement of radium implants. Some investigators have found that transtubal dissemination might also occur during diagnostic hysteroscopy, which involves distending the uterine cavity with fluid,^{51,52} but others have found no evidence for this when lower pressures were used (<80 mm Hg).⁵³ Laparoscopically assisted vaginal hysterectomy, applied with increasing frequency in the management of women with endometrial cancer, requires a uterine manipulator that might also cause iatrogenic dissemination of endometrial cancer cells.⁵⁴ Some investigators, however, have found no increase in positive PWC when certain manipulators were used.⁵⁵ Clamping the fallopian tubes before hysteroscopy appears to prevent dissemination of malignant endometrial cells.⁵⁶

The most common histologic subtype of endometrial cancer, accounting for approximately 85% of cases, is the endometrioid type. These tumors range from

well-differentiated adenocarcinomas (the most common) with abundant gland formation and only slight nuclear atypia, to poorly differentiated tumors with little or no glandular differentiation and marked nuclear pleomorphism.

Peritoneal washings positive for the common *endometrioid* type of endometrial cancer show clusters and isolated malignant cells. Nuclei are round and enlarged,



Cytomorphology of endometrial cancer (endometrioid)

- cell clusters and isolated cells
- enlarged nuclei
- coarse chromatin
- nuclear pleomorphism (high-grade tumors)
- scant or vacuolated cytoplasm

with coarsely textured chromatin and prominent nucleoli ([Fig. 5.17](#)). Cytoplasm is scant or moderate in amount. Nuclear pleomorphism is usually mild but can be marked in high-grade tumors. Cell block sections can be helpful by demonstrating tubular glands and squamous morule formation similar to that seen in histologic sections from the primary endometrial tumor ([Fig. 5.18A and B](#)).

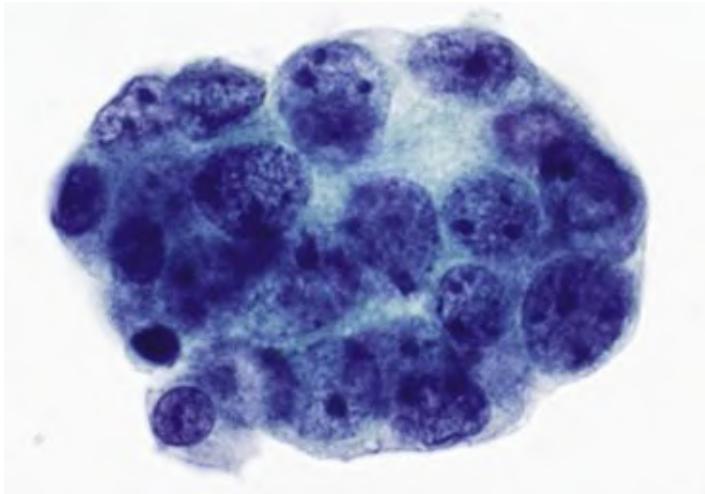


FIGURE 5.17 Endometrial carcinoma, endometrioid type.

The cells are enlarged, crowded, and have coarsely textured chromatin. Because well-differentiated endometrial cancers have round nuclei and only mild atypia, they can be difficult to distinguish from reactive mesothelial cells. The absence of intercellular windows is a helpful feature (Papanicolaou stain).

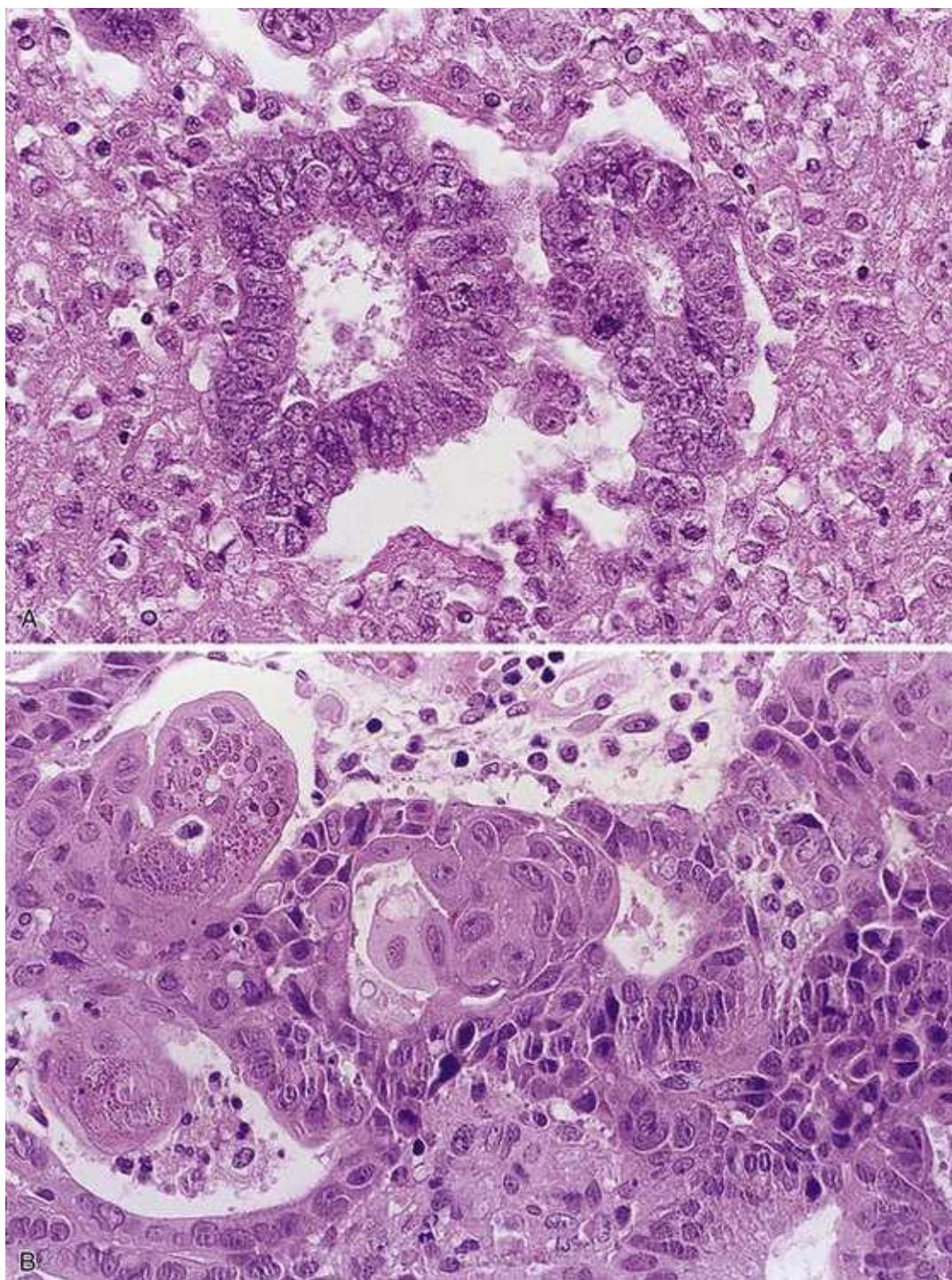


FIGURE 5.18 Endometrial carcinoma, endometrioid type.

A, Cell block sections occasionally show well-formed neoplastic glands. A mitotic figure is present (hematoxylin-eosin [H & E] stain). B, Cell block sections may show squamous morule formation by the neoplastic cells (H & E stain).

Some of the less common types of endometrial cancer, such as the *papillary serous* and *clear cell* types, are more aggressive, tending to spread to the

peritoneum more often than the endometrioid type. These tumors have a high nuclear grade and resemble high-grade ovarian cancers ([Fig. 5.19](#)). Carcinosarcoma (malignant mixed müllerian tumor) of the uterus can spread to the peritoneum. In most cases the washings demonstrate the adenocarcinoma component only, but they can contain both adenocarcinoma and sarcoma, or the sarcomatous component alone.^{57,58} As with ovarian cancers, there is significant overlap in the morphologic features of the various endometrial cancer subtypes when they appear in peritoneal washings, such that distinction among them is not reliable (or necessary) based on PWC. It is sufficient to report results as “positive for malignant cells; consistent with adenocarcinoma.” Alternatively, if the concurrent endometrial tumor resection has been histologically classified (e.g., as a clear cell adenocarcinoma), one can report the washings as “positive for malignant cells; consistent with endometrial adenocarcinoma, clear cell type.”

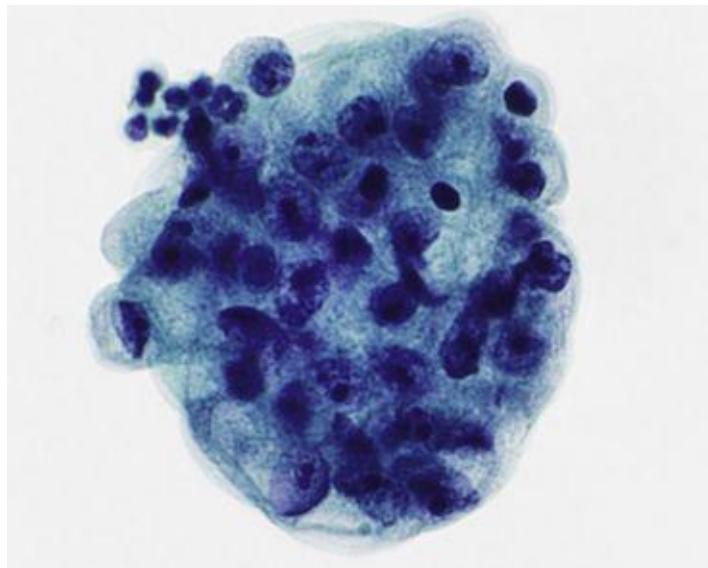


FIGURE 5.19 Endometrial carcinoma, clear cell type.
High-grade endometrial carcinomas like the clear cell type have large nuclei and prominent nucleoli. Abundant clear cytoplasm is characteristic of but not specific for clear cell cancers (Papanicolaou stain).

The management of a woman with endometrial cancer and positive PWC is controversial. It is not clear whether positive cytologic findings alone indicate an increased risk for recurrence. Perhaps positive PWC, in the absence of other poor prognostic markers, merely represents a harmless dissemination of cells that are not even viable. This hypothesis has been challenged; exfoliated

endometrial cancer cells, collected in run-off fluid through the fallopian tubes by flushing the uterus with saline after hysterectomy, do grow in culture in some cases and thus appear to be functionally viable.⁵⁹ By univariate analysis, positive PWC is associated with poor outcome in women with endometrial cancer, but positive PWC is often associated with other indicators of poor outcome, like lymph node metastases and adnexal involvement. Results from multivariate analyses have been decidedly mixed ([Table 5.2](#)). Some gynecologic oncologists maintain that management should not be determined on the basis of a positive PWC result alone, and positive PWC was dropped from the surgical staging system for endometrial cancer in 2009.^{43,60} A subsequent analysis of data from the Surveillance, Epidemiology, and End Results database of more than 14,000 women with endometrial cancer, however, found that PWC was indeed an independent predictor of lymph node metastasis and poor survival.^{61,62} A randomized clinical trial, with adjudicated cytologic diagnoses, might one day resolve this controversy.

TABLE 5.2
PERITONEAL WASHING CYTOLOGY IN WOMEN WITH ENDOMETRIAL CANCER: IS IT AN INDEPENDENT PROGNOSTIC VARIABLE?

First Author, Year	Number of Endometrial Cancers	FIGO Stages Examined	Positive Cytology (%)	Independent Variable?
Konski, 1988 ⁴⁸	134	Clinical I	14	NO
Grimshaw, 1990 ⁴⁷	322	Clinical I	5	NO
Kadar, 1992 ⁶⁰	269	Clinical I, II	13	NO
Ebina, 1997 ⁸¹	114	Surgical I, II, III, IV	35	NO
Takeshima, 2001 ⁸²	534	Non-FIGO subgroups	22	NO
Mariani, 2002 ⁸³	51	Surgical IIIA	82	NO
Fadare, 2005 ⁷	220	Surgical I, II	8.6	NO
Turner, 1989 ⁴⁵	567	Surgical I	4.9	YES
Morrow, 1991 ⁸⁴	895	Clinical I, II	11	YES
Descamps, 1997 ⁸⁵	144	Clinical I, II	9.7	YES
Kashimura, 1997 ⁸⁶	199	Clinical I, II, III, IV	15	YES
Obermair, 2001 ⁴⁹	369	Clinical I	3.5	YES
Santala, 2003 ⁸⁷	43	Surgical II, III, IV	35	YES
Garg, 2013 ⁶²	14,704	Surgical I, II	3.3	YES

Cervical Cancer

The results of PWC are not included in the FIGO staging system for cervical cancer.⁴³ The incidence of positive cytologic findings in patients who undergo primary definitive surgery for all stages of cervical cancer combined is relatively low (8%).⁶³ In patients with early-stage disease (FIGO stage IB) the incidence is even lower (1.2%).⁶³ For this reason, PWC is probably unnecessary in patients with early-stage cervical cancer.⁶³ Some investigators report a strong association

between positive cytologic findings and other indicators of a poor prognosis, such as lymph node involvement, and contend that patients with positive cytology have a high recurrence rate in the peritoneal cavity.^{64,65} These investigators recommend using PWC as a guide for planning therapy for patients with advanced disease.

Positive PWC results are more often found in patients with adenocarcinoma than with squamous cell carcinoma (SQC) of the cervix.⁶⁴⁻⁶⁷ Adenocarcinoma of the cervix in peritoneal washings can take one of several patterns, ranging from rare isolated cells to abundant clusters and isolated cells with enlarged nuclei and prominent nucleoli.⁶⁷ SQC is easily recognized when the tumor cells have the characteristic cytoplasmic orangeophilia seen in keratinized tumors. Nonkeratinized tumors are harder to identify and mimic sheets of crowded mesothelial cells.⁶⁷ They have a tendency to exfoliate either as large clusters with smooth, rounded contours or as dense sheets with elongated, irregular outlines. Nuclei are enlarged, with coarsely textured chromatin, and nucleoli can be prominent. The abnormal nuclei and the high nuclear-to-cytoplasmic ratio helps distinguish these sheets from benign mesothelial cells.

Other Malignancies

Fallopian tube cancers resemble ovarian cancers in many ways: They have similar risk factors, share most of the same histologic tumor types, and have a similar biologic behavior. For these reasons, the staging system for fallopian tube cancers is very similar to that for ovarian cancers.⁴⁵ Positive peritoneal washings modify stage I and II tumors to stage Ic and IIc, respectively.

PWC is sometimes used to document peritoneal spread of gastric and pancreatic cancers. Positive results are common in patients with pancreatic (21% to 30%)⁶⁸⁻⁷⁰ and gastric cancers (21% to 43%).^{20,69,71-74} A lower frequency has been reported for patients with colorectal cancer (3% to 15%),^{69,75} although some investigators have found higher rates.⁷⁶

Staging of pancreatic cancers aims to identify patients with resectable disease. Contrast-enhanced computed tomography (CT) is reliable when positive for metastases, but in many patients with a negative CT scan, the tumor is found to be unresectable at surgical exploration. Laparoscopy allows identification of metastatic disease beyond the resolution of CT: 24% of patients with stage 1 or 2, localized pancreatic cancer have visible metastases by laparoscopy; another 10% have microscopic disease detected by PWC.⁶⁸

Similarly, laparoscopic lavage identifies patients with gastric cancer who are

unlikely to be cured by resection. Between 4% and 16% of patients without visible metastases have positive PWC.^{[20,77,78](#)} By multivariate analysis, PWC is an independent prognostic factor for survival in patients with gastric cancer^{[71,74](#)} and is included in the Japanese staging system for gastric cancer.^{[74,79](#)}

Monitoring Response to Treatment (“Second-Look Procedures”)

Women with advanced ovarian cancer may undergo subsequent surgery to assess their response to primary cytoreductive surgery and adjuvant therapy. Such “second-look” procedures (followed by “third-look,” and so on) are sometimes needed because radiographic studies and measurement of serum CA-125 levels are insufficiently accurate as measures of residual disease. After the incision is made, peritoneal washings are obtained, and the peritoneum is inspected and palpated. If no tumor is visible, random biopsies are taken from various peritoneal locations, the omentum, and the retroperitoneal lymph nodes. More than 50% of women prove to have residual disease. Unfortunately, earlier initiation of treatment does not affect survival; therefore second-look procedures are currently recommended only for women in research protocols.¹³

PWC in this setting is hampered by low sensitivity for malignancy; findings are negative in 31% to 86% of biopsy-proven residual disease^{9,11,21,22,24-26} most likely because adhesions cause poor distribution of the lavage fluid. In addition, chemotherapy and radiotherapy produce alterations in normal mesothelial cells that may cause them to be misinterpreted as malignant.



Cytomorphology of treatment effect

- enlarged, multinucleated mesothelial cells
- large nuclei
- normochromatic
- prominent nucleoli
- abundant cytoplasm

Normal mesothelial cells altered by chemotherapy or radiation show frequent multinucleation and marked variation in nuclear size (anisonucleosis) ([Fig. 5.20](#)). Despite the often prominent nuclear enlargement, cytoplasm is usually abundant, and thus the normal nuclear-to-cytoplasmic ratio is unchanged. Chromatin is usually pale and finely granular. Nucleoli may be prominent. In contrast, malignant cells often have a highly increased nuclear-to-cytoplasmic ratio and more coarsely textured chromatin.⁸⁰ In ambiguous cases it is often

helpful to compare the washings with histologic sections from the patient's known malignancy. The distinction is important, because the decision to continue therapy depends, in part, on the results of PWC.

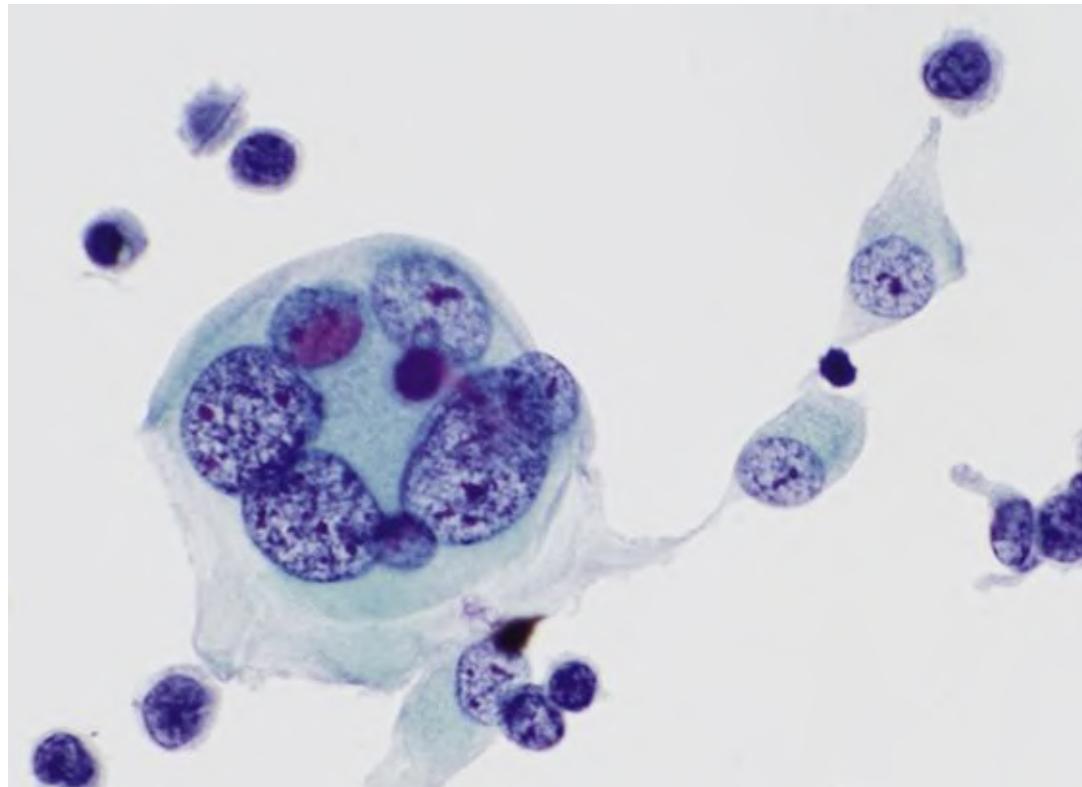


FIGURE 5.20 Mesothelial cell atypia due to chemotherapy.
Features include multinucleation and marked anisonucleosis but only slight hyperchromasia.
Cytoplasm is abundant (Papanicolaou stain).

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CHAPTER 6

Cerebrospinal Fluid

Edmund S. Cibas

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[Other Tumors of the Central Nervous System](#)

The lumbar puncture (LP) (spinal tap) was introduced in 1891,^{1,2} and in 1904 a French neurologist first described malignant cells in cerebrospinal fluid (CSF).³ Since then, preparatory methods have been refined and diagnostic features described in a number of monographs and atlases,⁴⁻⁷ attesting to the importance of CSF cytology for excluding leptomeningeal metastasis in a patient with neurologic symptoms.

Anatomy and Physiology

The brain contains four *ventricles*, fluid-filled cavities that communicate with each other and the subarachnoid space surrounding the brain and spinal cord. The ventricles are lined by a cuboidal ciliated cell layer called the *ependyma*. In some areas the ependyma differentiates into a complex villous structure called the *choroid plexus*, composed of a single layer of cuboidal nonciliated cells overlying a vascular core. The choroid plexus produces most of the CSF by filtering plasma across capillary walls and actively secreting fluid. After leaving the ventricles via the midline foramen of Magendie and the two lateral foramina of Luschka, CSF circulates in the *subarachnoid space*, formed by the *leptomeninges* (composed of the pia mater and arachnoid mater), over cerebral and spinal surfaces. Fluid is then reabsorbed by the arachnoid granulations into the venous system, and the cycle begins anew.⁸⁹

The total volume of CSF in the adult is about 150 mL. Because 500 mL/day is produced (0.35 mL/min), CSF is renewed three or four times daily.

Obtaining and Preparing the Specimen

Most CSF specimens are obtained by LP, in which a needle is passed through the intervertebral space at L3 to L4 or L4 to L5. Rarely, because of inflammation at these sites or a bony abnormality, the specimen must be obtained from the cisterna magna at the base of the brain. Cisternal CSF sometimes yields positive results when LP is negative.¹⁰ CSF is sometimes aspirated directly from a lateral ventricle during a neurosurgical procedure; such specimens often contain microscopic fragments of normal brain. In patients undergoing chemotherapy for leptomeningeal metastasis, a silicone pouch (Ommaya reservoir) is implanted in subcutaneous tissue. A cannula leads from the pouch into a lateral ventricle through a 3-mm burr hole (Fig. 6.1). This is an efficient way to introduce chemotherapeutic drugs and withdraw CSF periodically for examination.

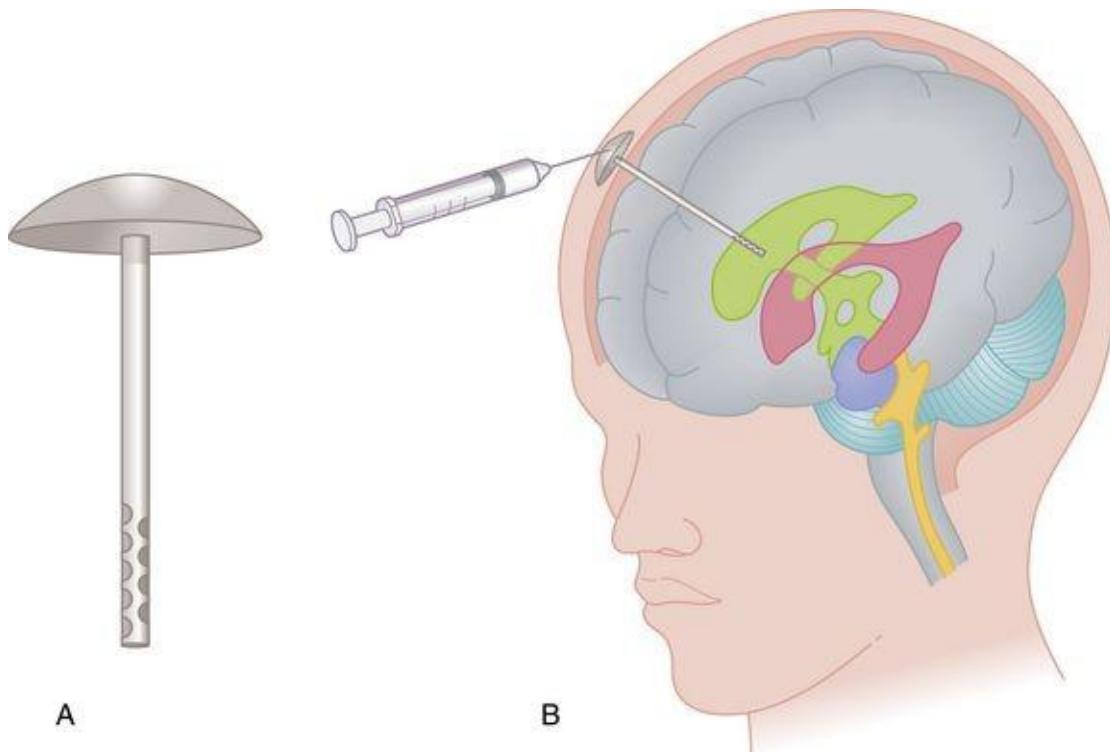


FIGURE 6.1 Ommaya reservoir.

A, The Ommaya reservoir consists of a pouch connected to a cannula with distal perforations. B, When implanted, the pouch is subcutaneous, and the cannula ends in one of the lateral ventricles. A needle penetrates the pouch to instill medication and withdraw cerebrospinal fluid (CSF) for examination of response to treatment.

A minimum of 1 mL should be collected for cytologic evaluation, but 3 mL or more is preferable,¹¹ and at least 10 mL is considered ideal.¹² Surprisingly, the amount of CSF removed does not affect the likelihood that a patient will develop a headache.^{13,14}

Fluid should be collected fresh and delivered to the laboratory as quickly as possible to prevent cellular deterioration. If the specimen cannot be prepared immediately, it should be refrigerated at 4° C. If a delay of more than 48 hours is anticipated, cytomorphology can be preserved by adding an equal volume of 50% ethanol or RPMI.¹⁵ A CSF sample for cytologic examination should never be frozen.

The most common ways to prepare CSF specimens are by cytocentrifugation (Cytospin) and thinlayer preparation. Cytocentrifugation has greater flexibility because both alcohol-fixed and air-dried slides can be prepared using this method. Lymphoid cells are best evaluated using air-dried preparations, thus it is advisable to prepare an air-dried Romanowsky-stained slide in addition to the traditional alcohol-fixed Papanicolaou-stained slide. Depending on the volume and cellularity of the specimen, additional unstained slides can be prepared and set aside for immunocytochemical studies if needed. Unstained air-dried slides can be kept at room temperature for 1 to 2 weeks without compromising cytomorphology or antigenicity.¹⁶

Blood is a common contaminant. The needle lacerates the veins that course along the crowded nerve roots of the cauda equina in 75% of LPs,¹⁷ and this can create diagnostic problems. Neutrophils and eosinophils, if accompanied by red blood cells, should not necessarily be interpreted as evidence of meningitis. Similarly, leukemic blasts from peripheral blood can contaminate the fluid, leading to an erroneous impression of meningeal involvement by leukemia. It takes only a minute amount of blood to alter the cellular composition of CSF significantly, an amount that can be invisible to the naked eye.¹⁸ Because alcohol fixation lyses red blood cells, contamination is best detected on air-dried preparations. If red blood cells are present, the possibility of contamination by peripheral blood blasts should be raised in a patient with leukemia.

Reporting Terminology

As with most cytologic specimens, CSF cytology results are commonly reported as either “negative for malignancy,” “atypical” (connoting a low degree of suspicion for malignancy), “suspicious” (connoting a high degree of suspicion of malignancy), or “positive.” Over 90% of CSF specimens are assigned a cytologic diagnosis of “negative for malignant cells.”¹⁹ Many show only a small number of lymphocytes and monocytes, essentially a normal CSF.

The primary role of CSF cytology is to exclude circulating malignant cells in CSF pathways. Although a specific diagnosis of some benign diseases (e.g., cryptococcosis) can be made cytologically, in most nonmalignant diseases of the central nervous system (CNS), CSF cytology is frustratingly unrevealing. The myriad diseases that cause aseptic meningitis, for example, have as their final common denominator nothing more than a lymphocytic and/or monocytic pleocytosis. The cause is identified by other clinical and laboratory methods.

Accuracy

The sensitivity of CSF cytology for detecting malignant cells is about 60%,^{20–22} but sensitivity depends on several factors. First and foremost, a positive CSF sample will occur only if a malignancy actually invades into a ventricle or the subarachnoid space ([Fig. 6.2](#)).

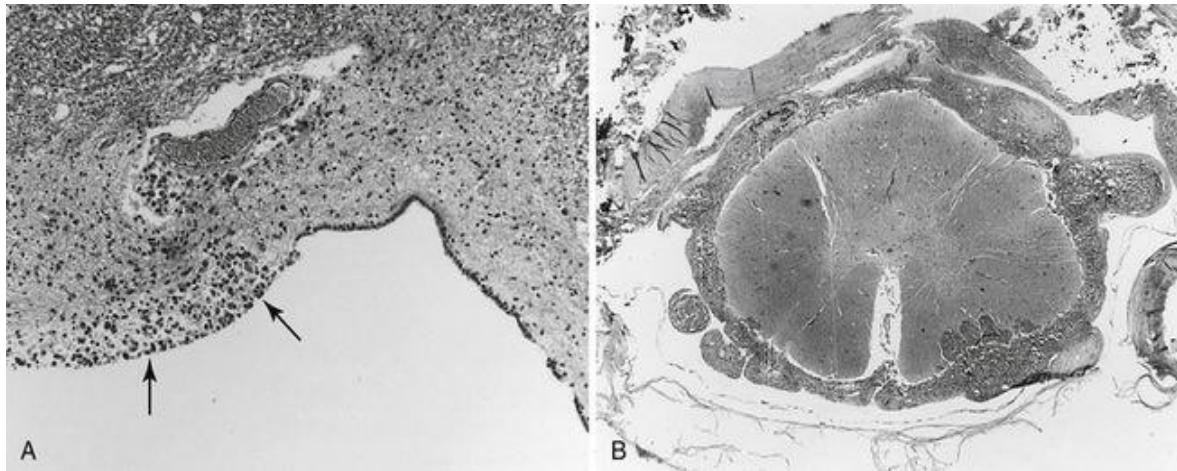


FIGURE 6.2 Tumor involvement of cerebrospinal fluid (CSF) pathways.
A, The arrows indicate a focus of astrocytoma cells that have disrupted the ependymal lining (hematoxylin-eosin [H & E] stain). B, The normally delicate subarachnoid membrane is filled and expanded by metastatic breast cancer cells, encasing the spinal cord. CSF was positive for malignant cells in both cases (H & E stain).

- The sensitivity of cerebrospinal fluid cytology depends upon
- the number of specimens examined
 - the volume of fluid submitted
 - the extent of leptomeningeal disease
 - the site from which the sample was obtained (lumbar versus cisterna magna)

The sensitivity of a single cytologic examination is 54% but increases to 84% with a second sample.²² Smaller incremental increases in sensitivity are observed

with more than two specimens.²³ Sensitivity is dependent of sample volume; sensitivity is greater if 10 mL are submitted rather than 3 mL.¹² Sensitivity also depends on the extent of leptomeningeal disease: 38% for focal and 66% for disseminated leptomeningeal tumor.²⁰ Similarly, only 50% of patients with early meningeal involvement by acute lymphoblastic leukemia (ALL) have a suspicious or positive CSF.²⁴ The site from which the sample is obtained can also affect the sensitivity; if LP specimens are negative, a tap from the cisterna magna sometimes yields malignant cells.¹⁰

The specificity of CSF cytology is high. False-positive diagnoses are estimated at 2% to 3%.²⁵ The most common cause is the overdiagnosis of lymphoma or leukemia, particularly in patients with herpes zoster meningitis,²⁰ cryptococcal meningitis,^{26,27} Lyme disease,²⁸ viral meningitis,²⁶ and in LP specimens contaminated with blood.²⁶

In light of these data, the American College of Physicians determined that cytologic examination of CSF for meningeal malignancy has moderate sensitivity and high specificity.²⁹

Normal Elements

Normal CSF is sparsely cellular and, in adults, contains less than five cells/mm³ (equivalent to 5000 cells/mL). In newborns the fluid is more cellular.³⁰



Benign cells in cerebrospinal fluid

- common
 - lymphocytes
 - monocytes
- rare
 - choroid plexus/ependymal cells
 - brain fragments
 - germinal matrix
 - chondrocytes
 - bone marrow

Normal CSF is composed of lymphocytes and monocytes ([Fig. 6.3](#)). Only small numbers of mature lymphocytes are present in normal CSF, but their number can increase markedly, particularly in viral meningitis and other inflammatory or infectious conditions. Such specimens can be highly cellular, with a minor population of so-called atypical lymphoid cells that are large and may show irregular nuclear contours, with clefting, blebs, and nucleoli.

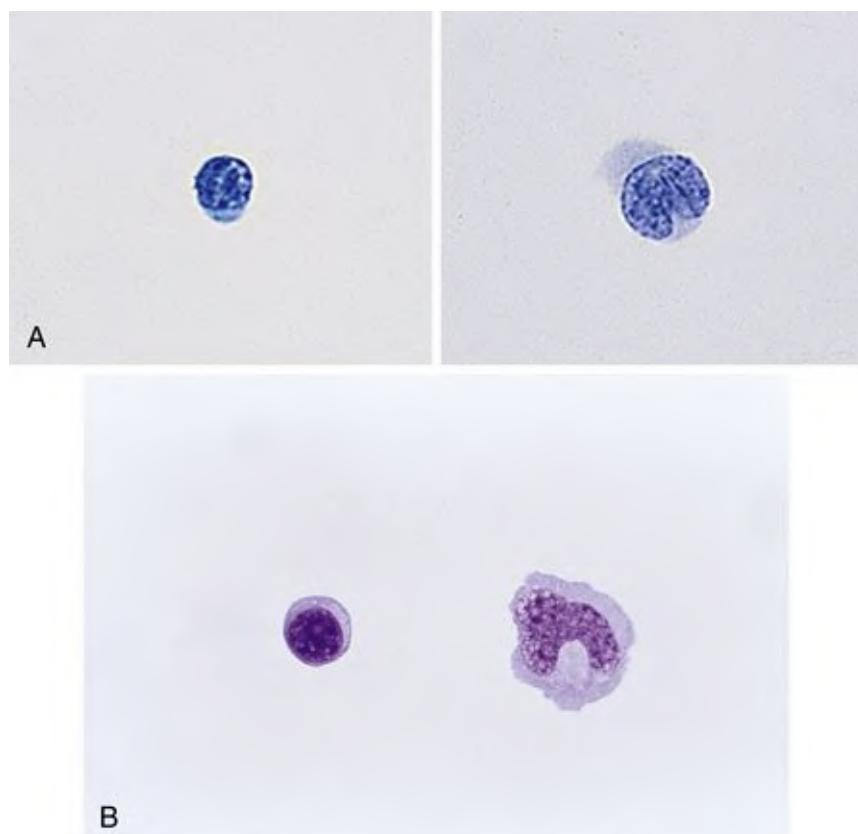


FIGURE 6.3 Normal cerebrospinal fluid (CSF).

Lymphocytes and monocytes are the usual components of normal CSF. A, Papanicolaou stain. B, Romanowsky stain.

Monocytes are also present in normal CSF. Larger than lymphocytes, they have folded, kidney bean–shaped nuclei and a moderate amount of cytoplasm.

Choroid plexus and ependymal cells are seen in less than 0.5% of LP specimens.³¹ These cells have a round to oval nucleus and a moderate amount of cytoplasm; they are isolated or in small clusters ([Fig. 6.4](#)). Microscopic brain fragments have a fibrillary texture and contain glial cells, neurons, and capillaries ([Fig. 6.5](#)). They are seen in samples taken directly from the ventricles, because the needle traverses brain parenchyma; they are not seen in LP samples. Rarely, isolated neurons are present ([Fig. 6.6A and B](#)).

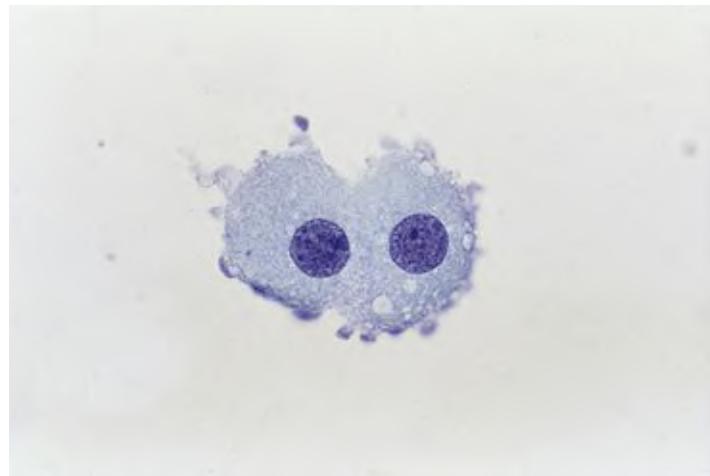


FIGURE 6.4 Choroid plexus/ependymal cells.

These cells are rare in cerebrospinal fluid (CSF); even when present, their number is usually small. They may be isolated or arranged in small clusters. Note the dispersed chromatin texture of the nuclei and the abundant cytoplasm (Papanicolaou stain).

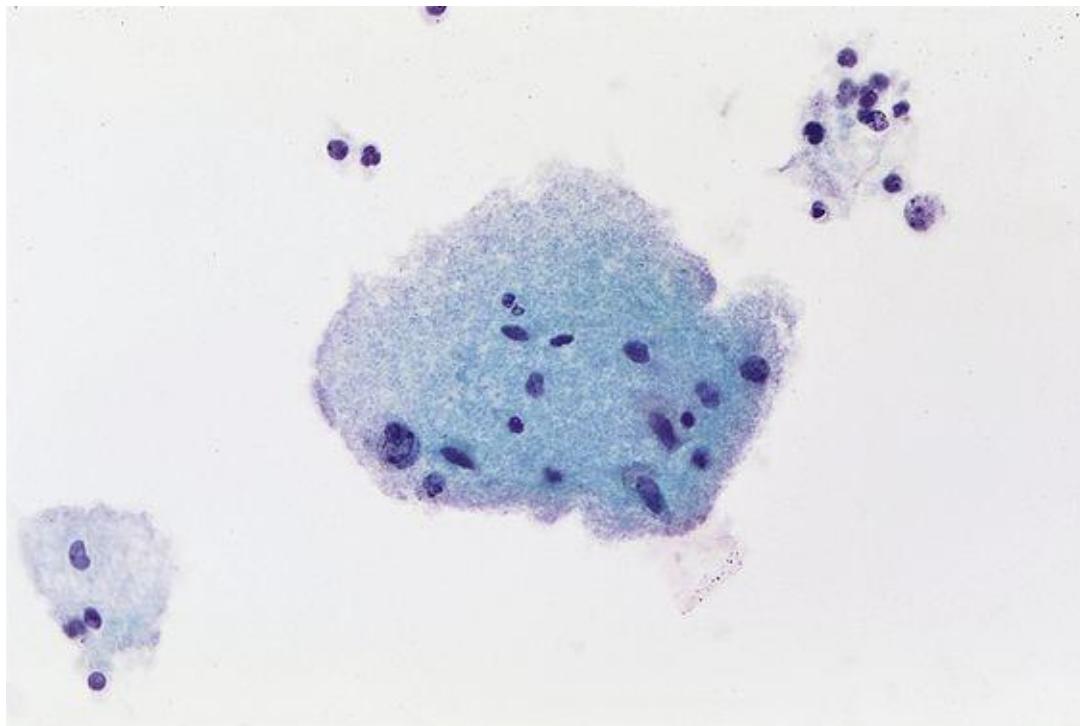


FIGURE 6.5 Brain tissue.

These fragments, obtained from a ventricular tap, have a fibrillary texture and contain normal glial cells. Some fragments may contain neurons and capillaries (Papanicolaou stain).

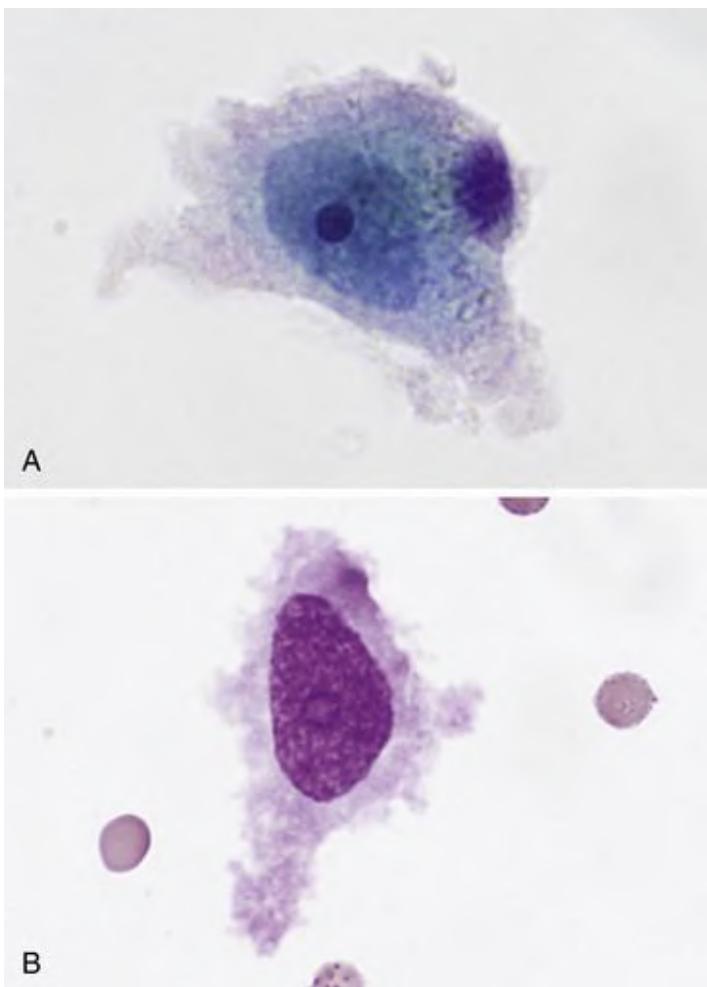


FIGURE 6.6 Neuron.

Many neurons have an angular shape, a round nucleus, and a prominent nucleolus. Some have lipofuscin pigment (A, Papanicolaou stain; B, Romanowsky stain).

In CSF from neonates, most commonly those born prematurely, one occasionally sees small, immature cells of germinal matrix origin.^{32,33} Germinal matrix cells lie beneath the ependyma in the wall of the lateral ventricles and exfoliate when there is subependymal and intraventricular hemorrhage ([Fig. 6.7](#)). They are often clustered and molded to one another, thus mimicking a small cell malignancy like medulloblastoma. Because of their frequent association with hemorrhage, these cells are often accompanied by hemosiderin-laden macrophages.

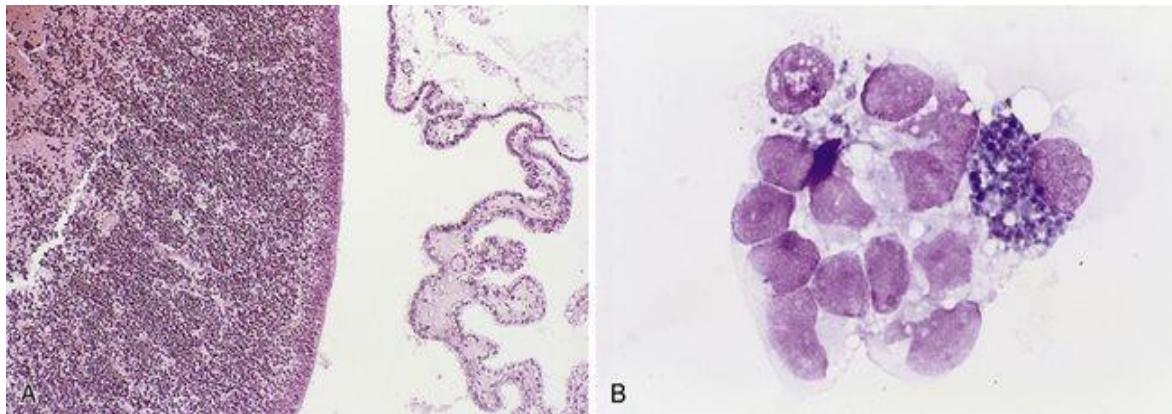


FIGURE 6.7 Germinal matrix.

A, In neonates, the small, dark, densely packed cells of the germinal matrix lie beneath the ependyma. Choroid plexus is seen on the right (hematoxylin-eosin [H & E] stain). *B*, When the ependyma is injured, clusters of small cells, accompanied by macrophages, are seen in cerebrospinal fluid (CSF) (Romanowsky stain).

If the LP needle is inserted too far anteriorly, CSF can be contaminated by chondrocytes ([Fig. 6.8](#)) or bone marrow cells ([Fig. 6.9](#)) from the intervertebral disc or vertebral body, respectively. These cells, seen in less than 1% of CSF specimens,^{[34,35](#)} should not be mistaken for malignant cells.^{[36](#)} Although mitoses are more common in malignant specimens, they are occasionally seen in benign conditions such as bacterial and viral meningitis.^{[37](#)}

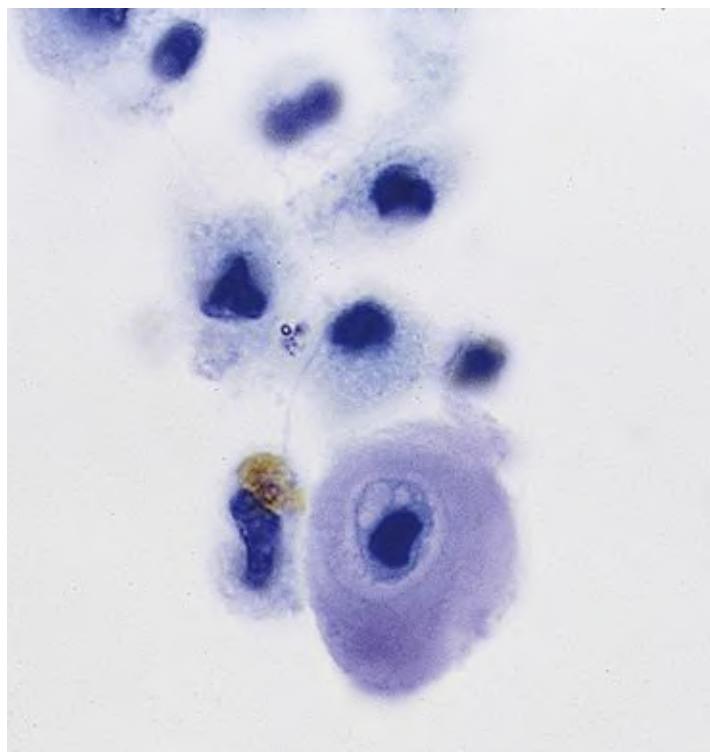


FIGURE 6.8 Chondrocyte.

Rarely seen in cerebrospinal fluid (CSF), chondrocytes have a pyknotic nucleus and are surrounded by a shell of extracellular mucopolysaccharide matrix that stains purple with the Papanicolaou stain. The adjacent cells are macrophages (Papanicolaou stain).

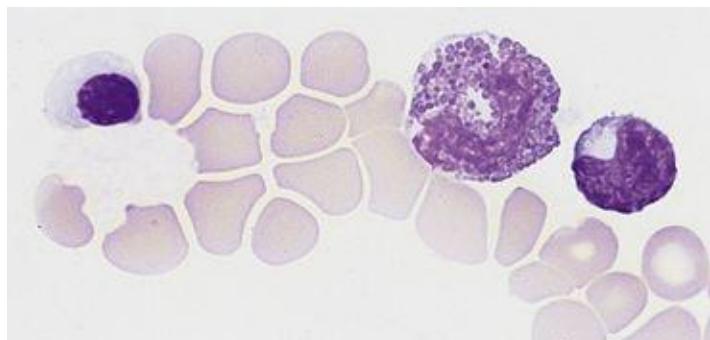


FIGURE 6.9 Bone marrow.

If the needle penetrates a vertebral body, immature erythroid and myeloid elements from normal bone marrow are sampled, as seen here (Romanowsky stain).

Abnormal Inflammatory Cells

Macrophages, plasma cells, and eosinophils are abnormal findings in CSF. They may accompany malignancy, but are also seen in a variety of nonneoplastic conditions.

Macrophages have abundant, vacuolated cytoplasm that sometimes contains ingested cells, organisms, or pigment (Fig. 6.10A and B).

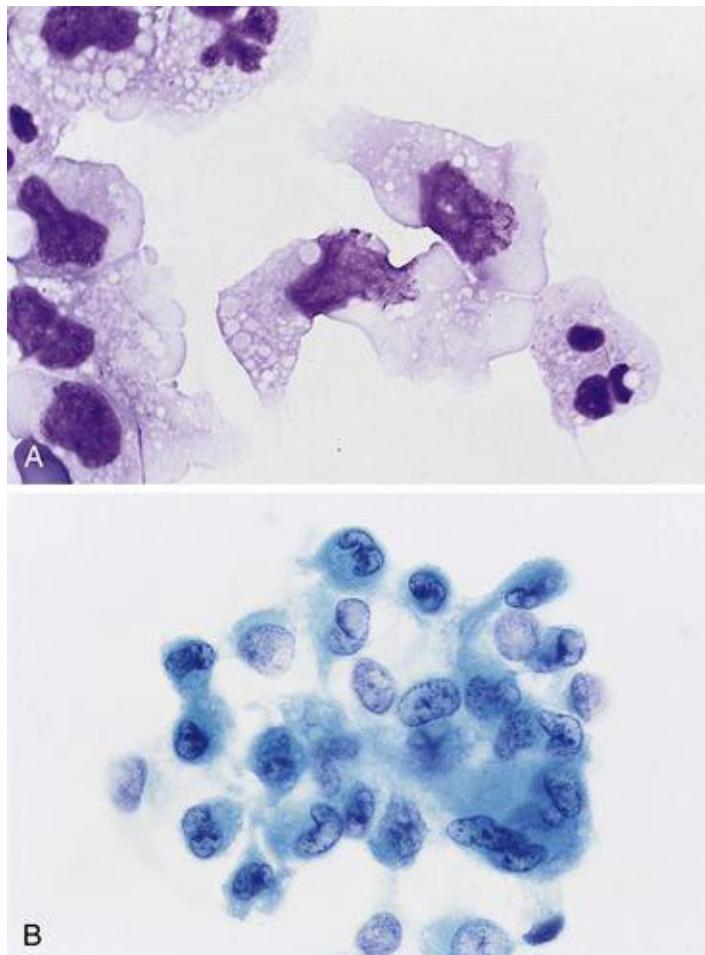


FIGURE 6.10 Macrophages.

Macrophages have abundant cytoplasm and are seen in benign and malignant conditions. A, Subarachnoid hemorrhage (Romanowsky stain). B, Chronic shunt infection (Papanicolaou stain).



Macrophages in cerebrospinal fluid are associated

with

- meningitis
- subarachnoid hemorrhage
- intraventricular hemorrhage
- cerebral infarction
- post-treatment inflammation
- multiple sclerosis³⁸

Plasma cells are also an abnormal but nonspecific finding in CSF ([Fig. 6.11](#)).

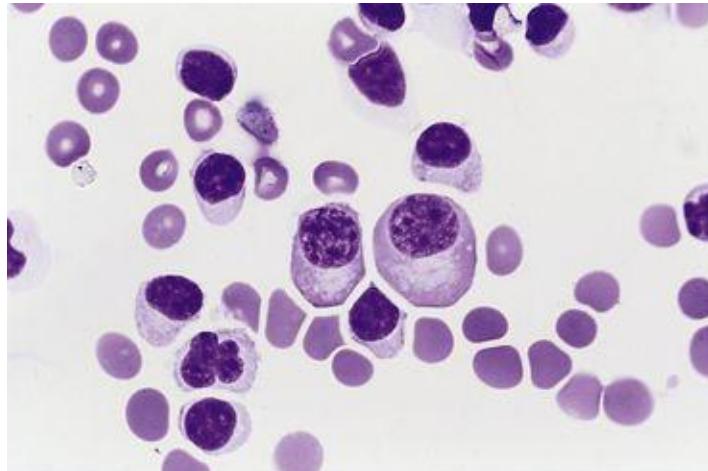


FIGURE 6.11 Plasma cells (Lyme disease).

Cerebrospinal fluid (CSF) from patients with aseptic meningitis, as in this case of Lyme meningitis, can be markedly hypercellular, with plasma cells as a prominent component. Such specimens resemble a lymphoproliferative disorder (Romanowsky stain).



Plasma cells in cerebrospinal fluid are associated with

- viral meningitis (e.g., enterovirus, human immunodeficiency virus [HIV])
- Lyme disease
- tuberculosis
- cysticercosis
- syphilis
- multiple sclerosis³⁸

Polymorphonuclear leukocytes are a normal finding if there is contamination by peripheral blood, but numerous neutrophils unaccompanied by a proportionate increase in red blood cells raise the possibility of acute meningitis ([Fig. 6.12](#)). In a patient with acquired immunodeficiency syndrome (AIDS), numerous neutrophils are highly suggestive of cytomegalovirus (CMV) radiculopathy. Viral cytopathic inclusions, however, are not seen.^{[39](#)}

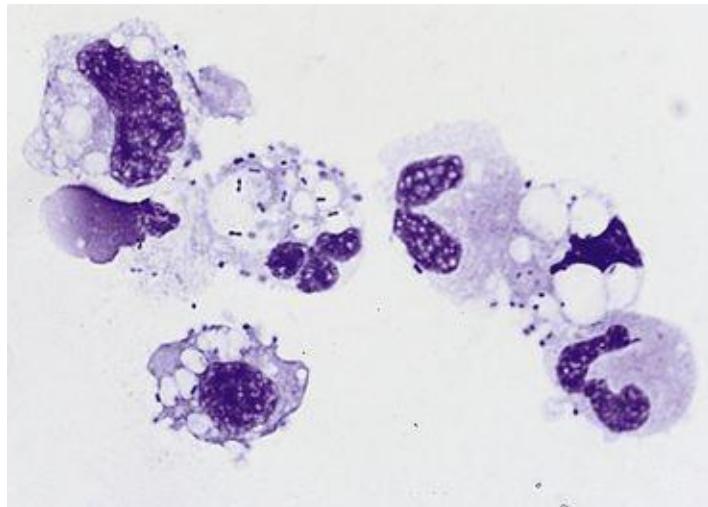


FIGURE 6.12 Acute bacterial (pneumococcal) meningitis.
There are numerous neutrophils and bacteria (Romanowsky stain).

Differential diagnosis of neutrophils in cerebrospinal fluid

- peripheral blood contamination
- acute bacterial meningitis
- CMV radiculopathy
- Toxoplasma meningoencephalitis
- viral meningitis (early stage)

Like polymorphonuclear leukocytes, eosinophils are a normal finding if there is contamination by peripheral blood. If peripheral blood contamination is not apparent, eosinophils, especially in large numbers ([Fig. 6.13](#)), suggest a parasitic infection, particularly *Taenia solium* and *Angiostrongylus cantonensis*.^{[40,41](#)}

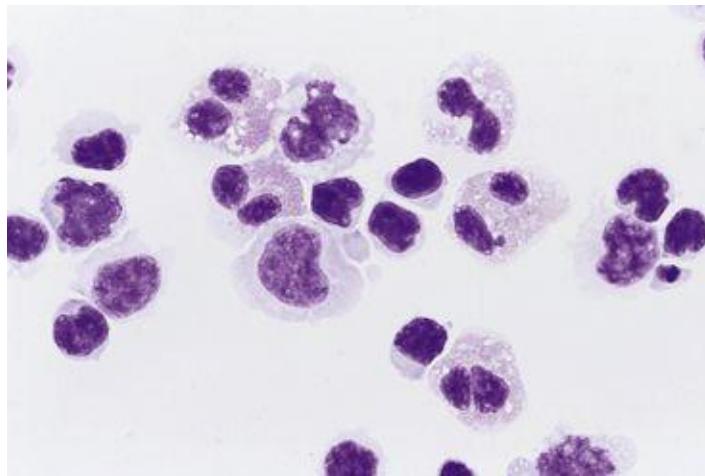


FIGURE 6.13 Eosinophils.

Except for a traumatic tap, eosinophils are an abnormal finding in cerebrospinal fluid (CSF). A parasitic infection should be considered (Romanowsky stain).

Differential diagnosis of eosinophils in cerebrospinal fluid

- parasites
- *Coccidioides immitis*
- ventriculoperitoneal shunts
- Rocky Mountain spotted fever
- malignancy
- medications

Eosinophilic meningitis is defined as 10 or more eosinophils/ μL or 10% or more of the total CSF leukocyte count.⁴¹ The most common cause is CNS invasion by helminthic parasites. Worldwide, the most common cause of eosinophilic meningitis is *Angiostrongylus cantonensis*. Other parasites that cause eosinophilic meningitis include *Gnathostoma spinigerum*, *Baylisascaris procyonis*, and *Taenia solium*. Meningitis caused by the fungus *Coccidioides immitis* occurs in up to 50% of patients with disseminated disease, and the meninges can be the only site of infection. Malignancies, especially Hodgkin and non-Hodgkin lymphoma and leukemia, have been associated with eosinophilic meningitis. Medications, either systemic or intrathecal, and ventriculoperitoneal shunts have been implicated in some cases of eosinophilic meningitis.⁴¹

NonNeoplastic Disorders

Acute Bacterial Meningitis

Many bacteria can cause meningitis, including *Neisseria meningitidis* (meningococcus), *Haemophilus influenzae*, *Streptococcus pneumoniae* (pneumococcus) (see [Fig. 6.12](#)), and *Listeria monocytogenes*.



Cytomorphology of acute bacterial meningitis

- numerous neutrophils
- bacteria (may or may not be seen)

Because bacterial meningitis can be fatal if not treated immediately, prompt diagnosis is crucial. Any CSF sample composed predominantly of neutrophils should be considered high probability for bacterial meningitis; precise identification of the organism depends on microbiologic cultures.



Differential diagnosis of acute bacterial meningitis

- traumatic tap
- *Toxoplasma* meningoencephalitis
- CMV radiculopathy
- aseptic meningitis (early)

Neutrophils admixed with red blood cells and other blood elements are a normal finding resulting from a traumatic tap. Abundant neutrophils are seen in other conditions like toxoplasmosis, CMV radiculopathy,³⁹ and the early stages of aseptic meningitis.

Aseptic Meningitis

Aseptic meningitis is a misnomer, but the term is ingrained in clinical practice.

Despite its name, it is most commonly caused by an infectious organism, usually a virus. The clinical course is less fulminant than that of acute bacterial (pyogenic) meningitis. Aseptic meningitis is usually self-limited and treated symptomatically. The most common pathogen is one of the enteroviruses (nonparalytic poliovirus, echovirus, and coxsackieviruses).



Cytomorphology of aseptic meningitis

- increase in lymphocytes and monocytes
- small proportion of atypical lymphocytes

The cytologic findings are nonspecific. There is an increased number of predominantly small, mature lymphocytes, but also monocytes, plasma cells, and enlarged (so-called atypical) lymphocytes, some of which have prominent nucleoli and irregular nuclear contours. In the early stages, neutrophils are present ([Fig. 6.14](#)). Viral inclusions are virtually never seen in CSF.⁴²

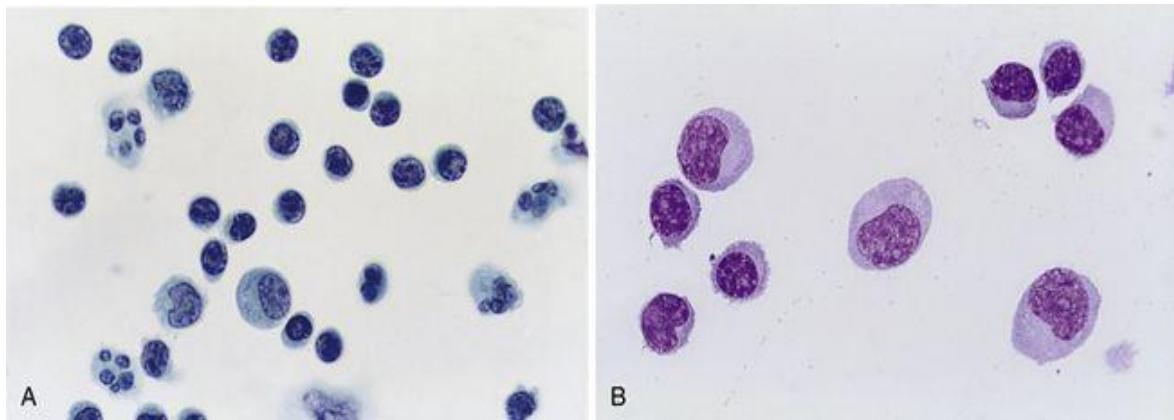


FIGURE 6.14 Aseptic meningitis (viral).

An increased number of lymphocytes is present, including occasional so-called atypical lymphoid cells: enlarged cells with large nuclei, some of which have irregular contours, more finely dispersed chromatin, and prominent nucleoli. Note that the predominant cell type is still the small, mature lymphocyte. *A*, Papanicolaou stain. *B*, Romanowsky stain.

Aseptic meningitis is caused by a wide range of organisms, systemic diseases, and miscellaneous conditions ([Table 6.1](#)), with identical cytologic findings. It is seen in about 10% of patients within 1 to 2 weeks of seroconversion resulting from human immunodeficiency virus-1 (HIV-1). Aseptic meningitis occurs in

some patients with Lyme disease.

TABLE 6.1
PARTIAL LIST OF CAUSES OF ASEPTIC MENINGITIS

VIRUSES
• enteroviruses
• herpes simplex viruses
• varicella-zoster virus
• mumps virus
• arboviruses
• arenavirus (lymphocytic choriomeningitis)
BACTERIA
• <i>Borrelia burgdorferi</i> (Lyme disease)
• <i>Treponema pallidum</i> (syphilis)
• <i>Mycobacterium tuberculosis</i>
• ehrlichiosis
• <i>Mycoplasma pneumoniae</i>
FUNGI
• <i>Cryptococcus neoformans</i>
• <i>Histoplasma capsulatum</i>
• <i>Coccidioides immitis</i>
SYSTEMIC DISEASES
• Behçet disease
• sarcoidosis
• lupus
OTHER
• drugs
• vaccines
• parainfectious syndrome (acute disseminated encephalomyelitis)
• vasculitis
• idiopathic (e.g., Mollaret meningitis)

A rare form of aseptic meningitis, *idiopathic recurrent aseptic meningitis*, also known as *Mollaret meningitis* after the man who first described the disease in 1944, is characterized by recurring attacks of fever, headache, and neck stiffness.

Symptoms appear suddenly, last for 5 to 7 days, and resolve spontaneously, but then recur days or years later. Herpes simplex virus (HSV) types 1 and 2 have been identified as the causative agents in some cases previously considered idiopathic; reactivation of latent HSV infection explains the periodic and self-limited nature of the illness in these cases.^{43–45} The diagnosis of Mollaret meningitis is made clinically after excluding other causes of aseptic meningitis. Cytologic findings are nonspecific, but there is often a marked predominance of monocytes.⁴⁶ So-called Mollaret cells—monocytes with deep nuclear clefts that impart a footprintlike appearance to the nucleus—are seen within the first 24 hours of the onset of symptoms.⁴⁶ They are characteristic of but not specific for Mollaret meningitis and can be seen in other diseases like sarcoidosis and Behçet disease.



Differential diagnosis of aseptic meningitis

- primary CNS lymphoma
- secondary involvement by lymphoma
- acute leukemia

A polymorphous lymphoid population supports the diagnosis of a benign, reactive process. Nevertheless, the presence of some atypical lymphocytes raises the possibility of malignant lymphoma. Some lymphomas in the CNS are accompanied by numerous small, reactive T lymphocytes and can, in fact, mimic an aseptic meningitis. Because the cells in most cases of aseptic meningitis are T cells,⁴⁷ immunocytochemistry and flow cytometry are useful in selected cases ([Fig. 6.15](#)). If virtually all the lymphoid cells are T cells, a malignant lymphoma is unlikely, because most lymphomas, including primary CNS lymphomas, are B-cell neoplasms.⁴⁸

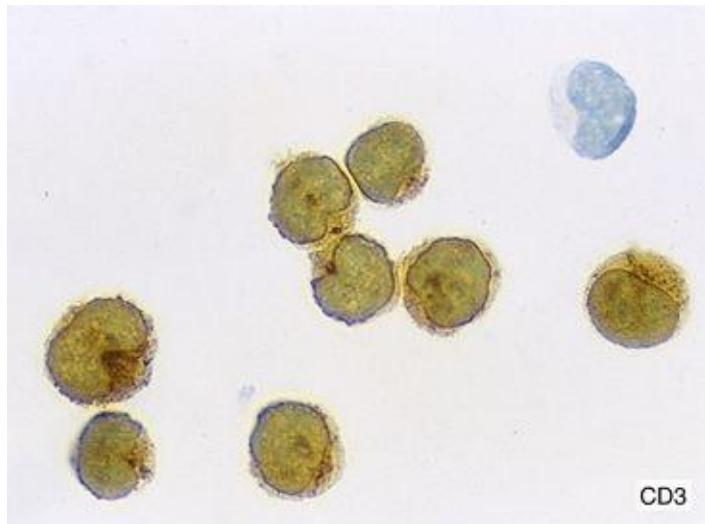


FIGURE 6.15 Aseptic meningitis.

In most cases of aseptic meningitis, the lymphoid infiltrate is mostly T cells (immunocytochemistry for CD3, cytocentrifuge preparation).

In contrast with viral meningitis, the meningeal infiltrate in Lyme disease is composed of B cells (see [Fig. 6.11](#)). In order to distinguish the florid pleocytosis in some cases of Lyme meningitis from lymphoma, demonstration of polyclonal versus monoclonal expression of κ and λ light chains is necessary.²⁸

Cryptococcal Meningitis

The only organism that is identified cytologically with any frequency in CSF is *Cryptococcus neoformans*, which causes disease in both healthy and immunocompromised people.



Cytomorphology of cryptococcal meningitis

- round yeast forms
- variable size: 5 to 15 μm diameter
- pink/purple (Papanicolaou stain)
- asymmetric, narrow-based budding
- mucin-positive capsule
- refractile artifact

The degree of inflammation is variable.⁴⁹ There can be a marked pleocytosis,

in which case organisms are hard to identify. Alternatively, there may be abundant organisms and very little inflammatory response ([Fig. 6.16A](#)). *C. neoformans* is sometimes perfectly round, but often indented. The indentations trap air under the coverslip, resulting in a crystal-like refractile artifact ([Fig. 6.16B](#)).

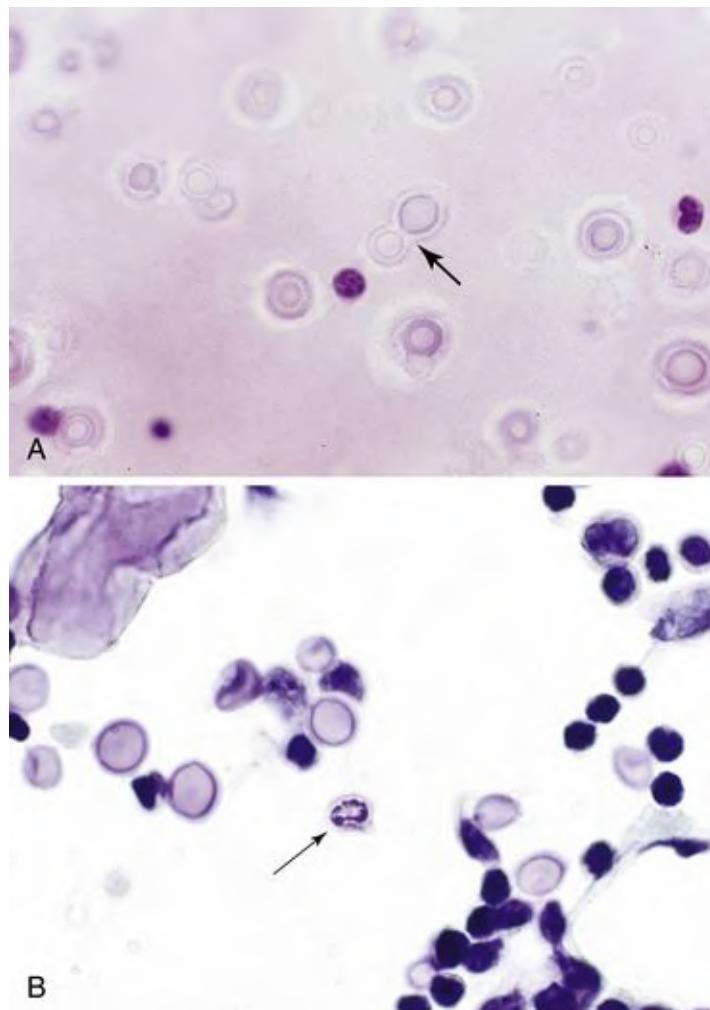


FIGURE 6.16 Cryptococcal meningitis.

A, The organisms have a mucopolysaccharide capsule. Note the characteristic thin-necked budding (arrow; Papanicolaou stain). B, Some organisms are cup-shaped, trapping air and producing a refractile artifact (arrow; Papanicolaou stain).

Toxoplasmosis

In immunocompromised patients, the protozoan *Toxoplasma gondii* can cause a

variety of diseases of the CNS, including meningoencephalitis.



Cytomorphology of *Toxoplasma* meningoencephalitis

- neutrophils
- mononuclear cells
- tachyzoites

Toxoplasma tachyzoites are small, crescent-shaped organisms, 3 to 6 μm in length, with a tiny, round nucleus⁵⁰⁻⁵¹ ([Fig. 6.17](#)). Although visible on routine cytologic preparations of CSF, tachyzoites are easily missed because of their small size. In patients who develop obstructive hydrocephalus, tachyzoites are more likely to be found in ventricular rather than in lumbar samples.⁵⁰

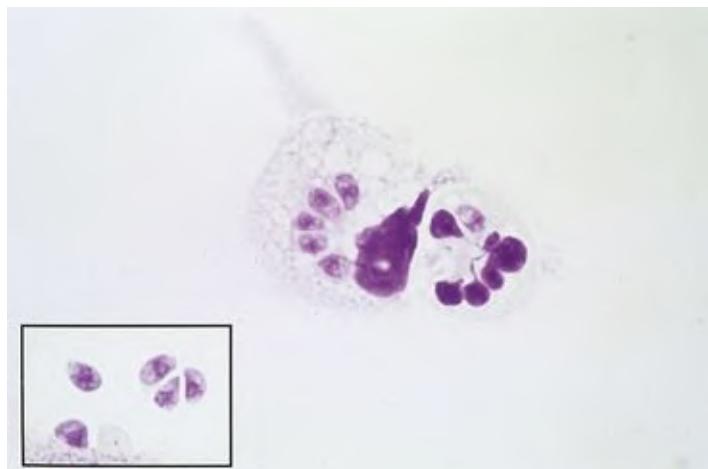


FIGURE 6.17 *Toxoplasma* meningoencephalitis.

T. gondii is a small bow-shaped organism with a single tiny nucleus. It can be intracellular or extracellular (inset) (Papanicolaou stain).

Cysticercosis

Cerebral cysticercosis results from colonization of the brain by larvae of the tapeworm *Taenia solium*. Clinical symptoms are nonspecific. Imaging studies usually show a focal lesion in the brain.



Cytomorphology of cysticercosis

- eosinophils
- mononuclear cells

Larvae are not seen in the CSF. Serologic tests confirm the diagnosis.

Angiostrongyliasis

The rat lungworm *Angiostrongylus cantonensis* is endemic to Asia, particularly the Pacific Basin (including Hawaii), but is found elsewhere, including several Caribbean nations.⁵² Humans become infected by consuming raw snails or contaminated vegetables. Infection, with subsequent migration of larvae to the CNS, results in an eosinophilic meningitis. Headache is the most common presenting symptom. The percentage of eosinophils in CSF is usually very high (20% to 70%). Larvae are occasionally seen in the fluid.⁵³ Focal lesions on computed tomography (CT) examination are usually absent, thus helping to distinguish angiostrongyliasis from cysticercosis. The disease is usually self-limited, and patients recover completely.

Other roundworm infections that usually present as eosinophilic meningitis are *Gnathostoma spinigerum*, a parasite of dogs and cats, and *Baylisascaris procyonis*, a parasite of raccoons.⁵³

Primary Amebic Meningoencephalitis

The free-living ameba *Naegleria fowleri* can cause acute meningoencephalitis. The organisms enter the subarachnoid space through the nose, often while the person is swimming in stagnant water or in an unchlorinated swimming pool. The organisms are best seen on wet preparations, where their motility can be appreciated.⁵⁴ With conventional cytologic preparations they can be difficult to distinguish from mononuclear cells. They have a relatively large nucleus and little cytoplasm, unlike *Entamoeba histolytica*. Fortunately, the disease is rare; it can be fatal within several days.

Primary amebic meningoencephalitis must be distinguished from an amebic brain abscess caused by *Entamoeba histolytica*. Amebae are not seen in the CSF with the latter infection.

Neoplasms

Involvement of the subarachnoid space by malignancy occurs in 5% to 8% of patients with cancer.⁵⁵ Some tumors, like small cell carcinoma of the lung (11%) and melanoma (20%), have a greater predilection than most for involving the leptomeninges.⁵⁶ Malignant cells gain access to the subarachnoid space by hematogenous dissemination, direct extension from a parenchymal brain lesion, or by tracking along spinal or cranial nerves. Involvement of the leptomeninges by tumor is often referred to as carcinomatous (or lymphomatous, etc.) meningitis, but the term *leptomeningeal metastasis* is best because it applies to all tumors that involve the meninges.

The clinical presentation of patients with leptomeningeal metastasis is highly variable. Symptoms can include headache, mental changes, gait difficulty, diplopia, back pain, and lower extremity weakness. Gadolinium-enhanced magnetic resonance imaging (MRI) often shows suspicious enhancement of the leptomeninges when malignancy is present,⁵⁷ but CSF for cytology is needed to confirm the diagnosis.

CSF examination is an important component in the diagnosis of leptomeningeal metastasis. Many patients have elevated opening pressure, with elevated protein and depressed glucose levels.⁵⁵ Cytologic examination of CSF, however, is essential for documenting leptomeningeal metastasis. Malignant cells are usually easily identified in CSF samples because they are strikingly different from the normal cells of CSF. Thus, most CSF samples are easily classified as either negative or positive for malignancy. When findings are inconclusive, often because of scant cellularity or poor preservation, a “suspicious” or “atypical” interpretation is warranted. Not surprisingly, the greatest difficulty is in the diagnosis of lymphoma and leukemia.

When malignant cells are identified, the clinical history often points to their site of origin. In most cases the patient is known to have a malignancy, and the findings simply confirm metastasis. The most commonly encountered malignancies in CSF are lung and breast cancer, melanoma, lymphoma, and leukemia.

In about 10% of patients with a positive CSF, cytologic examination provides the first documentation of a neoplasm.⁵⁸ The lung is by far the most common occult primary site,⁵⁹⁻⁶¹ followed by gastric cancer and melanoma. Patients with lymphoma, leukemia, or a primary CNS tumor can also present with a positive CSF examination. Curiously, a primary breast cancer is very rarely occult when

CSF involvement is detected.



Likely primaries in a patient with positive cerebrospinal fluid and no history of cancer

- lung
- stomach
- melanoma
- lymphoma
- CNS

Metastatic tumors are much more frequent than primary CNS tumors in CSF; primary CNS tumors account for only 6% of all positive CSF samples.⁵⁸

Immunocytochemistry is not needed to diagnose most cases of leptomeningeal metastasis.⁶² In selected cases, however, it is useful for establishing a likely primary site, for example, in a patient whose cancer first manifests as a positive CSF. Epithelial markers can establish a diagnosis of carcinoma and exclude the possibility of lymphoma, melanoma, and a primary glioma. Glial fibrillary acidic protein (GFAP) differentiates glial from nonglial tissue and can be used to confirm a primary CNS origin for malignant cells in CSF⁶³ ([Fig. 6.18A and B](#)). GFAP is useful in establishing a diagnosis of malignancy when the cytologic examination is equivocal,⁶⁴ because normal CSF obtained by LP does not contain GFAP-positive cells.⁶⁵

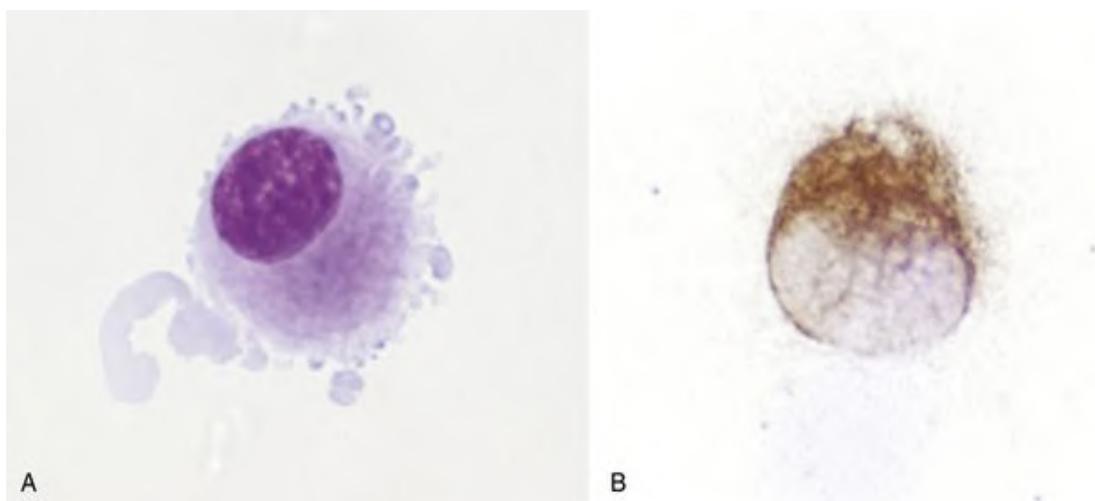


FIGURE 6.18 Astrocytic neoplasm (high grade).

A, The large cell has an oval nucleus, cytoplasmic blebs, and a long cytoplasmic extension (Romanowsky stain). B, An immunostain for glial fibrillary acidic protein (GFAP) is positive, confirming the glial nature of this neoplasm.

Fluorescence in situ hybridization (FISH) can be performed successfully on CSF preparations and provides useful clinical information in selected cases.⁶⁶

The median survival of untreated patients with leptomeningeal metastasis is 1 month.⁵⁵ Treatment stabilizes or improves about 75% of patients, but it is almost always palliative rather than curative, because most current treatments cannot eliminate tumors from the subarachnoid space. The median survival of treated patients ranges from 4 to 10 months and depends in part on the tumor type; breast cancer and lymphoma respond better than most other tumors.⁵⁵ Radiation therapy is often considered in patients with bulky and/or symptomatic leptomeningeal metastasis and is especially helpful in relieving pain and other symptoms. Intrathecal chemotherapy, administered by Ommaya reservoir, has been the mainstay of treatment, but its palliative effects are usually short-lived.⁵⁵ The most commonly used intrathecal agents are methotrexate, cytarabine, and thiotepa. Patients with leptomeningeal involvement by breast and gastric cancers that overexpress human epidermal growth factor receptor 2 (HER2) have shown symptomatic relief and disappearance of tumor cells from CSF after treatment with intrathecal trastuzumab.^{66,67} Systemic chemotherapy is gaining acceptance for treating leptomeningeal metastasis because of its greater ability to penetrate bulkier tumor deposits.⁵⁵

Metastatic Solid Tumors

Carcinoma of the Lung

All four of the common histologic subtypes of lung cancer (adenocarcinoma, squamous cell carcinoma [SQC], large cell carcinoma, and small cell carcinoma) can metastasize to CSF pathways. Adenocarcinomas of the lung are common and SQCs uncommon in CSF.



Cytomorphology of adenocarcinoma of the lung

- isolated cells and/or small clusters
- large cells
- abundant cytoplasm
- eccentric nucleus (some cases)

The differential diagnosis of metastatic adenocarcinoma of the lung to CSF ([Fig. 6.19](#)) includes macrophages, plasma cells, and ependymal/choroid plexus cells. Macrophages have smaller nuclei that are pale and often folded or curved, with granular and microvacuolated cytoplasm (see [Fig. 6.10](#)). Plasma cells are smaller than the cells of an adenocarcinoma of the lung, with more condensed chromatin and a tell tale perinuclear hof. Ependymal/choroid plexus cells are rare in CSF; when present, they are few in number, usually with a round, centrally placed nucleus.

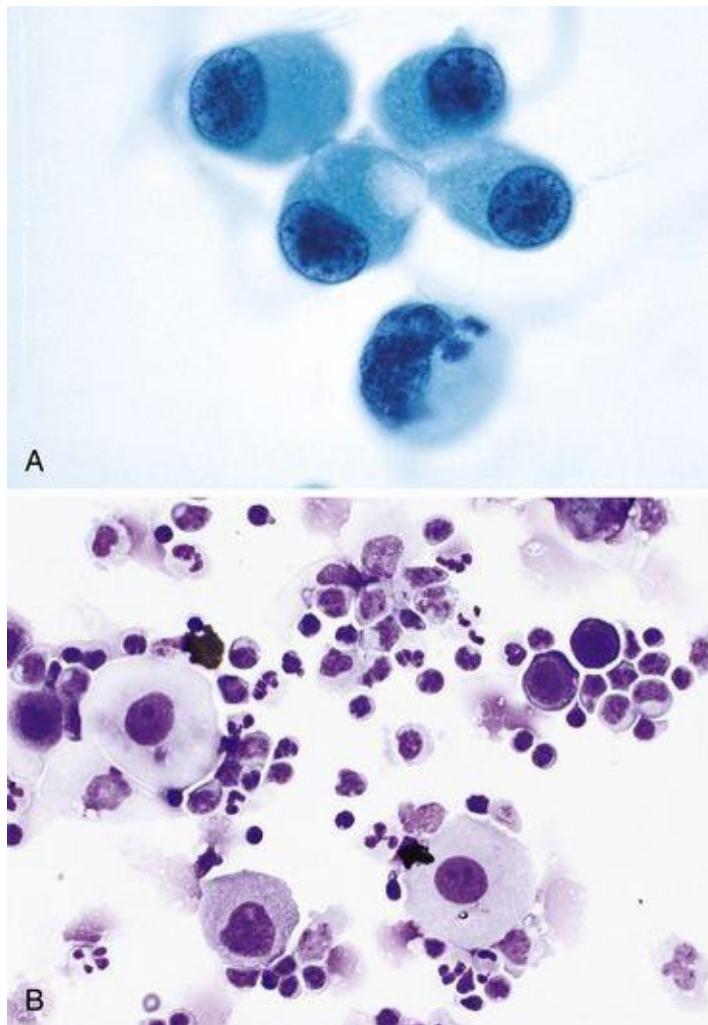


FIGURE 6.19 Adenocarcinoma of the lung.

A, The malignant cells have large hyperchromatic nuclei and abundant cytoplasm. Note the eccentric placement of nuclei, a characteristic feature of some lung adenocarcinomas (Papanicolaou stain). B, The large malignant cells are admixed with lymphocytes, neutrophils, and monocytes (Romanowsky stain).



Cytomorphology of small cell carcinoma of the lung

- isolated cells and/or clusters
- small cells
- nuclear molding
- karyorrhexis
- mitoses

Small cell carcinomas of the lung appear as small isolated or clustered cells in CSF. When isolated, they are easily mistaken for lymphocytes. Finding clusters with their characteristic molding is often essential for diagnosis ([Fig. 6.20](#)). Linear, molded arrangements with a “vertebral body” appearance are seen. The differential diagnosis includes other small cell malignancies, most of them pediatric, like medulloblastoma. A cytomorphologic distinction is impossible, but this rarely presents a problem, because patients usually have a history of small cell carcinoma or a suspicious lung mass.

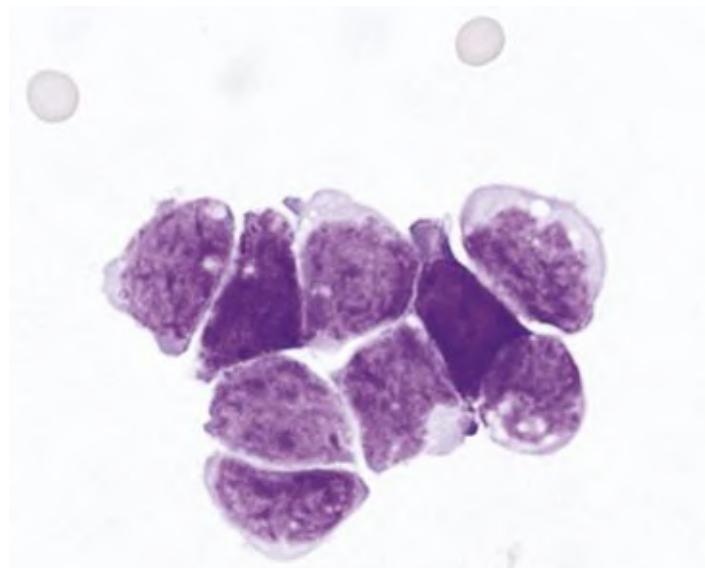


FIGURE 6.20 Small cell carcinoma of the lung.

The small cells have dispersed chromatin, indistinct nucleoli, and scant cytoplasm. Nuclear molding is prominent (Romanowsky stain).

Carcinoma of the Breast

Breast cancer not uncommonly metastasizes to the CSF. In contrast with lung cancer, it is rare that a positive CSF is the initial presentation of malignancy in a woman with breast cancer.



Cytomorphology of ductal breast cancer

- isolated cells or small groups
- linear rows, rings (rare)
- large cells
- round nucleus
- prominent nucleolus
- scant cytoplasm (often)

The large cannonball-like arrangements typical of breast cancer cells in pleural fluid (see [Fig. 4.22A](#)) are almost never seen in CSF. Instead, the malignant cells are usually scattered and isolated ([Fig. 6.21](#)). Nuclei are round or irregular, and some cells can be binucleated.

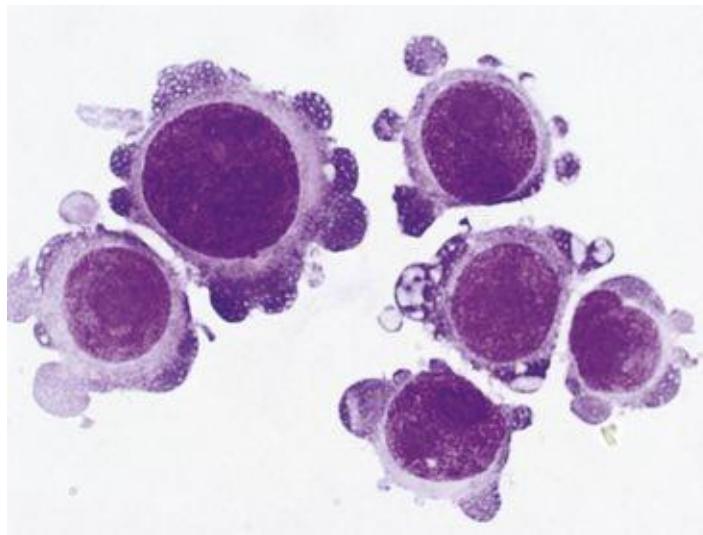


FIGURE 6.21 Ductal carcinoma of the breast.

The malignant cells of this grade 3 ductal cancer are round, large, and highly variable in size. Cytoplasmic blebs are commonly seen (Romanowsky stain).



Cytomorphology of lobular breast cancer

- medium-sized cells
- isolated cells
- signet ring shapes

Lobular breast cancers are morphologically similar to ductal cancers in CSF except the cells are somewhat smaller ([Fig. 6.22](#)). The differential diagnosis of ductal and lobular breast cancers is similar to that for adenocarcinoma of the lung and includes macrophages and ependymal/choroid plexus cells. Macrophages have smaller, pale, often folded or curved nuclei, with abundant granular and microvacuolated cytoplasm (see [Fig. 6.10](#)). Ependymal/choroid plexus cells are rare in CSF; when present, they are few in number and usually have a round, centrally placed nucleus. Some lobular breast cancer cells in CSF form linear arrangements reminiscent of the vertebral body-like structures seen with small cell carcinoma.

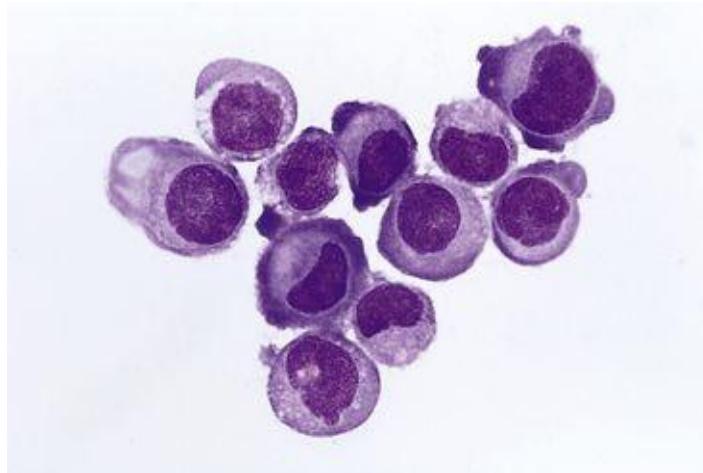


FIGURE 6.22 Lobular carcinoma of the breast.

These malignant cells have round or semilunar nuclei, and some have prominent nucleoli (Romanowsky stain).

Melanoma

Melanoma can metastasize to the meninges from a primary in the skin or other site, but it can also (rarely) arise in the leptomeninges as a primary CNS neoplasm. In a condition known as melanosis cerebri, the meninges contain

melanocytes; presumably, it is these cells that give rise to primary leptomeningeal melanomas.^{68,69}



Cytomorphology of melanoma

- large cells
- macronucleolus
- melanin (not always)
- melanophages

With a history of melanoma, the diagnosis is straightforward in most cases ([Fig. 6.23](#)). Rarely, as in a patient without a documented melanoma whose malignant cells lack visible melanin, the distinction from a carcinoma may require immunocytochemistry. Stains for HMB45 and S-100 are generally positive, whereas stains for keratin and epithelial membrane antigen are negative.

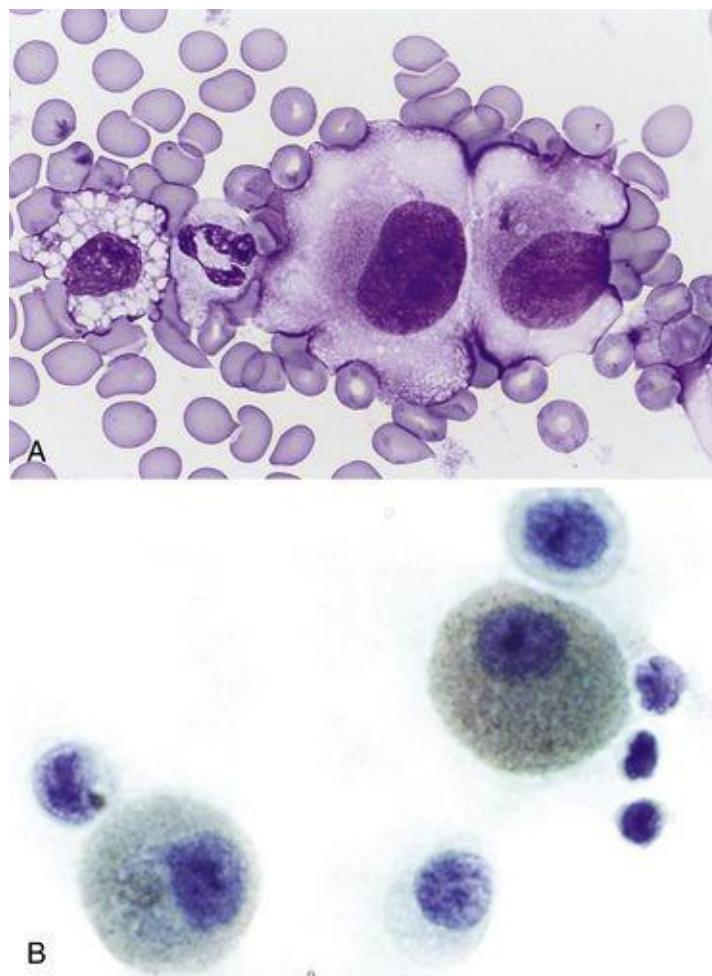


FIGURE 6.23 Melanoma.

A, Melanoma cells tend to be isolated or only loosely aggregated. The two cells shown here are amelanotic (Romanowsky stain). B, Melanin pigment is finely granular in malignant cells (Papanicolaou stain).

Leukemia

Acute Lymphoblastic Leukemia

ALL, a malignancy of lymphocyte precursors that arises from the bone marrow, is the most common malignancy in children. The peak incidence is between the ages of 2 and 7 years, but adults are also affected. Patients often present with pallor and weakness because of anemia and bleeding resulting from thrombocytopenia. They can have lymphadenopathy, hepatosplenomegaly, and an anterior mediastinal mass. The diagnosis is made by bone marrow aspiration and biopsy, which shows replacement of normal elements by lymphoblasts. CNS involvement is present at initial diagnosis in 5% of cases and is usually occult.²⁰

In fact, 75% of ALL patients with leptomeningeal metastasis are asymptomatic.²¹ Very rarely, patients with ALL or acute myeloid leukemia (AML) present with blasts in CSF but not in the peripheral blood.²²

Most childhood ALLs are B-cell neoplasms. T-cell ALL comprises about 15% of childhood ALL and about 25% of adult cases.²³

The appearance of ALL cells is variable. According to the French-American-British (FAB) classification system, ALL is divided into types L1, L2, and L3 based on the cytomorphologic appearance of blasts on air-dried Romanowsky-stained preparations ([Fig. 6.24A-C](#)).²⁴ Because this morphologic classification has no clinical or prognostic relevance, the World Health Organization (WHO) replaced the FAB classification with an immunophenotypic and cytogenetic classification that carries prognostic significance.²⁵ In the WHO classification, for example, ALL L3 is considered the leukemic variant of Burkitt lymphoma and not a subset of ALL. Nevertheless, knowledge of the spectrum of morphologic appearances of ALL can be very useful in evaluating CSF, particularly in correlating CSF findings with prior bone marrow aspirates.

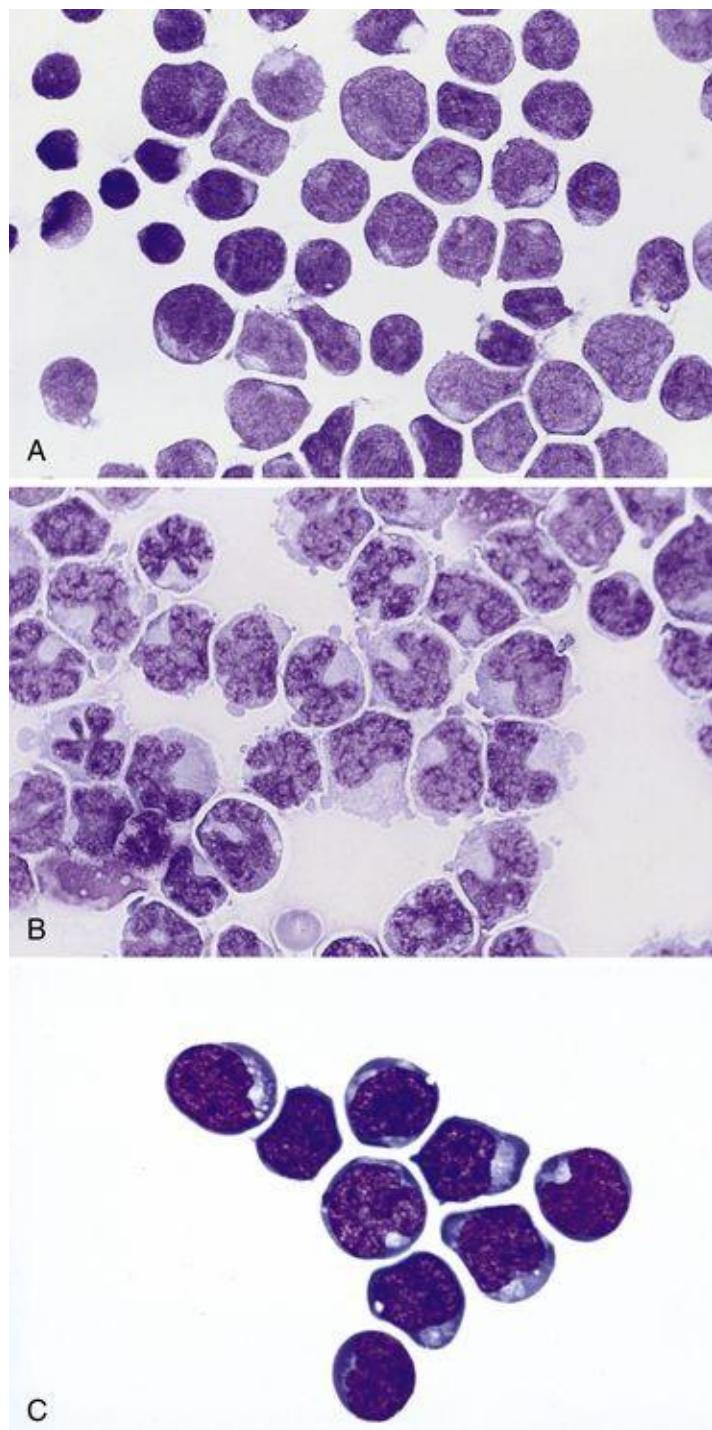


FIGURE 6.24 Acute lymphoblastic leukemia (ALL) and the leukemic variant of Burkitt lymphoma.

Depending on the subtype, the blasts of ALL may have several different appearances, which are best appreciated on air-dried preparations. A, L1 blasts are small, with predominantly round nuclei and scant cytoplasm. B, L2 blasts are larger, with irregular nuclei and more abundant cytoplasm. C, L3 blasts have coarse chromatin, dark-blue cytoplasm, and small lipid vacuoles. In the World Health Organization (WHO) classification, this is considered a leukemic variant of Burkitt lymphoma and not ALL. (A-C, Romanowsky stain.)



Cytomorphology of acute lymphoblastic leukemia (with Romanowsky stains)

- L1
 - small blasts
 - round nucleus (rare clefts)
 - fine chromatin
 - inconspicuous nucleolus
 - scant, slightly basophilic cytoplasm
- L2
 - larger blasts
 - irregular nucleus
 - fine chromatin
 - prominent nucleolus
 - abundant cytoplasm
- L3 (leukemic variant of Burkitt lymphoma)
 - coarse chromatin
 - multiple nucleoli
 - dark-blue cytoplasm
 - small cytoplasmic vacuoles (lipid)

In a small percentage of cases, the cells of ALL contain azurophilic cytoplasmic inclusions that resemble myeloid granules.

The treatment of ALL in children is one of the great successes of chemotherapy. At one time ALL was almost invariably fatal; today nearly 60% of children are long-term survivors of the disease. CNS prophylaxis is a mainstay of therapy. Without CNS prophylaxis, more than 50% of patients would develop CNS leukemia. Although prophylactic treatment of the CNS with radiotherapy to the cranium and intrathecal methotrexate has reduced the incidence of such relapses dramatically, they still occur in 5% to 10% of cases. Therefore, periodic monitoring of CSF for the presence of blasts is essential.



Differential diagnosis of acute lymphoblastic leukemia

- reactive lymphocytes (e.g., aseptic meningitis)
- peripheral blood contamination

When the sample is hypercellular, the diagnosis is usually straightforward, particularly with air-dried Romanowsky-stained slides, which accentuate the characteristic morphologic features. Sparsely cellular samples can be difficult. Immunocytochemistry for terminal deoxytransferase (Tdt), a nuclear enzyme found in 90% to 95% of L1 and L2 leukemias (but not in type L3), can be extremely useful in distinguishing blasts from reactive lymphocytes, which are consistently negative for this marker. An aliquot of fluid is also often sent to the hematology laboratory, and blasts, if present, are noted in the differential count. Contamination of CSF by blasts in peripheral blood must be excluded,¹⁸ because this can result in a false-positive diagnosis.²⁶ If CSF contains leukemic blasts admixed with red blood cells, the specimen is essentially noncontributory. The only exception is a patient in remission, whose peripheral blood counts are negative for blasts. In such a patient, a traumatic tap with blasts is diagnostic of a CSF recurrence.

Acute Myeloid Leukemia

AMLs are neoplastic proliferations of the myeloid progenitor cells: immature granulocytes, monocytes, erythrocytes, and megakaryocytes. They are more common in adults than in children. AML can involve CSF either at presentation or subsequently,^{22,25} although CSF involvement is less common than in ALL.

The FAB system recognizes eight morphologic types of AML based on bone marrow aspirates²⁶:

- AML undifferentiated (M0)
- AML without maturation (M1)
- AML with maturation (M2)
- acute promyelocytic leukemia (M3)
- acute myelomonocytic leukemia (M4)
- acute monocytic leukemia (M5)
- erythroleukemia (M6)
- acute megakaryoblastic leukemia (M7)

These subtypes have somewhat different clinical presentations. For example, it is more common for patients with acute myelomonocytic leukemia (AML M4) to have CNS involvement at the time of presentation than for those with other types of AML.²⁵ As with ALL, the WHO classification has replaced the FAB classification.



Cytomorphology of acute myeloid leukemias

- round or highly irregular nucleus

- fine chromatin
- prominent nucleolus
- azurophilic granules (sometimes)
- mature granulocytes (some subtypes)

for AML with a system that relies more on clinical and cytogenetic criteria.^{73,77}

As with ALL, characteristic features of AML are best seen on Romanowsky-stained preparations (Fig. 6.25). Although azurophilic granules can be present, they are not diagnostic of AML because they are also seen in some cases of ALL. An important finding in many but not all cases of AML is the Auer rod. This linear structure is a crystallization of azurophilic granules and is pathognomonic of a myeloid disorder. Mature granulocytes can be present. In evaluating the CSF, it is usually sufficient simply to identify blasts, because definitive diagnosis of ALL or AML and their subtypes depends on the evaluation of the bone marrow aspirate and biopsy.

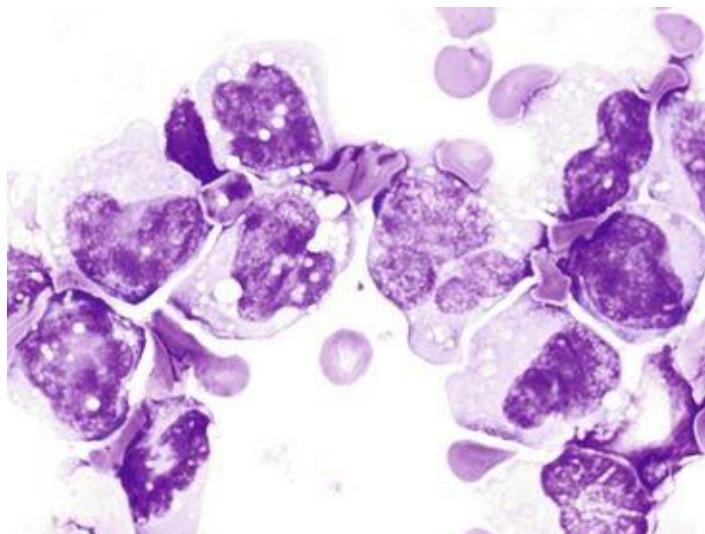


FIGURE 6.25 Acute myelomonocytic leukemia (AML M4).

In cerebrospinal fluid (CSF) from patients with leukemia, it is usually sufficient simply to identify the presence of blasts; further typing (myeloid versus lymphoid and their subtypes) is done on peripheral blood and bone marrow specimens (Romanowsky stain).

Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) predominantly affects adults and has a long and protracted clinical course. Despite this, leptomeningeal metastasis is extremely uncommon.^{71,78,79} The cells of CLL are morphologically indistinguishable from small, mature lymphocytes.⁸⁰ It is very important, therefore, to exclude peripheral blood contamination from a traumatic tap; if the sample contains a significant number of red blood cells, and the patient is known to have circulating leukemic cells at the time of specimen procurement, the CSF sample is essentially noncontributory. Even if red blood cells are scant or absent, immunophenotyping of the lymphoid cells must be performed⁸¹ to exclude meningitis resulting from a compromised immune system.

Myeloproliferative Neoplasms

The myeloproliferative neoplasms are clonal hematopoietic stem cell disorders characterized by a bone marrow proliferation of granulocytic, erythroid, or megakaryocytic cells. Clinically heterogeneous, they include chronic myelogenous leukemia; chronic neutrophilic leukemia; polycythemia vera; primary myelofibrosis; essential thrombocythemia; chronic eosinophilic leukemia; mastocytosis; and myeloproliferative neoplasm, unclassifiable.⁷³ In the initial, chronic phase of the disease, the neoplastic cells are minimally invasive; confined to bone marrow, blood, liver, and spleen; and they virtually never involve the CSF. Unfortunately, most patients eventually develop “blast crisis,” a transformation to acute leukemia that is almost always fatal, involving the CNS in some patients.⁸² As with other leukemias, opportunistic infections are common and must be suspected when a specimen is examined.

Malignant Lymphoma

Leptomeningeal metastasis occurs in 5% to 10% of patients with non-Hodgkin lymphoma, either at presentation, at a later point during the disease course, or at relapse.⁷¹ Leptomeningeal involvement is more common than parenchymal brain involvement by lymphoma. Some histologic types have a higher incidence of CNS involvement than others. Diffuse large B-cell, lymphoblastic, Burkitt, and Burkitt-like lymphomas have an especially high affinity for the CNS. On the other hand, some lymphomas like Hodgkin lymphoma and small lymphocytic

lymphoma are almost never seen in CSF. Patients with leptomeningeal metastasis due to lymphoma have a higher frequency of cranial nerve signs and symptoms.²¹



Cytomorphology of lymphoma in cerebrospinal fluid

- dispersed cells
- larger than normal lymphocytes
- irregular nuclear contours
- abnormal chromatin
- prominent nucleolus

Typically, CSF shows a monomorphic population of highly atypical cells, with perhaps only a small percentage of normal lymphocytes ([Fig. 6.26](#)). In some cases, however, the lymphoma cells are a minority of the total cell population.

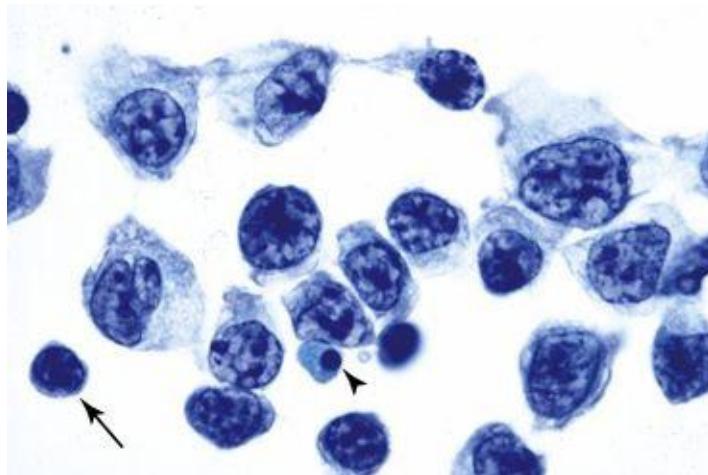


FIGURE 6.26 Diffuse large B-cell lymphoma.

Tumor cells are dispersed as isolated cells with irregular nuclei, coarsely textured chromatin, and scant to abundant cytoplasm. Pyknosis (arrowhead) and karyorrhexis are common and can be prominent. A small, benign lymphocyte is also present (arrow; Papanicolaou stain).

The differential diagnosis is a reactive lymphocytosis caused by meningitis. A reactive lymphocytosis is composed of a heterogeneous population of small, mature lymphocytes as well as larger, activated, so-called atypical lymphocytes.

The distinction from lymphoma, however, is not always straightforward.

Because lymphomas have many different appearances, knowledge of the histologic subtype and comparison with a previous biopsy of a lymph node or other site are helpful. In some cases, definitive diagnosis is not possible without lymphoid marker studies.⁸³ These can be performed on duplicate air-dried cytospins stained with antibodies against a pan B-cell marker (e.g., CD20), a pan T-cell marker (e.g., CD3), and κ and λ immunoglobulin light chains ([Fig. 6.27A-C](#)). Alternatively, fluid can be sent fresh for flow cytometric assessment of lymphocyte surface markers.^{84,85} When used selectively, successful results are obtained by flow cytometry in up to 75% of cases. In combination with CSF cytology, flow cytometry improves sensitivity for lymphoma diagnosis.⁸⁶ Most reactive and inflammatory fluids are composed primarily of T cells (Lyme disease is an exception), whereas the great majority of lymphomas that involve the CNS are B-cell neoplasms. Thus, a predominantly B-cell proliferation in CSF is highly suspicious for lymphoma; the diagnosis can be established by demonstrating light chain restriction (predominance of either κ or λ expression) ([Fig. 6.28](#)). The polymerase chain reaction is useful in selected cases and can be performed on archival as well as fresh CSF samples.^{87,88}

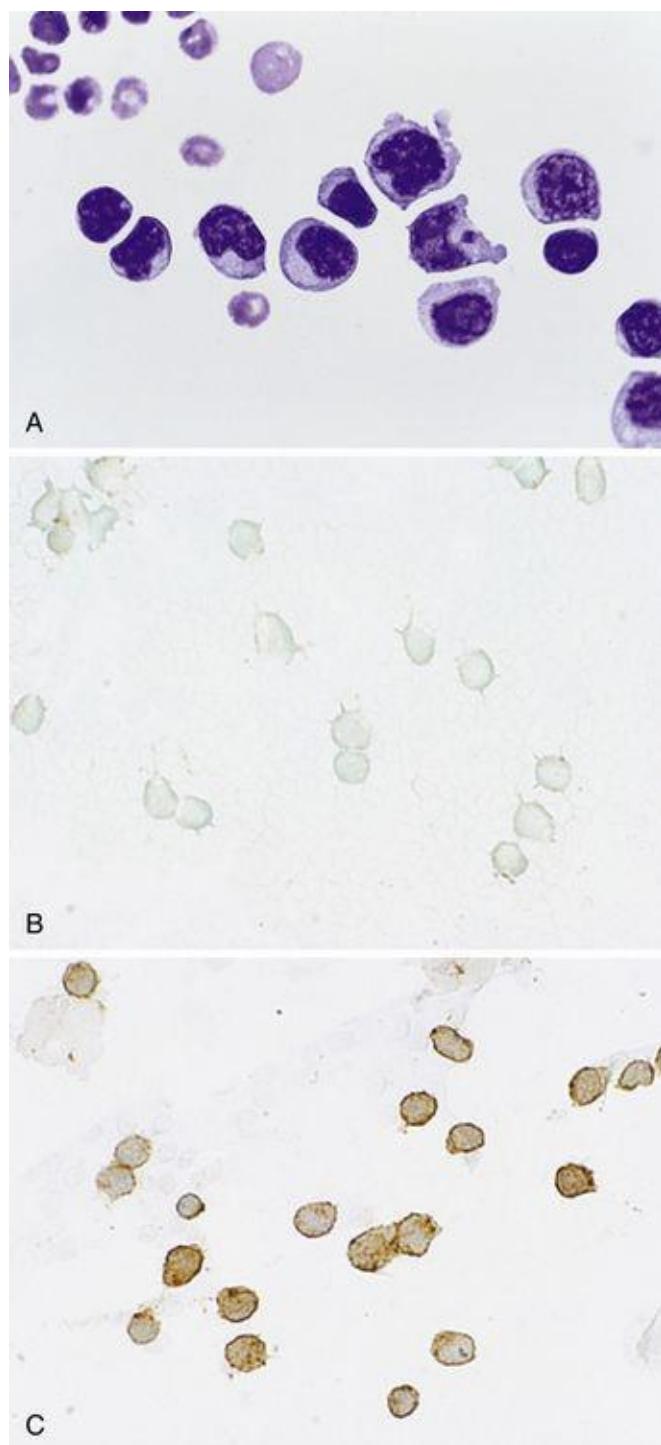


FIGURE 6.27 Follicular lymphoma.
The malignant cells are relatively small and have irregular nuclear contours. The cells show monotypic expression of immunoglobulin light chains. *A*, Romanowsky stain. *B*, κ . *C*, λ .

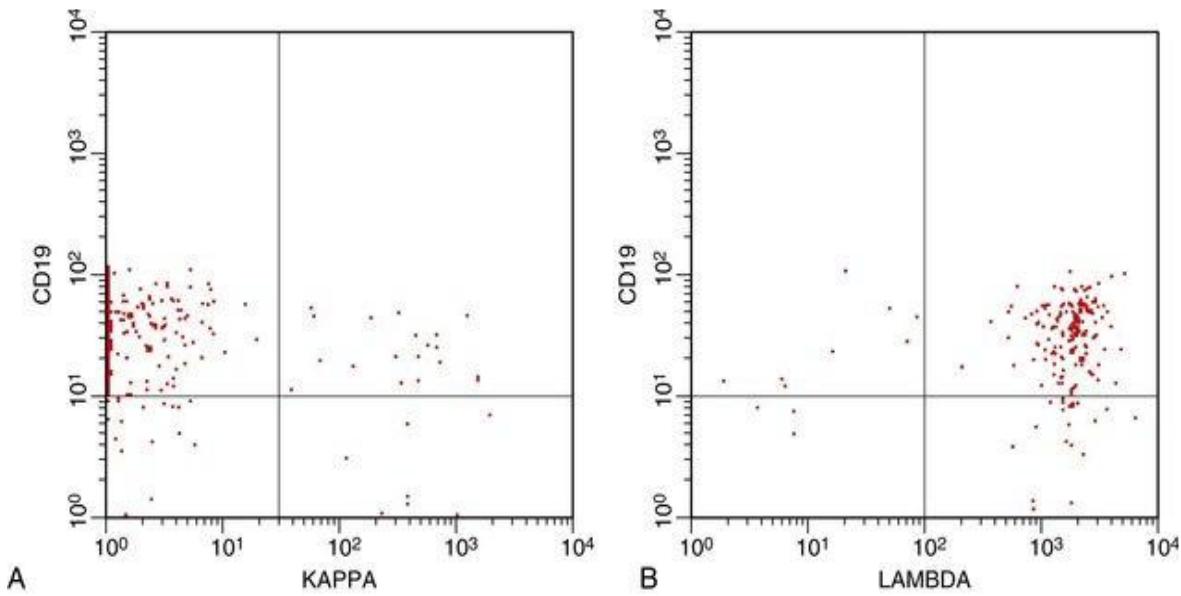


FIGURE 6.28 Flow cytometric analysis of cerebrospinal fluid (CSF) (non-Hodgkin lymphoma).

There is a population of CD19-positive B cells that are negative for κ and positive for λ light chains.

Primary Central Nervous System Tumors

CSF cytology is an important test for documenting leptomeningeal metastasis of a primary CNS tumor, at the time of either presentation or recurrence.⁸⁹ Certain CNS tumors have a greater predilection for involving the leptomeninges than others. These include medulloblastoma, ependymoma, germinoma, pineoblastoma, primitive neuroectodermal tumors (PNETs), atypical teratoid/rhabdoid tumor (ATRT), choroid plexus tumors, astrocytomas and glioblastomas, and primary CNS lymphomas. The clinical features of some of these are summarized in [Table 6.2](#).

TABLE 6.2
CLINICAL FEATURES OF PRIMARY CENTRAL NERVOUS SYSTEM TUMORS THAT SPREAD VIA CEREBROSPINAL FLUID PATHWAYS

Tumor	Predominant Age	Preferred Location(s)
Medulloblastoma	Children	Cerebellum
Glioblastoma	Adults	Cerebral hemispheres
Ependymoma	Children and adolescents	Fourth ventricle (children); spinal cord (adults)
Choroid plexus tumors	Children	Ventricles

Pineoblastoma	Children	Pineal gland
Germ cell tumors	Children and adolescents	Pineal, suprasellar regions
Atypical teratoid/rhabdoid tumor	Infants and children	Cerebellum, brainstem, cerebral hemispheres
Primary central nervous system lymphoma	Adults	Cerebral hemispheres, cerebellum, brainstem, spinal cord

Primary Central Nervous System Lymphoma

Primary CNS lymphoma represents up to 7% of all primary CNS tumors and occurs in both immunocompetent and immunocompromised patients.⁹⁰ Epstein-Barr virus (EBV) is detected in most primary CNS lymphomas in immunocompromised patients and rarely in those arising in immunocompetent individuals.^{90,91} Diffuse large B-cell lymphoma is the most common type, accounting for over 90% of primary CNS lymphomas⁹⁰; the indolent small cell lymphomas and lymphomas of T-cell phenotype occur much less frequently.^{92,93} In most patients with primary CNS lymphoma, the tumor involves the brain parenchyma, with or without leptomeningeal involvement.⁹² But about 8% of cases involve the leptomeninges only (“primary leptomeningeal lymphoma”).^{92,93}

CSF cytology is positive in one third of patients.⁹¹ The diagnosis is difficult because in some cases the large atypical cells are outnumbered by small reactive T cells, thus mimicking aseptic meningitis ([Fig. 6.29](#)). Flow cytometric analysis can be helpful by proving clonality and is highly sensitive; the technique can detect malignant cells that represent as few as 2% of the total cell population.⁸⁵ It is not practical to do flow cytometric analysis on all samples that show a lymphocytic pleocytosis, but flow cytometry should be considered in patients with suspicious clinical symptoms. Localized neurologic signs like cranial nerve palsies are common in patients with primary CNS lymphoma and rare in patients with aseptic meningitis.

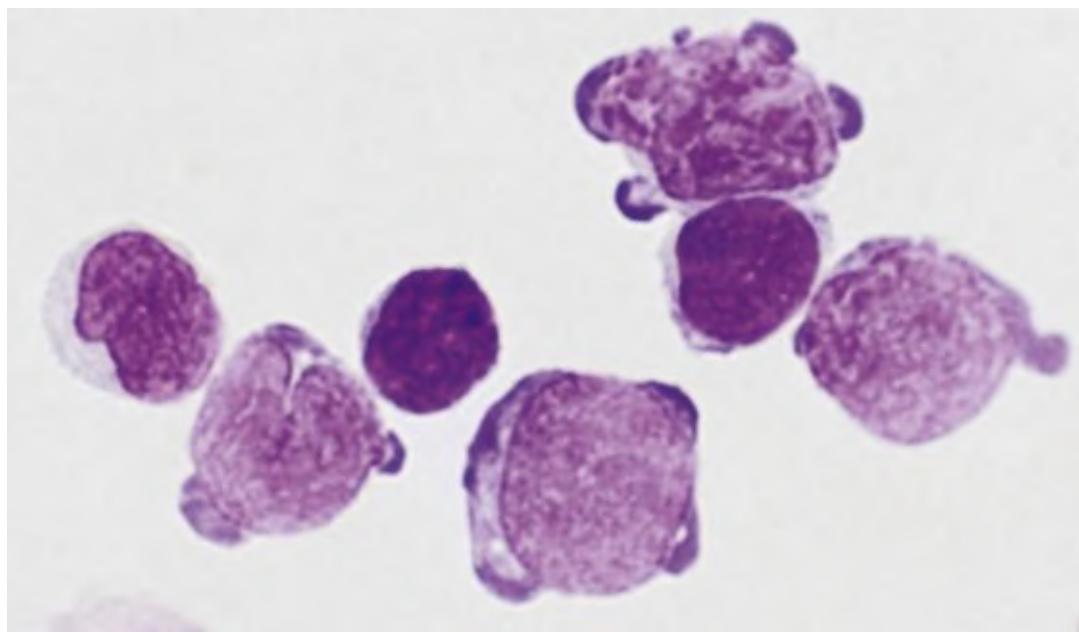


FIGURE 6.29 Primary central nervous system (CNS) lymphoma. Five large, atypical lymphoid cells are present in this field, one with a prominent nuclear cleave. (Two benign lymphocytes are smaller, with darker nuclei.) In some cases of primary CNS lymphoma, the malignant B cells are outnumbered by small reactive T cells, and the findings mimic aseptic meningitis. (Compare with [Fig. 6.14B](#).) In this case there was a high clinical suspicion of lymphoma because the patient had cranial nerve findings (diplopia and facial droop). A portion of the sample was sent for flow cytometry, which showed immunoglobulin light chain restriction by the large cells (Romanowsky stain).



Cytomorphology of medulloblastoma

- small to medium-sized cells
- hyperchromatic nucleus
- scant cytoplasm
- nucleolus may be prominent
- nuclear molding



Differential diagnosis of medulloblastoma

- poorly preserved lymphocytes ([Fig. 6.31](#))

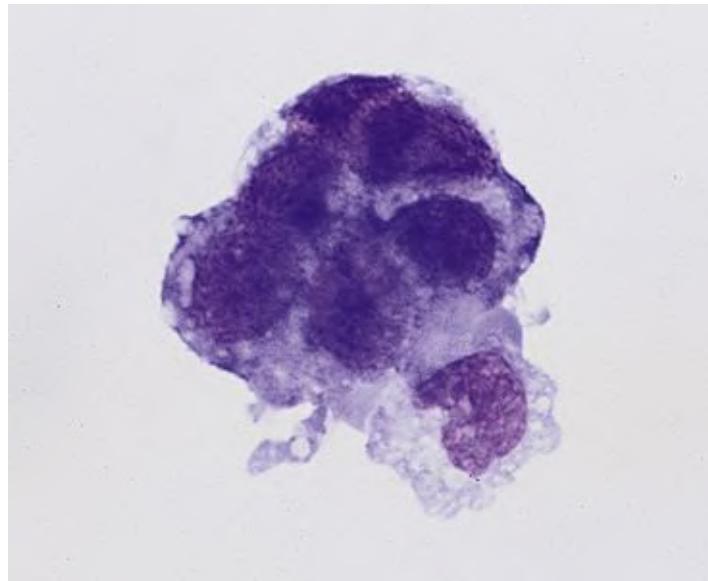


FIGURE 6.31 Poorly preserved lymphocytes and monocytes mimicking medulloblastoma.

Particularly in cerebrospinal fluid (CSF) samples older than 48 hours, lymphocytes and monocytes undergo degeneration; they cluster together uncharacteristically and even show nuclear molding (Romanowsky stain).

- germinal matrix
- small cell carcinoma
- pineoblastoma
- neuroblastoma
- retinoblastoma
- anaplastic ependymoma

Medulloblastoma

Medulloblastoma is a poorly differentiated small cell tumor of uncertain histogenesis that arises in the cerebellum. It is predominantly a disease of children (peak age at presentation is 7 years) but is sometimes seen in adults.⁹⁰ It tends to invade the adjacent fourth ventricle and/or meninges; approximately 25% of patients with medulloblastoma have positive CSF cytology (Fig. 6.30A and B).⁹⁴ At autopsy, leptomeningeal involvement is discovered in more than 50% of cases.⁹⁵ Morphologically similar tumors in the cerebrum or suprasellar region are called supratentorial PNETs.

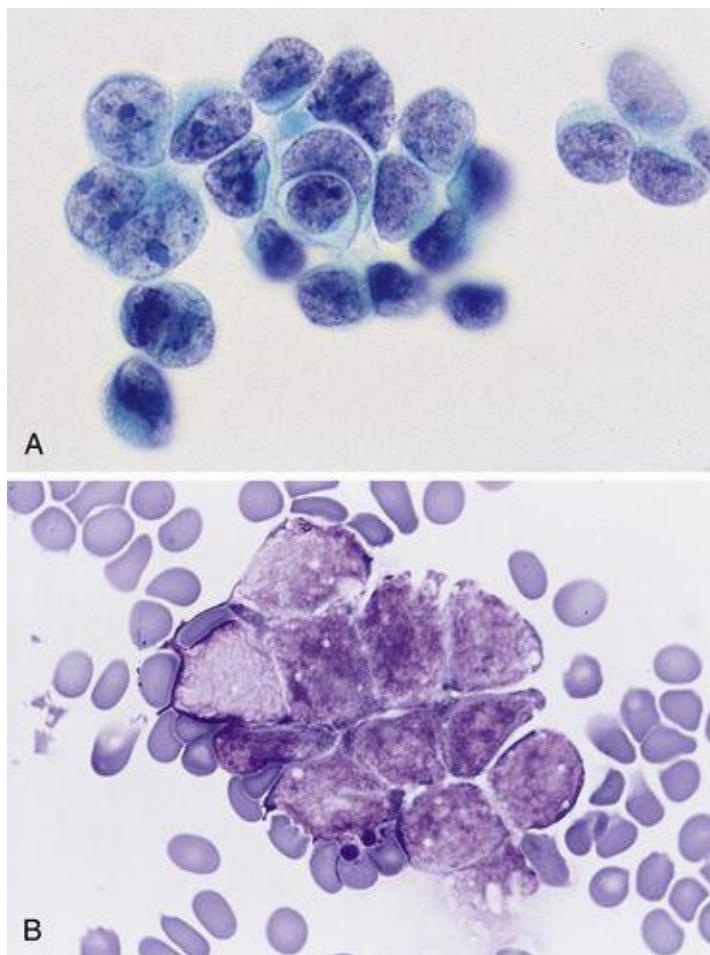


FIGURE 6.30 Medulloblastoma.

The tumor cells are small, with hyperchromatic nuclei and scant cytoplasm. Nuclear molding is prominent. *A*, Papanicolaou stain. *B*, Romanowsky stain.

When clusters of small cells are seen in a neonate, especially one born prematurely, they most likely represent cells of germinal matrix origin³² (see Fig. 6.7B). These cells mimic a poorly differentiated small cell malignancy like medulloblastoma. Clinical correlation is very important; the possibility of benign cells of germinal matrix origin should be considered in all neonates with hydrocephalus and in premature infants with intraventricular hemorrhage. In cases associated with hemorrhage, hemosiderin-laden macrophages are numerous. Morphologically, medulloblastoma cells are indistinguishable from those of other anaplastic small cell tumors like small cell carcinoma of the lung (see Fig. 6.20), but clinical and radiographic findings help in establishing the diagnosis. Anaplastic ependymoma of the fourth ventricle may be clinically and cytologically impossible to distinguish from medulloblastoma, however.

Astrocytomas and Glioblastoma

This group of tumors is the most commonly encountered primary CNS malignancy in adults. They arise from astrocytes, the supporting cells of the CNS, and are subtyped (and graded) as pilocytic astrocytoma (WHO grade I), diffuse astrocytoma (WHO grade II), anaplastic astrocytoma (WHO grade III), glioblastoma (WHO grade IV), gliomatosis cerebri, and a few other rare types, based in part on the degree of nuclear pleomorphism, necrosis, and vascular proliferation.⁹⁰ These tumors can arise virtually anywhere in the CNS (cerebrum, cerebellum, brainstem, and spinal cord), and all types can spread to the ventricles and subarachnoid space.

Glioblastoma, the highest-grade astrocytoma, is the most common of all brain tumors. It comprises 12% to 15% of all intracranial tumors and more than half of all astrocytic neoplasms.⁹⁰ *Pilocytic astrocytoma* is a circumscribed, slowly growing, often cystic tumor that arises in children and young adults. Subarachnoid space involvement is a characteristic feature of pilocytic astrocytomas, and CSF is positive in 11% of patients in whom it is examined,⁹⁶ but dissemination along CSF pathways is uncommon. An uncommon type of astrocytic neoplasm, *gliomatosis cerebri*, presents as a diffuse astrocytic proliferation without an obvious tumor mass. It often spreads to involve the subarachnoid space, and CSF examination is useful in establishing a diagnosis.⁹⁷



Cytomorphology of anaplastic astrocytoma and glioblastoma

- large pleomorphic cells, or
- smaller, anaplastic cells with hyperchromatic nuclei

Anaplastic astrocytomas and glioblastomas appear as isolated cells and small clusters. They have hyperchromatic, highly pleomorphic nuclei with coarse chromatin, irregular nuclear outlines, and prominent nucleoli⁹⁸ ([Fig. 6.32](#)). Cytoplasmic extensions are seen in some cases (see [Fig. 6.18A](#)), and the cells are often immunoreactive for GFAP (see [Fig. 6.18B](#)).

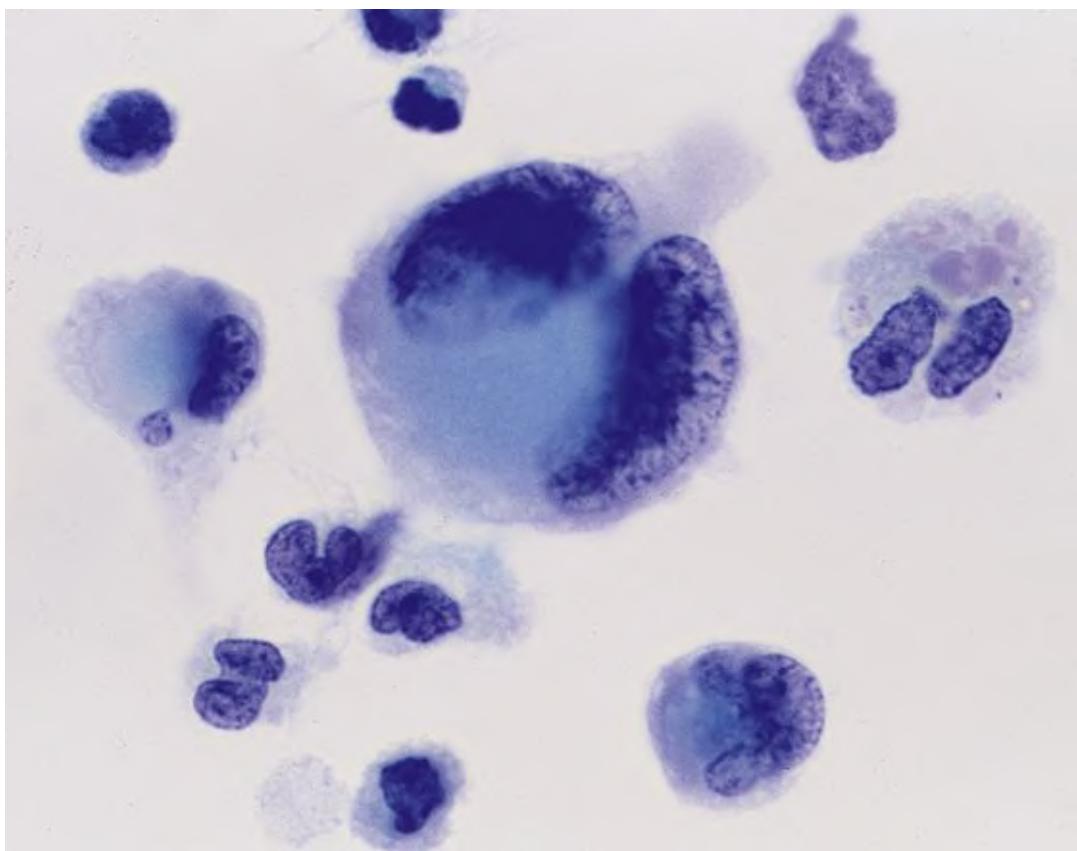


FIGURE 6.32 Glioblastoma.

The malignant cells are highly pleomorphic, with hyperchromatic nuclei and abundant cytoplasm (Papanicolaou stain).

Pilocytic astrocytomas appear in CSF as isolated cells or clusters. The isolated cells have long, hairlike cytoplasmic processes, and clustered cells appear epithelioid, often with finely textured chromatin and cobweblike cytoplasm.⁹⁶

Ependymoma

Ependymomas arise from the lining cells of the ventricles and can occur anywhere in the CNS, but the fourth ventricle and spinal cord are the most common sites. Although more common in children and adolescents, they are also seen in adults. Histologically, ependymomas are considered WHO grade II neoplasms and are comprised of monomorphic cells that form perivascular pseudorosettes and ependymal rosettes, and mitoses are rare. The prognosis is poor because their location makes complete surgical excision challenging. Of the patients who undergo CSF cytologic examination, 11% have either suspicious or positive cytology.⁹⁹ Clinically significant leptomeningeal metastasis is uncommon, however, most likely because of the inability of the seedlings to

adhere and proliferate.⁹⁵

The *myxopapillary ependymoma* is a slowly growing, WHO grade I tumor with a favorable prognosis. It accounts for 9% to 13% of all ependymomas, has a predilection for young adults, and is virtually always located at the terminal end of the spinal cord.⁹⁰ *Anaplastic ependymomas* represent the other end of the spectrum of ependymomas. Considered WHO grade III tumors, anaplastic ependymomas are poorly differentiated, with brisk mitotic activity.



Cytomorphology of ependymomas

- isolated cells or small groups
- round, eccentrically placed nucleus

The appearance of ependymoma cells in CSF depends on the histologic subtype.⁹⁹ The cells of the usual type of ependymoma are cuboidal or columnar (Fig. 6.33), with a round or oval, bland nucleus and a moderate amount of cytoplasm.^{98,99} They can be difficult to distinguish from benign ependymal cells.^{4,100,101} In patients with a tanycytic ependymoma, CSF samples show bipolar cells with long, hairlike glial processes.⁹⁹ Anaplastic ependymomas are cytologically indistinguishable from medulloblastomas.

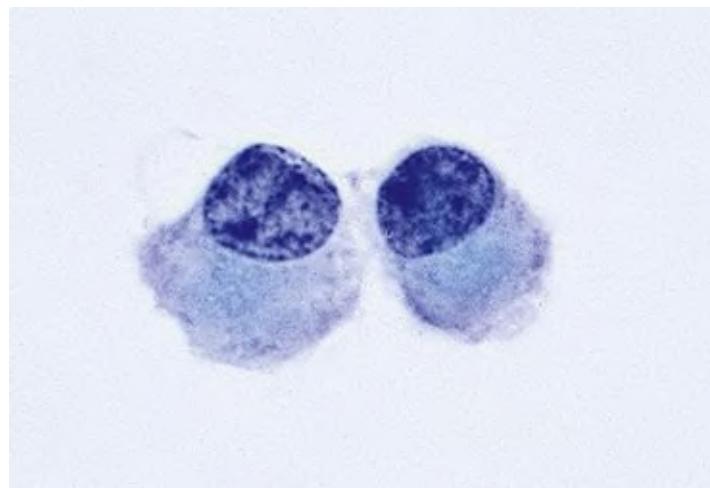


FIGURE 6.33 Ependymoma.

The tumor cells have a round, eccentrically placed nucleus (Papanicolaou stain).

Oligodendrolioma

This tumor of oligodendrocytes is more common in adults but is also seen in children. The great majority occur in the cerebral hemispheres. Spread to the CSF can be either rapid and fatal or chronic and sustained. The tumor is composed of uniform polygonal cells with round nuclei. In tissue sections there is a pronounced perinuclear cytoplasmic clearing that imparts a characteristic “fried egg” or “honeycomb” appearance to the tumor cells. Positive CSF has been reported,^{[101,102](#)} but the cytologic features have not been described in depth.



Cytomorphology of oligodendrolioma

- uniform round cells
- distinct cell outlines
- abundant clear cytoplasm
- round nucleus
- fine chromatin
- prominent nucleolus

Atypical Teratoid/Rhabdoid Tumor

The ATRT is a CNS tumor of unknown histogenesis that predominantly affects infants and children. More than 20% of patients have seeding of CSF pathways at presentation.^{[90](#)} Histologically and cytologically, the tumor contains rhabdoid cells: medium-sized to large cells with a round, eccentrically placed nucleus and a prominent nucleolus. The cytoplasm is homogeneous and may contain a large, poorly defined, dense, inclusion-like structure that pushes aside the nucleus ([Fig. 6.34](#)). Binucleated cells can be seen. Two thirds of cases have a poorly differentiated small cell component that resembles medulloblastoma cells in CSF. ATRT cells are immunoreactive for epithelial membrane antigen and vimentin, and they may express smooth muscle actin, GFAP, neurofilament protein, and keratin.

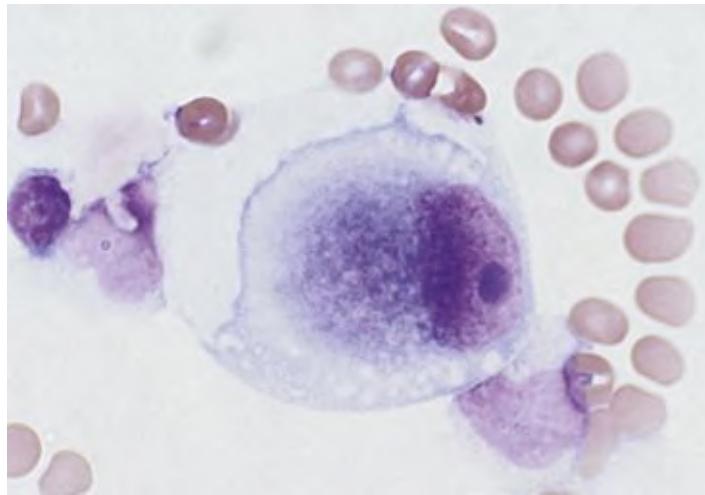


FIGURE 6.34 Atypical teratoid/rhabdoid tumor (ATRT).

This large rhabdoid cell has a dense cytoplasmic “body” that pushes the nucleus to the side. A lymphocyte, two monocytes, and some red blood cells are also present (Romanowsky stain).

Choroid Plexus Tumors

Tumors of the choroid plexus account for 0.3% to 0.6% of intracranial tumors. Predominantly seen in children, they also occur in adults. They arise in the lateral, fourth, and third ventricles. Histologically, they are subtyped as choroid plexus papilloma (WHO grade I), atypical choroid plexus papilloma (WHO grade II), and choroid plexus carcinoma (WHO grade III). The great majority are choroid plexus papillomas. Histologically, the tumors are papillary, composed of a fibrovascular core covered by a single layer of cuboidal or columnar epithelium.



Cytomorphology of choroid plexus papilloma

- large clusters
- uniform cuboidal cells
- round nucleus

The individual cells of a choroid plexus papilloma can be indistinguishable from normal choroid plexus or ependymal cells ([Fig. 6.35](#)). When present in abundance and in large clusters, the diagnosis of a papilloma is suggested.¹⁰³

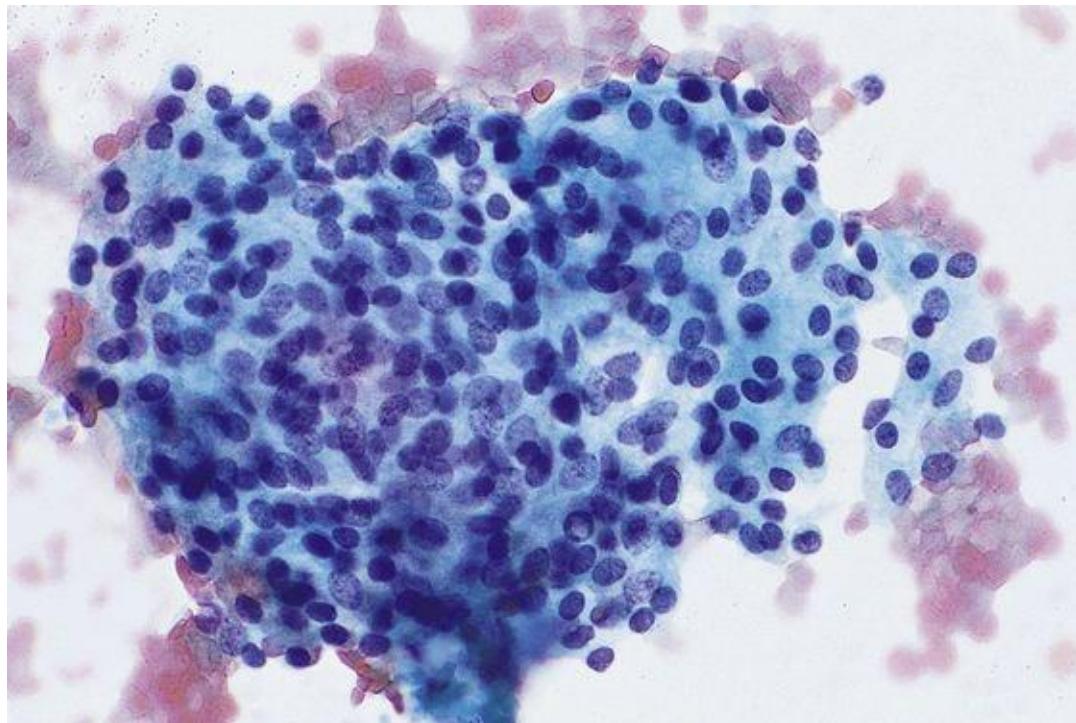


FIGURE 6.35 Choroid plexus papilloma.

The tumor cells are arranged in large, three-dimensional clusters of uniform cuboidal cells with a round or oval nucleus (Papanicolaou stain).

Choroid plexus carcinomas are rare, arising almost exclusively in infants and children.^{104,105} Their existence in adults has been questioned.⁹⁵



Cytomorphology of choroid plexus carcinoma

- isolated cells or clusters
- pleomorphic nuclei
- prominent nucleolus
- indistinguishable from adenocarcinoma

In adults, a metastasis from an occult lung adenocarcinoma must be excluded clinically before the diagnosis can be considered.

Pineal Tumors

Pineal region tumors are rare, accounting for less than 1% of intracranial

tumors.⁹⁰ More than half of the tumors that arise here are germ cell tumors. The rest arise from astrocytes or from specialized neurons called *pineocytes*. Tumors of pineocyte origin affect children more often than adults. They are subclassified as pineocytoma (WHO grade I), pineoblastoma (WHO grade IV), pineal parenchymal tumor of intermediate differentiation (WHO grade II or III), and the rare papillary tumor of the pineal region.

Pineocytomas are typically localized neoplasms that do not metastasize. CSF metastases do occur in a minority of the tumors of intermediate differentiation.⁹⁰ Pineoblastomas, on the other hand, commonly spread to CSF; in a study of 11 cases, all showed leptomeningeal involvement at autopsy.¹⁰⁶ Pineoblastoma is cytomorphologically indistinguishable from medulloblastoma.

Germ Cell Tumors

Germ cell neoplasms in the brain are typically midline lesions, most often in the pineal and suprasellar areas. The entire spectrum of testicular and ovarian germ cell tumors can be seen. The most common is the germinoma, histologically identical to the testicular seminoma and the ovarian dysgerminoma and equally radiosensitive. It occurs most commonly in children and young adults and is more common in males than in females. It is likely to infiltrate ventricles and meninges and spread via CSF.¹⁰⁷⁻¹⁰⁹ In one study, two of seven patients had positive CSF cytology.¹¹⁰ CSF elevations of α -fetoprotein, β -HCG, and placental alkaline phosphatase are considered strong presumptive evidence in patients suspected of harboring a CNS germ cell tumor.



Cytomorphology of germinoma ([Fig. 6.36](#))

- isolated cells
- large, round nucleus

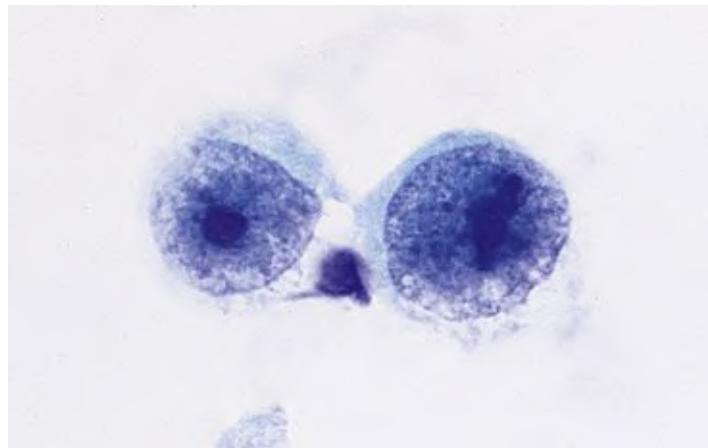


FIGURE 6.36 Germinoma.

The malignant cells are large, with a round nucleus and a prominent nucleolus (Papanicolaou stain).

- prominent nucleolus
- moderate amount of cytoplasm

Other types of germ cell tumors, including embryonal carcinoma, endodermal sinus tumor, teratoma, choriocarcinoma, and various combinations of these tumors can also occur and can be identified in CSF.¹¹¹

Other Tumors of the Central Nervous System

Some of the very common tumors of the CNS rarely spread by CSF pathways and are therefore rarely diagnosed by CSF cytology. Meningiomas constitute 14% of intracranial tumors, and almost all are benign. Their spread via CSF is extremely uncommon¹¹² and probably not distinguishable by cytologic methods.^{21,100} Pituitary tumors represent 10% to 15% of intracranial tumors. Most are benign adenomas, but the very small percentage with CSF or systemic metastases are defined as pituitary carcinomas.¹¹³ In a study of 20 pituitary tumors, CSF was positive in both of the patients whose tumors behaved aggressively.¹¹⁴ Exfoliated cells are arranged in clusters that mimic metastatic adenocarcinoma.⁴

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CHAPTER 7

Gastrointestinal Tract

Helen H. Wang and Gamze Ayata

Clinical Indications

Sampling a Wider Area and Reaching Deep Organs

Better Recognition of Lymphoid Cells

Less Invasiveness

Shorter Turnaround Time

Sample Collection and Preparation

Sample Collection

Processing the Sample

Accuracy

Review of Morphologic Findings

Esophagus

Infections

Epithelial Repair

Barrett's Esophagus

Dysplasia in Barrett's Esophagus

Adenocarcinoma of the Esophagus

Squamous Cell Carcinoma of the Esophagus

Uncommon Tumors of the Esophagus

Stomach

Infections

Epithelial Repair

Dysplasia and Gastric Adenomas

Adenocarcinoma of the Stomach

Neuroendocrine Tumors

Non-Hodgkin Lymphoma

[Gastrointestinal Stromal Tumor](#)
[Duodenum](#)
[Infections](#)
[Adenoma and Adenocarcinoma](#)
[Colon](#)
[The Anal Pap Test](#)

The advent of improved sampling and visualization devices has cemented cytology as a valuable method for the detection of gastrointestinal (GI) malignancies, premalignant lesions, and infections. Direct visualization of the mucosa, coupled with imaging of submucosal structures, allows for the collection of mucosal brushings, submucosal fine-needle aspirates, and mucosal biopsies, all with a single procedure.¹ Indeed, GI cytology is increasingly seen as a rapid, efficient, and cost-effective means for the evaluation of a variety of GI tract lesions.¹

Clinical Indications



Clinical indications

- suspected malignancy
- screening for dysplasia (e.g., Barrett's esophagus)
- suspected infection

A suspected malignancy is by far the most common and important indication for cytologic examination. With the aid of direct mucosal visualization, brush cytology is complementary to biopsy for detecting adenocarcinoma, dysplasia, and infections.¹ Cytologic sampling has the following advantages over biopsy.

Sampling a Wider Area and Reaching Deep Organs

In the case of a diffuse lesion, where multiple biopsies must often be taken to ensure adequate sampling, a single brushing is capable of sampling a wide area of interest. Brushings are particularly useful for the evaluation of suspected preinvasive neoplastic lesions and high-grade dysplasia, where only a poorly defined area of irregular, rough, or vaguely nodular mucosa is seen rather than an obvious gross lesion.²⁻⁴ For this reason, cytologic sampling is ideal for the surveillance of dysplasia in patients at increased risk of squamous or adenocarcinoma of the esophagus.⁵⁻⁷ Cytology is also superior to biopsy for stenotic lesions, such as tumors of the gastric cardia or pylorus, which cannot be reached easily with biopsy forceps.¹ Brush cytology specimens are also more sensitive than biopsies in detecting fungal infections.⁸

Better Recognition of Lymphoid Cells

Small endoscopic biopsies are commonly distorted and squeezed. A properly prepared cytologic smear, on the other hand, yields well-preserved, isolated lymphoid cells that are easier to recognize and interpret than the crushed, distorted cells on small biopsies. Cytologic features alone are often sufficient to render a definitive diagnosis of a large cell lymphoma.^{1,9} Two types of lymphoma pose special problems in diagnosis: a low-grade lymphoma arising from mucosa-associated lymphoid tissue (MALT) and CD30-positive anaplastic large cell

lymphoma.⁹ The former needs to be distinguished from a benign lymphoid infiltrate, and the latter mimics a large cell carcinoma because its cells can be cohesive, immunoreactive for epithelial membrane antigen (EMA), and negative for leukocyte common antigen (LCA). A high level of awareness and a panel of marker studies are usually needed in such cases before a definitive diagnosis can be made. Whereas brushings sometimes obtain limited material, endoscopic fine-needle aspiration (FNA) often yields sufficient material for immunohistochemistry, flow cytometry, and cytogenetic assessment of lymphoproliferative disorders.¹⁰

Less Invasiveness

Obtaining a brushing sample is less traumatic than taking a grasp biopsy. This is particularly relevant for patients with clotting abnormalities and for those with very vascular tumors. A diagnosis can be made on cytologic material in such patients while avoiding the risks associated with a biopsy.

Shorter Turnaround Time

Once smears have been made, they can either be air-dried and stained with a Romanowsky-type stain or immediately fixed in alcohol and stained with toluidine blue–eosin or a modified Papanicolaou stain.^{11,12} As a result, the slides are available for interpretation in a few minutes. Although a short turnaround time may not be required in most cases, it can be crucial when urgent decisions concerning patient management need to be made.

Sample Collection and Processing

Various instruments and techniques for collecting material have been developed—and abandoned—over the years. The most common today is the direct brushing of a visible lesion via a fiberscope. Lesions confined to the lamina propria (e.g., signet ring cell carcinoma) or the submucosa/muscularis (e.g., gastrointestinal stromal tumor [GIST], neuroendocrine tumor [NET], and lymphoma), however, can be difficult to sample by brush cytology. For such lesions, endoscopic FNA enables sampling of lesions hidden beneath necrotic debris or normal mucosa.^{13–18} Endoscopic FNA is further enhanced by ultrasound guidance (endosonography). This allows the needle to reach even deeper lesions, including those that are extrinsic to the GI tract but impinging on the lumen, like a metastatic malignancy.¹⁹ Endosonography is useful for identifying the extent of the lesion and detailing regional anatomy to permit an assessment of the safest and most appropriate site for FNA.¹⁹

Although not in routine use, balloon or encapsulated samplers can be used to screen for esophageal cancer. These have the advantages of sampling the entire esophagus at low cost.^{5,7,20–22} Three modes of collection have been designed for this purpose: esophageal balloon cytology, encapsulated sponge cytology, and encapsulated sponge-mesh cytology.^{22–24} The esophageal balloon cytology sampler consists of a disposable, single-lumen plastic catheter attached to an expandable latex balloon. When inflated with 15 to 20 mL of air, it turns into a near-perfect sphere with a diameter of 35 mm. The other two samplers consist of a gelatin capsule attached to a flexible plastic stylet. The gelatin capsule contains a compressed polyurethane sponge in the sponge sampler, and a polyurethane sponge covered with a cotton mesh in the sponge-mesh sampler. The patient swallows the sampler; once the sampler reaches the stomach, it is inflated (as with the balloon sampler), or the sponge is exposed by waiting 10 minutes for the gelatin to dissolve. Epithelial cells are collected as the sampler is pulled out of the patient. Although all three samplers obtain satisfactory yields of squamous and glandular cells, it is easier for patients to accept the encapsulated samplers.

Sample Collection

To obtain a *brushing* specimen, a brush is enclosed inside a transparent Teflon sheath and passed through the endoscope. When a lesion is visualized, the brush is expelled from the sheath and firmly and briskly plunged into the mucosa 5 to

10 times. To ensure an adequate sample, the lamina propria should be penetrated. After the sample has been obtained, the brush is retracted into its Teflon sheath, removed from the patient, extruded from the sheath again, and rinsed in a preservative solution for liquid-based preparations or pressed against glass slides as direct smears.

The *endoscopic FNA* involves introducing the needle through the biopsy channel of a fiberoptic endoscope. The lesion is localized either by direct vision or by ultrasound. Once the needle is in the lesion, negative pressure is applied to the needle using a 10 or 20 mL syringe. With the negative pressure maintained, the needle is moved back and forth within the lesion. Finally, the pressure is released, the needle removed from the scope channel, and the material prepared as smears or liquid-based preparations for cytologic examination.

Processing the Sample

The needle or brush is rinsed in transport/preservative medium (buffered saline solution, 50% ethanol, CytoLyt, or CytoRich).^{1,25,26} Alternatively, the material on the brush, needle, or sampler is rolled or spread onto one or more clean slide(s). The slide is immersed immediately in 95% alcohol for fixation. The importance of immediate fixation cannot be overemphasized, because cells that are thinly spread out on a slide dry out very quickly. The rolling process is repeated until no more cellular material can be rolled onto a slide. Some slides can be left to air-dry for a Romanowsky-type stain, if preferred. Additional cellular material can still be salvaged from the brush by vortexing it in an appropriate transport or preservation medium.

Slides are prepared from the transport medium sample most commonly using automated cell concentration methods like ThinPrep or SurePath. The advantages of these methods include better cell preservation, a cleaner background, a reproducible preparation, and a thin layer of cells.²⁶⁻²⁸ These advantages lead to shorter screening time. Another important advantage is the availability of material for ancillary studies. After routine slides have been prepared, there is usually residual material for any needed ancillary studies (e.g., immunostains).²⁸ Disadvantages include the cost of the equipment and reagents and the labor involved in preparing slides. In addition, there is a transition period for obtaining expertise in the interpretation of liquid-based preparations.^{26,29}

Accuracy

The sensitivity and specificity of brushing cytology for the detection of malignancies of the GI tract vary according to the location and sampling method. The sensitivity of brushings for the diagnosis of GI adenocarcinomas ranges from 77% to 94%, and the specificity is generally greater than 95%.³⁰⁻⁴¹ These figures are comparable to those for biopsies. The combined use of biopsy and cytology yields the highest detection rate ([Table 7.1](#)).^{8,33,36,41,42} The accuracy of brushing cytology is significantly higher when the brushing is performed before rather than after biopsy.^{43,44}

TABLE 7.1
DIAGNOSTIC SENSITIVITY OF ENDOSCOPIC BRUSHING AND BIOPSY IN DETECTING UPPER GASTROINTESTINAL TUMORS*

Author(s)	Diagnostic Sensitivity (%)		
	Cytology Alone	Histology Alone	Combined
Behmard et al., 1978	92	86	NA
Cook et al., 1988	85	86	91
Kobayashi and Kasugai, 1978	84	77	88
Lan, 1990	94	91	98
O'Donoghue et al., 1995	88	79	98
Prolla et al., 1971	91	81	100
Qizilbash et al., 1980	89	93	95
Shanghai Group, 1982	77	74	88
Vidyavathi et al., 2008	87	77	98
Witzel et al., 1976	85	83	96
Young and Hughes, 1980	92	69	NA

NA, Data not available.

*Table modified with permission from: Wang HH, Jonasson JG, Ducatman BS. Brushing cytology of the upper gastrointestinal tract. Obsolete or not? *Acta Cytol.* 1991;35(2):195-8.

Due to sampling error and the presence of confounding “pseudogoblet” cells, cytology is less accurate in identifying goblet cells (for the diagnosis of Barrett’s esophagus [BE]), with a sensitivity of 41% and specificity of 84%.⁴ The use of cytology for detecting dysplasia in BE, however, is more promising. Brush cytology detects high-grade dysplasia with high sensitivity and specificity.^{2,45-48} Nonendoscopic screening of the esophagus has a sensitivity of approximately 90% and a specificity of over 90% for squamous dysplasia or carcinoma and a sensitivity of 80% and specificity of 95% for high-grade glandular dysplasia or adenocarcinoma.^{2,5,6,20,48} Prospective outcome studies of balloon cytology in a high-risk area in China showed that cytology results are highly predictive of the subsequent risk of esophageal and gastric cardiac cancer.⁴⁹⁻⁵¹

Some consider cytology an unnecessary duplication of the biopsy and argue

that cytology has a higher false-positive rate (lower specificity), particularly in the setting of reactive or reparative conditions.^{32,38,52} Increased experience and strict adherence to criteria for diagnosing malignancy minimize the false-positive rate and maximize the diagnostic yield of the endoscopic procedure, with a predictive value of a positive result that approaches 100%. The predictive value of a negative result is more variable, depending both on the patient population and on the sampling technique of the endoscopist.

Review of Morphologic Findings



Features noted on low-power magnification

- cellularity
- cellular arrangements
- background

Examination of the slides under low magnification is tremendously important. Features to note are cellularity, cellular arrangements (flat sheets, three-dimensional clusters, and isolated cells), and background features (clean, inflammatory, or necrotic). Cells in reactive processes often exfoliate as flat cohesive sheets, whereas neoplastic cells (both benign and malignant) tend to aggregate in three-dimensional clusters. Cells from a malignant neoplasm are arranged as either tight or loose three-dimensional clusters or, due to the loss of cellular cohesion, as isolated cells. Whereas a clean background often indicates a benign process, a dirty, inflammatory background can indicate either a benign or a malignant process. Ulceration, whether due to inflammation or malignancy, is accompanied by many inflammatory cells and cellular debris. Inflammatory cells are usually numerous and predominate in reactive/reparative conditions. When many necrotic ghost cells are present without numerous inflammatory cells, a malignancy should be suspected.



Features noted on high-power magnification

- cytoplasmic characteristics
- nuclear details

High magnification is important for the evaluation of cellular and nuclear details. In general, a benign process is characterized by a uniformity of cellular arrangement, nuclear size and shape, and the number of nucleoli. A malignant neoplasm, on the other hand, is characterized by a haphazard cellular arrangement and marked irregularities in cell size and shape, nuclear chromatin pattern, and number of nucleoli. Lymphomas and endocrine tumors are exceptions to the rule, because they are often composed of very similar cells.



Special features with liquid-based preparations

- cells appear smaller
- fewer cells in sheets or groups; more single cells
- less obvious gland formation
- tumor diathesis appears as small clumps of amorphous, acellular debris

Esophagus

Infections

Esophageal infections occur most often (but not exclusively) in immunocompromised patients.⁵³⁻⁵⁷



Cytomorphology of *Candida* infection

- pseudohyphae and yeast forms
- reactive squamous cells

Fungal infection with *Candida* species is the most common esophageal infection (Fig. 7.1). Brushings are more sensitive than biopsies in the diagnosis of esophageal candidiasis.¹⁸ The number of organisms varies from few to many. Contamination by oral *Candida* is not a problem because the brush is contained within a sheath, which protects it while in transit to and from the lesion.

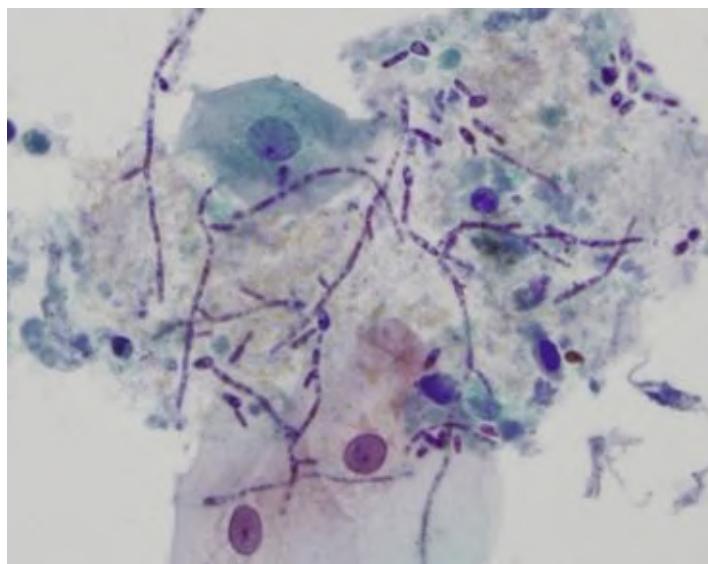


FIGURE 7.1 *Candida* (esophageal brushings).

Infection by *Candida* species is recognized by identifying both the ovoid yeast forms and elongated pseudohyphal forms (ThinPrep, Papanicolaou stain).



Cytomorphology of cells infected with herpes simplex virus

- multinucleation
- nuclear molding
- ground-glass chromatin
- Cowdry A bodies
- [Figure 7.2](#)

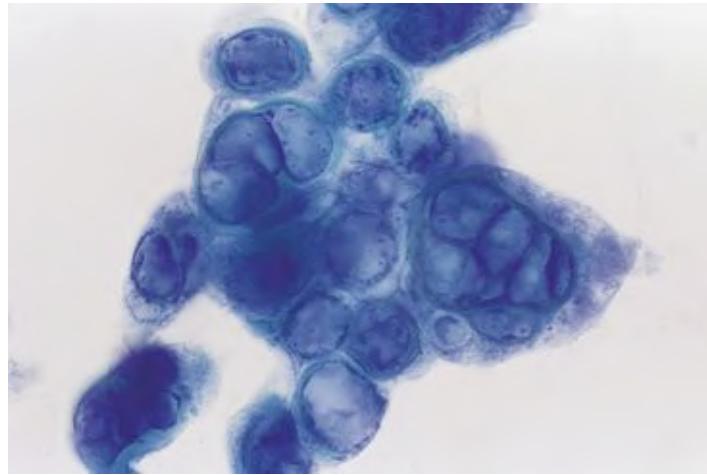


FIGURE 7.2 Herpes simplex infection (esophageal brushings). Multinucleation, molding of nuclei, and a ground-glass chromatin pattern are diagnostic features (ThinPrep, Papanicolaou stain).



Cytomorphology of cells infected with cytomegalovirus

- mononucleation
- large single basophilic intranuclear inclusion
- perinuclear halo
- intracytoplasmic textured inclusions



Differential diagnosis of viral infections

- epithelial repair
- carcinoma

The large intranuclear inclusions of many viral infections can be mistaken for the macronucleoli of repair or malignancy.^{58,59} Molecular biologic techniques (e.g., *in situ* hybridization, polymerase chain reaction) can be applied to confirm the presence of virus.^{60,61} Both are equivalent to culture in their sensitivity in detecting cytomegalovirus (CMV), and both are more sensitive than morphology alone.⁶²



Cytomorphology of epithelial repair

- cohesive sheets with a flowing or streaming pattern, better seen on smears, usually absent on liquid-based preparations ([Fig. 7.3A](#))
- uniform nuclei with:
 - enlargement
 - smooth and thin nuclear borders
 - fine chromatin
 - prominent nucleoli
- evident mitoses
- background inflammation
- atypical stromal cells

Epithelial Repair

Any injury to the mucosa, especially ulceration, evokes a cellular reaction known as epithelial repair. It is sometimes difficult to determine whether the reparative epithelium is of glandular or squamous origin.

Although epithelial repair is characterized by prominent nucleoli ([Fig. 7.3B](#)), they are usually not huge or numerous (more than three or four). The atypical stromal cells or their stripped nuclei from granulation tissue can be quite alarming. Although their nuclei are strikingly enlarged, they are not hyperchromatic; instead they have fine, homogeneous chromatin, a thin nuclear membrane, and a smooth nuclear border.

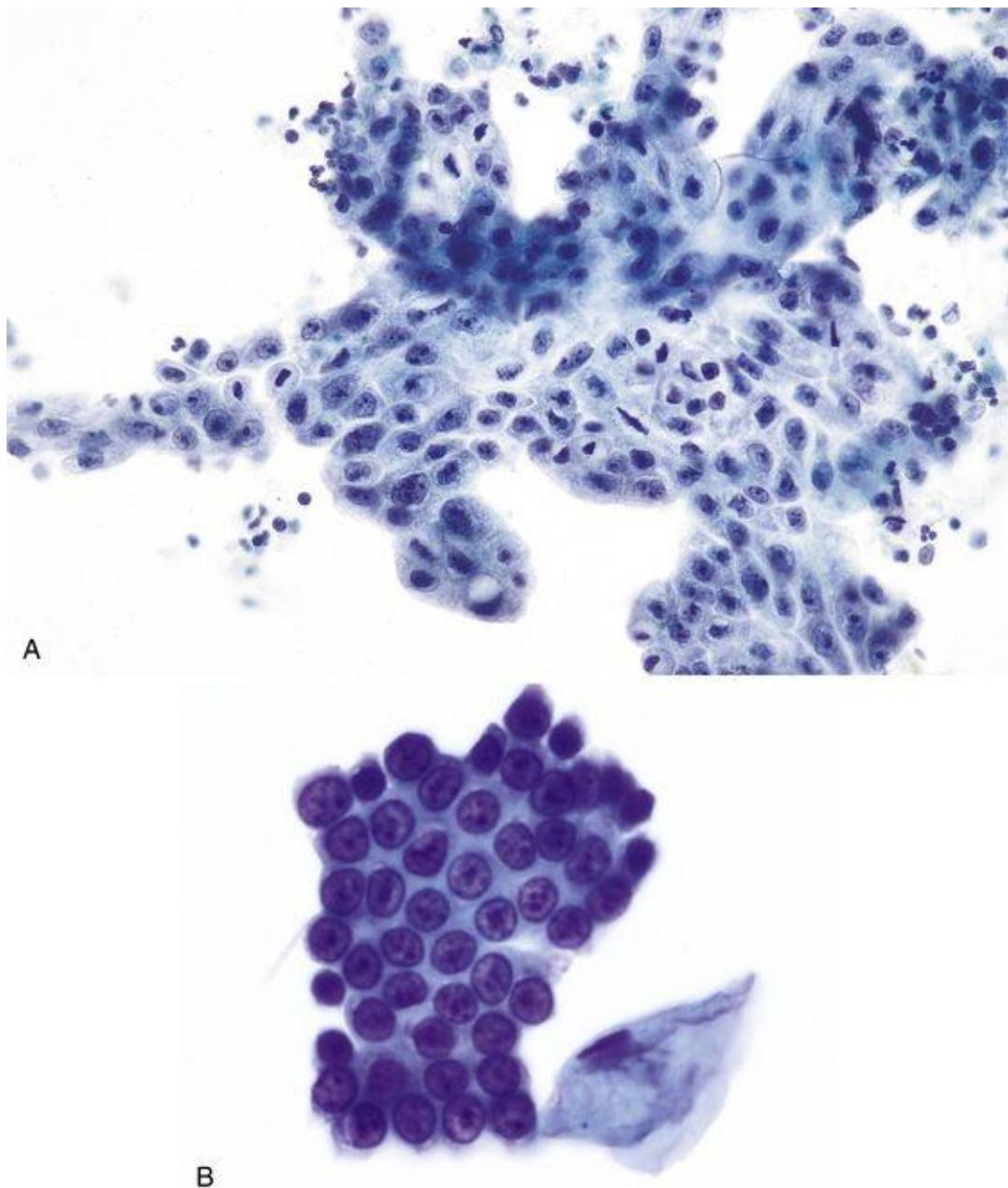


FIGURE 7.3 Epithelial repair.

A, A slight flowing or streaming pattern is noted. The cells have slightly enlarged nuclei with regular nuclear borders, finely dispersed chromatin, and one or a few prominent nucleoli (gastric brushings, ThinPrep, Papanicolaou stain). B, Nuclei are enlarged but round and regular with prominent nucleoli. Cells are somewhat crowded but very cohesive (esophageal brushings, ThinPrep, Papanicolaou stain).

Radiation-induced changes represent a special type of reactive change or repair. There is proportional cellular and nuclear enlargement, multinucleation, metachromatic cytoplasm, and vacuolization of both cytoplasm and nuclei ([Fig. 7.4](#)).

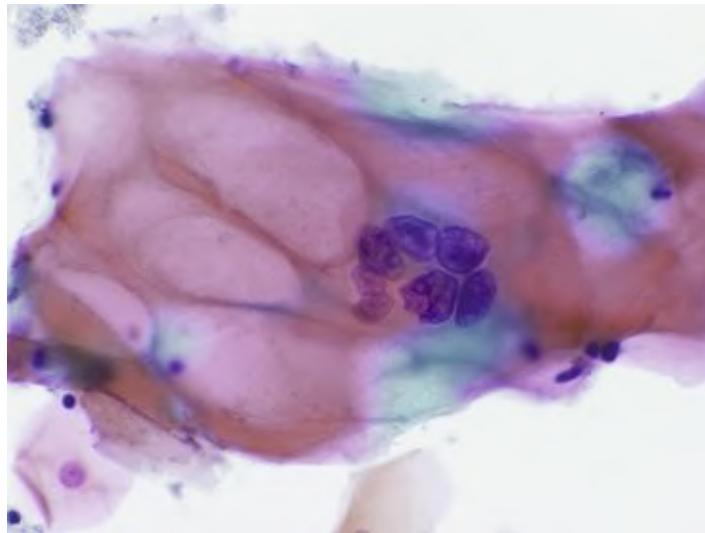


FIGURE 7.4 Radiation-induced changes (esophageal brushings). Cellular and nuclear enlargement, multinucleation, and vacuolization of cytoplasm are characteristic (ThinPrep, Papanicolaou stain).

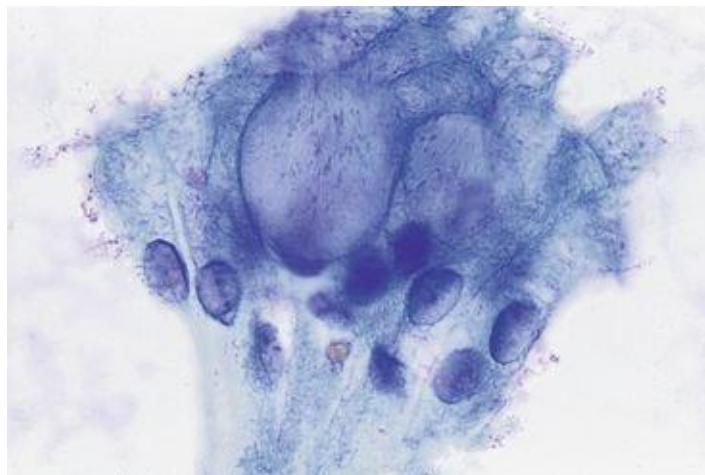


FIGURE 7.5 Barrett's epithelium with goblet cells. A single large cytoplasmic vacuole expands the apical portion of the cytoplasm and displaces the nucleus and shapes it into a crescent against the basal cell membrane (ThinPrep, Papanicolaou stain).



Differential diagnosis of repair

- dysplasia
- carcinoma

Both cellular arrangements and individual cell features are useful in this distinction. Cells with reactive or reparative changes are usually in flat sheets and there is little cell dyshesion. By contrast, neoplasms frequently show dyshesion by the “feathering” (dissociation of cells) at the periphery of cell clusters or by the dispersion of numerous isolated cells. Three-dimensionality is most often associated with neoplastic lesions. In addition, although reactive or reparative cell nuclei may be enlarged, they are often uniform within the same sheet and have a similar number of regular nucleoli. In contrast, neoplastic cells, especially malignant ones, show more variation in nuclear size and shape as well as more nuclear membrane irregularity and chromatin clumping and/or clearing.

Barrett's Esophagus

BE is an acquired condition in which the normal stratified squamous epithelium of the distal esophagus is replaced by columnar epithelium.^{63,64} It develops as a complication in 8% to 12% of patients with chronic gastroesophageal reflux.^{64,65} Estimates of the prevalence of BE range from 262 to 376 per 100,000.^{66,67} Followup studies of patients with BE show that their risk of developing esophageal adenocarcinoma is 0.12% to 0.29% per year, approximately 11 times the rate in the general population.^{68–70} Risk factors include male gender, long segment of BE, smoking, alcohol use, and dysplasia. The columnar epithelium that replaces the squamous epithelium in the distal esophagus can be of cardiac, fundic, or intestinal type.⁷¹ Although the increased risk of adenocarcinoma was once thought to be associated only with intestinal-type epithelium,^{72–74} this may be an oversimplification.⁷⁵



Cytomorphology of Barrett's esophagus

- epithelial repair
- goblet cells

When obtained by brushing the esophagus, goblet cells, characterized cytologically by a single large cytoplasmic vacuole displacing the nucleus and shaping it into a crescent against the cell membrane ([Fig. 7.5](#)), are indicative of BE. Multiple goblet cells impart a Swiss cheese appearance to a honeycomb sheet of glandular cells. On liquid-based preparations, they are sometimes

present as isolated cells. When columnar cells, with or without goblet cells, are seen on an esophageal brushing specimen obtained more than 3 cm from the gastroesophageal junction, it is reasonable to report the findings as “consistent with” or “suggestive of” BE.⁷⁶

Significant disparities are sometimes seen between the results of brushings and biopsies in the diagnosis of BE. By this measure, cytology is not entirely sensitive or specific for the diagnosis of BE.^{4,48} Much of the disparity can be accounted for by sampling. Another explanation may apply in some cases that are diagnostic of BE by cytology but negative by biopsy. Cells resembling goblet cells (“pseudogoblet” cells) have been described in the gastric cardia.^{4,77} The vacuoles of pseudogoblet cells are pink (containing neutral mucin) with hematoxylin-eosin (H & E) staining, whereas the vacuoles in goblet cells are pale blue (acid mucin). The Papanicolaou stain, however, does not distinguish between acid and neutral mucin—both are dissolved in the staining process and appear as an empty space. Alcian blue stains theoretically might help with this distinction, but they are not commonly applied to cytologic preparations.⁴ Immunocytochemical staining of cytologic specimens for villin and hepatocyte antigen is also helpful for identifying goblet cells on cytologic specimens but is not routine in most laboratories.^{78,79}

Dysplasia in Barrett's Esophagus

Adenocarcinomas in the setting of Barrett's esophagus (BE) arise through a sequence of mucosal alterations that include premalignant (dysplastic) esophageal epithelium.⁸⁰ Dysplastic cells have some but not all of the features of malignant cells.^{2,45,81,82}



Cytomorphology of esophageal glandular dysplasia

- background of Barrett's epithelium
- scattered atypical cells with some but not all features of adenocarcinoma

Preparations from an esophageal dysplasia contain fewer abnormal cells than those from an adenocarcinoma. In addition, dysplastic cells show incomplete features of malignancy (e.g., nuclear enlargement, hyperchromasia, increased

nuclear-to-cytoplasmic ratio), but they generally show variation in nuclear size and shape, irregular cellular spacing, crowding, stratification, and dyshesion. The degree of these changes is proportional to the grade of dysplasia.

Although cytology is not perfect in detecting BE, it is useful in identifying dysplasia.⁴⁴⁵⁻⁴⁸ Grading dysplasia is clinically important because patients with low-grade dysplasia are managed with followup and surveillance, whereas patients with high-grade dysplasia are managed with more frequent surveillance or resection.⁸³ To date, however, well-accepted criteria for diagnosing low-grade and high-grade dysplasia on cytology have not been established.⁸⁴ The diagnosis of low-grade dysplasia is still controversial even on histology.⁸⁵ Based on the features described below, one may suggest that the lesion is low or high grade.⁴⁴⁸ In practice, when a low-grade dysplasia in BE is suspected, the lesion is reported as “atypical,” with a comment that the findings are suggestive of, or consistent with, low-grade dysplasia, depending on the degree of certainty. When a high-grade dysplasia is suspected, it is reported as “suspicious,” with a comment that the findings are suggestive of, or consistent with, high-grade dysplasia. The phrase “invasion cannot be excluded” is added if the cytologic findings include prominent dyshesion and many atypical cells.



Cytomorphology of low-grade dysplasia ([Fig. 7.6](#))

- crowded groups with stratification

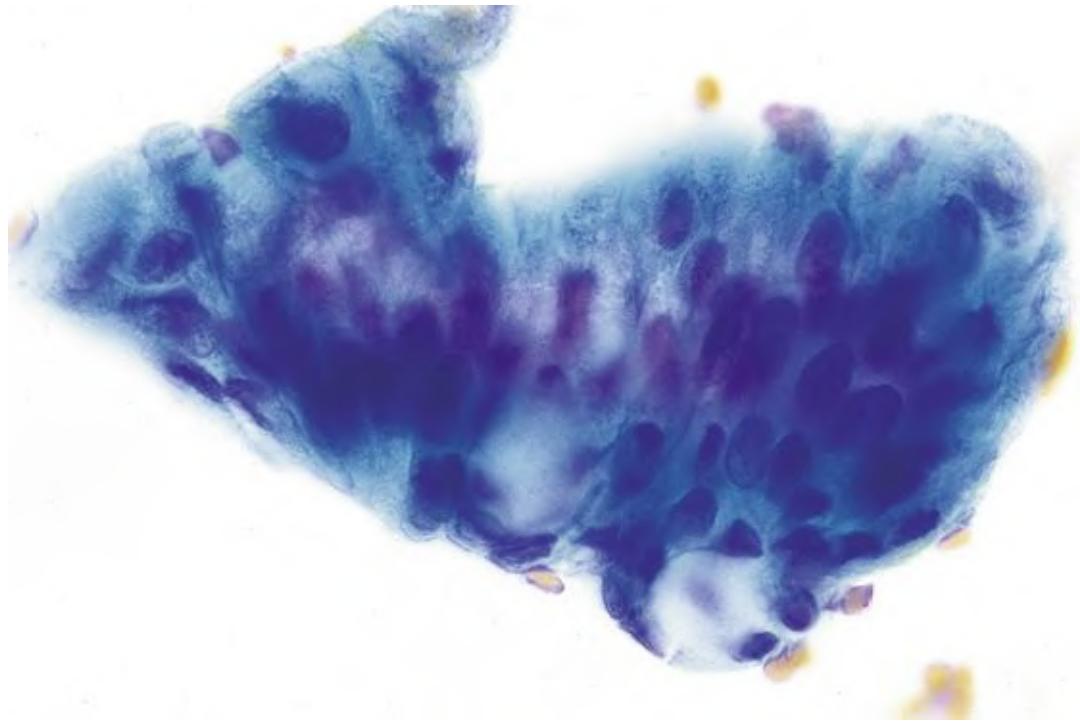


FIGURE 7.6 Low-grade dysplasia in Barrett's epithelium.
A fragment of glandular epithelium with stratified elongated nuclei is seen.
Although mucin depletion and slight nuclear enlargement are seen, significant
nuclear atypia is absent (direct smear, Papanicolaou stain).

- mild nuclear atypia and pleomorphism

● **Cytomorphology of high-grade dysplasia (Fig. 7.7)**

- crowded groups or isolated cells

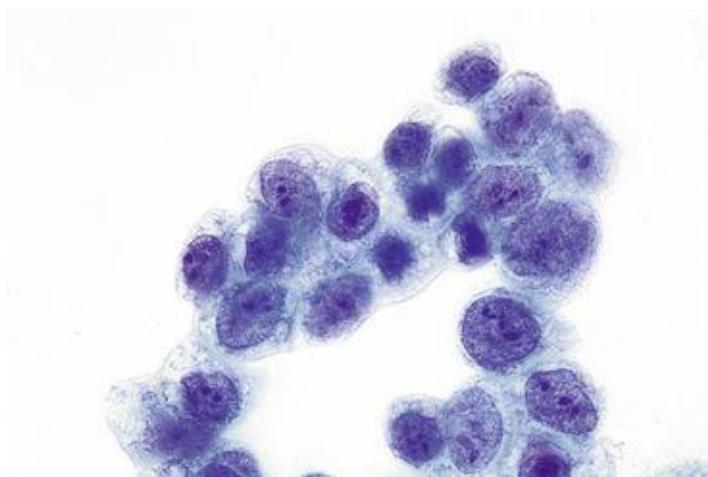


FIGURE 7.7 High-grade dysplasia in Barrett's epithelium.

A sheet of irregularly arranged cells with variably enlarged nuclei is present without evident dyshesion. In spite of the increased nuclear-to-cytoplasmic ratio, nuclear membrane irregularities, and slight hyperchromasia, the atypia is insufficient for a definitive diagnosis of malignancy (ThinPrep, Papanicolaou stain).

- higher degree of nuclear atypia and pleomorphism



Differential diagnosis of esophageal dysplasia

- epithelial repair
- adenocarcinoma

The diagnostic criteria for distinguishing dysplasia from reactive or reparative changes and adenocarcinoma are displayed in [Table 7.2](#).

TABLE 7.2

COMPARISON OF CYTOLOGIC FEATURES OF REACTIVE, PREMALIGNANT, AND MALIGNANT GLANDULAR LESIONS OF UPPER GASTROINTESTINAL TRACT

Cytologic Feature	Reactive	Dysplasia and Adenoma	Adenocarcinoma
Tight three-dimensional clusters	Absent to rare	Absent to moderate	Moderate to many
Loose clusters	Absent to rare	Absent to moderate	Moderate to many
Dyshesion	Absent to slight	Absent to moderate	Prominent
Single atypical cells	None to few	None to moderate	Moderate to many
Mitoses	Present	Present	Present
Atypical mitoses	None	None to few	Present
Chromatin	Vesicular	Variable	Variable
Nuclear pleomorphism	Absent to slight	Slight to moderate	Moderate to marked
Nuclear overlap	Absent to slight	Slight to moderate	Moderate to marked
Irregular nuclear spacing	Absent to slight	Slight to moderate	Moderate to marked within tight clusters
Necrosis	Absent	Absent	Occasionally present

Low-grade dysplasia is a strong predictor for the development of high-grade dysplasia and adenocarcinoma.^{86,87} Due to the difficulties in the morphologic diagnosis of low-grade dysplasia and the lack of any visible endoscopic abnormality, molecular alterations have been investigated for the purpose of risk stratification. In this regard, DNA content abnormalities (aneuploidy/tetraploidy) and overexpression of p53 are the strongest predictors of disease progression.^{87,88} Indeed, endoscopic esophageal brushing specimens are suitable for DNA ploidy

analysis, fluorescence in situ hybridization (FISH), and loss of heterozygosity studies,^{89–91} but none of these has yet found routine application.

Adenocarcinoma of the Esophagus

The incidence of esophageal adenocarcinoma in the United States increased four-fold between 1973 and 2002, surpassing squamous cell carcinoma (SQC), which showed a 30% drop over the same period.⁹² Most adenocarcinomas are located in the mid-or distal third of the esophagus, presumably arising in Barrett's esophagus (BE).^{72,93–95}



Cytomorphology of esophageal adenocarcinoma

- increased cellularity
- abnormal cellular arrangement
 - numerous isolated cells
 - “feathering” at the edges of cellular groups
 - haphazard, crowded arrangement
 - gland formation
- atypical nuclear features ([Fig. 7.8](#))

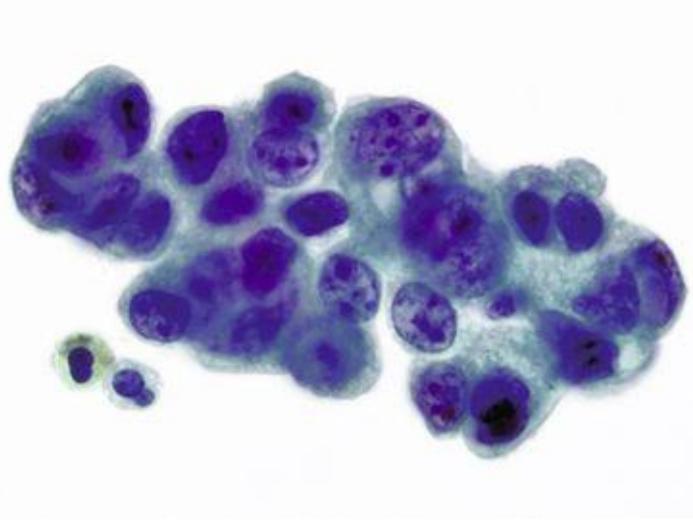


FIGURE 7.8 Adenocarcinoma of the esophagus.

In contrast to [Figure 7.7](#), the nuclei show more hyperchromasia, with chromatin clumping and clearing and large prominent nucleoli. Dyshesion was evident elsewhere on the slide (Papanicolaou stain).

- enlargement

- hyperchromasia
- uneven and irregular nuclear membrane
- pleomorphism
- variable amount of vacuolated cytoplasm
- tumor diathesis
- Barrett's epithelium may or may not be present in the background



Differential diagnosis of esophageal adenocarcinoma

- epithelial repair
- dysplasia in Barrett's epithelium, particularly high grade
- poorly differentiated squamous cell carcinoma

The distinction between reactive, dysplastic, and malignant esophageal lesions is challenging. Reactive or reparative cells sometimes appear more atypical than dysplastic cells, especially those from a low-grade lesion. Whereas low-grade dysplasia consists of crowded, stratified cells with elongated nuclei, reactive cells are in sheets with a streaming pattern. Nucleoli are inconspicuous in low-grade dysplasia, whereas prominent nucleoli are characteristic of repair. High-grade dysplasia has more malignant features than low-grade dysplasia (e.g., abnormal chromatin, irregular nuclear membranes, and dyshesion), which help to distinguish the dysplasias from repair. The difference between a high-grade dysplasia and carcinoma is quantitative rather than qualitative; a smear from an adenocarcinoma has more abnormal cells than one from a high-grade dysplasia, and the nuclear atypia in carcinoma is more marked. A tumor diathesis is a very useful feature for the diagnosis of a carcinoma, but it is not present in all cases. Endoscopic findings are also important. Abnormal cells from a fungating, ulcerating lesion most likely represent carcinoma, whereas those from an indistinct flat lesion most likely represent dysplasia. It is best to report a case as "high-grade dysplasia" when a small number of very atypical cells are present in a clean background from a patient undergoing surveillance for Barrett's esophagus, particularly if endoscopy shows only granular mucosa and no definite mass. Ultimately, a biopsy is necessary for definitive diagnosis.

Squamous Cell Carcinoma of the Esophagus

Squamous cell carcinoma (SQC) is the most common malignancy of the esophagus worldwide.^{96,97} Isolated malignant cells are more commonly seen in SQCs, especially well-differentiated ones, than in adenocarcinomas. Cellular features depend on the degree of differentiation.



Cytomorphology of well-differentiated squamous cell carcinoma

- hyperchromatic/pyknotic nucleus
- completely obscured chromatin
- various cell shapes (round, oval, or spindled)
- irregular, angulated nucleus
- keratinized cytoplasm (“hard” or “glassy” orangeophilia)
- sharp cytoplasmic border
- prominent necrosis/tumor diathesis
- [Figure 7.9](#)

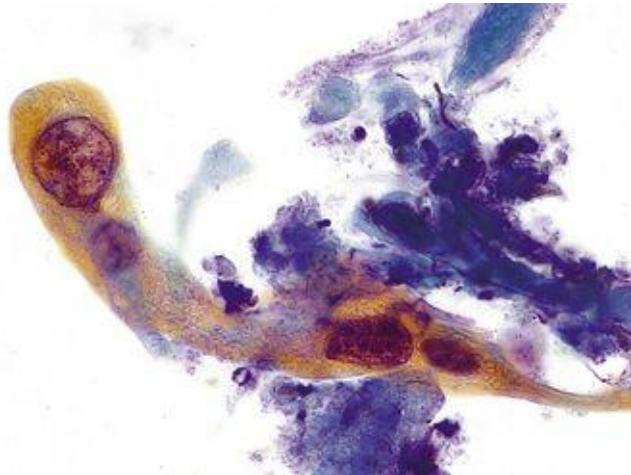


FIGURE 7.9 Well-differentiated squamous cell carcinoma of the esophagus. Two spindled-shaped keratinized malignant squamous cells with dense orange cytoplasm and hyperchromatic nuclei show markedly abnormal chromatin distribution. Degenerated cells with pyknotic nuclei are in the background (ThinPrep, Papanicolaou stain).



Cytomorphology of poorly differentiated squamous cell carcinomas

- less keratinization, nuclear angularity, pyknosis
- indistinct cell borders
- coarsely textured chromatin
- prominent nucleoli
- [Figure 7.10](#)

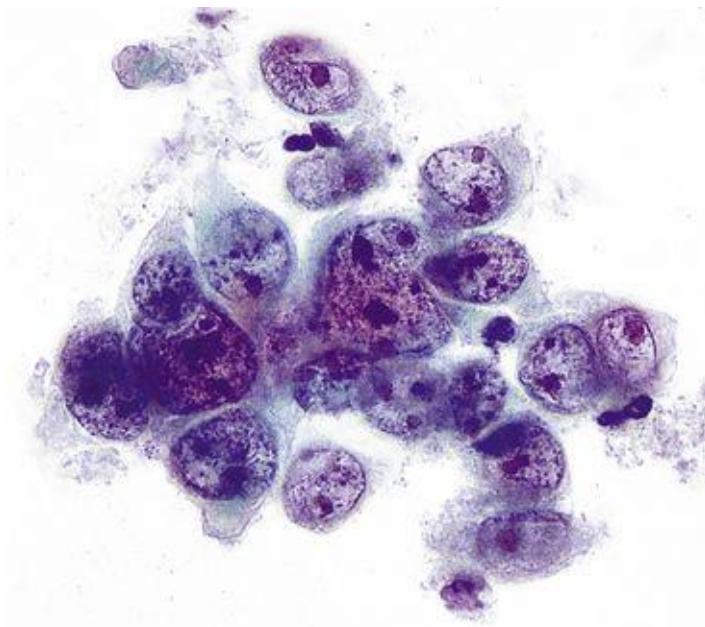


FIGURE 7.10 Poorly differentiated squamous cell carcinoma (SQC) of the esophagus.

Squamous differentiation is not discernible in this group of cells. Only scant cytoplasm is present. The nuclei show markedly abnormal chromatin distribution and prominent nucleoli (ThinPrep, Papanicolaou stain).



Differential diagnosis of squamous cell carcinoma of the esophagus

- epithelial repair
- squamous dysplasia
- poorly differentiated adenocarcinoma

Squamous dysplasia of the esophagus is similar to the squamous intraepithelial lesion of the uterine cervix. Its features include an increased

nuclear-to-cytoplasmic ratio, nuclear enlargement, hyperchromasia, coarse chromatin, and nuclear membrane irregularity. Tumor diathesis and keratinization of tumor cells are useful features to distinguish squamous dysplasia from invasive SQC. Precise distinction between a poorly differentiated SQC and a poorly differentiated adenocarcinoma is not always possible. While adenocarcinoma cells tend to have abundant cytoplasm and eccentric nuclei, SQC cells have dense cytoplasm and central nuclei. When in doubt, the tumor may be reported as “carcinoma, not otherwise specified.”

Uncommon Tumors of the Esophagus

Uncommon tumors of the esophagus include adenosquamous carcinoma, mucoepidermoid carcinoma, basaloid squamous cell carcinoma, adenoid cystic carcinoma, and neuroendocrine tumors (NETs). A complete discussion is beyond the scope of this chapter, but a few points are worth noting. NETs of the esophagus are very rare. When encountered, they are analogous to their counterparts in the stomach and intestine. The cells of a small cell neuroendocrine carcinoma are difficult to detect and recognize because they mimic lymphocytes. Often they are dispersed as isolated cells or small groups of only two or three cells ([Fig. 7.11](#)).

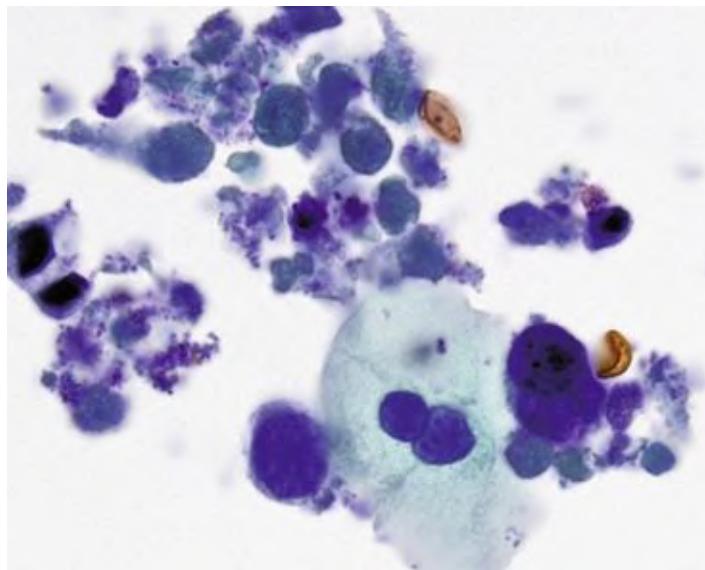


FIGURE 7.11 Small cell carcinoma of the esophagus.

Malignant cells are scattered in small groups and notable for their very high nuclear-to-cytoplasmic ratio and dark, coarsely granular chromatin (ThinPrep, Papanicolaou stain).

Leiomyoma is the most common mesenchymal tumor of the esophagus. Because they are submucosal and usually covered by an intact mucosa, leiomyomas are more often successfully sampled by endoscopic FNA rather than brushing (Fig. 7.12A and B). Leiomyomas are strongly immunoreactive for desmin (Fig. 7.12C), in contrast to GISTs, which are negative for desmin but immunoreactive for c-kit and DOG1.

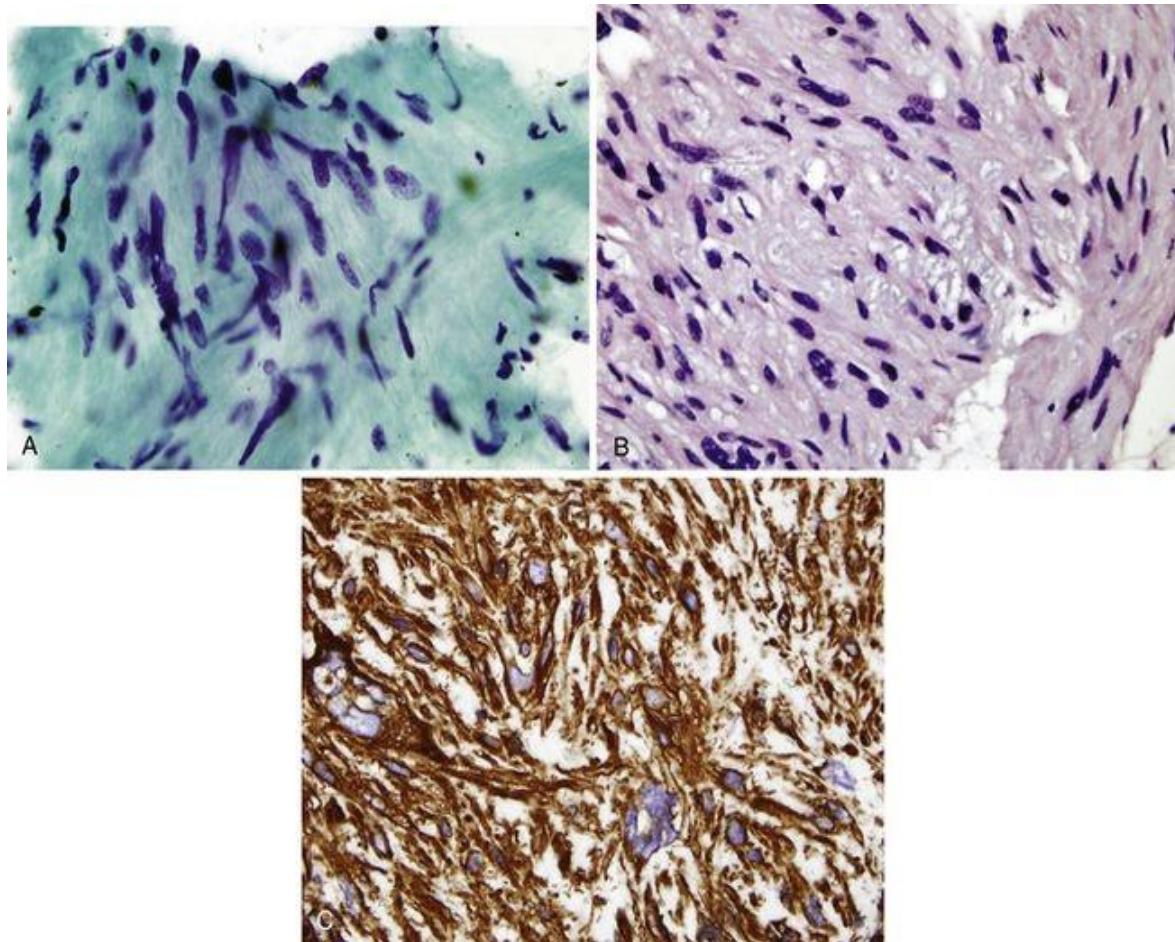


FIGURE 7.12 Leiomyoma of the esophagus.

A, Spindle-shaped cells have abundant wiry (fibrillar) cytoplasm (ThinPrep, Papanicolaou stain). B, Cell block sections reveal tumor fragments made up of cells with abundant eosinophilic cytoplasm (hematoxylin-eosin [H & E] stain). C, Leiomyomas are strongly and diffusely immunoreactive for desmin.

Stomach

Infections

Fungal and viral infections are similar to those previously described for the esophagus. Unique to the stomach is infection by *Helicobacter pylori*. The prevalence rates of *H. pylori* vary by region.^{98,99} In the United States, prevalence peaks at 30 years of age and remains stable at 10% to 15%.⁹⁹ Although most infections are asymptomatic, *H. pylori* is associated with chronic active gastritis, peptic ulcers, and gastric intestinal metaplasia, and inversely related to Barrett's esophagus.⁹⁹ Both *H. pylori* and peptic ulcer disease have decreased significantly in the past decade,^{100–102} probably due to increased awareness and treatment. *H. pylori* infection may be a cofactor in the development of gastric carcinoma and lymphoma,^{103–105} but most infected patients never develop gastric cancer. The organisms can be demonstrated either on imprint smears of gastric biopsies or on brush specimens.^{106–109} Compared to histologic examination of H & E–stained and modified Giemsa–stained sections, the imprint and brushing cytology are at least comparable in sensitivity (88% to 95%) and specificity (61%).^{106,108,110} The benefits of imprint and brushing cytology are rapid results, high specificity, and low cost.^{106,109} When done properly, imprint cytology does not adversely affect the quality of the biopsy specimen.¹¹¹ With the Papanicolaou stain, *H. pylori* is a faintly basophilic, S-shaped rod admixed with mucus in the vicinity of glandular cell clusters (Fig. 7.13).^{109,112} A triple stain, combining silver, H & E, and Alcian blue at pH 2.5 has a higher yield for *H. pylori* than the Papanicolaou stain.¹¹³

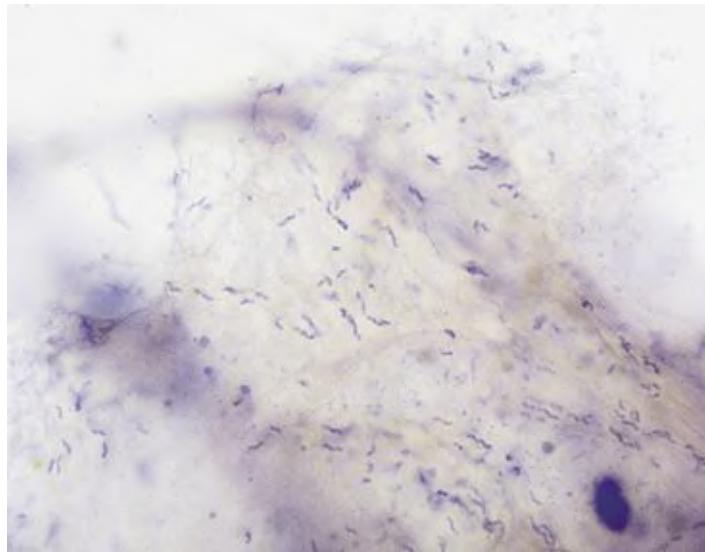


FIGURE 7.13 *Helicobacter pylori* (gastric brushings).

Numerous faintly basophilic S-shaped rods are entrapped in mucus (direct smear, Papanicolaou stain).

Atypical mycobacteria have a characteristic appearance on cytologic preparations. Because they accumulate within macrophages, the presence of many isolated foamy histiocytes ([Fig. 7.14](#)) should raise the suspicion of an atypical mycobacterial infection. On Romanowsky-stained smears, the intracytoplasmic mycobacteria form numerous rod-shaped negative images within the histiocytes.¹¹⁴ Special stains for acid-fast bacilli are necessary to confirm the diagnosis. In general, the burden of organisms is very high, and they are easily seen on the acid-fast stain. Careful screening is necessary when the stain appears negative and there is a high index of suspicion on clinical or cytologic grounds.

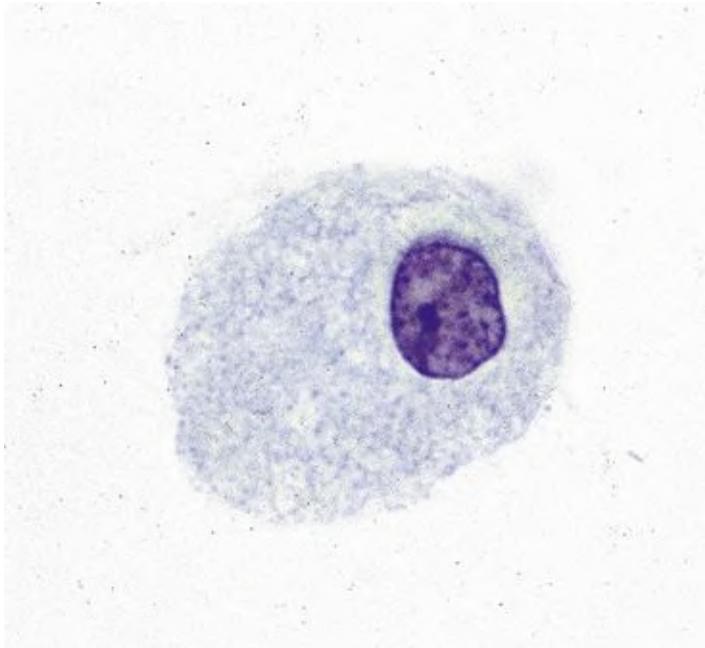


FIGURE 7.14 Atypical mycobacteria (duodenal brushings).

An isolated histiocyte with abundant foamy cytoplasm is present. The presence of numerous acid-fast bacilli was confirmed with the acid-fast stain (direct smear, Papanicolaou stain).

Epithelial Repair

Morphologically identical to its twin in the esophagus, repair in the stomach is a reactive epithelial response to gastritis and ulceration.

Dysplasia and Gastric Adenomas

Gastric dysplasia and gastric adenomas have a similar cytologic appearance, and both are precursor lesions to carcinoma. The lesion is termed “dysplasia” when it is flat and “adenoma” when polypoid. Gastric dysplasia is associated with atrophic gastritis and is rare in the United States. Adenomas of the stomach are also rare, unlike adenomas of the colon.



Cytomorphology of gastric dysplasia and adenoma

- cohesive three-dimensional clusters
- uniformly enlarged nucleus
- increased nuclear-to-cytoplasmic ratio
- crowded but regular nuclear spacing
- absent or inconspicuous nucleoli

As with dysplasia in Barrett's epithelium, the cytologic appearance of gastric dysplasia and adenoma depends on the degree of dysplasia.



Differential diagnosis of gastric dysplasia and adenoma

- epithelial repair
- adenocarcinoma

Crowding, a three-dimensional arrangement, and the absence of large eosinophilic nucleoli are typical of dysplasia and adenomas and help distinguish them from reparative epithelium. Cohesive groups with a regular arrangement of cells and without marked nuclear atypia or pleomorphism are typical of dysplasia/adenomas and allow distinction from a carcinoma. Some dyshesion, irregular cellular arrangement, and nuclear atypia, however, are present in high-grade dysplasia with or without a polypoid lesion. It is common for dysplasia and adenoma to coexist with adenocarcinoma. The difference between the two is often quantitative rather than qualitative and may be difficult to establish with certainty. The distinction between dysplasia and adenoma, dysplasia and adenoma with coexistent carcinoma, and carcinoma is discussed below (see "Adenoma and Adenocarcinoma" under "Duodenum"). Other gastric polyps, such as the hyperplastic polyp, inflammatory fibroid polyp, and fundic gland polyp, do not have distinctive cytologic features.

Adenocarcinoma of the Stomach

Gastric adenocarcinomas account for 90% to 95% of gastric malignancies and are commonly divided into two types, intestinal and diffuse. The intestinal type morphologically resembles the usual type of esophageal and colorectal adenocarcinoma. The malignant cells of the diffuse (signet ring cell) type infiltrate the lamina propria and are thus difficult to sample with a brush unless ulceration is present. Persistent symptoms unresponsive to treatment for peptic ulcer disease and thickened folds on radiologic or endoscopic evaluation are suspicious for a diffuse type adenocarcinoma.



Differential diagnosis of gastric adenocarcinoma,

intestinal type

- gastric dysplasia and gastric adenoma
- epithelial repair

The most significant difference between dysplasia and the intestinal type of adenocarcinoma is cellularity and dyshesion. Tumor diathesis is also very helpful when present. A smear from an adenocarcinoma is usually highly cellular, with many isolated cells as well as tight and loose clusters of cells in a dirty background. The evidence of malignancy is also seen in the arrangement of cells in groups and in the cytologic features of each cell, as described for esophageal adenocarcinoma.

The diffuse type of gastric cancer is composed predominantly of signet ring cells.



Cytomorphology of gastric adenocarcinoma, signet ring cell type

- small groups or isolated cells with small cell size
- vacuolated cytoplasm, often a single large vacuole
- crescent-shaped, angulated, hyperchromatic nucleus
- [Figure 7.15](#)

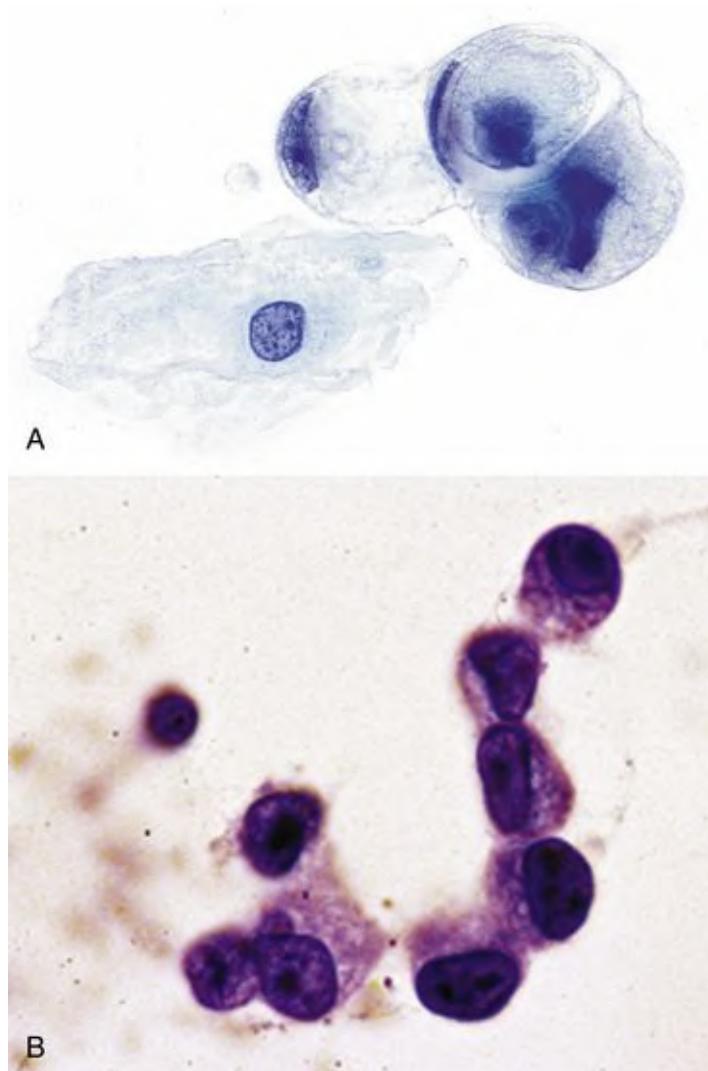


FIGURE 7.15 Signet ring cell carcinoma (gastric brushings). *A*, A group of malignant signet ring cells is seen in the upper right, with a normal squamous cell from the mouth or esophagus (*lower left*). The large vacuoles of the signet ring cells shape the nucleus into a crescent against the cell membrane. In contrast to benign goblet cells, the nuclei in malignant signet ring cells are hyperchromatic and angulated (ThinPrep, Papanicolaou stain). *B*, Signet ring carcinoma cells sometimes resemble histiocytes, but the nuclear-to-cytoplasmic ratio is much higher, and nucleoli are too prominent (Papanicolaou stain).

Signet ring cell adenocarcinoma can be very difficult to detect on cytologic and histologic preparations. Inflammatory cells often obscure the scattered, isolated neoplastic cells. Reactive or reparative epithelial cells associated with ulceration can distract attention from the real lesion. Some signet ring cells have such a bland nucleus that they are confused with histiocytes. A high degree of suspicion is the best safeguard against failure to detect a signet ring cell carcinoma.



Differential diagnosis of gastric adenocarcinoma, signet ring cell type

- inflammation (macrophages)
- intestinal metaplasia

Cells of signet ring adenocarcinoma are distinguished from histiocytes because adenocarcinoma cells lack phagocytosis and generally have some nuclear hyperchromasia and pleomorphism. A careful search for clusters of atypical cells helps to confirm the epithelial nature of the cells; histiocytes generally do not show cellular cohesion. When in doubt, immunostains can be used to determine the epithelial versus histiocytic nature of signet ring-type cells. Signet ring cells are positive for epithelial markers (e.g., keratin, EMA). Histiocytes are negative for these markers but express CD68 and CD163. Goblet cells from intestinal metaplasia are rarely isolated cells, except in liquid-based preparations. They do not show nuclear atypia like angulation and hyperchromasia.

Neuroendocrine Tumors

Neuroendocrine tumors (NETs) are derived from the diffuse (neuro)endocrine system of the GI tract, which is composed of amine-and peptide-producing cells.^{115,116} For prognostication and clinical management, GI NETs are classified into two major categories on the basis of the mitotic index or Ki-67 (MIB-1) proliferation index: *well-differentiated (encompassing low-and intermediate-grade) NETs* and *poorly differentiated (high-grade) neuroendocrine carcinomas (NECs)*, the latter further divided into small cell and large cell types.¹¹⁷ The term *well-differentiated NET* is synonymous with carcinoid tumor. By definition, a grade 1 NET has less than 2 mitoses per high-power field (HPF) or a proliferation index less than 3%. A grade 2 NET has between 2 and 10 mitoses per HPF or a proliferation index of 3% to 20%. A grade 3 NEC has greater than 20 mitoses per HPF or a proliferation index greater than 20%. A majority of gastric NETs are well differentiated.¹¹⁸ The GI tract is the most common site for carcinoid tumors.¹¹⁹ The small intestine is the most common site for GI NETs,¹²⁰ followed by the rectum and appendix; the stomach is a distant fourth.¹¹⁹ They

account for less than 1% of all gastric malignancies.^{96,121} Cytologic specimens are rarely, if ever, obtained from the ileum, rectum, or appendix.



Cytomorphology of neuroendocrine tumors

- dyshesive, monomorphic cells
- plasmacytoid
 - eccentric, round to oval nucleus
 - moderate amount of basophilic, dense cytoplasm
 - no perinuclear clear zone (“hof”)
- many stripped nuclei
- finely granular (“salt and pepper”) chromatin
- [Figure 7.16](#)

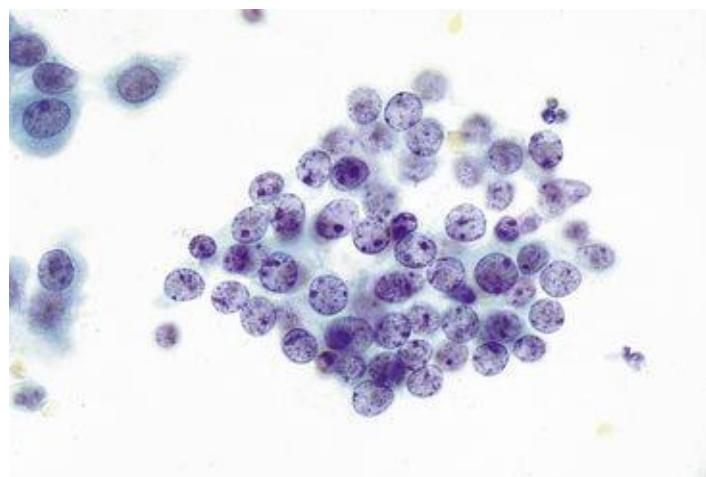


FIGURE 7.16 Well-differentiated neuroendocrine (carcinoid) tumor (duodenal brushings).

