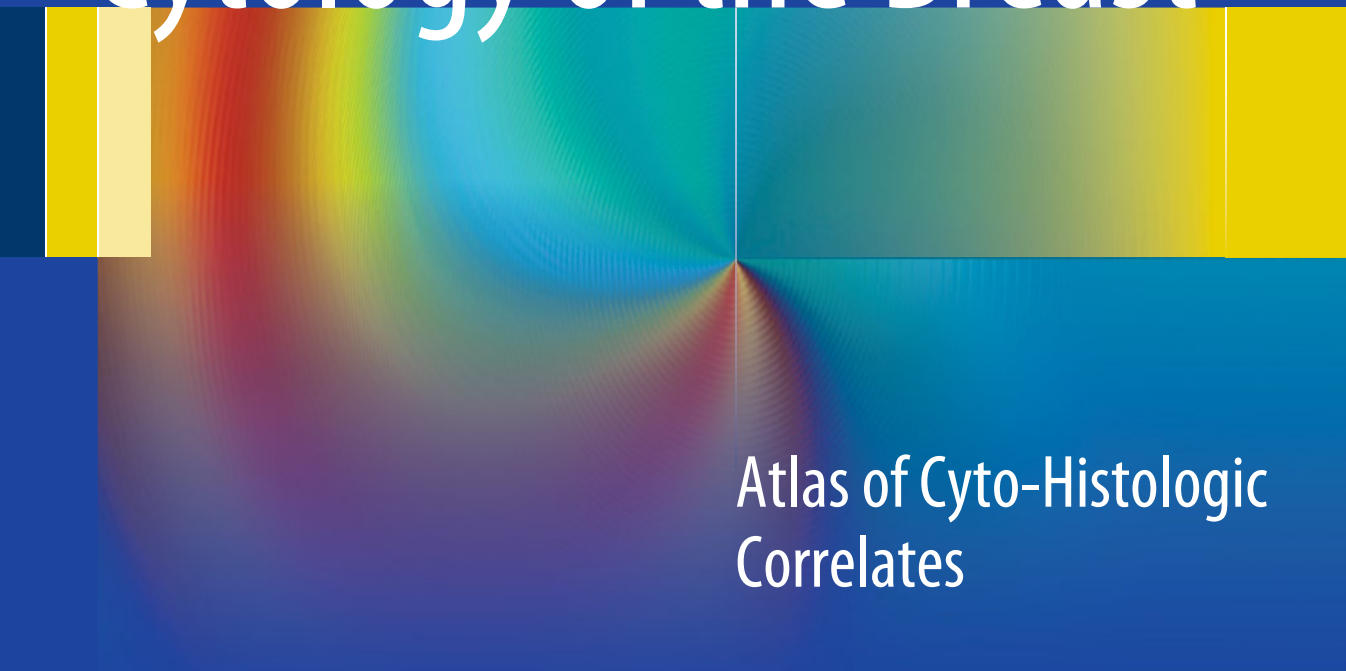
Gary Tse
Puay Hoon Tan
Fernando Schmitt

Fine Needle Aspiration Cytology of the Breast



Atlas of Cyto-Histologic Correlates

Springer

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Foreword

Fine needle aspiration cytology (FNAC) of the breast, although introduced in the 1930, became popular in the 1970s. As with many other techniques in medicine, it started slowly but gained greater acceptance when European investigators reported large series of breast FNAs proving the technique to be useful and accurate. Since then, the technique has gained acceptance throughout the world, although more recently a decline in the utilization of FNAC for some screen-detected lesions and greater reliance on core needle biopsy have taken place.

This book entitled *Fine Needle Aspiration Cytology of the Breast* incorporates state-of-the-art knowledge in the field, and the wealth of information was contributed by Dr. Gary Tse, Dr. Puay Hoon Tan, and Dr. Fernando Schmitt.

Chapters 1 and 2 cover anatomy and physiology of the breast and basic breast pathology. Chapter 3 describes the aspiration techniques emphasizing the aspiration procedure, reporting of results and diagnostic accuracy. Liquid-based cytology and cell block preparations are presented in Chap. 4. The various diagnostic entities are covered in Chaps. 5, 6, 7, 8, 9, 10, 11, 12, and 13. These chapters provide a concise and practical approach to challenges cytologists face in daily practice, with cyto/histologic correlation, differential diagnosis, and management of results.

Immunocytochemistry and molecular techniques, very important parts of current cytopathology practice, are described in detail in Chaps. 14 and 15.

Color photomicrographs provide excellent visual images of a variety of lesions throughout the book.

Comparison between FNAC and core biopsy is presented in Chap. 16, and the authors emphasize that when dealing with non-palpable screen-detected lesion, needle biopsies excel over FNAC as more material and better retrieval of calcifications are achieved.

Let us enjoy this impressive textbook and marvel at the beauty of the illustrations, a book that presents in a lucid fashion the current knowledge in breast FNAC.

Philadelphia, PA, USA — Marluce Bibbo, MD

Preface

We are experiencing extraordinary advances in medicine. Surgery is less invasive, and image guidance has become a fundamental tool in targeting the lesions of interest. The availability of cells or tissues from patients remains crucial for disease diagnosis, and identifying molecular changes or surrogate markers important for prognosis and predicting therapeutic response. With this backdrop, it is not difficult to conclude that cytology continues to play a central role in modern medicine. Currently, breast cytology is being replaced by core needle biopsy (CNB) in many centers of the western world under a perception that CNB is superior to cytology in all respects. In actual fact, fine needle aspiration cytology (FNAC) of the breast is an excellent way to diagnose breast lesions and can be accomplished during a routine doctor's office or clinic visit or at the patient's bedside. It utilizes inexpensive equipment and can be performed, interpreted, and reported in a matter of minutes, expediting the patient's entry into treatment. FNAC and CNB are not mutually exclusive but are complementary methods. This is extensively discussed in this textbook in the different chapters. In the practice of all authors, FNAC still has an important role as a first-line cost-effective method to investigate breast lesions. One of the causes of decline of this technique is the decreasing familiarity and experience of pathologists with breast cytology. This book aims to demonstrate the different aspects of breast cytology, including discussion of the technical aspects, description of the morphological characteristics of diverse lesions, and the harnessing of ancillary techniques on cytologic material that can gather more information for pathologists. Moreover, with the development of new treatment protocols for breast cancer patients, the use of FNAC is increasingly used to rule in or out multicentric disease. In combination with axillary lymph node and distant site aspirations, disease staging and planning of suitable therapy can be more quickly and economically achieved. FNAC is also being used to obtain cells to assess molecular markers that can guide treatment, especially in metastatic lesions. Recently, in recognition of the importance of FNA in assessment of breast cancer, a chapter on this subject was included in the latest 4th edition of the WHO Classification of Tumors of the Breast.

More than 50 years after the reintroduction of FNAC as a diagnostic method by the "Karolinska Hospital" school, this method still represents a near-perfect test. It is relatively easy to perform requiring no "high-tech" gadgetry; costs are low and are substantially less expensive than open biopsy. The procedure is safe, yielding material that provides for a high-diagnostic accuracy in an

extremely short time frame. The ability to perform either FNA and/or CNB, based on a given set of clinical/radiological/pathologic findings, allows one to take advantage of the benefits that both procedures have to offer.

Our wish is that you, the reader, for which this book is written, can use it in daily practice. We hope this book can serve as a ready resource for obtaining helpful information to solve cases and to help your patients.

Gary Tse
Puay Hoon Tan
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We are also very grateful to our families for being so understanding and supportive during this period.

Contents

Anatomy and Physiology of the Breast

1

1.1 Anatomy of the Breast

The anatomy of the breast has been well documented (Bannister 1995). The adult breast is a fibroadipose organ that sits on the anterior upper thorax, extending from the second through the sixth ribs and from the sternum to the anterior axillary line, with an axillary tail in the upper outer portion that can be palpated along the outer border of the pectoralis major muscle. It forms a secondary sexual characteristic of females, providing nutrition to their young, while it is rudimentary in males. It lies upon the deep pectoral fascia, overlying the pectoralis major and serratus anterior. The nipple protrudes from the center of the breast anteriorly, while the areola is the discoidal skin that encircles the nipple. The areolar surface appears rough because of the presence of large modified sweat glands called the glands of Montgomery, which are located beneath the skin and whose fatty secretions serve to lubricate the nipple. Smooth muscle bundles in the areolar tissue help to stiffen the nipple for better grasp during suckling by the infant. The breast consists of 15–20 segments and has components of epithelial glandular tissue, fibrous connective tissue that surrounds the glandular elements, and interlobar adipose tissue. The relative amount of fibrous and adipose tissue determines the size and consistency of the breast in the nonpregnant, nonlactating female. The fibrous connective tissue is continuous with the pectoralis fascia and also sends strands, called the suspensory ligaments of Cooper, into the overlying skin. Each lobe or segment of the breast comprises branching ducts and terminal secretory lobules which converge into 15–20 larger lactiferous ducts that open onto the apex of the nipple. The number and size of these terminal secretory lobules vary greatly in different individuals and at different periods of life – they are most numerous during the reproductive age and are fully developed only during pregnancy and lactation. The lactiferous sinuses or ampullae are short dilated portions of the lactiferous ducts that lie partly within and deep to the nipple, in which milk may be stored.

1.2 Physiology of the Breast

1.2.1 Development

Mammary development occurs during the 15th week of fetal gestation, with condensation of mesenchyme around the epithelial stalk of the breast bud (Rosen 2009). Together with dermal papillary and reticular layers which form the fibrous connective tissue of the breast, the epithelial stalk becomes the ductal system of the breast lobe. Myoepithelial cells form from basal cells during the 23–28th weeks of gestation. The mesenchyme differentiates into fat between the 20th and 32nd weeks. Fetal breast development depends on a combination of growth and differentiation factors as well as bcl2 and testosterone. During the late third trimester, maternal and placental steroid hormones and prolactin result in secretory activity in the fetal breast. After birth,

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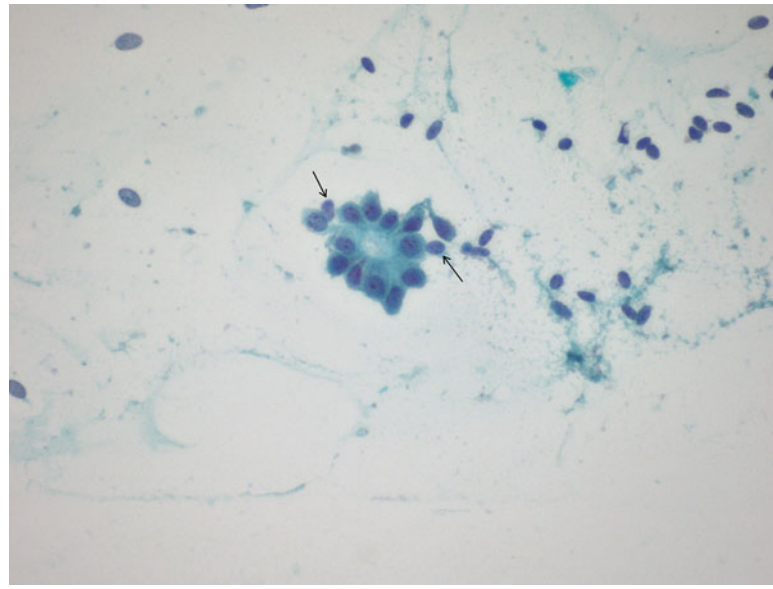

Fig. 1.1 Normal breast acinus comprising a wreath of luminal epithelial cells and occasional myoepithelial cells (*arrows*). Scattered bipolar naked nuclei are seen in the background

the neonate may have palpable breast enlargement which subsides after maternal hormones disappear from the infant's bloodstream. During childhood, the breast remains in an inactive state with histological ducts without alveolar or acinar differentiation.

In premature thelarche, there is unilateral or bilateral breast enlargement before puberty due to abnormal levels of endogenous hormones. The nodular breast tissue of premature thelarche tends to regress spontaneously. It is important to recognize this condition, as surgical excision will lead to amastia. Histologically, premature thelarche shows gynecomastoid appearances with ducts devoid of acini, demonstrating solid and micropapillary patterns of usual ductal hyperplasia. FNAC is generally paucicellular, with scattered benign bimodal aggregates within a background containing a few bipolar stromal cells and myxoid material (Fig. 1.1).

1.2.2 Puberty and Menarche

At puberty, there is onset of cyclical estrogen and progesterone secretion that initiates adolescent breast development. Estrogen, growth hormone, and glucocorticoids stimulate ductal growth, while periductal stromal development is dependent on estrogen. Lobular and acinar formation depends on insulin, progesterone, and growth hormone. Lobular growth and development continue from puberty until adulthood and are further enhanced during pregnancy and lactation.

1.2.3 Menstrual Cycle

During the menstrual cycle, physiologic variation of hormonal levels can lead to changes in the breast, including size and consistency. It is believed that the breast is less nodular at the mid-phase of the menstrual cycle as well as in the later follicular phase and is thus optimal for clinical breast examination during this period. Radiologically, the breast is also least dense during the follicular than luteal phase of the menstrual cycle. The breast is more voluminous in the luteal phase due to increased parenchymal water content.

Histologically, the *proliferative phase* of the menstrual cycle is accompanied by increased mitoses and apoptosis in the breast epithelium, without luminal formation or secretory activity. Myoepithelial cells are difficult to discern. The lobular stroma is dense. The *follicular phase* shows decreased mitotic activity and more discernible myoepithelial cells that may assume clear cytoplasm. Lumens are better visualized, and the intralobular stroma becomes loose.

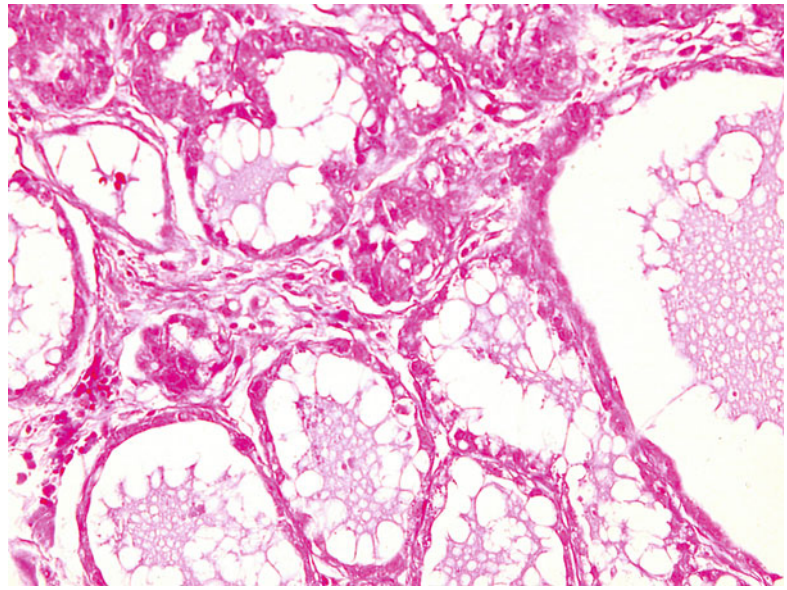

Fig. 1.2 Lactational changes. Corresponding histology shows dilated acini lined by cells with variably sized nuclei, containing luminal pink secretions

During the *luteal phase*, myoepithelial cells are increasingly prominent with cytoplasmic glycogen accumulation and clearing. Modest secretory activity is noted. In the secretory part of the cycle, there is distention of glands with secretions. Lobular stroma is maximally edematous. The *menstrual phase* is marked by loss of stromal edema with infiltration by inflammatory cells into the lobule. Glandular lumens may be obscured, and mitotic activity is absent.

Detection of estrogen and progesterone receptors in breast epithelium is reported to vary during the menstrual cycle with highest expression of estrogen receptor and progesterone receptor in the proliferative and follicular phases, respectively (Silva et al. 1983). There are also reports of greatest expression of both estrogen and progesterone receptors in the follicular phase (Fabris et al. 1987). Immunohistochemical detection of estrogen receptor was noted in 31 % of samples obtained on FNAC during the first half compared to its absence in the second half of the menstrual cycle (Markopoulos et al. 1988). Similarly, estrogen receptor in breast cancer is reported more frequently in the follicular than in the ovulatory or luteal phases, while progesterone receptor is more expressed in the ovulatory than in the follicular and luteal parts of the menstrual cycle (Pujol et al. 1998), though these observations are not statistically significant and have not been confirmed in other studies (Markopoulos et al. 1988; Weimer and Donegan 1987; Smyth et al. 1988).

1.2.4 Pregnancy and Lactation

Pregnancy results in hypertrophy and hyperplasia of lobular acini with secretory changes which can occur unevenly in the breast (Rosen 2009). Pregnancy-related hyperplasia is most prominent in the third trimester and can present as palpable lumps that are histologically lactational adenomas. Lobular enlargement commences in early pregnancy with accompanying reduction in stroma. Increased stromal vascularity and influx of inflammatory cells are noted. In the second and third trimesters, there is progressive lobular growth. Microscopically, luminal epithelial cells show cytoplasmic vacuolation, while myoepithelial cells become indistinct. Luminal secretions are seen in acini of the markedly expanded lobules. Stroma is diminished (Fig. 1.2). Alterations continue into lactation. FNAC of the pregnant or lactating breast shows a cellular yield with epithelial cells harboring open vesicular nuclei and conspicuous nucleoli, accompanied by lipoproteinaceous secretory material in the background (Fig. 1.3). Epithelial cell cytoplasm tends to be frayed, and these cytologic changes may

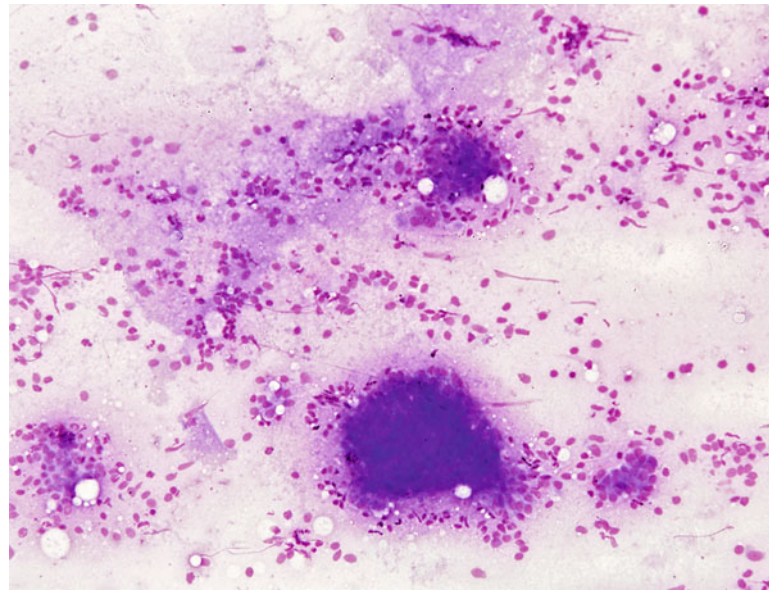

Fig. 1.3 Lactational changes. The smear shows cohesive epithelial clusters with many dispersed naked nuclei in a lipoproteinaceous background

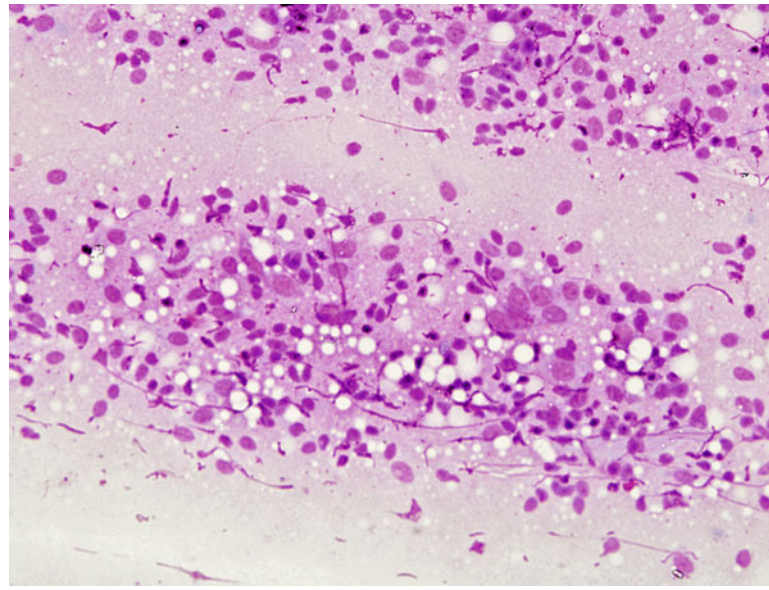

Fig. 1.4 Lactational changes. Higher magnification of the dispersed cells which show slight nuclear enlargement and variability in size, with discernible nucleoli. Cytoplasm is indistinct, and the lipoproteinaceous background incorporates some debris

occasionally be overinterpreted as atypical, suspicious, or even malignant, especially when superimposed on an underlying lesion such as a fibroadenoma (Fig. 1.4).

Involution after cessation of lactation takes about 3 months. Secretion of milk stops when prolactin levels decreases. Epithelial cells undergo desquamation and phagocytosis, and the number of lobules and acini diminishes. Some macrophages are seen in the lobule. The breast eventually returns to its fibrofatty consistency.

1.2.5 Menopause

Breast changes during menopause occur in response to decreased estrogen and progesterone levels whereas androgen levels are not reduced

(Rosen 2009). There is a decrease in the number of lobules and acini with epithelial atrophy accompanied by thickening of acinar basement membranes and occasional calcifications. Like pregnancy and lactation, the changes are uneven throughout the breast. Myoepithelial cells are relatively spared by the process of menopausal atrophy and appear prominent. Administration of hormone replacement therapy may attenuate these alterations.

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Basic Histopathology of Breast Lesions

2

2.1 Introduction

The clinical presentation of breast diseases can be multiple, including breast lump, breast "lumpiness," nipple discharge, pain and redness of the overlying skin, or axillary lymph node enlargement. Nowadays, many breast lesions are asymptomatic, being detected by imaging or breast screening or as incidental findings during surgical removal for a different lesion. In general, the presentation may give some hints to the nature of the underlying pathology. A solitary breast lump may represent either a benign or malignant breast tumor; typically, benign breast tumors are not fixed to the underlying structures, being freely mobile within the breast parenchyma, and palpation or imaging shows rounded borders. A malignant tumor, on the contrary, shows irregular borders, and the tumor desmoplasia may render the mass firm to hard on palpation and appear fixed to the underlying parenchymal tissue or the overlying skin. Malignant breast masses are usually painless. Breast "lumpiness" gives a sensation of many small nodularities upon palpation, and this is characteristic of fibrocystic changes with the multiple small cysts being felt. There may be associated pain, which typically is related to the menstrual cycle. Nipple discharge is an uncommon and alarming symptom, particularly when the discharge is blood stained. Nipple discharge can be multiorificial or uniorificial. The former usually occurs when there is diffuse change in the breast, as occurs in fibrocystic changes, particularly when there is duct ectasia, when the secretions are accumulated within the ductal system, whereas in uniorificial discharge, there is usually an intraductal lesion or growth. The typical example in this situation is a papillary lesion. Both benign (duct papilloma) and malignant (papillary carcinoma) papillary lesions may give rise to blood-stained discharge. Pain and redness of the overlying skin are characteristic symptoms for acute inflammation or abscess of the breast. Rarely patients with breast cancer may present not with a clinicoradiological breast mass but with enlarged axillary lymph nodes; such cases are referred to as 'occult primary' and warrant a diligent search for the primary tumor. Increasingly asymptomatic breast lesions are detected as a result of mammographic screening, either because of architectural distortion or calcifications on imaging. The lesions may range from benign fibrocystic changes, radial sclerosing lesions and columnar cell changes to atypical lesions and tumor precursors, notably flat epithelial atypia (FEA) or atypical epithelial hyperplasia, to carcinoma in situ or small invasive cancers.

2.2 Inflammatory Breast Lesions

Mastitis or inflammation of the breast is uncommon. Mastitis can be divided into acute and chronic, and acute mastitis may be associated with abscess formation. Acute mastitis usually occurs at the postpartum period, when the lactating breast tissue is swollen, and sometimes the ducts are obstructed, with inspissation of the secretion.

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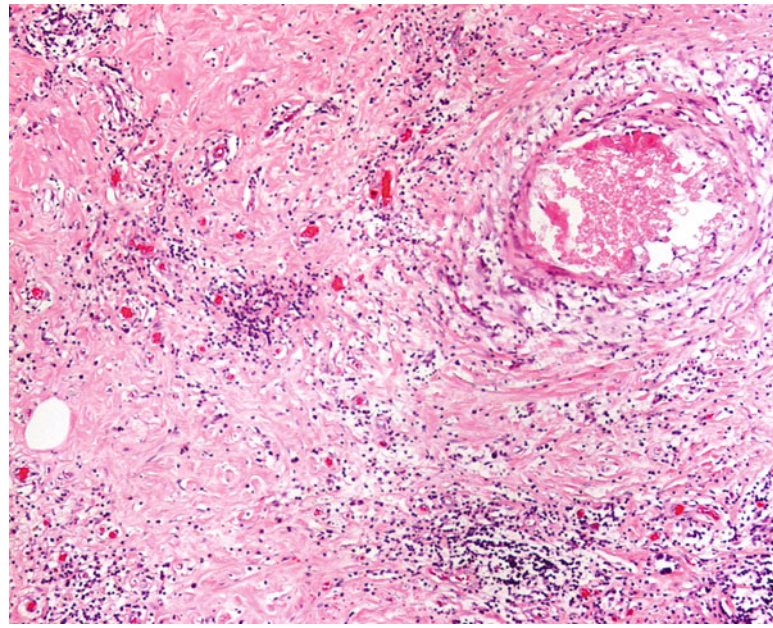

Fig. 2.1 Breast tissue with chronic inflammatory cells within a congested background. There is also accumulation of inflammatory cells within the ductal lumen

In addition, breast-feeding may cause trauma and cracks to the nipple, resulting in ascending infection of the commensals either originating from the skin or from the suckling baby's oral cavity. More severe cases would result in abscess formation. Histologically, there is infiltration of acute inflammatory cells, mostly neutrophils within the breast parenchyma. When there is abscess formation, significant necrosis is present with collection of necrotic debris and the exudates; at the same time, inflammatory reaction will result in granulation tissue formation and fibrotic tissue around the collection (Fig. 2.1). Duct ectasia is a common cause of chronic inflammation. In some cases of fibrocystic changes, the partially or totally blocked ducts may be distended by the accumulation of secretions from the lobules, resulting in cyst formation, and extravasation of the contents into the breast parenchyma will incite inflammatory response. This type of inflammation is aseptic and tends to be chronic, and sometimes, patients may present with nipple discharge. The histologic changes include infiltration of chronic inflammatory cells, including lymphocytes, plasma cells, and histiocytes; granulomas can sometimes be seen. Fat necrosis is a specific type of inflammatory change that occurs in the breast due to trauma or surgical procedure. Traumatic injury to the breast tissue causes disruption of the adipocytes, resulting in the release of the lipid into the stroma, eliciting an inflammatory response, with mostly histiocytes ingesting the fat. In addition, there is also fibrotic tissue reaction, resulting in the formation of a dense fibrotic scar that is hard on palpation, thus mimicking carcinoma on clinical examination. Depending on the stage of the evolution of the lesion, fat necrosis may present as a palpable nodule or just a focal area of pain. As a nodule, fat necrosis is often firm, and there may be skin retraction, thickening, or tethering. At ultrasound, in acute fat necrosis, there is an area of hyperechogenicity, which may have a central decrease in echogenicity. For more long-standing lesions, fat necrosis forms a circumscribed to ill-defined mass and may show posterior acoustic shadowing. At mammography, especially when there is calcification or a spiculated mass, fat necrosis can be indistinguishable from carcinoma. Lipid cysts on mammography are diagnostic of fat necrosis. Similar effects could be seen in cases with breast augmentation, as some of the augmented materials may penetrate the breast parenchyma, eliciting a similar inflammatory response.

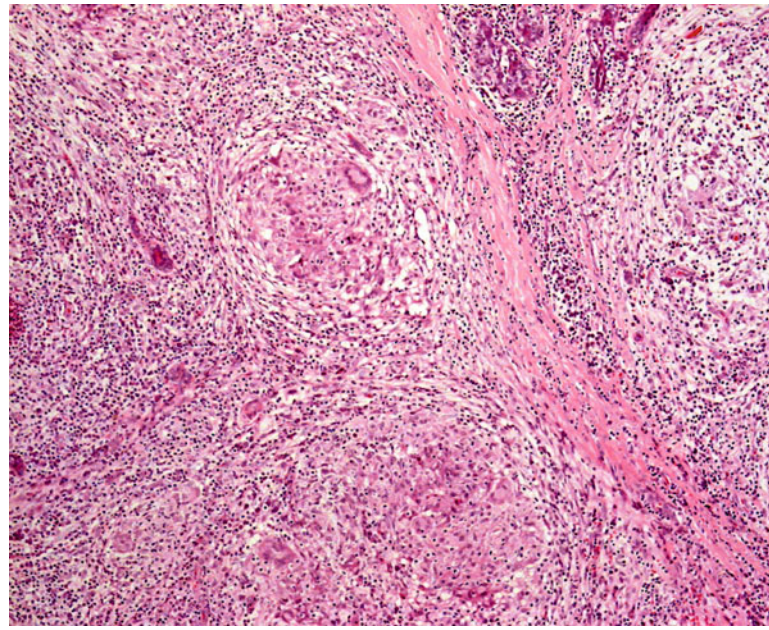

Fig. 2.2 Granulomatous mastitis showing well-formed granulomas composing of epithelioid histiocytes and occasional Langhans giant cells. No caseation necrosis is seen. Alymphocytic infiltrate is noted in the background

Chronic mastitis due to specific microorganisms is rare, and among these, granulomatous mastitis due to *Mycobacterium tuberculosis* is probably the most common, particularly in locations where tuberculosis is endemic. Clinically, tuberculous mastitis presents as progressively enlarging breast lumps that are of variable sizes and may be fixed to the adjacent breast tissue; radiologically, it shows an ill-defined mass, also mimicking carcinoma (Bakaris et al. 2006). Histologically, tuberculous mastitis shows epithelioid histiocytes, plasma cells, lymphocytes, eosinophils, and multinucleated histiocytic giant cells; caseation necrosis may or may not be present. In some but not all cases of tuberculous mastitis, microbiological investigations can confirm the diagnosis; but for the microbiologically negative cases, the diagnosis may have to be based on the appropriate treatment response, particularly in endemic areas or when there are systemic symptoms. Another granulomatous mastitis that is noninfectious, termed idiopathic granulomatous mastitis, can be confused with tuberculous mastitis, as the clinical presentation and imaging findings are very similar (Akcan et al. 2006). Diagnosis of idiopathic granulomatous mastitis is based on elimination of other causes of granulomatous inflammation, particularly tuberculosis. Histologically, idiopathic granulomatous mastitis is very similar to that of tuberculous mastitis, with only subtle differences of more plasma cells in idiopathic granulomatous mastitis and more eosinophils and necrosis in tuberculous mastitis (Fig. 2.2).

2.3 Benign Breast Lesions and Benign Breast Tumors

Fibrocystic changes represent the most common lesions of the breast. The clinical presentation is variable, ranging from asymptomatic to mastalgia that is related to the menstrual cycle. Histologically, a wide range of lesions are seen within fibrocystic changes, including epithelial metaplasia, hyperplasia of benign or usual type, adenosis, cyst formation, inflammatory changes, and fibrosis (Fig. 2.3). Apocrine metaplasia is common in fibrocystic changes. The apocrine cells possess abundant eosinophilic cytoplasm, and by electron microscopy, they are mitochondria rich. The apocrine cells may also line cysts, mostly containing clear serous fluid, but some may be blood stained. Various forms of epithelial hyperplasia may be present, but most common are mild to moderate

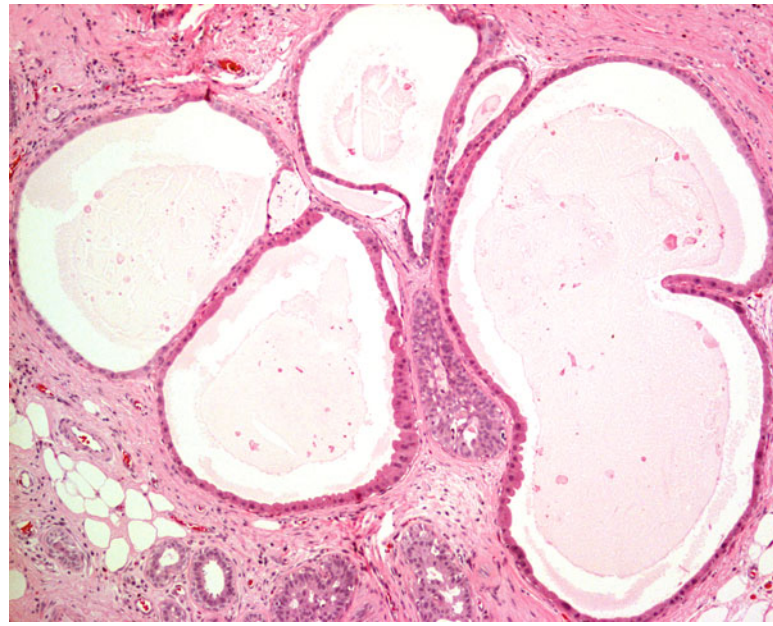

Fig. 2.3 Fibrocystic changes with apocrine cyst formation and mild epithelial hyperplasia in the adjacent duct. An intact myoepithelial cell layer can be discerned

and florid epithelial hyperplasia, as well as columnar cell changes; the latter may be associated with calcification. As fibrocystic changes are very common, they represent the majority of the FNAC in many centers. A detailed discussion on the clinical, radiological, and histologic aspects of fibrocystic changes is given in correlation with the cytologic features in Chap. 6.

Sclerosing adenosis is a variant of breast proliferation. This is an important entity to recognize, as clinically it is characterized by an irregular hard mass that is fixed to the adjacent structures, and by imaging, it also shows significant architectural distortion, rendering this indistinguishable from carcinoma. Histologically, there is proliferation of epithelial and myoepithelial cells in the small ducts and ductules, and these are present within a densely fibrotic stroma. At times, the dense fibrosis or sclerosis will cause architectural distortion and compression of these ductal structures, giving rise to an infiltrative pattern (Fig. 2.4). Careful histologic examination to establish the benign nature of the epithelial cells, as well as to identify the presence of an intact or attenuated myoepithelial cell layer, with the judicious use of immunohistochemistry for identification of the latter, helps to establish the diagnosis.

Other variants include blunt duct adenosis (columnar cell changes), microglandular adenosis, apocrine adenosis, and nodular adenosis. These will be discussed in greater details in Chap. 6.

Fibroadenomas are probably the most common benign breast tumor, presenting as solitary painless, mobile, and well-defined nodules. Multiple lesions are less frequent. Use of the immunosuppressant cyclosporine in transplant patients has resulted in an increased risk of fibroadenoma development. Macroscopically, fibroadenoma is ovoid, rubbery, and well circumscribed; the cut surface is grayish and may be lobulated. Microscopically, it shows a mixed epithelial and stromal proliferation, giving rise to the pericanalicular and intracanalicular patterns, with the former formed by stromal proliferation around the ducts and the latter formed by compression of the ductal elements by the proliferating stromal component into slit-like spaces (Fig. 2.5). These patterns have little prognostic significance. Occasionally stromal giant cells, myxoid changes, dystrophic calcifications, and other mesenchymal metaplasia have been described. Complex fibroadenomas are those that show cysts larger than 3 mm, sclerosing adenosis, epithelial calcifications, or papillary apocrine changes, and this group of fibroadenomas

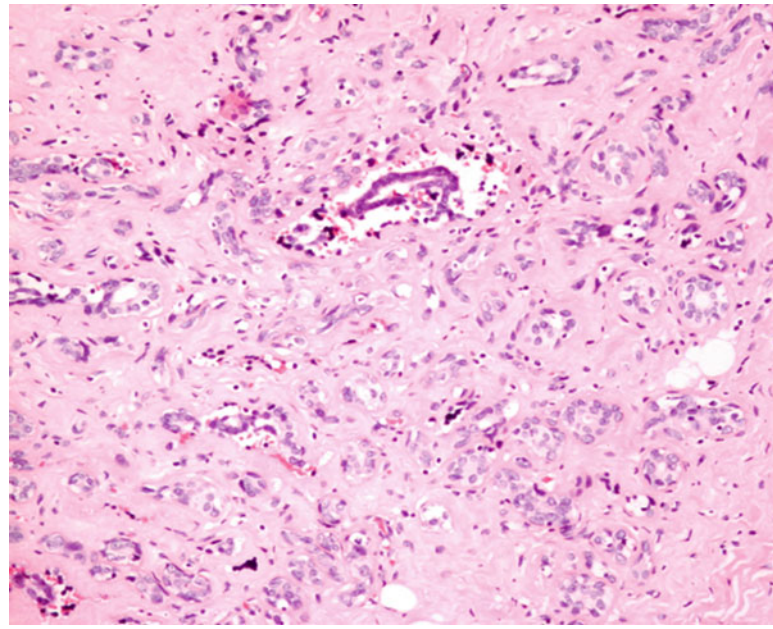

Fig. 2.4 Sclerosing adenosis with fibrosis of the intralobular stroma resulting in compression of the epithelial structures to give an pseudoinfiltrative growth pattern

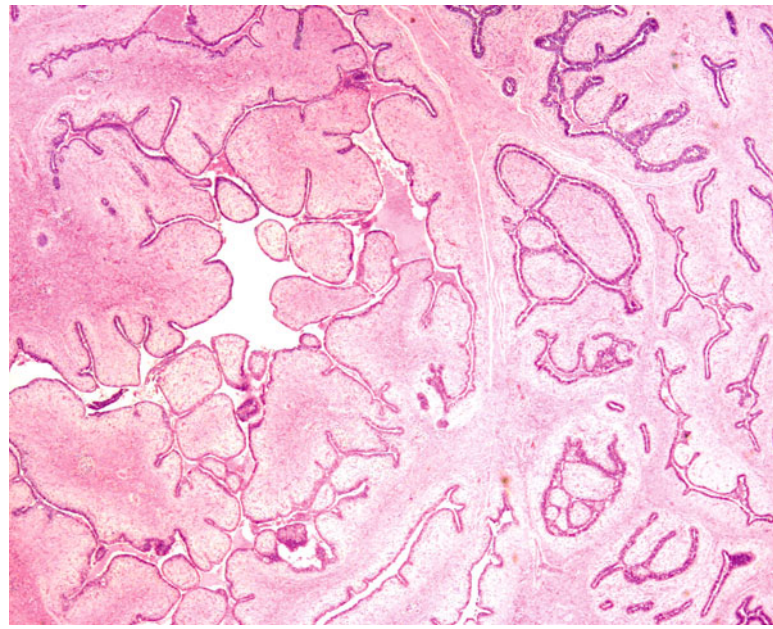

Fig. 2.5 Fibroadenoma showing proliferation and expansion of the stroma that is of usually low cellularity, and the ductal element sometimes forms an intracanalicular pattern with a "leaflike" pattern

shows slightly higher (1.6×) cancer risk compared to the usual fibroadenomas. Fibroadenomas are benign, and most do not recur after surgical excision.

Hamartoma may present as a soft palpable mass or as breast asymmetry, and is usually round to oval and lobulated. Histologically, it shows ducts, lobules, interlobular fibrosis, smooth muscle, and adipose tissue in varying proportions (Tse et al. 2002). This is a benign tumor and rarely recurs (Fig. 2.6).

Diabetic mastopathy is an inflammatory disorder of the breast characterized by a perilobular and perivascular lymphocytic infiltrate. It usually

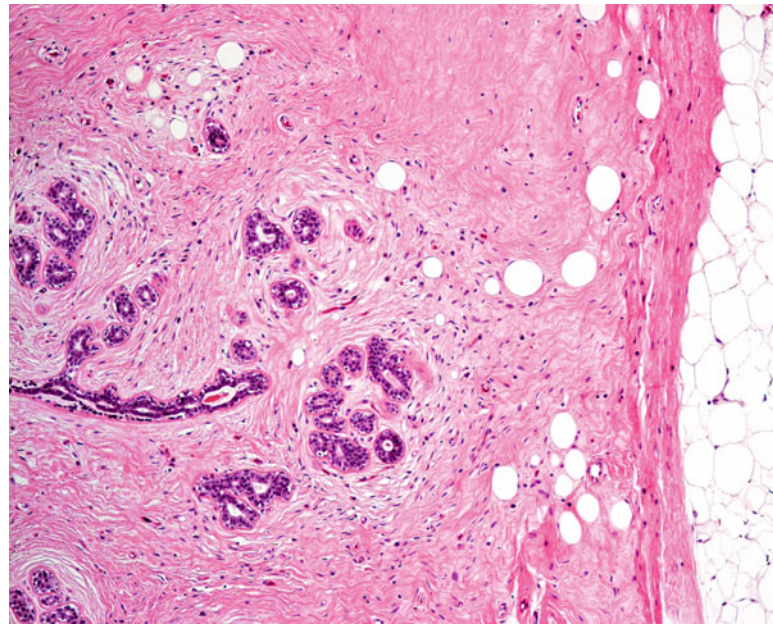

Fig. 2.6 Hamartoma showing a rounded border, with "encapsulated" adipocytes within a fibrotic stroma. There is also intralobular fibrosis

presents as a mass and is most common in women aged 25–60 years. It is characteristically associated with long-standing insulin-dependent diabetes mellitus and sometimes other autoimmune diseases (also known as sclerosing lymphocytic lobulitis). The radiologic features are variable, and mammography shows a dense parenchymal pattern with no specific mass, but sometimes, there is an asymmetric density and occasionally a circumscribed mass. Ultrasound often shows a hypoechoic mass. The characteristic histological features are the presence of circumscribed aggregates of lymphocytes, with some plasma cells, around lobules, ducts, and vessels. The interlobular stroma is fibrotic with plump epithelioid fibroblasts. In the stroma, keloidal fibrosis is seen.

Phyllodes tumor is uncommon fibroepithelial neoplasm that resembles fibroadenoma grossly. Patients with phyllodes tumors usually are older than patients with fibroadenomas, and there may be a history of a rapidly growing mass. Multifocality and bilateriality are rare. Imaging may show a rounded, well-defined mass with clefts or compressed cystic spaces, and occasionally coarse calcifications are noted. Macroscopically, phyllodes tumor is a well-circumscribed, firm, bulging mass, and the cut section shows a fleshy mass with curved spaces resembling leaves or leaf buds. There may be hemorrhage or necrosis. Microscopically, phyllodes tumor shows a prominent intracanalicular growth pattern with leaflike patterns projecting into lumens. The epithelial component is usually benign, with an intact myoepithelial cell layer separating it from the stroma. The stroma is of higher cellularity than fibroadenoma and may show geographic variation within the lesion. These stromal cells are bland looking, with scanty mitotic figures (Fig. 2.7a, b). Within the tumor, stromal areas of low cellularity, hyalinization, or myxoid changes are not uncommonly seen. Some examples of phyllodes tumors show stromal cell atypia and pleomorphism, increased mitotic activity, stromal overgrowth, and infiltrating margins, and these are considered phyllodes tumors of borderline or frank malignancy. Malignant phyllodes tumor behaves like a sarcoma rather than carcinoma and is further discussed in the section on sarcomas. Most of the phyllodes tumors are benign based on microscopy. Outcome of phyllodes tumor is dependent on the histologic grade. Benign phyllodes tumor may rarely recur but does not metastasize. Malignant phyllodes tumors including those of borderline or frank

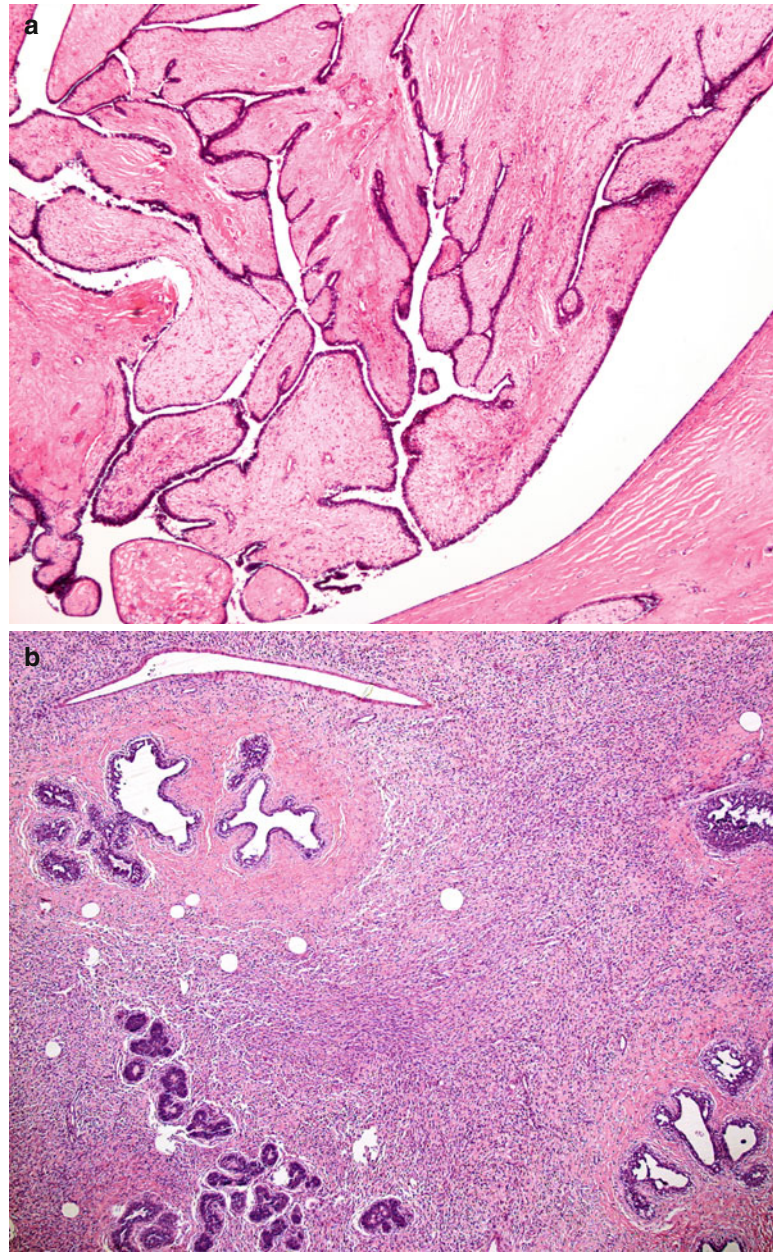

Fig. 2.7 (**a**) Benign phyllodes tumor showing a fronded appearance, with variable stromal cellularity, and a benign epithelial lining on the surface. (**b**) Benign phyllodes tumor at higher magnification showing areas of stromal expansion with moderately cellular stroma

malignancy may both recur or metastasize, more commonly with the latter group (Tse and Tan 2005).

Papillomas can be divided into solitary or multiple. Solitary papilloma is usually located beneath the nipple, whereas the multiple papillomas are more peripherally located. The former is more likely to present as nipple discharge and the latter is usually asymptomatic. Mammography may show a mass in solitary papilloma but multiple nodules or calcifications in peripheral papillomas. Ultrasound may highlight the cystic component particularly in the palpable examples. Microscopically, papillomas are characterized by an arborescent growth derived from the wall of a dilated duct, and these stromal tissue fibrovascular

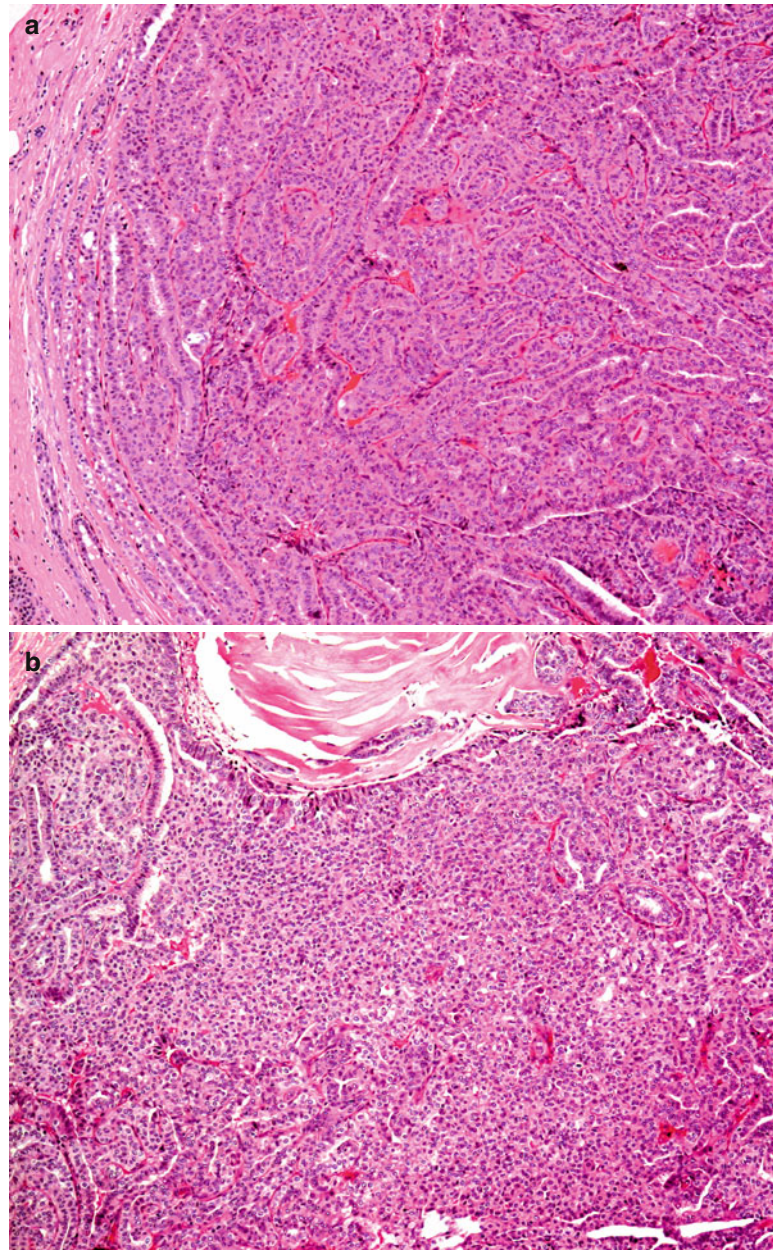

Fig. 2.8 (**a**) A benign papilloma showing a network of fibrovascular cores lined on the outside by benign ductal epithelial cells. The lesion is rounded and is present within a large ductal space. (**b**) A benign papilloma with a solid area of epithelial hyperplasia. The hyperplastic epithelium shows spindled nuclei with nuclear streaming, a feature characteristic of florid epithelial hyperplasia

cores are lined by epithelial cells, together with an intervening layer of myoepithelial cells (Fig. 2.8a). The epithelial cells are benign, but apocrine or squamous metaplasia may be seen. Frequently, there is superimposed epithelial hyperplasia particularly of the florid type, and this will lead to complex architecture and obliteration of the ductal lumen (Fig. 2.8b). The fibrovascular cores may also show sclerotic changes resulting in compression and entrapment of the benign glandular component to yield a pseudo-invasive pattern. Demonstration of a layer of residual myoepithelial cells as well as the absence of cellular atypia of these entrapped epithelial cells would help to differentiate this phenomenon from malignancy (Mulligan and O'Malley 2007). On the whole, papillomas are benign but are associated with slightly increased risk for cancer, more for the multiple papillomas than for the solitary papilloma (Lewis et al. 2006).

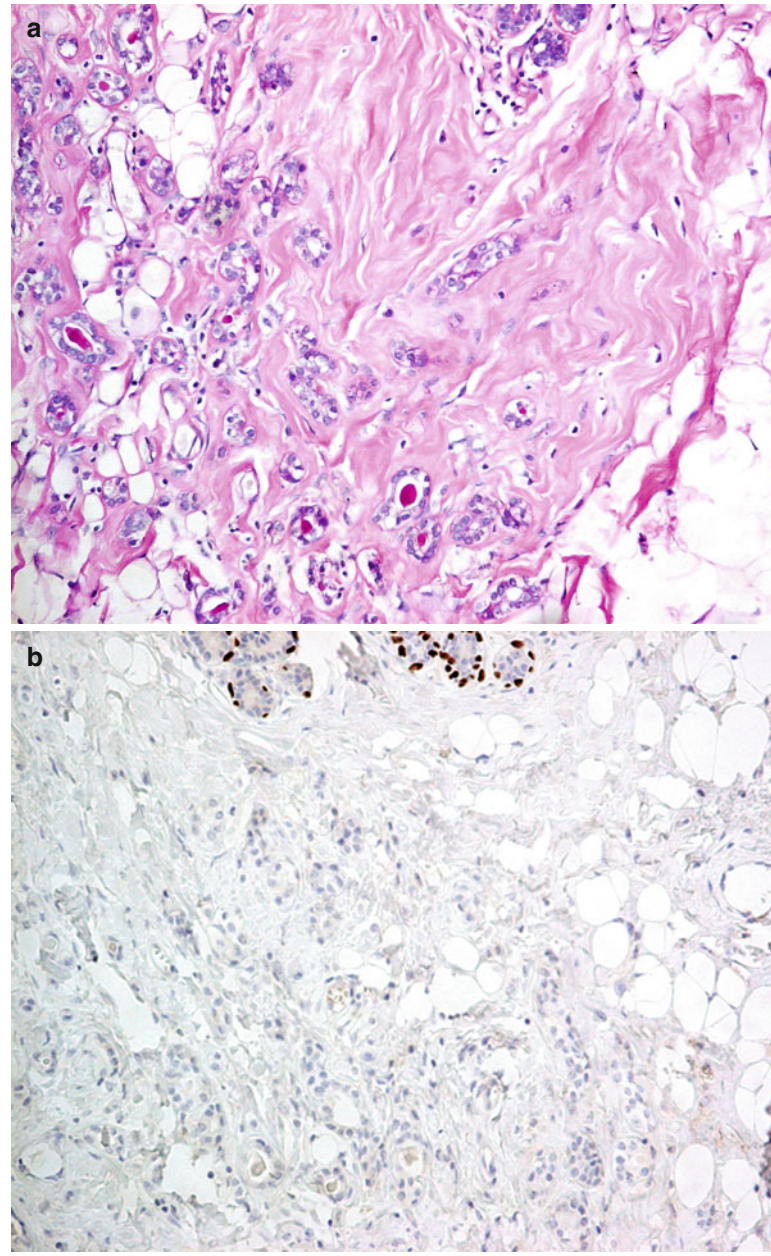

Fig. 2.9 (**a**) Microglandular adenosis showing permeation of the breast stromal tissue by small tubules formed by bland-looking epithelial cells with rounded lumens. Eosinophilic material may be seen within the lumens. (**b**) Immunohistochemical staining of microglandular adenosis for the myoepithelial marker p63 shows a lack of myoepithelial lining around the tubules (terminal duct lobular units intact layer of myoepithelial)

2.4 Epithelial Proliferative Lesions

Microglandular adenosis is an uncommon form of glandular proliferation. The proliferating tubules are lined by a single layer of epithelial cells devoid of a myoepithelial cell layer (Fig. 2.9a, b). Within the lesions, the epithelial cells form irregular tubules, and these epithelial cells are cytologically benign. Although myoepithelial cells are absent, these epithelial tubules have been shown to have an intact basement membrane. Microglandular adenosis has previously been considered benign (Millis and Eusebi 1995), however, recent studies showed that it might be a nonobligate precursor of triple-negative breast cancer.

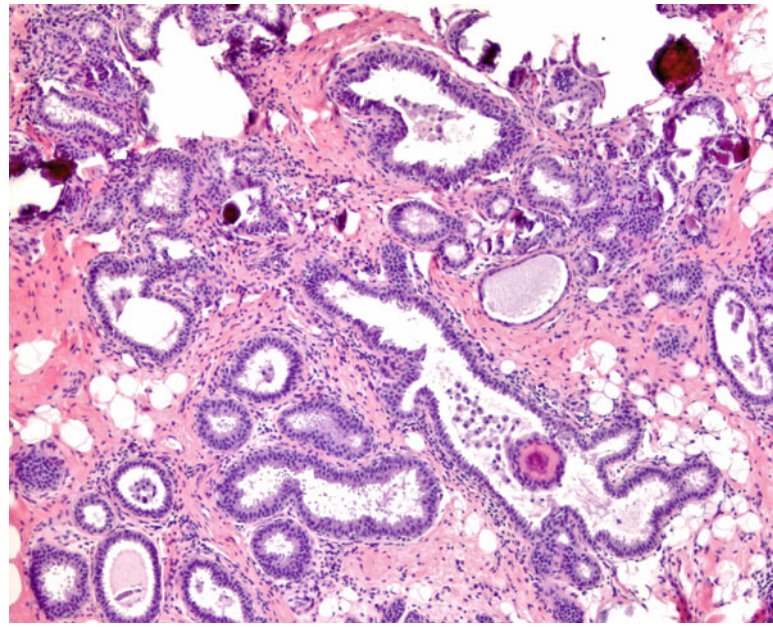

Fig. 2.10 Columnar cell changes showing dilatation of the acini with the cells demonstrating columnar morphology, with apical cytoplasmic snouts and the presence of flocculent material and calcifications within the lumens

Columnar cell changes and columnar cell hyperplasia represent another spectrum of breast epithelial changes. These are characteristically non-palpable and are frequently detected either as incidental findings or by mammography for the associated calcifications. Microscopically, these lesions show well-maintained lobular architecture, with dilated acini. The luminal cells are columnar, and they show apical snouting. Flocculent material is seen within the dilated lumens, and these are frequently associated with calcifications (Fig. 2.10). The lesion is called columnar cell change when there are only one to two layers of epithelial cells and termed columnar cell hyperplasia when there are more than two layers. The term flat epithelial atypia (FEA) is used when the epithelial cells show mild cytological atypia (Fig. 2.11). Some studies suggested that at least some FEA may represent precursors for low-grade ductal carcinoma in situ (DCIS), but the risk of local recurrence or progression to invasion is very low (Schnitt 2003).

Epithelial hyperplasia is the designation for proliferation of epithelial cells within preexisting ductal or lobular spaces. In general, epithelial hyperplasia can be divided into usual hyperplasia or atypical hyperplasia. Interestingly, usual hyperplasia has only been described with ductal morphology (intraduct hyperplasia, epitheliosis, hyperplasia of the usual type, or usual duct hyperplasia), whereas for atypical epithelial hyperplasia, both ductal and lobular morphologies have been described (atypical ductal hyperplasia (ADH) or atypical lobular hyperplasia (ALH)).

Usual ductal hyperplasia may be of variable size and extent, and although the morphology can be somewhat similar to low-grade DCIS, such a concept of progression is not generally accepted. The changes in usual ductal hyperplasia range from mild epithelial hyperplasia, in which the number of epithelial cell layers increased to two to four to florid epithelial hyperplasia, in which there are solid epithelial clusters within and obliterating ductal lumens, with formation of slit-like irregular peripherally located secondary lumens. The epithelial cells show mostly oval nuclei with irregular and streaming arrangement (Fig. 2.12). Apocrine metaplasia may be present.

ADH, when properly defined using stringent criteria (Page and Rogers 1992), carries a significant

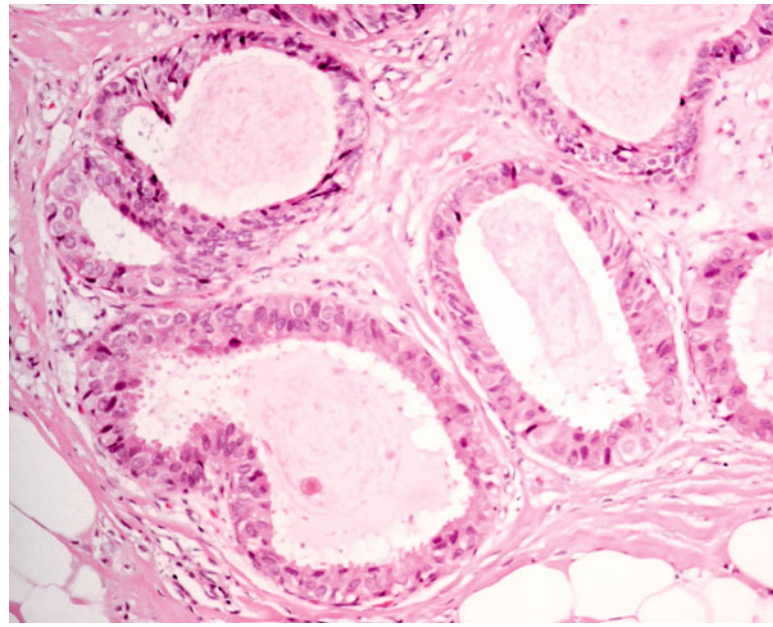

Fig. 2.11 Flat epithelial atypia showing dilated acini containing secretory material. The spaces are lined by one to two layers of columnar epithelial cells with apical snouts and monomorphic nuclei. The nuclei are similar in appearance to those of ADH or low-grade DCIS

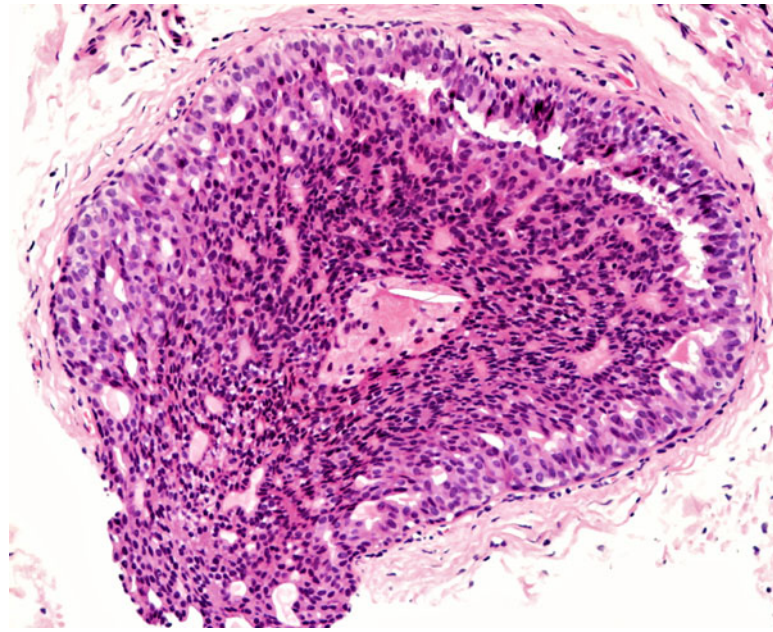

Fig. 2.12 Florid epithelial hyperplasia showing distension of ductal space by an epithelial proliferation, with the epithelial cells showing nuclear streaming and forming irregular and slit-like secondary lumens

cancer risk. The differentiation of atypical ductal hyperplasia from low-grade DCIS remains controversial and poorly standardized, with some authors reporting involvement of less than two glandular spaces or less than 2 mm being indicative of atypical ductal hyperplasia (Page and Rogers 1992; Tavassoli and Norris 1990). On the whole, the histologic features of ADH may be viewed as a smaller version of low-grade DCIS (Fig. 2.13). Where the cutoff is drawn is still unsettled although the WHO consensus is to use 2 mm as the threshold (Lakhani et al. 2012).

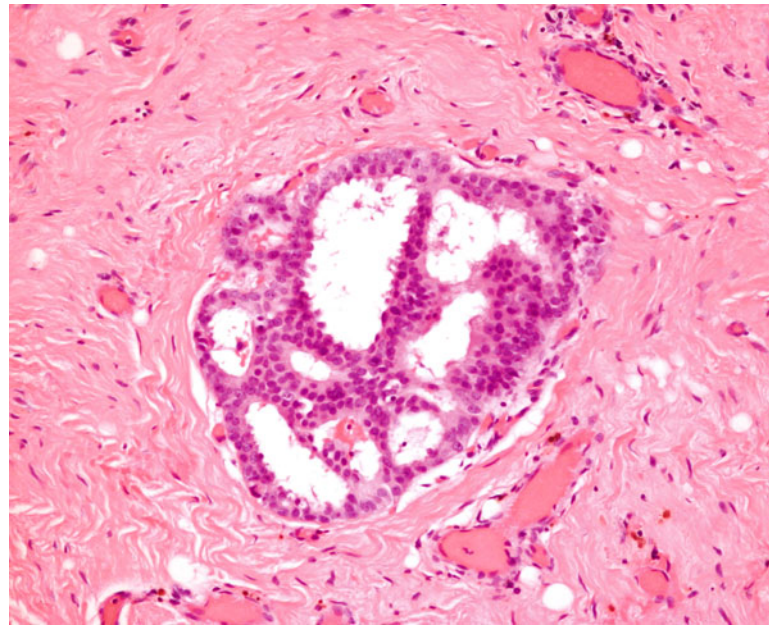

Fig. 2.13 Atypical ductal hyperplasia showing proliferation of a monomorphic population of epithelial cells forming a cribriform structure. The cellular monotomy and architectural pattern are similar to those seen in low-grade DCIS

2.5 Malignant Breast Tumors

2.5.1 Carcinoma In Situ

With the increasing use of mammographic screening, ductal carcinoma in situ (DCIS) is increasingly diagnosed as a non-palpable lesion. The traditional classification based on the architectural pattern is now out of favor. Newer classifications/grading always use nuclear grade as one of the defining features for DCIS. Other histologic features being used are necrosis and the presence of tumor cell polarization – the organization of the nuclei around lumens within the tumor, resulting in rosette or cribriform structures (Silverstein et al. 1995; Holland and Hendricks 1994).

High-grade DCIS is easily differentiated from benign lesions, with the highly pleomorphic tumor cells present within the enlarged ducts associated with central comedo necrosis. The associated calcifications within the necrotic debris produce a characteristic casting or branching pattern in mammography (Fig. 2.14). The ducts may be so distended that aggregation of these ducts may become palpable. Low-grade DCIS, on the other hand, shows monotonous and uniform tumor nuclei that may sometimes be difficult to distinguish from benign epithelial hyperplasia. Common histologic patterns include cribriform, with geometric punched out lumens within the tumor cell proliferation, or micropapillary with the proliferating tumor cells extending into the lumen without fibrovascular stalks (Fig. 2.15). The solid pattern is less common. Secretions may be seen within the ductal lumen and should not be confused with necrosis. Classifications, if present, are associated with the secretions and are usually smaller, rounded, and psammomatous. Intermediate-grade DCIS usually shows features in between high- and low-grade lesions.

Lobular carcinoma in situ is sometimes grouped with ALH under the umbrella term of lobular neoplasia. In both cases, the lobular architecture is essentially preserved, but individual acini are enlarged and distended with obliterated lumens. The neoplastic cells are small and uniform, smaller than the ductal lesions, with higher nuclear cytoplasmic ratio, mild nuclear pleomorphism, rare mitoses, and occasional cytoplasmic vacuoles (Fig. 2.16). Calcifications are much less

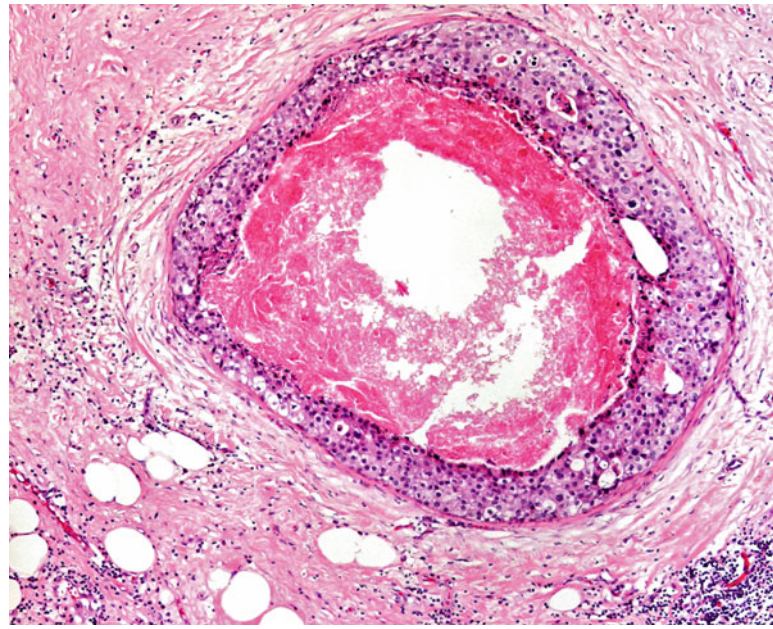

Fig. 2.14 High-grade ductal carcinoma in situ with highly pleomorphic tumor cells present within and distending the duct space, associated with central comedo necrosis

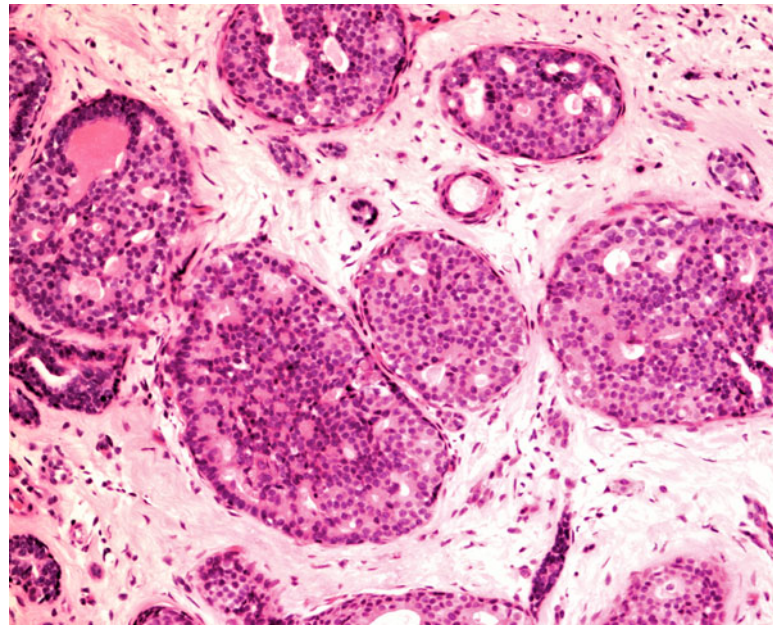

Fig. 2.15 Low-grade ductal carcinoma in situ with monotonous tumor cells distending ductal spaces and forming cribriform structures with geometric lumens

frequent than ductal lesions. The differentiating features between ALH and lobular carcinoma in situ are not well defined, with the former showing generally less lobular distension and lesser extent of involvement, usually limited to part of a ductal-lobular unit.

2.5.2 Papillary Carcinoma

Papillary carcinomas are uncommon malignant lesions, representing several different morphological entities, all possessing a common papillary architecture, characterized by epithelial proliferation

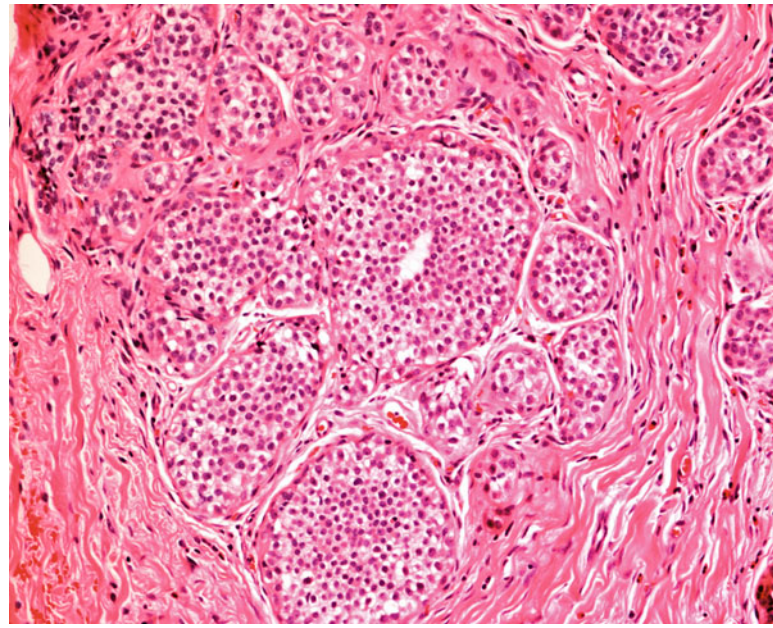

Fig. 2.16 Lobular neoplasia showing distension of the acini by a uniform population of small round cells with bland nuclear features. The growth pattern is solid

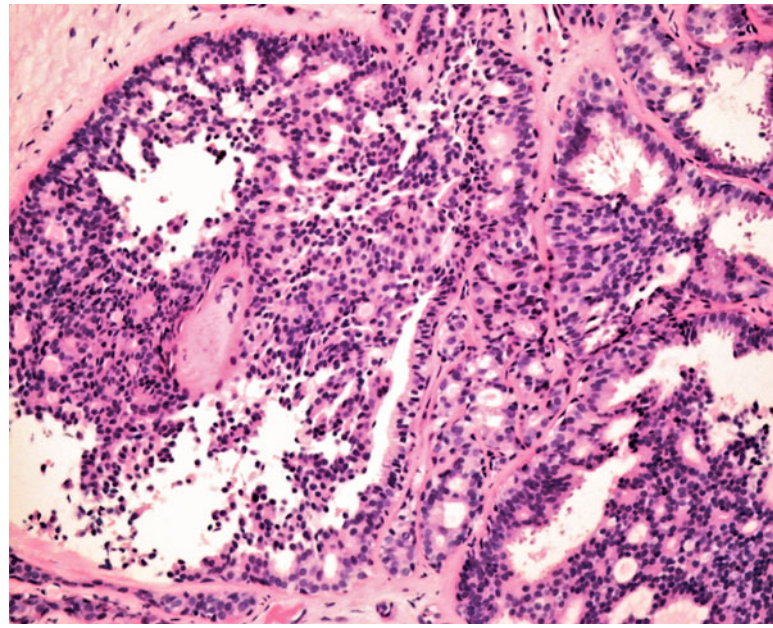

Fig. 2.17 Papilloma involved by atypical ductal hyperplasia. There are areas of atypical ductal hyperplasia separating the papillary fibrovascular stromal tissue cores. The atypical ductal hyperplasia shows typical morphology with rounded cells forming geometric structures

overlying elaborate fibrovascular cores. Papilloma with DCIS and intracystic papillary carcinoma are typical examples of papillary lesions with malignancy. Papilloma with DCIS usually occurs when there is a focus of atypical epithelial proliferation within an otherwise benign papilloma. This phenomenon is not unusual, and the atypical epithelial focus usually possesses the usual histomorphology of uncomplicated ADH or low-grade DCIS (Fig. 2.17). The differentiation between papilloma with ADH or papilloma with DCIS is arbitrary, with a size criterion of 3 mm being used. If the atypical focus is larger than 3 mm, the lesion should be termed as papilloma with DCIS, and

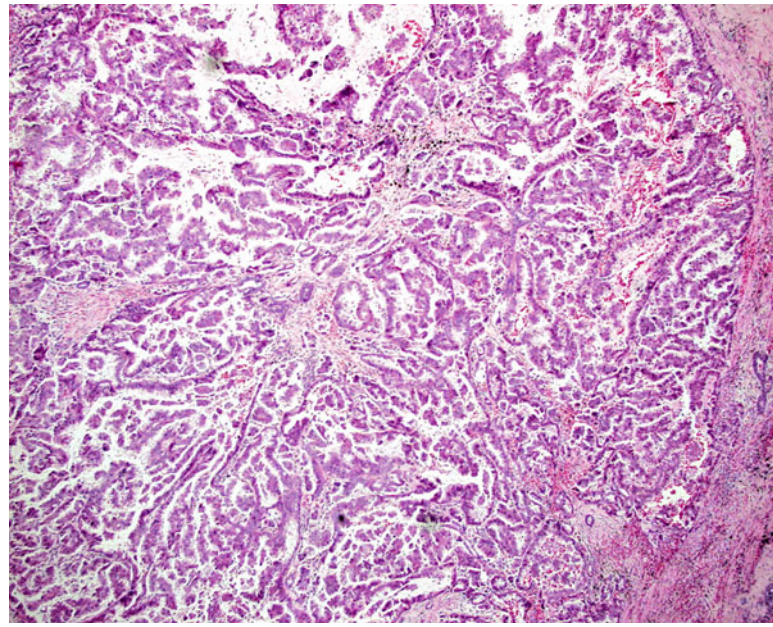

Fig. 2.18 Intracystic papillary carcinoma showing delicate fibrovascular cores within a rounded tumor, with the absence of an outer myoepithelial cell layer

when the focus is 3 mm or less, it should be termed papilloma with ADH (Page et al. 1996).

Intracystic or encapsulated papillary carcinoma is rare, usually occurring in older women, and may present as a breast mass. Microscopically, it is predominantly papillary but may also exhibit cribriform or micropapillary patterns as minor components. The characteristic feature of this tumor is the essentially absence of a complete layer of myoepithelial cells on the outside and the delicate nature of the papillary fronds (Collins et al. 2006) (Fig. 2.18). Intracystic papillary carcinoma has a good prognosis, having better outcome than mixed intracystic papillary/nonpapillary tumors (Carter et al. 1983; Lefkowitz et al. 1994). Most recommend a treatment protocol more akin to that of an in situ disease.

2.5.3 Invasive Carcinoma

Invasive breast carcinomas are classified based on their histological features, and this classification also reflects their clinical behavior. Incidence increases with patient's age, with family history being one of the most common risk factors. Clinically, it presents as an ill-defined mass, sometimes adherent to the skin or underlying muscle.

Among the tumor types, the most common is invasive ductal carcinoma, not otherwise specified (IDC, NOS). The tumor is of varying size and may be associated with calcification. It is usually firm, fibrotic, or stellate. The histomorphology of the tumor is also highly variable, ranging from a low-grade tumor showing mildly pleomorphic tumor cells arranged in tubules with little mitotic activity to a high-grade tumor showing highly pleomorphic tumor morphology, with the tumor cells arranged in solid sheets and groups, showing brisk mitotic activity and abundant tumor necrosis (Fig. 2.19). Most IDCs are graded based on three microscopic features, namely, the degree of tubule formation, nuclear pleomorphism, and mitotic count. The tumor cells in higher-grade cancer are usually arranged in sheets or are discohesive, showing very little tubule formation, whereas lower-grade cancer shows significant tubule formation generally. Nuclear morphology is also evaluated in terms of variation in nuclear size, regularity of the nuclear border, hyperchromasia, and prominence of nucleolus. Mitoses are usually counted per 10 high-power microscopic fields, taking into account the size

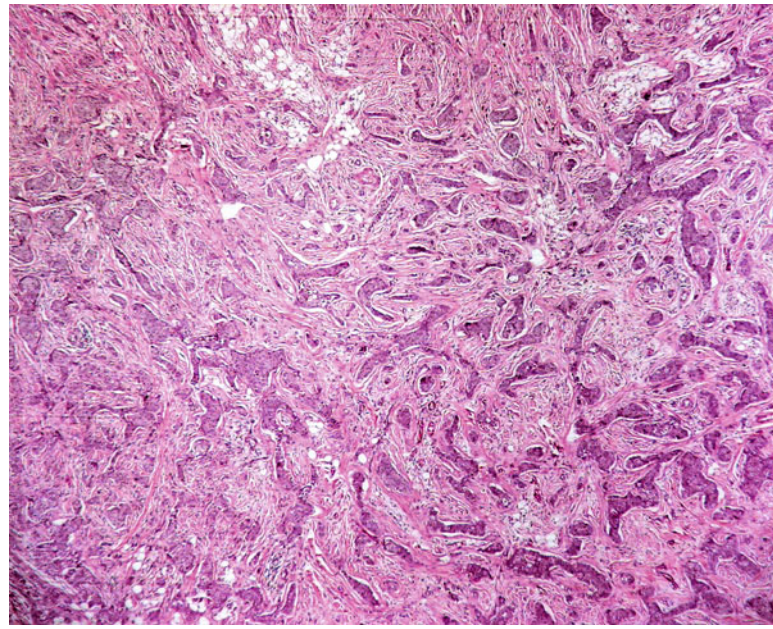

Fig. 2.19 Infiltrating duct carcinoma showing irregular groups of malignant cells invading a desmoplastic stroma. The normal ductal-lobular architecture is obliterated

of the microscopic high power field. A combination score on these three components reflects tumor grade and prognosis. Furthermore, hormone receptor assessment is also mandatory in tumor evaluation. ER, PR, and HER2 protein expression are routinely performed by immunohistochemistry, and the results carry prognostic and predictive significance. Tumors expressing hormone receptors have better prognosis and are highly responsive to hormonal therapy, whereas tumors expressing HER2/neu have poor prognosis but may respond to specific anti-HER2/neu targeted therapy.

Invasive lobular carcinoma represents 5–10 % of the invasive breast tumors. It is usually seen as an irregular thickening and with a lower density in mammography and less likely to be associated with calcification. The tumor can be relatively small and possesses poorly defined edges. The tumor cells are small and subtle, containing small amounts of cytoplasm. There is no definite configuration except the formation of cords or single file pattern (Fig. 2.20). The tumor cells usually show loss of E-cadherin, an epithelial cell adhesion molecule, and this can be demonstrated immunohistochemically, a fact that can be utilized in diagnosis. The loss of E-cadherin expression in ILC seems to be associated with evidence of impaired integrity of the E-cadherin catenin membrane complex. Whether invasive lobular carcinoma has a worse prognosis than invasive ductal carcinoma is still controversial, with numerous studies presenting contrasting results. However, contralateral invasive carcinoma is reported to be more frequent in patients with a history of invasive lobular carcinoma.

Tubular carcinoma is a rare breast tumor, accounting for less than 3 % of all the tumor types. Most of these tumors are detected via the screening program with a characteristic mammographic appearance of an irregular spiculated mass usually lacking calcification. The tumor is usually small and composed of angulated tubules embedded in a desmoplastic stroma (Fig. 2.21). This is one of the well-differentiated tumors that are commonly misdiagnosed as benign at fine needle aspiration or biopsy. The absence of myoepithelial cells helps to confirm a diagnosis of tubular carcinoma. Lymph node metastasis is rare and 5-year survival rate of patients with this tumor type is more than 90 %.

Mucinous carcinoma occurs commonly in postmenopausal women, presenting at the clinic with a palpable mass. The tumor is usually circumscribed and associated with radiologic microcalcification. Grossly, a large gelatinous

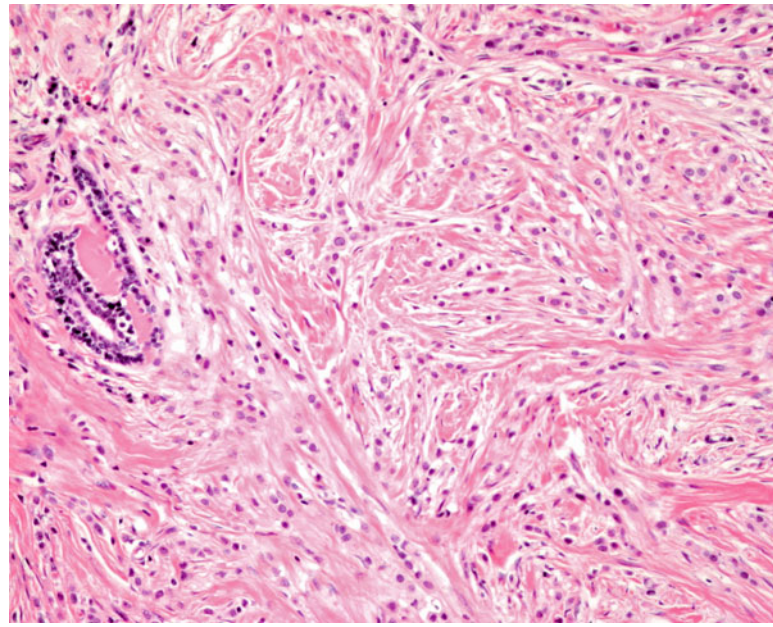

Fig. 2.20 Infiltrating lobular carcinoma showing poorly cohesive small round tumor cells arranged in single files within a fibrotic stroma

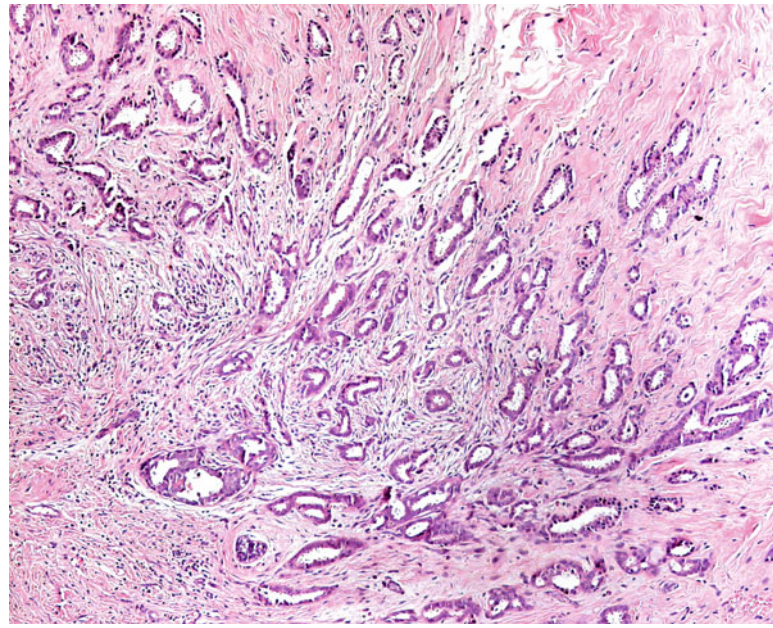

Fig. 2.21 Tubular carcinoma showing bland-looking tumor cells arranged in tubules that possess widely patent lumens. Myoepithelial cells are absent, and the malignant tubules are arranged in an irregular stellate pattern. The intervening stroma is densely fibrotic

appearance is appreciated. Microscopically, the tumor cells are usually in tubules, clusters, and small sheets floating in pools of mucin. They have a characteristic eosinophilic cytoplasm with low nuclear and mitotic grade. The amount of tumor cells varies, and hypercellular tumors may sometimes show neuroendocrine differentiation (Fig. 2.22). Furthermore, neuroendocrine differentiation in mucinous carcinoma is associated with favorable histologic and immunohistochemical parameters. The presence of the malignant cells is a prerequisite in the diagnosis in order to distinguish it from benign mucocele-like lesions. Generally, mucinous carcinoma confers good

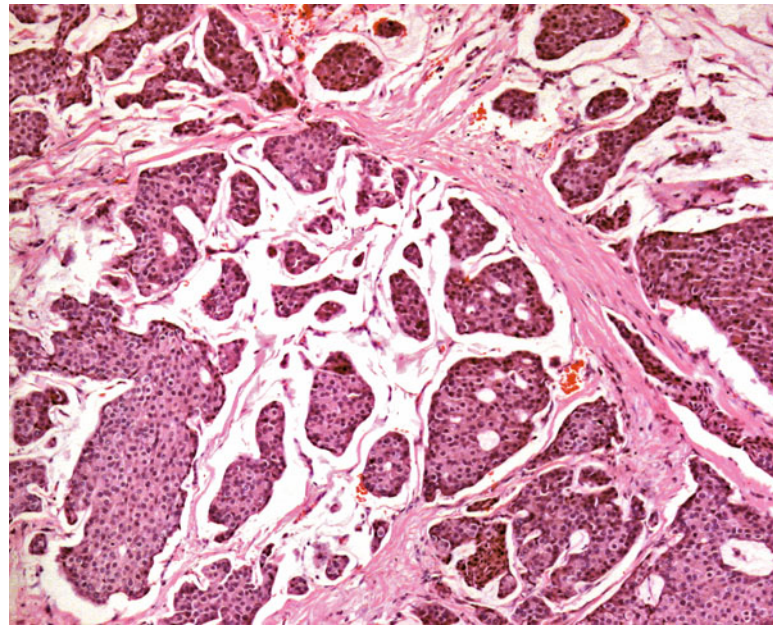

Fig. 2.22 Mucinous carcinoma showing large sheets of low-grade malignant cells present within pools of accumulated extracellular mucinous material

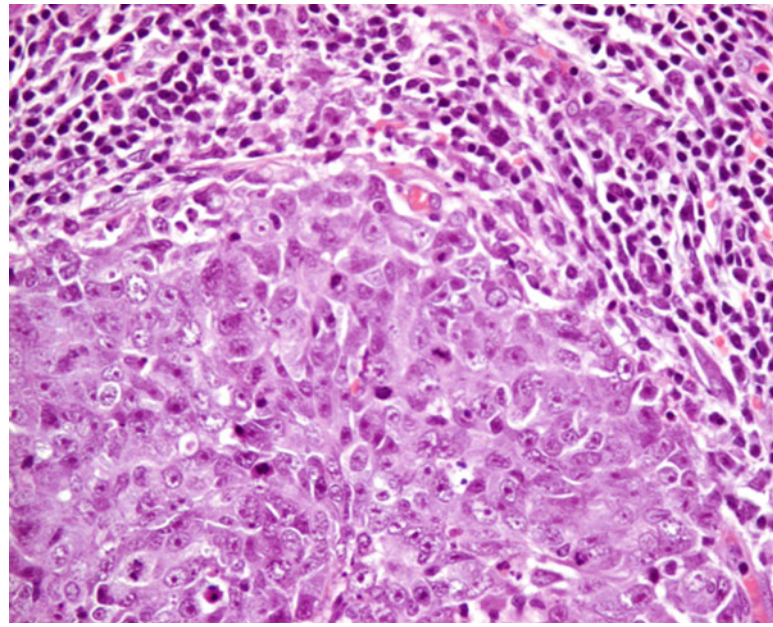

Fig. 2.23 Carcinoma with medullary features showing a rounded margin and an intense lymphocytic infiltrate at the periphery. The tumor cells form a sheetlike pattern with high degree of nuclear pleomorphism and brisk mitotic activity

prognosis, mostly diagnosed as grade I or II with 5-year survival of 90 %.

Carcinoma with medullary features has a distinctive morphological appearance. Grossly the tumor is a sharply circumscribed mass with soft and fleshy consistency which may be confused with fibroadenoma, having a gray and solid cut surface. The tumor cells are seen in a syncytial growth pattern, with pushing margins and prominent lymphoplasmacytic infiltrates in the perimeter (Fig. 2.23). Morphological appearance of the cells is high grade and usually with more than 20 mitotic figures per 10 high-power fields. Furthermore, this histologic type is also

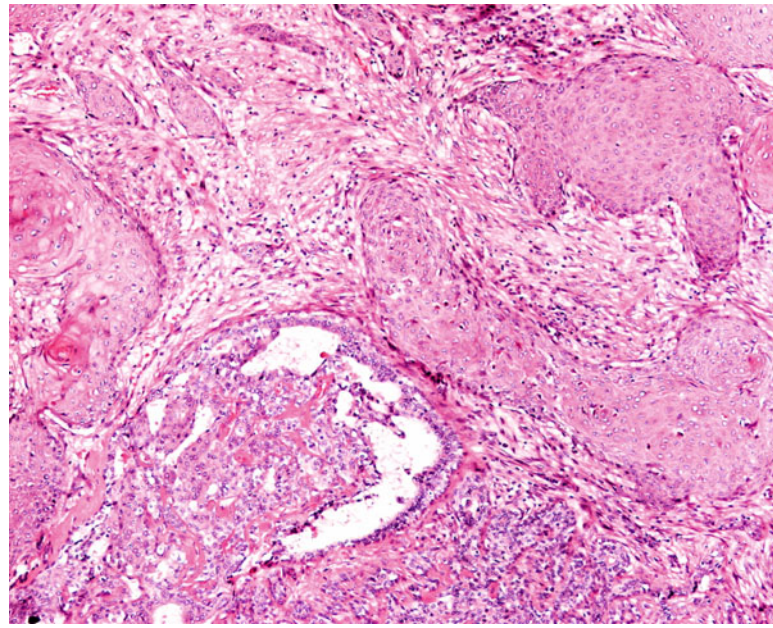

Fig. 2.24 Metaplastic carcinoma showing mixed squamous cell carcinoma and ductal carcinoma components. The intervening areas show malignant spindle cell proliferation

seen among patients with BRCA1-mutation-related tumors. However, it does not translate that all BRCA1-related tumors should be called medullary like. Nevertheless, these morphological features should prompt the search for a family history of a genetic predisposition, especially when a young patient is involved. BRCA1 is an independent predictor of disease-free interval, and its alteration may play a role in the development and progression of breast cancer (Rakha et al. 2008). The tumor cells are generally negative with ER, PR, and HER2 stains.

Metaplastic carcinoma encompasses a mixed range of uncommon cancers and has been used by pathologists to describe breast carcinomas of mixed epithelial and mesenchymal appearance. Overall, tumors in this group represent less than 1 % of all invasive breast tumors. These tumors are usually larger compared to other types, with ill-defined shapes and surface. Malignant squamous cells and glands are often the histological components encountered mixed with spindle cells (Fig. 2.24). They are further categorized based on the predominance of the epithelial and/or mesenchymal cells. Generally, the prognosis is poor, showing increased incidence of recurrence and metastasis.

Invasive micropapillary carcinoma accounts for approximately 2 % of all invasive breast carcinomas and appears associated with a poor prognosis. The tumor cells are seen as tubular nests surrounded by clear spaces which may be related to an artifactual tissue shrinkage. The tubules lack true fibrovascular cores and exhibit reverse polarity of cells (luminal markers on the periphery of islands) (Fig. 2.25). This tumor is predominantly of histologic grade III with a higher incidence of lymphatic invasion and lymph node metastasis.

The eosinophilic and granular cytoplasm of apocrine carcinoma is its distinguishing feature (Fig. 2.26). This is an uncommon tumor that exhibits the usual tubular or solid arrangement of the tumor cells. Similarly, the size, grade, and lymph node stage are the same with that of invasive ductal carcinoma. The tumor cells stain positively with androgen receptor (AR) and GCDFP-15.

Inflammatory carcinoma is a clinical description of the tumor which on presentation shows skin redness, warmth, and an edematous appearance. This tumor mimics an infection and may transiently respond to medications; thus, clinical caution is important. The prognosis is usually poor and usually associated with dermal lymphatic permeation.

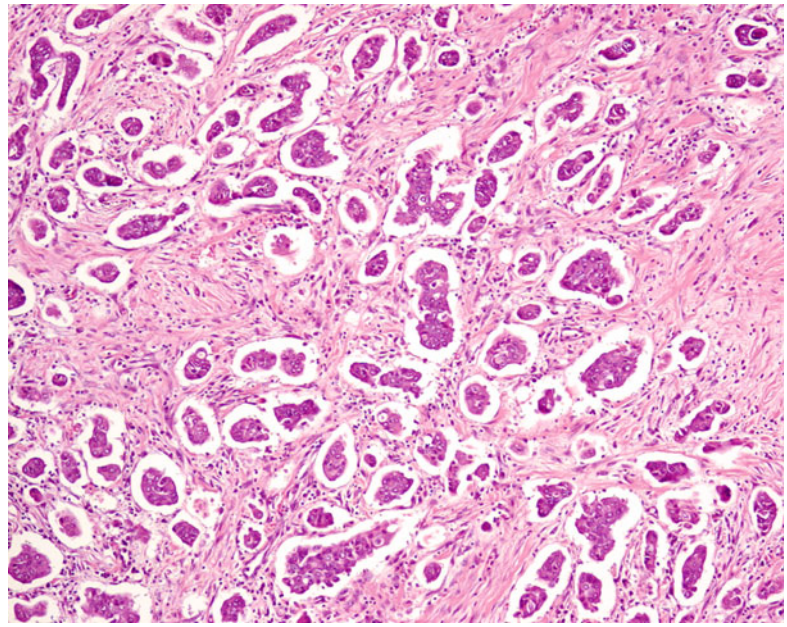

Fig. 2.25 Invasive micropapillary carcinoma showing rounded groups of tumor cells with a peripheral clear rim

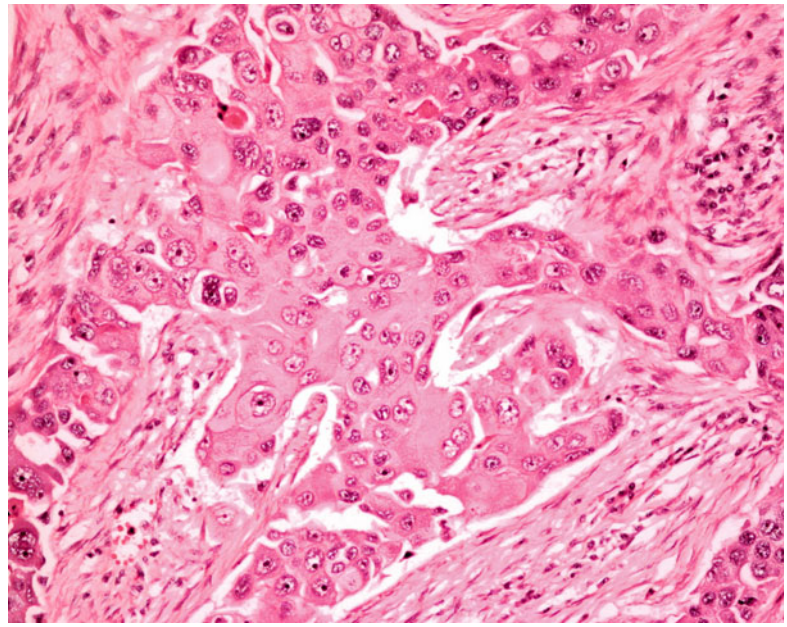

Fig. 2.26 Apocrine carcinoma showing tumor cells with the characteristic appearance of abundant eosinophilic and granular cytoplasm

Angiosarcoma of the breast is tumor arising from the blood vessels and is grossly seen as a poorly defined mass with hemorrhage. When an old patient is involved, it is most of the time secondary to chronic lymphedema or radiotherapy of prior breast cancer. Microscopically, vasoformative cells with endothelial tufting with atypia are seen. Necrosis and hemorrhage are common in aggressive tumors (Fig. 2.27).

Malignant phyllodes tumor is distinguished from the benign tumor based on the microscopic features of the stromal element. Stromal hypercellularity, overgrowth, atypia, and mitotic activity of 10 or more per ten high-power fields are the

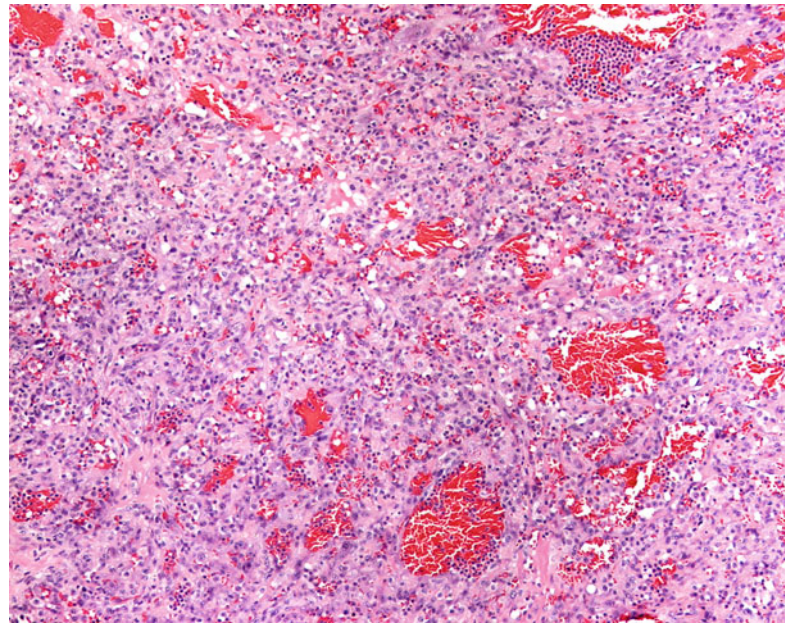

Fig. 2.27 Angiosarcoma showing malignant spindle cells with moderate nuclear pleomorphism forming vascular channels of varying sizes and shapes. Some of these lumens are blood filled

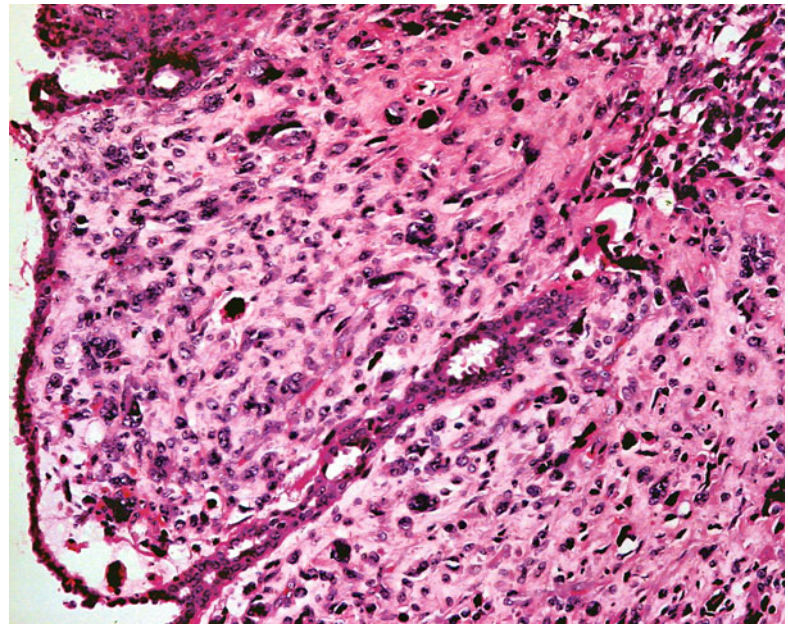

Fig. 2.28 Malignant phyllodes tumor showing highly pleomorphic malignant stromal cells, with bizarre cells adjacent to benign ductal epithelium. Atypical mitotic figures are seen within the malignant stromal cells

features seen in malignant phyllodes which will also show an infiltrative margin (Fig. 2.28). Management is usually difficult, and most surgeons will perform mastectomy once a malignant diagnosis is made. Incidence of recurrence and metastasis is also increased.

When entertaining metastatic tumors in the breast, a full knowledge of the clinical history is important to avoid misdiagnosis. The tumor will commonly present as small nodules which on microscopy will reveal a peculiar architecture that is different from the common breast histo-

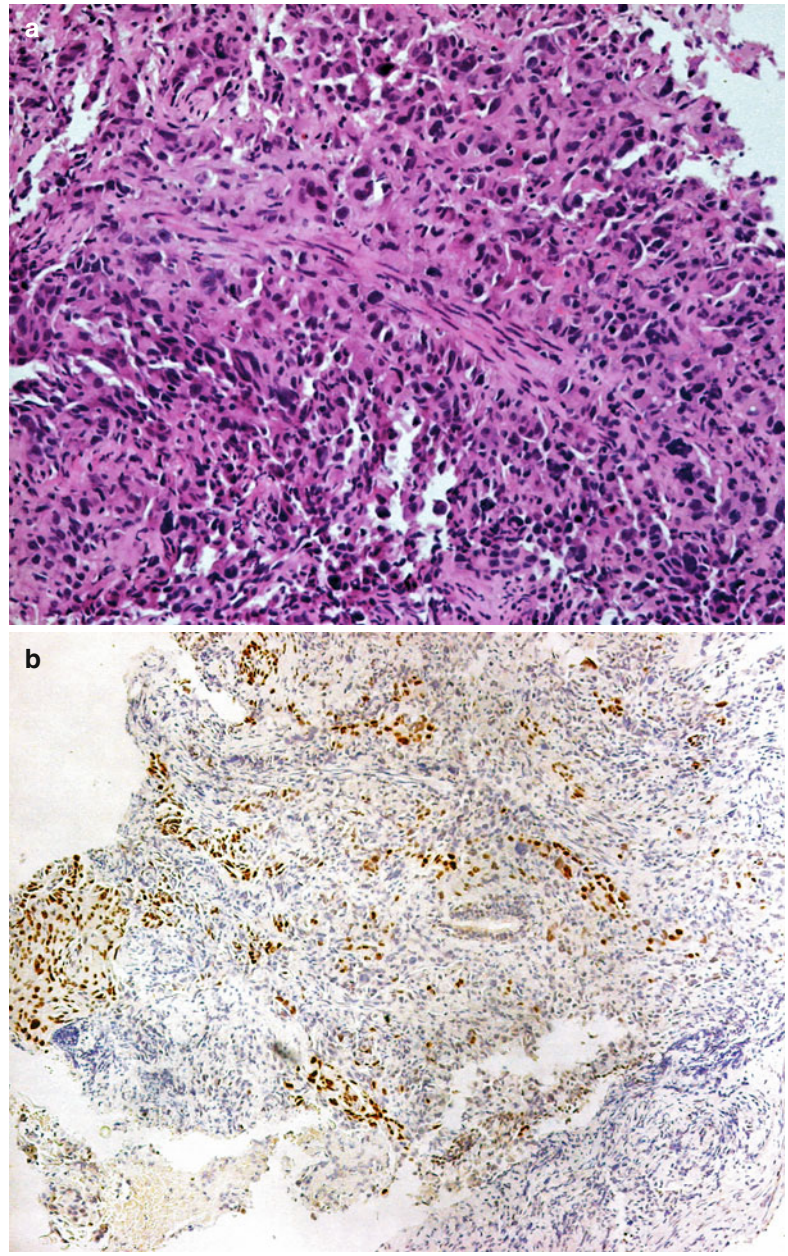

Fig. 2.29 (**a**) High-grade malignant cells presenting as a tumor nodule in the breast. The tumor cells show hyperchromatic nuclei and moderate amounts of cytoplasm. (**b**) The same tumor showing positivity for TTF1, confirming a metastasis from a lung primary

logic types. The common metastatic tumors in the breast are lung carcinoma and malignant melanoma. The tumor cells will usually be high-grade or anaplastic, making it more difficult to decipher the similarity within its origin. Presence of an in situ carcinoma may give a hint of a primary breast tumor, but its absence will also not rule it out. The use of special stains to identify tumor origin is most helpful, such as TTF-1 in detecting metastasis from lung adenocarcinoma (Fig. 2.29a, b).

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Aspiration Techniques

3

3.1 Introduction

Fine needle aspiration cytology (FNAC) is regarded as a minimally invasive, cost-effective technique with high diagnostic accuracy. In the era of high expenses in medical care, with costs due to the great complexity of the diagnostic procedures and high-priced equipment, FNAC runs opposite to this trend (Arkoumani and Wells 2006). FNAC is safe, gives rapid reporting, and demonstrates high sensitivity and specificity for the diagnosis of malignancy. In addition, this technique requires little equipment, causes minimal discomfort to the patients, is an outpatient procedure, and reduces bed occupancy and the incidence of additional surgical explorations. An aspiration can be performed during a routine doctor's office or clinic visit or at the patient's bedside. An intraoperative or preoperative aspiration can avoid the need for a frozen section or histologic examination of tissues, respectively, which is the first step in a traditional "two-step surgical procedure." However, it is important to stress that aspiration technique requires practice and skill and the interpretation of the results requires experience. FNAC is a multistep procedure, and when the same procedure is done by the same person (the pathologist), the final results are usually better (Stanley and Lowhagen 1993). Correctly executed, FNAC has the best safety record of any method of obtaining material for a morphological diagnosis.

3.2 Role of Breast FNAC in the Clinical Practice

FNAC forms part of the triple assessment of breast lesions: clinical, imaging, and morphology. It is relatively easy to perform and does not require high-tech equipment; costs are lower than core needle biopsy (CNB) and substantially lower than open biopsy. The procedure is safe, yielding tissues material that usually provides a high diagnostic accuracy in an extremely short time frame. Nowadays, in many centers, breast FNAC has been replaced by CNB (Kocjan et al. 2006). Both FNAC and CNB can be performed as an outpatient procedure with little significant negative cosmetic effects, for example, scarring and dimpling of the breast due to loss of tissue.

FNAC has some advantages over CNB, and these are listed as follows:

- Greater mobility of the needle during aspiration, allowing an increased area of sampling (CNB obtains tissue in one plane only).
- Greater sensitivity of the physical nature (palpation) of the lesion and therefore better needle localization.
- Better evaluation of the texture of the lesion during the aspiration, which helps to determine the need for additional needle passes and diagnosis. For example, gritty, rubbery, or "fatty" resistance feelings suggest possibilities of carcinoma, fibroadenoma, and fat necrosis, respectively.

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- Ability to immediately and accurately assess the adequacy of the specimen obtained, avoiding unnecessary repetition of the procedure and reducing time for diagnosis by the immediate identification of an inadequate aspirate.
- Processing time is significantly less when compared to both paraffin embedding and frozen section processing of needle biopsies, thereby permitting a more timely immediate assessment and handling of a larger number of patients.
- Shorter time for final diagnosis.

Disadvantages of FNAC when compared to CNB include:

- Limited tissue available for ancillary studies and research endeavors (paraffin embedded, cutting needle cores of tissue can yield hundreds of slides for analysis, whereas the average aspirate might have between four and ten slides). However, as demonstrated in other chapters of this book, many ancillary techniques can be performed in cytological material with results comparable to histology.
- Less familiarity/experience among pathologists. Maybe this is one of the main reasons to replace FNAC by CNB. In many centers, pathologists are not trained adequately to read cytological slides and as a result are more comfortable with histology. This, allied with a lack of skills of the aspirator, is one of the main reasons responsible for the decline of breast FNAC.
- Low cellular yield due to the nature of the lesion, despite the performance of an appropriate aspiration, for example, desmoplastic stroma, in some types of carcinomas.
- Difficulty in classifying proliferative lesions that have a degree of atypia but lack unequivocal features of malignancy.
- Inability to distinguish in situ versus invasive carcinoma, a significant drawback when neoadjuvant chemotherapy or sentinel lymph node biopsy is considered. However, many of these cases can be resolved by using the triple assessment (Kocjan 2006; Kocjan et al. 2006, 2008; Staerkel and Sneige 2006).
- High rate of insufficiency when aspirating microcalcifications or other non-palpable lesions under imaging guidance due to a paucity of epithelial tissue present. As a result, there is a general agreement that microcalcifications should be assessed by CNB and not by FNAC.

These drawbacks have led to a decline in FNAC of the breast, particularly in the evaluation of a patient's primary breast lesion. However, with the development of new treatment protocols for the care of breast cancer patients, FNAC is increasingly used to confirm or rule out multicentric disease. In combination with regional lymph nodes and distant site aspirations, the staging of a patient's disease and planning of suitable therapy can be achieved more quickly and economically (Titus 2010). Lastly, FNAC can now be used to obtain tissues for assessing proliferative breast changes and for the performance of molecular marker studies to better assess patient risk for the development of breast cancer. At this moment, many countries, due the high incidence of breast cancer and the advantages that a rapid diagnosis can bring to the management of patients, have seen the emergence of one-day one-stop clinics that demand a diagnosis on the same day. FNAC is the ideal technique to be applied in this setting (Kocjan et al. 2008).

The decision to perform either FNAC or CNB should be based on a given set of clinical/radiologic/pathologic findings, thus allowing one to take advantage of the benefits that both procedures have to offer.

3.3 Breast FNAC Procedure

3.3.1 Equipment

The equipment necessary to perform an FNAC is quite simple. In our practice, breast FNAC is performed in an FNAC clinic, which is an outpatient service offered to patients with lesions that require investigation. Patients are usually booked in advance by letter of referral or telephone. The minimum staff and equipment required are an assistant (especially if the FNAC was image guided), an examination couch, a writing desk, a work surface, an examination tray for instruments, and good lighting. The aspirator will need a bench, preferentially with a sink, a cotton, and

Fig. 3.1 Basic equipment for performing breast FNA

an antiseptic solution to perform a quick antisepsis of the skin overlying the lesion; gloves, a syringe, a needle, and a syringe holder for the aspiration procedure; glass slides for smear preparation; liquid fixatives, preferentially 95 or 100 % alcohol; a small bandage; and a disposal box to put the potentially infected material (Fig. 3.1). As one can see, it is easy to assemble these materials in a small box and place it on a small table or bench during the FNAC procedure at the radiologist's room, doctor's clinical office, or ward, if there is no designated room for the FNAC clinic. Preferentially, the pathologist who performs FNAC should have a room at the laboratory with the appropriate conditions to perform the FNAC and also a microscope to examine the smears after quick staining, in order to ensure adequate quality and quantity of the sample.

The diameter of the needles used to perform FNAC is a crucial element of the procedure. Most aspirations are performed with 23G–27G needles. Experienced cytopathologists advocate that aspirations performed with needles thicker than 23G cannot be considered FNAC. Small diameter needles are usually well tolerated by the patients, referring to the procedure as almost painless; they also result in low rates of complications such as hemorrhage. The adequate length of the needle depends on the target lesion. Most of breast lesions can be reached with a 25 or 30 mm 23G needle. Sterile and disposable syringes made of transparent plastic are preferred for FNAC. Most pathologists and clinicians use 10 ml syringes, but the 20 ml ones can also be used, mainly in FNAC of large cystic lesions, in order to empty the cyst with less needle punctures. It is important to remember that larger syringes will not provide larger or better samples; on the contrary, in highly vascularized lesions, larger syringes may result in more hemorrhage, and thus inadequate, samples. In summary, syringe choice should be based on availability and personal preference. Commonly a metal or plastic pistol-grip syringe holder is used during aspiration. Such a syringe holder permits suction and release of suction of the syringe to be accomplished with one hand, while the other hand can be made available in locating and stabilizing the palpable lesions. The authors prefer the metal syringe holder, which weighs less than 200 g and has higher durability. The suction in the syringe is enough to make the plunger return to its original position at the end of aspiration. Moreover, in the majority of aspirations, needle placement and application, as well as quality of material, are not compromised by the use of the syringe holder. One has to remember that the good quality aspiration sample fills the needle hub and not the syringe.

3.3.2 Lesion Localization

Palpable and non-palpable breast lesions can be submitted to FNAC procedure. Although breast aspiration should preferably be done guided by imaging, it is largely accepted that palpable lesions can be sampled using palpation as a guide for their localization.

3.3.2.1 Palpation

The localization of a nodule can be obvious, and a thorough palpation of the breast is not necessary. In contrast, when the lesion is not easily identified, a good breast clinical examination should be done. In this situation, it is best to examine the patient in the supine position, forearm up and underneath the head. Examine the breast systematically, nipple to axillary tail in a circular fashion or vice versa. The examining hand should be kept flat with no spacing between fingers. A pillow or towel placed under the shoulder can aid in the appreciation of a subtle nodule. It may be easier to palpate masses deep within the breast or close to the chest wall when the patient is supine with the arm of the side being aspirated raised above and under the head. However, aspiration in this position can at times be more difficult. This difficulty is due to the fact that the breast in this position will flatten out, which in turn places the nodule in question closer to the chest wall. The aspirator senses the added risk for pneumothorax and has a tendency to take a more tangential approach to the mass. Instead, it may be easier to locate the nodule in the supine position and then place the patient in an upright position for aspiration. This is due, in part, to the fact that a mass within the breast when in an upright position will become suspended (pulled downward by gravity) and more tethered to the surrounding connective tissue. This added immobilization of the mass enhances material acquisition. In addition, the nodule moves away from the chest wall, so the risk for pneumothorax decreases and the aspirator will feel more comfortable in performing this FNAC (Staerkel and Sneige 2006).

In contrast to deep organ sites, breast aspirations benefit from the fact that they are not impeded by overlying structures such as muscle. Masses within the breast are often found within adipose tissue and are therefore mobile. It is however this feature of mobility that frequently hinders adequate sampling. Consequently, it is crucial to immobilize the mass so that when a needle is introduced, a coring action is achieved. To achieve total immobilization, one should flatten the breast tissue between the mass and overlying skin by stretching the skin between the immobilizing fingers on each side of the mass. If properly done, almost any mass can be reached with a one- or one-and-a-half-inch needle. When possible, the mass should be fixed with the fingers along the axis of greatest mobility. Sometimes, because of the tumor's depth within the breast, the large size of the breast, and/or the infiltrative nature of the mass, the lesion lacks definition. On palpation, only a vague firmness may be appreciated over a large portion of the breast tissue. Multiple aspirations yield little significant tissue. The problem typically encountered here is that the aspirator has not placed the needle deep enough. The aspirator erroneously thinks that most of the deeply seated firmness is chest wall instead of tumor and not wanting to risk a pneumothorax, the aspirates are made too superficial.

An additional difficulty arises from the tendency of most aspirators to approach lesions with the needle positioned tangentially to the skin, rather than with a near vertical or perpendicular approach. This tangential positioning is more comfortable to the aspirator but may not be optimal for small deep-seated nodules. The problem that can occur with this approach is that by the time the needle has reached the level of the lesion, little of the nodule is in the path of the needle. Therefore, a more vertical approach is recommended, albeit uncomfortable for some aspirators. The best approach to fix the nodule appropriately is to use the middle and index fingers, instead of the commonly used thumb and index finger, for immobilization (Staerkel and Sneige 2006).

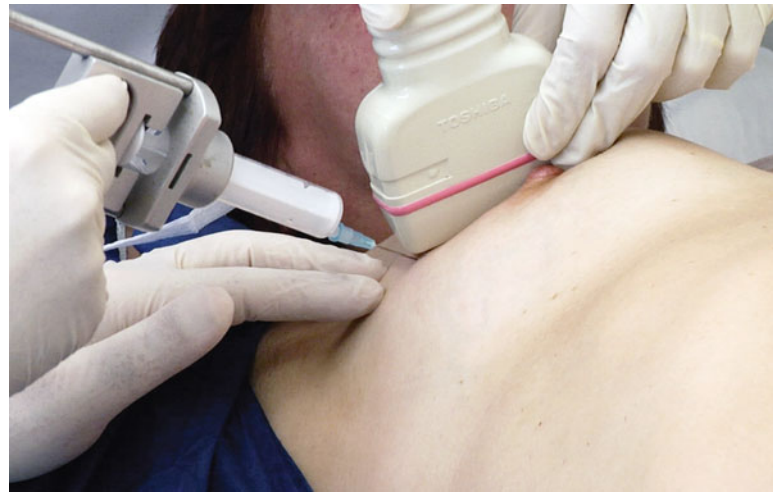

Fig. 3.2 Ultrasound-guided breast FNA. The transducer probe locates the lesion in one of the edges of the ultrasound field; the aspirator passes the needle through the skin, in parallel with the transducer probe in the edge where the lesion is located in

3.3.2.2 Ultrasound Guided FNAC

Nowadays, ultrasound is frequently used to guide breast FNAC, in order to choose the right area for aspiration, for example, solid areas, instead of cystic or necrotic areas (Liao et al. 2004). Currently, many FNAC procedures performed in routine practice are guided by ultrasound, even if the lesion is palpable. There are two guidance methods, depending on the site of the lesion: in the first one the transducer probe locates the lesion in the middle of the ultrasound field and makes a mark, by skin pressure, circumscribing the puncture site; the aspirator then passes the needle through the skin and advances it slowly into the lesion, in a way that the transducer probe can be placed perpendicular to the needle and guides the needle's movements. In the other method, the FNAC procedure is guided step-by-step so that the ultrasound field visualizes the needle's movements since the aspirator passes the needle through the skin until the needle is removed from the lesion. The transducer probe locates the lesion in one of the edges of the ultrasound field; the aspirator passes the needle through the skin, in parallel with the transducer probe, as well as with the sound waves, in the edge where the lesion is located in. It is possible, then, to guide the needle into the lesion, avoiding passing through vascular structures that might be in the way of the needle to the lesion. This latter method is preferred to guide the needle in breast lesions (Fig. 3.2).

3.3.3 Aspiration Procedure

FNAC of the breast is a simple procedure. Aspiration of most lesions is painless, and the patient feels only the initial pinprick through the skin. Anesthesia is not required for most breast aspirations. One of the keys to performing an adequate aspiration is the immobilization of the lesion by the aspirator's free hand as better cutting or coring of the mass can be achieved. The needle, with syringe and holder attached, is then inserted into the mass. The syringe plunger is pulled back, creating negative pressure, as the needle is advanced forward and backward. It is not the suction that directly results in obtaining a sample but rather the cutting action of the needle. The suction helps to pull tissue into the cutting path of the needle and to move the resulting fragments up into the needle's shaft (Fig. 3.3). Pumping the syringe plunger does not enhance sampling; in fact, sampling can be reduced as a result of increased bleeding. In general, needle movements that are more frequent, longer in length, and kept within the tumor during the

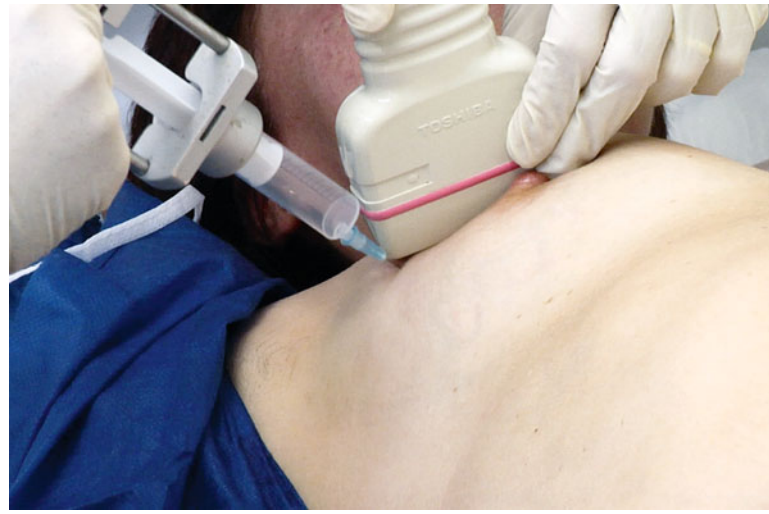

Fig. 3.3 Applying suction while moving the needle helps to pull cells into the needle. In general, needle movements that are more frequent, longer in length, and kept within the tumor during the entire aspiration yield more tissue. A blood-tinged specimen will appear in the hub of the needle. Suction is then released, and the needle is withdrawn

entire aspiration yield more tissue. Movement and frequency will depend on the size of the lesion and the aspirator's ability to maintain control. Typically, 30–50 excursions with the needle are made over a 10- to 20-s period. A blood-tinged specimen will appear in the hub of the needle. Suction is then released, and the needle is withdrawn. Although little bleeding occurs in a fine needle aspiration, it is best to avoid all bleeding, as cellular yield decreases and lesion localization/demarcation is less distinct with increased soft tissue hemorrhage. Therefore, after withdrawing the needle, applying a gauze pad with one's fingertips pressing directly over the puncture site for 1–3 min is recommended (a longer time is applied for individuals with an easy bruising history or for those currently taking blood-thinning agents).

After removing the needle from the patient, the syringe is detached from the needle, filled with air, and reattached. The aspirator expresses a drop of the sample acquired onto one or more slides. The drop is then smeared, fixed with 95 % ethanol or air-dried, then stained for interpretation. Non-palpable masses require radiological imaging (via ultrasound or stereotactic instruments) for correct needle placement; however, the procedure from acquisition to interpretation remains the same.

A modification of this aspiration technique is the acquisition of tissue using a needle only, the so-called capillary method. The syringe and the syringe holder are not used in this method. This modification allows greater sensitivity for the aspirator. Fingers are placed on the hub of the needle, allowing for a heightened appreciation of tissue density. Virtually no bleeding occurs. Because of the lack of suction, frequently no material is seen in the needle hub. Therefore, the aspiration is voluntarily stopped after 15–20 s. The needle is withdrawn, and an air-filled syringe is attached. Material is expelled onto a slide. Disadvantages of this method include the low cellular yield with fibrotic and sparsely cellular lesions and the rapid leakage of fluid from the end of the needle due to the pressure associated with cysts.

Under the following conditions, additional aspirations are recommended (Arkoumani and Wells 2006):

- Lesions that are fibrous in nature; this may represent a desmoplastic carcinoma which will have a low cell yield.
- Small lesions in which the risk of missing the target is great.
- A specimen that clots immediately when placed on a glass slide; evidence that significant bleeding has occurred and a diluted/hypocellular sample has been obtained.
- If the sample looks as smooth as a peripheral blood smear, without visible particles.
- The aspirate is yellow and slightly oily in appearance; this specimen is primarily composed of adipose tissue with frequently little or no epithelium; this situation would be

acceptable if one is relatively confident that a nonmalignant process is being evaluated, that is, a nodule which is soft on palpation and offers no resistance to needle penetration.

Often a cyst within the breast will present as a firm mass, mimicking malignancy, due to the buildup of pressure within the cyst from fluid accumulation. When using the needle only method (no attached syringe), the aspirator should always have a cup or syringe immediately available to capture the fluid which will otherwise rapidly leak onto the patient. Breast cysts require complete drainage. The drained area should be reevaluated by palpation or imaging for any residual solid mass, which will in turn require a separate needle aspiration.

3.3.4 Preparation of Smears

The glass slides should be clean and ready to use. The slides should be labeled preferentially with a pencil at the frosted end. The labeling can include the patient's name (initials), identification number, or site of aspiration, being safer if at least two identifiers are used.

Preparation of high-quality smears is one of the most important parts of the aspiration procedure itself (Stanley and Lowhagen 1993). It does not matter how adequate is the specimen aspirated or how experienced is the cytopathologist; if the smears are not interpretable, then a reliable diagnosis cannot be made. In a smear, cells should be spread over the slide surface by gentle pressure so that the cells are not crushed. The aim of preparing a smear is to obtain a homogenous layer of well-preserved cells, concentrated in a small area of the slide, which makes the microscopic analysis easier and quicker. Remember that an FNAC smear is not a blood cells analysis smear. One does not need the thinnest smear, but a smear that can maintain some of the lesion architecture, without being thick or crushing the cells.

There are two basic methods of smearing: one-step and two-step method (Stanley and Lowhagen 1993). The one-step method is preferentially used on small volume specimens obtained from solid lesions. To perform the smear, the slides should be held as shown in Fig. 3.4. The specimen should be placed near the slide label. The slide that contains the specimen droplet is held by the physician's left hand in a vertical

Fig. 3.4 Preparation of smears using the direct one-step technique. The lower slide holds the material, while the upper slide is used as a spreader slide. The spreader slide is poised over the material droplet, with its lower edge forming a hinge-like contact with the lower slide

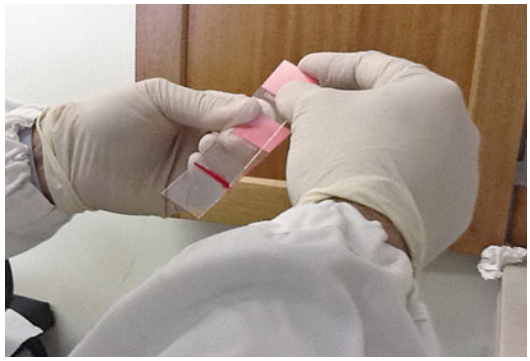

Fig. 3.5 Preparation of smears using the two-step method. Observe the concentration of the material in the middle of the slide that will be after smeared according to the one-step technique

position, while the spreader slide is held by the right hand, perpendicular and over the other slide. The spreader slide is held at an angle so that its superior edge is poised above the specimen droplet. Then, smoothly, the spreader slide touches the lower slide, homogenizes the specimen droplet, and, with a constant and gentle pressure, makes the specimen drawn along the length of the lower slide. The surface of the spreader slide must always be parallel to the surface of the specimen slide, and the smear should finish before the end of the specimen slide. It is also important that the smear occupies only a small area of the slide.

The two-step method is used for liquid or hemorrhagic specimens within which cells and tissue particles are suspended. To perform the smear, the slides should be held as shown in Fig. 3.5. The fluid sample is placed from the middle to the labeled edge of the slide. The spreader slide is held at a 45º angle to the specimen-bearing slide, and its end is brought into contact with the fluid. Then, the spreader slide advances toward the specimen slide's label, carrying the fluid and suspended particles. The surface tension causes the fluid to spread out in a line behind the edge of the spreader slide. The spreader slide, thus, returns in the opposite direction, stopping in the middle of the specimen slide, where the tissue particles remain concentrated. The spreader slide is quickly pulled away from the specimen slide, which is turned to one side in order to drain the fluid excess. After that, the spreader slide is turned perpendicular to the specimen slide, and the line of sediment tissue particles is smeared as it is in the one-step method. This technique is more complex and the smears are not that good-looking as in the one-step method, but it allows a better smear quality to fluid or hemorrhagic samples: the tissue particles are concentrated in the middle of the slide, which makes microscopic observation easier, and the excess of fluid or blood is removed, allowing rapid drying and a better fixation of the slide.

For different reasons, sometimes it may be necessary to prepare more than one smear from a single droplet of specimen, for example, when performing aspirations from tumor masses, which usually yield very cellular material. If too much sample is placed on one slide, the sample may be too thick for microscopic examination. Alternatively, sometimes even though a small amount of specimen is obtained by fine-needle aspiration, but for some reason, it may not be feasible to perform another aspiration, yet the physician knows that immunohistochemistry or special stains are needed, it may then be of interest to split the specimen droplet in additional slides. In such cases, the entire sample is expelled onto a glass slide, and the slides are held as described to the maneuver in the one-step method. The end of the spreader slide is placed in the sample droplet at a 45° angle. The spreader slide quickly touches the sample and carries off its edge. This can be done at least four times with the same specimen slide. Each portion of the original sample in the spreader slide is smeared in a new slide, while the original sample is smeared with the spreader slide, following the one-step method.

Fluid or bloody specimens may be expelled directly to a liquid-based vial: in the former the aim is to concentrate the amount of cells in a smaller portion of the slide, and in the latter the aim is to reduce the number of erythrocytes, allowing a better microscopic examination. Liquid-based specimens may also be used to perform additional techniques, such as immunohistochemistry and flow cytometry. Some reports in the literature, especially from surgical departments, claimed that the processing of FNAC specimens using liquid-based cytology offers an

accurate diagnostic tool (Kontzoglou et al. 2005), but this was probably a result from the lack of experience in preparing smears. In the authors' experience, processing of aspirated materials by liquid-based technique is restricted to very specific situations. It is preferred to have the aspirated specimen in a smear preparation, since the preservation of at least some architectural features of the tissues in the smears helps in the interpretation of the cytological findings.

3.3.5 Fixation and Staining

Although Papanicolaou stain is the most widely used staining method in cytology, most cytopathologists prefer to use a combination of two complementary stains in FNAC: Papanicolaou and Romanowsky. Papanicolaou staining includes the hematoxylin nuclear stain and two cytoplasmic counterstains, orange G and EA. There are at least three types of stains under the designation of the Romanowsky method – Giemsa, May-Grünwald-Giemsa, and Diff-Quik – but all three present the same staining pattern that characterizes the Romanowsky method. Some cytopathologists prefer the classical histologic hematoxylin and eosin (H&E) method, mainly due to easier and quicker comparison between the histologic and cytologic characteristics of nuclei, cytoplasm, and stroma.

The smears prepared after the aspiration procedure can be either intentionally air-dried for further Romanowsky staining or immediately fixed in 97 or 70 % ethanol for Papanicolaou or H&E stains. The air-dried smears are submitted to post-fixation with methanol.

The best staining is obviously the staining that the cytopathologist is most familiar, but each staining method has its advantages and works better together. The air-drying required for the Romanowsky method results in cell swelling, and the cells tend to look larger than in Papanicolaou or H&E methods. Romanowsky stains have the ability to react with several tissue components in a metachromatic way, giving them a reddish-purple color. This can be observed in nucleic acids, mucins, and extracellular matrix components, such as in fibroepithelial tumors and metaplastic carcinoma.

On the other hand, nuclear details are not the strength of the Romanowsky method. As already mentioned, the nuclei usually look larger in Romanowsky stains, but they also lack chromatin and membrane contour details. For this reason, Papanicolaou stain may be performed whenever the nuclear detail is important for the diagnosis or the subclassification of tumors.

While in a Papanicolaou-stained sample, nuclear detail provides important diagnostic clues, one has to bear in mind that FNAC usually provides highly cellular smears in which the tissue architecture is almost always maintained. The tumor cells are closely related to the stromal and extracellular matrix elements, and sometimes, evaluation of other additional parameters such as smear pattern, cellularity, and nuclear size may be sufficient to allow a specific diagnosis. The assessment of an FNAC may be supplemented by Romanowsky stains. H&E stain has the same characteristics in FNAC as it has in histologic sections, and for this reason, many pathologists prefer to use this method. Nevertheless, as Romanowsky and Papanicolaou stains are complementary stains, they are recommended to be used preferentially in cytological specimens.

3.3.6 Reporting of the Results

Reporting of the results is one of the most important parts of the FNAC procedure. The cytopathologist should have in mind the consequences of the report to the patient and be cautious when reporting suspicious or inconclusive diagnoses. Comments and explanatory notes should be used judiciously if these are thought to be helpful to the clinicians in making a therapeutic decision. Negative results should always be correlated with clinical and imaging findings because if the aspirated lesion is suspicious for malignancy in the clinical or imaging setting, a negative result in the FNAC specimen should not interfere with the clinical approach, and an alternative method, such as core needle biopsy or surgical excision, should be performed in order to obtain a reliable diagnosis.

Many countries and centers use similar reporting systems for breast FNAC (C1–C5) in keeping with European guidelines for quality assurance in breast screening and diagnosis. In this terminology, C1 means unsatisfactory; C2 means benign; C3 means atypical/suspicious, probably benign; C4 means suspicious, probably malignant; and C5 means malignant (Kocjan et al. 2008). In the United States a similar approach is used, based on the rec ommendations of the National Cancer Institute (NCI) consensus conference. FNAC is classified into one of five categories: benign, atypical/indeterminate, suspicious/probably malignant, malignant, and unsatisfactory (National Cancer Institute Sponsored Conference 1997). Despite subtle differences, both systems are essentially similar. In addition, adopting similar systems allows standardized reporting for breast cytology, and this could be very useful for a uniform clinical management of the different lesions. In addition, some authors prefer using descriptive reports for breast FNAC. This is also reflected in the recommendations of the NCI conference that each category should be further described and classified as appropriate, with an attempt to place the findings into a specific pathologic entity such as that used in a surgical pathology diagnosis. Furthermore, it was stated that "the report for an FNA of the breast should closely resemble the format for a surgical pathology report of a breast biopsy specimen. When a surgical pathology format is used, the opinion or diagnosis section should be like that of a surgical pathology report. The diagnosis section should have a description of the site of lesion, location, and microscopic findings. This section should be followed by FNA biopsy finding in parenthesis and then the opinion of the pathologist as to the nature of the lesion. Although a greater degree of flexibility is possible, the diagnostic categories should follow those as recommended in the Diagnostic Terminology section or give a precise pathologic diagnosis. It is clear that the use of this nomenclature is useful. When it is possible to diagnose a cyst, fibroadenoma, or carcinoma on cytological material, the diagnosis should not be replaced by categories of benign or malignant only. Standardization can be quite useful in a series of situations of the pathology practice, but to restrict the breast cytological diagnosis into limited categories, like in gynecological cytology (a screening and not a diagnostic method), will not bring clear advantages to the clinical practice. So preferably, breast FNAC should be reported as a surgical pathological report, giving all the clinical information, including specimen type and localization technique, followed by a microscopic description and the diagnostic conclusions. An additional space for comments is present, including a section for recommendation to correlate the cytology with clinical and imaging findings and the need for clinical follow-up or biopsy of the lesion for histologic assessment.

3.4 Clues to Enhance Diagnostic Accuracy

Some additional information is essential to enhance breast FNAC diagnostic accuracy, such as:

- Patient age. Advancing age increases suspicion for carcinoma and decreases the likelihood of fibroadenoma.
- Lesion location. If the location is subareolar, one should consider the possibility of a papillary neoplasm, nipple adenoma or subareolar abscess.
- A cystic lesion suggests fibrocystic disease. Exception occurs when the aspirate is markedly cellular with single columnar cells; then one should consider a papillary neoplasm. In addition, the acquisition of thin, watery green-gray fluid typical of benign cyst fluid of fibrocystic changes should caution against an overdiagnosis of carcinoma even when some of the cells present show degenerative nuclear atypia.
- Previous trauma or surgery at the aspiration site requires careful exclusion of a reactive/reparative process, such as fat necrosis, before a malignant diagnosis is made.
- Past history of another malignancy. The breast can be involved by other neoplasias such as melanomas, lymphomas, or metastatic carcinomas from other sites.

- Needle penetration findings are also helpful. Soft, low-resistance aspiration suggests benign disease, fat necrosis, or mucinous carcinoma, whereas firm, rubbery texture favors fibrocystic changes or fibroadenoma. A firm, gritty sensation suggests carcinoma.

The diagnosis derived from breast FNAC should always be correlated with both clinical and radiological findings to determine patient management (the so-called triple test approach) (Kocjan et al. 2008; Arkoumani and Wells 2006). A benign triple test results in the patient being followed clinically with a return visit in 6 months or 1 year. A patient with malignant triple results is referred for definitive therapy. A mixed (inconclusive) triplet requires excisional biopsy of the lesion in question.

3.5 Complications of Breast FNAC

Complications occurring in breast aspirations are rare and, when they occur, are usually bleeding, infection, and pneumothorax. The most likely complication is soft tissue bleeding with a resulting hematoma; however, this can be avoided if, after aspiration, firm directed pressure is placed over the puncture site. Infection is uncommon but when present is of little consequence and can be treated with antibiotics. Pneumothorax is extremely rare but, when present, may require chest tube insertion. In individuals with small breasts where an underlying rib can be palpated, one can position a mobile nodule over a rib for protection. Also, for deep-seated lesions adjacent to the chest wall, the use of a 25 gauge needle instead of a larger bore needle minimizes injury and therefore the risk of pneumothorax.

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Liquid-Based Cytology and Cell Block in Breast Lesions

4

4.1 Liquid-Based Cytology

Liquid-based cytology (LBC) is a method of retrieving and processing cytologic material for assessment. Developed primarily for cervical cytology screening in an effort to reduce inadequacy rates, the use of LBC has extended to FNAC cytology as well. In LBC, cytologic material is placed in a fixative solution and, depending on the system, then centrifuged or filtered to produce monolayers of cells on glass slides which can enhance and facilitate interpretation. These monolayered preparations can also be subjected to adjunctive studies like immunohistochemistry.

There are many ways of achieving monolayered cell preparations, which may be through automated platforms like ThinPrep and SurePath, or via alternative manual techniques that are less costly and that can produce similar monolayered preparations (Wauters et al. 2009). One study found that monolayered preparations of breast aspirates gave definitive diagnoses significantly more often than aspirates subjected to conventional smear preparations (72.8 % vs. 58.5 %) and that the benefit was most frequently observed in malignant breast lesions (Wauters et al. 2009).

LBC, however, has not been widely applied in FNAC of breast lesions, but its greater use in aspirates of other organ sites has allowed a reflection of some of its advantages and pitfalls.

4.1.1 Advantages

LBC provides optimal cellularity for evaluation, and in an assessment of thyroid lesions (Saleh et al. 2008), LBC using ThinPrep slide preparations was found to have a greater cellularity for diagnosis and the ability to detect atypical/neoplastic thyroid lesions as compared to the cell-block technique. Wauters et al. (2009) also found that centrifugation of aspirated cells that are subsequently concentrated on a single slide results in higher cellularity, as compared to conventional smears in which the cells are dispersed over multiple slides. LBC allows direct fixation of the cells, eliminating air-drying and smearing artifact, as well as avoiding interfering background material.

ThinPrep smears are described to be consistently devoid of obscuring elements, with adequately preserved and dispersed cells (Michael and Hunter 2000). The background is clean. Issues with excess blood, inflammatory exudates, mucus, air-drying, and variable smear thickness are lessened.

Cytologic specimens, traditionally considered to be less amenable to further workup using special stains and immunohistochemistry, can now be optimized for these tools to be performed on LBC to the same degree of effectiveness as on cell blocks (Leung and Bedard 1996; Rossi et al. 2005).

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4.1.2 Disadvantages

There are some limitations that need to be noted in the interpretation of LBC smears, as they can hamper cytologic interpretation by the unwary. Cytomorphologic changes specific to LBC smears include diminished cell cluster sizes, fragmentation of epithelial cell sheets, and an increased number of single discohesive cells. Cells tended to be smaller and more spindled with attenuated chromatin detail and prominent nucleoli, with difficulty in identifying intranuclear inclusions. There is also alteration in the amount and quality of background material. In particular for the breast, the number of myoepithelial cells is reduced (Michael and Hunter 2000). Apart from their reduction in number, myoepithelial cells in LBC have also been reported to localize at the periphery of the smears and acquire intact spindled cytoplasm causing difficulties in interpretation.

While an advantage of LBC is the reduction in background material, there are some types of breast lesions such as the mucinous carcinoma, for which background mucin is essential to the correct diagnosis, and diminished amounts or altered quality may hamper the diagnosis.

Epithelial fragmentation may lead to inability to recognize the architectural papillarity of papillary lesions; cellular discohesion can simulate malignant dispersion.

4.2 Cell Block

Cell blocks are obtained from centrifugation of needle aspirates, or from tissue or blood clots obtained during FNAC. They can be prepared by embedding centrifuged cell samples in agar, thrombin, or other gels. The centrifuged material can also be transferred directly to formalin and routinely processed in the histopathology laboratory, after which a paraffin block containing the cellular material is produced, which can be subjected to histologic examination and ancillary investigations (Figs. 4.1a–c and 4.2a–e).

There are many methods that have been described that can produce optimal cell blocks

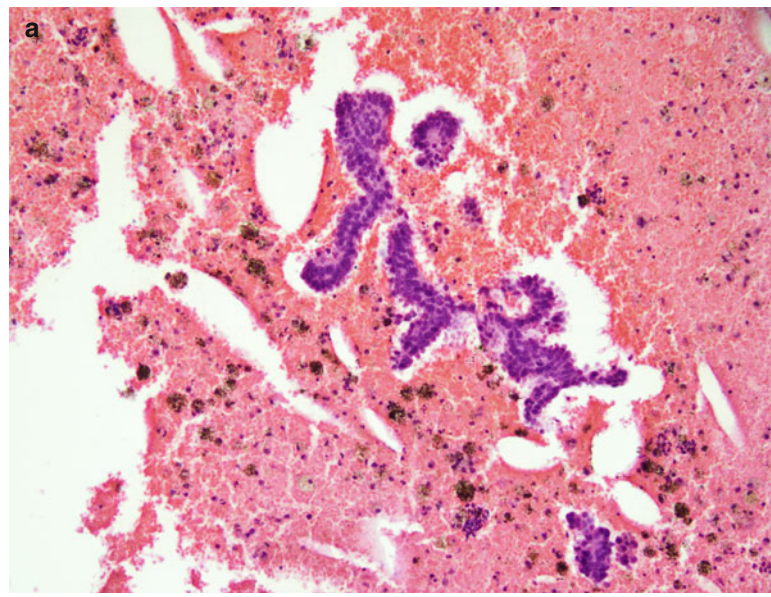
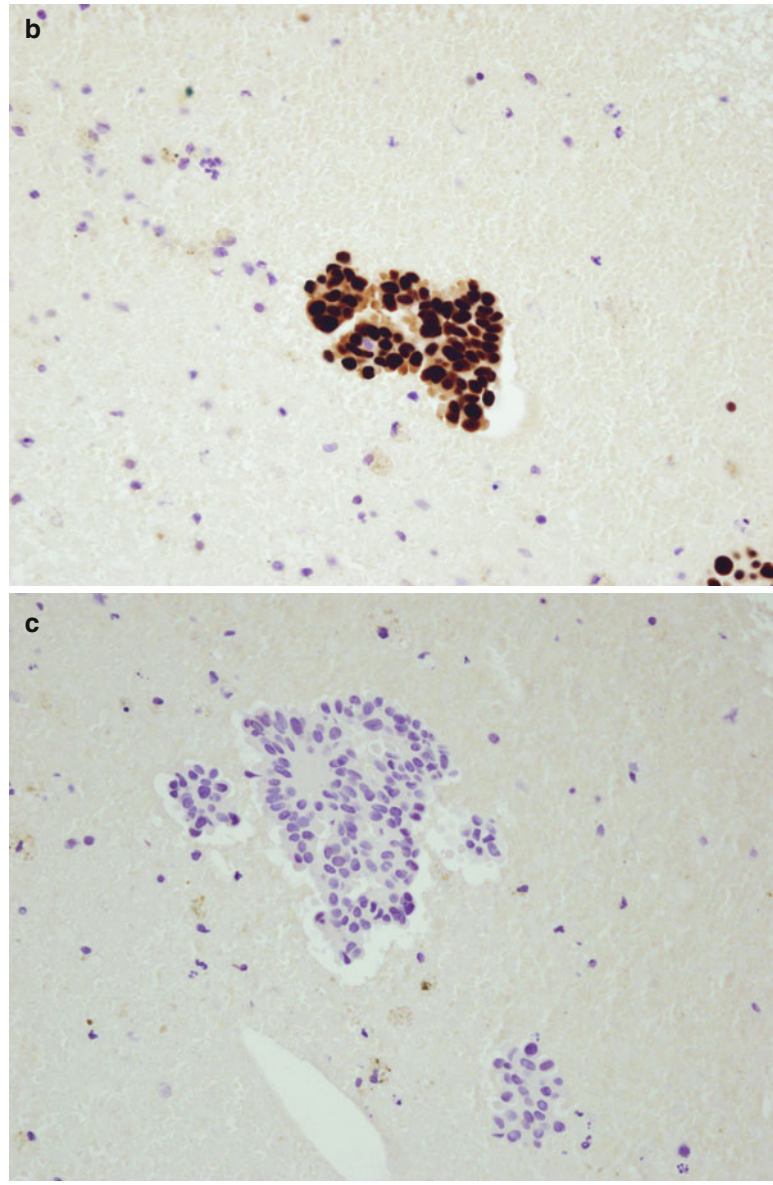


Fig. 4.1 (**a**–**c**) Papillary carcinoma. (**a**) Cell block shows papillary fragments within a bloody background. (**b**) Immunohistochemistry on cell block shows positive nuclear staining for estrogen receptor. (**c**) Immunohistochemistry on cell block shows no staining for CK14

Fig. 4.1 (continued)



for subsequent histologic examination. Some of these are proprietory and require the use of specialized equipment that can be costly.