A group of monomorphic stripped nuclei with a “salt and pepper” chromatin pattern is evident. A few intact cells with a plasmacytoid appearance are seen at the edge (direct smear, Papanicolaou stain).

Poorly differentiated NECs have a more variable appearance.



Differential diagnosis of neuroendocrine tumors

- adenocarcinoma

- small cell carcinoma
- non-Hodgkin lymphoma

NETs resemble a low-grade lymphoma because cytologic preparations from both often show isolated, monomorphic cells or even bare nuclei. One way to avoid this pitfall is to identify intact cells and base a diagnosis on these cells only. Intact cells of NETs have more abundant cytoplasm than those of a lymphoma.

It can be difficult to distinguish a poorly differentiated large cell NEC from an adenocarcinoma. Nuclear monomorphism and finely granular chromatin raise the suspicion of endocrine differentiation, which can be confirmed by immunocytochemistry for the neuroendocrine markers chromogranin, synaptophysin, and CD56.

The distinction between poorly differentiated small cell NEC and lymphoma can be difficult; nuclear molding and a finely dispersed chromatin pattern are features that favor small cell carcinoma. Immunocytochemical stains for keratin, chromogranin, and LCA are helpful. NETs are positive for keratin and negative for LCA. Chromogranin is often expressed in a well-differentiated NET but may be absent in NEC.

Non-Hodgkin Lymphoma

Non-Hodgkin lymphoma is the second most common malignancy of the stomach, accounting for approximately 5% of all gastric malignancies.⁹⁶ The stomach is, in fact, the most common site for extranodal non-Hodgkin lymphomas.^{122–124} In 1983, the connection between extranodal B-cell lymphomas and mucosa-associated lymphoid tissue (MALT) was recognized, and the MALT lymphoma concept was born.^{105,125} An association between *H. pylori* infection and gastric MALT lymphoma has been established,^{104,126} although the vast majority of patients with *H. pylori* infection do not develop lymphoma. Given the decrease in *H. pylori* prevalence, the incidence of MALT lymphomas has significantly decreased.¹²⁷

By definition, MALT lymphomas are small cell, low-grade lymphomas. The existence of a “large cell, high-grade MALT lymphoma” is controversial. Although some investigators believe that MALT lymphomas may undergo high-grade transformation,^{128–130} most agree to restrict the term *MALT lymphoma* to

low-grade B-cell lymphomas. In doing so, one avoids implying that “high-grade” MALT lymphomas respond to antibiotic therapy, as low-grade MALT lymphomas often do.¹³¹ As a result, MALT lymphoma is characterized by a diffuse infiltrate of small to medium-sized lymphoid cells of B-cell phenotype. A key histologic feature of MALT lymphoma is the lymphoepithelial lesion, an epithelial structure infiltrated by neoplastic B cells that is generally not recognized as such by cytologic methods.

The most common lymphoma of the stomach is diffuse large B-cell lymphoma (DLBL), which constitutes 55% to 65% of all gastric lymphomas.¹³²⁻¹³⁴ DLBL consists of large lymphoid cells; lymphoepithelial lesions are not as frequent as in MALT lymphoma.¹²⁵ With gastric brushings, the challenge is to recognize atypical cells as lymphoid. The final subtyping often depends upon subsequent histologic sampling with immunophenotyping and/or molecular studies.

The cellularity and the background elements of a gastric brushing specimen from a non-Hodgkin lymphoma vary depending on the presence or absence of ulceration. Because the tumor cells are predominantly in the lamina propria, cellularity is high only when ulceration exposes them to the brush. Otherwise, if the mucosa is intact, diagnostic cells may be sparse or absent altogether. As a result, only two thirds or fewer of biopsy-proven gastric lymphomas are detected on cytologic preparations.^{9,135,136} Ulceration also yields a dirty background, with many reactive or reparative epithelial cells. Endoscopic FNA may increase diagnostic yield by providing sufficient cells for immunohistochemistry, flow cytometry, and cytogenetics.^{10,19,137,138}



Cytomorphology of non-Hodgkin lymphoma

- numerous isolated cells
- scant cytoplasm

Because histologic preparations often suffer from crush artifact, it is sometimes easier to recognize malignant lymphoid cells on a cytologic preparation. The cytologic appearance varies according to the subtype of lymphoma. DLBL does not usually pose any diagnostic difficulty because the cells have large atypical nuclei, with a single large nucleolus or multiple smaller nucleoli ([Fig. 7.17](#)).^{9,139}

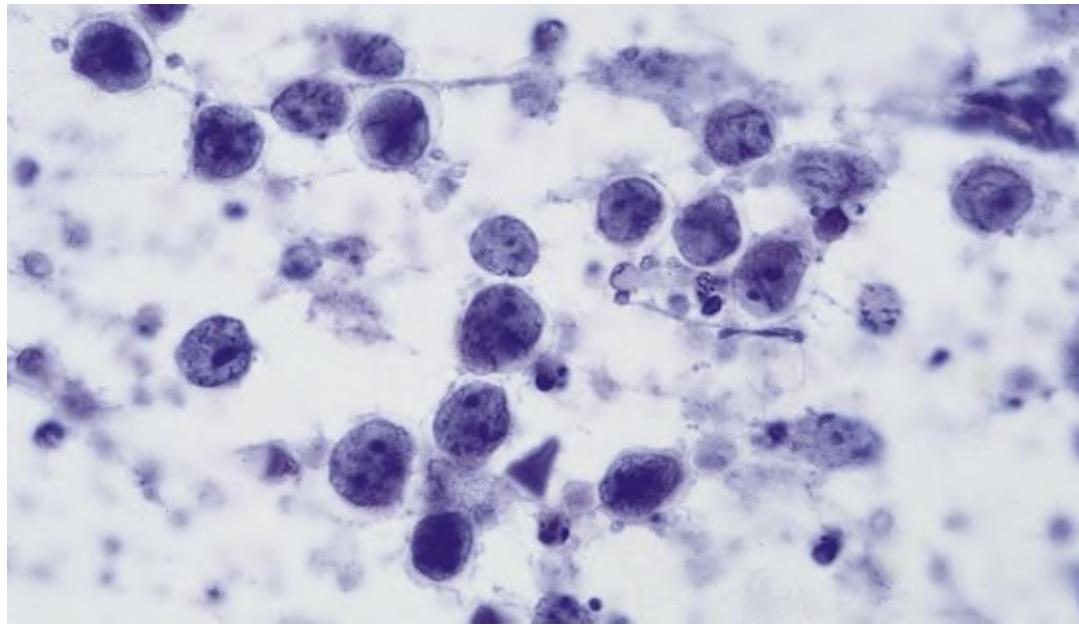


FIGURE 7.17 Diffuse large B-cell lymphoma (DLBL) (endoscopic fine-needle aspiration [FNA] of a gastric mass).

Scattered isolated large atypical cells with scant cytoplasm are seen. The nuclei have vesicular chromatin with one or a few prominent nucleoli. Necrotic debris and apoptotic bodies are present in the background (direct smear, toluidine blue and eosin stain).



Differential diagnosis of large cell lymphoma

- poorly differentiated carcinoma



Differential diagnosis of small cell lymphoma

- chronic inflammation
- neuroendocrine tumor (NET)

Low-grade lymphomas (see [Fig. 7.18](#)) are more difficult to diagnose and may be confused with an inflammatory condition. The absence of cellular cohesion is the principal diagnostic feature of lymphoma. The cells of a poorly differentiated carcinoma, in addition to being more cohesive, often have more abundant vacuolated cytoplasm and a greater degree of nuclear pleomorphism than a large cell lymphoma. Although a poorly differentiated carcinoma may sometimes present a pattern of isolated cells or bare nuclei, a careful search will usually uncover small groups or fragments of malignant cells that are recognizably epithelial. Immunocytochemistry can facilitate the distinction. Carcinomas are

positive for keratins and EMA and negative for LCA; the reverse is true for lymphomas. Because the majority of lymphomas in the GI tract are B-cell lymphomas, they stain for CD20, a pan B-cell marker. Anaplastic large cell lymphoma, however, can be positive for EMA and negative for LCA.⁹

A small cell lymphoma, whether MALT or follicular lymphoma, can be mistaken for chronic inflammation.¹⁴⁰ A predominance of lymphoid cells over neutrophils should raise the suspicion of lymphoma. Numerous monomorphic, atypical, small, or mixed small and large lymphoid cells strongly suggest a lymphoma. Immunophenotyping is almost always required to confirm the diagnosis.

The cells of an NET generally have more cytoplasm than those of a small cell lymphoma; their nuclei are round and have a more finely granular chromatin than those of lymphoid cells.

Gastrointestinal Stromal Tumor

Virtually all mesenchymal tumors of the GI tract were once considered to be smooth muscle tumors. A subgroup has been redefined as gastrointestinal stromal tumors (GISTs), based in large part on their immunoreactivity for c-kit (CD117).¹⁴¹ GISTs recapitulate differentiation toward the interstitial cells of Cajal, the normal, c-kit-expressing cells that act as the peristaltic pacemakers of the GI tract.¹⁴² Immunoreactivity for c-kit and DOG1 distinguishes GISTs from smooth muscle tumors, which are positive for desmin and smooth muscle actin but negative for c-kit and DOG1. Positive staining for c-kit (CD117) or DOG1 is now virtually essential for the diagnosis of a GIST.¹⁴³ DOG1 (“discovered on GIST-1”) is a protein of unknown function that was identified from gene expression data as a highly sensitive and specific marker for GIST.¹⁴⁴ It is more sensitive than c-kit, i.e., some GISTs are negative for c-kit but positive for DOG1.¹⁴⁵

Most GISTs arise because of a mutation in the c-kit gene, which encodes a transmembrane receptor for a growth factor called *stem cell factor*. Mutations generally occur in the DNA encoding the intracellular part of the receptor, which acts as a tyrosine kinase to activate other enzymes. Mutations make c-kit function independently of stem cell factor, leading to increased cell division. Additional mutations are likely required, but the c-kit mutation is probably the first step in the pathogenesis of a GIST.

Most GISTs are composed of spindle-shaped cells, but some are predominantly epithelioid. All are

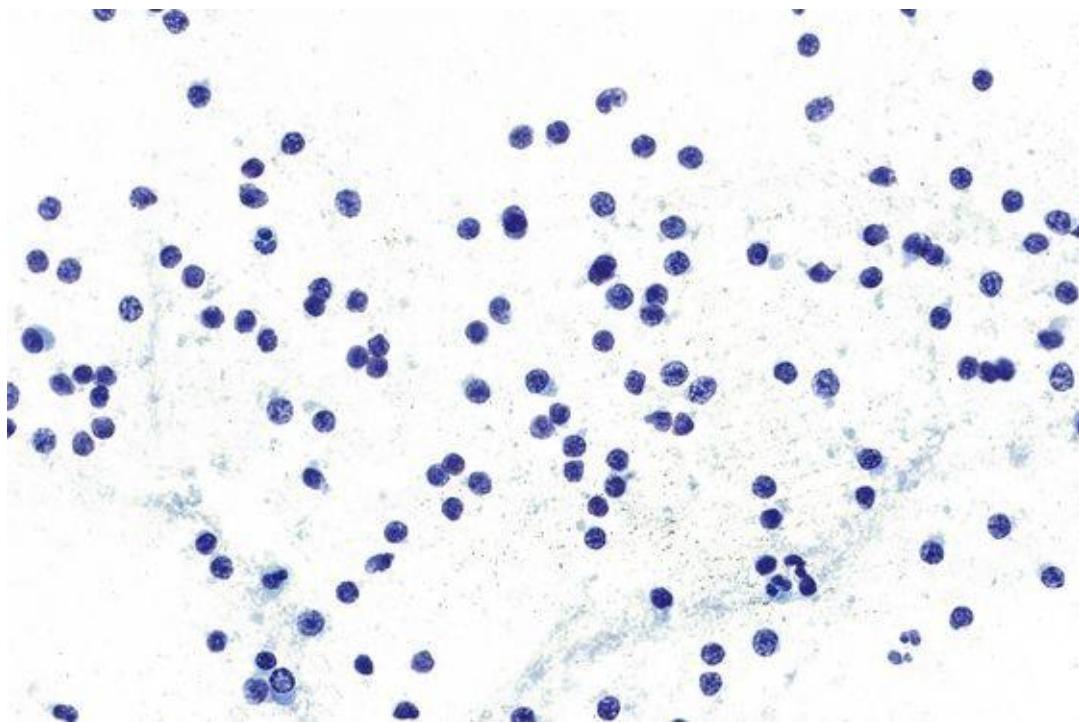


FIGURE 7.18 Lymphoma of mucosa-associated lymphoid tissue (MALT) type (gastric brushings). Scattered lymphocytes are seen without intermixed neutrophils or plasma cells (direct smear, Papanicolaou stain).

considered potentially malignant; the risk of progression depends in part on the location of the tumor, its size, and its mitotic rate.¹⁴⁶ The c-kit tyrosine kinase inhibitor imatinib (Gleevec), a drug initially marketed for chronic myelogenous leukemia, is useful in treating GISTS, leading to significant response rates in metastatic or inoperable cases. Patients who develop resistance to imatinib are treated with other tyrosine kinase inhibitors like sunitinib.



Cytomorphology of gastrointestinal stromal tumor

- irregularly outlined clusters of cells
- spindle-shaped or epithelioid
- wispy cytoplasm with long extensions
- mitoses, necrosis (occasionally)

Due to its mural location, a GIST is usually not accessible to the endoscopic brush unless the overlying mucosa is ulcerated. FNA is the preferred method of sampling.^{19,147} Specimens reveal isolated cells and large tissue fragments of crowded, spindle-shaped or epithelioid cells (Fig. 7.19). The individual cells often lose their wispy cytoplasm and become stripped nuclei.^{148–150} Perinuclear or paranuclear vacuoles are present in some cells.¹⁴⁸ Delicate cytoplasm and prominent nuclear palisading have also been noted.^{149,151} Immunocytochemistry for c-kit (CD117) and/or DOG1 can be applied to the aspirated material to confirm the diagnosis.^{145,149,152}

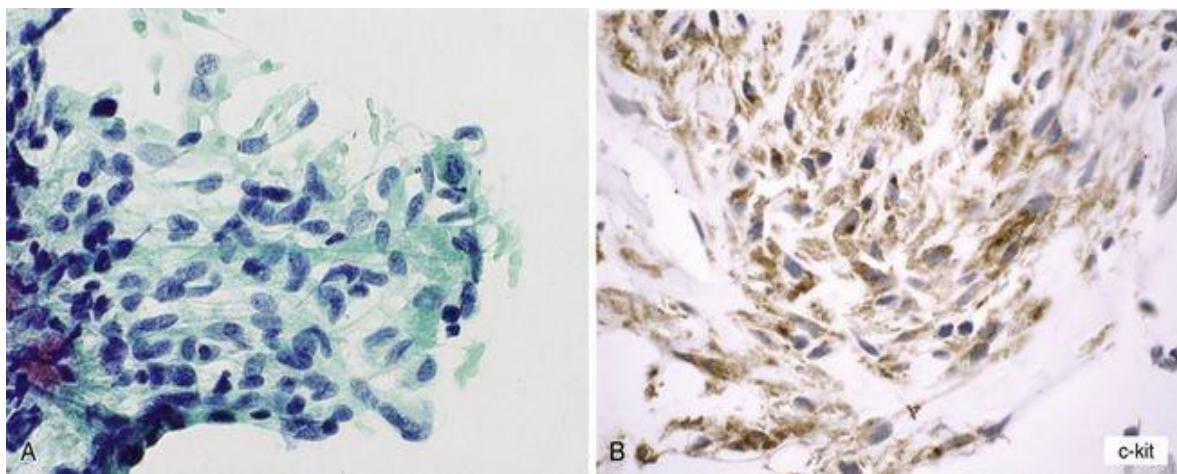


FIGURE 7.19 Gastrointestinal stromal tumor (GIST) (ultrasound-guided endoscopic fine-needle aspirate [FNA] of a gastric mass).
A, The cells have oval nuclei with bland chromatin and inconspicuous nucleoli (direct smear, Papanicolaou stain). B, The corresponding cells in a cell block preparation are immunoreactive for c-kit, some showing a characteristic dotlike cytoplasmic pattern.

Differential diagnosis of gastrointestinal stromal tumor

- leiomyoma
- leiomyosarcoma

Leiomyomas of the GI tract usually involve the esophagus (see Fig. 7.12) and colorectum. They are less common in the stomach. Endoscopic FNAs yield spindle-shaped cells. Leiomyosarcomas tend to show more significant nuclear

pleomorphism, nuclear atypia, and mitoses, as well as a less prominent vascular pattern than GIST. The distinction between a smooth muscle tumor and a GIST depends on differential immunocytochemical expression of c-kit (CD117), DOG1, desmin, and smooth muscle actin.¹⁴³

Duodenum

Infections

Brush cytology of the duodenum is useful to detect *Giardia lamblia*, an intestinal parasite that is usually acquired from contaminated drinking water and causes diarrhea.^{153,154} The organism is flat, gray, pear-shaped, and binucleate, with four pairs of flagella. The organisms are immunoreactive for c-kit, making this immunostain a sensitive detection method.¹⁵⁵

Intestinal infection with *Microsporidium*, an obligate intracellular spore-forming protozoan, occurs as an opportunistic infection in acquired immune deficiency syndrome (AIDS) patients.⁵⁷ Microsporidia can be detected on cytologic specimens such as urine, stool, nasal secretions, duodenal aspirates, bile, and brushings from the duodenum and biliary tract.^{156–158} With the Papanicolaou stain they appear in aggregates as brightly eosinophilic rod-shaped or ovoid organisms measuring 1 to 3 μm in diameter ([Fig. 7.20](#)). When intracellular, they are in the supranuclear portion of the cytoplasm. They may be missed for two reasons: (1) The pathologist may be distracted by the reactive changes as a result of the infection, and (2) they are in a slightly different plane of focus from that of the nucleus. This possibility should be kept in mind when examining a specimen from an immunocompromised patient. Their presence can be confirmed with the Brown-Brenn stain.

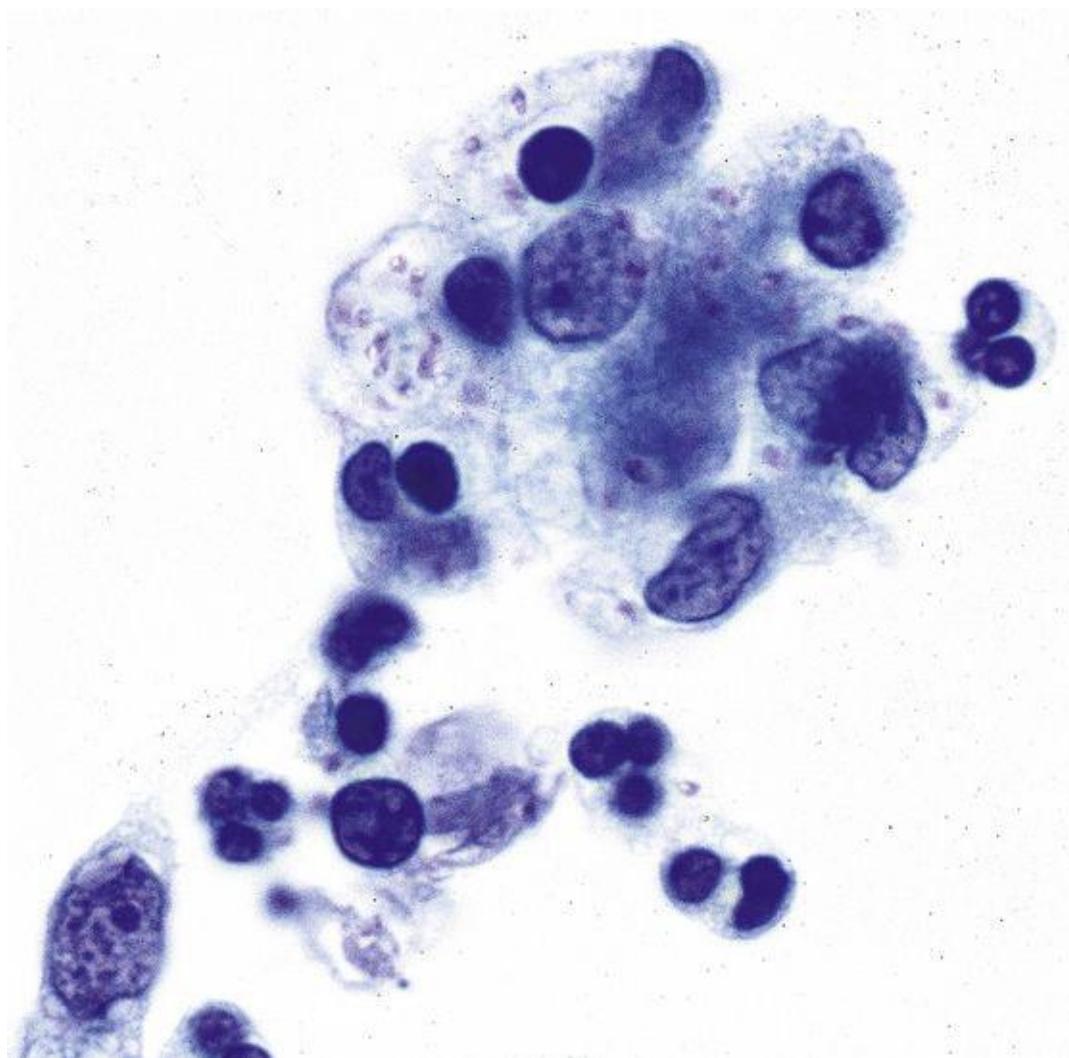


FIGURE 7.20 Microsporidia.

Aggregates of tiny, brightly eosinophilic rod-shaped or ovoid organisms are seen in the apical portion of glandular epithelial cells (ThinPrep, Papanicolaou stain).

Cryptosporidium can involve any glandular epithelium (stomach, bile duct, small and large intestine) of the GI tract in patients with human immunodeficiency virus (HIV) infection.^{57,159} Like microsporidia, cryptosporidia can be detected by light-microscopic examination of cytologic samples and stool. They are round basophilic bodies, 2 to 5 µm in diameter, on the luminal surface of the epithelial cells (Fig. 7.21). They are seen only when the plane of focus is shifted to the surface of the cells where the organisms reside. When in doubt, a confirmatory Grocott methenamine silver stain can be applied.

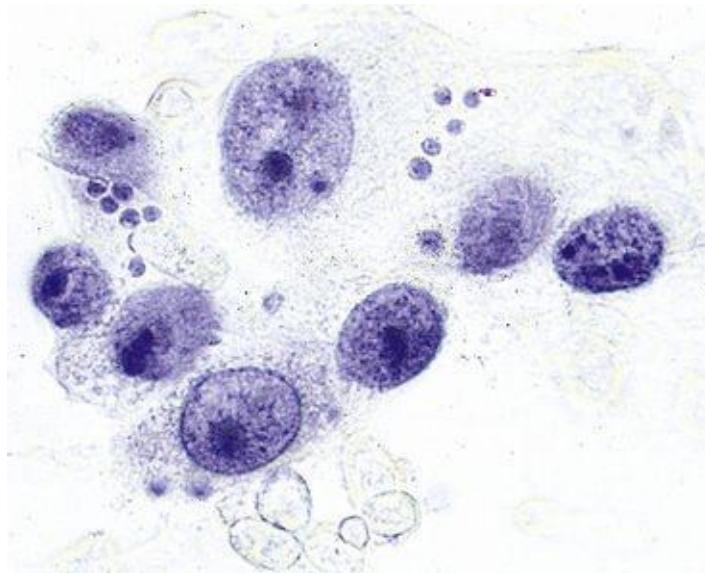


FIGURE 7.21 Cryptosporidia (gastric brushings).

Aggregates of small, round basophilic bodies are seen on the surface of glandular epithelial cells (direct smear, Papanicolaou stain).

Other common lesions of the duodenum, including epithelial repair, adenocarcinoma, NETs, and infections, are similar to their counterparts in the stomach.

Adenoma and Adenocarcinoma

Premalignant lesions, including adenomas, are more common in the colon than in the stomach or small bowel. A majority (60% to 70%) of adenomas in the duodenum are associated with an adenocarcinoma,^{160–162} especially those with high-grade dysplasia or of a size greater than 2 cm.¹⁶³ Adenomas of the duodenum resemble those found in the stomach and colon ([Fig. 7.22](#)).

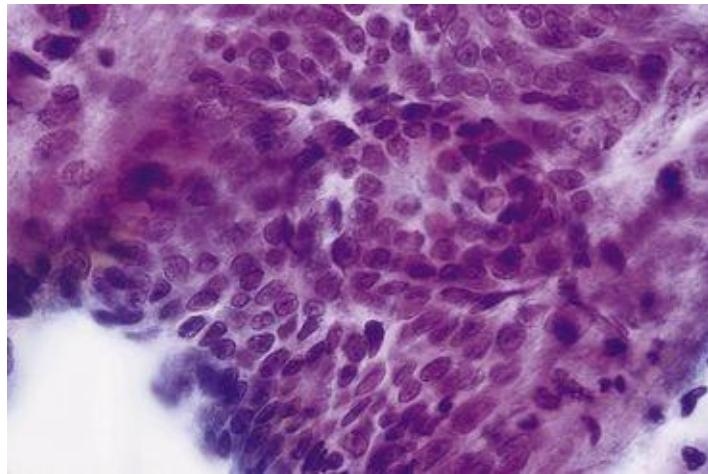


FIGURE 7.22 Ampullary adenoma (ampullary brushings).

A crowded group of glandular cells with mucin depletion and an increased nuclear-to-cytoplasmic ratio is present. A gland opening is apparent. In spite of the crowding, the arrangement is orderly. The nuclei are enlarged and elongated, but significant atypia is absent (ThinPrep, Papanicolaou stain).



Cytomorphology of duodenal adenoma

- cohesive three-dimensional clusters of crowded epithelial cells
- increased nuclear-to-cytoplasmic ratio
- absent goblet cells
- palisading and molding of elongated nuclei
- fine chromatin and absent or small nucleoli

If the findings are diagnostic of an adenoma, it is appropriate to report the case as “neoplastic cells present, consistent with adenoma.” If the findings are suggestive of but not conclusive for an adenoma, “atypical glandular cells present, suggestive of an adenoma” is more appropriate. A duodenal adenoma may coexist with adenocarcinoma. It is easier to distinguish a low-grade premalignant lesion from a carcinoma; a high-grade premalignant lesion is quite difficult to distinguish from an invasive malignancy.¹⁶⁴ A high-grade lesion is also more likely to coexist with a carcinoma. The degree of cellularity, dyshesion, and nuclear atypia helps to distinguish a high-grade premalignant lesion from a frankly malignant one, but the difference is quantitative, not qualitative.⁸² Malignant lesions show greater cellularity, more dyshesion, and more marked nuclear atypia and pleomorphism. A comparison of the cytologic features of reactive, premalignant, and malignant glandular lesions is shown in [Table 7.2](#).

Even when only a low-grade premalignant lesion is present on the cytologic specimen, an unsampled coexisting carcinoma elsewhere in the lesion cannot be excluded. Therefore, surgical resection of the entire lesion is indicated when a diagnosis of a premalignant lesion of the small bowel is suspected or rendered on cytology. When the lesion is diffuse or ill defined, multiple biopsies should be taken.

Colon

The cytomorphology of the common lesions of the colon, including epithelial repair, adenomas ([Fig. 7.23](#)), adenocarcinomas, lymphomas, and infections, are similar to those described previously for the same lesions elsewhere in the GI tract. The cytologic findings in ulcerative colitis have been described, but it is not customary to obtain cytologic specimens in this setting.^{[165–169](#)}

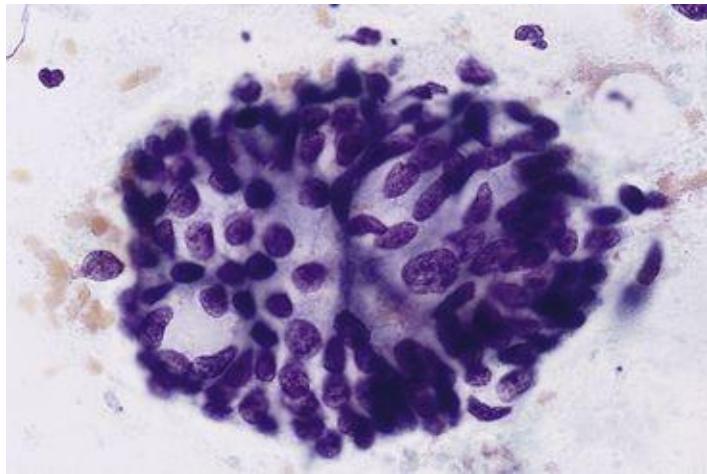


FIGURE 7.23 Colonic adenoma (colonic brushings).
A cohesive group of stratified but orderly glandular cells with elongated nuclei is seen (direct smear, Papanicolaou stain).

Cytology of the colon is less commonly used than cytology of the upper GI tract. One reason may be that colonoscopic examinations are widely performed for colitis, particularly inflammatory bowel disease, and cytologic examination is not useful in establishing the diagnosis of active or chronic colitis.^{[165](#)} In addition, the cytologic distinction between repair, dysplasia, and carcinoma in the setting of inflammatory bowel disease is challenging.^{[165,166](#)} Nonetheless, data suggest that, as with lesions of the upper GI tract, the combination of biopsy and cytology renders the highest detection rates for colon cancer.^{[42,170–175](#)}

The Anal Pap Test

The estimated annual incidence of cancers of the anus, anal canal, and anorectum (excluding rectum) in the United States is 6230 (2250 in men, 3980 in women).¹⁷⁶ Annual mortality is 780 (300 in men, 480 in women). The incidence has been increasing since early 1960s and continues to increase.¹⁷⁷⁻¹⁷⁹ The most important risk factor for anal neoplasia is infection by human papillomavirus (HPV).¹⁸⁰ Other risk factors include receptive anal intercourse; number of lifetime sexual partners; history of cervical, vaginal, and/or vulvar cancer; HIV infection; immunosuppression after solid-organ transplantation; and smoking.^{180,181} The annual incidence of anal cancer in men who have sex with men (MSM) is 37 per 100,000, comparable to the incidence of cervical cancer before the introduction of Pap screening.^{182,183} The annual incidence in HIV-positive MSM ranges from 42 to 137 per 100,000.¹⁸⁴ The HPV vaccine reduces the incidence of anal intraepithelial neoplasia among MSM.¹⁸⁵ Antiretroviral therapy does not seem to affect the risk of anal cancer in the HIV-positive population.¹⁸⁰

Eighty-five percent of invasive cancers are squamous cell carcinomas.¹⁸⁶ HPV-associated anal lesions are biologically and morphologically similar to their cervical counterparts, and testing results are reported using the same terminology.¹⁸⁷ Data show progression from low-to high-grade intraepithelial lesion and from anal intraepithelial lesions to invasive carcinoma, especially in HIV-positive or otherwise immunocompromised patients,¹⁸⁸⁻¹⁹¹ although the rates of progression or regression have not been as well established as for cervical intraepithelial neoplasia.

Screening for anal cancer and its precursors was implemented in the 1990s, with performance characteristics similar to those for cervical lesions.^{183,184} Screening with anal cytology has increased life expectancy by several months, depending on screening frequency and HIV status.^{192,193} In 2007, the New York State of Public Health AIDS Institute recommended digital anal-rectal examination and anal cytology at baseline and annually thereafter in HIV-infected MSM, patients with a history of anogenital condyloma, and women who have abnormal cervical and/or vulvar histology.¹⁸⁴

Sample procurement is simple and does not require an anoscope. Anal cytology specimens should sample the entire anal canal including keratinized and nonkeratinized squamous epithelium and the anorectal transformation zone. The sample is collected with the patient in the lateral recumbent or dorsal lithotomy position. A Dacron swab moistened with tap water is inserted blindly

5 to 6 cm into the anal canal past the anal verge and into the rectal vault. The swab is rotated with firm lateral pressure and slowly withdrawn. Smears or liquid-based slide preparations are equally acceptable.

Anal squamous intraepithelial lesions and carcinomas are morphologically identical to their counterparts in the cervix ([Fig. 7.24](#)). For this reason, Bethesda 2001 terminology (e.g., NILM, ASC-US, ASC-H, LSIL, HSIL; see [Chapter 1](#)) is commonly used for reporting results. Adequacy criteria differ slightly from those for cervical cytology. The minimum cellularity is approximately 2000 to 3000 nucleated squamous cells, which is equivalent to one or two nucleated squamous cells per $\times 40$ field with a ThinPrep slide, or 3 to 6 nucleated squamous cells per $\times 40$ field with a SurePath slide.¹⁹⁴ The presence of squamous metaplastic cells and rectal columnar cells should be reported to indicate anal transformation zone sampling.

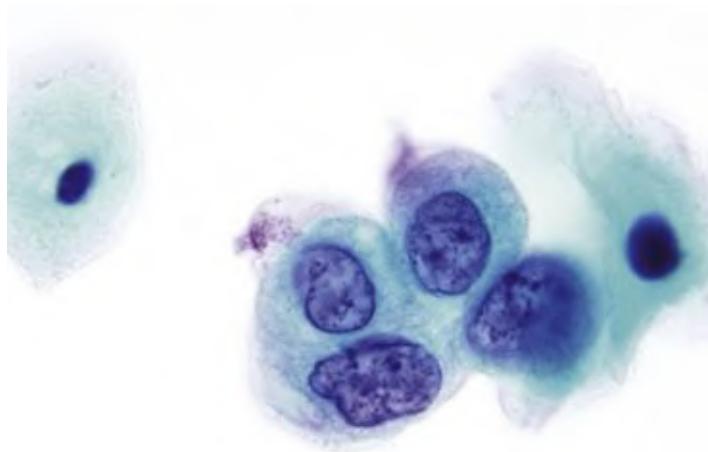


FIGURE 7.24 High-grade squamous intraepithelial lesion (anal Pap).
Cells show nuclear enlargement, nuclear membrane irregularity, and coarse chromatin, analogous to the changes seen in cervical specimens (ThinPrep, Papanicolaou stain).

The role of HPV testing is uncertain. Although preliminary data suggest a potential role in primary screening¹⁹⁵ and adjunct¹⁹⁶ and reflex testing,¹⁹⁷ its routine use requires further investigation. The prevalence of HPV infection is high (70% in one study) in HIV-positive patients¹⁹⁶ and as high as 84% in HIV-positive MSM.¹⁹⁸ In addition, the prevalence of high-risk HPV is not associated with lesion grade in the HIV-positive population,^{196,198} although the HPV 16 genotype seems to be more prevalent in patients with high-grade lesions.^{196,199} Therefore, HPV genotyping might one day prove useful in the management of patients at high risk for anal neoplasia.

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CHAPTER 8

Fine-Needle Aspiration Biopsy Technique and Specimen Handling

Amy Ly

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Introduction

Fine-needle aspiration (FNA) is widely used for evaluating palpable superficial masses and cysts as well as deep-seated, nonpalpable radiologic abnormalities under image-guidance. The capabilities and limitations of FNA, specific to the evaluation of a specific organ or anatomic site, are discussed in other chapters in this book.

In 1930, Martin and Ellis published the first significant North American description of FNA methodology for palpable lesions.¹ In spite of the long history of FNA and its application to the care of patients, there is no best practice for performing an FNA, and rigorous comparisons of biopsy techniques are lacking. Although the most common techniques for performing an FNA of a palpable mass are applicable to all superficial sites, there are nuances in method—some idiosyncratic—that depend on geographic or institutional custom and/or previous training and experience. Even for individual pathologists, details learned in training are often modified in practice by factors such as height, handedness, hand size, and finger strength.

Hands-on practical training in FNA technique is critical to developing the hand-eye coordination required. In comparison with physicians who had no formal training in FNA technique, those who received such training obtained diagnostic samples more frequently.² The best way to become proficient is to perform procedures under the direct supervision of someone who is proficient and provides feedback. Good training is important, but continued performance of procedures is necessary to maintain competence.

Materials and Supplies

All the equipment needed to perform an FNA ([Table 8.1](#)) is small and lightweight enough to be hand-carried in one container ([Fig. 8.1A](#) and [B](#)). This portability allows FNAs to be performed on demand and in virtually any setting. The equipment occupies only a small area of counter space when arranged for specimen preparation ([Fig. 8.1C](#)).

TABLE 8.1

FINE-NEEDLE ASPIRATION EQUIPMENT LIST

1. Syringe holder (10-mL Cameco syringe pistol or equivalent) (see Fig. 8.1A)
2. Disposable sterile 10-mL plastic syringes
3. Disposable sterile needles with transparent hubs (23 and 25 gauge, up to 1.5 inches long)
4. Alcohol swabs
5. Sterile gauze pads
6. Glass slides with frosted end for labeling
7. Fluid transport medium (e.g., RPMI, saline)
8. Local anesthesia and needle/syringe to administer (e.g., 2% lidocaine, 27 gauge needle/1 mL syringe)
9. Alcohol fixative (commercial spray fixative or Coplin jar filled with 95% ethanol)
10. Gloves
11. Pen/pencil for labeling slides (e.g., Leica pen, Sakura Tissu-Tek pencil)
12. Plastic slide holders or slide trays (for transporting slides)



FIGURE 8.1 FNA equipment.

The syringe holder (A) and other items (B) are small enough to fit into a basket. They can be quickly assembled on a small amount of counter space (C). (B and C courtesy Sara Monaco, MD, University of Pittsburgh Medical Center, Shadyside Hospital.)

Procedure for Performing a Fine-Needle Aspiration of a Palpable Mass

The essential steps involved in performing an FNA of a palpable mass are demonstrated in the video that accompanies this chapter. Standard safety precautions must be observed during the biopsy procedure and in handling the harvested specimen.



Steps for a successful FNA

1. Determine if the FNA is warranted,
2. Consent the patient for the procedure.
3. Position the patient and immobilize the lesion.
4. Sample the targeted lesion adequately.
5. Prepare the sample for evaluation, including appropriate allocation of material for ancillary studies as necessary.
6. Provide post-procedure instructions to the patient.

Determining If a Fine-Needle Aspiration Is Warranted

A patient presenting for an FNA has almost certainly been referred by another physician. The patient's clinical history should be reviewed when available, preferably before seeing the patient. A focused physical examination should be performed, confirming that the lesion is indeed palpable (and an FNA appropriate) and ensuring that the correct site is aspirated. It is helpful to ask the patient to point to the mass. If the lesion is not palpable or cannot be safely sampled, it should not be aspirated. By reviewing the results of imaging studies and performing a focused physical examination, the operator should assess the size, shape, and relationship of the lesion to nearby structures like large blood vessels. Imaging studies document the internal qualities of the lesion (e.g., vascularity and the relative proportion of solid versus cystic areas) and its distance from the skin surface. This information guides the approach to the lesion (e.g., the length of needle to use). During the brief examination, the operator should inquire about a significant bleeding disorder or use of anticoagulants.

Obtaining Patient Consent

The consent process involves a detailed description of the procedure, including its purpose and potential complications, allowing for questions from the patient. The consent form needs to be signed by a witness. (The physician performing the FNA can act as the witness.)

Sample Explanation of the Procedure

“Hello, Ms. Doe, I’m Dr. Ly from the Department of Pathology. I understand that Dr. Jones has sent you here for a fine-needle aspiration of a mass in your neck. The goal is to determine the diagnosis for the mass. Let me explain what the procedure involves. Feel free to ask questions at any time.

“I will use a very thin needle to take a sample of the mass. The needle is the same size or smaller than the ones used to draw blood. I will insert the needle into the mass and move the needle back and forth for about 15 to 20 seconds. I usually do this two or three times, which means two or three separate needle sticks. Sometimes I will need to do it a fourth time if I think I will need additional material to make a diagnosis. I usually take a small break after the first or second needle stick and do a quick check with a nearby microscope to see how much material there is. I will not make a diagnosis at this time—I am only checking to ensure that I am in the mass and getting enough material to make a diagnosis.

“Most patients feel a pinprick when the needle goes through the skin like during a blood draw, and while the needle is moving back and forth, most patients feel a dull pressure, pulling, or soreness. I can inject lidocaine into the skin over the lump if you like. It will be another small needle, and you may feel a burning sensation for a few seconds. If you cannot tolerate the procedure because of pain, I will stop and take the needle out.

“This procedure has a few minor risks that you should know about. Bleeding and infection are the most common, but I clean the skin with alcohol before each needle stick to minimize the chances of infection. You will probably experience a small amount of bleeding and possibly bruising. After each needle stick, my assistant will apply firm pressure with a gauze pad to minimize this.

“Do you understand the purpose of this procedure and the risks involved, and do you agree to the procedure? If so, please verify for me your full name and date of birth, and I’ll ask you to sign this consent form” (see Video 8-1  found on expertconsult.com).

Readyng the Equipment

The biopsy apparatus is assembled by loading a syringe onto the syringe holder and attaching a needle. Needles 22 gauge or smaller are considered “fine.”³ Commonly, 23 and 25 gauge needles measuring 1.0 to 1.5 inches long are used for palpable lesions. Larger-gauge needles (19 to 22 gauge) are used for aspirating abdominal fat to test for amyloid deposition.⁴⁻⁸ The shortest needle that reaches the furthest area of the lesion from the skin should be chosen. Shorter needles (less than 1.0 inch long) are sufficient for small nodules close to the skin surface. Needles vary in design; those with beveled tips are preferred, but FNA does not require a specific needle type to be successful. Once set up, the needle cap is loosened, and the equipment placed conveniently. If local anesthesia is to be given, it should be prepared at this time.

Because the sample must be prepared immediately before it dries/clots, several clean glass slides should be prelabeled with at least two patient identifiers (e.g., name, date of birth, medical record number), and alcohol slide fixative and a container filled with liquid transport medium should be at hand. These will be used to make smears, rinse the needle, and allocate material for special studies if necessary.

Positioning the Patient and Immobilizing the Lesion

The patient is positioned so that he or she is comfortable and the lesion can be palpated and immobilized. The patient should lie on their back when feasible; this is a safe position if there is a vasovagal response. Pillows, rolled towels, and foam shapes can be used for support. Changing the patient’s body position can dramatically affect the accessibility of the lesion. Breast masses, for example, are often best appreciated with the patient’s arm raised above the head. Neck nodules easily palpated in the sitting position may seem to disappear when the patient lies back. Becoming ambidextrous at FNA allows more flexibility in how the patient is positioned.

If the mass is beneath a band of skeletal muscle like the sternocleidomastoid, position the patient such that the muscle is relaxed, and move the muscle aside. In addition to being painful, passing a needle through skeletal muscle clogs the needle with skeletal muscle.

The exact methodology for stabilizing and immobilizing the mass varies by the site, the size of the mass, and the characteristics of the operator’s hands.

Once the method of immobilization and the needle trajectory have been determined, the skin is cleaned with an alcohol swab and local anesthesia

injected (if desired). Buffered lidocaine solution tends to be less painful than unbuffered.⁹ Local anesthetic is advisable if the mass is tender to palpation or if the procedure involves a sensitive site like the nipple/areola. It is best not to inject so much local anesthetic that excessive skin swelling obscures the mass. This is particularly true with smaller nodules. The local anesthetic agent requires a few minutes to take effect.

Sampling the Lesion

Once the mass is fixed with the nonaspirating hand, the skin is cleaned with an alcohol swab at the planned needle entry site (see  Video 8-1 found on [expertconsult.com](#)). The loosened needle cap is removed and the aspirating hand stabilized by resting the syringe barrel against the thumb or forefinger of the nonaspirating hand. This guards against any physiologic hand tremor and ensures precise needle placement but is not needed after insertion of the needle. The needle is inserted into the lesion, and the syringe plunger pulled back to generate several cubic centimeters of vacuum. The vacuum is maintained until the needle is removed from the patient. With a straight wrist, the needle is moved back and forth quickly and repeatedly in a sawing motion (“excursions”) for a dwell time no longer than 15 to 20 seconds (approximately 40 to 60 excursions) along the original needle trajectory, alternately advancing into the mass and withdrawing to a superficial location without exiting the patient. Slower needle action will yield less material. A shorter dwell time (2 to 5 seconds) is recommended for vascular lesions like thyroid nodules.¹⁰ Some practitioners also rotate the hand in a clockwise or counterclockwise fashion while it is moved within the lesion to achieve a “coring” effect, but this is not necessary. Each time the needle advances into the lesion, its cutting tip dislodges small tissue fragments; this cutting action is essential for a successful FNA. Negative pressure alone without needle movement will not procure enough tissue for diagnosis in solid lesions.¹ The vacuum in the syringe helps conduct the tissue fragments into the needle shaft and hub. A slight acceleration of the needle as it advances into the mass enhances the cutting action of the needle tip. Keeping the needle tip within the mass avoids diluting the specimen with adjacent nonlesional tissue. Material can be seen accumulating in the needle hub, although absence of visible material does not signify an inadequate sample. If blood is rapidly entering the hub, withdraw the needle immediately, especially in a vascular site like the thyroid gland.

There are nuances to the technique for different sites and types of lesions.

Sampling with thinner needles (25 or 27 gauge) is preferred for vascular organs like the thyroid, as well as for fibrous lesions such as fibroadenoma of the breast.^{3,10,11} For sampling a sclerotic lesion, the needle should be moved more vigorously.

To sample more of the mass with one needle pass, withdraw the needle tip to a superficial location while maintaining vacuum, then redirect it to a different area by changing the angle of entry. “Fanning” allows for sampling of a larger area: After each excursion, when the needle tip is in a superficial location, change its angle of entry slightly until the entire region of interest is sampled. Avoid changing direction when the needle is deep in the lesion. This would result in tissue tearing and hemorrhage, which compromises the diagnostic yield of subsequent passes.

Remove the needle from the patient after the last excursion is completed or when material or blood is visible in the needle hub, which can occur in less than 15 seconds. Withdraw the needle from the patient in a controlled manner and *only after release of the vacuum in the syringe*. Failure to release suction before withdrawing the needle from the patient pulls the aspirated material into the barrel of the syringe, making it difficult to expel for smear preparation. Pressure to the site is applied immediately with gauze to minimize bleeding. The patient or an assistant should perform this step, because the operator needs to prepare the sample immediately.

An FNA procedure typically involves inserting the needle into the mass two or three times (“passes”) to obtain several samples. The center of the mass is often sampled on the first needle pass with the needle approximately perpendicular to the skin. Other areas of the mass are sampled on subsequent passes, especially if the initial material is necrotic, cystic, or otherwise nondiagnostic. Sampling the mass along its long axis tends to yield more cellular specimens compared with moving the needle along the short axis.¹²

“Feeling with the Needle”

Learning to feel with the needle is an important skill. Cancer is often described as “gritty to needle,” like pushing a needle through the flesh of a pear. Aspirating a fibroadenoma of the breast feels like pushing a needle into a rubber stopper or leather. Fat is “soft to needle,” meaning that the needle encounters minimal or no resistance. Calcifications are rock-hard. The closer the aspirating hand is to the mass, the more tactile information is perceived. Feeling with the needle allows detection of differences within a mass and is useful for sensing whether the

needle has entered or exited the mass. This information is very useful for clinical-pathologic correlation.

Preparing the Sample

Making Smears

To prepare smears, the needle is detached from the syringe, the plunger drawn back to fill the syringe barrel with air, and the needle reattached. This is a very important step but one that carries the risk of a needle stick. Although the risk is lower with use of Luer-Lok syringes, attaching and detaching needles from these syringes take more effort and time. One way to minimize this is to detach and reattach the needle by clamping the hub with a hemostat.

To expel the material, the plunger is depressed back into the syringe. It is advisable to hold the needle hub securely on the syringe during this step to avoid having the needle detach and fly away under the increased pressure. Touching the needle tip, bevel down, to the glass slide minimizes spraying of the material. Spraying causes the sample to dry, which is counter productive if one is planning to wet-fix the slide in alcohol, and it may aerosolize infectious particles. Aspirated material can also be squirted directly into a liquid medium.

If the amount of material is small, one smear is made. If there is more material or blood, additional smears should be made. Because drying begins the moment material is expressed onto a slide, it is important that smears be made as quickly as possible.

The “one-smear” method ([Fig.8.2A-D](#)). The slide with the expelled material is held in one hand (usually the nondominant hand), frosted side up. The thumb rests on the frosted section, and the remaining fingers are placed along the back side of the slide. The dominant hand holds a clean slide (the “spreader” slide) at a 90-degree angle to the first slide. The lower edge of the spreader slide is brought into contact with the first slide, and the leading edge of the spreader slide is gently lowered (rotated) until the slide contacts the first slide and compresses the expelled material. Maintaining contact between the slides, the spreader (top) slide is smoothly pulled over the material to distribute the material evenly. If done correctly, the smeared material forms an oval shape on the slide. The spreader slide contains minimal material and, for this reason, is discarded in some laboratories.

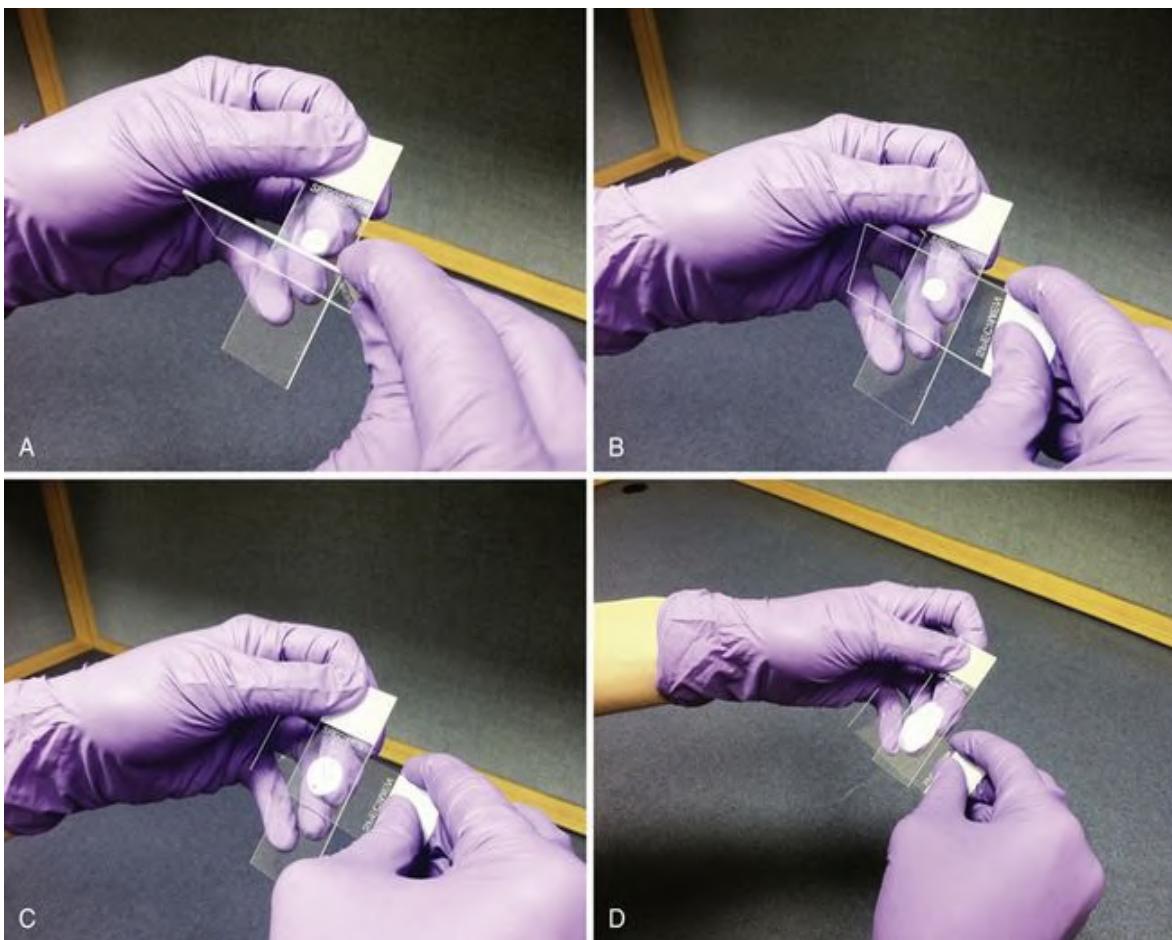


FIGURE 8.2 The “one-smear” preparation method.

A single smear is made by *A*, applying a spreader slide perpendicular to the slide with the aspirated material and *B*, rotating the spreader slide until *C*, it comes in contact with the bottom slide and slightly compresses the expelled material. *D*, The spreader slide is gently and quickly pulled back, maintaining contact with the bottom slide.

The “two-smear” method (Fig. 8.3A-C). The slide with the specimen is oriented horizontally with the frosted side up, and a second slide is placed over it, frosted side down. The two slides are gently pressed together until the material is flattened between them, and the slides are pulled apart to make two identical smears. It is more difficult to maintain the parallel orientation of the slides with this method, and there is a higher likelihood of preparation artifact (e.g., uneven distribution of material on the slide or scraping of material by the slide edge).

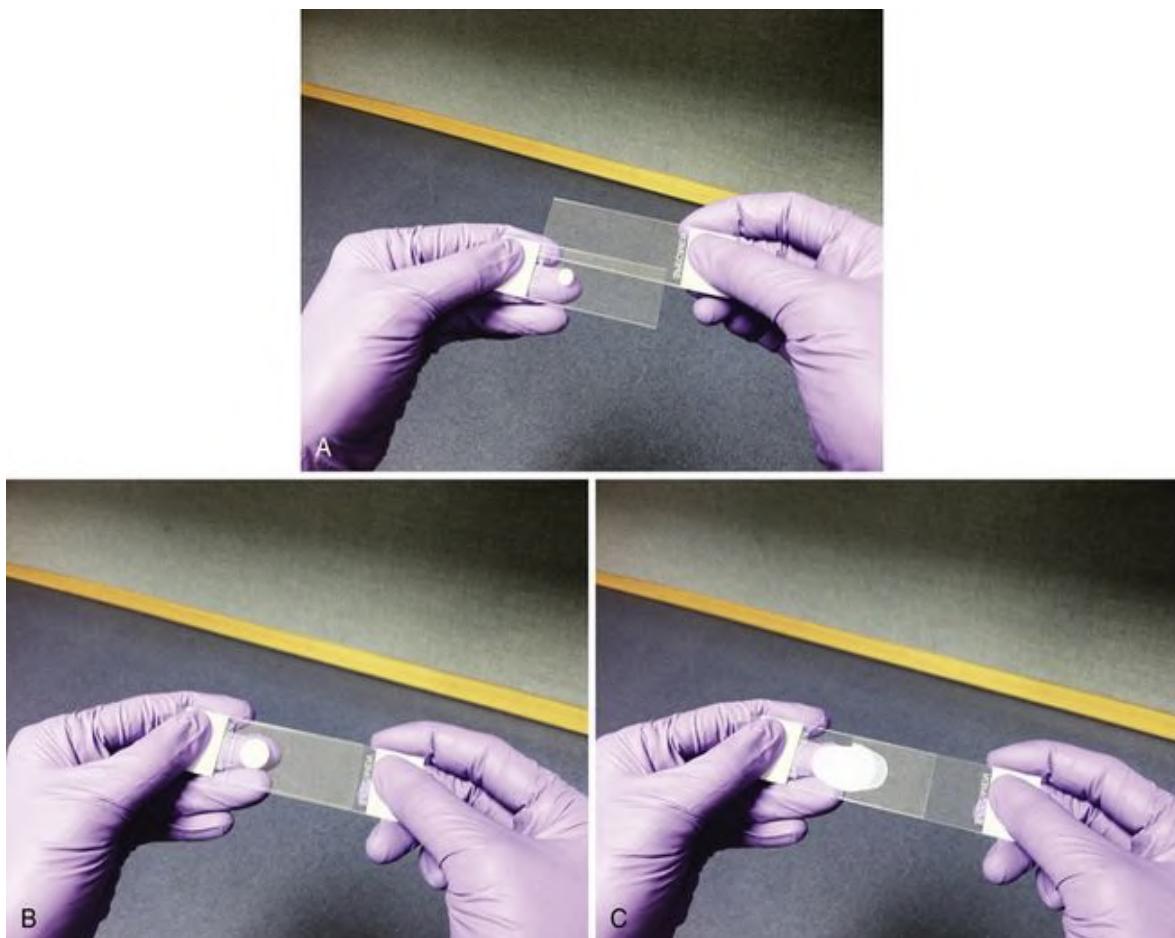


FIGURE 8.3 The “two-smear” preparation method.

A, Two identical smears are made by holding the slide that has the specimen horizontally, with its frosted side up. A second slide is positioned over it, frosted side down. B, The two slides are gently pressed together to compress the material. C, The slides are smoothly pulled apart.

Splitting Material for Multiple Smears

An abundant harvest can be divided to make two or more smears.

Method 1 (Fig. 8.4A-F). This process is similar to the “one-smear” method. The slide with the expelled material is held in one hand (usually the nondominant hand), frosted side up. The slide is positioned at about a 45-degree angle from vertical with the thumb on the frosted area, and all other fingers along the back of the slide. With the other (dominant) hand, the spreader slide is held at 90° above the first slide. The lower edge of the spreader slide is brought into contact with the first slide, and its leading edge is lowered (rotated) until the slide contacts and slightly compresses the expelled material. Some material will transfer to the spreader slide. The spreader slide is then separated from the first slide. The first slide is set aside and a clean slide picked up while held in the

same newspaper-reading position below the spreader slide. A smear with this second slide is made in the same way. This process can be repeated to make multiple smears.

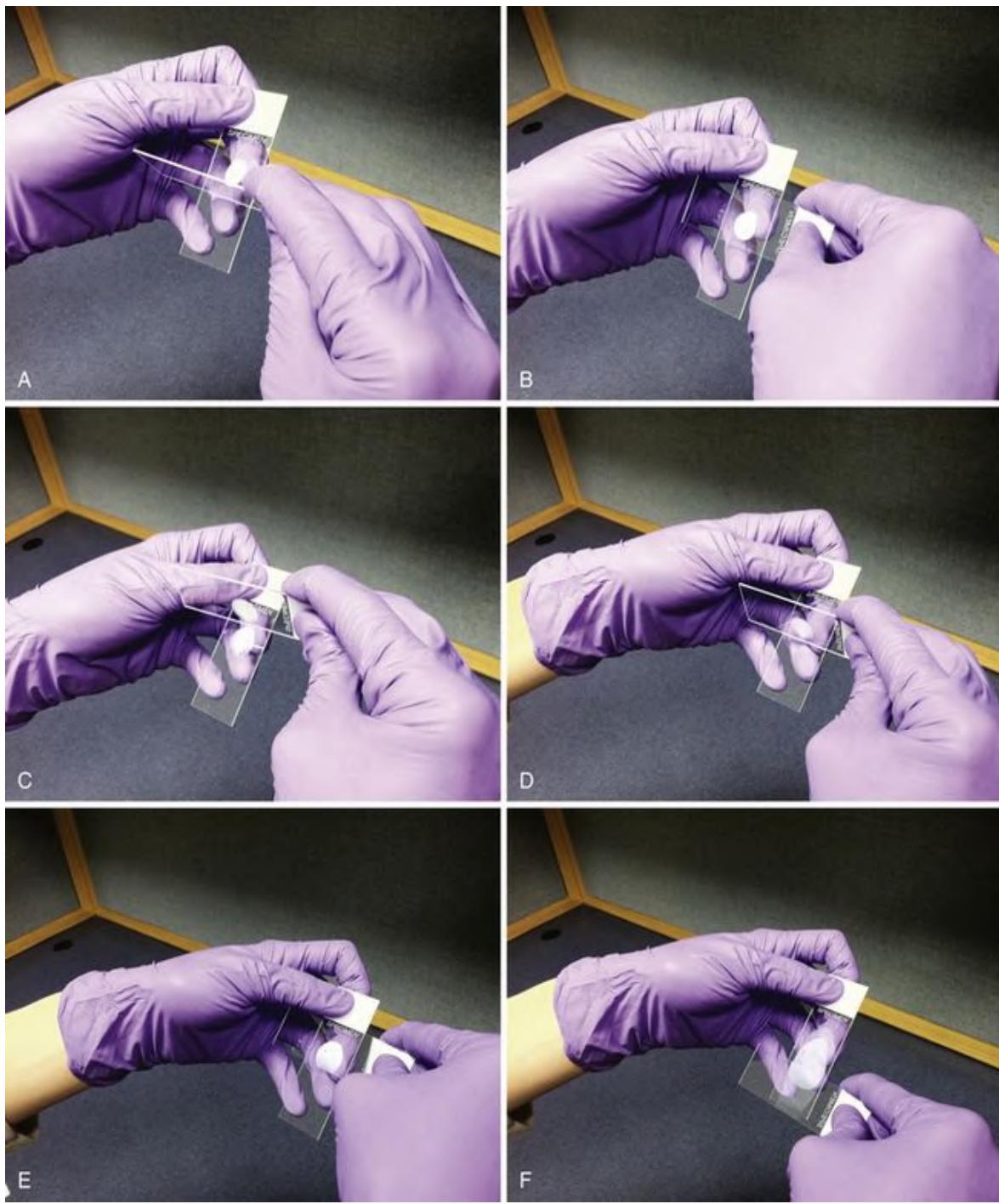


FIGURE 8.4 Splitting abundant material for multiple smears.

A, One edge of the spreader (top) slide engages the bottom slide containing the aspirated material at right angles, below the level of the aspirated droplet. *B*, The spreader slide is lowered (rotated) until it contacts the bottom slide and slightly compresses the expelled material. *C*, The two slides are separated. Some material will have transferred to the spreader slide. *D*, After setting aside the first bottom slide, a clean slide is picked up and the spreader slide engaged at right angles to the clean, bottom slide. *E*, A smear is made by rotating (lowering) the spreader slide until it contacts the bottom slide and compresses the material. *F*, The spreader slide is smoothly pulled back over the material. This process can be repeated to make multiple smears.

Method 2. Instead of expelling all of the aspirated material onto one slide, a small drop of material is expressed onto multiple slides. This method requires more control of the plunger. With a single spreader slide, a smear is made on each slide.

Fixing the Smears

Generally, at least one alcohol-fixed and one air-dried smear should be made from each needle pass. The advantages and disadvantages of each type are shown in [Table 8.2](#). Alcohol fixation provides better nuclear detail, whereas air-drying allows better visualization of cytoplasmic quality and extracellular material like mucin and cartilage. For rapid evaluation, an air-dried smear can be stained with a Romanowsky-type stain and examined without a cover slip. Although more time-consuming, an alcohol-fixed slide can be stained with either a rapid Papanicolaou or toluidine blue stain and examined with a coverslip. Air-dried smears should be dried quickly to avoid slow-dry artifacts. Thick, wet slides are often fanned to expedite drying. When preparing alcohol-fixed smears, the slide should be immersed in 95% ethanol or sprayed with an alcohol-based fixative without delay after the smear is made to avoid air-dry artifacts.

TABLE 8.2
RELATIVE ADVANTAGES OF AIR-DRIED VERSUS ALCOHOL-FIXED SMEARS

	Air-Dried Smear	Alcohol-Fixed Smear
Primary uses	Rapid on-site evaluation Evaluation of lesions with background material	Superior nuclear detail Evaluation of squamous lesions. Rapid on-site evaluation (if a modified ultrafast Papanicolaou stain, toluidine blue, or rapid hematoxylin and eosin stain is used)
Advantages	Fast and easy to perform Enhances pleomorphism	Superior nuclear detail Less air-drying artifact and enlargement

Accentuates cytoplasm and background material
Can visualize some mycobacteria (“negative images”) and other organisms

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Handling Cystic Masses

If the mass is cystic, fluid fills the syringe barrel under negative pressure. As much fluid as possible should be aspirated. Applying pressure on the mass can assist with this. The fluid can be discarded if appropriate (e.g., clear fluid from a breast cyst) or expelled directly into a container for subsequent processing. The patient is reexamined, and any residual mass sampled on a subsequent pass. Some cystic lesions are best aspirated under ultrasound guidance, as this allows the operator to target the solid area of a heterogeneous mass.

Retrieving Material from the Needle Hub

Some aspirated material may remain in the needle hub after attempts at expulsion onto glass slides. To extract this material, the needle is detached from the syringe and its tip pushed into the rubber top of a blood draw tube or a needle safety device such that the needle tip is not exposed ([Fig. 8.5A-D](#)). The needle hub is placed in the middle of a clean glass slide. In a coordinated manner, the glass tube is rocked back and forth with one hand, while the other hand flicks the needle hub. This technique takes some practice. Alternatively, with the needle tip firmly in the rubber top or safety device, the needle is inverted and its hub tapped against a clean glass slide to dislodge tissue fragments.

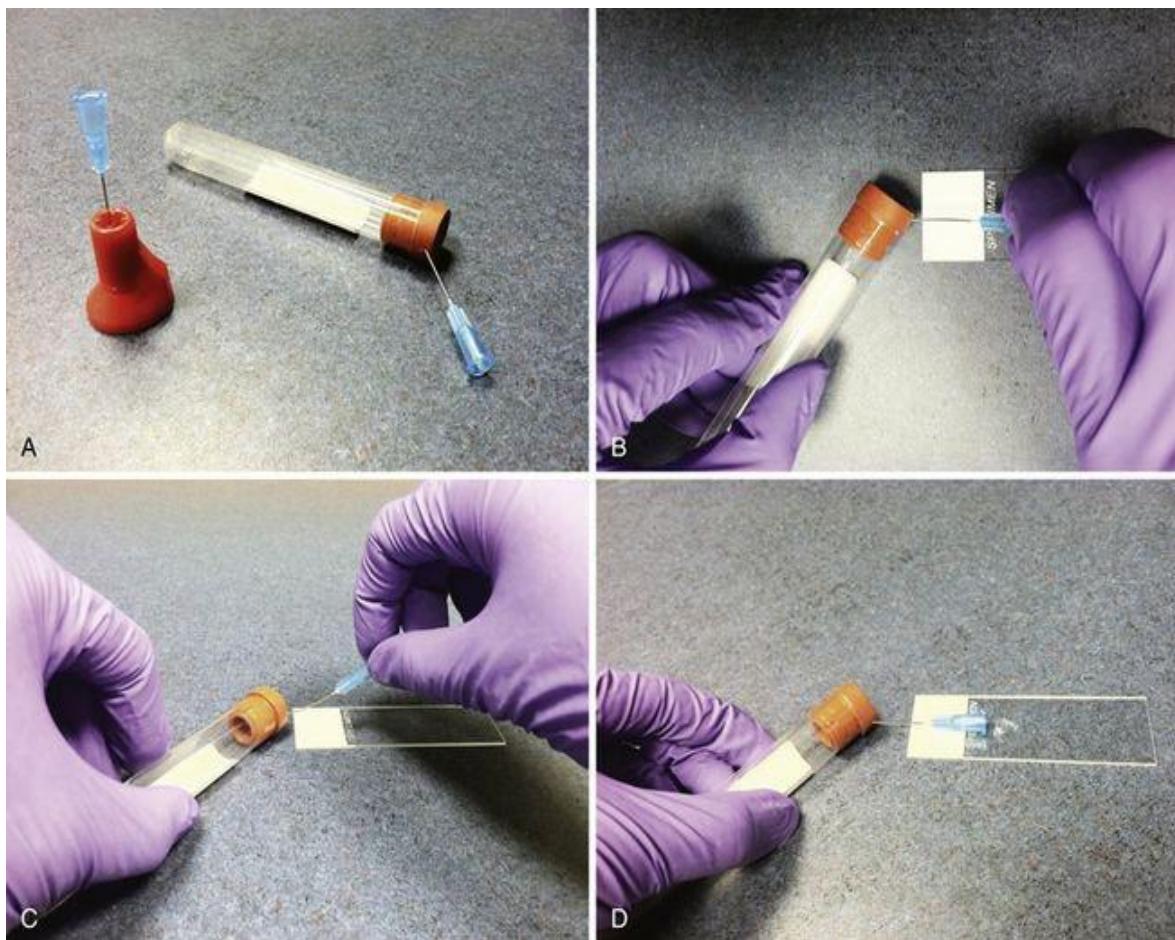


FIGURE 8.5 Retrieving material from the needle hub.

A, The needle is detached from the syringe and pushed into a needle safety device or rubber top of a blood draw tube. B, The needle hub is positioned over the middle of a glass slide. C, The hub is lifted and dropped repeatedly onto the slide while the blood draw tube is rocked side to side in a coordinated fashion.

In most cases, an FNA should not be performed on a given site more than three to four times at one clinic visit. Repeated biopsies increase tissue hemorrhage, reducing diagnostic yield. It is better to have the patient return in several days for a repeat FNA.

Rinsing the Needle and Reserving Material for Ancillary Studies

To harvest as much of the sample as possible, the needle should be rinsed using a liquid medium (see Video 8-1 found on expertconsult.com). The needle tip is placed in a container filled with the medium; a small amount of liquid is drawn into the syringe by pulling back on the plunger, then expelled back into the container by pushing in the plunger. This is repeated several times. Excessive

force should be avoided as this may mechanically damage the cells. Note that smears should be made before the needle is rinsed to avoid drying artifacts.

The needle can be rinsed with saline, a culture medium like RPMI (Roswell Park Memorial Institute medium), or a commercial hemolytic transport solution (e.g., CytoLyt, CytoRich Red) for cell block and/or liquid-based preparations (e.g., ThinPrep, SurePath). For routine assessment, the accuracy of thinlayer preparations is comparable to that obtained with smears. One should be aware of slight differences in the cytologic appearances of cells in thinlayer preparations, however, such as smaller and more three-dimensional cell clusters, smaller and darker nuclei, and a cleaner background.^{13–17}

The choice of medium for sample collection depends also on what ancillary studies are needed for diagnosis (Fig. 8.6, Table 8.3). The most versatile sample is the needle rinse in saline or culture medium like RPMI; such samples can be submitted for flow cytometric assessment of lymphoid markers in the case of a suspected lymphoma, or for a karyotype in the case of a suspected soft tissue neoplasm. On the other hand, some techniques like fluorescence in situ hybridization (FISH) and molecular assays that amplify DNA are very robust and can be performed successfully regardless of the cytologic substrate, whether a smear, cell block, or liquid-based preparation, so long as certain cellular adequacy criteria are met.

TABLE 8.3
TYPES OF FINE-NEEDLE ASPIRATION SAMPLE PREPARATIONS AND THEIR APPLICABILITY FOR ANCILLARY STUDIES

Preparation	Ancillary Test					
	Flow Cytometry	Cell Block	Karyotype	FISH	PCR-Based, DNA-Based Molecular Assay	Immunohistochemistry
Smear	No	Yes	No	Yes	Yes	Yes
Formalin-fixed cell block	No	Yes	No	Yes	Yes	Yes
RPMI/saline needle rinse	Yes	Yes	Yes	Yes	Yes	Yes
Commercial hemolytic fixative	No	Yes	No	Yes	Yes	Yes

FISH, fluorescence in situ hybridization; PCR, polymerase chain reaction.

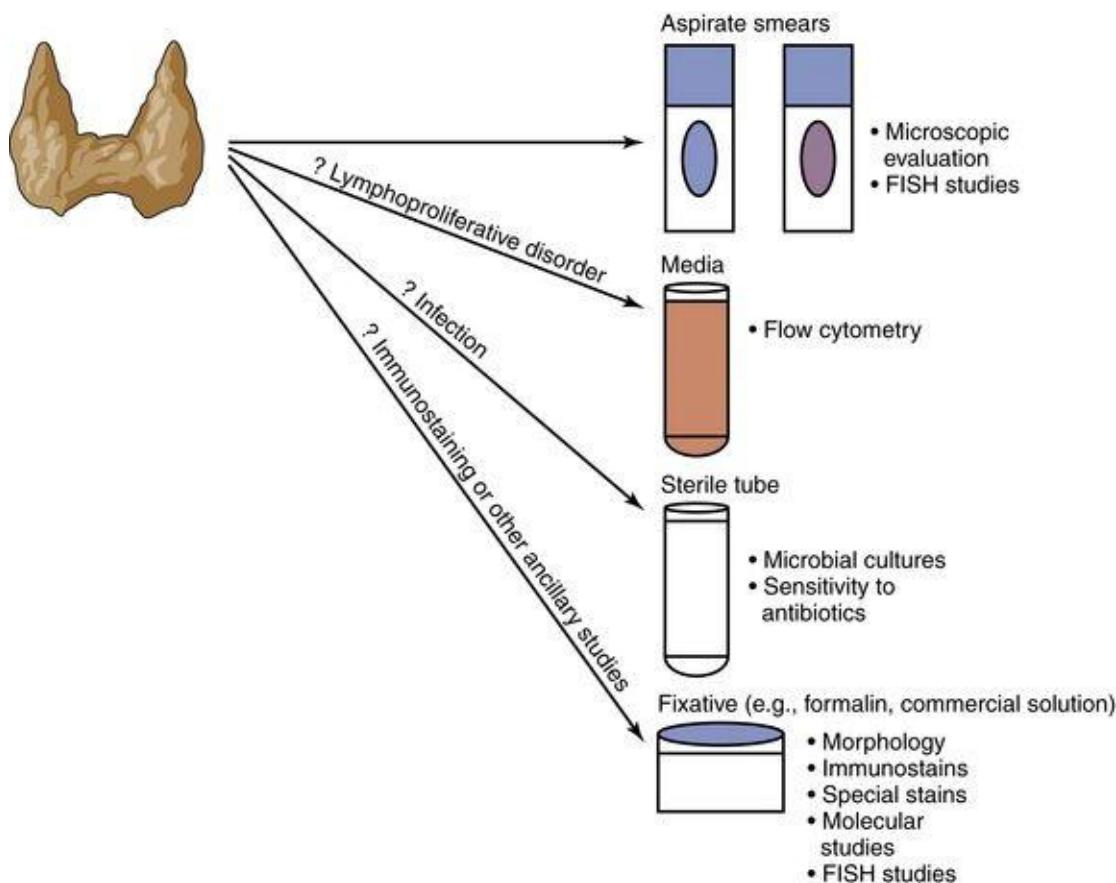


FIGURE 8.6 Rapid on-site evaluation allows appropriate triage of the material for ancillary studies. (Modified figure courtesy Sara Monaco, MD, University of Pittsburgh Medical Center, Shadyside Hospital.)

Making a Cell Block from a Smear

If an adequate cell block was not obtained after sedimenting the needle rinse sample, the material on a smear, even if previously stained, can be repurposed into a cell block (see Video 8-1 found on expertconsult.com). A cellular smear containing large tissue particles is best for this purpose. The slide is soaked in xylene until the coverslip falls off on its own. (This may take several days.) The material is scraped off the slide with a scalpel blade onto lens paper or a sponge and submitted for usual processing as a paraffin-embedded cell block.¹⁸

Post-Procedure Information for the Patient

Evaluate the patient before allowing him or her to stand up. If the patient is dizzy or light-headed, have the patient remain supine longer. Apply firm pressure for several minutes to the procedure site to minimize bruising, or longer if the patient has a history of coagulopathy or is on blood thinners.

Your parting words to the patient might go something like this: “The final report should be ready in a couple of days, and the results will go to Dr. Jones, who will then contact you. The report may take a little longer if additional tests are needed. You can go about your daily routine, including showering and swimming. You might feel some soreness; this should go away in a few days. In the meantime, apply an ice pack and/or take Tylenol if needed. Also, the lump might seem bigger than it was; this is due to some swelling from the procedure. The lump will return to its original size within a week or so. If you see signs of infection such as fever, or redness/pain/discharge at the biopsy site, call Dr. Jones. He knows you have had this procedure and will know what to do” (see  Video 8-1 found on expertconsult.com).

Variations on Biopsy Technique

Vacuum suction can be created without a syringe holder in one of two ways. The syringe barrel can be gripped with all the fingers of the aspirating hand while the thumb pulls back the plunger ([Fig. 8.7A](#)). Alternatively, the plunger is gripped with all fingers and the barrel pushed by the index finger ([Fig. 8.7B](#)). The latter method requires more finger strength and is a bit easier for those with longer fingers.

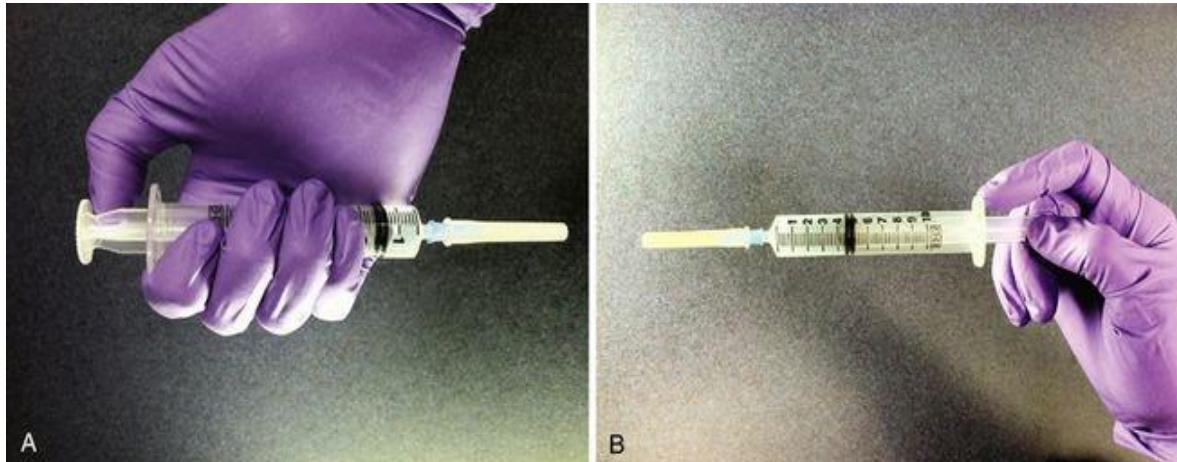


FIGURE 8.7 Vacuum suction without a syringe holder.

Suction can be created without a syringe holder by pulling the plunger back with the thumb (A) or the index finger (B).

The FNA biopsy can be performed without suction (the Zajdela or “French” technique),¹⁹ using only the needle or the needle attached to a syringe with the plunger either pulled out part way or completely removed. The needle or syringe is held like a pencil in the aspirating hand, which is stabilized by resting the wrist on an available fixed surface (e.g., the examining table or, with his or her permission, the patient). Without suction, the amount of material harvested is generally lower, but the preparations are also less bloody. This method places the aspirating hand closer to the sampled mass, which allows greater ability to “feel with the needle” and more needle control. It is especially useful in sampling very small or very vascular lesions.

Complications

Most complications associated with FNA of superficial sites are similar to those of a blood draw: minor pain, bleeding, and bruising.¹⁰ A hematoma develops occasionally. Bleeding is stopped by applying firm pressure to the biopsy site for several minutes. This is sufficient even in patients with a coagulopathy.²⁰ Some patients experience a vasovagal response. Positioning the patient supine while performing the biopsy prevents falls in the rare case that the patient faints. If the needle encounters a nerve, the patient may experience radiating/referred pain.²¹ In such situations, the needle should be removed and the patient observed. The condition is transient, and its resolution should be documented in the patient's record.

More serious complications are rare. A pneumothorax can occur with aspiration of a lesion near the chest wall, including the breast, supraclavicular area, and axilla. It occurs in less than 1 in 200 FNAs (Table 8.4).²²⁻²⁷ The risk is greater in thin patients and can be reduced by choosing a needle trajectory that is tangential to the rib cage. The clinical manifestations are immediate chest and shoulder pain. Mesothelial cells (see Fig. 2.3) may be seen on the slides.

TABLE 8.4
FREQUENCY OF PNEUMOTHORAX AS A COMPLICATION OF FINE-NEEDLE ASPIRATION OF BREAST AND OTHER SUPERFICIAL (PALPABLE) PERITHORACIC LESIONS

Study	Cases of Pneumothorax	Total Fine-Needle Aspirations	Incidence of Pneumothorax (%)
Catania et al ²⁴	13	74,000	1:5693 (0.018)
Gateley et al ²⁵	7	Not given	1:1000 (0.10)
Goodson et al ²⁶	1	285	1:285 (0.35)
Kaufman et al ²⁷	4	1666	1:417 (0.24)

There have been case reports of death resulting from FNA sampling of a carotid body tumor and carotid artery dissection after "blind" FNA of a neck mass.^{28,29} Ultrasound guidance can be helpful when sampling near a major vessel.

Seeding of malignant tumor cells along the needle tract has been reported. When large core biopsy needles (e.g., 17 gauge) are used, the rate of needle tract seeding can be as high as 12.5%.³⁰ The risk with FNA, however, is exceedingly low, ranging from 0 to 0.009%.³¹⁻³³ The risk increases with the depth of the lesion, needle size, and number of passes.^{33,34} Although the limited data available suggest that needle tract seeding by malignant tumor cells does not have clinical

implications, disease progression and decreased survival have been ascribed to FNA and core biopsies.³⁵⁻³⁹ Seeding of the tract by benign tumor cells can also occur.^{40,41}

FNA procedures pose a risk to the operator as well. Whenever the needle tip is exposed, the operator is at risk for a needle stick injury. Although the frequency is not well documented (and such injuries probably are underreported), the most frequent site of needle stick injury is the index finger of the nondominant hand (59.6%), which typically occurs during sampling of the lesion.⁴² Vigilance and attention to the location of the needle tip is important to avoid a needle stick injury. Standard safety precautions must always be exercised, including wearing a properly fitted N95 mask when performing an FNA on a patient with known or suspected tuberculous infection.

Management of Adverse and Unexpected Events

1. If the needle enters an artery, bright red blood shoots into the syringe barrel in a pulsatile fashion. The needle is immediately removed and firm pressure applied to the site for several minutes. The blood in the syringe is best submitted for cell block preparation.
2. If the patient experiences unusual symptoms (e.g., radiating tingling or pain), the procedure is stopped and the needle removed. Any specimen already procured is prepared, and the patient is observed. It is advisable to wait until symptoms have resolved before performing additional passes. If the patient experiences extreme pain (e.g., schwannomas are typically painful when aspirated), the procedure should be abandoned. Repeat sampling under sedation/general anesthesia should be considered if tissue biopsy is still desired.
3. Familiarity with emergency procedures (e.g., calling a code) is advisable in the very unusual event that a patient has a change in heart rate or experiences difficulty breathing.
4. If the patient moves unexpectedly during the procedure, the risk of a needle stick injury can be reduced by removing the needle from the patient only when it is safe to do so.
5. Needle stick injuries must be documented and appropriate medical care sought immediately as per institutional guidelines. Many institutions have a 24-hour hotline and/or dedicated beeper for handling needle stick injuries.
6. Complications of FNA and their resolution should also be documented in the medical record.

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Additional Resource

Video demonstration of FNA Technique by Dr. Britt-Marie Ljung, Papanicolaou Society website (<http://www.papsociety.org/fna.html>).

CHAPTER 9

Breast

Barbara S. Ducatman and Helen H. Wang

Specimen Types

Fine-Needle Aspiration

Nipple Discharge Cytology

Reporting Terminology

Evaluation of the Specimen

The Normal Breast

Benign Conditions

Cysts

Fibrocystic Changes

Fibroadenoma

Pregnancy-Related and Lactational Changes

Fat Necrosis

Radiation Change

Mastitis

Subareolar Abscess

Gynecomastia

Papillary Neoplasms

Phyllodes Tumor

Breast Cancer

Invasive Ductal Carcinoma

Invasive Lobular Carcinoma

Medullary Carcinoma

Mucinous (Colloid) Carcinoma

Tubular Carcinoma

Metaplastic Carcinoma

Uncommon Breast Tumors

Apocrine Carcinoma

Adenoid Cystic Carcinoma

Non-Hodgkin Lymphoma

Sarcoma

Metastatic Tumors

Specimen Types

Breast cytology includes the nipple discharge and fine needle aspiration (FNA). The more common specimen by far is the FNA sample.

Fine-Needle Aspiration

FNA is used to evaluate palpable breast masses and cysts as well as nonpalpable mammographic abnormalities. FNA is highly accurate for palpable lesions,¹⁻² although its accuracy is limited with lesions smaller than 1 cm. Despite competition from the automated core needle biopsy (CNB) under stereotactic guidance, FNA delivers good results, especially in a multidisciplinary setting with on-site radiologists and pathologists.³⁻⁷ Complications of FNA are rare, the most common being bleeding. Occasionally, FNA causes partial infarction of the lesion, particularly fibroadenomas, which can hinder histologic confirmation of the diagnosis.⁸

For nonpalpable lesions, FNA is a useful technique for sampling cystic lesions with ultrasound guidance, whereas the CNB is more often used for mammographically identified calcifications.⁹ In pregnant or postpartum patients, FNA is preferred in order to avoid a draining, nonhealing wound that can result after a core or incisional biopsy. FNA is also useful for assessment of recurrent lesions.

The accuracy of FNA of the breast, as with most things, is operator-dependent: Sensitivity for malignancy ranges from 65% to 98%, and specificity from 34% to 100%.^{1,10-16} False-positive results occur in 0 to 2% of cases.¹⁷ False-suspicious result rates are higher, ranging from 1% to 13%. In general, the sensitivity of FNA for palpable and nonpalpable malignant lesions (i.e., those sampled with mammographic or ultrasound guidance) is comparable.¹⁸⁻³⁴ False-negative results occur because of errors in sampling, interpretation, or both.^{14,35} Some studies show that satisfactory specimens are more likely when pathologists rather than clinicians perform the aspiration.^{14,36-41} Whether clinician or pathologist, however, practice makes perfect, and the physician with more FNA experience obtains the more accurate result.⁴²⁻⁴⁴ The use of p63 and CK5/6 immunostaining increases the accuracy of FNA by helping distinguish well-differentiated carcinomas from benign lesions.⁴⁵⁻⁴⁸

A major advantage of FNA is the ease with which the sample can be assessed for adequacy.^{49,50} Although a touch imprint or wash of a CNB specimen can be

done for rapid diagnosis, their utility is debatable.⁵¹⁻⁵⁶ The use of FNA and/or CNB significantly decreases health care costs by decreasing the number of open surgical biopsies per breast cancer identified, without sacrificing early detection.⁵⁷ When the diagnosis is benign, such as a lactating adenoma in a pregnant patient, FNA spares a patient with a solid and palpable lesion an open biopsy. A diagnosis of malignancy allows preoperative evaluation of available therapeutic options (lumpectomy with irradiation versus mastectomy), or it might persuade a reluctant patient to undergo surgical biopsy.

FNA of the breast has its limitations. Although sensitive in detecting ductal carcinomas, it cannot distinguish between an *in situ* and an invasive ductal carcinoma. It cannot identify the presence of lymphatic or vascular invasion. It is less sensitive in tumors with low-grade cancer histology (e.g., tubular and lobular), papillary proliferations, and mucinous lesions.⁵⁸⁻⁶⁰ The diagnosis of lobular carcinoma and tubular carcinoma requires considerable experience in FNA interpretation^{25,59} even so, equivocal findings are common because of the benign cytologic appearance of such tumors.⁶⁰ As with FNA of other sites, considerable discrepancy in performance exists among laboratories.⁶¹

In comparison with CNB, the nondiagnostic rate for FNA is higher, and FNA has a lower negative predictive value.^{55,58,62} Nevertheless, some authors have reported excellent results with breast FNA, and the combination of FNA and CNB may be superior to either alone.^{3,5,16,63-66}

Although atypical ductal proliferative lesions are more amenable to classification by CNB than by FNA, false-negative diagnoses and underestimates of malignancy have been reported in a considerable percentage of cases even with CNB.⁶⁷⁻⁷⁰ Prognostic markers can be assessed with either technique.^{2,71}

Many practitioners favor CNB over FNA^{62,72,73} because of the superior negative predictive value and wider acceptance of histopathology. The resulting increased use of CNB, particularly with stereotactic or ultrasound guidance, has led to diminished use of FNA,^{16,74} although some practitioners continue to use FNA for patients with radiologically and clinically negative lesions.^{75,76} The cost-effectiveness of FNA versus CNB is still debated.⁷⁷⁻⁷⁹

The increasing use of neoadjuvant chemotherapy in higher-stage cancers has further limited the use of FNA. Because an open biopsy is not performed, CNB, usually under ultrasonographic guidance, is superior because of its more robust sensitivity and ability to distinguish between ductal carcinoma *in situ* and invasive carcinoma. Furthermore, a CNB sample usually contains more abundant material for the determination of estrogen receptor (ER), progesterone receptor (PR), and HER2 status. Although a cell block prepared from an FNA

sample permits multiple immunohistochemical stains, staining for PR in cell blocks may be discordant with tissue results, and staining for HER2 may be unreliable.⁸⁰

Breast tumors are now classified in part on their molecular expression profile (luminal A, luminal B, HER2-positive, basal-like),⁸¹ and molecular-based risk stratification and prognostic testing by commercial products like Oncotype DX (Genomic Health) and MammaPrint (Agendia) is playing an increasing role in the management of patients.⁸¹ Entrance into many current neoadjuvant clinical trials stipulates core biopsy material for molecular testing. As new molecular tests are validated, the use of FNA may decline further unless studies incorporate FNA specimens in their design.

FNA of axillary lymph nodes remains indispensable in the evaluation of lymph node status prior to neoadjuvant treatment or for preoperative staging,⁸²⁻⁹² although some institutions are using CNB instead of FNA.

Nipple Discharge Cytology

A spontaneous nipple discharge not related to lactation or pregnancy is an abnormal finding. It may result from a breast lesion like a papilloma or a carcinoma or from a hormonal abnormality like that produced by a prolactin-secreting pituitary adenoma. Cytologic examination of a nipple discharge is generally used when the patient has no palpable or mammographic abnormality and is helpful in identifying small breast cancers and papillomatosis.^{93,94} If a palpable or mammographic abnormality is present, either FNA, CNB, or excisional biopsy is usually performed.⁹⁵ The sensitivity of nipple discharge cytology ranges from 41% to 60%.⁹⁶⁻⁹⁸ Nipple discharge cytology is not useful as a screening test for breast cancer because a discharge can be obtained in only 7% to 14% of asymptomatic, nonpregnant, nonlactating women.^{96,98,99} In the future, biomarker analysis of nipple discharge fluid might help increase the sensitivity of cytology.¹⁰⁰

Nipple discharge specimens are prepared by gently massaging the breast in the direction of the nipple. A glass slide is then touched to the secreted drops; the discharge need not be smeared unless it is abundant. The slides are fixed by spray fixation or by immersion in 95% ethyl alcohol and stained with the Papanicolaou stain. An alternative method is to air-dry the slide and stain it with a Romanowsky-type stain.

A nipple discharge can be unilateral or bilateral; unilateral discharges are more likely to be malignant.⁹⁶ The secretion can be milky, serous, purulent, or bloody.

Cancer is most prevalent when the discharge is macroscopically bloody (4%) and less prevalent when it is purulent (0.8%), serous (0.2%), or milky (0.1%).⁹⁶ Because bloody discharges are more likely than nonbloody discharges to contain malignant cells,^{94,101} some authors recommend cytologic examination of bloody secretions only, which represent 11% of all secretions.⁹⁶ Others recommend examination of all discharges.⁹⁸ Most patients with an intraductal papilloma do have a discharge,⁹⁸ which may or may not be bloody. Although most patients with breast cancer do not have a discharge from the nipple, about 2% of breast cancers are detected by this method and by no other.⁹⁸



Cytomorphology of benign nipple secretions

- usually sparsely cellular
- ductal cells
- foam cells
- inflammatory cells
- red blood cells

Benign ductal cells are arranged in tight clusters that are small and spherical or large and branching; isolated benign ductal cells are very uncommon. Usually, the cells are small and have scant cytoplasm, but occasionally they are larger and have abundant cytoplasm. It is common for benign ductal cells to mold themselves around one another, giving the cluster a scalloped appearance. Foam cells are large histiocytes that are usually dispersed as isolated cells. They contain abundant vacuolated cytoplasm and a round or oval nucleus that is sometimes degenerated ([Fig. 9.1A](#)) When a secretion contains numerous groups of benign ductal cells, especially large, branching clusters, it is likely that the patient has an intraductal papilloma or a florid intraductal hyperplasia, lesions that can only be distinguished histologically.

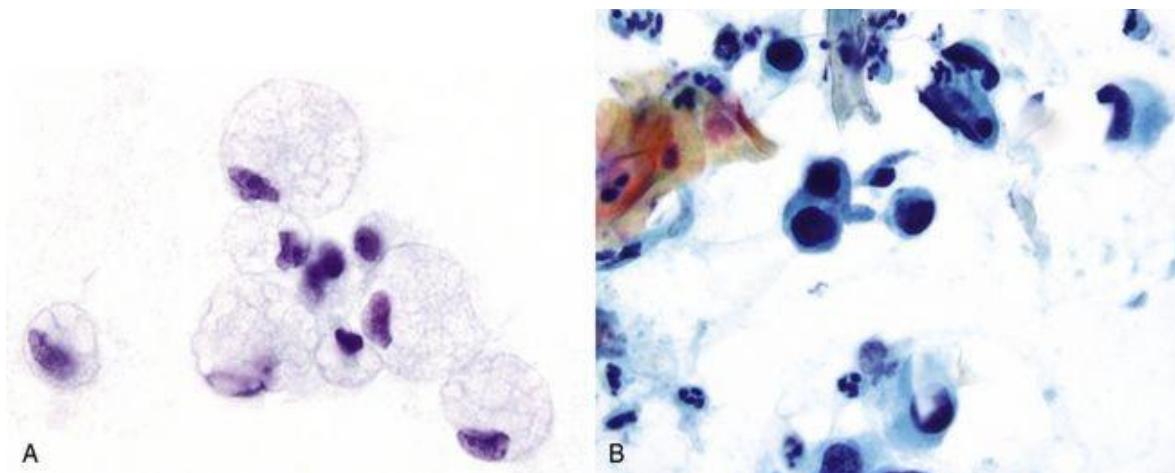


FIGURE 9.1 Nipple discharge cytology.

A, Benign nipple discharge. Histiocytes (foam cells) have a kidney bean–shaped nucleus and abundant vacuolated cytoplasm (Papanicolaou stain). *B*, Suspicious nipple discharge. The sample contains very atypical cells with enlarged nuclei. The subsequent biopsy showed a high-grade comedocarcinoma (Papanicolaou stain).

Cytomorphology of malignant nipple secretions ([Fig. 9.1B](#))

- enlarged ductal cells, isolated and in clusters
- variable nuclear size and shape
- stripped nuclei
- nucleoli
- acute inflammation
- blood
- necrotic debris

Reporting Terminology

To report FNA and nipple discharge results, the use of general categories with an implicit probability/risk of malignancy, followed by a specific diagnosis, is recommended.^{75,102-110} As might be expected, interobserver reproducibility is excellent for the positive category, poor for atypical, and fair to good for other categories.¹¹¹



General categories for reporting results of breast fine-needle aspiration

- negative for malignant cells (*or:* no malignant cells seen)
- atypical
- suspicious
- positive for malignant cells
- nondiagnostic (*or:* unsatisfactory, insufficient)

The exact wording for each category or diagnosis is less important than having a probabilistic (risk-based) system that clearly communicates the likelihood of malignancy. One pathologist might use the term “nondiagnostic” and another, “insufficient,” but the fact that this specimen is unsatisfactory is understood by clinician and pathologist alike. Nondiagnostic specimens contain too few well-preserved cells to permit an adequate evaluation—fewer than six epithelial cell clusters of at least 5 to 10 cells or less than 10 intact bipolar cells per 10 medium-power fields ($\times 200$).^{112,113}

A negative (benign) diagnosis should be reserved for an adequate specimen with a minimum of five to six well-preserved benign ductal cell groupings. A negative FNA result is more reliable when a specific diagnosis corroborates a clinical and radiologic impression (e.g., fibroadenoma, lactating adenoma).¹⁰² The clinician should always correlate the cytologic result with the clinical findings and the mammographic impression (these constitute the so-called “triple test”) to reduce the risk of an undiagnosed malignancy, and clinical followup is indicated.¹¹³⁻¹¹⁵ The false-negative rate is greatly reduced when the triple-negative test is implemented.¹¹⁶ Needless to say, a negative cytologic result is by no means a guarantee that the nodule is benign, and a mammographically or clinically

suspicious lesion necessitates a biopsy despite a negative cytologic result.

The atypical category is used for a specimen with a low probability of malignancy. This category is unavoidable due to the significant overlap in the cytologic features of some benign and malignant entities. It generally requires biopsy assessment.¹¹⁷

The suspicious diagnosis is used for lesions that are probably malignant, but the atypical cells are too few, too poorly preserved, or too obscured by blood or inflammation for a definitive diagnosis, or when the findings suggest a type of breast cancer with minimal cytologic atypia, such as lobular carcinoma, tubular carcinoma,¹¹⁸ or papillary carcinoma. A histologic specimen should be obtained with any FNA sample that is deemed suspicious.

Positive diagnoses are reserved for specimens with unequivocal features of malignancy. Although confirmation of all positive results with frozen section before definitive surgery is wise, some surgeons proceed directly to mastectomy or wide local excision. Therefore, it is prudent to diagnose any case for which the pathologist is not comfortable with definitive surgery as “suspicious.”

Evaluation of the Specimen



Low-power evaluation

- cellularity
- cellular arrangements
- background elements

Cellularity is important, although there is considerable overlap between categories. Hypocellular aspirates can be obtained from a fibroadenoma, fibrocystic changes (FCCs), fat necrosis, radiation changes, pregnancy/lactation, and carcinoma, both *in situ* and invasive (particularly scirrhous, tubular, and lobular types). Moderately cellular aspirates are seen with a fibroadenoma, phyllodes tumor, pregnancy/lactation, FCCs, and carcinoma. Hypercellular aspirates are seen in some fibroadenomas, phyllodes tumors, and invasive carcinomas.

Cellular arrangements provide important clues to the diagnosis. Cells can be arranged in sheets, tightly or loosely cohesive three-dimensional clusters, branching papillary clusters, or as isolated cells. The spacing of nuclei in cell clusters varies in different breast lesions. Regular nuclear spacing suggests a benign process; irregular spacing is characteristic of malignancy.



Lesions associated with architectural patterns

Sheets:

- fibrocystic changes
- fibroadenoma
- lobular carcinoma *in situ*

Tightly cohesive three-dimensional aggregates:

- fibroadenoma and phyllodes tumor
- intraductal papilloma
- lobular carcinoma *in situ*
- ductal proliferative lesions, from intraductal hyperplasia to ductal carcinoma *in situ*
- well-differentiated ductal carcinomas
- mucinous carcinoma

Loosely cohesive three-dimensional clusters:

- phyllodes tumor
- ductal carcinoma in situ
- invasive carcinoma
 - Branching papillary clusters:
- fibroadenoma
- intraductal papilloma
- papillary carcinoma
- Numerous isolated cells:
 - carcinoma
 - pregnancy/lactation
 - non-Hodgkin lymphoma
 - intramammary lymph node

Background elements include inflammatory cells, amorphous debris, fresh and old blood, and mucin. Acute inflammatory cells are seen with mastitis and necrotic carcinomas. Lymphocytes are noted with intramammary lymph nodes, medullary carcinoma, and lymphoproliferative disorders. Although amorphous granular debris suggests malignancy, it does not provide sufficient grounds for making a positive diagnosis, as it may also be observed with pregnancy/lactation and apocrine metaplasia. Presence of blood is a clue to an intraductal papilloma, papillary or another carcinoma, and angiosarcoma. Mucin and myxoid material are seen with fibroadenoma, mucinous carcinoma, and mucocele.



High-power examination

- types of isolated cells
- nuclear characteristics
- cytoplasmic characteristics

Isolated cells are epithelial or mesenchymal in origin and may be intact or stripped of cytoplasm (naked nuclei). Isolated epithelial cells are seen during pregnancy and lactation and with carcinoma. Mesenchymal cells are noted in fibroadenoma, phyllodes tumor, invasive carcinoma, and sarcoma. Naked nuclei are common in pregnancy, lactation, and fibroadenoma. Inflammatory cells are

commonly seen in fat necrosis, FCCs, mastitis, lymphoma, and intramammary lymph nodes. Histiocytes are seen in fat necrosis, radiation, FCCs, granulomas, and status post silicone injection or a ruptured silicone implant.

Nuclear atypia is assessed on the basis of nuclear location, size, and shape; the chromatin pattern; and the quality of nucleoli. Although the standard cytologic criteria for malignancy (eccentrically placed, large, angulated, pleomorphic nuclei with irregular and large nucleoli) apply with moderately and poorly differentiated ductal carcinomas, some malignant tumors, including tubular, lobular, and mucinous carcinoma, show little nuclear atypia. The recognition of other features, like the architectural arrangement or the presence of abundant extracellular mucin, is important in the diagnosis of these tumors.

Cytoplasmic characteristics are useful in classifying some breast lesions. Distinctive features include apocrine change and cytoplasmic vacuolization. Apocrine cytoplasm is seen in apocrine metaplasia and apocrine carcinoma. Vacuolated cytoplasm is observed with pregnancy and lactation, fat necrosis, radiation effect, mucinous carcinoma, lobular carcinoma, lipid-rich carcinoma, secretory carcinoma, and status post silicone injection or ruptured silicone implant.

The Normal Breast

The breast contains 15 to 25 lactiferous ducts, which begin at the nipple, then branch into smaller ducts, and end in the terminal duct lobular unit (or lobule). The lobule is composed of a terminal duct and many small ductules (or acini). An inner layer of cuboidal or columnar epithelial cells and an outer layer of myoepithelial cells line all ducts and ductules. The connective tissue within the lobule is a hormonally responsive mixture of fibroblasts, occasional lymphocytes, and histiocytes, in a background of collagen and acid mucin. The interlobular stroma is hypocellular and contains fibroadipose tissue. During pregnancy there is a marked proliferation of ductules, which results in very large lobules, and the epithelial cells have abundant cytoplasm filled with secretory vacuoles. These secretory changes usually disappear after lactation; in some instances, however, they persist for years after pregnancy and are sometimes seen even in patients who have not been pregnant. The cause in such cases has been ascribed to pharmaceutical agents.

Benign Conditions

Cysts

The aspiration of breast cysts and the need for cytologic analysis is controversial. Aspiration collapses a cyst and is thereby therapeutic. The great majority of cyst fluids are benign; only about 2% prove to be carcinoma.¹¹⁹ Even complex cystic lesions are virtually always benign; in one study, only 0.3% were malignant.¹²⁰ Furthermore, atypical cells can be seen in a cyst fluid, resulting in overdiagnosis and overtreatment when conservative followup would have been adequate.^{120,121} On the other hand, a small number of carcinomas are cystic and yield fluid that looks grossly much like that from benign cysts.¹²² If the fluid is not submitted for cytologic evaluation, these carcinomas will remain undiagnosed and untreated. It has been suggested that symptomatic complicated cysts, cystic lesions with thick indistinct walls and/or thick septations, intracystic masses, and predominantly solid masses with cystic degeneration are more likely to be malignant and thus merit cytologic or histopathologic examination.¹²³

Fibrocystic Changes

FCCs are the most common breast disorder. Findings may include any combination of small or large cysts, apocrine metaplasia, focal fibrosis, adenosis, and ductal hyperplasia. These changes can result in palpable, sometimes painful, masses. Cysts arise from the terminal duct lobular units by an unfolding and simplification of adjacent ductules. Moderate and florid ductal hyperplasia, which is seen in some but not all cases, is a marker for increased cancer risk. For this reason, FCC is usually categorized as nonproliferative or proliferative, depending on whether ductal hyperplasia is present.

Nonproliferative Fibrocystic Changes



Cytomorphology of nonproliferative fibrocystic changes

- apocrine cells
- foam cells

- small ductal cells

Nonproliferative lesions yield a scant specimen when the lesion is predominantly fibrous. When a cyst is present, the specimen is a fluid that may be thin and yellow or thick and darker in color. Apocrine cells line many but not all benign cysts. Apocrine cells have abundant granular cytoplasm that stains pink or green with the Papanicolaou stain and gray with a Romanowsky stain ([Fig. 9.2](#)). The nucleus is centrally located and round, with a prominent nucleolus, and moderate anisonucleosis is present in some cases. Benign apocrine cells are arranged as flat sheets; isolated cells are rare. Foam cells have abundant cytoplasm that is vacuolated rather than granular (see [Fig. 9.1](#)). Ductal epithelial cells are arranged in sheets and three-dimensional clusters ([Fig. 9.3](#)).

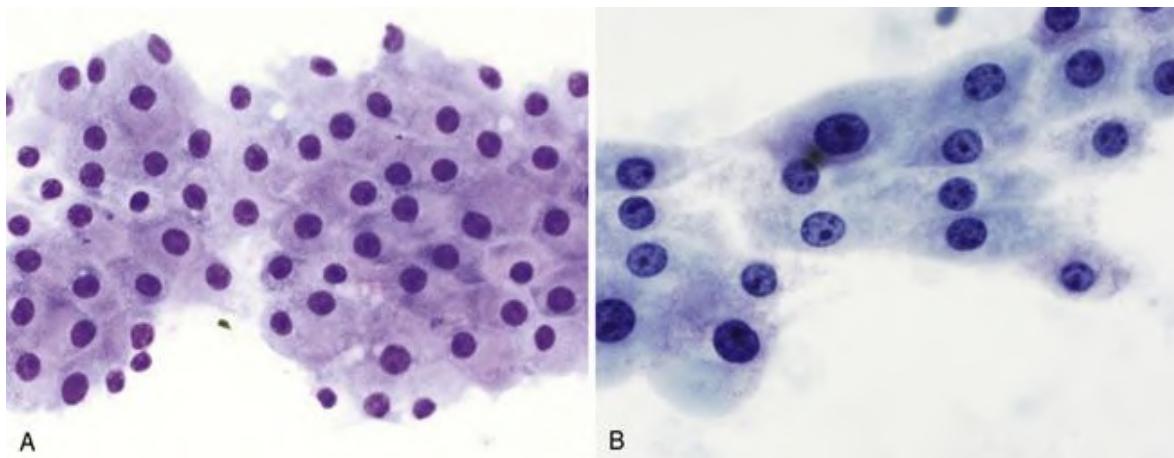


FIGURE 9.2 Apocrine metaplasia.

Large, flat sheets of apocrine cells have distinct cytoplasmic borders, a centrally located nucleus, and a prominent nucleolus. The abundant granular cytoplasm is gray-purple with a Romanowsky-type stain (A) and green with the Papanicolaou stain (B).

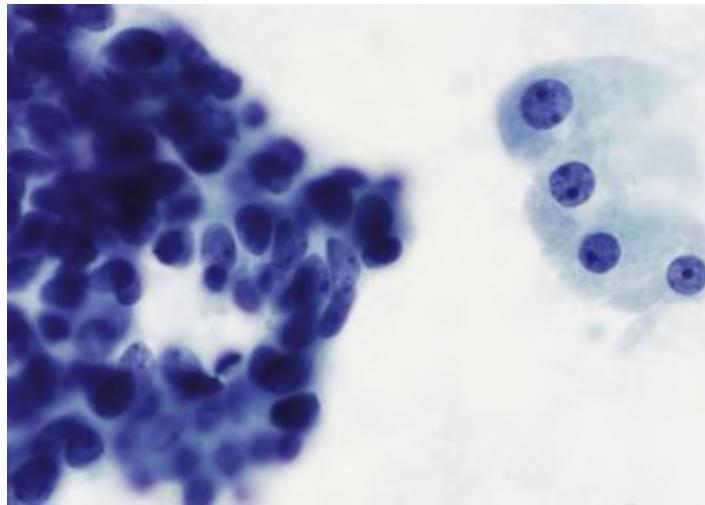


FIGURE 9.3 Benign ductal epithelium (nonproliferative fibrocystic changes). A tightly cohesive cluster of ductal epithelial cells without atypia is noted adjacent to apocrine metaplastic cells (Papanicolaou stain).

Differential diagnosis of nonproliferative fibrocystic changes

- granular cell tumor
- apocrine carcinoma

Granular cell tumors of the breast are rare. The cells of this tumor, thought to be of Schwann cell origin, have a very low nuclear-to-cytoplasmic ratio, a small nucleus, and a coarsely granular cytoplasm.¹²⁴ The background is usually clean (Fig. 9.4). An apocrine carcinoma should be suspected when there is hypercellularity, marked nuclear atypia, and pronounced cell dyshesion, but a cautious diagnostic approach is advisable, because marked variation in nuclear size is also seen in apocrine metaplasia. Apocrine adenosis, although rare, can also manifest with nuclear atypia, but there is less nuclear hyperchromasia than with carcinoma, and there are many naked nuclei.¹²⁵

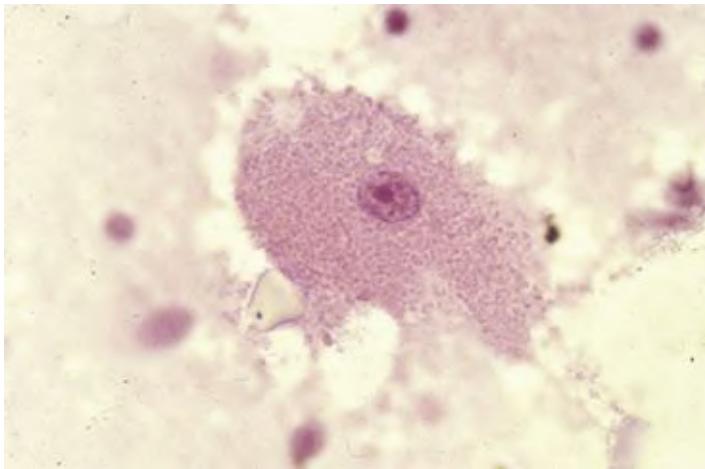


FIGURE 9.4 Granular cell tumor.

Tumor cells have a low nuclear-to-cytoplasmic ratio because of an abundance of granular cytoplasm (hematoxylin and eosin [H & E] stain).

Proliferative Fibrocystic Changes

Ductal proliferative lesions (i.e., proliferative FCC) comprise a group of lesions that vary in severity and degree of atypia. The spectrum includes proliferative lesions without atypia (“usual ductal hyperplasia”), atypical ductal hyperplasia, and atypical lobular hyperplasia. The criteria that define these entities and distinguish them from carcinoma *in situ* are histologic, not cytologic.^{126,127} Nevertheless, the degree of crowding and nuclear atypia allows for separation of the higher end of this spectrum from the lower. In one study, lesions reported cytologically as “proliferative lesion with atypia” were associated with a significantly higher frequency of histologically confirmed malignancy¹²⁸ than those reported as “proliferative lesion without atypia” (36.5% versus 1.7%).¹²⁸ A lesion diagnosed as atypical by FNA should be considered for excision, because the morphologic features of well-differentiated invasive and *in situ* carcinomas overlap with those of benign entities.¹¹⁷



Cytomorphology of ductal proliferative lesion without atypia (Fig. 9.5)

- sheets and tight clusters of cells without significant nuclear overlap
- regular cellular spacing
- finely granular chromatin pattern
- inconspicuous to small nucleolus

Cytomorphology of ductal proliferative lesion with atypia (Fig. 9.6)

- sheets and tight clusters of cells with significant nuclear overlap
- regular to irregular cellular spacing
- finely to coarsely granular chromatin
- prominent and/or multiple nucleoli

Differential diagnosis of ductal proliferative lesion without atypia

- intraductal papilloma
- fibroadenoma
- carcinoma in situ

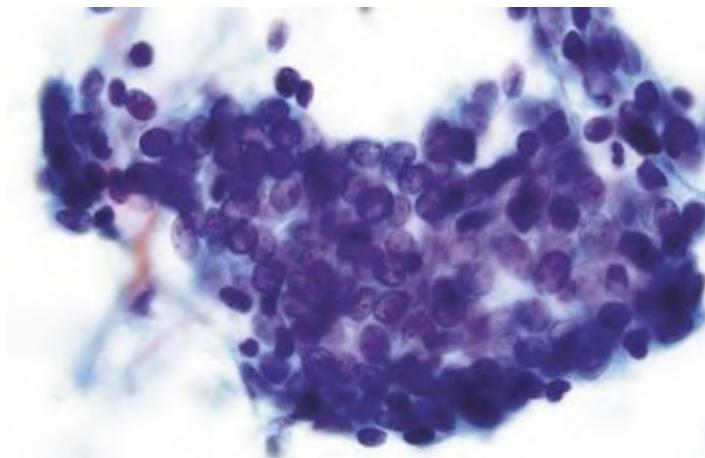


FIGURE 9.5 Ductal proliferative lesion without atypia.
Note the interspersed myoepithelial cells, which stand out like sesame seeds on a bun
(Papanicolaou stain).

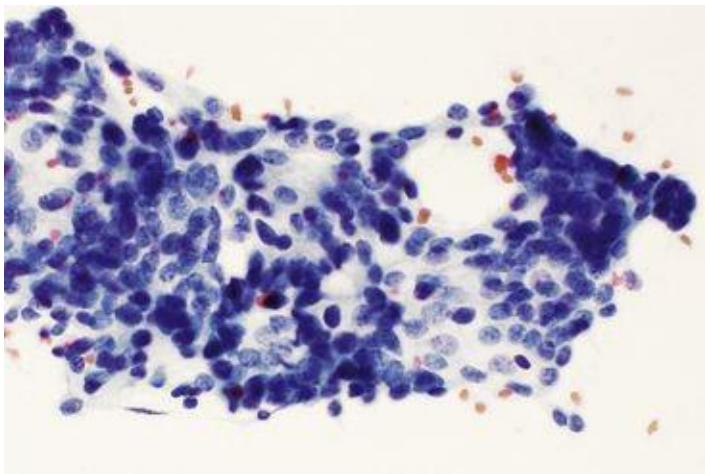


FIGURE 9.6 Ductal proliferative lesion with atypia.

In contrast with [Figure 9.5](#), there is less regular nuclear spacing, more overlapping, and more prominent nuclear atypia, with a suggestion of cribriform spaces. Such proliferative lesions cannot be categorized precisely by FNA. Histologic examination revealed atypical ductal hyperplasia bordering on non comedo ductal carcinoma in situ (Papanicolaou stain).

An intraductal papilloma is cytologically indistinguishable from proliferative FCC. Unlike proliferative FCC, however, many patients with an intraductal papilloma present with a nipple discharge or a discrete subareolar mass. Proliferative FCC may be impossible to distinguish from a fibroadenoma or phyllodes tumor,¹²⁹ except that stromal fragments are fairly common in the latter two, and rarely seen in proliferative FCC.

Pleomorphic carcinoma cells, calcium, necrosis, large nucleoli, and macrophages are indicative of comedo-type ductal carcinoma in situ and are usually diagnosed as either “suspicious” or “positive” ([Fig. 9.7](#)).^{130,131} Ductal carcinomas in situ of low and intermediate grade are usually interpreted as “atypical.”¹⁰² Some but not all well-differentiated ductal carcinomas in situ show more dyshesion than proliferative FCC. Still, distinguishing between ductal hyperplasia, atypical ductal hyperplasia, and well-differentiated ductal carcinoma in situ by cytology is difficult,^{19,104,117,131-135} even with the aid of image cytometry.^{136,137} Although ductal carcinomas in situ are more likely than papillomas and other benign ductal lesions to have numerical chromosomal aberrations as detected by fluorescence in situ hybridization (FISH),¹³⁸ it is unlikely that ductal proliferative lesions will be easily categorized by FNA in the near future. This limitation is not surprising, because primarily architectural, not nuclear or cellular, features define these lesions. The use of cell blocks may help in the cytologic classification of these entities.¹³⁹

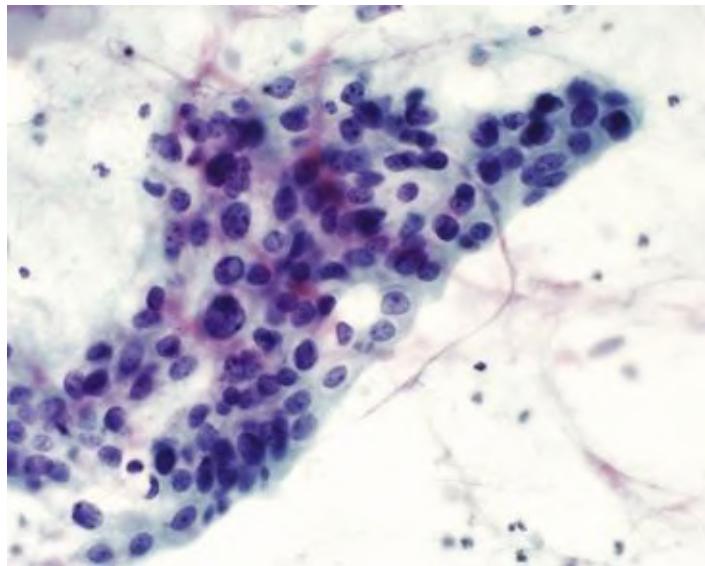


FIGURE 9.7 Suspicious for malignancy.

The cells are loosely cohesive, with marked nuclear pleomorphism, prominent nucleoli, and a dirty background. Such specimens cannot be distinguished from invasive carcinoma by FNA. Histologic examination revealed comedo-type ductal carcinoma in situ (Papanicolaou stain).

Fibroadenoma

Fibroadenoma is the most common benign tumor of the female breast. Although more common in young women, it is seen in women of any age, including those who are postmenopausal. Fibroadenomas are well-circumscribed, freely movable, rubbery masses that result from both stromal and glandular proliferation.



Cytomorphology of fibroadenoma

- hypercellular
- large sheets (see [Fig 9.8](#))

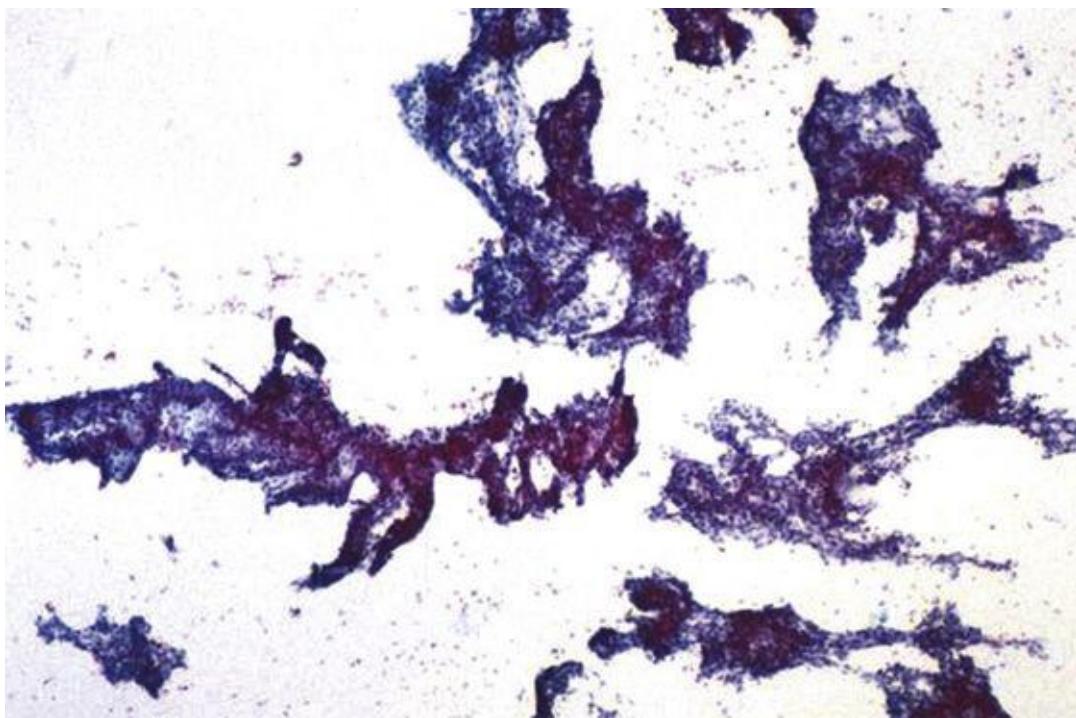


FIGURE 9.8 Fibroadenoma, low magnification.

The specimen is hypercellular, with many folded sheets and antler-horn clusters (Papanicolaou stain).

- three-dimensional clusters with an antlerlike configuration ([Fig. 9.9](#))

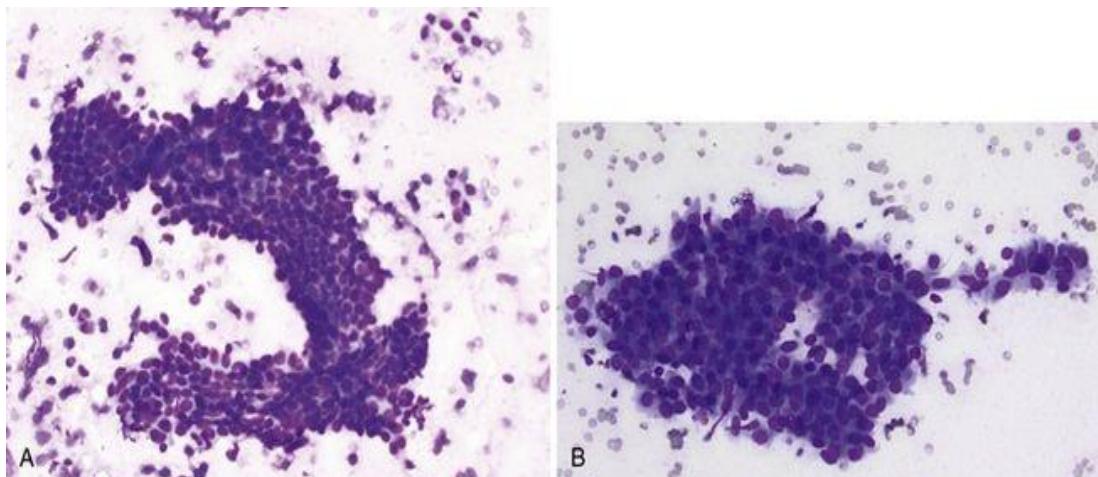


FIGURE 9.9 Fibroadenoma.

A, Branching antler-horn clusters are the predominant arrangement of cells. There are rare stripped naked nuclei and bipolar cells in the background, but they are not prominent in this case, making it difficult to distinguish from a ductal proliferative process (Romanowsky stain). B, Clusters of tightly cohesive cells with minimal nuclear atypia are characteristic of fibroadenomas (Romanowsky

stain).

- bipolar cells and spindled/oval naked nuclei ([Fig. 9.10](#))

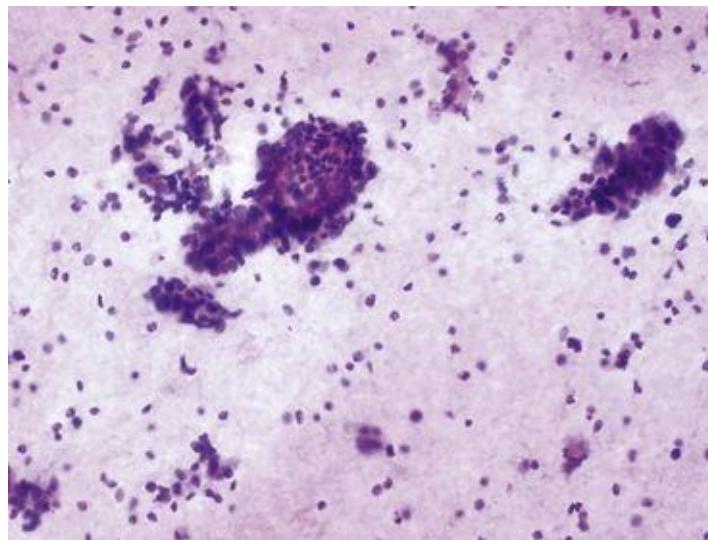


FIGURE 9.10 Fibroadenoma.

Clusters of epithelial cells in a background of numerous stripped, elongated naked nuclei are characteristic of fibroadenomas. When stromal cells are absent, the diagnosis is more difficult (Papanicolaou stain).

- fibrillar stromal fragments
 - bluish-gray with the Papanicolaou stain
 - intensely red-purple with a Romanowsky-type stain
- nuclear atypia ([Fig. 9.11](#))

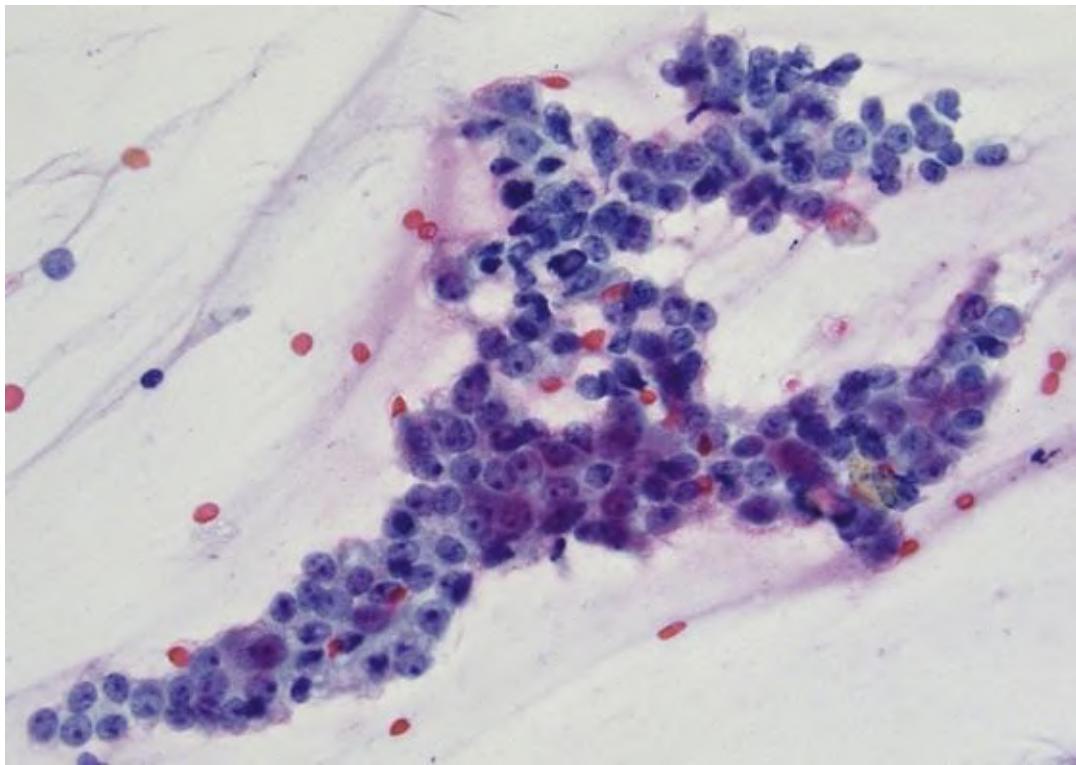


FIGURE 9.11 Fibroadenoma.

Note the presence of nuclear atypia and prominent nucleoli. The tightness of the cluster is an important clue for avoiding an overdiagnosis of malignancy (Papanicolaou stain).

- some loss of epithelial cohesion
- regular nuclear spacing
- finely granular chromatin pattern
- small, round nucleolus



Differential diagnosis of fibroadenoma

- ductal proliferation with or without atypia
- phyllodes tumor
- ductal carcinoma

Although no single criterion distinguishes a fibroadenoma from the ductal proliferations, a combination of features permits a distinction in most cases.¹⁴⁰ In general, fibroadenomas are more cellular. Naked nuclei, although more abundant in fibroadenomas, are seen in both conditions. Stromal fragments and papillary

antlerlike configurations, seen in many (but not all) fibroadenomas, are very uncommon in FCCs.^{140,141}

The distinction between fibroadenoma and phyllodes tumor is difficult. Numerous individual, long, plump, spindle-shaped nuclei are characteristic of a phyllodes tumor.^{142,143} Also characteristic of phyllodes tumors are fibromyxoid stromal fragments with spindle-shaped nuclei and “fibroblastic pavements”: small fragments of cohesive fibroblasts forming a flat “pavement.”¹⁴⁴ Hypercellular stromal fragments are more common in phyllodes tumor,¹⁴³ but can be seen in fibroadenoma as well.

The distinction between fibroadenoma and ductal carcinoma is usually straightforward; the most helpful diagnostic features are stromal fragments, antlerlike epithelial configurations, and honeycomb sheets of ductal cells, all of which are uncommon in ductal carcinomas. Some features can be misleading, however. Cytologic atypia is prominent in some fibroadenomas,^{140,145–147} and isolated cells with intact cytoplasm, a highly characteristic feature of ductal carcinoma, are seen in about 20% of fibroadenomas.^{8,140,147–149} Conversely, some ductal carcinomas masquerade as fibroadenomas. The greatest mimics are well-differentiated invasive ductal carcinoma and ductal carcinoma in situ. Naked nuclei, characteristic of fibroadenomas, are seen in some ductal carcinomas,¹⁴⁸ although usually in fewer numbers than in a fibroadenoma. Nuclear hyperchromasia favors a diagnosis of malignancy, whereas nuclei with small, uniform nucleoli suggest fibroadenoma.¹⁴⁸ The distinction is not discernible in all cases, and the differential diagnosis may be difficult, especially in older women.^{140,148,149} Another mimic of fibroadenoma is papillary carcinoma.¹⁵⁰ Most of the isolated epithelial cells from carcinomas are round to oval with eccentrically placed nuclei, whereas those from fibroadenomas are elongated or columnar, with cytoplasm on both sides of the nucleus. Papillary carcinomas, however, can have isolated spindle-shaped or columnar epithelial cells on FNA. Smears with equivocal findings should be reported as atypical or suspicious.

Pregnancy-Related and Lactational Changes

During pregnancy and lactation, the ductules of the terminal duct lobular unit become hyperplastic and manifest cytoplasmic vacuolization and luminal secretion. Occasionally, this change results in a discrete nodule, called a lactating adenoma, which is difficult to distinguish clinically from a malignancy. Because carcinoma is occasionally diagnosed in the setting of pregnancy, this diagnosis must be excluded in a pregnant or lactating woman. FNA in this setting may be

especially useful, because a diagnosis of pregnancy-related or lactational changes could at least postpone and even spare the woman an excisional biopsy.



Cytomorphology of pregnancy and lactational changes (Fig. 9.12)

- moderately cellular specimen
- numerous isolated epithelial cells and/or stripped nuclei
- nuclear enlargement without variation in size/shape
- prominent nucleolus
- mitoses
- abundant delicate and wispy granular or finely vacuolated cytoplasm
- cytoplasm easily stripped away, revealing:
 - foamy proteinaceous background
 - many naked nuclei
- occasional small ductal cell clusters and portions of lobules



Differential diagnosis of pregnancy and lactational changes

- carcinoma, lobular or ductal
- non-Hodgkin lymphoma

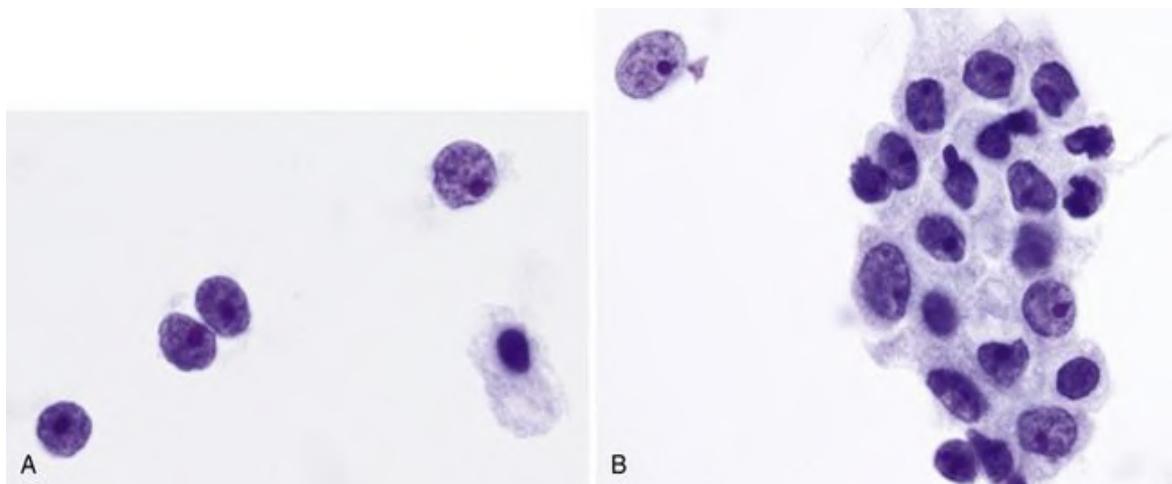


FIGURE 9.12 Pregnancy/lactational changes.

A, Numerous stripped (“naked”) nuclei are seen. B, Cells in loose clusters can also be seen. Nuclei are round or oval, with prominent nucleoli (ThinPrep, Papanicolaou stain).

The cells of invasive lobular carcinoma are similar in size to those of a lactating adenoma, but the foamy background, intact acini and lobules of benign breast tissue, and the prominent nucleoli of a lactating adenoma are absent in invasive lobular cancer. The nuclear size and shape of lactating adenoma cells can also resemble those of some well-differentiated ductal cancers, and the cytoplasmic features can overlap with secretory carcinoma.¹⁵¹ In general, ductal cancers do not have the foamy background characteristic of a lactating adenoma, and the cohesive groups of malignant cells in ductal cancers are not arranged in normal acinar structures. Also, the nuclei in ductal cancers are more hyperchromatic, the nucleoli less prominent, and the cytoplasm less wispy than in lactating adenoma.

Non-Hodgkin lymphoma can resemble the changes of pregnancy and lactation. Both can have many isolated cells with prominent nucleoli, but the cytoplasmic features, proteinaceous background, and intact benign breast tissue typical of lactating adenomas are helpful. The isolated cells of lymphoma vary more in size and shape than those of a lactating adenoma.¹⁵²

Fat Necrosis

Fat necrosis can mimic carcinoma both clinically and mammographically and is commonly seen in patients who have had a previous surgical biopsy or other trauma to the breast. Fat necrosis is also encountered in male patients.¹⁵³



Cytomorphology of fat necrosis (Fig. 9.13)

- hypocellular
- predominantly histiocytes with fine to coarse cytoplasmic vacuoles
- round to kidney bean-shaped nucleus
- low nuclear-to-cytoplasmic ratio
- multinucleated and atypical cells
- background of neutrophils, lymphocytes, and plasma cells



Differential diagnosis of fat necrosis

- silicone granuloma
- infection

- ductal carcinoma
- lipid-rich carcinoma

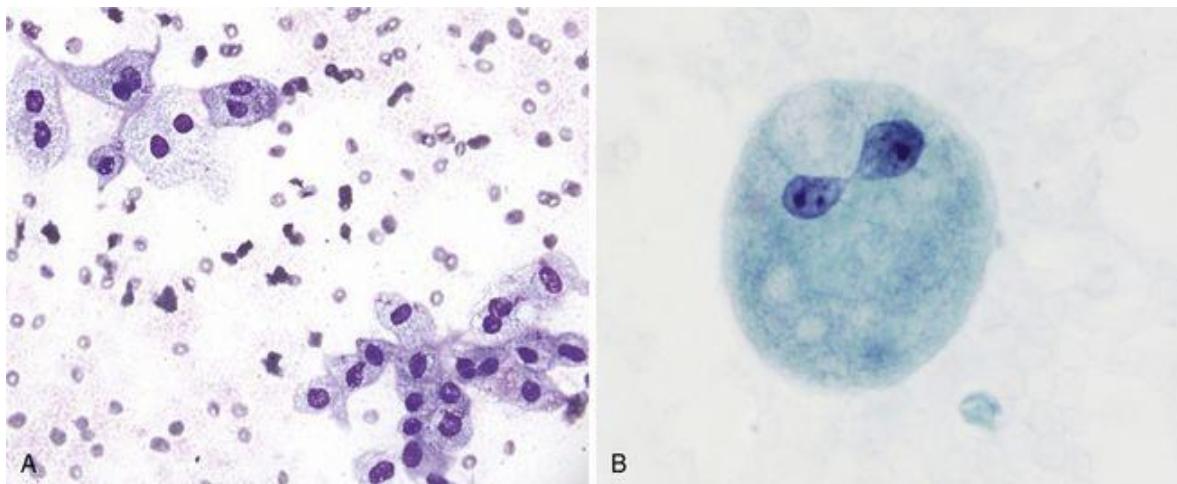


FIGURE 9.13 Fat necrosis.

A, Histiocytes with abundant foamy (microvacuolated) cytoplasm are present (Romanowsky stain). B, An isolated histiocyte has abundant vacuolated cytoplasm. Smears of fat necrosis are typically sparsely cellular (Papanicolaou stain).

The histiocytes seen in reactions to silicone injection or a ruptured silicone implant contain vacuoles that are larger than those seen in fat necrosis and often have a signet-ring appearance.¹⁵⁴⁻¹⁵⁵ In cryptococcosis of the breast, which can occur in an immunosuppressed patient, histiocytes have large cytoplasmic vacuoles containing refractile, budding yeast forms ([Fig. 9.14](#)).

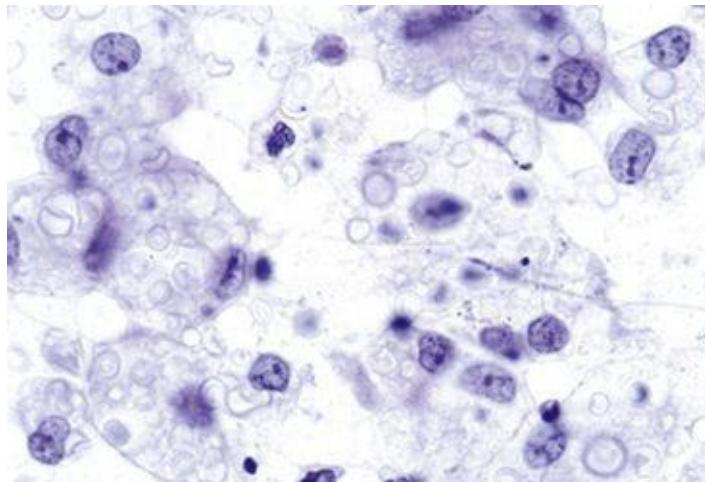


FIGURE 9.14 Cryptococcal infection.

There are numerous intracellular yeast forms within macrophages (hematoxylin and eosin [H & E] stain).

Some ductal carcinomas coexist with fat necrosis; they are identified by the presence of a distinct population of malignant cells in addition to histiocytes. The exceptionally rare lipid-rich carcinoma can also be confused with fat necrosis because the tumor cells have abundant vacuolated cytoplasm. Unlike fat necrosis, however, a lipid-rich carcinoma is hypercellular and shows marked nuclear atypia ([Fig. 9.15](#)).

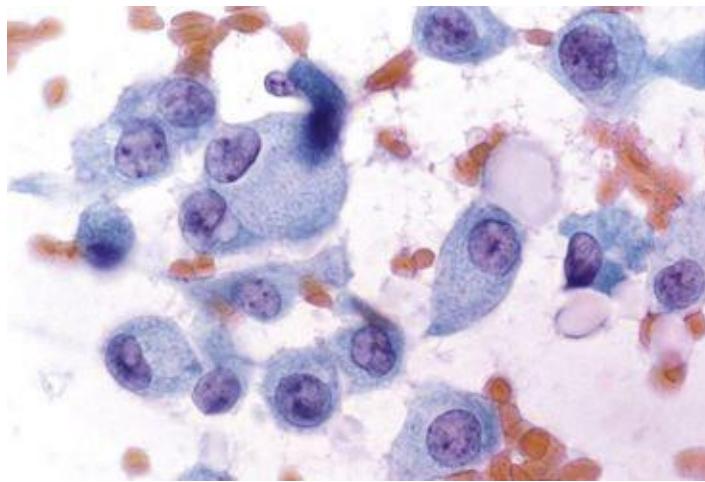


FIGURE 9.15 Lipid-rich carcinoma.

The cells are finely vacuolated and resemble histiocytes, but there is nuclear atypia with an increased nuclear-to-cytoplasmic ratio (Papanicolaou stain).

Radiation Change

Radiation change in normal breast epithelium is observed with increasing frequency because of the widespread use of lumpectomy and irradiation to treat patients with breast cancer. Radiation change is often seen in conjunction with fat necrosis.



Cytomorphology of radiation change ([Fig. 9.16](#))

- hypocellular
- proportionate nuclear and cellular enlargement (low nuclear-to-cytoplasmic ratio)
- hyperchromatic nuclei with a round, regular outline and prominent nucleoli
- coarse cytoplasmic vacuoles, some containing inflammatory cells
- multinucleation

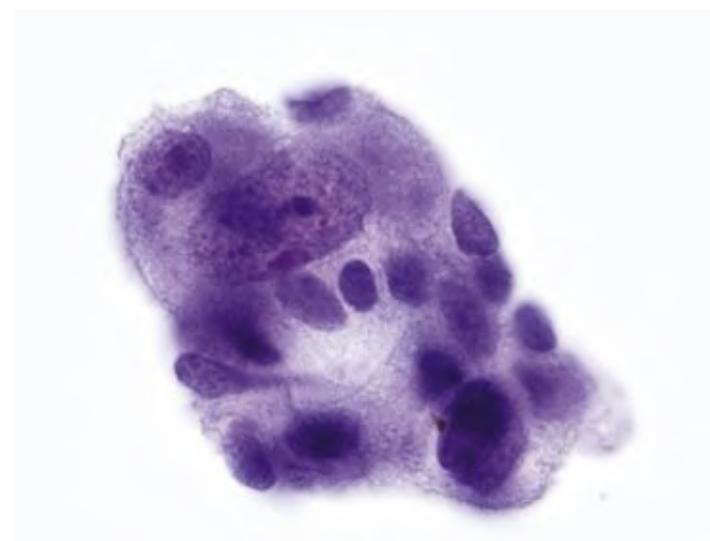


FIGURE 9.16 Radiation change.

The cells show pronounced nuclear enlargement with concomitant cytomegaly. The nuclear-to-cytoplasmic ratio is thus maintained (ThinPrep, Papanicolaou stain).



Differential diagnosis of radiation change

- fat necrosis
- recurrent carcinoma ([Fig. 9.17](#))

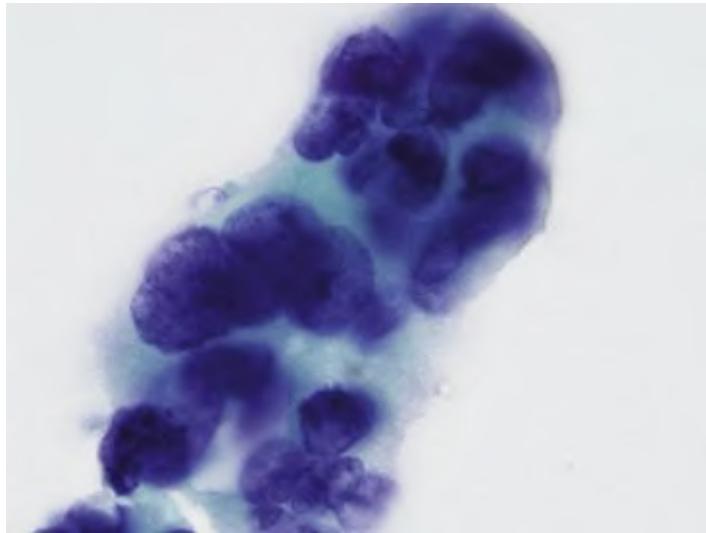


FIGURE 9.17 Recurrent carcinoma after radiation treatment.
In contrast to [Figure 9.16](#), the nuclei are irregular in contour and the nuclear-to-cytoplasmic ratio is increased (ThinPrep, Papanicolaou stain).

The histiocytic nuclei of fat necrosis are smaller than those of epithelial cells altered by radiation. Postsurgical and radiation-induced changes are a recognized pitfall but can usually be distinguished reliably from breast cancer.^{156–159} Most recurrent/persistent carcinomas demonstrate a moderately cellular or hypercellular specimen with many very atypical cells. Because radiation change also contains highly atypical epithelial cells, however, comparison with the morphology of the original tumor is recommended. Histologic confirmation may be necessary when the cytologic diagnosis is equivocal. The absence of isolated cells and necrotic cell debris is a useful finding, as these are more commonly seen in carcinomas.

Mastitis

Acute mastitis usually is due to a bacterial infection and is seen most commonly in the postpartum period. Bacteria invade the breast through the small erosions in the nipple of a lactating woman, and formation of an abscess can result. *Chronic mastitis* can be a sequel to acute mastitis or, more commonly, associated with duct ectasia. Chronic mastitis is a disease of unknown etiology that results

in the dilatation of large and intermediate-size ducts with a surrounding inflammatory infiltrate of lymphocytes and plasma cells. Some patients have a palpable mass that mimics carcinoma. A variant of chronic mastitis, characterized by an infiltrate composed predominantly of plasma cells, is called *plasma cell mastitis*. *Granulomatous mastitis* has the usual cytologic picture of granulomas and can be infectious (i.e., tubercular or fungal) in origin.^{160–162} The term *granulomatous lobular mastitis* has been given to a distinct clinical syndrome years after pregnancy. These lesions, which manifest as firm masses in the periphery of the breast, can be large and may suggest malignancy. The aspirate may contain cohesive clusters of histiocytes with “kidney bean” or boomerang-shaped nuclei. Other chronic inflammatory cells such as lymphocytes and plasma cells may be prominent. Special stains for bacteria, fungi, and acid-fast organisms are mandatory to rule out infection.



Cytomorphology

Acute mastitis:

- abundant neutrophils
- occasional groups of reactive ductal cells with enlarged nuclei and prominent nucleoli

Chronic mastitis:

- abundant, amorphous, granular debris from inspissated ducts
- inflammatory infiltrate composed of lymphocytes and plasma cells

Granulomatous mastitis:

- clustered epithelioid histiocytes
- abundant vacuolated cytoplasm
- round, indented, spindle-shaped, boomerang-shaped, and “kidney bean” nuclei
- dispersed chromatin texture
- large nucleoli
- giant cells, lymphocytes, plasma cells, and eosinophils
- rare clusters of benign ductal cells

Subareolar Abscess

Often called “recurring subareolar abscess,” this inflammatory condition arises in the areola as a result of squamous metaplasia of lactiferous ducts, with subsequent keratin plugging, dilatation, and rupture of the ducts. Without complete excision, the lesion can recur, potentially resulting in the formation of

sinus tracts.

Cytomorphology of subareolar abscess¹⁶³

- numerous anucleate squames admixed with neutrophils
- histiocytes and multinucleated giant cells
- occasional groups of atypical reactive ductal cells
- fragments of granulation tissue

Gynecomastia

Gynecomastia is the most common abnormality of the male breast. It is an enlargement of the breast that can be diffuse or nodular and is frequently bilateral.

Cytomorphology of gynecomastia¹⁶⁴

- resembles fibroadenoma ([Fig. 9.18](#))

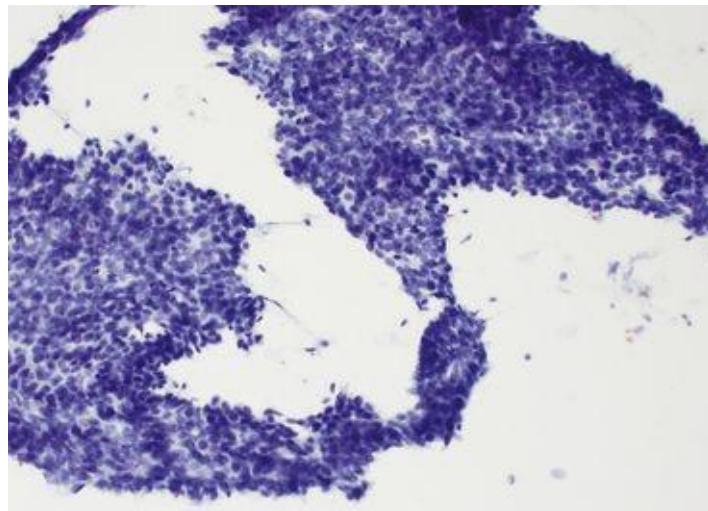


FIGURE 9.18 Gynecomastia.

Note the cohesive flat sheets that are identical to those of fibroadenoma and ductal proliferative processes (Papanicolaou stain).

- low, moderate, or (rarely) high cellularity
- groups of ductal cells with small oval nuclei, scant cytoplasm and little variation in size and shape
- isolated bipolar cells

- naked nuclei



Differential diagnosis of gynecomastia

- carcinoma

FNA is useful in the diagnosis of lesions of the male breast.^{165–168} Carcinoma arising in the male breast is usually of the invasive ductal type. Other subtypes are recognized, but lobular carcinoma is extremely rare.¹¹⁹ The cytologic features are identical to their counterparts in women.

Papillary Neoplasms

Intraductal papillomas are usually solitary retro areolar tumors, but they can occur anywhere in the breast. Because most central papillomas manifest with a discharge (often bloody) from the nipple, nipple discharge cytology is the customary method of evaluation. Less commonly, a patient presents with a palpable central mass that is evaluated by FNA.

Papillary carcinoma is a heterogeneous family of uncommon breast cancers that includes intraductal papillary carcinoma, encapsulated papillary carcinoma, solid papillary carcinoma, invasive papillary carcinoma, and invasive micropapillary carcinoma.¹²⁷

The distinction between intraductal papilloma and papillary carcinoma is virtually impossible to establish with certainty by FNA, although application of the “triple test” can help.^{169,170} Instead of a “positive” or “negative” interpretation, it is best to diagnose a “papillary lesion” and then specify, if possible, “favor benign” or “favor malignant.” Papillary carcinomas generally show a monomorphic population of abundant isolated columnar cells with intact cytoplasm. Papillary clusters of cells and tall, columnar cells in a hemorrhagic background should raise the suspicion of malignancy.¹⁷¹ Although a pure intraductal papilloma is more cohesive and less monomorphic than a papillary carcinoma,⁹³ some intraductal papillomas contain foci of intraductal papillary carcinoma. Sclerosing papillary lesions may manifest as suspicious lesions on FNA.¹⁷² It is best, therefore, to recommend an excisional biopsy when a breast mass has papillary cytomorphology. Papillary lesions can also present difficulty on core biopsy,¹⁷³ even with the assistance of immunohistochemistry for CK5/6, p63, myoepithelial cell markers, and the proliferation marker Ki67.¹⁷⁴



Cytomorphology of papillary neoplasm, “favor benign” (Fig. 9.19)

- moderate to high cellularity
- three-dimensional papillary groups with fibrovascular cores or flat sheets with myoepithelial cells
- only rare isolated cells with intact cytoplasm
- polymorphic small, cuboidal, or columnar cells
- round or oval nucleus with finely granular chromatin
- nucleolus may be present
- foam cells, apocrine metaplasia, and inflammation may be present

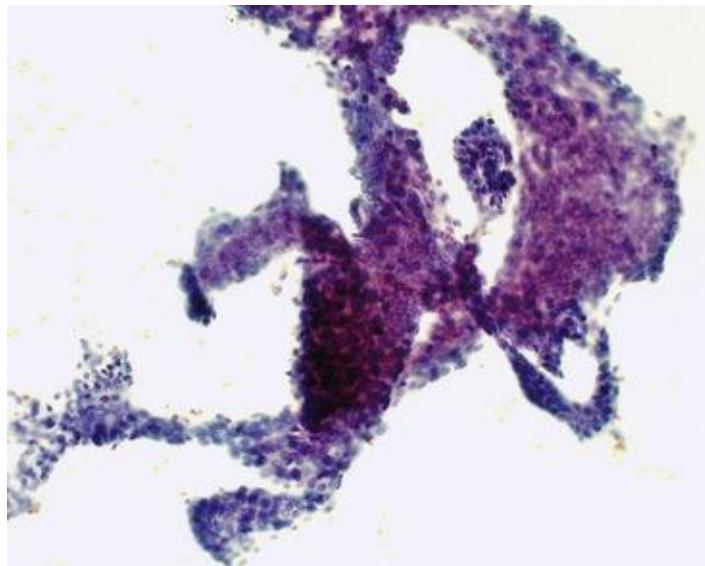


FIGURE 9.19 Papillary neoplasm.

An excisional biopsy showed that this lesion was an intraductal papilloma. Note the complex branching structure (Papanicolaou stain).



Cytomorphology of papillary neoplasm, “favor malignant” ([Fig. 9.20](#))

- moderate to marked cellularity
- papillary clusters and cribriform or tubular architecture
- absence or paucity of myoepithelial cells ([Fig. 9.21](#))

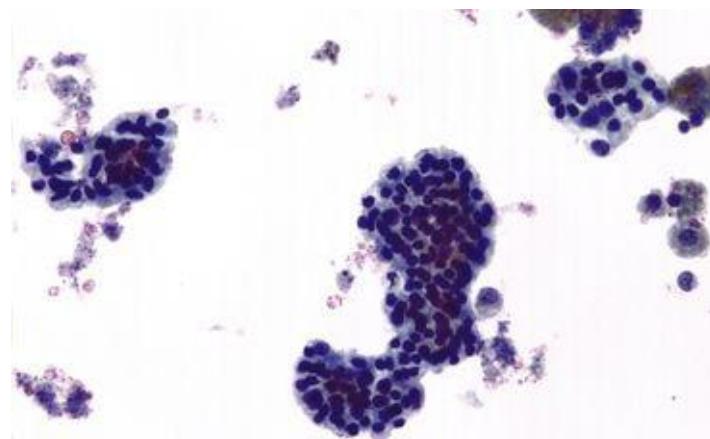


FIGURE 9.21 Invasive micropapillary carcinoma.

Well-differentiated neoplasms such as this invasive micropapillary carcinoma can be very difficult to diagnose (ThinPrep, Papanicolaou stain).

- numerous isolated cells

- often uniform tall, columnar cells
- elongated, uniform nuclei
- many naked nuclei but no bipolar cells
- blood and hemosiderin-laden macrophages common

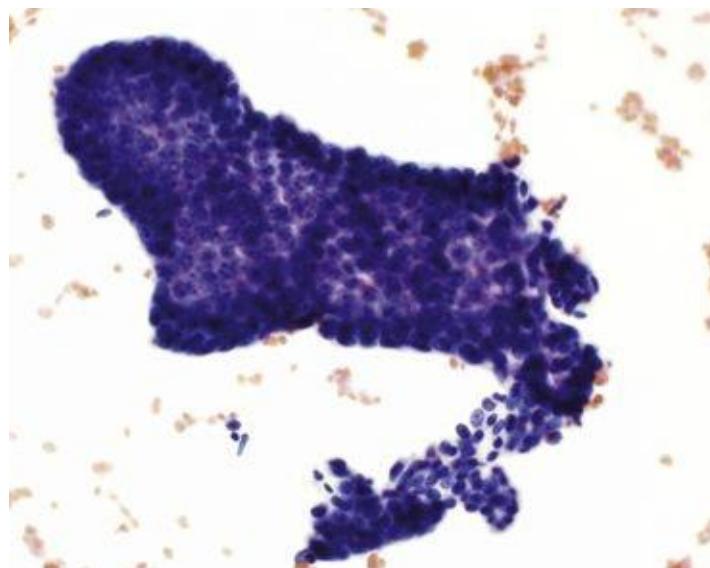


FIGURE 9.20 Papillary neoplasm.

In contrast with [Figure 9.19](#), this lesion proved to be a papillary carcinoma. They are very similar cytologically, and both are best diagnosed as “papillary lesion,” with the definitive diagnosis deferred to an excisional biopsy (Papanicolaou stain).



Differential diagnosis of a papillary neoplasm

- ductal hyperplasia
- fibroadenoma

The architectural pattern and the quantity of myoepithelial cells and bipolar naked nuclei are useful features in distinguishing among these lesions.[150,175](#) Although ductal hyperplasia is cytologically indistinguishable from an intraductal papilloma, it is rare for a patient with ductal hyperplasia to present

with a discharge from the nipple or with a subareolar mass. These cases usually show two-dimensional arrangements with myoepithelial cells.

The similarity of papillary carcinoma to a fibroadenoma can be striking.¹⁵⁰ Hemorrhage, a common feature of papillary carcinoma, is very uncommon in fibroadenomas, however. In papillary carcinoma, the frondlike clusters are composed of considerably noncohesive tumor cells that have a tendency to fall away from adjacent cells. Fibroadenoma more often manifests with folded branching clusters. There is little loss of cohesion, but moderately abundant to numerous bipolar nuclei often form doublets in the background. Prominent loss of cohesion occurs only rarely with fibroadenomas. A cellular stroma can also be seen.

Phyllodes Tumor

Like fibroadenomas, phyllodes tumors are biphasic, composed of an epithelial and stromal proliferation, but with more pronounced stromal cellularity. Phyllodes tumors are much less common than fibroadenomas, however, accounting for less than 1% of all breast tumors. Often growing to massive proportions, they can mimic carcinoma by distorting the breast and even ulcerating the overlying skin. Phyllodes tumors are classified as benign, borderline, or malignant using a combination of histologic criteria.¹²⁷ The distinction cannot be made on FNA, although cell blocks are helpful.¹³⁹ Stromal hypercellularity and a markedly increased stromal-to-epithelial ratio favor malignancy,¹⁷⁶ but histologic confirmation is necessary inasmuch as an invasive margin, among other features, is important in evaluating malignant potential.¹¹⁹



Cytomorphology of phyllodes tumor

- similar to fibroadenoma (Fig. 9.22) but more cellular (Fig. 9.23), with a more cellular stromal component (Fig. 9.23)

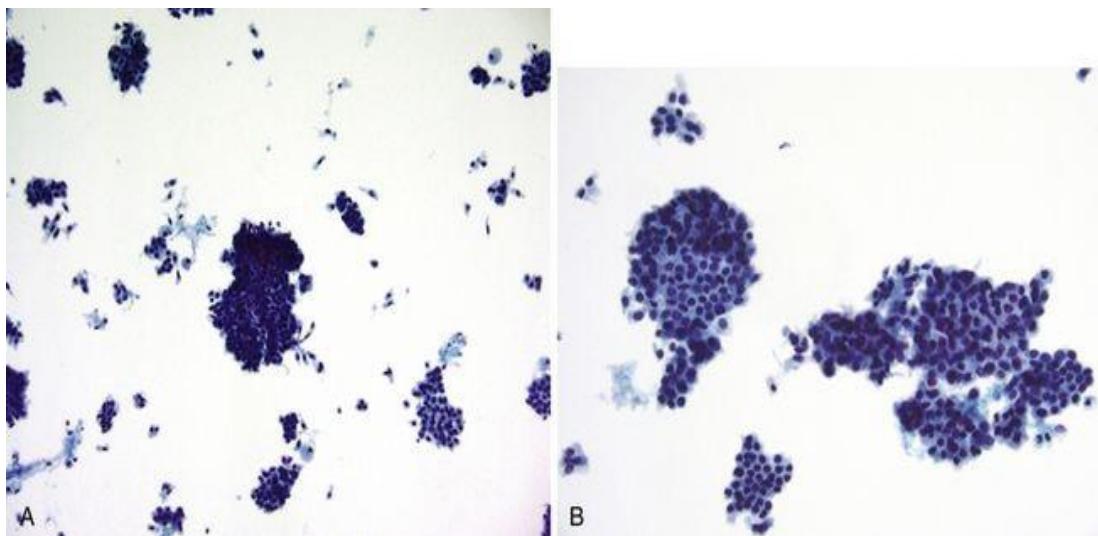


FIGURE 9.22 Phyllodes tumor.

A, Similar cytologically to a fibroadenoma, a phyllodes tumor has greater cellularity. B, The difference between stromal and epithelial cells is subtle and can be lost in a liquid-based preparation (ThinPrep, Papanicolaou stain).

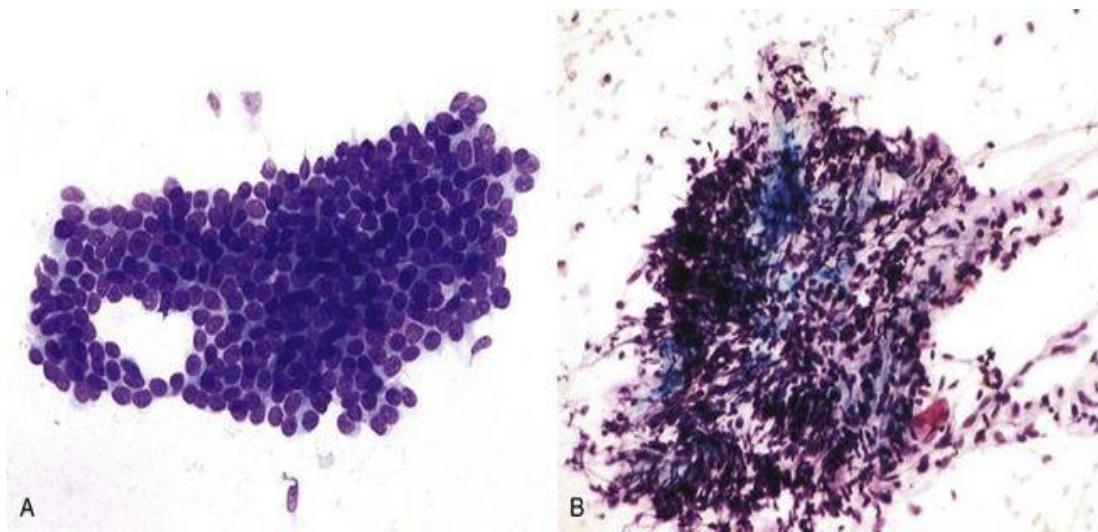


FIGURE 9.23 Phyllodes tumor.

A, Epithelial clusters in a phyllodes tumor resemble those of fibroadenoma but may be more crowded (Romanowsky stain). B, Stromal clusters can be very cellular (Papanicolaou stain).

- sometimes marked stromal atypia with numerous mitotic figures ([Fig. 9.24](#))

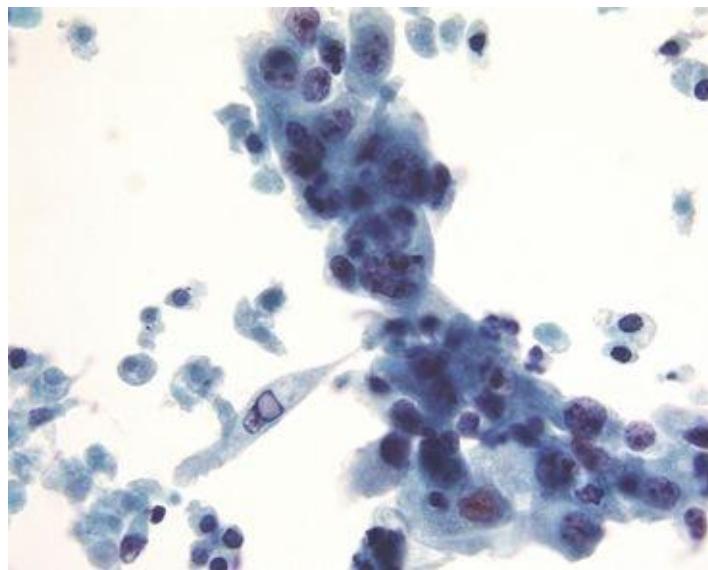


FIGURE 9.24 Phyllodes tumor.

Very atypical stromal and epithelial cells are noted. The background is necrotic. Although cytologically more alarming than the lesion in [Figure 9.23](#), this tumor was benign, and the previous one was malignant (ThinPrep, Papanicolaou stain).

- pronounced epithelial atypia mimicking carcinoma



Differential diagnosis of phyllodes tumor

- fibroadenoma
- ductal carcinoma
- metaplastic carcinoma
- primary sarcoma of the breast

In contrast with fibroadenoma, the stromal fragments of a phyllodes tumor are more cellular and contain larger and more atypical spindle cells, particularly dispersed cells and long, spindled nuclei.^{142,177–180} Although fibroblastic pavements (small fragments of cohesive fibroblasts forming a flat “pavement”), fibromyxoid fragments with spindle cells, and appreciable spindle cells among dispersed cells are reportedly seen only in phyllodes tumors,¹⁴⁴ a definite distinction is not possible, and core or excisional biopsy is usually necessary.¹⁸¹ Because epithelial atypia can be pronounced, some phyllodes tumors are mistaken for ductal carcinomas and thus constitute a significant cause of false-positive diagnosis.^{135,177,182–184} The presence of stromal fragments and some benign ductal cells supports a diagnosis of phyllodes tumor.¹⁸⁵ Metaplastic carcinoma can mimic a phyllodes tumor because of a prominent spindle cell component,¹⁸⁶ but the benign epithelial component of a phyllodes tumor is absent.

Breast Cancer

In the United States, breast cancer accounts for 29% of all new cancer cases and causes 14% of all deaths from cancer in women.^{[187](#)}

Invasive Ductal Carcinoma

Invasive ductal carcinoma is the most common malignant tumor of the breast, accounting for 40% to 75% of all breast cancers.^{[127](#)} The World Health Organization (WHO) has replaced “invasive ductal carcinoma” with “invasive carcinoma of no special type,” because use of the term *ductal* perpetuates the incorrect concept that these tumors are derived from the breast ducts.^{[127](#)} In fact, the terminal duct–lobular unit is the site of origin of most breast cancers. We have, however, retained the term *invasive ductal carcinoma*, because it is so entrenched in common practice.

Invasive ductal carcinoma is almost invariably solid and can be detected by palpation or mammography. Many invasive ductal carcinomas have a characteristically gritty consistency, appreciable during FNA. Although most are pure ductal carcinomas, limited foci of tubular, papillary, mucinous, or medullary differentiation can be present. Invasive ductal carcinoma ranges from well to poorly differentiated, and is usually graded by a combination of nuclear and architectural features. Thus, FNA is of limited use in grading breast carcinomas, despite some degree of correlation between cytologic and histologic grading.^{[188–190](#)} Some invasive ductal carcinomas are associated with dense fibrosis; such tumors may result in a nondiagnostic FNA despite multiple passes.^{[191](#)} In this circumstance, a tissue biopsy is needed for diagnosis.



Cytomorphology of invasive ductal carcinoma

- hypercellular ([Fig. 9.25](#))

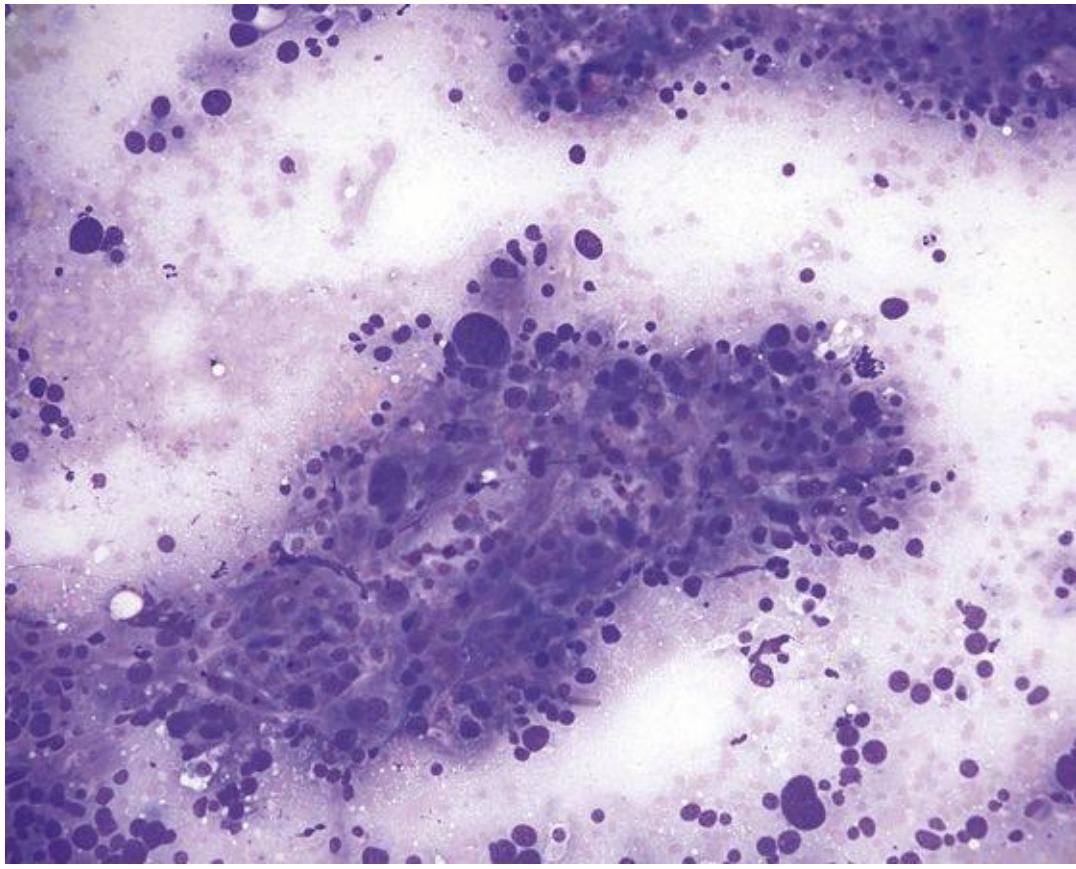


FIGURE 9.25 Ductal carcinoma.

A major criterion for the diagnosis of ductal carcinoma is hypercellularity. Even at low magnification, nuclear atypia is prominent (Romanowsky stain).

- isolated cells and poorly cohesive clusters of cells ([Fig. 9.26](#))

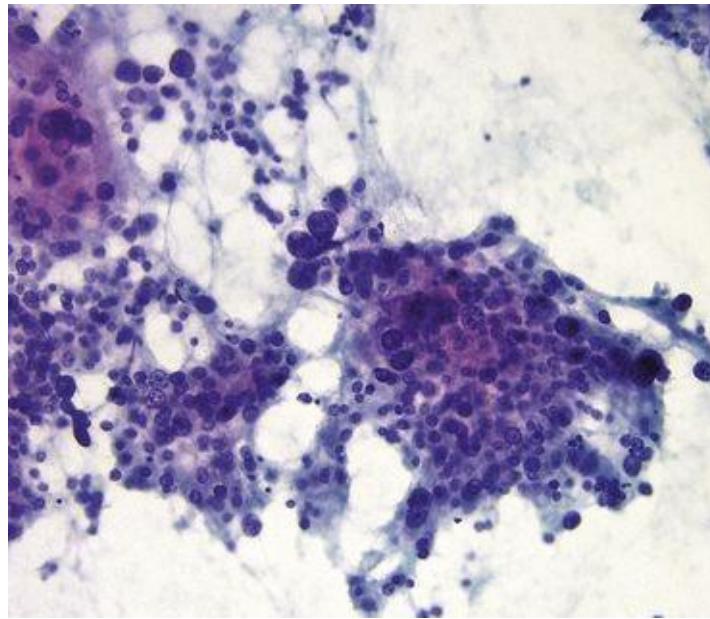


FIGURE 9.26 Ductal carcinoma.

The specimen is very cellular, and the cells are dispersed both as isolated cells and as loosely cohesive clusters (Papanicolaou stain).

- eccentric nucleus often protruding from the cytoplasm (“comet cells”) ([Fig. 9.27](#))

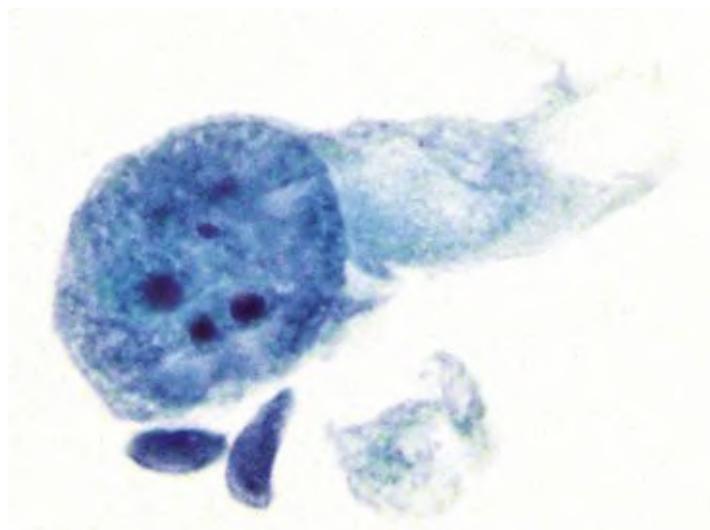


FIGURE 9.27 Ductal carcinoma.

Many of the isolated cells of ductal cancers are comet-shaped, with a nucleus that protrudes from the cytoplasm. Whether the nuclear atypia is marked or not, a protuberant nucleus suggests carcinoma (ThinPrep, Papanicolaou stain).

- enlarged, variably hyperchromatic nuclei, but can vary considerably in size and shape ([Fig. 9.28](#))

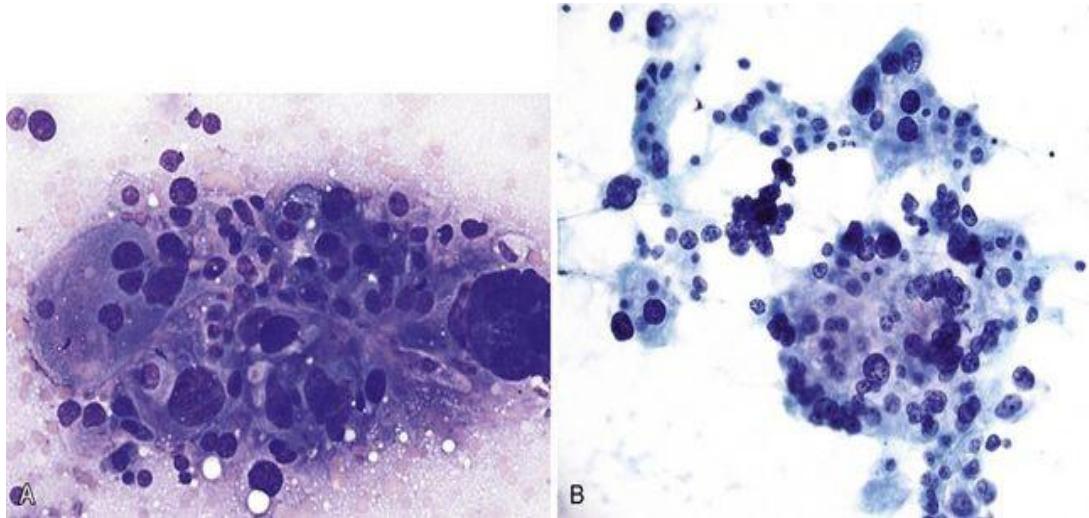


FIGURE 9.28 Ductal carcinoma.

Note the pronounced nuclear pleomorphism and atypia, apparent with both the Romanowsky (A) and Papanicolaou (B) stains.

- finely or coarsely granular chromatin pattern
- small or large, irregularly shaped nucleolus
- usually clean background, but can see inflammation, blood, and granular debris ([Fig. 9.29](#))

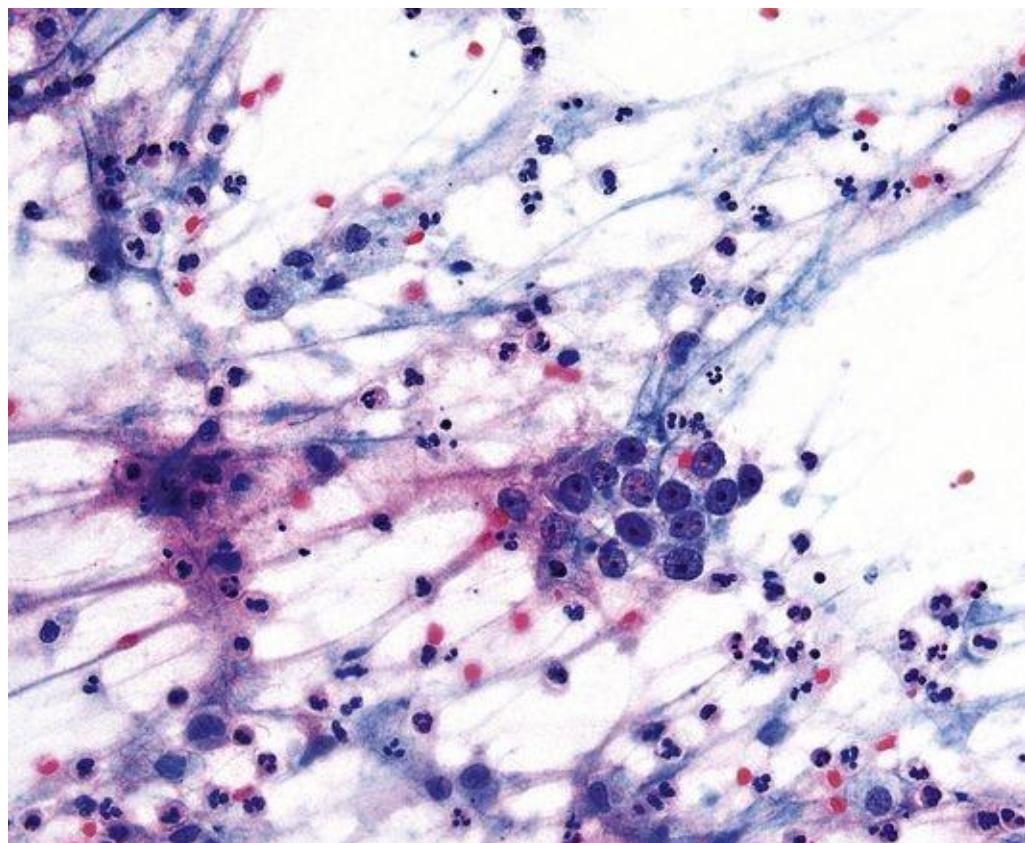


FIGURE 9.29 Ductal carcinoma.

The tumor cells are buried in a background of marked acute inflammation. In samples with abundant acute inflammation, a careful search must be conducted to exclude malignant cells (Papanicolaou stain).



Differential diagnosis of invasive ductal carcinoma

- ductal carcinoma in situ
- fibroadenoma/phyllodes tumor
- proliferative fibrocystic changes
- pregnancy or lactational changes

The differential diagnosis includes ductal carcinoma in situ, the presumed precursor of invasive ductal carcinoma. Not surprisingly, invasive ductal carcinoma and ductal carcinoma in situ appear identical on cytologic examination.^{134,192,193} The significance of malignant cells embedded in fat or stroma is controversial.¹⁹⁴ Some authors believe that invasion can be suggested if strict criteria (e.g., identification of “true infiltration” of fibrofatty tissue) are applied

to smears.^{130,195} Others have found this finding unreliable, because it is seen in the majority of cases of ductal carcinoma in situ.¹⁹⁶ In fact, benign ductal cells are commonly seen in association with fatty tissue.¹⁹⁶ This finding should not be taken as a sign of malignancy; rather, it is a mechanical artifact of aspiration and smear preparation. The cohesiveness of some invasive tumor cells, and the lack of tubular structures can suggest *in situ* carcinoma.¹⁹⁷ Important clues to the presence of invasion are cell clusters with a tubular structure, cytoplasmic lumen formation in malignant cells, fibroblast proliferation, and fragments of elastoid stroma.¹⁹⁸ Although these features may be specific, their sensitivity is low (48%). Like invasive carcinoma, ductal carcinoma *in situ* can present as a palpable mass or a nonpalpable mammographic abnormality. Due to these inherent difficulties, it has been suggested that cell blocks can aid in the diagnosis of invasion.¹³⁹

Well-differentiated ductal carcinomas are an important cause of false-negative results and can masquerade as fibroadenomas and phyllodes tumors.^{117,135,148,149,177,182,184,199,200} Although morphometry can statistically separate large cohorts of benign from malignant lesions,²⁰¹ it is not sufficiently robust or accurate for clinical application to individual cases.^{202,203} Because isolated cells with intact cytoplasm can be seen in both benign and malignant lesions, their presence alone is not diagnostic of malignancy. Isolated cells with nuclear atypia, however, are highly characteristic of malignancy. Nuclear hyperchromasia suggests ductal carcinoma; nuclei with a single, small, uniform nucleolus are more typical of fibroadenoma.¹⁴⁸ Stromal fragments and bipolar cells, particularly in pairs, are more common in fibroadenoma and relatively uncommon in ductal cancer.²⁰⁴ Although marked epithelial atypia can be seen in phyllodes tumors,¹⁸⁴ stromal fragments with spindle cell atypia are not seen in ductal cancers. The double stain for cytokeratin and smooth muscle actin or p63 to detect myoepithelial cells is useful.^{45,205} Immunocytochemistry for the proliferation markers Ki-67 and p27 (Kip1) have been attempted to separate fibroadenoma and FCCs from carcinoma.²⁰⁶ Despite a statistically significant difference between cohorts of benign and malignant lesions, the wide ranges observed for each marker render them useless in individual cases.

Pregnancy and lactational changes may mimic carcinoma because of the presence of numerous isolated cells with prominent nucleoli. The absence of nuclear hyperchromasia, nuclear size variation, and coarse chromatin favors a benign diagnosis. Focal nuclear atypia is seen in fat necrosis, radiation change, mastitis, and subareolar abscess, but the atypia is usually mild, and other background features provide clues to the diagnosis.

Invasive Lobular Carcinoma

Invasive lobular carcinoma constitutes 5% to 15% of invasive breast cancers.¹²⁷ It is composed of small or medium-sized uniform cells that have a characteristic pattern of infiltration. Tumor cells usually grow in a linear, swirling fashion and often evoke a marked desmoplastic stromal reaction, although solid growth patterns are occasionally seen. The distension of cytoplasm with mucus gives some tumor cells a signet ring shape.



Cytomorphology of invasive lobular carcinoma

- often sparsely cellular because of marked stromal fibrosis
- predominantly isolated cells with small groups or linear arrays ([Fig. 9.30A](#))

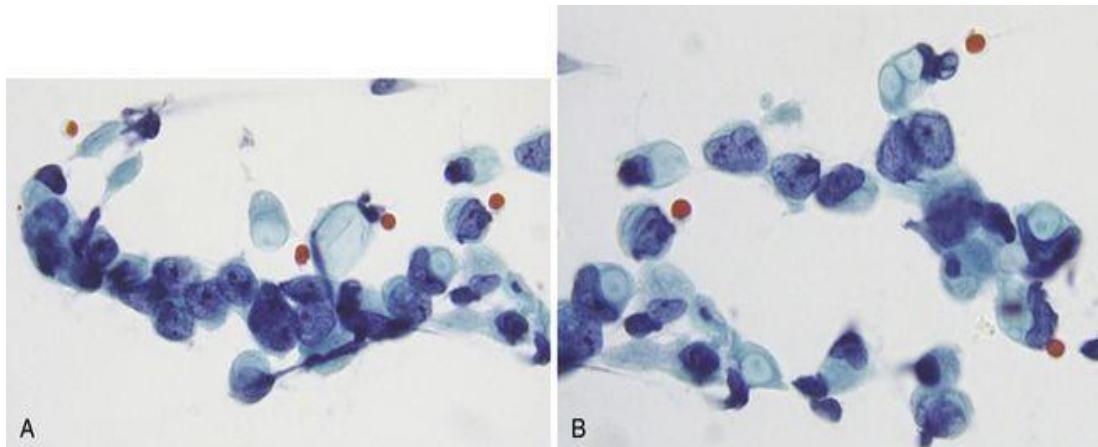


FIGURE 9.30 Lobular carcinoma.

A, A loose, single-file arrangement is apparent. B, Large, solitary intracytoplasmic vacuoles are present, imparting a signet ring cell appearance (Papanicolaou stain).

- small to mid-sized tumor cells
- large cytoplasmic vacuole (signet ring appearance) ([Fig. 9.30B](#))
- hyperchromatic, often kidney bean-shaped nucleus
- usually small nucleolus, rarely large



Differential diagnosis of invasive lobular carcinoma

- well-differentiated ductal carcinoma

This is one of the most difficult breast cancers to diagnose by FNA.^{[118,207](#)} Because of scant cellularity, cases are more often diagnosed as “atypical” or “suspicious,” especially by pathologists with less experience.^{[208](#)} The differential diagnosis includes invasive ductal carcinoma, which is usually more cellular and pleomorphic. Nevertheless, some well-differentiated invasive ductal carcinomas are impossible to distinguish from invasive lobular carcinomas. As with invasive ductal carcinomas, it is not possible to distinguish invasive lobular carcinoma from its precursor lesion, lobular carcinoma in situ (LCIS), by FNA. Most cases of LCIS are moderately cellular, with cohesive clusters of cells. LCIS cells have a mildly enlarged nucleus ([Fig. 9.31](#)), and LSILs may occasionally demonstrate isolated epithelial cells, a prominent nucleolus, an intracytoplasmic lumen, and two distinct epithelial cell populations.^{[209](#)} Many are diagnosed as atypical, but cases of LCIS with abundant isolated cells can be overdiagnosed as invasive lobular carcinoma ([Fig. 9.32](#)).

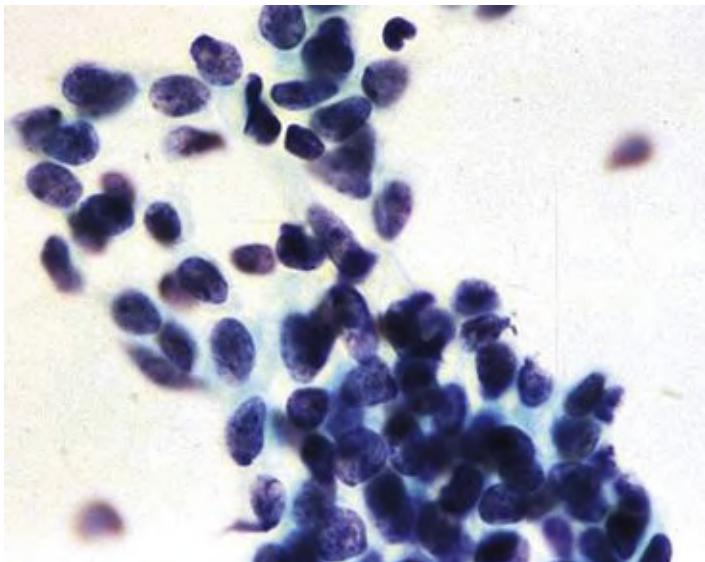


FIGURE 9.31 Lobular carcinoma in situ.

The cells are present in loosely cohesive sheets (Papanicolaou stain).

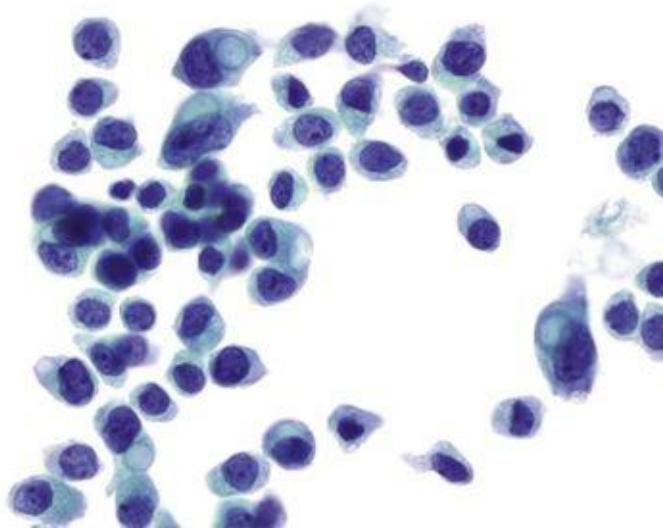


FIGURE 9.32 Lobular carcinoma in situ.

In contrast with [Figure 9.31](#), the cells are dispersed, and signet ring cell forms are noted. Such a case is likely to be over diagnosed as invasive lobular carcinoma (Papanicolaou stain).

Medullary Carcinoma

Classic medullary carcinoma accounts for less than 1% of breast cancers, although higher rates have been reported depending on the stringency of the criteria used for diagnosis.^{[127](#)} This breast cancer subtype, particularly when “triple negative” for ER, PR, and HER2, is associated with mutations of the *BRCA1* gene.^{[210, 211](#)} Thus, its diagnosis in a woman with a family history of breast cancer should lead to consideration of genetic analysis. Medullary carcinoma is a well-circumscribed tumor composed of large, poorly differentiated cells with syncytial architecture, scant stroma, and a prominent lymphoid infiltrate. Hemorrhage and necrosis occur in some cases. As a result, medullary carcinomas can be cystic.^{[122, 212](#)} Because they are well circumscribed, they can be mistaken clinically for a cyst or fibroadenoma.



Cytomorphology of medullary carcinoma ([Fig. 9.33](#))

- hypercellular
- numerous isolated cells and loose clusters
- markedly enlarged, vesicular nucleus
- prominent, often irregular macronucleolus
- numerous mitoses
- granular, scarce to abundant cytoplasm
- many lymphocytes and some plasma cells ([Fig. 9.34](#))

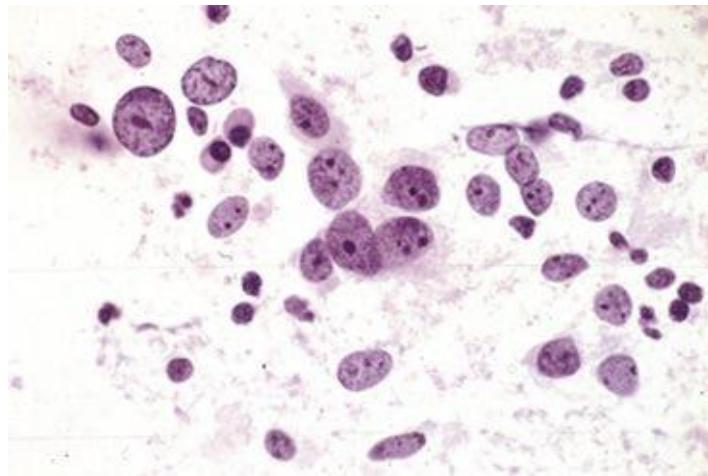


FIGURE 9.34 Medullary carcinoma.
Lymphocytes and plasma cells are often noted in the background (hematoxylin and eosin [H & E] stain).

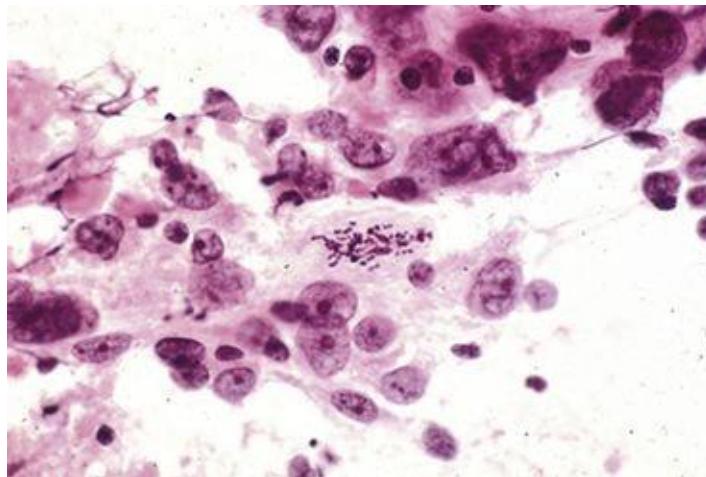


FIGURE 9.33 Medullary carcinoma.
The cells are very large, with prominent nucleoli and frequent mitoses (hematoxylin and eosin [H & E] stain).



Differential diagnosis of medullary carcinoma

- chronic mastitis
- intramammary lymph node

- lymphoma
- invasive ductal carcinoma

Chronic mastitis and an intramammary lymph node lack the abundant large, poorly differentiated tumor cells of medullary carcinoma.²¹³ The tumor cells of medullary carcinoma are larger and more variable than those of large cell lymphoma, and they often show some clustering, an uncommon feature in most lymphomas. In ambiguous cases, immunocytochemistry for leukocyte common antigen, keratin, and epithelial membrane antigen (EMA) are helpful. The difference between medullary carcinoma and poorly differentiated ductal carcinoma is virtually impossible to discern cytologically.^{213, 214} The distinction is based on histologic criteria such as circumscription of the tumor. FNA can only suggest the possibility of medullary carcinoma. The distinction is important, because patients with medullary carcinoma have a significantly better prognosis than those with usual ductal carcinoma, whereas a poorly differentiated carcinoma that does not meet the criteria carries a worse prognosis. In the absence of an excisional biopsy (such as in the setting of neoadjuvant chemotherapy), however, this distinction is usually not possible.

Mucinous (Colloid) Carcinoma

This distinct type of invasive carcinoma is composed almost entirely of aggregates of uniform cells floating in abundant extracellular mucus. Pure mucinous carcinomas, defined as carcinomas composed of greater than 90% mucinous carcinoma, constitute about 2% of invasive breast cancers.¹²⁷ These slow-growing tumors carry a better prognosis than that of the usual invasive ductal carcinoma. It has been suggested that FNA is significantly less sensitive than CNB in this setting.²¹⁵ Better performance for this type of carcinoma has been found with modified Giemsa-stained and ThinPrep slides than with Papanicolaou-stained conventional smears.²¹⁶ A mucinous carcinoma can be suggested in an FNA report (e.g., “carcinoma with prominent mucinous features”),²¹⁷ but the distinction between a pure mucinous carcinoma and a ductal carcinoma with focal mucinous change requires extensive sampling of a resected specimen and is thus not possible by FNA.²¹⁸



Cytomorphology of mucinous carcinoma

- tightly cohesive three-dimensional balls of cells ([Fig. 9.35](#))

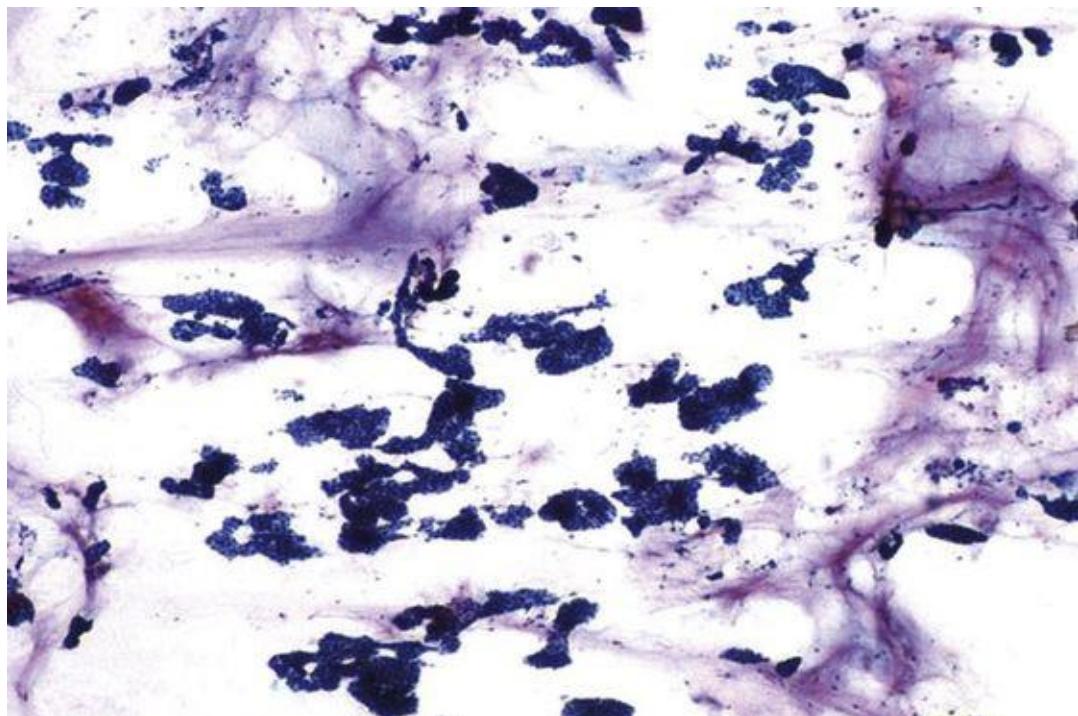


FIGURE 9.35 Mucinous carcinoma.

At low magnification, numerous tightly cohesive clusters are dispersed in a mucinous background (Papanicolaou stain).

- mucinous background (red-violet with a Romanowsky stain; green-purple with Papanicolaou)
- branching capillary structures ([Fig. 9.36](#))

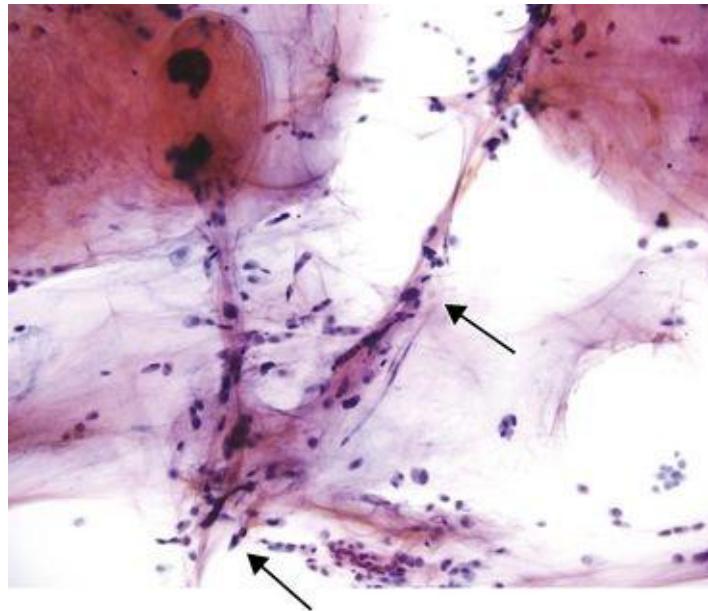


FIGURE 9.36 Mucinous carcinoma.
Branching capillary structures in a mucinous background suggest the diagnosis.
Isolated cells can be seen in addition to cellular balls (Papanicolaou stain).

- uniform, unremarkable nucleus ([Fig. 9.37](#))

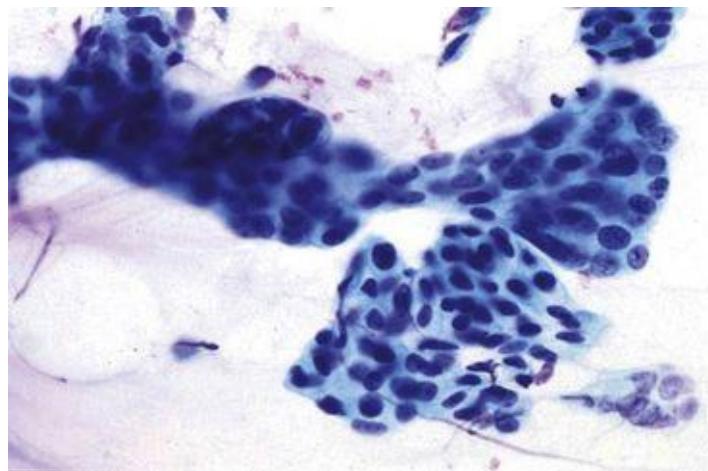


FIGURE 9.37 Mucinous carcinoma.
At higher magnification, the low-grade, round, regular nuclei are noted
(Papanicolaou stain).

- small vacuoles in the cytoplasm
- plasmacytoid cells with an eccentric nucleus^{[219,220](#)}
- rarely psammoma bodies^{[219,220](#)}



Differential diagnosis of mucinous carcinoma

- mucocele
- fibroadenoma
- lobular carcinoma
- invasive carcinoma with focal mucinous features

A mucocele is a benign mucin-filled cyst that often ruptures. Mucoceles lack the abundant three-dimensional balls of neoplastic cells that are typical of mucinous carcinoma.^{221,222} Although a fibroadenoma can have a myxoid background, it is more cellular, and the cells are arranged in large groups or occur in antlerlike configurations rather than as balls.²²³ In addition, fibroadenomas may have many single stromal/bipolar cells or stripped nuclei as well as myxoid stromal fragments. Lobular carcinoma is often composed of vacuolated cells, but these are commonly arranged as isolated cells and not as balls, and a mucinous background is absent. Because the distinction between a pure mucinous carcinoma and a mixed mucinous and typical ductal carcinoma is not possible by FNA, descriptive terminology like “carcinoma with prominent mucinous features” (rather than an outright diagnosis of “mucinous carcinoma”) is preferred for reporting results.²¹⁸

Tubular Carcinoma

Tubular carcinoma, a well-differentiated tumor accounting for about 2% of invasive breast carcinomas,¹²⁷ is composed of well-defined tubules lined by a single layer of neoplastic cells and surrounded by a dense fibrous stroma. Because some usual ductal carcinomas can have foci of tubular carcinoma, this specific diagnosis is reserved for cases in which well-defined tubules constitute more than 90% of the tumor.¹²⁷ When the condition is defined in this way, the prognosis for patients with tubular carcinoma is more favorable than for those with invasive ductal carcinoma. The sensitivity for the diagnosis of tubular carcinoma is lower for FNA than for core biopsy (50% versus 91%, respectively), and fewer cases receive an outright diagnosis of malignancy (42% versus 73%, respectively).^{224,225}



Cytomorphology of tubular carcinoma

- hypocellular (because of the dense fibrosis)
- predominantly cohesive, often angular clusters (sometimes described as comma shaped or cornucopia shaped) ([Figs. 9.38, 9.39](#))

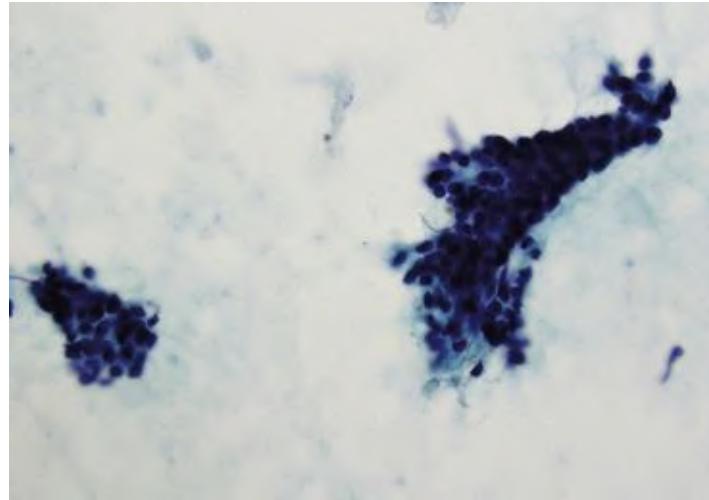


FIGURE 9.38 Tubular carcinoma.

Cells in tightly cohesive clusters often have rigid borders. Nuclear atypia is minimal, and isolated cells are usually not seen (Papanicolaou stain).

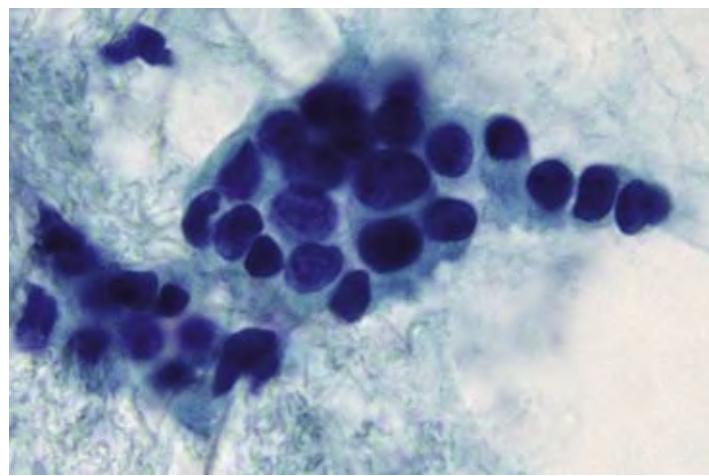


FIGURE 9.39 Tubular carcinoma.

Clusters of cells typically come to a sharp point (comma or cornucopia formations). By contrast, fibroadenomas tend to have more rounded and less rigid outlines (Papanicolaou stain).

- peripheral perpendicular cells around tubular clusters^{[226](#)}

- some dyshesion
- uniform, medium-sized cells with round, uniform nuclei
- finely granular chromatin
- small nucleolus
- occasional cells with a large cytoplasmic vacuole



Differential diagnosis of tubular carcinoma

- fibroadenoma
- proliferative ductal lesions
- invasive ductal carcinoma
- invasive lobular carcinoma

Tubular carcinoma is well known to yield false-negative FNA results. Tumor cells tend to be arranged in smaller groups than those of fibroadenoma or ductal proliferative lesions. The presence of angular epithelial groups, isolated epithelial cells, and nuclear atypia, warrants consideration of the diagnosis of tubular carcinoma.^{[225-227](#)} The cytologic features of tubular carcinoma overlap significantly with well-differentiated ductal carcinoma and lobular carcinoma, both of which can have a minor component of tubular carcinoma. A definitive diagnosis depends on a surgical biopsy.^{[118](#)}

Metaplastic Carcinoma

Metaplastic carcinoma, which comprises less than 1% of breast carcinomas, is a heterogeneous group of carcinomas with mesenchymal or squamous differentiation. In some cases, evidence of ductal differentiation is entirely lacking, and the tumor is composed exclusively of squamous or sarcomatoid elements. Some tumors can be cystic.



Cytomorphology of metaplastic carcinomas

- moderate to marked cytologic atypia
- isolated cells and clusters
- large, pleomorphic, and spindle-shaped cells ([Fig. 9.40](#))

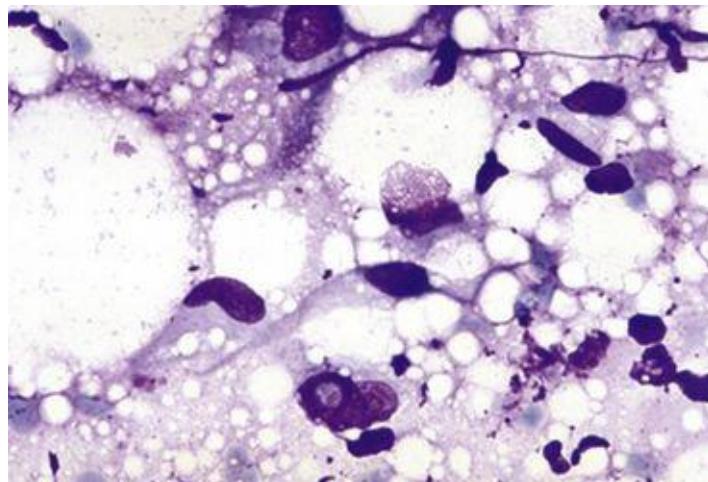


FIGURE 9.40 Metaplastic carcinoma.
Isolated highly atypical spindle cells are evident (Romanowsky stain).

- malignant squamous and/or glandular cells
- benign multinucleated giant cells²²⁸
- rarely, malignant cartilage or bone²²⁹
- background of amorphous debris and inflammatory cells



Differential diagnosis of metaplastic carcinomas

- fibrocystic changes
- subareolar abscess
- benign squamous metaplasia following lumpectomy and irradiation
- phyllodes tumor
- sarcoma
- solid papillary carcinoma
- angiosarcoma

Metaplastic carcinoma should be considered when there are high-grade malignant features with mesenchymal or combined epithelial and mesenchymal elements, and especially with two or more elements.^{186,230–235} Cystic tumors can yield few if any malignant cells, and some multinucleated giant cells resemble the foam cells seen in cysts of FCCs or osteoclasts.²²⁸ A squamous component with inflammation may suggest a subareolar abscess, but a peripheral location favors the diagnosis of metaplastic carcinoma. Squamous metaplasia following lumpectomy and irradiation is more problematic in that significant atypia can be

encountered.²³⁶ With a phyllodes tumor, the mesenchymal element is usually arranged in stromal fragments rather than as isolated cells, and abundant benign epithelium is present. A primary breast sarcoma can be cytologically indistinguishable from a sarcomatoid metaplastic carcinoma.²³⁷ Because metaplastic carcinoma is an epithelial neoplasm, immunocytochemistry can be helpful, because metaplastic carcinoma is immunoreactive for cytokeratin and EMA. Solid papillary carcinoma (formerly “spindle cell ductal carcinoma *in situ*”) is a rare entity that can be indistinguishable from metaplastic carcinoma.²³⁸ Angiosarcoma can manifest as a secondary lesion after radiation treatment for breast cancer, and tumor cells can be spindle shaped or epithelioid. Immunohistochemical stains for the endothelial markers CD31, CD34, and ERG are a useful adjunct.^{239,240}

Uncommon Breast Tumors

Apocrine Carcinoma

As its name indicates, apocrine carcinoma is composed predominantly of apocrine cells, that is, cells that resemble the apocrine metaplasia often seen in FCCs. Focal apocrine differentiation is common in invasive ductal carcinomas; extensive apocrine differentiation is seen in about 4% of invasive breast cancers. Apocrine carcinoma is clinically indistinguishable from the usual invasive ductal carcinoma.



Cytomorphology of apocrine carcinoma

- hypercellular ([Fig. 9.41](#))

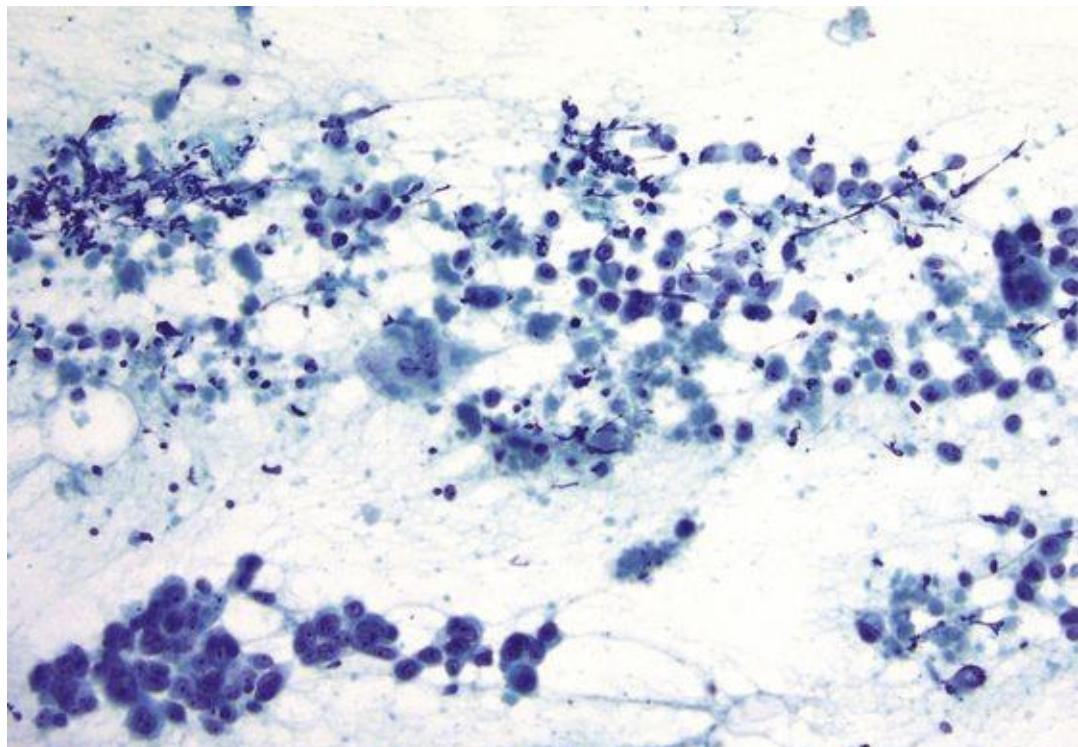


FIGURE 9.41 Apocrine carcinoma.

A variant of ductal carcinoma, apocrine carcinoma is also typified by hypercellularity, nuclear atypia, and many isolated cells (Papanicolaou stain).

- isolated cells; clusters and sheets
- abundant granular cytoplasm with indistinct cell borders
- enlarged nucleus with irregular contours
- prominent nucleolus ([Fig. 9.42](#))

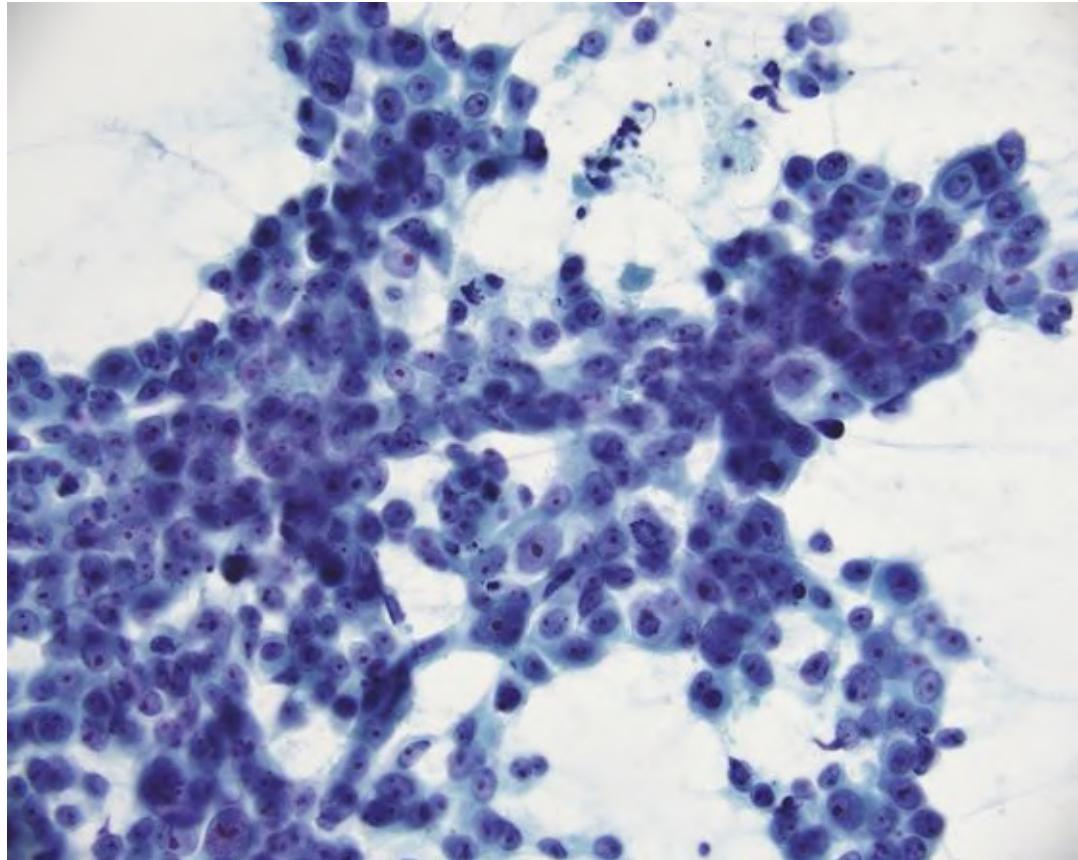


FIGURE 9.42 Apocrine carcinoma.

Note the abundant granular cytoplasm, pronounced nuclear atypia, and prominent nucleoli, which are round and centrally located (Papanicolaou stain).

- necrotic debris



Differential diagnosis of apocrine carcinoma

- apocrine metaplasia
- apocrine adenosis

Although apocrine metaplasia can demonstrate significant variation in nuclear size, marked nuclear atypia (e.g., hyperchromasia, irregular nuclear contours) is generally not observed.²⁴¹ Protruding nuclei (comet-shaped cells) are useful because they are seen in carcinoma, whereas nuclei are centrally located in apocrine metaplasia. Apocrine carcinoma is almost invariably a solid mass clinically and radiologically, and necrosis is not present in FCC. Apocrine adenosis may overlap morphologically with apocrine carcinoma but usually has many naked nuclei and less nuclear hyperchromasia.¹²⁵

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma comprises less than 0.1% of breast cancers and is morphologically identical to its namesakes in the salivary glands and other sites.²⁴²⁻²⁴⁸ An immunostain for collagen IV stains the background material of adenoid cystic carcinoma.²⁴⁹ Adenoid cystic carcinoma of the breast, unlike its salivary gland counterpart, is associated with an excellent prognosis.



Cytomorphology of adenoid cystic carcinoma

- hypercellular
- nests of cohesive small cells ([Fig. 9.43](#))

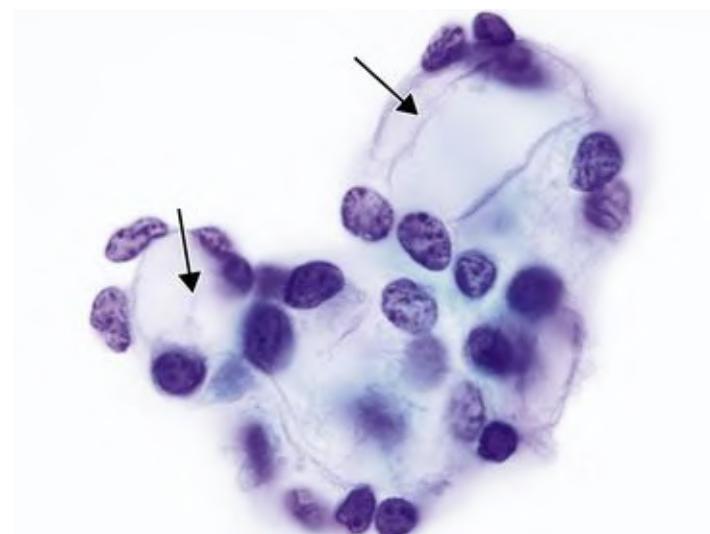


FIGURE 9.43 Adenoid cystic carcinoma.

Tightly cohesive clusters of cells with round, uniform nuclei are noted. Globules are subtle in this case (ThinPrep, Papanicolaou stain).

- uniform round or oval nucleus
- hyperchromatic nucleus with coarsely granular chromatin and small nucleolus
- scant cytoplasm
- round globules (bright red or purple with a Romanowsky stain; pale green with Papanicolaou, [Fig. 9.44](#))

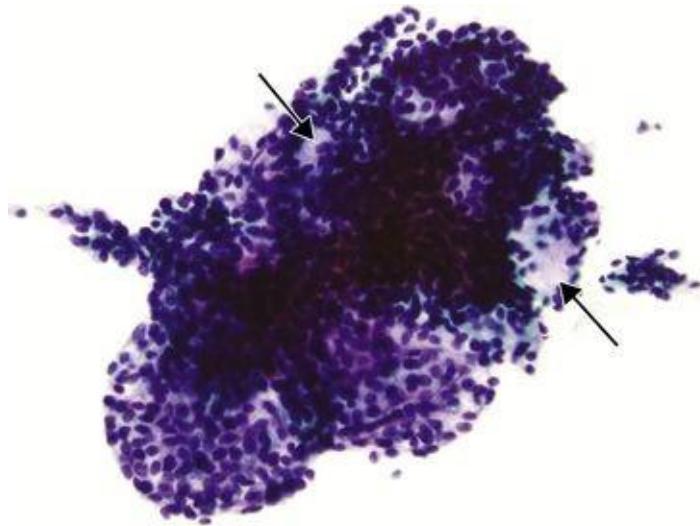


FIGURE 9.44 Adenoid cystic carcinoma.

Hyaline globules (arrows) are somewhat more prominent within aggregates of cells. They stain pale green with Papanicolaou stain (ThinPrep).

- p63 stains tumor cells in 85% of cases of adenoid cystic carcinoma ([Fig. 9.45](#))²⁵⁰

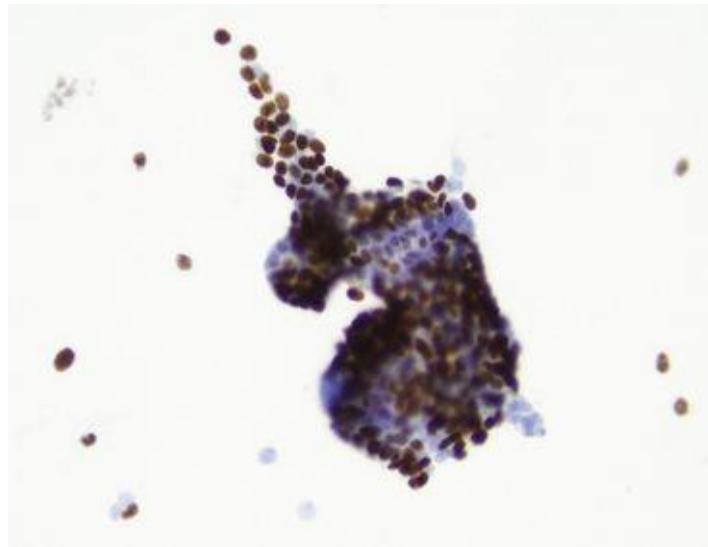


FIGURE 9.45 Adenoid cystic carcinoma.
Nuclear immunoreactivity for p63 is a useful adjunct test in considering this diagnosis (ThinPrep).



Differential diagnosis of adenoid cystic carcinoma

- collagenous spherulosis
- adenomyoepithelioma

Similar globules are seen in collagenous spherulosis associated with benign ductal hyperplasia.^{247,251–253} Adenomyoepithelioma is a rare tumor composed of myoepithelial cells surrounding small epithelium-lined spaces. The cells of adenomyoepithelioma are arranged in tightly cohesive clusters with scant stromal material, but lack the typical hyaline globules of adenoid cystic carcinoma.^{254,255}

Non-Hodgkin Lymphoma

Non-Hodgkin lymphoma can involve the breast either as a primary neoplasm or secondary to systemic disease that also involves lymph nodes. A majority of cases exhibit a B-cell phenotype, and the most common subtypes are diffuse large B-cell lymphoma, follicular lymphoma, and lymphomas of mucosa-associated lymphoid tissue (MALT).²⁵⁶ The cytologic features are identical to those of lymphomas that arise in lymph nodes. The use of flow cytometry can

improve subclassification significantly.^{[257,258](#)} Rarely, plasmacytoma or myeloma can involve the breast.^{[259,260](#)}



Cytomorphology of non-Hodgkin Lymphoma

- isolated cells
- lymphoglandular bodies (best seen with Romanowsky stains)
- monomorphic, atypical cells, often with irregular nuclear contours and a prominent nucleolus



Differential diagnosis of non-Hodgkin lymphoma

- chronic mastitis
- intramammary lymph node
- lactating adenoma

Sarcoma

Primary sarcomas of the breast are rare and include angiosarcoma, liposarcoma, osteosarcoma, leiomyosarcoma, and rhabdomyosarcoma. The most common is angiosarcoma.^{[256,261](#)} Cytomorphologic features recapitulate those of tumors arising in soft tissue.^{[239,262-266](#)}



Cytomorphology of angiosarcoma (Fig. 9.46)

- isolated cells and clusters
- hyperchromatic nuclei
- coarsely granular chromatin
- nucleolus often prominent
- blood or hemosiderin may be present

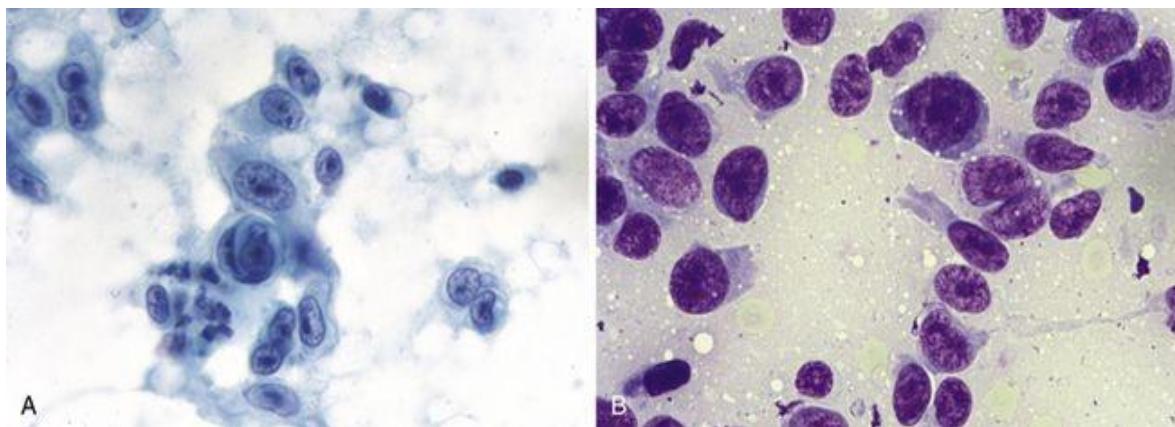


FIGURE 9.46 Postradiation angiosarcoma.

A, Very atypical isolated cells are noted, some with a cell-in-cell pattern (Papanicolaou stain).
B, The cells are round to spindle-shaped, with an eccentric nucleus and prominent nucleolus (Romanowsky stain.) (Case courtesy Dr. James E. Orr, formerly at A.O. Fox Memorial Hospital, Oneonta, NY; currently at Resurrection Medical Center, Chicago, IL.)



Differential diagnosis of angiosarcoma

- phyllodes tumor
- metaplastic carcinoma

Metastatic Tumors

A wide range of nonmammary tumors can metastasize to the breast, and in some cases the primary tumor is occult.²⁶⁷ Common types include lymphoma; melanoma; carcinomas of the lung, ovary, prostate, kidney, and stomach; and carcinoid tumors.^{119,268} A metastasis should be considered whenever the patient has a known extramammary malignancy or when cytologic findings are not typical of breast carcinoma (Figs. 9.47 and 9.48). If there is a history of a primary tumor elsewhere, comparison with slides from a prior specimen is often helpful.

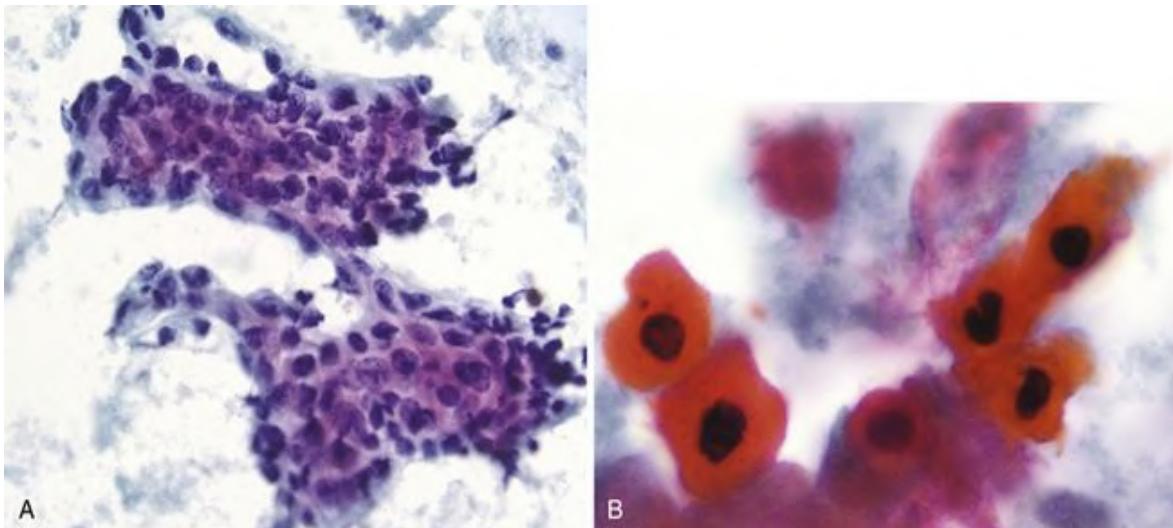


FIGURE 9.47 Metastatic squamous cell carcinoma (SQC).

A, The loosely cohesive aggregate of markedly atypical cells resembles a poorly differentiated breast carcinoma (Papanicolaou stain). *B*, Malignant keratinized squamous cells raise the possibility of an extramammary tumor. This was a metastasis from a squamous carcinoma of the vulva (Papanicolaou stain).

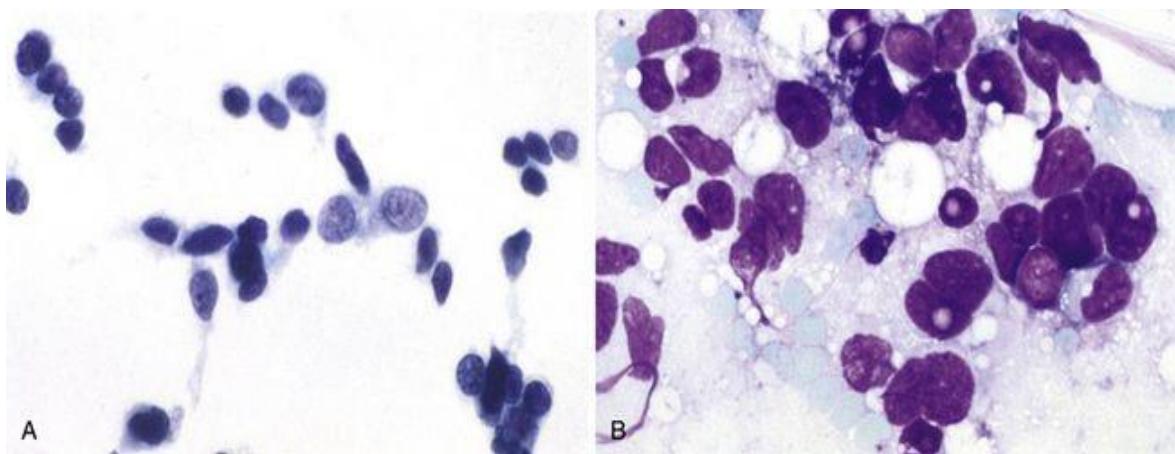


FIGURE 9.48 Metastatic small cell carcinoma of the lung.

Metastatic small cell carcinoma might be mistaken for lobular carcinoma, but there is greater nuclear atypia and pleomorphism, less cytoplasm, and very pronounced nuclear molding (*A*, Papanicolaou stain; *B*, Romanowsky stain).

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CHAPTER 10

Thyroid

Edmund S. Cibas

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Parathyroid Tumors

The main indication for fine-needle aspiration (FNA) of the thyroid is a thyroid nodule. The prevalence of thyroid nodules depends on how carefully one looks for them. Palpable nodules are found in 4% to 7% of adults,^{1,2} but the prevalence is much higher (20% to 70% of adults) when nonpalpable nodules are included, like those detected by ultrasonography or at autopsy.³⁻⁶

Although a thyroid nodule raises the suspicion of cancer, less than 5% are malignant.^{4,7} Given the high prevalence of nodules, combined with the impracticality of surgically excising all nodules, FNA plays a vital role as a screening test. Few cytology tests have so effectively decreased unnecessary surgery while increasing the yield of malignancy.⁸⁻¹⁰ At the Mayo Clinic, the percentage of patients requiring thyroid surgery dropped from 67% to 43% 1 year after FNA was introduced (around 1980); the percentage of excised nodules that were malignant rose from 14% to 29%; and the cost of a workup of a thyroid nodule decreased by 25%.⁹ Since then, the reduction in unnecessary surgery has been even greater: The percentage of excised nodules that are malignant is now 45% to 56%.^{11,12}

Every patient with a palpable thyroid nodule is a candidate for FNA. Palpation is not always accurate in assessing the presence and extent of thyroid nodularity, however. After ultrasound examination, 20% of patients with a palpable thyroid nodule prove not to have a nodule greater than 1 cm, and conversely, additional significant nodules are often found that were not appreciated on palpation.¹³ For this reason, a thyroid ultrasound is often obtained before (or at the time of) the FNA to confirm that the palpated nodule meets biopsy criteria.¹⁴

FNA can be avoided in patients who have a hyperfunctioning (“hot”) nodule (about 5% of all nodules) by also obtaining a serum thyrotropin (TSH) level.¹⁴ If the TSH level is normal or elevated, an FNA is usually performed. But if the TSH level is depressed, a radionuclide thyroid scan is obtained. If the scan confirms that the nodule is indeed hot, an FNA is not indicated because a hot nodule is very rarely malignant.^{15,16}

An increasing number of thyroid nodules are being detected incidentally on imaging studies of the neck (thyroid “incidentalomas”). The various imaging modalities include ultrasound (for carotid artery disease), sestamibi scans (for hyperparathyroidism), computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET). It is not practical or advisable to perform an FNA on all incidentalomas. Evidence suggests that PET-avid

thyroid nodules carry a high risk of malignancy and should undergo FNA. Nodules detected by CT, MRI, and sestamibi, however, should first undergo a dedicated ultrasound examination. Nodules less than 1.0 cm in maximum diameter on the ultrasound image are usually not biopsied unless they have sonographically suspicious features (e.g., microcalcifications).^{14,17}

Aspiration Technique and Slide Preparation

The aspiration can be guided either by palpation or ultrasound. The benefits of palpation guidance include its reduced cost and logistical efficiency. Ultrasound guidance permits the operator to be certain that the nodule of interest is aspirated ([Fig. 10.1A](#)), reduces the number of unsatisfactory FNA specimens, and improves accuracy.^{14,18–20} For these reasons, ultrasound guidance is preferred for nonpalpable nodules, nodules that have a significant cystic component (greater than 25%), and nodules that were previously aspirated and yielded an unsatisfactory sample.¹⁴

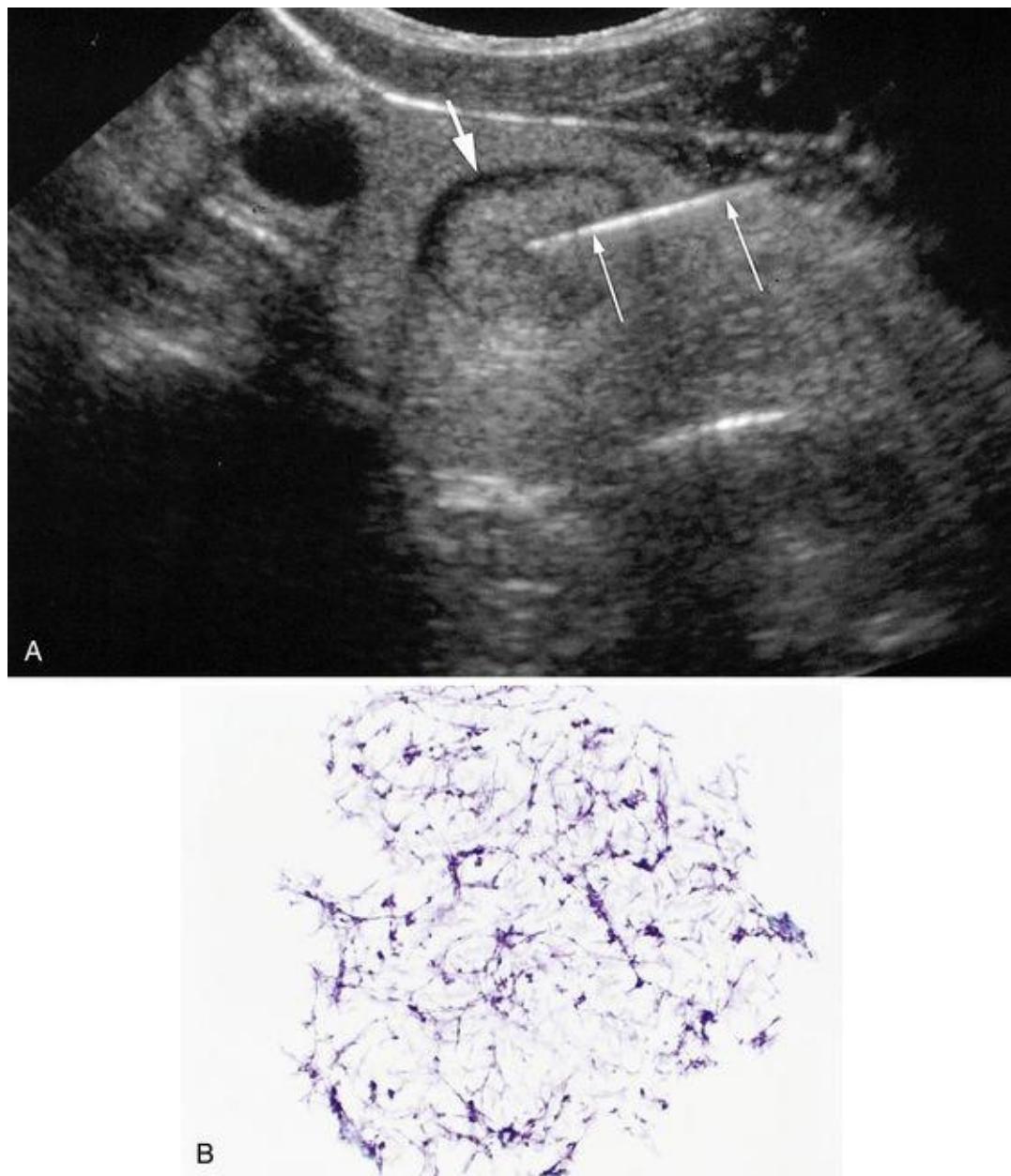


FIGURE 10.1 A, Transverse sonogram of a thyroid nodule. The tip of a needle (*thin arrows*) is in the middle of a nodule (*large arrow*). (Courtesy Dr. Carol Benson, Brigham and Women's Hospital, Boston, Massachusetts.) B, Ultrasound gel. Note its purple, meshlike or spider web–like appearance. If not wiped off the skin before aspiration, it can cover large areas of the slide surface and interfere with cellular evaluation (Papanicolaou stain).

The aspiration technique is essentially the same whether palpation or ultrasound is used for guidance. A very fine (25 or 27gauge) needle is ideal for most thyroid nodules, because the thyroid gland is very vascular.²¹ Depending on the circumstances and operator preference, the aspiration can be performed with or without suction applied by a syringe. Local anesthesia obtained by

subcutaneous lidocaine injection is commonly used but is optional.²¹ The skin is wiped clean with an alcohol swab. If ultrasound guidance is used, the needle should not pass through gel, which, when aspirated, obscures cytomorphology (Fig. 10.1B).²¹ The dwell time of each pass should be 2 to 5 seconds, with rapid back-and-forth oscillations of the needle (three per second) within the nodule.²¹ If suction is used, it should be released before the needle is withdrawn from the nodule. The number of passes needed to ensure adequacy varies. If a cytotechnologist or pathologist is called to evaluate the specimen for adequacy, one or two passes may be sufficient. On-site evaluation is time-consuming,²² however, and many aspirations that are performed in outpatient settings are too far removed from a laboratory for such evaluation. Most practitioners recommend between two and six passes for each nodule aspirated.^{13,21,22} Even minor complications such as a transient hematoma are uncommon; site infections are almost never seen. Post-FNA infarction of the nodule occurs to some degree in up to 10% of cases.²³ Other histologic alterations in the remaining gland include pseudoinvasion, vascular proliferation, and cytologic atypia, but these are usually easily recognized by virtue of their proximity to the linear biopsy tract.²⁴ Serious complications like needle tract seeding are virtually nonexistent.

Slides are prepared by expelling and smearing the cells on a slide. Alternatively, or as an adjunct to smears, the needle is rinsed, and the resulting cell suspension used for cytocentrifuge, thinlayer, and/or cell block preparations. The advantages of thinlayer ("liquid-based") preparations over smears include reduced blood; ease in preparation of consistently well-fixed slides, particularly when the FNA is not performed by a pathologist; and a concentrated specimen that requires less screening time. When liquid-based preparation is used, one slide is usually sufficient.²⁵ Architectural features (macrofollicles, microfollicles) are retained,²⁶ and adequacy rates and accuracy are similar for smears and thinlayer slides.²⁷⁻³¹ Some adjustment to minor morphologic alterations with liquid-based preparations is needed. For example, chronic lymphocytic thyroiditis is more subtle on thinlayer preparations because the lymphoid cells are intermingled with contaminating blood leukocytes.²⁸

Thinlayer and cytocentrifuge preparations are stained with the Papanicolaou stain. Smears can be alcohol-fixed and Papanicolaou-stained or air-dried and stained with a Romanowsky-type stain. Although most cytologists prefer one over the other, it is helpful to be familiar with both stains. Nuclear features such as inclusions, grooves, and especially chromatin texture are better appreciated with the Papanicolaou stain. The Romanowsky-type stains are especially useful for the evaluation of extracellular material, particularly colloid and amyloid, and

for cytoplasmic detail such as granules.

Terminology for Reporting Results

The Bethesda System for Reporting Thyroid Cytopathology has been widely adopted in the United States and abroad for reporting the results of thyroid FNAs.^{22–24} It recommends that every thyroid aspirate interpretation begin with designation of a general diagnostic category ([Table 10.1](#)). Each of the categories has an implied cancer risk and is linked to an evidence-based clinical management guideline: Suspicious and malignant nodules are likely to be resected, whereas patients with a benign result are instructed to return for a followup examination at an appropriate interval. There are six general categories. Three of them come with a choice of two names; ideally, a laboratory chooses one of the options and uses it exclusively for reporting results that fall into that category.

TABLE 10.1
THE BETHESDA SYSTEM FOR REPORTING THYROID CYTOPATHOLOGY

Diagnostic Category	Risk of Malignancy (%)	Usual Management*
Nondiagnostic (or unsatisfactory)	1–4	Repeat fine-needle aspiration with ultrasound guidance
Benign	<1–3	Clinical followup
Atypia of undetermined significance (or follicular lesion of undetermined significance)	~5–15	Repeat fine-needle aspiration
Suspicious for a follicular neoplasm (or follicular neoplasm); specify if Hürthle cell type	15–30	Surgical lobectomy
Suspicious for malignancy	60–75	Surgical lobectomy or thyroidectomy
Malignant	97–99	Near-total thyroidectomy

*Actual management may depend on other factors besides the fine-needle aspiration result.

“Nondiagnostic (ND)” (or “unsatisfactory”) applies to specimens that are unsatisfactory due to obscuring blood, overly thick smears, air-drying of alcohol-fixed smears, or an inadequate number of follicular cells. For a thyroid FNA specimen to be satisfactory for evaluation (and benign), at least six groups of benign, well-visaulized follicular cells are required, each group composed of at least 10 cells.^{35–37} Tissue fragments with multiple follicles can be split up and

counted as separate and distinct groups.³⁸ The minimum size requirement for the groups (at least 10 cells) allows determination (by the evenness of the nuclear spacing) of whether or not they represent fragments of macrofollicles rather than the more worrisome microfollicles. There are several exceptions to this adequacy requirement. A specimen with abundant colloid is adequate (and benign), even if six groups of follicular cells are not identified. A sparsely cellular specimen with abundant colloid is, by implication, a predominantly macrofollicular nodule and therefore almost certainly benign. Also, whenever a specific diagnosis (e.g., Hashimoto thyroiditis [HT]) can be rendered, and whenever there is any atypia, the specimen is considered adequate. ND results occur in 2% to 30% of cases.^{11,39,40}

Specimens that consist only of cyst contents (macrophages) ([Fig. 10.2](#)) are problematic. Many laboratories have traditionally considered a macrophages-only sample unsatisfactory and included them in the ND category. (Because the parenchyma of the nodule has not been sampled, it is not possible to exclude a cystic papillary carcinoma.) In such laboratories, macrophages-only often constituted the great majority of ND cases, with rates that range from 15% to 30%.^{12,40–42} Other laboratories have considered the risk of a false-negative result negligible and reported macrophages-only as benign.^{11,41} In the Bethesda System, cyst fluid–only (CFO) cases are a subset of the ND category. The significance (and clinical value) of a CFO result depends on sonographic correlation. If the nodule is almost entirely cystic, with no worrisome sonographic features, an endocrinologist might proceed as if it were a benign result. In a study that segregated CFO cases and analyzed them separately, the risk of malignancy was 4%.⁴⁰ The risk of malignancy for the ND category (excluding CFO cases) is 1% to 4%.^{11,39,40}

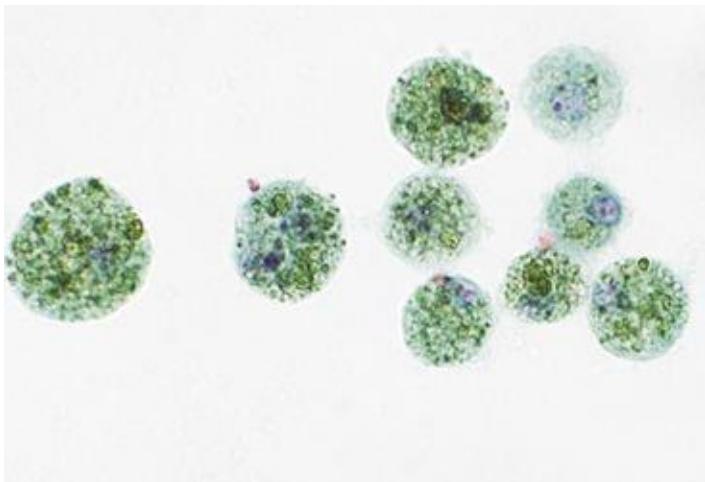


FIGURE 10.2 Macrophages.

These cells have abundant cytoplasm that may be filled with hemosiderin or red blood cell fragments. They are a nonspecific finding, seen in both benign and malignant thyroid nodules (Papanicolaou stain).

A repeat aspiration with ultrasound guidance is recommended for ND cases.⁴³ The repeat FNA is diagnostic in 50% to 88% of cases,^{12,35,40,41,44,45} but some nodules remain persistently nondiagnostic. Because about 10% of persistently nondiagnostic nodules are malignant,⁴⁴ excision is often considered.

A benign result is obtained in about 70% of thyroid FNAs. The most common benign specimen is the benign follicular nodule. The false-negative rate for a benign interpretation is very low (less than 1% to 3%), but patients nevertheless should undergo repeat assessment by palpation or ultrasound at 6-to 18-month intervals.^{43,46} If the nodule shows significant growth or suspicious sonographic changes, a repeat FNA is considered.⁴³

About 10% to 15% of cases are classified as “suspicious for a follicular neoplasm”; “suspicious for a follicular neoplasm, Hürthle cell type”; or “suspicious for malignancy.”^{11,12,32,42} Although virtually all patients with specimens classified as suspicious are referred for surgery, the predictive value for malignancy is different for these subcategories (see [Table 10.1](#)). The cases that fall into the “suspicious for malignancy” category are further subclassified as “suspicious for papillary carcinoma,” “suspicious for medullary carcinoma,” “suspicious for metastatic carcinoma,” “suspicious for lymphoma,” or other.⁴⁷

Approximately 3% to 7% of thyroid FNA specimens are interpreted as malignant, most as papillary thyroid carcinoma (PTC).^{11,12,41,42}

Accuracy

The accuracy of a benign thyroid FNA result is difficult to establish, because most patients with a benign result do not have surgery. Those who do undergo surgery represent a highly selected population of patients who have worrisome symptoms, larger nodules, or nodules exhibiting substantial growth. Nevertheless, data indicate that for FNA performed by experienced operators, a benign result is highly reliable. In a long-term followup study of 439 patients with benign cytology at the Mayo Clinic, only three proved to have a malignancy, for a false-negative rate of 0.7%, and none of the patients died of their disease.³⁶ Current estimates place the false-negative rate between less than 1% and 3%.³² The most common cause of a false-negative is papillary thyroid carcinoma, followed by follicular carcinoma.^{12,36,41,48} Errors are due in equal part to sampling and interpretation.⁴⁸

A malignant interpretation is likewise highly reliable. Experienced practitioners have false-positive rates of 1% to 3%,^{12,42,49} although higher rates have been reported.⁴¹ The most common benign tumors to cause a false-positive result are follicular adenoma (including the follicular adenoma with papillary hyperplasia) and hyalinizing trabecular tumor.^{12,41,49,50}

Ancillary Molecular Testing

The three categories “atypia of undetermined significance (AUS),” “suspicious for a follicular neoplasm (including the Hürthle cell type),” and “suspicious for malignancy” are often referred to by clinicians as the “indeterminate” thyroid FNA categories. Although they are associated with reasonably well defined malignancy risks and management guidelines (see [Table 10.1](#)), they have inspired the development of molecular tests to triage patients more effectively for conservative versus surgical management and to further reduce unnecessary surgery for patients who have a benign nodule. Results suggest that molecular testing, particularly in AUS cases, can play a role similar to that of reflex human papillomavirus (HPV) testing for a woman with an atypical Pap test.

Among the most promising single markers is BRAF. A BRAF mutation is found in 44% of papillary thyroid carcinomas and virtually never in benign thyroid nodules.⁵¹ Testing AUS nodules for selected genetic markers of thyroid cancer—point mutations in *BRAF* and *(H,K,N)RAS* and gene rearrangements in *RET/PTC* and *PAX/PPAR γ* —has a negative predictive value of 94% and positive predictive value of 88%⁵² and is available commercially as the Inform Thyroid Panel (Asuragen Inc., Austin, TX). A gene expression classifier (the Afirma test, Veracyte, Inc., San Francisco, CA) has been developed to optimize the detection of benign nodules. The Afirma test was evaluated in a prospective, multicenter study at 49 clinical trial sites and found to have a negative predictive value of 95% for AUS, 94% for suspicious for a follicular neoplasm, and 85% for suspicious for malignancy.⁵³ It has been estimated that the test could allow up to two thirds of patients with indeterminate FNA results to safely avoid surgery.⁵⁴

Evaluation of the Specimen

Evaluation of the specimen begins with a review of the slide(s) under scanning magnification, which quickly provides a wealth of information. Most benign follicular nodules are sparsely cellular, consisting predominantly of colloid. *Colloid* can be very thin and translucent (“watery”); thick and opaque, with sharp outlines; or extremely thick and sticky (“bubble gum” colloid). Smears that have a high ratio of colloid to follicular cells generally indicate a benign thyroid nodule. The benign nature can be confirmed by documenting a predominance of intact macrofollicles and macrofollicle fragments (flat sheets comprised of evenly spaced follicular cells).

Neoplasms, on the other hand, are usually highly cellular specimens notable for significant *architectural atypia*, with cell crowding and overlap, and the formation of abnormal arrangements like microfollicles, trabeculae, or papillae. Microfollicles are small circles of crowded follicular cells. A predominantly microfollicular pattern is suspicious for a follicular neoplasm or a follicular variant of papillary carcinoma. Papillae—abnormal cells surrounding a fibrovascular core, often with a branching pattern—are highly characteristic of papillary thyroid carcinoma.

Examination of the slide under high magnification is important, particularly for the *nuclear changes* of papillary thyroid carcinoma, which at times are subtle and incompletely displayed.

Hürthle cells (also called *oncocyes* or *oxyphilic cells*) are metaplastic follicular cells characterized by abundant mitochondria. Why follicular cells transform into Hürthle cells is poorly understood, but they have a striking appearance on cytologic preparations: polygonal, with abundant cytoplasm that is finely granular and green or orange with the Papanicolaou stain, and smooth and pale purple with Romanowsky-type stains. Nuclei are enlarged and sometimes pale, and nucleoli can be inconspicuous or prominent.

A predominantly *noncohesive (isolated) cell pattern* is a nonspecific finding but is almost never seen in benign follicular nodules or papillary carcinoma. Instead, it is common in lymphocytic thyroiditis, medullary carcinoma, Hürthle cell neoplasms, poorly differentiated carcinoma, undifferentiated (anaplastic) carcinoma, and lymphoma.

Too much emphasis should not be placed on a single cytologic finding. Many features that are highly characteristic of some neoplasms can be seen sporadically in other conditions. Intranuclear pseudoinclusions, nuclear grooves,

and even psammoma bodies—characteristic features of papillary carcinoma—are occasionally encountered in other conditions. Some features are entirely nonspecific: *Multinucleated giant cells* are seen in subacute thyroiditis, other granulomatous diseases, benign follicular nodules with cystic degeneration, papillary carcinoma, and undifferentiated (anaplastic) carcinoma.

Rarely, a thyroid FNA sample is contaminated by nonthyroid cells. Accidental penetration of the larynx or trachea, virtually always announced by a cough reflex, occurs in less than 1% of thyroid FNAs,⁵⁵ and cytologic samples contain *ciliated columnar cells*.

Benign Conditions

The most commonly encountered benign thyroid nodules are follicular cell proliferations—multinodular goiter (MNG) and follicular adenoma—that account for about 70% of thyroid FNAs. Less commonly, benign nodules or pseudonodules are encountered in patients with inflammatory diseases such as Hashimoto and subacute thyroiditis. Some benign conditions (e.g., black thyroid and radiation changes) do not cause nodules, but produce benign cellular alterations that might be confused with malignancy.

Benign Follicular Nodules

The cytologic term *benign follicular nodule* was coined to encompass a group of benign histopathologic entities that have identical cytologic features.⁵⁶ The most common are the nodule in multinodular goiter (MNG) and the macrofollicular type of follicular adenoma.

MNG is a common thyroid disorder characterized by an enlarged thyroid gland (goiter) with multiple areas of nodularity. Worldwide, it is the most common endocrine abnormality, affecting more than 500 million people.⁵⁷ Its prevalence varies depending on regional iodine intake: Lower dietary iodine correlates with an increased prevalence of MNG. In the United States, despite iodine supplementation, the prevalence of MNG is roughly 4% to 7%.

The pathogenesis of MNG is best understood in cases associated with iodine deficiency. Because iodine is required for thyroid hormone synthesis, a deficiency of iodine results in decreased thyroid hormone production and a compensatory elevation in serum TSH levels. Chronically elevated TSH levels stimulate a diffuse follicular cell hyperplasia. Over time, somatic mutations within the follicular cells (possibly as a result of hydrogen peroxide [H_2O_2] production and free radical generation that occurs with thyroid hormone synthesis) confer a survival advantage over selected clones, which results in nodule formation.⁵⁸ The mechanism of follicular cell hyperplasia in iodine-sufficient regions is less well understood but is likely to be related to other stimuli (e.g., smoking, radiation, drugs, naturally occurring goitrogens) superimposed on a genetic susceptibility.⁵⁸

MNG is 5 to 15 times more common in women than in men, and prevalence increases with age. Patients are usually asymptomatic and euthyroid, but 5% to 10% progress to hyperthyroidism (toxic MNG).⁵⁷ Most cases are discovered

incidentally by palpation or imaging studies. MNG varies in severity, from the minimally enlarged, asymptomatic gland with only one or two nodules, to the extremely enlarged nodular gland that extends from the neck into the mediastinum. Patients with large goiters can have compressive symptoms (e.g., shortness of breath, cough, dysphagia), and large goiters can be disfiguring. The growth of nodules is unpredictable and highly variable, but, on average, MNG nodules grow by about 4.5% per year.¹⁶

The treatment of MNG is varied and individualized. Choices include thyroid surgery, ¹³¹I (radioactive iodine) therapy, and TSH suppression with exogenous thyroxine (T_4) administration. Surgery is generally recommended for younger patients and those with a large goiter.¹⁶

Histologically, the nodules of MNG (commonly referred to as *adenomatous* or *adenomatoid nodules*) are usually not encapsulated. The follicles within the nodules vary in size, but most are larger than normal follicles (macrofollicles) and filled with colloid. Some nodules are composed of such enormous macrofollicles that they are virtually all colloid (*colloid nodules*). Less commonly, a nodule may be very cellular, composed of smaller follicles that contain little colloid. The rapid growth of some nodules leads to hemorrhage, scarring, cystic degeneration, and dystrophic calcification. *Uninodular* MNG is a controversial entity, but some histopathologists acknowledge its existence.

Unlike MNG, a *follicular adenoma* is usually a solitary nodule that measures 1 to 3 cm in diameter, but it can be much larger.⁵⁹ The histologic distinction between an adenomatous nodule in MNG and a follicular adenoma is somewhat arbitrary. In general, follicular adenoma is a solitary nodule with a well-defined fibrous capsule, and the follicular cells within the nodule are morphologically distinct from those in the surrounding gland.⁵⁹ Capsular and vascular invasion are absent. Follicular adenomas have a wide variety of predominant histologic patterns: *macrofollicular* (large follicles distended by colloid); *microfollicular* (follicles smaller than normal); and *trabecular* (follicular cells are arranged in crowded ribbons).⁵⁹ Uncommon variants include follicular adenoma with papillary hyperplasia (mostly in children and adolescents), signet ring cell follicular adenoma, mucinous follicular adenoma, lipoadenoma, clear cell follicular adenoma, and follicular adenoma with bizarre nuclei. Only the predominantly macrofollicular follicular adenomas are interpreted as benign follicular nodules by FNA. The others are likely to be interpreted as suspicious because of their unusual architectural patterns.

MNG and macrofollicular follicular adenoma are essentially indistinguishable by FNA, but this is of no clinical consequence. Patients with a benign result are instructed to return for a followup examination at 6-to 18-month intervals for at

least 3 to 5 years to ensure that the nodule is behaving in a benign fashion.^{43,46}



Cytomorphology of a benign follicular nodule

- predominantly macrofollicles
 - fragmented (flat sheets)
 - intact spheres
- low to moderate cellularity
- cohesive cells
- uniform, evenly spaced follicular cells
- coarse chromatin
- colloid (abundant and often watery, but not always visible)
- Hürthle cells (usually a minor component)
- macrophages and cyst lining cells (in cases of cystic degeneration)

By definition, *benign follicular nodule* applies to a sample that is adequate for evaluation and consists predominantly of colloid and benign-appearing follicular cells in varying proportions. The benign follicular nodule has a predominantly macrofollicular architecture. During aspiration and slide preparation, most macrofollicles break into fragments; intact macrofollicles are relatively uncommon ([Fig. 10.3A](#)). Instead, one commonly sees flat sheets that represent fragments of macrofollicles ([Fig. 10.3B](#)). A macrofollicle fragment is defined by its flatness and the even spacing of follicular cell nuclei. (If there is conspicuous cell crowding and overlapping, the sheet is not a macrofollicle fragment.) Although the term *macrofollicle fragment* implies large size, and many are indeed large, some macrofollicle fragments are small (10 or even fewer cells). A macrofollicular pattern is defined by architecture and not size. Evenly spaced follicular cells (not crowded or overlapped) constitute a macrofollicle fragment, no matter the size of the cell sheet.

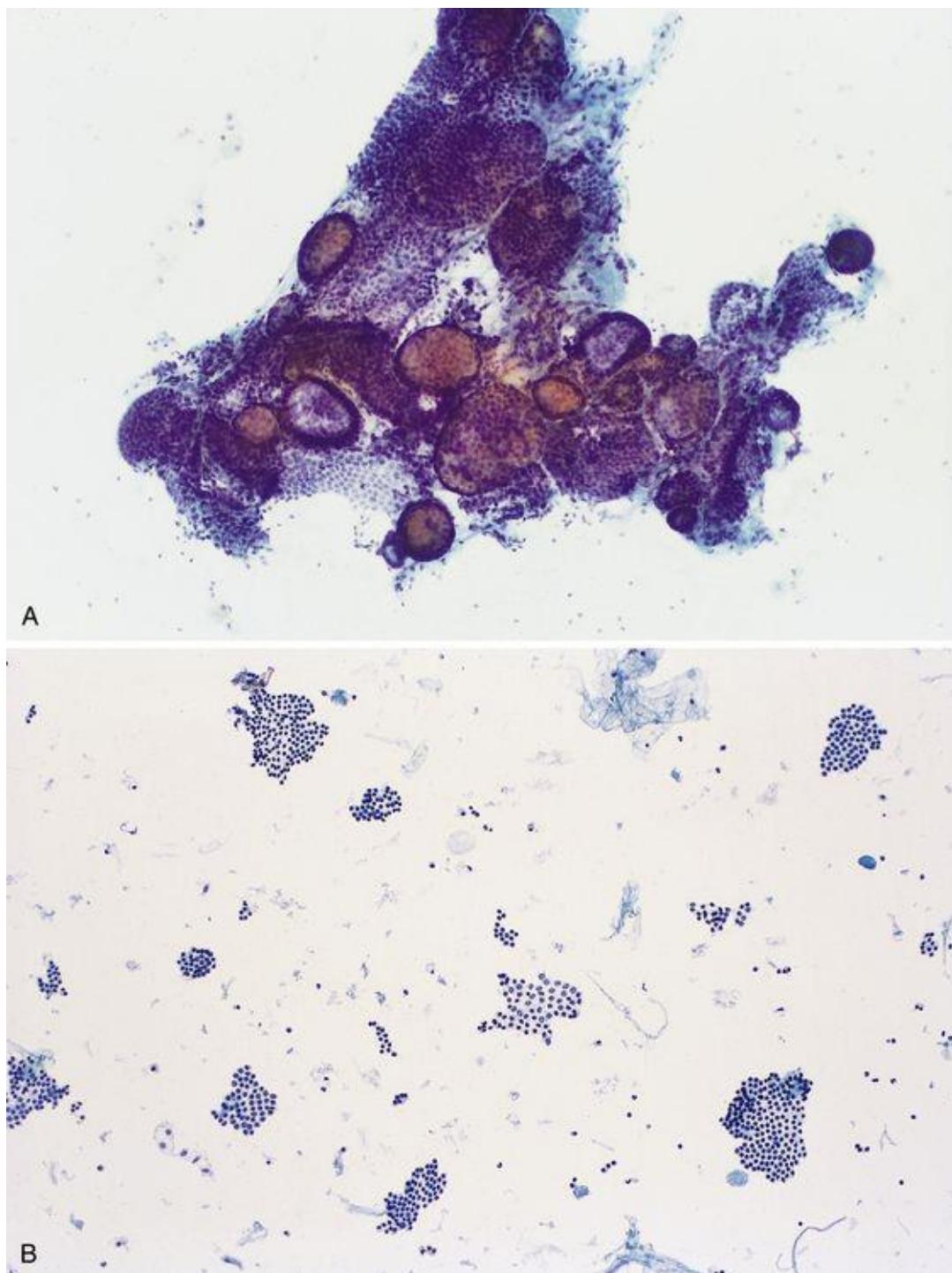


FIGURE 10.3 Benign follicular nodule.

A, The FNA sample contains small tissue fragments containing intact macrofollicles (Papanicolaou stain). B, Macrofollicles often break into fragments and appear as sheets of various sizes. Although colloid is scant in this case, a predominantly macrofollicular architecture can be inferred from these fragments (Papanicolaou stain).

The macrofollicle fragment is made up of medium-sized cells, each with a

round nucleus, coarsely granular chromatin, an inconspicuous nucleolus, and scant or a moderate amount of cytoplasm ([Fig. 10.4A](#) and [B](#)). Cytologic atypia is generally absent, but some nuclear size variation can be seen, and in some cases the nucleoli are moderate in size. Some follicular cells contain *hemosiderin pigment*, which is golden brown with the Papanicolaou stain and blue with Romanowsky-type stains.⁶⁰ In a benign follicular nodule, finding a minority of the follicular cells arranged in microfollicles or crowded groups is acceptable.

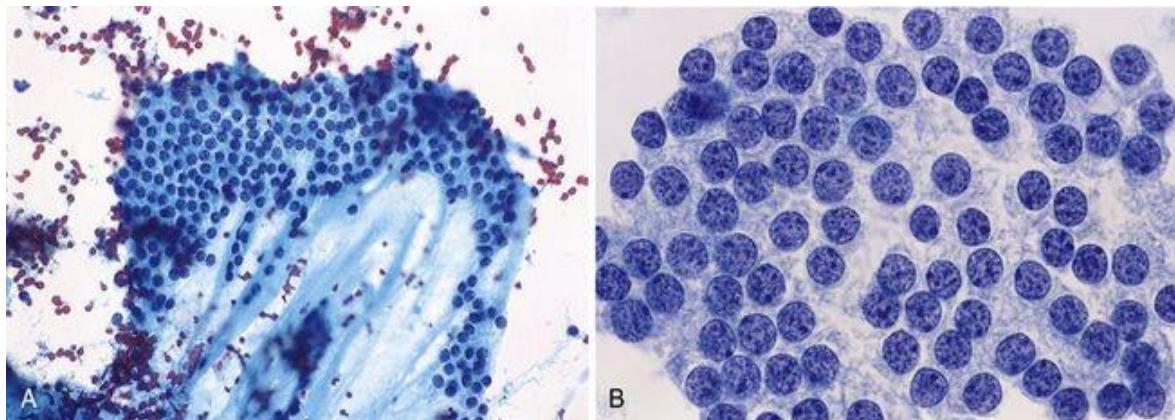


FIGURE 10.4 Benign follicular nodule.

A, The follicular cells comprising this unfolded macrofollicle are evenly spaced. Pale green-blue colloid has escaped from the follicle (Papanicolaou stain). B, Normal follicular cells have coarsely granular chromatin, and cytoplasm can be scant or moderately abundant (Papanicolaou stain).

Colloid is usually abundant and can take the appearance of dense blobs with a hyaline texture, hard edges, and cracking artifact ([Fig. 10.5](#)). These thick colloid chunks have a similar appearance on smears and liquid-based preparations. More often, colloid is mostly thin and watery, covering large areas of the smear as a translucent film with bubbles, folds, cracks, and a circular “chicken wire” artifact ([Fig. 10.6A](#)). On liquid-based preparations, this watery colloid has a characteristic “folded tissue paper” appearance²⁶ ([Fig. 10.6B](#)). Colloid stains pale pink, pale green, or orange with the Papanicolaou stain, and magenta with Romanowsky-type stains.



FIGURE 10.5 Colloid—benign follicular nodule.

Colloid can congeal into opaque, irregularly shaped hyaline chunks with hard edges that are clearly visible on smears and liquid-based preparations (Papanicolaou stain).

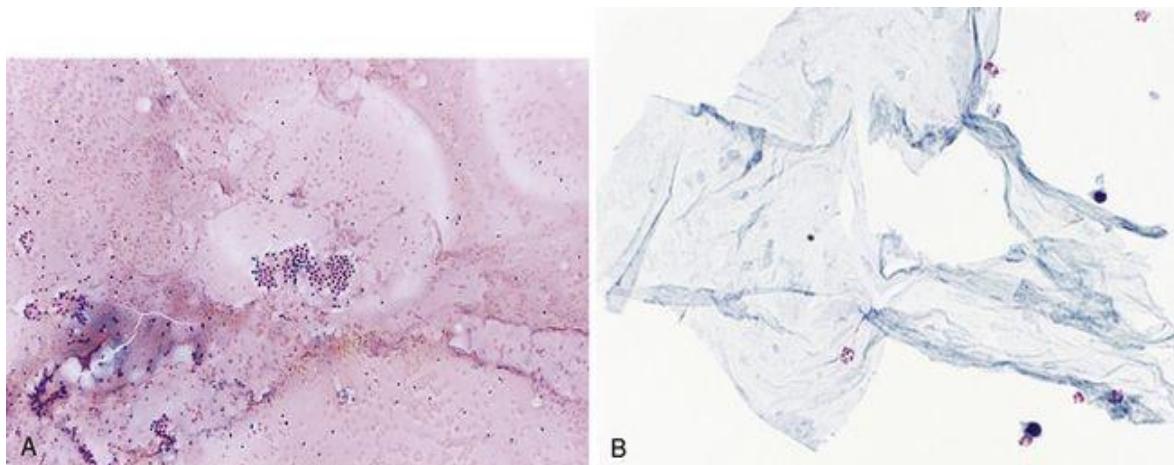


FIGURE 10.6 Colloid—benign follicular nodule.

A, On smears, watery colloid often covers the entire slide as a thin, translucent film (pink in this image) and is often admixed with blood (Papanicolaou stain). B, On liquid-based preparations, watery colloid has a transparent, tissue-paper appearance with numerous linear folds (Papanicolaou stain).

Because most of the volume of a benign follicular nodule is occupied by colloid and not follicular cells, cytologic preparations from them tend to be sparsely cellular. But exceptions occur, and a minority are moderately cellular, with numerous macrofollicle fragments.

Hürthle cells are seen in up to 50% of cases of MNG⁶⁰ (Fig. 10.7). Macrophages with foamy or hemosiderin-laden cytoplasm, including

multinucleated giant cells, are often present and are a nonspecific finding indicating cystic degeneration⁶⁰ (see [Fig. 10.2](#)). A minor population of elongated, large “cyst lining cells” with pale and grooved nuclei are sometimes present. Because of their streaming (“tissue culture”) arrangement, they resemble reparative epithelial changes and likely represent altered follicular cells lining areas of cystic degeneration⁶¹ ([Fig. 10.8](#)).

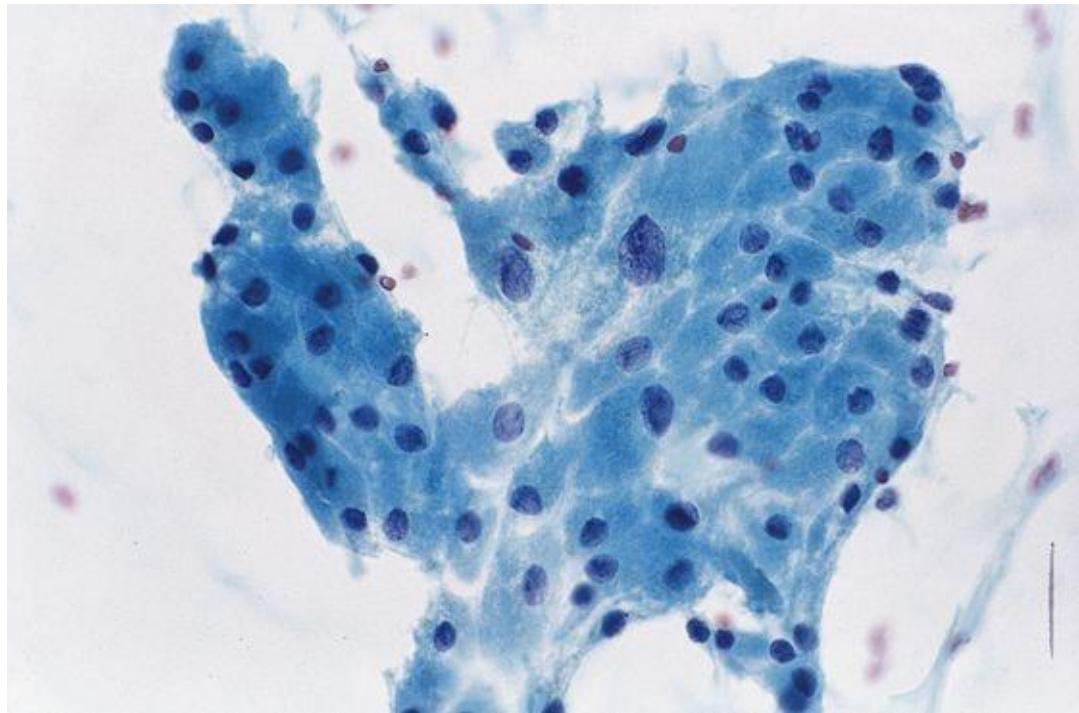


FIGURE 10.7 Focal Hürthle cell metaplasia, multinodular goiter (MNG). Hürthle cells are a common finding in some cases of MNG. The cells are large and polygonal and have abundant, finely granular cytoplasm. Variation in nuclear size, as seen here, is common in nonneoplastic Hürthle cell proliferations and does not signify neoplasia (Papanicolaou stain).

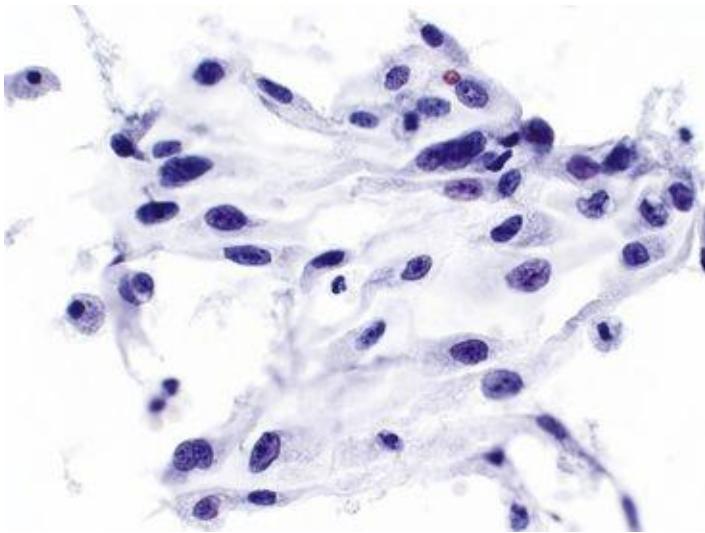


FIGURE 10.8 Cyst lining cells.

Reactive follicular cell changes are often seen adjacent to areas of cystic degeneration. They typically have a pulled-out appearance that mimics reparative epithelium (Papanicolaou stain).



Differential diagnosis of a benign follicular nodule

- suspicious follicular nodule
- suspicious Hürthle cell nodule
- papillary thyroid carcinoma

The diagnosis of a benign follicular nodule can be made confidently when there is a mostly orderly macrofollicular architecture. Follicular carcinomas are virtually never composed of orderly macrofollicles, but instead have a preponderance of microfollicles and/or trabeculae of crowded, overlapping cells.

A minor population of crowded follicular cells and/or microfollicles is often present in benign follicular nodules and does not alter the benign interpretation. If macrofollicles (intact and/or fragmented) outnumber the crowded, microfollicular/trabecular groups, the findings are consistent with a benign nodule. Follicular cells trapped in fibrin clots are often disrupted and architecturally distorted, conferring a crowded appearance that may potentially lead to an erroneous suspicious interpretation. An interpretation should not be rendered on the basis of the presence of clotted follicular cells.⁶²

It is important to note that not all MNGs and follicular adenomas are predominantly macrofollicular. Some nodules in MNG and some follicular adenomas are comprised predominantly of microfollicles and/or trabeculae and

are inevitably interpreted as suspicious by FNA. This is a well-known limitation. Of note, morphometry and image analysis have not demonstrated sufficient reliability for classifying individual cases.^{63–66} Although some separation of MNG, follicular adenoma, and carcinoma is possible,⁶⁷ these methods are not sufficiently accurate to be useful for clinical diagnosis.⁶⁸ Neither is DNA quantitation by flow cytometry useful, because 25% of follicular adenomas are aneuploid.^{69–72} Immunohistochemistry for MIB-1 is of limited value because there is significant overlap in the proliferative activity between benign and malignant follicular nodules.⁷³ Early reports on the application of immunohistochemistry for galectin-3 appeared promising,⁷⁴ but subsequent studies have not been able to demonstrate reliable results for FNA. An understanding of the underlying genetics of these diseases may eventually provide the key to more accurate distinction, and encouraging inroads have been made. The translocation t(2;3) (q13;p25), which results in a *PAX8-PPAR γ* gene fusion, is a relatively specific marker of follicular carcinoma that is virtually never present in follicular adenoma, MNG, or papillary carcinoma, but it has low sensitivity (26% by fluorescence *in situ* hybridization [FISH]).⁷⁵

Focal cytologic atypia is present in some benign nodules and may present diagnostic difficulties. Hürthle cell (oncocytic) metaplasia is common in MNG, and the Hürthle cells sometimes show marked nuclear atypia. Such changes raise the possibility of a Hürthle cell neoplasm. If Hürthle cells are admixed with ordinary follicular cells arranged in macrofollicles, particularly if there is abundant colloid, it is appropriate to interpret the findings as benign. By contrast, Hürthle cell neoplasms yield an exclusive population of oncocytic cells. A suspicious interpretation should be rendered only when the sample is composed exclusively (or virtually exclusively) of Hürthle cells, particularly when many isolated cells are noted.⁷⁶

The flat sheets of fragmented macrofollicles are reminiscent of the flat sheets of papillary thyroid carcinoma. The distinction between a benign nodule and papillary carcinoma depends primarily on careful examination of nuclear features. The distinction can be difficult in some cases, particularly with the macrofollicular variant of papillary carcinoma, in which the nuclear changes are often subtle and focal.^{77,78} Besides differences in the nuclei, the malignant cells of papillary carcinoma are arranged more haphazardly, with more crowding, than the follicular cells of benign nodules. In rare cases, macrophages in MNG can aggregate, and their large pale nuclei might be mistaken for papillary carcinoma.⁷⁹

The large, pale nuclei of cyst lining cells bear a resemblance to the cells of papillary carcinoma. When the nuclear changes are mild, and the great majority

of the sample looks benign, the benign, reactive nature of these cells is easily recognized. In some cases, nuclear atypia is marked and papillary carcinoma cannot be excluded. Depending on the degree of atypia, such cases are reported as AUS or even “suspicious for PTC.”⁶¹

Chronic Lymphocytic (Hashimoto) Thyroiditis

Chronic lymphocytic (Hashimoto) thyroiditis (HT) is a common autoimmune disease in which thyroid follicles are destroyed by a marked lymphoid infiltrate. Almost 95% of HT occurs in women, with a peak incidence between the ages of 40 and 60 years. The disease results in a diffuse, painless goiter with or without nodularity. Ultrasonography reveals a gland with a heterogeneous appearance. Most patients are hypothyroid. Histologic examination reveals an intense, patchy lymphoid infiltrate with germinal centers, follicular atrophy, and Hürthle-cell metaplasia. Fibrosis is prominent in a minority of cases. The diagnosis is established by correlating clinical findings with serologic test results. One or more of a variety of circulating autoantibodies are identified in almost all patients. The most common are anti thyroglobulin and anti thyroid peroxidase (TPO). (TPO was originally called the *microsomal antigen*.) In a patient with HT, FNA is performed only if there is a suspicious nodule that raises the possibility of a coexisting malignancy.



Cytomorphology of chronic lymphocytic (Hashimoto) thyroiditis

- mixed population of lymphocytes
- tingible-body macrophages
- lymphohistiocytic aggregates
- Hürthle cells ± follicular cells
 - occasional follicular/Hürthle cells with mild nuclear pallor and occasional grooves
- scant colloid

The aspirate is usually very cellular, with numerous lymphoid cells, including small lymphocytes, centrocytes, centroblasts, and dendritic cells ([Fig. 10.9A and B](#)). With liquid-based preparations, the dispersed lymphoid component is

admixed with the white blood cells from contaminating blood. Dendritic-lymphocytic aggregates (germinal center fragments containing dendritic cells) are also present, as are tingible-body macrophages. Colloid is usually absent or scant. Normal follicular cells are infrequent or absent altogether; instead, there may be occasional clusters of Hürthle cells ([Fig. 10.9C](#)). The Hürthle cells and any residual follicular cells occasionally display focal chromatin clearing and nuclear grooves. Multinucleated giant cells can be present but are usually not as numerous as in subacute thyroiditis.

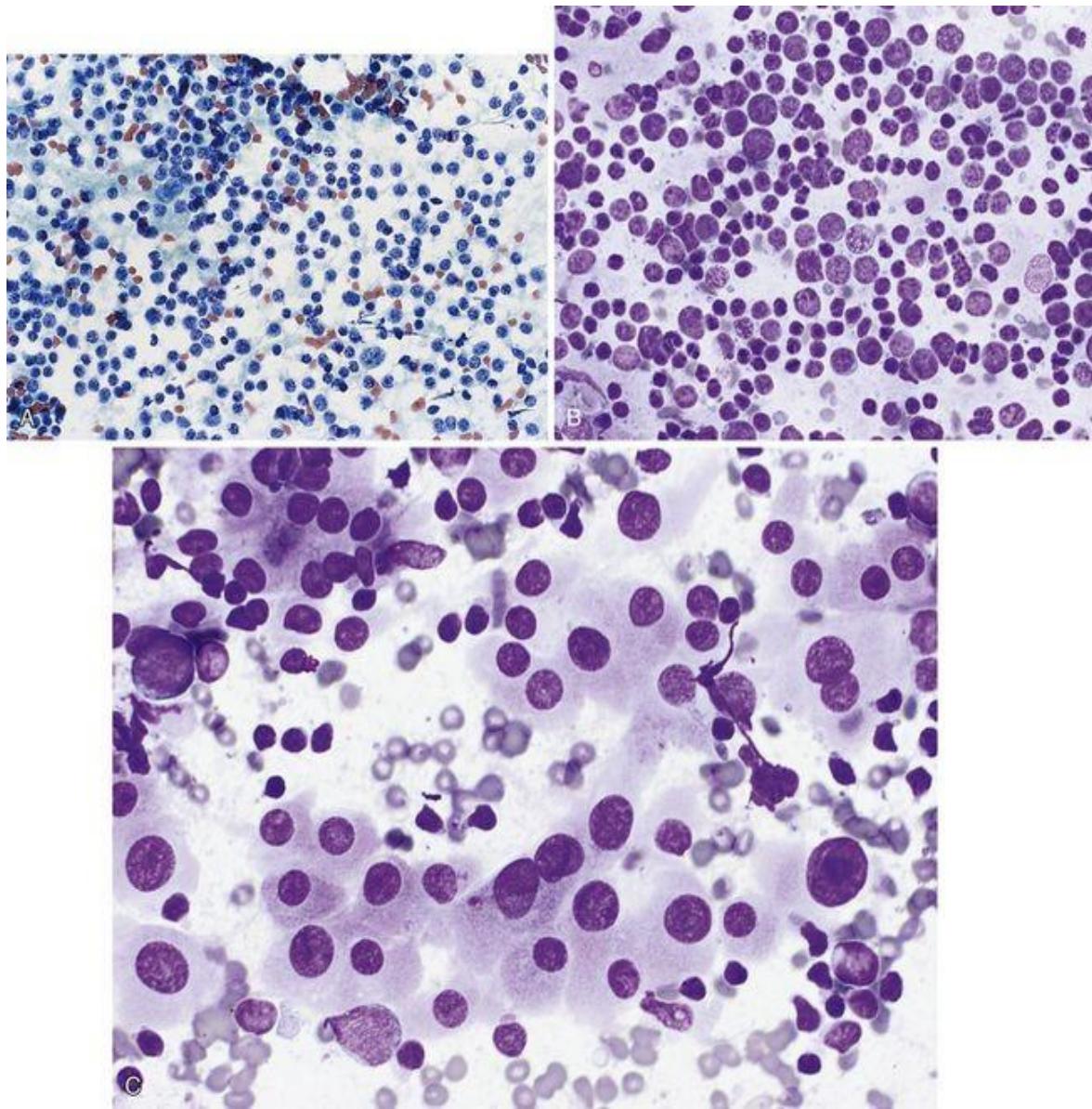


FIGURE 10.9 Chronic lymphocytic (Hashimoto) thyroiditis (HT).

A, Lymphoid cells are the predominant feature. Most are small, mature lymphocytes (Papanicolaou stain). *B*, The heterogeneity of the lymphoid cells is better appreciated on air-dried preparations (Romanowsky stain). *C*, Hürthle cells with abundant cytoplasm are usually identified in clusters in HT. Note the scattered lymphoid cells (Romanowsky stain).



Differential diagnosis of chronic lymphocytic (Hashimoto) thyroiditis

- reactive lymph node
- MNG with prominent Hürthle cell change
- primary thyroid lymphoma
- Hürthle cell neoplasm
- papillary carcinoma

The cytologic diagnosis of HT is usually straightforward and is confirmed clinically by serologic tests for antibodies against the aforementioned thyroid antigens. Pitfalls include aspiration of a reactive lymph node (unlikely if the FNA was ultrasound-guided) and MNG with prominent Hürthle cell changes. Prominent Hürthle cell change occurs in some cases of MNG, but a lymphoplasmacytic infiltrate is absent or sparse.

Primary thyroid lymphoma is an uncommon malignancy that must be considered in the differential diagnosis of HT. Almost all cases of thyroid lymphoma arise in patients with longstanding HT, and patients with HT have a significantly higher relative risk for developing thyroid lymphoma compared with the general population.⁸⁰ Histologically, primary thyroid lymphomas are either extranodal marginal zone B-cell lymphomas, diffuse large B-cell lymphomas, or a mixture of the two.⁵⁹ Cytologically, thyroid lymphomas are composed of a uniform population of either small or large lymphoid cells. Because lymphoma is uncommon, immunophenotyping is not performed routinely on cases of typical HT and might actually be counterproductive. Some HT specimens contain clonal B-cell populations (demonstrated by a restricted κ/λ light chain ratio by flow cytometry or an IgH gene rearrangement by the polymerase chain reaction) that do not equate to malignancy and thus can be misleading.^{81,82} For this reason, immunophenotyping and/or molecular genetics are recommended only if the clinical presentation (rapid growth, large nodule) or cytomorphology (monomorphic and/or atypical lymphoid population) suggest lymphoma.

Hyperplastic Hürthle cell nodules occur in HT and mimic a Hürthle cell

tumor. The cells of a Hürthle cell tumor, however, usually have a more prominent nucleolus than those of HT, and a prominent lymphoid infiltrate is absent. In a patient with a history of HT, a nodule is more likely to represent a hyperplasia of Hürthle cells rather than a Hürthle cell neoplasm. In a minority of cases this distinction may nevertheless be hard to make, and definitive classification may depend on histologic examination.

The Hürthle cells (and any residual follicular cells) of HT occasionally display focal chromatin clearing and nuclear grooves. When mild and focal, such changes usually do not correlate with a histologic papillary carcinoma and can be disregarded on an FNA. If they are widespread and associated with other features of papillary carcinoma, however, a suspicious or malignant interpretation is warranted.

Subacute (de Quervain) Thyroiditis

Subacute (de Quervain) thyroiditis (ST) is a rare, painful enlargement of the thyroid gland in which thyroid follicles are damaged by a chronic granulomatous reaction, possibly due to a viral infection. Early, transient hypothyroidism is very common, but permanent hypothyroidism occurs only in a minority of patients. ST usually lasts several months and is self-limited, but it can recur in about 4% to 9% of patients.^{83,84} Patients are treated with nonsteroidal anti-inflammatory medications and, in more severe cases, with corticosteroids. In most cases, FNA plays no role in the diagnosis, which is usually established clinically. Because the gland can appear inhomogeneous, however, an FNA is sometimes performed to rule out a malignant nodule.



Cytomorphology of subacute thyroiditis

- multinucleated giant cells
- granulomas (rare)
- lymphocytes

Aspirates can be sparsely, moderately, or highly cellular.⁸⁵ Multinucleated giant cells are usually a prominent, striking finding. They are numerous and very large, with bizarre, angular shapes ([Fig. 10.10A](#)). In ST, they tend to have densely granular but not vacuolated cytoplasm, in contrast with the frothy

cytoplasm typical of the multinucleated cells in MNG nodules with cystic degeneration.⁸⁶ Some multinucleated cells have ingested colloid droplets.⁸⁶ Granulomas (Fig. 10.10B) are the hallmark of the disease but are not always present. Granulomas are crowded aggregates of epithelioid histiocytes that have an oval, spindle-shaped, kidney bean-shaped, or curved (“boomerang”) nucleus and abundant granular or vacuolated cytoplasm. Cell membranes are poorly defined, and as a result, granulomas have a syncytium-like appearance. Granulomas, if present, are rare and need to be hunted for in most cases.⁸⁵ Scant unremarkable macrofollicle fragments and colloid are usually present.⁸⁵

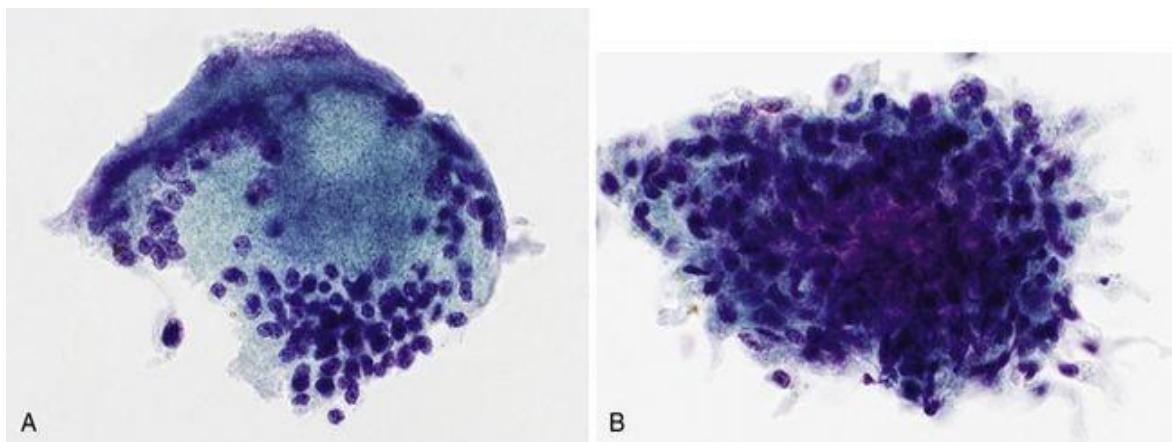


FIGURE 10.10 Subacute thyroiditis.

A, The most conspicuous finding is an abundance of multinucleated giant cells with bizarre shapes (Papanicolaou stain). B, Granulomas are often few in number. The nuclei of epithelioid histiocytes have a variety of elongated and curved shapes. Cytoplasm is abundant, clear, and poorly demarcated (Papanicolaou stain).

The cytologic differential diagnosis includes other granulomatous conditions like sarcoidosis and tuberculous infection, which can be excluded clinically and with microbiologic studies. Multinucleated giant cells by themselves are not diagnostic of ST, because they are seen in a variety of benign and malignant conditions, including papillary carcinoma and undifferentiated (anaplastic) carcinoma. The pale, elongated or folded nuclei of the epithelioid histiocytes of a granuloma may potentially be misinterpreted as papillary carcinoma nuclei. Undifferentiated (anaplastic) carcinoma can usually be ruled out owing to the absence of significant nuclear atypia.

Riedel Disease

Riedel disease, a very rare entity of unknown cause, manifests with a hard thyroid mass that clinically mimics undifferentiated (anaplastic) carcinoma. It is characterized by a dense fibrosis that replaces the thyroid parenchyma and extends beyond the thyroid to surrounding structures. The gland may be of normal size or slightly enlarged and is often asymmetric or nodular on clinical examination.⁸⁷ Because of the dense fibrosis, aspiration often results in a dry tap,⁴⁹ and surgical biopsy is often necessary for a diagnosis. FNA sometimes reveals fragments of fibrous tissue and plump myofibroblasts with abundant cytoplasm.⁸⁸ A mixed inflammatory infiltrate of lymphocytes, plasma cells, neutrophils, and rare eosinophils is seen. A fibrosing variant of HT is excluded by clinical correlation and the absence of germinal center fragments and Hürthle cells. Multinucleated giant cells and granulomas are absent, excluding subacute thyroiditis.

Amyloid Goiter

Amyloid goiter is a focal or diffuse enlargement of the thyroid gland, sometimes with alarming clinical symptoms: rapid growth, dyspnea, dysphagia, or hoarseness. Most patients have a chronic illness predisposing them to systemic amyloidosis. The thyroid interstitium is infiltrated by masses of amyloid, some surrounded by multinucleated giant cells. The diagnosis can be established by FNA.^{89,90} Amyloid is similar to colloid, except that the amyloid in amyloid goiter contains stretched and distorted fibroblast nuclei⁸⁹ ([Fig. 10.11](#)). A conclusive diagnosis rests on identifying the characteristic apple-green dichroism with the Congo red stain. Follicular cells are scant.

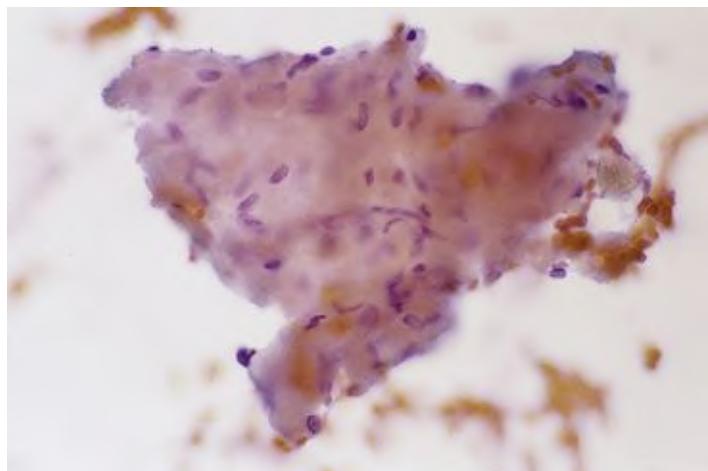


FIGURE 10.11 Amyloid goiter.

Like colloid, amyloid forms opaque masses with irregular, sharp edges. Amyloid is deposited in the interstitium in patients with amyloid goiter; hence aspirated amyloid fragments often contain entrapped fibroblasts (Papanicolaou stain).

Amyloid often accompanies medullary carcinoma and in some cases overshadows the cellular component (amyloid-rich medullary carcinoma).

Black Thyroid

A benign but striking dark brown pigmentation of thyroid follicular cells occurs in patients who take antibiotics of the tetracycline group (e.g., minocycline) for long periods of time, most commonly for acne. This pigmentation is so prominent in some cases that the gland is black on gross inspection. Cytologic preparations show abundant dark brown pigment granules within the cytoplasm of follicular cells ([Fig. 10.12](#)). The pigment is a darker brown than hemosiderin. Because the pigment stains with the Fontana stain, it is thought to represent a type of melanin. Recognizing this as a benign condition may prevent unnecessary surgery in these patients.^{[91](#)⁹²}

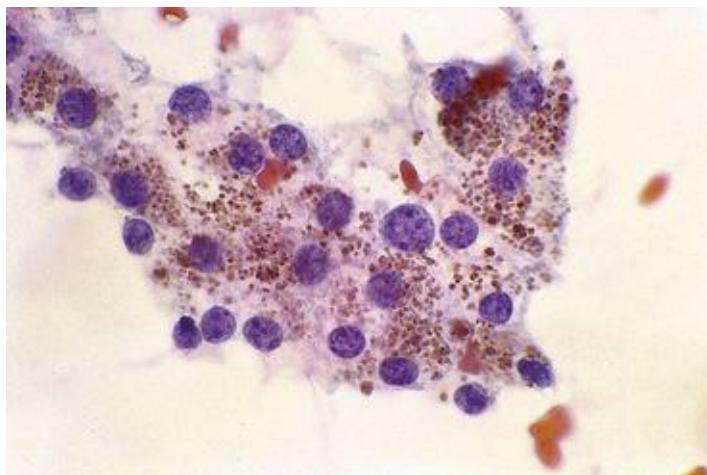


FIGURE 10.12 Black thyroid.

Follicular cells contain abundant coarse, brown cytoplasmic granules (Papanicolaou stain).

Radiation Changes

Both external radiation to the neck and systemic administration of radioactive iodine (^{131}I) can cause long-term morphologic changes in the thyroid gland.

External radiation is used in low doses to treat a variety of benign conditions, and in high doses for malignancies like Hodgkin disease. Radioactive iodine is administered to treat hyperthyroidism, whether due to Graves disease, MNG, or a functioning thyroid carcinoma.



Cytomorphology of radiation changes

- sheets (macrofollicle fragments)
- enlarged cells
- normal nuclear-to-cytoplasmic ratio
- Hürthle cell change
- cytoplasmic vacuolization
- marked nuclear atypia
 - marked size variation
 - hyperchromasia
 - smudged chromatin
 - grooves, pseudoinclusions
 - naked nuclei

Follicular cells altered by radiation are arranged in sheets, and nuclei vary greatly in size, some reaching giant proportions ([Fig. 10.13](#)). Chromatin is dark, coarsely granular, and nucleoli can be prominent. Cytoplasm is abundant, suggestive of Hürthle cell change, and sometimes vacuolated.

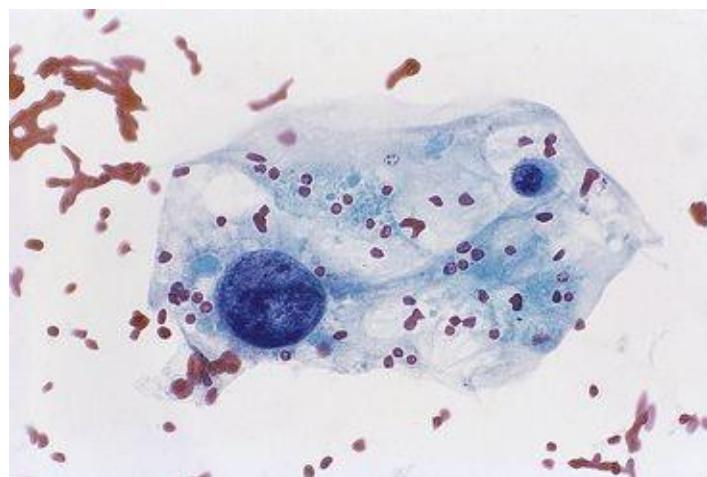


FIGURE 10.13 Radioactive iodine (^{131}I) effect.

Follicular cells show marked variation in cellular and nuclear size, sometimes with conspicuous cytoplasmic vacuolization (Papanicolaou stain).

The differential diagnosis includes follicular carcinoma, papillary carcinoma, and undifferentiated (anaplastic) carcinoma^{93,94}—important considerations in as much as external irradiation has been associated with an increased risk of thyroid cancer. (No association between ^{131}I and cancer has been demonstrated.) Although the atypia seen with irradiation is marked, there is little in the way of microfollicular architecture, a hallmark of follicular neoplasms. The isolated cell pattern of an undifferentiated (anaplastic) carcinoma is not seen. Although some of the nuclear features characteristic of papillary carcinoma are occasionally seen (grooves, pseudoinclusions), in other ways (marked hyperchromasia and nuclear size variation) the findings are not typical. Similar changes are induced by other drugs used to treat Graves disease, such as methimazole and carbimazole.⁹⁵

Suspicious for a Follicular Neoplasm

The general diagnostic category “suspicious for a follicular neoplasm” or simply “follicular neoplasm” (the terms are interchangeable, and either one is considered acceptable for this category) identifies a nodule with significant architectural alterations of follicular cells, raising the possibility of a follicular carcinoma and thus triaging the nodule for surgical excision.⁹⁶ FNA is *diagnostic* of many thyroid conditions (e.g., papillary carcinoma, Hashimoto thyroiditis), but with regard to follicular carcinoma, it is better considered a *screening* test.

Follicular carcinoma is the second most common malignant thyroid cancer and accounts for 10% to 15% of thyroid malignancies.⁵⁹ Together with papillary carcinoma and Hürthle cell carcinoma, follicular carcinoma is by definition one of the differentiated carcinomas, but it is biologically more aggressive than papillary carcinoma, tending to metastasize to the lung and bones and less often to regional lymph nodes. Follicular carcinoma is divided into two subtypes: the minimally invasive carcinoma with an excellent prognosis and the widely invasive malignancy with a poor prognosis.

Histologically, follicular carcinoma is distinguished from follicular adenoma by the presence of capsular penetration, vascular invasion, or both. Because the defining histologic criteria of invasion cannot be assessed by FNA, one cannot make a diagnosis of follicular carcinoma from a cytologic specimen. Historically, when a “histologic” diagnosis of follicular adenoma or follicular carcinoma was valiantly attempted using FNA, the results were disappointing: one third of cases diagnosed as follicular carcinoma turned out to be follicular adenomas, and 1 in 10 specimens identified as follicular adenoma on FNA proved to be carcinomas after histologic examination.⁴⁹ Nevertheless, follicular carcinomas have distinctive neoplastic features that are recognizable by cytology, resulting in a suspicious diagnosis that leads to a diagnostic lobectomy.^{42,46,97} About 15% to 30% of cases reported as suspicious for a follicular neoplasm prove to be malignant.^{11,12,48,98} Of those that prove to be malignant, some are follicular carcinomas, but many (55% to 67%) prove to be the follicular variant of papillary carcinoma.^{12,39,41,98} A significant proportion (35% or more) of cases reported as suspicious for a follicular neoplasm prove not to be neoplasms at all, but rather adenomatoid nodules in MNG,⁹⁸ which is why many investigators prefer “suspicious for a follicular neoplasm” rather than “follicular neoplasm” as the designation for this category.

After surgery, if histologic examination reveals an adenoma (or other benign

nodule), the patient requires no further therapy. If carcinoma is found, a completion thyroidectomy may be necessary, because a large thyroid remnant obscures subsequent ^{131}I uptake studies used to detect recurrent and/or metastatic follicular carcinoma. Follicular carcinoma metastases accumulate ^{131}I optimally only in the absence of normal thyroid tissue.⁹⁷



Cytomorphology of ‘suspicious for a follicular neoplasm’

- marked cellularity
- predominantly microfollicles and/or trabeculae
- enlarged, crowded follicular cells
- scant colloid

By definition, this category refers to a “cellular aspirate comprised of follicular cells, most of which are arranged in an altered architectural pattern characterized by significant cell crowding and/or microfollicle formation. Cases that demonstrate the nuclear features of papillary carcinoma are excluded from this category.”⁹⁶ Cytologic preparations typically have high cellularity, and colloid is scant or absent. The follicular cells are arranged predominantly in microfollicular ([Fig. 10.14A](#)) or trabecular ([Fig. 10.14B](#)) arrangements. Cellular crowding and overlapping are conspicuous, and the follicular cells are usually larger than normal. Nuclear atypia/pleomorphism and mitoses are uncommon. A minor population of macrofollicles (intact spheres and fragments) can be present.

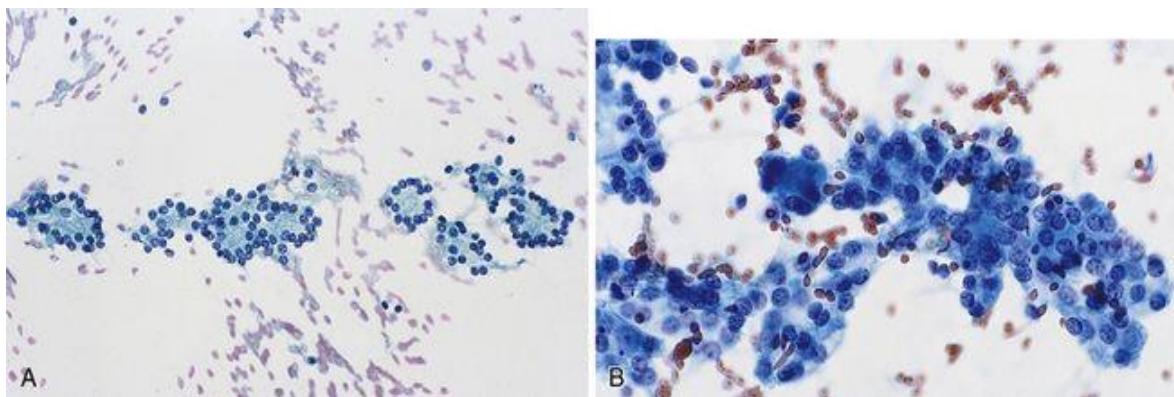


FIGURE 10.14 “Suspicious for a follicular neoplasm.”

A, A neoplasm should be suspected whenever a specimen is composed predominantly of microfollicles (Papanicolaou stain). B, The cells of some follicular neoplasms are crowded haphazardly into ribbons (trabeculae) (Papanicolaou stain).

Differential diagnosis of ‘suspicious for a follicular neoplasm’

- benign follicular nodule
- papillary thyroid carcinoma
- metastatic renal cell carcinoma
- parathyroid adenoma/carcinoma

Moderate cellularity alone does not qualify the nodule for a suspicious interpretation.¹⁰ If the sample is moderately cellular but mostly macrofollicular (intact spheres and flat fragments), a benign interpretation is appropriate. Benign follicular nodules often have a small population of microfollicles and crowded groups. As a minority of the total population, they have little significance, and the FNA specimen can be interpreted as benign. A suspicious interpretation is rendered when a majority of the follicular cells are arranged in abnormal architectural groupings (microfollicles, crowded trabeculae).

The differential diagnosis includes other thyroid neoplasms. If features of papillary carcinoma (e.g., nuclear grooves, pseudoinclusions) are present, the category “suspicious for a follicular neoplasm” is not appropriate. The case should instead be reported as “suspicious for malignancy (papillary carcinoma)” or malignant, depending on the quantity and quality of the cytomorphologic changes. Metastatic renal cell carcinoma can mimic a follicular neoplasm. A clinical history of renal cell carcinoma, very helpful to alert the cytopathologist to this possibility, is ideally provided on the requisition that accompanies the FNA.¹⁴ Some renal cell carcinomas, however, are occult at the time of thyroid metastases. The rare *clear cell variant* of follicular carcinoma is composed of cells with abundant cytoplasm and is morphologically indistinguishable from metastatic renal cell carcinoma and some parathyroid tumors.⁹⁹ Parathyroid adenomas are virtually impossible to distinguish from a follicular neoplasm by cytomorphology alone. A clinical suspicion of a parathyroid neoplasm is invaluable for the cytopathologist, as it may prompt additional evaluation with immunohistochemistry. Immunostains for thyroglobulin and thyroid transcription factor-1 (TTF-1) are positive in follicular neoplasms and negative

in parathyroid adenomas, which are immunoreactive instead for parathyroid hormone.

Suspicious for a Follicular Neoplasm, Hürthle Cell Type

The diagnosis “suspicious for a follicular neoplasm, Hürthle cell type” or simply “follicular neoplasm, Hürthle cell type” (either phrase is acceptable for this category) identifies a nodule with features that raise the possibility of an oncocytic (Hürthle cell) variant of follicular carcinoma, triaging the nodule for surgical excision.

The oncocytic (Hürthle cell) variant of follicular carcinoma accounts for 3% to 4% of thyroid cancers. In contrast with the typical follicular carcinoma, which rarely manifests with lymph node metastases, the oncocytic (Hürthle cell) variant is associated with nodal metastases in 30% of cases.⁵⁹ Histologically, the oncocytic (Hürthle cell) variants of follicular carcinoma show follicular, trabecular, or solid growth patterns. The nuclei have a prominent nucleolus, and there is abundant granular cytoplasm. Colloid, when present, sometimes undergoes a curious basophilic transformation with concentric calcification. These structures are difficult to distinguish from psammoma bodies. Clear cell change is prominent in some cases. The oncocytic (Hürthle cell) variants of follicular carcinoma are immunoreactive for thyroglobulin and TTF-1.

As with its conventional follicular counterpart, the histologic diagnosis of an oncocytic (Hürthle cell) variant of follicular carcinoma rests on identifying capsular and/or vascular invasion. Invasion cannot be established by FNA, but a suspicion of neoplasia based on the criteria outlined next can be raised that leads to a definitive diagnosis after lobectomy. The initial management is almost identical to that for patients with a cytologically suspicious follicular nodule: Unless there is clinical evidence of invasion, a lobectomy is advised. If the nodule proves to be malignant after histologic examination, a completion thyroidectomy is recommended.⁹⁷

Most nodules interpreted as “suspicious for a follicular neoplasm, Hürthle cell type” prove to be neoplasms, but 10% to 26% turn out to be nonneoplastic nodules of MNG or Hashimoto thyroiditis.^{49,100-102} From 15% to 45% of the nodules are malignant (see [Table 10.1](#)), and the remainder of the neoplasms prove to be adenomas.^{48,101,102}



Cytomorphology of ‘suspicious for a Hürthle cell neoplasm’

- pure Hürthle cell population
- usually noncohesive cells
- prominent nucleolus
- pseudopsammoma bodies

By definition, the interpretation “suspicious for a follicular neoplasm, Hürthle cell type” refers to “a cellular aspirate that consists exclusively (or almost exclusively) of Hürthle cells. Oncocytic cells with nuclear features of papillary carcinoma are excluded from this category.”⁷⁶ These are often isolated cells ([Fig. 10.15](#)), but groups, usually crowded and syncytium-like, can be seen.^{100,103} A large nucleolus is more typical of neoplastic than hyperplastic Hürthle cells.^{100,103} Colloid can be present, but abundant lymphocytes and normal follicular cells are absent. Basophilic structures with concentric lamellae are seen in some cases and are often indistinguishable from psammoma bodies.

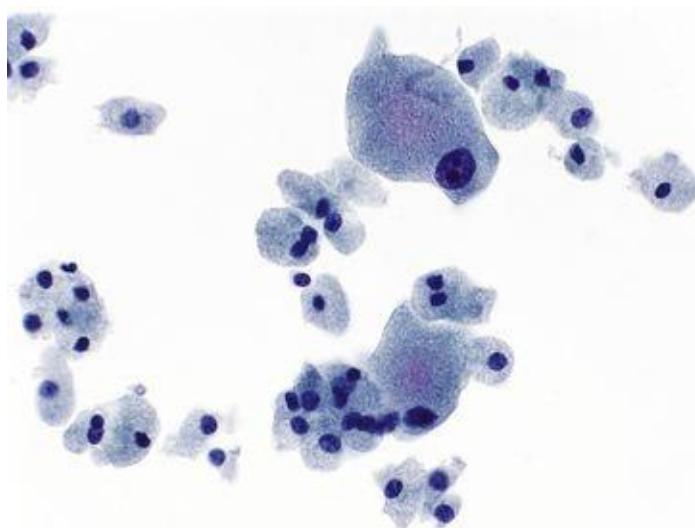


FIGURE 10.15 “Suspicious for a follicular neoplasm, Hürthle cell type.”
The sample is composed of numerous Hürthle cells and little else (Papanicolaou stain).

It has been suggested that the diagnostic criteria for this category should be narrowed to include only those cases in which the Hürthle cells show additional abnormalities, either cytologic (small cell dysplasia, large cell dysplasia) or architectural (crowding or marked dyshesion).^{102,104} The goal of the stricter criteria is to reduce the number of patients undergoing unnecessary lobectomy.

Differential diagnosis of ‘suspicious for a Hürthle cell neoplasm’

- Hashimoto thyroiditis
- MNG
- macrophages
- papillary carcinoma
- metastatic renal cell carcinoma
- medullary carcinoma
- parathyroid adenoma/carcinoma
- granular cell tumor

Hürthle cells are not neoplastic but are merely a component of Hashimoto thyroiditis when they are admixed with numerous lymphoid cells (see [Fig. 10.9A-C](#)). Similarly, they are not neoplastic when admixed with macrofollicles and colloid, a heterogeneous mixture that is typical of MNG. Cellular features of the Hürthle cells themselves can help in this regard. Neoplastic Hürthle cells are often monomorphic and have a prominent nucleolus. In Hashimoto thyroiditis and MNG, the cells can be pleomorphic, with marked anisonucleosis and hyperchromasia, but macronucleoli are usually absent. Macrophages can sometimes be confused with Hürthle cells and vice versa, particularly with liquid-based preparations, where the usually granular cytoplasm of Hürthle cells appears microvacuolated. But the pseudovacuolization of Hürthle cells in liquid-based preparations is very fine and does not have the coarsely vacuolated appearance of macrophage cytoplasm. In addition, Hürthle cells generally lack hemosiderin and are more polygonal, rather than rounded like macrophages.

If an FNA sample consists exclusively of Hürthle cells but the nodule is relatively small (less than 2.5 cm), and the patient has Hashimoto thyroiditis, the nodule is most likely a hyperplastic nodule. In that circumstance, downgrading the interpretation to AUS (with an explanatory note) can be considered. The same applies to a patient known to have multiple nodules, in whom the (small) nodule is likely to represent Hürthle cell transformation of an adenomatoid nodule.

The differential diagnosis includes variants of papillary carcinoma (tall cell and oncocytic), but these can be excluded by the absence of nuclear features diagnostic of papillary carcinoma. It is worth noting that the nuclei of Hürthle

cells can sometimes be paler than those of normal follicular cells. This can create diagnostic difficulty, but if there are no other convincing features of papillary cancer, the cells are most likely Hürthle cells. Similar confusion with papillary carcinoma may occur because some Hürthle cell tumors have calcific structures that resemble psammoma bodies.⁵⁹ Because Hürthle cell tumors can have clear cell features, metastatic renal cell carcinoma mimics a Hürthle cell neoplasm to perfection (Fig. 10.16). A clinical history of renal cell carcinoma can alert the cytopathologist to this possibility and should be provided on the requisition.¹⁴ Immunohistochemistry for thyroglobulin and TTF-1 are helpful because the cells of renal cell carcinoma are negative for these markers.

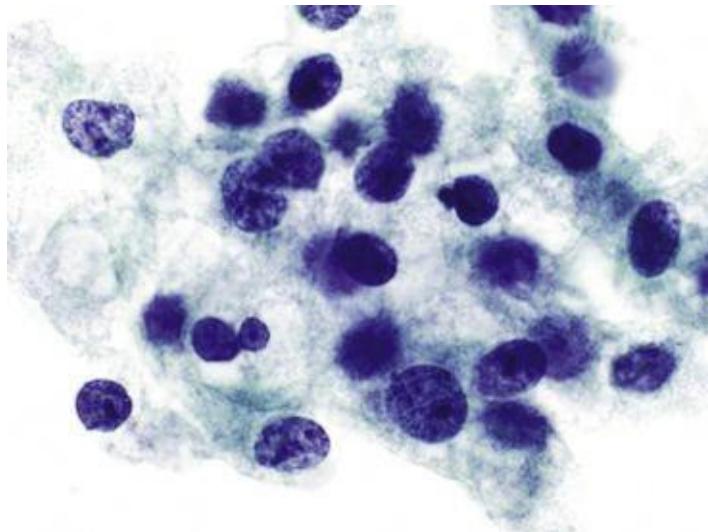


FIGURE 10.16 Renal cell carcinoma metastatic to the thyroid.

The prominent nucleoli and abundant granular cytoplasm of renal cell carcinomas mimic the features of a Hürthle cell neoplasm (Papanicolaou stain).

The differential diagnosis includes medullary thyroid carcinoma. A dispersed, noncohesive cell pattern is common in both, and the cells of both tumors can have a plasmacytoid appearance. A prominent nucleolus, however, is rare in medullary carcinoma. With Romanowsky stains, the cytoplasmic granules of Hürthle cells are blue, whereas those of medullary carcinoma are usually red. Immunostains are helpful: Hürthle cells are thyroglobulin-positive and TTF-1–positive but calcitonin-negative.

Parathyroid adenomas and carcinomas can have oncocytic cytoplasm and are thus virtually impossible to distinguish from a Hürthle cell neoplasm by cytomorphology alone. A clinical suspicion of a parathyroid neoplasm is

invaluable to the cytopathologist, as it may prompt additional evaluation with immunohistochemistry. Immunostains for thyroglobulin and TTF-1 are positive in Hurthle cell neoplasms and negative in parathyroid adenomas, which are immunoreactive instead for parathyroid hormone.

A granular cell tumor in the neck can resemble a Hürthle cell neoplasm. It can be distinguished from that lesion on the basis of strong immunoreactivity for S100.

Malignant Conditions

Papillary Thyroid Carcinoma

PTC is the most common thyroid malignancy, accounting for 80% of all thyroid cancers in the United States.^{105,106} It can occur at any age, including in children, but most patients are between 20 and 50 years old. There is a female-to-male ratio of 4:1. PTC manifests as a solitary nodule or a distinct nodule within a nodular goiter. Some patients present with cervical lymphadenopathy due to a metastatic tumor.

PTCs are defined histologically on the basis of their nuclear features. The nuclei are enlarged and can be oval, elongated, or irregular in contour. Profound changes in the nuclear skeleton make them less stiff and much more deformable than normal.¹⁰⁷ The increased plasticity of the nucleus results in nuclear grooves (resulting from a nucleus folded on itself) and pseudoinclusions (from invagination of cytoplasm into the nucleus). The nuclei are paler than normal, but nuclear pallor may be patchy within the tumor. Nuclear crowding and overlapping are common. Papillary architecture (tumor cells arranged around a fibrovascular core) is seen in some but not all tumors. Focal squamous metaplasia is common. Some PTCs are partly or predominantly cystic. Psammoma bodies are characteristic but not diagnostic of PTC.

The classic PTC has true papillary architecture, but a large number of PTC variants have been described. The most common is the *follicular variant*, which contains virtually no papillary structures. Others include the *macrofollicular variant*, comprised predominantly of macrofollicles; the *oncocytic variant*, composed of cells with abundant cytoplasm resembling Hürthle cells; the *Warthin-like PTC*, with its abundant lymphoid infiltrate and strong association with Hashimoto thyroiditis; the *clear cell variant* (self-explanatory); the *diffuse sclerosing variant*, which tends to occur in young adults and infiltrates in a diffuse rather than nodular pattern, with abundant squamous metaplasia and numerous psammoma bodies; the *tall cell variant*, whose cells are at least three times as tall as they are wide; the *columnar cell variant*, which has pseudostratified columnar cells; the *solid variant*, with solid growth but typical nuclear features of PTC; the *cryptophytic carcinoma*, which occurs in patients with familial adenomatous polyposis and Gardner syndrome; and *PTC with fasciitis-like stroma*. Additional variants that represent mixtures of PTC and other thyroid cancers have been reported. Awareness of these variants is important for the

cytopathologist, who might otherwise confuse them with other neoplasms. Several of these, such as the tall cell and columnar variants, have a tendency toward more aggressive clinical behavior than the classic PTC.

PTCs are immunoreactive for keratins, thyroglobulin, TTF-1, and PAX8. Many contain one of several chimeric oncogenes called *RET/PTC*, which result from gene rearrangements involving the ret proto-oncogene on chromosome 10.^{59,108} Other associated genetic changes include rearrangements of the *TRK* gene, point mutations of *RAS* proto-oncogenes, and point mutations of the *BRAF* gene.⁵⁹

Together with follicular carcinoma and its oncocytic (Hürthle cell) variant, PTC is considered a differentiated thyroid cancers. The prognosis for patients with PTC is excellent because surgical resection (usually thyroidectomy) is often curative. The 10-year survival rate is greater than 90%. Many patients are treated with a so-called near-total thyroidectomy,¹⁰⁸ in which the lobe with the dominant tumor mass is entirely removed, the contralateral lobe is nearly completely removed, and a small amount of thyroid tissue is left in order to preserve the vascular supply to the parathyroid glands. More limited resections (e.g., unilateral lobectomy) result in a higher recurrence rate, because many papillary carcinomas are multifocal.⁹⁷



Cytomorphology of papillary thyroid carcinoma

- sheets, papillae, microfollicles
- nuclear changes
 - powdery chromatin
 - grooves
 - pseudoinclusions
 - nucleolus (small or large)
 - membrane thickening and irregularity
- nuclear crowding/molding
- variable cytoplasm (scant, squamoid, Hürthle-like, or vacuolated)
- psammoma bodies
- histiocytes, including multinucleated giant cells

Typical architectural features of PTC include papillae ([Fig. 10.17](#)) and sheets ([Fig. 10.18](#)). Microfollicles are seen in the follicular variant ([Fig. 10.19](#)). The diagnosis of PTC rests on nuclear and not architectural changes. These include

longitudinal grooves, intranuclear pseudoinclusions (“holes”), and, perhaps most significantly, a pale, finely textured (“powdery”) chromatin pattern ([Fig. 10.20](#)). In addition, nuclei are enlarged and crowded, and some may be molded to one another. A nucleolus is usually present and may be small or large. The cytoplasm can be inconspicuous or abundant and is not a helpful diagnostic feature. It can appear squamoid, oncocytic ([Fig. 10.21](#)), or vacuolated, often confusing the cytodiagnostic picture. In the diffuse sclerosing variant, for example, many of the tumor cells have a squamoid appearance.^{[109](#)} *Multinucleated giant cells* are common in PTC; they are CD68-positive histiocytes and not tumor giant cells.^{[110](#)}

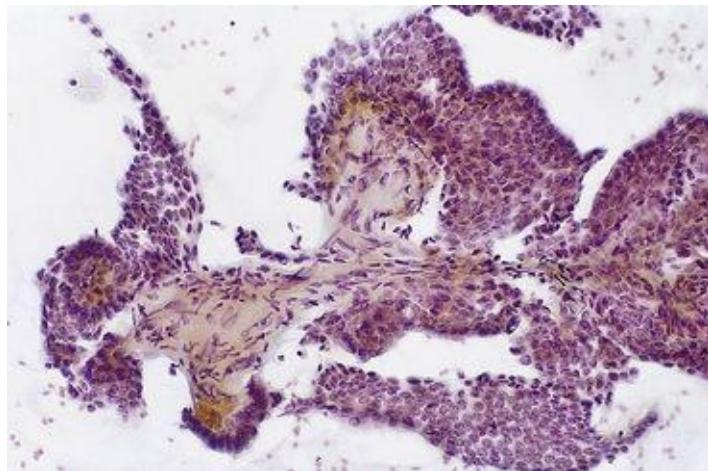


FIGURE 10.17 Papillary thyroid carcinoma (PTC).
In some cases, the tell-tale fibrovascular cores are seen (Papanicolaou stain).

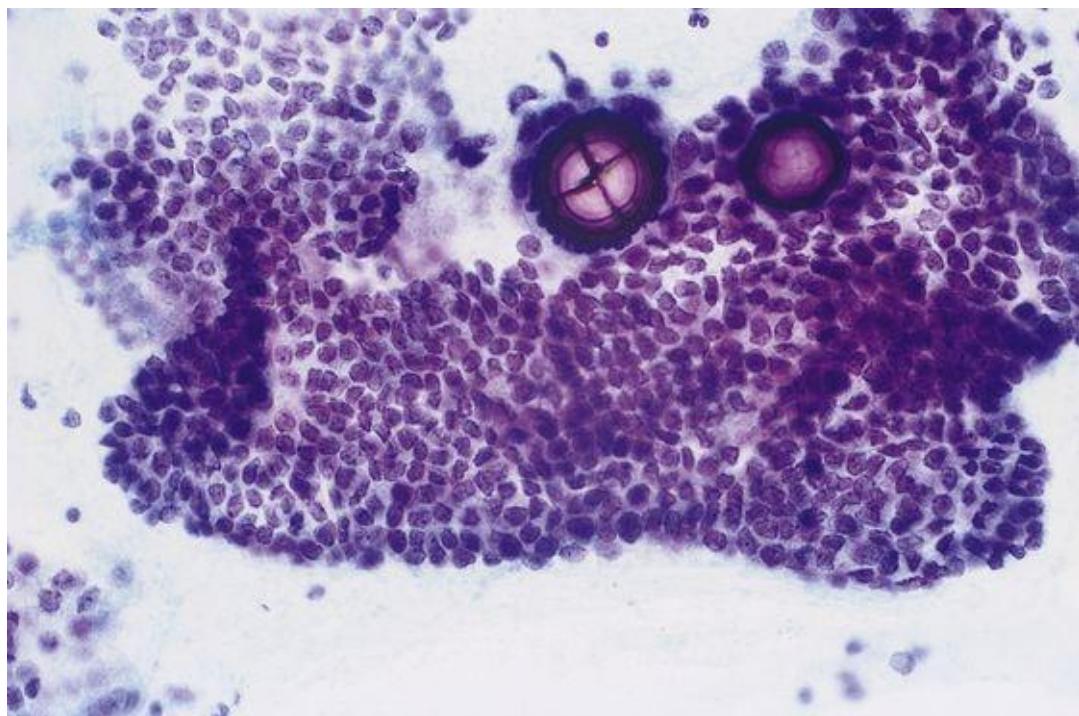


FIGURE 10.18 Papillary thyroid carcinoma (PTC).
In some cases, papillae are absent, and the neoplastic cells are arranged in crowded sheets.
Psammoma bodies are present (Papanicolaou stain).

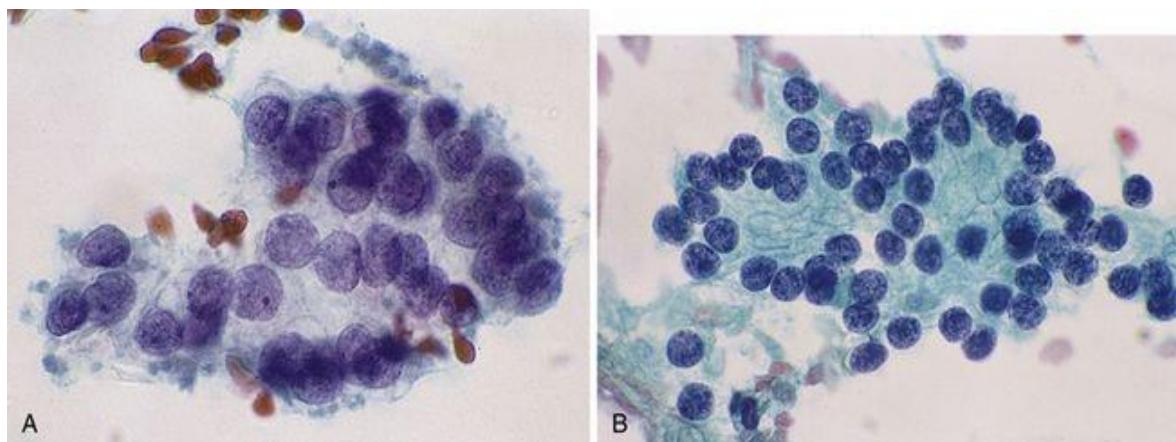


FIGURE 10.19 A, Papillary thyroid carcinoma (PTC), follicular variant. B, Follicular neoplasm (follicular adenoma).
Some papillary carcinomas show microfollicular arrangements that mimic a follicular neoplasm, but the pale chromatin of a papillary carcinoma (A) is different from the coarse chromatin granularity of a follicular neoplasm (B) (Papanicolaou stain).

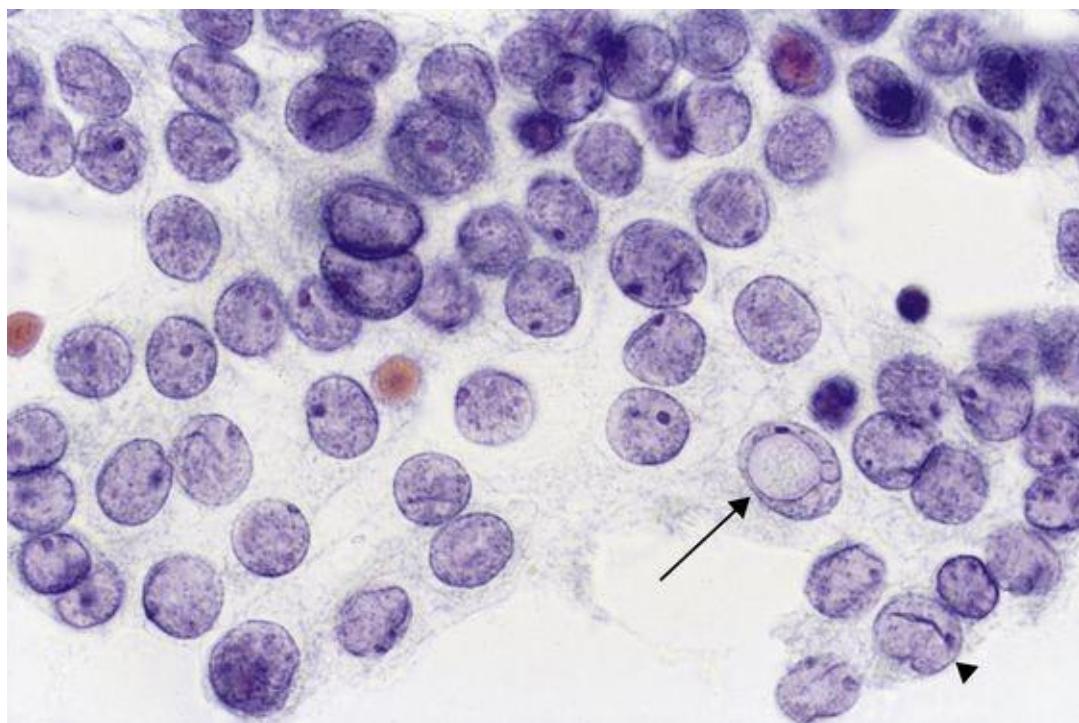


FIGURE 10.20 Papillary thyroid carcinoma (PTC).

The neoplastic cells have pale, powdery chromatin. Other important nuclear changes are the circular, sharply defined intranuclear pseudoinclusion (*arrow*) and the nuclear groove (*arrowhead*). Note that the pseudoinclusion has the same color and consistency as those of the surrounding cytoplasm (Papanicolaou stain).

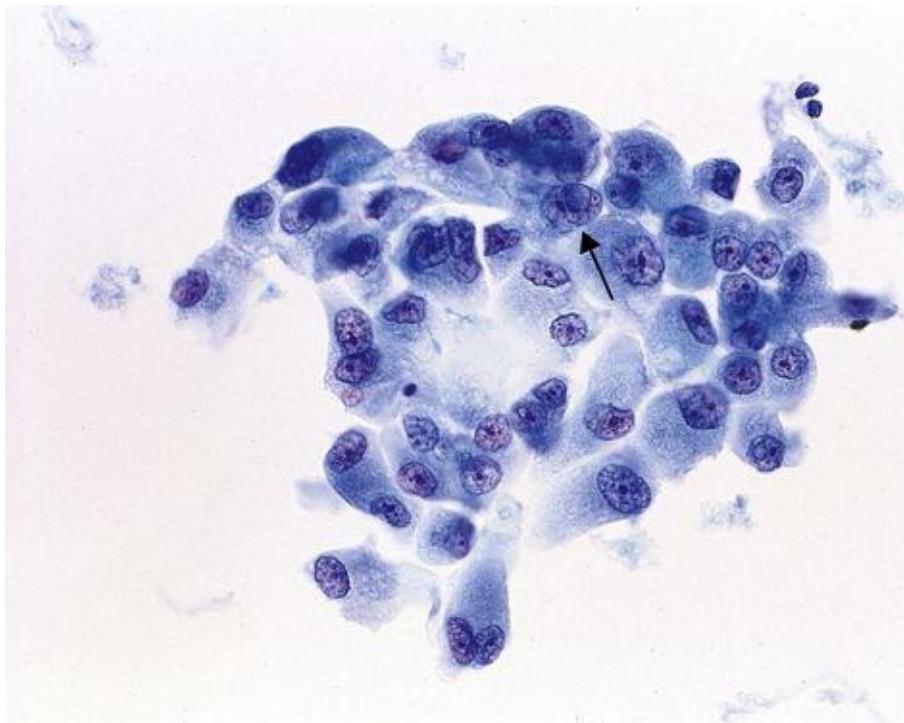


FIGURE 10.21 Papillary thyroid carcinoma (PTC), oncocytic variant.

The neoplastic cells of this variant have abundant granular cytoplasm resembling Hürthle cell cytoplasm. The nuclear features (arrow) are typical of papillary carcinoma (Papanicolaou stain).

Colloid is present and may be abundant. Sometimes it has an abnormal viscosity (“bubble gum colloid”) and may take the shape of long strands or dense blobs.

Psammoma bodies are a useful diagnostic feature (see Fig. 10.18). Although they are identified in about 50% of resected PTCs,¹⁰⁶ these concentric calcifications are less frequent on cytologic preparations and most numerous in the diffuse sclerosing variant.¹⁰⁹ They must be distinguished from nonspecific, usually dystrophic calcifications, which are not laminated. Psammoma bodies should raise the suspicion of PTC, especially in a cystic background, but because they are also seen in MNG¹¹¹ and Hürthle cell neoplasms,¹⁰⁶ a conclusive diagnosis must be based on diagnostic cells. By themselves, the positive predictive value of psammoma bodies for PTC is only 50%.¹¹¹

Cystic degeneration is especially common in PTC and is a significant cause of false-negative diagnoses.¹¹² Macrophages, with or without hemosiderin, are the cytologic hallmark of cystic degeneration.

Some cases contain a population of enlarged “histiocytoid” tumor cells. They resemble histiocytes and have abundant vacuolated cytoplasm, but they are more atypical, with a larger and darker nucleus. They may or may not have nuclear

grooves or inclusions. Rarely, such atypical (for PTC) cells comprise the entire specimen. They are uncommon in benign thyroid nodules and thus relatively specific for PTC.¹¹³

In addition to the follicular variant of PTC, two other variants deserve additional mention because of their distinctive cytologic features: The tall cell variant can be suspected when cells with PTC nuclei have abundant, elongated, granular cytoplasm (Fig. 10.22). The columnar cell variant can be suspected when the cells are elongated (columnar), with scant cytoplasm and crowded, cigar-shaped nuclei.

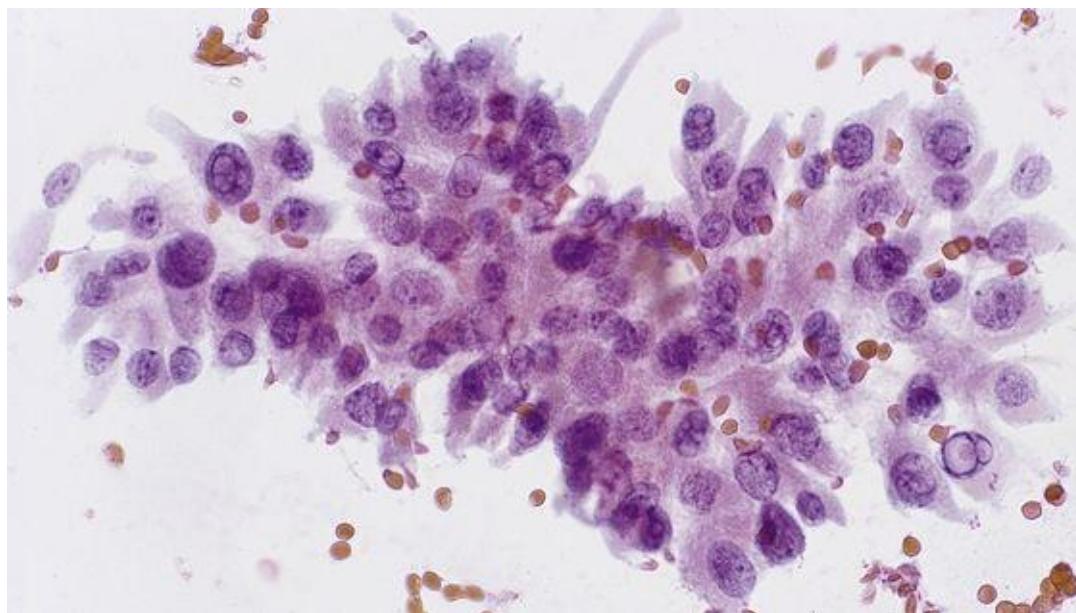


FIGURE 10.22 Papillary thyroid carcinoma (PTC), tall cell variant. The cells are large and have abundant elongated (“tall”) cytoplasm. Nuclear features typical of papillary carcinoma, including numerous intranuclear holes, are apparent (Papanicolaou stain).



Differential diagnosis of papillary thyroid carcinoma

- benign follicular nodule
- follicular neoplasm
- Hashimoto thyroiditis
- ^{131}I treatment effect
- cyst lining cells
- hyalinizing trabecular tumor

A majority of PTCs are straightforward to diagnose, because most or all of the nuclear and architectural changes described previously are clearly identifiable and widespread. Such cases are reliably interpreted as malignant (see [Table 10.1](#)). In some cases, however, the nuclear changes are subtle and focal. This is particularly true of the macrofollicular variant, which can be difficult to distinguish from a benign follicular nodule.⁷⁷ Other PTCs may be incompletely sampled and yield only a small number of abnormal cells.¹¹⁴ If only one or two characteristic features of PTC are present, if they are only focal and not widespread throughout the follicular cell population, or if the sample is sparsely cellular, a malignant diagnosis cannot be made with certainty. Such cases occur with some regularity, and they are best classified as suspicious for malignancy, qualified as suspicious for papillary carcinoma (see [Table 10.1](#)).

Care must be taken not to overinterpret nonspecific findings as malignant or suspicious for PTC. None of the aforementioned features is diagnostic of PTC by itself, so a diagnosis (or even suspicion) of PTC cannot be made on the basis of a single finding. If a well-sampled nodule has the characteristics of a benign follicular nodule, but a few grooves are present, they have little significance and can be ignored. The same applies to psammoma bodies, which occur not just in PTC but also in MNG.¹¹⁵ The cytotechnologist or pathologist must beware of overinterpreting nuclear pallor that is not accompanied by other nuclear changes: It can result from a technical staining problem. A variety of small nuclear holes, whether due to a fixation artifact or overlying red blood cells, mimic the pseudoinclusions of PTC. Most nonspecific nuclear holes are easily recognized as follows: they are small, not sharply etched, white (rather than the color of cytoplasm), and irregular in shape.

In patients with Hashimoto thyroiditis, some allowance needs to be made for mild nuclear atypia (e.g., focal pallor and occasional grooves). In small amounts, such changes are encountered with some regularity in thyroiditis, but they do not correlate with a histologic PTC. In benign follicular nodules affected by ¹³¹I treatment, there is usually hyperchromasia and marked nuclear size variation, which are not at all typical of PTC. Cyst lining cells sometimes have large pale and grooved nuclei, and in cystic lesions they may be the predominant cell type. In such cases a suspicious for malignancy interpretation cannot be avoided.⁶¹

The *hyalinizing trabecular tumor* is a rare and controversial entity that may be a variant of PTC. It has all the nuclear features of a PTC, and most cases are interpreted as suspicious for PTC or malignant by FNA.¹¹⁶ Cytologic clues to the diagnosis include a whorling, parallel array of elongated cells, amorphous hyaline material that is not amyloid, cytoplasmic yellow bodies (which stain light green with the Papanicolaou stain), and a perinucleolar clear zone, but most

cases are identified by histologic, not cytologic, examination.¹¹⁷ Encapsulation, marked intratrabecular hyalinization, and trabecular architecture are important criteria. The discovery that some cases show rearrangement of *RET/PTC* has led some investigators to hypothesize that hyalinizing trabecular tumors are variants of PTC.^{118,119} Others remain unconvinced and prefer to call them tumors/neoplasms rather than carcinomas.^{59,120}

Nodules reported as suspicious for papillary carcinoma are resected, by either lobectomy or thyroidectomy. Most (60% to 75%) prove to be PTCs, and the rest are usually follicular adenomas.^{11,12,42,121} Malignant nodules are typically removed by thyroidectomy. The positive predictive value of a malignant diagnosis is 97% to 99%.

Poorly Differentiated Thyroid Carcinoma

Some carcinomas derived from follicular cells are not readily classified as one of the differentiated carcinomas (follicular, Hürthle cell, papillary) nor as an undifferentiated (anaplastic) carcinoma. They fall somewhere in between, based on an intermediate degree of nuclear and architectural atypia. These tumors are called poorly differentiated thyroid carcinomas (PDTCs) and account for 4% to 7% of thyroid carcinomas.^{59,122,123} Nodal, pulmonary, and bone metastases are common at the time of diagnosis. Patients with a PDTC fare worse than those with one of the differentiated thyroid cancers, but better than those with an undifferentiated (anaplastic) carcinoma.¹⁰⁶ The 5-year survival rate is about 50%.

The PDTCs are a heterogeneous group, with three principal histologic patterns: insular, trabecular, and solid. In the insular pattern, the malignant cells are arranged in well-defined nests (*insulae*) surrounded by thin fibrovascular septae. Other PDTCs have a predominantly trabecular (cords or ribbons) or solid growth pattern without nests. In all three patterns, some microfollicles (with or without colloid) can be seen. Tumor cells are generally small to intermediate in size and uniform, with some hyperchromasia, but pleomorphism is absent or only focal. Mitoses and necrosis are present. Nuclear features of papillary thyroid carcinoma are common, but some PDTCs more closely resemble follicular carcinoma or its oncocytic (Hürthle cell) variant.¹⁰⁶ They are immunoreactive for thyroglobulin, TTF-1, and PAX8.



Cytomorphology of poorly differentiated thyroid carcinoma

- highly cellular

- noncohesive cells
- trabeculae, solid spheres
- monomorphic round nuclei
- mitoses, necrosis

The diagnosis of PDTC rests on histologic evaluation and cannot be made with certainty by FNA. Nevertheless, some general comments can be made. Cytologic preparations are usually highly cellular ([Fig. 10.23A](#)), with numerous isolated tumor cells ([Fig. 10.23B](#)) as well as clusters of overlapping cells. Microfollicles are often present. In many cases the tumor cells are uniform in size and shape,¹²² but a greater degree of atypia is seen in some cases ([Fig. 10.24](#)). Cytoplasm is poorly defined, and occasional cytoplasmic vacuoles are present. Intranuclear inclusions and nuclear grooves also may be seen.^{122,124} Colloid is scant.

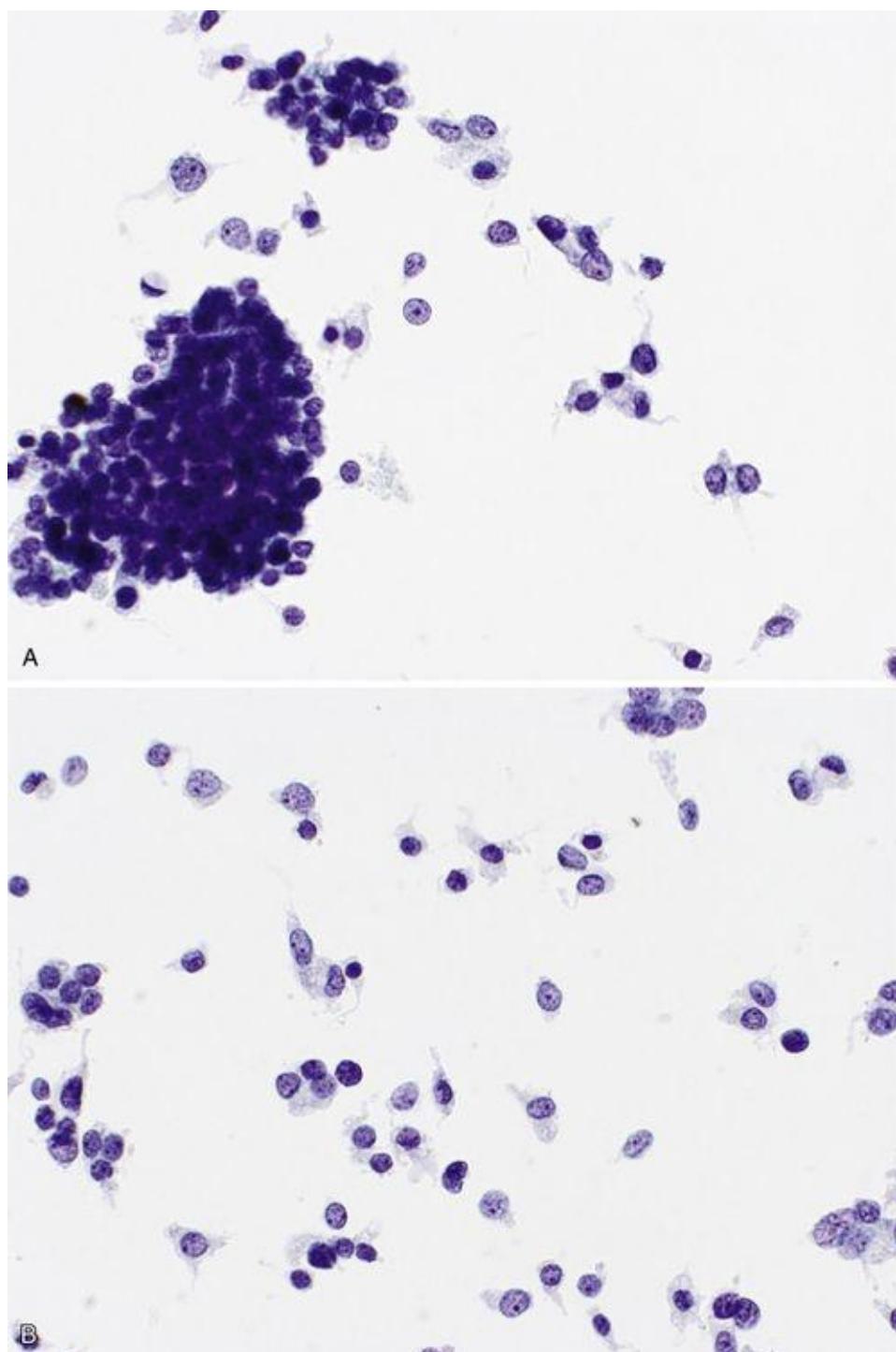


FIGURE 10.23 Poorly differentiated (insular) carcinoma.

A, The architectural pattern is variable, and may include crowded groups, microfollicles, and numerous isolated cells (Papanicolaou stain). B, The isolated malignant cells resemble those of medullary thyroid carcinoma (MTC) (Papanicolaou stain).

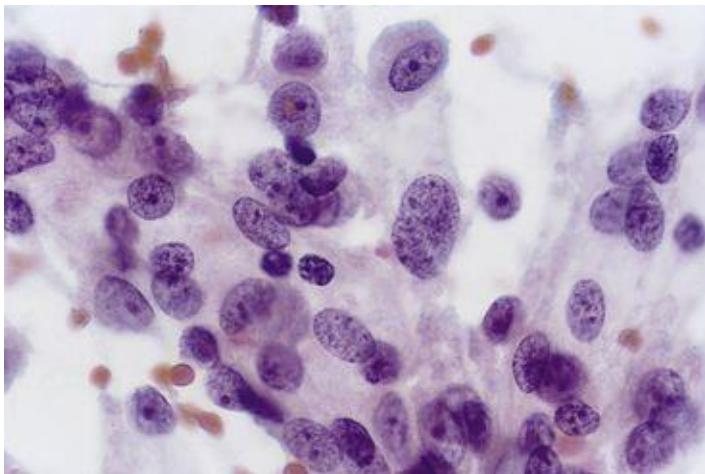


FIGURE 10.24 Poorly differentiated thyroid carcinoma (PDTC). These tumors have a greater degree of nuclear atypia than the differentiated thyroid carcinomas (Papanicolaou stain).

The possibility of PDTC can be supported by the immunocytochemical proliferation index. The Ki67/MIB1 index in well differentiated carcinomas is usually low (percentage of positive cells is less than 10%), whereas the index in PDTC is in the range of 10% to 30%.⁵⁹

The differential diagnosis is broad in scope. The presence of intranuclear inclusions and grooves in many of these tumors suggests a papillary thyroid carcinoma, but the prominence of isolated cells is unusual and might suggest PDTC. Nuclear pleomorphism, if present, raises the possibility of an undifferentiated (anaplastic) carcinoma. Most notably, PDTC has a striking resemblance to MTC, but microfollicles with colloid support the diagnosis of PDTC. Immunohistochemistry can be useful: PDTCs are thyroglobulin-positive and calcitonin-negative.

A definitive preoperative (i.e., FNA) diagnosis of PDTC is not possible and can be made only with certainty by histologic examination.¹²⁵ FNA specimens are usually diagnosed as follicular neoplasms^{124,125} or as metastatic carcinoma.¹²⁶

Undifferentiated (Anaplastic) Carcinoma

One of the most rapidly fatal of all cancers, undifferentiated (or anaplastic) carcinoma of the thyroid is uncommon and represents less than 5% of malignant thyroid tumors.⁵⁹ Despite its relatively low prevalence, it accounts for more than half of all deaths from thyroid cancer in the United States. Most patients are over the age of 60. Clinically, undifferentiated carcinoma manifests as a rapidly growing neck mass that has often spread to adjacent structures by the time it is

diagnosed. Many patients present with hoarseness and dysphagia. Histologically, undifferentiated carcinomas are composed of spindle-shaped and epithelioid cells admixed with pleomorphic or osteoclast-type giant cells. Extensive necrosis is common, mitoses are numerous, and the Ki67/MIB1 proliferation index is high (often greater than 30% positive cells).⁵⁹ Some cases have a prominent neutrophilic component. In about one third of cases, undifferentiated carcinoma is associated with a differentiated thyroid cancer such as papillary carcinoma, suggesting that many if not all undifferentiated carcinomas represent a dedifferentiation of a pre existing differentiated thyroid cancer.^{127,128} Undifferentiated carcinomas are usually positive for keratins and PAX8 but negative for thyroglobulin and usually negative for TTF-1.¹²⁹

By the time they are diagnosed, many have extended beyond the thyroid into adjacent structures. Although surgery is often considered for palliation, complete excision is often impossible. Radiotherapy is of benefit for controlling local disease. The 5-year survival rate is 0 to 14%.



Cytomorphology of undifferentiated (anaplastic) carcinoma

- many noncohesive cells
- large cells
- epithelioid or spindle-shaped
- marked nuclear pleomorphism
- multinucleated giant cells
 - pleomorphic, tumor type
 - osteoclast-type

The cytologic appearance is variable, reflecting the diversity of histologic patterns possible.¹³⁰ The cells can be arranged as large fragments, small clusters, or isolated cells ([Fig. 10.25](#)). Often a tumor diathesis, including many necrotic cells, is noted. Tumor cells can be spindle-shaped or epithelioid, with squamoid features. Nuclei are hyperchromatic and vary widely in size and shape. Extremely large, pleomorphic, and bizarrely shaped cells are seen, along with multinucleated tumor giant cells. Some FNA specimens contain numerous osteoclast-type giant cells. The nuclear features are unmistakably malignant: The large, pleomorphic nuclei have irregular nuclear membranes, coarse and irregular chromatin clumping, and macronucleoli. Intracellular inclusions are

common, and mitotic figures may be numerous. In some cases, undifferentiated carcinoma cells are accompanied by a distinct differentiated thyroid cancer component such as papillary carcinoma.

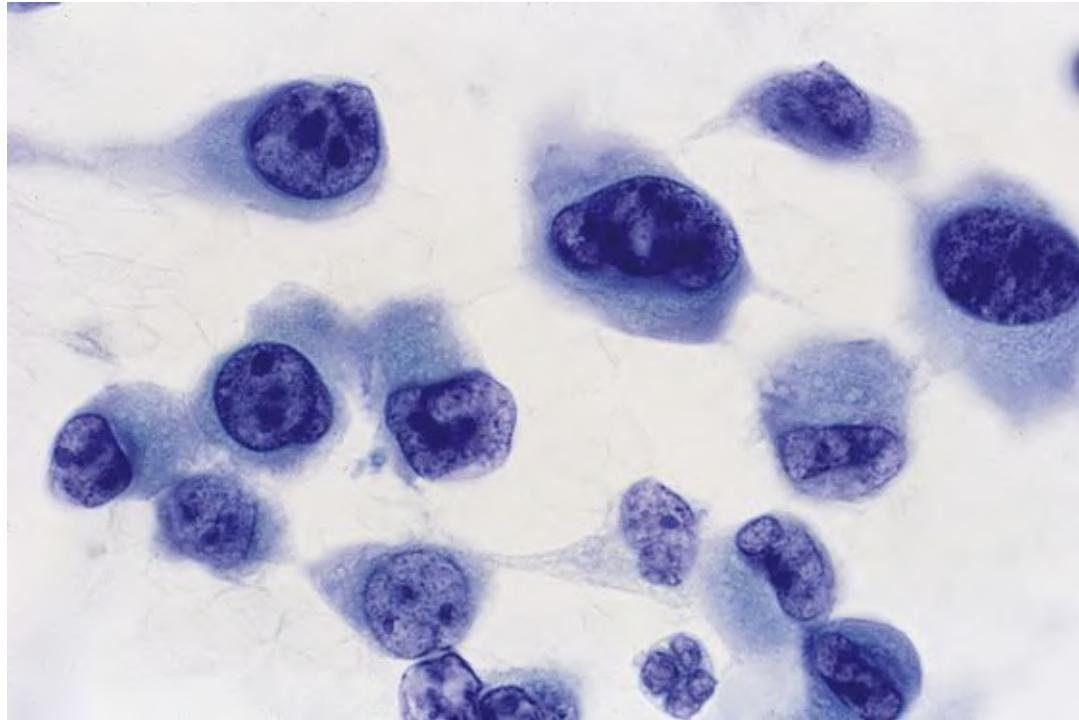


FIGURE 10.25 Undifferentiated (anaplastic) carcinoma.
Tumor cells are dispersed as isolated cells. Nuclei are large, hyperchromatic, and irregularly shaped (Papanicolaou stain).



Differential diagnosis of undifferentiated (anaplastic) carcinoma

- atypia of cyst lining cells
- ^{131}I treatment effect
- medullary thyroid carcinoma
- sarcoma
- metastatic carcinoma

MNGs and other benign follicular nodules with cystic degeneration contain a small number (usually) of atypical cells. Although the nuclei of these cyst lining

cells are enlarged, they have smooth and regular nuclear membranes, finely granular chromatin, and small, regular nucleoli. Radioiodine effect can produce wild variations in the nuclear size of affected follicular cells, but the pleomorphism of nuclear shape, mitotic activity, and necrosis of undifferentiated carcinoma are not seen.

The differential diagnosis includes medullary carcinoma, sarcoma, and metastatic tumors to the thyroid. Sarcomas can be excluded with an immunohistochemical panel that includes cytokeratins, muscle markers (desmin, Myo-D1, myogenin), and vascular markers (CD31, CD34, ERG). Metastatic malignancy is probably the most important consideration. Most undifferentiated carcinomas are immunoreactive for keratins and PAX8 and negative for thyroglobulin and TTF-1.^{59,127,128} This immunoprofile is not especially helpful in excluding metastatic carcinoma. The distinction rests heavily on clinical correlation and imaging studies; undifferentiated carcinoma is ultimately a diagnosis of exclusion.

Squamous Cell Carcinoma

Squamous cell carcinoma (SQC) of the thyroid accounts for 1% or less of thyroid cancers. Like undifferentiated carcinoma, it occurs in the elderly, and it has a similarly dismal prognosis. Histologically, it is defined as a tumor with squamous differentiation throughout. Most are poorly differentiated. Cytologic preparations show pleomorphic keratinized cells ([Fig. 10.26](#)). The differential diagnosis includes undifferentiated carcinoma and metastatic SQC. Clinical correlation and imaging studies are critical for excluding a metastasis.

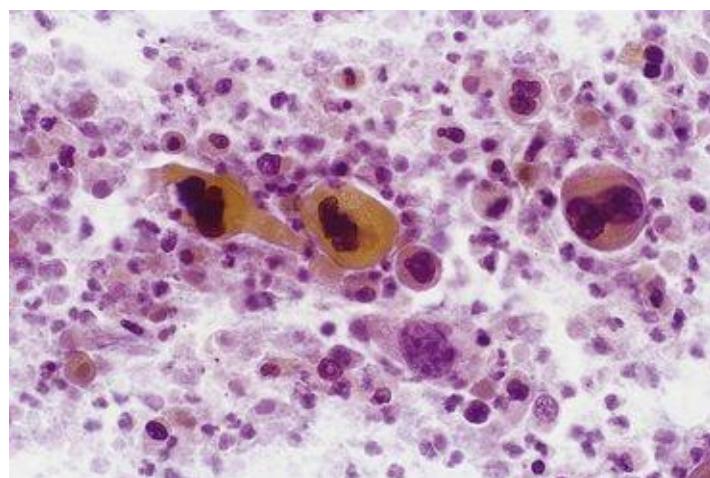


FIGURE 10.26 Squamous cell carcinoma (SQC).

A pleomorphic malignancy in the thyroid that is comprised of keratinized cells is considered an SQC, but it has the same clinical behavior as an undifferentiated carcinoma (Papanicolaou stain).

Medullary Thyroid Carcinoma

MTC accounts for 5% to 10% of all thyroid carcinomas.⁵⁹ Unlike the previously discussed thyroid neoplasms, it arises not from the follicular cells but from the parafollicular (C) cells, whose function is to synthesize and secrete calcitonin, the calcium-regulating hormone. About 80% to 90% are sporadic and occur in adults, with a mean age of 50 years. The rest occur in children in association with genetic syndromes such as the multiple endocrine neoplasia (MEN) syndromes. Patients with MEN 2a (Sipple syndrome) develop MTC and pheochromocytoma, sometimes with a hyperplasia or an adenoma of the parathyroid. Patients with MEN 2b (mucosal neuroma syndrome) have MTC, pheochromocytoma, multiple mucosal neuromas, and a marfanoid habitus. Because close to 90% of MTCs secrete calcitonin, serum can be tested as a screen for these tumors.

Up to 50% of patients with MTC present with regional node metastases. For this reason, MTC should be considered in the differential diagnosis for a patient with a positive neck node and an unknown primary. Histologically, MTC is highly variable. Tumor cells are arranged in sheets, nests, or ribbons and can be polygonal, round, plasmacytoid, or spindle-shaped. Nuclei are round or oval, and nucleoli are usually not prominent. Intranuclear pseudo-inclusions, indistinguishable from those in papillary carcinoma, are seen. Occasional cells can be markedly enlarged and pleomorphic. Amyloid deposits are seen in 80% of cases and can be confirmed with the Congo red stain. MTCs are immunoreactive for calcitonin, TTF-1, CEA, and chromogranin; they are negative for thyroglobulin.

MTCs are usually treated with total thyroidectomy with excision of regional lymph nodes.¹⁰⁸



Cytomorphology of medullary thyroid carcinoma

- numerous noncohesive cells
- loose clusters
- epithelioid, plasmacytoid, and/or spindle-shaped cells
- nuclei
 - round or elongated

- granular chromatin
- inconspicuous nucleolus
- pseudoinclusions (seen in 50% of cases)
- multiple nuclei
- red cytoplasmic granules (seen in 70% of cases)
- amyloid

The cytologic picture is variable.¹³¹ Tumor cells are predominantly isolated ([Fig. 10.27A](#)), but some loose clusters and rosettes are seen ([Fig. 10.27B](#)). They are usually uniform in size and shape, but many cases contain at least a few alarmingly large cells. Cytoplasm is moderate or abundant and finely granular. Red cytoplasmic granules are observed with Romanowsky-type stains in some, but not all cases² (see [Fig. 10.27B](#)). Nuclei are eccentrically placed, which gives these cells a plasmacytoid appearance, and binucleation and multinucleation are common. In some cases the cells are decidedly spindle-shaped, and the eccentrically placed nucleus makes the cell look like a comet with a long cytoplasmic tail. Nuclei have a coarsely granular, “salt and pepper” chromatin texture and inconspicuous nucleoli. Intranuclear inclusions are found in about one half of the cases.¹³¹ Amyloid is present in most but not all cases ([Fig. 10.27A](#)). It is similar to colloid but can be identified using the Congo red stain, which shows apple-green dichroism with polarized light.

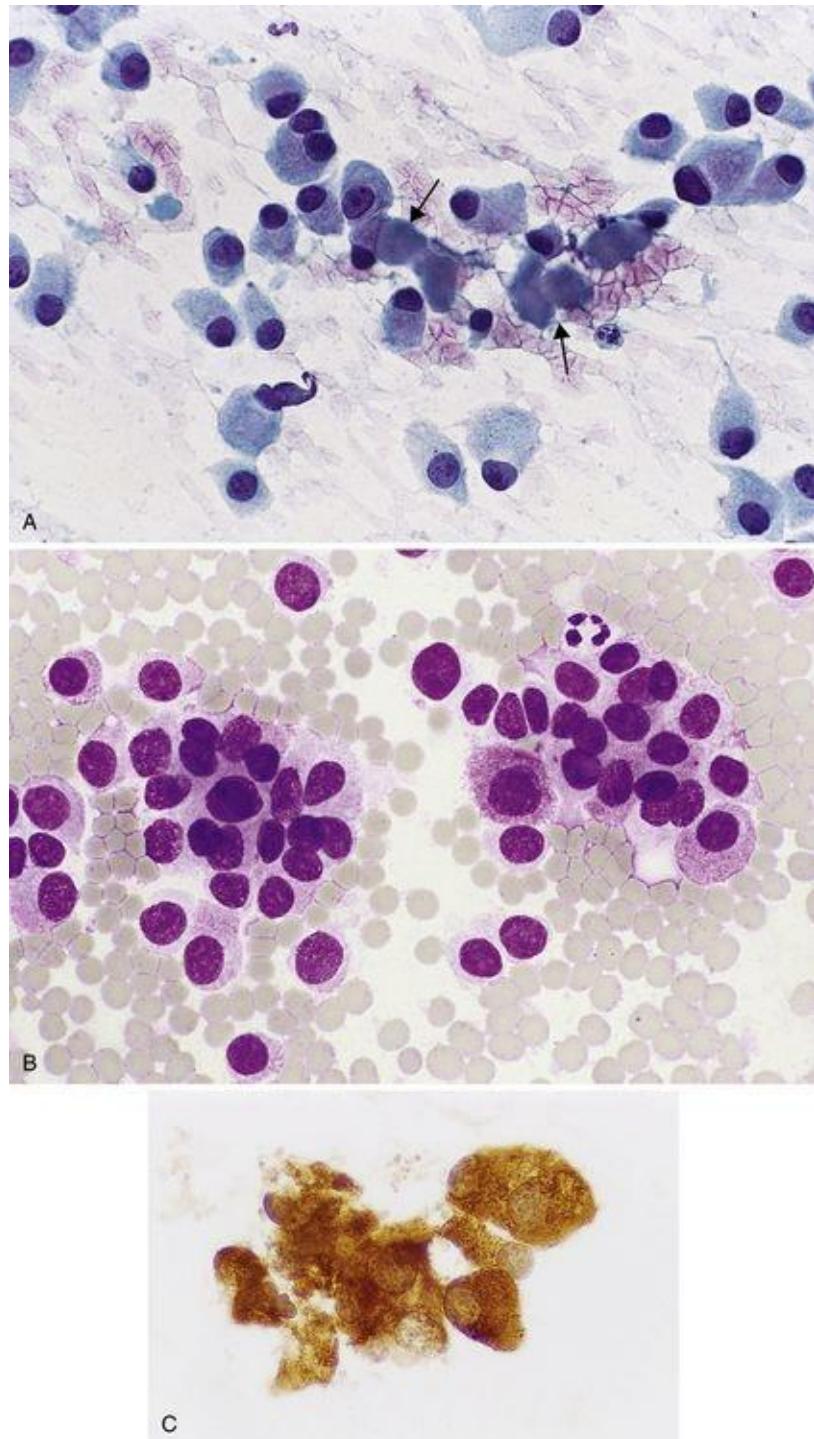


FIGURE 10.27 Medullary thyroid carcinoma (MTC).
A, Smears show numerous isolated cells and small blobs of amyloid (arrows) (Papanicolaou stain). B, Air-dried Romanowsky-stained preparations show fine red cytoplasmic granules, a helpful diagnostic feature. (Courtesy Dr. James Cappellari IV, Wake Forest University, Winston-Salem, NC, USA.) C, The malignant cells are strongly immunoreactive for calcitonin.



Differential diagnosis of medullary thyroid carcinoma

- undifferentiated carcinoma
- Hürthle cell neoplasm
- papillary thyroid carcinoma
- poorly differentiated carcinoma
- metastatic tumor

The large, pleomorphic cell component of some MTCs raises the possibility of an undifferentiated carcinoma, but the more prominent well-differentiated component of MTCs is not typical of undifferentiated carcinoma. A Hürthle cell neoplasm is a frequent consideration, because the cells of both neoplasms are often dispersed as isolated cells with moderate to abundant cytoplasm. But macronucleoli are rarely seen in MTC, and Hürthle cell neoplasms do not have “salt and pepper” chromatin. The color of the cytoplasmic granules with the Romanowsky-type stains can provide an additional clue: If red, the tumor is more likely to be an MTC. Papillary thyroid carcinoma and MTC have intranuclear inclusions, but other nuclear and architectural features help distinguish the two. MTC and poorly differentiated thyroid carcinoma can be difficult to distinguish by cytromorphology alone. The same applies for certain metastatic tumors, particularly melanoma. Immunohistochemistry is helpful to confirm the diagnosis of MTC ([Fig. 10.27C](#)). Virtually all cases of MTC are immunoreactive for calcitonin, TTF-1, chromogranin, and CEA. If immunohistochemistry cannot be performed, a serum calcitonin level can be obtained. Serum calcitonin levels are elevated in virtually all patients with MTC.⁵⁹

Lymphoma

Primary thyroid non-Hodgkin lymphoma represents 5% of thyroid cancers.⁵⁹ It typically occurs in older-aged women, and virtually all lymphomas arise in the setting of Hashimoto thyroiditis (HT). The relative risk for a patient with HT is about 40:1 to 80:1 compared with the general population, but primary thyroid non-Hodgkin lymphoma is still a very rare complication.⁸⁰ When it does occur, it is usually 20 to 30 years after the onset of HT, and the mean age of patients is 65 years. Most patients present with a noticeably enlarging mass, and compressive

symptoms (dyspnea, dysphagia, hoarseness) occur in about one third.¹³² The average size is 7 cm, but tumors as big as 20 cm have been reported.¹³² In some patients, cervical lymph nodes are involved.

These cases fall into three general categories: extranodal marginal zone B-cell lymphoma, diffuse large B-cell lymphoma, and mixed marginal zone lymphoma/diffuse large B-cell lymphoma type. Other types have been reported but are distinctly less common.^{59,132}



Cytomorphology of thyroid lymphomas

Extranodal marginal zone B-cell lymphoma

- small lymphoid cells
 - centrocytes
 - plasma cells
 - monocytoid B cells
- interspersed large lymphoid cells

Diffuse large B-cell lymphoma

- large lymphoid cells
- centroblasts
- immunoblasts
- Burkitt-like cells

Cytologic preparations are highly cellular and composed of lymphoid cells ([Fig. 10.28](#)). Lymphoepithelial lesions, characteristic of marginal zone lymphomas, are difficult to identify in cytologic preparations.

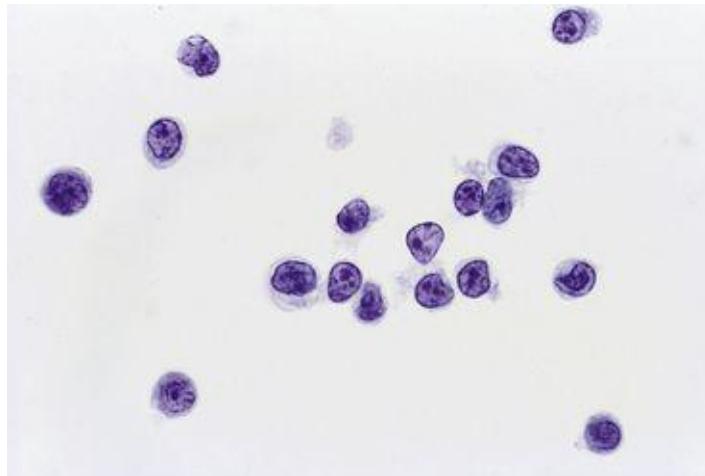


FIGURE 10.28 Extranodal marginal zone B-cell lymphoma of the thyroid. The neoplastic lymphoid cells are uniformly small, with irregularly shaped nuclei and a moderate amount of cytoplasm (Papanicolaou stain).

A lymphoma should be suspected in any patient with longstanding thyroiditis and a large or enlarging thyroid mass. The main consideration in the differential diagnosis is HT. Primary non-Hodgkin lymphomas that have a significant component of diffuse large B-cell lymphoma are the easiest to distinguish from HT because of the large, highly atypical lymphocytes. Marginal zone lymphoma is more difficult to distinguish cytomorphologically from HT. For this reason, demonstration of clonal restriction—by flow cytometry, immunocytochemistry, or molecular diagnostic methods—can be useful when lymphoma is suspected on clinical or cytomorphologic grounds. Caution is advised, because some HT specimens contain clonal B-cell populations that do not equate to malignancy and are thus misleading.^{81,82} For this reason, immunophenotyping and/or molecular genetic studies are recommended only if the clinical presentation (rapid growth, large nodule) or cytomorphology (monomorphic and/or atypical lymphoid population) suggests lymphoma. If immunophenotyping or molecular diagnostics are not available and the cytomorphology is equivocal, the interpretation “suspicious for malignancy,” qualified as “suspicious for non-Hodgkin lymphoma,” might be most appropriate (see [Table 10.1](#)). In such cases, a repeat FNA with allocation of cells for flow cytometry and/or molecular studies can be considered.

Metastatic Carcinoma

Metastatic tumors to the thyroid are encountered in 0.1% to 0.3% of thyroid aspirates.^{133,134} The lungs, esophagus, breast, and kidney are the most common

primary sites.¹³⁵ The possibility of a metastasis should be considered whenever the cytologic picture does not conform with common thyroid neoplasms and/or when the patient has a history of cancer elsewhere in the body. A clinical history of a known malignancy should be provided on the requisition.¹⁴ In 25% to 50% of cases, however, there is no previous history of malignancy, and the thyroid metastasis is the first manifestation of an occult malignancy.^{133,134} The distinction between a primary thyroid cancer and a metastatic lesion can be difficult. Some lung cancers can mimic undifferentiated carcinomas and squamous cell carcinomas of the thyroid; melanoma can mimic medullary carcinoma; and metastatic clear cell carcinoma of the kidney is a particularly good mimic of a follicular or Hürthle cell neoplasm (see [Fig. 10.16](#)).⁹⁹ Comparing the aspirated specimen with any previous histologic or cytologic material is very helpful, as is immunohistochemistry for thyroglobulin, calcitonin, and TTF-1.

Atypia of Undetermined Significance (or Follicular Lesion of Undetermined Significance)

Thyroid FNAs that are not easily classified into the benign, suspicious, or malignant categories are reported as “atypia of undetermined significance” (AUS) or “follicular lesion of undetermined significance” (the two terms are synonymous). This category is reserved for cases that “contain cells (follicular, lymphoid, or other) with architectural and/or nuclear atypia that is not sufficient to be classified as suspicious for a follicular neoplasm, suspicious for malignancy, or malignant. On the other hand, the atypia is more marked than can be ascribed confidently to benign changes. A contributing factor is often (but not always) a compromised specimen.”¹³⁶

The Bethesda System outlines nine AUS scenarios^{33,136} ([Table 10.2](#)). Examples are a sparsely cellular specimen but one that shows a predominance of microfollicles ([Fig. 10.29A](#)), a specimen with atypical cells that are difficult to classify because of obscuring blood ([Fig. 10.29B](#)), and a case with rare highly atypical cells that might represent cyst lining cells, but whose atypia extends beyond the usual, readily identifiable benign features.⁶¹ Similarly, a specimen composed exclusively of Hürthle cells is usually interpreted as suspicious, but in a patient with Hashimoto thyroiditis it is most likely to represent a benign hyperplastic Hürthle cell nodule and can be downgraded to AUS with an explanatory note.¹³⁷

TABLE 10.2
ATYPIA OF UNDETERMINED CYTOMORPHOLOGIC PATTERNS **SIGNIFICANCE:**

1. Predominance of microfollicles but sparsely cellular specimen
2. Predominance of Hürthle cells but sparsely cellular specimen
3. Interpretation of atypia hindered by preparation artifact
4. Cellular Hürthle cell sample in a patient with thyroiditis or nodular goiter
5. Focal nuclear atypia in an otherwise benign-appearing sample
6. Atypical cyst lining cells
7. Focal marked anisonucleosis
8. Atypical lymphoid infiltrate
9. Other

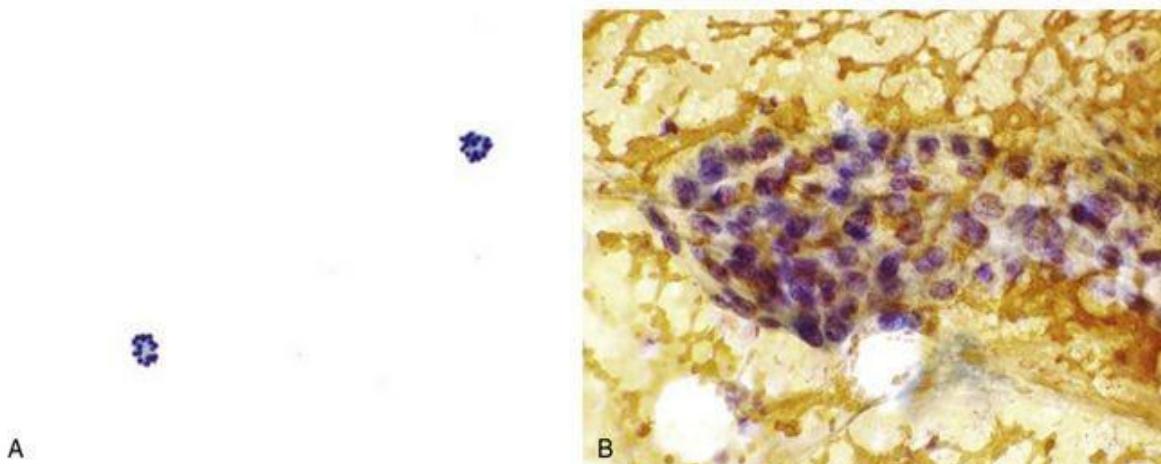


FIGURE 10.29 Atypia of undetermined significance (AUS).

A, This sample is composed mostly of microfollicles, but it is sparsely cellular (Papanicolaou stain). B, In another case, the follicular cell nuclei are enlarged and irregular in contour, but they are challenging to classify because of obscuring blood and clotting elements (Papanicolaou stain).

In reporting an AUS result, it is best to avoid phrases associated with malignancy like *pseudoinclusions*, *pale chromatin*, and *microfollicles*. Better to substitute generic phrases like *focal cytologic atypia* or *architectural atypia* so as to avoid confusion with the suspicious or malignant categories, which might prompt surgery rather than the intended more conservative management.¹³⁶

The frequency of an AUS result varies from laboratory to laboratory and

pathologist to pathologist, ranging from 2% to 29% of thyroid FNAs.^{11,12,138-140} An effort should be made to use this category as a last resort and limit its use to fewer than 7% of all thyroid FNAs.¹³⁶

The recommended management of an initial AUS interpretation is a repeat FNA after a reasonable interval (often 3 to 6 months), although in specific clinical settings, other management options might be more appropriate.^{12,46,136} In most cases, a repeat FNA results in a more definitive interpretation; only a minority of nodules are repeatedly AUS.^{12,140,141}

The risk of malignancy of an AUS nodule is difficult to ascertain because only a minority of patients with an initial AUS interpretation have surgical followup. Those that are resected represent a selected population of patients with worrisome clinical/sonographic findings or repeatedly atypical results. In this selected population, 20% to 41% of patients prove to have cancer after surgery, but this is undoubtedly an overestimate for all AUS nodules.^{11,12,54,139,141} Extrapolating for all AUS nodules, the risk of malignancy is probably closer to 5% to 15% (see [Table 10.1](#)).

Parathyroid Tumors

Parathyroid adenomas and the rare parathyroid carcinoma can be mistaken clinically for thyroid nodules. Cytologic preparations are cellular and show cohesive sheets, ribbonlike cords, and occasional microacini ([Fig. 10.30](#)). Nuclei are round and have a coarsely granular chromatin pattern; nucleoli are small or prominent. Cytoplasm is moderately abundant and granular. Isolated cells and naked nuclei can be present. Focal nuclear pleomorphism is seen, but it is not prominent. Colloid is absent.

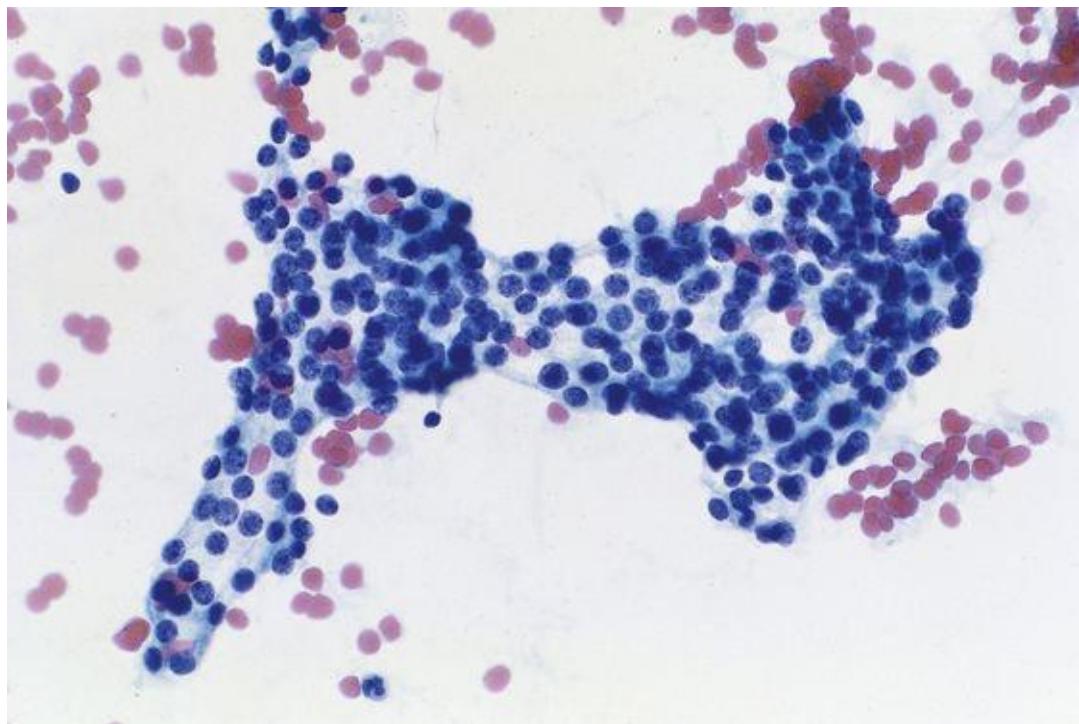


FIGURE 10.30 Parathyroid adenoma.

Smears are often highly cellular, and the cells are arranged in groups that resemble macrofollicle fragments or, as seen here, crowded trabeculae (Papanicolaou stain).

Parathyroid adenomas are frequently mistaken cytologically for a follicular lesion of the thyroid.[142,143](#) Most patients with a parathyroid adenoma have hypercalcemia, which is a clue to the correct diagnosis. Immunohistochemistry for thyroglobulin and parathyroid hormone can be helpful if a nonthyroid origin is suspected.

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CHAPTER 11

Salivary Gland

Jeffrey F. Krane and William C. Faquin

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Rationale, Indications, and Technical Considerations

Any unexplained salivary gland mass is an indication for fine-needle aspiration (FNA). FNA is the preferred biopsy method because incisional biopsy is associated with an increased risk of infection and potential contamination of surgical planes. FNA, by contrast, is a cost-effective technique¹⁻¹⁰ that poses minimal risk to the patient. The few contraindications to FNA are a bleeding disorder (predisposing the patient to hematoma formation) and acute sialadenitis (associated with aspiration-related pain); obscuring blood or inflammatory cells, in these settings, can limit diagnostic interpretation.

Clinical and imaging findings cannot always establish the origin of tumors in the head and neck region. Thus, a primary goal of FNA is to distinguish salivary gland lesions from nonsalivary head and neck masses, especially those of lymph node origin, but also soft tissue lesions and skin and skin adnexal masses. Because the normal parotid gland contains more than 20 intraparotid and periparotid lymph nodes,¹¹ primary lymphomas and metastatic tumors mimicking salivary gland neoplasms are commonly seen.

Some authors have argued that FNA is superfluous, because mass lesions in the head and neck always require excision.^{12,13} Such an approach would result in the unnecessary excision of a number of lesions (up to one third of salivary gland masses) that in fact do not necessitate surgery. Some patients have a nonneoplastic lesion (e.g., chronic sialadenitis, granulomatous disease, lymphoepithelial cyst); others have a benign neoplasm, and for those who are poor surgical candidates, knowledge of its benignity provides reassurance that surgery can be avoided. Still others have a malignancy for which surgical excision is not appropriate (e.g., lymphoproliferative disease, metastasis). Even when surgery is indicated, FNA guides preoperative strategy (e.g., partial or total parotidectomy, facial nerve resection, neck dissection), providing reassurance to both the patient and surgeon.^{2,12} Surgical management is often influenced by cytologic findings. High-grade malignancies are treated more aggressively than low-grade malignancies and benign neoplasms. In the superficial parotid gland, the most common site of salivary gland neoplasms, benign tumors and low-grade malignancies are treated with superficial parotidectomy alone, whereas high-grade carcinomas are treated with total parotidectomy requiring facial nerve sacrifice. Furthermore, lymph node neck dissection and neoadjuvant therapy are often indicated for high-grade tumors.

Aspirations are performed using a 25 or 23 gauge needle, small enough to

reduce the risk of tissue trauma but large enough to obtain an adequately cellular sample. Evaluation of smears at the time of the procedure can guide the number of passes required by assessing adequacy and also facilitate appropriate triage of the specimen, like allocation of material for flow cytometry (in the case of lymphoid lesions) or cell block preparation (in the case of diagnostically challenging lesions like oncocytic, spindle cell, and clear cell tumors).

Although FNA was first described in the 1930s,^{14–16} salivary gland FNA is a relatively new discipline, having gained wide acceptance only in the past 40 years.^{1,2,5,9,17–27} Metaanalysis of published series indicates 96% sensitivity and 98% specificity for neoplasia, whereas distinction between benign and malignant neoplasms has 79% sensitivity and 96% specificity.²⁸ Because accuracy varies greatly among published studies, the utility of salivary gland FNA is highly practitioner-dependent.²⁸ For nondiagnostic or indeterminate aspirates, results improve with repeat FNA²⁹ and ultrasound guidance.³⁰ Limited data indicate that core biopsy offers improved accuracy but at a cost: increased requirement for anesthesia, more discomfort for the patient, and a greater risk of complications, including nerve damage, tumor spillage, and needle tract seeding.^{28,31–33}

Criteria for assessing adequacy have yet to be established. False-negative results are principally the consequence of inadequate sampling of the lesion,³⁴ most frequently those that are cystic. Cystic neoplasms (e.g., Warthin tumor [WT], low-grade mucoepidermoid carcinoma, metastatic squamous cell carcinoma) are the most likely causes of false-negative diagnoses. Cysts frequently yield nonspecific fluid not representative of the underlying lesion. When a cystic salivary gland lesion is aspirated, any residual mass should be resampled after fluid is withdrawn. Surgical excision is indicated if the cyst does not resolve with aspiration or if it recurs.

False-negatives resulting from an interpretation error are most common with low-grade mucoepidermoid carcinoma, adenoid cystic carcinoma, and non-Hodgkin lymphoma. False-positive diagnoses are seen with cystic lesions, particularly WT, which sometimes contains atypical squamous cells, and pleomorphic adenoma (PA), in which nuclear atypia or stromal spheres result in an incorrect diagnosis of carcinoma ex pleomorphic adenoma or adenoid cystic carcinoma, respectively. Multiple passes help to minimize sampling and interpretative errors resulting from variation in cytologic atypia and cellular constituents within a tumor.

Complications are infrequent. Tumor seeding of the needle tract is extremely rare.^{12,35} The most common complications are bleeding, infection, and facial nerve pain. In some cases, FNA leads to partial or, rarely, complete infarction of the neoplasm.^{36–38} Particularly susceptible neoplasms are oncocytoma, WT, and acinic

cell carcinoma.^{12,39} With use of a 25 gauge needle, significant infarction or hemorrhage is seen in up to 10% of cases, but such changes rarely hinder histologic diagnosis.³⁹

Both Romanowsky-type and Papanicolaou stains are important, because many neoplasms contain a combination of epithelial and stromal components. Air-dried Romanowsky-stained smears highlight diagnostically useful features of the stromal component that are poorly visualized in alcohol-fixed preparations of lesions such as PA basal cell tumors, and adenoid cystic carcinoma. Romanowsky stains also aid in the evaluation of lymphoid lesions. Papanicolaou-stained preparations are especially useful for evaluating nuclear features and cytoplasmic differentiation.

Either smears or liquid-based preparations can be utilized,⁴⁰⁻⁴² but conventional smears are preferred. With liquid-based preparations, extracellular constituents are less prominent, cellular shrinkage is greater, and tissue fragmentation is more pronounced, with possible decreased sensitivity and specificity.⁴⁰ Although the role of immunohistochemical stains is limited, a cell block is valuable for histochemical stains such as periodic acid–Schiff (PAS) and mucicarmine. Cell block preparations also better demonstrate architectural patterns and some cellular features, particularly serous acinar differentiation.⁴³ In challenging cases, cytogenetic evaluation is also valuable. Characteristic chromosomal translocations have been identified in PA mucoepidermoid carcinoma, adenoid cystic carcinoma, mammary analogue secretory carcinoma, and clear cell carcinoma.⁴⁴⁻⁴⁹

Diagnostic Overview

Precise classification of salivary gland neoplasms by FNA is possible for many of the commonly encountered lesions but remains problematic for a number of the less common entities. Salivary gland FNA, in fact, poses a number of challenges to the cytopathologist. First, there are more than 35 salivary gland tumors of epithelial type,^{50,51} many of which are rare, placing familiarity with them out of reach for most practitioners. Second, most salivary gland malignancies are low-grade, displaying few overt cytologic features of malignancy. Third, the less common, high-grade malignancies are readily recognizable as malignant but are difficult to distinguish from one another. Fourth, some benign tumors (e.g., basal cell adenoma) have a malignant counterpart (e.g., basal cell adenocarcinoma) that is morphologically identical except that there is an infiltrative growth pattern, something that cannot be assessed cytologically. Finally, many different salivary gland tumors share similar cellular constituents; it is the architectural relationship and relative abundance of these constituents that ultimately determine the tumor type. With some tumors such as PA there is great variability not just within a given tumor but from one tumor to the next.

Fortunately for the cytopathologist, there are two mitigating factors. First, the two most common neoplasms, PA and WT, which together account for greater than 80% of salivary gland tumors, have distinctive cytomorphologic features and are readily identified. Second, conservative excision is used for both benign tumors and low-grade malignancies. By contrast, radical surgical approaches with combined-modality therapy are reserved for high-grade malignancies. Thus, although a specific diagnosis may not be feasible, low-grade neoplasms usually can be distinguished from high-grade ones, and then an appropriate differential diagnosis is sufficient for clinical management. This chapter provides a diagnostic approach and suggested reporting terminology for such cases.

The large group of so-called basaloid neoplasms are a special case, however. These neoplasms encompass the entire spectrum of biologic behavior, from benign neoplasms through low-grade malignancies to the aggressive solid variant of adenoid cystic carcinoma. A precise cytologic diagnosis is not possible with many FNA specimens showing basaloid cells only. A frozen section is often necessary to guide appropriate surgical management. This and other diagnostic dilemmas are listed in the following box. Suggested diagnostic approaches are discussed in detail in the remainder of the text.

Diagnostic dilemmas in salivary gland fine-needle aspiration

- basaloid neoplasms (especially basal cell adenoma and the solid variant of adenoid cystic carcinoma)
- oncocytic lesions (especially oncocytoma and acinic cell carcinoma)
- mucus-containing cysts (especially mucoepidermoid carcinoma, mucocele, and mucinous metaplasia)
- high-grade carcinomas (including mucoepidermoid carcinoma, salivary duct carcinoma, and carcinoma ex pleomorphic adenoma)
- clear cell neoplasms
- spindle cell lesions (especially myoepithelioma and schwannoma)

Clinical information, such as knowledge of a previous malignancy, is often helpful. Aggressive signs, symptoms, and imaging findings, such as rapid growth, pain (suggestive of neural invasion), and infiltrative growth on radiological studies, are indicative of malignancy. Most salivary gland neoplasms are firm and painless, although WT has a characteristically doughy consistency.

The presence of cystic change and bilaterality can also help narrow down diagnostic possibilities.

Tumors that are often cystic

- Warthin tumor (WT)
- mucoepidermoid carcinoma
- acinic cell carcinoma

Lesions that are sometimes bilateral

- sialadenitis
- amyloidosis
- lymphoepithelial cyst
- Warthin tumor (WT)
- acinic cell carcinoma
- lymphoma

The epidemiologic features of salivary gland neoplasms are also helpful. Most salivary gland neoplasms are more common in women, but WT, salivary duct carcinoma, and metastatic squamous cell carcinoma occur more frequently in men. Whereas 68% to 85% of parotid gland tumors are benign, 80% to 90% of sublingual and minor salivary gland neoplasms are malignant.⁵² Some tumors are almost site-specific (e.g., WT in the parotid gland, polymorphous low-grade adenocarcinoma [PLGA] in the minor salivary glands of the palate).

Attention to the constituents of an aspirate is the key to identifying the neoplastic, inflammatory, lymphoid, or cystic nature of a lesion. *Hypercellular specimens* are typical of neoplastic lesions. *Inflammatory cells* are prominent in sialadenitis and cystic lesions. *Stone fragments* are diagnostic of sialolithiasis. *Abundant lymphoid cells* can be seen in a variety of salivary gland lesions, not all of them lymphoid in nature.



Numerous lymphocytes are seen in:

- intraparotid or periparotid lymph node
- lymphoma
- lymphoepithelial cyst
- Warthin tumor (WT)
- mucoepidermoid carcinoma
- acinic cell carcinoma
- lymphoepithelial carcinoma

The presence and character of matrix material provide important diagnostic information. *Mucin* (pale magenta in Romanowsky preparations, translucent blue or purple on Papanicolaou smears) suggests a mucoepidermoid carcinoma, mucocele, retention cyst, or mucinous metaplasia. A *chondromyxoid matrix* is characteristic of PA and *stromal spheres* are typical of adenoid cystic carcinoma, but neither finding is entirely specific. Such findings, along with a more detailed impression of the cell type(s) seen (myoepithelial, duct-lining epithelial, basaloid, oncocytic, mucinous, squamous, acinic, sebaceous, and clear cells) enable one to refine the differential diagnosis.

A variety of crystalloids are seen in the salivary glands.^{53,54} Of importance, none of them is specific for any particular salivary gland lesion or neoplasm. *Tyrosine crystalloids* are floret-shaped and often encountered in pleomorphic adenomas, but they can be seen in other lesions, both benign and malignant ([Fig.](#)

11.1A).^{53–58} Amylase crystalloids (“nontyrosine crystalloids”) are polygonal, platelike, or needle-shaped and are most often seen in benign, nonneoplastic conditions, especially infections and cysts.^{59–62} (Fig. 11.1B).

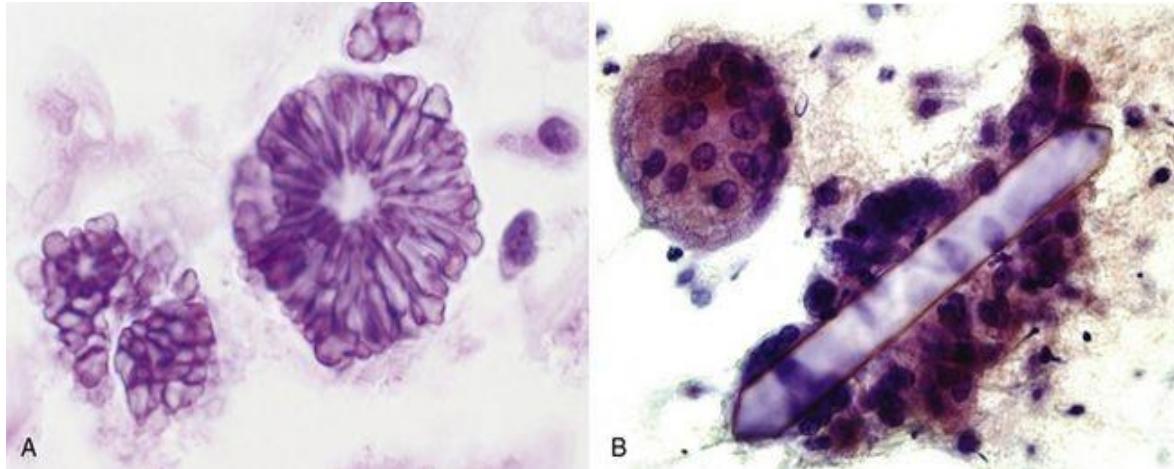


FIGURE 11.1 Salivary gland crystalloids.

A, Tyrosine crystalloids are clustered in flowerlike arrangements (hematoxylin and eosin [H & E] stain). B, Amylase crystalloids, needle-shaped or platelike, are occasionally encountered in salivary gland fine-needle aspirations (FNAs), usually in association with inflammatory conditions (Papanicolaou stain; Courtesy of Dr. David Kaminsky, Palm Springs Pathology Services, Palm Springs, CA).

The Normal Aspirate

In some instances, often as a result of sampling error, the FNA specimen shows only normal salivary gland elements.



Cytomorphology of normal salivary gland elements

- rounded clusters of serous or mucinous acinar cells
- flat sheets and tubules of ductal cells
- adipose tissue

Aspirates of normal salivary gland tissue are sparsely cellular, composed of acinar cells, ductal cells, and admixed adipose tissue⁴⁵³ ([Fig.11.2A](#)). Occasionally, naked acinar nuclei that mimic lymphocytes and scattered myoepithelial cells are present. Acinar cells are usually arranged in cohesive, grapelike clusters. Ductal cells are smaller and less conspicuous, arranged as tubules or honeycomb-like flat sheets. The acinar cells are of serous type in the parotid gland, a mixture of serous and mucinous types in the submandibular gland, and predominantly mucinous in the minor salivary glands. The serous-type acinar cells ([Fig. 11.2B](#)) are evenly spaced, with basally placed nuclei. They are large, pyramidal cells with abundant foamy and granular, basophilic cytoplasm and small, eccentrically placed, round to oval nuclei with indistinct nucleoli. Normal (and neoplastic) acinar cells have characteristic coarse, basophilic, PAS-positive and diastase-resistant cytoplasmic zymogen granules. In contrast with the pyramidal serous cells, mucinous cells are columnar, with pale cytoplasm that indents a bland nucleus. Ductal cells come in several varieties. Intercalated duct cells are uniform, small, and cuboidal, with scant, dense cytoplasm and uniform nuclei; occasional large branching ductal fragments are present. Ductal cells derived from the larger striated ducts are oncocytic, whereas those from the collecting ducts are columnar and ciliated. Ductal cells sometimes exhibit mucinous or squamoid changes. Mature lymphocytes can also be seen, owing to the abundance of intraparotid and periparotid lymphoid tissue. A mixture of ductal cells, adipose tissue, and acinar cells distinguishes normal salivary gland tissue from acinic cell carcinoma, which usually consists of a monomorphic population of neoplastic acinar cells.

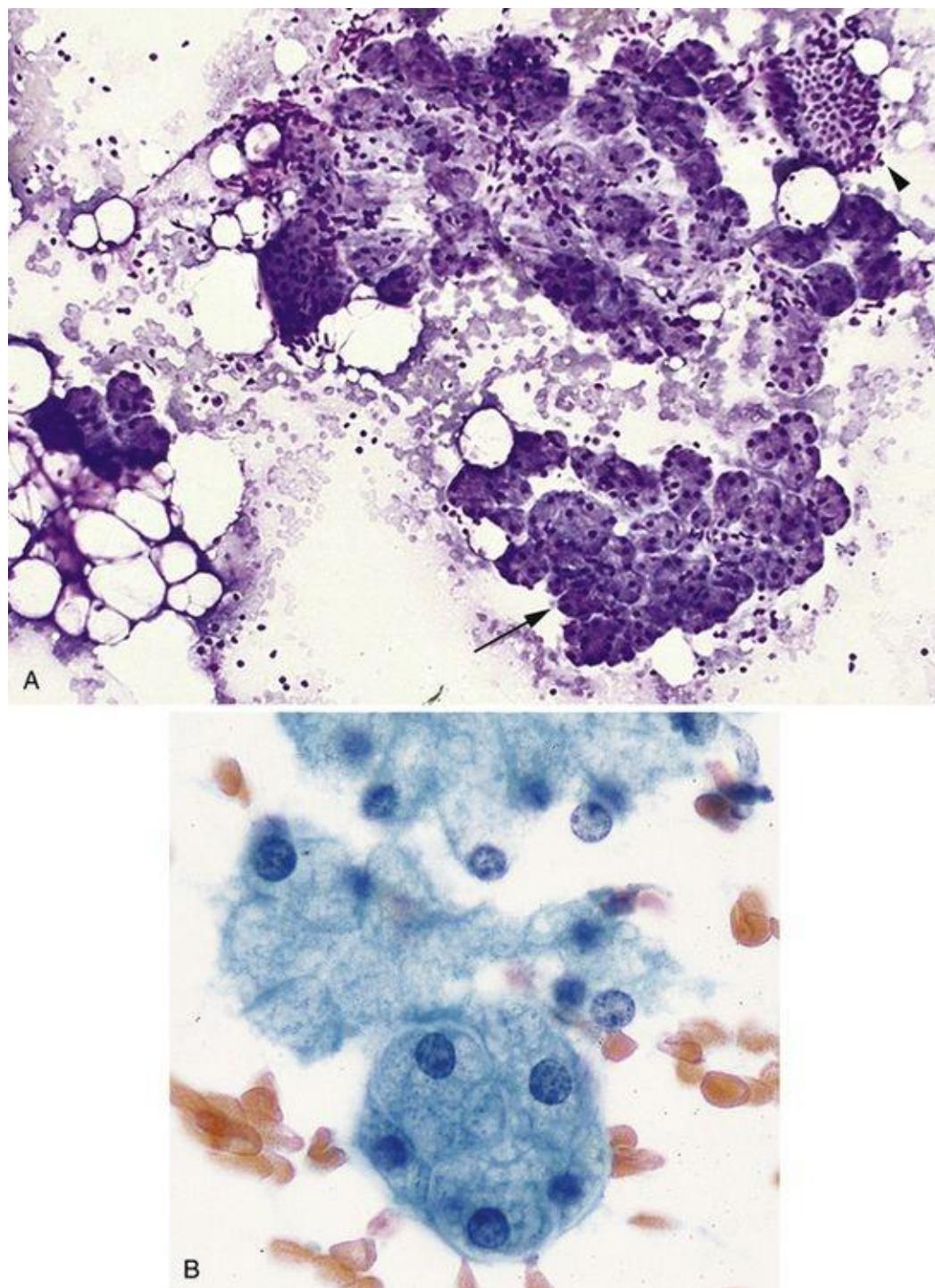


FIGURE 11.2 Normal salivary gland.

A, Normal acinar cells are aggregated in grapelike bunches (arrow) with admixed adipose tissue and an occasional flat sheet of ductal cells (arrowhead) (Romanowsky stain). B, Acinar cells have uniform, round nuclei and abundant vacuolated cytoplasm (Papanicolaou stain).

Up to 20% of salivary gland aspirates yield only normal tissue.^{5,63} A finding of only normal salivary gland elements in the FNA specimen warrants clinical

correlation to exclude the possibility of sampling error. Other explanations for a normal elements-only result include a prominent but normal salivary gland, sialadenosis, hamartoma, and lipoma.

NonNeoplastic Conditions

Acute and Chronic Sialadenitis

In acute sialadenitis, aspiration is rarely performed, because the disorder is usually diagnosed clinically as a postoperative complication, viral or fungal infection, or secondary bacterial infection due to an obstruction such as that caused by sialolithiasis⁶⁴⁻⁶⁷ (Fig. 11.3). There is usually no discrete mass, and in most cases the condition involves the parotid gland.

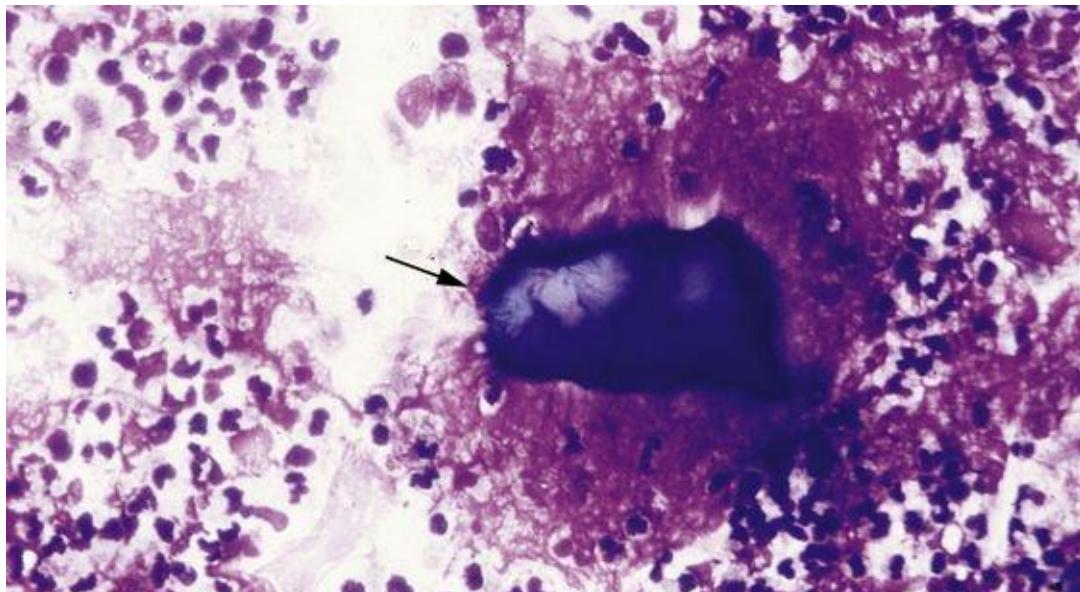


FIGURE 11.3 Sialolithiasis with acute sialadenitis.

Stone fragments (arrow) are blue, irregularly shaped, jagged structures of varying sizes that are diagnostic of sialolithiasis. The presence of numerous neutrophils signifies an accompanying acute sialadenitis (Romanowsky stain).



Cytomorphology of acute sialadenitis

- neutrophils, necrotic debris
- stone fragments (if sialolithiasis also present)
- scant ductal cells

Fine-needle aspirates from patients with acute sialadenitis show abundant neutrophils, necrotic cells, and fibrin.⁶⁴ Small groups of ductal cells, some with reactive atypia, are present. A malignant neoplasm is excluded based upon the hypocellularity and limited atypia. When an infectious etiology is suspected, a portion of the material should be sent for a microbiologic work-up. If there is a clinical suspicion of a neoplastic process, reaspiration after treatment and resolution of the acute infection may potentially be helpful.

As with acute sialadenitis, FNA in *chronic sialadenitis* can be associated with pain. Chronic sialadenitis is more likely to manifest as a clinically discrete mass, often in the submandibular gland. Common causes include sialolithiasis and radiation therapy for head and neck cancer (usually squamous cell carcinoma).



Cytomorphology of chronic sialadenitis

- scant cellularity
- small sheets and tubules of basaloid ductal cells with sharp borders
- paucity of acinar elements
- blood, proteinaceous debris, mature lymphocytes
- fragments of fibrous tissue

Aspirates of chronic sialadenitis are sparsely cellular. Clusters of small, basaloid ductal cells with sharp borders are admixed with blood, proteinaceous debris, mature lymphocytes, and small amounts of fibrous tissue ([Fig. 11.4](#)). Acinar cells are sparse or absent.⁶⁴ *Sialolithiasis* can be diagnosed when stone fragments are identified (see [Fig.11.3](#)). Atypical squamous metaplasia, mucinous metaplasia, radiation atypia, abundant histiocytes, extracellular mucin, crystals, and (rarely) psammoma bodies may be present.⁶⁸ *Chronic sclerosing sialadenitis (Kuttner tumor)* is a form of chronic sialadenitis affecting the submandibular gland and manifesting clinically as a firm mass that can be mistaken for a neoplastic process.^{50,69-71} The morphologic findings are nonspecific and similar to those with a conventional chronic sialadenitis. In some cases, lymphoid cells are abundant and lymphoma must be excluded.⁷¹ In a subset of chronic sclerosing sialadenitis cases, an increased proportion of IgG4-positive plasma cells is seen, and the condition is a form of systemic IgG4-related disease.⁷²

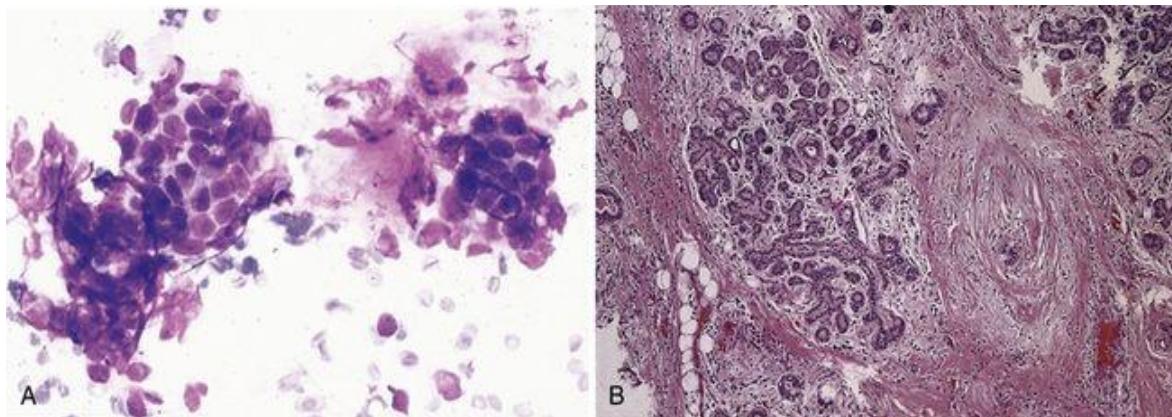


FIGURE 11.4 Chronic sialadenitis.

A, Sparsely cellular smears show small cohesive clusters of basaloid ductal cells that can be mistaken for the cells of a basaloid neoplasm (Romanowsky stain). B, The corresponding histologic specimen shows marked fibrosis, chronic inflammation, acinar atrophy, and residual ductal elements (hematoxylin and eosin [H & E] stain).



Differential diagnosis of chronic sialadenitis

- normal salivary gland
- lymphoepithelial sialadenitis (LESA)
- Warthin tumor (WT)
- basaloid neoplasms
- mucoepidermoid carcinoma
- squamous cell carcinoma

Normal salivary gland has a greater proportion of acinar to ductal cells than chronic sialadenitis. Chronic sialadenitis is distinguished from lymphoepithelial sialadenitis (LESA) by the absence of lymphoepithelial islands and germinal center fragments. Unlike WT, chronic sialadenitis lacks cohesive groups of oncocytes. The basaloid ductal cells of chronic sialadenitis can resemble the cells of basaloid neoplasms but are less numerous and arranged in smaller groups than those of neoplasms. The intermediate cells of a mucoepidermoid carcinoma resemble the basaloid ductal cells of chronic sialadenitis, but mature squamous cells and mucus cells are absent. Chronic sialadenitis can be separated from a squamous cell carcinoma by virtue of its scant cellularity; the absence of marked atypia, mitotic activity, and necrotic tumor cells; and the paucity of isolated epithelial cells.

Granulomatous Sialadenitis

Granulomatous sialadenitis can be caused by infection (fungal, mycobacterial, toxoplasmosis, or cat scratch disease), sarcoidosis, cyst rupture, and rarely neoplasia (Hodgkin lymphoma, T-cell lymphoma, or metastatic carcinoma).⁷³⁻⁷⁵ Aspirates are characterized by a variable background of granular, necrotic cell debris and inflammation, together with aggregates of epithelioid histiocytes, frequently with multinucleated forms. Epithelioid histiocytes have abundant eosinophilic cytoplasm and cytologically bland, elongated, folded nuclei with indistinct nucleoli. Asteroid bodies, Schaumann bodies, and calcium oxalate crystals can be present in sarcoidosis, but these findings are not specific, and an infectious etiology must be excluded by special stains and/or microbiologic cultures.⁷⁶

Sialadenosis

Sialadenosis is a nonneoplastic, noninflammatory enlargement of the salivary gland that more commonly affects the parotid gland and is often bilateral.^{64,77} It results from acinar cell hypertrophy, and has been associated with a wide range of etiologies, including endocrine abnormalities like diabetes mellitus, nutritional deficiencies, alcoholism, cirrhosis, and certain drugs, especially antihypertensives.^{50,65,77}

Aspirates of sialadenosis appear normal except that the constituent acinar cells are significantly larger than normal acinar cells, and inflammatory cells tend to be absent. Normal acinar cells are approximately 50 µm in diameter, whereas acinar cells of sialadenosis can measure up to 100 µm.^{50,64,65,77} In practice, these size differences are difficult to assess. Clinical correlation to exclude a discrete mass or neoplastic lesion is important before making a diagnosis of sialadenosis.

Lymphoepithelial Sialadenitis

LESA has been known by a variety of names, including Mikulicz disease, benign lymphoepithelial lesion, and myoepithelial sialadenitis.^{78,79} Resulting in part from the discovery that the cells composing the lymphoepithelial islands (formerly called *epimyoepithelial islands*) of this disorder are almost entirely epithelial, the term *lymphoepithelial sialadenitis* has emerged as the preferred designation for this disorder.^{78,80} LESA most frequently affects women and manifests as diffuse, often bilateral, enlargement of the parotid gland, as well as the submandibular gland in a minority of cases. Minor salivary glands show chronic inflammatory

changes related to LESA but lack the lymphoepithelial islands. LESA is believed to be an autoimmune disorder and is seen in virtually all patients with Sjögren syndrome. Approximately 50% of patients with LESA do not have Sjögren syndrome, however. They may have some other connective tissue disorder or no disease whatever.⁸⁰



Cytomorphology of lymphoepithelial sialadenitis

- cellular aspirate
- mixed population of lymphocytes, plasma cells, tingible-body macrophages
- germinal center fragments
- lymphoepithelial islands

Aspirates are cellular and show a mixed population of mature lymphocytes, plasma cells, tingible-body macrophages, germinal center fragments, and characteristic lymphoepithelial islands: large, cohesive sheets of pale, overlapping, ductal-type cells infiltrated by lymphocytes ([Fig. 11.5](#)). The ductal cells often exhibit reactive and squamous metaplastic changes. Acinar cells are rarely present.

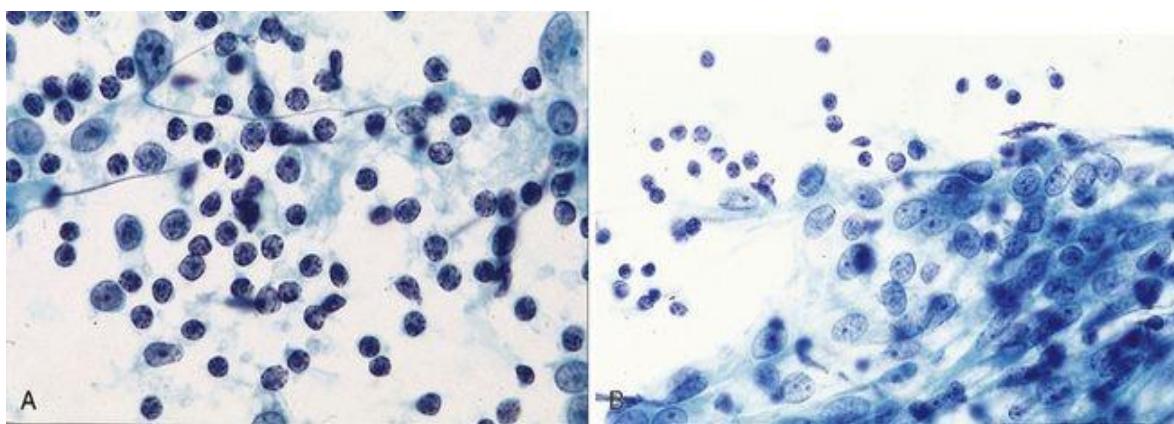


FIGURE 11.5 Lymphoepithelial sialadenitis (LESA).

A, Lymphoid cells, predominantly small lymphocytes, are usually abundant (Papanicolaou stain). B, The characteristic lymphoepithelial islands are composed of reactive epithelial cells admixed with lymphocytes (Papanicolaou stain).



Differential diagnosis of lymphoepithelial sialadenitis

- chronic sialadenitis
- simple lymphoepithelial cyst
- HIV-associated cystic lymphoepithelial lesions
- Warthin tumor (WT)
- extranodal marginal zone B-cell lymphoma

In chronic sialadenitis, the aspirate tends to be sparsely cellular, with fewer lymphocytes and germinal center fragments, and the characteristic lymphoepithelial islands of LESA are lacking. Simple lymphoepithelial cysts and human immunodeficiency virus (HIV)-associated cystic lymphoepithelial lesions are cytologically similar to the cystic form of LESA and are discussed further in the next section. WT is distinguished from LESA in that the former contains oncocytic epithelium. Perhaps most important, extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT) should be suspected if large numbers of monocyteid B cells are encountered. Ancillary studies to assess for clonality (flow cytometry, immunocytochemistry) can be invaluable in such circumstances.

NonNeoplastic Cysts

Cystic lesions account for approximately 5% of salivary gland FNAs. A wide variety of nonneoplastic and neoplastic lesions can be cystic.⁸¹ Nonneoplastic cysts, both congenital and acquired, occur either adjacent to or within the salivary glands.^{50,64} Diagnostically, these cysts can be broadly categorized into squamous-lined cysts and mucus-containing cysts.

Squamous-Lined Cysts

Squamous-lined cysts include *congenital cysts*, encompassing dermoid and branchial cleft cysts. Also in this category are the sporadic *simple lymphoepithelial cysts*, usually found within the parotid glands of middle-aged men.⁸⁰ Simple lymphoepithelial cysts are not related to HIV infection or Sjögren syndrome. Typically unilateral and solitary, they probably arise from either entrapped salivary duct tissue within intraparotid lymph nodes or branchial cleft remnants.⁸⁰ By contrast, *HIV-associated cystic lymphoepithelial lesions* are usually multiple and often bilateral.⁸² Squamous-lined cysts yield clear to turbid

yellow-brown fluid.



Cytomorphology of squamous-lined cysts

- cellular aspirate
- histiocytes
- keratin debris and anucleate squames
- small clusters of squamous (or columnar) cells
- mixed population of lymphocytes
 - with or without germinal center fragments with tingible-body macrophages
- absence of lymphoepithelial islands (except in HIV-associated cases and cystic LESA)

The aspirate findings are nonspecific, showing a mixed population of lymphocytes, often with germinal center fragments, tingible-body macrophages, keratin debris, squamous or columnar cyst lining cells, histiocytes, and proteinaceous debris^{83,84} ([Fig. 11.6A](#)). Within this group of lesions, finding epithelial cell clusters with interspersed lymphocytes (called *lymphoepithelial islands*) is indicative of either a cystic LESA or an HIV-associated cystic lymphoepithelial lesion. Clinical and radiographic correlation is helpful in this distinction.

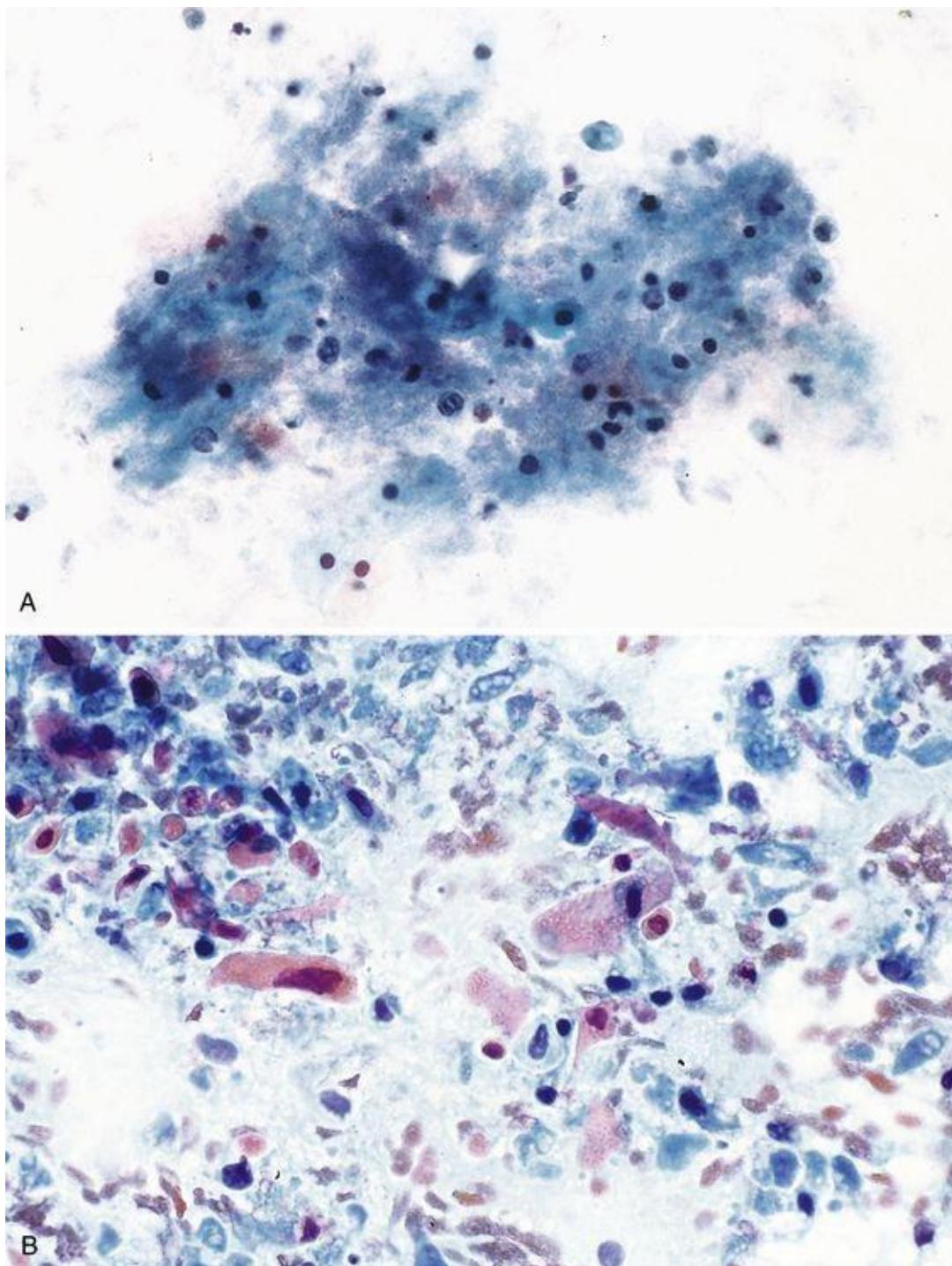


FIGURE 11.6 Squamous epithelial-lined cyst compared to squamous cell carcinoma (SQC). *A*, In a benign squamous-lined epithelial cyst, degenerated and anucleate squamous cells with minimal nuclear atypia are present. These findings are nonspecific (Papanicolaou stain). *B*, In contrast, keratinizing SQC has marked nuclear atypia, bizarre cell shapes, and necrosis (Papanicolaou stain).



Differential diagnosis of squamous-lined cysts

- pure lymphoid lesions (intraparotid lymph node, lymphoma)
- cystic salivary gland neoplasms (especially Warthin tumor, acinic cell carcinoma, and mucoepidermoid carcinoma)
- cystic metastatic squamous cell carcinoma (SQC)

The presence of an epithelial component distinguishes these cysts from a lymph node. Lymphoid elements do not help in the distinction from a cystic salivary gland neoplasm, because some neoplasms have a prominent lymphoid infiltrate. A lymphoma, especially of MALT type, can be excluded using flow cytometry, which demonstrates a polyclonal population of lymphoid cells in these benign cystic lesions. Distinction from a Warthin tumor (WT) can be especially difficult, because the oncocytes of a WT can show extensive squamoid differentiation. Identification of a salivary gland neoplasm rests on the recognition of the characteristic cell type(s) associated with that tumor (e.g., oncocytes, acinar cells, mucus, intermediate, and squamous cells). Most important, a cystic metastatic SQC must also be considered. Although squamous-lined nonneoplastic cysts can exhibit reactive atypia, metastatic SQC ([Fig. 11.6B](#)) contains necrotic cells, mitotic activity (including atypical mitoses), and more severe nuclear atypia. Nevertheless, a cystic well-differentiated SQC is occasionally difficult to distinguish from a benign squamous-lined cyst. Beware of diagnosing a nonmidline developmental cyst in an older adult. Such aspirates should be thoroughly screened to exclude malignant features. Even in the absence of definitive evidence of malignancy, it is prudent to report benign-appearing squamous-lined cysts descriptively and include the differential diagnosis of a developmental and lymphoepithelial cyst. An accompanying explanatory note can emphasize the need for clinical correlation to exclude a more significant lesion and a recommendation that any persistent mass be excised to exclude malignancy. The tonsil is often the primary site for SQC manifesting as a cystic lesion. Because tonsillar SQCs are frequently associated with human papillomavirus (HPV) infection, HPV testing of the aspirate can be helpful both in confirming the diagnosis and in identifying a likely primary site.^{[85,86](#)}

Mucin-Containing Cysts

The umbrella term *mucin-containing cysts* refers to a heterogeneous group of

lesions that include a malignant neoplasm (mucoepidermoid carcinoma), inflammatory conditions (chronic sialadenitis with mucinous metaplasia), and acquired cysts. Acquired cysts, comprising the mucocele and the retention cyst, occur more commonly in the submandibular and sublingual glands than in the parotid.⁵⁰ *Mucoceles* are pseudocysts because they lack an epithelial lining, whereas *retention cysts* are lined by squamous, columnar, or oncocytic epithelium. Both result from obstruction, usually by stones.



Cytomorphology of mucin-containing cysts

- sparsely cellular
- extracellular mucin
- histiocytes
- amylase crystalloids (nonspecific)
- scattered inflammatory cells

Aspirates are sparsely cellular and composed of histiocytes, some with intracellular mucin (“muciphages”), granular debris, extracellular mucin, amylase crystalloids (see [Fig. 11.1B](#)), and inflammatory cells ([Fig. 11.7](#)). Occasional metaplastic epithelial cells and normal salivary gland cells are also seen.

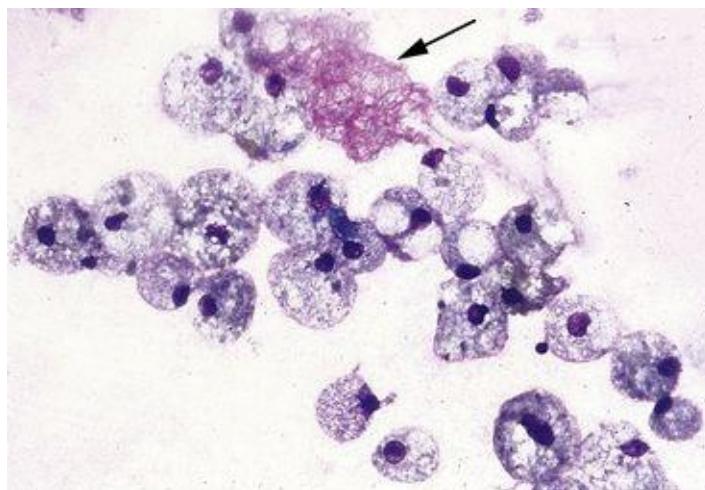


FIGURE 11.7 Mucus-containing cyst.

The combination of histiocytes and magenta-colored extracellular mucin (arrow) is a nonspecific finding. This example proved to be a retention cyst, but identical findings can be seen in a low-grade mucoepidermoid carcinoma (Romanowsky stain).



Differential diagnosis of mucin-containing cysts

- mucocele
- retention cyst
- chronic sialadenitis with mucinous metaplasia of ducts
- mucoepidermoid carcinoma

Chronic sialadenitis with mucinous metaplasia of ducts has a similar appearance to the acquired cysts; ciliated columnar cells suggest the diagnosis. The nonspecific constellation of findings described herein is also seen in some *low-grade mucoepidermoid carcinomas*, which, like mucoceles and retention cysts, contain muciphages. The following features, typical of a low-grade mucoepidermoid carcinoma, can help distinguish it from one of its benign mimics: a residual mass after aspiration, greater cellularity, more severe cytologic atypia, and at least an occasional cluster of intermediate and epidermoid cells.

Whenever a specimen contains extracellular mucin but minimal (or even absent) cellular atypia, an atypical interpretation is desirable nevertheless. Mucoepidermoid carcinoma MEC can be mentioned in the differential diagnosis, with an educational note. This diagnostic approach helps avoid a false-negative interpretation in the case of a low-grade mucoepidermoid carcinoma.



Sample report of a mucin-containing cyst

ATYPICAL.

Histiocytes (and epithelial elements) in a background of abundant mucin (see Note).

note: The differential diagnosis includes a mucocele, mucus retention cyst, chronic sialadenitis with mucinous metaplasia, and a mucoepidermoid carcinoma. If the lesion persists or recurs, excision should be considered.

Amyloidosis

Amyloidosis is a rare, often bilateral cause of focal or diffuse salivary gland enlargement.⁸⁷⁻⁸⁹ Characterized by variable amounts of acellular, eosinophilic extracellular material, the FNA amyloidosis specimen stains pale red with the

Congo red stain and exhibits a characteristic apple-green birefringence under polarized light. The remainder of the smear is often hypocellular, with scant or absent acinar cells and scattered groups of ductal cells such as are seen in chronic sialadenitis. Amyloid is also seen in association with an extramedullary plasmacytoma.⁹⁰

Benign Neoplasms

Pleomorphic Adenoma

PA is the most common tumor in all salivary glands, both in children and in adults. Two thirds of parotid tumors and 50% of all salivary gland tumors are PAs. The most commonly encountered site is the superficial parotid, often in the tail of the gland at the angle of the jaw. On physical examination, the nodule is firm, reflecting the abundance of chondromyxoid matrix. The aspiration is typically painless.



Cytomorphology of pleomorphic adenoma

- epithelial cells
- myoepithelial cells
- chondromyxoid matrix
- tyrosine crystalloids (nonspecific)

The epithelial cells are recognizable by their cohesive groupings, usually in a honeycomb pattern. When present as individual cells, epithelial cells are indistinguishable from myoepithelial cells. The myoepithelial cells have a variety of appearances: spindle-shaped (the most common), epithelioid, clear cell, and plasmacytoid. The plasmacytoid morphology is particularly common in palatal tumors. Unlike epithelial cells, myoepithelial cells are commonly found individually, within matrix material, in loose clusters, or in larger, haphazardly arranged clusters. The matrix material is the most characteristic finding.^{91,92} Fibrillary and “chondromyxoid” in appearance, it stains pale purple or blue in Papanicolaou-stained preparations and can be difficult to see and to distinguish from other extracellular material such as mucin ([Fig. 11.8A](#)). It is best appreciated on air-dried preparations stained with a Romanowsky-type stain, which gives it an intense magenta color that is “metachromatic,” meaning that the dye in the stain changes color through its interaction with the matrix material ([Fig. 11.8B](#)). Although the matrix of adenoid cystic carcinoma stains with a similar intensity, the features are otherwise distinctive.^{91,93} The characteristic fibrillary nature of the matrix in a PA can be appreciated by its frayed, indistinct

margins. Myoepithelial cells are readily identified embedded within it.