We recently reported a simple technique using readily available and inexpensive consumables in a routine cytology laboratory and FNAC clinic, which can result in excellent cell blocks that are amenable for detailed histologic interpretation and immunohistochemical studies (Al Jajeh et al. 2012).

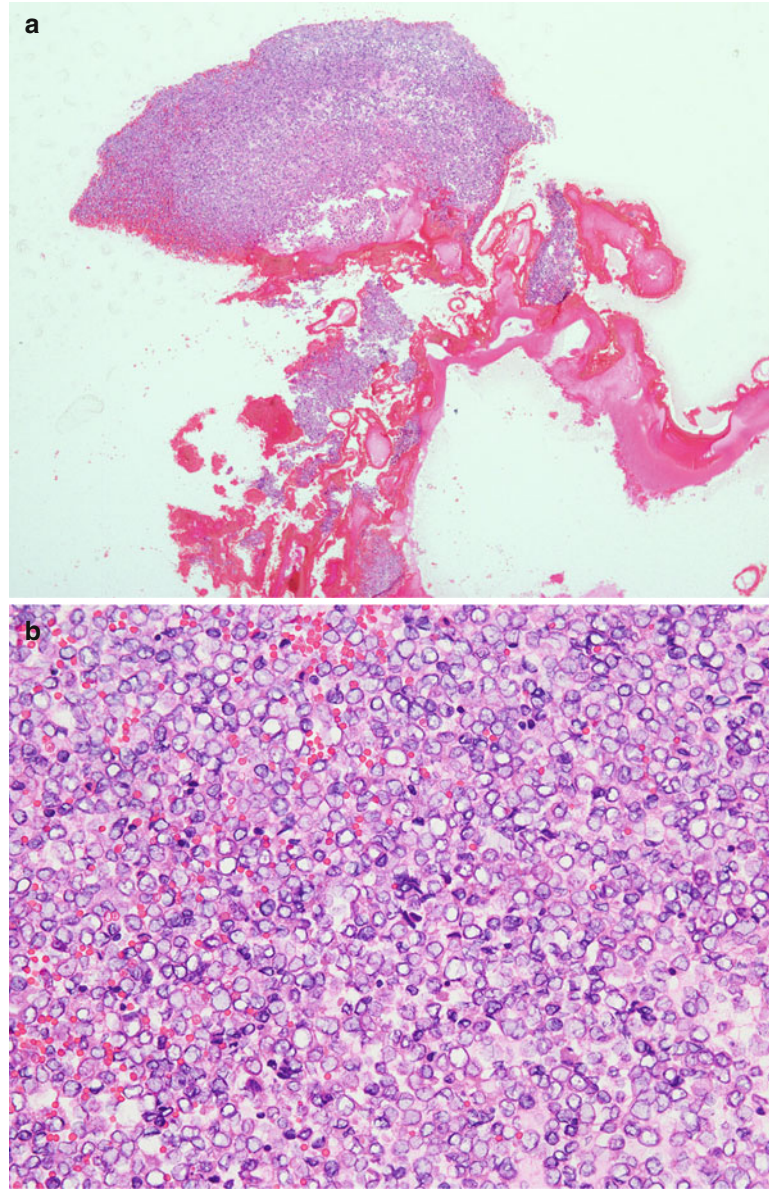
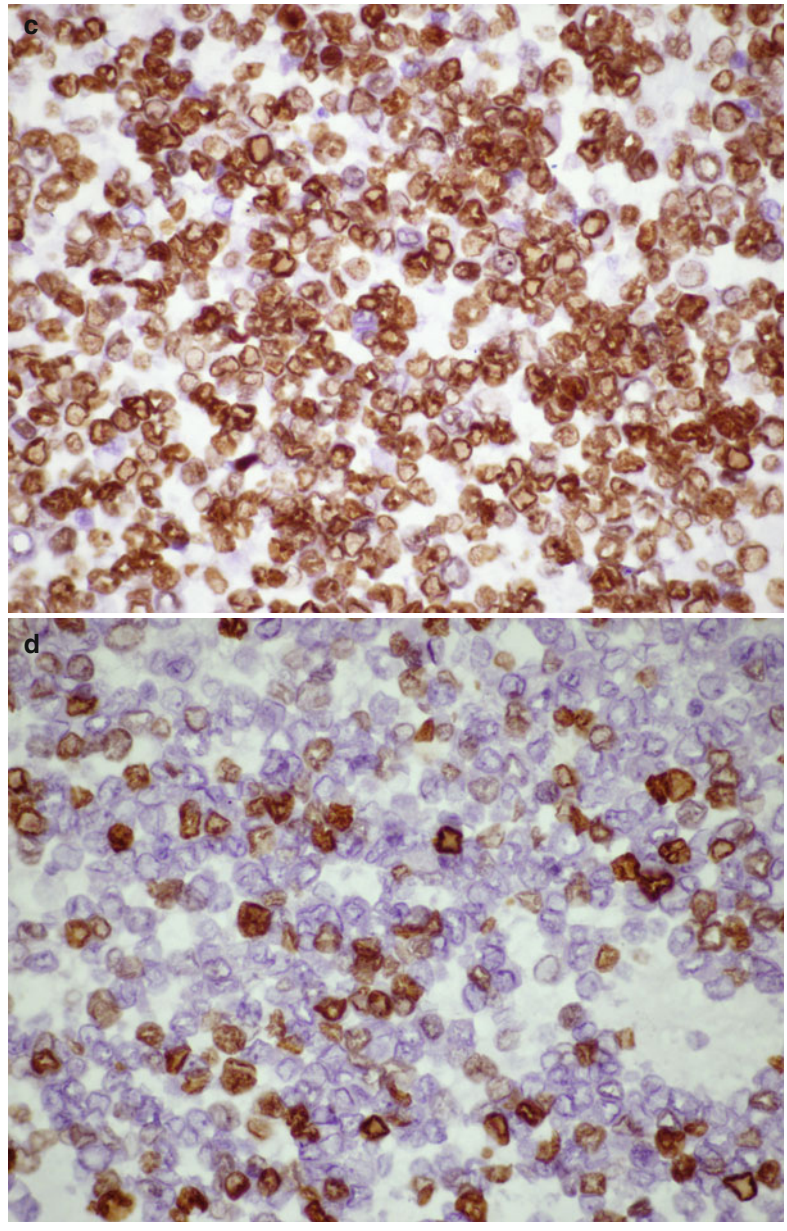


Fig. 4.2 (**a**, **b**) Breast ductal carcinoma. (**a**) Cell block shows abnormal cells admixed with blood and fibrin. (**b**) High magnification of cell-block preparation shows nuclei with variable sizes and occasionally discernible nucleoli. (**c**–**e**) Cell block immunohistochemistry of breast ductal carcinoma. (**c**) Estrogen receptor positivity. (**d**) Progesterone receptor positivity. (**e**) HER2 Immunohistochemistry shows 1+ staining along cytoplasmic membranes of some tumor cells, indicating a negative result

Fig. 4.2 (continued)



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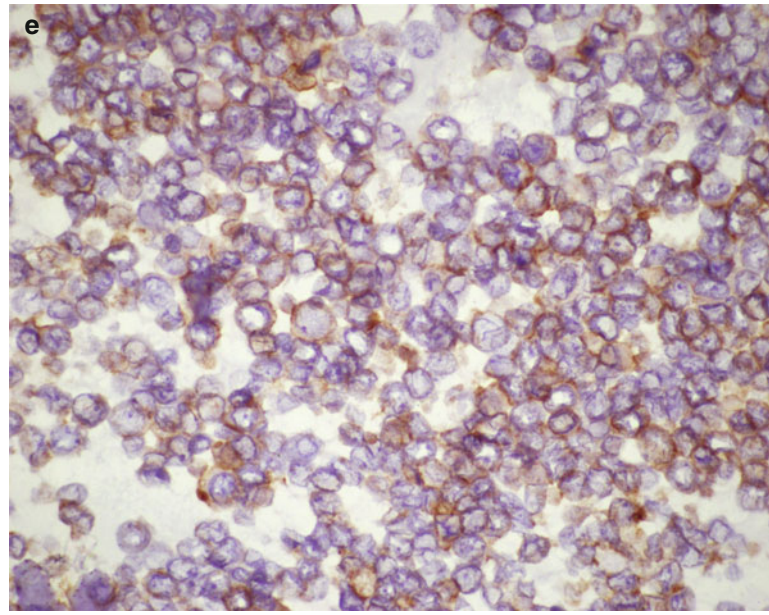

4.3 Summary

Whether LBC or cell-block method is used, it is recommended that the cytology laboratory of the institution acquires experience with the technique, recognizing any artifacts that are different from conventional smears. It would also be prudent to allocate a suitable time frame for validating the technique against conventional methods, in order to determine diagnostic accuracy as compared with routine smears, as well as to familiarize with any pitfalls encountered.

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Inflammatory Lesions of the Breast

5

5.1 Clinical Findings

The breast can be the site of acute and chronic inflammatory diseases. These diseases can be infectious in etiology (acute mastitis, breast abscess, and tuberculosis, among others), others are related to local changes in the nipple and breast (subareolar abscess and duct ectasia), some have posttraumatic causes (fat necrosis), and there are inflammatory processes related to hypersensitivity reaction (granulomatous mastitis) or even idiopathic. The clinical presentation of all of these entities is quite variable, and in many situations the diagnosis is clinical with immediate treatment (antibiotics in infectious cases), but otherwise, these lesions can cause tumorlike nodules that need to be sampled by fine-needle aspiration for definitive diagnosis. In this chapter the cytological aspects of the main inflammatory lesions that can affect the mammary gland are discussed.

5.2 Breast Abscess and Acute Mastitis

Breast abscesses and acute mastitis occur most frequently in the puerperium. As a result of frequent fissure formation in the nipple during breast feeding, there is direct penetration and retrograde ductal spread of bacteria into the breast parenchyma. This results in an acute inflammatory process, which may progress to abscess formation, and can be either solitary or multiple. Sometimes breast abscess occurs outside the lactation period and also usually affects the area near the nipple, as in cases of dermatitis or secondary bacterial infection of other lesions (see Sect. 5.3). The breast is swollen, painful, with redness of the skin. Most of the time the diagnosis is clinical, and the patient is treated with antibiotics. Occasionally resolution does not occur, and a diagnostic procedure is needed to rule out the possibility of inflammatory carcinoma. The bacteria most often associated with abscess are Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, and Bacteroides species.

During FNAC, the material aspirated is purulent. The smears are cellular, with predominantly neutrophils and fibrin (Fig. 5.1). Single- or multinucleated histiocytes with vacuolated cytoplasm can be present. The epithelial and mesenchymal cells may exhibit reactive atypia characterized by enlarged nuclei and prominent nucleoli. Granulation tissue and fat necrosis changes can also be observed (Das et al. 1992).

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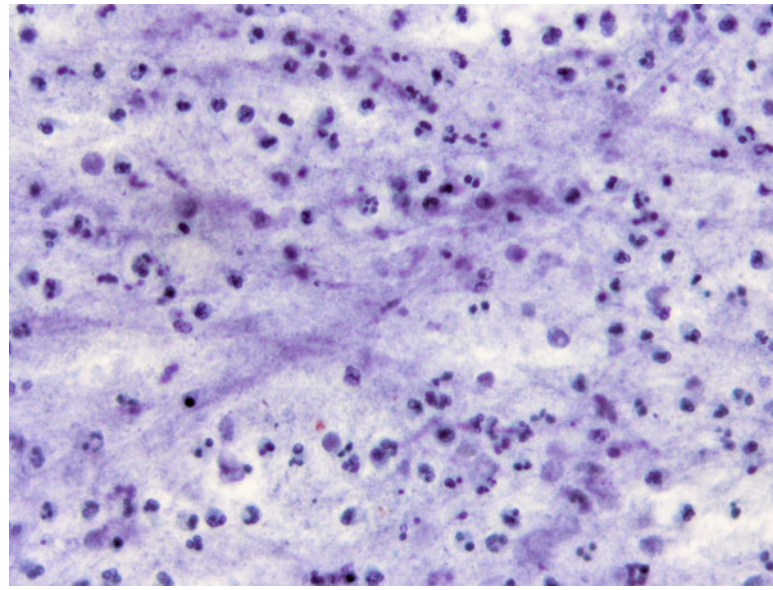

Fig. 5.1 Acute mastitis. Cellular smear showing predominantly neutrophils and fibrin (Pap stain)

5.3 Subareolar Abscess

This condition is more common in women of reproductive age but may occur after menopause and even in men. The majority of patients are smokers, and the lesion presents initially as a subareolar or periareolar painful swelling with skin erythema, suggesting an infectious process. Typically this is a relapsing condition (especially if treated only with incision and drainage) in which at each relapse a sinus tract is formed and opens to the skin at the areolar edge. Often there is nipple inversion due to the subsequent fibrotic scarring. The pathogenesis of the lesion is related to squamous metaplasia of the large ducts under the nipple, and the accumulation of keratin in the ductal system causes ductal dilatation and subsequent rupture. As a consequence of the presence of keratin in the stroma, there is an inflammatory response with abscess and sinus tract formation. Because subareolar abscess occurs predominantly in smokers, it is believed that the chronic action of tobacco may alter the epithelium of the lactiferous sinuses.

FNAC consists of primarily inflammatory exudates with neutrophils, histiocytes, and multinucleated giant cells, which may possess cytoplasmic keratin (Santos and Schmitt 1991). Metaplastic squamous cells and anucleated squames are frequently observed, and these findings, combined with the clinical characteristics, allow the definitive diagnosis to be made (Fig. 5.2). The presence of reactive nuclear atypia in the epithelial cells can be confused with malignancy by the unwary.

5.4 Duct Ectasia

Duct ectasia is an inflammatory disorder that affects women between the ages of 40 and 85 years. In the initial phase, there is a focal painful area near the nipple, accompanied by nipple discharge, which may be serous, creamy, or blood stained. After the acute episode, there usually remains a firm palpable mass in the subareolar or periareolar region. When there is severe periductal fibrosis, the clinical picture is dominated by skin retraction, nipple inversion, and palpable mass, simulating breast cancer. Mammography may show calcifications. In this situation, FNA is essential to rule out the possibility of malignancy. Duct ectasia affects the larger breast ducts and is characterized by duct dilatation with periductal

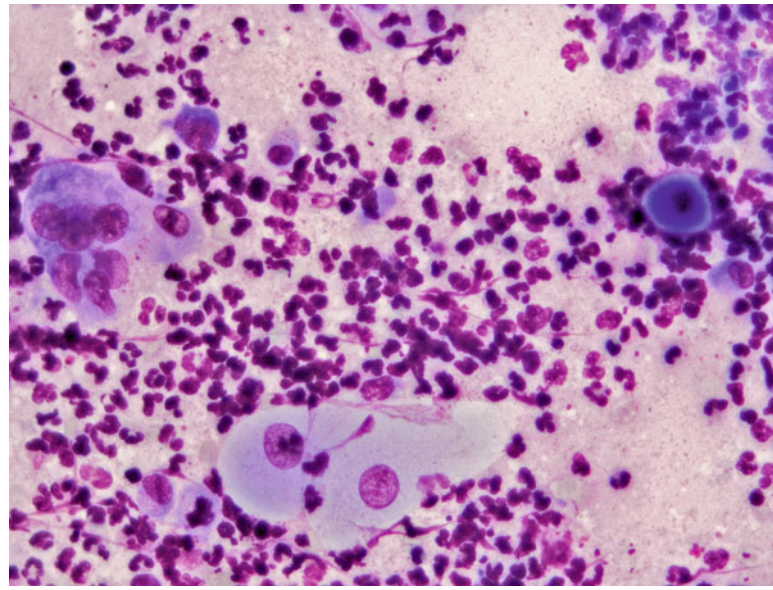

Fig. 5.2 Subareolar abscess. The smear shows the presence of inflammatory cells with multinucleated giant cells and metaplastic squamous cells (MGG stain)

inflammation and fibrosis. It is believed that the process starts with periductal inflammation (periductal mastitis) in younger patients, with subsequent destruction of the elastic layer of the duct. Eventually the duct becomes dilated accompanied by periductal fibrosis (duct ectasia). In a few cases, the inflammatory changes result in fibrosclerosis (obliterative mastitis) at the late stage. The cause of periductal inflammation is unknown, although some authors suggested bacterial infection (especially anaerobic organisms) may play a role. Unlike subareolar abscess, duct ectasia is not associated with the smoking habit. Duct ectasia has also to be distinguished from ductal dilatation frequently found in postmortem studies in women over 60 years, the latter occurring as part of normal breast involution in aging and has no apparent relationship with periductal mastitis/duct ectasia.

5.5 Chronic Granulomatous Mastitis

This group can be subdivided in two: those related to specific infections, like tuberculosis, fungi, or syphilis, and those that are noninfectious, which are grouped under the term idiopathic granulomatous mastitis. Tuberculosis of the breast is rare in western countries but still common in developing countries. Clinically it simulates a breast tumor, sometimes with skin fistula formation. Patients may or may not show any systemic symptoms of the disease. The typical cytologic appearance is the presence of epithelioid granulomas, Langhans giant cells, lymphocytes, and neutrophils. Necrosis is common (Fig. 5.3a, b) (Tse et al. 2003). For a definitive diagnosis, alcohol-acid-resistant bacilli should be demonstrated by Ziehl-Neelsen staining or have a positive PCR reaction for Mycobacterium tuberculosis (Fig. 5.4). However, sometimes even with negative results, in endemic areas, the morphological picture may be sufficient for starting antituberculosis therapy. If the patient fails to respond, it is important to exclude other infections with similar morphological features, such as syphilis, parasitic infestation, and fungal infection. In the latter two settings, the demonstration of the infectious agent in histology or cytology is possible. All systemic mycoses can affect the breast, and the fungus can be demonstrated using silver staining. Sometimes unusual fungal organisms can cause granulomatous inflammation, including paracoccidioidomycosis that may form a breast mass

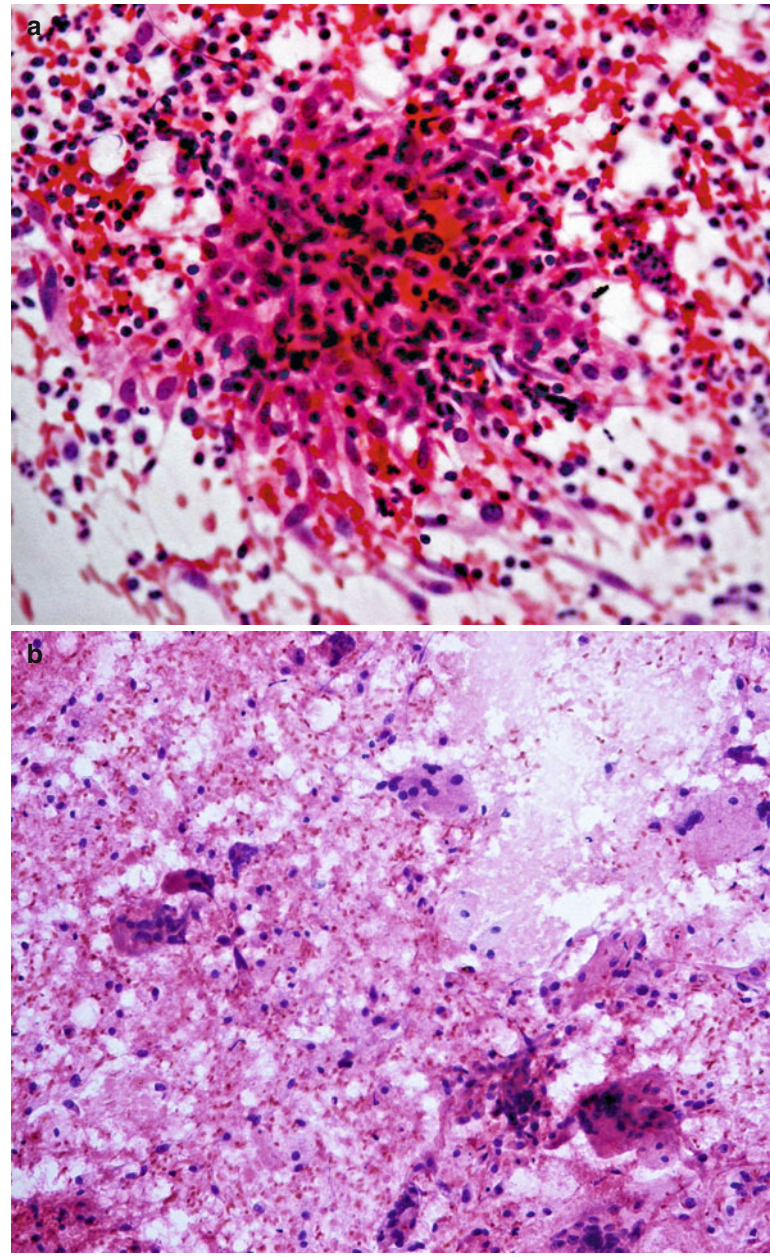

Fig. 5.3 (**a**, **b**) Mammary tuberculosis. (**a**) Cellular smear shows epithelioid granulomas, with lymphocytes and neutrophils in the background. (**b**) Cellular smear highlights Langhans giant cells and necrosis

(Fig. 5.5). Sarcoidosis rarely affects the breast, and in these cases usually there is systemic involvement. Presentation is as a single or multiple masses, and the morphological pattern is similar to all granulomatous disease except for necrosis which is absent in sarcoidosis. This diagnosis requires exclusion of other causes of granulomatous diseases (in particular tuberculosis) and clinical evidence of disease elsewhere.

When all other causes of granulomatous infections of the breast are excluded, the diagnosis of idiopathic granulomatous mastitis can be made. This condition affects young women between the ages of 20 and 40 years old and is frequently associated with

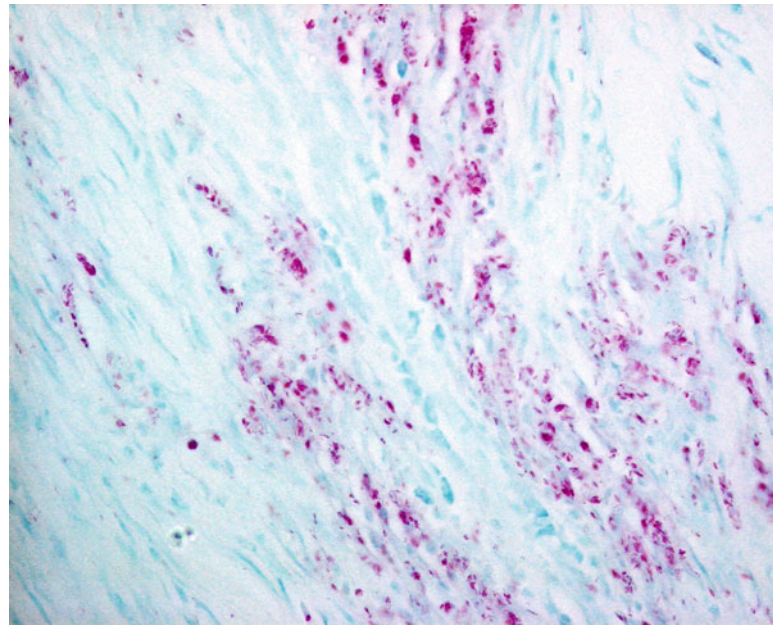

Fig. 5.4 Mammary tuberculosis. Alcohol-acid-resistant bacilli are demonstrated in a tissue section by Ziehl-Neelsen staining

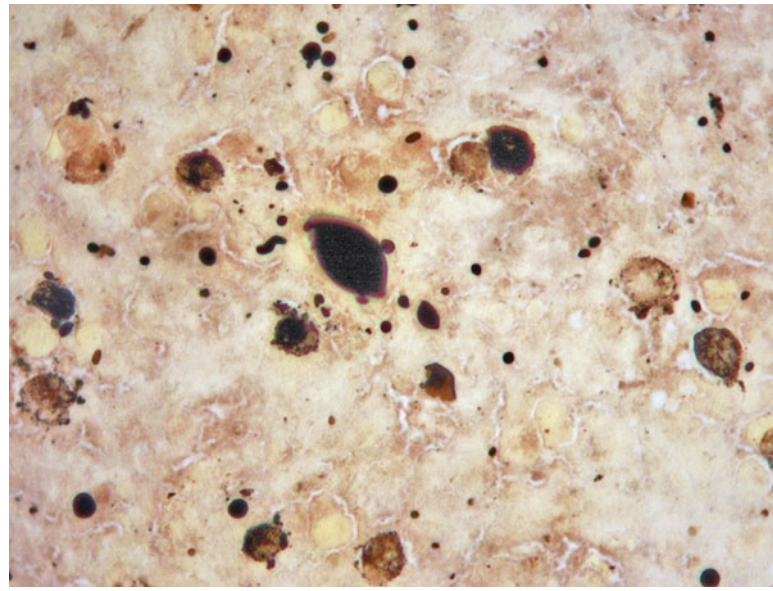

Fig. 5.5 Breast paracoccidioidomycosis. Note the yeast cells with multiple budding "steering wheels"

a recent pregnancy. The usual presentation is as a palpable mass, and in half of the cases, the clinical impression is carcinoma. In 25 % of the cases, the disease is bilateral. Mammography and ultrasound show an ill-defined, hypoechoic mass. FNAC smears show epithelioid macrophages, giant cells, and neutrophils, but necrosis is absent (Fig. 5.6) (Gupta 2010). However, to make a definitive diagnosis on cytology is difficult because this is a diagnosis of exclusion. It is important to emphasize that granulomatous reaction is sometimes observed in breast carcinomas and an FNAC may be taken from this area, thus missing the malignant lesion.

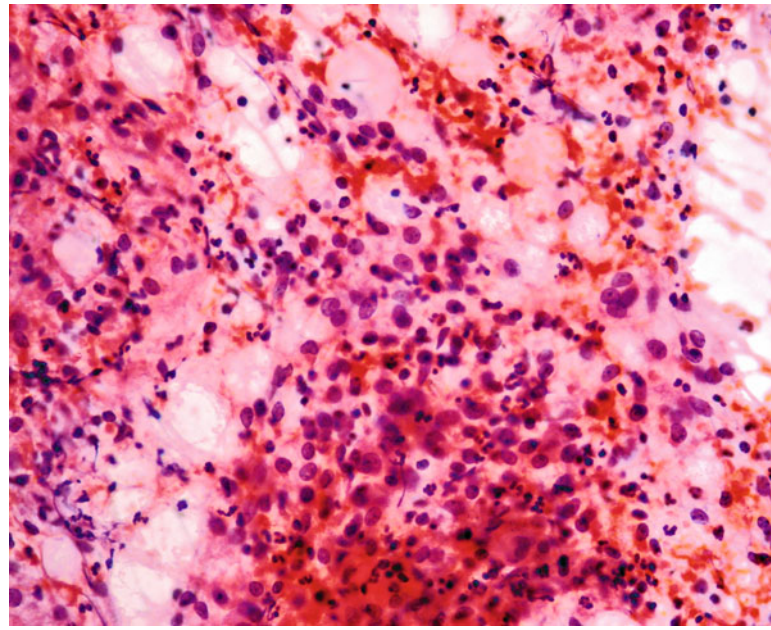

Fig. 5.6 Idiopathic granulomatous mastitis. FNAC smears show epithelioid macrophages, giant cells, and neutrophils, but necrosis is absent

5.6 Diabetic Mastopathy

Diabetic mastopathy is a mass-forming inflammatory disorder of the breast characterized by a perilobular and perivascular lymphocytic infiltrate (Miralles et al. 1998). The cytological picture is not specific, showing lymphocytes, benign epithelial cells, and fragments of stroma with epithelioid myofibroblasts. Frequently, due to the fibrosis, the material obtained is scarce and non-diagnostic.

5.7 Fat Necrosis

Fat necrosis of the breast commonly affects large breasts and presents as a mass. As fat necrosis is often mass forming and is firm to hard to palpation, it can simulate carcinoma clinically and radiologically. Thus, FNAC plays a very important role in the diagnosis and differentiation from cancer. The cytological aspects of fat necrosis are variable according to the stage of the lesion. At aspiration, viscous, oily-looking material is obtained. At early stages, the smears are moderately to highly cellular, with foamy mononuclear macrophages (always present), sometimes multinucleated macrophages, disintegrated or collapsed fat cells, neutrophils, and fibrofatty tissue fragments (Fig. 5.7). Oleic acid crystals may appear, in groups associated with degenerate fat cells, and they have needle-shaped morphology. They are not birefringent. In advanced stages, the aspiration is more difficult with scant cellularity. Macrophages with sparse finely vacuolated cytoplasm are still present, together with some mononuclear inflammatory cells, in a fatty and watery background. Fibrofatty tissue is frequent as well as stromal fragments with elongated fibroblasts. Sometimes macrophages appear atypical and may mimic carcinomatous cells. In general there are no epithelial cells, although their detection may be associated with reactive atypia. In later stages, lipid cysts may be formed. The liquefied fat tends to dissolve during the staining process, and the smears can only be interpreted correctly if the cytopathologist aspirates the patient or receives very good information from the aspirator.

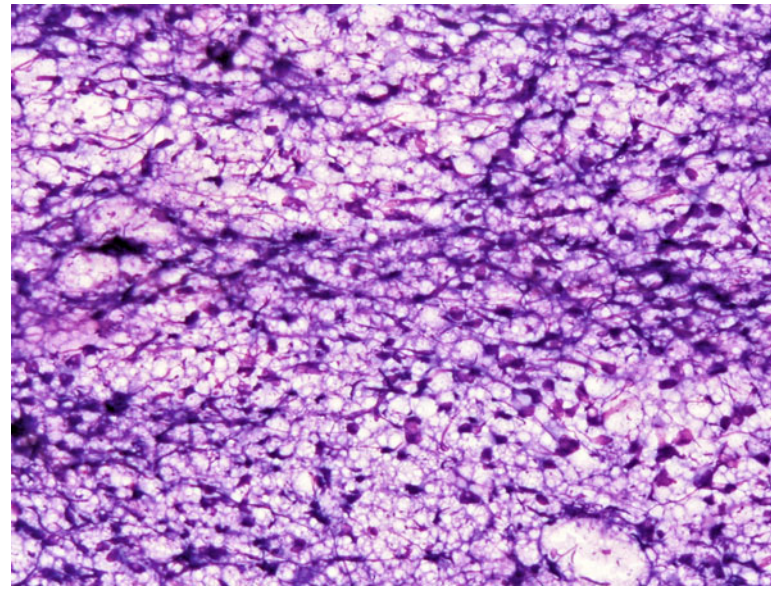

Fig. 5.7 Fat necrosis. Moderately cellular smears with foamy mononuclear macrophages, disintegrated or collapsed fat cells, neutrophils, and fibrofatty tissue fragments (MGG stain)

5.8 Summary

- Most inflammatory lesions of the breast can simulate malignancy clinically and radiologically, especially the chronic ones, due to the presence of fibrosis. FNAC is a good diagnostic tool to rule out carcinoma and confirm the inflammatory nature of the lesions.
- Ductal epithelium can display reactive changes, such as presence of nucleoli and enlargement of the nuclei, in the presence of inflammation.
- The combination of clinical findings with recurrence, formation of sinus tract, and presence of giant cells and squamous metaplasia on at smears allows the definitive diagnosis of subareolar abscess on FNAC.
- In duct ectasia the granular material with cellular debris, present in the lumen of the dilated ducts, is similar to necrosis, but without necrotic epithelial cells. If there is only amorphous material on the smears, without epithelial cells and inflammatory cells, we need to repeat the aspiration at the edges of the lesion to rule out the possibility of necrotic breast carcinoma.
- The absence of necrosis and foam cells and caseous necrosis, without any evidence of causal agents in smears with a granulomatous pattern, suggests the diagnosis of idiopathic granulomatous mastitis. This condition appears following pregnancy, and the classic histological picture is of a granulomatous inflammatory reaction around lobules.
- Diabetic mastopathy and sclerosing lymphocytic lobulitis are conditions frequently associated with insulin-dependent diabetes mellitus and other autoimmune disorders and produce variable clinical presentations. FNAC frequently gives an nonspecific picture, with lymphocytes and fibroblasts.
- Fat necrosis often mimics cancer clinically and on mammography. History of trauma is frequently found, and the cytological aspects are variable according to the stage of the lesion. Macrophages with vacuolated cytoplasm (foam cells) are uncommon in breast carcinomas and are the key for a correct diagnosis of fat necrosis. In addition, the presence of fat tissue associated with inflammatory cells is an important feature of the lesion.

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Fibrocystic Changes and Cysts

6

6.1 Clinical Findings

Fibrocystic changes (FCC) encompass a group of benign breast changes that are considered to represent normal, but exaggerated, hormonally mediated breast tissue responses. They are the most common benign breast conditions and are frequent causes of palpable breast lumps. More than one-third of women between the third and fifth decades of life show some evidence of FCC. Although FCC are frequently multifocal and bilateral, often the initial presentation is as a solitary lesion. The symptoms including premenstrual swelling, pain, and tenderness vary with the menstrual cycle. The risk of developing FCC appears to be increased in women who have disorders related to a preponderance of estrogenic hormones. The clinical manifestations are more prominent during the reproductive cycle, and in the absence of hormonal replacement therapy, the symptoms of FCC generally cease in the first 2 years postmenopause.

6.2 Radiologic Findings

Due to the heterogeneous nature of the FCC, there are no specific mammographic abnormalities for these lesions. The breast tissue may show increased density secondary to stromal fibrosis. Indeterminate calcifications may also be present, especially in cases of sclerosing adenosis. The cysts, which are better evaluated at ultrasound, show circumscribed margins, sharp anterior and posterior walls, and no internal echoes and posterior enhancement (Fig. 6.1). Some cysts are called complex and contain internal echoes reflecting the presence of debris within the cyst fluid. Lesions that do not exhibit all the characteristics of a simple cyst require further workup, commonly with FNAC in clinical practice.

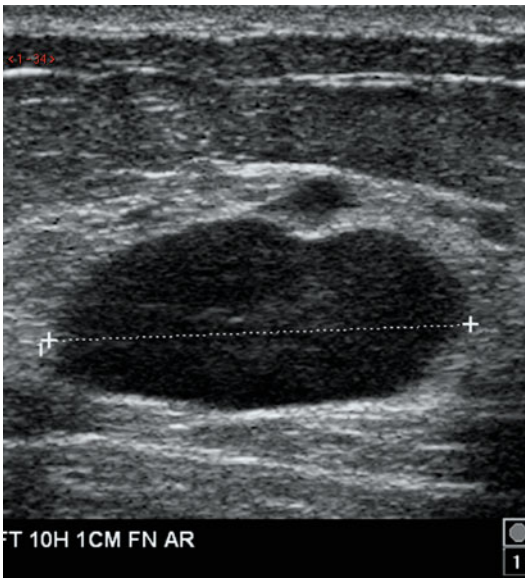

Fig. 6.1 Cystic lesion at ultrasound. Note the posterior enhancement of the ultrasound beam

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Table 6.1 Cytological findings in FCC

Predominant pattern	
Cystic	Solid
Low cellularity of epithelial cells	Moderate to high cellularity
Foam cells and apocrine metaplasia frequently present	Cohesive epithelial groups without or with mild nuclear overlapping and presence of myoepithelial cells
Fluid background	Heterogeneous cell population: mild variation in the size and shape of the nuclei (oval, round, or spindle)
Inflammatory cells can be present	Bipolar naked nuclei in the background
	Foam cells, apocrine metaplasia, and stromal fragments can be observed

6.3 Cytologic Findings

6.3.1 General Findings

Due to the heterogeneity of FCC, aspirates can yield smears with variable cellularity. In general, the clinical impression obtained during the aspiration may help to predict the type of cytologic material at microscopy. Ill-defined, sclerotic areas produce a scanty cellular smear, while areas with cysts can give a thick or liquid material. In FCC, there is a mixture of features including cysts, fibrosis, and epithelial proliferation. Areas of proliferation are highly cellular, areas of fibrosis are rubbery and yield few cells, and cysts yield fluid. Small regular ductal cells are seen in monolayer sheets with apocrine cells and bare myoepithelial nuclei. There may be thin proteinaceous fluid coating the slide. The nuclei of benign ductal cells have smooth nuclear membranes, fine and even chromatin, and inconspicuous nucleoli, and are 1.5–2 times bigger than the size of a red blood cell. The hallmark of a benign FNAC is the presence of bipolar myoepithelial nuclei. Myoepithelial nuclei are oval, the chromatin is evenly distributed, the nuclear membrane is smooth, the nuclei are devoid of nucleoli, and these cells are strongly positive for p63 (Reis-Filho et al. 2003). Despite the heterogeneity, some cytologic findings are characteristics of FCC and are summarized in Table 6.1.

6.3.2 Non-proliferative Lesions

6.3.2.1 General Considerations

In general, predominantly solid lesions show a variable moderate to high cellularity. Presence of stromal fragments, foamy histiocytes, apocrine cells, and clusters of ductal epithelial cells are common findings in non-proliferative FCC (Fig. 6.2). The epithelial ductal cells are usually arranged in monolayer clusters with a "honeycomb" pattern. Myoepithelial cells are disposed as isolated cells or overlapping with the epithelial clusters. Apocrine cells can be present, and these cells are polygonal with abundant, well-defined, finely granular cytoplasm and round nuclei, sometimes with prominent nucleoli. Apocrine cells are usually found arranged in cohesive monolayer sheets or as isolated, single cells. Occasionally, nuclear atypia – karyomegaly and irregularity of nuclear contours – can be seen in apocrine cells (Fig. 6.3). In these cases, correlation with the clinical and imaging findings helps in the correct diagnosis (Frost et al. 1997; Pogackik and Us-Krasovec 2004).

6.3.2.2 Cysts

Cysts are the most common lesions of the breast. The aspirated fluid from a breast cyst may be clear or turbid with variable color (yellowish, greenish, brownish). Such cysts have no epithelial lining, and the cytological smears are characterized by an amorphous,

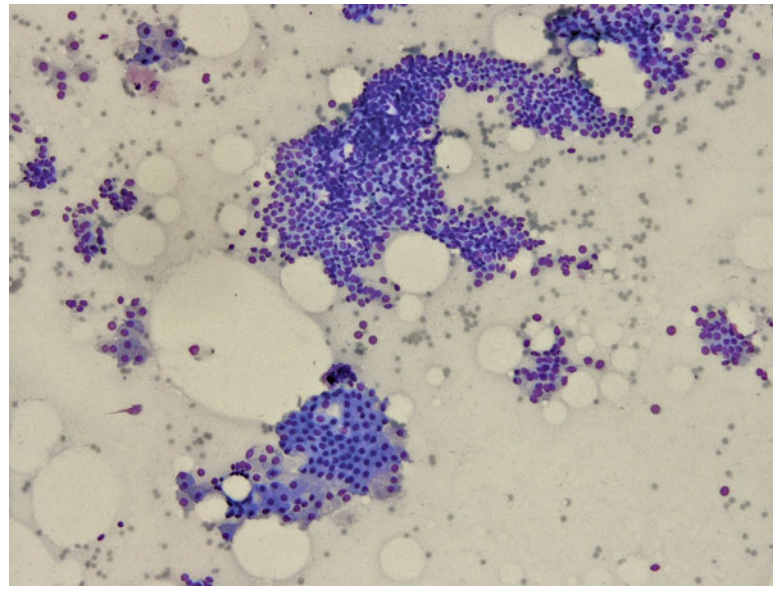

Fig. 6.2 Fibrocystic changes. Presence of apocrine cells and clusters of ductal epithelial cells are common findings in non-proliferative FCC (MGG stain)

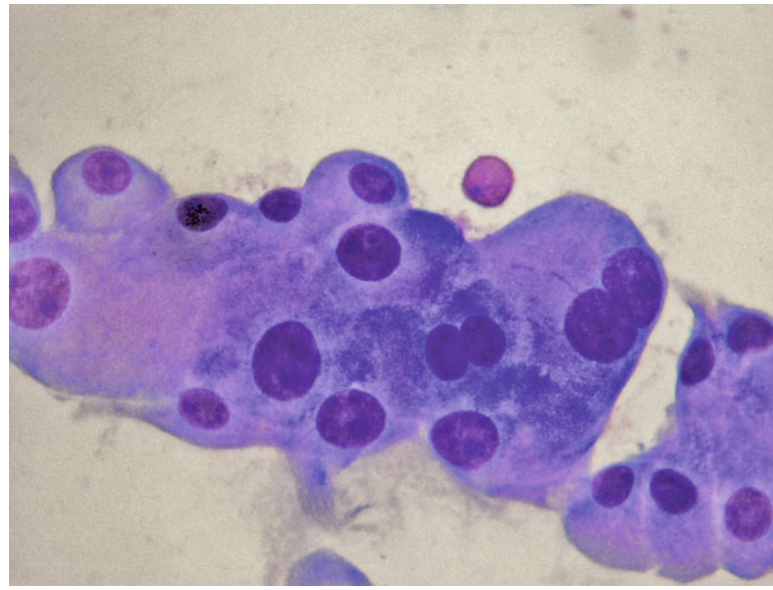

Fig. 6.3 Nuclear atypia in apocrine metaplastic cells (MGG stain)

proteinaceous material and variable amounts of foam cells. Some cystic lesions of the breast may be lined by epithelium with apocrine metaplasia (apocrine cysts). These cysts generally exhibit a thick content and variable amounts of apocrine cells that are isolated or are arranged in groups of varying sizes. Ductal epithelium can be present, usually in sparse numbers, and often

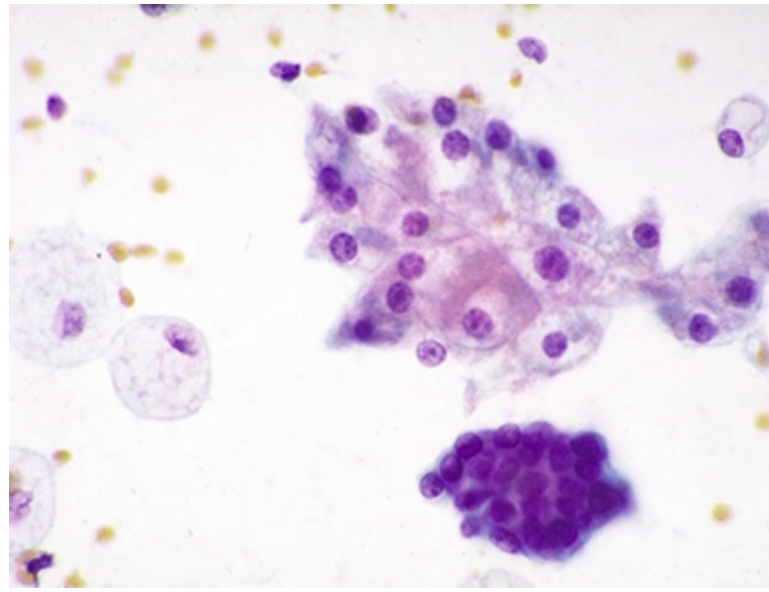

Fig. 6.4 Breast cyst. Note the presence of histiocytes, apocrine metaplasia, and a benign group of epithelial cells (MGG stain)

occur as flat sheets (Fig. 6.4). Three-dimensional clusters may raise the possibility of the presence of an intracystic papillary lesion and need further investigation.

6.3.3 Proliferative Lesions

6.3.3.1 General Considerations

This group includes epithelial proliferative lesions without atypia (adenosis, radial scar, usual and florid ductal epithelial hyperplasia) and epithelial proliferative lesions with atypia (ADH and low-grade DCIS). Columnar cell changes are also included in proliferative lesions. The cytologic features of these epithelial proliferative changes will be described in Chap. 9.

The distinction between epithelial proliferative lesions with and/or without atypia is clinically useful on cytology, because patients with atypical lesions may be referred to surgical biopsy for a definitive diagnosis, while those with lesions without atypia may be managed more conservatively (Zhao et al. 2009).

6.3.3.2 Adenosis Variants

Among the group of adenosis, sclerosing adenosis is probably one of the most frequently encountered whose cytologic features are described in Chap. 9. Among other adenosis lesions, microglandular adenosis is particularly difficult, sometimes impossible to distinguish from a low-grade carcinoma. The smears of these lesions show abundant cellularity, epithelial cells in small groups and cohesive three-dimensional elongated tubular arrangements, with the absence of myoepithelial cells both among the epithelial groups and in the background (Fig. 6.5). This characteristic, which is also present in histology, makes the differential diagnosis with tubular carcinoma very difficult. The regular honeycomb pattern of the epithelial cell groups of microglandular adenosis is probably the best clue for this differential diagnosis.

Radial scar/complex sclerosing lesions are one of the main causes of discrepancies in the preoperative workup of breast abnormalities with suspicious mammography findings and benign cytology. On many occasions, the emphasis is leaned more toward the clinical and imaging findings than the cytological findings, resulting in false-positive interpretations. Moreover, since epithelial hyperplasia and even in situ and invasive carcinomas can be associated with radial scars, they also paradoxically represent an important cause of false-negative results. For these reasons, many authors recommend surgical excision of the lesion for complete histological assessment. However, with

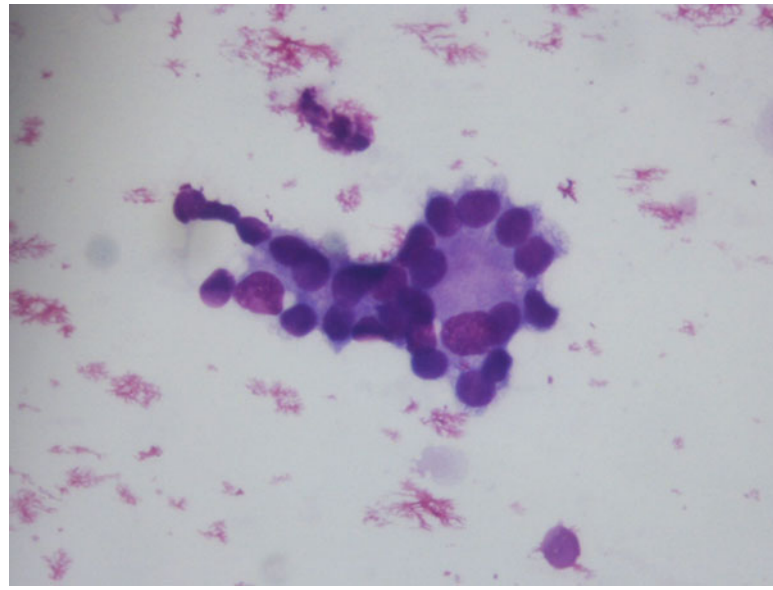

Fig. 6.5 Microglandular adenosis. Note tubular arrangement MGA (MGG stain)

the increasing use of mammographic screening and improvement of imaging facilities, these lesions are detected at an earlier stage and at smaller sizes, so that some clinicians may prefer follow-up rather than excision. Although difficult, the cytological diagnosis of radial scar/complex sclerosing lesions is possible in the context of the triple assessment. In cases where the diagnosis is possible, the smears show variable cellularity, composed of large and small epithelial groups, sometimes with nuclear crowding, tubular epithelial structures, apocrine cells, and bipolar naked nuclei. The presence of stromal fragments, partly as cell-poor elastoid fragments, can be observed. Single fibroblasts, macrophages, and mucoid material can also be seen. Although debatable, some authors (Field and Mak 2007) demonstrated that myoepithelial cells are present in most of the epithelial groups.

Cytologic diagnosis and classification of FCC with proliferative lesions can be very difficult because there is a significant overlap of cytological features among the different lesions, notably usual ductal hyperplasia, sclerosing and tubular adenosis, radical scar/complex sclerosing lesions, atypical hyperplasia, and even tumors such as papillomas and low-grade DCIS. Thus, in cases where the cytological features are not classical of FCC (such as hypercellularity or some loss of cohesiveness of the cells and some degree of cellular atypia), the most important diagnostic decision is to define if the cytological pattern is benign, indeterminate, or malignant instead of trying to allocate the cytologic preparation into a specific histologic diagnosis or pattern. In such situations, the definition of benign proliferative epithelial lesions (or proliferative FCC without atypia) and atypical proliferative epithelial lesions (or proliferative FCC with atypia) may be useful and conveys the appropriate clinical significance. In the former situation, the recommendation is clinical and radiologic follow-up, but in the latter situation, further investigation is necessary, using, for example, core needle biopsy or surgical biopsy.

6.4 Histologic Correlations

6.4.1 Gross Findings

The specimen generally shows fibrosis with blue-domed or clear cysts, most of which do not exceed 1–2 mm in size. The cysts are randomly distributed and can reach up to 2 cm in diameter. These changes are variable in extent but are usually multifocal and bilateral.

6.4.2 Histology: General Considerations

The histological spectrum of FCC is very broad and includes cyst formation, apocrine metaplasia, fibrosis of the stroma, adenosis and epithelial hyperplasia of various degrees. Based on the presence or not of epithelial proliferation, FCC can be subdivided into non-proliferative and proliferative categories.

6.4.2.1 Non-proliferative FCC

Non-proliferative FCC includes cysts, apocrine metaplasia, and fibrosis. Cysts are formed by dilated lobular units of the breast. Most cysts are microscopic, but they may coalesce to form macrocysts visible to imaging and palpation. These cysts are lined by flat or atrophic epithelium and contain fluid secretion, sometimes with a blue color. Frequently, the lining epithelium shows apocrine metaplasia, an alteration characterized by cells with abundant, granular, and acidophilic cytoplasm and round regular nuclei with prominent nucleoli. The apocrine cell layer may be flat or papillary (papillary apocrine changes). Fibrosis is characterized by the overgrowth of the interlobular and intralobular stroma that compresses ducts and lobules. Fibrosis is considered a secondary event to cyst rupture with release of the secretion into the adjacent stroma that stimulates chronic inflammation and fibroblastic proliferation.

6.4.2.2 Proliferative FCC

Proliferative FCC consists of a heterogeneous group of lesions accompanied by an increased number of acini, termed adenosis, or proliferation of the lining epithelium inside the ducts and acini, referred to as ductal epithelial hyperplasia.

Adenosis is characterized by an increase in the number of acini or ductules per lobular unit, resulting in increased size of the lobule. Adenosis appears as a physiological phenomenon during pregnancy and lactation, leading to diffuse increase of the breast lobules. In non-pregnant women, adenosis occurs as a focal change and represents about 25 % of all benign breast biopsies. Simple adenosis is the term used to describe the increased number of acini per lobule. Adenosis can also acquire special morphological characteristics constituting the variants: sclerosing adenosis, apocrine adenosis, blunt duct adenosis, microglandular adenosis, and nodular adenosis. Sclerosing adenosis arises in association with the terminal duct lobular unit, and this "lobulocentric" pattern is the key for the correct diagnosis. The glands and tubules are distorted by proliferating fibrosis of the intralobular stroma. Sclerosing adenosis may be confused with invasive carcinoma because it can induce architectural distortion and harbor a pseudoinfiltrative pattern. Myoepithelial cells and proliferating stroma can assume a spindle cell appearance and sometimes are more abundant than the glandular component. Immunohistochemistry for epithelial (cytokeratins) and myoepithelial markers (p63, calponin, actin) shows both epithelial and myoepithelial components of the lesion, confirming its benign nature. Apocrine adenosis is a form of adenosis whose cells undergo apocrine metaplasia. When there is significant atypia, differential diagnosis with apocrine type of DCIS is difficult. In blunt duct adenosis, the ductules branch out and expand causing increased size of the lobules. Ductules or acini when lined by single layer of columnar epithelium. Luminal epithelium can have mildly enlarged nuclei but without atypia, are currently included in the group of lesion, called columnar cell changes (see Chap. 9). Microglandular adenosis is a rare and very special form of adenosis. It is formed by small round ductules without myoepithelial lining, which are arranged in a disorderly fashion, diffusely infiltrating stroma and adipose tissue. This pattern simulates invasive tubular carcinoma. In microglandular adenosis, there are acidophilic secretions in the lumens of the tubules. Intact basement membranes (more evident in the reticulin staining, PAS, and immunohistochemistry for laminin and collagen IV), no atypia (round nuclei without prominent nucleoli), and vacuolated and clear cytoplasm of the epithelial cells can be observed. The tubular structures of tubular carcinoma are more irregular and angled, often associated with a component of DCIS. The stroma of tubular carcinoma is usually denser

and desmoplastic. Genetic alterations are present in some cases of microglandular adenosis, suggesting that this lesion can be a nonobligatory precursor of triple-negative carcinomas. The confluence of areas of adenosis in any of the subtypes can produce a well-defined nodule, referred to as nodular or tumoral adenosis. Radial scar is a benign sclerosing breast lesion characterized by a central fibroelastotic core with radiating ducts and lobules containing various proliferative changes and cysts. The ducts always have an outer myoepithelial cell layer that can be highlighted by myoepithelial cell markers such as p63, calponin, and actin, which are useful adjunctive markers in challenging cases where the differential diagnosis with invasive tubular carcinoma is posed. The term of complex sclerosing lesion is used if these lesions are larger than 1 cm, and sometimes they mimic invasive carcinomas clinically and mammographically.

Epithelial hyperplasias are characterized by the proliferation of epithelial cells in breast ducts or ductules. In general, they do not produce a palpable mass or macroscopic lesion and are diagnosed incidentally in biopsies from other lesions detected by mammography or are seen adjacent to carcinomas. Ductal epithelial hyperplasia is defined as an increase in the number of cells above the basement membrane. The ducts and ductules/acini are normally lined by two layers of cells, luminal and myoepithelial in contact with the basement membrane. Hyperplasia is characterized when there are three or more cells lining a duct or ductular space. The lesion does not, therefore, increase the number of units or terminal ductlobular acini, which, as already mentioned, is defined as adenosis. The ductal hyperplasias are divided into two major groups: without atypia or usual, and atypical. Ductal hyperplasia without atypia is designated as usual ductal hyperplasia (UDH) in the WHO classification (2012) and includes the mild usual ductal hyperplasia and moderate/florid ductal hyperplasia without atypia in the classification proposed by Page and Anderson (Page and Anderson 1987). Mild usual ductal hyperplasia is the mildest form of hyperplasia, characterized by the presence of three to four cell layers above the basement membrane. It is a very common lesion without an increased risk of developing breast cancer and clinical significance, and may be omitted in the pathology reports. In moderate/florid usual ductal hyperplasia, cells proliferate in three to four layers and they tend to fill and distend the ducts involved by forming bridges and peripheral irregular slits of different shapes and sizes. The lesion has varied architectural and cellular patterns, containing epithelial cells, myoepithelial, and sometimes cells with apocrine metaplasia. The cell orientation varies, sometimes forming curls, arches, or bridges in which the cells are positioned in parallel along the long axis or irregularly. The cell borders are not evident, and the cells may have oval, rounded or elongated nuclei with thin, evenly distributed chromatin. Mitoses are occasional. Moderate and florid UDH may be associated with a 1.5–2 times risk of developing cancer. Molecular changes (loss of expression of TGFb-RII and the estrogen receptor) are found in usual hyperplasia without atypia and may be related to proliferative activity, which predisposes to cancer development. Ductal hyperplasia with atypia is characterized by the proliferation of monomorphic cells with regular distribution, forming regular secondary lumens which are rounded and uniform. The lesions are small; the cells partially involve two or more ducts measuring less than 2 mm. It is a diagnosis of exclusion that should be considered when defining elements of low-grade DCIS are present, but incompletely. The criteria that define low-grade DCIS are (1) uniform population of cells, (2) geometrically regular spaces between the cells or rigid micropapillary formation, and (3) hyperchromatic nuclei. For the diagnosis of ADH, the lesions should contain one of the first two criteria, or have both, but not completely surround or involve two ducts, or measures less than 2 mm. Hyperchromasia contributes to the diagnosis, but is neither specific nor sufficient. The architectural patterns recognized in the ADH are similar to those of low-grade DCIS: cribriform, micropapillary, solid, and mixed. ADH and columnar cell changes will be discussed in detail on Chap. 9.

6.5 Summary

- Proliferative and non-proliferative FCC may present as palpable or non-palpable masses and are frequently sampled by FNA.
- Non-proliferative FCC and FCC with mild epithelial hyperplasia are not a risk for subsequent cancer development, whereas FCC with moderate/florid epithelial hyperplasia have a slightly increased relative.
- Even histology is difficult in establishing the boundaries between ADH and low-grade DCIS, and in cytology, these lesions are grouped under the category of proliferative lesions with atypia.
- Sclerosing adenosis (found in association of FCC) may have overlapping cytological findings with usual ductal hyperplasia.
- Radial scar/complex sclerosing lesion is one of the more frequent causes of discrepancy between the radiological image (suspicious/malignant) and cytological diagnosis (benign) in the preoperative workup of breast lesions; however, in many cases it is possible to characterize the benign nature of these lesions on cytology.
- Since reliable identification and subclassification of FCC with proliferative lesions on cytology can be very difficult due to the overlap of cytological features among the different lesions, it is more important to define the cytological pattern as benign, indeterminate, or malignant instead of giving a specific "histological" diagnosis. In our practice, we use the terms benign proliferative epithelial lesion (or proliferative FCC without atypia) and atypical proliferative epithelial lesion (or proliferative FCC with atypia). In the first group, follow-up is recommended, and in the second group, further investigation is necessary, using, for example, core needle biopsy or surgical biopsy.

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Fibroadenoma

7

7.1 Clinical and Epidemiological Findings

The fibroadenoma is the most common benign breast neoplasm that usually occurs in young women of child-bearing age, though it can occur at any age. It often presents as a localized tumor which can be clinically palpable as a mobile rounded and rubbery lump, but asymptomatic lesion has been detected by mammography where it is seen as a well-defined mass.

7.2 Cytologic Findings

Aspirates from the fibroadenoma are usually cellular with antler- or staghorn-shaped epithelial clusters and honeycomb monolayered sheets, set within a clean background with many naked bipolar nuclei, giving an appearance of "sesame seeds strewn among epithelial fragments" (Fig. 7.1). The bimorphic or bimodal epithelial clusters feature ductal epithelial cells with generally bland and banal vesicular nuclei with smooth nuclear contours, often accompanied by a second population of myoepithelial cells with their angular small dark nuclei interspersed among the ductal epithelial cells or inconspicuously punctuating the periphery of epithelial sheets (Fig. 7.2). Presence of usual ductal hyperplasia within the fibroadenoma can lead to the presence of larger branched proliferative epithelial aggregates in the aspirates (Fig. 7.3a, b). Apart from bipolar nuclei in the background, there are scattered stromal clumps which can be associated with myxoid material. These fibromyxoid stromal clumps are usually paucicellular with a few spindled nuclei and sometimes occur in proximity to the epithelial clusters (Fig. 7.4).

FNAC of the cellular and juvenile fibroadenoma shows similar cytological features as the conventional fibroadenoma, with the possibility of an increased population of stromal cells (Fig. 7.5). The fibroadenoma is the most common cause of a false-positive diagnosis in FNAC. The reasons for this include the frequent presence of occasional isolated intact cells with dissociation, epithelial nuclear atypia, and high cellularity. Apocrine metaplasia, multinucleation, and paucicellularity in hyalinized fibroadenomas are additional pitfalls (Kollur and El Hag 2006). Pregnancy and lactation, infarction, and prominent myxoid stroma in a fibroadenoma can aggravate worrisome cytological findings. Pregnancy or lactational atypia when superimposed on the cellularity of fibroadenoma aspirates can lead to a troublesome cytologic appearance with a potential false-positive result (Novotny et al. 1991). It has been observed that the majority of aspirates with cytologic features suggesting fibroadenomas but harboring atypia are often correlated with benign fibroadenomas on histology (Simsir et al. 2001). FNAC of fibroadenomas accounted for the majority of benign lesions in proliferative breast

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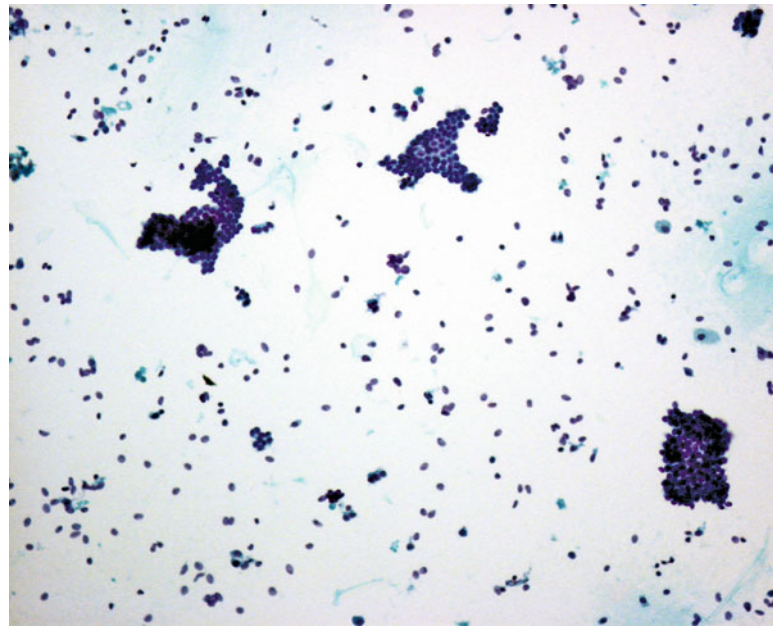

Fig. 7.1 Fibroadenoma. Aspirates are cellular with antler- or staghorn-shaped epithelial clusters in monolayered sheets, set within a clean background with many naked bipolar nuclei

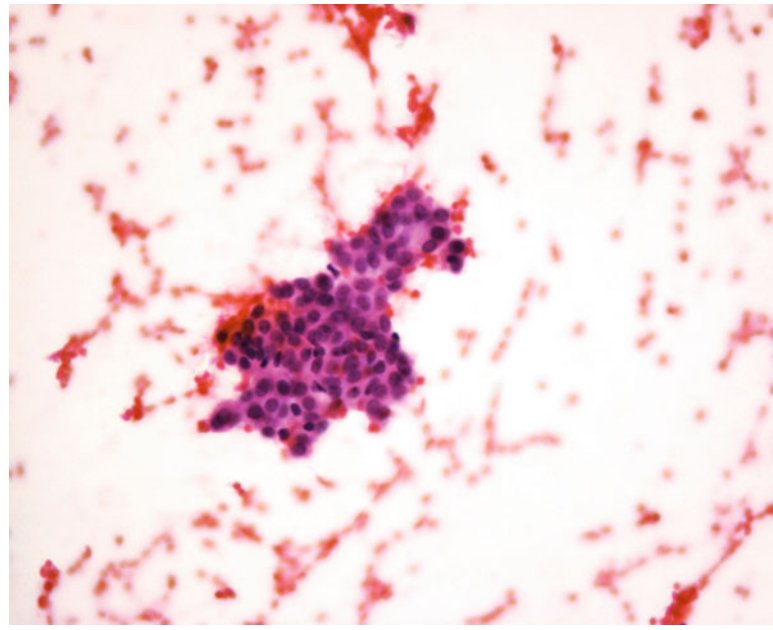

Fig. 7.2 Fibroadenoma. A closer view of the bimodal epithelial aggregate with small dark nuclei of myoepithelial cells at the periphery of the cluster, as well as intermixed among the ductal epithelial cells

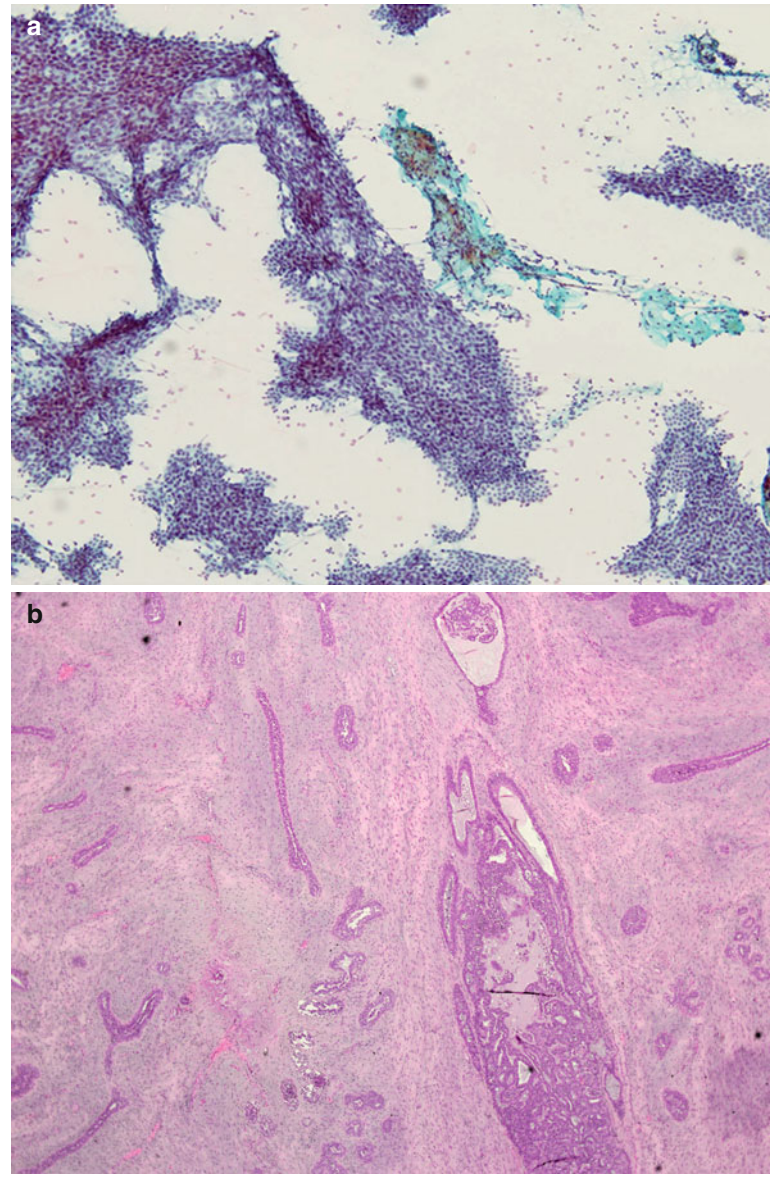

Fig. 7.3 (**a**, **b**) Fibroadenoma with usual ductal hyperplasia. (**a**) Large and branched cohesive epithelial sheets are seen in a clean background containing a few stromal clumps. (**b**) Corresponding histology shows a mixed intra- and pericanalicular growth pattern with the epithelial component demonstrating usual ductal hyperplasia

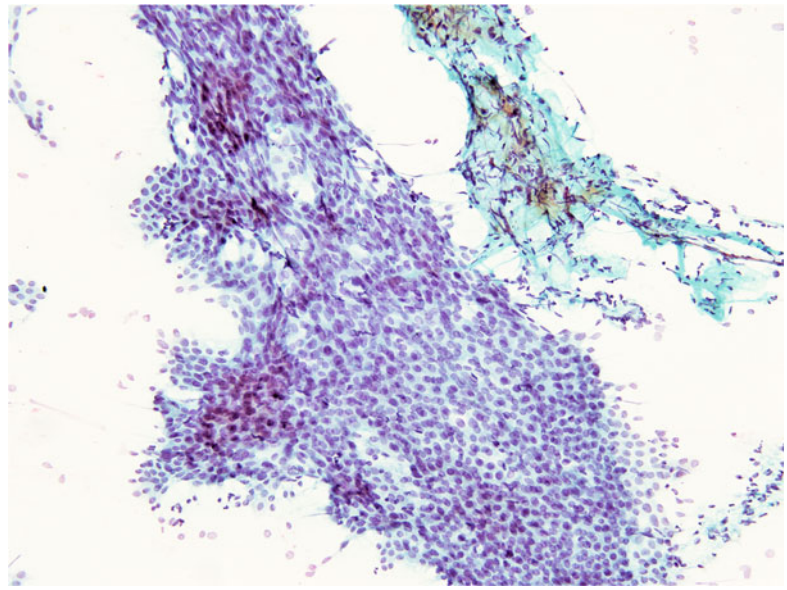

Fig. 7.4 Fibroadenoma with usual ductal hyperplasia. Large bimodal epithelial aggregate shows ductal epithelial cells admixed with darker nuclei of myoepithelial cells. A loose myxoid stromal clump with elongated nuclei is noted adjacent to the epithelial cluster

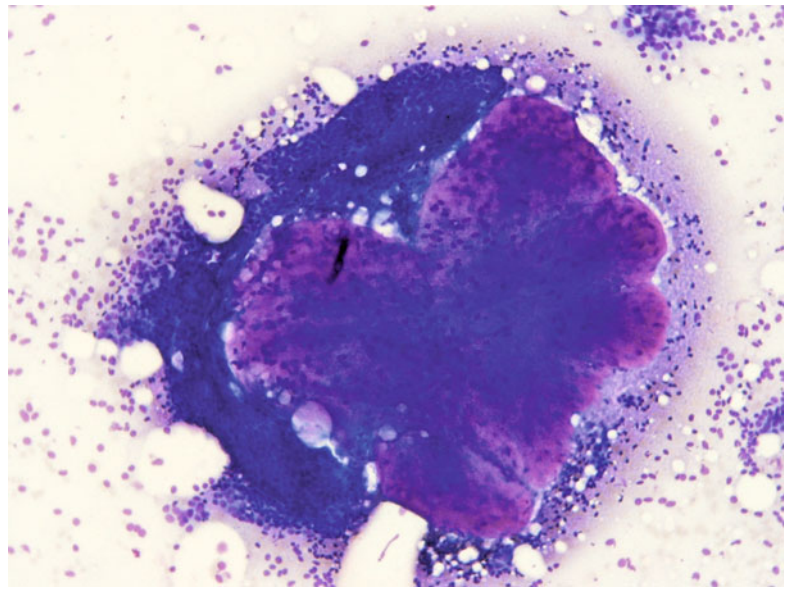

Fig. 7.5 Cellular fibroadenoma. Diff-Quik smear shows cohesive epithelial clusters accompanied by more cellular myxoid stromal clumps

lesions with and without atypia (Zhao et al. 2009). Nevertheless, caution must be exercised in this equivocal or atypical category as there are also cancers that can mimic fibroadenomas on cytology, leading to false-negative diagnoses. The role of the triple approach has to be underlined.

7.3 Differential Diagnosis

7.3.1 Fibrocystic Changes

While fibrocystic change can usually be readily distinguished from fibroadenoma by the lesser cellularity and presence of foam and apocrine cells, occasional aspirate yields from fibroadenoma can show similar appearances. Presence of fibromyxoid stroma, staghorn-shaped epithelial clusters, and higher cellularity are considered key cytologic criteria to separate fibroadenoma from fibrocystic change (Bottles et al. 1988).

7.3.2 Pseudoangiomatous Stromal Hyperplasia (PASH)

PASH is often encountered incidentally in breast biopsies for other conditions, but it may also present as a breast mass in some women. FNAC of PASH can be moderately cellular with clusters of bland epithelial cells that can be branched and staghorn-like, in a background of single naked nuclei as well as spindle cells. Loose hypocellular stromal fragments can be identified, and these cytologic features are similar to those found in fibroadenoma (Ng et al. 2003).

7.3.3 Mucocele-Like Lesions

Aspirates from a myxoid fibroadenoma can mimic a mucocele-like lesion due to the presence of mucoid material in the background of the smears. The myxoid fibroadenoma demonstrates greater cellularity than the benign mucocele-like lesion, and it is reported that the mucoid material of myxoid fibroadenoma shows a brighter pink coloration than the magenta hue of the mucocele-like lesion (Yeoh et al. 1999).