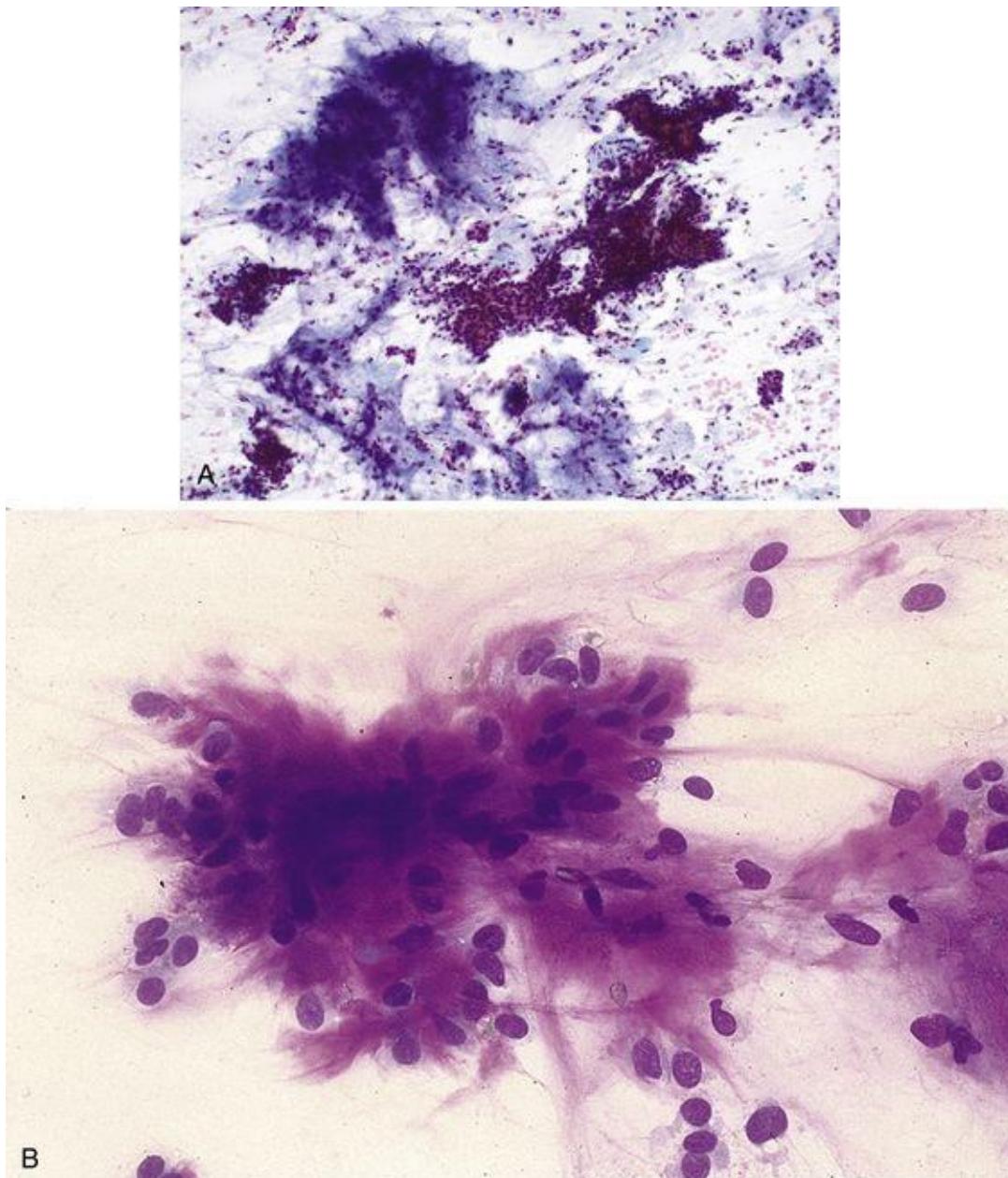


FIGURE 11.8 Pleomorphic adenoma (PA).

A, Pale blue matrix, myoepithelial cells, and sheets of ductal cells are identified at low power (Papanicolaou stain). B, In the air-dried preparation, magenta-colored fibrillary matrix has frayed edges. Isolated plump spindle-shaped myoepithelial cells are embedded within the matrix (Romanowsky stain).

The proportions of the cellular constituents are extremely variable from one

PA to another, and within an individual tumor—hence the importance of sampling different areas of the tumor with multiple passes. In at least two thirds of cases, the typical constituents are readily appreciated, without unusual features, and the diagnosis is straightforward.⁹⁴

Pitfalls Associated with Pleomorphic Adenomas

Sparse or absent matrix. The absence or paucity of matrix may preclude a definitive diagnosis of PA,^{92,95,96} but an appropriate differential diagnosis can still result in appropriate clinical management. The differential diagnosis includes basal cell adenoma or adenocarcinoma (in the case of an epithelial-rich lesion) and myoepithelioma (in the case of a myoepithelial-rich lesion).

Adenoid cystic–like matrix. Cylindromatous areas, raising the possibility of adenoid cystic carcinoma, are encountered in approximately 5% of PAs⁹⁴ ([Fig. 11.9A and B](#)). If a well-sampled lesion is otherwise typical for a PA, and these cylindromatous areas are rare, this finding is of no concern. If extensive, the diagnosis of adenoid cystic carcinoma, or the rare adenoid cystic carcinoma ex pleomorphic adenoma,⁹⁷ should be considered.

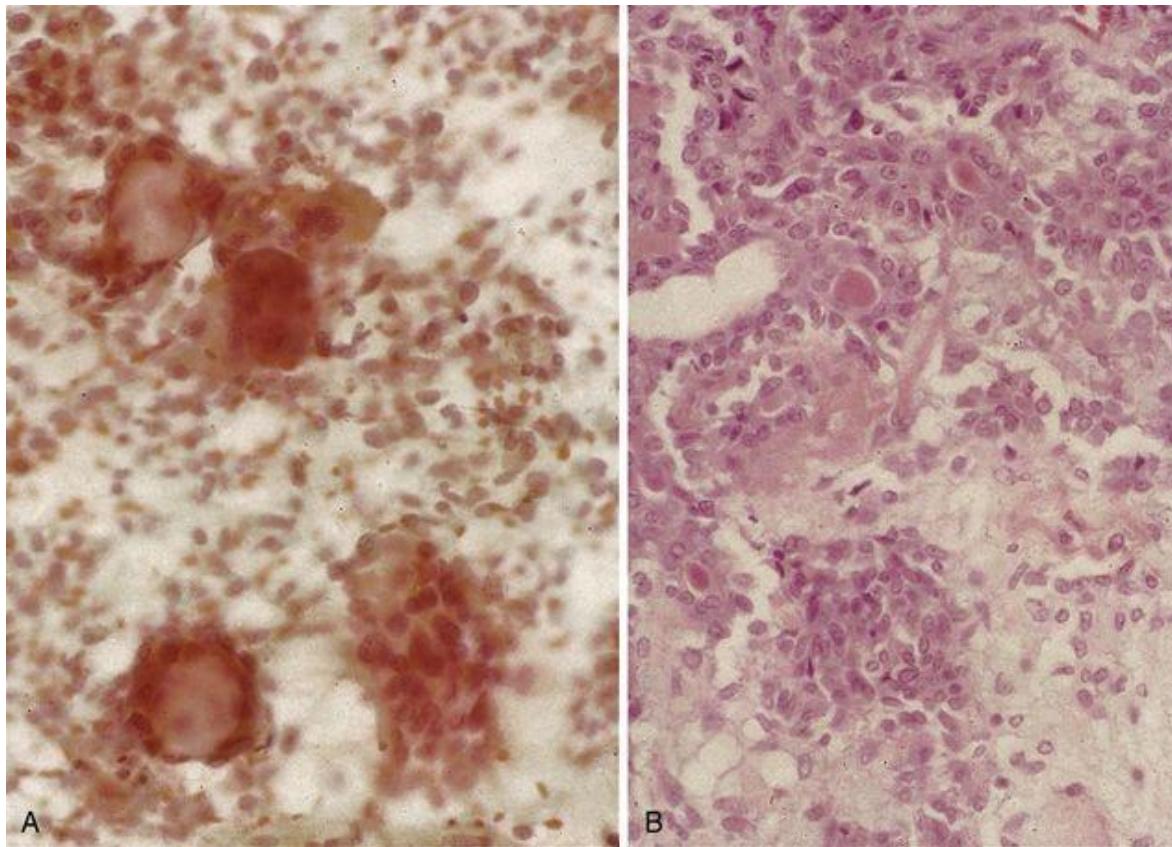


FIGURE 11.9 Pleomorphic adenoma (PA) with adenoid cystic-like foci.

A, Lucent cylinders are present in a background of myoepithelial cells (Papanicolaou stain). B, In the corresponding histologic specimen, cylinders are also seen. The findings in the aspirate and resected specimen were otherwise typical of a PA (hematoxylin and eosin [H & E] stain).

Cytologic atypia. Focal, mild cytologic atypia is seen in 20% of PAs⁹⁴ and causes little concern. Severe atypia, however, particularly if accompanied by necrosis or abundant mitoses, suggests the possibility of carcinoma ex pleomorphic adenoma. Most carcinomas ex pleomorphic adenoma are cytologically overtly malignant, and the challenge is recognizing the preexisting PA. Extensive atypia without overt malignant features ([Fig.11.10](#)) justifies the diagnosis of a *PA with atypia*, with an accompanying comment that the finding could be indicative of early malignant change.

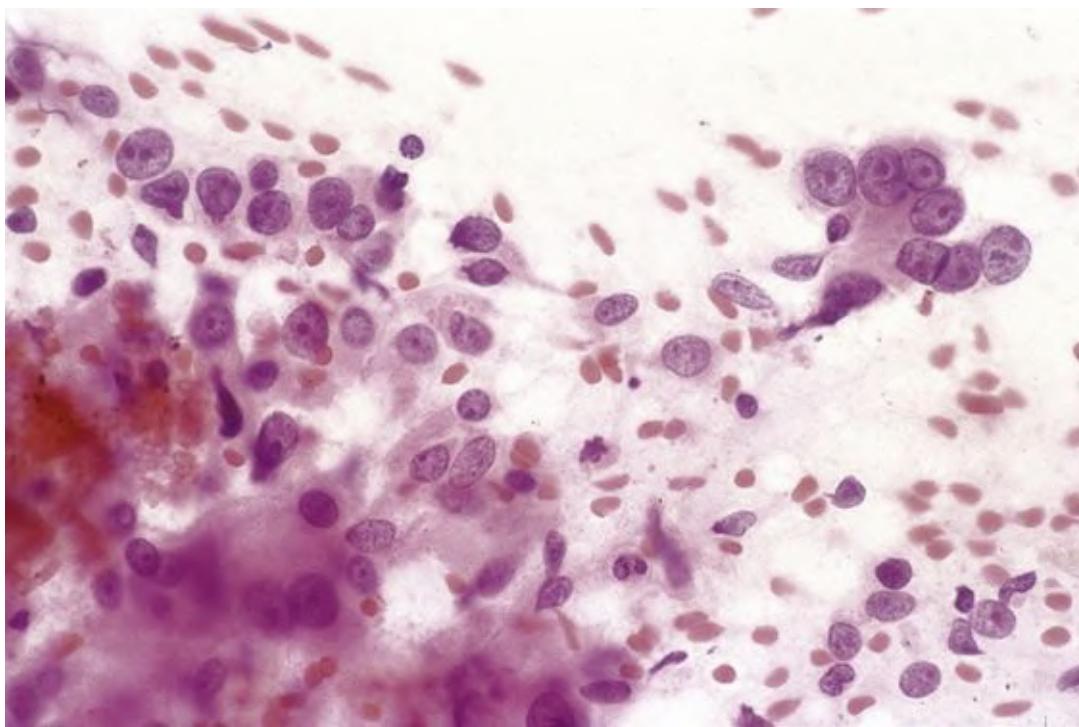


FIGURE 11.10 Pleomorphic adenoma (PA) with atypia.

Atypical cells with enlarged nuclei and prominent nucleoli are present in an aspirate that is otherwise typical of a pleomorphic adenoma. When few and far between, such cells are not concerning for malignancy. More extensive atypia, as in this case, merits the interpretation PA with atypia. The resected specimen revealed a noninvasive carcinoma ex PA (hematoxylin and eosin [H & E] stain).

Squamous or mucinous metaplasia. These changes are of little concern when present focally in an otherwise typical PA. If such changes are extensive, the differential diagnosis includes mucoepidermoid carcinoma, possibly arising in a preexisting PA.^{98,99} Mucoepidermoid carcinoma MEC ex pleomorphic adenoma is exceedingly rare and usually a high-grade malignancy.¹⁰⁰

Myoepithelioma

Myoepithelioma is a rare and unusual salivary gland tumor that can be considered a monomorphic variant of pleomorphic adenoma (PA). The clinical features and natural history of myoepitheliomas and PAs are similar.⁵⁰



Cytomorphology of myoepithelioma

- loose aggregates and isolated cells
- spindle cell, clear cell, epithelioid, or plasmacytoid morphology
- lack of honeycomb-like sheets of epithelial cells

- lack of chondromyxoid matrix

The myoepithelial cells of a myoepithelioma are identical to those of a PA. The absence of chondromyxoid matrix material and epithelial cells distinguishes a myoepithelioma from a PA. A smear composed exclusively of myoepithelial cells, however, often results from incomplete sampling of a myoepithelial-cell–rich pleomorphic adenoma⁹⁵ (Fig. 11.11) and can result in a misdiagnosis of myoepithelioma. Although this error has no clinical consequence, a generic interpretation of a “myoepithelial cell-rich neoplasm” (accompanied by a differential diagnosis) is prudent in such circumstances.

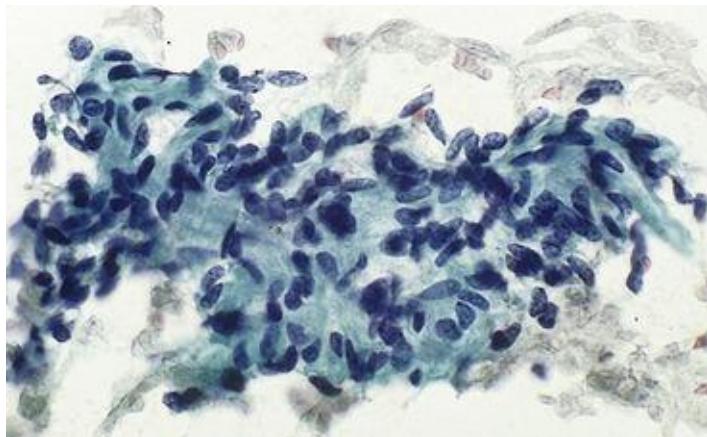


FIGURE 11.11 Myoepithelial cell–rich neoplasm.

Loosely cohesive, spindle-shaped myoepithelial cells are the sole constituents of this aspirate. The resected specimen proved to be a myoepithelial cell–rich pleomorphic adenoma, but the aspiration findings were indistinguishable from those of a myoepithelioma (Papanicolaou stain).

A spindle cell myoepithelioma can be difficult to distinguish from a schwannoma.^{95,101–103} Both can have nuclear palisading, although this is more common in a schwannoma, in which rows of palisaded nuclei are called *Verrocay bodies*. Immunohistochemistry is helpful; although S-100 is diffusely positive in schwannoma and often positive in myoepithelial cells, myoepithelial cells also stain for one or more of the following: p63, keratins, smooth muscle actin, glial fibrillary acidic protein, and calponin. Of these, p63 has the greatest value among the current immunohistochemical markers of myoepithelial differentiation. An important caveat: p63 also stains cells showing squamous

differentiation (e.g., the epidermoid cells of a mucoepidermoid carcinoma).

Plasmacytoid myoepithelial tumors^{[104,105](#)} ([Fig. 11.12](#)) resemble plasmacytomas, but myoepithelial cells lack the “clock-face” chromatin and perinuclear hof of plasma cells. Immunohistochemistry and serum electrophoresis are useful in difficult cases.

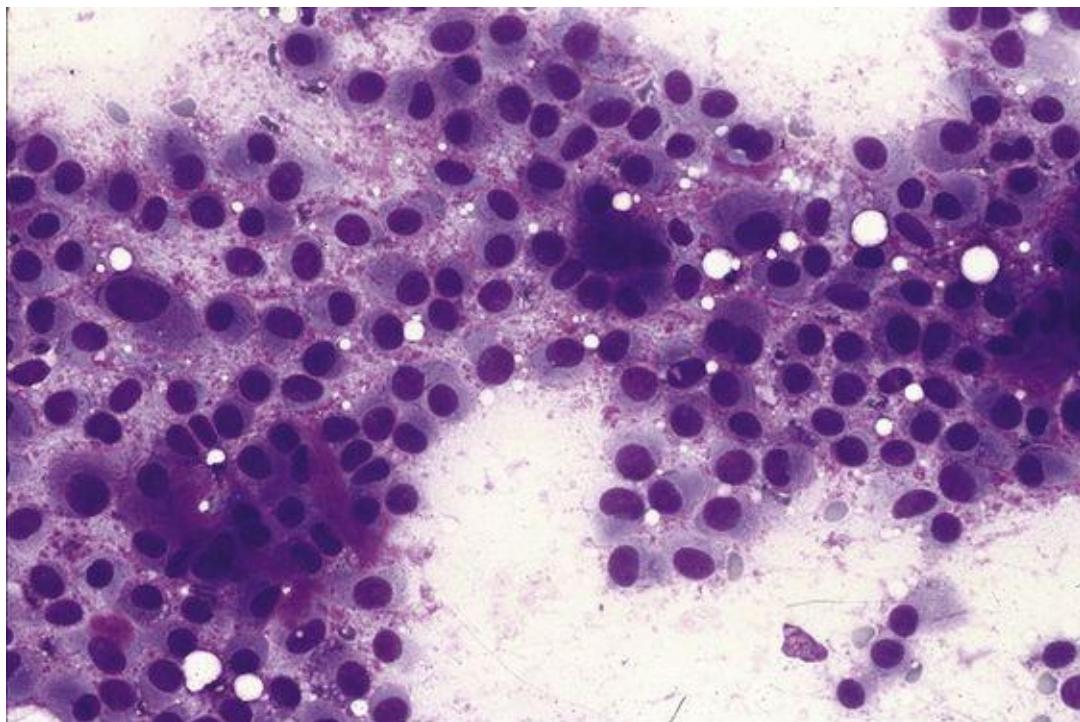


FIGURE 11.12 Plasmacytoid myoepithelial cells in a pleomorphic adenoma (PA). Round to oval myoepithelial cells with eccentric nuclei mimic plasma cells, but wisps of matrix material are present, and the perinuclear hof and “clock-face” chromatin of plasma cells are absent (Romanowsky stain).

Clear cell myoepithelioma resembles other neoplasms with clear cell differentiation, like epithelial-myoepithelial carcinoma, acinic cell carcinoma, mucoepidermoid carcinoma, and metastatic renal cell carcinoma.

Myoepithelial carcinoma is the rare malignant counterpart. Necrosis, pleomorphism, mitotic activity, coarse chromatin, and prominent nucleoli are distinguishing features.^{[106](#)}

Basal Cell Adenoma

Basal cell adenoma occurs most frequently in the parotid gland and clinically is

usually mistaken for a pleomorphic adenoma (PA). It can exhibit a variety of histologic patterns: solid, tubular, trabecular, membranous, and mixed patterns. The canalicular adenoma is a distinct entity because of its propensity for the upper lip. The membranous type of basal cell adenoma also has clinically distinct features: unlike the other subtypes, it is associated with an autosomal dominant syndrome that includes the development of other synchronous cutaneous tumors.^{[107](#)}



Cytomorphology of basal cell adenoma

- small and intermediate-sized basaloid cells with peripheral palisading
- dense, nonfibrillary stroma at the periphery of cell groups

Sheets and cords of neoplastic cells are arranged in large, geographic configurations with a sharply demarcated epithelial-stromal interface. The matrix of a basal cell adenoma stains brightly cyanophilic or eosinophilic with the Papanicolaou stain, and basophilic to metachromatic with Romanowsky-type stains. The neoplastic cells, of epithelial and myoepithelial derivation, have a “basaloid” appearance (i.e., scant cytoplasm and uniform round to oval nuclei). Bare nuclei can be abundant. Two populations of basaloid cells, arranged in variable-sized clusters, are often present, sometimes with peripheral palisading of the smaller cells^{[108-110](#)} ([Fig. 11.13A](#)). Many cell groups show a peripheral band of hyaline material and sparse intercellular hyaline matrix that is not fibrillar (as in PA).^{[110](#)} Smooth-contoured hyaline globules may be present.^{[111-113](#)} The globules or cylinders, small and often interdigitated with basaloid myoepithelial cells, are a nonspecific finding associated with any myoepithelial-containing tumor ([Fig. 11-13B](#)) The small globules or cylinders of a basal cell adenoma differ from the variably sized, often large, individual spheres commonly seen in adenoid cystic carcinoma.

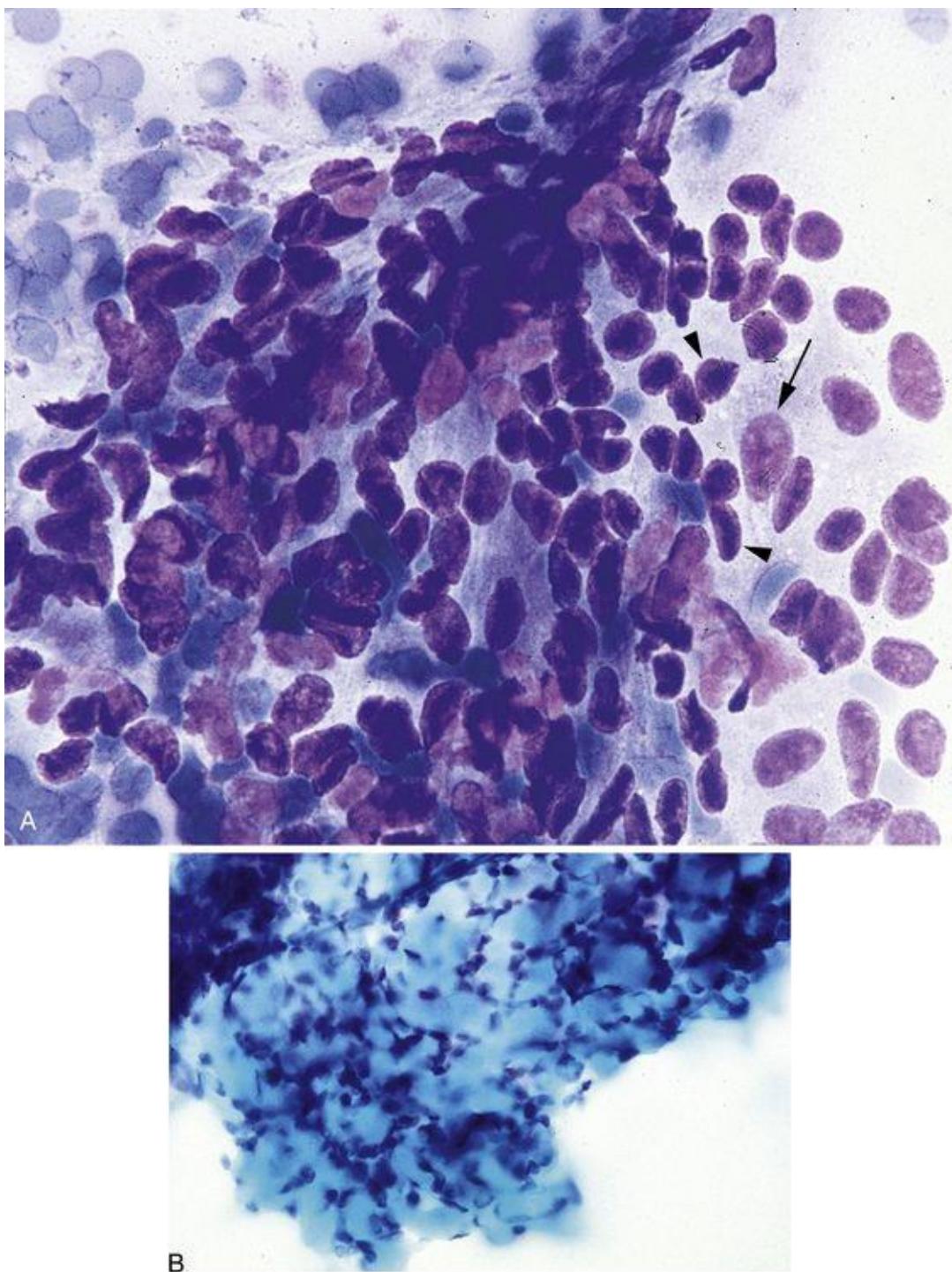


FIGURE 11.13 Basaloid neoplasms.

A, Two populations of loosely cohesive cells are present: cells with larger nuclei and moderate amounts of cytoplasm (arrow) and a second population of basaloid cells with dark nuclei and scant cytoplasm (arrowheads). A more specific interpretation than basaloid neoplasm is not possible. Histologic examination revealed a basal cell adenocarcinoma (Romanowsky stain).

B, A latticelike network of myoepithelial cells surrounds coalescing extracellular matrix globules. This pattern is nonspecific and seen in a variety of matrix-containing basaloid neoplasms. Histologic examination revealed a pleomorphic adenoma (PA) (Papanicolaou

stain).

The *membranous type* (also called the *dermal analogue tumor*) is a cytologically and clinically distinct variant in which coalescing spheres of matrix material are prominent, and aggregates of basaloid cells are surrounded by a thick ribbon of matrix material^{111,114-116} (Fig. 11.14A and B). It is indistinguishable from a dermal cylindroma and resembles a basaloid squamous cell carcinoma (SQC). In most instances, however, a basaloid SQC exhibits cytologic features of malignancy, including necrosis, marked atypia, and mitotic activity. HPV is associated with a subset of basaloid SQCs, and in difficult cases a positive HPV test helps make the distinction.

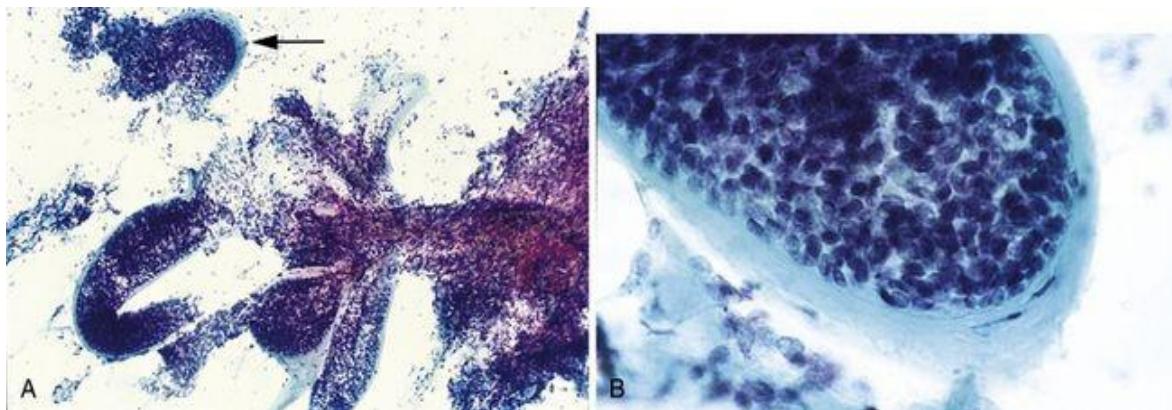


FIGURE 11.14 Basal cell adenoma, membranous type.
A, Clusters of basaloid cells are surrounded by a ribbon (“cuticle”) of dense aqua-colored matrix material (arrow) (Papanicolaou stain). B, The ribbon of matrix is better seen in this high-magnification image (Papanicolaou stain).



Differential diagnosis of basaloid neoplasms

- chronic sialadenitis
- basal cell adenoma
- basal cell adenocarcinoma
- adenoid cystic carcinoma (solid variant)
- cellular pleomorphic adenoma
- metastatic basal cell carcinoma
- metastatic basaloid squamous cell carcinoma
- small cell carcinoma

Evaluating and interpreting the basaloid neoplasms constitute the most difficult problem in salivary gland FNA. The differential diagnosis includes chronic sialadenitis, benign tumors, low-grade malignancies, and high-grade malignancies. The solid variant of adenoid cystic carcinoma, basal cell carcinoma of the skin,¹¹⁷ metastatic and primary small cell carcinomas, and rare malignant basaloid tumors—basaloid SQC^{118–120} and basal cell adenocarcinoma^{109,121–124}—are all in the differential diagnosis.

Unlike the other conditions on this list, chronic sialadenitis is typically sparsely cellular and has a background of chronic inflammation. It is usually a diffuse process rather than a discrete mass. Although cytologic atypia, necrosis, and significant mitotic activity exclude basal cell adenoma, the absence of these malignant features does not exclude a malignancy like adenoid cystic carcinoma^{112,113} and basal cell adenocarcinoma. By FNA, basal cell adenoma cannot be distinguished from its low-grade malignant counterpart (basal cell adenocarcinoma), as the diagnosis rests on the histologic demonstration of an infiltrative growth pattern. Both basal cell adenoma and the solid variant of adenoid cystic carcinoma can contain occasional stromal cylinders like those of the usual type of adenoid cystic carcinoma. Stromal material in basal cell adenomas often surrounds the neoplastic cells, in contrast with adenoid cystic carcinoma, in which the neoplastic cells almost always surround stroma. The matrix of basal cell adenoma can be hyalinized and show dense staining characteristics with the Papanicolaou stain, whereas in adenoid cystic carcinoma the matrix is more typically transparent. Squamous whorls are a histologic hallmark of basal cell adenoma, but they are an uncommon finding in FNA samples.¹²⁵ Nevertheless, when present, squamous whorls help to exclude an adenoid cystic carcinoma, which never exhibits squamous differentiation. Clinical evidence of malignancy as well as a complete history can also be helpful in distinguishing these entities.

In many cases a specific diagnosis is not possible, but a differential diagnosis can help guide clinical management (see sample reports presented in the following box). Frozen section diagnosis at the time of excision can also assist in guiding the extent of surgery.



Reporting basaloid neoplasms—sample reports

1. If the tumor has characteristic features of basal cell adenoma/adenocarcinoma (e.g., a peripheral band of hyaline material):
NEOPLASTIC CELLS PRESENT.

Basal cell neoplasm of salivary gland origin (see Note).

note: The cytologic features are compatible with either basal cell adenoma or basal cell adenocarcinoma. Distinction between the two cannot be made on cytologic material. Excision is recommended for precise classification.

2. If the tumor lacks distinctive cytologic features:
NEOPLASTIC CELLS PRESENT.

Basaloid neoplasm (see Note).

note: The differential diagnosis is broad in scope and includes basal cell adenoma, basal cell adenocarcinoma, the solid variant of adenoid cystic carcinoma, and nonsalivary gland neoplasms. Excision is recommended for precise classification, including intraoperative frozen section examination if clinically indicated.

Warthin Tumor

WT occurs primarily in the parotid gland and periparotid region and is believed to arise from salivary duct remnants entrapped within salivary gland-associated lymph nodes.⁵⁰ It is the second most common salivary gland neoplasm, representing approximately 5% to 10% of all salivary gland tumors. It occurs most often in the 50-to 79-year age range, is more common in men, and can be bilateral.⁵⁰ WTs feel “doughy” on palpation and contain distinctive thick, brown-green, granular fluid grossly resembling motor oil.



Cytomorphology of Warthin tumor

- lymphocytes
- oncocytes
- granular debris

The typical aspirate is composed of lymphocytes, oncocytes, and granular debris⁹² ([Fig. 11.15](#)). The lymphocyte population usually predominates and is composed mostly of small lymphocytes, with some admixed larger, reactive forms. Mast cells are present and are easiest to appreciate on Romanowsky-stained preparations.¹²⁶ Oncocytes, which may be scant, form cohesive flat sheets with an orderly arrangement of cells, usually without crowding. Infrequently, papillary groups and even a bilayered arrangement, as seen in histologic

sections, are found. The abundant cytoplasm of oncocytes looks different depending on which stain is used. The cytoplasm is granular, orange-pink to gray-blue with the Papanicolaou stain, but waxy (non granular) and deep blue with Romanowsky-type stains. Nuclei are usually uniform, round, and eccentrically located, with evenly textured chromatin and small nucleoli, but some can be enlarged, with prominent nucleoli. Nuclear enlargement and prominent nucleoli are not signs of malignancy, however.

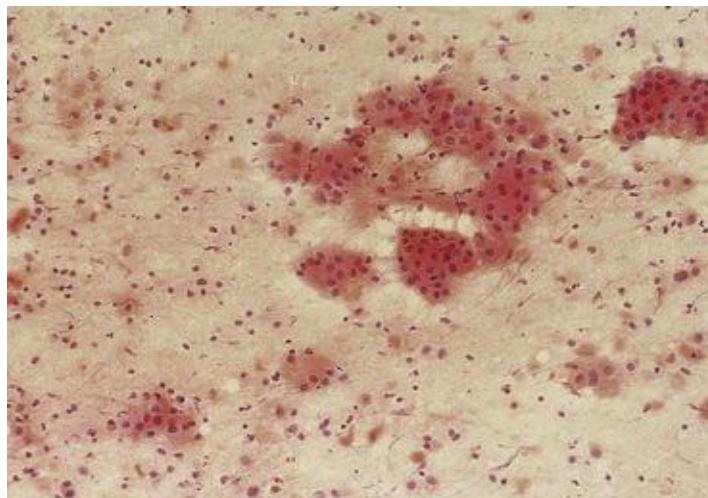


FIGURE 11.15 Warthin tumor (WT).

Scattered, small cohesive clusters of oncocytes are present in a background of lymphocytes (Papanicolaou stain).

In some cases, especially those associated with marked inflammation, squamous or mucinous metaplasia is present, suggesting a diagnosis of squamous cell carcinoma or mucoepidermoid carcinoma.^{68,127} In such cases, a careful search reveals the characteristic duo of lymphocytes and oncocytes. Other entities in the differential diagnosis include oncocytoma, acinic cell carcinoma, and metastatic renal cell carcinoma. Occasionally, aspirates of WTs are unsatisfactory or result in a nonspecific diagnosis. Because WT is a cystic neoplasm, sampling sometimes yields cyst contents only. In other cases, a spontaneous, partial or complete infarction may have occurred, resulting in necrosis and few, if any, intact, viable cells.

Oncocytoma

Oncocytoma is a rare benign salivary gland neoplasm accounting for 1% to 3%

of all salivary gland tumors.⁵⁰ In contrast to oncocytic metaplasia and oncocytosis, oncocytoma is a discrete, clinically detectable nodule. It is well circumscribed and demarcated from the surrounding salivary gland parenchyma by at least a partial fibrous capsule.⁵⁰ Most oncocytomas occur in the parotid gland, and the peak incidence is in the seventh to ninth decades; these tumors are rare in patients under 50 years of age.^{50,128}



Cytomorphology of oncocytoma

- cellular specimen
- oncocytes
- clean background
- no lymphocytes

Aspirates are moderately cellular and consist of large polygonal oncocytes whose cytoplasm, like that of the Warthin tumor (WT) oncocytes, contains an abundance of mitochondria¹²⁸ ([Fig.11.16A and B](#)). Oncocytes are present as sheets, cords, dyshesive clusters, and single cells with sharp cellular outlines. As with the oncocytes of a WT, the abundant cytoplasm looks different depending on what stain is used. The cytoplasm is granular, orange-pink to gray-blue with the Papanicolaou stain, but waxy (non granular) and deep blue with Romanowsky-type stains. Nuclei are usually small, round to oval, and centrally placed. Nuclear enlargement and distinct nucleoli can be seen, but mitotic activity is absent. The background lacks the granular proteinaceous debris and lymphocytes of a WT.

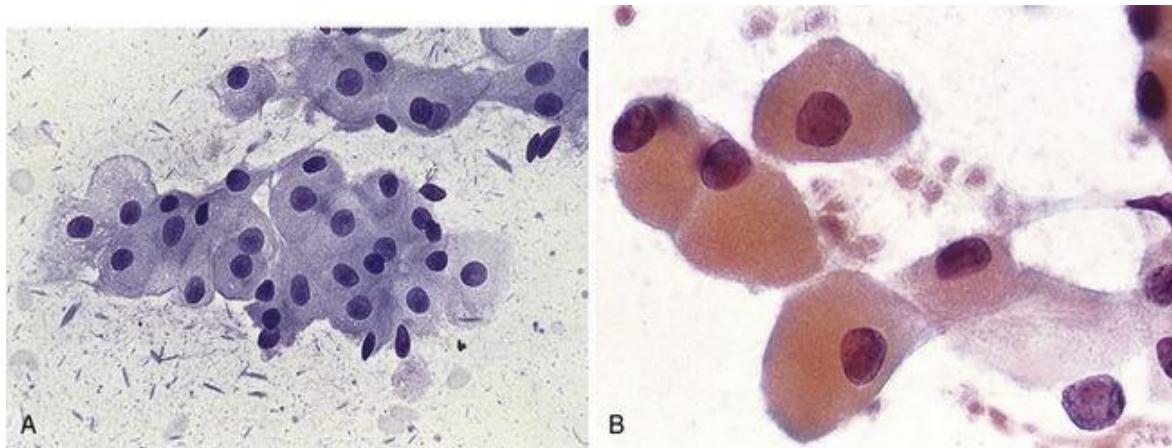


FIGURE 11.16 Oncocytoma.

A, Cohesive sheetlike clusters of oncocytes with distinct cell borders are present in a clean background (Romanowsky stain). B, Oncocytes have uniform round to oval nuclei and abundant densely granular cytoplasm (Papanicolaou stain).



Differential diagnosis of oncocytic lesions

- oncocytoma
- oncocytosis
- Warthin tumor (WT)
- pleomorphic adenoma (PA)
- mucoepidermoid carcinoma
- acinic cell carcinoma
- oncocytic carcinoma
- metastatic renal cell carcinoma

Because oncocytes and oncocytic change are present in normal salivary glands and a variety of salivary gland neoplasms, the differential diagnosis is broad in scope.¹²⁹ Oncocytes occur in oncocytic metaplasia, oncocytosis, and oncocytoma, and in some cases, it can be difficult or impossible to distinguish among these entities cytologically, particularly oncocytosis and oncocytoma. WT is distinguished from an oncocytoma by virtue of its abundant lymphocytes and relatively minor component of oncocytes. With regard to the rare oncocytic carcinoma, cytologic features alone do not always allow for distinction from an oncocytoma, although marked cytologic atypia, mitotic activity, necrosis, or appropriate clinical symptoms alert one to the possibility of an oncocytic carcinoma.^{130,131} Oncocytic carcinoma is a diagnosis of exclusion and must be

distinguished from other tumors with oncocytic change. The rare oncocytic variant of mucoepidermoid carcinoma is identified by a mixture of mucin-producing and intermediate cell types.

Although the finely granular or waxy cytoplasm of oncocytomas and WT differs from the vacuolated cytoplasm of acinic cell carcinoma, these tumors have overlapping cytologic features, especially at low magnification, where acinic cell carcinomas appear to consist of sheets of cells with dense cytoplasm and uniform nuclei. The challenge is exacerbated by the existence of clear cell variants of both oncocytoma and acinic cell carcinoma. Special stains can be helpful: The mitochondria of oncocytes are strongly positive with a phosphotungstic acid hematoxylin (PTAH) stain, whereas acinic cell carcinoma is negative; conversely, oncocytes lack the characteristic PAS-positive, diastase-resistant cytoplasmic granules of an acinic cell carcinoma. We have seen several instances in which the distinction between acinar and oncocytic differentiation was not possible by examining just the smears, but characteristic acinar cell cytoplasm was readily apparent in cell block material, without the need for special stains (see [Fig. 11.19D](#)). In difficult cases, a diagnosis of a *low-grade salivary gland neoplasm* with the appropriate differential diagnosis is justified.

Carcinomas of Salivary Gland Origin

Mucoepidermoid Carcinoma

MEC is the most common salivary gland malignancy in children and adults, and the most common malignancy of the major and minor salivary glands. Clinical, histologic, and cytologic features differ between the low-and high-grade tumors. Low-grade MECs are more commonly cystic, whereas high-grade MECs are solid and infiltrative. Modest surgical excision is curative for low-grade MECs; high-grade MECs require aggressive surgical treatment, and sometimes adjunctive radiotherapy and lymph node dissection.



Cytomorphology of mucoepidermoid carcinoma

- mucus cells (predominant in low-grade tumors)
- epidermoid cells (predominant in high-grade tumors)
- intermediate cells
- extracellular mucin
- overt cytologic malignancy (high-grade tumors)

The diagnostic feature of MEC is the combination of mucous cells, epidermoid (squamoid) cells, and intermediate cells, with clear cells often present as well.^{132–135} Mucous cells have a columnar or signet ringlike appearance; the epidermoid cells are polygonal and squamoid, with dense cyanophilic cytoplasm; and the intermediate cells are smaller, resembling immature squamous metaplastic cells. In some instances, individual cells can combine features of both mucinous and epidermoid differentiation; such cells are considered diagnostic of MEC. Clear cells in MEC represent a morphologic variant of squamous differentiation.⁵⁰ A subset of intermediate-grade MECs are predominantly oncocytic and diagnostically challenging.¹³⁶

Low-grade MEC is commonly cystic, and mucous cells predominate ([Fig. 11.17A](#)). Individual cells with features of both mucinous and epidermoid differentiation are seen and are considered diagnostic ([Fig. 11.17B](#)). Overt cytologic evidence of malignancy is not seen, and, because the low-grade tumors are cystic, the neoplastic cells are sometimes undersampled. As a result, low-

grade MEC is the most frequent cause of a false-negative diagnosis in salivary gland FNA.¹³³ In some instances, only extracellular mucin—pale purple with Romanowsky stains, pale blue-purple with the Papanicolaou stain—is seen. Scattered, round neoplastic mucinous cells may be mistaken for histiocytes.

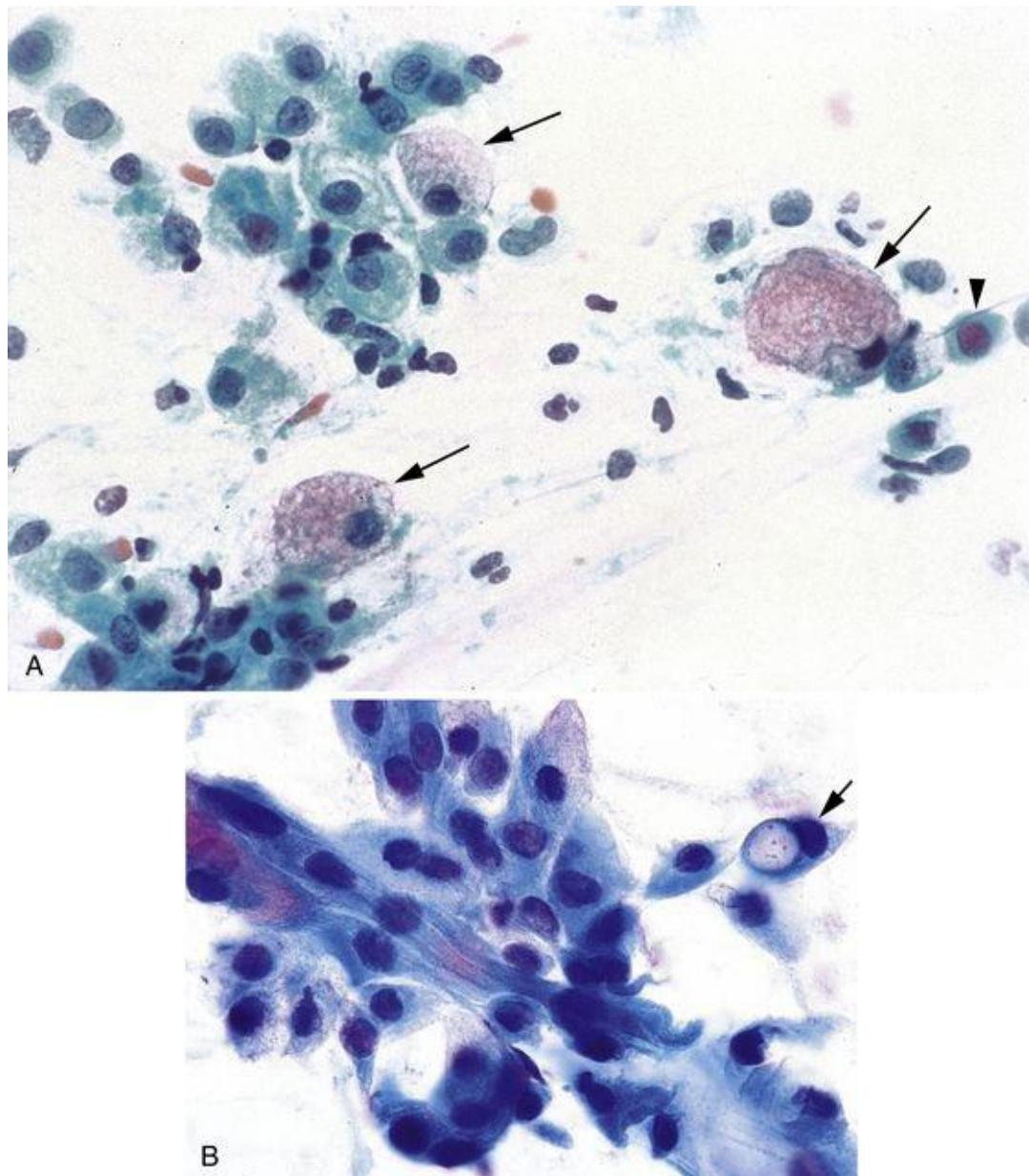


FIGURE 11.17 Low-grade mucoepidermoid carcinoma (MEC).
A, In a low-grade MEC, mucus cells (*arrows*) and intermediate cells (*arrowhead*) predominate (Papanicolaou stain). B, In this low-grade MEC, squamoid cells predominate. There is an isolated cell with features of both squamoid and mucinous differentiation (*arrow*) (Papanicolaou stain).

Differential diagnosis of low-grade mucoepidermoid carcinoma

- acquired cyst (mucocele or retention cyst)
- chronic sialadenitis with mucinous metaplasia of ducts
- Warthin tumor (WT)
- pleomorphic adenoma (PA)
- mammary analogue secretory carcinoma

The differential diagnosis of low-grade MEC includes an acquired cyst (mucocele or retention cyst) and ductal mucinous metaplasia in chronic sialadenitis (commonly associated with sialolithiasis). The presence of intermediate or epidermoid cells excludes an acquired cyst. Stone fragments are diagnostic of sialolithiasis, and ciliated columnar cells are a metaplastic change seen with duct obstruction. If these features are not apparent, a low-grade MEC cannot be excluded. Thus, whenever a specimen contains extracellular mucin, an atypical (or suspicious) interpretation is warranted, with an appropriate explanatory note. As with all cystic lesions, any residual mass should be reaspirated after drainage, and excision should be considered if the lesion persists.

Distinction of low-grade MEC from a WT is more problematic, because oncocytic variants of MEC do occur. Atypia and non-oncocytic epithelial cells can be seen in both but are more common in MEC. In an otherwise typical PA, PA small foci of squamous or mucinous differentiation are not significant. *MEC ex pleomorphic adenoma* is extremely rare and is usually a high-grade malignancy.⁹⁸⁻¹⁰⁰ Like MEC, mammary analogue secretory carcinoma may contain mucous cells,¹³⁷ but it lacks epidermoid cells. Mammary analogue secretory carcinoma is immunoreactive for S-100 and mammaglobin, whereas MEC is diffusely p63-positive. Both entities have characteristic chromosomal translocations, involving *MAML2* in MEC and *ETV6-NTRK3* in mammary analogue secretory carcinoma.^{46,48,137}

High-grade MEC has a greater proportion of squamoid cells, with more cytologic atypia, and a less prominent cystic component. Recognizing the tumor as a malignancy is straightforward ([Fig. 11.18](#)); precise identification as a high-grade MEC can be difficult.

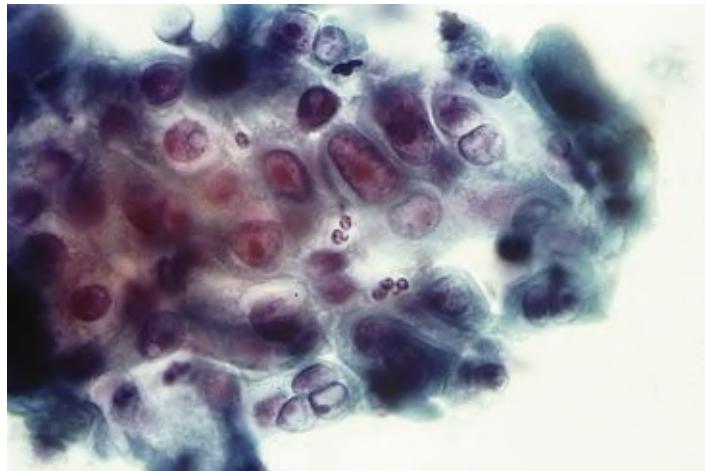


FIGURE 11.18 High-grade mucoepidermoid carcinoma (MEC). In a high-grade MEC, squamoid cells predominate. Marked nuclear atypia is present (Papanicolaou stain).



Differential diagnosis of high-grade mucoepidermoid carcinoma

- carcinoma ex pleomorphic adenoma
- salivary duct carcinoma
- oncocytic carcinoma
- metastatic carcinoma

High-grade carcinomas in the salivary gland include high-grade MEC, carcinoma ex pleomorphic adenoma, salivary duct carcinoma, oncocytic carcinoma, and metastatic carcinoma, most frequently metastatic SQC. On rare occasions, any salivary gland carcinoma, in fact, can undergo dedifferentiation and take on the appearance of a high-grade malignancy. All high-grade carcinomas of the salivary gland are treated similarly, so the distinction is not critical for patient management. A high-grade MEC is recognized by the combination of squamoid, mucinous, and intermediate-type cells. Distinction from a nonkeratinizing SQC is dependent on the identification of a mucinous component. A mucicarmine stain is diagnostically useful. Carcinoma ex PA is identified if the benign component (PA) is apparent. Salivary duct carcinoma has extensive necrosis and can have a papillary architecture. Oncocytic carcinoma resembles the oncocytic variant of MEC, but the cells lack intracellular mucin. Clinical history is essential to exclude metastatic squamous cell carcinoma.

(SQC). Extensive keratinization (see [Fig. 11.6B](#)) is not a feature of high-grade MEC^{[4,135](#)} and should prompt a search for a primary SQC elsewhere, especially in the head and neck region. When specific diagnostic features are absent, the interpretation “high-grade carcinoma” is sufficient to ensure appropriate therapy.

Acinic Cell Carcinoma

Acinic cell carcinoma is the second most common salivary gland malignancy, representing approximately 4% to 6% of all salivary gland neoplasms and up to 17% of malignancies.^{[50,138](#)} It is usually a low-grade malignancy that can recur locally or metastasize to regional lymph nodes and distant sites. A majority arise in the parotid gland, most commonly in women. The mean age is 44 years, although the range is broad and includes children and the elderly.^{[50,139](#)} Acinic cell carcinoma typically manifests as a circumscribed, mobile, slowly growing mass that is occasionally painful. It is bilateral in up to 3% of cases.



Cytomorphology of acinic cell carcinoma

- cellular smear with serous-type acinar cells
 - sheets, crowded clusters, isolated cells
 - large polygonal cells, abundant vacuolated cytoplasm
 - PAS-positive diastase-resistant cytoplasmic zymogen granules
 - indistinct cell borders
 - bland round nuclei
- naked nuclei with or without lymphocytes

FNA yields a cellular aspirate containing large, polygonal serous cells with delicate, vacuolated, basophilic cytoplasm ([Fig. 11.19A-D](#)). In the background are scattered naked nuclei and sometimes lymphocytes (approximately 10% of cases).^{[140-142](#)} The neoplastic cells have indistinct cell borders and are loosely arranged in three-dimensional groups and sheets. In some cases, a papillary or follicular microarchitecture is present. The cytoplasm may contain occasional coarse zymogen granules that are blue on Papanicolaou-stained preparations and metachromatic with Romanowsky stains. Some cells show marked cytoplasmic vacuolization (see [Fig. 11.19C](#)). The cytoplasmic granules are PAS-positive and diastase-resistant, a characteristic feature that is particularly well appreciated

with hematoxylin and eosin (H & E)-stained cell block sections. The nuclei are usually bland and round, and nucleoli are sometimes present. Intranuclear pseudoinclusions can be seen; mitoses are infrequent. Some cases contain a predominance of intercalated duct cells. Uncommonly, high-grade transformation occurs in acinic cell carcinoma, manifested by more marked nuclear atypia and less acinar cell differentiation.^{[143,144](#)} This subgroup is particularly difficult to distinguish from other high-grade carcinomas that involve the salivary gland. The rare papillary cystic variant is characterized by papillary groups with vacuolated, histiocyte-like cells that may contain pigment, granular cells, and cyst fluid. These subtle tumors may result in a false-negative interpretation or a mistaken diagnosis of mucoepidermoid carcinoma.^{[145,146](#)} Acinic cell carcinomas are richly vascular neoplasms, and aspirates often contain delicate blood vessels surrounded by neoplastic cells (see [Fig. 11.19A](#)); care should be taken not to mistake these vascular structures for normal ducts. Uncommon features include marked clear cell change, psammoma bodies, and cystic change.

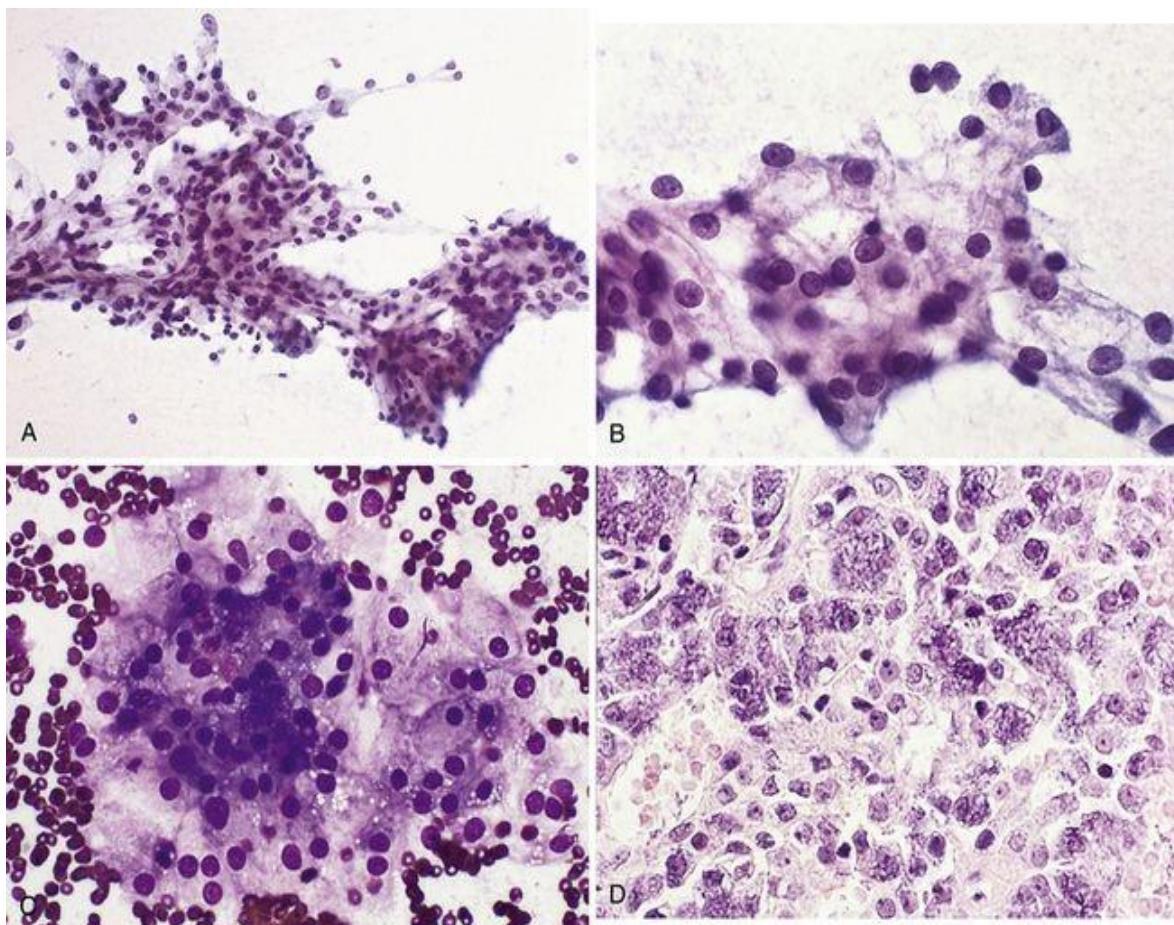


FIGURE 11.19 Acinic cell carcinoma.

A, Loosely cohesive, crowded clusters of cells with acinar differentiation surround thin-walled blood vessels. Note the absence of tightly formed grapelike clusters, adipose tissue, and ductal elements typical of a normal aspirate (compare with Fig. 11.2B) (Papanicolaou stain). B, Neoplastic cells have abundant delicate cytoplasm and cytologically bland nuclei (Papanicolaou stain). C, A well-differentiated acinic cell carcinoma displaying abundant vacuolated cytoplasm and small round to oval nuclei. Ductal cells are absent (Romanowsky stain). D, In some instances, acinic cell differentiation is more apparent in cell block preparations, which reveal the typical purple, granular cytoplasm of these cells (hematoxylin and eosin [H & E] stain).



Differential diagnosis of acinic cell carcinoma

- normal salivary gland
- sialadenosis
- oncocytoma
- Warthin tumor (WT)
- mucoepidermoid carcinoma
- sebaceous neoplasms
- clear cell neoplasms

- MASC

Acinic cell carcinoma is distinguished from normal salivary gland and sialadenosis by the looser clustering, overlapping, and dyshesion of the neoplastic acinar cells. Neoplastic cells lack the fibroadipose tissue and cohesive grapelike arrangement of peripherally polarized normal acinar cells surrounding ductal structures that is typical of normal salivary gland tissue. The distinction from oncocytic neoplasms like WT has been previously discussed. Although the cells of acinic cell carcinoma can be vacuolated or clear and mimic the mucin-containing cells of mucoepidermoid carcinoma, the cells of acinic cell carcinoma are negative for mucin. Similarly, the vacuolated cells of acinic cell carcinoma resemble the sebaceous cells of sebaceous lymphadenoma and sebaceous carcinoma, but sebaceous cells contain lipid and are PAS-positive and diastase-sensitive. Acinic cell carcinoma can have a prominent clear cell appearance and thus can mimic other clear cell neoplasms. MASC also has cells with abundant vacuolated cytoplasm, sometimes with papillary architecture. PAS-positive, diastase-resistant granules are present in acinic cell carcinoma. Mammary analogue secretory carcinoma is immunoreactive for S-100 and mammaglobin,^{48,137} whereas acinic cell carcinoma is positive for DOG 1 (“discovered on gastrointestinal stromal tumors” protein 1).^{137,147}

Adenoid Cystic Carcinoma

Adenoid cystic carcinoma trails mucoepidermoid carcinoma and acinic cell carcinoma in frequency in the major salivary glands, although it occurs at a higher frequency in the submandibular gland. In minor salivary glands, the frequency of adenoid cystic carcinoma is less than previously thought, with the recognition of polymorphous low-grade adenocarcinoma as a distinct pathologic entity.⁵⁰ Although the clinical course of adenoid cystic carcinoma is often protracted, long-term (10-to 20-year) survival is poor. Three variants are recognized and often present in combination: tubular, cribriform, and solid. Recognition of the solid pattern is important because of its more aggressive clinical course. Owing to a tendency to invade nerves, this tumor manifests itself clinically as a painful mass or as pain during the FNA, which should increase suspicion for malignancy.



Cytomorphology of adenoid cystic carcinoma

- variably sized, often large, three-dimensional, acellular hyaline matrix globules and linear branching structures
- matrix is acellular with sharp borders
- basaloid cells
- *solid variant*: numerous basaloid cells (with or without atypia) and scant matrix

The cells are basaloid, with scant cytoplasm and hyperchromatic, often angulated, nuclei.¹⁴⁸⁻¹⁵⁰ Nucleoli, mitoses, and necrosis are not prominent except in rare high-grade examples. Most tumors have abundant acellular matrix that is arranged in discrete globules and cylinders with sharp borders ([Figs. 11.20A and B](#), [11.21](#)). The globules are intensely metachromatic with a Romanowsky-type stain but nearly invisible with the Papanicolaou stain. The solid variant of adenoid cystic carcinoma has scant matrix, and basaloid cells predominate ([Fig. 11.22](#)). Consequently, distinguishing the solid variant of adenoid cystic carcinoma from basal cell adenoma is often impossible.^{111-113,150,151} Most adenoid cystic carcinomas have translocations involving the MYB gene and demonstrate MYB protein overexpression even in the absence of a detectable translocation.⁴⁷ Thus, immunohistochemistry for MYB overexpression and/or fluorescence in situ hybridization (FISH) for the MYB gene rearrangement are potentially useful for diagnosis.^{152,153}

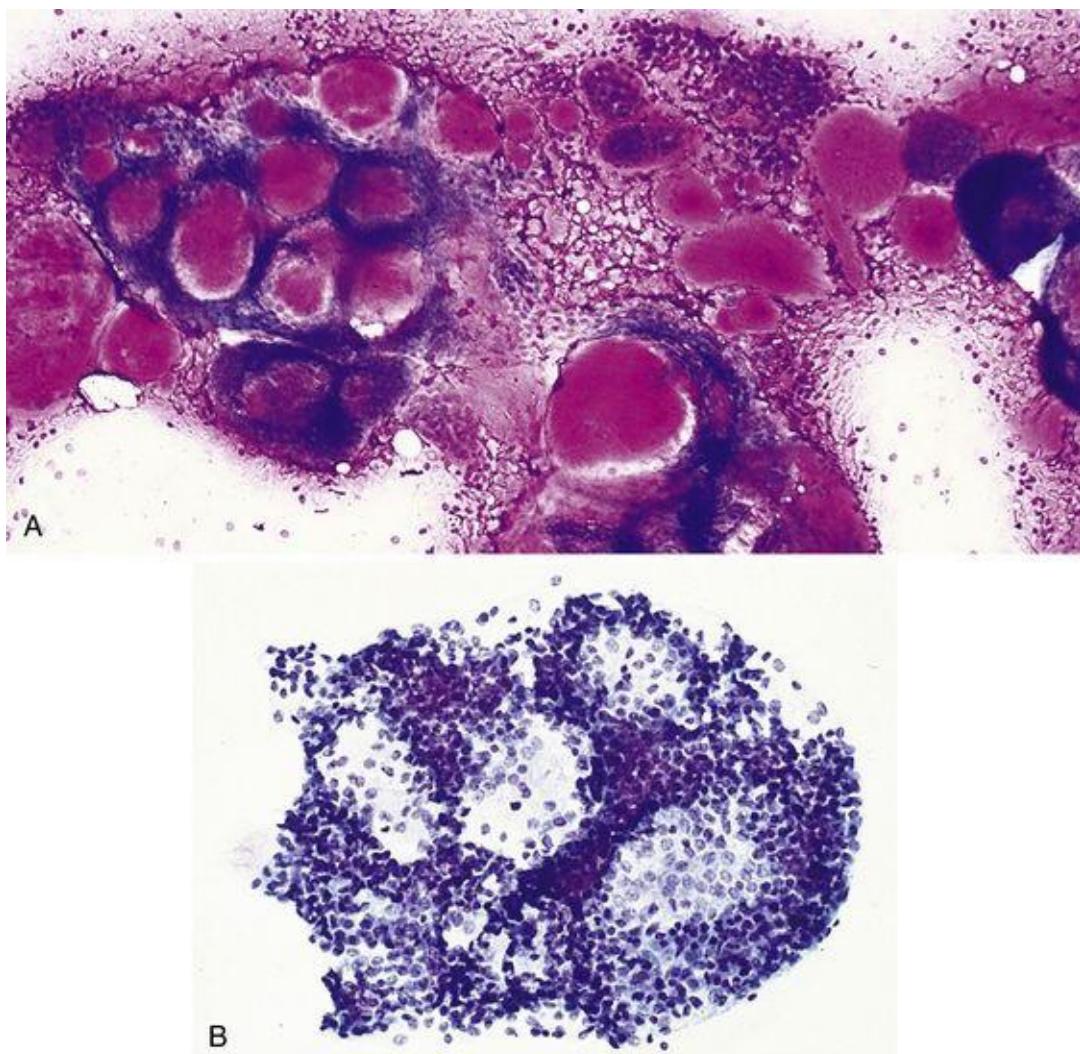


FIGURE 11.20 Adenoid cystic carcinoma.

A, Numerous variably sized stromal spheres are evident (Romanowsky stain). B, With the Papanicolaou stain, the same tumor shows cribriform architecture. The basaloid tumor cells are readily apparent, but the matrix material is transparent (Papanicolaou stain).

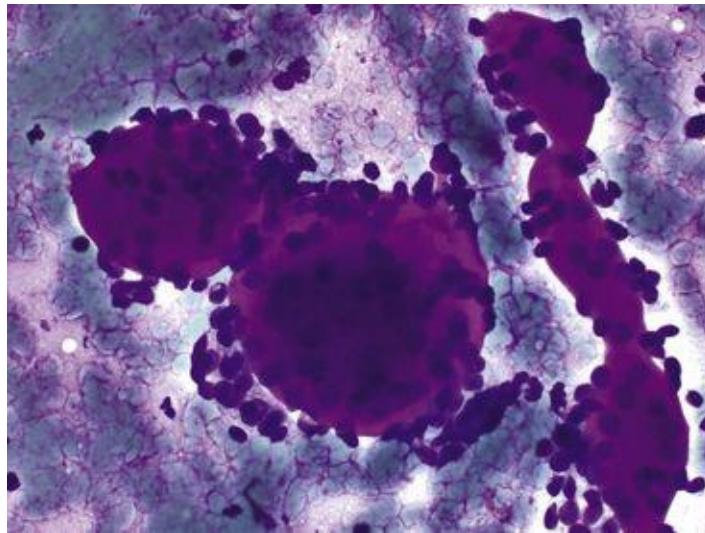


FIGURE 11.21 Adenoid cystic carcinoma.

Basaloid cells surround spheres and fingerlike projections of matrix with sharp borders and a “cookie-cutter”-like appearance (Romanowsky stain).

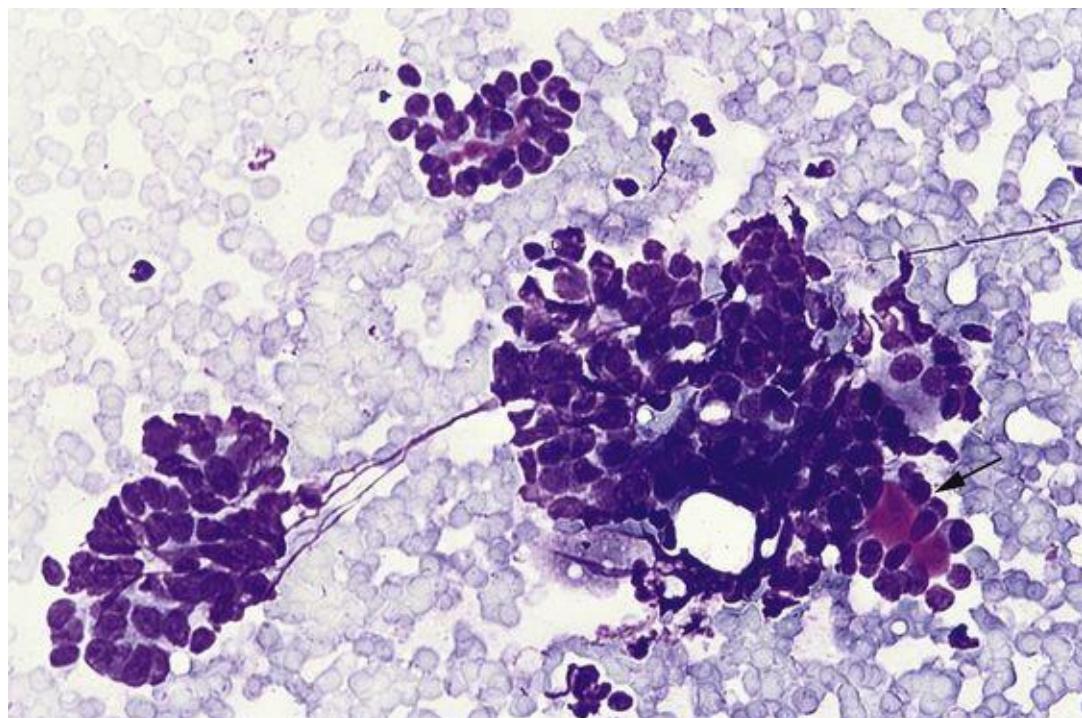


FIGURE 11.22 Adenoid cystic carcinoma, solid variant.

Basaloid cells predominate in this aggressive variant. The identification of rare stromal spheres (arrow) is needed to make the diagnosis (Romanowsky stain).



Differential diagnosis of adenoid cystic carcinoma

- pleomorphic adenoma (PA)
- other basaloid neoplasms
- PLGA
- epithelial-myoepithelial carcinoma
- eccrine cylindroma of the skin

Although most adenoid cystic carcinomas are straightforward to diagnose by FNA, occasional diagnostic challenges arise. The characteristic acellular spheres are seen in a variety of other salivary gland tumors, including PA, PA basal cell adenoma, polymorphous low-grade adenocarcinoma, and epithelial-myoepithelial carcinoma,¹⁵⁰ but usually they are less numerous or smaller in size in these other neoplasms.

Some PAs contain scattered stromal spheres that resemble those of an adenoid cystic carcinoma. There are other commonalities. For example, the matrix of an adenoid cystic carcinoma is intensely metachromatic with a Romanowsky stain, as is the more usual, chondromyxoid matrix of a PA. With Papanicolaou-stained preparations, the matrix of both tumors can be nearly invisible. In contrast with the fibrillary quality and embedded myoepithelial cells of the chondromyxoid matrix of a PA however, the matrix of adenoid cystic carcinoma has sharp edges, as if it were cut out with a cookie cutter. Moreover, the neoplastic cells of adenoid cystic carcinoma surround the matrix material but are not embedded in it. This results in discrete islands of variable-sized globules and fingerlike projections of matrix material in adenoid cystic carcinoma. Less frequently, the matrix of adenoid cystic carcinoma stains densely with Papanicolaou preparations, similar to that of other basaloid tumors like basal cell adenoma and myoepithelioma. In basal cell adenoma and myoepithelioma, however, the matrix islands and surrounding cells form incompletely separated, interdigitating strands.

PLGA can have stromal spheres, such as with adenoid cystic carcinoma, but the nuclei are not hyperchromatic. In addition, adenoid cystic carcinoma tends to contain a greater proportion of myoepithelial cells. The key to identifying an epithelial-myoepithelial carcinoma is recognizing a biphasic cell population, an abundance of large clear myoepithelial cells, and the peripherally located acellular basement membrane material. The distinction of adenoid cystic carcinoma from benign dermal eccrine cylindroma can only be made by tumor location (either clinically or radiologically) and not on cytological features.¹⁵⁴

Given the numerous mimics of adenoid cystic carcinoma and the significant

clinical consequences of a diagnosis of adenoid cystic carcinoma, many experts recommend that this diagnosis not be made without clinical evidence of malignancy.^{[113,155](#)}

Malignant Mixed Tumor

In the salivary glands, there are three types of malignant mixed tumor: carcinoma arising in a PA (carcinoma ex pleomorphic adenoma), metastasizing mixed tumor, and carcinosarcoma.^{[50](#)}

Carcinoma ex pleomorphic adenoma is the most common form. Unless excised, a significant proportion of untreated PAs eventually undergo malignant transformation, the estimated risk increasing with the duration of the adenoma: 1.5% for PAs present for less than 5 years, increasing to 9.5% for those present more than 15 years.^{[156](#)} Typically, a longstanding painless mass has undergone rapid enlargement with associated pain (suggestive of perineural involvement by malignancy). In most instances, carcinoma ex pleomorphic adenoma is a high-grade adenocarcinoma of ductal subtype. These tumors are readily recognized as malignant, but they are difficult to distinguish from other high-grade carcinomas such as high-grade mucoepidermoid carcinoma, salivary duct carcinoma, and metastatic carcinoma. A preexisting PA is necessary for the diagnosis^{[157](#)} ([Fig. 11.23](#)). Conversely, recognition of a preponderance of benign elements should lead to caution in rendering a definitive diagnosis of malignancy.^{[157](#)} The typical clinical history may suggest this diagnosis.

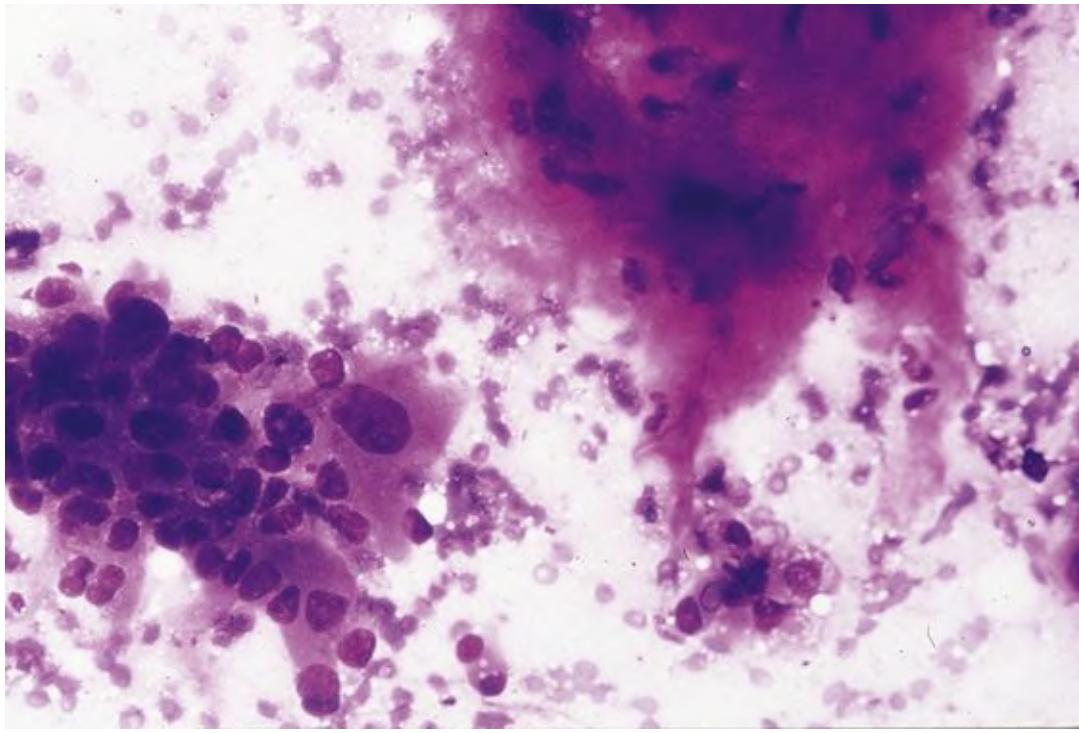


FIGURE 11.23 Carcinoma ex pleomorphic adenoma (PA).

Carcinoma, typically high-grade (*lower left*), juxtaposed with residual PA (*upper right*), is needed for definitive diagnosis, but the benign component (well seen here) is often inapparent (Papanicolaou stain).

A metastasizing mixed tumor is a cytologically benign PA in a distant site. Invariably, the primary PA of the salivary gland had undergone surgical manipulation, often with multiple local recurrences.¹⁵⁸ The cytologic features are indistinguishable from PA.¹⁵⁹ Despite its benign appearance, it is often fatal.

True carcinosarcoma is rare and represents a metaplastic carcinoma. Cytologically, pleomorphic malignant epithelial and mesenchymal cells are identified.^{160–162} The most common differentiated mesenchymal components are chondrosarcoma and osteosarcoma.

Salivary Duct Carcinoma

Salivary duct carcinoma is an uncommon, clinically aggressive malignancy most common in the parotid gland, occurring primarily in elderly men. It resembles a high-grade, comedo-type ductal carcinoma of the breast.⁵⁰ A low-grade variant similar in appearance to low-grade ductal carcinoma in situ of the breast has been described,^{163,164} but the preferred term is *low-grade cribriform cystadenocarcinoma*,³⁹ and reports of cytologic findings are rare.¹⁶⁵



Cytomorphology of salivary duct carcinoma

- overtly malignant cytology
- polygonal cells, abundant granular or vacuolated cytoplasm, prominent nucleoli
- sheets, clusters, papillae, and cribriform groupings
- necrosis

Papillary and cribriform groupings of large, cytologically malignant cells with abundant necrosis strongly suggest the diagnosis (Fig. 11.24A and B).^{166–172} Distinction from other high-grade malignancies is frequently impossible by cytomorphology alone. Immunohistochemistry can be very helpful: Salivary duct carcinoma is usually immunoreactive for HER2 and androgen receptor.^{173–175} This immunoprofile can also help with the distinction from a high-grade metastasis from a primary elsewhere.

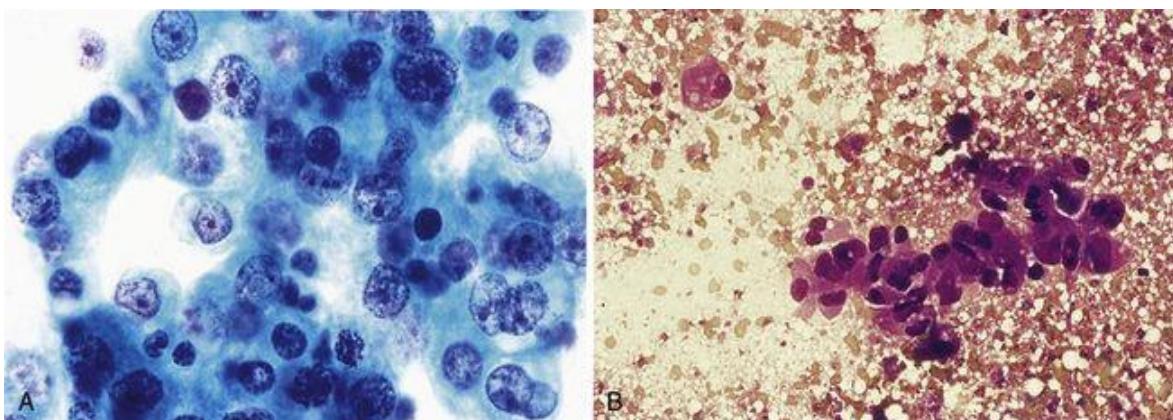


FIGURE 11.24 High-grade carcinoma (salivary duct carcinoma). A, A high-grade carcinoma is evident on the basis of large, round nuclei with prominent nucleoli and nondescript cytoplasm (Papanicolaou stain). B, A salivary duct carcinoma can be suspected when there is abundant necrosis (Romanowsky stain).

Polymorphous Low-Grade Adenocarcinoma

PLGA is a low-grade malignancy characterized by a predilection for perineural invasion, but with an overall favorable prognosis.^{176,177} PLGA is the second most common salivary gland malignancy of the minor salivary glands, where this

tumor is encountered almost exclusively. Consequently, FNA specimens are rarely obtained from these neoplasms. The histologic hallmark is cytologic uniformity with architectural diversity. Tubules, cords, and linear cell groupings (like those of lobular breast carcinoma) are seen.^{[178–180](#)} The tumor can have stromal spheres like adenoid cystic carcinoma, but the nuclei are normochromatic, not hyperchromatic as in adenoid cystic carcinoma. In addition, adenoid cystic carcinoma contains a greater proportion of myoepithelial cells. A PA can be misdiagnosed as a PLGA if fibrillary chondromyxoid stroma is absent. Basal cell adenoma is also in the differential diagnosis, but the clinical features of these entities are distinctive.

Rare Malignant Neoplasms

Basal Cell Adenocarcinoma

Basal cell adenocarcinoma is the rare malignant counterpart of basal cell adenoma,^{121,181,182} accounting for 2% of malignant epithelial salivary gland tumors. Most occur in the parotid gland, although occasional cases are encountered in the submandibular and minor salivary glands. Basal cell adenocarcinoma is a low-grade malignancy with a good prognosis but a tendency for local recurrence; metastatic disease is uncommon.

In most instances, basal cell adenocarcinoma is cytologically identical to basal cell adenoma¹⁰⁹; it is distinguished from the latter only by infiltrative growth. Accordingly, the reader is referred to the previous discussion of basal cell adenoma for the cytologic features and differential diagnosis. A subset of these neoplasms show nuclear atypia, mitotic activity, or necrosis, and are more easily recognized as malignant.⁵⁰ With these tumors the differential diagnosis is that of basaloid neoplasms with overt malignant features: adenoid cystic carcinoma, metastatic basal cell carcinoma of the skin, metastatic basaloid squamous carcinoma, and less commonly, sebaceous carcinoma, Merkel cell carcinoma, and small cell undifferentiated carcinoma.

Epithelial-Myoepithelial Carcinoma

Epithelial-myoepithelial carcinoma is a locally aggressive, low-grade tumor of ductlike structures composed of two cell types: small, inner duct lining cells and larger, peripheral myoepithelial cells. It represents 1% of all salivary gland neoplasms, and over 75% occur in the parotid gland.^{12,50} Epithelial-myoepithelial carcinomas have a broad age range (average age, 62 years). It is twice as common in women as in men.^{12,183}

Three-dimensional clusters of cells are surrounded by small amounts of homogeneous acellular basement membrane material^{184,185} ([Fig. 11.25](#)). In some cases, cell clusters surround concentrically laminated

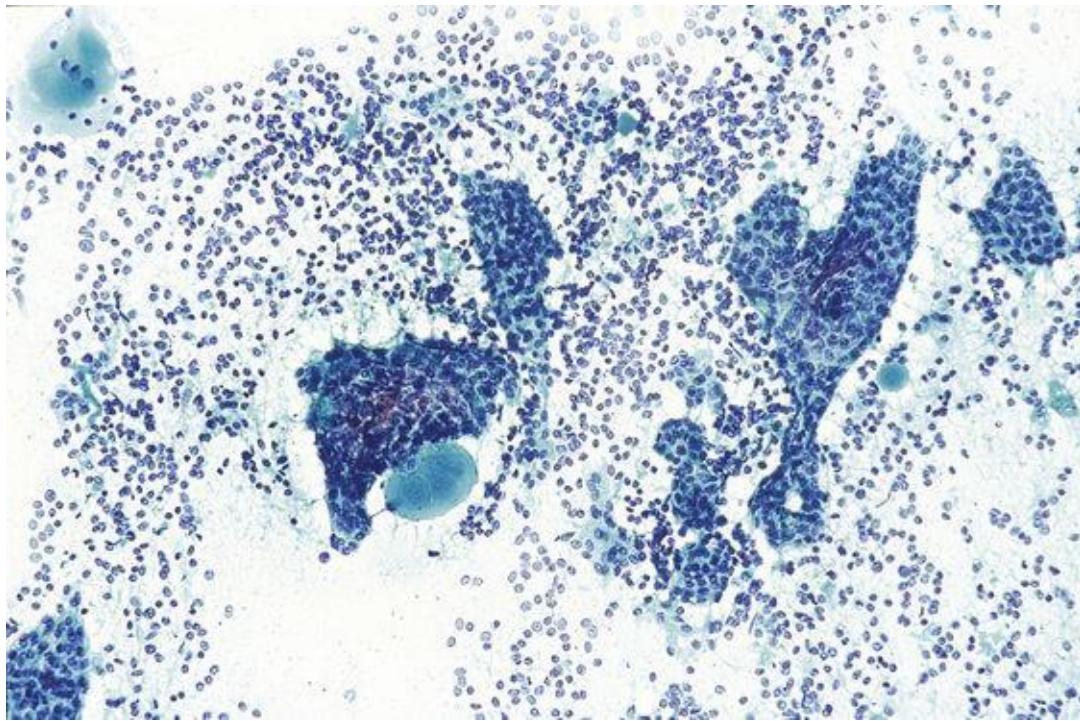


FIGURE 11.25 Epithelial-myoepithelial carcinoma.

Irregular cohesive clusters of epithelial cells are present in a background of bare myoepithelial cell nuclei and acellular hyalinized stromal material (Papanicolaou stain).



Cytomorphology of epithelial-myoepithelial carcinoma

- cellular aspirate
- biphasic cell population:
 - large, clear myoepithelial cells
 - small, dark ductal cells
- basement membrane material
- naked nuclei

proteinaceous spheres, somewhat resembling the matrix spheres of adenoid cystic carcinoma. The epithelial population includes small, dark ductal cells with scant cytoplasm and round to oval nuclei. These are accompanied by a second, often predominant population of large, clear myoepithelial cells with moderate or abundant glycogen-rich

cytoplasm, round vesicular nuclei, and small, distinct nucleoli. The background may contain abundant isolated clear cells and naked nuclei, potentially masking the biphasic pattern of the tumor.¹⁸⁶



Differential diagnosis of epithelial-myoepithelial carcinoma

- adenoid cystic carcinoma
- basaloid neoplasms
- myoepithelial carcinoma
- pleomorphic adenoma (PA)
- PLGA
- metastatic renal cell carcinoma
- other clear cell neoplasms

The key to the diagnosis of an epithelial-myoepithelial carcinoma is recognizing a biphasic cell population, an abundance of large clear myoepithelial cells, and the peripherally located acellular basement membrane material. The architectural features seen in cell block preparations can be helpful in distinguishing epithelial-myoepithelial carcinoma from other biphasic, epithelial-and myoepithelial-derived neoplasms of the salivary gland with overlapping cytologic features.

Clear Cell Carcinoma, Not Otherwise Specified

A number of salivary gland neoplasms can, on rare occasions, exhibit prominent clear cell change.¹⁸⁷ The very rare clear cell carcinoma, not otherwise specified (NOS), a diagnosis of exclusion, is probably the purest example.^{188,189} Clear cytoplasm can be due to mitochondrial condensation or an accumulation of glycogen, mucin, or fat. The differential diagnosis of a clear cell neoplasm in a salivary gland aspirate is broad in scope.^{187,190,191}



Differential diagnosis of clear cell carcinoma

- pleomorphic adenoma (PA)
- myoepithelioma or myoepithelial carcinoma

- oncocytoma
- lipoma
- acinic cell carcinoma
- epithelial-myoepithelial carcinoma
- mucoepidermoid carcinoma
- metastatic renal cell carcinoma
- sebaceous adenoma or carcinoma

Distinction between these entities is based on an assessment of nuclear atypia, the detection of a second cell population admixed with the clear cells, and a careful search for cells without clear cell features.¹⁸⁷ Cell block preparations for histochemical stains, to assess the nature of the clear cytoplasm (e.g., glycogen, mucin, zymogen granules), can be especially helpful. Immunohistochemistry for squamous and myoepithelial differentiation is also helpful.

Mammary Analogue Secretory Carcinoma

MASC is a rare salivary gland neoplasm first described in 2010.⁴⁸ It resembles the secretory carcinoma of the breast. Before its recognition as a separate entity, it was classified as acinic cell carcinoma or adenocarcinoma, NOS. MASC occurs more commonly in the parotid gland in middle-aged and older individuals and is more frequent in men than in women.^{187,192} The prognosis is similar to that for other low-grade salivary gland tumors.¹⁹³ There may be an increased propensity for lymph node metastasis, and surgeons may consider including a limited lymph node dissection in addition to complete excision of the tumor with negative margins.¹⁹³



Cytomorphology of mammary analogue secretory carcinoma

- cellular aspirate
- crowded cell clusters, papillary groups, and isolated cells
- large polygonal cells
- abundant vacuolated cytoplasm
- absence of cytoplasmic zymogen granules
- indistinct cell borders

- bland round nuclei with distinct nucleolus
- extracellular mucoid material

MASC is characterized by a dispersed or loosely cohesive population of cells, often in a papillary or pseudopapillary arrangement.^{137,192,194,195} Individual cells have abundant vacuolated eosinophilic cytoplasm (Fig. 11.26). Nuclei are uniform, round, and often eccentrically placed, with a distinct nucleolus. The background features abundant pale-staining mucoid material. Both intracytoplasmic vacuoles and the extracellular secretory material are positive for mucin.

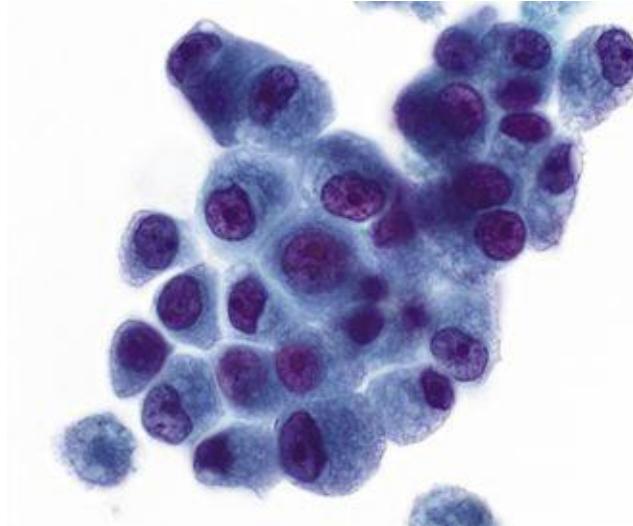


FIGURE 11.26 Mammary analogue secretory carcinoma. Loosely cohesive cells have abundant vacuolated cytoplasm, round nuclei, and distinct nucleoli (Papanicolaou stain).

Differential diagnosis of mammary analogue secretory carcinoma

- normal salivary gland
- oncocytoma
- Warthin tumor (WT)
- acinic cell carcinoma
- mucoepidermoid carcinoma
- sebaceous neoplasms

- clear cell neoplasms
- salivary duct carcinoma
- adenocarcinoma, NOS

The differential diagnosis of MASC includes other tumors with eosinophilic cytoplasm: acinic cell carcinoma; adenocarcinoma, NOS; mucoepidermoid carcinoma; and salivary duct carcinoma. The diagnosis of MASC should be considered when a cytologic pattern of zymogen granule–poor acinar-like cells are present in a background of abundant mucoid secretory material. Aspirates of MASC lack the organized arrangement of acinar cells in lobules seen in the normal salivary gland. A positive immunohistochemical reaction for gammaglobulin and S-100 helps to differentiate MASC from other entities. Acinic cell carcinoma contains cytoplasmic PAS-positive, diastase-resistant zymogen granules not found in MASC, and acinic cell carcinoma is strongly immunoreactive for DOG-1. Mucoepidermoid carcinoma lacks the many multivacuolated cells found in MASC and is usually diffusely positive for p63. Salivary duct carcinoma exhibits marked atypia and extensive necrosis and lacks the prominent vacuolated cells of MASC. MASC is the only primary salivary gland tumor with an ETV6 gene rearrangement.¹³⁷

Primary Small Cell Carcinoma

Primary small cell carcinoma is a rare malignant tumor composed of small undifferentiated cells, although neuroendocrine features are often present.^{196,197} Because of the morphologic similarity to the more common small cell carcinoma of the lung and cutaneous Merkel cell carcinoma, primary small cell carcinoma of the salivary gland is a diagnosis of exclusion. It represents 1% to 2% of all major salivary gland malignancies. The mean age at presentation is 56 years, and it is much more common in men.^{198–200} These are high-grade neoplasms with a poor long-term prognosis.¹⁹⁸



Cytomorphology of primary small cell carcinoma

- cellular smear
- noncohesive cells
- small cells

- round to oval, hyperchromatic nuclei
- indistinct nucleoli
- high nuclear-to-cytoplasmic ratio
- nuclear molding
- frequent mitoses
- necrosis

Aspirates are cytomorphologically indistinguishable from those of a small cell carcinoma of pulmonary origin. Isolated cells and loosely cohesive clusters of cells slightly larger than lymphocytes have a high nuclear-to-cytoplasmic ratio and a hyperchromatic nucleus. Nuclei are round to oval, with dispersed chromatin and indistinct nucleoli. Nuclear molding, mitotic activity, and necrotic material are characteristic.^{[201,202](#)}

Special studies are helpful. Immunocytochemistry shows that most cells are positive for cytokeratins, usually with a dotlike pattern. In addition, many tumors demonstrate reactivity for the neuroendocrine markers synaptophysin, chromogranin, Leu-7, and neuron-specific enolase.^{[198](#)}

The differential diagnosis includes lymphoma, the solid variant of adenoid cystic carcinoma, basaloid squamous cell carcinoma, metastatic small cell carcinoma from the respiratory tract, and Merkel cell carcinoma. Most primary small cell carcinomas of the salivary gland (and Merkel cell carcinomas) show positive immunoreactivity for cytokeratin 20, which distinguishes them from small cell carcinomas of pulmonary origin.^{[202,203](#)} Despite the many similarities with Merkel cell carcinoma, primary salivary gland small cell carcinoma is not associated with the Merkel cell polyomavirus.^{[204](#)}

Lymphoepithelial Carcinoma

Lymphoepithelial carcinomas are clinically aggressive undifferentiated tumors most common among the inhabitants of Greenland and Southern China and those of North American Inuit descent. This tumor comprises less than 0.5% of salivary gland neoplasms^{[50](#)} and is cytologically and histologically similar to its nasopharyngeal counterpart (see [Fig. 12.32](#)).^{[51](#)} The median age of patients is 40 years.



Cytomorphology of lymphoepithelial carcinoma

- syncytial sheets of undifferentiated cells
- pleomorphic, vesicular nuclei
- large nucleoli
- numerous mitoses
- lymphocytes and plasma cells

Aspirates contain a mixture of lymphocytes, plasma cells, and syncytial sheets of large, oval to spindle-shaped cells, with malignant cytologic features: markedly pleomorphic, vesicular nuclei with prominent nucleoli, small to moderate amounts of cytoplasm, and abundant mitotic activity.^{17,205–207} The differential diagnosis includes other undifferentiated carcinomas. In particular, metastatic nasopharyngeal carcinoma must be excluded; this is based upon clinical rather than cytologic features. Both neoplasms are strongly associated with the Epstein-Barr virus (EBV). Keratin immunostains are helpful to exclude lymphoma and malignant melanoma.

Adenocarcinoma, Not Otherwise Specified

Salivary gland adenocarcinomas without specific features are categorized as adenocarcinoma, NOS. With the increased recognition of specific entities, this category is diminishing.²⁰⁸ Low-grade adenocarcinomas may not be recognized as malignant, because the diagnosis rests on the histologic identification of infiltrative growth. High-grade adenocarcinoma, NOS is recognizably malignant and defined by the absence of the distinctive features of other, specified salivary gland malignancies.

Other Malignancies

Squamous Cell Carcinoma

SQC is a rare primary malignancy in the salivary gland. Metastasis to the salivary gland or intraparenchymal lymph nodes is far more common and must be excluded.

Metastases from head and neck SQCs are often cystic and therefore constitute a potentially significant source of false-negative diagnoses. The differential diagnosis was previously discussed. High-grade nonkeratinizing SQC may be indistinguishable from other high-grade salivary gland carcinomas, especially mucoepidermoid carcinoma. Identification of mucin is helpful for the diagnosis of mucoepidermoid carcinoma.

Lymphoma Involving the Salivary Gland

Primary and secondary malignant lymphomas occur in the salivary glands and intraparotid lymph nodes, and constitute 2% to 5% of salivary gland neoplasms.^{78,209} The parotid region is the most frequently involved. Most lymphomas are B-cell non-Hodgkin lymphomas; the most common are extranodal marginal zone lymphoma of the MALT type, follicular lymphoma, and diffuse large B-cell lymphoma.^{51,78} Follicular lymphomas mainly involve periparotid lymph nodes rather than the salivary gland itself. Hodgkin lymphoma rarely involves the salivary glands. A more detailed discussion of the cytologic criteria for diagnosing lymphomas is found in [Chapter 12](#).



Cytomorphology of malignant lymphomas

Extranodal marginal zone lymphoma of MALT type:

- small to intermediate-size lymphocytes with pale cytoplasm (monocytoid B cells)
- round to slightly irregular nuclei
- occasional immunoblasts
- CD45+, CD20+, CD23-, CD10-, CD5-, bcl2+, bcl6-, cyclin D1-

Diffuse large B-cell lymphoma:

- large markedly atypical lymphocytes

- CD45+, CD20+, keratin–, S-100–

Extranodal marginal zone lymphoma of MALT type is a low-grade B-cell lymphoma that typically develops in a background of LESA or Sjögren syndrome.^{210,211} It constitutes a majority of primary salivary gland lymphomas. Aspirates contain a mixed population of small to intermediate-sized lymphocytes (monocytoid B cells) with a moderate amount of pale staining cytoplasm, round to slightly irregular nuclei, condensed chromatin and indistinct nucleoli, admixed with occasional immunoblasts^{212,213} (Fig. 11.27A). Tingible-body macrophages are generally scant to absent, but their presence should not be used to exclude the diagnosis of lymphoma. In some cases, the aspirate can exhibit a polymorphous population of lymphocytes, making the cytologic diagnosis of lymphoma quite difficult; immunophenotyping is recommended when considering the diagnosis of any lymphoma, particularly one of MALT type. The cells of MALT lymphoma are typically CD20+, CD5–, CD10–, CD23–, bcl2+, bcl6–, and cyclin D1–.^{214,215} The differential diagnosis includes a reactive intraparotid lymph node, LESA, cystic lymphoid hyperplasia, and chronic sialadenitis. Some epithelial lesions have a prominent lymphoid component (WT sebaceous lymphadenoma, mucoepidermoid carcinoma, acinic cell carcinoma, and lymphoepithelial carcinoma) and raise the possibility of lymphoma. MALT lymphomas are distinguished from follicular lymphomas by their round to only slightly irregular nuclei and more monomorphic population of cells. In addition, follicular lymphomas are bcl2+, bcl6+ and have a characteristic translocation.

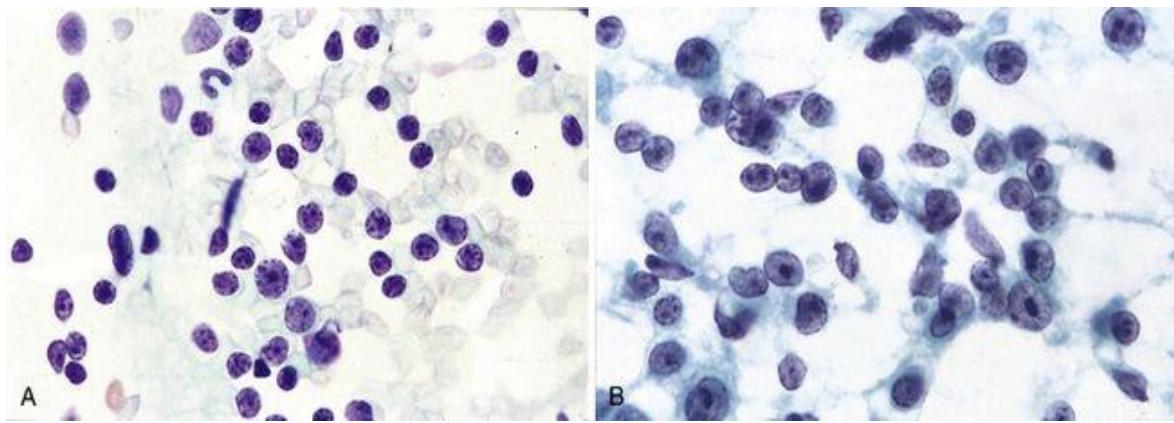


FIGURE 11.27 Lymphomas of the salivary gland.

A, Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) type. A monomorphic population of small to intermediate-sized lymphocytes with a moderate amount of pale cytoplasm (monocytoid B cells) is characteristic, but confirmatory marker studies are necessary (Papanicolaou stain). *B*, Diffuse large B-cell lymphoma. There is a predominant population of large, markedly atypical lymphoid cells with distinct nucleoli and nuclear pleomorphism (Papanicolaou stain).

Diffuse large B-cell lymphoma is composed of an overtly malignant population of large centroblastic and/or immunoblastic cells ([Fig. 11.27B](#)). Small cell and undifferentiated malignancies such as lymphoepithelial carcinoma, malignant melanoma, and small cell carcinoma must be excluded. Demonstration of lymphoid markers like leukocyte common antigen (CD45) and the B-cell markers CD19 and CD20, along with the absence of cytokeratins and other nonlymphoid markers, is especially helpful in difficult cases.

Miscellaneous

Metastatic tumors to the salivary gland or intraparenchymal lymph nodes are not uncommon and are readily documented by FNA.^{214,215} In most instances, the primary site is in the head and neck; metastatic squamous cell carcinoma and malignant melanoma account for the vast majority.

In children, PA is the most common neoplasm; mucoepidermoid carcinoma and acinic cell carcinoma are the most common malignancies. *Hemangiomas* are the most common salivary gland neoplasm in infants. Aspirates are seen to contain blood and bland spindle cells.^{216,217}



Summary of salivary gland fine-needle aspiration

- Salivary gland FNA is an effective tool that helps in the classification and clinical management of and surgical planning for salivary gland lesions.
- Precise classification is possible for many of the commonly encountered lesions, especially pleomorphic adenoma and Warthin tumor.
- Even in the absence of a specific diagnosis, an appropriate differential diagnosis provides clinically useful information.
- The basaloid neoplasms pose the biggest dilemma in diagnosis and clinical management.
- Cystic lesions may result in a false-negative diagnosis, but appropriate clinical communication and followup ultimately result in the correct diagnosis for these lesions.
- For all lesions (cystic and solid), a well-sampled, cellular specimen is vital. With sparsely cellular specimens, a conservative approach—a differential diagnosis rather than an unequivocal interpretation—is strongly encouraged.

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CHAPTER 12

Lymph Nodes

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Lymphomas of Small Cells

Follicular Lymphoma

Marginal Zone (Mucosa-Associated Lymphoid Tissue) Lymphoma

Small Lymphocytic Lymphoma

Mantle Cell Lymphoma

Differential Diagnosis of Small Cell Lymphomas

Lymphomas of Large Cells

Diffuse Large B-Cell Lymphoma

B-Cell Lymphoma, Unclassifiable, with Features Intermediate Between Diffuse Large B-Cell Lymphoma and Burkitt Lymphoma

Burkitt Lymphoma

Plasmablastic Lymphoma

T-Cell Lymphomas

Peripheral T-Cell Lymphoma, Unspecified

Anaplastic Large Cell Lymphoma

Mycosis Fungoides

Adult T-Cell Leukemia/Lymphoma

Precursor T-and B-Cell Lymphoblastic Lymphoma

PostTransplant Lymphoproliferative Disorders

Differential Diagnosis of Large Cell Lymphomas

Nonlymphoid Neoplasms

Carcinomas

Malignant Melanoma

Seminoma/Germinoma

Sarcomas

Enlarged lymph nodes are a prime target for fine-needle aspiration (FNA). In an adult, lymph nodes greater than 1 to 2 cm are an immediate source of concern and, unless the cause is evident, the enlarged node should be aspirated. Although FNA is readily applicable to children also,¹ lymphadenopathy in children and young adults is common and usually due to reactive hyperplasia; for this reason,

it is often watched and not aspirated unless the node is very large or persistent.



Indications for lymph node fine-needle aspiration

- confirm a clinical impression of reactive hyperplasia
- diagnose a suspected malignancy
 - Hodgkin lymphoma
 - non-Hodgkin lymphoma
 - metastatic tumor of unknown primary
- document a metastasis or recurrence in a patient with a known malignancy
- diagnose a suspected infection
- confirm transformation of a known lymphoma to one of higher grade

FNA is accepted by most patients as a minimally invasive method for evaluating lymphadenopathy. It has advantages compared to surgical excision and preserves lymph node architecture should an excision be necessary.



Advantages of lymph node fine-needle aspiration

- rapid turnaround time
- low cost
- easily provides cells for immunophenotyping and molecular diagnostic tests
- less morbidity

FNA is particularly useful in patients with deep-seated lymphadenopathy (e.g., mediastinal, retroperitoneal, abdominal), for which surgical intervention carries the risk of significant morbidity. Even with superficial (e.g., cervical) lymphadenopathy, using FNA avoids uncommon but serious morbidity associated with lymph node excision, like accessory spinal nerve injury.²

FNA has been successfully used for the primary diagnosis of lymphoma in many institutions.³⁻⁵ In some individuals FNA is ideal for this indication, particularly those with rapidly progressive disease, an oncologic emergency (e.g., spinal cord compression, airway compromise, superior vena cava syndrome), deep or surgically inaccessible nodes, advanced age, and/or comorbid clinical conditions that preclude surgical biopsy or excision.⁶

Conversely, when excisional biopsy is less problematic, a larger amount of tissue might be desired at academic institutions for correlative research studies involving emerging technologies like proteomics and genomics, although FNA may sometimes be able to obtain material for these studies as well.⁷



Limitations of lymph node fine-needle aspiration

- sampling error
 - small or deep-seated lymph node
 - nodal fibrosis
 - excessive necrosis or inflammation
 - partial involvement of lymph node by the lesion
- important architectural and/or vascular patterns are lost in some entities:
 - progressive transformation of germinal centers (PTGC)
 - Human immunodeficiency virus lymphadenopathy (HIVAL)
 - toxoplasma lymphadenitis
 - Castleman disease (CD)
 - nodular lymphocyte–predominant Hodgkin lymphoma (NLPHL)
 - T-cell–rich large B-cell lymphoma (TCRLBL)
 - angioimmunoblastic T-cell lymphoma
 - diffuse large B-cell lymphoma (DLBL) arising in follicular lymphoma

Technical Aspects

The principle guiding the cytopathologist is the same as that guiding the surgical pathologist: the integration of clinical information, light microscopic analysis, and results of ancillary studies into a final diagnosis. The full value of FNA is only achieved with this integrated approach.⁸ The cytopathologist who performs the FNA, in fact, has a great opportunity (and responsibility) to incorporate clinical history and physical findings into the final diagnosis⁹ and to judiciously set aside material for ancillary studies when indicated. Indeed, although FNAs are performed by pathologists and clinicians, pathologists have an advantage in that they can perform an on-site adequacy assessment and repeat the procedure until adequate material is obtained for both light microscopy and ancillary studies, which, for suspected lymphoma cases, may include flow cytometry, cell block (for immunocytochemistry), fluorescence in situ hybridization (FISH), karyotypic analysis, and molecular genetic studies. If there is evidence of an infection (neutrophils, granulomas, necrosis, or visible organisms), a portion of the specimen can be submitted for microbiologic culture.

Care in the preparation of smears is necessary. Lymphocytes are fragile and easily crushed if too much pressure is applied during the making of smears. Most slides are air-dried and stained with a Romanowsky (Wright-Giemsa, May-Grünwald-Giemsa, DiffQuik®, Hemacolor®) type of stain. A smaller percentage are stained with a modified Papanicolaou stain. Most pathologists rely heavily on the Romanowsky stain because it highlights cytoplasmic details of lymphoid cells and lymphoglandular bodies. Furthermore, because it is the same stain used for bone marrow aspirates and peripheral blood, it makes for better comparison with other hematologic specimens. The Papanicolaou stain highlights nuclear details (chromatin texture, nucleoli, convolutions, and knobs) and the orangeophilia of a metastatic squamous cell carcinoma (SQC). Because these stains complement each other, both should be used whenever possible. Every needle pass is rinsed in a balanced salt solution after material is expelled onto slides; the needle rinse is useful for ancillary studies (flow cytometry, cytospins, paraffin-embedded cell blocks) as needed. RPMI-1640 cell culture medium is recommended by some practitioners, but normal saline is also acceptable.

Although all slides should be carefully screened, primary attention to the overall low-power pattern is paramount. In particular, an immediate assessment can usually be made as to whether a slide shows a small cell pattern, a large cell pattern, or a mixed cell pattern. These patterns constitute major branch points in

differential diagnosis, as described below.

The major relative contraindication is a severe coagulation disorder; however, in patients with mild coagulopathy, pressure can be applied to a superficial node after aspiration to prevent a hematoma, and appropriate blood products can be given in the case of a deep-seated node aspiration. Complications of lymph node FNA are rare. The most common is a hematoma. Data are sparse on the frequency of a hematoma, but experience suggests that it occurs in less than 1% of cases. Tissue artifacts attributable to FNA (focal hemorrhage with organization; segmental or total infarction) are seen in only 4% of excised lymph nodes¹⁰ and rarely preclude histologic analysis.¹¹ A pneumothorax can result from aspiration of a deep axillary, low cervical, or supraclavicular lymph node if the pleural space is entered inadvertently.

Reporting Terminology and Accuracy

Terminology for reporting lymph node aspiration results is similar to that used for most cytologic specimens. Results are classified into several broad categories: nondiagnostic (unsatisfactory), negative, atypical, suspicious, or positive for malignant cells. The frequency of nondiagnostic (unsatisfactory) results ranges from 5% to 15% of cases¹²⁻¹⁵ and is site and size dependent. For example, it is more difficult to obtain adequate material from a small, deep axillary node than a large cervical lymph node.

A negative result does not absolutely exclude malignancy. For this reason, an explanatory note is helpful, such as “Clinical correlation is advised to ensure that the sample is representative. If clinical suspicion of malignancy persists, additional tissue sampling should be considered.”

FNA of lymph nodes has high sensitivity and specificity in the distinction between a benign and malignant lesion. Accuracy estimates for lymph node FNA vary because of local variations in technique and referral patterns, but most investigators report over 90% accuracy in the diagnosis of metastatic tumor to lymph nodes, and a positive predictive value of almost 100%.¹⁵

Some oncologists question the utility of FNA for the primary diagnosis of lymphoma,¹⁶ even though most studies performed since flow cytometry became readily available show a sensitivity for recognizing non-Hodgkin lymphoma that is higher than 80%,^{3-5,8,17,18} with specificity greater than 90%. The importance of using modern practice guidelines when evaluating an FNA for the possibility of lymphoma can not be overemphasized. At a minimum, the cytologist must have a working knowledge of the 2008 and any subsequent World Health Organization (WHO) classification;¹⁹ some of the sample must be allocated for immunophenotyping (IP); and a close interaction with hematopathologists must be fostered. Under such circumstances, the accuracy of FNA for the diagnosis of non-Hodgkin lymphoma is excellent. Accuracy is even higher when FNA is performed for recurrent, as opposed to newly diagnosed, non-Hodgkin lymphoma.

Regarding subclassification, some lymphomas are easier to subtype precisely than others. IP either by flow cytometry^{20,21} (preferred) or immunocytochemistry plays a vital role in the diagnosis and subtyping of lymphomas; without it, the accuracy of FNA is severely diminished. Lymphomas that have characteristic immunophenotypes, like small lymphocytic lymphoma (SLL), mantle cell lymphoma (MCL), and lymphoblastic lymphoma, are easier to recognize than

those that do not.¹² Differentiating the so-called “small cell lymphomas” from each other and from reactive hyperplasia by morphology alone is not recommended.¹²⁻¹⁴ In addition, FISH and molecular techniques (discussed below) are playing an increasingly important role as adjuncts to FNA, particularly in the diagnosis and subtyping of non-Hodgkin lymphoma.

Ancillary Studies

In evaluating a lymph node FNA for a possible lymphoproliferative disorder, a variety of specialized studies are indispensable. These require additional effort and may increase the turnaround time of the case, but the extra effort and time are often rewarded by precise diagnosis and classification of the neoplastic process.



Ancillary studies help to

- distinguish lymphoid from nonlymphoid lesions
- distinguish non-Hodgkin lymphomas from reactive lesions by confirming clonality
- subclassify a lymphoma

Clonality in B-cell lymphomas is usually documented by demonstrating light chain restriction: a clonal expansion of B cells that preferentially express either κ or λ immunoglobulin light chains. Clonal B-cell proliferations identified by flow cytometry almost never occur in reactive lymph nodes, but exceptions have been documented.²² Therefore, it cannot be overemphasized that all aspects of the case—morphology, immunophenotype, and clinical features—must be taken into consideration before issuing a diagnosis of lymphoma.

Lymphomas are subclassified into biologically meaningful subtypes based on clinical features, expression of immunophenotypically detectable markers (e.g., differential expression of CD5, CD10, bcl-1, and CD23 in the small cell lymphomas), and by genetic changes detectable by FISH or karyotypic analysis. Because aberrant results occasionally occur with every assay, it is advisable to use a panel of antibodies and appropriate genetic studies (when indicated), rather than any one in isolation.

Immunophenotyping to determine B-cell clonality and document antigen expression can usually be performed by flow cytometry,^{13,17,21,23-25} although immunocytochemistry on cell block preparations can also be used.^{26,27} Although flow cytometry is preferred, each method has its advantages ([Table 12.1](#)).

TABLE 12.1

COMPARISON OF FLOW CYTOMETRY AND IMMUNOCYTOCHEMISTRY FOR THE ANALYSIS OF LYMPHOID MARKERS

	Flow cytometry	Immunocytochemistry (cell block)
Morphology	lost	preserved
Sensitivity	high	lower
Specimen requirement	few cells, fresh (unfixed)	many cells, fixed
Detection of small monoclonal population	good	poor
Multiple labeling	easy	laborious
Hodgkin lymphoma	useless	useful
Turnaround time	2 hours	24–48 hours



Advantages of flow cytometry

- rapid turnaround (2 hours)
- quantitative (number of cells and intensity)
- multiple stains per cell
- superior detection of small monoclonal populations



Advantages of immunocytochemistry

- morphology is preserved
- more markers available (including nuclear markers)
- useful in detecting Reed-Sternberg cells and other large cells

To fully characterize a lymphoma, between 12 and 15 antibodies may be needed,²⁸ but often fewer are sufficient. If the sample is sparsely cellular, the cytologist can assist the flow cytometry or immunocytochemistry laboratory by using cell morphology to select an appropriate, more limited panel of antibodies.

Flow Cytometry

The routine application of flow cytometry has markedly improved the diagnostic accuracy of FNA.^{29,30} In a flow cytometer, a liquid suspension of single cells is made to flow in single file through a laser beam. As each cell traverses the beam, it scatters light in different directions.



The amount of light scattered in different directions gives useful information about the cell

- forward scatter: proportional to cell size
- side scatter: proportional to cell complexity (e.g., shape of nucleus and cytoplasmic granularity)

Using forward and side scatter alone, a flow cytometer easily distinguishes normal lymphocytes, monocytes, and polymorphonuclear leukocytes.

Along with these morphologic properties, lymphoid cells are characterized by their expression of different immunophenotypic markers, which are identified using antibodies conjugated to a fluorescent molecule (fluorochrome). When excited by a laser beam, a fluorochrome emits a pulse of light of a specific wavelength (color). The intensity is proportional to the amount of fluorochrome-conjugated antibody attached to that cell. For example, if we incubate a suspension of cells with fluorochrome-conjugated antibodies to CD19, a marker of B cells, each B cell will emit a bright pulse of light, but T cells, which lack CD19, will show only background fluorescence.



Commonly used fluorochromes, which emit pulses of different-colored light when excited by a laser beam

- fluorescein isothiocyanate (FITC)
- phycoerythrin (PE)
- peridinin chlorophyll protein (PerCP)

With two fluorochromes that have different emission spectra, one can incubate cells with two antibodies, each conjugated with a different fluorochrome. The intensity of staining of cells for the two antibodies is plotted as a two-

dimensional dot-plot (scattergram).



Advantages of using multicolor cytometry

- fewer tubes needed
- multiple antigens can be examined on each cell.

Four-color flow cytometry is standard, but many laboratories use more fluorochromes for comprehensive immunophenotyping. Being able to analyze multiple antigens per cell improves the sensitivity in the detection of clonal B cells. For example, a small proportion of CD5-positive, κ-restricted B cells, in a background of many reactive polytypic B cells, can be detected by four-color flow cytometry when the sample is simultaneously labeled with four antibodies (CD19, CD5, κ, λ), but will go undetected with fewer antibodies. For most clinical applications, four-color flow cytometry is adequate. For a full lymphoma panel, 10 aliquots of 1×10^5 cells each, or a total of 1×10^6 cells is usually sufficient.

Immunocytochemistry

With immunocytochemistry, the presence of cell markers is probed on cells attached to a glass slide. We prefer to perform immunohistochemistry on formalin-fixed, paraffin-embedded sections from cell block preparations (because this is technically similar to the more familiar procedure for tissue specimens), but some antigens, notably κ and λ immunoglobulin light chains, are difficult to evaluate in fixed specimens. For this reason, unfixed (air-dried) cytocentrifuge preparations can be attempted on lymph node FNAs when lymphoma is a consideration, but, even in such preparations, background staining can be high. Commonly, slides are prepared by cytocentrifugation onto a 6-mm circle on a glass slide. The limited area to be evaluated conserves antibodies and facilitates interpretation of results and is particularly appropriate when the specimen is too scant for cell block preparation. Some of the most commonly used markers are listed in [Table 12.2](#). Immunostaining methods vary slightly from one laboratory to another.^{26,27}

TABLE 12.2

USEFUL CORE IMMUNOCYTOCHEMICAL MARKERS FOR THE DIAGNOSIS OF LYMPHOPROLIFERATIVE DISORDERS

CD3	T cells
CD5	T cells, coexpressed in some B-cell lymphomas (small lymphocytic lymphoma and mantle cell lymphoma)
CD19	B cells
CD20	B cells
CD10	follicular lymphoma
bcl-6	follicular center cells/follicular lymphoma
CD23	small lymphocytic lymphoma (negative in mantle cell lymphoma)
PAX5/BSAP	B cells (recurrent/rituximab treated patients)
CD45	most lymphoid cells
κ	B-cell immunoglobulin light chain (assessing clonality)
λ	B-cell immunoglobulin light chain (assessing clonality)
cyclin D1	Mantle cell lymphoma
Ki67	proliferative rate (for grading)
CD15 (LeuM1)	Reed-Sternberg cells (except nodular lymphocyte predominant Hodgkin lymphoma)
CD30 (Ki-1)	Reed-Sternberg cells, anaplastic large cell lymphoma
Anaplastic lymphoma kinase	anaplastic large cell lymphoma

Molecular Genetic Studies

Just as immunocytochemistry localizes specific proteins in cells, molecular genetic studies identify specific DNA sequences. They are especially helpful when an immunophenotype is inconclusive in establishing clonality and/or cell lineage. For example, T-cell clonality can usually only be established by these methods.



Molecular techniques available

- polymerase chain reaction (PCR)
- FISH
- gene expression profiling

The *polymerase chain reaction (PCR)* amplifies tiny amounts of a defined region of DNA, provided that sequences surrounding the region of interest are known. The technique requires repeated cycles of denaturation and DNA synthesis using oligonucleotide primers, a heat-resistant DNA polymerase, and the four nucleotides. The oligonucleotide primers are synthesized to complement DNA sequences flanking the region of interest. Typically, 20 to 60 cycles of denaturation and synthesis over several hours result in a tremendous amplification of a homogeneous set of the DNA sequence of interest. PCR is fast (1 to 2 days), does not require radioactive probes, and can be performed on formalin-fixed cell blocks. Owing to its extraordinary sensitivity, however, it is prone to false-positive results; rigorous controls and critical review of the results are vital. PCR is a useful test for immunoglobulin and T-cell receptor rearrangements as markers of clonality.^{25,31-35} PCR for clonality is redundant for B-cell neoplasms that are shown to be monoclonal by immunophenotyping, but it is useful in select circumstances: It can be used as a confirmatory test when flow cytometry suggests a T-cell neoplasm, when cells fail to survive flow cytometry processing, or when unfixed cells are unavailable for flow cytometry.^{31,36}

PCR can be used to detect specific breakpoints in lymphomas. This is especially helpful if (1) the patient has a known lymphoma with a characteristic translocation, and the FNA is carried out to rule out recurrence; or (2) in the case of a primary diagnosis of lymphoma, if the morphology suggests a specific subtype of lymphoma with a characteristic translocation. Because a translocation characteristic of a particular type of lymphoma can occur at different breakpoints in different patients, PCR may have low sensitivity if only one breakpoint is probed.

With *FISH*, DNA probes for specific chromosomal regions, labeled with a fluorochrome such as rhodamine, are hybridized with intact nuclei. Smears, cytocentrifuge preparations, and, especially, thinlayer slides are ideal substrates, because the intact cells on cytologic preparations allow optimal signal detection. DNA probes are particularly useful for demonstrating translocations,³⁷ deletions, and gene amplifications (e.g., trisomies). Most practitioners recommend that cells be allocated for PCR and FISH, in case they are needed, in addition to flow cytometry, in every case of suspected lymphoma.³⁸

Gene expression profiling using microarray analysis may find a role in the cytologic diagnosis of lymphoma. Aspirates of suspected non-Hodgkin lymphoma can be rinsed in a ribonucleic acid (RNA)-stabilization reagent, and gene expression profiling performed by hybridizing RNA to gene chips.³⁹ Among other potential applications, gene expression profiles can distinguish among different prognostic subtypes of diffuse large B-cell lymphoma (DLBL).⁴⁰

NonNeoplastic Lesions

The normal microanatomy of a lymph node consists of a cortex, paracortex, and medulla, each with a different mix of cells that include small B and T lymphocytes, follicular center cells (centrocytes and centroblasts), plasma cells, plasmacytoid lymphocytes, immunoblasts, follicular dendritic cells, interdigitating reticulum cells, tingible-body macrophages, sinus histiocytes, endothelial cells, mast cells, and eosinophils. Some of these cells are morphologically similar (but immunophenotypically different), while others show distinctive morphologic features. Truly normal lymph nodes are rarely aspirated, because they are too small to produce a palpable mass. Most normal but enlarged lymph nodes are examples of hyperplasia, in which various cellular components of a normal node are increased.



Two consistent findings in aspirated lymphoid tissue

- a dispersed cell pattern
- lymphoglandular bodies

A dispersed, isolated cell pattern is typical of lymphoid cells, but there are exceptions. Smear thickness, clotting, and suboptimal spreading technique may cause pseudo-clustering of lymphocytes. Aggregates of lymphocytes adherent to follicular dendritic cells occur in follicular hyperplasia and follicular lymphoma (FL),^{41,42} where they are known as dendritic-lymphocytic aggregates or *intact follicles*. Conversely, some nonlymphoid tumors (e.g., melanoma) lack cohesion and mimic malignant lymphoma.

Detached fragments of lymphoid cell cytoplasm, visible in most smears of lymphoid (both benign and malignant) tissue, are given the fanciful (and imprecise) name *lymphoglandular bodies*.⁴³ In Romanowsky-stained smears they are a pale blue or blue-gray, and may contain tiny vacuoles; they are much more difficult to see in Papanicolaou-stained smears. Rarely, similar findings are seen in a small number of nonlymphoid lesions such as small cell neuroendocrine carcinoma,⁴⁴ but, in general, the absence of lymphoglandular bodies makes it unlikely that the cells are of lymphoid origin.

Reactive Hyperplasia (without Specific Etiology)

Reactive lymphoid hyperplasia is a common nonspecific form of lymphadenopathy due to a variety of causes and usually presents in the pediatric and young adult population with a single moderately enlarged node. Most cases of reactive lymphoid hyperplasia are examples of follicular rather than paracortical hyperplasia. The excursions of an aspirating needle capture cells from the expanded cortex, paracortex, and/or medulla which, randomly commingled within the barrel of the needle, are then expelled onto the slide as a rich, heterogeneous mixture.



Cytomorphology of reactive hyperplasia

- polymorphous population
- small lymphocytes
- plasmacytoid lymphocytes
- centrocytes
- centroblasts
- immunoblasts
- tingible-body macrophages
- dendritic cells
- dendritic-lymphocytic aggregates (“intact follicles”)
- capillaries, eosinophils, mast cells

There is great variety in the cell types encountered ([Fig.12.1](#)). *Small lymphocytes* with round nuclei and coarsely textured chromatin usually predominate, but they are admixed with other cells. *Centrocytes* are intermediate-sized lymphocytes with irregular or cleaved nuclei, inconspicuous nucleoli, and scant cytoplasm. *Centroblasts* are large cells with round, vesicular nuclei, one to three peripheral nucleoli, and a narrow rim of basophilic cytoplasm. *Immunoblasts* are large cells with fine, open chromatin; one very prominent, centrally located nucleolus; and moderate to abundant, pale to basophilic cytoplasm. Some have a perinuclear clear zone. *Tingible-body macrophages* are very large phagocytic cells with a spherical or ovoid nucleus, finely granular chromatin, a small distinct nucleolus, and voluminous debris-laden cytoplasm. *Dendritic cells* are also large, with pale oval nuclei, small nucleoli, and cytoplasmic processes; they are usually binucleated or

multinucleated and may appear epithelioid if the cytoplasmic processes are not prominent.

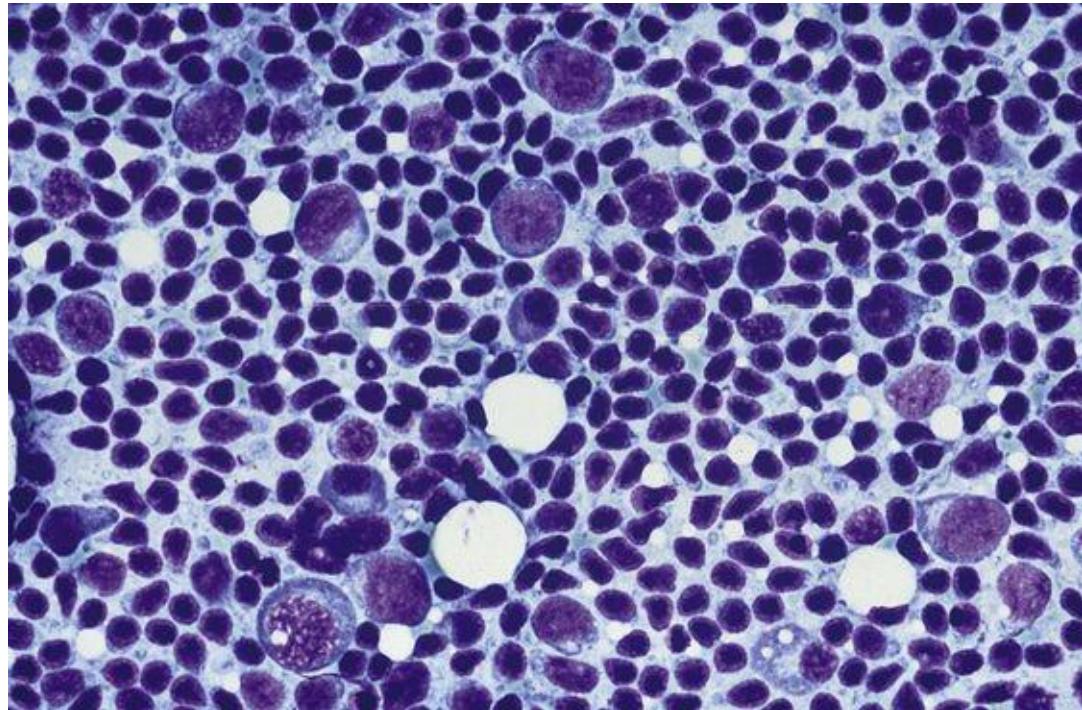


FIGURE 12.1 Reactive lymphoid hyperplasia.

Immunoblasts and plasmacytoid lymphocytes are interspersed throughout a smear dominated by small, round lymphocytes, creating a polymorphous cell picture (Romanowsky stain).

Dendritic-lymphocytic aggregates (also known as *follicular center fragments* or *intact follicles* when tingible-body macrophages and capillaries are present), are clusters of dendritic cells, small lymphocytes, centrocytes, and centroblasts⁴¹ ([Figs. 12.2, 12.3](#)). Distinguishing between fragments that do or do not contain tingible-body macrophages and capillaries does not appear to have diagnostic value. These aggregates break the rule that lymphoid lesions lack cell cohesion; they may be seen when follicular hyperplasia is a prominent component of the lymphoid proliferation and must not be confused with a cohesive metastatic neoplasm.

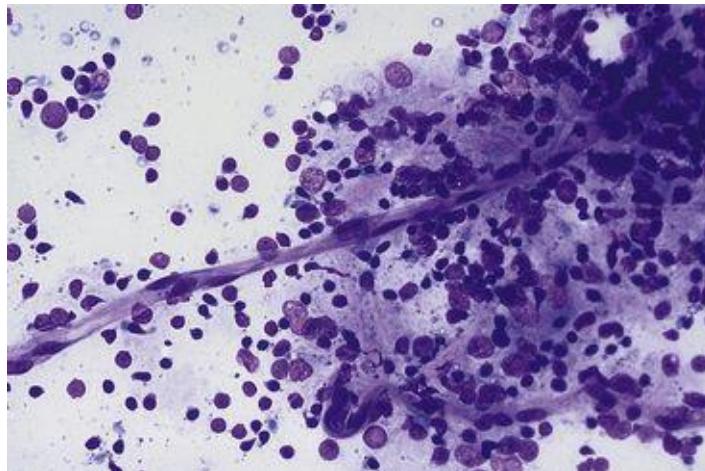


FIGURE 12.2 Reactive lymphoid hyperplasia.

A capillary emanates diagonally from this follicular center fragment (dendritic-lymphocytic aggregate), which contains a mixture of dendritic cells and a heterogeneous lymphocyte population (Romanowsky stain).

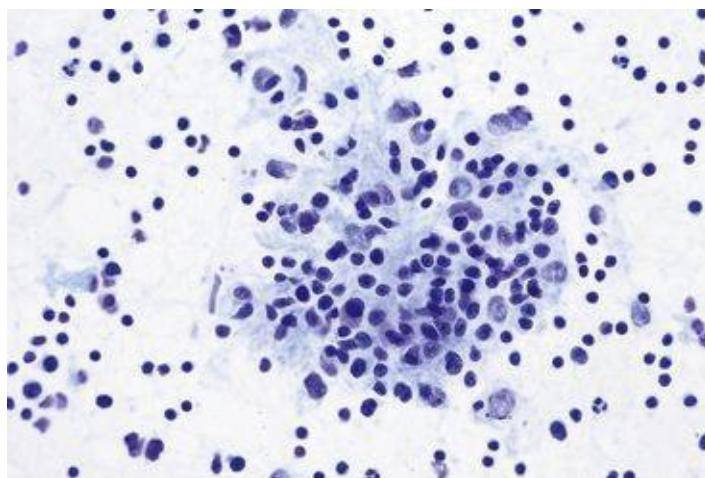


FIGURE 12.3 Reactive lymphoid hyperplasia.

This dendritic-lymphocytic aggregate is a loose collection of small round lymphocytes and dendritic cells. The latter have pale nuclei with delicate cytoplasmic extensions (Papanicolaou stain).

Because little stroma exists to retain lymphoid cells during aspiration, large numbers of cells are obtained, resulting in highly cellular smears. Thus, high cellularity in a lymph node FNA does not correlate with malignancy. In most examples of reactive lymphoid hyperplasia, small lymphocytes vastly outnumber centroblasts and centrocytes, which are themselves more numerous than immunoblasts, plasmacytoid lymphocytes, and tingible-body macrophages.



Differential diagnosis of reactive hyperplasia

- non-Hodgkin lymphoma, especially:
 - follicular lymphoma (FL)
 - marginal zone lymphoma (MZL)
 - T-cell lymphoma
 - T-cell-rich large B-cell lymphoma (TCRLBL)
- Hodgkin lymphomas
- posttransplant lymphoproliferative disorder (PTLD)
- other benign lymphadenopathies

The major challenge is to exclude a lymphoma or one of the benign lymphadenopathies with specific etiology. Knowledge of the clinical background is critical; an FNA diagnosis of reactive hyperplasia should be avoided in the elderly⁴⁵ and in patients with markedly enlarged (e.g., greater than 3 cm) or deep-seated nodes and/or multiple enlarged nodes. Because architectural pattern is not available, cell size, smear composition, and, (most importantly) immunophenotype are used as discriminators. An often cited, helpful morphologic feature is the heterogeneity of the cell pattern: In non-Hodgkin lymphoma the range of lymphoid forms and other cell types is sometimes more narrow. In practice, however, there are many exceptions. Hodgkin lymphoma, follicular lymphoma (FL), marginal zone lymphoma (MZL), posttransplant lymphoproliferative disorders (PTLDs), some T-cell lymphomas, and T-cell-rich large B-cell lymphoma (TCRLBL) have a heterogeneous appearance that mimics reactive lymphoid hyperplasia. The distinction in such cases relies on identifying rare atypical forms (e.g., Reed-Sternberg cells or lymphocytic and histiocytic [L & H] cells in Hodgkin lymphoma) or the immunophenotype, which is useful whenever non-Hodgkin lymphoma is a consideration (clinically or cytologically).

In the special case of the patient who has received a solid organ or bone marrow transplant, *in situ* hybridization for Epstein-Barr virus (EBV)-encoded RNA (EBER) can be helpful in identifying a PTLD.⁴⁶

Benign Lymphadenopathies with Specific Etiology

Some benign lymphadenopathies have distinct architectural features that allow for a specific diagnosis in tissue sections. These features are not readily apparent in smears, and therefore, in most circumstances, these lymphadenopathies cannot be reliably distinguished from reactive lymphoid hyperplasia by FNA.



Lymphadenopathies that cannot be reliably distinguished from reactive lymphoid hyperplasia by fine-needle aspiration

- Castleman disease (CD)
- toxoplasma lymphadenitis
- progressive transformation of germinal centers (PTGC)

Castleman disease (CD) is a rare form of lymph node hyperplasia and is divided into hyaline-vascular type (usually unicentric and asymptomatic) and plasma cell type (usually multicentric and often associated with constitutional symptoms, elevated interleukin-6, and human immunodeficiency virus [HIV] and human herpesvirus 8 [HHV8] infection). The most common sites of involvement in the unicentric type are the mediastinum and lung, but peripheral lymphadenopathy is the rule for patients presenting with the multicentric type. In the hyaline-vascular type, tissue sections reveal small, hyalinized germinal centers and broad expansion of the mantle zone. Large, pleomorphic follicular dendritic cells are seen. In the plasma cell type, the interfollicular areas contain sheets of mature plasma cells. Although these findings are characteristic, they are not specific, and ultimately CD is a diagnosis of exclusion.

There are few descriptions of the cytomorphology of CD in FNA specimens, but some investigators suggest that dysplastic follicular dendritic cells may be a clue to the diagnosis.^{47,48} The presence of tissue fragments containing branching capillaries and/or hyaline material has been proposed as a cytologic hallmark, but similar vascular branching can be seen in reactive lymphoid hyperplasia. Many believe that a specific diagnosis by FNA is not possible even with immunophenotypic analysis.⁴⁷ Fortunately, the clinical significance of this difficulty is minimal: In the unicentric type, a nonspecific FNA diagnosis in a symptomatic patient would eventuate in excision of the node, curing the disease, whereas in the multicentric type, blood tests for HHV8 and/or interleukin-6 (or an HHV8 immunostain of an FNA cell block) could provide the diagnosis (in the

appropriate clinical context). A caveat, however, for the FNA cytologist is that HHV8 preferentially infects immunoglobulin M (IgM) λ -expressing B cells, so that the resulting virally driven proliferation could appear clonal by immunophenotyping—simulating lymphoma—but is in reality polyclonal,⁴⁹ as could be demonstrated by molecular (PCR) testing for immunoglobulin gene rearrangement.

Lymphadenopathy caused by *Toxoplasma gondii* is most commonly cervical and usually self-limited, but it can mimic lymphoma clinically. The histologic changes are highly suggestive of this entity: follicular hyperplasia with small aggregates of epithelioid histiocytes that hug the periphery of germinal centers, and zones of monocyteoid B-cell hyperplasia. These architectural features are largely unappreciated in smears, which resemble reactive hyperplasia. The diagnosis is usually confirmed by serologic titers. It is only when organisms are seen—a rare occurrence—that a confident cytologic diagnosis can be issued.^{50,51} The organisms occur as bradyzoites within enlarged finely granular histiocytes, or as free tachyzoites lying within an exudate, and their presence can be confirmed by immunocytochemistry⁵¹ or PCR.

Progressive transformation of germinal centers (PTGC) is a condition marked by follicle expansion, disruption, and/or replacement by mantle cells, which are small-to intermediate-sized lymphocytes with round or irregular nuclei, inconspicuous nucleoli, and scant cytoplasm. As an architectural disruption, PTGC is impossible to identify by FNA. By flow cytometry, one-third of PTGC cases show a significant proportion of CD4+CD8+ (“double positive”) T-cells, which can be a clue to the diagnosis, but such populations can also be seen in T-cell lymphoma, thymoma, and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL).⁵² In fact, PTGC is often seen in patients with NLPHL, but most patients with PTGC do not develop lymphoma.

Inflammatory/Infectious Conditions with Characteristic Fine-Needle Aspiration Findings

Sarcoidosis

Sarcoidosis is a systemic granulomatous disease of unknown cause affecting young to middle aged adults that is more prevalent in African Americans. A wide variety of tissues may be involved, but the lung and lymph nodes of the mediastinum are most often affected. Peripheral lymphadenopathy is most common in the head and neck.⁵³ Noncaseating granulomas are a characteristic but

nonspecific feature of sarcoidosis—they are also encountered in fungal, bacterial, and mycobacterial infections; hypersensitivity conditions; neoplasms such as Hodgkin lymphoma and T-cell lymphoma; the drainage pathway of a malignancy; and exposures to foreign material—and the diagnosis (whether by surgical pathology or FNA) is always one of exclusion and clinical correlation. An important practical point is that granulomas are often sparse in FNAs in the late phase of sarcoidosis (due to fibrosis); such aspirates are usually very hypocellular. Indeed, sarcoidosis is one of the commonest explanations for the paradoxical finding of a very hypocellular or acellular aspirate from a large node (provided a targeting error has been excluded).



Cytomorphology of sarcoidosis

- granulomas
- epithelioid histiocytes
- multinucleated giant cells
- lymphocytes
- hypocellular aspirate (late phase)
- clean background

The key component of a granuloma is the epithelioid histiocyte, with its elongated, elliptical or spindle-shaped, curved or slightly indented nucleus. This produces a nuclear outline described as a “footprint,” “C-shape,” “V-shape,” or “boomerang shape” ([Fig. 12.4](#)). The number of epithelioid histiocytes in a granuloma varies from 5 to 50 or more, and they typically appear as a syncytium.

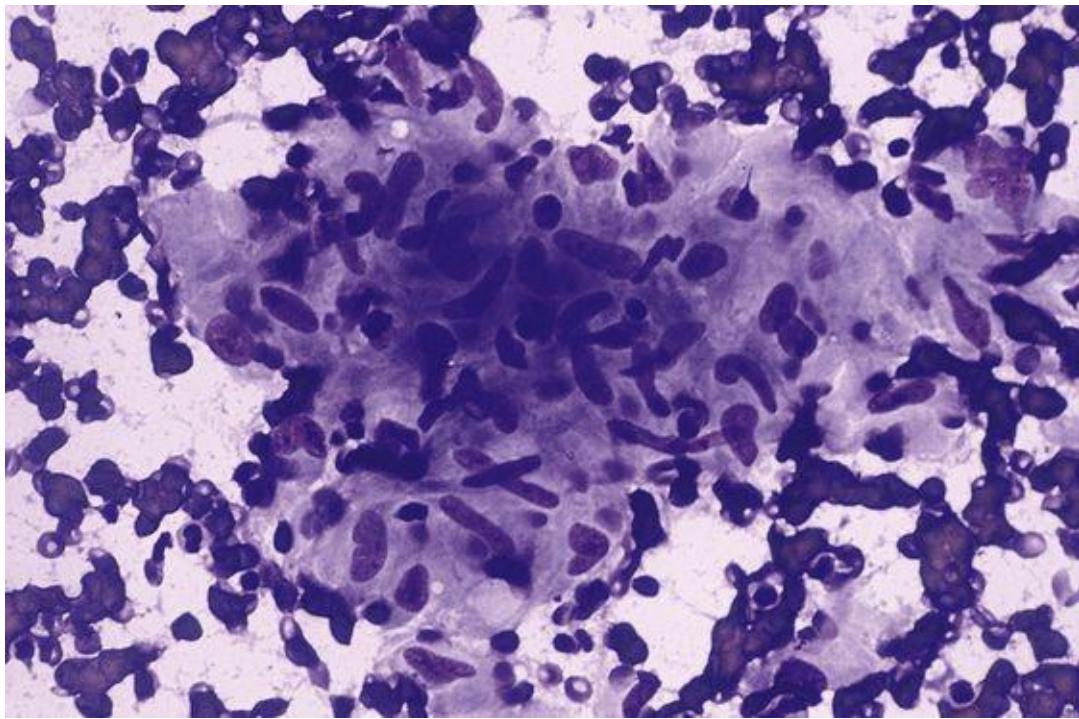


FIGURE 12.4 Sarcoidosis.

In sarcoidosis, the granulomas are tight aggregates of epithelioid histiocytes. The cells have oval or curved nuclei and abundant cytoplasm with indistinct borders (Romanowsky stain).



Differential diagnosis of sarcoidosis

- infections
- foreign-body reaction
- spindle cell neoplasm
- granulomas accompanying a malignant neoplasm
- dendritic-lymphocytic aggregates

Infectious organisms must be excluded by special stains and/or microbiologic culture results, but most fungal and mycobacterial infections are not purely granulomatous; rather, they are accompanied by necrotic debris and a neutrophilic, lymphocytic, and/or plasmacytic infiltrate. The cells of spindle cell sarcoma, although often in aggregates, have atypical nuclei with coarsely textured chromatin, and footprint-shaped nuclei are uncommon in spindle cell tumors. The dendritic cells in dendritic-lymphocytic aggregates have round to oval nuclei rather than elongated, curved nuclei, and germinal center elements (centrocytes, centroblasts, and tingible-body macrophages) are usually seen in

association.

Granulomas accompany some malignancies (e.g., squamous cell carcinoma, Hodgkin lymphoma, T-cell lymphoma, seminoma) more often than others (adenocarcinoma, sarcoma). This is generally not problematic because the malignant cells are clearly seen and distinct from the granulomas. Infrequently, however, the granulomatous response is so pronounced it obscures malignant cells, or the malignant cells resemble epithelioid histiocytes.⁵⁴

Bacterial and Fungal Lymphadenitis

Aspirates of acute bacterial lymphadenitis are very cellular and composed of nearly a pure population of variably degenerated neutrophils. Pus is often seen in the hub or barrel of the syringe at the time of the procedure. One should submit a part of any purulent biopsy for culture, expelling it into a sterile container or culturette device. This should be done even if a patient is receiving antibiotics, because the organisms may be resistant to the patient's current antibiotic therapy. In some examples, the organisms are seen directly on the Romanowsky-stained smear ([Fig. 12.5](#)).

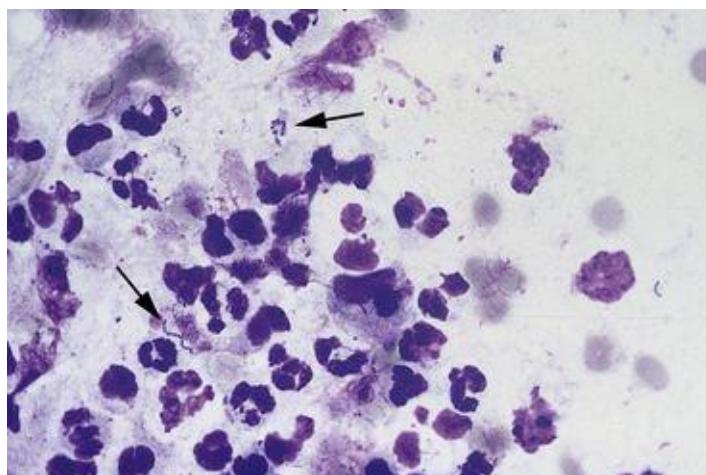


FIGURE 12.5 Acute lymphadenitis.

Neutrophils are mixed with small, round lymphocytes. Note the chains of bacterial cocci (arrows) (Romanowsky stain).

Aspirates of fungal lymphadenitis are variable in morphology. Some have only a pure neutrophilic infiltrate, some only granulomas, some a mixture of the two, and in some only the fungal organisms are present with few if any accompanying inflammatory cells. Among the more common fungi are

Cryptococcus neoformans, *Histoplasma capsulatum*, and *Coccidioides immitis*. Definitive diagnosis depends on culture and histochemical stains, including methenamine silver, periodic acid-Schiff (PAS), and mucicarmine (for encapsulated *Cryptococcus*), which can be performed directly on destained or unstained smears or cell block sections.

In both bacterial and fungal lymphadenitis, identifying an organism by morphology or culture is important for more than just antibiotic selection, because the identification of an infectious agent aids in exclusion of a neoplastic cause for the necrosis and inflammatory reaction in an FNA specimen.

Cat Scratch Disease

Cat scratch disease is a self-limited infection by *Bartonella henselae*, which primarily affects lymph nodes in the axilla, inguinal region, and neck of children and adolescents and resolves in 1 to 4 months. Nodes are typically tender and may be matted together. The clinical history reveals a recent cat bite or scratch (often from a newly acquired kitten) in about 50% to 75% of cases. Early lesions begin with a proliferation of plasmacytoid monocytes, with small foci of necrosis and neutrophils. The necrotic foci enlarge and coalesce to form necrotizing granulomas called *stellate microabscesses*.



Cytomorphology of cat scratch disease

- neutrophils
- loose or tightly clustered granulomas
- necrosis

Smears show numerous neutrophils and variably sized granulomas⁵⁵ ([Fig. 12.6](#)). If only small granulomas are present, they may be obscured by the abundant neutrophils. The causative agent, the Gram-negative bacillus *Bartonella henselae*, may be stained using the Steiner method, but results are highly variable.⁵⁶ Unless the organism is identified or isolated, the cytologic diagnosis is presumptive, not definitive, but serologic studies can be helpful.

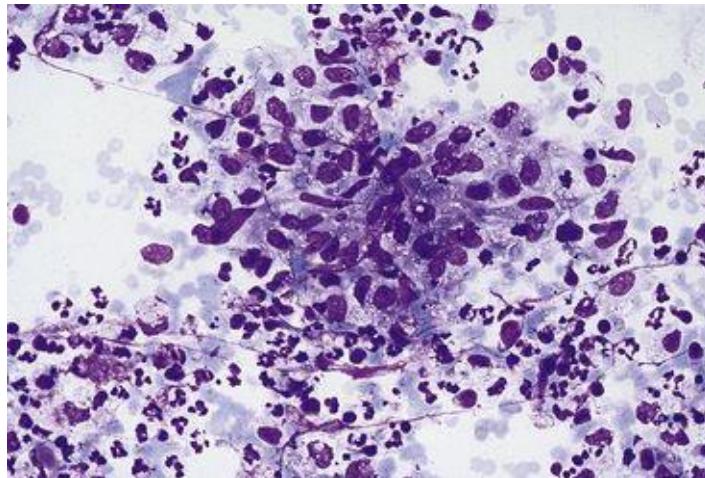


FIGURE 12.6 Cat scratch disease.

A discrete aggregate of epithelioid histiocytes is surrounded by large numbers of neutrophils (Romanowsky stain).



Differential diagnosis of cat scratch disease

- other causes of suppurative granulomatous lymphadenitis
- reactive lymphoid hyperplasia

Other organisms can produce an identical cytologic picture including *Francisella tularensis*, which causes tularemia, *Chlamydia trachomatis*, which causes lymphogranuloma venereum, *Yersinia enterocolitica* or *Y. pseudotuberculosis*, certain fungi, and less commonly *Mycobacterium tuberculosis*. In most cases, microbiologic and/or serologic analysis are required for specific diagnosis. Early stages of cat scratch disease display very few granulomas and mimic reactive lymphoid hyperplasia.⁵⁵

Mycobacterial Lymphadenitis

Mycobacterial infections occur in immunocompetent and immunosuppressed individuals. FNA is particularly efficacious in those countries where mycobacterial infection is endemic, but its accuracy may be lower in the United States because of the lower prevalence of disease, atypical clinical presentations,⁵⁷ and better treatment of HIV-infected patients (who would otherwise constitute the majority of patients with mycobacterial infections).



Cytomorphology of mycobacterial lymphadenitis

- necrosis
- granulomas
- histiocytes
- neutrophils
- intra-and extracellular bacilli (“negative images”)
- acid-fast staining of bacilli

Smears may show granulomas with necrosis, granulomas without necrosis, or sometimes necrosis only⁵⁸ (Fig. 12.7A). In immunocompromised patients, there may be only loose aggregates of histiocytes rather than true granulomas. Aspirates from patients with nontuberculous mycobacterial infection (typically *Mycobacterium avium* complex) may show the “negative image” phenomenon,⁵⁹ which occurs because the lipid coat of the bacilli resists staining with any Romanowsky stain; thus, the bacilli are seen as optically clear rods/striations surrounded by stained proteinaceous or necrotic material. These rods may be extracellular or within macrophages, where they appear as multiple linear striations resembling the crinkled tissue paper appearance of the storage cells of Gaucher disease. This phenomenon is not visible with the Papanicolaou stain.

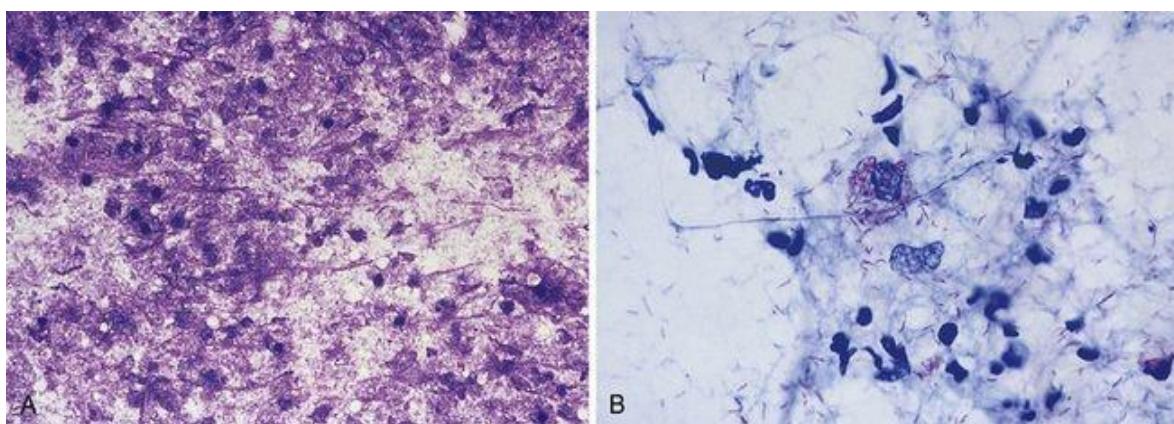


FIGURE 12.7 Mycobacterial lymphadenitis.

A, Necrotic material and only few degenerating nuclei (Romanowsky stain). B, A stain for acid-fast bacilli shows many extracellular bacilli and a macrophage filled with organisms (Ziehl-Neelsen stain).



Differential diagnosis of mycobacterial lymphadenitis

- sarcoidosis
- granulomatous lymphadenitis due to other organisms
- lymph node infarction

Cases of mycobacterial lymphadenitis with little or no necrosis resemble sarcoidosis. Special stains for bacteria, acid-fast bacilli, and fungi are important whenever granulomas, necrosis, and/or a neutrophilic infiltrate is present. Importantly, organisms are best seen with special stains in necrotic material ([Fig. 12.7B](#)), but the sensitivity of acid-fast bacilli stains is low, especially for *M. tuberculosis*. Molecular techniques, however, can detect and speciate mycobacteria. Alternatively, if necrosis or granulomas are seen at the time of the rapid evaluation of specimen adequacy, a portion of the needle rinse can be submitted for microbiologic cultures.

Complete or subtotal lymph node infarction is uncommon, but occurs in malignant lymphoma, systemic lupus erythematosus (SLE), vascular thrombosis, trauma, and infection. In such cases, smears may contain amorphous proteinaceous material with or without inflammatory cells, and the outlines of cell “ghosts” may be seen, but stains for organisms are negative.

Rosai-Dorfman disease (Sinus Histiocytosis with Massive Lymphadenopathy)

Rosai-Dorfman disease (RDD) is an uncommon nodal and extranodal disease primarily of young children and adolescents, although a wide age spectrum is affected. The classic clinical presentation is bilateral, painless cervical lymphadenopathy. Accompanying symptoms include fever, joint pain, night sweats, and even weight loss—all of which can mimic lymphoma clinically. Laboratory findings include polyclonal hypergammaglobulinemia and leukocytosis. Histologic sections show marked distension of sinuses by large histiocytes.



Cytomorphology of Rosai-Dorfman disease

- small lymphocytes
- histiocytes with emperipoleisis

Small lymphocytes vastly outnumber transformed lymphocytes, plasmacytoid lymphocytes, and immunoblasts. The histiocytes are large, with large pale nuclei, nucleoli, and abundant vacuolated cytoplasm; they can simulate the cells of an epithelial neoplasm. The key cytomorphologic feature is emperipoleisis, the engulfment of lymphocytes by histiocytes ([Fig. 12.8](#)), but it may be difficult to appreciate, because engulfed lymphocytes can be so numerous that they obscure the underlying histiocyte nucleus.^{[60,61](#)} The large histiocytes are immunoreactive for S-100 protein and CD68.

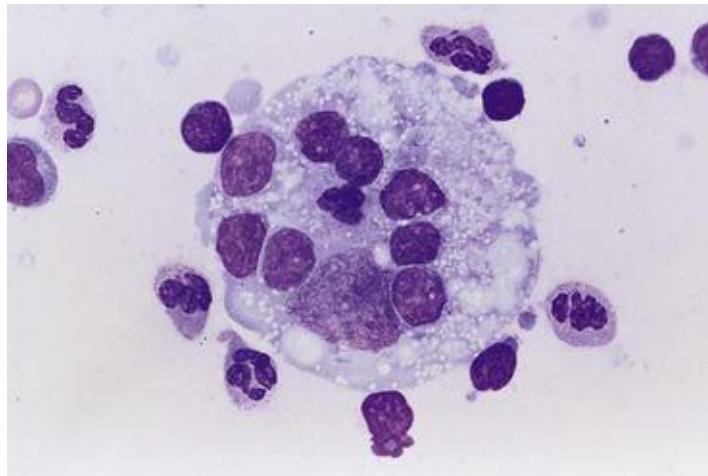


FIGURE 12.8 Rosai-Dorfman disease (RDD).

An enormous histiocyte has engulfed many small lymphocytes (Romanowsky stain).

(Courtesy of Dr. Harry Kozakewich, Children's Hospital Medical Center, Boston, MA, USA.)



Differential diagnosis of Rosai-Dorfman disease

- reactive lymphoid hyperplasia
- Langerhans cell histiocytosis

Dendritic-lymphocytic aggregates in reactive lymphoid hyperplasia mimic histiocytes with emperipoleisis. Instead of being engulfed by histiocytes, however, lymphocytes overlie and commingle with them in reactive hyperplasia. Since many of the lymphocytes “spill” from the cytoplasm as a consequence of smearing in RDD, they are often not strictly within the cytoplasm, but adjacent to it, thus adding to the confusion. An additional helpful feature is that histiocytes are much more numerous in RDD than in a reactive lymph node.

Also, the histiocytes in dendritic-lymphocytic aggregates are negative for S-100 protein.

Langerhans cell histiocytosis, primarily a disorder of children, uncommonly involves lymph nodes without simultaneously or previously also involving bone, lung, or another site. The neoplastic cells, like the cells of RDD, are positive for S-100 protein but, unlike RDD, are also positive for CD1a. Cytologic features are helpful in this distinction: Unlike the round nuclei of RDD histiocytes, those of Langerhans cell histiocytosis have reniform and contorted shapes, lack nucleoli, and display partial or complete linear grooves. There is no emperipoleisis. Smears of Langerhans cell histiocytosis may also contain increased numbers of eosinophils.

Kikuchi Lymphadenitis

Kikuchi lymphadenitis (KL), also known as *histiocytic necrotizing lymphadenitis*, is a self-limited adenopathy of unknown cause that occurs more often in Asian patients. Patients are usually young and often present with cervical adenopathy, fever, lymph node tenderness, and an atypical peripheral lymphocytosis. Laboratory test results are nonspecific. Clinically, the patient is often suspected of having an acute bacterial lymphadenitis, abscess, or even lymphoma. The cytologic diagnosis of KL can be very reassuring, because the disease is self-limited, and antibiotics are unnecessary.



Cytomorphology of Kikuchi lymphadenitis

- necrotic debris, karyorrhexis
- small phagocytic histiocytes with sharply angulated (crescent-shaped) nuclei
- cytoplasmic tingible bodies
- an increased number of immunoblasts and plasmacytoid monocytes
- absence of neutrophils

Smears from patients with KL have a characteristic appearance that permits diagnosis by FNA in the proper clinical context.⁶² Granular proteinaceous and nuclear debris is admixed with distinctive histiocytes with a contorted, sharply angulated, often crescent-shaped, peripherally placed nucleus^{62,63} ([Fig. 12.9](#)). These

histiocytes contain karyorrhectic debris but are readily distinguished from tingible-body macrophages, which are larger and have round nuclei. Plasmacytoid monocytes—medium-sized cells with an eccentrically placed round nucleus, condensed chromatin, and a moderate amount of cytoplasm—are also present, as are immunoblasts. Neutrophils are sparse or absent. No lymphocytic emperipoleisis is present.

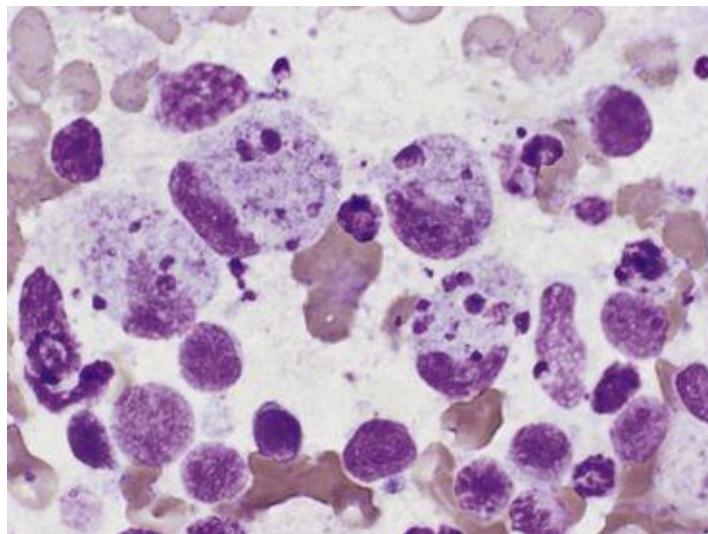


FIGURE 12.9 Kikuchi lymphadenitis (KL).

Histiocytes with contorted nuclei, including one with a C shape, contain ingested debris. These cells are significantly smaller than tingible-body macrophages (Romanowsky stain).



Differential diagnosis of Kikuchi lymphadenitis

- reactive follicular hyperplasia (with tingible body macrophages)
- tuberculous lymphadenitis
- necrotizing (suppurative) granulomatous lymphadenitis
- SLE

The combination of necrosis and contorted small phagocytic histiocytes, in the absence of neutrophils, is very striking. The characteristic small, contorted phagocytic histiocytes are easily overlooked on casual examination, but they are highly characteristic of KL and only rarely occur in other conditions.⁶² These cells are usually numerous and easily found if specifically sought, and, in the

proper clinical context, the FNA report can strongly suggest the diagnosis of KL because the small phagocytic histiocytes are morphologically distinguishable from the larger tingible-body macrophages, which typically have round, centrally placed nuclei. Small phagocytic histiocytes can be seen in tuberculous lymphadenitis,⁶² a diagnosis that should be excluded with the help of histochemical stains and microbiologic cultures. A variety of bacterial and fungal infections can result in a necrotizing, suppurative, and granulomatous lymphadenitis, but a suppurative lymphadenitis contains abundant neutrophils which are not seen in KL. The epithelioid histiocytes of a granulomatous lymphadenitis have elliptical, indented nuclei with smooth, rounded contours that are distinct from the angulated edges of the histiocytic nuclei of KL, and epithelioid histiocytes do not contain karyorrhectic debris. Lupus lymphadenitis is morphologically similar to KL,⁶⁴ but smears from lupus lymphadenitis may contain hematoxylin bodies—darkly stained clumps of nuclear debris measuring 1 to 100 µm in diameter that have not been described in patients with KL.⁶⁴ Even if hematoxylin bodies are absent from smears, it is prudent to exclude lupus with serologic testing whenever the FNA findings suggest KL.

Infectious Mononucleosis

Infectious mononucleosis is caused by the Epstein-Barr virus (EBV) and spread through person-to-person contact, most commonly in adolescents and young adults. The clinical presentation may include fever, malaise, pharyngitis, rash, and peripheral lymphadenopathy (typically cervical), but axillary and inguinal lymphadenopathy also occur. Lymph nodes are often tender and movable. Splenomegaly is common, and splenic rupture is a potential complication. Laboratory findings include peripheral blood atypical lymphocytosis, and a positive heterophile (Monospot) test.



Cytomorphology of infectious mononucleosis

- increased percentage of immunoblasts, plasmacytoid lymphocytes, and plasma cells
- few dendritic-lymphocytic aggregates and tingible-body macrophages

Aspirates contain predominantly small lymphocytes, accompanied by an increased number of immunoblasts, centroblasts, plasmacytoid lymphocytes, and occasionally plasma cells^{65,66} (Fig. 12.10). Binucleated immunoblasts simulating Reed-Sternberg cells may be found but are rare. Immunophenotyping confirms the polyclonal nature of the B-cells and often shows a reversed CD4/CD8 ratio.

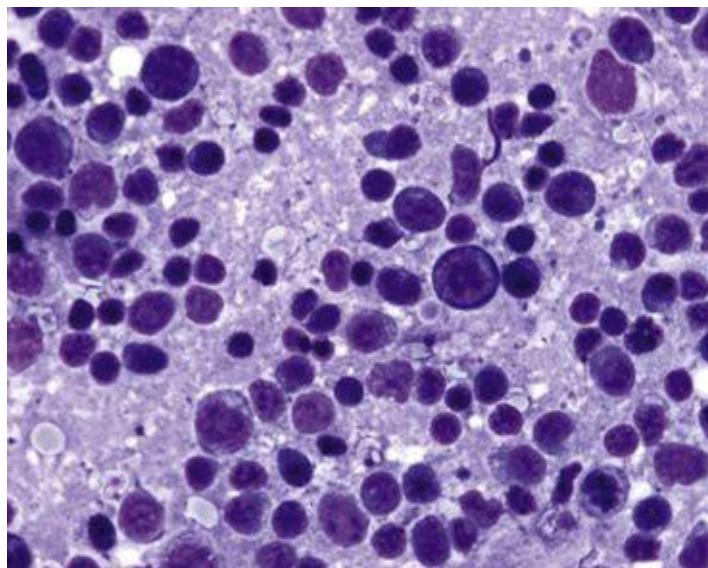


FIGURE 12.10 Infectious mononucleosis.

Many immunoblasts are mixed with small, round lymphocytes and plasmacytoid lymphocytes (Romanowsky stain).



Differential diagnosis of infectious mononucleosis

- reactive lymphoid hyperplasia
- other lymphadenopathy associated with increased immunoblasts
- large cell lymphoma
- Hodgkin lymphoma

A high percentage of immunoblasts, centroblasts, and plasmacytoid lymphocytes is typical of infectious mononucleosis, but the amount depends on the stage of the disease. In the early stages of infectious mononucleosis, immunoblast proliferation is barely perceptible, and FNA is indistinguishable from reactive lymphoid hyperplasia.⁶⁶ The immunoblast proliferation in other lymphadenopathies (e.g., anticonvulsant-associated lymphadenopathy, herpes

simplex, cytomegalovirus (CMV), and postvaccinal lymphadenitis) and drug hypersensitivity is indistinguishable from that of infectious mononucleosis. A detailed clinical history, serologic studies, or excisional biopsy may be necessary to exclude these other entities. Most patients who develop anticonvulsant-related lymphadenopathy (principally to phenytoin) usually do so within 6 months of the onset of therapy. Herpetic lymphadenitis usually occurs in the setting of disseminated infection in an immunosuppressed patient, and diagnosis is usually made by association with the cutaneous lesions. In addition to increased immunoblasts, herpes simplex and CMV lymphadenitis may contain cells with diagnostic viral inclusions.

Because infectious mononucleosis induces a discernible increase in large cells and there is a scarcity of tingible-body macrophages and dendritic-lymphocytic aggregates, one of the large cell non-Hodgkin lymphomas needs to be considered. Immunophenotypic studies are helpful in problematic cases to confirm the polyclonal nature of the immunoblasts in infectious mononucleosis. Immunoblasts are occasionally mistaken for mononuclear Reed-Sternberg cell variants. Unlike Reed-Sternberg cells, immunoblasts are smaller, have smaller nucleoli, greater cytoplasmic basophilia, and sometimes contain a perinuclear zone of clearing. Binucleated immunoblasts mimicking classic Reed-Sternberg cells are extremely rare. Immunohistochemistry on cell block sections can aid by showing that the Reed-Sternberg mimics in mononucleosis are negative for CD15.

Human Immunodeficiency Virus–Associated Lymphadenopathy

FNA is extremely helpful in the identification of infectious and neoplastic causes of lymphadenopathy in acquired immune deficiency syndrome (AIDS) patients.^{67,68} By contrast, an FNA diagnosis of HIV-associated lymphadenopathy (HIVAL) (defined as a primary, persistent nodal enlargement in an HIV-infected patient that is not due to an opportunistic infection or neoplasm) is one of exclusion. Nevertheless, in the early stages of HIV infection, a striking follicular hyperplasia pattern (including intact follicles, large cells, and plasma cells) is notable on FNA ([Fig. 12.11](#)) and may be suggestive in the appropriate clinical setting, even if a serologic diagnosis of HIV has not yet been made.⁶⁹ Cytologically, one is unable to document the follicular involution, partial lymphocyte depletion, and Castleman-like changes of the mid phase, or the small depleted follicles and paracortical vascular hyperplasia of late phase reactions.⁷⁰

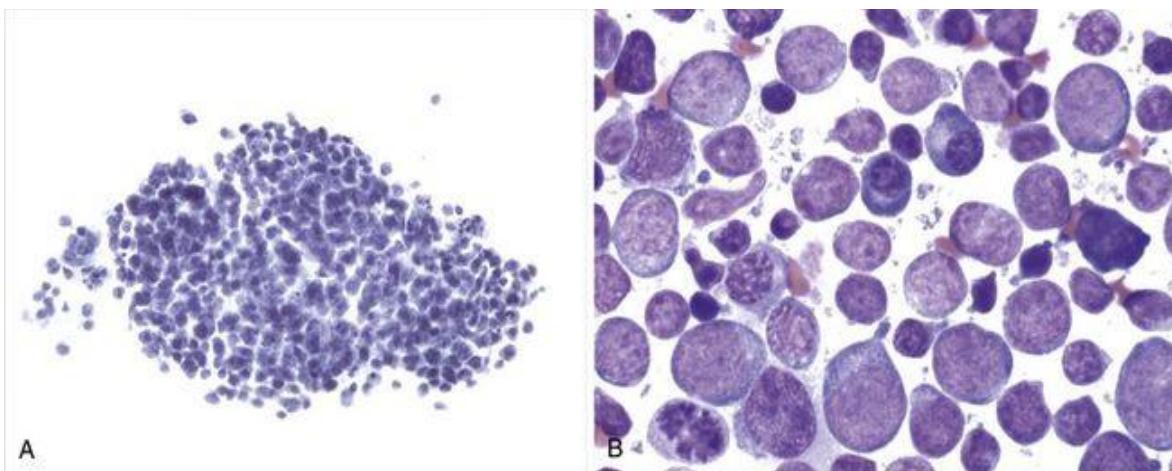


FIGURE 12.11 Human immunodeficiency virus (HIV) lymphadenitis. The fine-needle aspiration (FNA) features are not specific, but HIV lymphadenitis may be suspected when (A) “giant” intact lymphoid follicles are seen (liquid-based preparation, Papanicolaou stain), and (B) numerous plasma cells and large lymphocytes are present (Romanowsky stain), in the appropriate clinical setting.

Dermatopathic Lymphadenitis

Dermatopathic lymphadenitis is a nonspecific nodal enlargement associated with many chronic dermatoses (including psoriatic erythroderma, exfoliative dermatitides, and mycosis fungoides) and skin/tissue injury or foreign material (e.g., tattoos). The marked paracortical expansion by lymphocytes, dendritic cells, and histiocytes, seen in tissue sections as large pale nodules, is undetectable in smears, but if numerous dendritic cells and pigment-laden macrophages are readily apparent ([Fig. 12.12](#)), and few or no tingible-body macrophages are seen, the diagnosis can be strongly suggested.²¹ Otherwise, most examples mirror reactive lymphoid hyperplasia. FNA, combined with molecular testing for T-cell receptor gene rearrangement, can play a role in patients with mycosis fungoides in distinguishing dermatopathic lymphadenitis from lymph node involvement by lymphoma.²²

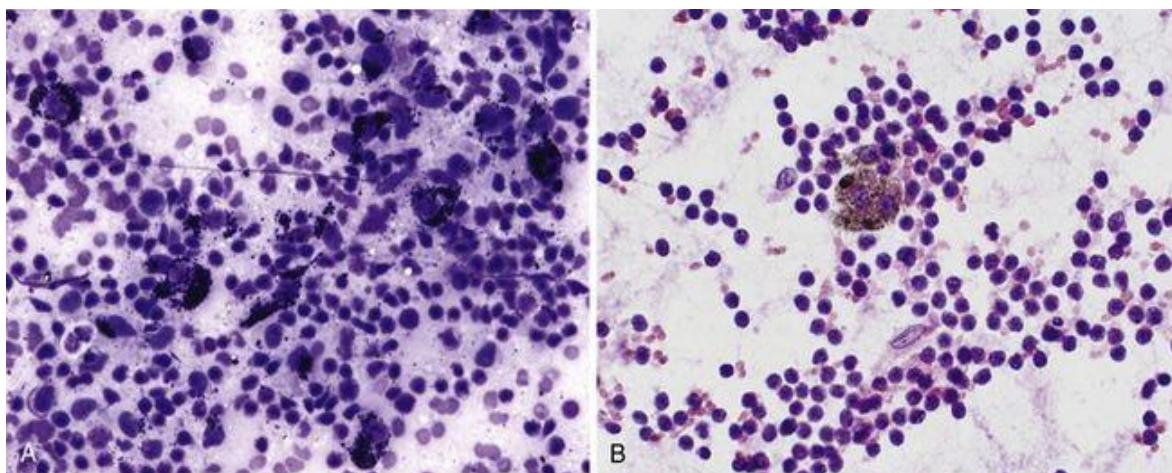


FIGURE 12.12 Dermatopathic lymphadenitis.

A, Several macrophages contain coarse granular pigment in this aspirate from an enlarged inguinal node adjacent to a large tattoo. A heterogeneous population of lymphocytes is also present (Romanowsky stain). B, Macrophages containing melanin pigment are characteristic of dermatopathic lymphadenitis associated with primary inflammatory dermatoses. Nonpigmented histiocytes and/or dendritic cells and small lymphocytes are also seen (hematoxylin and eosin [H & E] stain).

Silicone Lymphadenitis

All silicone breast implants eventually rupture, and, when they do, the silicone may travel to lymph nodes, especially in the axilla. FNA of such lymph nodes shows large multinucleate giant cells with vacuoles containing hyaline material (on direct smear) or empty-appearing vacuoles (after laboratory processing) ([Fig. 12.13](#)). Foreign-body giant cells containing implant shell fragments may also be seen. The cytologic diagnosis is immediate if the appropriate history is provided or sought (based on the cytologic findings).²³

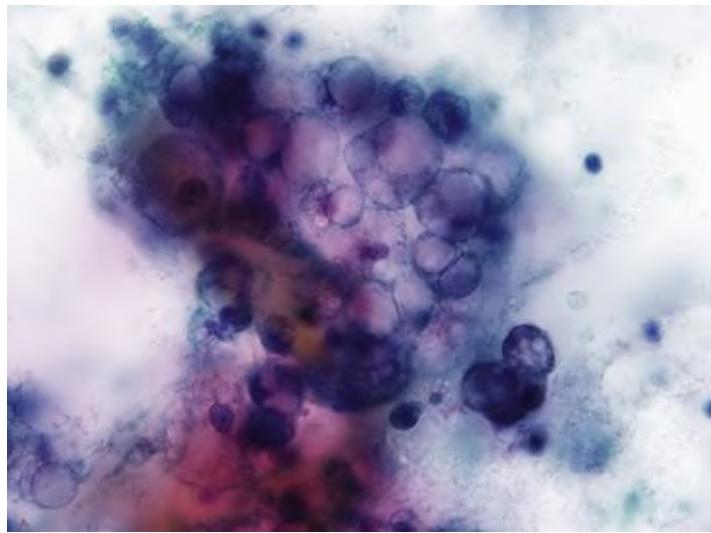


FIGURE 12.13 Silicone lymphadenitis.

This cluster of macrophages contains cells with multiple cytoplasmic vacuoles of varying size (liquid-based preparation, Papanicolaou stain).

Neoplasms

Lymph node FNA has always been well accepted for the diagnosis of metastatic tumors and recurrent lymphoma. In recent years, there is a broad consensus among cytopathologists that FNA is also a valuable tool in the primary diagnosis and classification of lymphoma,^{4,21,24,25,74} but only if used by individuals who apply immunophenotyping, develop a thorough understanding of the 2008 (or any subsequent) WHO classification, and understand the limitations of the technique.¹⁶



Fine-needle aspiration is a valuable tool in the primary diagnosis and classification of lymphoma if the cytopathologist

- understands modern lymphoma classification
- uses special studies (immunophenotyping, molecular genetics)
- works closely with a hematopathologist

According to the 2008 WHO classification of hematologic malignancies, lymphoid neoplasms are defined by a combination of morphology, immunophenotype, genetic features, and clinical findings.¹⁹ There is no single gold standard for all lymphomas; instead, for each disease, one or more of these may be more important for diagnosis than the others. Architectural pattern (e.g., follicular versus diffuse), important in previous classification systems, is no longer of paramount importance. This plays into the strengths of FNA, and the new classification has been embraced by cytopathologists.²⁵

A primary diagnosis of lymphoma is not clinically useful for treatment without precise classification. In particular, unqualified diagnoses such as malignant lymphoma, non-Hodgkin lymphoma, or even small cell lymphoma are usually not refined enough for patient management, and, given such nonspecific diagnoses, an oncologist will correctly demand an open biopsy.¹⁶ Thus, in contrast to a diagnosis like metastatic squamous cell carcinoma, typically accomplished by light microscopic examination of routinely stained cytologic preparations only, lymphoma diagnosis by FNA requires more time and effort, special handling, and the use of ancillary markers applied in a systematic

fashion.²⁶

Hodgkin Lymphoma

Hodgkin lymphoma is a common cause of malignant lymphadenopathy, representing approximately 30% of all lymphomas. [Table 12.3](#) shows the current 2008 WHO classification of Hodgkin lymphoma. It comprises two distinct diseases: classical Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). They are bound by a shared characteristic: Within a lesion, the neoplastic cells are outnumbered by nonneoplastic inflammatory cells. They differ, however, in their clinical features, immunophenotype, genetics, and natural history.

TABLE 12.3
2008 WORLD HEALTH ORGANIZATION CLASSIFICATION OF HODGKIN LYMPHOMA

CLASSICAL HODGKIN LYMPHOMA (NOT FURTHER SUBDIVIDED FOR TREATMENT/PROGNOSIS)
<ul style="list-style-type: none">• Nodular sclerosis classical Hodgkin lymphoma• Lymphocyte-rich classical Hodgkin lymphoma• Mixed cellularity classical Hodgkin lymphoma• Lymphocyte-depleted classical Hodgkin lymphoma
NODULAR LYMPHOCYTE PREDOMINANT HODGKIN LYMPHOMA



A comprehensive lymph node fine-needle aspiration workup includes

- clinical history
- rapid evaluation
- good smears
- needle rinse for
 - flow cytometry or immunocytochemistry
 - molecular genetic studies (selected cases)
 - tissue fragments for architecture, immunohistochemistry (selected cases)

Classical Hodgkin lymphoma accounts for 95% of Hodgkin lymphoma. Epidemiologic studies show a bimodal age curve with a peak at 15 to 35 years of age and a smaller peak later in life. The malignant cells—large multinucleated Reed-Sternberg (RS) cells and their mononuclear variants—reside in an infiltrate of nonneoplastic small lymphocytes, eosinophils, neutrophils, histiocytes, plasma cells, and fibroblasts. The proportions of the various nonneoplastic elements vary. In virtually all cases, the RS cells are monoclonal B cells as demonstrated by immunophenotyping and molecular genetics. RS cells are positive for CD30 in almost all cases, and for CD15 in 75% to 85%. CD20 is detected in up to 40% of cases but often expressed weakly in only a minority of the RS cells. In practice, because CD15 reactivity can be hard to interpret (due to focal staining of the RS cells and numerous admixed positive granulocytes) and because CD30 is nonspecific, nuclear positivity of the Reed-Sternberg cells for the broad spectrum B cell marker PAX5/BSAP (which marks Reed-Sternberg cells in approximately 95% of Hodgkin lymphoma cases⁷⁷) is a key finding. Epithelial membrane antigen (EMA) positivity is seen in less than 5% of cases, and CD45 is usually negative in the Reed-Sternberg cells (although difficult to interpret due to abundant positive background lymphocytes).

Four histologic variants of classical Hodgkin lymphoma have been recognized ([Table 12.3](#)), but distinction among these variants has no current clinical relevance, and thus it is no practical loss that they cannot be separated by FNA. In the past, most oncologists required that a newly diagnosed patient have an excisional biopsy for confirmation of the FNA diagnosis. Today an FNA diagnosis of classical Hodgkin lymphoma that is confirmed with immunostains requires no further tissue confirmation or subclassification for patient management.^{78,79}

NLPHL is much less common than classical Hodgkin lymphoma, with different clinical features and natural history. Most patients are male, presenting between 30 and 50 years of age with cervical, axillary, or inguinal (not mediastinal) lymphadenopathy. The malignant cells are monoclonal B cells called L & H cells: large cells with one large, often folded or multilobate nucleus. The nuclei of these cells are so contorted they are commonly called popcorn cells. The nucleoli are usually smaller than those in classical Reed-Sternberg cells. Like mature B cells and unlike Reed-Sternberg cells, L & H cells are uniformly positive for CD20 and CD45 in almost all cases and for EMA in up to 50% of cases (though the latter stain may be of lower practical utility).⁸⁰ Moreover, the L & H cells are almost always negative for CD15 and CD30, a further point of distinction from classical Hodgkin lymphoma. The architecture is partially or totally nodular. This architectural feature is important in the

distinction from a T-cell-rich large B-cell lymphoma and thus limits the ability of FNA to make this distinction. The FNA features of NLPHL are not well documented because the disease is so uncommon.



Cytomorphology of Hodgkin lymphoma

- small lymphocytes
- eosinophils (especially in the mixed cellularity subtype)
- Reed-Sternberg cells, classic and mononuclear variants (classical Hodgkin lymphoma)
- L & H cells (NLPHL)
- no follicular aggregates or tingible-body macrophages (exceptions: partial node involvement and lymphocyte-predominant Hodgkin lymphoma)

The diagnosis of classical Hodgkin lymphoma is established by finding the rare Reed-Sternberg cells with the appropriate immunophenotype in a mixed background of small lymphocytes and eosinophils ([Fig. 12.14A](#)). Thus, FNA samples with conspicuous eosinophils should be carefully screened for Reed-Sternberg cells, particularly in a young patient with marked lymph node enlargement. In fact, concern for Hodgkin lymphoma is the main practical reason for screening lymph node FNA slides, because most other diagnoses are evident upon low-power examination of the cytologic pattern, rather than high-power examination of individual cells. Diagnostic binucleated and multinucleated Reed-Sternberg cells have at least two nuclei or nuclear lobes, each with an enormous nucleolus, but binucleated and multinucleated Reed-Sternberg cells are infrequent in many cases. A clue to the diagnosis of classical Hodgkin lymphoma in such cases is the more frequently encountered mononuclear Reed-Sternberg cell variant ([Fig. 12.14B](#)). The mononuclear variant is three to four times the size of a small lymphocyte and has a round, irregular, or polylobated nucleus.⁸¹ Large, bare nuclei of the mononuclear Reed-Sternberg cell variants are sometimes prominent at medium power; their presence is helpful in raising the possibility of classical Hodgkin lymphoma. Sometimes, however, they are masked in a densely lymphocytic smear. Granulomas are sometimes present⁸² but are typically small, inconspicuous, and poorly formed.

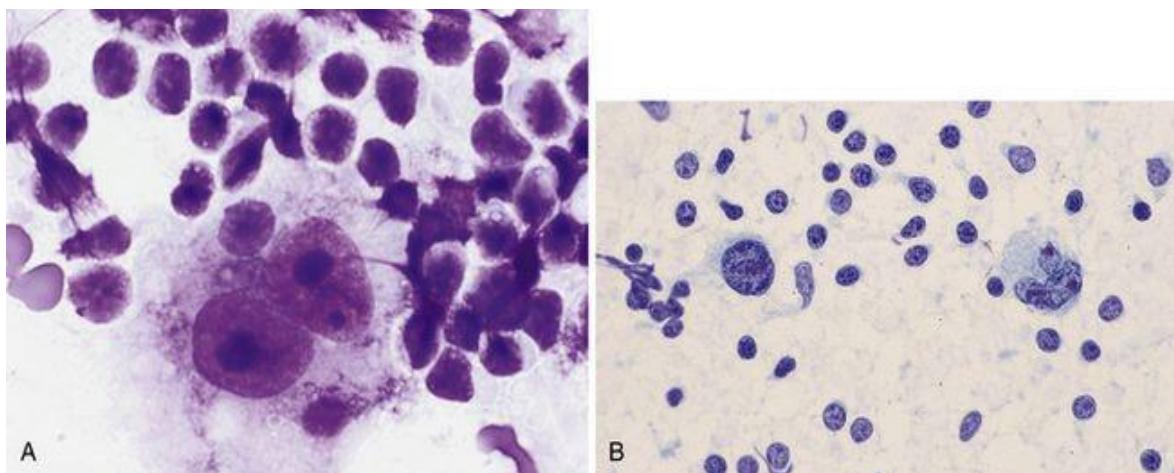


FIGURE 12.14 Classical Hodgkin lymphoma.

A, A classic binucleated Reed-Sternberg cell (Romanowsky stain). B, Two conspicuous mononuclear Reed-Sternberg cells are surrounded by lymphocytes. The Reed-Sternberg nucleus at the right is lobulated, whereas the one to the left has only slight bosselation. Both have macronucleoli (Papanicolaou stain).

The cytologic findings in NLPHL are not as well documented. In many ways, NLPHL resembles classical Hodgkin lymphoma in that the neoplastic L & H (popcorn) cells are outnumbered by a nonneoplastic, reactive population of small B and T lymphocytes and miscellaneous cell types. L & H cells are large cells with a single, large, highly folded or multilobate nucleus that often look like popcorn ([Fig. 12.15](#)). Nucleoli are not as prominent as in Reed-Sternberg cells. In practice, the diagnosis of NLPHL is extremely challenging to make by FNA; the most important point is to at least consider the diagnosis, because false-negative interpretations can be a pitfall.⁵² Therefore, if L & H-like cells are seen on smear in a patient with clinically significant lymph node enlargement, it is prudent to describe the findings as atypical or suspicious and suggest excision. By flow cytometry, NLPHL may have a significant proportion of CD4+CD8+ (double positive) T cells, which is rare in most reactive lymphadenopathies and can be a clue to the diagnosis,⁵² but a double-positive T-cell population is also sometimes seen in progressive transformation of germinal centers and T-cell lymphomas. In any event, the finding of such a double-positive population by flow cytometry should prompt consideration of excisional biopsy.

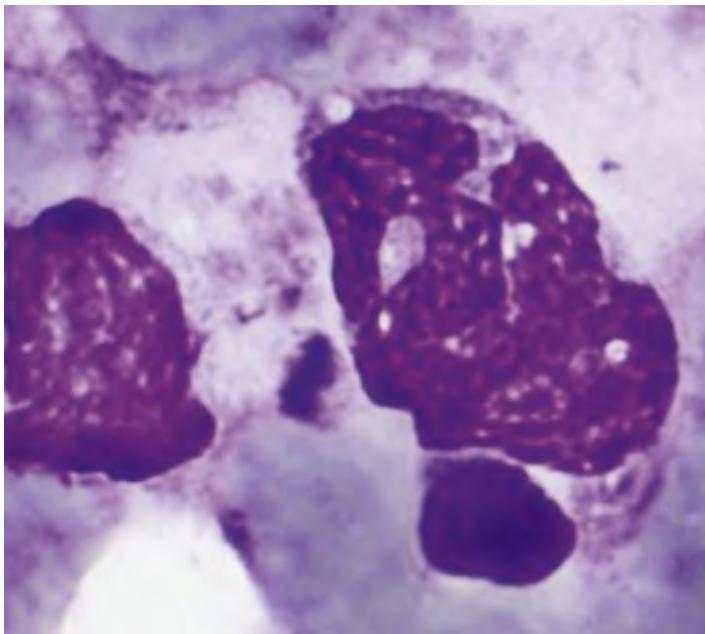


FIGURE 12.15 Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). The characteristic cell is the lymphocytic and histiocytic (L & H, or “popcorn”) cell, which has a large, folded nucleus. One must hunt for these cells, because they are vastly outnumbered by small lymphocytes and other nonneoplastic lymphoid cells (Romanowsky stain).



Differential diagnosis of Hodgkin lymphoma

- reactive lymphoid hyperplasia
- infectious mononucleosis
- T-cell-rich large B-cell lymphoma (TCRLBL)
- anaplastic large cell lymphoma (ALCL)
- acute lymphadenitis (rare)
- nasopharyngeal carcinoma (NPC)

A major pitfall is a hypocellular smear. Because nodular sclerosis is the most common classical Hodgkin lymphoma subtype, an inability to aspirate sufficient numbers of diagnostic Reed-Sternberg cells is a source of false-negative interpretations.⁸³ There may be very few RS (or L & H) cells in smears that otherwise resemble *reactive lymphoid hyperplasia* (e.g., lymphocyte-rich Hodgkin lymphoma, NLPHL). A search for Reed-Sternberg cells and L & H cells is warranted whenever a lymph node has the features of reactive lymphoid hyperplasia or conspicuous eosinophils, particularly if the node is clinically

suspicious.

Mononuclear Reed-Sternberg cells resemble immunoblasts, and therefore benign lesions with conspicuous immunoblasts, like *infectious mononucleosis*, must be considered in the differential diagnosis. Immunoblasts are smaller than Reed-Sternberg cells and often can be distinguished simply on the basis of their size, but clinical correlation, serologic testing, and special stains (such as CD15) can be helpful in difficult cases. EBER in situ hybridization testing will not distinguish classical Hodgkin lymphoma from infectious mononucleosis, as both conditions may show positivity in this assay.

TCRLBL mimics Hodgkin lymphoma in that the neoplastic cells are outnumbered by abundant nonneoplastic small lymphocytes. Immunostains are helpful, because the malignant cells of TCRLBL are usually uniformly positive for CD20 and negative for CD30 and CD15, making classical Hodgkin lymphoma highly unlikely. The distinction between TCRLBL and NLPHL is difficult if not impossible by FNA and usually requires nodal excision with assessment of nodular versus diffuse architecture.

The neoplastic cells in *anaplastic large cell lymphoma* (ALCL) usually lack the inclusion-like macronucleoli of Reed-Sternberg cells but are sometimes indistinguishable from Reed-Sternberg and L & H cells. In ALCL, the neoplastic cells may be much more numerous, whereas in Hodgkin lymphoma, Reed-Sternberg and L & H cells represent only 0.1% to 10% of the total cell population.⁸⁴ In borderline cases, immunostains for EMA (positive in ALCL, negative in classical Hodgkin lymphoma) and CD15 and B-cell-specific activator protein (BSAP) (positive in classical Hodgkin lymphoma⁷⁷ and negative in ALCL). If necessary, gene rearrangement studies for T-cell receptors are helpful.

Classical Hodgkin lymphoma associated with suppuration can mimic *acute lymphadenitis* due to the abundance of neutrophils and cell debris.⁸⁵ A painstaking search reveals large Reed-Sternberg cell variants scattered among the acute inflammatory cells.

Metastatic nasopharyngeal carcinoma (NPC) has an abundant lymphoid background and shows a predilection for cervical lymph nodes, with neoplastic cells that resemble Reed-Sternberg cells. Immunostaining for keratins is helpful in this distinction.

Non-Hodgkin Lymphoma

The 2008 WHO classification divides non-Hodgkin lymphomas into B-and T-

cell types. In the United States, the great majority (90%) are B-cell neoplasms ([Table 12.4](#)). Two B-cell neoplasms—diffuse large B-cell lymphoma and follicular lymphoma—account for up to two thirds of all B-cell neoplasms. About 10% of non-Hodgkin lymphomas are T-cell neoplasms, and a small minority are of null cell type. Non-Hodgkin lymphomas are also segregated by morphology and can be loosely subdivided into those composed of small cells versus large cells (or a mixture of cell sizes). Morphology is integrated with immunophenotypic and genetic results and clinical findings for a final subclassification.

TABLE 12.4
2008 WORLD HEALTH ORGANIZATION CLASSIFICATION OF B-CELL NEOPLASMS RELEVANT TO FINE-NEEDLE ASPIRATION*

Precursor B lymphoblastic leukemia/ lymphoma
Mature B-cell neoplasms
B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma
Mantle cell lymphoma
Follicular lymphoma
Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue type
Nodal marginal zone B-cell lymphoma with or without monocytoid B-cells
Splenic marginal zone B-cell lymphoma
Diffuse large B-cell lymphoma
Subtypes: mediastinal (thymic); T cell/histiocyte-rich; intravascular; primary effusion; B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma; double hit lymphoma
Burkitt lymphoma
Plasmacytoma
Plasma cell myeloma

*More common entities are in bold.

In discussing FNA diagnosis of non-Hodgkin lymphoma, it is helpful to review the small cell lymphomas separately from the large cell lymphomas. The differential diagnosis for each will be discussed at the end of each section.

Lymphomas of Small Cells

The clinical features of the four major non-Hodgkin lymphomas comprised of small lymphoid cells are summarized in [Table 12.5](#).

TABLE 12.5

CLINICAL FEATURES OF SMALL B-CELL LYMPHOMAS

Type	Median age/gender	Stage I	Extranodal*	Clinical Course
Small lymphocytic	65/ m>f	None	Incidental	Indolent, incurable
Mantle cell	60/ m>f	Rare	Many	Aggressive, incurable
Follicular	59/ m=f	Rare	Variable	Indolent, rarely curable
Marginal zone/ mucosa-associated lymphoid tissue	61/ m=f	Often	Usually	Indolent, curable

*excluding bone marrow

Follicular Lymphoma

FL accounts for about 35% of adult non-Hodgkin lymphoma in the United States. Patients are generally older than 50 years, and the vast majority (more than 80%) have disseminated disease (spleen, multiple lymph node sites, and/or bone marrow involvement) at the time of diagnosis. In 25% to 35% of patients with FL, transformation to a large B-cell lymphoma occurs. This usually portends rapid disease progression that is refractory to treatment.¹⁹

The CD5–, CD10+ immunophenotype is characteristic of FL (CD23 can be + or –), but as many as 40% of FLs are negative for CD10²⁰ ([Table 12.6](#)), especially when testing is performed solely by flow cytometry. The tumor cells more reliably show nuclear immunoreactivity for bcl-6.

TABLE 12.6

DIFFERENTIAL IMMUNOPHENOTYPE AND GENETICS OF SMALL B-CELL LYMPHOMAS

	Small Lymphocytic	Mantle Cell	Follicular	Marginal Zone
CD5	+	+/-	–	–
CD10	–	-/+	+/-	–
CD20	+ (dim)	+	+	+
CD23	+	-/+	-/+	–
bcl-6	–	–	+	–
cyclin D1	–	+	–	–
CD43	+	+	–	-/+
terminal deoxynucleotidyl transferase genetics	–	–	–	–
	trisomy 12 (30%), others	t(11;14)	t(14;18)	trisomy 3 or 18, t(11;18), others

+/-, Majority of cases are positive, small percentage negative.

-/+, Majority of cases are negative, small percentage positive.

FL is characterized genetically by a t(14:18)(q32;q21) translocation, with rearrangement of the bcl-2 gene, in up to 95% of cases. This translocation can be detected by FISH on cytologic material in over 80% of cases,⁸⁶ and FISH appears to be more sensitive than flow cytometry in diagnosing FL.⁸⁷ Antibody staining with bcl-2, which is extremely helpful in distinguishing FL from follicular hyperplasia in tissue sections, is ineffective in smears because the spatial relationship of follicle center versus paracortical staining cannot be evaluated, unless a two-color bcl-6/bcl-2 double stain is used.



Cytomorphology of follicular lymphoma

- predominantly small irregular/cleaved lymphocytes
- large cleaved/noncleaved lymphocytes (more in high-grade FL)
- few tingible-body macrophages
- lymphoid cell aggregates (one third or more of cases)

Although some have claimed that a follicular pattern can be appreciated in well-made smears, our experience leads us to conclude that this is uncommon. Aspirates may be composed almost exclusively of small centrocytes but are more apt to show a mixture of centrocytes and some centroblasts (large noncleaved lymphocytes).⁷⁴ Cell nuclei are commonly contorted into notches and grooves. In some aspirates the clefts are so exaggerated as to appear to bisect or trisect the nucleus, while in others the nuclear folds are more subtle ([Fig. 12.16](#)). Follicular aggregates occur in smears of FL in about one third of cases,⁷⁴ but the dendritic cells are sometimes difficult to discern.^{42,74} Several variants of FL have been described: FL with monocyteid B cells, FL with rosettes, floral pattern of FL, and FL with cerebriform nuclei. These variants have no clinical significance and generally are not recognizable by FNA. A signet ring cell variant of FL, however, is cytologically distinct because the cells have a single, large, optically clear cytoplasmic vacuole filled with immunoglobulin that can create confusion with a signet ring cell carcinoma.

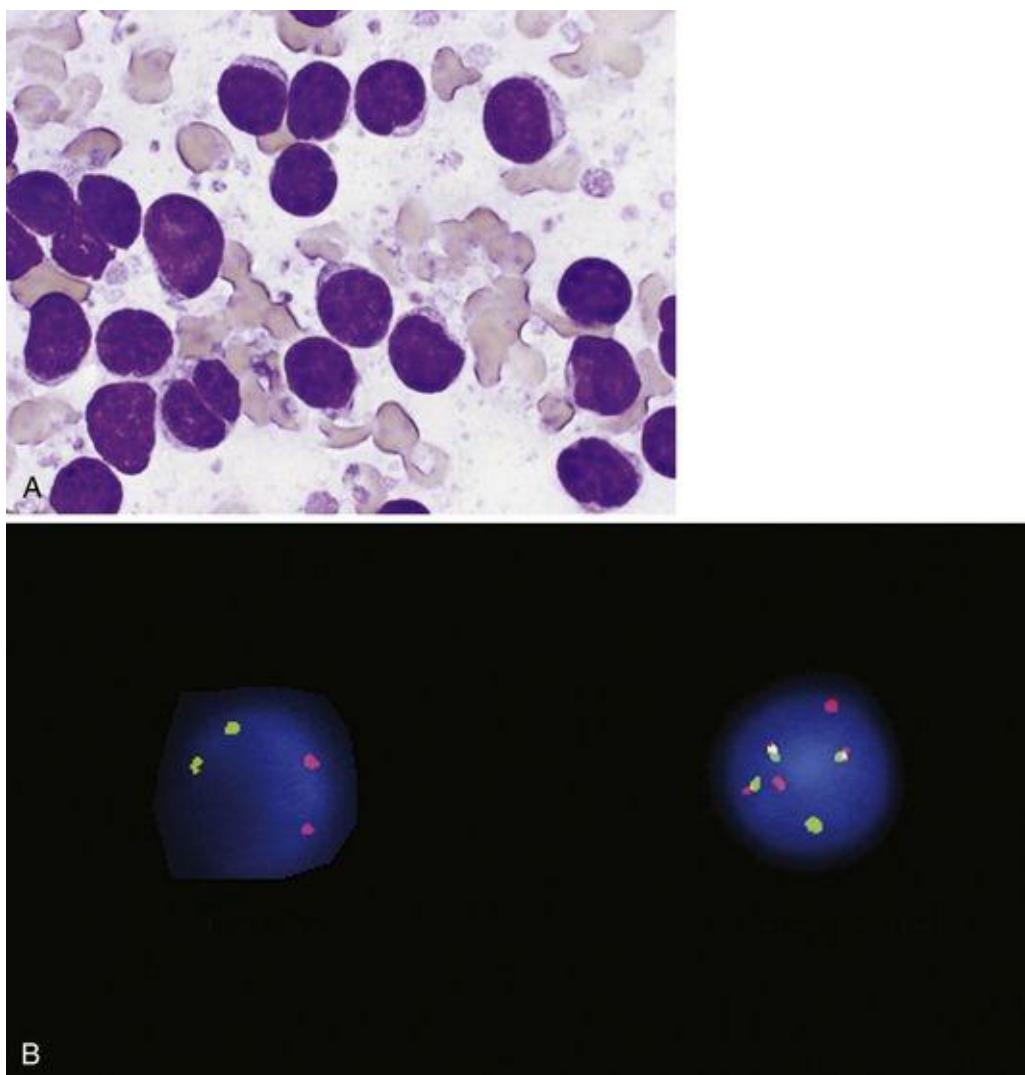


FIGURE 12.16 Follicular lymphoma.

A, Note the nuclear irregularities and occasional clefted nucleus. Lymphoglandular bodies are numerous (Romanowsky stain). B, Fluorescence in situ hybridization (FISH) reveals a (14;18) translocation in the abnormal cell on the right. A green probe for immunoglobulin heavy chain (IgH) at 14q32 and a red probe for bcl-2 at 18q21 come together to give a yellow signal when there is a translocation. Note that the green and red signals are far apart in the normal cell. (Dual color interphase FISH. Courtesy of Paola dal Cin, PhD, Brigham and Women's Hospital, Boston, MA, USA.)

For practical therapeutic purposes, FLs are divided into low-grade (a combination of WHO grades 1 and 2) and high-grade (WHO grade 3) tumors based on the quantity of large nucleolated cells (centroblasts) in tissue sections. Unfortunately, there is no standardized method for grading an FL obtained by FNA.^{88,89} As a practical matter, grading is typically attempted by visual estimation of the percentage of small and large lymphocytes,⁹⁰ with low-grade FL favored if the percentage of large cells is less than 40%.⁹¹ The distinction between grade 3 FL and DLBCL is usually not possible by FNA but fortunately may not be

clinically relevant for choice of therapy.

Marginal Zone Lymphoma

MZL is an indolent, low-grade B-cell lymphoma that is divided into nodal and extranodal types. The extranodal type is synonymous with mucosa-associated lymphoid tissue (MALT) lymphoma and is more common than the nodal type, accounting for 7% to 8% of all B-cell lymphomas. Although MALT lymphomas occur in a variety of sites, FNA cytologists typically only encounter them in specimens from the salivary gland, lung, lacrimal gland, thyroid, breast, and skin. There is a strong association with autoimmune disorders such as Hashimoto thyroiditis and Sjögren syndrome. A variety of genetic alterations are seen, but the incidence of these varies by site, and none are sufficiently common in FNA sites to be useful. Patients with MALT lymphoma often have stage I or II disease, and thus are potentially curable by surgery or regional radiotherapy.

Histologically, a mixed background including reactive follicular hyperplasia is the rule. The neoplastic cells are small lymphocytes, some of which have more abundant cytoplasm, leading to a monocyteoid appearance. Neoplastic cells with the appearance of plasma cells are present in many cases. In glandular organs, nests of neoplastic cells invade the epithelium, resulting in characteristic lymphoepithelial lesions.



Cytomorphology of marginal zone lymphoma

- polymorphous population
- small lymphocytes
- round or slightly irregular nuclei
- monocyteoid cells (moderate amount of pale cytoplasm)
- plasma cells
- follicular dendritic cells, tingible-body macrophages, follicular aggregates, immunoblasts (reactive elements)

The heterogeneous cell population makes it difficult to recognize the lesion as a lymphoma.⁹² Unfortunately, the diagnostically useful lymphoepithelial lesions are difficult (or impossible) to appreciate in smears. A mixed population of small lymphocytes with pale staining cytoplasm (monocyteoid cells) and plasma cells,

together with reactive elements in a site where lymphoid tissue is normally sparse, should raise the suspicion of MALT lymphoma⁹³ ([Fig.12.17](#)). Immunophenotyping (for clonality) is critical to establish the diagnosis in most cases. The neoplastic cells are usually negative for CD5 and CD10.

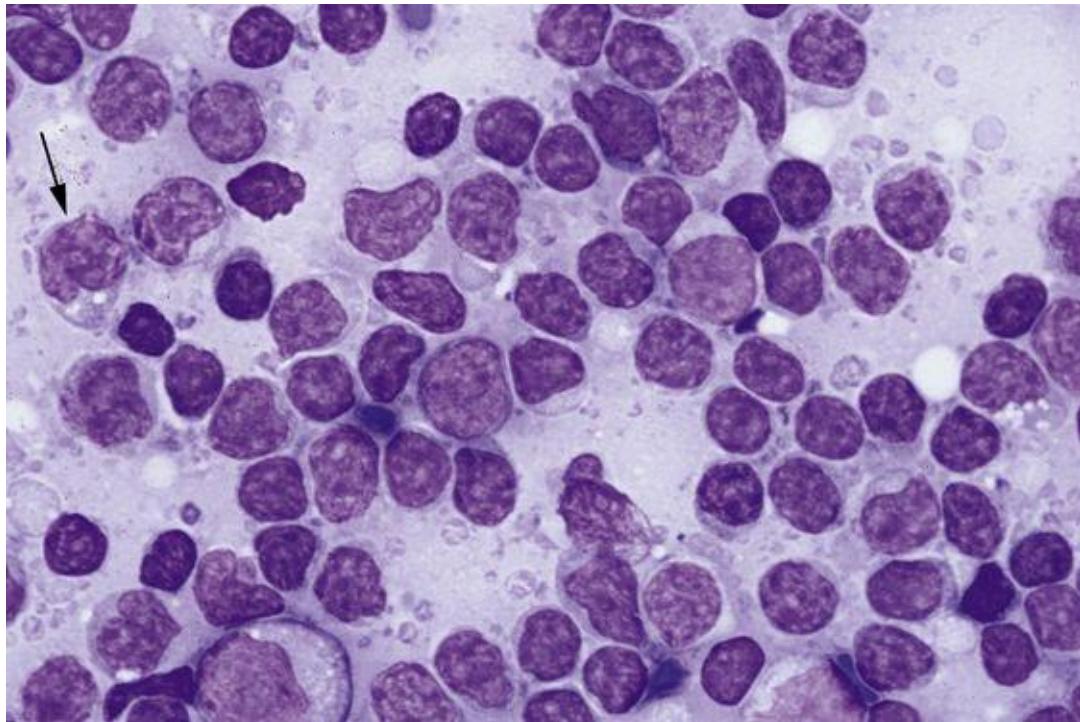


FIGURE 12.17 Marginal zone lymphoma (MZL).

A mixture of small-and intermediate-size lymphocytes have irregular nuclear contours, and occasional monocyteid cells are present (arrow). Lymphoglandular bodies are numerous (Romanowsky stain).

Small Lymphocytic Lymphoma

SLL comprises about 6% of non-Hodgkin lymphoma. It is an indolent and incurable neoplasm of older adults. Disease is widespread (nodally and extranodally) at the time of diagnosis, usually including peripheral blood and bone marrow involvement (the latter perversely referred to by a different name: chronic lymphocytic leukemia [CLL]). Tissue sections show effacement of nodal architecture by the neoplastic cells, with pseudofollicular proliferation centers that contain prolymphocytes and paraimmunoblasts. The CD5+, CD23+, CD10– immunophenotype is highly characteristic of SLL/CLL, but it is important to know that CD5 expression (and CD20 and light chain expression) is usually dim

or weak.



Cytomorphology of small lymphocytic lymphoma

- monomorphic small lymphocytes
- clumped (soccer-ball-like) chromatin
- smooth or minimally irregular nuclear contour
- inconspicuous or absent nucleoli
- scant cytoplasm
- prolymphocytes and paraimmunoblasts
- rare or absent tingible-body macrophages and follicular aggregates

Nuclei are round and have coarse, clumped chromatin—so-called clotted chromatin. Nuclear borders are smooth and usually round. So-called smudge cells (crushed cells) may be common on smears. Prolymphocytes are larger, have a distinct nucleolus and a modest amount of pale cytoplasm, while paraimmunoblasts are larger still, with a more prominent nucleolus, and more basophilic cytoplasm ([Fig.12.18](#)). These two types of transformed lymphocytes are not easily distinguished from each other in smears, and need not be.

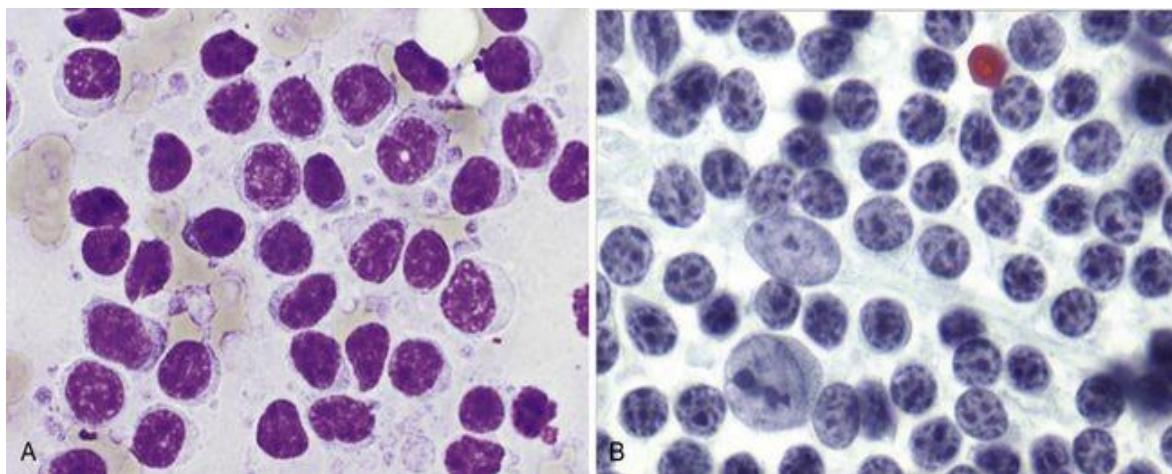


FIGURE 12.18 Small lymphocytic lymphoma.

A, The monomorphic neoplastic small lymphocytes have scant cytoplasm; some bare nuclei are present. The mostly round nuclei have very dense (“clotted”) chromatin. Lymphoglandular bodies are present in the background (Romanowsky stain). B, “Soccer-ball-like” chromatin is more evident with alcohol-fixed smears. A few (large) paraimmunoblasts are also present (Papanicolaou stain).

Morphologic transformation to DLBCL occurs in up to 20% of SLL/CLL patients and is an important indicator of prognosis. In fact, clinical concern for transformation is the most common indication for lymph node FNA in SLL patients. Transformation is assessed by determining the proportion of large cells and identifying necrosis. Immunocytochemical staining with the Ki-67 proliferation marker can be a valuable adjunct to morphologic assessment of transformation. A Ki-67 index of greater than 30% strongly suggests transformation.⁹⁴ Transformation to Hodgkin lymphoma is also observed in heavily treated patients and may be diagnosable by FNA,⁹⁵ provided one takes care to identify immunophenotypically and morphologically typical Reed-Sternberg cells in the appropriate background. Reed-Sternberg-like cells can occur in untransformed SLL, but the appropriate background is lacking.

Mantle Cell Lymphoma

MCL is a biologically aggressive B-cell lymphoma comprising 3% to 10% of non-Hodgkin lymphoma. It occurs in adults over 50 years of age with a male predominance. MCL is commonly extranodal, and most patients have disseminated disease (including variably subtle peripheral blood involvement) at initial diagnosis. MCL is resistant to standard lymphoma therapeutic regimens and has a poor prognosis, with a median survival of 3 to 5 years. A t(11;14) (q13;q32) translocation is present in the vast majority of patients, resulting in overexpression of the protein cyclin D1, which acts at the G1 to S phase checkpoint of the cell cycle to drive cell proliferation. This translocation is readily detectable by FISH on cytologic material.^{96,97} The CD5+, CD23–, CD10– immunophenotype is characteristic of MCL.



Cytomorphology of mantle cell lymphoma

- monomorphic small-to intermediate-sized cells
- fine nuclear chromatin
- irregular nuclear contours
- inconspicuous/absent nucleoli
- scant cytoplasm
- no centroblasts or immunoblasts
- “pink” histiocytes
- lymphoid cell aggregates (one third of cases)
- blastoid variant

Aspirates of MCL are comprised of dispersed, uniform cells that have a very high nuclear to cytoplasmic ratio and somewhat resemble centrocytes,^{98,99} although the nuclei are more often subtly notched than deeply clefted ([Fig. 12.19A](#)). Lymphoid cell aggregates occur in about one third of cases.⁷⁴ They are similar to dendritic-lymphocytic aggregates, except that dendritic cells are inconspicuous.^{42,74} Unlike other small cell lymphomas, there are virtually no transformed lymphocytes, and monotony is the rule. Occasional histiocytes with moderately abundant eosinophilic cytoplasm (“pink histiocytes”) are noted. In typical cases, the cytologic interpretation can be confirmed by demonstrating the characteristic CD5+, CD23–, CD10– immunoprofile by flow cytometry, but approximately 10% to 20% of cases deviate from this immunophenotype,¹⁰⁰ therefore, demonstration of immunoreactivity for cyclin D1 on cytocentrifuge or cell block preparations, and/or the t(11;14) translocation by FISH ([Fig. 12.19B](#)) may be necessary for primary diagnosis/subclassification.¹⁰¹ Rarely, the blastoid variant of MCL is observed; this variant has a more aggressive course,¹⁹ and the cells either resemble lymphoblasts (with immature-appearing chromatin),¹⁰² or are pleomorphic (with nuclear clefts and nucleoli).

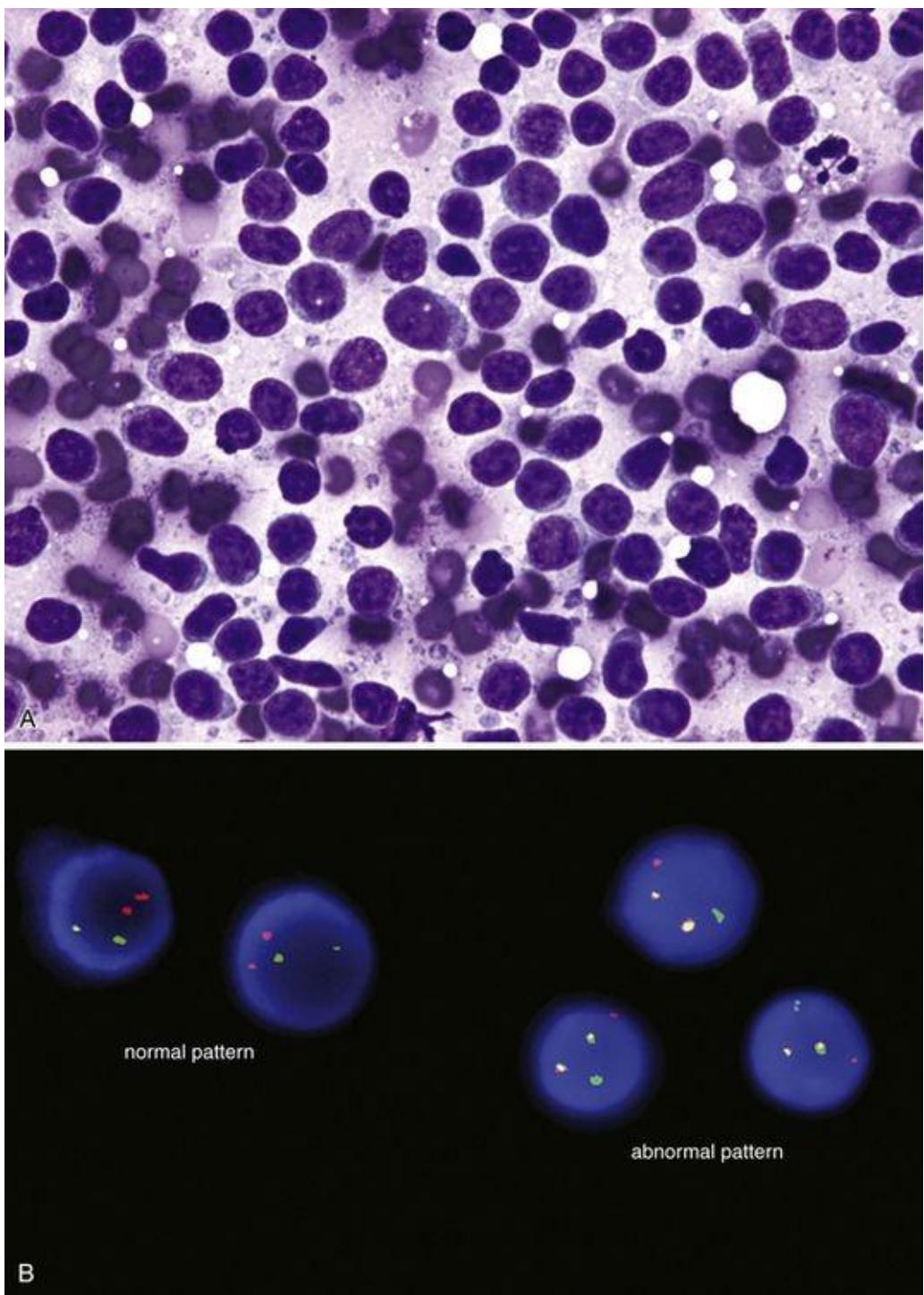


FIGURE 12.19 Mantle cell lymphoma (MCL).