7.3.4 Phyllodes Tumors

Phyllodes tumor is a fibroepithelial neoplasm that is closely related to fibroadenoma. Cytological distinction can be particularly problematic since both lesions possess overlapping characteristics. In particular, aspirates from cellular fibroadenomas are both cytologically and histologically difficult to distinguish from the benign phyllodes tumor. Fibromyxoid stromal clumps that are cellular and contain spindled nuclei, fibroblastic pavements (El Hag et al. 2010), reduced epithelial-stromal ratio (Tse et al. 2002), larger epithelial clusters with wavy or folded shapes (Shimizu and Korematsu 2002), and stromal cytologic atypia (Scolyer et al. 2001) favor phyllodes tumor. One study has advocated that recognition of long spindled nuclei in more than 30 % of the dispersed stromal cell population is the most reliable feature to favor phyllodes tumor over fibroadenoma (Krishnamurthy et al. 2000). While multinucleated giant cells have been described in fibroadenomas as well, it has been reported that these cells are found more frequently in phyllodes tumors (Tse et al. 2002; Simi et al. 1988).

7.3.5 Hamartoma

As compared with the fibroadenoma, aspirate of the breast hamartoma is less cellular and shows cytologically intact lobular units with lack of stromal elements (Herbert et al. 2006). Distinction is often possible radiologically.

7.3.6 Tubular Adenoma

Cytological features of tubular adenoma resemble the fibroadenoma. More specific appearances of tubular adenoma are three-dimensional cohesive epithelial balls and tubular aggregates in a cellular background, with relative lack of

staghorn epithelial clusters (Kumar et al. 1998). Epithelial cells are uniform. Intracytoplasmic magenta granules (observed in Giemsa smears), straight tubules, and closely placed acini have been described as findings that are not seen in aspirates from fibroadenomas (Shet and Rege 1998).

7.3.7 Adenomyoepithelioma

The adenomyoepithelioma of the breast is a neoplasm that consists of a biphasic proliferation of both ductal epithelial and myoepithelial components. FNAC of this lesion is often moderate to highly cellular with clusters of both epithelial and myoepithelial cells. Myoepithelial cells are also seen as naked bipolar nuclei. Intranuclear and intracytoplasmic vacuoles have been described in the myoepithelial cell population (Iyengar et al. 2006). Cytologic recognition of adenomyoepithelioma is difficult, and the lesion may be initially diagnosed as fibroadenoma or other lesions including cancer. Differences from fibroadenoma include the higher volume of dispersed myoepithelial cells as well as epithelioid myoepithelial cells with discernible nucleoli (Iyengar et al. 2006; Loh et al. 2004).

7.3.8 Pleomorphic Adenoma

Pleomorphic adenoma is a very rare neoplasm in the breast, sharing similarities to the adenomyoepithelioma. Cytological features of this tumor are difficult to recognize and may resemble the fibroadenoma (Iyengar et al. 2005). Myxoid and squamous material may be present.

7.3.9 Papillary Lesions

FNAC of papillary lesions can mimic the fibroadenoma (Simsir et al. 2003; Michael and Buschmann 2002). The intraduct papilloma is characterized by epithelial aggregates with broad ruffled branching and scalloped contours, smaller tonguelike projections, and columnar cells. Fibrovascular cores when identified are helpful in corroborating a papillary lesion. Myoepithelial cells tend to be fewer than in the fibroadenoma (Michael and Buschmann 2002).

7.3.10 Carcinoma

Some breast cancers can be underdiagnosed as fibroadenomas on FNAC. These may occur in young women where there often is a hesitation to make an outright diagnosis of cancer on aspirate smears unless cytologic changes are overt, and in pregnant and lactating women where presence of atypia can be erroneously attributed to physiological alterations. It is prudent to recommend histological confirmation when cytologic atypia is discovered, in order not to overlook a cancer (Maygarden et al. 1991).

Carcinomas with osteoclastic giant cells can mimic fibroadenoma on cytology (Jogai et al. 2004). Mucinous carcinoma with the background mucinous material can be difficult to distinguish from myxoid fibroadenoma. The tubular cancer can also be mistaken for fibroadenoma with its low-grade features and presence of angular epithelial groups. Careful scrutiny of isolated or dispersed cells in an aspirate can assist in correct classification of malignant disease (Benoit et al. 1992), in conjunction with the triple approach.

Presence of carcinoma within a fibroadenoma can lead to potentially confusing cytological appearances, with pleomorphic abnormal cells of the malignant component superimposed on a background population of bland epithelial cells derived from the fibroadenoma (Psarianos et al. 1998).

7.4 Histologic Correlations

Macroscopically circumscribed, encapsulated, and lobulated, the fibroadenoma shows two main histological patterns of intracanalicular and pericanalicular growth which are without clinical significance. The admixture of epithelial and stromal elements gives rise to the cytological appearances of epithelial aggregates within the

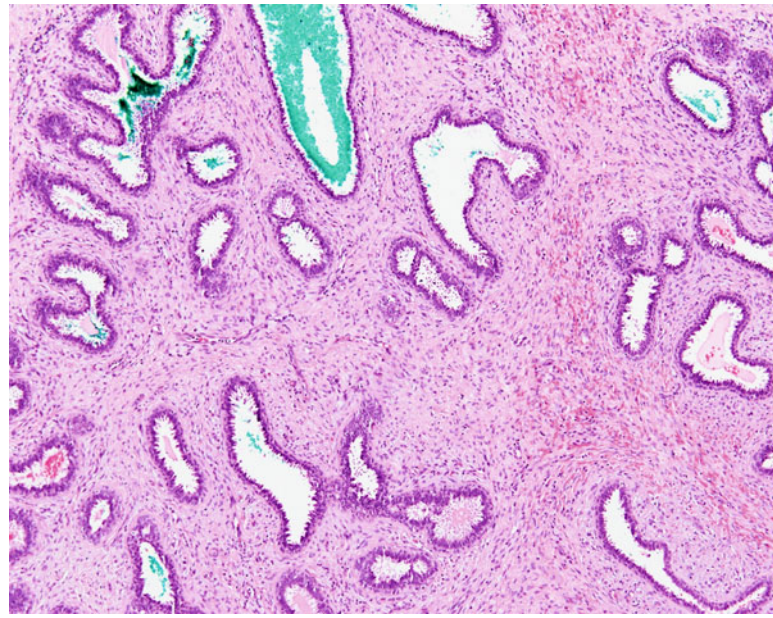

Fig. 7.6 Cellular fibroadenoma. Cellular stromal component intermixed with epithelial elements

background of scattered naked nuclei of bipolar myoepithelial and stromal cells. Presence of usual ductal hyperplasia and fibrocystic changes within the fibroadenoma leads to more complex and branched epithelial fragments, histiocytes, columnar cells, and apocrine cells on cytology.

Histological variants include the cellular fibroadenoma which discloses increased stromal cells (Fig. 7.6); the juvenile fibroadenoma which is usually diagnosed in adolescents and are characterized by increased stromal cellularity with usual epithelial hyperplasia; and the complex fibroadenoma that contains cysts larger than 3 mm, sclerosing adenosis, epithelial calcifications, or papillary apocrine hyperplasia.

7.5 Management

A definitive FNAC diagnosis of fibroadenoma, when concordant with clinicoradiological features, can be observed nonoperatively. In the presence of any doubt or discordance, histological confirmation must be pursued. For some institutions, a large lesional size despite a benign fibroadenomatous cytological conclusion will prompt excision through surgical or mammotome approaches in view of inherent sampling issues and possibility of phyllodes tumor for larger masses. When atypia is observed in an aspirate that is otherwise in keeping with a fibroadenoma, there ought to be a discussion to determine if close surveillance or excision is required. In certain situations when both clinical and radiological opinions are benign and the cytologic atypia is mild and focal, the option for follow-up and later review may be acceptable. However, if atypia is more worrisome or if there is clinicoradiological concern, excision is advised.

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Other Fibroepithelial Lesions

8

While fibroadenomas constitute most of the fibroepithelial lesions of the breast as seen in routine clinical and pathological practice, other fibroepithelial lesions, albeit less common, can also be encountered in the routine clinical pathology practice, and recognizing these other fibroepithelial lesions is important, as some of these may potentially behave in a biologically aggressive manner. Accurate preoperative identification of these is important to allow for proper treatment. The lesions so included are phyllodes tumors, hamartoma, and pseudoangiomatous stromal hyperplasia.

8.1 Phyllodes Tumors

8.1.1 Cytologic Grading in Phyllodes Tumors

In the cytology of phyllodes tumor, the main concern is the differentiation of phyllodes tumors from the much more common fibroadenoma. The cytologic criteria have been investigated fairly extensively, and these are discussed in Chap. 7. The other role that cytology can play in phyllodes tumor is its grading. The cytologic grading of phyllodes tumor is fraught with uncertainty. In general, it is accepted that the cytologic changes from benign to malignant phyllodes tumors are subtle and gradual, and discrete categorization of the lesions into these diagnostic labels is not possible. Given the fact that the grading of phyllodes tumors is in a continuum, even based on histologic criteria, it is not surprising that the differentiation relying on the less concrete cytologic criteria is even more difficult. Very few reports in the literature have evaluated the usefulness of different cytologic criteria, and overall, no clear-cut conclusion could be drawn from these reports. In these studies, the same criteria had been used as in the histologic diagnosis of phyllodes tumors and their differentiation from fibroadenomas. The presence and characteristics of the stromal fragments are one of the mainstays of the differentiating features. It was reported that benign phyllodes tumors tend to show stromal fragments of low to moderate cellularity (Figs. 8.1a, b), whereas borderline to malignant phyllodes tumors show moderate to high stromal cellularity (Bhattarai et al. 2000) (Figs. 8.2a, b and 8.3a, b), even though other authors reported high stromal cellularity irrespective of the grade of the phyllodes tumors (Shabb 1997). In addition, it was further reported that pleomorphism of the stromal cells was minimal in benign phyllodes tumors but more prominent in malignant examples. Single cells and mitotic figures were not seen in benign phyllodes tumors but were present in malignant phyllodes tumors (Bhattarai et al. 2000; Shabb 1997) (Fig. 8.4). However, the stromal nuclear size had not been found to be different among different grades of phyllodes tumors (Shabb 1997). In addition, in malignant phyllodes tumors, the stromal fragments tended to be large and show trabecular, anastomosing, and arborizing patterns (Jayaram and Sthaneshwar 2002).

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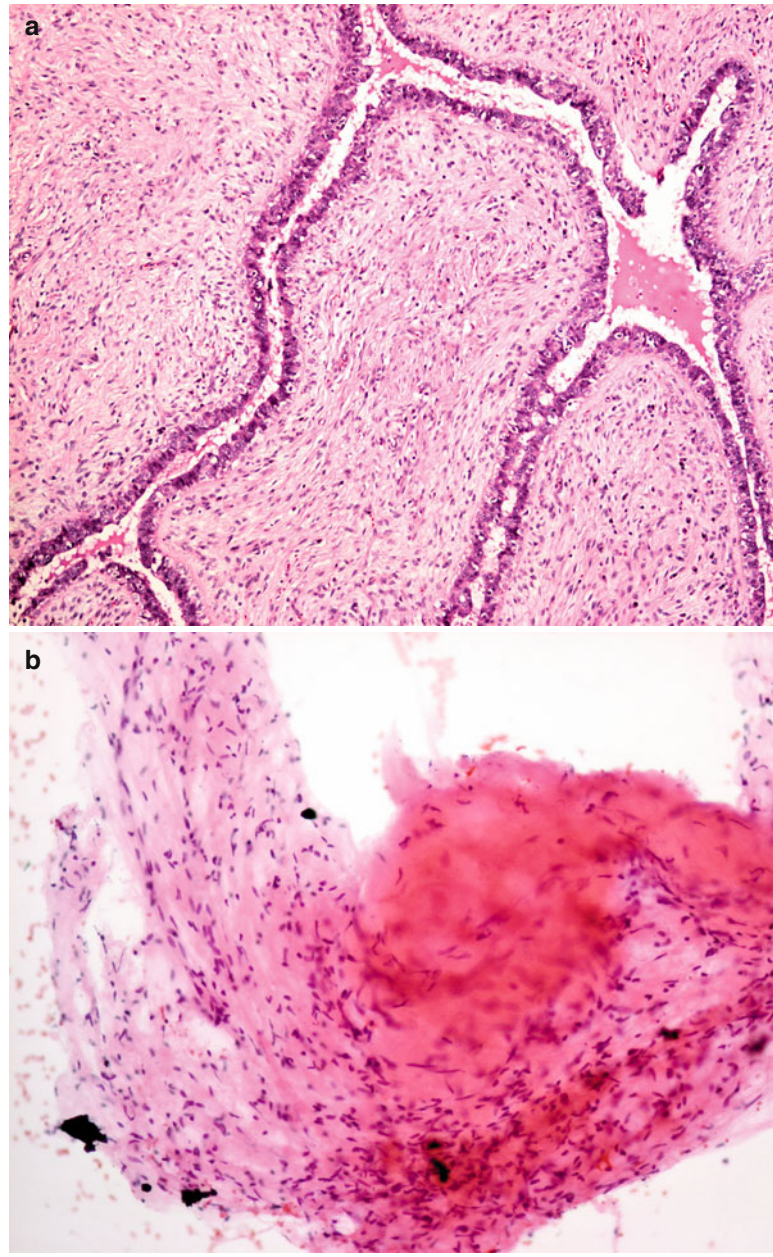


Fig. 8.1 (**a**, **b**) Benign phyllodes tumor. (**a**) Histology shows mild stromal hyper cellularity. (**b**) Cellular stromal fragments are of low to moderate cellularity on cytology

The other frequently used defining criteria were the presence of atypical single cells in the background. In benign phyllodes tumors, such single cells were present in much less quantity, devoid of atypia and mitotic activity, whereas in malignant phyllodes tumors, these background single cells were more numerous, with visible atypia and mitotic activity (Bhattarai et al. 2000; Shabb 1997; Jayaram and Sthaneshwar 2002) (Fig. 8.5). Some authors reported the use of p53

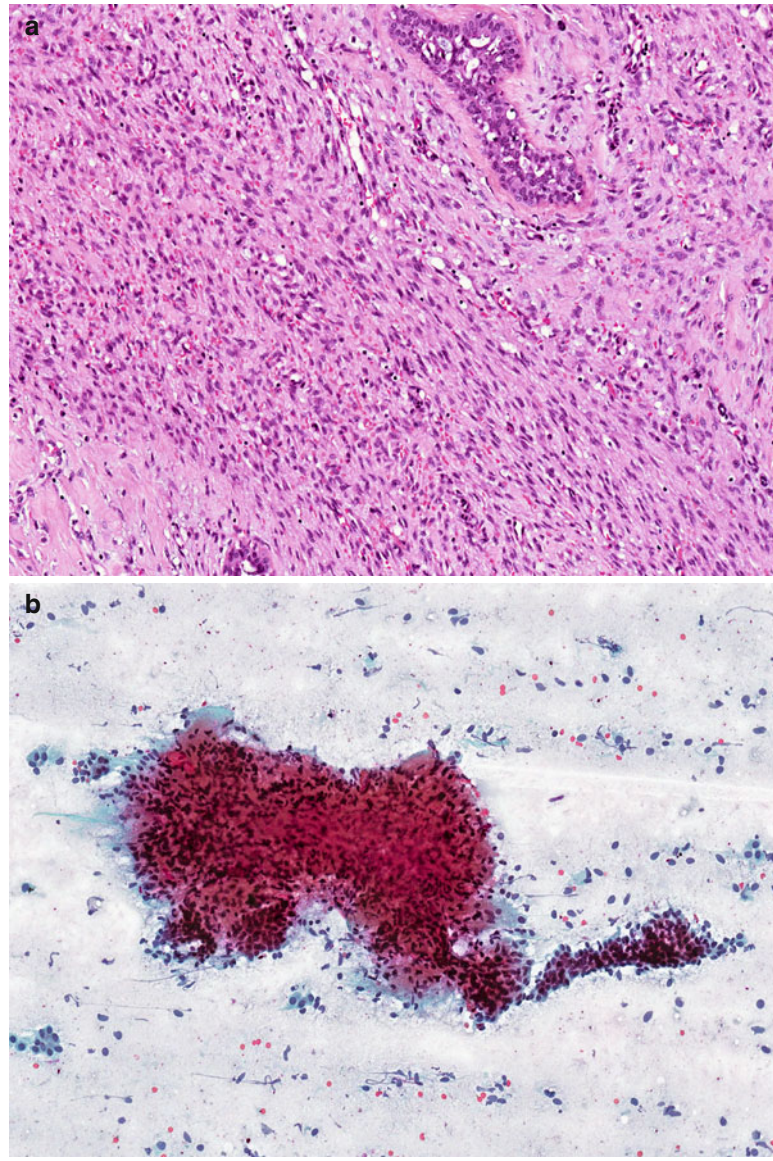


Fig. 8.2 (**a**) Phyllodes tumor of borderline malignancy. Cellular stroma with mild to moderate stromal atypia and scattered mitoses. (**b**) Phyllodes tumor. Cellular stromal clump is closely associated with an epithelial cell aggregate

staining to be useful, with borderline to malignant phyllodes tumors showing up to 55 % staining, whereas benign phyllodes tumors did not show any staining (Shabalova et al. 1997); however, this observation was made on a small series of phyllodes tumors and has not been confirmed nor widely accepted. Another cytologic parameter that had been reported includes the presence of apocrine metaplastic cells, which were present in all grades of phyllodes tumors, but in greater numbers in benign phyllodes. This again requires further experience to confirm its usefulness (Jayaram and Sthaneshwar 2002).

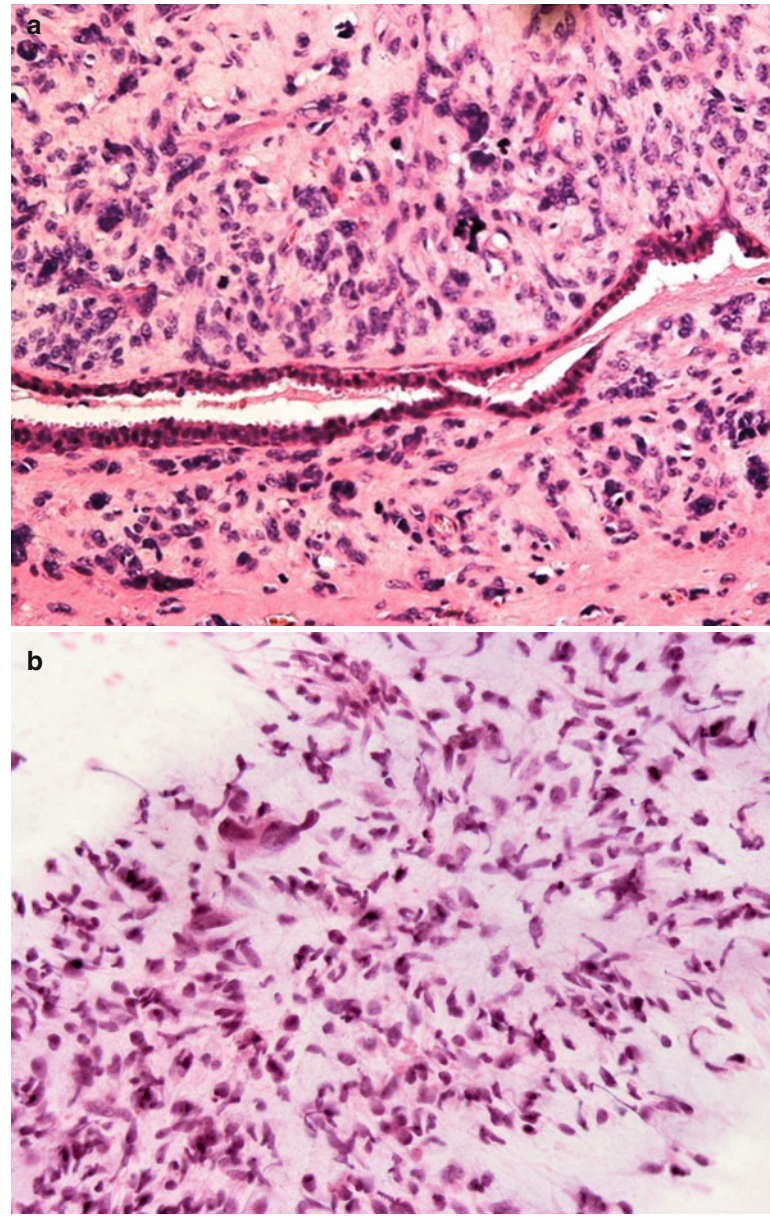


Fig. 8.3 (**a**, **b**) Malignant phyllodes tumor. (**a**) Stroma is of high cellularity and prominent pleomorphism. (**b**) Stromal fragments are of high cellularity and prominent pleomorphism on cytology

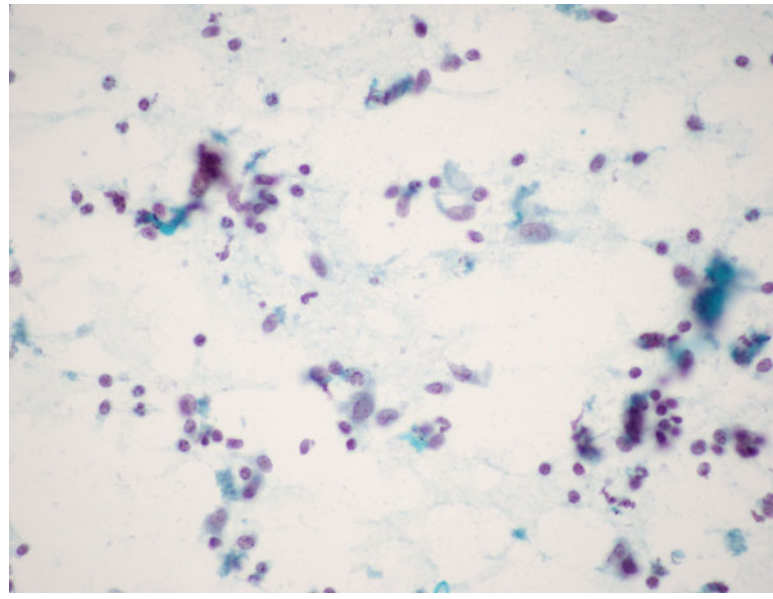


Fig. 8.4 High magnification of the plump and spindled cells, some of which show elongated cytoplasmic extensions in a smear of a malignant phyllodes tumor

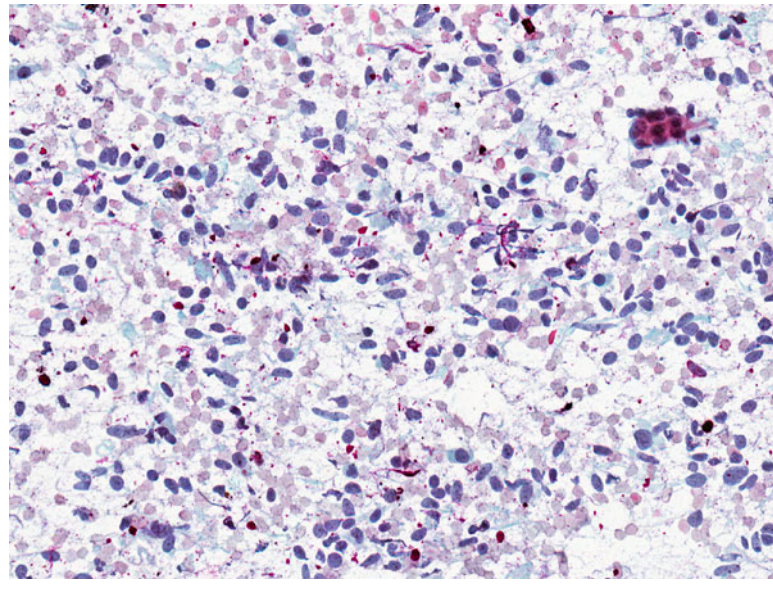


Fig. 8.5 Phyllodes tumor. Dispersed cells show both spindle and ovoid nuclei with variability in nuclear sizes

8.2 Hamartoma

8.2.1 Clinical and Epidemiological Findings

Hamartomas are thought to comprise about 5 % of all benign breast tumors, and they occur mostly in women in their 40s but can be found at any age. Hamartomas are usually well circumscribed and comprise variable amounts of admixed benign epithelial elements, fibrous tissue, and fat. It may show characteristic radiological features, but as it is composed of an admixture of normal breast tissue components, they are under-recognized pathologically, particularly in small core needle biopsies or FNAC (Tse et al. 2002). The cytologic features of hamartomas are not widely described.

8.2.2 Cytologic Findings

The cytologic features of hamartoma usually show mild to moderate cellularity, composed of benign ductal and epithelial cells arranged in branching sheets. Many lobules are also seen. All these epithelial elements are seen in close proximity to adipose tissue, fibrous tissue, or even skeletal muscle (Fig. 8.6a, b). These can be present in up to 55 % of the cases (Gomez-Aracil et al. 2003). In the majority of the cases, bare nuclei can also be noted in the background, but the amount is usually scanty. The fibrotic stromal fragments, when present, are less prominent compared to other fibroepithelial lesions (Herbert et al. 2003). Occasionally, the presence of apocrine cells has been reported in a small proportion of cases.

8.2.3 Differential Diagnosis

The cytologic differential diagnosis is with other fibroepithelial lesions, notably fibroadenomas. While the basic cytologic pattern is very similar for both entities, with usually moderate cellularity, the presence of ducts, bipolar nuclei, and stromal/fibrous tissue fragments, there exist subtle differences. In hamartomas, the aspirates tend to show relatively less or a paucity of stromal fragments compared to fibroadenomas, and also aspirates of hamartomas may show more intact lobules, which are absent in fibroadenomas (Singh and Nawaz 1998; Herbert et al. 2006).

8.2.4 Management

A cytologic diagnosis of hamartoma is rarely made, as most cases would be diagnosed as fibroadenoma. The biologic behavior of hamartomas is totally benign, and excision may be considered in selected cases.

8.3 Pseudoangiomatous Stromal Hyperplasia (PASH)

8.3.1 Clinical and Epidemiological Findings

PASH can affect patients of all ages, but more commonly occurs in premenopausal women. This can also occur in men, associated with gynecomastia. Smaller lesions may be incidental, whereas larger nodules can present as a breast lump, which is usually solitary, firm, well circumscribed, and freely mobile, resembling a fibroadenoma. Pathologically, PASH can be nodular or can be seen as a component occurring in other lesions (fibroadenoma, phyllodes tumor, hamartoma) (Virk and Khan 2010).

8.3.2 Cytologic Findings

Cytologic preparations of PASH typically show low to moderate cellularity, with some authors reporting a relatively lower cellularity than fibroadenomas (Vicandi et al. 1998; Lui et al. 2004). The epithelial clusters are variable, and they are usually solid and without branching (Lui et al. 2004) or only with very rare branching

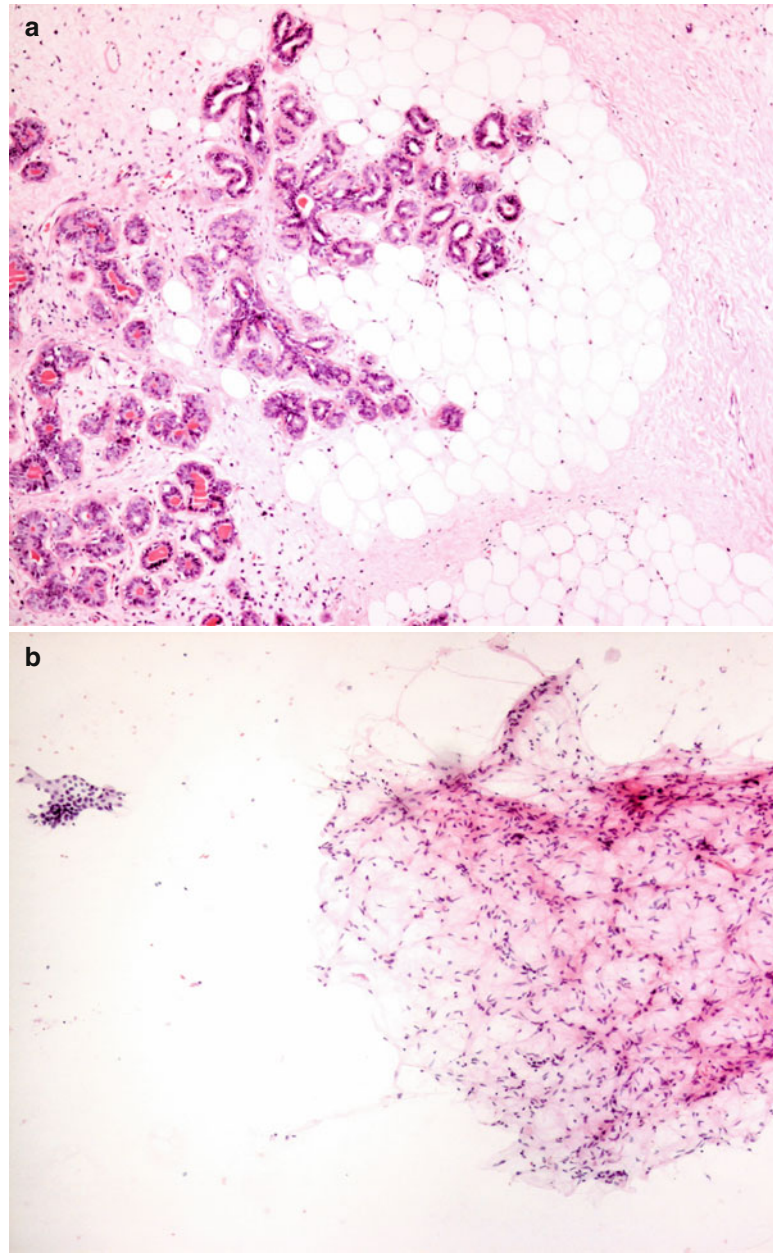


Fig. 8.6 (**a**, **b**) Hamartoma. (**a**) Histology shows mature adipose tissue with admixed breast lobules. (**b**) Cytology shows epithelial cells arranged in branching sheets, in proximity to fibrofatty tissue

(Ng et al. 2003). These epithelial sheets can be two-dimensional (Spitz et al. 1999). Sometimes, some of the epithelial cells show a mild degree of atypia, and atypia had been reported in about 20 % of the cases in one series (Levine et al. 2005). Stromal fragments, on the other hand, tend to be present in much less quantity, and they are usually hypocellular (Lui et al. 2004;

Ng et al. 2003; Spitz et al. 1999). Sometimes crush artifact is seen, and elongated stromal cells are identified at the edge of these stromal fragments (Lui et al. 2004). Rarely, bipolar nuclei are also seen in the background (Fig. 8.7a–c).

8.3.3 Differential Diagnosis

The cytologic diagnosis of PASH is highly difficult. In one series, all ten cases of PASH were not diagnosed as such (Levine et al. 2005), whereas in another series, more than

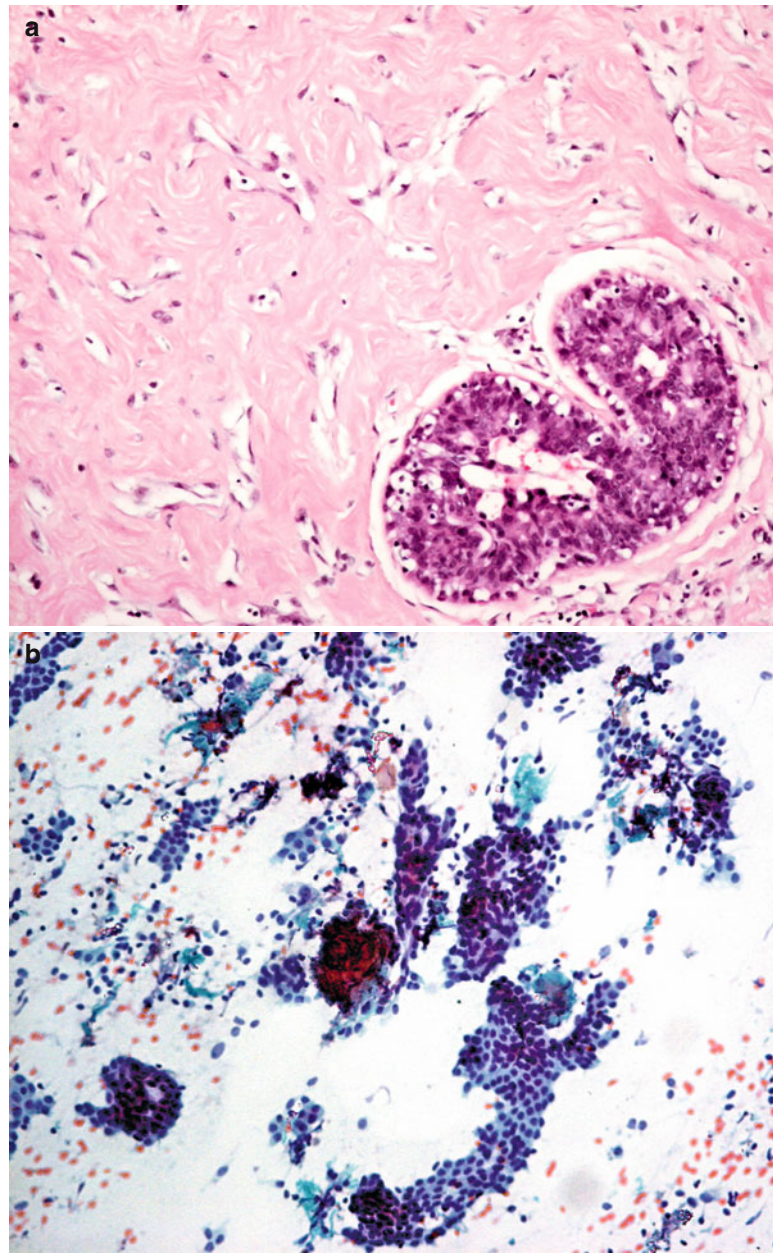
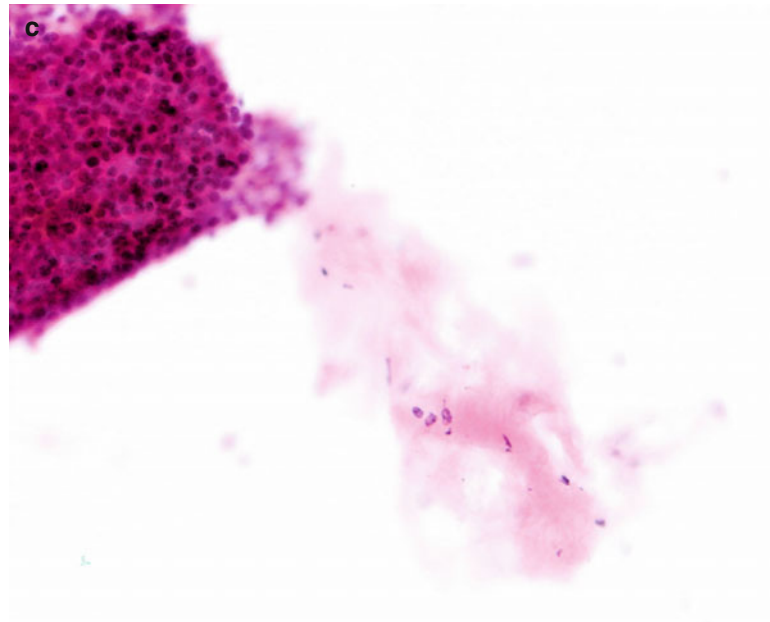


Fig. 8.7 (**a**) PASH. Histology shows anastomosing empty slits lined by myofibroblasts resembling vascular spaces. (**b**) Cytologic preparation of PASH shows moderate cellularity. The variable epithelial clusters are solid or with very rare branching. (**c**) Cytology of PASH shows hypocellular stromal fragments

Fig. 8.7 (continued)



80 % of these cases were diagnosed as either fibroadenomas or fibrocystic changes, and the remainder as suspicious (Vicandi et al. 1998). The differential diagnosis is obviously with other fibroepithelial lesions, notably the much more common fibroadenomas. This differentiation is very difficult. Cytologically, most cases of PASH were diagnosed as fibroadenomas (Levine et al. 2005; Vicandi et al. 1998). Some authors suggested lower overall cellularity and less cellular stromal fragments in PASH, but this is probably a moot point as both lesions are benign, and the clinical management is the same.

8.3.4 Management

A cytologic diagnosis of hamartoma is rarely made, as most cases would be diagnosed as fibroadenoma. The biologic behavior of hamartomas is totally benign, and excision may be considered in selected cases.

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9 Cytology of Epithelial Proliferative Lesions and High-Grade Ductal Carcinoma In Situ

Nour Sneige and Gary Tse

9.1 Introduction

With the advent of mammographic screening, non-palpable lesions are increasingly detected. As the central dogma of triple assessment is frequently regarded as the gold standard in breast lesion assessment, some form of pathological evaluation is often performed. Both FNAC and core needle biopsy (CNB) are routinely used. CNB is generally considered superior to FNAC in dealing with these clinically elusive lesions that are only detected by either architectural distortion, densities/masses, or more commonly by the presence of calcifications. CNB, compared to FNAC, has a higher sensitivity, specificity, and positive predictive value for malignancy and lower inadequacy rate (Tse and Tan 2010). As a result, many centers preferentially perform CNB for such evaluation, and the pure cytologic experience in dealing with these lesions is limited. In the literature, there are sporadic reports confirming the usefulness of FNAC when the clinical and radiological findings are taken into consideration (Zardawi et al. 1999). In this group of screen detected lesions, however, the clinical and radiological findings can be nonspecific, and the clinical non-palpability infers that they are clinically inapparent. Radiologically, these lesions are identified mostly due to calcifications or they may produce a mass or asymmetric density. The calcifications associated with these lesions are usually small and nonspecific, reflecting the secretory type of calcifications that one may see in a wide range of lesions from fibrocystic changes to low-grade malignancy (Tse et al. 2008). Pathologically this group of lesions encompasses the specific entities of epithelial hyperplasia without or with atypia. The group with epithelial hyperplasia without atypia can be further divided into those with non-proliferative changes (mild epithelial hyperplasia) to proliferative changes (moderate epithelial hyperplasia, florid epithelial hyperplasia, and columnar cell changes), whereas the atypical category includes columnar cell changes with atypia (FEA), ADH, low-grade DCIS and LN (atypical lobular hyperplasia and lobular carcinoma in situ).

9.2 Non-proliferative Breast Disease

9.2.1 Clinical and Epidemiological Findings

These lesions are asymptomatic and, in some cases, may be detected by mammographic screening with calcifications.

9.2.2 Cytologic Findings

Cytologically mild epithelial hyperplasia shows simple, small- to medium-sized epithelial cell groups with occasional intercellular spaces and

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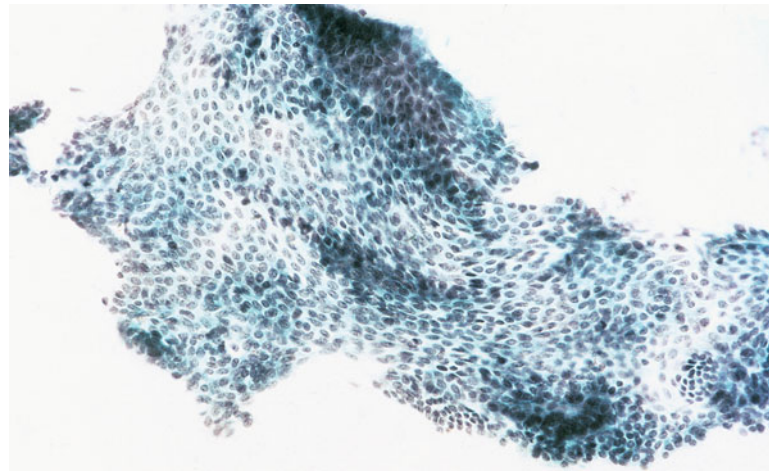

Fig. 9.1 Large sheets of benign ductal epithelial cells are seen, with the individual epithelial cells showing bland and normochromatic nuclei with a regular architecture

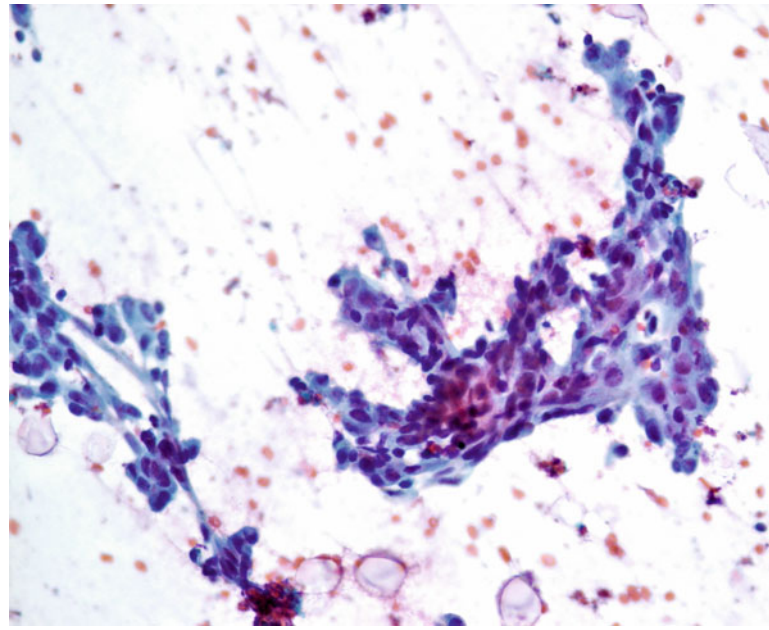

Fig. 9.2 Small fragments of epithelial cells showing minimal nuclear enlargement and crowding, admixed with myoepithelial cells. A vague lobular architecture can be discerned

conspicuous myoepithelial cells (Figs. 9.1, 9.2, and 9.3). Occasionally apocrine metaplastic cells may also be seen.

9.2.3 Histologic Correlations

The vast majority of breast biopsy samples have histologic features of non-proliferative breast diseases, and this does not entail any increased risk for malignancy for the patients. In addition to mild epithelial hyperplasia, other common changes of apocrine metaplasia and cyst formation are described in Chap. 6.

Mild epithelial hyperplasia is recognized by the stratification of epithelial cells of up to three to four cell layers above the basement membrane, with intact myoepithelial cells separating the epithelium from the basement membrane. The involved ductal spaces show minimal distension, and there is little intraluminal cell mass formation (Sneige 2000; Ducatman et al. 1992).

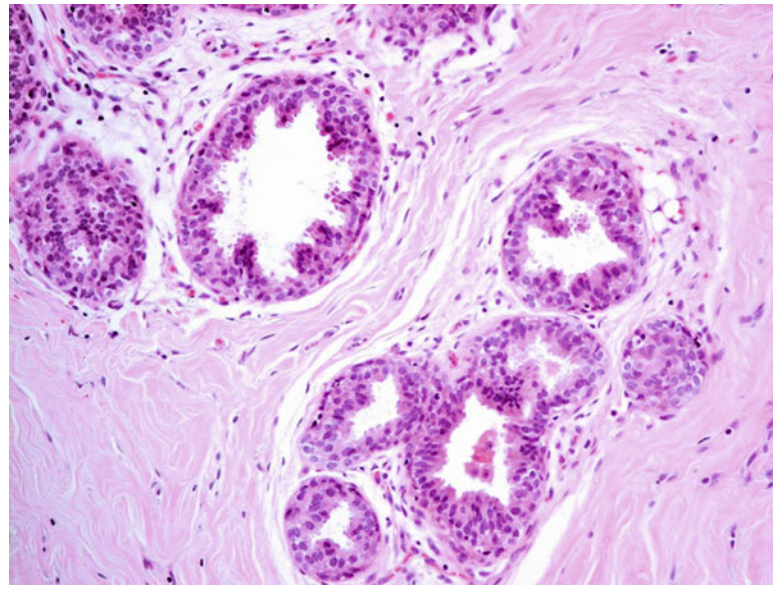

Fig. 9.3 Histologic picture of mild epithelial hyperplasia with mild increase in the number of epithelial cell layers within the lobules. The epithelial cells show bland morphology

9.3 Benign Breast Proliferative Lesions

These lesions are asymptomatic and, in some cases, may be detected by mammographic screening with calcifications. In many of these cases, most FNAC diagnoses will fall into the benign category, allowing the correct characterization and management of the patients. However, there exists, inevitably, a very small number of false-positive cases due to cytologic overcall. Although such false-positive cases are in general much less common than false-negative cases in breast FNAC, a false-positive cytologic diagnosis will result in inappropriate overtreatment for the patients. In the literature, the reported false-positive rates ranged from 0 to 2 % (Mendoza et al. 2011; Rosa and Masood 2011; Arisio et al. 1998; Ishikawa et al. 2007; Feichter et al. 1997). The reported benign entities that gave rise to false-positive FNAC include ductal and lobular hyperplasia as well as pregnancy changes. In most instances, the radiological findings are indeterminate. The cytological differential diagnoses include all those lesions within this proliferative breast lesion category. Histologically, these lesions show higher degree of epithelial hyperplasia, sometimes associated with specific architectural changes. These include moderate to florid epithelial hyperplasia, sclerosing adenosis, and papillomas. One may also include columnar cell changes (including columnar cell hyperplasia) to this category. The cytological changes of sclerosing adenosis and papillomas are discussed in Chaps. 6 and 10 and are not included here.

9.3.1 Moderate and Florid Epithelial Hyperplasia

9.3.1.1 Clinical and Epidemiological Findings

For the proliferative breast lesions without atypia, moderate epithelial hyperplasia and florid epithelial hyperplasia are probably the most commonly encountered.

9.3.1.2 Cytologic Findings

Cytologically, the proliferating cell masses may show large complex structures with visible intercellular spaces or cell bridges (Thomas et al. 1995; Sneige and Staerkel 1994; Dawson et al. 1995). In florid epithelial hyperplasia, the architectural characteristics can be well demonstrated in the FNAC, with the irregular stretched out intercellular spaces or tapered bridges composing of epithelial cells that are aligned parallel to the

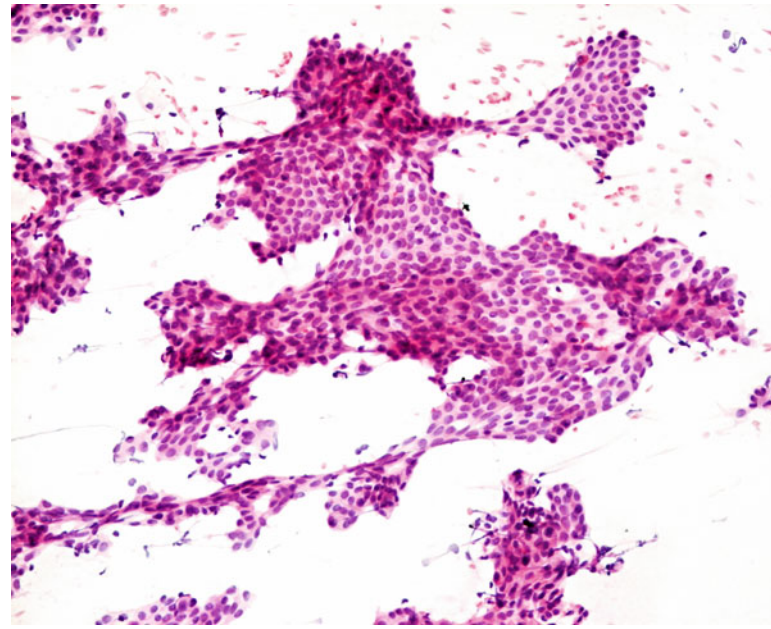

Fig. 9.4 Florid epithelial hyperplasia showing a sheet of benign ductal epithelial cells. Myoepithelial cells can be identified at the periphery. The epithelial cells possess spindled nuclei, with some suggestion of nuclear streaming

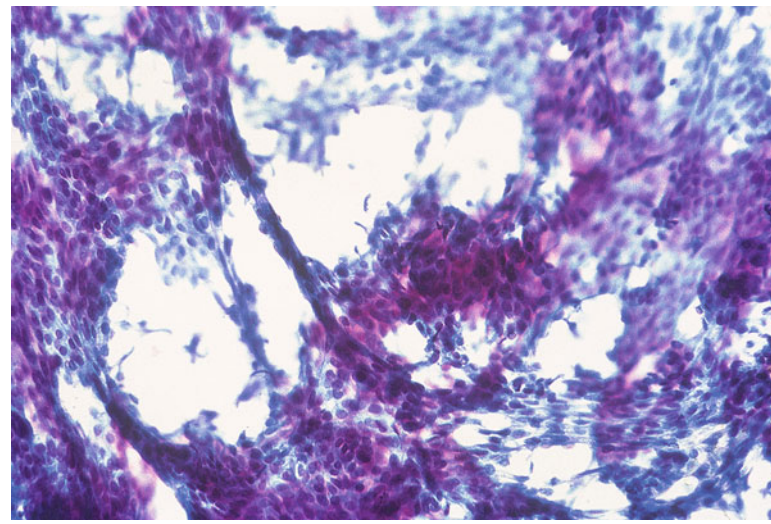

Fig. 9.5 Florid epithelial hyperplasia showing large sheets of benign epithelial cells with somewhat spindled nuclei, with cells that are aligned along the periphery of the fragment in a parallel direction

long axis of the bridges (Figs. 9.4, 9.5, and 9.6). However, in any aspirate the changes may only be limited to one or a few groups of epithelial cells. In moderate epithelial hyperplasia, the cells are smaller, with oval nuclei and indistinct cytoplasmic borders. Myoepithelial cells are well represented. In the background, variable numbers of single cells may be present. The cellularity of these background cells does not reflect the degree of epithelial hyperplasia. The main differential diagnosis is with low-grade carcinoma, which may also show mildly atypical single cells as well as large complex epithelial structures with secondary architecture (Sneige and Staerkel 1994).

9.3.1.3 Histologic Correlations

Histologically moderate to florid epithelial hyperplasia shares similar cellular morphology, characterized by proliferating cell masses distending ducts. The cells show variable nuclear

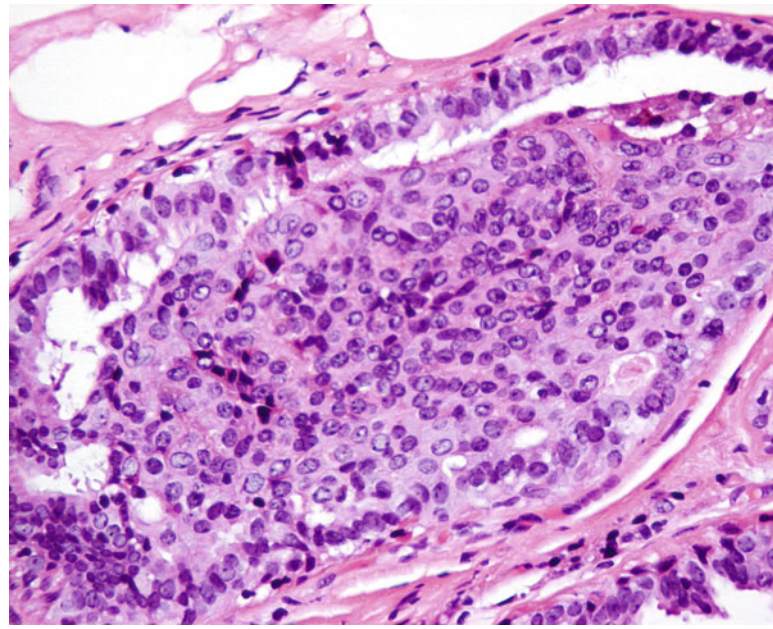

Fig. 9.6 Histology of florid epithelial hyperplasia showing cohesive proliferation of benign epithelial cells with nuclear streaming. The secondary lumina are peripherally located and slit-like

roundedness, and the nuclei also display variable chromatin patterns, delicate nuclear membranes, and small nucleoli. The cell borders are unapparent. Occasionally apocrine cells may also be present. The main difference between moderate and florid hyperplasia is architectural. Moderate hyperplasia may form small luminal proliferating masses but with little secondary lumens, whereas in florid epithelial hyperplasia, the cells form slit-like intercellular spaces or secondary lumens, which are more abundant in the periphery of the involved ductal spaces. The cellular orientation of the spindled, proliferative cells is parallel to the long axis of the intercellular spaces or the cellular bridges between these spaces.

9.3.2 Sclerosing Adenosis

9.3.2.1 Clinical and Epidemiological Findings

Sclerosing adenosis represents another proliferative lesion that causes problem at FNAC.

9.3.2.2 Cytologic Findings

Cytologically, sclerosing adenosis shows moderate to high cellularity with tightly cohesive cell groups/tubules surrounded by myoepithelial cells and intact basement membranes, and in the background many single cells or bare nuclei may be seen (Silverman et al. 1989). The cytologic features closely reflect the histologic appearances (i.e., compressed and attenuated tubules and sclerotic stroma), but may cause overinterpretation on FNAC smears (Fig. 9.7).

9.3.2.3 Histologic Correlations

Histologically sclerosing adenosis can be defined as a lesion showing increased numbers of deformed, sclerotic glandular elements in a lobulocentric pattern usually more than two times that of the adjacent ductal lobular units. There may or may not be associated epithelial hyperplasia, and it entails a two-time increased cancer risk for the patients (Jensen et al. 1989) (Fig. 9.8).

9.3.3 Columnar Cell Changes

9.3.3.1 Clinical and Epidemiological Findings

Columnar cell changes are common, and they are usually asymptomatic, only detected by their associated calcification at mammography or as incidental findings.

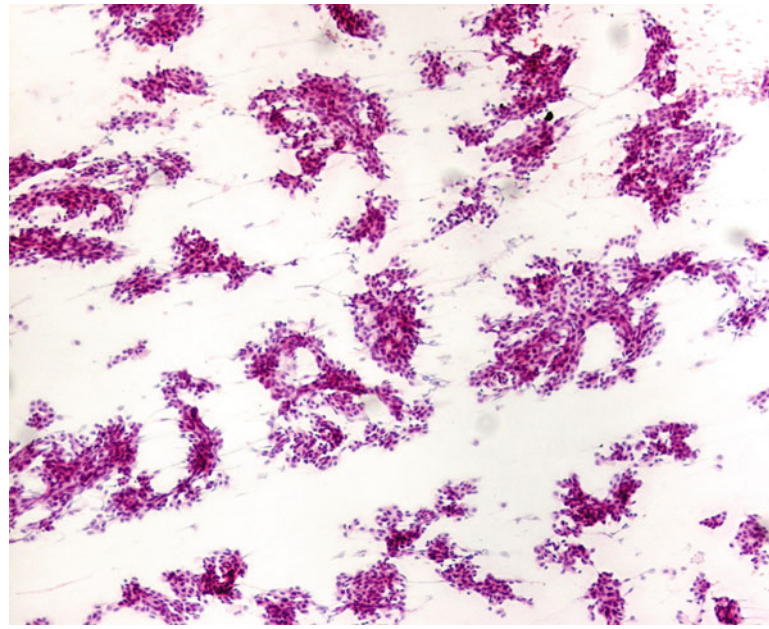

Fig. 9.7 Sclerosing adenosis showing a smear with high cellularity and the epithelial cell clusters show cohesive tubules surrounded by myoepithelial cells. Some single cells and bare nuclei are noted in the background

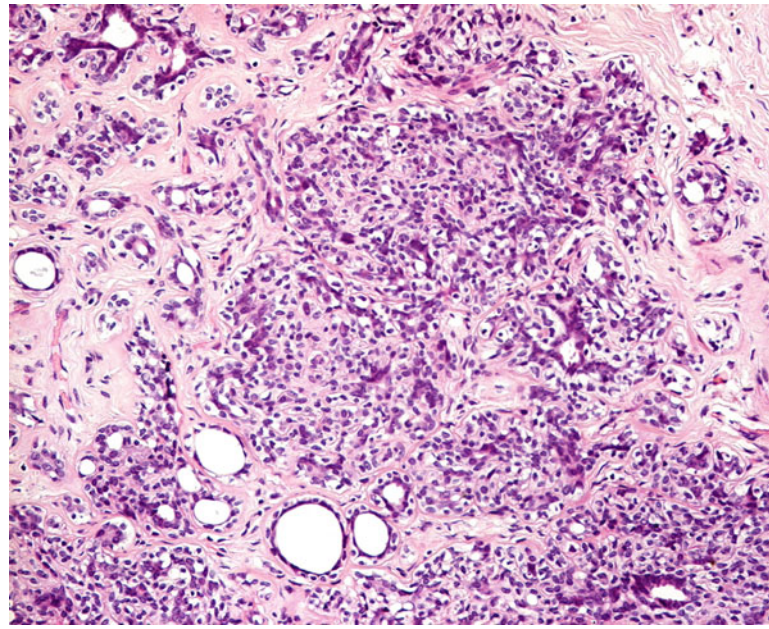

Fig. 9.8 Histology of sclerosing adenosis, with pseudoinfiltrative tubules of epithelial cells surrounded by myoepithelial cells in a densely fibrotic stroma

9.3.3.2 Cytologic Findings

The cytologic changes of columnar cell changes have not been described extensively. The aspirates usually show a uniform cell population with mildly enlarged nuclei, mild nuclear pleomorphism, variable nucleoli, focal nuclear membrane irregularity, and a paucity of myoepithelial cells. There is usually minimal cellular discohesion. Cellular fragments may be seen, and the cells within the center of these three-dimensional cell groups tend to show crowding, overlapping, and loss of polarity. There may be peripheral palisading of the epithelial cells (Fig. 9.9). Background single cells occurred in about 70 % of the cases, and these cells ranged from cuboidal to columnar (Saqi et al. 2004).

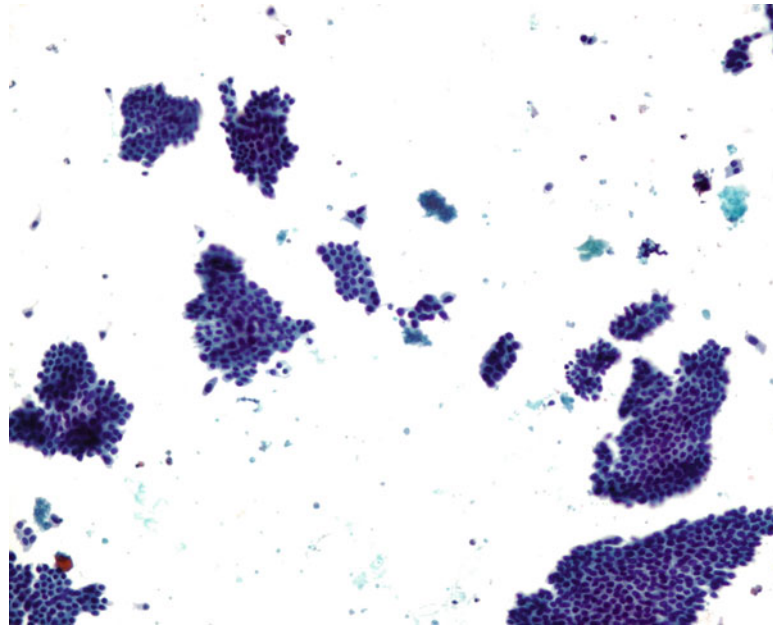

Fig. 9.9 Aspirate of columnar cell changes showing a relatively uniform cell population with mildly enlarged and variable nuclei. These cells form three-dimensional cell groups and show crowding and some loss of polarity. A vague suggestion of peripheral palisading is also seen

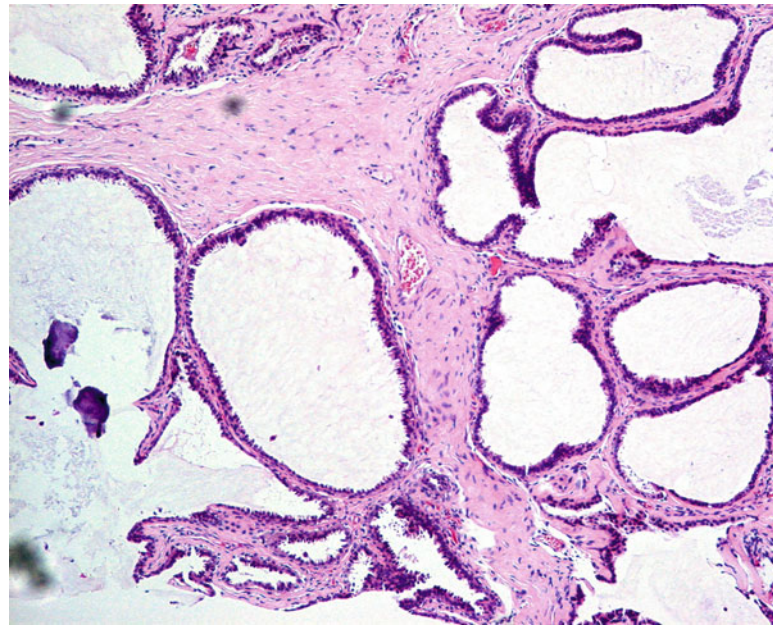

Fig. 9.10 Histologic features of columnar cell changes showing dilated ductal lobular spaces that are lined by epithelial cells with dense nuclei and columnar cytologic appearances

9.3.3.3 Histologic Correlations

Columnar cell changes typically shows dilated ductal lobular units, with the lining cells being tall and columnar, with cytoplasmic apical snouts and basally located nuclei. Within the dilated lumens, flocculent material as well as calcification may be present (Fig. 9.10). In some cases there is epithelial hyperplasia to more than four layers. In other cases, the epithelial cells may adopt a monotonous, rounded appearance with scanty cytoplasm, reminiscent of ADH. Under such situations the lesions are called columnar cell changes with atypia or FEA. The architecture however remains flat, devoid of intraluminal mass formation.

9.4 Atypical Epithelial Proliferations

For epithelial proliferative lesions with atypia, it must be emphasized that the histologic and cytologic diagnosis of atypia may not be totally concordant – with a significant proportion of histologically atypical lesions being diagnosed as either benign or malignant at cytology and also a significant number of cytologically atypical lesions turning out to be either histologically benign or malignant entities. This can be construed as strong evidence of overlapping diagnostic criteria for these categories of lesions. Using the probabilistic approach to categorize breast lesions (Sneige 1993; NIH 1997), these would be grouped into the cytologically atypical (C3) category. The cytologic features for this category classically show most of the characteristics of a benign smear, but with some worrisome features including cellular crowding, pleomorphism, and discohesion. Alternatively these may represent smears with very scanty highly atypical cells. It has been reported that low cellularity accompanied about 60 % of these atypical aspirates, whereas interpretative errors may account for about 20 % of these (Al-Kaisi 1994).

In the cytologically atypical category, most of these cases actually represent benign lesions, with a quoted range of 55–70 % (Tran et al. 2010; Lim et al. 2004; Al-Kaisi 1994; Wang and Ducatman 1997; Mulford and Dawson 1994). Conversely, 30–45 % of these cases turned out to be malignant. Interestingly very low numbers of these, if at all, turned out to be atypical hyperplasia on histology.

Many of the atypical histologic lesions may also be underdiagnosed at FNAC. In the literature, false-negative FNAC ranged from 1.2 to 10 % (Rosa and Masood 2011; Arisio et al. 1998; Ishikawa et al. 2007; Feichter et al. 1997; Park and Ham 1997). Apart from the smaller proportion of diagnostic errors, most of these false-negative cases were attributable to true false-negative factors, and among these small lesion size and minimal atypia are significant causes (Mendoza et al. 2011; Arisio et al. 1998). Non-palpable lesions as a group contribute significantly to the false negativity of FNAC. The histologic entities that constitute this group included ductal carcinoma, not otherwise specified, and low-grade tumors including lobular carcinoma (Park and Ham 1997; Bulgaresi et al. 2005). Even if one were to be able to identify this group of lesions on FNAC, thus avoiding the fallacy of a false-negative diagnosis, the actual cytologic differentiation of these atypical lesions is also fraught with difficulty. The underlying reason for this is readily understandable when one looks at the basic histology in details. In the entire group of atypical epithelial proliferation, including FEA, ADH, low-grade DCIS and LN, they all share the common features of having low-grade cytomorphology, typically characterized by uniform, rounded, monotonous nuclei with small nucleoli, and moderate amounts of cytoplasm with rather distinct cell borders. Mitotic activity is not markedly increased, and there is usually no significant necrosis. The main differentiating features among all these entities lie within their respective architecture and size. In FEA, the typical architecture is the presence of these atypical cells around ductal lumens, without forming significant intraluminal mass. In ADH and in low-grade DCIS, both show similar cells, but these cells form intraluminal cell masses. The cells are arranged in specific and geometric architectures and may adopt solid or cribriform patterns. The cell orientation is often perpendicular to the intervening cell bridges between the secondary lumens and lie perpendicular to the basement membranes. The difference between ADH and DCIS is purely based on the difference in size or extent, using either 2, 3 mm, or two membrane-bound spaces as cutoffs. A similar situation also occurs in lobular carcinoma, with the term LN being used by many to denote the group of lesions that range from atypical lobular hyperplasia to lobular carcinoma in situ, the differentiation of which is also dependent on the lesional involvement of terminal duct lobular units. To put this into perspective, this group of

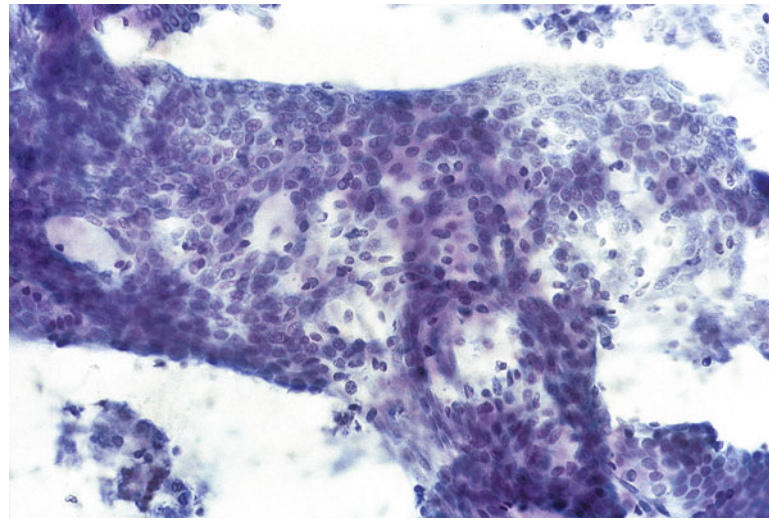

Fig. 9.11 Cytologic preparation of atypical ductal hyperplasia showing sheets of monotonous, evenly spaced epithelial cells exhibiting round, slightly enlarged nuclei with fine chromatin and inconspicuous nucleoli. Occasional myoepithelial cells may be seen. The epithelial cells are arranged in a vague cribriform pattern

lesions is differentiated based on the architecture and size, or degree of involvement, and not cellular morphology. In fact there is also molecular evidence that these lesions show progressive but similar genetic changes, leading a proposal of "low-nuclear-grade breast cancers and their precursors" (Abdel-Fatah et al. 2008). As a result, cytologic differentiation of these lesions is difficult and very often necessitates the assessment of tissue fragments within the FNAC so that some of the architectural characteristics can be evaluated. The correct differentiation remains a challenging task.

9.4.1 Atypical Ductal Hyperplasia

9.4.1.1 Clinical and Epidemiological Findings

ADH is usually asymptomatic, only detected by the associated calcifications at mammography or as incidental findings.

9.4.1.2 Cytologic Findings

The FNAC of ADH shows variable cytology. The cells show atypical proliferative changes characterized by monotonous, evenly spaced epithelial cells harbouring round, slightly enlarged nuclei with fine chromatin and inconspicuous nucleoli. Cellular cohesion may be reduced, resulting in variable to high numbers of single epithelial cells. The cell arrangement for the most part is cribriform, but solid and micropapillary patterns may be encountered. Rarely myoepithelial cells may be identified. In the background, a mixed proliferative epithelial cells population with or with atypia may be present. Scattered foamy histiocytes and calcifications may be seen (Figs. 9.11, 9.12, and 9.13).

9.4.1.3 Differential Diagnosis

Differentiation of ADH and florid epithelial hyperplasia in FNAC can be made by the presence of cellular monotony, even nuclear placement and slight nuclear enlargement and hyperchromasia in the former, while the latter tends to show nuclear variability, cell streaming, and prominence of myoepithelial cells or the presence of apocrine metaplasia (Sneige 2000).

Low-grade DCIS and ADH only differs in their sizes, with similar cytomorphology and architecture. As quantitative separation of these lesions is not possible at FNAC, it has been recommended that ADH should be categorized as indeterminate (C3) and biopsy recommended (NCI 1996).

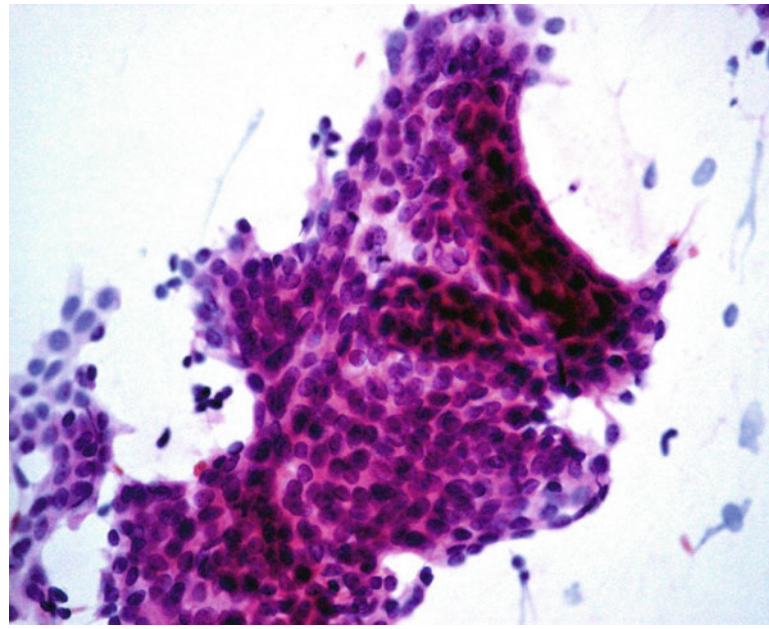

Fig. 9.12 Aspirate of atypical ductal hyperplasia showing monotonous epithelial cells with slight nuclear pleomorphism and nuclear crowding. Myoepithelial cells can be discerned in the periphery

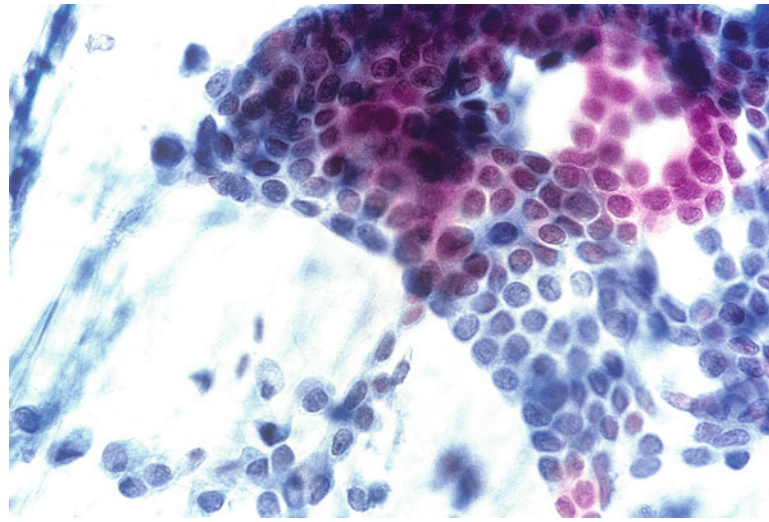

Fig. 9.13 Aspirate of atypical ductal hyperplasia showing epithelial cells with fine chromatin and inconspicuous nucleoli

9.4.1.4 Histologic Correlations

ADH is defined histologically as having some but not all of the features of low nuclear grade DCIS. Both are characterized by the presence of uniform cells and cribriform or micropapillary architecture. ADH is diagnosed when the size criterion of low grade ductal carcinoma is not fulfilled, or when cytoarchitectural atypia does not involve affected

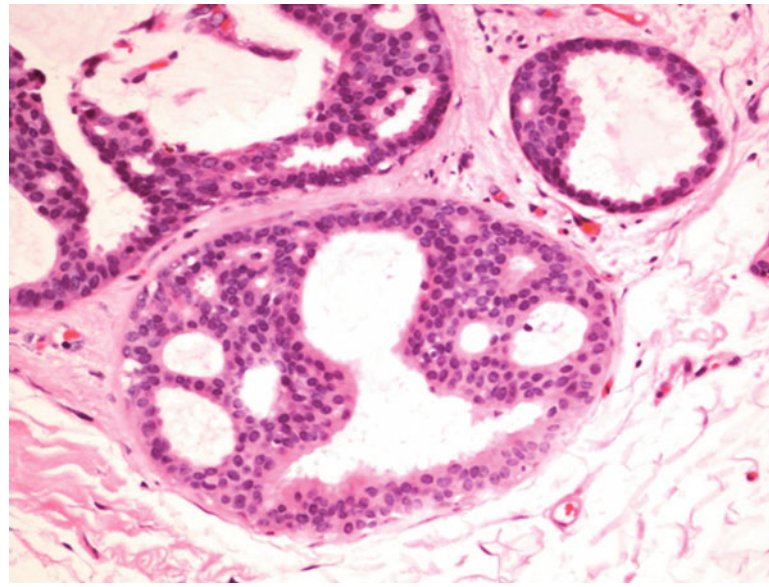

Fig. 9.14 Histology of atypical ductal hyperplasia showing intraductal luminal epithelial proliferation. The cells show round and dark staining nuclei and form rounded lumens with a geometric pattern

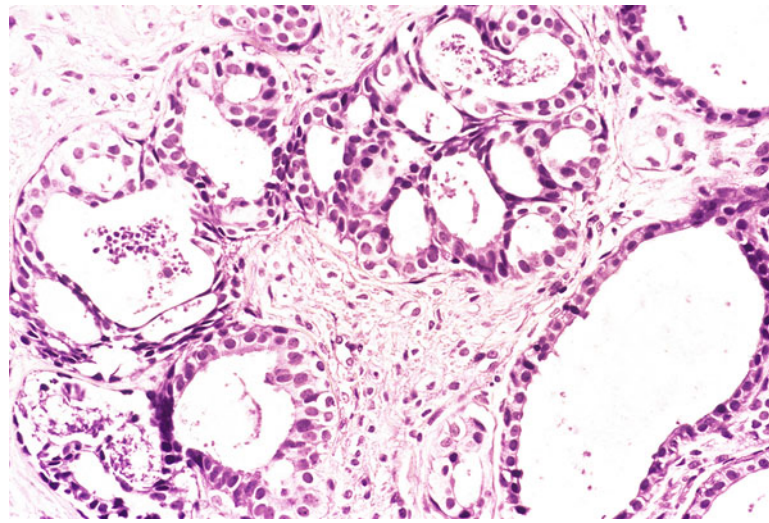

Fig. 9.15 Atypical ductal hyperplasia showing uniform epithelial cells forming small lumens

ducts uniformly. An alternative definition is when both cytologic and architectural changes of either cribriform or micropapillary DCIS are present, but the extent is less than two completely involved membrane-bound spaces (Page et al. 1985) (Figs. 9.14, 9.15, and 9.16). Hence, the differentiation between ADH and DCIS, is quantitative, and not qualitative.

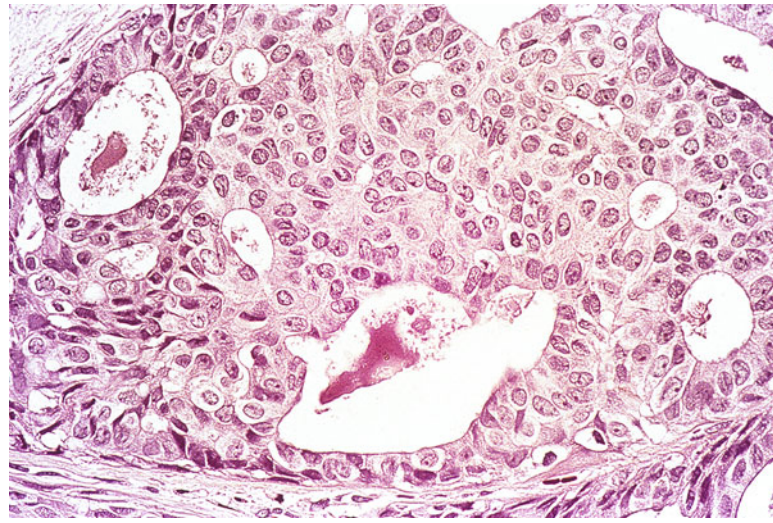

Fig. 9.16 Atypical ductal hyperplasia showing relatively uniform atypical epithelial cells forming a solid mass, with rounded lumen formation

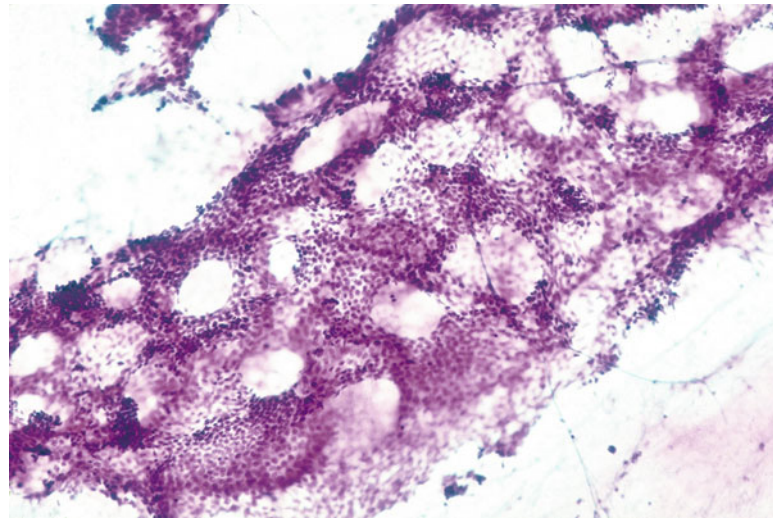

Fig. 9.17 Ductal carcinoma in situ in an aspirate showing a sheet of malignant cells with monotonous appearances forming a large cribriform tissue fragment, which is apparent in the FNAC preparation

9.4.1.5 Management

A cytologic diagnosis of ADH is considered C3, and biopsy is recommended.

9.4.2 Ductal Carcinoma In Situ, Low Grade

9.4.2.1 Clinical and Epidemiological Findings

Some low-grade DCIS may be mass forming, whereas others may not form discrete masses. They may be detected at mammography as calcifications, but the type of calcifications is usually indeterminate, as opposed to the coarse, pleomorphic, and branching type of calcifications that one sees in high-grade DCIS with comedo necrosis.

9.4.2.2 Cytologic Findings

Cytology of low-grade DCIS is characterized by a monomorphic cell population of small to intermediate epithelial cells that may be arranged in clusters or singly. These cells are polygonal to cuboidal and may contain small nucleoli. Mitotic figures may be identified (Shin and Sneige 1998). Occasionally myoepithelial cells may be seen. For the cell clusters, they may show variable architectural arrangements, including papillary, cribriform, or solid (Figs. 9.17, 9.18, 9.19, 9.20, 9.21, and 9.22). As the degree of loss of

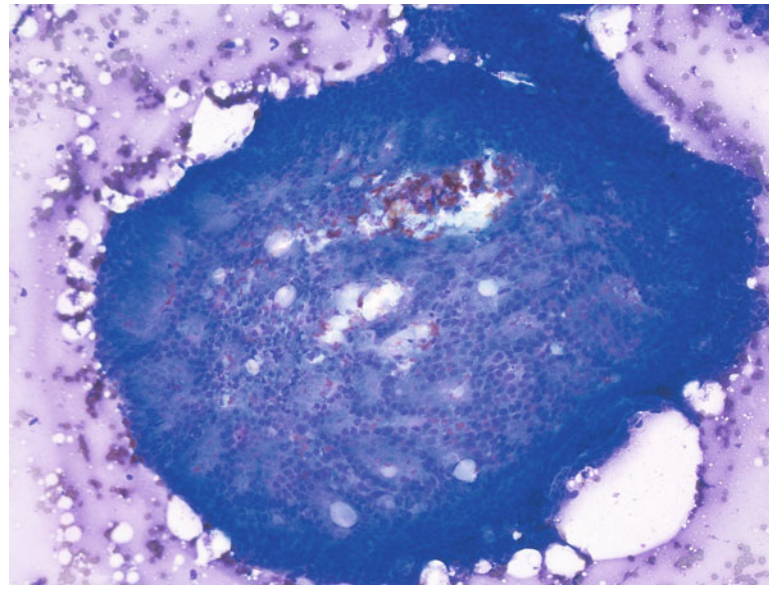

Fig. 9.18 Ductal carcinoma in situ in FNAC showing an essentially solid pattern with rare central lumen formation. Cribriforming can be identified in part of the epithelial structure

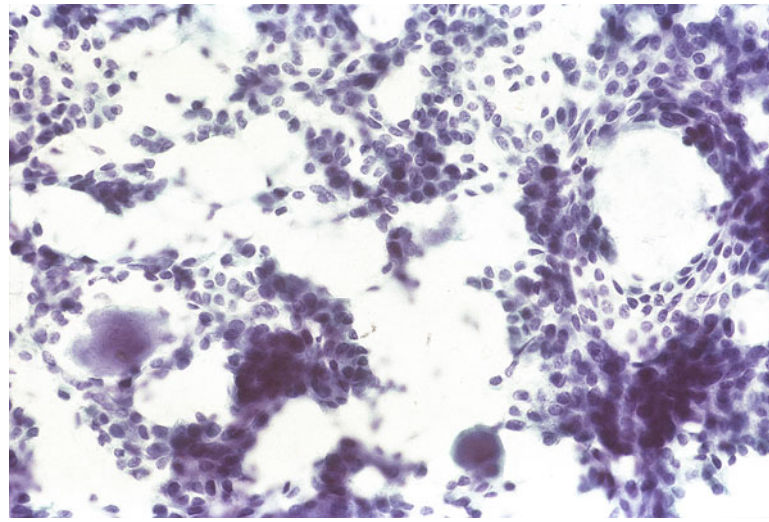

Fig. 9.19 Ductal carcinoma in situ in an aspirate showing details of cribriforming, with small luminal calcifications identified

cellular cohesion is variable, the background cellularity of the lesional single cells is also variable. Because of this variation, a significant proportion of these lesions was diagnosed cytologically as atypical epithelial proliferation (Lilleng et al. 1992).

9.4.2.3 Differential Diagnosis

Cytologically, low-grade DCIS and ADH cannot be differentiated with any certainty, as these lesions differ by their sizes only. However, in aspirates that are more cellular with a monotonous cell population and the presence of numerous single cells, particularly in at least two separate smears, the diagnosis of low-grade DCIS over ADH can be favored. When a cytologic diagnosis of DCIS is rendered, it is to be put into indeterminate or suspicious categories and tissue biopsy is recommended (NCI 1996).

9.4.2.4 Histologic Correlations

Low-grade DCIS histologically tends to show monomorphic malignant cells that are oval to cuboidal, and these cells are arranged in a

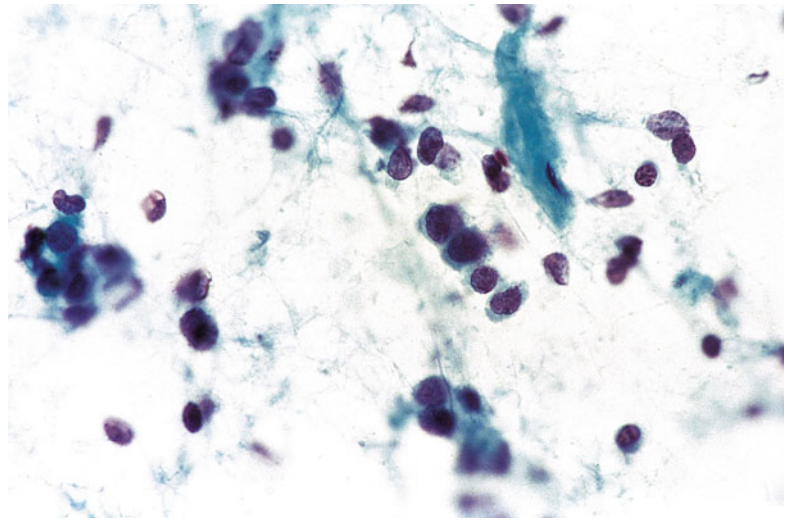

Fig. 9.20 Ductal carcinoma in situ showing single low-grade malignant cells with mild nuclear variation and a clean background

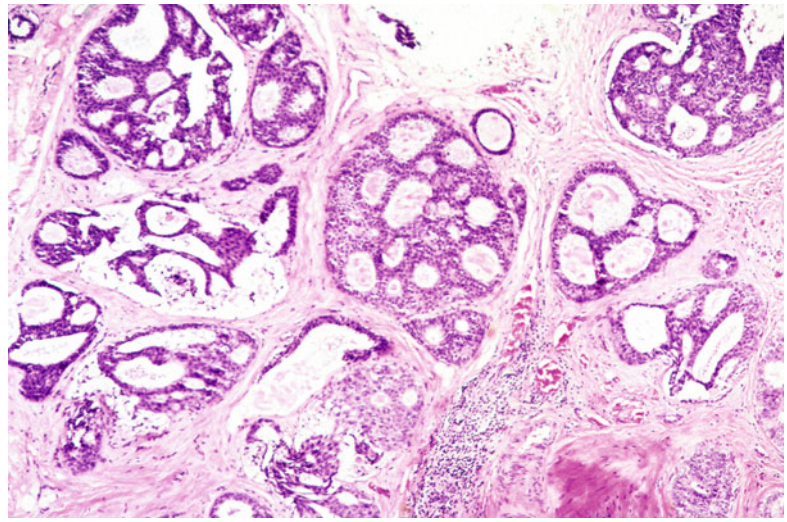

Fig. 9.21 Histologic appearance of low-grade ductal carcinoma in situ showing distension of the ducts and lobules by a monotonous cell population forming sievelike, geometric patterns

geometric and regular manner. The individual cells show moderate amounts of cytoplasm, and the degree of nuclear atypism is mild to moderate. Occasionally mitotic figures can be seen. These low-grade DCIS may have different architectural patterns, most commonly solid, cribriform, or papillary, and these patterns may be mixed within one lesion (Figs. 9.21 and 9.22). Necrosis is not a characteristic feature of low-grade DCIS.

9.4.2.5 Management

In a low-grade malignant aspirate, invasion cannot be diagnosed with certainty. When such a diagnosis is rendered, histologic assessment is imperative to assess invasion, which would have significant treatment implications.

9.4.3 Lobular Neoplasia

9.4.3.1 Clinical and Epidemiological Findings

LN encompasses both atypical lobular hyperplasia (ALH) and lobular carcinoma in situ (LCIS). Both lesions differ by the degree of involvement, with LCIS often being more extensive than ALH. Clinically most of these lesions are asymptomatic, and calcifications tend to be seen associated

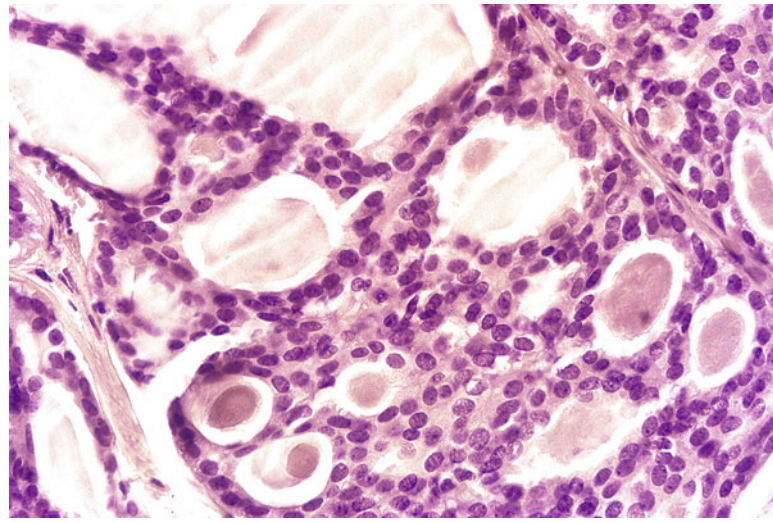

Fig. 9.22 Histologic appearance of ductal carcinoma in situ showing monotonous tumor cells with only mild nuclear atypism

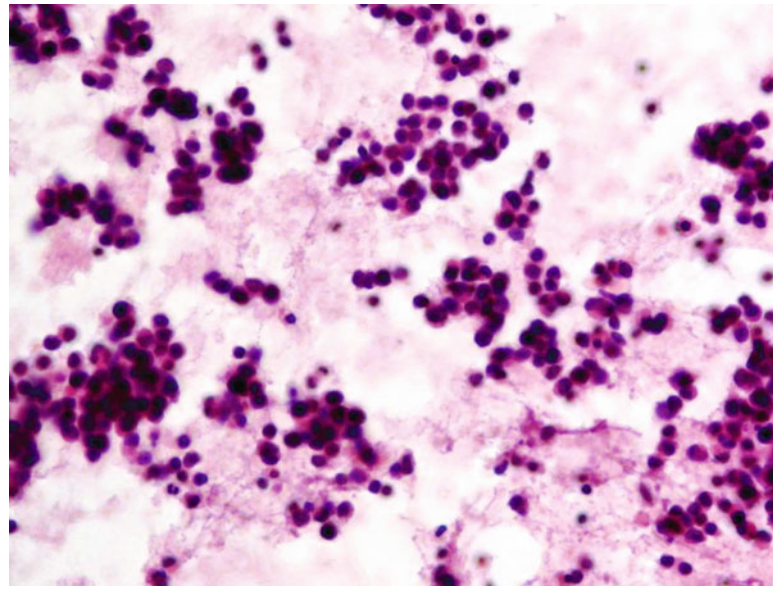

Fig. 9.23 Lobular neoplasia showing poorly cohesive abnormal cells with mild nuclear atypism. Vague single-cell strands can be identified

with the adjacent benign changes rather than with the lesion itself.

9.4.3.2 Cytologic Findings

The characteristic cytologic features reflect the typical histology. The cells appear in tight clusters, singly or both. They tend to be small, uniform, with eccentric nuclei and may also show intracytoplasmic vacuoles (Figs. 9.23 and 9.24)

9.4.3.3 Differential Diagnosis

The cellular features are similar in ALH and LCIS and in invasive lobular carcinoma, but as ALH and LCIS are usually clinically asymptomatic, their aspirates tend to show materials from coexisting lesions (many of which are probably benign) and, as a result, make a definitive diagnosis for ALH and LCIS difficult.

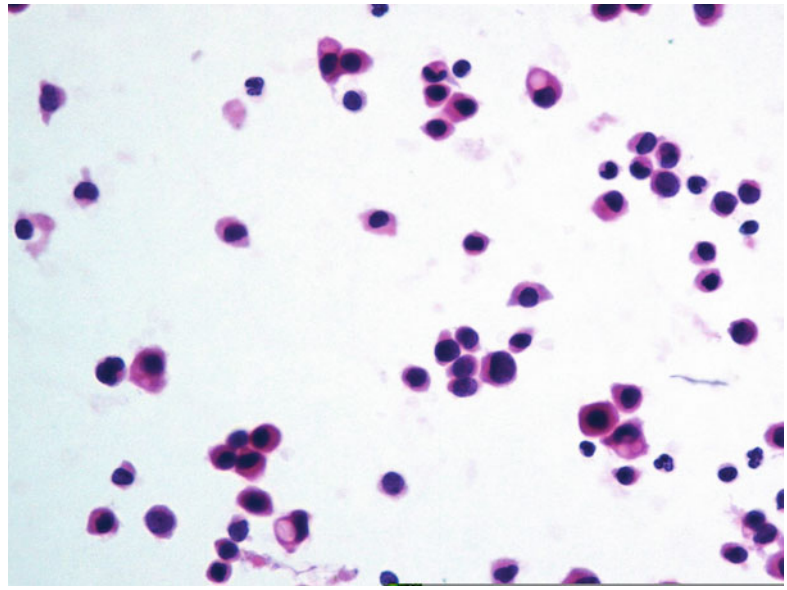

Fig. 9.24 Lobular neoplasia showing malignant cells with mild nuclear atypism. They tend to be small, uniform, with eccentric nuclei and also show intracytoplasmic vacuoles. Vague single cell files can be discerned

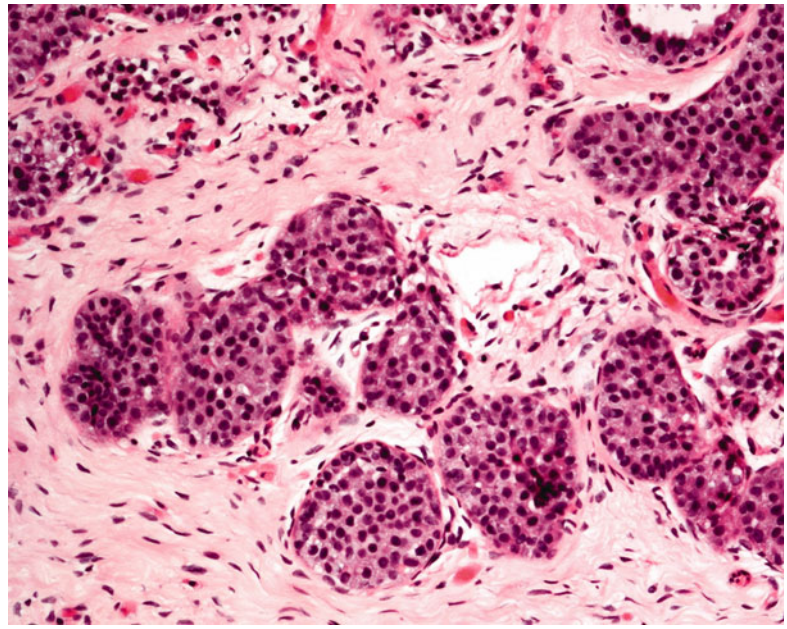

Fig. 9.25 Histology of LCIS showing distension of the ducts and lobules by uniform malignant cells. These cells form solid epithelial proliferations with the absence of lumen formation

9.4.3.4 Histologic Correlations

Histologically LCIS tends to show proliferation of small rounded cells that distend ducts and lobules in a solid nested pattern, without significant lumen or glandular formation or necrosis (Fig. 9.25). The cells show rounded nuclei with fine chromatin and inconspicuous nucleoli. The cytoplasm is eosinophilic to somewhat clear and may contain intracytoplasmic vacuoles.

9.4.3.5 Management of the Results

When a cytologic diagnosis of ALH or LCIS is made, it is to be put into indeterminate or suspicious categories and tissue biopsy is recommended.

9.5 Ductal Carcinoma In Situ, High Grade

The cytologic diagnosis of carcinoma in situ of the breast is controversial, with many authors and practicing pathologists considering that a reliable diagnosis of carcinoma in situ cannot be made, particularly in the differentiation from invasive carcinoma. As a result, most practicing pathologists usually report malignant aspirates as carcinoma and refrain from making a proclamation as to whether the lesions is considered in situ or invasive, leaving this responsibility to the subsequent histologic assessment.

The cytologic features of carcinomas in situ of the breast had not been extensively studied. There are few reports in the literature on DCIS and much fewer for lobular carcinoma in situ. As lobular carcinoma in situ and invasive lobular carcinoma share the same cytologic morphology, these lesions show very little specific architectural arrangement, with the former forming a solid tumor mass within ductal spaces and the latter showing single cells with file formation invading into a fibrotic stroma. It is felt that a discussion on the cytologic diagnosis of lobular carcinoma in situ and its differentiation from invasive lobular carcinoma is futile. For DCIS, it should be noted that the low-grade DCIS shares many overlapping morphologic and molecular similarities with ADH and low-grade invasive ductal carcinoma. This group of lesions has been previously discussed.

9.5.1 Cytologic Findings

The cytologic features of DCIS in general reflect the heterogeneity of the histologic features. In terms of grading, good correlation has been reported with an accuracy rate of more than 90 %, particularly in high-grade DCIS. The cytologic features that had been reported to be useful in the differentiation between high- and low-grade DCIS include large and pleomorphic tumor cells, calcification, necrosis (either focal or diffuse) in the background and macrophages (McKee et al. 1999), large nuclei and nucleoli (Malamud et al. 1992), as well as solid to cribriform aggregates of epithelial cells (Sauer et al. 2005). In contrast, the cytologic features of low-grade DCIS included only moderate to high cellularity (as compared to mostly high cellularity in high-grade DCIS), the presence of cohesive three-dimensional sheets of uniform cells with small nuclei and inconspicuous nucleoli. Sometimes these tumor cells are arranged around a central lumen, forming punched out spaces, and a cribriform pattern may be discerned. In the background, abundant single cells are seen (Cangiarella et al. 2003). In addition, myoepithelial cells have been reported to be more frequently in low-grade DCIS (51 %) than in high-grade DCIS (27 %).

9.5.2 Differential Diagnosis

The main cytologic differential diagnosis of high-grade DCIS is with infiltrating duct carcinoma. An accurate and reliable differentiation between high-grade DCIS and infiltrating duct carcinoma is not widely considered feasible, and it is not recommended to attempt to differentiate high grade in situ and invasive ductal carcinoma in most circumstances. An attempt may be made under exceptional cases, for example, when most of the cytologic features that are considered relevant (to be discussed in the following paragraphs) are present, and even in such instances, a note should be made in the report alluding to the potential false positivity or negativity regarding the assessment of invasion. It is actually better not to attempt to make a conclusive statement in the assessment of a malignant breast aspirate.

In the literature, many features have been examined to address the issue of presence or absence of invasion:

1. The presence of stromal fragments associated with tumor cells or tumor cells invading into stromal fragments has been considered one of the most reliable features for confirmation of invasion in a cytologic aspirate (Fig. 9.26). This feature has been observed in 69–72 % of invasive carcinoma aspirates but 0–33 % of in situ disease aspirates (McKee et al. 2001; Shin and Sneige 1998). However, some other

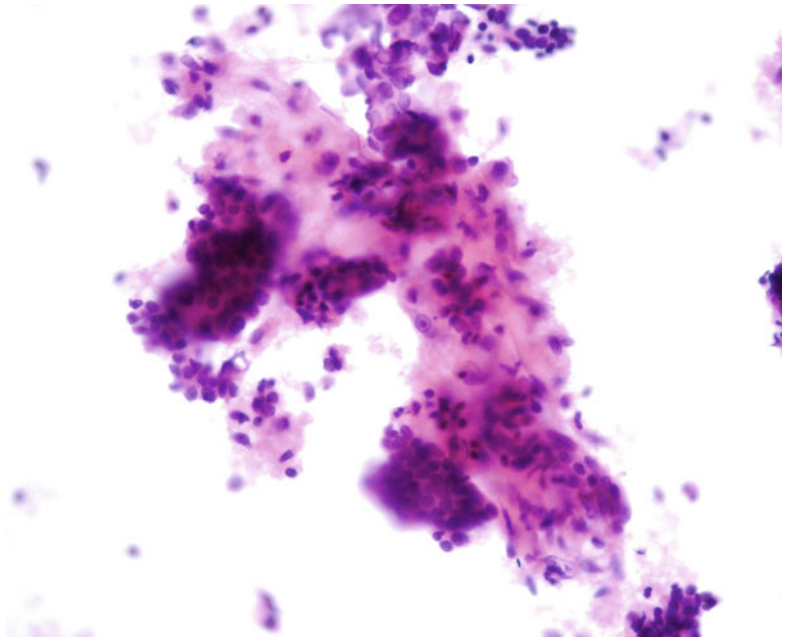

Fig. 9.26 Cytologic preparation of a high-grade invasive ductal carcinoma showing tumor cells invading into stromal fragments

authors did not find this feature to be useful (Maygarden et al. 1997).

2. Tubular structures formed by tumor cells have been described in 24–34 % of invasive aspirates but 0–7 % of in situ aspirates (McKee et al. 2001; Shin and Sneige 1998; Bonzanini et al. 2001). In addition, it was also reported that most of the invasive cancers showing tubular aggregates in the aspirate were of low to intermediate grade. Tubular aggregates were less likely to be found in the aspirates of high-grade infiltrating duct carcinoma.
3. Cellular cohesion has also been considered a differentiating feature. Invasive aspirates showed lower degree of cellular cohesiveness of the malignant cells. Cellular cohesion has been defined as the presence of tight tumor cell clusters, some of which may show cribriform or papillary patterns, and loose clusters in which the tumor cells are poorly cohesive (Bonzanini et al. 2001). It was reported to be useful as high cellular cohesion was seen in 88 % of aspirates of in situ carcinoma but only 28 % of invasive carcinoma aspirates. Others also found significantly more cellular dissociation with invasive carcinoma compared to carcinoma in situ (Bofin et al. 2004).
4. Tumor cell presence within adipose tissue fragments has been reported by some authors to be useful (Fig. 9.27), with such being seen in 42–72 % of invasive aspirates but only in up to 20 % of the aspirates of in situ carcinoma (McKee et al. 2001; Bofin et al. 2004). Other authors did not however find this feature to be useful (Maygarden et al. 1997).
5. The presence of myoepithelial cells in the aspirate as a differentiating feature is somewhat controversial. Myoepithelial cells have been found overlying clusters of tumor cells in 50–86 % of in situ carcinoma aspirates but in only 7–27 % of aspirates of invasive carcinoma. Another study using p63 staining to highlight the myoepithelial cells demonstrated myoepithelial cells in 60 % of aspirates of in situ carcinoma, but none of the invasive carcinoma aspirates. Nevertheless, the authors concluded that using the presence of myoepithelial cells to rule out invasion was not reliable due to the high percentage (60 %) of myoepithelial cells in invasive breast cancer (Reis-Filho et al. 2002).
6. Other cytomorphologic features have been reported to be useful, and these included higher degrees of nuclear pleomorphism, coarse chromatin patterns, and the presence of nucleoli, all of these were thought to be

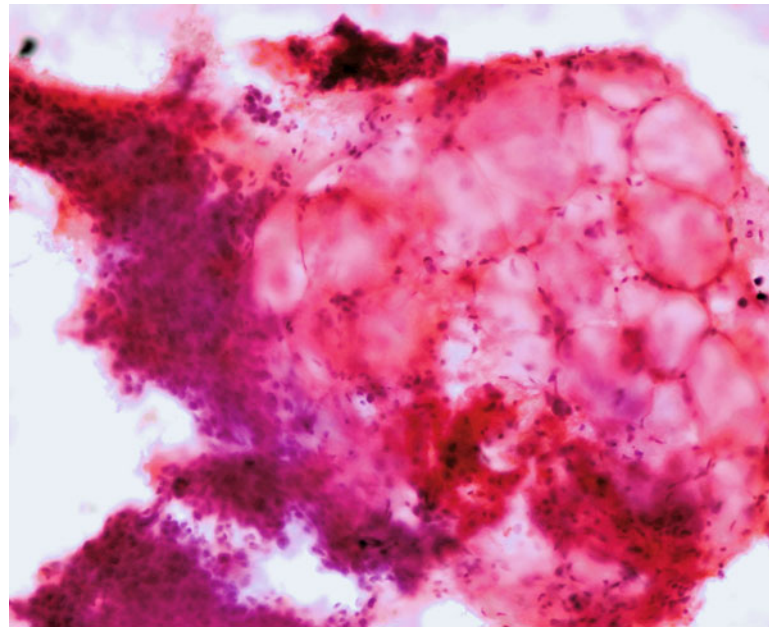

Fig. 9.27 Cytologic preparation of a high-grade invasive ductal carcinoma showing the presence of tumor cells within adipose tissue fragments

more likely to occur in invasive carcinoma aspirates and were useful as differentiating features from in situ carcinoma aspirates (Bofin et al. 2004). These features however were mostly evaluated as differentiating features in the grading of ductal carcinoma in situ aspirates rather than in the identification of invasion.

7. Background calcifications had also been reported to be useful. Calcifications are reported to be more common in in situ diseases aspirates (50–71 %) than invasive carcinoma aspirates (15–20 %) (Bofin et al. 2004; McKee et al. 2001).
8. Necrosis and the presence of bipolar nuclei have been reported by some to be useful to differentiate invasion in aspirates, but these had not been adequately evaluated.

In non-palpable breast lesions, FNAC has also been reported to be useful in the diagnosis of malignancy and in the prediction of invasiveness in a malignant aspirate. Similar to the palpable/high-grade carcinoma aspirates, the presence of tubule formation, cytoplasmic vacuolation formation, fibroblastic proliferation, and elastoid stromal fragments has been reported to be useful to predict invasion. Experience on the FNAC of non-palpable breast malignancy remains limited, and the usefulness of these features requires further substantiation (Bondeson and Lindholm 1997).

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Papillary Lesions of the Breast

10

Andrew S. Field and Gary Tse

10.1 Introduction

The diagnosis of papillary lesions of the breast by FNAC is a contentious and confusing area, and this is due at least in part to the varying surgical pathology definitions of papillary lesions, and these usually include benign, borderline, and malignant papillary lesions, as discussed previously.

In the FNAC diagnosis of breast papillary lesions, the differentiation of such lesions into at least benign or malignant may be useful, as the current recommendations for any "papillary" lesion diagnosed on core or FNAC biopsy is wide excision (Ueng et al. 2009), whereas lumping all these lesions into a single category of "papillary lesions" (Masood et al. 2003) may not be desirable. In the past, many of these lesions were given a diagnosis of "proliferative lesions" or "papillary lesions," with a hope that these would be biopsied or excised for accurate diagnosis. With increasing experience in mammography including tomographic mammography, diagnostic ultrasound, and MRI assessment of the breast, radiologists have become more specific in their diagnoses. Attempts should be made for more specific cytological diagnoses to highlight potential discrepancies between imaging and FNAC findings and to improve the accuracy of the "triple test" which correlates clinical, imaging, and cytological findings.

It would be useful to define at the outset the terms of "a true papillary tissue fragment," as a tissue fragment with a fibrovascular core covered by epithelium, and "micropapillary," as a rounded epithelial tissue fragment without a fibrovascular core that has a narrow neck attaching it to a larger epithelial fragment. The term "papillary" can be used to describe a rounded epithelial fragment, which on occasion may contain a microcalcification.

As papillary lesions or papillomas are frequently complicated by epithelial proliferation of different types, recognition of epithelial hyperplasia and its distinction from low- and intermediate-grade in situ or invasive ductal carcinomas are crucial in diagnosing papillary lesions. Other cytological features such as the nature of stromal fragments present greatly assist in diagnosing intraduct papillomas and distinguishing them from fibroadenomas.

Another important criterion is cellularity, which depends not only on the nature of the lesion but also to a great degree on the skill of the operator and how the smears are made. Thus, cellularity is best assessed in a well-made smear, by looking at the center of the smeared material because crush artifact will cause dispersal in the tail of the smear away from the label. Crucially in breast FNACs, the most common benign lesions such as fibroadenomas may produce marked epithelial cellularity, and unlike other body sites, where high cellularity usually is associated with malignancy, this is frequently not so in the breast. However, cellularity is an essential part of the diagnostic assessment of breast lesions and also provides a measure of the confidence that can be put in a diagnosis: the more material as highlighted by high epithelial cellularity, the more confident is the diagnosis.

G. Tse et al., *Fine Needle Aspiration Cytology of the Breast*,
DOI 10.1007/978-3-642-35000-9_10,

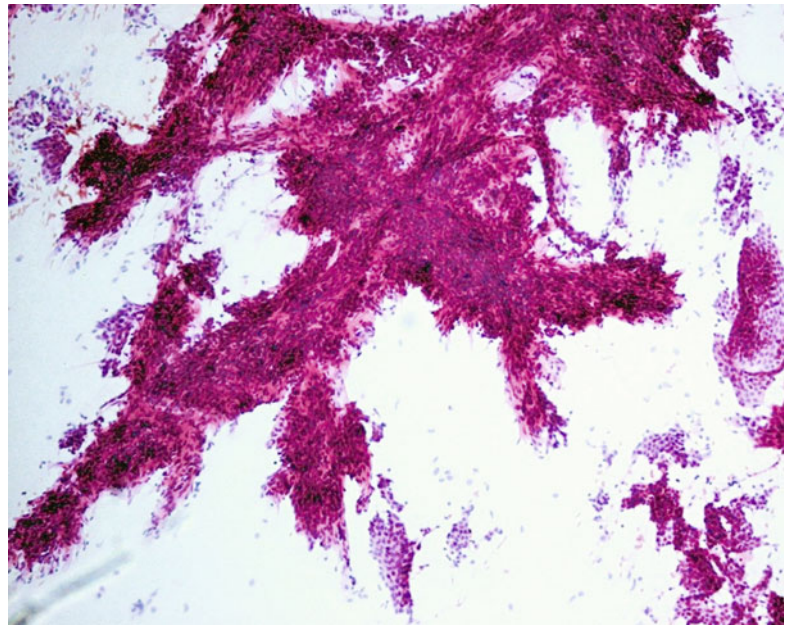

Fig. 10.1 Cytologic preparation showing true papillary firagments with discernible fibrovascular cores. Sheets of benign hyperplastic epithelial cells are seen in the background

10.2 Intraduct Papilloma

10.2.1 Clinical and Epidemiological Findings

Papilloma can be central and solitary and may present with nipple discharge. Imaging may show a rounded lesion with varying echogenicity on ultrasound within a recognizable dilated duct, and there may be demonstrable blood flow into the lesion. Papillomas have a minimally increased risk of subsequent carcinoma in the same or tralateral breast of up to 2×, while multiple smaller peripheral papillomas arising in a background of fibrocystic change with epithelial hyperplasia, florid epithelial hyperplasia, or radial scars have an increased risk similar to the background proliferative changes.

10.2.2 Cytologic Findings

Papillomas usually show the diagnostic meshwork, stellate tissue fragments, and less commonly, true papillary tissue fragments in FNAC. The meshwork tissue fragments have a criss-crossing mesh of usually sclerotic and elastotic thin stromal strands containing tubules of bland ductal cells and myoepithelial cells resembling adenosis (Figs. 10.1 and 10.2). The stellate tissue fragments may have a similar sclerotic or a more fibroblastic branching stellate cores, including thin nonstaining elastotic fibrils, covered in hyperplastic sheets of ductal cells with myoepithelial cells (Figs. 10.3 and 10.4). The stellate tissue fragments represent the entire small papillomas or portions of larger papillomas most typically seen deep to the nipple. The two types of tissue fragments can merge.

The number of dispersed cells and bare bipolar nuclei varies and may be masked by the proteinaceous background or blood, and columnar cells as well as apocrine metaplasia may be prominent. Siderophages may be more numerous than histiocytes, due to hemorrhage into the duct or even papilloma infarction, the latter presenting as nipple discharge.

Once the meshwork and stellate tissue fragments are recognized, the attached epithelium

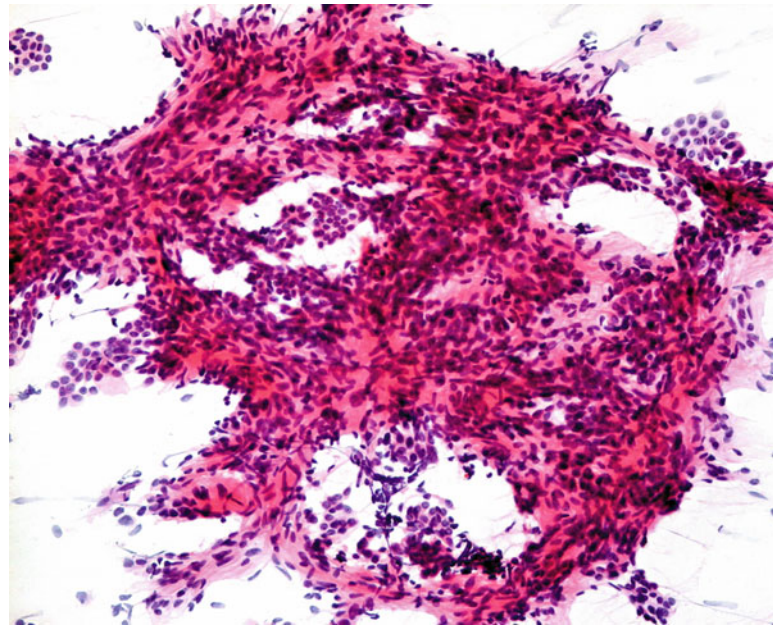

Fig. 10.2 Cytologic smear of a meshwork fragment displaying stromal strands covered by epithelial cells

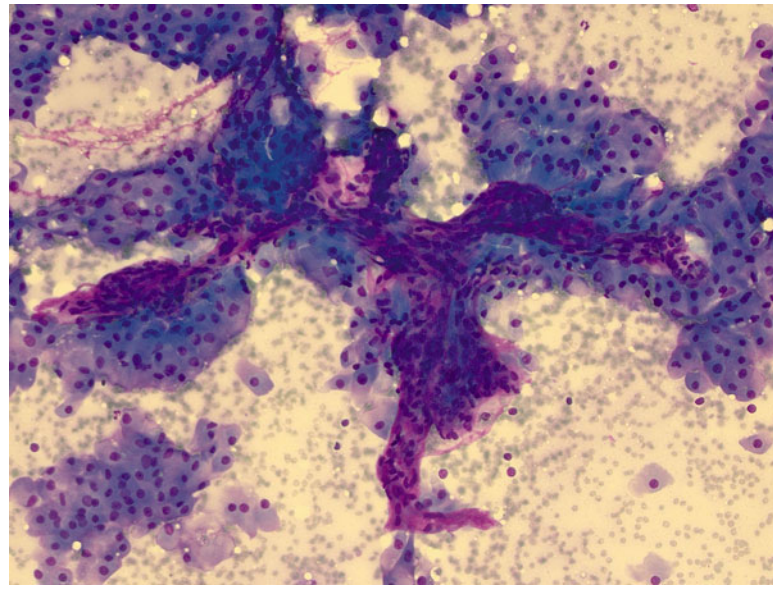

Fig. 10.3 Papillary fragment with sclerotic fibrovascular cores and showing fibroblasts, blood vessels, and myoepithelial cells. The epithelial cells show apocrine changes

and dispersed cells should be assessed for atypia, using the criteria of nuclear enlargement, anisonucleosis, hyperchromasia or pleomorphism, and architectural arrangement with overlapping.

In the epithelial fragments, there should be no micropapillary or distinct cribriform architecture, but rather the pattern of typical florid epithelial hyperplasia. Micropapillae have narrow necks and bulbous rounded, hard-edged tips showing

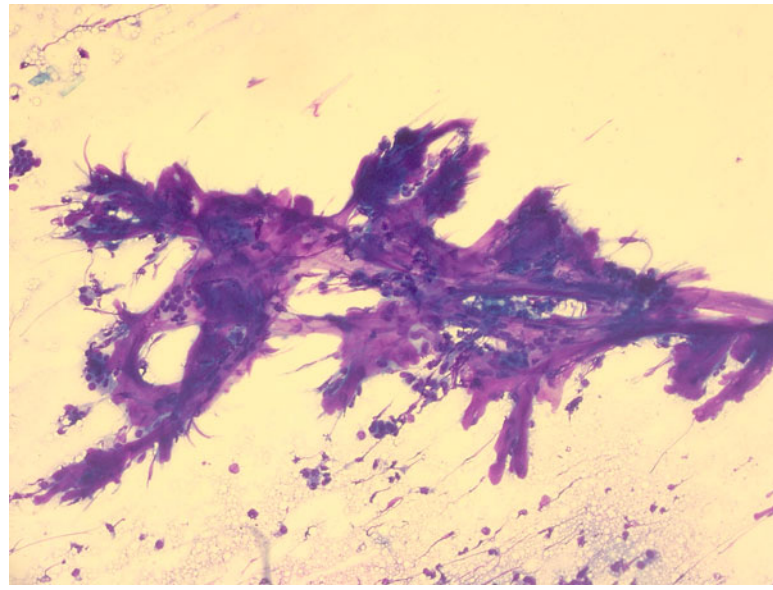

Fig. 10.4 Papillary fragment showing the fibrotic core with branching

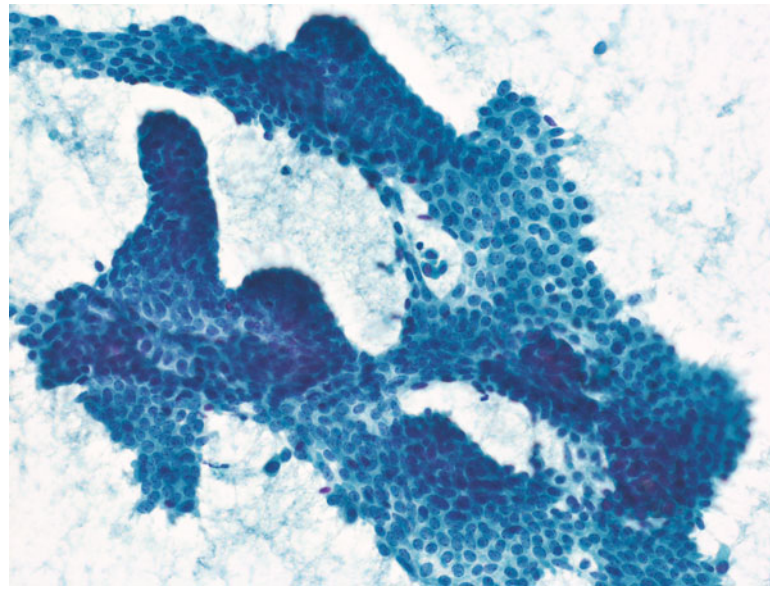

Fig. 10.5 Epithelial fragments showing micropapillae formation. Micropapillae are devoid of genuine fibrovascular cores. The epithelial cells show slight nuclear pleomorphism and crowding

nuclear crowding and overlapping, but without fibrovascular cores (Figs. 10.5 and 10.6). True papillae are relatively uncommon, although tufting of epithelium presenting in cytology as irregular ductal tissue fragments may occur.

Infarction of papillomas can produce atypical, small rounded epithelial fragments, but recognition of the other features of intraduct papilloma and the presence of partial cellular degeneration contributing to the nuclear hyperchromasia and atypia in a background of old blood and siderophages should prevent a false-positive diagnosis of malignancy.

The background may show fibrocystic changes and papillary apocrine hyperplasia, with true papillae covered in columnar metaplastic apocrine cells showing low N:C ratio and round nuclei showing minimal nuclear atypia, and these may form large micropapillary tissue fragments. Despite the high cellularity and the

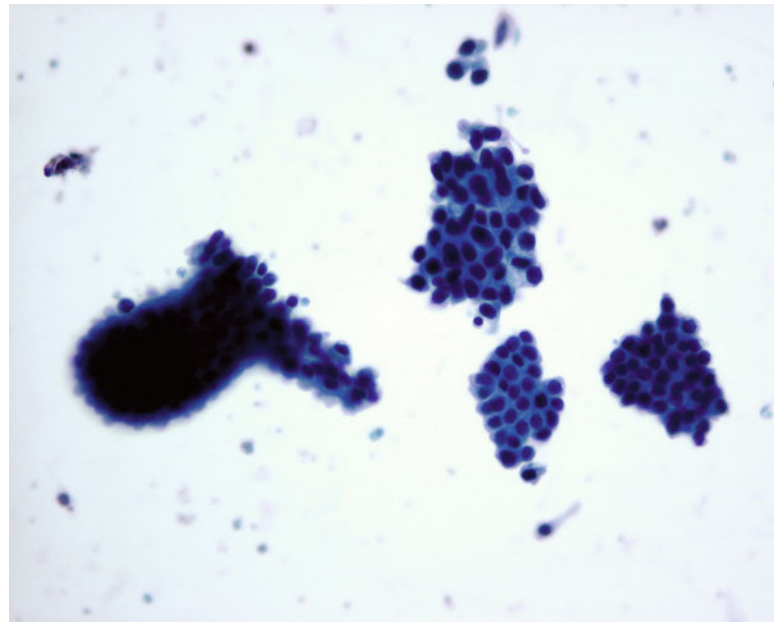

Fig. 10.6 Small epithelial fragments of micropapillae. These epithelial cells show rounded and slightly hyperchromatic nuclei, and fibrovascular cores are absent

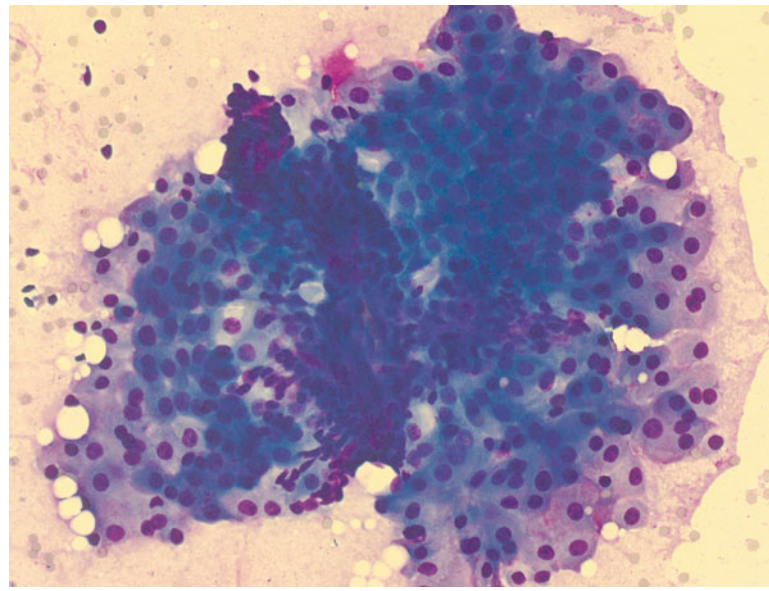

Fig. 10.7 Juvenile papillomatosis showing a large fragment with identifiable fibrovascular cores in the center, surrounded by hyperplastic epithelial cells, many of which show apocrine metaplasia

micropapillary tissue fragments, these cases can be reported as benign, due to the lack of atypia in the apocrine cells.

In juvenile papillomatosis, there is high cellularity with a pattern of large sheets of metaplastic apocrine cells and sometimes hyperplastic ductal tissue fragments, and fragments resembling stellate tissue fragments but having very thin, magenta fibrovascular strands as cores, often with prominent myoepithelial cells (Figs. 10.7 and 10.8). These cores lack the elastotic fibrils of papilloma stellate fragments. There is a proteinaceous background with variable but usually scant histiocytes.

In some cases, although the meshwork, stellate, and true papillary tissue fragments may be present, the degree of epithelial cellularity, especially in a postmenopausal patient not receiving

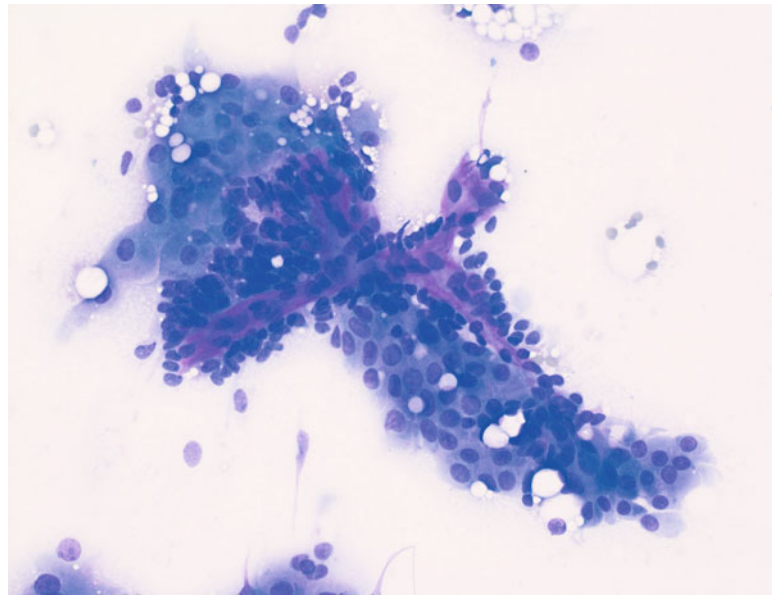

Fig. 10.8 Another papillary fragment in juvenile papillomatosis showing hyperplastic epithelial cells, some of which show apocrine metaplasia

hormonal replacement therapy, the degree of cellular dispersal, and the presence of significant nuclear or architectural atypia will be of a degree that the lesions should be reported as "intraduct papillomas with epithelial hyperplasia with atypia" and excision rather than a core biopsy recommended. The recognition of the intraduct papilloma criteria assists in avoiding a false-positive diagnosis of malignancy, and ductectomy or other appropriate surgery can be recommended.

10.2.3 Differential Diagnosis

The low-power features of papillomas are the same as epithelial hyperplasia and radial scars, but these lesions lack the meshwork, stellate, and true tissue fragments of papilloma. It is appropriate to discuss these differential diagnoses in detail.

10.2.3.1 Epithelial Hyperplasia

FNAC of epithelial hyperplasia has been previously discussed. The salient features are highlighted here. There are usually large epithelial fragments showing a biphasic pattern with myoepithelial nuclei recognizable on the well-ordered sheets and three-dimensional fragments of ductal epithelial cells. The recognition of myoepithelial cells as bare oval bipolar nuclei in the background, showing uniform fine chromatin without nucleoli, is crucial in making the diagnosis of epithelial hyperplasia (Fig. 10.9).

10.2.3.2 Atypical Epithelial Hyperplasia

FNAC may show some degree of cytologic atypia with nuclear crowding, overlapping, enlargement, hyperchromasia, and irregularities, and these may become marked, either focally or throughout the epithelial material. Architectural atypia of prominent intact cell dispersal or complex tissue fragments may with possible cribriform, or micropapillary areas, may also be seen (Fig. 10.10).

10.2.3.3 Columnar Cell Changes

In cytology, the dilated terminal ducts and ductules have a ballooned tissue fragment appearance, resembling a "conquistador's helmet," while smaller tissue fragments and

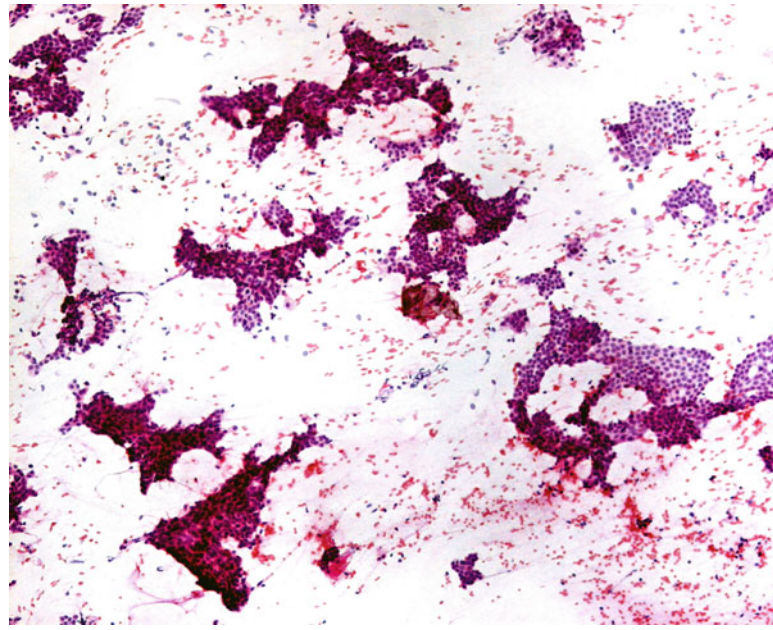

Fig. 10.9 Aspirate of epithelial hyperplasia showing sheets of benign-looking ductal epithelial cells admixed with myoepithelial cells. Some of the epithelial sheets show three-dimensional architecture

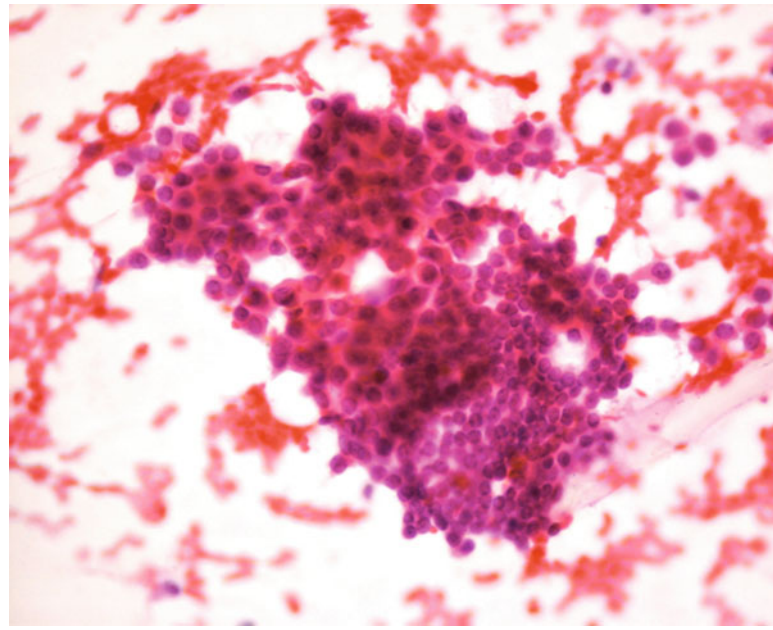

Fig. 10.10 Aspirate of ADH showing hyperplastic epithelial cells forming vague structures of lumen formation and rudimentary cribriforming

dispersed cells have columnar cell features, with myoepithelial cells and bare bipolar nuclei. There may be an increased number of dispersed columnar cells in the usually proteinaceous background, and some tissue fragments consisting of columnar cells may show nuclear enlargement, crowding, and atypia, corresponding to the spectrum in surgical pathology of these lesions and warranting an atypical diagnosis. Calcifications may be present (Fig. 10.11).

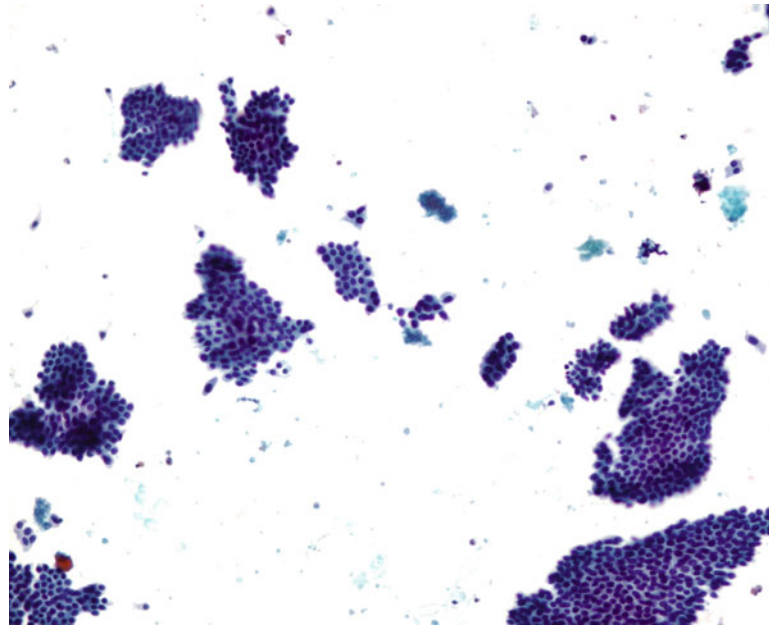

Fig. 10.11 Columnar cell changes showing ballooned tissue fragments with uniform monotonous epithelial cells. Occasional single columnar cells are noted in the background

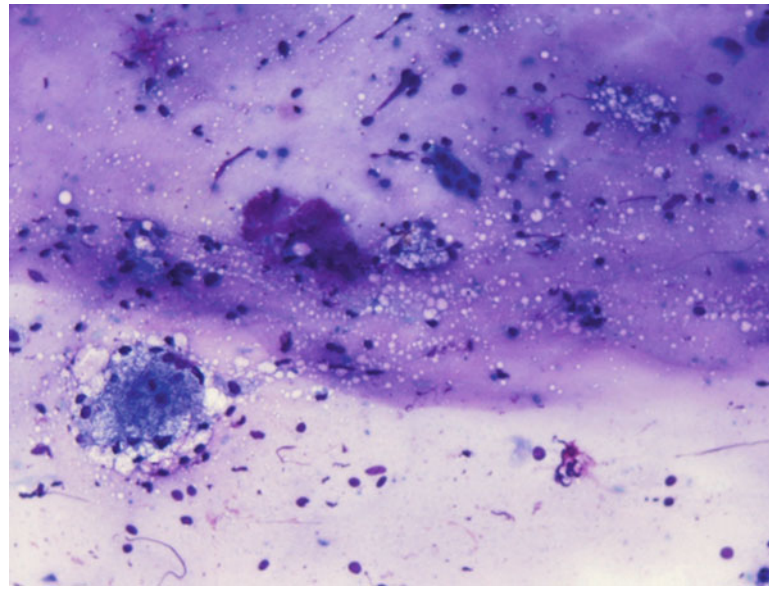

Fig. 10.12 Radial scar shows dispersed ductal cells and hyperplastic tissue fragments, but lack of meshwork or stellate tissue fragments

10.2.3.4 Radial Scar

Radial scars often show marked cellularity, increased dispersed ductal cells, and hyperplastic tissue fragments in FNAC. They have the features of "florid epithelial hyperplasia with fibrocystic change" with accentuated cellularity and plentiful large hyperplastic ductal tissue fragments. In this setting, the low-power pattern is crucial to the correct diagnosis. The low-power pattern is very similar to that of a papilloma, and the distinction from papilloma is made on the absence of meshwork or stellate tissue fragments (Field and Mak 2007) (Fig. 10.12).

10.2.4 Histologic Correlations

The histological features of intraduct papillomas vary from a simple single lesion with fibrovascular cores covered in a ductal cell layer with intervening

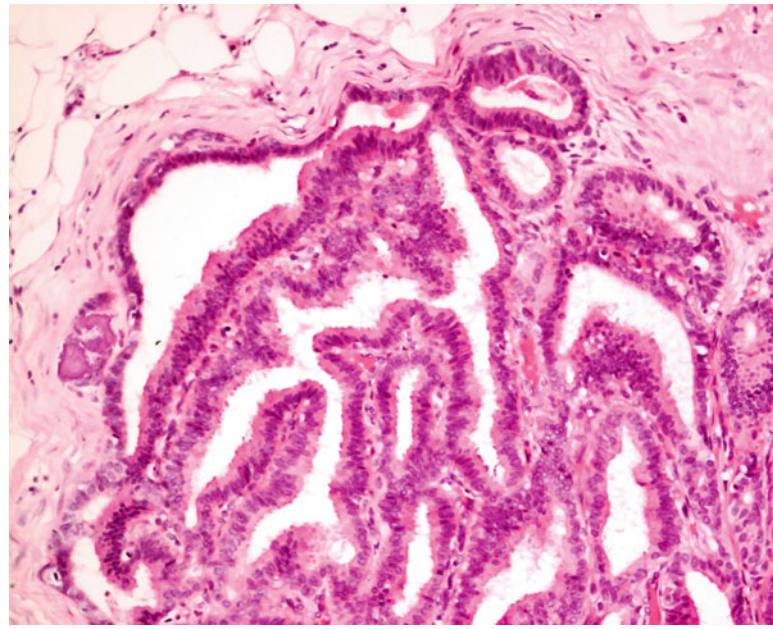

Fig. 10.13 Histologic picture of a papilloma showing broad fibrovascular cores covered by a ductal cell layer with intervening myoepithelial cells, within a dilated duct lined by benign ductal epithelium

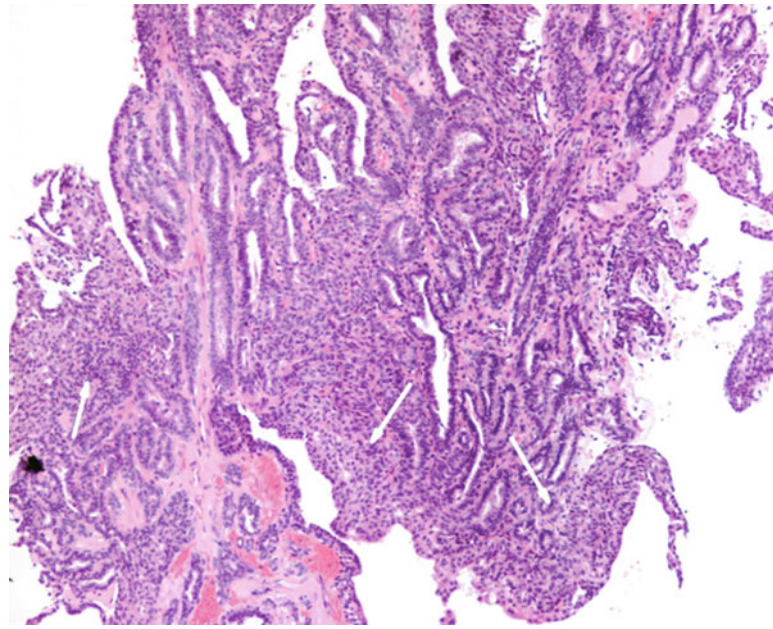

Fig. 10.14 Histologic picture of a papilloma showing broad fibrovascular cores composed of loose fibrotic tissue. The branching of the fibrovascular cores is irregular. Foci of solid florid epithelial hyperplasia are seen within the papilloma (*arrow*)

myoepithelial cells, within a duct lined by similar epithelium, to papillomas that ramify through a network of adjacent ducts (Figs. 10.13 and 10.14). The fibrovascular cores may be sclerotic, or very broad, and the intervening epithelial component may show changes ranging from marked epithelial hyperplasia, ADH, or low- to intermediate-grade DCIS, which may be papillary. Recognition of these lesions in surgical pathology and their distinction from papillary intraductal and invasive lesions in most situations may be straightforward. Recent work suggests that FNAC can reliably recognize this spectrum of intraduct papillomas and their variable epithelial hyperplasia, which is not usually prominently papillary but rather sheet-like with epithelial streaming and slit-like spaces.

However, true papillary tissue fragments with thin fibrovascular cores covered in bland ductal and myoepithelial cell layers do occur in papillomas (Field and Mak 2007).

10.3 Papillary Carcinoma In Situ

10.3.1 Clinical and Epidemiological Findings

The clinical presentation of papillary carcinoma in situ is similar to intraduct papilloma. The radiological appearance is also similar to papilloma. Because of the significant clinical and radiological overlap, accurate differentiation will be based on pathologic assessment.

10.3.2 Cytologic Findings

In papillary carcinoma in situ or intracystic papillary carcinoma, the true papillary tissue fragments have thin fibrovascular cores and a crowded, overlapping, and multilayered, often columnar epithelium showing moderate to marked nuclear enlargement and atypia. Myoepithelial nuclei are absent in the tissue fragments. These tissue fragments are seen amid a large number of dispersed similar cells in a proteinaceous background lacking bare bipolar nuclei. There is no meshwork or stellate tissue fragments as seen in intraduct papilloma, where the nuclear arrangement in the sheets is more ordered and lacks significant overlapping and nuclear atypia is minimal, with myoepithelial cells and bare bipolar nuclei present (Figs. 10.15, 10.16, 10.17, 10.18, and 10.19).

Microcalcifications associated with papillary intraductal carcinoma may be psammomatous or more irregular and angulated and may be faintly birefringent, rather than granular as seen in high-grade DCIS, but this is rarely helpful in the differential diagnosis. However, if calcifications are seen, this should be reported to assist with correlation with imaging findings.

There will be cases where the degree of nuclear or architectural atypia is greater than expected in the range of epithelial hyperplasia associated with a papilloma, but there will be no

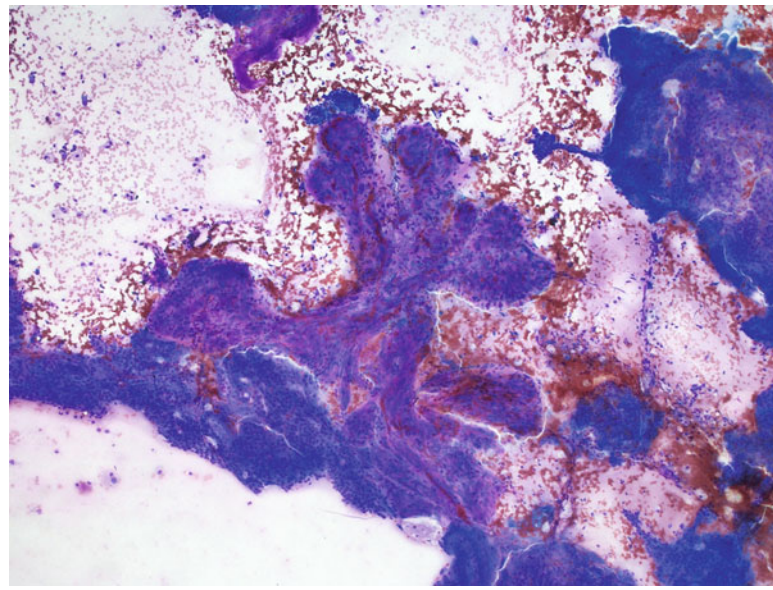

Fig. 10.15 Papillary carcinoma in situ showing large overlapping sheets of epithelium

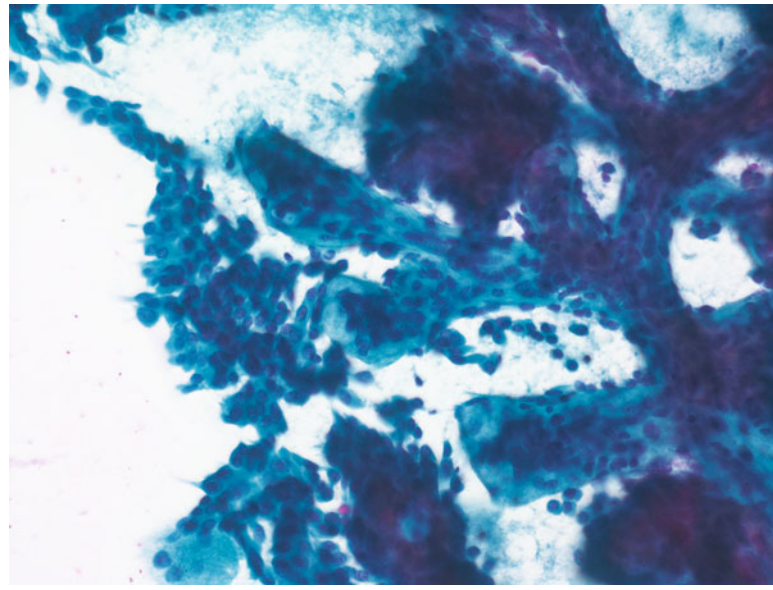

Fig. 10.16 Papillary fragments in papillary carcinoma in situ showing thin fibrovascular cores that are lined by mildly atypical epithelial cells showing nuclear crowding

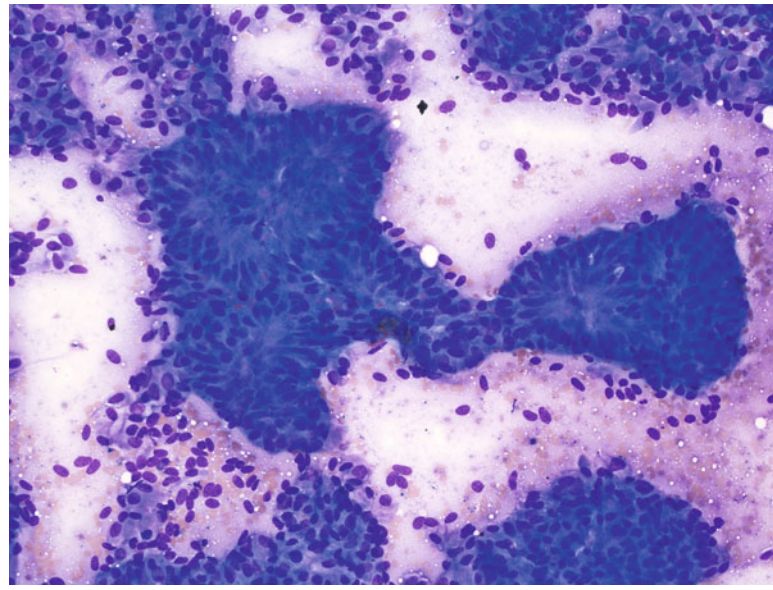

Fig. 10.17 Epithelial papillary fragments in papillary carcinoma in situ showing mildly atypical epithelial cells and the paucity of myoepithelial cells

micropapillary or cribriform tissue fragments. These should be signed out as "papilloma with epithelial hyperplasia with atypia" or, if the degree of atypia is more marked, "suspicious of low- to intermediate-grade intraduct carcinoma." It is not appropriate to attempt to make a cytological diagnosis of "ADH." In addition, some of these cases of atypia will be found to be low-grade invasive carcinomas, and evidence of invasive carcinoma such as the presence of crowded, rigid atypical tubules, tufted sclerotic stromal fragments infiltrated by atypical epithelial strands, and prominent dispersal of atypical epithelial cells should be searched for.