A, The smear shows an isomorphic population of small cells with round-to-oval nuclei and scant cytoplasm. Note the neutrophil at upper right for size comparison (Romanowsky stain). B, Fluorescence in situ hybridization (FISH) reveals an (11;14) translocation in the abnormal cells on the right. A green probe for immunoglobulin heavy chain (IgH) at 14q32 and a red probe for the cyclin D1 gene come together to give a yellow signal when there is a translocation. Note that the green and red signals are far apart in the normal cells. (Dual color interphase FISH. Courtesy of Paola dal Cin, PhD, Brigham and Women's Hospital, Boston, MA, USA.)

Differential Diagnosis: Small Cell Lymphomas

The differential diagnosis of the small cell lymphomas includes reactive hyperplasia, Hodgkin lymphoma, and each of the four small cell lymphomas themselves.



Differential diagnosis of small cell lymphomas

- reactive hyperplasia
- Hodgkin lymphoma
- follicular lymphoma (FL)
- MZL/MALT lymphoma
- small lymphocytic lymphoma (SLL)
- mantle cell lymphoma (MCL)

The four small cell lymphomas must, first and foremost, be distinguished from a *reactive hyperplasia*. There are helpful morphologic clues, but they are subtle, and, in practice, immunophenotyping (IP) is commonly relied upon to make this distinction. If a smear is comprised of a monomorphous population of small lymphoid cells, lymphoma is likely, but a reactive lymph node cannot be excluded without IP, because a minimally reactive lymph node can also show little lymphocyte polymorphism.¹⁰³ Although small, round lymphocytes predominate in reactive hyperplasia, in most cases there is an expanded range of lymphoid cells, including plasmacytoid lymphocytes, plasma cells, and immunoblasts. Dendritic-lymphocytic aggregates and tingible-body macrophages, while common in reactive hyperplasia, are rare or absent in the small cell lymphomas, with the exception of FL.

MZL, in particular, is an exception to the rule of morphologic homogeneity in the small cell lymphomas. MZL resembles a reactive hyperplasia because of its associated nonneoplastic polymorphous infiltrate of small lymphocytes, centrocytes, and monocytoid B cells.¹⁷ Ultimately, the distinction between lymphoma and reactive hyperplasia rests on IP, either by flow cytometry or immunocytochemistry.

IP also helps in the distinction between non-Hodgkin lymphoma and a *Hodgkin lymphoma* with very few Reed-Sternberg cells: The small B-cells of

Hodgkin lymphoma show polyclonal expression of immunoglobulin light chains.

The small cell lymphomas are distinguished from one another by a combination of clinical, morphologic, immunophenotypic, and genetic features. Clinical features are summarized in [Table 12.5](#). An extranodal location is common in MZL and MCL and may be seen in FL (particularly in the head and neck region), but is rarely the presenting site of SLL.

Morphologically, SLL and MCL are more monomorphic than FL and MZL. In particular, SLL is composed of uniform small round cells, with relatively infrequent larger cells. MCL is also composed of monomorphic small cells, with more finely textured chromatin and more irregular nuclear membranes than SLL. Monocytoid B cells are a clue to the diagnosis of MZL, and cleaved nuclei are characteristic of FL. An infiltrate that includes plasma cells and plasmacytoid cells is characteristic of MZL. Although morphologic and clinical features are helpful, the immunophenotype, particularly the expression of CD5, CD10, and CD23, is paramount in subclassifying the small cell lymphomas.



Algorithm for discriminating among small cell lymphomas based on flow cytometry immunophenotype

- CD5+ (SLL and MCL)
 - CD23+ SLL
 - CD23– MCL
 - light chain,CD20 dim favor SLL
- CD5- (FL and MZL)
 - CD10+ FL
 - CD10-unresolved

The CD5+, CD23+, CD10– immunophenotype is relatively specific for SLL, and the CD5+, CD23–, CD10– immunophenotype is relatively specific for MCL. With SLLs, fluorescence for light chains and CD20 is characteristically weak (commonly referred to as dim). In addition, increased cyclin D1 (bcl-1) expression, which can be measured by immunocytochemistry, is highly specific for MCL. The CD5–, CD10+ immunophenotype is characteristic of FL (CD23 can be + or –), but many cases of FL are negative for CD10 (at least by flow cytometry, which seems to have imperfect sensitivity for detection of CD10). The CD5–, CD10– profile is therefore nonspecific, as it does not distinguish

between FL and MZL; immunohistochemistry on cell block for CD10 and bcl-6 is recommended in such cases.

Aberrant immunophenotypes occur, so the immunophenotype must be correlated with morphologic, clinical, and, where appropriate, molecular genetic studies, if available. Molecular genetic studies are most helpful ([Table 12.6](#)) when the immunophenotype is aberrant or ambiguous. About 30% of SLLs show trisomy 12, and other aberrations may also be seen (e.g., del 13q14.3, del 11q22-23, or del 17p13). The t(11;14)(q13;q32) translocation is a hallmark of MCL and is detected in most cases. FL is characterized by a t(14;18)(q32;q21) translocation in up to 95% of cases. These characteristic abnormalities can be identified by FISH on cytologic preparations.^{[37,96,97](#)}

Lymphomas of Large Cells

A broad and heterogeneous group of neoplasms that arise from B cells, T cells, or natural killer (NK) cells, this category is nonetheless dominated by a single common entity: diffuse large B-cell lymphoma (DLBL). Unlike the previous group of small B-cell lymphomas, which are tumors of adults, many of the large cell lymphomas occur in children as well as adults. In children, the most common lymphomas are DLBL, lymphoblastic lymphoma, and Burkitt lymphoma.^{[104](#)}

Note that the designation “large cells” for the entities described in this section is used here as a convenience; indeed, *large* in this context refers to nuclear size relative to histiocyte nuclei, and therefore large lymphocytes often are actually smaller than the epithelial cells that cytologists are more familiar with. Moreover, although some of the neoplasms discussed below are indeed comprised predominantly of large lymphoid cells, others contain a mixture of small and large neoplastic cells, or large neoplastic cells with reactive (nonlesional) small cells. Some are actually comprised of intermediate-sized cells (e.g., Burkitt lymphoma). Lymphoblastic lymphoma cells are often smaller cells, but this entity is considered here under the broader heading of T-cell lymphomas. Also note that not all posttransplant lymphoproliferative disorders are even lymphomatous, but they are discussed here because most of them are neoplastic, and those that are neoplastic usually contain at least some, if not a preponderance of, large cells. The differential diagnosis of the lymphomas of large cells is discussed in a dedicated section after the descriptions of the individual entities.

Diffuse Large B-Cell Lymphoma

DLBL is an aggressive but potentially curable subtype of non-Hodgkin lymphoma that comprises 35% of adult lymphomas. It is also one of the three major lymphomas in children. About 40% of patients present with extranodal disease.

The histologic hallmark of DLBL is the presence of confluent areas of large malignant B cells. Several morphologic subtypes have been recognized (e.g., centroblastic, immunoblastic, and anaplastic large B-cell), but subclassification is usually not clinically relevant¹⁰⁵ except as noted below. Through gene expression profiling, different genetic subtypes of DLBL—germinal center B-cell-like and activated B-cell-like—have been identified.¹⁰⁶ These may have clinical significance in that they may predict survival after chemotherapy.¹⁰⁷ Evidence suggests that gene expression profiling can be performed on FNAs.⁴⁰

The bcl-2 gene rearrangement is found in 30% of cases.⁸⁷ Another 30% show abnormalities of the 3q27 region involving bcl-6.



Cytomorphology of diffuse large B-cell lymphoma

- predominantly large cells (2.5 to 5 times the size of a small lymphocyte; nucleus larger than histiocyte nucleus)
- distinct to large nucleoli
- lymphoglandular bodies
- variable number of tingible-body macrophages
- paucity or absence of dendritic-lymphocytic aggregates

The cellularity of smears from DLBL is variable. Aspirates of mediastinal DLBL and extranodal sites can be very sparsely cellular due to the extensive sclerosis and/or sparse distribution of neoplastic cells common to these tumors. Most FNAs of DLBL are at least moderately cellular, however, and smears are usually easily recognizable as abnormal because of the abundance of large atypical cells ([Fig. 12.20](#)). They can have a round or highly irregular nucleus with multiple nucleoli and scant cytoplasm (centroblastic type); a round or irregular nucleus with a single very prominent nucleolus and more abundant cytoplasm (immunoblastic type); or a bizarre, pleomorphic nucleus with frequent multinucleation that resembles the nucleus of Reed-Sternberg cells (anaplastic type). A population of small, nonneoplastic T cells is always present but usually

represents a minority of the cell sample. When T cells or histiocytes are few in number (or preparation artifact is present), it may be difficult to judge the size of the neoplastic cells. Attention to the presence of nucleoli helps to exclude one of the lymphomas of small cells in such cases. Conversely, diagnostic difficulty can be encountered when small T cells and/or histiocytes outnumber the large, neoplastic B cells (See discussion of T-cell/histiocyte rich DLBCL below).

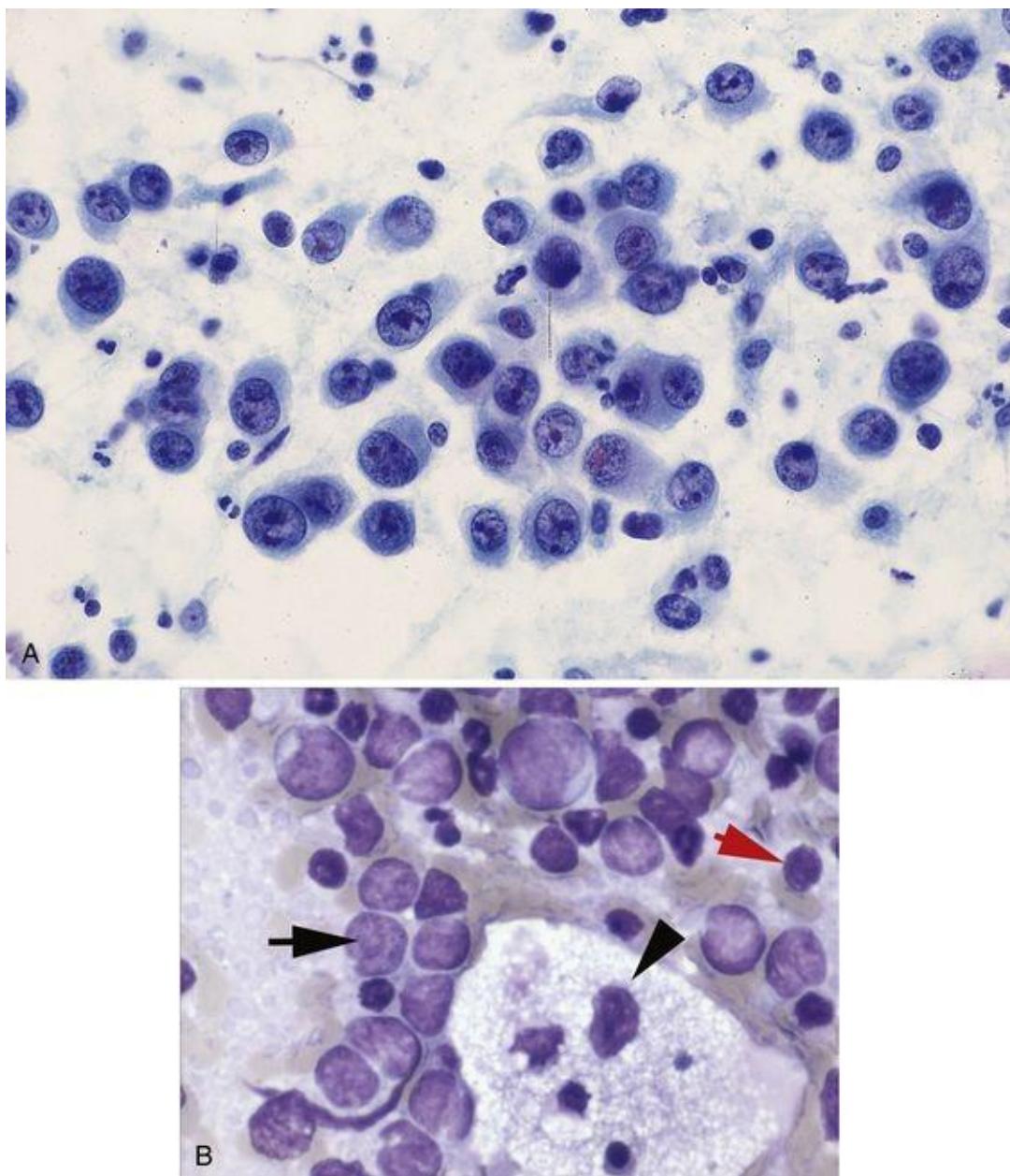


FIGURE 12.20 Diffuse large B-cell lymphoma (DLBL).
A, A uniform population of lymphocytes with large, almost vesicular nuclei, obvious nucleoli,

and a moderate amount of cytoplasm is admixed with neutrophils and small lymphocytes (Papanicolaou stain). *B*, By definition, the nucleus of DLBL cells (*black arrow*) is at least the size of a histiocyte nucleus (*black arrowhead on foam-cell nucleus*); the background T-cell nuclei (*red arrow*) are much smaller (Romanowsky stain).

Characterization of DLBL by flow cytometry can be challenging (requiring careful correlation of cytomorphology with flow results), because the large, atypical cells may appear outside the usual “lymphoid gate” on flow cytometry scattergrams. Less commonly, the large cells may not survive processing due to fragility.¹⁰⁸ Additionally, DLBL often lacks detectable surface light chain expression, hampering confirmation of clonality. This apparent stumbling block, however, can in fact be a clue to the correct diagnosis, because lack of surface light chain on B cells outside the marrow is an abnormal finding, usually (but not invariably¹⁰⁹) associated with neoplasia.¹¹⁰ Nevertheless, determination of clonality by PCR may be required if doubt remains and if the neoplastic nature of the cells is not evident morphologically. With respect to other markers, DLBL cells usually show bright CD20 staining by flow cytometry and/or immunohistochemistry, but rituximab-treated patients (i.e., recurrences) may show reduced CD20 staining; in these circumstances PAX5/BSAP staining by immunohistochemistry may be helpful to confirm the B-cell phenotype of the cells.

Note that the distinction between grade 3 follicular lymphoma (FL) and DLBL by FNA is not generally possible due to the loss of tissue architecture.¹⁷ FLs that have a vastly predominant population of centroblasts (i.e., grade 3 FL) are generally treated like DLBL; thus, determination of follicular versus diffuse architecture may not be critical in such cases.

Variants of Diffuse Large B-Cell Lymphoma

Several clinically distinct variants of DLBL are worth noting. Primary mediastinal (thymic) large B-cell lymphoma predominates in young adult females and may cause superior vena cava obstruction. FNA has been used successfully in diagnosing this variant,^{33,111} but the sclerosis may limit FNA sampling to a few inconspicuous cells. Tumor cell expression of CD30, IRF4/MUM1, TRAF1, and nuclear c-Rel is distinctive.¹¹²

Another emerging entity, double hit lymphoma^{113–115} (lymphoma with both IgH–bcl-2 and c-myc translocations) deserves mention because of its newly recognized high incidence in older patients with advanced/aggressive disease and its poor prognosis. Clinically, a subset of these patients may have a history of low-grade FL, with sudden progression, but many present de novo. In fact,

double hit lymphoma may form a discrete clinicopathologic entity within the otherwise heterogeneous WHO category “B-cell lymphoma, unclassifiable, with features intermediate between DLBL and Burkitt lymphoma (BL).” In addition, lymphomas which exhibit a three-way translocation involving bcl-2, IgH, and c-myc, such as t(8;14;18), or the combination of a bcl-6 and c-myc translocation may also form part of the spectrum of double hit lymphoma. Although this entity shares some morphologic and immunophenotypic features with BL, double hit lymphoma usually shows immunohistochemical expression of bcl-2 and a moderately high Ki67 index (usually greater than 80%), and it may show reduced CD20 expression by flow cytometry.¹¹⁶ The cytologic features are not specific (Fig. 12.21), but in an older patient, a highly aggressive and/or proliferative bcl-2-positive B-cell lymphoma with some features of BL should prompt consideration for c-myc immunohistochemistry,^{117,118} with confirmatory FISH or cytogenetic studies to detect IgH-bcl-2 and c-myc translocations (Table 12.7).

TABLE 12.7
DIFFERENTIAL FEATURES OF DIFFUSE LARGE B-CELL LYMPHOMA AND MIMICS

	Diffuse Large B-Cell Lymphoma	Double Hit Lymphoma	Burkitt Lymphoma	Lymphoblastic Lymphoma
CD20	+	+	+	-/+
CD10	+/-	+	+	+
bcl-6	+/-	+	+	NR
bcl-2	+/-	+/-	-/+	NR
IRF4/MUM1	-/+	-	-	NR
slg	+/-	+/-	+	-
nuclear c-myc ^{117,118}	-/+	+	+	NR
terminal deoxynucleotidyl transferase	-	-	-	+
Ki67	40–100%	80–100%	100%	NR
cytogenetics	variable	c-myc translocation and bcl-2 or bcl-6 translocation	c-myc translocation	variable

+/-, Majority of cases are positive, minority negative.

-/+, Majority of cases are negative, minority positive.

NR=not relevant for differential diagnosis

*CD20 may be weak in double hit lymphoma by flow cytometry

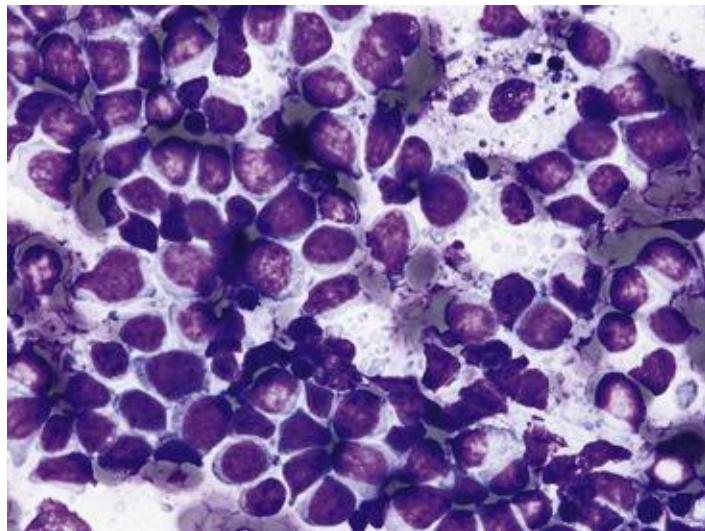


FIGURE 12.21 Double-hit lymphoma.

A monomorphic population of intermediate-sized cells closely mimics Burkitt lymphoma (BL). Note the tingible-body macrophage at upper right (Romanowsky stain).

A third DLBL variant, T-cell/histiocyte-rich large B-cell lymphoma,¹¹⁹ is rarely (if ever) specifically diagnosed by FNA because of the small proportion of neoplastic large B cells in a background of nonneoplastic cells, but careful screening for large atypical cells should at least prompt further tissue sampling. Two other DLBL variants are primary effusion large B-cell lymphoma (see [Chapter 4](#)) and intravascular large B-cell lymphoma (see [Chapter 15](#)).

Burkitt Lymphoma

BL is a highly aggressive but potentially curable B-cell malignancy with a characteristic morphology and a cytogenetic translocation that constitutively activates the c-myc oncogene. The diagnosis of BL has great clinical importance because it necessitates a chemotherapeutic regimen that is more aggressive than that used for other high-grade B-cell lymphomas.

BL occurs in three clinical settings: (1) an *endemic* form found in Africa and Asia and rare in the United States and Europe; (2) a *sporadic* form, which occurs in the United States and other countries; and (3) an *immunodeficiency-associated* form. The endemic and sporadic forms are more common in children, and the immunodeficiency-associated form is more common in adults. Virtually all the endemic cases show latent infection of the tumor cells by EBV, whereas only a subset of the sporadic and immunodeficiency-associated cases are EBV-associated.

The malignant cells involve lymph nodes and/or extranodal tissues (the

gastrointestinal tract, liver, and bone marrow are common sites in the nonendemic form), and cerebrospinal fluid is involved in 20% to 30% of cases at presentation.



Cytomorphology of Burkitt lymphoma

- uniform intermediate-sized cells
- round nuclei, coarse chromatin
- 2 to 5 small nucleoli per nucleus
- scant blue cytoplasm with small vacuoles (Romanowsky stain)
- apoptosis and numerous mitoses
- numerous tingible-body macrophages

Smears are monotonously hypercellular. Tingible-body macrophages are randomly dispersed throughout the smear, mimicking the “starry sky” pattern of tissue sections. Because individual cell necrosis (apoptosis) is common, a “dirty” background is typical. The cells are intermediate in size, with round nuclei that have coarse chromatin, multiple (but small) nucleoli, and scant cytoplasm (Fig. 12.22A). With Romanowsky-type stains, the cytoplasm is a deep blue with the often-mentioned small lipid vacuoles,¹²⁰ but small vacuoles are common in DLBCL as well.

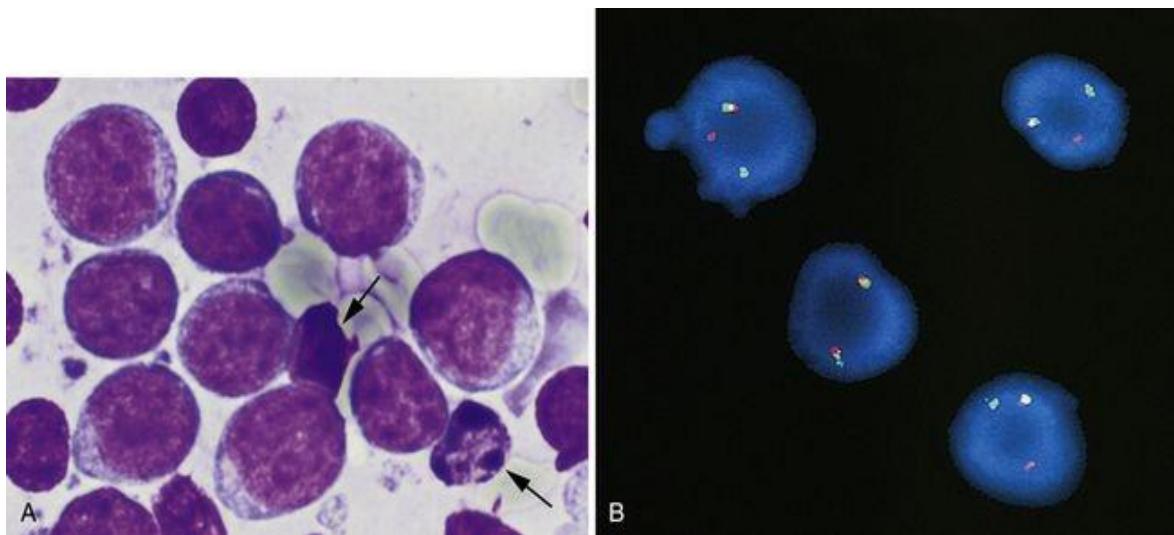


FIGURE 12.22 Burkitt lymphoma (BL).

A, The intermediate-sized neoplastic lymphocytes have round nuclei, multiple indistinct nucleoli, blue cytoplasm, and small cytoplasmic vacuoles. Apoptotic bodies (arrows) are present (Romanowsky stain). B, Detection of c-myc translocation by fluorescence in situ

hybridization (FISH). Red and green fluorescent probes to regions flanking c-myc are split apart. This occurs in all three of the common c-myc translocations. (Dual color interphase FISH. Courtesy of Paola dal Cin, PhD, Brigham and Women's Hospital, Boston, MA, USA.)

BL cells express B-cell-specific surface antigens such as CD19, CD20, the immunoglobulin heavy chain (IgH), and monotypic surface immunoglobulin light chains. BL also expresses CD10 and bcl-6 but is negative for bcl-2 and terminal deoxynucleotidyl transferase (Tdt). Nearly 100% of cells are positive for Ki67.

BL is almost invariably associated with a chromosomal translocation involving the *c-myc* proto-oncogene on chromosome 8 and either the immunoglobulin G (IgG) heavy chain or κ or λ light chain genes. The most reliable method for identifying the specific translocation in BL is karyotyping by conventional cytogenetics, but the presence of any of these three translocations can also be suggested by immunohistochemistry for nuclear c-myc^{117,118} or documented by FISH.^{121,122} In the latter assay, probes to the flanking regions of the c-myc locus show a split signal, indicating a rearrangement ([Fig. 12.22B](#)).

A c-myc rearrangement is highly sensitive but not specific for BL, as it is occasionally seen in large B-cell lymphoma, rare cases of FL, some late-stage multiple myelomas, and (more commonly) in the provisional entity “B-cell lymphoma, unclassifiable, with features intermediate between DLBL and BL.” For this reason, the diagnosis is based on an integration of clinical, morphologic, immunophenotypic, and genetic findings ([Table 12.7](#)).

Plasmablastic Lymphoma

Plasmablastic lymphoma is a rare tumor, occurring in HIV-positive or otherwise immunodeficient patients, typically in the oral cavity or other mucosal sites, but also in lymph nodes. This tumor is unique among hematolymphoid neoplasms because the constituent cells resemble B immunoblasts (i.e., large, noncohesive cells with large central nucleoli) ([Fig. 12.23](#)) but have an immunophenotype similar to plasma cells (i.e., CD138+, CD38+, IRF4/MUM1+, CD45-, CD20-, and PAX5/BSAP-, but, unlike myeloma, CD56-) and express EBER by *in situ* hybridization.¹²³ Caution should be used before overinterpreting CD138 positivity in a large cell neoplasm, however, because this antigen is widely distributed in nonhematolymphoid neoplasms, including carcinomas.

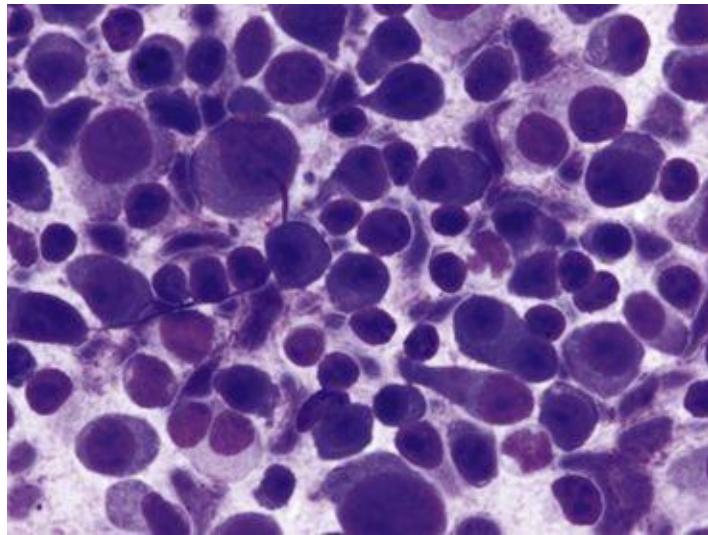


FIGURE 12.23 Plasmablastic lymphoma.

Large malignant cells are dispersed in a noncohesive pattern. The eccentric position of the nucleus is the only “plasmacytic” feature. Because this image is virtually impossible to distinguish from a plasmablastic myeloma or melanoma, correlation with clinical history and ancillary testing are necessary for definitive diagnosis (Romanowsky stain).

T-Cell Lymphomas

T-cell lymphomas comprise about 10% of all non-Hodgkin lymphomas in Western countries. The 2008 WHO classification lists numerous subtypes ([Table 12.8](#)) that are broadly divided into precursor T-cell lymphoblastic lymphoma and mature T-cell and NK-cell lymphomas.

TABLE 12.8

2008 WORLD HEALTH ORGANIZATION CLASSIFICATION OF T-AND NATURAL KILLER-CELL NEOPLASMS RELEVANT TO FINE-NEEDLE ASPIRATION[‡]

Precursor T-cell lymphoblastic leukemia/lymphoma
Mature T-cell and natural killer-cell neoplasms
Aggressive natural killer-cell leukemia
Extranodal natural killer/T-cell lymphoma, nasal-type
Mycosis fungoides
Sézary syndrome
Angioimmunoblastic T-cell lymphoma
Peripheral T-cell lymphoma, unspecified
Adult T-cell leukemia/lymphoma (human T-cell leukemia-virus 1 positive)
Anaplastic large cell lymphoma, anaplastic lymphoma kinase positive
Anaplastic large cell lymphoma, anaplastic lymphoma kinase negative
Subcutaneous panniculitis-like T-cell lymphoma
Hepatosplenic T-cell lymphoma

*More common entities are in bold.

The mature T-cell and NK-cell neoplasms comprise a diverse group of neoplasms defined primarily by their clinical features. In contrast to B-cell lymphomas, most T-cell lymphomas are not associated with specific immunophenotypic profiles. With the exception of anaplastic large cell lymphoma (ALCL), which is sometimes associated with the t(2;5) translocation, specific genetic abnormalities have not been identified for many T-cell and NK-cell neoplasms.

Unlike B-cell lymphomas, where light chain restriction is a marker of malignancy, there are no convenient antigenic markers of monoclonality. The diagnosis is suggested by an aberrant T-cell immunophenotype, such as loss of one or more of the pan T-cell markers CD2, CD3, CD5, CD7; loss of both CD4 and CD8; or coexpression of both CD4 and CD8.³¹ Care must be taken, however, not to base the diagnosis of T-cell lymphoma solely on the presence of a double positive (CD4+CD8+) T-cell population, which can be seen in NLPHL⁵² or in the lymphoid population accompanying thymoma, nor on the presence of a double negative (CD4–CD8–) population, which can also be observed in the lymphoid population accompanying thymoma.¹²⁴ Molecular genetic studies, most commonly PCR for rearrangement of the T-cell receptor genes, are often required to confirm the clonality of a T-cell proliferation.⁷²

Peripheral T-cell lymphoma, unspecified; ALCL; lymph node involvement by mycosis fungoides; and adult T-cell leukemia/lymphoma are the most common

mature T-cell lymphomas encountered by the cytologist.

Peripheral T-cell lymphoma, unspecified

Peripheral T-cell lymphoma of unspecified type is much more common in Asia than Europe or North America. In the United States, these patients are typically elderly and systemically ill, with fevers, night sweats, and bulky lymphadenopathy.



Cytomorphology of peripheral T-cell lymphoma, unspecified

- monomorphous small or large lymphocytes, or a mixture of small and large lymphocytes
- irregular nuclei
- histiocytes, plasma cells, and eosinophils
- Reed-Sternberg-like cells

Peripheral T-cell lymphoma cases show marked variety in cell composition. Most aspirates (70%) have a polymorphous cell population with histiocytes, eosinophils, plasma cells, and intermediate and large lymphocytes ([Fig. 12.24](#)), whereas others have the morphology of a pure large cell lymphoma, and a smaller number have a monomorphic, small lymphocytic lymphoma (SLL)-like appearance.^{25,31,125} Nuclei of both small and large lymphocytes frequently display nuclear indentations, grooves, and knoblike projections.

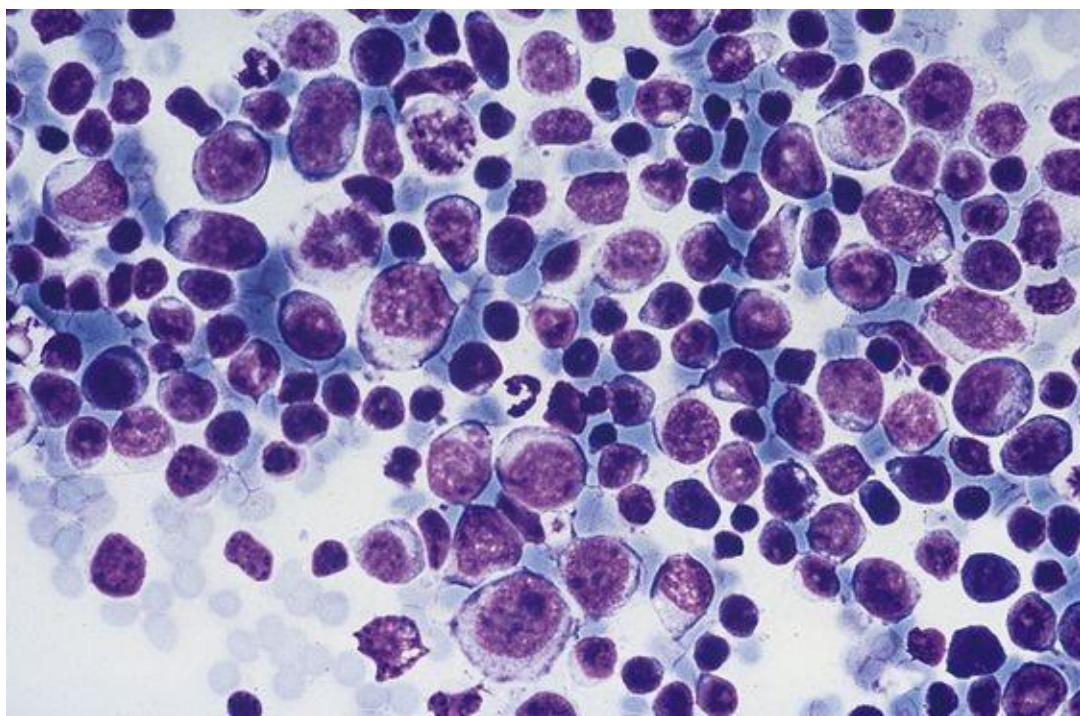


FIGURE 12.24 Peripheral T-cell lymphoma, unspecified.

A picture of lymphocyte heterogeneity is produced by this mixture of small, intermediate, and large cells. In other fields of the smear the large cells were more numerous, but taken in isolation this image is easily misinterpreted as representing reactive hyperplasia (Romanowsky stain).

Anaplastic large cell lymphoma

ALCL is a subtype of non-Hodgkin lymphoma that was recognized after the discovery of the CD30 (Ki-1) antigen. In the past, many of these were misdiagnosed as carcinomas and other nonlymphoid malignancies, or as undifferentiated malignant neoplasms. Initially they were called *Ki-1 lymphomas*, but Ki-1 (CD30) expression is not limited to ALCL.

ALCL accounts for about 3% of adult non-Hodgkin lymphoma and 10% to 30% of childhood lymphomas.¹⁹ It is usually a T-cell neoplasm and often expresses one or more T-cell antigens. Some cases have an apparent “null cell” phenotype, but their T-cell lineage is revealed by demonstrating clonal T-cell receptor rearrangement.

They show a broad morphologic spectrum, but in the common type the cells are large and pleomorphic. Histologically, a bizarre cell with a horseshoe-shaped nucleus (the so-called hallmark cell) is characteristic. Reflecting the fact that lymph nodes are often only partially involved (with a sinusoidal growth pattern in histologic sections), the FNA sample can contain a mixed lymphoid

background that obscures the hallmark cells. In addition to this classic form, there is a *lymphohistiocytic variant*, characterized by a large number of histiocytes that may mask the malignant cells, and a *small cell variant*, which resembles a peripheral T-cell lymphoma.

Some cases of ALCL are associated with a t(2;5)(p23;q35) or similar anaplastic lymphoma kinase (ALK) translocation, which fuses the ALK gene with the nucleophosmin (NPM) gene to produce the ALK protein, but many of these tumors in adults lack this translocation. Immunostaining for the ALK protein (detectable in 60% to 85% of cases) is helpful both in diagnosis and prognosis; patients with ALK positivity have a better prognosis than ALK-negative patients.¹²⁶ This prognostic difference and the tendency of ALK-negative ALCL to occur in older patients has led the WHO to divide ALCL into two provisionally separate entities: *ALCL*, *ALK-negative* and *ALCL, ALK-positive*.



Cytomorphology of anaplastic large cell lymphoma

- intermediate and large cells
- irregular nuclei: horseshoe, donut, and embryoid shapes
- Reed-Sternberg-like cells with smaller nucleoli
- histiocytes, neutrophils
- no tingible-body macrophages or dendritic-lymphocytic aggregates
- few or absent lymphoglandular bodies

Aspirates show moderate to high cellularity; necrosis and inflammation can be prominent. The hallmark cell of ALCL has a horseshoe-shaped nucleus, but a spectrum of abnormal large cells varying in number, shape, and lobation of nuclei, particularly ring-shaped (“donut”) nuclei, are seen^{127–129} (Fig. 12.25). Necrosis can be a prominent feature.¹³⁰ The tumor cells are positive for CD30 in a membranous pattern and in the Golgi region, but CD30 is nonspecific. Many cases are positive for ALK, EMA, and clusterin, but negative results do not exclude the diagnosis. EBV RNA sequences are absent. A break-apart FISH probe permits identification of the t(2;5) translocation in FNAs,¹³¹ but immunohistochemistry is generally sufficient for confirming ALK-positivity. Flow cytometry results have been described,¹³² but flow cytometry is not usually useful in practice. Confirmation of a T-cell receptor gene rearrangement may be necessary in more difficult cases.

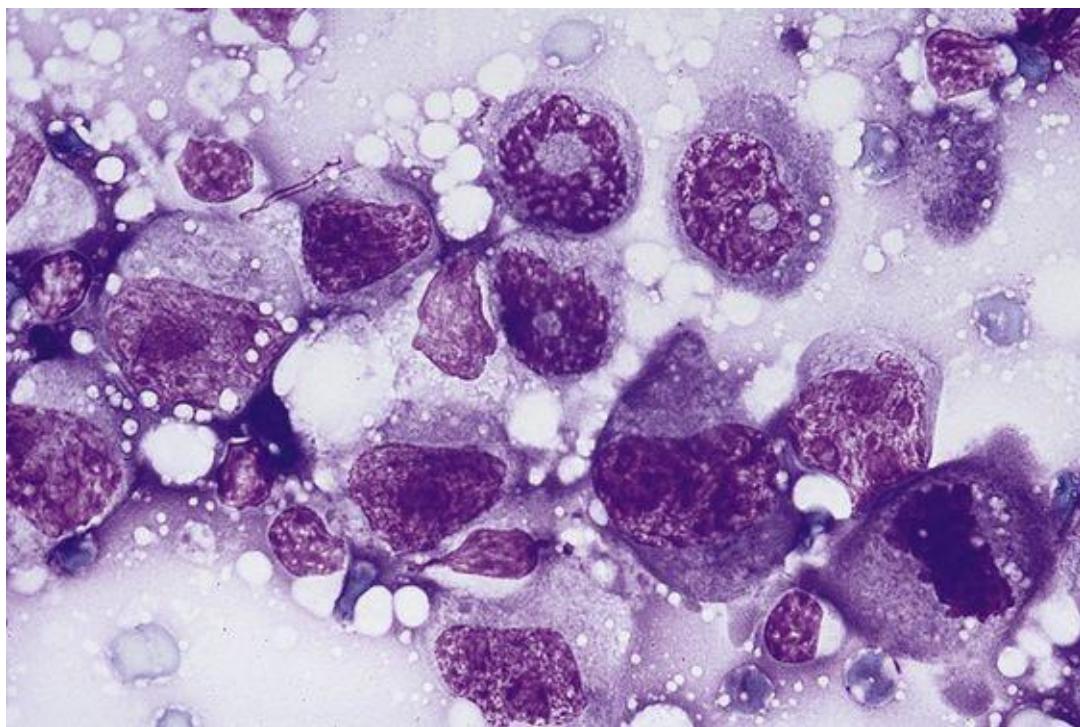


FIGURE 12.25 Anaplastic large cell lymphoma (ALCL).

Very large cells with marked variation in nuclear shape including “donut” forms are seen (Romanowsky stain).

Lymph node involvement by mycosis fungoides

Mycosis fungoides is a primary cutaneous T-cell lymphoma, but, in some late-stage patients, lymph nodes and viscera may be involved. Such nodes may exhibit small or large lymphocytes with cerebriform nuclei on FNA, but the cytologic features alone are insufficient for accurate diagnosis of lymph node involvement.¹³³ FNA is, however, a useful tool for confirming such involvement, provided that flow cytometry and molecular study for clonality is also performed on the FNA specimen.³⁶

Adult T-cell leukemia/lymphoma

Adult T-cell leukemia/lymphoma is a disease of adult patients in endemic regions (including the Caribbean islands in the Western Hemisphere) caused by latent infection by the retrovirus HTLV-I. Adult T-cell leukemia/lymphoma usually presents with lymph node involvement, and the FNA cytologist may be called upon to make the initial diagnosis if the characteristic circulating cells are absent or not specifically appreciated on routine peripheral blood testing. Large

cells with multilobulated “floretlike” nuclei, prominent nucleoli, and deeply basophilic cytoplasm with occasional vacuoles are characteristic (Fig. 12.26), but in practice the cytomorphologic spectrum is wide. Although most aspirates appear malignant, specific diagnosis rests on appreciation of the characteristic clinical features (ethnic background, hypercalcemia, and atypical lymphocytosis in the peripheral blood) coupled with flow cytometry (showing the CD2+, CD3+, CD4+, CD5+, CD25+, CD7–, CD8– immunophenotype) and followup serologic testing for HTLV-I.¹³⁴

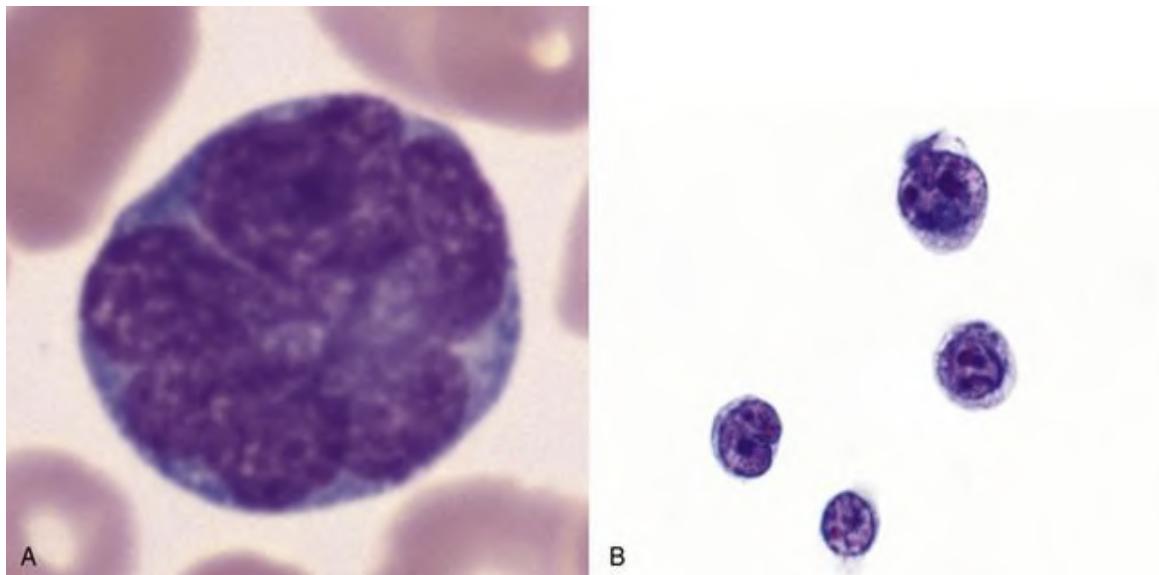


FIGURE 12.26 Adult T-cell leukemia/lymphoma.

A, The most distinctive cell is the floret cell, a large lymphocyte with multiple radiating nuclear lobes (Romanowsky stain), but B, a wide range of neoplastic forms are typically seen, including large nucleolated cells (liquid-based preparation, Papanicolaou stain).

Precursor T-and B-cell lymphoblastic lymphoma

Lymphoblastic lymphoma is an aggressive lymphoma that comprises almost one half of childhood non-Hodgkin lymphoma and is more common in males. Lymphadenopathy almost always occurs above the diaphragm. An anterior mediastinal mass is commonplace (up to 80% of patients) and can induce clinical symptoms mimicking bronchial asthma (due to tracheal compression) or superior vena cava syndrome. Because these patients often have a palpable neck mass and are critically ill (many with some degree of respiratory compromise), the expediency of FNA makes it the procedure of choice to establish the

diagnosis. Combined with immunophenotypic studies, FNA has near perfect accuracy.^{[135-137](#)}



Cytomorphology of lymphoblastic lymphoma

Lymphoblasts:

- round or convoluted nuclei
- finely granular chromatin
- inconspicuous nucleoli
- scant or moderate amount of cytoplasm

Smears are nearly always very cellular and contain monotonous lymphoblasts to the near exclusion of any other cell type. Blasts are twice the size of a small lymphocyte, and nuclei are round or irregular. Nucleoli are either absent or inconspicuous ([Fig.12.27](#)). Cytoplasm is usually scant and may contain tiny vacuoles. Mitotic figures are variable and depend on smear thickness. Tingible-body macrophages are variably present. About 90% of cases are of T-cell derivation; the remainder derive from B-cells. In nearly all cases, blasts are positive for Tdt (a nuclear enzyme) and CD10 and negative for light chains. Immunophenotyping, coupled with cell morphology and clinical features, is nearly always diagnostic. The clinical scenario usually aids in distinction from thymoma, an important clinical and immunophenotypic mimic that features lymphocytes with an “immature” immunophenotype (but typically mature cytomechanics). The epithelial component of a thymoma is often inconspicuous on FNA smears, requiring immunocytochemistry (for keratin) for confident identification.

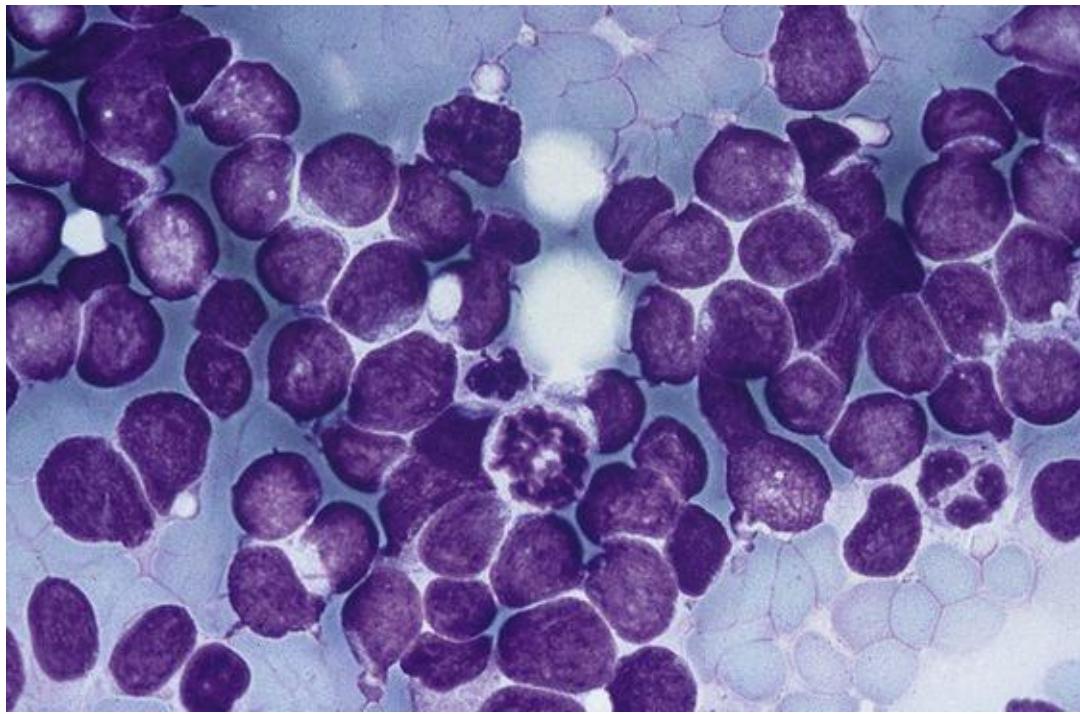


FIGURE 12.27 Lymphoblastic lymphoma.

A pure population of blasts is present with finely dispersed chromatin and nuclear molding. A mitotic figure is seen. Compare the size of the blasts with the neutrophil at the lower right (Romanowsky stain).

PostTransplant Lymphoproliferative Disorders

PTLDs are a heterogeneous group of lesions that include both nonneoplastic lymphoid proliferations as well as a variety of lymphomas. They occur in about 1% to 2% of solid organ (e.g., kidney, liver, lung) and bone marrow transplant recipients.¹⁹ Eighty percent of PTLDs are associated with EBV infection and represent EBV-induced monoclonal or (less often) polyclonal B-cell proliferations. A small percentage are T-cell proliferations. PTLDs usually occur within the first year of transplantation, but they can also occur many years later. PTLDs tend to involve lymph nodes and the gastrointestinal tract, but other extranodal sites can also be involved, including the allograft itself. A significant proportion of PTLDs resolve after reduction of immune suppression. Some fail to regress, however, and are treated with cytotoxic chemotherapy.

Histologically, PTLDs are divided into four major categories.¹⁹ (1) “*Early*” *lesions* are polyclonal, nonneoplastic lymphoid proliferations that preserve the underlying lymph node architecture and show plasma cell hyperplasia or an infectious mononucleosis-like picture. (2) *Polymorphic PTLD* is a destructive, neoplastic proliferation that shows the full range of B-cell maturation, including

immunoblasts, plasma cells, centrocytes, and small and medium-sized lymphocytes. Immunophenotyping by flow cytometry may appear polytypic, but molecular tests reveal clonal rearrangements of the IgHs and monoclonal episomal EBV. (3) *Monomorphic PTLD* is also a destructive, neoplastic proliferation, but it is composed of large, cytologically malignant lymphoid cells with prominent nucleoli resembling non-Hodgkin lymphoma. Most monomorphic PTLDs are B-cell neoplasms like DLBL, but some arise from T cells. (4) *Hodgkin lymphoma and Hodgkin-lymphoma-like PTLD*.

The cytologic features mirror the varied histology of PTLD.⁴⁶ Early lesions and polymorphic PTLD have overlapping features cytologically (a mixture of immunoblasts, plasma cells, centrocytes, and small-and medium-sized lymphocytes) ([Fig. 12.28A](#)) but can be distinguished by molecular studies for clonality. The monomorphic PTLDs are cytologically malignant and are classified according to the 2008 WHO classification system of non-transplant-related lymphomas, but the term *PTLD* should be included in the diagnosis. The PTLD moniker is important because it prompts the oncologist to include reduction of immunosuppression in the therapeutic armamentarium.

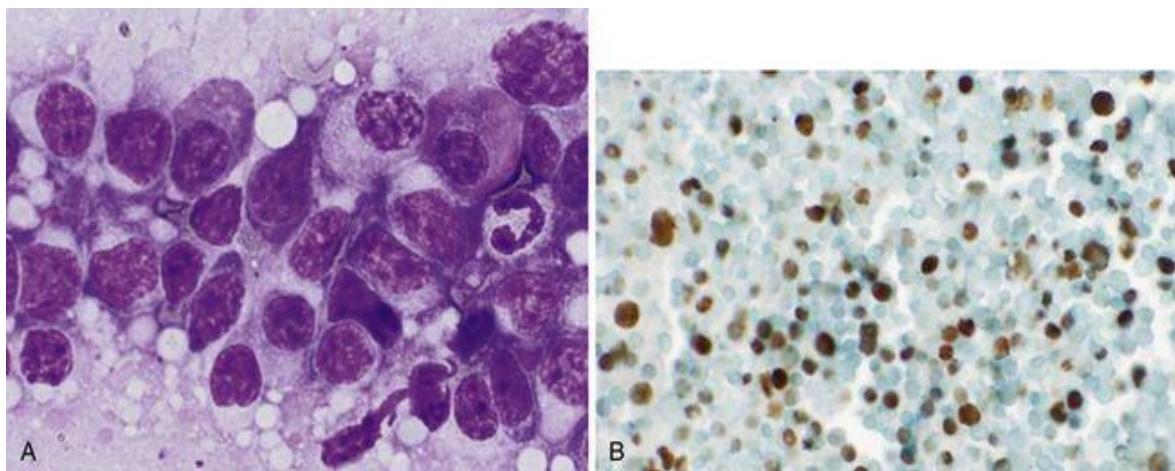


FIGURE 12.28 Posttransplant lymphoproliferative disorder (PTLD), polymorphic type. A, The smear shows a polymorphic population of small lymphocytes, plasmacytoid lymphocytes, plasma cells, and centrocytes (Romanowsky stain). B, In situ hybridization for Epstein-Barr virus (EBV)-encoded RNA (EBER), performed on cell block sections, shows a positive reaction in many of the lesional cells.

Light chain restriction is sometimes absent by flow cytometry or immunocytochemistry in polymorphic and monomorphic PTLDs; molecular testing is often needed to demonstrate clonality. Thus, the absence of light chain

restriction does not exclude the diagnosis of PTLD. In situ hybridization for EBER can be very helpful: A positive result for EBER in a majority of the lesional cells provides strong support for the diagnosis of PTLD⁴⁶ (Fig. 12.28B). About 20% of PTLDs are EBV-negative, however. In patients who received a bone marrow transplant for lymphoma, the differential diagnosis includes recurrent lymphoma. A positive result for EBER in a majority of the lesional cells helps establish the diagnosis of PTLD in this setting.

Differential Diagnosis: Large Cell Lymphomas



Differential diagnosis of large cell lymphomas

- nonhematopoietic tumors
- reactive hyperplasia
- Hodgkin lymphoma
- diffuse large B-cell lymphoma (DLBL)
- Burkitt lymphoma (BL) and Burkitt-like lymphoma
- B-cell lymphoma, unclassifiable, with features intermediate between DLBL and BL (subtype: double hit lymphoma)
- peripheral T-cell lymphoma, not otherwise specified
- anaplastic large cell lymphoma (ALCL)
- precursor T-and B-cell lymphoblastic lymphoma
- thymoma
- posttransplant lymphoproliferative disorder (PTLD)
- myeloid sarcoma (synonymous with granulocytic sarcoma and chloroma)
- histiocytic and dendritic cell neoplasms

The group of large cell lymphomas can mimic and be mimicked by *nonhematopoietic neoplasms*. Poorly differentiated large cell carcinomas, epithelioid sarcomas, melanoma, and germ cell tumors all can resemble DLBL. With rare exceptions (nasopharyngeal carcinoma and seminoma), the nonlymphoid tumors lack lymphoglandular bodies.

Conversely, ALCL is very carcinoma-like.



Anaplastic large cell lymphoma is carcinoma-like because of

- a paucity of lymphoglandular bodies¹³⁰
- cell clustering
- marked pleomorphism
- spindle cells¹³⁸
- immunoreactivity for EMA
- scant smaller lymphocytes

Immunostains are particularly useful in the distinction between lymphoma and a nonhematopoietic neoplasm, particularly keratins, S-100 protein, MelanA, OCT3/4 (for germ cell tumors), CD45, CD3, and CD20. Of note, some ALCLs are negative for CD45, but these will usually stain for CD3, CD30, clusterin, and/or the ALK protein.

Nonhematopoietic neoplasms are also in the differential diagnosis of lymphoblastic lymphoma and BL. The cells of these lymphomas are intermediate in size rather than truly large. For this reason, and because they are common in children, the differential diagnosis includes small round-cell malignancies such as *neuroblastoma*, *rhabdomyosarcoma*, and *Ewing sarcoma/primitive neuroectodermal tumor (PNET)*. Morphologic features are helpful: The nonhematopoietic neoplasms lack lymphoglandular bodies, and the cells form aggregates in smears. The cells of neuroblastoma form rosettes, have fibrillary neuropil, and show nuclear molding. The cells of rhabdomyosarcoma have a greater degree of anisonucleosis than lymphoblastic lymphoma or BL; more abundant, tapered cytoplasm; and they are frequently binucleated or multinucleated.¹³⁹ Immunostains for neural, muscle, and lymphoid markers help to confirm the morphologic impression.

Occasionally, one of the large cell lymphomas resembles *reactive lymphoid hyperplasia*. This is particularly true of a subtype of DLBL, the T-cell/histiocyte rich DLBL.¹² In this tumor, the neoplastic large cells are outnumbered by reactive T cells. To identify the monoclonal population by flow cytometry, it is necessary to gate on the large cells using forward scatter. Even so, flow may give a false-negative result. Some cases of peripheral T-cell lymphoma, those composed of a mixture of small and large cells, also mimic reactive hyperplasia. In most cases, however, there is a sufficient number of atypical small and large cells, with nuclear membrane irregularities (indentations, grooves, knobs), to prompt consideration of neoplasia.^{25,125} Immunophenotyping often demonstrates aberrant patterns of expression of T-cell antigens, supporting the neoplastic nature of the lesion.

Mononuclear and classical (binucleate) Reed-Sternberg cells are frequently

seen in ALCL. The sheer number of these pleomorphic cells,⁸⁴ and the paucity of inclusion-like macronucleoli help to exclude *Hodgkin lymphoma*. In borderline cases, immunostaining for CD15 and BSAP/PAX5 (positive in Reed-Sternberg cells and negative in the hallmark cells of ALCL) and, if necessary, T-cell receptor rearrangements (detectable by molecular testing in ALCL) can be helpful.

Extensive sclerosis in some cases of DLBL, particularly those in the mediastinum, may yield sparsely cellular smears. The few lymphoid cells present are often crushed, and the remaining fragments of fibrous tissue might be mistaken for a *spindle cell neoplasm*¹¹¹ or *granulomatous inflammation*. Knowledge of this pitfall (and thus carefully searching for large, atypical lymphoid forms, especially in young adult female patients) is usually rewarding.

The large cell type of peripheral T-cell lymphoma is morphologically identical to *DLBL*; the distinction depends on immunophenotyping. The distinction between DLBL, particularly T-cell/histiocyte rich DLBL, and Hodgkin lymphoma can also be problematic. Again, immunomarkers are useful: In general, Reed-Sternberg cells (except in the nodular lymphocyte predominant Hodgkin lymphoma) are positive for CD15 and CD30, and most non-Hodgkin lymphomas are negative.¹⁹

ALCLs express a T-cell or null cell phenotype, and may or may not express the ALK protein, thus the diagnosis is not restricted to the ALK-positive cases. Rather, a diagnosis is based on both morphology, immunophenotype, and the exclusion of mimics. Morphologically similar B-cell tumors exist, and some of these may even express CD30, but because the anaplastic B-cell neoplasms have clinical characteristics and behavior similar to other DLBLs, they are categorized as DLBL, not as ALCL. An exception is the extremely rare ALK-positive large B-cell lymphoma, which is usually negative for CD30.

Lymphoblastic lymphoma is almost always positive for Tdt, a highly specific and sensitive marker of lymphoblasts that is not present in other lymphomas or reactive lymphocytes in a lymph node. With an aspiration of a mediastinal mass, however, the differential diagnosis includes *thymoma*, particularly type B1 thymoma (lymphocyte-rich thymoma), in which the neoplastic epithelial cells are frequently inconspicuous. Not only are the lymphocytes of a B1 thymoma Tdt positive, like the cells of lymphoblastic lymphoma, but also the two cell types—B1 thymoma lymphocytes and lymphoblasts—are often morphologically indistinguishable.^{124,140} Flow cytometry can be helpful in this distinction, because T-cell (and other antigen) expression patterns are different,¹²⁴ and immunostaining for keratin should reveal the obscured thymic epithelial cells.

The diagnosis of *BL* is based on a combination of clinical, morphologic,

immunophenotypic, and cytogenetic features. The endemic, sporadic, and HIV-associated forms have characteristic clinical presentations: Extranodal sites are involved in the endemic (jaw/orbit) and sporadic (abdomen) forms, as well as the immunodeficiency-associated forms. BLs express B-cell antigens as well as CD10 and bcl-6 and are negative for bcl-2 and Tdt (the latter two antibodies aiding in exclusion of DLBL and lymphoblastic lymphoma, respectively). The morphologic features (cellular uniformity, intermediate cell size, round nuclei, clumped chromatin, small nucleoli, high mitotic rate, apoptosis, tingible-body macrophages) are highly characteristic. If any morphologic features of BL are present, or the Ki67 index is high, immunohistochemistry for nuclear c-myc^{[117,118](#)} with confirmatory FISH for a c-myc translocation may be helpful, albeit nonspecific, as these studies will detect both BL and double hit lymphoma (with the latter diagnosis confirmed by followup FISH testing for IgH-bcl-2 translocation).^{[114,115](#)}

PTLDs occur only in solid organ or bone marrow transplant recipients. In such patients, this diagnosis should always be considered. In situ hybridization for EBER is very helpful: A positive result for EBER in a majority of the lesional cells provides strong support for the diagnosis of PTLD, although a negative result does not exclude this diagnosis, as about 20% are EBV-negative.

Myeloid sarcomas, tumors of immature myeloid cells that occur in bones or extramedullary locations, may mimic non-Hodgkin lymphoma ([Fig. 12.29](#)).^{[141,142](#)} These tumors may be identifiable on routine lymphoma flow cytometry, because blasts typically form a population with dim CD45. Further immunophenotyping for myeloid antigens (CD13, CD33, CD117) and/or histochemistry for myeloid enzymes (myeloperoxidase, chloroacetate esterase) is, however, essential for final diagnosis. Once a diagnosis of myeloid sarcoma is established, full genetic analysis, by conventional cytogenetics, FISH, and molecular assays, is essential for clinical management, because the genetic profile stratifies the acute myeloid leukemias and myeloid sarcomas into prognostically and therapeutically relevant subgroups.^{[19](#)} A repeat FNA can easily be performed to obtain material for such genetic studies if none was available from the initial specimen.

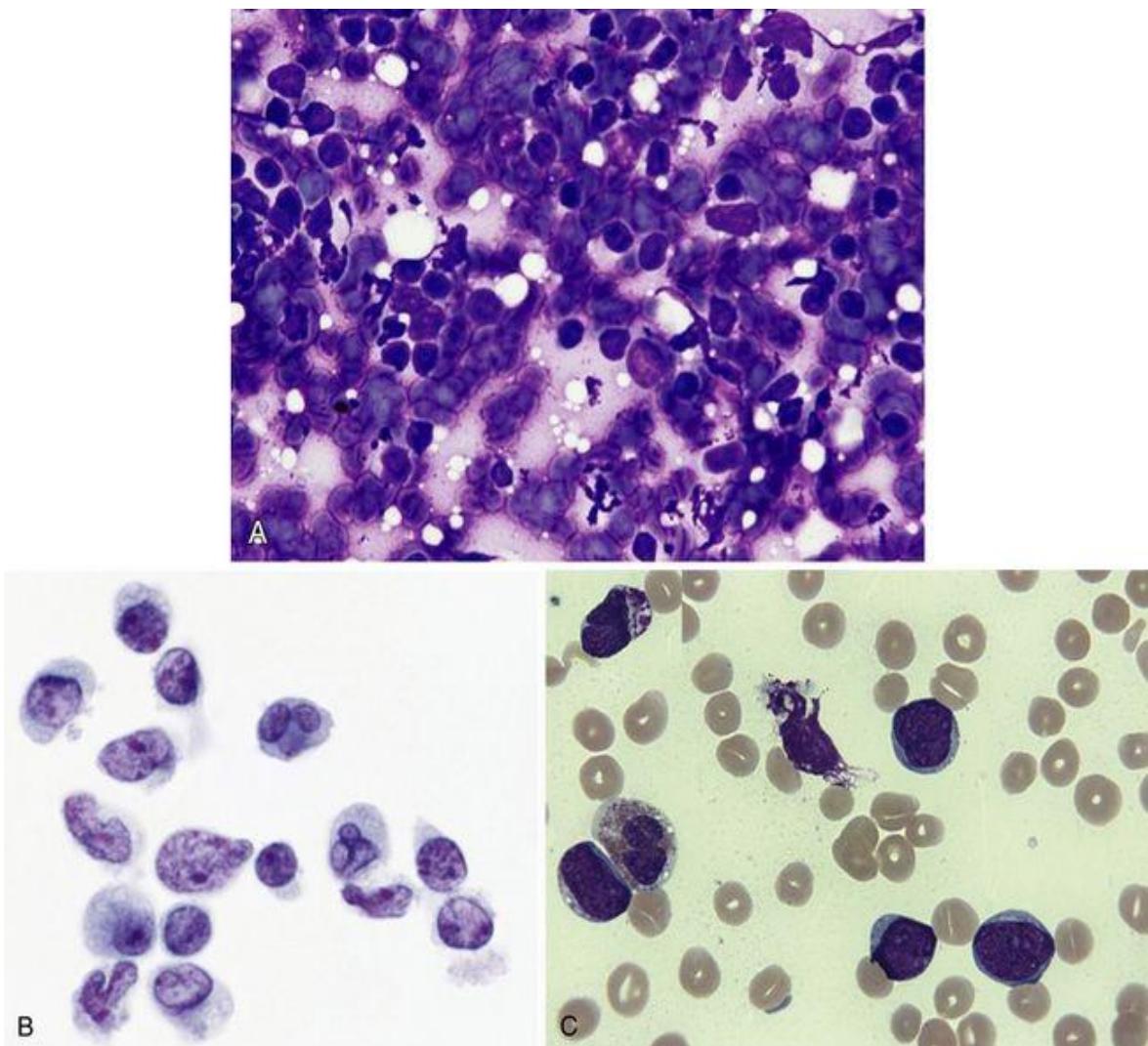


FIGURE 12.29 Myeloid sarcoma.

A, At low and medium magnification, blasts mimic lymphoma cells. Recognition of these cells as myeloid is often precluded when a tumor lacks immature granulocytes (Romanowsky stain). B, Myeloid blasts are also difficult to identify as such with the Papanicolaou stain, even if they show characteristic (but nonspecific) folded nuclear membranes and dispersed chromatin (liquid-based preparation, Papanicolaou stain). C, Blasts are more easily recognized with Romanowsky stains if the characteristic chromatin pattern, nucleoli, cytoplasmic granules (*upper left*), and accompanying granulocytic/eosinophilic forms are all present (Romanowsky stain).

Finally, the differential diagnosis includes the rare *histiocytic and dendritic cell neoplasms* (Table 12.9), which together represent less than 1% of tumors in lymph nodes.¹⁹ They are diagnosed on the basis of their phenotypic resemblance to their presumed cell of origin. Histiocytic sarcoma can be morphologically indistinguishable from a DLBCL or an ALCL. Unlike the latter two tumors, however, it expresses the histiocyte markers CD68 and CD163. Because myeloid

lesions, like myeloid sarcomas, are also positive for CD68, the absence of myeloid markers (e.g., myeloperoxidase) and demonstration of a more mature histologic appearance and immunophenotype (e.g., CD34 negativity) must be established before the diagnosis of histiocytic sarcoma is made.

TABLE 12.9
HISTIOCYTIC AND DENDRITIC CELL NEOPLASMS AND THEIR IDENTIFYING MARKERS

Tumor	Identifying Markers
Histiocytic sarcoma	CD68, CD163
Langerhans cell histiocytosis	S-100, CD1a, langerin
Langerhans cell sarcoma	S-100, CD1a, langerin
Interdigitating dendritic cell sarcoma	S-100
Follicular dendritic cell sarcoma	CD21, CD23, CD35

The family of dendritic cells, whose function is antigen presentation to other cells of the immune system, includes the Langerhans cells (in the skin), interdigitating dendritic cells (in the lymph nodes), and follicular dendritic cells (in lymph node follicles). Both Langerhans cells and interdigitating dendritic cells are positive for S-100 protein, but in other ways they are phenotypically distinct. *Langerhans cell histiocytosis* can affect children or adults and be unifocal or multifocal. Common sites are bones, skin, and lungs. The key to diagnosis is recognizing the Langerhans cell, which has a grooved, indented, or lobulated vesicular nucleus and abundant cytoplasm (imparting an epithelioid appearance), in a background of eosinophils, histiocytes, neutrophils, and lymphocytes. Immunohistochemical positivity for CD1a and langerin confirm the diagnosis. Tumors with overtly malignant cytologic features are *Langerhans cell sarcomas*.¹⁹

Interdigitating dendritic cell sarcoma/tumor is an extremely rare tumor that occurs predominantly in adults.¹⁴³ The cells can have an epithelioid and/or spindle-cell appearance. Like the Langerhans cells, interdigitating dendritic cells are positive for S-100 protein, but they lack CD1a, CD21, CD35, B-and T-cell markers, and keratin.

Because *follicular dendritic sarcomas* usually have a spindle-cell appearance, they are discussed under “Sarcomas” below.

Nonlymphoid Neoplasms

Carcinomas, melanomas, germ cell tumors, and sarcomas can all metastasize to lymph nodes; carcinomas are the most frequent. Because one may have a clinical history of a malignancy, and because the cytomorphology of these tumors is so alien to that of the normal lymph node milieu, metastases to lymph nodes are readily recognized as malignant on FNA. Moreover, the metastatic tumor cells are usually present in abundance relative to the background lymphocytes (if any); rare atypical cells in a sea of lymphocytes (e.g., cells found only by screening) are likely to be benign cells (such as dendritic cells, histiocytes, or large lymphocytes) rather than metastatic tumor cells.

Carcinomas

Small cell carcinoma of the lung ([Fig. 12.30](#)) commonly metastasizes to lymph nodes. Its principle mimic is metastatic *Merkel cell carcinoma* ([Fig. 12.31](#)), which is easily distinguished by its CK20 positivity. Large cell carcinomas of the aerodigestive tract, lung, thyroid, breast, kidney, pancreas, and colorectal region also metastasize to the lymph nodes, and in some instances the primary tumor is occult. In such instances, a useful rule of thumb is that primaries below the diaphragm tend to metastasize to the left supraclavicular lymph node,¹⁴⁴ rather than the right, because of the thoracic duct anatomy. Thus, the primary site for a right supraclavicular lymph node metastasis should be sought above the diaphragm. More generally, smears may contain clues that point to a likely primary site.

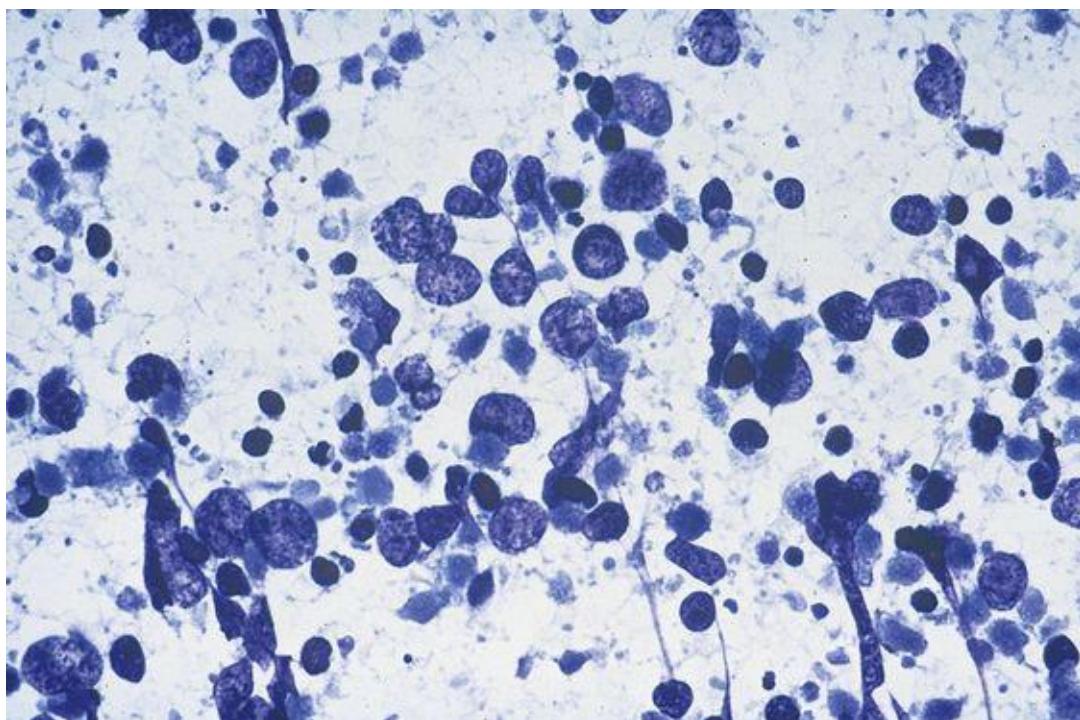


FIGURE 12.30 Small cell carcinoma.

When cell clusters are sparse and there is smearing of cell nuclei in a background of necrotic debris, the smears mimic lymphoma. Fragments of cytoplasm from necrotic cell “ghosts” can be misinterpreted as lymphoglandular bodies (Papanicolaou stain).

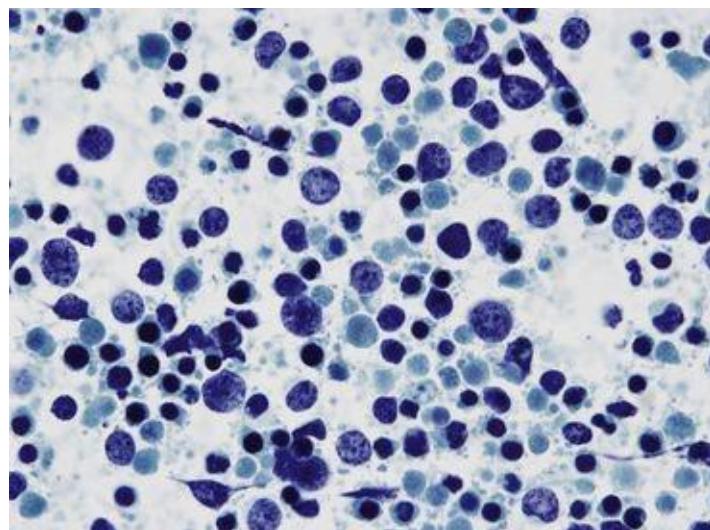


FIGURE 12.31 Merkel cell carcinoma.

Meager cytoplasm and round nuclei with finely granular chromatin instead of nucleoli characterize these malignant cells. Smear background shows fragments of cells in various stages of necrosis that can be confused for large lymphoglandular bodies (Papanicolaou stain).



Cytomorphology of large cell carcinomas

- cells predominantly in clusters
- no lymphoglandular bodies
- abundant necrosis: favor colorectal and lung
- signet ring cells: favor gastric and breast carcinoma
- abundant clear cytoplasm: favor renal cell carcinoma and ovarian carcinoma
- intranuclear inclusions: favor papillary thyroid carcinoma and melanoma

Some aspirates from metastatic *squamous cell carcinomas (SQCs)* with cystic degeneration contain only necrotic parakeratotic cells and amorphous debris, and thus imitate a branchial cleft cyst or epidermal inclusion cyst. In the absence of convincingly malignant cells, smudgy nuclear hyperchromasia and pyknosis should prompt the diagnosis “suspicious for SQC.”

Nasopharyngeal carcinoma (NPC) commonly manifests itself, like lymphoma, as an enlarged cervical lymph node.



Cytomorphology of nasopharyngeal carcinoma

- clusters of undifferentiated large cells
- large nuclei with pale chromatin
- ± prominent nucleolus
- moderate amount of cytoplasm
- lymphocytes, often commingled with epithelial cells
- lymphoglandular bodies

An overwhelming population of lymphocytes can sometimes obscure the clusters of malignant epithelial cells of metastatic NPC, which then resemble dendritic-lymphocytic aggregates¹⁴⁵ ([Fig. 12.32](#)). The malignant cells are not keratinized, thus the cytoplasm is thin and transparent in Papanicolaou-stained smears. In lymph node samples with few metastatic NPC cells, the rare large malignant cells with their prominent nucleoli can be mistaken for the Reed-

Sternberg cells of Hodgkin lymphoma. Immunostains can be very helpful. The cells of NPC are cytokeratin-positive and CD45-negative. Virtually all NPCs are positive for EBV DNA or RNA ([Fig. 12.32 inset](#)). Similarly, metastatic *human papillomavirus (HPV)-associated squamous cell carcinoma* (often from an occult primary in the base of tongue, tonsil, or oropharynx) may have morphologically subtle (i.e., nonkeratinizing) cells on lymph node FNA, with obscuring lymphoid cells also present. Awareness of this entity and use of keratin and p16 immunostains and HPV in situ hybridization testing should resolve any difficulty.

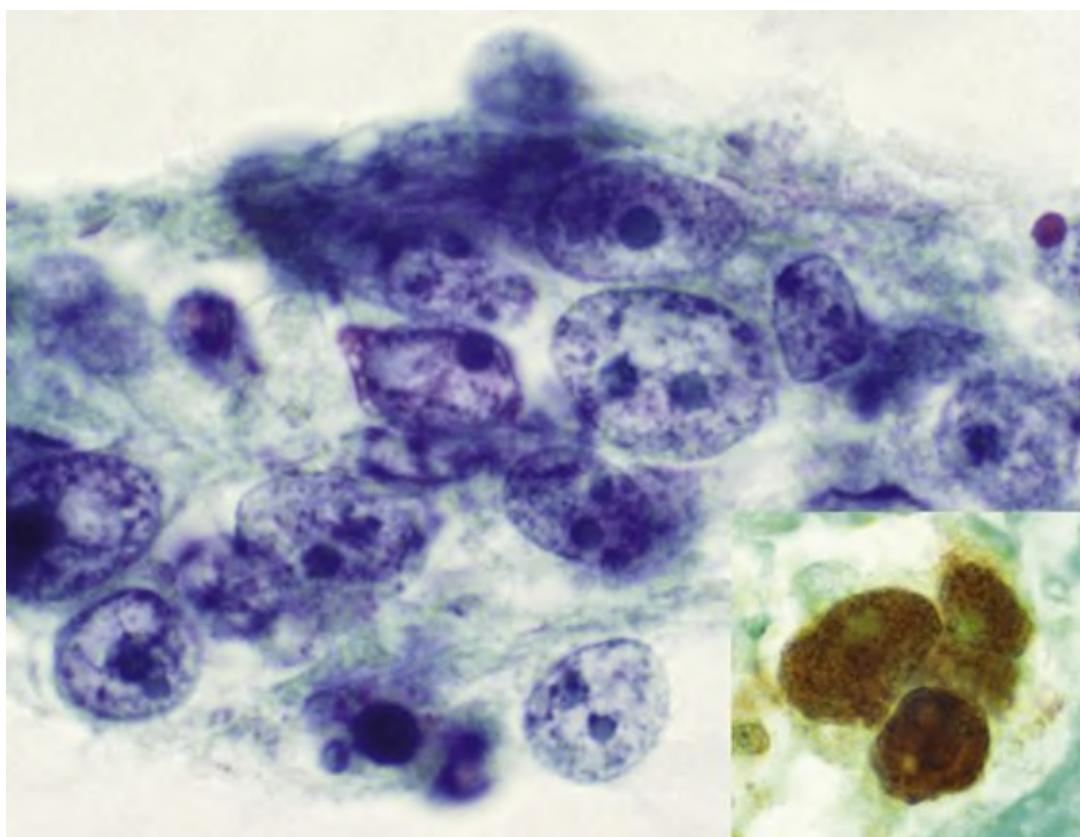


FIGURE 12.32 Nasopharyngeal (undifferentiated) carcinoma.

This loose but definite cluster of epithelial cells was almost obscured by a large number of small round lymphocytes (Papanicolaou stain). The malignant cells show nuclear staining for Epstein-Barr virus (EBV) encoded ribonucleic acids (RNAs) (EBER) by in situ hybridization (*inset*).

Malignant Melanoma

Malignant melanoma is aptly named *the great masquerader*. Lymph nodes are

among the most commonly aspirated metastatic sites.¹⁴⁶



Cytomorphology of melanoma

- dispersed single cells and loose clusters
- epithelioid, spindle, and/or pleomorphic shapes
- nuclei eccentrically placed, commonly binucleated
- nuclear inclusions
- single small to large nucleoli
- cytoplasm: melanin pigment variable, vacuoles variable
- no lymphoglandular bodies

Melanin pigment is seen in less than 50% of aspirate smears.¹⁴⁶ The differential diagnosis of amelanotic malignant melanoma includes DLBL, because both show a single-cell pattern of monomorphic large cells. The eccentric nuclear placement (plasmacytoid nuclei), lack of lymphoglandular bodies, and frequent binucleation are clues to the diagnosis of malignant melanoma (Fig. 12.33), as is the presence of abundant tiny vacuoles in the cytoplasm (best visualized on air-dried smears).

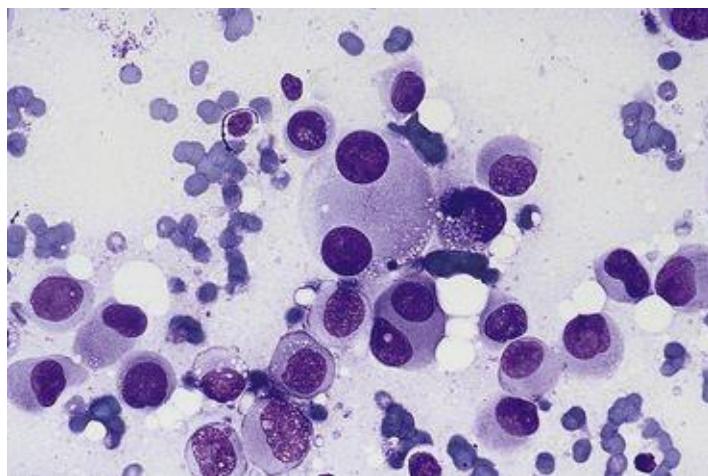


FIGURE 12.33 Malignant melanoma.

Large nonpigmented cells are distributed in a noncohesive pattern. Tiny cytoplasmic bubbles (best seen with Romanowsky stains) and binucleated cells with wide spacing between the mirror-image nuclei are clues to the diagnosis (Romanowsky stain).

Seminoma/Germinoma

Testicular and mediastinal seminomas/germinomas commonly metastasize to deep lymph nodes of the abdomen and chest respectively.



Cytomorphology of seminoma/germinoma

- dispersed large cells
- macronucleolus
- voluminous cytoplasm, peripherally placed vacuoles with large blisterlike quality
- small round lymphocytes and lymphoglandular bodies
- granulomas
- tigroid background

Much of the literature regarding seminoma has focused on the smear background, in which strands of cytoplasm and proteinaceous fluid are arranged in a reticular or linear network termed the *tigroid pattern* ([Fig. 12.34](#)). Although helpful in the proper clinical context, it is not entirely specific for seminoma; it is also seen with clear cell carcinomas. Lymphocytes are common in seminoma, and thus lymphoglandular bodies may be present, so that confusion with a lymphoma is possible. Foci of cell clustering and the marked vacuolization of germ cells are helpful in this regard. FNA smears of other malignant germ cell tumors (e.g., yolk sac tumor and embryonal carcinoma) mimic those of nonsmall cell carcinoma. The tigroid pattern is not evident, nor is a background population of lymphocytes present. Sometimes, one finds hyaline globules in aspirates of yolk sac tumor where they appear as spherical glassy structures. Immunohistochemical positivity for OCT3/4 is relatively specific for seminoma and embryonal carcinoma.^{[147](#)}

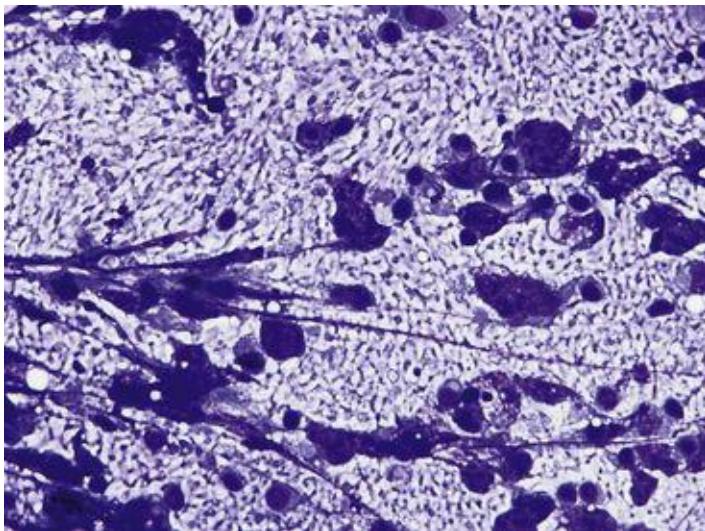


FIGURE 12.34 Seminoma.

Partially vacuolated detached strips of cytoplasm create a striking “tigroid” background. Large germ cells are mixed with small lymphocytes (Romanowsky stain).

Sarcomas

Most sarcomas tend not to metastasize to lymph nodes: Less than 3% of patients with sarcoma develop lymph node metastases.¹⁴⁸ A subset of sarcomas breaks rank with the “anti-lymph node” imperative, however.



Sarcomas that more commonly involve lymph nodes include

- synovial sarcoma
- epithelioid sarcoma
- angiosarcoma
- rhabdomyosarcoma
- Kaposi sarcoma
- follicular dendritic cell sarcoma

One of the more common sarcomas to metastasize to lymph nodes, *synovial sarcoma*, is usually seen on smears as large, loosely cohesive syncytia of small cells with very monomorphic, bland-appearing ovoid nuclei with finely granular chromatin, smooth nuclear outlines, and scant amounts of tapering cytoplasm. Rarely, acinar structures reflecting the glandular epithelial component are found.

Lymph node involvement by *Kaposi sarcoma* occurs in an endemic form, and sporadically in immunodeficient states such as renal transplantation and AIDS. Smears are variably cellular with abundant red cells, and the cytomorphology is that of a nondescript spindle cell sarcoma.¹⁴⁹ Hyaline globules, a typical histologic feature, are usually difficult to find in smears. Definitive diagnosis nearly always requires immunocytochemistry. The spindle cells are positive for CD31, CD34, and (more specifically) HHV8.

Follicular dendritic cell sarcoma is a rare tumor of young to middle-aged adults that arises in lymph nodes as well as in extranodal sites. It is a slow growing tumor with metastatic potential. Smears show loose, flat aggregates and single cells with oval and spindle shapes.¹⁵⁰ Intermediate-sized nuclei have smooth borders and small, inapparent nucleoli ([Fig. 12.35](#)). Background lymphocytes are usually present. The neoplastic cells are positive for one or more of the follicular dendritic cell markers CD21, CD23, and CD35, and are negative for CD45.

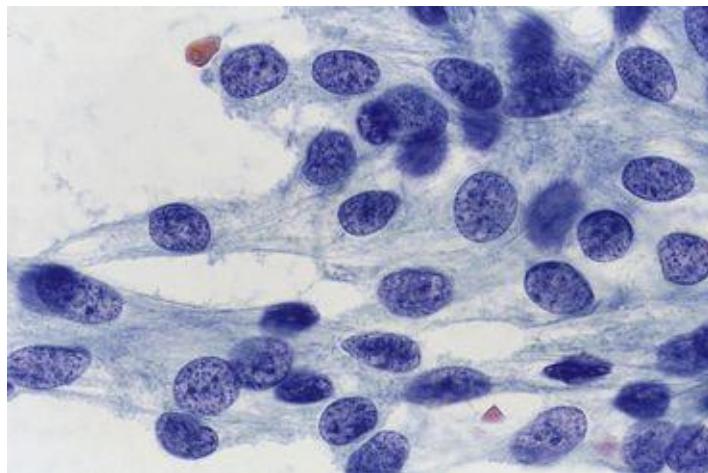


FIGURE 12.35 Follicular dendritic cell sarcoma.
Delicate cytoplasmic processes extend from these clustered cells with smooth, ovoid nuclei (Romanowsky stain).

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CHAPTER 13

Liver

Barbara S. Ducatman

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Fine-needle aspiration (FNA) is a mainstay in the evaluation of liver masses. It is usually performed percutaneously with guidance by computed tomography (CT), ultrasound, or magnetic resonance imaging (MRI), and its principal value is in

the diagnosis of malignancies. (The large core needle biopsy is generally reserved for diffuse liver diseases such as hepatitis and cirrhosis, for which large-scale architectural details are important.) The sensitivity of FNA ranges from 71% to 94% and specificity from 87% to 100%, with an accuracy of 90% to 94%.¹⁻²¹ False-positive diagnoses are very uncommon; hepatic dysplasia, bile duct hamartoma, and focal nodular hyperplasia (FNH) have been misdiagnosed as malignant.^{7,22,23} Cell block preparations facilitate the subtyping of tumors.²⁴

Obtaining brushings during endoscopic retrograde cholangiopancreatography (ERCP) is preferred for diagnosing tumors at the hilum of the liver, particularly cholangiocarcinoma.^{25,26} When brushings are negative or inconclusive, endosonography-guided FNA is used.^{16-18,25,27, 28} In a recently described technique, a sample is obtained using a small forceps biopsy; histology is combined with cytology of tissue squashed between two slides (smash technique).²⁹

FNA has also been used in the routine monitoring of liver transplants for acute cellular rejection.³⁰⁻³⁶ Slides are air-dried and stained with a Romanowsky stain. Lymphocytes are examined for evidence of activation (enlargement, “blast” forms), and hepatocytes for signs of injury (swelling, vacuolization, necrosis) and cholestasis.³⁵ The technique is not useful for diagnosing causes of chronic rejection.³²

Complications of liver FNA are rare and include hemorrhage,^{18,37,38} pain,¹⁸ bile peritonitis, infection,³⁹ and anaphylactic shock (after aspiration of an echinococcal cyst). FNA is associated with a very low risk (0.1% to 0.6%) of tumor seeding^{38,40-45} and recurrence of hepatocellular carcinoma (HCC) after liver transplantation.⁴⁶ Procedure-related death is very uncommon (mortality rate of 0.6%).^{18,47-50}

The Normal Liver

Benign liver cells are often seen in aspirates of liver masses and even, occasionally, in aspirates of adjacent organs such as the pancreas, right kidney, right adrenal gland, and the right lower lobe of the lung, when the liver is penetrated en route to the target organ. The components of a normal liver aspirate commonly include hepatocytes, bile duct epithelium, Kupffer cells, and sheets of mesothelial cells.



Cytomorphology of hepatocytes ([Fig.13.1A and B](#))

- large polygonal cells
- isolated cells, thin ribbons (trabeculae), or large tissue fragments
- centrally placed, round to oval, and variably sized nuclei
- binucleation common
- prominent nucleolus
- intranuclear pseudoinclusions
- abundant granular cytoplasm
- pigment:
 - lipofuscin (common; a normal pigment related to cellular aging): golden with the Papanicolaou stain and green-brown with a Romanowsky-type stain
 - hemosiderin (uncommon; when present in large quantities it suggests a disorder of iron metabolism): dark brown with the Papanicolaou stain and blue with a Romanowsky-type stain.
 - bile (seen in cholestasis): dark green with both Papanicolaou and Romanowsky stains ([Fig. 13.2](#))

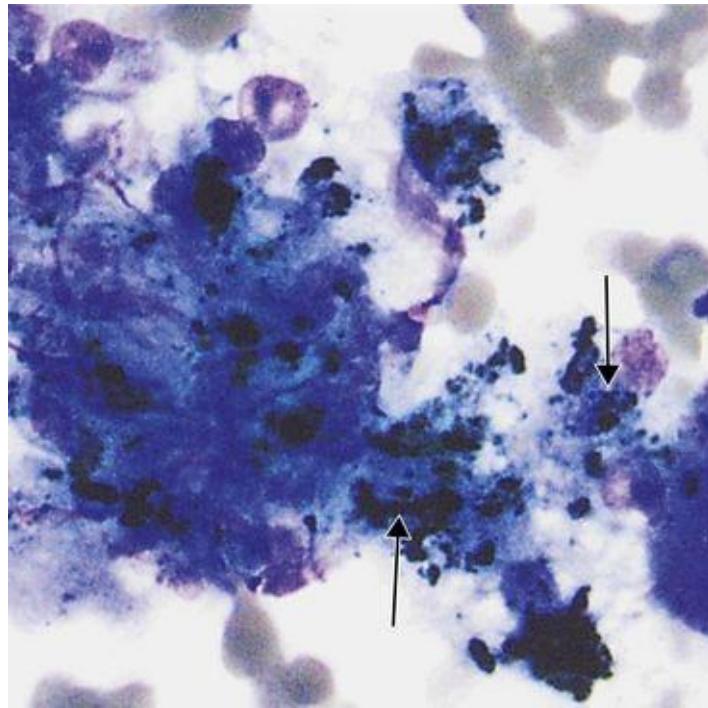


FIGURE 13.2 Bile.

Bile pigment is not usually seen unless there is bile stasis. With the Romanowsky stains, bile pigment is a dense, dark greenish-black pigment in the cytoplasm of hepatocytes (*arrows*). This preparation is from a patient with cirrhosis (Romanowsky stain).



Cytomorphology of bile duct epithelium

- cohesive flat sheets
- cuboidal cells smaller than hepatocytes
- evenly spaced nuclei (“honeycomb” appearance)



Cytomorphology of Kupffer cells

- resemble macrophages
- vacuolated cytoplasm and may contain pigment, most commonly hemosiderin



Differential diagnosis of normal liver cells

- hepatic adenoma
- focal nodular hyperplasia
- regenerative nodule in cirrhosis
- nodular regenerative hyperplasia
- steatosis

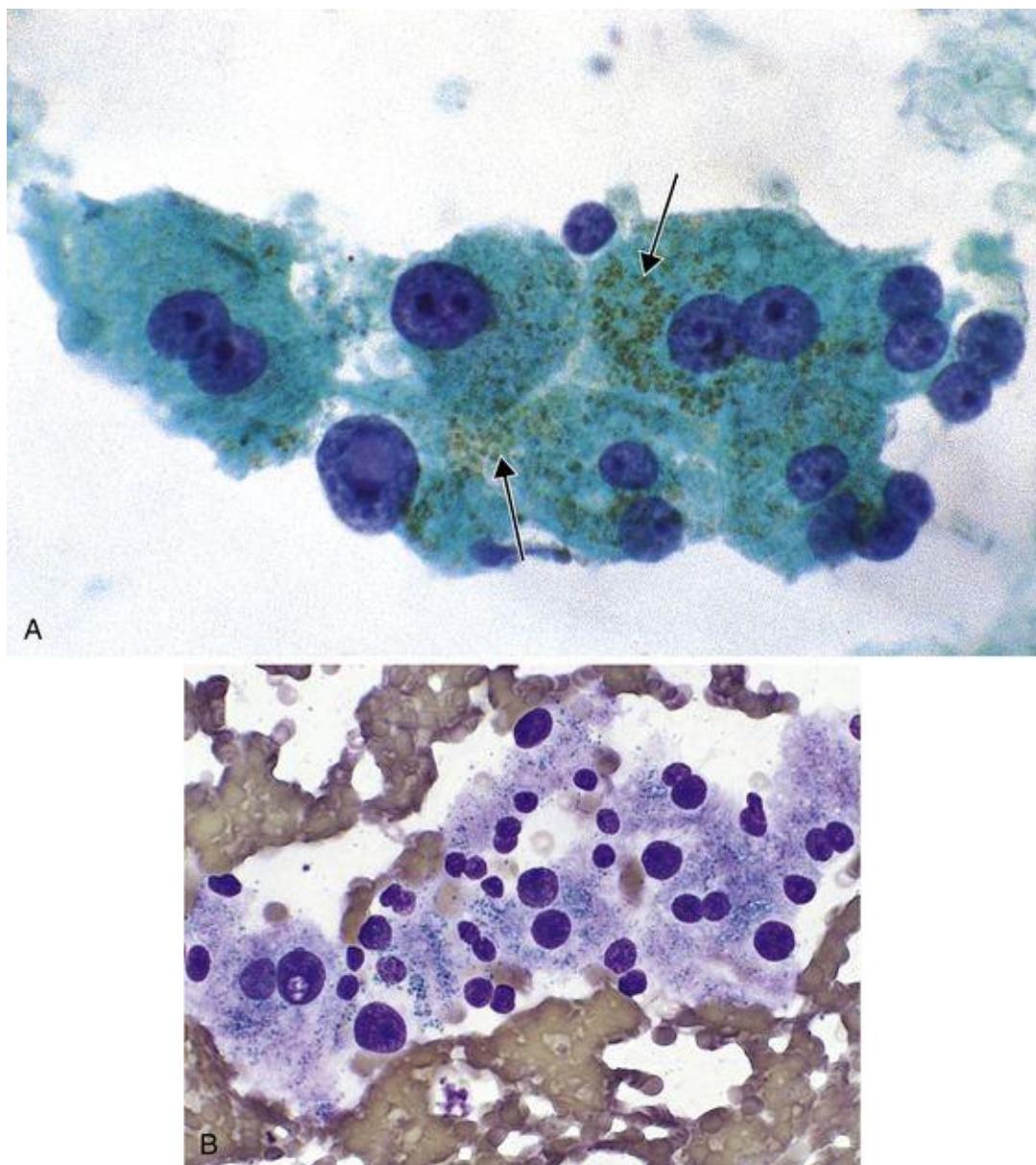


FIGURE 13.1 Normal hepatocytes.

A, Hepatocytes have abundant granular cytoplasm, a round and regular nucleus (or two), and a prominent nucleolus. They are arranged as a ribbon of two cells' width. Lipofuscin, the normal “wear and tear” pigment, is present (arrow) (Papanicolaou stain). B, Lipofuscin appears greenish with the Romanowsky stains. A pseudoinclusion is present (Romanowsky stain).

The first four conditions listed in the differential diagnosis have characteristic clinical and/or imaging findings and should be considered when an FNA of the liver is composed entirely of normal liver cells (predominantly hepatocytes) and the patient has a focal lesion. The possibility of sampling error must also be considered. In steatosis (fatty metamorphosis), many hepatocytes have large cytoplasmic vacuoles filled with lipid ([Fig. 13.3](#)). This alteration of the liver is

seen with toxic-metabolic injuries such as those caused by alcohol consumption, diabetes, obesity, drugs (e.g., methotrexate), total parenteral nutrition, post jejunoileal bypass surgery, and hepatitis C. Although this is usually a diffuse liver abnormality, some cases of steatosis show areas of low attenuation on CT scan that suggest an infiltrative tumor. Steatosis can be a component of focal nodular hyperplasia and hepatic adenoma; thus correlation with imaging and clinical findings is essential.

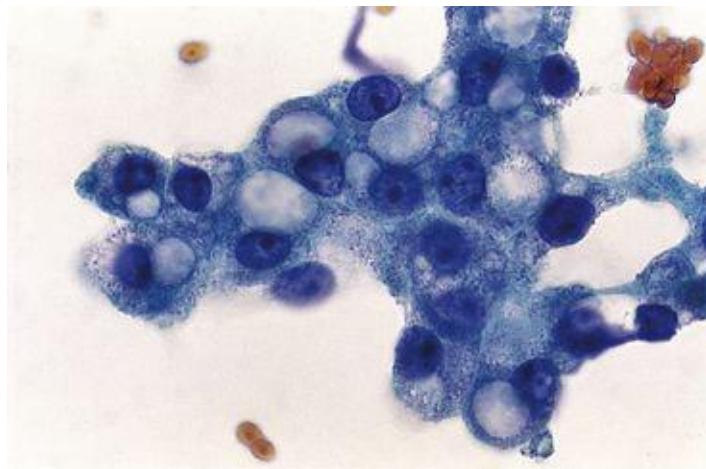


FIGURE 13.3 Steatosis.

The patient had a longstanding history of alcohol abuse, and diffuse nodularity was noted on computed tomography (CT). Fine-needle aspiration (FNA) demonstrated abundant fatty changes (Papanicolaou stain).

Infections

Hepatic Abscess

Hepatic abscesses can be bacterial, fungal, or amebic, the last principally due to *Entamoeba histolytica*. Bacterial abscesses result from ascending cholangitis and sepsis. The most common organisms are streptococci, staphylococci, and enteric bacteria. Fungal abscesses are most common in immunocompromised patients. *Candida* species are the most common pathogens.



Cytomorphology of bacterial and fungal abscesses

- abundant polymorphonuclear leukocytes and necrotic debris
- may see bacteria and fungi with routine stains, but special stains (i.e., silver or Gram) and culture are helpful

Amebic abscesses are rare in the United States but common in other countries, particularly developing nations. They are caused by the protozoan *Entamoeba histolytica* and are usually a sequela of colonic infection.



Cytomorphology of amebic abscess

- “anchovy paste” (macroscopic appearance)
- necrotic debris
- little if any acute inflammation
- amebic trophozoites resemble histiocytes:
 - round nucleus
 - peripheral chromatin margination
 - abundant ovoid cytoplasm containing ingested red blood cells

A careful search for malignant cells is essential whenever an aspirate shows abundant necrotic debris or acute inflammation, or both. Microbiologic culture helps to confirm a presumptive diagnosis of infection. For this reason, an on-site

evaluation is crucial to ensure that aspirated material is sent for culture.

Echinococcal Cyst (Hydatid Cyst)

The larval form of *Echinococcus granulosus*, a dog tapeworm, causes infection in a variety of organs in humans, chiefly the liver. In one series of hepatic cysts 4 cm or larger in diameter, 10% were echinococcal cysts.⁵¹ The disease is endemic in the countries bordering the Mediterranean and Baltic seas, in South America, and in Australia and New Zealand. It is also seen in North America. Infection can be asymptomatic. Imaging studies reveal a solitary cyst, often with a fluid level. An outer, acellular, laminated membrane lines the cyst. The internal, germinal layer gives rise to daughter cysts, each of which contains scolices with numerous hooklets. Hooklets resist degeneration, but scolices can be lost in longstanding cysts with degeneration.



Cytomorphology of echinococcal cyst

- fragments of the laminated membrane ([Fig. 13.4](#))

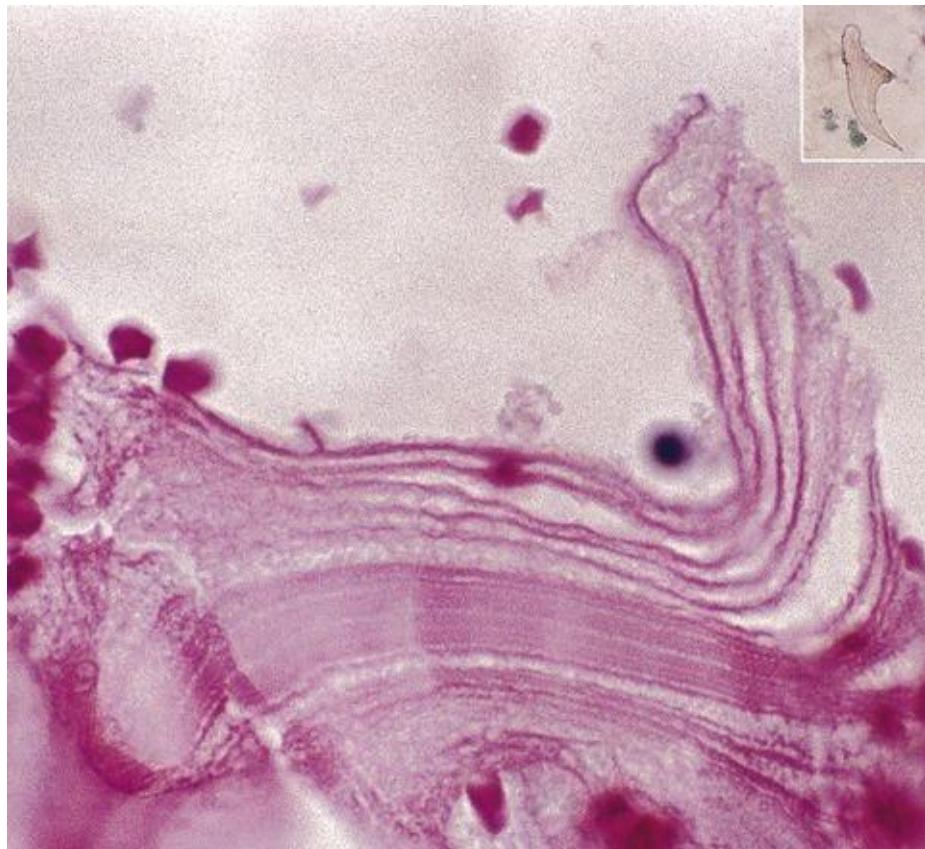


FIGURE 13.4 Echinococcal cyst.

Fragments of the laminated membrane of this organism appear as parallel, acellular striations, which are diagnostic (hematoxylin-eosin [H & E]-stained cell block). *Inset:* Some cysts contain mostly acellular debris without scolices. A diligent search uncovers the pale, daggerlike hooklets, which do not stain with the Papanicolaou stain (Papanicolaou stain).

- scolices
- hooklets (see [Fig. 13.4 inset](#))

Although anaphylactic shock has been reported as an occasional complication of FNA,⁴⁷ its incidence has not been established, and successful aspiration without serious complications has been observed.^{34,52–54} Other parasitic diseases that manifest with a liver mass include schistosomiasis, clonorchiasis, and visceral larva migrans.⁵⁵

Other Infections

Infection by the hepatotropic viruses (hepatitis A, B, and C viruses) is generally not evaluated by FNA, because the diagnostic changes of acute, subacute, and

chronic hepatitis caused by these viruses are primarily architectural and not cytologic. Immunocompromised patients can develop hepatitis caused by cytomegalovirus (CMV) and herpesviruses, but such cases also result in diffuse liver disease and are rarely diagnosed by FNA.

Granulomas are seen in miliary tuberculosis, sarcoidosis, primary biliary cirrhosis, Hodgkin lymphoma, and drug reactions. Cytologic features are described elsewhere (see [Figs. 2.15](#) and [2.4](#)). The differential diagnosis of granulomatous inflammation includes a hepatic angiomyolipoma (AML) because the myoid cells of an AML have a syncytium-like appearance similar to that of epithelioid histiocytes. The presence of adipocytes and extramedullary hematopoiesis are clues to the diagnosis of AML.

Benign Lesions

Solitary Cysts

Solitary, nonparasitic cysts of the liver include the simple unilocular cyst, which is lined by cuboidal or columnar epithelium that resembles biliary epithelium. The aspirated fluid is typically hypocellular and nondiagnostic.

The ciliated foregut cyst is also solitary and unilocular and is lined by respiratory-type epithelium.



Cytomorphology of ciliated foregut cyst

- ciliated columnar cells
- mucus cells



Differential diagnosis of ciliated foregut cyst

- bile duct cystadenoma
- bile duct cystadenocarcinoma

Bile duct cystadenomas and cystadenocarcinomas are multilocular tumors lined by mucinous epithelium and filled with watery or viscous fluid. The cystadenoma is lined by a single layer of benign mucin-producing cells, whereas the cystadenocarcinoma shows nuclear pleomorphism and stromal infiltration; the latter cannot be evaluated on cytologic preparations.

Cirrhosis

Cirrhosis, whether caused by alcoholic hepatitis, viral hepatitis, or other diseases, results in a disruption of normal liver architecture, with bands of fibrosis separating nodules of regenerating hepatocytes. Some regenerative nodules are larger than others and, on imaging studies, raise the specter of malignancy, primarily that of hepatocellular carcinoma (HCC), because patients with cirrhosis are at increased risk for developing HCC. Focal lesions in the setting of cirrhosis are often biopsied by FNA, although accuracy may be higher

with needle biopsy or a combination of the two.²



Cytomorphology of cirrhosis

- normal-appearing hepatocytes, sometimes with steatosis (see [Fig. 13.3](#))
- focal atypia in some cases
 - marked variation in nuclear size
 - prominent nucleolus
 - binucleation



Differential diagnosis of cirrhosis

- hepatocellular carcinoma
- hepatic adenoma
- focal nodular hyperplasia
- normal liver
- nodular regenerative hyperplasia

The distinction between a cirrhotic nodule and a well-differentiated HCC can be problematic. When hepatocyte atypia is due to cirrhosis, there is a wide morphologic spectrum ranging from normal hepatocytes to markedly atypical ones, whereas an HCC is generally more monomorphic. Other features of HCC, rare or absent in cirrhosis, include an increased nuclear-to-cytoplasmic ratio, a thickened trabecular arrangement of hepatocytes surrounded by endothelial cells, acinar architecture, and atypical naked nuclei. Larger tissue fragments composed of normal hepatocytes are characteristic of cirrhosis, whereas trabeculae of variable thickness are seen in HCC.⁵⁶ The hepatocyte dysplasia of cirrhosis features loose hepatocyte clusters with minimal or no endothelial wrapping, cellular enlargement with preservation of a normal nuclear-to-cytoplasmic ratio, and cytoplasmic basophilia.⁵⁷ A combination of features permits the diagnosis in most cases of HCC.^{23,58,59}

Specimens composed of hepatocytes without atypia are cytologically indistinguishable from the remainder of the conditions listed in the differential diagnosis; the diagnosis requires clinical-pathologic correlation. Nodular regenerative hyperplasia is a poorly understood condition in which small nodules of regenerating liver are scattered diffusely throughout the liver. Unlike

cirrhosis, these nodules are not separated by bands of fibrosis, but patients often have portal hypertension and ascites.

Focal Nodular Hyperplasia

FNH is a benign liver lesion that possibly results from focal vasculopathy. It usually manifests as a solitary mass or, less commonly, several masses. Most patients are women in their third or fourth decade. The nodules usually contain a central scar, which can be appreciated on imaging studies. Histologic examination reveals nodules of hepatocytes that are separated by radiating fibrous septae that contain bile ductules.



Cytomorphology of focal nodular hyperplasia

- hepatocytes without significant atypia ([Fig. 13.5A](#))

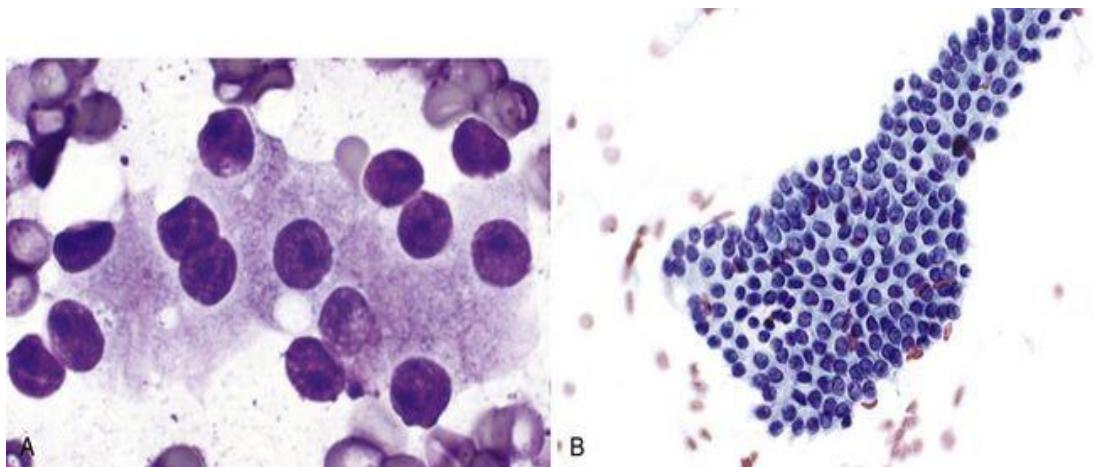


FIGURE 13.5 Focal nodular hyperplasia (FNH).

A, Findings include benign hepatocytes, indistinguishable from those of normal liver (Romanowsky stain). B, In some cases, there may be numerous benign ductal cells. This constellation of findings is nonspecific, however (Papanicolaou stain).

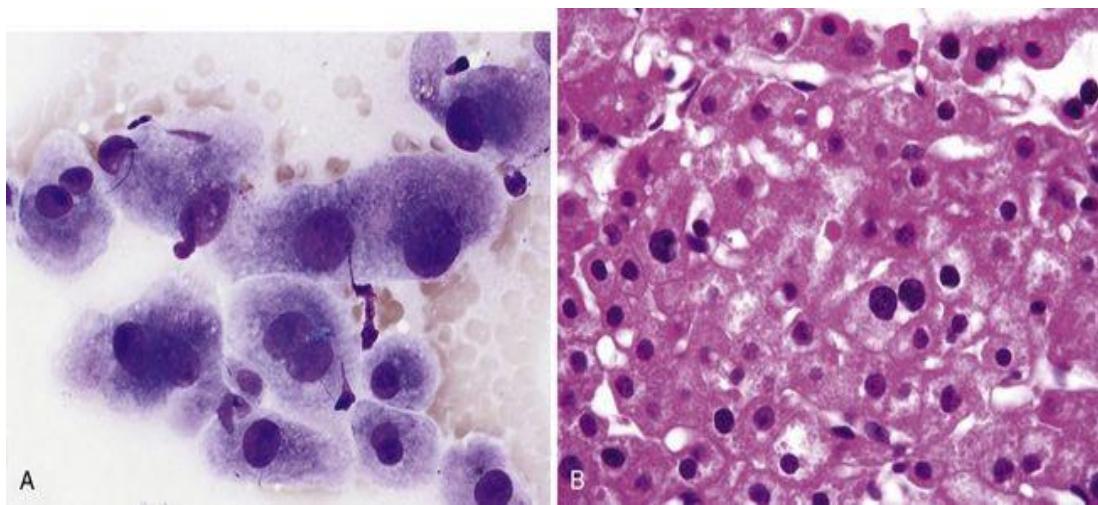


FIGURE 13.6 Hepatic adenoma.

Anisonucleosis and binucleation are no different from that seen with normal hepatocytes. (A, smear, Romanowsky stain, B, cell block, hematoxylin-eosin [H & E] stain).

- normal trabeculae (two cells thick)
- steatosis (some cases)
- bile ductular cells ([Fig. 13.5B](#))



Differential diagnosis of focal nodular hyperplasia

- hepatic adenoma
- regenerating nodule in cirrhosis
- normal liver

These conditions are cytologically indistinguishable, and radiologic and clinical correlation is necessary. FNHs express the usual markers of hepatocellular differentiation, including HepPar1, thyroid transcription factor-1 (TTF-1) (cytoplasmic staining), arginase-1 (ARG-1), and CAM5.2, and canalicular staining for polyclonal carcinoembryonic antigen (CEA). A core biopsy can help by suggesting or confirming the diagnosis of FNH if bile ductules are present within the lesion: Bile ductules rule out an adenoma. Thus if FNH is suspected on the basis of imaging findings, and the rapid on-site assessment shows only normal hepatocytes and/or ductal cells, the cytologist can recommend a concurrent core biopsy.

The distinction between FNH and hepatic adenoma is a difficult but important one, inasmuch as hepatic adenomas, unlike FNH, have an increased risk of life-threatening hemorrhage and are linked to HCC. Molecular studies have

confirmed the distinction between FNH and hepatic adenoma and have identified at least three different subtypes of hepatic adenoma.^{60,61} Data from molecular studies have led to the application of selected immunohistochemical markers (glutamine synthetase, liver fatty acid-binding protein, serum amyloid A or C-reactive protein, and β -catenin) in the distinction between FNH and hepatic adenoma in needle biopsies.⁶² For example, glutamine synthetase has a characteristic maplike staining pattern in FNH. They have not been widely applied to FNAs, however, and interpretation of these markers, especially in limited samples, can be problematic and is best deferred to specialists with experience in liver biopsies.

Hepatic Adenoma

Hepatic adenomas are rare benign neoplasms usually encountered in women under the age of 30, especially those with a history of long-term use of oral contraceptives. Patients often present with abdominal pain.⁶³ These lesions may rupture through the liver capsule, resulting in intraperitoneal hemorrhage, and they have been linked to HCC. Malignant transformation to HCC is rare but well documented.⁶¹ Histologically, they lack portal triads and are composed exclusively of hepatocytes. So-called naked arterioles—arterioles surrounded by scant connective tissue without bile ducts—are characteristic.



Cytomorphology of hepatic adenoma (Fig. 13.6A and B)

- normal-appearing hepatocytes
- steatosis (some cases)
- normal trabeculae (two cells thick)
- “naked arterioles” (in cell blocks and core biopsy specimens)



Differential diagnosis of hepatic adenoma

- normal liver
- focal nodular hyperplasia
- regenerating nodule in cirrhosis
- hepatocellular carcinoma

Hepatic adenomas express the usual markers of hepatocellular differentiation, including HepPar1, TTF-1 (cytoplasmic staining), ARG-1, and CAM5.2, and canalicular staining for polyclonal CEA. With regard to the distinction between FNH and hepatic adenoma, the presence of bile duct epithelium in the lesion helps to exclude hepatic adenoma,⁶⁴ but in practice clinical and radiologic correlation is necessary. Molecular studies have confirmed the distinction between FNH and hepatic adenoma and have identified several different subtypes of hepatic adenoma.^{60,61} If needed, immunohistochemical markers (glutamine synthetase, liver fatty acid binding protein, serum amyloid A or C-reactive protein, and β-catenin) can aid in the distinction between FNH and hepatic adenoma in needle biopsy specimens,⁶² but interpretation is best deferred to specialists with experience in liver biopsies.

The cells of HCC have a higher nuclear-to-cytoplasmic ratio and more architectural derangement than is seen in adenomas.

Bile Duct Hamartoma and Adenoma

The bile duct hamartoma (von Meyenberg complex) is characterized by multiple small nodules dispersed throughout the liver and composed of haphazardly arranged bile ductules and fibrous stroma. The bile duct adenoma is usually a solitary subcapsular nodule less than 1 cm in diameter.



Cytomorphology of bile duct hamartoma and adenoma

- hypocellular specimens
- benign ductal cells in tubules and cohesive sheets
- benign hepatocytes

Hemangioma

The hemangioma is the most common benign tumor of the liver. Histologically, dilated vascular spaces are lined by benign endothelial cells. Most hemangiomas are recognized radiologically with sufficient accuracy and an FNA is not necessary. Occasional hemangiomas have unusual imaging features, however, and an FNA is performed to exclude a more significant lesion. If the lesion is well sampled, cytologic findings are characteristic. Some of these tumors, however, show only blood or benign hepatocytes.⁶⁵ If a spindle cell lesion of the

liver is encountered, it is most likely a hemangioma. The next most likely lesions are metastatic gastrointestinal stromal tumor (GIST), metastatic leiomyosarcoma, and granulomatous hepatitis.⁶⁶ Epithelioid hemangioendothelioma is rare in the liver, with larger, epithelioid cells and greater cytologic atypia and dyshesion.⁶⁷



Cytomorphology of hemangioma

- blood only (some cases)
- hepatocytes only (some cases)
- three-dimensional arcades of bland elongated spindle cells
- compact, dense coils of spindle cells ([Fig. 13.7A](#))

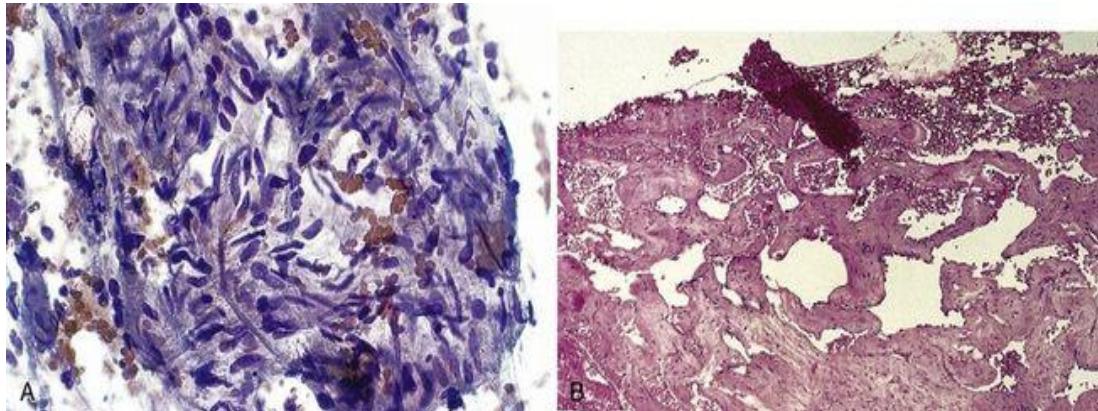


FIGURE 13.7 Hemangioma.

A, Smears show occasional tangles of spindle-shaped cells that are difficult to classify (Papanicolaou stain). B, The diagnosis is easier with cell block sections, where the vascular architecture is more readily apparent (hematoxylin-eosin [H & E] stain).

- rare isolated spindle-shaped cells
- vascular channels lined by endothelial cells (cell block sections) ([Fig. 13.7B](#)).

Angiomyolipoma

AML is the most common benign tumor of the kidney. It is seen less often in other locations but does occur in the liver, mediastinum, heart, spermatic cord, vaginal wall, fallopian tube, oral cavity, pharynx, nasal cavity, skin, and parotid gland. Of the extrarenal sites, the liver is the most common; to date, more than

100 hepatic AMLs have been reported worldwide.⁶⁸

Hepatic AML shares many clinical features with its renal counterpart. The average age at diagnosis is about 50 years. Although 60% of patients are symptomatic, many tumors, particularly small ones, are diagnosed incidentally by imaging studies. Hepatic AMLs can rupture spontaneously, just like their renal counterparts, but this is uncommon. Some patients with a hepatic AML have tuberous sclerosis as do some patients with a renal AML.

AMLs of the liver range in size from 0.3 to 36 cm in diameter (mean 9 cm) and are well circumscribed. Hemorrhage, necrosis, and calcification are rare. As with AMLs of the kidney, many are not biopsied, because their high fat content permits an accurate diagnosis by CT or MRI studies. Only the radiologically atypical AMLs (usually due to low fat content) undergo biopsy. One study of 49 sporadic hepatic AMLs found no loss of heterozygosity or microsatellite instability in any of the cases.⁶⁹

The proportions of the components vary considerably from patient to patient. Some tumors are composed predominantly of adipose tissue, whereas others have virtually no fat and are almost exclusively myoid. The myoid cells may cause diagnostic difficulty because they can be epithelioid and markedly pleomorphic with bizarre giant cells, and they may show mitotic activity. In contrast with renal AMLs, extramedullary hematopoiesis is a frequent feature, seen in 40% of cases.



Cytomorphology of hepatic angiomyolipoma

- clusters of epithelioid and/or spindle cells (myoid cells) ([Fig. 13.8](#))

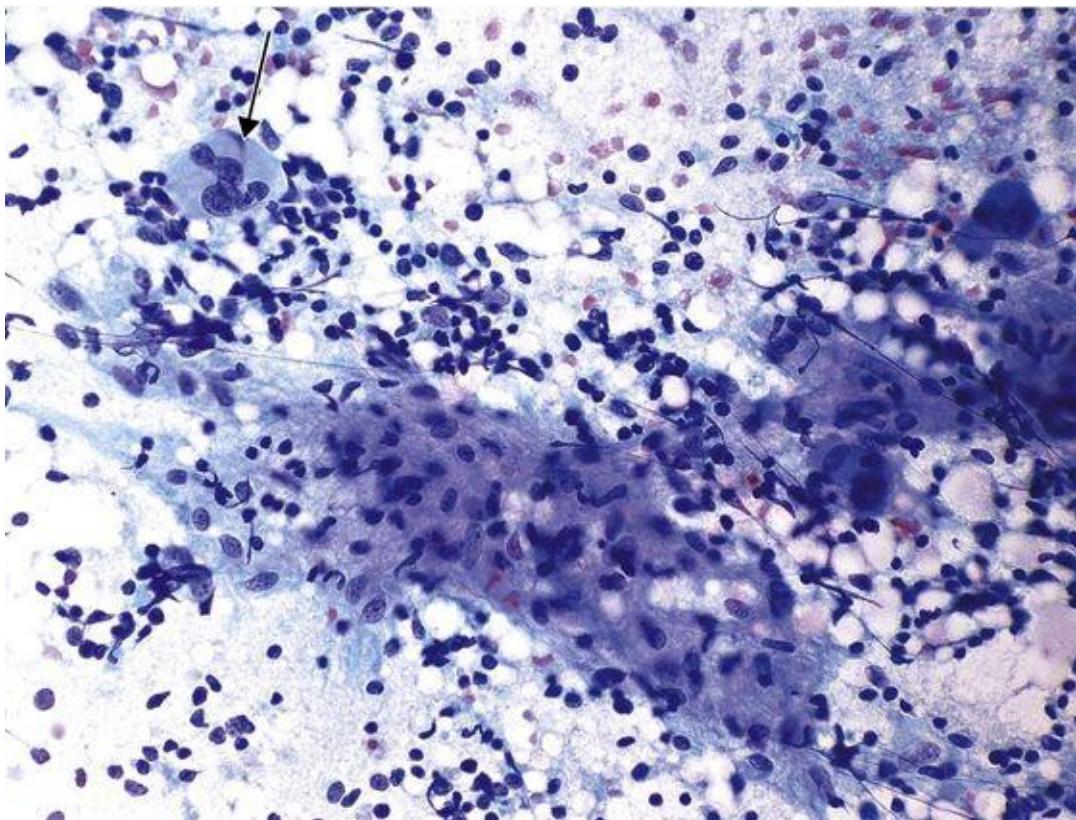


FIGURE 13.8 Angiomyolipoma (AML) of the liver.

The myoid cells are sometimes epithelioid, as in this case, and have indistinct cell borders. Note the prominent component of extramedullary hematopoiesis, including megakaryocytes (Papanicolaou stain).

- fat cells
- blood vessels
- extramedullary hematopoiesis (see [Fig. 13.8](#))

Hepatic AMLs can be diagnosed accurately by FNA.²⁰ The correct diagnosis rests on identifying the triad of fat, vessels, and smooth muscle, but the myoid component is the only specific and diagnostic component of AML. The myoid cells have indistinct cell membranes and abundant granular cytoplasm. Sheets of myoid cells, therefore, have a syncytium-like appearance that resembles that of a granuloma. Adipose tissue is not consistently present.²¹ The positivity of the myoid cells for HMB-45 and Melan-A is a very helpful diagnostic feature (see [Fig. 15.4D](#)).²² Almost one half of AMLs of the liver have extramedullary hematopoiesis, a striking finding that should make one consider the diagnosis of

an AML.



Differential diagnosis of hepatic angiomyolipoma

- hepatocellular carcinoma
- other carcinomas
- granulomatous inflammation
- myelolipoma of the liver
- nodular hematopoiesis
- sarcoma

The differential diagnosis includes hepatocellular carcinoma (HCC). The cells of HCC have distinct cytoplasmic borders, unlike the myoid cells of AML, which have a fibrillar cytoplasm with indistinct cell membranes. Because hematopoietic elements are seen in many hepatic AMLs, the differential diagnosis also includes myelolipoma and nodular hematopoiesis of the liver, but cytologic samples from these lesions lack the myoid cells of AML. Because the myoid cells vary widely in their appearance, they can mimic a variety of spindle and epithelioid cell neoplasms of the liver. Their indistinct cell membranes and arrangement in nodular aggregates may cause them to be mistaken for the epithelioid histiocytes of granulomas, especially when hematopoietic elements are interspersed. Ultimately, the diagnosis rests on showing that the large neoplastic cells are positive for HMB-45 and/or MelanA (Mart-1).

Because a small percentage of hepatic AMLs rupture, a patient who is symptomatic or has a large tumor or does not have a definitive diagnosis may be considered for resection. Nevertheless, the majority of patients are spared surgical resection, and their tumors are monitored by periodic imaging studies.

Malignant Tumors

Hepatocellular Carcinoma

HCC accounts for 90% of all primary cancers of the liver. It is less common in the United States and Western Europe than in Africa and Asia. In the United States, a majority of cases are seen in the setting of cirrhosis. Most patients are over the age of 50, but children and younger adults can be affected. An alpha-fetoprotein (AFP) serum level greater than 4000 ng/mL is highly suggestive of HCC, but elevated titers are not present in all cases.

The tumor can manifest as a solitary nodule, as multiple nodules, or as diffuse liver enlargement. Histologically, HCCs are divided into classical and “special types.”⁶¹ The classical HCC has three architectural patterns that often occur in combination and can be appreciated cytologically, especially with cell block preparations: *trabecular*, *pseudoglandular (acinar)*, and *compact*. In addition, classical HCC has myriad cytologic variants, including pleomorphic cells, clear cells, spindle cells, and fatty change. Bile is prominent in a minority of tumors, and a variety of cytoplasmic inclusions are occasionally encountered (Mallory hyaline bodies, globular hyaline bodies, pale bodies, and ground glass). The special types of HCC include fibrolamellar carcinoma, scirrhouous HCC, undifferentiated carcinoma, lymphoepithelioma-like carcinoma, and sarcomatoid HCC.

There is a wide range of differentiation, from well-differentiated tumors that resemble normal liver, to poorly differentiated ones with marked nuclear pleomorphism and tumor giant cells ([Fig. 13-9A](#) and [B](#)). Because the cytomorphology and differential diagnosis are distinctly different at the two ends of the spectrum of HCC (i.e., well-to-moderately versus poorly differentiated), they are presented separately. HCCs express the usual markers of hepatocellular differentiation, including HepPar1, TTF-1 (cytoplasmic staining), ARG-1, and CAM5.2, and show canalicular staining for polyclonal CEA.

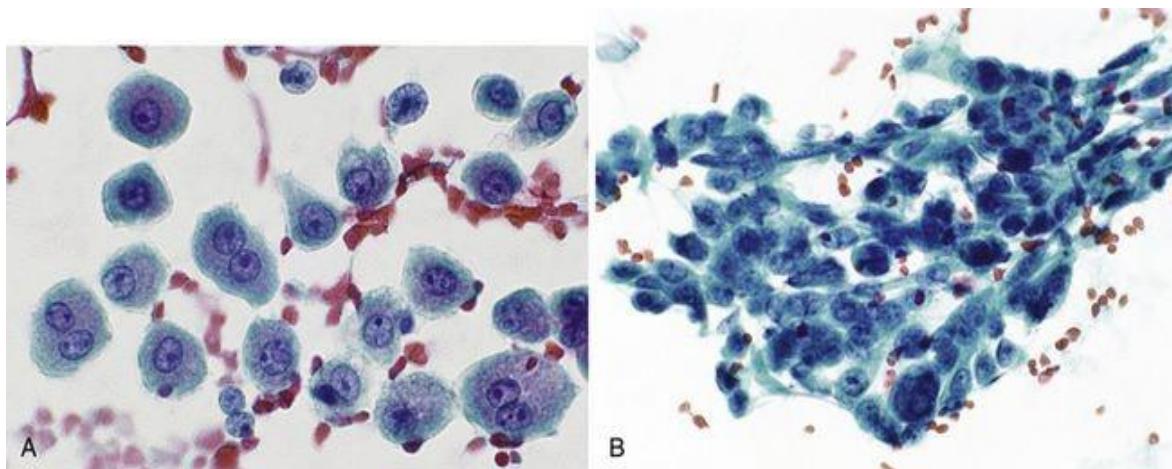


FIGURE 13.9 The spectrum of hepatocellular carcinoma (HCC). *A*, Well-differentiated tumors are clearly of hepatocyte derivation but can be difficult to distinguish from benign hepatocytes (Papanicolaou stain). *B*, Poorly differentiated tumors show little if any hepatocellular differentiation; they resemble poorly differentiated cholangiocarcinomas and metastatic carcinomas (Papanicolaou stain).

● Cytomorphology of well and moderately differentiated hepatocellular carcinoma

- highly cellular smears
- isolated cells and large naked nuclei
- thickened cell cords (trabeculae) wrapped by endothelial cells
- pseudoglandular structures (acini)
- transgressing vessels
- increased nuclear-to-cytoplasmic ratio
- large, round nucleus with prominent nucleolus
- intranuclear pseudoinclusions
- intracellular bile

The cells of well and moderately differentiated HCCs are often recognizably hepatocytic in origin: They are polygonal, with moderate to abundant amounts of granular cytoplasm, and their nuclei are round, centrally placed, with prominent nucleoli and occasional pseudoinclusions. The cells of HCCs are often dispersed as numerous isolated cells (see Fig. 13.9A) or naked nuclei (Fig. 13.10). Thickened trabeculae of neoplastic hepatocytes are a prominent feature of some HCCs. In contrast with normal liver, cirrhotic nodules, focal nodular hyperplasia, and hepatic adenoma, where hepatocytes are predictably arranged in thin ribbons

no more than two or three cells across, the trabeculae in HCC can be markedly thickened ([Fig. 13.11A](#) and [B](#)). Thick trabeculae, often outlined by flattened endothelial cells, are virtually pathognomonic of HCC.^{56,73} Some HCCs have a predominantly acinar pattern: Instead of thickened trabeculae wrapped by endothelial cells, the cords of neoplastic cells form acini. The acinar pattern is more easily recognized on cell block preparations ([Fig. 13.12A](#) and [B](#)). An increased nuclear-to-cytoplasmic ratio is characteristic of HCC (see [Figs. 11A](#) and [12A](#)), although it may not be prominent in some well-differentiated HCCs (see [Fig. 13.9A](#)).^{58,74-79} Cellular monomorphism, nuclear crowding, macronucleoli, multinucleated cells, mitoses, and capillaries traversing neoplastic tissue fragments ([Fig. 13.13](#)) are also characteristic features of HCC; some features are better seen on cell block sections than on smears.^{5,78,80} Irregularly arranged, overlapping cells in sheets are also typical of HCC, in contrast with the evenly spaced arrangement of benign lesions.⁸¹

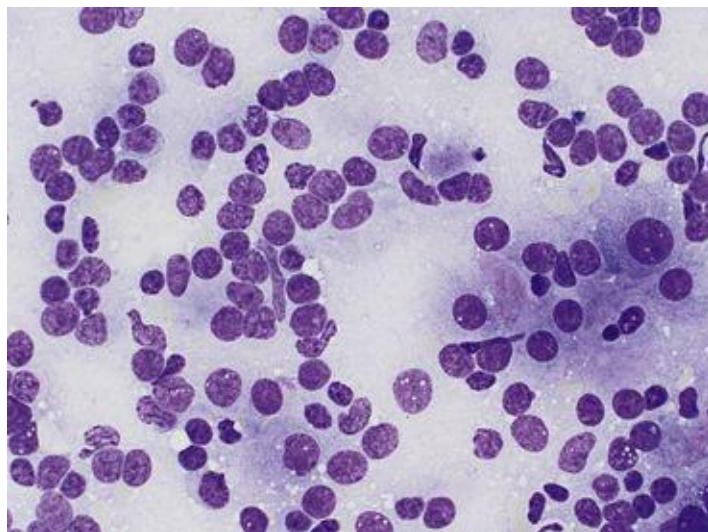


FIGURE 13.10 Hepatocellular carcinoma (HCC).

A markedly cellular preparation with abundant naked nuclei is characteristic of many HCCs (Romanowsky stain).

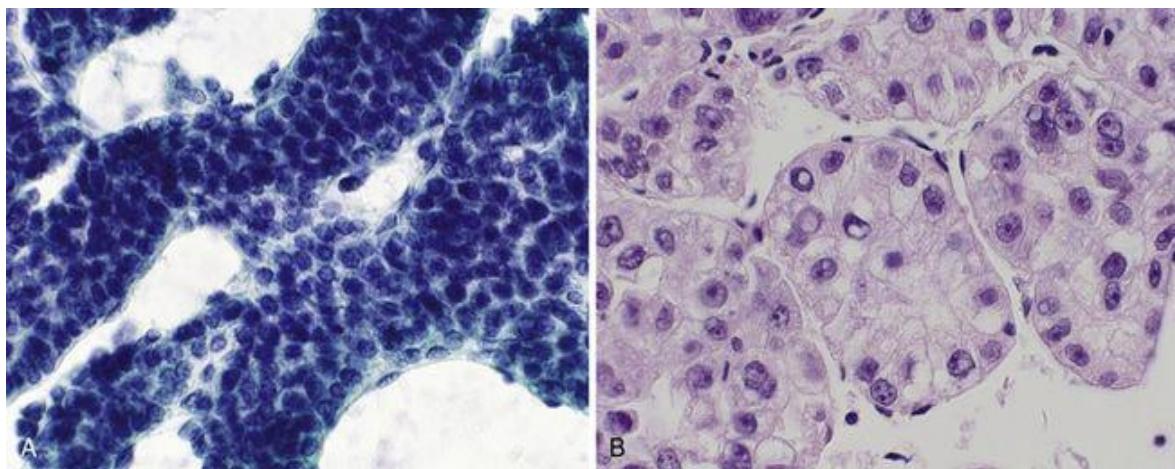


FIGURE 13.11 Hepatocellular carcinoma (HCC), trabecular type.

A, Thick (greater than 2 cells across) trabeculae are a characteristic feature of the classic HCC with a prominent trabecular pattern. Note the increased nuclear-to-cytoplasmic ratio of the neoplastic hepatocytes and the flat endothelial cells with spindle-shaped nuclei that wrap the trabeculae (Papanicolaou stain). B, Thickened trabeculae are cut across in cell block sections. Note the prominence of the wrapping endothelial cells, a helpful diagnostic feature (hematoxylin-eosin [H & E] stain).

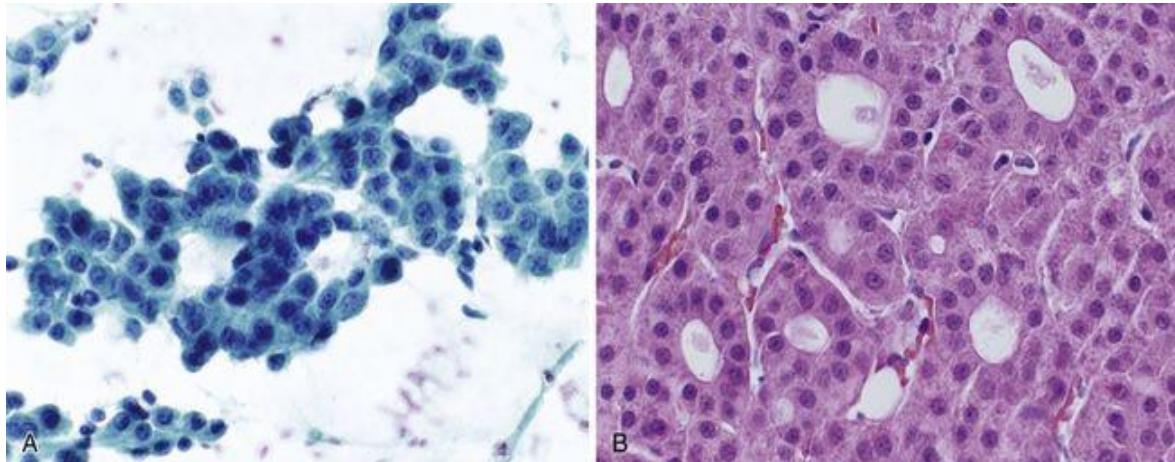


FIGURE 13.12 Hepatocellular carcinoma (HCC), acinar type.

A, The cords of neoplastic cells are arranged in acini. Note the increased nuclear-to-cytoplasmic ratio, a key feature in the diagnosis of HCC (Papanicolaou stain). B, Acini are easily appreciated in cell block preparations (hematoxylin-eosin [H & E] stain).

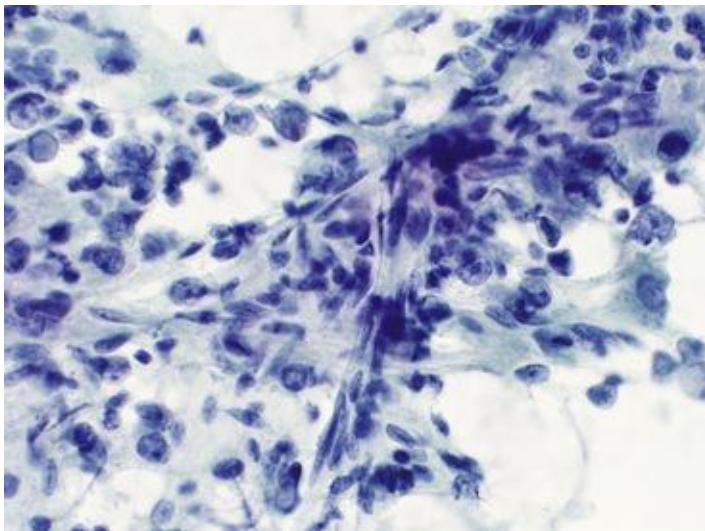


FIGURE 13.13 Hepatocellular carcinoma (HCC).
Prominent transgressing vessels are characteristic of HCC (Papanicolaou stain).



Differential diagnosis of well and moderately differentiated hepatocellular carcinoma

- regenerating nodule in cirrhosis
- hepatic adenoma
- focal nodular hyperplasia

The distinction between benign conditions and a well or moderately differentiated HCC can be challenging. Abundant bare hepatocyte nuclei are rarely seen in benign conditions and should prompt a search for other characteristic features, such as intact cells with an increased nuclear-to-cytoplasmic ratio and thickened trabeculae lined by endothelial cells. Although endothelial cells can be seen within large fragments of benign hepatocytes, thickened trabeculae are not apparent.⁸²

Histochemistry and immunohistochemistry techniques can be helpful.⁸³ A reticulin stain outlines the normal, thin ribbons of hepatocytes in benign lesions versus thickened trabeculae in HCC (Fig. 13.14A and B).^{78,84-86} CD34 highlights the endothelial cells that wrap the thickened cords of HCC.^{76,87} Immunohistochemistry for glypican-3 (GPC3), an oncofetal protein important in cell growth and differentiation, is also useful: Benign hepatocytes are negative, and most HCCs are positive (Fig. 13.15).⁸⁸⁻⁹² The definite distinction between a benign nodule and HCC is not always possible, and the interpretation

“suspicious for HCC” is appropriate in some cases.

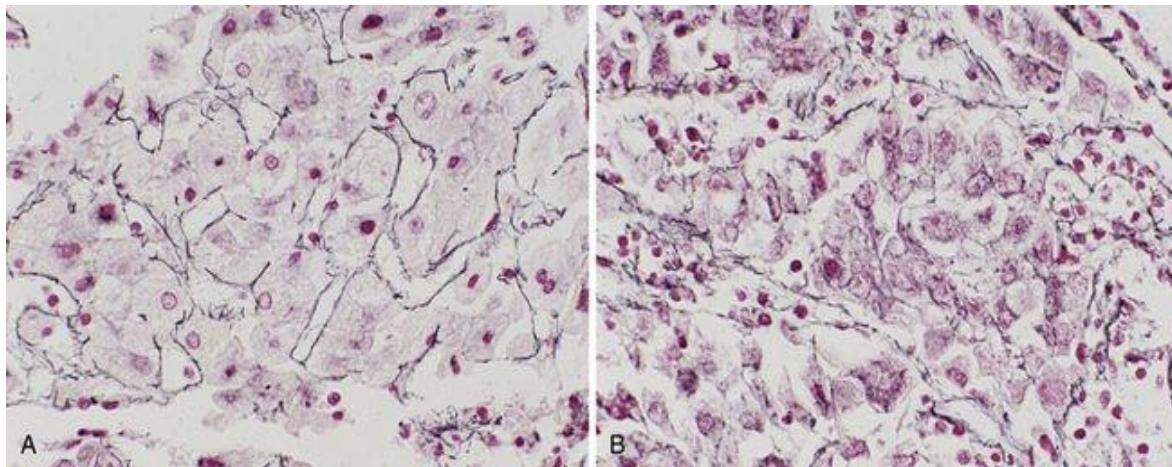


FIGURE 13.14 Reticulin for distinguishing benign from malignant hepatocellular proliferations.
A, Benign liver. The reticulin stain sharply outlines the normal, two-cell-thick hepatic cords. *B*, Hepatocellular carcinoma (HCC). The reticulin strands are attenuated and outline large, chunky masses of neoplastic cells (*A* and *B*, reticulin stain).

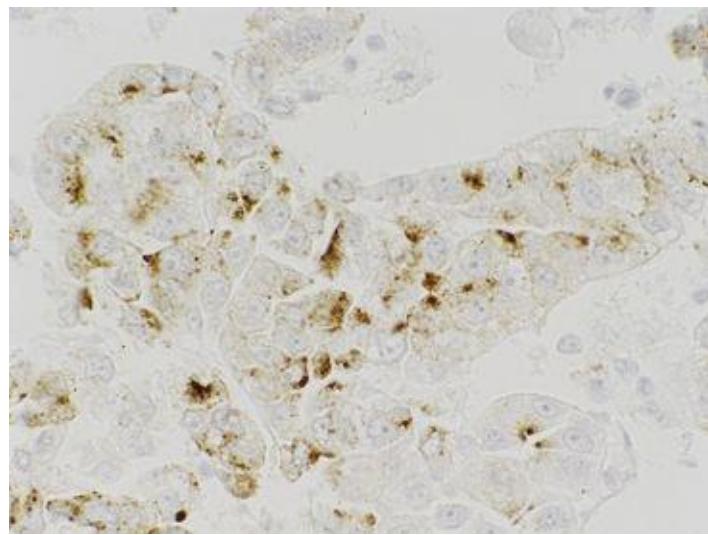


FIGURE 13.15 Hepatocellular carcinoma (HCC).
Glypican-3 (GPC3) is a useful marker that distinguishes benign from malignant hepatocytes: Benign hepatocytes are virtually always negative, whereas most HCCs show cytoplasmic and membranous staining (immunostain for GPC3).



Cytomorphology of poorly differentiated hepatocellular carcinoma

- highly cellular smears
- isolated cells or clusters
- moderate to marked pleomorphism
- atypical mitoses
- spindle-shaped cells
- tumor giant cells
- bile

The most notable features of hepatocytic differentiation—polygonal cell shape, a centrally placed nucleus, cytoplasmic bile ([Fig. 13.16](#)), and trabecular architecture—are either lost or barely apparent in poorly differentiated HCC. Atypia and pleomorphism can be marked ([Fig. 13.17A and B](#)).

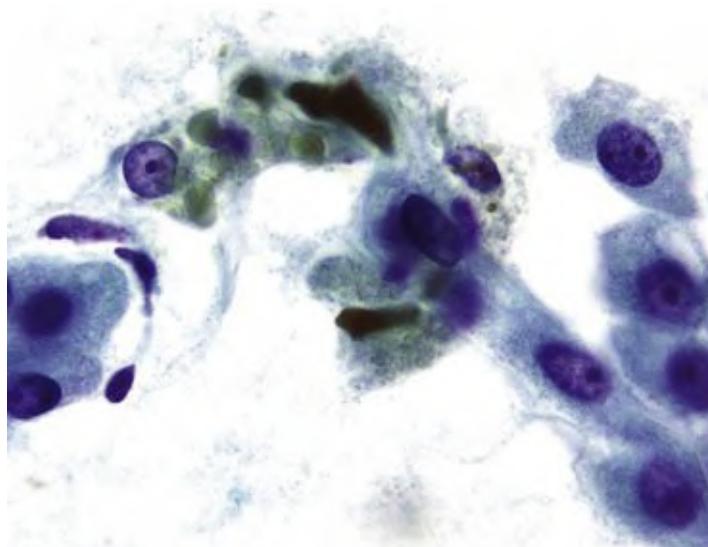


FIGURE 13.16 Hepatocellular carcinoma (HCC).
Bile stains dark green-brown and is a useful marker of hepatocyte differentiation
(Papanicolaou stain).

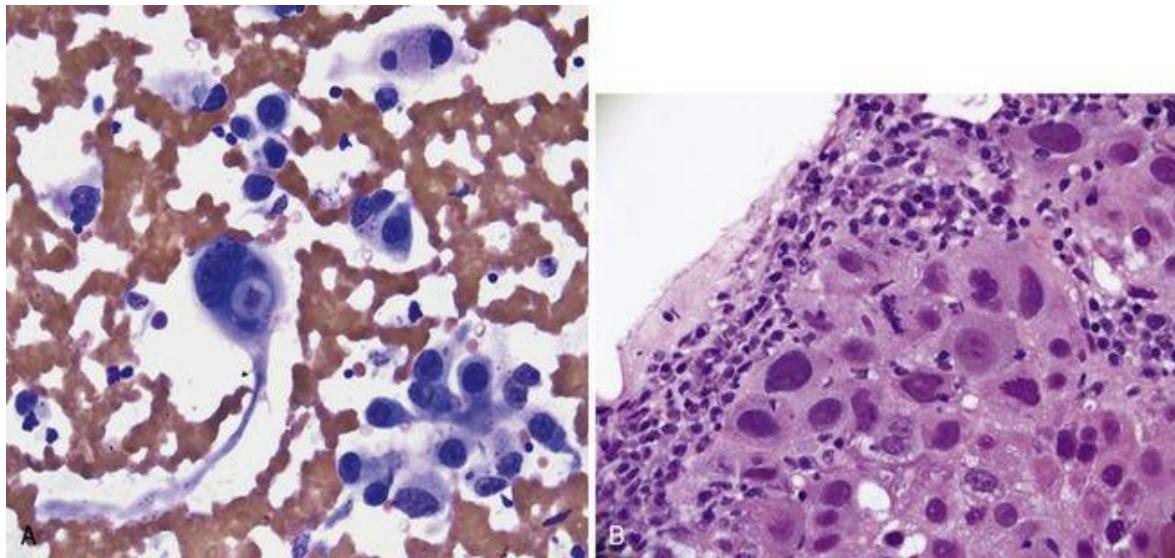


FIGURE 13.17 Hepatocellular carcinoma (HCC), poorly differentiated.

A, Bizarre, atypical forms including spindle cells with intracytoplasmic globules are noted (smear, Romanowsky stain). B, Pronounced nuclear atypia and numerous mitoses are seen (cell block, hematoxylin-eosin [H & E] stain).

Differential diagnosis of poorly differentiated hepatocellular carcinoma

- cholangiocarcinoma
- metastatic carcinoma

Poorly differentiated HCCs, if well sampled, are usually obviously malignant lesions. The difficulty is in distinguishing them from cholangiocarcinoma and metastatic carcinoma. To complicate matters, the existence of a rare combined HCC and cholangiocarcinoma presents a special diagnostic challenge.⁹³⁻⁹⁵ The clear cell (Fig. 13.18A and B) and fatty change (Fig. 13.19) variants of HCCs mimic metastatic tumors, most notably those of renal, adrenal, or germ cell origin.^{96,97} Hyaline globules are seen in HCC, but also in other tumors like renal cell carcinoma.⁹⁸

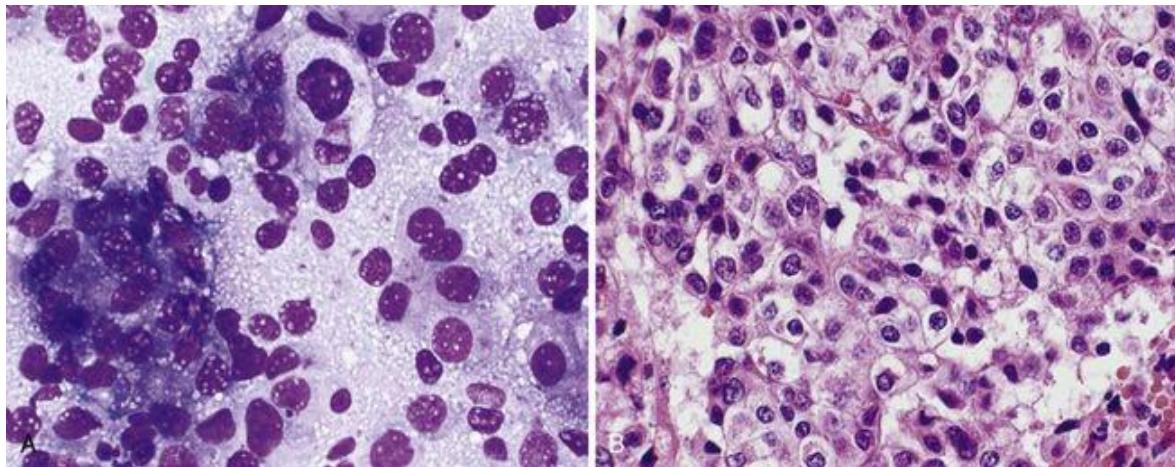


FIGURE 13.18 Hepatocellular carcinoma (HCC), clear cell variant.

A, As with many HCCs, abundant bare nuclei are present. The cells have been disrupted, and the abundant spilled cytoplasmic glycogen gives a mottled appearance to the background that is reminiscent of the “tigroid” background seen with another glycogen-rich tumor, the seminoma (see Fig. 2.45) (Romanowsky stain). B, Intact clear cells are better seen in the cell block sections (hematoxylin-eosin [H & E] stain).

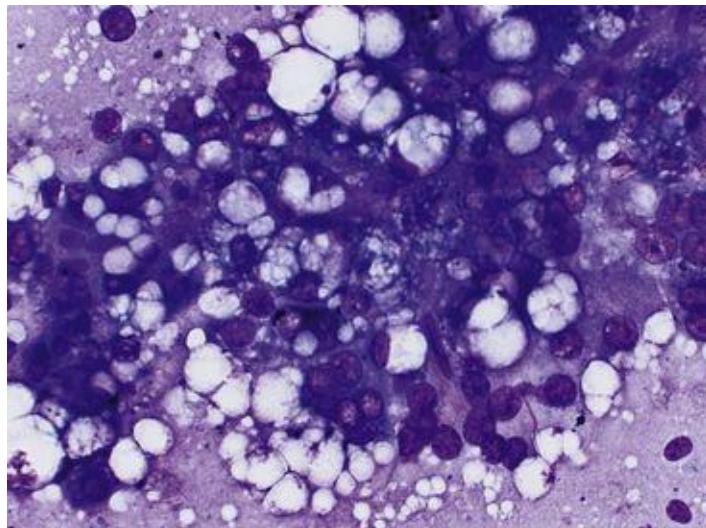


FIGURE 13.19 Hepatocellular carcinoma (HCC), fatty change variant.

The malignant cells are distended by sharply outlined, large fat vacuoles (Romanowsky stain).

Special stains are helpful.^{99,100} Intracytoplasmic mucin is extremely uncommon in HCC but present in most adenocarcinomas, including cholangiocarcinoma. The markers of hepatocellular differentiation—canalicular staining for polyclonal CEA, HepPar1, TTF-1 (cytoplasmic staining), ARG-1, and GPC3—are very useful in distinguishing HCC from cholangiocarcinoma and metastatic

adenocarcinoma (Fig. 13.20A-D). Polyclonal CEA show a distinctive linear staining pattern outlining bile canaliculi (see Fig. 13.20A); this is not seen with adenocarcinomas, which instead show diffuse cytoplasmic staining.¹⁰¹⁻¹⁰⁷ HepPar1 has high sensitivity and specificity for HCC (see Fig. 13.20B), but there may not be uniform staining,^{102,108-110} and aberrant HepPar1 staining is sometimes seen in tumors other than HCC.¹⁰⁹⁻¹¹¹ GPC3 is another very useful marker for HCC,¹¹²⁻¹¹⁵ and may be a more sensitive marker of hepatocellular differentiation than HepPar1 (see Fig. 13.15).¹¹⁶ TTF-1 stains normal hepatocytes and HCCs in a granular cytoplasmic (rather than nuclear) staining pattern and is a useful marker of hepatocytic differentiation (see Fig. 13.20C).¹¹⁷⁻¹¹⁹ The cytoplasmic staining of TTF-1 appears to be mitochondrial, similar to that of HepPar1.^{120,121} ARG-1 is the newest marker of hepatocellular differentiation and effectively distinguishes between HCC and metastatic carcinomas (see Fig. 13.20D).¹²²⁻¹²⁴

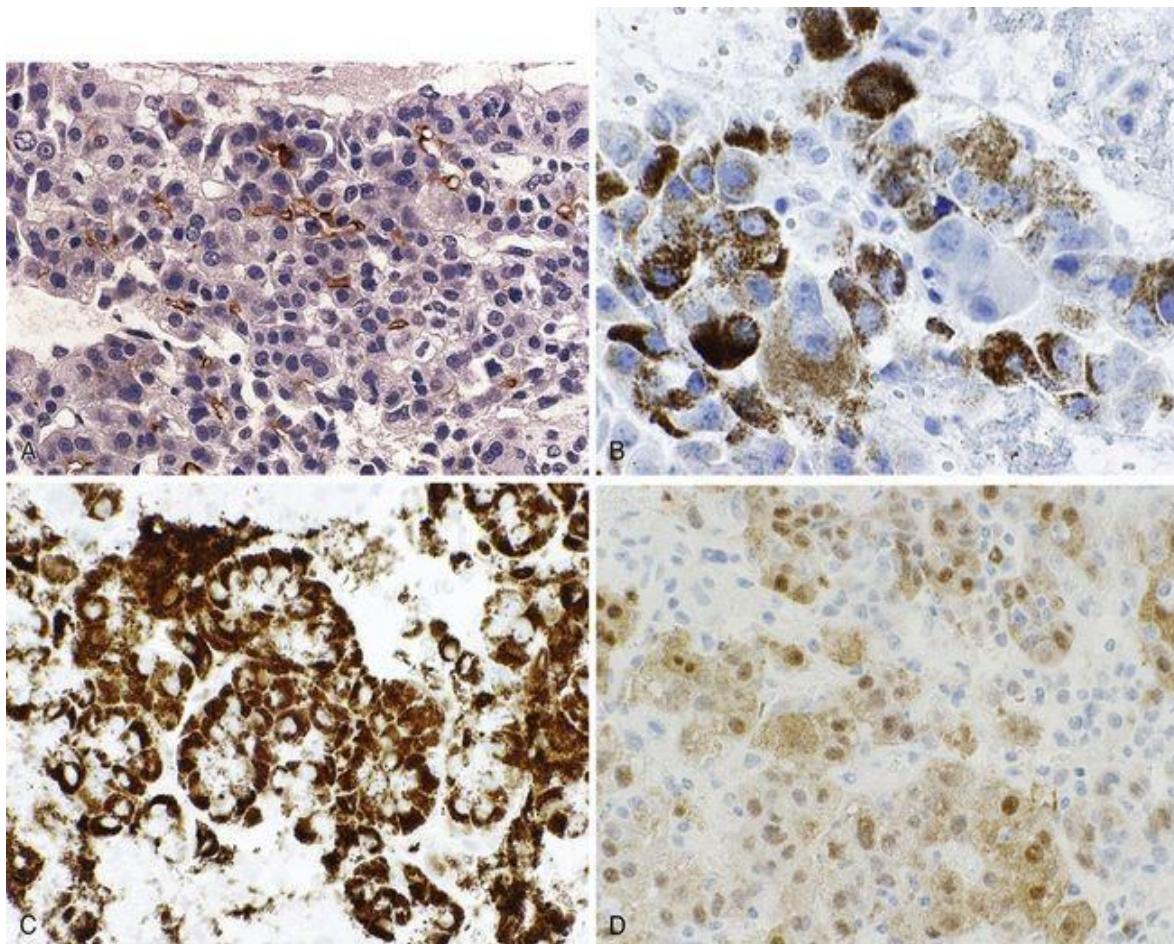


FIGURE 13.20 Immunomarkers of hepatocytic differentiation.

The markers illustrated here help distinguish hepatocellular carcinoma (HCC) from

cholangiocarcinoma and metastatic carcinoma. (All images are of HCCs.) *A*, Immunoreactivity with polyclonal antibodies to carcinoembryonic antigen (CEA) leaves the malignant cells unstained but outlines the bile canaliculi as short, discontinuous linear threads and small lumina. *B*, Most HCCs are immunoreactive for HepPar1. *C*, Most HCCs show cytoplasmic immunoreactivity for thyroid transcription factor-1 (TTF-1). *D*, Most HCCs show cytoplasmic and sometimes nuclear reactivity for arginase-1 (ARG-1).

It is important to note that polyclonal CEA, HepPar1, cytoplasmic TTF-1, and ARG-1 are merely markers of hepatocytic differentiation: They do not distinguish benign from malignant hepatocytes.

CD34 is useful for highlighting the endothelial cells that wrap the thickened cords of HCC.^{76,87} Numerous spider-shaped, vimentin-positive Kupffer cells are noted in HCCs and not in metastatic carcinomas.¹²⁵ Organ-specific immunohistochemical markers (e.g., PAX8, prostate-specific antigen [PSA]) can be helpful in selected circumstances as dictated by clinical and imaging findings.

The **fibrolamellar variant of HCC** deserves special mention because of its unusual cyto-and histomorphology and clinical presentation. It is seen predominantly in young patients (average age, mid-20s) who do not have cirrhosis, and the prognosis is favorable. Histologically, the malignant cells are large and polygonal, with abundant eosinophilic cytoplasm. Dense bands of fibrosis surround tumor cells.



Cytomorphology of fibrolamellar hepatocellular carcinoma

- large cells with abundant cytoplasm
- large nucleus
- very prominent nucleolus
- mostly isolated cells or loose clusters
- hyaline intracytoplasmic globules (some cells)
- bands of fibrosis separating neoplastic cells

The cells of fibrolamellar HCC are much larger than normal hepatocytes and larger than the cells of a well-differentiated HCC ([Fig. 13.21A and B](#)).¹²⁶ Notably, the nuclear-to-cytoplasmic ratio is lower in fibrolamellar HCC than in ordinary HCC.¹²⁶ In contrast with typical HCC, fibrolamellar HCC and pediatric HCCs are more likely to stain positively with CK7 than adult HCCs.¹²⁷⁻¹²⁹

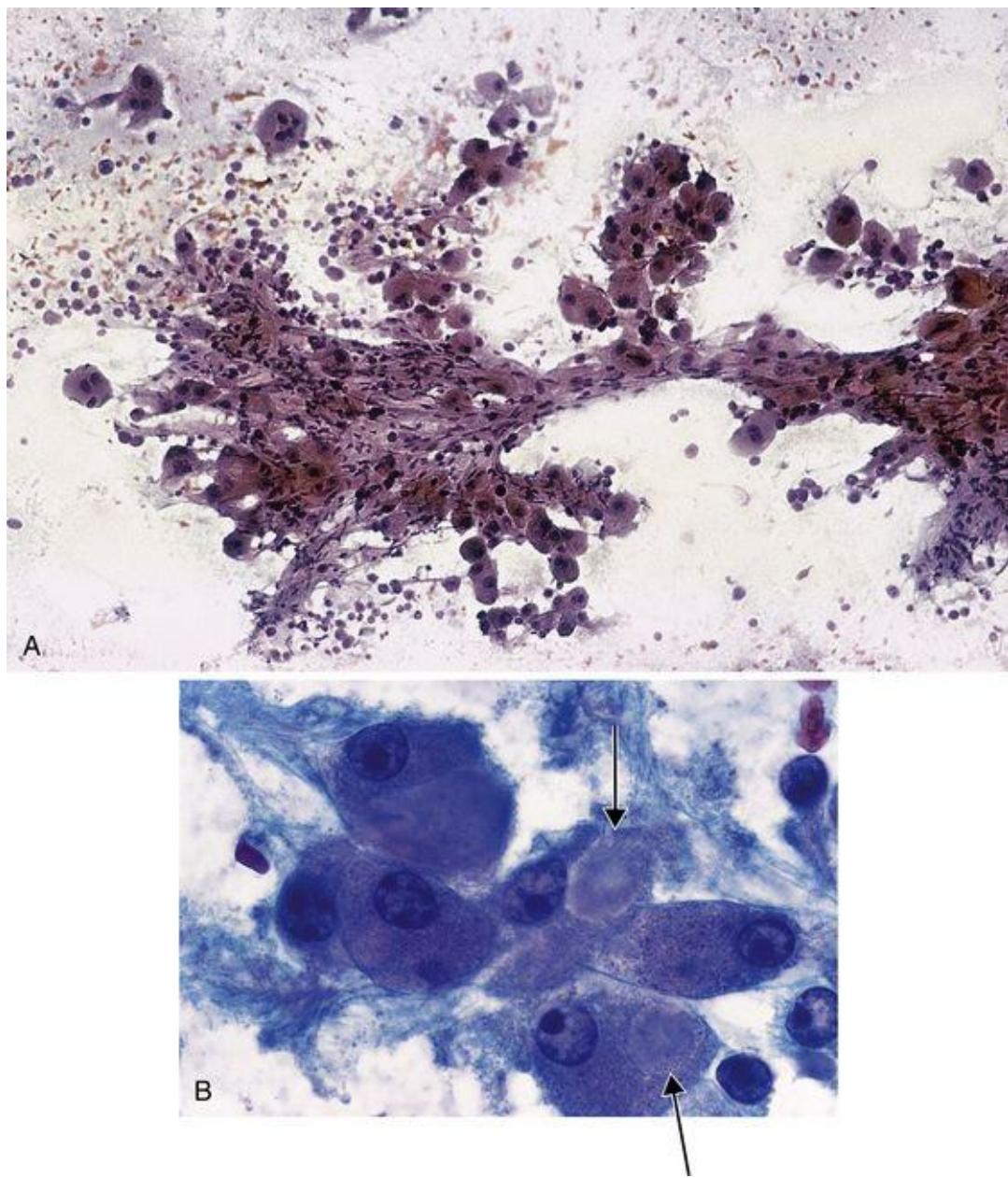


FIGURE 13.21 Fibrolamellar hepatocellular carcinoma (HCC).
A, Large, atypical hepatocytes are accompanied by fibrous tissue. B, The malignant cells have abundant granular cytoplasm and intracytoplasmic hyaline globules (arrows) (Papanicolaou stain).

Cholangiocarcinoma

Cholangiocarcinoma is the second most common primary hepatic malignancy after HCC, reaccounting for 5% to 15% of primary liver cancers.⁶¹ Risk factors include primary sclerosing cholangitis, hepatolithiasis (more common in the Far East), parasitic infestation of the liver by *Clonorchis sinensis*, nonbiliary

cirrhosis, and deposition of thorotrast. Cholangiocarcinomas can arise from the intrahepatic and extrahepatic bile ducts; a tumor arising from the right or left hepatic ducts near their junction is called a hilar cholangiocarcinoma (or Klatskin tumor). Obstructive jaundice is the most common presentation in patients with extrahepatic tumors. Significant elevation of Ca19-9 and CEA can be seen. The diagnosis is made in 60% to 70% of patients using bile cytology, biliary brush cytology, or FNA.^{130, 131} Increasingly, endoscopic ultrasound-guided FNA (EUSFNA) is used to diagnose this tumor,^{132–140} and transperitoneal biopsy has been associated with an increased risk of peritoneal metastases.¹⁴¹



Cytomorphology of cholangiocarcinoma

- isolated cells, crowded sheets, and cell clusters
- glandular differentiation (e.g., mucin vacuoles, acinar structures)
- nuclear enlargement, with variation in size and shape

Histologically and cytologically, cholangiocarcinomas resemble adenocarcinomas of the pancreas and demonstrate a similar variety of morphologic types and range of differentiation (Fig. 13.22A and B). Smears typically show crowded sheets of cells with marked anisonucleosis, and atypical glands can be seen in cell block sections.

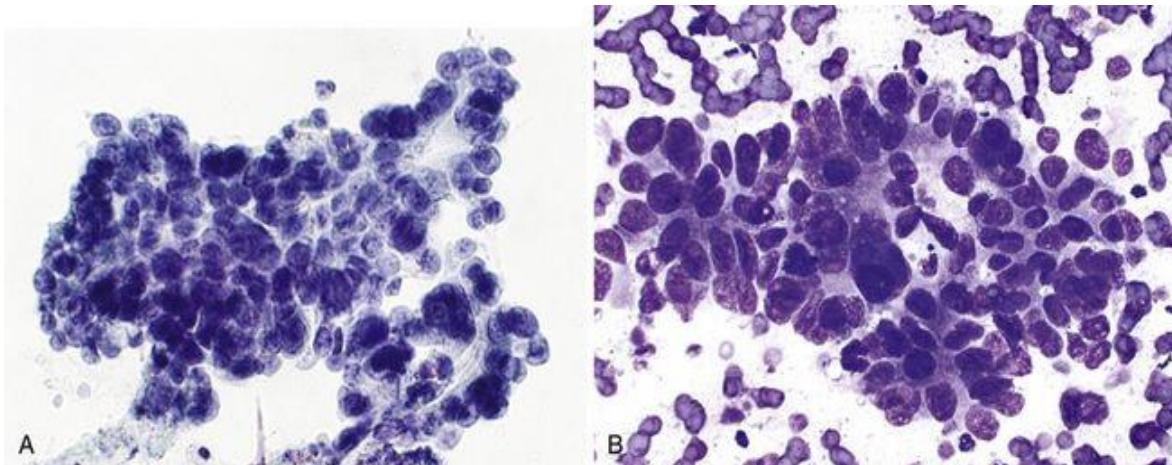


FIGURE 13.22 Cholangiocarcinoma.

A, The tumor cells are in crowded sheets. Haphazard cellular arrangement and glandular differentiation are apparent (Papanicolaou stain). B, Anisonucleosis and nuclear membrane

irregularity are apparent (Romanowsky stain).

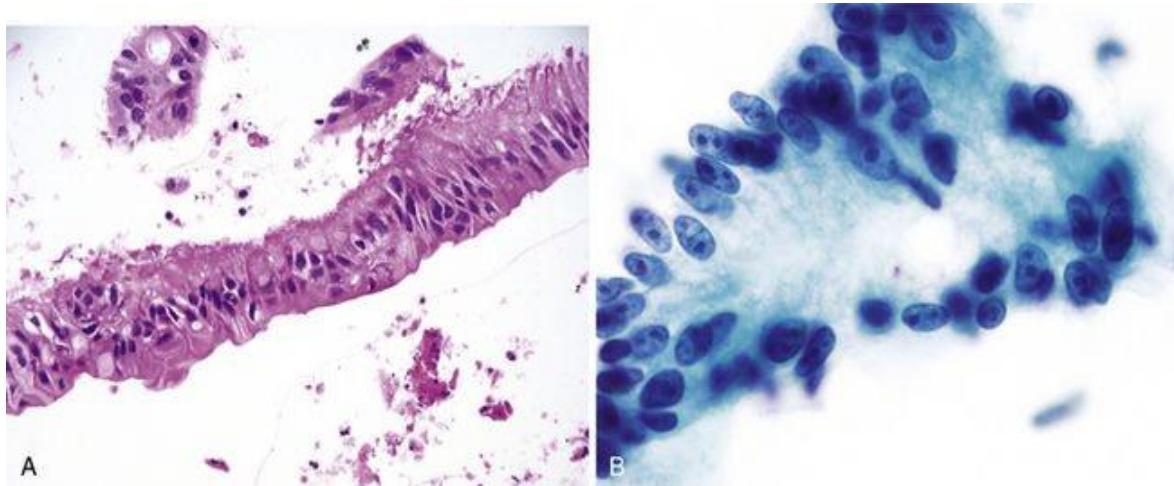


FIGURE 13.23 Metastatic colonic carcinoma.

A, Colorectal cancer cells often have a “picket fence” arrangement and are associated with a dirty, necrotic background (cell block, hematoxylin-eosin [H & E] stain). B, The columnar, “picket fence” arrangement is apparent on smears as well. Nuclei are enlarged and ovoid (smear, Papanicolaou stain).



Differential diagnosis of cholangiocarcinoma

- hepatocellular carcinoma
- metastatic adenocarcinoma

The cells of most cholangiocarcinomas are cuboidal or columnar rather than polygonal. Neither bile nor endothelial wrapping of thickened trabeculae, so typical of HCC, is seen with cholangiocarcinoma. Cholangiocarcinoma is a gland-forming tumor; mucin production may be abundant.¹⁴² Positive staining for mucicarmine and AE1 keratin and diffuse cytoplasmic staining for polyclonal CEA are characteristic of cholangiocarcinoma and very uncommon in HCC. (The canalicular staining pattern for polyclonal CEA is not seen in cholangiocarcinoma.) Immunoreactivity for CK7, CK17, and CK19 is typical of cholangiocarcinomas and less so for HCC, whereas the reverse is true for CK8/18.¹⁴³ Hepatitis B viral antigens are uncommon in cholangiocarcinoma.

The distinction between cholangiocarcinoma and metastatic adenocarcinoma is challenging. Because cholangiocarcinomas and pancreatic cancers are morphologically and immunophenotypically similar, the distinction between

these entities is based on anatomic location rather than pathologic characteristics. Of note, overexpression of p53 and loss of SMAD4 by immunohistochemistry are seen in both cholangiocarcinomas and pancreatic adenocarcinomas.¹⁴⁴ The presence of proliferating ductules, in particular more than ten ductular clusters has been suggested as a cytologic feature to distinguish cholangiocarcinoma from metastatic adenocarcinoma.¹⁴⁵ In general, the diagnosis of cholangiocarcinoma is made only after ruling out other primary sites clinically.

Hepatoblastoma

Hepatoblastoma is a rare tumor of infancy and childhood. Cytologic findings mimic those in either HCC, with even larger and more anaplastic cells, or a small, round-cell tumor of childhood.¹⁴⁶⁻¹⁴⁸

Angiosarcoma

Angiosarcoma is an uncommon malignant tumor of endothelial cells that accounts for less than 1% of primary hepatic malignancies. Most cases arise in adults, but the tumor can occur in people of any age. Like HCC, angiosarcoma is associated with cirrhosis: About one third of adult cases arise in this setting. It is seen with increased frequency in workers who are exposed to polyvinyl chloride and in patients who received thorium dioxide (Thorotrast) as a radiographic contrast agent. Histologically, these are spindle or epithelioid cell neoplasms that range from well-differentiated tumors with well-formed vascular channels to poorly differentiated tumors. Massive bleeding is a potential complication of FNA.¹⁴⁹



Cytomorphology of angiosarcoma

- well-differentiated tumors: elongated cells; isolated cells, tightly cohesive clusters, and syncytia
- poorly differentiated tumors: large, spindle-shaped or epithelioid cells; pleomorphic nuclei; multinucleated giant cells
- “rhabdoid” forms in some epithelioid angiosarcomas¹⁵⁰
- abundant finely or coarsely vacuolated cytoplasm, often with intracytoplasmic lumina¹⁵¹
- cell blocks useful in demonstrating the characteristic anastomosing vascular pattern

Angiosarcoma is a rare neoplasm and difficult to recognize by cytomorphology. The epithelioid variant is especially tricky, inasmuch as it is often misinterpreted, at least initially, as a carcinoma.^{[152](#)}



Differential diagnosis of angiosarcoma

- epithelioid hemangioendothelioma
- other malignant neoplasms

Angiosarcoma cells are larger, more atypical and more monomorphous than those of an epithelioid hemangioendothelioma, and they are more likely to be cohesive.^{[153](#)} They may have a large nucleolus and intracytoplasmic vacuoles.^{[153](#)} Positive immunoreactivity for the endothelial markers CD34, CD31, and ERG is helpful for confirming the diagnosis of a vascular neoplasm.^{[151,152,154-158](#)}

Epithelioid Hemangioendothelioma

This uncommon tumor arises in the liver and at other sites. Like angiosarcoma, it is a tumor of endothelial cells, but its course is less aggressive. The cytologic findings are suggestive,^{[67,153,159](#)} and immunostains for endothelial markers are very useful in confirming the diagnosis.



Cytomorphology of epithelioid hemangioendothelioma

- hypocellular (some cases) with a clean background
- isolated, large polymorphous cells with a folded nuclear outline
- fragments of metachromatic stroma^{[67](#)}
- frequent binucleated or multinucleated giant cells
- round or irregular nucleoli and prominent nucleoli
- abundant lacy or dense cytoplasm, sometimes with intracytoplasmic lumina^{[67,160](#)}
- See [Figure 4.18](#)

Metastatic Tumors

The liver is a common site for metastases, and a majority of liver masses sampled by FNA prove to be metastatic malignancies, most often metastatic

carcinomas.¹⁶¹ Common primary tumors include those of the colon and rectum, lung, pancreas, stomach, and breast. As one might expect, the cytologic picture varies greatly depending on the tumor. If the patient has a known primary and the aspirate demonstrates malignant cells, establishing a morphologic match by comparing the FNA sample with available histologic or cytologic slides from the original tumor is very helpful, and the cost of immunohistochemistry can be avoided in such cases. If there is no history of a primary outside the liver, cytologic features may suggest a specific primary site or a limited differential diagnosis. Correlating the cytomorphology with clinical findings allows one to select a tailored immunohistochemical panel to further refine the interpretation. In some cases, the cytomorphology and immunoprofile prove relatively nonspecific. In such cases, it can be helpful to suggest the most likely primary sites.



Cytomorphology of common metastatic malignancies to the liver

- colorectal cancers
 - tall, dark, and necrotic ([Fig. 13.23A and B](#))
 - immunoreactive for CK20, CDX2, and SATB2
- gastric cancer
 - intestinal or signet ring cell morphology
- breast cancer
 - ductal or lobular, variable differentiation
 - signet ring cells
 - immunoreactive for mammaglobin, GCDFP-15, estrogen receptor, progesterone receptor, HER2neu
- prostate ([Fig. 13.24A and B](#))

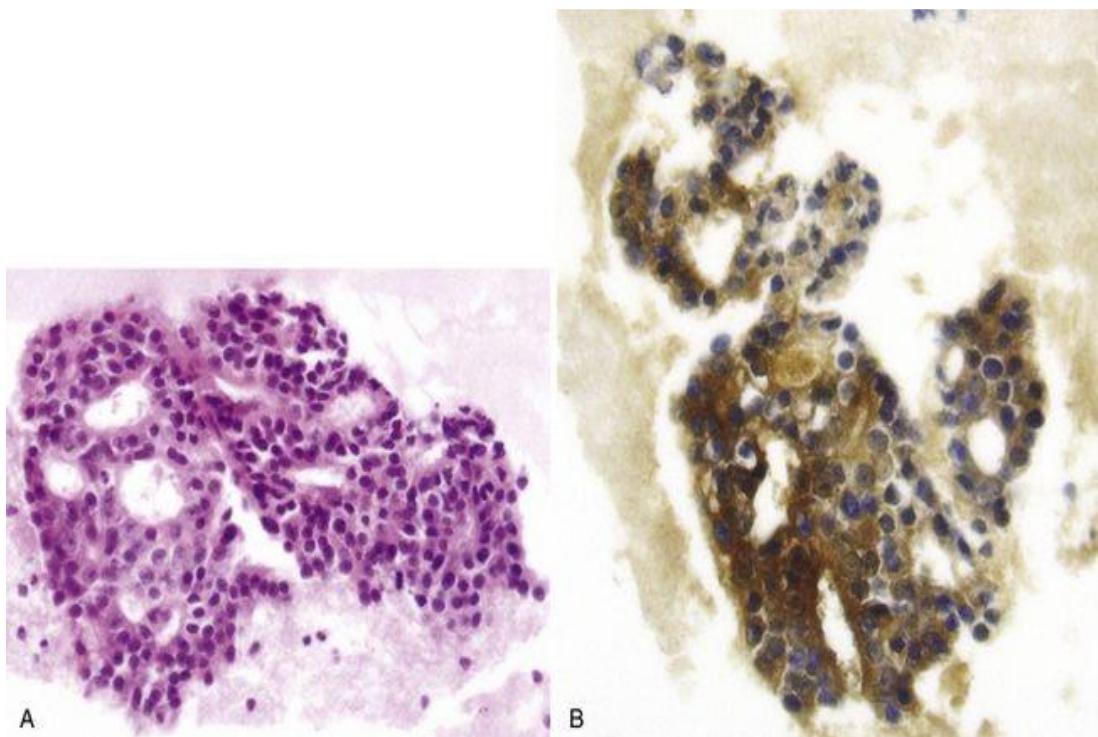


FIGURE 13.24 Metastatic prostatic carcinoma.

A, Acinar structures are characteristic of prostate cancers (cell block, hematoxylin-eosin [H & E] stain). *B*, Immunoreactivity for prostate specific antigen (PSA) confirms the diagnosis (cell block).

- microacini
- prominent nucleoli
- immunoreactive for prostatic acid phosphatase and prostate specific antigen
- well-differentiated neuroendocrine tumor ([Fig. 13.25](#))

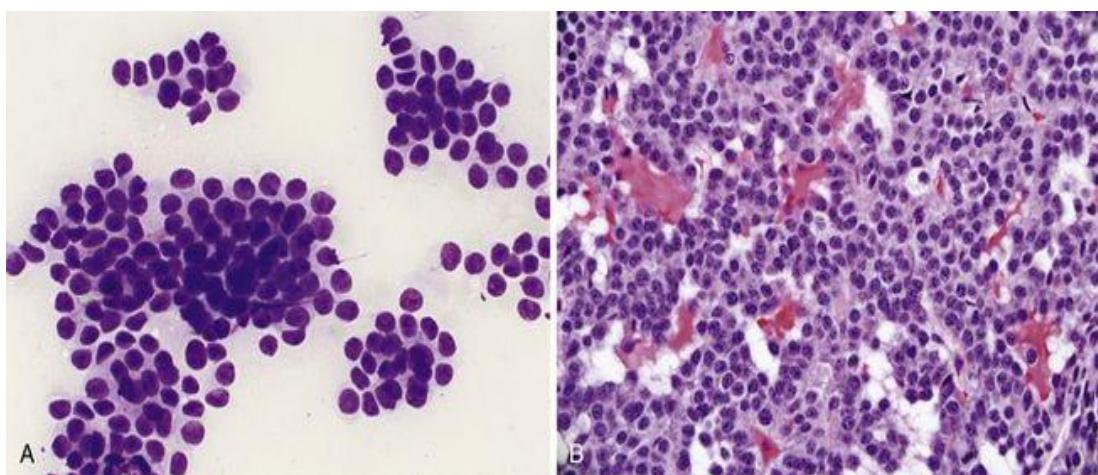


FIGURE 13.25 Metastatic carcinoid tumor.

A, The tumor cells are monomorphic and arranged in loose clusters. This appearance should raise the possibility of a neuroendocrine tumor (NET) (Romanowsky stain). B, The cell block shows cords and trabeculae of cells with a granular chromatin pattern (hematoxylin-eosin [H & E] stain).

- eccentrically placed nucleus
- “salt and pepper” chromatin pattern
- abundant granular cytoplasm
- isolated cells, loosely cohesive clusters and rosettes
- immunoreactive for synaptophysin, chromogranin, and CD56
- immunoreactive for islet-1 and PAX8 (if pancreatic endocrine tumor)
- small cell carcinoma ([Fig. 13.26](#))

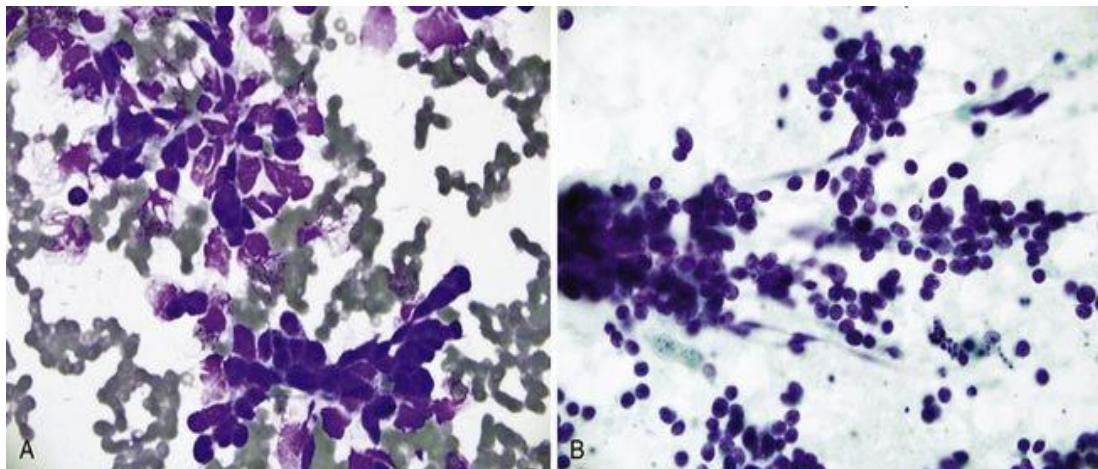


FIGURE 13.26 Metastatic small cell carcinoma.

A, Note the abundant small blue cells with scant cytoplasm and pronounced molding. Nuclear crush artifact is prominent (Romanowsky stain). B, The cells are isolated and arranged in loosely cohesive clusters. The nuclear-to-cytoplasmic ratio is very high, nuclear molding is seen, and the chromatin is finely granular without nucleoli (Papanicolaou stain).

- isolated and loosely cohesive cells
- nuclear molding
- hyperchromatic nucleus with finely granular chromatin and small nucleoli
- round, polygonal, or spindled cells
- paranuclear blue bodies (see [Fig. 2.41B](#))
- squamous cell carcinoma (SQC)
 - small, dark nucleus without discernible texture

- abundant cytoplasm with a hard, glassy appearance (orange with Papanicolaou stain, gray with Romanowsky-type stain)
- poorly differentiated, nonkeratinized: hard to distinguish from other poorly differentiated malignancies
- immunoreactive for keratins and p63
- malignant melanoma ([Fig. 13.27](#))

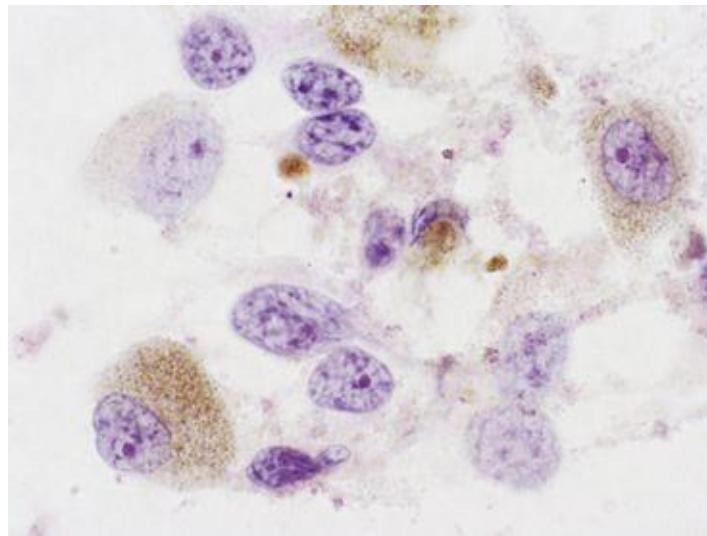


FIGURE 13.27 Metastatic melanoma.

Melanin is more finely granular than bile. Melanoma cells are usually isolated rather than clustered. The nucleoli of melanoma cells are usually prominent and solitary (Papanicolaou stain).

- prominent isolated cell pattern
- intranuclear pseudoinclusions, macronucleolus
- abundant cytoplasm, sometimes pigmented
- melanin pigment is finely granular (bile pigment more variable in size)
- immunoreactive for S-100, HMB-45, Mart-1
- sarcomas ([Fig. 13.28A and B](#))

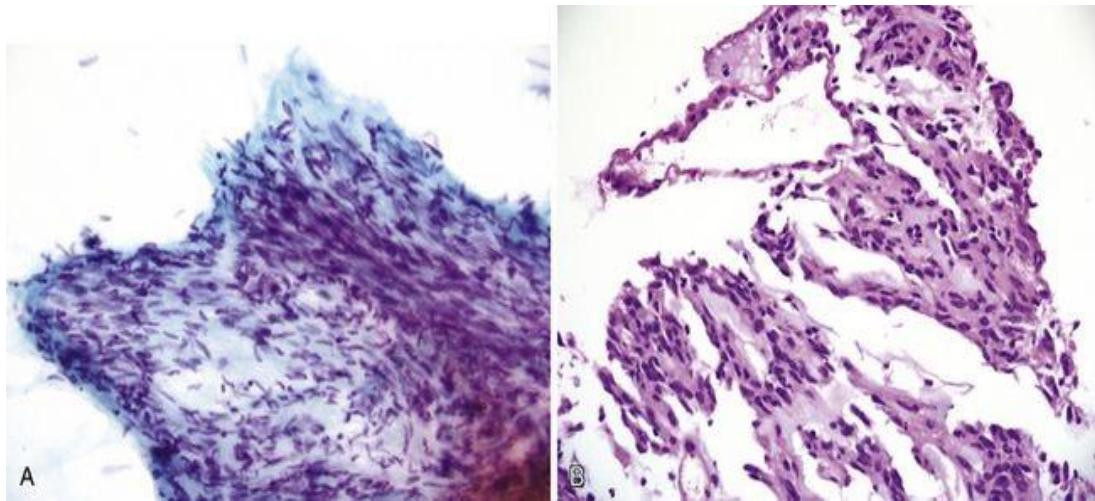


FIGURE 13.28 Metastatic gastrointestinal stromal tumor (GIST).

A, There are cohesive groups of spindle-shaped cells. Nuclear atypia is minimal in this case (Papanicolaou stain). B, The spindle-cell proliferation is also apparent on cell block sections. The tumor cells were immunoreactive for CD117 (c-kit) (not shown) (hematoxylin-eosin [H & E] stain).

- spindle-shaped, round, or pleomorphic cells

Colorectal cancers have a predilection for metastasizing to the liver, and some patients present with liver metastases and an unknown primary. These tumors have a characteristic cytomorphology: The cells are large and columnar (“tall”), nuclei are hyperchromatic (“dark”), and necrosis with abundant nuclear debris (“dirty necrosis”) is often present—hence the familiar mnemonic for FNAs from colorectal cancers: “tall, dark, and necrotic.” The impression can be confirmed by demonstrating the typical immunoprofile of colon cancer: positive for CK20, CDX2, and STAB2, and negative for CK7.

Esophageal and gastric adenocarcinomas resemble colorectal cancers. A larger proportion, however, have a prominent component of signet ring cells. Gastric and esophageal adenocarcinomas have a similar immunoprofile and cannot be distinguished from each other by FNA of a metastatic lesion. Gastric cancers are heterogeneous in their CK7, CK20, and CDX2 profile, although CDX2 immunoreactivity, when present, is often patchy and weaker than the strong and diffuse staining seen in most colorectal cancers.

Both ductal and lobular breast cancers are encountered as metastases to the liver. It is very uncommon for a breast primary tumor to be occult when the patient presents with metastases to the liver.

Well-differentiated (low-and intermediate-grade) neuroendocrine tumors (NETs), whether carcinoids (of gastrointestinal or lung origin) or pancreatic neuroendocrine tumors, all have a similar cytomorphology, and all are usually positive for the generic neuroendocrine markers synaptophysin, chromogranin, and/or CD56. Islet 1 and PAX8 are relatively specific for NETs of the pancreas, however, and thus helpful in confirming pancreatic origin if one or both is positive. (Of course, the presence of a pancreatic mass by imaging is also helpful!) The proliferative rate is used to grade NETs because it provides useful prognostic information and may influence clinical management. In some patients, the only pathologic specimen might be an FNA of a NET metastatic to the liver, so grading of liver FNA samples is appropriate. The proliferative rate of gastrointestinal and pancreatic NETs is assessed as the number of mitoses per 10 high power fields (hpfs): Low-grade tumors have less than 2 mitoses per 10 hpf, intermediate-grade tumors 2 to 20 per 10 hpf, and high-grade tumors greater than 20 per 10 hpf. Staining for Ki67 (MIB-1) is a useful surrogate because it provides an accurate assessment of the proliferative rate and is readily applicable to smaller specimens. The Ki67 index is less than 3% for low-grade, 3% to 20% for intermediate-, and greater than 20% for high-grade tumors¹⁶² (see [Fig. 14-14A](#) and [B](#)). A grade assigned on the basis of an FNA or core biopsy, however, needs to take into account the possibility that a higher-grade focus may not have been sampled.

GISTs of the stomach and small intestine have a propensity to metastasize to the liver. Immunoreactivity for c-kit and DOG1 (“discovered on GIST-1”) distinguishes GISTs from smooth muscle tumors, which are positive for desmin and smooth muscle actin but negative for c-kit and DOG1. Positive staining for c-kit (CD117) and/or DOG1 is virtually essential for the diagnosis of a GIST. DOG1 is a protein of unknown function that was identified from gene expression data as a highly sensitive and specific marker for GIST. It is more sensitive than c-kit; that is, some GISTs are negative for c-kit but positive for DOG1.¹⁶³

Extramedullary hematopoiesis in the liver, seen in patients with myeloproliferative disorders, can be diagnosed when megakaryocytes, erythroid precursors, and myeloid precursors are identified. Lymphomas occur as metastatic or primary tumors of the liver,¹⁶⁴ and features are described in [Chapter 12](#).

Germ cell tumors may metastasize to liver. Seminomas and dysgerminomas are immunoreactive for placental alkaline phosphatase (PLAP) (cytoplasmic and membrane staining), CD117 (c-kit; membrane staining), and the stem cell-related proteins Oct-3/4 (synonymous with Oct-4), NANOG, and SALL4 (nuclear staining for all three) (see [Fig. 16.16B-D](#)). PLAP and SALL4 are

expressed in most germ cell tumors, whereas Oct-3/4 and NANOG are only expressed in dysgerminoma/seminoma and embryonal carcinoma. The membranous staining pattern for CD117 is very characteristic of seminoma/dysgerminoma and not seen in embryonal carcinoma or yolk sac tumor. Unlike the embryonal and yolk sac tumors, dysgerminomas generally show no immunoreactivity for keratin proteins, epithelial membrane antigen, AFP, or CEA.

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CHAPTER 14

Pancreas and Biliary Tree

Martha Bishop Pitman

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Indications

An unexplained pancreatic mass and a bile duct stricture are the major indications for cytologic evaluation of the pancreas and bile ducts. Imaging studies contribute useful information on the location, distribution (solitary, multiple, or diffuse), and nature (cystic versus solid) of a lesion, but a cell sample is usually necessary for definitive diagnosis. In the case of a potentially resectable mass that is malignant by imaging, however, the value of preoperative aspiration or brushings is still debated. The increased cost, potential delay in diagnosis, and imperfect negative predictive value attributed to aspiration and brushings are cited as arguments to proceed with surgical resection without preoperative cytology in this scenario.¹ Still, many clinicians request a tissue diagnosis even in this circumstance, because radiologic accuracy is not 100%.²⁻⁵ Despite improvements in surgical technique, the morbidity of pancreatic surgery is significant,⁶ and not all malignancies are surgically managed (e.g., lymphoma). Moreover, nonsurgical management of patients with a benign neoplasm or premalignant disease is increasingly common.^{3,7-10}

Sampling Techniques

A fine-needle aspiration (FNA) of the pancreas can be performed percutaneously (by a radiologist) or endoscopically (by a gastroenterologist). A *percutaneous pancreatic FNA* is performed using computed tomography (CT). Percutaneous needle placement techniques vary depending on the location of the lesion and the trajectory of the needle. The tandem technique involves placing a guide needle to serve as a reference point for the second, biopsy needle and is most useful in CT-guided FNA when real-time visualization of needle insertion is impossible. The coaxial technique involves inserting a larger caliber needle to localize the lesion. A smaller caliber needle is then inserted through the larger needle to sample the lesion. This method permits multiple sampling attempts without the increased risk to local structures created by repeated needle passes.¹¹

Percutaneous FNA has been largely replaced by *endoscopic ultrasound (EUS)-guided FNA* because it provides real-time visualization of the needle tip, better visualization of small lesions than with CT, and the ability to identify local metastases or invasion of local structures, thus permitting simultaneous diagnosis and staging.^{5,12,13} EUS equipment consists of an image guidance system and an echoendoscope that is placed into the stomach or duodenum. A high-frequency ultrasound transducer on the tip of the echoendoscope guides a 19 to 25 gauge needle through the gut wall into the pancreatic mass or cyst. Pancreatic head masses benefit from a transduodenal approach, and body and tail masses from a transgastric approach. The pathologist should be aware of the approach so that contaminating normal gastric or duodenal mucosa is recognized and not misinterpreted as lesional. Once in the lesion, the stylet is removed and the needle oscillated under suction to dislodge cells and pull them into the needle.

With cystic lesions, as much fluid is drained as possible. Depending on volume, cyst fluid can be submitted for routine cytology and biochemical and molecular analysis. Any visible mural nodule or solid component should be separately sampled.

Suspicious pancreatic and extra pancreatic biliary lesions can be sampled by *brushing the pancreatic and common bile ducts*, which is particularly useful when there is a duct stricture without a discernible mass. Brushings are obtained endoscopically, sometimes in conjunction with endoscopic retrograde cholangiopancreatography (ERCP); they can also be obtained during percutaneous transhepatic cholangiography (PTC). Because pancreatic ductal adenocarcinoma tends to invade the main pancreatic or common bile ducts, this

sampling method is highly effective. Brushings can also diagnose accessible cholangiocarcinomas and hepatocellular carcinomas (HCCs).¹³

Complications

The most common major complication of FNA is acute pancreatitis, with an incidence of 1% to 3%; mortality is less common.¹⁴⁻¹⁹ Other, rare major complications include pancreatic duct leaks, massive hemorrhage, and septic shock.²⁰ Perforation associated with a malignant luminal stenosis is a specific complication of EUS-guided FNA.²⁰ Post aspiration needle tract seeding by tumor cells or mucin is exceedingly rare.²¹⁻²³ Complications of bile duct brushing are minor and include cholangitis and mild pancreatitis.

Rapid On-Site Evaluation

Rapid on-site evaluation of the specimen by a cytologist provides feedback to the operator on the adequacy of individual passes, allowing the operator to obtain additional passes until the sample is judged adequate. It has potential utility with solid masses, where some have found that on-site evaluation reduces the rate of nondiagnostic results,^{24–28} others have questioned its value when experienced operators perform the procedure, finding no difference in nondiagnostic rates with and without on-site evaluation.²⁹ On-site evaluation offers little benefit with cysts, because smearing a small drop of cyst fluid results in a paucicellular sample poorly suited for rapid examination.

Sample Preparation and Cyst Fluid Analysis

Aspirates and brushings can be prepared as smears, a liquid-based preparation (e.g., ThinPrep, SurePath), a CytoSpin, a cell block, or some combination. If smears are employed, proper smear technique is vitally important; even the most cellular sample is useless if the cells cannot be evaluated due to a preparation-related artifact. Clotted tissue in needle casts (“worms”) should be gently lifted from the glass slides and placed into formalin for a cell block. (Small formalin vials like those used for bone marrow cores are recommended.) Smears can be air-dried and Romanowsky-stained or alcohol-fixed and Papanicolaou-stained, depending on the preference of the laboratory.

Bile duct brushings are best prepared by a liquid-based method (e.g., ThinPrep, SurePath), cytocentrifugation (i.e., CytoSpin), and/or cell block, because a liquid collection method, used by all these methods, allows for less mechanical artifact when dislodging the cells from the brush.

A cell block from the sedimented fluid can be invaluable for immunohistochemical and molecular studies. An aliquot for microbiologic cultures or flow cytometry might be considered based on the on-site evaluation findings.

Biochemical and molecular analysis can help in classifying cyst fluids. [Table 14.1](#) shows the usual (expected) results for the 4 most useful tests. Fresh, unfixed cyst fluid is, therefore, often apportioned among cytology, biochemistry, and molecular studies to establish if the cyst is mucinous, and if so, if it is malignant. Grossly viscous white fluid, cytologic evidence of colloidlike extracellular mucin, and an elevated carcinoembryonic antigen (CEA) level support a mucinous cyst.³⁰ Cytomorphologic assessment, however, is required to establish malignancy.³¹⁻³⁴ The two most useful biochemical tests are CEA and amylase. If CEA is low, KRAS molecular testing is helpful for the identification of a mucinous cyst but adds no value if CEA is elevated, which by itself supports a mucinous cyst.³⁵ GNAS may add value by distinguishing an intraductal papillary mucinous neoplasm (IPMN) from a mucinous cystic neoplasm (MCN), given that the latter is resected irrespective of grade.³⁶ KRAS and GNAS do not help in distinguishing benign from malignant lesions.

TABLE 14.1
BIOCHEMICAL AND MOLECULAR TESTS FOR CLASSIFYING PANCREATIC CYSTS

Cyst	CEA	Amylase	KRAS	GNAS
Pseudocyst	↓	↑↑	-	-
Serous cystadenoma	↓	↓	-	-
Intraductal papillary mucinous neoplasm	↑	↑	+	+
Mucinous cystic neoplasm	↑	↓↑	+	-

Accuracy and Limitations

To best guide patient management, the cytologic diagnosis should be correlated with clinical findings and radiologic and ancillary laboratory test results.³⁷⁻³⁸ Given the technical challenges in obtaining an adequate sample and the cytomorphologic interpretive challenges, the sensitivity of EUS-FNA of the pancreas is variable, averaging 80%. Specificity ranges from 60% to 100%.³⁹⁻⁵⁵ Sensitivity improves with increasing technical expertise.^{56, 57} Diagnostic accuracy for solid pancreatic masses is greater than 90%, lower for cystic lesions.^{32,55,58}

Brush cytology has almost 100% specificity but only about 50% sensitivity because of false-negatives due to sampling and interpretation.¹³ Indeterminate (i.e., atypical or suspicious) interpretations result not only from preparation artifact but also from the high threshold needed for an unequivocal malignant interpretation, given that marked atypia on brush cytology is often associated with an inflamed and/or stented duct.^{59,60}

Reporting Terminology

There is no universal standard for reporting pancreatic cytology results, but the following modification of conventional cytologic nomenclature offers a useful framework:

- Negative for malignant cells

- Atypical cells present
- Suspicious for malignancy
- Neoplastic cells present
- Positive for malignant cells
- Nondiagnostic specimen

A *negative* sample is one that contains adequate cellular and/or extracellular tissue to evaluate or define a nonneoplastic lesion identified on imaging. An *atypical* diagnosis reflects a mild degree of cytologic atypia, often in the setting of inflammation, previous duct instrumentation, or sparse cellularity. This interpretation implies a low suspicion of malignancy. A *suspicious* interpretation implies a strong concern for malignancy, usually for ductal adenocarcinoma, but a definitive interpretation is not possible, usually because the cytologic features are quantitatively or qualitatively insufficient. A second opinion can be helpful before issuing a report in such cases; without a definitive diagnosis, an additional procedure might be needed. A *positive* interpretation indicates that the cytologic features meet the criteria for the diagnosis of a malignancy of some type, most commonly ductal adenocarcinoma. A *nondiagnostic* specimen is one that provides no useful information about the lesion sampled. Any cellular atypia precludes a nondiagnostic report.

The *neoplastic* category is for cases that clearly represent a neoplasm. These include premalignant neoplasms (e.g., IPMN; MCN with low-, intermediate-, or high-grade dysplasia) and neoplasms whose risk for metastasis cannot be predicted from cytologic parameters (e.g., pancreatic neuroendocrine tumor (PanNET) and solid-pseudopapillary neoplasm [SPN]). The same is true for a biliary tract counterpart, now called *intraductal papillary neoplasm of the bile ducts* (previously, *intraductal papillary mucinous neoplasm* or *papillomatosis* or *papillary cholangiocarcinoma*).

Normal Pancreas and Bile Duct

The pancreas is rich in parenchyma and poor in stroma. Exocrine acinar tissue is arranged in lobules around a ductal system that increases in caliber as it progresses toward the ampulla. Acinar cells secrete digestive enzymes into this excretory ductal system, which is lined by epithelium that progressively increases in size from cuboidal (in the smaller intralobular ducts) to tall columnar cells (in the larger interlobular ducts). The epithelial cells lining the pancreatic ductal system are generally nonmucinous, although goblet cells are occasionally noted in the main pancreatic duct. Mucinous differentiation, therefore, is evidence of pancreatic intraepithelial neoplasia. Loose connective tissue separates the lobules, and a zone of connective tissue surrounds the ducts and islets.⁶¹



Cytomorphology of benign pancreatic acinar cells

- cohesive, grapelike aggregates, isolated and attached to fibrovascular stroma
- scattered, stripped naked nuclei
- eccentrically placed, round nucleus
- evenly distributed, finely granular chromatin
- small nucleolus; larger in reactive acinar cells
- abundant granular cytoplasm
- indistinct cell borders in clusters

Acinar cells are normally arranged in small rounded (“grapelike”) groups (acini) with a small lumen. They are polygonal, with ample dense, blue-green cytoplasm with a Papanicolaou stain ([Fig. 14.1A](#)) and punctate and purple with a Romanowsky stain. Nuclei are round, with finely textured, evenly distributed chromatin and small nucleoli that enlarge markedly with reactive changes. Nucleoli help to distinguish naked acinar cell nuclei from lymphocytes.

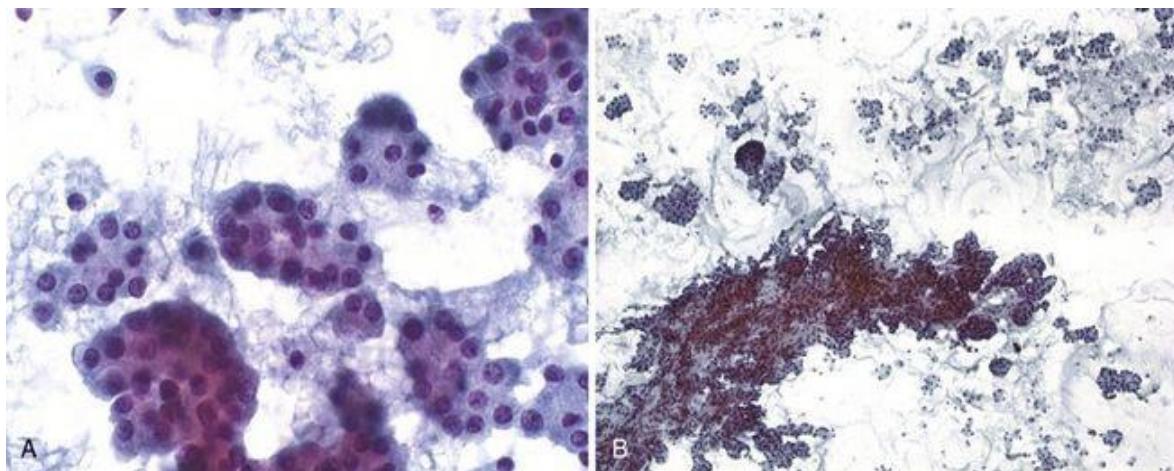


FIGURE 14.1 Normal pancreatic acinar cells.

A, Acinar cells are polygonal cells with abundant granular cytoplasm and eccentrically placed round nuclei (Papanicolaou stain). B, Organoid clustering around loose vascular connective tissue, like “grapes on a vine,” is a characteristic architectural feature (Papanicolaou stain).

Aspirates of benign acinar tissue are often very cellular, which can result in a mistaken impression of a neoplasm. Isolated acini (“grapes”) and clusters of acini attached to fibrovascular tissue (“grapes on a vine”) ([Fig. 14.1B](#)) are characteristic of benign pancreatic acinar tissue. Single cells are also present, but are less prominent than in acinar cell carcinoma.



Cytomorphology of benign ductal cells

- flat, cohesive sheets (few isolated cells)
- even nuclear spacing within sheets
- round to oval nucleus
- evenly distributed, finely granular chromatin
- inconspicuous nucleolus
- well-defined cytoplasmic borders

An FNA specimen of normal pancreas displays fewer ductal cells than acinar cells, whereas pancreatic and bile duct brushings consist mostly of ductal cells, and acinar cells are absent. Ductal cells are cuboidal or columnar and usually arranged as monolayer sheets or strips of cells with a luminal edge of nonmucinous cytoplasm. Monolayer sheets have a uniform, latticelike (“honeycomb”) appearance, with evenly spaced, round, regular nuclei and well-defined cytoplasmic borders ([Fig. 14.2](#)). Nuclei have evenly distributed, finely

granular chromatin and inconspicuous nucleoli that enlarge significantly in reactive conditions. The cytoplasm varies in amount, from scant to moderate, and is dense and nonmucinous with routine stains. Any visible cytoplasmic mucin is pathologic; overtly mucinous ductal cells warrant careful examination to exclude pancreatic intraepithelial neoplasia and well-differentiated adenocarcinoma. With liquid-based preparations, nuclei may appear crenated, a common artifact that is easily recognized with experience.

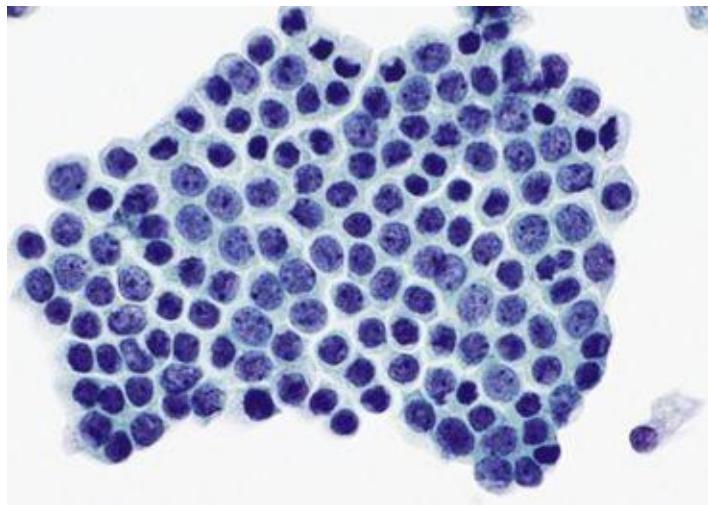


FIGURE 14.2 Normal pancreatic ductal cells.

Normal ductal cells are often arranged in large, cohesive sheets with evenly spaced nuclei that impart a uniform, honeycomb-like appearance (Papanicolaou stain).

Normal islet cells are rarely identified owing to their relative paucity and fragility.

The pancreas is nestled in the retroperitoneum, with its head surrounded by the second portion of the duodenum. It is close to the liver, transverse colon, stomach, spleen, and kidneys; thus it is not unusual for a CT-guided needle to procure normal tissue elements from adjacent organs on its way to the pancreas. Mesothelial cells, hepatocytes, and renal tubular cells are occasionally encountered. Mesothelial cells resemble benign ductal cells: They have a similar flat, monolayer sheetlike arrangement. Because they are relatively large cells that vary in size, they are prone to misinterpretation as adenocarcinoma. Recognizing the characteristic slitlike spaces (“windows”) between adjacent mesothelial cells helps identify them as such.



Cytomorphology of gastrointestinal mucosa

Cytomorphology of duodenal mucosa

- flat, cohesive monolayer sheets with a honeycomb pattern; occasionally papillary groups (intact villi), smaller groups, single cells
- nonmucinous glandular cells with brush border
- sporadically placed goblet cells appear as “fried eggs” within the sheet
- lymphocytes (“sesame seeds”) in the epithelium

Cytomorphology of gastric mucosa

- small sheets and strips; occasional isolated cells and gastric pits
- visible mucin (foveolar cells)
- grooved naked nuclei

With EUS-FNA, cells from the stomach and duodenum are ever-present contaminants.⁶²⁻⁶³ Both duodenal and gastric epithelial cells appear as flat, monolayer sheets with a honeycomb pattern. Duodenal enterocytes are nonmucinous epithelial cells in large sheets studded with goblet cells, which have the appearance of a fried egg on alcohol-fixed preparations, because the centrally placed goblet cell nucleus is surrounded by a clear halo ([Fig. 14.3](#)). Intraepithelial lymphocytes (“sesame seeds”) are sprinkled among the enterocytes. Because most branch-duct IPMNs are lined by gastric-foveolar type cells, the absence of visible mucin and the presence of intraepithelial lymphocytes and a brush border help identify the fragment as duodenal in origin.