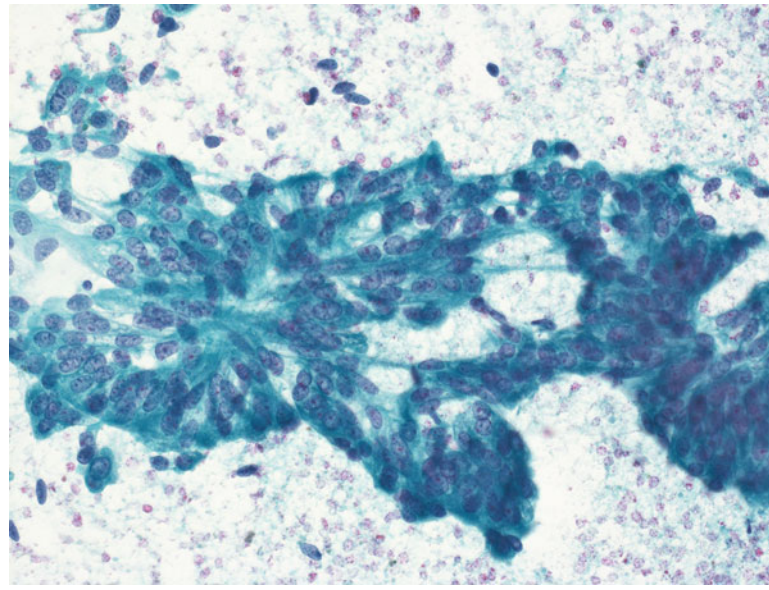

Fig. 10.18 Cytologic details of the epithelial cells in papillary carcinoma in situ, showing a single-cell population of epithelial cells with mild nuclear atypia. No myoepithelial cells identified

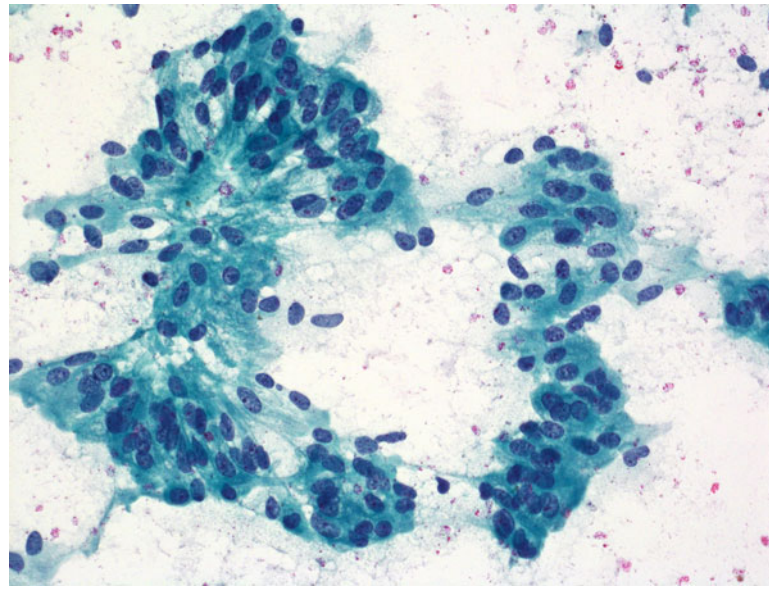

Fig. 10.19 Cytologic details of the epithelial cells in papillary carcinoma in situ, showing a single-cell population of epithelial cells with mild nuclear atypia. No myoepithelial cells identified

10.3.3 Differential Diagnosis

10.3.3.1 Benign Papillary Lesions

Within the telltale tissue fragments present within the FNAC, the increased nuclear size, mild atypia, and disordered arrangement are subtle but good clues to suggest papillary carcinoma in situ, which can then be confirmed by the lack of myoepithelial cells on the tissue fragments and the lack of bare bipolar nuclei in the background. Care should be taken when assessing the presence of myoepithelial cells: only those nuclei which are perfectly oval and lacking nucleoli should be accepted, as degenerate nuclei and apoptotic debris can mimic myoepithelial cells.

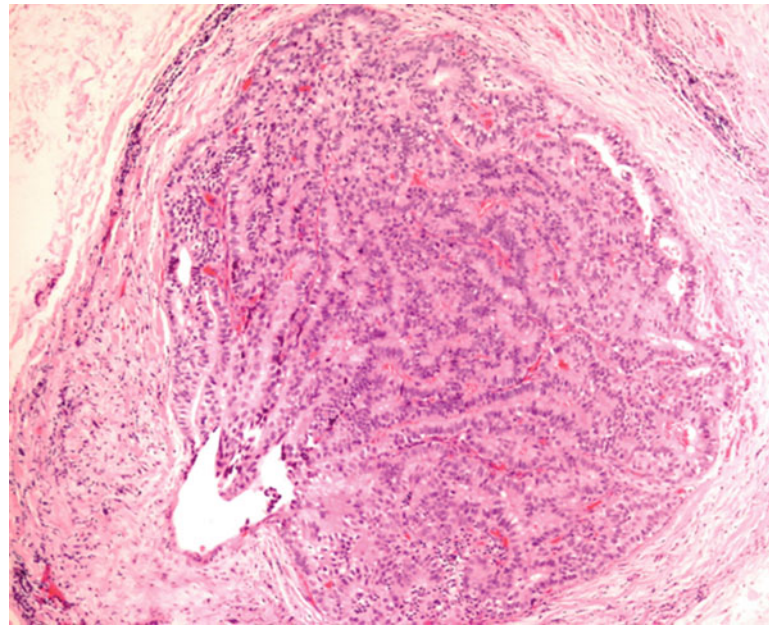

Fig. 10.20 Papillary carcinoma in situ showing proliferation of monotonous epithelial cells lining fine and elaborate fibrovascular cores

10.3.3.2 Other Types of Low-Grade Carcinoma In Situ

The surgical management of most low-grade carcinoma in situ, including papillary carcinoma in situ, is similar; in addition, very often these different patterns of carcinomas in situ are present in a mixed pattern. The clinical significance of the exact differentiation between different patterns may not be high. Typically, the characteristic cribriform pattern is best highlighted in the cribriform tissue fragments in Papanicolaou-stained smears where the punched out holes with radiating nuclei can be easily recognized and the degree of nuclear atypia more easily assessed. Distinguishing cribriform holes from secondary slit-like lumina in epithelial hyperplasia tissue fragments in Giemsa-stained smears is more difficult, but in some cases, almost every tissue fragment will show frequent craterlike or pockmark depressions which represent a cribriform architecture.

10.3.3.3 Invasive Low-Grade Ductal Carcinoma

Distinguishing papillary carcinoma in situ (and other intraductal carcinomas of low and intermediate grade with cribriform or solid sheet patterns) from invasive low-grade ductal carcinoma is problematic. Features that suggest intraductal carcinoma and those that suggest invasion (tubules, tufts of stroma, stromal fragments infiltrated by carcinoma, marked dispersal) should be noted, but often a firm diagnosis cannot be made.

10.3.4 Histologic Correlations

Papillary carcinoma in situ usually shows low-grade carcinoma cells present within and distending ducts. These cells are arranged in a fine and aborizing papillary pattern with elongated and thin fibrovascular cores. Calcifications may be seen, but necrosis is distinctly rare. The epithelial cell population is monotonous, with mild pleomorphism and fine chromatin pattern (Figs. 10.20 and 10.21). Myoepithelial cells are absent, in contradistinction to the benign counterpart of a duct papilloma where there is a complete layer of myoepithelial cells between the fibrovascular cores and the epithelium. Furthermore, when compared to duct papilloma, papillary carcinoma in situ tends to show higher epithelial cellularity and finer and thinner fibrovascular cores with more elaborate patterns.

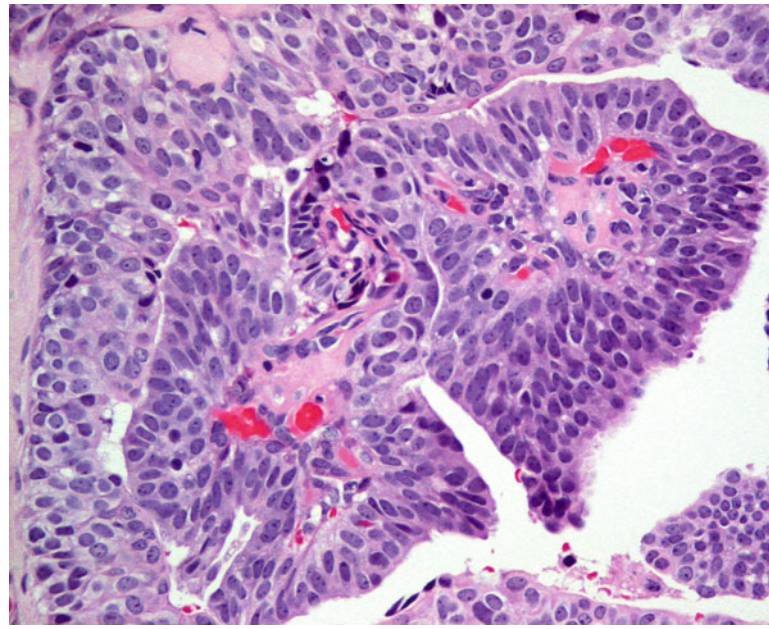

Fig. 10.21 Histologic details of papillary carcinoma in situ showing layers of mildly atypical epithelial cells over thin and delicate fibrovascular cores, devoid of an intervening myoepithelial cell layer

10.4 Solid Papillary Carcinoma

10.4.1 Clinical and Epidemiological Findings

Solid papillary carcinoma usually occurs in elderly patients, presenting with a nodular mass that is slow growing. The tumor nodules are usually well demarcated and rounded, and an infiltrative border is not a common characteristic. These features are well demonstrated at imaging. While solid papillary carcinoma is regarded as in situ (stage Tis) disease by the WHO Classification of breast tumors, there are occasions when it may be considered invasive.

10.4.2 Cytologic Findings

In FNAC material, there is high cellularity, prominent dispersal as well as irregular tissue fragments and sheets, and distinctive fibrovascular tissue fragments in which the coiled anastomosing capillaries have bulbous tips formed by the capillaries as they curve around, lined by endothelial cells and often containing red cells. Sheets of discohesive epithelium are usually loosely attached, with very prominent dispersal and demonstrating low to intermediate nuclear grade. Actual micropapapillae are not seen, although cribriform tissue fragments can be present (Figs. 10.22, 10.23 and 10.24).

10.4.3 Differential Diagnosis

The differential diagnosis is with other low-grade carcinomas in situ including papillary carcinoma in situ.

10.4.4 Histologic Correlations

Solid papillary carcinoma has cribriform intraductal components. It shows multiple ducts filled with a solid proliferation of atypical cells, with single or multiple capillary coils in the midst of the epithelial cells or occasionally taking origin from the duct wall. A proportion of these solid papillary carcinomas will show nuclear streaming and granular nuclear chromatin (Figs. 10.25 and 10.26). Many may express neuroendocrine

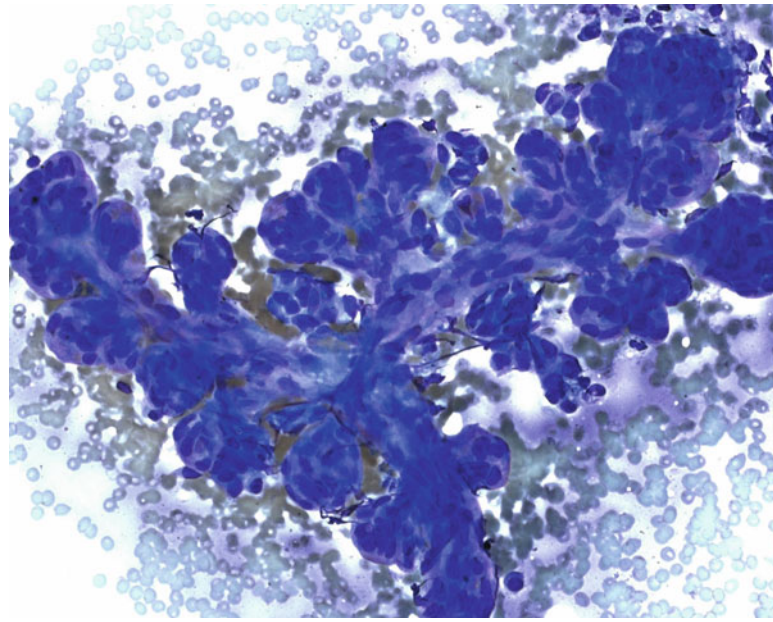

Fig. 10.22 Solid papillary carcinoma showing epithelial fragments with distinctive fibrovascular tissue fragments with bulbous tips

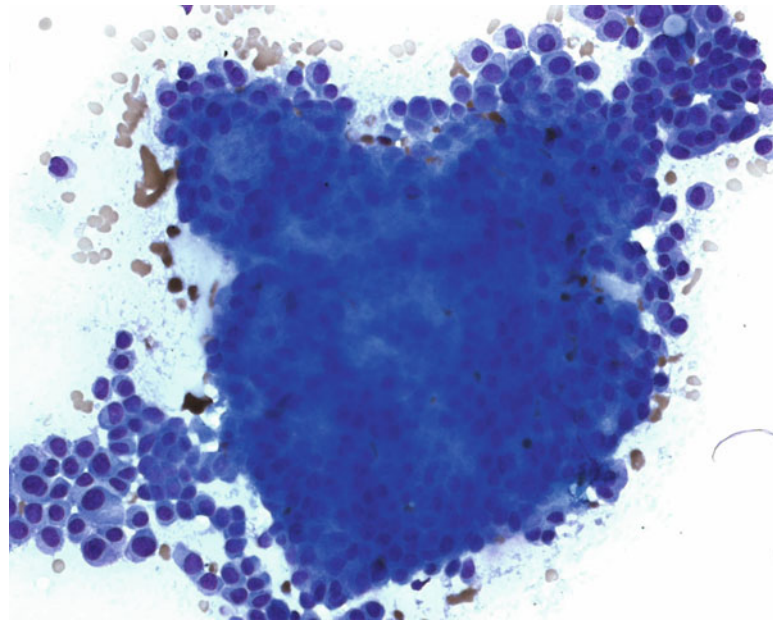

Fig. 10.23 Large cellular fragment of solid papillary carcinoma with rounded and blunt bulbous tips. The lining epithelium shows eccentric nuclei devoid of prominent nucleoli, and granular cytoplasm, suggestive of neuroendocrine differentiation

markers including synaptophysin and chromogranin. The ducts may expand markedly, and in these cases myoepithelial cells around the rim and covering the capillary loops may become scant or even absent. When the solid papillary islands have jagged shapes and are completely devoid of rimming myoepithelial cells, with accompaniment by a desmoplastic stroma, some authors regard these histological alterations as reflecting an invasive disease.

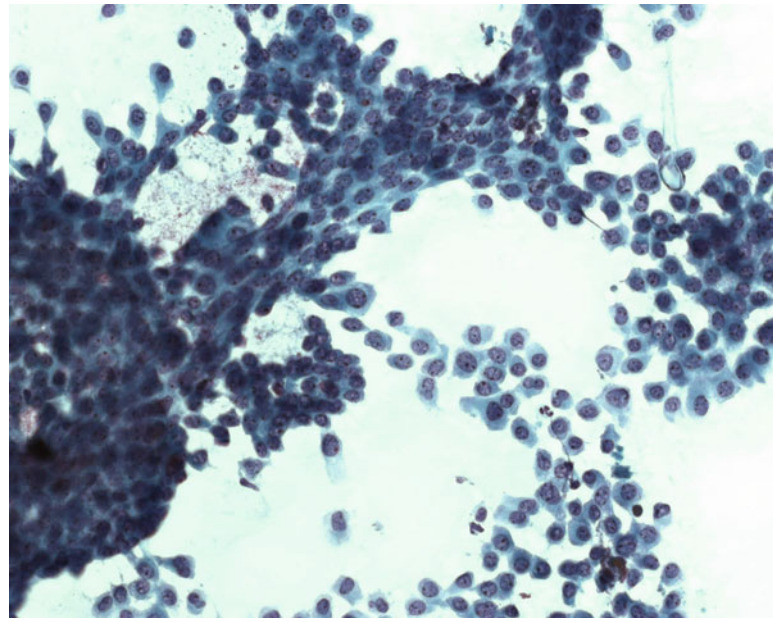

Fig. 10.24 Cellular details of solid papillary carcinoma showing epithelial cells with eccentric nuclei and moderate to abundant granular cytoplasm, suggestive of neuroendocrine differentiation. Abundant single cells with similar morphology are usually seen in the background

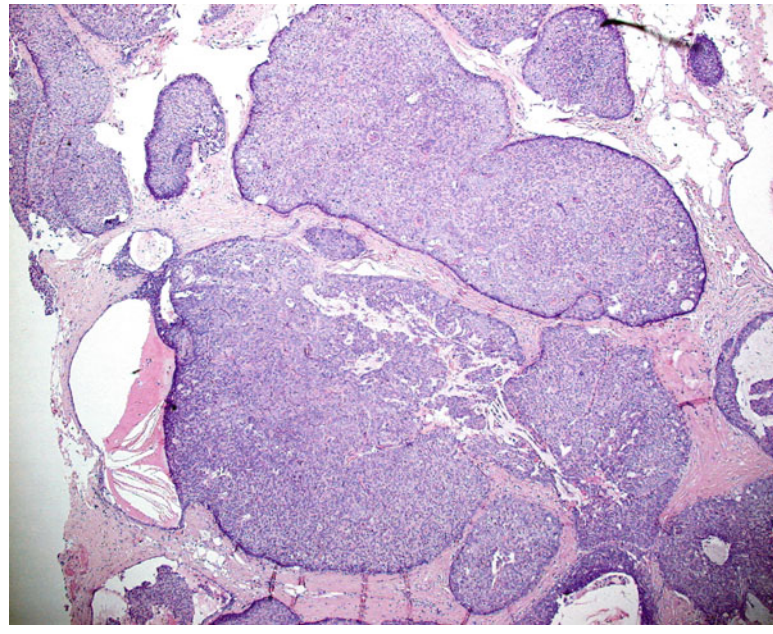

Fig. 10.25 Histology of a solid papillary carcinoma showing ducts filled with a solid proliferation of atypical cells, with single or multiple capillary coils in the midst of the epithelial cells. Fibrovascular cores are infrequent within the epithelial proliferation

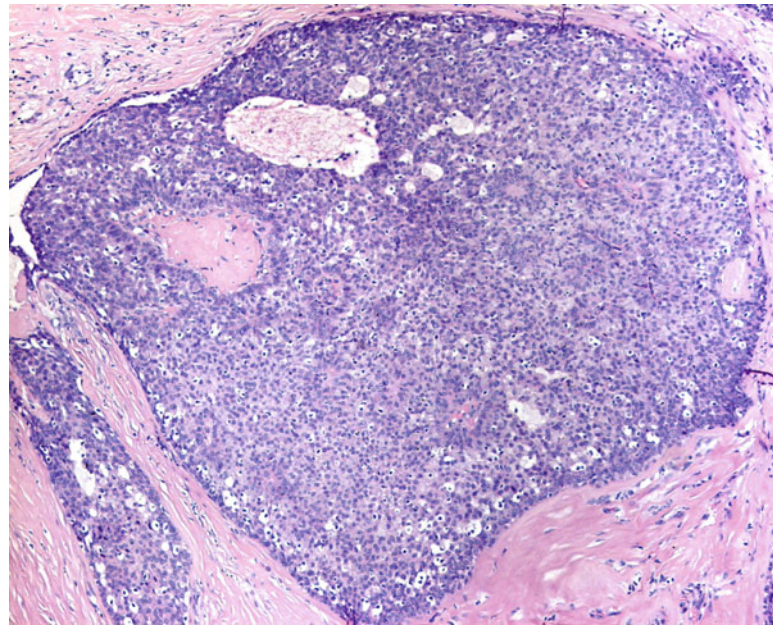

Fig. 10.26 Solid papillary carcinoma with epithelial cells filling up the entire ductal space; only several small fibrovascular cores can be identified. The cells show spindled nuclei, and the architectural pattern may give an impression of nuclear streaming

10.5 Encapsulated Papillary Carcinoma (Intracystic Papillary Carcinoma, Encysted Papillary Carcinoma)

10.5.1 Clinical and Epidemiological Findings

The clinical and epidemiological characteristics of encapsulated papillary carcinoma are similar to other low-grade papillary carcinomas. On ultrasound imaging encapsulated papillary carcinomas usually present as somewhat irregular cystic structures with heterogeneous echoing with or without foci of "wall thickening," and although usually less than 3 cm, they can be larger. Owing to the absence of myoepithelial cells in the cystic wall and papillary fronds of the encapsulated papillary carcinoma, it has been suggested that this tumor may represent an indolent form of invasive cancer with a rounded pushing front, or a carcinoma in transition from the in situ to invasive phase. The Working Group of the WHO Classification of Breast Tumors (2012) regard encapsulated papillary carcinoma as in situ (Tis) disease.

10.5.2 Cytologic Findings

Low-grade or "clinging" intraduct carcinoma has cytologic smears can produce very distinctive mildly crowded micropapillary tissue fragments. In these cases the degree of nuclear enlargement and atypia is mild, and myoepithelial cells on the tissue fragments and bare bipolar nuclei in the background are scant (Figs. 10.27 and 10.28). A diagnosis of "suspicious of low-grade intraduct carcinoma, with or without an invasive component," can be made.

In some cases, large cystic structures may lack epithelium in the FNAC material, which will consist of old blood and granular proteinaceous material with siderophages. Careful examination for epithelial cells is required (cytospin preparations of the "cyst fluid" are recommended); however, in some cases the epithelium may be degenerate or apocrine in type and the distinction from hemorrhage into a benign cyst with reactive atypia becomes problematic. Correlation with imaging findings is essential, and simple excision may be appropriate.

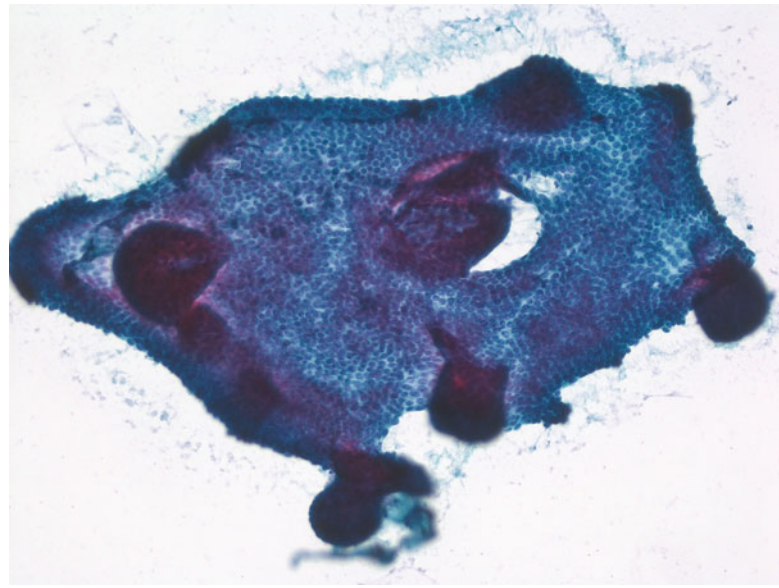

Fig. 10.27 Intracystic papillary carcinoma showing micropapillary tissue fragments with mild degree of nuclear crowding and distinctive rigid micropapillae

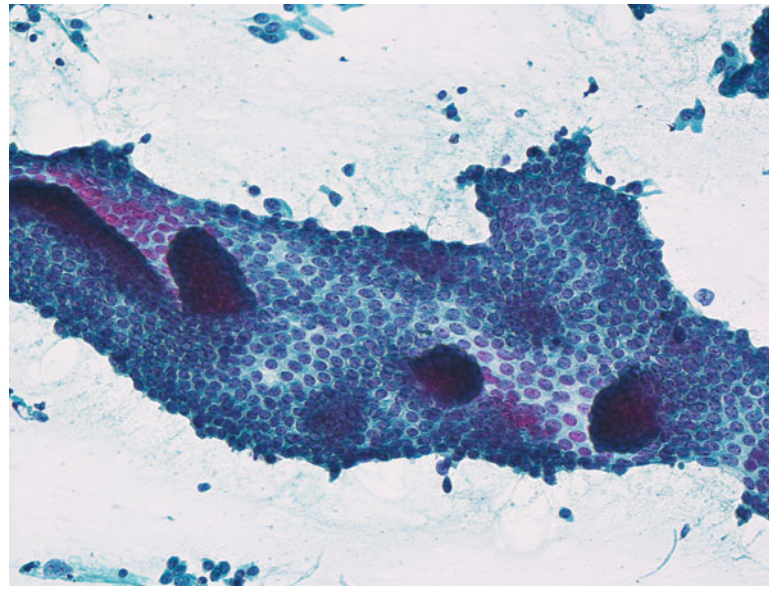

Fig. 10.28 Cytologic cellular details of intracystic papillary carcinoma showing mild nuclear atypia, together with rigid micropapillae

10.5.3 Differential Diagnosis

1. High-grade DCIS can have a tufted or rudimentary micropapillary architecture, again, usually in a mixed pattern with other ducts showing solid, centrally necrotic or cribriform patterns. These lesions produce FNAC smears with a high degree of dispersal, high nuclear grade in large pleomorphic cells, and there may be necrosis or calcifications. True papillary tissue fragments are not seen, and these cases will be signed out as "suspicious of high-grade DCIS with or without aninvasive component".
2. Carcinoma of varying types, including tubular, invasive cribriform, infiltrating ductal, mucinous, and rarely lobular, can arise from intraductal papillary carcinoma and infiltrate the often fibrotic periductal tissue. The features on FNAC reflect the infiltrating component although there may be an admixture of papillary or cystic components.

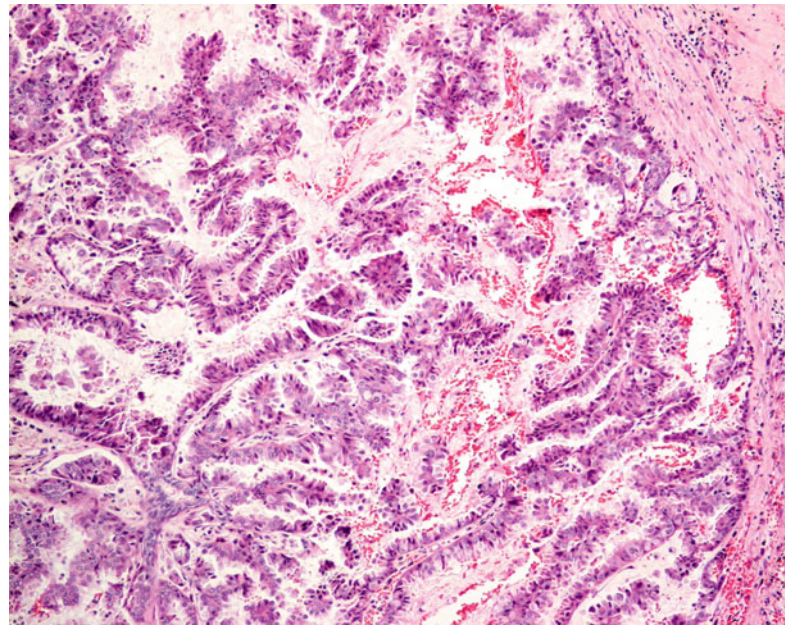

Fig. 10.29 Histology of intracystic papillary carcinoma showing papillary epithelium lacking myoepithelial cells on delicate fibrovascular fronds taking up a variable amount of the cystic space, which is devoid of myoepithelial cell lining

10.5.4 Histologic Correlations

Surgical pathology of these uncommon lesions usually shows papillary epithelium lacking myoepithelial cells on delicate fibrovascular fronds taking up a variable amount of the cystic space, sometimes crowded with the papillary fronds and sometimes showing only focal arborescent papillary structures (Figs. 10.29). The epithelial layer lining the cystically dilated duct is usually considered to be devoid of myoepithelial cells, despite using multiple myoepithelial markers (Collins and Schnitt 2008; Collins et al. 2006). To a certain extent this may be reflected, in a subtle manner, in the cytologic preparations. Most authors will consider this lesion to behave in a manner similar to carcinoma in situ and should be managed as such.

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Mucinous Lesions 11

11.1 Introduction

Mucinous breast lesions refer to a broad spectrum of entities, ranging from benign fibrocystic changes with luminal mucin to mucocele-like lesions and invasive mucinous carcinoma (Tan et al. 2008). There are also lesions with stromal mucinous or myxoid material that mimic the more conventional "mucinous" entities.

Mucins are complex carbohydrates secreted by specialized epithelial and occasionally by connective tissue cells. "Mucoid" and "myxoid" are traditionally used to refer to extracellular mucosubstances of epithelial and mesenchymal origins, respectively.

Apart from lesions associated with extracellular mucin, LN, DCIS and infiltrative lobular carcinomas can demonstrate intracytoplasmic mucin. Stromal myxoid changes can be observed in fibroadenomas and phyllodes tumors, as well as in the less common lesions of pleomorphic adenoma and nodular mucinosis.

While FNAC of mucinous lesions show overlapping features and can potentially be mistaken for one another, there are some distinctive cytological features apart from the presence of mucinous material in the background.

11.2 Mucocele-Like Lesions

11.2.1 Clinical and Epidemiological Findings

Mucocele-like lesions (MLL) were initially described as benign lesions analogous to mucoceles of minor salivary glands (Rosen 1986). They can present as palpable lumps, though impalpable lesions have become increasingly diagnosed with mammographic screening as many MLL are associated with calcifications that form within the extracellular mucin.

11.2.2 Cytologic Findings

FNAC of MLL shows a paucicellular yield with abundant pools of mucin within which sparse bimorphic epithelial clusters float. Scattered bipolar nuclei of myoepithelial cells are observed (Fig. 11.1). If there is ADH or DCIS associated with the MLL, there may be abnormal architectural patterns such as cribriforming or bud-like epithelial forms, though the diagnosis of ADH or DCIS accompanying MLL is extremely difficult to conclude on needle aspirates and requires histological verification.

11.2.3 Histologic Correlations

Histologically, cystically dilated glands distended with mucin are present, often with flattened attenuated epithelium. The cysts rupture and mucin extrudes into the surrounding stroma. MLL can be associated with atypical epithelial changes that range from ADH to DCIS and invasive mucinous carcinoma.

The diagnosis of ADH and DCIS in the context of MLL relies on conventional cytoarchitectural

G. Tse et al., *Fine Needle Aspiration Cytology of the Breast*,
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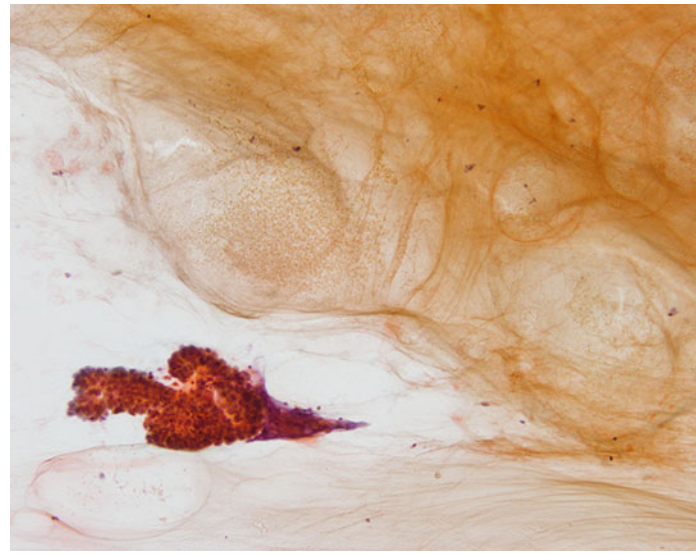

Fig. 11.1 Mucocele-like lesion. Benign bimodal epithelial cluster floats within abundant mucinous material

criteria, with a smaller extent (<2 mm) of involvement by a monomorphic uniform epithelial proliferation with architectural atypia representing ADH and a more extensive lesion (>2 mm) defining low-nuclear-grade DCIS. As their distinction depends on extent criteria, it is clear that this diagnosis cannot be made on FNAC, and it may be that the cytologic conclusion is of an atypical epithelial population within a possible MLL, and to recommend histological confirmation. Even on histology, there are potential challenges in their separation, as some cytoarchitectural abnormalities may not affect the entire duct wall, and the distension of the ducts makes it difficult to ascertain the actual extent of involvement.

11.2.4 Management

Consideration of MLL on preoperative FNAC should prompt surgical excision, as it is difficult to rule out any more sinister associated lesions. MLL on FNAC or core biopsies are categorized as equivocal or borderline (C3, B3). The advent of large core mammotome biopsies has led to debate regarding whether small microscopic extravasated mucin pools in limited quantities should mandate subsequent open excision. On aspirates, however, it is not likely that a microscopic MLL will be removed by the needling procedure and should therefore be followed with surgical excision.

There is also the difficulty of ensuring that a paucicellular mucinous carcinoma is not the underlying lesion. Close clinicoradiological and pathological correlation will be helpful.

11.3 Mucinous Carcinoma

11.3.1 Clinical and Epidemiological Findings

Mucinous carcinoma, also known as mucoid, colloid, or gelatinous carcinoma, is a special subtype of invasive breast carcinoma with an excellent prognosis, with 10-year survivals in excess of 80 %. It constitutes about 2 % of all breast cancers, occurring in women of older age group (Lakhani et al. 2012). Mammographically, it mimics a benign process with its rounded contours. On ultrasound, it is seen as a hypoechoic mass. Clinically, it can present as a palpable soft lump.

11.3.2 Cytologic Findings

Aspirates from mucinous carcinoma can range from low epithelial cellularity to those that reflect a higher cellular yield. Mucinous pools are observed in the background (Fig. 11.2). The epithelial cells can be bland and mimic benign clusters, but the presence of intact cells with retained cytoplasm is often seen (Fig. 11.3). Nuclear size

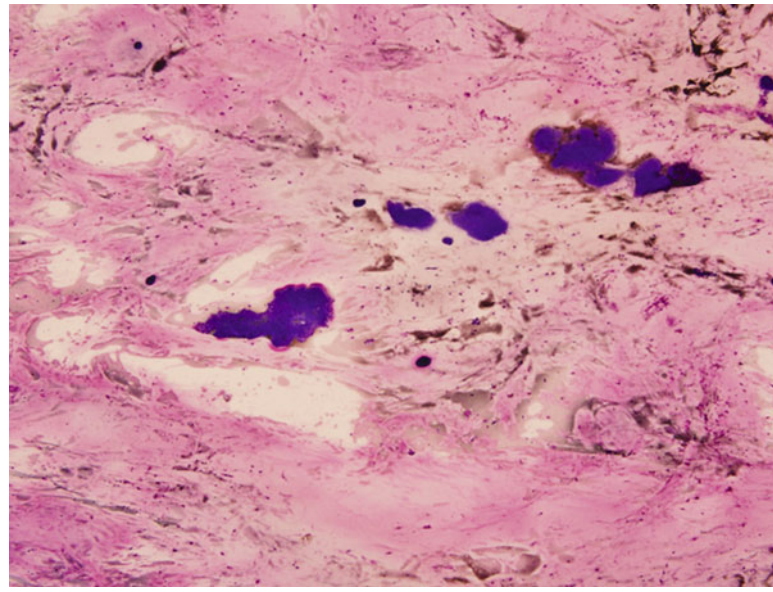

Fig. 11.2 Mucinous carcinoma. Diff-Quick stain shows cohesive groups of epithelial cells within a mucoid background

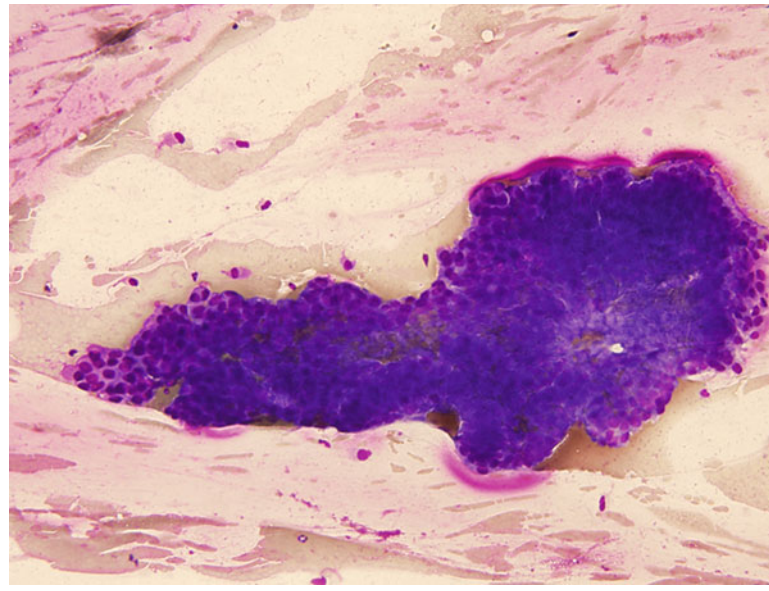

Fig. 11.3 Mucinous carcinoma. High magnification of an epithelial cluster showing relatively uniform nuclei. Metachromatic mucoid material is noted. A few dissociated cells with retention of cytoplasm are seen

enlargement and variation can be noted. Some of the epithelial cells have plasmacytoid appearances with eccentric nuclei and amphophilic cytoplasm with occasional granularity, and these cases may be correlated with histological evidence of neuroendocrine differentiation.

11.3.3 Histologic Correlations

Defined as a tumor composed of malignant epithelial cells floating within extracellular mucin lakes, there needs to be at least 90 % pattern purity to be classified as a mucinous carcinoma with excellent outcome (NHS Breast Screening Programme 2005) (Fig. 11.4a, b). Some pathologists require low nuclear grade accompaniment (grade 1 or sometimes grade 2 nuclei) for its diagnosis, precluding grade 3 nuclear changes for the diagnosis of mucinous carcinoma. The excellent outcome for conventional mucinous carcinoma has been postulated to be related to the barricade formed by mucin enclosing the malignant epithelial cells preventing their spread, as

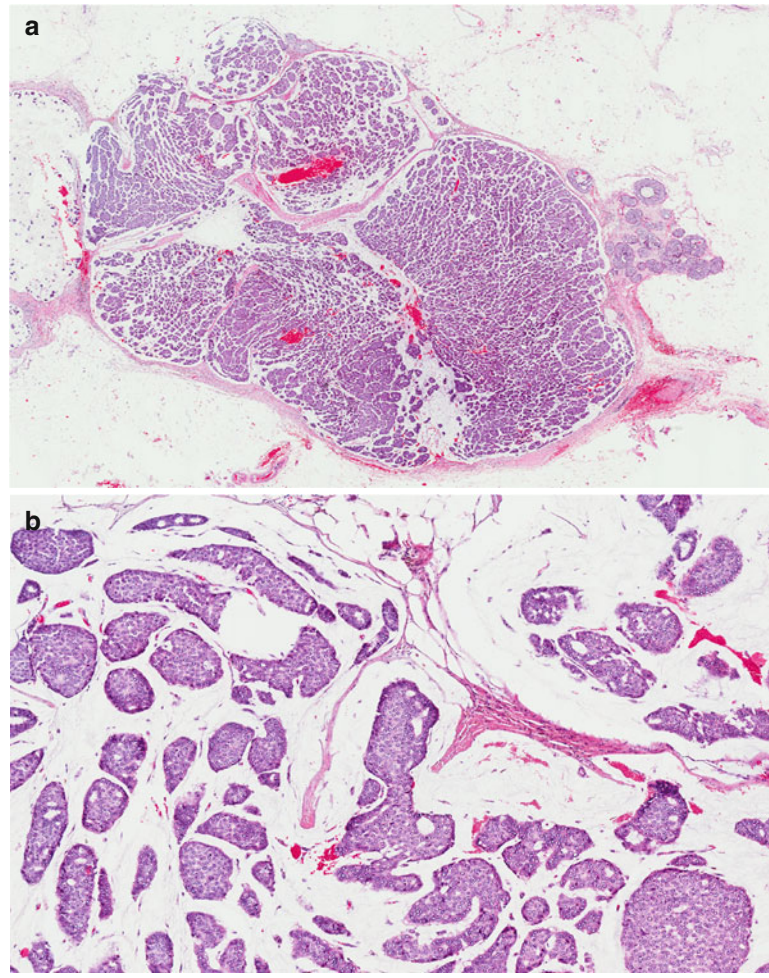

Fig. 11.4 (**a**) Scanning view of the circumscribed mucinous carcinoma, with islands of DCIS at the periphery. (**b**) Higher magnification shows solid nests of tumor cells within mucin pools

well as the diminished tumor cell burden especially in the paucicellular variant, decreased angiogenesis, and increased cytotoxic T lymphocyte activity induced by the extracellular mucin.

Two main forms of mucinous carcinoma have been described by Capella et al. in 1980 – type A and type B, with type AB having transitional features. Type A mucinous carcinoma, considered the classical variety, harbors copious amounts of extracellular mucin, and these are the lesions that will produce paucicellular mucin-rich smears that can potentially mimic benignity. Type B mucinous carcinoma is the more cellular form with endocrine differentiation and sometimes signet ring cells, and these lesions are less likely to be underdiagnosed as benign. Recent findings of expression studies using genome-wide oligonucleotide microarrays suggest that mucinous B and neuroendocrine carcinomas are part of the same spectrum of lesions, while mucinous A cancer is a discrete entity (Weigelt et al. 2009).

When classical invasive mucinous carcinoma forms between 50 and 90 % of the tumor, with the rest comprising ductal (no special type/not otherwise specified) carcinoma, the term mixed mucinous-ductal carcinoma is used, with an attendant less favorable prognosis. FNAC of these mixed tumors are unlikely to be overlooked, as the ductal component will demonstrate cytological features of ductal cancer.

11.3.4 Management

The diagnosis of mucinous cancer on FNAC, in conjunction with corroborating clinicoradiological findings, can be therapeutically managed accordingly.

11.4 Mucinous Papillary Neoplasms

11.4.1 Clinical and Epidemiological Findings

Papillary neoplasms range from benign intraduct papillomas to those harboring ADH or DCIS, and malignant papillary tumors which include papillary DCIS, encapsulated papillary carcinoma, solid papillary carcinoma and invasive papillary carcinoma (Lakhani et al. 2012). The intraduct papilloma constitutes about 5.5 % of benign breast biopsies and can present as a central or peripheral lump or with nipple discharge. The incidence of the malignant counterparts is difficult to determine, with papillary carcinoma accounting for less than 2 % of all breast cancers. The clinicoradiological presentation of malignant papillary lesions is similar to that of the intraduct papilloma, with more frequent blood-stained discharge, and occasional growth to large sizes in the elderly. Mucin in papillary neoplasms is found either in the cystically dilated duct within which the papillary lesion projects, or it can be noted within the cytoplasm of lesional cells, or as small puddles among the epithelial proliferation.

11.4.2 Cytologic Findings

FNAC of mucinous papillary lesions contains mucin in the background, admixed with branched epithelial clusters with club-shaped contours and occasional fibrovascular cores (Fig. 11.5). Columnar cells that are sometimes in small groups or individually disposed are seen (Fig. 11.6). There may be foamy histiocytes and some stripped naked ovoid nuclei of myoepithelial cells in the benign intraduct papilloma. Spindled and plasmacytoid cells that correlate with neuroendocrine differentiation can be discovered in solid papillary carcinoma. Cytological findings of a papillary lesion often fall into the indeterminate or suspicious category, as appearances of benign papillomas and those that harbor malignancy show substantial overlap (see Chap. 10).

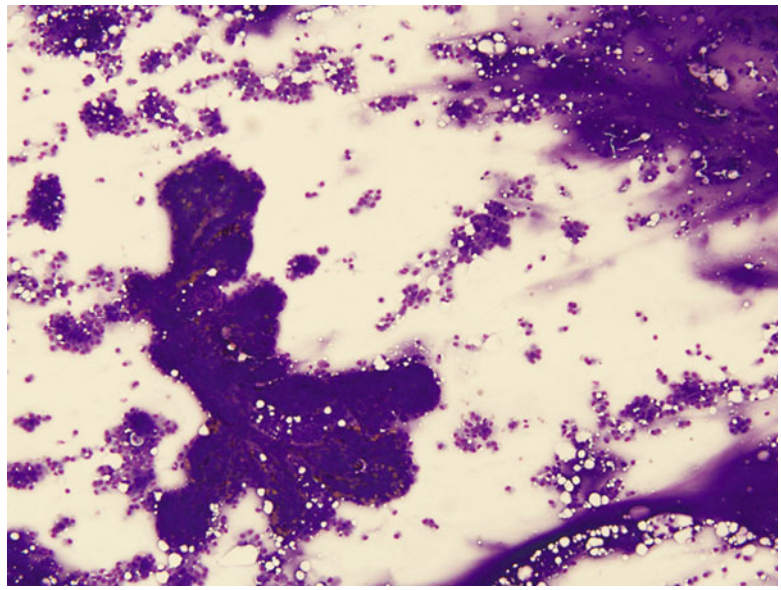

Fig. 11.5 Diff-Quick stain of the fine needle aspirate shows papillary epithelial structures within a background containing mucin as well as dispersed intact cells

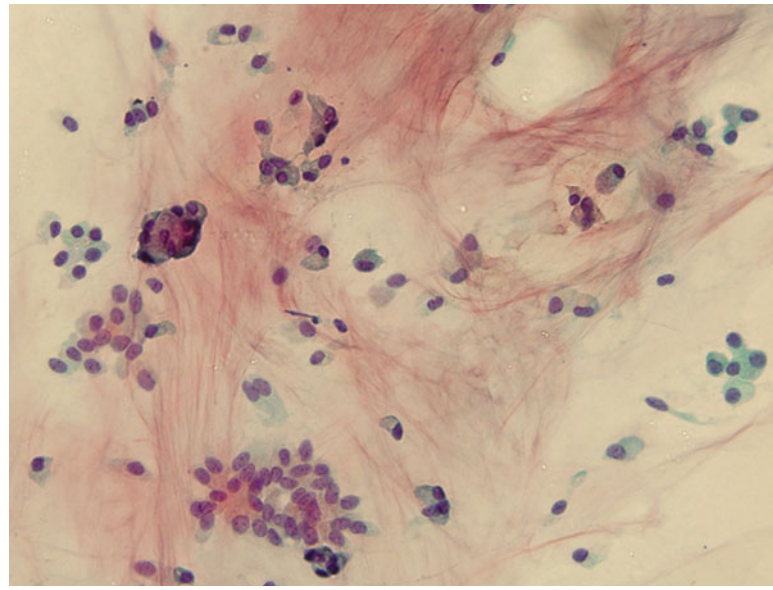

Fig. 11.6 Polygonal and columnar cells within a mucinous background

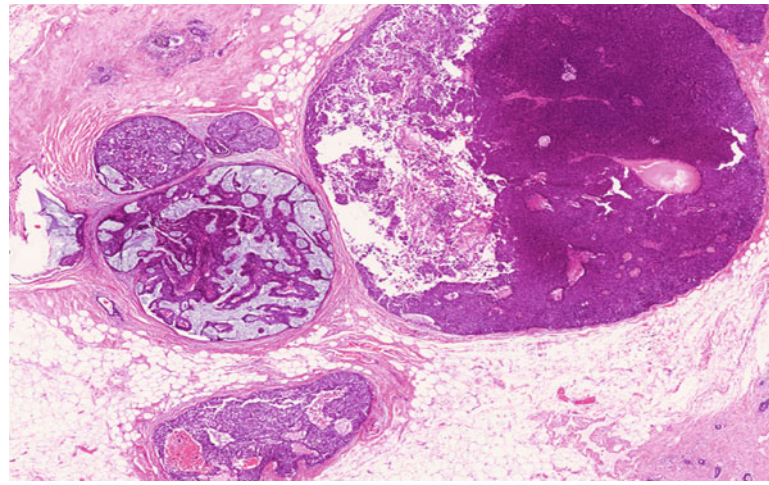

Fig. 11.7 Low magnification of solid papillary carcinoma with luminal mucin in a few distended duct spaces

11.4.3 Histologic Correlations

Histologically, the findings reflect the various papillary neoplasms. Those associated with extracellular mucin tend to be of solid papillary architecture, with a significant proportion disclosing neuroendocrine differentiation and occasional spindle epithelial morphology (Fig. 11.7). The histological appearances of intra- and extracellular mucin in a solidified papillary neoplasm should raise the possibility of an in situ malignancy with possible neuroendocrine differentiation. Confirmation of the malignant in situ papillary tumor in such cases can be assisted with immunohistochemical workup using a combination of antibodies to CK5/6, CK14, and ER. For CK5/6 and CK14, an in situ malignant papillary neoplasm would display diminished or absent staining of the epithelial cell population, with positively stained myoepithelial cells confined to the periphery of the duct wall. In benign papillomas with usual ductal hyperplasia, these antibodies would demonstrate a heterogeneous mosaic pattern of staining. ER immunohistochemistry shows diffuse nuclear staining of malignant papillary lesions, with patchy staining in benign counterparts.

11.4.4 Management

The diagnosis of a mucinous papillary lesion on FNAC should prompt surgical biopsy for confirmatory diagnosis.

11.5 Differential Diagnosis

11.5.1 Myxoid Fibroadenoma

The myxoid fibroadenoma can show cytological appearances that resemble a mucinous epithelial neoplasm. Clues to the correct diagnosis are the staghorn bimodal epithelial aggregates with many bipolar naked nuclei within the myxoid background and the presence of stromal clumps. The clinicoradiological appearances may not be contributory since the mucinous carcinoma can be resemble benignity, though the age group of the fibroadenoma is in younger women compared to mucinous carcinoma which occurs in older individuals. For MLL, there are usually accompanying radiological calcifications, and when mass lesions are seen, they tend to be noted as multiple small nodules corresponding to the multiple distended mucin-filled ducts (Khodeza Nahar Begum et al. 2009).

11.5.2 Fibrocystic Changes with Luminal Mucin

Cytological appearances resemble those of fibrocystic change except with the presence of mucin in the background. It is difficult to distinguish this from benign MLL cytologically.

11.5.3 Polyacrylamide Gel Injection

Women with polyacrylamide gel injection for breast augmentation may present with a breast lump due to the material. On FNAC, these tend to be hypocellular smears with sparse but benign epithelial clusters, sometimes accompanied by histiocytes and foreign-body-type multinucleated giant cells, within a gelatinous acellular background. The staining pattern of this gelatinous material with PAP and DQ is reported to consistently show polychromasia and appear magenta violet with bubbly vacuoles, respectively (Lau et al. 2009).

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Carcinoma and Variants

12

12.1 Clinical Findings

Invasive breast carcinoma is a malignant epithelial lesion derived from the terminal duct lobular unit of the breast. Breast carcinoma is common, and it is estimated that one in nine women will develop breast cancer in their lifetime. Breast cancer arises anywhere in the breast parenchyma or accessory breast tissue, although most common in the upper outer quadrant. The main purpose of the identification of specific types of invasive breast carcinoma is to refine the prediction of likely behavior and response to treatment offered by the other major prognostic parameters such as lymph node stage, histological grading, tumor size and lymphovascular invasion. A wide range of clinical behavior is seen with different morphologies. Some patients with small tumors are cured by surgery, while others will die of metastatic disease within a few years. There is an increasing frequency of breast cancer with increasing patient age. Breast carcinoma is rare before 30 years without a family history of breast cancer. Clinically, breast carcinomas present most commonly with an ill-defined mass, sometimes adherent to skin or underlying muscle. When impalpable, breast cancer can be detected by mammographic screening programs.

12.2 Radiologic Findings

Most commonly, breast cancer is identified as a mass lesion, often ill-defined or as a spiculated mass on the mammography. Associated microcalcifications may be present. Ultrasound shows an irregular mass with ill-defined margins and an inhomogeneous echotexture. Some variants have special radiologic features, for example, invasive lobular carcinoma has a lower density mammographically and may be occult. Mucinous and medullary carcinomas usually present as well-circumscribed masses on mammography and ultrasound.

12.3 Pathologic Findings

12.3.1 Gross Findings

The tumors are in general moderately or ill-defined with a nodular or stellate configuration. The cut surface is gray/white and firm. Some special variants have distinct macroscopic appearances. Lobular carcinomas are poorly defined; tubular carcinomas are in general small, moderately defined with a stellate appearance and gray in color; mucinous carcinomas are well-circumscribed with a gelatinous cut surface; medullary carcinomas are usually circumscribed and soft with a pale gray/tan cut surface; metaplastic carcinomas are often cystic with hemorrhage and necrosis.

12.3.2 Histologic Findings

The WHO classification requires a nonspecialized pattern in over 50 % of the tumor area to classify a breast carcinoma as "no special type" (NST). If the NST is present in 10–49 % of the

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tumor area, the tumor is classified as mixed NST and special type (Lakhani et al. 2012). NST tumors are named as *invasive ductal carcinomas* and show variable combinations of morphology, with trabecular, sheetlike, acinar, and nesting arrangements. The cells show variable atypia, often with focal necrosis and associated inflammation. Accompanying DCIS is commonly present. The stroma can be desmoplastic, elastotic, or may be minimal. There is increasing evidence that within the group of grade III ductal NST tumors, there is a distinct subset showing evidence of basaloid differentiation. In these cases, necrosis and associated inflammation are frequent, as well as a circumscribed macroscopic appearance. The special types show some distinct characteristics that are reflect in their cytological aspects (see later in this chapter). The cell-type characteristic of *invasive lobular carcinoma* is non-cohesive, with relatively regular, round or oval, eccentrically placed nuclei with small nucleoli. There is a small amount of cytoplasm in which intracytoplasmic lumina may be identified. *Tubular carcinoma* shows irregular infiltrative angulated tubules, with a single layer of epithelial cells showing apical snouts. Frequently, there is a central hyalinized area with a paucity of tubules compared to an increased abundance at the periphery. The tubular structures in pure tubular carcinoma are formed from only mild to moderately pleomorphic cells. *Cribriform carcinoma* is characterized by the presence of infiltrating islands of tumor cells with cribriform arrangement, and the constituent cells have amphophilic cytoplasm with low- to intermediate-grade nuclei. *Mucinous carcinoma* is characterized by the presence of nests, trabeculae, acini, or sheets of epithelial cells, usually with some glandular lumen formation, within pools of extracellular mucin. The cells have granular eosinophilic cytoplasm, sometimes with intracellular mucin. The histologic features defining *carcinoma with medullary* features have been debated for many years. The WHO classification described five classical traits of medullary carcinoma, namely, syncytial growth pattern in more than 75 %, absence of glandular structures, diffuse moderate/marked lymphoplasmacytic infiltrate, grade 2–3 nuclear pleomorphism, and histologic circumscription. The cells are vesicular, with prominent nucleoli and indistinct cell borders. Squamous metaplasia, necrosis, and a giant cell reaction are all consistent with a diagnosis of carcinoma with medullary features. The *invasive micropapillary carcinoma* is characterized by the presence of solid/tubular epithelial structures composed of eosinophilic cells surrounded by a clear space. The nests lack a true fibrovascular core. This carcinoma has been described as having an "inside-out" appearance as the polarity of the cells is reversed with luminal marker expression by EMA on the periphery of the cell islands. The tumors are predominantly of high histologic grade. *Metaplastic carcinomas* comprise a heterogeneous group of tumors. The actual proposed classification of these tumors is descriptive and divides them in the following groups: low-grade adenosquamous carcinoma, fibromatosis-like metaplastic carcinoma, squamous cell carcinoma, spindle cell carcinoma, carcinoma with mesenchymal differentiation (chondroid, osseous, other), and mixed. Low-grade adenosquamous carcinomas show well-developed glandular and tubular formation intimately admixed with solid nests of squamous cells in a spindle cell background. Clusters of lymphocytes are often observed at the periphery, sometimes in a "cannonball" pattern. Fibromatosis-like metaplastic tumors of the breast are characterized by bland spindle cells with pale eosinophilic cytoplasm and slender nuclei with tapered edges and finely distributed chromatin embedded in stroma with varying degrees of collagenization. Nuclear atypia is mild or absent. The spindle cells are often arranged in wavy, interlacing fascicles, the form long fascicles with fingerlike extensions infiltrating the adjacent breast parenchyma. Squamous cell carcinomas usually present as a cystic lesion, where the cavity is lined by squamous cells with varying degrees of nuclear atypia and pleomorphism. The neoplastic cells infiltrate the adjacent stroma in the form of sheets, cords, and nests, which elicit a conspicuous stromal reaction. Inflammatory infiltrate is usually prominent. The acantholytic variant of squamous cell carcinoma, characterized by the formation of irregular spaces lined by atypical

squamous cells leading to a pseudoglandular or pseudoangiosarcomatous appearance, should be borne in mind as a potential differential diagnosis with angiosarcoma. Spindle cell carcinomas are characterized by atypical spindle cells, arranged in a multitude of architectural patterns ranging from long fascicles in herringbone or interwoven patterns to short fascicles in a storiform (cartwheel) pattern. The cytoplasm ranges from elongated to plump spindle. Nuclear pleomorphism is usually moderate to high. Metaplastic breast carcinomas with mesenchymal elements are often composed of an admixture of mesenchymal components, including chondroid, osseous, rhabdomyoid, and even neuroglial differentiation, with epithelial carcinoma areas, which can form glandular tubules, solid clusters, and/or foci of squamous differentiation. It should be noted that upon extensive sampling, a large proportion of metaplastic breast cancers display a mixture of different elements. These cases should be reported as metaplastic carcinomas and the distinct elements recorded in the final report. The distinct aspect of the *apocrine carcinomas* is the constituent cells with eosinophilic granular cytoplasm with enlarged nuclei and prominent nucleoli. There is no specific architectural growth pattern. *Invasive papillary carcinomas* are characterized by the presence of islands of malignant cells centered on fibrovascular cores. While more tubular structures may be seen, these often have papillary structures at the periphery. *Secretory carcinoma* may have different architectural patterns, with cells showing abundant clear/vacuolated amphophilic granular cytoplasm and prominent intra- and extracellular secretion. Histologically, *acinic cell carcinoma* is composed of cells arranged in a complex admixture of solid, microcystic, and microglandular areas. These cells have round to ovoid nuclei containing a single, conspicuous nucleolus. Their cytoplasm is usually abundant, amphophilic-to-eosinophilic, and granular. The granules can be bright eosinophilic, resembling those seen in Paneth cells. *Glycogen-rich carcinomas* tend to have either an infiltrative or circumscribed margin and are high grade. There are sheets or nests of cells and acinar formation is rare. Mitoses are numerous and necrosis may be identified. *Lipid-rich carcinoma* shows an infiltrating margin with cells having clear or bubbly cytoplasm. The tumors are usually high grade. *Adenoid cystic carcinoma* has at least focally infiltrative margins, and the morphology of the tumor is tubular, cribriform, solid, or mixed. The component cells are biphasic with the majority of the tumor composed of small hyperchromatic cells with sparse cytoplasm (basaloid cells). There are pseudolumens that contain amorphous eosinophilic basement membrane material. True glandular lumens are present lined by the luminal epithelial component with more abundant eosinophilic cytoplasm and round nuclei. Although *Paget's disease* is not a specific variant of breast carcinoma, it is mentioned here because it is often sampled by cytology. The defining histologic feature of Paget's disease is the presence of malignant glandular epithelial cells within the squamous epithelium of the nipple. Cytologically, these cells are large, with pleomorphic nuclei and abundant eosinophilic cytoplasm. The nipple may vary from being macroscopically normal to being erythematous and ulcerated. Underlying a Paget's disease can be an in situ or invasive carcinoma.

12.4 Cytologic Findings

12.4.1 General Findings

Breast carcinoma is a heterogeneous entity from clinical, radiological, morphological, and molecular perspectives. Despite that, there is a general criterion of malignancy on breast cytology that can be applied to most tumors. Most breast carcinomas show aspirates with moderate to abundant cellularity, with some discohesiveness of the cells. Lack of cell-to-cell adhesion, although not diagnostic per se, is one of the strong criteria of malignancy on breast FNA smears. These isolated cells in general have preserved cytoplasm in contrast with the naked nuclei observed in benign lesions. In most invasive carcinomas, myoepithelial cells are missing, both in the background as well as in the middle and periphery of tumor cell groups. However, in some cases of

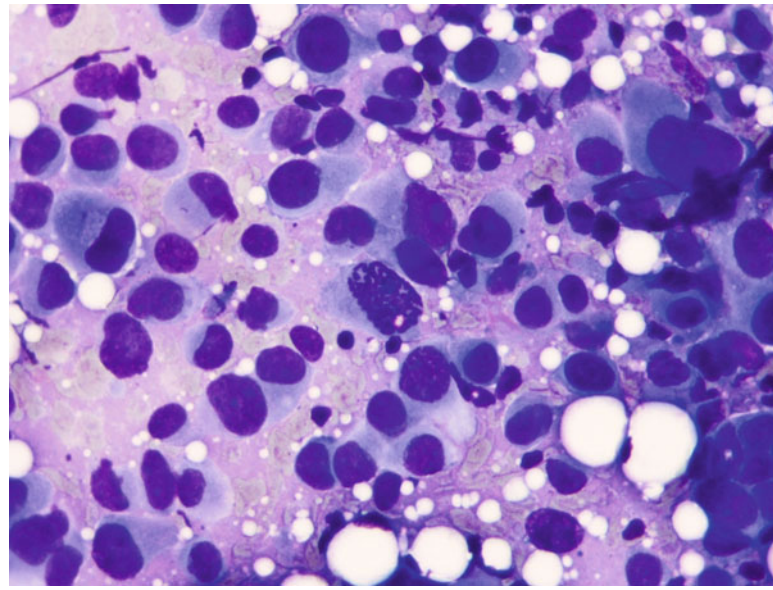

Fig. 12.1 Invasive breast carcinoma. Observe the main criteria of malignancy in breast aspirates: lack of cohesiveness, nuclear pleomorphism, and presence of mitotic figures (MGG stain)

DCIS, tubular carcinomas, and low-grade ductal carcinomas, few myoepithelial cells can be observed. Nuclear pleomorphism, presence of nucleoli, nuclear membrane irregularity, and presence of mitotic figures are other criteria of malignancy that can be variably found in breast carcinoma aspirates (Fig. 12.1). Presence of intracytoplasmic lumina is more common in malignant lesions although they can be rarely seen in benign lesions. The characteristics of the background can be useful when we find cell debris, necrosis, and inflammatory cells that usually are associated with malignancy. The cytological diagnosis of DCIS and the distinction of DCIS from invasive carcinoma have been a subject of much discussion and controversy. As a result, in many institutions, CNB and vacuum-assisted biopsy are replacing FNAC to investigate non-palpable breast lesions, but this will be discussed in Chapter 16. There are some criteria suggestive of invasion such as presence of elastoid stromal fragments, invasion of stromal or fatty tissue by neoplastic cells, presence of intracytoplasmic vacuoles, and presence of tubular structures, but none of them are definitive. Despite this limitation, as discussed previously, this is not reason to abandon FNA in the investigation of breast lesions. As in all breast lesions, breast carcinomas should be studied using the triple approach: clinical, imaging, and cytology. In cases where the distinction between DCIS and invasive cancer is not clear in cytology, imaging is very useful to solve this problem (Kocjan et al. 2008).

12.4.2 Invasive Breast Carcinoma of No Special Type (Ductal)

The cytology appearance of invasive breast carcinoma of no special type (ductal) varies according to the degree of differentiation, the presence or absence of necrosis, and the extent of stromal proliferation. A definitive diagnosis of ductal carcinoma can be made when the breast aspirates display the following cytological characteristics: cellular smears, monomorphic cell population with variable cell patterns, loss of cellular cohesion, numerous isolated single epithelial cells with preserved cytoplasm, and anisonucleosis (Fig. 12.1). The cellular arrangement includes irregular three-dimensional clusters, syncytial groupings, or occasionally, acinic-like pattern. Tumor cells are often larger than normal ductal cells, with pleomorphism and frequently with prominent nucleoli. Nuclei may be eccentric, which lends a plasmacytoid appearance to the cells. The cytoplasm is well-defined and varies from dense to granular to vacuolated. The background can be bloody, with occasional necrotic debris, or rarely

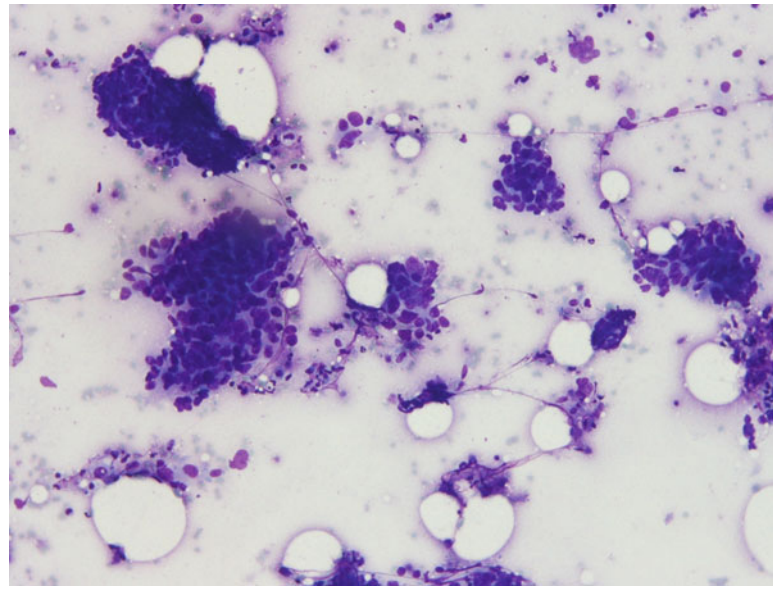

Fig. 12.2 Invasive ductal carcinoma. Groups of malignant epithelial cells with nuclear atypia. Note the background with necrotic debris and inflammatory cells (MGG stain)

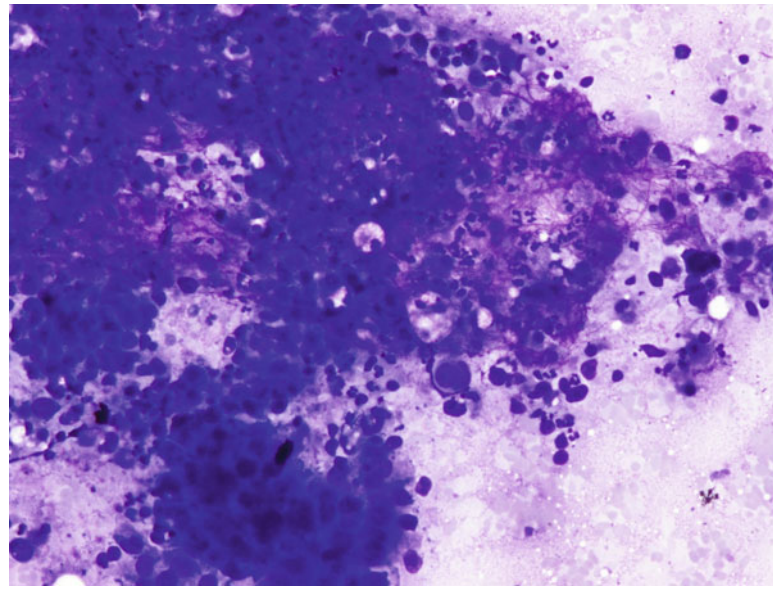

Fig. 12.3 High-grade invasive ductal carcinoma with necrosis and inflammatory cells in the background. The histology of this case revealed a triple-negative breast carcinoma (MGG stain)

clean (Fig. 12.2). Myoepithelial cells and stromal cells are in general absent. Poorly differentiated carcinomas are characterized by highly pleomorphic cells with readily identified mitotic figures, whereas aspirates of well-differentiated carcinoma display monomorphic cell populations with features similar to those of lobular carcinoma, although the cellularity is usually greater. High-grade ductal carcinomas, with prominent nucleoli, necrosis, and neutrophils, in general are associated with a hormone receptor negative phenotype (Fig. 12.3) (Dufloth et al. 2009). As mentioned before, it is difficult to distinguish invasive carcinomas from DCIS. In these cases, the presence of criteria of malignancy, absence of myoepithelial and stromal cells, and correlation with imaging findings (triple test) are crucial to make the correct diagnosis (Kocjan et al. 2008). Cases suspected to be low-grade carcinoma should be confirmed by CNB prior to definitive surgery.

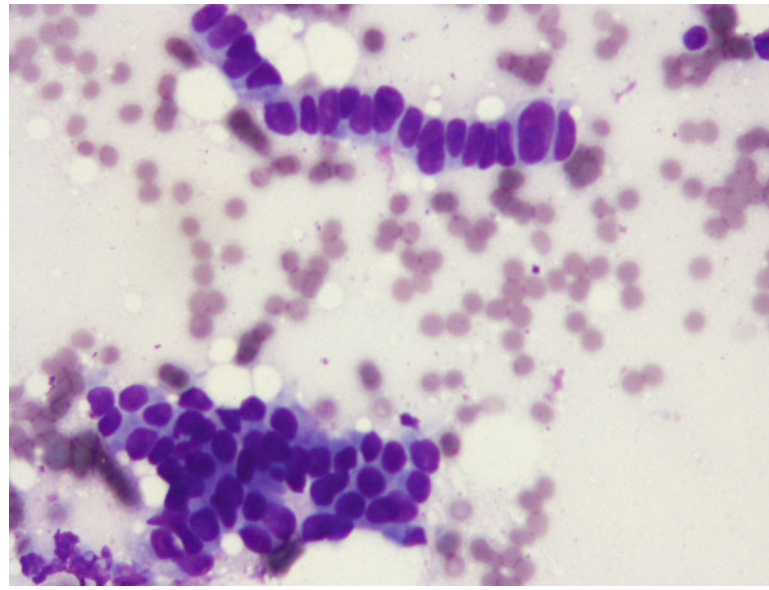

Fig. 12.4 Invasive lobular carcinoma. Note the malignant cells forming *small chains* in the aspirates (MGG stain)

12.4.3 Invasive Lobular Carcinoma

FNAC of invasive lobular carcinoma (ILC) usually yields a paucicellular smear with subtle atypia and rare single intact epithelial cells. A most valuable clue for the diagnosis is the tendency of the cells to form small chains in the aspirates (Fig 12.4). Nuclei are often eccentric, round, or oval with finely dispersed chromatin and small, distinct nucleoli. The cytoplasm is scanty, clear or vacuolated, or may contain a mucin droplet that gives it a target-like appearance. Occasional signet ring cells may be present. The nuclear/cytoplasmic ratio is high. Pleomorphic lobular carcinoma of the breast is a subtype of lobular carcinoma that is well recognized clinically and histologically (Simpson et al. 2008). The recognition of this variant on FNAC is important because pleomorphic lobular carcinoma pursues an aggressive clinical behavior as compared to classic lobular carcinoma. The pleomorphic variant features larger cell size with more nuclear atypia and may be misclassified as ductal in up to 25% of FNAC smears. ILC is one of the main reasons for false-negative diagnosis in breast aspirates. This is due to the low cell yield and the small cell size. According to the literature, the overall sensitivity in detection of malignancy in ILC cases is 76 %. The discrepant cytologic findings with clinical and imaging findings are the key to avoid a false-negative diagnosis (Menet et al. 2008). Such cases should be followed by CNB for a definitive diagnosis. A correct malignant diagnosis in the pleomorphic variant is significantly more frequent than in classic types. In these cases, the differential diagnosis with ductal carcinoma can be a problem.

12.4.4 Tubular Carcinoma

Tubular carcinoma shows aspirates with variable cellularity, with many cohesive clusters of uniform, bland epithelial cells. The cells are arranged mostly in tubular structures with an angular appearance or comma-like pattern (Fig. 12.5). At low magnification, a pattern somewhat similar to that of fibroadenoma may be visible, but detailed examination shows the tubular structures to be three-dimensional with central lumens. These epithelial cells show loss of polarity with absence of myoepithelial cells. In some cases, bare nuclei or bipolar cells are present on the smears raising the differential diagnosis with benign lesions. Single cells with vacuolated cytoplasm similar to the cells in lobular carcinoma also can occur sparsely. Because of the minimal cytologic atypia and cohesiveness of the cells, tubular carcinoma may be mistaken for fibroadenoma or fibrocystic change. Tubular

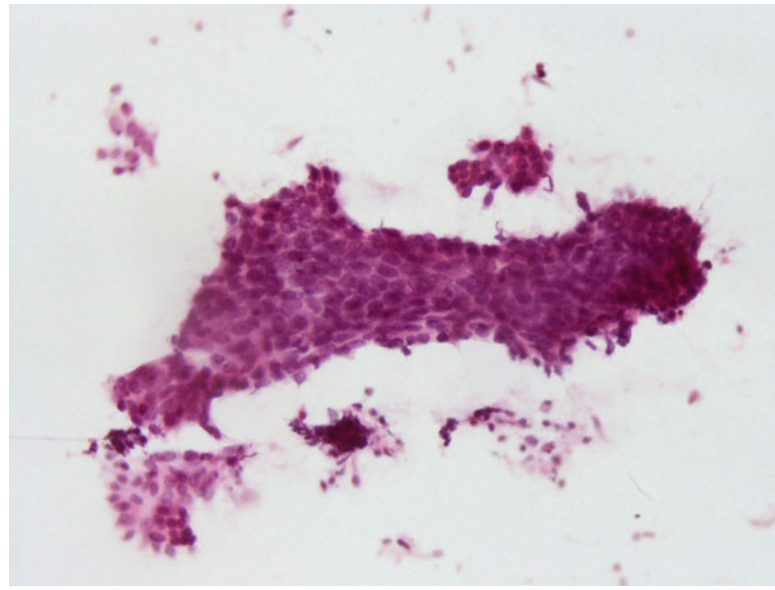

Fig. 12.5 Tubular carcinoma. Aspirate shows cohesive clusters of uniform, mildly pleomorphic tumor cells. The cells are arranged mostly in tubular structures with an angular appearance or comma-like pattern

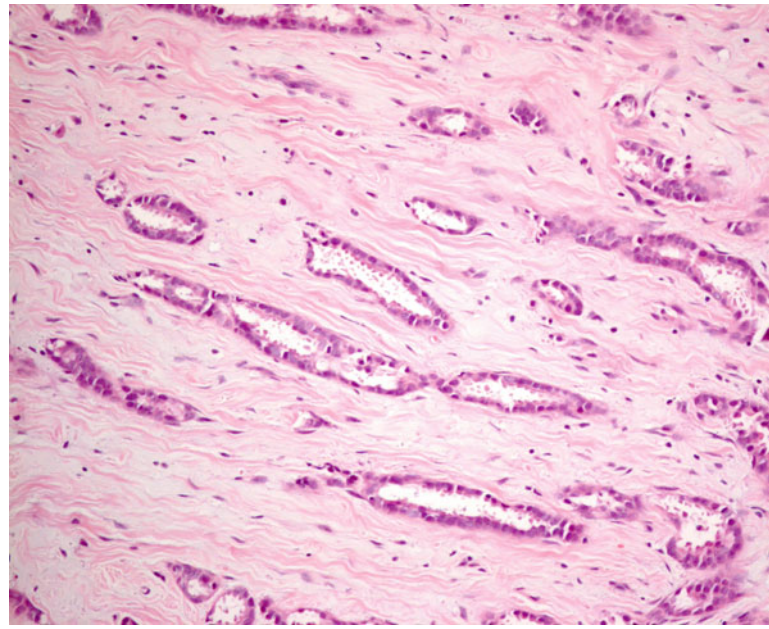

Fig. 12.6 Tubular carcinoma. Histology demonstrates angular tubules lacking significant cytonuclear atypia

carcinoma accounts for some false-negative cytological diagnoses as the malignant characteristics are subtle. The characteristic angular tubular structures, coupled with clinical and mammographic findings, should be the clue to the correct diagnosis (Fig. 12.6). Aspirates of radial scars may reveal a monomorphic cell population with a tubular arrangement that mimics tubular carcinoma (De la Torre et al. 1994). Cases suspected to represent a tubular carcinoma or radial scar should be followed by excisional biopsy for a definitive diagnosis.

12.4.5 Invasive Cribriform Carcinoma

FNAC from invasive cribriform carcinoma shows cohesive sheets and three-dimensional cribriform clusters of bland-looking and mitotically

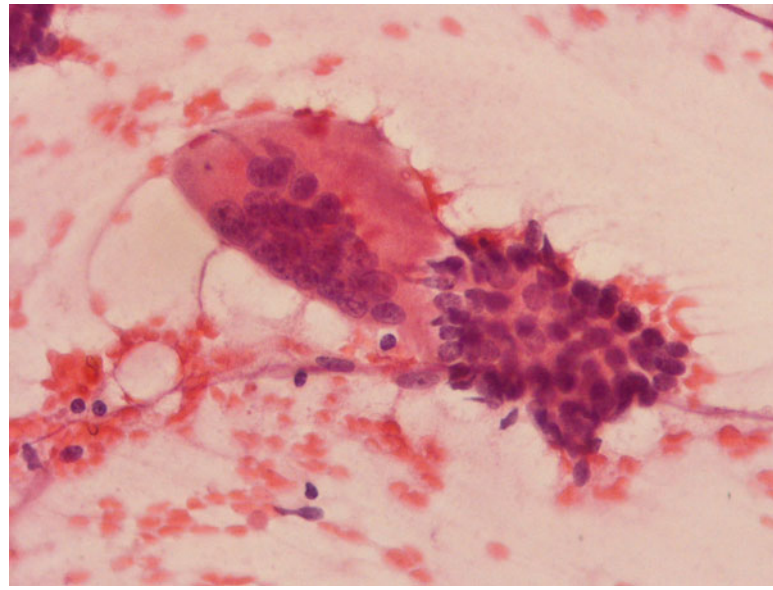

Fig. 12.7 Invasive cribriform carcinoma. Note osteoclast-like multinucleated cells at the periphery of a group of mildly pleomorphic malignant cells with round to oval nuclei (HE stain)

inactive ductal cells in a bloodstained background. The ductal cells have regular round to oval nuclei, evenly dispersed chromatin, inconspicuous nucleoli, and a small amount of amphophilic cytoplasm. Myoepithelial cells and naked nuclei usually are not seen. It is not rare that this variant of breast carcinoma is sometimes accompanied by osteoclast-like multinucleated cells at periphery of the epithelial cells or in the background of the smears. These giant cells have multiple (usually 10–20) oval nuclei; fine chromatin; small, mostly solitary and distinct nucleoli; and ample, dense, amphophilic cytoplasm (Fig. 12.7). Sometimes, hemosiderin pigment is seen in the cytoplasm of giant cells. Groups of plump spindle cells and stroma can be present in this variant of low-grade breast cancer raising the differential diagnosis with fibroepithelial tumors.

12.4.6 Mucinous Carcinoma

FNAC of mucinous carcinoma often produces gelatinous material with variable cellularity. The cells are distributed in three-dimensional groups surrounded by abundant extracellular mucinous material that stains metachromatically with the Diff-Quick stain or appears as linear strands or filmy, wispy blue-green material on Papanicolaou's stain (Figs. 12.8 and 12.9). Branching, thin-walled blood vessels may be prominent. The cell groups are mostly tightly cohesive cell balls, although flat sheets and loosely cohesive cell clusters are present. Single cells are present in moderate to great numbers and are small to medium sized with round, often eccentric nuclei. In general, nuclear pleomorphism is minimal in mucinous carcinoma (Stanley et al. 1989). The relatively bland appearance of tumor cells coupled with decreased cellularity secondary to the abundant extracellular mucinous material can potentially lead to a false-negative diagnosis. The diagnosis should be suspected when extracellular mucinous material is seen and individually malignant cells are present. Mucinous carcinoma should be distinguished from mucocele-like lesion. The aspirates from mucocele-like lesions show abundant extracellular mucin indistinguishable from that in mucinous carcinoma; however, the epithelial cells are present in a few flat sheets with few or no single cells. Myoepithelial cells are also present. A mucoid background may be present in aspirates from fibroadenomas. The concomitant presence of naked bipolar nuclei and normal ductal epithelium serves to distinguish the mass from a mucinous carcinoma.

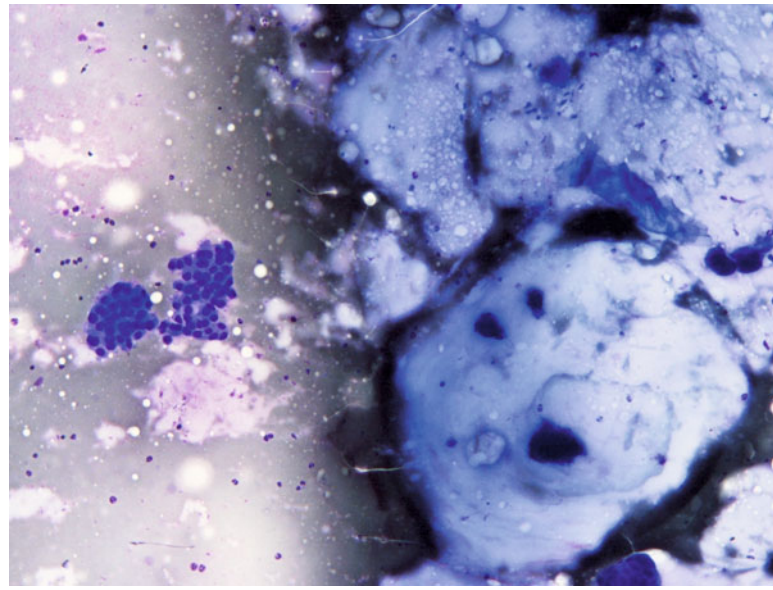

Fig. 12.8 Mucinous carcinoma. Note three-dimensional groups of neoplastic cells surrounded by abundant extracellular mucinous material which is metachromatic on MGG staining (MGG stain)

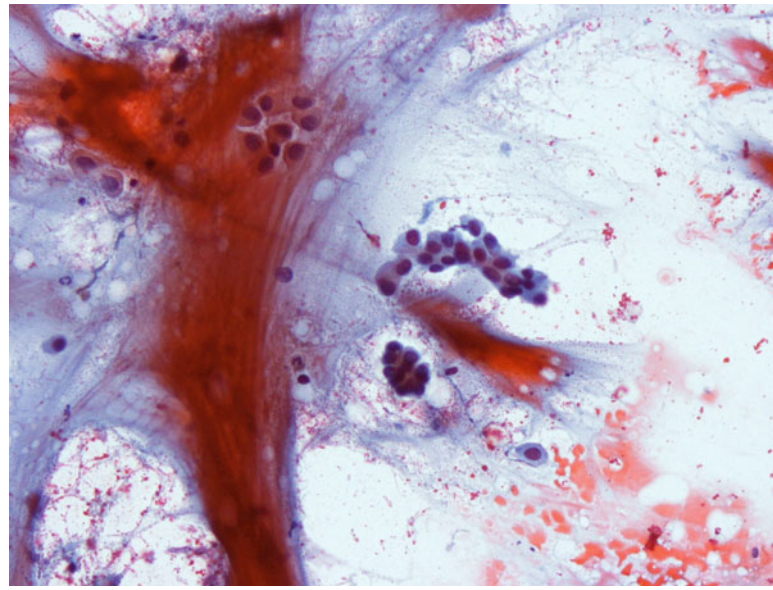

Fig. 12.9 Mucinous carcinoma. Papanicolaou staining highlights the background mucin as a wispy blue-green material (Pap stain)

12.4.7 Carcinoma with Medullary Features

The definitive diagnosis of breast carcinoma with medullary features cannot be achieved based only on cytological aspects. There are many and controversial criteria to diagnose a "typical" and an "atypical" medullary carcinoma that include macroscopic and histological features (Fig. 12.10). A definitive diagnosis of carcinoma with medullary features requires evaluation of tissue sections to determine margin circumscription and other parameters, so such a diagnosis on FNAC can only be suggested when the clinical and imaging findings – well-circumscribed, mobile mass – coupled with the cytologic results, are suggestive of this carcinoma. However, "medullary features" can be recognized in aspirates, and this can be clinically useful because

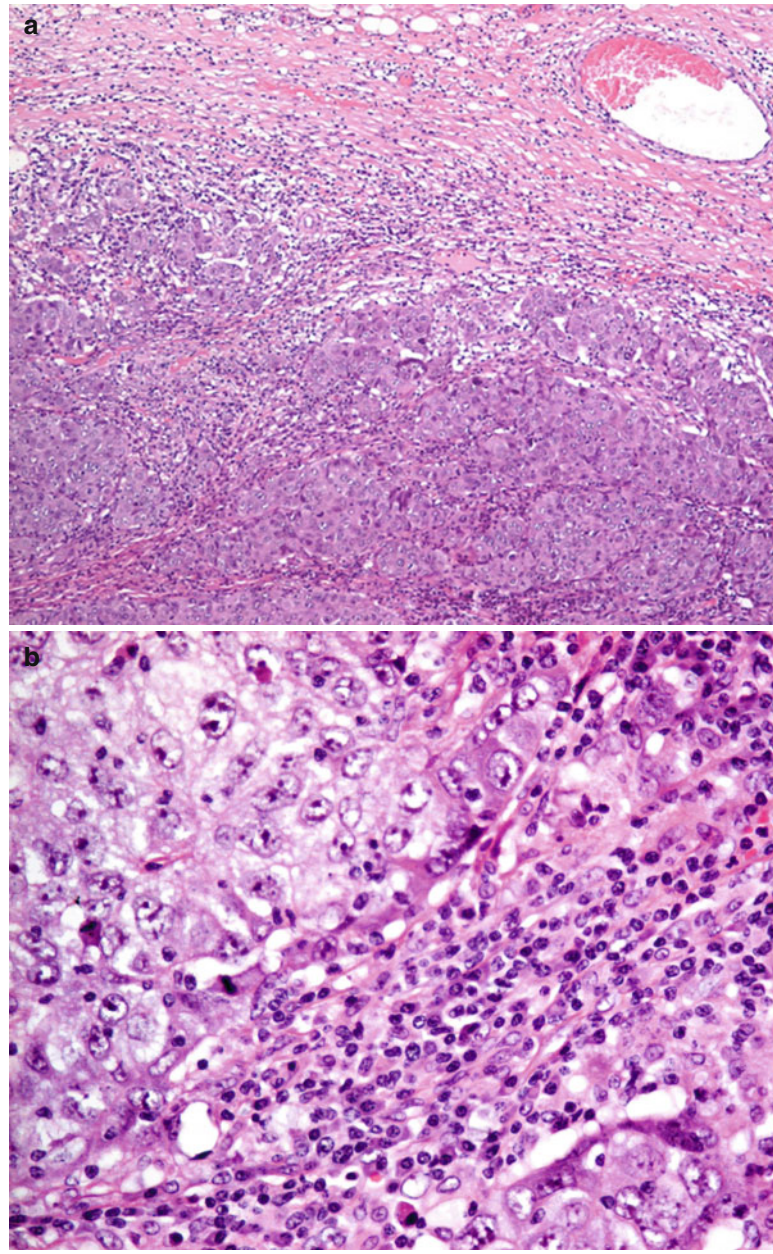

Fig. 12.10 (**a**, **b**) Carcinoma with medullary features. (**a**) Histology shows well-defined margin, syncytial growth pattern, and prominent lymphocytic infiltrates (HE stain). (**b**) High-power histology shows pleomorphic high-grade vesicular nuclei with prominent nucleoli, as well as numerous mitoses (HE stain)

these tumors are associated with BRCA 1 mutation and basal-like phenotype (Lakhani et al. 2012). Aspirates from carcinoma with medullary features are usually cellular with large pleomorphic tumor cells in a background of lymphocytes and plasma cells (Fig. 12.11). The large cells are displayed in clusters, syncytial groupings, or individually. The cytoplasm is homogeneous or granular, and poorly demarcated, and the nuclei are irregular with clumped chromatin and macronucleoli (Akbulut et al. 2009). In some instances, the smears may show predominantly lymphoid tissue and a few small groups of atypical epithelial cells, or the tumor nuclei may appear bare without cytoplasm or with only a rim of cytoplasm. Based on cytological features, the differential diagnosis includes poorly differentiated ductal carcinoma with inflammatory infiltrate. In this case, the aspirates consist predominantly of pleomorphic

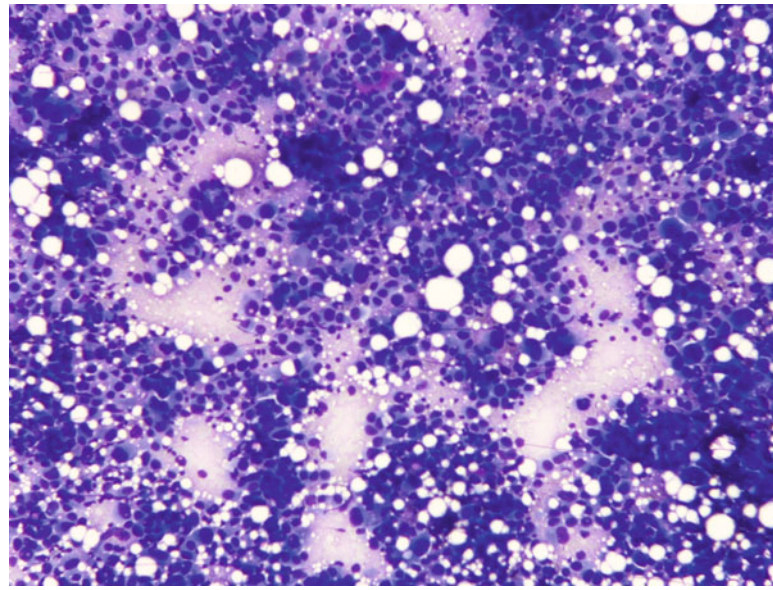

Fig. 12.11 Carcinoma with medullary features. Note large pleomorphic tumor cells in a background of lymphocytes (MGG stain)

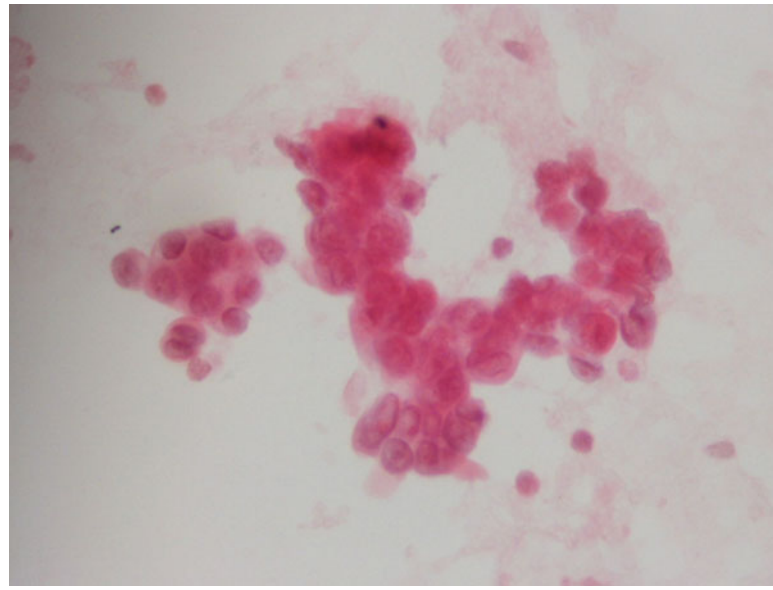

Fig. 12.12 Invasive micropapillary carcinoma. Tightly cohesive tumor cell clusters with angulated borders demonstrating a morular appearance (HE stain)

cells arranged in three-dimensional clusters rather than syncytial groups. Carcinoma with medullary features presenting at the tail of the breast must be distinguished from metastatic carcinoma to the lymph node. In such cases, correlation with clinical findings may be helpful for establishing the correct diagnosis. Malignant lymphoma involving primarily or secondarily the breast is one of the differential diagnoses. Lymphomas present as a discohesive population of malignant lymphoid cells, devoid of neoplastic epithelial cells.

12.4.8 Invasive Micropapillary Carcinoma

Aspirates from invasive micropapillary carcinoma usually show moderate to high cellularity with multiple tightly cohesive tumor clusters with rare isolated cells (Lui et al. 2007a). Tumor morules with angulated or scalloped borders are the main architectural arrangement (Figs. 12.12 and 12.13). The nuclear atypia is usually high grade. Well-developed papillary fronds with fibrovascular cores are not seen.

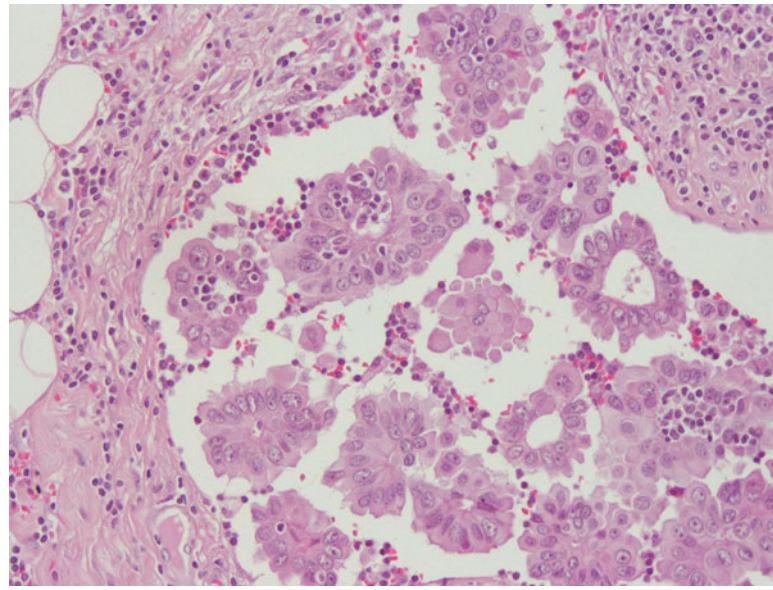

Fig. 12.13 Invasive micropapillary carcinoma. Tumor cell clusters with central spaces residing within empty stromal spaces (HE stain)

In cytology, invasive micropapillary carcinoma may mimic breast papillary lesions, metastatic carcinoma (especially ovarian serous carcinoma), or even benign proliferative lesions. In papillary neoplasms, papillary fronds with fibrovascular cores are usually seen. In metastatic serous carcinoma, psammoma bodies are more often present, but the final differential diagnosis should be made based on clinicopathological findings.

12.4.9 Metaplastic Carcinoma

Metaplastic breast carcinomas (MBC) comprise a very heterogeneous group of malignant breast tumors that are characterized by a complex admixture of usual types of breast cancer (i.e., invasive ductal or lobular carcinomas) with metaplastic elements (Lakhani et al. 2012). The metaplastic elements can be subclassified as homologous (i.e., squamous and spindle cells) or heterologous (i.e., chondroid, osseous, and rhabdomyoid elements). These tumors account for <1–3 % of all breast carcinomas, depending on the definition and the type of metaplasia. Cytomorphology of MBC has not been extensively reported in the literature. The cytological features of MBC described reflect the morphological heterogeneity of these tumors (Lui et al. 2007b). FNAC findings in MBC may include the presence of different neoplastic cell types, including ductal, spindle-shaped, and squamous cells (Fig. 12.14). A liquid necrotic aspirate and a proteinaceous or myxoid background are helpful but not specific for these neoplasms, as they may be found in poorly differentiated carcinomas and sarcomas of the breast. Multinucleated giant cells are also a relatively common but nonspecific finding (Fig. 12.15). Occasionally, the sparse cellularity of liquid aspirates, a background of histiocytes and inflammatory cells, or the relatively monotonous appearance of ductal or spindle cells may cause problems in the diagnosis of malignancy (Fig. 12.16). In this situation, p63 is an extremely useful marker that can be used in cytological smears. p63 is positive in MBC and is always negative in benign spindle cell proliferations such as fibromatosis or nodular fasciitis (Reis-Filho and Schmitt 2003). A definite cytological diagnosis of MBC requires a convincing demonstration of at least two different neoplastic components, either ductal or squamous or epithelial and mesenchymal components. Frequently, smears are composed of a predominant cell population and the focal presence of other cell types or mesenchymal fragments may easily be disregarded, thereby missing the correct diagnosis (Fig. 12.17). Squamous metaplasia is the most common feature in metaplastic carcinomas, and a careful scrutiny of all smears

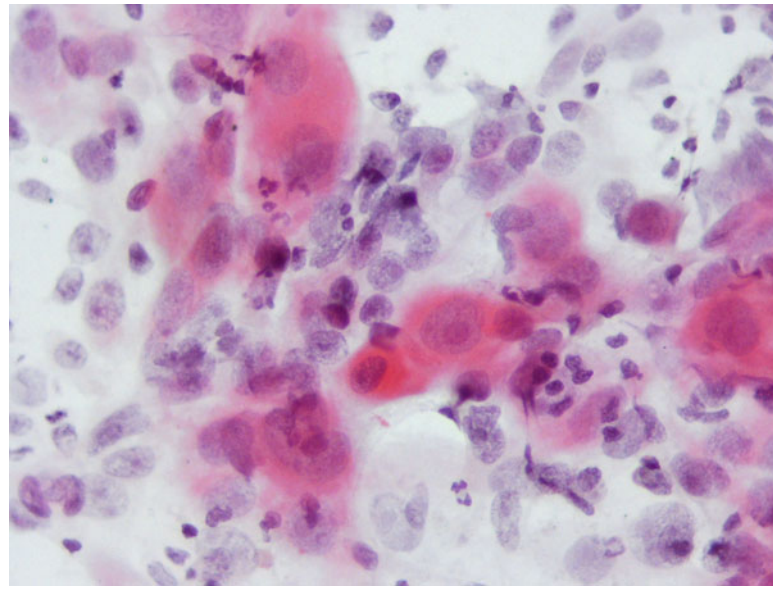

Fig. 12.14 Metaplastic breast carcinoma showing squamous cell differentiation (HE stain)

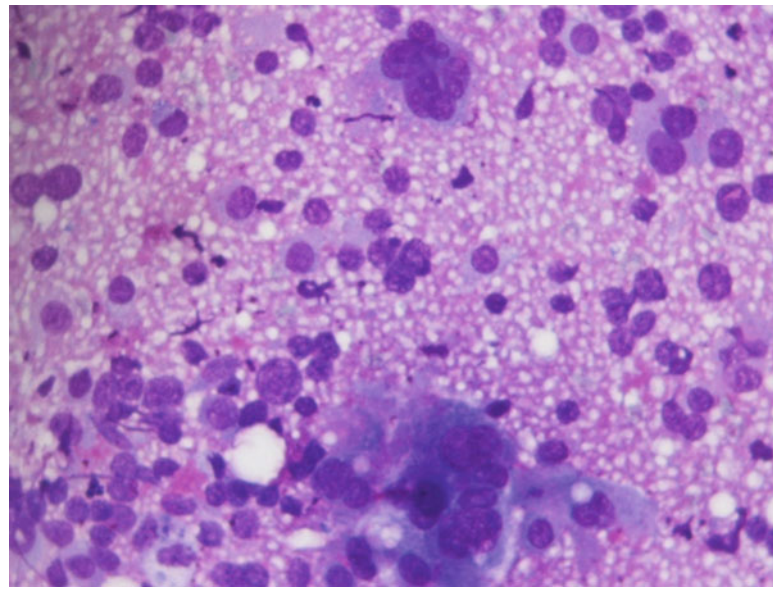

Fig. 12.15 Metaplastic breast carcinoma displaying malignant multinucleated giant cells (MGG stain)

is necessary as squamous cells are often scarce. Sarcomas of the breast excluding malignant phyllodes tumors are extremely uncommon. Therefore, in smears with a predominance of neoplastic spindle cells, the possibility of metaplastic carcinoma should be considered and immunocytochemistry performed, as cytokeratin and p63 positivity of neoplastic cells confirms this diagnosis. In some cases, the finding of a myxoid background is the key to identification of the mesenchymal component of the neoplasia. Differential diagnosis in breast FNAC smears showing myxoid ground substance includes phyllodes tumor, fibroadenoma, mixed tumor, and stromal sarcoma (Lui et al. 2007b). In phyllodes tumor and fibroadenoma, the presence of sheets of bland epithelial cells and bipolar naked nuclei should prevent a false-positive diagnosis. Mixed tumors frequently showed bland spindle cells or groups of epithelial cells without significant atypia. Breast sarcomas are generally

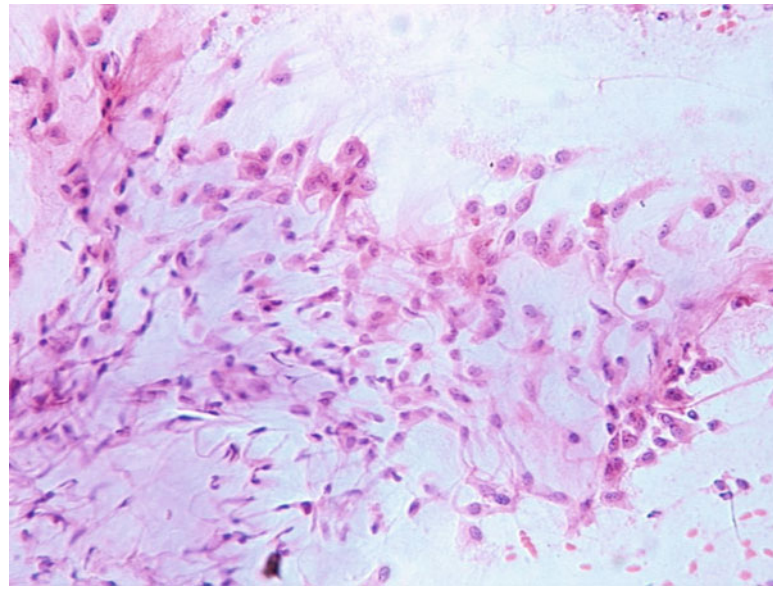

Fig. 12.16 Metaplastic breast carcinoma. Hypercellular smears with isolated spindle cells with bland appearance (HE stain)

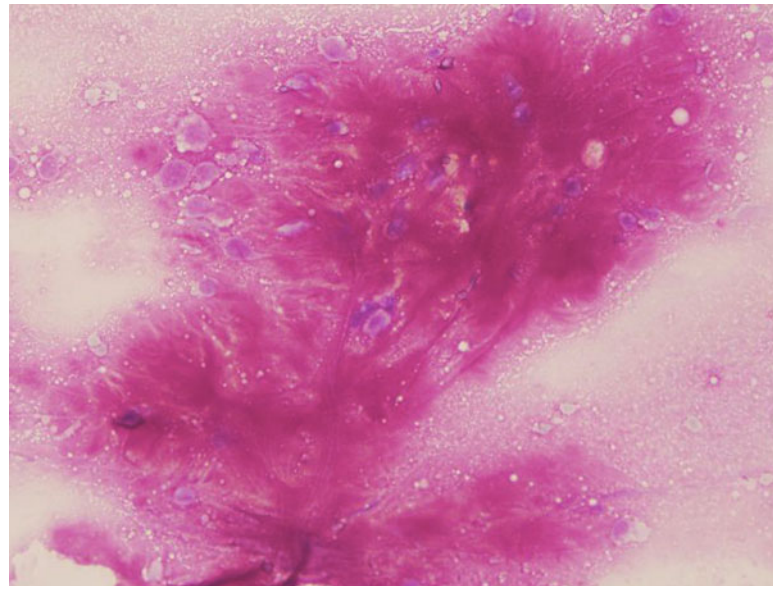

Fig. 12.17 Metaplastic breast carcinoma. Note dispersed malignant cells mixed with stroma resembling chondroid matrix (MGG stain)

more pleomorphic and anaplastic and neoplastic epithelial component is absent. In summary, several FNAC cytology findings may suggest the diagnosis of metaplastic carcinoma of the breast, namely, a liquid aspirate, a proteinaceous or chondromyxoid background, and poorly differentiated malignant tumor cells with multinucleated giant cells. A definite diagnosis, however, requires a convincing demonstration of different neoplastic components, consisting of either ductal and squamous cells or simultaneous epithelial and mesenchymal differentiation. Immunocytochemistry can be helpful, to confirm the presence of different neoplastic components.

12.4.10 Apocrine Carcinoma

Aspirates from apocrine carcinoma are characterized by high tumor cellularity. The tumor cells are arranged singly and in syncytial tissue fragments. Both the cells and the nuclei are enlarged

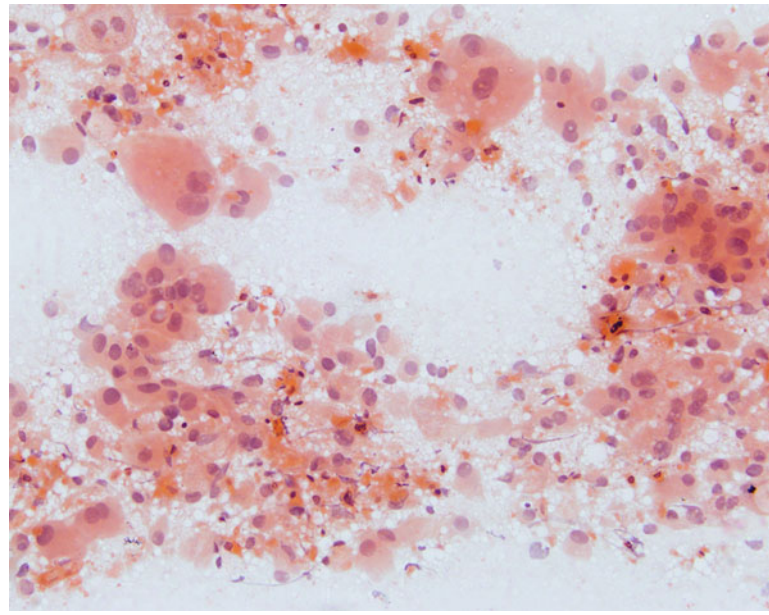

Fig. 12.18 Apocrine carcinoma. Pap-stained smear reveals abnormal cells with pleomorphic nuclei and copious cytoplasm, interspersed with degenerate necrotic cells with pyknotic nuclei

and pleomorphic. The cytoplasm is abundant, finely granular, and amphophilic (Fig. 12.18). The cell outline is polygonal. The nuclei are oval to round and contain prominent nucleoli. Pleomorphic nuclei, poorly defined cell borders, and cell discohesion are criteria that favor malignancy when compared with benign apocrine lesions. Also, the presence of an accompanying polymorphous cell population should be the clue to the correct diagnosis of benign lesions. Apocrine metaplasia with atypia may be difficult to distinguish from well-differentiated apocrine carcinoma.

12.4.11 Papillary Carcinoma

Papillary carcinoma of the breast is a rare variant of breast cancer with good prognosis. Because of the possibility of a papillary carcinoma being entirely in situ, this is one of the variants of breast carcinoma where the definitive diagnosis is not possible on cytology. Additionally, breast papillary proliferations constitute a group of lesions that show a broad spectrum of morphological changes, ranging from benign to malignant and posing challenges at all diagnostic levels. Moreover, a significant portion of lesions displaying a papillary pattern on FNA are non-papillary on histology follow-up. Fibrocystic change and fibroadenoma may closely simulate papilloma on cytology. The details have been discussed in Chapter 10. Suffice to summarize that in FNAC material, the presence of papillary architecture, spherical papillae (cell balls), columnar cells coating the papillae or as dissociated cells, is a sign of a papillary tumor which could be benign or malignant. Although samples of papillary carcinomas are characterized by an abundance of material, three-dimensional papillary clusters, small papillae arranged in cell balls, isolated columnar cells, and absence of bipolar naked nuclei and apocrine metaplasia, most cytologic features overlap in benign and malignant papillary lesions (Fig. 12.19). The aspirates may be associated with blood. Presence of ball cells and absence of bipolar naked nuclei are two of the most distinctive findings favoring a malignant conclusion. Ancillary techniques can be useful in the distinction of benign and malignant papillary tumors. In our experience, p63, a p53-homolog nuclear transcription factor, is a reliable myoepithelial cell marker in cytological smears (Reis-Filho et al. 2003). p63 could be better than other myoepithelial markers as its localization in the nuclei of cells overcomes the cytoplasmic fragility of myoepithelial cells in FNAC. Moreover, p63 highlighted all the bipolar naked nuclei in the background of the smears, which prove their

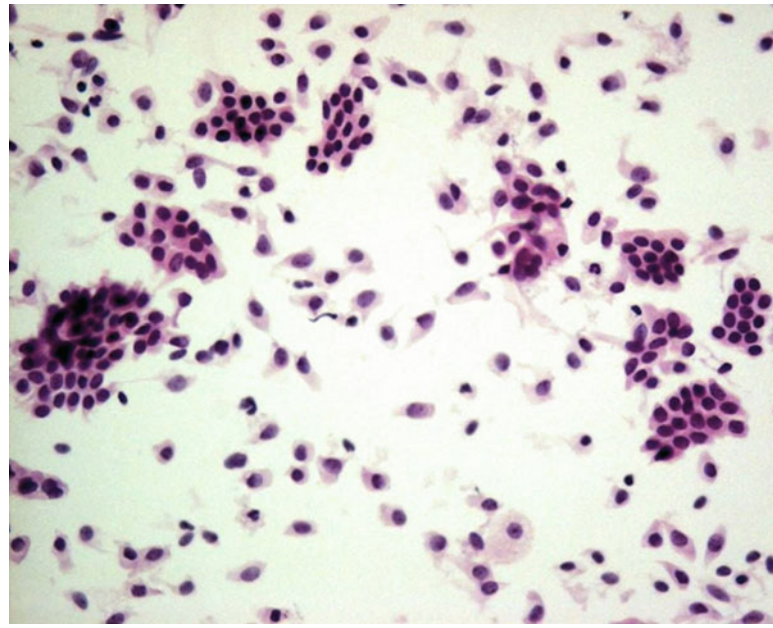

Fig. 12.19 Papillary carcinoma. Cytology shows papillae arranged in cell balls, isolated columnar cells, and absence of bipolar naked nuclei

myoepithelial origin. Therefore, p63 is a potential adjunct in the interpretation of papillary lesions of the breast, being positive in benign tumors.

12.4.12 Secretory Carcinoma

Aspirates of secretory carcinoma are characterized by the presence of globular structures consisting of small centrally located, mucoid material with covering epithelia, usually composed of two or three, and occasionally more, cells (Shinagawa et al. 1994). The globular structures are generally uniform in size. The nuclei are crescentic or ovoid with no atypia. The presence of grapelike clusters of vacuolated cells may also be a helpful cytologic feature. Other findings of secretory carcinoma include prominent intracytoplasmic vacuolization and occasional signet ring cells. Abundant colloid-like material with cracking artifact can be present.

12.4.13 Acinic Cell Carcinoma

This is a variant of breast carcinoma, similar to the acinic cell carcinoma (ACC) of the salivary gland and pancreas in morphology. ACCs are reported to have a rather indolent behavior. The cytological diagnosis of ACC is difficult even when the tumor is primary from salivary glands. The smears of the well-differentiated cases show acinic cells with small and uniform nuclei abundant and granular cytoplasm, and tendency to form glandular structures. Groups of cells can be traversed by large and prominent blood vessels. Poorly differentiated cases can have cells with more vacuolated cytoplasm, which are less cohesive, and harbor moderate atypia (Fig. 12.20). In the breast, differentiation from tumors with similar histological appearances should be considered here, namely, apocrine carcinoma, glycogen-rich carcinoma, oncocytic carcinoma, and secretory carcinoma. Due to the low-grade appearance of the ACC, some benign conditions should also enter the differential diagnoses, such as lactating adenoma. Despite the absence of nuclear atypia, the nucleoli are more prominent in lactating adenoma than in ACC. The homogeneous aspect of the cells and the clinical history are helpful for this differential diagnosis. Apocrine carcinoma cells have abundant granular cytoplasm, but in this variant of breast cancer, the nucleoli are consistently prominent in contrast with ACC. In the glycogen-rich carcinoma, we observe the presence of plasmacytoid appearances in cells with

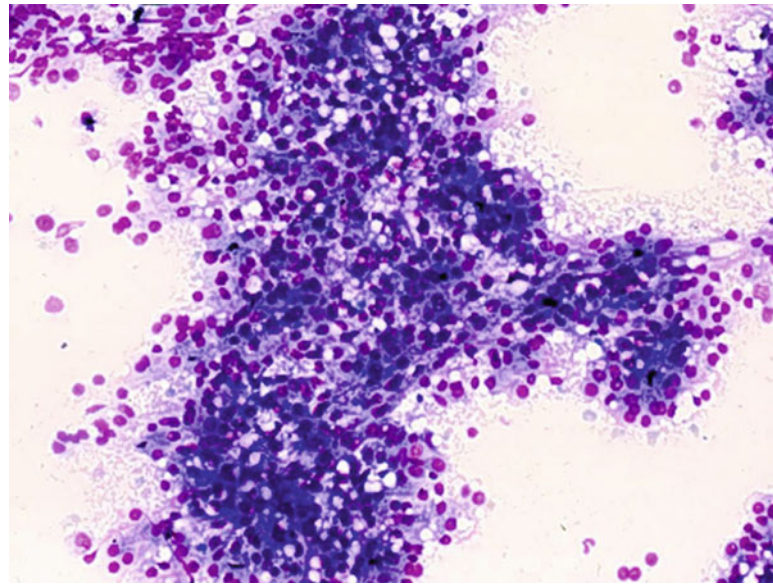

Fig. 12.20 Acinic cell carcinoma. Presence of vacuolated cells with moderate atypia can be seen. (MGG stain)

abundant granular eosinophilic to finely vacuolated or clear cytoplasm that is PAS-positive. Nuclei are in general high grade. Secretory carcinoma is characterized by the presence of large amounts of intracellular and extracellular secretions in a clear background. Large vacuoles containing proteinaceous material are present. Some authors hypothesized that ACC and secretory carcinoma are identical lesions. However, secretory carcinoma and ACC differ in terms of their clinical presentation: no cases of ACC affecting prepubertal patients or males have been reported to date. Furthermore, cytologically, there are differences between secretory carcinoma and ACC. In addition, as recently demonstrated, the absence of ETV6 gene rearrangements in ACCs provides strong circumstantial evidence to suggest that ACC of the breast is not a variant of secretory carcinoma and should be considered a separate entity. Metastatic acinic cell adenocarcinomas originating in the salivary glands or in the pancreas should be excluded before rendering a diagnosis of primary acinic cell-like carcinoma of the breast.

12.4.14 Glycogen-Rich Carcinoma

This tumor is characterized by the presence of clear cells that are filled with glycogen. Aspirates are cellular with tumor cells in groups, clusters, or as isolated cells. The cytoplasm is ample, clear, and fragile, containing a central nucleus with moderate to marked pleomorphism. Metastatic clear cell carcinoma from the kidney should be differentiated from this variant.

12.4.15 Lipid-Rich Carcinoma

Aspirates of lipid-secreting carcinoma are moderately cellular with loosely cohesive tumor cells (Insabato et al. 1993). The cells display well-demarcated cytoplasm containing many large and small vacuoles. The number of vacuoles varies from single one occupying most of the cytoplasm to so many as to give the cytoplasm a foamy appearance. The nuclei are slightly pleomorphic, with distinct nuclear membranes, coarse or fine chromatin, and small nucleoli. Some tumor cells show deeply indented nuclei as well as nuclear vacuoles.

12.4.16 Adenoid Cystic Carcinoma

Adenoid cystic carcinoma is a rare variant of breast cancer with an excellent prognosis and often does not have lymph node metastasis. The FNAC smears of this tumor may show different

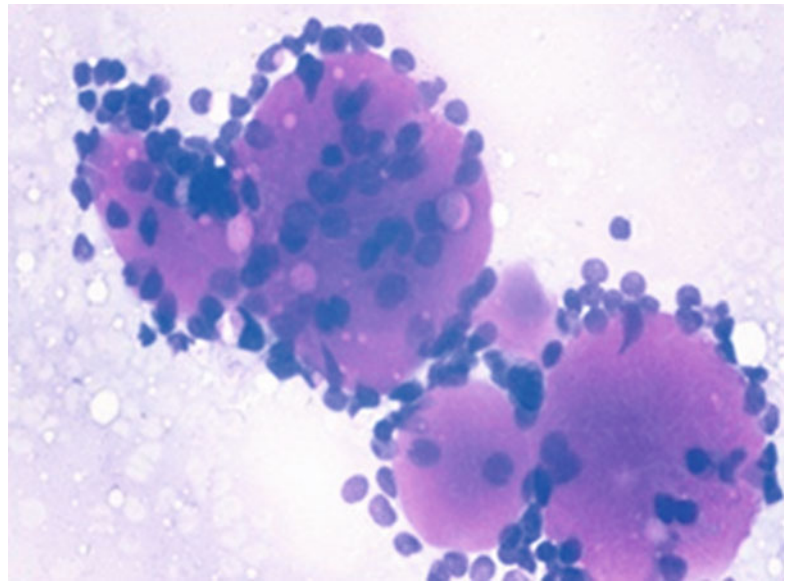

Fig. 12.21 Adenoid cystic carcinoma. Note the malignant cells surrounding the hyaline spheres

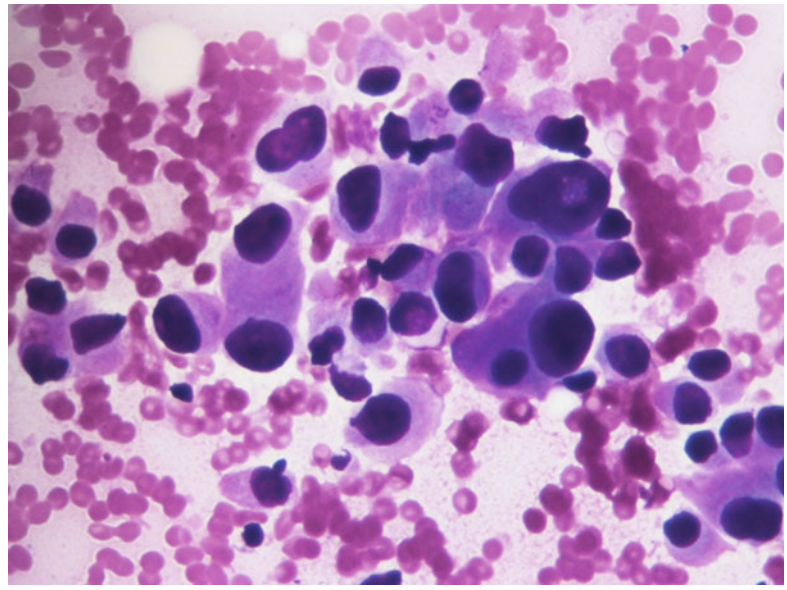

Fig. 12.22 Paget's disease. Tumor cells are large, with abundant cytoplasm and large nuclei with prominent nucleoli

patterns. The most common is clusters of cohesive small uniform cells arranged around magenta-stained hyaline globules associated with tubular structures covered with uniform epithelial cells (Fig. 12.21). The individual cells are small and have round or ovoid nuclei which in smears often are naked but a narrow rim of cytoplasm may be present. In less differentiated cases, there is a predominance of solid fragments of tumor cells.

12.4.17 Paget's Disease

Paget's disease is an eczema-like change of the nipple and areola usually associated with an underlying in situ or invasive breast carcinoma. The cytological diagnosis can be made either by scraping the nipple or by FNAC. Tumor cells are large, with abundant cytoplasm and large nuclei with prominent nucleoli (Fig. 12.22). The tumor cells are arranged individually and in clusters.

The differential diagnoses include malignant melanoma (in general S100 and HMB-45 positive) and squamous cell carcinoma. The malignant cells of Paget's disease in general are positive for CK7, EMA, and HER2.

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Assessment of Axillary Nodes

13

Jill Su Lin Wong, Gay Hui Ho, and Puay Hoon Tan

13.1 Background

The status of the axillary nodes represents the single most important prognostic factor in the assessment of a newly diagnosed breast cancer. Traditionally, this was assessed by performing axillary lymph node dissection (ALND) and submitting the tissue for histologic examination. This approach is considered the gold standard for assessing the axilla. However, ALND incurs significant morbidity such as lymphedema, sensory loss, and shoulder immobility. With the advent of breast screening and the detection of smaller and more favorable cancers, a large number of newly diagnosed cancers are node negative, and full axillary clearance in these patients would lead to unnecessary long-term morbidity.

13.2 Sentinel Node Biopsy

The development of sentinel node techniques addresses the issue of avoiding unnecessary ALND. The premise of the procedure is that the sentinel node is an accurate surrogate for the rest of the axilla. It represents the first node to receive lymphatic drainage from a malignancy in the breast. If this node is negative, there is a low probability that the rest of the axilla is involved by tumor. Most studies have found that the sentinel node is an accurate predictor of the rest of the axillary nodes in >95 % of cases (Mabry and Giuliano 2007; Straver et al. 2010).

Sentinel node biopsy is performed by injecting blue dye or a technetium-labeled sulfur colloid, either alone or together, in the dermis close to the tumor, peritumorally, or in the subareolar complex. The tracers are taken up by lymphatics and drain into the sentinel node (Fig. 13.1). There is often more than one sentinel lymph node.

At surgery, the sentinel node is localized by direct visualization and/or identified by a gamma probe and excised.

If the sentinel node is positive for malignant cells, completion ALND is performed. Sentinel node biopsy is not indicated in patients with clinically palpable nodes. Such patients require axillary clearance. However, clinical palpation is often not accurate; the false-negative rate has been reported at 33 % (Sacre 1986). Ultrasound (US) evaluation is a more accurate method of assessing non-palpable nodes. Those nodes which demonstrate indeterminate or suspicious sonographic features could be further evaluated by FNAC or core biopsy. If the lymph node is positive for malignancy, the patient would not undergo SLNB but proceed to ALND as part of the initial surgery. This would obviate the need for a second surgical procedure.

There is a small number (<5 %) of cases where the sentinel node procedure fails as the node is not identified. This is usually due to extensive tumor involvement of the node which prevents adequate accumulation of radioactive tracer or dye. These cases in particular would benefit from ultrasound evaluation preoperatively as their abnormal sonographic appearance can be assessed

G. Tse et al., *Fine Needle Aspiration Cytology of the Breast*,
DOI 10.1007/978-3-642-35000-9_13,

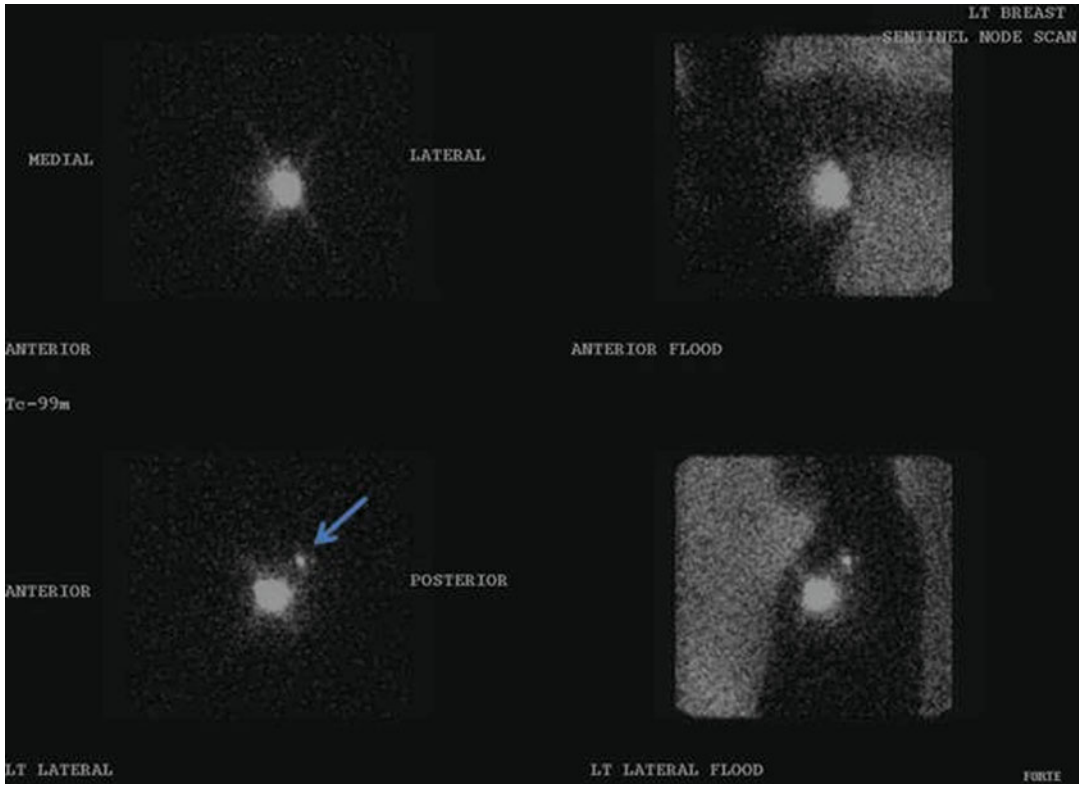

Fig. 13.1 A 68-year-old lady presented with a screen-detected invasive carcinoma. Mammography and US of the nodes were unremarkable. Prior to surgery, Tc99m sulfur colloid was injected into the dermis overlying the tumor. The larger area of tracer accumulation occurs over the tumor; the *arrow* points to the sentinel node superior and posterior to the tumor. Histologic examination of the sentinel nodes was negative for malignancy

and needle biopsy could then be performed (De Kanter et al. 2006).

13.3 Normal Versus Abnormal Nodes: Imaging Appearances

Normal axillary nodes are small bean-shaped structures with a thin cortex and a central fatty hilus. They are commonly seen in mammography (Fig. 13.2). Ultrasound also demonstrates normal nodes which typically have a smooth thin hypoechoic cortex and an echogenic central hilus (Fig. 13.3). Suspicious nodes are generally larger than 5 mm and demonstrate either focal or diffuse cortical thickening (greater than 2 mm), focal nodularity, and/or replacement of their normal fatty hilus. On mammography, as the low-density fat is replaced, the node becomes more dense (Fig. 13.4a). Their characteristic bean shape is lost, and they appear round with or without focal nodularity (Figs. 13.4b, c and 13.5a–c).

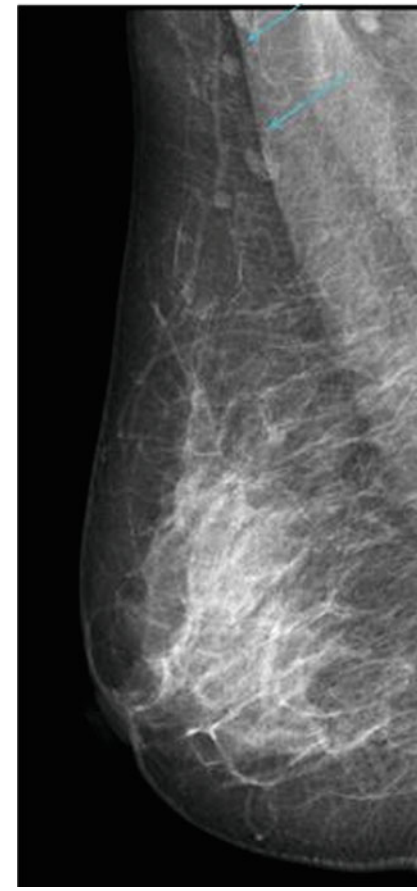

Fig. 13.2 A normal right mammogram shows normal small axillary nodes (*arrows*). They are small, bean-shaped opacities with low-density fat within a hilar concavity

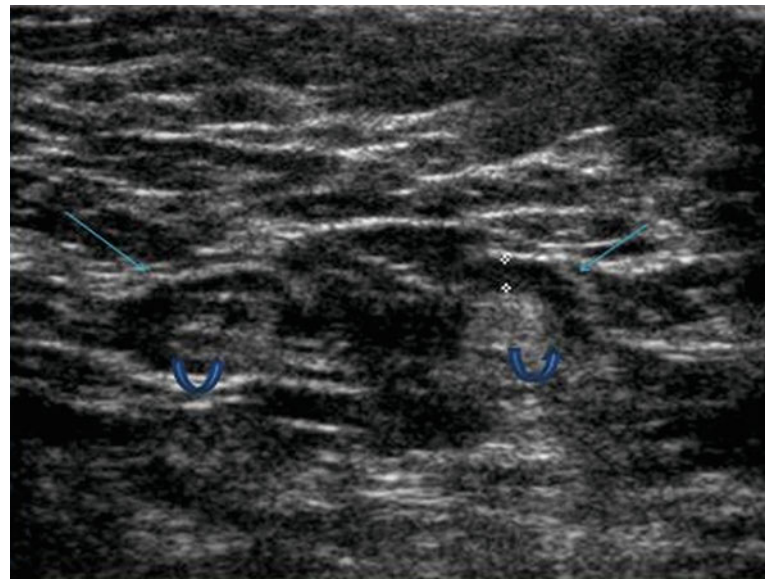

Fig. 13.3 Two axillary nodes (*blue arrows*) with normal appearances. The *calipers* outline the cortical thickness which is under 2 mm. The central echogenic fatty hilus is preserved in both nodes (*curved arrows*)

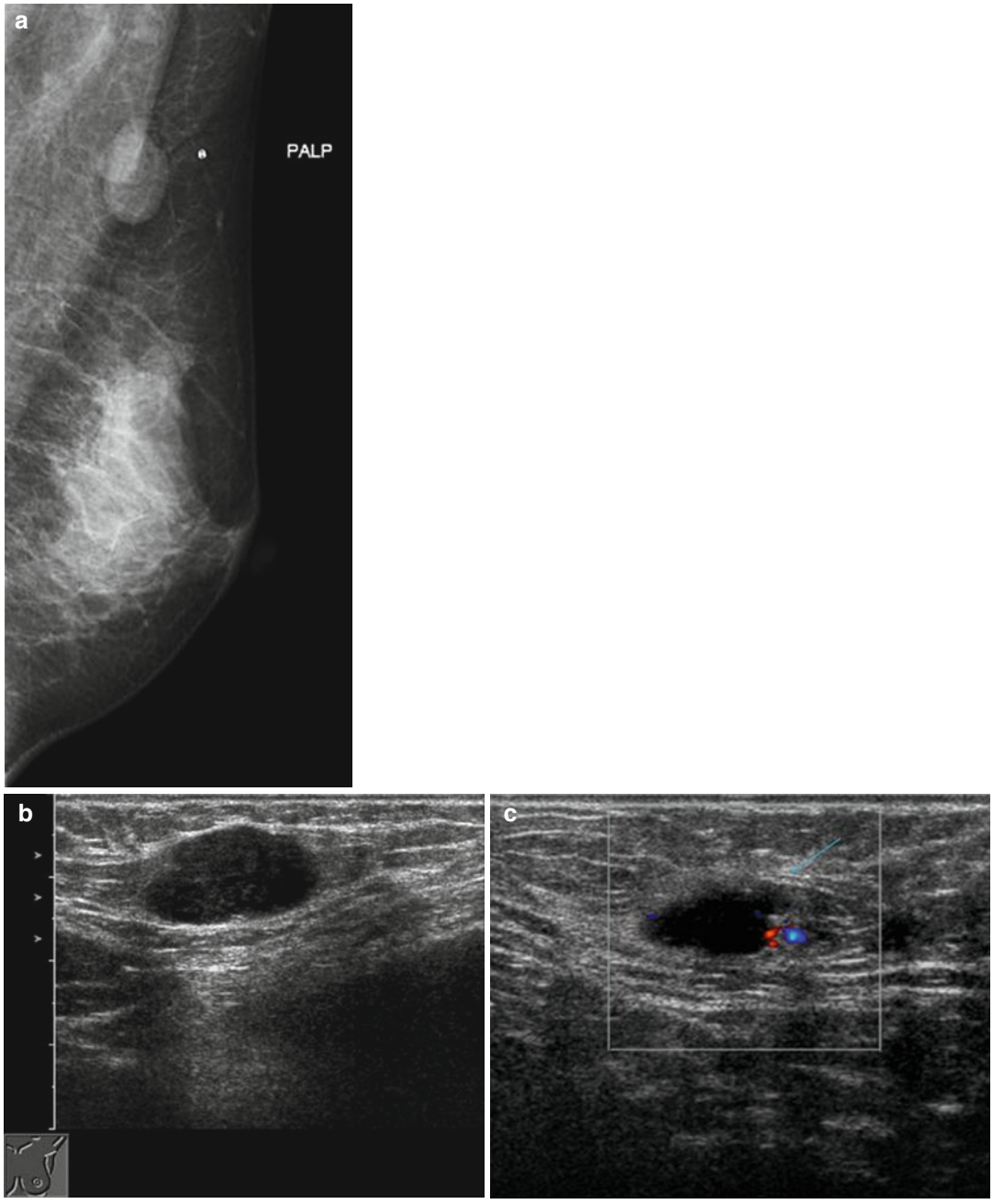

Fig. 13.4 (**a**) Left mammogram in a patient with newly diagnosed invasive carcinoma which cannot be seen on mammography. A skin marker has been placed over a palpable enlarged dense lymph node. The normal fatty hilus has been completely replaced in this node which has lost its normal bean shape and no longer contains any lucency. (**b**) Ultrasound of the same node shown in (**a**) demonstrates a 22 mm hypoechoic nodule; its normal echogenic fatty hilus has been replaced. This yielded malignant cells on FNAC. (**c**) Another node in the same patient is not particularly large (14 mm); however, its fatty hilus is replaced. There is a nodule projecting from its surface (*arrow*) which is not seen in normal nodes

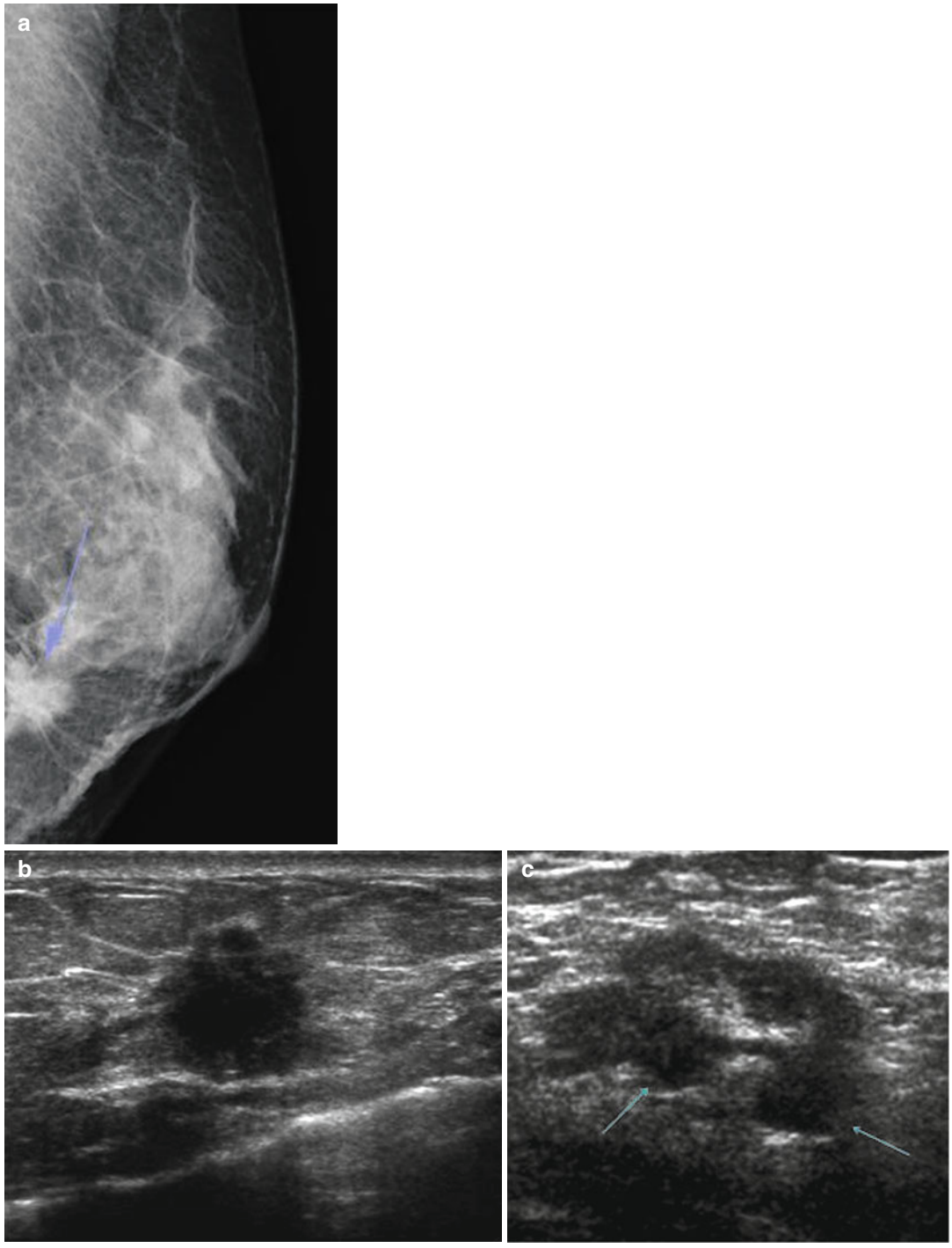

Fig. 13.5 (**a**) A 51-year-old patient presented with a screen-detected spiculated mass in the inferior aspect of the left breast (*arrow*). No enlarged nodes are seen on mammography. (**b**) Demonstrates the sonographic appearance of the breast mass which was biopsy-proven invasive carcinoma. (**c**) Ultrasound of the ipsilateral axilla demonstrates a morphologically abnormal node with at least two nodules protruding from its inferior surface (*arrows*). FNAC failed to obtain malignant cells; however; at surgery, the sentinel node as well as 19 axillary nodes were positive with extracapsular extension

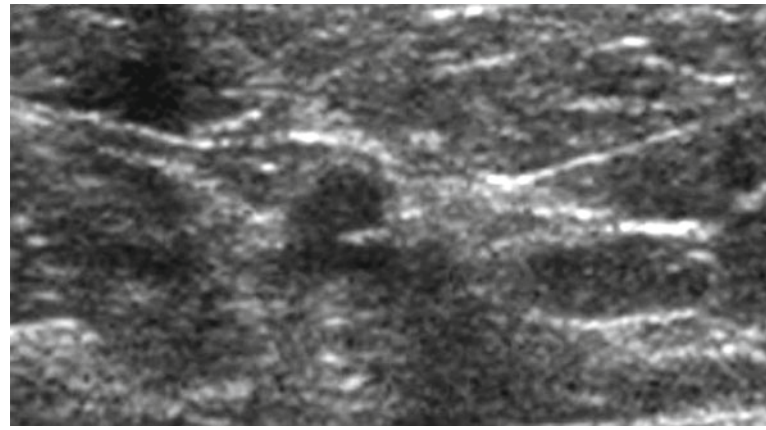

Fig. 13.6 A 58-year-old patient with a newly diagnosed invasive cancer. There is a non-palpable but sonographically suspicious node. This underwent FNA which yielded malignant cells. The needle is kept as horizontal as possible, that is, parallel to the transducer. This allows the length of the needle as well as its tip to be visualized in the same plane

13.4 Ultrasound-Guided Needle Biopsy of Axillary Nodes

US-guided FNAC or core biopsy is indicated in a newly diagnosed breast cancer if the axillary nodes appear suspicious or if the physical examination is equivocal. Occasionally, needle biopsy is requested when the nodes are palpable but thought to be reactive. Another scenario for needle biopsy is in the setting of locally advanced breast cancer when neoadjuvant chemotherapy is planned. Nodal status is difficult to assess after the completion of therapy.

Ultrasound-guided needle biopsy is performed either using 23 G fine needle aspiration and cytologic examination or 14-16G core biopsy can be undertaken if the nodes are sufficiently remote from the vascular structures so that biopsy can be safely performed.

13.5 Fine Needle Aspiration Cytology

FNAC is usually performed using a 23 G needle attached to a 10 ml syringe. Local anesthesia may or may not used. If considered desirable, a small amount (5 mls) of 1 % lignocaine may be infiltrated into the area. In most cases of aspiration, a hypodermic needle is used. However, in deeply sited nodes, a 23 G spinal needle may be used instead. The needle is inserted under ultrasound guidance and is kept as parallel as possible to the transducer (Fig. 13.6). If a spinal needle is used, the stylet should be kept in place during insertion into the node to stiffen the needle. Once the node is pierced, the stylet is removed, and a syringe is fitted to the end of the needle taking care not to dislodge the tip from the node. (The stylet should not be removed prior to insertion as the needle will flex; once the needle bends, its tip is no longer visible.) Color flow imaging can be switched on to check the position of the node relative to the vessels.

Once the tip is inserted into the abnormal node, its position is documented in two planes. When the needle is in satisfactory position, the syringe is aspirated, and the needle and syringe assembly is moved back and forth, keeping the tip within the node. Care should be taken to avoid aspirating a large amount of blood which will clot and block the needle. If a large amount of blood is aspirated, the whole assembly should be changed. The aspirate is placed on slides, and direct smears from the aspirate are either fixed in alcohol or air-dried for Pap and Diff-Quik stains (Figs. 13.7 and 13.8).

The procedure should ideally be performed with a cytotechnologist in attendance who can indicate the adequacy of the samples obtained. If a cytotechnologist is not available, some may consider making more passes into the suspicious node.

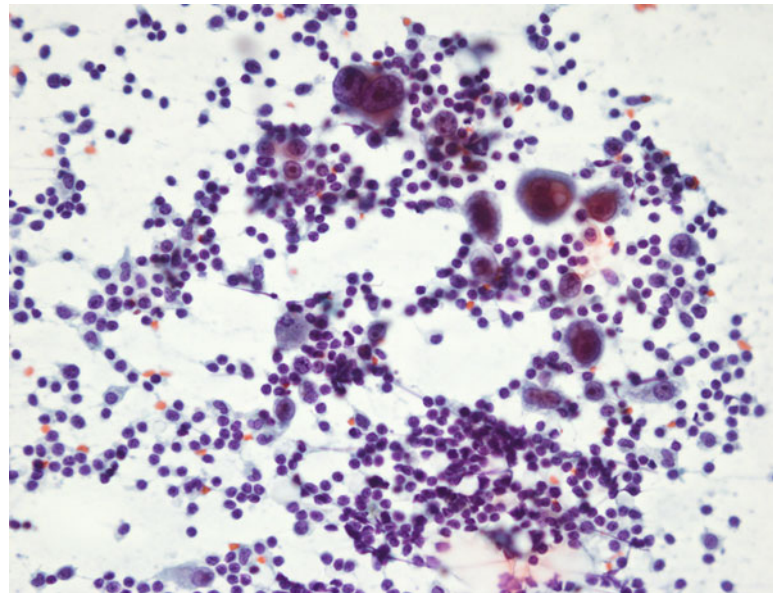

Fig. 13.7 Cytologic smear of a sentinel lymph node that is positive for metastatic carcinoma cells

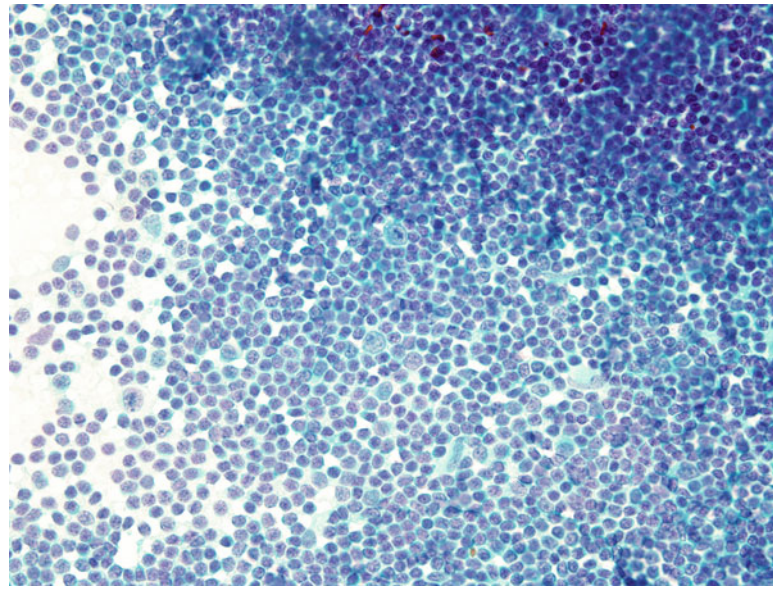

Fig. 13.8 Imprint smear (PAP) of a sentinel lymph node shows predominantly medium-sized lymphocytes with a few interspersed larger activated lymphoid cells

13.6 Accuracy of the Preoperative Ultrasound-Guided Needle Biopsy

Assessment of axillary nodes with ultrasound and ultrasound-guided needle biopsy have been performed for many years. Alvarez et al. reviewed 16 prior studies evaluating the role of sonography in axillary metastases in breast cancer and concluded there was a 37 % false-negative rate using ALND or SNLB as the gold standard. These included cases where the nodes were not seen or visualized on ultrasound or were not suspicious and in whom no biopsy was performed but were found to have metastases (Alvarez et al. 2006). The common causes of discrepancy between the initial and final axillary lymph node status include failure to visualize all lymph nodes during US examination, small-sized metastases, and preoperative neoadjuvant chemotherapy (Krishnamurthy et al. 2002). The published studies suggest that sonographic evaluation of nodes with ultrasound-guided needle biopsy is moderately accurate. If the result is positive for malignancy, the patient will proceed for full axillary clearance at the first surgery; the SNLB procedure is avoided. If the needle biopsy is negative, the patient will proceed to SNLB as a negative needle biopsy does not confidently exclude nodal metastasis.

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Special Ancillary Techniques: Immunohistochemistry

14

The role of immunohistochemistry in the assessment of breast lesions, in particular breast cancers, cannot be overemphasized. Currently, there is a whole gamut of markers that are being used, aiding in the differentiation between benign and malignant breast lesions, prognostication of breast cancers, and prediction of responses to therapy. While most of the routine immunohistochemistry studies are being done on the paraffin-embedded histologic samples using either the resection specimen or the preoperative biopsy, the use of cytologic preparations in immunohistochemistry is gaining popularity and experience.