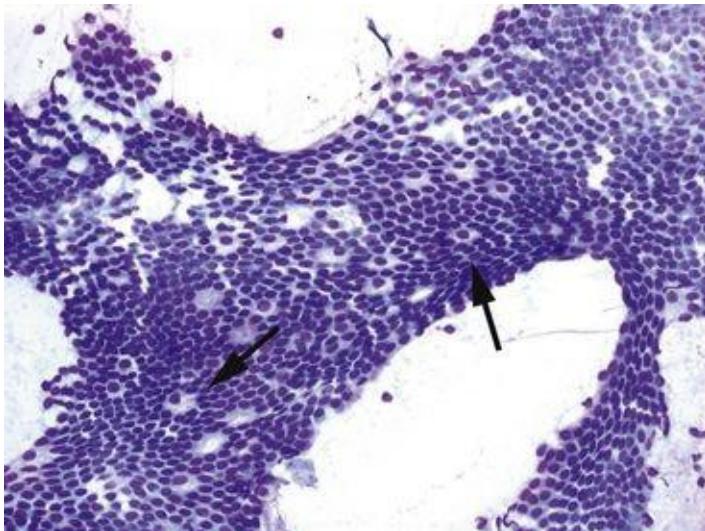


FIGURE 14.3 Duodenal epithelial cells.

Duodenal enterocytes are nonmucinous epithelial cells in large sheets. The sheets are studded with occasional goblet cells that impart a “fried egg” appearance (arrows) (Romanowsky stain).

Gastric surface foveolar cells appear as sheets of cells with mucinous cytoplasm; larger groups contain intact crypts ([Fig. 14.4](#)). Stripped naked nuclei with grooves are also seen. The distinction from low-grade neoplastic mucinous epithelium, particularly that seen in branch-duct cysts that are typically lined by gastric-foveolar type cells, is virtually impossible. Gastrointestinal (GI) epithelium is readily distinguished from a high-grade adenocarcinoma and a high-grade dysplasia of mucinous cysts, however. Reactive epithelium with atypia can resemble adenocarcinoma, leading to a false-positive interpretation. Conversely, a specimen consisting exclusively of epithelial contaminants should be interpreted as *nondiagnostic*; misinterpreting epithelial contaminants as benign ductal cells, for example, can result in a false-negative interpretation.

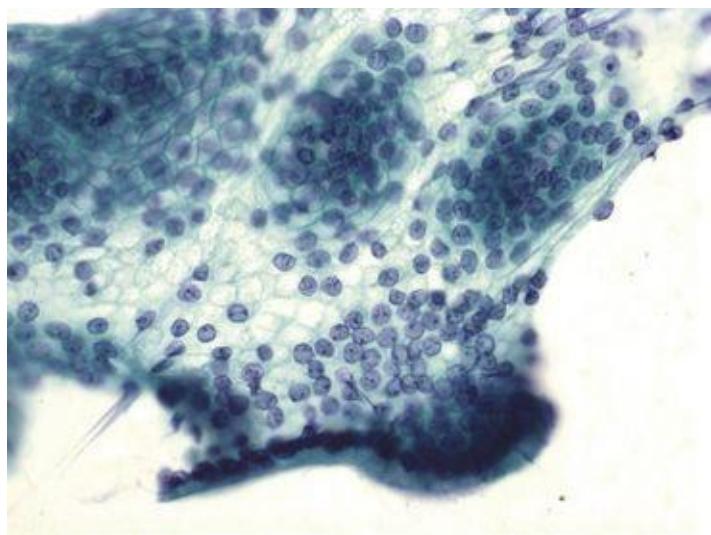


FIGURE 14.4 Gastric epithelial cells.

Surface gastric foveolar cells have mucinous cytoplasm; large sheets may display intact crypts (Papanicolaou stain).

Pancreatitis and Reactive Changes

Acute pancreatitis results from the enzymatic auto-destruction of pancreatic parenchyma. In the United States, it is most often caused by alcohol abuse. Other causes include biliary stones, trauma, and surgery. The diagnosis of acute pancreatitis is based on clinical findings coupled with laboratory evidence, including an elevated white blood cell count and elevated plasma amylase and lipase levels. The radiologic appearance is seldom suggestive of a mass lesion. For these reasons, acute pancreatitis is rarely aspirated. Aspirates demonstrate a background of necrotic debris composed of degenerating cells, foamy histiocytes, fat necrosis, calcifications, and acute inflammation ([Fig. 14.5](#)).

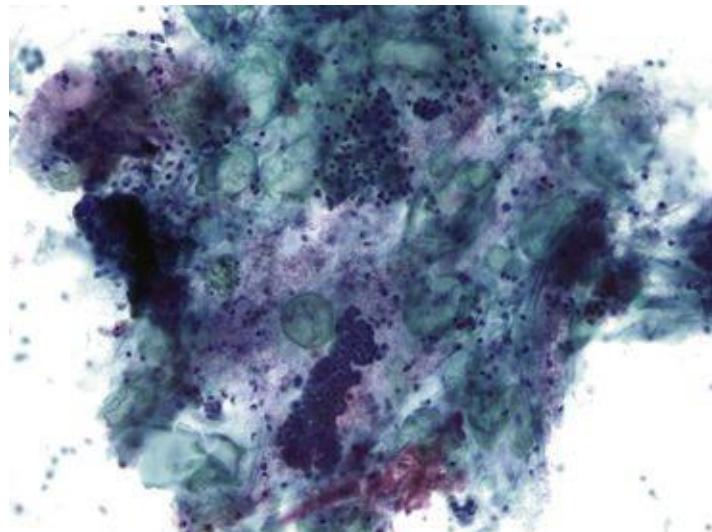


FIGURE 14.5 Acute pancreatitis.

Benign-looking ducts and acini are associated with fat necrosis and inflammation (Papanicolaou stain).

In contrast, late stage *chronic pancreatitis* with mass-forming fibrosis is often indistinguishable radiologically from a neoplastic process. Fibrosis is particularly prominent in *autoimmune pancreatitis* (lymphoplasmacytic sclerosing pancreatitis), a duct-centric inflammatory process dominated by lymphocytes and plasma cells. Autoimmune pancreatitis is important to recognize, as it responds to corticosteroid therapy and does not require surgery.⁶⁴



Cytomorphology of chronic pancreatitis and reactive

ductal atypia

- low cellularity
- background inflammation, fat necrosis, calcific debris
- cellular stromal fragments (especially autoimmune pancreatitis)
- flat, cohesive sheets with evenly spaced or slightly crowded nuclei
- absent (or only rare) isolated atypical cells
- enlarged nuclei, but little variation in size in the same sheet (less than 4:1 diameter ratio)
- round to oval nucleus and smooth nuclear membranes
- prominent nucleoli but not macronucleoli
- occasional mitoses
- low nuclear-to-cytoplasmic ratio

Because its features are not very distinctive, autoimmune pancreatitis is a difficult diagnosis to make by FNA, and the results are rarely definitive. Clues include hypocellularity, cellular stromal fragments with crush artifact, and chronic inflammation with plasma cells ([Fig. 14.6](#)).⁶⁵ Some forms of autoimmune pancreatitis are associated with a predominantly neutrophilic infiltrate.⁶⁶ There can be marked reactive ductal cell atypia, with nuclear crowding and cytoplasmic mucin from an associated pancreatic intraepithelial neoplasia.^{65,67}

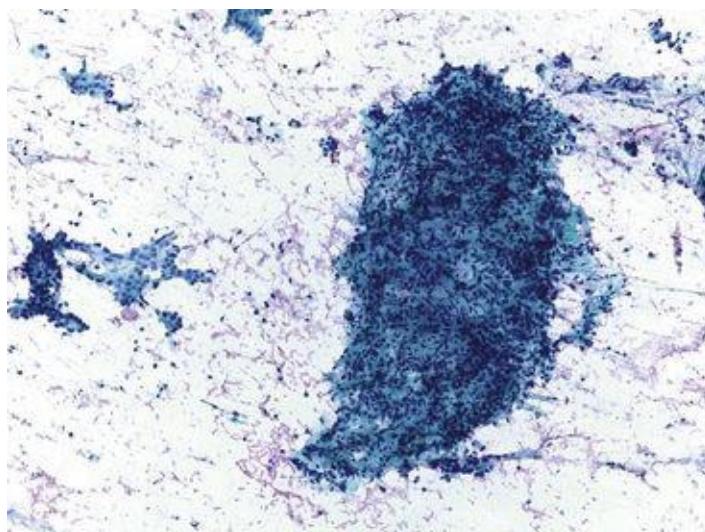


FIGURE 14.6 Autoimmune pancreatitis.

Cellular stromal fragments containing inflammatory cells are a clue to the diagnosis (Papanicolaou stain).

The reactive ductal epithelium with atypia ([Fig. 14.7](#)) that is associated with pancreatitis and cholangitis should be distinguished from ductal adenocarcinoma. A high threshold for malignancy is especially needed for brushings from patients with primary sclerosing cholangitis, primary biliary cirrhosis, stones, or an indwelling stent.^{[13](#)[68](#)[69](#)} Mitoses, prominent nucleoli, and nuclear enlargement are common to both reactive and malignant epithelium. Characteristic malignant features include irregularly distributed chromatin with parachromatin clearing, nuclear membrane irregularity, loss of polarity, nuclear crowding, and significant anisonucleosis: Variation in nuclear diameter more than 3- or 4-fold within a group of cells favors a malignant interpretation.^{[70](#)[71](#)} Because morphologic features of reactive and malignant cells overlap, in borderline cases the most appropriate diagnosis is either “atypical cells present” or “suspicious for malignancy,” the choice depending on the quality and quantity of atypia. Immunohistochemical analysis for *SMAD4* (DPC4) and p53 is helpful in selected cases, inasmuch as half of pancreatic adenocarcinomas show loss of *SMAD4*, and most demonstrate an accumulation of p53, features not seen in chronic pancreatitis.^{[72](#)[73](#)}

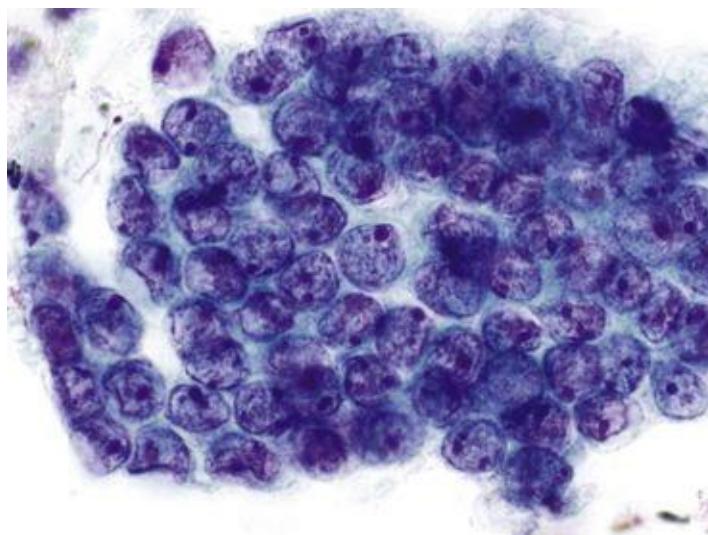


FIGURE 14.7 Reactive (benign) ductal cell atypia.
Nuclear enlargement, crowding, and prominent nucleoli are evident, but there is little variation in nuclear size (Papanicolaou stain).

Pancreatic Intraepithelial Neoplasia

Pancreatic intraepithelial neoplasia (PanIN) is a premalignant mucinous change of pancreatic ducts that includes low-grade atypia (PanIN-1), moderate atypia with stratification and tufting of the epithelium but maintained polarity (PanIN-2), and severe atypia, with malignant-appearing cells confined to the duct (PanIN-3).²⁴ These changes are subclinical and do not create a mass that can be targeted by FNA. But, as a common finding in the setting of chronic pancreatitis and other conditions, PanIN can “contaminate” aspirates and cause false-positive interpretations.^{24,25}

Ductal Adenocarcinoma

Pancreatic ductal adenocarcinoma is the most common tumor of the pancreas, accounting for 85% to 90% of all pancreatic neoplasms.²⁶ Smoking is a well-established risk factor. Ductal adenocarcinoma is a highly aggressive tumor occurring predominantly in individuals aged 60 to 80 years. Presenting symptoms include abdominal pain, jaundice, pruritus, and unexplained weight loss. Radiologically, most ductal adenocarcinomas appear as poorly defined, hypodense (on CT) or hypoechoic (on EUS) masses that distort the normal lobular architecture of the pancreas.²⁷ Because most (60% to 70%) are located in the pancreatic head, they are commonly associated with pancreatic and bile duct stricture, with downstream dual dilatation of both ducts (“double-duct” sign).²⁷ Secondary cystic change occurs sometimes, obscuring the solid appearance and causing confusion with cystic tumors, primarily IPMN.

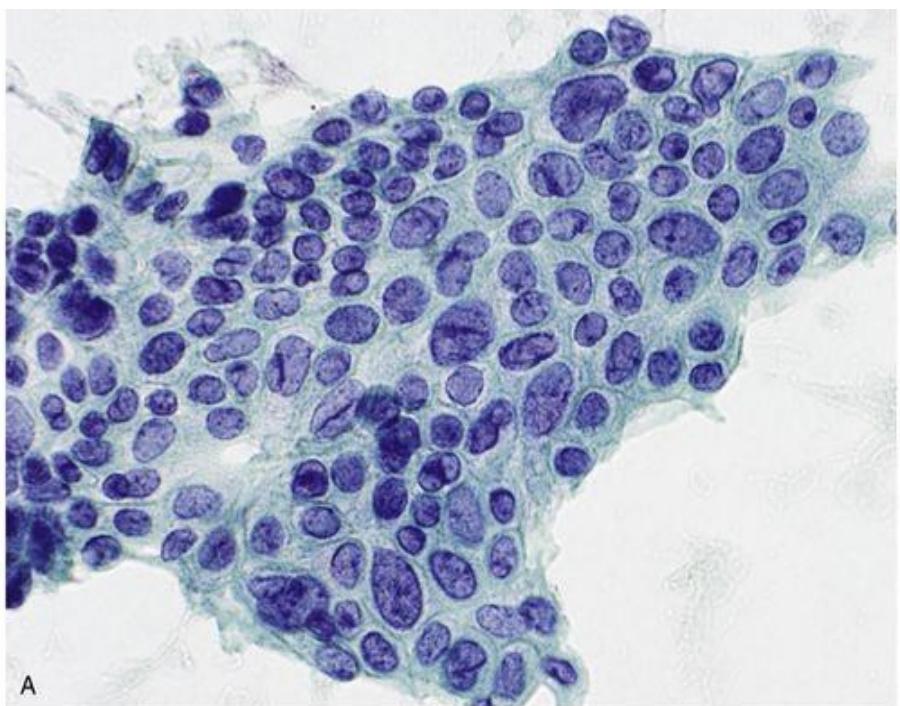
Histologically, most ductal adenocarcinomas are well to moderately differentiated, consisting of large, medium-sized, or small malignant ducts that infiltrate a desmoplastic stroma. Infiltrating tubular architecture is most common, and reactive myxoid or desmoplastic stroma is a key to correct interpretation.



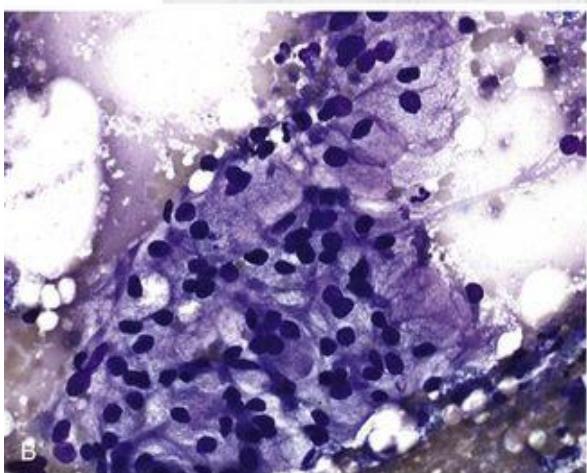
Cytomorphology of ductal adenocarcinoma

- moderate to high cellularity
- irregularly sized and shaped clusters and isolated cells
- uneven distribution of ductal cells within a sheet (“drunken honeycomb”)
- isolated malignant cells
- irregular nuclear contours (notches, grooves, convolutions)
- nuclear enlargement
- anisonucleosis (greater than 4:1 variation in diameter within a single sheet)
- irregular chromatin distribution (clumping and parachromatin clearing)
- mitoses
- scant or abundant mucinous cytoplasm

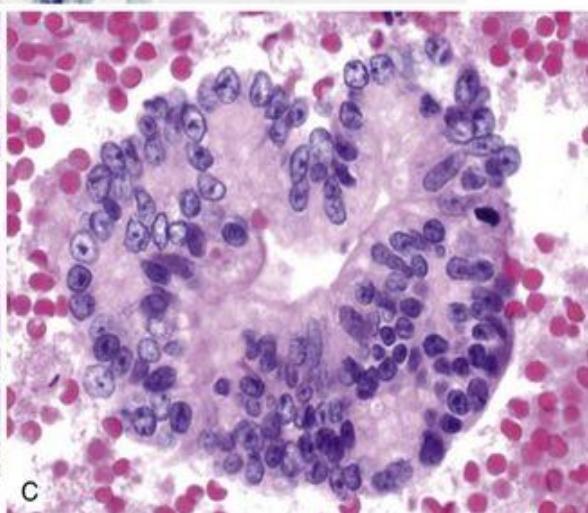
The cells of a well-differentiated ductal adenocarcinoma are large and columnar, often with abundant pale, mucinous cytoplasm. Cells in sheets lose the evenly spaced, latticelike distribution of benign ductal cells, becoming disarranged (dubbed a “drunken honeycomb”).⁷⁸ Marked anisonucleosis (4:1 or higher variation in diameter among nuclei within a group or sheet) and visible cytoplasmic mucin are very helpful features^{70,79} ([Fig. 14.8A](#)). Some well-differentiated carcinomas have abundant, “foamy” cytoplasm, yielding a deceptively low nuclear-to-cytoplasmic ratio (“foamy gland adenocarcinoma”)^{80,81} ([Fig. 14.8B](#)). Cell blocks from sedimented fluid and/or core biopsy specimens help by highlighting cribriform architecture ([Fig. 14.8C](#)) and invasion of desmoplastic stroma ([Fig. 14.8D](#)).



A



B



C

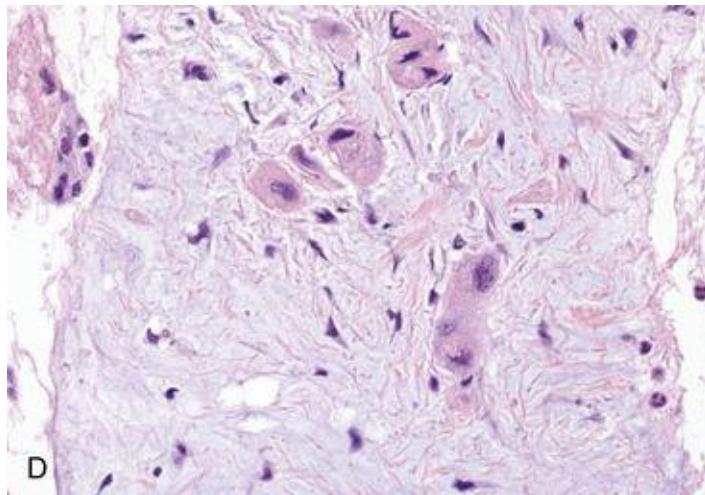


FIGURE 14.8 Ductal adenocarcinoma.

A, The uneven distribution of nuclei in the sheet (“drunken honeycomb”) and the variation in nuclear diameters (anisonucleosis) exceeding a ratio of 4:1 are characteristic features (Papanicolaou stain). B, “Foamy gland” adenocarcinoma is deceptively bland owing to the abundance of mucinous cytoplasm and resulting in a low nuclear-to-cytoplasmic ratio (Romanowsky stain). C, Cell block preparations may demonstrate cribriform glands with nuclear atypia (hematoxylin-eosin [H & E] stain). D, Cell blocks can also show invasive glands or cells in a desmoplastic stroma (H & E stain).

High-grade ductal adenocarcinoma ([Fig. 14.9](#)) shows more overt features of malignancy and is more easily distinguished from reactive ductal epithelium and GI contamination. Slides reveal marked variation in cell size, loss of polarity, large nucleoli, numerous isolated cells, necrosis, and mitoses.

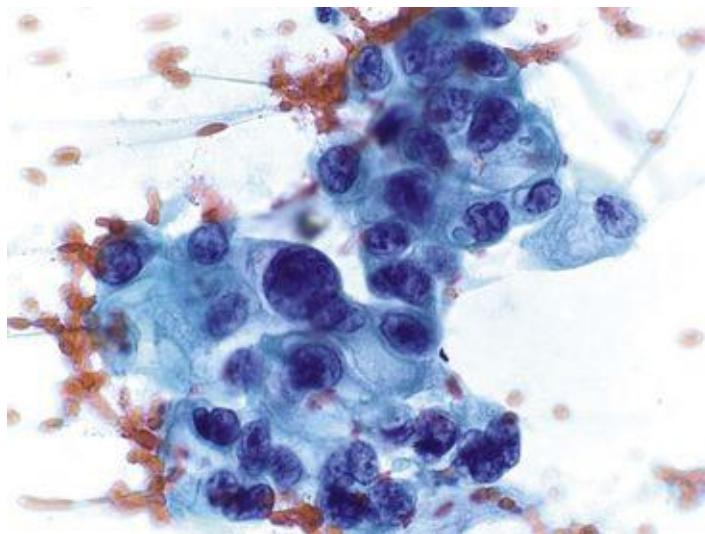


FIGURE 14.9 Ductal adenocarcinoma.

High-grade carcinoma shows more overt features of malignancy with marked nuclear

pleomorphism, hyperchromasia, and irregular nuclear membranes (Papanicolaou stain).

Cholangiocarcinomas are morphologically indistinguishable from pancreatic ductal adenocarcinomas.



Differential diagnosis of ductal adenocarcinoma

- chronic pancreatitis, especially autoimmune pancreatitis
- radiation changes
- reactive and reparative atypia
- metastatic adenocarcinoma

Several benign conditions cause ductal cell atypia that mimics ductal adenocarcinoma. Chronic pancreatitis, especially autoimmune and radiation-induced, often results in ductal atypia, including nuclear enlargement and prominent nucleoli. Background elements like fat necrosis and calcific, inflammatory debris, should raise the possibility of pancreatitis.

Biliary and pancreatic duct stents cause irritation and reactive/reparative ductal cell changes, with enlarged nuclei and nucleoli (see [Fig. 14.7](#)). A high threshold for malignancy is required in the evaluation of specimens from patients with a current or recent stent and those with primary sclerosing cholangitis and primary biliary cirrhosis. Nuclear membrane irregularity, an increased nuclear-to-cytoplasmic ratio, isolated atypical cells, and marked anisonucleosis (4-fold or greater differences in nuclear size) are the more reliable indicators of malignancy. A definite distinction between reactive atypia and ductal adenocarcinoma is not possible in all cases. For cases with equivocal findings, an atypical or suspicious interpretation is appropriate.

Immunohistochemistry for p53 and SMAD4 can help establish a malignant interpretation, because half of pancreatic adenocarcinomas show loss of SMAD4, and most demonstrate an accumulation of p53, features not seen in normal cells or chronic pancreatitis ([Fig. 14.10A and B](#)).^{82,83} Loss of nuclear expression of SMAD4 can also distinguish pancreatic adenocarcinoma from other adenocarcinomas, because SMAD4 is rarely lost in adenocarcinomas of the ovary, colon, endometrium, and lung.²³ The evaluation of these markers can be difficult, however, in limited samples. Fluorescence *in situ* hybridization (FISH) is also useful but not commonly available.^{71,84-86} To date, molecular tests have not found wide application to diagnosis. KRAS mutations, however, are

found in just under half of low-grade PanIN cases, and their frequency increases with increasing degrees of dysplasia.⁸⁷

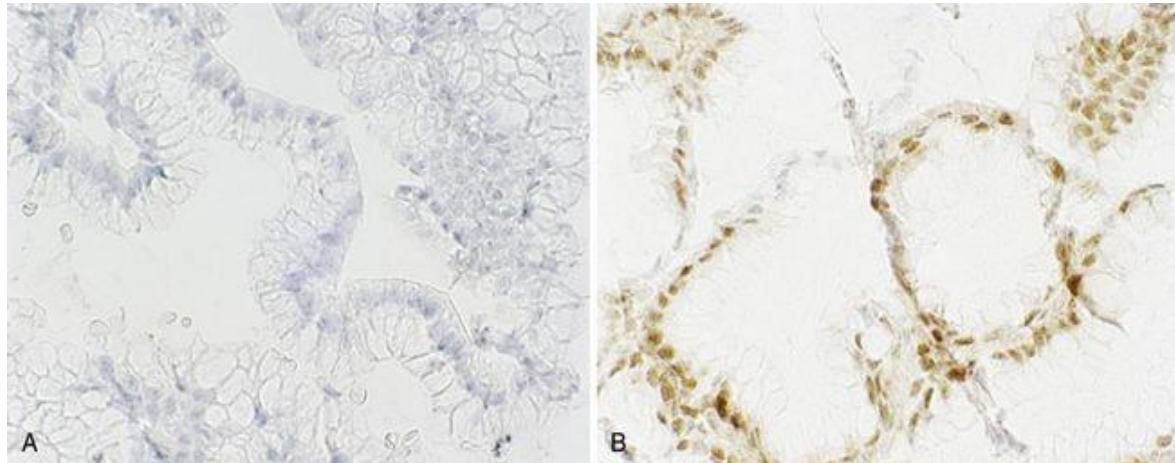


FIGURE 14.10 SMAD4 as a helpful marker of ductal adenocarcinoma.

A, The loss of SMAD4 expression in these atypical cells supports the diagnosis of adenocarcinoma. B, Elsewhere in the cell block from this case, benign gastric foveolar cells act as an internal control by showing normal nuclear SMAD4 expression.

Variants of Ductal Adenocarcinoma

The two most common variants of pancreatic ductal adenocarcinoma are *adenosquamous carcinoma* (Fig. 14.11A), accounting for 3% to 4% of all pancreatic malignancies, and *undifferentiated (anaplastic) carcinoma* (Fig. 14.11B), accounting for 0.3% to 10%.²² The proportions of the glandular and squamous components of an adenosquamous carcinoma vary widely from one tumor to another, and a metastasis from a primary elsewhere needs to be considered.²³ Undifferentiated carcinomas lack glandular differentiation. FNAs yield highly cellular specimens with pleomorphic epithelioid and spindled cells and bizarre multinucleated tumor giant cells.²⁴ The giant cell nuclei resemble those of the mononuclear tumor cells. In contrast, *undifferentiated carcinoma with osteoclast-like giant cells* is characterized by benign osteoclast-type giant cells admixed with usually pleomorphic mononuclear cells. In some cases, the mononuclear malignant component of the tumor appears very bland. The mononuclear tumor cells generally express vimentin, but they can also express cytokeratin. KRAS mutations, however, are found in about 90% of the mononuclear component of the tumors, and TP53 mutations in about half, whereas these mutations are not found in the giant cell component.^{22,24} The cytologic features of the other variants (*colloid carcinoma*, *hepatoid carcinoma*, *medullary carcinoma*, and *signet ring cell carcinoma*) demonstrate features that recapitulate their histopathology.

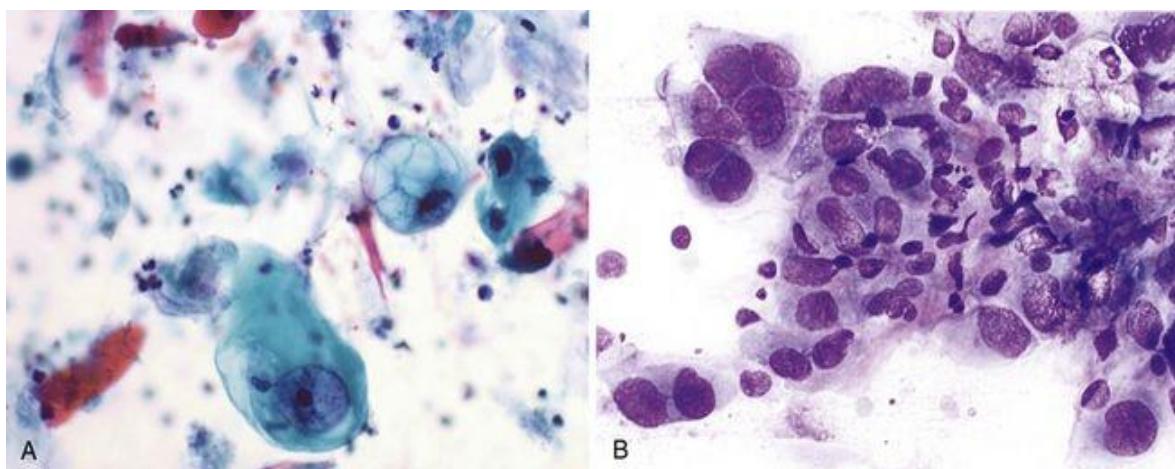


FIGURE 14.11 Ductal carcinoma variants.

A, Adenosquamous carcinoma is composed of a combination of malignant glandular and

squamous cells. The malignant squamous component, which sometimes predominates, is characterized by dense, sometimes orange cytoplasm; the glandular component is manifest by cells with vacuolated, mucinous cytoplasm (Papanicolaou stain). *B*, Undifferentiated carcinomas consist of large epithelioid or spindle-shaped cells (Romanowsky stain).

Pancreatic Neuroendocrine Tumor

Also known as *islet cell tumor*, *pancreatic endocrine neoplasm (PEN)*, and *pancreatic endocrine tumor*, this tumor is called *pancreatic neuroendocrine tumor (PanNET)* in the WHO 2010 Classification of Neoplasms of the Digestive System, to be consistent with similar tumors elsewhere in the GI tract.⁹¹ Uncommon tumors, they represent only 1% to 2% of all pancreatic neoplasms. They are histologically separated into well-differentiated (low-and intermediate-grade) tumors and poorly differentiated (high-grade) neuroendocrine carcinomas. By histopathologic definition, a carcinoma has greater than 20 mitoses per 10 high-power fields (hpf) and is subclassified into small cell and large cell types.

PanNETs can be functional or nonfunctional. A majority of functional tumors secrete one of the following hormones: insulin, glucagon, somatostatin, vasoactive intestinal polypeptide (VIP), pancreatic polypeptide, serotonin, adrenocorticotrophic hormone (ACTH), or calcitonin. Owing to excess hormone secretion, patients with a functional tumor can develop life-threatening signs and symptoms such as hypoglycemia, GI ulcers, and diarrhea with dehydration. Functional tumors are typically detected earlier than nonfunctional ones and are smaller at resection. Insulinomas are often less than 1 cm in size and usually follow a benign course. Most PanNETs, however, are biologically aggressive, 65% to 80% demonstrating unequivocal features of malignancy.⁹¹

Although PanNETs occur at any age, they are most common in adults (mean age around 40 years). They tend to be small, usually around 1 to 3 cm in diameter, but biologic behavior is not correlated with tumor size. Like acinar cell carcinoma and solid pseudopapillary neoplasm (and unlike ductal adenocarcinomas), PanNETS tend to be circumscribed masses. They can be partially cystic but are completely cystic in only 4% of cases, which can lead to misclassification as a primary pancreatic cyst by imaging studies.⁹²

Most PanNETs are well differentiated, and the unqualified term *PanNET* is assumed to mean a well-differentiated tumor. Although certain histologic features predict malignant behavior (e.g., invasion of adjacent structures and vessels), no features are capable of predicting benign behavior. Of note, cytologic atypia does not correlate with malignant behavior, with the exception of high-grade small and large cell neuroendocrine carcinomas.

Although all neuroendocrine tumors of the pancreas larger than 0.5 cm in diameter are potentially malignant,⁹³ it is common cytologic practice to use

“neoplasm” or “neoplastic cells present” in reporting these tumors, for consistency with the histologic classification. The most reliable indicator of malignancy in the case of a well-differentiated PanNET is the presence of metastasis or invasion of adjacent structures.^{22,91}

Histologically, tumor cells show a variety of growth patterns: solid, trabecular, and gland-forming. Cells are usually monomorphic, with a round or oval nucleus. Nucleoli are usually indistinct, but they can be prominent. The cytoplasm is typically granular, but rare cases have predominantly clear and vacuolated or dense and oncocytic cytoplasm. Amyloid is seen in some cases, more commonly with insulinomas. Somatostatinomas, particularly those that arise in the duodenum, often show gland formation with psammoma bodies.



Cytomorphology of pancreatic neuroendocrine tumor

- highly cellular aspirate with solid-cellular smear pattern
- predominantly isolated cells, bare nuclei
- pseudorosettes and small clusters
- uniform, round/oval nuclei
- eccentric nuclei (plasmacytoid appearance)
- finely stippled (“salt and pepper”) chromatin
- moderate to abundant cytoplasm, typically granular but may be vacuolated or oncocytic

PanNETs are usually sampled by FNA rather than duct brushings, because these tumors rarely invade the pancreatic ducts. FNA of a well-differentiated PanNET yields a highly cellular “solid-cellular” smear pattern with abundant isolated cells and bare nuclei ([Fig. 14.12A](#)), loosely cohesive groups, and occasional pseudorosettes ([Fig. 14.12B](#)). Multilayered fragments with delicate capillaries can also be seen. The cells are monomorphic, with an intermediate-size, round to oval nucleus that is often eccentrically positioned within the cell, imparting a plasmacytoid appearance. The chromatin is finely stippled and evenly dispersed (“salt and pepper”) ([Fig. 14.12C](#)). Nucleoli can be prominent. The cytoplasm is usually finely granular or dense, with ill-defined cell borders; red cytoplasmic granules can be seen with Romanowsky stains. A rare mitotic figure and/or an occasional very large, pleomorphic cell can be present (“endocrine atypia”), but cytologic atypia does not predict malignant behavior. Diffusely vacuolated cytoplasm is a hallmark of the “lipid-rich” PanNET ([Fig.](#)

[14.13A](#)),²⁴ which resembles metastatic renal cell carcinoma ([Fig. 14.13B](#)).

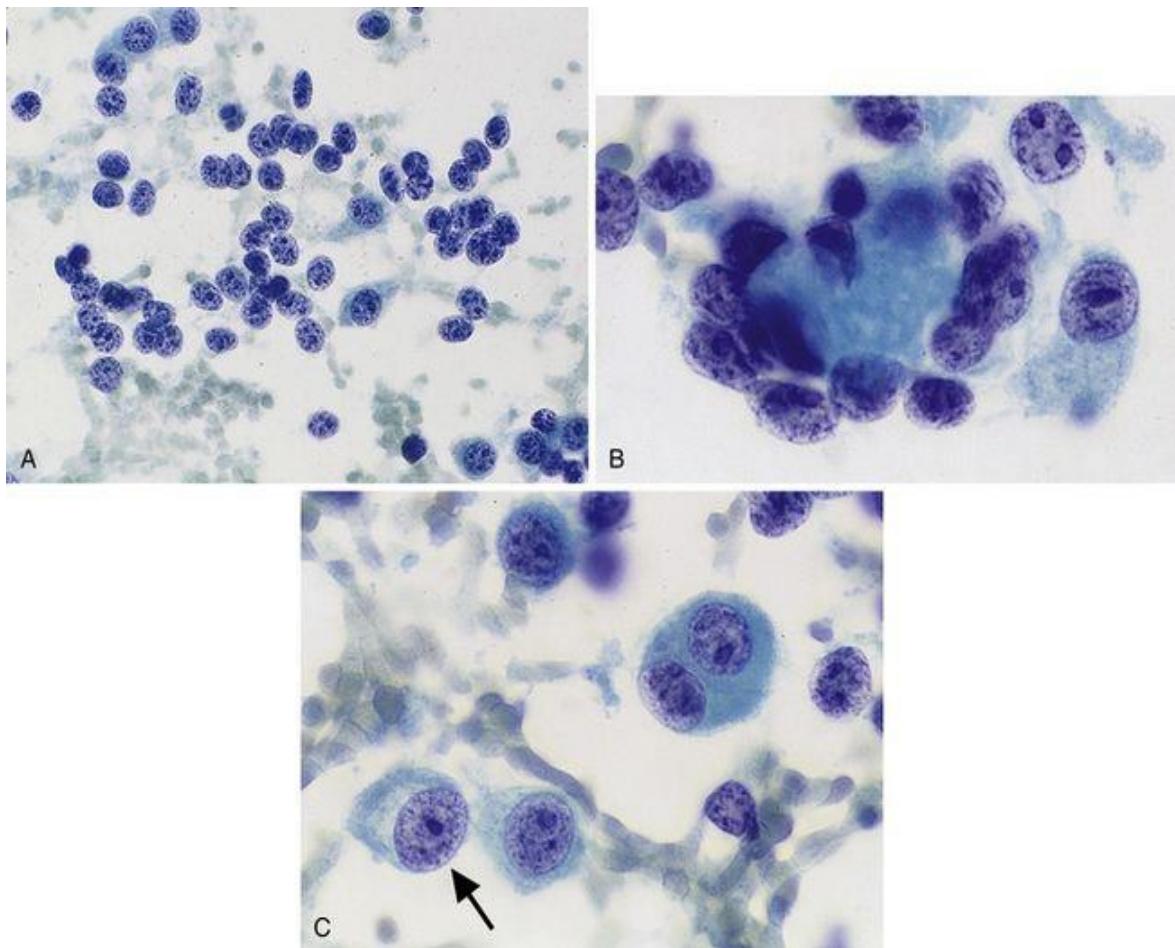


FIGURE 14.12 Pancreatic neuroendocrine tumor (PanNET).

A, Tumor cells are dispersed as isolated cells and bare nuclei (Papanicolaou stain). B, Some cells are arranged in a pseudorosette (Papanicolaou stain). C, Finely stippled (“salt and pepper”) chromatin, occasional binucleation, and a plasmacytoid appearance (arrow) are characteristic (Papanicolaou stain).

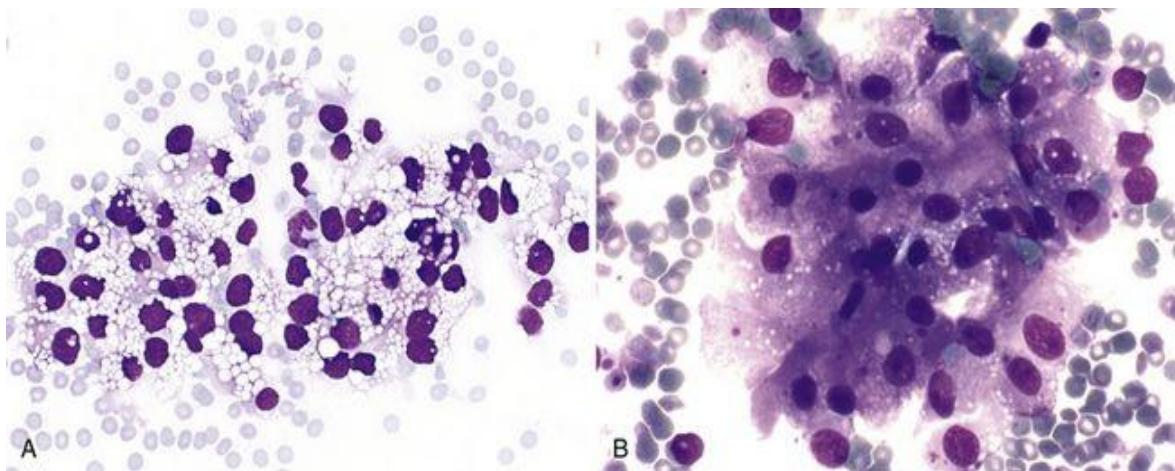


FIGURE 14.13 *A*, Lipid-rich pancreatic neuroendocrine tumor (PanNET). Abundant vacuolated cytoplasm is a hallmark of the lipid-rich variant; care must be taken to distinguish this tumor from metastatic renal cell carcinoma (Romanowsky stain). *B*, Metastatic renal cell carcinoma. Polygonal cells with round central nuclei, prominent nucleoli, and punched-out cytoplasmic vacuoles are characteristic. The features are similar to those of the lipid-rich pancreatic PanNET (Romanowsky stain).

The proliferative rate is used to grade PanNETs because it provides useful prognostic information and may influence clinical management. The proliferative rate of PanNETs is assessed as the number of mitoses per 10 hpfs: Low grade tumors have less than 2 mitoses per 10 hpfs, intermediate grade tumors 2 to 20 per 10 hpfs, and high-grade tumors greater than 20 per 10 hpfs. The recommendation is to count 40 or 50 hpfs, more than is found in most cell blocks from FNAs. Staining for Ki67 (MIB-1) is a useful surrogate because it provides an accurate assessment of the proliferative rate and is readily applicable to smaller specimens. The Ki67 index is less than 3% for low-grade, 3% to 20% for intermediate-, and greater than 20% for high-grade tumors⁹⁵ (Fig. 14.14A and B). A grade assigned on the basis of FNA or core biopsy findings, however, needs to take into account the possibility that a higher grade focus may not have been sampled.

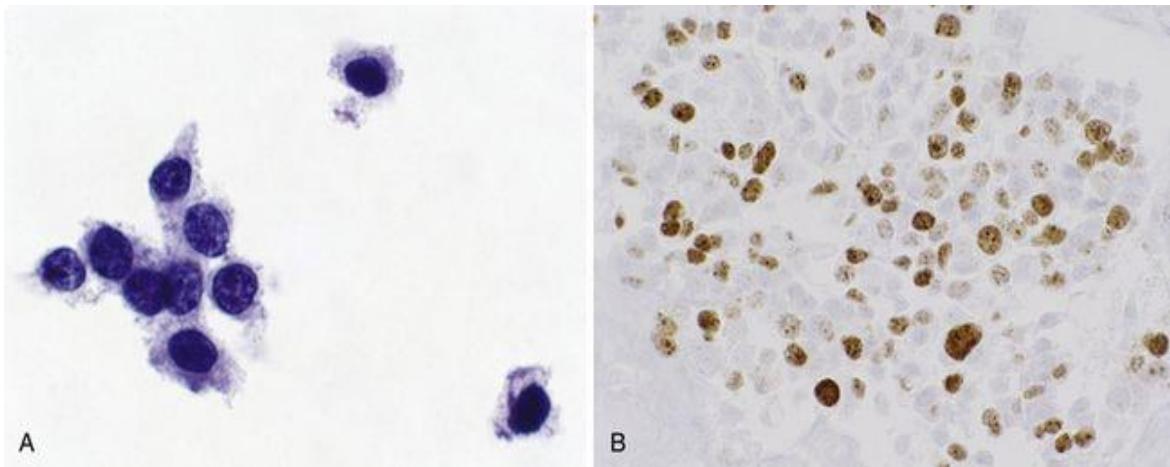


FIGURE 14.14 High-grade pancreatic neuroendocrine tumor (PanNET) (carcinoma). *A*, Monomorphic plasmacytoid cells are present, some showing degenerative changes. *B*, The Ki-67 (MIB-1) proliferation index of this tumor was estimated at 50% to 60%, placing it in the high-grade category.

Differential diagnosis of well-differentiated (low-and intermediate-grade) pancreatic neuroendocrine tumor

- acinar cell carcinoma (ACC)
- solid-pseudopapillary neoplasm (SPN)
- metastatic neuroendocrine carcinoma
- non-Hodgkin lymphoma
- melanoma
- plasmacytoma
- metastatic renal cell carcinoma

The differential diagnosis of a well-differentiated PanNET includes other parenchyma-rich, stroma-poor pancreatic neoplasms, primarily ACC, pancreatoblastoma, and SPN. Given the overlap in the cytologic appearance of these neoplasms, the distinction often relies on immunohistochemistry ([Table 14.2](#)). PanNETs are positive for the endocrine markers chromogranin, synaptophysin, and CD56. Some PanNETs also express pancreatic hormones (e.g., insulin, glucagon, somatostatin, VIP, or gastrin), but correlation between immunohistochemical expression and clinical symptoms is imprecise, and tissue documentation of hormone expression is rarely needed.

TABLE 14.2

DIFFERENTIAL IMMUNOHISTOCHEMICAL PROFILES OF THE SOLID-CELLULAR PANCREATIC TUMORS

Marker	Pancreatic Neuroendocrine Tumor	Acinar Cell Carcinoma	Solid-Pseudopapillary Neoplasm	Pancreatoblastoma
Pankeratin	+	+	-/focal	+
Trypsin	-	+	-	+
Chromogranin	+	-/focal	-	+/-
Synaptophysin	+	-/focal	-	+/-
CD56	+	-/focal	+	+/-
β -Catenin	-	weak/focal	+ (nuclear)	weak/focal

Islet 1 and PAX8 are relatively specific markers of PanNET as compared with other NETs and thus particularly helpful in distinguishing PanNETs from small bowel neuroendocrine tumors (i.e., carcinoids) in metastatic foci like the liver.^{96,97}

The differential diagnosis also includes other tumors that manifest with a dispersed, plasmacytoid cell pattern, like non-Hodgkin lymphoma, plasmacytoma, and melanoma. PanNETs lack the lymphoglandular bodies typical of lymphoid proliferations, and lymphoid cells have much less cytoplasm and a more finely dispersed chromatin pattern. Immunohistochemistry for S-100 protein, HMB45, leukocyte common antigen, and κ and λ light chains helps distinguish PanNET from melanoma and plasmacytoma.

Poorly differentiated neuroendocrine carcinomas are uncommon. Small cell neuroendocrine carcinoma of the pancreas resembles small cell carcinoma of the lung and, like its lung counterpart, may express thyroid transcription factor-1 (TTF-1).

Acinar Cell Carcinoma

ACC phenotypically resembles pancreatic acini and produces pancreatic enzymes. It accounts for less than 2% of pancreatic exocrine tumors and tends to occur in older adults (mean age, 62 years) but is occasionally seen in children and adolescents. It carries a poor prognosis, with an overall 5-year survival of less than 10%.⁹⁸ Tumors vary in size and can be bulky. ACCs differ from ductal adenocarcinomas in that they are usually well circumscribed, occasionally multinodular, and rarely cystic. Histologically, ACCs show an acinar or solid growth pattern. Tumor cells contain scant to moderate amounts of granular cytoplasm. Nuclei are round or oval, quite uniform, typically with a single prominent nucleolus.



Cytomorphology of acinar cell carcinoma

- highly cellular
- solid-cellular pattern of monomorphic cells:
 - numerous isolated cells
 - loose cell aggregates
 - naked tumor nuclei
 - loose granules in the background
- round or oval nucleus
- smooth nuclear contour
- prominent nucleolus (usually)
- delicate granular cytoplasm

FNA yields loose aggregates, crowded acinar-like arrangements, and isolated polygonal cells with moderately abundant granular cytoplasm ([Fig. 14.15A](#)).^{99,100} Stripped naked nuclei are common, and the spilled zymogen granules are noted in the background, a distinctive feature that helps to distinguish ACC from other solid and cellular neoplasms like neuroendocrine tumors and solid-pseudopapillary neoplasms ([Fig. 14.15B](#)). Nuclei are round to oval, eccentrically placed, and generally larger than those of normal acinar cells. They have smooth contours, coarse chromatin, and usually (but not always) one prominent nucleolus. Toluidine blue and periodic acid–Schiff (PAS) stains highlight the cytoplasmic granules. Immunohistochemical stains for pancreatic enzymes trypsin, lipase, chymotrypsin, and phospholipase A2 are typically positive. The

most specific marker, however, is trypsin, usually the only marker necessary to confirm exocrine differentiation.¹⁰¹

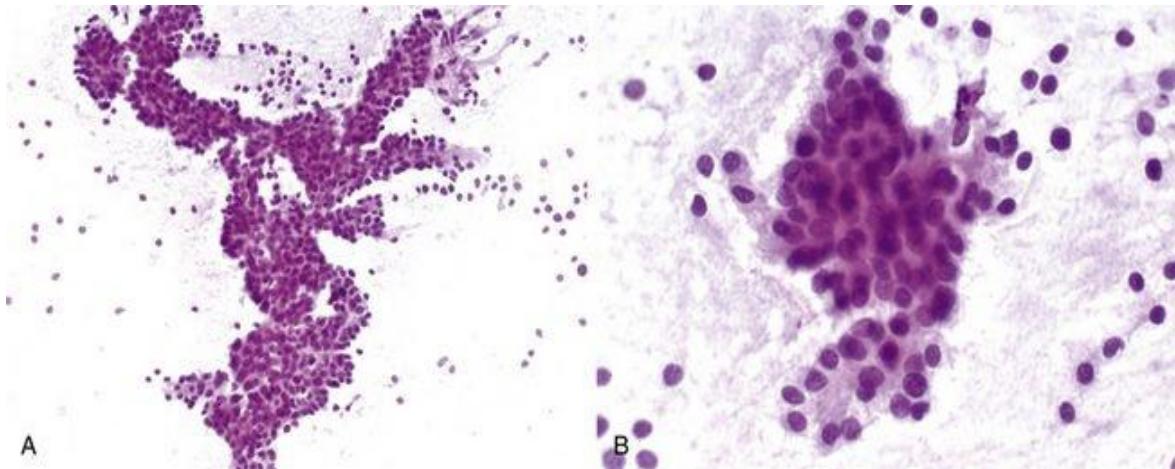


FIGURE 14.15 Acinar cell carcinoma (ACC).

A, ACCs recapitulate acinar architecture, but the sharp “grapelike” clustering of normal acini is blunted, resulting in solid, irregularly shaped cell clusters (hematoxylin-eosin [H & E stain]). B, Acinar carcinoma cells are fragile; spilled zymogen granules from disrupted cytoplasm are characteristic and help distinguish this tumor from a neuroendocrine tumor (NET) (H & E stain).



Differential diagnosis of acinar cell carcinoma

- benign acinar cells
- PanNET
- solid-pseudopapillary neoplasm (SPN)

Because of its rarity, ACC can be a challenge to recognize. First, one must distinguish the cells of ACC from *benign acinar cells*. This is relatively straightforward, because the cells of ACC are markedly noncohesive: The tight, “grapelike” clustering of normal acinar tissue is lost (see Fig. 14.1B). Moreover, benign acinar cells, unlike their malignant counterparts, usually have small, inconspicuous nucleoli. More difficult is the distinction from the other solid-cellular neoplasms, *PanNET* and *SPN*,^{37,99,100} which, like ACC, consist of monomorphic, mostly noncohesive, isolated cells. Both ACC and PanNET have relatively abundant granular cytoplasm and prominent nucleoli, and the loosely acinus-like aggregates of ACC cells resemble the rosettes of a PanNET. The

distinctive vascular and papillary pattern of SPNs is not always apparent, but these neoplasms generally have small chromocenters without a prominent nucleolus, and cytoplasm is usually scant and ill-defined.

Immunohistochemistry plays an essential role in diagnosis ([Table 14.2](#)). Both ACCs and PanNETs are usually strongly reactive for cytokeratin, whereas SPNs are generally negative. ACCs are distinguished by reactivity for pancreatic enzymes, especially trypsin; other exocrine markers like chymotrypsin, antichymotrypsin, and alpha-1-antichymotrypsin are less specific. PanNETs are almost always strongly reactive for at least one of the neuroendocrine markers synaptophysin, chromogranin, and CD56, and they are often reactive for islet1 and polyclonal PAX8. Weak or focal staining for neuroendocrine markers is not sufficient to confirm the diagnosis of PanNET, as this may be seen in some ACCs. CD56 is positive in both SPNs and PanNETs. The distinguishing marker of SPNs is nuclear staining for β -catenin.

Solid-Pseudopapillary Neoplasm

SPN is an uncommon tumor that accounts for 1% to 2% of pancreatic exocrine neoplasms. SPNs occur almost exclusively in young women (about 90%; mean age 28 years), but they are seen in men and older adults. There is no preferential location in the pancreas. Although SPNs are often incidental findings, presenting symptoms include abdominal pain and discomfort, early satiety, and nausea and vomiting.²¹ SPN is a tumor of low malignant potential.²² Histologic features of aggressive behavior include perineural invasion, vascular invasion, and invasion of adjacent structures, but some tumors without these features also metastasize. Most tumors are treated successfully with conservative surgical resection.¹⁰²

Like acinar cell carcinoma, SPN is usually a well-circumscribed mass with variable amounts of solid and cystic components. By imaging, the cystic spaces of SPN show no septations, in contrast with the mucinous cystic neoplasm, a principal differential diagnostic consideration in a young woman with a pancreatic cyst. Histologically, solid and pseudopapillary cell arrangements are admixed with cyst debris from necrosis. Cholesterol crystals, calcifications, and (rarely) ossification are present. Delicate vessels with a hyalinized or myxoid stroma, surrounded by loosely arranged tumor cells, are a common feature, sometimes resembling ependymal rosettes. The neoplastic cells show little pleomorphism or anisonucleosis: They have round or oval, occasionally bean-shaped nuclei, finely textured chromatin, nuclear grooves, and pale or granular cytoplasm. The cytoplasm is often finely vacuolated, with perinuclear vacuoles and scattered PAS-positive, diastase-resistant hyaline globules.



Cytomorphology of solid-pseudopapillary neoplasm

- highly cellular aspirate with solid-cellular pattern
- myxoid or hyalinized vascular stalks lined by neoplastic cells
- delicate, finely vacuolated cytoplasm with indistinct cell borders:
 - perinuclear vacuoles
 - PAS-D-positive hyaline globules
- round/oval, bean-shaped nuclei
- nuclear grooves
- inconspicuous nucleoli
- foam cells and necrotic debris

FNA yields abundant monomorphic cuboidal nonmucinous cells arranged as loosely cohesive groups, isolated cells, and, most characteristically, a single or multiple layer around vascular structures expanded by myxoid or hyaline material ([Fig. 14.16A](#)). The tumor cells have delicate vacuolated cytoplasm with indistinct cell borders. The nuclei are round to oval with finely dispersed chromatin, smooth or grooved nuclear contours, and indistinct nucleoli ([Fig. 14.16B](#)). The background may contain abundant blood, foam cells, globules of amorphous myxoid material, and necrotic debris.

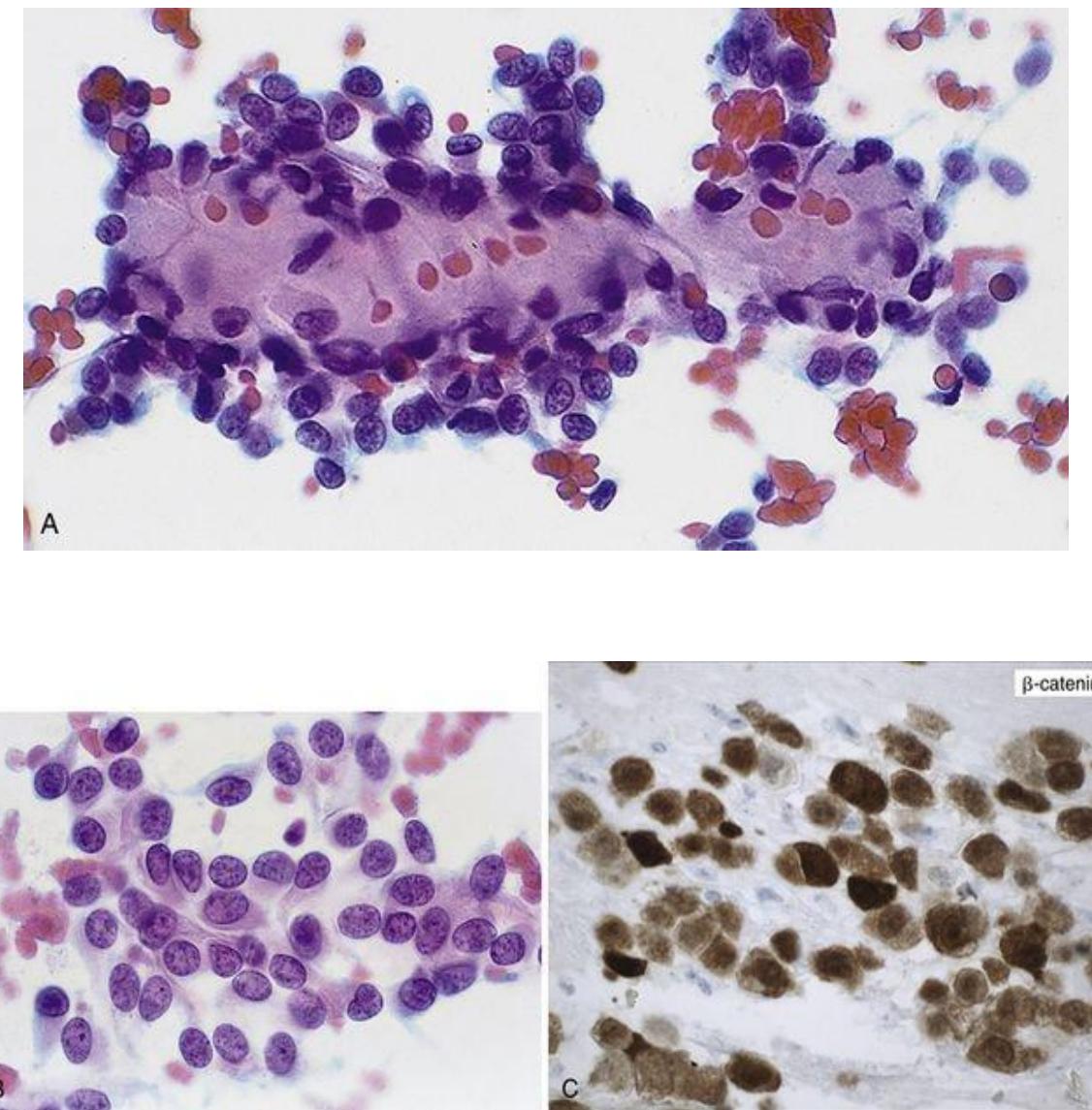


FIGURE 14.16 Solid-pseudopapillary neoplasm (SPN).

A, Tumor cells surround a thick stalk with myxoid change (Romanowsky stain). B, Tumor cells have finely granular chromatin and an indistinct nucleolus (Papanicolaou stain). C,

Tumor cells show nuclear immunoreactivity for β -catenin.

SPNs are immunoreactive for $\alpha 1$ -antitrypsin, $\alpha 1$ -antichymotrypsin, progesterone receptor, CD56, CD10, and *KIT* (CD117).^{22,103} Inconsistent results have been reported for epithelial markers (cytokeratin can be positive, but staining is usually weak and focal), synaptophysin, pancreatic enzymes, and islet cell hormones. An alteration in the *APC*/ β -catenin pathway leads to cytoplasmic and nuclear accumulation of the protein β -catenin in over 95% of tumors¹⁰⁴ ([Table 14.2](#)). Thus, nuclear immunoreactivity for β -catenin is the key diagnostic marker of SPN ([Fig. 14.16C](#)).

The differential diagnosis includes other solid and cellular neoplasms, in particular acinar cell carcinoma and PanNET (see box, “[Differential diagnosis of acinar cell carcinoma](#),” earlier). Pseudopapillary structures with myxoid or hyalinized vascular stalks and the typical clinical presentation (usually young women) are keys to the correct interpretation. Nuclear immunoreactivity for β -catenin helps support the cytomorphologic impression.

Pancreatoblastoma

Pancreatoblastoma is a rare malignant epithelial tumor primarily affecting young children¹⁰⁵ and the most common malignant pancreatic neoplasm of childhood.⁷² Histologically, the tumor is composed of solid nests of polygonal cells with acinar, endocrine, and ductal differentiation, with intervening cellular, fibrous stromal bands. The acinar component usually predominates, making distinction from acinar cell carcinoma a challenge; this distinction, however, has little or no impact on management.



Cytomorphology of pancreatoblastoma

Epithelial component

- syncytial groups and isolated cells
- monomorphic cell with moderate amount of cytoplasm
- squamoid corpuscle

Stromal component

- primitive spindle-shaped cells
- occasionally heterologous elements (e.g., cartilage)

The diagnostic feature is the squamoid corpuscle, an aggregate of cells with squamous differentiation ([Fig. 14.17](#)). Squamoid corpuscles are typically not immunoreactive but may show focal staining for cytokeratin, endocrine markers, and CEA.⁷²

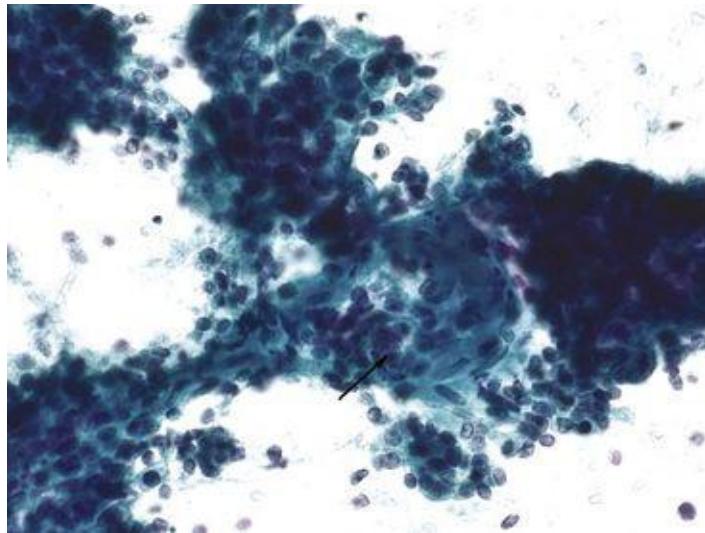


FIGURE 14.17 Pancreatoblastoma.

The diagnostic feature is the squamoid corpuscle (*arrow*) (Papanicolaou stain).



Differential diagnosis of pancreatoblastoma

- acinar cell carcinoma (ACC)
- PanNET
- solid-pseudopapillary neoplasm (SPN)
- Wilms tumor
- neuroblastoma

In addition to ACC, the differential diagnosis includes the other solid cellular neoplasms of the pancreas, PanNET, and SPN. Morphologically similar tumors of the adjacent kidney such as Wilms tumor and neuroblastoma also warrant consideration.

Pancreatic Cysts

Pancreatic cysts are a heterogeneous group of lesions that includes nonneoplastic and malignant cysts. Until the 1980s, cysts of the pancreas were thought to be relatively rare, but the routine use of ever-improving cross-sectional imaging has seen a dramatic increase in the detection of pancreatic cysts in general and asymptomatic cysts in particular.^{15,51,106-111} It is now estimated that 1.2% of the general population and up to 8% of the elderly have a pancreatic cyst requiring followup.^{8,108} Clinical management algorithms recommend a multidisciplinary, multimodal approach to preoperative diagnosis in which cytology plays a key role.^{3,9,10,34,37,38,111-117} Surgical intervention is dictated by the type of cyst and the risk of malignancy.

Pseudocyst

Pancreatic pseudocysts result from the autodigestion of pancreatic parenchyma, often in the setting of acute pancreatitis. Historically, they have accounted for a majority of pancreatic cysts, but this has changed in recent decades with the increasing detection of asymptomatic cysts, many of which represent small branch-duct IPMNs.⁷²

A pseudocyst, by definition, lacks an epithelial lining and is composed instead of an inflammatory fibrous capsule enclosing a region of tissue necrosis. Imaging demonstrates a unilocular, thick-walled cyst without septations.^{118,119} FNA yields either thin and cloudy or dark and turbid fluid. Cyst fluid analysis demonstrates a markedly elevated amylase level (generally in the thousands), and CEA is not elevated (less than 192 ng/mL).^{120,121}



Cytomorphology of pseudocyst

- thin, nonmucinous cyst fluid (barring GI contamination)
- inflammatory cells (mixed) and histiocytes
- yellow, hematoidin-like pigment
- no epithelial cells (barring GI contamination)

The cyst fluid is composed principally of granular debris, macrophages (some

of which contain hemosiderin), and yellow hematoidin-like pigment¹²² ([Fig. 14.18](#)). Aspirates may also contain adjacent normal pancreas (ductal and acinar cells), fibroblasts from the fibrous capsule, mesothelial cells, and gastric or duodenal epithelium, which should not be mistaken for cyst lining cells.

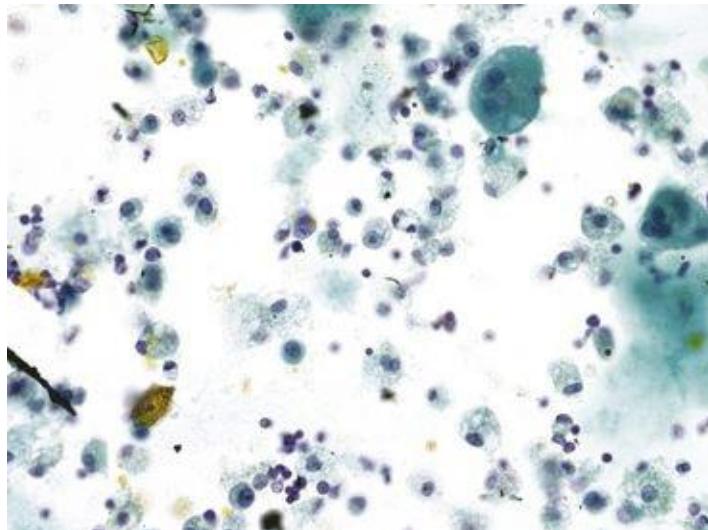


FIGURE 14.18 Pseudocyst.

Granular debris, histiocytes, and yellow hematoidin-like pigment are characteristic features of pseudocyst fluid (Papanicolaou stain).

The differential diagnosis includes the neoplastic mucinous cystic lesions of the pancreas (see discussion of mucinous cystic neoplasm and intraductal papillary mucinous neoplasm, further on). This distinction is especially difficult with EUS-guided specimens, which are almost always contaminated by benign gastric or duodenal epithelium. A neoplastic mucinous cyst should be considered if the patient has no history or signs of pancreatitis, because these are almost always present in a patient with a pseudocyst.

Serous Cystadenoma

Serous cystadenoma is a rare neoplasm that accounts for 1% to 2% of pancreatic tumors. It is subclassified based on the number and size of its cysts. *Serous microcystic adenoma* is composed of numerous small cysts, whereas as *serous oligocystic adenoma* has fewer but larger cysts. A unilocular variant has also been reported. Serous microcystic adenoma is the more common of the two types. It has a predilection for women, with a mean age at presentation of 66

years, and occurs most often in the body and tail of the pancreas. It is almost always benign, although rare cases of serous cystadenocarcinoma have been reported.¹²³⁻¹²⁵ Some patients present with abdominal pain or other nonspecific symptoms, but most tumors are discovered incidentally. The numerous small cysts impart a spongy appearance to the tumor on imaging studies, and about 30% to 40% are associated with a central stellate scar. Serous oligocystic adenomas are much less common and are encountered in children as well as adults. Serous oligocystic adenomas are often confused with mucinous cysts on imaging studies. Histologically, the cysts are filled with serous fluid and lined by uniform cuboidal cells with clear, glycogen-rich cytoplasm. Cytologic atypia is generally absent.



Cytomorphology of serous cystadenoma

- sparse cellularity
- clean or bloody background
- flat sheets and loose clusters
- cuboidal cells
- clear, finely vacuolated or granular cytoplasm with indistinct borders
- bare nuclei
- small, round nucleus
- fine chromatin
- inconspicuous nucleolus
- +/- hemosiderin-laden macrophages

Cytologic preparations are characterized by low cellularity and a relatively clean background, although aspirates can be bloody because of septal vascularity. Hemosiderin-laden macrophages may be noted in an otherwise sparsely cellular preparation.¹²⁶ When cells survive the slide preparation process, they are arranged in small clusters or flat sheets. Cytoplasm is clear and finely vacuolated or occasionally granular, with indistinct borders. The presence of glycogen is demonstrated by PAS positivity that is abolished by predigestion with diastase. Nuclei are small and round, with evenly distributed chromatin and an inconspicuous nucleolus. Occasionally, mild nuclear atypia is noted.^{126,127}

The cytologic diagnosis of serous cystadenoma is very challenging. Because FNA specimens are often sparsely cellular, a nondiagnostic or nonspecific negative interpretation is common.¹²⁷ The epithelium is bland and nondistinctive.

Cyst fluid analysis demonstrates low amylase and low CEA (less than 192 ng/mL) levels; in most cases, CEA is greater than 5 ng/mL.^{[120,121](#)}



Differential diagnosis of serous cystadenoma

- benign pancreatic ductal and acinar cells
- lymphangioma and hemangioma
- mucinous cystic neoplasm with low-grade dysplasia
- cystic PanNET
- multicystic mesothelioma
- metastatic renal cell carcinoma

Benign acinar cells are arranged in tighter groups than the loose clusters of a serous cystadenoma. Normal ductal cells from the smaller ducts resemble the cells of a serous cystadenoma, but radiologic correlation helps exclude the possibility that only normal ductal cells were aspirated. Lymphangiomas and hemangiomas are generally lined by flat rather than cuboidal cells. Sometimes the cells of a serous cystadenoma are taller rather than cuboidal and can be mistaken for those of a mucinous neoplasm.^{[127](#)} This is especially problematic if the FNA was done endoscopically, and the sample is contaminated with mucin of gastric or intestinal origin. A cystic PanNET can be excluded if there is sufficient material for immunohistochemistry, but serous cystadenomas rarely yield sufficient material for an adequate cell block. A descriptive diagnosis, including a note highlighting pertinent negative findings (e.g., absence of extracellular mucin and high-grade cellular atypia), coupled with the chemistry results and imaging findings, can be helpful in the proper clinical setting.

Lymphoepithelial Cyst

Lymphoepithelial cyst is a rare benign squamous-lined cyst with subepithelial nonneoplastic lymphoid tissue. It is much more common in middle-aged men, with a 4:1 male to female ratio.^{[22,128,129](#)} Lymphoepithelial cysts occur anywhere in the pancreas, with a wide size range up to 17 cm (mean 5 cm).^{[22,128,129](#)} On imaging studies, these cysts are unilocular or multilocular, or they may appear solid due to thick internal debris.



Cytomorphology of lymphoepithelial cyst

- anucleate squames and abundant keratinous debris

- mature superficial squamous cells
- lymphocytes (variable cellularity)
- +/- cholesterol clefts

The cytologic appearance is similar to that of an epidermal inclusion cyst, with nucleated and anucleated squamous cells and keratinous and cholesterol debris. Histiocytes and lymphocytes may be present ([Fig. 14.19](#)).

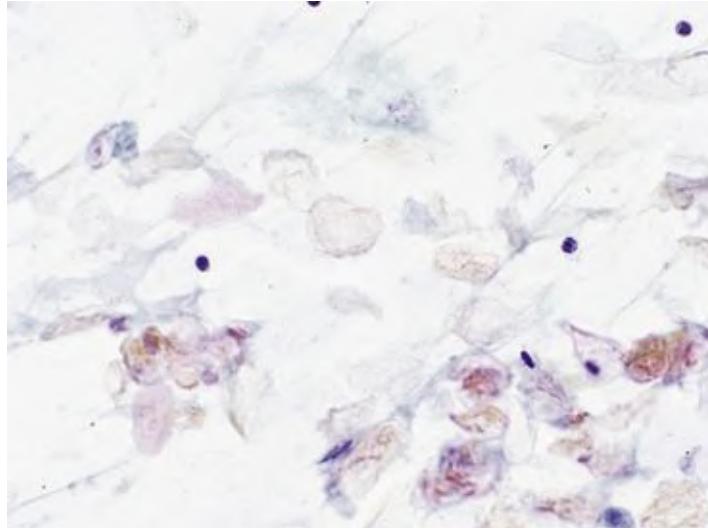


FIGURE 14.19 Lymphoepithelial cyst.

These cysts are filled with mature squamous cells; lymphocytes can be scant (Papanicolaou stain).



Differential diagnosis of lymphoepithelial cyst

- complicated pseudocyst
- dermoid and epidermoid cysts

The differential diagnosis is with other squamous-lined cysts such as a dermoid cyst of the pancreas and splenic epidermoid cyst, both rarer than lymphoepithelial cyst.^{[128](#)[130](#)} The distinction may not be possible, but this is not clinically relevant. A pseudocyst is also a consideration owing to the necrotic

appearance of the keratinous debris. A conservative resection is curative.

Mucinous Cysts: Mucinous Cystic Neoplasm and Intraductal Papillary Mucinous Neoplasm

There are two distinct neoplastic mucinous cysts of the pancreas that share common morphologic features, the *mucinous cystic neoplasm (MCN)* and the *intraductal papillary mucinous neoplasm (IPMN)*. The distinction between these entities is generally not possible by cytomorphology alone and requires correlation with clinical and imaging findings. The distinction is important, however, for clinical management.¹⁰

MCNs account for 5% of pancreatic neoplasms.²² It is found almost exclusively in women (mean age, 49 years), and most are located in the body and/or tail of the pancreas. Imaging studies reveal a sharply defined mass with one or more locules separated by fine septa; calcification is sometimes present. An MCN is lined by mucinous epithelium that almost always forms a closed cyst that does not communicate with the ductal system. The defining feature is ovarian-type stroma that forms a layer of variable thickness beneath the mucinous epithelium and expresses estrogen and progesterone receptors.^{22,114,131} Histologically, MCNs are subclassified as premalignant (with low-, intermediate-, or high-grade dysplasia) if there is no evidence of invasion and as malignant if there is.⁹¹ Most MCNs are premalignant and are cured with complete resection.¹¹⁴ The current recommendation is to resect all MCNs irrespective of grade, because intervention at diagnosis avoids long-term and costly annual surveillance.¹⁰

IPMNs account for 3% to 5% of pancreatic tumors.²² They differ from ductal adenocarcinoma and MCNs in that they are “open-ended” neoplasms that grow intraluminally along the pancreatic ducts, producing thick mucus that blocks secretions and causes cyst formation. Like MCNs, IPMNs are subclassified as premalignant (with low-, intermediate-, or high-grade dysplasia) or malignant depending on the presence of invasion.⁹¹ IPMN occurs almost equally in men and women, and about 70% are located in the head.²² There are three main types: (1) IPMN involving only the main pancreatic duct; (2) IPMN involving only the branch ducts; and (3) IPMN involving both. Imaging and EUS features are diagnostic if a markedly distended main pancreatic duct has filling defects or there are multiple branch-duct cysts.¹³²⁻¹³⁴ Copious mucus from a patulous ampulla is pathognomonic of IPMN.¹³⁵ An isolated branch-duct IPMN, however, is almost impossible to distinguish from other cysts by imaging alone.^{109,136,137} In contrast

with MCNs, branch-duct IPMNs are most common in the pancreatic head or uncinate process and are nonseptate, unilocular cysts often less than 5 cm across.^{9,137-139} An associated mural nodule is an independent predictor of malignancy.¹³³

The most important prognostic factor is evidence of invasion; thus the goal is preoperative detection of preinvasive lesions. The prognosis for patients with a noninvasive neoplasm with any degree of cytologic atypia is excellent.²² Because IPMN patients are typically older and often have comorbid conditions, the risk of surgical resection must be balanced against the risk of malignancy. Branch-duct IPMNs are most often located in the pancreatic head or uncinate process. The risk of malignancy is low and does not justify the morbidity of a Whipple resection, especially in a high-risk surgical candidate. The prognosis for patients with an invasive MCN or IPMN depends on the depth of invasion, but survival is generally better than for patients with a ductal adenocarcinoma. Patients with unresectable disease due to local invasion or metastasis have a prognosis similar to that for ductal adenocarcinoma.

Surgical management guidelines take into consideration symptoms, cyst size, presence of a dilated main pancreatic duct (as surrogate marker for main duct involvement), presence of a mural nodule, and cytologic evidence of malignancy. The risk of malignancy is higher for mainduct IPMN than branch-duct IPMN, in part because the more common intestinal-type lining of mainduct IPMN carries a higher risk of malignancy than the more common gastric-type lining of branch-duct IPMN.^{110,140,141} Determination of cell type and the distinction between low-grade dysplasia and GI contamination are not possible by cytology in most cases. The most important task for cytology is the recognition and reporting of high-grade dysplasia or carcinoma.³²⁻³⁴



Cytomorphology of cystic mucinous neoplasm and intraductal papillary mucinous neoplasm

- usually hypocellular specimen
- variable amounts of extracellular mucin
- thick, colloidlike mucin with or without cyst lining epithelium is diagnostic of a mucinous cyst
- *low-grade dysplasia*: benign-appearing mucinous epithelium in sheets and groups, often indistinguishable from gastric epithelium
- *high-grade dysplasia*: small, tight, budlike clusters or isolated cells with increased nuclear-to-cytoplasmic ratio, irregular nuclear membranes, and variably vacuolated cytoplasm

- *malignant*: three-dimensional groups with marked anisonucleosis, irregular nuclear membranes, prominent nucleoli, and parachromatin-clearing, necrosis
- cellular debris/necrosis

In general, an MCN cannot be distinguished from an IPMN by cytology. Among other things, the subepithelial ovarian type stroma of an MCN is typically not appreciated on FNA. Aspiration of both an MCN and an IPMN produces highly variable amounts of extracellular mucin and epithelium, depending on the size of the cyst and the presence or absence of a solid component. In addition, the cells sampled may not represent the most atypical areas of the cyst, underestimating the final histologic grade.¹⁴² When thick mucus that is difficult to draw into or express from the needle is encountered, a mucinous cyst should be suspected. This is reflected on the slide as a thick sheet of colloidlike mucin, which frequently covers most of the slide ([Fig. 14.20A](#)). (Mucin from the GI lumen is not colloidlike.) This type of mucin is sufficient by itself (i.e., even in the absence of epithelial cells) for the diagnosis of a neoplastic mucinous cyst. Not all MCNs and IPMNs have abundant thick mucus, however. The extracellular mucin can appear as focal thick clumps or thin wisps, and as a focal or diffuse thin background. Liquid-based preparations dilute and attenuate the mucin, making it more difficult to appreciate. An aliquot of the cyst fluid, if sufficient in quantity, can be used to prepare Cytospin slides for mucicarmine and/or Alcian blue pH 2.5 to assess for mucin. Negative mucin stains do not exclude a mucinous cyst, but detecting mucin can be very helpful.

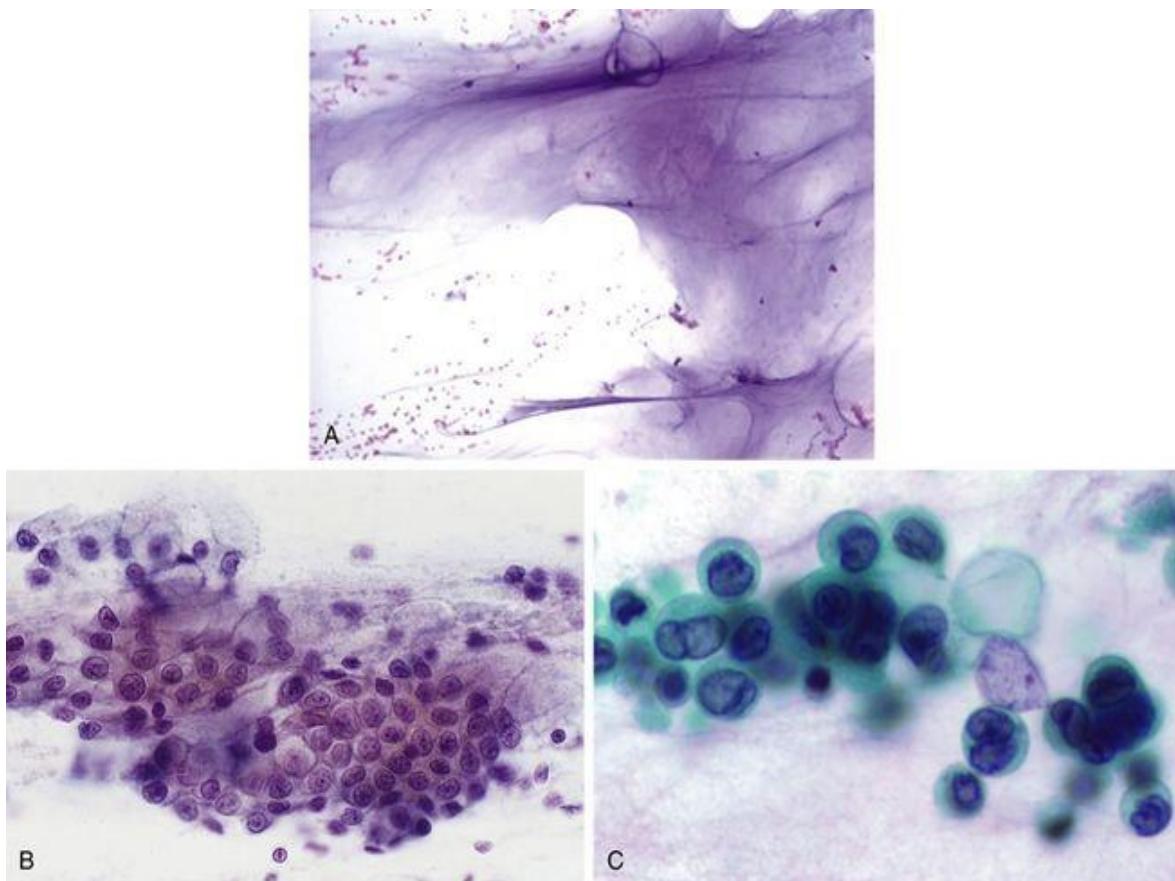


FIGURE 14.20 Mucinous cystic neoplasm.

A, Thick, colloidlike mucin is indicative of a neoplastic mucinous cyst and not gastrointestinal (GI) contamination (Papanicolaou stain). B, Flat sheets of columnar mucinous cells with distinct cytoplasmic borders are characteristic, but virtually impossible to distinguish from normal gastric mucosa (compare with Fig. 14.4). C, The cells of an intermediate-grade dysplasia have morphological features in between low-grade and high-grade dysplasia (Papanicolaou stain).

Mucinous cysts with *low-grade dysplasia* yield sparsely cellular aspirates; sometimes muciphages (foamy histiocytes) are the only cells seen. Benign-appearing mucinous epithelium procured via a transduodenal FNA can usually be interpreted as lesional (Fig. 14.20B). In the absence of high-grade atypia (high-grade dysplasia or carcinoma), an interpretation of cyst fluid with low-grade–appearing mucinous epithelium may be sufficient for patient management.

Cysts with *high-grade dysplasia* contain atypical epithelial cells with nuclear crowding, loss of polarity, nuclear elongation or rounding, hyperchromasia, and increased nuclear-to-cytoplasmic ratio. Cells are dispersed and isolated or arranged in small, tight, budlike clusters with or without mucin.^{31-34,37} Crowded groups of cells with open chromatin, irregular nuclear membranes and nucleoli, and necrosis support the interpretation of carcinoma. Because it can be difficult

to distinguish between high-grade dysplasia and invasive carcinoma in cyst fluid specimens, the term high-grade atypia encompasses both and indicates a cyst at high-risk for invasive carcinoma.¹⁴³ Cysts with intermediate grade dysplasia ([Fig. 14.20C](#)) are difficult to classify accurately but are best grouped in the low-grade category using a two-tiered grading system.³⁴

The data obtained from EUS and cyst fluid analysis are essential.^{9,10,31-34,37,109} CEA analysis is best at detecting a mucinous cyst, and cytology is best at discerning malignancy.³⁰ Detecting *KRAS* point mutation and loss of heterozygosity is helpful for the diagnosis of a mucinous cyst if the CEA level is low, but neither distinguishes among grades of dysplasia, nor do they differentiate benign from malignant cysts.^{35,144-146} *GNAS* mutations do help differentiate MCN from IPMN.³⁶ This may be helpful in patient management, because MCNs, unlike IPMNs, are usually resected regardless of grade.

Secondary Pancreatic Neoplasms and Ectopic Splenic Tissue

FNA of the pancreas is particularly useful in documenting a metastatic malignancy to the pancreas: The diagnosis of a metastasis averts an unnecessary pancreatic resection and prompts a search for the primary site (if unknown).

The neoplasms that most often metastasize to the pancreas include carcinomas of the lung, breast, and kidney, and lymphoma.¹⁴⁷ Rare cases of metastatic adenocarcinoma from the ovary, colon, gallbladder, stomach, and prostate have also been reported. Immunohistochemical staining for TTF-1, positive in a large percentage of lung adenocarcinomas and negative in pancreatic adenocarcinomas, helps distinguish these two adenocarcinomas.

Because pancreatic ductal adenocarcinoma is by far the most common malignancy of the pancreas, any aspirate that demonstrates unusual cytologic features should raise the possibility of a metastasis. Primary squamous cell carcinoma and small cell carcinoma of the pancreas are exceedingly rare; an alternate primary site should be excluded clinically if these interpretations are a consideration. *Renal cell carcinoma* is the most common tumor that mimics a primary pancreatic neoplasm. It can manifest as a solitary pancreatic mass or multiple masses.^{148,149} The primary renal cell carcinoma may be not yet have been detected, or it may have been treated many years (even decades) earlier and thus be a distant memory to the patient and the treating physician. The cells of renal cell carcinoma are large and polygonal, with eccentrically placed nuclei, prominent nucleoli, and vacuolated cytoplasm (see [Fig. 14.13B](#)). The differential diagnosis includes a lipid-rich PanNET (see [Fig. 14.13A](#)).¹⁴⁹

Ectopic splenic tissue is occasionally encountered in the pancreas, and FNA can play an important role by establishing the diagnosis, thereby averting an unnecessary pancreatic resection. The term *ectopic spleen* encompasses *accessory spleen*, a congenital defect, and *splenosis*, an acquired condition defined as one or more focal deposits of auto-implanted splenic tissue after abdominal trauma or splenectomy. Ectopic splenic tissue is highly vascular and mimics an pancreatic neuroendocrine tumor on imaging studies. The diagnosis should be considered whenever cytologic preparations reveal numerous lymphocytes ([Fig. 14.21A](#)).^{150,151} Cell block sections can be indispensable by demonstrating the tell-tale thin-walled blood vessels that are immunoreactive for CD8 ([Fig. 14.21B](#)).

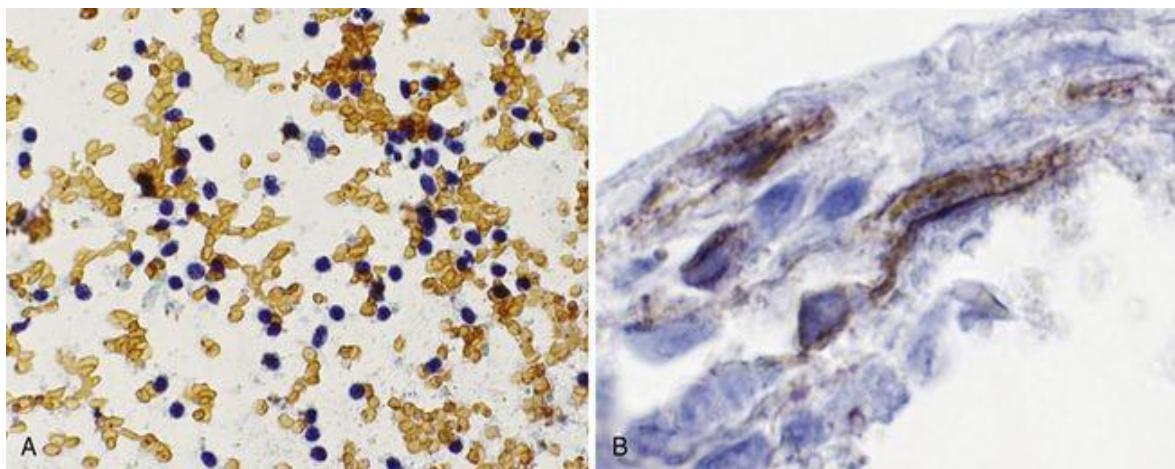


FIGURE 14.21 Ectopic spleen.

A, Preparations reveal numerous small lymphocytes (Papanicolaou stain). *B*, The diagnosis can be confirmed by demonstrating immunoreactivity of thin-walled vessels for CD8.

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CHAPTER 15

Kidney and Adrenal Gland

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The Kidney

Fine-needle aspiration (FNA) of the kidney is a useful technique for the diagnosis of selected renal lesions. FNA, as it turns out, is not necessary for most renal masses. In adults, the great majority of renal lesions are either radiologically benign cysts requiring no treatment or radiologically malignant masses for which FNA is redundant. Cross-sectional imaging like ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI) are remarkably accurate in diagnosing most benign cysts and renal cell carcinomas (RCCs). Thus, only a small percentage of adult renal lesions, perhaps less than 10%, are candidates for FNA,¹ but in these cases FNA plays a vital role in patient management.

Until recently, aspiration of a suspected Wilms tumor, the most common renal tumor in children, resulted automatically in clinical upstaging, and thus a renal FNA in the pediatric population was rarely done. The staging criteria have changed, however, and FNA no longer leads to clinical upstaging in patients with Wilms tumor. This has resulted in an increase in the use of FNA in pediatric patients in some centers. Interpretation of these specimens is challenging²⁻⁴ and discussed more fully in the section “Fine-Needle Aspiration in Pediatric Patients.”



A fine-needle aspiration is indicated when:

- radical nephrectomy is contraindicated
 - probable advanced-stage (unresectable) RCC
 - possible metastasis to the kidney (e.g., history of lung cancer)
 - patient has coincident medical problems
 - patient desires an investigational treatment (e.g., lesion ablation *in vivo*)
- radiologic findings are equivocal
 - atypical cyst
 - small solid mass (“low-fat” angiomyolipoma versus oncocytoma versus RCC)
- a partial nephrectomy is considered
 - small lesion and/or young patient
 - decreased renal function
- an infection (pyelonephritis or abscess) is suspected

For patients who are not candidates for nephrectomy, FNA provides the easiest method to obtain a diagnosis. A mass might be unresectable for a variety of reasons: (1) Based on imaging features, it is probably an advanced-stage RCC; (2) there is a history or suspicion of a primary malignancy elsewhere, and the kidney mass might be a metastasis; or (3) the patient is a poor operative risk because of a comorbid condition. A benign FNA diagnosis (e.g., oncocytoma, angiomyolipoma) would avoid unnecessary surgery in such a high-risk patient. In addition, recent investigational protocols involve ablating small RCCs using cryotherapy, radiofrequency, or ethanol injections; in these patients an FNA specimen is obtained to confirm malignancy before the ablation procedure.⁵

Some patients have a radiographically indeterminate lesion. These include atypical cysts and small, homogeneously enhancing masses. Atypical, radiologically indeterminate cysts, by definition, contain more than a few septations, thickened septa, thick walls, and/or non-border-forming calcifications. Extensive histologic sampling is needed before one can conclude that an atypical cyst is benign. This requirement significantly limits the utility of FNA in this setting. Still, FNA is sometimes performed, even though the results need to be viewed with some skepticism⁶ (see “[Renal Cysts](#)”). Small, homogeneously enhancing renal masses are also difficult to classify as benign or malignant by imaging studies alone.⁵ Many turn out to be angiomyolipomas with little or no fat content, but some are small RCCs. Because angiomyolipoma can be reliably distinguished from RCC by FNA in most cases, FNA plays an important role in the evaluation of small, homogeneously enhancing renal masses.⁶

Some carcinoma subtypes, namely, papillary RCC, chromophobe RCC, and mucinous tubular and spindle cell carcinoma, have a good prognosis and are amenable to treatment by partial nephrectomy. This operation is increasingly common, as the incidence of smaller lesions and lesions in younger patients increases.⁷ Patients with the von Hippel-Lindau (VHL) syndrome, who are at risk for bilateral tumors, and those with decreased renal function are also candidates for nephron-sparing surgery. Although the ultimate decision to treat by partial nephrectomy depends upon the size and location of the lesion as well as other clinical factors, FNA is valuable because it discloses whether or not the tumor is one of the good-prognosis neoplasms, which may be most appropriately treated this way.

In the patient with focal bacterial pyelonephritis or a renal abscess, needle placement permits both diagnosis and therapeutic drainage. These lesions can appear masslike and mimic a renal tumor. Signs and symptoms of a urinary tract infection (UTI) are usually present, and thus most renal infections can be

diagnosed clinically, without the need for an FNA. Some renal infections are clinically subtle, however. Imaging findings sometimes help in the distinction from a neoplasm: Pyelonephritis with abscess formation tends to show ill-defined margins and perinephric stranding. If, after clinical and radiographic assessment there is still doubt, FNA can be useful to confirm infection and exclude malignancy.

Specimen Collection and Preparation

Virtually all renal aspirations are performed percutaneously by radiologists using US, CT, or MRI for guidance. Rarely, the FNA is performed endoscopically with US guidance⁸ or intraoperatively. Complications are uncommon but may include bleeding, hematuria, pneumothorax, infection, arteriovenous fistula, and urinoma. Needle tract seeding is extremely rare with the use of small (less than 18 gauge) needles. There have been fewer than 10 reported cases of needle track seeding associated with a renal mass FNA, for an estimated incidence of less than 0.01%.⁹

Slides are air-dried and stained with a Romanowsky stain, and/or alcohol-fixed and stained with the Papanicolaou or hematoxylin-eosin (H & E) stain. Cell blocks are particularly helpful for identifying architectural features like papillae and provide an ideal platform for immunocytochemical studies (e.g., HMB-45 to confirm the diagnosis of an angiomyolipoma). Some authors have recommended the use of agar microbiopsies to improve diagnostic yield.⁹ Recent studies have focused on the value of core needle biopsy in improving diagnostic yield and allowing for immunohistochemical evaluation.¹⁰ The combination of FNA and core has better yield than either technique alone,¹¹⁻¹³ and it is advisable to accept a core biopsy as an adjunct to FNA if the aspirator judges that it is safe to perform one. Some tumors, including some clear cell RCCs, are so tightly cohesive that they are almost impossible to aspirate, yet straightforward to interpret given a tissue core or cell block material. Conversely, with scant material (insufficient for immunohistochemistry), it is often easier to make a diagnosis with a smear as opposed to a core biopsy.¹⁴

Cytogenetics and molecular cytogenetics are useful adjuncts, particularly in subtyping RCCs.¹⁵⁻¹⁸ A karyotype can be obtained from short-term culture of fresh, needle-rinse fluid. Fluorescence in situ hybridization (FISH) can be performed on unstained cytospins, thinlayers, smears, and cell block sections.

Accuracy

Experienced cytologists find that renal FNA accurately distinguishes benign from malignant lesions in 73% to 94% of cases.^{13,19-36} Correct subclassification of RCC by cytomorphology is achieved in 74% to 80% of cases,^{12,35,37-41} and in up to 90% of cases for the most common subtypes of RCC.^{34,41} Accuracy increases to 99% for the most common subtypes of RCC by using cores and immunohistochemistry for carbonic anhydrase IX, CD117, AMACR, CK7, and CD10.³⁴ Unfortunately, there is no immunohistochemical stain specific for oncocytoma, making this a diagnosis of exclusion, one that is less reliable than a diagnosis of RCC.³⁹

Because kidney FNAs are relatively uncommon, it can be difficult for some cytologists to obtain expertise in this area. False-positive results occur when xanthogranulomatous pyelonephritis, angiomyolipoma, benign hepatocytes, benign tubular cells, glomeruli, and benign adrenal cortical cells are misinterpreted as RCC.⁶ Cellularity is important to consider when interpreting a kidney FNA; benign mimics of malignancy can contain atypical cells, but they are usually few in number or the sample itself is hypocellular. Most hypocellular kidney aspirates, therefore, should not be diagnosed as positive even if they contain some atypical cells.

Adequacy

Up to 30% of renal aspirates are nondiagnostic (inadequate); a repeat aspiration is helpful in approximately one half of cases.^{19-20,23,25-28,32} Most inadequate specimens are related to a technical failure in obtaining cells representative of the lesion.⁴² Although there is no consensus on adequacy criteria, it is reasonable to consider a renal FNA specimen adequate if a specific (benign or malignant) diagnosis can be made, or if there is sufficient cellularity to suggest a limited differential diagnosis. A specimen composed exclusively of macrophages (typically from a cystic lesion) is best reported as nondiagnostic rather than negative, because a cystic RCC cannot be excluded.

Normal Elements

Glomeruli and Tubular Cells



Cytomorphology of glomeruli

- large, dense, globular structures
- capillary loops



Differential diagnosis of glomeruli

- papillary RCC



Cytomorphology of proximal tubular cells

- rare cells, abundant granular cytoplasm



Differential diagnosis of proximal tubular cells

- oncocytoma
- chromophobe RCC



Cytomorphology of distal tubular cells

- rare cells, scant granular cytoplasm



Differential diagnosis of distal tubular cells

- low-grade clear cell or papillary RCC

Normal elements are occasionally encountered, particularly when the radiologist is sampling a small lesion, and the needle excursions traverse normal kidney. It is vital not to misinterpret normal elements as tumor cells. Glomeruli ([Fig. 15.1](#)) are highly cellular globular structures that mimic the papillae of papillary RCC, especially at low magnification. Glomeruli lack atypia, but so do low-grade papillary RCCs. In contrast with papillary RCC, however, the cells in a glomerulus are not evenly distributed, but instead are much more dense in the center than at the periphery; most important, close inspection of the edges of a glomerulus reveals its distinctive capillary loops.

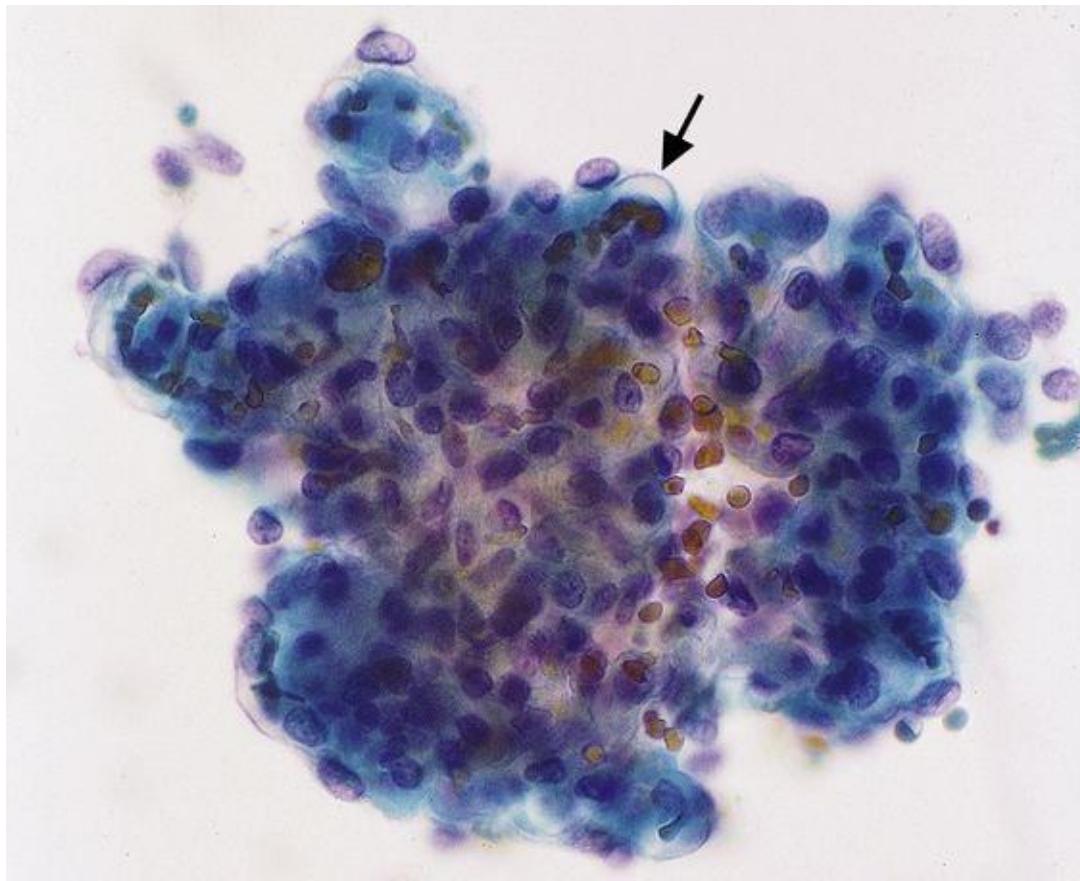


FIGURE 15.1 Glomerulus.

Glomeruli are densely cellular spherical structures with scalloped edges. Note the characteristic capillary loops (arrow) (Papanicolaou stain).

Proximal tubular cells have a round, bland nucleus; a small but easily seen nucleolus; and abundant, granular cytoplasm (Fig. 15.2A). The cells lack a well-defined cell border, and the granules often appear to be spilling out of the cells. They are very similar to the cells of an oncocytoma and chromophobe RCC. FNA preparations of both tumors, however, are usually more cellular, and their cells are frequently binucleate, often with some variation in cell and nuclear size and shape. Also, the cell borders of an oncocytoma and chromophobe RCC are usually sharply defined, whereas those of proximal tubular cells are torn and irregular.

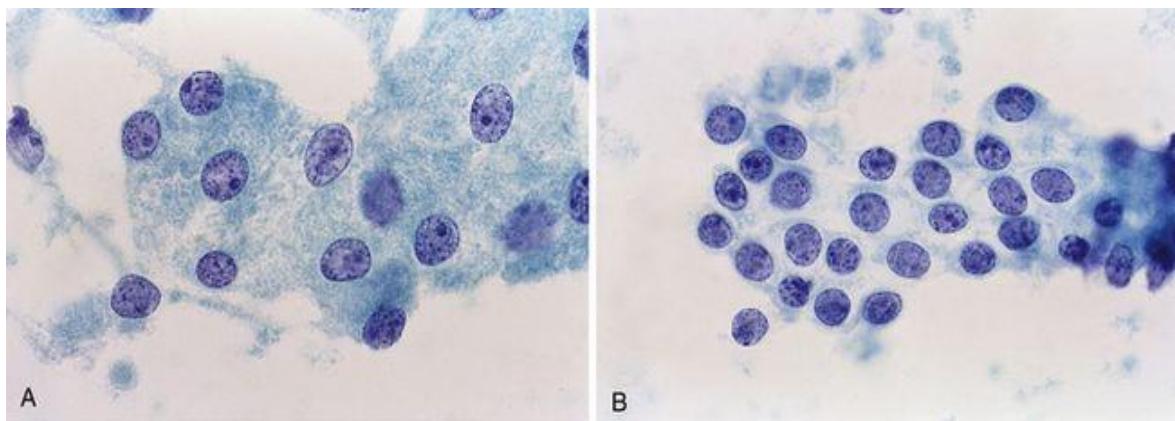


FIGURE 15.2 Tubular cells.

A, Proximal tubular cells have abundant granular cytoplasm and poorly demarcated cell membranes (Papanicolaou stain). B, Distal tubular cells are smaller and have less cytoplasm (Papanicolaou stain).

Distal tubular cells are small, isolated or cohesive cells, with less cytoplasm than proximal tubular cells (Fig. 15.2B). Their cytoplasm is clear to slightly granular, and they have a small, round nucleus and an inconspicuous nucleolus. The cell borders are well defined, and the cytoplasm is not vacuolated.⁴³ Distal tubular cells are very similar to the malignant cells of a low-grade clear cell or papillary RCC. Aspirates of those tumors are usually more cellular, however, and the cells of low-grade papillary RCC form papillae and spherules, something distal tubular cells do not.

Benign Lesions

Oncocytoma

Oncocytoma, a benign tumor of oncocytes (cells with abundant granular cytoplasm), comprises 3% to 5% of all renal tumors^{44–50}; rare metastases have been reported.⁵⁰ They have a wide age distribution, with a median size of 6 cm, and most are incidental findings in patients undergoing imaging studies for unrelated reasons. Radiologic findings, unfortunately, cannot be relied upon to make a confident diagnosis of oncocytoma. Histologically, tumor cells have abundant granular cytoplasm and uniform round nuclei, with small but distinct nucleoli equivalent to those of a Fuhrman grade 2.⁵¹ Occasional cases contain scattered large, sometimes bizarre nuclei, but mitoses are absent or very rare. Electron microscopy reveals abundant mitochondria, which account for the characteristically granular cytoplasm by light microscopy.⁵² The arrangement of

the neoplastic cells in rounded nests is distinctive and contrasts with the trabeculae (ribbons) of a chromophobe RCC.⁵³ Cytogenetics reveals a mosaic of normal and abnormal karyotypes. The most common abnormalities are losses of chromosomes 1 and Y, but deletions and rearrangements also occur.⁵⁴ Unfortunately, these findings are nonspecific and therefore not useful for confirming the diagnosis of an oncocytoma.



Cytomorphology of oncocytoma

- highly cellular
- rounded nests (cell block or core)
- cohesive fragments and dyshesive cells (smears)
- abundant uniformly granular cytoplasm
- Fuhrman grade 2 nucleoli

Cytologically, smears from a well-sampled oncocytoma reveal numerous isolated cells with abundant, eosinophilic, granular cytoplasm; well-demarcated cell borders; and round nuclei with small or medium-sized nucleoli⁵⁵⁻⁵⁹ ([Fig. 15.3A, B](#)). In occasional cases, isolated pleomorphic or bizarre nuclei occur and can be quite alarming,⁵⁷ but these are thought to represent degenerative changes and are not indicative of malignancy. Necrosis is absent, and mitoses are either absent or very infrequent.

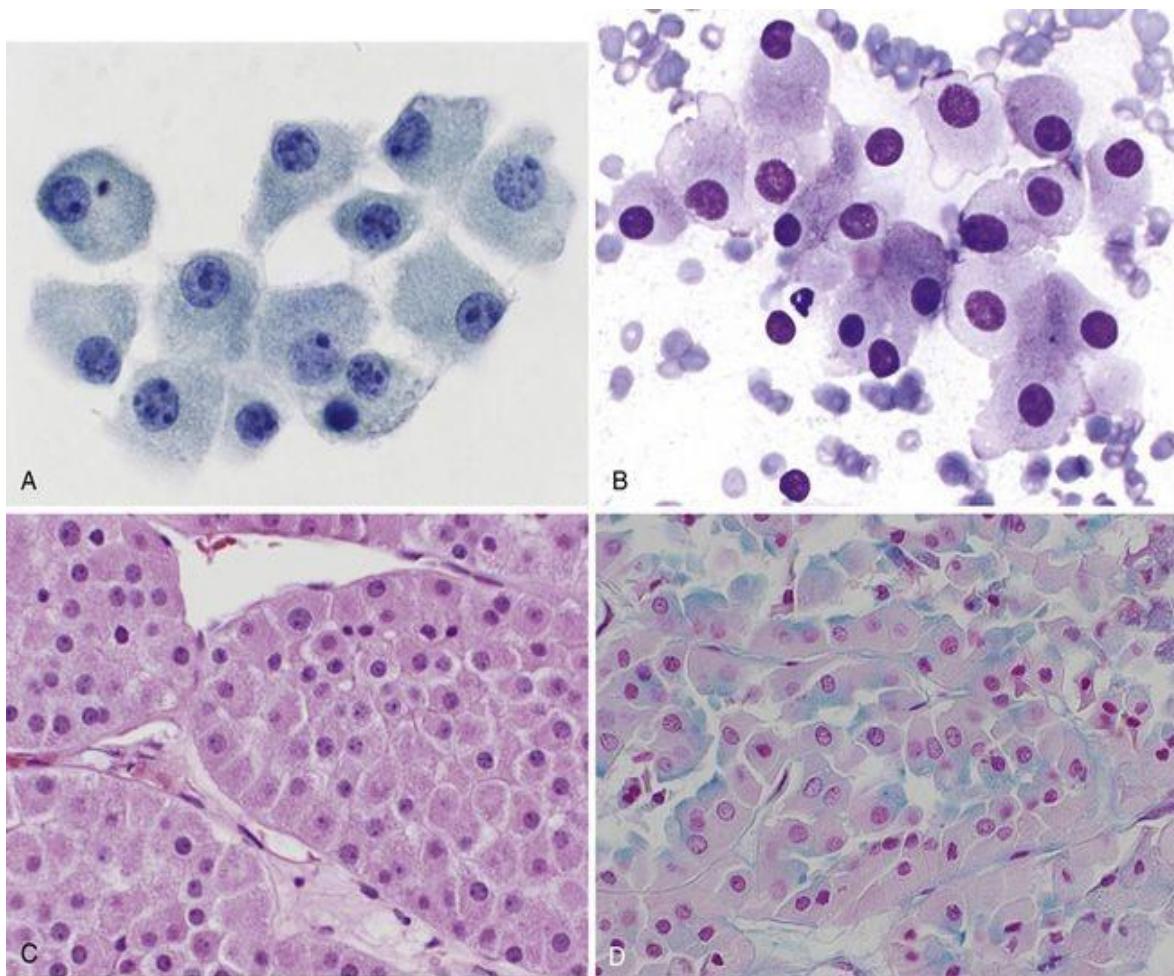


FIGURE 15.3 Oncocytoma.

A, Smears show noncohesive cells with abundant granular cytoplasm and well-demarcated cell membranes. Nucleoli are evident (Papanicolaou stain). B, Well-demarcated cell membranes are also apparent with air-dried smears (Romanowsky stain). C, Circumscribed cell nests are characteristic of these tumors, best seen with cell block sections or core biopsies (hematoxylin-eosin [H & E] stain). D, An apical staining pattern with Hale's colloidal iron (HCl) stain is sometimes encountered with oncocytomas.



Differential diagnosis of oncocytoma

- hepatocytes
- clear cell RCC
- papillary RCC
- chromophobe RCC

When examining cells with abundant granular cytoplasm from a kidney FNA, one should consider inadvertent sampling of the liver, which happens with FNAs of a right renal mass if the needle traverses the liver on its way to the kidney.

Hepatocytes have abundant granular cytoplasm, but they often contain lipofuscin pigment and show more variation in nuclear and cellular size (see [Fig. 13.1](#)). If hepatocytes are the only finding, the sample is nondiagnostic (insufficient).

One also needs to exclude a RCC. Several different subtypes of RCC can have granular cytoplasm and therefore mimic an oncocytoma. Some clear cell RCCs are composed mostly of granular (rather than clear) cytoplasm. In comparison with the cells of an oncocytoma, those of a clear cell RCC, including the granular cell variant, are more cohesive, usually with more nuclear atypia and less uniformly granular cytoplasm. The rare eosinophilic variant of papillary RCC has a similar cytologic appearance to an oncocytoma, but papillae and foamy macrophages, typical of papillary RCC, are not features of oncocytomas. The cells of chromophobe RCCs have abundant granular cytoplasm, but it is usually less uniformly granular (there is often a patchy, perinuclear clearing), and they may have greater nuclear outline irregularity. Nevertheless, in any individual case the distinction between a chromophobe RCC and an oncocytoma can be extremely difficult on smear preparations alone. Tissue fragments in cell block sections, fortunately, are tremendously helpful: The cells of an oncocytoma are arranged in round nests (you can draw a circle around them) ([Fig. 15.3C](#)), whereas the cells of a chromophobe RCC are in endless trabeculae. Hale's colloidal iron (HCl) stain is helpful because it usually shows diffuse cytoplasmic staining in chromophobe RCCs, and most oncocytomas are negative. Unfortunately, some oncocytomas show a positive staining reaction with HCl, but usually only focally and in an apical pattern,⁶⁰ and therefore care in interpreting the result is necessary ([Figure 15.3D](#)). Immunohistochemical markers might be helpful in the distinction between oncocytoma, chromophobe RCC, and clear cell RCC, but their reliability in routine practice is not yet known.⁶¹ To make the situation even more complicated, rare hybrid renal tumors composed of oncocytoma and chromophobe RCC do occur, either sporadically or in association with the Birt-Hogg-Dube syndrome⁶²; FNA findings depend upon the areas sampled.

If only smears were prepared, the wisest (and most honest) interpretation in most cases is "oncocytic neoplasm (oncocytoma versus chromophobe RCC)," with a note that, if clinically indicated, a partial nephrectomy should be considered. This may not satisfy the urologist, who was hoping for an unequivocal interpretation. It is unwise, however, to make a definitive diagnosis of oncocytoma without an assessment of tissue architecture with the help of a cell block or core needle biopsy, because a rounded (nested) architecture is essentially the only finding relatively specific for an oncocytoma. Virtually all the other features of oncocytoma can be mimicked by several subtypes of RCCs,

not just the chromophobe RCC.

Renal Cortical Adenoma

Except for their size, renal cortical adenomas are histologically, immunohistochemically, and cytogenetically indistinguishable from low-grade papillary RCCs.⁶³⁻⁶⁴ Renal adenomas are by definition very small lesions, always less than 0.5 cm and usually less than 0.2 cm.⁶⁵ They are too small to be aspirated, and therefore the diagnosis “renal cell adenoma” is not appropriate for an FNA specimen.

Angiomyolipoma

Angiomyolipomas (AMLs) are benign mesenchymal tumors that arise from the so-called “perivascular epithelioid cell” and compose between 0.7% and 2.0% of all renal tumors. They occur in two distinct clinical settings.⁶⁶⁻⁶⁸ Approximately one half occur in young adults with tuberous sclerosis (TS), an autosomal dominant disease caused by mutation of one of the two TS-associated genes and manifested by mental retardation, seizures, and skin changes. In TS patients, the AMLs are usually multiple and bilateral. The other half are usually solitary and occur in young and middle-aged women with no known clinical syndrome. Some patients present with flank pain, but a majority of AMLs are detected incidentally during a radiologic work-up for an unrelated disorder. They vary widely in size and can be tiny or up to 20 cm in diameter. Large lesions can bleed, and some are resected to prevent this from occurring.

Histologically, an AML is composed of three elements: mature fat, blood vessels, and smooth-muscle cells. The latter can have moderate to marked atypia. Mature fat makes up the bulk of most AMLs, but these common, fatty AMLs are reliably identified by imaging studies, precluding the need for an FNA, except perhaps when well-differentiated liposarcoma is a consideration based on imaging findings.⁶⁹ FNA is needed only for the subset of AMLs that have very little adipose tissue (the “fat-free” or “low-fat” AMLs), which cannot be distinguished from a RCC by imaging alone. In virtually all cases, the neoplastic cells, most conspicuously the smooth muscle cells but also the lipid-distended fat cells, are immunoreactive for melanoma-related antigens like HMB-45 and MART-1 (Melan-A).⁷⁰⁻⁸⁰ An uncommon subtype, the epithelioid AML, is composed of large epithelioid rather than spindle smooth muscle cells. Epithelioid AMLs have metastatic potential, but predicting which will behave in

a malignant fashion is problematic.⁷⁰



Cytomorphology of angiomyolipoma

- spindled cells (\pm nuclear atypia) with stringy cytoplasm
- fat cells
- thick-walled blood vessels (rare)
- epithelioid cells with marked atypia (epithelioid angiomyolipoma)

AMLs can be diagnosed confidently by FNA, but they do have their challenges: specimens are usually paucicellular; the adipose tissue component is scant or nowhere to be found; and the thick vessels are rarely seen. The smooth muscle cells, sometimes with moderate-to-marked nuclear atypia, usually dominate the picture (Fig. 15.4A-C). Atypical spindle cells and their very large nuclei, if present, are usually interspersed among numerous much less atypical cells. The cytoplasm of the smooth muscle cells has a stringy or crystalline appearance, quite different from the vacuolated or granular cytoplasm of RCC. Scattered large, clear fat vacuoles can be present in the smooth muscle cells and impart a resemblance to RCC. The differential diagnosis includes sarcoma⁸¹ and sarcomatoid RCC. Immunostains for HMB-45 and MART-1 (i.e., Melan-A) are extremely helpful, because RCCs and most sarcomas are negative for these markers (Fig. 15.4D).

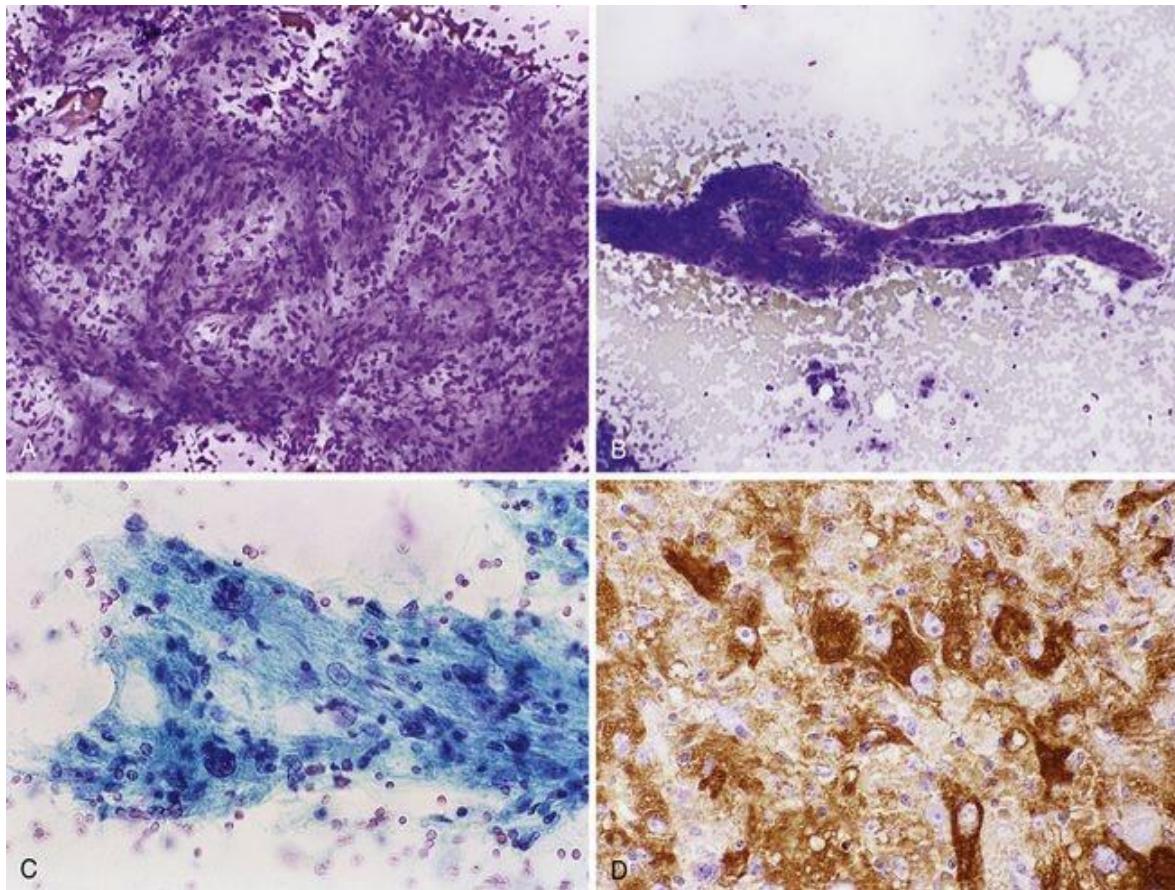


FIGURE 15.4 Angiomyolipoma.

A, Cohesive tissue fragments comprised of spindled-shaped, smooth muscle cells are usually the predominant finding (Papanicolaou stain). B, Occasionally, thick-walled blood vessels are seen (Romanowsky stain). C, Nuclear atypia is commonly encountered in benign angiomyolipomas (Papanicolaou stain). D, The tumor shows cytoplasmic immunoreactivity for HMB-45, a helpful marker in difficult cases.

The cells of an epithelioid AML are round and range from medium-sized to huge; because of their abundant cytoplasm and enormous nucleoli, they resemble ganglion cells. Necrosis and mitoses can be seen. The differential diagnosis of an epithelioid AML includes clear cell RCC.^{20,80,82} Here, too, immunostains are extremely helpful in distinguishing an epithelioid AML from RCC.

Of note, AML is one of the lesions most likely to yield a sparsely cellular, difficult-to-interpret sample composed of rare spindle-shaped cells and stromal fragments. Nevertheless, it is often possible to demonstrate immunoreactivity for HMB45 and make a definitive diagnosis on this basis. For this reason, it is prudent to obtain immunostains on any atypical and/or paucicellular spindle cell or epithelioid cell lesion in the kidney.

Metanephric Adenoma

Metanephric adenoma (MA) is a rare, essentially benign kidney tumor,^{83–86} although the occasional case with metastases has been described.⁸⁷ It can be found at any age but most commonly occurs in women in the fifth decade. About one half are discovered incidentally; the rest are detected because of hematuria, flank pain, or polycythemia. They range from small to very large lesions and can measure up to 15 cm in diameter. Histologically, MA is composed of tight, uniform tubules lined by bland cells with small round nuclei and inconspicuous nucleoli. Mitoses are absent or very infrequent. Psammoma bodies are common. They resemble low-grade papillary RCCs and differentiated Wilms tumors. Unlike papillary RCC, they are negative for epithelial membrane antigen (EMA) and have a normal karyotype. Like Wilms tumor, MAs show nuclear immunoreactivity for Wilms tumor 1 (WT1).



Cytomorphology of metanephric adenoma

- tight cell clusters or sheets
- small cells
- scant cytoplasm
- round, uniform nuclei
- small nucleoli

On cytologic preparations, the cells form short tubules, tight balls, and loose sheets; they have scant cytoplasm, round monotonous nuclei, fine, even chromatin, and rare small nucleoli⁸⁸ ([Fig. 15.5A-C](#)).

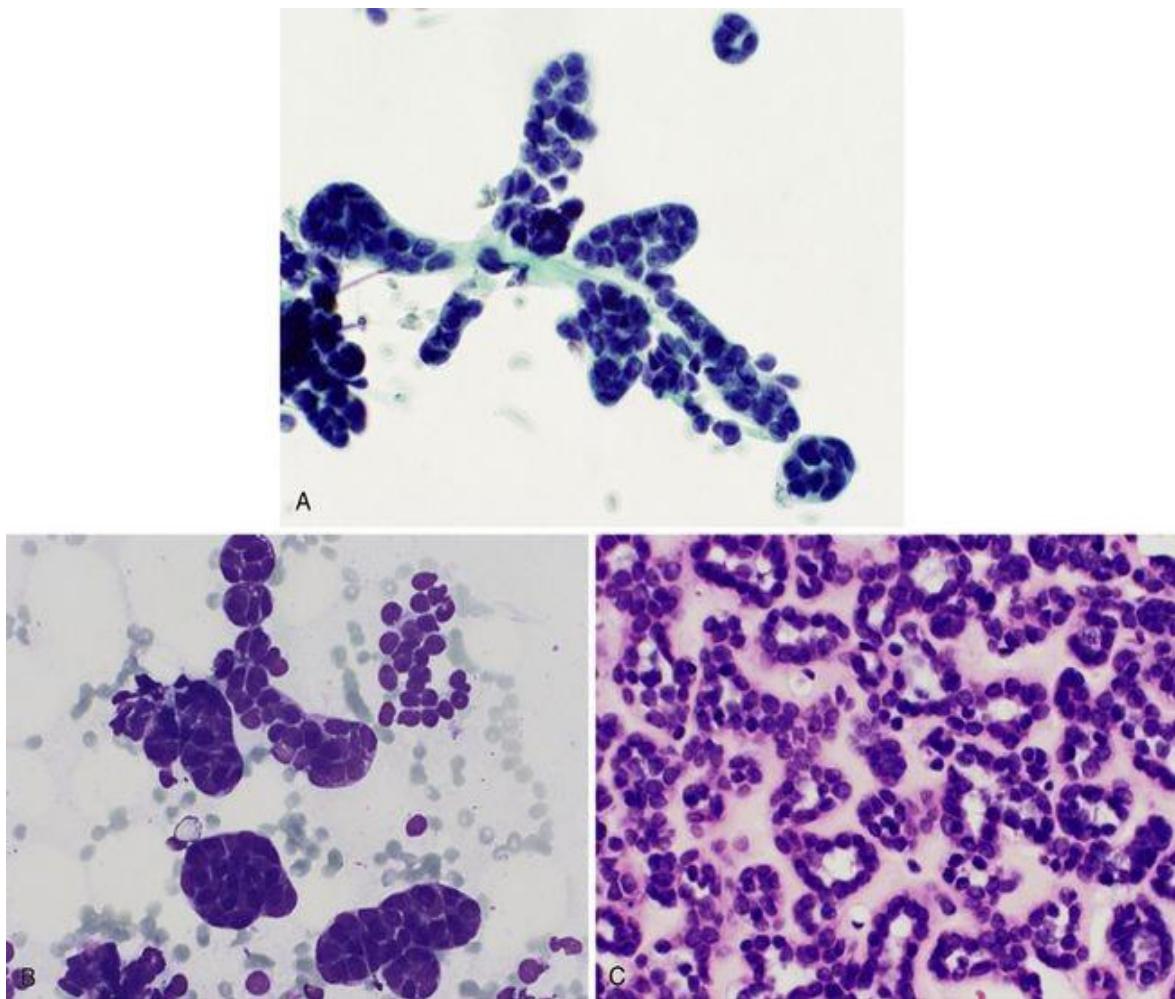


FIGURE 15.5 Metanephric adenoma (MA).
A and B, Smears show short tubules, cords, and tight balls of cells with scant cytoplasm (A, Papanicolaou stain; B, Romanowsky stain). C, Cell block sections show numerous small tubules (hematoxylin-eosin [H & E] stain).



Differential diagnosis of metanephric adenoma

- differentiated, epithelial-predominant Wilms tumor
- low-grade papillary RCC
- metastatic tumor

Most Wilms tumors are easily distinguished from MA in that Wilms tumors are triphasic neoplasms that contain a blastemal component (small, closely packed, mitotically active cells) as one of their three constituents. An epithelial-predominant Wilms tumor, with little or no blastema, can be virtually impossible

to distinguish from an MA,⁸⁹ although some claim—on the basis of histologic material—that the cells of a Wilms tumor are larger, with more hyperchromasia and mitoses.⁸³ Low-grade papillary RCCs have more cytoplasm than MA and, unlike MA, are positive for EMA and negative for WT1.^{64,83,90} Finally, because of the high nuclear-to-cytoplasmic ratio of MA cells, a metastasis should be considered. Most metastases are EMA-positive, and a metastasis is unlikely in the absence of a known or suspected primary tumor elsewhere.

Cystic Nephroma/Mixed Epithelial and Stromal Tumor

Cystic nephroma/mixed epithelial and stromal tumor is a benign cystic neoplasm, also known as *multilocular cyst*, that most commonly occurs as a solitary tumor in boys and middle-aged women. Although some argue that these are distinct tumors, many pathologists disagree, and the cytologic features are identical.⁹¹⁻⁹² It is composed of stroma and small cysts lined by atypical epithelium.⁶⁸ Although cystic, this lesion appears solid radiographically. FNA samples are usually misdiagnosed as either RCC, angiomyolipoma, or sarcoma.^{29,93-94} Specimens are generally hypocellular, but contain some epithelial cells with clear to vacuolated cytoplasm, nuclear membrane irregularity, and prominent nucleoli.⁹⁵⁻⁹⁶ Alternatively, specimens consist of large, pleomorphic spindle cells, admixed with cells with intracytoplasmic vacuoles simulating fat.⁹⁷ As a rule, complete excision is required to make the diagnosis. The only predictable cytologic feature is sparse cellularity. This adds support to the wisdom that hypocellular specimens with atypical cells should be reported as suspicious rather than positive.

Renal Abscess

Focal bacterial pyelonephritis and a renal abscess can appear masslike radiologically.^{1,98} Aspirates contain necrotic material and numerous neutrophils, sometimes with rare atypical cells that are easily confused with those of a clear cell RCC. The atypical cells are few in number, however, and in the context of abundant acute inflammation should be reported (at most) as suspicious for malignancy.

Xanthogranulomatous Pyelonephritis

Xanthogranulomatous pyelonephritis is an atypical host reaction to a bacterial infection and usually presents as a mass lesion.²⁸ Histologically and cytologically,

the lesion is composed of histiocytes and multinucleated giant cells. The histiocytes can form aggregates and resemble the cells of clear cell RCC, but they lack nuclear atypia, and their cytoplasm has a more microvacuolated appearance than that of typical RCCs. Differential immunoreactivity for EMA and CD68 is helpful in difficult cases.

Renal Infarct

Rarely, renal infarcts have a radiographic appearance suggestive of malignancy.⁹⁹ Specimens are sparsely cellular and composed of necrotic material, which can contain rare atypical cells resembling those of clear cell RCC. A diagnosis of malignancy should be avoided when the atypical cells are few in number, which is usually the case with renal infarcts.

Renal Cysts

Renal cysts are common. Of all renal lesions, 70% to 85% are cysts, and 50% of men over the age of 50 years have at least one cyst.¹⁰⁰⁻¹⁰¹ A majority are benign, acquired, and solitary; only 1% to 4% of cysts are cystic RCCs,¹⁰²⁻¹⁰⁸ usually of clear cell or papillary type.⁶⁸ The prognosis of a patient with a cystic RCC is generally excellent,¹⁰⁹⁻¹¹⁰ but metastases do occur,¹¹¹ and resection, either by partial or radical nephrectomy, is indicated.

The pretest probability that a renal cyst is malignant depends, in part, on the radiologic appearance. Cysts are classified according to the Bosniak system.¹¹²⁻¹¹⁷ Most lesions are category 1 (benign); category 4 lesions are frankly malignant and are resected directly; and categories 2 and 3 are indeterminate. Between 5% and 57% of indeterminate cysts are malignant. Because the pretest probability of malignancy is as high as 57%, many urologists believe that all indeterminate cysts should be resected.

How helpful is an FNA specimen that lacks atypical cells (contains only macrophages)? Does this result change the pretest probability of an RCC? Much of the data on this subject predates CT and MRI^{26,98,101,104-105,118-120} but implies that a negative diagnosis supports the diagnosis of a benign cyst. Closer examination, however, reveals that the vast majority of the lesions in these studies were simple cysts (Bosniak category 1 lesions) that would not be aspirated today. Unfortunately, no study has examined the sensitivity of FNA for Bosniak 2 and 3 lesions. Indirect evidence provides some insights. First, 10% of all RCCs yield a nondiagnostic or falsely negative FNA result.^{20,22,26,29,37,121-122} Second, most RCCs

incorrectly interpreted as negative by FNA are cystic.³⁷ Third, most radiographically suspicious cysts prove to be RCC.^{20,22,29} Finally, in the largest series of cystic RCCs (11 cases), only two cases had atypical cells on cytologic examination, and repeat aspirates in both patients were negative.¹²³ From these data, it can be inferred that the sensitivity of FNA for Bosniak category 2 and 3 cysts is low, and certainly no higher than 10% to 20%. Given that the pre-FNA probability is 5% to 57%, a negative FNA result (macrophages only) in this setting has little effect on the patient's risk of disease and is best reported as nondiagnostic ([Fig. 15.6](#)).

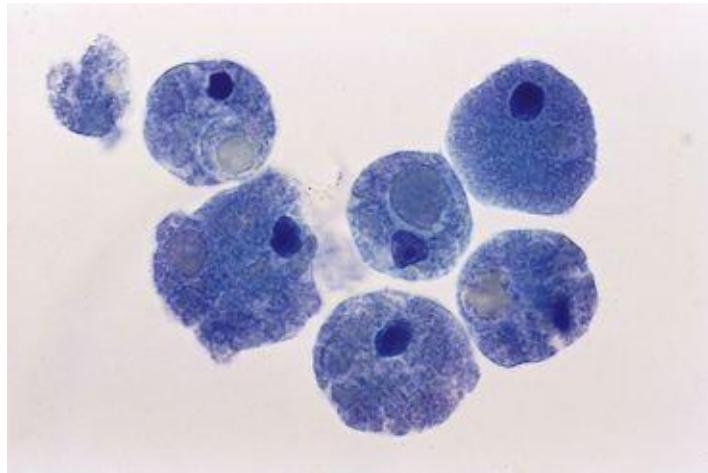


FIGURE 15.6 Renal cyst.
Macrophages are a nonspecific finding (Papanicolaou stain).

Complicating the matter further is the fact that some benign cysts are virtually impossible to distinguish from an RCC by FNA. Patients on renal dialysis for renal failure often acquire cysts, and 9% develop RCC, often a multifocal tumor.¹²⁴⁻¹²⁷ Patients with adult polycystic kidney disease, an autosomal dominant disease, are also at an increased risk for developing RCC, which, like the tumors in renal dialysis patients, can be multifocal. In both settings, papillary hyperplasia occurs within the cysts,¹²⁸ and distinction between this hyperplasia and carcinoma is not possible by FNA. (Histopathologic criteria for this distinction are controversial as well.) The best approach is simply to avoid aspirating cysts in these patients.

When confronted by an FNA specimen of a cystic renal mass, the cytopathologist can be aided by a review of the imaging findings, especially with a radiologist who has expertise with renal cysts. Some urologists aspirate benign, Bosniak

category 1 cysts merely for symptomatic relief. In this setting, where the radiographic probability of RCC is so low, one should take a very conservative approach in interpreting any atypical cells. If, on the other hand, the aspirate is performed for an indeterminate (Bosniak category 2 or 3) lesion and even a few atypical cells are seen, a suspicious diagnosis is appropriate ([Fig. 15.7](#)). If the result is nondiagnostic (macrophages only), an explanatory note can be helpful, such as “The findings are consistent with a cystic renal lesion. Because parenchymal elements have not been sampled, the possibility of a neoplasm cannot be excluded.”

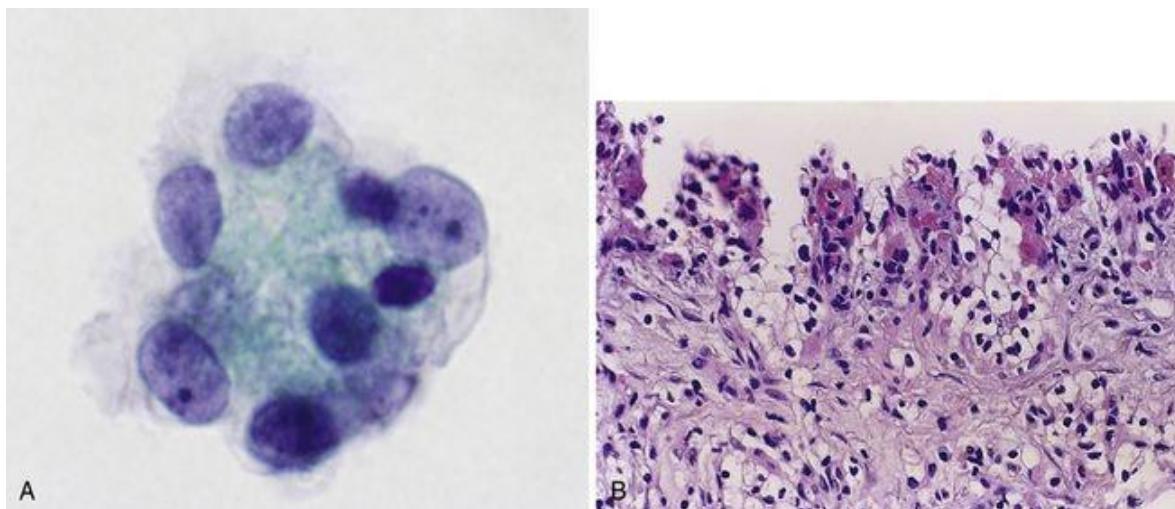


FIGURE 15.7 Cystic renal cell carcinoma (RCC).

A, Fine-needle aspiration (FNA) specimen. There were only a few groups of cells with clear or granular cytoplasm on the slide. In the setting of a radiologically atypical cyst, this finding is suspicious for carcinoma. B, Surgical resection specimen. The tumor was almost entirely cystic. Malignant cells were found in the wall in only a few areas (hematoxylin-eosin [H & E] stain).



Summary of the challenge of renal cysts

- Renal cysts are common, and most are benign.
- RCC can be cystic.
- Adequate sampling of a cystic RCC is difficult.
- Some benign cysts are difficult to distinguish from RCC:
 - cysts due to renal failure
 - adult polycystic kidney disease
 - cystic nephroma
- The value of a negative diagnosis is limited.



Summary: atypical cells on a kidney FNA

- Benign entities that sometimes have atypical cells:
 - cystic nephroma
 - renal abscess
 - xanthogranulomatous pyelonephritis
 - renal infarct
 - atypical cysts
 - angiomyolipoma
- Best diagnostic clue:
 - hypocellular specimen
- Best cytologic interpretation:
 - atypical or suspicious (rather than positive)

Renal Cell Carcinoma

65,000 malignant tumors of the kidney are diagnosed in the United States every year, with more than 13,000 deaths.¹²⁹ Men are more commonly affected than women. In men, tumors of the kidney are the 10th most frequent cause of cancer-related mortality in the United States, accounting for 3% of all cancer deaths.¹²⁹

Of all the malignant tumors of the kidney, RCC, a malignancy of the renal tubules, is the most common. Risk factors include tobacco smoking and obesity. A minority of RCCs occur in the setting of one of several inherited cancer syndromes, the most common being VHL disease. In patients with VHL disease, the mean age at manifestation of RCC is 37 years, as compared with 61 years for sporadic RCC.

Patients sometimes present with the classic triad of hematuria, flank pain, and/or a palpable mass, but the tumor is often discovered incidentally. The grade of an RCC has prognostic significance second only to tumor stage, and RCCs are graded using the Fuhrman system.⁵¹ Because it is based on nuclear features, it is easily applied to cytologic preparations, with good cytologic-histologic correlation.¹³⁰⁻¹³² With heterogeneous RCCs, the highest grade is assigned.

The most recent histologic classification system,⁶⁵ strongly influenced by genetic evidence, divides RCCs into various subtypes. The most common types

for which cytologic features have been described are listed in [Table 15.1](#). Cytologists should be familiar with these subtypes, which differ in their morphologic features and prognosis. When performed on a portion of the cytologic specimen, cytogenetic and/or molecular genetic analysis, including conventional karyotyping and FISH, are a helpful adjunct in subclassifying renal neoplasms.

TABLE 15.1

SUBTYPES OF RENAL CELL CARCINOMA

Subtype	Percent of All RCCs	Cytogenetics	Other
Clear cell/conventional	75	loss of 3p	—
Papillary	15	trisomies 7, 17	good prognosis, multifocal
Chromophobe	3-5	extensive loss of entire chromosomes (most common: 1, 2, 3, 6, 10, 13, 17, 21)	—
Collecting duct	< 1	not well characterized	—
Sarcomatoid	3	complex karyotypes	poor prognosis
Xp11.2 translocation-associated	< 1	translocations of X	immunoreactive for TFE3
Mucinous tubular and spindle cell carcinoma	< 1	not well characterized	—

RCC, renal cell carcinoma; TFE3, transcription factor E3.

Clear Cell Renal Cell Carcinoma

Clear cell (also called *conventional*) RCC accounts for 75% to 80% of all RCCs and is strongly associated with a variety of deletions on the short arm of chromosome 3 (3p), the site of the VHL gene.¹³³⁻¹³⁶ The average size of a clear cell RCC is 7 cm, but small tumors are being detected with increasing frequency due to the increasing use of cross-sectional imaging techniques. Size is not a determinant of malignancy, but the frequency of metastases does correlate with increasing size of the primary tumor. Necrosis, hemorrhage, cystic degeneration, and calcification are common; these features give the clear cell RCC its characteristic heterogeneous appearance on imaging studies. Histologically, clear cell RCC is composed of cells with abundant cytoplasm that is clear, granular, or a mixture of both. The clear and granular appearance is due to the presence of lipid and glycogen in the cytoplasm. A distinction used to be made between RCCs of predominantly clear or granular type, but this is no longer believed to have clinical relevance, and the granular type is now folded into the clear cell category. The tumor cells have a rich network of delicate, thin-walled blood vessels, which accounts for the contrast enhancement pattern on imaging studies and the frequent bloodiness of FNA samples.



Cytomorphology of clear cell renal cell carcinoma

- blood
- large cohesive cell groups
- abundant wispy cytoplasm with ill-defined edges
- cytoplasmic vacuoles
- large, round, eccentrically placed nucleus

- nucleoli vary in size depending on Fuhrman grade

Aspirates from a clear cell RCC are often very bloody. Smears can be highly cellular, but in fact sometimes reveal only blood; in such cases tissue fragments are often recovered in cell block sections. With cellular smears, the tumor cells are displayed as large tissue fragments and isolated cells. The malignant cells have abundant cytoplasm and thus a low nuclear-to-cytoplasmic ratio, with a round to slightly irregular, eccentrically placed nucleus^{26,28,43,137} ([Fig. 15.8A](#) and [B](#)). The eccentrically positioned, round nucleus gives the cells a plasmacytoid appearance ([Fig. 15.8C](#)). The nucleus is sometimes so far off-center that it appears partially extruded. The size of the nucleolus depends on the grade of the tumor: small and inconspicuous in grade 1 tumors, progressively more prominent in grade 2, 3, and 4 tumors. Cytoplasm is thin and wispy, and cell membranes are poorly defined. Small cytoplasmic vacuoles are often peripherally placed, and the remaining, more granular, cytoplasm is central. Higher-grade tumors have more isolated cells, less cytoplasmic vacuolization, and, as mentioned, larger nucleoli ([Fig. 15.8D](#)). Pink, strandlike fibrillary material, possibly basement membrane material, is seen with Romanowsky stains and is highly characteristic (see [Fig. 15.8B](#)). Cytologic preparations from roughly one half of RCCs demonstrate so-called transgressing vessels;¹³⁸ their diagnostic significance is unclear, however, as some other tumors show similar features. Low-grade tumors are challenging because fragments of the tumor are extremely cohesive: One encounters large, highly cellular aggregates in which the individual cells are difficult to identify. A careful search around the perimeter of these chunks reveals the characteristic cells. Grade 1 and 2 clear cell RCCs, sometimes a challenge to interpret correctly on direct smears, are often surprisingly straightforward on cell block sections, where the clear cell morphology is especially easy to recognize ([Fig. 15.8E](#)).

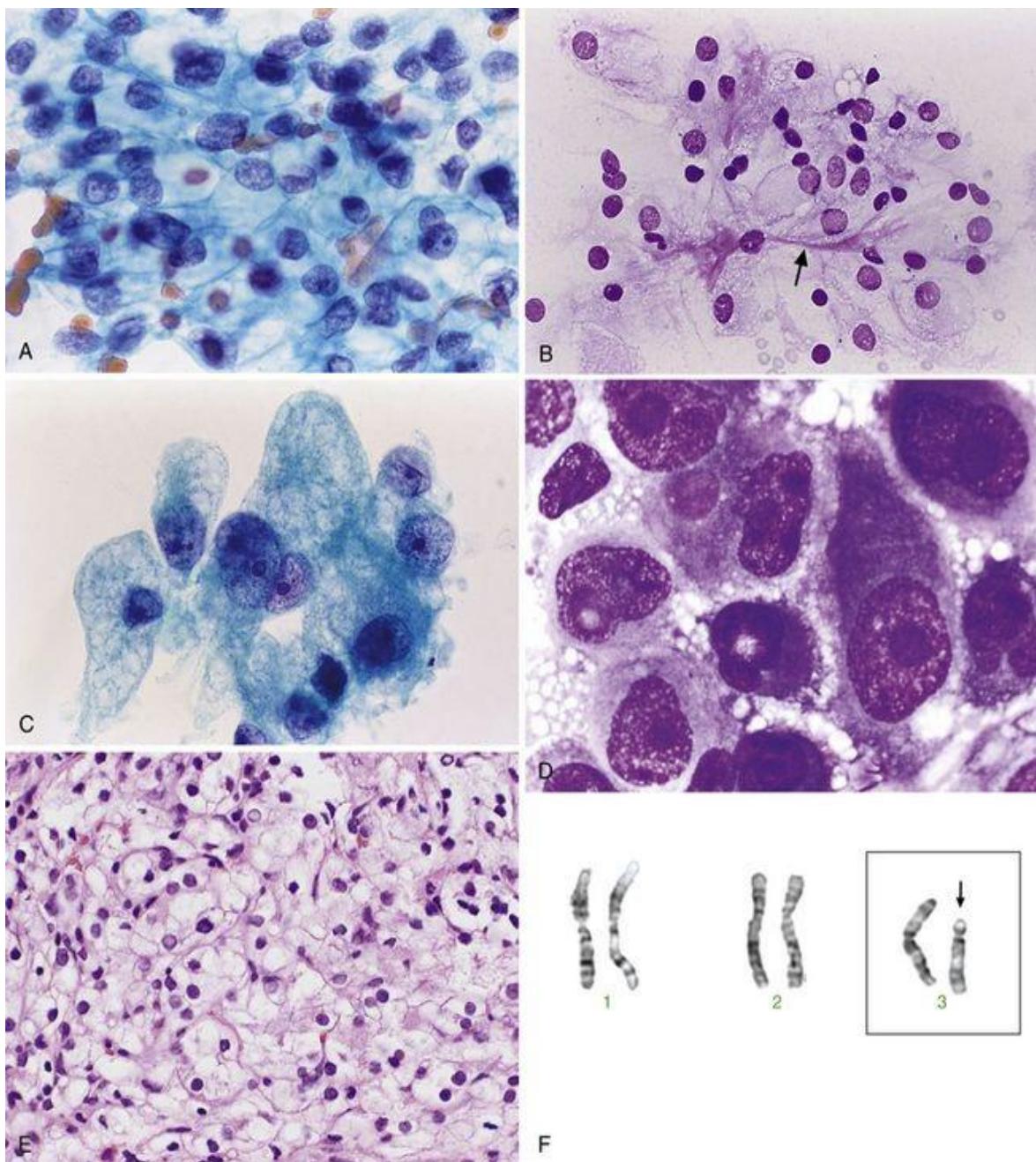


FIGURE 15.8 Clear cell renal cell carcinoma (RCC).

A, The tumor cells are in a broad sheet and have abundant clear and granular cytoplasm (Papanicolaou stain). B, Fine cytoplasmic vacuoles and pink, strandlike material (arrow) are well seen with the Romanowsky stain. C, Nuclei are often eccentrically placed (Papanicolaou stain). D, The cytoplasm of this grade 4 tumor is more opaque, the cells are less cohesive, and nucleoli are prominent (Romanowsky stain). E, Cell block sections reveal the characteristic clear cell morphology (hematoxylin-eosin [H & E] stain). F, A karyotype reveals a 3p deletion (arrow).



Differential diagnosis of clear cell renal cell carcinoma

- normal distal tubular cells
- macrophages
- adrenal cortical cells
- hepatocytes
- cystic nephroma
- angiomyolipoma (epithelioid)
- papillary RCC
- oncocytoma

The differential diagnosis includes benign tubular cells, which rarely emerge as large groups and do not have vacuolated cytoplasm. Macrophages, as in xanthogranulomatous pyelonephritis, mimic the cells of RCC but are more dispersed rather than aggregated and more uniformly microvacuolated. Also, they lack nuclear atypia and an extruded nucleus. Adrenal cortical cells are more uniform, have smaller vacuoles, and are frequently stripped of their cytoplasm. Hepatocytes have a more uniformly granular cytoplasm containing lipofuscin and round nuclei of various sizes that are almost always centrally placed. Specimens from a cystic nephroma are generally hypocellular but sometimes contain occasional atypical cells with clear to vacuolated cytoplasm, nuclear membrane irregularity, and prominent nucleoli. An unequivocal diagnosis of RCC should be avoided if the sample is sparsely cellular, particularly if a cell block is unavailable. Some angiomyolipomas have an epithelioid morphology, potentially misdiagnosed as RCC even after histopathologic examination of a nephrectomy specimen. It is advisable to consider the diagnosis of an epithelioid angiomyolipoma whenever a kidney tumor has an epithelioid morphology; the diagnosis of an angiomyolipoma can be excluded by demonstrating negative staining for HMB45 and MART1. Similarly, some high-grade papillary RCCs are virtually indistinguishable from clear cell RCC by conventional morphology. Cytogenetic analysis is particularly helpful,⁵⁴ because clear cell RCC is characterized by deletion of 3p (Fig. 15.8F; see Table 15.1). Clear cell RCCs with predominantly granular cytoplasm can look like oncocytomas (see “Differential Diagnosis of Oncocytoma” earlier).

Papillary Renal Cell Carcinoma

Papillary RCC represents between 7% and 15% of all RCCs.^{64,139–140} This subtype

is associated with trisomies of chromosomes 7, 16, and 17;^{54,63,141-142} renal cortical adenomas;¹⁴³ and multifocality.¹⁴³ Tumors measure as large as 23 cm, and large tumors can be cystic and necrotic. Contrast-enhanced imaging studies reveal an avascular or hypovascular lesion,¹⁴⁰ in contrast to clear cell RCCs, which are hypervascular. Patients with a low-grade/low-stage papillary RCC have an excellent prognosis,^{139-140,142} whereas those with high-grade/high-stage papillary RCC have a poor prognosis.^{142,144} Low-grade papillary RCCs are often small and peripheral and are amenable to partial nephrectomy. Despite the high rate of multifocality, patients do not appear to be at increased risk for local recurrence after partial nephrectomy.

An RCC is assigned the papillary subtype histologically if at least 50% of it is composed of true papillae. In some cases the papillae are so closely packed that the architecture is obscured, and the cells appear to grow in solid sheets.⁶⁴ Papillary RCCs are further divided into two basic morphologic subtypes, called types 1 and 2, which correlate with tumor grade. Thus, type 1, the more common type, is a low-grade tumor composed of small cells with scant cytoplasm. Nuclei are small and round and nucleoli are inconspicuous (Fuhrman grade 1 or 2). Type 2 is a less common, high-grade tumor composed of large cells with abundant granular cytoplasm. Nuclei of type 2 tumors are large, and nucleoli are prominent (grade 3). As can be seen from this description, type 2 papillary RCCs resemble higher-grade clear cell RCCs. Papillary RCCs are immunoreactive for EMA, low-molecular-weight keratins, and cytokeratin 7^{64,145} and negative for high-molecular-weight keratin 34BE12 and WT-1.



Cytomorphology of papillary renal cell carcinoma (two types)

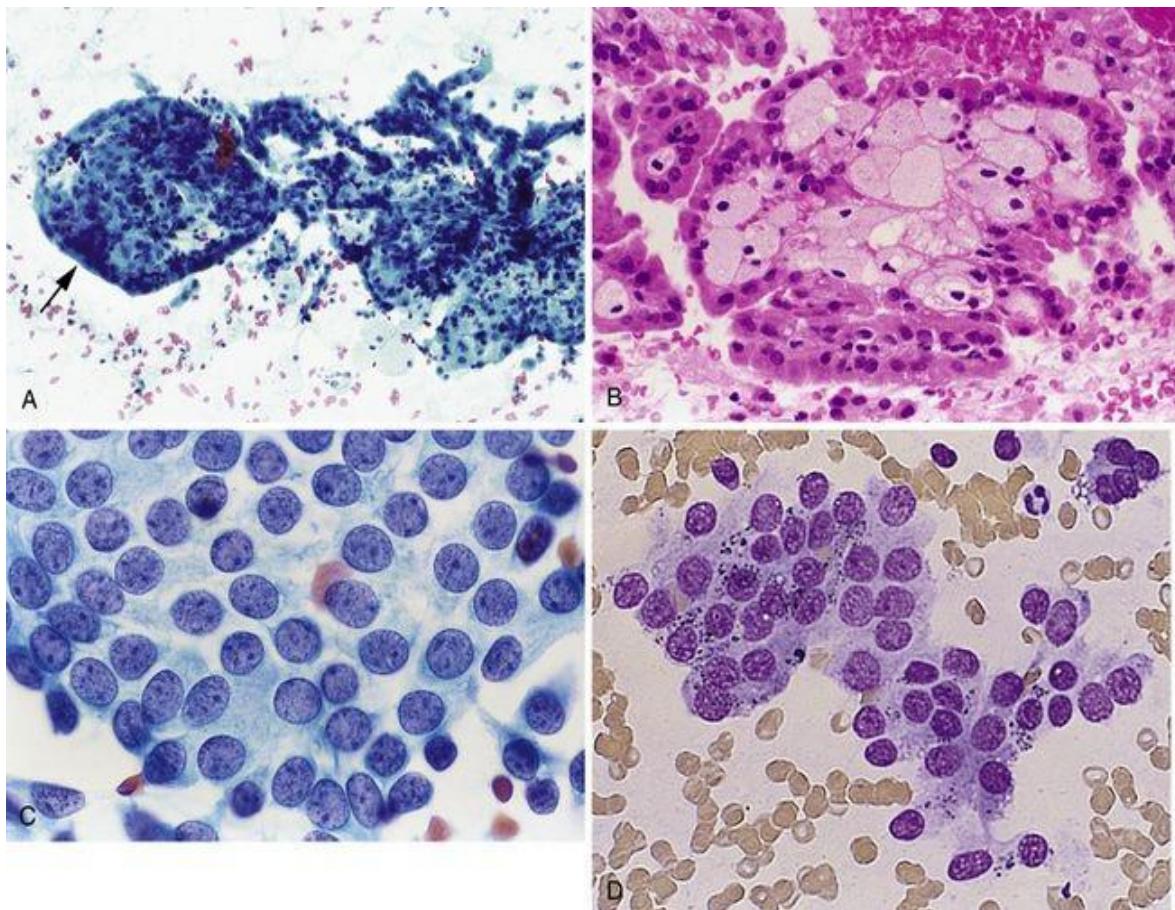
Type 1 (low grade)

- papillae and spherules
- small to medium sized cuboidal cells
- uniform nuclei
- scant to moderate amount of cytoplasm
- abundant intracytoplasmic hemosiderin
- foamy macrophages

Type 2 (high grade)

- large cells
- large nuclei with grade 3 nucleoli
- abundant granular cytoplasm

Papillary RCCs have a number of characteristic cytologic features.^{146–149} These include papillae with true fibrovascular cores,¹³⁸ spherules, and tubules (Fig. 15.9A). Fibrovascular cores distended with foamy macrophages are common and especially well seen on cell block preparations (Fig. 15.9B); indeed, the cell block is often the easiest way to correctly identify a papillary RCC. The tumor cells can be extremely uniform (Fig. 15.9C) when the tumor is type 1 (low grade). In some cases there is abundant intracytoplasmic hemosiderin, a characteristic feature of papillary RCC (Fig. 15.9D). Psammoma bodies occur but only in a small minority of papillary RCCs.¹⁴⁶ Rare cases with hyaline globules have been described.¹⁵⁰



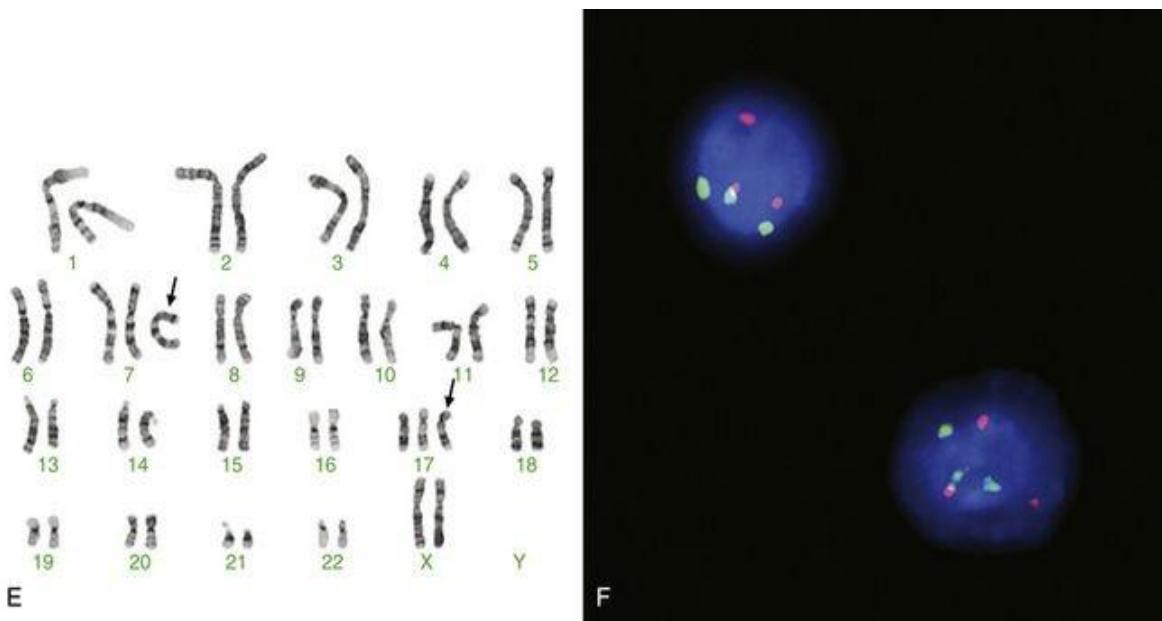


FIGURE 15.9 Papillary renal cell carcinoma (RCC).

A, A complex papillary architecture is apparent on smears. Note the large sphere (arrow), a characteristic finding. Foamy macrophages are scattered in the background (Papanicolaou stain). B, Foamy macrophages stuff the fibrovascular cores of the papillae and are often best seen on cell block sections (hematoxylin-eosin [H & E] stain). C, Note how round and monomorphic the nuclei are in a low-grade papillary RCC (Papanicolaou stain). D, Cytoplasmic hemosiderin is a common finding (Romanowsky stain). E, A karyotype reveals trisomies of chromosomes 7 and 17 (arrows). F, Fluorescence in situ hybridization (FISH) shows trisomies of chromosomes 7 (green signal) and 17 (red signal).



Differential diagnosis of papillary renal cell carcinoma

- distal tubular cells
- glomeruli
- metanephric adenoma
- papillary hyperplasia in cysts associated with dialysis or adult polycystic kidney disease
- clear cell RCC

The cells of a low-grade (type 1) papillary RCC resemble benign distal tubular cells, but benign tubular cells are rarely present in large numbers and never show papillae or spherule formation. Glomeruli, with their densely cellular, spherical arrangement, can be misinterpreted as a papillary RCC, but close observation reveals the capillary loops that identify them as glomeruli. Papillary hyperplasia occurs within the cysts of dialysis patients and those with adult polycystic

disease, and distinction between this hyperplasia and carcinoma is not possible by FNA. (The distinction is controversial even histologically.) As previously mentioned (see “[Renal Cysts](#)”), the best approach is to avoid aspirating cysts in dialysis patients and those with adult polycystic kidney disease.

The differential diagnosis also includes clear cell RCC. Indeed, many high-grade (type 2) papillary RCCs are mistaken for clear cell RCCs.³⁷ Conventional cytogenetics or FISH can serve as an adjunct to cytology in distinguishing clear cell from papillary RCC based on their distinctive genetic aberrations (see [Fig. 15.8F](#); [Table 15.1](#)).

Chromophobe Renal Cell Carcinoma

Chromophobe RCC is a distinctive variant that makes up 3% to 5% of all RCCs^{151–153} and is associated with numerous chromosomal monosomies.^{54,154–156} Patients have an excellent prognosis^{153,157–158} unless their tumor is large or multifocal.¹⁵⁹

Histologically, the tumor is composed of trabeculae of cells with abundant, flocculent cytoplasm, distinct cell borders, frequent binucleation, dark chromatin, and irregular, “raisinoid” nuclear outlines. The cytoplasm is either pale (hence “chromophobic;” classic variant), eosinophilic (eosinophilic variant), or both (mixed variant). The cytoplasmic pallor of the classic variant is due to the abundance of microvesicles, a characteristic ultrastructural feature.⁵² The microvesicles push the remaining organelles to the periphery, so the pallor tends to be accentuated around the nucleus. Their prominent cell borders, characteristic nuclear changes, and cytoplasmic clearing make them resemble koilocytes, although the cytoplasmic clearing in chromophobe RCC is patchier and less sharply defined than it is in koilocytes. With the Hale’s colloidal iron stain, the cytoplasm stains blue in a reticular (meshlike) or coarse, dropletlike pattern.⁶⁰



Cytomorphology of chromophobe renal cell carcinoma

- trabecular arrangement
- koilicytoid cells:
 - abundant cytoplasm
 - well-defined cell borders
 - binucleation
 - nuclear size variation
 - hyperchromatic chromatin without nucleoli

- mitoses

Aspirates are highly cellular. In general, the cells are less cohesive than those of clear cell RCCs.¹⁶⁰⁻¹⁶² Abundant, irregularly granular, fluffy cytoplasm is characteristic (Fig. 15.10A). Nuclei show significant variation in size, occasional binucleation, hyperchromasia, irregular outlines and grooves, and pseudoinclusions.¹⁶³⁻¹⁶⁴ Rarely, calcifications are present.¹⁶⁵ With Romanowsky-stained smears, the perinuclear reticulated or vacuolated zone is particularly well seen (Fig. 15.10B). When fully developed and widespread, the koilicytoid appearance is unique to chromophobe RCCs.

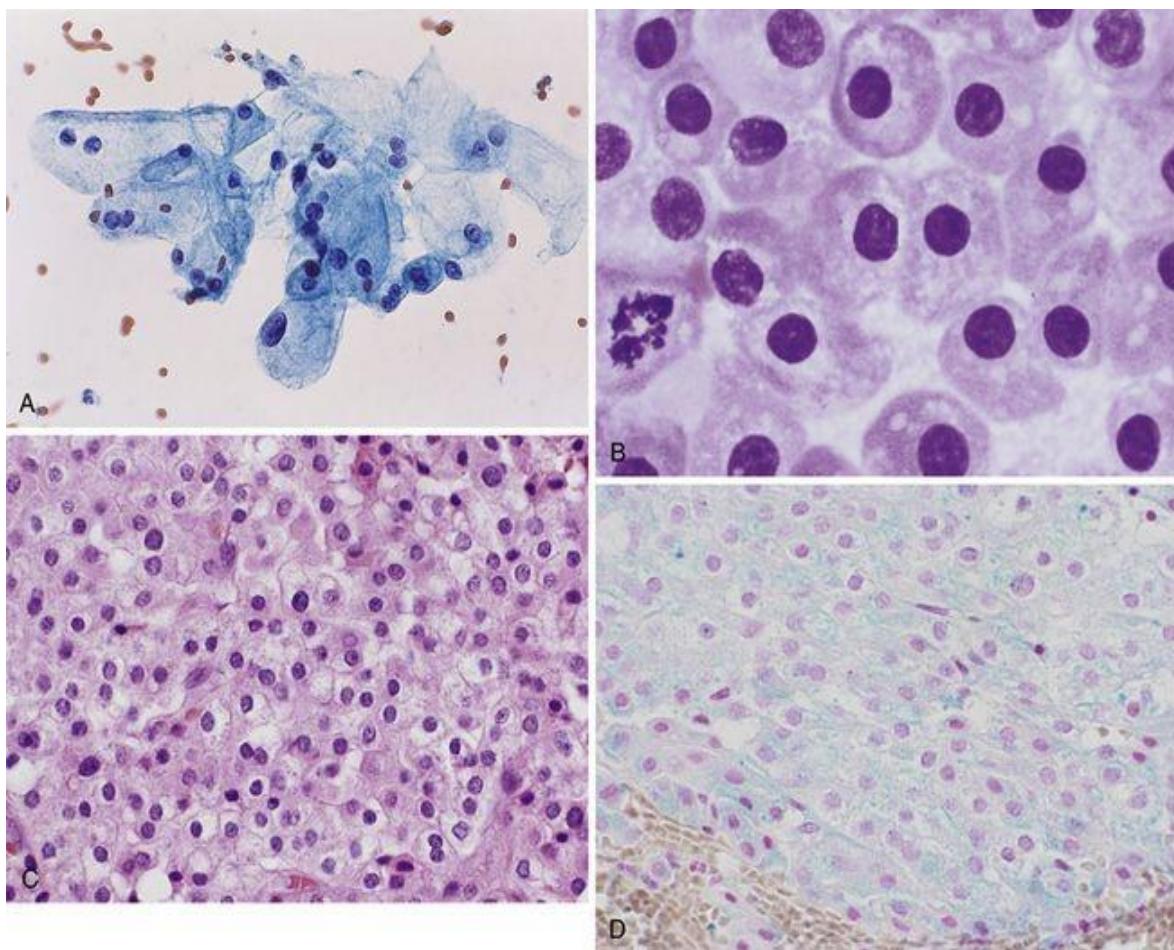


FIGURE 15.10 Chromophobe renal cell carcinoma (RCC), typical variant.
A, The tumor cells have a koilicytoid appearance, with abundant cytoplasm, prominent cell

borders, binucleation, and dark nuclei that vary in size. Nucleoli are hard to see (Papanicolaou stain). *B*, The fluffy, irregularly vacuolated cytoplasm has a moth-eaten appearance (Romanowsky stain). *C*, In cell block sections, the tumor cells are arranged in an endless sheet (hematoxylin-eosin [H & E] stain). *D*, Hale's colloidal iron (HCI) stain shows a diffuse, reticulated staining pattern.



Differential diagnosis of chromophobe renal cell carcinoma

- clear cell/conventional RCC
- oncocytoma

The classic variant of chromophobe RCC is easily confused with a clear cell RCC, and the eosinophilic variant with an oncocytoma. In comparison with clear cell RCCs, chromophobe RCCs have more variation in cell and nuclear size, darker chromatin without nucleoli, and much more irregular nuclear outlines. Compared with oncocytoma, chromophobe RCCs have more nuclear size variation and nuclear outline irregularity, but in some cases the distinction is impossible by routine cytologic stains alone. A tissue fragment in the cell block sections can be helpful. The cells of oncocytomas are arranged in rounded nests: One can draw a circle around groups of cells (see [Fig. 15.3C](#)), whereas the cells of a chromophobe RCC are arranged in endless trabeculae that are impossible to circumscribe ([Fig. 15.10C](#)). In many oncocytomas, there is more stroma between the nests of cells, whereas the trabeculae of a chromophobe RCC are so closely packed that there is little room for intervening stroma. Diffuse reticular or focal coarse, dropletlike cytoplasmic staining with Hale's colloidal iron is characteristic of chromophobe RCC ([Fig. 15.10D](#)). This contrasts with the negative or focal apical staining of oncocytomas (see [Fig. 15.3D](#)). Immunohistochemistry might also be helpful but is not yet more reliable than Hale's colloidal iron staining alone.⁶¹ Fortunately, in the appropriate clinical setting, a partial nephrectomy is the preferred treatment for both lesions. In equivocal cases, a reasonable approach is to defer the distinction between oncocytoma and chromophobe RCC to extensive histologic sampling of the resected tumor, and interpret the FNA specimen as "oncocytic neoplasm (oncocytoma versus chromophobe RCC)," with a comment that a partial nephrectomy should be considered if clinically appropriate.

Sarcomatoid Renal Cell Carcinoma

Sarcomatoid RCC accounts for 3% of all RCCs. It is a high-grade tumor with a poor prognosis. Many are unresectable at presentation and therefore ideal candidates for FNA,¹⁶⁶⁻¹⁶⁷ although more are being identified at a lower stage. Most are found in association with clear cell RCC, but sarcomatoid transformation of chromophobe, collecting duct, and papillary RCCs also occurs.

Histologically and cytologically, sarcomatoid RCC is a high-grade spindle cell neoplasm, with or without epithelioid differentiation¹⁶⁸ (Fig. 15.11). If an epithelioid component is not identified, a sarcomatoid tumor must be immunoreactive for keratin proteins or EMA to merit a diagnosis of RCC. The differential diagnosis depends on sampling. If only the spindle cell area is sampled, a sarcoma and an angiomyolipoma are considered; if only the epithelioid area is sampled, the tumor may be incorrectly classified as a clear cell RCC.

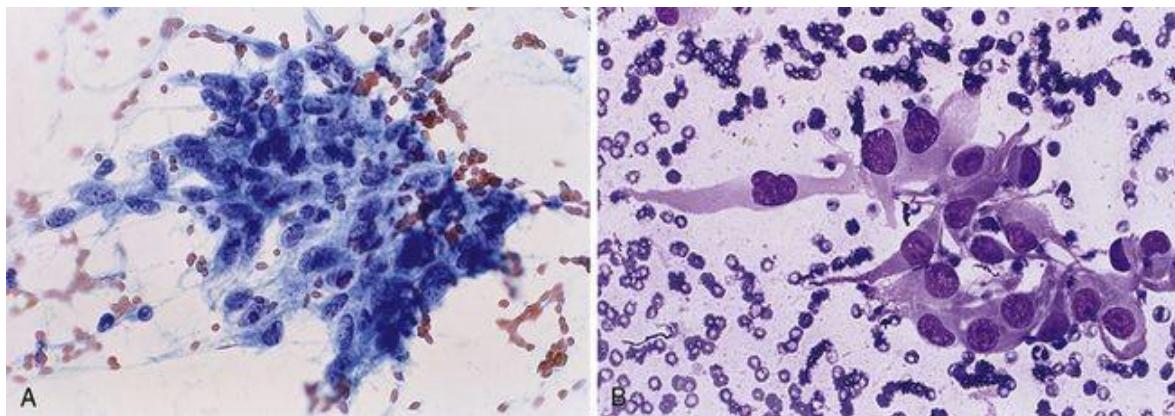


FIGURE 15.11 Sarcomatoid renal cell carcinoma (RCC).

The malignant cells are haphazardly arranged and very elongated. *A*, Papanicolaou stain. *B*, Romanowsky stain.

Collecting Duct Carcinoma (Bellini Tumor)

Collecting duct carcinoma is a rare, poorly defined tumor characterized by its medullary location, tubulopapillary histology, high-grade cytology, and prominent desmoplasia.¹⁶⁹⁻¹⁷⁹ It resembles other tumors, especially renal medullary carcinoma¹⁸⁰ and papillary RCC.^{171, 174, 176, 181-182} Characteristically, it is immunoreactive for high-molecular-weight cytokeratin 34BE12.^{169, 175}

Cytologic features resemble those of a metastatic tumor: Cells have large,

hyperchromatic nuclei and scant cytoplasm.^{183–187} A renal metastasis in a patient without a known primary is unusual, however, and should prompt consideration of an unusual primary renal tumor. Resection may be necessary to accurately diagnose this tumor.

Xp11.2 Translocation–Associated Renal Cell Carcinoma

Xp11.2 translocation–associated RCC was described in 1999 as a distinct histologic lesion that occurs primarily in children.¹⁸⁸ Subsequent genetic studies confirmed the histologic features, identified cases in adults, and showed a strong association with translocations on chromosome X involving the transcription factor E3 (TFE3) gene.^{189–190} Several distinct cytogenetic rearrangements have since been described, and an association with different histologic and clinical features has been proposed. Histologically, the tumor has a mixed nested and papillary architecture made up of cells with abundant voluminous cytoplasm and frequent calcifications.¹⁸⁸ The diagnosis is confirmed by demonstrating immunoreactivity for TFE3 and relatively scant staining for EMA and keratin. Cytologic preparations reveal cells with abundant clear and granular cytoplasm.^{191–193} Strong clinical suspicion (young patient), immunocytochemistry, and/or cytogenetics are necessary to distinguish this tumor from clear cell and other RCCs.

Mucinous Tubular and Spindle Cell Carcinoma

Mucinous tubular and spindle cell carcinoma is a rare tumor with an excellent prognosis.¹⁹⁴ Tumors are composed of a mixture of tubular and spindle cells, often in a mucoid matrix. Tumor cells are immunoreactive for EMA, AMACR, and CK7.¹⁹⁵ Loss of chromosomes has been reported.¹⁹⁵ Cytologically, aspirates consist of aggregates of relatively uniform, oval to spindle-shaped cells with abundant myxoid matrix (Fig. 15.12).^{195–196} The myxoid matrix is relatively unique to this tumor, although it can be seen with some metastases (e.g., adenoid cystic carcinoma). In the absence of this matrix, the tumor can be confused with a low-grade clear cell or papillary RCC. Papillary RCCs are also positive for CK7.

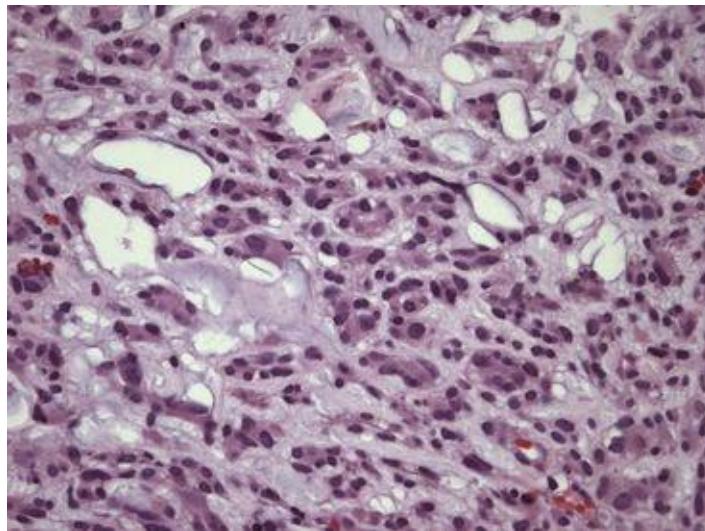


FIGURE 15.12 Mucinous tubular and spindle cell carcinoma.
Compressed tubules, spindle cells, and pale-blue myxoid material are apparent in this cell block section (hematoxylin-eosin [H & E] stain).

Urothelial Carcinoma

Urothelial carcinoma (UC) of the kidney is histologically and clinically similar to its bladder counterparts. It accounts for 5% to 10% of all renal tumors, and multifocality is common. There is a strong association with tobacco smoking and long-term use of analgesics, especially phenacetin. Other risk factors include urinary tract infections, urinary stones, papillary necrosis, Balkan nephropathy, and radiologic contrast material that contains thorium. The most common presenting signs or symptoms are hematuria and flank pain.

Distinguishing UCs from RCCs is important because the management of a patient with a UC of the kidney requires resection of the ureter along with the kidney. The distinction between a UC and an RCC can sometimes be made on the basis of imaging features, but this is more difficult with large and centrally placed tumors.



Cytomorphology of urothelial carcinoma

- large cells
- dark nuclei
- dense, smooth cytoplasm
- elongated cells
- cercariform cells

The cytologic appearance depends upon the grade of the UC.^{22,24,26,197} Smears from low-grade tumors contain sheets, papillae, and isolated cells with a moderate or abundant amount of smooth cytoplasm without vacuolization. In many cases, elongated cells with long cytoplasmic tails are prominent. Some of the tails are narrow in the middle and wide and flat at the end. An intracytoplasmic vacuole may be present at the end of the tail. These so-called *cercariform cells*¹⁹⁸⁻²⁰⁰ are characteristic of UCs and, when present in large numbers, are helpful in distinguishing UC from tumors of other sites ([Fig.15.13](#)). High-grade tumors are composed of isolated cells and small clusters with a scant to moderate amount of cytoplasm, dense hyperchromatic chromatin, and irregular nuclei. Cercariform cells may be present.

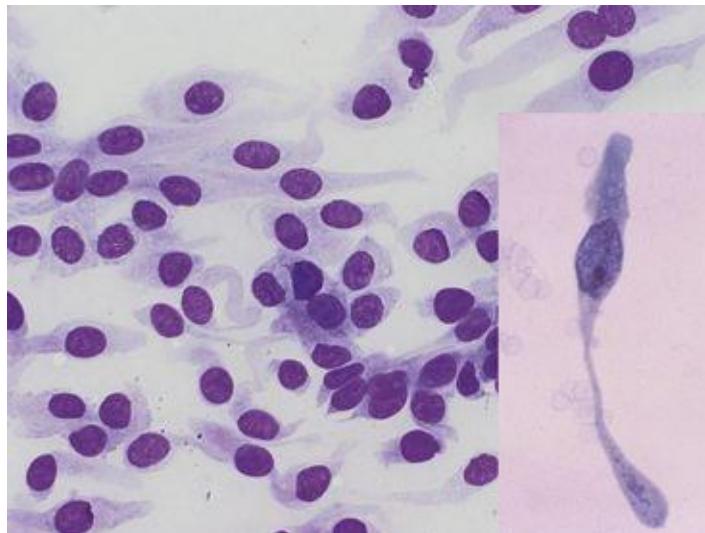


FIGURE 15.13 Urothelial carcinoma (UC).

UCs typically contain numerous cells with elongated cytoplasm, some with flared ends, called *cercariform cells* (Romanowsky stain). *Inset:* cercariform cell (Papanicolaou stain).



Differential diagnosis of urothelial carcinoma

- renal cell carcinoma
- metastasis

In difficult cases, immunocytochemical studies are helpful. UCs are variably positive for keratins 34BE12, CK20, GATA3, carcinoembryonic antigen (CEA),

and mucin; RCCs are negative for these markers. Conversely, RCCs are usually positive for CD10, RCCma, PAX-2, and PAX-8, whereas UCs are not. Distinction between UC and a metastasis can be difficult, but a history of a primary elsewhere; immunostaining for thyroid transcription factor-1 (TTF-1) (lung), CDX-2 (colon), or other relatively specific marker; and presence or absence of cercariform cells are of value.

Metastatic Tumors

Metastases to the kidney are present in 7% of all cancer patients at autopsy,²⁰¹ but most are clinically silent. It is extremely uncommon for a metastasis to the kidney to be the initial manifestation of malignancy. Most metastases without a known primary probably represent unusual primary renal tumors.²⁰²

The lung is the most common primary site. Most cases show cells with dark nuclei and irregular nuclear outlines^{22,26,37,203-205} (Fig. 15.14), but some lung cancer metastases have abundant clear cytoplasm and prominent nucleoli and thus mimic high-grade clear cell RCC. Metastases like these can only be recognized as such by knowledge of the clinical history and judicious application of immunocytochemistry. Many metastases from the lungs are positive for mucin, CEA, and TTF-1. The distinction from typical variants of RCC is usually not difficult, particularly with immunocytochemistry techniques, because RCCs are often positive for CD10, RCCma, PAX-2, and PAX-8.²⁰⁶ A variety of rare primary renal tumors, however, including collecting duct carcinoma, mucin-positive RCC,²⁰⁷ small cell carcinoma of the kidney,²⁰⁸ and primary lymphoma of the kidney,²⁰⁹ are impossible to distinguish from a metastatic tumor by cytomorphology alone.

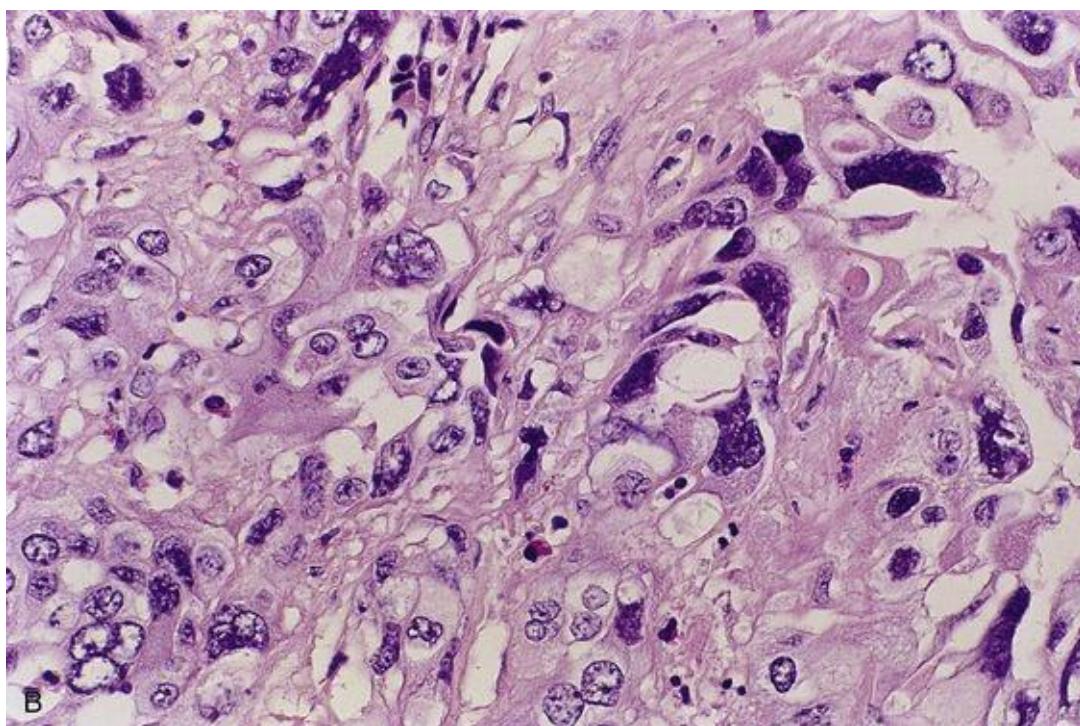
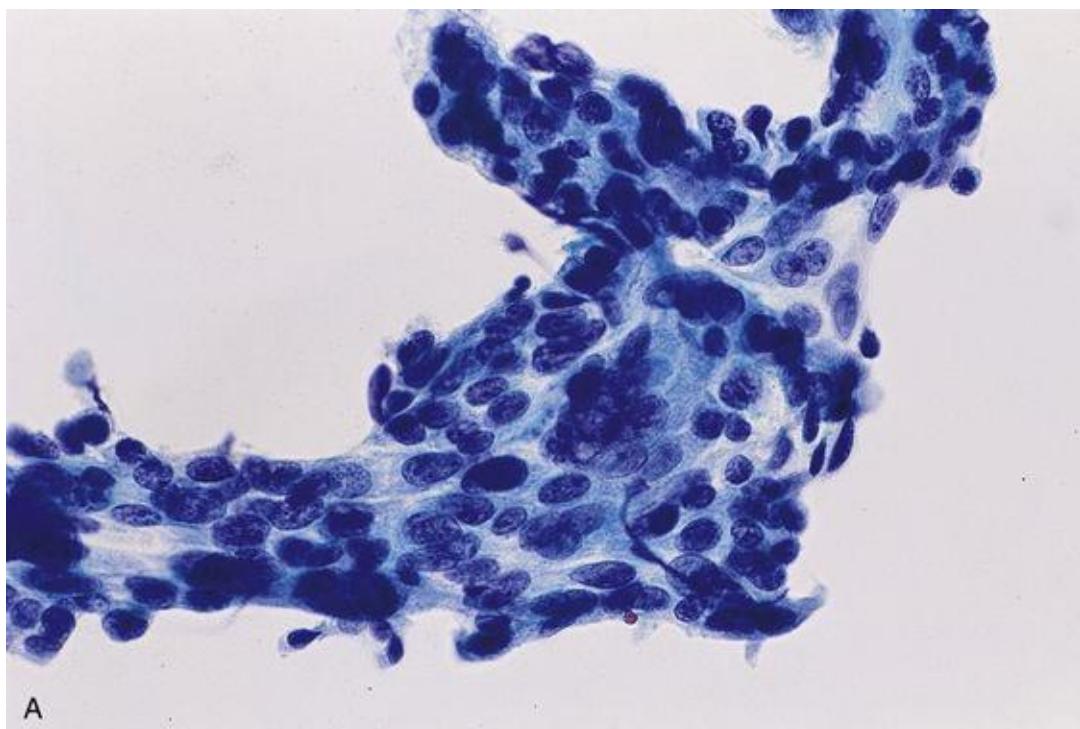


FIGURE 15.14 Metastatic lung cancer to the kidney.

These hyperchromatic, crowded malignant cells are not typical of renal cell carcinoma (RCC). In a patient with a history of lung cancer, the findings are most consistent with a metastasis from that site. *A*, Papanicolaou stain. *B*, Hematoxylin-eosin [H & E]-stained cell block.



Primary renal tumors easily misinterpreted as a metastatic tumor

- metanephric adenoma
- collecting duct carcinoma
- mucin-positive RCC
- primary small cell carcinoma of the kidney
- primary lymphoma of the kidney
- urothelial carcinoma
 - Best diagnostic clue:
- clinical history (i.e., is there evidence of a primary tumor elsewhere?)

Although exceedingly rare, an unusual primary kidney tumor should be considered when one is confronted with the aforementioned features in a patient with no evidence of malignancy elsewhere. If there is a history of malignancy, comparison with the original tumor is often helpful in confirming that the renal tumor is in fact a metastasis ([Fig. 15.15](#)).

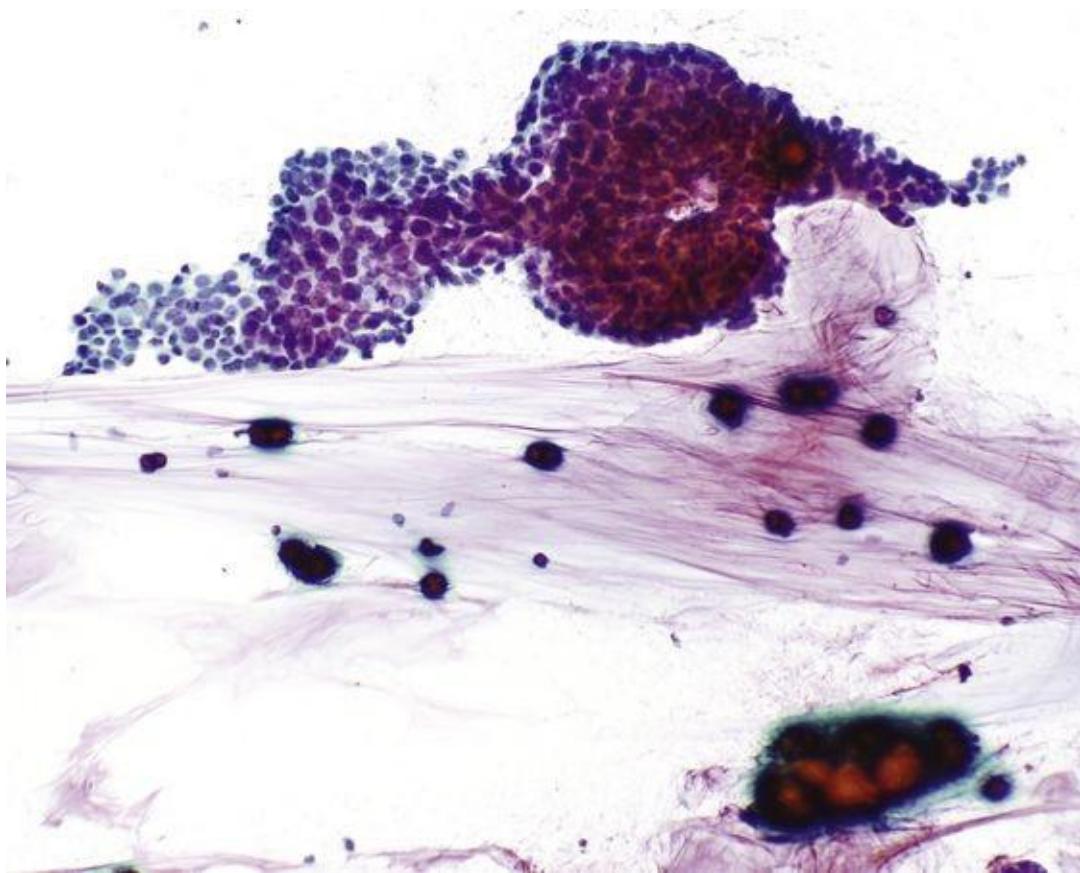


FIGURE 15.15 Metastatic breast cancer to the kidney.

There is abundant extracellular mucin, with clusters of malignant cells and occasional psammoma bodies. The resemblance to the patient's colloid carcinoma of the breast, resected 13 years earlier, was striking (Papanicolaou stain).

Fine-Needle Aspiration in Pediatric Patients

Until recently, FNA of renal masses in children was uncommon, in large part because Wilms tumor, the most common tumor in this age group, was automatically upstaged if an FNA had been performed in the patient. The staging system has since been revised, however, and FNA, no longer an adverse factor in staging, is becoming more common in the pediatric population, with accuracy rates as high as 90%.²¹⁰⁻²¹¹ Nevertheless, aspirates in these patients can be quite challenging to interpret.³ Although Wilms tumor is the most common pediatric renal neoplasm, there are mimics: metanephric adenoma mimics the epithelial component of Wilms, and cellular mesoblastic nephroma mimics the stromal component.²¹²⁻²¹³ In addition, pediatric tumors such as clear cell sarcoma of the kidney and rhabdoid tumor have protean histologic and cytologic appearances.²¹⁴ Clear cell sarcoma of the kidney has myxoid, sclerosing, cellular, epithelioid,

spindled, and palisading histologic features, only some of which have been described for cytologic preparations.^{2,213,215} Rhabdoid tumor can manifest cytologically as single cells or groups of cells which can be spindled, round, rhabdoid, or epithelioid.²¹⁴ Consideration of these tumors is the best defense against misdiagnosis.²¹¹ Other uncommon pediatric conditions to be considered in this setting are Ewing sarcoma/primitive neuroectodermal tumor (PNET),²¹⁶ adrenocortical carcinoma,²¹⁷ and extramedullary hematopoiesis.²¹⁸



Cytomorphology of Wilms tumor

One or more of three components

- *blastema*, present in virtually all cases
 - small, round cells
 - round-to-oval nuclei
 - finely dispersed chromatin
 - scant cytoplasm
 - mitoses
 - apoptosis
 - glomeruloid bodies (infrequent)
- *tubules*
- *stroma*

The cytology of Wilms tumor has been well described.^{4,210} Most aspirates demonstrate at least two of the three components (blastema, tubules, and stroma), which facilitates diagnosis, and atypia (anaplasia) can be appreciated when present. Owing to sampling error, however, aspirates from some triphasic tumors demonstrate only blastema (Fig. 15.16). In such circumstances, cytokeratin stains can be helpful in identifying any tubules present. Blastema-only aspirates can be difficult to distinguish from those from other round blue cell tumors of childhood that (rarely) manifest as kidney masses. Immunohistochemistry and cytogenetics help in some cases. Glomeruloid bodies—immature glomeruli that appear as tight, three-dimensional clusters or crescents of immature cells surrounded by a basal lamina—are infrequent. The uncommon aspirate that is predominantly epithelial is difficult to distinguish from metanephric adenoma.^{213,219}

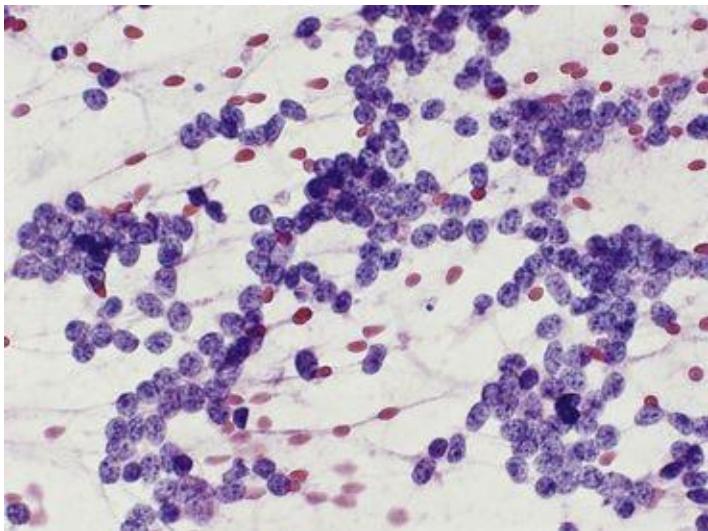


FIGURE 15.16 Wilms tumor.

This image illustrates only the blastemal component, which often predominates. Blastema cells are small, round cells with scant cytoplasm, arranged as isolated cells or in loose clusters (hematoxylin-eosin [H & E] stain). Case courtesy of Dr. Harry Kozakewich, Children's Hospital Boston, Boston, Massachusetts.

The Adrenal Gland

The adrenal glands are a pair of triangular organs on top of the kidneys. They regulate metabolism by secreting cortisol; salt and water balance by secreting aldosterone; and response to stress by secreting a variety of hormones including epinephrine. Primary tumors of the adrenal gland fall into two principal categories: those that arise from the cortex (adrenal cortical adenoma and adrenal cortical carcinoma) and those that arise from the medulla (pheochromocytoma). Of importance, the adrenal gland is also a common site of metastasis from tumors elsewhere in the body. With the widespread use of CT, an increasing number of adrenal nodules are being detected, most commonly during a staging workup for cancer elsewhere but also for other complaints unrelated to the adrenal gland (adrenal “incidentalomas”).²²⁰ FNA is an especially effective method for distinguishing benign adrenal nodules from metastatic tumors during staging procedures.²²¹⁻²²⁴ It is also useful for the diagnosis of some infectious diseases, such as disseminated histoplasmosis manifesting as bilateral adrenal enlargement.²²⁵ Its value in the assessment of an adrenal incidentaloma in a patient without a history of malignancy, however, is unclear.²²⁶⁻²²⁷

Specimen Collection, Preparation, and Accuracy

Virtually all adrenal aspirations are performed percutaneously by radiologists using CT or US imaging guidance.²²⁰ A transhepatic approach is sometimes used, more commonly for right than for left adrenal nodules; in such cases, hepatocytes can be seen on FNA preparations. Complications are usually mild and include minimal hematuria and asymptomatic, self-limited hypotension and bradycardia. More serious complications are uncommon and include pneumothorax (2%) and hemothorax (1%) that may require a chest tube.²²⁰ Because episodic hypertension and even death have occurred with FNA of pheochromocytoma, FNA is generally avoided when a pheochromocytoma is suspected.²²⁸ Preprocedural evaluation for elevations in the urinary levels of catecholamines and their metabolites is the best screening test for pheochromocytoma.

Slides are air-dried and stained with a Romanowsky stain and/or alcohol-fixed and stained with the Papanicolaou or H & E stain. Cell blocks are helpful and provide an ideal platform for immunocytochemical studies.

Adrenal FNA has an accuracy of 96% to 98%^{220,224} and very good negative

predictive value, particularly for lesions larger than 3 cm.²²⁰ False-positive results are uncommon: Some authors report no false-positives in their experience.²²⁰ Rarely, the cells of a benign adrenal nodule/adenoma have been misinterpreted as metastatic small cell carcinoma.²²⁹⁻²³⁰ The nondiagnostic rate is 14%.^{220,231}

Myelolipoma

Myelolipoma, an uncommon benign neoplasm of the adrenal gland, is composed of adipose tissue and benign hematopoietic elements. Although myelolipomas are usually incidental findings, they vary considerably in size, and tumors larger than 30 cm in diameter have been reported. Aspirated material reveals fat with interspersed marrow elements including nucleated red blood cells, megakaryocytes, and granulocytes and their precursors.²³² The differential diagnosis includes an angiomyolipoma of the kidney, because both often contain fat, but the presence of hematopoietic elements combined with the absence of smooth muscle cells and negative immunoreactivity for HMB45 confirms the diagnosis.

Adrenal Cortical Neoplasms

Adrenal cortical adenomas are very common, occurring in approximately 5% of adults, and their frequency increases with age. Adenomas are usually unilateral, solitary masses, in contrast with adrenal cortical hyperplasia, which is a bilateral, diffuse or multinodular enlargement. More than 85% of adenomas are nonfunctioning—they do not secrete cortisol, aldosterone, or other hormones. A minority are functioning adenomas, and the most frequent endocrine abnormalities associated with them are primary aldosteronism (Conn syndrome), Cushing syndrome, virilization, and feminization. Histologically, there is no difference among the different types of functioning and nonfunctioning adenomas. Adenomas are composed of varying proportions of clear (lipid-filled) and granular cells, some with lipofuscin, arranged in cords and nests.

Adrenal cortical carcinomas are much less common than adenomas, with an incidence of 1 case per 1 million per year. In contrast with adenomas, 80% are functional; they can secrete cortisol, androgens, or a combination of both. They tend to be large tumors (larger than 5 to 6 cm) that spread most commonly to the liver, lungs, retroperitoneum, and bones. Histologically, adrenal cortical carcinomas, like adenomas, are composed of a mix of clear and granular cells. Nuclear atypia can be minimal or pronounced. The mitotic rate varies widely.

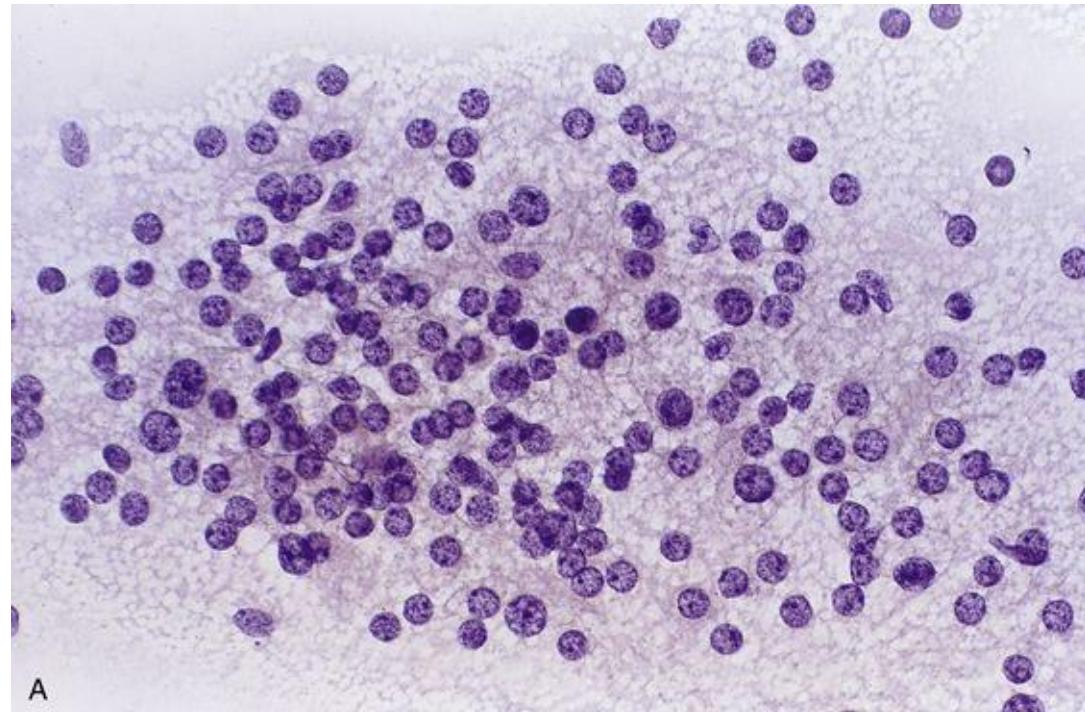
Several systems have been proposed for distinguishing adenomas from carcinomas histologically. They are multifactorial, and most include an evaluation of nuclear grade, necrosis, invasion, mitotic rate, and atypical mitoses.²³³⁻²³⁵ Because some of the features included in these systems cannot be assessed by FNA (e.g., capsular, sinusoidal invasion), they are impossible to apply rigorously to a cytologic specimen. Nevertheless, many adrenal tumors can be correctly classified as benign or malignant by FNA.



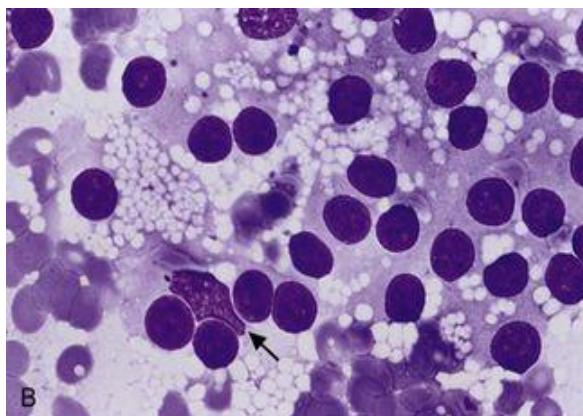
Cytomorphology of benign adrenal cortical nodule/adenoma

- numerous naked nuclei
- “frothy,” granular background
- occasional intact cells with bubbly cytoplasm

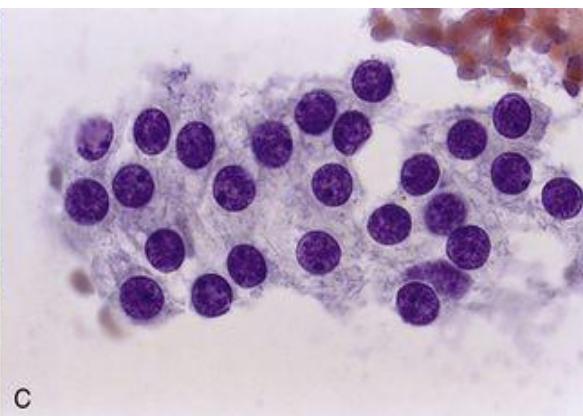
By imaging and cytomorphology it is not possible to distinguish an adrenal cortical adenoma from a hyperplastic nodule. For this reason, we refer to them henceforth as *benign adrenal cortical nodules/adenomas*. Benign adrenal cortical nodules/adenomas yield moderately cellular smears that have a characteristic pattern.²³⁶ Smears show numerous isolated naked nuclei dispersed in a bubbly and granular background ([Fig. 15.17A](#)). Some overlapping nuclei show slight molding ([Fig. 15.17B](#)). There may be some variation in nuclear size, with occasional large nuclei, but mitoses are usually absent. Occasional cells with intact, bubbly (microvesicular) cytoplasm can be identified ([Fig. 15.17C](#)). Most cases contain large tissue fragments with benign spindled endothelial cells.²³⁶ Some adenomas contain abundant intracellular lipofuscin pigment. Hepatocytes sometimes contaminate an adrenal FNA specimen²³¹ and can be confused with the cells of a benign adrenal cortical nodule/adenoma.²³⁷ Both can have lipofuscin pigment, but hepatocytes have uniformly granular cytoplasm as opposed to the microvesicular cytoplasm of most adrenal cortical cells.



A



B



C

FIGURE 15.17 Benign adrenal cortical nodule/adenoma.

A, Nuclei are stripped of cytoplasm and float in a sea of frothy cytoplasmic remnants (Papanicolaou stain). B, Adjacent nuclei occasionally show molding (arrow) and thus mimic small cell carcinoma. (Romanowsky stain). C, Intact cells have indistinct cell borders (Papanicolaou stain).



Cytomorphology of adrenal cortical carcinoma

- numerous isolated cells with intact cytoplasm
- moderate to marked nuclear atypia
- mitoses
- necrotic debris

Adrenal cortical carcinomas range from well-differentiated to poorly differentiated tumors. In general, the pattern of isolated naked nuclei that is characteristic of benign adrenal cortical nodules/adenomas is not seen in carcinomas. Instead, smears show numerous isolated cells with intact cytoplasm that is granular or vacuolated ([Fig. 15.18A](#)). Nuclei are enlarged and pleiomorphic, especially when the tumor is moderately or poorly differentiated, and mitoses can be seen ([Fig. 15.18B](#)). Necrotic debris may be present in the background. Adrenal cortical carcinomas are at most only focally positive for keratins other than Cam5.2. Adrenal cortical carcinomas are reactive for SF1 (NR5A1), Melan-A, inhibin, and calretinin,²³⁹ but negative results for these markers do not exclude a primary tumor.

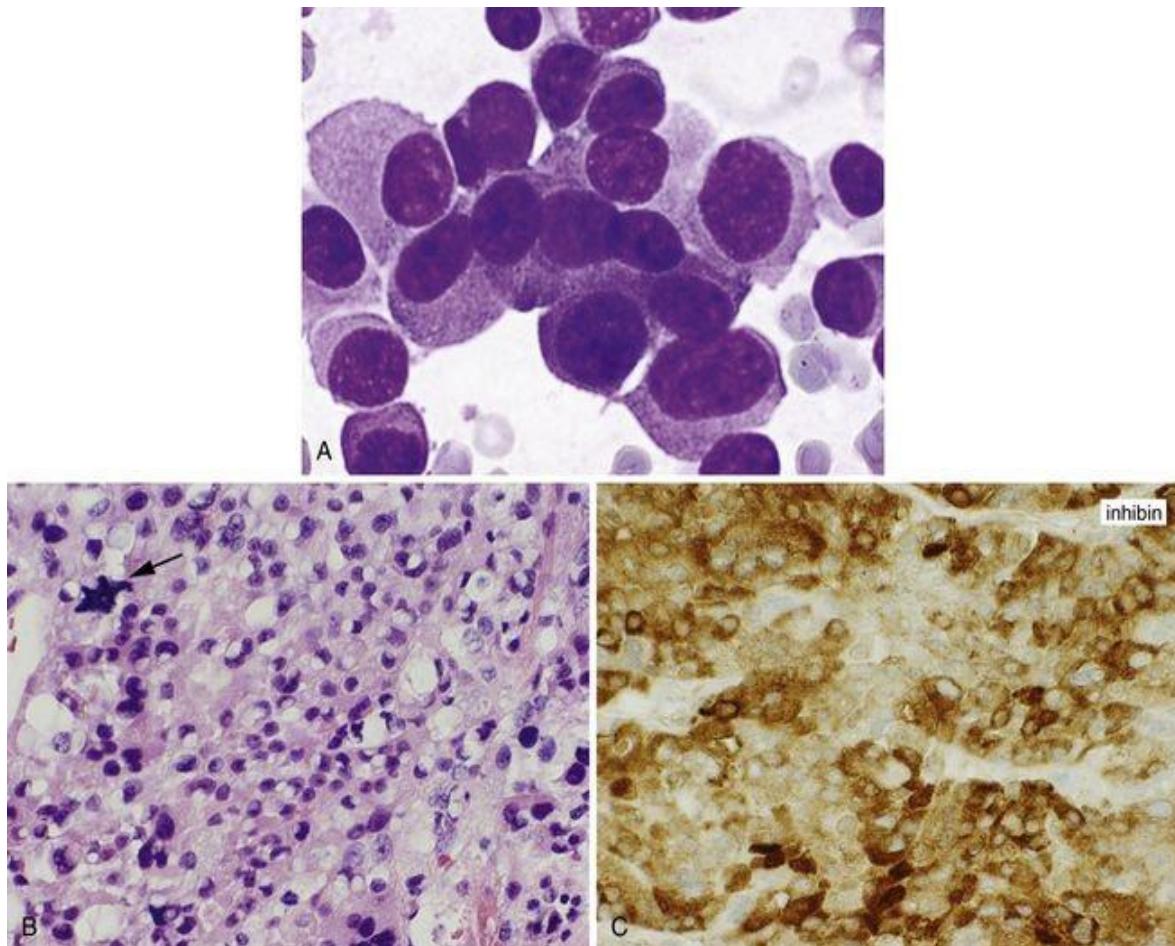


FIGURE 15.18 Adrenal cortical carcinoma.

A, The malignant cells are large and have intact cytoplasm (Romanowsky stain). B, Many tumors are highly pleiomorphic and contain atypical mitoses (arrow). The mixture of clear and granular cytoplasm resembles that of a clear cell renal cell carcinoma (RCC) (hematoxylin-

eosin [H & E]-stained cell block). C, Adrenal cortical carcinomas show cytoplasmic immunoreactivity for inhibin.

Small (less than 5 cm) tumors without atypia, necrosis, or mitoses pose no problems in diagnosis. Neither do large tumors with pleomorphism, abundant mitoses, atypical mitoses, necrosis, and/or clinical evidence of metastases. Distinguishing between a benign adrenal cortical nodule/adenoma and carcinoma can be perplexing, however, in tumors confined to the adrenal gland that show only mild or moderate nuclear atypia. When the findings are ambiguous, the interpretation “adrenal cortical neoplasm of uncertain malignant potential” is reasonable.²³¹ Surgical excision is advised for precise classification of such nodules.

The differential diagnosis includes metastatic tumors²³⁶ (see “[Metastatic Tumors](#)” further on). Immunohistochemistry for SF1 (NR5A1), inhibin, Melan-A, and calretinin is helpful.

Pheochromocytoma

Pheochromocytomas arise from cells of the adrenal medulla and can cause paroxysmal hypertension as a consequence of excessive catecholamine production. In 10% to 20% of cases, they are associated with familial neoplastic syndromes such as the multiple endocrine neoplasia 2a and 2b (MEN2), neurofibromatosis, and the VHL syndrome. A presumptive diagnosis of pheochromocytoma is based on the combination of an adrenal mass, hypertension, and elevated blood and urinary levels of catecholamines and their metabolites such as vanillylmandelic acid. From 10% to 20% of tumors are bilateral, most often in familial cases. Although 97% arise in the adrenal gland, a small percentage occur elsewhere, such as in the organs of Zuckerkandl; extra-adrenal pheochromocytomas are called paragangliomas. Because episodic hypertension, hemorrhage, and even death have occurred with FNA, the procedure is generally avoided when a tumor is suspected of being a pheochromocytoma.²³⁸

Cytologic preparations are highly cellular and contain cells arranged in loose clusters and as isolated cells. Cellular pleomorphism can be marked, with small polygonal cells admixed with large spindle shaped and/or epithelioid cells ([Fig. 15.19A and B](#)). Nuclei are pleomorphic and irregular in contour. The chromatin is finely stippled; intranuclear cytoplasmic pseudoinclusions ([Fig. 15.19B](#)) and prominent nucleoli are sometimes present. Romanowsky-type stains show red cytoplasmic granules. On alcohol-fixed, Papanicolaou-stained preparations, the

cytoplasm has a characteristic, deep red-to-purple, granular appearance.

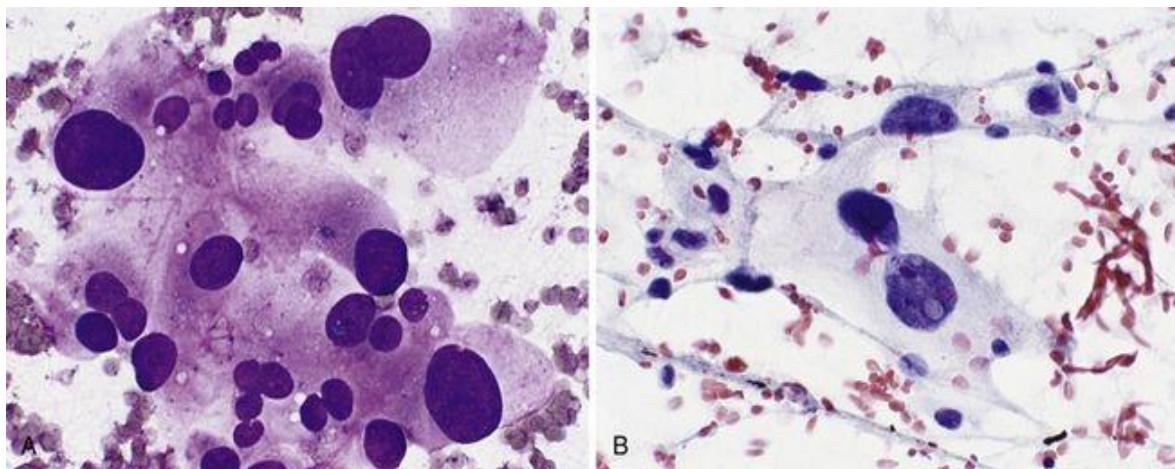


FIGURE 15.19 Pheochromocytoma.
A, In some cases there is marked anisonucleosis (Romanowsky stain). B, Intranuclear cytoplasmic pseudoinclusions, a characteristic but nonspecific feature, are sometimes seen (Papanicolaou stain).

Pheochromocytoma can resemble an adrenal cortical neoplasm. Immunostains assist in making the identification: Pheochromocytomas are positive for chromogranin and synaptophysin; adrenal cortical tumors are positive for SF1 (NR5A1), inhibin, Melan-A, and calretinin.²³⁹

Metastatic Tumors

FNA of the adrenal gland truly shines when, with minimal invasiveness, it is used to confirm or rule out an adrenal metastasis in a patient with a history of cancer. The most common metastases encountered on FNA are from lung cancers, melanoma, and RCC.^{231,240} Certain tumors such as lung cancer and RCC have a predilection for producing a solitary adrenal metastasis that mimics a primary adrenal lesion on imaging studies. In this setting FNA plays a key role in patient management, because it allows, in most cases, the distinction between a primary adrenal lesion (usually a benign adrenal cortical nodule/adenoma) and a solitary metastasis.

Comparison of cytologic or histologic material from the primary tumor, if available, to the FNA specimen can be particularly helpful in establishing whether or not a nodule is a metastasis. Immunostains are helpful (e.g., TTF-1 for metastatic adenocarcinoma or small cell carcinoma of the lung; S-100 and

HMB-45 for melanoma). One pitfall is worth pointing out: The dispersed, focally molded nuclei of a benign adrenal cortical nodule/adenoma resemble the cells of a small cell carcinoma of the lung²²⁹⁻²³⁰ (see Fig. 15.17B). The bubbly background and the absence of mitoses, necrosis, and elongated cells confirm that the lesion is a benign adrenal cortical nodule/adenoma and not metastatic small cell carcinoma.

Metastatic adenocarcinomas from the lung, kidney, breast, and other sites can look like an adrenal cortical carcinoma. Stains for mucin, CEA, keratin, and EMA, which are negative in adrenal cortical carcinomas, are usually positive in a metastatic adenocarcinoma. Adrenal cortical carcinoma can be particularly difficult to distinguish from a solitary RCC metastasis. Immunostains for inhibin, A103/Melan-A, SF1 (NR5A1), and calretinin are helpful. Adrenal cortical tumors are positive, whereas RCCs are negative.^{239,241-245} Conversely, RCCs are positive for EMA, keratins, CD10, RCCma, PAX-2, and PAX-8, and adrenal cortical tumors are negative or only weakly positive for low-molecular-weight keratins.²⁴⁶

Some metastatic malignancies mimic a pheochromocytoma. The former can be identified by their characteristic immunoreactivity for, for example, keratin proteins (in the case of metastatic carcinoma) and S-100 and HMB-45 (in the case of metastatic melanoma).