14.1 Markers Useful for Differentiating Between Benign and Malignant Aspirates

Markers previously evaluated in this category can be grouped into proliferation markers related (Ki-67 and proliferating cell nuclear antigen/PCNA), tumor suppressor gene related (p53), and myoepithelial cell markers related (p63). The prototypical scenario of malignant aspirates is that the carcinoma cells tend to show increased p53 expression, higher Ki-67 index and PCNA expression due to high cellular proliferation, and absence of p63 staining owing to loss of myoepithelial cells.

Ki-67 is a cellular marker for proliferation which is detected during the active phases of the cell cycle (G1, S, G2, M), but not G0 phase. In the non-mitotic phase, the protein is detected in the nuclei, and in the mitotic phase, the protein is detected on the surface of the chromosomes. The uncontrolled cellular proliferation in breast cancer renders the immunohistochemical staining for nuclear Ki-67, the most widely used method for assessing proliferative activity of the tumor, and this assessment has potential use in prognostication and prediction of response to chemotherapy or endocrine therapy (Dowsett et al. 2011). In the realm of cytology, Ki-67 has also been investigated as a diagnostic marker, being expressed in most breast cancer cases that are evaluated cytologically, with staining performed on FNAC smears. Some authors reported that Ki-67 is useful in the differentiation between benign and malignant aspirates, with the former showing a lower Ki-67 expression (Midulla et al. 2002) (Fig. 14.1). In most of the reported series, there was established correlation of Ki-67 with mitotic count, grade, and S phase fraction (Ostrowski et al. 2001; Pelosi et al. 1994; Dalquen et al. 1997). Very little is known regarding the comparison of Ki-67 expression in different breast lesions. Interestingly, there was one previous report (Dalquen et al. 1997) that showed the expression of Ki-67 in cytologic smears to be significantly different between ductal and lobular carcinoma, with the former showing higher degree of expression.

Mutations in *p53* gene are among the most common changes in human cancers, including breast cancer, resulting in an increased posttranscriptional stability of the p53 protein, allowing it to be detected by immunohistochemistry. It has

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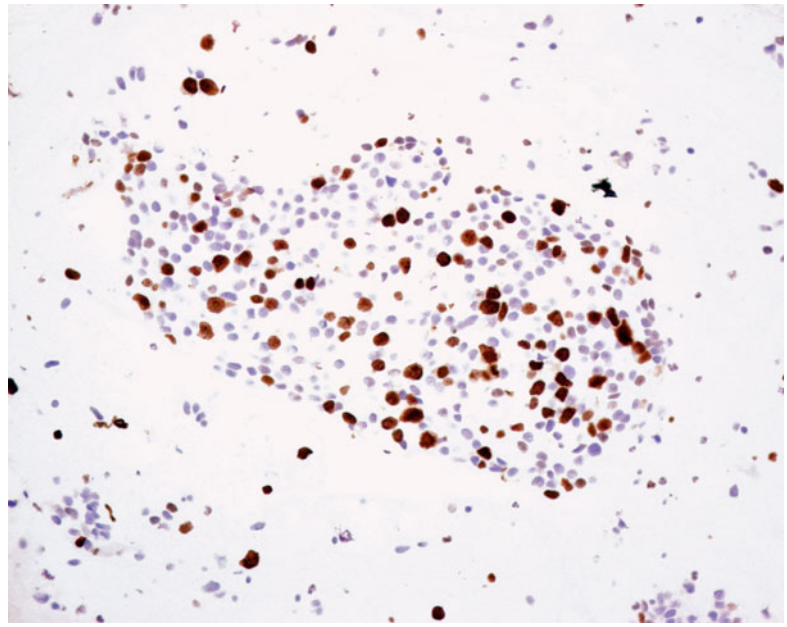

Fig. 14.1 Cell block of invasive breast carcinoma. Ki-67 expression was seen as nuclear staining in some of the tumor cells

been reported that staining in the cytologic preparations for p53 was positive in 28–70 % of malignant cases but was mostly negative (or only up to 11 %) in benign aspirates (Pelosi et al. 1994; Koutselini et al. 1991; Colecchia et al. 1995; Stephenson et al. 1994). Thus, p53 may potentially be useful in the characterization of cytologic preparations into either benign or malignant categories (Fig. 14.2). It was also further reported that p53 expression was related to the grade of the tumor, with higher grade showing higher p53 expression, but the expression was not related to patients' age, lymph node status, or tumor size (Koutselini et al. 1991; Colecchia et al. 1995).

Cyclin D1 is involved in the mediation of G1–S phase transition of the cell cycle through phosphorylation and inactivation of retinoblastoma (Rb) protein and has been found to be overexpressed in many human cancers including breast (Jares et al. 1997). Despite the many studies evaluating cyclin D1 and breast cancer, only very few were done in cytologic preparations. In one study on FNAC smears, cyclin D1 was found to be expressed in 72.5 % of carcinoma aspirates and 40 % for benign aspirates (Fig. 14.3). Furthermore, in the malignant aspirates, the expression of cyclin D1 was not related to tumor size and tumor grade, but increased expression was found in tumors with increased S phase fraction (Park et al. 2001).

The fundamental guiding principle in differentiating benign from malignant breast aspirates is the presence of myoepithelial cells in benign lesions and the absence of such in malignant lesions. This is a widely adopted central dogma in diagnostic histopathology, with only very few exceptions. A parallel has been drawn in cytology, and studies have been carried out to evaluate the role of myoepithelial staining in cytologic preparations differentiating between benign and malignant aspirates. Among the myoepithelial markers, *p63* has been the most widely investigated, likely related to its higher specificity and easier interpretation, as it stains the nuclei rather than the cytoplasm, compared to other markers. In general, there is a high percentage of staining of p63 in benign aspirates, ranging from 75 to 86 % (Aiad et al. 2011; Harton et al. 2007). Some authors differentiated staining patterns between single cells or in cell clusters, but the proportion of positivity was very similar (Harton et al. 2007). In malignant smears, the positivity rate ranged from 11 to 60 % (Aiad et al. 2011; Harton et al. 2007; Reis-Filho et al. 2002), widely affected by both the staining pattern and the presence of in situ component. There was less staining with the sin-

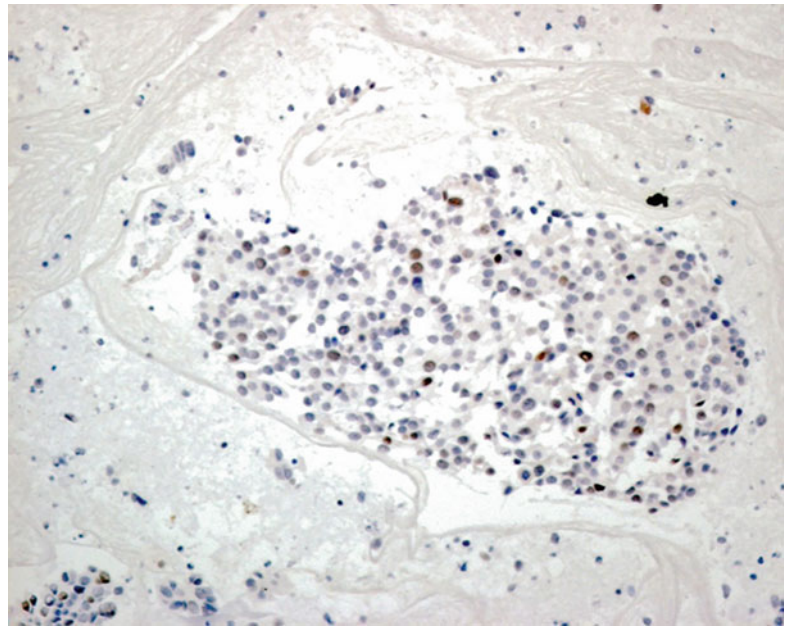

Fig. 14.2 Cell block of invasive breast carcinoma. Scattered p53 expression was seen as nuclear staining in some of the tumor cells

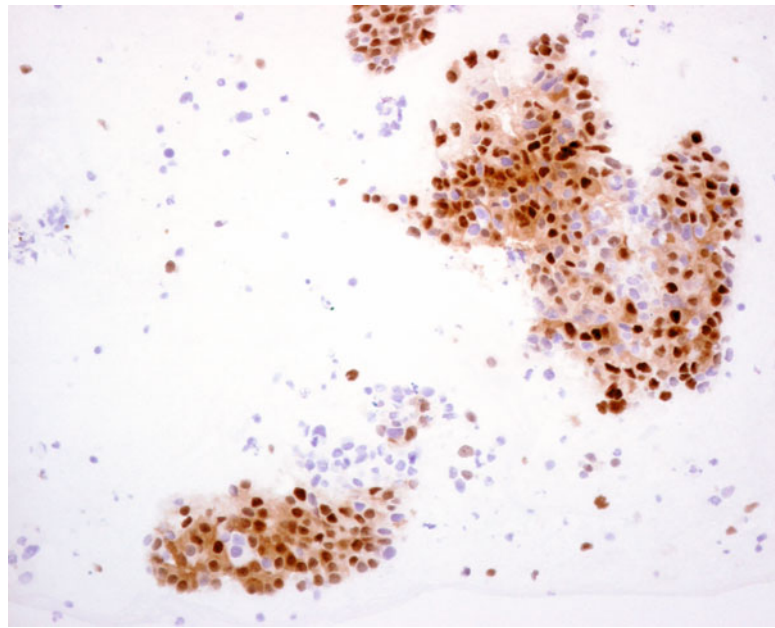

Fig. 14.3 Cell block of invasive breast carcinoma. Cyclin D1 was seen as nuclear staining in some of the tumor cells

gle cells compared to that of cell clusters (Harton et al. 2007), and in one series, all cases of DCIS showed some staining (Reis-Filho et al. 2002) with more substantial staining in pure DCIS. Thus, the specificity of using p63 in cytologic differentiation between benign and malignant lesions is not absolute, with a high margin of uncertainty, especially when there is significant in situ component. Another potential source of error is due to the positive staining of some of the epithelial cells observed in up to 20 % of invasive carcinoma and 37 % of in situ carcinoma, thus adding to the confusion of interpretation (Reis-Filho et al. 2002). Therefore, p63 should only be used as a soft sign,

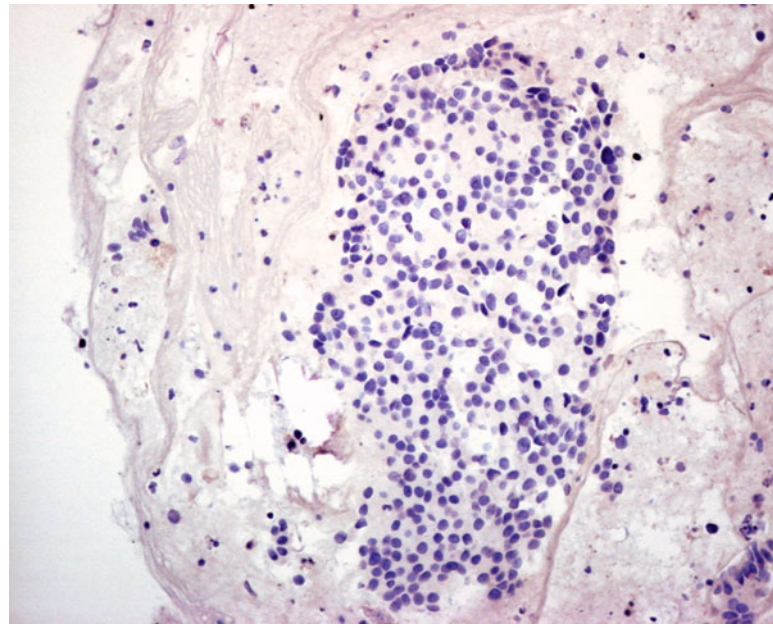

Fig. 14.4 Cell block of invasive breast carcinoma, showing absence of p63 nuclear staining in all of the tumor cells

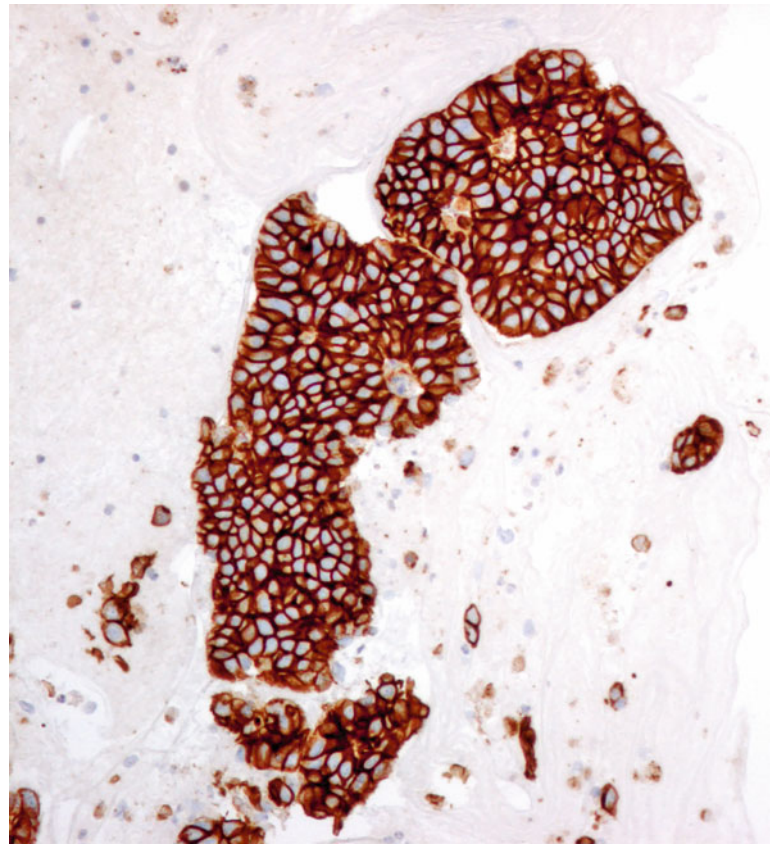

Fig. 14.5 Cell block of invasive breast carcinoma. E-cadherin expression as membrane staining was detected in most of the tumor cells

and the staining results have to be corroborated with other diagnostic considerations (Fig. 14.4).

Another marker increasingly used in diagnosing breast cancer is *E-cadherin* which is an "epithelial" class of the cellular adhesion molecules. It is a transmembrane glycoprotein that is present at the cellular adherens junction of the epithelium and mediates tight cell-cell adhesion. In most breast cancers, E-cadherin expression is retained, with the notable exception of invasive lobular carcinoma and lobular neoplasia (lobular carcinoma in situ and atypical lobular hyperplasia). Hence, the detection of E-cadherin is expected in most aspirates from ductal carcinoma. In fact, E-cadherin was found to be expressed in 66 % of invasive ductal carcinoma aspirates (Fig. 14.5). Furthermore, decreased expression of E-cadherin was shown to be associated with higher tumor grade, lymph node metastases, negative ER status, and negative bcl-2 staining. Hence, E-cadherin staining was associated with adverse biological parameters (Kalogeraki et al. 2003).

14.2 Markers Useful for Classification of Breast Cancers

Gene profiling studies have enabled the establishment of the molecular classification of breast cancers, with the respective categories showing robust grouping and prognostic significance (Perou et al. 2000). Despite the original classification being based on gene profiling, there has been a plethora of literature investigating the use of immunohistochemical markers as surrogates in performing this molecular classification. In essence, the generally accepted surrogate immunohistochemical markers for the various molecular classes of breast cancer include *luminal A* (ER and/or PR positive, HER2 negative), *luminal B* (ER and/or PR positive, HER2 positive; or ER and/or PR positive, HER2 negative, high Ki-67 (>15 %)), *HER2* (ER negative, PR negative, HER2 positive), and *basal* (ER negative, PR negative, HER2 negative, basal markers (CK5/6, CK14, p63 or EGFR) positive (Schnitt 2010). It has been recently investigated whether or not performing the immunohistochemical staining in cytologic preparations correlated with the staining in histologic sections, and preliminary data suggested that using CK5/6, CK8/18, and smooth muscle actin could allow reduplication of such classification in cytologic preparations (Delgallo et al. 2010).

14.3 Markers Useful for Prognostication and Prediction to Response to Treatment

In the evaluation of breast cancers, *hormone receptors' (ER and PR)* status is one of the most important predictive factors for response to hormonal therapy. Initially, these were evaluated by biochemical methods, and the assessment of these hormone receptors was limited by the requirement of fresh tumor tissue of sufficient quantity. Currently with the wide availability and adoption of monoclonal antibodies, assessment for ER and PR has become a simple immunohistochemical test that is mandatorily performed in all malignant breast cancers diagnosed histologically. Hence, performing these immunochemical tests in cytologic preparations has become an issue of great interest and relevance to the clinical management of breast cancer patients, particularly in selected cases and on many centers where FNAC may be the only procedure for harvesting tumor cells. Several studies have evaluated the accuracy of ER and PR immunohistochemistry in FNAC in comparison to those performed either biochemically or immunohistochemically with the corresponding histologic material. FNAC had a higher sensitivity and specificity when compared to biochemical assay, achieving 90 % sensitivity and specificity for immunohistochemistry on both ER and PR (Marrazzo et al. 1995). Interestingly, an earlier report showed no false-positive testing for ER but a false-negative rate of about 12 % attributed to the presence of a prominent stromal component (Reiner et al. 1987). Comparing ER and PR immunohistochemistry on FNAC with that of the histology showed a false positivity of 24 % (Jayaram and Elsayed 2005). Other investigators have evaluated the general overall ER/PR concordance of FNAC with biochemical assay or histology. Earlier

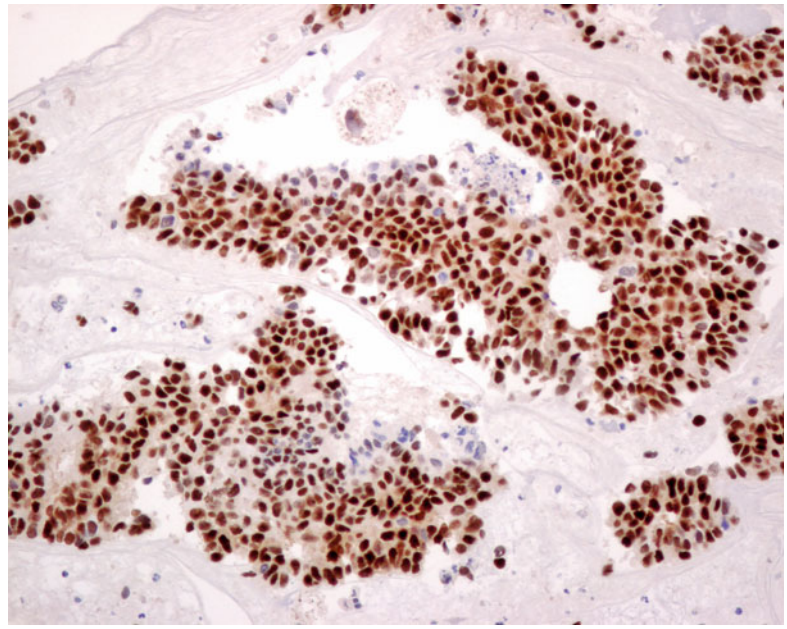

Fig. 14.6 Cell block of invasive breast carcinoma. ER was expressed as nuclear staining in most of the tumor cells

reports demonstrated a concordance rate of about 87 % for both ER and PR (Nizzoli et al. 1994), and a similar figure of 80 % was also reported when FNAC immunohistochemistry was compared to enzyme immunoassay (Lofgren et al. 2003). Recently with the increasing popularity and experience of FNAC, the concordance has increased to 95–98 % when compared to immunohistochemistry on histologic materials (Moriki et al. 2004). As experience with immunohistochemistry in FNAC breast cancers increased, it is now apparent that the correlations of ER and PR with the staining in the histologic samples are not equal. In general, the correlation of ER was higher than that of PR (Tafjord et al. 2002; Zoppi et al. 2002; Cano et al. 2003). In these series, the reported concordance rates for ER were in the range of 89–94 %, whereas those for PR were between 63 and 78 %. Some authors also reported a correlation of ER between FNAC and histology immunohistochemistry, but not for PR (Railo et al. 1996). Thus, currently, the evidence is that hormone receptor assessment on cytologic materials is accurate, more so for ER than PR (Figs. 14.6 and 14.7).

HER2 represents another mandatory biomarker that is routinely assessed in breast cancer. HER2 overexpression, either by gene amplification or protein expression, can be detected in about 15 to 25 % of all breast cancers by in situ hybridization or immunohistochemistry, respectively. Breast cancers showing HER2 gene amplification reveal a high predictive value for response to targeted treatment directed at the HER2/neu domain, and to date, immunohistochemistry has shown good correlation with the gene amplification. Assessment of HER2 expression by immunohistochemistry on FNAC may have an important bearing on the proper treatment approach and preoperative chemotherapy. Many studies have assessed immunohistochemical staining on either FNAC smears (Moriki et al. 2004; Nizzoli et al. 2003; Jorda et al. 1994; Corkill and Katz 1994) or cell blocks (Shabaik et al. 2011; Klorin and Keren 2003; Williams et al. 2009), and most of them demonstrated good correlation with the histologic immunohistochemical staining, with a concordance rate ranging from 84 to 100 %. Other authors also showed that using cell block, the immunohistochemical staining of HER2 on primary and metastatic breast cancers detected in serous effusions showed 100 % correlation between these tumors, indicating the usefulness of immunohistochemical staining on cell block of metastases in serous fluid (Shabaik et al. 2011) and lymph node

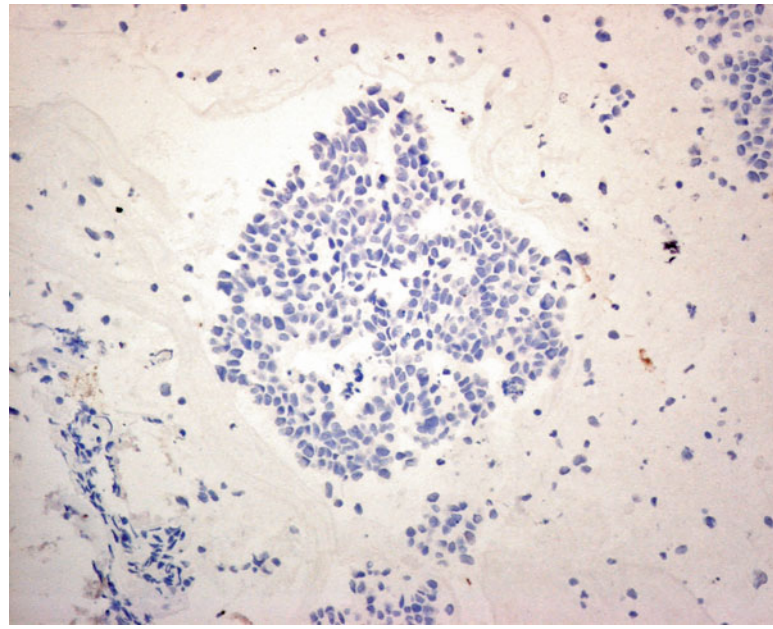

Fig. 14.7 Cell block of invasive breast carcinoma. PR was negative in the same case

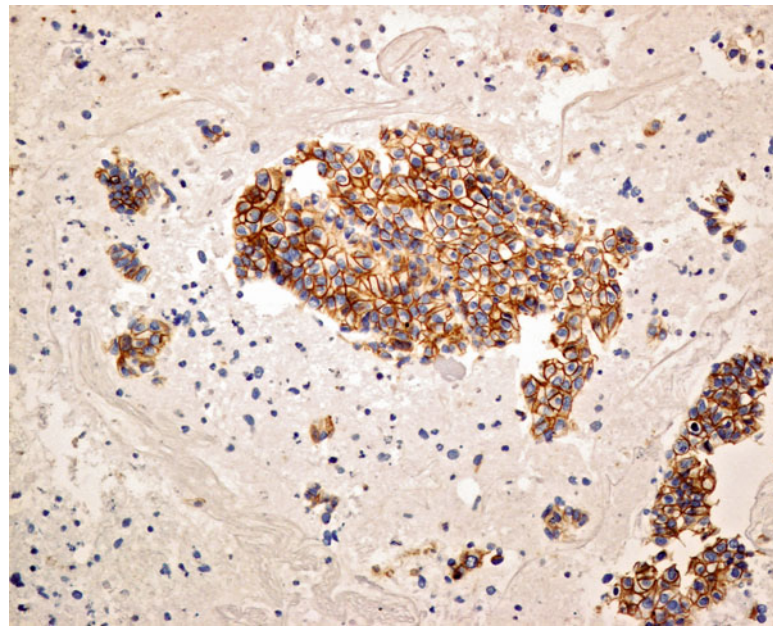

Fig. 14.8 Cell block of invasive breast carcinoma. Complete and strong membrane staining of HER2 was seen in most of the tumor cells

(Briffod et al. 2000) (Fig. 14.8). In those cases which reported discordance, the false-positive results were attributed to poor cytologic preparation (Nizzoli et al. 2003; Jorda et al. 1994) and stronger staining intensity in the cytologic preparations in comparison to the histologic preparations (Corkill and Katz 1994). This phenomenon has also been reported by other authors (Slamon et al. 1989; Gusterson et al. 1988), who attributed this observation to the weakening of the antigens during formalin fixation of the tissue in histologic preparations. Alcohol fixation of the cytology cell block may also contribute to the higher positivity rate for HER2 (Hanley et al. 2009).

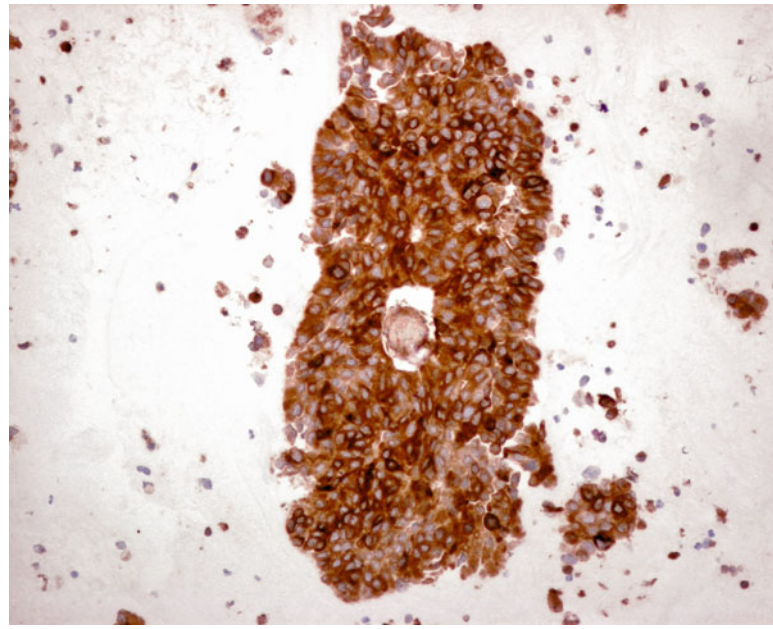

Fig. 14.9 Cell block of invasive breast carcinoma. Bcl2 was highly expressed in the cytoplasm of the tumor cells

14.4 Markers Useful for Correlation with Response to Treatment and Outcome

The prognostic and predictive markers for breast cancer are well established, and these are routinely assessed in the histologic assessment of resection specimens. At times, it may be beneficial for treatment planning to assess these factors in the initial diagnostic pathologic samples. This may entail such assessments in the FNAC or the corresponding cell block (Makris et al. 1997). In a prospective study, it was shown that immunohistochemical staining on cell blocks of breast cancer aspirates showing ER and PR negative or "triple negativity" (ER, PR, and HER2 negativity) was associated with tumor regression at neoadjuvant chemotherapy (Becette et al. 2011). In addition, *Bcl2,* which is involved in cell death regulation, may also act as a modulator for breast cancer response to chemotherapy or hormonal therapy. It has been reported that using immunohistochemistry on FNAC smears, the expression of bcl2 was correlated with ER and PR expression and negatively with p53, Ki-67, and high nuclear grade, thus affirming the good prognostic value of bcl2, and this can be demonstrated using cytologic preparations (Fig. 14.9).

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Molecular Studies 15

15.1 Introduction

The application of molecular techniques in pathology has changed the practice of cytopathology. Currently the use of molecular techniques on cytology as adjuncts to morphology for diagnosis and prognosis is widely accepted. Moreover, the study of markers of therapeutic response has been helpful in some types of tumors. There are many advantages in the use of cytological material over histology to perform molecular studies: ease of obtaining fresh material, ability to check the quality of the material immediately after harvest, and better preservation of DNA and RNA (Schmitt et al. 2008; Schmitt and Barroca 2011, 2012). The possibility of using genomic and proteomic studies in small amounts of material obtained, for example, by FNAC, can minimize invasive procedures and allow the monitoring of cancer, including therapeutic response, with repeated testing. The introduction of liquid-based cytology offered the possibility of preservation of cells in an environment of excellent quality, especially when compared to formalin-fixed and paraffin-embedded tissues.

The molecular techniques most commonly used on cytology include polymerase chain reaction (PCR) and in situ hybridization (ISH). However, other techniques such as in situ PCR, microarrays, proteomic and sequencing (including next-generation sequencing) methodologies are now being validated (Di Lorito and Schmitt 2011). PCR methods are ideal for cytology material, and some applications are for detection of gross chromosomal alterations as deletions and translocations or even point mutations in individual genes. RT-PCR uses cDNA as a template for primers exon sequences to flank rupture points of translocations. PCR applications are centered in diagnosis of solid tumors detecting gene mutations or detecting clonal gene rearrangements. PCR analysis can be performed directly with freshly collected material from FNAC, in liquid-based cytology samples, or even with cells scraped from FNAC slides. In the first instance, the needle should be washed in ethanol, methanol, or culture mediums like RPMI. The amount and quality of DNA obtained by FNAC for PCR assay do not seem to be a problem, and 50–100 cells are adequate to obtain good PCR results. FNAC-obtained tumor cells provide excellent representative samples, with less contamination by stroma or local structures. In fact, studies on molecular profiling using cDNA microarrays have demonstrated that cytological material compared with histological material has less stromal and inflammatory contaminants.

ISH can also be applied to cytology, with either fluorescent or chromogenic markers, to detect numerical or structural aberrations of chromosomes. This technique is reliable, and is particularly useful in cytology as it can be applied directly in smears. Monolayer smears are ideal for ISH techniques. Slides with ethanol or air-dried fixed preparations, as well as cell blocks, are equally suitable. These techniques are used to

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detect deletions, insertions, or translocations but are more frequently used routinely to detect gene amplifications like HER2 in breast carcinoma.

The main challenges for the application of molecular techniques on cytology are to select the proper test for a limited sample quantity, to avoid jumping from a technique adapted from histology directly to cytology, and to use appropriate controls for cytological material. Validation is an essential step for any molecular test applied on cytology. Comparison with a standard procedure as in paired samples with histological biopsies is a good example of validation (Schmitt and Barroca 2012; Schmitt 2011; Pang et al. 2011). In settings of metastatic tumors, cytological samples may be the only material available for testing. Therefore, comparison between the same technique on cytology and histology is crucial to validate the technique. Controls are another important concern when one uses cytological material. In more than 50 % of published papers on immunocytochemistry applied on cytological material, controls are not even mentioned (Colasacco et al. 2011).

Introduction of molecular techniques brings also another important point: how to preserve good quality material maintaining cellular morphology and DNA/RNA integrity. Previous work demonstrated that liquid-based cytology preparations are suitable for preserving cell samples and DNA and RNA with sufficient quality to be used in several molecular analyses such as PCR, RFLP, and even sequencing (Longatto-Filho et al. 2009; Wholschlaeger et al. 2009). However, the horizons for using molecular techniques on cytology specimens have been expanded with studies showing the applicability of these techniques on archival FNA samples, which are extremely useful in situations where the diagnostic material may be limited to certain slides. In the face of the increasing importance of minimally invasive methods to obtaining samples from metastatic sites, limited cytological samples may be the only material available for mutation analysis (Pang et al. 2011, Schmitt and Barroca 2011, 2012). On the other hand, most of the standardized high-throughput molecular methods for measuring gene expression, such as gene expression profiling, require sufficient quantity and high-quality RNA obtained from fresh or frozen tissues. High-quality fresh-frozen human neoplastic and normal tissues may be stored in tumor banks through validated procedures for collection, storage, retrieval, shipping, and tracking of samples. Recently, it is demonstrated that cells obtained from fine-needle sampling of breast cancer surgical specimens are an effective tissue-sparing method for cell collection and banking with preservation of high-quality RNA (Eloy et al. 2009). This methodology is a very useful alternative to keep material for molecular studies from small tumors in which we need to include all the material for histological evaluation. The role of the cytopathologist is mandatory in the collection and selection of cells, with microdissection in some cases being valuable for enriching the tumor cell population.

15.2 Molecular Classification of Breast Cancer

Breast cancer is a heterogeneous disease, and this term encompasses a variety of entities with distinct morphological features and clinical behavior. In recent years, it has become apparent that this diversity is the result of distinct genetic, epigenetic, and transcriptomic alterations (Curtis et al. 2012; Perou et al. 2000; Reis-Filho and Pusztai 2011; Tabchy et al. 2010; Weigelt et al. 2011). Although morphology is often associated with the pattern of molecular aberrations in breast cancers, it is also clear that tumors of the same histological type show remarkably different clinical behavior. This is most evident in invasive ductal carcinomas of no special type, where tumors of the same histological grade may have distinct outcomes and dramatically different responses to therapy. Using high-throughput technologies, particularly microarray analysis, several groups have proposed a new taxonomy for breast cancer based on their molecular features. The gene expression microarray-based discovery studies have led to the identification of at least five molecular breast cancer subtypes: luminal A, luminal B, normal breast-like, HER2, and basal-like (Perou et al. 2000; Reis-Filho and Pusztai 2011).

Although based on the analysis of a limited number of samples and with somewhat different definitions for the various molecular groups in these studies, this approach to the classification of breast cancer has captured the attention of oncologists, pathologists, and scientists alike. Nowadays this classification has been updated and modified. The normal breast-like subgroup is considered by most of the studies as an artifact of the microarray studies. New subgroups are emerging among the so-called triple-negative tumors (ER−, PR−, and HER2−), such as the claudin low and molecular apocrine. Among the ER-positive tumors, the subgroups luminal A and luminal B are distinguished because of the low and high proliferative index, or the absence or presence of HER2 co-expression, respectively. It should be noted, however, that this taxonomy has identified subgroups of breast cancer that were to some extent already known, and that the stability of the assignments of molecular subtypes by microarray-based methods has been called into question (Badve et al. 2011). Indeed, the most robust distinction observed by microarray analysis is between the transcriptome of ER-positive (ER+) and ER-negative (ER−) breast cancers.

Microarrays have undoubtedly contributed to our understanding of breast cancer. They have provided direct evidence to demonstrate that breast cancer is a heterogeneous disease at the molecular level, that ER-positive and ER-negative diseases are fundamentally different, that molecular subtypes of breast cancer do exist, and that some special histological types of breast cancer are distinct entities at the molecular level. Furthermore, they have led to the development of a molecular taxonomy that is currently being tested in clinical trials and of prognostic "gene signatures," some of which have already been approved by clinical use in the USA and Europe. However, this classification has important limitations, and for the microarray-based molecular taxonomy of breast cancer to be incorporated into clinical practice, standardization of the definitions and the methodologies for the identification of the molecular subtypes and prospective clinical trials to validate the contribution of these molecular subtypes are still required. Despite the huge amount of resources allocated to translational research, only three predictive markers are used to define the therapy of breast cancer patients: ER and PR, the predictive markers of response to endocrine therapy, and HER2, the molecular target of trastuzumab and lapatinib.

15.3 Molecular Studies on FNAC from Primary Breast Tumors

The current clinical management of breast cancer still relies on traditional prognostic and predictive factors, like histology, clinical parameters, and well-defined biologic factors like ER, PR, as well as HER2, all of which present an association with prognosis and treatment outcome. However, this classification system fails at taking into account the tumor heterogeneity, as even tumors that apparently present the same characteristics can have markedly different responses to therapy and present distinct outcomes. The use of high-throughput molecular technologies has enabled the better understanding of this complexity, by allowing the classification of breast tumors into biologically and clinically distinct groups based on their gene expression patterns. From the point of view of treatment, breast cancer patients fall into three categories: the hormone receptor positive cases that can be treated with hormone receptor targeted therapies with or without adding chemotherapy, the HER2 positive cases that will receive HER2-directed therapy either with the monoclonal antibody trastuzumab or the tyrosine kinase inhibitor lapatinib, or those that are negative for hormone receptors and HER2 that are solely treated with chemotherapy. The expression of ER is an important prognostic and predictive factor in breast cancer and has relevant implications for the biology of this type of carcinomas. Patients with tumors that express ER have a longer disease-free interval and overall survival than patients with tumors lacking ER expression. In fact these tumors are not homogenous and can be divided at least in two types: luminal A and luminal B, based on the co-expression of HER2 or a high-proliferative index. This subdivision

is important from the therapeutic point of view because the luminal B tumors are more aggressive, develop resistance more frequently, and should be treated with chemotherapy.

In a preoperative setting, as well as during the treatment of inoperable patients, alcohol-fixed smears obtained by FNAC from breast cancer patients are suitable for determination of the hormonal status using immunocytochemistry. This is reliable and even allows semiquantification of the results. With ER expression in 1 % or more of breast cancer nuclei being considered the criterion of positivity according to the ASCO/CAP guidelines, cytological material can be used to perform ER and PR assessment in a preoperative setting. More recently the utility of proliferative markers, like Ki-67, to stratify ER + breast cancer patients for chemotherapy was also demonstrated.

The clinical use of drugs such as trastuzumab (Herceptin) or lapatinib requires evaluation of HER2 overexpression or amplification on tumors from every potentially eligible patient. FISH is currently regarded as the gold standard method for detecting HER2 amplification. The main difficulty for adopting FISH in a clinical setting is the need for additional equipment for analysis, such as fluorescence microscopy and multiband fluorescence filters. Silver in situ hybridization (SISH), which was developed to overcome the aforementioned disadvantages of FISH, has been used with excellent concordance with FISH. A study showed an overall concordance rate between CISH and FISH was higher than 95 % (Di Palma et al. 2008). Performing HER2 immunocytochemistry studies on FNA material remains problematic, because HER2 scoring is not validated in this material. However, HER2 assessment using FISH or SISH is now possible and useful in FNA with excellent correlation with the histological specimens. The recent approval for Herceptin in adjuvant therapy can expand the use of ISH in aspirates obtained from the primary tumor.

Triple-negative breast cancers (TNBC), defined as tumors that are negative for ER, PR, and HER2, nowadays represent the focus of increasing interest at the clinical, biological, and epidemiological level due to their aggressive behavior, poor prognosis, and current lack of targeted therapies. A better understanding of the pathologic mechanisms of TNBC onset and progression, including the as yet unclarified association with BRCA1 mutation, and the causes of phenotypic heterogeneity, may allow improvement in planning prevention and designing novel individualized treatments for this breast cancer subgroup. Immunohistochemistry is frequently used to explore the distribution of the molecular subtypes by using formalin-fixed, paraffin-embedded tissues from larger cohorts of breast cancer patients. The ultimate selection of surrogate markers is an ongoing debate, and a consensus for an appropriate panel still has to be reached. Triple negativity is often used to identify basal-like tumors although these tumors are not synonymous, and a supplement of additional markers is needed to define basal-like expression. Immunocytochemistry based studies use different markers to define their basal-related tumors, and the lack of a systematic classification scheme makes comparison of results difficult. Although triple negativity coupled with positivity for CK5 and/or EGFR are the panel more frequently used, it was recently demonstrated that adding P-cadherin, vimentin, and CK14 is possible to detect basal-like carcinomas that were negative for CK5 and EGFR (Sousa et al. 2010). Another study has found that a tripanel of CK14, 34BE12 and EGFR is able to identify the basal-like subtype in TNBC with optimal sensitivity and specificity (Thike et al. 2010). Due to the awareness of the aggressive nature of the triple-negative tumors, it is of great clinical interest to establish its diagnosis as early as possible. In the presence of cytological findings of necrosis, prominent nucleoli, and abundant cellularity associated with negativity for ER and HER2, it is advisable to investigate the possibility of dealing with a basal breast carcinoma and, if possible, try to confirm this diagnosis through the immunohistochemical analysis for basal markers (Dufloth et al. 2009).

More than one decade ago, the feasibility of using FNAC material obtained from primary breast cancer to characterize the expression profiling of the tumors was demonstrated. Although this approach is not so important for assessing the molecular subtype of breast cancer as we can translate the classification using surrogate immunocytochemistry, its advantage lies in its application on repeated FNAs of primary

tumors in breast cancer patients undergoing neoadjuvant therapy. Changes in these markers may relate to the clinical outcome of the patients, allowing the selection, optimization, or monitoring of treatment. Some studies conducting repeat sampling of tumors for molecular markers have involved multiple pretreatment and on-treatment samples. There are indications that the optimal time points for the analysis of changes in gene expression may vary between genes, between treatments, and possibly between patients. Multiple sampling episodes will be required to optimize detection of changes in such time-dependent profiles. This will only be possible using sampling techniques that are sufficiently atraumatic to be acceptable to the patient and that minimally perturb tumor gene expression by the sampling procedure itself. For these reasons, FNAC rather than core biopsies, or other incisional approaches, is better for tissue access for molecular markers (Annaratone et al. 2012). Recently, an international randomized clinical trial demonstrated that it was feasible to perform a prospective expression analysis for response prediction of chemotherapy using material obtained by FNA from primary breast cancer patients (Tabchy et al. 2010). Seventy-five percent of the FNAC specimens mailed to a central laboratory yielded adequate RNA for genomic analysis. A 30-gene molecular test was predictive of response to T/FAC and not to FAC chemotherapy. Like most other currently used molecular response predictors, which rely on measuring molecular equivalents of clinical phenotype, this first-generation genomic predictor derives its predictive value from detecting the large-scale gene expression differences that distinguish ER-negative from ER-positive tumors and high-grade from low-grade cancers. To improve their clinical utility, second-generation genomic predictors will need to be developed separately for the different molecular and phenotypic subsets of breast cancers.

15.4 Molecular Studies on FNAC from Metastatic Breast Tumors

Another important field to use molecular assessment on FNAC material is metastatic breast cancer, which is usually diagnosed by a combination of clinical and imaging findings. Once diagnosed, the choice of systemic therapy is based on the ER, PR, and HER2 status from the patient's primary tumor. Biopsy of suspected metastatic lesions is rarely done. Intra-tumor heterogeneity at both the genetic and protein levels is well described in breast cancer. It is, therefore, not surprising that discordance in tumor characteristics between primary and recurrent breast cancers has been observed. Retrospective studies show discrepancies between primary and metastasis with variations of up to 30 % for the hormonal receptors and 5–10 % for HER2 status (Amir et al. 2011). Recent studies obtained from clinical trials with tissue confirmation of disease recurrence showed rates of discordance between the primary tumor and the recurrence for ER, PR, and HER2 in 12.6, 31.2, and 5.5 %, respectively. For ER and HER2, there were similar rates of gain and loss of receptor expression, and for PR, loss of receptor was seen more commonly than gain (Amir et al. 2011).

The results of these studies highlighted the need of obtaining tissue confirmation of recurrence in breast cancer. Since surgical biopsy of metastasis might be associated with negative outcomes such as anxiety, pain, treatment delay, and high costs, FNAC can be a safe, trustworthy, and cheaper alternative to obtain cells from metastatic sites to study cell characteristics. (Wilking U et al. 2011) using FISH for HER2 in FNAC samples from metastatic sites of breast cancer showed an intra-patient agreement in HER2 status of 76 % and a disagreement of 10 %. The multivariable Cox analysis showed a significantly increased risk of dying in the patient group with changed HER2 status compared to patients with concordant positive HER2 status. The unstable status for HER2 in breast cancer is clinically significant and should motivate more frequent testing of recurrences. In our own experience, we observed 15 % of disagreement between HER2 assessment in primary and respective metastases of breast cancer, using FISH on FNAC material (data not published). So, in conclusion, FNAC is a less traumatic method that provides a good source of breast cancer cells, including from metastatic sites, to perform ISH for HER2 with excellent

quality preservation of integrity of the nuclei and signals. Moreover, discordance in gene or protein expression between primary and recurrent breast cancer may extend beyond ER, PR, and HER2 and include other potential drug targets. Additional mutation analysis of phosphatase and tensin homolog (PTEN) and phosphoinositide 3-kinase (PI3K) has been proposed in patients with HER2-amplified tumors to detect therapeutically resistant tumors. FISH to detect loss of heterozygosity (LOH) for PTEN as well as RT-PCR and sequencing to detect PTEN or PI3K mutations can be done in FNAC material.

15.5 Molecular Studies on FNAC Material Used for Frozen Tissue Banking of Breast Cancer

Molecular breast cancer characterization methods such as expression microarrays require fresh-frozen tissues as RNA sources. High-quality fresh-frozen human neoplastic and normal tissues may be stored in tumor banks through validated procedures for collection, storage, retrieval, shipping, and tracking of samples. Various approaches to banking research tissue specimens have been described. Most facilities use either tissue fragments storage within cryovial tubes or embedding in cryosection molds using cryopreservation media. In some instances (small tumors or after neoadjuvant volume-reducing treatments), it is impossible to collect the 0.5 cm^3 minimum recommended sample without compromising diagnosis. As discussed before, breast cancer molecular studies have already been successfully performed in cytological samples as smears obtained from scraping fresh breast tumors or fine-needle samples. In an attempt to spare tissue for histological diagnosis, fine-needle sampling can be performed on fresh tumors to obtain representative tissue (Eloy et al. 2009). After collection, the needle can be rinsed with phosphate-buffered saline (PBS) in a labeled Eppendorf tube. The tube can be frozen and stored at −70 °C, and RNA can be successfully extracted from this material. So, fine-needle sampling of breast cancer surgical specimens is an effective tissue-sparing method for tissue collection and banking. High-quality RNA can be obtained from this material. This methodology is a very useful alternative to keep material for molecular studies from small tumors in which we need to include all the material for histological evaluation.

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Comparison of Aspiration and Core Needle Biopsy

16

16.1 Introduction

In the assessment of breast lesions, the time-honored triple approach utilizing clinical, radiologic, and pathologic assessment cannot be overemphasized. In the pathologic assessment of breast lesions, small sample assessments (FNAC and needle biopsy) have largely replaced excisional biopsy, as the former do not require general anesthesia and are associated with lower morbidity and complication rate. Many centers now use both FNAC and needle biopsy as the initial investigations of choice.

FNAC can be performed either by free hand or by ultrasound guidance. The technique has been described in a previous chapter and is summarized here. In either case, the needle is inserted into the lesion, then a mild suction is applied, with to and fro movements made. Cellular materials are thus aspirated into the syringe hub by capillary action. Each needle insertion is considered one pass. The operator may elect to perform one pass or multiple passes. The aspirated materials may be spread onto glass slides to makes smears, which may either be air-dried or alcohol fixed, or put into alcohol fixative to make cell blocks.

Needle biopsy includes core needle biopsy and vacuum-assisted breast biopsy. Core needle biopsy is a percutaneous procedure that involves removing small samples of breast tissue using a hollow large-core needle, usually gauge 14 or 16. Palpable lesions may be biopsied manually by fixing the lesion with one hand, and both palpable and non-palpable lesions may be biopsied under stereotactic mammography or ultrasound image guidance. As a single sample is obtained each time the device is inserted, multiple insertions are needed to obtain sufficient breast tissue for diagnosis.

Vacuum-assisted biopsy, on the other hand, is a procedure that relies on stereotactic mammography or ultrasound imaging using a gauge 11 core needle. It allows for the removal of multiple tissue samples by vacuum aspiration and unlike core needle biopsies, only requires a single insertion of the special biopsy probe into the breast through a small nick made into the skin.

16.2 Comparison Between FNAC and Core Biopsy

Comparing the methodologies, FNAC is in general much quicker and can be performed in the office setting without special equipment. It is safe and inexpensive. Complications are minimal, with possibly minor hemorrhage being the most common complication. Needle biopsies, on the other hand, require special equipment, and the biopsy needles are considerably more expensive than a syringe for FNAC. As more tissue is removed, needle biopsy is associated with significantly more complications including hemorrhage. The time required is significantly more. This is particularly so for vacuum-assisted biopsy. In terms of cost, which is an important consideration as breast services are usually high volume, FNAC is much cheaper than needle biopsy.

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In terms of pathologic diagnosis, in general, both FNAC and needle biopsies are accepted to be highly accurate in the assessment of breast lesions. Nevertheless, there exist subtle differences between these sampling modalities. An awareness of these differences is essential in appropriately choosing between FNAC and needle biopsies, as well as in the correct interpretation of the results. In the literature, there are very few reviews comparing the efficacy of FNAC and needle biopsies, and some series that assessed these modalities performed on the same lesions by the same groups of operators showed that needle biopsy demonstrated higher specificity, sensitivity, and lower suspicious and inadequacy rates (Barra Ade et al. 2008; Shannon et al. 2001). Furthermore, the interpretation of needle biopsy, when compared to FNAC, appeared to be less operator dependent, probably because the skill level required is less, as the diagnosis can be based on more lesional tissues, the availability of architectural features, as well as having more material for further studies (e.g., immunohistochemistry) including assessment of prognostic and predictive factors like hormone receptors and HER2 expression (Shannon et al. 2001). As a result, in many centers, needle biopsies are gaining popularity over FNAC. However, in centers handling high patient volumes and with limited resources, FNAC is very often still the investigation of choice, mainly because of its low cost and the ease of performance. Another problem for needle biopsy is that it cannot be done in some anatomical locations, like areas near to the skin, chest wall, or in the supraclavicular fossa. Some authors also argued that for small lesions, needle biopsy (particularly vacuum-assisted biopsy) may completely remove the lesions, making eventual assessment of margins difficult (Tse and Tan 2010).

In the realm of diagnostic breast pathology, there are specific areas or groups of lesions that cause significant diagnostic difficulties. These include non-palpable, screen-detected calcifications, low-grade malignancy and borderline lesions, DCIS and invasive carcinoma, papillary lesions, and selected fibroepithelial lesions. Many of these lesions are seen commonly and are likely to be encountered by practicing cytologists.

16.3 Non-palpable, Screen-Detected Calcifications

With the increasing availability and performance of breast screening, more and more clinically silent, non-palpable breast lesions, whether they present with architectural distortion or calcifications, are being encountered. As these radiological features are worrisome, most of these lesions require pathologic diagnosis. The efficacy of both FNAC and needle biopsies has been the subject of many studies. In general it has been shown that FNAC had a lower sensitivity and specificity (about 70 %) in this group of lesions, as compared to needle biopsies which attained a sensitivity and specificity of about 90 % (Leifland et al. 2003). If one is to take out the uncertain categories in FNAC, that is, the atypical and suspicious categories, the accuracy of FNAC may improve to about 90–95 % (Leifland et al. 2003). High inadequacy rate is often considered the main problem of FNAC in dealing with non-palpable breast lesions, with the reported inadequacy rates ranging from 10 % to up to 58 %, whereas the reported inadequacy rate was up to 20 % for core needle biopsies (Ibrahim et al. 2001; Boerner et al. 1999). It would thus appear that needle biopsy shows a much better performance than FNAC. However, if one considers the issue of inadequacy further, it becomes apparent that while the definition of inadequacy is easily established in FNAC, with widely used quantitative criteria of less than six groups of epithelial cells in all slides (Boerner et al. 1999), the same is not readily available for needle biopsy. A needle biopsy showing only benign fibrous tissue is likely to be reported as benign rather than inadequate. As a result, the quoted inadequacy rate of needle biopsies may represent an underestimation. Nevertheless, it is not difficult to envisage that performing an FNAC on a non-palpable lesion is fraught with challenges. These include difficulties in localization of the lesion, and if the lesion is associated with hyalinization or is hypo-

cellular, yielding adequate materials for diagnosis is unlikely.

Among the different needle biopsies, the results are also different. Vacuum-assisted biopsies appear to give higher sensitivity and specificity over smaller gauge core needle biopsies, even though the performance of the latter improved with correlation with radiological localization. In the detection of calcifications, in which FNAC is not useful, vacuum-assisted biopsies also demonstrate a higher detection rate of calcifications in nonpalpable breast lesions over needle core biopsies (Lacambra et al. 2011).

16.4 Low-Grade Malignancy and Borderline Lesions

The role of FNAC in the diagnosis of low-grade malignant lesions and in the differentiation of this group of lesion from atypical epithelial hyperplasia is limited. Nevertheless, this differentiation is important as there is significant difference in clinical impact of the diagnostic labels. In current clinical practice, a DCIS, even if it is of low grade, is considered and treated as a more "severe" disease as compared to ADH. In addition a cytologic diagnosis of atypia has no direct relationship with these "atypical or borderline" lesions on histology. The cytologic diagnosis of "atypia" is again a nonspecific category. About 30–45 % of atypical aspirates turn out to be malignant, whereas 55–70 % are benign. To view from another perspective, a significant proportion of malignant lesions are diagnosed as atypia, and also, up to 23 % of the benign lesions were reported as atypia on FNAC (Tran et al. 2010). So far no cytologic features in an atypical aspirate had been reported to be consistently useful in prediction of the histologic outcome to be either benign or malignant (Lim et al. 2004). The reasons for the existence of this cytologically atypical group are many and can be categorized into technical, interpretative, and intrinsic. Technical causes include samples with bloodstained background, scanty materials, or drying artifact, affecting correct interpretation. Interpretative causes usually refer to the inexperience of the interpreter. The most significant reason however is the intrinsic cause, which is related to the overlapping cytologic features of many lesions belonging to this category (Lim et al. 2004). This is inevitable, as our understanding of the borderline lesions increases, particularly with the unfolding of the molecular mechanisms. It is now believed that many of the borderline lesions, ranging from flat epithelial atypia, ADH, and lobular neoplasia to low-grade duct carcinoma (in situ or invasive, including tubular carcinoma), are all different phases of a similar molecular progression model of the so-called low-nuclear-grade neoplasia family. This group of low-grade lesions is characterized by similar atypical cytomorphology, with the differentiating features being complex architecture and larger size for ADH and low grade DCIS respectively.

Needle biopsies, in general, are more accurate in diagnosing borderline lesions. Nevertheless, the accuracy is not total. A needle biopsy of ADH may actually reflect partial sampling of a more extensive DCIS of the same morphology. Indeed, up to 45 % of low-grade DCIS cases may be diagnosed as ADH on needle biopsy (Wagoner et al. 2009), particularly in those DCIS that are of smaller sizes and low nuclear grade. It was further reported that a needle biopsy having more than two foci of ADH or showing a micropapillary pattern of ADH is associated with an increased chance of DCIS on excision (Wagoner et al. 2009). Comparing vacuum-assisted biopsy and core needle biopsy, it appears that the adequacy and accuracy of vacuum-assisted biopsy is independent on the number of cores taken, whereas for trucut core needle biopsy, a minimum of three cores is required (Lacambra et al. 2011), indicating that inadequate sampling may occur in core needle biopsy but not in vacuum-assisted biopsy.

16.5 Ductal Carcinoma In Situ and Invasive Carcinoma

One of the fundamental questions of breast FNAC is whether or not invasion can be diagnosed in a malignant breast aspirate. This has great clinical significance, as the treatment

offered to the patients differ significantly. DCIS, in general, only requires local excision, whereas invasive carcinoma usually requires both local excision and some form of lymph node assessment (axillary dissection or sentinel lymph nodes assessment). In daily practice, most cytologists will refrain from proclaiming the invasion status of a malignant breast aspirate. Are there any cytologic parameters that would allow one to predict invasion? Several features, including proliferation of fibroblasts, elastoid stromal fragments, tubular structures, infiltration into fragments of fat, and infiltration into fibrous tissue fragments, have been reported to be useful in predicting invasion (Sauer et al. 2006; Klijanienko et al. 2004; Mckee et al. 2001). Among these features, infiltration into fibrous tissue fragment has been considered the most reliable (Klijanienko et al. 2004), but false-positive results had been reported using this criterion alone. Even if one were to use two to three of these parameters, this set of criteria is still hampered by low sensitivity and significant false positivity, which is a highly undesirable outcome (Sauer et al. 2006; Mckee et al. 2001). Given this inherent problem of diagnosing invasion in FNAC, and the importance of knowing the invasion status preoperatively, should needle biopsy be considered the sole alternative? Interestingly, if one looks at DCIS diagnosed at needle biopsy and comparing this with the subsequent excision, up to 44 % of these cases are upgraded to invasive carcinomas in the final excision, that is, the needle biopsy also misses invasion significantly (Dillon et al. 2006). What is more problematic is that despite efforts by many investigators, there are no good predictors for invasion in needle biopsies showing DCIS only. The predictors that have been evaluated include radiological features (mass, density, calcifications), histologic features (size, grade, necrosis, calcifications, architecture, lobular extension, periductal inflammation, and periductal fibrosis), and sampling adequacy (sampled core numbers). None of these had been shown to be reliable and consistent predictors. Nevertheless, the extent of the disease as indicated by the percentage of positive cores was a reasonable predictor (Go et al. 2010). Other than that, currently there is still no reliable predictor for invasion for needle biopsies showing DCIS only.

16.6 Papillary Lesions

Papillary lesions of the breast represent a highly problematic area for accurate cytologic diagnosis. The cytologic details of and problems associated with papillary lesions have been covered in a previous chapter. In short, papillary lesions include a wide range of breast lesions with the biological behavior ranging from benign to malignant. This results in a wide morphologic spectrum and attempts to accurately diagnose and categorize these lesions in small samples, either in FNAC or needle biopsies, can be problematic. Apart from the common prototypic benign duct papilloma and papillary carcinoma, papillomas can also be complicated by superimposed florid epithelial hyperplasia, atypical epithelial hyperplasia, and DCIS. What is more daunting is that the dividing line between papilloma with ADH and papilloma with DCIS is based purely on size, with a 3 mm atypical focus being used as a cutoff. As there is no qualitative difference between these entities, it would not be possible to attain cytologic differentiation. Given the difficulty in cytologic diagnosis, the National Cancer Institute (NCI) guideline has placed papillary lesions into an intermediate category (Cytology Subgroup of the National Coordinating Committee for Breast Cancer Screening Pathology 1994). In the literature, the diagnostic accuracy of FNAC in papillary lesions is reported to be low, when compared to most other breast lesions, and the accuracy ranges from 27 to 88 % (Tse et al. 2008). The generally low accuracy of FNAC in diagnosing papillary lesions notwithstanding, would it be possible to differentiate between benign and malignant papillary lesions cytologically? The differences in the cytologic features are subtle, with malignant papillary lesions being associated with longer, more slender, and elaborate papillary fronds, higher overall cellularity, more epithelial cell balls without fibrovascular cores, and atypical

cells in the background. Nevertheless, these features may not be unanimously present in all cases (Choi et al. 2006), and the overall accuracy of FNAC in diagnosing malignant papillary lesions remains low and in some series reported to be less than 50 %. At needle biopsy, diagnosing papillary lesions is relatively easy, and this represents a distinct advantage of needle biopsy over FNAC. However, in the detailed categorization of papillary lesions, needle biopsy faces the same problems as encountered in FNAC. In fact, up to 35 % of benign papillomas diagnosed at needle biopsy are upgraded to either atypical or malignant lesions in some series (Rizzo et al. 2008). In the differentiation between benign and atypical or even malignant papillary lesions, there are established histologic criteria, but unfortunately the differentiating features are frequently quantitative rather than qualitative. Hence, in a biopsy sample, full assessment of the lesion may be hampered by the size of the available sample, thus resulting in significant under-calling in the biopsy diagnosis. Interestingly when one looks at needle biopsy and excision results for papillary lesions, the false-negative (under-calling) rate is usually higher than the false-positive (over-calling) rate.

16.7 Fibroepithelial Lesions

Fibroepithelial lesions usually refer commonly to fibroadenomas and phyllodes tumors. Fibroadenomas are generally accepted to be benign, whereas phyllodes tumors may behave in a variable fashion, from running a totally benign clinical course, to a propensity for local recurrences, to giving rise to rare distant metastases. Complete excision with adequate margins is considered the optimal treatment for phyllodes tumors, but there is no similar margin requirement for fibroadenomas. The recent WHO Classification of Breast Tumors (2012) has recommended favoring a diagnosis of fibroadenoma in benign fibroepithelial lesions that show some but not all characteristic features of benign phyllodes tumors, in order to avoid overtreatment. This highlights the importance of preoperative accurate diagnosis of these fibroepithelial lesions to aid optimal treatment planning. There are two major problems associated with the FNAC assessment of phyllodes tumors, namely, (1) the differentiation of phyllodes tumors from fibroadenomas and (2) the grading of phyllodes tumors in FNAC. To date, the diagnostic accuracy of FNAC for phyllodes tumors is suboptimal, with a reported accuracy rate ranging from 25 to 70 % (Dusenbery and Frable 1992; Bhattarai et al. 2000). The underlying reasons for this poor cytologic performance are multifold, including overlapping features of phyllodes tumors and fibroadenomas, as well as the heterogeneous growth pattern of phyllodes tumors. The cytologic features that have been reported to be useful for phyllodes tumors are increased stromal fragments or stromal to epithelial fragment ratio, the presence of more cellular stromal fragments, columnar cells in the background, and higher overall cellularity (Bhattarai et al. 2000). Similar problems are being encountered in needle biopsies, which in some instances also fail to differentiate phyllodes tumors from fibroadenomas (Jara-Lazaro et al. 2010). Some histologic parameters have been cited to be useful in the setting of needle biopsy differentiation of phyllodes tumors from fibroadenomas, and these include stromal hypercellularity, stromal nuclear atypia, stromal overgrowth, the presence of >2 mitotic figures per 10 high-power fields, and the presence of pseudoangiomatous stromal hyperplasia (PASH) (Tsang et al. 2011). The detection of malignancy in aspirates that are diagnosed as phyllodes tumors is even more problematic. Although some reported the presence of hypercellular smears, mitotic figures, phyllodal fragments, and atypia of the stromal cells as helpful (Vladescu et al. 2004), others cautioned that assessment of mitotic figures in FNAC of phyllodes tumors may not be reliable (Jayaram and Sthaneshwar 2002). As malignant phyllodes tumors are rare, FNAC experience with their diagnosis remains scant.

16.8 Summary

Both FNAC and needle biopsy are very useful and accurate in diagnosing most breast lesions, particularly when coupled with the triple

assessment approach. FNAC is cheaper, quicker, and easier to perform but generally obtains less material for further ancillary testing, particularly in malignant cases. Due to the inherent nature of the sampling, FNAC is also associated with higher inadequacy and insufficient rates. In specific lesions, FNAC and needle biopsy show different efficacy in achieving the correct diagnosis, and this should be borne in mind by all breast health professionals in choosing between FNAC or needle biopsy, and in evaluating the pathologic results. When dealing with non-palpable screen-detected lesions, needle biopsies excel over FNAC as more material and better retrieval of calcifications are achieved. Vacuum-assisted biopsy is also significantly better than core needle biopsy in this respect. When dealing with atypical and borderline lesions, FNAC is unable to provide differentiation between lesions within this group, due to the overlapping and frequently similar cytomorphology, as the distinction is frequently based on architecture and size, parameters that are not assessable in FNAC. Needle biopsy does allow a diagnosis of borderline lesions to be made, but again this is also associated with significant "under-grading" of the lesions. The accuracy of core needle biopsy is dependent on a minimal number of cores sampled, but not the vacuum-assisted biopsy, indicating that the latter provides adequate sampling even in a single core sample. One of the fundamental limitations of FNAC in breast cancer is the inability to predict invasion reliably. Despite repeated assessment, no cytology criterion has been reliably demonstrated to be consistently useful. Needle biopsy, while highly accurate in confirming invasion in breast cancer when invasive foci are seen in the biopsy, is much less useful in predicting invasion when the biopsy shows only carcinoma in situ. Again no histologic criterion has been shown to be consistently useful. The FNAC diagnosis of papillary lesions and their grading are problematic, whereas using needle biopsy, papillary lesions can be diagnosed with greater accuracy and certainty, but there is still a significant false negativity with missing of papillary carcinoma. Within the fibroepithelial group, FNAC cannot reliably differentiate between phyllodes tumors and fibroadenoma, and when a phyllodes tumor is suspected, its grading is also problematic cytologically. As discussed previously, many of these problems are related to the inherent nature of the FNAC; thus, these problems are likely to persist even under optimal FNAC conditions. This should be borne in mind when one is choosing FNAC as an investigative procedure and also in the interpretation of the FNAC results.

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Future Directions

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Breast cancer has become a major health problem globally, being the most common female cancer in many parts of the world. In developed countries, the high incidence shows some evidence of plateauing, whereas in developing countries, the incidence is generally lower but is on the increase. Whether this will "catch up" with the developed countries remains unknown. As the population base in developing countries is much larger, even a slight increase in cancer incidence means a significant increase in patient numbers. Henceforth, the investigation and diagnosis of breast lesions is likely to continue to be a major health-care and planning issue. Currently the time-honored triple assessment dictates pathologic evaluation being one of the mandatory tools of assessment of breast lesions. FNAC is expected to play a critical role as it is a much cheaper and quicker investigative modality, and the performance of FNAC does not require special instruments. Widespread use of core needle biopsy may not be financially feasible in some of the health-care systems; hence, FNAC will likely be the method of choice for many of these regions expecting a larger increase in the number of breast cancer cases. Compared to core biopsy, FNAC is more operator dependent, requiring higher skill levels in the proper interpretation (Westenend et al. 2001). Correlation of the aspiration impression is also crucial; thus, optimally the interpreter should also be the aspirator. Under experienced hands, FNAC achieves high sensitivity and specificity (Gordon et al. 1993). In the routine setting, FNAC is highly useful in the vast majority of the cases that are likely to be encountered. Virtually all the benign lesions, including inflammatory lesions, fibrocystic changes, and other benign tumors including fibroadenomas, can very often be diagnosed with confidence in an FNAC sample. The same also applies to most of the malignant lesions. Bearing in mind that most of the malignant breast cancers are infiltrating ductal carcinoma not otherwise specified, these can be diagnosed without difficulty on FNAC. One long-standing problem associated with FNAC diagnosis of cancers is the inability to reliably differentiate between noninvasive (in situ) and invasive carcinomas. This has significant bearing on the subsequent surgical management of the patients.

Our increased understanding of the underlying molecular mechanisms of breast carcinogenesis has led to the widespread interest in molecular classification of breast cancers, which is in contrast to the historic histologic classification, and has demonstrated utility in the management of the patients. As the molecular classification is essentially based on the expression profile of genes related to hormone receptors, HER2, and other basal cytokeratins, knowledge of the "molecular status" of the tumor confers advantage in the management, particularly in the choice of therapy (including personalized targeted therapy). In the routine day-to-day practice, using immunohistochemistry to assess these "molecular signatures" has become a common practice. Breast cytology also possesses the ability for allowing these immunohistochemical tests to be

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done on the cytology materials, thus obviating a more aggressive, traumatic, needle core biopsy that requires more time and even larger instrument (mammotome). The assessment of hormone receptors and HER2 can be easily done in the cytology preparation, especially with making a thrombin clot or cell block. These would then be processed routinely and immunohistochemical stainings performed. Theoretically, this would allow the commencement of neoadjuvant chemotherapy without waiting for needle core or excisional biopsy. There is good evidence in the literature that both ER and RP staining are accurate when compared to routine histologic assessment (Moriki et al. 2004), particularly for ER (Cano et al. 2003) rather than PR.

Furthermore, breast cytology has developed to such a state in which the performance of many investigations that were not feasible previously could now be done with ease. Immunohistochemistry can now be performed in the cell-block material. A variety of markers have been previously investigated to aid in the differentiation of benign and malignant breast lesions, including Ki-67, p53, p63, E-cadherin, and cyclin D1. As mentioned previously, hormone receptors and HER2 had prognostic and predictive value and can now be assessed in the FNAC preparation, and this is particularly relevant in the neoadjuvant setting or in the assessment of recurrences. Furthermore, in addition to ER, PR, and HER2, there is preliminary evidence that bcl2 can also be a useful biomarker to predict tumor response to therapy (Becette et al. 2011). Thus, it is expected that a multitude of clinically important tests can be performed in the FNAC samples, providing a comprehensive profile of the breast cancer.

There are specific groups of breast lesions that are difficult to be diagnosed at FNAC. These include the non-palpable borderline lesions, the papillary lesions, and some fibroepithelial lesions. Among the non-palpable lesions, the most common lesions that are encountered are florid epithelial hyperplasia, columnar cell lesions including flat epithelial hyperplasia and ADH or low-grade DCIS, as well as lobular neoplasia. In general, although the detailed cytologic differentiating features have been covered in the previous chapters, in actual daily practice, a confident classification and differentiation of these entities is still unachievable. However, one needs to put this into the proper clinical perspective. Firstly, as most of these lesions are non-palpable, it is difficult to ensure direct sampling of these lesions at FNAC. Even with imaging guidance, it may not be possible to sample these lesions adequately by cytologic means. The consequence of imprecision in the diagnoses of misdiagnosing these lesions may also not be very critical. Our current understanding of the biological behavior of these borderline lesions is that they tend to evolve slowly, and only some of these may eventually become cancers, which are usually low grade. Furthermore, the alternative means of preoperative diagnosis, by histology with any forms of needle core biopsies, is also associated with significant (albeit less than FNAC) degree of inaccuracy (Tse and Tan 2010). Hence, currently there is no good answer to the evaluation of borderline lesions of the breast. As the finer differentiation of these entities may depend on the actual size of the lesions, histologic assessment of the completely excised lesions still remains the gold standard.

The second category of problematic diagnoses is papillary lesions. It is well recognized that papillary lesions as a group encompass a variety of lesions with varying biological behavior, ranging from benign to low-grade malignancy, with many lesions behaving in between these ends. An accurate diagnosis is always difficult, both in histologic core biopsy and cytology. The underlying reasons are multiple, the main considerations being that firstly, papillary lesions can be heterogeneous, with admixed benign and malignant (or atypical) elements present within the same lesions, and secondly, the assessment of the size of the lesion has a determinate role in the ultimate diagnosis – while a small focus of atypical hyperplasia would be diagnosed as atypical hyperplasia, a similar but larger focus will necessitate a diagnosis of carcinoma in situ. In FNAC preparation, one cannot assess the lesional size; even if the atypical epithelial changes were successfully

identified, reaching a correct diagnosis would still be impossible. Rarely there could be exceptions, when the aspirate was done with a wide-bore needle, giving adequate tissue fragments for correct identification of the papillary nature and the confirmation of degree of epithelial atypia.

The other category of problematic diagnosis is fibroepithelial lesions. While they are mostly benign fibroadenomas, some of these may actually represent phyllodes tumors, which may show varying degrees of biological behavior, with malignant tumors behaving in a sarcomatous fashion. In general, phyllodes tumors show higher degrees of stromal cellular proliferation, atypia, and mitotic activity. Correct preoperative identification of these lesions is ideal, but it may not be possible in many situations. The underlying reason is the often heterogeneous stromal morphology of these lesions, with some areas being indistinguishable from the less cellular benign fibroadenoma.

Cytology is also playing an increasingly important role in the molecular studies of breast lesions. While the most common molecular platforms that are amenable to cytologic samples are PCR and in situ hybridization, other techniques such as in situ PCR, microarrays, proteomic and sequencing (including next-generation sequencing) methodologies are now being validated. In situ hybridization (ISH) permits either fluorescent or chromogenic markers to detect numerical or structural aberrations of chromosomes. This technique is reliable and is particularly suited to direct smears that are either ethanol- or air-dried fixed or cell-block slides (Schmitt 2011). HER2 assessment using FISH or SISH is now possible and useful in FNA with excellent correlation with the histological specimens. The advantages of using cytological materials are many, including the ease of obtaining fresh material, ability to check the quality of the material immediately after harvest, and better preservation of DNA and RNA. Hence, easier monitoring of cancer, including therapeutic response, with repeated testings, can be achieved (Schmitt and Vielh 2012). In addition, liquid-based cytology offers the possibility of preservation of cells in an environment of excellent quality, especially when compared to formalin-fixed and paraffin-embedded tissues. The development of cDNA microarrays allows high-throughput, genome-wide analysis for potentially relevant molecular markers of disease (Di Lorito and Schmitt 2011). As our knowledge of molecular progresssion and carcinogenesis increases, additional targets for therapeutic interventions will no doubt be identified. Cytological testing will assume an increasingly important position in patient management in the identification of additional biomarker targets in patients and the close monitoring of patients' response to specific targeted therapy.

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