Finally, the adrenal glands can be involved by Hodgkin and non-Hodgkin lymphomas.^{231,236,247} Adrenal involvement is usually found in the context of widespread disease, but rare examples of primary lymphoma of the adrenal glands have been reported. A rare and unusual lymphoma, the intravascular large B-cell lymphoma, has a predilection for involving the adrenal glands at presentation. It is an uncommon subtype of extranodal diffuse large B-cell lymphoma (DLBL), characterized by lymphoma cells confined to the lumina of blood vessels. This can result in significant enlargement, detectable by CT, of one or both adrenal glands. Morphologically, the malignant cells are indistinguishable from an DLBL.



Kidney and adrenal gland: summary and helpful hints

- FNA is useful for the diagnosis of selected kidney masses.
- Never call a renal FNA specimen positive on the basis of a small number of atypical cells. Benign lesions can have rare highly atypical cells. (The kidney will likely be resected even if you report the results as suspicious.)
- FNA has very low sensitivity for the diagnosis of a cystic RCC.

- RCCs can and should be subclassified by FNA.
- Both oncocytoma and chromophobe RCC are candidates for partial nephrectomy. You may not always be able to distinguish between them.
- Renal adenoma is not a diagnostic consideration in evaluating an FNA from a radiographically visible mass.
- The FNA diagnosis of an angiomyolipoma is challenging because only the radiographically indeterminate masses (i.e., those with low fat content) are selected for FNA.
- Metanephric adenomas are benign and negative for EMA, and they may be difficult to distinguish from a Wilms tumor.
- Urothelial carcinoma cells often have tails (cercariform cells) and are frequently immunoreactive for GATA3, CEA, and CK20.
- The cells of most metastases to the kidney have scant cytoplasm and dark, dense chromatin.
- Avoid diagnosing a metastasis to the kidney if there is no evidence for a primary elsewhere.
- Be wary of diagnosing Wilms tumor without at least a biphasic sample.
- Adrenal cortical lesions are immunoreactive for SF1 (NR5A1), inhibin, A103/Melan-A, and calretinin.

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CHAPTER 16

Ovary

Edmund S. Cibas

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Fine needle aspiration (FNA) is a safe, minimally invasive, and relatively inexpensive procedure for the diagnosis of selected ovarian lesions. Indeed, with the widespread use of ultrasound and laparoscopy, many more ovarian lesions are being detected and aspirated than ever before.



Indications for fine-needle aspiration of the ovary

- confirm the benign nature of an incidental cyst discovered during:
 - an infertility workup
 - pregnancy
- confirm malignancy in a patient with a suspicious pelvic mass
- confirm recurrence of an ovarian tumor treated conservatively
- drain a tubo-ovarian abscess

FNA of the ovary is often used for evaluation of small, incidental cystic masses that appear benign on sonographic or laparoscopic examination.^{1–3} Incidental ovarian cysts are often discovered in women with infertility or during pregnancy.⁴ Aspiration cytology, in combination with a benign ultrasound appearance, is used to reassure the patient that an oophorectomy is not necessary.⁵ In some laboratories, estradiol (E2) levels in the fluid are also measured, as these are usually elevated in follicle-derived cysts but not in epithelial lesions.^{6,7} Thus, ultrasound, cytology, and E2 levels can form an effective “triple test” for distinguishing benign from malignant ovarian cysts.



Ultrasound features of a benign ovarian cyst

- small
- thin-walled
- no septations
- no papillarity
- no solid areas
- low echogenicity (sonolucency)

In part because of this preselection, a majority of ovarian FNAs are benign.⁸ The diagnosis of endometriosis is sometimes established by this means as an unsuspected cause of infertility.⁴

If imaging features suggest a malignancy, the clinician will often forego aspiration and recommend surgery. Because borderline and malignant ovarian tumors are treated by surgical resection, FNA is perceived as unnecessary in many such circumstances. Aspiration of suspicious ovarian masses is therefore uncommon, although it is used to confirm malignancy in patients with inoperable or metastatic disease.^{3,9} Nevertheless, on the basis of their positive experience, some authors advocate cytologic assessment for suspicious, potentially early stage ovarian lesions.¹⁰

Obtaining the Specimen

The aspiration can be carried out transrectally, transvaginally, percutaneously, intraoperatively,¹¹ or during laparoscopy. The method used depends on the size of the lesion, its location, and the resources available to the aspirator. Percutaneous transabdominal aspiration can be performed by palpation¹² or, more commonly, with imaging guidance. Transvaginal and transrectal aspiration can be done by palpation using the Franzen needle guide,¹² originally developed for FNA of the prostate. Transvaginal aspiration under ultrasound guidance, with the probe placed in the vagina, can be performed in the outpatient setting with only intravenous sedation.⁴⁸ Depending on the trajectory of the needle, the cyst fluid may be contaminated with squamous cells, mesothelial cells, urothelial cells, or intestinal epithelium. Therefore, knowledge of the route taken is important in evaluating the specimen.^{8,13-15} Aspirations are also performed when cysts are discovered during laparoscopy and laparotomy.

Complications are uncommon. Two decades ago, a pair of authors argued that aspiration of a malignant ovarian tumor causes seeding of the peritoneal cavity,¹⁶ but their evidence consisted of only two cases, both with ambiguous circumstances. Since then, there has been no documentation of tumor seeding; on the contrary, practitioners with considerable experience have reported no such cases.^{17,18} The risk of peritoneal seeding, therefore, is unknown but probably very low.¹⁸ Severe pelvic infection after transvaginal and transrectal FNA is seen in 1.3% of cases, however.¹⁷ Acute abdominal or pelvic pain is a contraindication to FNA, because it may delay treatment of a serious condition such as torsion of a cyst.¹

Preparing the Specimen and Reporting Results

Specimens are usually cyst fluids. Standard methods are used in the preparation of direct smears, cytocentrifuge preparations, thinlayer preparations, and/or cell block sections. A portion of the fresh fluid can be submitted to the clinical laboratory to measure the level of E2 or tumor-associated antigens CA-125, carcinoembryonic antigen (CEA), and alpha-fetoprotein. Elevated levels are typical of some ovarian lesions and can be a useful adjunct to cytologic examination.^{17,19}

Specimens that are virtually acellular or uninterpretable for other reasons are reported as nondiagnostic.^{20,21} The percentage of FNAs of the ovary that are nondiagnostic ranges widely, from a low of 13% to a high of 72%.^{14,13,14,20-22} This variation is likely related to the type of lesion selected for aspiration and the definition of a nondiagnostic specimen. The cytology report should state that malignant cells are either absent (“no malignant cells identified”) or present (“positive for malignant cells”). If the findings are equivocal, the report should state that atypical or suspicious cells are present. If sufficient benign lesional cells (granulosa cells, epithelial cells) are seen, descriptive terms can further categorize the cyst as a follicular, simple, serous, benign mucinous, endometriotic, or dermoid cyst.³ Benign ovarian FNA results fall into two broad categories: (1) follicular/lutein cysts and (2) epithelial cysts. This has significant clinical relevance, because surgery is unnecessary for follicle-derived cysts, which usually regress over time. Surgery may be indicated, however, for a benign-appearing epithelial cyst, particularly if the sonographic findings are worrisome. The lining of an epithelial cyst can vary from one area to another, and some cysts contain benign, borderline, and frankly malignant foci; sampling is not always representative of this heterogeneity. For this reason, an explanatory note accompanying the diagnosis of a benign epithelial cyst is helpful (e.g., “clinical correlation is advised to ensure that the sample is representative of the underlying lesion”).²³

Accuracy

Reported sensitivities for malignancy of 84% and 93% are inflated because borderline tumors were excluded from analysis.^{13,17} In fact, FNA of a borderline tumor often yields a false-negative result.^{4,17,24,25} Much of the lesion is acellular cyst fluid, and neoplastic epithelium may not be sampled. When borderline tumors are included, sensitivity is low, ranging from 26% to 40%.^{24–26} Low sensitivity is another argument against the aspiration of clinically suspicious ovarian masses.

False-positive results have been reported.^{13,26} Follicle cysts are a notorious cause of false positives, because they can yield a cellular and highly mitotic specimen.^{9,27,28} Familiarity with the laparoscopic and sonographic findings should be taken into account when making a diagnosis. Knowing that a lesion appears benign clinically can help avoid a false-positive interpretation.

FNA of the ovary, therefore, has its limitations. Not only is there a high false-negative rate, particularly for borderline tumors, but the method cannot reliably distinguish between borderline tumors and carcinomas.^{20,29,30} (Because both lesions are treated by surgical resection, the inadequacy of FNA in this instance is not very significant.) Furthermore, benign lesions cannot always be histologically categorized by cytologic evaluation.^{19,29} Although in some cases FNA can establish a specific diagnosis such as endometriosis,⁹ at present it is most valuable for confirming a clinical impression that an ovarian cyst is benign, thus sparing the patient unnecessary surgery.^{1,20,31}

Benign Tumorlike Lesions of the Ovary

NonNeoplastic Cysts

Benign ovarian cysts are common. Most are discovered incidentally by ultrasonography, laparoscopy, or laparotomy. They fall into two major categories: The most common are the so-called “functional cysts” (i.e., cysts of follicular origin—follicle and corpus luteum cysts). The rest are nonfunctional cysts derived from ovarian surface epithelium or endometriosis. Precise classification by cytologic examination is not always possible, particularly when only cyst contents (fluid and macrophages) are obtained.³²

Cystic Follicle and Follicle Cyst

These two lesions have a similar histogenesis and are distinguished only on the basis of size. The arbitrary size cut-off is variably given as 2 cm, 2.5 cm, and 3 cm. If the size cut-off is exceeded, a cystic follicle is called a *follicle cyst*. One of the most common, if not the most common, cystic lesions of the ovary, they arise from an ovarian follicle and are physiologic, not neoplastic. Ovarian follicles follow a predictable development from primordial follicle (oocyte surrounded by a flattened layer of granulosa cells), to primary follicle (increased size and mitotic activity of granulosa cells), to secondary follicle (stratification of granulosa cells), to the graafian follicle (characterized by a distinct zone of fluid, an antrum for the oocyte, and proliferation of surrounding theca cells). Cystic follicles and follicle cysts occur when a graafian follicle does not rupture but rather persists for a variable period of time. The remainder of this discussion focuses on the follicle cyst which, because of its size, is more likely to manifest clinically.

Follicle cysts can be solitary or multiple, and they range in size up to 8 cm or more in diameter. They look benign on sonographic and laparoscopic examination. They are unilocular, smooth-surfaced, translucent, and thin-walled. Most regress spontaneously within a few months. Multiple follicle cysts are common in juvenile hypothyroidism, ovarian hyperstimulation syndrome, and polycystic ovary disease.

Histologically, a follicle cyst is lined by an inner layer of attenuated or stratified granulosa cells and an outer layer of theca cells ([Fig. 16.1](#)). Both may show luteinization. Fluid is clear or hemorrhagic. It may be sparsely cellular,

containing only histiocytes and blood, or markedly cellular (sometimes alarmingly so), with numerous granulosa cells that are isolated and in large clusters.

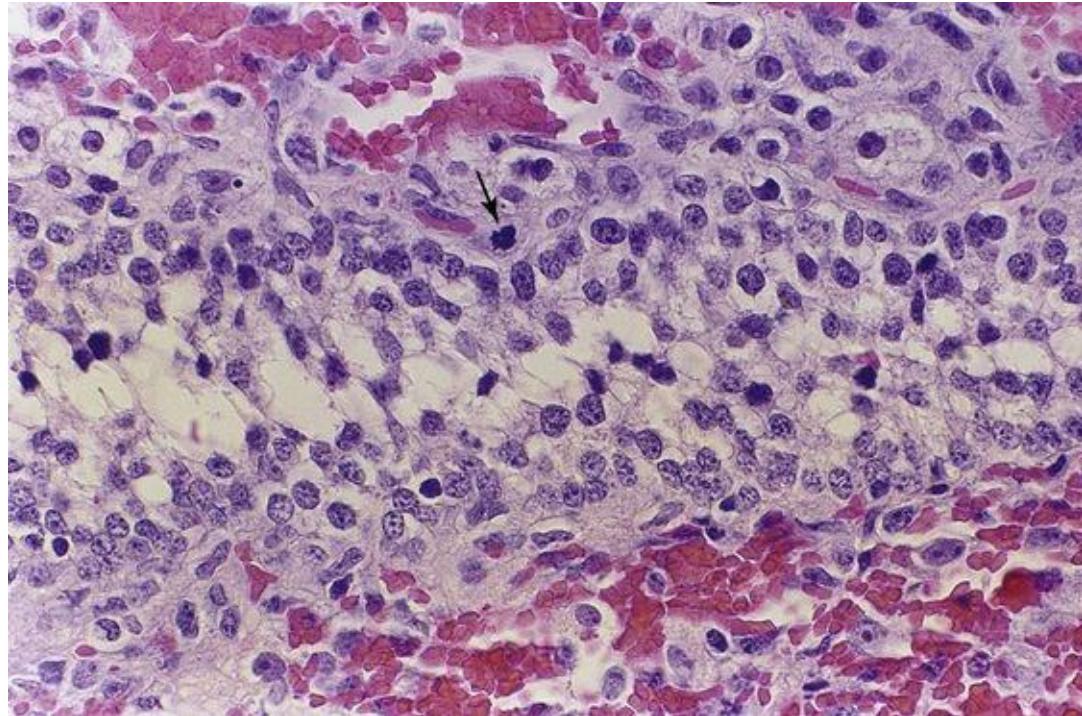


FIGURE 16.1 Follicle cyst.

In this histologic section, the follicle cyst is collapsed and the cavity slitlike. Follicle cysts are lined by one or several layers of granulosa cells. Mitotic activity (arrow) is common (hematoxylin-eosin [H & E] stain).

Because follicle cysts appear benign sonographically and laparoscopically, they are managed conservatively to preserve the ovary. FNA is commonly used as a minimally invasive method to confirm the diagnosis.



Cytomorphology of follicle cyst

- sparsely or highly cellular
- isolated cells and clusters
- coarsely granular chromatin
- mix of viable and pyknotic nuclei
- foamy cytoplasm
- mitoses

FNA specimens range from sparsely to highly cellular. The highly cellular cases are termed *cellular follicle cysts* and account for between 23% and 76% of all follicle cysts.^{20,33} The granulosa cells can be isolated, but they have a tendency to cluster haphazardly in loose spherical aggregates (Fig. 16.2). They have a round nucleus with coarsely granular chromatin and one or two small nucleoli. Nuclear grooves are not usually seen.²⁷ Some nuclei are dark, pyknotic, and eccentrically placed, whereas others are round and vesicular with a prominent nucleolus. Mitotic figures can be absent or numerous (Fig. 16.3). Luteinized granulosa cells have foamy cytoplasm that may contain yellow pigment. Theca interna cells have an eccentrically placed ovoid nucleus and a moderate amount of cytoplasm. Theca externa cells are indistinguishable from spindle-shaped ovarian stromal cells.³⁴

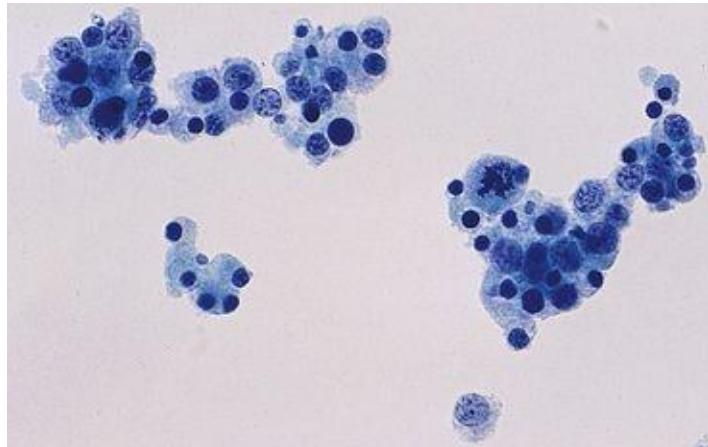


FIGURE 16.2 Follicle cyst.

Granulosa cells are clustered in loose aggregates (Papanicolaou stain).

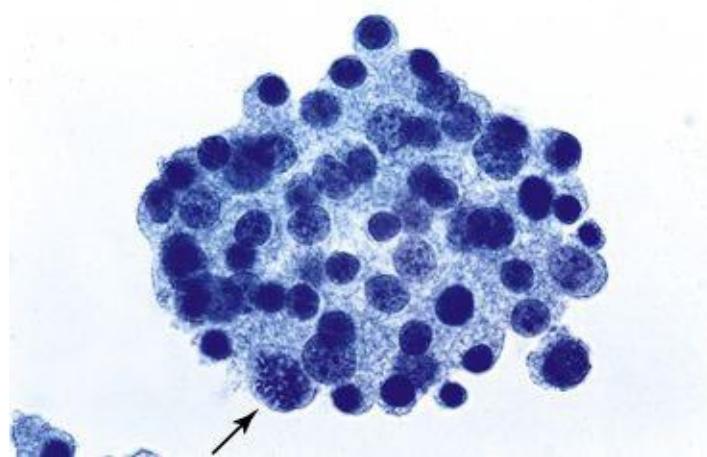


FIGURE 16.3 Follicle cyst.

Granulosa cells have round nuclei and a moderate amount of granular cytoplasm. Degenerated granulosa cells with pyknotic nuclei are a common finding, as are mitoses (arrow) (Papanicolaou stain).



Differential diagnosis of follicle cyst

- epithelial cysts
 - simple cyst
 - serous cyst
 - mucinous cyst
 - endometriotic cyst
- granulosa cell tumor
- carcinoma

Follicle cysts can be a challenge to distinguish from epithelial cysts. When ciliated or mucinous epithelium is identified, the cyst is of surface epithelial origin and not a follicle cyst. Immunostaining for α -inhibin, epithelial membrane antigen (EMA), and CK7 can be very helpful. Granulosa cells are often immunoreactive for inhibin and negative for EMA and CK7; the reverse is true of epithelial cysts.³⁵ E2 levels in cyst fluid are also helpful. Elevated concentrations correlate strongly with cysts of follicular origin^{1,6,7,17}: 81% to 90% of follicle cysts have an E2 content greater than 20 nmol/L as measured by radioimmunoassay, and in 97% to 99% of nonfollicular cysts it is less than 20 nmol/L. Also helpful are fluid CEA and CA-125 levels, which are low in follicle cysts; one or more of these is usually elevated in serous, mucinous, and endometriotic cysts.^{19,31} The differential diagnosis also includes a cystic granulosa

cell tumor. Fluid from this lesion is usually highly cellular.³⁶ Unlike the cells of a follicle cyst, those of a granulosa cell tumor have nuclei with pale, finely dispersed chromatin. Cellular follicle cysts represent a potential diagnostic pitfall: Because of their cellularity and conspicuous mitotic activity, they may raise the possibility of a granulosa cell tumor or carcinoma.^{9,27,28} Knowledge of the invariably benign sonographic and laparoscopic findings of follicle cysts, including the cellular variant, is reassuring and diagnostically helpful.

Corpus Luteum Cyst

Corpus luteum cysts are unilocular and histologically similar to follicle cysts except that the wall is composed of large luteinized granulosa and theca interna cells.



Cytomorphology of corpus luteum cyst

- isolated very large cells
- abundant foamy cytoplasm
- intact or pyknotic nuclei
- macrophages
- hyaline bodies, calcification (pregnancy)

The fluid may be hemorrhagic and composed of numerous, predominantly isolated luteinized granulosa cells with round or pyknotic nuclei. The cytoplasm is abundant and finely vacuolated and contains lipid ([Fig. 16.4](#)). Macrophages with hemosiderin or the yellow hematoidin pigment and smaller luteinized theca interna cells are also seen.³⁷ Cysts associated with pregnancy contain hyaline bodies and calcifications.²⁰ Fluid from a hemorrhagic corpus luteum cannot always be distinguished cytologically from that of other benign cysts, including endometriotic cysts.

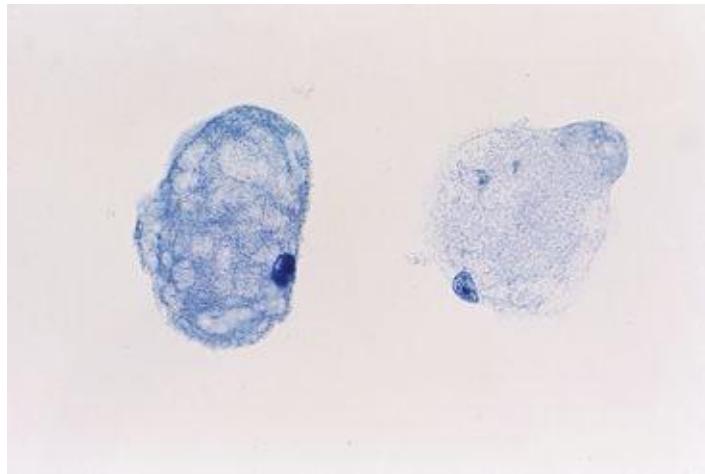


FIGURE 16.4 Corpus luteum cyst.

These cysts are lined by very large cells that have a small nucleus and abundant finely vacuolated cytoplasm (Papanicolaou stain).

Endometriotic Cyst

Endometriosis affects women of reproductive age and is often associated with infertility. It can result in a cystic tumorlike mass of the ovary, known as an endometriotic cyst or endometrioma. As many as one half of ovarian endometriomas are bilateral. For an unequivocal diagnosis, identification of two of the following features is required: endometrial glands, endometrial stroma, and hemosiderin.



Cytomorphology of endometriotic cyst

- hemosiderin-laden macrophages
- endometrial glandular cells
- endometrial stromal cells

The aspirated cyst fluid contains hemolyzed old blood the color of chocolate (“chocolate cyst”). Hemosiderin-laden macrophages are numerous ([Fig. 16.5A](#)). Endometrial epithelial cells are usually arranged in clusters or sheets. The cells are small and have indistinct borders; nuclei are round or oval; nucleoli are inconspicuous; and cytoplasm is scant and sometimes vacuolated. Nuclear molding may be seen. Stromal cells with oval nuclei and scant cytoplasm may also be present. Epithelial and stromal cells are best appreciated as such on cell

block preparations ([Fig. 16.5B](#)).

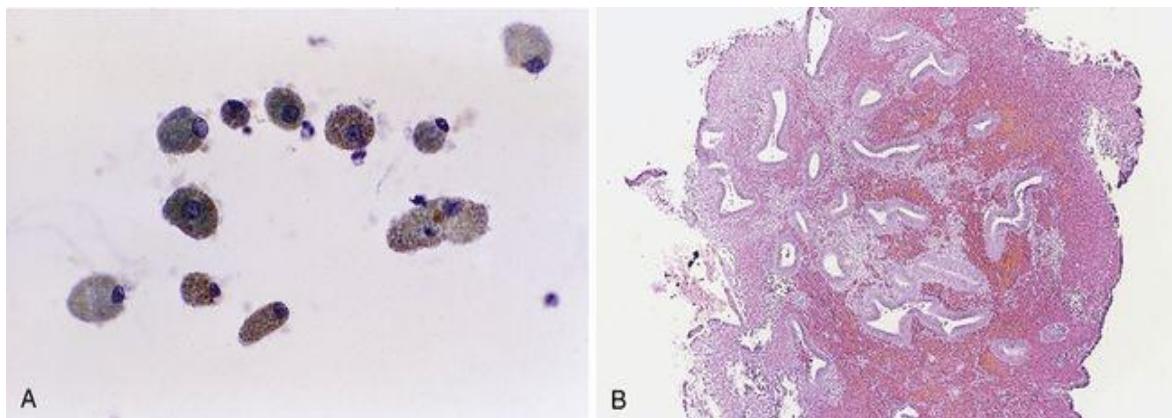


FIGURE 16.5 Endometriotic cyst.

A, Cytologic preparations often show only macrophages with abundant intracytoplasmic hemosiderin (Papanicolaou stain). B, In cell block sections, fragments containing endometrial glands and stroma are diagnostic (hematoxylin-eosin [H & E] stain).

The diagnosis of endometriosis is easily made when both cytologically benign epithelial and stromal cells are present. It is impossible to distinguish an endometriotic cyst from a hemorrhagic corpus luteum when epithelial cells are absent, because lutenized granulosa and theca cells resemble histiocytes.³⁸ Occasional endometriotic cysts have marked cytologic atypia. Flow cytometric DNA measurements have demonstrated aneuploidy in 50% of such cases,³⁹ causing some investigators to speculate that they may be precursors to ovarian carcinoma. A suspicion of malignancy may be unavoidable in these atypical endometriotic cysts.

Simple Ovarian, Parovarian, and Paratubal Cysts

Simple cysts of the ovary or fallopian tube are most common in postmenopausal women and result from an invagination of mesothelium or the surface epithelium. They are usually small and multiple and lined by a single layer of benign columnar, cuboidal, or flattened epithelium. When the epithelium is ciliated, the cyst is called a *serous cyst*.



Cytomorphology of simple cysts

- scant cellularity
- mesothelial-like cells

- sheets and clusters

The cytologic features of simple ovarian, parovarian, and paratubal cysts are identical. They contain clear fluid that is sparsely cellular. When small cuboidal epithelial cells are seen (rarely), they are usually arranged in tight clusters or sheets. The cells resemble benign mesothelial cells ([Fig. 16.6](#)), having a small round or oval nucleus, finely granular chromatin, and an inconspicuous nucleolus. The quantity of cytoplasm varies. If ciliated cells are present, however, the lesion is either a serous cyst or a hydrosalpinx.

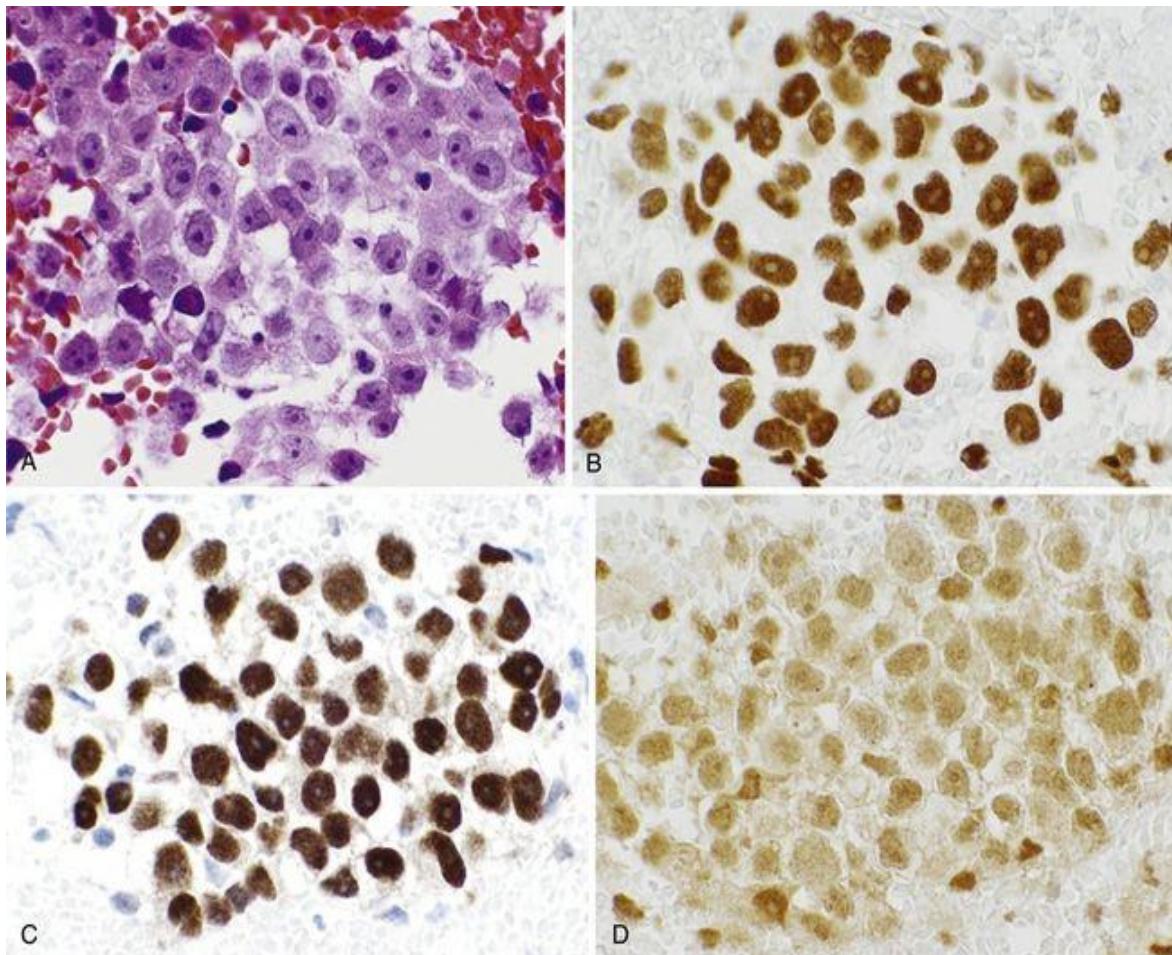


FIGURE 16.16 Dysgerminoma.

A, Tumor cells have a large, round nucleus with a prominent nucleolus and clear cytoplasm (hematoxylin-eosin [H & E]-stained cell block). They show nuclear immunoreactivity for the stem cell proteins SALL4 (B), OCT-3/4 (C), and NANOG (D).

Hydrosalpinx

Hydrosalpinx, a complication of salpingitis, manifests as a large cystic adnexal mass. The distended fallopian tube is filled with clear fluid and is lined by ciliated epithelium. The fluid is hypocellular and may contain histiocytes, ciliated epithelial cells, and/or detached ciliary tufts ([Fig. 16.7](#)). These findings are identical to those in serous ovarian and parovarian cysts.

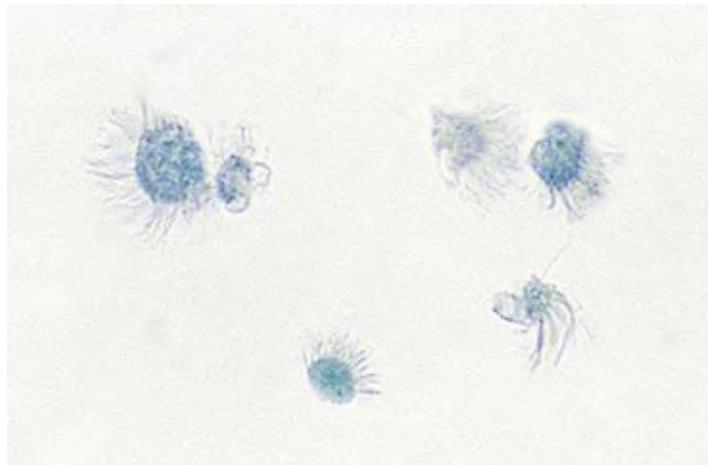


FIGURE 16.7 Detached ciliary tufts.

These decapitated tips of ciliated cells are diagnostically very useful because they exclude a follicle cyst. They are seen in serous cysts, cystic teratomas, and hydrosalpinx (Papanicolaou stain).

Tubo-ovarian Abscess

Tubo-ovarian abscess is an advanced complication of acute salpingitis, known clinically as *pelvic inflammatory disease*. It manifests as palpable adnexal mass that can be visualized by ultrasonography or computed tomography (CT). Most cases result from an ascending infection of the lower genital tract by sexually transmitted pathogens such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and *Mycoplasma genitalium*.

Aspirated material is thick and yellow and composed of numerous polymorphonuclear leukocytes and abundant necrotic debris. When these elements are seen during a rapid interpretation of the aspirated material, additional passes should be performed to obtain samples for microbiologic study to identify the causative organism. Surgery is considered for those patients who

do not respond to antibiotic therapy. Laparoscopic or radiographically guided drainage is helpful in some cases.

Benign Surface Epithelial-Stromal Tumors

Tumors derived from the ovarian surface epithelium are the most common neoplasms of the ovary. They are divided histologically into serous (the most common), mucinous, endometrioid, clear cell, transitional, squamous, mixed, and undifferentiated types. Most of these have a benign, borderline, and malignant counterpart. Because FNA is rarely used to diagnose these neoplasms, only the more common types are discussed here.

Benign Serous Tumors

Benign serous tumors (e.g., serous cystadenoma, serous adenofibroma, serous cystadenofibroma) constitute about 16% of benign ovarian neoplasms.⁴⁰ They are defined as tumors composed of epithelium resembling either the fallopian tube lining or ovarian surface. They are usually cystic, either unilocular or multilocular, and the cysts contain clear fluid. The cyst wall may be entirely smooth and round or nodular. They are lined by ciliated or sometimes nonciliated columnar cells. Some benign serous tumors have an additional stromal component, hence *adenofibroma* and *cystadenofibroma*. A majority of ovarian adenofibromas are serous, but endometrioid, mucinous, and clear cell differentiation is occasionally seen.²³



Cytomorphology of benign serous tumor

- ciliated cells
- cuboidal cells
- detached ciliary tufts
- psammoma bodies (rare)

FNA of a benign cystic serous tumor yields clear fluid that is usually sparsely cellular but occasionally highly cellular. The cuboidal cyst lining cells, if present, have a uniformly round or oval nucleus and are arranged in crowded clusters. Ciliated columnar cells ([Fig. 16.8](#)), psammoma bodies, and detached ciliary tufts (see [Fig. 16.7](#)) are sometimes present.⁴¹ If the needle has collected a sample from a solid area of an adenofibroma, stromal cells may be seen.

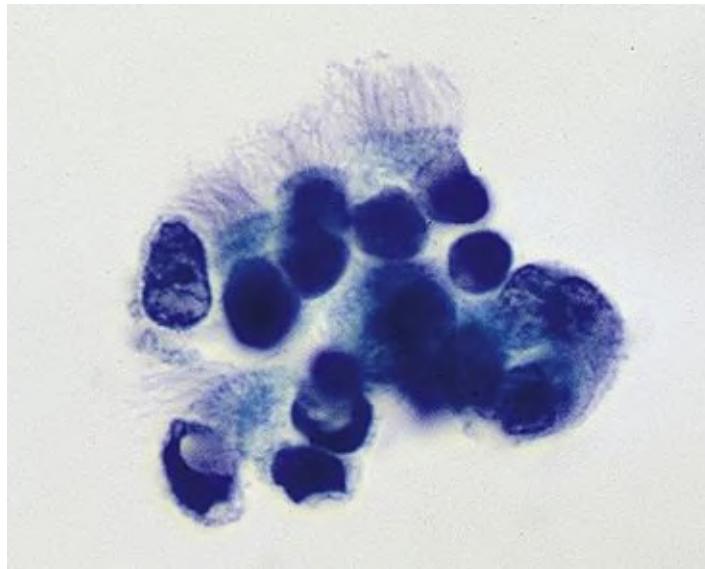


FIGURE 16.8 Serous cystadenoma.

The cyst is lined by benign ciliated cells that have basally placed nuclei, terminal bars, and cilia (Papanicolaou stain).

If the cyst fluid does not contain ciliated cells or detached ciliary tufts, the findings are nonspecific. Analysis of tumor markers may be helpful, because elevated CA-125 levels are characteristic of serous cysts.^{1,17,19,31}

Benign Mucinous Tumors

Twenty percent of benign ovarian neoplasms are mucinous tumors (mucinous cystadenoma, cystadenofibroma, adenofibroma). Most are seen in women of reproductive age. The most common subtype by far is the mucinous cystadenoma, which is usually large and multiloculated. The wall is smooth and lined by a single layer of endocervical-like columnar cells or intestinal-type cells, predominantly goblet cells.



Cytomorphology of mucinous cystadenoma

- mucinous cells
 - endocervical-like cells
 - goblet cells
- isolated cells, ribbons, sheets
- macrophages
- extracellular mucin

The fluid is either gelatinous or watery. The lining cells are isolated, often retaining their columnar shape, or arranged in clusters and honeycomb-like sheets with sharply defined cell membranes. They resemble either benign endocervical cells ([Fig. 16.9](#)) or goblet cells ([Fig. 16.10](#)). Mucin-filled macrophages and extracellular mucin are present.

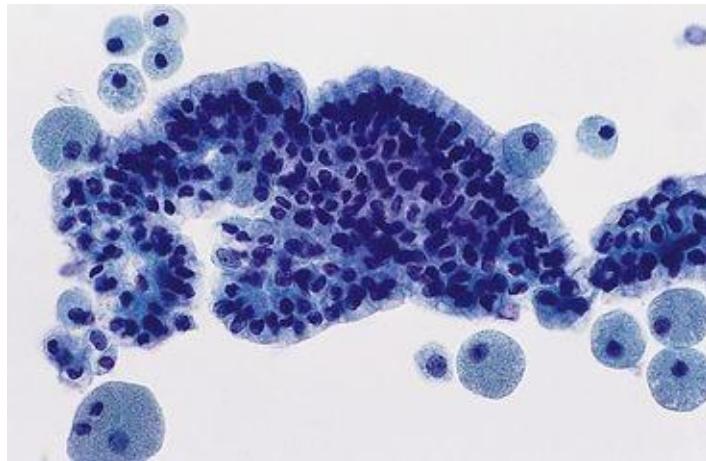


FIGURE 16.9 Mucinous cystadenoma.

Admixed with macrophages are fragments of benign mucinous epithelium resembling endocervial epithelium (Papanicolaou stain).

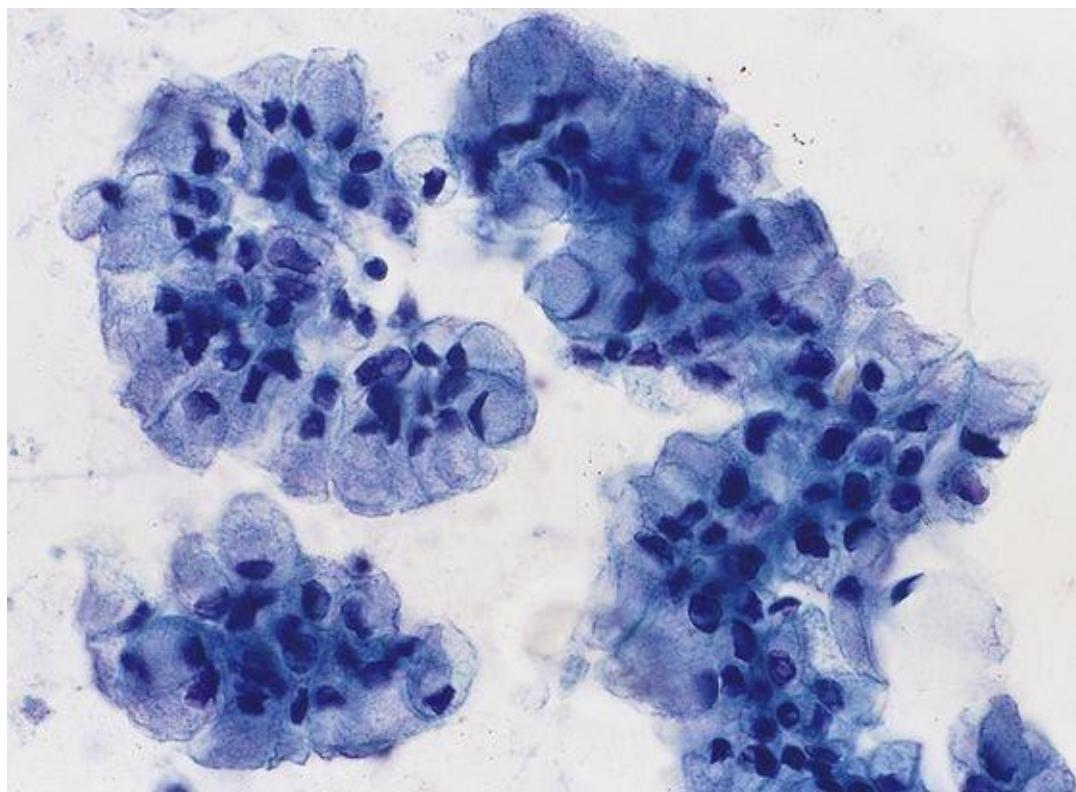


FIGURE 16.10 Mucinous cystadenoma.

Some mucinous cystadenomas exhibit a mixture of endocervical-type cells and intestinal-type goblet cells (Papanicolaou stain).

Diagnosing a mucinous cystadenoma is straightforward when mucinous epithelium is identified. If needed, chemical analysis of the fluid can be helpful: A high CEA level and low E2 and CA-125 levels are characteristic of mucinous cysts and help to distinguish them from follicle and serous cysts.^{1,17,19,31} The differential diagnosis also includes a mucinous carcinoma and a mucinous borderline tumor. If nuclear atypia is present, a borderline tumor or carcinoma should be suspected. Surgical excision of the cyst should be considered even when the cytologic findings are benign, because the borderline or malignant areas can be focal and may not be sampled by FNA.^{17,24,25}

Benign Brenner Tumor

Brenner tumors are a subtype of the transitional cell neoplasms of the ovary and account for about 2% of ovarian neoplasms. Most are benign, and less than 10% of cases occur bilaterally. Most Brenner tumors are solid, but some contain small or large cystic areas which may show mucinous differentiation.

Benign Brenner tumors are composed of nests of transitional epithelium

embedded in a dense fibrous stroma.

Aspirates show sheets of transitional cells, the nuclei of which resemble coffee beans, because they are oval and have a prominent longitudinal groove. Globular hyaline structures that stain bright orange with the Papanicolaou stain are seen.⁴² Their composition and significance are not known. The differential diagnosis includes a granulosa cell tumor, the cells of which also have longitudinal grooves. Granulosa cell tumors, unlike Brenner tumors, are negative for keratin proteins.

Malignant Surface Epithelial-Stromal Tumors

FNA is rarely used to diagnose borderline and malignant ovarian tumors, as noted earlier: Most of these tumors already have a suspicious laparoscopic or imaging appearance that merits surgery, and FNA has a significant false-negative rate for these tumors. Ovarian tumors, however, sometimes manifest as pelvic masses so large that they significantly alter pelvic anatomy. In such cases, an FNA is performed because a soft tissue, lymphoid, or other tumor is suspected rather than ovarian cancer. FNA is also used to diagnose the recurrence or metastasis of a previously documented ovarian cancer.^{43,44}

Immunohistochemistry is useful in the diagnosis and classification of ovarian tumors. Virtually all epithelial tumors of the ovary express CK7; therefore, the absence of CK7 staining suggests a non epithelial tumor of the ovary or a metastasis. Most ovarian tumors are negative for CK20 and CDX2, with the exception of the intestinal type of mucinous ovarian tumor, which sometimes expresses both. WT1 is expressed by serous carcinomas of the ovary, fallopian tube, and peritoneum; serous carcinomas of the endometrium tend to be negative. Mesotheliomas are also positive for WT1, as are other uncommon ovarian tumors, including transitional cell carcinoma, small cell carcinoma of hypercalcemic type, and some sex cord–stromal tumors. PAX8 is a highly sensitive marker of serous tumors of the ovary, fallopian tube, and peritoneum, but it is also expressed in renal cell carcinomas, thyroid cancers, and pancreatic endocrine neoplasms. PLAP, SALL4, Oct-3/4, and NANOG are sensitive and relatively specific markers of germ cell tumors and are discussed in greater detail further on. Inhibin is a sensitive and relatively specific marker of sex cord–stromal tumors but it is also expressed in adrenocortical neoplasms. Calretinin is a more sensitive but less specific marker of sex cord–stromal tumors than inhibin. CA125, on the other hand, is so promiscuous—it is expressed by tumors of the breast, colon, lung, and pancreas, as well as gynecologic cancers—that it has little value in the cytologic diagnosis of ovarian cancer.

Serous Borderline Tumor and Adenocarcinoma

The most common malignant ovarian neoplasm is the serous adenocarcinoma. Often bilateral, serous adenocarcinomas commonly contain cystic and solid foci. Histologic samples show pleomorphic cells lining the cystic areas, forming papillary projections and invading the stroma. About 30% contain psammoma

bodies.

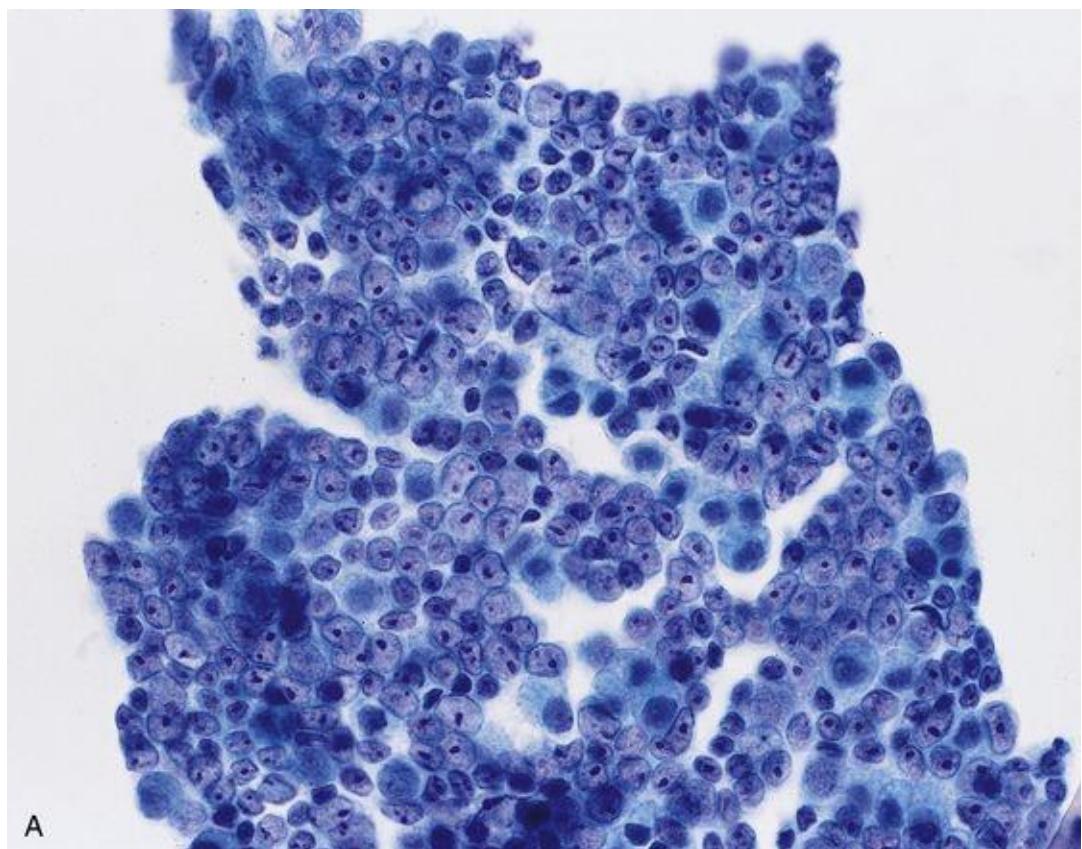
Like serous adenocarcinoma, the serous borderline tumor can be both solid and cystic. Although the degree of nuclear atypia is usually less pronounced in a borderline tumor than in a carcinoma, it is not possible to distinguish between the two cytologically.^{29,30} The diagnosis of adenocarcinoma is based on identifying stromal invasion. Nevertheless, because borderline tumors are generally of lower grade than carcinomas, some generalizations are possible.



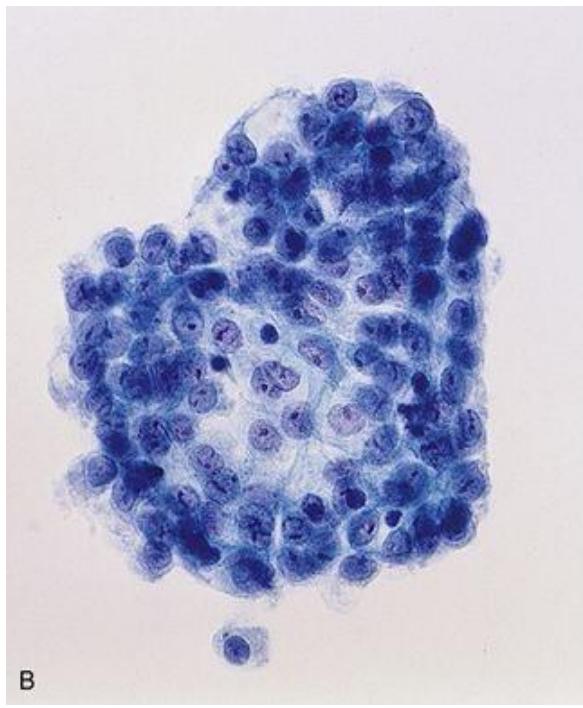
Cytomorphology of serous borderline tumor

- twisted sheets and spheres
- branching clusters
- mild to moderate nuclear atypia
- large cytoplasmic vacuoles (some cells)
- psammoma bodies
- stripped fibrovascular cores

Aspirates from serous borderline tumors are often sparsely cellular because the needle samples only cyst contents, resulting in a false-negative diagnosis.^{17,24,25} When sampled adequately, the specimen is composed of atypical cells in spheres, branching clusters, and sheets ([Fig. 16.11](#)).



A



B



C

FIGURE 16.11 Serous borderline tumor.

A, The cells are arranged in a crowded sheet. There is mild to moderate atypia. *B*, In this tight spherical aggregate, some cells have large cytoplasmic vacuoles. *C*, Psammoma bodies are a common finding (Papanicolaou stain).



Cytomorphology of serous adenocarcinoma

- clusters and isolated cells
- large pleomorphic cells
- round nuclei
- prominent nucleoli
- psammoma bodies

Aspirates from serous adenocarcinomas are usually very cellular, composed of atypical cells in papillary clusters. The nuclei are large and pleomorphic, and nucleoli are prominent ([Fig. 16.12](#)). Many atypical bare nuclei are present.⁴² The cytoplasm, like that of serous borderline tumors, can have large vacuoles. Psammoma bodies accompanied by a rim of malignant cells are seen in some but not all cases.²⁹

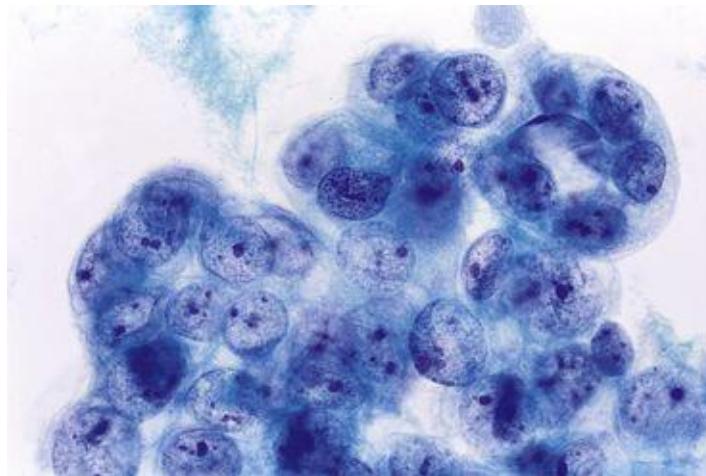


FIGURE 16.12 Serous adenocarcinoma.

The malignant cells often have large, round, and pleomorphic nuclei, and nucleoli are prominent (Papanicolaou stain).

Nuclear atypia distinguishes serous borderline tumors and adenocarcinomas from benign serous cystadenomas. Although aspirates of cellular follicle cysts, like those of serous adenocarcinomas, can be highly cellular and show mitotic

activity, follicle cysts have uniform nuclei and negative sonographic and/or laparoscopic findings. As mentioned above, the distinction between a borderline tumor and a serous adenocarcinoma is possible only on the surgically excised specimen. The distinction between serous adenocarcinoma and other epithelial malignancies like mucinous adenocarcinoma and clear cell carcinoma, especially with poorly differentiated tumors, is sometimes difficult.²⁹ Serous borderline tumors and adenocarcinomas are almost always immunoreactive for PAX8, which helps to distinguish them from PAX8-negative neoplasms like mesothelioma.^{45,46} The other epithelial tumors of the ovary described in this section are also often positive for PAX8, however, so this marker is not useful in distinguishing them from serous tumors.

Mucinous Borderline Tumor and Adenocarcinoma

Mucinous adenocarcinomas are less common than serous adenocarcinomas. They are usually large, multilocular cysts with solid areas and papillary excrescences. Metastatic intestinal adenocarcinomas to the ovary, particularly from the appendix, are histologically very similar to primary mucinous ovarian cancers and need to be excluded by a combination of clinical features, extensive histologic sampling, and immunophenotyping with CDX2 and cytokeratins CK7 and CK20.



Cytomorphology of mucinous adenocarcinoma

- columnar mucinous cells with mild atypia and/or groups of pleomorphic large cells with prominent nucleoli
- cytoplasmic vacuolization
- extracellular mucin
- macrophages

Aspirates from mucinous adenocarcinomas are cellular, composed of isolated cells and cells in sheets or grouped in irregular clusters. Cells from well-differentiated tumors are columnar, contain mucin, and have mild nuclear atypia. The nuclei of poorly differentiated tumors are pleomorphic and indistinguishable from poorly differentiated serous adenocarcinomas; some tumors show a range of differentiation ([Fig. 16.13](#)).

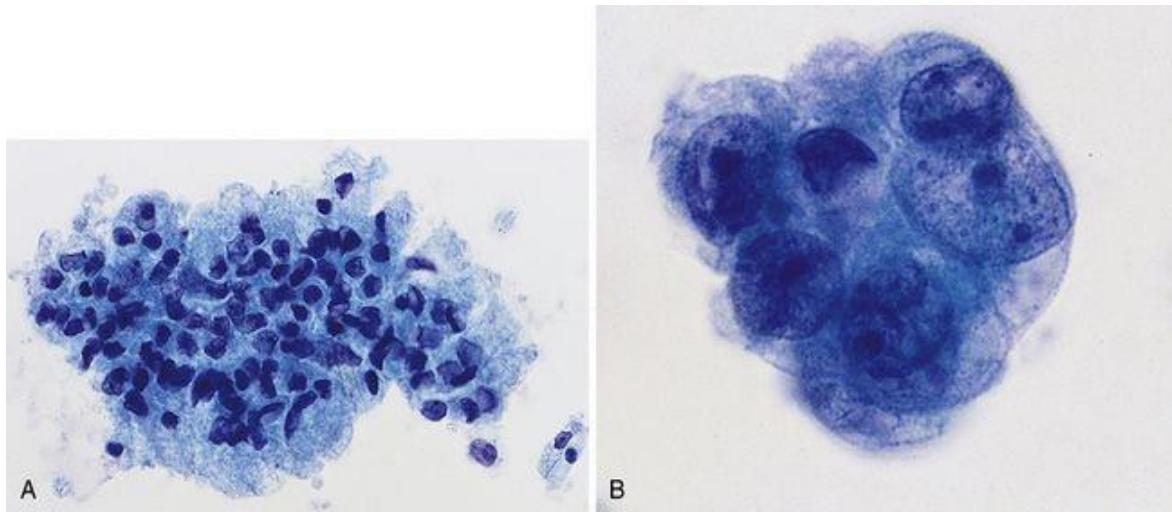


FIGURE 16.13 Mucinous adenocarcinoma.
A, Some sheets of mucinous cells show only mild atypia (compare with [Figs 16.9, 16.10](#)). B, Other cells are markedly atypical, with little if any mucinous differentiation (Papanicolaou stain).

As is the case for serous tumors, cytologic samples cannot accurately distinguish a borderline mucinous tumor from a well-differentiated mucinous adenocarcinoma; histologic examination for the presence of invasion is needed. Aspirates from mucinous borderline tumors are a common cause of false-negative results.

Spread of a mucinous ovarian tumor into the peritoneum can lead to pseudomyxoma peritonei, but most ovarian tumors associated with pseudomyxoma peritonei are actually metastases from an occult appendiceal primary. Aspirates from pseudomyxoma peritonei are hypocellular, composed predominantly of abundant mucin.⁴⁷ Atypical mucinous glands are identified in some but not all cases.

Endometrioid Carcinoma

Endometrioid carcinoma accounts for 10% to 20% of ovarian carcinomas and is bilateral in 28% of cases.⁴⁰ Up to 42% are associated with endometriosis, and 15% to 20% with a coexisting adenocarcinoma of the endometrium.⁴⁰ Endometrioid carcinomas are usually cystic and solid tumors with foci of necrosis and hemorrhage. Morphologically similar to the usual type of adenocarcinoma of the endometrium, they are graded using the same criteria. Squamous differentiation may be seen.



Cytomorphology of endometrioid carcinoma

- numerous isolated cells
- strips and/or crowded glands
- palisading
- elongated columnar shape

Aspirates yield numerous isolated elongated cells, as well as occasional short strips of cells and intact or “broken” glands ([Fig. 16.14A](#)). Cell block sections are helpful in revealing the resemblance of the neoplasm to endometrial glands ([Fig. 16.14B](#)). The background is usually bloody and contains hemosiderin-laden macrophages. It may be impossible to distinguish an endometrioid carcinoma from other surface epithelial malignancies, such as a serous or mucinous adenocarcinoma, especially with high-grade tumors.^{1,13,29,48}

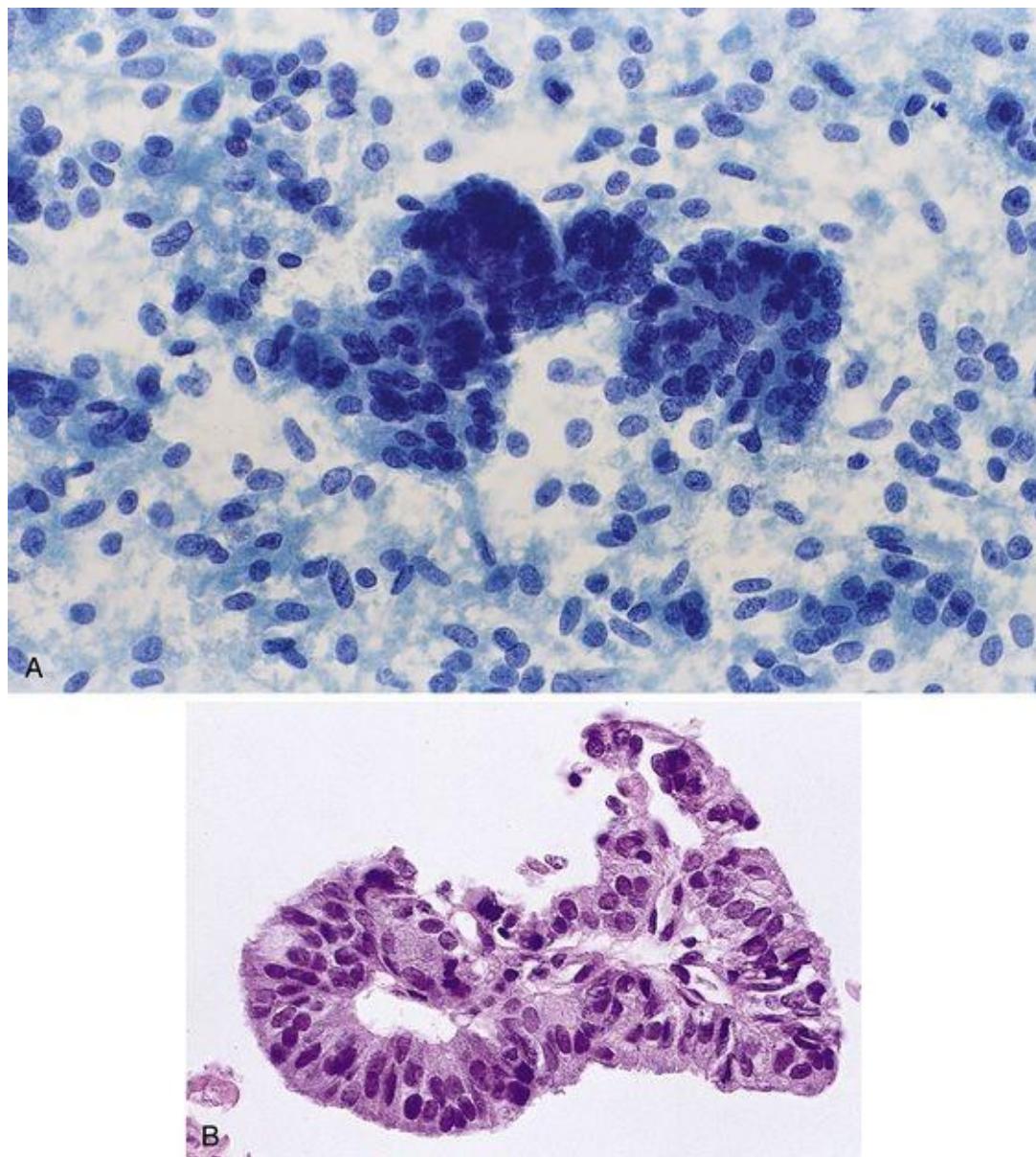


FIGURE 16.14 Endometrioid adenocarcinoma.

A, The cells have elongated nuclei and a narrow columnar shape. Some are arranged in pseudostratified strips and glands (Papanicolaou stain). B, Cell block sections reveal endometrial-like glands with atypia (hematoxylin-eosin [H & E] stain).

Clear Cell Carcinoma

Clear cell carcinoma of the ovary resembles the clear cell carcinomas of the endometrium, cervix, and vagina. Tumor cells have large, pleomorphic, often eccentrically placed nuclei with prominent nucleoli. The cytoplasm is abundant and vacuolated or scant and eosinophilic. Within or adjacent to the tumor cell clusters there may be hyaline extracellular material that stains pink-purple with

Romanowsky stains.⁴⁹ The background shows necrosis.

Germ Cell Tumors

Germ cell tumors are most common in the ovary and testis, but can also be seen in the retroperitoneum, mediastinum, and the midline of the central nervous system. Neoplasms identical to those of the ovary occur in the testis and extragonadal sites. Germ cell tumors can occur at any age but are most common during the reproductive years. They account for 30% of all ovarian tumors. The vast majority of those seen in adults (up to 95%) are benign cystic teratomas (dermoid cysts). In contrast, up to one third of germ cell tumors in children are malignant.

Although some germ cell tumors are “pure” tumors, such as the pure dysgerminoma, many are composed of a mixture of germ cell subtypes.

Teratoma

Mature Teratoma

Mature teratomas are the most common of the germ cell tumors. Although they are seen at any age, they usually occur during the reproductive years. Most are cystic (mature cystic teratoma or dermoid cyst) and composed of tissue derived from ectoderm, endoderm, and mesoderm, with ectodermal derivatives such as skin and hair the most common. Sebaceous glands are prominent. Struma ovarii, a variant of mature teratoma, is ectopic thyroid tissue in the ovary. Mature teratomas are rarely aspirated, because their sonographic features (particularly the tooth) are diagnostic.

FNA yields cellular material containing predominantly anucleated squamous cells ([Fig. 16.15A](#)). Ciliated cells, detached ciliary tufts, mucinous cells, and hair are seen in some cases ([Fig. 16.15B](#)).⁴¹ With transvaginal aspirations, contamination of a benign epithelial cyst by normal vaginal squamous cells mimics this picture.³ A confident diagnosis of a mature teratoma can be made, however, if some other route was taken to obtain the specimen. In rare cases, a mature cystic teratoma may undergo malignant transformation, most commonly to squamous cell carcinoma.

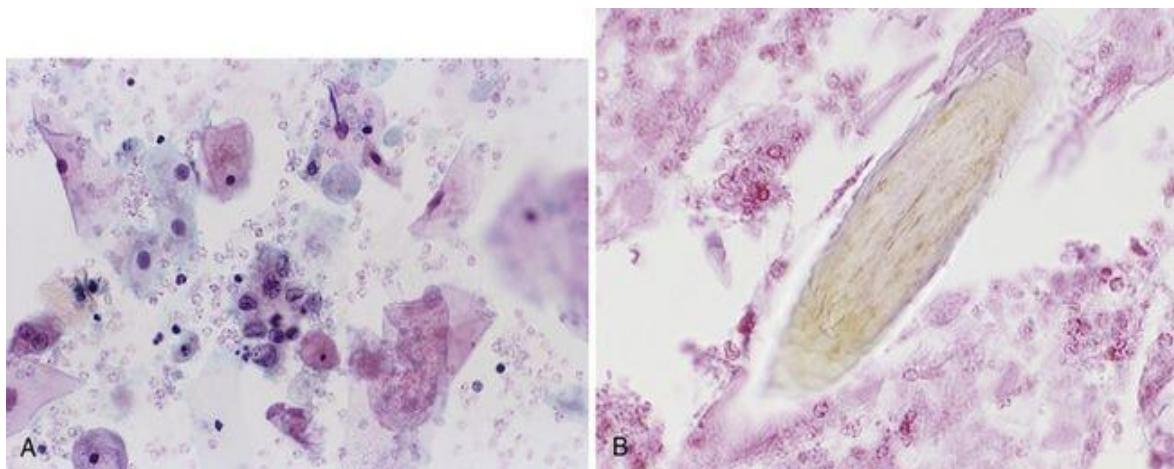


FIGURE 16.15 Mature cystic teratoma (dermoid cyst).

A, Mature nucleated and anucleate squamous cells and occasional glandular cells are present (Papanicolaou stain). B, This cell block section contained several transected hair shafts, recognizable based on their size, shape, and pigmentation (hematoxylin-eosin [H & E]-stained cell block).

Immature Teratoma

Immature teratomas are rare, malignant, rapidly growing neoplasms, usually solid and unilateral, most commonly in the first two decades of life. These tumors are composed of derivatives from all three germ cell layers and contain a variable amount of immature or embryonal-type tissue. Microscopically, immature or embryonal tissue, usually admixed with benign mature elements, is characteristic of these tumors. Immature neuroectoderm is particularly prominent.

Carcinoid Tumor

Carcinoid tumors of the ovary are usually a component of mature teratomas, but may also occur in a pure form. It is important to distinguish a primary ovarian tumor from a metastatic carcinoid tumor to the ovary, a distinction that requires clinical correlation and is not possible by cytologic examination. Metastatic carcinoid tumors to the ovary almost always originate from a gastrointestinal primary and are most often bilateral.

FNA reveals numerous isolated cells and loose clusters. Occasionally, rosettes are seen. Tumor cells have an eccentrically placed round or oval nucleus and the chromatin has a granular, “salt and pepper” appearance. The cytoplasm is eosinophilic and granular, with distinct outlines. Intracytoplasmic neurosecretory

granules are identified by immunocytochemistry for chromogranin and other neuroendocrine markers.

Dysgerminoma

Dysgerminomas are the ovarian analogue of the testicular seminoma. They constitute 1% to 5% of all malignant ovarian tumors and 40% of all malignant ovarian germ cell tumors. They are often large, solid tumors, and are bilateral in 15% to 20% of cases. They are most frequent in women under the age of 30. Because they are highly radiosensitive, accurate diagnosis is crucial so that appropriate therapy may be instituted. Most tumors have rounded or bosselated contours. Large tumors may show cystic areas associated with necrosis and hemorrhage. Histologic examination reveals sheets, nests, and cords of uniform, large, polyhedral tumor cells with centrally placed round nuclei; prominent, sometimes bizarre, eosinophilic nucleoli; and coarsely granular chromatin. The cytoplasm of these cells is abundant, well defined, and eosinophilic or vacuolated. It contains both lipid and glycogen and stains positively with the periodic acid–Schiff (PAS) reaction. The tumor cells are separated by fibrous septa infiltrated by lymphocytes, some arranged as lymphoid follicles with germinal centers. Granulomas are seen in some cases. A small percentage (3%) contain syncytiotrophoblastic cells that secrete human chorionic gonadotropin.⁵⁰ Ultrastructurally, dysgerminoma cells contain intracytoplasmic annulated lamellae, lipid, and glycogen.⁵¹

Aspirates from dysgerminomas are usually highly cellular, composed predominantly of isolated tumor cells, with some loose syncytium-like clusters. In the background are small lymphocytes and, in some cases, granulomas. A characteristic “tiger stripe” background, similar to that seen in seminomas, is seen on air-dried preparations (see [Fig. 2.45](#)). Necrosis and hemorrhage may be present. The tumor cells have a large, round, centrally placed nucleus with one or more prominent, irregularly shaped nucleoli ([Fig. 16.16A](#)). The cytoplasm may be clear or granular. Mitoses are present.

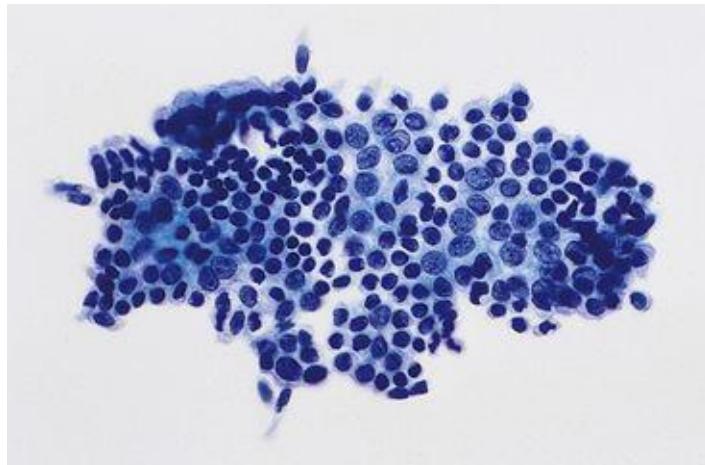


FIGURE 16.6 Simple ovarian cyst.

This sheet of evenly arranged cuboidal cells resembles a sheet of mesothelial cells (Papanicolaou stain).

Dysgerminomas are immunoreactive for placental alkaline phosphatase (PLAP) (cytoplasmic and membrane staining), CD117 (c-kit) (membrane staining), and the stem cell-related proteins Oct-3/4 (synonymous with Oct-4), NANOG, and SALL4 (nuclear staining for all three) ([Fig. 16.16B-D](#)). PLAP and SALL4 are expressed in most germ cell tumors, whereas Oct-3/4 and NANOG are expressed only in dysgerminoma/seminoma and embryonal carcinoma. The membranous staining pattern for CD117 is very characteristic of dysgerminoma and not seen in embryonal carcinoma or yolk sac tumor. Unlike the embryonal and yolk sac tumors, dysgerminomas generally show no immunoreactivity for keratin proteins, EMA, alpha-fetoprotein, or CEA.

Embryonal Carcinoma and Other Malignant Germ Cell Tumors

In contrast with their testicular counterparts, embryonal carcinomas, endodermal sinus tumors, and choriocarcinomas of the ovary are extremely rare. They are aggressive neoplasms that often present at an advanced stage of disease. Histologically, they are primitive large cell neoplasms with marked nuclear anaplasia. Necrosis and hemorrhage are prominent features.

The tumor cells of embryonal carcinoma have a centrally placed, large, round or highly irregular nucleus with several nucleoli. The cytoplasm is indistinct and pale. Bizarrely shaped cells and mitoses are common. Cells of yolk sac tumors resemble those of poorly differentiated adenocarcinomas. They are cohesive, pleiomorphic cells with prominent nucleoli.¹⁴ Some tumor cells contain intracytoplasmic dense hyaline globules composed of alpha-fetoprotein; others

have vacuolated cytoplasm.⁵² Mucoid and basement membranelike material may be present in the background.⁵³ Choriocarcinomas are composed of malignant cytotrophoblast and syncytiotrophoblast. These tumors secrete human chorionic gonadotropin.

A cytologic diagnosis of malignancy in this group of tumors is straightforward, but correct subtyping and distinction from a poorly differentiated epithelial malignancy require correlation with serologic markers and immunocytochemical studies. Embryonal carcinoma, yolk sac tumor, and choriocarcinoma are keratin positive but can be distinguished from epithelial malignancies because of their staining for PLAP and SALL4. Additionally, embryonal carcinoma, like dysgerminoma, is immunoreactive for Oct-3/4 and NANOG. Dysgerminoma can be distinguished from embryonal carcinoma because the former has a characteristic membranous staining pattern for CD117.

Sex Cord–Stromal Tumors

Sex cord–stromal tumors constitute approximately 8% of all ovarian neoplasms and include tumors derived from granulosa cells, theca cells, Sertoli cells, Leydig cells, and the fibroblasts of the ovarian stroma.⁴⁰ Many of these tumors produce and secrete steroid hormones, which result in clinically apparent estrogenic or, less commonly, androgenic changes.

Granulosa Cell Tumors

Granulosa cell tumors account for about 1.5% of all ovarian tumors. There are two major subcategories: the adult and juvenile types.

Adult Granulosa Cell Tumor

The adult granulosa cell tumor (AGCT) accounts for more than 95% of all granulosa cell tumors and occurs in middle-aged and postmenopausal women. Typically unilateral, the tumor grows slowly but is capable of metastasis. Many AGCTs secrete estrogens; prolonged elevated levels of estrogen may result in endometrial hyperplasia or carcinoma. Rarely, androgens are secreted, with the resultant signs of virilization. Although about 10% of these tumors are accompanied by ascites, the fluid does not usually contain malignant cells.³⁶ Although most commonly solid and cystic, with foci of hemorrhage and necrosis, purely solid or cystic tumors are also seen. The tumor is composed of neoplastic granulosa cells arranged in a variety of architectural patterns. Most characteristic is the microfollicular pattern, which shows Call-Exner bodies: tumor cells arranged around small cavities containing eosinophilic fluid. Granulosa cell tumors are typically strongly immunoreactive for inhibin and calretinin. Other immunostains that are often or occasionally positive include estrogen receptor, progesterone receptor, CD56, CD99, WT1, cytokeratins, S-100, and smooth muscle actin. AGCTs are negative for EMA.



Cytomorphology of adult granulosa cell tumor

- highly cellular
- isolated cells, clusters, sheets, trabeculae
- Call-Exner bodies

- small to medium-sized cells
- round, monomorphic nuclei
- nuclear grooves
- ill-defined cytoplasm
- globular basement membrane-like material (best seen with Romanowsky-type stains)

FNA samples are highly cellular. Small to medium-sized cells have a centrally placed, round or oval, monomorphous nucleus ([Fig. 16.17A](#)). Nuclear chromatin is pale and finely dispersed. Naked nuclei are common.⁵⁴ Nuclear grooves (“coffee bean nuclei”) are present to some degree in virtually all cases⁵⁴ but are easily visible in only a minority.^{44,54–56} Nucleoli are prominent in some cases.⁵⁴ Cytoplasm is scant, poorly defined, and pale.³⁶ Mitoses are rare. Call-Exner bodies (small rings of granulosa cells surrounding fluid and/or pyknotic nuclei) are seen in some cases.^{54,56} In some cases, Romanowsky-type stains reveal globular basement membrane-like material.⁴⁴ Almost one half of cases show a prominent, arborizing vascular pattern.^{54,56} Cell block sections are helpful in highlighting one or more of the different growth patterns of granulosa cell tumors: microfollicular, macrofollicular, trabecular, insular, diffuse, and moiré silk (watered silk) ([Fig. 16.17B](#)).

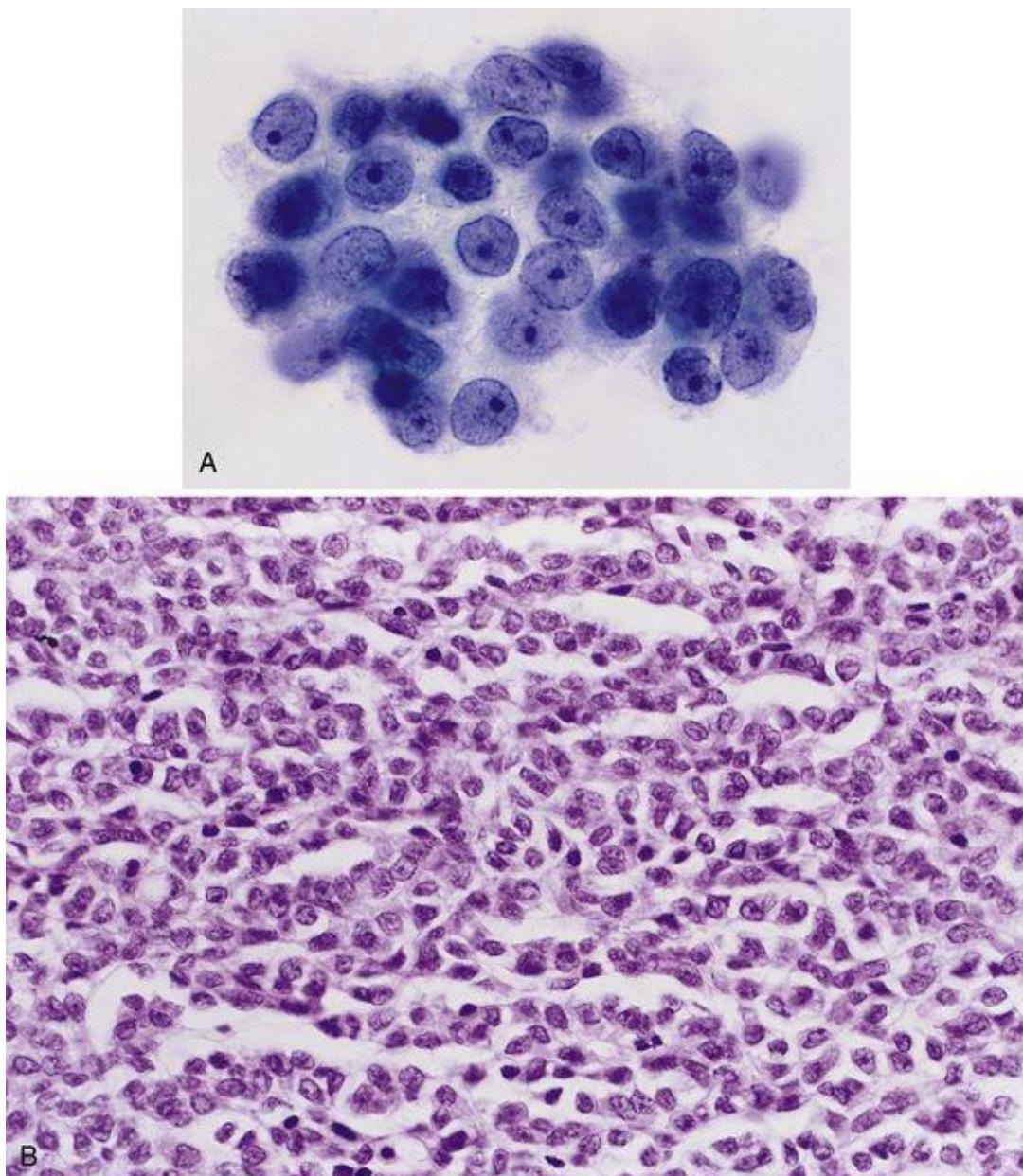


FIGURE 16.17 Adult-type granulosa cell tumor (AGCT).

A, The small to medium-sized cells are arranged in loose clusters. They are difficult to distinguish from normal granulosa cells (compare with [Figs 16.2, 16.3](#)) (Papanicolaou stain). B, Large fragments of tumor in cell block sections may show one of the characteristic architectural patterns, in this case the watered-silk pattern (hematoxylin-eosin [H & E]-stained cell block).



Differential diagnosis of adult granulosa cell tumor

- cellular follicle cyst
- other sex cord–stromal tumors
- carcinoid tumor

- endometrioid carcinoma
- small cell carcinoma, pulmonary type

The cytomorphology of a cellular follicle cyst (see [Figs. 16.1-16.3](#)) mimics that of an AGCT.³⁷ Correlation with sonographic and laparoscopic findings can be key to avoiding an erroneous interpretation: Cellular follicle cysts are usually unilocular and clinically entirely benign. Other sex cord–stromal tumors like the Sertoli-Leydig cell tumor and the sex cord tumor with annular tubules cannot be reliably distinguished from an AGCT by cytology or immunohistochemistry.^{43,44,55} A carcinoid tumor lacks nuclear grooves and is immunoreactive for chromogranin. Call-Exner bodies mimic the appearance of endometrioid tubules, but endometrioid carcinomas lack nuclear grooves and often show squamous metaplasia.

Two rare ovarian tumors are composed partly or completely of undifferentiated small cells: *small cell carcinoma, hypercalcemic type*, and *small cell carcinoma, pulmonary type*.⁴⁰ The hypercalcemic type is more common in young women and therefore needs to be considered in the differential diagnosis of juvenile granulosa cell tumor (JGCT) (discussed next). The small cell carcinoma, pulmonary type, resembles small cell carcinoma of the lung ([Fig. 16.18](#)). It typically occurs in postmenopausal women and needs to be considered in the differential diagnosis of AGCT.

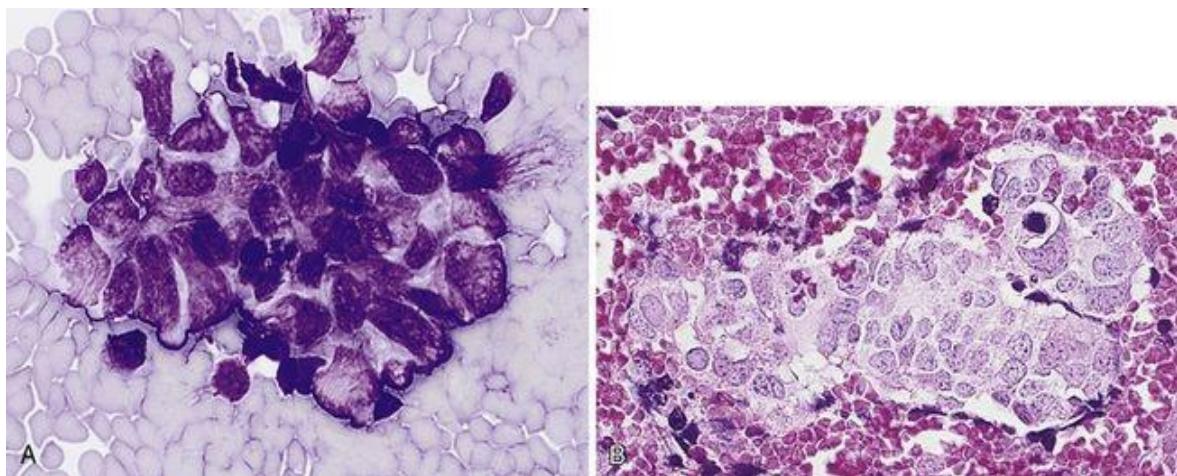


FIGURE 16.18 Small cell carcinoma of the ovary, pulmonary type. This tumor resembles the small cell carcinoma of neuroendocrine type of the lung. A, Wright–

Giemsa-stained smear; *B*, hematoxylin-eosin (H & E)-stained cell block section.

Juvenile Granulosa Cell Tumor

JGCTs occur predominantly in childhood and adolescence. Like AGCT, JGCTs secrete estrogens and thus are often associated with sexual pseudoprecocity. JGCTs are usually solid and cystic, unilateral, and restricted to the ovary. Patients may present with an acute abdomen after rupture of the tumor capsule. The tumor forms follicles lined by granulosa cells and luteinized theca cells. In contrast with AGCT, almost all nuclei lack grooves. Despite the presence of pleomorphism and frequent mitoses, only 10% behave aggressively. The cells of JGCT are positive for cytokeratin, inhibin, and vimentin⁵⁸ and lack reactivity for CEA, human chorionic gonadotropin, and alpha-fetoprotein.

On FNA preparations, tumor cells are seen in loose clusters and single cells.⁵⁶ Nuclei are round, with fine chromatin and small to prominent nucleoli. There may be nuclear protrusions, but nuclear grooves are absent. Some mitoses are seen. There is a moderate amount of granular cytoplasm, and the oil red O stain demonstrates intracytoplasmic lipid. Call-Exner bodies are usually not found.

JGCTs should be distinguished from the small cell carcinoma, hypercalcemic type, which occurs in the same age range. The clinical association of JGCT with estrogenic manifestations and small cell carcinoma with hypercalcemia are helpful clues. Immunostaining for inhibin (positive in JGCT, negative in small cell carcinoma) can provide confirmation.

Thecoma

Thecomas, benign tumors of theca cells, are more common in postmenopausal women and are usually solid and unilateral tumors. They may be functional, secreting estrogens or androgens, or nonfunctional. Microscopically, the tumor is composed of monomorphic oval or spindle-shaped cells with cytoplasm that shows variable degrees of luteinization.

Unlike aspirates from granulosa cell and Sertoli-Leydig cell tumors, aspirates from thecomas are usually hypocellular.³⁰ They are composed of isolated elongated cells and loose clusters. The nuclei are spindle-shaped, with finely granular chromatin. The cytoplasm is clear and contains numerous lipid vacuoles.

Fibroma

Fibromas are benign, non functional, stromal tumors that constitute 4% of ovarian neoplasms. They are more common during reproductive years. These tumors may be accompanied by ascites and pleural effusion (Meigs' syndrome). Histologically, fibromas are composed of bundles of spindle-shaped cells arranged in a whorling or storiform pattern. The cells have a fibroblastic appearance, and collagen is usually abundant. Intracellular edema and intracytoplasmic lipid are sometimes observed.

Aspirates are hypocellular and contain bundles of fibroblastic spindle-shaped cells similar to those seen in thecomas. The distinction between a fibroma and a thecoma by FNA cytology is impossible because luteinized cells can be a component of both tumors.

Uncommon Primary Ovarian Tumors

Benign and malignant mesenchymal tumors, bone-producing and cartilage-producing tumors, vascular tumors, and primary lymphomas of the ovary are rare, as are tumors of neural origin and those derived from the mesothelium (adenomatoid tumors). Leiomyomas may exhibit prominent cystic degeneration; aspirates from such tumors contain benign-appearing foam cells suggestive of a benign cyst. When leiomyomas are not cystic, the aspirates are usually very scant and contain only occasional smooth muscle cells. Aspirates from lymphomas yield numerous isolated cells that are usually easily recognizable as lymphoid in origin.

Metastatic Tumors

The most common tumors that metastasize to the ovaries originate in the colon, stomach, appendix, and breast and elsewhere in the female genital tract. Approximately 15% to 20% of bilateral ovarian malignancies are metastatic. Tumors that occur as multiple nodules on the ovarian surface are likely to be metastatic.

Krukenberg tumors are characterized by mucin-filled signet ring-shaped cells metastatic to the ovary.³⁰ Most arise in the stomach, but tumors of the colon, appendix, and breast also cause this pattern of spread. Because signet ring cells are rare in primary ovarian tumors, the possibility of a metastasis should be considered when an ovarian tumor is rich in signet ring cells.

In many cases, distinguishing between a primary ovarian carcinoma and a metastasis is impossible by cytomorphology alone. Because primary ovarian epithelial tumor are generally CK7-positive and CK20-negative, the reverse immunophenotype (CK7-negative, CK20-positive) suggests a metastasis, probably from the appendix or intestine. Measuring the concentration of tumor-associated antigens in cyst fluid is useful: Unlike ovarian carcinomas, metastatic colon cancers have a high CEA level combined with a low CA-125 level.¹⁹

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CHAPTER 17

Soft Tissue

Xiaohua Qian

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Few areas in cytology arouse such passionate discourse as fine-needle aspiration (FNA) of soft tissue masses. Critics jump at the opportunity to point out the limitations of the technique but pepper their few valid arguments with outdated fallacies and academic concerns that do not necessarily consider the best interests of individual patients. Over the years, as needle core biopsy has largely replaced incisional biopsy as the primary diagnostic modality for soft tissue lesions, FNA has begun to be accepted as an adjuvant tool to assess the adequacy of the needle core biopsy, as an essential element for proper triage of precious small biopsy tissue and as an alternative diagnostic tool in certain settings.

One undeniable difficulty with soft tissue FNA is the lack of experience most cytopathologists have with the greater than 130 different soft tissue lesions, including more than 30 types of sarcoma.¹ Because the morphologic heterogeneity is so astounding, it has been suggested that at least five soft tissue lesions ought to be sampled weekly to maintain practitioner experience.² With sarcomas constituting only 0.6% of all malignancies in the United States,³ this recommendation is unrealistic for most cytopathologists. Although it might be desirable to restrict FNA of primary soft tissue lesions to centers with cytologic expertise and the capability for a multidisciplinary approach, most patients are first seen in community practices. A systematic approach to differential diagnosis, specimen triage, recommendations, and referrals can enhance the efforts of the cytopathologist, regardless of his or her level of experience. For this reason, this chapter focuses on pattern recognition and is organized into sections on adipocytic, myxoid, spindle cell, fibrohistiocytic, round cell, epithelioid, and pleomorphic neoplasms.

Sampling error is a problem FNA shares with core and even incisional biopsies.⁴⁻⁶ Ancillary techniques such as immunohistochemistry and cytogenetics are often indispensable for definitive classification.⁷⁻¹⁰ Needle tract seeding, a concern in the era of larger needles, is exceedingly rare with FNA.¹¹ To prevent this potential complication, use of a single needle insertion point is recommended for several passes in cases with a high suspicion for sarcoma.¹²

FNA is a useful screening tool for evaluating soft tissue masses. It is the least invasive method for sampling a heterogeneous lesion, does not compromise tissue planes for a subsequent excision, and is cost-effective.^{13,14} Although not widely accepted by most soft tissue pathologists as the sole sampling modality for the primary classification of sarcomas, it plays an important role in triaging patients—the initial clinical differential diagnosis for many soft tissue masses is broad in scope and might include lymphoma and even metastatic carcinoma. FNA can be indispensable as the initial step toward narrowing the possibilities. FNA is especially valuable for confirming a recurrence or metastasis of a

sarcoma.¹⁵⁻¹⁷ With recent advances in our understanding of mesenchymal lesions and the availability of an increasing number of ancillary diagnostic tests, FNA is suitable for sampling a growing number of primary soft tissue tumors, including most small round cell tumors, which have well-defined cytomorphologic, immunophenotypic, and/or cytogenetic features.¹⁸

The location, size, and degree of invasiveness of a lesion and its relationship to surrounding structures are important clues to the correct diagnosis. With a few exceptions, benign lesions are generally small, circumscribed, and subcutaneous or superficial masses, whereas malignant lesions are more often large (greater than 5 cm), infiltrative, and deep-seated (inter-or intramuscular or retroperitoneal).^{2,13-15,19-22} Patients with a benign mesenchymal mass lesion, which is at least 100 times more common than a sarcoma, benefit the most from a definitive benign diagnosis provided by FNA, the fastest, least expensive, and least invasive diagnostic procedure.



Advantages of fine-needle aspiration for soft tissue lesions

- technical ease
- less invasive; less morbidity
- allows for immediate assessment of specimen adequacy and allocation of tissue for special studies
- provides suitable material for ancillary studies
- no contamination of tissue planes
- cost-effective

Most (87% to 100%) cases are correctly diagnosed as benign or malignant, with an average sensitivity and specificity for malignancy of approximately 95%.^{2,13,14,16,19-21,23,24} The distinction among lymphoma, carcinoma, and sarcoma has specificity rates for sarcoma of 54% to 98% and a positive predictive value of 91% to 99%.^{16,23,25} Owing to the rarity of most soft tissue tumors, data on sensitivity and specificity for a specific entity are just emerging.^{18,26} The three main reasons for a false diagnosis are sampling error, a technically limited specimen, and misinterpretation.¹³ The false-positive rate for soft tissue FNA is relatively low (0% to 5%). False-negative rates vary a little more (2% to 15%), but any clinically suspicious lesion should be further evaluated by biopsy.^{2,6,13,14,16,17,21,23}

Rapid evaluation of specimen adequacy at the time of the procedure, along with triage of tissue for ancillary studies, will decrease the rate of inadequate specimens.^{2,13-15,19,27}



Summary of statistical performance characteristics of soft tissue fine-needle aspiration

- sensitivity rates: as high as 95%
- specificity for sarcoma: 54% to 98%
- false-positive rate: 0% to 5%
- false-negative rate: 2% to 15%

Specimen Collection and Preparation

Cytomorphologic evaluation should be based on both alcohol-fixed, Papanicolaou-stained preparations (for nuclear details) and air-dried, Romanowsky-type stained preparations (for cytoplasmic details and matrix material). Thinlayer preparations offer good nuclear detail, optimize results from aspirates carried out without the benefit of rapid evaluation, and can be used for adjunct studies, but cells can appear smaller, rounder, and falsely epithelioid, and useful background information (e.g., vascular patterns, chondromyxoid material) is sometimes lost.^{2,14,24,28-32} Cell block preparations are especially helpful for immunohistochemical studies and for mini-tissue architecture. Unstained smears or cytospin slides are suitable for immunocytochemistry, fluorescence in situ hybridization (FISH), and even molecular studies. A concurrent core biopsy, taken with a larger gauge needle (18G or larger), is commonly obtained when imaging characteristics or the rapid, on-site evaluation of smears suggests a primary soft tissue tumor.



Specimen collection and preparation

- 22 to 25 gauge needles
- separate passes for ancillary studies
- alcohol-fixed, Papanicolaou-stained smears for nuclear detail
- air-dried, Romanowsky-stained smears for cytoplasmic and matrix details
- cell block and core needle sample for immunohistochemical studies
- air-dried, unstained cytospin slides for FISH studies
- limited use of thinlayer preparations for morphologic evaluation

Ancillary Studies

Ancillary techniques like immunohistochemistry, cytogenetics, and molecular genetics are often indispensable for the classification of soft tissue lesions. Electron microscopy is only occasionally used for assistance in classifying a

poorly differentiated neoplasm or soft tissue tumor that exhibits specific ultrastructural features, like the Weibel-Palade bodies of vascular tumors.^{2,33,34} Cell block sections are preferred for immunohistochemical studies, but air-dried or alcohol-fixed direct smears and cytocentrifuge or thinlayer preparations can also be used. Flow cytometry is useful when lymphoma is in the differential diagnosis. Ideally, one makes a dedicated pass for each desired ancillary study.^{23,34-37}

An increasing number of reproducible, relatively specific cytogenetic abnormalities can be identified with conventional chromosomal analysis (karyotyping), FISH, and reverse transcriptase-polymerase chain reaction (RT-PCR) techniques (Table 17.1).^{10,21,23,38,39} Conventional chromosomal analysis offers the advantages of a full karyotype, but this is counterbalanced by its low sensitivity and longer turnaround time as compared with molecular methods such as FISH.^{9,21,38,40}

TABLE 17.1
SOFT TISSUE TUMORS, THEIR ASSOCIATED CHROMOSOMAL CHANGES, AND FLUORESCENCE IN SITU HYBRIDIZATION (FISH) PROBES

TUMOR	CYTOGENETIC ABERRATION(S)	FLUORESCENCE IN SITU HYBRIDIZATION PROBE(S)
Angiofibroma of soft tissue	t(5;8)(p15;q13)	N/A
Angiomatoid fibrous histiocytoma	t(12;16)(q13;p11) t(12;22)(q13;q12) t(2;22)(q34;q12)	<i>FUS</i> <i>EWSR1</i> <i>EWSR1</i>
Alveolar soft part sarcoma	der(X)t(X;17)(p11;q25)	<i>TFE3</i>
Clear cell sarcoma	t(12;22)(q13;q12) t(2;22)(q33;q12)	<i>EWSR1</i> <i>EWSR1</i>
Dermatofibrosarcoma protuberans/giant cell fibroblastoma	r(17;22)(q21;q13)/t(17;22)(q21;q13)	<i>PDGFB</i>
Desmoid fibromatosis	Trisomy 8 and 20	<i>CEP8/CEP20</i>
Desmoplastic small round cell tumor	t(11;22)(p13;q12)	<i>EWSR1</i>
Epithelioid hemangioendothelioma	t(1;3)(p36.3;q25)	N/A
Epithelioid sarcoma	t/der(22)(q11.2)	N/A
Ewing sarcoma	t(11;22)(q24;q12) t(21;22)(q22;q12) t(2;22)(q33;q12) t(7;22)(p22;q12)	<i>EWSR1</i> <i>EWSR1</i> <i>EWSR1</i> <i>EWSR1</i>

	t(17;22)(q12;q12) t(20;22)(q13;q12)	<i>EWSR1</i> <i>EWSR1</i>
Extraskeletal myxoid chondrosarcoma	t(9;22)(q22;q12) t(9;17)(q22;q11) t(9;17)(q22;q11) t(3;9)(q11;q22)	<i>EWSR1</i> <i>NR4A3</i> <i>NR4A3</i> <i>NR4A3</i>
Hibernoma	11q13 rearrangement	N/A
Inflammatory myofibroblastic tumor	2p23 rearrangement	<i>ALK</i>
Infantile fibrosarcoma	t(12;15)(p13;q26)	<i>ETV6</i>
Lipoblastoma	8q12 rearrangement or polysomy 8	N/A
Lipoma, chondroid	t(11;16)(9q13;p12-13)	N/A
Lipoma, ordinary	12q14.3 rearrangement	<i>HMGA2</i>
Lipoma, spindle cell and pleomorphic	Deletions of 13q and 16q	N/A
Liposarcoma, well-differentiated/dedifferentiated	Ring/giant marker chromosomes from chromosome 12q	<i>HMGA2/MDM2</i>
Liposarcoma, myxoid	t(12;16)(q13;p11) t(12;22)(q13;q12)	<i>DDIT3/FUS</i> <i>DDIT3/EWSR1</i>
Low-grade fibromyxoid sarcoma	t(7;16)(q34;p11) t(1;16)(p11;p11)	<i>FUS</i> <i>FUS</i>
Myoepthelioma, soft tissue	t(19;22)(q13;q12) t(1;22)(q23;q12) t(6;22)(p21;q12)	<i>EWSR1</i> <i>EWSR1</i> <i>EWSR1</i>
Myxoinflammatory fibroblastic sarcoma	der(10) t(1;10)(p22;q24)	N/A
Nodular fasciitis	t(17;22)(p13.1;q12.3)	N/A
Rhabdomyosarcoma, alveolar	t(2;13)(q35;q14) t(1;13)(p36;q14), double minutes 2q35 rearrangement	<i>FOXO1</i> <i>FOXO1</i> N/A
Rhabdomyosarcoma, embryonal	Trisomies 2q, 8, and 20 Loss of heterozygosity at 11p15	N/A
Schwannoma	Deletion of 22q	N/A
Synovial sarcoma	t(X;18)(p11;q11)	<i>SS18</i>
Tenosynovial giant cell tumor	t(1;2)(p13;q37)	N/A

N/A, Not available, i.e., either the probe is not commercially available or the gene is unknown.

From: Dal Cin P, Qian X, Cibas ES. The marriage of cytology and cytogenetics. Cancer Cytopathol 2013, with permission from Wiley.

FISH accurately labels targeted chromosomal regions, and virtually all cytologic preparations (cytocentrifuge preparations, thinlayer slides, and smears)

are ideal substrates for FISH, because they contain intact cells and nuclei free of sectioning artifact and truncation. When the diagnostic chromosomal aberration is known for a suspected soft tissue tumor, FISH is preferred because the number of cells needed is far less than for conventional karyotyping.²⁴¹ A FISH test is especially useful in the differential diagnosis of lipomatous tumors, myxoid tumors, and small round cell tumors. Because only a specified chromosomal abnormality is targeted, however, a negative result provides minimal information.⁹⁴²

Reporting Terminology

As with other cytologic specimens, the use of general category headings on the report (e.g., benign, atypical, suspicious, positive, nondiagnostic), along with a descriptive interpretation, is useful for clarity of communication. Although histologic typing is desired whenever possible, when the findings are not conclusive for a specific entity, a descriptive interpretation with differential diagnosis is appropriate.^{2,15,16,21,23,43}

The most important prognostic consideration with soft tissue tumors is the histologic grade.⁴⁴ Grading should only be attempted when a specific diagnosis can be unequivocally established, and the tumor is gradable based on cellularity, pleomorphism, mitoses, and necrosis. For many sarcomas, additional grading is not necessary, as a specific diagnosis itself is indicative of a grade: Ewing sarcoma, synovial sarcoma, and angiosarcoma are high-grade malignancies, whereas well-differentiated liposarcoma and dermatofibrosarcoma protuberans are low-grade ones. Some sarcomas are not readily gradeable, such as epithelioid sarcoma and clear cell sarcoma. If grading is applied, a two-tiered grading system of low versus high is probably sufficient in a majority of cases.^{2,13,17,44,45} Low-grade lesions exhibit mild nuclear atypia, minimal or absent necrosis, low cellularity with minimal nuclear overlap, and rare or absent mitoses (less than 3 per 10 high-power fields [hpfs]). High-grade lesions show moderate to marked nuclear atypia, intermediate to high cellularity with conspicuous to prominent nuclear overlap, definite necrosis, and frequent mitoses.^{17,45}

The presence or absence of mitoses and necrosis is an objective finding worthy of separate mention in the report. A mitotic count based on field unit is rarely possible on cytologic preparations. A statement such as “mitoses are absent” or “mitoses are numerous” suffices to avoid falsely high or low counts.^{17,44}



Reporting soft tissue fine-needle aspiration results

- general category
- histologic subtype or description with differential diagnosis
- grade
 - mitoses
 - necrosis
- ancillary study results
- notes and recommendations

Adipocytic and Lipogenic Neoplasms

Neoplasms with lipogenic differentiation occur over a broad age range, but malignant tumors occur almost exclusively in adults. Lesions with well-developed adipocytic morphology are difficult to classify accurately.⁴⁶ Some authors discourage the use of FNA for large, deep-seated, intramuscular or retroperitoneal lesions that radiographically appear predominantly fatty.²³ The diagnosis of a well-differentiated liposarcoma can be missed if the (focal) diagnostic areas are not sampled, and this has led to the suggestion that potentially lipogenic lesions need to be excised and thoroughly sampled for definitive diagnosis. This approach does not take into consideration the newer therapeutic options (tumors pretreated before surgery) or the value of ancillary studies (e.g., cytogenetics), which are useful in the classification of most fatty lesions (see [Table 17.1](#)).^{14,21-23,47}

Lipoma

Benign lipomas, occurring mainly in adults older than 30 years of age, are very common and account for about half of all soft tissue tumors. Most are slowly growing, subcutaneous or intramuscular tumors that occur over a wide anatomic distribution but spare the hands and feet; rarely exceed 10 cm in size; and manifest as soft, painless lumps. Retroperitoneal lipomas are exceedingly rare and usually need molecular/genetic confirmation. Benign-appearing adipose tissue in a retroperitoneal mass aspirate should evoke the differential diagnosis of well-differentiated liposarcoma, myelolipoma, angiomyolipoma, and retroperitoneal fibrosis.



Cytomorphology of lipoma

- small tissue fragments
- large, univacuolate adipocytes of uniform size
- small, bland nuclei without atypia

Smears are composed of small tissue fragments; isolated cells are rarely present. Larger fragments contain thin capillaries, and striated or atrophic muscle

fibers can be seen in juxta- or intramuscular tumors. The large, univacuolate adipocytes are of uniform size and shape ([Fig. 17.1](#)). Their nuclei are small, round, and regular, with evenly distributed chromatin.^{29,48} Scattered foamy macrophages with ingested lipid (called “lipophages”) are common in tumors with regressive changes ([Fig. 17.2](#)). Occasionally, myxoid stroma and metaplastic bone are seen.

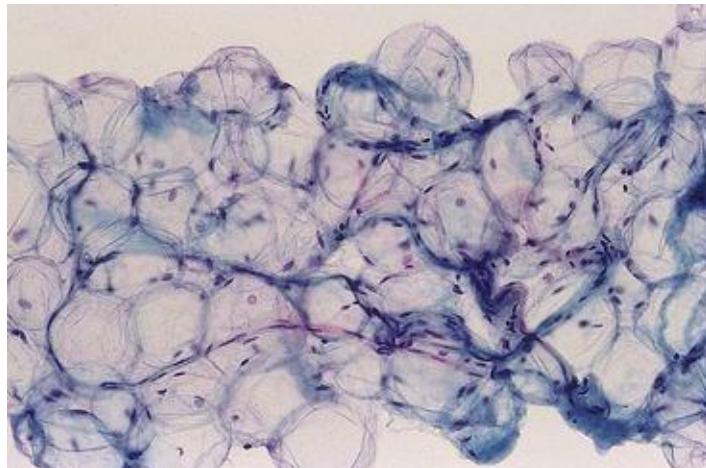


FIGURE 17.1 Lipoma.

Benign lipomas show occasional capillaries, but they are not as numerous as in hibernomas (Papanicolaou stain).

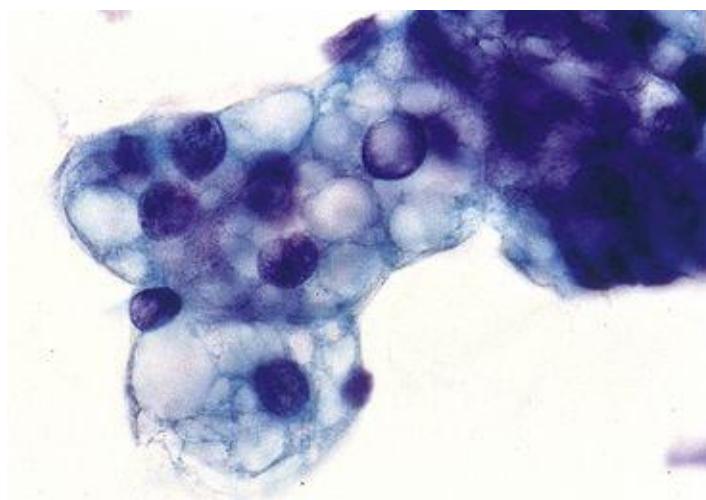


FIGURE 17.2 Fat necrosis.

Multivacuolated histiocytes accompanying areas of fat necrosis or regressive change lack the

more distinct, larger cytoplasmic vacuoles, well-demarcated cytoplasmic membranes, and scalloped nuclei of true lipoblasts (Papanicolaou stain).



Differential diagnosis of lipoma

- subcutaneous adipose tissue
- pleomorphic lipoma
- well-differentiated liposarcoma

Normal subcutaneous adipose tissue lacks the masslike clinical presentation of a lipoma, thus the distinction between the two is most confidently achieved when the cytopathologist performs the aspiration, especially under ultrasound guidance. Atrophic muscle fibers in some lipoma aspirates can mimic the floret cells of a pleomorphic lipoma, but atypical mononucleated stromal cells are absent. Lipomas lack the variable cell size and atypical stromal cells of well-differentiated liposarcoma.^{21,29,48-50}

Hibernoma

The hibernoma is a rare benign tumor of brown fat that occurs predominantly in adults aged 20 to 50 years. The most common site is the thigh, followed by the trunk/chest, upper extremity, and head and neck. Although most often subcutaneous, it can be intramuscular and large (greater than 10 cm), mimicking an atypical lipomatous tumor radiologically.⁵¹ It can also occur in the intraabdominal cavity, mediastinum, and retroperitoneum.



Cytomorphology of hibernoma

- large tissue fragments containing many delicate capillaries
- numerous hibernoma cells with multiple small cytoplasmic vacuoles
- variable cytoplasm ranging from granular to microvesicular to containing larger fat vacuoles
- small, bland nuclei

Hibernomas yield moderately cellular aspirates of fatty tissue fragments

containing many delicate capillaries and numerous hibernoma (brown fat) cells of variable size. These round to polygonal cells have finely multivacuolate to granular cytoplasm and small, bland nuclei. Some large cells with two or more larger fat vacuoles indenting the small nucleus may resemble lipoblasts ([Fig. 17.3](#)). Regular fat, myxoid stroma, and bland spindle cells may also be present.

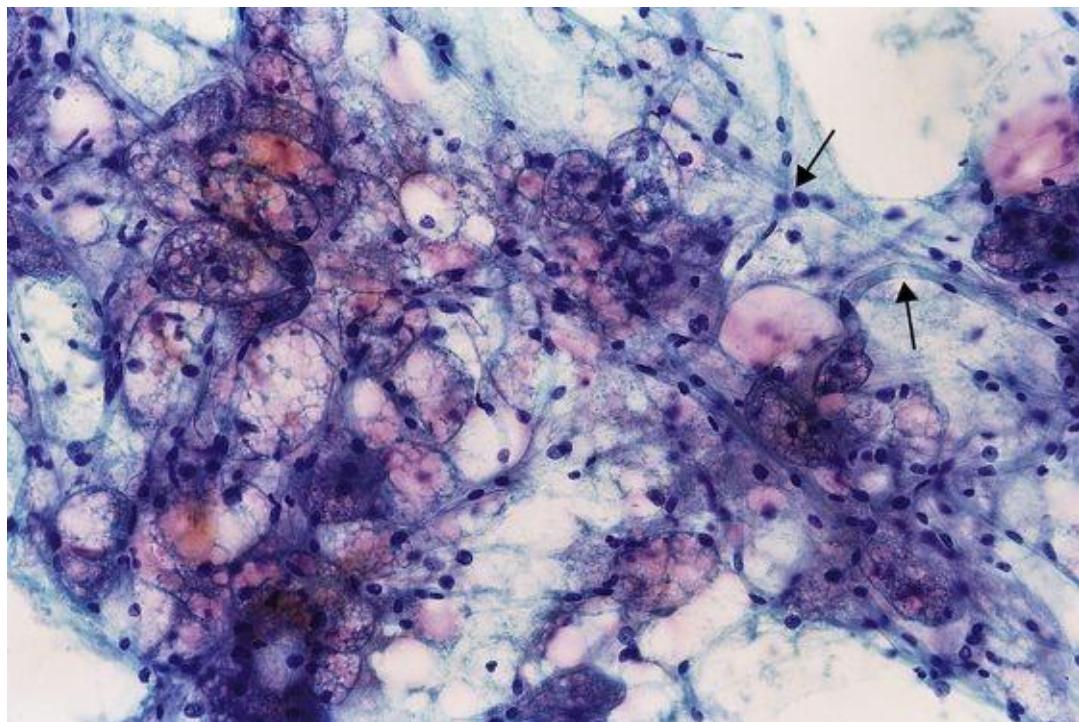


FIGURE 17.3 Hibernoma.

Delicate capillaries (arrows) can be numerous and are closely associated with tumor cells that have distinct granular and multivacuolate cytoplasm. (Papanicolaou stain).



Differential diagnosis of hibernoma

- well-differentiated liposarcoma and atypical lipomatous tumor
- myxoid liposarcoma
- spindle cell/pleomorphic lipoma
- chondroid lipoma
- lipoma with fat necrosis
- granular cell tumor
- adult rhabdomyoma
- pilosebaceous units

Although lipoblast-like cells are seen in a hibernoma, the atypical lipoblasts (see [Fig. 17.7](#)) characteristic of well-differentiated liposarcoma and atypical lipomatous tumor are not present. Capillary vessels can be numerous in hibernomas, but they are less prominent than in a myxoid liposarcoma. Spindle cell lipomas lack the characteristic microvacuolate hibernoma cells. The myxoid stroma and chondromyxoid matrix of a chondroid lipoma are not prominent in a hibernoma. Lipophages in a lipoma generally have a larger, plumper nucleus, and their fat vacuoles are more variable in size (see [Fig. 17.2](#)). Granular cell tumors lack adipocytic differentiation, and rhabdomyomas have larger cells with dense, granular cytoplasm.^{49,51,52} Normal pilosebaceous units can contaminate any FNA that traverses hair-bearing skin. They are recognized by the admixture of finely vacuolated sebaceous cells with ductlike epithelial cells that have nonvacuolated cytoplasm.

Spindle Cell Lipoma and Pleomorphic Lipoma

Spindle cell and pleomorphic lipomas are benign lipoma variants with overlapping clinical, morphologic, and cytogenetic features; they likely represent a single entity with variable morphology. Both are solitary, painless, well-circumscribed, and slowly growing lesions that often arise in the subcutis or dermis of the upper back, shoulder, neck or anterior head and neck regions in middle-aged to older men. They rarely exceed 5 cm in greatest dimension. Recent studies have shown that spindle cell lipoma is related to cellular angiofibroma and mammary-type myofibroblastoma, with overlapping morphology and an association with deletions involving 13q.⁵³



Cytomorphology of spindle cell and pleomorphic lipoma

- fragments of mature adipose tissue
- often myxoid background
- occasional multivacuolate lipoblast-like cells
 - Spindle cell lipoma
 - bland and uniform spindle cells
 - ropy collagen fibers
 - mast cells
- Pleomorphic lipoma
 - “floret” cells
 - smudged chromatin

The spindle cell lipoma is characterized by a mixture of mature adipocytes and bland spindle cells in short fascicles. Hyaline, ropy collagen fibers and mast cells are typically seen (Fig. 17.4).⁵⁴ The pleomorphic lipoma shows predominantly mature adipocytes admixed with pleomorphic large, atypical stromal cells that have multiple hyperchromatic, floret-type nuclei (Fig. 17.5).^{29,55} Hybrids of spindle cell and pleomorphic lipomas are quite common.

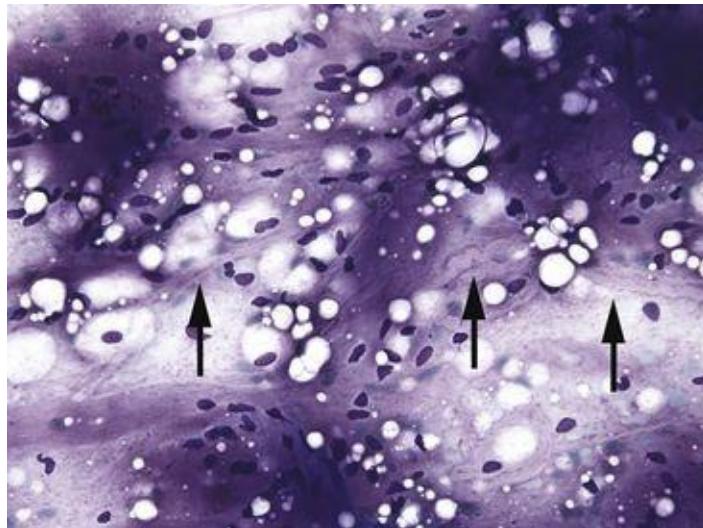


FIGURE 17.4 Spindle cell lipoma.

This tumor is characterized by a mixture of dispersed bland spindle cells, mature adipocytes, and long, ropy collagen fibers (*arrows*) in a myxoid background (Romanowsky stain).

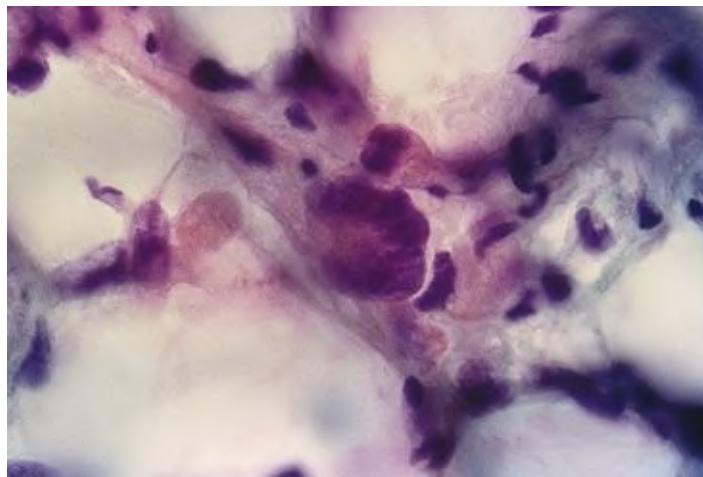


FIGURE 17.5 “Floret” cell.

Multinucleated giant cells with nuclei in a wreathlike configuration are a characteristic but nonspecific finding in pleomorphic lipomas (Papanicolaou stain).



Differential diagnosis of spindle cell and pleomorphic lipoma

- well-differentiated liposarcoma and atypical lipomatous tumor
- schwannoma
- myxoid liposarcoma
- myxofibrosarcoma, low grade
- solitary fibrous tumor
- dermatofibrosarcoma protuberans

A myxoid background and multivacuolate lipoblast-like cells are well-recognized diagnostic pitfalls associated with both variants, particularly the pleomorphic lipoma, that can lead to an erroneous diagnosis of a myxoid or well-differentiated liposarcoma or other myxoid or spindle cell neoplasm.^{49,55} This is particularly likely in cases that lack the classic floret cells and/or mature adipose tissue component. Spindle cell and pleomorphic lipomas are distinguished from a liposarcoma by their typical anatomic distribution and superficial location, along with supportive immunohistochemistry (CD34 positivity in spindle cells) and cytogenetic findings (chromosomal aberrations of 13q and 16q) (see [Table 17.1](#)). The positivity for CD34 and lack of S-100 protein in the spindle cells also help to distinguish it from a schwannoma. Myxoid liposarcoma and myxofibrosarcoma have more prominent blood vessels and lack mature adipose tissue and collagen fibers. With a spindle cell–predominant variant, the distinction from a dermatofibrosarcoma protuberans (DFSP) and a solitary fibrous tumor, especially the fat-forming variant, can be difficult, because both mimics can have mature adipose tissue and CD34-positive spindle cells. Clinical correlation and cytogenetic findings are essential in difficult cases.^{29,54,56}

Well-Differentiated Liposarcoma and Atypical Lipomatous Tumor

Liposarcoma is the most common soft tissue sarcoma of adults. It has three major forms that are biologically distinct: (1) well-differentiated and dedifferentiated; (2) myxoid; and (3) pleomorphic. The terms *well-differentiated liposarcoma* and *atypical lipomatous tumor* are essentially synonymous. Well-

differentiated liposarcoma applies to deep-seated lesions in the retroperitoneum, spermatic cord, and mediastinum, where recurrence and local aggressiveness are common because a resection with clear margins is difficult to achieve at these locations; atypical lipomatous tumor applies to lesions of the limbs and trunk that do not recur if adequately excised.



Cytomorphology of well-differentiated liposarcoma and atypical lipomatous tumor

- clusters of lipogenic cells with lipid vacuoles of varying size
- atypical stromal cell nuclei
- lipoblasts
- occasional floret cells
- nonlipogenic pleomorphic or spindle-cell tissue fragments suggestive of dedifferentiation

In general, tissue fragments of large but variably sized, univacuolate adipocytes ([Fig. 17.6](#)) are admixed with blood and extruded lipid. Smaller multivacuolate cells with atypical, scalloped nuclei (lipoblasts) are present in some cases ([Fig. 17.7](#)). Lipoblasts are not necessary for the diagnosis of liposarcoma, with the exception of pleomorphic liposarcoma. The nuclei of larger adipocytes range from small and compressed to large, round to oval, and hyperchromatic ([Fig. 17.8](#)). They are often multilobated or convoluted. Similar atypical nuclei are seen in fragments of collagenous stromal tissue.^{[46,48,57,58](#)}

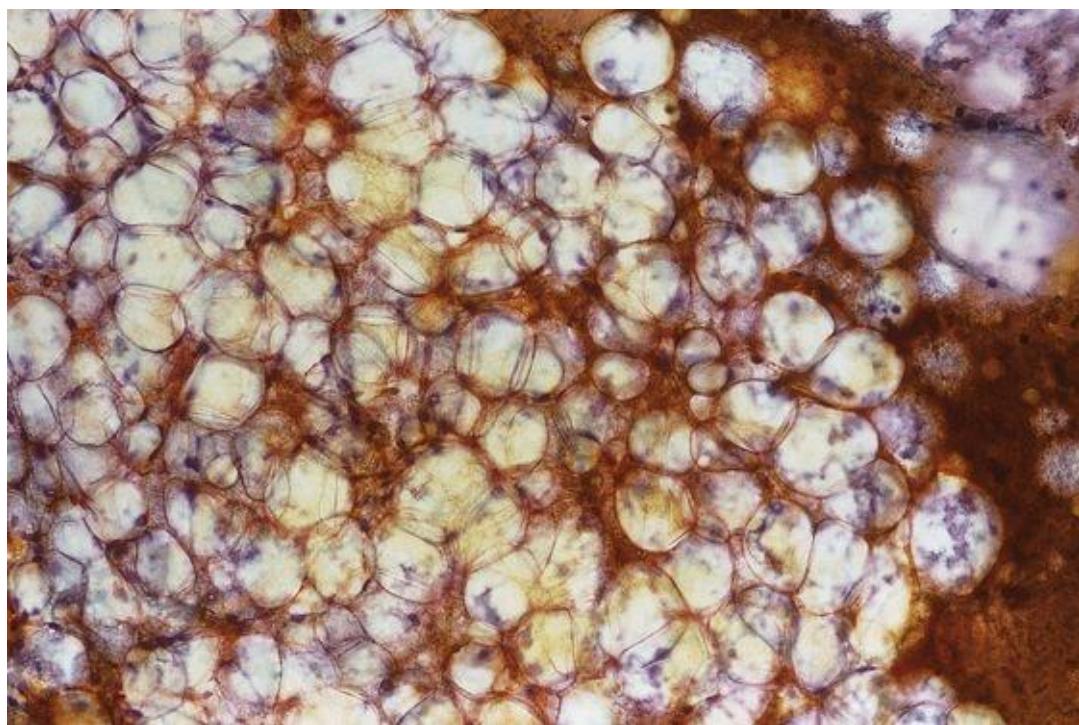


FIGURE 17.6 Well-differentiated liposarcoma.

The much-touted variation in adipocyte size can be subtle and easily overlooked (compare with [Fig. 17.8](#)) (Papanicolaou stain).

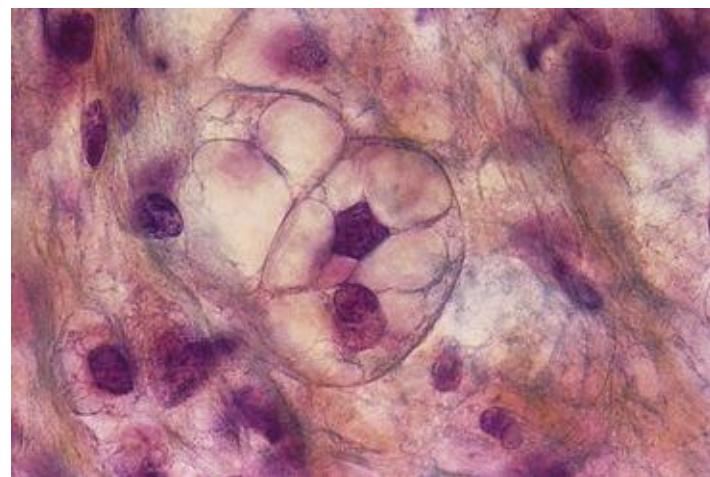


FIGURE 17.7 Lipoblast.

The hyperchromatic nuclei of lipoblasts are scalloped owing to multiple impinging cytoplasmic lipid droplets (Papanicolaou stain).

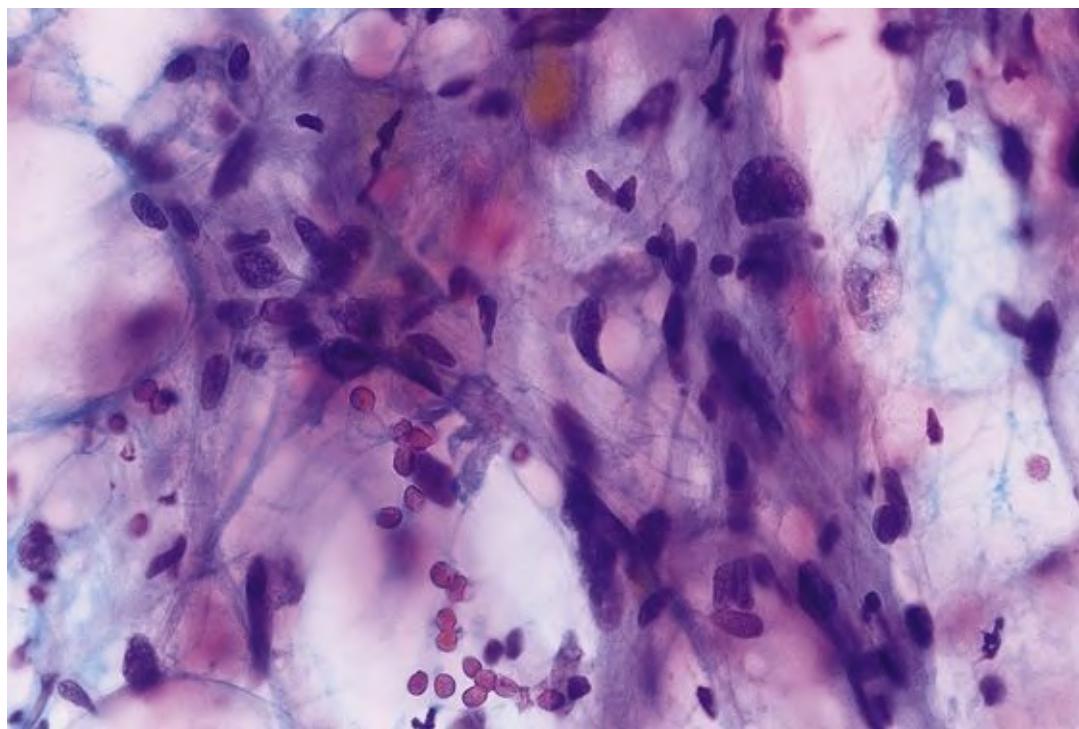


FIGURE 17.8 Well-differentiated liposarcoma.

Atypical adipocyte nuclei in deep-seated lesions denote malignancy even when classic lipoblasts are not readily identified (Papanicolaou stain).



Differential diagnosis of well-differentiated liposarcoma and atypical lipomatous tumor

- lipoma with fat necrosis (see [Fig. 17.2](#))
- extruded lipid from normal fat
- spindle cell and pleomorphic lipomas
- hibernoma

Extensive sampling of a large liposarcoma is necessary because diagnostic areas can be extremely focal.^{29,48,50,55} The first impression might be that of a lipoma, but the clinical presentation of lipomas and liposarcomas is generally so different that this rarely poses a significant dilemma. Nevertheless, careful examination of the specimen is important in order to identify the sometimes subtle nuclear and cellular alterations of a well-differentiated liposarcoma (see [Fig. 17.6](#)). Benign lipid droplets entrapped in blood are a common finding whenever any fatty tissue, including normal adipose tissue, is sampled. Its nonspecific nature is revealed by the absence of nuclei ([Fig. 17.9](#)). Fat necrosis is comprised of lipophages (see [Fig. 17.2](#)), whose cytoplasm is foamy and filled with small

vacuoles, whereas the cytoplasm of adipocytes and lipoblasts is optically clear. Some of the multilobated hyperchromatic cells of a liposarcoma can resemble the floret cells of a pleomorphic lipoma; clinical and radiographic correlation is the key to this distinction. Giant marker and ring chromosomes derived from 12q13-15 are characteristic of well-differentiated liposarcoma (see [Table 17.1](#)) and help to differentiate it from its benign mimics like hibernoma and spindle cell or pleomorphic lipoma. The latter have their own characteristic cytogenetic alterations. With lipomatous tumors, however, one rarely gets enough material from an FNA for a conventional karyotype. They are more amenable to molecular diagnostic or FISH analysis. Overexpression of MDM2 and CDK4 from amplification of the *MDM2* and *CDK4* genes on chromosome 12q13-15, a characteristic genetic abnormality in atypical lipomatous tumor, well-differentiated liposarcoma, and dedifferentiated liposarcoma, can be detected by immunohistochemistry, which can be diagnostically helpful.⁵⁹

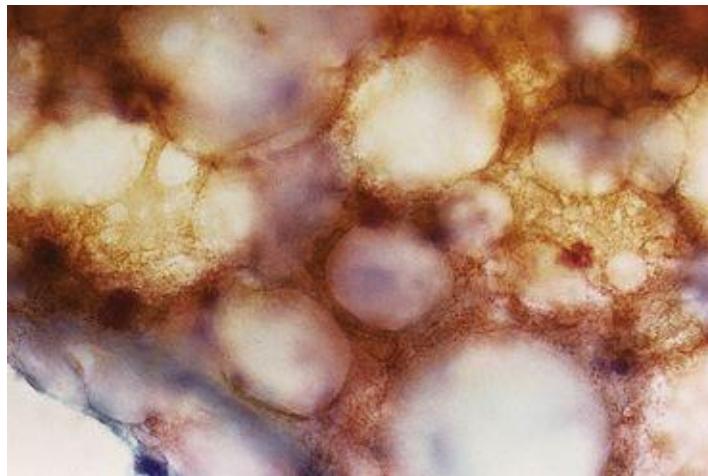


FIGURE 17.9 Extruded lipid.

A high clinical suspicion for liposarcoma might lead to an overinterpretation of extruded lipid from normal fat at the time of rapid onsite evaluation (Papanicolaou stain).

A rare variant of well-differentiated liposarcoma, the *inflammatory liposarcoma*, yields moderately cellular smears with abundant small lymphocytes, larger lymphoid cells, and plasma cells. The scattered large, atypical cells with multiple or multilobate nuclei of this variant evoke the unusual differential diagnosis of Hodgkin lymphoma, anaplastic large cell lymphoma, inflammatory myofibroblastic tumor (IMT), and metastatic carcinoma.⁶⁰

Pleomorphic Liposarcoma

Pleomorphic liposarcoma is a rare malignancy, accounting for about 5% of liposarcomas. It commonly occurs in the extremities of elderly adults and carries a bleak prognosis. It is distinguished from other high-grade pleomorphic sarcomas by the presence of multivacuolate, highly atypical lipoblasts.



Cytomorphology of pleomorphic liposarcoma

- hypercellular smear
- dispersed cells and three-dimensional clusters
- pleomorphic cells with marked nuclear atypia
- variable number of atypical lipoblasts
- mitoses and necrosis

Smears are moderately to highly cellular, with dispersed, markedly anaplastic and pleomorphic cells and three-dimensional tumor cell clusters. The cells range in contour from polygonal to spindle-shaped. Nuclei are bizarre and occasionally scalloped, with coarse chromatin and prominent nucleoli. The abundant cytoplasm varies from homogeneous to multivacuolate, with vacuoles of varying sizes ([Fig. 17.10](#)). Mitoses and necrosis are usually present.^{58,61,62} Extensive sampling to identify unequivocal atypical lipoblasts is necessary to separate pleomorphic liposarcoma from a pleomorphic sarcoma of another lineage. Immunohistochemistry, though helping to exclude some cytologic mimics such as poorly differentiated signet ring cell adenocarcinoma, has a very limited role in establishing the diagnosis of pleomorphic liposarcoma. Cytogenetically, pleomorphic liposarcoma exhibits increased chromosome numbers with complex rearrangements, a profile, unfortunately, of no diagnostic value.^{29,58,62}

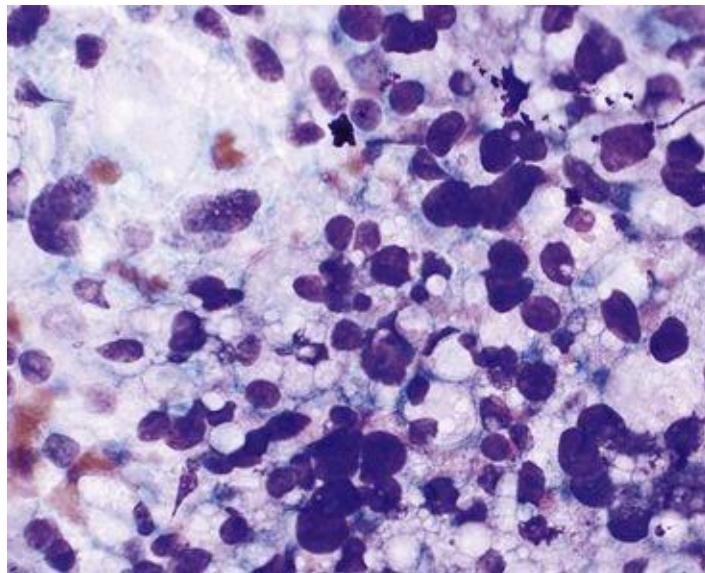


FIGURE 17.10 Pleomorphic liposarcoma.

Clusters of pleomorphic cells, including numerous atypical lipoblasts, are seen (Romanowsky stain).

Myxoid Neoplasms

The diverse and mostly unrelated myxoid soft tissue lesions share one (often striking) morphologic feature: abundant, homogeneous, myxoid matrix. In most instances, it appears as an amorphous, granular film that stains pale blue-green with the Papanicolaou stain ([Fig. 17.11](#)) and a striking blue-violet with Romanowsky stains. A myxoid picture is produced by a very broad spectrum of lesions that includes nodular fasciitis, benign myxoid tumors, and myxoid sarcomas. Clinically, most benign lesions are superficially located except for intramuscular myxoma; whereas sarcomas are deep-seated with the exception of myxofibrosarcoma. Morphologically, benign lesions generally have lower cellularity and less nuclear atypia than malignant neoplasms, but exceptions do occur. Limited sampling may yield hypocellular smears with abundant matrix and result in an erroneous low-grade assignment. Despite the challenges, myxoid lesions, particularly the low-grade ones, can often be subtyped based on cytomorphology alone with accuracy rates up to 81%.⁶³ Cytogenetic analysis is helpful in resolving the differential diagnosis of some of the malignant lesions, such as myxoid liposarcoma, low-grade fibromyxoid sarcoma (LGFMS), and extraskeletal myxoid chondrosarcoma (EMC) (see [Table 17.1](#)).⁹

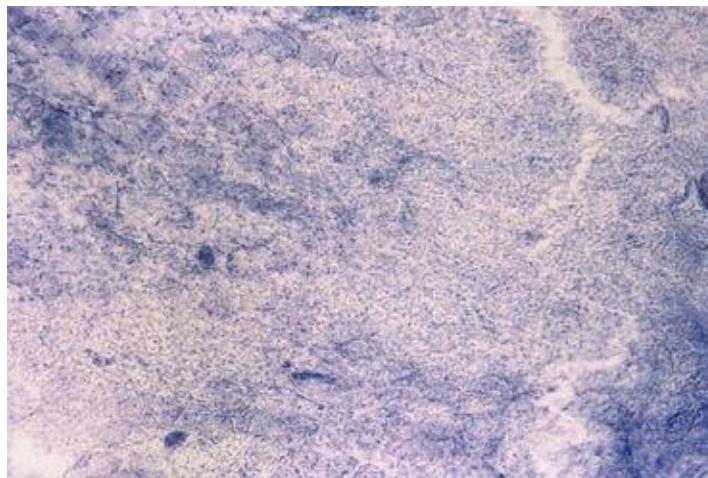


FIGURE 17.11 Myxoid matrix (ganglion cyst).

The copious matrix material of a ganglion cyst has a granular appearance common to most myxoid soft tissue lesions (Papanicolaou stain).

Intramuscular Myxoma

Intramuscular myxoma is a benign, painless, slowly growing, well-circumscribed but unencapsulated neoplasm of adults aged 40 to 70 years, usually involving the muscles of the thigh, shoulder, buttocks, and upper arm. A *cellular myxoma* is a morphologic variant that has increased cellularity and a vascular component but lacks the nuclear atypia and hyperchromasia of low-grade myxofibrosarcoma. It carries a slight risk of local recurrence. The *juxta-articular myxoma* is cytologically similar to a cellular myxoma but occurs adjacent to large joints, especially the knee.



Cytomorphology of intramuscular myxoma

- hypocellular
- a film of granular matrix material
- absent or very scant vascular component
- uniform cells with a bland nucleus
- long, fibrillary cytoplasmic processes
- multinucleated atrophic muscle fibers
- macrophages with vacuolated cytoplasm

FNA specimens from intramuscular myxomas are hypocellular, with abundant, granular myxoid matrix. Rare fibroblast-like cells, isolated and in loose clusters, are uniform in size and display a variety of shapes: round to oval, spindle-shaped, stellate, and polygonal. Their small, round to oval nuclei lack atypia and have fine, uniform chromatin with small nucleoli. The cytoplasm is finely granular with long, fibrillary cytoplasmic processes and occasional small vacuoles. Macrophages and mast cells can be present. The vascular component, if present, consists of very rare short vessels ([Fig. 17.12](#)). Often, multinucleated skeletal muscle fibers, sometimes atrophic, are strewn about. Mitoses are virtually never observed.⁶⁴⁻⁶⁶

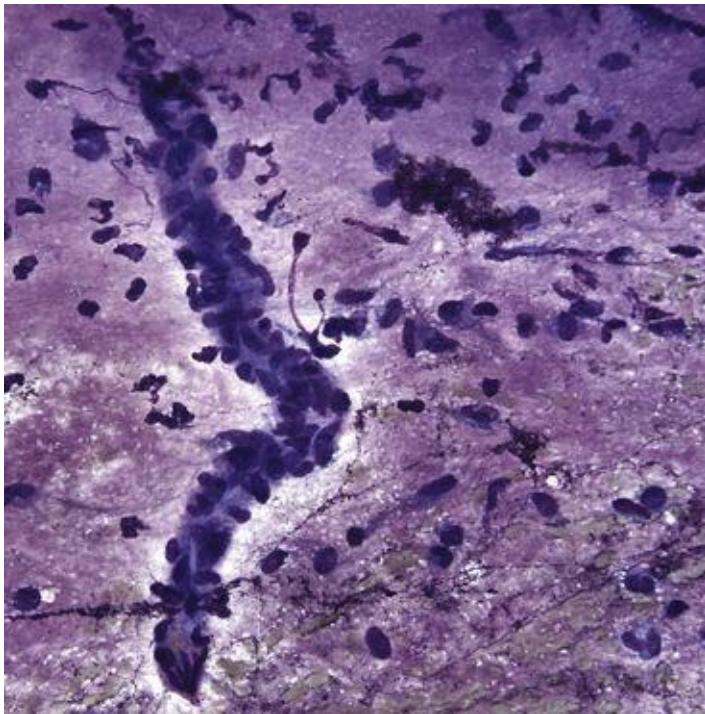


FIGURE 17.12 Intramuscular myxoma.

Loose aggregates of fibroblast-like cells appear to swim through a sea of myxoid ground substance. Stubby vessels can be seen, especially in the cellular myxoma (Romanowsky stain).



Differential diagnosis of intramuscular myxoma

- ganglion cyst
- nodular fasciitis
- soft tissue perineurioma with prominent myxoid stroma
- low-grade myxofibrosarcoma
- low-grade fibromyxoid sarcoma
- myxoid liposarcoma
- extraskeletal myxoid chondrosarcoma

Aspirates from ganglion cysts contain only abundant granular myxoid material and occasional macrophages; the spindle-shaped, stellate, or polygonal myxoma cells are absent. Nodular fasciitis shows a more haphazard cellular arrangement of polymorphic myofibroblasts and ganglion-like cells, often with interspersed inflammatory cells. Soft tissue perineurioma has more slender perineural cells ([Fig. 17.13](#)) and characteristically expresses EMA and claudin-1. Both myxofibrosarcoma and myxoid liposarcoma have a more prominent

vascular component than the typical intramuscular myxoma. Although low-grade myxofibrosarcoma and low-grade fibromyxoid sarcoma have notable nuclear atypia, their distinction from cellular myxoma can be difficult. Adequate sampling from different parts of the mass is essential to avoid missing a low grade fibromyxoid sarcoma, which has alternating areas of myxoid and collagenous stroma. The stroma in extraskeletal myxoid chondrosarcoma is more brightly magenta and fibrillar rather than blue-purple and granular as seen in a myxoma, and the lacelike arrangement of tumor cells is more uniform than that of myxoma cells (see [Fig. 17.20A](#)).

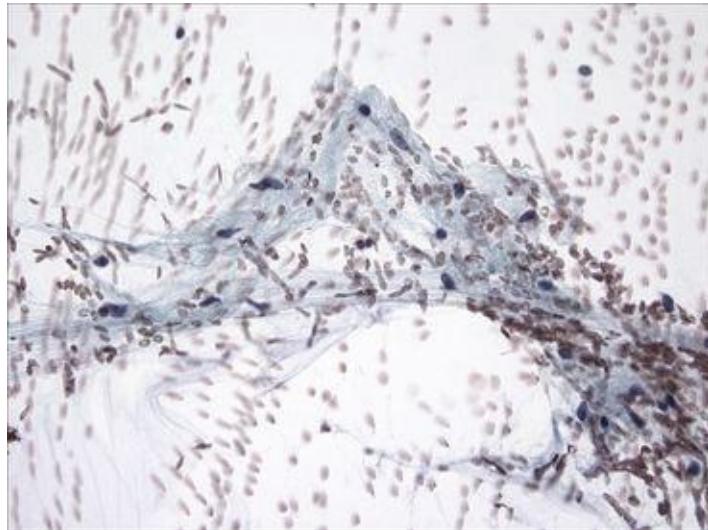


FIGURE 17.13 Soft tissue perineurioma.

Loose aggregates of bland spindle cells with long, thin cytoplasmic processes in a myxoid stroma are a typical finding (Papanicolaou stain).

Soft Tissue Perineurioma

Soft tissue perineurioma is a benign peripheral nerve sheath neoplasm composed of perineurial cells. It usually occurs in the subcutaneous tissue in the limbs of middle-aged adults. The distinctive histologic growth patterns, such as storiform, perivascular whorled, lamellar, or reticular, are imperceptible in FNA smears. Nevertheless, the presence of slender cells with bipolar, long, thin cytoplasmic processes, characteristic of perineurial cells, is a reproducible finding in smears.^{67,68} Perineurial cells are positive for EMA, claudin-1, and CD34 (in two thirds of cases) and negative for S-100 protein, a useful immunoprofile to support the diagnosis.



Cytomorphology of soft tissue perineurioma

- variable cellularity
- myxoid or collagenous stroma
- absent or very scant vascular component
- slender cells with a bland nucleus and bipolar, thin, long cytoplasmic processes

The aspirate cellularity is variable, as is the type of stroma. Prominent myxoid stroma is common, leading to the impression of a “low-grade myxoid spindle cell neoplasm.” The typical slender cells are either isolated or arranged in loose clusters (see [Fig. 17.13](#)). Stripped nuclei and strings of cytoplasmic processes are common. Nuclei are bland, with finely granular, evenly dispersed chromatin.



Differential diagnosis of soft tissue perineurioma

- dermatofibrosarcoma protuberans (DFSP)
- intramuscular myxoma, cellular type
- neurofibroma and schwannoma
- low-grade fibromyxoid sarcoma (LGFMS)
- low-grade myxofibrosarcoma
- low-grade malignant peripheral nerve sheath tumor

For superficial lesions, the main differential diagnosis is DFSP, which can be distinguished from soft tissue perineurioma by the absence of expression of EMA and claudin-1. Cellular myxoma, LGFMS, and perineurioma with prominent myxoid stroma can be indistinguishable from each other in small biopsies without the aid of ancillary studies. Other benign peripheral nerve sheath tumors like neurofibroma and schwannoma show more diffuse immunoreactivity for S100 protein, although hybrid tumors with both perineurioma and schwannoma components exist.⁶⁹ The tumor cells in low-grade sarcomas have more cytologic atypia and lack the long, thin cytoplasmic processes characteristic of perineurioma.

Myxofibrosarcoma, Low-and High-Grade

Myxofibrosarcoma, also previously known as *myxoid malignant fibrous histiocytoma* (MFH), is one of the most common sarcomas. It typically occurs in the extremities of elderly patients, principally during the sixth to eighth decades of life. Usually dermal or subcutaneous, it involves deeper soft tissues in one third to one half of cases. Up to 60% of cases recur locally regardless of grade, and they tend to become progressively higher-grade with each recurrence. Only higher-grade lesions have the potential to metastasize to lungs, bones, and lymph nodes.



Cytomorphology of myxofibrosarcoma

- variable amounts of myxoid matrix
- short segments of curved, collagenized vessels
- mildly atypical spindle and stellate cells (low-grade tumors)
- marked nuclear pleomorphism, multinucleation, and necrosis (high-grade tumors)
- pseudolipoblasts

Myxofibrosarcomas are morphologically quite variable, ranging from low-to high-grade tumors. In general, the amount of myxoid material is inversely proportional to cellularity and grade. The curvilinear vessels that are characteristic of these tumors in histologic sections are absent from smears in many cases, but when present they appear as short segments of collagenized capillaries best seen in paucicellular areas ([Fig. 17.14A](#)). The neoplastic cells are randomly distributed and noncohesive. Occasional small aggregates occur, and higher-grade lesions contain large cell groups and tissue fragments. A majority of cells in a low-grade tumor are spindle-shaped, with occasional tadpole, stellate, and polygonal forms. Nuclear pleomorphism and atypia increase with grade. In higher-grade lesions, binucleation and multinucleation are more prominent. Irrespective of grade, tumor cells with vacuolated cytoplasm, resembling lipoblasts but containing acid mucins rather than lipid, are found ([Fig. 17.14B](#)).^{23,63,64,70,71} Mitotic activity is uncommon.

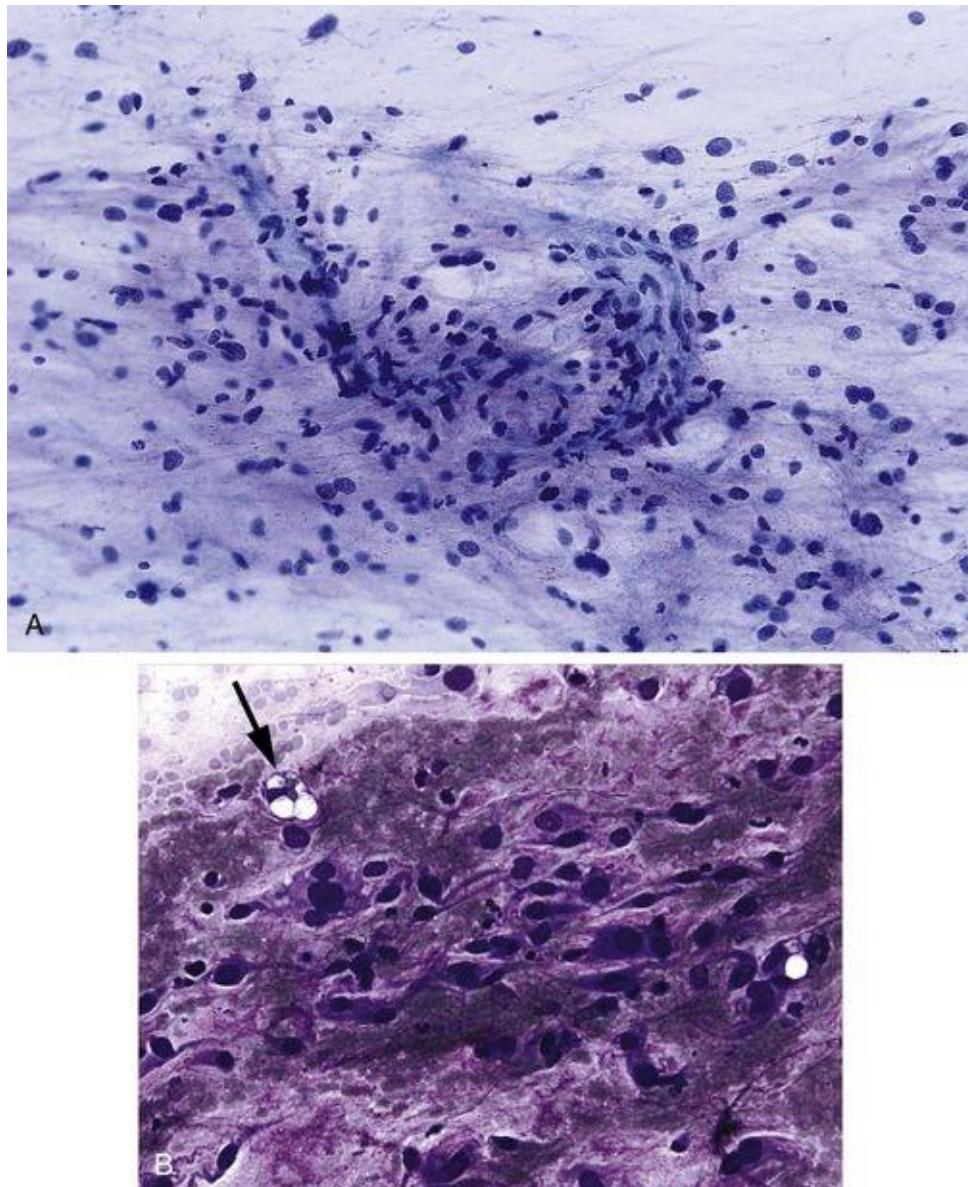


FIGURE 17.14 Myxofibrosarcoma.

A, The vascular component, when present, manifests itself as short segments of thick-walled vessels with adherent matrix and pleomorphic tumor cells (Papanicolaou stain). B, Pseudolipoblasts (arrow) contain acid mucins rather than lipid (Romanowsky stain).



Differential diagnosis of myxofibrosarcoma

- cellular myxoma
- nodular fasciitis
- myxoid liposarcoma
- low-grade fibromyxoid sarcoma (LGFMS)
- dedifferentiated liposarcoma
- other high-grade sarcomas with a clear line of differentiation

Cellular myxoma and nodular fasciitis lack the curvilinear vessels and nuclear atypia of myxofibrosarcoma. Myxoid liposarcoma contains delicate branching vessels and true lipoblasts and usually does not have much nuclear atypia or pleomorphism. The distinction between low-grade myxofibrosarcoma and the similarly named LGFMS is difficult but possible, especially in conjunction with clinical and cytogenetic findings. Other high-grade sarcomas with myxoid changes but with a clear line of differentiation, like dedifferentiated liposarcoma, should be excluded in a deep-seated tumor.

Low-Grade Fibromyxoid Sarcoma

LGFMS, first described by Evans, is clinically, morphologically, and cytogenetically different from a low-grade myxofibrosarcoma.²² It affects mainly younger adults in the third to fifth decades and arises in the deep soft tissue of the thigh or trunk. Despite its deceptively bland morphology, up to 45% of cases eventually metastasize after a long indolent course.⁴⁹



Cytomorphology of low-grade fibromyxoid sarcoma

- abundant myxoid matrix
- uniform fibroblast-like spindle cells with mild nuclear atypia
- no significant vascularity or nuclear pleomorphism

Histologically, LGFMS exhibits a characteristic whorled arrangement of bland spindle cells with alternating areas of myxoid and collagenous stroma, but the cytologic findings, described in only a few case reports and small series,²³⁻²⁶ are somewhat nonspecific. Often the myxoid, hypocellular areas are sampled, yielding a homogeneous population of fibroblast-like spindle cells with slightly plump but bland, uniform nuclei in a myxoid background. Vascularity is variable depending on the area sampled ([Fig. 17.15](#)).

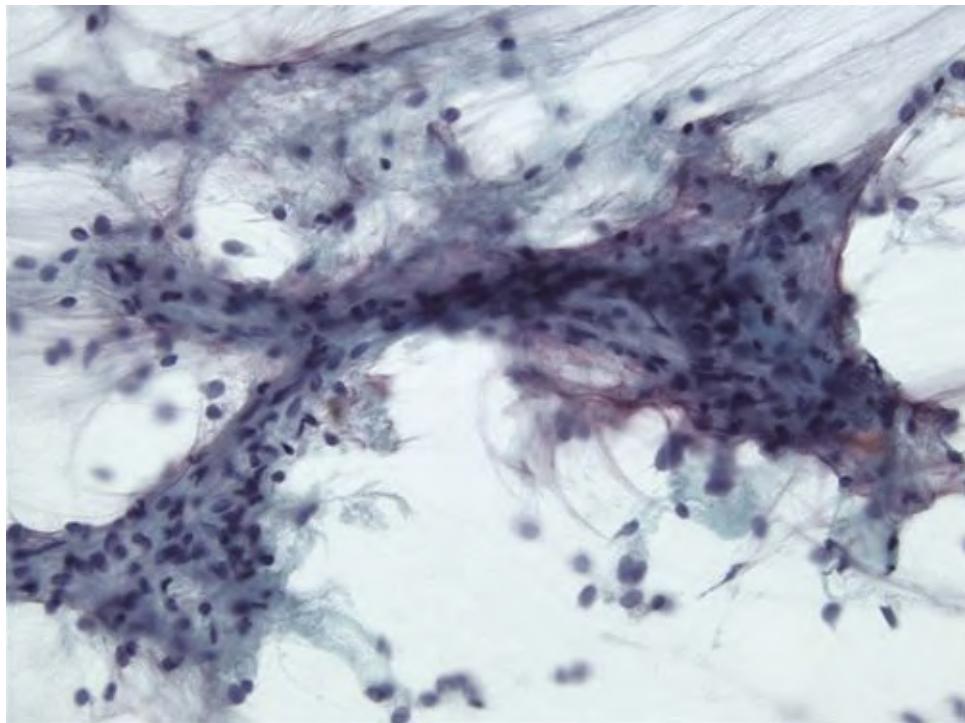


FIGURE 17.15 Low-grade fibromyxoid sarcoma (LGFMS). Uniformly bland fibroblasts are embedded in a myxoid matrix (Papanicolaou stain).

➊ Differential diagnosis of low-grade fibromyxoid sarcoma

- cellular myxoma
- soft tissue perineurioma
- low-grade myxofibrosarcoma
- low-grade malignant peripheral nerve sheath tumor (MPNST)
- extraskeletal myxoid chondrosarcoma (EMC)
- solitary fibrous tumor (SFT)
- desmoid fibromatosis

A cellular myxoma is usually less cellular and poorly vascularized. Perineurioma cells have longer cytoplasmic processes. Low-grade myxofibrosarcoma and malignant peripheral nerve sheath tumor (MPNST) have more notable nuclear atypia. Clinically, low-grade myxofibrosarcoma tends to occur in the superficial soft tissues of elderly patients, whereas LGFMS is usually situated in the deep soft tissues of young adults. As with LGFMS, EMC

occurs in young to middle-aged adults and involves the deep soft tissue of extremities. Similar to LGFMS cytologically, EMC also yields smears with uniform oval to spindled cells in a myxoid background. Immunohistochemistry can be helpful: MUC4 is a highly sensitive and specific marker of LGFMS.⁷⁸ The chromosomal abnormality t(7;16)(q34;p11), typical of LGFMC, is also a reliable diagnostic aid (see [Table 17.1](#)).^{78,79}

Myxoid Liposarcoma

Myxoid liposarcoma and round cell liposarcoma represent two ends of the morphologic spectrum of a single entity. They share the same reciprocal chromosomal translocations (see [Table 17.1](#)). Pure myxoid liposarcomas form the low-grade end of the spectrum; an increase in the round cell or hypercellular component culminates in the high-grade, pure round cell liposarcoma. They occur in a slightly younger population than the other liposarcomas, primarily in the fourth and fifth decades, and have a predilection for the deep soft tissue of the lower limbs, especially the thigh.



Cytomorphology of myxoid liposarcoma

- abundant granular myxoid matrix in tissue fragments
- delicate, branching, thin-walled capillaries
- round to oval primitive cells with occasional small cytoplasmic vacuoles
- univacuolate and bivacuolate lipoblasts (“signet ring cell”–like lipoblasts), especially along capillaries
- increased number of uniform round cells with scant cytoplasm and prominent nucleoli (round cell liposarcoma)

Smears are moderately to markedly cellular, with a strikingly monotonous appearance. Lower-grade myxoid liposarcomas contain tissue fragments ([Fig. 17.16A](#)) with a prominent myxoid stroma, thin-walled vessels, and numerous round to oval tumor cells with scant cytoplasm and indistinct cell borders. Regardless of grade, myxoid and round cell liposarcomas have abundant granular myxoid matrix with arborizing, thin-walled capillaries of uniform caliber ([Fig. 17.16B](#)). The lipoblasts are variable in number from case to case

and usually small, univacuolate or bivacuolate, resembling signet ring cells (signet ring cell–like lipoblasts), and are often present along the capillaries (Fig. 17.16C).

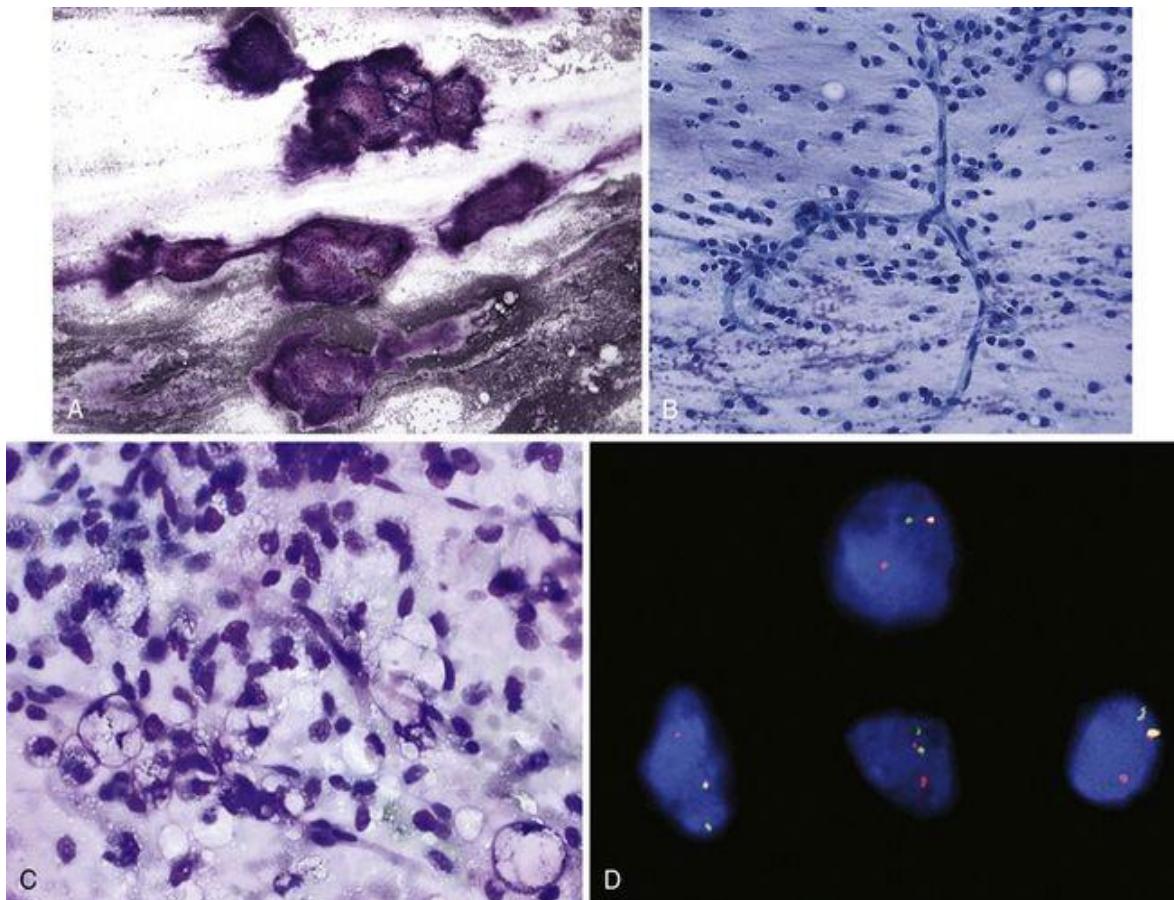


FIGURE 17.16 Myxoid liposarcoma.

A, Discrete tissue fragments contain myxoid matrix material, vessels, and tumor cells. B, Delicate “chicken-wire” capillaries are easily obtained by aspiration and are seen in abundance on smears (Papanicolaou stain). C, The lipoblasts in myxoid liposarcomas resemble signet ring cells and histiocytes and are often associated with the vessels (Romanowsky stain). D, Fluorescence in situ hybridization (FISH) is often performed with centromeric (red) and telomeric (green) probes that flank the *CHOP* (*DDIT3*) gene breakpoint. There is one normal chromosome (probes adjacent = yellow), but the split-apart green and red signals indicate a translocation on the other chromosome in the four tumor cells in this field. (Courtesy Dr. Paola Dal Cin, Brigham and Women's Hospital, Boston, Massachusetts, USA.)

In contrast, higher-grade tumors have a greater component of isolated and clustered cells.^{24,29,48,50,63,64,71} These are substantially larger and rounder, with more anisokaryosis of the centrally placed nuclei with vesicular chromatin and multiple, prominent nucleoli. The cytoplasm is scant, more densely staining, but

rarely vacuolated. The prominent vessels are often obscured by densely packed tumor cells. Owing to its significance, the degree of round cell or hypercellular component should be documented.



Differential diagnosis of myxoid liposarcoma

- low-grade myxofibrosarcoma
- spindle cell lipoma with myxoid changes
- low-grade myxofibrosarcoma
- extraskeletal myxoid chondrosarcoma
- myoepithelioma of soft tissue

The cytomorphology of myxoid liposarcomas is often distinctive and allows for a correct diagnosis. Immunohistochemistry is usually not necessary and of limited value. The detection of the typical chromosomal abnormality $t(12;16)$ (q13;p11) or $t(12;22)(q13;q12)$ by either karyotype or FISH ([Fig. 17.16D](#)) can help distinguish it from other myxoid and round cell tumors (see [Table 17.1](#)).^{9,24,29,48,50,63,64}

Lipoblastoma

Lipoblastoma is a rare, benign tumor affecting mainly boys 3 years of age or younger. It involves the superficial subcutaneous tissues of the limbs, especially the lower extremities, as a slowly growing circumscribed mass. More deeply seated lesions tend toward larger size and a diffusely infiltrative growth pattern (lipoblastomatosis). Local recurrence is uncommon and is primarily restricted to the infiltrative lesions.



Cytomorphology of lipoblastoma

- myxoid matrix with lipid vacuoles
- prominent thin, branching capillaries
- three types of cells: bland lipoblasts, primitive spindle cells, and mature adipocytes

Smears are moderately cellular, with cohesive clusters and sheets of bland lipoblasts and primitive spindle-shaped cells embedded in a myxoid background with fatty vacuoles. Myxoid material can be abundant or sparse and restricted to small to medium-sized tissue fragments with irregular borders. Delicate branching capillaries are conspicuous. Bare nuclei can be widespread, and variable numbers of mature adipocytes are seen, depending on the degree of maturation. The lipoblasts are generally small, round, and uniform in appearance. Chromatin is delicate or moderately coarse, and nucleoli are largely absent.^{48,80,81} Nuclear atypia is limited to focal minimal nuclear membrane irregularities; it is not as prominent as that seen with the lipoblasts of a liposarcoma.

In addition to affecting a much younger-aged population, lipoblastoma is distinguished from its primary morphologic mimic, myxoid liposarcoma, by the absence of nuclear atypia, less abundant myxoid stroma, and the presence of solid sheets of tumor cells. Cytogenetic analysis is helpful in difficult cases (see [Table 17.1](#)).^{48,80,81}

Myxofibrosarcoma-Like Dedifferentiated Liposarcoma

Dedifferentiation, a concept first introduced by Evans in 1979, refers to “tumors containing distinct areas of well-differentiated liposarcoma and cellular nonlipogenic spindle cell or pleomorphic sarcoma.” Dedifferentiation is not limited to liposarcomas.

Dedifferentiated liposarcoma has a variable morphologic picture, but most cases resemble either an undifferentiated pleomorphic (MFH-like) sarcoma or a myxofibrosarcoma. When the typical morphology of a myxofibrosarcoma is encountered in the retroperitoneum, the diagnosis of a dedifferentiated liposarcoma should be given serious consideration.^{82,83}



Cytomorphology of myxofibrosarcoma-like dedifferentiated liposarcoma

- abundant granular myxoid matrix
- thick-walled branching vessels
- spindle cells with mild to moderate nuclear atypia and sometimes, vacuolated cytoplasm
- occasional multinucleated tumor cells

Smears are moderately to highly cellular, with a prominent granular, myxoid background. Dispersed spindled to stellate cells surround loosely cohesive tumor cell clusters arrayed around long vascular channels. The vessels show complex branching similar to the delicate capillaries of myxoid and round cell liposarcoma, but their walls are notably thicker ([Fig. 17.17](#)). Most individual cells have round to oval, hyperchromatic nuclei with evenly distributed chromatin and indistinct nucleoli. The cytoplasm is scant to moderate and sometimes vacuolated. Other cells have more abundant, stellate-shaped cytoplasm, slightly larger and irregular nuclei with smudgy chromatin, and a greater tendency toward binucleation or multinucleation. Rare diagnostic lipoblasts may be seen.⁶¹ The presence of heterologous osteosarcomatous or rhabdomyosarcomatous differentiation can be misleading in small biopsies. Cytogenetic analysis and clinical details are important for the correct diagnosis. As for atypical lipomatous tumor and well-differentiated liposarcoma, demonstration of MDM2 and CDK4 positivity by immunohistochemistry is helpful.

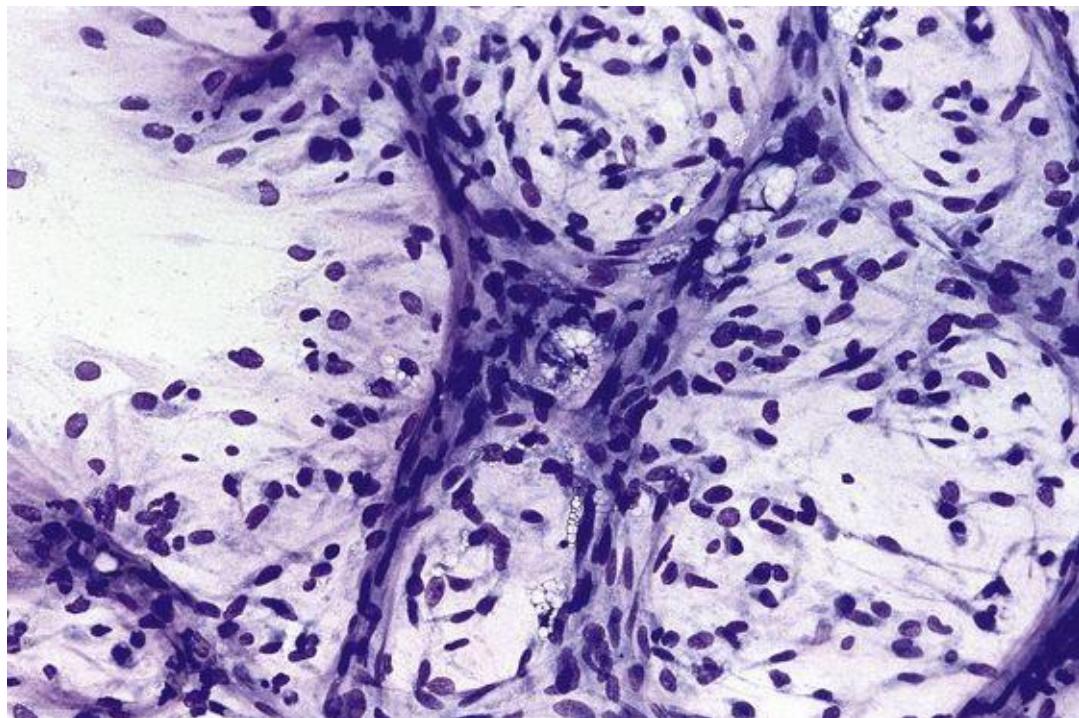


FIGURE 17.17 Myxofibrosarcoma-like dedifferentiated liposarcoma. Noticing the complex, branching, thick-walled vasculature of a dedifferentiated liposarcoma can aid in its distinction from other myxoid lesions (Romanowsky stain).

Myxoinflammatory Fibroblastic Sarcoma

Myxoinflammatory fibroblastic sarcoma (MIFS) is a locally aggressive fibroblastic neoplasm of the distal extremities in middle-aged adults. When first described in 1998, it was called “inflammatory myxohyaline tumor of distal extremities with virocytes or Reed-Sternberg–like cells,” “acral MIFS,” or “inflammatory myxoid tumor of the soft parts with bizarre giant cells.”¹ Although most lesions arise in the subcutaneous tissue of the feet and hands, proximal sites can be affected. The finding of a t(1;10) translocation involving TGFBR3 and MGEA5 in both MIFS and hemosiderotic fibrolipomatous tumor has recently linked these two rare entities to each other.⁸⁴



Cytomorphology of myxoinflammatory fibroblastic sarcoma

- myxoid matrix
- mixed population of bland spindle cells and mononuclear epithelioid cells
- large atypical Reed-Sternberg–like cells with macronucleoli
- mixed inflammatory cells with hemosiderin and macrophages
- pseudolipoblasts

Typical cytologic features include a cellular smear with a prominent myxoid background, spindle cells with bipolar processes, mononuclear epithelioid cells, scattered large atypical ganglion-like or Reed-Sternberg–like cells with inclusion-like nucleoli, and mixed inflammatory cells (Fig. 17.18). The hypervacuolate epithelioid cells may resemble lipoblasts.^{85–88} Given the heterogeneity of the tumor, FNA samples may not contain all of the above elements, posing significant diagnostic challenges.

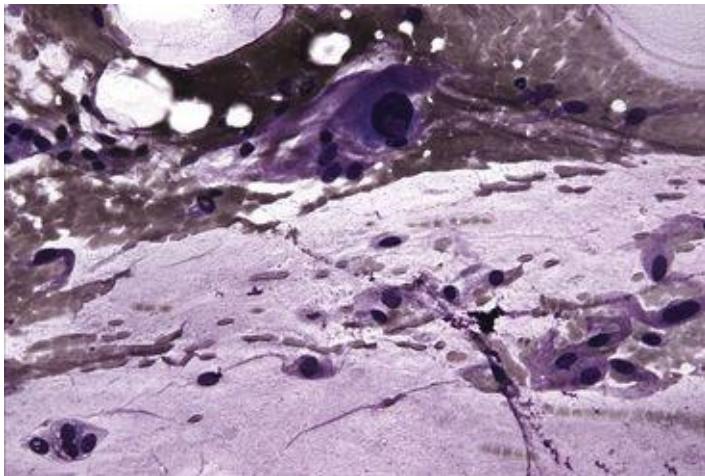


FIGURE 17.18 Myxoinflammatory fibroblastic sarcoma (MIFS).

The occasional large ganglion-like or Reed-Sternberg-like cells in a background of otherwise bland spindle cells, vacuolated epithelioid cells, and inflammatory cells may lead to confusion with a high-grade sarcoma or Hodgkin lymphoma (Romanowsky stain).

Differential diagnosis of myxoinflammatory fibroblastic sarcoma

- nodular fasciitis
- fibroinflammatory reactive lesion
- myxofibrosarcoma
- liposarcoma
- inflammatory myofibroblastic tumor
- Hodgkin lymphoma

MIFS is a low-grade sarcoma with a risk for frequent local recurrence but infrequent distant metastases. It is important not to misinterpret the bland fibroblastic proliferation admixed with inflammation as a reactive process or nodular fasciitis. Conversely, the large atypical cells in a background of mixed inflammation should not be confused with a high-grade sarcoma⁸⁹ or Hodgkin lymphoma. Finally, the pseudolipoblasts should not be construed as indicative of a myxoid liposarcoma or an inflammatory well-differentiated liposarcoma. Immunohistochemistry is of limited value in establishing the diagnosis of MIFS but is helpful in excluding some mimics like Hodgkin lymphoma.

Chordoma

Chordoma is the only malignant neoplasm derived from notochordal elements. This rare, midline lesion usually occurs at one of the two ends of the axial skeleton. Patients can have symptoms for months to years before diagnosis. Chordoma has a relatively indolent, protracted course, often with multiple recurrences. Metastases occur in a minority of cases.



Cytomorphology of chordoma

- granular and fibrillary myxoid matrix
- cohesive clusters and cords of neoplastic cells
- comparatively large cells
- physaliphorous cells

Cohesive clusters and cords of round to cuboidal cells are found in a rich myxoid matrix. The cells are larger than those of other myxoid neoplasms. Mild nuclear pleomorphism is present, with more prominent nucleoli in the more pleomorphic cells. The cytoplasm contains conspicuous cytoplasmic vacuoles ([Fig. 17.19](#)). Some cells have an abundance of vacuolated cytoplasm and are called *physaliphorous* (from the Greek *physallis*, meaning *bubble*); the edges of the vacuoles in physaliphorous cells resemble thin spider legs extending from the nucleus to the cell borders. Univacuolate signet ring-like cells and nonvacuolated smaller cuboidal cells with moderate amounts of granular cytoplasm are also present.

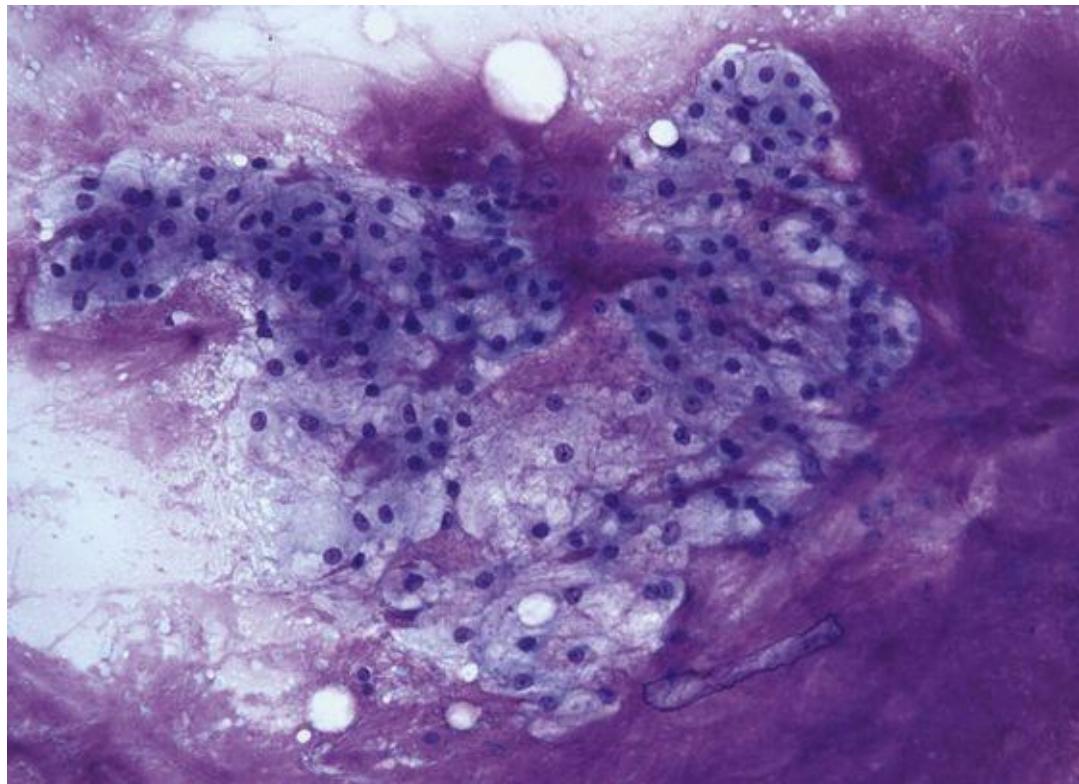


FIGURE 17.19 Chordoma.

The cells are much more cohesive and epithelioid than the cells of other myxoid sarcomas (Romanowsky stain).



Differential diagnosis of chordoma

- myxoid chondrosarcoma
- myxopapillary ependymoma
- metastatic mucinous adenocarcinoma

The cytologic differential diagnosis includes chondrosarcoma, mucinous adenocarcinoma, and myxopapillary ependymoma. In difficult cases, immunohistochemical studies are of help. Both chordomas and adenocarcinomas are positive for cytokeratins, but chordomas are also positive for S-100 protein. Chondrosarcomas and ependymomas are negative for cytokeratins, but chondrosarcomas are positive for S-100, and ependymomas express glial fibrillary acid protein (GFAP).^{24,71,90-92} Brachyury is more sensitive than the conventional panel of S-100 and keratins.⁹³

Extraskeletal Myxoid Chondrosarcoma

EMC is a slowly growing malignant tumor that occurs in adults, with a median age at diagnosis of 50 years. It involves the deep soft tissues of the proximal limbs, especially the thigh and popliteal fossa. Despite the name *chondrosarcoma*, there is no evidence of cartilaginous differentiation. It is regarded as a tumor of unknown differentiation in the current World Health Organization (WHO) classification.¹



Cytomorphology of extraskeletal myxoid chondrosarcoma

- consistent appearance from case to case
- bright magenta fibrillary stroma on Romanowsky-stained preparations
- variable degree of lacunar formation
- cords and lacelike arrangements
- uniform, bland epithelioid to spindled cells

Smears are moderately cellular, with a chondromyxoid matrix background ([Fig. 17.20A](#)) and a monotonous population of plump epithelioid to spindle-shaped tumor cells arranged in a lacelike pattern of anastomosing, loosely cohesive cords and nests. The malignant cells are uniform and lack nuclear pleomorphism ([Fig. 17.20B](#)). Nuclei are round or oval and hyperchromatic, with finely stippled chromatin. The single nucleolus is small and inconspicuous. Nuclear grooves and clefts are common. Cytoplasm is homogeneous and scant to moderately abundant and often appears wispy and tapered, with well-defined cell borders.^{94,95}

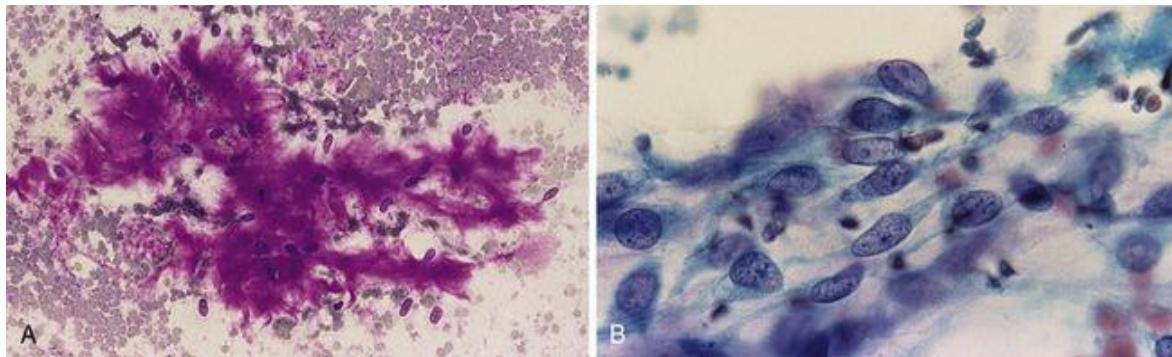


FIGURE 17.20 Extraskeletal myxoid chondrosarcoma (EMC).

A, The EMC has a distinctive fibrillar chondromyxoid matrix seen only to a lesser degree in some chordomas (Romanowsky stain). B, Long cytoplasmic extensions link one cell to another, often conferring a lacelike appearance (Papanicolaou stain).

Differential diagnosis of extraskeletal myxoid chondrosarcoma

- low-grade fibromyxoid sarcoma
- myoepithelial carcinoma of soft tissue
- myxoid liposarcoma, high grade
- ossifying fibromyxoid tumor

Of all the myxoid soft tissue neoplasms, EMC shows the least morphologic variation from one tumor to another. The myxoid matrix has a fibrillary texture that is distinct from the granular appearance of most of the other myxoid lesions. It stains bright magenta on air-dried preparations ([Fig. 17.20A](#)). Among the myxoid sarcomas, EMC has the fewest vascular structures. Chondroblast-like lacuna formation can occur, but no definite hyaline cartilage differentiation has been described.⁹⁵

There is no specific immunoprofile; less than 20% of cases show immunoreactivity for S-100 protein. An *NR4A3* gene rearrangement with different fusion partners (including *EWSR1*) is considered a hallmark genetic event and is of diagnostic value (see [Table 17.1](#) and [Fig. 17.34](#)).^{94,96}

Spindle Cell Neoplasms

The benign tumors in this group yield intact tissue fragments with few or no dispersed cells, whereas the malignant lesions feature more cell dissociation, greater cellularity, necrosis, and mitotic activity. One important distinction is determining whether the mass is of smooth muscle, nerve sheath, myofibroblastic, or other mesenchymal origin. Although some characteristic cytomorphologic features can suggest a specific entity or line of differentiation, a definitive distinction depends on immunohistochemical studies using a panel of antibodies ([Table 17.2](#)).^{19,23,47,97} Spindle cell carcinoma and melanoma often need to be considered in the differential diagnosis as well.

TABLE 17.2

IMMUNOPROFILE OF SPINDLE CELL NEOPLASMS

TUMOR	SMA AND DESMIN	S-100 PROTEIN	CD34	C-KIT AND DOG1	CK AND EMA	β -CATENIN (NUCLEAR)
Spindle cell lipoma	-	+ adipocytes	+ spindle cells	-	-	-
Schwannoma	-	+ diffuse	-	-	-	-
MPNST	-	+ focal	-/+	-	-/+	-
Synovial sarcoma*	-	-/+	-	-	+	-/+
GIST	-/+	-	+	+	-	-
Leiomyoma and leiomyosarcoma	+ diffuse	-	-	-	-/+	-
Solitary fibrous tumor*	-	-	+	-	-	-/+
Fibromatosis	-	-	-	-	-	+

CK, Cytokeratin; EMA, epithelial membrane antigen; GIST, gastrointestinal stromal tumor; MPNST, malignant peripheral nerve sheath tumor; SMA, smooth muscle actin.

*CD99 and bcl-2 are also positive; TLE1 and STAT6 are useful, newer markers for synovial sarcoma and solitary fibrous tumor, respectively.

Leiomyosarcoma

Leiomyosarcomas are subdivided into four clinical subtypes: intraabdominal, subcutaneous or deep soft tissue, cutaneous, and vascular. Intraabdominal tumors (40% to 45%) occur in older females in the retroperitoneum, mesentery, or omentum. Metastases to lung and liver are common. Subcutaneous and deep soft tissue tumors (20% to 30%) involve the extremities, particularly the thigh, with a slight male predominance. Cutaneous tumors (15% to 20%) affect younger adults, also with a male predominance. They are often painful lesions of the limbs and typically recur locally. Because it is now widely believed that a cutaneous leiomyosarcoma never metastasizes, the term *atypical intradermal smooth muscle neoplasm* is favored over *cutaneous leiomyosarcoma*.⁴⁹ Finally, vascular leiomyosarcomas (5%) occur adjacent to muscular-walled blood vessels, particularly the inferior vena cava and large veins of the lower limbs in older adults. Leiomyosarcomas are immunoreactive for smooth muscle actin (SMA), desmin, and caldesmon. They are negative for S-100 protein. About one third of cases exhibit positivity for cytokeratins and EMA.



Cytomorphology of leiomyosarcoma

- fascicular tissue fragments
- spindle-shaped cells
- “cigar-shaped” nuclei, some with indentation

- naked nuclei
- abundant homogeneous, finely granular cytoplasm
- mitoses

The cellular arrangement is variable; loosely cohesive clusters, closely packed tissue fragments with rigid edges ([Fig. 17.21A](#)), and short fascicles with elongated cells are seen. The percentage of isolated cells increases with tumor grade. In the *myxoid variant*, occasional hyaline stromal fragments (with blood vessels) contain a diffuse, granular ground substance. A parallel, side-by-side arrangement of tumor cells is common. The centrally placed nucleus is generally oval to elongated, with blunted or rounded ends (“cigar-shaped”) ([Fig. 17.21B](#)) that are often indented. Naked nuclei are common. Anisokaryosis, pleomorphism, and multinucleation increase with tumor grade and are prominent features of the *pleomorphic variant*. The cytoplasm is generally abundant, bipolar, and homogeneously granular. Mitoses are common, and atypical forms are seen in higher-grade lesions. The presence of even a single mitosis in a deep-seated smooth muscle tumor is highly suspicious for malignancy. Necrosis can be prominent in poorly differentiated tumors. [19,23,47,98,99](#)

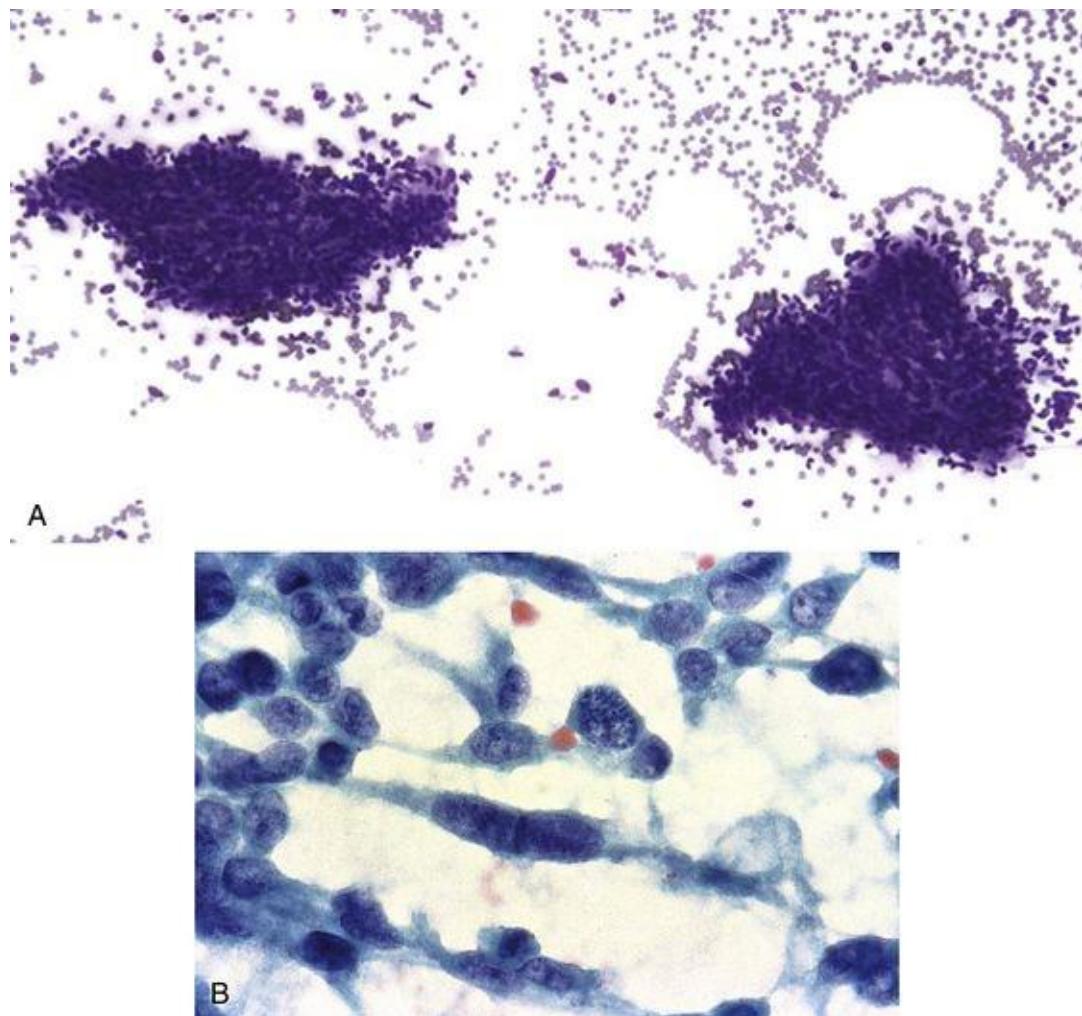


FIGURE 17.21 Leiomyosarcoma.

A, Spindle cells are packed in cohesive tissue fragments with well-circumscribed, rigid edges (Romanowsky stain). B, Nuclei are hyperchromatic, with finely textured chromatin in lower-grade tumors and more coarsely clumped chromatin in the higher-grade neoplasms. (Papanicolaou stain).



Differential diagnosis of leiomyosarcoma

- leiomyoma
- schwannoma
- malignant peripheral nerve sheath tumor
- gastrointestinal stromal tumor
- solitary fibrous tumor
- desmoid fibromatosis

Most leiomyosarcomas can be distinguished from a leiomyoma in that the former has more generalized nuclear atypia, more mitotic activity, and necrosis. Unless attached to the uterus or the gastrointestinal tract, any deep-seated smooth muscle tumor should be considered malignant until proven otherwise. Nerve sheath tumors have wavy, “fishhook” nuclei and fibrillary cytoplasmic processes, with immunoreactivity for S-100 protein. The gastrointestinal stromal tumor (GIST) is characterized by a prominent vascular component, cellular clusters with irregular outlines, and spindle cells with delicate cytoplasmic processes (see [Fig. 7.19A](#) and [B](#)). Solitary fibrous tumor and desmoid fibromatosis are distinguished from leiomyosarcoma by their characteristic immunoprofiles (see [Table 17.2](#)) and by the absence of nuclear atypia.^{[98–100](#)}

Schwannoma

Schwannomas are benign tumors of nerve sheath origin that primarily affect adults. They have a wide anatomic distribution but usually involve the limbs, head and neck, retroperitoneum, and posterior mediastinum.



Cytomorphology of schwannoma

- large, cohesive fragments
- wavy, “fishhook” nuclei
- pointed nuclear ends
- nuclear palisading
- filamentous cytoplasm

Schwannomas often elicit pain during aspiration. Smears contain large tissue fragments that have irregular rather than sharp edges and vary greatly in their cellularity—they can be highly or only sparsely cellular. Antoni A and Antoni B areas can be hard to distinguish with smears. Verocay bodies (palisades of nuclei alternating with anucleate expanses of fibrillar cytoplasm) are present and are especially well-seen on cell block preparations. The elongated, spindle-shaped cells, arranged in syncytium-like fragments, have minimally atypical nuclei that range in shape from oval to wavy to fishhooklike and taper to pointed ends ([Fig. 17.22A](#)). In “ancient” schwannomas, nuclear pleomorphism is marked but focal and scattered. Cytoplasm is abundant and filamentous ([Fig. 17.22B](#)). Mitotic

figures are rarely seen. Aspirates from cystic, myxoid, or densely collagenous schwannomas yield few cells and are often nondiagnostic.^{19,47,101,102}

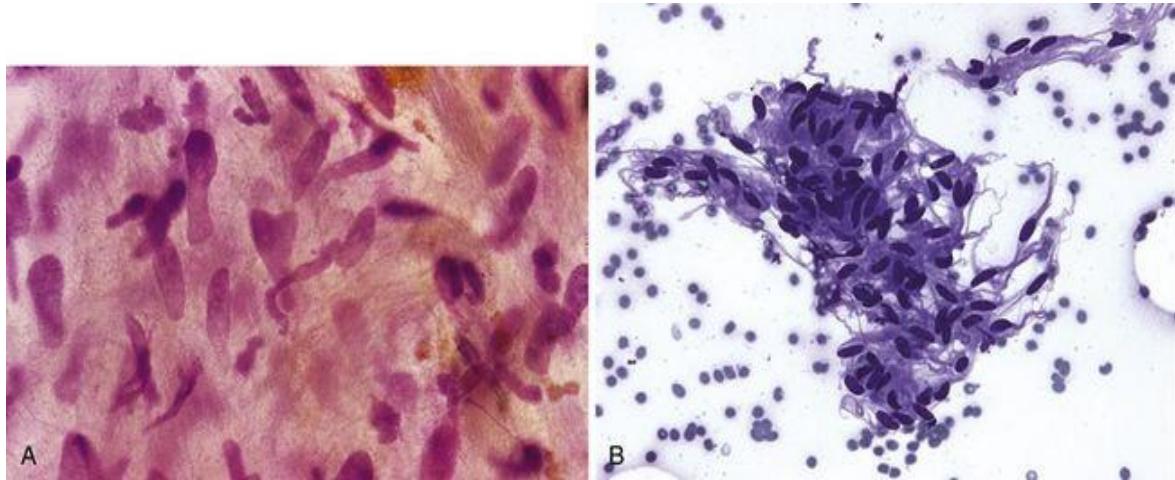


FIGURE 17.22 Schwannoma.

A, The cells of a benign schwannoma have a syncytium-like appearance, with indistinct cell borders (Papanicolaou stain). B, The neoplastic cells have abundant filamentous cytoplasm and a wavy nucleus (Romanowsky stain).



Differential diagnosis of schwannoma

- spindle cell lipoma
- malignant peripheral nerve sheath tumor (MPNST), low-grade
- leiomyosarcoma, low-grade
- solitary fibrous tumor
- neurofibroma
- desmoid fibromatosis

A schwannoma often raises the possibility of a malignancy because the rather thick aspirated tissue fragments appear very cellular on smears, particularly in the *cellular schwannoma variant*. This erroneous impression of malignancy is heightened with an ancient schwannoma and its large, bizarre nuclei.^{103,104} Diffuse and strong immunoreactivity for S-100 protein is characteristic of schwannomas and can be helpful in distinguishing it from malignancies like a low-grade MPNST. An MPNST, except for the epithelioid variant, stains only focally or even not at all for S-100 protein. A spindle cell lipoma can be distinguished from

schwannoma by the presence of mature adipose tissue and its positivity for CD34 in spindle cells. A solitary fibrous tumor exhibits more complex cellular clusters with wavy collagen fibers and bland spindle cells with oval rather than wavy nuclei. Immunoreactivity for CD34, not S-100 protein, is also helpful. Neurofibroma contains spindle-shaped fibroblasts and scattered axons in addition to wavy Schwann cells, and a myxoid background is a common finding. Desmoid fibromatosis yields less cellular smears with more abundant collagenous stroma.^{[102](#),[104](#),[105](#)}

Malignant Peripheral Nerve Sheath Tumor

MPNSTs occur sporadically and in patients with neurofibromatosis (NF-1). Often arising from a large peripheral nerve or a preexisting neurofibroma, they also occur in soft tissue and over a wide anatomic distribution. The limbs are most commonly affected, followed by the trunk and the head and neck.



Cytomorphology of malignant peripheral nerve sheath tumor

- hypercellular
- increased number of dispersed cells
- spindle-shaped cells with comma-shaped nuclei and nuclear pleomorphism with tapered, pointed, or rounded ends
- scant fibrillar cytoplasm
- round or polygonal cells in epithelioid variant
- mitotic figures
- heterologous elements (bone, cartilage, glands, rhabdomyoblasts, angiosarcoma)

Cytomorphology varies depending on the grade and subtype. Aspirates are moderately to highly cellular, with variably sized fascicles, isolated cells, and naked nuclei. Occasional rosettelike or pseudoglandular configurations are seen. Tumor cells are relatively large and predominantly spindled to oval but can be pleomorphic and/or rounder in higher-grade lesions. Mild to marked nuclear pleomorphism, with occasional bizarre giant nuclei, hyperchromasia, and inconspicuous to prominent nucleoli, is seen. The nuclear-to-cytoplasmic ratio is

increased, and nuclear palisading is rarely seen. The cells have scant, tapering, and fibrillar cytoplasm and wavy or comma-shaped nuclei ([Fig. 17.23](#)). Mitoses are usually readily identified. Necrosis and apoptosis can be present. MPNSTs, except for the *epithelioid variant* (diffuse S-100 staining), show only focal and patchy staining for S-100 protein in less than 50% of cases and for GFAP in 20% to 30% of cases. The cytomorphology, though not specific enough by itself for the initial (primary) diagnosis, usually displays sufficient features for documentation of recurrence or metastasis.^{[106–109](#)}

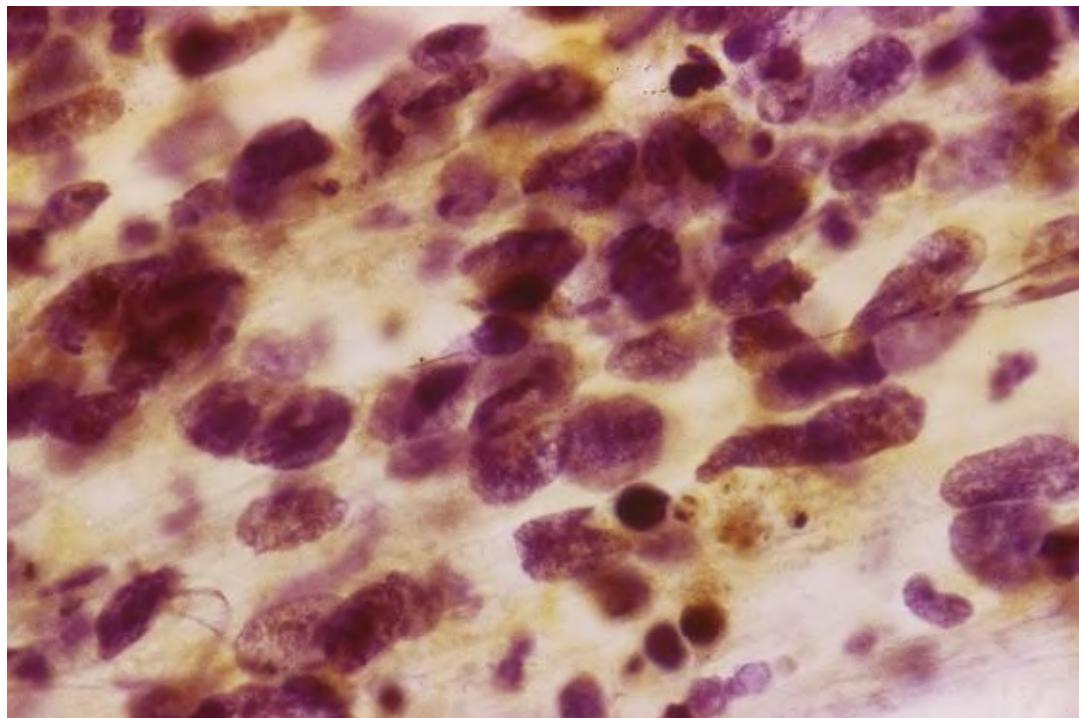


FIGURE 17.23 Malignant peripheral nerve sheath tumor (MPNST).

Although some of the elongated, wavy or fishhook nuclei of benign nerve sheath tumors are extant, in general, they give way to plumper forms with less pointed ends (Papanicolaou stain).



Differential diagnosis of malignant peripheral nerve sheath tumor

- schwannoma (especially with “ancient” change)
- synovial sarcoma
- leiomyosarcoma
- spindle cell carcinoma
- spindle cell melanoma

- malignant solitary fibrous tumor
- dedifferentiated liposarcoma (retroperitoneum)

Densely cellular fascicles alternating with hypocellular myxoid zones, geographic necrosis, and heterologous differentiation, though favoring an MPNST on histology, are rarely seen in FNA smears. Low-grade MPNSTs can be difficult to distinguish cytologically from benign peripheral nerve sheath tumors such as cellular schwannomas and neurofibromas. A traumatic neuroma following the excision of a sarcoma can be mistaken for recurrent sarcoma. The spindle cell component of synovial sarcoma has less morphologic variability and nuclear pleomorphism, but up to 25% of MPNSTs express cytokeratins. The nuclei of a leiomyosarcoma have blunted/truncated (rather than pointed) ends, and cytoplasm is denser. In general, diagnosing MPNST on FNA is extremely difficult, even with the help of ancillary studies, due to the lack of reliable specific makers for its line of differentiation, diverse histopathology, and complex karyotype. Clinical details are often as important (if not more so) for a correct diagnosis of MPNST.^{[49](#),[102](#),[107](#)}

Synovial Sarcoma

Synovial sarcoma is a malignant tumor that occurs at any age but mainly in young adults (aged 10 to 35 years) and accounts for up to 10% of all sarcomas. It has a wide anatomic distribution and, despite its name, no particular relationship to synovium. Most are deep-seated and arise in the lower limbs, especially the thigh. Others occur on the trunk and a wide variety of visceral organs. Radiologic evidence of calcification is common.

Histologically, synovial sarcomas can be biphasic (both spindle cell and epithelial components present) or monophasic (spindle cell), and there is a poorly differentiated type which can mimic a small round blue cell malignancy. Synovial sarcomas are focally immunoreactive for EMA and cytokeratins, especially in areas of epithelial differentiation, but also focally in the spindle cell areas. Up to two thirds of synovial sarcomas express CD99 (O13), and about 30% are positive for S-100 protein. Bcl-2 positivity is seen in most cases. All forms of synovial sarcoma share a reproducible, tumor-specific chromosomal translocation t(X;18)(p11.2;q11.2), which results in an SS18-SSX fusion gene. Immunocytochemical studies may not be conclusive in difficult cases. In such

cases FISH and/or RT-PCR are the most effective tools for definitive diagnosis (see [Table 17.1](#)).^{[10,39,110,111](#)}



Cytomorphology of synovial sarcoma

- high cellularity
- cell clusters alternating with dispersed cells
- cell clusters with shaggy edges and thin, branching capillaries
- extreme uniformity of cells
- bland, oval nucleus
- scant, delicate, tapering cytoplasm
- occasional epithelial elements
- mitoses
- mast cells

FNA specimens are moderately to markedly cellular, with occasional fragments of either myxoid or fibrous extracellular matrix. Irrespective of subtype, at low magnification there is a distinctive pattern of dispersed cells alternating with cohesive cell clusters that contain delicate branching capillaries ([Fig. 17.24](#)). The clusters can be large, with prominent fascicular and whorled growth patterns and shaggy edges. Epithelial structures (glands, acini, tubules, and papillae) can be seen in biphasic tumors, but often the epithelial differentiation is subtle ([Fig. 17.25](#)).^{[112-115](#)}

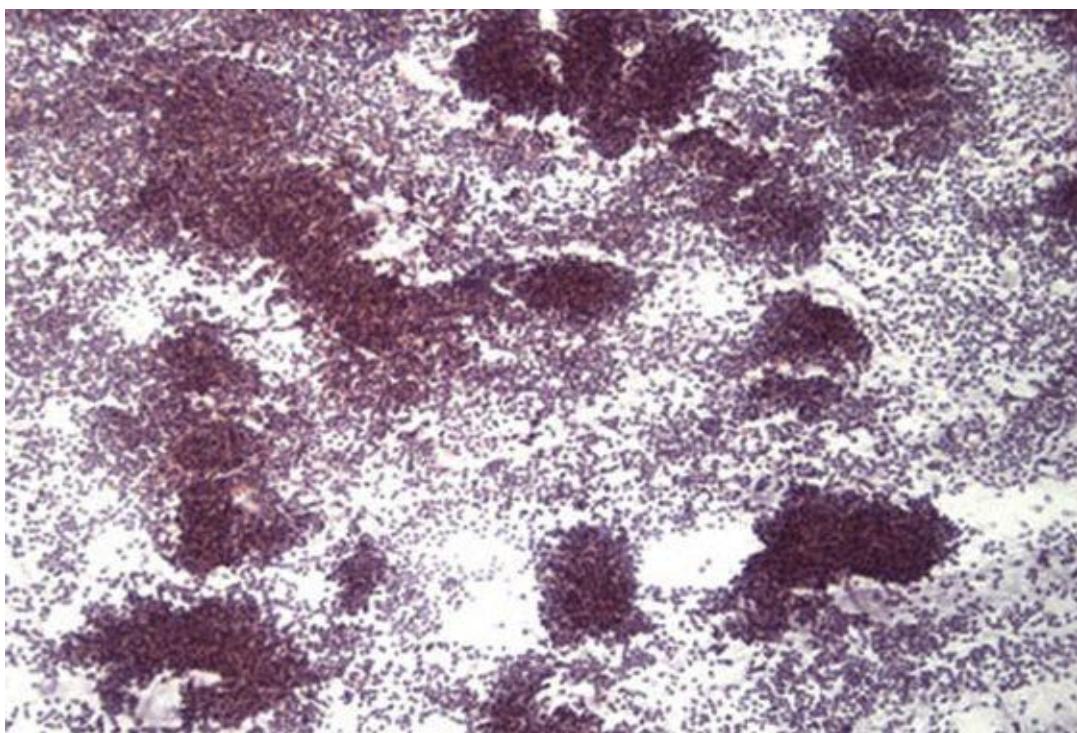


FIGURE 17.24 Synovial sarcoma.

There is a distinctive pattern of dispersed cells alternating with cohesive cell clusters.
(Papanicolaou stain).

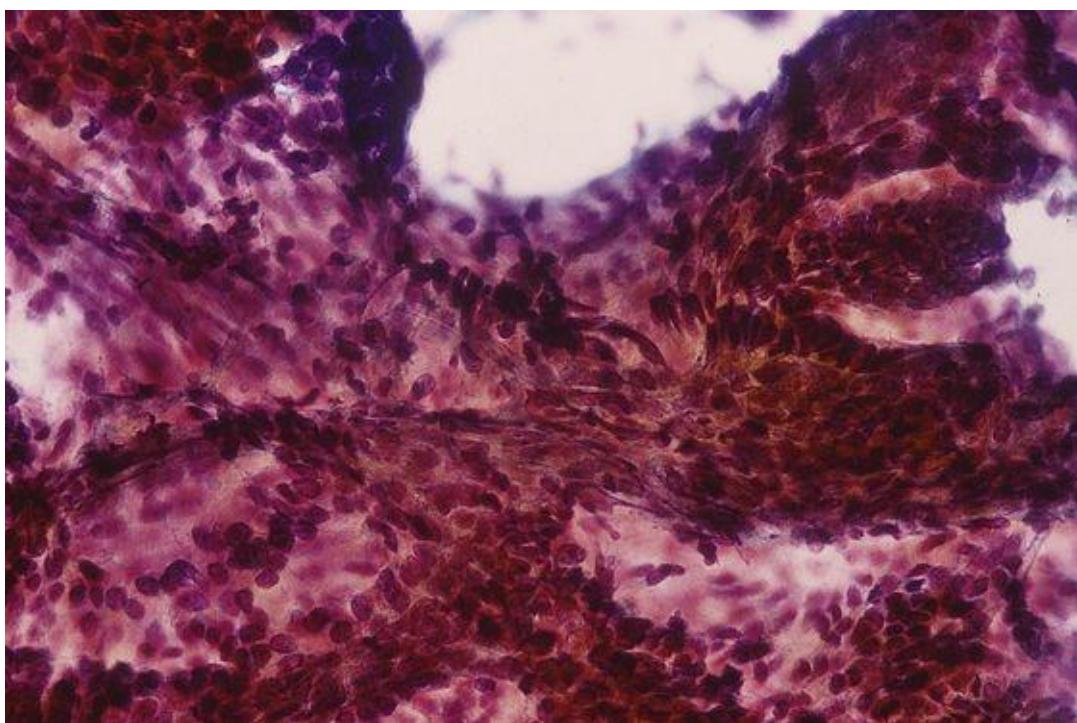


FIGURE 17.25 Synovial sarcoma, biphasic.

Epithelial differentiation is recognized as only a vague palisading of nuclei along the edges of tissue fragments or as clusters of more epithelioid-appearing cells among the spindle cells. (Papanicolaou stain).

Synovial sarcomas are remarkable for the striking uniformity of the cells (Fig. 17.26). They are small to medium-sized and round or fusiform. Epithelial differentiation, if present, confers a rounded, polygonal, or even cuboidal to columnar outline, and occasional epithelial cells are flattened as they stretch around three-dimensional cell clusters. The nucleus is bland and round to oval, with finely granular chromatin and a small nucleolus. Nucleoli are occasionally prominent in the epithelial component. In general, the cells have a high nuclear-to-cytoplasmic ratio, with scant, delicate, tapering cytoplasm that is denser and more abundant in the epithelial cells. Mitoses and mast cells are present. Occasionally, necrosis is identified.¹¹²⁻¹¹⁵

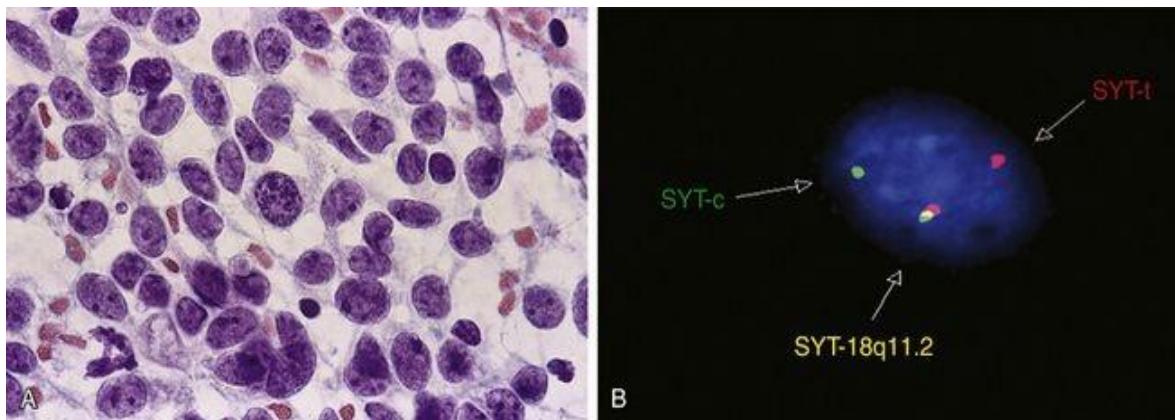


FIGURE 17.26 Synovial sarcoma, monophasic.

A, The oval to round nuclei are uniform in size and chromatin texture, although rare elongated, blunt-ended, and bent forms are seen (Papanicolaou stain). B, Fluorescence in situ hybridization (FISH) is often performed with centromeric (SYT-c) (green) and telomeric (SYT-t) (red) probes that flank the SYT gene breakpoint. There is one normal chromosome (*probes adjacent = yellow*), but the split-apart green and red signals indicate a translocation on the other chromosome. (Courtesy Dr. Paola Dal Cin, Brigham and Women's Hospital, Boston, Massachusetts.)



Differential diagnosis of synovial sarcoma

- leiomyosarcoma
- malignant peripheral nerve sheath tumor (MPNST)
- solitary fibrous tumor (SFT)
- Ewing sarcoma
- metastatic carcinoma

- ectopic hamartomatous thymoma
- carcinosarcoma

The nuclei of a leiomyosarcoma have blunted/truncated (rather than rounded) ends, and cytoplasm is denser. Some nuclei of a MPNST are more curved and pointed, and there is greater nuclear pleomorphism. A solitary fibrous tumor contains fewer dispersed cells and more complex cellular clusters, withropy collagen fibers and blood in the background. Immunohistochemistry can be helpful, because a solitary fibrous tumor is usually immunoreactive for CD34 and negative for EMA (see [Table 17.2](#)). At least 20% of synovial sarcomas have a poorly differentiated round cell morphology resembling Ewing sarcoma ([Fig. 17.27](#)). In such cases (indeed, in most cases when a primary diagnosis is being rendered), immunohistochemistry and genetic analysis are indispensable for confirming the diagnosis of synovial sarcoma ([Fig. 17.26B](#)). Ectopic hamartomatous thymoma is a rare benign biphasic tumor composed of sheets of bland spindle cells, squamoid epithelial islands, and mature adipose tissue, showing more diffuse keratin positivity and focal CD34 positivity in small spindle cells. Finally, the cells of synovial sarcoma are much more uniform than those of a metastatic sarcomatoid carcinoma or carcinosarcoma.^{[112-116](#)}

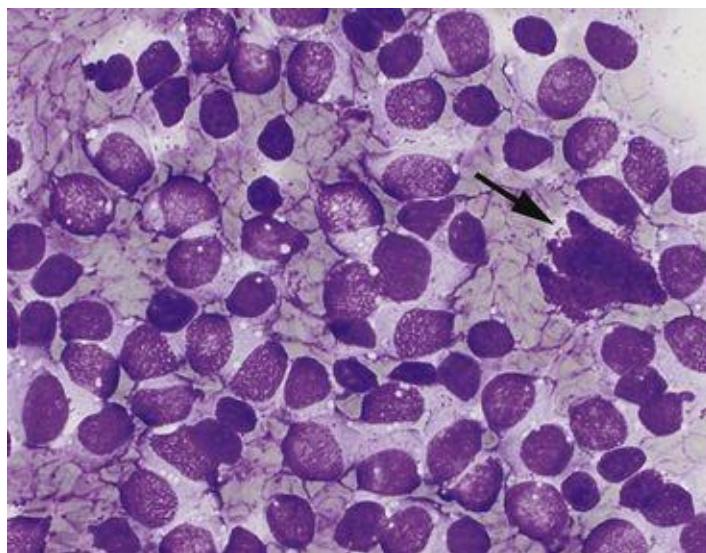


FIGURE 17.27 Synovial sarcoma, poorly differentiated round cell type. The tumor cells have a round cell morphology resembling that of a Ewing sarcoma. Mast cells are a characteristic feature (arrow) (Romanowsky stain). (Case courtesy Dr. Bastiaan de Boer, PathWest, QE II Medical Centre, Nedlands Perth, Australia.)

Solitary Fibrous Tumor

SFT is a fibroblastic neoplasm that involves the pleura as well as a wide variety of extrapleural sites, including the peritoneum, mediastinum, retroperitoneum, upper respiratory tract, orbit, deep soft tissues, indeed, almost any human organ. Many SFTs were formerly called *hemangiopericytomas*. SFT affects adults and manifests as a slowly growing mass. The majority are benign, but about 5% to 10% behave in a malignant fashion.¹¹⁷ Immunohistochemistry offers valuable support for the diagnosis of an SFT. SFTs are almost invariably positive for CD34 and only slightly less often for CD99 and bcl-2. They are negative for S-100 protein, actin, desmin, and the keratins (see [Table 17.2](#)).



Cytomorphology of solitary fibrous tumor

- variable yield with bloody background
- a meshwork of irregular fascicles and individual cells
- bland spindle cells with a fusiform nucleus
- scant elongated cytoplasmic processes
- ropy collagen
- naked nuclei

The aspirate exhibits scant to moderate cellularity, with a bloody background that contains ropy collagen fibers. Cellular fragments with a meshwork of irregular fascicles alternate with isolated spindle-shaped cells ([Fig. 17.28A](#)). The cells are uniformly bland, with a fusiform nucleus, finely dispersed chromatin, an inconspicuous nucleolus, and scant elongated cytoplasm. Naked nuclei are common. Cell block preparations are very helpful in capturing the characteristic bland, monomorphic spindle-shaped cells insinuated between collagen bundles ([Fig. 17.28B](#)) and hemangiopericytoma-like (i.e., thin, branching, staghornlike) vessels.^{2,118-120} Highly cellular clusters with nuclear pleomorphism, prominent nucleoli, necrosis, and noticeable mitotic activity should raise the suspicion of a malignant SFT.¹¹⁹⁻¹²¹ A fat-forming variant of SFT, initially named *lipomatous hemangiopericytoma*, shows variably prominent adipocytes, even lipoblast-like cells, a diagnostic pitfall that can lead to the misinterpretation of an SFT as any of the benign and malignant lipomatous tumors.¹²²

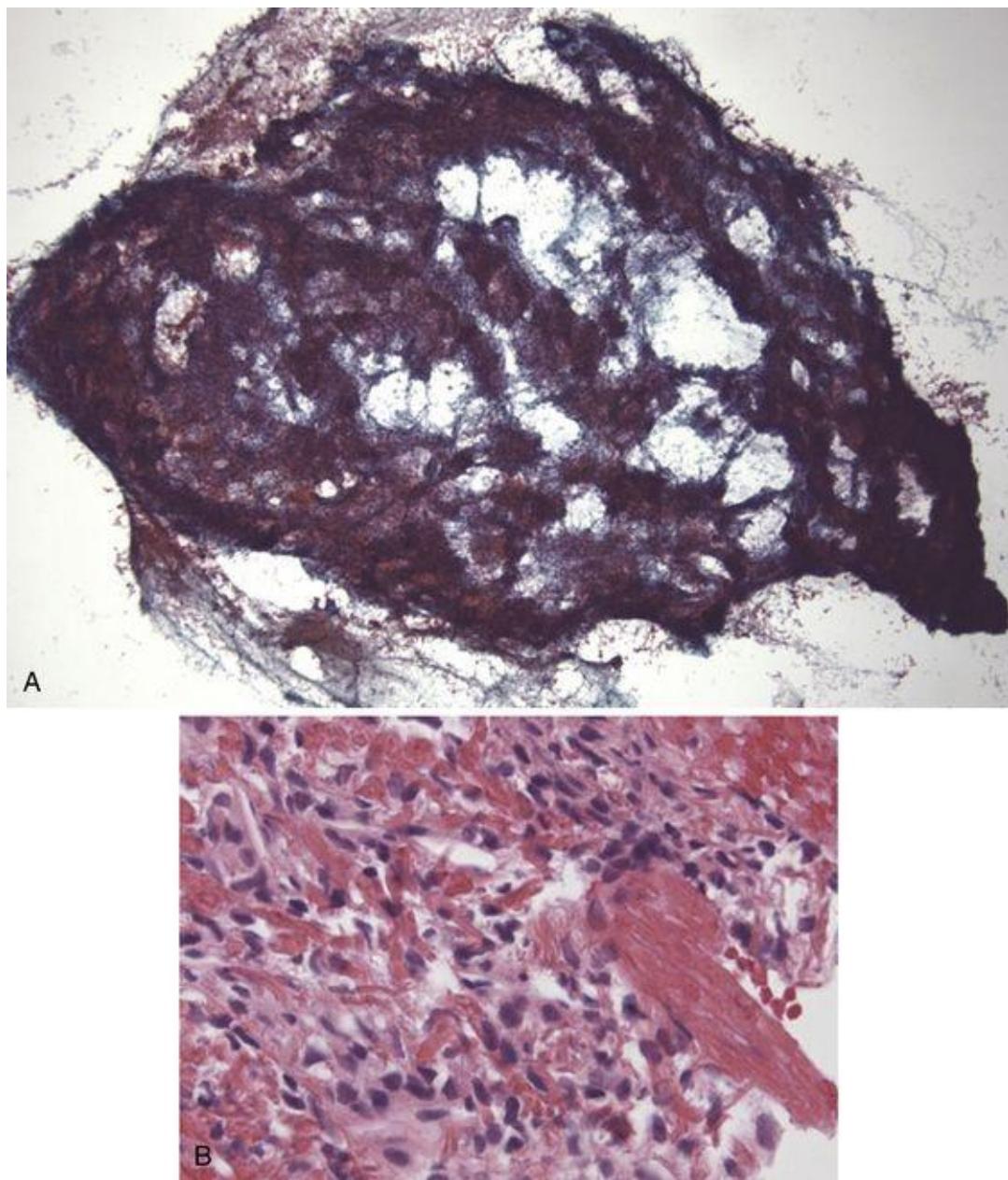


FIGURE 17.28 Solitary fibrous tumor (SFT).

A, Cellular fragments with a meshwork of irregular fascicles alternate with isolated spindle-shaped cells (Papanicolaou stain). B, Cell block preparations are very helpful for capturing the characteristic bland, monomorphic spindle-shaped cells insinuated between collagen bundles (hematoxylin-eosin [H & E] stain).



Differential diagnosis of solitary fibrous tumor

- malignant peripheral nerve sheath tumor (MPNST), low-grade
- monophasic synovial sarcoma
- desmoid fibromatosis
- sarcomatoid mesothelioma

- spindle cell thymoma
- benign or malignant lipomatous tumor (for fat-forming variant)

MPNST has more nuclear pleomorphism and atypia. Synovial sarcoma exhibits even more striking uniformity in the cell population and cellular arrangements than SFT. Desmoid fibromatosis yields hypocellular smears, with more abundant collagenous stroma. Sarcomatoid mesothelioma and spindle cell thymoma are positive for cytokeratins and negative for CD34.

Fibromatoses

The fibromatoses are a broad spectrum of locally aggressive fibroblastic neoplasms that display an infiltrative growth pattern and can recur but never metastasize. They are divided into two groups based on their location: The superficial or fascial fibromatoses include the palmar, plantar, and penile variants; and the deep or desmoid fibromatoses include the abdominal, extraabdominal, and intraabdominal variants. In childhood and adolescence, desmoid fibromatosis can be the initial manifestation of familial adenomatous polyposis.



Cytomorphology of fibromatosis

- variable, often low cellularity
- bland spindle-shaped cells, isolated or in slender fascicles
- fragments of dense collagenous stroma
- oval to elongated nuclei, frequently with crush artifact
- multinucleated degenerated muscle cells (if intramuscular)
- rare mitotic activity

FNA preparations exhibit low but variable cellularity, with long fascicular clusters, isolated bland, spindle-shaped fibroblasts, and scattered degenerated skeletal muscle fibers ([Fig 17.29A](#)). Other features include an oval to elongated nucleus with bipolar ends and even chromatin, often with a crush artifact when embedded in the collagenous stroma. Some have tapering cytoplasmic processes,

whereas others are stellate. Rare inflammatory cells are present, but cytologic atypia is absent, and mitotic activity is rare. Fragments of dense collagenous stroma are common and large long fascicular tissue fragments may be seen.^{26,123} Occasionally, myxoid stroma is present. As with the cytomorphology, the immunoprofile is not entirely specific. Positive staining for β -catenin (aberrant nuclear staining, Fig. 17.29B) and negative staining for CD34, SMA, and c-kit support the diagnosis of a desmoid fibromatosis in the right clinical setting (see Table 17.2).

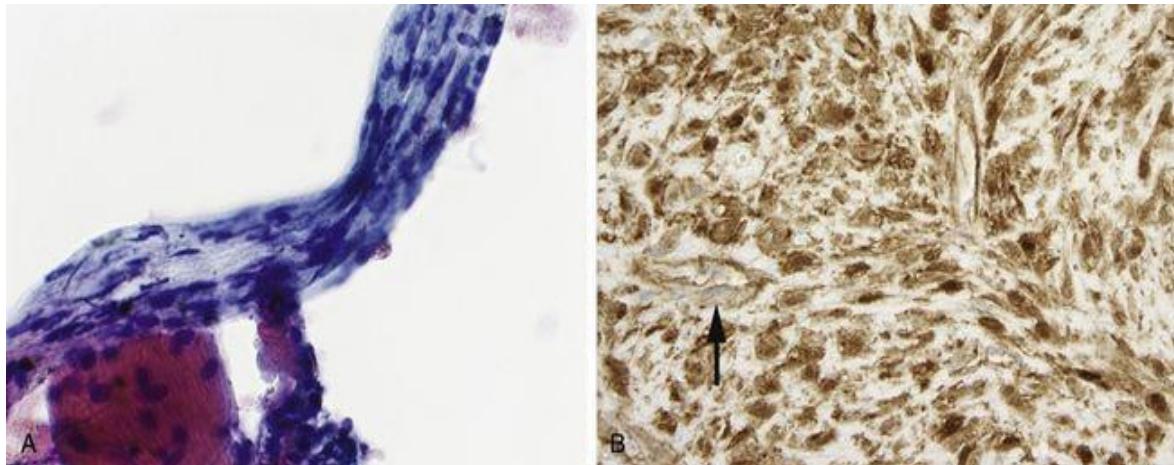


FIGURE 17.29 Desmoid fibromatosis.

A, Long fascicular arrangements of spindle-shaped fibroblasts and scattered degenerated skeletal muscle fibers are characteristic (Papanicolaou stain). B, Aberrant nuclear staining for β -catenin is seen in tumor cells but not in adjacent endothelial cells (arrow).



Differential diagnosis of fibromatosis

- nodular fasciitis
- low-grade fibromyxoid sarcoma (LGFMS)
- scar tissue
- nerve sheath tumor
- smooth muscle tumor
- solitary fibrous tumor
- gastrointestinal stromal tumor (GIST)

Nodular fasciitis produces more cellular and pleomorphic smears with a

haphazard cellular arrangement and ganglion-like cells. LGFMS exhibits alternating myxoid and fibrous areas. Scars have less cellularity and greater cohesion. The nuclei in nerve sheath tumors are longer and wavier. Those in smooth muscle tumors are blunted, whereas those of fibromatosis have pointed, bipolar ends. SFT and GIST should be considered in the differential diagnosis of an intraabdominal desmoid fibromatosis. These two mimics have more complex, irregular tissue fragments and a more prominent vascular component. Immunohistochemical studies are usually indispensable in classifying these spindle cell neoplasms (see [Table 17.2](#)). Because of its dense collagenous nature, desmoid fibromatosis yields paucicellular or nondiagnostic smears in more than half of cases. A larger needle or core biopsy is usually required to obtain a diagnostic sample.^{[26,123](#)}

Nodular Fasciitis

Nodular fasciitis is a relatively common, self-limiting, fibroblastic and myofibroblastic pseudosarcomatous proliferation that typically arises in subcutaneous tissue. It occurs in all age groups but is most common in young adults. The most common sites are the upper extremities, trunk, and head and neck (including salivary gland), but any part of the body can be affected. It has a typical clinical presentation: rapid growth over a relatively short period of time, usually no more than 2 months. The lesion usually measures 2 to 3 cm in greatest dimension and is rarely bigger than 5 cm. It can be sore and/or tender. Only a minority of patients report a history of trauma to the affected area. Because of the recent demonstration of clonal gene rearrangements involving the *USP6* locus, the traditional view of nodular fasciitis as a nonneoplastic, reactive lesion has been challenged.^{[124](#)}



Cytomorphology of nodular fasciitis

- myxoid background (early phase)
- numerous dispersed or clusters of polymorphic (e.g., spindle-shaped, stellate) myofibroblasts
- lack of significant hyperchromasia
 - uni- or binucleate ganglion-like cells with eccentric nuclei and prominent nucleoli
 - inflammatory cells

The moderately hypercellular aspirates have a heterogeneous background of myxoid material, inflammatory cells, and delicate branching vessels ([Fig. 17.30A](#)). Prominent myxoid stroma is more commonly seen in early-phase lesions ([Fig. 17.30B](#)), whereas fragments of collagenous stroma are more common in longstanding lesions. There are numerous solitary spindle-shaped and stellate myofibroblastic cells (see [Fig. 17.30A](#)), but some also form cohesive groups. The presence of uninucleate or binucleate ganglion-like cells is a characteristic finding in the conditions known as *proliferative fasciitis* and *proliferative myositis*. Nuclei are uniform and lack significant atypia. They are small in the fusiform cells and somewhat larger in the plumper stellate cells. Their chromatin is delicate and open, and the inapparent nucleolus of the spindle-shaped cells is more distinct in the larger cells. Cytoplasm is generally abundant, but wispy with tapering ends and ill-defined borders. Mitotic figures can be alarmingly numerous. Osteoclast-like giant cells are occasionally present. The myofibroblasts are positive for SMA and pan-muscle actin, but negative for desmin and S-100 protein, compatible with their myofibroblastic nature.

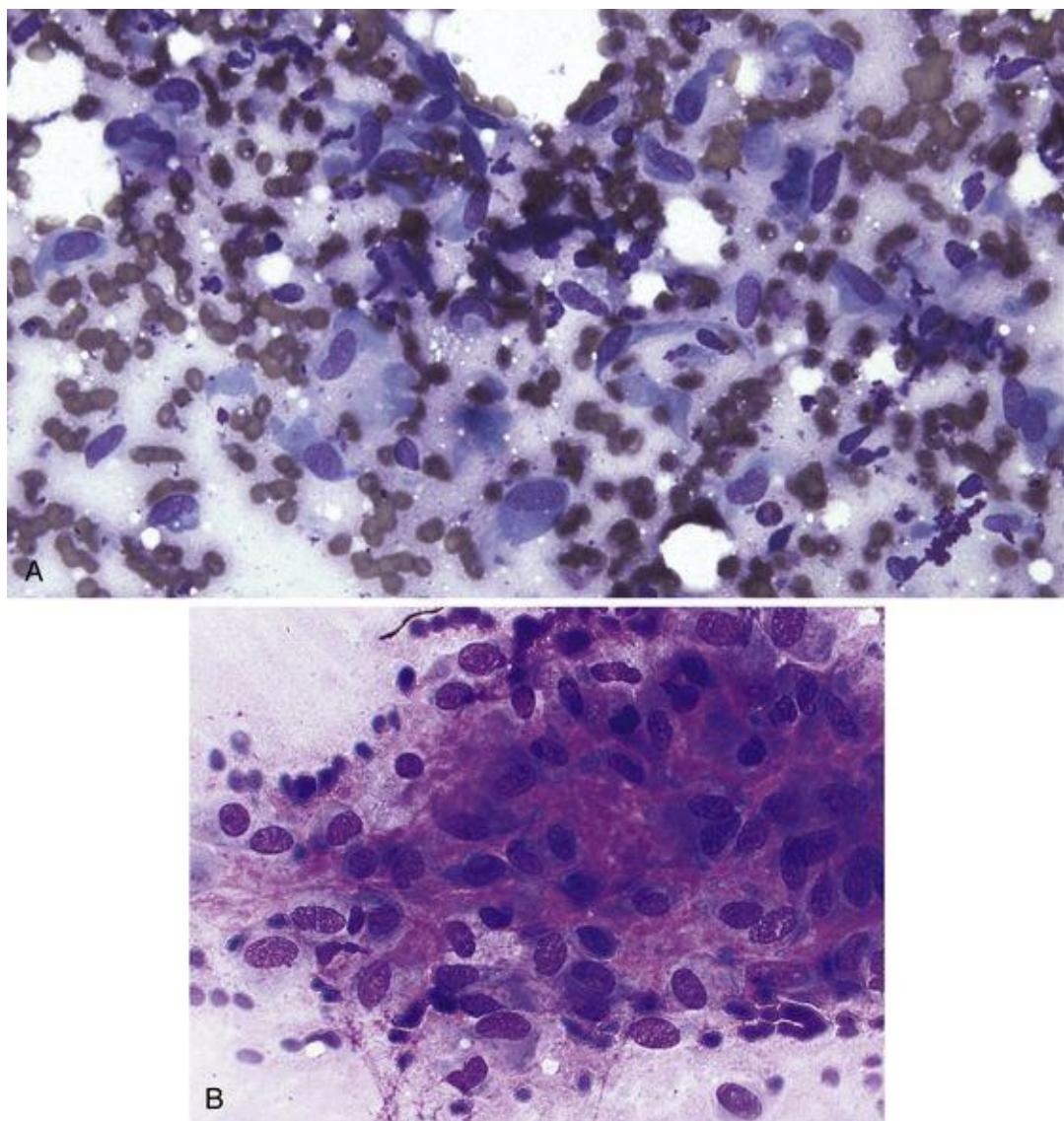


FIGURE 17.30 Nodular fasciitis.

A, Numerous dispersed, polymorphic myofibroblasts are characteristic (Romanowsky stain).

B, Prominent myxoid stroma and hypercellularity are common in the early phase

(Romanowsky stain).

Nodular fasciitis is one of the most notorious mimics of malignancy among all the soft tissue lesions. The differential diagnosis includes a variety of sarcomas. The key to arriving at the correct (benign!) diagnosis is the correlation of clinical information with the FNA findings. The diagnosis is often suggested just by the clinical presentation (e.g., a rapidly growing subcutaneous mass smaller than 3 cm), and the typical FNA findings serve simply to confirm this clinical impression.

Dermatofibrosarcoma Protuberans

DFSP is a slowly growing fibroblastic tumor of the dermis and subcutis in young adults, often preexisting its diagnosis by 5 years or even decades. It occurs mainly on the trunk, proximal extremities, and groin, but a small percentage occur on the scalp or elsewhere in the head and neck region. Fibrosarcomatous progression occurs in 10% to 15% of cases. DFSP and the related giant cell fibroblastoma in children carry the characteristic *COL1A1-PDGFB* fusion gene.¹



Cytomorphology of dermatofibrosarcoma protuberans

- dense, storiform cell clusters in a collagenous stroma
- dispersed or loose clusters of spindle cells in a myxoid stroma (*myxoid variant*)
- mildly atypical spindle cells with minimal pleomorphism
- occasional entrapped adipose tissue

Aspirates yield moderately cellular smears composed of dense cellular clusters with a prominent storiform growth pattern. Occasional fragments of metachromatic fibrillary stroma are demonstrable with a Romanowsky stain. Adipose tissue entrapped within fascicles of spindle cells is a useful but inconsistent finding. Isolated fibroblast-like and stellate cells are minimally pleomorphic, with bland, oval nuclei. The chromatin is fine and homogeneous, and nuclear membranes are smooth and round. Small, distinct nucleoli are usually present. The cytoplasm is moderate in amount, pale, and finely granular. Cell borders are ill defined within tissue fragments, but bipolar cytoplasmic processes are prominent on dispersed cells. In the *myxoid variant*, dispersed or loose clusters of spindled to stellate cells are more prominent than the typical storiform clusters.^{125–127} Tumor cells are strongly and diffusely positive for CD34 and negative for S-100 protein, EMA, and desmin. Detection of the *COL1A1-PDGFB* fusion gene by FISH or RT-PCR is helpful in difficult cases (see [Table 17.1](#)).⁹



Differential diagnosis of dermatofibrosarcoma protuberans

- benign fibrous histiocytoma
- scar
- nodular fasciitis
- perineurioma
- neurofibroma
- low-grade fibromyxoid sarcoma (LGFMS)

Clinical features such as the anatomic location and rate of growth distinguish benign fibrous histiocytoma (dermatofibroma) from DFSP, but the mitotic rate is variable and unhelpful in the diagnosis of these lesions. Scars have less cellularity and greater cohesion with more angulated nuclei. Nodular fasciitis shows a more haphazard cellular arrangement, spindle-shaped and ganglion-like cells, less rounded nuclei, and more prominent nucleoli, often with an inflammatory infiltrate. Nerve sheath tumors are excluded by the lack of neural nuclear features and the absence of immunoreactivity for S-100 protein or EMA. LGFMS may be confused with a myxoid DFSP, but the former is usually more deep-seated, negative for CD34, and positive for MUC-4.^{[97,125,126](#)}

Inflammatory Myofibroblastic Tumor

IMT is a rare low-grade neoplasm of children and adolescents that was once regarded as a reactive inflammatory pseudotumor. IMT has a local recurrence rate of 10% to 25%, and metastases occur in just under 5% of cases. It has a predilection for visceral organs including lung and liver, as well as the abdominal cavity. Most IMTs show fascicular proliferation of plump myofibroblasts with a prominent infiltrate of plasma cells and lymphocytes, whereas a small subset of aggressive IMTs exhibit an epithelioid cytomorphology with myxoid stroma and a neutrophil-rich inflammatory infiltrate.^{[128](#)}



Cytomorphology of inflammatory myofibroblastic tumor

- inflammatory cells
- mildly pleomorphic spindle cells
- large polygonal cells

- elongated cytoplasmic tails

Most IMTs yield highly cellular smears with a prominent inflammatory population composed mostly of plasma cells and lymphocytes. Isolated spindle-shaped tumor cells are admixed with loose clusters and dense aggregates. The tumor cells exhibit typical myofibroblastic cytomorphology: slightly eccentrically placed, plump nuclei with finely granular chromatin and conspicuous small nucleoli. Fine cytoplasmic vacuolization and cytoplasmic tails or extensions are typical. Large ganglion-like myofibroblasts with abundant cytoplasm and enlarged, sometimes binucleate, vesicular nuclei with prominent nucleoli are also common.^{[129-131](#)} Like other myofibroblastic tumors, IMTs are positive for SMA (80%), desmin (60%), and keratins (30%). Although not entirely specific for IMT, and seen in only 50% of cases, immunoreactivity for *anaplastic lymphoma receptor tyrosine kinase* (ALK), which results from an ALK gene rearrangement, can be diagnostically helpful (see [Table 17.1](#)).^{[132](#)}



Differential diagnosis of inflammatory myofibroblastic tumor

- inflammatory pseudotumor
- inflammatory leiomyosarcoma
- desmoid fibromatosis
- gastrointestinal stromal tumor
- dedifferentiated liposarcoma
- follicular dendritic cell sarcoma
- angiomyxoid fibrous histiocytoma

Inflammatory pseudotumor is usually associated with a clinical history of trauma, a surgical procedure, or infection. Most other morphologic mimics can be distinguished from IMT by their absence of ALK reactivity and the presence of specific immunoreactivities: desmoid fibromatosis (for β -catenin), GIST (for c-KIT, DOG1, and CD34), dedifferentiated liposarcoma (for MDM2 and CDK4), and follicular dendritic cell sarcoma (for CD21, CD35, and D2-40). Positivity for multiple smooth muscle markers including caldesmon favors

leiomyosarcoma. Demonstration of an *EWSR1* gene rearrangement by either FISH or RT-PCR is helpful to separate angiomyomatoid fibrous histiocytoma (AFH) from an ALK-negative IMT.^{[133](#)}

Hemangiopericytoma and Fibrosarcoma

Hemangiopericytoma is a controversial entity lacking uniformly accepted diagnostic criteria. There was substantial clinical and pathologic heterogeneity among Stout's original cases. Today, most would be reclassified as other neoplasms, a majority as solitary fibrous tumors. Like hemangiopericytoma, adult fibrosarcoma is best regarded as a diagnosis of exclusion. Most cases diagnosed before the era of immunohistochemistry and electron microscopy would be reclassified today as either malignant peripheral nerve sheath tumor or monophasic synovial sarcoma. The true fibrosarcomas that remain are the infantile type and the fibrosarcoma arising in DFSP in adults. Neither hemangiopericytoma nor fibrosarcoma is reliably diagnosed by FNA.^{[1,134](#)}

So-Called Fibrohistiocytic Neoplasms

The tumors in this group are very heterogeneous but bound together morphologically by a mixture of histiocytoid stromal cells, histiocytes, and multinucleated giant cells. They are some of the most commonly targeted neoplasms by FNA. The fibrohistiocytic differentiation of some of these tumors has been challenged over the past 20 years. The so-called pleomorphic and storiform MFH, once the most frequently diagnosed sarcoma in adults (40% of adult sarcomas), is nowadays a diagnosis of exclusion and synonymous with *undifferentiated high-grade pleomorphic sarcoma* (less than 5% of adult sarcomas). *Myxofibrosarcoma* (also known as *myxoid MFH*) and *angiomatoid fibrous histiocytoma* (AFH) (formerly known as *angiomatoid MFH*) remain distinct entities. *Myxofibrosarcoma* and *undifferentiated high-grade pleomorphic sarcoma* are discussed in other sections of this chapter. The remaining fibrohistiocytic neoplasms, discussed in this section, are the localized and diffuse types of tenosynovial giant cell tumor and the AFH.

Tenosynovial Giant Cell Tumor, Localized and Diffuse Types

The localized and diffuse types of tenosynovial giant cell tumor share similar cellular compositions and cytomorphology but differ in their growth pattern, clinical features, and biological behavior. The *localized type*, also known as *giant cell tumor of tendon sheath*, most commonly manifests as a slowly growing, circumscribed, and at least partially encapsulated nodule in close proximity to the synovium of the tendon sheaths of the hands and feet. It occurs more commonly in females between the ages of 30 and 50 years. The *diffuse type*, also known as *pigmented villonodular synovitis* (PVNS), is a locally destructive lesion with an infiltrative growth pattern, occurring at intra-and extra-articular sites. The diffuse lesion tends to affect younger patients than its localized counterpart. Intra-articular lesions affect predominantly the knee (75% of cases), followed by the hip (15%), ankle, elbow, and shoulder. The extra-articular lesions are usually located in periarticular soft tissue with or without involvement of the adjacent joint. Both types have a *COL6A3-CSF1* gene fusion in a small subset of tumor cells, mainly the larger epithelioid cells.



Cytomorphology of tenosynovial giant cell tumor

- mononuclear histiocytoid cells of varying shapes (ovoid, polygonal, and spindle-shaped)
- foamy histiocytes
- osteoclast-type giant cells
- extracellular and intracytoplasmic hemosiderin
- moderate anisocytosis and minimal anisokaryosis

Aspirates from localized and diffuse type tumors have moderate to high cellularity. The predominant cells, including mononuclear histiocytoid cells with folded nuclei and larger epithelioid cells with eccentric nuclei, are dispersed as isolated cells and arranged in irregular clusters. The nuclei show minimal variation. They are ovoid with smooth contours, finely granular chromatin, inconspicuous nucleoli, frequent grooves, and occasional intranuclear inclusions ([Fig. 17.31](#)). Binucleation is not uncommon. Although mitotic figures are occasionally present, nuclear atypia and necrosis are very rare. Variable numbers of foamy histiocytes and osteoclast-type giant cells are admixed with the mononuclear histiocytoid cells. The giant cells vary widely in size and in the number of nuclei (3 to 50 or more).^{[135,136](#)} The absence of giant cells does not exclude the diagnosis, especially for the diffuse-type tumors. Hemosiderin deposition in mononuclear cells and in macrophages is inevitably present.

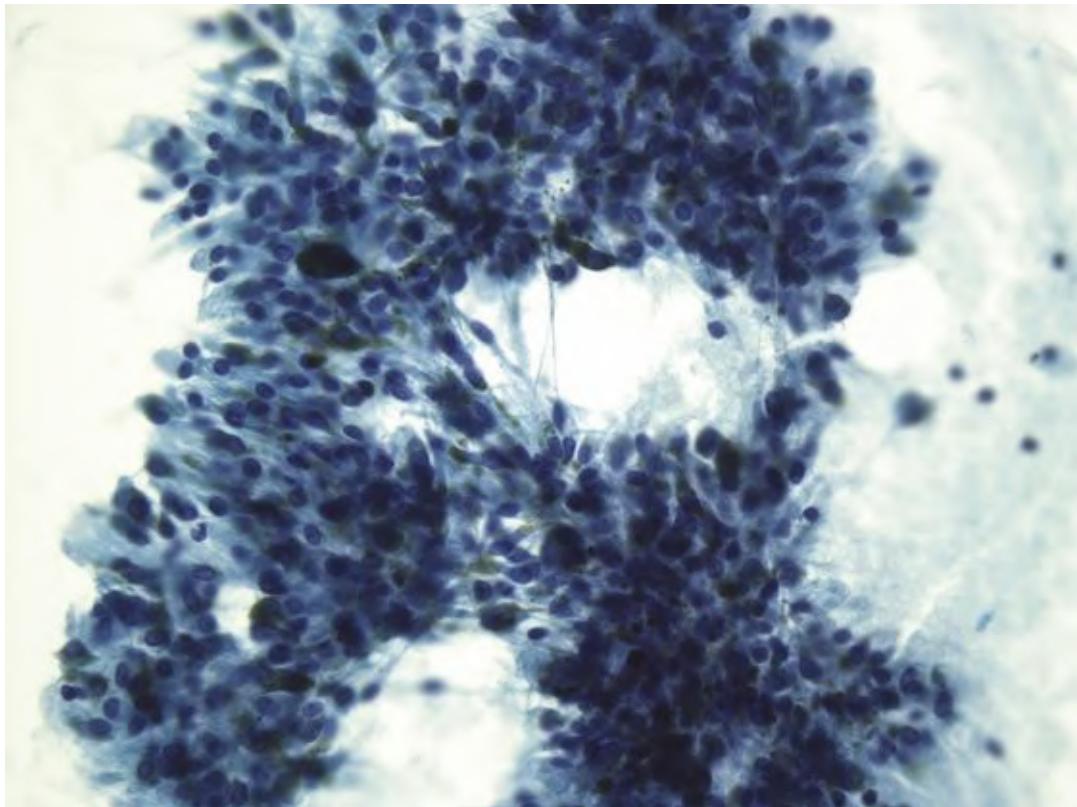


FIGURE 17.31 Tenosynovial giant cell tumor.

Mononuclear histiocytoid cells are arranged in irregular clusters and admixed with foamy histiocytes, giant cells, and prominent hemosiderin deposition (Papanicolaou stain).



Differential diagnosis of tenosynovial giant cell tumor

- gouty tophi
- chronic synovitis with synovial hyperplasia
- metastatic melanoma
- giant cell–rich sarcoma

Gouty tophi can have many giant cells, but the lack of mononuclear cells and the presence of birefringent needle-shaped crystals on polarizing microscopy ([Fig. 17.32A and B](#)) are diagnostic. Chronic synovitis is characterized by more inflammation and sometimes necrosis, but giant cells and hemosiderin deposition are usually not prominent. Metastatic melanoma and giant cell–rich sarcoma exhibit more pleomorphism, nuclear atypia, and necrosis. In difficult cases, immunohistochemistry can be helpful: both the mononuclear cells and the giant cells are positive for the histiocytic markers CD68 and CD163.

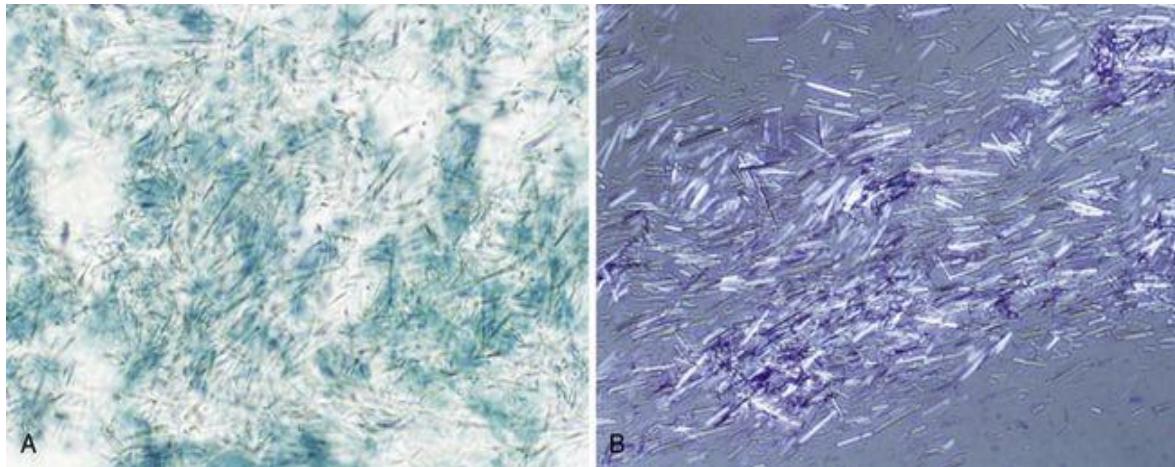


FIGURE 17.32 Gout.

A, The sample is composed of innumerable needle-shaped crystals in a background of granular debris (Papanicolaou stain). B, Examination under polarized light demonstrates the crystals more clearly.

Angiomatoid Fibrous Histiocytoma

AFH, formerly known as *angiomatoid MFH*, is a distinctive mesenchymal neoplasm of uncertain lineage. It is a slowly growing tumor arising in the deep dermis and subcutis of the extremities in children, adolescents, and young adults but may also occur in middle-aged patients and rarely may affect other sites such as the lung and bone. It has an indolent behavior: Local recurrences occur in 2% to 10% and metastases in less than 1% of patients. Histologically AFH has characteristic features: nodules and sheets of histiocytoid cells arranged in ill-defined fascicles or in a whorled pattern; cystic spaces with hemorrhage; and a peripheral, thick fibrous pseudocapsule containing a lymphoplasmacytic infiltrate.



Cytomorphology of angiomatoid fibrous histiocytoma

- at least moderately cellular
- dispersed cells or clusters
- whorled arrangement of tumor cells
- ovoid to spindled histiocytoid cells
- capillaries with spindled endothelial cells in cellular clusters
- bloody background and hemosiderin granules
- occasional lymphocytes and plasma cells

Most aspirate smears are at least moderately cellular. Occasionally, hypocellular, bloody smears are encountered as a result of sampling from the cystic pseudovascular spaces. The tumor cells, isolated or in clusters, have a histiocytoid appearance: ovoid to spindled in shape, ill-defined cell borders, wispy cytoplasm, and often folded nuclei with finely granular chromatin and inconspicuous nucleoli. Some cellular clusters contain capillaries with spindled endothelial cells and a whorled arrangement of tumor cells ([Fig. 17.33](#)). A bloody background and hemosiderin granules are common, whereas the lymphoplasmacytic infiltrate (at the periphery of the lesion) is rarely seen in FNA samples. Occasional nuclear atypia, pleomorphism, and mitoses are present, but these lack prognostic implications.^{[133,137,138](#)}

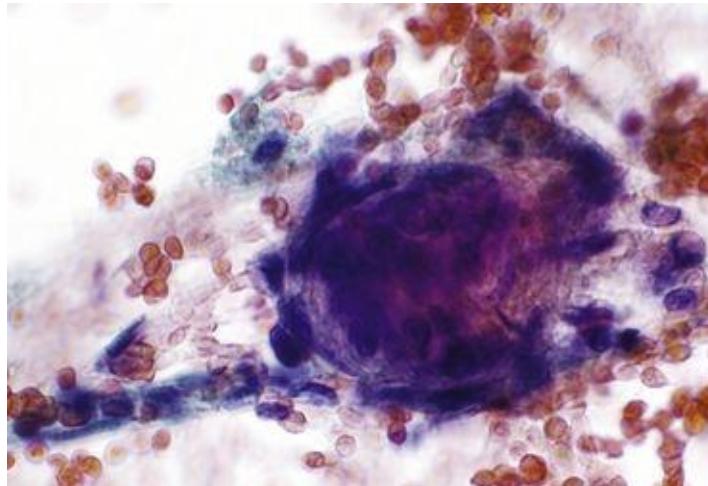


FIGURE 17.33 Angiomatoid fibrous histiocytoma.
Ball-like clusters of histiocytoid cells are commonly associated with capillaries lined by spindle-shaped endothelial cells.

Differential diagnosis of angiomatoid fibrous histiocytoma

- epithelioid hemangioma
- benign fibrous histiocytoma
- nodular fasciitis
- inflammatory myofibroblastic tumor (IMT)
- follicular dendritic cell sarcoma

Epithelioid hemangiomas express endothelial markers (CD31, ERG, and to a lesser extent CD34). Benign fibrous histiocytoma, especially the aneurysmal type, exhibits more variable cytomorphology and Touton-like multinucleated cells. The neoplastic cells of nodular fasciitis and inflammatory myofibroblastic tumor are myofibroblasts, which have plumper nuclei and more distinctive cytoplasmic tails/extensions. A bloody background, hemosiderin, and capillary-rich smears favor AFH over follicular dendritic cell sarcoma. In addition, AFH expresses EMA, desmin, CD68, and CD99 in about 50% cases and lacks reactivity for keratin, S-100 protein, CD34, and follicular dendritic cell markers (CD21, CD35). The presence of an EWSR1 or FUS rearrangement detected by FISH is valuable to confirm the diagnosis of AFH in the right setting (see [Table 17.1](#) and [Figure 17.34](#)).¹³³

Round Cell Neoplasms

The round cell neoplasms are primarily tumors of young patients and account for a majority of solid malignancies in the pediatric age group. Most are high-grade malignancies. Great diagnostic success is attainable in this group of tumors using FNA. The sensitivity and specificity of FNA for the round cell neoplasms exceed 90%, and, with an adequate specimen, a diagnosis of a specific histologic subtype is accurately rendered in 93% of cases. Accuracy is even higher with the use of ancillary techniques (see [Table 17.1](#) for cytogenetic alterations).^{18,34,35,47,139}

Neuroblastoma

Neuroblastoma is the third most common malignant tumor of childhood and the most common malignancy in the neonate. A majority of cases are diagnosed before the age of 5 years. Most cases arise in the adrenal gland or along the intraabdominal portion of the sympathetic chain, but they can occur anywhere along the sympathetic chain.



Cytomorphology of neuroblastoma

- fibrillary matrix
- mostly noncohesive, small undifferentiated cells
- rare neurogenic rosettes
- “salt and pepper” chromatin
- rare ganglion-like cells

Smears are cellular, with variable amounts of fibrillary, metachromatic matrix (neuropil) in the background and between the cells. Necrotic debris and dystrophic calcifications are sometimes observed. Tumor cells are predominantly isolated; cohesive aggregates are less common. Homer-Wright rosettes are present. Most cells are uniformly small and undifferentiated in appearance. Those undergoing differentiation are larger and have a moderate amount of cytoplasm. In better differentiated lesions, schwannian-type spindle cells are present. The nuclei are round to oval, with only slight irregularities, finely granular (“salt and pepper”) chromatin, and small nucleoli. Occasional nuclear

molding is noted. In better differentiated lesions, binucleated and multinucleated ganglion-like cells have a more coarsely granular chromatin, prominent nucleoli, and a moderate amount of cytoplasm. Neuroblastomas are invariably positive for neuron specific enolase and show occasional staining for neurofilament protein, synaptophysin, and GFAP. In cases with schwannian differentiation, immunoreactivity for S-100 is seen. Cytogenetic analysis has become essential for prognosis and clinical management.^{[34,35,47,139,140](#)}

Ewing Sarcoma

Ewing sarcoma is a primitive neoplasm showing varying degrees of neuroectodermal differentiation. It is the second most common sarcoma of bone in children and young adults, after osteosarcoma. The extraskeletal Ewing sarcoma accounts for 10% to 20% of cases and presents as a rapidly enlarging, often painful mass of the deep soft tissues, with a predilection for the thigh, pelvis, paravertebral region of the trunk, and foot. Ewing sarcoma is characterized by an *EWSR1-FLI1* (85%) or *EWSR1-ERG* (10%) gene fusion, or fusions between *EWSR1* and other members of the *ETS* family of transcription factors.^{[1](#)}



Cytomorphology of Ewing sarcoma

- “tigroid” background
- hypercellular smears with dispersed isolated round cells
- “light” cells and “dark” cells
- nuclear molding
- vacuolated cytoplasm

The highly cellular smears often have numerous naked nuclei and a “tigroid” background (a spotted or striped appearance due to innumerable small cytoplasmic fragments). The cells are predominantly isolated, with scattered small cohesive clusters ([Fig. 17.34A](#)). Pseudorosettes are a fairly specific but rare finding. The cells of Ewing sarcoma are approximately two to three times the size of a small lymphocyte and have a high nuclear-to-cytoplasmic ratio. Nuclear molding is prominent.^{[18,34,35,47,141](#)}

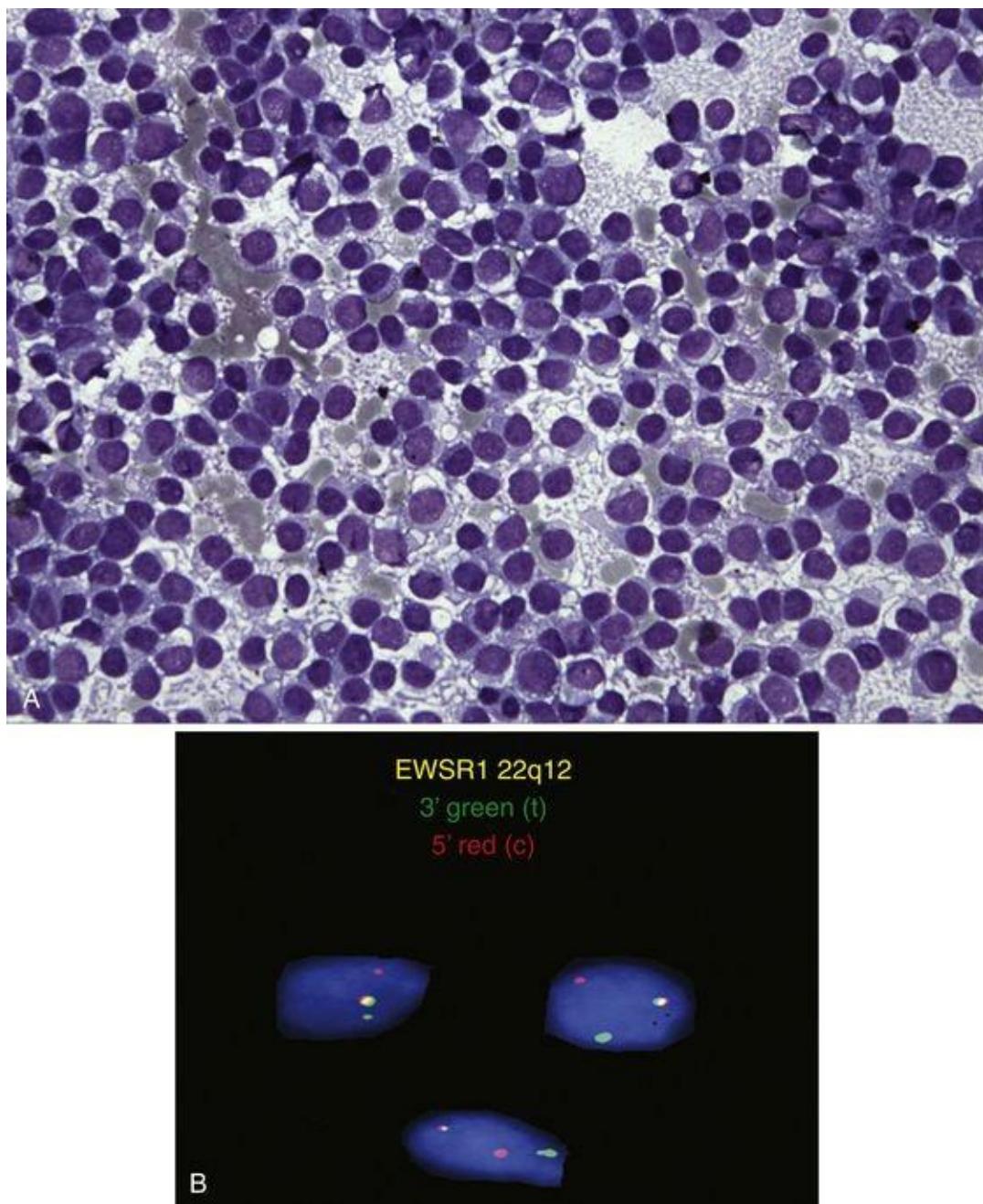


FIGURE 17.34 Ewing sarcoma.

A, The tigroid background, characteristic light and dark cells, and the occasional cytoplasmic vacuoles are better appreciated with air-dried Romanowsky-stained preparations (Romanowsky stain). B, Fluorescence in situ hybridization (FISH) with centromeric (red) and telomeric (green) probes that flank the EWS gene breakpoint show one normal chromosome (*adjacent probes = yellow*), but the split-apart green and red signals indicate a translocation on the other chromosome in all three cells. FISH cannot distinguish the *EWS* gene rearrangements in Ewing sarcoma, desmoplastic small round cell tumor (DSRCT), clear cell sarcoma, angiomyxoid fibrous histiocytoma (AFH), and extraskeletal myxoid chondrosarcoma (EMC), but these tumors are morphologically and immunophenotypically distinct. (Courtesy Dr. Paola Dal Cin, Brigham and Women's Hospital, Boston, Massachusetts, USA.)

Traditionally, two distinct cell types have been described, but these just represent a mixture of viable and dying tumor cells. The “light” (viable) cells have paler nuclei with finely granular, evenly dispersed chromatin and distinct nuclear membranes with slight contour irregularities. One or two small nucleoli or chromocenters are readily identifiable, as is an increased amount of pale cytoplasm. The “dark” (dying) cells are smaller, with dark, irregularly distributed, and smudged chromatin and unappreciable nucleoli. In both cell types, the nucleus is round to oval. No multinucleated or ganglion-type cells are present. A thin rim of cytoplasm contains small intracytoplasmic vacuoles that contain glycogen. Perinuclear cytoplasmic clearing is seen in some cases. Cell borders are typically ill-defined, and fine, wispy cytoplasmic tags can sometimes be seen on Papanicolaou-stained smears.^{[18](#),[34](#),[141](#),[142](#)}



Differential diagnosis of Ewing sarcoma

- alveolar rhabdomyosarcoma
- poorly differentiated synovial sarcoma, small cell type
- desmoplastic small round cell tumor
- precursor lymphoblastic leukemia/lymphoma
- neuroblastoma
- small cell osteosarcoma
- mesenchymal chondrosarcoma

The differential diagnosis includes all small round cell neoplasms but, most important, alveolar rhabdomyosarcoma (RMS), the poorly differentiated small cell type of synovial sarcoma (see [Fig. 17.27](#)); desmoplastic small round cell tumor (DSRCT); neuroblastoma; and precursor lymphoblastic leukemia or lymphoma. RMS usually has larger rhabdomyoblasts with more abundant, denser cytoplasm and multinucleated and spindle-shaped cells in addition to small round cells. Positive staining for EMA and/or cytokeratins along with cytogenetic analysis aids in the correct recognition of the poorly differentiated synovial sarcoma. DSRCT has smaller and more cohesive cells and exhibits a polyphenotypic immunoprofile. Small cell osteosarcomas show more nuclear pleomorphism and hyperchromasia. The cells of precursor lymphoblastic leukemia or lymphoma are accompanied by lymphoglandular bodies and are immunoreactive for lymphoid markers.^{[141](#),[143](#)}

Expression of the *MIC-2* gene product is demonstrable by staining with CD99

(O13), but the diffuse membranous staining pattern is not entirely specific for Ewing sarcoma. Positivity for CD99 in other small round cell tumors, such as mesenchymal chondrosarcoma and lymphoblastic lymphoma or leukemia, poses significant diagnostic problems. Anti-FLI1 antibody has proved to be more specific, but both CD99 and FLI1 can be positive in lymphoblastic leukemia or lymphoma. Up to 30% of Ewing sarcomas show some reactivity for cytokeratins, but they are nonreactive for muscle markers, leukocyte common antigen, and κ and λ light chains. Cytogenetic or molecular genetic analysis is the most important ancillary study for confirming the diagnosis. The detection of the gene fusion transcript *EWSR1/FLI1* or another variant gene fusion, not just an *EWSR1* rearrangement, by FISH, is diagnostically important for Ewing sarcoma (see [Table 17.1](#) and [Fig. 17.34B](#)).^{18,144}



Tumors with *EWSR1* rearrangements

- Ewing sarcoma
- desmoplastic small round cell tumor
- extraskeletal myxoid chondrosarcoma
- clear cell sarcoma of soft tissue
- clear cell sarcoma-like tumor of GI tract
- angiomyomatoid fibrous histiocytoma
- myxoid liposarcoma (small subset)
- myoepithelial tumor of soft tissue
- hyalinizing clear cell carcinoma of salivary gland
- unclassified round cell sarcoma

Desmoplastic Small Round Cell Tumor

DSRCT is an aggressive malignant neoplasm of uncertain histogenesis with a striking predilection for forming large and small tumor masses on the serosal surfaces. Most cases are intraabdominal, but the tumor can involve the pelvis, retroperitoneum, scrotum, and pleura. DSRCT affects mainly male adolescents and young adults between the ages of 15 and 35 years.



Cytomorphology of desmoplastic small round cell tumor

- sheets and clusters of small to intermediate-sized cells
- fragments of desmoplastic stroma

- uniformly round, oval, or slightly angulated cells
- nuclear molding
- vague, small acinar structures

Smears are variably cellular, with sheets and clusters of loosely cohesive, small to intermediate-sized cells. The groups often recapitulate the irregular shapes of the nests and islands seen in histologic sections. Occasional isolated cells are present among fragments of variably cellular and collagenous (desmoplastic) stroma. The tumor cells are uniformly undifferentiated and predominantly round to oval, with rare polygonal or spindled forms ([Fig. 17.35](#)). Nuclei are hyperchromatic, with finely granular chromatin and inconspicuous nucleoli. Nuclear molding can be striking, particularly at the edges of groups. The scant cytoplasm is pale and amphophilic to eosinophilic, and occasional rhabdoid cells are present. Tumors in postchemotherapy cases can have isolated larger cells with conspicuous nucleoli.^{[145,146](#)}

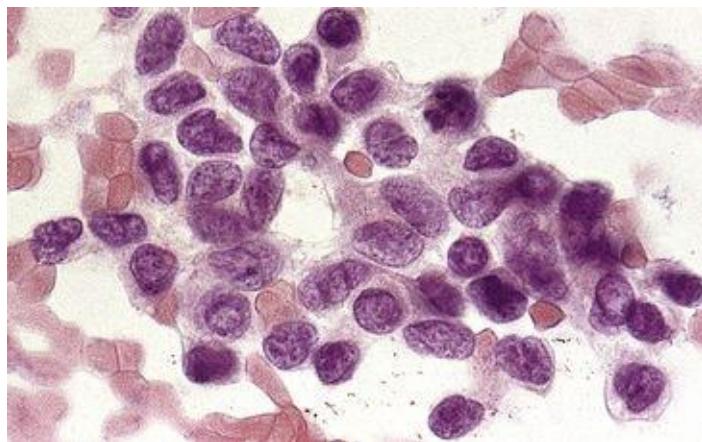


FIGURE 17.35 Desmoplastic small round cell tumor (DSRCT). This tumor differs morphologically from the other round cell neoplasms because its cells retain some cohesiveness and are rarely dispersed as isolated cells (Papanicolaou stain).

The differential diagnosis is similar to that for Ewing sarcoma. DSRCT cells demonstrate a peculiar but characteristic polyphenotypic differentiation, with positive immunoreactivity for low-molecular-weight cytokeratins, EMA, neuroendocrine markers, and desmin, the latter in a characteristic dotlike pattern. They are also positive for Wilms tumor (WT1) (antibody against C-terminal of

WT1) and variably positive for CD99 (cytoplasmic). DSRCT demonstrates a specific cytogenetic abnormality: a t(11;22)(p13;q12) translocation involving the *EWS* gene on 22q12 and the *WT1* gene on 11p13 (see [Fig. 17.34](#) and [Table 17.1](#)).^{35,145,147,148}

Embryonal Rhabdomyosarcoma

Four main subtypes of rhabdomyosarcoma (RMS) are recognized in the current WHO classification: *embryonal*, *alveolar*, *pleomorphic*, and *spindle cell/sclerosing*.¹ The embryonal and alveolar forms occur mainly in children, whereas pleomorphic RMSs occur almost exclusively in adults, and the spindle cell/sclerosing RMSs affect both children and adults. RMS is the most common sarcoma of childhood, and embryonal RMS accounts for a majority in children (60%). The most common sites are the head and neck region (including the orbit and meninges), the genitourinary tract, and the trunk. Because the distinction between its two principal forms, embryonal and alveolar, has significant prognostic implications, and cytomorphology is usually insufficient for this distinction, ancillary diagnostic methods, especially cytogenetic and molecular studies, have become the standard of care.^{2,149}



Cytomorphology of embryonal rhabdomyosarcoma

- predominantly isolated cells
- round and/or spindle-shaped cells
- cellular pleomorphism
- variable number of rhabdomyoblasts
- occasional inclusion-like cytoplasmic condensation (myogenic differentiation)

Aspirates are moderately to highly cellular and composed predominantly of isolated cells, with occasional loose clusters and, uncommonly, a frothy “tigroid” or myxoid background. Tumor cells vary from small-to intermediate-sized and are round, polygonal, or spindle shaped. Larger cells show rhabdomyoblastic differentiation, with elongated, strap-or tadpole-shaped cytoplasm and eccentric nuclei. Anisonucleosis and cellular pleomorphism are especially pronounced among the larger cells. Nuclei have dense hyperchromatic chromatin and

irregular membranes. Occasional cells have inclusion-like cytoplasmic condensations indicating myogenic differentiation. Cytoplasmic cross-striations are rarely seen.^{[35,149–153](#)}

Embryonal RMS cells show myogenic differentiation manifested by positive immunoreactivity for desmin, muscle-specific actin, and the protein products of the specific myogenic nuclear transcription factors myo-D1 and myogenin (myf-4).

Alveolar Rhabdomyosarcoma

Alveolar RMS, a subtype with unfavorable prognosis, is a tumor of older children that occurs most frequently in adolescents. The limbs, head and neck region, and trunk are the most common sites.



Cytomorphology of alveolar rhabdomyosarcoma

- larger cells than in embryonal RMS
- uniformly round to polygonal cells
- variable number of rhabdomyoblasts
- multinucleated tumor giant cells with wreathlike nuclei
- mitoses

Aspirates are highly cellular and infrequently have a “tigroid” background. Most cells are undifferentiated, with uniformly round to polygonal outlines ([Fig. 17.36](#)). Compared to the tumor cells of the embryonal variant, alveolar RMS cells are rounder, with larger and more irregular nuclei. Variable number of rhabdomyoblasts and multinucleated giant tumor cells, with or without wreathlike nuclei, are helpful diagnostic features when present. Mitoses are common.^{[2,149,151,153](#)}

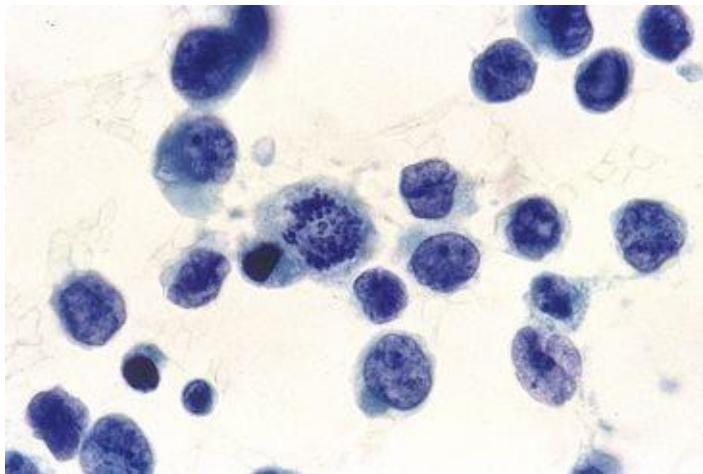


FIGURE 17.36 Alveolar rhabdomyosarcoma (RMS).

The cells disperse individually and are generally larger and more uniformly round to polygonal than those seen in embryonal RMS (Papanicolaou stain).



Differential diagnosis of alveolar rhabdomyosarcoma

- Ewing sarcoma
- neuroblastoma
- poorly differentiated synovial sarcoma
- precursor lymphoblastic lymphoma/leukemia
- extrarenal malignant rhabdoid tumor

Like its embryonal cousin, alveolar RMS is immunoreactive for desmin, muscle-specific actin, myo-D1, and myogenin. In recent years, cytogenetic and/or molecular genetic detection of *PAX3-FOXO1* or *PAX7-FOXO1* have become essential for confirming and refining the diagnosis of RMS (see [Table 17.1](#)).^{9,149}

Extraskeletal Mesenchymal Chondrosarcoma

Extraskeletal mesenchymal chondrosarcoma (MC) is an uncommon bimorphic malignant tumor composed of primitive small round cells and well-differentiated cartilage. It primarily occurs in adolescents and young adults, accounting for about 1% to 2% of all chondrosarcomas. Approximately one third are extraskeletal and affect the soft tissues of the orbit, cranial and spinal meninges, and lower limbs. Rare cases occur in the mediastinum, hand musculature,

retroperitoneum, kidney, and lungs. Recently, a recurrent *HEY1-NCOA2* gene fusion has been identified in all MCs tested, which is of potential diagnostic value.¹



Cytomorphology of extraskeletal mesenchymal chondrosarcoma

- cartilaginous matrix
- two components: small round primitive cells and well-differentiated cartilaginous tissue
- cellular uniformity in the primitive component
- frequent mitoses

Smears are hypercellular and remarkable for two distinct components. One is well-differentiated, hyaline cartilage, with polygonal cells that have vacuolated cytoplasm, a round nucleus, granular chromatin, and a prominent nucleolus. This component has features of a well-differentiated chondrosarcoma. The second is made up of primitive small round cells that aggregate into compact groups, with some isolated cells. The primitive cells are uniform in appearance, each with a round to oval nucleus, granular chromatin, a thick nuclear membrane, an inconspicuous nucleolus, and scant cytoplasm. Mitoses are frequent and often atypical. There can be necrosis and inflammation. A concurrent core biopsy can provide valuable information by preserving the distinctive architectural relationship between the two components. The tumor is S-100-positive in the cartilaginous component. The small round cells can be variably positive for CD99 and desmin. SOX-9 positivity in both components can be helpful to separate MC from other small round cell tumors like Ewing sarcoma and small cell osteosarcoma.¹⁵⁴⁻¹⁵⁸

Epithelioid Neoplasms

Epithelioid soft tissue neoplasms are tumors composed of medium-sized or large, round or polygonal cells with ample cytoplasm. Metastatic carcinoma and melanoma are always in the differential diagnosis. Epithelioid neoplasms yield cellular smears with cohesive tumor cell aggregates as well as numerous individually dispersed neoplastic cells. This group of tumors includes the epithelioid variants of many benign and malignant soft tissue neoplasms like epithelioid schwannoma, epithelioid angiosarcoma as well as specific, well-defined entities like granular cell tumor, epithelioid sarcoma, and alveolar soft part sarcoma (ASPS). Immunohistochemical studies play a decisive role in establishing the diagnosis of most epithelioid soft tissue tumors and excluding metastatic carcinoma, melanoma, and even large cell lymphoma (see [Table 17.3](#)).

TABLE 17.3

IMMUNOPROFILE OF EPITHELIOID CELL NEOPLASMS

TUMOR	CK/EMA	S-100 PROTEIN	HMB-45	DESMIN	CD34	CD31 AND ERG	INI1
Carcinoma and mesothelioma	+	-	-	-	-	-	Retained
Melanoma and CCS	-	+	+	-	-	-	Retained
Chordoma*	+	+	-	-	-	-	Retained
Epithelioid sarcoma	+	-	-	-	+	-	Lost (90%)
Epithelioid angiomyxoma	-/+	-	-	-	+	+	Retained
Epithelioid MPNST	-	+	-	-	-	-	Lost (50%)
Myoepithelial carcinoma	+	+	-	-	-	-	Lost (10%-40%)
PEComa	-	-	+	+	-	-	Retained

CCS, Clear cell sarcoma; MPNST, malignant peripheral nerve sheath tumor; PEComa, perivascular epithelioid cell tumor.

*Brachyury is also positive.

Epithelioid Sarcoma

Epithelioid sarcoma is a rare malignant mesenchymal neoplasm with an epithelioid cytomorphology and immunophenotype. It affects the distal extremities (especially the hands and wrists) of young adults with a predilection for males. The tumors are generally superficial, involving the dermis and subcutis, or tendo aponeurotic. They present as slowly growing masses often of long duration. Pain and ulceration are frequent. A subset of epithelioid sarcomas are termed *proximal type*; they affect the deep soft tissue of the pelvis and perineum and are more aggressive than the classical (distal) form. Both types are characterized by aberrations of the *INI1* gene on 22q, which results in the nuclear loss of INI1 protein expression in a majority of cases.¹⁵⁹



Cytomorphology of epithelioid sarcoma

- numerous dispersed round, polygonal, or spindle-shaped cells
- loosely cohesive clusters of spindle-shaped cells associated with central fibrillary matrix
- eccentrically placed nuclei with vesicular chromatin and small nucleoli
- dense cytoplasm with small vacuoles
- well-defined cell borders
- rhabdoid cells (proximal type)
- binucleation and multinucleation
- necroinflammatory debris

Smears are dominated by isolated cells admixed with small, loosely cohesive clusters of cells associated with central fibrillary matrix. The tumor cells are round, polygonal, or spindle shaped, with mild to moderate nuclear pleomorphism. The nuclei are large, round, and eccentrically placed, with vesicular, occasionally clumped chromatin and one or more small nucleoli. Binucleation and multinucleation are common. The moderate to abundant cytoplasm is dense and can contain vacuoles ([Fig. 17.37](#)). Large atypical epithelioid cells with a rhabdoid appearance are common in the proximal type, and so are necrosis and mitoses (normal or atypical). The vaguely pseudogranulomatous growth pattern on histology is imperceptible on FNA smears.^{[160-163](#)}

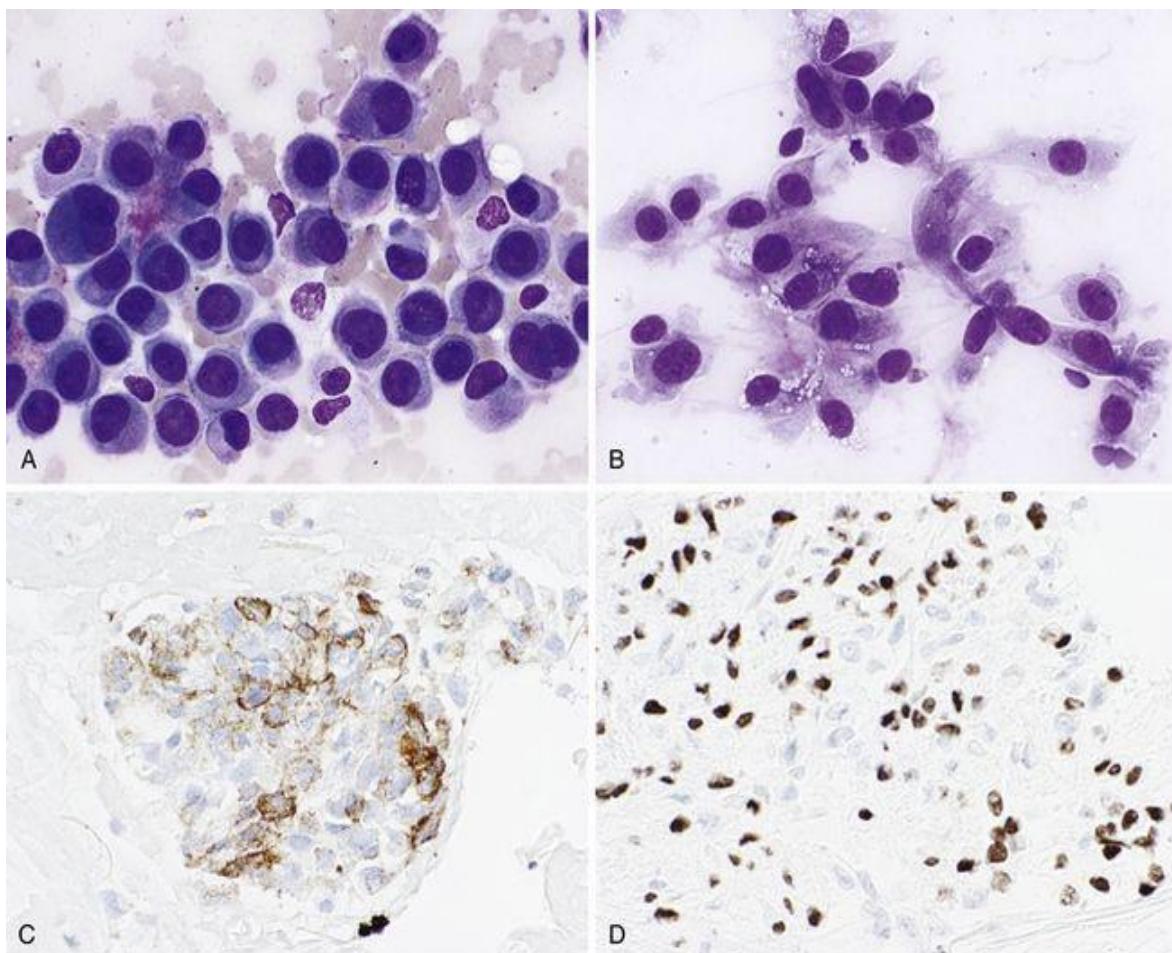


FIGURE 17.37 Epithelioid sarcoma.

A, The smears show dispersed epithelioid cells with eccentrically placed nuclei and dense cytoplasm with sharply defined cell borders (Romanowsky stain). B, The smear shows polygonal, rounded, and spindle-shaped cells, some with cytoplasmic vacuoles (Romanowsky

stain). C, Tumor cells are EMA-positive. D, There is loss of INI1 nuclear expression in tumor cells, which is characteristic of epithelioid sarcoma. Note that the admixed normal endothelial cells and inflammatory cells have retained nuclear expression.



Differential diagnosis of epithelioid sarcoma

- metastatic carcinoma and melanoma
- epithelioid hemangioendothelioma
- epithelioid angiosarcoma
- epithelioid malignant peripheral nerve sheath tumor (MPNST)
- myoepithelial carcinoma of soft tissue
- extrarenal malignant rhabdoid tumor

Even with the help of immunohistochemistry, epithelioid sarcoma is difficult to distinguish from a metastatic carcinoma because it is often diffusely positive for EMA and cytokeratins. The clinical presentation of an extremity mass in a young patient is an important clue to the correct diagnosis. Although most epithelioid sarcomas are negative for vascular markers like CD31 and ERG, they are often positive for CD34, a potential problem in distinguishing them from an epithelioid hemangioendothelioma (EHE) (see [Fig. 4.18](#)) and epithelioid angiosarcoma (see [Fig. 2.37A and B](#)). Positive staining for S-100 protein favors metastatic melanoma, epithelioid MPNST, and myoepithelial carcinoma of soft tissue. Loss of INI1 nuclear expression favors epithelioid sarcoma (both types) and excludes most mimics except for the epithelioid MPNST (50%) and extrarenal malignant rhabdoid tumor (98%), which show INI1 protein loss as well. Extrarenal malignant rhabdoid tumor occurs in younger patients, is CD34-negative, and has mutations in *INI1* gene (see [Table 17.3](#)).¹⁵⁹

Clear Cell Sarcoma of Soft Tissue

Clear cell sarcoma (also known as *malignant melanoma of soft tissues*) is a rare sarcoma with melanocytic differentiation. The tumor affects primarily adolescents and young adults. Manifesting as a slowly growing, often painful mass of the deep tissues of the extremities (foot/ankle), it is usually closely associated with fascia, tendons, or aponeuroses. Most lesions are relatively small (less than 5.0 cm). It is characterized by the presence of a recurrent *EWSR1-ATF1* fusion gene.



Cytomorphology of clear cell sarcoma

- dispersed round, polygonal, and/or spindle-shaped cells
- prominent nucleolus
- wreathlike multinucleated giant cells
- intranuclear cytoplasmic pseudoinclusions
- clear or pale cytoplasm
- tigroid background

Cytologic preparations are moderately to highly cellular, with a remarkably clean or tigroid background. The cells are mostly isolated, with occasional small clusters. They vary in size and shape and can be round, polygonal, and fusiform. Most nuclei are uniform in size, large, round to oval, and eccentrically placed ([Fig. 17.38](#)). They are slightly hyperchromatic, with finely granular, irregularly distributed, vesicular chromatin. The nucleolus is usually central, prominent, and solitary, but two or three smaller nucleoli can be seen. Intranuclear cytoplasmic pseudoinclusions are almost invariably present. Binucleated cells are ubiquitous, and wreathlike multinucleated giant cells are rare but diagnostically important. The moderately abundant cytoplasm is clear and pale, even imperceptible in some cells, and rarely finely vacuolated. Most of the scant intracytoplasmic melanin is found in macrophages. Occasional mitoses are seen, but necrosis is not a typical feature. Most cases are strongly positive for S-100 protein and HMB-45. Clinical and cytogenetic findings (see [Table 17.1](#) and [Fig. 17.34](#)) help distinguish it from metastatic cutaneous melanoma.^{[164,165](#)}

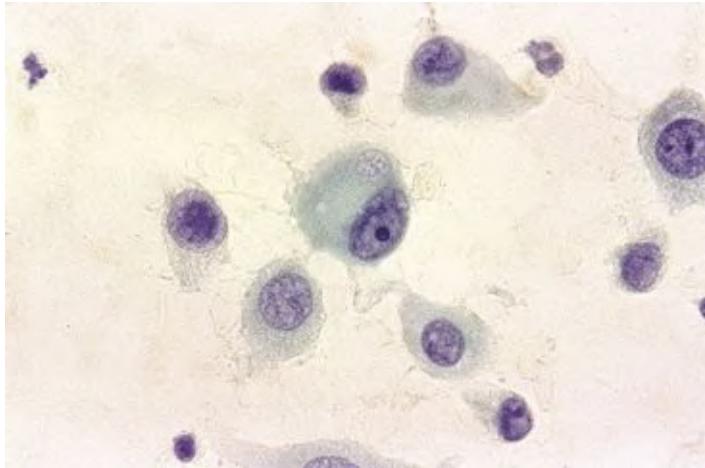


FIGURE 17.38 Clear cell sarcoma of soft tissue.

Cell borders are clearly defined, and delicate cytoplasmic processes extend from a number of the cells (Papanicolaou stain).

Alveolar Soft Part Sarcoma

Accounting for less than 1% of all sarcomas, ASPS is a tumor of older adolescents and young adults, with a slight female predominance. It is a slowly growing, deep-seated, painless mass most commonly found in the lower extremities or limb girdle, especially the anterior thigh. In children, the head and neck region is mainly affected. These tumors are negative for cytokeratins, EMA, chromogranin, and synaptophysin. ASPS is associated with a characteristic genetic anomaly, an unbalanced (X;17) translocation (see [Table 17.1](#)) that results in an *ASPSCR1-TFE3* fusion gene and nuclear overexpression of TFE3 protein.



Cytomorphology of alveolar soft part sarcoma

- naked nuclei
- large, round to polygonal cells
- prominent nucleolus
- abundant granular and fragile cytoplasm

Aspirates show slight to moderate cellularity, with numerous naked nuclei. Often, a background of disrupted cytoplasmic contents and capillary networks devoid of tumor cells is seen. The cells are largely noncohesive but sometimes aggregate in large groups. Pseudoalveolar arrangements surrounded by a thin vascular structure, though a characteristic feature on histology, is a very rare

finding on smears. The cells are uniformly round to polygonal with some variation in size. The mildly pleomorphic, hyperchromatic, large nuclei are round to oval, with vesicular, finely stippled chromatin; smooth nuclear contours; and a central, prominent, round nucleolus. The fragile cytoplasm is abundant and finely granular ([Fig.17.39](#)). Intracytoplasmic and extracellular periodic acid–Schiff (PAS)–positive rhomboid crystals are a helpful feature but are rarely identified. Mitotic activity is low.^{[166,167](#)}

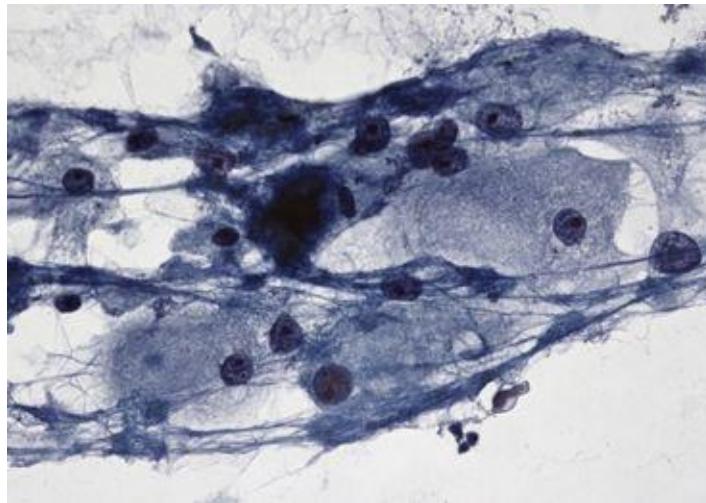


FIGURE 17.39 Alveolar soft part sarcoma (ASPS).

Large polygonal cells have abundant granular cytoplasm, large nuclei, vesicular chromatin, smooth nuclear contours, and a central, prominent, round nucleolus (Papanicolaou stain).



Differential diagnosis of alveolar soft part sarcoma

- granular cell tumor
- melanoma
- rhabdomyoma
- paraganglioma
- renal cell carcinoma
- PEComa

Renal cell carcinoma, paraganglioma, rhabdomyoma, melanoma, granular cell tumor, and PEComa resemble ASPS, but all lack rhomboid crystals and all have a different immunoprofile.^{[167](#)} ASPS tumor cells show strong nuclear staining with

TFE3 and variable staining with desmin and S-100 protein, and are negative for cytokeratins, EMA, chromogranin, and HMB45. A specific diagnosis can usually be made on the basis of its distinctive cytomorphology, immunoprofile, and characteristic chromosomal translocation or gene fusion.

Epithelioid Hemangioendothelioma

EHE is a rare malignant vascular neoplasm, affecting adults older than 20 years of age. It occurs in the extremities, head and neck region, trunk, mediastinum, bone, and visceral organs such as lung and liver. About half arise from a large or medium-sized vein (angiocentric). Multicentricity is frequent. EHE is characterized by cords of epithelioid endothelial cells embedded in a myxohyaline stroma. Recently, a *WWTR1-CAMTA1* gene fusion has been identified in most EHE cases.¹



Cytomorphology of epithelioid hemangioendothelioma

- round to polygonal plasmacytoid cells
- only slight nuclear pleomorphism and frequent binucleation
- frequent nuclear grooves and pseudoinclusions
- dense cytoplasm with occasional intracytoplasmic lumina
- hyaline stroma
- few mitoses

Smears are variably cellular, with fragments of metachromatic, hyaline, or chondroid stroma. Although predominantly isolated, the cells can form small, loose aggregates and rosettelike, pseudoacinar arrangements. The cells are monomorphic, with round, oval, or polygonal contours; a plasmacytoid appearance, and moderate amounts of dense cytoplasm (see Fig. 4.18). Rare sharply demarcated intracytoplasmic lumina (vacuoles) distort the nucleus. Nuclei are round to oval with frequent binucleation and minimal nuclear pleomorphism. The chromatin is unevenly distributed, and one or two small nucleoli are seen. Occasional larger cells have hyperchromatic, irregular nuclei with nuclear membrane clefts and protrusions. Mitoses are seldom seen.^{168,169} Tumor cells are strongly and diffusely positive for the vascular markers CD31, CD34, and ERG. About 20% to 30% are immunoreactive for cytokeratin, but

they are negative for EMA.

Differential diagnosis of epithelioid hemangioendothelioma

- metastatic carcinoma
- melanoma
- epithelioid sarcoma
- angiosarcoma
- mesothelioma
- epithelioid hemangioma

The tumor is distinguished cytologically from metastatic carcinoma, melanoma, epithelioid sarcoma, and angiosarcoma by its lower degree of nuclear atypia and mitotic activity. EHEs of the lung have a predilection for spreading to the pleural surfaces, clinically mimicking a mesothelioma and not infrequently manifesting with a pleural effusion. The similarity extends to morphologic evaluation: EHEs strongly resemble an epithelioid mesothelioma. The distinction rests with immunohistochemistry. Tumor cells of epithelioid mesothelioma are positive for mesothelial cell markers (calretinin, WT1) whereas those of EHE are reactive for endothelial cell markers (CD31, CD34, ERG). Epithelioid hemangioma is more superficially located and more obviously vasoformative. It has histiocytoid endothelial cells and lacks hyaline or chondroid stroma and is often associated with an inflammatory infiltrate.

Epithelioid Angiosarcoma

Epithelioid angiosarcoma, another rare but highly aggressive malignant vascular tumor, has a marked predilection for males, usually adults in middle to late life. It is a rapidly growing tumor of deep soft tissues, but it can be encountered in a variety of other sites, including the lower extremities, retroperitoneum, and abdominal cavity.¹⁷⁰

Cytomorphology of epithelioid angiosarcoma

- extensively bloody background
- large, noncohesive epithelioid cells

- moderate to marked nuclear pleomorphism
- prominent nucleolus
- occasional angioformative structures: pseudoacini with central erythrocytes or intracytoplasmic lumina containing erythrocytes
- numerous mitoses

Smears are variably cellular, depending on the degree of dilution by blood. The mostly noncohesive cells infrequently aggregate into small clusters and sometimes even form pseudoacini. They are large, rounded epithelioid cells with moderately to markedly pleomorphic nuclei and smooth nuclear contours (see [Fig. 2.37A and B](#)). Binucleation and especially multinucleation are rare. Mitoses are frequent. The nuclei are eccentrically placed within an abundant, finely granular cytoplasm. Intracytoplasmic hemosiderin is detected in many cases. Angioformative structures (e.g., pseudoacini with central erythrocytes; intracytoplasmic lumina [vacuoles] containing intact or fragmented erythrocytes) are rare but very useful findings.[^{171–173}](#)



Differential diagnosis of epithelioid angiosarcoma

- metastatic carcinoma
- metastatic melanoma
- malignant mesothelioma
- epithelioid hemangioendothelioma
- large cell lymphoma
- germ cell tumor
- epithelioid sarcoma
- epithelioid variant of other sarcomas

Epithelioid angiosarcoma should be considered in the differential diagnosis of any poorly differentiated neoplasm with epithelioid cytomorphology, especially in the abdominal cavity. Epithelioid angiosarcoma cells stain with endothelial markers CD31, CD34, ERG, and FLI1, and with cytokeratins in up to 50% cases (see [Table 17.3](#)).

Granular Cell Tumor

Granular cell tumors are relatively common, slowly growing neoplasms with neuroectodermal differentiation. With rare exceptions, they are benign. They occur most commonly in the head and neck region, including the tongue of middle-aged adults. Other common sites include the extremities, gastrointestinal tract, respiratory tree, breast, and thyroid. Many granular cell tumors are sampled by FNA during the workup of breast lesions.¹⁷⁴



Cytomorphology of granular cell tumor

- bare nuclei in a granular background
- uniform cellular appearance
- small nuclei
- abundant granular cytoplasm

Smears show numerous naked nuclei and a finely granular background derived from the abundant, fragile cytoplasm of the tumor cells. Intact cells are uniform and occur as syncytial groups and isolated cells ([Fig.17.40](#)). The small, round to oval nuclei are minimally pleomorphic, with finely granular chromatin and a small but conspicuous nucleolus. Very rare intranuclear cytoplasmic invaginations can be seen, and cell borders are typically ill-defined. Mitoses and necrosis are not seen in aspirates of benign lesions.¹⁷⁴ Malignant granular cell tumors are exceedingly rare, and it is difficult to definitively separate them from their benign counterparts. The cytologic features that are suggestive of malignancy include spindle cell morphology, cellular pleomorphism, enlarged nucleoli, and mitoses.^{175,176} Tumor cells in both benign and malignant forms show strong cytoplasmic staining for S-100 protein. They are also immunoreactive for other melanocytic markers, like microphthalmia transcription factor (MiTF) and NKI/C3, a melanoma-associated antigen. Of interest, they are also positive for CD68, TFE3, inhibin, and calretinin. They are negative for muscle markers, GFAP, HMB-45, and cytokeratins.

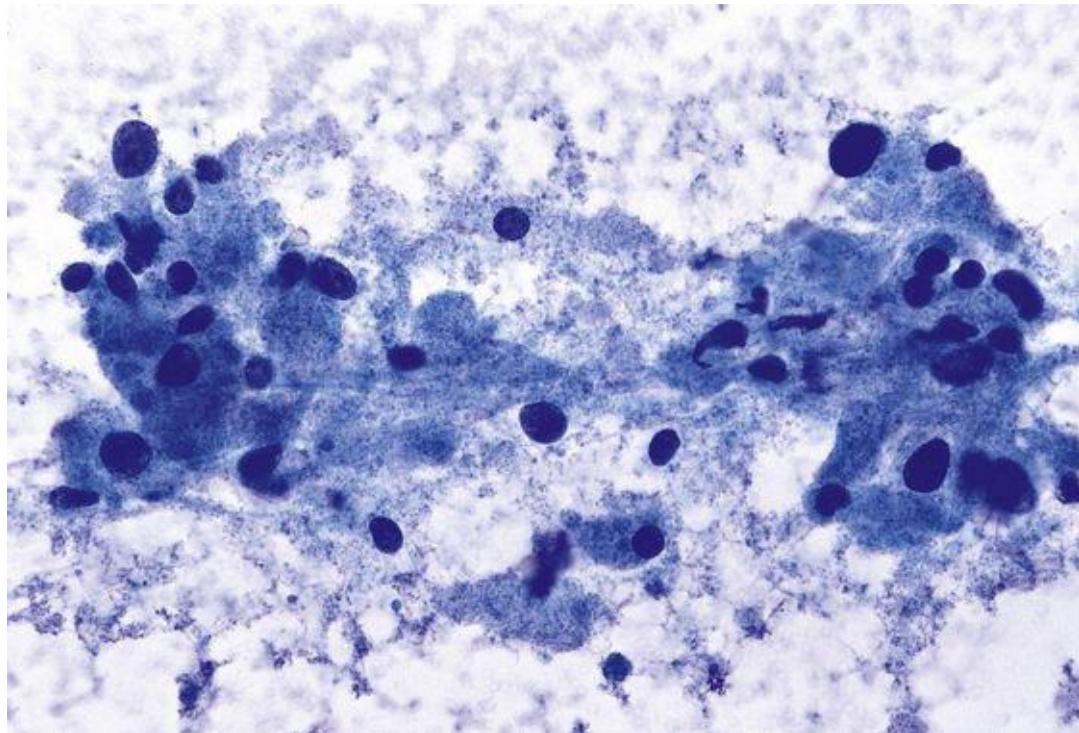


FIGURE 17.40 Granular cell tumor.

Small nuclei within abundant cytoplasm result in a low nuclear-to-cytoplasmic ratio (Papanicolaou stain).



Differential diagnosis of granular cell tumor

- fat necrosis
- Whipple disease
- rhabdomyoma
- alveolar soft part sarcoma (ASPS)
- renal cell carcinoma

Fat necrosis exhibits bean-shaped or oval histiocytes with foamy cytoplasm, multinucleated giant cells, and other inflammatory cells. A similarly mixed cell population is typical of Whipple disease. Rhabdomyoma may contain multinucleated cells and is negative for S-100 protein. ASPS has prominent nucleoli, frequent bi-and multinucleation, and pseudoacinar or pseudoalveolar arrangements. Renal cell carcinomas usually have some cells with clear cytoplasm, and they are positive for PAX8.

Pleomorphic Neoplasms

Many pleomorphic soft tissue tumors are poorly differentiated or dedifferentiated forms of soft tissue tumors that have a clear line of differentiation (e.g., forming adipose tissue, skeletal muscle). Pleomorphic and dedifferentiated liposarcomas have been discussed above, and pleomorphic RMS is discussed in this section. With the exception of atypical fibroxanthoma and pleomorphic lipoma, pleomorphic soft tissue tumors are aggressive and considered high-grade for management purposes. The most commonly reported pleomorphic sarcoma is pleomorphic MFH, now known as *undifferentiated pleomorphic sarcoma*. With wide sampling and immunoprofiling, most “first impression” pleomorphic MFHs prove to be other poorly differentiated malignant neoplasms (e.g., pleomorphic osteosarcomas, leiomyosarcomas, rhabdomyosarcomas, liposarcomas, or high-grade myxofibrosarcomas). In those instances when all efforts fail to reveal a line of differentiation, *undifferentiated pleomorphic sarcoma* is preferable to the histogenesis-implying term *malignant fibrous histiocytoma*.¹

Undifferentiated Pleomorphic Sarcoma

In the most recent WHO classification of soft tissue tumors, undifferentiated pleomorphic sarcomas are considered part of the morphologic spectrum of all undifferentiated soft tissue sarcomas (USTSs).¹ USTSs include undifferentiated sarcomas with round cell, spindle cell, pleomorphic, epithelioid, or “not otherwise specified” cytomorphology. The undifferentiated pleomorphic sarcomas probably account for 5% to 10% of sarcomas in adults older than age 40 and occur mostly in the extremities and trunk; some are radiation-associated.



Cytomorphology of undifferentiated pleomorphic sarcoma

- hypercellular smears
- variable proportions of cellular clusters and dispersed cells
- marked cellular and nuclear pleomorphism and anaplasia
- giant cells with solitary or multiple nuclei
- numerous mitoses including atypical forms
- necrosis

The highly cellular smears contain variable proportions of cellular clusters and dispersed cells in a necrotic background. All the tumors in this group share impressive morphologic features that include markedly pleomorphic, spindle-shaped, and/or epithelioid cells with abundant cytoplasm; giant cells with solitary or multiple nuclei; prominent necrosis; and brisk mitotic activity with atypical forms (Fig. 17.41). It is difficult to identify any cytomorphic clues to suggest a line of differentiation in these tumors. The tumor cells can be focally positive for keratin, actin, desmin, or EMA but are usually negative for all other specific immunomarkers. Cytogenetics usually reveals a highly complex karyotype with marked intratumoral heterogeneity.

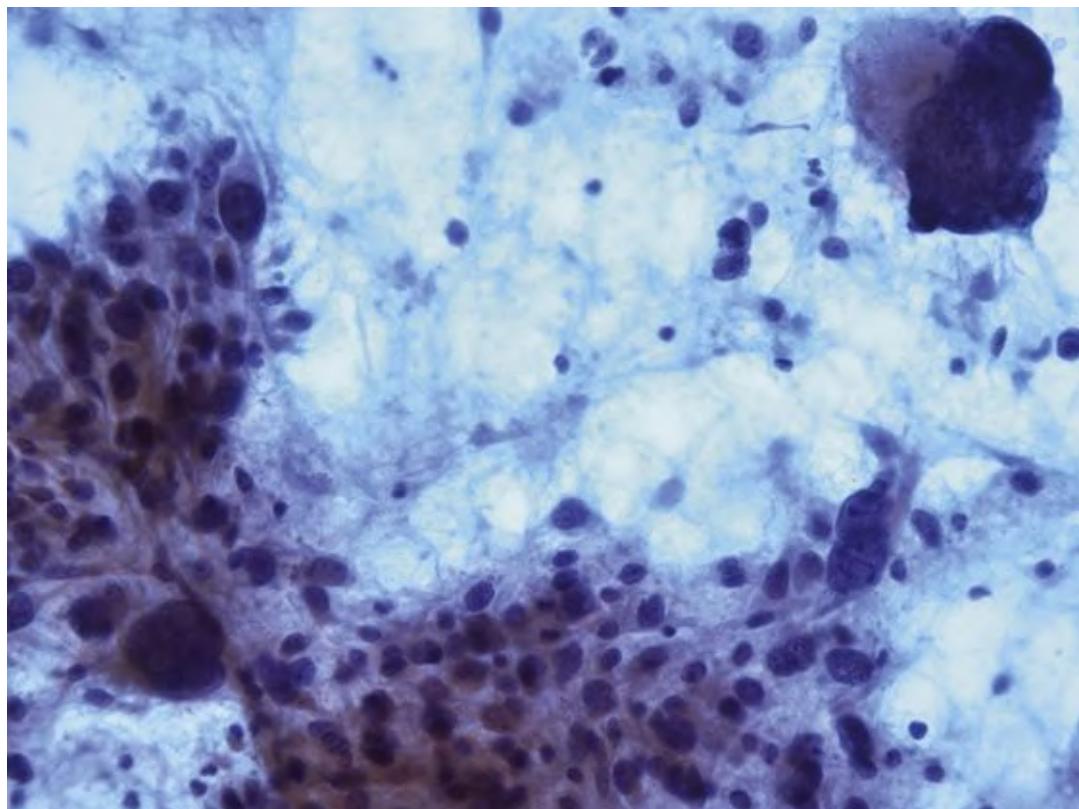


FIGURE 17.41 Undifferentiated high-grade pleomorphic sarcoma. The bizarre tumor cells and necrotic background are nonspecific findings seen with any pleomorphic soft tissue neoplasm (Papanicolaou stain).

Differential diagnosis of undifferentiated pleomorphic sarcoma

- sarcomatoid carcinoma

- sarcomatoid mesothelioma
- melanoma
- anaplastic large cell lymphoma (ALCL)
- pleomorphic sarcoma with a specific line of differentiation (e.g., rhabdomyosarcoma, liposarcoma)
- dedifferentiated sarcoma

Sarcomatoid carcinoma, sarcomatoid mesothelioma, malignant melanoma, and anaplastic large cell lymphoma (ALCL) can have the same pleomorphism, including a mixture of highly atypical spindle cells, epithelioid cells, and giant cells. Immunohistochemical studies are usually necessary to make the distinction. Sarcomatoid carcinoma and mesothelioma should have at least focal positivity for one or more cytokeratins, and mesothelioma is additionally positive for mesothelial cell markers (calretinin, WT1, and D2-40). ALCL shows noncohesive large, anaplastic, round cells with an embryo-like nucleus (see [Fig. 12.25](#)). ALCL cells are positive for CD30 and in some cases for ALK as well. Melanoma often has melanin pigment in tumor cells and in adjacent macrophages, and the diagnosis can be confirmed by immunoreactivity for melanocytic markers. A differentiated pleomorphic sarcoma, like pleomorphic liposarcoma or pleomorphic rhabdomyosarcoma (see [Fig. 17.10](#)) is confirmed by demonstrating a clear line of differentiation with or without ancillary techniques. Clinical history and the presence of a differentiated area are essential for the diagnosis of a dedifferentiated sarcoma. Finally, the diagnosis of a radiation-induced, high-grade sarcoma should be considered when an undifferentiated high-grade pleomorphic sarcoma occurs at the site of prior radiation therapy.

Because an FNA represents only a portion of the neoplasm, excluding carcinoma, mesothelioma, melanoma, and lymphoma might not be possible due to sampling error. A pleomorphic neoplasm that is negative for differentiation markers might still be best reported as “pleomorphic malignant neoplasm, cannot classify further.”

Pleomorphic Rhabdomyosarcoma

Pleomorphic RMS is a high-grade sarcoma showing large polygonal cell cytomorphology and skeletal muscle differentiation. It most commonly occurs in the lower extremities of elderly patients.



Cytomorphology of pleomorphic rhabdomyosarcoma

- hypercellular smears with predominantly dispersed cells
- marked cellular and nuclear pleomorphism
- **large rhabdoid cells:** eccentrically located nuclei, prominent nucleoli, abundant cytoplasm with a perinuclear density
- frequent binucleation and multinucleation
- numerous mitoses including atypical forms
- necrosis

Smears are hypercellular, with numerous dispersed cells and occasional small clusters of polygonal cells. Cellular and nuclear pleomorphism is marked, with frequent very large cells. Most cells exhibit a typical rhabdoid cytomorphology: eccentrically located nuclei with prominent nucleoli and abundant cytoplasm with a perinuclear globoid density. Binucleation and multinucleation are very common. Mitoses and necrosis are also present.



Differential diagnosis of pleomorphic rhabdomyosarcoma

- heterologous rhabdoid differentiation of other sarcomas
- pleomorphic leiomyosarcoma
- proximal-type epithelioid sarcoma
- metastatic melanoma
- metastatic carcinoma

The rhabdoid cytomorphology is nonspecific ([Fig. 17.42](#)) and can be seen focally in many nonmyogenic tumors, including melanoma, carcinoma (e.g., renal cell carcinoma) and sarcomas like the proximal type of epithelioid sarcoma. Demonstration of nuclear expression of myogenin (myf4) in addition to diffuse desmin positivity is critical for establishing rhabdomyoblastic differentiation. In a small needle biopsy, a heterologous rhabdoid component of other sarcomas like malignant peripheral nerve sheath tumor and dedifferentiated liposarcoma should be given serious consideration before rendering a diagnosis

of pleomorphic RMS.

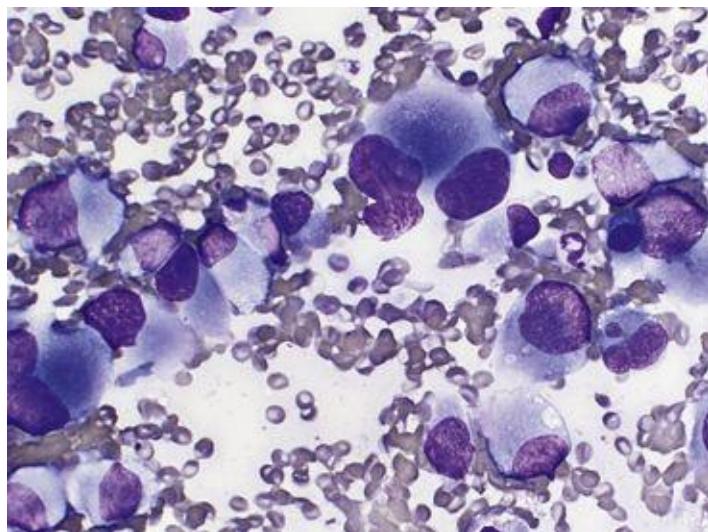


FIGURE 17.42 Pleomorphic sarcoma with rhabdoid features.

Numerous dispersed rhabdoid cells have eccentrically located nuclei with prominent nucleoli, abundant cytoplasm, and a perinuclear cytoplasmic density (Romanowsky stain).

Dedifferentiated Sarcomas

Three different soft tissue tumors can undergo dedifferentiation: *well-differentiated liposarcoma*, *well-differentiated chondrosarcoma*, and *chordoma*. Recent rapid growth of a previously stable mass is typical of all three tumors, and the correct classification of each hinges upon identifying an adjacent well-differentiated component. For this reason, cytologists must boldly request additional, wider sampling when confronted with an undifferentiated pleomorphic or giant cell lesion during rapid onsite evaluation.

Dedifferentiation occurs with approximately 10% of all chondrosarcomas, usually in patients about 10 years older than those with a conventional chondrosarcoma. Dedifferentiated chordoma is an exceedingly rare event.

Dedifferentiated liposarcoma is the most common of the three and accounts for up to 10% of liposarcomas. It is usually retroperitoneal or intraabdominal. Its morphology is variable, but most cases resemble either an undifferentiated pleomorphic sarcoma or a myxofibrosarcoma (see [Fig. 17.14](#)). While the transition between the dedifferentiated and well-differentiated components usually occurs in an abrupt fashion, in rare cases it can be a gradual intermingling mimicking pleomorphic liposarcoma in limited samples. In exceptional cases, rare lipoblasts are present in otherwise nonlipogenic areas, also mimicking pleomorphic liposarcoma.⁶¹ Cytogenetic analysis can assist with the diagnosis, because dedifferentiated liposarcoma, in addition to complex aberrations, retains the same giant marker and ring chromosomes as its well-differentiated counterpart. When a karyotype cannot be obtained, detection of *MDM2* and *CDK4* gene amplifications by molecular cytogenetic approaches (FISH and/or PCR) can be of great diagnostic value.^{9,59}

NonNeoplastic Soft Tissue Lesions

Idiopathic Retroperitoneal Fibrosis

Idiopathic retroperitoneal fibrosis (IRF) is an enigmatic lesion affecting mostly males in their fourth to fifth decades of life. The typical lesion shows bilateral periureteral fibrosis at the level of the L4 vertebra, often encasing the aorta.^{[177](#)} A subset of IRF cases are now recognized to be associated with IgG4-related disease.^{[178](#)}



Cytomorphology of idiopathic retroperitoneal fibrosis

- mixed inflammatory infiltrate
- prominent crush artifact
- fragments of fibrous tissue
- spindle-shaped fibroblastic cells

The moderately cellular smears contain rare fragments of fibrous tissue and inflammatory cells, including small lymphocytes, plasma cells, histiocytes, rare eosinophils, and mast cells. Neutrophils are also occasionally present. The inflammatory cells are partially obscured by prominent crush artifact. Sparse, benign-looking, spindle-shaped fibroblastic cells are visible in thicker fragments of the fibrous tissue. Nuclei lack atypia; nucleoli are inconspicuous; and mitoses and necrosis are absent. The inflammatory infiltrate exhibits a slight predominance of B cells over T cells (60% versus 40%).^{[177,179,180](#)}



Differential diagnosis of idiopathic retroperitoneal fibrosis

- Hodgkin disease
- non-Hodgkin lymphoma
- well-differentiated inflammatory liposarcoma
- metastatic carcinoma with desmoplastic stroma
- sclerosing mesenteritis

The differential diagnosis includes any lesion with prominent fibrosis and inflammation. The nodular sclerosis variant of Hodgkin lymphoma has distinguishing Reed-Sternberg cells, lacunar cells, and associated clinical features. The lymphoid component of non-Hodgkin lymphoma is usually more monomorphic. Well-differentiated inflammatory liposarcoma should have lipoblasts, more cytologic atypia, and immunoreactivity for MDM2 and CDK4. The desmoplastic stroma of a metastatic carcinoma can resemble the fibrotic areas of idiopathic retroperitoneal fibrosis, and thus immunocytochemistry for keratins and EMA are helpful in excluding this possibility. Sclerosing mesenteritis usually involves the small bowel mesentery, with more prominent fat necrosis.

Elastofibroma

Elastofibroma is a relatively uncommon lesion thought to represent a reactive process, possibly from repeated trauma to the tissues between the scapulae and the chest wall. Elderly individuals, particularly women, are most commonly affected. It manifests as a slowly growing subscapular mass attached to the rib periosteum.



Cytomorphology of elastofibroma

- hypocellular, waxy matrix
- linear, dentate or serrated, rodlike structures
- serrated globular bodies
- scant uniform bland fibroblasts

Cytologic preparations are remarkably hypocellular. Rare fibroblasts with bland nuclear features are seen within a homogeneous, waxy, hyalinized matrix. These stromal fragments have sharp borders. Linear, slightly curved or angulated, rodlike elastic fibers of various sizes, lengths, and widths typically have dentations or serrations along the long edge and should not be mistaken for degenerated fungi. The globular bodies also have serrated borders and vary in diameter. Most are solitary, but occasional loose or tight clusters are found. A nonspecific diagnosis is avoided only if the elastic fibers and globular bodies are noted.¹⁸¹

Amyloidoma (Tumoral Amyloidosis)

An amyloidoma is an uncommon mass lesion caused by extensive amyloid deposition. Some but not all cases are associated with systemic amyloidosis. It can occur in many visceral organs and extremely rarely in bone and soft tissues.



Cytomorphology of amyloidoma

- fragments of waxy, amorphous material with sharply defined edges and embedded fibroblasts and capillaries
- multinucleated giant cells
- scant inflammatory cells including plasma cells

Cytologic preparations are hypocellular and contain abundant fragments of acellular, waxy, amorphous material with sharply defined edges and bland mesenchymal cells ([Fig. 17.43](#)). It is a metachromatic blue-purple with Romanowsky stains and pink, orange, or green with the Papanicolaou stain. Scattered multinucleated giant cells are common. Inflammatory cells including plasma cells may or may not be present. A nonspecific diagnosis is avoided only if the amyloid deposition is confirmed by a Congo red stain and/or ultrastructural analysis.^{[182,183](#)}

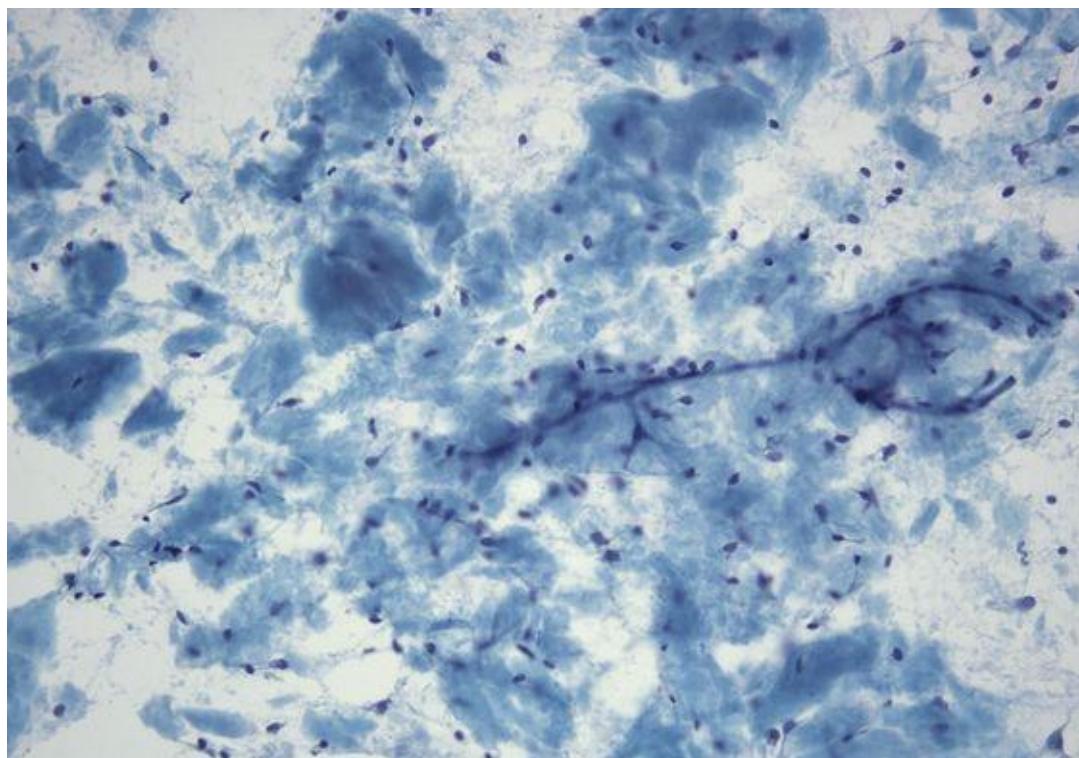


FIGURE 17.43 Amyloidoma.

Fragments of waxy, amorphous material have sharply defined edges with embedded fibroblasts and capillaries (Papanicolaou stain).

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CHAPTER 18

Laboratory Management

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In the United States, the cytology laboratory is one of the most regulated of all the laboratories involved in clinical testing. To effectively manage and work in a cytology laboratory, personnel must be familiar with the relevant regulatory agencies and professional organizations (“the players”) and their licensure, accreditation, quality control, billing, and safety regulations (“the rules”).

Agencies and Organizations

Centers for Medicare & Medicaid Services

The United States does not have a national, single-payer health insurance system. Rather, U.S. citizens obtain health insurance from a variety of federal or private carriers. The Centers for Medicare & Medicaid Services (CMS), formerly called the Health Care Financing Administration (HCFA), is a federal agency that provides health insurance to qualified individuals through the **Medicare** and **Medicaid** programs. Together, Medicare and Medicaid provide health care to one in three Americans (more than 100 million people). Besides Medicare and Medicaid, CMS also administers the **Clinical Laboratory Improvement Amendments of 1988 (CLIA 88)** and **Health Insurance Portability and Accountability Act of 1996 (HIPAA)** programs, as well as some other national health programs.

CMS is 1 of 11 divisions of the U.S. Department of Health and Human Services (DHHS). Some of the other divisions of DHHS are the National Institutes of Health (NIH), the U.S. Food and Drug Administration (FDA), and the Centers for Disease Control and Prevention (CDC). DHHS is a Cabinet-level department created in 1953, originally as the Department of Health, Education, and Welfare (HEW). In 1979, HEW became DHHS when the Department of Education split off as a separate department.

The Joint Commission

The Joint Commission (formerly, the Joint Commission on Accreditation of Healthcare Organizations) is an independent, not-for-profit organization that evaluates and accredits more than 20,000 health care organizations and 3000 laboratories in the United States. It is governed by a Board of Commissioners that includes physicians, consumers, and administrators, and its corporate members include professional societies like the American Medical Association (AMA) and the American Hospital Association.

Founded in 1951, The Joint Commission has developed standards for evaluating hospitals, assisted living facilities, outpatient services, and clinical laboratories. It has been accrediting hospitals since 1953 and laboratories since 1979. Laboratories surveyed by The Joint Commission have been deemed certifiable under CLIA 88 requirements. To earn accreditation, a hospital or

laboratory undergoes an onsite survey. To maintain accreditation, hospitals are surveyed every 3 years and laboratories every 2 years. Surveys have been unannounced since 2006.

In 2004, The Joint Commission initiated a new survey process that uses patient “tracers,” an evaluation method that focuses on service processes and traces patients through the care they have received. Surveyors also conduct systems tracers to analyze key operational systems that directly impact the quality and safety of patient care. The new process has shifted the emphasis from survey preparation to continuous improvement of operational systems that enhance safety and reduce medical errors.

College of American Pathologists

The College of American Pathologists (CAP) is a professional society of pathologists that offers a Laboratory Accreditation Program that inspects more than 7000 laboratories in the United States and worldwide. Surveys are performed every 2 years and have been unannounced since 2006. Volunteer surveyors use inspection checklists that undergo regular revision to reflect federal regulations and professional standards. Like those inspected by The Joint Commission, CAP-inspected laboratories are eligible for a CLIA certificate.

Commission on Accreditation of Allied Health Education Programs

The Commission on Accreditation of Allied Health Education Programs (CAAHEP) (formerly, the Committee on Allied Health Education and Accreditation [CAHEA]), is an independent certifying agency for health education programs. It accredits cytotechnology training programs in the United States upon the recommendation of the Cytotechnology Programs Review Committee of the American Society of Cytopathology.

Occupational Safety and Health Administration

The Occupational Safety and Health Administration (OSHA) was created by Congress in 1971 under the Occupational Safety and Health Act, also known as “the safety bill of rights,” a response to public outcry in the 1960s against rising workplace-related injuries and deaths. A division within the Department of Labor, OSHA’s mission is to prevent injuries, illnesses, and deaths on the job.

OSHA conducts inspections in response to reports of high injury rates or imminent dangers, fatalities or serious accidents, and employee complaints. Violations of standards can result in stiff monetary penalties.

Of particular relevance to cytology laboratories are the **OSHA Bloodborne Pathogens Standard** and the **OSHA Laboratory Standard**, which are available on the OSHA website.

National Fire Protection Association

Despite its name, the National Fire Protection Association (NFPA) is an international nonprofit organization, founded in 1896, and serving as the premier advocate of fire protection. Its more than 300 safety codes and standards influence the design and construction of every building in the United States. The cytologist must be familiar with applicable safety codes to ensure that they are being observed.

Of the numerous standards published by NFPA, the two of greatest relevance to cytology laboratories are the **Standard for Health Care Facilities** and the **Standard on Fire Protection for Laboratories Using Chemicals**.

Regulations

Clinical Laboratory Improvement Amendments of 1988

In the 1980s there was an extraordinary flurry of media attention on the problem of false-negative Papanicolaou tests (Paps). The sentinel article appeared on the front page of the *Wall Street Journal*.¹ These media reports were a driving force behind legislation enacted by the U.S. Congress called the Clinical Laboratory Improvement Amendments of 1988 (CLIA 88). (A less stringent act, CLIA 67, had been passed in the 1960s, which mandated rescreening of 10% of negative Paps.) The final regulations of CLIA 88 were published in 1992, and minor modifications appeared in 2003.^{2,3} They can be found at <http://www.cdc.gov/clia/regs/toc.aspx>. Congress charged CMS with the implementation of these standards.



Clinical Laboratory Improvement Amendments of 1988 established:

- daily workload limits for cytotechnologists
- new quality control procedures
 - 5-year retrospective rescreening
 - cytologic-histologic correlation
- pathologist review of
 - all abnormal Paps and “reactive and reparative changes”
 - all nongynecologic specimens
- proficiency testing for cytology
- unannounced specialized surveys

To bill for and receive Medicare or Medicaid payments, a clinical laboratory must have a CLIA certificate. To obtain a certificate, a laboratory must be accredited by one of two approved accrediting organizations, The Joint Commission or the CAP. In a few states like Washington and New York, a laboratory may obtain a state license in lieu of a CLIA certificate.⁴

Specialized surveys, required by CLIA 88, are performed by the American Society for Cytotechnology (ASCT) on a small proportion of cytology

laboratories with CLIA certificates.⁵ A laboratory is selected either at random or if a complaint has been lodged against it with the CMS.⁴ The survey team evaluates the laboratory's operations and reviews at least 100 Pap cases. If applicable, a statement of deficiencies is forwarded to the laboratory via the CMS Regional Office, and the laboratory is given an opportunity to respond with a plan of correction. For example, between 1988 and 2006 a total of 616 laboratories were surveyed, and 32% were found to be noncompliant with CLIA. After filing a plan of correction, only 4% of laboratories received sanctions, had their certificates revoked, or voluntarily withdrew from the program.⁶

An advisory committee known as the Clinical Laboratories Improvement Advisory Committee (CLIA) consists of 20 members, including laboratorians and consumer advocates. CLIA meets at least twice yearly and advises CMS and other governmental agencies on the need for and nature of any revisions to the standards that govern laboratory testing.

Health Insurance Portability and Accountability Act of 1996

HIPAA is a comprehensive law that regulates several unrelated areas of health care, like the protection of (1) health care coverage for those who change jobs and (2) the privacy of medical information. The law asked Congress to pass comprehensive privacy legislation by August 1999. Because Congress did not do so, the law required DHHS to write the Privacy Rule/Regulation. DHHS published the Final Rule on December 28, 2000, which took effect on April 14, 2001, requiring compliance by health care providers by April 14, 2003.

The Rule governs the use of so-called individually identifiable health information and is intended to ensure that a patient's health information is used only for health purposes, unless permission is obtained for other purposes. This information includes, for example, a cytologic diagnosis linked to an identifier such as a social security number, medical record number, and/or accession number. The DHHS Office of Civil Rights (OCR) is responsible for implementing the Rule and has issued written guidelines for health care providers which can be viewed at <http://www.hhs.gov/ocr/hipaa>.

HIPAA also requires that every provider who does business electronically use the same health care transactions, code sets, and identifiers. Code sets (e.g., current procedural terminology [CPT], Healthcare Common Procedure Coding System [HCPCS], and International Classification of Diseases, 9th Revision, Clinical Modification [ICD-9-CM]) are the codes used to identify specific diagnoses and procedures on claims and encounter forms.

Laboratory Personnel

Personnel in a cytology laboratory include the cytotechnologists (CTs), cytopathologists (CPs), cytopreparatory staff, administrative staff, and clerical personnel. The qualifications and responsibilities of some of these have been defined by CLIA 88. Many of the personnel titles codified by CLIA, particularly *technical supervisor* (TS), sound strange to cytologists' ears because they were not in common usage before CLIA. In addition, the CLIA personnel designations often do not coincide with institution-based job titles, which, in many cases, predate CLIA 88 and were not changed to conform with CLIA titles—a confusing situation that often means an individual has one CLIA-defined title but a different institution-designated title.

Laboratory Director

Every laboratory performing so-called high-complexity testing (cytology falls into this category under CLIA 88 regulations) must have a laboratory director. This is the individual who is named the laboratory director on the CLIA certificate. (Institutions might also designate individuals as laboratory directors of Cytology, Microbiology, etc., but they are not the “laboratory director” in the eyes of CMS if they do not hold the CLIA certificate.) He or she may direct more than one laboratory but no more than five. It is the laboratory director who has ultimate responsibility for the work performed in the laboratory.



Responsibilities of the laboratory director

- overall operation and administration, including employment of personnel
- may also perform duties of the technical supervisor and general supervisor



Qualifications of the laboratory director

- licensed to practice medicine or osteopathy
- anatomic pathologist or, if not, must employ an anatomic pathologist as technical supervisor
- must possess a license as a laboratory director issued by the state in

which the laboratory is located, if such licensing is required

Technical Supervisor

The TS, as defined by CLIA 88, is the pathologist responsible for the “technical and scientific” oversight of the laboratory. He or she is not required to be on site at all times but must be available on an as-needed basis. The TS may perform the duties of a general supervisor (GS) and a cytotechnologist (CT).



Responsibilities of the technical supervisor

- establishing quality control procedures
- resolving technical problems
- evaluating competency of personnel
- monitoring test results
- performing semiannual evaluations of CTs
- establishing a workload limit for each CT and reassessing it at least every 6 months

The TS of a cytology laboratory is usually someone that the institution (but not necessarily CLIA) considers the “laboratory director” of cytology. The TS may delegate some of his or her responsibilities to a trainee in the final year of training in anatomic pathology.



Qualifications of the technical supervisor

- licensed to practice medicine or osteopathy
- certified in anatomic pathology, or
- certified by the American Society of Cytopathology to practice cytopathology

The American Society of Cytopathology no longer certifies cytopathologists; the last examination was in 1978. The TS need not have specialty qualification (“boards”) in cytopathology. In any given laboratory, more than one individual

may qualify as a TS.

General Supervisor

Each cytology laboratory must have a GS. The GS is responsible for the day-to-day oversight of operations and personnel. He or she must be accessible to provide onsite, telephone, or electronic consultation to resolve problems in accordance with procedures established by the TS. The same individual may fulfill the roles of both TS and GS.



Qualifications of the general supervisor

- same as for technical supervisor, or
- CT with at least 3 years of full-time experience within the past 10 years

Cytotechnologist

The job description of a CT varies depending on the laboratory where he or she works. Most are involved in slide examination. In some laboratories, they are also involved in quality control (QC) activities, teaching, research, cytopreparation, and assistance with preparation of smears and adequacy assessments during fine-needle aspirations (FNAs). The following responsibilities of a CT are the minimum established by CLIA 88.



Responsibilities of the cytotechnologist (defined by the Clinical Laboratory Improvement Amendments)

- documenting slide interpretation results
- recording the number of slides examined each day
- recording the number of hours worked each day



Qualifications of the cytotechnologist (at least one of the following)

- graduated from a school of cytotechnology accredited by CAAHEP
- certified in cytotechnology by an approved agency (American Society of Clinical Pathologists or HEW)
- grandfathered based on experience (opportunities for grandfathering

ended September 1, 1994)

Cytotechnologists are not required under CLIA 88 to be certified by a certifying agency, so long as they have graduated from an approved school. But the **Board of Certification** (BOC) (formerly the Board of Registry) of the **American Society of Clinical Pathologists** offers two certificates: “cytotechnologist (CT)” and “specialist in cytotechnology” (SCT). The SCT certificate requires a higher-level examination. As of 1988, a bachelor’s degree, plus graduation from an accredited cytotechnology program, is required for eligibility for a certificate. The BOC used to offer an “experience route,” but this is no longer the case, and CTs who have not attended an approved school of cytotechnology can no longer sit for the BOC examination.

A few years ago, the certificates offered by the American Society for Clinical Pathology (ASCP) BOC became time-limited. As a result the ASCP BOC now offers a Certification Maintenance Program (CMP). The CT certifications that were issued effective 2004 and beyond are valid only for a 3-year period. The same expiration applies to the SCT certifications issued effective 2006 and beyond. For these individuals, recertification is required every 3 years to maintain valid certification. Voluntary participation in the CMP is encouraged for those certified as CT before 2004.

The former Department of Health, Education, and Welfare (HEW, now DHHS) once offered CT certification, but this ended many years ago. Certification by the International Academy of Cytology (IAC) is not sufficient for those who wish to practice in the United States, because the IAC is not an approved certifying agency.

Some states in the United States require licensure of CTs, but most do not.

Policy and Procedure Manuals

The cytology laboratory is responsible for maintaining two types of procedure manuals: a client service manual and a laboratory procedure manual. The client service manual is a written or electronic guide to providers on proper methods for obtaining, storing, and transporting specimens to the cytology laboratory.



Client service manual—required policies

- preparation of patients
- specimen collection
- specimen labeling
- specimen preservation
- conditions for transportation

For example, policies specify when refrigeration is recommended for body cavity fluids and how to obtain a proper urine sample for cytology.

Every laboratory must also maintain a laboratory procedure manual. The Clinical and Laboratory Standards Institute publishes a document that outlines steps for preparing and maintaining such manuals.⁷ It does not, however, address many of the specific policies and procedures of cytology laboratories. Specific tips for compiling a cytology laboratory procedure manual can be found in other references.⁸



Laboratory procedure manual

The procedure manual must include:

- requirements for specimen collection and processing
- criteria for specimen rejection
- procedures for microscopic examination
- step-by-step description of the performance of a procedure

Procedures must be:

- approved, signed, and dated by the director
- reapproved, signed, and dated if the directorship changes

Each change in a procedure must be approved, signed, and dated by the director. Records of discontinued procedures must be kept for 2 years.

A manufacturer's package insert or operator manual may be used as a procedure, but any of the required items not provided by the manufacturer must be provided by the laboratory. Electronic manuals are acceptable, so long as they are readily available.

Workflow

The flow of work in a cytology laboratory follows an established pathway, and the CLIA 88 regulations specify certain requirements in the process for quality control purposes.



Steps in the flow of work of a cytology laboratory

- specimen collection and transportation
- accessioning
- slide preparation
- slide examination
- reporting results
- record retention

To ensure proper handling of specimens and documentation, the CLIA 88 regulations specify certain mandatory procedures.

For starters, samples must be accompanied by a cytology requisition form that is completed by a physician or other authorized individual. **Requisitions** must be retained for at least 2 years.



The requisition must include:

- patient's name or other unique identifier
- patient's age or date of birth
- patient's gender
- name and address of the authorized person ordering the test or the name and address of the laboratory submitting the specimen, with contact person
- date of specimen collection
- test to be performed
- specimen source
- for Pap tests:
 - last menstrual period
 - indication whether there has been a previous abnormal Pap, treatment, or biopsy

- any additional information relevant and necessary to a specific test to ensure accuracy

The laboratory must have a policy that documents criteria for the rejection of specimens (e.g., broken slides, missing patient identifiers, lack of medical necessity).

All Pap specimens must be stained using a Papanicolaou or modified Papanicolaou staining method.

Measures must be in place to prevent cross-contamination between Pap and nongynecologic specimens during the staining process. In addition, nongynecologic specimens with a high potential for cross-contamination must be stained separately, and the stains filtered or changed after use. Direct smears from sediments of highly cellular specimens are especially problematic; cytocentrifuge, filter, and thinlayer preparations are less likely to lead to cross-contamination during staining. Highly cellular specimens can be identified using a toluidine blue or other rapid stain on a wet preparation.

All cytology slides must be evaluated on the premises of a laboratory certified for cytologic testing. Slides may not be taken home for evaluation. The test record system must include the identity of the personnel who performed the test and the date and time the specimen was received.

CLIA 88 requires that a technical supervisor (TS), i.e., a pathologist, review every Pap interpreted as showing reactive or reparative changes, as well as any squamous or glandular abnormality at the level of atypical squamous cells (ASC) or atypical glandular cells (AGC) and higher. A TS does not need to review a case that a CT judges to be negative and lacking in reactive and reparative changes. In addition, a TS must review all nongynecologic cytology cases. A TS's written or electronic signature must be on the cytology report, and CLIA 88 specifies that the report must use narrative, descriptive terminology. A numerical reporting system (e.g., "Class IV") is not acceptable.



The test report must include:

- the name and address of the laboratory
- the test performed
- result
- pathologist's signature

In case a corrected (amended) report needs to be issued, the corrected report must state the reason for the correction.

Reports must be retained for 10 years. This may be in electronic or hard copy format. **Cytology slides** must be retained for at least 5 years. The requirement is the same whether the sample is gynecologic or nongynecologic, normal or abnormal. [Table 18.1](#) summarizes the retention requirements for cytology records and slides. Note that these are federal regulations; state regulations may be more stringent. In Massachusetts, for example, cytology slides must be retained for 7 years.

TABLE 18.1
FEDERAL RETENTION REQUIREMENTS FOR CYTOLOGY LABORATORIES

Requisitions	2 years
Worksheets*	2 years
Slides	5 years
Reports	10 years

*Includes quality control, quality assurance, and proficiency testing results.

Billing

Billing is one of the most complex aspects of laboratory management in the United States. A cynic might say that the labyrinthine regulations were designed to be difficult so that payers could deny payment to providers. Certainly, some of the rules defy logic, but notwithstanding this, all providers/laboratories are bound to comply or else risk forfeiting payment or incurring stiff penalties for fraud and abuse.

In this section, the rules for filing claims to government agencies for cytology-related services provided to Medicare, Medicaid, and TriCare beneficiaries are outlined.⁹ The claim policies of private insurers and managed care companies are likely to be different in some respects. Of importance, the rules are different for Pap tests, nongynecologic/non-FNA specimens, FNAs, and consultations, and therefore each is discussed separately. But first the reader needs to understand the “languages” known as CPT and ICD-9-CM, two different but complementary coding systems that are required for all medical billing in the United States.

To bill for a test, the laboratory must submit a claim that includes (1) the date and location of service; (2) a procedure code (to describe what was done); and (3) an ICD-9-CM code (to justify medical necessity).

Procedure Codes

A medical bill submitted to an insurer for payment needs to describe the medical procedure/service that is being billed. The common language that is used in the United States to communicate the vast majority of procedures is called **CPT**, for ***current procedural terminology***, a registered trademark of the AMA. The AMA owns and maintains CPT. The first edition was published by the AMA in 1966 when the (then new) Medicare program needed a terminology for describing medical services. To this day, CMS, which administers the Medicare program for DHHS, agrees via contract with the AMA to use the CPT codebook as the main source of codes and descriptors for processing medical claims. With the implementation of the HIPAA regulations in 2003, CPT became the language that must be used by all providers, government agencies, and private insurers.

According to the AMA, the objective of CPT is to provide “a uniform language that will accurately describe medical, surgical, and diagnostic services, and will thereby provide an effective means for reliable nationwide

communication among physicians, patients, and third parties.”¹⁰ A CPT code has been assigned to virtually every type of physician and laboratory service, including cytologic slide preparation and interpretation. (For example, CPT code 10021 describes the procedure of performing an FNA without image guidance.) CPT codes describe even the most complex of medical procedures in the form of a simple 5-digit numeric code. Tell a knowledgeable person, for example, that you just performed an 88164, and he or she will know immediately that this was a manual screening of a cervical/vaginal smear (not a liquid-based preparation); that Bethesda terminology was used to report the result; and that the procedure included only the so-called technical component (staining, coverslipping, CT review, but not a pathologist’s interpretation)—all this from a 5-digit code!

CPT codes are the foundation for determining facility (“technical”) and physician (“professional”) payments, in conjunction with Medicare’s Resource-Based Relative Value System (RBRVS) or its clinical laboratory fee schedule (CLFS). The RBRVS is a system for comparing the relative value of medical services across all specialties, based on work, practice expense, and other factors. By doing so, the RBRVS establishes a relative value unit (RVU) for every current medical procedure. The dollar value of any given medical service or procedure is determined by its composite relative weight, multiplied by a nationally set (by CMS) dollar conversion factor. The RVUs and conversion factor for physician services are published annually in the *Federal Register* by CMS. Various geographic cost-of-living adjustments and other factors are also applied to obtain the specific allowed charge for any given procedure and locale, and therefore the process is not so simple as multiplying an RVU by the conversion factor. (It is not within the scope of this chapter to elaborate on the detailed formula.) CMS provides an allowed charge lookup system on its website. Medicare’s CLFS is briefly described below in conjunction with Pap test technical services.

HCPCS codes are a separate set of codes used to describe drugs, supplies, and certain other services not included in CPT. Like CPT codes, HCPCS codes have 5 characters, but the first is a letter and the rest are numbers (e.g., G0123). The HCPCS codes are administered not by the AMA but by CMS. Responsibility for maintaining and updating them is vested in a national panel composed of representatives from CMS, the Blue Cross and Blue Shield Association, and America’s Health Insurance Plans. Cytologists need to be concerned with only a small number of HCPCS codes, those for routine and high-risk Pap tests for Medicare beneficiaries.

In some circumstances, CPT and HCPCS codes require the use of modifiers to avoid filing a false claim and to assure accurate and prompt payment by payers.

A complete discussion of modifiers is beyond the scope of this chapter, but familiarity with the concept of modifiers is important. Some commonly used modifiers for cytology cases deserve mention.

CPT Modifier 26. This is the most widely used in pathology. It denotes that only the physician professional component of the service is being billed.

CPT Modifier 52. This modifier denotes a reduced service from the customary procedure. In cytology, a good example is the manual review of a slide that was intended for evaluation by the ThinPrep Imaging System but rejected for technical reasons. A laboratory can still bill the automated screening code 88175, but with modifier 52 (i.e., 8817552).

CPT Modifier 59. Modifier 59 denotes a “separate procedure,” such as a different specimen (e.g., washing versus brushing) or anatomic site. Payers often require this modifier when two or more codes are considered mutually exclusive or duplicative. For example, reporting 8810459 for a direct smear bronchial brushing with 88108 for a cytopsin bronchial washing is often necessary to avoid having the former charge denied.

HCPCS Modifier GC. Teaching physicians must append modifier GC to CPT and HCPCS codes on Medicare claims when a resident or fellow actively participates in performing the underlying medical service. The modifier declares that the teaching physician personally performed the “critical” portion of the procedure and is thus entitled to bill for it.

HCPCS Modifiers GA, GY and GZ. These modifiers are applied to Pap test HCPCS codes when billing Medicare. They clarify the laboratory’s right (or lack thereof) to bill the Medicare beneficiary for the charge if it is denied by the contractor.

HCPCS Modifier TC. This modifier denotes the facility technical component of the service being billed, and thus is the counterpart of the CPT 26 modifier.

A few points about procedure codes are worth noting: 1. **Just because a code is printed in CPT or HCPCS does not mean it is a covered service.** Coverage decisions are made by the U.S. Congress, state legislatures, and private insurers. Coverage limits might also be imposed by participation agreements made with managed care companies and private insurers.

2. **The AMA and CMS sometimes have conflicting interpretations on the scope and meaning of the CPT codes.** Historically, the AMA was the sole authority everyone, including CMS, looked to for guidance in using CPT codes. In 1996, CMS launched its National Correct Coding Initiative (NCCI). Since then, the AMA and CMS have diverged in ways that affect a number of pathology-related procedure codes. The result: “AMA-CPT rules” and “CMS-CPT rules.” The nongynecologic cytology procedure codes 88104 (direct

smears) and 88108 (cytospin) are a good example. CMS says it is not medically necessary to use both types of preparations for one nongynecologic cytology specimen, and therefore you are only permitted to bill 88108 to CMS, even if you examined both preparations. By contrast, the AMA considers both procedures billable, even when they relate to the same specimen. How should one deal with such discrepancies? You should adhere to CMS policy if billing a service to a Medicare Administrative Contractor (MAC) (“render unto Caesar...”). You should also adhere to CMS policy for Medicaid, TriCare, Medicare Advantage, and private insurer accounts if they specify that you should adhere to Medicare CPT policies. If they do not, follow their specific instructions (if any), or follow the AMA rules if the insurer does not name a CPT authority.

3. You should always use only the most recent version of the CPT codebook. The so-called Category I CPT codes that account for the vast majority of the codes you will use are updated effective January 1 every year, and every year some edits are made that affect pathology codes.

International Classification of Diseases, 9th Revision, Clinical Modification Codes

The ICD-9 is the taxonomy used by all health care professionals and insurers in the United States when discussing medical conditions.¹¹ The version of ICD-9 used for billing purposes in the United States is “clinical modification” (CM). (For example, in ICD-9-CM “speak,” hematuria is 599.7x [the fifth character denotes gross, microscopic, or unspecified], and a solitary thyroid nodule is 241.0.) ICD-9-CM coding is used to determine whether or not a procedure billed to an insurer is medically necessary, in which case it is a covered benefit for the patient. With the passage of the Medicare Catastrophic Coverage Act of 1988, diagnostic coding using ICD-9-CM became mandatory for Medicare claims, and when HIPAA was implemented in 2003, ICD-9-CM coding became universal, meaning that private insurers as well as government agencies are required to use it. For convenience, hereinafter we refer to ICD-9-CM simply as ICD-9.

In the United States, all health care providers must furnish an ICD-9 code to justify the medical necessity of a diagnostic test. For example, if a urine cytology test is ordered for a patient with hematuria, the patient’s physician will furnish ICD-9 code 599.7x (which, as noted, denotes hematuria, with the fifth digit delineating gross, microscopic, or unspecified) on the requisition form that accompanies the urine specimen. Similarly, when your laboratory bills an insurer

for having performed a cytologic examination of a specimen, the bill must include an appropriate ICD-9 code to justify the medical necessity of the test. (It might be the same “clinical code” you received from the ordering physician, or it might be different, a “pathologic code,” as discussed further on.) Payers have lists of approved ICD-9 codes for many laboratory tests, and will deny payment if a nonapproved code is provided. In the earlier example, if you supply a wrong ICD-9 code, such as 784.0 (denoting “headache”) with the bill for the urine cytology, it is likely that Medicare will deny the claim, because 784.0 does not justify the medical necessity of urine cytology. Pointers for selecting the appropriate ICD-9 code for cytology specimens are provided further on.

The provider who sends a cytology sample to the laboratory does not have to provide a literal ICD-9 code. It is acceptable for the referring physician to write a narrative diagnosis (e.g., “hematuria”) on the requisition form, which the laboratory can then translate into an ICD-9 code (in this example, 599.7x) by consulting the codebook.

The ICD-9 codebook consists of three volumes. The entire three-volume set is used by hospitals to bill for *inpatient services*. All other providers, and hospitals billing for *outpatient services* use only the first two volumes of the set. Volume 1 is a tabular list of hundreds of 3-4-, and 5-digit numeric codes, each accompanied by a description of the corresponding condition. Volume 1 is arranged by systems. Volume 2 is an alphabetical list of the conditions included in volume 1. Volumes 1 and 2 are distributed by the National Center for Health Statistics, a branch of the CDC. You need to be alert for changes in the ICD-9 codes (i.e., watch the CMS and professional associations’ websites), because the codes are eligible for update twice a year (April 1 and October 1). Volume 3, with rules for inpatient coding, is not of concern to pathologists and independent labs.

A few points about ICD-9 coding for nongynecologic cytology services, including FNAs, are worth noting:

- 1. The pathologic diagnosis is the principal diagnosis used for ICD-9 coding.** For example, if you interpret a urine specimen as “positive for” or “consistent with” transitional cell carcinoma, the code for “transitional cell carcinoma, site not specified” (188.9) should be reported on the insurance claim. You can think of this as the “pathologic code,” as opposed to the “clinical code” that came from the patient’s attending physician.

- 2. The clinical code is used for coding if there is no specific pathologic diagnosis.** In many circumstances, a specific pathologic diagnosis cannot be assigned to a specimen. This applies, for example, to a negative urine specimen. In this circumstance you must fall back on the clinical code that was provided

with the specimen (e.g., 599.71 for gross hematuria).

3. Do not report an uncertain diagnosis. In cytology (or surgical pathology, for that matter), your interpretation will occasionally be “suspicious for” or “cannot rule out.” In such circumstances, do not report the uncertain diagnosis as if it were confirmed. For example, do not use code 193 (malignant neoplasm of the thyroid) if you interpret a thyroid FNA as “suspicious for papillary carcinoma.” Instead, code it to the highest degree of certainty for that patient visit (e.g., 241.0: solitary thyroid nodule).

4. Report codes to the greatest level of specificity. For example, there are ICD-9 codes for a malignant neoplasm of the upper (162.3), middle (162.4), and lower lobes (162.5) of the lung, as well as a code for an “unspecified” site (162.9). If you diagnose a small cell carcinoma in an FNA specimen of an upper lobe lesion, use 162.3, not 162.9. You already know from its prior appearance herein that hematuria requires five digits for accurate reporting, with the fifth digit denoting gross (599.71), microscopic (599.72), or unspecified (599.70).

5. As with the CPT codebook, always use the most recent version of the ICD-9 codebook. As earlier mentioned, the ICD-9 codebook is updated twice a year, so it is very important that you use only the most recent text.

The ICD-9 codebook includes a series of so-called health status codes called *V-codes* because they always start with the letter V. They are used when a patient receives health services in the absence of any current sign or symptom of disease or injury. One example is a Pap test for a healthy woman. In such a circumstance, you most likely will report a code such as V76.2 (screening Pap test in absence of sign, symptom, or history) because the Pap is normal and the referring physician furnished no information regarding abnormal signs, symptoms, or history.

Parenthetically, CMS has been planning for some years to transition from ICD-9-CM to ICD-10-CM for Medicare reporting purposes. At present, the transition effective date set by CMS is October 1, 2014. Notwithstanding, provider representatives continue to encourage CMS to further delay the conversion date, or to stay with ICD-9-CM, or to adopt some other diagnosis coding system owing to the complexities of ICD-10-CM, among other issues.

Coding Pap Tests

Because of recent advances in technology, it is impossible to talk about a generic Pap test, certainly with regard to procedure coding. One or two codes would be inadequate to describe the variety of ways in which a Pap might be evaluated

today—as a smear or liquid-based preparation; manually or with computer assistance; computerized screening only or with manual review; with or without physician interpretation. Thus, assigning a procedure code to a Pap test follows a rather complex algorithm. As of 2013, there are 21 different CPT/HCPCS codes for a Pap test. Those most commonly used are described in [Table 18.2](#).

TABLE 18.2
PROCEDURE CODES FOR PAP TESTS (as of 2013)

Preparation Method	Component	Manual Screening Only	ThinPrep Imager-Assisted Screening	FocalPoint (Instrument Only)	FocalPoint (with Manual Screening)
PROCEDURE CODES (HCPCS) FOR SCREENING PAP TESTS (ROUTINE AND HIGH RISK)					
Smear	Technical	P3000	NA	G0147	G0148
	Professional	P3001	NA	G0141	G0141
Liquid-based	Technical	G0123	G0145	G0144	G0145
	Professional	G0124	G0141	G0124	G0141
PROCEDURE CODES (CPT) FOR DIAGNOSTIC PAP TESTS					
Smear	Technical	88164*	NA	88147	88148
	Professional	88141	NA	88141	88141
Liquid-based	Technical	88142	88175	88174	88175
	Professional	88141	88141	88141	88141

NA, Not applicable.

*Substitute 88150 if Bethesda System terminology is not used to report results.

Anal Pap tests are considered nongynecologic specimens and are discussed as such.

Another aspect of Paps that makes them unique is that, most of the time, they are ordered as a screening test in the absence of any history, signs, or symptoms related to the cervix or vagina. For Medicare beneficiaries, Pap tests are therefore divided into three categories according to medical indication, thus very significantly affecting CPT/HCPCS procedure and ICD-9 diagnosis coding. These categories have implications for the frequency of beneficiary coverage and direct payment to the laboratory by Medicare (as opposed to beneficiary financial liability), and thus the categories affect procedure coding, as seen in [Table 18.2](#).

The three categories of Pap tests and their implications for Medicare beneficiary coverage are:

- **Screening, routine:** No more than one test every 24 months is covered.

- **Screening, high-risk:** No more than one test every 11 months is covered.
- **Diagnostic:** No frequency limit, but test must be medically indicated.

The coding rules for these three categories of Pap tests are described in the sections that follow. Note that most Medicaid agencies and private insurers do not differentiate between routine and high-risk screening Pap tests, although screening versus diagnostic testing distinction is often made, including to the extent of insured coinsurance requirements.

The Screening (Routine) Pap Test

The “routine screening Pap test” is defined by Medicare as follows.



Definition of a screening (routine) Pap test

- no current sign, symptom, or complaint referable to the reproductive organs
- no previous abnormal Pap
- no high-risk factors for cervical or vaginal cancer

A screening (routine) Pap is one ordered solely as part of a preventive health care visit (e.g., periodic check-up). If the woman has not had a Pap paid for by Medicare within the past 2 years, the laboratory can expect payment by Medicare and should post one of the four accepted ICD-9 codes, **V72.31**, **V76.2**, **V76.47**, or **V76.49** (see [Table 18.3](#) for definitions), as it has been supplied (hopefully!) by the referring physician. (Should the pathologist report an abnormality not expected by the referring physician, the ICD-9 code for that atypia would be posted as a secondary diagnosis on the claim. This is how Medicare becomes aware that the beneficiary is to move to a high-risk or diagnostic category, because in the future she will have a history of an abnormal Pap.) If the woman has already had a Pap paid for by Medicare within the past 2 years, the test is not payable by Medicare. The laboratory may then bill the patient directly, but only if it has a signed Advance Beneficiary Notice of Noncoverage (ABN) from the patient on file. By signing an ABN, the patient authorizes the laboratory to bill her directly if a service is not covered by Medicare. The laboratory generally relies on the referring physician to obtain the ABN signatures it needs, because it almost never has direct contact with the patient in advance of a Pap test. (Medicare states that an ABN is valid—and the patient is financially liable for the service—only if the ABN is signed in advance of the test; post-test ABN signatures are not valid or binding on the beneficiary.) It is advisable, however, not to rely on the referring physician to store ABNs for you. Many laboratories have solved this problem by making the ABN a part of the requisition form itself. The content and specific wording of the ABN form is prescribed by CMS and changes from time to time, so you should periodically

check with your compliance advisor to make sure you are using the latest authorized form.

TABLE 18.3
COMMONLY-USED ICD-9 CODES FOR PAP TESTS

V76.2	Screening Pap of cervix in the absence of signs, symptoms, or history
V72.31	Screening Pap of cervix in the absence of signs, symptoms, or history, collected as part of gynecologic examination
V76.47	Screening Pap of vagina in the absence of signs, symptoms, or history
V76.49	Screening Pap in the absence of signs, symptoms, or history, woman without a cervix
V15.89	Screening Pap, woman at high risk for developing cervical or vaginal cancer
616.0	Cervicitis
616.10	Vaginitis
622.10	Histologic cervical intraepithelial neoplasia (CIN), unspecified
622.11	Histologic CIN I (mild dysplasia)
622.12	Histologic CIN II (moderate dysplasia)
233.1	Histologic CIN III (severe dysplasia)
795.00	Abnormal glandular Pap
795.01	Abnormal Pap: atypical squamous cell, undetermined significance (ASC-US)
795.02	Abnormal Pap: atypical squamous cell, cannot exclude high-grade squamous intraepithelial lesion (ASC-H)
795.03	Abnormal Pap: low-grade squamous intraepithelial lesion (LSIL)
795.04	Abnormal Pap: high-grade squamous intraepithelial lesion (HSIL)
795.05	High-risk human papillomavirus DNA-positive (cervix)
795.06	Malignant cells, without histologic confirmation
795.08	Unsatisfactory Pap
795.09	Other abnormal Pap smear (includes reactive/reparative changes)
112.1	Candidiasis
623.5	Vaginal discharge
626.2	Menometrorrhagia
626.4	Irregular menstrual cycle
626.6	Metrorrhagia
626.8	Dysfunctional uterine bleeding
627.1	Postmenopausal bleeding

Laboratories frequently append modifier *GA* or *GZ* to the HCPCS code when billing Medicare for screening or high-risk Paps. The modifiers declare whether or not the laboratory has an ABN on file to permit billing the beneficiary for the charge if Medicare denies it. Modifier *GA* says that a beneficiary-signed ABN is

available, so the laboratory can bill the beneficiary if Medicare denies the charge. Modifier GZ says the laboratory did not obtain an ABN, so the beneficiary cannot be billed when Medicare denies the charge. (Medicare contractors are instructed to automatically deny charges filed with modifier GZ, which is a consideration that your billing office should take into account when deciding whether or not to regularly use that modifier.) Consult with your compliance officer for additional information about modifiers *GA* and *GZ*, as well as modifier *GY*, which is not herein explained owing to infrequent usage by laboratories.

The laboratory is responsible for ensuring that referring physicians distribute an ABN advisory to all Medicare patients informing them that Medicare does not pay for all laboratory tests ordered under all circumstances.

Medicare expects one of the four ICD-9 V-codes to appear on the laboratory's claim for a routine screening Pap. The referring physician is not always so cooperative as to provide one of those codes on the requisition form. In these instances the laboratory is advised to contact the referring physician to get a conforming ICD-9 code, because the laboratory could be charged with filing a false claim if it unilaterally adds a missing code or changes a code provided by the referring physician. Similarly, if the routine screening Pap shows an unexpected abnormality, the referring physician can be contacted to determine if something in the patient's history or current visit might have been overlooked when ordering the test; otherwise, Medicare may not cover the test, even though it turned out to be abnormal. ICD-9 reporting tips for routine screening Pap tests are provided below.

The Screening (High-Risk) Pap Test

The "screening (high-risk) Pap test" is defined by Medicare as follows.



Definition of a screening (high-risk) Pap test

- early onset of sexual activity (before 16 years of age)
- multiple sexual partners (five or more in a lifetime)
 - history of sexually transmitted disease (including human immunodeficiency virus [HIV])
 - fewer than three negative Paps in the previous 7 years
 - daughter of a woman given diethylstilbestrol during pregnancy
 - abnormal Pap in the past 3 years (childbearing-age women only)

As is evident from the foregoing definition, to properly assign a clinical ICD-9 code when submitting a Pap to a laboratory, the referring physician must obtain a detailed history of risk factors. The Medicare-approved clinical code for a high-risk Pap is **V15.89**, with one exception: If the high-risk factor is early onset of sexual activity, the ICD-9 code is **V69.2**. These codes must be supplied by the referring physician, not assigned unilaterally by the laboratory. Medicare will cover the test (i.e., pay the laboratory directly) only if it has not paid for another Pap for the patient within the past 11 months. If it has, the test is not covered, and the laboratory can bill the patient directly, provided it has a properly executed ABN form on file.

The Diagnostic Pap Test

The diagnostic Pap test is defined by Medicare as follows.



Definition of a diagnostic Pap test

- previously diagnosed cancer of the vagina, cervix, or uterus
- previous abnormal Pap
- current abnormal findings of vagina, cervix, uterus, ovaries, or adnexae
- significant complaint referable to the female reproductive system
- any sign or symptom that might be related to a gynecologic disorder

As defined, a diagnostic Pap test is always covered by Medicare, whether or not it is abnormal, so long as the clinical ICD-9 code provided by the referring physician is on the local Medicare contractor's limited coverage list. (General criteria for medical necessity always apply.) Medicare contractors publish their local medical review policies (called *LCDs*, for *local coverage determinations*) in bulletins and their Web sites. An LCD contains a list of ICD-9 codes and other factors that justify the medical necessity of a Pap test in the opinion of the local Medicare contractor. (Ironically, even though Medicare is a national program, it happens all too often that a Pap or other laboratory test that is covered in one contractor's jurisdiction is not covered in another locale!) Not all Medicare contractors have a published LCD for Pap tests, so do not become alarmed if you

cannot find one on your contractor's Web site. Some commonly accepted diagnostic Pap test ICD-9 codes are listed in [Table 18.3](#).

A few points worth noting regarding Pap test coding: 1. **Only the referring physician can classify a Pap as screening, high risk, or diagnostic.** The laboratory has no right to unilaterally classify the Pap, or reassign it to a different class from that given by the referring physician. This noncompliant activity is equivalent to "code jamming." Nevertheless, the laboratory may have information (previous medical history) available to it that suggests that the referring physician has misclassified the medical necessity of the test. Under these circumstances, the laboratory may amend the order, but only after contacting the referring physician and obtaining authorization to do so.

2. **ICD-9 coding rules for screening (routine and high-risk) Paps include instructions for "primary" and "secondary" codes.** In submitting a claim to Medicare for a screening Pap test (either routine or high risk), the clinical ICD-9 code submitted by the ordering physician (a V-code, as earlier enumerated) must appear in claim field position one (i.e., the "primary" diagnosis). If an abnormality is discovered after cytologic review of the slide, the ICD-9 code for that abnormality is reported in claim field position two (i.e., a "secondary" diagnosis). As mentioned previously, this is how Medicare becomes aware that the beneficiary is to move to the high-risk or diagnostic category, because in the future she will have a history of an abnormal Pap.

Official ICD-9 guidebook reporting instructions for screening tests are fundamentally the same just described for Medicare. Therefore, for a non-Medicare patient, you should report the screening code (V-code) supplied by the ordering physician as the primary diagnosis and report any abnormality determined during the laboratory examination process as a secondary diagnosis. It is conceivable that a Medicaid agency or private insurer may, however, prescribe a different reporting protocol.

3. **You can bill for an unsatisfactory Pap under certain circumstances.** A small percentage of Paps are reported as "unsatisfactory," usually by a cytotechnologist (CT). If the slide was deemed unsatisfactory before it was reviewed (e.g., broken beyond repair, insufficient identifying information), a charge should not be submitted on that case. If, however, the slide was reviewed and deemed unsatisfactory because of obscuring blood, insufficient squamous cells, or the like, then a charge should be reported, accompanied by ICD-9 code 795.08.

4. **A physician interpretation charge should be billed only if the pathologist interpreted the slide owing to an atypia or question identified by the CT.** When a pathologist interprets a Pap, his or her signature (handwritten or

electronic) must appear on the report. Conversely, the pathologist's signature should not appear on a Pap report when he or she did not personally review the slide. (The pathologist's name, as lab director or staff, may appear in the masthead of the report alongside the name and address of the laboratory, so long as it is clear that it is not a signature.) A separate professional fee is billable by the pathologist if his or her interpretation is precipitated by a finding of abnormal, atypical, or reactive cells by the CT. A pathologist's professional fee is legitimate in this instance even though he or she ultimately signs out the smear as "normal." Pathologist review of a high-risk patient smear judged to be "normal" by the CT is not a separately billable professional service; similarly, a pathologist review that is conducted as part of the laboratory's quality assurance/control program is not separately billable. When a pathologist screens a smear in place of a CT and no abnormality/atypia is identified, the test is billable with the regular technical code (e.g., 88142, G0142) alone.

5. Medicare payment of the technical component of a Pap test is via the clinical laboratory fee schedule. Payment for Pap test technical preparation and screening services (i.e., all services except pathologist interpretation) is made by Medicare via its clinical lab test fee schedule (CLFS), the same as for other routine clinical laboratory tests (e.g., glucose, complete blood count, urinalysis). The amount paid to the laboratory provider represents 100% of the allowed charge, with no coinsurance due from the beneficiary. This payment provision applies to both screening (routine and high-risk) and diagnostic Pap tests. When a pathologist interprets an atypical or suspect Pap test, the physician interpretation—but not the underlying technical preparation and screening—is payable by Medicare via the physician fee schedule. Recall that two CPT/HCPCS codes, one technical (e.g., 88142) and one professional (e.g., 88141), are always required to report a "complete" Pap test that includes a physician interpretation component.

Coding Nongynecologic, Non–Fine-Needle Aspiration Cases

This section focuses on all nongynecologic cytology specimens, excluding FNAs. (The rules for FNA billing are different and discussed in the next section.) Anal Pap tests are considered nongynecologic specimens and therefore coded according to the rules in this section.

The rules for coding nongynecologic/non-FNA specimens are much simpler than those for Paps. The procedure (charge) codes are based not on specimen type (e.g., urine, cerebrospinal fluid [CSF], bronchial brushing), but on the

preparation method, and the choices are rather limited ([Table 18.4](#)). Each separate nongynecologic/non-FNA specimen is assigned one or more procedure codes, depending on the preparations that are ordered by the pathologist. Direct smears, cytospins, thinlayer smears, and cell block slides are distinct preparations and are separately coded per specimen. Thus, a nongynecologic/non-FNA specimen prepared as one or more direct smears is coded as **88104**. The cytospin and Saccomanno methods are considered “concentration” methods (code **88108**), and the ThinPrep and SurePath nongynecologic preps are considered “enriched/concentration” methods (code **88112**). A cell block has a separate charge code (**88305**).

TABLE 18.4
PROCEDURE CODES FOR NONGYNECOLOGIC/NON–FINE-NEEDLE ASPIRATION SPECIMENS

Preparation	Code
Direct smear	88104
Concentrated prep (cytospin or Saccomanno)	88108
Enriched/concentrated prep (e.g., ThinPrep, SurePath)	88112
Cell block	88305
“Other source” (received as stained smear)	88160
“Other source” (received as unstained smear)	88161
Extended cytology study of “other source” specimen	88162
Special stains for microorganisms	88312
Other histochemical stains (e.g., periodic acid–Schiff)	88313
Immunohistochemical stains (qualitative)	88342

Unlike some surgical pathology specimens, where certain separately identified specimens cannot be billed separately but must be “bundled” (e.g., breast and axillary lymph nodes from a modified radical mastectomy procedure), rarely are cytology samples bundled. For example, right and left pleural fluids from the same patient received on the same day are billed separately, as are bronchial brushings and washings. Bundling is required only in those unusual situations when a sample such as sputum (or bronchial washings from the same site) arrives in multiple vials; there is no clinical basis for splitting such a sample, and charge reporting is impacted accordingly. But if two samples are submitted from the same patient on the same day, and they are *from different sites*, they can and should be billed as separate specimens.

In many cases, multiple preparations are made, and a charge code for each is

appropriate, except when the patient is a Medicare beneficiary. For example, if a pleural fluid is prepared as a smear, cytopspin, and cell block, codes 88104, 88108, and 88305 are considered appropriate by the AMA. But Medicare permits billing only the 88108 and 88305 codes in this instance; the 88104 code is considered medically unnecessary in light of the 88108. (This is an example of the dichotomy between “AMA-CPT rules” and “Medicare-CPT rules” alluded to earlier.) The AMA and CMS agree, however, that you cannot report 88112 and 88108 together for the same specimen, because 88112 already includes “concentration.”

A small number of cytology specimens are considered to be “other source” nongynecologic samples in CPT parlance. These are, principally, sputum, nipple discharge, and anal-rectal cytology. If one of these specimens arrives as a stained slide or slides (presumably a rare event), report **88160**; but if the slide(s) is/are stained in your lab, report **88161** for the specimen. Note that 88160 or 88161 replaces 88104 for an “other source” specimen only if the preparation is a direct smear; report the regular 88108 or 88112 code if a concentrated or enriched/concentrated preparation is made. There is also an “extended study” “other source” code, **88162**, but the circumstances under which it may be reported seldom arise these days.

A few additional points are worth noting regarding coding nongynecologic/non-FNA specimens:

- 1. The number of smears, cytopspins, ThinPreps, or SurePath slides you examine for a specimen does not affect the charge code.** Codes 88104, 88108, and 88112 are applied once per specimen no matter how many slides of each preparation type were examined. These codes can be used in combination (with certain restrictions, as mentioned earlier), but they cannot be multiplied for any one specimen.

- 2. Only one 88305 code may be posted for each separate specimen.** The same principle applies to cell blocks as to other preparation methods: If multiple cell blocks are prepared for a specimen, you should report 88305 only once.

- 3. Each histochemical and immunohistochemical study is charged per stain or antibody used.** Special stain codes 88312 and 88313 and immunohistochemical stain code 88342 are charged and reported for each different stain or antibody used per specimen (e.g., Gomori methenamine silver [GMS] and periodic acid–Schiff [PAS] on one sample constitute two separate charges). However, if the same special stain or immunohistochemical antibody reagent is applied to both the cytologic preparation (e.g., smear or cytopspin) and a cell block from the same specimen, that stain or reagent may be billed as two units of charge with proper medical report documentation, assuming that both applications are medically necessary.

4. Each preparation method, special stain, and immunohistochemical antibody that was used must be named in the report for proper billing. Third-party payer auditors assume that billed procedures were not actually performed if they are not properly documented in the medical report. Unsupported charges are denied, and additional sanctions can be imposed if the auditor deems the problem to be egregious.

Coding Fine-Needle Aspirates

Like the rules for nongynecologic/non-FNA specimens, the coding rules for FNA specimens are relatively straightforward. When coding an FNA, the specimen type does not play a role. It does not matter, for example, if the FNA sample was from the breast, thyroid, or salivary gland. What matters is what procedures were used to obtain and examine the specimen. The procedure (charge) codes for coding FNAs are given in [Table 18.5](#). There are four basic codes: extraction by a pathologist without image guidance (**10021**), extraction by a pathologist with image guidance (**10022**), immediate study for sample adequacy (**88172** and **88177**), and diagnosis (**88173**).

TABLE 18.5

PROCEDURE CODES FOR FINE-NEEDLE ASPIRATIONS

Procedure	Code
Extraction by a pathologist without image guidance	10021
Extraction by pathologist with image guidance	10022*
Immediate study (adequacy assessment), first episode	88172
Each additional episode per lesion/site	88177
Diagnosis	88173
"Add-On" Procedures:	
• Cell block	88305
• Special stains for microorganisms	88312
• Other histochemical stains (e.g., periodic acid-Schiff)	88313
• Immunohistochemical stains (qualitative)	88342

*A radiology needle placement code may also be billable.

Several points regarding billing for FNAs deserve emphasis.

1. The fundamental unit for billing the extraction codes (10021, 10022) and the diagnosis code (88173) is the specimen, not the “pass.” Only one extraction code (10021 or 10022) may be billed per lesion or anatomic site, even though it involved multiple passes. Similarly, only one diagnosis code (88173) may be billed per specimen, even when each pass from one lesion is received in a separate vial. If separate anatomic sites are aspirated (e.g., left and right neck masses or distinct nodules from the right neck), then separate 10021 (or 10022) charges may be billed for each site, and separate diagnosis charges (88173) are warranted, too.
2. To bill code 10021 or 10022, the report must state that the pathologist personally extracted the specimen. An extraction code may be charged for a procedure performed by a resident or fellow so long as the senior pathologist is physically present in the procedure or operating suite to personally supervise the procedure. A pathologist cannot bill 10021 or 10022 for simply assisting another physician (e.g., radiologist, surgeon) who performs the FNA.
3. The fundamental unit for billing an immediate assessment (88172 and 88177) is the “evaluation episode.” Two CPT codes are available for reporting in relation to pathologist intraprocedural evaluation of FNA specimen adequacy: 88172 is for the first evaluation episode of the cytologic material from a lesion or anatomic site; and 88177 is for each evaluation episode beyond the first of additional cytologic material from the same lesion or anatomic site. An evaluation episode may focus on the cytologic material in a single aspiration syringe (i.e., one pass), or it may take the aggregate material from multiple passes into account. Multiple units of 88172 may be reported for a case only if distinct lesions or anatomic sites are under medical investigation (e.g., right and left breast lesions). The pathology report must clearly distinguish one evaluation episode from each other episode for a case. The following two clinical examples demonstrate the proper use of FNA immediate adequacy evaluation codes 88172 and 88177.

Clinical Example #1: The pathologist is called to radiology where a clinician is performing an FNA on a right thyroid nodule. Two syringes, labeled A1 and A2, are handed to the pathologist as he walks into the radiology suite. The pathologist conducts an adequacy evaluation on the two aspirates and reports “inadequate material” to the clinician. The clinician then extracts a third aspirate (A3), and, after examining it, the pathologist reports “adequate specimen” to the clinician. The clinician terminates the procedure and releases the patient. Two evaluation episodes are represented by the pathologist’s immediate study work in

this instance: (1) the episode consisting of the assessment of passes A1 and A2 (code this as one unit of 88172); and (2) the episode consisting of the assessment of pass A3 (code this as one unit of 88177).

Clinical Example #2: The pathologist is called to radiology where a clinician is performing an FNA on a right thyroid nodule. One syringe, labeled A1, is handed to the pathologist as she walks into the radiology suite. The pathologist conducts an adequacy evaluation on the aspirate and reports “blood only” to the clinician. The clinician then extracts a second aspirate (A2) and hands it to the pathologist. While the second pass is being evaluated by the pathologist, the clinician decides to take a third pass at the lesion. The third syringe is given to the pathologist, who examines the contents under the microscope. A few minutes later the pathologist tells the clinician that the second pass is satisfactory for definitive diagnosis, but the third pass is “blood only.” The clinician terminates the procedure and releases the patient. Two evaluation episodes are represented by the pathologist’s immediate study work in this instance: (1) the episode consisting of the assessment of pass A1 (code this as one unit of 88172); and (2) the episode consisting of the assessment of passes A2 and A3 (code this as one unit of 88177). Note that, while the pathologist expected to influence the clinician’s activity via individual assessment of pass A2, the clinician unilaterally proceeded to perform a third pass, which negated the pathologist’s interventional influence.

Codes 88172 and 88177 pertain solely to adequacy evaluation of cytologic material generated by an FNA interventional procedure. Different CPT codes are available for pathologist interpretive work in relation to other types of surgical procedures (e.g., bronchial brushing, or surgical biopsy touch preparation), especially codes 88333 and 88334.

4. The “immediate study” may be limited to an assessment of adequacy. The immediate study does not need to be a diagnosis; it may involve just an adequacy assessment. Codes 88172 and 88177 apply in either case.

5. The facility (e.g., hospital) may also report 88172 and 88177. The resources of the facility (e.g., microscope, cart, slides, stain, room depreciation, and interest) are being used to support the work of the pathologist. Hence, if the pathologist is entitled to report 88172 (and 88177 too, if applicable), the hospital or independent lab that supports the procedure is justified in reporting that code(s) too, including multiple units of the code(s) when warranted.

6. Codes 88172 and 88177 apply for any immediate study, no matter who performed the FNA. It does not matter if the extraction was performed by a pathologist, surgeon, or radiologist.

7. Codes 88172 and 88177 are appropriate for cases in which the slides are

rushed to the cytology laboratory while the patient is kept on the radiology table, not just those performed in the radiology suite. Once the patient leaves the procedure room, however, there is no longer any value to an immediate assessment, and 88172 (and 88177, if applicable) should not be reported if the immediate study is performed after that point.

8. The CAP advises that codes 88172 and 88177 are not reportable if a cytotechnologist (CT) alone performs the immediate study. The CAP offers no rational explanation for why you may not bill for the services of CTs to perform adequacy readings on FNAs, even though it is within their scope of practice and commonly done. (The efficacy of CAP's advice in these regards is hotly contested at the time of this writing, so you should consult with your compliance advisor before establishing a charge policy covering this situation.) Of course, if a CT alone renders the specimen adequacy assessment (i.e., no pathologist involvement), only the technical component charge (e.g., 88172TC) may be billed.

9. Do not report interpretation code 88173 if an FNA specimen is completely acellular. This includes "blood only" samples, when the apparent cause is technique-based. On the other hand, if the sample is scanty but some cellular elements were identified and examined, it is appropriate to report 88173. Be careful when using the word "nondiagnostic" in the report. Payers may deny the charge when they see that word, as it connotes, in layman terms, the absence of medical necessity. You can minimize disputes in such cases by elaborating on your findings in the report with phrases like "occasional histiocytes present," or "fragments of skeletal muscle and bone."

10. According to the CAP, codes 88108 and 88112 may not be reported in addition to code 88173 for the same FNA specimen. According to the CAP, 88173 covers all slide preparations for an FNA, excluding only cell block slides. This advice is contrary to conventional wisdom. You will remember from the earlier discussion that the AMA allows reporting 88104 and 88108 together for a nongynecologic/non-FNA specimen. It seems illogical to permit multiple preparation code billing for nongynecologic specimens but not for FNAs. Medicare, it is true, does not permit reporting 88108 or 88112 in addition to 88173; in general, CMS deems any two similar preparations to be "duplicative" when used in relation to a single specimen or anatomic site. For non-Medicare payers that do not explicitly abide by Medicare CPT rules, you must decide (in consultation with legal counsel and your compliance officer) whether you will report 88108 or 88112 with 88173 when the case circumstances otherwise warrant.

11. The "extended cytology study" code (88162) never applies to FNA

specimens. No matter how many slides are prepared or how many different routine stains (Papanicolaou and Wright-Giemsa) are used, do not report 88162 with an FNA specimen, either as an add-on code or instead of 88173.

12. In the event that only concentrated (e.g., cytospin) or enriched/concentrated (e.g., ThinPrep) preparations are made (no smears), the “base” code 88173 is retained. Code 88173 applies to an FNA specimen, regardless of the base preparation. An add-on code (88108 or 88112) is not used under such circumstances.

13. As with nongynecologic, non-FNA specimens, special stains, and immunohistochemical stains are charged per stain or antibody used, and each must be named in the report. A cell block (88305) is also separately chargeable, following the same rules as were mentioned previously under “Coding Nongynecologic/Non-FNA Specimens.” By contrast, routine stains (i.e., those used solely to enable microscopic evaluation of cell morphology) are never separately chargeable. For example, the DiffQuik stain applied during the specimen adequacy evaluation procedure is not separately chargeable.

Additional codes are available for charging what are called *Evaluation and Management (E/M) services* in conjunction with an FNA. Several preconditions must apply to justify these additional codes. The referring physician must explicitly request such a consultation; the pathologist must obtain a focused clinical history and perform an appropriate physical examination; and the cytology report must go beyond the usual FNA report by including a consultation summary. A detailed discussion of E/M codes as they relate to FNAs is beyond the scope of this chapter, but it is important for the reader to know that they are available and applicable to pathologist-performed FNAs.

Coding Consultation Cases

In pathology, accurate diagnosis sometimes rests on the input of a consultant who is especially knowledgeable in a particular area. Payers and regulators acknowledge that, under certain circumstances, this is a medically justified activity and should be separately paid. The following three codes are used for billing a consultation service. These apply to surgical pathology as well as cytopathology consultations.



Codes for consultation on outside slides or materials

- 88321 Outside slides
- 88323 Outside slides + in-house H & E or other slide preparation

- 88325 Outside slides + “much more”

Code 88321 applies when you do not need to make any *routine* preparations (e.g., hematoxylin-eosin [H & E]) or special stains (e.g., PAS or immunohistochemistry) in addition to the slides prepared elsewhere. This code accounts for a majority of pathology/cytology consultations. It applies whether you are reviewing just one slide or many more, including the special stains and immunohistochemistry preparations that were developed at the outside laboratory.

Code 88323 is used when a consultation case requires additional routine (e.g., H & E) slide preparation, usually from a submitted paraffin block. (A slide prepared primarily for the consultant’s file does not warrant an upgrade from 88321 to 88323.) It also applies if the consultant must order a special stain, immunohistochemistry preparation, or other special study from his or her laboratory to complete the consultation.

Code 88325 is known as the “comprehensive” consultation code and applies when a consultation requires the review of medical records and other materials (e.g., clinical lab results, oncologist’s report) beyond the pathology report and slides.

Several points regarding billing for consultations deserve emphasis.

1. **A consultation does not need to be initiated by an outside pathologist.** A patient may be coming to see another physician at your hospital, and that physician may request that you review the outside materials.
2. **Standard add-on procedures** for special stains and immunohistochemistry are separately chargeable when ordered by the consultant from his or her lab, if medically indicated. Separate procedure modifier 59 must be appended to the add-on codes in that instance, and the consultation report must clearly distinguish those preparations from others that came with the case from the outside laboratory.
3. **A consultation fee cannot be charged for intragroup consultations.**
4. **You cannot consult with yourself** by charging an extra consultation fee for reviewing prior materials on a patient whose current specimen you are examining. The consultation ordinarily must be initiated by a physician unrelated to your practice.
5. **You must generate some sort of report to bill a consultation charge.** This can be a formal cytology report or a letter to the outside pathologist.

6. The basic (charge) unit of service for consultation cases is “each case.” Often, multiple specimens from a patient are received for consultation (e.g., five Pap tests over a 7 year-period). According to AMA rules, you are entitled to report 88321 five times for reviewing these cases, even if your review is resulted as a single consultation report (as is commonly done) that lists all five cases. If, on the other hand, you receive three specimens that were part of a single outside case (e.g., S08-1234A, S08-1234B, and S08-1234C), then you are entitled to bill only one consultation code. Notwithstanding the AMA’s advice, effective Oct. 1, 2007, Medicare permits you to bill only one unit of one consultation code per beneficiary per date of consultation. The various permutations that govern this rule can get rather complex, and a complete discussion is beyond the scope of this chapter.

We have set forth in the foregoing discussion the current coverage and coding policies for the most frequent cytopathology service scenarios. Be aware, however, that billing policy changes can occur at any time. It is thus important to periodically consult with your coding advisor to determine the latest accepted charge coding rules.

Quality Control and Quality Assurance

All tests have inherent limitations, and cytology is no exception. Even in the hands of well-trained and conscientious clinicians and cytologists, false-negative and false-positive results can happen. Potential sources of error are particularly well understood when it comes to Pap testing.



False-negative Pap interpretations result from:

- “sampling error”
 - The collection device does not sample lesional cells.
 - There is inefficient transfer of lesional cells from the collection device to the glass slide.¹²
- laboratory error
 - screening (i.e., abnormal cells are missed)
 - interpretation (i.e., abnormal cells are misinterpreted as benign)

The CLIA 88 Final Rule established quality control (QC) standards to minimize the laboratory component of such errors. Some of the QC standards have been reviewed previously. In addition, three forms of slide reexamination are required in the United States, all related to gynecologic specimens.



Cytology slide reexamination requirements

- prospective rescreening of 10% of negative Pap cases (“10% rescreen”)
- retrospective review of all negative Paps from women with a newly diagnosed high-grade squamous intraepithelial lesion (HSIL) (“5-year lookback”)
- review of discrepancies between Pap and biopsy results

Each of these is discussed in turn in the sections that follow. Documenting compliance with and generating statistics from QC activities is greatly aided by

robust reports within one's laboratory information system.

Prospective 10% Rescreen

The prospective rescreening of 10% of negative Paps has been a QC requirement of all laboratories in the United States since the 1960s, predating CLIA 88 but reaffirmed by it.¹³ Some of the negative Pap cases for review must be selected randomly and others by targeting high-risk women, most commonly those with a history of a squamous intraepithelial lesion (SIL). The laboratory must specify how the cases are selected at random and how cases from high-risk women are identified. The rescreening must be carried out prospectively, so that errors in diagnosis can be corrected before the report is issued. The rescreening must be done by a technical supervisor, i.e., pathologist, a general supervisor, or a cytotechnologist (CT) with 3 years of full-time experience in the past 10 years.

There is one exception: For laboratories with a solo pathologist and no CT, this rescreen need not be performed.

The rationale for this requirement is to identify CTs whose work is unreliable. The drawback is that, given the prevalence of disease in most populations, it could take more than 10 years using this method to identify someone with a high error rate if the threshold for an error is set at SIL.¹⁴ The percentage of rescreened cases upgraded to SIL or invasive cancer ranges from 0.2% to 5%.¹⁵⁻¹⁷ Even when defined as an upgrade to ASC or worse, some have reported a frequency as low as 0.18%.¹⁸ These calculations, whereby the number of errors is divided by the number of slides rescreened, provides only a crude error "rate" that does not control for the prevalence of disease in a population. The strictly defined "false-negative rate" (FNR) is the proportion of *abnormal Paps* that are falsely called negative. The FNR and its use in evaluating performance are discussed later in the chapter.

Retrospective Rescreen ("5-Year Lookback")

Retrospective rescreening, which targets archived negative Paps from women with a new Pap interpretation of HSIL or cancer, was introduced by CLIA 88. By rescreening all previous negative Paps during the 5-year period before a new Pap diagnosis of HSIL or cancer, the likelihood of detecting an error is greater than with prospective rescreening of negative Paps. The percentage of diagnoses reclassified as unsatisfactory (UNS), ASC, or worse ranges from 12% to 94%¹⁹⁻²⁷ ([Table 18.6](#)). Not surprisingly, the higher the intensity of rescreening, the greater

the likelihood of detecting an abnormality on review.²¹ The estimated frequency of errors in many published reports is inflated, however, because the Paps were reviewed with the knowledge that all the women had a current HSIL or worse Pap result. When controlled for retrospective bias, the percentage of cases reclassified is lower.²³

TABLE 18.6
RESULTS OF 5-YEAR RETROSPECTIVE RESCREENING

Study	Number of Slides Rescreened	Percent of Cases Reclassified as ASC or worse (incl. UNS)
Allen et al ¹⁹	80	18
Djemli et al ²⁷	751	15
Gatscha et al ²⁰	422	29
Hatem and Wilbur ^{*21}	17	94
Nick et al ²⁴	351	71
Jones ²²	3762	20
Tabbara et al ^{25**}	58	38
Montes et al ^{23***}	100	12

ASC, Atypical squamous cells; UNS, unsatisfactory.

*Only 2 years reviewed.

**Rescreened seven cases originally called atypical squamous cells of undetermined significance (ASC-US) or atypical glandular cells (AGC). The ASC-US and AGC categories were not used for reclassification.

***Included controls for retrospective bias.

If a significant discrepancy is found that might affect *current* patient care, the patient's physician must be notified and an amended report issued. Such instances are vanishingly rare, because the review is prompted by a current abnormality at the level of HSIL or higher.

Cytologic-Histologic Correlation

Cytologic-histologic correlation is the mainstay of QC in cytology, the foundation for developing and refining cytologic diagnostic criteria. Histologic

outcome is a common gold standard against which cytologic interpretations, gynecologic and nongynecologic, are measured.

It is important to recognize, however, that perfect cytologic-histologic correlation is not realistic. Discrepancies between Paps and biopsies, in particular, are not uncommon (ranging from 11% to 32%) ([Table 18.7](#)). The majority of discrepancies are due to so-called sampling error,^{17,28-31} implicated when a review of the discrepant Pap-biopsy pair confirms both original diagnoses. Sometimes biopsies are negative because colposcopy has an inherent false-negative rate (i.e., the lesion is simply not detected colposcopically). The ASCUS/LSIL Triage Study (ALTS) found that 33% to 36% of histologic cervical intraepithelial neoplasia (CIN) 2 and worse lesions were missed by colposcopy.^{32,33} Even when detected colposcopically and biopsied, sometimes the lesion is not properly embedded in paraffin or adequately sectioned. Errors in cytologic and histologic interpretation do occur, however, but are less common than sampling errors.

TABLE 18.7
ANALYSES OF DISCREPANCIES BETWEEN PAPS AND BIOPSIES

	Joste et al ³¹	Tritz et al ⁴¹	Rohr ¹⁷	Ibrahim et al ^{29*}	Jones and Novis ³⁴
Frequency of a discrepancy (%)	11	11	32	14	16
Number of discrepancies reviewed	175	69	104	481	2971
Reasons for discrepancy					
Sampling error** (%)	93	61	75	93	86
Pap errors					
Screening (%)	0.6	2.9	4.8	4	2
Interpretation (%)	2.8	11	15	1.6	8
Biopsy errors					
Interpretation (%)	5.7	13	3.8	1.6	8
Histochemical processing (%)	0	4.3	0	0.2	0
Combination (%)	0	7.2	0	0	0

*Compared only concurrent Pap-biopsy pairs.

**Sampling error includes biopsy and cytologic sampling.

The positive predictive value (PPV) of the Pap test is directly proportional to the degree of the Pap abnormality. Women with a Pap diagnosis of ASC have a 44% to 62% chance of having histologic SIL.³⁴⁻³⁶ For a Pap diagnosis of low-grade SIL (LSIL), the PPV for histologic SIL is 50% to 86%.^{34,36-39} When the biopsy from a woman with an LSIL Pap shows a lesion, it is usually low grade, but in 12% to 17% it is high grade.^{36,38,39} For a Pap diagnosis of high-grade SIL (HSIL), the PPV for histologic SIL is around 82%.³⁶

A discordance in lesion grade accounts for approximately 43% of discrepant Pap-biopsy interpretations.⁴⁰ The woman with an HSIL Pap but an LSIL biopsy is a particular dilemma for the clinician, who almost always relies on the biopsy

to guide management. Nevertheless, data suggest that the HSIL Pap interpretation in such cases cannot be entirely discounted: There is a higher prevalence of high-risk human papillomavirus (HPV) types in these women, and a greater risk of HSIL on followup.⁴⁰

CLIA 88 requires that laboratories compare Pap and cervical biopsy reports and determine the cause of any discrepancies in interpretation.¹³ At a minimum, CLIA 88 requires that all Paps reported as HSIL, adenocarcinoma, or other malignant neoplasms be correlated with subsequent histopathologic reports.² The way in which Pap-biopsy discrepancies are reviewed, however, varies from laboratory to laboratory. The variables include (1) the time interval between the Pap test and the biopsy that defines case inclusion; (2) the definition of a discrepancy; (3) the timing of the review (e.g., during or after the biopsy signout); and (4) the individual(s) assigned responsibility for discrepancy resolution. Some laboratories choose to review only concurrent Paps and biopsies²⁹; others include Paps that precede a biopsy, provided the Pap specimen was obtained within a 3-month period before the biopsy.³⁴ Some laboratories consider an ASC Pap discordant if the biopsy shows SIL²⁹; others do not.⁴¹ In some laboratories, all discrepant Pap-biopsy pairs are reviewed by the pathologist responsible for the current biopsy interpretation. In others, the responsibility falls on the pathologist who made the original Pap interpretation.²⁸ In yet others, all Paps and biopsies are reviewed independently by several pathologists, and the review interpretation is an average of several interpretations.^{17,41} Some laboratories perform discrepancy resolution at a weekly consensus conference.³¹ Having so many choices allows a laboratory to select the one that fits its workflow best. Some of the variables, and how laboratories handle them, are summarized in a CAP Q-Probes study.³⁴

Annual Statistics

The CLIA 88 regulations require that cytology laboratories compile annual statistics.



Annual statistics must document:

- the number of cytology cases examined
- the number of specimens by specimen type (e.g., urine, sputum)
- the volume of cases by diagnosis (e.g., negative, atypical, suspicious, positive)
- the number of unsatisfactory cases

- the number of Pap tests with discrepant histologic results
- the number of negative Pap tests that were reclassified as abnormal
- the number of Paps reported as HSIL, adenocarcinoma, or other malignant neoplasm with no histologic followup

Workload Records

The CLIA 88 regulations established, for the first time, workload limits for cytotechnologists (CTs) in the United States. The maximum number of slides is 100 per 24-hour period. This applies to anyone who does primary screening, whether pathologist or CT. The minimum amount of time spent examining this maximum is 8 hours (average 12.5 slides per hour). If a CT spends less than 8 hours screening, the maximum number of slides is prorated using the formula: number of hours examining slides \times 100/8.

The maximum of 100 slides per 24 hours is not intended as a performance target, but rather as an absolute maximum allowed by law.

Each CT is responsible for keeping records of the total number of slides examined each day and the number of hours spent screening. If a CT works at one or more other laboratories that day, the hours spent working at the other laboratories and the number of slides screened there must also be recorded and taken into account by the technical supervisor of the primary laboratory, to ensure that the total number of slides at all laboratories does not exceed the maximum allowed.

The limit on slides includes gynecologic and nongynecologic cases, as well as slides rescreened for QC purposes. Not all slides are created equal. Smears are counted as one slide, but nongynecologic slides in which the cellular material covers one half or less of the slide surface are counted as a half slide.



Slides that typically count as half slides

- cytocentrifuge preparations
- cell block sections
- ThinPrep slides (applies only to nongynecologic cases; gynecologic cases count as a full slide)²
- SurePath slides (applies only to nongynecologic cases; gynecologic cases count as a full slide)²

According to CLIA 88, in performing evaluations using automated or semi-automated screening devices, the calculations of slide equivalencies must follow the manufacturer's instructions.

The 100 slides per day rule is an absolute maximum, but the actual workload limit is set by the technical supervisor based on performance evaluations. The actual workload limit may be 100 slides per day, or it may be lower, but there must be evidence that the performance of each CT is evaluated every 6 months and that the limit is established in accordance with performance measures.

Custom-designed or off-the-shelf software within one's laboratory information system (LIS) is the easiest and most accurate way to document compliance with workload limits. A good LIS can automatically track the number of slides examined when the CT enters his or her provisional (or final, in the case of negative Paps) interpretation. The CT need only enter the number of hours spent screening, the number of slides screened, and the time spent screening at another laboratory, if applicable, for the LIS to calculate the daily workload.

Competency Assessment

CLIA 88 requires that all testing personnel, cytotechnologists and pathologists, undergo documented competency assessment to ensure that they are fulfilling their duties as required by federal regulation. Evaluating and documenting competency are required at least semiannually during the first year the individual tests patient specimens. Thereafter, competency assessment must be performed at least annually.

Note that training and personnel evaluation are not the same as competency assessment. Training happens before someone begins testing, whereas competency assessment confirms that the person is doing the testing correctly. Personnel evaluations evaluate other behaviors and attributes as they relate to the position or job, like customer service.

Six procedures are the minimal requirements for assessment of competency.



Minimal requirements for competency assessment

1. Direct observations of routine patient test performance, including specimen handling, processing, and testing
2. Monitoring the recording and reporting of test results
3. Reviewing of intermediate test results or worksheets, QC records, proficiency testing (PT) results, and preventive maintenance records
4. Direct observations of performance of instrument maintenance and function checks
5. Assessment of test performance through testing previously analyzed specimens, internal blind testing samples, or external PT samples
6. Assessment of problem-solving skills

Competency assessment must be performed for each test that the individual is approved to perform. In the cytology laboratory, the technical supervisor is responsible for performing and documenting competency assessments. This responsibility can be delegated, in writing, to a general supervisor. Competency assessment can be done throughout the entire year by coordinating it with routine practices and procedures to minimize impact on workload. Proficiency testing (PT) performance may be used as part of the competency assessment, but

PT performance alone is not sufficient to meet all six required elements.

Proficiency Testing

Despite provisions in CLIA 88 for cytology PT, a national cytology PT program was not approved until 2004. (CMS had approved an examination offered by the **State of Maryland**, but this test was only available to practitioners in that state.) The first vendor to obtain approval by CMS to offer a national PT program was the **Midwest Institute for Medical Education (MIME)**. Approval of the MIME program was granted in the fall of 2004 for testing in 2005. A year later, in September of 2005, CMS approved the **CAP Pap** PT program for testing beginning in 2006. In 2006, the **ASCP** acquired the complete cytology product line of MIME, including its PT program.

A general description of cytology PT follows:

1. The test is administered onsite every year. Under most circumstances, it is an announced test that has been scheduled at least 30 days in advance.
2. Each participant evaluates 10 gynecologic slides.
3. Each participant is allowed 2 hours to complete the test.
4. Participants are allowed to select the type of Pap preparation (smear, ThinPrep, SurePath) they are most accustomed to.
5. Pathologists who ordinarily examine Pap slides after they are prescreened by a cytotechnologist (CT) may choose to screen a set of test slides that have been previously screened and dotted by a CT. If the pathologist chooses to examine a prescreened set, the CT's diagnosis accompanies the test set.
6. Participants are proctored during the 2-hour period.
7. The laboratory documents the slide handling, proctoring, and testing process.
8. A copy of all records is maintained by the laboratory director.

Scoring:

There are only four possible answers (“response categories”) to each test case:

- a. Unsatisfactory for diagnosis.
- b. Negative. (This may include infectious organisms and/or inflammatory/reactive changes, but not HPV effect.)
- c. LSIL.
- d. HSIL or cancer.

Each test set must contain at least one slide from each of these four response categories.

The scoring grid is different for CTs ([Table 18.8](#)) and for CPs ([Table 18.9](#)).

TABLE 18.8

SCORING GRID FOR CYTOTECHNOLOGISTS

Correct Response	Participant Response			
	A. UNSAT	B. NEG	C. LSIL	D. HSIL
A. UNSAT	10	0	5	5
B. NEG	5	10	5	5
C. LSIL	5	0	10	10
D. HSIL/CA	0	-5	10	10

CA, Cancer; *HSIL*, high-grade squamous intraepithelial lesion; *LSIL*, low-grade squamous intraepithelial lesion; *NEG*, negative; *UNSAT*, unsatisfactory.

TABLE 18.9

SCORING GRID FOR CYTOPATHOLOGISTS

Correct Response	Participant Response			
	A. UNSAT	B. NEG	C. LSIL	D. HSIL
A. UNSAT	10	0	0	0
B. NEG	5	10	0	0
C. LSIL	5	0	10	5
D. HSIL/CA	0	-5	5	10

CA, Cancer; *HSIL*, high-grade squamous intraepithelial lesion; *LSIL*, low-grade squamous intraepithelial lesion; *NEG*, negative; *UNSAT*, unsatisfactory.

Results:

To pass, a participant must attain an overall score of 90% or higher. An individual who passes the test does not need to be tested until the following year.

If a passing score of 90% is not achieved on the initial test, the participant is allowed to take a second 10-slide test within 45 days after notification of test failure. When an individual passes the second 10-slide test, he or she has successfully participated for the year and need not be tested again until the following year. If the participant fails the retest (second event):

- The individual must obtain documented, remedial training in the area of test failure. (The “area of test failure” is noted on the test results letter.)
- All Pap slides screened by the individual after notification of failure must be reevaluated.

• The individual must successfully participate in a 4-hour, 20-slide test.

If the individual fails the third testing event (20-slide test):

- The individual must cease examining Pap slides immediately upon notification of failure.

- The individual must obtain at least 35 hours of documented, formally structured, continuing education in diagnostic cytopathology that focuses on the examination of gynecologic cytology specimens.

- The individual must successfully pass a 20-slide proficiency test.

This final cycle would continue until the individual successfully participates in a 20-slide proficiency test.

Information on aggregate annual pass-fail rates can be found on the CMS web site at <http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/CytologyProficiencyTesting.html>.

For example, in 2005 a substantial difference was found in the first-test failure rates of pathologists who practice with CTs (10%) versus those without (33%). Failure

rates for pathologists and CTs decreased during the first 4 years of testing.

Performance Evaluation

Competency assessment and PT help confirm that personnel are *adequately* performing their laboratory duties and have the basic skill set to continue to do their job. Details of performance evaluation, as described in this section, can be used to fulfill some of the elements of competency assessment but often go beyond the assessment of competence and proficiency by measuring higher-level performance parameters.

Of importance, objective measures of cytotechnologist (CT) and pathologist performance can be distilled from the CLIA-mandated QC activities described previously. In many cases, the data are not absolute measures of accuracy, but rather allow comparison of individuals to their peers as reflected in the laboratory average. The technical supervisor (TS) can then identify outliers who may need educational enhancement and/or an adjustment in their workload limit. As mentioned, the TS has responsibility for evaluating the performance of all CTs every 6 months and determining the actual workload limit of each CT for the next 6-month period. According to CLIA 88, the TS's assessment of the CT must be based on (1) results from the 10% rescreen of negative Paps and (2) the level of pathologist concordance with the CT's interpretations on the cases referred for pathologist review.

Performance evaluation is more problematic in small laboratories with only one or two CTs and/or pathologists because an intralaboratory comparison of the CT with his or her peers is not feasible. In this setting, performance can be evaluated in part by using commercially available, glass slide-based gynecologic and nongynecologic testing modules available from professional societies like CAP and ASCP. Results from many laboratories are tabulated, allowing comparison with one's peers across the country. Information on these programs can be found at the CAP and ASCP websites.

Measures of Cytotechnologist Performance

A technical supervisor (TS), i.e., pathologist, works very closely with CTs and, particularly through daily case review of referred Paps and nongynecologic specimens, develops a subjective impression of their continued performance. These subjective impressions are valuable, but it is wise to temper them with objective measures.



Rationale for objective measures of performance

- reinforce subjective impressions
- rectify misperceptions
- carry more weight with regulatory agencies
- may be more acceptable to the employee than subjective evaluations as justification for educational enhancement

A CT's role in slide review can be broadly divided into two functions: **screening**, or the detection of significant cells, and **interpretation** of cellular findings, with documentation of a provisional (in the case of abnormal Paps and all nongynecologic cases) or final (in the case of negative Paps) interpretation. Both skills should be evaluated. Objective measures of a CT's performance are tabulated at periodic intervals, for example, monthly or every 6 months.

Screening Skills

A CT's screening skills can be assessed from (a) the false negatives detected during the rescreening of 10% of negative Paps and (b) the percentage of cases called abnormal ("abnormal rate") by the CT.

False Negatives. Inevitably, when enough cases interpreted as negative by one CT are rescreened by another, abnormal cells are identified in some of them. Even the most conscientious, well-trained, and alert CT eventually misses abnormal cells. These cases are called false negatives. The prospective rescreen of 10% of negative Paps, originally enacted in the 1960s, was intended as a tool for identifying CTs with less-than-optimal performance. As mentioned earlier, the 10% rescreen is not particularly effective in documenting performance, because the number of missed SILs, at least over a 6-month period, is very low and does not allow meaningful comparison among CTs.¹⁴ For this reason, when the CLIA 88 regulations were submitted for public comment, many argued for abolishing this QC requirement.¹³ It was nevertheless retained, and most laboratories continue to monitor false negatives for performance evaluation. In fact, CLIA regulations now require that performance be evaluated using, in part, the results of the 10% rescreen.²

The 10% rescreening requirement has some merit: It puts CTs on notice that each Pap slide they screen has a chance (at least 1 in 10) of being reviewed by

someone else. This likely improves vigilance.

False negatives are truly uncommon when the threshold for a false negative is set at SIL. An editorial published in 1994 suggested lowering the threshold to ASC.⁴² At this lower threshold, the authors argued, errors are detected frequently enough to make statistically meaningful comparisons among CTs. This argument has merits and drawbacks. Even with a lower threshold, errors may not be frequent enough for statistical significance.^{18,43} The argument is also predicated on the assumption that ASC is a reproducible diagnosis, a tenuous thesis at best.^{44–46} To improve statistical significance, at least, the definition of an error could be expanded even further to include an incorrect statement of adequacy or a missed infectious agent.⁴⁷

In addition to tallying false negatives as raw numbers per CT over time, a statistically useful FNR (also known as the *false negative fraction* or *false negative proportion*) can be calculated. The FNR is defined as *the proportion of all abnormal Paps that are falsely negative*.

Thus, **FNR = FN/(TP+FN)**, where *FN* is *false negatives* and *TP* is *true positives*. A sample calculation is given in [Table 18.10](#). In this example, 5% of negative Paps were rescreened at random. (Another 5% were rescreened from the high-risk pool.) Not all interpretation errors are detected if only 5% of negative Paps are randomly rescreened. Therefore, a total “estimated FNs” must be calculated by multiplying the number of FNs detected (four in this example) by 20, thereby extrapolating to a 100% rescreening effort (i.e., if 100% of the negative Paps had been rescreened, 80 FNs would have been detected).

TABLE 18.10

SAMPLE FALSE-NEGATIVE RATE CALCULATION

Total Paps screened	10,000
Abnormal Paps diagnosed	500
5% random rescreen	4 FNs
Estimated total FNs	$4 \times 20 = 80$
FNR	Total estimated FNs/total abnormals = $80/(500 + 80) = 14\%$

FN, False negative; *FNR*, false-negative rate

FNRs in published literature range from 0% to 28%.⁴²⁻⁴⁸ FNRs can be calculated for individual CTs and used for performance evaluation, particularly if the lab information system is programmed to do this calculation.⁴⁹ The challenge is to establish benchmarks and action thresholds. Although there are no national benchmarks,⁴³ a technical supervisor who uses FNRs for CT performance evaluation can set a threshold for performance and define followup action when this threshold is crossed.⁴⁸

Note that FNRs cannot be used to compare one laboratory with another, because the rescreening effort may vary in its thoroughness from one laboratory to another. Ironically, a laboratory that does a more thorough job of rescreening will have a higher FNR than one that is less thorough. An FNR that corrects for the accuracy of the rescreening process is a better measure of the accuracy of a laboratory,⁵⁰ but this method requires added effort and is not widely applied.

Data generated from the retrospective rescreen (“5-year lookback”) are difficult to apply to performance evaluation. Among other reasons, many of the errors detected were made 4 or 5 years previously, and it is difficult to justify evaluating someone’s current performance based on errors made several years earlier.⁵¹

Abnormal Rate. The abnormal rate is the percentage of abnormal cases (ASC, AGC, SIL, and carcinoma) diagnosed by a CT divided by the total number of cases examined. The abnormal rate of each CT is useful for performance evaluation, because it allows comparison with the laboratory average. A lower-than-average abnormal rate suggests that significant lesions are being missed and can be used as a double-check against the FNR, another measure of screening competency. In fact, CLIA 88 requires that the “case reviews” of individual CTs be evaluated against the laboratory’s annual statistics

and that discrepancies be documented and addressed.

The significance of any individual's variance from the lab average can be measured using statistical measures of variance like the Z score, also called the *standard normal deviate*.⁵² A Z score greater than 2.0 shows an abnormal rate more than two standard deviations (SD) above the average. More importantly, a Z score less than –2.0 indicates an abnormal rate two SDs lower than the lab average, suggesting that there may be a problem in detecting abnormalities.

Note that statistical comparisons are reliable only if there is an equal distribution of abnormalities in the cases screened by the CTs.

Interpretative Skills

Cytotechnologist/Cytopathologist Discrepancy Log. CLIA 88 requires that the performance of a CT be based, in part, on an evaluation of the cases submitted to the pathologist for review. In addition to the valuable subjective impressions obtained from the daily interchange between CT and pathologist, performance can be evaluated using quantitative measures of the degree of agreement.⁵¹⁻⁵³ A well-designed lab information system permits easy tracking of concordance rates. Software programs can tabulate the frequency and severity of discordance and provide statistical measures such as κ values. The κ value, a statistical measure of the degree of concordance between two observers, here the CT and the pathologist, ranges from 0 to 1.0, with 0 being chance agreement and 1.0 being perfect concordance.⁵⁴ Monitoring such measures is useful in identifying CTs with diagnostic interpretative difficulties.⁵³

Unsatisfactory Rate. The unsatisfactory rate is the proportion of all Paps that are interpreted as unsatisfactory. A low unsatisfactory rate suggests that insufficiently stringent adequacy criteria are being applied. As with the abnormal rate, calculating the significance of any variance from the laboratory average can be helpful.

Measures of Cytopathologist Performance

Atypical Squamous Cell-to–Squamous Intraepithelial Lesion Ratio

Because ASC is essentially a diagnosis of uncertainty, it is reasonable for a laboratory to monitor its ASC/SIL ratio and that of its individual pathologists to see if it (or any individual) may be using the ASC interpretation too frequently. To this end, useful benchmarks exist: There is a proposed upper limit (3:1) based

on the collective wisdom of the authors of the Bethesda System⁵⁵ and nationwide percentile rankings for ASC/SIL ratios obtained from laboratory surveys.⁵⁶ Periodic confidential feedback to pathologists on their ASC/SIL ratio can have a beneficial effect, particularly on outliers.^{57,58}

The ASC/SIL ratio is a useful measure of cytotechnologist (CT) as well as pathologist performance. As a measure of CT performance, the ratio must be calculated from the CT's provisional interpretation, not the final one that was resulted by the pathologist.

High-Risk Human Papillomavirus Positivity Rates for Atypical Squamous Cells of Undetermined Significance

Another performance measure of pathologists is based on the commonly used reflex test for high-risk HPV (HR HPV) in women with a cytologic interpretation of ASC of undetermined significance (ASC-US).⁴⁴ A laboratory and, by extension, an individual pathologist, can assess their aggregate ASC-US interpretations over time by seeing if the frequency of positive HR HPV test results conform with accepted benchmarks like those from the ALTS trial (50.6%).⁵⁹ In this manner, HPV test results are used to adjudicate aggregate morphologic interpretations.⁶⁰⁻⁶²

There is little correlation between the ASC/SIL ratio and the percentage of positive HR HPV test results for ASC-US interpretations.⁶⁰ The lack of correlation is not surprising, because these two variables measure different aspects of a cytopathologist's (CP's) performance. The ASC/SIL ratio is a measure of the degree of uncertainty in the interpretation of cytologic findings, expressed as a ratio of the number of diagnoses of uncertainty (ASC) to a number of diagnoses of certainty (SIL). By contrast, the percentage of ASC-US cases that are positive for HR HPV DNA is an objective assessment of the risk of dysplasia in an aggregate population of ASC-US cases, because aggregate ASC-US cases are measured against an external standard, the presence or absence of an oncogenic virus. Importantly, a benchmark for the HR HPV-positive rate of ASC-US cases adjudicated by experienced CPs exists in the ALTS trial database.⁶³

Drift from the desired norm, in practice, comes in a variety of combinations of an ASC/SIL ratio (low, average, high) and a HR HPV-positive rate for ASC-US (low, average, high).⁶² It is important for the pathologist to understand the underlying forces that can cause indicators to shift from the norm. A working schema that explains the possible alterations from the desired ASC/SIL ratio

and/or HR HPV-positive rate for ASC-US can be useful for understanding the forces that perturb these indicators (Fig. 18.1).⁶² Identifying such patterns and finding an explanation for perturbations in one or both measures can be useful to the pathologist, who can make an informed decision to adjust a diagnostic threshold.

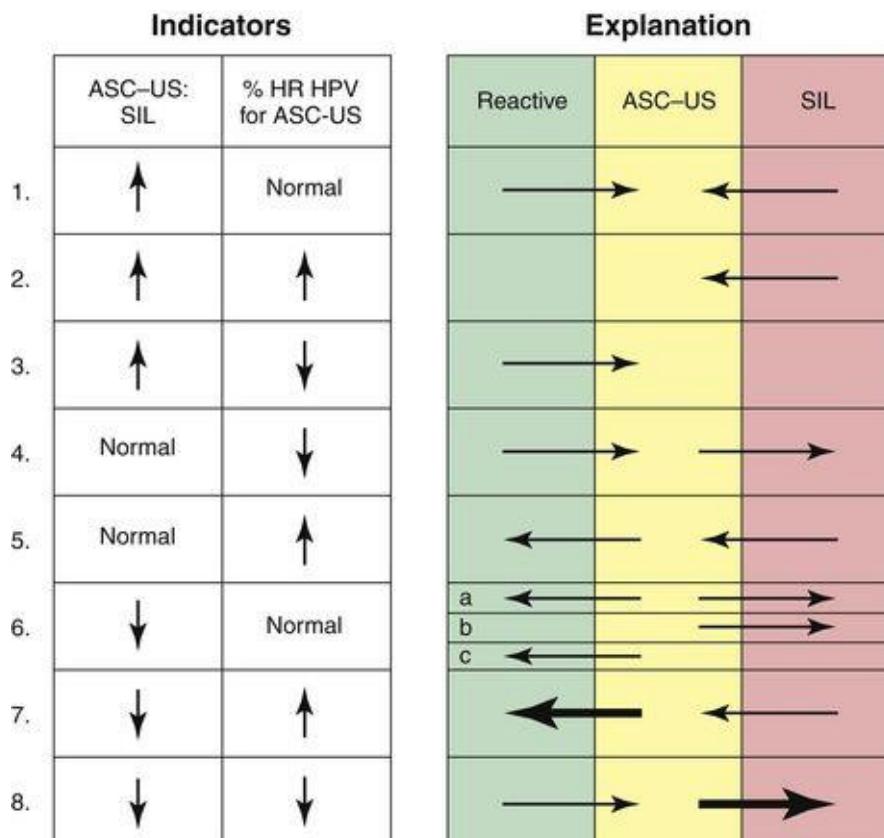


FIGURE 18.1 Behavioral explanations of variation from the expected atypical squamous cells, undetermined significance (ASC-US)/squamous intraepithelial lesion (SIL) ratio and high-risk human papillomavirus (HR HPV) positivity rate for ASC-US.

Eight different anomalies with regard to these two variables are illustrated. For example, an individual with pattern 1 has an elevated ASC-US/SIL ratio but a normal HR HPV positivity rate for ASC-US. The horizontal arrows show how movement of interpretations from one diagnostic category to another can affect the ASC-US/SIL ratio and the rate of HR HPV positivity for ASC-US in different ways.

(With permission from Cibas ES, Zou KH, Crum CP, Kuo F. Using the rate of positive HR HPV test results for ASC-US together with the ASC-US/SIL ratio in evaluating the performance of the cytopathologist (CP). Am J Clin Pathol 2008;129:97-101. © 2008-13 American Society for Clinical Pathology; © 2008-13 American Journal of Clinical Pathology.)

It is important to note that neither the ASC/SIL ratio nor the HR HPV-positive rate for ASC-US is a measure of diagnostic accuracy in gynecologic cytology. Although useful for calibrating interpretive thresholds, neither allows the identification of true-positive, false-positive, true-negative, and false-negative

results, all of which are necessary for calculations of sensitivity and specificity, which are prerequisites for an accuracy measurement. The accuracy of a pathologist can be measured after unbiased review of slides or by correlation with histologic followup.

Cytology-Biopsy Correlation

Cytology-biopsy correlation provides information on a pathologist's diagnostic accuracy in a variety of ways. Data from histologic followup can be used to generate different statistical measures of accuracy, such as the positive predictive value (PPV). When a pathologist makes a positive cytologic diagnosis, how often is it confirmed by a subsequent biopsy?

The formula is

$$\text{PPV} = \text{TP}/(\text{TP} + \text{FP}),$$

where *TP* is the number of cases with a positive biopsy (true positive), and *FP* is the number of cases with a negative biopsy (false positive).

Because cytologic diagnoses are not binary but are expressed in several probabilistic categories (negative, atypical, suspicious, positive), a positive diagnosis for the purposes of biopsy correlation can be defined in several ways. For example, one can combine suspicious with positive diagnoses, but exclude atypical diagnoses. A PPV can be calculated for any specimen type, whether gynecologic or nongynecologic, but a positive diagnosis must be precisely defined.

A related measure is the specificity, which also takes into account FP diagnoses. The formula is

$$\text{specificity} = \text{TN}/(\text{TN} + \text{FP}),$$

where *TN* (true negatives) is the number of cases called negative and confirmed as such. It is a more difficult measure to apply to pathologist performance evaluation because negative diagnoses are more difficult to verify.

The PPV and specificity measure only one side of accuracy—that related to false positives (“overcalls”). FNs (“undercalls”) by the pathologist are equally important and are often expressed as the sensitivity:

$$\text{sensitivity} = \text{TP}/(\text{TP} + \text{FN}).$$

Sensitivity and specificity are highly negatively correlated, meaning that improvements in specificity often come at the expense of sensitivity.⁶⁴ An ideal measure of a pathologist's abilities evaluates both. In fact, sensitivity and specificity can be expressed simultaneously in a **likelihood ratio** (LR), the probability of a given test result if disease is present divided by the probability of the same result if disease is absent.⁶⁵

$$\text{LR} = \text{sensitivity}/(1 - \text{specificity}).$$

Sensitivity is sometimes called the *true-positive fraction*, and $(1 - \text{specificity})$ is called the *false-positive fraction*.⁶⁶

LRs can be expressed at different decision levels (e.g., negative, atypical, suspicious, positive). When LRs are calculated for a test at several different decision levels, the result is a **receiver operating characteristic (ROC) curve**. ROC curves can be generated for individual pathologists, and thresholds can be set for acceptable performance ([Fig. 18.2](#)).

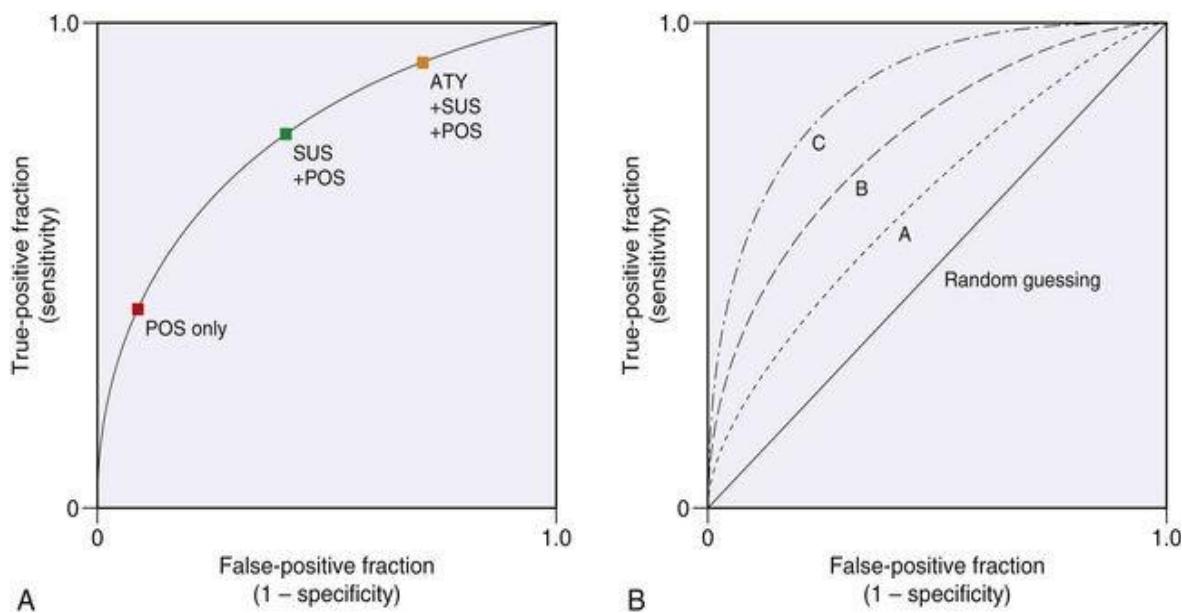


FIGURE 18.2 Receiver operating characteristic (ROC) curves.

A, Cytologists use different decision thresholds—"atypical" (ATY), "suspicious" (SUS), "positive for malignancy" (POS)—in diagnosis. Sensitivity and specificity can be measured at each decision threshold. At the most stringent decision threshold, when only positive

diagnoses are considered true positives, a point closest to the origin is generated. As the criteria become less stringent, the points move higher on the curve. *B*, Random guessing produces a 45-degree angle line. The accuracy of observers A, B, and C is the area under their curves. Perfect accuracy is an area of 1.0. Of the three observers, C has the best accuracy.

ROC curves have been used to evaluate the accuracy of pathologists interpreting breast FNA findings and have shown that pathologists with greater training and experience have greater accuracy.⁶⁵ ROC curves have shown that pathologists have better accuracy evaluating bronchial brushings when they are provided with clinical history.⁶⁷ Finally, ROC curves have been used to evaluate the accuracy of pathologists reviewing Pap test findings, with ASC, LSIL, and HSIL treated as discrete diagnostic thresholds of increasing stringency.⁶⁸

ROC curves, although useful for performance evaluation, have limitations, especially when applied to gynecologic cases. Biopsy data are sparse for women with negative Paps, and most negative Paps are not reviewed by a pathologist. If a PPV or ROC curve is generated from routine work, the group of Paps each pathologist reviews is different.

Finally, the biopsy is not a perfect gold standard. Note, however, that these limitations are less important if one is comparing individuals with the norm, rather than looking for absolute accuracy. For example, the imperfections of the biopsy as a gold standard are less troublesome if its imperfections affect all individuals equally. This is likely to be the case if the sample size is sufficiently large, for example, if an ROC curve is calculated for a 1- or 2-year period.

Safety

Occupational Safety and Health Administration Bloodborne Pathogens Standard

The OSHA Bloodborne Pathogens Standard was published in 1991 to control the health risk associated with exposure to blood and other potentially infectious materials. Of primary concern are the human immunodeficiency virus (HIV) and the hepatitis B and C viruses, and needlestick injuries are a major culprit. An estimated 600,000 to 800,000 needlestick and other percutaneous injuries occur every year among health care workers. In response to this public health concern, Congress passed the Needlestick Safety and Prevention Act that became law on November 6, 2000. To meet its requirements, OSHA revised its Bloodborne Pathogen Standards in 2001. The revised standard clarifies the need for employers to select safer needle devices and to involve employees in identifying and choosing these devices. The updated standard also requires employers to maintain a log of injuries from contaminated sharps.

Exposure Control

Each employer must establish a written exposure control plan, the goal of which is to eliminate or minimize occupational exposures. The plan needs to be updated annually.



The written exposure control plan includes:

- exposure determination
- methods of compliance
- hepatitis B vaccination and postexposure evaluation
- communication of hazards to employers
- recordkeeping

“Exposure determination” contains a list of all job classifications in which all employees have occupational exposure, and a list of all job classifications in which some employees have exposure. It also contains a list of tasks or

procedures in which exposure occurs.

Methods of compliance include:

- universal precautions
- engineering and work practice controls

Because it is impossible to identify all patients whose blood may contain HIV, hepatitis B, or hepatitis C, it is advisable that blood and certain body fluids of all patients are considered to be potentially infectious. This principle of **universal precautions** was emphasized in the 1980s and is still in effect today.⁶⁹

Potentially infectious human materials include:

- blood
- semen
- vaginal secretions
- CSF
- synovial fluid
- pleural, pericardial, and peritoneal fluids
- amniotic fluid
- saliva in dental procedures
- any fluid visibly contaminated with blood
- all body fluids in situations where it is difficult or impossible to differentiate between body fluids
- any unfixed human tissue

Engineering and work practice controls include:

- handwashing facilities
- sharps disposal methods
- storage and transport methods
- personal protective equipment
- housekeeping practices
- regulated waste removal
- safer laundry practices

Employers are encouraged to provide handwashing facilities. Employees must wash hands after removal of gloves or other personal protective equipment. If blood or other potentially infectious materials come in contact with skin or mucous membranes, the skin should be washed with soap and water, and mucous membranes must be flushed with water immediately.

Proper precautions are advised regarding the handling of contaminated needles and other “sharps” (scalpel blades, glass). Needles should not be bent or recapped unless no alternative is feasible, in which case the procedure must be accomplished by mechanical device or a one-handed technique.



Contaminated sharps are discarded immediately in containers that are:

- closable
- puncture-resistant
- leakproof on sides and bottom
- labeled or color-coded
- easily accessible during use
- maintained upright
- replaced routinely and not allowed to overfill

Eating, drinking, smoking, applying cosmetics or lip balm, and handling contact lenses are prohibited in the cytopreparatory area or during an FNA procedure, when there is a reasonable likelihood of occupational exposure.

Specimens must be placed in a leakproof container during collection, handling, processing, storage, or transport. If outside contamination of the primary container occurs, it should be placed inside a second leakproof container.

The employer is required to provide **personal protective equipment (PPE)** to the employee. PPE includes gloves, mask, eye protection, and face shield. Gloves should be worn whenever the employee may have contact of the hands with blood or other potentially infectious materials. Masks in combination with eye protection devices (goggles or glasses with solid side shields) or chin-length face shields should be worn whenever splashing of blood or potentially infectious materials may occur and contaminate the eyes, nose, or mouth.

Contaminated work surfaces should be decontaminated with a disinfectant after completion of procedures, after surfaces are overtly contaminated, and at

the end of the work shift. Protective coverings such as imperviously backed absorbent paper should be removed and replaced after they are overtly contaminated or at the end of the work shift. Broken glassware must be picked up by mechanical means (brush and dust pan) and not with the hands.

Regulated waste, such as empty specimen containers, must be placed in containers that are closable, leak proof, and labeled. Contaminated laundry must be handled as little as possible and bagged at the location it was used.

Hepatitis B Vaccination

The employer must offer free hepatitis B vaccination to all employees with potential occupational exposure, unless the employee has been previously immunized, is immune based on antibody testing, or the vaccine is contraindicated for medical reasons. Employees must either accept or sign a statement to say that they decline the vaccine. After a report of an exposure, the employer must provide the employee with an immediate confidential medical evaluation and followup.

Communication of Hazards to Employees

Warning labels must be applied to containers of waste and refrigerators containing blood or other potentially infectious material. Specimen containers that are labeled as to their contents are exempted from this requirement. The labels must be fluorescent orange or orange-red, with lettering and symbols in a contrasting color ([Fig. 18.3](#)).



FIGURE 18.3 Biohazard warning label.
The label is brightly colored, and the lettering is easy to read.

Employers need to ensure that all employees with occupational exposure participate in a training program, at no cost, at the time of initial assignment and at least annually thereafter.

Recordkeeping

The employer must establish and maintain a record of each employee with occupational exposure.



The record for each employee with occupational exposure must include:

- employee's name and social security number
- hepatitis B vaccination status
- postexposure and followup
- written opinion of health care professional
- confidentiality

The employer must also keep a record of all training sessions attended by the employee as well as a sharps injury log.

Occupational Safety and Health Administration Laboratory Standard

OSHA recognizes the hazards of the laboratory as a workplace and has developed a standard, often referred to as the *Laboratory Standard*, for occupational exposure to hazardous chemicals. The list of hazardous chemicals in a cytology laboratory is relatively short. Nevertheless, all laboratories are required to produce a chemical hygiene plan that specifically addresses the hazards on its premises. Numerous chemical hygiene plans from universities and health care facilities can be found on the Internet, and many are listed on OSHA's Web site.

Excluded from this standard are chemicals used in building maintenance and

the production of chemicals for commercial sale; these are regulated under other OSHA standards.

The laboratory director must determine which hazardous chemicals are present in the laboratory. He or she must also monitor the exposure of employees to chemicals that might exceed the action level. OSHA provides a summary of the properties of hazardous chemicals, their health effects, and procedures for sampling the levels of exposure. **Permissible exposure limits (PELs)** have been established for hazardous chemicals. These are expressed as longer (typically 8-hour) and shorter (15-minute) time-weighted averages (TWAs). An action level is the PEL calculated as an 8-hour TWA. Exceeding it initiates required activities such as exposure monitoring.

The current OSHA PEL for xylene is 100 parts per million (ppm) for an 8-hour TWA. The odor threshold for xylene is 1 ppm. Because the odor threshold is below the PEL, xylene is considered to have adequate warning properties.

For every hazardous chemical, OSHA requires that the manufacturer develop warning labels for containers, as well as **material safety data sheets (MSDSs)**. MSDSs provide comprehensive technical information, such as PELs, and serve as a reference for exposed workers, their employer, and their health care provider. By contrast, labels provide a brief synopsis of the hazards. Training in the reading of labels and MSDSs is essential so that employees know what to wear to protect themselves and what actions are necessary in case of emergency.

All laboratories that generate hazardous waste must set up a **satellite accumulation area (SAA)**. An SAA is a designated area where the hazardous waste will be stored until it is sent out for processing. An SAA can be a bench top, a section of the hood, or an empty cabinet, but it must be near the point where waste is generated.



The satellite accumulation area must have a label with:

- the words *hazardous waste*
- the chemical name in words (not formula)
- the type of hazard (e.g., ignitable, toxic)
- a place to write the date when the container is full

A designated person is called to pick up the container when it is full and arrange for its disposal. Hazardous waste cannot be poured down sinks or in

waste baskets; it must be treated, disposed of in a chemical landfill, or burned in a hazardous waste incinerator approved by the Environmental Protection Agency (EPA).

National Fire Protection Association Standard for Health Care Facilities

This standard, known as *NFPA 99*, contains requirements for minimizing hazards, particularly due to fire in health care facilities. Of particular relevance to cytology laboratories, it sets the standards for the storage and use of flammable and combustible liquids in laboratories.

National Fire Protection Association Standard on Fire Protection for Laboratories Using Chemicals

This standard, known as *NFPA 45*, outlines the maximum allowable quantities of flammable and combustible liquids and the requirements for laboratory ventilation and hoods.

NFPA has also published a standard (*NFPA 704*) for recognizing and identifying specific hazards that may present as short-term exposure as a result of a fire or a spill. The system uses a diamond-shaped placard (“fire diamond”) that incorporates colors and numbers. Hazard severity ranges from zero (0), which indicates a minimal hazard, to 4, the most severe hazard. The diamond is subdivided into four squares of different colors.



Each color in the fire diamond identifies a different hazard:

- blue: health hazard
- red: flammability
- yellow: instability/reactivity
- white: special hazards

An example is shown in [Fig. 18.4](#). The “special hazards” are *W*, which indicates unusual reactivity with water and is a caution against using water for either fire or spill control, and *OX*, which indicates that the material is an

oxidizer. These placards are posted on exterior walls of a facility, at any access to a room, or at each principal means of access to an exterior area. It is especially important that they be visible to emergency responders. If there are many chemicals in a laboratory, professional judgment is advised to increase or decrease the severity ratings by assessing the relative quantities and their synergistic effects.

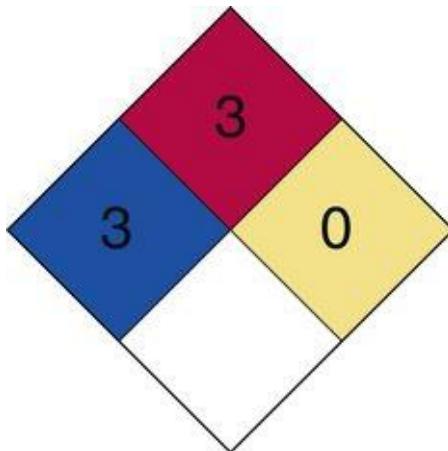


FIGURE 18.4 Fire safety placard.

In this example, the health hazard (*blue*) and fire hazards (*red*) are rated 3 (out of 4). The instability hazard (*yellow*) is zero and there are no special hazards (*white*).

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